STATE OF OKLAHOMA CONTRACT WITH WATERS TECHNOLOGIES CORPORATION

This State of Oklahoma Contract ("Contract") is entered into between the State of Oklahoma by and through the Oklahoma Department of Agriculture, Food and Forestry ("State") and Waters Technologies Corporation ("Supplier") and is effective as of the effective date set forth on a properly issued purchase order or, if no effective date is listed, the date of last signature ("Effective date"). The term of the Contract is one (1) year with no renewal options.

Purpose

The State is awarding the Contract to Supplier for the purchase of a High-Performance Liquid Chromatograph with Vacuum Degasser equipped with QQQ Mass Spectrometer, Autosampler and Instrument Controller, as more particularly described in certain Contract Documents. Supplier submitted a proposal with no exceptions, vendor documents or confidentiality requests. Supplier did include a best and final offer. This Contract Document memorializes the agreement of the parties with respect to terms of the Contract that is being awarded to Supplier.

Now, therefore, in consideration of the foregoing and the mutual promises set forth herein, the receipt and sufficiency of which are hereby acknowledged the parties agree as follows:

1. The parties agree that Supplier has not yet begun performance of work under the Contract. Issuance of a purchase order is required prior to payment to a Supplier.

2. The following Contract Documents are attached hereto and incorporated herein:

   2.1. Attachment A - Solicitation;
   2.2. Attachment A1 - Non-Negotiable Terms;
   2.3. Attachment B – Negotiable Terms;
   2.4. Attachment C - Reserved;
   2.5. Attachment D - IT Terms;
   2.6. Attachment E1 -Pricing;
   2.7. Attachment F - Requested Exceptions; and
   2.8. Attachment E1 - Quote No. 23953257
   2.9. Attachment E2 - Additional Bidder Terms - Waters Licenses, Warranties, and Support Services;
   2.10. Attachment E3 - Bidder's Statement of Coverage;
   2.11. Attachment F Exceptions and Add Terms;
   2.12. Exhibit 1 - Specifications and Requirements;
   2.13. Exhibit 2 - Response to Specifications and Requirements;

2.14. Exhibit 3 - Micro Specifications and Instrument Capabilities;
2.15. Exhibit 4 - Response to State of Oklahoma Questions;
2.16. Exhibit 5 - Implementation & Transfer Method to Water Platform;
2.17. Exhibit 6 - Application Note; and
2.18. Amendment No. 1 to Solicitation.

3. The parties additionally agree:

3.1. Except for information deemed confidential by the State pursuant to applicable law, rule, regulation or policy, the parties agree Contract terms and information are not confidential and are disclosable without further approval of or notice to Supplier.

3.2. To the extent any term or condition in any Contract Document, including via a hyperlink or uniform resource locator, conflicts with an applicable Oklahoma and/or United States law or regulation, such term or condition is void and unenforceable. By executing any Contract Document which contains a conflicting term or condition, the State or Customer makes no representation or warranty regarding the enforceability of such term or condition and the State or Customer does not waive the applicable Oklahoma and/or United States law or regulation which conflicts with the term or condition.

4. The parties recognize that while the State of Oklahoma is executing this contract, payment obligations rest solely with the Office of the Oklahoma Department of Agriculture, Food and Forestry and OMES shall not be responsible for such. Please send invoices and billing inquiries to:

Laurie Mercer
Oklahoma Department of Agriculture,
Food and Forestry 2800 N. Lincoln Blvd.
Oklahoma City, OK 73105
(405) 522-4381
Email: Laurie.Mercer@ag.ok.gov

5. Any reference to a Contract Document refers to such Contract Document as it may have been amended. If and to the extent any provision is in multiple documents and addresses the same or substantially the same subject matter but does not create an actual conflict, the more recent provision is deemed to supersede earlier versions.

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SIGNATURES

The undersigned represent and warrant that they are authorized, as representatives of the party on whose behalf they are signing, to sign this Agreement and to bind their respective party thereto.

STATE OF OKLAHOMA
by and through the OKLAHOMA DEPARTMENT OF AGRICULTURE, FOOD AND FORESTRY :

By: Daniel C. Ridings
Daniel C. Ridings (Mar 24, 2025 13:06 CDT)

Name: Daniel C. Ridings

Title: Director of Administrative Services

Date: Mar 24, 2025

WATERS TECHNOLOGIES CORPORATION

By: Timothy D'Souza
Timothy D'Souza (Mar 21, 2025 14:26 EDT)

Name: Timothy D'Souza

Title: Contract Manager

Date: Mar 21, 2025

OKLAHOMA DEPARTMENT OF AGRICULTURE FOOD AND FORESTRY GENERAL COUNSEL:

By: James W. Rucker
James W. Rucker (Mar 24, 2025 08:22 CDT)

Name: James W. Rucker

Title: Deputy General Counsel

Date: Mar 24, 2025

OKLAHOMA DEPARTMENT OF AGRICULTURE FOOD AND FORESTRY
Purchasing Officer:

By:
Mitch Broiles (Mar 24, 2025 07:43 CDT)

Name: Mitch Broiles

Title: APO IV

Date: Mar 24, 2025

The State Purchasing Director is signing solely to ensure state agency compliance with provisions of the Oklahoma Central Purchasing Act pursuant to 74 O.S., 85.5 concerning acquisitions by state agencies.

By: Amanda L Otis
Amanda Otis (Mar 24, 2025 13:28 CDT)

Name: Amanda Otis

Title: State Purchasing Director

Date: Mar 24, 2025

Attachment A

This Solicitation is a Contract Document and is a request for proposal in connection with the Contract awarded by the Office of Management and Enterprise Services as more particularly described below. Any defined term used herein but not defined herein shall have the meaning ascribed in the General Terms or other Contract document.

I. PURPOSE

The Office of Management and Enterprise Services (OMES), Central Purchasing Division, is seeking responses on behalf of the Oklahoma Department of Agriculture, Food and Forestry from potential Suppliers to provide a contract for the purchase of a High-Performance Liquid Chromatograph with Vacuum Degasser equipped with QQQ Mass Spectrometer, Autosampler and Instrument Controller. A Contract resulting from this Solicitation may be designated for use as a Statewide Contract.[1]

The Contract is awarded on behalf of Oklahoma Department of Agriculture, Food and Forestry for High-Performance Liquid Chromatograph with Vacuum Degasser equipped with QQQ Mass Spectrometer, Autosampler and Instrument Controller. All state agencies and state affiliates may avail themselves of this contract.

1. Contract Term and Renewal Options:

1.1. The initial Contract term, which begins on the effective date of the Contract, is one year with no renewal options.

2. Solicitation Criterion:

2.1. The Bid will be evaluated using a best value criterion, based on the following:

i. Price.
ii. Ability to meet specifications.

2.2 Scope and Description:

i. The Bid shall show the ability of the Bidder to meet or exceed the specifications as listed in Exhibit 1. Bidders shall respond to each line of specifications on Exhibit 1 indicating product bid meets that specification or explain any variation or exception to these minimum specifications in detail and item by item.

ii. Pricing shall be proposed as a single total firm, fixed cost and include all information concerning fees, other costs and any other information relevant to the total cost.

[1] 74 O.S. 85.5(G)(3)

II. STATE OF OKLAHOMA NON-NEGOTIABLE GENERAL TERMS

In addition to other terms contained in an applicable Contract document, Supplier and State agree to the following General Terms:

1 Scope and Contract Renewal

1.1 Supplier may not add products or services to its offerings under the Contract without the State's prior written approval. Such request may require a competitive bid of the additional products or services. If the need arises for goods or services outside the scope of the Contract, Supplier shall contact the State.

1.2 At no time during the performance of the Contract shall the Supplier have the authority to obligate any Customer for payment for any products or services (a) when a corresponding encumbering document is not signed or (b) over and above an awarded Contract amount. Likewise, Supplier is not entitled to compensation for a product or service provided by or on behalf of Supplier that is neither requested nor accepted as satisfactory.

1.3 If applicable, prior to any Contract renewal, the State shall subjectively consider the value of the Contract to the State, the Supplier's performance under the Contract, and shall review certain other factors, including but not limited to the: a) terms and conditions of Contract documents to determine validity with current State and other applicable statutes and rules; b) current pricing and discounts offered by Supplier; and c) current products, services and support offered by Supplier. If the State determines changes to the Contract are required as a condition precedent to renewal, the State and Supplier will cooperate in good faith to evidence such required changes in an Amendment. Further, any request for a price increase in connection with a renewal or otherwise will be conditioned on the Supplier providing appropriate documentation supporting the request.

1.4 Upon mutual agreement, the Parties may extend the Contract for ninety (90) days beyond a final renewal term. The Parties may to the extent allowable by law, choose to exercise subsequent ninety (90) day extensions.

1.5 Supplier understands that supplier registration expires annually and, pursuant to OAC 260:115-3-3, Supplier shall maintain its supplier registration with the State as a precondition to a renewal of the Contract.

2 Contract Effectiveness

2.1 Unless specifically agreed in writing otherwise, the Contract is effective upon the date last signed by the parties. Supplier shall not commence work, commit funds, incur costs, or in any way act to obligate the State until a proper purchase order has been issued.

2.2 Any Contract document shall be legibly written in ink or typed. All Contract transactions, and any Contract document related thereto, may be conducted by electronic means pursuant to the Oklahoma Uniform Electronic Transactions Act.

3 Modification of Contract Terms and Contract documents

3.1 The Contract may only be modified, amended, or expanded by an Amendment. Any change to the Contract, including the addition of work or materials, the revision of payment terms, or the substitution of work or materials made unilaterally by the Supplier, is a material breach of the Contract. Unless otherwise specified by applicable law or rules, such changes, including without limitation, any unauthorized written Contract modification, shall be void and without effect and the Supplier shall not be entitled to any claim under the Contract based on those changes. No oral statement of any person shall modify or otherwise affect the terms, conditions, or specifications stated in the Contract.

3.2 Any additional terms on an ordering document provided by Supplier are of no effect and are void unless mutually executed. OMES bears no liability for performance, payment or failure thereof by the Supplier or by a Customer other than OMES in connection with an Acquisition.

3.3 Except for information deemed confidential by the State pursuant to applicable law, rule, regulation, or policy, the parties agree Contract terms are not confidential and are disclosable without further approval of or notice to Supplier.

3.4 Unless mutually agreed to in writing by the State of Oklahoma by and through the Office of Management and Enterprise Services, no Contract document or other terms and conditions or clauses, including via a hyperlink or uniform resource locator, shall supersede or conflict with the terms of this Contract or expand the State's or Customer's liability or reduce the rights of Customer or the State.

3.5 To the extent any term or condition in any Contract document, including via a hyperlink or uniform resource locator, conflicts with an applicable Oklahoma and/or United States law or regulation, such term or condition is void and unenforceable. By executing any Contract document which contains a conflicting term or condition, the State or Customer makes no representation or warranty regarding the enforceability of such term or condition and the State or Customer does not waive the applicable Oklahoma and/or United States law or regulation which conflicts with the term or condition.

4 Pricing

4.1 Pursuant to 68 O.S. §§ 1352, 1356, and 1404, State agencies are exempt from the assessment of State sales, use, and excise taxes. Further, State agencies and political subdivisions of the State are exempt from Federal Excise Taxes pursuant to Title 26 of the United States Code. Any taxes of any nature whatsoever payable by the Supplier shall not be reimbursed.

4.2 Pursuant to 74 O.S. §85.40, all travel expenses of Supplier must be included in the total Acquisition price.

4.3 The price of a product offered under the Contract shall include and Supplier shall prepay all shipping, packaging, delivery and handling fees. All product deliveries will be free on-board Customer's Destination. No additional fees shall be charged by Supplier for standard shipping and handling. If Customer requests expedited or special delivery, Customer may be responsible for any charges for expedited or special delivery

4.4 Any product to be delivered pursuant to the Contract shall be subject to final inspection and acceptance by the Customer at Destination. The Customer assumes no responsibility for a product until accepted by the Customer. Title and risk of loss or damage to a product shall be the responsibility of the Supplier until accepted. The Supplier shall be responsible for filing, processing, and collecting any and all damage claims accruing prior to acceptance

4.5 Pursuant to OAC 260:115-9-1, payment for an Acquisition does not constitute final acceptance of the Acquisition. If subsequent inspection affirms that the Acquisition does not meet or exceed the specifications of the order or that the Acquisition has a latent defect, the Supplier shall be

notified as soon as is reasonably practicable. The Supplier shall retrieve and replace the Acquisition at Supplier's expense or, if unable to replace, shall issue a refund to Customer. Refund under this section shall not be an exclusive remedy.

5 Invoices and Payments

5.1 Supplier shall be paid upon submission of a proper invoice(s) at the prices stipulated in the Contract in accordance with 74 O.S. §85.44B which requires that payment be made only after products have been provided and accepted or services rendered and accepted This section shall not prohibit the payment of membership dues or payment for subscriptions to magazines, periodicals or books or for payment to vendors providing subscription services under 74 O.S. 85.44B.
The following terms additionally apply:

A. An invoice shall contain the purchase order number, description of products or services provided and the dates of such provision.

B. Failure to provide a timely and proper invoice may result in delay of processing the invoice for payment. Proper invoice is defined at OAC 260:10-1-2.

C. Payment of all fees under the Contract shall be due NET 30 days but shall not be deemed late until 45 days. Payment and interest on late payments are governed by 62 O.S. §34.72. Such interest is the sole and exclusive remedy for late payments by a State agency and no other late fees are authorized to be assessed pursuant to Oklahoma law.

D. The date from which an applicable early payment discount time is calculated shall be from the receipt date of a proper invoice. There is no obligation, however, to utilize an early payment discount.

E. If an overpayment or underpayment has been made to Supplier any subsequent payments to Supplier under the Contract may be adjusted to correct the account. A written explanation of the adjustment will be issued to Supplier.

F. If the Supplier accepts payment by Purchase Card they shall do so according to Oklahoma law.

6 Oklahoma Open Records Act

Supplier acknowledges that all State agencies and certain other Customers are subject to the Oklahoma Open Records Act set forth at 51 O.S. §24A-1 et seq. Supplier also acknowledges that compliance with the Oklahoma Open Records Act and all opinions of the Oklahoma Attorney General concerning the Act is required. Customer may be provided access to Supplier Confidential Information. State agencies are subject to the Oklahoma Open Records Act and Supplier acknowledges information marked confidential information will be disclosed to the extent permitted under the Open Records Act and in accordance with this section. Nothing herein is intended to waive the State Purchasing Director's authority under OAC 260:115-3-9 in connection with Bid information requested to be held confidential by a Bidder. Notwithstanding the foregoing, Supplier Confidential Information shall not include information that: (i) is or becomes generally known or available by public disclosure, commercial use or otherwise and is not in contravention of this Contract; (ii) is known and has been reduced to tangible form by the receiving party before the time of disclosure for the first time under this Contract and without other obligations of confidentiality; (iii) is independently developed without the use of any of Supplier Confidential Information; (iv) is lawfully obtained from a third party (without any confidentiality obligation) who has the right to make such disclosure or (v) pricing provided to the State. In addition, the obligations in this section shall not apply to the extent that the applicable law or regulation requires disclosure of Supplier Confidential Information, provided that the Customer provides reasonable written notice, pursuant to Contract notice provisions, to the Supplier so that the Supplier may promptly seek a protective order or other appropriate remedy.

7 Conflict of Interest

In addition to any requirement of law or of a professional code of ethics or conduct, the Supplier, its employees are required to disclose any outside activity or interest that conflicts or may conflict with the best interest of the State. Prompt disclosure is required under this section if the activity or interest is related, directly or indirectly, to any person or entity currently under contract with or seeking to do business with the State, its employees or any other third-party individual or entity awarded a contract with the State. Further, as long as the Supplier has an obligation under the Contract, any plan, preparation or engagement in any such activity or interest shall not occur without prior written approval of the State. Any conflict of

interest shall, at the sole discretion of the State, be grounds for partial or whole termination of the Contract.

8 State Shall Not Indemnify

The State of Oklahoma cannot lawfully agree to indemnify a private contractor. The credit of the State shall not be given, pledged, or loaned to any individual, company, corporation, or association, municipality, or political subdivision of the State pursuant to Oklahoma Constitution article 10, Section 15, OAC 260:115-7-32(k)(3)(A) and Attorney General Opinion 2012-18.

9 Indemnification Coordination of Defense

9.1 In connection with indemnification obligations under the Contract, when a State agency is a named defendant in any filed or threatened lawsuit, the defense of the State agency shall be coordinated by the Attorney General of Oklahoma, or the Attorney General may authorize the Supplier to control the defense and any related settlement negotiations; provided, however, Supplier shall not agree to any settlement of claims against the State without obtaining advance written concurrence from the Attorney General. If the Attorney General does not authorize sole control of the defense and settlement negotiations to Supplier, Supplier shall have authorization to equally participate in any proceeding related to the indemnity obligation under the Contract and shall remain responsible to indemnify the applicable Indemnified Parties.

10 Termination for Funding Insufficiency

10.1 Notwithstanding anything to the contrary in any Contract document, the State may terminate the Contract in whole or in part if funds sufficient to pay obligations under the Contract are not appropriated or received from an intended third-party funding source. In the event of such insufficiency, Supplier will be provided at least fifteen (15) calendar days' written notice of termination. Any partial termination of the Contract under this section shall not be construed as a waiver of, and shall not affect, the rights and obligations of any party regarding portions of the Contract that are not terminated. The determination by the State of insufficient funding shall be accepted by, and shall be final and binding on, the Supplier.

10.2 Upon receipt of notice of a termination, Supplier shall immediately comply with the notice terms and take all necessary steps to minimize the incurrence of costs allocable to the work affected by the notice. If a purchase order or other payment mechanism has been issued and a product or service has been accepted as satisfactory prior to the effective date of termination, the

termination does not relieve an obligation to pay for the product or service but there shall not be any liability for further payments ordinarily due under the Contract or for any damages or other amounts caused by or associated with such termination. Any amount paid to Supplier in the form of prepaid fees that are unused when the Contractor certain obligations are terminated shall be refunded.

10.3 The State's exercise of its right to terminate the Contract under this section shall not be considered a default or breach under the Contract or relieve the Supplier of any liability for claims arising under the Contract.

11 Suspension of Supplier

11.1 Supplier may be subject to Suspension without advance notice and may additionally be suspended from activities under the Contract if Supplier fails to comply with confidentiality, privacy, security, environmental or safety requirements applicable to Supplier's performance or obligations under the Contract.

11.2 Upon receipt of a notice pursuant to this section, Supplier shall immediately comply with the notice terms and take all necessary steps to minimize the incurrence of costs allocable to the work affected by the notice. If a purchase order or other payment mechanism has been issued and a product or service has been accepted as satisfactory prior to receipt of notice by Supplier, the Suspension does not relieve an obligation to pay for the product or service but there shall not be any liability for further payments ordinarily due under the Contract during a period of Suspension or suspended activity or for any damages or other amounts caused by or associated with such Suspension or suspended activity. A right exercised under this section shall not be an exclusive remedy but shall be in addition to any other rights and remedies provided for by law. Any amount paid to Supplier in the form of prepaid fees attributable to a period of Suspension or suspended activity shall be refunded.

11.3 Such Suspension may be removed, or suspended activity may resume, at the earlier of such time as a formal notice is issued that authorizes the resumption of performance under the Contract or at such time as a purchase order or other appropriate encumbrance document is issued. This subsection is not intended to operate as an affirmative statement that such resumption will occur.

12 Certification Regarding Debarment, Suspension, and Other Responsibility Matters

The certification made by Supplier with respect to Debarment, Suspension, certain indictments, convictions, civil judgments and terminated public contracts is a material representation of fact upon which reliance was placed when entering into the Contract. A determination that Supplier knowingly rendered an erroneous certification, in

addition to other available remedies, may result in whole or partial termination of the Contract for Supplier's default. Additionally, Supplier shall promptly provide written notice to the State Purchasing Director if the certification becomes erroneous due to changed circumstances.

13 Certification Regarding State Employees Prohibition From Fulfilling Services

Pursuant to 74 O.S. § 85.42, the Supplier certifies that no person involved in any manner in development of the Contract employed by the State shall be employed to fulfill any services provided under the Contract.

14 Notices

All notices, approvals or requests allowed or required by the terms of any Contract shall be in writing, reference the Contract with specificity and deemed delivered upon receipt or upon refusal of the intended party to accept receipt of the notice. Notice information may be updated in writing to the other party as necessary.
In addition to other notice requirements in the Contract and the designated Supplier contact provided in a successful Bid, notices shall be sent to the State at the email address set forth below.

Notwithstanding any other provision of the Contract, confidentiality, breach and termination-related notices shall be delivered to the address below in addition to e-mail.

If sent to the State:
State Purchasing Director
2401 North Lincoln Blvd., Second Floor
Oklahoma City, Oklahoma 73105

With a copy, which shall not constitute notice, to:
Purchasing Division Deputy General Counsel
2401 North Lincoln Blvd., Second Floor

Oklahoma City, Oklahoma 73105

15 Miscellaneous

15.1 Choice of Law and Venue

Any claim, dispute, or litigation relating to the Contract documents, in the singular or in the aggregate, shall be governed by the laws of the State of Oklahoma without regard to application of choice of law principles. Pursuant to 74 O.S. §85.7(F), where Federal awards are involved, applicable federal laws, rules and regulations shall govern to the extent necessary to insure ensure compliance with the terms of the Federal award. Venue for any action, claim, dispute, or litigation relating in any way to the Contract documents, shall be in Oklahoma County, Oklahoma. The State expressly declines any terms that minimize its rights under Oklahoma Law, including but not limited to, Statutes of Limitations.

15.2 Employment Relationship

The Contract does not create an employment relationship. Individuals providing products or performing services pursuant to the Contract are not employees of the State or Customer and, accordingly are not eligible for any rights or benefits whatsoever accruing to such employees.

15.3 Failure to Enforce

Failure by the State or a Customer at any time to enforce a provision of, or exercise a right under, the Contract shall not be construed as a waiver of any such provision. Such failure to enforce or exercise shall not affect the validity of any Contract document, or any part thereof, or the right of the State or a Customer to enforce any provision of, or exercise any right under, the Contract at any time in accordance with its terms. Likewise, a waiver of a breach of any provision of a Contract document shall not affect or waive a subsequent breach of the same provision or a breach of any other provision in the Contract.

15.4 Invalid Term or Condition

To the extent any term or condition in the Contract conflicts with a compulsory applicable State or United States law or regulation, such Contract term or condition is void and unenforceable. By executing any Contract document which contains a conflicting term or condition, no

representation or warranty is made regarding the enforceability of such term or condition. Likewise, any applicable State or federal law or regulation which conflicts with the Contract or any non-conflicting applicable State or federal law or regulation is not waived.

15.5 Severability

If any provision of a Contract document, or the application of any term or condition to any party or circumstances, is held invalid or unenforceable for any reason, the remaining provisions shall continue to be valid and enforceable and the application of such provision to other parties or circumstances shall remain valid and in full force and effect. If a court finds that any provision of this contract is invalid or unenforceable, but that by limiting such provision it would become valid and enforceable, then such provision shall be deemed to be written, construed, and enforced as so limited.

15.6 Section Headings

The headings used in any Contract document are for convenience only and do not constitute terms of the Contract.

15.7 Sovereign Immunity

Notwithstanding any provision in the Contract, the Contract is entered into subject to the State's Constitution, statutes, common law, regulations, and the doctrine of sovereign immunity, none of which are waived by the State nor any other right or defense available to the State; provided, however, that the parties hereby agree that the doctrine of sovereign immunity does not apply to actions grounded in contract and therefore does not prohibit Supplier from pursuing claims arising under the Contract against the State and Customers.

15.8 Survival

As applicable, performance under all license, subscription, service agreements, statements of work, transition plans and other similar Contract documents entered into between the parties under the terms of the Contract shall survive Contract expiration. Additionally, rights and obligations under the Contract which by their nature should survive including, without limitation, certain payment obligations invoiced prior to expiration or termination; confidentiality obligations; security incident and data breach

obligations and indemnification obligations, remain in effect after expiration or termination of the Contract.

15.9 Gratuities

The Contract may be immediately terminated, in whole or in part, by written notice if it is determined that the Supplier, its authorized employee, agent, or another representative acting within the scope of their authority violated any federal, State or local law, rule or ordinance by offering or giving a gratuity to any State employee directly involved in the Contract. In addition, Suspension or Debarment of the Supplier may result from such a violation.

15.10 Import/Export Controls

Neither party will use, distribute, transfer or transmit any equipment, services, software or technical information provided under the Contract (even if incorporated into other products) except in compliance with all applicable import and export laws, conventions and regulations.

ATTACHMENT B

STATE OF OKLAHOMA NEGOTIABLE GENERAL TERMS

This State of Oklahoma General Terms ("General Terms") is a Contract document in connection with a Contract awarded by the Office of Management and Enterprise Services on behalf of the State of Oklahoma.

In addition to other terms contained in an applicable Contract document, Supplier and State agree to the following General Terms:

1 Contract Order of Priority

1.1 Contract documents shall be read to be consistent and complementary. Any conflict among the Contract documents shall be resolved by giving priority to Contract documents in the following order of precedence:

A. any Amendment.

B. terms contained in this Contract document.

C. any Contract-specific State terms contained in a Contract document including, without limitation, information technology terms and terms specific to a statewide Contract or a State agency Contract.

D. any applicable Solicitation.

E. any successful Bid as may be amended through negotiation and to the extent the Bid does not otherwise conflict with the Solicitation, Contract or applicable law.

F. any statement of work, work order, or other mutually agreed Contract documents.

1.2 If there is a conflict between the terms contained in this Contract document or in Contract-specific terms and an agreement provided by or on behalf of Supplier including but not limited to linked or supplemental documents which alter or diminish the rights of Customer or the State, the conflicting terms provided by Supplier shall not take priority over this Contract document or Acquisition-specific terms. In no event will any linked document alter or override such referenced terms except as specifically agreed in an Amendment.

2 Definitions

In addition to any defined terms set forth elsewhere in the Contract, the Oklahoma Central Purchasing Act and the Oklahoma Administrative Code, Title 260, the parties agree that, when used in the Contract, the following terms are defined as set forth below and may be used in the singular or plural form:

2.1 **Acquisition** means items, products, materials, supplies, services and equipment acquired by purchase, lease purchase, lease with option to purchase, value provided or rental under the Contract.

2.2 **Amendment** means any mutually executed, written modification to a Contract document or a written change, addition, correction or revision to a Solicitation.

2.3 **Bid** means an offer a Bidder submits in response to the Solicitation.

2.4 **Bidder** means an individual or business entity that submits a Bid in response to the Solicitation.

2.5 **Contract** means the written, mutually agreed and binding legal relationship resulting from the Contract documents and an appropriate encumbering document as may be amended from time to time, which evidences the final agreement between the parties with respect to the subject matter of the Contract.

2.6 **Customer** means the entity receiving goods or services contemplated by the Contract.

2.7 **Debarment** means action taken by a debarring official under federal or state law or regulations to exclude any business entity from inclusion on the Supplier list; bidding; offering to bid; providing a quote; receiving an award of contract with the State and may also result in cancellation of existing contracts with the State.

2.8 **Destination** means delivered to the receiving dock or other point specified in the applicable Contract document.

2.9 **Federal award** means the Federal financial assistance that a recipient receives directly from a Federal awarding agency or indirectly from a pass-through entity

2.10 **Governmental Entity** means any governmental entity specified as a political subdivision of the State pursuant to the Governmental Tort Claim Act including any associated institution, instrumentality, board, commission, committee, department, or other entity designated to act on behalf of the state.

2.11 **Indemnified Parties** means the State and Customer and/or its officers, directors, agents, employees, representatives, contractors, assignees and designees thereof.

2.12 **Inspection** means examining and testing an Acquisition (including, when appropriate, raw materials, components, and intermediate assemblies) to determine whether the Acquisition meets Contract requirements.

2.13 **Moral Rights** means any and all rights of paternity or integrity of the Work Product and the right to object to any modification, translation or use of the Work Product and any similar rights existing under the judicial or statutory law of any country in the world or under any treaty, regardless of whether or not such right is denominated or referred to as a moral right.

2.14 **OAC** means the Oklahoma Administrative Code.

2.15 **OMES** means the Office of Management and Enterprise Services.

2.16 **Solicitation** means the document inviting Bids for the Acquisition referenced in the Contract and any amendments thereto.

2.17 **State** means the government of the state of Oklahoma, its employees and authorized representatives, including without limitation any department, agency, or other unit of the government of the state of Oklahoma.

2.18 **Supplier** means the Bidder with whom the State enters into the Contract awarded pursuant to the Solicitation or the business entity or individual that is a party to the Contract with the State.

2.19 **Suspension** means action taken by a suspending official under federal or state law or regulations to suspend a Supplier from inclusion on the Supplier list; be eligible to submit Bids to State agencies and be awarded a contract by a State agency subject to the Central Purchasing Act.

2.20 **Supplier Confidential Information** means certain confidential and proprietary information of Supplier that is clearly marked as confidential and agreed by the State Purchasing Director or Customer, as applicable, but does not include information excluded from confidentiality in provisions of the Contract or the Oklahoma Open Records Act.

2.21 **Work Product** means any and all deliverables produced by Supplier under a statement of work or similar Contract document issued pursuant to this Contract, including any and all tangible or intangible items or things that have been or will be prepared, created, developed, invented or conceived at any time following the Contract effective date including but not limited to any (i) works

of authorship (such as manuals, instructions, printed material, graphics, artwork, images, illustrations, photographs, computer programs, computer software, scripts, object code, source code or other programming code, HTML code, flow charts, notes, outlines, lists, compilations, manuscripts, writings, pictorial materials, schematics, formulae, processes, algorithms, data, information, multimedia files, text web pages or web sites, other written or machine readable expression of such works fixed in any tangible media, and all other copyrightable works), (ii) trademarks, service marks, trade dress, trade names, logos, or other indicia of source or origin, (iii) ideas, designs, concepts, personality rights, methods, processes, techniques, apparatuses, inventions, formulas, discoveries, or improvements, including any patents, trade secrets and know-how, (iv) domain names, (v) any copies, and similar or derivative works to any of the foregoing, (vi) all documentation and materials related to any of the foregoing, (vii) all other goods, services or deliverables to be provided by or on behalf of Supplier under the Contract and (vii) all Intellectual Property Rights in any of the foregoing, and which are or were created, prepared, developed, invented or conceived for the use of benefit of Customer in connection with this Contract or with funds appropriated by or for Customer or Customer's benefit (a) by any Supplier personnel or Customer personnel or (b) any Customer personnel who then became personnel to Supplier or any of its affiliates or subcontractors, where, although creation or reduction-to-practice is completed while the person is affiliated with Supplier or its personnel, any portion of same was created, invented or conceived by such person while affiliated with Customer.

3 Additional Pricing

3.1 The price of a product offered under the Contract shall include and Supplier shall prepay all shipping, packaging, delivery and handling fees. All product deliveries will be free on-board Customer's Destination. No additional fees shall be charged by Supplier for standard shipping and handling. If Customer requests expedited or special delivery, Customer may be responsible for any charges for expedited or special delivery.

3.2 Supplier shall have no right of setoff.

3.3 Because funds are typically dedicated to a particular fiscal year, an invoice will be paid only when timely submitted, which shall in no instance be later than six (6) months after the end of the fiscal year in which the goods are provided or services performed.

4 Ordering, Inspection, and Acceptance

4.1 Any product or service furnished under the Contract shall be ordered by issuance of a valid purchase order or other appropriate payment mechanism, including a pre-encumbrance, or by use of a valid Purchase Card. All orders and transactions are governed by the terms and conditions of the Contract. Any purchase order or other applicable payment mechanism dated prior to termination or expiration of the Contract shall be performed unless mutually agreed in writing otherwise.

4.2 Services will be performed in accordance with industry best practices and are subject to acceptance by the Customer. Notwithstanding any other provision in the Contract, deemed acceptance of a service or associated deliverable shall not apply automatically upon receipt of a deliverable or upon provision of a service.

Supplier warrants and represents that a product or deliverable furnished by or through the Supplier shall individually, and where specified by Supplier to perform as a system, be substantially uninterrupted and error-free in operation and guaranteed against faulty material and workmanship for a warranty period of the greater of ninety (90) days from the date of acceptance or the maximum allowed by the manufacturer. A defect in a product or deliverable furnished by or through the Supplier shall be repaired or replaced by Supplier at no additional cost or expense to the Customer if such defect occurs during the warranty period.

Any product to be delivered pursuant to the Contract shall be subject to final inspection and acceptance by the Customer at Destination. The Customer assumes no responsibility for a product until accepted by the Customer. Title and risk of loss or damage to a product shall be the responsibility of the Supplier until accepted. The Supplier shall be responsible for filing, processing, and collecting any and all damage claims accruing prior to acceptance.

Pursuant to OAC 260:115-9-1, payment for an Acquisition does not constitute final acceptance of the Acquisition. If subsequent inspection affirms that the Acquisition does not meet or exceed the specifications of the order or that the Acquisition has a latent defect, the Supplier shall be notified as soon as is reasonably practicable. The Supplier shall retrieve and replace the Acquisition at Supplier's expense or, if unable to replace, shall issue a refund to Customer. Refund under this section shall not be an exclusive remedy.

4.3 Supplier shall deliver products and services on or before the required date specified in a Contract document. Failure to deliver timely may result in liquidated damages as set forth in the applicable Contract document. Deviations, substitutions, or changes in a product or service, including changes of personnel directly providing services, shall not be made unless expressly authorized in writing by the Customer. Any substitution of personnel directly providing services shall be a person of comparable or greater skills, education and experience for performing the services as the person being replaced. Additionally, Supplier shall provide staff sufficiently experienced and able to perform with respect to any transitional services provided by Supplier in connection with termination or expiration of the Contract.

4.4 Product warranty and return policies and terms provided under any Contract document will not be more restrictive or more costly than warranty and return policies and terms for other similarly situated customers for a like product.

5 Maintenance of Insurance, Payment of Taxes, and Workers' Compensation

5.1 As a condition of this Contract, Supplier shall procure at its own expense, and provide proof of, insurance coverage with the applicable liability limits set forth below and any approved subcontractor of Supplier shall procure and provide proof of the same coverage. The required insurance shall be underwritten by an insurance carrier with an A.M. Best rating of A- or better.

Such proof of coverage shall additionally be provided to the Customer if services will be provided by any of Supplier's employees, agents or subcontractors at any Customer premises and/or employer vehicles will be used in connection with performance of Supplier's obligations under the Contract. Supplier may not commence performance hereunder until such proof has been provided. Additionally, Supplier shall ensure each insurance policy includes a notice of cancellation and includes the State and its agencies as certificate holder and shall promptly provide proof to the State of any renewals, additions, or changes to such insurance coverage. Supplier's obligation to maintain insurance coverage under the Contract is a continuing obligation until Supplier has no further obligation under the Contract. Any combination of primary and excess or umbrella insurance may be used to satisfy the limits of coverage for Commercial General Liability, Auto Liability and Employers' Liability. Unless agreed between the parties and approved by the State Purchasing Director, the minimum acceptable insurance limits of liability are as follows:

A. Workers' Compensation and Employer's Liability Insurance in accordance with and to the extent required by applicable law.

B. Commercial General Liability Insurance covering the risks of personal injury, bodily injury (including death) and property damage, including coverage for contractual liability, with a limit of liability of not less than $2,000,000 per occurrence.

C. Automobile Liability Insurance with limits of liability of not less than $2,000,000 combined single limit each accident.

D. If the Supplier will access, process, or store state data, then Security and Privacy Liability insurance, including coverage for failure to protect confidential information and failure of the security of Supplier's computer systems that results in unauthorized access to Customer data with limits $5,000,000 per occurrence; and

E. Additional coverage required in writing in connection with a particular Acquisition.

5.2 Supplier shall be entirely responsible during the existence of the Contract for the liability and payment of taxes payable by or assessed to Supplier or its employees, agents and subcontractors of whatever kind, in connection with the Contract. Supplier further agrees to comply with all state and federal laws applicable to any such persons, including laws regarding wages, taxes, insurance, and Workers' Compensation. Neither Customer nor the State shall be liable to the Supplier, its employees, agents, or others for the payment of taxes or the provision of unemployment insurance and/or Workers' Compensation or any benefit available to a State or Customer employee.

5.3 Supplier agrees to indemnify Customer, the State, and its employees, agents, representatives, contractors, and assignees for any and all liability, actions, claims, demands, or suits, and all related costs and expenses (including without limitation reasonable attorneys' fees and costs required to establish the right to indemnification) relating to tax liability, unemployment insurance and/or Workers' Compensation in connection with its performance under the Contract.

6 Compliance with Applicable Laws

6.1 As long as Supplier has an obligation under the terms of the Contract and in connection with performance of its obligations, the Supplier represents its present compliance, and shall have an ongoing obligation to comply, with all applicable federal, State, and local laws, rules, regulations, ordinances, and orders, as amended, including but not limited to the following:

A. Drug-Free Workplace Act of 1988 set forth at 41 U.S.C. §81.

B. Section 306 of the Clean Air Act, Section 508 of the Clean Water Act, Executive Order 11738, and Environmental Protection Agency Regulations which prohibit the use of facilities included on the EPA List of Violating Facilities under nonexempt federal contracts, grants or loans.

C. Prospective participant requirements set at 45 C.F.R. part 76 in connection with Debarment, Suspension and other responsibility matters.

D. 1964 Civil Rights Act, Title IX of the Education Amendment of 1972, Section 504 of the Rehabilitation Act of 1973, Americans with Disabilities Act of 1990, and Executive Orders 11246 and 11375.

E. Anti-Lobbying Law set forth at 31 U.S.C. §1325 and as implemented at 45 C.F.R. part 93.

F. Requirements of Internal Revenue Service Publication 1075 regarding use, access and disclosure of Federal Tax Information (as defined therein).

G. Obtaining certified independent audits conducted in accordance with Government Auditing Standards and Office of Management and Budget Uniform Guidance, 2 CFR 200 Subpart F §200.500 et seq. with approval and work paper examination rights of the applicable procuring entity.

H. Requirements of the Oklahoma Taxpayer and Citizen Protection Act of 2007, 25 O.S. §1312 and applicable federal immigration laws and regulations and be registered and participate in the Status Verification System. The Status Verification System is defined at 25 O.S. §1312, includes but is not limited to the free Employment Verification Program (E-Verify) through the Department of Homeland Security, and is available at **Home | E-Verify**;

I. Requirements of the Health Insurance Portability and Accountability Act of 1996; Health Information Technology for Economic and Clinical Health Act; Payment Card Industry Security Standards; Criminal Justice Information System Security Policy and Security Addendum; and Family Educational Rights and Privacy Act; and

J. Be registered as a business entity licensed to do business in the State, have obtained a sales tax permit, and be current on franchise tax payments to the State, as applicable.

6.2 The Supplier's employees, agents and subcontractors shall adhere to applicable Customer policies including, but not limited to acceptable use of Internet and electronic mail, facility and data security, press releases, and public relations. As applicable, the Supplier shall adhere to the State Information Security Policy, Procedures, Guidelines set forth at **Information Security Policy, Procedures, Guidelines (oklahoma.gov)** Supplier is responsible for reviewing and relaying such policies covering the above to the Supplier's employees, agents and subcontractors.

6.3 At no additional cost to Customer, the Supplier shall maintain all applicable licenses and permits required in association with its obligations under the Contract.

6.4 In addition to compliance under subsection 6.1 above, Supplier shall have a continuing obligation to comply with applicable Customer-specific mandatory contract provisions required in connection with the receipt of federal funds or other funding source.

6.5 The Supplier is responsible to review and inform its employees, agents, and subcontractors who provide a product or perform a service under the Contract of the Supplier's obligations under the Contract and Supplier certifies that its employees and each such subcontractor shall comply with minimum requirements and applicable provisions of the Contract. At the request of the State, Supplier shall promptly provide adequate evidence that such persons are its employees, agents or approved subcontractors and have been informed of their obligations under the Contract.

6.6 As applicable, Supplier agrees to comply with the Governor's Executive Orders related to the use of any tobacco product, electronic cigarette or vaping device on any and all properties owned, leased, or contracted for use by the State, including but not limited to all buildings, land and vehicles owned, leased, or contracted for use by agencies or instrumentalities of the State.

6.7 The execution, delivery and performance of the Contract and any ancillary documents by Supplier will not, to the best of Supplier's knowledge, violate, conflict with, or result in a breach of any provision of, or constitute a default (or an event which, with notice or lapse of time or both, would constitute a default) under, or result in the termination of, any written contract or other instrument between Supplier and any third party.

6.8 Supplier represents that it has the ability to pay its debts when due and it does not anticipate the filing of a voluntary or involuntary bankruptcy petition or appointment of a receiver, liquidator or trustee.

6.9 Supplier represents that, to the best of its knowledge, any litigation or claim or any threat thereof involving Supplier has been disclosed in writing to the State and Supplier is not aware of any other litigation, claim or threat thereof.

6.10 If services provided by Supplier include delivery of an electronic communication, Supplier shall ensure such communication and any associated support documents are compliant with Section 508 of the Federal Rehabilitation Act and with State standards regarding accessibility. Should any communication or associated support documents be non-compliant, Supplier shall correct and re-deliver such communication immediately upon discovery or notice, at no additional cost to the State. Additionally, as part of compliance with accessibility requirements where documents are only provided in non-electronic format, Supplier shall promptly provide such communication and any associated support documents in an alternate format usable by individuals with disabilities upon request and at no additional cost, which may originate from an intended recipient or from the State.

7 Audits and Records Clause

7.1 As used in this clause and pursuant to 67 O.S. §203, "record" includes a document, book, paper, photograph, microfilm, computer tape, disk, record, sound recording, film recording, video record, accounting procedures and practices, and other data, regardless of type and regardless of whether such items are in written form, in the form of computer data, or in any other form.

7.2 Supplier agrees any pertinent federal or State agency or governing entity of a Customer shall have the right to examine and audit, at no additional cost to a Customer, all records relevant to the execution and performance of the Contract except, unless otherwise agreed, costs of Supplier that comprise pricing under the Contract.

7.3 The Supplier is required to retain records relative to the Contract for the duration of the Contract and for a period of seven (7) years following completion or termination of an Acquisition unless otherwise indicated in the Contract terms. If a claim, audit, litigation or other action involving such records is started before the end of the seven-year period, the records are required to be maintained for two (2) years from the date that all issues arising out of the action are resolved, or until the end of the seven (7) year retention period, whichever is later.

7.4 Pursuant to 74 O.S. §85.41, if professional services are provided hereunder, all items of the Supplier that relate to the professional services are subject to examination by the State agency, State Auditor and Inspector and the State Purchasing Director.

8 Confidentiality

8.1 The Supplier shall maintain strict security of all State and citizen data and records entrusted to it or to which the Supplier gains access, in accordance with and subject to applicable federal and State laws, rules, regulations, and policies and shall use any such data and records only as necessary for Supplier to perform its obligations under the Contract. The Supplier further agrees to evidence such confidentiality obligation in a separate writing if required under such applicable federal or State laws, rules and regulations. The Supplier warrants and represents that such information shall not be sold, assigned, conveyed, provided, released, disseminated or otherwise disclosed by Supplier, its employees, officers, directors, subsidiaries, affiliates, agents, representatives, assigns, subcontractors, independent contractors, successor or any other persons or entities without Customer's prior express written permission. Supplier shall instruct all such persons and entities that the confidential information shall not be disclosed or used without the Customer's prior express written approval except as necessary for Supplier to render services under the Contract. The Supplier further warrants that it has a tested and proven system in effect designed to protect all confidential information.

8.2 Supplier shall establish, maintain and enforce agreements with all such persons and entities that have access to State and citizen data and records to fulfill Supplier's duties and obligations under the Contract and to specifically prohibit any sale, assignment, conveyance, provision, release, dissemination or other disclosure of any State or citizen data or records except as required by law or allowed by written prior approval of the Customer.

8.3 Supplier shall immediately report to the Customer any and all unauthorized use, appropriation, sale, assignment, conveyance, provision, release, access, acquisition, disclosure or other dissemination of any State or citizen data or records of which it or its parent company, subsidiaries, affiliates, employees, officers, directors, assignees, agents, representatives, independent contractors, and subcontractors is aware or have knowledge or reasonable should have knowledge. The Supplier shall also promptly furnish to Customer full details of the unauthorized use, appropriation, sale, assignment, conveyance, provision, release, access, acquisition, disclosure or other dissemination, or attempt thereof, and use its best efforts to assist the Customer in investigating or preventing the reoccurrence of such event in the future. The Supplier shall cooperate with the Customer in connection with any litigation and investigation deemed necessary by the Customer to protect any State or citizen data and records and shall bear all costs associated with the investigation, response and recovery in connection with any breach of State or citizen data or records including but not limited to credit monitoring services with a term of

at least three (3) years, all notice-related costs and toll free telephone call center services.

8.4 Supplier further agrees to promptly prevent a reoccurrence of any unauthorized use, appropriation, sale, assignment, conveyance, provision, release, access, acquisition, disclosure or other dissemination of State or citizen data and records.

8.5 Supplier acknowledges that any improper use, appropriation, sale, assignment, conveyance, provision, release, access, acquisition, disclosure or other dissemination of any State data or records to others may cause immediate and irreparable harm to the Customer and certain beneficiaries and may violate state or federal laws and regulations. If the Supplier or its affiliates, parent company, subsidiaries, employees, officers, directors, assignees, agents, representatives, independent contractors, and subcontractors improperly use, appropriate, sell, assign, convey, provide, release, access, acquire, disclose or otherwise disseminate such confidential information to any person or entity in violation of the Contract, the Customer will immediately be entitled to injunctive relief and/or any other rights or remedies available under this Contract, at equity or pursuant to applicable statutory, regulatory, and common law without a cure period.

8.6 The Supplier shall immediately forward to the State Purchasing Director, and any other applicable person listed in the Notices section(s) of the Contract, any request by a third party for data or records in the possession of the Supplier or any subcontractor or to which the Supplier or subcontractor has access and Supplier shall fully cooperate with all efforts to protect the security and confidentiality of such data or records in response to a third party request.

9 Assignment and Permitted Subcontractors

9.1 Supplier's obligations under the Contract may not be assigned or transferred to any other person or entity without the prior written consent of the State which may be withheld at the State's sole discretion. Should Supplier assign its rights to payment, in whole or in part, under the Contract, Supplier shall provide the State and all affected Customers with written notice of the assignment. Such written notice shall be delivered timely and contain details sufficient for affected Customers to perform payment obligations without any delay caused by the assignment.

9.2 Notwithstanding the foregoing, the Contract may be assigned by Supplier to any corporation or other entity in connection with a merger, consolidation, sale of all equity interests of the Supplier, or a sale of all or substantially all of the assets of the Supplier to which the Contract relates. In any such case, said

corporation or other entity shall by operation of law or expressly in writing assume all obligations of the Supplier as fully as if it had been originally made a party to the Contract. Supplier shall give the State and all affected Customers prior written notice of said assignment. Any assignment or delegation in violation of this subsection shall be void.

9.3 If the Supplier is permitted to utilize subcontractors in support of the Contract, the Supplier shall remain solely responsible for its obligations under the terms of the Contract, for its actions and omissions and those of its agents, employees and subcontractors and for payments to such persons or entities. Prior to a subcontractor being utilized by the Supplier, the Supplier shall obtain written approval of the State of such subcontractor and each employee, as applicable to a particular Acquisition, of such subcontractor proposed for use by the Supplier. Such approval is within the sole discretion of the State. Any proposed subcontractor shall be identified by entity name, and by employee name, if required by the particular Acquisition, in the applicable proposal and shall include the nature of the services to be performed. As part of the approval request, the Supplier shall provide a copy of a written agreement executed by the Supplier and subcontractor setting forth that such subcontractor is bound by and agrees, as applicable, to perform the same covenants and be subject to the same conditions and make identical certifications to the same facts and criteria, as the Supplier under the terms of all applicable Contract documents. Supplier agrees that maintaining such agreement with any subcontractor and obtaining prior written approval by the State of any subcontractor and associated employees shall be a continuing obligation. The State further reserves the right to revoke approval of a subcontractor or an employee thereof in instances of poor performance, misconduct or for other similar reasons.

9.4 All payments under the Contract shall be made directly to the Supplier, except as provided in subsection A above regarding the Supplier's assignment of payment. No payment shall be made to the Supplier for performance by unapproved or disapproved employees of the Supplier or a subcontractor.

9.5 Rights and obligations of the State or a Customer under the terms of this Contract may be assigned or transferred, at no additional cost, to other Customer entities.

10 Background Checks and Criminal History Investigations

Prior to the commencement of any services, performance of background checks and criminal history investigations of the Supplier's employees and subcontractors who will be providing services may be required. If required, the Suppler agrees to provide the State with a description of the background check process to include any vendor's

used to gather information. Supplier will further attest that each employee and subcontractor providing services has passed the background check. Supplier's access to facilities, data and information may be withheld prior to completion of background verification acceptable to the State. The costs of additional background checks beyond Supplier's normal hiring practices shall be the responsibility of the Customer unless such additional background checks are required solely because Supplier will not provide verification of results of its otherwise acceptable normal background checks; in such an instance, Supplier shall pay for the additional background checks. Supplier will coordinate with the State and its employees to complete the necessary background checks and criminal history investigations. Should any employee or subcontractor of the Supplier who will be providing services under the Contract not be acceptable as a result of the background check or criminal history investigation, the Customer may require replacement of the employee or subcontractor in question and, if no suitable replacement is made within a reasonable time, terminate the purchase order or other payment mechanism associated with the project or services.

11 Patents and Copyrights

Without exception, a product or deliverable price shall include all royalties or costs owed by the Supplier to any third party arising from the use of a patent, intellectual property, copyright or other property right held by such third party. Should any third party threaten or make a claim that any portion of a product or service provided by Supplier under the Contract infringes that party's patent, intellectual property, copyright or other property right, Supplier shall enable each affected Customer to legally continue to use, or modify for use, the portion of the product or service at issue or replace such potentially infringing product, or re-perform or redeliver in the case of a service, with at least a functional non-infringing equivalent. Supplier's duty under this section shall extend to include any other product or service rendered materially unusable as intended due to replacement or modification of the product or service at issue. If the Supplier determines that none of these alternatives are reasonably available, the State shall return such portion of the product or deliverable at issue to the Supplier, upon written request, in exchange for a refund of the price paid for such returned goods as well as a refund or reimbursement, if applicable, of the cost of any other product or deliverable rendered materially unusable as intended due to removal of the portion of product or deliverable at issue. Any remedy provided under this section is not an exclusive remedy and is not intended to operate as a waiver of legal or equitable remedies because of acceptance of relief provided by Supplier.

12 Indemnification

12.1 Acts or Omissions

A. Supplier shall defend and indemnify the Indemnified Parties, as applicable, for any and all liability, claims, damages, losses, costs, expenses, demands, suits and actions of third parties (including without limitation reasonable attorneys' fees and costs required to establish the right to indemnification) arising out of, or resulting from any action or claim for bodily injury, death, or property damage brought against any of the Indemnified parties to the extent arising from any negligent act or omission or willful misconduct of the Supplier or its agents, employees, or subcontractors in the execution or performance of the Contract.

B. To the extent Supplier is found liable for loss, damage, or destruction of any property of Customer due to negligence, misconduct, wrongful act, or omission on the part of the Supplier, its employees, agents, representatives, or subcontractors, the Supplier and Customer shall use best efforts to mutually negotiate an equitable settlement amount to repair or replace the property unless such loss, damage or destruction is of such a magnitude that repair or replacement is not a reasonable option. Such amount shall be invoiced to, and is payable by, Supplier sixty (60) calendar days after the date of Supplier's receipt of an invoice for the negotiated settlement amount.

12.2 Infringement

Supplier shall indemnify the Indemnified Parties, as applicable, for all liability, claims, damages, losses, costs, expenses, demands, suits and actions of third parties (including without limitation reasonable attorneys' fees and costs required to establish the right to indemnification) arising from or in connection with Supplier's breach of its representations and warranties in the Contract or alleged infringement of any patent, intellectual property, copyright or other property right in connection with a product or service provided under the Contract. Supplier's duty under this section is reduced to the extent a claimed infringement results from: (a) a Customer's or user's content; (b) modifications by Customer or third party to a product delivered under the Contract or combinations of the product with any non-Supplier-provided services or products unless Supplier recommended or participated in such modification or combination; (c) use of a product or service by Customer in violation of the Contract unless done so at the direction of Supplier, or (d) a non-Supplier product that has not been provided to the State by, through or on behalf of Supplier as opposed to its combination with products Supplier provides to or develops for the State or a Customer as a system.

12.3 Notice and Cooperation

In connection with indemnification obligations under the Contract, the parties agree to furnish prompt written notice to each other of any third-party claim. Any Customer affected by the claim will reasonably cooperate with Supplier and defense of the claim to the extent its interests are aligned with Supplier. Supplier shall use counsel reasonably experienced in the subject matter at issue and will not settle a claim without the written consent of the party being defended and where applicable the Attorney General of Oklahoma, which consent will not be unreasonably withheld or delayed, except that no consent will be required to settle a claim against Indemnified Parties that are not a State agency, where relief against the Indemnified Parties is limited to monetary damages that are paid by the defending party under indemnification provisions of the Contract.

12.4 Limitation of Liability

A. With respect to any claim or cause of action arising under or related to the Contract, neither the State nor any Customer shall be liable to Supplier for lost profits, lost sales or business expenditures, investments, or commitments in connection with any business, loss of any goodwill, or for any other indirect, incidental, punitive, special or consequential damages, even if advised of the possibility of such damages.

B. Notwithstanding anything to the contrary in the Contract, no provision shall limit damages, expenses, costs, actions, claims, and liabilities arising from or related to property damage, bodily injury or death caused by Supplier or its employees, agents or subcontractors; indemnity, security or confidentiality obligations under the Contract; the bad faith, negligence, intentional misconduct or other acts for which applicable law does not allow exemption from liability of Supplier or its employees, agents or subcontractors.

C. The limitation of liability and disclaimers set forth in the Contract will apply regardless of whether Customer has accepted a product or service. The parties agree that Supplier has set its fees and entered into the Contract in reliance on the disclaimers and limitations set forth herein, that the same reflect an allocation of risk between the parties and form an essential basis of the bargain between the parties. These limitations shall apply notwithstanding any failure of essential purpose of any limited remedy.

13 Termination for Cause

13.1 Supplier may terminate the Contract if (i) it has provided the State with written notice of material breach and (ii) the State fails to cure such material breach within thirty (30) days of receipt of written notice. If there is more than one Customer, material breach by a Customer does not give rise to a claim of material breach as grounds for termination by Supplier of the Contract as a whole. The State may terminate the Contract in whole or in part if (i) it has provided Supplier with written notice of material breach, and (ii) Supplier fails to cure such material breach within thirty (30) days of receipt of written notice. Any partial termination of the Contract under this section shall not be construed as a waiver of, and shall not affect, the rights and obligations of any party regarding portions of the Contract that are not terminated.

13.2 The State may terminate the Contract in whole or in part immediately without a thirty (30) day written notice to Supplier if (i) Supplier fails to comply with confidentiality, privacy, security, environmental or safety requirements applicable to Supplier's performance or obligations under the Contract; (ii) Supplier's material breach is reasonably determined to be an impediment to the function of the State and detrimental to the State or to cause a condition precluding the thirty (30) day notice or (iii) when the State determines that an administrative error in connection with award of the Contract occurred prior to Contract performance.

13.3 The State may terminate the Contract if the scope includes PR Vendor services and the Supplier, or Supplier's employee, violate the lobbying clause. PR Vendor services is defined to include a contract for public relations (PR), marketing or communication services. The State may immediately terminate the Contract with no more than 10-day notice under this section.

13.4 Upon receipt of notice of a termination, Supplier shall immediately comply with the notice terms and take all necessary steps to minimize the incurrence of costs allocable to the work affected by the notice. If a purchase order or other payment mechanism has been issued and a product or service has been accepted as satisfactory prior to the effective date of termination, the termination does not relieve an obligation to pay for the product or service but there shall not be any liability for further payments ordinarily due under the Contract or for any damages or other amounts caused by or associated with such termination. Such termination is not an exclusive remedy but is in addition to any other rights and remedies provided for by law. Any amount paid to Supplier in the form of prepaid fees that are unused when the Contract or certain obligations are terminated shall be refunded. Termination of the Contract under this section, in whole or in part, shall not relieve the Supplier of liability for claims arising under the Contract.

13.5 The Supplier's repeated failure to provide an acceptable product or service; Supplier's unilateral revision of linked or supplemental terms that have a materially adverse impact on a Customer's rights or obligations under the Contract (except as required by a governmental authority); actual or anticipated failure of Supplier to perform its obligations under the Contract; Supplier's inability to pay its debts when due; assignment for the benefit of Supplier's creditors; or voluntary or involuntary appointment of a receiver or filing of bankruptcy of Supplier shall constitute a material breach of the Supplier's obligations, which may result in partial or whole termination of the Contract. This subsection is not intended as an exhaustive list of material breach conditions. Termination may also result from other instances of failure to adhere to the Contract provisions and for other reasons provided for by applicable law, rules or regulations; without limitation, OAC 260:115-9-1 is an example.

14 Termination for Convenience

14.1 The State may terminate the Contract, in whole or in part, for convenience if it is determined that termination is in the State's best interest. In the event of a termination for convenience, Supplier will be provided at least thirty (30) days' written notice of termination. Any partial termination of the Contract shall not be construed as a waiver of, and shall not affect, the rights and obligations of any party regarding portions of the Contract that remain in effect.

14.2 Upon receipt of notice of such termination, Supplier shall immediately comply with the notice terms and take all necessary steps to minimize the incurrence of costs allocable to the work affected by the notice. If a purchase order or other payment mechanism has been issued and a product or service has been accepted as satisfactory prior to the effective date of termination, the termination does not relieve an obligation to pay for the product or service but there shall not be any liability for further payments ordinarily due under the Contract or for any damages or other amounts caused by or associated with such termination. Such termination shall not be an exclusive remedy but shall be in addition to any other rights and remedies provided for by law. Any amount paid to Supplier in the form of prepaid fees that are unused when the Contract or certain obligations are terminated shall be refunded. Termination of the Contract under this section, in whole or in part, shall not relieve the Supplier of liability for claims arising under the Contract.

15 Suspension of Supplier

15.1 Supplier may be subject to Suspension without advance notice and may additionally be suspended from activities under the Contract if Supplier fails

to comply with confidentiality, privacy, security, environmental or safety requirements applicable to Supplier's performance or obligations under the Contract.

15.2 Upon receipt of a notice pursuant to this section, Supplier shall immediately comply with the notice terms and take all necessary steps to minimize the incurrence of costs allocable to the work affected by the notice. If a purchase order or other payment mechanism has been issued and a product or service has been accepted as satisfactory prior to receipt of notice by Supplier, the Suspension does not relieve an obligation to pay for the product or service but there shall not be any liability for further payments ordinarily due under the Contract during a period of Suspension or suspended activity or for any damages or other amounts caused by or associated with such Suspension or suspended activity. A right exercised under this section shall not be an exclusive remedy but shall be in addition to any other rights and remedies provided for by law. Any amount paid to Supplier in the form of prepaid fees attributable to a period of Suspension or suspended activity shall be refunded.

15.3 Such Suspension may be removed, or suspended activity may resume, at the earlier of such time as a formal notice is issued that authorizes the resumption of performance under the Contract or at such time as a purchase order or other appropriate encumbrance document is issued. This subsection is not intended to operate as an affirmative statement that such resumption will occur.

16 Certification Regarding State Employees Prohibition From Fulfilling Services

Pursuant to 74 O.S. § 85.42, the Supplier certifies that no person involved in any manner in development of the Contract employed by the State shall be employed to fulfill any services provided under the Contract.

17 Force Majeure

17.1 Either party shall be temporarily excused from performance to the extent delayed as a result of unforeseen causes beyond its reasonable control including fire or other similar casualty, act of God, strike or labor dispute, war or other violence, or any law, order or requirement of any governmental agency or authority provided the party experiencing the force majeure event has prudently and promptly acted to take any and all steps within the party's control to ensure continued performance and to shorten duration of the event. If a party's performance of its obligations is materially hindered as a result of a force majeure event, such party shall promptly notify the other party of its best reasonable assessment of the nature and duration of the force majeure event and steps it is taking, and plans to take, to mitigate the effects of the force majeure event. The party shall use commercially reasonable best efforts to

continue performance to the extent possible during such event and resume full performance as soon as reasonably practicable.

17.2 Subject to the conditions set forth above, non-performance as a result of a force majeure event shall not be deemed a default. However, a purchase order or other payment mechanism may be terminated if Supplier cannot cause delivery of a product or service in a timely manner to meet the business needs of Customer. Supplier is not entitled to payment for products or services not received and, therefore, amounts payable to Supplier during the force majeure event shall be equitably adjusted downward.

17.3 Notwithstanding the foregoing or any other provision in the Contract, (i) the following are not a force majeure event under the Contract: (a) shutdowns, disruptions or malfunctions in Supplier's system or any of Supplier's telecommunication or internet services other than as a result of general and widespread internet or telecommunications failures that are not limited to Supplier's systems or (b) the delay or failure of Supplier or subcontractor personnel to perform any obligation of Supplier hereunder unless such delay or failure to perform is itself by reason of a force majeure event and (ii) no force majeure event modifies or excuses Supplier's obligations related to confidentiality, indemnification, data security or breach notification obligations set forth herein.

18 Security of Property and Personnel

In connection with Supplier's performance under the Contract, Supplier may have access to Customer personnel, premises, data, records, equipment and other property. Supplier shall use commercially reasonable best efforts to preserve the safety and security of such personnel, premises, data, records, equipment, and other property of Customer. Supplier shall be responsible for damage to such property to the extent such damage is caused by its employees or subcontractors and shall be responsible for loss of Customer property in its possession, regardless of cause. If Supplier fails to comply with Customer's security requirements, Supplier is subject to immediate suspension of work as well as termination of the associated purchase order or other payment mechanism.

19 Miscellaneous

19.1 Transition Services

If transition services are needed at the time of Contract expiration or termination, Supplier shall provide such services on a month-to-month basis, at the contract rate or other mutually agreed rate. Supplier shall provide a proposed transition plan, upon request, and cooperate with any successor

supplier and with establishing a mutually agreeable transition plan. Failure to cooperate may be documented as poor performance of Supplier.

19.2 Publicity

The existence of the Contract or any Acquisition is in no way an endorsement of Supplier, the products or services and shall not be so construed by Supplier in any advertising or publicity materials. Supplier agrees to submit to the State all advertising, sales, promotion, and other publicity matters relating to the Contract wherein the name of the State or any Customer is mentioned or language used from which, in the State's judgment, an endorsement may be inferred or implied. Supplier further agrees not to publish or use such advertising, sales promotion, or publicity matter or release any informational pamphlets, notices, press releases, research reports, or similar public notices concerning the Contract or any Acquisition hereunder without obtaining the prior written approval of the State.

19.3 Mutual Responsibilities

A. No party to the Contract grants the other the right to use any trademarks, trade names, other designations in any promotion or publication without the express written consent by the other party.

B. The Contract is a non-exclusive contract and each party is free to enter into similar agreements with others.

C. The Customer and Supplier each grant the other only the licenses and rights specified in the Contract and all other rights and interests are expressly reserved.

D. The Customer and Supplier shall reasonably cooperate with each other and any Supplier to which the provision of a product and/or service under the Contract may be transitioned after termination or expiration of the Contract.

E. Except as otherwise set forth herein, where approval, acceptance, consent, or similar action by a party is required under the Contract, such action shall not be unreasonably delayed or withheld.

19.4 Entire Agreement

The Contract documents taken together as a whole constitute the entire agreement between the parties. The Contract documents include this Contract, any Amendments to this Contract, applicable Solicitation, and any successful bid as may be amended or limited through negotiation. No statement, promise,

condition, understanding, inducement or representation, oral or written, expressed or implied, which is not contained in a Contract document shall be binding or valid. The Supplier's certifications, including any completed electronically, are incorporated by reference into the Contract.

ATTACHMENT C

AGENCY TERMS

SOLICITATION NO. (INSERT NUMBER)

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ATTACHMENT D

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Dr. Johnathan White
State of Oklahoma
Dept of Agriculture
2800 N Lincoln Blvd
Oklahoma City,OK,73105-4207
US

Telephone : 405 522 5443
Email : johnathan.white@ag.ok.gov

Sales Proposal
Please reference this Quotation when Purchase Order is issued

Quotation No: 23953257 - Expiration Date: 03/24/2025

Dear Dr. Johnathan White,

Thank you for your interest in Waters! Please find the enclosed Sales Quotation for the products you inquired about. We look forward to working with you and your team for all of your laboratory needs.

To place an order for products and services on this quotation, you may send your hard copy purchase order via email to waters_quotes@waters.com

You may also contact Waters Sales Support to place your order via telephone at 800-252-4752 Ext.8023, fax your purchase order to 508-482-8532 or 508-482-8834.

If you have any questions regarding this quotation, please contact your local Account Representative: Dennis Karote. Dennis may be reached by telephone at , or via Email at DENNIS_KAROTE@WATERS.COM, or visit us online at www.waters.com.

Waters Sales Support
Tel: 800-252-4752 Ext.8023
Email: waters_quotes@waters.com

EHS

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Account	: State of Oklahoma
Quotation number	: 23953257
Creation date	: 02/05/2025
Expiration date	: 03/24/2025

Sales Proposal
Please reference this Quotation when Purchase Order is issued

Item	Product#	Qty	Description	Unit Price	Discount/ Surcharge	Net Price
1	176850043	2	Xevo TQ-S micro System	455,112.00	- 243,153.75	423,916.50
			With the following configuration:			
	176003468	2	Xevo TQ-S micro			
	176002526	2	MassLynx Workstation with TL			
	668000273	2	MONITOR, 24"			
	176002685	2	Vac Rough Pump + Freq Conv			
	176003469	2	MS Ref Stds Xevo TQ-S micro			
	176003949	2	APcI Probe Option			
			Arc HPLC System			
	176017041	2	Arc HPLC Core System			
	176004978	2	Arc HPLC CH-30A Column Heater			
			Installation, Training and Plans			
	741000321	2	TQD SYSTEM INSTALLATION CERT			
	176003950	2	Analytical LC-MS Solvent Install Kit			
2	750000488	1	NA - Professional Srv Training - 3 Days	14,590.00	- 8,120.45	6,469.55
3	176850055	2	Nitrogen Generator Options	24,514.00	- 13,328.00	22,372.00
			With the following configuration:			
	186009339	2	Nitrogen Generator, Genius XE35, 120V			
4	725000473	2	Power Supply, Uninterruptable 5.2KV	12,772.00	- 6,944.00	11,656.00
5	750000805	3	1 Day Custom Web based training	2,430.00	- 1,341.95	3,264.15

Net Total	467,678.20
Estimated Freight	10,000.00
Total Quotation in USD	477,678.20
(Taxes are extra if applicable)	

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Account	: State of Oklahoma
Quotation number	: 23953257
Creation date	: 02/05/2025
Expiration date	: 03/24/2025

Sales Proposal
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Waters Standard Terms and Conditions

Delivery:	60 Days
Freight Terms:	FOB Shipping Point
Payment Terms:	NET 30 DAYS
	Payment Terms Subject to Credit Review

Additional notes:

A training certificate will be shipped and invoiced at the same time your instrument ships.
The certificate will be valid for one year.

For Finance and Leasing Options, please contact our Waters Leasing Account Manager, Jonathan Bennett at (800) 252-4752, extension 8206.

Sales Proposal
Please reference this Quotation when Purchase Order is issued

Detail Product Description(s)

Product# Description

176003468 Xevo TQ-S micro

The Xevo® TQ-S micro is a sensitive but compact tandem quadrupole mass spectrometer featuring reliable performance with a wide dynamic range and high rates of data acquisition. Robust sensitivity is enabled by proven ZSpray™ and StepWave™ which facilitate the detection of analytes at low concentrations in complex matrices and enable low volume injections with consistent, precise and accurate results. Xtended dynamic range™ (XDR) technology provides accessible sensitivity and method transfer. The Xevo® TQ-S micro makes it easier to confidently quantify more analytes using reproducible high acquisition rates with Xcellerated Ion Transfer™ (XIT). Using RADAR, which enables rapid switching between MS full scan and MS/MS acquisition modes, analysts can understand sample complexity and improve method development.

The following items are included as part of the standard system:
Z SPRAY™ API interface...Dual orthogonal interface for robust LC/MS
Electrospray (ESI) inlet probe...for efficient ionisation of a wide range of compounds
ESCi™ ionisation capability...rapid switching source for both ESI and APCI in the same run
IntelliStart™ fluidics...Automated tuning, calibration and method development
TargetLynx XS™ ...Application manager (requires license as provided with PC, below)
OpenLynx™ ...Application manager (requires license as provided with PC, below)

The standard system does not include the following items, which must be specified separately:
Additional inlet probes and ion source options (detailed below).
Acquisition PC data system and monitor
Additional Workstation data system terminals.
Printers.
Additional MassLynx™ options (detailed below).
Vacuum backing pump options (rotary or oil-free combinations)
HPLC systems or other inlet options.

1. Z SPRAY™ API INTERFACE
This instrument is equipped with an atmospheric pressure ionisation (API) LC interface. The source and spraying elements are visible through a transparent window in the enclosure and are easily accessible via a quick-release mechanism. The source elements may be wiped clean in situ or removed for cleaning without the need for tools and without breaking vacuum. The nebulized spray is orientated orthogonally and positioned off axis for maximum source longevity and analyser protection against 'dirty' samples. The source also includes facilities for de-clustering ions formed at atmospheric pressure. Positive and negative capability is included. Positive ion, negative ion and ESCi™ capability is available as standard (allowing rapid switching between ESI and APCI, positive and negative in the same run). All source voltages and gases are under data system control.

2. INTELLISTART™ FLUIDICS
The instrument is equipped with an on-board infusion system capable of delivering reference solutions from 3 built-in vial locations. The on-board fluidics system is controlled by the IntelliStart software to provide automated instrument setup, mass calibration and method development. The reference solutions are delivered via switching valves for either direct or combined (into an LC flow) infusion into the API source. The valves can in addition be programmed from the software to function as an LC flow divert. If required, the fluidics can be controlled manually via the system Console.

3. TANDEM QUADRUPOLE ANALYSER

Sales Proposal
Please reference this Quotation when Purchase Order is issued

Detail Product Description(s)

Product#	Description
176003468	Xevo TQ-S micro - Continued

The instrument is equipped with two high performance quadrupole mass analysers with inter-element beam focusing and a mass range of 2-2000 amu. Pre-filters are fitted to the mass analysers to maximise resolution and transmission. The pre-filters also eliminate the need for cleaning of the quadrupole mass analysers. All lens and analyser voltages are digitally controlled. Analyser parameters may be programmed with respect to mass for optimal performance. Analyser parameters used for data acquisition are automatically recorded and appended to the relevant data file.

3. StepWave™ ION TRANSFER OPTICS
This instrument is equipped with patented, off-axis StepWave ™ ion transfer optics. Uniquely the StepWave technology both dramatically increases the efficiency of ion transfer from the ion source to the quadrupole MS analyser at the same time as efficiently eliminating undesirable neutral contaminants. The technology employed allows Xevo TQ-S micro to deliver unprecedented levels of sensitivity, speed, and selectivity.

4. COLLISION CELL
The collision cell can be operated as a high efficiency travelling wave (T-Wave) device for collision induced dissociation. The travelling wave enables rapid cell clearance and refill for fast MRM transition switching while maintaining optimum signal to noise.

T-Wave™ :
The cell can be operated as a high efficiency travelling wave (T-Wave) device for collision induced dissociation. The travelling wave enables rapid cell clearance and refill for fast MRM transition switching while maintaining optimum signal to noise.

RADAR™ :
An information-rich acquisition approach that allows you to collect highly specific quantitative MRM data for target compounds while providing additional spectral data to help visualize all other components in the sample.

5. VACUUM SYSTEM
Clean, differentially pumped, automated vacuum system comprising:
Air-cooled Pfeiffer splitflow turbomolecular drag pump evacuating both the source & analyser.
Vacuum read backs and system vent/pump cycles are digitally monitored and controlled, to provide total software control and ensure fail-safe operation in the event of power failure.
The backing option must be ordered separately (See Backing Pump Options for part numbers and descriptions)

6. DETECTOR - Xtended Dynamic Range™ (XDR)
The instrument is equipped with a low noise dynolite photomultiplier detector. The detector is positioned after the second analyzer. A High Voltage conversion dynode and phosphor are positioned at 90° off-axis to the analyser for the elimination of neutral noise. The detector features novel, integral focusing optics, which provides a detection efficiency approaching 100% for single ions. New XDR electronics incorporate 40 MS/s and 16 bit ADC to increase the dynamic range. The photomultiplier is enclosed in its own vacuum envelope for long life. The detector operates in both positive and negative ion mode, which can be switched rapidly under software digital control.

7. MASSLYNX™ SOFTWARE / MS Workstation
The MS Workstation and MassLynx™ License for the application software for instrument control, data acquisition and processing must be ordered separately (See MS Workstation Variant Configurator section for part numbers and descriptions)

Sales Proposal
Please reference this Quotation when Purchase Order is issued

Detail Product Description(s)

Product#	Description

176003468 Xevo TQ-S micro - Continued

Post acquisition processing and general data manipulation can be carried out by an additional computer workstation and software installation (See the MassLynx™ Process-only Workstations section of the MassLynx Variant configurator 176706000).

176002526 MassLynx Workstation with TL

MassLynx™ Workstation with License
MassLynx Application Software License includes key discs for
TargetLynx™ and OpenLynx™
Contains the standard MS workstation as defined in the MassLynx Variant configurator 176706000 and Product Ordering Guide

668000273 MONITOR, 24"

22" Flat Panel Monitor
Lenovo ThinkVision L2250p - LCD display - TFT - 22" - Widescreen - 1680 x 1050 / 75 Hz - 250 cd/m2 - 1000:1 - 5 ms -0.282 mm DVI-D, VGA - business black

176003949 APcI Probe Option

Dedicated tool-free probe option for Xevo TQ-XS atmospheric pressure chemical ionisation (APCI). The interface can be used at up to 2 ml/min without the need for flow splitting.

176017041 Arc HPLC Core System

The Arc HPLC System configuration includes quaternary solvent delivery (QSM-R) and sample introduction via a direct inject sample manager (SM FTN-R) with temperature control. The system may be configured for a broad range of applications supported by choice of either one, of the two available HPLC optical detectors.
The following items are included as part of this configuration:

- 186017016 Arc HPLC Quaternary Solvent Manager (QSM-R)
- 186017017 Arc HPLC Sample Manager FTN (SM FTN-R) with Temp Control
- 205000505 Leak Sensor Assembly (QTY = 3)
- 186003031 XBridge C18 Column, 3.5µm 4.6 x 50 mm
- 186000307c 2 mL Glass Vial with pre-slit Septa (Pack of 100)
- 186008093 Arc HPLC Start-up solution
- 716006651 Info Set, Arc HPLC System
- 667006352 Waters Driver Pack 2019 R2 SR1 (Analytical)
- 205002445 Kit, Accessories Arc HPLC System
- 289008967 Assy, Bottle Tray

741000321 TQD SYSTEM INSTALLATION CERT

Waters TQ Detector MS System Installation

Sales Proposal
Please reference this Quotation when Purchase Order is issued

Detail Product Description(s)

Product#	Description

741000321 TQD SYSTEM INSTALLATION CERT - Continued

Includes:
-System Set up and Specification Testing
-Product Familiarization Training
-1 Year Manufacturers Warranty
- Insight Remote Intelligent Services

176003950 Analytical LC-MS Solvent Install Kit

Analytical LC-MS Solvent Install Kit
The Analytical LC-MS Solvent Install Kit is intended to assist in effectively installing new LC-MS systems by minimizing sources of possible contamination. This kit includes the LC-MS Grade Solvent Kit (186008715) as well as the Waters Certified Container Kit (186007088). Additional kit components also include a formic acid additive, aqueous ammonia additive and nitrile gloves.
Note: Because this is a hazardous material and GHS requirements vary from country to country, Waters Corporation is only able to ship this item to specific countries worldwide. If the destination for this order (i.e. the ship to country) is changed to a non-permitted country at any point after this quote was generated, this item will be excluded from the final order.

725000473 Power Supply, Uninterruptable 5.2KV

Power Supply, uninterruptible 5.2kVA
(This unit is modified from the standard to allow an overload capacity on Instrument Start-up)
Please Note that this requires an L6-30 wall socket

Waters
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Account	: State of Oklahoma
Quotation number	: 23953257
Creation date	: 02/05/2025
Expiration date	: 03/24/2025

Sales Proposal
Please reference this Quotation when Purchase Order is issued

Waters General Sales Terms and Conditions

THIS TRANSACTION IS EXPRESSLY CONDITIONED UPON AND SUBJECT TO ALL OF THE FOLLOWING TERMS AND CONDITIONS:

1. Warranty - The products and/or services shall be covered by the applicable Waters standard warranty, a copy of which is supplied with the products and/or services or upon request. NO OTHER WARRANTY, WHETHER EXPRESS OR IMPLIED, IS MADE WITH RESPECT TO THE PRODUCTS AND/OR SERVICES. WATERS EXPRESSLY EXCLUDES THE IMPLIED WARRANTIES OF MERCHANTABILITY AND OF FITNESS FOR A PARTICULAR PURPOSE. Any model or sample furnished to the Buyer is merely illustrative of the general types and quality of goods and does not represent that the products will conform to the model or sample.
2. Technical Advice - Waters may, at Buyer's request furnish technical assistance, advice and information with respect to the products if and to the extent that such advice, assistance and information is conveniently available.
3. Fair Labor Standards - The products or services provided hereunder were produced and/or performed in compliance with the requirements of all sections of the Fair Labor Standards Act of 1938 as amended.
4. Equal Employment - Waters is an Equal Opportunity Employer. It does not discriminate in any phase of the employment process against any person because of race, color, creed, religion, national origin, sex, age, veteran or handicapped status.
5. Software - To the extent there is any software included with the products, the software is being licensed, not sold and all rights, title and interest therein shall remain with Waters. Use of the software shall be in accordance with the applicable software license delivered with the products. U.S. Government Restricted Rights - RESTRICTED RIGHTS LEGEND. Use, duplication or disclosure by the Government is subject to restrictions as set forth in subparagraph (c)(1)(ii) of the Rights in Technical Data and Computer Software clause at DFARS 252.227-7013 or subparagraphs (c)(1) and (2) of the Commercial Computer Software - Restricted Rights clause at 48 CFR 52.227-19, as applicable.
6. Diagnostic Products - Buyer acknowledges and agrees that only those products which are labeled and identified as in vitro diagnostic ("IVD")

Sales Proposal
Please reference this Quotation when Purchase Order is issued

Waters General Sales Terms and Conditions

devices are intended to be used for IVD purposes. Buyer acknowledges and agrees that any products that are not labeled and identified as IVDs are general laboratory products intended for research and other general scientific uses and are not for use in IVD procedures.
7. Software as a Service (SaaS) - Notwithstanding any other term of this quotation to the contrary, all purchases of and access to Waters′ SaaS offerings contained within this quotation

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Attachment E2 to
STATE OF OKLAHOMA CONTRACT WITH Waters Technologies Corporation
RESULTING FROM SOLICITATION NO. EV00000549
ADDITIONAL BIDDER TERMS

Waters Licenses, Warranties, and Support Services

71500029510
Revision G

Company information

The term "Waters" shall mean Waters Corporation and/or a Related Company of Waters Corporation. A Related Company of Waters Corporation means any corporation or other business entity that is directly controlled by Waters Corporation. Control means direct or indirect ownership of or other beneficial interest in fifty percent (50%) or more of the voting stock, other voting interest, or income of a corporation or other business entity.

Copyright notice

The information in this document is subject to change without notice. Waters Corporation assumes no responsibility for any typographic errors that may appear in this document. This document is believed to be complete and accurate at the time of publication.

Trademarks

NuGenesis®, Waters®, Empower®, MassLynx®, THE SCIENCE OF WHAT'S POSSIBLE.®, and UNIFI®, are registered trademarks of Waters Corporation, and Breeze™, Waters NuGenesis SDMS™, Waters SDMS Vision Publisher™, Waters Analytical Workflow Manager™ are trademarks of Waters Corporation.

Other registered trademarks or trademarks are the sole property of their owners.

Contacting Waters

Contact Waters® with technical questions regarding the use, transportation, removal, or disposal of any Waters product. You can reach us via the Internet, telephone, or conventional mail.

Waters contact information:

Contacting medium	Information
Internet	The Waters Web site includes contact information for Waters locations worldwide. Visit www.waters.com.
Telephone and fax	For all worldwide location phone and fax numbers, please visit the Waters Web site.
Conventional mail	Waters Corporation 34 Maple Street Milford, MA 01757 USA
Online Service Request System - iRequest	Contact Waters online at www.waters.com/irequest

Table of Contents

License, Warranty, Support, Shipment, Damage, Claim, and Return Information

1.1 Software license and warranty

All use of the Waters Software shall be governed by the terms and conditions of the Oklahoma Statewide Contract resulting from Solicitation # EV00000549.

The term "Software" includes the object code version of the MassLynx software, Breeze software, Empower software, Waters NuGenesis Scientific Data Management System (SDMS) software, Waters SDMS Vision Publisher software, Waters Analytical Workflow Manager (AWM) software, Waters UNIFI software and/or such other software licensed to you by Waters.

1.2 Instrumentation and service warranty

1.2.1 Limited warranty: instrumentation

Waters warrants from the date of shipment for the applicable warranty period that its instrumentation identified and/or marketed as Waters products shall be free from defects in design, material, and workmanship and shall conform to and perform materially in accordance with the specifications set forth in the applicable operator or user manual when used in the proper operating environment under normal use and service. This warranty covers all new instrumentation products manufactured by Waters. Any warranty that may be applicable to third-party instrumentation products and accessories shall be provided by the respective manufacturers or suppliers of such third-party components.

Waters instruments are for use only by properly qualified personnel. Waters instruments labeled and identified as *in vitro* diagnostic ("IVD") devices may be used for IVD purposes. Such IVD uses must be in accordance with the instrument's intended use statement provided in the product literature. Any patient diagnosis or treatment determination made as a result of data generated using a Waters IVD instrument must be made by a qualified health care professional. Waters instruments that are not labeled and identified as IVDs are general laboratory instruments intended for research use only and are not for use in IVD procedures. Customer shall not use any such general laboratory instruments for IVD purposes.

1.2.1.1 Exclusions

The foregoing limited warranty does not apply to any material deviation from the specifications by any instrumentation product that results from (a) use of the instrumentation for any purpose other than general purpose use unless specifically expressed otherwise in the product literature; (b) use of the instrumentation products for investigational use with or without confirmation of diagnosis by another, medically established diagnostic product or procedure; (d) modification of the instrumentation products by anyone other than Waters; (e) failure by customer to install any standard enhancement or update in accordance with

an update procedure or release of firmware or any operating system release; (f) any willful or negligent action or omission of customer; (g) any misuse, or incorrect use, of the instrumentation product; (h) any malfunction of any non-Waters information system or instrument with which the instrumentation product may be connected; or (i) failure to establish or maintain the operating environment for the instrumentation product in accordance with the applicable operator or user manual.

1.2.1.2 Remedy

In the event of any failure of a Waters' instrumentation product to perform in accordance with the foregoing limited warranty, Waters' liability and customer's remedy, shall at Waters' be the repair or replacement of the instrumentation product or refund of amounts paid by customer for the instrumentation product that does not meet the limited warranty.

1.2.1.3 Warranty service

Warranty service is performed at no charge and at Waters' option and in Waters' in one of four ways:

- With your authorization, a service representative will access your system remotely.
- A service representative is dispatched to the customer facility.
- The instrumentation product is returned for repair at a Waters facility.
- Replacement parts with appropriate installation instructions are sent to the customer.

Warranty service is performed only if the customer notifies Waters during the applicable warranty period.

Unless otherwise agreed in writing at the time of sale, if the instrumentation product for which warranty service is sought has been removed from the initial installation geographic site, no warranty service will be provided.

Warranty service is provided during normal business hours (8:00 A.M. to 5:00 P.M., Monday through Friday). Service is not available when Waters offices are closed in observance of legal holidays.

1.2.1.3.1 Warranty service exceptions for instrumentation

Warranty service is not performed on

- any instrumentation product or part that has been repaired by others, improperly installed, altered, or damaged in any way.
- any instrumentation product or parts not manufactured by Waters.
- any instrumentation product that malfunctions because the customer has failed to perform maintenance, calibration checks, or observe good operating procedures.
- any instrumentation product that malfunctions due to the use of unapproved maintenance, or repair parts, or operating supplies and computers not meeting minimum hardware requirements, or as a result of network-related problems.

Repair or replacement is not made

- for expendable items such as lamps, panel lights, fuses, batteries, filters, seals, and other items contained in a Performance Maintenance Kit, when such items were operable at the time of initial use.

- because of decomposition due to chemical action.
- because of poor facilities, operating conditions, or inadequate utilities.

1.2.1.3.2 Limited warranty: repair and maintenance service

Waters warrants repairs and maintenance services for a duration of the contract. Waters also warrants the parts used shall be free from defects in design, material and workmanship and shall conform to and perform materially in accordance with the specifications set forth in the applicable operator or user manual when used in the proper operating environment under normal use and service.

Repair and maintenance warranty service is not provided for

- any instrument, maintenance or repair part that has been repaired by others, improperly installed, altered, or damaged in any way.
- any instrumentation product that malfunctions because the customer has failed to perform maintenance, calibration checks, or observe good operating procedures.
- any instrumentation product that malfunctions due to the use of unapproved maintenance or repair parts or operating supplies and computers not meeting minimum hardware requirements or as a result of network related problems.
- any system component or assembly that falls outside the scope of the repair or maintenance service that fails either during a service event.

Repair or replacement is not made

- because of decomposition due to chemical action.
- because of poor facilities, operating conditions, or inadequate utilities.

1.2.2 Warranty disclaimers

TO THE EXTENT PERMITTED BY APPLICABLE LAW, THE LIMITED WARRANTIES SET FORTH HEREIN ARE EXCLUSIVE AND IN LIEU OF ALL OTHER REPRESENTATIONS, WARRANTIES AND GUARANTEES, EXPRESS OR IMPLIED, INCLUDING WITHOUT LIMITATION ANY IMPLIED WARRANTIES OF MERCHANTABILITY, FITNESS OF THE PRODUCTS FOR A PARTICULAR PURPOSE, INCLUDING FITNESS FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES OR FOR INVESTIGATIONAL USE WITH OR WITHOUT CONFIRMATION OF DIAGNOSIS BY ANOTHER MEDICALLY ESTABLISHED DIAGNOSTIC PRODUCT OR PROCEDURE, OR NONINFRINGEMENT, AND ANY WARRANTIES ARISING OUT OF COURSE OF DEALING OR COURSE OF PERFORMANCE. CUSTOMER EXPRESSLY ACKNOWLEDGES THAT BECAUSE OF THE COMPLEX NATURE OF THE PRODUCTS AND THEIR MANUFACTURE, WATERS CANNOT AND DOES NOT WARRANT THAT THE OPERATION OF THE PRODUCTS WILL BE WITHOUT INTERRUPTION OR ERROR-FREE OR WITHOUT DEFECT. CUSTOMER EXPRESSLY ACKNOWLEDGES THAT CUSTOMER IS RESPONSIBLE FOR USE OF THE PRODUCTS IN CLINCIAL DIAGNOSTIC PROCEDURES OR FOR INVESTIGATIONAL USE WITH OR WITHOUT CONFIRMATION OF DIAGNOSIS BY ANOTHER MEDICALLY ESTABLISHED DIAGNOSTIC PRODUCT OR PROCEDURE.

1.2.3 Transfer of warranty

The warranty is not transferable from the original owner or original installation site to another geographic location without the written consent of Waters. In the event that the instrument(s) must be relocated within the same company and country during the warranty period, Waters offers relocation services to ensure proper care is taken when de-installing, packing and re-installing in

order to maintain the warranty coverage.

1.2.4 Warranty periods

Note that warranty periods begin when products are shipped.

Instrumentation:

Warranted item	Component or components	Warranty period
Electronic and mechanical assemblies	Entire instrument, except for maintenance parts, operating supplies, and expendable components.	One year (12 months) from the date of shipment, unless otherwise stated in the instrument's accompanying user documentation.
Mechanical and electronic assemblies	Instruments that have served as demonstration models.	Ninety (90) days from date of shipment.
Normal wear and maintenance parts	As defined in the instrument Performance Maintenance Kit, if available.	Ninety (90) days from date of shipment.
Operating supplies and expendables	Autosampler vials, solvent and sample filters, and fuses.	Warranted to function properly when delivered.

Service:

Warranted item	Component or components	Warranty period
Parts installed during a demand service repair	Mechanical and electronic assemblies	Ninety (90) days from date of shipment.
Service labor	Service workmanship	Ninety (90) days from date of service delivery.

1.2.5 Warranty returns

No returns may be made without prior notification and authorization from Waters. If it is necessary to return products to Waters, contact Waters Customer Service, the Waters subsidiary nearest you, or your Waters representative for a return merchandise authorization (RMA) number and forwarding address.

1.2.6 Warranty: non-Waters hardware

Waters does not assemble, configure, or install software on any computer or computer peripheral that has not been purchased from Waters.

The warranty for hardware not manufactured by Waters follows the warranty, if any, of the original equipment manufacturer.

1.3 Support and extended coverage

Waters' USA and Canadian customers seeking service and support may contact Waters Technical Service (800 252-4752). Others may phone their local Waters subsidiary or Waters corporate headquarters in Milford, Massachusetts (USA), or they may visit the Waters Web site (http://www.waters.com) and click Support.

Total Assurance Warranty is available during the first 90 days of system ownership and receives the same discount as that of the system purchased. It provides full support coverage for two years and guarantees a scheduled Performance Maintenance (PM) visit in year two.

Total Assurance Plan is available at the end of the Warranty and is renewable annually, it also provides annual scheduled PM visit.

Both TAW, and TAP provide technical telephone support, priority service, repair visits and replacement parts as needed to ensure your system is running at peak performance.

1.4 Installation and extended training

1.4.1 Instrument startup

As part of the purchased installation charge, Waters offers familiarization training for a single, designated primary operator.

Instrument startup consists of these procedures:

- Assembling computer hardware and connecting a printer purchased from Waters.
- Connecting computer hardware to the system instruments.
- Configuring and testing a system for proper instrument function and data collection.

Optional installation services are available to purchasers of workstation add-on kit software products. The services consist of software installation, system configuration, and primary operator familiarization training. During this day of system installation service, a certified Waters field

service technician will configure the customer's computer, load software, and interface the computer with the system.

1.4.2 Extended training

Waters Educational Services provides instrument and software training beyond that which is provided at startup. Courses are available at the customer site, our worldwide campus in Milford, Mass., U.S.A., in our Regional Training Centers in Europe and Asia, and at most Waters subsidiaries. Programs can be generic or customized to address specific challenges.

For details about the training and extended support programs, visit the Waters Web site (http://www.waters.com), and click Education, or Services & Support.

1.5 Shipments, damages, claims, and returns

All shipments are made free on board (FOB) shipping point. Waters suggests that you authorize insurance for all shipments. Instruments and major components are packed and shipped via ground transportation unless otherwise required. Supplies and/or replacement parts are packed and shipped via a ground courier, air parcel post, or parcel post, unless otherwise requested.

1.5.1 Damages

The U.S. Interstate Commerce Commission (ICC) has determined that carriers are as responsible for concealed damages that occur during transit as they are for obvious damages. Concealed damage is damage that occurs to the contents of a shipping package where the package exterior remains apparently undamaged. Therefore, unpack the instrument or component promptly after receiving it, aware that it may have sustained concealed damage while in transit.

1.5.2 Claims

If you discover the item shipped has sustained concealed damage, do not continue to unpack it. Instead, request the local agent or carrier to immediately inspect the unit, and secure a written (inspection) report of his or her findings to support the claim. You must make this request within 15 days of receiving the damaged unit, otherwise, the carrier will not honor the claim. Do not return damaged goods to Waters without first securing the inspection report and contacting Waters for a return merchandise authorization (RMA) number.

Ensure the shipment is protected and secure after you receive it. Components removed from the shipment, or damaged while awaiting installation, are the responsibility of the customer.

After a damage inspection report is secured, Waters cooperates fully in supplying replacements and handling a claim, which either party may initiate.

1.5.3 Returns

No returns may be made without prior notification and authorization, If for any reason it is necessary to return material to Waters, contact Waters Customer Service, the Waters subsidiary nearest you, or your local Waters representative for a return merchandise authorization (RMA) number and forwarding address.

A Waters Software License Agreement

This is a legal agreement ("Agreement") between you (the "Customer") and Waters Corporation and/or a Related Company of Waters Corporation (collectively, "Waters"). A Related Company of Waters Corporation means any corporation or other business entity that is directly controlled by Waters Corporation. Control means direct or indirect ownership of or other beneficial interest in fifty percent (50%) or more of the voting stock, other voting interest, or income of a corporation or other business entity.

By using Waters' Software including any Upgrades (as defined below), you represent that you have the power and authority to enter into this Agreement on behalf of your company. In such event, “you” refers to your company.

1. **Definitions**.

a. The term “Software” includes the object code version of the MassLynx software, Breeze software, Empower software, Waters NuGenesis Scientific Data Management System (SDMS) software, Waters SDMS Vision Publisher software, Waters Analytical Workflow Manager (AWM) software, Waters UNIFI software and/or such other software indicated on the Waters' Quotation and accepted by you on your Purchase Order ("PO") and licensed to you by Waters. The Software is comprised of the computer programs, media containing the computer programs (including Oracle® Network Embedded Software, where applicable), user documentation, and any Upgrades that Waters may provide to you. You acknowledge and agree that the Software constitutes Waters' confidential information.

b. “Upgrades" shall mean and include any changes, additions, or corrections made by Waters to the Software.

c. “Quotation” shall mean a document provided by an authorized representative of Waters which describes the Software, Waters' products, and/or those certain Waters Partners' product(s), if any, that you, the Customer, may purchase, including pricing. All such Quotations shall include and be subject to the terms and conditions contained in this Agreement unless otherwise agreed in writing.

d. “Purchase Order” shall mean a written authorization from you, the Customer, to Waters for the purchase of Software and products. All such Purchase Orders shall reference a Quotation and be subject to the terms and conditions contained in this Agreement.

2. License and Usage of Software

Subject to the terms and conditions of this Agreement and upon payment of the applicable license fees, Waters hereby grants to you a non-exclusive, non-transferable, non-sublicenseable right and license during the Term (as defined below) to use the Software in connection with Waters' products and/or those certain Waters' Partner product(s) authorized by Waters, if any. In this regard, you may install, copy, operate and transmit the Software in whole or in part: (i) for single-seat licenses, only as necessary to use the Software either on a single personal computer or workstation, and (ii) for client/server licenses, in a reasonable manner to ensure that the number of users does not exceed the number of users for which you have paid license fees. The Software is protected by the copyright laws of the United States and international treaties. A "Waters' Partner" is an entity with which Waters has a business alliance.

3. Ownership of the Software.

The Software is licensed to you, not sold. Subject to the rights granted above, Waters and the manufacturers of any third-party software included within the Software retain all right, title and interest in and to the Software. You acknowledge that the Software is licensed in object code for use solely in conjunction with Waters' products. Use of the Software in conjunction with non-Waters products, other than those certain Waters' Partner product(s) authorized by Waters, if any, is not licensed hereunder and is prohibited.

4. General Usage Restrictions.

a. You may not use the Software for any purpose beyond the scope of the license granted in this Agreement.

b. Without limiting the generality of the foregoing, you will not: (i) authorize or permit use of the Software by persons not authorized to do so; (ii) market or distribute the Software; (iii) assign, sublicense, sell, lease or otherwise transfer, convey or pledge as security or otherwise encumber, your rights under the license granted in Section 2 above; (iv) use the Software in any time-sharing, subscription, rental or service bureau arrangement, including, without limitation, any use to provide services or process data for the benefit of, or on behalf of, any third party; (v) modify the Software; (vi) combine or integrate the Software with hardware, software or technology not provided to you by Waters; (vii) decompile, disassemble, reverse engineer (unless required by law for interoperability) or otherwise attempt to obtain or perceive the source code from which any component of the Software is compiled or interpreted, and you hereby acknowledge that nothing in this Agreement shall be construed to grant you any right to obtain or use such source code; (viii) disclose the results of any benchmark tests run on the Software (whether or not the results were obtained with assistance from Waters) to any party; or (ix) make copies of the Software other than a reasonable number of copies solely for archival purposes, provided that you reproduce and include Waters' and any third party manufacturer's copyright notices on any backup, disaster recovery or archival copies of the Software and on copies of any user documentation. It is understood and agreed that you may temporarily move, install and operate the Software at a different computer or workstation in the event of computer or workstation malfunction.

c. The Software is not for use by individuals other than properly qualified personnel. Generally, the Software is not intended for use in *in vitro* diagnostic ("IVD") procedures,

but is general laboratory software intended for research use only. Customer shall not use any such general laboratory software for IVD purposes. Notwithstanding the foregoing, certain Software may be labeled and identified as an IVD device ("IVD Software") and as such may be used for IVD purposes. Customer shall not use such IVD Software except in accordance with the IVD Software's intended use statement provided in the product literature. Any patient diagnosis or treatment determination made as a result of data generated using the IVD Software must be made by a qualified health care professional.

5. Oracle Software.

a. Waters has provided, as part of the Software, access to certain Oracle embedded software as a convenience. To the extent that the Software contains Oracle software, you acknowledge that Oracle has no express or implied obligation to provide any technical or other support to you for such software. Please contact Waters directly for technical support and customer service related to the Oracle software.

b. Oracle may provide to its own customers, who may include you, as part of an Oracle software package source code identical to the Oracle source code embedded in the Software. Regardless of whether Oracle does do so, the Oracle source code embedded in the Software shall be governed solely by the terms of this Agreement. If you have obtained an Oracle software license, you must not attempt to use the Oracle software to access, use, reproduce, modify reverse, engineer or otherwise tamper with the Software.

c. Third party technology that is appropriate or necessary for use with some Oracle software, if any, is specified in the Software documentation or otherwise by Waters and

such third party technology is licensed to you only for use with the Software under the terms of the third party license agreement specified in the Software documentation or otherwise by Waters and not under the terms of this Agreement.

d. Oracle is a third party beneficiary of the rights and obligations of this Agreement.

6. Exclusion of the Uniform Computer Information Transactions Act ("UCITA").

It is understood and agreed that the provisions of the UCITA do not apply to this Agreement and the license contained herein.

7. Warranties; Disclaimers.

a. **Representations and Warranties.** Each party to this Agreement hereby represents and warrants (i) that it is duly organized, validly existing and in good standing under the laws of its jurisdiction of incorporation; and (ii) that the first installation or use of the Software in the designated operating environment constitutes a valid and binding obligation between you and Waters and will be enforceable against you in accordance with the terms of this Agreement.

b. Waters Limited Warranty.

i. Waters warrants that the Software will, when used in the designated operating environment, perform substantially in accordance with the operating specifications set forth in the user manual as amended by any release notes issued during the Warranty Period and that the Software will be free of defects in materials and workmanship (the "Limited Warranty"). The Limited Warranty shall apply only to the most current version of the Software that was supplied to you by Waters.

ii. The Limited Warranty is subject to the conditions set forth below:

(a.) You must give written notice to Waters during the Warranty Period with an explanation of the circumstances of any claim that the Software fails to conform to this Limited Warranty.

(b.) Your remedy in the event of any such failure is o the correction or replacement of the defective Software or the refund of the fees paid for the defective Software.

(c.) The Limited Warranty shall not apply to any Software delivered to you that has been improperly installed or modified or that has been the subject of neglect, misuse, abuse, misapplication or alteration.

(d.) No representative of Waters is authorized to commit Waters to any warranty other than the Limited Warranty contained herein.

c. **Disclaimer.** SUBJECT TO THE LIMITED WARRANTY SET FORTH ABOVE, TO THE MAXIMUM EXTENT PERMITTED BY APPLICABLE LAW, WATERS AND ORACLE DISCLAIM ANY AND ALL PROMISES, REPRESENTATIONS AND WARRANTIES, WHETHER EXPRESS, IMPLIED OR STATUTORY, INCLUDING, BUT NOT LIMITED TO, ANY WARRANTIES OF MERCHANTABILITY, FITNESS FOR A PARTICULAR PURPOSE, DATA ACCURACY, SYSTEM INTEGRATION, AND/OR QUIET ENJOYMENT, AND THE SOFTWARE, DOCUMENTATION AND ANY OTHER INFORMATION OR MATERIALS OTHERWISE PROVIDED ARE PROVIDED "AS IS" AND ARE SUBJECT TO NO OTHER WARRANTY. NO WARRANTY IS MADE BY

WATERS AND/OR ORACLE ON THE BASIS OF TRADE USAGE, COURSE OF DEALING OR COURSE OF TRADE. NEITHER WATERS NOR ORACLE WARRANTS THAT THE SOFTWARE WILL MEET YOUR REQUIREMENTS OR THAT THE OPERATION OF THE SOFTWARE WILL BE UNINTERRUPTED OR ERROR-FREE. YOU ACKNOWLEDGE THAT WATERS' AND/OR ORACLE'S OBLIGATIONS UNDER THIS AGREEMENT ARE FOR YOUR BENEFIT ONLY.

8. Relationship of the Parties; No Agency.

Nothing contained herein shall be construed to place Waters and you in a relationship of partners, joint ventures, principal agent or employer employee, and neither party shall have any authority to obligate or bind the other whatsoever, except as specifically provided by the terms of this Agreement. In no event shall either party hold itself out to be an agent of the other with the authority to bind such other party to any agreement, contract or obligation.

Waters
THE SCIE CE OF WHAT'S POSSIBLE:"

Attachment E3 to
STATE OF OKLAHOMA CONTRACT WITH Waters Technologies Corporation
RESULTING FROM SOLICITATION NO. EV00000549
BIDDER'S STATEMENT OF COVERAGE TERMS
Waters FlexCHOICE Coverage

STATEMENT OF COVERAGE

Waters™ FlexCHOICE™ Coverage (the "Plan") is a flexible, configurable approach to provide the Customer with entitlements focused on service needs of identified systems within its laboratory environment. It is intended to let the Customer choose the level of coverage for its laboratory needs. Entitlements herein indicated as "standard" are provided in the baseline configuration and pricing of the Waters FlexCHOICE Coverage.

Plan entitlements listed as "optional" or "non-standard" entitlements are offered for an additional charge. Inclusion of Optional and Non-Standard entitlements and their quantities will be reflected in the Customer's quotation.

For the coverage period and price set forth in the quotation for Waters Instruments and System Coverage, Waters shall provide the Customer with the maintenance services, repair services and other STANDARD OR OPTIONAL on-site and off-site services AS PURCHASED AND SPECIFIED IN THE QUOTATION to keep Waters instruments and software performing in accordance with the operating specifications set forth in the applicable user documentation. Each new customer will receive this Statement of Coverage, which details the materials, parts and services provided under the terms of the Plan. For so long as Waters offers coverage this Plan will be renewable at a minimum of successive one-year periods, at the charges and on the terms in effect at the time of renewal. As part of each renewal, Waters will provide the Customer with any modified Plan terms.

Please note that when the Plan is procured at either Point of Sale (POS) or within the first 90 days of the system order purchase order issuance, the 90-day full coverage warranty is absorbed within the total coverage period set forth in the quotation, entitling the customer to a seamless continuance of coverage. If the Plan is procured after the first 90 days of the system order purchase order issuance, the Customer will receive the warranty provisions set forth in the standard limited warranty terms for the remainder of the first year.

Any services provided under this Plan do not ensure uninterrupted operation of the Customer's systems or instruments contained therein.

SERVICE AVAILABILITY

During the Plan coverage period, Waters will provide scheduled maintenance service where required and available, as well as any additional repair and maintenance services required for the listed systems and/or instruments including optional components installed within the system(s) and/or instrument(s). Service availability and requirements will be determined for each geographical location by the customer's local Waters office.

Service will be delivered at Waters option in one of three ways:

1. **A** Waters representative will be dispatched to the Customer's site.
2. The instrument will be serviced at an off-site Waters repair facility.
3. Replacement parts with appropriate installation instructions will be shipped to the Customer.

Performance Maintenance will be delivered at Waters option in one of two ways:

1. A service representative will be dispatched to the Customer's site.
2. The instrument will be serviced at an off-site Waters repair facility.

RESPONSE TIMES

Waters will respond to all service calls in a timely manner during normal business hours (9:00 AM to 5:00 PM Monday through Friday). Service is not available when local Waters offices are closed in observance of local legal holidays.

Standard Response Time

For all FlexCHOICE Coverage Customers, scheduling of services and resources will take precedence over non-plan customers.

Option: Priority Response Time

Coverage entitlements include priority on-site response time of 48 hours and call back within four hours of call receipt.
These response times are over a five day working week. Please verify the availability of this option as it is not available in all regions. This option where available is at an additional charge.

Clinical Systems 48-Hour Response Time

For clinical systems, Waters guarantees two-hour response time for call back and 48-hour (2 business days) on-site response time over a standard five day working week during normal business hours. If Waters fails to meet the on-site response time stated Waters will extend the time frame of the Plan by one month. Clinical services include confirmation of system performance after every Field Service Engineer intervention to verify the system is functioning properly.

TELEPHONE TECHNICAL SUPPORT

During the Plan coverage period, the Customer shall receive standard telephone technical support from the applicable Waters office during such office's normal business hours. Telephone technical support addresses troubleshooting, part number identification, and basic operation information. Coverage does not guarantee resolution to all issues reported via phone support.

The technical support specialist may, at their discretion, limit technical information shared if there is a potential safety risk to the Customer or the expertise of a Certified Waters Field Service Engineer is required.

Option: Priority Phone Support

Priority phone support will be provided to Customers (and flagged for escalation). Waters will guarantee a two-hour response time for call back during normal business hours.

MATERIALS, PARTS, AND SERVICES PROVIDED FOR WATERS SYSTEMS

All Waters FlexCHOICE Coverage Plans utilize genuine Waters Quality Parts™ which can be delivered by Waters Certified Field Service Engineers to maintain optimal operational performance and qualification of instruments and systems identified in the system quotation.

Instrument Upgrades

During the Plan coverage period, as Waters releases instrument performance upgrades, the Customer may purchase the upgrade at a 20% discount from the local list price.

Firmware Updates

During the Plan coverage period, updates to instrument modules and systems may be required to maintain adaptability with changing technologies. As firmware updates are released, the Customer will receive notice of the new firmware including release notes indicating changes which have been made and the potential impact of the change to their system. It is the Customer's option to either perform these updates or maintain the current version of firmware based upon their release notes evaluation.

If firmware updates are requested to be performed by Waters personnel, this will be scheduled and may coincide with instrument qualification or performance maintenance activities (if one of these optional entitlements are selected). If the qualification option is not purchased, the firmware update will be provided on a billable basis. Likewise, if the Customer has not selected the qualification or performance maintenance option and the release notes recommend such, the qualification will be performed on a billable basis.

If an instrument or system has been purchased new from Waters and FlexCHOICE Coverage Plan has been purchased at the point of sale, this qualification option will be considered standard and be provided for the first year at no additional charge. If the qualification option is not selected at point of sale then any qualification activities will be performed on a billable basis.

Option: Performance Maintenance

Performance Maintenance (PM) is offered to maintain the overall operational performance of the instrument or system. This service is offered on either a single basis, or as requested by the Customer. PM visits will be provided at a mutually agreed upon time, during which PM Kits (where available) will be installed.

These PM Kits contain the general maintenance parts (subject to changes related to product improvements) listed below:

Solvent Management Systems/Pumps

- Plunger replacement
- Plunger seal replacement
- Check valve replacement/rebuilding
- Solvent filter(s) replacement
- Draw off and reference valve rebuilding

Sample Management Systems (Auto-Injectors and Injectors)

- Seal pack and needle rebuilding or replacement
- Fluid pack rebuilding or assembly replacement
- Fluid pack syringe replacement
- Manual injector rebuild kit

Non-Mass Spectrometry Detectors

- Source lamps
- Lens/window replacement as required

Mass Spectrometry Based Systems

- Vacuum pump maintenance parts
- System cleaning materials
- Fan filters, valve rebuild kits, o-rings

HPLC Instrument Inlets

If optional instrument calibration service is selected for use in a system including a mass spectrometer, the instrument functional verification testing will be completed and the results log completed.

Waters Educational Services Courses

During the Plan coverage period, Customers are entitled to receive a 15% discount from local list price on Waters Educational Services.

Option: Ion Source Cleaning Services

Based on need, Customer may wish to include optional ion source cleaning services for their mass spectrometry-based systems. During each visit a Waters trained Field Service Engineer will provide an on-site visit and perform the following services:

1. Benchmark system performance.
2. Clean the ion source and ion transfer regions.
3. Perform vacuum pump maintenance as required per operating procedure.
4. Benchmark system performance once cleaning is complete.
5. Additional cleaning visits may be purchased and scheduled as appropriate.

MATERIALS, PARTS, AND SERVICES PROVIDED FOR NON-WATERS SYSTEMS

Waters FlexCHOICE Coverage Plan provides several service options utilizing Waters Certified Field Service Engineers to maintain the overall operational performance and qualification status of Agilent'" instruments identified on the quotation.

Option: Performance Maintenance

Performance Maintenance (PM) for Agilent systems is offered by Waters in order to maintain the overall operational performance of the instrument or system. This service is offered on either a single basis or as requested by the Customer.

PM's will be provided at a mutually agreed upon time, during which an Agilent PM kit (where available) will be installed.

Agilent system coverage includes all parts and service labor required for the maintenance of the covered components. In the event of heavy use or internal operating practices, additional scheduled Performance Maintenance visits are available as an extra charge option.

During the Plan coverage period, all parts and service labor required for the repair of non-functioning instrumentation including the supply (and installation if not Customer Installable) of additional maintenance parts as required.

Option: Qualification

During the Plan coverage period, instrument systems may require qualification activities in order to maintain compliance with regulatory bodies. This optional service is available for Agilent instruments or systems.

The supply and installation of parts as required to maintain the level of performance required to meet Waters Compliance/ Qualification testing requirements will be provided as entitled.

Policies and provisions for qualification of each instrument will be provided at the time qualification activities are executed or prior to execution for review and approval by the customer.

COVERAGE EXCLUSIONS

Waters FlexCHOICE Coverage excludes any service or supply of material (s) that may be occasioned by (i) the Customer's failure to continuously provide a suitable operating environment, (ii) the Customer's failure to follow Waters' installation, operation or maintenance instructions, (iii) Customer abuse, misuse or neglect or (iv) use of a Customer generated calibration/performance verification/qualification procedure. Waters Corporation has no responsibility or liability for failure to deliver services during the coverage entitlement period due to (i) the lack of a Customer request for such services or (ii) the Customer not providing adequate time or access to the equipment.

A. Operating Supplies and Consumables

The Customer is responsible for purchasing and installing and or de-installing operating supplies and instrument consumable components without any on-site assistance of a Waters Field Service Engineer.

The following is a list (but not inclusive to) operating supplies and consumables not covered by any Waters coverage:

- Solvents, mobile phase
- Columns, column packings (except for column provided during qualification service visit only for ion source)
- MS system water chillers
- Calibration standards (System Suitability Standards) for clinical and diagnostic (except for calibration chemistry kits used during qualification service visits only for ion source cleaning)
- Glassware, sample vials and holders, priming syringes
- Reagents, sample, and solvent filters
- Printer cartridges and toner, integrator and printer paper, computer diskettes, CDs, (except those supplied during qualification service visits only in ion source), tapes
- Computer and peripherals
- Non-Waters software related firmware and updates

B. Installation of Customer Installable Maintenance Parts and Operating Supplies

Only during a Performance Maintenance or inspection visit will Waters Field Service Engineer install customer-installable normal wear and maintenance parts. Normal wear and maintenance parts are defined as parts and components in PM kits.

The following is a list (but not inclusive) of normal wear and maintenance parts that are considered to be customer-installable:

- Pump plunger seals
- Pump check valve cartridges
- Injector syringes
- Filters
- Fuses
- Absorbance detector source lamps
- MS detector sample cones, probe capillaries and fittings, o-rings
- Vacuum pump oil, filters and seals
- Tubing and tubing connectors
- Columns

C. Non-Waters System Exclusions

Waters warranties and coverage are not included for non-Waters instruments and accessories connected to Waters instrumentation that are entitled. **It is** the Customer's responsibility to obtain coverage for such components from the original equipment manufacturer.

The following is a list (but not inclusive) of non-Waters system accessories and instruments:

- Computer and computer peripherals
- Non-Waters software, related firmware, and updates
- Non-Waters detectors
- MS system water chillers

For non-Waters systems with the option for qualification, should any test fail on the first qualification, the system will be considered to have a pre-existing condition. In case of such a failure, Waters Corporation will supply the labor to repair the system and the Customer will be responsible to purchase required parts to repair the system.

Waters shall be under no obligation to provide repair and maintenance services if (i) the instrument has been modified without the prior written approval of Waters, (ii) the instrument has been worked on or serviced by person(s) not certified by Waters, (iii) the instrument contains parts other than Waters Quality Parts for maintenance or repair, or (iv) the instrument is contaminated with radiological, chemical or biological hazards.

FLEXCHOICE PLAN COVERAGE TRANSFERABILITY

All Waters FlexCHOICE Plan Coverage, including those for non-Waters systems and technical telephone support, is provided solely to the Customer and cannot be assigned or transferred.

LIMITED WARRANTY

Waters warrants to the Customer that the services performed under all Waters FlexCHOICE Plan Coverage will be of a quality conforming to generally accepted industry standards and practices and that its personnel performing services under the coverage shall have appropriate skills. Except as set forth herein, WATERS MAKES, AND THE CUSTOMER RECEIVES, NO WARRANTIES OF ANY KIND, EXPRESS, IMPLIED OR STATUTORY, ARISING IN ANY WAY OUT OF, RELATED TO, OR UNDER THIS PLAN OR THE PROVISION OF SERVICES THEREUNDER, AND WATERS SPECIFICALLY DISCLAIMS ANY IMPLIED WARRANTY OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE. The obligation of Waters shall be to repair or replace Waters non-conforming product or part entitled during the term of the coverage. This warranty shall not be deemed to have failed its essential purpose as long as Waters is able to repair or replace any Waters non-conforming product or part *covered* by the plan selected by the customer. In the *event* that an instrument *covered* by a service plan cannot be repaired, Waters shall provide to the customer: (a) Prorated refund or credit of the purchase price, or (b) Prorated credit towards the purchase of a replacement system or instrument.

DISCLAIMER AND LIMITATION OF LIABILITY

IN NO EVENT WILL WATERS BE LIABLE FOR ANY INDIRECT, INCIDENTAL, SPECIAL OR CONSEQUENTIAL DAMAGES, INCLUDING BUT NOT LIMITED TO, DAMAGES DUE TO LOSS OF PROFITS, REVENUE, DATA, INFORMATION, OR USE, EVEN IF WATERS HAS BEEN ADVISED OF THE POSSIBILITY OF SUCH DAMAGES, NOR SHALL WATERS HAVE ANY LIABILITY FOR ANY CLAIM OF ANY THIRD PARTY. Waters' total liability to the Customer for damages, from any cause whatsoever, and regardless of the form of action, whether in contract or in tort, including negligence, shall be limited to the charges the Customer paid to Waters for *every* Waters Coverage during the period when the cause of action arose.

Waters

THE SCIENCE OF WHAT'S POSSIBLE:"

Waters Corporation
34 Maple Street
Milford, MA 01757 U.S.A.
T: 1 508 478 2000
F: 1 508 872 1990
www.waters.com

Attachment F to
STATE OF OKLAHOMA CONTRACT WITH Waters Technologies Corporation
RESULTING FROM SOLICITATION No. EV00000549
Negotiated Exceptions and Additional Terms to the Contract

The Contract is hereby amended to include the terms as set forth below and supersedes all prior terms and exceptions submitted by **Waters Technologies Corporation** or discussed by the parties. Requested Exceptions and Additional Terms not addressed below are declined by the State of Oklahoma.

Section	Exception
ATTACHMENT B, STATE OF OKLAHOMA NEGOTIABLE GENERAL TERMS, Section 12.4	**The parties agree that the following paragraph will be added to the State's Section 12.4 - Limitation of Liability terms as paragraph D:** TO THE EXTENT PERMITTED BY APPLICABLE LAW, IN NO EVENT SHALL SUPPLIER BE LIABLE FOR ANY INDIRECT, INCIDENTAL, SPECIAL, EXEMPLARY, PUNITIVE OR CONSEQUENTIAL DAMAGES INCLUDING, WITHOUT LIMITATION, OR PECUNIARY LOSS ARISING OUT OF THE USE OR INABILITY TO USE THE PRODUCTS AND/OR SERVICES, EVEN IF SUPPLIER HAS BEEN ADVISED OF THE POSSIBILITY OF SUCH DAMAGES. SUPPLIERS' TOTAL LIABILITY IN ANY EVENT SHALL NOT EXCEED THE PURCHASE PRICE OF THE GOODS AND/OR SERVICES WITHIN 12 MONTHS OF THE DAMAGE AND, THE PARTIES AGREE THAT SUCH LIMITED LIABILITY IS A REASONABLE ALLOCATION OF THE RISKS INVOLVING THE PRODUCTS AND/OR SERVICES.
ATTACHMENT B, STATE OF OKLAHOMA NEGOTIABLE GENERAL TERMS, Section 13	**The parties agree that the following paragraph will be added to the State's Section 13 - Termination for Cause terms as paragraph 13.6:** "Supplier may terminate a software license for cause in the same manner as termination of the Contract pursuant to Section 13.1. In such event, Customer shall cease all further use of the software and return to Supplier or destroy all copies of the software in its possession or control."
ATTACHMENT B, STATE OF OKLAHOMA NEGOTIABLE GENERAL TERMS, Section 7	**The parties agree that the following paragraph will be added to the State's Section 7 - Audits and Records Clause terms as paragraph 7.5:** "Upon Supplier's request, no more frequently than annually, Customer shall provide reasonable information as necessary to verify that Customer's usage of the licensed software complies with applicable licensing terms such as usage limits regarding maximum number of users or seats."

EXHIBIT 1
SPECIFICATIONS

Bid Specifications: High-Performance Liquid Chromatograph with Vacuum Degasser equipped with QQQ Mass Spectrometer, Autosampler and Instrument Controller.

For the bid to qualify for consideration the following minimum specifications must be met entirely and without exception OR the vendor must explain any variation or exception to these minimum specifications in detail and item by item. The vendor shall provide product brochures and/or published literature detailing the instrument specifications.

Bidders shall initial each line of specifications indicating product bid meets that specification.

1. High-Performance Liquid Chromatograph with Vacuum Degasser must include the following features:

___ **1.1.** Overall Instrument System Requirements, Safety and Environmental:

- _____ **1.1.1.** Instrument system must be of a modular design using stackable, self-contained units with solvent resistant material used in all areas that may have contact with the mobile phase. - **Meets**
- _____ **1.1.2.** All modular components must form a fully integrated instrument system, with functional communication passing between all modular components and the instrument controller. -**Meets**
- _____ **1.1.3.** System must be entirely electric and operate on US standard current of 120 VAC at 60 Hz. -**Meets**
- _____ **1.1.4.** System must be able to operate in an environment with a temperature range of 18-30 ^{0}C and with a relative humidity range of 20-80% non-condensing. -**Meets**
- _____ **1.1.5.** Leak detection and safe leak handling design, including leak sensors, must be incorporated in the module housings. -**Meets**
- _____ **1.1.6.** Design must ensure isolation of electrical components from liquid flow path. -**Meets**
- _____ **1.1.7.** User maintenance areas must be physically isolated from areas of high voltages. -**Meets**
- _____ **1.1.8.** The following able includes target compounds and desired concentrations routinely tested by our laboratory. Please indicate with “YES” or “NO” your instrument’s ability to analyze these compounds at the concentration specified in sample matrices including soil, vegetation, and water: Meets

Vendor Response	Analyte	Concentration in sample
Yes	2,4 Dichlorophenoxyacetic Acid (2,4-D)	1-5 ppb
Yes	4-(2,4-dichlorophenoxy)butyric Acid (2,4-DB)	1-5 ppb
Yes	Clopyralid	1-5 ppb
Yes	Dicamba	1-5 ppb
Yes	Dichlorprop (2,4-DP)	1-5 ppb
Yes	2-methyl-4-chlorophenoxyacetic acid (MCPA)	1-5 ppb
Yes	Methylchlorophenoxypropionic acid (MCPP)	1-5 ppb
Yes	Picloram	1-5 ppb
Yes	Triclopyr	1-5 ppb
Yes	Bensulfuron-methyl	1-9 ppb
Yes	Chlorimuron-ethyl	1-9 ppb
Yes	Chlorsulfuron	1-9 ppb
Yes	Halosulfuron-methyl	1-9 ppb
Yes	Metsulfuron-methyl	1-9 ppb
Yes	Nicosulfuron	1-9 ppb
Yes	Primisulfuron-methyl	1-9 ppb
Yes	Prosulfuron	1-9 ppb
Yes	Rimsulfuron	1-9 ppb
Yes	Sulfometuron-methyl	1-9 ppb
Yes	Sulfosulfuron	1-9 ppb
Yes	Triasulfuron	1-9 ppb
Yes	Imazamox	1-9 ppb
Yes	Imazapic	1-9 ppb
Yes	Imazapyr	1-9 ppb
Yes	Imazaquin	1-9 ppb
Yes	Imazethapyr	1-9 ppb
Yes	Glyphosate	1-9 ppb
Yes	Aminopyralid	10-99 ppb
Yes	Paraquat	100-999 ppb

___ **1.2.** Liquid Chromatograph

- _____ **1.2.1.** Must include a solvent cabinet with four 1-liter or other recommended mobile phase bottles with caps and filters. -**Meets**
- _____ **1.2.2.** Quaternary pump design with variable stroke volume for pulse-free flow delivery or other mechanism to minimize pulsation in flow. -**Meets**
- _____ **1.2.3.** Mobile phase flow must be user settable from at least 0.02-8 ml/min in steps of 0.001 ml/min at a minimum. -**Exceed the lower flow rate, but does not meet the high flow rate as Arc HPLC system have a flow rate range of 0.001 to 5ml/min**
- _____ **1.2.4.** Flow precision must be no more than 0.1% RSD at a flow rate of 1 ml/min, based on retention time matching. -**Meets**
- _____ **1.2.5.** Flow accuracy must be +/- 1% RSD. -**Meets**
- _____ **1.2.6.** The mobile phase composition precision from mixing must be no more than 0.20% RSD at 0.2 ml/min and 5 ml/min. -**Meets**
- _____ **1.2.7.** Flow path must be able to tolerate solvent pH from 2-12.-**Exceeds as Arc HPLC have 1 to 12.5**
- _____ **1.2.8.** Gradient delay volume must not exceed 1100 μL.-**Exceeds less than 1000ul**
- _____ **1.2.9.** System must incorporate a purge valve for convenient flushing and mobile phase change. -**Meets**
- _____ **1.2.10.** An optional seal wash must be included. -**Meets**
- _____ **1.2.11.** It must be possible to store time progression plots for the following instrument parameters: -**Meets**
  - ___ **1.2.11.1.** Flow-M**eets**
  - ___ **1.2.11.2.** %A, %B, %C, %D -mobile phase constituents. -**Meets**
  - ___ **1.2.11.3.** System Pressure-**Meets**
- _____ **1.2.12.** User-programmable functions must include %B, %C, %D, flow rate, stop time, maximum pressure, minimum pressure, and primary channel. -**Meets**
- _____ **1.2.13.** Must be time programmable for instrument set-points of % B, % C, %D and flow rate. -**Meets**
- _____ **1.2.14.** The instrument must log and display information for diagnostic purposes including resettable counters for: -**Meets**
  - ___ **1.2.14.1.** The number of injections and number of valve cycles. -**Meets**
  - ___ **1.2.14.2.** Liters of pumped mobile phase and seal wear. -**Meets**
- _____ **1.2.15.** The instrument must allow remote access through an internet connection for off-site diagnosis and troubleshooting. -**Meets**

___ **1.3.** Vacuum Degasser

- _____ **1.3.1.** Must use a gas permeable membrane with continuous vacuum degassing design to degas the mobile phases. -**Meets**
- _____ **1.3.2.** Must operate at a maximum flow rate of at least 8 ml/min per channel. -**NA, as max flow rate is 5ml/min and can operate at that flow rate**
- _____ **1.3.3.** Must degas independent mobile phase supply lines. -**Meets**

_____ **1.3.4.** Material in contact with solvent must be non-metallic and contamination -free to prevent artificial peaks in trace analysis. -**Meets**

_____ **1.3.5.** Must have Power and Status indicator to inform the operator of the operational state of the degasser including error log. -**Meets**

_____ **1.3.6.** Must provide a pressure reading output signal. -**Meets**

___ **1.4.** Column Heater

_____ **1.4.1.** Must be thermostatically controlled for regulating the column compartment temperature in the range of 20 ^{0}C below ambient to 80 ^{0}C.-**Exceeds as range is to 90C**

_____ **1.4.2.** Temperature stability must be controlled within +/- 0.15 ^{0}C.-**Meets**

_____ **1.4.3.** Temperature accuracy must be controlled within +/- 0.8 ^{0}C-**Meets**

Must identify installed columns and transmit the column information to the instrument controller. - **Meets**

___ **1.5.** Automated Sampler

_____ **1.5.1.** Must include sample preparation functions, including sample dilution and internal standard addition. -**Meets**

_____ **1.5.2.** Injection precision must typically be no more than 0.1% RSD of peak area across the injection range. -**Meets**

_____ **1.5.3.** Replicate injections from a single vial must be allowed. -**Meets**

_____ **1.5.4.** Sample capacity must be at least 80 **–** 2 mL vials in a single tray. -**Meets**

_____ **1.5.5.** Must accommodate injection volumes in the range of at least 0.1- 100 μL in 0.1 μL increments. -**Meets**

_____ **1.5.6.** Carryover must be ≤ 0.05%. -**Exceeds, less than 0.002% on Arc HPLC**

_____ **1.5.7.** Must allow random access to vials. -**Meets**

_____ **1.5.8.** Must be electrically driven and require no gases for operation. -**Meets**

___ **1.6.** One (1) capillary detector of the following type: QQQ Mass Spectrometer.

_____ **1.6.1.** Must include Nitrogen generator to support this system including battery backup. -**Meets**

_____ **1.6.2.** Must include UPS Battery Backup capable of supporting entire system - (HPLC, QQQ, and computer) through satisfactory shutdown. -**Meets**

_____ **1.6.3.** The mass spectrometer shall have a scan speed of ≥ 15,000 amu/second.-**Exceeds as Xevo TQ-S Micro have scan rate of 20,000 Da/s**

_____ **1.6.4.** The mass spectrometer shall have the following scan types available: MS scan, MS SIM, MS/MS product ion scan, MRM, MS/MS neutral loss/gain scan and parent ion scan. Scan modes are time programmable for both polarity (Positive and Negative Ion) and type

of scan. -**Meets**

_____ **1.6.5.** The mass spectrometer shall allow 450 MRM transitions per time segment, > 10,000 MRMs possible in a method. -**Meets**

_____ **1.6.6.** The mass spectrometer shall be able to switch from positive ion mode to negative ion mode in 25 ms.-**Exceeds , Xevo TQ-S Micro can switch 15ms**

_____ **1.6.7.** The mass spectrometer shall provide for time programming of the following parameters: -**Meet**

___ **1.6.7.1.** Polarity changes in time segment-**Meets**

___ **1.6.7.2.** Solvent diversion through calibrant delivery system valve-**Meets**

___ **1.6.7.3.** Possible 99-time segments with potential 200 MRMs per time window-**Meets**

_____ **1.6.8.** The Detector must include the following features:

___ **1.6.8.1.** Inert ion source, programmable up to 350°C-**Meets**

_____ **1.6.9.** Mass range: 5 u to 3000 u- **Does not Meet, Xevo TQ-S Micro have a range of 2-2048m/z, which should cover all the small molecules**

___ **1.6.9.1.**

___ **1.6.9.2.** Autotune-**Meet**

___ **1.6.9.3.** Automated peak integration with built-in peak validation-**Meet**

___ **1.6.9.4.** Pesticide database spectral library of at least 900 compounds-**Meet**

___ **1.6.9.5.** Ion gauge controller-**Meet**

___ **1.6.9.6.** Ionization modes: -**Meet**

___ **1.6.9.6.1.** Electrospray (ESI)-**Meet**

___ **1.6.9.6.2.** Atmospheric pressure chemical ionization (APCI)-**Meet**

2. Instrument Controller and Software

___ **2.1.** The instrument controller hardware must include:

_____ **2.1.1.** Operating System software and OS Restore Media-**Meet**

_____ **2.1.2.** Instrument controller software media-**Meet**

_____ **2.1.3.** All additional communications hardware necessary for use with the system modular components included in this bid-**Meet**

_____ **2.1.4.** Dual monitors included Meet

_____ **2.1.5.** Pesticide database spectral library of at least 900 compounds-**Meet**

___ **2.2.** Instrument Controller Software

_____ **2.2.1.** Must be designed for use and operate within the industry-standard -Windows graphical user environment. -**Meet**

_____ **2.2.2.** Must integrate completely with and provide full functional control of all modules included in the bid. This includes all actions related to downloading instrument set-points from stored methods, full automation of batch runs, acquiring data, evaluating chromatograms, and reporting analytical results. -**Meet**

_____ **2.2.3.** Must be able to run at the same time as other programs on the computer such as word processors, spreadsheets and small relational databases such as Microsoft Access. -**Meet**

_____ **2.2.4.** Must include Trace Ion Detection and Deconvolution Reporting Software. -**Meet**

_____ **2.2.5.** Must include an integrated tutorial using the software to teach the operator how to use the software. -**Meet**

_____ **2.2.6.** Must include a task-oriented graphical user interface including task diagrams for easy comprehension and fast access toolbars. -**Meet**

_____ **2.2.7.** Must store each analytical method as a discreet instrument method, including instrument set points, data acquisition conditions, and data evaluation and reporting parameters. -**Meet**

_____ **2.2.8.** Must monitor the instrument during acquisition and recording both instrument performance parameters (temperature, pressure, flow, etc.) and any unusual or unexpected events that would affect the integrity or quality of the results. -**Meet**

_____ **2.2.9.** Must include graphical zoom, overlay and alignment features to allow visual comparison of signals. -**Meet**

_____ **2.2.10.** Must allow the users to annotate chromatograms or spectra. -**Meet**

_____ **2.2.11.** Must use a robust peak integration algorithm that gives reliable and reproducible results without users having to set specific integration events or use manual integration for satisfactory data evaluation. -**Meet**

_____ **2.2.12.** Must allow users to interactively integrate chromatograms by manually specifying the baseline points graphically on the screen. -**Meet**

_____ **2.2.13.** Must be able to identify individual compounds based on default or compound specific retention time windows and the ratios between signals if specified (qualifiers). -**Meet**

_____ **2.2.14.** Must be able to set both default calibration curve fits for all compounds and specific fits for each compound. The calibration weighting for each calibration level must be configurable as none (no weighting) and calibrator specific, inverse concentration (1/Conc.) or inverse concentration squared (1/Conc.^2.)-**Meet**

_____ **2.2.15.** Must be user selectable to generate quantitative results based on peak area and peak height. -**Meet**

_____ **2.2.16.** Must include a standard set of report formats that allow users to report results with or without graphics. -**Meet**

_____ **2.2.17.** Must include a report designer/editor that allows users to specify their own report layouts including graphical elements. -**Meet**

_____ **2.2.18.** Must be able to send reports to the screen, to a file and to a printer. -**Meet**

_____ **2.2.19.** Must allow users to set up the system for unattended operation in a tabular editing manner, much like a spreadsheet, with easy, standard entry or editing. -**Meet**

_____ **2.2.20.** Must allow the user to preview the execution order of the runs on the screen before they are executed. **-Meet**

_____ **2.2.21.** Must include summary reporting capabilities that produce graphical trend diagrams. -**Meet**

_____ **2.2.22.** Must allow modification of set points and time programs even during a run. -**Meet**

_____ **2.2.23.** Must provide method and run control, online signal display, and system diagnostics, for both the individual detector and the complete system. -**Meet**

3. GLP and Regulatory Conformity:

In order to comply with federal and regulatory requirements for quality system accreditation the manufacturer must meet the following specifications:

___ **3.1.** Manufacturer must be certified under ISO 9001 for the instrument manufacturing, service/support and software. -**Meet**

___ **3.2.** Manufacturer must provide a declaration of conformity describing tests performed and instrument serial numbers for all modular components. -**Meet**

___ **3.3.** Manufacturer must be able to supply protocols or procedures describing the software development process and the instructions for verifying the system performance at the customer's site. -**Meet**

___ **3.4.** The manufacturer must include the following services for the system in this bid:

_____ **3.4.1.** Installation Qualification- fully test and document that the system meets -manufacturing specifications when placed in the customer's environment. -**Meet**

_____ **3.4.2.** Operational/Performance Qualification **–** fully test and document the system performance when configured to the customer's operating conditions. -**Meet**

_____ **3.4.3.** The system must have electronic records or logs of maintenance and system errors (audit trails).-**Meet**

_____ **3.4.4.** The system must provide the following features to meet quality system and regulatory requirements including: -**Meet**

___ **3.4.4.1.** Mechanisms to automatically read instrument serial numbers for traceability. -**Meet**

___ **3.4.4.2.** Mechanisms to automatically read or specify the analytical column used in the analysis. -**Meet**

___ **3.4.4.3.** An electronic log of each functional step the software executes (audit trails). -**Meet**

4. Delivery, Installation, Training, Support, and Service

___ **4.1.** Specifications:

- _____ **4.1.1.** Shipping costs must be included with FOB to the destination agency located in Oklahoma City, Oklahoma. - **Meet**
- _____ **4.1.2.** Installation and Initial onsite operator training on system use, including system setup, data analysis, and report generation as well as preventative maintenance must be included and shall be a minimum of 3 days. This training shall also include example analysis from extraction to report generation. -**Meet**
- _____ **4.1.3.** During installation the vendor must verify the system meets manufacturing performance criteria by analyzing a manufacturer designated compound under standard conditions. -**Meet**
- _____ **4.1.4.** Manufacturer must provide all resource support information including contact information for technical support, method development, and sample preparation. - Meet
- _____ **4.1.5.** Manufacturer must provide telephone support between the hours of 7:00 am and 6:00 pm Central Time. Call back times shall not exceed 4 business hours. -**Meet**
- _____ **4.1.6.** Manufacturer must provide a minimum of a 1 year on-site warranty including parts, travel, labor and instrument control software updates. -**Meet**
- _____ **4.1.7.** Manufacturer must have factory trained service personnel within the physical boundaries of the state of Oklahoma or adjoining states. -**Meet**

October 21 2024

Oklahoma Office of Management & Enterprise Services
Department of Agriculture
Food and Forestry
Richard Williams
Richard.Williams@omes.ok.gov

RE: Solicitation # EV00000549: High-Performance Liquid Chromatography with QQQ Mass spectrometer

Dear Richard Williams

Waters is pleased to submit our proposal in response to your request for the GSA RFQ1715313 for the acquisition of a Liquid Chromatography Tandem Quadrupole Mass spectrometer (LC-MS/MS) system. We understand your technical requirement that is precise and reliable mass spectrometer system play in your day-to-day operations and we are confident that our solution will not only meet but exceed your expectations. At Waters corporation, we specialize in providing cutting edge analytical solutions tailored to the specific demands of our clients.

The Micro System Mass Spectrometer with Arc HPLC System, please see below for Waters' responses to each specification outlined in the Statement of Work on Exhibit. Waters' response to each specification will be one of the following responses: EXCEEDS for exceeding the technical specification requirement, MEETS to be meeting the requirement or DOES NOT MEET for falling short.

Response to Specifications and Requirements- Overall Exceeds

EXHIBIT 1
SPECIFICATIONS

Bid Specifications: High-Performance Liquid Chromatograph with Vacuum Degasser equipped with QQQ Mass Spectrometer, Autosampler and Instrument Controller.

For the bid to qualify for consideration the following minimum specifications must be met entirely and without exception OR the vendor must explain any variation or exception to these minimum specifications in detail and item by item. The vendor shall provide product brochures and/or published literature detailing the instrument specifications.

Bidders shall initial each line of specifications indicating product bid meets that specification.

1. High-Performance Liquid Chromatograph with Vacuum Degasser must include the following features:

___ **1.1.** Overall Instrument System Requirements, Safety and Environmental:

____ **1.1.1.** Instrument system must be of a modular design using stackable, self-contained units with solvent resistant material used in all areas that may have contact with the mobile phase. - **Meets**

____ **1.1.2.** All modular components must form a fully integrated instrument system, with functional communication passing between all modular components and the instrument controller. - **Meets**

____ **1.1.3.** System must be entirely electric and operate on US standard current of 120 VAC at 60 Hz. -**Meets**

____ 1.1.4. System must be able to operate in an environment with a temperature range of 18-30 ^{0}C and with a relative humidity range of 20-80% non-condensing. -**Meets**

____ 1.1.5. Leak detection and safe leak handling design, including leak sensors, must be incorporated in the module housings. -**Meets**

____ 1.1.6. Design must ensure isolation of electrical components from liquid flow path. -**Meets**

____ 1.1.7. User maintenance areas must be physically isolated from areas of high voltages. -**Meets**

____ 1.1.8. The following able includes target compounds and desired concentrations routinely tested by our laboratory. Please indicate with "YES" or "NO" your instrument's ability to analyze these compounds at the concentration specified in sample matrices including soil, vegetation, and water: Meets

Vendor Response	Analyte	Concentration in sample
Yes	2,4 Dichlorophenoxyacetic Acid (2,4-D)	1-5 ppb
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Yes	Dicamba	1-5 ppb
Yes	Dichlorprop (2,4-DP)	1-5 ppb
Yes	2-methyl-4-chlorophenoxyacetic acid (MCPA)	1-5 ppb
Yes	Methylchlorophenoxypropionic acid (MCPP)	1-5 ppb
Yes	Picloram	1-5 ppb
Yes	Triclopyr	1-5 ppb
Yes	Bensulfuron-methyl	1-9 ppb
Yes	Chlorimuron-ethyl	1-9 ppb
Yes	Chlorsulfuron	1-9 ppb
Yes	Halosulfuron-methyl	1-9 ppb
Yes	Metsulfuron-methyl	1-9 ppb
Yes	Nicosulfuron	1-9 ppb
Yes	Primisulfuron-methyl	1-9 ppb
Yes	Prosulfuron	1-9 ppb
Yes	Rimsulfuron	1-9 ppb
Yes	Sulfometuron-methyl	1-9 ppb
Yes	Sulfosulfuron	1-9 ppb
Yes	Triasulfuron	1-9 ppb
Yes	Imazamox	1-9 ppb
Yes	Imazapic	1-9 ppb
Yes	Imazapyr	1-9 ppb
Yes	Imazaquin	1-9 ppb
Yes	Imazethapyr	1-9 ppb
Yes	Glyphosate	1-9 ppb
Yes	Aminopyralid	10-99 ppb
Yes	Paraquat	100-999 ppb

___ 1.2. Liquid Chromatograph

____ 1.2.1. Must include a solvent cabinet with four 1-liter or other recommended mobile phase bottles with caps and filters. -**Meets**

____ 1.2.2. Quaternary pump design with variable stroke volume for pulse-free flow delivery or other mechanism to minimize pulsation in flow. -**Meets**

____ **1.2.3.** Mobile phase flow must be user settable from at least 0.02-8 ml/min in steps of 0.001 ml/min at a minimum. -**Exceed the lower flow rate, but does not meet the high flow rate as Arc HPLC system have a flow rate range of 0.001 to 5ml/min**

____ **1.2.4.** Flow precision must be no more than 0.1% RSD at a flow rate of 1 ml/min, based on retention time matching. -**Meets**

____ **1.2.5.** Flow accuracy must be +/- 1% RSD. -**Meets**

____ **1.2.6.** The mobile phase composition precision from mixing must be no more than 0.20% RSD at 0.2 ml/min and 5 ml/min. -**Meets**

____ **1.2.7.** Flow path must be able to tolerate solvent pH from 2-12.-**Exceeds as Arc HPLC have 1 to 12.5**

____ **1.2.8.** Gradient delay volume must not exceed 1100 µL.-**Exceeds less than 1000ul**

____ **1.2.9.** System must incorporate a purge valve for convenient flushing and mobile phase change. -**Meets**

____ **1.2.10.** An optional seal wash must be included. -**Meets**

____ **1.2.11.** It must be possible to store time progression plots for the following instrument parameters: -**Meets**

___ **1.2.11.1.** Flow-M**eets**

___ **1.2.11.2.** %A, %B, %C, %D -mobile phase constituents. -**Meets**

___ **1.2.11.3.** System Pressure-**Meets**

____ **1.2.12.** User-programmable functions must include %B, %C, %D, flow rate, stop time, maximum pressure, minimum pressure, and primary channel. -**Meets**

____ **1.2.13.** Must be time programmable for instrument set-points of % B, % C, %D and flow rate. -**Meets**

____ **1.2.14.** The instrument must log and display information for diagnostic purposes including resettable counters for: -**Meets**

___ **1.2.14.1.** The number of injections and number of valve cycles. -**Meets**

___ **1.2.14.2.** Liters of pumped mobile phase and seal wear. -**Meets**

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____ **1.3.1.** Must use a gas permeable membrane with continuous vacuum degassing design to degas the mobile phases. -**Meets**

____ **1.3.2.** Must operate at a maximum flow rate of at least 8 ml/min per channel. -**NA, as max flow rate is 5ml/min and can operate at that flow rate**

____ **1.3.3.** Must degas independent mobile phase supply lines. -**Meets**

____ **1.3.4.** Material in contact with solvent must be non-metallic and contamination -free to prevent artificial peaks in trace analysis. -**Meets**

____ **1.3.5.** Must have Power and Status indicator to inform the operator of the operational state of the degasser including error log. -**Meets**

____ **1.3.6.** Must provide a pressure reading output signal. -**Meets**

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____ **1.4.1.** Must be thermostatically controlled for regulating the column compartment temperature in the range of 20 ^{0}C below ambient to 80 ^{0}C.-**Exceeds as range is to 90C**

____ **1.4.2.** Temperature stability must be controlled within +/- 0.15 ^{0}C.-**Meets**

____ **1.4.3.** Temperature accuracy must be controlled within +/- 0.8 ^{0}C-**Meets**

Must identify installed columns and transmit the column information to the instrument controller. - **Meets**

___ **1.5.** Automated Sampler

____ **1.5.1.** Must include sample preparation functions, including sample dilution and internal standard addition. -**Meets**

____ **1.5.2.** Injection precision must typically be no more than 0.1% RSD of peak area across the injection range. -**Meets**

____ **1.5.3.** Replicate injections from a single vial must be allowed. -**Meets**

____ **1.5.4.** Sample capacity must be at least 80 – 2 mL vials in a single tray. -**Meets**

____ **1.5.5.** Must accommodate injection volumes in the range of at least 0.1- 100 µL in 0.1 µL increments. -**Meets**

____ **1.5.6.** Carryover must be ≤ 0.05%. -**Exceeds, less than 0.002% on Arc HPLC**

____ **1.5.7.** Must allow random access to vials. -**Meets**

____ **1.5.8.** Must be electrically driven and require no gases for operation. -**Meets**

___ **1.6.** One (1) capillary detector of the following type: QQQ Mass Spectrometer.

____ **1.6.1.** Must include Nitrogen generator to support this system including battery backup. -**Meets**

____ **1.6.2.** Must include UPS Battery Backup capable of supporting entire system - (HPLC, QQQ, and computer) through satisfactory shutdown. -**Meets**

____ **1.6.3.** The mass spectrometer shall have a scan speed of ≥ 15,000 amu/second.-**Exceeds as Xevo TQ-S Micro have scan rate of 20,000 Da/s**

____ **1.6.4.** The mass spectrometer shall have the following scan types available: MS scan, MS SIM, MS/MS product ion scan, MRM, MS/MS neutral loss/gain scan and parent ion scan. Scan modes are time programmable for both polarity (Positive and Negative Ion) and type of scan. -**Meets**

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____ **1.6.6.** The mass spectrometer shall be able to switch from positive ion mode to negative ion mode in 25 ms.-**Exceeds , Xevo TQ-S Micro can switch 15ms**

____ **1.6.7.** The mass spectrometer shall provide for time programming of the following parameters: -**Meet**

___ **1.6.7.1.** Polarity changes in time segment-**Meets**

___ **1.6.7.2.** Solvent diversion through calibrant delivery system valve-**Meets**

___ **1.6.7.3.** Possible 99-time segments with potential 200 MRMs per time window-**Meets**

____ **1.6.8.** The Detector must include the following features:

___ **1.6.8.1.** Inert ion source, programmable up to 350°C-**Meets**

____ **1.6.9.** Mass range: 5 u to 3000 u- **Does not Meet, Xevo TQ-S Micro have a range of 2-2048m/z, which should cover all the small molecules**

___ **1.6.9.1.**

___ **1.6.9.2.** Autotune-**Meet**

___ **1.6.9.3.** Automated peak integration with built-in peak validation-**Meet**

___ **1.6.9.4.** Pesticide database spectral library of at least 900 compounds-**Meet**

___ **1.6.9.5.** Ion gauge controller-**Meet**

___ **1.6.9.6.** Ionization modes: -**Meet**

___ **1.6.9.6.1.** Electrospray (ESI)-**Meet**

___ **1.6.9.6.2.** Atmospheric pressure chemical ionization (APCI)-**Meet**

2. Instrument Controller and Software

___ **2.1.** The instrument controller hardware must include:

____ **2.1.1.** Operating System software and OS Restore Media-**Meet**

____ **2.1.2.** Instrument controller software media-**Meet**

____ **2.1.3.** All additional communications hardware necessary for use with the system modular components included in this bid-**Meet**

____ **2.1.4.** Dual monitors included Meet

____ **2.1.5.** Pesticide database spectral library of at least 900 compounds-**Meet**

___ **2.2.** Instrument Controller Software

____ **2.2.1.** Must be designed for use and operate within the industry-standard -Windows graphical user environment. -**Meet**

____ **2.2.2.** Must integrate completely with and provide full functional control of all modules included in the bid. This includes all actions related to downloading instrument set-points from stored methods, full automation of batch runs, acquiring data, evaluating chromatograms, and reporting analytical results. -**Meet**

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____ **2.2.5.** Must include an integrated tutorial using the software to teach the operator how to use the software. -**Meet**

____ **2.2.6.** Must include a task-oriented graphical user interface including task diagrams for easy comprehension and fast access toolbars. -**Meet**

____ **2.2.7.** Must store each analytical method as a discreet instrument method, including instrument set points, data acquisition conditions, and data evaluation and reporting parameters. -**Meet**

____ **2.2.8.** Must monitor the instrument during acquisition and recording both instrument performance parameters (temperature, pressure, flow, etc.) and any unusual or unexpected events that would affect the integrity or quality of the results. -**Meet**

____ **2.2.9.** Must include graphical zoom, overlay and alignment features to allow visual comparison of signals. -**Meet**

____ **2.2.10.** Must allow the users to annotate chromatograms or spectra. -**Meet**

____ **2.2.11.** Must use a robust peak integration algorithm that gives reliable and reproducible results without users having to set specific integration events or use manual integration for satisfactory data evaluation. -**Meet**

____ **2.2.12.** Must allow users to interactively integrate chromatograms by manually specifying the baseline points graphically on the screen. -**Meet**

____ **2.2.13.** Must be able to identify individual compounds based on default or compound specific retention time windows and the ratios between signals if specified (qualifiers). -**Meet**

____ **2.2.14.** Must be able to set both default calibration curve fits for all compounds and specific fits for each compound. The calibration weighting for each calibration level must be configurable as none (no weighting) and calibrator specific, inverse concentration (1/Conc.) or inverse concentration squared (1/Conc.^2.)-**Meet**

____ **2.2.15.** Must be user selectable to generate quantitative results based on peak area and peak height. -**Meet**

____ **2.2.16.** Must include a standard set of report formats that allow users to report results with or without graphics. -**Meet**

____ **2.2.17.** Must include a report designer/editor that allows users to specify their own report layouts including graphical elements. -**Meet**

____ **2.2.18.** Must be able to send reports to the screen, to a file and to a printer. -**Meet**

____ **2.2.19.** Must allow users to set up the system for unattended operation in a tabular editing manner, much like a spreadsheet, with easy, standard entry or editing. -**Meet**

____ 2.2.20. Must allow the user to preview the execution order of the runs on the screen before they are executed. -**Meet**

____ 2.2.21. Must include summary reporting capabilities that produce graphical trend diagrams. -**Meet**

____ 2.2.22. Must allow modification of set points and time programs even during a run. -**Meet**

____ 2.2.23. Must provide method and run control, online signal display, and system diagnostics, for both the individual detector and the complete system. -**Meet**

3. GLP and Regulatory Conformity:

In order to comply with federal and regulatory requirements for quality system accreditation the manufacturer must meet the following specifications:

___ 3.1. Manufacturer must be certified under ISO 9001 for the instrument manufacturing, service/support and software. -**Meet**

___ 3.2. Manufacturer must provide a declaration of conformity describing tests performed and instrument serial numbers for all modular components. -**Meet**

___ 3.3. Manufacturer must be able to supply protocols or procedures describing the software development process and the instructions for verifying the system performance at the customer's site. -**Meet**

___ 3.4. The manufacturer must include the following services for the system in this bid:

____ 3.4.1. Installation Qualification- fully test and document that the system meets -manufacturing specifications when placed in the customer's environment. -**Meet**

____ 3.4.2. Operational/Performance Qualification – fully test and document the system performance when configured to the customer's operating conditions. -**Meet**

____ 3.4.3. The system must have electronic records or logs of maintenance and system errors (audit trails).-**Meet**

____ 3.4.4. The system must provide the following features to meet quality system and regulatory requirements including: -**Meet**

___ 3.4.4.1. Mechanisms to automatically read instrument serial numbers for traceability. -**Meet**

___ 3.4.4.2. Mechanisms to automatically read or specify the analytical column used in the analysis. -**Meet**

___ 3.4.4.3. An electronic log of each functional step the software executes (audit trails). -**Meet**

4. Delivery, Installation, Training, Support, and Service

___ 4.1. Specifications:

____ 4.1.1. Shipping costs must be included with FOB to the destination agency located in Oklahoma City, Oklahoma. - **Meet**

____ 4.1.2. Installation and Initial onsite operator training on system use, including system setup, data analysis, and report generation as well as preventative maintenance must be included and shall be a minimum of 3 days. This training shall also include example analysis from extraction to report generation. -**Meet**

____ 4.1.3. During installation the vendor must verify the system meets manufacturing performance criteria by analyzing a manufacturer designated compound under standard conditions. -**Meet**

____ 4.1.4. Manufacturer must provide all resource support information including contact information for technical support, method development, and sample preparation. - Meet

____ 4.1.5. Manufacturer must provide telephone support between the hours of 7:00 am and 6:00 pm Central Time. Call back times shall not exceed 4 business hours. -**Meet**

____ 4.1.6. Manufacturer must provide a minimum of a 1 year on-site warranty including parts, travel, labor and instrument control software updates. -**Meet**

____ 4.1.7. Manufacturer must have factory trained service personnel within the physical boundaries

of the state of Oklahoma or adjoining states. -**Meet**

Thank you for considering Waters corporation as your partner in advancing your analytical capabilities, our proposed solution **Quote # 23840068** not only meet your technical requirement but will exceed your expectations for your application. We look forward to the possibilities of collaborating with you on this important project.

Although, we had two "Does not Meet" for max flow rate and max mass range, both are irrelevant for your application. Our Arc HPLC have a max flow rate of 5mL/min, which is the standard for HPLC applications, lastly, Xevo TQ-S micro mass range of 2-2048m/z is excellent mass range for all small molecule and large molecule assay. Finally, we had seven "exceed" RFP requirement. We look forward to working with you to bring our solution and expertise for these assay.

Sincerely,

Donna D'Amico
Contract Manager
Waters Corporation
BID EMAIL bid_desk@waters.com
Email donna_damico@waters.com
34 Maple Street Milford MA 01757

Technical Contacts:
Dennis Karote
Account Manager
Email: Dennis_Karote@Waters.com
Phone: 551-689-9268

Jorge Smith
MS Specialist
Email: Jorge_smith@waters.com
Phone: 608-234-2061

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Xevo TQ-S micro

Xevo™ TQ-S micro is a sensitive but compact, advanced tandem quadrupole mass spectrometer featuring reliable performance with a wide dynamic range and high rates of data acquisition. Robust sensitivity is enabled by proven ZSpray™ and StepWave™ which facilitate the detection of analytes at low concentrations in complex matrices and enable low volume injections with accurate, precise, and consistent results. Xtended Dynamic Range™ (XDR) technology provides accessible sensitivity and method transfer. The Xevo TQ-S micro makes it easier to confidently quantify more analytes using reproducible high acquisition rates with Xcelerated Ion Transfer™ (XIT). Using RADAR,™ which enables rapid switching between MS full scan and MS/MS acquisition modes, analysts can understand sample complexity and improve method development.

SYSTEM HARDWARE SPECIFICATIONS

API sources and ionization modes	High performance ZSpray dual-orthogonal API sources: 1) Multi-mode source – tool-free ESI/APCI/ESCi™* (standard) NB – Dedicated APCI requires an additional probe (optional) 2) UniSpray™ ion source (optional) 3) Tool-free APCI probe (optional) 4) nanoFlow™ ESI source* (optional) 5) ASAP* (optional) 6) APGC ion source* (optional) 7) ionKey™/MS™ source* (optional) Optimized gas flow dynamics for efficient ESI desolvation Tool-free source exchange Vacuum isolation valve Tool-free access to customer serviceable elements Plug-and-play probes De-clustering cone gas Software control of gas flows and heating elements
UniSpray ion source option	UniSpray is an ionization technique designed to broaden the scope of compounds which can be analyzed in a single run, including those which typically optimize in ESI, APCI or APPI. Enhanced ionization efficiency and desolvation allow the potential to combine several methodologies into one, or simply enable the operator to keep the same source for multiple methods, requiring less time performing set-up and routine maintenance, and more time delivering results

** Not available with waters_connect.™*

Ion source transfer optics	StepWave ion transfer optics delivering class leading UPLC™-MS/MS sensitivity. The unique off-axis design dramatically increases the efficiency of ion transfer from the ion source to the quadrupole MS analyzer at the same time as actively eliminating undesirable neutral contaminants
Mass analyzer	Two high resolution, high stability quadrupole analyzers (MS1/MS2), plus pre-filters to maximize resolution and transmission while preventing contamination of the main analyzers
Collision cell	T-Wave™ enabled for optimal MS/MS performance at high data acquisition rates. Allows use of Nitrogen or Argon as the collision gas.
Detector	Low noise, off axis, long life photomultiplier detector
Vacuum system	One split-flow air-cooled vacuum turbomolecular pump evacuating the source and analyzer; One vacuum backing pump
Dimensions	Width: 35.6 cm (14.0 in) Height: 60.0 cm (23.6 in) Depth: 93 cm (36.6 in)
Regulatory approvals/marks	CE, CB, NRTL (CAN/US), RCM

SYSTEM SOFTWARE SPECIFICATIONS

Software	Systems supported on waters_connect and MassLynx™ version 4.2 or later
System setup and method development	System parameter checking and alerts Integrated sample/calibrant delivery system + programmable divert valve Automated mass calibration Automated sample tuning Automated MRM method development UPLC-MS/MS System Check – automated on-column performance test
Automated MRM scheduling (acquisition window assignment)	Dwell time, inter-channel delay time, and inter-scan delay time for individual channels in a multiple MRM experiment can be automatically assigned (using the Auto-Dwell feature) to ensure that the optimal number of MRM data points per chromatographic peak is acquired. The Auto-Dwell feature can dynamically optimize MRM cycle times to accommodate retention time windows that either partially or completely overlap. This greatly simplifies MRM method creation, irrespective of the number of compounds in a single assay, while at the same time ensuring the very best quantitative performance for every experiment
waters_connect Software	The waters_connect Software provides a modern user experience with a HUB design and apps that provide a consistent connected user experience across all applications. It is built for applications with convenient access to scientific apps allowing accelerated time-to-results and result quality. There are common utilities that complete the end-to-end workflow and help increase productivity and efficiency. Confidently report results with accurate, reliable, regulation-standard data from application-focused quantitative workflows with built in traceability for utmost integrity

PERFORMANCE CAPABILITIES

Acquisition modes	Full scan MS Product ion scan Precursor ion scan Constant neutral loss Multiple reaction monitoring (MRM) Simultaneous full scan and MRM (RADAR)
RADAR	An information rich acquisition approach that allows you to collect highly specific quantitative data for target compounds while providing the ability to visualize all other components
Mass range	2 to 2048 *m/z*

For more detailed instrument performance specifications, please contact your local sales representative.

For patent information, please see waters.com/patents

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RE: Solicitation # EV00000549 High-Performance Liquid Chromatography with QQQ Mass spectrometer

Dear Richard Williams

Thank you for the opportunity to participate in the Request for proposals (RFP) process for the solicitation # EV00000549 for a High-Performance Liquid Chromatography (HPLC) System with Vacuum Degasser Equipped with QQQ Mass Spectrometer, Autosampler and instrument controller. We appreciate the request for clarity on key aspects of our LC-MS/MS system. Given the opportunity, Waters will ensure the transfer of the method to Waters platform and assist in successful implementation of these methods. Below is our response to the questions posed:

Question 1: Please provide documentation for Xevo TQ-S Micro instrument demonstrating capabilities to meet specifications.

Attached is the specification sheet for Xevo TQ-S Micro Instrument, also, please see Table 1 for application notes on Waters Platform.

Question 2: Please provide documentation for Arc HPLC instrument demonstrating capabilities to meet specifications.

Attached is the specification sheet for Arc HPLC instrument, Since the introduction of UPLC, Waters application notes are generally done with UPLC inlet for Mass spec, however the RFP requires HPLC, attaching ASMS poster highlighting comparison of HPLC vs UPLC.

Question 3: Please provide documentation for Masslynx Software demonstrating capabilities to meet specifications.

Here is the link to Masslynx Software page, highlighting the capabilities of the software, the application notes listed in Table 1 is completed by Masslynx software.

Question 4: Please provide documentation of Demonstration of Capability for Chlorophenoxy Herbicides, and for Sulfonylurea Herbicides to meet specifications.

Please refer to Table 1, the application notes have hyperlink that will take you to Waters Application notes.

Thank you once again for considering Waters corporation for this opportunity. We look forward to collaborating and delivering.

34 Maple Street Milford, MA [T] 508.478.2000 [T] 1.800.252.4752 [F] 508.872.1990 [W] www.waters.com

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Table 1: Waters Application Note for the list of Compounds listed in RFP Solicitation #EV00000549

Vendor Response	Analyte	Concentration in sample	Application Notes
Yes	2,4 Dichlorophenoxyacetic Acid (2,4-D)	1-5 ppb	Attachment 1
Yes	4-(2,4-dichlorophenoxy)butyric Acid (2,4-DB)	1-5 ppb	Attachment 1
Yes	Clopyralid	1-5 ppb	Attachment 1
Yes	Dicamba	1-5 ppb	Attachment 1
Yes	Dichlorprop (2,4-DP)	1-5 ppb	Attachment 1
Yes	2-methyl-4-chlorophenoxyacetic acid (MCPA)	1-5 ppb	Attachment 1
Yes	Methylchlorophenoxypropionic acid (MCPP)	1-5 ppb	Attachment 2
Yes	Picloram	1-5 ppb	Attachment 2
Yes	Triclopyr	1-5 ppb	Attachment 1
Yes	Bensulfuron-methyl	1-9 ppb	Attachment 3
Yes	Chlorimuron-ethyl	1-9 ppb	Attachment 3
Yes	Chlorsulfuron	1-9 ppb	Attachment 3
Yes	Halosulfuron-methyl	1-9 ppb	Attachment 3
Yes	Metsulfuron-methyl	1-9 ppb	Attachment 3
Yes	Nicosulfuron	1-9 ppb	Attachment 4
Yes	Primisulfuron-methyl	1-9 ppb	Attachment 3
Yes	Prosulfuron	1-9 ppb	Attachment 4
Yes	Rimsulfuron	1-9 ppb	Attachment 3
Yes	Sulfometuron-methyl	1-9 ppb	Attachment 5
Yes	Sulfosulfuron	1-9 ppb	Attachment 3
Yes	Triasulfuron	1-9 ppb	Attachment 4
Yes	Imazamox	1-9 ppb	Attachment 6
Yes	Imazapic	1-9 ppb	Attachment 6
Yes	Imazapyr	1-9 ppb	Attachment 1
Yes	Imazaquin	1-9 ppb	Attachment 3
Yes	Imazethapyr	1-9 ppb	Attachment 6
Yes	Glyphosate	1-9 ppb	Attachment 7
Yes	Aminopyralid	10-99 ppb	Attachment 9
Yes	Paraquat	100-999 ppb	Attachment 8

Sincerely,

Dennis Karote
Account Manager
Waters Corporation
551-689-9268
Dennis_karote@waters.com

Waters™

Application Note

Determination of Acidic Herbicides in Water Using Liquid Chromatography-Tandem Quadrupole Mass Spectrometry

Renata Jandova, Simon Hird, Euan Ross, Marijn Van Hulle

Waters Corporation

Abstract

This application note describes a rapid method for the determination of a range of acidic herbicides in water samples using large volume direct injection by LC-MS/MS using the ACQUITY UPLC I-Class System coupled to the Xevo TQ-XS to achieve best degree of performance and ultra-high sensitivity.

Benefits

Specific, targeted method for the determination of 20 acidic herbicides in water samples that is suitable for monitoring both drinking water and surface waters with minimal sample preparation, for compliance with European regulatory limits.

Introduction

The removal of weeds from agricultural crops is an important contribution to maintaining the productivity and the quality of those crops. Herbicides are a specific group of plant protection products used to treat a variety of weeds integrated with other control practices such as crop rotation, tillage, and fallow systems. Acidic herbicides comprise families of compounds that include derivatives of benzoic acid (e.g. dicamba), acetic acid (e.g. 2,4-dichlorophenoxyacetic acid [2,4-D]), propanoic acid (e.g. fluazifop), butanoic acid (e.g. 4-(2,4-dichlorophenoxy)butanoic acid [2,4-DB]), picolinic acid (e.g. clopyralid), and other miscellaneous acids such as thiadiazine dioxide (bentazone), and imidazolinones (e.g. imazapyr). Some of the acidic herbicides have been commercially available for almost 80 years and are widely approved for use in agriculture and recreational areas such as golf courses and water courses worldwide. Herbicides can enter water bodies either directly through spray or spray drift, or indirectly via surface water runoff or leaching and sub-surface draining. In the aqueous environment, residues of phenoxyacetic acid herbicides are most commonly found in the form of the free acid.

The presence of pesticides in water is monitored globally, modelled after international norms from the World Health Organization (WHO) which are typically used as the basis for regulations worldwide.[1] Presence of pesticides in European waters is regulated through different directives. The EU Drinking Water Directive sets a maximum limit of 0.1 µg/L for individual pesticide residues present in a sample (0.5 µg/L for total pesticides).[2] The Water Framework Directive (WFD) deals with surface waters, coastal waters, and groundwater.[3] Member States must identify River Basin Specific Pollutants and set their own national

environmental quality standards (EQSs) for substances that may have a harmful effect on biological quality and have been identified as being discharged into the water environment in significant quantities. Values for these EQS vary across Europe; for example, the annual average EQS for 2,4-dichlorophenoxyacetic acid (2,4-D) was reported as 0.1 µg/L in France and Germany but 26 µg/L in the Netherlands.[4] In the USA, drinking water is regulated under the Safe Drinking Water Act, where there are varying maximum contaminate levels for each residue.[5]

Other environmental waters are regulated under the Clean Water Act.[6] Although regulations vary from country to country, many look to guidelines established by the WHO, EU, or USA.

There is a need for reliable analytical methods for monitoring acidic herbicides in drinking and surface waters. Historically used methods are liquid-liquid extraction or solid-phase extraction (SPE) as a concentration step. Liquid chromatography with tandem mass spectrometry (LC-MS/MS) has now supplanted gas chromatography-mass spectrometry (GC-MS) due to ease of use and no need or minimal sample preparation.

This application note describes a rapid method for the determination of a range of acidic herbicides in water samples using large volume direct injection by LC-MS/MS on Waters ACQUITY UPLC I-Class System coupled to the Xevo TQ-XS to achieve best degree of performance and ultra-high sensitivity.

Experimental

Sample preparation

Aliquots of surface water samples (10 mL) were centrifuged and passed through a syringe PVDF filter (0.2 µm). Aliquots (1.5 mL) from each water sample were then transferred to deactivated glass vials and acidified (30 µL of 5% formic acid) prior to analysis. The accuracy (trueness and precision) of the method was assessed by analysis of the water samples. Two different samples of drinking (DW) and surface waters (SW), previously shown to be blank, were spiked with the compounds of interest at 0.02 and 0.1 µg/L three times; n=6 for each water type at each concentration. Solutions of standards were prepared over the range 0.01 to 1.0 µg/L in UPLC water, drinking and surface water, to evaluate linearity of response and to determine the concentration of analytes in the spikes (using bracketed calibration).

UPLC conditions

UPLC system:	ACQUITY UPLC I-Class with FTN Sample Manager equipped with a 250 µL extension loop, 500 µL sample syringe and 15 µL sample needle
Column:	ACQUITY UPLC HSS T3, 1.8 µm, 2.1 × 150 mm
Mobile phase A:	0.02% formic acid (aqueous)
Mobile phase B:	Methanol
Flow rate:	0.4 mL/min
Injection volume:	250 µL
Loop offline:	Automatic
Column temp.:	40 °C
Sample temp.:	10 °C
Run time:	15 min

Gradient

Time (min)	%A	%B	Curve
0.0	80	20	–
9.0	0	100	6
12.0	0	100	6

Time (min)	%A	%B	Curve
15.0	80	20	1

MS conditions

MS system:	Xevo TQ-XS
Ionization:	ESI +/-
Capillary voltage:	+2.0/-1.0 kV
Desolvation temp.:	300 °C
Desolvation gas flow:	1000 L/Hr
Source temp.:	120 °C
Cone gas flow:	150 L/Hr
Collision gas flow:	0.14 mL/min
Nebulizer gas pressure:	7 Bar

The data was acquired using MassLynx MS Software v. 4.2, and processed using TargetLynx XS Application Manager. The selection of MRM transitions and optimization of critical parameters was performed by infusion of individual solutions of all the analytes and evaluation of the data by IntelliStart Software to automatically create acquisition and processing methods. Table 1 summarizes conditions for all MRM transitions including the retention times. Soft ionization mode was enabled for MCPB, dicamba, 2,4-DB and triclopyr.

Compound	Retention time (min)	Polarity	MRM	Cone (V)	CE (eV)
Clopyralid	2.91	ESI+	**192>110** 192>146	30 30	30 20
Imazapyr	4.19	ESI+	**262>149** 262>202	30 30	25 22
Dicamba	5.45	ESI-	**175>145** 219>175	20 20	5 5
Fluroxypyr	5.79	ESI-	**253>233** 253>175	30 30	8 22
Bentazone	5.99	ESI-	**239>132** 239>175	30 30	25 20
Bromacil	6.17	ESI-	**259>203** 259>160	30 30	18 18
Imazaquin	6.31	ESI+	**312>267** 312>199	30 30	20 25
2,4-D	6.92	ESI-	**219>161** 219>125	30 30	13 25
MCPA	7.12	ESI-	**199>141** 201>143	30 30	13 13
Ioxynil	7.29	ESI-	**370>127** 370>215	30 30	32 30
Dichlorprop	7.66	ESI-	**233>161** 233>125	30 30	13 25
Triclopyr	7.49	ESI-	**256>198** 254>196	20 20	12 12
Fluazifop	7.74	ESI+	**328>282** 328>254	30 30	16 25
Mecoprop	7.76	ESI-	**213>141** 213>71	30 30	10 15
2,4,5-T	7.77	ESI-	**253>195** 253>159	30 30	15 25
2,4-DB	8.13	ESI-	**161>125** 247>161	30 30	10 15
MCPB	8.17	ESI-	**227>141** 229>163	20 20	15 15
Fenoprop	8.37	ESI-	**267>195** 269>197	30 30	15 15
Haloxyfop	8.64	ESI+	**362>288** 362>272	30 30	25 32

Table 1. MRM parameters for acid herbicides (quantitative transitions in bold).

Results and Discussion

Fragile compounds and soft ionization

Some of the compounds of interest, 2,4-DB, dicamba, MCPB and triclopyr, exhibited fragmentation within the source region under typical settings. Therefore, the temperature of the source block and the desolvation gas was reduced to 120 °C and 300 °C, respectively, which increased the response of the deprotonated molecular ion. These compounds were also acquired in soft ionization mode, a function enabled in the MS acquisition file that applies a shallower gradient of voltages to the StepWave XS ion transfer optics to reduce fragmentation during transmission of ions to the first quadrupole. Reducing fragmentation can result in significant improvements in sensitivity as shown in Figure 1.

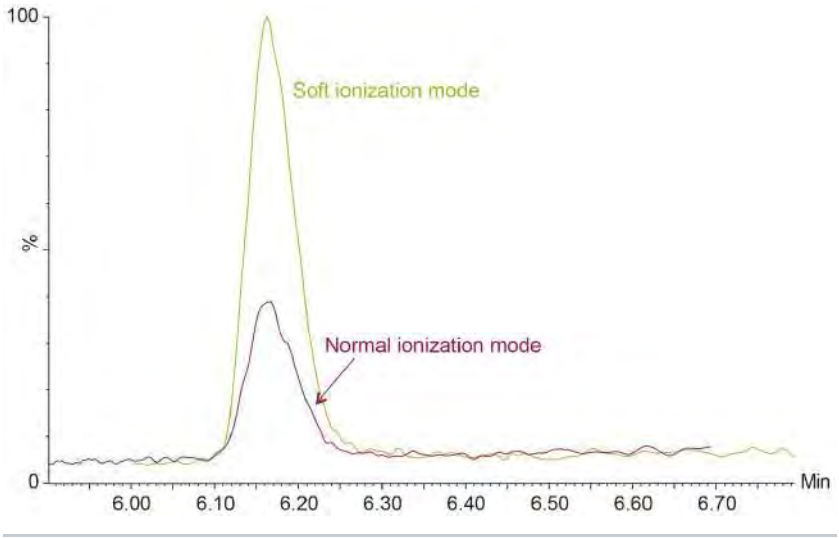

Figure 1. Chromatogram for MRM transition of dicamba 219>175 (fragment originating from deprotonated molecular ion) in soft ionization mode and normal mode.

Sensitivity and selectivity

Excellent sensitivity and selectivity were demonstrated by the response for each compound detected from the analysis of drinking and surface waters spiked at 0.02 µg/L, which is well below the maximum limits. Figure 2 shows a representative example for surface water, while the drinking water provided comparable or better results for all of the herbicides. Laboratories are expected to provide methods with lower limits of quantification (LLOQ) of at least one third of the EQS. The sensitivity observed suggests that detection and

quantification of all compounds at lower concentrations should be possible.

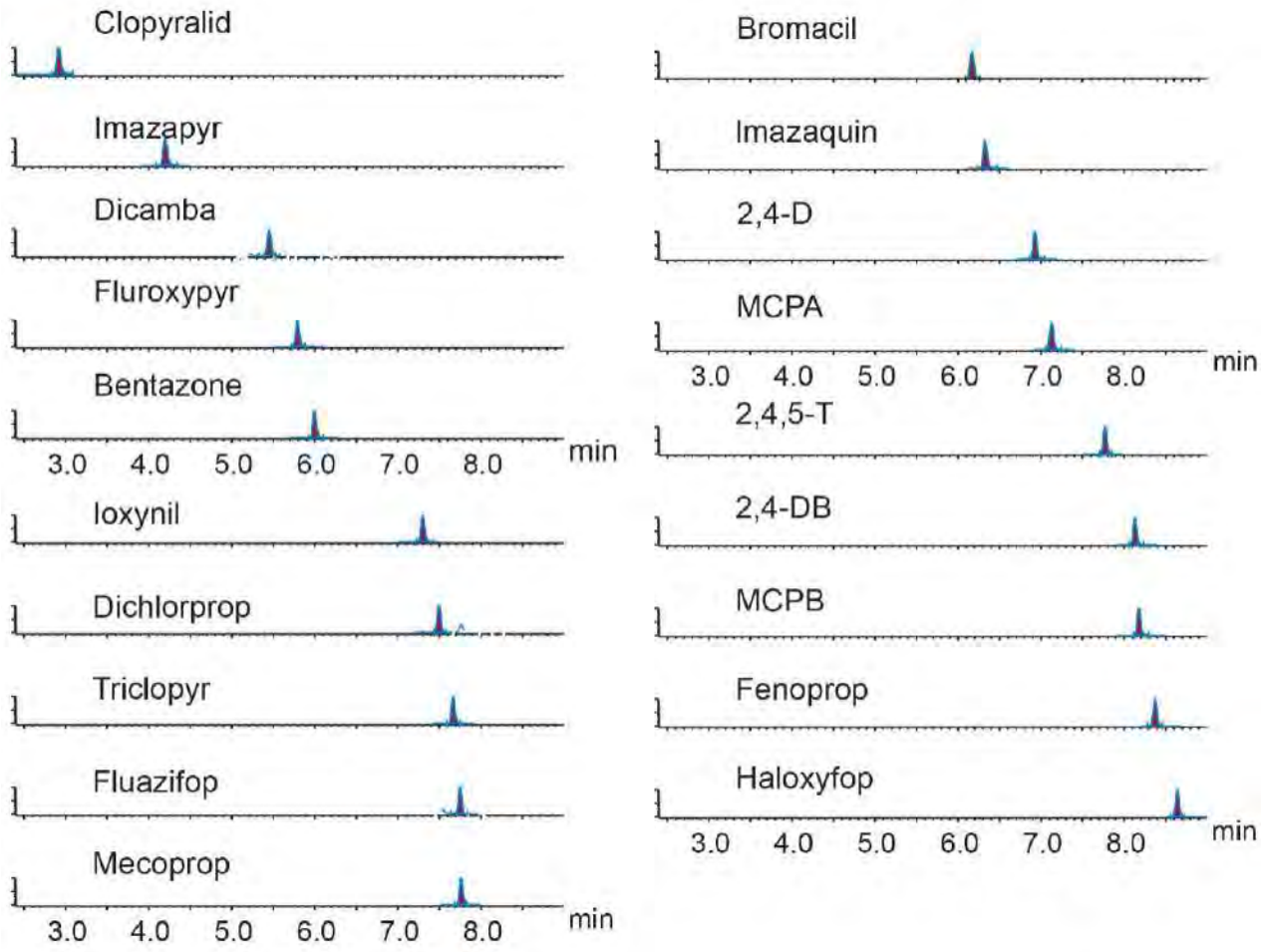

Figure 2. Chromatograms showing the quantitative transitions for acidic herbicides from the analysis of surface water spiked at 0.02 µg/L.

Quantification and accuracy

Standard solutions, prepared in drinking and surface waters at seven concentrations: 0.01, 0.02, 0.05, 0.10, 0.20, 0.50, and 1.0 µg/L, were used for calibration. The response was linear for most compounds, and where non-linearity was observed (e.g. bentazone), a second-order regression (quadratic) was applied to the compound's calibration points. In all cases, the correlation coefficients (r^2) were >0.99 with residuals of <20%, as shown in Figure 3.

The accuracy of the method was determined from the analysis of spiked water samples. The measured values were compared with the expected values from spiking and found to be within the range 88% to 120%

(Table 2). Repeatability was good with RSDs ≤7% at 0.1 µg/L and ≤20% at 0.02 µg/L.

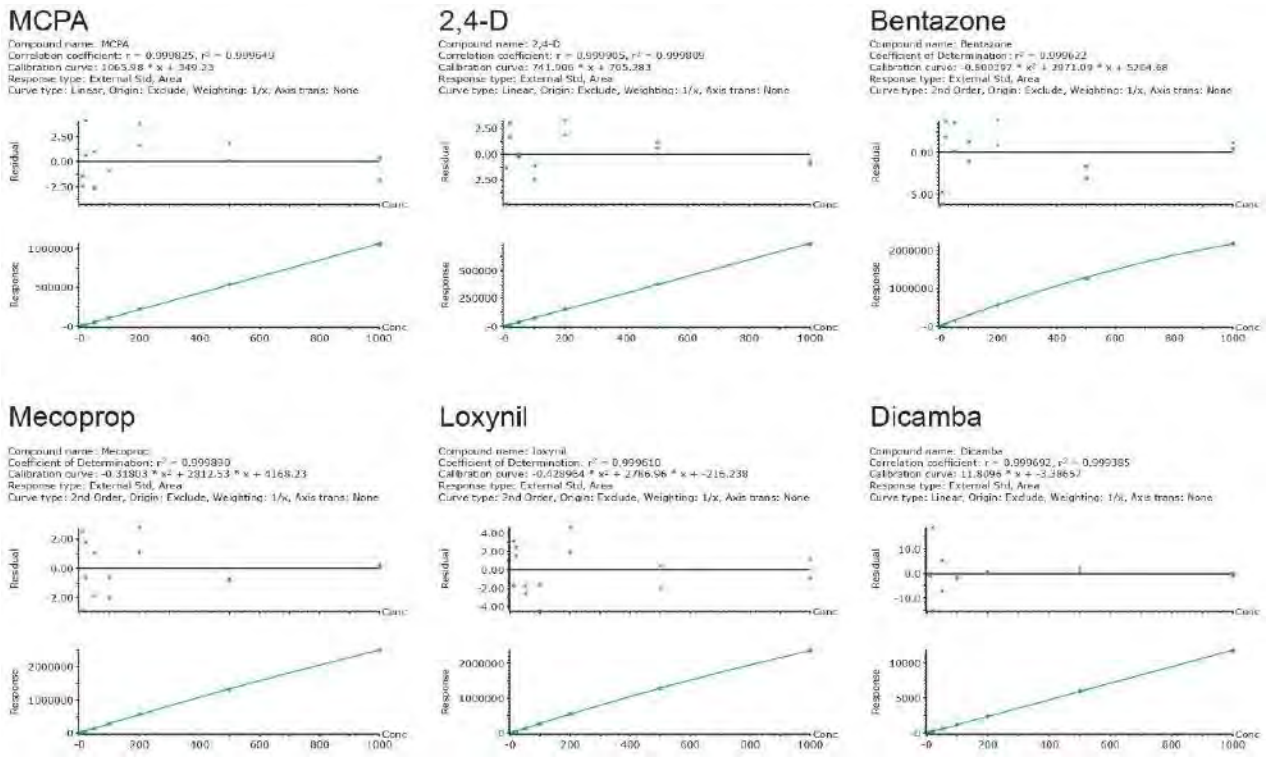

Figure 3. Calibration graphs for a selection of acidic herbicides prepared in drinking water.

Compound	DW 0.02 µg/L	DW 0.1 µg/L	SW 0.02 µg/L	SW 0.1 µg/L
2,4,5-T	99 (4)	104 (5)	102 (5)	100 (4)
2,4-D	105 (4)	104 (4)	111 (13)	101 (6)
2,4-DB	102 (3)	104 (4)	102 (9)	99 (2)
Bentazone	112 (1)	119 (1)	100 (4)	105 (6)
Bromacil	104 (6)	105 (5)	100 (3)	96 (2)
Clopyralid	88 (20)	99 (1)	100 (3)	98 (3)
Dicamba	98 (11)	99 (7)	95 (5)	96 (6)
Dichlorprop	101 (3)	101 (5)	102 (5)	103 (2)
Fenoprop	103 (4)	102 (3)	104 (2)	104 (2)
Fluazifop	106 (3)	100 (2)	103 (9)	100 (3)
Fluroxypyr	105 (3)	101 (5)	109 (14)	98 (2)
Haloxyfop	101 (2)	100 (2)	108 (14)	101 (3)
Imazapyr	106 (2)	108 (3)	105 (9)	102 (3)
Ioxynil	99 (2)	105 (2)	99 (2)	101 (1)
Imazaquin	103 (1)	104 (1)	107 (11)	102 (1)
MCPA	103 (4)	104 (4)	105 (8)	101 (6)
MCPB	102 (6)	103 (6)	102 (7)	99 (3)
Mecoprop	103 (4)	104 (4)	101 (9)	99 (3)
Triclopyr	102 (3)	103 (3)	98 (5)	99 (1)

Table 2. Trueness (%) and precision (%RSD) from measurements of spiked water samples (n=6 per each concentration).

Identification criteria, ion ratios, and retention times, were all within acceptance tolerances (±30% of the reference and ±0.1 min, respectively). Figure 4 shows the transitions for a selection of acidic herbicides from analysis of surface water spiked at 0.02 µg/L.

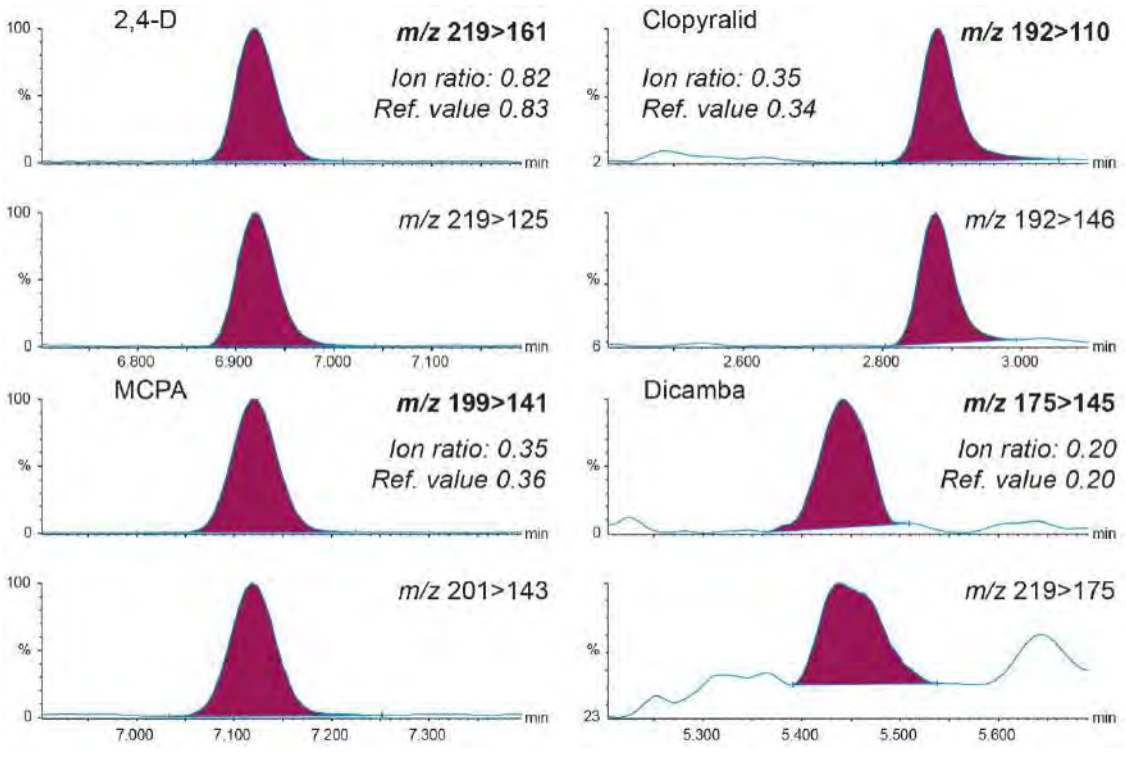

Figure 4. Chromatograms showing both transitions for a selection of acidic herbicides from the analysis of surface water spiked at 0.02 µg/L (quantitative transitions in bold).

Conclusion

This application note describes the performance of a method for the determination of 20 acidic herbicides by direct, large-volume injection of water samples by UPLC-MS/MS on an ACQUITY UPLC I-Class System coupled to the Xevo TQ-XS. The method is simple, fast, and reliable which avoids the time, costs, and potential losses of recovery associated with various types of sample preparation. The results of our internal validation indicate that the method is suitable for the determination of acidic herbicides in both drinking and surface waters for monitoring purposes. Calibration characteristics, linearity, and residuals were very good over the concentration range studied and accuracy of the method was shown to be excellent. Scientists must validate the method in their own laboratories and demonstrate that the performance is fit for purpose and meets the needs of the relevant analytical control assurance system.

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720006307, June 2018

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Direct Quantification of Acidic Herbicides in Drinking Water Samples Using Ultra-Sensitive UPLC/MS/MS Analysis

Claude R. Mallet, Dimple Shah, Jennifer Burgess
Waters Corporation, Milford, MA, U.S.A.

APPLICATION BENEFITS

- Fast UPLC® analysis with ACQUITY UPLC® BEH C_{18} Column
- Sensitive and selective UPLC/MS/MS analysis with 5 ppt LOQ
- Requires no sample handling or sample preparation

WATERS SOLUTIONS

ACQUITY UPLC I-Class System

Xevo® TQ-S Mass Spectrometer

ACQUITY UPLC BEH C_{18} Column

RADAR™ Technology

Quanpedia™ Database

KEY WORDS

Phenoxyacetic acids, drinking water, direct injection

INTRODUCTION

Phenoxyacetic acids are classified as herbicides. They were first introduced in the 1940's and were in widespread use in agriculture by the middle of the 1950's. Phenoxyacetic acids are widely used in forestry applications, and to some extent in home gardening, and they account for approximately 70% of the weed killers used in agriculture.[1] Consequently, these herbicides are of interest for environmental monitoring in surface and ground waters and are also monitored in drinking water supplies.

When using chemicals for crop protection, toxicity is a crucial factor and these chemicals will often be subject to health evaluations and risk assessments. For example, 2,4-D is used for a variety of crop protection (fruit and vegetable), as well as for turf and lawn care. This herbicide is registered in the United States because it has a favorable environmental profile, and exposures are expected to be minimal in both terrestrial and aquatic environments. 2,4-D is also rapidly broken down by microbial action in soil and it does not persist, accumulate, or leach into groundwater under proper use.[2] In 2005, the U.S. EPA approved the continued use of 2,4-D,[3] with a maximum contaminant level goal (MCLG) of 70 ug/L. The European Union has also evaluated 2,4-D and included it on the list of approved pesticides, since residue levels do not produce any measurable harmful health issues in humans or animals.[4]

Not all phenoxyacetic acid herbicides exhibit low toxicity levels. For example, the toxicity of 2,4,5-T came to light during the Vietnam War. Because phenoxyacetic acids exhibit a rapid activity against broad-leaf plants, they were extensively used as a fast-acting defoliant under the code name "Agent Orange".[5] The formulation was equal parts of 2,4,5-T and 2,4-D. Its toxicity was linked to the contamination of 2,4,5-T with an extremely toxic dioxin.[6] In 1985, the U.S. EPA banned all remaining uses of 2,4,5-T within the United States.

For those phenoxyacetic acids currently registered for commercial use, the EU council directive[7] states that water intended for human consumption should not contain more than 100 ng/L for individual pesticides, and must not exceed 500 ng/L for the sum of all pesticides. In the U.S., they are monitored with EPA methods 515.4 (GC/ECD) with minimum detection limits (MDL's) at 50 ng/L, and method 555 (LC/UV) with MDL's at 100 ng/L.

EXPERIMENTAL

UPLC conditions

UPLC system:	ACQUITY UPLC I-Class
Runtime:	8.0 min
Column:	ACQUITY UPLC BEH C_{18}, 2.1 x 100 mm, 1.7 µm
Column temp.:	60 °C
Mobile phase A:	0.5 % Formic acid in water
Mobile phase B:	0.5 % Formic acid in acetonitrile
Elution:	5-min linear gradient from 5% (B) to 95% (B)
Flow rate:	0.5 mL/min
Injection volume:	100 µL

MS conditions

MS system:	Xevo TQ-S
Ionization mode:	ESI negative
Capillary voltage:	2.0 kV
Cone voltage:	20.0 V
Source temp.:	140 °C
Desolvation temp.:	550 °C
Desolvation gas:	1100 L/hr
Cone gas:	50 L/hr

This application note presents a novel analytical approach for the analysis of phenoxyacetic herbicides in drinking water by direct injection using Waters® highly sensitive Xevo TQ-S tandem quadrupole Mass Spectrometer with the ACQUITY UPLC System. The option of direct injection on the ACQUITY UPLC I-Class System permitted trace level analysis as low as 2.5 ng/L; without the traditional requirement of high volume enrichment during sample preparation. This resulted in faster analysis times and the ability to rapidly report results.

The chemical structures and MRM conditions used for the phenoxyacetic acid herbicides are listed in Figure 1 and Table 1, respectively.

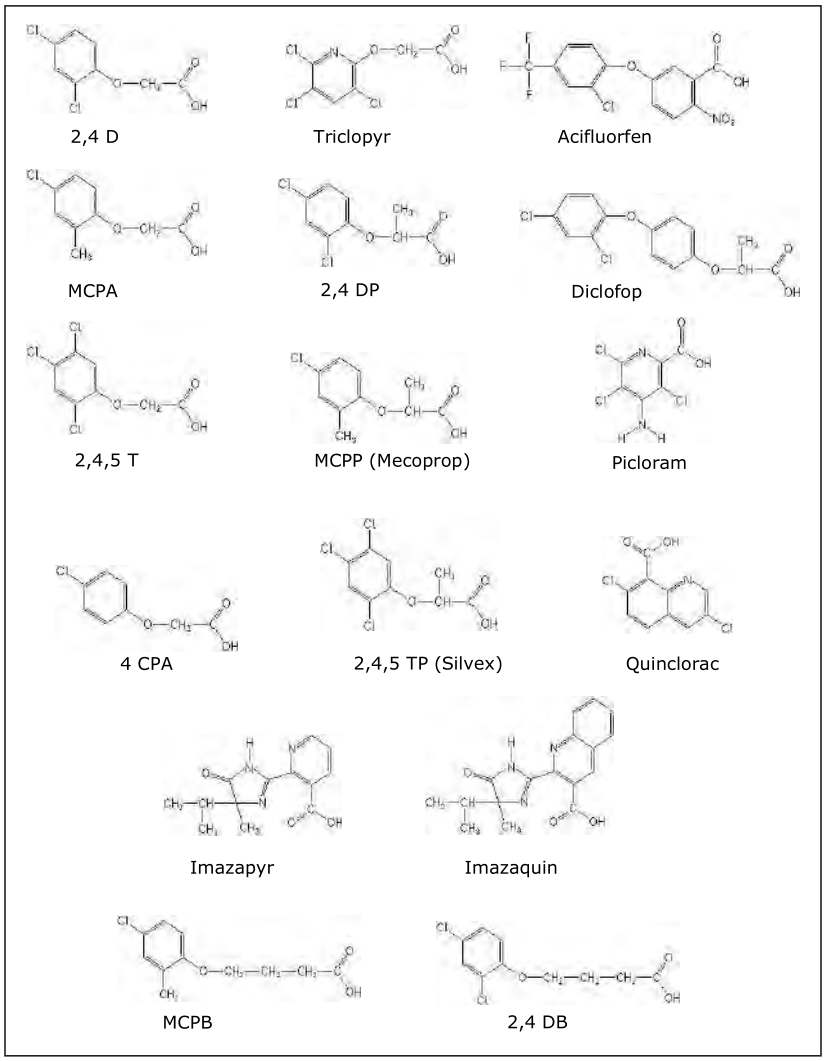

Figure 1. Chemical structure of phenoxyacetic acids used in this study.

The application began by selecting MRM transitions stored in the Quanpedia database and using IntelliStart™ Technology to optimize conditions for one additional compound. Quanpedia is an extensible and searchable database for quantitative LC/MS and LC/MS/MS method information that simplifies and accelerates quantitative analytical method creation. IntelliStart's intuitive software is used to start up, tune, and calibrate Waters' mass spectrometers and, more importantly, to automate analyte tuning and MS method building. Chromatographic separation was performed on an ACQUITY UPLC I-Class System, equipped with an ACQUITY UPLC BEH C_{18} 2.1 x 100 mm Column. A 5-minute linear water/acetonitrile gradient with 0.5 % formic acid was used. The detection was performed using the Xevo TQ-S Mass Spectrometer. The phenoxyacetic acids standards were purchased from Sigma Aldrich (St-Louis, MO, U.S.A.). MilliQ water was used to produce calibration standards. The deuterated 2,4 D was selected as the internal standard. Water samples were collected from natural spring water sources.

Herbicides	Precursor	Product	Cone	Collision
4CPA	185.0	90.9	20	40
	185.0	127.0	20	15
MCPA	199.0	105.0	20	40
	199.0	141.0	20	15
MCPP	213.0	105.0	20	40
	213.0	141.0	20	15
2,4 D	218.9	125.0	20	40
	218.9	160.9	20	15
MCPB	227.0	105.0	20	40
	227.0	141.0	20	15
2,4,5 T	254.9	160.9	20	40
	254.9	196.9	20	15
2,4DP	233.0	125.0	20	40
	233.0	160.9	20	15
2,4,5 T	254.9	160.9	20	40
	254.9	196.9	20	15
Triclopyr	255.9	175.7	20	30
	255.9	197.8	20	10
Imazapyr	260.0	173.0	20	20
	260.0	216.0	20	10
2,4,5 TP	268.9	160.9	20	40
	268.9	196.9	20	15
Imazathapyr	288.0	201.1	20	25
	288.0	244.1	20	15
Diclofop	324.9	145.0	20	25
	324.9	253.0	20	15
Haloxyfop	359.0	252.0	20	25
	359.0	288.0	20	15
Acifluorfen	359.9	194.9	20	20
	359.9	315.9	20	10

Table 1. Phenoxyacetic acids MRM conditions.

RESULTS AND DISCUSSION

When dealing with trace level analysis in the ng/L range, the extraction protocol incorporates an enrichment factor to reach the targeted level of sensitivity. This sensitivity requirement means processing a large sample volume (up to 1000 mL), which translates into long and laborious sample handling. With the introduction of the novel StepWave™ ion optics, the Xevo TQ-S Mass Spectrometer offers unsurpassed performance for trace-level analysis. Its high sensitivity allows for the option to bypass the tedious sample concentration requirement associated with trace-level detection of contaminants in drinking water. A clean water sample can be pre-concentrated directly on-column by simply increasing the injection volume (up to 100 µL) using the ACQUITY UPLC I-Class System with Xevo TQ-S.

Quantification

In this application, MilliQ water was used to prepare calibration standards for quantification of natural spring water. As shown in Figure 2, the calibration curve for 2,4-D and 2,4,5-T for natural spring water showed excellent linearity from 5 ng/L to 1000 ng/L (r^2 at 0.995). The other phenoxyacetic acids in the mix showed similar linear regression with r^2 ranging from 0.995 to 0.999 for the same dynamic range, with the exception of Triclopyr, which showed good linearity from 25 ng/L to 1000 ng/L with an r^2 value of 0.995.

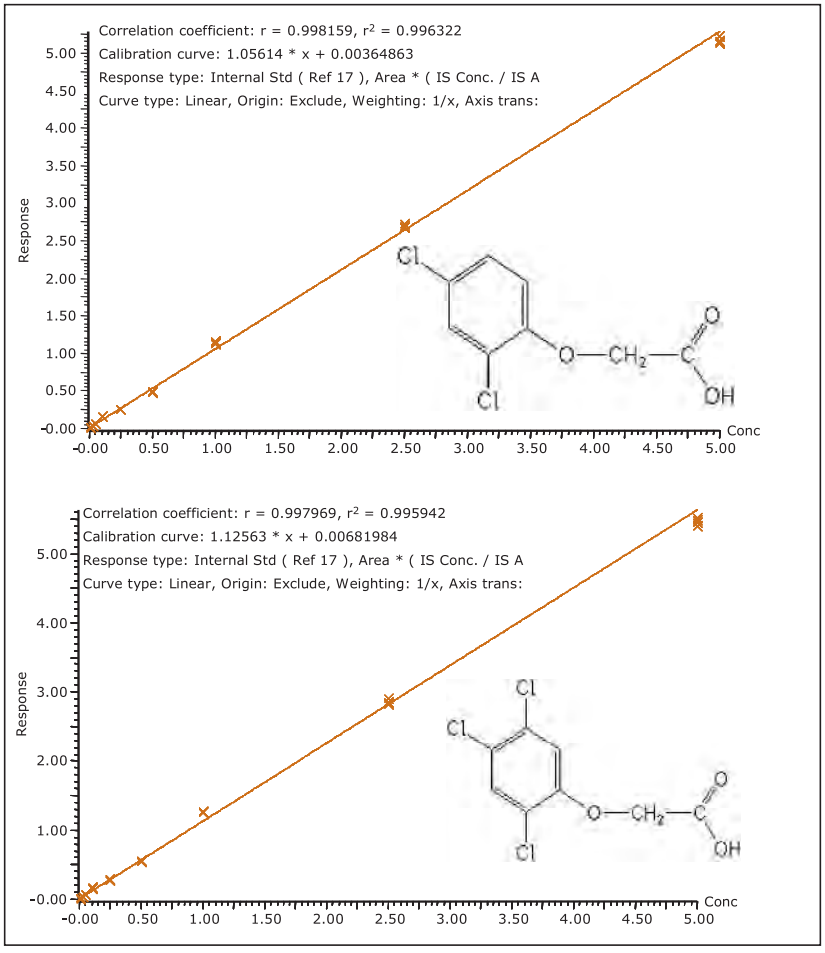

Figure 2. Calibration curve for 2,4 D and 2,4,5 T in natural spring water from 5 to 1000 ppt.

The limit of detection (LOD) was 2.5 ng/L for all phenoxyacetic acids, except for Triclopyr, which had an LOD value of 5 ng/L. The MRM chromatograms at the LOD for a selection of the analytes are shown in Figure 3. The recoveries for a 100 ng/L spike for the phenoxyacetic acids are shown in Table 2. The fortified natural spring water samples were measured against a MilliQ water standard curve and showed recoveries in the range of 107% to 117%. For the majority of the herbicides, the average coefficient of variation (CV's) was well below 5% in natural spring water samples.

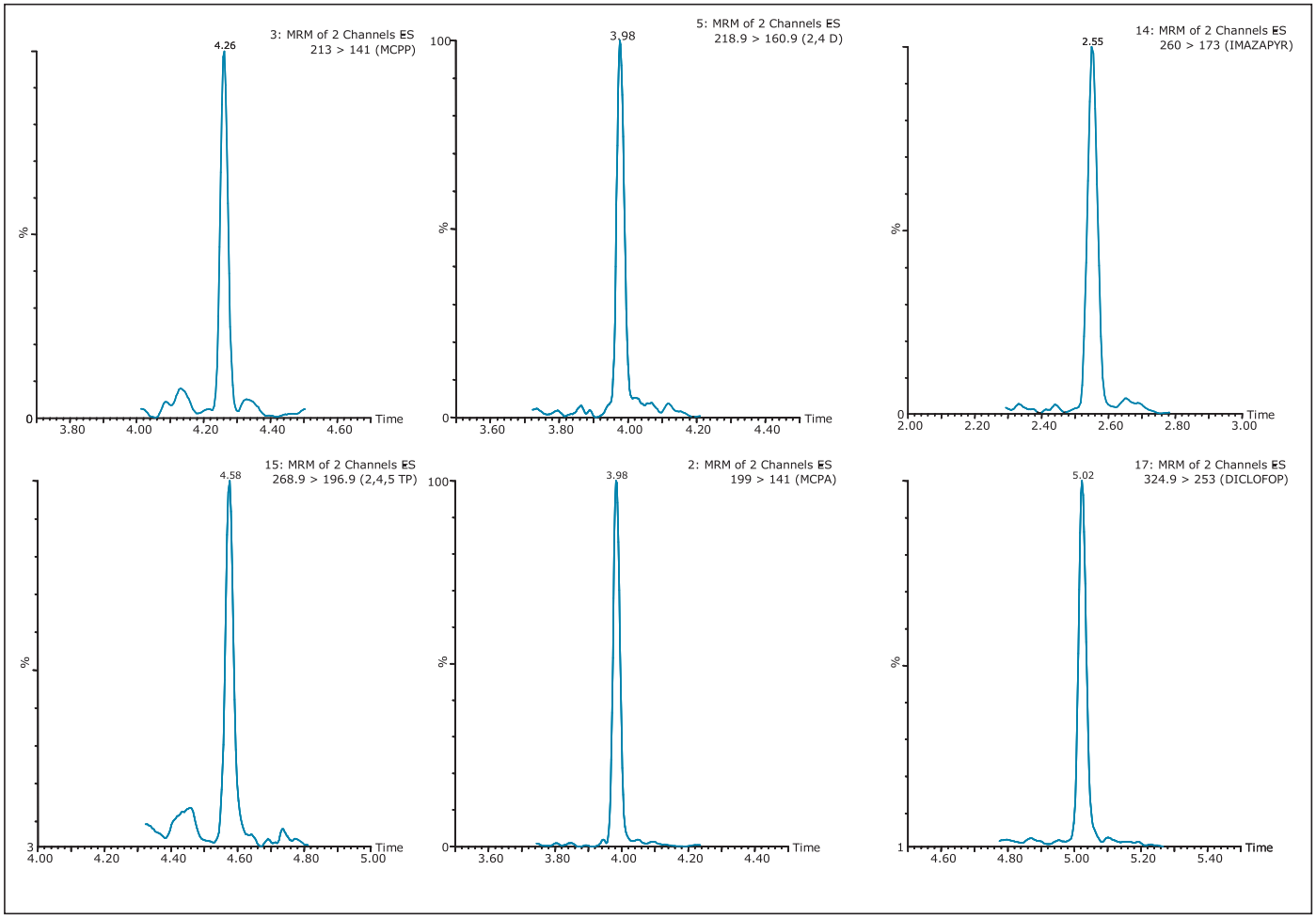

Figure 3. MRM chromatograms at 2.5 ppt for seletected phenoxyacetic acids.

Herbicides	MilliQ	Natural spring
4 CPA	0.14 (3.3)	0.15 (1.5)
MCPA	0.14 (0.9)	0.16 (1.3)
MCPP	0.15 (1.3)	0.16 (1.2)
2,4 D	0.14 (1.4)	0.16 (1.5)
MCPB	0.12 (3.6)	0.14 (2.1)
2,4 DP	0.14 (2.2)	0.16 (1.3)
2,4,5 T	0.14 (2.1)	0.15 (2.1)
Triclopyr	0.12 (1.5)	0.14 (5.2)
IMAZAPYR	0.12 (1.0)	0.14 (0.7)
2,4,5 TP	0.14 (0.9)	0.16 (0.6)
Diclofop	0.14 (0.9)	0.17 (2.4)
ACIFLUORFEN	0.12 (1.9)	0.14 (1.6)
QUINCLORAC	0.13 (4.7)	0.14 (2.3)
PICLORAM	0.13 (1.1)	0.14 (3.8)
2,4 DB	0.13 (1.8)	0.14 (4.6)
IMAZAQUIN	0.12 (0.7)	0.14 (1.0)

Table 2. Recoveries and (coefficient of variations) at 100 ppt in natural spring water samples (n=3).

RADAR TECHNOLOGY

RADAR Technology, is a unique capability of Waters' Xevo tandem quadrupole Mass Spectrometers that enables the simultaneous acquisition of full scan MS data and MRM transitions. This functionality leads to the ability to make informed decisions during the method development process. Using RADAR mode, crucial information can be collected, such as an overview of the water sample's complexity, which will ultimately impact the lifetime of the analytical column and the robustness of the method. Figure 4 shows the MRM chromatograms for a standard of the herbicides at 100 ng/L.

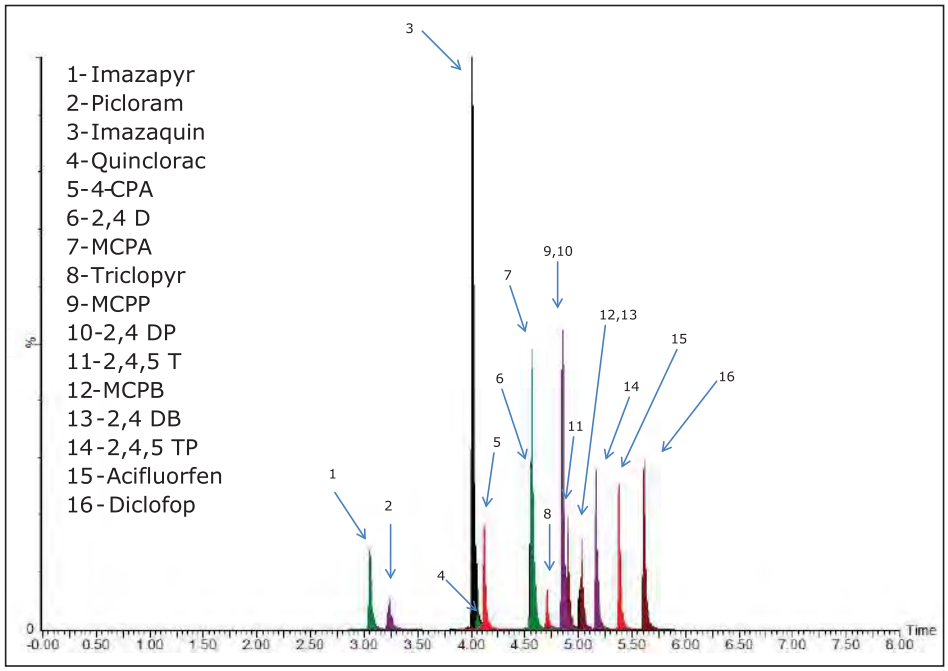

Figure 4. MRM chromatogram of phenoxyacetic acids standards in MilliQ water at 100 ppt.

Overall, the chromatogram showed well-resolved peaks with a Gaussian distribution for all analytes, which is a key parameter for peak integration during quantitation. The chromatography also showed three co-elution zones. With mass spectrometry detection, analytes co-eluting during chromatography are resolved according to their mass-to-charge ratio. The RADAR data – which is acquired simultaneously – can be used to identify whether the herbicides are eluting in regions of potential matrix interferences. The TIC chromatogram of the RADAR data for MilliQ, and natural spring water samples are shown in Figure 5. The MilliQ and natural spring water, shown in Figure 5, detail a potential matrix effect zone during the last minute of the gradient, with a retention time range from 4.2 to 5.2 minutes.

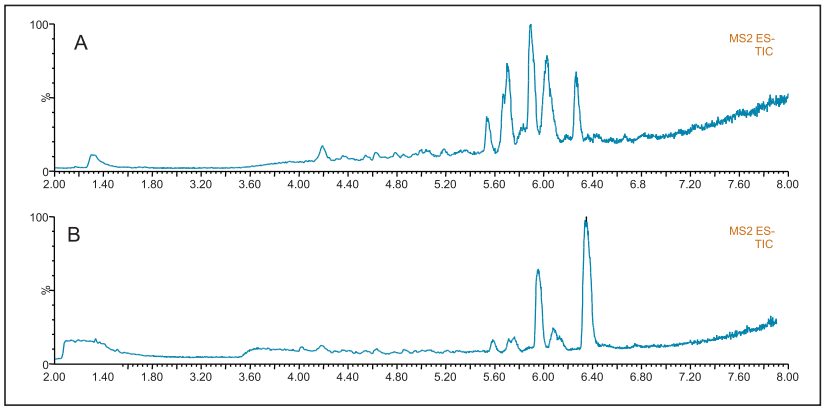

Figure 5. RADAR Full scan total ion chromatograms for A) MilliQ water, B) natural spring water.

By overlaying the MRM chromatograms of the earliest and latest eluting herbicides with the RADAR data, shown in Figure 6, it can be seen that the herbicides eluted at least 30 seconds ahead of the matrix zone. With the information gleaned from the use of RADAR Technology, confidence in the robustness of the method is gained, and changes in the matrix over time can be monitored for any new potential interference.

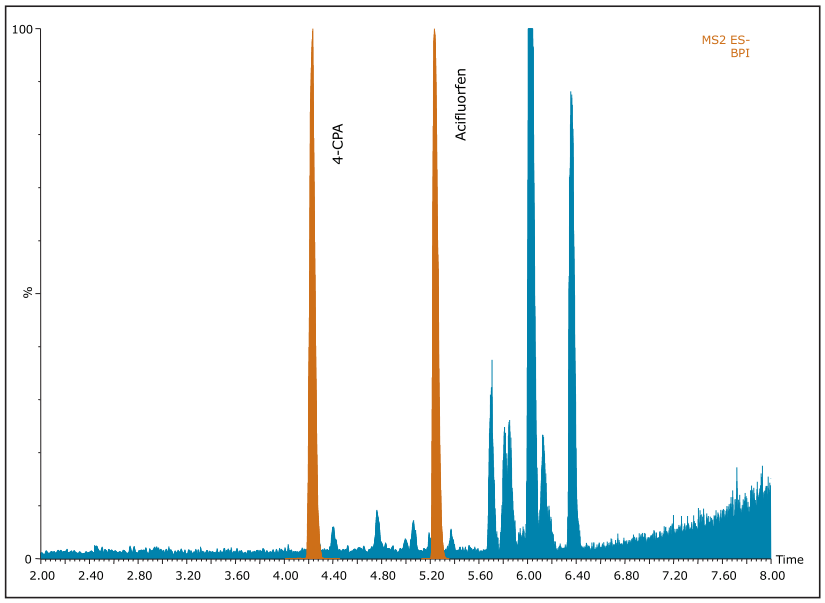

Figure 6. RADAR Full scan BPI chromatogram versus MRM's chromatograms for the earliest and latest eluting analytes.

Lifetime and robustness

The results for a column lifetime study using natural spring water are shown in Figure 7. Samples were injected onto the same analytical column, and demonstrated that even after 500 injections, the analytical performance was not compromised. As shown in Figure 7, the peak shape from the first and the 500th injections for both water samples show no indication of distortion or tailing.

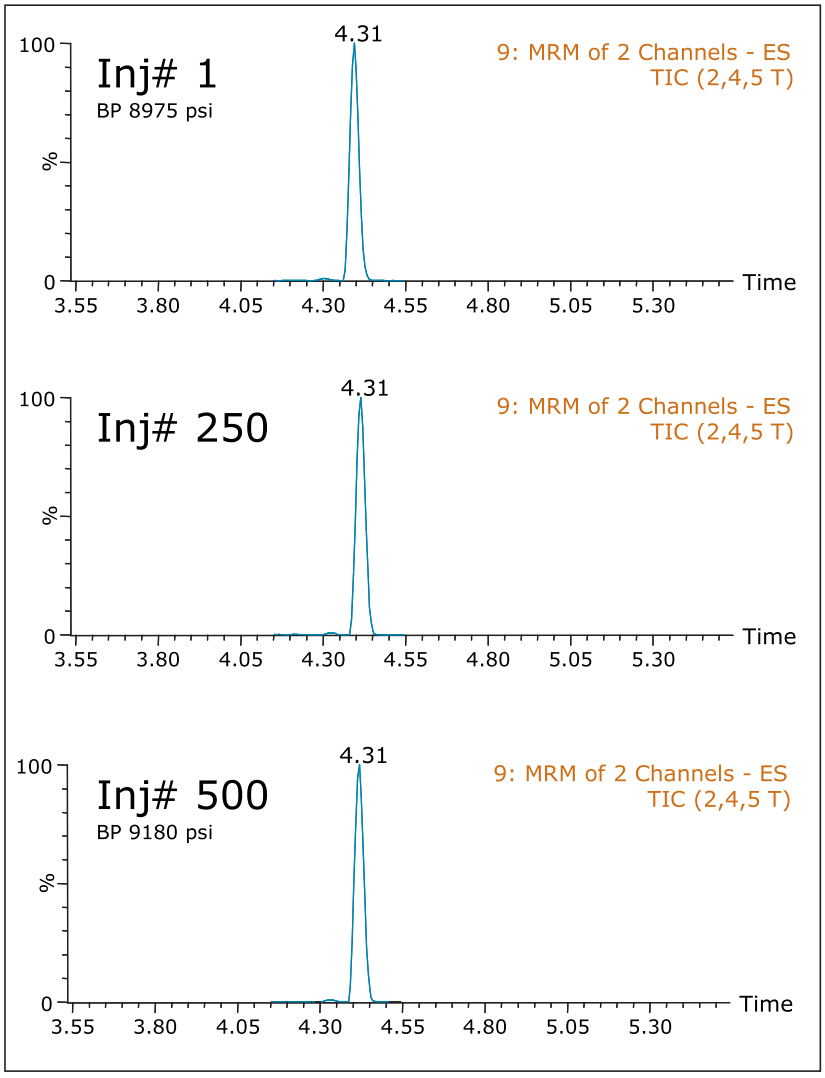

Figure 7. Example MRM chromatograms over the column lifetime study. Injections 1, 250, and 500 are shown for 2,4,5-T for natural spring water samples.

An example of TrendPlot™ Software, shown in Figure 8, gives an overall perspective of the excellent reproducibility for 150 injections of 2,4,5-T in natural spring water.

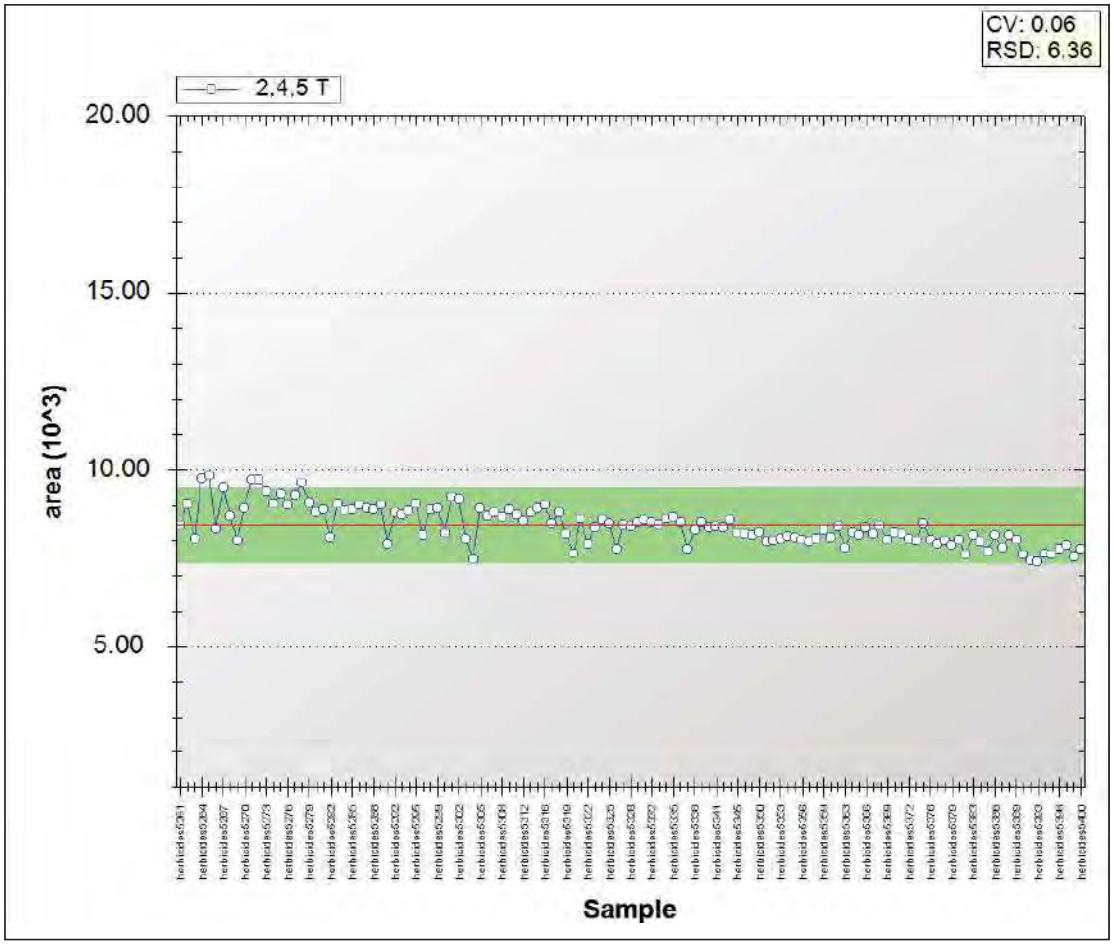

Figure 8. TrendPlot showing peak area of 2,4,5-T for 150 injections in natrual spring water.

Monitoring the analytical column's backpressure throughout its use provides a good indicator of the analytical performance of the column. With UltraPerformance Liquid Chromatography (UPLC) using sub-2 µm particles in an analytical column that is pressure rated at 18,000 psi, the direct injection technique showed reliable results for these drinking water samples.

For example, in this application, the backpressure for natural spring water showed an increase of less than 200 psi after 500 injections. Overall, for this application, the peak shape for phenoxyacetic acids in natural spring water samples showed excellent peak shape with no distortion after 500 injections. The chromatography indicates that the system is still operating at peak performance.

CONCLUSIONS

Trace level analysis using legacy analytical instrumentation (*e.g.* HPLC) will always be associated with tedious and laborious standard operating procedures (SOPs). This application note has demonstrated the versatility of direct injection using the ACQUITY I-Class UPLC System combined with the Xevo TQ-S Mass Spectrometer for the analysis of phenoxyacetic herbicides in natural spring water. The limit of detection (LOD) for the majority of the phenoxyacetic acids in this study was 2.5 ng/L, which exceeded the detection requirements of the U.S. EPA and European Union Directives. The high sensitivity of the Xevo TQ-S enabled excellent quantitation for acidic herbicides using a 100-µL injection without any pre-treatment prior to injection. The recovery data showed good results with excellent CV's below 5% for natural spring water samples. RADAR Technology proved its value during the chromatography optimization process by identifying potential interferences or matrix effect zones.

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Additional Information

Please contact the application note authors for additional information required for this application.

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[APPLICATION NOTE]

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Routine UPLC-MS/MS Quantification of Pesticide Residues in Okra with Simultaneous Acquisition of Qualitative Full-Spectrum MS and MS/MS Data

Dimple Shah,[1] Mark Benvenuti,[1] Antonietta Gledhill,[2] P. M. N. Rajesh,[3] and Jennifer A. Burgess[1]
[1]Waters Corporation, Milford, MA, USA
[2]Waters Corporation, Manchester, UK
[3]Waters Corporation, Bangalore, India

APPLICATION BENEFITS

- Multiple pesticide residues can be detected simultaneously at legislative limits in okra samples using the ACQUITY UPLC® H-Class System coupled with Xevo® TQD MS.
- Quantitative and qualitative information can be achieved in a single injection.
- RADAR™ technology enables simultaneous full-scan data to be acquired, providing important information on matrix background ions that could potentially interfere with the analysis.
- PICS (product ion confirmation scan) provide additional confirmation for compound identification through acquisition of MS/MS spectra in the same injection.

WATERS SOLUTIONS

ACQUITY UPLC H-Class System

Xevo TQD

ACQUITY UPLC HSS T3 Column

MassLynx® Software

KEY WORDS

Pesticides, okra, QuEChERS, food safety

INTRODUCTION

Okra is an important vegetable of the tropical countries and a popular diet component in several countries including India. According to the Food and Agriculture Organization of the United Nations (FAO),[1] India is one of the largest okra producers in the world and it produced approximately 5,800 tons of okra in 2010 and 2011. Okra is susceptible to a variety of pests and diseases[2] and a wide-range of pesticides are used to treat okra plants in India. Legislative limits are in place for the presence of pesticides in domestically produced, imported, or exported okra.[3] It is, therefore, very important to monitor the presence of commonly used pesticides in okra at legislative limits.

According to the PRiF (Pesticide Residues in Food) report, import controls under regulation (EC) No 669/2009 have been increased for okra imported from India because of the frequent detection of pesticide residues, mainly monocrotophos. The consignment is supposed to be rejected if it is non-compliant with MRLs (Maximum Residue Limits). Since July 1, 2012, the frequency of testing consignments has been increased from 10% to 50%. With this frequent testing, monocrotophos, triazophos, and acetamiprid were found at 0.02 mg/kg in okra samples from India, while the MRL for these compounds is 0.01 mg/kg.[4]

In this application note, a multi-residue analysis method for the detection of 212 pesticides in okra is presented. For a complete list of all pesticides, see Appendix A.

Methods

A multi-residue MS method for the pesticides was created using Waters® Xevo TQD Quanpedia™ database. All of the pesticides were analyzed under ESI+ or ESI- mode using rapid polarity switching. Full-scan data were acquired in order to assess any matrix effects and the use of two MRMs and product ion confirmation scans were acquired to confirm and quantify the pesticide residues.

EXPERIMENTAL

UPLC conditions

LC system:	ACQUITY UPLC H-Class
Column:	ACQUITY HSS T3 2.1 X 100 mm, 1.8 µm
Column temp.:	45 °C
Injection volume:	10 µL
Flow rate:	0.45 mL/min
Mobile phase A:	10 mM ammonium acetate (pH 5) in water
Mobile phase B:	10 mM ammonium acetate (pH 5) in methanol
Weak needle wash:	50/50 Water/methanol (v/v)
Strong needle wash:	10/90 Methanol/water (v/v)
Seal wash:	90/10 water/methanol

Time (min)	Flow rate (mL/min)	%A	%B	Curve
Initial	0.450	98	2	6
0.25	0.450	98	2	6
12.25	0.450	1	99	6
13.00	0.450	1	99	6
13.01	0.450	98	2	6
17.00	0.450	98	2	6

Table 1. UPLC method for pesticide analysis.

MS conditions

MS system:	Xevo TQD
Ionization mode:	ESI+/ESI-
Capillary voltage:	3 kV
Desolvation temp.:	500 °C
Desolvation gas flow:	1000 L/Hr
Source temp.:	150 °C

Standard preparation

Pesticide standards were purchased either from Sigma-Aldrich, Fisher Scientific, or AccuStandard. A mix of all pesticides at 400 ng/mL was prepared in acetonitrile and stored at 4 °C.

Sample preparation

QuEChERS is a popular method worldwide for the multi-residue analysis of pesticides in fruits and vegetables. The AOAC official method 2007.01, was used to prepare okra samples that were purchased at a local supermarket. Briefly, okra samples were homogenized in water and 15 grams of homogenate was collected into a 50-mL centrifuge tube. Samples were extracted with acidified acetonitrile and mixed with $MgSO_4$ and NaCl (Tube 1). The tube was shaken for a minute and centrifuged at 1500 rcf for 1 minute. After centrifugation, the matrix cleanup was accomplished by dispersive solid phase extraction (d-SPE) by using 50 mg of primary secondary amine (PSA), 50 mg of C_{18} bonded silica, 150 mg of $MgSO_4$, and 7.5 mg of graphitized carbon black (GCB).[5] 1 mL of supernatant from Tube 1 was added to d-SPE cleanup tube and centrifuged at 1500 rcf for 1 minute. 1 mL of this extract was evaporated to dryness and reconstituted in 200 µL of 40/60 acetonitrile/water spiked with internal standard.

RESULTS AND DISCUSSION

All of the pesticides were successfully detected at 10 ppb (0.01 mg/kg) in okra sample. For all of the pesticides, Appendix A lists the ionization mode, retention time, and whether or not the compound was detected in a pre-spike 1 ppb sample, as well as the 10 ppb pre-spike sample. Figure 1 shows an overlay of the total ion chromatogram (TIC) of all the pesticides at 10 ppb in okra sample.

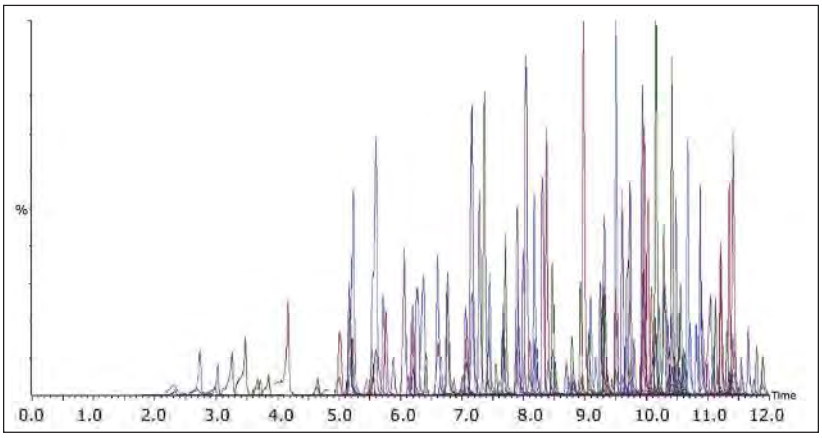

Figure 1. Overlay of MRM chromatograms of all pesticides at 10 ppb (0.01 mg/kg) in okra.

Solvent and matrix match spiked calibration (MMS) curves were prepared at concentrations that equated to the range 1 ppb to 50 ppb (*i.e.* 0.001 to 0.05 mg/kg of okra) and injected in triplicate. The majority of the compounds showed linearity with R^2 values greater than 0.99 in both the solvent and MMS curves. Ethoxyquin, milbemectin A3, and A4, oxadiazon, spiromesifen, and terbufos showed R^2 values greater than 0.970 for both solvent and MMS curves. However, fipronil, phorate, and thiabendazole showed R^2 values greater than 0.970 in MMS curves only. Figures 2 and 3 show calibration curves and residuals for an example compound (triazophos) in solvent and matrix respectively.

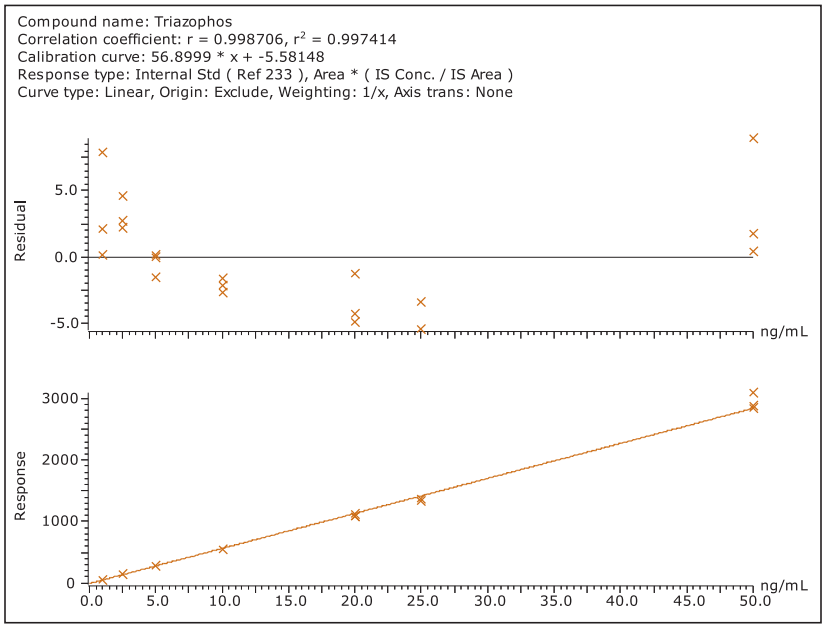

Figure 2. Calibration curve of triazophos in solvent from 1 ppb to 50 ng/mL (0.001 to 0.05 mg/kg).

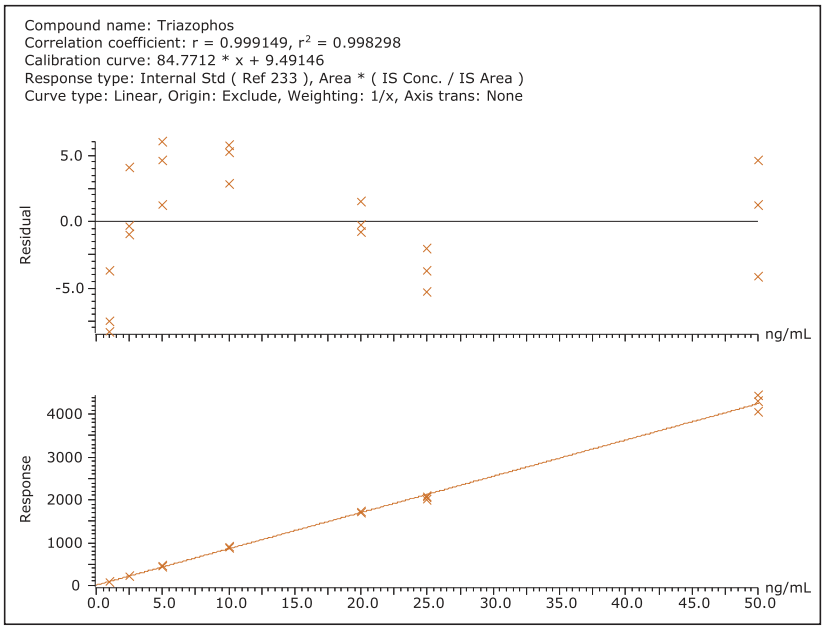

Figure 3. Matrix-match spiked calibration curve of triazophos in okra sample from 1 ppb to 50 ppb (0.01 to 0.05 mg/kg).

To evaluate the recovery, accuracy, and precision of the method, studies were carried out on spiked samples. Okra samples were pre-spiked with all the pesticides at 10 ppb (0.01 mg/kg) in triplicate, extracted, and quantified against the MMS calibration curve. Recoveries were calculated using TargetLynx™ Software. The recoveries reported are without any internal standard correction. As shown in Figure 4 (A, B, C, and D), recoveries for all of the pesticides ranged from 25% to 150%. Relative standard deviations (RSDs, shown as error bars in Figure 4) for most compounds were <20%. The RSDs for 34 compounds were found to be higher than 20%. Use of an internal standard would be likely to significantly improve repeatability for those analytes.

Figure 4. %Recovery for 212 pesticides in okra sample at 10 ppb (0.01 mg/kg).

Matrix effects

Matrix effects for all of the pesticides were calculated by taking the ratio of the slope of the MMS calibration curve to the slope of solvent calibration curve. A percent variation of + 20% was considered as no matrix effect as this variation is close to the repeatability values.[6] Values between + 20% to + 50% were considered as a medium matrix effect, and a strong matrix effect was considered to be values greater than + 50%.[7] Figure 5 shows levels of the matrix effect that were observed in the analysis of okra for all pesticides. A strong matrix effect was observed for the majority of compounds, demonstrating that the analysis of okra samples poses a challenge in regards to high matrix complexity. Even with these high matrix effects, all compounds can easily be detected at legislative limits and quantified using the matrix-matched calibration curve.

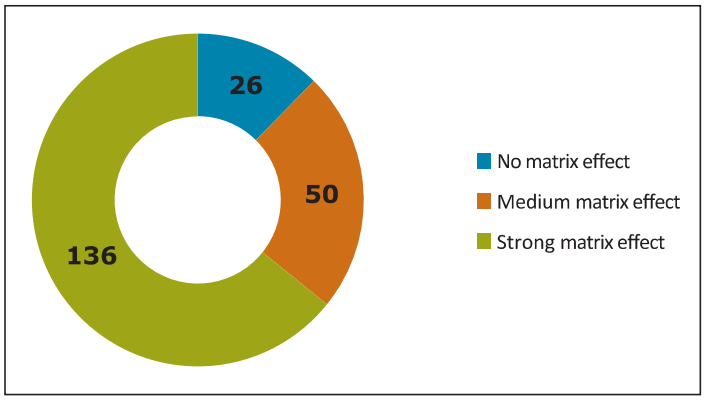

Figure 5. Matrix effects observed for okra sample.

Understanding matrix effects – RADAR

To further understand the impact of co-eluting matrix components that can compete with an analyte of interest during the ionization process, RADAR technology enables the simultaneous acquisition of full spectrum data during quantitative MS/MS analysis. Figure 6 shows an example of the use of RADAR technology. In Figure 6A, the base peak intensity (BPI) chromatogram from the full-scan background data for the okra sample is shown. At 5.08 minutes, close to the retention time of dimethoate (Figure 6B and 6C), high matrix interference was observed. The spectrum at 5.08 minute showed an intense ion at *m/z* 217.1 (Figure 6D).

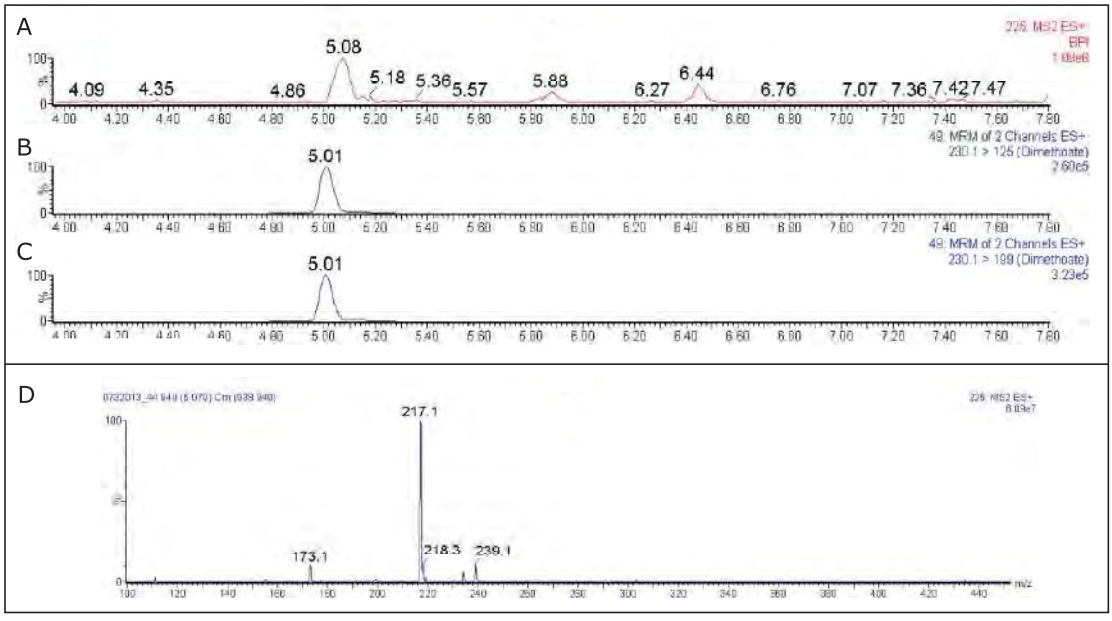

Figure 6. Use of RADAR Technology: (A) Full scan background data for okra sample, (B) and C) MRM transitions of dimethoate, (D) spectrum at retention time of dimethoate.

This interferent potentially has a large impact on the detection of dimethoate and a 48% ion suppression effect was observed for dimethoate. In the case of aldicarb, however, matrix interference was minimal (0.4%) and the RADAR data (Figure 7) showed no evidence of interferences at the retention time of aldicarb (6.13 minutes). The spectrum at the retention time of aldicarb has been expanded and zoomed in the inset (Figure 7D), clearly demonstrating that there was a much higher response from co-extracted matrix ions at the retention time of dimethoate compared to aldicarb. These data clearly demonstrate the usefulness of RADAR technology in assessing the matrix background and its potential effect on ion enhancement or suppression.

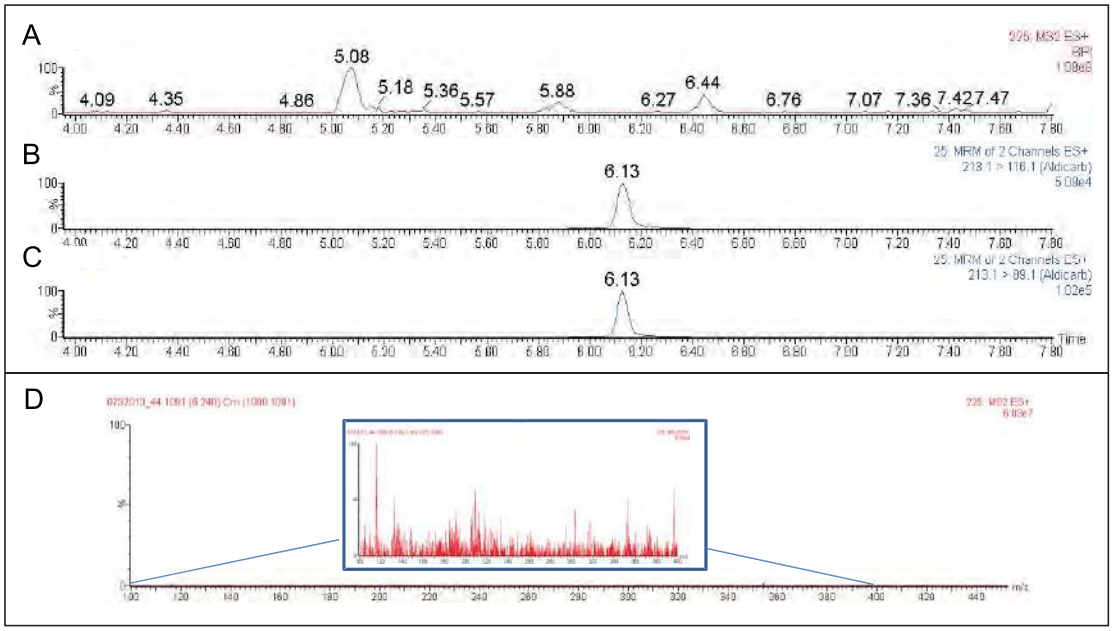

Figure 7. Use of RADAR technology. (A) Full-scan background data for okra sample, (B) and (C) MRM transitions of aldicarb, (D) Spectrum at retention time of aldicarb. The inset has been zoomed to show lower level response compared to the spectrum at the retention time of dimethoate.

Product ion confirmation (PICs)

In complex matrices, situations arise where closely-related compounds such as metabolites or matrix interferences show responses for the target compounds of interest, even in MRM mode. This can lead to ambiguity and may require an additional qualitative experiment. An alternative is to employ a product ion confirmation scan (PICs) within the quantitative MRM experiment. PICs can be used to confirm peak identity through automatic acquisition of an MS/MS spectrum after the apex of the peak has eluted. PICs, in combination with TargetLynx, provides additional confirmation of the compounds of interest through comparison of the acquired MS/MS spectrum to a reference spectrum. Figure 8 shows the TargetLynx results from the comparison of the atrazine MS/MS spectrum obtained from PICS in an okra sample versus the reference spectrum, which was obtained from MS/MS analysis of the standard in solvent.

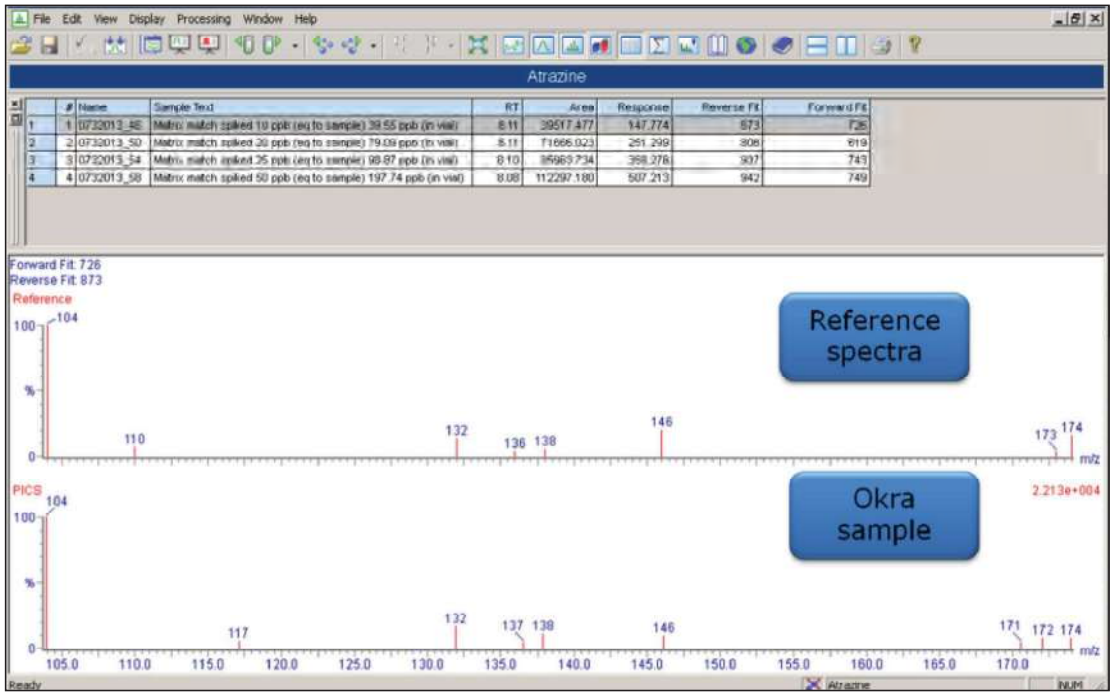

Figure 8. Product ion confirmation (PICs) data for atrazine in okra sample.

CONCLUSIONS

- The combination of ACQUITY UPLC H-Class System with the Xevo TQD tandem mass spectrometer can detect pesticides below the legislative limit in okra samples.
- Even though a strong matrix effect was observed for many compounds, detection and quantification at the legislative limit was achieved.
- Simultaneous acquisition of MRMs and RADAR full-scan data provides quantitative and qualitative information in single injection.
- Product ion confirmation (PICs) increases confidence in compound assignments, which proves highly useful when working with complex matrices.

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Appendix A

In order to determine that the method was fit-for-purpose for the analytes listed, the analysis of pre-spiked samples at 1 ppb (0.01 mg/kg) and 10 ppb (0.01 mg/kg) was undertaken. All compounds were detected at 10 ppb. Those compounds that were also detected at 1 ppb are indicated in the fourth column. Some early eluting compounds showed compromised peak shapes, owing to the sample diluent (40% acetonitrile). Signal-to-noise improvements (and therefore lower LODs) can be gained from reducing the organic content of the sample diluent, however, some risk lies with ensuring that non-polar analytes remain in solution. For this work 40% organic was utilized. Atrazine desethyldesisopropyl, dinotefuran, methamidophos, and oxydemeton methyl showed compromised chromatographic peaks. In addition, for seven compounds, the second transition peak was not apparent at the lowest level. These compounds are shown by an asterisk in the table below.

Name	Ionization mode	Retention time (minute)	Pre-spike level detected
2,4-D	ESI -	6.10	10 ppb
6-Benzyl Adenine*	ESI +	6.63	1 ppb
Acephate	ESI +	2.33	10 ppb
Acetachlor	ESI +	9.80	10 ppb
Acetamiprid	ESI +	5.19	1 ppb
Acifluorfen*	ESI -	8.49	10 ppb
Aldicarb	ESI +	6.16	1 ppb
Aldicarb sulfone	ESI +	3.27	1 ppb
Aldicarb Sulfoxide	ESI +	3.00	1 ppb
Allethrin	ESI +	11.73	1 ppb
Atrazine	ESI +	8.10	1 ppb
Atrazine desethyldesisopropyl	ESI +	1.81	10 ppb
Atrazine desisopropyl	ESI +	4.33	1 ppb
Atrazine-desethyl	ESI +	5.61	1 ppb
Azoxystrobin	ESI +	8.98	1 ppb
Barban/Barbamate*	ESI +	9.25	1 ppb
Bendiocarb	ESI +	7.19	1 ppb
Benalaxyl	ESI +	10.41	1 ppb
Benfuracarb	ESI +	11.20	1 ppb
Bensulfuron methyl	ESI +	8.51	1 ppb
Bifenazate	ESI +	9.53	1 ppb
Bitertanol	ESI +	10.50	1 ppb
Boscalid	ESI +	9.19	1 ppb
Bromacil	ESI +	7.03	1 ppb
Bromodialone	ESI +	10.11	10 ppb
Buprofezin	ESI +	11.37	1 ppb
Butachlor	ESI +	11.43	1 ppb
Carbaryl	ESI +	7.42	1 ppb
Carbendazim	ESI +	5.61	1 ppb

Name	Ionization mode	Retention time (minute)	Pre-spike level detected
Carbofuran	ESI +	7.18	1 ppb
Carbofuran 3 keto	ESI +	6.22	1 ppb
Carbofuran-3-hydroxy	ESI +	5.23	1 ppb
Carbosulfan	ESI +	12.46	1 ppb
Carboxin	ESI +	7.46	1 ppb
Chlorantraniliprole	ESI +	8.73	1 ppb
Chlorfenvinphos	ESI +	10.51	1 ppb
Chlorimuron ethyl	ESI +	7.94	1 ppb
Chlorpyriphos /Dursban	ESI +	11.52	1 ppb
Chlorsulfuron	ESI +	5.46	1 ppb
Clothianidin	ESI +	4.68	1 ppb
Coumachlor	ESI +	8.57	1 ppb
Coumatetralyl	ESI +	7.49	1 ppb
Cruformate	ESI +	9.98	1 ppb
Cyazofamide/ cyazofamid	ESI +	9.81	1 ppb
Cycloxidim	ESI +	10.17	1 ppb
Cymoxanil	ESI +	5.55	1 ppb
Cyprazine	ESI +	8.19	1 ppb
Cyproconazole I	ESI +	9.36	1 ppb
Cyproconazole II	ESI +	9.52	1 ppb
Diafenthiuron	ESI +	11.89	10 ppb
Diazinon	ESI +	10.43	1 ppb
Dichlofluanid	ESI +	9.64	10 ppb
Dichlorvos	ESI +	6.89	10 ppb
Diclofop methyl	ESI +	11.19	10 ppb
Difenconazole I	ESI +	10.35	10 ppb
Difenconazole II	ESI +	10.74	1 ppb
Difenoxuron	ESI +	8.32	1 ppb
Diflubenzuron	ESI +	10.15	1 ppb
Diflufenican	ESI +	10.82	1 ppb
Dimethoate	ESI +	5.04	1 ppb
Dimethomorph I	ESI +	9.09	1 ppb
Dimethomorph II	ESI +	9.29	1 ppb
Diniconazole	ESI +	10.62	1 ppb
Dinotefuran	ESI +	2.99	10 ppb
Dioxathion	ESI +	11.25	1 ppb
Diuron	ESI +	8.20	1 ppb
DMSA	ESI +	6.23	1 ppb
Edifenfos	ESI +	10.28	1 ppb

Name	Ionization mode	Retention time (minute)	Pre-spike level detected
Emamectin Benzoate	ESI +	11.81	1 ppb
Ethiofencarb	ESI +	7.68	1 ppb
Ethion	ESI +	11.42	1 ppb
Ethoxyquin	ESI +	9.79	10 ppb
Ethoxysulfuron	ESI +	7.73	1 ppb
Etrimphos	ESI +	10.25	1 ppb
Famoxadone	ESI +	10.37	1 ppb
Fenamidone	ESI +	9.11	1 ppb
Fenamiphos	ESI +	9.97	1 ppb
Fenarimole	ESI +	9.64	1 ppb
Fenazaquin	ESI +	12.11	1 ppb
Fenchlorphos-oxon	ESI +	9.61	10 ppb
Fenobucarb	ESI +	8.79	1 ppb
Fenoxaprop-p-ethyl	ESI +	11.16	1 ppb
Fenoxycarb	ESI +	9.95	1 ppb
Fenpropathrin	ESI +	11.79	1 ppb
Fenpyroximate	ESI +	11.91	1 ppb
Fenthion	ESI +	10.21	10 ppb
Fenthion sulfoxide	ESI +	7.45	1 ppb
Fenthion-sulfone	ESI +	7.67	1 ppb
Fipronil*	ESI +	10.01	1 ppb
Fipronil carboximide	ESI -	8.54	1 ppb
Fipronil desulfinyl	ESI -	9.81	1 ppb
Fipronil sulfone	ESI +	8.77	10 ppb
Fipronil sulphide	ESI -	10.12	1 ppb
Flonicamid	ESI +	3.69	1 ppb
Fluazifop	ESI +	7.65	1 ppb
Fluazifop-p-butyl	ESI +	11.24	1 ppb
Flubendazole	ESI +	8.42	1 ppb
Flufenacet	ESI +	9.76	1 ppb
Flufennoxuron (flufenoxuron)	ESI +	11.66	1 ppb
Flufenzine *	ESI +	10.06	1 ppb
Fluopicolide	ESI +	9.32	1 ppb
Fluopyram	ESI +	9.61	1 ppb
Flusilazole	ESI +	9.94	1 ppb
Halosulfuron-methyl	ESI +	6.82	1 ppb
Haloxyfop	ESI +	8.85	10 ppb
Hexaconazole	ESI +	10.37	1 ppb
Hexazinone	ESI +	7.17	1 ppb

Name	Ionization mode	Retention time (minute)	Pre-spike level detected
Hexythiazox	ESI +	11.54	1 ppb
Imazalil	ESI +	10.06	1 ppb
Imazaquin	ESI +	5.28	10 ppb
Imazosulfuron	ESI +	6.69	1 ppb
Imidachloprid	ESI +	4.65	1 ppb
Indoxacarb	ESI +	10.81	1 ppb
Iodosulfuran-methyl	ESI +	6.64	1 ppb
Iprobenfos	ESI +	10.15	1 ppb
Iprodione	ESI +	9.91	10 ppb
Iprovalicarb	ESI +	9.72	1 ppb
Isoprothiolane	ESI +	9.32	1 ppb
Isoproturon	ESI +	8.18	1 ppb
Linuron	ESI +	8.83	1 ppb
Lufenuron	ESI -	11.28	1 ppb
Malaoxon	ESI +	7.37	1 ppb
Malathion	ESI +	9.33	1 ppb
Mandipropamid	ESI +	9.25	1 ppb
Mesosulfuron methyl	ESI +	7.31	1 ppb
Metaflumizone	ESI -	11.08	10 ppb
Metalaxyl	ESI +	8.38	1 ppb
Methabenzthiazuron	ESI +	8.09	1 ppb
Methamidophos	ESI +	1.76	10 ppb
Methidathion	ESI +	8.47	1 ppb
Methiocarb	ESI +	8.92	1 ppb
Methomyl	ESI +	3.71	1 ppb
Metolachlor + S-metolachlor	ESI +	9.94	1 ppb
Metoxuron	ESI +	6.30	1 ppb
Metribuzin	ESI +	7.08	10 ppb
Metsulfuron methyl	ESI +	5.19	1 ppb
Mevinphos I	ESI +	5.22	1 ppb
Mevinphos II	ESI +	5.88	1 ppb
Milbemectin A3*	ESI +	12.26	10 ppb
Milbemectin A4 *	ESI +	12.53	10 ppb
Molinate	ESI +	9.37	1 ppb
Monocrotophos	ESI +	4.18	1 ppb
Monolinuron	ESI +	7.55	1 ppb
Mycobutanil	ESI +	9.38	1 ppb
Novaluron	ESI +	10.99	1 ppb
Omethoate	ESI +	2.73	1 ppb

Name	Ionization mode	Retention time (minute)	Pre-spike level detected
Oryzalin	ESI +	9.69	10 ppb
Oxadiargyl	ESI +	10.52	10 ppb
Oxadiazon	ESI +	11.38	1 ppb
Oxamyl	ESI +	3.49	1 ppb
Oxycarboxin	ESI +	5.56	1 ppb
Oxydemeton methyl	ESI +	3.78	10 ppb
Oxyfluorfen	ESI +	8.83	1 ppb
Paclobutrazole	ESI +	9.26	1 ppb
Parathion ethyl	ESI +	9.98	10 ppb
Paraxon methyl	ESI +	6.42	1 ppb
Penconazole	ESI +	10.16	1 ppb
Pencycuron	ESI +	10.67	1 ppb
Pendimethalin	ESI +	11.57	10 ppb
Phenthoate	ESI +	10.09	1 ppb
Phorate	ESI +	5.36	10 ppb
Phorate sulfone	ESI +	8.04	1 ppb
Phorate sulfoxide	ESI +	7.93	1 ppb
Phosalone	ESI +	10.56	1 ppb
phosmet	ESI +	8.70	1 ppb
Phosphamidon	ESI +	6.77	1 ppb
Picoxystrobin	ESI +	10.02	1 ppb
Pirimiphos methyl	ESI +	10.65	1 ppb
Pretilachlor	ESI +	11.04	1 ppb
Primicarb	ESI +	8.06	1 ppb
Prochloraz	ESI +	10.55	10 ppb
Profenofos	ESI +	11.11	1 ppb
Propanil	ESI +	8.81	1 ppb
Propetamphos	ESI +	9.44	1 ppb
Propiconazole (Tilt)	ESI +	10.36	1 ppb
Propoxur	ESI +	7.09	1 ppb
Pyraclostrobin	ESI +	10.48	1 ppb
Pyridalyl	ESI +	12.91	1 ppb
Pyrimethanil	ESI +	8.97	10 ppb
Pyriproxyfen	ESI +	11.40	1 ppb
Pyrithiobac sodium	ESI +	7.01	10 ppb
Quinalphos	ESI +	10.11	1 ppb
Quizalfop free acid	ESI +	8.53	10 ppb
Quizalfop-p-ethyl	ESI +	11.14	1 ppb
Rimsulfuron	ESI +	5.79	1 ppb

Name	Ionization mode	Retention time (minute)	Pre-spike level detected
Simazine	ESI +	7.09	1 ppb
Spinosad A	ESI +	12.40	1 ppb
Spinosad D	ESI +	12.62	1 ppb
Spiromesifen	ESI +	11.76	10 ppb
Spirotetramat	ESI +	9.75	10 ppb
Spiroxamine	ESI +	10.20	1 ppb
Sulfosulfuron	ESI +	6.26	1 ppb
Tebuconazole	ESI +	10.21	1 ppb
Temephos	ESI +	11.33	1 ppb
Terbufos	ESI +	11.26	10 ppb
Tetraconazole	ESI +	9.68	1 ppb
Tetradifon	ESI +	9.40	1 ppb
Thiabendazole	ESI +	6.38	1 ppb
Thiacloprid	ESI +	5.73	1 ppb
Thiobencarb	ESI +	10.59	1 ppb
Thiodicarb	ESI +	7.87	1 ppb
Thiomethoxam (Thiamethoxam)	ESI +	3.87	1 ppb
Thiophanate methyl	ESI +	7.09	10 ppb
Tralkoxydim	ESI +	10.52	1 ppb
Triademefon	ESI +	9.41	1 ppb
Triademenol	ESI +	9.52	1 ppb
Triallate	ESI +	11.61	10 ppb
Triazophos	ESI +	9.50	1 ppb
Trichlorfon	ESI +	5.04	1 ppb
Tricyclazole	ESI +	6.07	1 ppb
Tridemorph	ESI +	12.84	1 ppb
Trifloxystrobin	ESI +	10.88	1 ppb
Triflumizole	ESI +	10.94	1 ppb
Triticonazole	ESI +	9.72	10 ppb
Vamidathion (Vamidothion)	ESI +	5.24	1 ppb

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Application Note

New Technologies for the Simultaneous Analysis of Multiple Pesticide Residues in Agricultural Produce

Catherine Ryan, Gordon Kearney

Waters Corporation

Abstract

This application note highlights recent advances in the chromatographic and mass spectrometric technologies via the analysis of a multi-component mixture for surveillance monitoring of pesticides in agricultural produce.

Introduction

Pesticides are often used in the production of foodstuffs. The concentrations of individual pesticides permitted in our food are controlled by legislation. There is, therefore, a requirement for surveillance monitoring of pesticide residues in foodstuffs. Analytical methods developed for this purpose must achieve limits of detection at or below the Maximum Residue Limit (MRL).

Given the large number of pesticides in existence and the variety of agricultural produce available, multi-residue pesticide screening methods can offer efficiency advantages over single residue and class specific methods. However, these multi-residue methods are limited both by the chromatographic separation of the analytes and the speed of data acquisition.

Tandem quadrupole mass spectrometry is often used as a detection system due to the high selectivity offered in multiple reaction monitoring (MRM) mode, which compensates for generic sample preparation methods involving minimal sample cleanup. Due to the number of potential analytes, the mass spectrometer chosen should be able to rapidly switch both between MRM channels and between positive and negative ionization modes, thereby offering the potential to achieve greater efficiency in the analysis of multi-component mixtures. Complementing these +/– ionization mode switching capabilities in the Waters Micromass Quattro Premier Mass Spectrometer is the revolutionary Waters ACQUITY UPLC System, offering improved chromatographic resolution and shorter analysis times resulting from the use of columns packed with novel 1.7 µm stationary phase particles.[1]

In this work, we highlight recent advances in these chromatographic and mass spectrometric technologies via the analysis of a multi-component mixture for surveillance monitoring of pesticides in agricultural produce.

Experimental

Method

Sample Preparation, Extraction and Cleanup Procedure

The raisin sample, Californian sun-dried seedless raisins (Thompson variety) was prepared using a procedure described below involving methanolic extraction and ChemElut cleanup, evaporation and reconstitution.[2]

The raisin sample was chopped to avoid loss of juice. A 5 g aliquot of the homogenized sample was transferred to a blender cup, to which 9 mL of water was added. After 10 minutes, 20 mL of methanol was added and the sample was blended for 2 minutes. 6 mL of the resultant extract was mixed with 2 mL of a solution of sodium chloride (20 g in 100 mL water). A 5 mL aliquot was then transferred to a ChemElut column containing 5 mL of diatomaceous earth. After 5 minutes, the ChemElut column was eluted with 16 mL of dichloromethane. The eluate was evaporated to dryness and the dry residue was reconstituted in 250 µL of methanol and further diluted with 1 mL of water. The final extract contained the residues of 0.5 g dry sample per mL. The extract was filtered through a 0.45 µm filter into a glass sample vial.

Blank matrix was prepared from organically grown sun-dried seedless raisins (Thompson variety) using the same extraction and cleanup procedure described above. Matrix-matched standards were prepared by spiking all analytes at 0.5, 1, 2.5, 5, 10 pg/L (equivalent to 1, 2, 5, 10, 20 µg/kg, respectively).

LC Conditions

LC system:	ACQUITY UPLC System
Mobile phase A:	MeOH/H_2O (1:4 v/v) + 5 mM $CH_3CO_2NH_4$
Mobile phase B:	MeOH/H_2O (9:1 v/v) + 5 mM $CH_3CO_2NH_4$
Column:	ACQUITY UPLC BEH C_{18}, 2.1 x 100 mm, 1.7 µm
Flow rate:	0.45 mL/min
Injection volume:	20 µL

Column temp.:	40 °C

Gradient elution

Time	%B
0 min	0%
8.5 min	100%
11.0 min	100%
11.1 min	0%
13.5 min	0%

MS Conditions

MS system:	Quattro Premier
Ionization mode:	ES+/ES-
Capillary voltage:	0.8 kV (+/- ionization)
Gas flow:	800 L/hr
Source temp.:	120 °C
Desolvation temp.:	400 °C
Cone voltage:	See Table 1
MS/MS:	Operated in MRM mode
Collision voltage:	See Table 1

Pesticide Residue	Retention Time (min)	Precursor Ion m/z	Product Ion m/z	Cone Voltage (V)	Collision Voltage (V)	Dwell Time (ms)	LOD (ppb)
Daminozid	0.50	161.1	143.1	18	12	200	0.01
Methamidophos	0.79	141.8	93.8	22	14	80	0.02
			124.9	22	13	80	
Acephate	0.89	184.1	143.0	16	8	40	0.04
Butoxycarboxim-sulfoxide	1.00	207.1	132.1	17	6	30	0.05
Dimethoate	1.01	214.0	183.0	20	12	30	0.01
			154.9	20	15	30	
Aldicarb-sulfoxide	1.11	207.1	132.0	16	10	30	0.1
			89.0	16	14	30	
Butoxycarboxim	1.20	240.1	106.1	10	14	30	0.04
Aldoxycarb	1.26	240.1	86.0	15	20	30	0.005
Oxamyl	1.32	237.1	71.9	12	10	30	0.003
Propamocarb	1.36	189.1	102.0	25	17	30	0.01
			144.0	25	12	30	
Oxydemeton-methyl	1.49	247.0	169.0	20	13	10	0.002
Pymetrozin	1.57	218.0	105.0	25	17	10	0.02
6-chloro-4-hydroxy-3-phenyl-pyridazin	1.60	207.1	77.0	35	30	10	0.04
			104.0	35	21	10	
Methamyl	1.60	162.9	87.8	15	8	10	0.01
			105.9	15	10	10	
Demeton-S-methyl-sulfon	1.61	263.1	169.1	28	16	10	0.02
			121.2	28	16	10	

Table 1. MRM method parameters, UPLC retention times and LODs achievable from solvent standards.

Table 1. (continued)

Pesticide Residue	Retention Time (min)	Precursor Ion m/z	Product Ion m/z	Cone Voltage (V)	Collision Voltage (V)	Dwell Time (ms)	LOD (ppb)
Quinmerac	1.69	222.0	141.0	22	33	10	0.008
Monocrotophos	1.78	224.0	126.9	20	15	10	0.005
Bendiocarb	1.78	224.1	109.0	18	18	10	0.01
			167.1	18	9	10	
Nicosulfuron	1.80	411.0	182.1	22	19	10	0.05
Amidosulfuron	1.84	370.0	261.2	18	14	10	0.02
Metsulfuron-methyl	2.00	382.0	167.0	22	15	10	0.02
Thifensulfuron-methyl	2.00	388.0	167.1	22	15	10	0.02
Ethiofencarbsulfon	2.04	275.1	107.1	10	20	10	0.006
Rimsulfuron	2.05	431.9	182.1	30	22	10	0.02
Ethiofencarbsulfoxide	2.13	242.1	107.0	18	18	10	0.003
Thiofanox-sulfoxide	2.14	252.1	104.0	10	12	10	0.3
Imidacloprid	2.14	256.1	209.2	22	16	10	0.02
			175.1	22	20	10	
Florasulam	2.28	360.1	129.0	30	20	10	0.09
5 Hydroxy-clethodim-sulfon	2.29	408.2	204.2	22	16	10	0.1
Thiofanox-sulfon	2.32	268.1	76.0	10	10	10	0.02
Clethodim-imin-sulfon	2.35	302.2	98.1	35	30	10	0.04
Metamitron	2.37	203.0	175.1	28	16	10	0.02
Cinosulfuron	2.42	414.1	183.1	25	18	10	0.05
Chlorsulfuron	2.43	358.1	141.1	25	16	10	0.08
			167.1	25	16	10	
Bromoxynil*	2.45	273.9	78.9	40	25	30	0.2
Dimethoate	2.48	230.1	125.1	17	20	10	0.03
			199.1	17	10	10	
Clethodim-imin-sulfoxide	2.49	286.2	208.2	25	17	10	0.03
Vamidothion	2.51	288.1	146.1	17	12	10	0.005
Carbofuran-3-hydroxy	2.56	220.1	163.1	25	10	10	0.007
Flazasulfuron	2.66	408.1	182.1	25	22	10	0.5
Triasulfuron	2.85	402.0	167.1	25	17	10	0.2
			141.0	25	20	10	
Clethodim-sulfon	2.90	392.1	300.2	20	12	10	0.04
Clethodim-sulfoxide	2.95	376.1	206.2	22	15	10	0.05
Carbendazim	2.98	192.1	160.1	25	18	10	0.05
			132.1	25	30	10	
Thiacloprid	3.05	253.0	126.0	28	22	10	0.01
Difenzoquat methylsulfate	3.12	249.2	193.1	45	28	10	0.03
Butocarboxim	3.32	213.1	75.0	20	14	10	0.005
Aldicarb	3.39	208.1	116.0	7	7	10	0.3
Ioxynil*	3.40	369.8	126.9	40	30	20	0.1
Carbofuran	3.41	222.3	165.2	25	15	10	0.1
Iodosulfuron	3.63	508.2	167.2	25	18	30	1
Thiabendazol	3.78	202.0	175.1	40	25	20	0.07
			131.0	40	32	20	
Propoxur	4.17	210.1	111.0	14	15	10	0.01

Table 1. (continued) MRM method parameters, UPLC retention times and LODs achievable from solvent standards.

Table 1. (continued)

Pesticide Residue	Retention Time (min)	Precursor Ion m/z	Product Ion m/z	Cone Voltage (V)	Collision Voltage (V)	Dwell Time (ms)	LOD (ppb)
Formetanate	4.23	222.1	165.2	20	12	10	0.005
Prosulfuron	4.46	420.0	141.1	25	20	10	0.2
			167.0	25	18	10	
Carbaryl	4.60	202.1	145.0	18	10	10	0.005
Bensulfuron-methyl	4.67	411.1	149.1	25	22	10	0.05
Ethiofencarb	4.76	226.1	107.1	15	15	10	0.01
			164.1	15	8	10	
Primisulfuron-methyl*	4.84	466.9	226.2	20	15	10	1
Triflusulfuron-methyl	4.86	493.0	264.2	28	20	10	0.8
Thiodicarb	4.88	355.1	87.9	15	16	10	0.02
Thiofanox	4.92	219.0	56.9	15	18	10	0.01
Pirimicarb	4.97	239.1	72.0	28	18	10	0.005
			182.1	28	15	10	
Atrazin	5.08	216.1	174.1	30	17	10	0.01
Isoproturon	5.26	207.1	72.1	25	18	10	0.008
Isoxaflutole	5.31	377.1	251.2	15	20	10	0.3
Metalaxyl	5.34	280.1	220.2	20	13	10	0.01
			192.2	20	17	10	
Diuron	5.35	233.1	72.1	25	18	10	0.02
3,4,5-Trimethacarb	5.41	194.1	137.1	18	10	10	0.01
Clethodim	5.52	360.2	164.1	20	19	10	0.05
Desmedipham	5.56	318.2	182.2	17	12	10	0.01
Phenmedipham	5.69	301.1	168.0	25	10	10	2
Linuron	5.92	249.1	160.0	28	16	10	0.02
			182.1	28	15	10	
Pyrimethanil	5.93	200.1	107.0	42	22	10	0.1
			82.0	42	25	10	
Azoxystrobin	5.97	404.1	372.2	22	15	10	0.02
			329.2	22	30	10	
Methiocarb	6.06	243.1	121.0	10	22	10	0.3
Fludioxonil*	6.20	247.0	180.1	45	28	20	0.1
			126.1	45	35	20	
Promecarb	6.23	208.1	151.0	20	9	10	0.03
			109.0	20	15	10	
Iprovalicarb	6.55	321.2	119.1	15	18	10	0.1
Fenhexamid	6.61	302.1	97.0	35	25	10	0.05
			55.1	35	35	10	
Metolachlor	6.81	284.1	176.1	20	25	10	0.01
			252.1	20	15	10	
Tebufenozide	7.01	353.2	133.0	13	20	10	0.4
			297.2	13	8	10	
Fenoxycarb	7.04	302.1	88.0	20	20	10	0.05
Cyprodinil	7.19	226.2	93.1	45	33	10	0.08
			108.1	45	25	10	
Tebuconazol	7.23	308.1	70.0	30	20	10	0.02

Table 1. (continued) MRM method parameters, UPLC retention times and LODs achievable from solvent standards.

Table 1. (continued)

Pesticide Residue	Retention Time (min)	Precursor Ion m/z	Product Ion m/z	Cone Voltage (V)	Collision Voltage (V)	Dwell Time (ms)	LOD (ppb)
Imazalil	7.24	297.1	159.0	30	20	10	0.3
			69.1	30	20	10	
Triflumuron	7.49	359.1	156.0	25	18	10	0.2
			139.0	25	37	10	
Haloxyfop-methyl	7.73	376.1	316.2	30	18	10	0.03
Indoxacarb	7.80	527.9	218.1	28	20	10	0.5
Hexaflumuron*	7.85	459.1	276.1	22	22	30	5
Quizalofop-ethyl	8.00	373.1	299.2	30	19	10	0.03
Fluazifop-P-butyl	8.07	384.1	282.2	32	22	10	0.02
			328.2	32	16	10	
Haloxyfop-ethoxyethyl	8.07	434.0	316.2	25	20	10	0.05
Spiroxamine	8.11	298.3	144.1	30	20	10	0.03
Furathiocarb	8.12	383.1	195.1	20	16	10	0.04
Diflubenzuron	8.14	311.0	158.1	30	14	10	0.1
Teflubenzuron*	8.31	379.0	196.0	18	25	10	0.6
			339.1	18	15	10	
Flufenoxuron	8.68	488.9	158.1	25	18	10	0.05
Pyridate		379.1	207.1	25	16	120	0.05
Fenpropimorph	9.38	304.2	147.2	45	30	120	0.02

Table 1. MRM method parameters, UPLC retention times and LODs achievable from solvent standards.

Results and Discussion

Method Development and Performance

The work details the development of a multi-residue method for the analysis of 100 pesticide residues by UPLC-MS/MS. The work is based upon a previously developed HPLC-MS/MS method using a Waters Alliance HT/Quattro Premier System, which had an overall cycle time of 25 minutes (HPLC Conditions: XTerra MS C_{18} Column, 2.1 x 100 mm, 3.5 µm, linear gradient from 0 to 100% B in 17 min).

Comparison of UPLC and HPLC chromatograms is shown below (Figure 1). Peak widths observed for the majority of pesticide residues analyzed under UPLC conditions are approximately 0.1 min (cf 0.3 min under HPLC conditions). The narrower peak widths often resulted in an increase in signal response over that achieved under HPLC-MS/MS conditions (Figure 1).

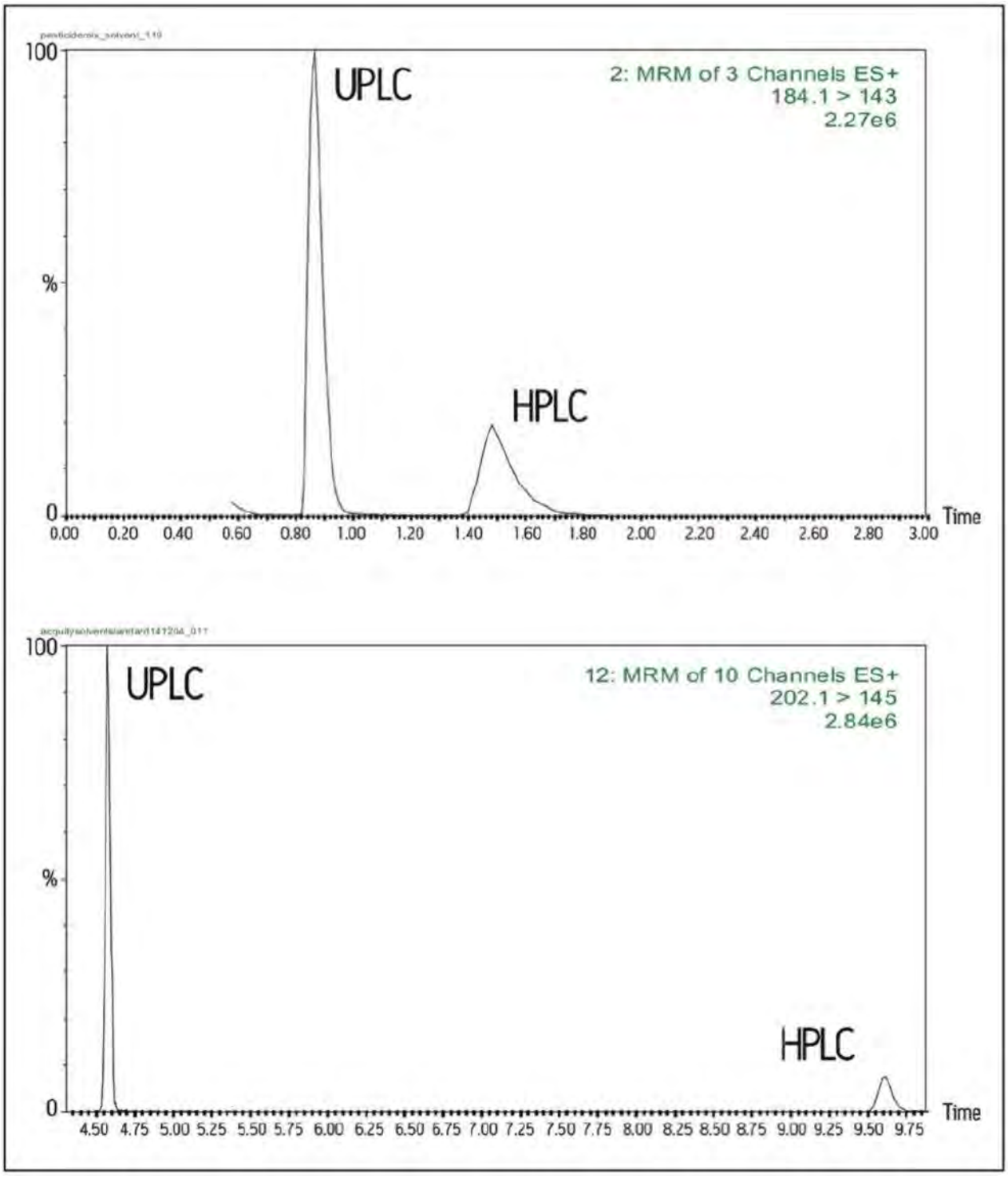


Figure 1. Comparison of UPLC (0.1 min) and HPLC (0.3 min) chromatograms. Data obtained for a) acephate and b) carbaryl (solvent standard) at 10 pg/µl.

Greater chromatographic resolution is achievable under UPLC conditions (cf. HPLC) and is illustrated in Figure 2. Butoxycarboxim sulfoxide and aldicarb sulfoxide have similar retention properties, with

butoxycarboxim sulfoxide eluting first, and the same MRM transition (*m/z* 207.1>132).

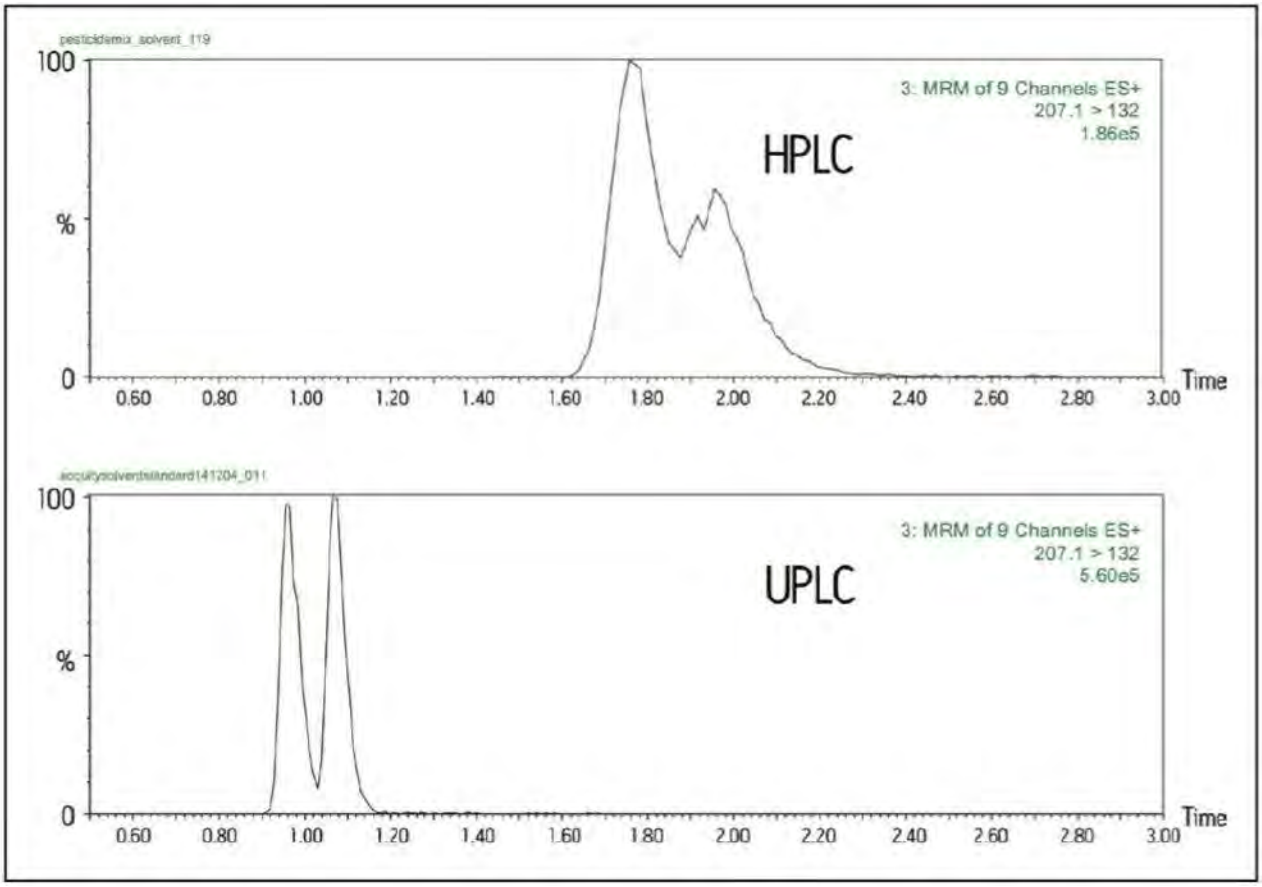


Figure 2. Increased resolution of UPLC over HPLC.

It can be seen that UPLC has the ability to separate complex mixtures. This is confirmed by considering the analysis of 100 pesticide residues in raisin matrix (Figure 3). All 100 pesticides elute within 10 minutes, and the overall cycle time is just 13.5 minutes.

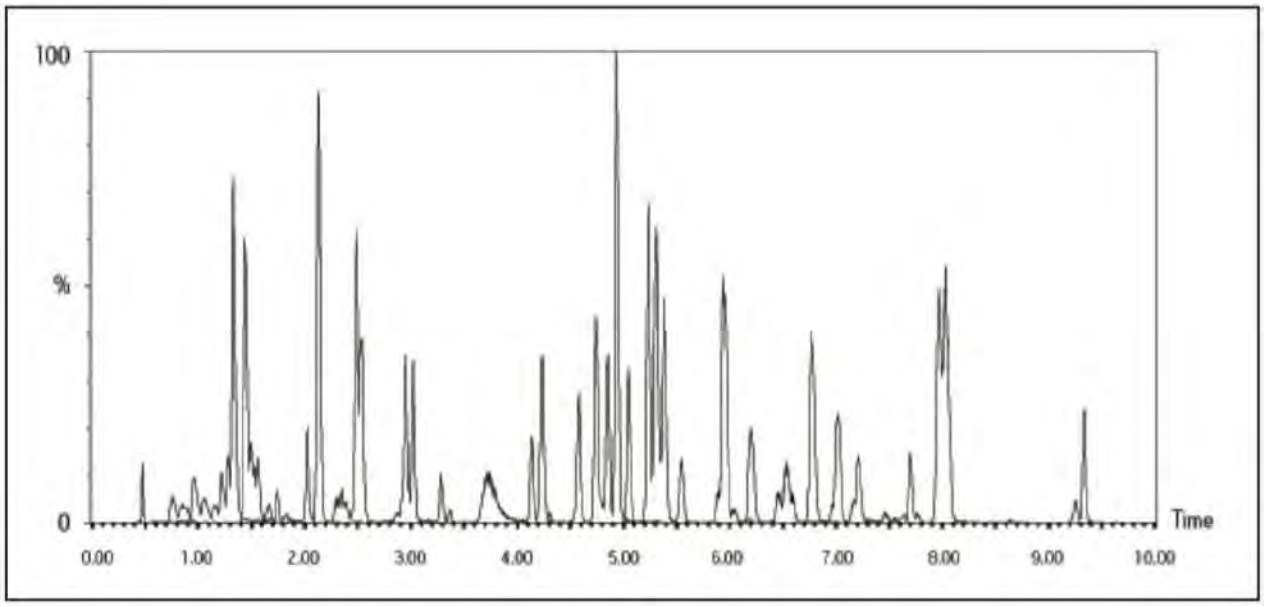


Figure 3. UPLC TIC chromatogram for the analysis of 100 pesticide residues in raisin extract (10 µg/kg).

Since the analytical method is intended for surveillance monitoring, it needs to be able to detect tens of pesticide residues; some of which are better detected under negative ES conditions (Table 1). The use of the ACQUITY UPLC System places added demands on the mass spectrometer due to the improved chromatographic resolution and short analysis times. For these reasons, the Quattro Premier Tandem Quadrupole Mass Spectrometer was selected as the detector for this application.

In order for accurate quantization to be performed, a minimum of 10 data points across each peak must be acquired. This requirement, coupled with the number of target analytes and narrow chromatographic UPLC peaks indicated that it would be advantageous if the MRM functions were arranged into time windows, based on analyte retention times (Figure 4). This system enabled the flexible use of dwell times (Table 1), such that those peaks with lower intensities can have their S/N ratios increased by employing longer dwell times, while retaining a minimal scan time.

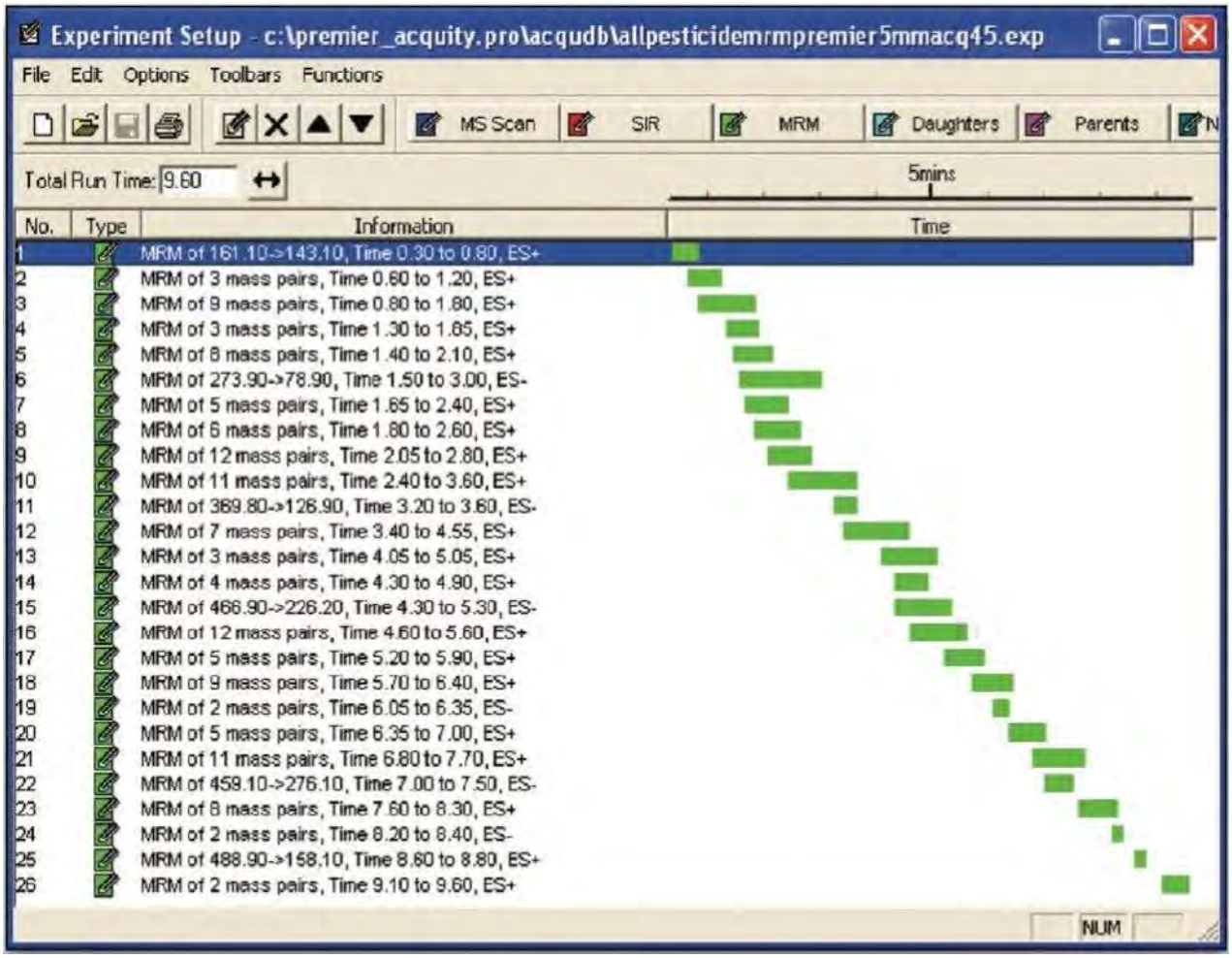


Figure 4. MRM functions arranged into time windows.

In addition to the primary MRM traces monitored for each analyte, confirmation MRM traces were incorporated into the method for the 31 most commonly found residues. In total, 131 MRM transitions were monitored in 26 time windows (Figure 4).

Six of the pesticides included within the method ionize under negative ES mode. The Quattro Premier can switch rapidly between positive and negative ionization modes, so that closely eluting analytes under both modes can be achieved within a single analytical run as illustrated above right (Figure 5), thereby minimizing the need to perform separate analyses.

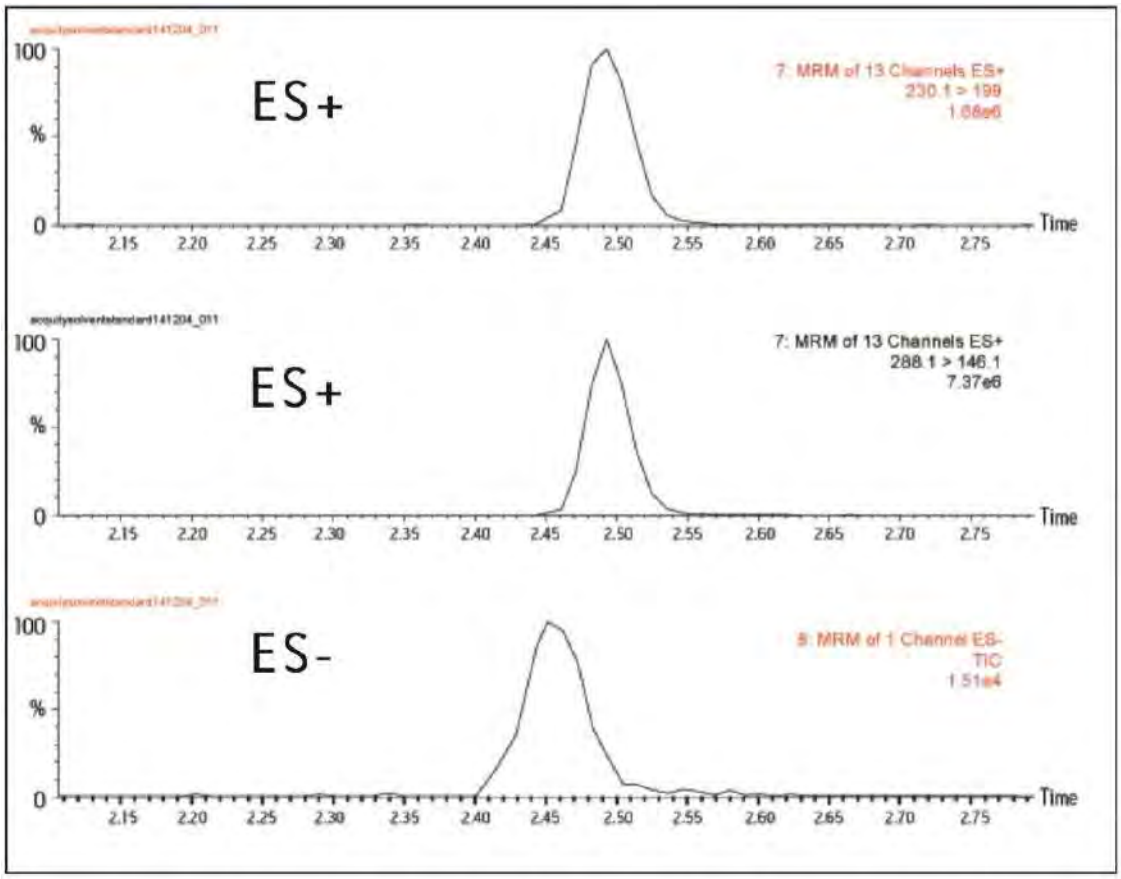


Figure 5. Chromatographic traces for dimethoate and vamidothion (ES+) and bromoxynil (ES-).

Analysis of standard solutions enabled LODs (based on 3 x S/N) to be determined (Table 1). All are well below the necessary reporting level of individual pesticides in food (10 µg/kg, 5 pg/L), indicating that this method could be applied to the analysis of pesticide residues in a variety of matrices.

Application

The analytical method was applied to the analysis of pesticide residues in raisins. The chromatogram (Figure 3) obtained for the analysis of a raisin sample containing the pesticides spiked at a level equivalent to the MRL demonstrates good signal response for all analytes at this reporting level. Since the analytical method is intended for surveillance monitoring, it needs to be able to detect tens of pesticide residues; some of which are better detected under ES- conditions (Table 1).

Good linearity in calibration was demonstrated over the range analyzed, 1-20 µg/kg (Figure 6).

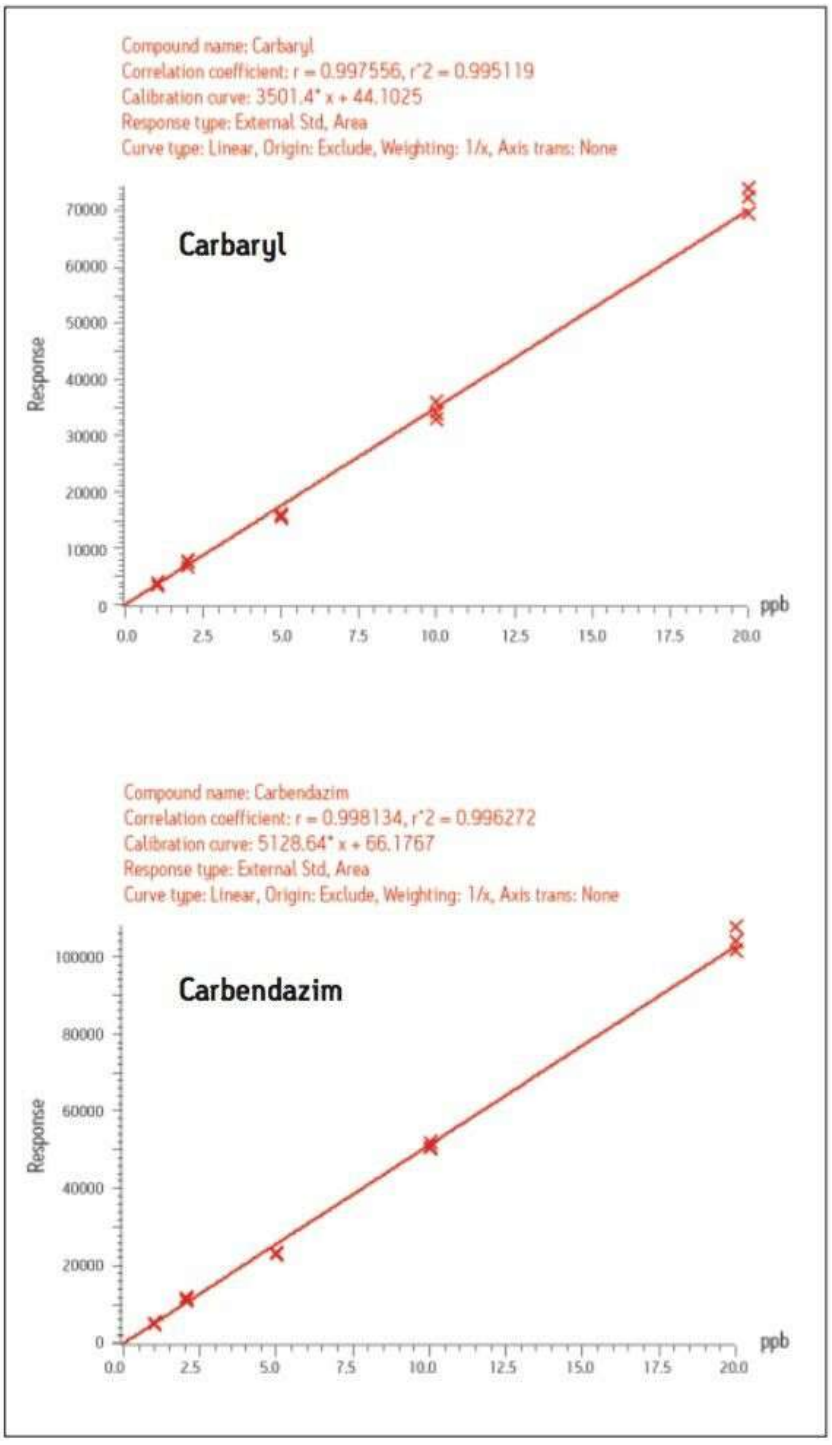


Figure 6. Representative calibration graphs for the analysis of carbaryl (top) and carbendazim (bottom) spiked into blank raisin matrix at a range of concentrations.

Inclusion of a second transition within the surveillance method enables unambiguous confirmation of the presence of a residue within the sample, without the need to perform a second confirmatory analytical run

(Figure 7) resulting in further efficiency gains. Two pesticide residues (imidacloprid and tebufenozide) were confirmed present within the raisin sample at levels below the MRL, 4.4 and 3.4 µg/kg, respectively.

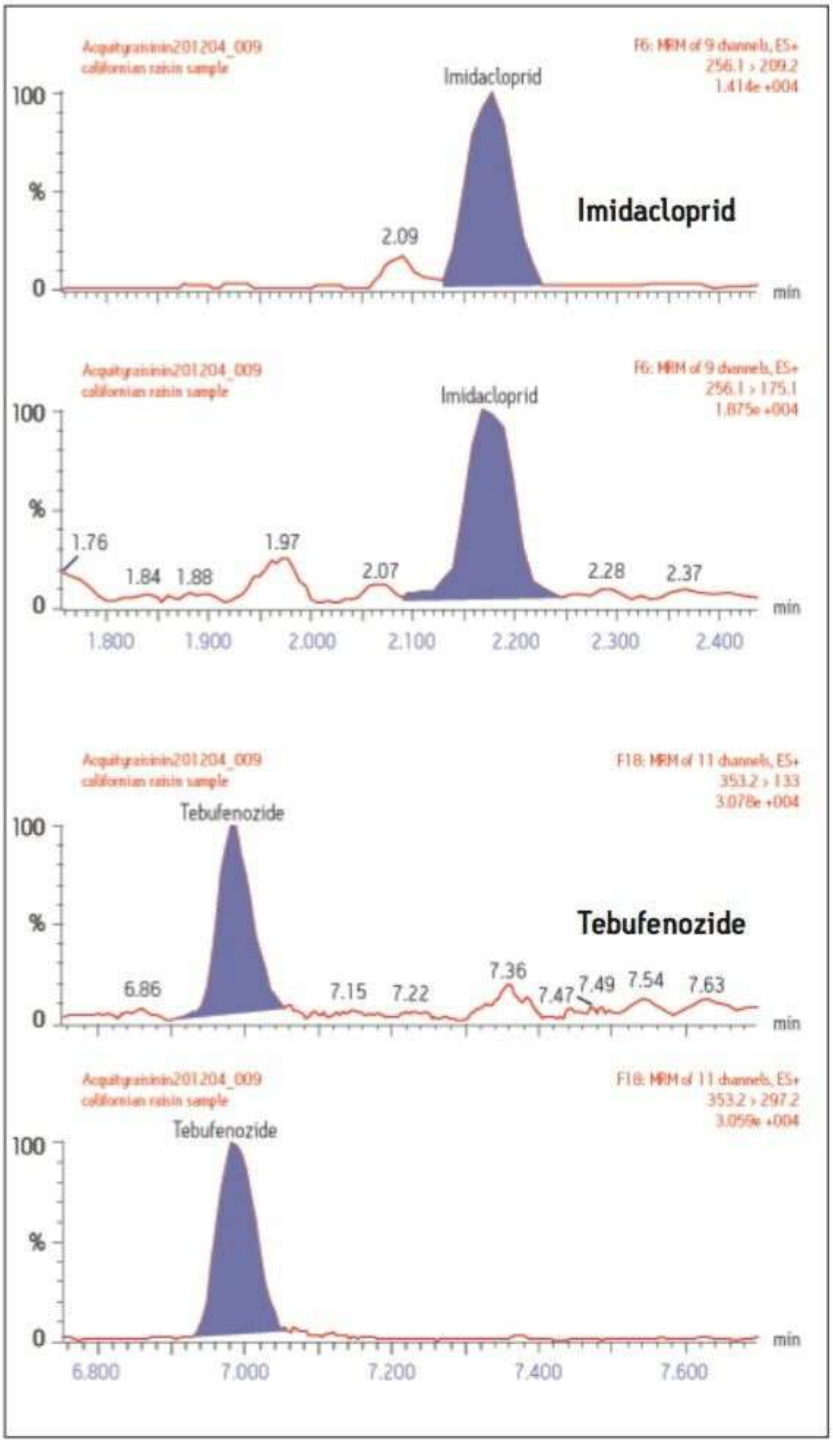


Figure 7. Confirmation that the Californian raisin sample contained imidacloprid (top) and tebufenozide (bottom).

Conclusion

- A rapid multi-residue UPLC-MS/MS method has been developed for surveillance monitoring of 100 pesticide residues and has been applied to the analysis of raisins.
- Improved efficiency and increased sample throughput has been realized through the combination of these UPLC and MS technologies which offer:
  - enhanced chromatographic resolution and short analysis times.
  - the ability to group MRM functions into time windows, enabling the incorporation of confirmatory MRM traces.
  - the capability to switch rapidly between MRM channels and between positive and negative ionization modes.
- The sensitivity achieved for the majority of pesticide residues indicates that this UPLC-MS/MS method could be applied to the analysis of pesticides in different matrices over the range analyzed.
- Given the chromatographic improvements afforded by the ACQUITY UPLC System coupled to the advances in data acquisition methods seen with the Quattro Premier Mass Spectrometer, it is feasible that this method could be extended to over three hundred compounds (provided efficient sample extraction).

References

1. Plumb R., Castro-Perez J., Granger J., Beattie I., Joncour K., Wright A. *Rapid Commun. Mass Spectrom*. 2004; 18: 2331–7.
2. A Multi-Residue LC-MS/MS Method for the Determination of 81 Pesticide Residues in Fruit and Vegetables: Part 1. Waters Application Note 720000686EN < https://www.waters.com/nextgen/us/en/library/application-notes/2003/a-multi-residue-lc-ms-ms-method-for-the-determination-of-81-pesticide-residues-in-fruit-and-vegetables-part-1-method-overview.html> . Method Overview.

Featured Products

ACQUITY UPLC System <https://www.waters.com/514207>

720001172, June 2007

Test Material:	Sulfometuron methyl
MRID:	49393601
Title:	Analytical Method for the Determination of Sulfometuron Methyl and Metabolites in Water Using LC/MS/MS
MRID:	49393602
Title:	Independent Laboratory Validation of DuPont-39340 "Analytical Method for the Determination of Sulfometuron Methyl and Metabolites in Water Using LC/MS/MS"
EPA PC Code:	122001
OCSPP Guideline:	850.6100

For CDM Smith

Primary Reviewer: Lynne Binari **Signature:** 

Date: 10/29/14

Secondary Reviewer: Lisa Muto **Signature:** 

Date: 10/29/14

QC/QA Manager: Joan Gaidos **Signature:** 

Date: 10/29/14

Analytical method for sulfometuron methyl and its products IN-00581, IN-X0993, and IN-D5803 in water

Reports: ECM: EPA MRID No.: 49393601. Henze, R., and J. Stry. 2013. Analytical Method for the Determination of Sulfometuron Methyl and Metabolites in Water Using LC/MS/MS. DuPont Project ID: DuPont-39340. Report prepared by E. I. du Pont de Nemours and Company, DuPont Crop Protection, Stine-Haskell Research Center, Newark, Delaware, sponsored and submitted by E. I. du Pont de Nemours and Company, Wilmington, Delaware; 81 pages. Final report issued December 3, 2013.
ILV: EPA MRID No. 49393602. Fiorito, B. 2014. Independent Laboratory Validation of DuPont-39340 "Analytical Method for the Determination of Sulfometuron Methyl and Metabolites in Water Using LC/MS/MS". Alliance Pharma Project No.: 140110. DuPont Project ID: DuPont-39497. Report prepared by Alliance Pharma, Malvern, Pennsylvania, sponsored and submitted by E. I. du Pont de Nemours and Company, Wilmington, Delaware; 133 pages. Final report issued May 20, 2014.

Document No.: MRIDs 49393601 & 49393602

Guideline: 850.6100
EC SANCO/825/00 rev. 8.1 (p. 11 of MRID 49393602)

Statements: ECM: The study director reported that the development work for this ECM was not required to be conducted in compliance with USEPA Good Laboratory Practice (GLP) standards; however, the work was conducted in a GLP compliant facility according to Standard Operating Procedures (p. 3 of MRID 49393601). Signed and dated Data Confidentiality, GLP, and Authenticity Certification statements were provided (pp. 2-4). A Quality Assurance statement was not provided.
ILV: The study was conducted in compliance with USEPA GLP standards (p. 3 of MRID 49393602). Signed and dated Data Confidentiality, GLP, Quality Assurance, and Authenticity Certification statements were provided (pp. 2-5).

Classification: This analytical method is classified as Supplemental. The ILV successfully duplicated the method with acceptable performance for all analytes in all test systems, however, the ECM performance data for 0.10 µg/kg (LOQ) fortified IN-00581 in ground water did not meet OCSPP Guideline 850.6100 criteria. The performance data were acceptable for the other three test analytes and were acceptable for IN-00581 in the other test media. In addition, ECM reported recoveries were corrected for any residues detected in the matrix control samples. The ILV test drinking water matrix was not characterized.

PC Code: 122001

Reviewer: Patricia Engel, Physical Scientist **Signature:** Patricia Engel
Date: 9-27-2018

Reviewer: Dena Barrett, Senior Fate Scientist **Signature:** Dena Barrett
Date: 9-27-2018

Executive Summary

This analytical method, DuPont-39340, is designed for the quantitative determination of sulfometuron methyl and its products IN-00581, IN-X0993, and IN-D5803 in ground water, surface water, and drinking water using HPLC/MS/MS. The method is quantitative for the analytes at the stated LOQ of 0.10 µg/kg. The LOQ is less than the current lowest toxicological level of concern in water. The independent laboratory validated the method for analysis of sulfometuron methyl, IN-00581, IN-X0993, and IN-D5803 in ground water, surface water, and drinking water after one trial. No major modifications were made by the independent laboratory. The ILV test ground and surface water matrices were characterized but the drinking water matrix was not characterized.

Table 1. Analytical Method Summary

Analyte(s) by Pesticide	MRID		EPA Review	Matrix[1]	Method Date (dd/mm/yyyy)	Registrant	Analysis	Limit of Quantitation (LOQ)
	Environmental Chemistry Method	Independent Laboratory Validation						
Sulfometuron methyl	49393601	49393602	9/27/18	Water	03/12/2013	E. I. du Pont de Nemours and Company	HPLC/MS/MS	0.10 µg/kg
IN-00581								
IN-X0993								
IN-D5803								

1 For the ECM, the surface, ground, and drinking water matrices were fully characterized (Appendix 4, pp. 76-81 of MRID 49393601). For the ILV, the surface water and ground water matrices were characterized, but not the drinking water (Appendix 2, pp. 131-133 of MRID 49393602).

I. Principle of the Method

Water (20.0 g, ± 1%) was acidified with concentrated formic acid (1.0 µL), then loaded under gravity flow onto a Waters Oasis HLB solid phase extraction (SPE) cartridge (1 g/20 mL) pre-conditioned with methanol and pH 3 water [HPLC grade water:concentrated formic acid (200:0.01, v:v); pp. 10-11, 13-14 of MRID 49393601]. The loaded cartridge was dried under vacuum for 10 minutes to remove water. Residues were eluted with 20 mL of basic acetonitrile [acetonitrile:1.0M ammonia hydroxide (98:2, v:v)]. The eluate was brought to volume (20 mL) with basic acetonitrile. An aliquot (10 mL) was concentrated to *ca*. 2 mL under nitrogen at 25-30°C, 0.5 mL of 0.01M aqueous ammonium acetate was added, and the sample reduced to <0.5 mL. The concentrated sample was amended with 50 µL methanol, brought to 1.0 mL with 0.01M aqueous ammonium acetate, and an aliquot filtered (syringe filter, pore size not specified) for LC/MS/MS analysis.

Samples were analyzed for sulfometuron methyl (DPX-T5648) and its products IN-00581, IN-X0993, and IN-D5803 by HPLC [Phenomenex C-18(2), 2.0 mm x 100 mm, 3 µm, 40°C] using a mobile phase of (A) 0.01M aqueous ammonium acetate and (B) methanol [percent A:B (v:v) at 0.0-0.3 min. 90:10, 7.0 min. 60:40, 7.1-8.5 min. 1:99, 8.6 min. 90:10] with MS/MS-ESI (AB Sciex API 5000 triple quadrupole MS, electrospray ionization, positive ion mode for sulfometuron methyl, IN-X0993, and IN-D5803, negative ion mode for IN-00581) detection and multiple reaction monitoring (MRM; pp. 9, 14-16 of MRID 49393601). For IN-D5803, ammonia adducts were used instead of the protonated compound, because IN-D5803 did not readily add a proton in the electrospray ion source, but did readily add NH_4^+. Injection volume was 10 µL. Analytes were identified using two ion transitions; one for quantitation (Q) and one for confirmation (C). Ion transitions monitored were as follows: *m/z* 365.0→150.1 (Q) and *m/z* 365.0→67.0 (C) for sulfometuron methyl, *m/z* 182.0→105.9 (Q) and *m/z* 182.0→61.9 (C) for IN-00581, *m/z* 124.1→67.0 (Q) and *m/z*

124.1→107.0 (C) for IN-X0993, and *m/z* 233.2→199.0 (Q) and *m/z* 233.2→77.1 (C) for IN-D5803. An additional confirmation ion transition was reported for sulfometuron methyl (*m/z* 365.0→77.0 , but this transition was not utilized in this ECM or the ILV.

The ILV performed the method as written with minor method modifications and equivalent equipment and instrumentation substitutions (pp. 14, 17-18 of MRID 49393602). Water samples were acidified with 10 µL concentrated formic acid prior to SPE. A Symmetry C18 (2.1 mm x 100 mm, 3 µm) HPLC column was substituted, and an AB Sciex Triple Quad 5500 MS was used. Strong background interference was found for the IN-D5803 confirmation ion transition *m/z* 233.2→77.1, therefore DuPont approved use of ion transition *m/z* 233.2→135.1 for confirmation (p. 13 of MRID 49393602).

LOQs and LODs for all analytes were the same in the ECM and ILV at 0.10 µg/kg and 0.03 µg/kg, respectively (pp. 8, 18 of MRID 49393601; p. 14 of MRID 49393602).

II. Recovery Findings

ECM (MRID 49393601): Mean recoveries and relative standard deviations (RSDs) were within guidelines (mean 70-120%; RSD ≤20%) for analysis of sulfometuron methyl and its products IN-00581, IN-X0993, and IN-D5803 in drinking (tap) water, ground (well) water, and surface (creek) water at fortification levels of 0.10 µg/kg (LOQ) and 1.0 µg/kg (10x LOQ), with the exception of 0.10 µg/kg (LOQ) IN-00581 in ground water (mean 123% and RSD 27.3% for quantitation ion analysis, mean 125% and RSD 27.8% for confirmation ion analysis; Tables 1-2, pp. 21-28 of MRID 49393601 and DER Attachment 2). The study authors excluded IN-00581 recovery results of 181% for the quantitation ion analysis and 187% for the confirmation ion analysis to yield mean recoveries (n =4) of 108% (RSD 8.6%) and 110% (RSD 1.7%), respectively, but did not provide supporting statistical tests to justify exclusion of the high recovery as an outlier. Recoveries were corrected for any residues detected in the matrix control samples (pp. 16-17; Appendix 2, pp. 58-63). Analytes were identified and quantified using two ion transitions; quantitation ion and confirmation ion recovery results were comparable. The water matrices were characterized (p. 13; Appendix 4, pp. 76-81).

ILV (MRID 49393602): Mean recoveries and relative standard deviations (RSDs) were within guidelines (mean 70-120%; RSD ≤20%) for analysis of sulfometuron methyl and its products IN-00581, IN-X0993, and IN-D5803 in drinking (tap) water, surface (river) water, and ground water at fortification levels of 0.10 µg/kg (LOQ) and 1.0 µg/kg (10x LOQ; Tables 1-12, pp. 27-50 of MRID 49393602). Analytes were identified and quantified using two ion transitions; quantitation ion and confirmation ion recovery results were comparable. The method was validated for all analytes in the three water matrices at both fortification levels after one trial, with minor method and instrument parameter modifications (pp. 13-14, 17-18, 22, 25). The surface water and ground water matrices were characterized, but not the drinking water (Appendix 2, pp. 131-133).

Table 2. Initial Validation Method Recoveries for Sulfometuron methyl and Its Products IN-00581, IN-X0993, and IN-D5803 in Water[1]

Analyte	Fortification Level (µg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
	Drinking (Tap) Water					
	Quantitation ion					
Sulfometuron methyl	0.10 (LOQ)	5	79-93	87	5.3	6.1
	1.0	5	67-85	75	6.7	8.8
IN-00581	0.10 (LOQ)	5	107-120	114	4.9	4.3
	1.0	5	80-91	85	4.5	5.3
IN-X0993	0.10 (LOQ)	5	88-113	102	9.9	9.6
	1.0	5	67-89	79	9.2	11.7
IN-D5803	0.10 (LOQ)	5	92-112	104	7.5	7.2
	1.0	5	90-110	102	8.1	7.9
	Confirmation ion					
Sulfometuron methyl	0.10 (LOQ)	5	76-93	84	6.1	7.3
	1.0	5	66-86	76	7.2	9.6
IN-00581	0.10 (LOQ)	5	107-126	115	7.8	6.8
	1.0	5	80-85	83	1.8	2.2
IN-X0993	0.10 (LOQ)	5	93-110	101	7.8	7.8
	1.0	5	72-112	91	18.2	19.9
IN-D5803	0.10 (LOQ)	5	96-128	108	12.2	11.2
	1.0	5	89-102	97	6.1	6.3
	Ground (Well) Water					
	Quantitation ion					
Sulfometuron methyl	0.10 (LOQ)	5	87-100	91	5.4	6.0
	1.0	5	80-103	90	10.2	11.4
IN-00581	0.10 (LOQ)	5	98-181	**123**[2]	33.5	**27.3**[2]
	1.0	5	83-114	97	14.0	14.4
IN-X0993	0.10 (LOQ)	5	85-106	95	9.5	10.0
	1.0	5	70-77	74	2.5	3.4
IN-D5803	0.10 (LOQ)	5	99-112	104	5.2	5.0
	1.0	5	95-118	105	9.2	8.7
	Confirmation ion					
Sulfometuron methyl	0.10 (LOQ)	5	82-95	87	5.1	5.9
	1.0	5	81-106	92	11.0	11.9
IN-00581	0.10 (LOQ)	5	108-187	**125**[2]	34.7	**27.8**[2]
	1.0	5	83-113	97	12.6	13.0
IN-X0993	0.10 (LOQ)	5	92-110	99	7.3	7.4
	1.0	5	63-79	72	8.0	11.2
IN-D5803	0.10 (LOQ)	5	92-115	106	9.7	9.2
	1.0	5	101-117	108	6.9	6.4
	Surface (Creek) Water					
	Quantitation ion					
Sulfometuron methyl	0.10 (LOQ)	5	87-102	97	5.9	6.2
	1.0	5	81-90	83	3.8	4.5
IN-00581	0.10 (LOQ)	5	76-113	98	15.2	15.5
	1.0	5	79-93	84	5.9	7.0
IN-X0993	0.10 (LOQ)	5	95-113	104	7.5	7.2
	1.0	5	84-102	91	8.2	9.0

Analyte	Fortification Level (µg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
IN-D5803	0.10 (LOQ)	5	91-101	97	3.7	3.9
	1.0	5	88-97	92	3.4	3.7
	Confirmation ion					
Sulfometuron methyl	0.10 (LOQ)	5	85-99	94	6.1	6.4
	1.0	5	81-96	85	6.4	7.5
IN-00581	0.10 (LOQ)	5	80-115	97	16.1	16.7
	1.0	5	74-90	82	6.1	7.4
IN-X0993	0.10 (LOQ)	5	95-114	105	8.1	7.8
	1.0	5	81-98	86	7.1	8.3
IN-D5803	0.10 (LOQ)	5	75-116	99	15.6	15.7
	1.0	5	81-103	91	8.6	9.5

Data (corrected for residues in matrix control samples) were obtained from Tables 1-2, pp. 21-28 of MRID 49393601 and DER Attachment 2 (standard deviations).

1 Matrix characterizations were provided (Appendix 4, pp. 76-81 of MRID 49393601). The surface water was identified as White Clay Creek, but the primary source was not further described. Primary sources for the well water and tap water were not reported.

2 Values in **red** text are outside the target recovery range of 70-120%. The study authors excluded the recovery results of 181% for the quantitation ion analysis and 187% for the confirmation ion analysis to yield mean recoveries (n =4) of 108% (RSD 8.6%, range 98-118%) and 110% (RSD 1.7%, range 108-112%), respectively (Table 1, p. 21; Table 2, p. 25 of MRID 49393601). However, no supporting statistical tests, such as Grubbs' and Dixon tests, were presented to justify exclusion of the high recovery as an outlier.

Table 3. Independent Validation Method Recoveries for Sulfometuron methyl and its products IN-00581, IN-X0993, and IN-D5803 in Water[1]

Analyte	Fortification Level (µg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
	Drinking (Tap) Water					
	Quantitation ion					
Sulfometuron methyl	0.10 (LOQ)	5	76-107	89	12.4	14
	1.0	5	66-81	75	5.3	7
IN-00581	0.10 (LOQ)	5	86-114	97	11.7	12
	1.0	5	79-98	92	7.5	8
IN-X0993	0.10 (LOQ)	5	86-105	96	7.4	8
	1.0	5	71-94	83	8.3	10
IN-D5803	0.10 (LOQ)	5	87-99	93	4.3	5
	1.0	5	83-104	94	7.5	8
	Confirmation ion					
Sulfometuron methyl	0.10 (LOQ)	5	75-106	89	12.1	14
	1.0	5	63-79	72	5.7	8
IN-00581	0.10 (LOQ)	5	82-119	98	14.9	15
	1.0	5	79-100	90	7.5	8
IN-X0993	0.10 (LOQ)	5	81-93	87	5.4	6
	1.0	5	77-88	84	4.9	6
IN-D5803	0.10 (LOQ)	5	87-97	93	4.4	5
	1.0	5	82-103	94	7.5	8
	Surface (River) Water					
	Quantitation ion					
Sulfometuron methyl	0.10 (LOQ)	5	92-98	94	2.3	2
	1.0	5	77-96	90	7.5	8
IN-00581	0.10 (LOQ)	5	67-74	71	3.4	5

Analyte	Fortification Level (µg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
	1.0	5	114-128	119	5.3	4
IN-X0993	0.10 (LOQ)	5	89-113	100	8.7	9
	1.0	5	84-107	96	8.4	9
IN-D5803	0.10 (LOQ)	5	78-89	82	4.0	5
	1.0	5	70-94	86	9.9	12
	Confirmation ion					
Sulfometuron methyl	0.10 (LOQ)	5	90-97	93	2.9	3
	1.0	5	75-95	87	7.6	9
IN-00581	0.10 (LOQ)	5	73-81	75	3.4	4
	1.0	5	111-120	116	3.9	3
IN-X0993	0.10 (LOQ)	5	85-107	94	8.5	9
	1.0	5	87-105	99	7.1	7
IN-D5803	0.10 (LOQ)	5	81-90	84	3.4	4
	1.0	5	70-92	85	8.9	11
	Ground Water					
	Quantitation ion					
Sulfometuron methyl	0.10 (LOQ)	5	76-84	80	3.6	5
	1.0	5	87-92	90	2.1	2
IN-00581	0.10 (LOQ)	5	85-94	88	3.5	4
	1.0	5	98-109	105	4.0	4
IN-X0993	0.10 (LOQ)	5	91-119	101	11.6	11
	1.0	5	97-116	111	7.8	7
IN-D5803	0.10 (LOQ)	5	89-103	96	5.2	5
	1.0	5	108-117	112	3.5	3
	Confirmation ion					
Sulfometuron methyl	0.10 (LOQ)	5	77-83	79	2.7	3
	1.0	5	86-95	90	3.3	4
IN-00581	0.10 (LOQ)	5	90-109	102	7.6	7
	1.0	5	104-114	108	5.3	5
IN-X0993	0.10 (LOQ)	5	80-88	84	3.7	4
	1.0	5	107-113	110	2.3	2
IN-D5803	0.10 (LOQ)	5	83-100	92	5.8	6
	1.0	5	107-115	110	3.2	3

Data (uncorrected recovery results) were obtained from Tables 1-12, pp. 27-50 of MRID 49393602.

1 Matrix characterizations were provided for the surface water and ground water, but not for the drinking water (Appendix 2, pp. 131-133 of MRID 49393602). The surface water was identified as Goose River, but the primary source was not further described. The primary source for the ground water was not reported. The drinking water was tap water from Alliance Pharma, Malvern, Pennsylvania (p. 17 of MRID 49393602).

III. Method Characteristics

LOQs and LODs for all analytes were the same in the ECM and ILV at 0.10 µg/kg and 0.03 µg/kg, respectively (pp. 8, 18 of MRID 49393601; p. 14 of MRID 49393602). The ECM defined the LOQ as the lowest fortification level at which acceptable average recoveries (70-120%, RSD <20%) were achieved, and the fortification level at which analyte peaks were consistently produced at a level *ca*. 10-20 times the signal at the corresponding retention time of the analyte in an untreated matrix control sample (p. 18 of MRID 49393601). The LOD was estimated as *ca*. 3x background noise at the corresponding retention time of the least responsive analyte, or *ca*. one-third of the LOQ.

Table 4. Method Characteristics for Sulfometuron methyl and Its Products IN-00581, IN-X0993, and IN-D5803 in Water

		Sulfometuron methyl	**IN-00581**	**IN-X0993**	**IN-D5803**
Limit of Quantitation (LOQ)		0.10 µg/kg			
Limit of Detection (LOD)		0.03 µg/kg			
Linearity (calibration curve r^2 and concentration range)[1]	ECM:	Q ion: r^2 = 0.9961 C ion: r^2 = 0.9982	Q ion: r^2 = 0.9986 C ion: r^2 = 0.9989	Q ion: r^2 = 0.9999 C ion: r^2 = 0.9993	Q ion: r^2 = 0.9999 C ion: r^2 = 0.9997
	ILV:	Q ion: r^2 = **0.994** C ion: r^2 = **0.980**	Q ion: r^2 = **0.993** C ion: r^2 = **0.992**	Q ion: r^2 = 0.997 C ion: r^2 = **0.994**	Q ion: r^2 = **0.992** C ion: r^2 = **0.988**
	Range:	0.50-25 ng/mL			
Repeatable	ECM:	Yes, except for 0.10 µg/kg (LOQ) IN-00581 in ground water.			
	ILV:	Yes			
Reproducible		Yes			
Specific		Yes			

Data were obtained from p. 9; Figure 2, pp. 33-36 of MRID 49393601; pp. 11-14 of MRID 49393602; DER Attachment 2.

1 ILV calibration curve r^2 values were derived from reported r values ($1/x^2$ weighting; DER Attachment 2). Linearity of provided ECM and ILV standard curves could not be verified by the reviewer because the individual calibration standard data were not provided. Coefficient of determination (r^2) values less than 0.995 are highlighted in **red** text.

IV. Method Deficiencies and Reviewer's Comments

1. For the ECM, the following fortifications did not meet OCSPP Guideline 850.6100 criteria for precision and accuracy (mean recoveries for replicates at each spiking level between 70% and 120% and relative standard deviations (RSD) ≤20%): 0.10 µg/kg (LOQ) fortified IN-00581 in ground water (mean 123% and RSD 27.3% for quantitation ion analysis, mean 125% and RSD 27.8% for confirmation ion analysis; Tables 1-2, pp. 21-28 of MRID 49393601 and DER Attachment 2). The study authors excluded recovery results of 181% for the quantitation ion analysis and 187% for the confirmation ion analysis to yield mean recoveries (n =4) of 108% (RSD 8.6%) and 110% (RSD 1.7%), respectively, but did not provide supporting statistical tests to justify exclusion of the high recovery as an outlier.

2. For the ECM, reported recoveries were corrected for any residues detected in the matrix control samples (pp. 16-17; Appendix 2, pp. 58-63 of MRID 49393601). For the ILV, example calculations indicate recoveries were not corrected (pp. 21-22; Tables 1-12, pp. 27-50 of MRID 49393602).

3. For the ECM, no chromatograms for ground water analyses (quantitation or confirmation) were provided. No chromatograms for surface and drinking water confirmation ion analyses were provided. Chromatograms of reagent blank samples were not provided.

4. For the ECM, the study authors reported interferences, under quantitation ion conditions, were <LOD at the retention times of sulfometuron methyl and its products in the matrix control samples (p. 18; Figure 4, pp. 43-52 of MRID 49393601). Low levels of sulfometuron methyl detected in matrix control samples were attributed to a low level of laboratory background contamination. Baseline noise was apparent under IN-X0993 conditions for the lower calibration standards (0.50 and 1.0 ng/mL) and LOQ fortifications in both the surface water and drinking water matrices (Figure 3, pp. 37, 39; Figure 4, pp. 45-46, 51-52 of MRID 49393601).

   For the ILV, the study author reported interferences were <30% of the LOQ for all analytes (p. 24 of MRID 49393602). Low levels of sulfometuron methyl were detected in reagent blank samples (Figure 6, p. 80; Figure 7, p. 84; Figure 8, p. 88). Low levels of IN-00581 were detected in drinking and surface water matrix control samples (Figure 9, p. 91; Figure 10, pp. 95, 97). Baseline noise was apparent for the lower calibration standards (0.5 and 1.0 mg/mL) under IN-00581 confirmation ion conditions and IN-X0993 quantitation and confirmation ion conditions (Figure 2, p. 60; Figure 4, pp. 69, 72). Baseline noise was also apparent for IN-00581 in ground water and IN-X0993 in all three water matrices (Figure 6, pp. 79, 81; Figure 7, pp. 83, 85; Figure 8, pp. 87, 89; Figure 9, pp. 91, 93; Figure 10, pp. 95, 97; Figure 11, pp. 99, 101; Figure 12, pp. 103, 105; Figure 13, pp. 107, 109; Figure 14, pp. 111, 113; Figure 15, pp. 115, 117; Figure 16, pp. 119, 121; Figure 17, pp. 123, 125).

5. For both the ECM and ILV, standard curves were provided, but the individual calibration standard data were not reported (Figure 2, pp. 33-36 of MRID 49393601; Figure 5, pp. 75-78 of MRID 49393602).

   For the ILV, Figure 2 (p. 58-62 of MRID 49393602) of IN-00581 calibration standards, Figure 3 (pp. 64-68) of IN-D5803 standards, and Figure 4 (pp. 70-74) of IN-X0993 standards are all incorrectly titled as Figure 1 sulfometuron methyl standards.

6. For the ILV, linearity (r^2) of the calibration standards was not always ≥0.995 (see Table 4 above).

7. For the ECM, matrix characterizations were provided, but the primary source of each water matrix was not adequately reported (Appendix 4, pp. 76-81 of MRID 49393601). The surface water was identified as White Clay Creek, but the primary source was not further described. Primary sources for the well water and tap water were not reported.

   For the ILV, the surface water and ground water matrices were characterized, but not the drinking water (Appendix 2, pp. 131-133 of MRID 49393602). The surface water was identified as Goose River, but the primary source was not further described. The drinking (tap) water was collected at the test facility (p. 17). The primary source for the ground water was not reported.

8. It was reported for the ILV that one analyst could complete a set of twelve samples (two matrix control samples and ten validation samples) in one 8-hour day with LC/MS/MS analysis performed unattended the same day (p. 25 of MRID 49393602).

9. The reported limit of quantitation (LOQ) was determined as the lowest level of method validation (LLMV). Further work could have been done to explore the actual LOQ. This means that concentrations can be reliably quantified at the LOQ (*i.e.*, LLMV), but whether lower concentrations may also be reliably quantified is uncertain. This is a minor deficiency because the LOQ satisfies the requirement that the LOQ is less than the current toxicological levels of concern.

   LOQs and LODs for the analytes were the same in the ECM and ILV. The LOQ and LOD were 0.10 µg/kg and 0.03 µg/kg, respectively, for sulfometuron methyl, IN-00581, IN-X0993, and IN-D5803 (p. 9 of MRID 49393601; p. 14 of MRID 49393602). The ECM defined the LOQ as the lowest fortification level at which acceptable average recoveries (70-120%, RSD <20%) were achieved, and the fortification level at which analyte peaks were consistently produced at a level *ca*. 10-20 times the signal at the corresponding retention time of the analyte in an untreated matrix control sample (p. 18 of MRID 49393601). The ECM estimated the LOD as *ca*. 3x background noise at the corresponding retention time of the least responsive analyte, or *ca*. one-third of the LOQ.

V. References

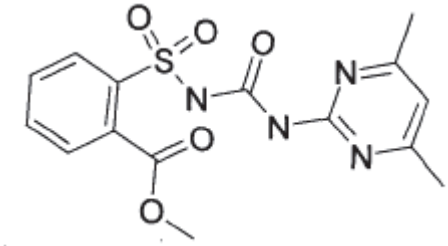
U.S. Environmental Protection Agency. 2012. Ecological Effects Test Guidelines, OCSPP 850.6100, Environmental Chemistry Methods and Associated Independent Laboratory Validation. Office of Chemical Safety and Pollution Prevention, Washington, DC. EPA 712-C-001.

40 CFR Part 136. Appendix B. Definition and Procedure for the Determination of the Method Detection Limit-Revision 1.11, pp. 317-319.

Attachment 1: Chemical Names and Structures

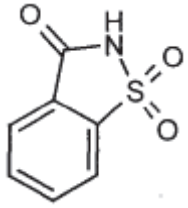
Sulfometuron methyl (DPX-T5648; Trade Name - Oust)

IUPAC Name: Not reported
CAS Name: Methyl 2-[[[[(4,6-dimethyl-2-pyrimidinyl)-amino]carbonyl]amino]-sulfonyl]benzoate
CAS Number: 74222-97-2
SMILES String: Not found



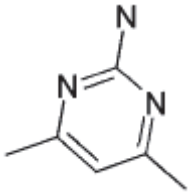
IN-00581 (Saccharin)

IUPAC Name: Not reported
CAS Name: 1,2-Benzisothiazol-3(2H)-one, 1,1-dioxide
CAS Number: 81-07-2
SMILES String: Not found



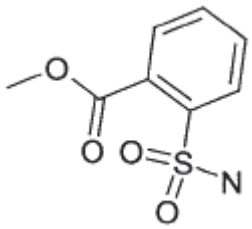
IN-X0993

IUPAC Name: Not reported
CAS Name: Not reported
CAS Number: Not reported
SMILES String: Not found



IN-D5803

IUPAC Name: Not reported
CAS Name: Not reported
CAS Number: Not reported
SMILES String: Not found



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A simple and efficient method for imidazolinone herbicides determination in soil by ultra-high performance liquid chromatography–tandem mass spectrometry

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ABSTRACT

The use of pesticides in agriculture has generated numerous consequences to the environment, requiring analysis of the persistent residues in soil, water and air. The variability of soil properties interferes in the extraction of pesticide residues with robustness and accuracy. The group of imidazolinones herbicides, widely used for weed control, becomes an additional task in multiresidue extraction procedures because of their low pK_a values. In order to determine these compounds in soil samples, different methods have been proposed, however they can be very laborious and require more time and well trained analysts. Thus, this study aimed to develop a simple and efficient method for determination of imidazolinones (imazamox, imazapic, imazapyr, imazaquin and imazethapyr) residues in soil, using an extraction with aqueous ammonium acetate solution (0.5 M) and clean-up with dispersive solid phase extraction employing PSA, followed by UHPLC–MS/MS analysis. Satisfactory values of accuracy (70–93%) and RSD ($\leq$17%) were achieved, as well as lower limit of quantification (5.0 $\mu g\,kg^{-1}$). Considering the matrix and compounds complexity, the developed and validated method proved to be an excellent tool for rapid analysis (20 min), with reliability for application in real samples with wide pH range. In the analysis of 22 real samples, the method allowed the quantification of imazapic (5.84 and 12.1 $\mu g\,kg^{-1}$), imazapyr (5.3 $\mu g\,kg^{-1}$) and imazethapyr (24.0 and 37.7 $\mu g\,kg^{-1}$) in three samples.

1. Introduction

Soil is a complex matrix with a porous structure that contains inorganic and organic substances. Furthermore residues and contaminants may be adsorbed at different concentrations depending on the compound and soil characteristics, such as: moisture, pH, texture and organic matter content. Thus, sample preparation step is critical in the analysis of residues and contaminants in soils [1]. Pesticides are widely applied in agriculture to control weeds or microorganisms and may reach the soil via different routes, even indirectly as the case of application in aerial parts of plants. The behavior of pesticides in soil depends mainly on adsorption/desorption, transformation and transport process [1,2] and can change according to soil properties such as pH, organic carbon content and ionic strength [3,4].

Single methods have been developed for the analysis of residues and contaminants in soil for different classes of pesticides, as organophosphorus [5,6] and organochlorines [7–10] as well for polycyclic aromatic hydrocarbons [7] and highly chlorinated polychlorinated biphenyls [11]. These methods are extremely important for residue determinations, since these classes of compounds require more attention when developing an extraction procedure, because of their special characteristics, being not possible insert them in a multiresidue method.

Imidazolinones herbicides is a class of pesticides relatively persistent in soil, which may persist on subsequent crops grown, with half-lives ranging from 30 to 150 days [1,3,12]. The most important use of these compounds is related to weed control in soybean, alfalfa, wheat and barley crops, and non-crop situations, once they display excellent activity when applied as to pre- or post-emergence control [13]. Imidazolinones are potent herbicides that inhibit essential enzymes for plants and are characterized by relative low application rates. The chemical analysis of these compounds in soil often shows problems due to the low detection limits required [3]. Sometimes it is necessary to develop specific analytical

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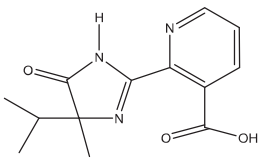
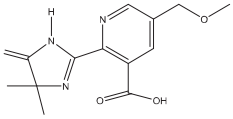
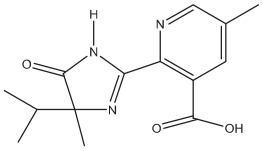
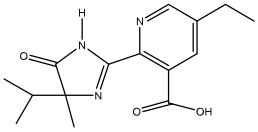
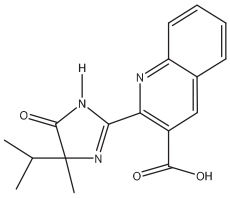
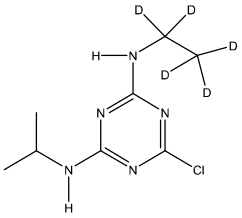
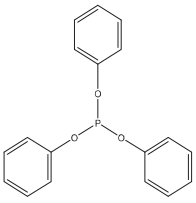
0021-9673/

Table 1
Retention time (t_R), chemical structure, molar mass (MM), pK_a, ion ratio and ion transitions of the selected imidazolinone herbicides.

RT (min)	Compound	Chemical structure	MM (g mol^{-1})	pK_a	Precursor Ion, m/z	CV (V)	Product ion quantitation m/z (CE, eV)	Product ion confirmation m/z (CE, eV)	Ion ratio (%)
1.81	Imazapyr		261.28	1.9, 3.6	262	27	69 (26)	86 (27)	46
1.90	Imazamox		305.33	2.3	306	25	218 (30)	278 (25)	41
1.92	Imazapic		275.30	2.1, 3.9	276	35	231 (20)	248 (35)	35
2.01	Imazetapir		289.33	2.1, 3.9	290	35	159 (38)	245 (35)	47
2.06	Imazaquin		328.37	1.8, 3.7	312	29	267 (20)	86 (29)	23
2.38	Atrazin d5 (SS)		220.71	–	221	26	179 (18)	101 (23)	–
2.39	Trifenilfosphate (IS)		326.28	–	325	40	169 (18)	226 (18)	–

t_R, retention time; MM, molar mass; CV, cone voltage; CE, collision energy; SS, surrogate standard; IS, internal standard.

methods to extract these herbicides in order to achieve suitable detection limits lower than 1 μg kg^{-1} [14,15]. The imidazolinones imazapyr, imazamox, imazaquin, imazethapyr and imazapic differ slightly in structure (Table 1). In general these herbicides present a carboxylic acid and a basic pyridine functional group with amphoteric chemical characteristic [1]. These compounds are weak acids, with pK_a ranging from 1.8 to 3.9 and may occur in cationic, neutral, or anionic forms (Table 1). In soil samples with pH higher than 6, these compounds are weakly sorbed. However, when the soil pH decreases greater amounts of these compounds are

strongly sorbed, since the compounds become less ionic [16]. The relationship between the pH and pK_a of these compounds makes this extraction a laborious task in residue chemical analysis. Soil organic matter content is a physico chemical parameter that can interfere in the pesticide residues analysis. Otherwise, according to Laganá et al. [19] the variation in organic matter content does not have a prominent influence on imidazolinones determination.

Different authors have been reported the difficulties in the extraction of imidazolinone herbicides from soil, considering their physico-chemical characteristics, which require additional steps of pH adjustment and extraction with a basic solution [1]. In the literature it is possible to find different methods for extraction of imadozolinones from soil, as supercritical fluid extraction (SFE) using CO_2 and solid-phase extraction (SPE) for extraction of imazethapyr [17]. Also, the use of modified QuEChERS method was reported for the analysis of imazapic and imazethapyr, using a saturated calcium hydroxide solution [1] and a methanol–phosphoric acid aqueous solution (pH 2) [18] for the extraction and analysis by LC–MS/MS [1]. Samples were also extracted with soil column extraction using potassium dihydrogen phosphate 0.1 M solution (pH 8) followed by a clean-up step in Carbograph-1 cartridge for the determination of imazapyr, imazethapyr and imazaquin by LC–MS/MS [19]. Extraction procedures for imidazolinones with sodium hydroxide solution (0.5 M) and clean-up with SPE cartridges (C18, SAX, SCX) are commonly used for imidazolinones analysis by LC–MS [20], HPLC–DAD [3] or HPLC–UV [21]. The available methods are time consuming, mainly in the clean-up step, with limits of detection from 0.1 to 1.36 $\mu g\,kg^{-1}$ [1,3,19].

In the last years, new approaches in terms of clean-up have been successfully used, especially based on dispersive solid phase extraction (d-SPE). In general, d-SPE involves the use of one or a mixture of different sorbents to remove co-extractives from the organic extract in combination with anhydrous magnesium sulfate for the removal of residual water [22]. However, liquid chromatography is a well-established tool for pesticide residues analysis. The introduction of ultra-high pressure liquid chromatography (UHPLC), promotes rapid-scan and sensitive mass spectrometry (MS) instruments led to a major change from traditional chromatographic techniques for residual contaminant analysis and thus more emphasis in sample preparation and/or data processing steps is necessary, particularly in complex applications [23,24]. Even developing a good method, a validation step is fundamental to ensure the quality, reliability and consistency of the analytical results [25]. According to Ruiz-Angel et al. [26], the validation is considered a complex and time-consuming process, despite this, it must be broken down in well-defined steps, like as: define method scope, define validation criteria, performance tests and ongoing routine tests.

Rice (*Oryza Sativa* L.) is widely planted in Brazil. However, *O. Sativa* f. spontanea, commonly known as weedy/red rice, it is one of the most difficult weeds to control in rice cultivation. Sometimes, herbicides such as imidazolinones (imazethapyr and imazapic) that are employed to eradicate weedy rice would also harm the cultivated one [18]. Otherwise, imazapic, imazaquin and imazamox are used extensively for broad-spectrum weed control in soybeans and other imidazolinone-resistant crops. They differ slightly in structure but have widely different potentials for carryover injury to subsequent crops [16]. Thereby, the present work shows the development and validation of a simple and effective extraction procedure for determination of five imidazolinones herbicides (imazapyr, imazamox, imazaquin, imazethapyr and imazapic) based on existent alkali extraction methods [1,19]. The procedure combines a fast extraction of these compounds in soil with ammonium acetate solution and a sensitive analysis by UHPLC–MS/MS.

2. Experimental

2.1. Reagents and materials

Neat standards of imazapyr (99.5%), imazaquin (99.5%), imazethapyr (99%), imazamox (99%) and imazapic (98%), atrazin d5 (99.8%) as surrogate standard and trifenilfosphate (99.5%) as internal standard were purchased from Dr. Ehrenstorfer (Augsburg, Germany). Selected pesticides are listed in Table 1, which presents their chemical properties. The extraction solution was prepared with ammonium acetate (99.3%) purchased from JT Baker (USA) and ultrapure water obtained from a Milli-Q Direct UV3® system (Millipore, USA). Among the solvents, acetonitrile LC grade (Mallinckrodt, USA) was used to prepare the stock solutions of 1000 $mg\,L^{-1}$ and mobile phase preparation. Other reagents and materials are hydrochloric acid p.a. (Isofar, Brazil) and primary secondary amine (PSA) sorbent of 40 μm (Agilent, USA). Nylon filters (13 mm) with porosity of 0.22 μm (Vertical Chromatography, Thailand), glass vials with capacity of 2 mL (Agilent, USA) and polypropylene tubes with screw caps with capacity of 15 and 50 mL (Sarstedt, Germany) were used.

2.2. Instrumentation

General apparatus consisted in a vortex mixer model QL-901 (Microtécnica, Brazil), analytical balances (AUW-220D and UX-420H from Shimadzu, Japan) and refrigerated centrifuge NT 825 (Novatécnica, Brazil). Chromatographic analyses were performed using a Waters Acquity UPLC system with Xevo TQ mass spectrometer equipped with electrospray source (Milford, MA, USA), nitrogen generator (Atlas Copco, Belgium) and argon gas 6.0, as collision gas for MS/MS system. The separation was achieved using an Acquity BEH C18 column (50 mm × 2.1 mm, 1.7 μm) with a mobile phase consisting of: (A) an aqueous solution containing 2% (v/v) methanol, 0.1% (v/v) formic acid and (B) methanol with formic acid 0.1% (v/v). The percentage of organic solution was changed linearly as follows: 0 min, 5%; 2.5 min, 100%; and 2.6 min, 5%. The flow rate was 0.225 $mL\,min^{-1}$ and the total chromatographic run time was 3 min. Injection volume was 10 μL and the column temperature was set at 40 °C. The MS parameters were optimized by infusion of individual pesticide solutions directly into the mass spectrometer. Nitrogen was used as desolvation gas with a flowrate of 600 $L\,h^{-1}$ and argon was used as collision gas with a flow of 0.15 $mL\,min^{-1}$. Table 1 shows precursor and products ions monitored and collision energies used in the chromatographic method by selected reaction monitoring (SRM) mode with positive ionization. The most abundant ion (1st transition) was selected for quantification and the 2nd transition was used as a qualitative ion. MassLynx V 4.1 software was used for qualitative and quantitative analysis.

2.3. Preliminary tests and extraction procedure

Once the imidazolinones are highly depend of the pH during the extraction, preliminary tests were conducted in order to achieve the optimum conditions to all analytes. Since the pH of the soil sample utilized was 5.5, different concentrations of aqueous ammonium acetate solution (0.1, 0.2, 0.5 and 1.0 $mol\,L^{-1}$) were tested for the imidazolinones extraction. From these solutions, different pH (6, 7, 8 and 9) were originated, respectively. Due to the complexity of the soil matrix, a clean-up step was introduced to the method to reduce the co-extractives amount. Based on that, 125 mg of PSA were used according to similar previous published applications and the extraction efficiency was evaluated [22,27–29]. After these tests, the proposed extraction procedure was validated based on the extraction of 5 g of soil in a 50 mL polypropylene tube with 10 mL of an aqueous solution of ammonium acetate 0.5 $mol\,L^{-1}$. The

tube was shaken for 1 min and centrifuged for 5 min (3400 rpm). For the clean-up step 2 mL of the extract were transferred to a 15 mL polypropylene tube containing 125 mg of PSA, shake 1 min and centrifuge 5 min (3500 rpm). The extract was filtered with a nylon filter of 0.2 μm and the pH was adjusted to 3.0 with HCl (6 mol L^{-1}). The extract was diluted 5× with ultrapurified water prior injection in the UHPLC–MS/MS system. Surrogate (added to the samples during the fortification step) and internal standards (added in the final diluted extract) were used in concentration of 20 μg L^{-1}, in order to evaluate sample preparation performance and instrumental response, respectively.

2.4. Method validation

For validation purposes, a blank soil sample was selected and different validation parameters were evaluated, including linear range (linearity), recovery, precision and method limits of detection (LODm) and quantification (LOQm). The linearity was evaluated using calibration curves at 0.2, 0.5, 1, 2, 5, 10 and 20 μg L^{-1} prepared in acetonitrile and matrix blank extract, corresponding to 2, 5, 10, 20, 50, 100 and 200 μg kg^{-1} in the sample (method factor of 10) (n = 3). The matrix effect was estimated comparing the slopes of the curves in matrix blank extract with those obtained from solvent (acetonitrile) curves. The difference in the slopes of the matrix extraction and solvent curves were divided by the slope of the solvent curve and was expressed as % of matrix effect [30]. Accuracy was evaluated through recovery tests, spiking the "blank" samples at 5, 10, 50 and 100 μg kg^{-1} with 7 extraction replicates performed for each spiked level to determine the method precision. Repeatability and intermediate precision of the method were also evaluated through relative standard deviation (RSD%) of seven replicates. LOQm was obtained as the lowest fortified concentration in the blank matrix which presented recoveries results between 70 and 120%, with RSD ≤ 20%. The LODm was obtained dividing LOQm by 3.33 [30]. Intermediate precision was evaluated by performing the analytical procedure on different days, by injecting the calibration curve and the blank sample spiked at the intermediate level (50 μg kg^{-1}). All the analyses were performed in an ISO/IEC 17025:2005 accredited laboratory following these quality criteria.

3. Results and discussion

3.1. UHPLC–MS/MS conditions

UHPLC–MS/MS conditions were optimized to enable the separation of all analyzed compounds with good efficiency in a short time. Precursor and product ions were obtained by infusion of each compound at 0.5 mg L^{-1} in the MS system, varying cone voltage and collision energy. These results are shown in Table 1. A proper separation was achieved using the gradient elution mentioned in Section 2.2. The gradient begins with higher aqueous content, thus the sample and the mobile phase are similar in the beginning of the chromatographic run. When organic content increases until 100% promotes the separation of the imidazolinones that is promoted from 1.8 to 2.1 min of analysis. In this work, the mobile phase consists of (A) an aqueous solution containing 2% (v/v) methanol, 0.1% (v/v) formic acid and (B) formic acid 0.1% (v/v) in methanol. The published methods for imidazolinones separation use acetic or formic acids as additives, acetonitrile and water with C8 or C18 columns [1,3,19,20]. In this work, a C18 column was employed and methanol was used in the mobile phase instead of acetonitrile, once it provided better peak shapes (Fig. 1). Besides the close retention times of the analytes (Fig. 1) the triple quadrupole shows its efficiency to detect and quantify each compound in the SRM mode without any interference. Also, we intend to develop a method

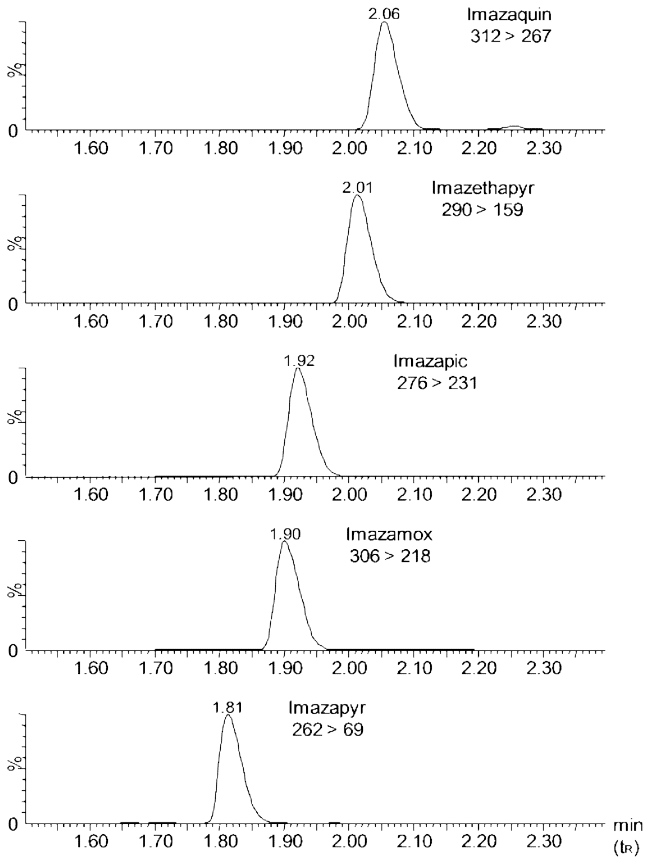


Fig. 1. UHPLC–MS/MS total ion chromatogram (1st transition) of imidazolinones matrix matched standard solution at 5 μg L^{-1}.

with low injection volume (10 μL), to minimize extract sample consumption and low waste of mobile phase with a low flow rate (0.225 mL min^{-1}). All these characteristics are adequated to the green chemistry concepts, and preserve the column and minimize UHPLC–MS/MS maintenance using an extract dilution 5× (v/v) with ultrapurified water.

3.2. Extraction procedure optimization

In the present work, 5 g of the soil sample were used in the extraction step. It can be considered an intermediate value when comparing to others amounts found in literature [19,20,9] and provides appropriate and representative results as some authors reported [1]. Different solutions can be used as extraction solvent for imidazolinones in soil. The most widely used solution is sodium hydroxide (0.5 mol L^{-1}). Ramezani et al. [3] and Bresnahan et al. [21] reported the use of 40 mL and 100 mL, to 10 g and 15 g of sample, respectively. Also, 20 mL of a saturated solution of calcium carbonate and 10 mL of acetonitrile were employed by Martins et al. [1] for extraction of imazapic and imazethapyr with QuEChERS based method. Laganá et al. [19] used 25 mL of aqueous potassium dihydrogen phosphate 0.1 mol L^{-1} (pH 8) to 1 g of soil in the procedure soil column extraction and obtained good results. The anionic form of imidazolinone herbicides predominates from pH 6 to 9. In higher pH, the anionic form of these herbicides will tend to be repulsed from the colloids produced by suspension of the humic acids [3] and the extraction will be favored. In the present work, the possibility of employing an aqueous ammonium acetate solution was investigated. Four concentration levels of this solution were tested (0.1, 0.2, 0.5 and 1.0 mol L^{-1}) and the results obtained showed that the solutions 0.1 and 0.2 mol L^{-1} presented pHs values of 6.0 and

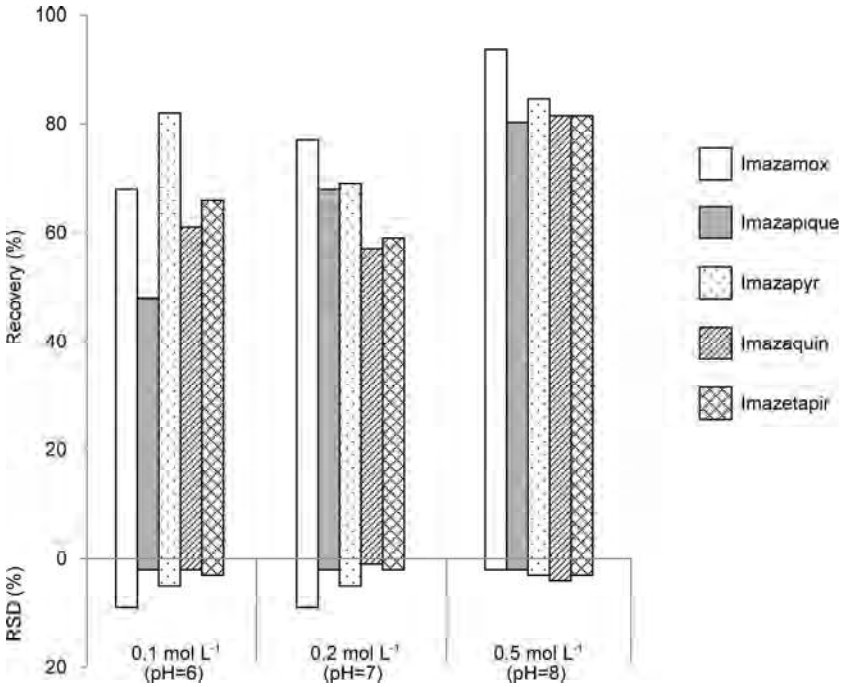


Fig. 2. Recovery and RSD results for a blank samples spiked with imidazolinones at 100 μg kg^{-1} (n = 3) and extracted with ammonium acetate solutions at different concentrations and their respective pH values.

7.0, respectively. These were not effective to extract the imidazolinones from soil matrix, presenting recoveries around 65% (Fig. 2). This probably occurred because when mixed with blank soil (pH 5.5) the final solution pH was below 6.0 and the ionic strength was not enough. When the solution 0.5 mol L^{-1} was used, the final pH of the solution to be injected in the UHPLC was around 8.0. Furthermore, it was observed average recovery results of 79% with a RSD ≤ 20% for the selected compounds, being chosen for the extraction procedure. The extract obtained with the solution 1.0 mol L^{-1} presented a pH value around 9.0. As the use of solution 0.5 mol L^{-1} presented good results, a higher pH was not necessary for imidazolinone extraction. The volume of the extracting solution was 10 mL and the centrifugation time was 5 min at 3400 rpm and 20 °C, resulting in a fast extraction with little waste generation [31].

3.3. Clean-up procedure optimization

Most available procedures for determination of imidazolinones in soil require a clean-up step with solid phase extraction (SPE) with different sorbents: C18, SAX and/or SCX [3,20,21]. However, the use of SPE can become a disadvantage in relation to costs, experience and long time required to perform the procedure. Thus, the use of dispersive solid phase extraction (d-SPE) is an option to simplify the process [32]. In this study, the use of d-SPE with PSA sorbent was the strategy for clean-up the extract (125 mg for 2 mL of extract), removing interferences from matrix, like as polar organic acid, polar pigments, some sugars and fatty acids, due to its chelating action. When no clean-up was applied, the average recoveries were satisfactory (81%), even higher than with PSA, but in routine analysis do not using clean-up may cause equipment damage. Recoveries using PSA were between 76 and 88%, being acceptable despite the capability of PSA to retain compounds, like imidazolinones [33]. As further advantages, the method does not require evaporation and reconstitution steps, so the extract can be injected after simple filtration, adjustment of pH to 3.0 and dilution in ultrapure water.

3.4. Method validation

3.4.1. Linearity and matrix effect

According to the results (Table 2), the compounds showed good response in the range of 0.2–20 μg L^{-1} with good linearity, evidenced by the values of r^2 > 0.99. Matrix effect was also evaluated in this work. Matrix effects occur in the LC system when molecules coeluting with the compounds of interest altering the ionization efficiency of the electrospray interface [34]. Many authors have been reported different values to infer about matrix effect. In the work of [35], they considered that a change in the chromatographic response above 10% means that the matrix effect starts to influence the analysis. To Ferrer et al. [36], values lower than 20% are considered as no matrix effect, because of this variation would be close to the repeatability values. In spite of these discussions, some strategies have been proposed to eliminate or minimize matrix effect. These strategies include improvement of chromatographic selectivity avoiding co-elution of the pesticides with interfering components present in the matrix and also, sample preparation methods aiming to remove matrix interferences and reduce the matrix effect in complex matrices [36]. In this study, d-SPE was used for clean-up and the values found for matrix effect were ≤11%. Some compounds showed negative matrix effect (%), as imazapic (−9), imazaquin (−11) and imazetapir (−4). These values represent a loss of the analytical signal (ion suppression) due to alterations in ionization efficiency. Imazamox and imazapyr showed a positive value (7) representing an enhancement in the analytical signal [30,34]. Despite the low changes in chromatographic system when using d-SPE, in the validation step, the calibration curves were obtained in matrix blank extract in order to compensate any matrix effect that may appear when soils with different characteristics are analyzed. Furthermore, the use of matrix matched calibration standards provides to the analysis a more realistic scale [30,37].

3.4.2. Repeatability, intermediate precision, LOD and LOQ

The precision, in terms of repeatability, evaluated at four concentration levels (5, 10, 50 and 100 μg kg^{-1}) with seven replicates

Table 2
Results of precision (repeatability, intermediate precision), accuracy (recovery), matrix effect linearity, LOD and LOQ of the validated method.

Compounds	Repeatability recovery (RSD)%*				Intermediate precision recovery (RSD)%*	Matrix effect (%)	Linearity (R^2)	LOD ($\mu g\,kg^{-1}$)	LOQ ($\mu g\,kg^{-1}$)
	5 $\mu g\,kg^{-1}$	10 $\mu g\,kg^{-1}$	50 $\mu g\,kg^{-1}$	100 $\mu g\,kg^{-1}$	50 $\mu g\,kg^{-1}$				
Imazamox	80 (5)	93 (17)	85 (9)	90 (9)	88 (2)	7	0.994	1.5	5.0
Imazapic	71 (3)	73 (16)	76 (7)	81 (7)	78 (3)	−9	0.998	1.5	5.0
Imazapyr	77 (6)	79 (14)	85 (5)	88 (9)	84 (4)	7	0.997	1.5	5.0
Imazaquin	73 (8)	72 (10)	76 (7)	79 (6)	76 (2)	−11	0.998	1.5	5.0
Imazetapyr	70 (3)	70 (9)	73 (10)	75 (5)	78 (4)	−4	0.999	1.5	5.0

* $n = 7$.

Table 3
Results obtained from the analysis of imidazolinones in soil samples.

Compound	Concentration of pesticides residues ($\mu g\,kg^{-1}$)						
	S11 (pH 5.1)*	S12 (pH 4.7)	S14 (pH 5.0)	S17 (pH 6.3)	S18 (pH 5.0)	S19 (pH 6.7)	S20 (pH 4.9)
Imazamox	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Imazapic	<LOQ	12.1	5.8	n.d.	n.d.	n.d.	<LOQ
Imazapyr	<LOQ	n.d.	n.d.	<LOQ	<LOQ	<LOQ	5.3
Imazaquin	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Imazethapyr	<LOQ	37.7	24.0	n.d.	n.d.	n.d.	n.d.

* Soil sample pH; n.d., not detected.

of each level, presented recoveries in the acceptable range of 70 to 93% and RSD value for repeatability ranged from 3 to 17%. In terms of intermediate precision, recovery ranged from 76 to 88% with RSD between 2 and 4%. Imidazolinone herbicides show some properties, as low pK_a and low hydrophobicity, that make the extraction from soil a very difficult task [4]. The use of deuterated atrazine as surrogate standard (SS) allows the evaluation of the extraction procedure. The recovery values of the SS remained practically constant in the range of 95–107% in all evaluated levels, so it is possible to conclude that no significant variations during the extraction procedure were observed. For the compounds in study, we considered that the limit of quantification (5.0 $\mu g\,kg^{-1}$) obtained by the proposed method is adequate because the values of regulatory guidance values found in literature (imazetapyr: 0.1–53,000 $mg\,kg^{-1}$ and imazaquin: 0.3–53,000 $mg\,kg^{-1}$) [38] are quite higher. The estimated limit of detection (LOD) of the method was 1.5 $\mu g\,kg^{-1}$. Validation results are presented in Table 2.

3.5. Comparison with previous studies

Compared with other reported methods for determination of imidazolinones residues in soil, this method presented similar recoveries and needed less steps, time and use of organic solvents that other methods [1,3,19]. In the last years, different methods were developed for extraction of imidazolinone herbicides from soil samples, but most of them showed recovery problems. As described by Arias et al. [22] it can be explained by their amphoteric character, which allows them to be found in the anionic, cationic or neutral state. As described before, the pK_a values of imidazolinone herbicides ranged from 1.3 to 3.9. Furthermore, most of these procedures have evaluated only one herbicide [39,40]. Laganá et al. [19] developed a method with good recovery values (78–92%) for extracting three herbicides (imazapyr, imazethapyr and imazaquin) from soil, but this method not covered imazapic and imazamox determination. Ramezani et al. [3] developed

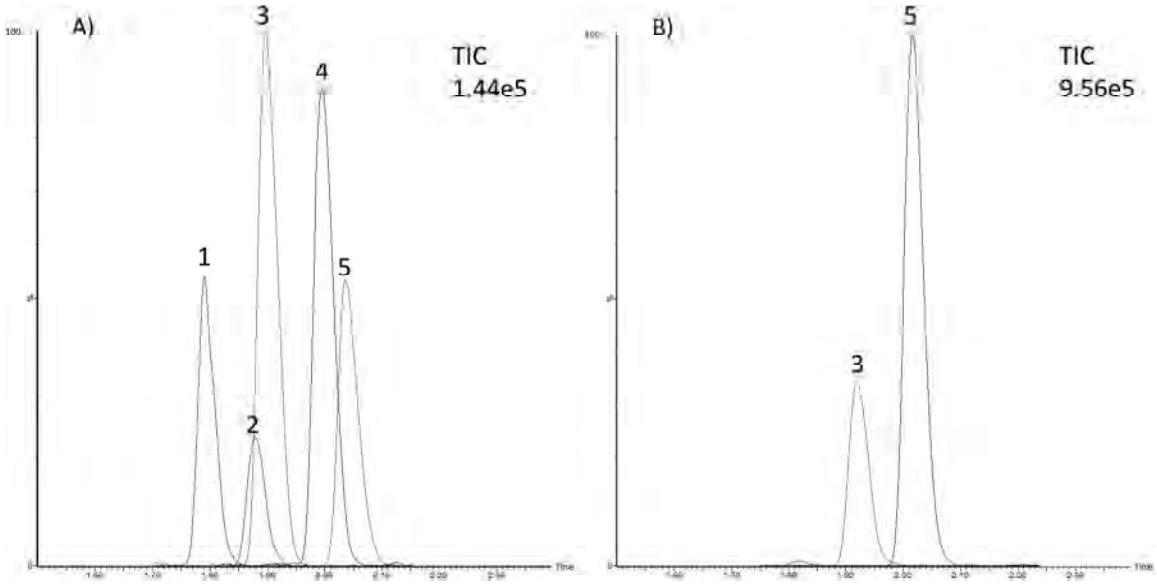


Fig. 3. (A) Total ion chromatogram obtained from soil blank extract spiked at the LOQ level (5 $\mu g\,kg^{-1}$), (1) imazapyr, (2) imazamox, (3) imazapic, (4) imazethapyr, (5) imazaquin. (B) Real sample chromatogram presenting imazapic and imazaquin at 12.1 and 37.7 $\mu g\,kg^{-1}$, respectively.

an aqueous extraction procedure and clean-up methods for imidazoline herbicides from soil. A series of solvent mixtures, pH conditions and sorbents had been tested. The procedure provided consistent high recovery (85%). Otherwise, this method is time consuming, need a SPE step and only three imidazolinone residues were analyzed. Still, Assalin et al. [18] developed a simple method for imazethapyr and imazapic residues determination, employing a modified QuEChERS extraction with ultrasonic bath and evaporation steps. As described by Bol'shakov et al. [29] the use of PSA sorbent during the d-SPE step was adequate and provides clean extracts. Thus, the method that we proposed for determination of imidazolinone residues shows differences from others found in literature, since it covers the five most used imidazolinone compounds (imazapyr, imazamox, imazaquin, imazethapyr and imazapic) and it is easy and simple to perform.

3.6. Method application

In this work, 22 samples from different sites of Rio Grande do Sul and Bahia states (Brazil), with different chemical compositions and pH values (4.3 and 7.3) were analyzed. The validated method was applied and seven samples presented at least one compound. In three of these samples (S12, 14 and 20) the compounds imazapic, imazapyr and imazethapyr were quantified, as shown in Table 3. Concentration values quantified in the samples (5.3–37.7 $\mu g\,kg^{-1}$) emphasize the need for development of analytical methods for determination of imidazolinones herbicides in soil, since its high persistence in soil can harm on subsequent crops grown (Fig. 3).

4. Conclusions

In this study an approach based on the efficacy of extraction with ammonium acetate solutions of imazapyr, imazamox, imazaquin, imazethapyr and imazapic residues in soil was developed to permit the analysis in UHPLC–MS/MS. The best accuracy and precision results were obtained with a 0.5 $mol\,L^{-1}$ ammonium acetate solution, employing a clean-up step with d-SPE using PSA sorbent, to minimize the effects in chromatographic system. It can be emphasized that the ammonium acetate solution has a high potential to be used in extraction of imidazolinone compounds in other matrices, a possibility that can be explored in the future. In this study, low quantification limits (5.0 $\mu g\,kg^{-1}$) were achieved with a combination of a quick and effective extraction procedure and a fast and sensitive determination in UHPLC–MS/MS system, resulting in a total of 20 min for execution. Thus, the validated method has been applied in routine analysis since it demonstrates its applicability in soil samples with different pH values, that is the mainly property to be considered when extracting imidazolinones from soil samples.

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Determination of Anionic Polar Pesticides in High Water Foodstuffs

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The compact Xevo TQ-S micro demonstrated robust system and method performance in the analysis of polar, anionic pesticides on a routine-level UPLC-MS/MS platform.

GOAL

To evaluate the performance of the Xevo™ TQ-S micro for key representative compounds to determine whether the solution can be used to meet the default MRL for key representative compounds.

BACKGROUND

Interest in the determination of highly polar, anionic pesticides in foodstuffs has noticeably increased over the last five years, resulting from concerns regarding the potential safety of glyphosate.[1] Because of this, the demand for surveillance has increased, leading to a desire for underivatized analysis of highly polar anionic compounds by many food safety laboratories.

It is the aim of most of these laboratories to have detection limits at or below 0.010 mg/kg for all pesticide/commodity combinations, facilitating more efficient, simplified workflows to accommodate compounds/commodity combinations with default MRLs[2] (0.010 mg/kg) as well as organic and infant foods, which have lower MRLs.

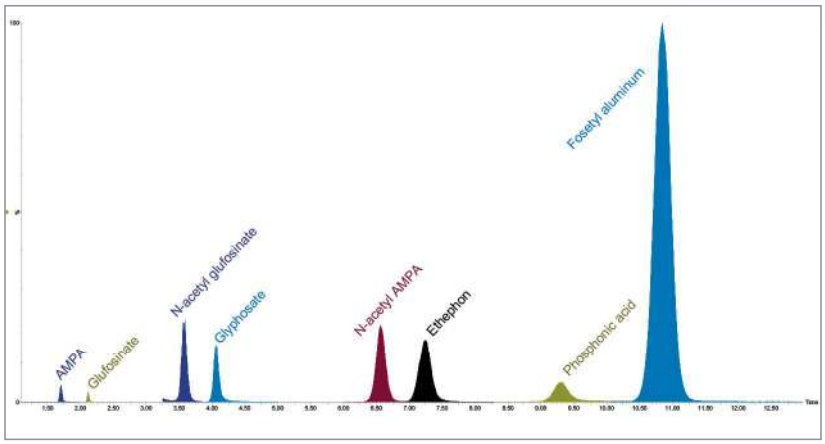

Figure 1. Example chromatography at 0.010 mg/kg spike level in a cucumber QuPPe extract sample.

The analysis of highly polar pesticides without derivatization typically requires either specialized liquid chromatography equipment or the use of the highest-performance, tandem quadrupole systems to meet the sensitivity requirements of this analysis. While these approaches allow for direct analysis, they do lead to additional laboratory costs and larger system footprints.

In previous work,[3] the method for underivatized determination of anionic polar pesticides has been presented on a Xevo TQ-XS Mass Spectrometer employing Waters™ Anionic Polar pesticide, 5 µm, 2.1x100 mm Column (p/n: 186009287) in HILIC mode, with excellent performance achieved. The aim of this technology brief is to evaluate the performance of the compact, refreshed Xevo TQ-S micro for key representative compounds to determine whether the solution can be used to meet the default MRL for key representative compounds, when evaluated against the SANTE guidelines.[4]

THE SOLUTION

To achieve the required retention and separation for this analysis, an underivatized HILIC-based method was used. The column stationary phase consisted of ethylene bridged hybrid (BEH) particles with tri-functionally bonded diethylamine (DEA) ligands. The combination of the hydrophilic surface and the anion-exchange properties of the ligands provides chromatographic characteristics well suited to the retention and separation of polar anionic compounds.

A panel of eight pesticides (AMPA, glyphosate, n-acetyl glufosinate, glufosinate, n-acetyl AMPA, ethephon, fosetyl aluminum, and phosphonic acid) were analyzed in different food commodities using electrospray negative ionization mode. All food commodities were extracted following the QuPPe methodology.

The SANTE guidelines specify that "the minimum acceptable retention time for the analyte(s) under examination should be at least twice the retention time corresponding to the void volume of the column" (SANTE 2018). The analytical column provided excellent retention of all compounds. Example chromatography for the analysis of the representative pesticides spiked into cucumber at 0.010 mg/kg can be seen in Figure 1. This method also provided excellent retention-time stability, in accordance with the SANTE guidelines tolerance of ±0.1 minute, across a selection of relevant commodities, as shown in Figure 2.

Excellent linearity (R^2 >0.99, residuals <20%) was found for calibration curves of all analytes in the absence of isotopically labelled standards. An example of matrix-matched, bracketed curves for AMPA and glyphosate in cucumber and tomato are shown in Figures 3a and 3b, where the concentration ranged from 2.5 ng/mL to 100 ng/mL in a vial (0.005 mg/kg–0.200 mg/kg matrix matched).

To evaluate the performance of the TQ-S micro for the routine analysis of anionic polar pesticides, the panel of eight representative compounds was spiked into tomato and cucumber at the targeted LOQ of 0.010 mg/kg as well as 2x LOQ and 5x LOQ, each at n = 6.

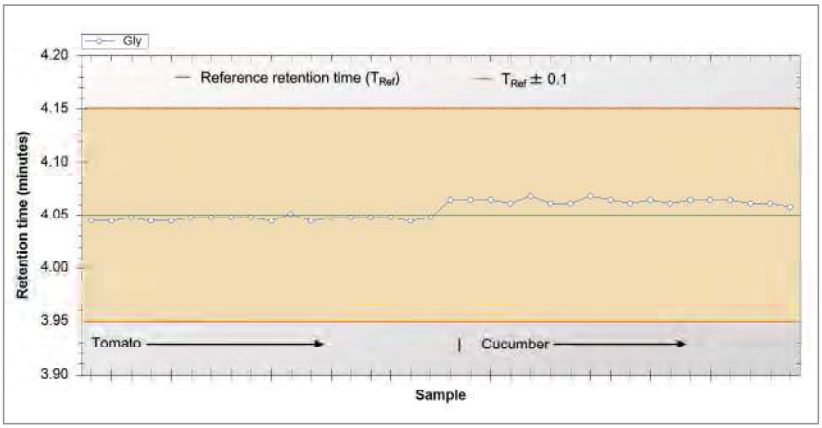

Figure 2. Retention time stability of glyphosate plotted in TrendPlot for the two commodities, each at n = 30, for samples spiked at various levels.

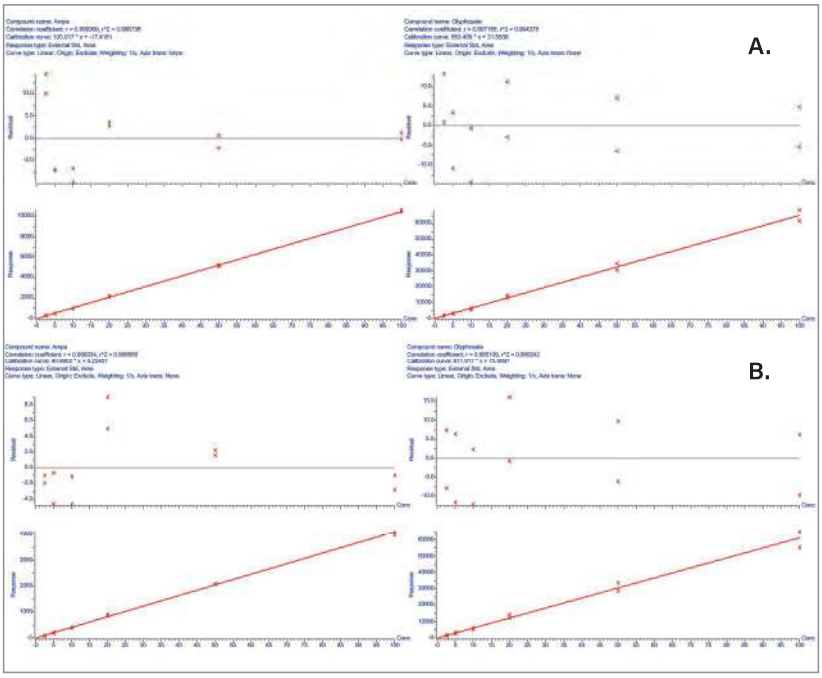

Figure 3. Example of matrix matched, bracketed calibration curves of AMPA and glyphosate in tomato (a) and cucumber (b) at 0.005 mg/kg–0.200 mg/kg.

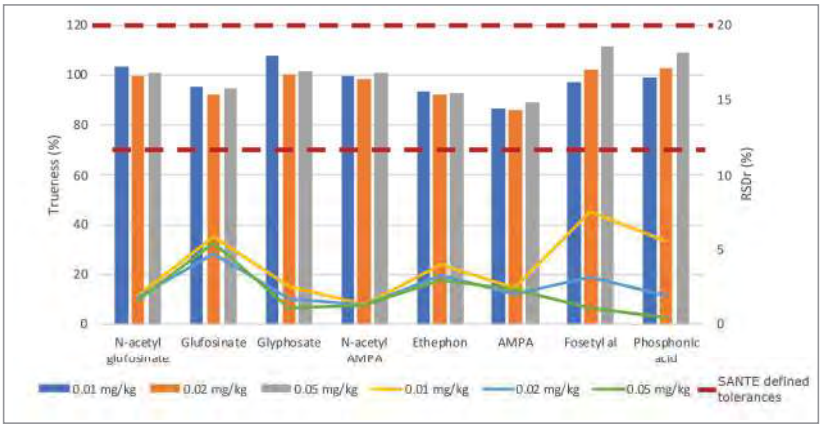

Figure 4. Percent trueness data at 0.010 mg/kg, 0.020 mg/kg, and 0.050 mg/kg spiking levels in tomato are plotted in the bar chart with the respective %RSDr plotted as lines on the secondary y-axis. The tolerances permitted by 11813/2017/SANTE are plotted in red for trueness (within 70 and 120%) and repeatability (< 20%).

Table 1. Method validation results are summarized for the tomato matrix against the criteria set out in the SANTE guidelines.

Compound	Retention time (±0.1 min)	% Trueness (70–120%)	% Precision (RSDr ≤20%)	Linearity (Residuals ≤ ±20%)	Ion Ratio (±30%)	LOQ 0.010 mg/kg
AMPA	✓	✓	✓	✓	✓	✓
Glufosinate	✓	✓	✓	✓	✓	✓
N-acetyl glufosinate	✓	✓	✓	✓	✓	✓
Glyphosate	✓	✓	✓	✓	✓	✓
N-acetyl AMPA	✓	✓	✓	✓	✓	✓
Ethephon	✓	✓	✓	✓	✓	✓
Phosphonic acid	✓	✓	✓	✓	✓	✓
Fosetyl aluminum	✓	✓	✓	✓	✓	✓

The spiked samples were quantified against a matrix matched calibration curve, as described above, to assess the capability of the Xevo TQ-S micro to reach the target LOQ level as well as the trueness (%) and precision (%RSDr) of the method. Figure 4 shows the trueness (%) data for the compounds at the spiking levels in tomato, where all compounds were within the range of 70–120% and the target LOQ was achieved for all compounds tested. The repeatability (%RSDr) of the method are also plotted in Figure 4, where all compounds at each level were <20% RSDr.

The overall results for tomato, compared against the analytical criteria defined in the 11813/2017/SANTE guidelines, are summarized in Table 1.

SUMMARY

The analysis of highly polar pesticides on a small footprint, routine-level tandem quadrupole has been demonstrated in this technology brief. The panel of eight compounds were spiked into various food matrices and excellent retention, retention time stability, and separation were achieved on a novel HILIC column. The Xevo TQ-S micro provided excellent performance in terms of sensitivity, linearity, and calibration range for the target compounds. The trueness and precision of the LC-MS/MS method determined at three levels was found to be acceptable for all compounds. Overall the performance data indicated that the configuration of the ACQUITY™ UPLC™ I-Class System coupled with Xevo TQ-S micro Mass Spectrometer, when used in combination with the Waters polar pesticides column, is suitable for checking MRL/tolerance compliance in a routine laboratory for these target compounds.

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Direct Quantification of Diquat and Paraquat in Drinking Water Samples Using Ultra-Sensitive UPLC/MS/MS Analysis

Claude R Mallet
Waters Corporation, Milford, MA, USA

APPLICATION BENEFITS

- Direct injection of clean water samples removes the need for sample extraction or concentration, saving valuable analyst time.
- Fast UPLC® analysis on an ACQUITY UPLC® BEH C_{18} Column decreases sample turn around time and improves lab productivity.
- The high sensitivity of Xevo TQ-S enables excellent trace-level quantification using a 100-µL direct injection, with no deterioration in performance apparent even after 250 sample injections.

WATERS SOLUTIONS

ACQUITY UPLC

Xevo® TQ-S
ACQUITY UPLC BEH C_{18} Column

TrendPlot™ MS Software

KEY WORDS

diquat, paraquat, drinking water, Xevo TQ-S, herbicide, bipyridyls

INTRODUCTION

Crop protection in countries around the globe is usually associated with the use of a wide range of pesticides, insecticides, or herbicides. These agricultural products can potentially have harmful effects on the environment and impact the health of both humans and animals. Despite the risk, they are a crucial part of the global economy[1] For example, the use of herbicides is important to control the growth of weeds, for if not suppressed weeds can reduce crop yields up to 80%.[2] In the herbicides family the bipyridyls are used extensively in agriculture to control broadleaf and aquatic weeds. The most common bipyridyls are diquat and paraquat. They constituted the largest share of the global market until recently overtaken by glyphosate.[3] Due to their high efficiency as pre-harvest desiccants and defoliants, diquat and paraquat are also classified as highly toxic.[4] The World Health Organization (WHO) has classified these compounds as moderately hazardous.[5] Even with a half-life in water of 48 hours, accidental or intentional ingestion can have serious health effects. For drinking water, the U.S. Environmental Protection Agency (U.S. EPA) has established a maximum contaminant level of 20 ppb for diquat and a desired goal of 3 ppb for paraquat[6] (not EPA regulated). The European Union (EU) has not regulated the levels of these compounds specifically in drinking water and continues to apply the value of 0.1 ppb.[7]

The analysis of bipyridylium herbicides can be difficult mainly because they are cationic molecules. Their inherent high polarity and positive charge, require the use of ion pairing additives when analyzing quaternary amines by reversed-phase chromatography. The U.S. EPA method 549.2 utilizes reversed-phase chromatography with ion pairing for the separation of diquat and paraquat using UV detection.[8] Ion pairing agents are typically avoided with ESI-MS applications owing to suppression of the ionization in the MS source. For MS applications, HILIC has provided suitable chromatography without the requirement of ion pairing agents.[9] However, recent advances in MS sensitivity have made the direct analysis of trace-level contaminants in water attainable and very attractive. The possibility of removing laborious and time-consuming solid phase extraction and sample concentration is highly desirable. Direct injection of an aqueous sample for RP chromatography is ideal as the sample matrix is similar to the initial mobile phase conditions. For HILIC, a water sample would first require dilution with the organic solvent.

EXPERIMENTAL

Diquat and paraquat standards were purchased from Sigma Alrich (St-Louis, MO, USA). HFBA (HPLC grade) was purchased from Thermo Scientific (Rockford, IL). MilliQ water was used to produce calibration standards. The water samples were collected from bottled and in-house tap water. The chemical structure and MRM conditions used for the quaternary herbicides are listed in Figure 1 and Table 1, respectively. MRM transitions stored in the Quanpedia™ database were selected for analysis. Chromatographic separation was performed on Waters® ACQUITY UPLC System equipped with an ACQUITY UPLC BEH C_{18} 2.1 x 30 mm Column. A one -minute linear water/methanol gradient with 10 mM HFBA was used. The detection was performed using a Xevo TQ-S.

UPLC conditions

UPLC system:	ACQUITY UPLC
Runtime:	3.0 min
Column:	ACQUITY UPLC BEH C_{18} 2.1 x 30 mm, 1.7 µm
Column temp.:	25 °C
Mobile phase A:	10 mM HFBA in water
Mobile phase B:	10 mM HFBA in methanol
Elution:	1 minute linear gradient from 2% (B) to 95% (B)
Flow rate:	0.6 mL/min
Injection volume:	100 µL

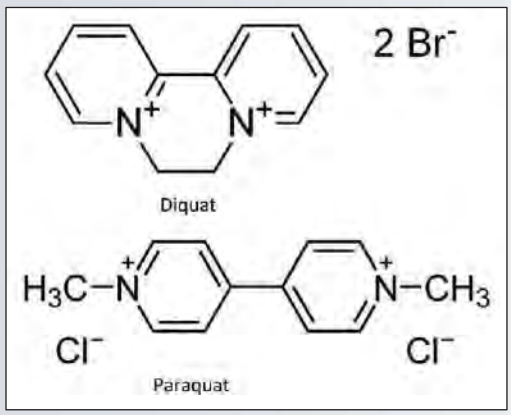

Figure 1. Chemical structure of diquat and paraquat.

MS conditions

MS system:	Xevo TQ-S
Ionization mode:	ESI positive
Capillary voltage:	3.0 kV
Cone voltage:	50.0 V
Source temp.:	140 °C
Desolvation temp.:	550 °C
Desolvation gas:	1100 L/hr
Cone gas:	50 L/hr

Herbicides	Precursor	Product	Cone	Collision
Diquat	183.0	157.0	50	20
	183.0	78.0	50	35
Paraquat	185.0	170.0	50	20
	185.0	107.0	50	30

Table 1. Diquat and paraquat MRM conditions.

This application note presents the analysis of diquat and paraquat herbicides in drinking water by direct injection using a volatile ion pairing reagent (heptafluorobutyric acid-HFBA), RP-UPLC, and the highly sensitive Xevo TQ-S.

RESULTS AND DISCUSSION

With the StepWave™ ion optics, Waters® Xevo TQ-S offers unsurpassed performance for trace-level analysis. The high sensitivity allows for the option to bypass the tedious sample concentration requirement associated with trace-level detection of contaminants in drinking water. With this high level of sensitivity, a clean water sample can be pre-concentrated directly on column by using a direct injection technique with the ACQUITY UPLC System. As shown in Figure 2, diquat and paraquat gave well-defined Gaussian peak shapes on the RP column. The vertical axes are linked in Figure 2 and show the difference in response of the two analytes. Even with the lower response seen for paraquat compared to diquat, the required levels of quantification for both compounds were achieved.

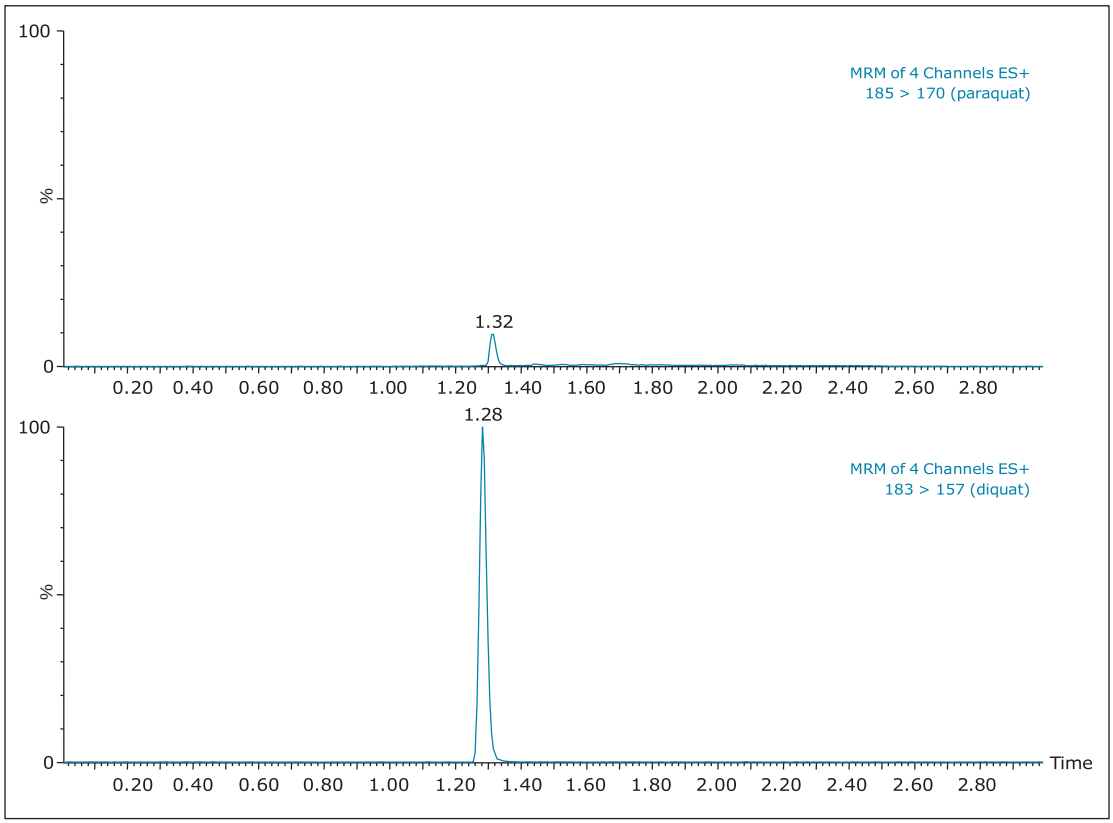

Figure 2. Reversed chromatograms of diquat and paraquat (1 ppb spike).

Quantification

Using the direct injection protocol, the quantification of bottled and tap water was measured against a calibration curve generated using standards made in MilliQ water. In this case, external calibration showed excellent results and an internal standard was not deemed necessary. As shown in Figure 3, the calibration curves for diquat and paraquat for tap water showed excellent linearity from 50 ppt to 100 ppb, with r^2 of 0.997 and 0.995 for diquat and paraquat, respectively. The recoveries for a 1 ppb spike are shown in Table 2, with recoveries in the range of 75% to 107%. The relative standard variation (RSD's) for diquat and paraquat was below 8% in both water samples.

Herbicides	Bottled water	Tap water
Diquat	107.0 (2.6)	75.1 (4.4)
Paraquat	99.0 (3.9)	76.5 (6.1)

Table 2. Recoveries and coefficient of variations at 1 ppb in bottle and tap water (n=3).

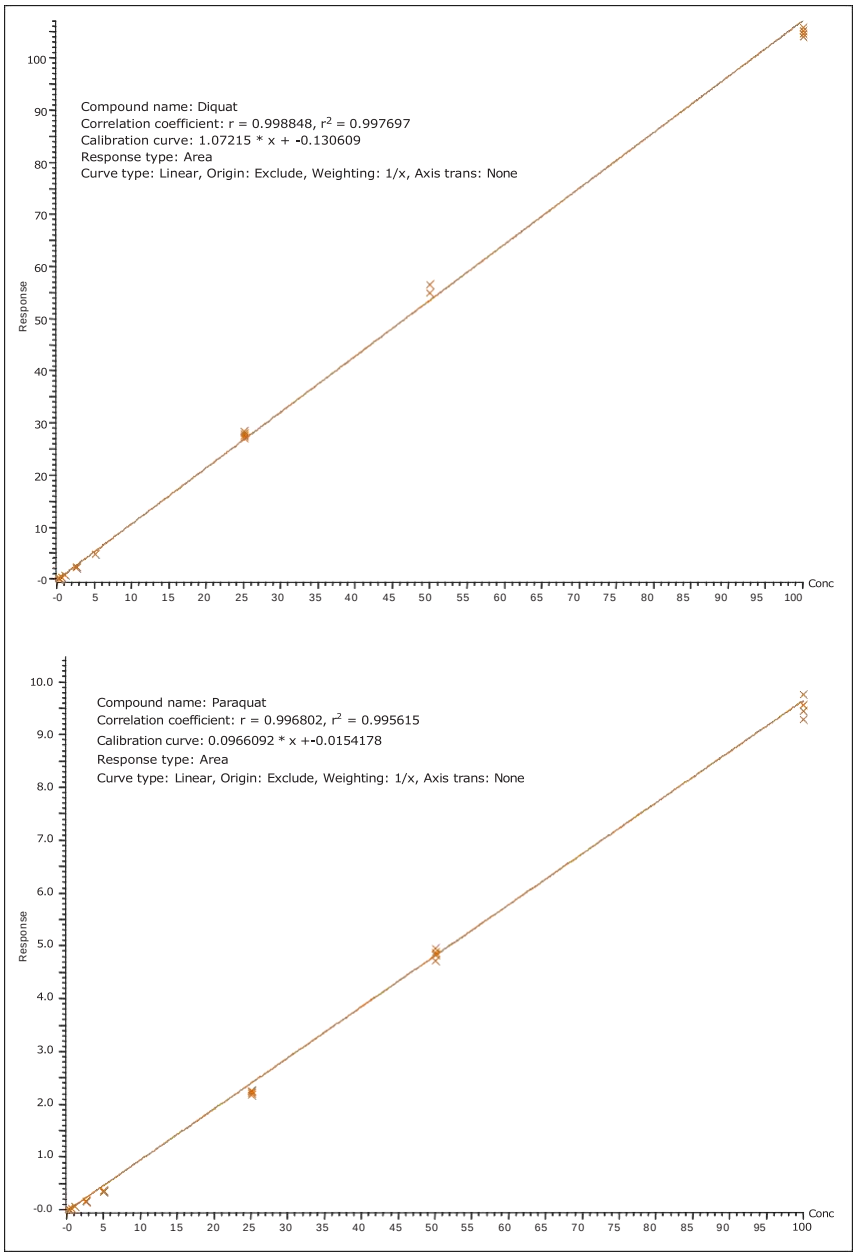

Figure 3A. Calibration curve for diquat from 50 ppt to 100 ppb.

Figure 3B. Calibration curve for paraquat from 50 ppt to 100 ppb.

In this application, since the ion pairing agent was added to both the mobile phases (aqueous and organic) and the sample, the purity of HFBA was crucial. During the development phase, the 185 → 170 *m/z* MRM transition for paraquat showed an interferent near the expected retention time of paraquat. It also showed high background levels which made it difficult to quantify paraquat below 500 ppt. This issue was attributed to the ion pair additive, most likely due to a lower purity grade that was employed. With a higher purity grade, the interferent was eliminated and the background noise was reduced to a satisfactory level. As a consequence, the limit of detection (LOD) of 50 ppt was achieved and the MRM chromatograms are presented in Figure 4 for bottled water. The ion ratios for both diquat and paraquat, calculated from the quantification and the confirmation MRM transitions (Figure 5) showed good correlation between the standard and spiked samples, further supporting the applicability of the direct injection method.

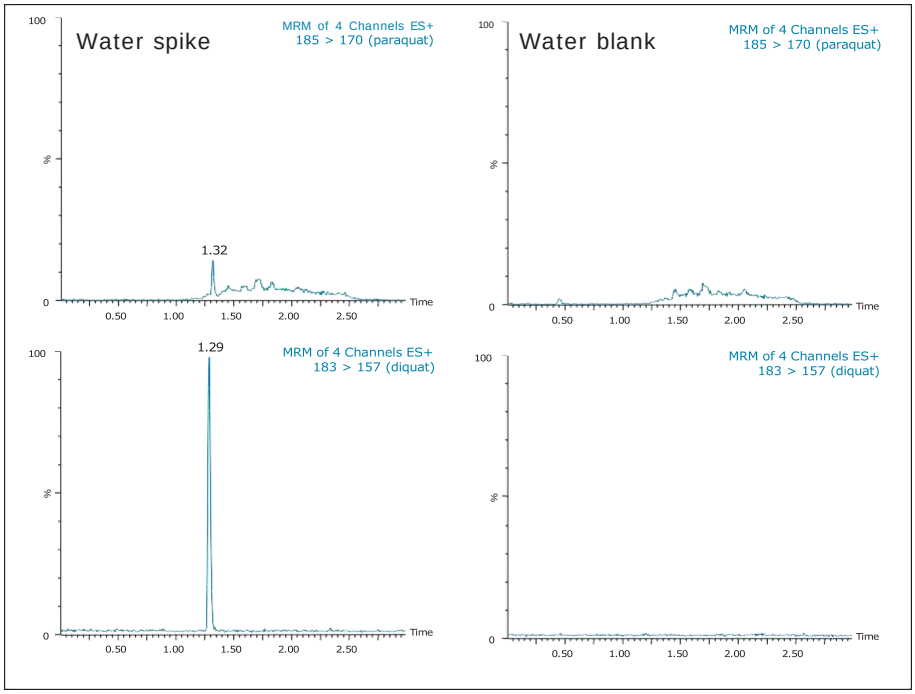

Figure 4. Chromatograms for paraquat and diquat at 50 ppt spike and blank (bottled water).

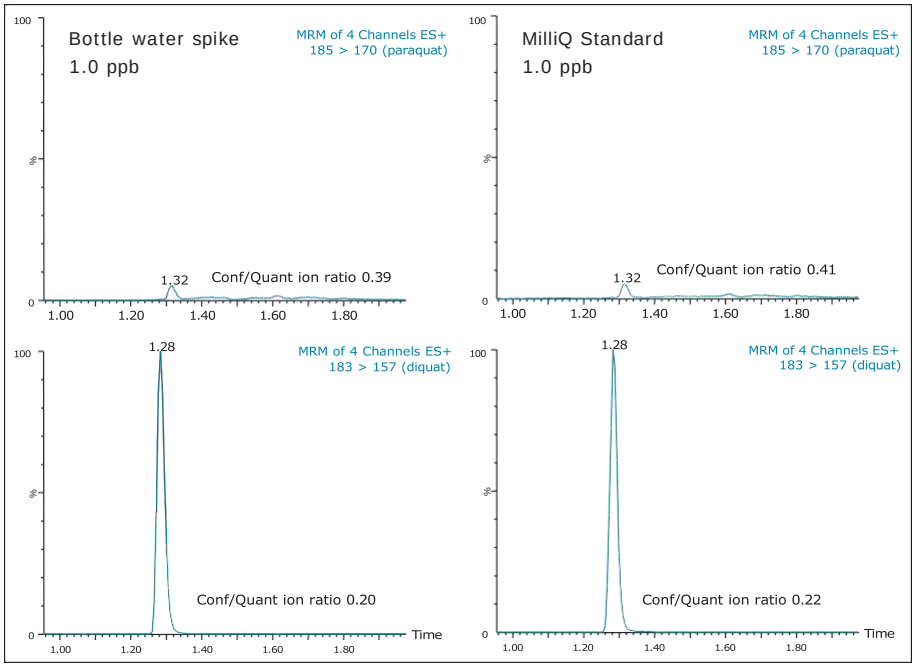

Figure 5. Ion ratio for diquat and paraquat using quantification and confirmation MRM transitions.

Lifetime and robustness

The direct injection approach is very efficient in term of speed and ease of use. However, the technique is not immune to potential situations which could affect the analytical performance over extended periods of time. The repeated injection and high injection volume of unfiltered and un-extracted samples could lead to peak distortion. During lifetime and robustness studies, the peak shape and column backpressure are excellent indicators of the column's overall performance. In this application, as seen in Figure 6, the peak shape of diquat and paraquat showed no noticeable distortion between the first and 250th injection. The initial column backpressure readout before injection of the first sample was recorded at 3500 psi. After 250 injections of tap water samples, the initial column backpressure shows a reading of 3900 psi, an increase of 400 psi. The key feature for quantification remains for the target analyte to elute with a Gaussian peak shape throughout the analysis.

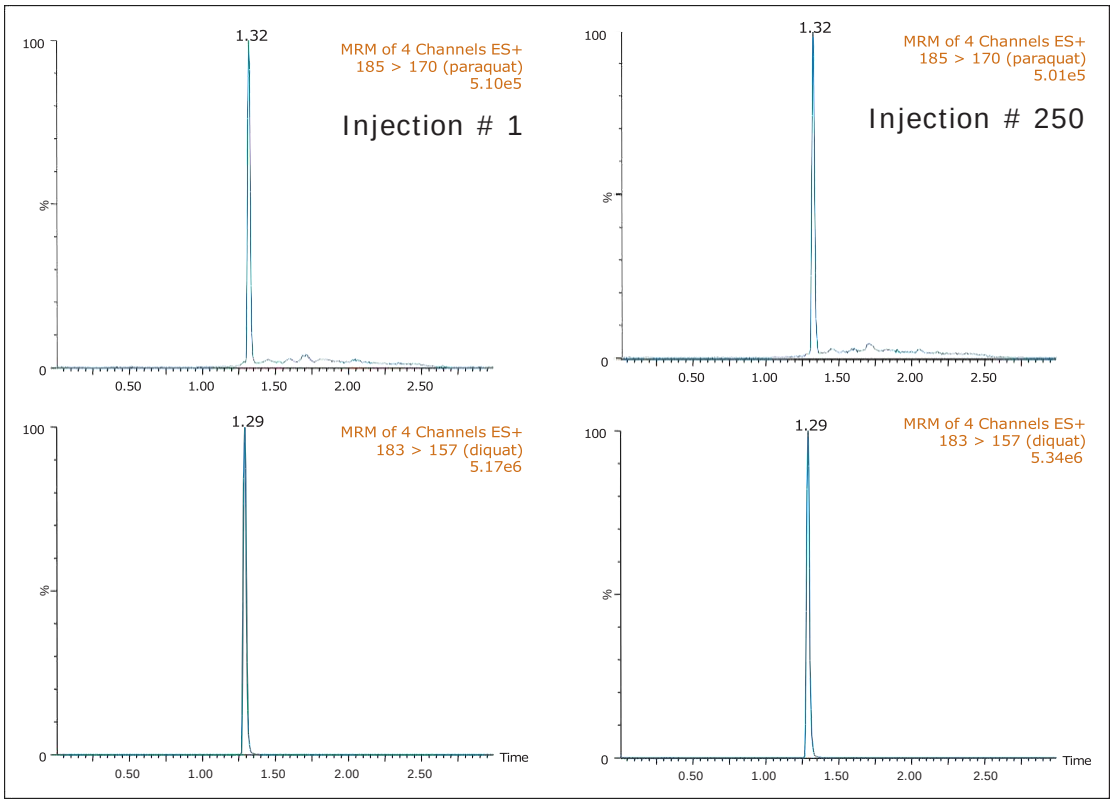

Figure 6. Example MRM chromatograms over the column lifetime study. Injections 1 and 250 are shown for diquat and paraquat in tap water.

In this application, the lifetime chromatograms for diquat and paraquat showed no signs of peak distortion and the RSDs on the quantification results were below 5%. Therefore, the small backpressure increase recorded for the tap water samples did not influence the overall analytical performance during this study. The TrendPlot™ profile report for diquat and paraquat are shown in Figure 7. As it can be seen in Figure 7, the TrendPlot shows excellent linearity for both compounds with RSDs at 4.7% and 7.5% for 100 injections, respectively.

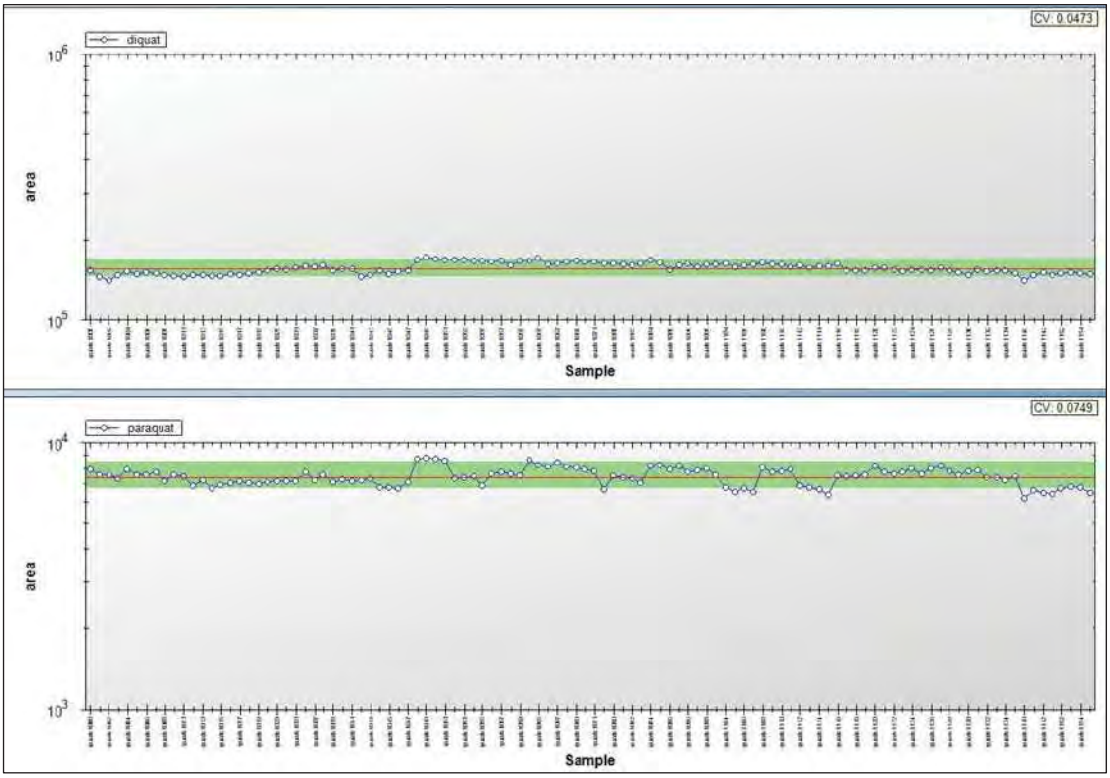

Figure 7. TrendPlot profiles of diquat and paraquat in tap water.

CONCLUSIONS

This application note has demonstrated the versatility of direct injection using the ACQUITY I-Class UPLC System with the Xevo TQ-S Mass Spectrometer for the analysis of diquat and paraquat in tap water and bottled water. The limit of detection in this study was 50 ppt, which is below the European Union Directive LOD of 100 ppt. The high sensitivity of Xevo TQ-S enabled excellent quantitation using a 100-µL injection without sample extraction or concentration prior injection. The recovery data showed good results with excellent RSD's below 8% for both water samples.

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Dutch mini-Luke ("NL-") extraction method followed by LC and GC-MS/MS for multi-residue analysis of pesticides in fruits and vegetables

CONTENTS

1. Aim and scope

This report describes the validation of a multiresidue method using LC-MS/MS and GC-MS/MS for the determination of 175 pesticides in lettuce and orange.

2. Short description

A homogeneous sample is extracted with acetone and partitioned with petroleum ether and dichloromethane, in the presence of sodium sulphate. The obtained extract is analysed by GC-MS/MS and LC-MS/MS.

3. Apparatus and consumables

- Automatic pipettes, suitable for handling volumes of 10 µL to 5000 µL and 1 mL to 3 mL
- 250 ml PTFE centrifuge tubes
- 40 ml glass tubes with caps
- Vortex
- Ultar-Turrax or Polytron homogeniser
- Centrifuge, suitable for the centrifuge tubes employed in the procedure and capable of achieving at least 3300 rpm
- Water bath
- Injection vials, 2 ml, suitable for LC and GC auto-sampler
- Volumetric flasks

4. Chemicals

- Acetone p.a.
- Petroleum ether
- Dichloromethane
- Anhydrous sodium sulphate
- Ammonium formate
- Ultra-pure water
- Methanol HPLC grade
- Isooctane
- Toluene
- Acetic acid
- Pesticides standards

5. Procedure

5.1. Sample preparation

According to Document No. SANCO/12571/2013, the sample was efficiently homogenised with a Stephan cutter at its arrival in the laboratory.

5.2. Recovery experiments for method validation

The samples employed in validation studies did not contain any of the pesticides analysed.

Individual pesticide stock solutions (1000 mg/L) were prepared in toluene or methanol and were stored in screw-capped glass vials in the dark at -20 °C.

For spiking, 15 g representative portions of previously homogenised sample were weighed in teflon tubes, where they were fortified homogenously with the appropriate amount of the working standard solution in methanol.

The validation was performed at three fortification levels (0.005, 0.01 and 0.02 mg/Kg). Six replicates were analysed at each level.

5.3. Extraction method

1. Weigh 15 g ± 0.1 g of sample in 250 mL PTFE centrifuge tube.
2. Add 75 µL of 10 µg/mL propoxur and PCB-153 (procedure internal standards), 20 mL of acetone and 15 g Na_2SO_4.
3. Blend the sample using a Turrax homogeniser for 30 s at 1500 rpm (extraction step).
4. Add 20 ml of petroleum ether and 10 ml of dichloromethane.
5. Blend it again by Turrax for 30 s at 1500 rpm (partitioning step).
6. Centrifuge for 3 min at 3300 rpm.
7. Transfer the supernatant into 40 mL glass tube.
8. Evaporate an aliquot of the extract carefully, until nearly dry, in a water bath programmed at 45°C and continuing to 63°C. The last part of the solvent was allowed to evaporate in the air in a fume hood.
    a. for LC analysis, evaporate 0.66 mL extract and reconstitute with 1 mL of 0.025% of acetic acid in methanol containing quinalphos at 0.04 µg/mL (Injection Internal Standard) for LC analysis.

b. for GC analysis, evaporate 5 mL extract and reconstitute with 1.5 mL of isooctane: toluene (9:1) containing 0.2 µg/mL of HCB-C13 and PCB-209 (Injection Internal Standards).

With this procedure, 1 mL of sample extract corresponds with 0.2 g of sample in LC and in GC the final matrix concentration is 1 g/mL.

5.4. Measurement

Both LC and GC systems were operated in multiple reaction monitoring mode (MRM). Selected reaction monitoring (SRM) experiments were carried out to obtain the maximum sensitivity for the detection of the target analytes. For identification of the studied compounds, two SRM transitions and a correct ion ratio of the detector responses of the two optimised SRM transitions (SRM2/SRM1) were used, together with retention time matching. The MRM transitions used are presented in Appendix I.

5.5. Instrumentation and analytical conditions for the LC- MS/MS system

5.5.1. Acquitty UPLC (Waters)

- Column: Acquity UPLC BEH C18, 2.1 mm x 100 mm and 1.7 µm particle size (Waters)
- Mobile phase A: 300 mg/L ammonium formate in milliQ water
- Mobile phase B: methanol
- Column temperature: 40°C
- Flow rate: 0.45 mL/min
- Injection volume: 1 µL
- Total chromatographic run time: 10 min

Mobile phase gradient for pesticides analyse

Time [min]	Mobile phase A	Mobile phase B
0	90%	10%
0.25	90%	10%
7.75	0%	100%
8.50	0%	100%
8.51	90%	10%

Re-equilibration with initial mobile phase: 1.5 minutes.

5.5.2. XEVO TQ-S triple quadrupole system (Waters)

- Ionisation mode: Positive mode
- ESI source gas temperature: 150 °C

- Desolvation temperature: 600 °C
- Desolvation gas flow: 1200 L/h
- Cone gas flow: 150 L/h
- Nebuliser gas: nitrogen
- Capillary voltage: 1.8 kV
- Collision gas: argon

5.6. Instrumentation and analytical conditions for the GC- MS/MS system

5.6.1. 436 GC (Bruker)

- Column: Varian VF-5ms 30 m × 0.25 mm ID and 0.25 µm
- Injection mode: LVI-PTV, solvent vent
- Open liner with carbofrit
- Injection volume: 5 µl
- Injector temperature: held at 80°C (0.1 min) and then ramped up to 300°C at 200°C/min. This temperature was held for 19.5 min.
- Carrier gas: helium at constant flow of 1 mL/min
- Carrier gas purity: 99.999%
- Oven temperature: 80°C for 1 min, programmed to 180°C at 25°C/min, then to 280°C at 8°C/min and finally to 300°C at 30°C/min and kept at 300°C for 3.17 min
- Total chromatographic run time: 22 min

5.6.2. Scion-TQ triple quadrupole system (Bruker)

- Ionisation mode: electron impact ionisation
- Temperature of the transfer line: 280 °C
- Temperature of ion source: 250 °C
- Temperature of manifold: 40 °C
- Collision gas: argon
- Collision gas purity: 99.999%
- Solvent delay: 3.3 minutes

6. Validation of the method

6.1. Recoveries and within-laboratory repeatability

The results for the mean recovery (n=6) and within-laboratory repeatability as relative standard deviation (RSD_r) at three fortification levels (0.005, 0.01 and 0.02 mg/kg) are summarized in Appendix II, Table 3.

Almost all the recovery results are within the range 70-120% except biphenyl, nitempyram, propamocarb in lettuce and aminopyralid, bifenazate, biphenyl, methamidophos, nitempyram and propamocarb in orange. This could be explained by the high water solubility of methamidophos, nitempyram and propamocarb, the high vapour pressure of biphenyl; bifenazate is a strong base and aminopiralid had a poor chromatographic behaviour.

6.2. Limits of quantitation

Document N° SANCO/12571/2013 defines limit of quantitation as the lowest validated spike level meeting the method performance acceptability criteria. LOQs are summarized in Appendix II, Table 4. The LOQ for 97% of the pesticides is 0.005 mg/kg in lettuce and 96% in orange.

6.3. Linearity

Linearity of the TQ-MS systems was evaluated by assessing the detector responses of the target analytes from matrix-matched calibration solutions prepared by spiking blank extracts at seven concentration levels, from 0.0005 to 0.100 mg/kg. In almost all cases, the coefficient of determination (r^2) was higher than 0.99 for most of the analytes. Linearity ranges for all pesticides are summarized in Appendix II, Table 4.

6.4. Matrix effects

Matrix effects were assessed by comparison of the slopes of seven-point calibration curves of matrix-matched standards with the slopes of the calibration curves of standards in solvent. The matrix effects data are summarized in Appendix II, Table 4.

This report aims to provide information to laboratories that analyse pesticide residues in fruits and vegetables or are interested in it.

7. References

- **Analytical quality control and method validation procedures for pesticide residues analysis in food and feed.** Document N° SANCO/12571/2013.
- **http://www.eurl-pesticides.eu**

- **Miniaturisation and optimisation of the Dutch mini-Luke ("NL-") extraction method for implementation in the routine multi-residue analysis of pesticides in fruits and vegetables.** Ana Lozano , Barbara Kiedroska, Jos Scholten, Marijke de Kroon, André de Kok and Amadeo R. Fernández-Alba, submitted to J. Chromatogr. A (2014).

APPENDIX I: MRM TRANSITIONS

Table 1. MS/MS detection and chromatographic parameters for the selected compounds analysed by LC-MS/MS.

No.	Name	t_R (min)	Cone voltage (V)	Precursor ion (m/z)	Product ion 1 (m/z)	Product ion 2 (m/z)	CE 1 (eV)	CE 2 (eV)
1	Acephate	1.25	20	183.9	142.8	94.6	10	25
2	Acetamiprid	3.13	35	223.1	125.9	90.0	22	35
3	Ametoctradin	6.77	20	276.3	176.1	149.0	38	36
4	Aminopyralid	0.58	25	207.1	189.0	161.0	14	18
5	Amisulbrom	6.89	25	468.1	228.9	148.0	26	50
6	Azoxystrobin	5.51	15	404.1	372.0	328.9	16	30
7	Benthiavalicarb-Isopropyl (R)	5.77	20	382.0	116.0	197.0	20	20
8	Benthiavalicarb-Isoprpoyl (S)	5.89	20	382	116.0	197	20	20
9	Bixafen	6.29	30	414.3	394.0	265.9	16	22
10	Bupirimate	6.22	35	317.2	107.9	166.0	25	25
11	Buprofezin	7.08	20	306.1	201.0	115.9	12	16
12	Carbaryl	4.56	14	202.1	144.9	126.9	12	24
13	Carbendazim	3.37	25	192.0	159.9	132.0	16	30
14	Carbofuran, 3 Hydroxy	3.20	28	238.1	163.0	181.0	16	10
15	Chlorfenvinphos	6.46+6.62	25	359.0	98.9	126.9	30	24
16	Clofentezine	6.58	20	303.0	137.9	101.9	14	35
17	Clothianidin	2.80	20	250.0	168.9	131.8	14	14
18	Cyflufenamid	6.56	25	413.3	295.0	240.9	16	22
19	Cyflumetofen	6.89	25	465.5	172.9	249.0	26	14
20	Cyproconazole	5.80+5.95	25	292.1	70.1	124.9	18	30
21	Cyprodinil	6.38	45	226.1	92.9	107.9	35	25
22	Demeton-S-methyl sulphone	2.24	30	263.0	168.9	108.9	16	30
23	Diazinon	6.43	25	305.1	169.0	96.9	22	35
24	Diethofencarb	5.48	20	268.1	123.9	152.0	30	22
25	Difenoconazole	6.70+6.73	35	406.1	250.9	187.8	25	40
26	Dimethoate	3.04	20	230.0	198.8	124.8	10	22
27	Dimethomorph	5.62+5.82	35	388.1	300.9	165.0	20	30
28	Dinotefuran	1.62	20	203.1	128.8	113.3	12	12
29	Dodine	6.60	45	228.2	56.9	59.8	23	23
30	EPN	6.73	30	324.1	156.9	295.9	23	15
31	Epoxiconazole	6.06	35	330.1	120.9	122.9	25	20
32	Ethion	7.11	20	385.0	198.9	96.9	11	45
33	Ethoprofos	6.05	30	243.0	131.0	173.0	20	15
34	Etoxazole	7.32	15	360.0	141.0	177.0	25	20
35	Fenamidone	5.62	25	312.1	92.0	236.1	25	14
36	Fenarimol	6.04	40	331.0	268.0	81.0	30	25
37	Fenazaquin	7.59	30	307.2	57.2	161.0	25	19
38	Fenbuconazole	6.16	35	337.1	70.1	124.9	18	30
39	Fenoxycarb	6.24	20	302.1	88.0	115.9	18	12
40	Fenpyroximate	7.41	30	422.3	366.1	137.9	17	31
41	Fenthion	6.37	25	279	168.9	104.9	18	25
42	Fenthion sulfoxide	4.55	40	295.0	108.9	78.9	30	24
43	Fludioxonil	5.67	15	266.0	228.9	157.9	10	35
44	Flufenoxuron	7.34	30	489.0	157.9	141.0	20	40
45	Fluopyram	5.94	35	397.1	172.8	207.9	29	23
46	Flutolanil	5.73	25	324.1	242.0	262.0	25	18

No.	Name	t_R (min)	Cone voltage (V)	Precursor ion (m/z)	Product ion 1 (m/z)	Product ion 2 (m/z)	CE 1 (eV)	CE 2 (eV)
47	Flutriafol	5.01	25	302.1	70.1	122.9	16	30
48	Fluxapyroxad	5.77	25	382.1	362.1	342.1	14	20
49	Furathiocarb	6.97	25	383.2	194.9	252.0	18	12
50	Halofenozide	5.69	34	275.0	104.8	138.8	8	16
51	Haloxyfop	5.63	25	362.0	91.0	316.0	20	30
52	Hexaconazole	6.56	30	314.1	70.1	158.8	20	40
53	Hexythiazox	7.22	20	353.1	167.9	228.0	27	17
54	Imazalil	6.35	35	297.1	69.1	158.9	18	24
55	Imazapic	1.85	30	276.2	231.1	86.1	20	24
56	Imidacloprid	2.79	25	256.1	174.9	209.0	20	12
57	Indoxacarb	6.75	30	528.1	202.9	217.9	40	25
58	Iprovalicarb	5.89+5.94	20	321.2	118.9	203.0	18	10
59	Linuron	5.51	20	249.0	159.9	181.9	20	16
60	Lufenuron	7.11	25	512.0	141.0	158.0	15	40
61	Malathion	5.82	20	331.0	126.9	285.0	12	10
62	Methamidophos	1.00	30	141.9	93.9	124.8	12	14
63	Methiocarb	5.59	20	226.1	121.0	168.9	20	10
64	Methiocarb sulfone	3.29	15	275.1	121.9	200.9	24	14
65	Methiocarb sulfoxide	3.00	25	242.1	121.9	184.9	14	30
66	Monocrotophos	2.42	20	224.0	126.8	97.9	16	14
67	Myclobutanil	5.87	30	289.1	70.1	124.9	18	30
68	Nitenpyram	2.02	25	271.1	125.9	224.9	25	12
69	Omethoate	1.47	25	214.0	124.8	182.8	22	10
70	Oxamyl-oxime	1.50	20	162.9	72.0	89.9	12	16
71	Oxydemeton-methyl	2.15	20	247.0	168.8	108.9	14	25
72	Penconazole	6.38	30	284.1	70.1	158.9	16	30
73	Pencycuron	6.66	30	329.1	124.9	218.0	30	16
74	Phosalone	6.57	25	368.0	182.0	111.0	15	40
75	Picolinafen	7.03	35	377.3	237.9	359.0	28	20
76	Pirimiphos-methyl	6.58	30	306.1	107.9	67.1	30	40
77	Prochloraz	6.59	20	376.0	307.9	70.1	12	25
78	Profenofos	6.95	30	372.9	302.6	127.9	20	40
79	Propamocarb	1.70	20	189.0	102.0	74.0	25	25
80	Propargite	7.32	20	368.3	231.1	175.0	11	15
81	Propiconazole	6.45+6.49	35	342.1	158.9	69.1	20	30
82	Propoxur (P.I.S)	4.36	20	210.1	110.9	92.9	14	24
83	Propyzamide	5.74	20	256.0	189.8	172.8	14	25
84	Proquinazid	7.50	25	373.2	330.9	288.9	16	24
85	Pymetrozine	2.25	30	218.1	104.9	78.3	18	35
86	Pyraclostrobin	6.52	25	388.1	163.0	193.9	25	12
87	Pyrimidafen	7.09	45	378.2	183.9	149.8	25	37
88	Quinalphos (I.I.S.)	6.25	20	299.0	243.0	271.0	15	15
89	Rotenone	6.20	40	395.2	213.0	191.9	25	25
90	Spinetoram I	7.63	45	748.9	141.9	114.9	30	46
91	Spinetoram II	7.82	45	761.0	141.9	114.9	30	48
92	Spinosyn A	7.44	50	732.6	142.0	98.1	31	59
93	Spinosyn D	7.64	45	746.5	142.0	98.1	31	53
94	Spirotetramat	5.97	30	374.4	330.1	302.1	16	16
95	Spiroxamine	6.27	30	298.3	144.0	100.0	20	30
96	Tebuconazole	6.39	30	308.2	70.1	124.9	24	40
97	Tebufenozide	6.22	16	353.3	132.7	297.0	16	8

No.	Name	t_R (min)	Cone voltage (V)	Precursor ion (m/z)	Product ion 1 (m/z)	Product ion 2 (m/z)	CE 1 (eV)	CE 2 (eV)
98	Teflubenzuron	7.12	25	381.0	141.0	158.0	15	30
99	Tembotrione	3.66	42	441.1	380.8	261.7	8	28
100	Thiabendazole	6.06	35	372.0	158.9	70.1	35	22
101	Thiacloprid	3.48	35	253.0	125.8	90.0	20	40
102	Thiamethoxam	2.27	20	292.1	210.9	180.9	12	22
103	Tolclophos-Methyl	6.58	35	301.1	125.0	174.9	17	29
104	Tolylfluanid	6.36	20	347.0	136.8	237.8	26	10
105	Triazophos	5.89	25	314.1	161.9	118.9	18	35
106	Triazoxide	4.94	40	248.2	68.1	95.0	24	24
107	Trifloxystrobin	6.77	25	409.2	185.9	145.0	14	40
108	Triflumizole	6.87	15	346.1	278.0	73.1	10	18
109	Zoxamide	6.46	30	336.0	186.9	158.9	25	40

Table 2. MS/MS detection and chromatographic parameters for the selected compounds analysed by GC-MS/MS.

No.	Name	t_R (min)	Precursor ion 1 (m/z)	Product ion 1 (m/z)	CE 1 (eV)	Precursor ion 2 (m/z)	Product ion 2 (m/z)	CE 2 (eV)
1	2-Phenylphenol	6.07	170	141	20	170	115	35
2	Acrinathrin	15.95	208	181	15	208	152	30
3	Azinphos-methyl	15.68	160	104	10	160	132	5
4	Bifenazate	14.85	300	158.0	10	300.0	196.0	20
5	Bifenthrin	14.58	181	166.0	15	181.0	165.0	25
6	Biphenyl	5.20	154	153.0	15	154.0	115.0	25
7	Bitertanol	16.93	170	115.0	30	170.0	141.0	20
8	Boscalid	17.99	140	112.0	15	140	76	25
9	Bromopropylate	14.74	341	155.0	30	341.0	183.0	20
10	Carbofuran	7.64	164	122.0	10	164.0	149.0	10
11	Chlorantraniliprole	15.05	278	249.0	25	278.0	215.0	25
12	Chlorfenapyr	12.24	247	227.0	15	247.0	200.0	20
13	Chlorothalonil	4.27	266	168.0	25	266.0	231.0	15
14	Chlorpropham	7.00	213	127.0	15	213.0	171.0	10
15	Chlorpyrifos-ethyl	9.78	314	258.0	15	314.0	286.0	10
16	Chlorpyrifos-methyl	8.93	286	208.0	15	286.0	271.0	20
17	Cypermethrin	17.96	181	152.0	30	163.0	127.0	10
18	Deltamethrin	19.12	172	93.0	10	253.0	172.0	10
19	Dichlorvos	4.29	185	93.0	15	185.0	109.0	20
20	Dicloran	7.72	206	176.0	15	206.0	148.0	20
21	Diniconazole	12.77	268	171.0	20	268.0	232.0	10
22	Diphenylamine	6.84	169	167.0	30	169.0	168.0	10
23	Endosulfan-alpha	11.48	241	206.0	15	241.0	170.0	25
24	Endosulfan-beta	12.83	241	206.0	15	241.0	170.0	25
25	Endosulfan-sulfate	13.57	272	237.0	18	387.0	253.0	10
26	Etofenprox	18.11	163	107.0	15	163.0	135.0	10
27	Famoxadone	19.45	196	167.0	15	330.0	224.0	10
28	Fenhexamid	13.76	97	55.0	10	177.0	113.0	10
29	Fenitrothion	9.55	277	109.0	20	277.0	260.0	10
30	Fenpropathrin	14.86	265	210.0	15	265.0	181.0	20
31	Fenpropimorph	9.85	128	110.0	5	303.0	128.0	10
32	Fenvalerate	18.58	225	147.0	10	225.0	119.0	15
33	Fipronil	10.65	367	213.0	25	367.0	255.0	20
34	Fluopicolide	13.68	347	172.0	30	347.0	136.0	40
35	Flusilazole	12.05	233	152.0	15	233.0	165.0	15
36	HCB-C13 (I.I.S.)	7.50	290	220.0	25	290.0	255.0	15
37	Iprodione	14.59	314	271.0	10	314.0	245.0	20
38	Kresoxim-methyl	12.08	206	116.0	15	206.0	131.0	15
39	lambda-Cyhalothrin	17.96	197	141.0	15	181.0	152.0	25
40	Mepanipyrim	11.55	222	158.0	20	222.0	207.0	10
41	Metalaxyl	9.15	206	132.0	15	149.0	190.0	10
42	Methidathion	11.17	125	79.0	10	145	85	10
43	Metrafenone	16.24	393	363.0	15	393.0	299.0	20
44	Oxadixyl	12.84	163	132.0	10	233.0	146.0	10
45	Parathion methyl	9.08	247	200.0	10	247.0	230.0	10
46	PCB-153 (P.I.S.)	13.10	360	290.0	25	360.0	325.0	12
47	PCB-209 (I.I.S.)	18.21	356	286.0	30	356.0	321.0	25
48	Pendimethalin	10.47	252	162.0	15	252.0	191.0	10

No.	Name	t_R (min)	Precursor ion 1 (m/z)	Product ion 1 (m/z)	CE 1 (eV)	Precursor ion 2 (m/z)	Product ion 2 (m/z)	CE 2 (eV)
49	Permethrin-cis	16.79	183	153.0	20	183.0	168.0	20
50	Permethrin-trans	16.96	183	153.0	20	183.0	168.0	20
51	Phenthoate	10.82	274	121.0	10	274.0	246.0	5
52	Picoxystrobin	11.33	335	173.0	10	335.0	303.0	10
53	Piperonyl-butoxide	14.33	176	131.0	15	176.0	161.0	10
54	Pirimicarb	8.45	238	72.0	25	238.0	166.0	10
55	Pirimicarb-desmethyl	8.69	152	96.0	15	224.0	152.0	10
56	Procymidone	10.93	283	96.0	15	283.0	255.0	10
57	Prothiofos	11.69	309	239.0	15	309.0	221.0	25
58	Pyridaben	17.03	147	117.0	25	147.0	132.0	15
59	Pyrimethanil	8.20	198	158.0	15	198.0	183.0	10
60	Pyriproxyfen	15.65	136	96.0	10	226.0	186.0	15
61	Quinoxyfen	13.56	307	237.0	25	307.0	272.0	10
62	Silthiofam	8.55	252	197.0	15	252.0	210.0	15
63	Spirodiclofen	16.62	312	109.0	20	312.0	259.0	10
64	Spiromesifen	14.22	272	209.0	10	272.0	254.0	10
65	tau-Fluvalinate	18.69	250	200.0	20	250.0	208.0	25
66	Tebufenpyrad	14.99	333	171.0	20	333.0	276.0	10
67	Terbuthylazine	7.97	214	132.0	15	214.0	104.0	20
68	Tetraconazole	10.03	336	191.0	20	336.0	218.0	15
69	Tetradifon	15.40	229	166.0	20	229.0	201.0	15
70	Triadimefon	10.08	208	111.0	20	208.0	127.0	15
71	Triadimenol	10.99	168	70.0	10	168.0	112.0	5

APPENDIX II: VALIDATION RESULTS FOR THE NL-METHOD

Table 3. Accuracy data (as % recovery) and precision data (as repeatability RSD_r, n=6) at spike levels of 0.005, 0.01 and 0.02 mg/kg for lettuce and orange.

No.	Pesticide	Lettuce						Orange					
		0.005 mg/kg		0.01 mg/kg		0.02 mg/kg		0.005 mg/kg		0.01 mg/kg		0.02 mg/kg	
		Rec. (%)	RSD_r (%)	Rec. (%)	RSD_r (%)	Rec. (%)	RSD_r (%)	Rec. (%)	RSD_r (%)	Rec. (%)	RSD_r (%)	Rec. (%)	RSD_r (%)
1	*2-Phenylphenol*	89	7.8	81	18.4	81	15.6	88	4.6	80	11.7	97	19.0
2	**Acephate**	100	11.4	94	5.5	92	3.3	79	19.5	79	10.6	70	15.2
3	**Acetamiprid**	109	4.2	109	2.2	107	1.6	110	11.2	111	7.4	108	8.0
4	*Acrinathrin*	101	13.8	87	19.3	87	19.3	105	3.4	102	5.9	119	13.7
5	**Ametoctradin**	105	3.9	107	6.9	106	5.0	102	11.4	99	5.9	104	10.1
6	**Aminopyralid**	n.d.		n.d.		n.d.		53	30.6	52	22.2	48	14.1
7	**Amisulbrom**	109	11.3	104	5.6	106	2.9	96	11.5	101	6.4	100	9.9
8	*Azinphos-methyl*	95	18.5	96	17.8	94	15.0	n.d.		n.d.		98	23.8
9	**Azoxystrobin**	106	5.3	104	4.5	103	5.5	103	12.8	112	10.2	105	7.2
10	**Benthiavalicarb-Isopropyl (R)**	109	3.6	105	5.6	105	5.1	95	11.4	100	5.4	97	4.7
11	**Benthiavalicarb-Isoprpoyl (S)**	107	3.1	100	4.5	106	4.8	98	11.6	99	13.0	95	7.2
12	*Bifenazate*	119	7.2	111	10.2	117	11.6	64	11.9	66	10.1	46	14.7
13	*Bifenthrin*	97	5.0	88	18.5	91	15.5	94	2.7	87	6.1	104	15.8
14	*Biphenyl*	66	14.4	50	27.0	50	18.3	74	3.9	64	7.8	76	19.1
15	*Bitertanol*	101	5.3	95	6.2	95	5.2	82	10.0	84	9.3	80	14.7
16	**Bixafen**	108	2.2	102	7.1	105	5.5	103	6.8	96	9.2	98	7.4
17	*Boscalid*	102	3.4	97	5.6	99	4.2	81	9.7	85	10.3	79	12.8
18	*Bromopropylate*	98	4.5	90	17.5	94	16.7	93	2.6	87	8.2	103	15.0
19	**Bupirimate**	105	4.4	105	5.0	105	6.2	104	6.4	99	7.8	97	7.0
20	**Buprofezin**	106	1.3	109	4.3	104	4.4	100	10.3	98	5.7	99	11.3
21	**Carbaryl**	111	9.5	103	8.9	104	6.9	112	15.5	116	6.2	106	6.3
22	**Carbendazim**	108	3.6	107	3.6	106	1.9	103	11.1	101	7.3	100	7.9
23	*Carbofuran*	99	19.8	83	18.0	79	18.2	86	10.9	87	9.8	84	14.8
24	**Carbofuran, 3 Hydroxy**	104	6.2	109	4.5	111	2.9	110	16.4	111	4.0	107	5.7
25	*Chlorantraniliprole*	95	11.7	91	10.0	94	10.6	86	12.7	71	11.1	71	16.3
26	*Chlorfenapyr*	92	9.8	94	12.9	94	17.1	95	9.5	89	14.0	103	16.6
27	**Chlorfenvinphos**	107	4.0	104	6.7	103	3.9	106	6.1	106	4.2	106	7.5
28	*Chlorothalonil*	100	18.9	82	19.1	73	10.3	105	11.4	88	14.7	100	25.0
29	*Chlorpropham*	97	4.5	91	10.0	92	9.6	89	6.2	87	6.3	93	16.9
30	*Chlorpyrifos-ethyl*	101	6.0	96	10.0	96	10.7	93	6.7	99	11.2	101	14.9
31	*Chlorpyrifos-methyl*	102	8.8	94	13.0	93	12.8	95	10.5	98	13.4	100	17.8
32	**Clofentezine**	73	5.5	80	9.1	70	6.5	92	8.1	90	7.2	90	10.5
33	**Clothianidin**	110	4.9	109	2.8	107	2.8	104	14.0	105	10.1	101	8.5
34	**Cyflufenamid**	109	4.5	107	4.5	105	3.9	104	7.9	96	6.4	104	8.1
35	**Cyflumetofen**	109	5.5	105	5.0	101	8.1	103	10.5	106	7.7	110	9.2
36	*Cypermethrin*	95	14.1	84	19.1	83	19.7	96	6.4	86	11.5	101	16.8
37	**Cyproconazole**	112	5.7	105	4.0	104	6.3	106	10.0	107	11.6	98	5.9
38	**Cyprodinil**	109	5.9	104	11.6	101	9.3	106	14.2	100	9.4	101	14.0
39	*Deltamethrin*	91	15.1	79	18.5	76	19.6	79	13.4	78	15.0	99	16.4
40	**Demeton-S-methyl sulphone**	109	5.1	106	3.0	103	5.1	110	14.0	112	14.9	100	13.2
41	**Diazinon**	108	3.6	107	4.9	106	4.3	108	9.1	102	7.7	103	7.8
42	*Dichlorvos*	80	10.4	71	14.3	71	16.6	75	11.7	80	6.4	76	18.8
43	*Dicloran*	96	6.6	86	19.4	87	18.2	89	4.4	80	13.0	96	18.2
44	**Diethofencarb**	101	6.4	101	8.1	104	4.9	96	11.9	100	17.7	101	7.4
45	**Difenoconazole**	105	6.5	104	6.4	104	3.7	106	9.4	97	4.6	103	6.2
46	**Dimethoate**	108	4.3	111	3.1	107	2.0	114	13.1	114	5.7	112	7.0
47	**Dimethomorph**	99	4.2	104	4.1	101	3.2	99	8.6	102	8.9	100	8.1

No.	Pesticide	Lettuce						Orange					
		0.005 mg/kg		0.01 mg/kg		0.02 mg/kg		0.005 mg/kg		0.01 mg/kg		0.02 mg/kg	
		Rec. (%)	RSD_r (%)	Rec. (%)	RSD_r (%)	Rec. (%)	RSD_r (%)	Rec. (%)	RSD_r (%)	Rec. (%)	RSD_r (%)	Rec. (%)	RSD_r (%)
48	*Diniconazole*	98	4.2	95	4.6	98	3.7	85	10.3	89	5.9	86	14.5
49	**Dinotefuran**	103	5.9	100	6.7	103	3.5	96	12.0	93	10.4	91	11.9
50	*Diphenylamine*	89	5.1	74	15.4	75	14.6	90	2.7	79	8.6	96	18.9
51	**Dodine**	100	7.3	98	5.1	100	2.4	80	17.5	82	5.9	78	6.1
52	*Endosulfan-alpha*	92	7.0	88	18.9	94	17.1	89	4.3	85	6.4	103	15.5
53	*Endosulfan-beta*	99	11.9	89	18.4	95	16.9	92	7.1	84	1.6	102	17.2
54	*Endosulfan-sulfate*	100	6.4	99	6.9	98	5.0	100	11.4	83	13.0	104	25.2
55	**EPN**	106	6.1	108	7.5	107	5.3	105	8.1	104	6.5	108	9.8
56	**Epoxiconazole**	110	4.0	104	7.3	106	4.3	97	4.4	105	8.6	100	5.5
57	**Ethion**	109	3.8	107	7.1	103	5.6	97	8.3	101	7.0	98	11.6
58	**Ethoprofos**	110	1.3	99	5.0	101	4.7	101	8.4	104	9.5	100	4.0
59	*Etofenprox*	101	3.4	93	5.8	95	4.8	97	13.2	96	5.7	85	14.3
60	**Etoxazole**	108	2.2	104	6.7	102	4.7	100	12.2	101	8.7	100	11.4
61	*Famoxadone*	107	19.4	87	17.8	99	17.7	89	14.4	85	10.7	74	19.6
62	**Fenamidone**	107	6.7	103	3.8	103	4.4	107	10.9	107	7.0	103	7.0
63	**Fenarimol**	114	11.4	105	10.4	111	3.0	92	19.6	112	15.1	91	16.6
64	**Fenazaquin**	105	6.6	105	9.2	103	4.5	107	12.9	107	6.1	98	6.7
65	**Fenbuconazole**	107	7.7	102	8.4	101	5.2	91	19.8	99	14.2	96	3.3
66	*Fenhexamid*	100	7.8	95	5.1	97	5.3	90	11.6	93	9.3	90	14.3
67	*Fenitrothion*	95	11.5	81	19.5	80	16.0	96	6.2	87	14.9	97	18.9
68	**Fenoxycarb**	108	3.7	110	6.2	104	4.2	103	2.9	101	8.6	94	7.7
69	*Fenpropathrin*	98	7.2	90	17.8	93	15.4	91	4.7	87	7.6	106	13.2
70	*Fenpropimorph*	99	5.8	86	12.0	98	6.4	85	11.8	87	8.3	87	19.6
71	**Fenpyroximate**	104	3.4	105	4.5	104	9.2	100	9.5	101	6.1	100	8.1
72	**Fenthion**	106	2.8	105	5.9	102	4.4	101	7.3	101	5.9	101	7.7
73	**Fenthion sulfoxide**	112	8.1	110	7.4	112	7.3	114	12.8	116	6.0	113	5.3
74	*Fenvalerate*	97	14.4	75	10.8	84	16.6	91	2.5	82	6.7	98	17.4
75	*Fipronil*	104	10.8	89	17.0	96	17.6	88	6.0	81	6.9	94	18.7
76	**Fludioxonil**	119	15.3	114	13.1	108	11.4	102	13.1	103	7.3	102	5.9
77	**Flufenoxuron**	102	3.3	104	5.0	102	4.4	106	8.6	102	6.4	96	7.8
78	*Fluopicolide*	99	4.7	92	18.3	97	15.2	86	5.8	79	13.2	95	18.2
79	**Fluopyram**	104	5.2	102	5.2	101	4.6	100	9.0	100	9.5	95	6.1
80	*Flusilazole*	103	4.3	98	5.6	100	4.3	82	10.5	84	8.6	82	16.3
81	**Flutolanil**	106	6.2	102	5.3	103	3.0	101	5.9	99	8.7	94	6.5
82	**Flutriafol**	120	13.7	115	15.6	107	16.2	109	13.6	114	8.1	105	7.0
83	**Fluxapyroxad**	107	4.1	101	4.4	101	4.5	101	11.2	101	9.1	93	6.2
84	**Furathiocarb**	107	4.5	104	3.7	103	3.4	103	8.6	100	5.3	98	9.0
85	**Halofenozide**	97	13.6	98	6.6	100	3.9	107	7.7	105	10.0	97	8.0
86	**Haloxyfop**	118	12.5	110	6.3	103	5.9	108	17.1	106	9.3	93	5.8
87	**Hexaconazole**	107	4.0	110	3.9	106	7.1	100	14.8	99	8.7	103	6.6
88	**Hexythiazox**	104	3.6	104	6.2	104	6.5	103	8.3	101	4.2	94	10.8
89	**Imazalil**	102	8.3	101	8.0	89	5.6	91	15.9	101	8.8	90	12.8
90	**Imazapic**	98	8.2	98	7.3	94	7.5	108	16.0	104	11.7	98	12.4
91	**Imidacloprid**	110	5.5	108	3.1	108	2.4	101	11.7	106	8.9	102	8.5
92	**Indoxacarb**	110	6.4	99	7.3	101	5.0	103	5.9	101	6.4	102	7.3
93	*Iprodione*	98	19.5	78	15.1	77	13.8	87	10.7	78	9.8	85	17.5
94	**Iprovalicarb**	106	9.0	104	6.0	104	5.4	101	8.8	101	10.4	93	7.8
95	*Kresoxim-methyl*	103	6.1	98	5.4	100	5.4	88	11.7	88	7.9	90	13.7
96	*lambda-Cyhalothrin*	96	9.1	76	8.2	83	17.9	90	2.3	83	6.3	99	17.5
97	**Linuron**	106	5.8	106	5.8	106	6.8	103	8.2	103	12.3	102	7.8
98	**Lufenuron**	111	26.8	98	18.4	115	12.9	87	19.2	92	18.6	94	16.6
99	**Malathion**	98	11.1	105	3.5	106	8.1	95	19.1	104	10.8	96	6.3
100	*Mepanipyrim*	100	3.4	98	4.8	99	3.4	90	8.6	88	7.6	87	13.0

No.	Pesticide	Lettuce						Orange					
		0.005 mg/kg		0.01 mg/kg		0.02 mg/kg		0.005 mg/kg		0.01 mg/kg		0.02 mg/kg	
		Rec. (%)	RSD_r (%)	Rec. (%)	RSD_r (%)	Rec. (%)	RSD_r (%)	Rec. (%)	RSD_r (%)	Rec. (%)	RSD_r (%)	Rec. (%)	RSD_r (%)
101	*Metalaxyl*	100	3.1	97	5.6	101	4.1	85	9.9	86	8.3	83	13.4
102	**Methamidophos**	88	7.4	81	4.5	81	2.3	64	26.4	64	18.2	59	23.4
103	*Methidathion*	100	13.5	97	6.4	98	10.5	91	13.2	95	13.0	92	22.8
104	**Methiocarb**	107	2.8	102	7.0	101	3.9	107	11.2	110	9.6	101	5.7
105	**Methiocarb sulfone**	107	3.5	107	5.5	109	3.8	110	12.6	111	7.5	108	6.4
106	**Methiocarb sulfoxide**	107	6.6	109	2.6	108	2.3	106	10.6	107	8.2	105	8.0
107	*Metrafenone*	98	3.3	91	18.5	94	15.4	88	2.6	82	8.8	98	15.8
108	**Monocrotophos**	108	4.5	105	2.6	103	6.2	98	13.0	105	14.3	93	11.7
109	**Myclobutanil**	100	10.4	105	7.4	104	9.1	97	10.9	96	13.2	93	2.9
110	**Nitenpyram**	66	6.6	71	5.6	66	2.5	44	15.8	45	16.7	37	16.8
111	**Omethoate**	105	5.7	99	4.6	98	2.8	90	12.4	86	14.1	77	14.9
112	*Oxadixyl*	99	3.8	96	5.0	97	7.5	75	11.8	76	13.1	73	15.4
113	**Oxamyl-oxime**	108	7.2	103	8.5	102	4.9	97	14.3	97	15.2	89	11.1
114	**Oxydemeton-methyl**	106	5.9	104	3.7	103	3.6	93	13.7	96	17.5	82	16.3
115	*Parathion methyl*	94	12.8	80	16.8	79	18.2	97	10.4	84	12.0	99	21.9
116	**Penconazole**	107	8.3	110	4.4	106	6.0	101	9.4	97	11.8	101	8.4
117	**Pencycuron**	103	2.6	104	5.2	103	4.2	76	6.7	72	5.4	75	8.5
118	*Pendimethalin*	96	9.1	81	18.9	82	19.7	90	6.4	80	6.7	98	18.3
119	*Permethrin-cis*	97	7.7	88	17.7	93	15.2	92	2.8	85	8.7	99	14.8
120	*Permethrin-trans*	98	4.1	90	17.8	90	14.6	91	5.0	80	4.6	99	15.6
121	*Phenthoate*	103	10.0	97	7.5	99	7.6	93	8.6	95	9.1	89	17.5
122	**Phosalone**	110	2.4	105	8.3	104	4.1	104	7.4	99	3.6	104	8.1
123	**Picolinafen**	106	4.4	107	8.0	104	5.1	108	10.0	101	5.4	101	12.0
124	*Picoxystrobin*	102	4.7	99	4.6	100	4.1	84	10.2	93	7.8	91	13.7
125	*Piperonyl-butoxide*	101	4.8	97	5.3	98	4.3	90	8.3	93	6.7	90	13.8
126	*Pirimicarb*	99	4.7	95	5.3	97	4.4	78	8.8	77	10.6	72	16.7
127	*Pirimicarb-desmethyl*	106	5.3	99	4.4	98	10.2	88	17.2	76	16.5	66	15.4
128	**Pirimiphos-methyl**	107	4.1	102	5.3	102	5.2	104	11.5	98	7.0	100	4.9
129	**Prochloraz**	109	5.3	108	6.0	106	6.0	99	6.3	94	5.9	96	7.2
130	*Procymidone*	98	6.8	90	18.2	94	16.3	86	9.6	79	11.9	99	19.1
131	**Profenofos**	108	3.1	106	5.9	103	3.9	105	7.9	103	4.5	104	9.0
132	**Propamocarb**	57	17.1	61	6.5	62	2.7	6	78.2	6	14.8	3	30.5
133	**Propargite**	112	12.2	101	6.4	102	8.2	110	9.4	111	8.5	109	7.2
134	**Propiconazole**	105	8.1	101	10.4	106	4.5	104	7.9	103	5.0	102	8.6
135	**Propyzamide**	107	4.3	104	7.9	101	5.1	99	6.3	93	8.1	93	5.8
136	**Proquinazid**	107	2.8	107	4.5	102	5.1	105	14.7	102	6.4	97	10.5
137	*Prothiofos*	101	4.7	93	18.8	95	16.3	96	2.9	89	7.4	107	17.3
138	**Pymetrozine**	97	3.6	93	3.5	88	4.3	37	31.2	37	23.7	31	14.3
139	**Pyraclostrobin**	106	4.6	105	4.9	105	4.9	105	7.4	97	7.4	103	6.9
140	*Pyridaben*	102	5.6	95	5.5	96	4.7	91	11.0	93	5.5	89	11.6
141	*Pyrimethanil*	96	5.6	88	4.9	92	5.1	82	8.8	82	8.3	78	16.0
142	**Pyrimidafen**	106	3.5	105	6.6	102	5.3	100	10.0	100	6.8	97	8.4
143	*Pyriproxyfen*	101	4.2	96	5.9	98	4.3	91	8.6	95	6.7	91	16.4
144	*Quinoxyfen*	101	4.4	97	5.1	100	3.4	85	8.5	90	4.7	85	13.0
145	**Rotenone**	107	7.8	98	9.3	101	7.5	96	12.1	99	7.9	100	7.6
146	*Silthiofam*	97	5.1	92	5.8	93	5.4	86	8.8	90	5.4	87	14.1
147	**Spinetoram I**	100	3.2	101	4.8	99	2.5	100	10.6	100	9.4	91	5.8
148	**Spinetoram II**	101	3.7	101	4.5	96	6.9	98	13.0	98	10.7	92	12.1
149	**Spinosyn A**	104	3.6	105	5.7	102	2.1	100	14.5	95	7.0	91	7.8
150	**Spinosyn D**	100	4.4	100	4.0	99	2.5	97	14.7	97	6.2	92	5.8
151	*Spirodiclofen*	119	7.1	111	10.6	115	15.7	112	11.0	120	1.4	117	13.6
152	*Spiromesifen*	104	5.9	98	5.4	100	8.2	98	12.0	98	5.5	92	16.4
153	**Spirotetramat**	99	9.7	93	4.9	98	3.2	99	11.3	108	12.7	100	5.6

No.	Pesticide	Lettuce						Orange					
		0.005 mg/kg		0.01 mg/kg		0.02 mg/kg		0.005 mg/kg		0.01 mg/kg		0.02 mg/kg	
		Rec. (%)	RSD_r (%)	Rec. (%)	RSD_r (%)	Rec. (%)	RSD_r (%)	Rec. (%)	RSD_r (%)	Rec. (%)	RSD_r (%)	Rec. (%)	RSD_r (%)
154	**Spiroxamine**	90	5.6	90	7.6	92	4.0	98	7.8	96	8.9	93	12.0
155	*tau-Fluvalinat*	97	12.4	79	19.9	78	19.7	94	3.3	85	9.9	102	17.6
156	**Tebuconazole**	109	5.7	109	4.8	104	2.3	101	10.6	101	3.4	99	9.1
157	**Tebufenozide**	99	13.4	97	12.5	103	8.3	106	13.6	99	19.1	102	8.8
158	*Tebufenpyrad*	103	3.9	100	5.6	100	3.8	87	10.2	93	5.0	90	14.1
159	**Teflubenzuron**	102	5.3	103	6.5	104	3.1	106	11.9	97	6.2	91	6.5
160	**Tembotrione**	106	6.7	103	5.6	106	4.2	116	12.6	113	5.8	119	7.3
161	*Terbuthylazine*	96	5.4	77	10.1	91	15.5	86	4.3	79	8.3	97	17.2
162	*Tetraconazole*	104	4.6	99	5.2	101	4.2	81	10.2	84	8.1	82	14.5
163	*Tetradifon*	97	5.9	90	19.7	94	17.2	91	5.7	85	8.1	102	16.5
164	**Thiabendazole**	112	5.1	107	5.7	103	4.4	96	19.1	109	7.3	111	6.2
165	**Thiacloprid**	107	3.9	107	3.5	106	2.5	114	15.7	111	6.1	113	7.5
166	**Thiamethoxam**	108	2.9	104	5.9	101	5.5	104	11.8	108	15.0	94	12.8
167	**Tolclophos-Methyl**	111	11.5	104	6.9	107	4.7	100	13.3	96	6.6	101	4.2
168	**Tolylfluanid**	109	3.8	103	5.4	102	4.8	103	9.0	100	7.7	100	8.6
169	*Triadimefon*	105	5.9	100	4.9	102	4.7	85	9.2	88	8.3	87	14.6
170	*Triadimenol*	104	7.4	97	6.3	103	5.4	84	7.6	83	9.6	79	18.9
171	**Triazophos**	104	3.3	102	5.2	102	5.9	98	7.7	105	8.3	99	4.5
172	**Triazoxide**	96	4.9	94	5.7	94	10.9	103	14.7	109	10.6	97	10.2
173	**Trifloxystrobin**	108	2.9	107	5.4	104	4.3	108	8.6	100	5.6	104	7.6
174	**Triflumizole**	111	9.5	116	2.5	109	6.8	112	13.9	104	4.7	101	8.9
175	**Zoxamide**	110	5.8	107	5.3	105	7.1	104	11.3	99	8.4	99	11.2

In **bold**, pesticides analysed by LC-MS/MS
In *italic*, pesticides analysed by GC-MS/MS
Underlined, pesticides with recovery lower than 70%.

Table 4. Limits of quantification, linearity range, coefficient of determination and matrix effects for the selected pesticides and matrices studied. Negative values of matrix effects mean suppression of the detector signal, and positives values enhancement.

No.	Pesticide	Lettuce				Orange			
		LOQ (mg/kg)	Linearity range (mg/kg)	R^2 matrix	ME (%)	LOQ (mg/kg)	Linearity range (mg/kg)	R^2 matrix	ME (%)
1	*2-Phenylphenol*	0.005	0.0005-0.1	0.9971	10	0.005	0.0005-0.1	0.9995	6
2	**Acephate**	0.005	0.002-0.1	0.9963	3	0.005	0.001-0.1	0.9963	-1
3	**Acetamiprid**	0.005	0.0005-0.1	0.9999	-1	0.005	0.0005-0.1	0.9875	-2
4	*Acrinathrin*	0.005	0.0005-0.1	0.9982	37	0.005	0.0005-0.1	0.9969	52
5	**Ametoctradin**	0.005	0.0005-0.1	0.9998	-7	0.005	0.0005-0.1	0.9968	-10
6	**Aminopyralid**	n.d.	0.01-0.1	0.9785	-22	n.f.r.	0.002-0.1	0.9988	-31
7	**Amisulbrom**	0.005	0.0005-0.1	0.9999	4	0.005	0.0005-0.1	0.9997	-2
8	*Azinphos-methyl*	0.005	0.005-0.1	0.9929	302	n.f.r.	0.01-0.1	0.9907	561
9	**Azoxystrobin**	0.005	0.0005-0.1	0.9999	-1	0.005	0.0005-0.1	0.9997	-29
10	**Benthiavalicarb-Isopropyl (R)**	0.005	0.0005-0.1	0.9999	-6	0.005	0.0005-0.1	0.9996	-62
11	**Benthiavalicarb-Isoprpoyl (S)**	0.005	0.0005-0.1	0.9995	-3	0.005	0.0005-0.1	0.9996	-51
12	*Bifenazate*	0.005	0.0005-0.1	0.9906	-71	n.f.r.	0.0005-0.1	0.9967	-2
13	*Bifenthrin*	0.005	0.0005-0.1	0.9971	14	0.005	0.0005-0.1	0.9999	16
14	*Biphenyl*	0.005*	0.0005-0.1	0.9864	8	0.005	0.0005-0.1	0.9966	-15
15	*Bitertanol*	0.005	0.0005-0.1	0.9981	42	0.005	0.0005-0.1	0.9987	74
16	**Bixafen**	0.005	0.0005-0.1	0.9996	3	0.005	0.0005-0.1	0.9999	-7
17	*Boscalid*	0.005	0.0005-0.1	0.9966	5	0.005	0.0005-0.1	0.9997	17
18	*Bromopropylate*	0.005	0.0005-0.1	0.9985	28	0.005	0.0005-0.1	0.9998	49
19	**Bupirimate**	0.005	0.0005-0.1	0.9987	-5	0.005	0.0005-0.1	0.9994	-11
20	**Buprofezin**	0.005	0.0005-0.1	0.9999	2	0.005	0.0005-0.1	0.9983	-3
21	**Carbaryl**	0.005	0.002-0.1	0.9995	1	0.005	0.002-0.1	0.9901	-6
22	**Carbendazim**	0.005	0.0005-0.1	1.0000	1	0.005	0.0005-0.1	0.9616	-10
23	*Carbofuran*	0.005	0.0005-0.1	0.9963	17	0.005	0.001-0.1	0.9998	37
24	**Carbofuran, 3 Hydroxy**	0.005	0.0005-0.1	0.9998	1	0.005	0.001-0.1	0.9882	-5
25	*Chlorantraniliprole*	0.005	0.001-0.1	0.9963	-14	0.005	0.0005-0.1	0.9887	-8
26	*Chlorfenapyr*	0.005	0.001-0.1	0.9972	29	0.005	0.0005-0.1	0.9998	27
27	**Chlorfenvinphos**	0.005	0.0005-0.1	0.9997	1	0.005	0.0005-0.1	0.9998	-5
28	*Chlorothalonil*	0.005	0.001-0.1	0.9942	79	0.005	0.005-0.1	0.9939	140
29	*Chlorpropham*	0.005	0.0005-0.1	0.9985	8	0.005	0.0005-0.1	0.9999	4
30	*Chlorpyrifos-ethyl*	0.005	0.0005-0.1	0.9990	12	0.005	0.0005-0.1	0.9999	17
31	*Chlorpyrifos-methyl*	0.005	0.0005-0.1	0.9982	24	0.005	0.0005-0.1	0.9993	70
32	**Clofentezine**	0.005	0.0005-0.1	0.9994	-1	0.005	0.0005-0.1	0.9994	-10
33	**Clothianidin**	0.005	0.0005-0.1	0.9999	3	0.005	0.0005-0.1	0.9881	13
34	**Cyflufenamid**	0.005	0.0005-0.1	0.9991	4	0.005	0.0005-0.1	0.9999	-8
35	**Cyflumetofen**	0.005	0.0005-0.1	0.9996	8	0.005	0.0005-0.1	0.9994	-4
36	*Cypermethrin*	0.005	0.0005-0.1	0.9981	20	0.005	0.0005-0.1	0.9990	41
37	**Cyproconazole**	0.005	0.002-0.1	0.9988	-1	0.005	0.002-0.1	0.9992	-16
38	**Cyprodinil**	0.005	0.0005-0.1	0.9987	-1	0.005	0.0005-0.1	0.9971	-8
39	*Deltamethrin*	0.005	0.0005-0.1	0.9981	19	0.005	0.0005-0.1	0.9982	45
40	**Demeton-S-methyl sulphone**	0.005	0.0005-0.1	0.9999	0	0.005	0.0005-0.1	0.9997	-7
41	**Diazinon**	0.005	0.0005-0.1	0.9997	3	0.005	0.0005-0.1	1.0000	-7
42	*Dichlorvos*	0.005	0.0005-0.1	0.9792	10	0.005	0.0005-0.1	0.9944	-6
43	*Dicloran*	0.005	0.0005-0.1	0.9974	9	0.005	0.0005-0.1	0.9993	11
44	**Diethofencarb**	0.005	0.0005-0.1	0.9995	3	0.005	0.001-0.1	0.9989	-30
45	**Difenoconazole**	0.005	0.0005-0.1	0.9992	5	0.005	0.001-0.1	0.9993	-7
46	**Dimethoate**	0.005	0.0005-0.1	0.9998	2	0.005	0.0005-0.1	0.9865	-9
47	**Dimethomorph**	0.005	0.0005-0.1	0.9986	3	0.005	0.001-0.1	0.9998	-13

No.	Pesticide	Lettuce				Orange			
		LOQ (mg/kg)	Linearity range (mg/kg)	R^2 matrix	ME (%)	LOQ (mg/kg)	Linearity range (mg/kg)	R^2 matrix	ME (%)
48	*Diniconazole*	0.005	0.0005-0.1	0.9984	31	0.005	0.0005-0.1	0.9998	52
49	**Dinotefuran**	0.005	0.002-0.1	0.9995	-1	0.005	0.001-0.1	0.9927	7
50	*Diphenylamine*	0.005	0.0005-0.1	0.9937	2	0.005	0.0005-0.1	0.9986	-5
51	**Dodine**	0.005	0.002-0.1	0.9996	-3	0.005	0.002-0.1	0.9995	0
52	*Endosulfan-alpha*	0.005	0.0005-0.1	0.9971	14	0.005	0.0005-0.1	0.9993	-20
53	*Endosulfan-beta*	0.005	0.0005-0.1	0.9972	56	0.005	0.0005-0.1	0.9998	1
54	*Endosulfan-sulfate*	0.005	0.0005-0.1	0.9985	17	0.005	0.0005-0.1	0.9989	44
55	**EPN**	0.005	0.0005-0.1	0.9998	1	0.005	0.0005-0.1	0.9975	-5
56	**Epoxiconazole**	0.005	0.0005-0.1	0.9997	-2	0.005	0.001-0.1	0.9999	-12
57	**Ethion**	0.005	0.0005-0.1	0.9993	-1	0.005	0.0005-0.1	0.9981	-2
58	**Ethoprofos**	0.005	0.0005-0.1	0.9997	-2	0.005	0.001-0.1	0.9991	-5
59	*Etofenprox*	0.005	0.0005-0.1	0.9985	4	0.005	0.0005-0.1	0.9998	15
60	**Etoxazole**	0.005	0.0005-0.1	0.9997	-1	0.005	0.0005-0.1	0.9923	-1
61	*Famoxadone*	0.005	0.005-0.1	0.9716	515	0.005	0.0005-0.1	0.9995	62
62	**Fenamidone**	0.005	0.0005-0.1	0.9999	4	0.005	0.0005-0.1	0.9992	-13
63	**Fenarimol**	0.005	0.0005-0.1	0.9992	2	0.005	0.001-0.1	0.9973	-8
64	**Fenazaquin**	0.005	0.0005-0.1	0.9994	3	0.005	0.0005-0.1	0.9914	-6
65	**Fenbuconazole**	0.005	0.0005-0.1	0.9996	-6	0.005	0.0005-0.1	0.9985	4
66	*Fenhexamid*	0.005	0.0005-0.1	0.9987	127	0.005	0.0005-0.1	0.9996	80
67	*Fenitrothion*	0.005	0.0005-0.1	0.9971	41	0.005	0.0005-0.1	0.9972	152
68	**Fenoxycarb**	0.005	0.0005-0.1	0.9998	-2	0.005	0.0005-0.1	0.9989	-9
69	*Fenpropathrin*	0.005	0.0005-0.1	0.9982	11	0.005	0.0005-0.1	0.9998	18
70	*Fenpropimorph*	0.005	0.001-0.1	0.9975	13	0.005	0.0005-0.1	0.9996	-5
71	**Fenpyroximate**	0.005	0.0005-0.1	0.9995	0	0.005	0.002-0.1	0.9880	-12
72	**Fenthion**	0.005	0.0005-0.1	0.9989	1	0.005	0.001-0.1	0.9957	-7
73	**Fenthion sulfoxide**	0.005	0.0005-0.1	0.9997	0	0.005	0.001-0.1	0.9965	-1
74	*Fenvalerate*	0.005	0.0005-0.1	0.9979	14	0.005	0.0005-0.1	0.9980	36
75	*Fipronil*	0.005	0.0005-0.1	0.9985	137	0.005	0.0005-0.1	0.9998	15
76	**Fludioxonil**	0.005	0.001-0.1	0.9989	3	0.005	0.001-0.1	0.9978	-16
77	**Flufenoxuron**	0.005	0.0005-0.1	0.9997	1	0.005	0.0005-0.1	0.9922	4
78	*Fluopicolide*	0.005	0.0005-0.1	0.9990	23	0.005	0.0005-0.1	0.9997	18
79	**Fluopyram**	0.005	0.0005-0.1	0.9998	1	0.005	0.0005-0.1	0.9999	-11
80	*Flusilazole*	0.005	0.0005-0.1	0.9988	10	0.005	0.0005-0.1	0.9996	5
81	**Flutolanil**	0.005	0.0005-0.1	0.9997	4	0.005	0.0005-0.1	0.9999	-36
82	**Flutriafol**	0.005	0.0005-0.1	1.0000	-38	0.005	0.0005-0.1	0.9950	-21
83	**Fluxapyroxad**	0.005	0.0005-0.1	0.9998	-3	0.005	0.0005-0.1	0.9999	-65
84	**Furathiocarb**	0.005	0.0005-0.1	0.9998	0	0.005	0.0005-0.1	0.9976	-3
85	**Halofenozide**	0.005	0.002-0.1	0.9993	0	0.005	0.001-0.1	0.9999	-5
86	**Haloxyfop**	0.005	0.001-0.1	0.9980	3	0.005	0.0005-0.1	0.9951	5
87	**Hexaconazole**	0.005	0.0005-0.1	0.9997	3	0.005	0.0005-0.1	0.9990	2
88	**Hexythiazox**	0.005	0.0005-0.1	0.9996	6	0.005	0.001-0.1	0.9944	2
89	**Imazalil**	0.005	0.0005-0.1	0.9998	1	0.005	0.0005-0.1	0.9993	-7
90	**Imazapic**	0.005	0.0005-0.1	0.9998	0	0.005	0.0005-0.1	0.9948	-4
91	**Imidacloprid**	0.005	0.0005-0.1	0.9997	3	0.005	0.0005-0.1	0.9902	28
92	**Indoxacarb**	0.005	0.0005-0.1	0.9994	2	0.005	0.0005-0.1	0.9989	-5
93	*Iprodione*	0.005	0.0005-0.1	0.9996	23	0.005	0.0005-0.1	0.9997	56
94	**Iprovalicarb**	0.005	0.0005-0.1	0.9999	0	0.005	0.002-0.1	0.9993	-33
95	*Kresoxim-methyl*	0.005	0.0005-0.1	0.9983	28	0.005	0.0005-0.1	0.9999	10
96	*lambda-Cyhalothrin*	0.005	0.0005-0.1	0.9972	16	0.005	0.0005-0.1	0.9987	37
97	**Linuron**	0.005	0.0005-0.1	0.9999	3	0.005	0.0005-0.1	0.9991	-12
98	**Lufenuron**	0.010	0.002-0.1	0.9971	2	0.005	0.002-0.1	0.9935	-1
99	**Malathion**	0.005	0.002-0.1	0.9992	-5	0.005	0.0005-0.1	0.9984	-38

No.	Pesticide	Lettuce				Orange			
		LOQ (mg/kg)	Linearity range (mg/kg)	R^2 matrix	ME (%)	LOQ (mg/kg)	Linearity range (mg/kg)	R^2 matrix	ME (%)
100	*Mepanipyrim*	0.005	0.0005-0.1	0.9983	28	0.005	0.005-0.1	0.9991	10
101	*Metalaxyl*	0.005	0.0005-0.1	0.9998	6	0.005	0.0005-0.1	0.9999	2
102	**Methamidophos**	0.005	0.0005-0.1	0.9998	-2	n.f.r.	0.0005-0.1	0.9997	-1
103	*Methidathion*	0.005	0.0005-0.1	0.9961	81	0.005	0.0005-0.1	0.9983	170
104	**Methiocarb**	0.005	0.0005-0.1	0.9997	-5	0.005	0.0005-0.1	0.9998	-8
105	**Methiocarb sulfone**	0.005	0.0005-0.1	1.0000	-3	0.005	0.0005-0.1	0.9748	-9
106	**Methiocarb sulfoxide**	0.005	0.0005-0.1	1.0000	-2	0.005	0.0005-0.1	0.9878	-7
107	*Metrafenone*	0.005	0.0005-0.1	0.9942	9	0.005	0.0005-0.1	0.9998	11
108	**Monocrotophos**	0.005	0.0005-0.1	0.9999	0	0.005	0.0005-0.1	0.9968	-6
109	**Myclobutanil**	0.005	0.0005-0.1	0.9982	11	0.005	0.0005-0.1	0.9999	-54
110	**Nitenpyram**	0.005*	0.001-0.1	0.9998	-1	0.005*	0.001-0.1	0.9988	6
111	**Omethoate**	0.005	0.0005-0.1	0.9993	2	0.005	0.0005-0.1	0.9929	2
112	*Oxadixyl*	0.005	0.0005-0.1	0.9972	10	0.005	0.0005-0.1	0.9991	-15
113	**Oxamyl-oxime**	0.005	0.001-0.1	0.9999	-4	0.005	0.0005-0.1	0.9908	7
114	**Oxydemeton-methyl**	0.005	0.0005-0.1	0.9997	0	0.005	0.0005-0.1	0.9997	-7
115	*Parathion methyl*	0.005	0.0005-0.1	0.9985	52	0.005	0.0005-0.1	0.9967	170
116	**Penconazole**	0.005	0.0005-0.1	0.9997	-1	0.005	0.0005-0.1	0.9999	-14
117	**Pencycuron**	0.005	0.0005-0.1	0.9997	-3	0.005	0.0005-0.1	0.9999	-5
118	*Pendimethalin*	0.005	0.0005-0.1	0.9987	26	0.005	0.0005-0.1	0.9984	31
119	*Permethrin-cis*	0.005	0.0005-0.1	0.9980	16	0.005	0.0005-0.1	0.9999	14
120	*Permethrin-trans*	0.005	0.0005-0.1	0.9985	15	0.005	0.0005-0.1	0.9998	15
121	*Phenthoate*	0.005	0.0005-0.1	0.9972	68	0.005	0.0005-0.1	0.9997	60
122	**Phosalone**	0.005	0.0005-0.1	0.9996	0	0.005	0.0005-0.1	0.9995	-10
123	**Picolinafen**	0.005	0.0005-0.1	0.9994	4	0.005	0.001-0.1	0.9939	1
124	*Picoxystrobin*	0.005	0.0005-0.1	0.9987	18	0.005	0.0005-0.1	0.9999	11
125	*Piperonyl-butoxide*	0.005	0.0005-0.1	0.9977	21	0.005	0.0005-0.1	0.9995	22
126	*Pirimicarb*	0.005	0.0005-0.1	0.9984	2	0.005	0.0005-0.1	0.9995	4
127	*Pirimicarb-desmethyl*	0.005	0.0005-0.1	0.9951	6	0.005	0.0005-0.1	0.9839	-15
128	**Pirimiphos-methyl**	0.005	0.0005-0.1	0.9992	0	0.005	0.0005-0.1	0.9995	-3
129	**Prochloraz**	0.005	0.0005-0.1	0.9995	-3	0.005	0.001-0.1	0.9996	-17
130	*Procymidone*	0.005	0.0005-0.1	0.9999	9	0.005	0.0005-0.1	0.9997	1
131	**Profenofos**	0.005	0.0005-0.1	0.9999	2	0.005	0.0005-0.1	0.9994	-3
132	**Propamocarb**	0.005*	0.002-0.1	0.9996	-8	n.f.r.	0.0005-0.1	0.9951	-10
133	**Propargite**	0.005	0.0005-0.1	0.9991	-5	0.005	0.001-0.1	0.9985	0
134	**Propiconazole**	0.005	0.0005-0.1	0.9998	4	0.005	0.002-0.1	0.9979	-3
135	**Propyzamide**	0.005	0.0005-0.1	0.9999	1	0.005	0.0005-0.1	0.9997	-62
136	**Proquinazid**	0.005	0.0005-0.1	0.9999	-3	0.005	0.0005-0.1	0.9842	3
137	*Prothiofos*	0.005	0.0005-0.1	0.9985	17	0.005	0.0005-0.1	0.9998	26
138	**Pymetrozine**	0.005	0.0005-0.1	0.9998	-2	n.f.r.	0.0005-0.1	0.9993	3
139	**Pyraclostrobin**	0.005	0.0005-0.1	0.9998	1	0.005	0.0005-0.1	0.9997	-7
140	*Pyridaben*	0.005	0.0005-0.1	0.9981	23	0.005	0.0005-0.1	0.9992	21
141	*Pyrimethanil*	0.005	0.0005-0.1	0.9989	6	0.005	0.001-0.1	0.9996	3
142	**Pyrimidafen**	0.005	0.0005-0.1	0.9998	1	0.005	0.0005-0.1	0.9976	-4
143	*Pyriproxyfen*	0.005	0.0005-0.1	0.9943	8	0.005	0.0005-0.1	0.9998	19
144	*Quinoxyfen*	0.005	0.0005-0.1	0.9992	19	0.005	0.0005-0.1	0.9996	9
145	**Rotenone**	0.005	0.0005-0.1	0.9998	9	0.005	0.0005-0.1	0.9993	6
146	*Silthiofam*	0.005	0.0005-0.1	0.9996	8	0.005	0.0005-0.1	0.9999	6
147	**Spinetoram I**	0.005	0.002-0.1	0.9997	-2	0.005	0.001-0.1	0.9813	-10
148	**Spinetoram II**	0.005	0.005-0.1	0.9991	-2	0.005	0.005-0.1	0.9791	-5
149	**Spinosyn A**	0.005	0.0005-0.1	0.9999	-2	0.005	0.0005-0.1	0.9879	-4
150	**Spinosyn D**	0.005	0.001-0.1	0.9998	-1	0.005	0.001-0.1	0.9807	-6
151	*Spirodiclofen*	0.005	0.0005-0.1	0.9977	16	0.005	0.0005-0.1	0.9992	17

No.	Pesticide	Lettuce				Orange			
		LOQ (mg/kg)	Linearity range (mg/kg)	R^2 matrix	ME (%)	LOQ (mg/kg)	Linearity range (mg/kg)	R^2 matrix	ME (%)
152	*Spiromesifen*	0.005	0.0005-0.1	0.9994	30	0.005	0.0005-0.1	0.9999	12
153	**Spirotetramat**	0.005	0.0005-0.1	0.9989	5	0.005	0.001-0.1	0.9998	6
154	**Spiroxamine**	0.005	0.0005-0.1	0.9978	6	0.005	0.0005-0.1	0.9968	-4
155	*tau-Fluvalinate*	0.005	0.0005-0.1	0.9968	33	0.005	0.0005-0.1	0.9971	73
156	**Tebuconazole**	0.005	0.001-0.1	0.9997	3	0.005	0.001-0.1	0.9997	-10
157	**Tebufenozide**	0.005	0.001-0.1	0.9994	-1	0.005	0.001-0.1	0.9961	-12
158	*Tebufenpyrad*	0.005	0.0005-0.1	0.9982	8	0.005	0.0005-0.1	0.9998	11
159	**Teflubenzuron**	0.005	0.0005-0.1	0.9995	0	0.005	0.001-0.1	0.9964	-5
160	**Tembotrione**	0.005	0.0005-0.1	0.9994	2	0.005	0.0005-0.1	0.9852	-15
161	*Terbuthylazine*	0.005	0.0005-0.1	0.9995	3	0.005	0.0005-0.1	0.9996	3
162	*Tetraconazole*	0.005	0.0005-0.1	0.9995	17	0.005	0.0005-0.1	0.9998	5
163	*Tetradifon*	0.005	0.0005-0.1	0.9985	6	0.005	0.0005-0.1	0.9998	1
164	**Thiabendazole**	0.005	0.0005-0.1	0.9996	1	0.005	0.0005-0.1	0.9897	-10
165	**Thiacloprid**	0.005	0.0005-0.1	0.9999	0	0.005	0.0005-0.1	0.9636	-16
166	**Thiamethoxam**	0.005	0.0005-0.1	0.9998	4	0.005	0.002-0.1	0.9996	6
167	**Tolclophos-Methyl**	0.005	0.0005-0.1	0.9999	0	0.005	0.0005-0.1	0.9982	-7
168	**Tolylfluanid**	0.005	0.001-0.1	0.9992	3	0.005	0.0005-0.1	0.9997	-6
169	*Triadimefon*	0.005	0.0005-0.1	0.9989	15	0.005	0.0005-0.1	0.9997	6
170	*Triadimenol*	0.005	0.0005-0.1	0.9986	17	0.005	0.0005-0.1	0.9998	27
171	**Triazophos**	0.005	0.0005-0.1	0.9997	-1	0.005	0.0005-0.1	0.9998	-30
172	**Triazoxide**	0.005	0.0005-0.1	0.9987	-5	0.005	0.0005-0.1	0.9938	-14
173	**Trifloxystrobin**	0.005	0.0005-0.1	0.9997	2	0.005	0.0005-0.1	0.9976	-4
174	**Triflumizole**	0.005	0.001-0.1	0.9993	-4	0.005	0.002-0.1	0.9930	-1
175	**Zoxamide**	0.005	0.0005-0.1	0.9998	-1	0.005	0.0005-0.1	0.9991	-5

In **bold**, pesticides analysed by LC-MS/MS
In *italic*, pesticides analysed by GC-MS/MS
* Lowest spike level detectable with good precision (RSD<20%), but recovery <70%
n.f.r.: not fulfilling requirements for quantitative method
n.d. : Not detectable

[APPLICATION NOTE]

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Single LC-MS/MS Method for Confirmation and Quantification of Over 400 Pesticides in a Complex Matrix Without Compromising Data Quality

Dimple Shah,[1] Eimear McCall,[2] and Gareth Cleland[1]
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[2]Waters Corporation, Wilmslow, UK

APPLICATION BENEFITS

- Single method for confirmation and quantification of a large number of pesticides in chili powder.
- The Xevo® TQ-S micro provides reliable, robust, and reproducible data at fast acquisition rates.
- Automated quantification of samples with Auto Addition and standard addition features.

WATERS SOLUTIONS

ACQUITY UPLC® H-Class System

Xevo TQ-S micro

ACQUITY UPLC BEH C_{18} Column

MassLynx® MS Software

DisQuE™ QuEChERS Dispersive Solid Phase Extraction

LC Multiresidue Pesticide Standards Kit

KEY WORDS

Pesticides, chili, QuECheRS, food safety

INTRODUCTION

Hundreds of compounds are routinely used for crop protection across the globe. With increasing global trade there is a requirement for rapid multi-residue screening and quantification methods to efficiently determine residue violations and protect consumers. Effective multi-residue methods rely on management of the acquisition of a large number of MRM transitions. Setting up overlapping MRM windows based around the retention time of each analyte ensures that no time is wasted acquiring other transitions for compounds that have yet to elute. This optimizes the time spent acquiring data to maximize sensitivity while ensuring a sufficient number of data points across peaks to give good precision. One of the objectives of this method was to implement relatively wide MRM windows without any loss in performance. This removes the need to make regular checks on retention time drift, and avoids having to make adjustments to the acquisition method before each analysis.

In this application note, we describe a single, fast method for confirmation and quantification of more than 425 pesticides. All pesticides were analyzed on a reverse phase column within 12 minutes. Two MRM transitions for each pesticide were monitored using both ESI positive and negative modes (deploying polarity switching). The excellent performance of the method has been demonstrated at very low concentrations in chili powder, which is a very complex matrix. Standard curves in solvent and matrix, easy-to-use instrument and software features to calculate the incurred residues in a chili sample, and data showing robustness after a large number of injections will be presented.

EXPERIMENTAL

UPLC conditions

LC system:	ACQUITY UPLC H-Class
Column:	ACQUITY BEH C_{18}, 2.1 x 100 mm, 1.7 µm
Column temp.:	45 °C
Injection volume:	1 µL
Flow rate:	0.45 mL/min
Mobile phase A:	10 mM Ammonium Acetate, pH 5, (P/N 186006693) in water
Mobile phase B:	10 mM Ammonium acetate in methanol
Weak needle wash	50/50 Water/methanol (v/v)
Strong needle wash:	90/10 Methanol/water (v/v)
Seal wash:	90/10 Water/methanol

Time (min)	Flow rate (mL/min)	%A	%B	Curve
Initial	0.450	98	2	6
0.25	0.450	98	2	6
12.25	0.450	1	99	6
13.00	0.450	1	99	6
13.01	0.450	98	2	6
17.00	0.450	98	2	6

Table 1. UPLC method for pesticide analysis.

MS conditions

MS system:	Xevo TQ-S micro
Ionization mode:	ESI +/-
Capillary voltage:	1 kV(+) and 0.5 kV(-)
Desolvation temp.:	500 °C
Desolvation gas flow:	1000 L/hr
Source temp.:	150 °C
Acquisition:	Multiple Reaction Monitoring (MRM)

MS methods and data acquisition

Two MRM transitions for each pesticide were generated using QUANPEDIA,™ except for fipronil, where 1 MRM transition was used. Methods are shown in Table 2. The data were acquired using MassLynx Software, and processed using TargetLynx XS Application Manager.

Method	MRM transitions	Dwell time	Average peak width (sec) n=3	Average data points across peak n=3
A	16	Autodwell	4.5	10
B	859	Autodwell	4.5	10

Table 2. Methods A and B showing the number of MRM transitions and dwell times in the respective methods. Also listed are the average peak widths and data points across the peak of furalaxyl for three replicates of a chili sample spiked at 10 µg/kg.

Standards

Waters® LC Multiresidue Pesticide Standards Kit (P/N 186007574) was used to make a mix of standards. The standards kit has a collection of 204 compounds in 10 different ampoules at individual concentrations of 100 µg/mL. The stock solution of 10 µg/mL was prepared by combining 100 µL from each ampoule. The working standards were further diluted with acetonitrile.

For the solvent calibration curve and standard addition work, acetonitrile was spiked with 204 pesticides at 1, 5, 10, 25, 50, 100, 250, 500, and 1000 µg/kg (ppb) (concentrations equate to sample).

Sample preparation

The sample preparation followed the protocol described in a previous application note.[1] A DisQuE QuEChERS (CEN method 15662)[2] was used to prepare all samples. Briefly, two grams of chili powder was weighed into a centrifuge tube. The sample was mixed with 8 mL of water and vortexed for 30 seconds. The mixture was extracted with 10 mL of acetonitrile followed by the addition of QuEChERS CEN material (4 g MgSO4, 1 g NaCl and 1.5 g sodium citrate). The resulting mixture was shaken for 1 minute. The tube was then centrifuged at 4000 rpm for 5 minutes, and the supernatant was placed into vials for analysis.

For the matrix match spiked (MMS) calibration, a chili sample was spiked with 204 pesticides at the same level as the solvent calibration range. These spiked levels equaled the concentrations in the chili sample.

RESULTS AND DISCUSSION

The chili sample extract was analyzed for the presence of pesticides using an ACQUITY UPLC H-Class System coupled with the Xevo TQ-S micro Mass Spectrometer. Novel SpaceWire technology facilitates faster acquisition speeds with Xcellerate Ion Transfer (XIT™). To achieve the additional sensitivity, the instrument is integrated with StepWave™ Ion Guide Technology. StepWave effectively removes the neutral molecules, providing additional sensitivity, and improving robustness. Figure 1 shows the Xevo TQ-S micro, along with the StepWave ion guide.

In order to demonstrate the fast acquisition rate and excellent data quality of the MS instrument, a chili extract was post spiked with pesticides at 10 µg/kg (concentration equates to sample) and analyzed using Methods A and B, as described in Table 2. Method A contains 8 pesticides having two MRM transitions for each compound (16 MRMs total). Method B contains 430 pesticides (1 SRM, and 429 with two MRM transitions, totaling 859 MRMs). Both methods were enabled with AutoDwell functionality, at the click of a button. AutoDwell allows MassLynx MS Software to optimize the dwell time automatically for each compound depending on its retention time, as well as the peak width and required data points across the peak, as defined by the user. In this experiment, 1-minute wide acquisition windows were selected for both methods which eliminates the regular checks for retention time drift (due to matrix interferences) and simplifies inter- and/or intra- laboratory method transfer.

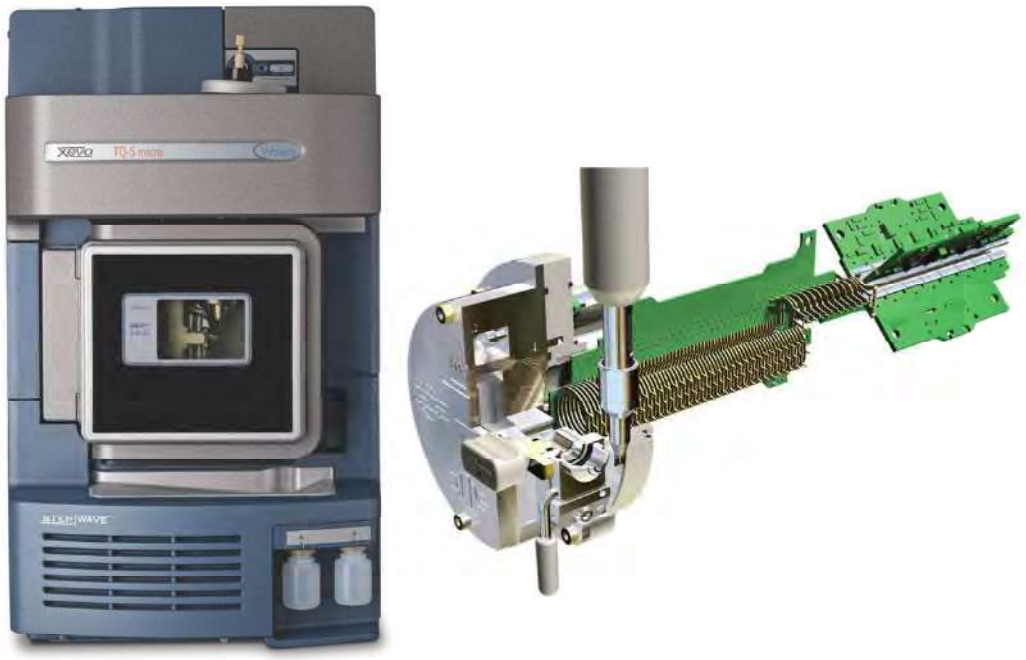

Figure 1. Xevo TQ-S micro (left) and StepWave ion guide diagram (right).

Figure 2 shows a screen shot of part of Method B. In this method, more than 100 pesticides (of the 430 being monitored) eluted between 9 and 10 minutes. Furalaxyl (RT 9.1 min) has eluted within this crowded region. As shown in Table 2, furalaxyl had an average peak width and data points across the peak of 4.4 sec and 10 data points respectively (Method B), for three replicates of the chili sample spiked at 10 µg/kg. Similar results were observed with Method A where fewer transitions (16) were monitored in the method. Despite the large number of compounds in Method B, the data quality was not compromised in the complex matrix.

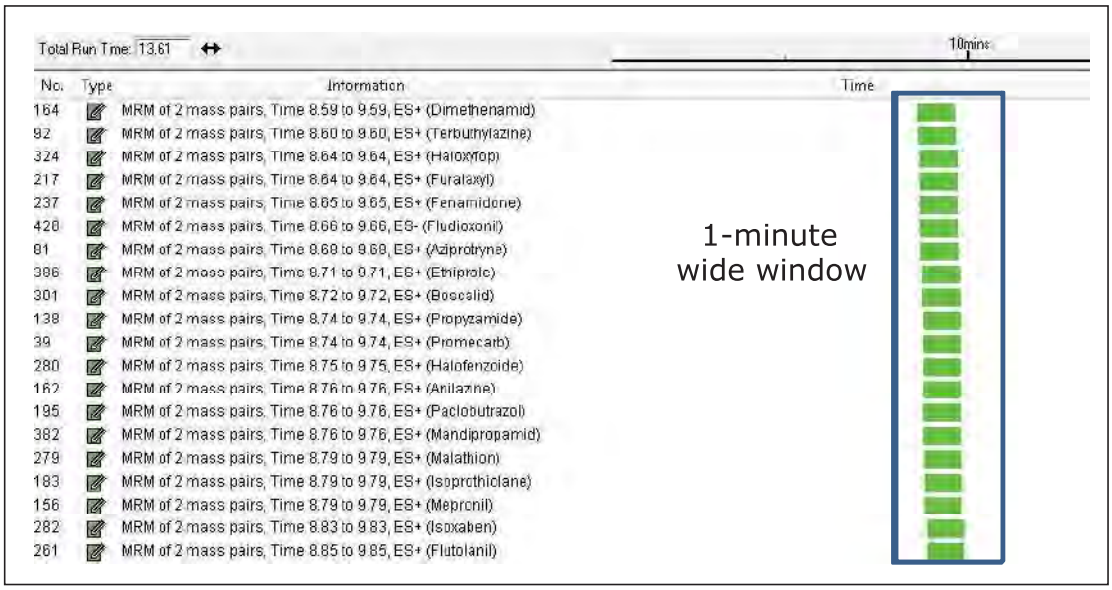

Figure 2. Screen capture of Method B.

Figure 3 shows chromatograms of furalaxyl acquired by Methods A and B. The peak area difference between Methods A and B which contained 16 and 859 MRM transitions respectively were minimal. Despite Method B having significantly more MRMs than Method A, both methods yielded similar area counts (<2% deviation), which is an acceptable deviation for injection of replicate matrix. Both methods have shown a minimum of 10 data points across the peak, a typical requisite for accurate quantification.

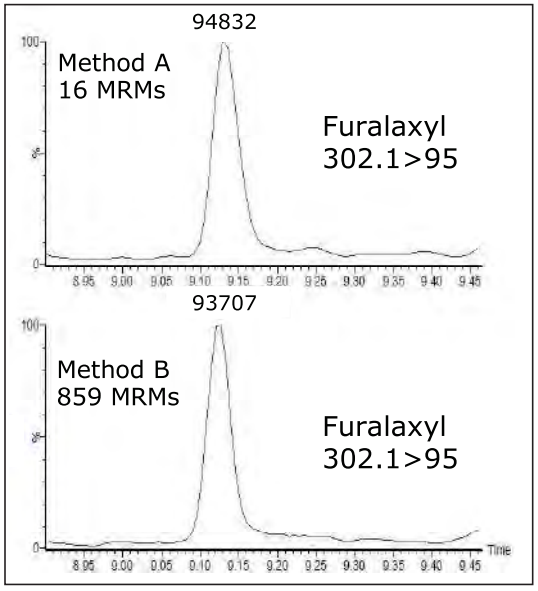

Figure 3. Chromatograms of furalaxyl showing peak area count (Methods A and B) in a spiked chili powder sample at 10 µg/kg.

Linearity

Linearity over the required working range was determined in solvent (data not shown) and matrix matched calibration curves using both MS methods. Figure 4 shows excellent agreement and linearity for matrix matched calibration curve of methoxyfenozide (RT 9.56 min) from 1 to 1000 µg/kg (ppb) using methods A and B. As can be seen in the Figure 4, Methods A and B showed good linearity with an r^2 value >0.999 over the entire specified range and similar slope of the equation.

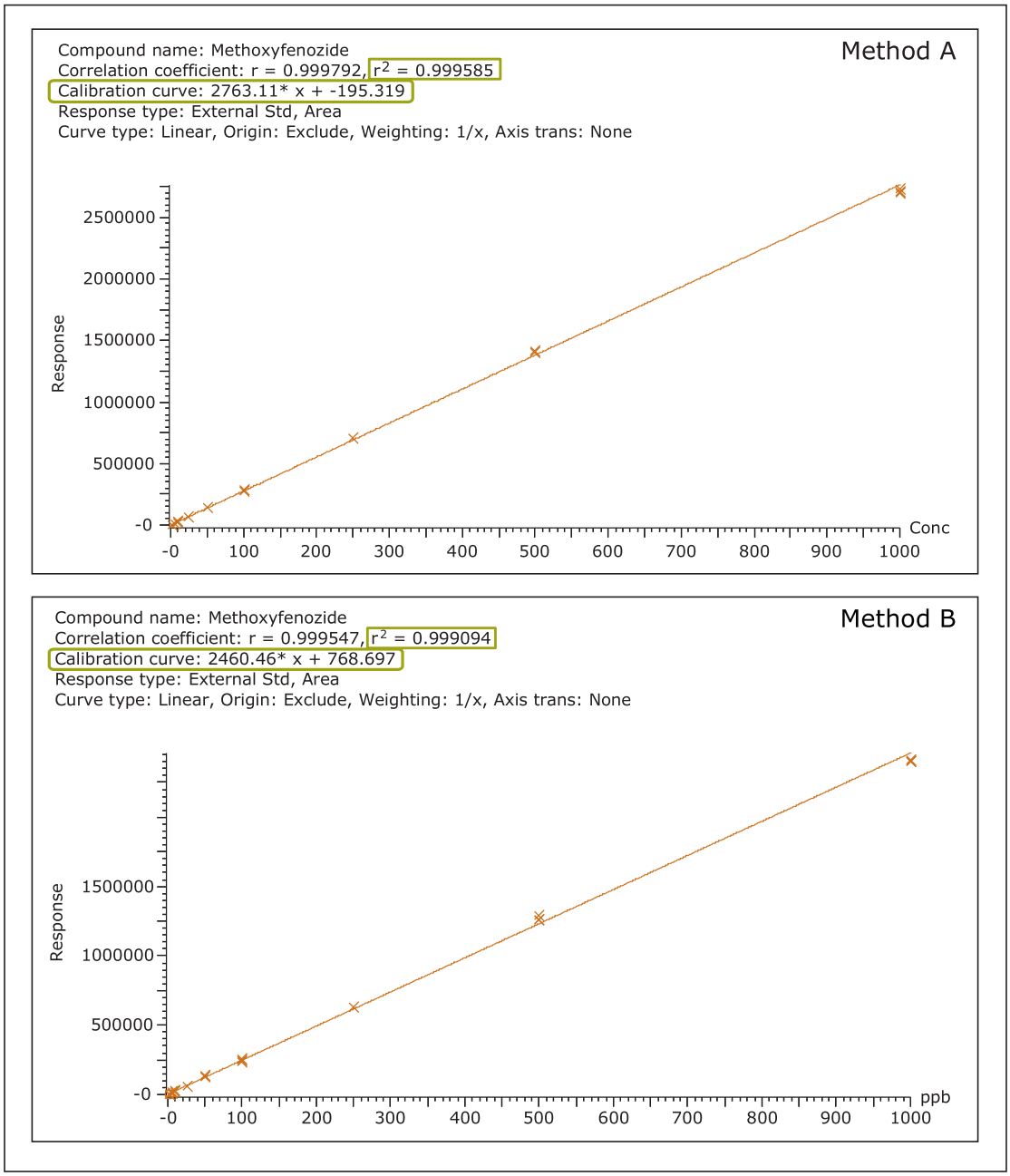

Figure 4. Matrix match spiked calibration curves of methoxyfenozide using Methods A and B.

Robustness study

To assess the repeatability and robustness of the method, 300 injections of the spiked chili sample (25 µg/kg) were analyzed by Method B. Figure 5 shows the %RSD of the peak area for example pesticide residues. As shown in Figure 5, all positive and negative ionized compound showed good %RSD (3.3 to 13.9) over 300 injections. Despite the large number of MRM transitions employing polarity switching, Method B showed excellent reproducibility and quantification at a lower concentration in a complex matrix like chili.

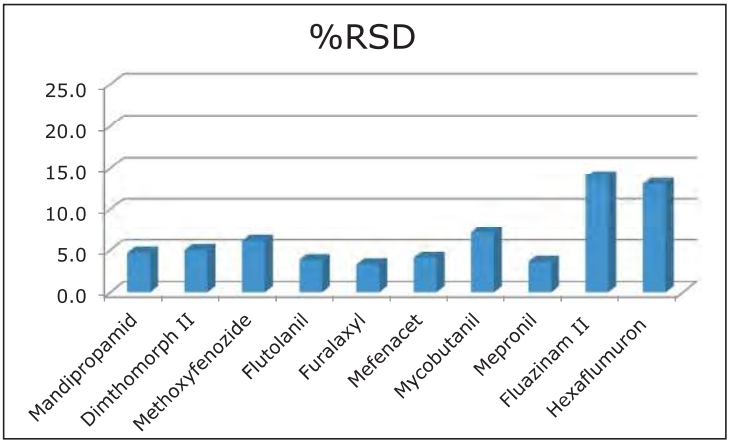

Figure 5. %RSD of 300 injections of the example pesticides spiked in chill sample at 25 µg/kg and analyzed by Method B.

Standard addition using the Auto Addition feature

A few incurred residues were found when the chili sample was screened with Method B. The standard addition method was employed to calculate the concentration of incurred residues in the chili sample. Matrix matched samples were prepared using the Auto Addition functionality of the UPLC® System, automatically enabling the repeatable mixing of multiple aliquots from several vials within a single injection. The extract was diluted directly in the sample loop before injection. In this study, Auto Addition provided an automated calibration curve for the chili sample by spiking blank chili extract with various concentration of pesticides. Figure 6 shows the Auto Addition setup sample list created in MassLynx Software.

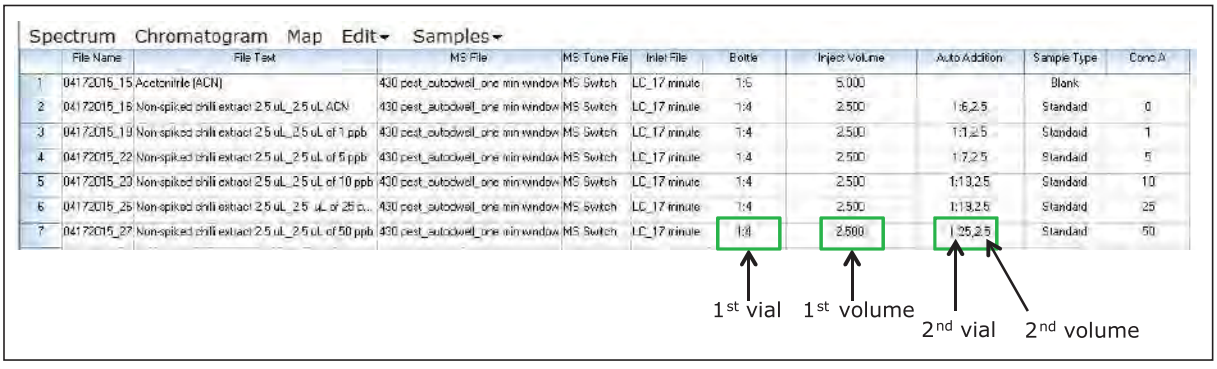

Figure 6. Sample list created in MassLynx showing Auto Addition setup.

As shown in Figure 6, vials 1:6 and 1:4 contain acetonitrile and a blank chili extract respectively. A mix of pesticide standards in acetonitrile, ranging from 1 to 50 µg/kg (ppb), were placed in individual vials (1:6 to 1:25). Figure 6 also shows the injection order that starts with drawing 2.5 µL of non-spiked extracted chili sample followed by 2.5 µL of acetonitrile. The rest of the injections start by drawing 2.5 µL of the non-spiked extracted chili sample and 2.5 µL of various concentrations of pesticide standards. In this instance, a 5-µL injection total volume was kept constant to ensure accurate and reproducible injections. It also maintains a constant amount of matrix to allow for accurate quantification.

After the data acquisition, incurred residues were then automatically quantified using the Standard Addition functionality in TargetLynx XS Software. Figure 7 shows an example of the calculated concentration of pyraclostrobin (12.35 ppb) in a chili sample using the standard addition approach.

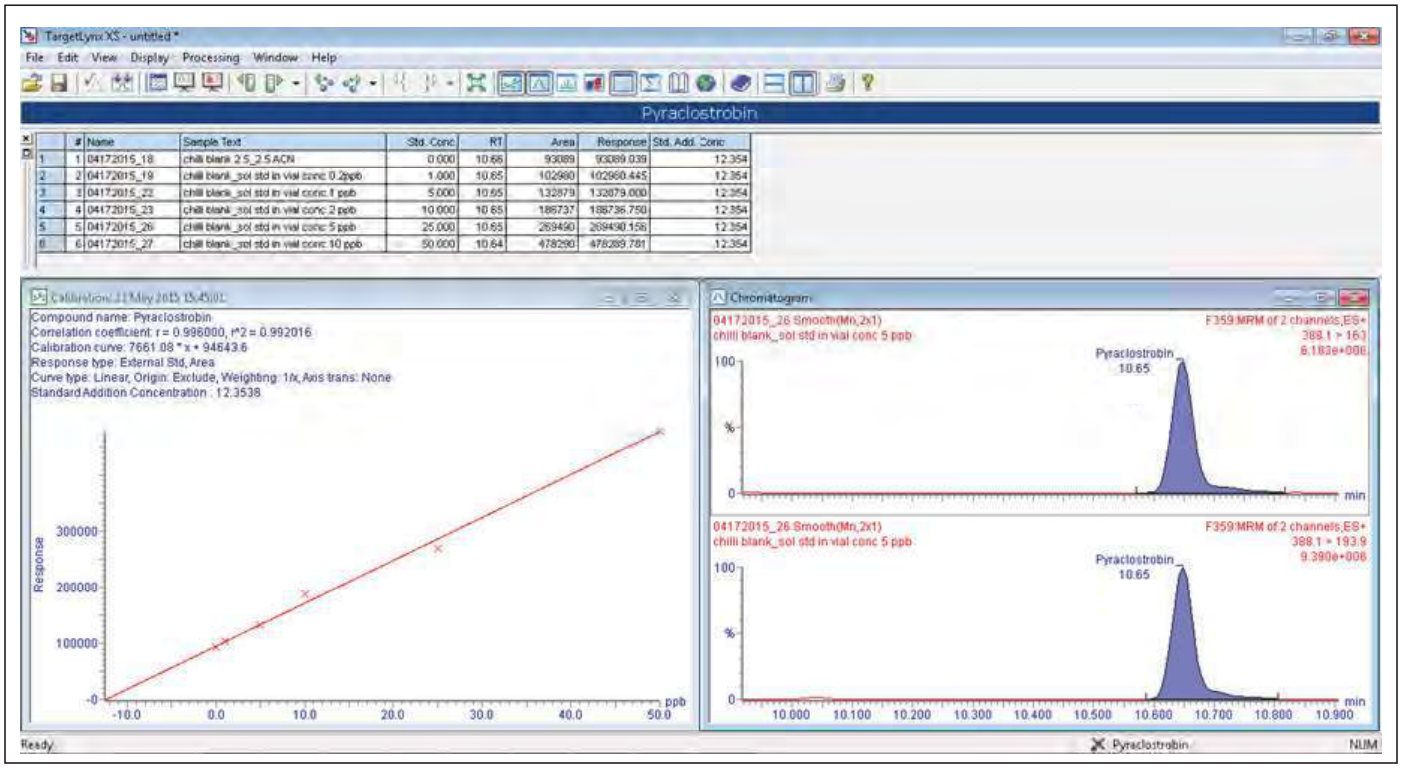

Figure 7. TargetLynx XS showing quantification of incurred pyraclostrobin in a chili sample by standard addition method.

CONCLUSIONS

- Waters LC Multi-Residue Pesticide Standards Kit is a quality assured collection of 204 compounds that eliminates the need for monotonous manual measurement of individual standards, thus saving the chemist time and laboratory resources.
- A large number of MRM transitions with a one-minute wide acquisition window in Method B eliminates the manual retention time check process and allows for easy transfer of methods between laboratories.
- The fast scanning speed of the Xevo TQ-S micro provides enough data points across the peak for accurate quantification within high volume multi-residue analysis.
- A combination of the Auto Addition and standard addition features facilitates automated quantification of incurred residues which reduces labor and the need for a blank sample.
- Excellent linearity, robustness, and sensitivity were achieved in a complex matrix such as chili powder.

References

1. D Shah, E Goh, J Burgess. Rapid analysis of Sudan and other prohibited dyes in chili powder using the ACQUITY UPLC H-Class System with Xevo TQD. Waters application note no. 720004975en. March, 2014.
2. DisQuE Dispersive Sample Preparation brochure no. 720003048en. July, 2015.

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Amendment of Solicitation

Date of Issuance: 10/3/24 **Solicitation No.** EV00000549

Requisition No. NA **Amendment No.** 1

Hour and date specified for receipt of offers is changed: ☒ No ☐ Yes, to: _____ CST

Pursuant to OAC 260:115-7-30(d), this document shall serve as official notice of amendment to the solicitation identified above. Such notice is being provided to all suppliers to which the original solicitation was sent.
Suppliers submitting bids or quotations shall acknowledge receipt of this solicitation amendment prior to the hour and date specified in the solicitation as follows:

(1) Sign and return a copy of this amendment with the solicitation response being submitted; or,
(2) If the supplier has already submitted a response, this acknowledgement must be signed and returned prior to the solicitation deadline. All amendment acknowledgements submitted separately shall have the solicitation number and bid opening date printed clearly in the subject line of the email.

RETURN TO: Supplier Portal (oklahoma.gov)

Richard Williams
Contracting Officer

Richard.Williams@omes.ok.gov
E-Mail Address

Description of Amendment:

a. This is to incorporate the following:

On behalf of the State of Oklahoma, the Office of Management and Enterprise Services (OMES) gives notice of the following questions concerning this solicitation, received during the Q&A period. All questions and procurement/agency responses are detailed below:

Q.1. Why is a capillary triple quadrupole detector required if other types could meet required specifications?

A.1. Capillary triple quadrupole detector is required to be compatible with our current methods and workflow.

Q.2. Is an upper mass range of 1250 acceptable? Or 2000 acceptable?

A.2. 3000 upper mass range is required to be compatible with current methods and capabilities.

Q.3. What application workflow would you need 10,000 MRMs? Is 4000 Acceptable?

A.3. We can evaluate the capabilities of submitted instrument to our capabilities.

Q.4. 1.6.8.2. Mass range: 5 u to 3000 u –one of the requirement of mass range 5m/z -3000m/z is sole source , there are no small molecule that are above the mass range of 2040 , and when molecules become larger, it have multiple charged state, that allows you to capture under 2040m/z, we have not seen compounds with that large m/z monitored, please let us know if this is acceptable.

A.4. We can evaluate the capabilities of submitted instrument to our capabilities.

Q.5. 1.2.6 . The mobile phase composition precision from mixing must be no more than 0.20% RSD at 0.2 ml/min and 5 ml/min. Specification question 1.2.3 and 1.2.6 both had different flow rate range, monitoring pesticide in various metrics require high performance advantage of separation technique, , UPLC or UHPLC , has narrow peak , tall peak,

3 sec wide (flow rate 0.010-2ml/min, this ultimately results in better separation and higher s/n performance, were as HPLC have wider peak 30 second (0-10ml/min). We were wanting to know the need for high flow rate 8ml/ min?

A.5. High flow rate is sometimes used in older analytical methods, to be compatible with current capabilities.

b. All other terms and conditions remain unchanged.

Waters Technologies Corporation		October 21, 2024
Supplier Company Name (**PRINT**)		Date
Timothy D'Souza	Vice President Americas Field Operations	DocuSigned by: Timothy D'Souza
Authorized Representative Name (**PRINT**)	Title	Authorized Representative Signature