ANALYTICAL METHOD FOR THE DETERMINATION OF CHLORSULFURON AND METABOLITES IN-JJ998, IN-A4097, IN-M6957, AND IN-A4098 IN WATER USING LC/MS/MS

James S. LeNoir

REASON FOR REVISION

The reason for revision of this report is that an additional metabolite, IN-A4098, was added to the method. There have been only minor changes to the method outlined in the previous version of this report. The minor modifications to the method have been added to the appropriate sections throughout the report and supporting data for the inclusion of the metabolite, IN-A4098, have been added as Appendix 5 to this report.

1.0 SUMMARY

The purpose of this study was to develop an analytical method for the detection, quantitative analysis, and confirmation of chlorsulfuron (DPX-W4189) and metabolites IN-JJ998, IN-A4097, IN-M6957, and IN-A4098 in water. This method was validated using ground, surface, and drinking water.

Chlorsulfuron, IN-JJ998, IN-A4097, IN-M6957, and IN-A4098 were extracted from the water samples by filtering 100 mL of water through an Oasis HLB solid phase extraction (SPE) cartridge. Following a wash step, the analytes were eluted in base-adjusted acetonitrile. The volume collected was diluted to 20 mL and a 10-mL aliquot was removed. Two hundred and fifty microliters of water was added and the extract was evaporated under a flow of nitrogen until the volume was less than 250 µL. The extracts were diluted to 2.5 mL using an aqueous ammonium acetate buffer. Chlorsulfuron, IN-JJ998, IN-A4097, IN-M6957, and IN-A4098 were separated from co-extracts by reversed phase liquid chromatography (LC) and were detected by positive ion electrospray mass spectrometry/mass spectrometry (MS/MS). The Limit of Quantitation (LOQ) was 0.050 µg/L (ppb). The Limit of Detection (LOD) was estimated to be 0.02 µg/L (ppb).

2.0 INTRODUCTION

The structure, CAS name, CAS registry number, and various physical properties of chlorsulfuron (DPX-W4189) and metabolites IN-JJ998, IN-A4097, IN-M6957, and IN-A4098 can be found in Appendix 1. The method was validated on ground, surface, and drinking water.

The analytes were concentrated onto a Waters Oasis solid-phase extraction cartridge (SPE). The SPE cartridge was washed prior to the elution of the analytes. The eluate volume was reduced under a flow of nitrogen and the final volume was adjusted using an aqueous buffer solution. Detection and quantitative analysis was performed using electrospray LC/MS/MS analysis.

The LOQ for chlorsulfuron and metabolites IN-JJ998, IN-A4097, IN-M6957, and IN-A4098 was 0.050 µg/L (ppb). The LOD was estimated to be 0.02 µg/L (ppb). During method validation, acceptable recoveries for water samples fortified at 1× and 10× the LOQ were generated.

Due to the selective nature of the LC/MS/MS method, a separate confirmation method was not necessary. Confirmation using LC/MS/MS of possible residues were based on the detection and relative ratios of two MS/MS ion fragments. Confirmation criteria and examples are discussed in this report.

3.0 MATERIALS

Equivalent equipment and materials may be substituted unless otherwise specified. Note any specification in the following descriptions before making substitutions. Substitutions should only be made *if equivalency/suitability has been verified with acceptable control and fortification recovery data.*

3.1 Equipment

Instrumentation

HPLC system, HP1100 (Hewlett-Packard, Wilmington, DE)

Mass Spectrometer System, Quattro II with ESI interface (Micromass Inc., Altrincham, UK)

VWR brand Vortex Geni 2 Mixer, Cat. No. 58815-178 (VWR Scientific Co., Bridgeport, NJ)

Biohit Proline Electronic Pipettors, Variable Volume with Tip Ejector, Vanguard, 5.0-100 µL Cat. No. 53495-200, 50-1000 µL Cat. No. 53495-205, and 0.10-5.0 mL Cat. No. 53495-290 (VWR Scientific Co., Bridgeport, NJ)

Evaporator - N-Evap® Model 111 laboratory sample evaporator/nitrogen manifold fitted with Teflon®-coated needles (Organomation Associates, South Berlin, MA). This unit is attached to a dry, clean nitrogen source.

Solid-Phase Extraction Equipment

Visiprep 12 port SPE vacuum manifold, PN 5-7030 (Supelco, Bellefonte, PA).

Solid-Phase Extraction Supplies

Oasis® HLB cartridge, 1g/20cc, PN 186000117 (Waters, Milford, MA) - **Do not substitute**

Solid Phase Extraction Plastic Reservoir – 75-mL size, Catalog No. 1213-1012 (Varian, Harbor City, CA)

Reservoir Adapters – Catalog No. 1213-1003 (Varian, Harbor City, CA)

Chromatographic Supplies

HPLC Column: 4.6 mm i.d. × 15 cm, Phenomenex Luna Phenyl-Hexyl analytical column with 3-µm diameter packing Part # 00F-4256-E0
(Phenomenex, Torrance, CA)

HPLC Vials, Target DP Amber Kit, T/S/T Septa, 100 PK, Part # 5182-0556
(Hewlett-Packard, Wilmington, DE)

Labware

Pyrex Brand Single Metric Scale Graduated Cylinders, 10-mL and 100-mL capacity, Cat. No. 24709-715 and 24709-748, respectively
(VWR Scientific Co., Bridgeport, NJ)

VWR brand Disposable Pasteur Pipettes, Borosilicate Glass, 9 in, Cat. No. 53283-914 equipped with 2 mL, 13 × 32 mm rubber bulbs, Cat. No. 56310-240
(VWR Scientific Co., Bridgeport, NJ)

Centrifuge tubes, Pyrex Brand 15-mL capacity, Cat. No. 21048-027
(VWR Scientific Co., Bridgeport, NJ)

Centrifuge tubes, Pyrex Brand Conical Centrifuge Tubes with Standard Taper Stopper, 50-mL capacity, Cat. No. 21048-050 (VWR Scientific Co., Bridgeport, NJ)

Centrifuge tubes, Polystyrene 50-mL capacity, Cat. No. 21008-939
(VWR Scientific Co., Bridgeport, NJ)

Centrifuge tubes, Polystyrene 15-mL capacity, Cat. No. 21008-930
(VWR Scientific Co., Bridgeport, NJ)

Erlenmeyer Flask, polycarbonate, 25-mL capacity, 29152-146, respectively
(VWR Scientific Co., Bridgeport, NJ)

Miscellaneous

Syringe filter - Acrodisc PTFE 0.2 µm, 13-mm diameter Filter Unit, Cat. No. 28143-985 (VWR Scientific Co., Bridgeport, NJ)

6 Port Electrically Actuated Valve, Valco Instruments Co. Inc., PN 1384
(Alltech, Deerfield, IL)

3.2 *Reagents and Standards*

Equivalent reagents may be substituted for those listed below. To determine if impurities in substituted reagents interfere with analyses, appropriate amounts of the solvents should be taken through the entire method using the chromatographic conditions specified in this report.

Acetic Acid - Baker Analyzed® glacial acetic acid, #9524-00
(J.T. Baker, Inc. Danvers, MA)

Ammonium Hydroxide Solution - 28-30%, #AX-1303-13
(EM Science, Gibbstown, NJ)

Acetonitrile (ACN) - EM Omni Solv®, HPLC-grade acetonitrile, #AX0142-1
(EM Science, Gibbstown, NJ)

Ethyl Acetate - EM Omni Solv®, HPLC-grade ethyl acetate, #EX0241-1
(EM Science, Gibbstown, NJ)

Formic Acid - Guaranteed Reagent 98% minimum, #FX0440-5
(EM Science, Gibbstown, NJ)

Hexanes - EM Omni Solv®, #HX0296-1 (EM Science, Gibbstown, NJ)

Methanol - EM Omni Solv®, HPLC-grade methanol, #MX0488-1
(EM Science, Gibbstown, NJ)

Phosphoric acid - EM Omni Solv®, #PX0995-6 (EM Science, Gibbstown, NJ)

Water - EM Omni Solv®, HPLC-grade water, #WX0004-1
(EM Science, Gibbstown, NJ)

Chlorsulfuron reference substances (Lot number 145, 99.2% pure) used for sample analysis: Analytical standard grade reagents (DuPont Crop Protection, Global Technology Division, E.I. du Pont de Nemours and Company)

IN-JJ998 reference substances (Lot number 1, Approximately 70% pure) used for sample analysis: Analytical standard grade reagents (DuPont Crop Protection, Global Technology Division, E.I. du Pont de Nemours and Company)

IN-A4097 reference substances (Lot number 10, 99.7% pure) used for sample analysis: Analytical standard grade reagents (DuPont Crop Protection, Global Technology Division, E.I. du Pont de Nemours and Company)

IN-M6957 reference substances (Lot number 3, 94.2% pure) used for sample analysis: Analytical standard grade reagents (DuPont Crop Protection, Global Technology Division, E.I. du Pont de Nemours and Company)

IN-A4098 reference substances (Lot number 5, 98.7% pure) used for sample analysis: Analytical standard grade reagents (DuPont Crop Protection, Global Technology Division, E.I. du Pont de Nemours and Company)

3.3 Safety and Health

No unusually hazardous materials are used in this method. All appropriate material safety data sheets should be read and followed, and proper personal protective equipment used. An MSDS sheet for the analytes is available from DuPont Crop Protection, Global Technology Division, E.I. du Pont de Nemours and Company.

4.0 METHOD

4.1 Principles of the Analytical Method

The analytes were concentrated from the water sample onto a Waters Oasis SPE. The SPE cartridge was washed prior to the elution of the analytes. The eluate

volume was reduced under a flow of nitrogen and the final volume was adjusted using water. Detection and quantitative analysis was performed using LC/MS/MS analysis.

4.2 Analytical Procedure

4.2.1 Glassware and Equipment

Cleaning

Glassware should be scrubbed with a brush using a laboratory soap solution, rinsed two to five times with tap water, rinsed with distilled or deionized water, and finally rinsed with acetone or another suitable solvent and allowed to air dry prior to each use.

4.2.2 Preparation of Solutions

The following solutions should be prepared weekly and stored at room temperature unless stated otherwise:

0.020 M aqueous Formic Acid Solution - Add 920 µL of formic acid to 1000 mL of water and mix the resulting solution to homogeneity.

1.0 M ammonium hydroxide. Add 6.9 mL of ammonium hydroxide solution (28-30% NH_3) to a volume of 93.1 mL of EM Science water. Mix the resulting solution to homogeneity.

1:10 (v/v) phosphoric acid solution. Add 10 mL of phosphoric acid into 90 mL of water. Mix the resulting solution to homogeneity.

Solution A -- 70:30 hexane:ethyl acetate. Add 700 mL of hexane to 300 mL of ethyl acetate. Mix the resulting solution to homogeneity. This solution may be prepared monthly.

Solution B -- Basic Acetonitrile - Add 20 mL of 1.0 M ammonium hydroxide to 980 mL of acetonitrile and mix the resulting solution to homogeneity. This solution may be prepared monthly.

Injection Solvent -- 0.005 M aqueous ammonium acetate. Add 0.385 g of ammonium acetate to a volume of 900 mL of EM Science water. Mix the resulting solution to homogeneity and dilute to 1000 mL. Adjust the pH of the solution to 6.5 using the 1:10 (v/v) phosphoric acid solution (approximately 1-3 drops will be required).

4.2.3 Preparation and Stability of Stock Standards

Use Class A volumetric flasks when preparing standard solutions.

Prepare standard stock solutions by accurately weighing 10.00 ± 0.1 mg of each analyte into individual 100-mL volumetric flask using an analytical balance. Record the accurate weight of the standard. Dissolve the standards in approximately 50 mL of HPLC-grade acetonitrile. After dissolving, bring the solutions to a volume of 100 mL using HPLC-grade acetonitrile and invert the

volumetric flask to mix the solutions to homogeneity. These standard solutions are stable for approximately 3-months when stored in a freezer at approximately -20°C immediately after each use. The concentration of each analyte in solution is 100 µg/mL.

4.2.4 *Preparation and Stability of Fortification Standards*

Use Class A volumetric flasks when preparing standard solutions.

Prepare a 1.0-µg/mL chlorsulfuron, IN-JJ998, IN-A4097, IN-M6957, and IN-A4098 fortification standard in acetonitrile by pipetting 1.00 mL of each of the 100.0-µg/mL stock standards into a 100-mL volumetric flask. Bring to volume using HPLC-grade acetonitrile and mix to homogeneity.

Prepare a 0.10-µg/mL chlorsulfuron, IN-JJ998, IN-A4097, IN-M6957, and IN-A4098 fortification standard in acetonitrile by pipetting 1.00 mL of the 1.00-µg/mL fortification standard into a 10-mL volumetric flask. Bring to volume using HPLC-grade acetonitrile and mix to homogeneity.

Alternate or additional solutions may be prepared as needed. All standard solutions prepared in acetonitrile are stable for approximately 3 months if stored in a freezer at approximately -20°C immediately after each use.

4.2.5 *Preparation and Stability of Calibration Standards*

Prepare the calibration standards by pipetting volumes of the 1.00-µg/mL and 0.10-µg/mL standard solutions shown in the following table into separate 10.0-mL volumetric flasks (alternative or additional standards may be prepared as needed):

DESIRED STANDARD CONCENTRATION (NG/ML)	VOLUME OF 1.00-µG/ML STANDARD REQUIRED (ML)	VOLUME OF 0.10-µG/ML STANDARD REQUIRED (ML)
15	0.150	-
10	0.100	-
5.0	0.050	-
1.0	-	0.100
0.60	-	0.060

Add the appropriate amount of injection solution to the volumetric flasks to dilute to 10.00 mL. These standard solutions should be freshly prepared with each sample set and stored approximately 4°C prior to use. Each of the calibration standards was vortexed for 30 seconds prior to injection.

4.2.6 *Source of Samples*

Water control samples were obtained from local water sources. All water sources are provided in the table below. Bottled water was purchased from a local grocery store.

ORIGIN	LOCATION
Lums Pond	Bear, Delaware
Brandywine River	Wilmington, Delaware
Kemblesville Well	Kemblesville, Pennsylvania
Bottle Spring	Vendor: Great Bear Source: Sasoonan Spring, South Coventry, PA

All samples were refrigerated until use.

4.2.7 *Storage and Preparation of Samples*

Water samples should be stored at approximately 4°C. The water samples were shaken by hand prior to use to ensure homogeneity. No additional filtration or purification was performed prior to sample processing.

4.2.8 *Sample Fortification Procedure*

All fortifications were made directly to the water following the measurement of the sample.

Fortified 100-mL samples were prepared using a 1.00-µg/mL and 0.10-µg/mL fortification standard solution.

FORTIFICATION LEVEL (µG/L)	VOLUME OF STANDARD (mL)	SPIKING STANDARDS CONCENTRATION (µG/mL)
0.050	0.050	0.10
0.50	0.050	1.00

The total amount of acetonitrile applied to the water should be less than 0.50 mL.

4.2.9 *Analyte Extraction and Purification Procedures*

1. Accurately measure 100.0 mL (± 1%) of water into a 125-mL polycarbonate Erlenmeyer flask. Fortify sample if necessary. Cap and shake the samples vigorously.
2. Using an adapter, place a 75-mL reservoir above a 20-cc, 1-g Oasis HLB cartridge and attach it to an SPE manifold. Precondition the cartridge with 20 mL of methanol; discard the conditioning solution. Do not let the cartridge go to dryness. Then condition the cartridge with 20 mL of HPLC grade water. Do not let the cartridge go to dryness.
3. Load the sample into the 75-mL reservoir. Using vacuum, pull the sample through the Oasis cartridges at a flow rate of 2-10 mL/min. Rinse the flask with 10 mL of HPLC grade water and load the rinse into the reservoir just before all of the sample would have passed through. Use vacuum to dry the cartridge for 1 minutes. Discard the eluate.
4. Wash the cartridge with 10 mL of Solution A. Use vacuum to dry the cartridge for 3 minutes. Discard the eluate.

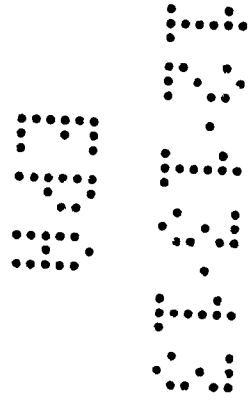
5. Elute the analytes with 20 mL of Solution B. Load Solution B onto the cartridge, vacuum or positive pressure may be required to start the flow but should be turned off once the flow has started. Once the dripping has stopped, use a small amount of vacuum to empty the remaining liquid in the cartridge into a centrifuge tube. Collect the eluate in a 50-mL centrifuge tube.
6. Adjust the volume of the eluate to 20 mL using Solution B. Transfer a 10-mL aliquot of the extract into a 15-mL centrifuge tube.
7. Evaporate the extract to approximately 3.0 mL using a flow of nitrogen in an N-Evap at 30-35°C. Pipette 0.250 mL of water into the centrifuge tube and continue evaporating until the volume reaches approximately 0.25 mL. Adjust the final volume to 2.5 mL using the injection solution. Vortex the centrifuge tube and filter an aliquot of the extract using a disposable syringe through a 0.2-µm Acrodisc filter into an HPLC vial. Analyze the solution by LC/MS/MS as described in the following section.

Extracts will be stable for approximately 48 hours if stored at 4°C.

4.3 Instrumentation for the Method

4.3.1 Chromatography

Reversed-phase chromatography was used to separate chlorsulfuron and metabolites from co-extracts. A Phenomenex phenyl hexyl column was selected. The column choice reflected experimental results indicating preferred separation from co-extractants. Alternative chromatographic conditions can be used, provided the analytical method is validated and provides acceptable recoveries as defined by regulatory method guidelines.



System:	Hewlett-Packard HP1100 HPLC			
Column:	4.6 mm i.d. × 15 cm, Phenomenex phenyl hexyl			
Column Temperature:	25°C			
Sample Temperature:	4°C			
Injection Volume:	0.200 mL			
Conditions:	A: 0.020 M aqueous Formic Acid B: Methanol			
	Time	%A	%B	Flow (mL/Min.)
	0.0	95	5	0.80
	1.0	95	5	0.80
	3.0	45	55	0.80
	11.0	15	85	0.80
	14.0	15	85	0.80
	14.2	5	95	1.00
	17.0	5	95	1.00
	17.5	95	5	1.00
	19.8	95	5	1.00
IN-A4098 Retention Time:	(7.1 minutes)*			
IN-JJ998 Retention Time:	8.1 minutes (11.3 minutes)			
IN-A4097 Retention Time:	8.3 minutes (11.3 minutes)			
IN-M6957 Retention Time:	10.7 minutes (13.8 minutes)			
Chlorsulfuron Retention Time:	13.6 minutes (15.0 minutes)			
Total Run Time:	25 minutes			

* Retention times in parenthesis represent the retention times as determined in the second method validation with the inclusion of IN-A4098.

A six-port electronically activated switching valve was used to direct the flow to waste prior to and following the elution of the compounds of interest. The use of this valve reduces source contamination and enables additional samples to be analyzed prior to source cleaning. The valve switching times are given in the following table.

TIME (MINUTES)	COLUMN ELUATE FLOW
0.00-7.0	Waste
7.00-14.5	MS source
14.5-End	Waste

Since electrospray LC/MS systems perform optimally at low flow rates, the eluate was split following the switching valve. Approximately 100 µL/min of eluate (10:1 split) flowed into the ion source with the remaining eluate flowing into a waste container.

4.3.2 *LC/MS/MS Analysis*

The quantitative analysis of chlorsulfuron and metabolites was performed using a Micromass Quattro II LC/MS/MS system. The system parameters were adjusted while a solution of each analyte was infused directly into the electrospray ion source. The solution composition was 50% methanol/50% water, so that it would approximate the composition of the mobile phase at the retention time of the analyte. The solution concentration was approximately 2 µg/mL. A summary of the experimental conditions is provided in the following table:

Micromass Quattro LC ESI-LC/MS/MS Mass Spectrometer Conditions

ANALYTES	IONS MONITORED	CONE VOLTAGE	COLLISION ENERGY	DWELL (SECONDS)
IN-JJ998	**319.5 → 85.8 ± 0.5 AMU**	**25V**	**21V**	**0.20**
	319.5 → 276.0 ± 0.5 AMU	25V	11V	0.10
IN-A4097	192.0 → 110.9 ± 0.5 AMU	26V	22V	0.50
	194.0 → 112.9 ± 0.5 AMU	26V	22V	0.50
IN-M6957	343.8 → 126.8 ± 0.5 AMU	26V	12V	0.20
	343.8 → 152.8 ± 0.5 AMU	26V	14V	0.20
Chlorsulfuron	357.8 → 140.9 ± 0.5 AMU	25V	18V	0.20
	357.8 → 166.9 ± 0.5 AMU	**25V**	**16V**	**0.20**
IN-A4098	140.80 → 84.8 ± 0.5 AMU	**28V**	**12V**	**0.30**
	140.80 → 56.8 ± 0.5 AMU	28V	12V	0.30
Ion Mode:	Positive			
Electrospray Voltage:	4.0 kV			
Detector Voltage:	750 V			
Source Temperatures:	100°C			
Collision Gas Pressure:	2.1e-3 mBar			
Nebulizing Gas Flow:	15 L/h			
Drying Gas Flow:	300 L/h			

A complete list of the experimental parameters is given in Appendix 4. Typical LC/MS and LC/MS/MS full scan spectrums are shown in Figure 1 and Figure 2, respectively.

The instrument was operated in MS/MS-(MRM) positive ion mode for quantitative analysis. Peak area was used for quantitation. **Quantitation of chlorsulfuron, IN-JJ998, and IN-A40908 was performed using the ion transition displayed in bold face print. Quantitation of IN-A4097 and IN-M6957 was performed using the TIC.** The relative ratio of the fragment ions was evaluated to confirm the presence of an analyte in an unknown sample.

4.3.3 *Calibration Procedure and Sample Analysis*

A 0.60-ng/mL chromatographic standard should be analyzed prior to the start of analyses to establish that the instrument is working properly. If a signal-to-noise

ratio of approximately 5-10 to 1 is not attained, the instrument must be tuned or cleaned prior to sample analysis. Operating parameters must be tailored to the particular instrument used, especially if it is to be an alternate vendor's instrument, and should be checked daily. Note that some ion channels other than those used for development of this method may need to be added or eliminated when utilizing this method on other instrumentation. Each ion channel used for sample analysis/quantitation must be checked to insure it is free of interference. The control will be used to demonstrate that baseline interference is less than signal-to-noise 3:1. Begin each sample set by injecting a minimum of 2 calibration standards. The first injection should always be disregarded.

4.4 Calculations

Due to the relatively high concentrated formic acid used in the mobile phase the IN-JJ998 and IN-A4097 calibration curves deviate from linearity at the higher concentrations. As a result, the recoveries were calculated based on the average response factor for the three standards closest in response to the fortification analyzed. The standards selected must include a minimum of one standard above and one standard below.

4.4.1 Method

Average Response Factor (RF_{avg}) was calculated as follows:

$$RF_{Ave} = \frac{(\text{Conc. A} \div \text{Area A}) + (\text{Conc. B} \div \text{Area B}) + (\text{Conc. C} \div \text{Area C})}{3}$$

ppb found was calculated as follows:

$$\text{ppb Found} = \frac{(\text{Peak Area}) \times (RF_{Ave}) \times (\text{Final Volume}) \times (\text{Aliquot Factor})}{(\text{Sample Volume})}$$

In the event a peak was detected in the control, a corrected peak area was used to calculate ppb found for freshly fortified samples. The corrected peak area is the area of the fortified sample minus the area of the control sample.

The percent recovery found was calculated as follows:

$$\%\ \text{Recovery} = \frac{(\text{ppb Found})}{(\text{Fortification level})} \text{ x } 100$$

4.4.2 Example

For a well water sample fortified with IN-M6957 at 0.05 µg/L (0.05 ppb) [Date Extracted 22-Oct-2001, H-128], the concentration found was calculated as follows:

Average Response Factor was calculated as follows:

$$RF_{Ave} = \frac{(0.60\ ng/\text{mL} \div 596\ \text{AC}) + (1.0\ \text{ng/mL} \div 976\ \text{AC}) + (5.0\ \text{ng/mL} \div 4785\ \text{AC})}{3}$$

(AC ≡ Area Counts)

$RF_{avg} = 1.02541e^{-3}$ ng/mL/AC

ppb found was calculated as follows[1]:

$$\text{ppb Found} = \frac{(1032\ \text{AC}) \times (1.02541\text{e-}3\ \text{ng/mL/AC}) \times (2.5\ \text{mL}) \times (2)}{(100\ \text{mL})} \times \frac{1\ \mu\text{g/L}}{1\ \text{ng/mL}}$$

ppb Found = 0.052911 µg/L

(ppb values are reported to two significant figures in Table 1 of this report. Rounding was performed using the Microsoft Excel version 7.0 for Windows 95 rounding function)

The percent recovery found was calculated as follows:

$$\%\ \text{Recovery} = \frac{(0.052911\ \mu\text{g/L})}{(0.050\ \mu\text{g/L})} \text{ x } 100$$

% Recovery = 106%

(percent recoveries are rounded to the nearest whole number in Table 1, without rounding the concentration or ppb found)

[1] Aliquot factor was equal to 2 since 10-mL of extract was remove from a 20-mL volume (20 mL/10 mL = 2).

5.2 Time Required for Analysis

Typically ten to twelve samples can be prepared during the course of an eight-hour day. LC/MS/MS analyses were run unattended overnight.

5.3 Modifications or Special Precautions

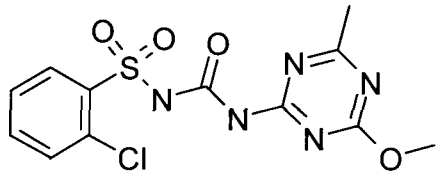
The calibration curve for IN-A4097 and IN-JJ998 may deviate from linearity over the calibration range. This was attributed to the relatively high concentration of formic acid (0.02 M) used in the mobile phase. The high formic acid concentration was needed to ionize IN-A4097 in the positive ion mode. IN-A4097 provides significantly less response then the other analytes in the ESI source. As a result of the calibration curves observed, the recovery data was based on the

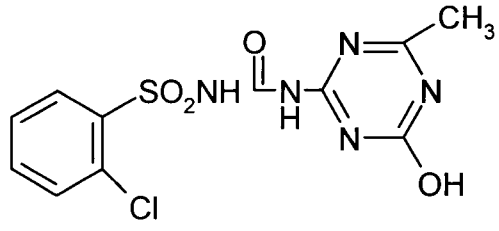
average response of three standards closest in concentration. The standards selected must include a minimum of one standard above and one standard below. If calculations are to be based on a curve fit or average response factor always check the standard linearity.

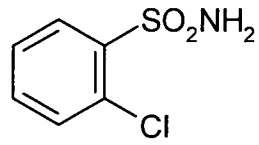
While conducting the second method with IN-A4098, an additional modification and precaution was noted. The response of the metabolite IN-A4097 has been found to improve by reducing the flow from the LC to the mass spectrometer from 100 µL/minute to approximately 75 µL/minute. The reduction in flow was achieved by modifying the splitter assembly used so that a higher split ratio was obtained. The reduction in flow has increased the signal for IN-A4097 by approximately a factor of 2 at the LOQ.

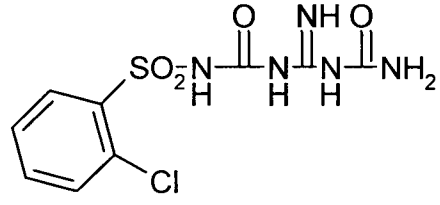
The additional note of precaution is in regards to the stability of the IN-M6957 stock standard. In Section 4.2.3, "Preparation and Stability of Stock Standards," the method outlines individually dissolving 10 mg of each analyte up in 100 mL of acetonitrile. During the method revision it was recognized that the metabolite IN-M6957 becomes less soluble when stored frozen. When making fortification or calibration standards from the original stock IN-M6957, one must be sure to warm the stock solution to room temperature and thoroughly shake. If a residue of IN-M6957 appears to be settled at the bottom of the 100 mL volumetric, then a new stock standard should be prepared before proceeding to prepare fortification or calibration standards.

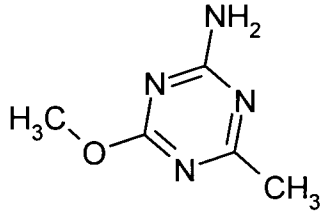
APPENDIX 1 STRUCTURE AND PROPERTIES OF CHLORSULFURON AND METABOLITES

Common Name	Chlorsulfuron
Structure	
DPX Number	DPX-W4189
Trade Names	Glean, Telar
CAS Chemical Name	2-Chloro-N-[4-methoxy-6-methyl-1,3,5-triazin-2-yl)aminocarbonyl]benzenesulfonamide
CAS Number	64902-72-3
Formula	$C_{12}H_{12}N_5O_4SCl$
Molecular Weight	357.78
Monoisotopic Weight	357.03
pKa	3.6

Common Name	None
Structure	
DPX Number	IN-M6957
Formula	$C_{11}H_{10}N_5O_4SCl$
Molecular Weight	343.75
Monoisotopic Weight	343.01

Common Name	None
Structure	
DPX Number	IN-A4097
Formula	$C_6H_6NO_2SCl$
Molecular Weight	191.64
Monoisotopic Weight	190.98

Common Name	None
Structure	
DPX Number	IN-JJ998
Formula	$C_9H_{10}N_5O_4SCl$
Molecular Weight	319.73
Monoisotopic Weight	319.01

Common Name	None
Structure	
IN Code	IN-A4098
Formula	$C_5H_8N_4O$
Molecular Weight	140.15 g/mole
Monoisotopic Weight	140.07 g/mole

APPENDIX 4 EXPERIMENTAL CONDITIONS

Acquisition Experiment Report
File:p:\chloro water chros\sjh10220109.09

Header
Acquired File Name: SJH10220122-Oct-2001
Acquired Date: 22-Oct-2001
Acquired Time: 18:55:58
Job code: 102201 S
Bottle Number: 88
Description: H-131

Instrument Calibration
Parameters
MS1 Static:
Mass 85 Da to 525 Da.
Resolution : 15.0/15.0
Ion Energy : 0.5
Reference File : peghnh4
Acquisition File : STATMS1
MS1 Scanning:
Mass 80 Da to 530 Da.
Resolution : 15.0/15.0
Ion Energy : 0.5
Reference File : peghnh4
Acquisition File : SCNMS1
MS1 Scan Speed:
Scan 46 to 223 amu/sec.
Resolution : 15.0/15.0
Ion Energy : 0.5
Reference File : peghnh4
Acquisition File : FASTMS1
MS2 Static:
Mass 85 Da to 525 Da.
Resolution : 15.0/15.0
Ion Energy : 0.5
Reference File : peghnh4
Acquisition File : STATMS2
MS2 Scanning:
Mass 80 Da to 530 Da.
Resolution : 15.0/15.0
Ion Energy : 0.5
Reference File : peghnh4
Acquisition File : SCNMS2
MS2 Scan Speed:
Scan 46 to 223 amu/sec.
Resolution : 15.0/15.0
Ion Energy : 0.5
Reference File : peghnh4
Acquisition File : FASTMS2
Calibration Time: 13:16
Calibration Date: 10/02/00
Coefficients
MS1 Static: -0.000000000014*x^4 + 0.000000026628*x^3 + -
0.000016179056*x^2 + 1.003854928591*x +-0.291897283930
MS2 Static: -0.000000000070*x^4 + 0.000000091827*x^3 + -
0.000042895346*x^2 + 1.008166472346*x +-0.429729717586

Instrument ID: OCP -v3.1_4 -QUAT2 4000

Tuning Parameters: ES+
Source Page (ESI)

Capillary	4.00	kVolts
HV Lens	0.70	kVolts
Cone	30	Volts

Skimmer Offset	8	Volts
Skimmer	1.8	Volts
RF Lens	0.4	Volts
Source Temp	100	oC

MS1

Ion Energy	2.0	Volts
Ion Energy Ramp	0.0	Volts
LM Resolution	5.0	
HM Resolution	5.0	
Lens 5	100	Volts
Lens 6	2	Volts
Multiplier 1	750	Volts

MS2

Ion Energy	1.5	Volts
Ion Energy Ramp	0.0	Volts
LM Resolution	10.0	
HM Resolution	10.0	
Lens 7	250	Volts
Lens 8	200	Volts
Lens 9	0	Volts
Multiplier	750	Volts

Pressures

Analyser Vacuum	2.4e-5	mBar
Gas Cell	2.1e-3	mBar

Acquisition Threshold
SIR or MRM Data
Baseline level: 1.0
General
Ion count threshold: 25
Prescan Statistics
Zero Level: 0
ADC zero: 82.11
ADC standard deviation: 0.83

Acquisition Threshold MS2
SIR or MRM Data
Baseline level: 1.0
General
Ion count threshold: 25
Prescan Statistics
Zero Level: 0
ADC zero: 78.44
ADC standard deviation: 0.88

---------- Run method parameters ----------

HP1100 LC Pump Initial Conditions

Solvents

A%	95.0
B%	5.0
C%	0.0
D%	0.0
Flow (ml/min)	0.800
Stop Time (mins)	25.0
Min Pressure (bar)	0
Max Pressure (bar)	350
Oven Temperature Left(°C)	25.0
Oven Temperature Right(°C)	25.0

HP1100 LC Pump Gradient Timetable

The gradient Timetable contains 9 entries which are :

Time	A%	B%	C%	D%	Flow	Pressure
0.00	95.0	5.0	0.0	0.0	0.800	400
1.00	95.0	5.0	0.0	0.0	0.800	400
3.00	45.0	55.0	0.0	0.0	0.800	400
11.00	15.0	85.0	0.0	0.0	0.800	400
14.00	15.0	85.0	0.0	0.0	0.800	400
14.20	5.0	95.0	0.0	0.0	1.000	400
17.00	5.0	95.0	0.0	0.0	1.000	400
17.50	95.0	5.0	0.0	0.0	1.000	400
19.80	95.0	5.0	0.0	0.0	1.000	400

HP1100 LC Pump External Event Timetable

The Timetable contains 7 entries which are :

Time	Column Switch	Contact 1	Contact 2	Contact 3	Contact 4
Initial	Off	Off	Off	Off	Off
0.00	Off	On	Off	Off	Off
0.10	Off	Off	Off	Off	Off
7.00	Off	Off	Off	On	Off
7.10	Off	Off	Off	Off	Off
14.50	Off	Off	Off	Off	On
14.60	Off	Off	Off	Off	Off

HP1100 Autosampler Initial Conditions

Injection Volume(µl)	200.0
Draw Speed	200.0
Eject Speed (µl/min)	200
Draw Position (mm)	0.00
Stop Time (mins)	25.00
Vial Number	88
Thermostat On	
Thermostat Temperature(°C)	4.0

---------- oOo ----------

Function 1

Scans in function:	96
Cycle time (secs):	0.230
Inter Channel delay (secs):	0.00
Retention window (mins):	7.500 to 9.500
Ionization mode:	ES+
Data type:	SIR or MRM data
Function type:	MRM of 2 channels

Chan	Reaction	Dwell(secs)	Cone Volt.	Col.Energy
1	: 319.50 > 85.80	0.20	25.0	21.0
2	: 319.50 > 276.00	0.10	25.0	11.0

Function 2

Scans in function:	80
Cycle time (secs):	0.530
Inter Channel delay (secs):	0.00
Retention window (mins):	7.600 to 9.500
Ionization mode:	ES+
Data type:	SIR or MRM data
Function type:	MRM of 2 channels

Chan	Reaction	Dwell(secs)	Cone Volt.	Col.Energy
1	: 192.00 > 110.90	0.50	26.0	22.0
2	: 194.00 > 112.90	0.50	26.0	22.0

```
Function 3
Scans in function:          323
Cycle time (secs):          0.230
Inter Channel delay (secs):0.00
Retention window (mins):    9.500 to 12.000
Ionization mode:            ES+
Data type:                  SIR or MRM data
Function type:              MRM of 2 channels
Chan Reaction               Dwell(secs) Cone Volt. Col.Energy
1   : 343.80 > 126.80           0.20        26.0        12.0
2   : 343.80 > 152.80           0.20        26.0        14.0

Function 4
Scans in function:          323
Cycle time (secs):          0.230
Inter Channel delay (secs):0.00
Retention window (mins):    12.000 to 14.500
Ionization mode:            ES+
Data type:                  SIR or MRM data
Function type:              MRM of 2 channels
Chan Reaction               Dwell(secs) Cone Volt. Col.Energy
1   : 357.80 > 140.90           0.20        25.0        18.0
2   : 357.80 > 166.90           0.20        25.0        16.0
```

APPENDIX 5 SUPPORTING DOCUMENTATION FOR IN-A4098

Experimental Conditions

Acquisition Experiment Report
File: d:\w4189.pro\data\jsl101003bw_09

Header
Acquired File Name: JSL101003BW_09
Acquired Date: 10-Oct-2003
Acquired Time: 22:13:54
Job code: JSL101003BW
Task code:
User Name: Administrator
Laboratory Name: Lab
Instrument: Inst
Conditions:
Submitter:
SampleID:
Bottle Number: 79
Description: 10 ng/mL

Instrument Calibration
Parameters
MS1 Static: None
MS1 Scanning: None
MS1 Scan Speed: None
MS2 Static: None
MS2 Scanning: None
MS2 Scan Speed: None
Calibration Time: 09:19
Calibration Date: 06/27/94
Coefficients
MS1 Static: None
MS2 Static: None
Function 1: None
Function 2: None
Function 3: None
Function 4: None
Function 5: None

Instrument ID: OCP -v3.1_4 -QUAT2 4000

Tuning Parameters: ES+
Source Page (ESI)

Capillary:	3.95	kVolts
HV Lens:	0.04	kVolts
Cone:	42	Volts
Skimmer Offset:	6	Volts
Skimmer:	1.4	Volts
RF Lens:	0.3	Volts
Source Temp:	130	øC

MS1

Ion Energy:	2.0	Volts
Ion Energy Ramp:	0.0	Volts
LM Resolution:	8.0	
HM Resolution:	8.0	
Lens 5:	100	Volts
Lens 6:	4	Volts
Multiplier 1:	750	Volts

MS2

Ion Energy:	3.5	Volts
Ion Energy Ramp:	0.0	Volts
LM Resolution:	8.0	
HM Resolution:	8.0	
Lens 7:	250	Volts

Lens 8:	44	Volts
Lens 9:	0	Volts
Multiplier:	750	Volts

Pressures

Analyser Vacuum:	2.8e-5	mBar
Gas Cell:	2.8e-3	mBar

Acquisition Threshold
SIR or MRM Data

Baseline level:	1.0

General

Ion count threshold:	0

Prescan Statistics

Zero Level:	16
ADC zero:	81.32
ADC standard deviation:	0.81

Acquisition Threshold MS2
SIR or MRM Data

Baseline level:	1.0

General

Ion count threshold:	0

Prescan Statistics

Zero Level:	20
ADC zero:	77.60
ADC standard deviation:	0.85

ACE Experimental Record

---------- Run method parameters ----------

HP1100 LC Pump Initial Conditions

Solvents

A%	95.0
B%	5.0
C%	0.0
D%	0.0
Flow (ml/min)	0.800
Stop Time (mins)	19.8
Min Pressure (bar)	0
Max Pressure (bar)	400
Oven Temperature Left(°C)	25.0
Oven Temperature Right(°C)	25.0

HP1100 LC Pump Gradient Timetable

The gradient Timetable contains 7 entries which are :

Time	A%	B%	C%	D%	Flow	Pressure
0.00	95.0	5.0	0.0	0.0	0.800	400
1.00	95.0	5.0	0.0	0.0	0.800	400
11.00	15.0	85.0	0.0	0.0	0.800	400
14.00	15.0	85.0	0.0	0.0	0.800	400
14.20	5.0	95.0	0.0	0.0	1.000	400
17.00	5.0	95.0	0.0	0.0	1.000	400
17.50	95.0	5.0	0.0	0.0	1.000	400

HP1100 LC Pump External Event Timetable

The Timetable contains 7 entries which are :

Time	Column Switch	Contact 1	Contact 2	Contact 3	Contact 4
Initial	Off	Off	Off	Off	Off
0.00	Off	On	On	Off	Off
0.10	Off	Off	Off	Off	Off
5.00	Off	Off	Off	On	Off
5.10	Off	Off	Off	Off	Off
17.00	Off	Off	Off	Off	On
17.10	Off	Off	Off	Off	Off

HP1100 Autosampler Initial Conditions

Injection Volume(µl)	200.0
Draw Speed	200.0
Eject Speed (µl/min)	200
Draw Position (mm)	0.00
Stop Time (mins)	25.00
Vial Number	79
Thermostat On	
Thermostat Temperature(°C)	4.0

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End of experimental record.
Solvent Delay
None

Function 1

Scans in function:	180
Cycle time (secs):	0.330
Inter Channel delay (secs):	0.00
Retention window (mins):	6.000 to 8.000
Ionization mode:	ES+
Data type:	SIR or MRM data
Function type:	MRM of 2 channels

Chan	Reaction	Dwell(secs)	Cone Volt.	Col.Energy
1	: 141.00 > 84.80	0.30	28.0	12.0
2	: 141.00 > 56.80	0.30	28.0	12.0

Function 2

Scans in function:	96
Cycle time (secs):	0.280
Inter Channel delay (secs):	0.00
Retention window (mins):	10.000 to 12.000
Ionization mode:	ES+
Data type:	SIR or MRM data
Function type:	MRM of 2 channels

Chan	Reaction	Dwell(secs)	Cone Volt.	Col.Energy
1	: 192.00 > 110.90	0.25	24.0	24.0
2	: 192.00 > 110.91	0.25	22.0	24.0

Function 3

Scans in function:	96
Cycle time (secs):	0.230
Inter Channel delay (secs):	0.00
Retention window (mins):	10.010 to 12.000
Ionization mode:	ES+
Data type:	SIR or MRM data
Function type:	MRM of 3 channels

Chan	Reaction	Dwell(secs)	Cone Volt.	Col.Energy
1	: 320.00 > 276.90	0.20	38.0	13.0
2	: 320.00 > 85.90	0.20	25.0	21.0
3	: 320.00 > 128.90	0.20	38.0	18.0

```
Function 4
Scans in function:          143
Cycle time (secs):          0.330
Inter Channel delay (secs):0.00
Retention window (mins):    13.000 to 15.000
Ionization mode:            ES+
Data type:                  SIR or MRM data
Function type:              MRM of 2 channels
Chan Reaction               Dwell(secs) Cone Volt. Col.Energy
1   : 343.80 > 126.80           0.30        26.0        14.0
2   : 343.80 > 152.80           0.30        26.0        14.0

Function 5
Scans in function:          181
Cycle time (secs):          0.230
Inter Channel delay (secs):0.00
Retention window (mins):    14.000 to 16.000
Ionization mode:            ES+
Data type:                  SIR or MRM data
Function type:              MRM of 2 channels
Chan Reaction               Dwell(secs) Cone Volt. Col.Energy
1   : 358.00 > 140.80           0.20        38.0        19.0
2   : 358.00 > 166.80           0.20        38.0        19.0
```