1.0 ABSTRACT

The purpose of this independent laboratory validation study is to evaluate the performance of the analytical method for atrazine, simazine, propazine, G30033, G28279, G28273, and metolachlor in water as described in Syngenta Method GRM014.02A. This study was designed to demonstrate the utility, ruggedness, efficiency, and identify potential points of clarification in the subject method as written. It fulfills the requirements of the U.S. EPA guidelines found in 850.7100, Data Reporting for Environmental Chemistry Methods, EPA, Washington, D.C. April 1996. This validation study also meets the European Commission Guidance Document requirements on Residue Analytical Methods, SANCO/825/00 rev.7, March 17, 2004. The subject method is designed to measure residues of atrazine, simazine, propazine, G30033, G28279, G28273, and metolachlor in water with limits of quantitation (LOQs) of 0.050 µg/L (ppb) for atrazine, simazine, propazine, G30033, and G28279, 0.50 µg/L (ppb) for G28273, and 0.10 µg/L (ppb) for metalochlor. The representative matrices examined in this validation study were groundwater (raw), tap water (finished) and surface water (raw).

The respective LOQ values reported in the analytical method were independently validated by obtaining average recoveries within the acceptable range of 70 to 120% for controls fortified five times each at the LOQ, and with a relative standard deviation (RSD) of less than 20%.

Performance of the subject method was successfully validated for the quantitation of atrazine, simazine, propazine, G30033, G28279, G28273, and metolachlor in groundwater, treated (finished) water, and surface water at the respective LOQ and 10X LOQ. A re-analysis of treated (finished) water at 10x LOQ was performed to correct apparent laboratory fortification error in one of the 10X replicates. This independent laboratory validation study demonstrated that the analytical method GRM014.02A is acceptable for the quantification of atrazine, simazine, propazine, G30033, G28279, G28273 and metolachlor in water.

Chromatograms of control (unfortified) water samples indicated there were no interferences at the elution regions of simazine, propazine, G30033, G28279, G28273 and metolachlor. Minimal interferences (<30% of LOQ) were noticed for atrazine in all control water samples.

Each validation set, consisting of 23 injections (which includes all samples, standards, and controls), was prepared in approximately 2 hours. HPLC/MS/MS analysis took approximately 4 hours. Data processing and verification required approximately 3 hours on the following day. The HPLC/MS/MS run time was the rate-determining step.

2.0 INTRODUCTION

Independent laboratory validation of enforcement methods is required by the U.S. EPA OPPTS 850.7100 (Reference 1) and EU Guidance document SANCO/825/00 rev. 7 (Reference 2).

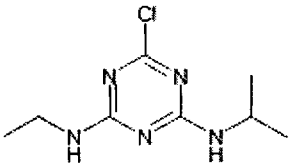
The subject method is applicable for the quantification of atrazine, simazine, propazine, G30033, G28279, G28273, and metolachlor in water, as described in Syngenta Method GRM014.02A (Reference 3). Groundwater, treated (finished) drinking water, and surface water were chosen to validate the analytical method as representative matrices.

Fortification levels in this study were chosen to provide method performance data at the method LOQs and 10×LOQ for each analyte in each matrix.

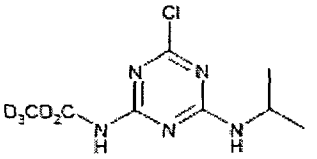
3.0 MATERIALS AND METHODS

3.1 Test and Reference Substances

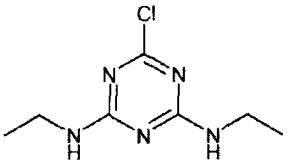
The structure and chemical name of each test material is shown below:



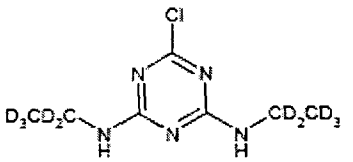
Code Name: Atrazine (G30027)
***Chemical Abstracts* Name**: 1,3,5-Triazine-2,4-diamine, 6-chloro-N-ethyl-N'-(1-methylethyl)-
CAS Registry No.: 1912-24-9
Lot No.: 455333
Purity: 96.2%
Storage: < 30° C



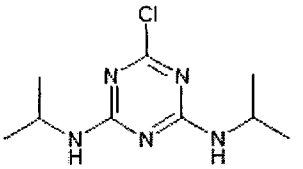
Code Name: Atrazine-d5
Lot No.: K136P100
Purity: >99%
Storage: Room Temperature



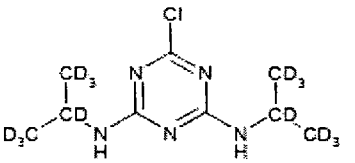
Code Name: Simazine (G27692)
***Chemical Abstracts* Name**: 1,3,5-Triazine-2,4-diamine, 6-chloro-N,N'-diethyl-
CAS Registry No.: 122-34-9
Lot No.: 486779
Purity: 98.8%
Storage: < 30° C



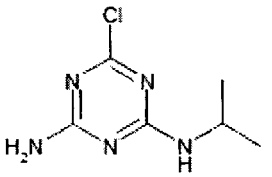
Code Name: Simazine-d10
Lot No.: R544P33
Purity: >99%
Storage: Room Temperature



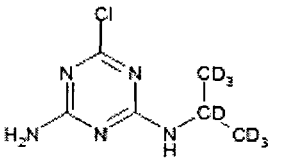
Code Name: Propazine (G30028)
***Chemical Abstracts* Name**: 1,3,5-Triazine-2,4-diamine, 6-chloro-N,N'-bis(1-methylethyl)-
CAS Registry No.: 139-40-2
Lot No.: 423440
Purity: 98.5%
Storage: < 30° C



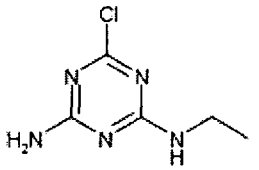
Code Name: Propazine-d14
Lot No.: Z188P7
Purity: >99%
Storage: Room Temperature



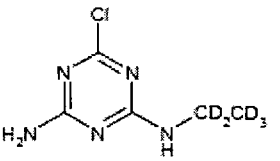
Code Name: Deethyl Atrazine (G30033)
***Chemical Abstracts* Name**: 1,3,5-Triazine-2,4-diamine, 6-chloro-N-(1-methylethyl)-
CAS Registry No.: 6190-65-4
Lot No.: DAH-XXXIII-48
Purity: 99.2%
Storage: Refrigerate



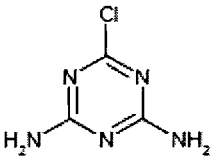
Code Name: G30033-d7
Lot No.: U437P33
Purity: >99%
Storage: Room Temperature



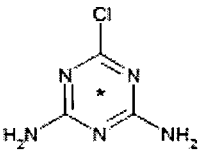
Code Name: Deisopropyl Atrazine (G28279)
***Chemical Abstracts* Name**: 1,3,5-Triazine-2,4-diamine, 6-chloro-N-ethyl-
CAS Registry No.: 1007-28-9
Lot No.: JAK-XXVI-61-1
Purity: 98.8%
Storage: Refrigerate



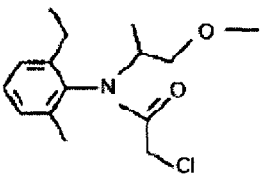
Code Name: G28279-d5
Lot No.: R488P25
Purity: 98.8%
Storage: Room Temperature



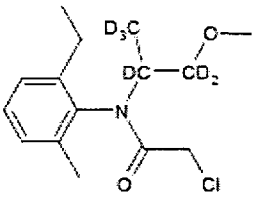
Code Name: Didealkyl Atrazine (G28273)
***Chemical Abstracts* Name**: 1,3,5-Triazine-2,4-diamine, 6-chloro-
CAS Registry No.: 3397-62-4
Lot No.: 456525
Purity: 97%
Storage: < -10° C



Code Name: G28273-$^{13}C_3$
Lot No.: SCGJ-022
Purity: 95.3%
Storage: Room Temperature



Code Name: Metolachlor (CGA24705)
***Chemical Abstracts* Name**: Acetamide, 2-chloro-N-(2-ethyl-6-methylphenyl)-N-[2-methoxy-1-methylethyl]-
CAS Registry No.: 51218-45-2
Lot No.: 412778
Purity: 98%
Storage: < 30° C



Code Name: Metolachlor-d6
Lot No.: G556P22
Purity: 99.3%
Storage: Room Temperature

The test substances were supplied by Syngenta Crop Protection, Inc. (Greensboro, NC). Information pertaining to the characterization and stability of each test substance is archived by Syngenta Crop Protection, Inc. (Greensboro, NC). Characterization data were provided by Syngenta Crop Protection, Inc. (Greensboro, NC). A Certificate of Analysis, including lot number and purity, is included with the study raw data file that will be archived by ABC Laboratories, Inc. and Syngenta Crop Protection, Inc.

The reference substance for G28723 was supplied by Cambridge Isotope Laboratories, Inc. (Andover, MA). The other six reference substances were supplied by CDN Isotopes, Inc. (Pointe-Claire, QC, Canada). The reference substances were characterized by their respective suppliers.

3.2 Test System

The subject method is applicable for the quantitation of atrazine, simazine, propazine, G30033, G28279, G28273, and metolachlor in water. Groundwater, treated (finished) water, and surface water were chosen to validate the analytical method as representative of the water types to be tested using the method.

The control matrices were acquired from Agvise Laboratories (Northwood, ND). These water samples were refrigerated prior to being analyzed. Groundwater was from a well owned by Bob Deutsh at Agvise Laboratories, treated (finished) water was tap water from Agvise Laboratories and surface water was from Goose River in Northwood, ND. The pertinent physical characteristics are summarized in the following table. Water samples were characterized at Agvise Laboratories (Northwood, ND). Characterization records are included in the raw data.

MEASUREMENT	GROUNDWATER	TREATED WATER	SURFACE WATER
pH	7.5	7.9	8.2
Calcium	158 ppm	32 ppm	133 ppm
Magnesium	67 ppm	8.7 ppm	74 ppm
Sodium	18 ppm	7.0 ppm	149 ppm
Hardness	677 mg equiv. CaCO3/L	117 mg equiv. CaCO3/L	643 mg equiv. CaCO3/L
Conductivity	1.12 mmhos/cm	0.26 mmhos/cm	1.47 mmhos/cm
Sodium Adsorption Ratio (SAR)	0.31	0.28	2.57
Total Dissolved Solids	814 ppm	114 ppm	1156 ppm
Turbidity	0.91 NTU	0.20 NTU	18.8 NTU

3.3 Equipment

The following equipment items were used in the conduct of this independent laboratory validation.

3.3.1 Instrumentation/Chromatography

HPLC/MS/MS System: Applied Biosystems/Sciex API 5000; Waters Acquity Column Manager, Sample Manager, Binary Solvent Manager, and Sample Organizer, Zorbax SB-Aq, 4.6 x 50 mm, 3.5 µm particle size diameter

3.3.2 General Lab Equipment/Devices

Balance: Mettler Toledo, Model XP205DR

Mixer: Vortex mixer

3.3.3 Labware

Volumetric flasks: various sizes, Class A certified

Pipettors/tips: Glass volumetric pipets, Class A certified; assorted volumes

Disposable Pasteur pipets, 146 mm, Fisher Scientific, Catalog No. 22-230-482

Gilson Positive Displacement pipets, 10µL -1000µL

Hamilton Air Displacement pipets

Centrifuge tubes: polypropylene, 15-mL, graduated with plastic screw cap, Fisher Scientific Catalog No. 4-959-53A

Glassware: Bottles, amber glass Boston round, 4 oz., with Polyseal-lined cap, Fisher Scientific Catalog. No. 02-911-895

Vials/enclosures: Auto-sampler vials, National Scientific C4011-6W, Fisher Scientific Catalog No. 03-395H

Auto-sampler vial enclosures, National Scientific C4011-55R , Fisher Scientific Catalog No. 03-396AA

3.4 Reagents

Reagents and standards used were of equivalent grade as specified in the analytical method.

3.5 Principles of the Analytical Method

After thermal equilibration to ambient temperature, a 900 µL aliquot of a thoroughly mixed water sample is transferred to an HPLC vial followed by addition of 100 µL of internal standard solution of known concentration. The internal standard solution applied in this method contains atrazine-d5, simazine-d10, propazine-d14, G30033-d7, G28279-d5, G28273-$^{13}C_3$ and metolachlor-d6 at a concentration of 50 pg/µL for each individual analyte. This results in a final concentration of internal standard of 5.0 pg/µL in every sample. After complete mixing, the sample is subjected to LC-MS/MS analysis. The data system uses the calibration plot and the respective peak response ratios relative to corresponding internal standards to calculate the amount of analyte in a sample.

3.6 Modifications, Interpretations, Critical Steps, and Deviations

The analytical method was run exactly as written except as follows:

The injection volume of the LC system was 35 µL instead of 50 µL to optimize instrument response.

3.7 Instrumentation

3.7.1 Chromatography

Reversed-phase liquid chromatography was used to achieve adequate analytical separation.

HPLC Conditions

System:	Applied Biosystems/Sciex API 5000, Waters Acquity UPLC
Column:	Zorbax SB-Aq 3.5 µm, 4.6 x 50 mm
Column Temperature:	25° C
Injection Volume:	35 µL
Flow Rate:	(0.5 – 1.0 mL/min)
Conditions:	A: 0.1% formic acid (aq.) B: 0.1% formic acid in MeOH
Total Run Time:	7.5 minutes

Gradient:	Time	%A	%B
	0.0	95.0	5.0
	0.50	95.0	5.0
	1.00	70.0	30.0
	1.50	70.0	30.0
	2.50	10.0	90.0
	5.50	10.0	90.0
	5.60	95.0	5.0
	6.50	95.0	5.0
	6.60	95.0	5.0
	7.50	95.0	5.0

3.7.2 LC/MS/MS Analysis

Analysis of atrazine, simazine, propazine, G30033, G28279, G28273, and metolachlor was performed using a Sciex API 5000 LC/MS/MS, equipped with a TurboIonSpray source, operated in MRM positive ion mode. Quantitation was based upon interpolation from a quadratic regression curve of analyte/internal standard peak area ratios versus analyte concentration calculated by Analyst software 1.5. Recovery calculations were performed using Microsoft Excel. A summary of representative experimental conditions is provided in the following table:

MDS Sciex API 5000 MS/MS Mass Spectrometer Conditions Positive Ion Mode

Analyte	Ions Monitored	CXP (Collision Cell Exit Potential)	DP (Declustering Potential)	Dwell Time (msec)	Collision Energy
Atrazine	216.0 → 174.0	13V	70V	50	26V
Atrazine-d5	221.0 → 179.0				
Simazine	202.0 → 132.0	13V	70V	50	27V
Simazine-d10	212.0 → 137.0				
Propazine	230.0 → 146.0	13V	70V	50	32V
Propazine-d14	244.0 → 148.0				
G30033	188.0 → 146.0	13V	70V	50	32V
G30033-d7	195.0 → 147.0				
G28279	174.0 → 104.0	13V	70V	50	32V
G28279-d5	179.0 → 137.0				26V
G28273	146.6 → 104.0	13V	70V	50	26V
G28273-$^{13}C_3$	149.6 → 106.0				
Metolachlor	284.0 → 176.0	13V	70V	50	36V
Metolachlor-d6	290.0 → 258.0				21V

NEB	CUR	TEM	CAD	IS	EP
50 psig	15 psig	500° C	6 psig	5500	10

3.7.3 Calibration Procedure

Calibration standards were interspersed throughout the batch with concentrations varying randomly.

3.8 Calculations

Residue values were calculated from a quadratic regression curve of analyte/internal standard peak area ratios versus analyte concentrations.

The equation used for the least squares fit is:

$$y = ax^2 + bx + c$$

Where:

y	=	peak area ratio
x	=	ppb found for peak of interest
a	=	x^2 coefficient
b	=	x coefficient
c	=	y-intercept

Example calculations for an untreated control sample and a fortified control sample are provided below.

The concentration (ng/mL) found in the samples was determined by the following calculation:

$$\text{ppb of analyte} = \frac{(-b + b^2 - 4 \times a\ (y - \text{intercept} - \text{peak area ratio}))^{1/2}}{2 \times a}$$

Where:
ppb of analyte = Determined amount of residue in nanograms per milliliter

peak area ratio = (analyte peak area/internal standard peak area) for the sample

For example, for atrazine in control groundwater (sample 66299-GW02):

$$\frac{(-0.2350278 + 0.2350278^2 - 4 \times -0.005954081(0.003050031 - 0.001688))^{1/2}}{2 \times -0.005954081} = 0 \text{ ppb } (< LOQ)$$

Percent Recovery was calculated as:

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

For example, for atrazine in groundwater fortified at 0.050 µg/kg (sample 66299-GW04):

$$\frac{(0.0478 - 0)}{0.050} \times 100 = 96\%\ \text{Recovery}$$

Water Matrix	Calibration Standard Range (ppb)						
	Atrazine	Simazine	Propazine	G30033	G28279	G28273	Metolachlor
Groundwater	0.020-10.0	0.020-10.0	0.020-10.0	0.020-10.0	0.020-10.0	0.20-10.0	0.050-10.0
Treated Water	0.020-10.0	0.020-10.0	0.020-10.0	0.020-10.0	0.020-10.0	0.20-10.0	0.050-10.0
Treated Water Re-analysis	0.020-5.0	0.020-5.0	0.020-5.0	0.020-5.0	0.020-5.0	0.20-5.0	0.050-5.0
Surface Water	0.020-5.0	0.020-5.0	0.020-5.0	0.020-5.0	0.020-5.0	0.20-5.0	0.050-5.0

The detector response suitability of each analyte was assessed using a calibration curve generated via regression analysis within each analysis sequence injected. All calibration plots exhibited correlation coefficients (r) $\geq$ 0.999. Detector response was stable throughout the course of the analytical run as was demonstrated by standard accuracy and precision values.

4.1.2 Control Samples

There were no interference peaks detected at the retention time for propazine, G28279, G28273, or metolachlor in duplicate unfortified control samples analyzed concurrently with the accepted validation trials. A small amount of atrazine, simazine, and G30033 residues (< 30% LOQ) were detected in unfortified control water samples. Analyte recovery values were corrected for the average amount found in the control samples.

4.1.3 Trial Results

The first trials for the independent laboratory validation of atrazine, simazine, propazine, G30033, G28279, G28273, and metolachlor in groundwater, treated (finished) water, and surface water (Trial 1) were successful, with the exception of the treated (finished) water samples at the 10XLOQ fortification level due to laboratory fortification errors. A re-analysis of the 10XLOQ fortification levels (5 replicates 10XLOQ) in treated (finished) water was successful.

A typical run sequence is presented in the table below (taken from groundwater analyses):

SAMPLE DESCRIPTION/ STANDARD CONCENTRATION
1.0 ng/mL calibration standard
0.20 ng/mol calibration standard
Injection Solvent
2.0 ng/mLcalibration standard
0.020 ng/mLcalibration standard
Reagent blank
control ground water
control ground water
0.10 ng/mL calibration standard
control ground water + LOQ
control ground water + LOQ
control ground water + LOQ
0.50 ng/mL calibration standard
control ground water + LOQ
control ground water + LOQ
control ground water + 10XLOQ
5.0 ng/mL calibration standard
control ground water + 10XLOQ
control ground water + 10XLOQ
control ground water + 10XLOQ
control ground water + 10XLOQ
0.050 ng/mL calibration standard
10.0 ng/mL calibration standard

4.2 Communications

For detailed communications refer to Appendix 2.

4.3 Time Requirements

Each validation trial, consisting of 23 injections (which includes all samples, standards, and controls), was prepared in approximately 2 hours. HPLC/MS/MS analysis took approximately 4 hours. Data processing and verification required approximately 3 hours.

5.0 CONCLUSIONS

This independent laboratory validation study was successful and it demonstrated that the analytical method GRM014.02A is acceptable for the quantitation of atrazine, simazine, propazine, G30033, G28279, G28273, and metolachlor residues in water, according to guidelines set forth by US EPA Ecological Effects Guidelines, OPPTS 850.7100 "Data Reporting for Environmental Chemistry Methods" (Reference 1) and European Commission Guidance Document on Residue Analytical Methods, SANCO/825/00 rev.7, March 17, 2004 (Reference 2). Acceptable recoveries were obtained from groundwater, treated (finished) water, and surface water samples fortified at respective LOQ and 10X LOQ levels of atrazine, simazine, propazine, G30033, G28279, G28273 and metolachlor.

6.0 RETENTION OF RECORDS

All raw data, including, but not limited to, the original chromatograms, worksheets, correspondence, and results shall be included with the data package submitted to the Sponsor after completion of the study. These will be sent for archiving, along with the original protocol, amendments, final report, and all pertinent information to:

Syngenta Crop Protection, Inc.
410 Swing Road
Greensboro, North Carolina 27409

Laboratory-specific or site-specific raw data such as personnel files, instrument, equipment, refrigerator, freezer raw data, exact copies of all raw data and pertinent information, including copies of the original protocol, any amendments, and the final report will be retained at:

ABC Laboratories, Inc.
7200 E. ABC Lane
Columbia, Missouri 65202

7.0 REFERENCES

1. United States Environmental Protection Agency (US EPA). Office of Prevention, Pesticides, and Toxic Substances (OPPTS). Ecological Effects Test Guidelines, OPPTS 850.7100, Data Reporting for Environmental Chemistry Methods. EPA, Washington, D.C. April 1996.
2. European Commission (Directorate General Health and Consumer Protection), Guidance Document on Residue Analytical Methods, SANCO/825/00 rev.7, March 17, 2004.
3. Huang, Sung-Ben. 2010. "Analytical Method for the Determination of Atrazine, Simazine, Propazine, G30033, G28279, G28273 and Metolachlor in Water Using Direct-Aqueous-Injection Liquid Chromatography-Electrospray Ionization Tandem Mass Spectrometry (LC-ESI/MS/MS) with Stable Isotope Analogues as Quantification Internal Standard", Method GRM014.02A, Syngenta Crop Protection, Inc. 410 Swing Road Greensboro, NC 27409 USA.