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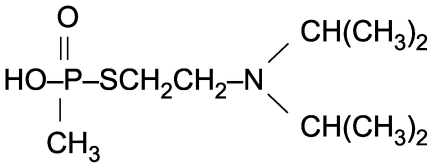
United States
Environmental Protection
Agency

Adaptation of the Conditions of U.S. EPA Method 538 for the Analysis of a Toxic Degradation Product of Nerve Agent VX (EA2192) in Water by Direct Aqueous Injection- Liquid Chromatography/Tandem Mass Spectrometry

FINAL REPORT



S-(2-Diisopropylaminoethyl)
methyl phosphonothioate
(EA 2192)

Office of Research and Development
National Homeland Security Research Center

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Office of Research and Development
National Homeland Security Research Center
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DISCLAIMER

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EXECUTIVE SUMMARY

The objective of this study was to evaluate U.S. EPA's Method 538 for the assessment of drinking water exposure to the nerve agent degradation product, EA2192, the most toxic degradation product of nerve agent VX. As a result of the similarities in sample preparation and analysis that Method 538 uses for nonvolatile chemicals, this method is applicable to the nonvolatile chemical warfare agent (CWA) degradation product, EA2192, in drinking water. The method might be applicable to other nonvolatile CWAs and their respective degradation products as well, but the method will need extensive testing to verify compatibility. Gaps associated with the need for analysis methods applicable to such analytes were addressed by adapting the EPA 538 method for this CWA degradation product. Many laboratories have the experience and capability to run the already rigorous method for nonvolatile compounds in drinking water. Increasing the number of laboratories capable of carrying out these methods serves to significantly increase the surge laboratory capacity to address sample throughput during a large exposure event. The approach desired for this study was to start with a proven high performance liquid chromatography tandem mass spectrometry (HPLC/MS/MS) method for nonvolatile chemicals in drinking water and assess the inclusion of a similar nonvolatile chemical, EA2192. Two analytes that are currently in Method 538, methamidophos and acephate, were used as reference standards to determine method acceptability. Methamidophos-d6 was used as an internal standard.

An HPLC/MS/MS assay for the quantitation of EA2192 in deionized (DI) water was evaluated in a series of studies reported here. DI water samples fortified with EA2192 were analyzed following Method 538 procedures. The samples were analyzed on an Applied Biosystems API-4000 Mass Spectrometer, coupled with a Shimadzu Liquid Chromatography system. The objectives and procedures used for sample preparation and analysis are described in EPA Method 538. The only modification to Method 538 was the inclusion of a flow diversion valve to reduce source contamination.

The method accuracy, precision, reproducibility, linearity, detection limit and quantitation limit for EA2192 in DI water were evaluated and found to be within the acceptance criteria of Method 538. Additionally, EA2192 was stable following 28 days at refrigerated temperatures (5 °C ± 3 °C) in all tested water types except chlorinated water.

The method was evaluated to determine if filtering water samples prior to analysis affected EA2192 concentrations. No loss of EA2192 was observed after filtering the spiked samples.

Preliminary method development was performed to determine if the current HPLC/MS/MS method could be transferred to ultra-high performance chromatography tandem mass spectrometry (UPLC/MS/MS). Modifying this method to incorporate UPLC analysis would drastically shorten the analytical run time from the current 30 minute method to 5 minutes or less. A method was developed for two of the analytes currently monitored in Method 538, methamidophos and acephate, along with EA2192. Methamidophos-d6 was used as an internal standard. Further method development efforts are required to determine the feasibility of transferring all Method 538 analytes to UPLC/MS/MS, followed by an Independent Demonstration of Capability to transfer the method.

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LIST OF ACRONYMS AND ABBREVIATIONS

ACC	% Accuracy
Acephate	N-(methoxy-methylsulfanylphosphonyl) acetamide
°C	degree(s) Celsius
CAL	Calibration Standard
CCC	Continuing Calibration Check
CWA	Chemical Warfare Agent
DI	Deionized (Water)
DAI-LC/MS/MS	Direct Aqueous Injection – Liquid Chromatography/Tandem Mass Spectrometry
DL	Detection Limit
EA2192	S,2-diisopropylaminoethyl methylphosphonothioic acid
EPA	Environmental Protection Agency
ESI	Electrospray Ionization
HPLC/MS/MS	High-Performance Liquid Chromatography/Tandem Mass Spectrometry
HR_{PIR}	Half Range for the Prediction Interval of Results
HSS	High Strength Silica
IDA	Initial Demonstration of Accuracy
IDC	Initial Demonstration of Capability
IDP	Initial Demonstration of Precision
IS	Internal Standard
IS PDS	Internal Standard Primary Dilution Standard
LC	Liquid Chromatography
LFB	Laboratory Fortified Blank
LFSM	Laboratory Fortified Sample Matrix
LFSMD	Laboratory Fortified Sample Matrix Duplicate
LRB	Laboratory Reagent Blank
MEOH PDS	Methanolic Analyte Primary Dilution Standard
Methamidophos	O,S-dimethyl phosphoramidothioate
MRL	Minimum Reporting Level
MRM	Multiple Reaction Monitoring
ms	millisecond(s)
NA	Not Applicable
PDS	Primary Dilution Standard
PIR	Prediction Interval of Result
PPE	Personal Protective Equipment
psi	Pounds per Square Inch
QC	Quality Control
QCS	Quality Control Sample
r	Correlation Coefficient
RE	Relative Error
RSD	Relative Standard Deviation
RT	Retention Time
SD	Standard Deviation
SDS	Safety Data Sheet

SOP	Standard Operating Procedure
SSS	Stock Standard Solution
TOC	Total Organic Carbon
UPLC/MS/MS	Ultra High Performance Chromatography Tandem Mass Spectrometry
V	Voltage
VX	O-ethyl S-[2-ethyl] methylphosphonothioate
WATER PDS	Aqueous Analyte Primary Dilution Standard

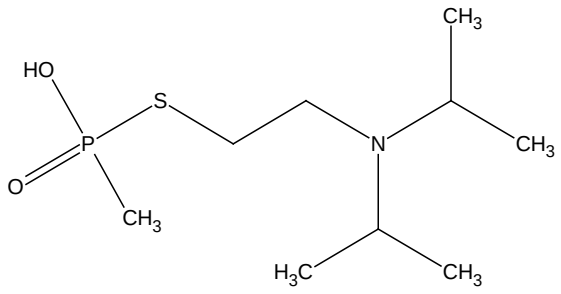
1. INTRODUCTION

1.1 The U.S. Environmental Protection Agency's (EPA's) Method 538 is a direct aqueous injection-liquid chromatography/tandem mass spectrometry (DAI-LC/MS/MS) method for the determination of selected nonvolatile chemical contaminants in drinking water. The purpose of this study was to evaluate EPA Method 538 for its applicability to the assessment of nerve agent degradation exposure by analyzing S,2-diisopropylaminoethyl methylphosphonothioic acid (EA2192), a degradation product of O-ethyl S-[2-ethyl] methylphosphonothioate (VX). EA2192 was evaluated following the criteria outlined in U.S. EPA Method 538 across a concentration range of 0.05-20 µg/L (See the Attachment 20.1 for U.S. EPA Method 538). The sample preparation, analysis, and quantitation were performed according to Method 538. The method accuracy, precision, reproducibility, linearity, and quantitation limits in deionized (DI) water were evaluated. Holding time studies in a variety of water types were evaluated for 28 days. Two chemicals that are currently included in Method 538, methamidophos and acephate, were included in the analysis as reference standards to verify method functionality. Methamidophos-d6 was used as the internal standard. EPA's Method 538 conditions can be used to analyze for EA2192.

2. SCOPE AND APPLICATION

2.1 The scope of this study was to determine if EA2192, a degradation product of VX, could be analyzed under similar conditions as reported in Method 538. Method 538 was evaluated for accuracy, precision, reproducibility, linearity, and quantitation limits for EA2192 in water. (See Table 1 for a summary of results.) The detection limit for EA2192 is 0.0130 µg/L. Holding time studies in DI water and drinking water were evaluated for a period of 28 days. Additionally, water samples were tested, representing a variety of water types (chlorinated, chloraminated, hard water, etc.), to determine the stability of EA2192. Methamidophos and acephate, compounds currently included in Method 538, were included in the testing as reference standards. Methamidophos-d6 was used as the internal standard. The following analyte was tested:

Chemical Name:	S-[2-(diisopropylamino) ethyl] methylphosphonic acid
Code Name:	EA2192
Empirical Formula:	$C_9H_{22}NO_2PS$
Lot Number:	NA
Purity:	94.2 % by NMR (Appendix C)
Storage Conditions:	2-8 °C
Structure:	



3. SUMMARY OF METHOD

3.1 A 40-mL water sample was collected in a bottle containing sodium omadine (antimicrobial agent) and ammonium acetate (to bind free chlorine in sample). An aliquot of the sample was placed in an autosampler vial with the internal standard added. A 50-µL injection was made into an LC equipped with a C18 column interfaced to an MS/MS operated in the electrospray ionization (ESI) mode. The analytes were separated and identified by comparing the acquired mass spectra and retention times to reference spectra and retention times for calibration standards acquired under identical LC/MS/MS conditions. The concentration of each analyte was determined by internal standard calibration using procedural standards.

4. DEFINITIONS

4.1 CALIBRATION STANDARD (CAL) – A solution prepared from the primary dilution standard solution and/or stock standard solution and the internal standard. The CAL solutions are used to calibrate the instrument response with respect to analyte concentration.

4.2 CONTINUING CALIBRATION CHECK (CCC) – A calibration standard containing the method analytes and internal standard. The CCC is analyzed periodically to verify the accuracy of the existing calibration for those analytes.

4.3 DETECTION LIMIT (DL) – The minimum concentration of an analyte that can be identified, measured, and reported with 99 % confidence that the analyte concentration is greater than zero. The DL is a statistical determination of precision and accurate quantitation is not expected at this level.

4.4 INTERNAL STANDARD (IS) – A pure chemical dissolved in a standard solution in a known amount and used to measure the relative response of other method analytes that are components of the same solution. The internal standard should be a chemical that is structurally similar to the method analytes, has no potential to be present in water samples, and is not a method analyte.

4.5 LABORATORY FORTIFIED BLANK (LFB) – A volume of reagent water or other blank matrix to which known quantities of the method analytes and all the preservation reagents are added in the laboratory. The LFB is analyzed exactly like a sample, and its purpose is to determine whether the methodology is in control, and whether the laboratory is capable of making accurate and precise measurements.

4.6 LABORATORY FORTIFIED SAMPLE MATRIX (LFSM) – A preserved field sample to which known quantities of the method analytes are added in the laboratory. The LFSM is processed and analyzed exactly like a sample, and its purpose is to determine whether the sample matrix contributes bias to the analytical results. The background concentrations of the analytes in the sample matrix must be

determined in a separate sample and the measured values in the LFSM corrected for background concentrations.

4.7 LABORATORY FORTIFIED SAMPLE MATRIX DUPLICATE (LFSMD) – A duplicate of the Field Sample used to prepare the LFSM. The LFSMD is fortified, and analyzed identically to the LFSM.

4.8 LABORATORY REAGENT BLANK (LRB) – An aliquot of reagent water or other blank matrix that is treated exactly as a sample including exposure to all glassware, equipment, solvents and reagents, sample preservatives, and internal standards that are used in the analysis batch. The LRB is used to determine if method analytes or other interferences are present in the laboratory environment, the reagents, or the apparatus.

4.9 MINIMUM REPORTING LEVEL (MRL) – The minimum concentration that can be reported as a quantitated value for a method analyte in a sample following analysis. This defined concentration can be no lower than the concentration of the lowest calibration standard for that analyte and can be used only if acceptable QC criteria for this standard are met.

4.10 PRIMARY DILUTION STANDARD SOLUTION – A solution containing the analytes prepared in the laboratory from stock standard solutions and diluted as needed to prepare calibration solutions and other needed analyte solutions.

4.11 QUALITY CONTROL SAMPLE (QCS) – A solution of method analytes of known concentrations that is obtained from a source external to the laboratory and different from the source of calibration standards. The QCS is used to check calibration standard integrity.

4.12 SAFETY DATA SHEET (SDS) – Written information provided by vendors concerning a chemical's toxicity, health hazards, physical properties, fire, and reactivity data including storage, spill and handling precautions.

4.13 STOCK STANDARD SOLUTION (SSS) – A concentrated solution containing one or more method analytes prepared in the laboratory using assayed reference materials or purchased from a reputable commercial source.

5. INTERFERENCES

5.1 Method interferences could be caused by contaminants in solvents, reagents (including reagent water), sample bottles and caps, and other laboratory supplies or hardware that lead to discrete artifacts and/or elevated baselines in the chromatograms. All items such as these were routinely demonstrated to be free from interferences (less than $^1/_3$ the DL) under the conditions of the analysis by analysis of an LRB. Subtracting blank values from sample results is not permitted.

5.2 Relatively large quantities of the preservatives were added to sample bottles. The potential existed for trace-level organic contaminants in these reagents. Interferences from these sources were monitored by analysis of LRBs.

6. HEALTH AND SAFETY

6.1 The toxicity or carcinogenicity of each reagent used in this method had not been defined precisely. Each chemical was treated as a potential health hazard and exposure to these chemicals was minimized through the proper use of personal protective equipment (PPE). A reference file of SDSs was made available to all personnel involved in the chemical analyses.

7. EQUIPMENT AND SUPPLIES

7.1 GLASSWARE AND SUPPLIES – all equipment used was calibrated and validated (if applicable) according to standard operating procedures (SOPs).

7.1.1 ANALYTICAL BALANCE – Balances used included:
Mettler Toledo AX26DR (Mettler-Toledo Inc., Columbus, OH)
Mettler Toledo XS205DU (Mettler-Toledo Inc., Columbus, OH)
Mettler Toledo UMX2 microbalance (Mettler-Toledo Inc., Columbus, OH)

7.1.2 AUTOPIPETTES – 10 µL, 100 µL, 1,000 µL ± 1 % accuracy

7.1.3 CLASS A VOLUMETRIC GLASSWARE – various sizes

7.1.4 SAMPLE COLLECTION CONTAINERS – Clean 100 mL Nalgene® (Thermo Fisher Scientific Inc., Waltham, MA) polypropylene containers

7.1.5 AUTOSAMPLER VIALS – 2-mL autosampler vials with pre-slit screw tops

7.1.6 COLORIMETER – Hach Pocket Colorimeter II, Chlorine, MR and HR, with Hach Voluette® Analytical Standards, chlorine concentration: 64.8 ± 0.2 mg/L (Hach Company, Loveland, CO)

7.1.7 pH PAPER - Fisher Brand™ (Pittsburgh, PA) pH paper rolls (catalog no. 13-640-507)

7.1.8 FILTERS – Acrodisc® filters (Pall Corporation, Port Washington, NY), GHP, 25 mm, 0.45 µm

7.1.9 REFRIGERATOR – 5 °C ± 3° C

7.1.10 FREEZER – −20 °C ± 10 °C

7.2 LC/MS/MS APPARATUS

7.2.1 LIQUID CHROMATOGRAPHY (LC) SYSTEM – The LC system had programmable solvent mixers capable of delivering a flow rate of 0.3 mL/min. The LC system had all requisite accessories including injection syringe, degasser, and temperature-controlled autosampler. The LC system used in this analysis was a Shimadzu Solvent Delivery Module (LC-10 ADvp) (Shimadzu Inc., Columbia, Maryland) with a SIL−5000 autosampler.

7.2.2 ANALYTICAL COLUMN – Waters (Milford, MA) Atlantis T3, 150 × 2.1 mm, 5 µm particle size.

7.2.3 TANDEM MASS SPECTROMETER (MS/MS) SYSTEM – The mass spectrometer for the analyses (Applied Biosystems API-4000) (Waltham, MA) utilized positive ion ESI ionization and was capable of performing MS/MS analyses, producing unique product ions with a minimum of 10 scans across each chromatographic peak.

7.2.4 DATA SYSTEM – Analyst Version 1.5.1 software was used to acquire, store, reduce and output mass spectral data. The computer software had the capability of processing stored LC/MS/MS data by recognizing an LC peak within any given retention time window. The software allowed integration of the ion abundance of any specific ion within specified time or scan number limits. The software was able to construct linear regression calibration curves and calculate analyte concentrations. The LC was controlled using Waters Acquity (Milford, MA) Version 1.40.

7.2.5 ULTRA-HIGH PERFORMANCE LIQUID CHROMATOGRAPH – The UPLC used for the method development was a Waters Acquity UPLC System (Milford, MA), which included the temperature-controlled autosampler, injection syringe, and degasser. The UPLC was capable of delivering a flow rate of 0.6 mL/min.

8. REAGENTS AND STANDARDS

8.1 STANDARDS, SOLVENTS, AND REAGENTS – All reagents used during the course of this study were analytical grade or equivalent.

8.1.1 STANDARDS – EA2192 was supplied by in-house supply. See the Attachment 20.2 for the EA2192 Certificate of Analysis. Methamidophos (CAS No. 10265-92-6, Lot No. SZBD011XV) and acephate (CAS No. 30560-19-1, Lot No. SZBA083XV) were supplied by Sigma-Aldrich (St. Louis, MO). Methamidophos-d6 (Lot No. 20515AC) was procured from EQ Laboratories (Atlanta, GA).

8.1.2 SOLVENTS AND CHEMICALS – Solvents utilized for this study were acetonitrile (Fisher, HPLC Grade) (Waltham, MA), methanol (Burdick and

Jackson, HPLC Grade) (Morristown, NJ), and DI water (in-house supply), and were demonstrated to be free of analytes and interferences. Chemicals included ammonium acetate (Sigma-Aldrich, ≥ 97 %), sodium omadine (Sigma-Aldrich, ≥ 96 %) and ammonium formate (Sigma-Aldrich, ≥ 99.995 %).

8.1.3 MOBILE PHASE A – Prepared by adding 1.26 g of ammonium formate, accurately weighed (±0.1 g) to a mobile phase bottle and dissolving in 1 L of high-purity water. The mobile phase was mixed well and stored at room temperature. The solution expired in 48 hours.

8.1.4 MOBILE PHASE B – 100 % methanol.

8.1.5 SODIUM OMADINE SOLUTION – Prepared by transferring ~ 0.8 g (±0.1 g) of sodium omadine, accurately weighed, into a 25 mL, Class A, volumetric flask. The compound was dissolved and diluted with DI water and mixed by inversion. The nominal concentration of the resulting solution was 32 g/L. The solution was stored at 5 °C (±3 °C). The solution was prepared fresh daily.

8.1.6 AMMONIUM ACETATE SOLUTION – Prepared by transferring ~ 15.4 g of ammonium acetate into a 100 mL, Class A, volumetric flask. The mixture was diluted to volume with DI water and mixed by inversion. This 2 mM solution was stored at 5 °C (±3 °C). The solution was prepared fresh daily.

8.1.7 10 % METHANOL IN WATER SOLUTION – Prepared by combining 10 mL of methanol with 90 mL of DI water. The solution was mixed well and stored at room temperature for up to 30 days.

8.1.8 NEEDLE WASH A – Prepared by transferring 500 mL of methanol into a mobile phase bottle and mixing with 500 mL of water. The wash solution was mixed well and stored at room temperature for up to 30 days.

8.1.9 NEEDLE WASH B – 100 % methanol.

8.2 STANDARD SOLUTIONS

8.2.1 STOCK STANDARD SOLUTIONS (SSS) – The methamidophos stock solution was prepared by transferring ~ 10.0 mg (± 0.5 mg) of methamidophos, accurately weighed into a weighing pan on a microbalance in an argon-purged glove box and transferred to a 10 mL, Class A, volumetric flask. The compound was dissolved and diluted with methanol and mixed by inversion. The nominal concentration of the resulting solution was 1 g/L. The stock solution was stored in amber 4-dram vials at –20 °C (±10 °C) for up to six months.

The acephate stock solution was prepared by transferring ~ 10.0 mg (± 0.5 mg) of acephate, accurately weighed into a weighing pan on a microbalance in an argon-purged glove box and transferred to a 10 mL, Class A, volumetric flask.

The compound was dissolved and diluted with methanol and mixed by inversion. The nominal concentration of the resulting solution was 1 g/L. The stock solution was stored in 4-dram amber vials at –20 °C (±10 °C) for up to six months.

The EA2192 stock solution was prepared by transferring ~ 10.0 mg (±0.5 mg) of EA2192, accurately weighed, into a 10 mL, Class A, volumetric flask. The compound was dissolved and diluted with acetonitrile and mixed by inversion. The nominal concentration of the resulting solution was 1 g/L. The stock solution was stored in 4-dram amber vials at 5 °C (±3 °C). Assessment of the stock solution stability of EA2192 was not part of the EPA Scope of Work for this project; however, EA2192 is known to be very stable. The EA2192 stock solution was 308 days old for the final stability testing batch.

To verify the stock solution preparation, a second EA2192 stock solution was prepared by transferring ~ 7.5 mg (± 0.5 mg) of EA2192, accurately weighed, into a 10 mL, Class A, volumetric flask. The compound was dissolved and diluted with acetonitrile and mixed by inversion. The nominal concentration of the resulting solution was 0.75 g/L. The stock solution was stored at 5 °C (±3 °C).

The two independent stock solutions were diluted to concentrations within the calibration curve, internal standard was added, and the solutions were then analyzed by LC/MS/MS. The analysis showed a ≤ 5 % difference between the concentrations of the stock solutions. Once verified, one stock solution was used for preparation of the standards.

8.2.2 METHANOLIC ANALYTE PRIMARY DILUTION STANDARD – The Methanolic Analyte Primary Dilution Standard (MEOH PDS) Solution was prepared by transferring 40 µL of the methamidophos stock solution, 40 µL of the acephate stock solution, and 40 µL of the EA2192 stock solution into a 1 mL, Class A, volumetric flask. The mixture was diluted to volume with methanol and mixed by inversion. The nominal concentration for each compound was 40 mg/L. The MEOH PDS solution was stored at –20 °C (±10 °C). Expiration was set at one month although stability was not tested for EA2192 in solution.

8.2.3 AQUEOUS ANALYTE PRIMARY DILUTION STANDARD – The Aqueous Analyte Primary Dilution Standard (WATER PDS) Solution was prepared by transferring 62 µL of the MEOH PDS into a 10 mL, Class A, volumetric flask. The mixture was diluted to volume with 10 % methanol in water and mixed by inversion. The nominal concentration of the resulting solution was 250 µg/L for each compound. The MEOH PDS solution was stored at –20 °C (±10 °C). Expiration was set at one month although stability was not tested for EA2192 in solution.

8.2.4 IS PRIMARY DILUTION STANDARD – The Internal Standard Primary Dilution Standard (IS PDS) was prepared by transferring 40 µL of methamidophos-d6 stock solution into a 10 mL, Class A, volumetric flask. The solution was diluted to volume with acetonitrile and mixed by inversion. The nominal concentration of the resulting solution was 400 µg/L. This solution was stored at 5 °C (±3 °C). Method 538 indicates that this solution is stable for up to six months.

8.2.5 CALIBRATION STANDARDS – The calibration (CAL) standards were prepared by transferring a set amount of the WATER PDS solution into 10 mL, Class A, volumetric flasks. The 2M ammonium acetate solution (100 µL) and 20 µL of the 32 g/L sodium omadine solution were added to each CAL standard. The CAL standard was diluted to volume with DI water and mixed by inversion. Table 2, Calibration Standards, details the dilution series.

8.2.6 CONTINUING CALIBRATION CHECK STANDARDS – The Continuing Calibration Check (CCC) Standards were prepared by transferring a set amount of the WATER PDS solution into 10 mL, Class A, volumetric flasks. The 2M ammonium acetate solution (100 µL) and 20 µL of the 32 g/L sodium omadine solution were added to each CCC standard. The CCC standard was diluted to volume with DI water and mixed by inversion. Table 3, Continuing Calibration Check Standards, details the dilution series. These dilution schemes were also used for the Detection Limit (DL), Minimum Reporting Limit (MRL), Initial Demonstration of Precision (IDP), and Initial Demonstration of Accuracy (IDA) study sample preparations.

8.2.7 MATRIX BLANKS – Matrix blanks from each water source were prepared without preservatives and analyzed. Blanks were prepared fresh for each analysis.

8.2.8 MATRIX SPIKES – A Laboratory Fortified Sample Matrix (LFSM) and a Laboratory Fortified Sample Matrix Duplicate (LFSMD) were prepared from each water source according to Table 4, then mixed well by inversion. Matrix spikes were prepared fresh for each analysis.

8.2.9 STABILITY SAMPLES – Stability studies were performed in accordance with Method 538 to determine if water samples from different sources (representing a variety of water conditions) spiked with EA2192 were stable for 28 days. Water samples were received from four different sources (determined by the EPA); see Table 5 for the representative water conditions. Water samples were received at the laboratory on blue ice (5 °C ± 3 °C) and stored under refrigerated conditions (5 °C ± 3 °C) prior to sample preparation. See Table 6 for the water source parameters (pH, turbidity, conductivity, alkalinity, hardness, free chlorine, chloramine, and total organic carbon) and their measurements for the four source waters. The free chlorine concentration was measured for each bulk water sample using a Hach colorimeter immediately

prior to Time 0, the time of the sample preparation (See Table 7); pH was also measured using pH strips.

9. SAMPLE COLLECTION, PRESERVATION, AND STORAGE

9.1 HOLDING TIME STUDY IN DEIONIZED WATER – A holding time study was performed using Method 538 conditions to determine if DI water samples that were spiked with EA2192 were stable for up to 28 days. The following preparation scheme was used for sample preparation:

1. Mix 1 mL of 2M ammonium acetate and 200 µL of sodium omadine (32 g/L) into 100 mL of DI water.
2. Confirm pH of this solution using pH paper.
3. Aliquot 10 mL of the solution into amber vials (n = 6).
4. Spike 20 µL of the WATER PDS solution into each vial and mix by inversion.
5. Prepare one vial immediately as Time 0 sample in accordance with the sample preparation procedure, n = 7 replicates from Time 0 sample.
6. Place remaining vials into 5 °C (±3 °C) conditions for stability testing.

Stability time points were taken on Day 7, Day 14, and Day 28. After the allotted storage, the vial was removed from the storage condition and allowed to reach room temperature. Seven (7) aliquots of each sample were then prepared and analyzed in accordance with the method.

9.2 WATER STABILITY STUDY IN TAP WATER– Water from four different sources (100 mL), representing a variety of water types (chlorinated, chloraminated, hard water, etc.) was transferred into two wide mouth iChem™ jars with caps (Thermo Scientific), one to represent the low concentration sample (at the CAL2 level, 0.125 µg/L) and one to represent the high concentration sample (at the CAL 6 level, 2.50 µg/L). One mL of 2M ammonium acetate and 200 µL of 32 g/L sodium omadine were added to each iChem™ jar and mixed well by inversion.

Each of the four low concentration samples was spiked with 50 µL of WATER PDS and mixed well. Each low concentration sample was then split into 10 mL aliquots (six per sample source). Five aliquots were stored at 5 °C (± 3 °C). The remaining aliquot (the Time 0 sample) was left at room temperature for immediate sample preparation.

Each of the four high concentration samples was spiked with 1,000 µL of WATER PDS and mixed well. Each high concentration sample was then split into 10 mL aliquots (six per sample source). Five aliquots were stored at 5 °C (± 3 °C). The

remaining aliquot (the Time 0 sample) was left at room temperature for immediate sample preparation. See Table 8, Water Sample Preparation, for the dilution series.

Stability analyses were performed at Time 0, Day 7, Day 14, and Day 28. After the allotted storage, the vial was removed from the storage conditions and allowed to reach room temperature. Seven (7) aliquots of each sample were then prepared and analyzed in accordance with the method.

9.3 EFFECTS OF RESIDUAL CHLORINE ON EA2192 – (NOTE: This is an additional investigation (not presented in Method 538) to investigate residual chlorine effects on EA2192.) The purpose of this study was to determine the stability of EA2192 in water samples with a free chlorine level of ~ 1 mg/L, representative of a common concentration of free chlorine in a distribution system. A water sample was received and stored at 5 °C (± 3 °C) until sample preparation. Immediately prior to sample preparation, the free chlorine level was adjusted to 1 mg/L using the Hach colorimeter chlorine standard kit using the following procedure:

1. Verify the calibration of the Hach colorimeter using the supplied Hach standards.
2. Measure 1 L of water and transfer to an iChem™ jar.
3. Add 10 mL of chlorine standard to the water sample. Mix well for ~ 30 seconds.
4. Transfer 10 mL of the chlorinated water to a Hach vessel and confirm the reading is 1 (± 0.2) mg/L.

The chlorinated water was transferred into two iChem™ jars (100 mL each), one to represent the low concentration sample and one to represent the high concentration sample.

The low concentration sample was spiked with 50 µL of WATER PDS and mixed well. The low concentration sample was then split into 10 mL aliquots (n=7). Five aliquots were stored at 5 °C (± 3 °C) for future time points. One aliquot was left at room temperature for a three-hour time point. The remaining aliquot (the Time 0 sample) was prepared immediately.

The high concentration sample was spiked with 1,000 µL of WATER PDS and mixed well. The high concentration sample was then split into 10 mL aliquots (n = 7). Five aliquots were stored at 5 °C (± 3 °C) for future time points. One aliquot was left at room temperature for the three-hour time point. The remaining aliquot (the Time 0 sample) was prepared immediately.

After the required storage time, 100 µL of 2 M ammonium acetate and 20 µL of 32 g/L sodium omadine were added to each sample and mixed well by inversion, followed by the preparation scheme.

9.4 WATER FILTRATION STUDY – A study was performed to determine if there was a loss of analyte upon filtration of water samples spiked with EA2192. DI water was transferred into four wide mouth iChem™ jars with caps (100 mL each), two to represent the low concentration samples (one filtered and one non-filtered) and two to represent the high concentration samples (one filtered and one non-filtered). One mL of 2 M ammonium acetate and 200 µL of 32 g/L sodium omadine were added to each iChem™ jar and mixed well by inversion.

The low concentration samples were spiked with 50 µL of WATER PDS and mixed well. Each of the four high concentration samples was spiked with 1,000 µL of WATER PDS and mixed well. The samples were then split into individual 10 mL aliquots (seven per sample source). For the filtered samples, the 10-mL aliquots were filtered individually and transferred to a syringe attached with a GHP Acrodisc prior to sample preparation (GHP Acrodisc, 25 mm, 0.45 µm).

10. QUALITY CONTROL

10.1 Quality control (QC) requirements include the Initial Demonstration of Capability (IDC) and ongoing QC requirements that were met when preparing and analyzing samples. This section describes the QC parameters, their required frequencies, and the performance criteria that were met to meet EPA quality objectives.

10.2 CALIBRATION CURVE – Calibration curves consisted of at least five nonzero samples (each at a different concentration) covering the nominal concentration range of 0.05-20 µg/L. A blank DI water sample (collected at the same time as the DI water sample used for standard preparation) was also analyzed. Plots of the peak area response versus gravimetric standard concentration were constructed using a best-fit line determined by a regression analysis. A curve-weighting factor of 1/x with linear regression was utilized.

10.3 CONTINUING CALIBRATION CHECK – The calibration was confirmed by analysis of a CCC at the beginning and end of a sample analysis batch. The beginning CCC was required to be at or below the MRL (typically at CCC2 level) (refer to Table 3) to verify instrument sensitivity. CCCs were then injected after every ten samples and after the last sample, alternating between a mid-level (CCC4) and a high-level (CCC7).

The following requirements were required to be met for a batch to meet acceptability criteria:

1. The absolute area counts of the IS had to be within 50-150 % of the average areas measured in the most recent calibration.
2. The calculated amount for each analyte for medium and high level CCCs had to be within ±30 % of the true value.
3. The calculated amount for each analyte for low level CCCs had to be within ±50 % of the true value.

10.4 INITIAL DEMONSTRATION OF CAPABILITY

10.4.1 DETECTION LIMIT DETERMINATION – The Detection Limit (DL) was verified with the preparation and analysis of seven (7) replicates of a standard at the CCC1 concentration (see Table 3) over the course of three (3) days. This concentration was estimated by selecting a concentration that was approximately two to five times the noise level. The DL was calculated using the following formula:

$$DL = s \times t_{(n\text{-}1,\ 1\text{-}\alpha=0.99)}$$

where: s = standard deviation of replicate analyses
$t_{(n\text{-}1,\ 1\text{-}\alpha=0.99)}$ = Student's t value for the 99 % confidence level with n-1 degrees of freedom
n = number of replicates

10.4.2 MINIMUM REPORTING LEVEL CONFIRMATION – Seven replicates of the Minimum Reporting Level (MRL) were prepared at the CCC2 level (see Table 3) and analyzed. The mean measured concentration and standard deviation for the method analytes in the seven replicates were calculated and the Half Range for the prediction interval of results (HR_{PIR}) was determined using the following formula (per Method 538):

$$HR_{PIR} = 3.963s$$

where: s = standard deviation
3.963 = a constant value for seven replicates

The upper and lower limits for the Prediction Interval of Result (PIR) were required to meet the following upper and lower recovery limits based on the following formulas:

The upper PIR limit requirement was ≤ 150 % recovery

$$\frac{\text{Mean} + HR_{PIR}}{\text{Fortified Concentration}} \times 100\% \leq 150\%$$

The lower PIR limit requirement was ≥ 50 % recovery

$$\frac{\text{Mean} - \text{HR}_{\text{PIR}}}{\text{Fortified Concentration}} \times 100\% \geq 50\%$$

10.4.3 INITIAL DEMONSTRATION OF LOW SYSTEM BACKGROUND – Any time a new lot of solvents, reagents, or autosampler vials was used, a Laboratory Reagent Blank (LRB) was prepared to demonstrate that the new lot was reasonably free of contamination. To demonstrate the freedom from contamination, an LRB was prepared by analyzing blank DI water prepared with the same additives as a standard (i.e., ammonium acetate and sodium omadine) and internal standards. To be acceptable, method analytes could not be detected in the LRB at concentrations > $^1/_3$ the DL.

10.4.4 INITIAL DEMONSTRATION OF PRECISION – Seven (7) replicates of CCC4 were prepared for the Initial Demonstration of Precision (IDP) study as described in Table 3 and analyzed. To pass acceptability criteria, the calculated relative standard deviation from the replicate analyses was required to be < 20 %.

10.4.5 INITIAL DEMONSTRATION OF ACCURACY – The same seven (7) replicates of CCC4 that were generated for the IDP study were used for the Initial Demonstration of Accuracy (IDA) study. To pass acceptability criteria, the calculated mean recovery from the replicate analyses was required to be ±30 %.

10.5 STABILITY STUDIES – The concentrations of the stored (stability) samples were compared to the concentrations of the samples analyzed at Time 0. To be reported as stable, the concentration of the stored samples could not deviate from the concentration of the samples analyzed at time 0 by more than ±30 %. In addition, replicate stability samples at a given stability condition must have a % RSD value of ≤ 15 % to be acceptable. For the stability batches to be acceptable, the batch must meet CCC requirements.

10.6 WATER FILTRATION STUDY – The concentrations of the filtered samples were compared to the concentrations of the non-filtered samples. To be reported as comparable, the concentration of the filtered samples could not deviate from the concentration of the non-filtered samples by more than ± 30 %. In addition, replicate samples at a given condition required a % RSD value of ≤ 15 % to be acceptable. For the batch to be acceptable, the batch had to meet the CCC standard requirements.

11. INSTRUMENT CALIBRATION AND STANDARDIZATION

11.1 HPLC INSTRUMENT AND PARAMETERS – The HPLC method parameters are listed in Table 9. The HPLC gradient is listed in Table 10.

11.2 ESI-MS/MS TUNING – The $[M+H]^+$ signal was optimized for each method analyte by infusing approximately 1 µg/mL of each analyte directly into the MS. The MS

parameters were varied until optimal analyte responses were determined. Once the MS parameters were optimized, the product ions and MS/MS parameters were determined. See Table 11 for the optimized ESI-MS/MS conditions and Table 12 for the Multiple Reaction Monitoring (MRM) transitions.

11.3 INITIAL CALIBRATION – The initial calibration curve consisted of seven CAL standards. The lowest CAL was required to be at or below the MRL. The curve was calibrated using the IS technique. The LC/MS/MS data system software was used to generate a linear regression calibration curve with 1/x weighting.

11.4 CALIBRATION ACCEPTANCE CRITERIA – Each calibration point (except the lowest point) should calculate to be within 70-130 % of its true value. The lowest CAL point should calculate to be within 50-150 % of its true value.

11.5 CONTINUING CALIBRATION CHECK (CCC) – The initial calibration was verified at the beginning and end of each group of analyses, and after every tenth sample. The beginning CCC of each analysis batch was required to be at or below the MRL to verify instrument sensitivity prior to any analyses. Subsequent CCCs alternated between a medium and high concentration CAL standard. The absolute areas of the quantitation ions of the IS had to be within 50 %-150 % of the average areas measured in the most recent calibration. Additionally, the calculated amount for each analyte for medium and high level CCCs had to be within ±30 % of the true value and ±50 % at the lowest calibration level.

12. ANALYTICAL PROCEDURE

The following procedure was used for the preparation of samples for analysis (i.e., CALs, CCC standards, IDC samples, Stability Samples, Water Matrix Blanks, LFSM, etc.). Volumes were delivered with calibrated adjustable pipettes:

12.1 Transfer 990 µL of sample into an autosampler vial (except for blanks—add 1,000 µL of sample).
12.2 Add 10 µL of IS PDS to each sample (do not add IS PDS to blanks).
12.3 Mix by inversion and cap for analysis.

13. DATA ANALYSIS AND CALCULATION

13.1 DESCRIPTIVE STATISTICS – Descriptive statistics [mean, standard deviation (SD), relative standard deviation (% RSD), percent accuracy (% ACC), relative error (% RE), and percent difference], were calculated for this method..”

The following formulas were used during the course of this study:

13.1.1 Results were expressed as a concentration based on the calibration curve. The concentration was calculated as follows:

$$\text{Sample Concentration (ng/mL)} = \left(\frac{(response - \text{y int})}{\text{Slope}} \right)$$

where: *response* = Peak area of the analyte versus IS in the sample
y int = y-intercept obtained from the calibration curve
Slope = slope obtained from the calibration curve

13.1.2 Method accuracy was expressed as percent relative error (% RE) which was calculated based on the gravimetric concentration as follows:

$$\%\ \text{Relative Error} = \frac{(D - E)}{E} \times 100$$

where: D = determined concentration
E = expected (gravimetric) concentration

13.1.3 Method precision was expressed as percent relative standard deviation (% RSD) when the number of samples (n) ≥ 3 and was calculated as follows:

$$\%\ \text{Relative Standard Deviation} = \left(\frac{\sigma}{X}\right) \times 100$$

where: σ = standard deviation
X = mean

13.1.4 To evaluate stability, the mean concentration after the storage time was compared to the mean concentration at Time 0 as follows:

$$\%\ \text{of Time 0} = \frac{X}{Y} \times 100$$

where: X = mean concentration after storage time
Y = mean determined concentration at Time 0

13.1.5 To evaluate percent difference between LFSM and LSFSMD samples, the determined concentration of individually prepared LFSM and LFSMD solutions were compared to each other:

$$\%\ \text{Difference} = \left[\left(\frac{(X - Y)}{(X + Y)/2}\right)\right] \times 100$$

where: X = determined concentration of LFSM
Y = determined concentration of LFSMD

14. METHOD PERFORMANCE

14.1 LINEARITY – Coefficients of correlation (r) were at least 0.9996 for EA2192. The percent accuracy (% RE) for EA2192 met Initial Demonstration of Capability (IDC) criteria ranging from 96.4 % to 105 % for CAL1 and from 92.4 % to 107 % at all other concentrations (see Table 13). A representative EA2192 calibration curve is shown in Figure 1, where linearity is demonstrated over the tested calibration range.

Coefficients of determination (r) were 0.9999 for methamidophos. The percent accuracy (% RE) for methamidophos ranged from 91.6 % to 107 % for CAL1 and from 91.8 % to 107 % at all other concentrations (Table 14). A representative methamidophos calibration curve is shown in Figure 2, where linearity is demonstrated over the tested calibration range.

Coefficients of determination (r) were at least 0.9988 for acephate. The percent accuracy (% RE) for acephate ranged from 94.1 % to 106 % for CAL1 and from 89.5 % to 109 % at all other concentrations (Table 15). A representative acephate calibration curve is shown in Figure 3, where linearity is demonstrated over the tested calibration range.

14.2 CONTINUING CALIBRATION CHECKS – Continuing calibration checks were analyzed during each batch to verify that the current calibration was still meeting acceptability criteria. A CCC2 sample (at the MRL) was initially analyzed to verify sensitivity, followed by a CCC4 and CCC7 to verify the accuracy of the sequence in comparison to the current calibration. CCCs were reanalyzed after every ten samples and/or at the end of the sequence to verify there was no loss in sensitivity.

For EA2192, CCCs in all batches passed the acceptability criteria. Methamidophos and acephate CCC results were calculated to verify instrument performance and to determine if the sensitivity and chromatography were acceptable. In two batches, the final grouping of CCCs for acephate failed the acceptability criteria of ±30 % of its true value. In both cases, the accuracy was within ± 40 % and in both cases, the value was a response that was higher than expected. The methamidophos CCCs passed acceptability criteria for all batches. Because EA2192 passed acceptability criteria for these batches, and because the purpose of this study was to incorporate EA2192 into the method, the batches were accepted. The acephate CCC failures could indicate that the instrument requires cleaning or that a new calibration curve is required. The failures were identified and corrective actions were taken to remedy the issue in subsequent batches by cleaning the instrument, analyzing a new calibration curve, and replacing the analytical column. Further steps included the addition of a diversion valve to remedy source contamination during longer analysis run sequences (section 14.9) and reduce the risk of further sample failures. After the IDC and initial holding time studies were completed, it was decided to eliminate acephate from the method for the subsequent studies.

14.3 INITIAL DEMONSTRATION OF LOW SYSTEM BACKGROUND – Method analytes were not detected in an LRB spiked with preservatives and internal standards at concentrations that were > $^1/_3$ of the DL. A representative chromatogram of a blank sample without internal standard is shown in Figure 4, and a representative chromatogram of a blank with internal standard is shown in Figure 5.

14.4 DETECTION LIMIT DETERMINATION – The EA2192 DL was determined from seven replicates of samples at the CCC1 level, batches prepared over three days. The DL was calculated to be 0.0130 µg/L, using a t value of 3.143. The DL calculation is presented in Table 16.

14.5 MINIMUM REPORTING LEVEL CONFIRMATION – MRL was determined from seven replicates of samples at the CCC2 level. The HR_{PIR} was determined to be 0.0353 µg/L. Based on this result, the Lower PIR was calculated to be 86.3 % and the Upper PIR was calculated to be 145 %. These values meet both the Upper and Lower PIR limit requirements of ≤ 150 % for the Upper PIR and ≥ 50 % for the Lower PIR. The MRL confirmation is shown in Table 17. A representative chromatogram of the CAL2 standard at the MRL is shown in Figure 6.

14.6 INITIAL DEMONSTRATION OF PRECISION – The IDP was determined from seven replicates at the CCC4 concentration level, calculated versus a calibration curve. The precision (% RSD) was 9.61 %. This value was within the % RSD acceptability criteria of ≤ 20 %. The IDP results are summarized in Table 18.

14.7 INITIAL DEMONSTRATION OF ACCURACY – The IDA was determined from the same seven CCC4 replicates that were used for the IDP study, calculated vs. a calibration curve. The IDA (% RE) was 21.8 %. This value was within the % RE acceptability criteria of ± 30 %. The IDA results are summarized in Table 18.

14.8 HOLDING TIME STUDY IN DEIONIZED WATER – The average concentration of EA2192 after storage under refrigerated conditions (5 °C ± 3 °C) when compared to Time 0 was 81.6 % (8, % RSD) after seven days, 97.4 % (3, % RSD) after 14 days, and 86.7 % (4, % RSD) after 28 days. The holding time study results are summarized in Table 19.

The Day 14 batch failed upon initial analysis. The response of the internal standard was less than 50 % of the average internal standard area counts of the initial calibration. Remedial action was taken by cleaning the instrument, replacing the analytical column, and preparing and analyzing a fresh calibration curve. The Day 14 samples were reanalyzed compared to the new calibration curve to obtain the reported value, but these samples were analyzed more than 24 hours after sample preparation. A stability study of the samples on the autosampler has not been performed on EA2192. Such an investigation would be necessary prior to including EA2192 in Method 538 due to Day 14 batch reanalysis past 24 hours; however, holding time study data in source water samples are sufficient for Method 538 holding time parameters.

14.9 WATER SAMPLE STABILITY STUDY IN TAP WATER – During the Time 0 analysis, CCC standards for EA2192 started to fail acceptance criteria after 48 injections. The IS area counts were decreasing throughout the analysis which caused the CCC standards to calculate higher than the acceptance criteria allowed. A diversion valve was added to divert waste for the first three minutes of the analysis (prior to analyte elution) as well as after 20 minutes into the analysis (after elution of the last analyte in Method 538). The source was thoroughly cleaned and the diversion was used to help keep the source clean through the longer analyses. After instituting these processes, the remaining analyses passed all acceptance criteria for both EA2192 and methamidophos (tested for up to 105 injections per batch).

Water was received from four individual utility companies. The associated water parameters are listed in Table 6. The water was spiked with EA2192, and the concentration of the compound was determined after storage under refrigerated conditions (5 °C ± 3 °C) for 28 days. The pH and free chlorine levels were measured immediately prior to sample preparation at Day 0 and are listed in Table 7. When compared to Time 0, the concentration was 81.7 %-117 % after seven days, 98.5 %-118 % after 14 days, and 79.7 %-119 % after 28 days. The highest Relative Standard Deviation (%RSD) of the triplicate samples was 11.9 %. The water sample stability study results are summarized in Table 20. Representative figures for each of the four water sample types are included in Figures 7-10, which include a matrix blank, low concentration sample and high concentration sample and water sample parameters are provided in Tables 5, 6, and 7.

The IS area counts for two of the four method blanks (from Water Sources No. 1 and No. 4) were not ± 50 % of the average IS response from the initial calibration (both instances failed low). This failure was observed in two separate preparations and analyses (Stability Day 0 and Day 7, data not shown). Method blanks were then prepared with ammonium acetate and sodium omadine for all four water types. The IS response passed acceptance criteria with the inclusion of additives.

14.10 EFFECTS OF RESIDUAL CHLORINE ON EA2192 – Water was received from Water Source No. 1 and the free chlorine concentration was adjusted to 0.93 mg/L, measured using a Hach Colorimeter, immediately prior to sample preparation. EA2192 was not detected in the Time 0 samples or the three-hour samples. Therefore, the remaining stability time points were not prepared. Because bleach is used for the decontamination of CWAs, it is assumed that the higher level of chlorine present in this water sample led to the rapid degradation of EA2192.

14.11 WATER FILTRATION STUDY – The average concentration of the filtered Low samples was 100 % of the non-filtered samples. The average concentration of the filtered High samples was 118 % of the non-filtered samples. The highest %RSD of the triplicate samples was 15.6 %. The water filtration study results are summarized in Table 21. Filtering the samples at either high or low concentrations did not affect the recovery of the target analyte.

15. POLLUTION PREVENTION

15.1 This method utilized ESI-LC/MS/MS for the analysis of method analytes in water. The method required the use of very small volumes of organic solvent and very small quantities of pure analytes, thereby minimizing the potential hazards to both the analyst and the environment.

16. WASTE MANAGEMENT

16.1 The analytical procedures described in this method generated relatively small amounts of waste since only small amounts of reagents and solvents were used. The matrices of concern were finished drinking water and/or source water. Laboratory waste management practices were conducted consistent with all applicable rules and regulations, and the laboratory protected the air, water, and land by minimizing and controlling all releases from fume hood and bench operations. Compliance with any sewage discharge permits and regulations, particularly the hazardous waste identification rules and land disposal restrictions, were followed.

17. REFERENCES

17.1 All data obtained from the study were evaluated in accordance with the following EPA methods or published SOPs:

17.1.1 U.S. EPA Method 538, "Determination of Selected Organic Contaminants in Drinking Water by Direct Aqueous Injection-Liquid Chromatography/Tandem Mass Spectrometry (DAI-LC/MS/MS)," Version 1.0, November 2009, EPA Document No. EPA/600/R-09-149

18. TABLES AND VALIDATION DATA

Table 1. Initial Demonstration of Capability Testing Summary

Test Article	EA2192	
Matrix	DI Water	
Quantitation	LC/MS/MS	
Regression Type	Linear (1/x)	
Linear Range	0.05 µg/L to 20 µg/L	
IDC Tests (EA2192)	**Acceptance criteria**	**Results**
Coefficient of Determination (r)	NA	≥0.9996
Minimum Reporting Level	≤ 150 % Upper PIR Limit ≥ 50 % Lower PIR Limit	Upper Limit = 145 % Lower Limit = 86.3 %
Detection Limit Determination	NA	0.0130 µg/L
Initial Demonstration of Low System Background	Background < 1/3 of minimum reporting level	Non-detect
Initial Demonstration of Precision	< 20 % RSD	9.61 %
Initial Demonstration of Accuracy	±30 % mean recovery (RE)	21.8 %
EA2192 Holding Time Study	**Acceptance criteria**	**% Recovery from Day 0**
Day 7	70-130 %	81.6 %
Day 14	70-130 %	97.4 %
Day 28	70-130 %	86.7 %

NA = Not applicable; RE = Relative Error; RSD = Relative Standard Deviation; PIR = Prediction Interval of Result.

Table 2. Calibration Standards

Calibration Solution Name	Source Solution	Source Solution Volume (µL)	(2M) Ammonium Acetate Volume (µL)	(32 g/L) Sodium Omadine Volume (µL)	Final Volume of Solution (mL)	Nominal Solution Conc. (µg/L)
CAL 1	WATER PDS	2	100	20	10	0.050
CAL 2	WATER PDS	5	100	20	10	0.125
CAL 3	WATER PDS	10	100	20	10	0.250
CAL 4	WATER PDS	20	100	20	10	0.500
CAL 5	WATER PDS	40	100	20	10	1.00
CAL 6	WATER PDS	100	100	20	10	2.50
CAL 7	WATER PDS	200	100	20	10	5.00
CAL 8	WATER PDS	400	100	20	10	10.0
CAL 9	WATER PDS	800	100	20	10	20.0

CAL = Calibration; PDS = Primary dilution standard.

Table 3. Continuing Calibration Check Standards

Solution Name	Source Solution Name	Source Solution Volume (µL)	(2M) Ammonium Acetate Volume (µL)	(32 g/L) Sodium Omadine Volume (µL)	Final Volume of Solution (mL)	Nominal Solution Conc. (µg/L)
CCC 1 (DL Study)	WATER PDS	2	100	20	10	0.050
CCC 2 (MRL Study)	WATER PDS	5	100	20	10	0.125
CCC 4 (IDP and IDA Studies)	WATER PDS	20	100	20	10	0.500
CCC 7	WATER PDS	200	100	20	10	5.00

CCC = Continuing calibration check; PDS = Primary dilution standard.

Table 4. Laboratory Fortified Sample Matrix Preparation

Solution Name	Source Solution	Source Solution Volume (µL)	Ammonium Acetate Volume (µL)	Sodium Omadine Volume (µL)	Final Volume (mL)	Nominal Solution Conc. (µg/L)
[Source Number] LFSM[1]	WATER PDS	5	100	20	10	0.125

[1] Each water source was prepared as listed in this table.

LFSM = Laboratory fortified sample matrix; PDS = Primary dilution standard.

Table 5. Water Conditions

Source Number	Representative Water Condition
1	Low TOC, chlorinated surface water
2	High TOC, chloraminated surface water
3	Low TOC, chloraminated surface water
4	High hardness, chlorinated ground water

TOC = Total organic carbon.

Table 6. Water Sample Parameters upon Collection

Source Number	pH	Turbidity (NTU)	Conductivity	Alkalinity (mg/L)	Hardness (mg/L)	Free Chlorine (mg/L)	Chloramine (mg/L)	Total Organic Carbon (mg/L)
1	8.7	0.02	525 µS/cm	88	157	1.21	--	0.80
2	9.18	0.10	414 µS/cm	--	--	3.10	3.40	7.98
3	7.4	0.07	400 µS/cm	118	165	0	3.5	1.3
4	7.24	0.15	998 µS/cm	333	461	0.38	--	-- [1]

[1] Reported to have total organic carbon below the detection limit as expected for groundwater not under the influence of surface water.

Parameters were measured upon water collection.

"--" indicates that a value was not reported.

Table 7. Measured Water Parameters at Time of Sample Preparation

Source Number	pH	Free Chlorine (mg/L)
1	6 to 6.5	0.74
2	6 to 6.5	0.03
3	6 to 6.5	0.08
4	6 to 6.5	0.19

Parameters were measured immediately prior to sample preparation.
pH was measured using pH strips.
Free chlorine was measured using Hach Colorimeter.

Table 8. Water Sample Preparation

Solution Name	Source Solution	Source Solution Volume (µL)	Ammonium Acetate Volume (µL)	Sodium Omadine Volume (µL)	Water Source Volume (mL)	Nominal Solution Conc. (µg/L)
[Source Number] Low Concentration [1]	WATER PDS	50	1,000	200	100	0.123
[Source Number] High Concentration [1]	WATER PDS	1,000	1,000	200	100	2.45

[1] Each water source was prepared as listed in this table, then split into six aliquots.

Table 9. HPLC Method Parameters

Setting Name	Value
Liquid Chromatograph	Shimadzu Solvent Delivery Module LC-10 ADvp
Autosampler	Shimadzu SIL-5000
Column	Waters Atlantis T3 5 µ, 150 × 2.1 mm
Mobile Phase A	20mM Ammonium Formate
Mobile Phase B	100 % Methanol
Flow Rate	0.3 mL/min
Injection Volume	50 µL
Run Time	30.0 min
Sample Temperature	5 °C
Needle Wash A	50/50 (v/v) Methanol/Water
Needle Wash B	100 % Methanol
Column Temperature	Ambient

Table 10. HPLC Gradient

Time (min)	%A	%B	HPLC Flow
0	90	10	To Waste
3.0	90	10	To Instrument (3 to 20 min)
5.0	70	30	
8.0	70	30	
20.0	30	70	
20.1	10	90	To Waste
25.0	10	90	
25.1	90	10	

Table 11. ESI-MS/MS Method Parameters

Setting Name	Value
Mass Spectrometer	Applied Biosystems API-4000
Software	Analyst V. 1.5.1
Ionization Mode	Turbo ionspray, positive
Scan Mode	Multiple Reaction Monitoring (MRM)
Curtain Gas	20 psi
Collision (CAD) Gas	4 psi
Ion Spray Voltage (IS)	5000 V
Temperature	450 °C
Ion Source (GS1)	30 psi
Ion Source (GS2)	30 psi
Interface heater (ihe)	on
Entrance Potential	10 V
Collision Cell Exit Potential	12 V

Table 12. MRM Transitions

Compound Name	Monitored Transition	Dwell Time (ms)	Declustering Potential (V)	Collision Energy (V)	Retention Time (min)
EA2192	240.4 > 128.1	100	45	25	5.6
Acephate	184.2 > 143.0	100	35	12	5.7
Methamidophos	142.0 > 94.0	100	30	20	3.6
Methamidophos-d6	148.0 > 97.0	100	30	20	3.7

Table 13. IDC Calibration Curve Standards—EA2192

Standard Name	Standard Concentration Level (µg/L)	Determined Concentration (µg/L)	% Accuracy
CAL1	0.0481	0.0464	96.4
		0.0467	97.1
		0.0479	99.6
CAL2	0.120	0.123	102
		0.124	104
		0.120	100
CAL3	0.241	0.242	100
CAL4	0.481	0.468	97.3
CAL5	0.962	0.961	99.9
CAL6	2.41	2.42	100
CAL7	4.81	5.02	104
CAL8	9.62	9.52	99.0
CAL9	19.2	19.1	99.4
correlation coefficient (r) value: 0.9998			

CAL = Calibration

Standard Name	Standard Concentration Level (µg/L)	Determined Concentration (µg/L)	% Accuracy
CAL1	0.0481	0.0505	105
CAL2	0.120	0.111	92.4
CAL3	0.241	0.236	98.0
CAL4	0.481	0.483	100
CAL5 *	0.962	1.35	140
CAL6	2.41	2.40	99.8
CAL7	4.81	5.15	107
CAL8	9.62	9.46	98.3
CAL9	19.2	19.0	99.2
r value: 0.9996			

CAL = Calibration; r = Correlation coefficient.
* Point excluded from calibration curve.

Table 14. IDC Calibration Curve Standards—Methamidophos

Standard Name	Standard Concentration Level (µg/L)	Determined Concentration (µg/L)	% Accuracy
CAL1	0.0472	0.0505	107
		0.0455	96.3
		0.0432	91.6
CAL2	0.118	0.127	107
		0.127	107
		0.119	101
CAL3	0.236	0.236	100
CAL4	0.472	0.440	93.3
CAL5	0.944	0.901	95.4
CAL6	2.36	2.35	99.7
CAL7	4.72	4.74	101
CAL8	9.44	9.40	99.6
CAL9	18.9	19.0	100
r value: 0.9999			

CAL = Calibration
r = Correlation coefficient.

Standard Name	Standard Concentration Level (µg/L)	Determined Concentration (µg/L)	% Accuracy
CAL1	0.0472	0.0505	107
CAL2	0.118	0.108	91.8
CAL3	0.236	0.227	96.4
CAL4	0.472	0.476	101
CAL5 *	0.944	1.21	128
CAL6	2.36	2.39	101
CAL7	4.72	4.89	104
CAL8	9.44	9.43	99.9
CAL9	18.9	18.7	99.0
r value: 0.9999			

CAL = Calibration; ; r = Correlation coefficient.
* Point excluded from calibration curve.

Table 15. IDC Calibration Curve Standards—Acephate

Standard Name	Standard Concentration Level (µg/L)	Determined Concentration (µg/L)	% Accuracy
CAL1	0.0496	0.0478	96.3
		0.0497	100
		0.0528	106
CAL2	0.124	0.125	101
		0.136	109
		0.129	104
CAL3	0.248	0.240	96.7
CAL4	0.496	0.475	95.7
CAL5	0.992	0.925	93.2
CAL6	2.48	2.42	97.4
CAL7	4.96	4.90	98.8
CAL8	9.92	9.93	100
CAL9	19.8	20.0	101
r value: 0.9999			

CAL = Calibration
r = correlation coefficient.

Standard Name	Standard Concentration Level (µg/L)	Determined Concentration (µg/L)	% Accuracy
CAL1	0.0496	0.0467	94.1
CAL2	0.124	0.111	89.5
CAL3	0.248	0.250	101
CAL4	0.496	0.522	105
CAL5 *	0.992	1.52	153
CAL6	2.48	2.54	102
CAL7	4.96	5.42	109
CAL8	9.92	10.2	103
CAL9	19.8	19.0	95.9
r value: 0.9988			

CAL = Calibration; r = Correlation coefficient.
* Point excluded from calibration curve.

Table 16. EA2192 Detection Limit Determination

Sample Name	Gravimetric Concentration Level (µg/L)	Determined Concentration (µg/L)
DL CAL 1 Day 1	0.0481	0.0553
DL CAL 1 Day 1		0.0561
DL CAL 1 Day 1		0.0605
DL CAL 1 Day 2		0.0558
DL CAL 1 Day 2		0.0591
DL CAL 1 Day 3		0.0622
DL CAL 1 Day 3		0.0667
Average		0.0594
Std. Dev.		0.00415
n		7
degrees of freedom		6
t value		3.143
Detection Limit		**0.0130**

DL CAL = Detection limit Calibration; n= number of samples; Std. Dev. = standard deviation; t value = Student's t-value

Table 17. EA2192 Method Reporting Limit Confirmation

Sample Name	Gravimetric Concentration Level (µg/L)	Determined Concentration (µg/L)
CAL 2 (MRL)	0.120	0.130
CAL 2 (MRL)		0.137
CAL 2 (MRL)		0.145
CAL 2 (MRL)		0.137
CAL 2 (MRL)		0.154
CAL 2 (MRL)		0.141
CAL 2 (MRL)		0.128
Average		0.139
Std. Dev.		0.00890
HR_{PIR}		0.0353
Lower PIR Limit		86.3 %
Upper PIR Limit		145 %

Table 18. EA2192 Initial Demonstration of Precision and Accuracy

Sample Name	Gravimetric Concentration Level (µg/L)	Determined Concentration (µg/L)
IDP/IDA Cal 4	0.481	0.584
IDP/IDA Cal 4		0.588
IDP/IDA Cal 4		0.634
IDP/IDA Cal 4		0.557
IDP/IDA Cal 4		0.497
IDP/IDA Cal 4		0.655
	Average	0.586
	Std. Dev.	0.0563
	Precision (%RSD)	9.61
	Accuracy (%RE)	21.8

IDP/IDA Cal = Initial demonstration of precision/initial demonstration of accuracy calibration

Table 19. EA2192 Holding Time Study—DI Water

Time, Days	Average* (µg/L) Standard Deviation % RSD	% of Day 0
0	0.513	--
	0.0181	
	3.52	
7	0.419	81.6
	0.0317	
	7.57	
14	0.500	97.4
	0.0147	
	2.95	
28	0.455	86.7
	0.0166	
	3.73	

NOTE: Gravimetric Concentration: 0.481 µg/L.
***NOTE:** Seven replicates were prepared and analyzed for each concentration at each time point.

Table 20. EA2192 Stability Study in Tap Water

Source No. 1						
Low Concentration (µg/L)				High Concentration (µg/L)		
Time, days	Average* / Std. Dev. / % RSD	% of Day 0		Time, days	Average* / Std. Dev. / % RSD	% of Day 0
0	0.120 / 0.004 / 3.7	--		0	2.43 / 0.170 / 7.0	--
7	0.104 / 0.005 / 4.7	86.9		7	2.44 / 0.220 / 9.2	101
14	0.131 / 0.006 / 4.3	110		14	2.39 / 0.060 / 2.5	98.5
28	0.099 / 0.004 / 4.4	82.9		28	2.42 / 0.070 / 2.9	99.7

Source No. 2						
Low Concentration (µg/L)				High Concentration (µg/L)		
Time, days	Average* / Std. Dev. / % RSD	% of Day 0		Time, days	Average* / Std. Dev. / % RSD	% of Day 0
0	0.125 / 0.011 / 9.0	--		0	2.28 / 0.030 / 1.4	--
7	0.102 / 0.003 / 2.6	81.7		7	2.62 / 0.160 / 6.0	115
14	0.132 / 0.005 / 3.8	106		14	2.27 / 0.150 / 6.5	99.5
28	0.100 / 0.003 / 2.7	79.7		28	2.44 / 0.040 / 1.8	107

***NOTE:** Seven replicates were prepared and analyzed for each concentration at each time point.

Table 20. EA2192 Stability Study in Tap Water (cont.)

Source No. 3						
Low Concentration (µg/L)				High Concentration (µg/L)		
Time, days	Average*	% of Day 0		Time, days	Average*	% of Day 0
	Std. Dev.				Std. Dev.	
	% RSD				% RSD	
0	0.121	--		0	2.22	--
	0.014				0.080	
	11.9				3.6	
7	0.104	85.6		7	2.57	116
	0.002				0.110	
	2.2				4.2	
14	0.126	104		14	2.32	105
	0.004				0.030	
	3.3				1.5	
28	0.105	87.0		28	2.49	112
	0.005				0.030	
	4.3				1.3	

Source No. 4						
Low Concentration (µg/L)				High Concentration (µg/L)		
Time, days	Average*	% of Day 0		Time, days	Average*	% of Day 0
	Std. Dev.				Std. Dev.	
	% RSD				% RSD	
0	0.106	--		0	2.27	--
	0.002				0.090	
	2.1				4.0	
7	0.124	117		7	2.53	112
	0.014				0.070	
	10.9				2.8	
14	0.125	118		14	2.35	104
	0.008				0.040	
	6.0				1.9	
28	0.112	106		28	2.69	119
	0.003				0.130	
	2.6				4.7	

***NOTE:** Seven replicates were prepared and analyzed for each concentration at each time point.

Table 21. Filtered Water Comparison Study (HPLC)

Low Concentration (µg/L)				High Concentration (µg/L)		
Condition	Average*	% of Non-Filtered		Condition	Average*	% of Non-Filtered
	Std. Dev.				Std. Dev.	
	% RSD				% RSD	
Non-Filtered	0.123	--		Non-Filtered	3.27	--
	0.0114				0.512	
	9.3				15.6	
Filtered	0.123	100 %		Filtered	3.85	118 %
	0.0113				0.202	
	9.2				5.2	

***NOTE:** Seven replicates were prepared and analyzed for each concentration at each time point.

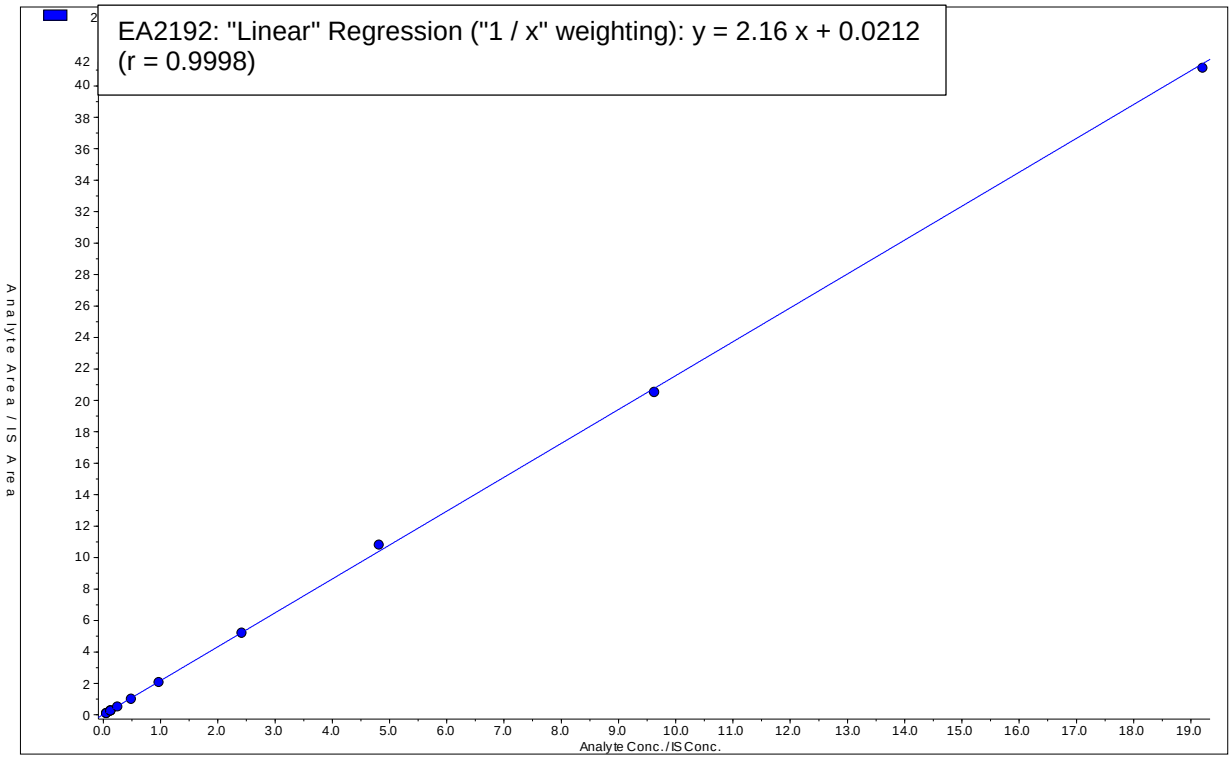


Figure 1. Representative EA2192 calibration curve: x-axis, analyte concentration/internal standard concentration.; y-axis, analyte area/internal standard area.

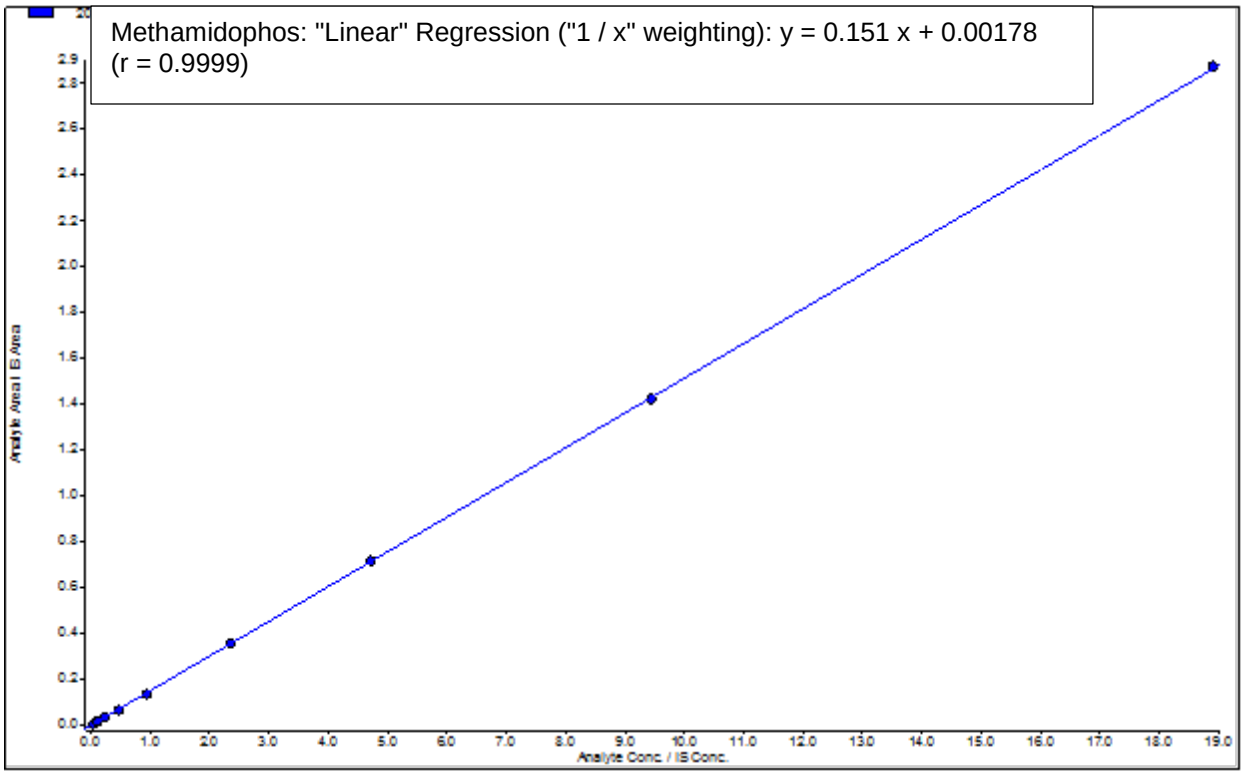


Figure 2. Representative Methamidophos calibration curve: x-axis, analyte concentration/internal standard concentration; y axis, analyte area/internal standard area.

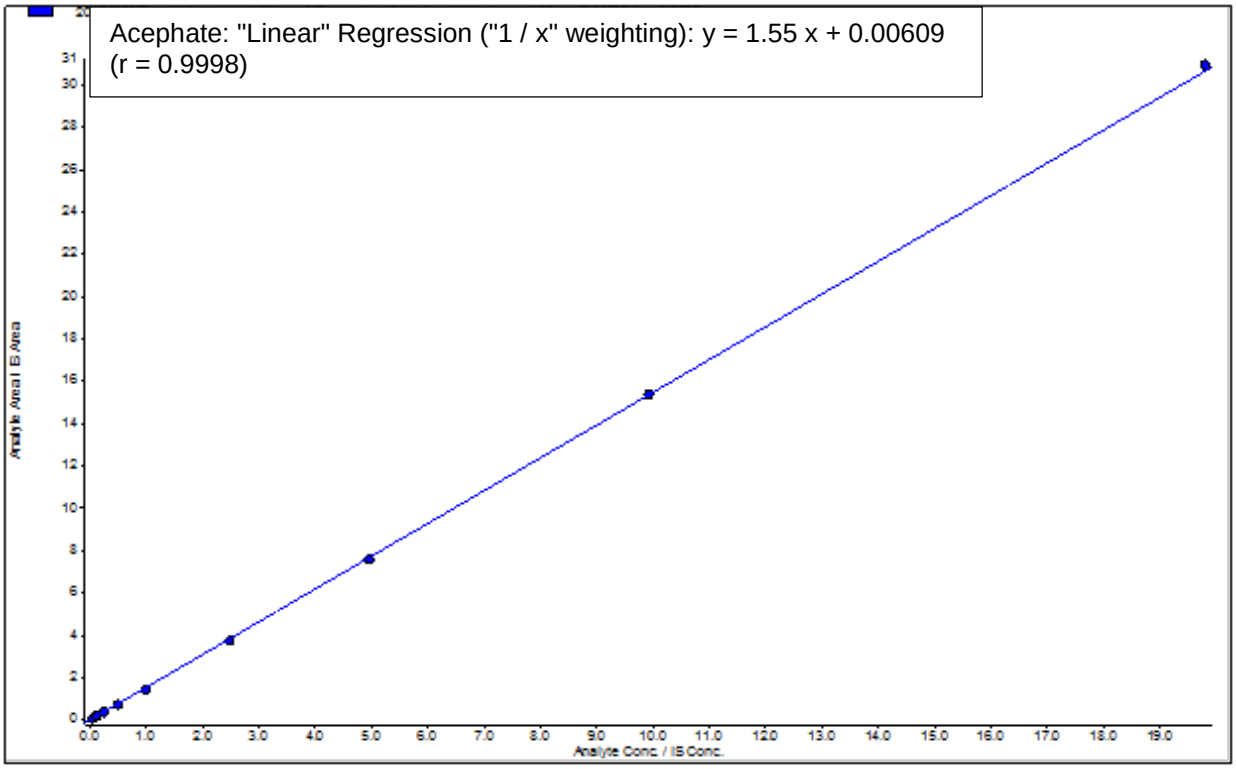


Figure 3. Representative Acephate calibration curve: x-axis, analyte concentration/internal standard concentration; y axis, analyte area/internal standard area.

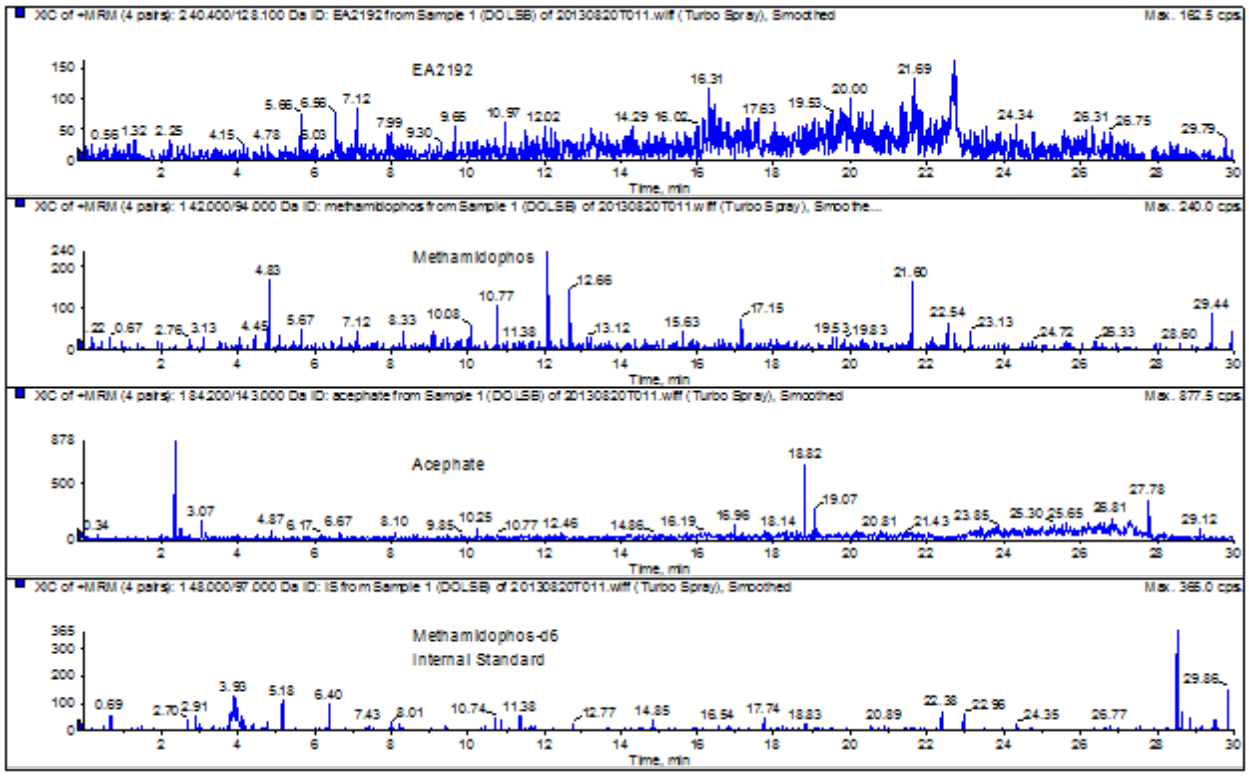


Figure 4. Representative Chromatogram of a Matrix Blank without Internal Standard

Chromatogram order:
EA2192 (top)
Methamidophos
Acephate
Methamidophos-d6 (bottom)

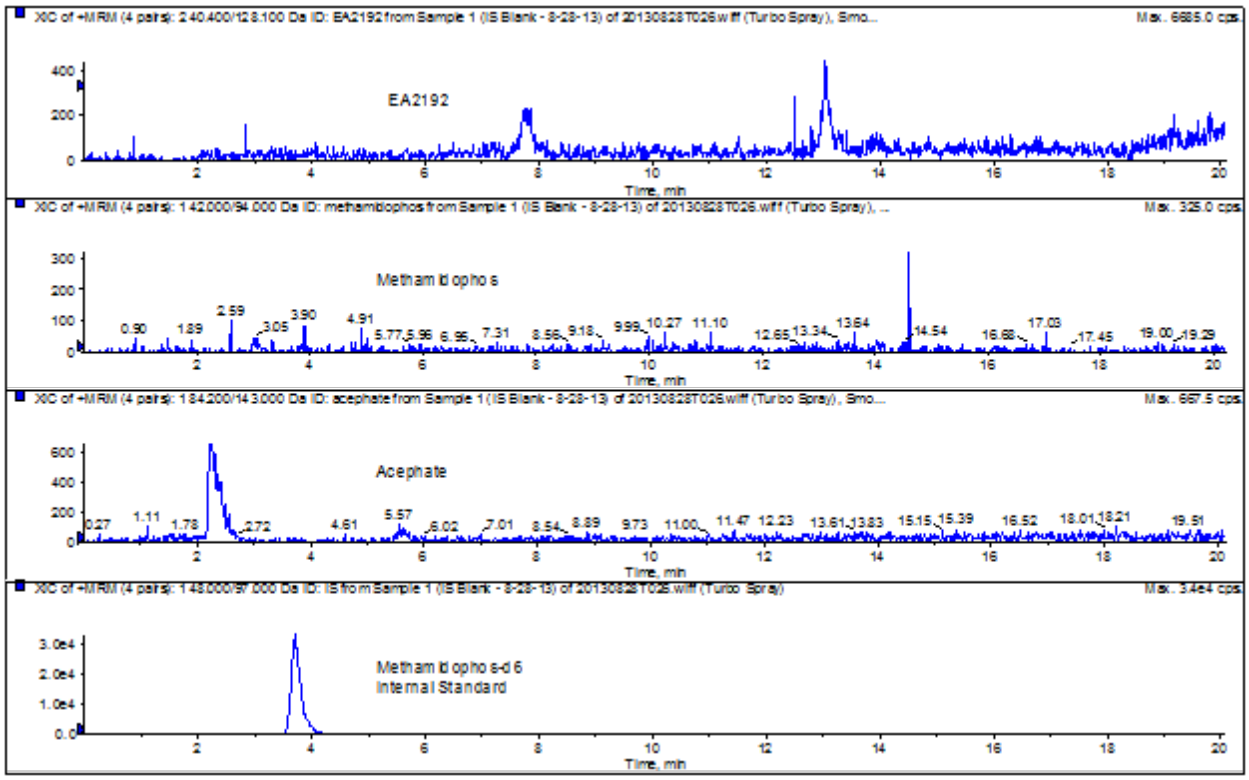


Figure 5. Representative Chromatogram of a Matrix Blank with Internal Standard: x-axis, time (minutes); y axis, intensity (counts)

Chromatogram order:
EA2192 (top)
Methamidophos
Acephate
Methamidophos-d6 (bottom)

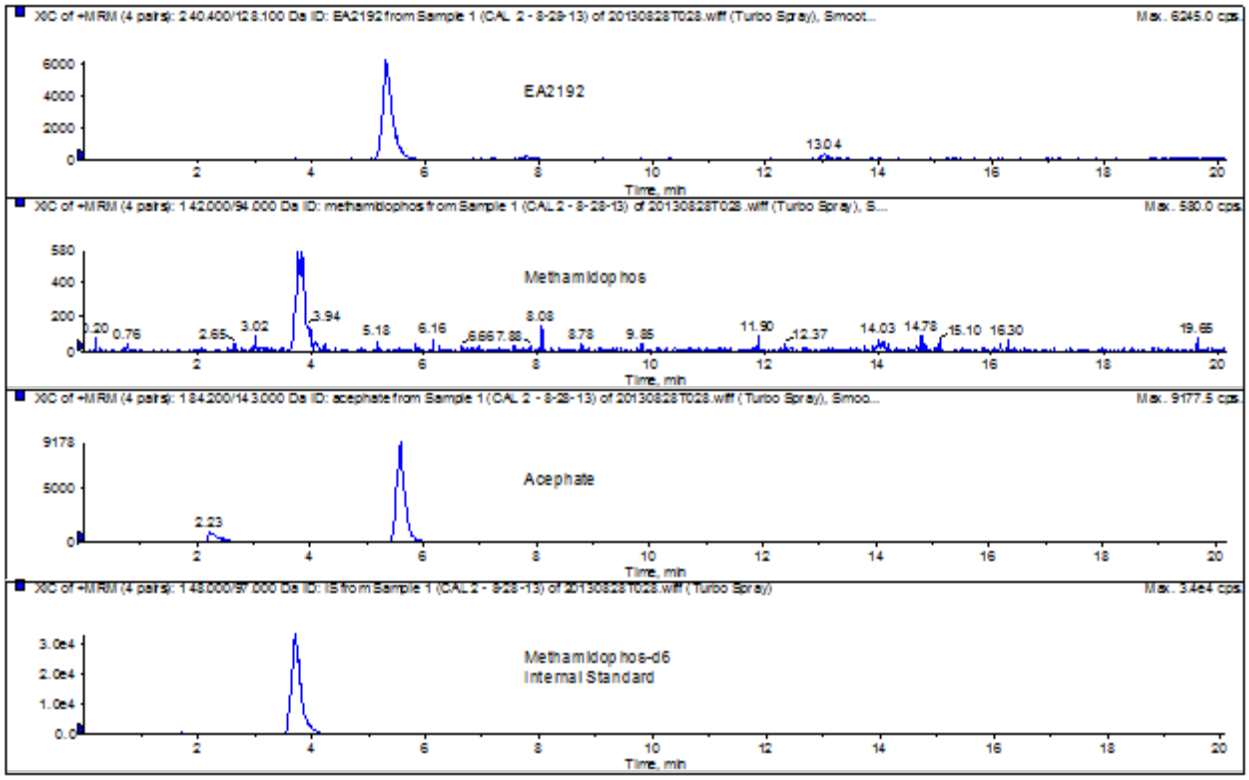


Figure 6. Representative Chromatogram of a Calibration Standard at the Minimum Reporting Level: x-axis, time (minutes); y axis, intensity (counts)

Chromatogram order:
EA2192 (top)
Methamidophos
Acephate
Methamidophos-d6 (bottom)

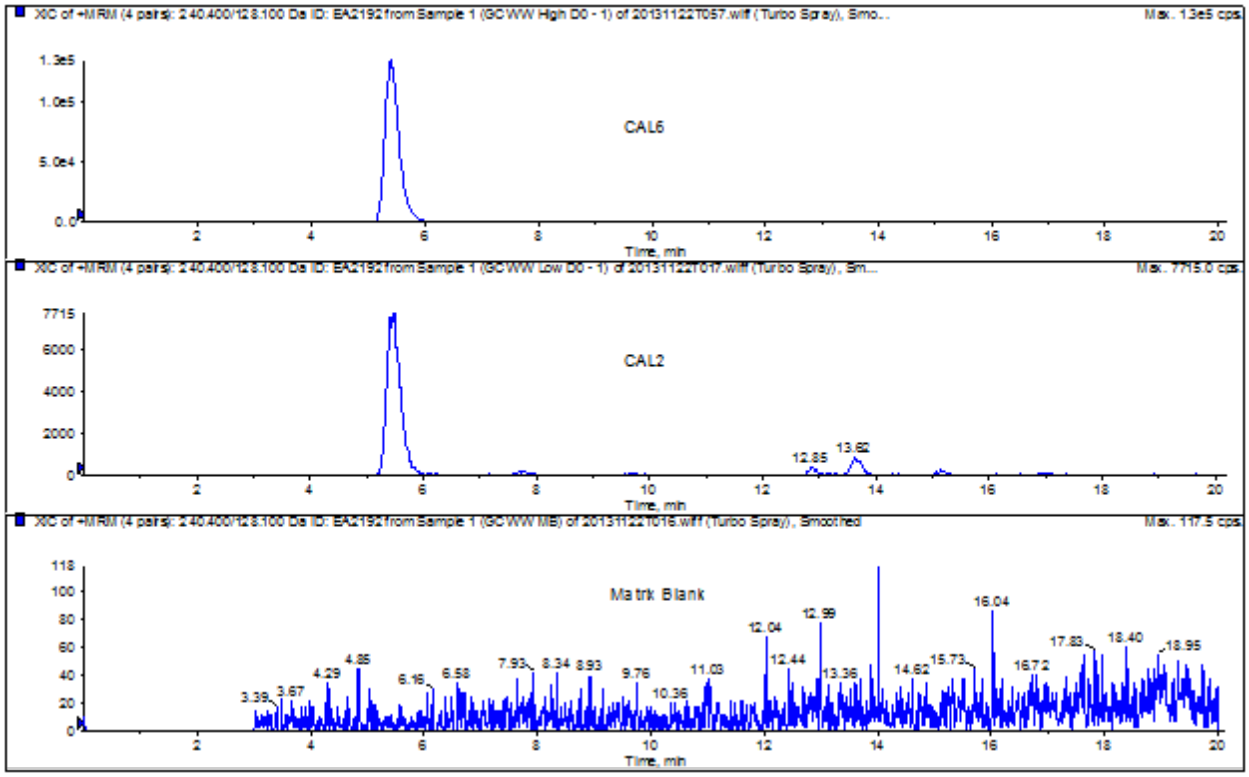


Figure 7. Representative EA2192 Chromatograms of Source No. 1 Water : x-axis, time (minutes); y axis, intensity (counts)

Chromatogram order:
High Concentration Time 0 (top)
Low Concentration Time 0 (middle)
Matrix Blank (bottom)

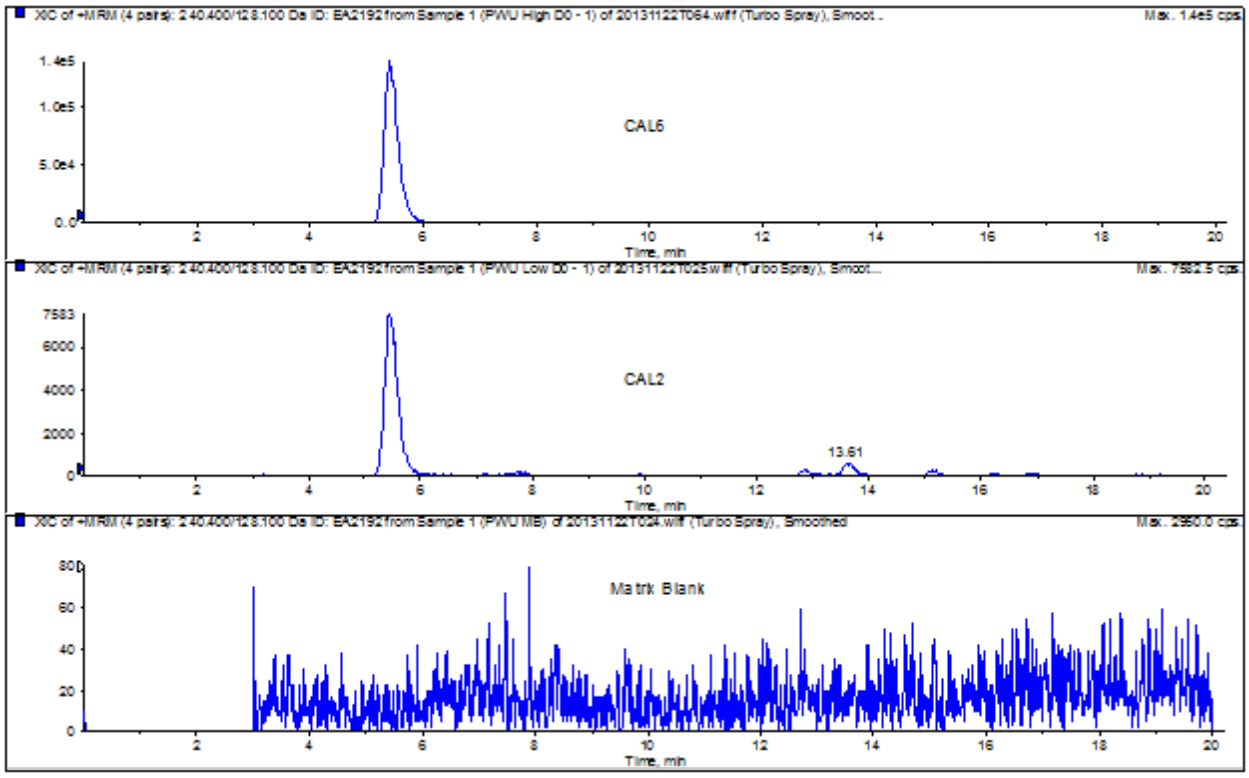


Figure 8. Representative EA2192 Chromatograms of Source No. 2 Water: x-axis, time (minutes); y axis, intensity (counts)

Chromatogram order:
High Concentration Time 0 (top)
Low Concentration Time 0 (middle)
Matrix Blank (bottom)

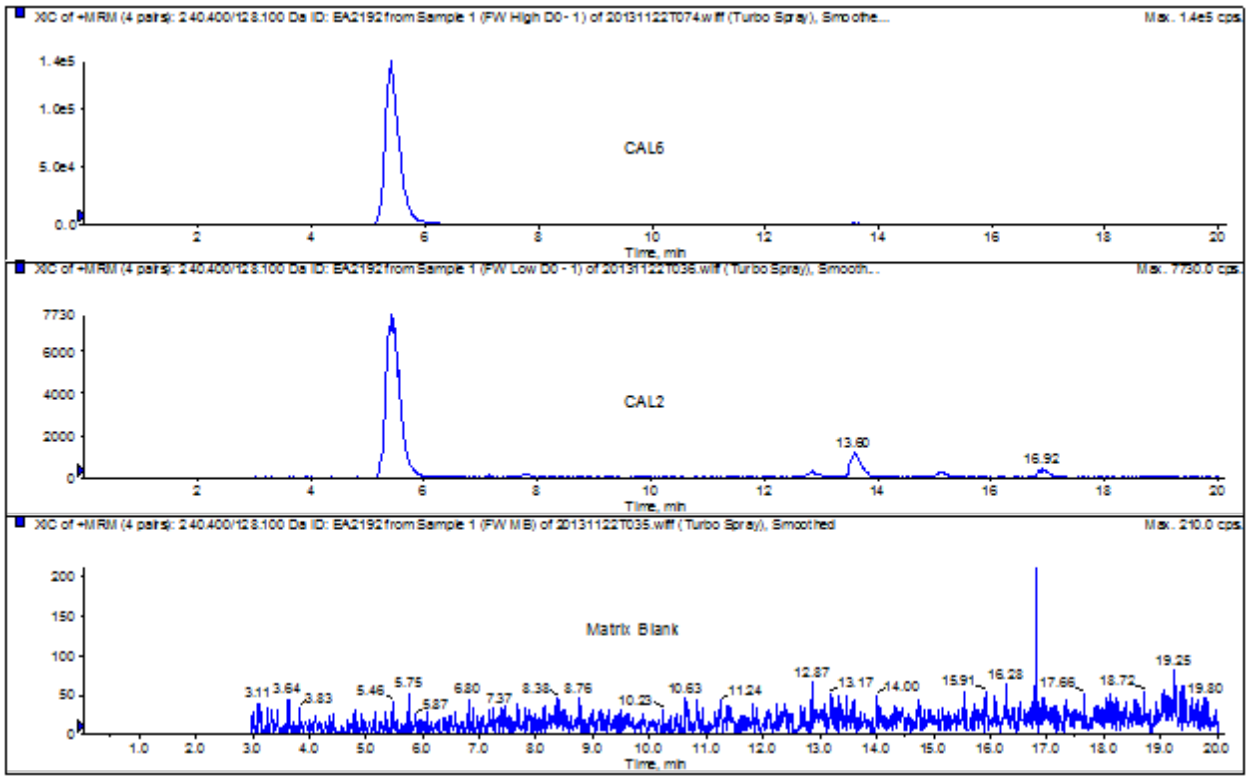


Figure 9. Representative EA2192 Chromatograms of Source No. 3 Water: x-axis, time (minutes); y axis, intensity (counts)

Chromatogram order:
High Concentration Time 0 (top)
Low Concentration Time 0 (middle)
Matrix Blank (bottom)

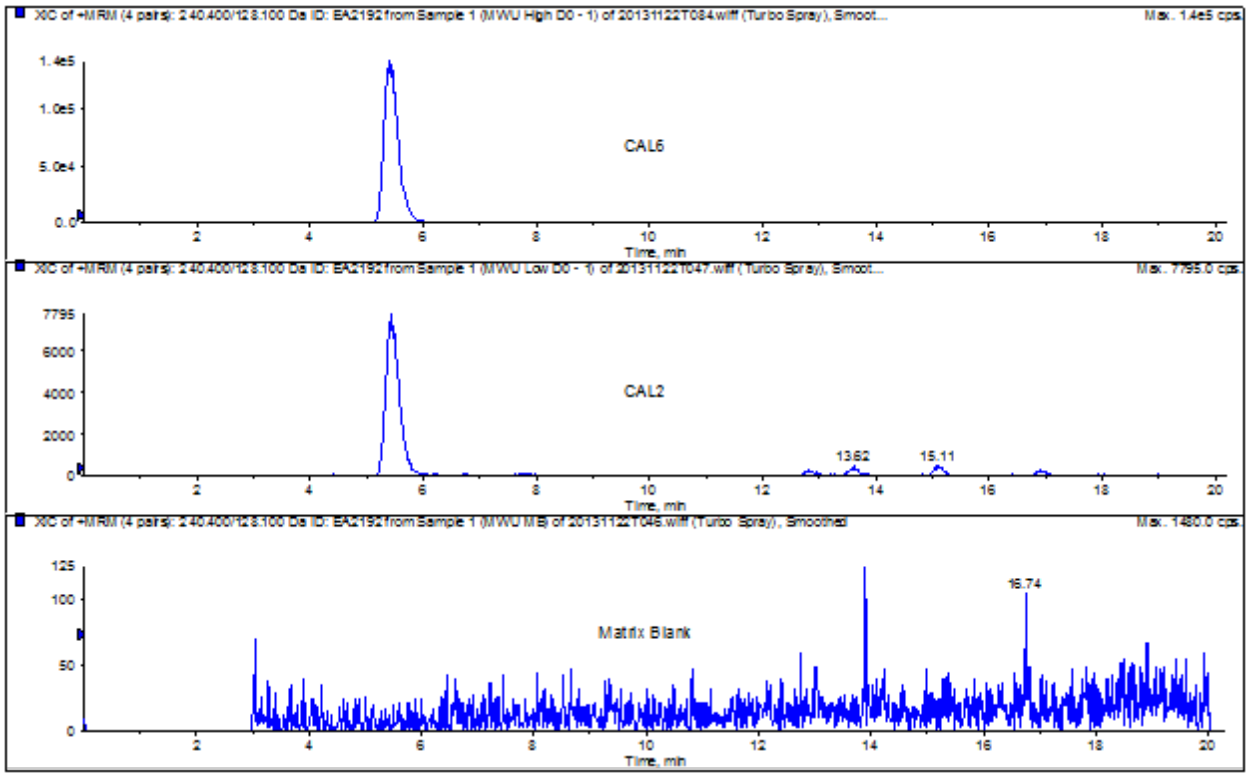


Figure 10. Representative EA2192 Chromatograms of Source No. 4 Water: x-axis, time (minutes); y axis, intensity (counts)

Chromatogram order:
High Concentration Time 0 (top)
Low Concentration Time 0 (middle)
Matrix Blank (bottom)

19. ATTACHMENTS

19.1 Ultra-High Performance Chromatography (UPLC) Method Development and Results by Adapting of the Conditions of U.S. EPA Method 538 for Ultra-High Performance Liquid Chromatography/Tandem Mass Spectrometry (UPLC/MS/MS) Analysis of EA2192 in Water

19.2 CERTIFICATES OF ANALYSIS – EA2192, Methamidophos, and Acephate

19.1 ULTRA-HIGH PERFORMANCE CHROMATOGRAHPY (UPLC) METHOD DEVELOPMENT AND RESULTS

19.1.1 UPLC/MS/MS METHOD DEVELOPMENT – A preliminary method was developed to transfer the adapted conditions from U.S. EPA Method 538 using High-performance liquid chromatography/tandem mass spectrometry (HPLC/MS/MS) to UPLC/MS/MS for the analysis of EA2192. Modifying this method to incorporate UPLC analysis would drastically shorten the analytical run time from the current 30 minute method to 5 minutes or less. In case of a time sensitive environmental incident, the shorter analysis time could be vital for increasing laboratory efficiency. The flow diversion valve was included in the method due to a decrease in sensitivity over the course of longer analyses. By switching to UPLC/MS/MS, the analysis of 100 injections could be accomplished in under ten hours, whereas by HPLC/MS/MS, this same analysis would take over 50 hours.

Standards of EA2192, methamidophos, acephate, and methamidophos-d6 were injected as a sub-set of the Method 538 analytes to determine the feasibility of transferring the method to UPLC. Once a method was developed, the samples prepared for the Water Filtration Study (Table 22) were injected using the developed UPLC/MS/MS method to compare the two analytical methods.

19.1.2 UPLC SYSTEM AND PARAMETERS – The following UPLC system and parameters were used in the UPLC/MS/MS method. The UPLC gradient program is detailed below. The gradient used was derived from the U.S. EPA Method 538 gradient and optimized for UPLC analysis.

UPLC:	Waters Acquity
Column:	Waters Acquity HSS (high strength silica) T3, 1.8 µ, 100 × 2.1 mm
Mobile Phase A:	20mM Ammonium formate in water
Mobile Phase B:	100 % Methanol
Flow Rate:	0.6 mL/min
Injection Volume:	30 µL
Run Time:	5.0 min
Sample Temperature:	5 °C
Column Temperature:	45 °C
Needle Wash A:	50/50 (v/v) Methanol/Water
Needle Wash B:	100 % Methanol

UPLC/MS/MS Gradient

Time	%A	%B
0	90	10
1.3	70	30
3.5	70	30
3.51	10	90
4.2	10	90
4.21	90	10
5	90	10

19.1.3 MASS SPECTROMETER SYSTEM AND PARAMETERS – The mass spectrometer parameters stayed consistent with the parameters used for HPLC/MS/MS analyses (Table 11 and Table 12).

19.1.4 UPLC/MS/MS METHOD DEVELOPMENT RESULTS – The retention times (RTs) of the four analytes are listed below. See Figure 11 and Figure 12 for representative chromatograms of UPLC analyses.

Analyte	HPLC RT (min)	UPLC RT (min)
Methamidophos	3.3	1.0
Acephate	5.2	1.2
EA2192	5.4	1.2
Methamidophos-d6	3.7	1.0

RT= retention time

The average concentration of the filtered low concentration samples was 105 % of the non-filtered samples. The average concentration of the filtered high concentration samples was 107 % of the non-filtered samples. The UPLC water filtration study results are summarized in Table 22. The results obtained from the UPLC and HPLC analyses (see Table 21) were very similar.

Further work is suggested to complete the transfer of Method 538 conditions for EA 2192 described here to UPLC to include:

- Additional method development for all remaining Method 538 analytes to confirm retention times and optimize the UPLC gradient.
- Initial Demonstration of Capability (IDC) testing to confirm method acceptability.
- Addition of other CWA-related chemicals to Method 538.
- Tap waters high in total organic carbon (TOC) and hardness will be evaluated under the UPLC conditions.

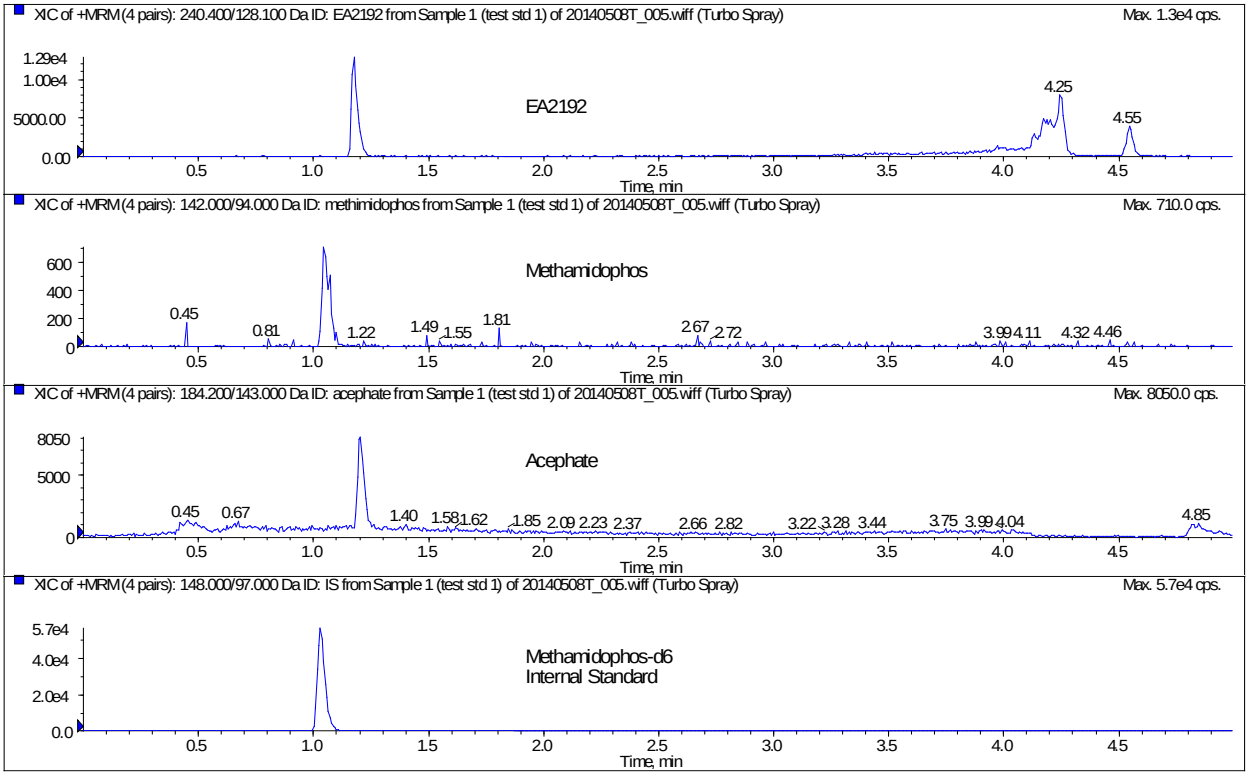


Figure 11. CAL1 Standard, UPLC/MS/MS Analysis

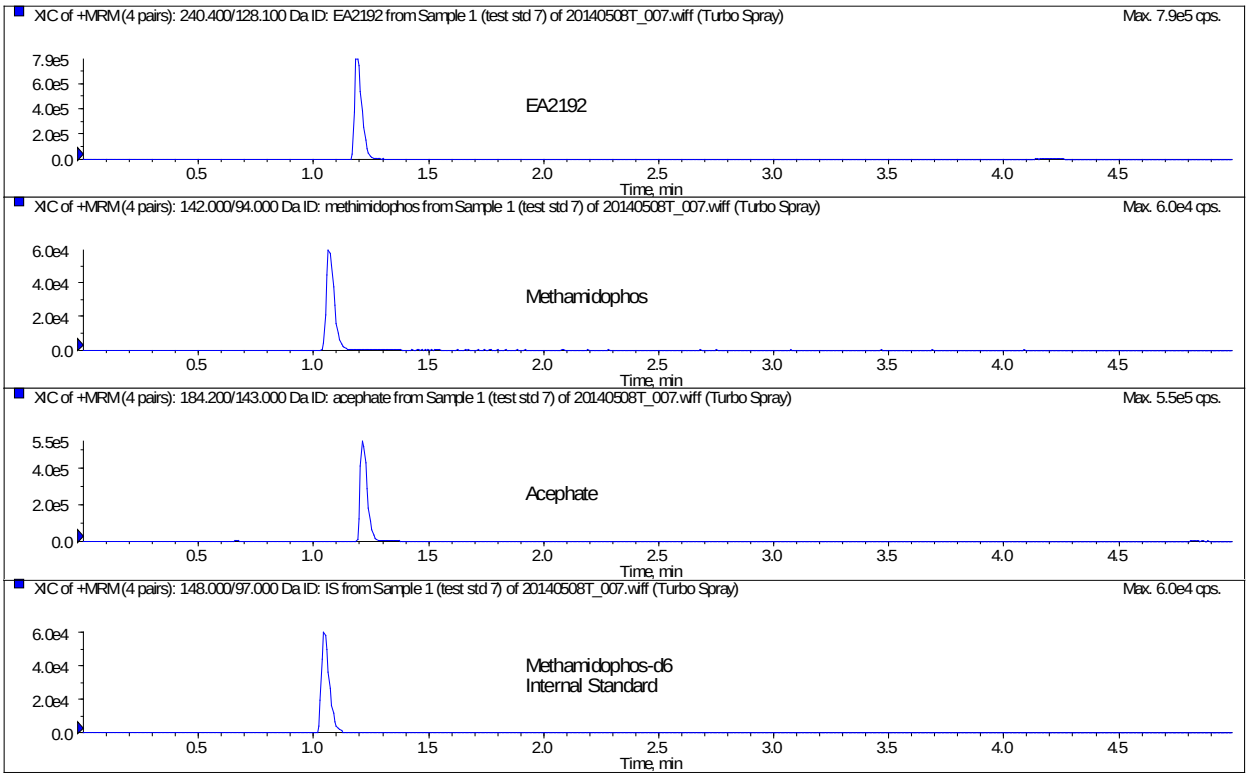


Figure 12. CAL7 Standard, UPLC/MS/MS Analysis

Table 22. Filtered Water Comparison Study (UPLC)

Low Concentration (µg/L)				High Concentration (µg/L)		
Condition	Average*	% of Non-Filtered		Condition	Average*	% of Non-Filtered
	Std. Dev.				Std. Dev.	
	% RSD				% RSD	
Non-Filtered	0.121	--		Non-Filtered	2.80	--
	0.0126				0.179	
	10.4				6.4	
Filtered	0.127	105 %		Filtered	2.99	107 %
	0.0110				0.152	
	8.7				5.1	

***NOTE:** Seven replicates were prepared and analyzed for each concentration at each time point.

19.2 Certificates of Analysis – EA2192, Methamidophos, and Acephate

MRIGlobal
National Solutions Worldwide Impact

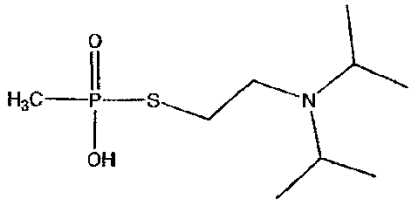
CERTIFICATE OF ANALYSIS

EA2192: [(S-diisopropylamino0ethyl]methylphosphonic acid

MRIGlobal Agent ID: EA2192110301-DOC-1

Original data is archived under MRIGlobal Project No. 610105.02.002.01

Compound Identification



Product:	[(S-diisopropylamino0ethyl]methylphosphonic acid
Empirical Formula:	$C_9H_{22}NO_2PS$
Molecular Weight:	239.32
ECBC Lot #:	NA
Primary Standard ID.:	12632-50-2
Solvent:	Acetonitrile-d3

Quality

Purity (%):	94.2%
Storage Conditions:	< 4 °C
Date of Analysis:	July 9, 2013
Expiration Date:	Requires re-assessment after July 9, 2014
Standard Operating Procedure:	MRI-5870, Rev. 5

Experimental Techniques

1) Nuclear Magnetic Resonance (^{31}P-NMR). See data package dated July 24, 2013, for complete details on the purity assessment.

Approved: _____ Date: 8/14/13

Jeff Smith
Chemical Agent Custodian
CA Group/Test and Evaluation Section/NSSI Division

Missouri - Colorado - Florida - Maryland - Virginia - Kansas - Washington, D.C.
Science Engineering Integration Management
425 Volker Blvd., Kansas City, MO 64110-2241 Phone: 816-753-7600 Fax: 816-753-8420 www.mriglobal.org

SIGMA-ALDRICH

CERTIFICATE OF ANALYSIS

Sigma-Aldrich Laborchemikalien GmbH D-30918 Seelze
Telefon: +49 5137 8238-150

Seelze, 21.01.2013/467129/13/01301

Order-No.:
Customer-No.:

Order-Code:

Quantity:

Production Date: 11.Jan.2013
Expiry Date: 11.Jan.2016

Article/Product: 33395 Batch : SZBD011XV

Methamidophos PESTANAL®

Reference Material (RM)

1. General Information

Formula: C2H8NO2PS
CAS-No.: [10265-92-6]
Usage : Acaricide/Insecticide

Molar mass: 141.13 g/Mole
Recomm. storage temp.: -20 °C

The estimated uncertainty of a single measurement of the assay can be expected to be 1 % relative (confidence level = 95%, n= 6) whereby the assay measurements are calculated by 100% minus found impurities.

2. Batch Analysis

Identity (NMR)	complying	
Assay (HPLC)	97.7	area %
Melting range	40.0-45.0	°C
Water (Karl Fischer)	0.2	%
Date of Analysis	18.Jan.2013	

3. Advice and Remarks

- The expiry date is based on the current knowledge and holds only for proper storage conditions in the originally closed flasks/ packages.
- Whenever the container is opened for removal of aliqout portions of the substance, the person handling the substance must assure, that the integrity of the substance is maintained and proper records of all its handlings are kept. Special care has to be taken to avoid any contamination or adulteration of the substance.
- We herewith confirm that the delivery is effected according to the technical delivery conditions agreed.
- Particular properties of the products or the suitability for a particular area of application are not assured.
- We guarantee a proper quality within our General Conditions of Sales.

Sigma-Aldrich Laborchemikalien GmbH
Quality Management SA-LC

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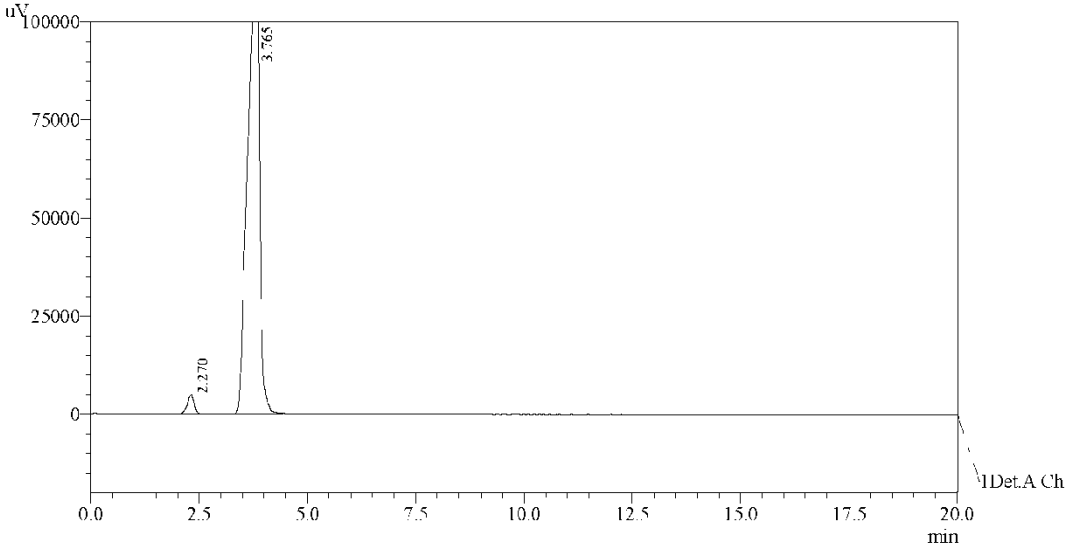
HPLC-Method

Article	: Methamidophos
Article-No	: 33395
Batch	: SZBD011XV
Column	: L=250mm, ID=4,6mm; Supelcosil LC-18 5µm
Eluent	: 40 % Acetonitrile 60 % Water
Flow	: 0,8ml/min
Detector	: UV-215nm
Injection-Volume	: 20µl
Sample-Preparation	: 2mg/ml Acetonitrile
Linearity	: checked
Evaluation	: Normalisation (uncorrected)
Operator	: Schowe

Chromatogram
Methamidophos C:\LabSolutions\Data\Project1\1300763.AIA\SIGNAL01.cdf



1 Det.A Ch1 /

PeakTable

Ch1

Peak#	Ret. Time	Area	Area %
1	2.270	56	2.298
2	3.765	2364	97.702
Total		2420	100.000

SIGMA-ALDRICH

CERTIFICATE OF ANALYSIS

Sigma-Aldrich Laborchemikalien GmbH D-30918 Seelze
Telefon: +49 5137 8238-150

Seelze, 21.12.2010/153344/10/06407

Order-No.:
Customer-No.:

Order-Code:

Quantity:

Production Date: 24.Mar.2010
Expiry Date: 24.Mar.2015

Article/Product: 45315 Batch : SZBA083XV

Acephate PESTANAL®

Reference Material (RM)

1. General Information

Formula: C4H10NO3PS
CAS-No.: [30560-19-1]
Usage : Insecticide

Molar mass: 183.17 g/Mole
Recomm. storage temp.: 2-8 °C

The estimated uncertainty of a single measurement of the assay can be expected to be 1 % relative (confidence level = 95%, n= 6) whereby the assay measurements are calculated by 100% minus found impurities.

2. Batch Analysis

Identity (NMR)	complying	
Assay (GC)	97.8	area %
Melting range	87.0-90.8	°C
Water (Karl Fischer)	0.07	%
Date of Analysis	15.Apr.2010	

3. Advice and Remarks

- The minimum shelf life is based on the current knowledge and holds only for proper storage conditions in the originally closed flasks/ packages.
- Whenever the container is opened for removal of aliqout portions of the substance, the person handling the substance must assure, that the integrity of the substance is maintained and proper records of all its handlings are kept. Special care has to be taken to avoid any contamination or adulteration of the substance.
- We herewith confirm that the delivery is effected according to the technical delivery conditions agreed.
- Particular properties of the products or the suitability for a particular area of application are not assured.
- We guarantee a proper quality within our General Conditions of Sales.

Sigma-Aldrich Laborchemikalien GmbH
Quality Management SA-LC

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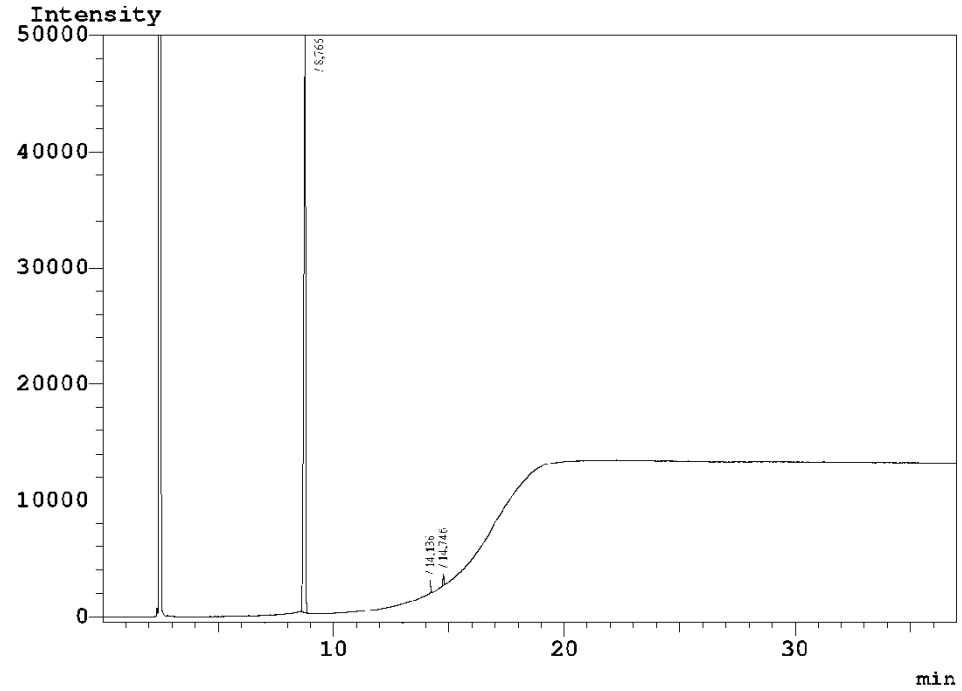
GLC-Method

Article : Acephate
Article-No : 45315
Batch : SZBA083XV

Column : MDN-5, 30m,fs cap.,I.D.=0,32mm,1,0micron df
Inj.-temp. : 280°C
Det.temp. : 330°C
Oven-temp. : 150°(4min)to 320°C(10°/min)hold 15min
Split : 1:100
Flow : 1ml He/min
Inj.v. : 1µl solution in Dichloromethane
Evaluation : Normalisation(uncorrected)
Operator : Schulz



Peak Table - Channel 1

Peak#	Ret.Time	Height	Area	Area%
1	8,766	53854	263888	97,8284
2	14,186	1120	3031	1,1236
3	14,746	1052	2827	1,0481
Total		56026	269746	100,0000

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