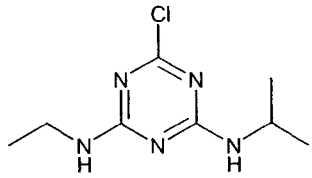
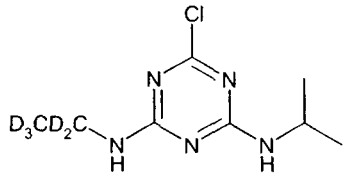
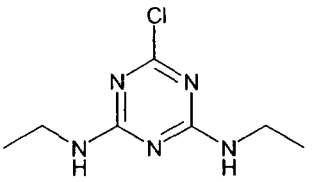
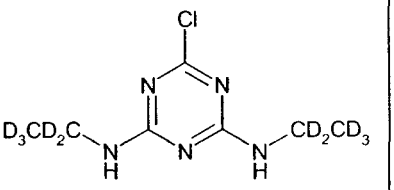
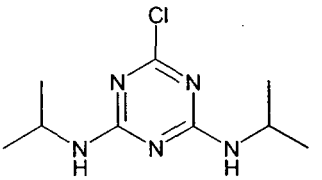
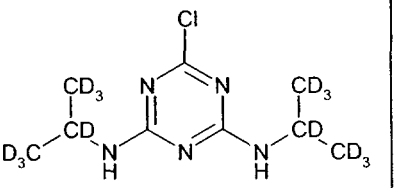
# 1.0 INTRODUCTION

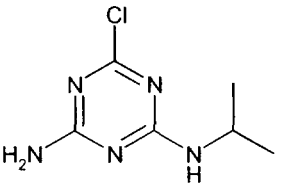
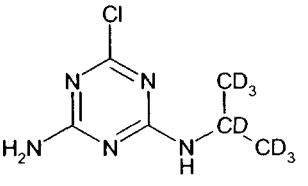
## 1.1 Scope and Chemical Structures

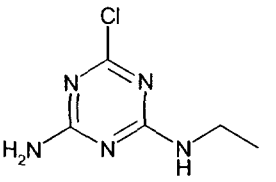
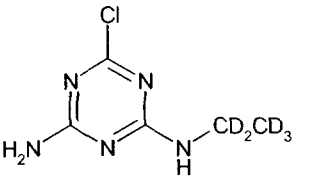
This method is for the residue determination of (1) the chlorotriazine compounds atrazine, simazine and propazine and the chlorotriazine degradates G30033, G28279, and G28273, and (2) metolachlor in various types of water samples. The limit of quantitation (LOQ) has been established at 0.050 ppb for atrazine, simazine, propazine, G30033 and G28279. The limits of quantitation (LOQ) for metolachlor and G28273 have been established at 0.10 ppb and 0.50 ppb, respectively. The instrument limit of detection (LOD) is 0.90 picogram (pg) for atrazine, simazine, propazine, G30033 and G28279. The instrument limit of detection (LOD) for metolachlor and G28273 are 2.25 and 9.0 picogram (pg), respectively. These LOD's are defined as the lowest concentration of standard injected (on-column injected amount) and used to construct the respective calibration plots. The s-metolachlor was used as reference material for metolachlor quantification; however, the chromatographic conditions employed in this method were not designed to resolve the stereoisomers in racemic mixtures. The types of water samples include laboratory de-ionized, ground, treated (finished), and surface water. The chemical structures of the analytes and the corresponding stable isotopic internal standards are listed as follows:

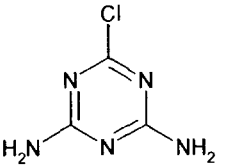
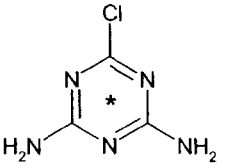
| **Synonym / Code Name:** | Atrazine (G30027) | Atrazine-d5 |
|---|---|---|
| **CAS Name:** | 1,3,5-Triazine-2,4-diamine, 6-chloro-N-ethyl-N'-(1-methylethyl)- | n/a |
| **CAS Number:** | 1912-24-9 | n/a |
| **Chemical Structure:** |  |  |
| **Molecular Formula:** | $C_8H_{14}ClN_5$ | $C_8H_9D_5ClN_5$ |
| **Molecular Mass:** | 215.09 | 220.12 |

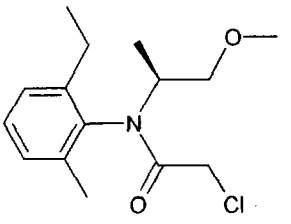
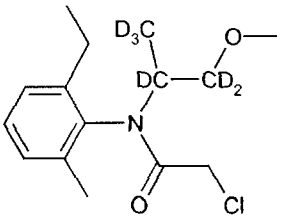
| **Synonym / Code Name:** | *Simazine (G27692)* | *Simazine-d10* |
|---|---|---|
| **CAS Name:** | 1,3,5-Triazine-2,4-diamine, 6-chloro-N,N'-diethyl- | n/a |
| **CAS Number:** | 122-34-9 | n/a |
| **Chemical Structure:** |  |  |
| **Molecular Formula:** | $C_7H_{12}ClN_5$ | $C_7H_2D_{10}ClN_5$ |
| **Molecular Mass:** | 201.08 | 211.14 |

| **Synonym / Code Name:** | Propazine (G30028) | Propazine-d14 |
|---|---|---|
| **CAS Name:** | 1,3,5-Triazine-2,4-diamine, 6-chloro-N,N'-bis(1-methylethyl)- | n/a |
| **CAS Number:** | 139-40-2 | n/a |
| **Chemical Structure:** |  |  |
| **Molecular Formula:** | $C_9H_{16}ClN_5$ | $C_9H_2D_{14}ClN_5$ |
| **Molecular Mass:** | 229.11 | 243.20 |

| **Synonym / Code Name:** | G30033 | G30033-d7 |
|---|---|---|
| **CAS Name:** | 1,3,5-Triazine-2,4-diamine, 6-chloro-N-(1-methylethyl)- | n/a |
| **CAS Number:** | 6190-65-4 | n/a |
| **Chemical Structure:** |  |  |
| **Molecular Formula:** | $C_6H_{10}ClN_5$ | $C_6H_3D_7ClN_5$ |
| **Molecular Mass:** | 187.06 | 194.11 |

| Synonym / Code Name: | G28279 | G28279-d5 |
|---|---|---|
| CAS Name: | 1,3,5-Triazine-2,4-diamine, 6-chloro-N-ethyl- | n/a |
| CAS Number: | 1007-28-9 | n/a |
| Chemical Structure: |  |  |
| Molecular Formula: | $C_5H_8ClN_5$ | $C_5H_3D_5ClN_5$ |
| Molecular Mass: | 173.05 | 178.08 |

| Synonym / Code Name: | G28273 | G28273-$^{13}C_3$ |
|---|---|---|
| CAS Name: | 1,3,5-Triazine-2,4-diamine, 6-chloro- | n/a |
| CAS Number: | 3397-62-4 | n/a |
| Chemical Structure: |  |  |
| Molecular Formula: | $C_3H_4ClN_5$ | $^{13}C_3H_4ClN_5$ |
| Molecular Mass: | 145.01 | 148.03 |

| Synonym / Code Name: | S-Metolachlor (CGA77102) | Metolachlor-d6 |
|---|---|---|
| CAS Name: | Acetamide, 2-chloro-N-(2-ethyl-6-methylphenyl)-N-[(1S)-2-methoxy-1-methylethyl]- | n/a |
| CAS Number: | 87392-12-9 | n/a |
| Chemical Structure: |  |  |
| Molecular Formula: | $C_{15}H_{22}ClNO_2$ | $C_{15}H_{16}D_6ClNO_2$ |
| Molecular Mass: | 283.13 | 289.17 |

Note: Although s-metolachlor and recemic metolachlor-d6 are used as reference materials for metolachlor quantification, the chromatographic conditions employed in this method are not designed to resolve the stereoisomers in racemic mixtures.

### 1.2 Method Summary

Typically, 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 at 5.0 pg/µL level in every water sample. After complete mixing, the sample is subjected to LC-MS/MS analysis. The data system (*e.g.* Xcalibur™) uses the calibration plot and the respective peak response ratios relative to corresponding internal standards to calculate the amount of analyte in a sample. Dilution may be required prior to internal standard addition for samples containing residue concentration levels greater than 10 ppb since this is the upper limit of the calibration plots.

## 2.0 MATERIALS AND APPARATUS

### 2.1 Apparatus

The recommended equipment and apparatus are listed in Appendix 1. Equipment with equivalent performance specifications may be substituted except in cases where it is noted that no substitution is allowed.

### 2.2 Reagents

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. A list of reagents and analytical standards used in this method along with details of preparation of solutions is included in Appendix 2.

### 2.3 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 gloves and laboratory coat.
3. Prevent inhalation and contact with mouth or skin.
4. Wash any contaminated area immediately.

In general, individual primary stock solutions are prepared at the 50 µg/mL (or 100 µg/mL) concentration level by dissolving 5.0 mg (or 10.0mg) of individual compound into individual 100-mL volumetric flasks followed by dilution to the mark with methanol. The amounts weighed for each compound should be corrected for its respective % purity. If sonication is

applied to help dissolution of analytes into solvents, allow the solution to return to room temperature before adjusting the final volume.

Alternatively, the appropriate volume of methanol is added to a known amount of standard material using the equation below. The concentration of the analytical standard is corrected for its chemical purity.

$$V(mL)=\frac{wt.(mg)\times P}{C(\mu g/mL)}\times 10^{3}$$

Where "*V*" is the volume of methanol needed; "*wt.*" is the weight, in mg, of the solid analytical standard; "*P*" is the purity, in decimal form, of the analytical standard; "*C*" is the desired concentration of the final solution, in µg/mL; and $10^3$ is a conversion factor. In this second case, the standard material is weighed directly into an amber glass storage bottle.

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 individual or 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.

### 2.3.1 Calibration Standards

Individual stock calibration solutions at the 100 µg/mL concentration level are prepared by dissolving 10.0 mg of each compound into individual 100-mL volumetric flasks followed by dilution to the mark with methanol (5.0 mg of G28273, G28279, or G30033 are used to give 50 µg/mL concentration stock standards due to limited solubility). The amounts weighed for each compound should be corrected for its respective % purity. A stock of mixed standard solution at concentration of 5.0 µg/mL is prepared by mixing 5.0 mL of each stock standard (10 mL for the G28273, G28279, and G30033 stock standards) into a 100-mL volumetric flask and filling to the mark with HPLC grade methanol. Minimum of five concentration levels of calibration solutions, ranging from 0.02 to 10 pg/µL concentrations, are prepared by serial dilutions of this stock mixed standard solution with 5/95 (v/v) methanol/HPLC water. These mixed working standards are used for both analytical and fortification purposes.

### 2.3.2 Stable Isotope Internal Standards

Individual stable isotope internal standard stock solutions at the 50 µg/mL concentration level (1.0 µg/mL for G28273-$^{13}C_3$) are prepared by dissolving 5.0 mg of each compound into individual 100-mL volumetric flasks followed by dilution to the mark with methanol (except G28273-$^{13}C_3$ which is prepared at 1.0 µg/mL concentration). The amounts weighed for each compound should be corrected for its respective % purity. Individual stable isotope internal standard stock solutions at the 1.0 µg/mL concentration are prepared by transferring 2.0 mL of the above stock solutions at 50 µg/mL concentration into a 100-mL volumetric flask and filling to the mark with HPLC grade methanol.

The stock solution of G28273-$^{13}C_3$ at 1.0 µg/mL concentration stock is prepared as described below since the standard is commercially available only dissolved in nonane at 100 µg/mL concentration. Therefore, 1.0 mL of the G28273-$^{13}C_3$ at 100 µg/mL concentration is carefully measured and transferred into a 100 mL volumetric flask with an air-tight syringe. The amounts of volume needed should be corrected for its respective % purity. The nonane is removed by evaporation under a gentle stream of $N_2$ at 30-35°C in a water bath. Upon complete removal of the nonane, re-dissolve the remaining residue in 80 mL of HPLC grade methanol and sonicate for approximate 5 minutes. Allow the solution to cool to room temperature prior to final adjustment of the final volume to the mark with additional HPLC grade methanol.

A mixed standard solution at the 50 pg/µL (*i.e.* 50 ppb) concentration level with 1% formic acid is prepared by mixing 5.0 mL of individual internal standard stock solution into a 100-mL volumetric flask with 1.0 mL of concentrated formic acid and filling to the mark with 1:1 (v/v) HPLC grade $H_2O$/methanol solution. This solution containing the mixed working internal standards is used for internal standard spiking for all samples, including calibration standards used for multi-points calibration, within the entire analytical set.

## 2.4 Safety Precautions and Hazards

The following information is included as an indication to the analyst of the nature and hazards of the reagents used in this procedure. If in any doubt, consult the appropriate MSDS or a monograph such as 'Hazards in the Chemical Laboratory', edited by S. G. Luxon, The Chemical Society, London (Reference 1).

**Reagent Hazards**:

| Solvent | MeOH | Formic Acid |
|---|---|---|
| Harmful Vapor | ✓ | ✓ |
| Highly Flammable | ✓ | X |
| Harmful by Skin Absorption | ✓ | ✓ |
| Irritant to Respiratory system & eye | ✓ | ✓ |
| Syngenta Divisional Toxicity Class | SHC-C,S | SHC-C,S |
| OES Short Term (mg $m^{-3}$) | 310 | N/A |
| OES Long Term (mg $m^{-3}$) | 260 | 9 |

In all cases avoid breathing vapor. Avoid contact with eyes and skin.

# 3.0 ANALYTICAL PROCEDURE

## 3.1 Sample Storage and Temperature Re-Equilibration

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

## 3.2 Sample Preparation

1. Carefully measure and transfer 900 µL aliquot of water sample into auto-sampler vials. Follow the same procedure for the calibration standards that will be used for multi-points calibration.
2. Carefully measure and spike 100 µL of the internal standard solution at 50 pg/µL concentration into the above pre-measured water and calibration samples.
3. Properly cap the vial and mix well with a vortex mixer.
4. Load the sample vials onto the injection tray for LC-MS/MS analysis.

Note: Accurate volume delivery and efficient mixing of samples and internal standard spiking solutions are critical for the method quantification results. The water sample should be subjected to centrifugation or filtration prior to analysis if the appearance of the water sample is not clear or if particulates are visible. Dilution with HPLC grade water and reanalysis may be required when (1) samples contain residues > 10 ppb and/or (2) earlier analytical runs indicated possible interferences.

## 3.3 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 0.00050 µg/mL working standard solution to a 9.0-mL aliquot portion of water sample produces a 0.050 ppb fortification of all the analytes. These fortified samples are then prepared and spiked with internal standards as described in Section 3.2 prior to LC-MS/MS analysis for method procedural recovery evaluation purposes. The fortification levels used in each set of analyses can vary but should always include one recovery sample at the LOQ level (*e.g.* 0.50 ppb for G-28273, 0.10 ppb for metolachlor and 0.050 ppb for all other analytes).

## 3.4 Time Required for Analysis

In the method validation, a typical validation set consisted of a batch of 23 samples from each type of water. A typical analytical sequence included these 23 samples and the required calibration standards. One skilled analyst can complete the sample preparation of two sets of 23 samples within a few hours. The analytical sequence was typically performed overnight on a LC-MS/MS system.

## 3.5 Method Stopping Points

Procedural stopping is typically not required due to the simplistic operational procedures of the method. It has been demonstrated, during the method development, that the internal standard spiked samples (and calibration standards) can be stored under refrigeration up to two weeks without repeat preparation of the samples. However, it is generally not recommended unless it deemed necessary when an instrumental difficulty is encountered during the time of analysis. In this case, the spiked samples and calibration standards should be stored in sealed vials at refrigerated temperatures when the analyses cannot be completed in a single working day.

## 3.6 Preparation of Calibration Standards for LC-MS/MS

As outlined in Section 2.3.1, standards for multi-point calibration should be prepared in 5/95 (v/v) methanol/water (HPLC grade). In general, it is recommended that a minimum of five levels of calibration standards be used for calibration plot establishment. In the method validation, the following concentration levels of standards were prepared for calibration plots: 0.02 pg/µL, 0.05 pg/µL, 0.10 pg/µL, 0.20 pg/µL, 0.50 pg/µL, 1.0 pg/µL, 2.0 pg/µL, 5.0 pg/µL, and 10 pg/µL. The effective final concentration after internal standard spiking (*i.e.* 90% of the original concentration before internal standard spiking when samples are prepared according to Section 3.2) should be used to construct the calibration curve.

The final concentration of the stable isotope internal standards should be kept constant within the given analytical set. The final concentration of the internal standards added to the sample for this method is recommended to be 5.0 pg/µL. Therefore, 100µL of a mixed internal standard solution at a concentration of 50 pg/µL is spiked into 900µL of calibration standards, resulting in a final concentration of 5.0 pg/µL of internal standard in the calibration samples (Section 3.2).

All the LC calibration standards and internal standards should be stored in amber glass bottles under refrigeration conditions (approximately 4°C). An expiration date of three months is recommended unless additional study data are generated that show a longer expiration date is appropriate.

## 4.0 FINAL DETERMINATION

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

### 4.1 LC System Description and Operating Conditions

**LC Instrumentation:**

The Surveyor Plus LC system consists of an analytical pump unit (a quaternary solvent system) and an autosampler. The solvent degasser, column oven, and sample tray temperature control are integral parts of the LC system. The system is controlled and data processed by Thermo Electron Xcalibur™ Software.

**LC Operating Conditions:**

Injection Volume: 50 µL
Sample Compartment Temp.: refrigerated at 15°C (recommended)
Column Temperature: 20°C (higher temperature settings are NOT recommended)
Column Zorbax SB-Aq, 4.6 x 50 mm, 3.5 µm (Agilent Catalog No. 835975-914)
Column Filter: ColumnSaver (MAC-MOD Catalog No. MMCS210)
Mobile Phase A: 0.1% formic acid in HPLC grade water
Mobile Phase B: 0.1% formic acid in HPLC grade methanol

| Step | Time (min) | %A | %B | Flow Rate (mL/min) | Gradient |
|---|---|---|---|---|---|
| 0 | 0.0 | 95 | 5 | 0.5 | --- |
| 1 | 0.5 | 95 | 5 | 0.5 | --- |
| 2 | 1.0 | 70 | 30 | 0.5 | --- |
| 3 | 1.5 | 70 | 30 | 0.5 | --- |
| 4 | 2.5 | 10 | 90 | 0.5 | linear |
| 5 | 5.5 | 10 | 90 | 0.5 | --- |
| 6 | 5.6 | 95 | 5 | 1.0 | linear |
| 7 | 6.5 | 95 | 5 | 1.0 | --- |
| 8 | 6.6 | 95 | 5 | 0.5 | --- |
| 9 | 7.5 | 95 | 5 | 0.5 | --- |

The typical retention time for the analytes are listed in Section 4.3 when using this instrumentation and conditions. The retention time may vary depending upon chromatographic conditions and systems.

Note: To help minimize instrument contamination, a timed event controlled switching valve may be used to divert the LC stream to waste during periods of no data collection.

## 4.2 Mass Spectrometer Conditions

A Thermo Electron TSQ Quantum Ultra mass spectrometer was used to establish and validate the method. The system is controlled and data processed by Thermo Electron Xcalibur™ Software. Electrospray ionization (ESI) source with positive ion detection mode is applied for all analytes.

The following are the typical instrumental parameters applied for this method during method validation. Alternative instrument with comparable sensitivity and performance criteria can be used for this method. The analyst should make necessary adjustments and tuning of the instrument parameters to obtain optimum operational conditions based on the actual instrument used for the specific study.

**Ion Source Parameters (Positive Mode):**

| | |
|---|---|
| Spray Voltage (V) | 3200 |
| Vaporization Temperature (°C) | 350 |
| Sheath Gas Pressure (psi) | 45 |
| Ion Sweep Gas Pressure (psi) | 5.0 |
| Aux Gas Pressure (psi) | 40 |
| Capillary Temperature (°C) | 300 |
| Tube Lens Offset (V) | 90 - 110 |
| Skimmer Offset (V) | 0 |
| Collision Pressure (mTorr) | 1.0 |

## 4.3 MRM (SRM) Operating Conditions:

*MS/MS Transitions*

| Analyte | MS/MS Transition* | Scan Width | Dwell (sec.) | CE (Volts) | Q1 PW | Q3 PW | RT (min.) |
|---|---|---|---|---|---|---|---|
| G28273 | 146.00 → 104.00 | 0.01 | 0.100 | 20 | 0.7 | 0.7 | 3.65 |
| G28273-$^{13}C_3$ | 148.95 → 106.00 | 0.01 | 0.050 | 20 | 0.7 | 0.7 | 3.65 |
| G28279 | 174.05 → 132.00 | 0.01 | 0.100 | 20 | 0.7 | 0.7 | 4.17 |
| G28279-d5 | 179.10 → 137.00 | 0.01 | 0.050 | 20 | 0.7 | 0.7 | 4.17 |
| G30033 | 188.05 → 145.95 | 0.01 | 0.050 | 20 | 0.7 | 0.7 | 4.30 |
| G30033-d7 | 195.10 → 146.95 | 0.01 | 0.050 | 20 | 0.7 | 0.7 | 4.30 |
| Simazine | 202.10 → 132.00 | 0.01 | 0.050 | 22 | 0.7 | 0.7 | 4.50 |
| Simazine-d10 | 212.10 → 137.00 | 0.01 | 0.020 | 22 | 0.7 | 0.7 | 4.50 |
| Atrazine | 216.10 → 174.10 | 0.01 | 0.020 | 20 | 0.7 | 0.7 | 4.60 |
| Atrazine-d5 | 221.10 → 179.10 | 0.01 | 0.010 | 20 | 0.7 | 0.7 | 4.60 |
| Propazine | 230.10 → 146.10 | 0.01 | 0.020 | 25 | 0.7 | 0.7 | 4.71 |
| Propazine-d14 | 244.20 → 148.00 | 0.01 | 0.010 | 28 | 0.7 | 0.7 | 4.71 |
| S-Metolachlor** | 284.10 → 252.10 | 0.01 | 0.010 | 20 | 0.7 | 0.7 | 4.87 |
| Metolachlor-d6 | 290.10 → 258.10 | 0.01 | 0.010 | 20 | 0.7 | 0.7 | 4.87 |

Data collection window is 2.5 – 5.5 minutes.

* The MS/MS transitions listed were the most sensitive and stable transitions for the corresponding analytes based on the optimal tuning parameters obtained prior to method validation with Thermo Electron TSQ Quantum Ultra instrument. Alternative MS/MS transitions may be used if different comparable instrument is applied or encounter interferences. Analysts should consult with instrument operation manuals for the specifics and adjustments when using instruments from different manufacturers to obtain optimum results.

** Although s-metolachlor is used as reference material for metolachlor quantification, the chromatographic conditions employed in this method are not designed to resolve the stereoisomers in racemic mixtures.

## 5.0 CALCULATION OF RESULTS

**Determination of Residues in Samples:**

Analyze the samples prepared as described in Section 3.2 on the LC-MS/MS system along with a selected range of calibration standards. Calibrate the instrument by intermittently injecting at least five (or more) concentration levels of the standard solutions and generate a calibration curve for the analyte using proper regression parameters (*e.g.* linear or quadratic regression with 1/X weighing) with internal standard calibration. Forcing the calibration curve through the origin is not recommended. The data system (*e.g.* Xcalibur™) uses the calibration plot and the respective peak response ratios relative to corresponding internal standards to calculate the amount of analyte in a sample. If the amount of analyte found in the sample exceeds 10% of the response for the highest valid calibration standard injected, the sample should be diluted with 5/95 (v/v) methanol/HPLC grade water and re-analyzed.

**Procedural Recoveries:**

The procedural recovery data for each set of sample analyses must fall within EPA's acceptance criteria of mean recoveries from 70 to 120% and standard deviations of ≤ 20%. Recovery samples are corrected for control values when detected. At the discretion of the study director, the procedural recoveries can be applied to further correct residues in a sample within the same analytical set if deemed to be appropriate.

**Calculations:**

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

$$(1) \qquad ppb\ of\ analyte = \frac{picogram\ of\ analyte\ found\ (pg)}{amount\ of\ sample\ injected\ (mg)} \quad x \quad \frac{1}{R}$$

Where R is the recovery factor expressed in decimal form (*i.e.*, 1.0 = 100%) and is calculated from equation *(3)*. Use a factor of 1.0 (*i.e.* R =1.0) for recoveries greater than 100% or when recovery correction is not applied.

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

$$(2) \quad amount\ of\ sample\ injected = V_I \left(\frac{1\,mL}{1000\,\mu L}\right)\left(\frac{1\,g}{1\,mL}\right)\left(\frac{1000\,mg}{1\,g}\right)\left(\frac{V_{initial}}{V_{final}}\right)$$

Where $V_I$ is the volume (µL) of sample injected, $V_{initial}$ is the initial volume (µL) of the sample and $V_{final}$ is the final volume (µL) of the sample. The weight/volume conversion factor used for water is 1.0 mL equals to 1.0 gram.

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

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

## 6.0 INTERFERENCES AND CONFIRMATION

Due to the highly selective nature of the detection technique using tandem mass spectrometry, interference arising from the sample matrix has not been observed in the validation study. Although residue determination by LC-MS/MS is considered to be highly specific, a secondary MS/MS transition for the specific analyte can be acquired for confirmatory purposes when needed.

It is recommended that each batch of solvent or reagents be checked for potential contamination prior to use. This method uses disposable labware, where possible. All reusable glassware should be detergent washed then rinsed with HPLC grade methanol or acetone and thoroughly dried prior to use.

Although the internal standard quantification method is designed to compensate for possible matrix interference and instrumental operational variations to achieve better quantification accuracy and precision, severe interference may still exist. It is strongly recommended the peak responses (*i.e.* peak areas) of the internal standards in all injections within the analytical set must not deviate by more than ±50% from the average areas measured within the given analytical set. If the internal standard area counts for a sample do not meet this criterion, sample re-analysis or further investigation is required.

## 7.0 MODIFICATIONS AND POTENTIAL PROBLEMS

It is possible that contaminants from chemicals, solvents, glassware, etc. may interfere with the analysis and give a false positive result with confirmatory measures in place. It is recommended that reagent blank samples be included in a sample set if contamination is suspected. During the method development, significant residue carryovers of simazine were observed when the HPLC column had been subjected to heavy usage. In this case, replacement with a fresh HPLC column is required. Minor carryover of analytes with some types of LC auto-injectors immediately after

high level standards or samples was also observed during the method development. If injector carryover is suspected, injection of 5/95 methanol/$H_2O$ (v/v; HPLC grade) samples containing the same level of internal standards can be applied immediate after the high level calibration standard to minimize the carryover. In general, the effective method LOQ should be adjusted and reported accordingly if the carryover issue cannot be resolved.

The quality of the calibration plot can deteriorate if the ESI source becomes too dirty. Thus, inspection of each calibration plot needs to be performed in order to maintain accurate and reliable quantification of each set of samples. In general, calibration plots exhibit good regression analysis characteristics with $R^2 \geq 0.99$ is considered acceptable. Further, the chromatographic conditions employed were not designed to resolve the stereoisomers in racemic mixtures.

In some cases, sample preservation may be required to prevent analytes from biological or chemical degradation in surface (raw) or finished water samples (Reference 2). These additives may exhibit additional matrix effects to the analysis accuracy and precision. The supplementary data presented in Appendix 3 demonstrate the applicability of this internal standard method for the field water samples in the presence of sodium omadine as preservative at a concentration of 64mg/L on a comparable instrument.

Any modifications to this method must be documented in the study raw data.

## 9.0 LIMITATIONS

The method has been tested on representative water samples. It can reasonably be assumed that the method can be applied for water samples obtained from other locations not tested in this method provided successful recovery tests at the relevant levels validate the suitability of the method.

## 10.0 CONCLUSIONS

Analytical Method GRM014.02A is a valid and accurate method for the determination of atrazine, simazine, propazine, G-30033, G-28279, G-28273 and metolachlor in deionized, ground (well), finished and surface water samples. The method limits of quantification (LOQ) have been established at 0.50 ppb for G28273, 0.10 ppb for metolachlor, 0.050 ppb for all other analytes. The method is cost effective since minimum sample manipulation is required prior to instrumental analysis. More than 90 injections can be performed overnight (within 14 hours period).

## 11.0 REFERENCES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2. "Preservation and Analytical Procedures for the Analysis of Chloro-s-Triazines and Their Chlorodegradate Products in Drinking Waters Using Direct Injection Liquid Chromatography Tandem Mass Spectrometry"; G.A. Smith, B.V. Pepich and D.J. Munch; *J. Chromatogr. A*, **2008**, *1202*, 138.

3. Syngenta Protocol No. T001943-09. Validation of Analytical Method GRM014.02A for the Determination of Atrazine, Simazine, Propazine, G30033, G28279, G28273 and *S* Metolachlor in Water Using Direct-Aqueous-Injection Liquid Chromatography Electrospray Ionization Tandem Mass Spectrometry (LC-ESI/MS/MS) with Stable Isotopic Analogues as Quantification Internal Standard, Syngenta Crop Protection, Inc., Greensboro, NC.

4. 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.

5. U.S. Environmental Protection Agency, Office of Pollution Prevention and Toxic Substances (OPPTS). 1996. "Public Draft" – Residue Chemistry Test Guidelines, OPPTS 860.1340. EPA 712-C-96-174.

## APPENDIX 1 Apparatus

General laboratory glassware (*e.g.* beakers, graduated cylinders, flat bottom flasks, round bottom flasks, pipet bulbs, etc.) are available from a general laboratory supply company.

1. Balance, analytical (Sartorius Model R160P). Electronic display of 0.01 mg, for weighing in preparation of the stock standard solutions.
2. Balance, laboratory (Mettler Model PB3002-S). Electronic display of 0.01 g, for weighing soil samples.
3. Refrigerated Centrifuge, Du Pond Instruments, Model Sorvall® RC-5B.
4. Fisher Variable-Speed Touch Mixer, Fisher Scientific, Catalog No. 12-812
5. Volumetric Pipets, glass, Class A certified, assorted volumes. (These pipets should be used for sample fortification and standard solution preparation)
6. Bottles, amber glass Boston round, 4 oz., with Polyseal-lined cap, Fisher Scientific Catalog. No. 02-911-895
7. Disposable centrifuge tubes, polypropylene, 50-mL, graduated with plastic screw cap, Fisher Scientific Catalog No. 05-538-68
8. Disposable centrifuge tubes, polypropylene, 15-mL, graduated with plastic screw cap, Fisher Scientific Catalog No. 4-959-53A
9. Brinkmann Eppendorf Pipettor Tips, 200 µL tip, Fisher Scientific Catalog No. 022491334
10. Brinkmann Eppendorf Pipettor Tips, 1000 µL tip, Fisher Scientific Catalog No. 022491351
11. Brinkmann Eppendorf Pipettor Tips, 5 mL, Fisher Scientific Catalog No. 022491385
12. Brinkmann Eppendorf Pipettor Tips, 10 mL, Fisher Scientific Catalog No. 05-403-119
13. Brinkmann Eppendorf 2100 Research Series Pipettor, range 20 - 200 µL, Fisher Scientific Catalog No. 05-402-89
14. Brinkmann Eppendorf 2100 Research Series Pipettor, range 100 - 1000 µL, Fisher Scientific Catalog No. 05-402-90
15. Brinkmann Eppendorf 2100 Research Series Pipettor, range 500 - 5000 µL, Fisher Scientific Catalog No. 05-402-91
16. Brinkmann Eppendorf 2100 Research Series Pipettor, range 1 - 10 mL, Fisher Scientific Catalog No. 05-403-121
17. Disposable Pasteur Pipets, 146 mm, Fisher Scientific, Catalog No. 22-230-482
18. Auto-sampler vials, National Scientific C4011-6W, Fisher Scientific Catalog No. 03-395H
19. Auto-sampler vial enclosures, National Scientific C4011-55R , Fisher Scientific Catalog No. 03-396AA

20. HPLC column filter, MAC-MOD Analytical Inc., P/N MMCS210
21. HPLC column: Agilent Zorbax SB-Aq, 4.6 x 50 mm, 3.5 µm, Agilent Catalog No. 835975-914

Note: Unless otherwise noted, other manufacturers' equivalents of the items listed above can be used; however, the use of the substitutes must be demonstrated by obtaining acceptable procedural recoveries. In general, Class A glass volumetric flasks and pipettes were utilized for standard solution preparation and are not individually listed.

## APPENDIX 2 Reagents

1. Water, HPLC grade, Fisher Scientific, Catalog No. W5SK-4
2. Methanol, HPLC grade, Fisher Scientific, Catalog No. A452SK-4
3. Formic Acid, 88%, HPLC grade, Fisher Scientific, Catalog No. A118P-500

   **Note: Equivalent reagents obtained from other manufacturers can be used instead of the reagents described above; however, it is important to verify the quality of the solvents to insure there are no interfering contaminants.**

4. Methanol/$H_2O$, 5/95 (v/v; HPLC grade); prepared by mixing 50 mL of HPLC grade methanol and 950 mL of HPLC water.
5. Methanol/$H_2O$, 50/50 (v/v; HPLC grade); prepared by mixing 500 mL of HPLC grade methanol and 500 mL of HPLC water.
6. "0.1%" Formic Acid in HPLC $H_2O$; prepared by mixing 1.0 mL of formic acid with 1000 mL of HPLC grade Water.
7. "0.1%" Formic Acid in HPLC Methanol; prepared by mixing 1.0 mL of formic acid with 1000 mL of HPLC grade methanol.
8. The calibration standards used in this method were supplied by the Analytical and Product Chemistry Department or Chemical Synthesis Group of Syngenta Crop Protection, Inc.

| | |
|---|---|
| Atrazine (G30027) | CAS RN: 1912-24-9<br>CAS Name: 1,3,5-Triazine-2,4-diamine, 6-chloro-N-ethyl-N'-(1-methylethyl)-<br>Syngenta standard code: 455333<br>Purity: 96.2% |
| G30033 | CAS RN: 6190-65-4<br>CAS Name: 1,3,5-Triazine-2,4-diamine, 6-chloro-N-(1-methylethyl)-<br>Syngenta standard code: DAH-XXXIII-48<br>Purity: 99.4% |
| G28279 | CAS RN: 1007-28-9<br>CAS Name: 1,3,5-Triazine-2,4-diamine, 6-chloro-N-ethyl-<br>Syngenta standard code: 45269<br>Purity: 94.0% |
| G28273 | CAS RN: 3397-62-4<br>CAS Name: 1,3,5-Triazine-2,4-diamine, 6-chloro-<br>Syngenta standard code: 456525<br>Purity: 97.0% |

| | |
|---|---|
| Simazine (G27692) | CAS RN: 122-34-9<br>CAS Name: 1,3,5-Triazine-2,4-diamine, 6-chloro-N,N'-diethyl-<br>Syngenta standard code: 486779<br>Purity: 98.4% |
| Propazine (G30028) | CAS RN: 139-40-2<br>CAS Name: 1,3,5-Triazine-2,4-diamine, 6-chloro-N,N'-bis(1-methylethyl)-<br>Syngenta standard code: 423440<br>Purity: 98.5% |
| S-Metolachlor (CGA77102) | CAS RN: 87392-12-9<br>CAS Name: Acetamide, 2-chloro-N-(2-ethyl-6-methylphenyl)-N-[(1S)-2-methoxy-1-methylethyl]-<br>Syngenta standard code: 410316<br>Purity: 97.9% |

9. The stable isotopic analogues used as internal standards in this method were ordered from C/D/N Isotopes, Inc. (88 Leacock Street, Pointe-Claire, Quebec, Canada H9R 1H1) or Cambridge Isotope Laboratories, Inc. (50 Frontage Road, Andover, MA 01810-5413 USA).

| | |
|---|---|
| Atrazine-d5 | CAS RN: 1912-24-9 (unlabelled)<br>CAS Name: N/A<br>Lot No.: K136P61<br>Purity: 99.0% |
| G30033-d7 | CAS RN: 6190-65-4 (unlabelled)<br>CAS Name: N/A<br>Lot No.: U437P23<br>Purity: 99.0% |
| G28279-d5 | CAS RN: 1007-28-9 (unlabelled)<br>CAS Name: N/A<br>Lot No.: R488P15<br>Purity: 99.0% |
| G28273-$^{13}C_3$ | CAS RN: 3397-62-4 (unlabelled)<br>CAS Name: N/A<br>Lot No.: SCGJ-022<br>Purity: 95.3% |

| | |
|---|---|
| Simazine-d10 | CAS RN: 122-34-9 (unlabelled)<br>CAS Name: N/A<br>Lot No.: R544P24<br>Purity: 99.0% |
| Propazine-d14 | CAS RN: 139-40-2 (unlabelled)<br>CAS Name: N/A<br>Lot No.: Z188P3<br>Purity: 99.0% |
| Metolachlor-d6 | CAS RN: 87392-12-9 (unlabelled)<br>CAS Name: N/A<br>Lot No.: G556P12<br>Purity: 99.3% |