Reference Substance Information:

Atrazine (G-30027)
CAS RN: 1912-24-9
Chemical Name: 1,3,5-Triazine-2,4-diamine, 6-chloro-*N*-ethyl-*N*-(1-methylethyl)-
Syngenta standard code: S96-1870, S02,2704
Purity: 97.9%, 97.2%

G-30033
CAS RN: 6190-65-4
Chemical Name: 1,3,5-Triazine-2,4-diamine, 6-chloro-*N*-(1-methylethyl)-
Syngenta standard code: S92-1618
Purity: 94%

G-28279
CAS RN: 1007-28-9
Chemical Name: 1,3,5-Triazine-2,4-diamine, 6-chloro-*N*-ethyl
Syngenta standard code: S87-1225
Purity: 96%

G-28273
CAS RN: 3397-62-4
Chemical Name: 1,3,5-Triazine-2,4-diamine, 6-chloro
Syngenta standard code: S87-1195
Purity: 97%

Simazine (G-27692)
CAS RN: 122-34-9
Chemical Name: 1,3,5-Triazine-2,4-diamine, 6-chloro-*N*,*N*'-diethyl-
Syngenta standard code: S86-1071
Purity: 99.7%

Ametryn (G-34162)
CAS RN: 834-12-8
Chemical Name: 1,3,5-Triazine-2,4-diamine, *N*-ethyl-*N*'-(1-methylethyl)-6-(methylthio)-
Syngenta standard code: S00-2434, S01-2517
Purity: 97.9%, 98.3%

Prometryn (G-34161)
CAS RN: 7287-19-6
Chemical Name: 1,3,5-Triazine-2,4-diamine, N,N'-bis(1-methylethyl)-6-(methylthio)-
Syngenta standard code: S89-1411
Purity: 99.7%

Reference Substance Information (continued):

GS-11354
CAS RN: 4147-57-3
Chemical Name: 1,3,5-Triazine-2,4-diamine,*N*-(1-methylethyl)-6-(methylthio)-
Syngenta standard code: S85-0804
Purity: 97%

GS-11355
CAS RN: 4147-58-4
Chemical Name: 1,3,5-Triazine-2,4-diamine,N-ethyl-6-(methylthio)-
Syngenta standard code: DAH-XXXI-29-1
Purity: 98%

GS-26831
CAS RN: 5397-01-3
Chemical Name: 1,3,5-Triazine-2,4-diamine,6-(methylthio)-
Syngenta standard code: S85-0802
Purity: 99%

Metolachlor
CAS RN: 51218-45-2
Chemical Name: Acetamide, 2-chloro-N-(2-ethyl-6-methylphenyl)-N-(2-methoxy-1-methylethyl)-
Syngenta standard code: S98-2315
Purity: 98.0%

ABBREVIATIONS AND SYMBOLS

Abbreviation	Definition
A	acre
a.i.	active ingredient
amt	amount
amu	atomic mass unit
C	Celsius or Centigrade
CAS	Chemical Abstract Services
CFR	Code of Federal Regulations
cm	centimeter
DA[#]A	days after application, [#] = 1, 2, 3 etc., if there are multiple applications
EPA	Environmental Protection Agency (U.S.)
EU	European Union
FIFRA	Federal Insecticide, Fungicide and Rodenticide Act (U.S.)
ft	foot (feet)
g	gram
gal	gallon
GC	gas chromatography
GLPs	Good Laboratory Practices
ha	hectare
HPLC	high performance liquid chromatography
i.d.	inside diameter
ID	identification
in.	inch
IUPAC	International Union of Pure and Applied Chemistry
kg	kilogram
L	liter
lb	pound
LC	liquid chromatography
LC/MS/MS	tandem liquid chromatography/mass spectrometry/mass spectrometry
LOD	limit of detection
LOQ	limit of quantitation
m	meter
m/z	mass to charge ratio
µg	microgram

ABBREVIATIONS AND SYMBOLS (continued)

Abbreviation	Definition
µL	microliter
µm	micrometer
MDL	method detection limit
mg	milligram
mL	milliliter
mm	millimeter
mmol	millimole
min	minute
mol	mole
MS	mass spectrometry
MS/MS	tandem mass spectrometry/mass spectrometry
mV	millivolt
MW	molecular weight
N/A	not applicable
ND or nd	nondetect (below limit of detection)
ng	nanogram
No.	number
oz	ounce
PMRA	Pest Management Regulatory Agency, Canada
ppb	parts per billion or micrograms per kilogram
ppm	parts per million or microgram per gram or milligrams per kilogram
pg	picogram
psi	pounds per square inch
QAU	quality assurance unit
R^2 (or r^2)	square of correlation coefficient
RSD	relative standard deviation
Rt	retention time
s	second
SD	standard deviation
USDA	United States Department of Agriculture
UV	ultraviolet
vol	volume
wt	weight

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.

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		

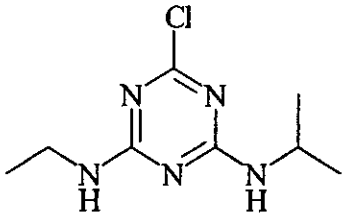
Analyte	MRM Transition	Ion Mode	DP	CE	XP	
G-28273	146.0/104.0	+	40	26	8	Period 1 dwell 3000
GS-26831	158.2/110.0	+	60	25	10	Period 2 dwell 100
G-28279	174.2/96.2	+	75	24.5	8	
GS-11355	186.1/96.0	+	80	30	9	
G-30033	188.3/146.1	+	65	24	10	
GS-11354	200.3/158.0	+	70	25	14	
Simazine	202.1/132.1	+	53	27	8	
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.

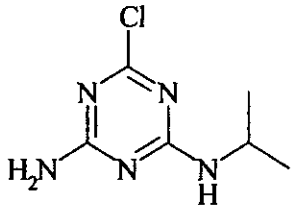
Note: Above parameters are typical for an API 4000. Parameters for an API 3000 are shown in Appendix 3. Optimization parameters for the above compounds must be established when using any LC/MS/MS for the first time for these compounds.

8.0 FIGURES

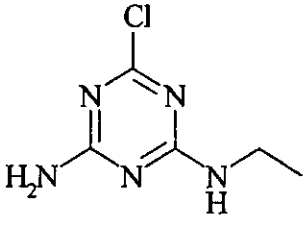
FIGURE 1. STRUCTURES OF ANALYTES



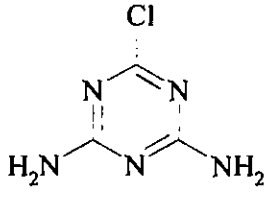
Atrazine
MW 215.7



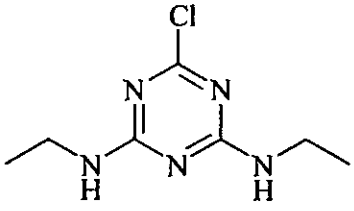
G-30033
MW 187.6



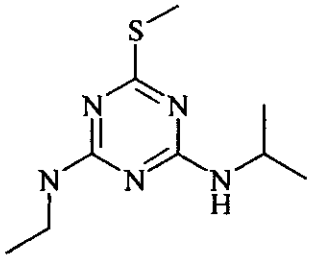
G-28279
MW 173.6



G-28273
MW 145.6

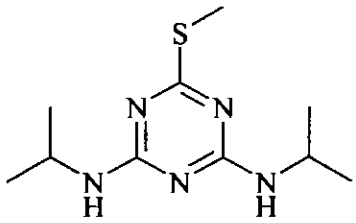


Simazine
MW 201.7

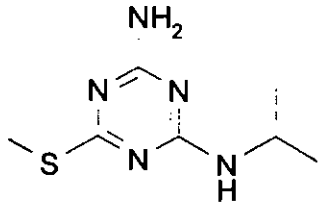


Ametryn
MW 227.3

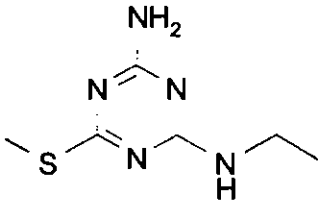
FIGURE 1. STRUCTURES OF THE ANALYTES (Continued)



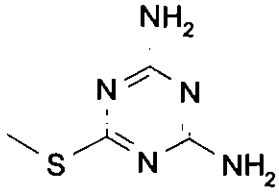
Prometryn
MW 241.4



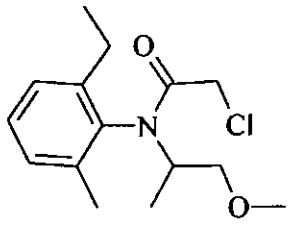
GS-11354
MW 199.1



GS-11355
MW 185.1

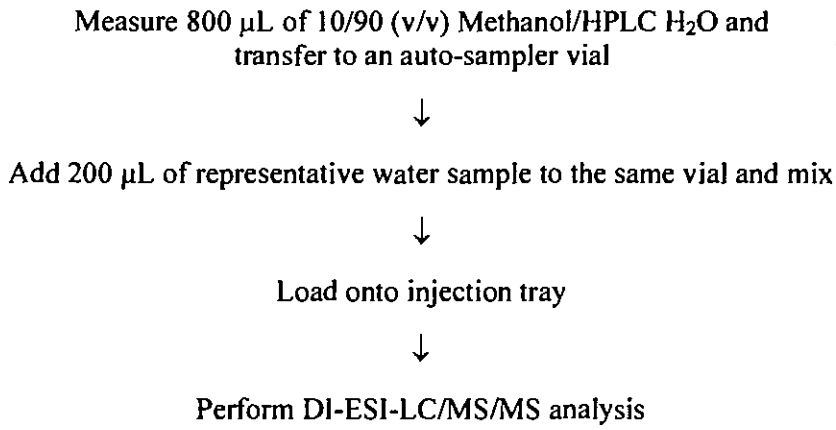


GS-26831
MW 157.0



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.