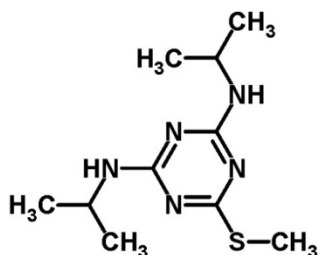


3.0 MATERIALS AND METHODS

3.1 Test/Reference Substance

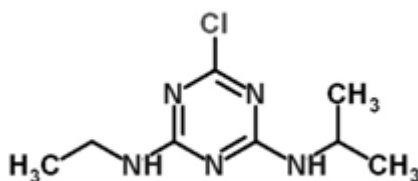
The test/reference substances were obtained from Syngenta Crop Protection, LLC. The following test/reference substances were used:

Compound Structure:



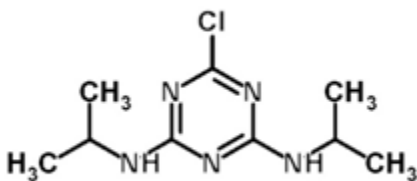
Syngenta Code:	G34161
Common Name:	Prometryn
CAS Name:	N,N'-Diisopropyl-6-(methylsulfanyl)-1,3,5-triazine-2,4-diamine
CAS Number:	7287-19-6
Molecular Weight:	241.4
Standard Reference:	410566
Storage Conditions:	<30°
Purity:	NA
Expiration Date:	01/2018

Compound Structure:



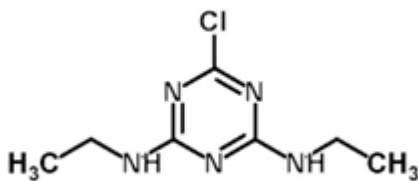
Syngenta Code:	G30027
Common Name:	Atrazine
CAS Name:	6-Chloro-N-ethyl-N'-isopropyl-1,3,5-triazine-2,4-diamine
CAS Number:	1912-24-9
Molecular Weight:	215.7
Standard Reference:	691260
Storage Conditions:	<30°
Purity:	97.1%
Expiration Date:	09/2016

Compound Structure:



Syngenta Code:	G30028
Common Name:	Propazine
CAS Name:	6-Chloro-N,N'-diisopropyl-1,3,5-triazine-2,4-diamine
CAS Number:	139-40-2
Molecular Weight:	229.7
Standard Reference:	423440
Storage Conditions:	<30°
Purity:	98.5%
Expiration Date:	09/2016

Compound Structure:



Syngenta Code:	G27692
Common Name:	Simazine
CAS Name:	6-Chloro-N,N'-diethyl-1,3,5-triazine-2,4-diamine
CAS Number:	122-34-9
Molecular Weight:	201.7
Standard Reference:	486779
Storage Conditions:	<30°
Purity:	98.8%
Expiration Date:	06/2016

Characterization data for the test/reference standard are maintained by the Sponsor, "Syngenta Crop Protection LLC". The Certificate of Analysis is included in Appendix 2.

The test/reference substance (analytical standard) used in this study was provided by the sponsor and stored as directed on "Analytical Standards Chain of Custody" documents. All solutions made from the reference substances (analytical standards) were stored according to the method.

3.2 Test System

The test system evaluated in this study was soil. The control sample used in this study was provided by the sponsor. The control soil sample was characterized by AGVISE Laboratories of Northwood, North Dakota and reported to Syngenta Archive under Syngenta Study Number TK0225755.

The control soil sample was received on 05/30/2014 and stored in the freezer (-20°C) at Primera Analytical Solutions Corp. (PASC).

The control sample was checked for contamination prior to use in this ILV study by employing the same extraction and detection method as described in Syngenta Method GRM071.01A “Analytical Method for the Determination of Atrazine, Simazine, Propazine, and Prometryn in Soil by LC-MS/MS”.

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

3.2.1 Equipment

- a) Analytical Balance: Mettler Toledo, Model MT5 (LETS# 169, Calibration Due: 05/2015, weight verification daily)
- b) pH Meter: ORION, Model 520A (LETS# 92, Calibration Due: 05/2015, pH calibration daily)
- c) Water Acquity UPLC (LETS# 196, Calibration Due: 05/2015)
- d) Mass spectrum: AB Sciex API4000, software: Analyst 1.4.2 (LETS#102-A, Calibration Due: 09/2014)
- e) Column : ACE 5 C18, 3.0 × 50mm, 5.0 μm
- f) Pipettes: Eppendorf, LETS# 305-5, Calibration Due 03/2015, 20-200 μL
Eppendorf, LETS#305-6, Calibration Due: 11/2014, 100-1000μL

3.2.2 Reagents

Name	Manufacture	Lot No.	Expiry Date
Acetonitrile	J.T.Baker	60450	06/10/2019
Methanol	J.T.Baker	71346	05/06/2019
Acetone	Alfa	C22Z724	08/30/2018
Acetic acid	J.T.Baker	K45803	06/17/2019
Water	Milli-Q System	N/A	Daily

3.2.3 Preparation of Reagents

Reagents were prepared and stored as recommended in the method and conducted as shown in the following ratio.

Extraction solvent:

Acetonitrile: Water (90:10)

Diluent:

Acetonitrile: Water: acetic acid (40:60:0.1)

Mobile Phase A:

0.1% acetic acid in Milli-Q water

Mobile Phase B:

0.1% acetic acid in acetonitrile

3.3 Preparation of Standard Solutions

Standard solutions were prepared and stored as recommended in the method and conducted as shown in the following tables.

Stock Standard

Stock Standard Solutions						
Analytical Standard	Stock standard No.	Amount Weighed (mg)	Solvent	Final Dilution Vol. (mL)	Final Conc. (mg/mL)	Prep. Date
Prometryn	S08071401	10.080	ACN	10	1.008	08/07/2014
Atrazine	S08071402	10.341	ACN	10	1.304	08/07/2014
Propazine	S08071403	11.151	ACN	10	1.115	08/07/2014
Simazine	S08071404	10.531	acetone:methanol (1:1, v:v)	10	1.053	08/07/2014

Fortification Standard

Stock Solution			Fortification Solution				
Standard Solution ID	Compound	Conc (µg/mL)	Aliquot Taken (mL)	Diluted to (mL)	Solvent	Conc (µg/mL)	Fortification Solution ID
S08071401	Prometryn	1008	0.992	10	ACN	100	F08071401
S08071402	Atrazine	1034	0.967				
S08071403	Propazine	1115	0.897				
S08071404	Simazine	1053	0.949				
F08071401	Mix	100	1	10	ACN	10	F08071402
F08071402	Mix	10	2	20	ACN	1	F08071403
F08071403	Mix	1	2	20	ACN	0.1	F08071404

3.3.1 Calibration Standard

Stock Solution			Calibration Solution				
Standard Solution ID	Compound	Conc (ng/mL)	Aliquot Taken (mL)	Diluted to (mL)	Solvent	Conc (ng/mL)	Calibration Solution ID
F08071404	Mix	100	1	10	ACN:Water:AA (40:60:0.1)	10	C08071401
F08071404	Mix	100	0.5	10	ACN:Water:AA (40:60:0.1)	5.0	C08071402
C08071401	Mix	10	2.5	10	ACN:Water:AA (40:60:0.1)	2.5	C08071403
C08071401	Mix	10	1	10	ACN:Water:AA (40:60:0.1)	1.0	C08071404
C08071402	Mix	5	1	10	ACN:Water:AA (40:60:0.1)	0.5	C08071405
C08071402	Mix	5	0.5	10	ACN:Water:AA (40:60:0.1)	0.25	C08071406
C08071404	Mix	1	1	10	ACN:Water:AA (40:60:0.1)	0.1	C080071407

3.4 Analytical Procedures and Modifications

The Analytical Method was independently validated as written.

3.4.1 Modifications

Two modifications were made to Section 3.0 of the analytical method during the ILV analysis, a 10mL glass tube was used to centrifuge sample instead of a 125mL Nalgene bottle and a Waters Sep-Pak C18 40mg 96-well plate was used for filtration instead of a 50mg CEC18 sorbent (UCT). E-mail authorization for the modifications is attached in Appendix 4.

3.4.2 Fortifications

Untreated control soil samples were fortified using microliter amounts of the appropriate fortification standard to LOQ and 10X LOQ concentrations as per method.

Fortifications used in this method validation are as follows:

Sample Type	Sample Weight	Concentration of Spiking Solution (ug/mL)	Volume of Spiking Solution (mL)	Level of Fortification in ppm (mg/kg)	Inject Conc.
Control	10.0 g	-	-	0	0
Fortification (LOQ)	10.0 g	0.1	1.0	0.01*	1.0 ng/mL
Fortification (10×LOQ)	10.0 g	1.0	1.0	0.10	10.0 ng/mL
Treated	10.0 g	-	-	-	

*Limit of quantification, wait 30 min after spiking before extraction solvent is added.

3.4.3 Sample preparation

As indicated by method GRM071.01A, the following extraction steps were performed:

1. If soil samples are received frozen (high moisture content) they should be allowed to defrost completely at room temperature. All samples should be prepared using an approved method of preparation to obtain a homogeneous sample prior to analysis.
2. Transfer 10.0 g of soil sample to be analysed into a 125 mL round bottom flask. Sample fortification is carried out at this time using combined fortification standards in acetonitrile, if required; and wait for 30 min for solvent to evaporate at room.
3. To each sample add 60 mL of acetonitrile: water (90/10 v/v) and sufficient boiling chips. Place samples on reflux/condenser apparatus and reflux for 1 hour (boil rime).
4. Transfer 10 mL aliquot to a glass tube and centrifuge at 3500 rpm for 5 minutes.
5. Filter supernatant through 40 mg C18 sorbent using 96-well plate format. Collect 0.6 mL in LV vial or deep well plate.
6. Add an additional 0.4 mL of 0.1% aqueous acetic acid to bring final volume to 1.0 mL, in which the final injection concentration of LOQ fortification is 1.0 ng/mL. Final solution should be mix thoroughly and submit for LC-MS/MS analysis.

3.5 Instrumentation

LC System:	Waters Acquity UPLC System
Detector:	AB Sciex 4000 QTRAP triple quadrupole mass spectrometer with Analyst software version 1.4.2
Column 1:	ACE 5 C18, 3.0 × 50nm, 5.0 μm
Column Oven Temperature:	30 °C
Injection Volume:	20 μL
Flow Rate:	1.0 mL/minute
Run Time:	3.5 minutes
Injection protocol:	Analyze calibration standard after 3 to 4 sample injections
Mobile Phase:	A (Solvent 1): 0.1% acetic acid in Milli-Q water B (Solvent 2): 0.1% acetic acid in HPLC ACN

Mobile Phase Composition:

Time (mins)	% A	% B
0.00	95	5
1.50	5	95
2.20	5	95
2.50	95	5
3.50	95	5

3.6 Data Acquisition

Peak integration and peak area count quantitation were performed by “Analyst 1.4.2”. A linear regression equation was derived and used in conjunction with the analyte response in each sample to calculate the concentration of analyte. The correlation coefficients (R) for the calibration curves for each analytical set was greater than 0.99. Recoveries were computed for fortified samples.

A statistical treatment of the data includes the calculation of averages, standard deviations, relative standard deviations. Mean percent recoveries, standard deviations, and relative standard deviations were calculated using a current Microsoft Office Excel package.