1.0 Introduction and Summary

This method is utilized for the determination of the chlorotriazine compounds atrazine and simazine and their applicable degradates G-30033, G-28279, and G-28273; ametryn and prometryn and their applicable degradates GS-11354, GS-11355, and GS-26831; and metolachlor in water at a limit of quantitation (LOQ) of 0.10 ppb for all analytes using the Applied Biosystems MDS Sciex API 4000. The limit of detection is 0.0005 ng injected for each analyte based on the lowest concentration of standard injected and used to construct the calibration plot using the API 4000. The compounds included in this method, chemical names, and the applicable LOQ for each are shown in Table 1. The compound structures are shown in Figure 1. Sample preparation is minimal and final measurement is accomplished using direct injection (DI) - electrospray ionization (ESI) - liquid chromatography/mass spectrometry/mass spectrometry (LC/MS/MS).

Gas chromatography/MS (GC/MS) methods are typically used for the analysis of water samples[1, 2] but these require the use of time-consuming sample preparation procedures, such as liquid/liquid extraction (LLE) and/or solid phase extraction (SPE), prior to final sample analysis. The method described in this report is a fast and relatively low cost alternative procedure for monitoring these compounds in water because sample preparation is minimal. The same low LOQ is achieved when using this direct injection procedure when compared to those using the GC/MS methods and analyte confirmatory capabilities are retained.

This is an amended report, which incorporates two changes. The first change is to correct typographical errors in the compound nomenclatures. The second change is to correct the HPLC conditions in Table 2.

1.1 Principle

Typically, 200 µL of water sample is mixed with 800 µL of 5/95 (v/v) methanol/H_2O in an HPLC vial (or 1 mL of water to 4 mL of 5/95 (v/v) methanol/H_2O) to obtain a final methanol/water ratio of 4%. Sample injection is typically performed overnight using DI-ESI-LC/MS/MS. Water samples can be analyzed without dilution if the analyst can demonstrate the absence of suppression causing components in the water. In this case, 40 µL of methanol can be added to 1.0 mL of water sample (methanol/water ratio of 3.8%) to increase the overall sensitivity of the measurement.

A flow diagram of the procedure is presented in Figure 2.

2.0 Materials and Apparatus

The recommended equipment and apparatus are listed in Appendix 1. Equipment with equivalent performance specifications may be substituted, as indicated.

2.1 Reagents and Analytical Standards

All solvents and other reagents must be of high purity, e.g. glass distilled/HPLC grade solvents and analytical grade reagents. Particular care must be taken to avoid contamination of the reagents used. Reagents of comparable purity may be substituted as long as acceptable performance is demonstrated. See Appendix 2 for a list of reagents and analytical standards used in this method.

2.2 Preparation of Analytical Standard Solutions

It is recommended that the following precautions should be taken when weighing the analytical materials.

1. Ensure good ventilation.
2. Wear approved eye protection, gloves and a laboratory coat.
3. Prevent inhalation and contact with mouth.
4. Wash any contaminated area(s) immediately.

Individual stock solutions containing single analytes at the 100 µg/mL concentration level are prepared by dissolving 10.0 mg of each compound into 100-mL volumetric flasks (5.0 mg of G-28273, G-28279, or G-30033 to give 50 µg/mL due to solubility) followed by dilution to the mark with methanol. The amounts weighed for each compound should be corrected for its respective % purity. For analysis using the API 4000, a mixed standard at the 2 ug/mL concentration level was prepared from each of the stock solutions into a 100-mL volumetric flask and filling the flask to the mark with 5/95 (v/v) methanol/HPLC water. Serial dilutions of these mixed standards are prepared in 5/95 (v/v) methanol/HPLC water to create working mixed standards in the appropriate range for the respective instrument to start with a standard at half the concentration equivalent to the LOQ and to an upper concentration range equivalent to 50 ppb or within the upper linear range of the instrument for each compound. The mixed working standards are used for analytical and fortification purposes. Preparation scheme for the API 3000 can be found in Appendix 3. Other standard preparation schemes may be utilized.

All standard solutions are stored in amber glass bottles in a refrigerator at approximately 4°C to prevent concentration changes due to photodecomposition of the analytes or solvent evaporation. Fresh mixed working standard solutions are typically prepared every three months and fresh mixed stock standard solutions are prepared every six months. In general, the expiration dates of the mixed stock and working standard solutions are not extended beyond the expiration date of the solid standard unless stability considerations or other pertinent information dictate otherwise.

2.3 Fortification

Water samples can be fortified for procedural recovery purposes by judicious choice of working solution concentration and volume. For example, the addition of 1.0-mL of a 0.01

µg/mL working standard solution to a 100-mL aliquot portion of water sample produces a 0.10 ppb fortification of each analyte. The fortification levels used in each set of analyses can vary but should always include one control and two recoveries in order to verify method performance. Recovery samples are prepared by fortifying a control sample at the LOQ level when necessary to establish the LOQ of the method or/and at another or higher concentration level.

No more than 0.5 mL (500 µL) of fortification solution should be added. Correct the control water volume used as recovery for the volume of fortification standard to be added. For example, if 500 µL of fortification standard will be used, dispense 9.5 mL of water. Do not use a glass syringe to make fortifications. If using an adjustable volume repeating pipette instead of a class "A" volumetric pipette for fortification, it is necessary to calibrate the device prior to using it.

2.4 Safety Precautions and Hazards

Whereas most of the chemicals in this method have not been completely characterized, general laboratory safety precautions are advised (e.g., safety glasses, gloves, etc.). The user(s) should consult the relevant MSDS for commonly used reagents and materials.

3.0 Analytical Procedure

Note: Due to the low detection limit of the method it is important that precautions be taken to avoid cross contamination in the laboratory.
Specifically:

- Where possible disposable glassware/plastic-ware has been specified, new glassware/plastic-ware should be used for each batch of samples. Do not use syringes for transfer or fortification of samples.
- Each solvent used in the method should be checked prior to use to verify that it is free from contamination.
- Existing glassware should be solvent (methanol, acetone or acetonitrile) rinsed, after washing and before use in the method.

3.1 Sample Storage and Temperature Re-Equilibration

Water samples are typically received chilled and then stored at refrigerator temperature (about 4°C) until removed for analysis. The sample should be allowed to re-equilibrate to room temperature before removing and measuring an aliquot portion for analysis.

In some cases sample filtration may be necessary, in such case, filter the water sample prior to sample preparation.

3.2 Sample Preparation

3.2.1 Transfer 800 µL of 5/95 (v/v) methanol/HPLC water to an auto-sampler vial.

3.2.2 Add 200 µL of representative water sample to the same vial from 3.2.1 and mix.

Note: If the water sample is not clear or if particulates are visible, the water sample should be subjected to centrifugation prior to dilution.

3.2.3 Load the sample vials onto the injection tray for DI-ESI-LC/MS/MS analysis.

3.3 Instrumentation for DI-ESI-LC/MS/MS

3.3.1 Description and Operating Conditions

Refer to Table 2 for the DI-ESI-LC/MS/MS operating parameters.

3.3.2 Calibration and Standardization

a) The mass spectrometer should undergo periodic mass calibration of both quadrupole analyzers according to manufacturer's recommendation. More frequent mass calibration is required only if drift from the expected masses is excessive.

b) The instrument is tuned and optimized by infusing individual standards (typically at a concentration of 5 ng/µL or less depending on the sensitivity of the instrument) directly into the source (ESI) at a flow rate of 60 µL/minute. And by Flow Injection Analysis (FIA) at the HPLC flow rate to be used in the instrument. The respective precursor and product ion pairs for each analyte are shown in Table 2.

c) Standardize the system using the conditions listed in Table 2 by injecting the appropriate µL portions of mixed standard at different concentration levels over a suggested range of 0.00001 to 0.0001 ng/µL (a calibration range of 0.001 to 0.010 ng injected). Other calibration ranges may be suitable depending on the instrument sensitivity. The Analyst data system software allows one to create a calibration plot (e.g. linear regression) from the standard concentrations and the measured peak responses obtained for each analyte. Typical standardization data shown in Table 3 are obtained from the Multiple Reaction Monitoring (MRM) chromatograms of mixed standards at various concentrations. Representative MRM chromatograms of standards at various concentrations are shown in Figures 3.

d) During each sequence, mixed standards are run at the various concentration levels to construct a calibration curve. Typical calibration plots are presented in Figure 4. The "ng (or pg) found" values can be manually or automatically transferred to an applicable worksheet such that the residue in units of ppb can be calculated using the equations described in Section 3.7.3.

3.4 Interferences

Interferences are generally not significant due to the highly selective nature of MS/MS analysis. If suppression or enhancement issues are encountered, the samples can be diluted further prior to injection, a lower amount injected or the retention times lengthened to reduce suppression or enhancement.

3.5 Confirmatory Techniques

Analysis using MS/MS is confirmatory.

3.6 Modifications and Potential Problems

Although procedural recoveries may fluctuate due to varying qualitative and quantitative dissolved sample components in different types of water samples, average recoveries should fall within the range of 70-120%.

The quality of the calibration plot can deteriorate if the ESI source becomes contaminated. Thus, inspection of each calibration plot needs to be performed in order to maintain accurate and reliable quantification with each set of samples.

To help minimize instrument contamination, it is highly recommended that a timed event controlled switching valve be used to divert the LC stream to waste during periods of no data collection, during initial period prior to the first peak and after the last peak has eluted from the HPLC column.

If suppression or enhancement issues (matrix effects) are encountered, follow suggestions in Section 3.4. Suppression or enhancement can be determined by fortifying a control sample ("needle spiking") with a known concentration of standard; doing the same with a solvent blank and comparing results. If after conditioning the column, the fortified sample analyte response is lower or higher than the fortified solvent blank, there is a matrix effect.

Any modification to the chromatography should be documented in the raw data and should be done only if the results are acceptable compared to the validated results obtained using the listed chromatographic conditions.

The instruments and conditions used in this method have been found to be suitable for this analysis. Other instruments may also be used, however optimization may be required to achieve the desired separation and sensitivity. Operating manuals for the instruments should always be consulted to ensure safe and optimum use.

3.7 Determination of Sample Residues

3.7.1 Samples

Calibrate the instrument with injections of at least four (or more) concentration levels of mixed standard and generate a calibration curve for each of the eleven analytes during the analysis run. The data system uses the calibration plots and the respective peak responses (e.g., area, height) to calculate the amount of each analyte in a sample. If the analyte response in the sample exceeds 10% of the response for the highest concentration standard injected, dilute the sample accordingly and re-inject. Typical chromatograms for the reagent blank, control, and fortified control water samples are presented in Figures 6 - 8.

3.7.2 Procedural Recoveries

Each set of sample analyses is validated by acquiring data for procedural recovery samples that should be within EPA's acceptance criteria of mean recoveries of 70 – 120% and standard deviations of ≤20%. Recoveries are corrected for control values.

3.7.3 Calculations

Calculate the concentration of the analytes in units of ppb from equation (1):

$$(1) \qquad \text{ppb of analyte} = \frac{\text{nanogram of analyte found (ng)}}{\text{gram of sample injected (g)}} \times \frac{1}{R}$$

where R is the recovery factor expressed in decimal form (i.e., 1.0 = 100%) and is calculated from equation (3). For R > 1, use a factor of 1.0.

The grams of sample injected is calculated from equation (2).

$$(2) \qquad \text{gram of sample injected} = \frac{\text{gram of sample extracted} \times V_I}{V_F}$$

where gram of sample extracted is the gram of sample used in extraction (for water, 1.0 ml = 1.0 g), V_I is the volume (ml) of sample injected, and V_F is the final volume (ml) of the extracted sample.

The recovery factor, expressed as a percentage (R%), is calculated from fortification experiments and is presented in equation (3).

$$(3)\ R\% = \left(\frac{\text{ppb of analyte found - ppb of analyte found in control}}{\text{ppb of analyte added}} \right) \times 100$$

3.8 Time Required for Analysis

The methodology is normally performed with a batch of 50 or more samples. One person can complete the sample preparation of 50 samples in less than an hour and LC/MS/MS analysis can be performed unattended overnight or during the day in about 10 to 14 hours.

3.9 Method Stopping Points

Should it be necessary to store the prepared samples overnight or over the weekend, the results of the fresh recovery sample will indicate whether acceptable results are obtained. Samples should be stored in sealed containers at a temperature of approximately 8°C (refrigerated) until samples can be analyzed by LC/MS/MS.

6.0 References

1. Yokley, Robert A. and Cheung, Max W., Analytical Method for the Determination of Atrazine and Its Dealkylated Chlorotriazine Metabolites in Water Using Gas Chromatography/Mass Selective Detection, J. Ag. and Food Chem., Vol. 48, No.10, pp 4500-4507, 2000

2. Huang, Sung-Ben, Stanton, Jeffrey S. Lin, Yi., and Yokley, Robert A., Analytical Method for the Determination of Atrazine and Its Dealkylated Chlorotriazine Metabolites in Water Using SPE Sample Preparation and GC/MSD Analysis, J. Ag. and Food Chem., Vol. 51, No. 25, pp 7252-7258, 2003.

3. Syngenta Protocol No. T010097-04. Method Validation of Syngenta Analytical Method T010097-04 Direct Injection ESI LC/MS/MS Multiresidue Triazine Analysis of Water Samples, Syngenta Crop Protection, Inc., Environmental Residue Group, Greensboro, NC (Includes amendment 1).

Table 2. DI-ESI-LC/MS/MS API 4000 Operating Parameters

HPLC: Perkin Elmer Series 200
Column: Zorbax SB-CN, 4.6 x 75 mm, 3.5 µm particle size (Agilent P/N 866953-905)

Flow rate: 0.40 mL/min

Column Temperature: 45°C (room temperature may also be used - provides better sensitivity and peak shape for G-28273).

Injection volume: 100 µL or 50 µL (generally, 50 µL is preferred)

Mobile Phase: A = HPLC grade water
B = HPLC grade methanol

Time (mins)	%A	%B	Description
0.0 – 0.5	95	5	Isocratic condition for 0.5 minutes.
0.5 – 2.5	35	65	Linear gradient for 2 minutes.
2.5 – 10.5	35	65	Isocratic conditions for 8.0 minutes.
10.5 – 10.6	95	5	Step gradient to initial conditions.
10.6 – 18.6	95	5	Isocratic conditions for 8.0 minutes for column equilibration.

MS/MS Conditions & Parameters:

Instrumentation: Applied Biosystems, MDS Sciex API 4000 triple quadrupole
Software: Analyst 1.4
Ionization: Electrospray (Positive mode)
Source Temperature: 700°C

Equivalent equipment, conditions or other columns may be used as long as equivalent recovery results are obtained. Equilibration time may be shortened by increasing the flow rate. Any changes must be documented in the raw data.

Table 2. DI-ESI-LC/MS/MS API 4000 Operating Parameters (Continued)

Acquisition parameters used:

CUR:	10	Scan Type:	MRM
GS1:	50	Polarity:	Positive
GS2:	50	Resolution Q1:	Unit
IS:	5500	Resolution Q2:	Unit
TEM:	700	Ion Source:	Turbo Spray
CAD:	2		
EP:	10		

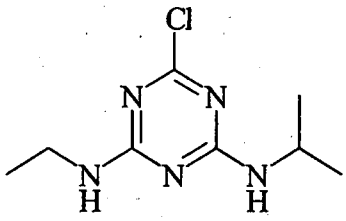
Analyte	MRM Transition	Ion Mode	DP	CE	XP		
G-28273	146.0/104.0	+	40	26	8	Period 1	dwell 3000
GS-26831	158.2/110.0	+	60	25	10	Period 2	dwell 100
G-28279	174.2/96.2	+	75	24.5	8		
GS-11355	186.1/96.0	+	80	30	9		
G-30033	188.3/146.1	+	65	24	10		
GS-11354	200.3/158.0	+	70	25	14		
Simazine	202.1/132.1	+	53	27	8		
Atrazine	216.1/174.2	+	70	25	11		
Ametryn	228.2/186.1	+	65	25	20		
Prometryn	242.0/158.2	+	70	32	12		
Metolachlor	284.2/176.3	+	55	40	17		

If significant interferences are encountered during sample analysis, alternate product ions can be used for each MRM transition by optimizing the MS parameters for the newly selected ion. The retention time may vary depending upon chromatographic conditions and systems.

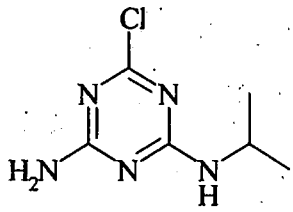
Note: Above parameters are typical for an API 4000. Parameters for an API 3000 are shown in Appendix 3. Optimization parameters for the above compounds must be established when using any LC/MS/MS for the first time for these compounds.

8.0 FIGURES

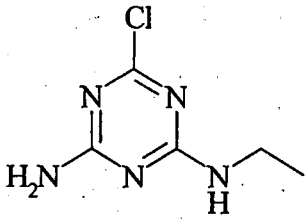
FIGURE 1. STRUCTURES OF ANALYTES



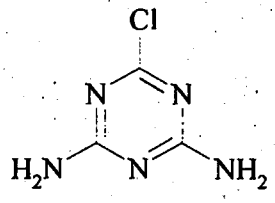
Atrazine
MW 215.7



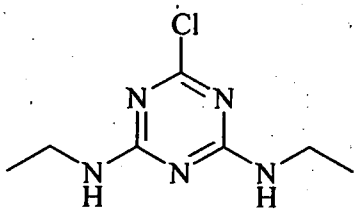
G-30033
MW 187.6



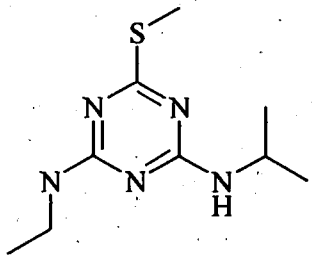
G-28279
MW 173.6



G-28273
MW 145.6

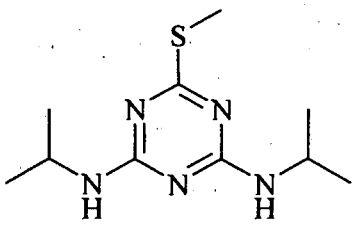


Simazine
MW 201.7

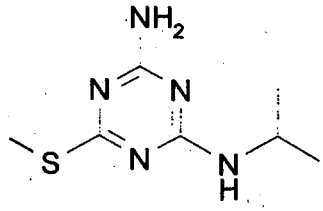


Ametryn
MW 227.3

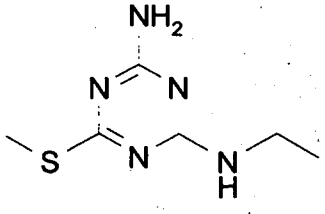
FIGURE 1. STRUCTURES OF THE ANALYTES (Continued)



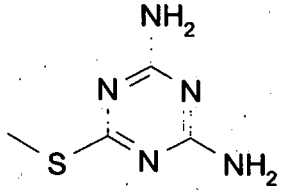
Prometryn
MW 241.4



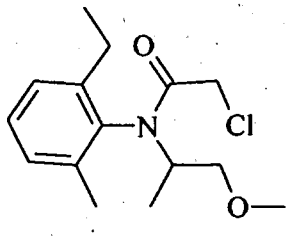
GS-11354
MW 199.1



GS-11355
MW 185.1

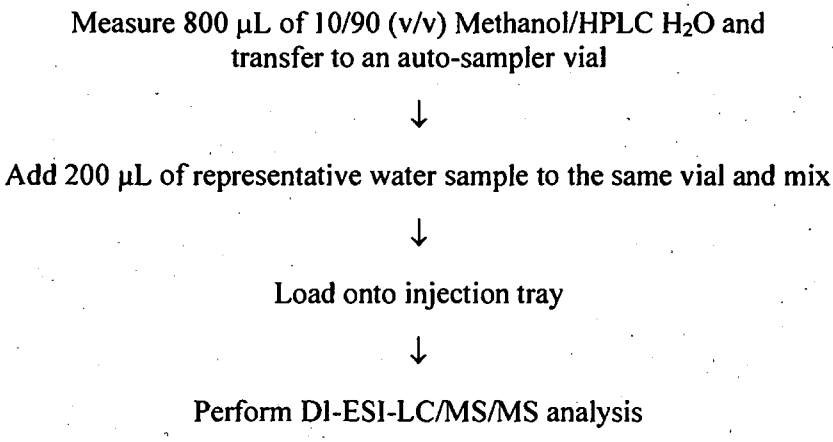


GS-26831
MW 157.0



Metolachlor
MW 283.8

FIGURE 2. FLOW DIAGRAM OF METHOD



9.0 Appendices

Appendix 1. Apparatus

General laboratory glassware (beakers, graduated cylinders, pipet bulbs, etc.) available from a general laboratory supply company.

Balance, analytical (Sartorius R160P), or equivalent. Electronic display of 0.01 mg, for weighing in preparation of the stock standard solutions.

Bottles, amber glass Boston round, 2 oz. and 4 oz., with Polyseal-lined cap (Fisher Scientific cat. nos. 03-320-4A and 03-320-4B) or equivalent.

Disposable culture tubes, 16 mm x 100 mm, borosilicate glass (Fisher Scientific cat. no. 14-961-29) or equivalent.

Mixer, Vortex-Genie 2 (Fisher Scientific cat. no. 12-812) or equivalent.

Pipets, Pasteur, (Fisher Scientific cat. no.13-678-7C) or equivalent.

Pipets, glass, Class A certified, assorted volumes.

Pipetter, Eppendorf Repeater, 100 – 1000 µL variable volume range (VWR cat. no. 53511-582) and 500-5000 µL variable volume range (VWR cat. no. 53513-412), or equivalent.

LC Vials, amber snap cap ID, 1.5 mL (National Scientific, Inc. cat no. C4001-6W) and pre-slit snap-it™ caps (National Scientific, Inc. cat no. C40011-55), or equivalent.

Appendix 2. Reagents and Analytical Standards

All solvents and other reagents must be of high purity, e.g. glass distilled/HPLC grade solvents and analytical grade reagents. Particular care must be taken to avoid contamination of the reagents used. All reagents are stored at room temperature. Solid analytical standards are stored in a freezer (temperature < -10°C) unless specified otherwise.

Standards are obtained from the Analytical and Product Chemistry Department, Syngenta Crop Protection, Inc., Greensboro, North Carolina.

Methanol, HPLC grade (Fisher cat. no. A452SK-1), or equivalent.

Water, ultra pure or HPLC grade (Fisher cat. no. W5SK-4) or equivalent.

Mobile Phases: Prepared as needed in one liter volumetric flask. Mix well. Store at room temperature.

Appendix 3. API 3000 Data

This method may be utilized for the determination of the chlorotriazine compounds atrazine and simazine and their applicable degradates G-30033, G-28279, and G-28273; ametryn and prometryn and their applicable degradates GS-11354, GS-11355, and GS-26831; and metolachlor in water at a limit of quantitation (LOQ) ranging from 0.20 to 1.0 ppb (see Table A3-1) using an Applied Biosystems MDS API 3000, provided that the instrument generates acceptable recovery data and calibration curves. Specifications for the API 3000 can be found in Table A3-1. The applicable LOQ for each compound using the API 3000 are shown in Table A3-2 through A3-4.

Although the same low LOQ obtained on the API 4000 might not be achievable on less sensitive triple quadrupole instruments such as the API 3000 when using this direct injection procedure, meaningful quantification levels at the higher LOQ are possible and analyte confirmatory capabilities can be retained.

Characterization of the water used in the API 3000 work is found in Table A3-5.

For the API 3000, mixed standards at the 4.0, 8.0 and 20.0 µg/mL concentration levels were prepared by pipetting 4.0 mL of Atrazine, Ametryn, Prometryn, GS-11355 and GS-11354 stock solutions; 8.0 mL of the Simazine, Metolachlor, GS-28631 and G-30033 stock solutions; 16.0 mL of the G-28279 stock solution; and 40.0 mL of the G-28273 stock solution into a 100-mL volumetric flask and filling the flask to the mark with 5/95 (v/v) methanol/HPLC water.

A summary of the results of recovery experiments conducted using the API 3000 mass spectrometer are given in Table A3-2 through A3-4.

Table A3-1. DI-ESI-LC/MS/MS API 3000 Operating Parameters

HPLC: Agilent Series 1100 LC
Column: Zorbax SB-CN, 4.6 x 75 mm, 3.5 µm particle size (Agilent P/N 866953-905)
Column Filter: ColumnSaver (MAC-MOD P/N MMCS210)
Flow rate: 0.40 mL/min

Column Temperature: 40°C
Injection volume: 50 µL
Mobile Phase: A = 5/95 (v/v) Methanol/HPLC Water, B = Methanol

Time	%A	%B
0.0	100	0
0.5	100	0
2.0	35	65
10.0	35	65
10.1	100	0
15.0	100	0

MS/MS Conditions & Parameters:

Instrumentation:	MDS Sciex API 3000 triple quadrupole
Software:	Analyst 1.4
Ionization:	Electrospray (Positive mode)
Source Temperature:	400°C

Equivalent equipment, conditions or other columns may be used as long as equivalent recovery results are obtained. Any changes must be documented in the raw data.

Table A3-1. DI-ESI-LC/MS/MS API 3000 Operating Parameters (Continued)

Analyte	MRM Transition[1]	Ion Mode	DP	FP	EP	CE	CXP	Dwell (ms)	Retention Time (min.)[2]	LOQ (ppb)
G-28273	**146.2 > 68.0**	+	30	100	15	35	35	500	4.87	0.50
GS-26831	**158.2 > 68.0**	+	35	110	8	40	2	200	5.24	0.20
G-28279	**174.2> 68.1**	+	35	145	10	35	2	200	5.56	0.20
GS-11355	**186.2 > 91.0**	+	40	140	8	27	4	50	5.86	0.10
G-30033	**188.1 > 145.9**	+	30	130	10	25	8	200	5.80	0.10
GS-11354	**202.1 > 68.0**	+	30	140	10	45	6	200	6.14	0.10
Simazine	**200.2 > 158.2**	+	40	150	8	25	10	50	6.25	0.20
Atrazine	**216.2 > 174.0**	+	41	130	10	25	10	200	6.59	0.10
Ametryn	**228.2 > 68.0**	+	45	180	10	40	12	50	7.08	0.10
Prometryn	**242.1 > 68.1**	+	40	170	15	50	12	50	7.51	0.10
Metolachlor	**284.1 > 252.2**	+	35	150	5	21	25	100	7.48	0.20

[1] If significant interferences are encountered during sample analysis, alternate product ions can be used for each MRM transition by optimizing the MS parameters for the newly selected ion.
[2] The retention time may vary depending upon chromatographic conditions and systems.

Note: Above parameters are typical for an API 3000. Optimization parameters for the above compounds must be established when using any LC/MS/MS for the first time for these compounds.

Appendix 4. Protocol and Protocol Amendments

STUDY PROTOCOL

Method Validation of Syngenta Analytical Method Number T010097-04
Direct Injection ESI LC/MS/MS Multiresidue Triazine Analysis of Water Samples

Data Requirement
EPA Residue Chemistry Test Guidelines;
OPPTS 860.1340, August 1996

Sponsor
Syngenta Crop Protection, Inc.
410 Swing Road
Greensboro, North Carolina 24719

Performing Laboratory
ADPEN Laboratories, Inc.
11757 Central Parkway
Jacksonville, Florida 32224

Syngenta Study Number T010097-04
***ADPEN* Study Number** ADPEN-2K5-0106

Page 1 of 10

PROTOCOL REVIEW AND APPROVAL

Study Title: Method Validation of Syngenta Analytical Method Number T010097-04
Method Title: Analytical Method T010097-04 for the Determination of Atrazine, Simazine, G-30033, G-28279, G-28273, Ametryn, Prometryn, GS-11354, GS-11355, GS-26831, and Metolachlor in Water Using Direct Injection LC-ESI/MS/MS Including Validation Data.

ADPEN Study No.:	ADPEN-2K5-0106
Syngenta Study No.:	T010097-04
Proposed Experimental Start Date:	January 14, 2005
Proposed Experimental Termination Date:	January 20, 2005
Proposed Study Completion Date:	February 1, 2005

Quality Assurance:

Nela Ovshak Janjic — 1-18-2005
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Project Manager, Syngenta Crop Protection, Inc.:

Andrew Merritt — Jan. 19 2005
Andrew Merritt — Date
Syngenta Project Manager

Study Director:

Rolando Perez — 1/19/2005
Rolando Perez — Date
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1.0 INTRODUCTION

The purpose of this study is to perform a Method Trial/ Method Validation of draft Syngenta Crop Protection, Inc. (SCP) Method Number T010097-04, entitled " Analytical Method T010097-04 for the Determination of Atrazine, Simazine, G-30033, G-28279, G-28273, Ametryn, Prometryn, GS-11354, GS-11355, GS-26831, and Metolachlor in Water Using Direct Injection LC-ESI/MS/MS Including Validation Data." [1] to determine the suitability of the method for the analysis of ground, surface and finished water for atrazine when using an Applied Biosystems/PE Sciex API 3000 LC/MS/MS tandem quadrupole instrument.

The United States Environmental Protection Agency (USEPA) issued a harmonized test guideline on August 1996 for residue methods (OPPTS 860.1340). This study will be conducted to satisfy guideline requirements described in the OPPTS, EPA Residue Chemistry Test Guidelines, OPPTS 860.1340 [2]. In addition, this study will be conducted in compliance with EPA FIFRA Good Laboratory Practice Standards, 40 CFR Part 160 [3].

The method trial/method validation of draft Method No. T010097-04 will be performed by ADPEN Laboratories, Inc. in Jacksonville, Florida. The study will be audited for compliance with EPA FIFRA Good Laboratory Practice Standards, 40 CFR Part 160, by the Quality Assurance Unit of ADPEN.

2.0 TEST MATERIALS

2.1 Test and Reference Substances

Common Name:	Atrazine (G30027)
Chemical Name:	6-chloro-N^2-ethyl-N^4-isopropyl-1,3,5-triazine-2,4-diamine
CAS No.:	1912-24-9
Purity:	97.9%
Expiration Date:	6/2006
Storage Conditions:	Room temperature
Source:	SCP

Common Name:	G-30033
Chemical Name:	1,3,5-Triazine-2,4-diamine, 6-chloro-N-(1-methylethyl)-
CAS No.:	6190-65-4
Purity:	94%
Expiration Date:	8/2008
Storage Conditions:	Freezer
Source:	SCP

Common Name:	G-28279
Chemical Name:	1,3,5-Triazine-2,4-diamine, 6-chloro-N-ethyl-
CAS No.:	1007-28-9
Purity:	96%
Expiration Date:	3/2007
Storage Conditions:	Freezer
Source:	SCP

Common Name:	G-28273
Chemical Name:	1,3,5-Triazine-2,4-diamine, 6-chloro-N-ethyl-
CAS No.:	1007-28-9
Purity:	97%
Expiration Date:	8/2008
Storage Conditions:	Freezer
Source:	SCP

Common Name:	Simazine (G-27692)
Chemical Name:	1,3,5-Triazine-2,4-diamine, 6-chloro-N,N'-diethyl-
CAS No.:	122-34-9
Purity:	99.7%
Expiration Date:	3/2007
Storage Conditions:	Room Temperature
Source:	SCP

Common Name:	Ametryn (G-34162)
Chemical Name:	1,3,5-Triazine-2,4-diamine, 6-chloro-N,N'-diethyl-
CAS No.:	122-34-9
Purity:	98.3%
Expiration Date:	10/2005
Storage Conditions:	Room Temperature
Source:	SCP

Common Name:	Prometryn (G-34161)
Chemical Name:	1,3,5-Triazine-2,4-diamine, N,N'-bis(1-methylethyl)-6-(methylthio)-
CAS No.:	7287-19-6
Purity:	99.7%
Expiration Date:	1/2005
Storage Conditions:	Room Temperature
Source:	SCP

Common Name:	GS-11354
Chemical Name:	1,3,5-Triazine-2,4-diamine, N,N'-bis(1-methylethyl)-6-(methylthio)-
CAS No.:	7287-19-6
Purity:	97%
Expiration Date:	4/2007
Storage Conditions:	Freezer
Source:	SCP

Common Name:	GS-11355
Chemical Name:	1,3,5-Triazine-2,4-diamine, N-ethyl-6-(methylthio)-
CAS No.:	4147-58-4
Purity:	98%
Expiration Date:	5/21/06
Storage Conditions:	Refrigerator
Source:	SCP

Common Name:	GS-26831
Chemical Name:	N/A
CAS No.:	N/A
Purity:	99%
Expiration Date:	4/2007
Storage Conditions:	Freezer
Source:	SCP

Common Name:	Metolachlor
Chemical Name:	Acetamide, 2-chloro-N-(2-ethyl-6-methylphenyl)-N-(2-methoxy-1-methylethyl)-
CAS No.:	51218-45-2
Purity:	98.0%
Expiration Date:	5/2009
Storage Conditions:	Room Temperature
Source:	SCP

Characterization data for the test and reference substance are maintained by SCP. 410 Swing Road, Greensboro, NC 24719. The chemical structures of these compounds are presented in Syngenta Method No. T010097-04.

A record of test and reference substances storage conditions and records of weights and dilutions will be maintained and checked by ADPEN.

Material Safety Data Sheets (MSDS) or other information necessary for proper and safe handling, shipping, and storage of the test materials will be provided to ADPEN by Syngenta Crop Protection.

2.2 Test Systems

The control water samples to be used for the validation are available at ADPEN and should be identified in the work orders and Final Report.

3.0 EXPERIMENTAL DESIGN

3.1 Method Parameters

Prior to performing the method trial / method validation, ADPEN will review the method; tune and optimize the API 3000 LC/MS/MS to get maximum sensitivity for all compounds; and determine the analytes retention time and instrument sensitivity.

3.2 Sample Identification

Each measured aliquot of sample used for validation will be assigned a unique code number. The code numbers will be assigned by ADPEN for this study (ie. 2K5-0106-01).

3.3 Validation Set

The validation will include but not be limited to using control ground, surface and finished water sources free of Atrazine and other selected analytes as much as possible as previously analyzed by GC/MS. A validation will consist of analysis of one control from each water type and five samples of each of the three water types fortified with Atrazine and the other analytes at three concentration levels LOQ, 2X and 3.0 ppb. Each sample will be fortified and then diluted to 5X and another dilution such as 2X prior to analysis. A total of 48 samples will be analyzed for this validation at two dilutions as noted above. LOQ is dependant on each analyte sensitivity and is defined in the method and below for each of the analytes.

Common Name	Proposed LOQ, ppb
Atrazine (G-30027)	0.1
Simazine (G-27692)	0.2
G-30033	0.1
G-28279	0.2
G-28273	0.5
Ametryn	0.1
Prometryn	0.1
GS-11354	0.1
GS-11355	0.1
GS-26831	0.2
Metolachlor	0.2

3.4 Method Performance

ADPEN will analyze the validation set following instructions in Syngenta Method No. T010097-04 and this protocol. The method will be tested using an Applied Biosystems, PE Sciex API 3000 LC/MS/MS tandem quadrupole instrument. Equivalent instrumentation, materials and reagents may be substituted for those specified in the method. Any modifications or substitutions will be clearly documented in the raw data. Average recoveries should be in the range of 70-120%. No historical recovery data are available on similar LC/MS/MS instrumentation. Correlation of calibration standard curve should be greater than 0.99 and the relative standard deviations should be less than 20%. Interferences and/or matrix suppression should be negligible. As stated in the method, to prevent matrix effects, samples may be diluted. Final LOQ values will be determined from this validation.

The results of the method trial / validation will be reviewed by the Study Director and Sponsor. If the results are determined to be acceptable, results of this validation will be included in the method by Syngenta.

3.5 Data Evaluation

Evaluation of the data will include but not be limited to calculations of percent recovery, mean percent recovery and standard deviation for the analyte.

4.0 FINAL REPORT

ADPEN Laboratories, Inc. will prepare a final report containing the results of the validation study. The report will include but not be limited to the reporting requirements outlined in the USEPA guidance documents cited in this protocol [2, 3, and 4] and in PR Notice 86-5 [5].

5.0 DATA RETENTION

In addition to the original protocol and final report, all original raw data generated during the study will be retained in the archives of ADPEN Laboratories, Inc., 11757 Central Parkway, Jacksonville, Florida as required by EPA FIFRA GLP following completion of the final report.

6.0 PROTOCOL CHANGES

Any required changes or revisions will be documented by issuing a protocol amendment. The amendment will include a description of the change, the reason for the change and the effective date of the change. The protocol amendment will be signed and dated by the Study Director. The amendment will be appended to the protocol and included in the final report.

7.0 QUALITY ASSURANCE

The Quality Assurance Unit of ADPEN Laboratories, Inc. will inspect one or more critical phases of the study to assure that facilities, equipment, personnel, procedures, and records conform to Good Laboratory Practice Standards, 40 CFR Part 160. The results of these inspections will be reported to the Study Director, the Sponsor and ADPEN management. The final report will be reviewed for protocol and GLP compliance, as well as to assure that the methods and standard operating procedures utilized were followed. A signed statement will be included in the final report specifying when inspections were made and when inspections were reported to the above named personnel.

8.0 REFERENCES

1. Sung-Ben Huang and Robert A. Yokley, " Analytical Method T010097-04 for the Determination of Atrazine, Simazine, G-30033, G-28279, G-28273, Ametryn, Prometryn, GS-11354, GS-11355, GS-26831, and Metolachlor in Water Using Direct Injection LC-ESI/MS/MS Including Validation Data.". Syngenta Method No. T010097-04.

2. U.S. Environmental Protection Agency, Office of Pollution Prevention and Toxic Substances (OPPTS). August 1996. Residue Chemistry Test Guidelines, OPPTS 860.1340, Residue Analytical Method. EPA 712-C-96-174.

3. U.S. Environmental Protection Agency, Office of Compliance Monitoring. 1989. Federal Insecticide, Fungicide and Rodenticide Act (FIFRA); Good Laboratory Practice Standards; Final Rule, 40 CFR, Part 160. Federal Register, Vol. 54, No. 158: pp. 34052-34074.

4. U.S. Environmental Protection Agency. July 1986. PR Notice 86-5. Standard format for data submitted under the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) and certain provisions of the Federal Food, Drug and Cosmetic Act (FFDCA).

PROTOCOL AMENDMENT

Study Title: Method Validation of Syngenta Analytical Method Number T010097-04 Direct Injection ESI LC/MS/MS Multiresidue Triazine Analysis of Water Samples.

Syngenta Study No.: T010097-04

1. **Description of Change:** Change to Protocol, pages 4,7 and 8.

   The references in these pages to API 3000, should be changed to read API 3000 and API 4000.

   Reason for Change: Syngenta has an opportunity window to run the validation of the method also on the API 4000 at their facility.

   Effect of Change on Study: No adverse effect.

2. **Description of Change:** Addition to Protocol, page 9, Section 7.

   During the validation trial at Syngenta, Syngenta's QA will perform an in-progress audit of the critical phases of the method, standard preparation, sample dilution and LC/MS/MS analysis. A data audit will be performed of the data generated at Syngenta, by Syngenta's QA. Response to any findings will be completed by Syngenta's Project Manager and the audit reports then submitted to the Study Director prior to completion of the Final Report. A QA statement will be completed by Syngenta's QAU in the Final Report. A Final Report GLP statement will be signed by the Sponsor Management.

   Reason for Change: Additional validation will be done outside of ADPEN Laboratories, Inc. under the direction of Syngenta's Project Manager.

   Effect of Change on Study: No adverse effect.

3. **Description of Change:** Change to Protocol, page 7 and 8.

   The references on page 7 to two dilutions should be changed to read for the API 4000 test, only a 5X dilution will be tested. The LOQ is dependant on each analyte sensitivity and the LOQ tested on the API 4000 will be 0.1 ppb as 1 X LOQ for each

analyte, 0.2 ppb (2X LOQ) and 3 ppb for the three matrices.

Reason for Change: Syngenta has an opportunity window to run the validation of the method on the API 4000 at their facility which may have better sensitivity and lower LOQ concentrations than the API 3000.

Effect of Change on Study: No adverse effect.

Approved by:

Project Manager, Syngenta Crop Protection, Inc.:

Andrew Merritt
Syngenta Project Manager

March 16, 2005
Date

Study Director, ADPEN Laboratories, Inc.:

Rolando Perez
Technical Director

3/14/05
Date

Management/Sponsor, Syngenta Crop Protection, Inc.

Sunmao Chen, Ph.D.
Manager, Environmental Residue; Environmental Fate Department

3/21/05
Date

PROTOCOL AMENDMENT

Study Title: Method Validation of Syngenta Analytical Method Number T010097-04 Direct Injection ESI LC/MS/MS Multiresidue Triazine Analysis of Water Samples.

Syngenta Study No.: T010097-04

1. **Description of Change:** Addition of Standard information
   The standards listed below were used during Syngenta's method validadion run.
   Reason for Change: Validation tests were also conducted at Syngenta
   Effect of Change on Study: No adverse effect.

Compound	Purity	Standard No.	Expiration
Atrazine	97.2	S02-2704	9/2005
G-30033	94.0	S92-1618	8/2008
G-28279	96.0	S87-1225	3/2007
G-28273	97.0	S87-1195	8/2008
Simazine	99.7	S86-1071	3/2007
Ametryn	97.9	S00-2434	10/2005
Prometryn	99.7	S89-1411	10/2005
Metolachlor	98.0	S98-2315	5/2009
G-26831	99.0	S85-0802	4/2007
G-11354	97.0	S85-0804	4/2007
G-11355	98.0	DAH-XXXI-29-1	5/2006

Approved by:

Project Manager, Syngenta Crop Protection, Inc.:

Andrew Merritt
Syngenta Project Manager

April 12, 2005
Date

Study Director, ADPEN Laboratories, Inc.:

Rolando Perez
Technical Director

4/13/05
Date

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4/12/05
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