

1.0 EXECUTIVE SUMMARY

The purpose of this study was to perform an independent laboratory validation of Syngenta Analytical Method T001681-06 entitled "Analytical Method for the Determination of Atrazine, Simazine, Propazine, G-30033, G-28279, G-28273, Ametryn, Prometryn, GS-11354, GS-11355, GS-26831, S-Metolachlor, Metolachlor-ESA, Metolachlor-OA and Mesotrione in Water Using Direct-Aqueous-Injection ESI-LC/MS/MS, Including Validation Data," dated October 4, 2006, using representative water samples.

EPA Guideline OCSPP 850.6100 (2012) and PR Notice 2011-3 (References 1 and 2). This study was conducted in compliance with EPA FIFRA Good Laboratory Practice Standards, 40 CFR Part 160.

This study was conducted by Morse Laboratories, LLC (Morse Labs) of Sacramento, California according to the protocol for Syngenta Study No. TK0049794 entitled "Atrazine, Simazine, Propazine, G-30033, G-28279, G-28273, Ametryn, Prometryn, GS-11354, GS-11355, GS-26831, S-Metolachlor, Metolachlor-ESA, Metolachlor-OA and Mesotrione - Independent Laboratory Validation of Residue Method (Syngenta Number T001681-06) for the Determination of Atrazine, Simazine, Propazine, G-30033, G-28279, G-28273, Ametryn, Prometryn, GS-11354, GS-11355, GS-26831, S-Metolachlor, Metolachlor-ESA, Metolachlor-OA and Mesotrione in Water by LC-MS/MS" (Appendix 1).

The method is suitable for the determination of atrazine, simazine, propazine, G-30033, G-28279, G-28273, ametryn, prometryn, GS-11354, GS-11355, GS-26831, S-metolachlor, metolachlor-ESA, metolachlor-OA and mesotrione in surface and ground water.

The first method validation trials conducted on surface and ground water were unsuccessful due to inconsistent recoveries for various analytes. It was determined the cause of the out-of-range recoveries was likely related to chromatography; therefore, as a result, a second validation trial was performed after completing instrument maintenance and rectifying sources of enhancement or suppression. The method was successfully validated on the second attempt for both surface and ground water at the LOQ and 10 × LOQ concentration levels using the method as written. The stated LOQ in the method is 0.05 µg/L (0.05 ppb) for all analytes, except for G-28273 which has an LOQ of 0.50 µg/L (0.50 ppb).

The successful validation results are summarized below. See Tables 2 and 3 for individual recovery data.

A single analyst completed a sample set consisting of 13 samples in approximately three hours with LC-MS/MS analysis performed overnight.

2.0 INTRODUCTION

The purpose of this study was to perform an independent laboratory validation of Syngenta Analytical Method T001681-06 entitled "Analytical Method for the Determination of Atrazine, Simazine, Propazine, G-30033, G-28279, G-28273, Ametryn, Prometryn, GS-11354, GS-11355, GS-26831, *S*-Metolachlor, Metolachlor-ESA, Metolachlor-OA and Mesotrione in Water Using Direct-Aqueous-Injection ESI-LC/MS/MS, Including Validation Data," dated October 4, 2006, using representative water samples.

This study was designed to satisfy guideline requirements described in EPA Guideline OCSP 850.6100 (2012) and PR Notice 2011-3 (References 1 and 2). This study was conducted in compliance with EPA FIFRA Good Laboratory Practice Standards, 40 CFR Part 160.

The method is suitable for the determination of atrazine, simazine, propazine, G-30033, G-28279, G-28273, ametryn, prometryn, GS-11354, GS-11355, GS-26831, *S*-metolachlor, metolachlor-ESA, metolachlor-OA and mesotrione in surface and ground water.

To summarize, water samples were received cold and then refrigerated until removed for analysis. The samples were allowed to re-equilibrate to room temperature before an aliquot sample of water was transferred to a suitable vessel (i.e. test tube) and fortified as necessary by judicious choice of standard solution concentration and sample volume (as permitted by the method). After fortification, a portion of the sample was transferred to an auto-sampler vial. The sample vials were loaded onto the injection tray for LC/MS/MS analysis. Two injection sequences for the positive and negative ESI detections were performed. Residue quantification was carried out using external standard calibrations. The limit of quantification of the method is 0.05 ppb for all analytes (except G-28273 which has an LOQ of 0.50 ppb) in surface and ground water.

The method was performed as written. Communication with the Study Sponsor occurred to discuss items such as 1) clarification/approval of the protocol and method, 2) acquisition of

analytical standards and control samples and 3) questions regarding preparation of reagents. A complete list of communications is provided in Appendix 6.

3.0 MATERIALS AND METHODS

3.1 Test Item/Reference Substances

The analytical (reference) standards used for this study are:

Atrazine (G-30027):

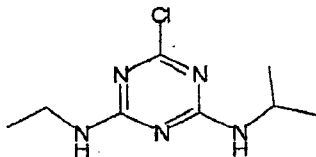
Chemical Name:

IUPAC:

CAS:

2-chloro-4-ethylamino-6-isopropylamino-s-triazine
1,3,5-triazine-2,4-diamine, 6-chloro-N-ethyl-N'-
(1-methylethyl)-

Structural Formula:



CAS No.:

Molecular Weight:

Source:

Purity:

Batch ID:

Receipt Date:

Expiration Date:

Storage:

1912-24-9

215.68 g/mol

Syngenta Crop Protection, LLC

96.2%

410313

April 3, 2013

End of August 2017

Refrigerated (2 °C to 8°C)

Simazine (G-27692):

Chemical Name:

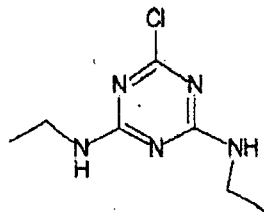
IUPAC:

CAS:

2-chloro-4,6-bis(ethylamino)-s-triazine

1,3,5-triazine-2,4-diamine, 6-chloro-N,N'-diethyl-

Structural Formula:



CAS No.:

Molecular Weight:

Source:

122-34-9

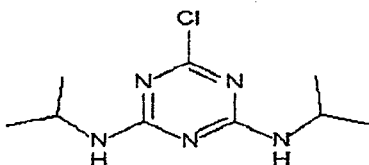
201.66 g/mol

Syngenta Crop Protection, LLC

Purity: 98.8%
Batch ID: 486779
Receipt Date: April 3, 2013
Expiration Date: End of June 2013, extended to end of June 2016
Storage: Refrigerated (2 °C to 8°C)

Propazine (G-30028):

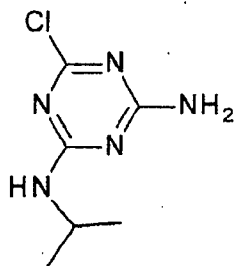
Chemical Name:
IUPAC: 2-Chloro-4,6 bis(isopropylamino)-s-triazine
CAS: 1,3,5-triazine-2,4-diamine, 6-chloro-N,N'-bis(1-methylethyl)-
Structural Formula:



CAS No.: 139-40-2
Molecular Weight: 229.71 g/mol
Source: Syngenta Crop Protection, LLC
Purity: 98.5%
Batch ID: 423440
Receipt Date: April 3, 2013
Expiration Date: End of September 2013, extended to end of September 2016
Storage: Refrigerated (2 °C to 8°C)

G-30033:

Chemical Name:
IUPAC: 2-amino-4-chloro-6-(isopropylamino)-s-triazin
CAS: 1,3,5-Triazine-2,4-diamine, 6-chloro-N-(1-methylethyl)-
Structural Formula:

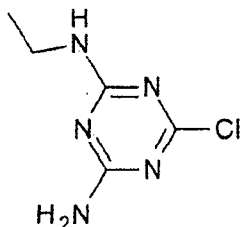


CAS No.: 6190-65-4
Molecular Weight: 187.63 g/mol
Source: Syngenta Crop Protection, LLC
Purity: 98.9%
Batch ID: DAH-XXXIV-77

Receipt Date: April 11, 2013
Expiration Date: February 28, 2015
Storage: Refrigerated (2 °C to 8°C)

G-28279:

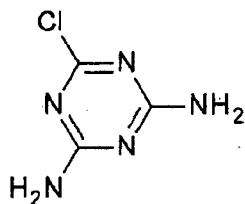
Chemical Name:
IUPAC: not available
CAS: 1,3,5-Triazine-2,4-diamine, 6-chloro-N-ethyl-
Structural Formula:



CAS No.: 1007-28-9
Molecular Weight: 173.60 g/mol
Source: Syngenta Crop Protection, LLC
Purity: 99.4%
Batch ID: DAH-XXXIII-62
Receipt Date: April 11, 2013
Expiration Date: December 31, 2014
Storage: Refrigerated (2 °C to 8°C)

G-28273:

Chemical Name:
IUPAC: not available
CAS: 1,3,5-Triazine-2,4-diamine, 6-chloro-
Structural Formula:

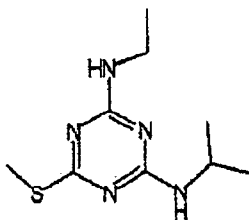


CAS No.: 3397-62-4
Molecular Weight: 145.55 g/mol
Source: Syngenta Crop Protection, LLC
Purity: 99.2%
Batch ID: DAH-XXXIV-65

Receipt Date: April 11, 2013
Expiration Date: February 28, 2015
Storage: Refrigerated (2 °C to 8°C)

Ametryn (G-34162):

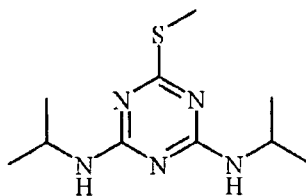
Chemical Name:
IUPAC: N-Ethyl-N'-isopropyl-6-methylsulfanyl-[1,3,5]triazine-2,4-diamine
CAS: 1,3,5-Triazine-2,4-diamine, N-ethyl-N'-(1-methylethyl)-6-(methylthio)-
Structural Formula:



CAS No.: 834-12-8
Molecular Weight: 227.33 g/mol
Source: Syngenta Crop Protection, LLC
Purity: 98.3%
Batch ID: 410430
Receipt Date: April 3, 2013
Expiration Date: October 2017
Storage: Refrigerated (2 °C to 8°C)

Prometryn (G-34161):

Chemical Name:
IUPAC: 2,4-Bis(isopropylamino)-6-methylthio-s-triazine
CAS: 1,3,5-Triazine-2,4-diamine, N,N'-bis(1-methylethyl)-6-(methylthio)-
Structural Formula:

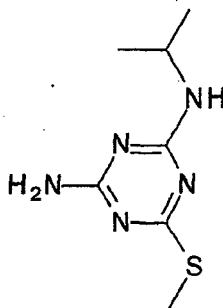


CAS No.: 7287-19-6
Molecular Weight: 241.36 g/mol
Source: Syngenta Crop Protection, LLC
Purity: 99.7%
Batch ID: 410566

Receipt Date: April 3, 2013
Expiration Date: End of January 2018
Storage: Refrigerated (2 °C to 8°C)

GS-11354:

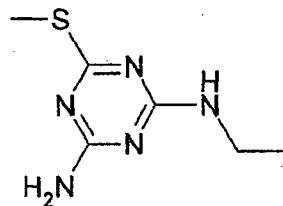
Chemical Name:
IUPAC: not available
CAS: 1,3,5-Triazine-2,4-diamine, N-(1-methylethyl)-6-(methylthio)-
Structural Formula:



CAS No.: 4147-57-3
Molecular Weight: 199.28 g/mol
Source: Syngenta Crop Protection, LLC
Purity: 98.7%
Batch ID: WFH-I-25
Receipt Date: April 11, 2013
Expiration Date: January 31, 2014
Storage: Refrigerated (2 °C to 8°C)

GS-11355:

Chemical Name:
IUPAC: not available
CAS: 1,3,5-Triazine-2,4-diamine, N-ethyl-6-(methylthio)-
Structural Formula:



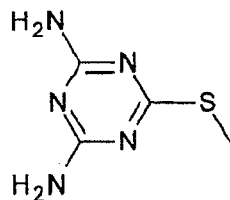
CAS No.: 4147-58-4
Molecular Weight: 185.25 g/mol
Source: Syngenta Crop Protection, LLC
Purity: 99.1%

Batch ID: DAH-XXXI-29-1
Receipt Date: April 11, 2013
Expiration Date: April 30, 2015
Storage: Refrigerated (2 °C to 8°C)

GS-26831:

Chemical Name:
IUPAC: not available
CAS: 1,3,5-Triazine-2,4-diamine, 6-(methylthio)-

Structural Formula:

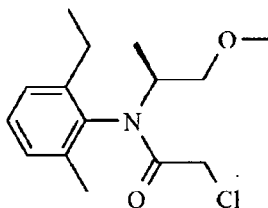


CAS No.: 5397-01-3
Molecular Weight: 157.20 g/mol
Source: Syngenta Crop Protection, LLC
Purity: 99.0%
Batch ID: DAH-XXXII-60
Receipt Date: April 11, 2013
Expiration Date: November 30, 2014
Storage: Refrigerated (2 °C to 8°C)

S-Metolachlor (CGA-77102):

Chemical Name:
IUPAC: (S)-2-ethyl-6-methyl-N-(2-methoxy-1-methylethyl)-chloroacetanilide
CAS: acetamide, 2-chloro-N-(2-ethyl-6-methylphenyl)-N-[2-methoxy-1-methylethyl]-, (S)-

Structural Formula:

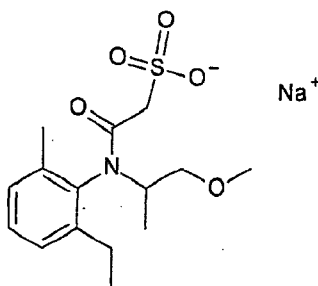


CAS No.: 87392-12-9
Molecular Weight: 283.79 g/mol
Source: Syngenta Crop Protection, LLC
Purity: 97.9%
Batch ID: 410316
Receipt Date: April 3, 2013
Expiration Date: End of August 2015
Storage: Refrigerated (2 °C to 8°C)

Metolachlor-ESA (CGA-354743):

Chemical Name:
IUPAC: not available
CAS: Ethanesulfonic acid, 2-[(2-ethyl-6-methylphenyl)(2-methoxy-1-methylethyl)amino]-2-oxo-, sodium salt

Structural Formula:

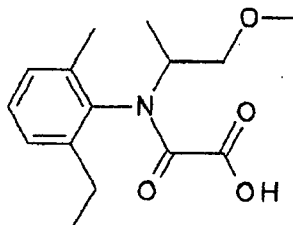


CAS No.: 171118-09-5
Molecular Weight: 351.40 g/mol
Source: Syngenta Crop Protection, LLC
Purity: 94.2%
Batch ID: DAH-XXVII-76
Receipt Date: April 11, 2013
Expiration Date: February 28, 2015
Storage: Refrigerated (2 °C to 8°C)

Metolachlor-OA (CGA-51202):

Chemical Name:
IUPAC: not available
CAS: Acetic acid, [(2-ethyl-6-methylphenyl)(2-methoxy-1-methylethyl)amino]oxo-

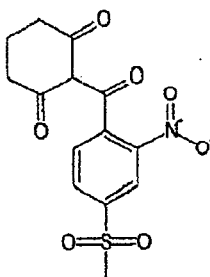
Structural Formula:



CAS No.: 152019-73-3
Molecular Weight: 279.33 g/mol
Source: Syngenta Crop Protection, LLC
Purity: 99.4%
Batch ID: DAH-XXVIII-40
Receipt Date: April 11, 2013
Expiration Date: March 31, 2014
Storage: Refrigerated (2 °C to 8°C)

Mesotrione (ZA-1296):

Chemical Name:
IUPAC: 2-[4-(methylsulfonyl)-2-nitrobenzoyl]-1,3-cyclohexanedione
CAS: 1,3-cyclohexanedione, 2-[4-(methylsulfonyl)-2-nitrobenzoyl]
Structural Formula:



CAS No.: 104206-82-8
Molecular Weight: 339.32 g/mol
Source: Syngenta Crop Protection, LLC
Purity: 99.5%
Batch ID: 492970
Receipt Date: April 3, 2013
Expiration Date: End of February 2016
Storage: Freezer (-10 °C to -25 °C)

Characterization data for the test/reference standards are maintained by the Sponsor. The Certificates of Analysis are included in Appendix 3.

3.2 Test System

The test systems evaluated in this study were surface and ground water. These matrices were chosen because they are representative of the matrices for which the method was designed.

The control samples used in this study were provided by the Sponsor. They were received at Morse Laboratories cold and in good condition on March 27, 2013. Upon receipt, the samples were placed in limited-access refrigerated storage (typically 2 °C to 8 °C), where they remained pending analysis for suitability. The samples were logged in according to Morse Labs SOPs. The sample was uniquely identified by the Sponsor. The laboratory assigned additional designations such as "control," and "fortified control" as appropriate. Refrigerator storage temperatures were monitored on a daily basis and were typically at 2 °C to 8 °C. Except for the periods during which the samples were removed for analysis, they were stored refrigerated.

3.3 Equipment and Reagents/Supplies

The equipment and reagents/supplies used for the method validation were as outlined in the method. Identical or equivalent equipment and materials were used, as permitted by the method. The equivalent equipment and reagents used were as follows:

3.3.1 Equipment

Autosampler vials:	Xpertek 2 mL, polypropylene vials 12 × 32-mm (Cobert Associates, P# 954002)
Autosampler vial caps:	Xpertek Cap Pk, screw caps, black with bonded PTFE/SIL Septa (Cobert Associates, P# 952228)
Disposable pasteur pipets:	5 ¾ inch, Fisher Scientific, Catalog #13-678-20A
Glass graduated pipets:	1-mL, KIMAX-51, No. 37033
Glass reservoir:	Mobile phase reservoir, 1-Liter, Wheatman
Pipets, electronic:	EDP electronic pipets, various sizes and corresponding pipet tips (Rainin Instrument Co., Inc., Ridgefield, NJ)
Vortexer:	VWR mini-vortexer (VWR Scientific, Bridgeport, NJ)

3.3.2 Reagents

Formic acid:	98% GR ACS, EMD Chemicals
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3.3.3 Preparation of Reagents

Reagent solutions were prepared as specified³⁸ in the method. Solutions where equivalent preparation methods were used were as follows:

1. Acetonitrile:HPLC Water 5:95 (v/v);
 - a. prepared by mixing 100-mL of HPLC water and 5-mL of HPLC acetonitrile.
 - b. prepared by mixing 475-mL of HPLC water and 25-mL of HPLC acetonitrile.
 - c. prepared by mixing 570-mL of HPLC water and 30-mL of HPLC acetonitrile.

3.4 Preparation of Standard Solutions

The preparation of standard solutions used for this study is described below. The solutions were stored as recommended in the method when not in use (refrigerated, 2 °C to 8 °C).

3.4.1 Stock Standard Solution

For all analytes with the exception of G-30033, G-28279 and G-28273, 10.0 mg (corrected for purity) of analytical standard were accurately weighed and quantitatively transferred to a 100-mL volumetric flask. The contents were brought to volume with HPLC methanol to make a stock standard solution having a concentration of 100 µg/mL.

For G-30033, G-28279 and G-28273, 5.0 mg (corrected for purity) of analytical standard were accurately weighed and quantitatively transferred to a 100-mL volumetric flask. The contents were brought to volume with HPLC methanol to make a stock standard solution having a concentration of 50 µg/mL.

3.4.2 Fortification/Intermediate Standard Solutions

5.0 µg/mL: 5.0 mL of each 100-µg/mL stock standard solution and 10 mL of each 50-µg/mL stock standard solution were transferred to a 100-mL volumetric flask. The contents were brought to volume with HPLC methanol and mixed well.

0.05 µg/mL: 500 µL of a 5.0-µg/mL mixed standard solution were transferred to a 50-mL volumetric flask. The contents were brought to volume with HPLC acetonitrile:HPLC water (5:95, v/v) and mixed well.

0.005 $\mu\text{g}/\text{mL}$: 50 μL of a 5.0- $\mu\text{g}/\text{mL}$ mixed standard solution were transferred to a 50-mL volumetric flask. The contents were brought to volume with HPLC acetonitrile:HPLC water (5:95, v/v) and mixed well.

3.4.3 HPLC (Calibration) Standard Solutions

4.0 $\text{pg}/\mu\text{L}$: 40 μL of a 5.0- $\mu\text{g}/\text{mL}$ mixed standard solution were transferred to a 50-mL volumetric flask. The contents were brought to volume with HPLC acetonitrile:HPLC water (5:95, v/v) and mixed well.

2.0 $\text{pg}/\mu\text{L}$: 25 mL of a 4.0- $\text{pg}/\mu\text{L}$ mixed standard solution were transferred to a 50-mL volumetric flask. The contents were brought to volume with HPLC acetonitrile:HPLC water (5:95, v/v) and mixed well.

1.0 $\text{pg}/\mu\text{L}$: 25 mL of a 2.0- $\text{pg}/\mu\text{L}$ mixed standard solution were transferred to a 50-mL volumetric flask. The contents were brought to volume with HPLC acetonitrile:HPLC water (5:95, v/v) and mixed well.

0.5 $\text{pg}/\mu\text{L}$: 25 mL of a 1.0- $\text{pg}/\mu\text{L}$ mixed standard solution were transferred to a 50-mL volumetric flask. The contents were brought to volume with HPLC acetonitrile:HPLC water (5:95, v/v) and mixed well.

0.2 $\text{pg}/\mu\text{L}$: 20 mL of a 0.5- $\text{pg}/\mu\text{L}$ mixed standard solution were transferred to a 50-mL volumetric flask. The contents were brought to volume with HPLC acetonitrile:HPLC water (5:95, v/v) and mixed well.

0.1 $\text{pg}/\mu\text{L}$: 25 mL of a 0.2- $\text{pg}/\mu\text{L}$ mixed standard solution were transferred to a 50-mL volumetric flask. The contents were brought to volume with HPLC acetonitrile:HPLC water (5:95, v/v) and mixed well.

0.05 $\text{pg}/\mu\text{L}$: 25 mL of a 0.1- $\text{pg}/\mu\text{L}$ mixed standard solution were transferred to a 50-mL volumetric flask. The contents were brought to volume with HPLC acetonitrile:HPLC water (5:95, v/v) and mixed well.

0.02 pg/ μ L: 20 mL of a 0.05-pg/ μ L mixed standard solution were transferred to a 50-mL volumetric flask. The contents were brought to volume with HPLC acetonitrile:HPLC water (5:95, v/v) and mixed well.

3.5 Analytical Method

The analytical method independently validated in this study was Syngenta Analytical Method T001681-06 entitled "Analytical Method for the Determination of Atrazine, Simazine, Propazine, G-30033, G-28279, G-28273, Ametryn, Prometryn, GS-11354, GS-11355, GS-26831, S-Metolachlor, Metolachlor-ESA, Metolachlor-OA and Mesotrione in Water Using Direct-Aqueous Injection ESI-LC/MS/MS, Including Validation Data," dated October 4, 2006. See Appendix 2 for the complete text of the method. The following is a summary of that method:

To summarize, water samples were received cold and then refrigerated until removed for analysis. The samples were allowed to re-equilibrate to room temperature before an aliquot sample of water was transferred to a suitable vessel (i.e. test tube) and fortified as necessary by judicious choice of standard solution concentration and sample volume (as permitted by the method). After fortification, a portion of the sample was transferred to an auto-sampler vial. The sample vials were loaded onto the injection tray for LC/MS/MS analysis. Two injection sequences for the positive and negative ESI detections were performed. Residue quantification was carried out using external standard calibrations. The limit of quantification of the method is 0.05 ppb for all analytes (except G-28273 which has an LOQ of 0.50 ppb) in surface and ground water.

The method was used as written.

Residue calculations were performed as specified in the analytical method, using the multi-point calibration procedure. Calculations were conducted using a validated software application (Applied BioSystems/ MDS Sciex Analyst Software, version 1.6) to create a standard curve based on linear regression and Microsoft Excel[®] 2007. All standards injected were used to generate the standard curve. The regression functions were used to calculate a best-fit line (from a set of standard concentrations in pg/ μ L versus peak response) and to determine concentration of the analyte found during sample analysis from the calculated best-fit line. Weighting (1/x) was used in construction of the standard curve. Equations used for calculation of residues and example calculations can be found in Appendix 4. The calculation spreadsheets can be found in Appendix 5.

3.6 Instrumentation Conditions

All samples were analyzed by HPLC employing tandem mass spectrometric (MS/MS) detection. Optimum operating conditions were established prior to analysis (as permitted by the method). Typical conditions were as follows:

Operating Conditions

Instrument: AB Sciex QTRAP 6500 detector with ACQUITY UPLC system. The system was controlled and data processed by Applied BioSystems/MDS Sciex Analyst Software (Version 1.6).

Analytical column: 50-mm × 4.6-mm i.d., Zorbax SB-AQ, 3.5 µm particle size

Guard cartridge: Wispore C18, 4 × 2.0 mm, Phenomenex, P# AJO-4320

Guard column: Analytical guard cartridge, protects 2-mm to 8-mm i.d. columns, Phenomenex, P# AJO-6071

Mobile phase: *Fisher* water, *Fisher* acetonitrile, EMD formic acid (all solvents HPLC grade)

Component A: 0.1% Formic Acid in HPLC water

Component B: 0.1% Formic Acid in HPLC acetonitrile

Gradient:

<u>Time (min.)</u>	<u>% A</u>	<u>% B</u>
0 - 2.00	95.0	5.0
2.00 - 4.00	5.0	95.0
4.00 - 6.00	95.0	5.0

Flow rate: 0.5 mL/minute from 0 - 3.00 minutes
1.00 mL/minute from 3.00 - 4.00 minutes
0.5 mL/minutes from 4.00 - 6.00 minutes

Interface: TIS (Turbo Ion Spray)

Ionization mode: Positive and Negative (+/-)

Acquisition mode: MRM

Injection volume: 10 µL

Column temperature: 25 °C

Retention times:

<u>Analyte</u>	<u>Retention Time</u>
Atrazine	~2.6 minutes
Simazine	~2.5 minutes
Propazine	~2.7 minutes
G-30033	~2.3 minutes
G-28279	~2.2 minutes
G-28273	~1.9 minutes
Ametryn	~2.3 minutes
Prometryn	~2.4 minutes
GS-11354	~2.1 minutes
GS-11355	~2.0 minutes
GS-26831	~1.8 minutes
S-Metolachlor	~2.8 minutes
Metolachlor-ESA	~2.4 minutes
Metolachlor-OA	~2.5 minutes
Mesotrione	~2.5 minutes

The detection method utilized was LC/MS/MS employing electrospray (TIS) interface in the positive and negative mode on a triple quadrupole instrument. The instrument was tuned by infusing the analytes into a TIS (turbo ion spray) source, then creating a tune file to maximize the response of each analyte using the TIS source. The acquisition method was adjusted to maximize the response of the fragment ions detected. The ion transitions for each analyte are shown in the table below:

MS Conditions: Surface and Ground Water

System	AB Sciex QTRAP 6500 detector with ACQUITY UPLC system							
Analyte Monitored	Ions Monitored (AMU)	Declustering Potential (volts)	Collision Energy (volts)	Dwell Time (seconds)	EP (volts)	CXP (volts)	Acquisition Timing (minutes)	
Atrazine	216 → 174	65	24	40	10	17	0 - 6	
	216 → 104	65	37	40	10	17	0 - 6	
	216 → 132	65	31	40	10	17	0 - 6	
Simazine	202 → 132	55	26	40	10	17	0 - 6	
	202 → 124	55	25	40	10	17	0 - 6	
	202 → 104	55	33	40	10	17	0 - 6	
Propazine	230 → 146	69	31	40	10	17	0 - 6	
	230 → 188	69	23	40	10	17	0 - 6	
G-30033	188 → 104	50	33	40	10	17	0 - 6	
	188 → 146	50	23	40	10	17	0 - 6	
	188 → 110	50	29	40	10	17	0 - 6	
G-28279	174 → 96	88	17	40	10	17	0 - 6	
	174 → 104	88	44	40	10	17	0 - 6	
	174 → 146	88	23	40	10	17	0 - 6	
G-28273	146 → 104	54	26	40	10	17	0 - 6	
	146 → 110	54	21	40	10	17	0 - 6	
	146 → 79	54	25	40	10	17	0 - 6	
Ametryn	228 → 186	76	25	40	10	17	0 - 6	
	228 → 116	76	36	40	10	17	0 - 6	
	228 → 138	76	29	40	10	17	0 - 6	
Prometryn	242 → 158	78	32	40	10	17	0 - 6	
	242 → 200	78	25	40	10	17	0 - 6	
	242 → 152	78	28	40	10	17	0 - 6	
GS-11354	200 → 158	68	25	40	10	17	0 - 6	
	200 → 116	68	33	40	10	17	0 - 6	
	200 → 110	68	31	40	10	17	0 - 6	
GS-11355	186 → 96	63	28	40	10	17	0 - 6	
	186 → 158	63	24	40	10	17	0 - 6	
	186 → 91	63	25	40	10	17	0 - 6	
GS-26831	158 → 110	56	24	40	10	17	0 - 6	
	158 → 116	56	25	40	10	17	0 - 6	
	158 → 85	56	23	40	10	17	0 - 6	

MS Conditions: Surface and Ground Water (continued)

System	AB Sciex QTRAP 6500 detector with ACQUITY UPLC system						
Analyte Monitored	Ions Monitored (AMU)	Declustering Potential (volts)	Collision Energy (volts)	Dwell Time (seconds)	EP (volts)	CXP (volts)	Acquisition Timing (minutes)
S-Metolachlor	284 → 176	40	35	40	10	17	0 - 6
	284 → 252	40	19	40	10	17	0 - 6
	284 → 212	40	26	40	10	17	0 - 6
Metolachlor-ESA	328 → 121	-120	-31	40	-10	-16	0 - 6
	328 → 192	-120	-28	40	-10	-16	0 - 6
	328 → 135	-120	-34	40	-10	-16	0 - 6
Metolachlor-OA	278 → 206	-10	-14	40	-10	-16	0 - 6
	278 → 174	-10	-22	40	-10	-16	0 - 6
	278 → 158	-10	-28	40	-10	-16	0 - 6
Mesotrione	338 → 291	-24	-12	40	-10	-16	0 - 6
	338 → 212	-24	-41	40	-10	-16	0 - 6
	338 → 248	-24	-38	40	-10	-16	0 - 6

Additional detector settings are shown in the table below:

Parameter	Setting
Acquisition Mode:	MRM
Ionization Modes:	Positive (+) and Negative (-)
Source Temp.:	550 °C
Nebulizer (GS1):	70
Auxillary Gas (GS2):	70
Curtain Gas:	20
CAD Gas:	Medium
Ion Spray Voltage:	5500 (+) and 4500 (-)

The instrument was operated in both the MS/MS (MRM) positive ion mode and negative ion mode for quantitative analysis. Single transition chromatograms for each analyte were integrated and the peak areas used for quantitation. Quantitation was performed using a single transition for each matrix.

Calibration/Sample Analysis

A standard curve consisting of eight standard concentrations for all analytes (with the exception of G-28273 which used a standard curve consisting of five standard concentrations) was prepared by injecting constant volumes of calibration standards at specified concentrations. Constant volume injections were used for sample extracts as well. A curve

check standard was injected every 2-4 sample injections. All standard injections were used to construct the standard curve.

3.7 Fortification Procedures

Surface and ground water samples were fortified for procedural recovery purposes with the addition of 1.0 mL of a 0.0005- $\mu\text{g}/\text{mL}$ spiking solution to a 9.0 mL aliquot of water sample which produced a 0.05 ppb fortification for all analytes (excluding G-28273), representative of the LOQ. Addition of 1.0 mL of a 0.005- $\mu\text{g}/\text{mL}$ spiking solution to a 9.0 mL aliquot of water sample produced a 0.50 ppb fortification of all analytes, representative of the LOQ for G-28273 and $10 \times \text{LOQ}$ for all other analytes. Addition of a 1.0 mL of a 0.05- $\mu\text{g}/\text{mL}$ spiking solution to a 9.0 mL aliquot of water sample produced a 5.0 ppb fortification for G-28273 only, representative of $10 \times \text{LOQ}$.

Untreated control samples were fortified according to the following scheme:

Matrix	Sample Type	Fortifying Compounds	Fortification Level	No. of Samples
Surface Water	Reagent Blank	none	N/A	1
	Control	none	N/A	2
	Fortified control	atrazine, simazine, propazine, G-30033, G-28279, ametryn, prometryn, GS-11354, GS-11355, GS-26831, <i>S</i> -metolachlor, metolachlor-ESA, metolachlor-OA, mesotrione	0.05 ppb (LOQ)	5
		G-28273	0.50 ppb (LOQ)	
	Fortified control	atrazine, simazine, propazine, G-30033, G-28279, ametryn, prometryn, GS-11354, GS-11355, GS-26831, <i>S</i> -metolachlor, metolachlor-ESA, metolachlor-OA, mesotrione	0.50 ppb ($10 \times \text{LOQ}$)	5
		G-28273	5.0 ppb ($10 \times \text{LOQ}$)	

Matrix	Sample Type	Fortifying Compounds	Fortification Level	No. of Samples
Ground Water	Reagent Blank	none	N/A	1
	Control	none	N/A	2
	Fortified control	atrazine, simazine, propazine, G-30033, G-28279, ametryn, prometryn, GS-11354, GS-11355, GS-26831, S-metolachlor, metolachlor-ESA, metolachlor-OA, mesotrione	0.05 ppb (LOQ)	5
		G-28273	0.50 ppb (LOQ)	
	Fortified control	atrazine, simazine, propazine, G-30033, G-28279, ametryn, prometryn, GS-11354, GS-11355, GS-26831, S-metolachlor, metolachlor-ESA, metolachlor-OA, mesotrione	0.50 ppb (10 × LOQ)	5
		G-28273	5.0 ppb (10 × LOQ)	

3.8 Modifications, Interpretations, and Critical Steps

The analytical method was run as written.

3.9 Statistics

Statistical methods used were limited to calculations of the mean, range, standard deviation and relative standard deviation. Validated software programs, Microsoft Excel® 2007 and Applied BioSystems/MDS Sciex Analyst software (version 1.6), were employed to develop all regression analysis and statistical data.

4.0 RESULTS

4.1 Pre-Validation Evaluations

Prior to analysis of actual validation samples, the control samples initially selected for use in the study were analyzed to determine if any interferences were present in the area of elution. The result of this evaluation indicated that the control samples provided were free of any interference that would affect the analyte responses. The results are summarized below. See Table 1 for more detailed data.

4.3 Communications

Communication with the Study Sponsor occurred to discuss items such as 1) clarification/approval of the protocol and method, 2) acquisition of analytical standards and control samples and 3) questions regarding preparation of reagents. A complete list of communications is provided in Appendix 6.

4.4 Time Requirements

A single analyst completed a sample set consisting of 13 samples in approximately three hours with LC-MS/MS analysis performed overnight.

4.5 Protocol/SOP/Method Deviations/Memos to the Study File

No method deviations were generated for this study.

Two memos to the study file were generated for this study. The first memo, dated August 7, 2013, documented the addition of a dilution factor in the calculation of analyte ppb. The second memo, dated January 3, 2014, acknowledged a transcription error found for some standard concentration units within the chromatographic runs generated in this study.

The deviations and memos described above were not considered to have a negative impact on the integrity of the study.

5.0 CONCLUSION

Morse Laboratories, LLC successfully independently validated Syngenta Analytical Method T001681-06 entitled "Analytical Method for the Determination of Atrazine, Simazine, Propazine, G-30033, G-28279, G-28273, Ametryn, Prometryn, GS-11354, GS-11355, GS-26831, S-Metolachlor, Metolachlor-ESA, Metolachlor-OA and Mesotrione in Water Using Direct-Aqueous-Injection ESI-LC/MS/MS, Including Validation Data."

The method was demonstrated to be suitable for the determination of atrazine, simazine, propazine, G-30033, G-28279, G-28273, ametryn, prometryn, GS-11354, GS-11355, GS-26831, S-metolachlor, metolachlor-ESA, metolachlor-OA and mesotrione in surface and ground water, the matrices for which the method was designed. An LOQ of 0.05 ppb was confirmed for all analytes with the exception of G-28273 which had a confirmed LOQ of 0.50 ppb.

6.0 REFERENCES

1. U.S. Environmental Protection Agency, Office of Prevention, Pesticide and Toxic Substances, Ecological Effects Test Guidelines EPA Documents EPA 712-C-001 (OCSP 850.6100, Environmental Chemistry Methods and Associated Independent Laboratory Validation, Jan, 2012. Internet Address: <http://www.epa.gov/ocpps/pubs/frs/home/guidelin.htm>
2. Pesticide Registration Notice 2011-3, U.S. Environmental Protection Agency, Office of Pesticide Programs: Washington, DC, January 6, 2012.

Equations

Calculations for instrumental analysis were conducted using a validated software application to create a standard curve based on linear regression. All standards injected were used in the generation of the standard curve. The regression functions were used to calculate a best fit line (from a set of standard concentrations in pg/ μ L versus peak response) and to determine concentrations of the analyte found during sample analysis from the calculated best fit line. For this study, the correlation coefficient for each calibration curve was equal to or greater than 0.990 (r^2 equal to or greater than 0.98). 1/x weighting was used.

The equation used for the least squares fit is:

$$y = mx + b$$

where:

y	=	peak response
m	=	slope
x	=	pg/ μ L found for peak of interest
b	=	y-intercept

1. The calculations used for pg found was:

$$pg\ found = injection\ volume\ (\mu L) \times pg/\mu L\ found$$

where:

injection volume (μ L) = constant volume injected of standard for each sample (10 μ L)

pg/ μ L found = pg/ μ L of analyte found as determined from the standard curve

2. The calculations for ppb found and percent recovery (for fortified samples) were:

a. The amount of analyte (in ppb) found in the sample was calculated according to the following equation:

$$Residue\ (ppb) = \frac{Analyte\ Found\ (pg)}{Amt.\ Samp.\ Inj.\ (mg)} \times HPLC\ Dil.\ Factor$$

where:

Analyte Found (pg) = injection volume (μL) multiplied by $\text{pg}/\mu\text{L}$ found as determined from the standard curve

Amt. Samp. Inj. (mg) = injection volume (μL) multiplied by the following conversion factors

1 mL/1000 μL = conversion factor

1 g/1 mL = conversion factor

1000 mg/1 g = conversion factor

HPLC Dil. Factor = dilution of sample extract required to produce analyte responses bracketed by standards

b. The percent recovery in fortified control samples is calculated as follows:

$$\% \text{ Recovery} = \frac{\text{ppb found in fortified control} - \text{average ppb found in control}}{\text{ppb added}} \times 100$$

Example Calculations

All analytes were calculated in an identical manner. Only examples of atrazine residue calculations were provided and thus serve to illustrate the calculations for all analytes in surface and ground water.

1. LIMS ID# 123488, Surface water, Atrazine, Set# 5, RIMV00312-0001, **Control 5** (Figure 1-2):

187388 peak response \rightarrow *0.0260 pg/ μL*

pg found = *10 μL* \times *0.0260 pg/ μL*

= 0.26

$$ppb = \times \frac{0.26 \text{ pg}}{10 \text{ mg}} \times 1$$

$$ppb = 0.026$$

$$\text{Reported } ppb = < 0.05$$

2. LIMS ID# 123488, Surface water, Atrazine, Set# 5, RIMV00312-0001,
Fortified Control 35 @ 0.05 ppb (Figure 1-3):

$$562592 \text{ peak response} \rightarrow 0.0804 \text{ pg}/\mu\text{L}$$

$$pg \text{ found} = 10 \mu\text{L} \times 0.0804 \text{ pg}/\mu\text{L}$$

$$= 0.804$$

$$ppb = \times \frac{0.804 \text{ pg}}{10 \text{ mg}} \times 1$$

$$ppb = 0.0804$$

$$\text{Reported } ppb = 0.0804$$

$$\% \text{ Recovery} = \frac{0.0804 \text{ ppb} - 0.0265 \text{ ppb}}{0.05 \text{ ppb}} \times 100$$

$$= 108\%$$