ANALYTICAL METHOD FOR THE DETERMINATION OF CHLORSULFURON AND METABOLITES IN SOIL USING LC/MS/MS

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1.0 SUMMARY

The purpose of this study was to develop an analytical method for the detection, quantitative analysis and confirmation of chlorsulfuron and metabolites IN-D5293, IN-A4097, IN-JJ998, IN-M6957, IN-UND13 and IN-A4098 residues in soil.

Chlorsulfuron, IN-D5293, IN-A4097, IN-JJ998, IN-M6957, IN-UND13 and IN-A4098 were extracted from soil samples using a solution of ammonium carbonate and acetonitrile. A 10-mL aliquot of the extracts were purified using a dispersive solid phase extraction (SPE) step using bulk Strong Ion Exchange (SAX) material. The extracts were centrifuged and the supernatant was passed through a graphitized carbon SPE cartridge. The eluate was collected and evaporated to a volume less than 4-mL. The extracts were diluted to 4-mL using 0.10 M aqueous ammonia carbonate. An aliquot of the extracts were transferred to an auto-sampler vial for analysis. Chlorsulfuron and its metabolites were separated from co-extracts by reversed phase liquid chromatography. Chlorsulfuron and IN-A4098 were detected by positive ion electrospray mass spectrometry/mass spectrometry (MS/MS). The metabolites IN-D5293, IN-A4097, IN-JJ998, IN-M6957 and IN-UND13 were detected by negative ion electrospray MS/MS. The Limit of Quantitation (LOQ) for each analyte was 1.0 µg/kg (ppb). The Limit of Detection (LOD) was estimated to be 0.3 µg/kg (ppb) for the least responsive analyte, IN-A4098.

2.0 INTRODUCTION

The structure, CAS name, CAS registry number, and various properties of chlorsulfuron and metabolites IN-D5293, IN-A4097, IN-JJ998, IN-M6957, IN-

UND13 and IN-A4098 can be found in Appendix 1. The method was validated on two soils from the United States.

Chlorsulfuron and metabolites IN-D5293, IN-A4097, IN-JJ998, IN-M6957, IN-UND13 and IN-A4098 were extracted from soil samples using a solution of ammonium carbonate and acetonitrile. An aliquot of the extracts was purified using a dispersive SPE step and by filtration through a graphitized carbon SPE cartridge. The volume of the purified extract was evaporated to less than 4-mL and diluted to 4-mL using aqueous ammonia carbonate. The purified extracts were analyzed using reversed phase liquid chromatography (LC) and electrospray mass spectrometry/mass spectrometry (MS/MS). The Limit of Quantitation (LOQ) was 1.0 µg/kg (ppb). The Limit of Detection (LOD) was estimated to be 0.3 µg/kg (ppb) for the least responsive analyte, IN-A4098.

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

LC system, HP1290 with temperature controlled autosampler (Agilent Technologies, Wilmington, DE)

Mass Spectrometer System, API 5000 triple quadrupole mass spectrometer using a Turbo Ion Spray and Analyst version 1.52 software (Applied Biosystems/MDS Sciex, Foster City, CA)

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)

Solid-Phase Extraction Equipment

Bondesil SAX Material, 100 g bottle, PN 12213042 (Agilent Technologies, Wilmington, DE). **Do not substitute.**

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

Supelco Supelclean Envi Carb Cartridge, 6-mL, 0.25 gram PN 57092 (Supelco, Bellefonte, PA)

Chromatographic Supplies

HPLC Column: 2.0 mm i.d. × 15 cm, Phenomenex Luna C18(2) analytical column with 3-µm diameter packing Part # 00F-4251-B0 (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 X 32 mm rubber bulbs, Cat. No. 56310-240 (VWR Scientific Co., Bridgeport, NJ)

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

Centrifuge tubes, Polystyrene 14-mL capacity, Cat. No. 21008-930 (VWR Scientific Co., Bridgeport, NJ)

Miscellaneous

Carbon Steel Balls, 1/4 inch, Catalog No. 00073254 (MSC Industrial Supply, Melville, NY)

Genoginder : Spex SamplePrep Model number 2000.

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.

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

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

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

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

Ammonium Carbonate - Baker Analyzed®, #0650-01 (J. T. Baker Inc., Danvers, MA)

Chlorsulfuron (DPX-W4189), GLP characterized material used (Dash 735, 99.4% pure) for sample analysis, prepared by DuPont Crop Protection, Global Technology Division, E. I. du Pont de Nemours and Company

IN-UND13 reference substance (Dash 001, 98.2% pure) used for sample analysis: Analytical standard grade reagent (DuPont Crop Protection, Global Technology Division, E. I. du Pont de Nemours and Company)

IN-D5293 reference substance (Dash 018, 99.2% pure) used for sample analysis: Analytical standard grade reagent (DuPont Crop Protection, Global Technology Division, E. I. du Pont de Nemours and Company)

IN-JJ998 reference substance (Dash 004, 90.0% pure) used for sample analysis: Analytical standard grade reagent (DuPont Crop Protection, Global Technology Division, E. I. du Pont de Nemours and Company)

IN-A4098 reference substance (Dash 005, 98.7% pure) used for sample analysis: Analytical standard grade reagent (DuPont Crop Protection, Global Technology Division, E. I. du Pont de Nemours and Company)

IN-M6957 reference substance (Dash 003, 82.9% pure) used for sample analysis: Analytical standard grade reagent (DuPont Crop Protection, Global Technology Division, E. I. du Pont de Nemours and Company)

IN-A4097 reference substance (Dash 010, 99.7% pure) used for sample analysis: Analytical standard grade reagent (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

Chlorsulfuron and metabolites IN-D5293, IN-A4097, IN-JJ998, IN-M6957, IN-UND13 and IN-A4098 were extracted from soil samples using a solution of ammonium carbonate and acetonitrile. An aliquot of the extracts was purified using a dispersive SPE step and by filtration through a graphitized carbon SPE cartridge. The volume of the purified extract was evaporated to less than 4-mL and diluted to 4-mL using aqueous ammonia carbonate. The purified extracts were analyzed using reversed phase liquid chromatography (LC) and electrospray mass spectrometry/mass spectrometry (MS/MS).

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 monthly and stored at room temperature unless stated otherwise:

0.1 M ammonium carbonate solution. Add 9.6 g of ammonium carbonate to a volume of 900 mL of EM science water. Mix the resulting solution to homogeneity and dilute to 1000 mL. The pH was not adjusted.

50:50 0.1 M ammonium carbonate: acetonitrile: In a 1-L bottle add 500 mL of 0.10 M ammonium carbonate solution and 500 mL of acetonitrile. Mix the resulting solution to homogeneity.

Mobile Phase A : 0.02 M aqueous formic acid solution - Add 920 µL of formic acid to 1000 mL of water mix the resulting solution to homogeneity.

Mobile Phase B : Methanol

4.2.3 *Preparation and Stability of Stock Standard*

Use Class A volumetric flasks when preparing standard solutions.

All standards that are less than 95% pure need to be corrected for purity.

Preparation of the chlorsulfuron, IN-D5293, IN-A4097, IN-M6957, IN-UND13 and IN-A4098 Stock Standards

Prepare standard stock solutions by accurately weighing 10 ± 0.01 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 solution to a volume of 100 mL using HPLC-grade acetonitrile and invert the volumetric flask to mix the solution to homogeneity. The 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.

Preparation of the IN-JJ998 Stock Standard

Prepare standard stock solutions by accurately weighing 10 ± 0.01 mg of IN-JJ998 into a 100-mL volumetric flask using an analytical balance. Record the accurate weight of the standard. Dissolve the standards in approximately 5 mL of DMSO. After dissolving, bring the solution to a volume of 100 mL using HPLC-grade acetonitrile and invert the volumetric flask to mix the solution to homogeneity. The 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 Intermediate and Fortification Standards*

Use Class A volumetric flasks when preparing standard solutions.

Prepare a 1.0-µg/mL chlorsulfuron, IN-D5293, IN-A4097, IN-JJ998, IN-M6957, IN-UND13 and IN-A4098 intermediate standard in acetonitrile by pipetting 1.00 mL of

each 100.0-µg/mL stock standard 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-D5293, IN-A4097, IN-JJ998, IN-M6957, IN-UND13 and IN-A4098 fortification standard in acetonitrile by pipetting 1.00 mL of the 1.0-µg/mL 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 as showed in the table below (alternative or additional standards may be prepared as needed):

Standard Used	Volume Pipetted (µL)	Volume of 0.1 M aqueous ammonium carbonate (µL)	Final Concentration (ng/mL)
0.10 µg/mL	75	925	7.5
0.10 µg/mL	50	950	5.0
0.10 µg/mL	25	975	2.5
7.5 ng/mL	100	900	0.75
5.0 ng/mL	100	900	0.50
2.5 ng/mL	100	900	0.25

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 vortex mixed for 30 seconds prior to placing them on the autosampler tray.

4.2.6 *Source of Samples*

Soil control samples were obtained from sites located in the United States. The soil characteristics are shown in the following table:

Soil Name	Country	Type	% Clay	% Sand	% Silt	pH_W	OM (%)	Notebook
Drummer	USA	Loamy Sand	18	23	59	6.1	3.9	L1058-130
Hildago	USA	Clay Loam	34	40	26	7.8	1.1	2004-008

4.2.7 *Storage and Preparation of Samples*

Soil samples should be stored frozen at approximately -20°C until use. The soil core was dived into segments based on depth. For method development purposes only the 0-5 cm cores were selected. Both cores were mixed by hand prior to analysis.

4.2.8 *Sample Fortification Procedure*

All fortifications were made directly to the 7.5-g soil sample after weighing the sample. Fortified samples were prepared using a 0.10-µg/mL standard solution.

FORTIFICATION LEVEL (µG/KG)	VOLUME OF STANDARD (ML)
1.00	0.075
10.0	0.750

4.2.9 *Analyte Extraction and Purification Procedures*

1. Accurately measure 7.5-g (± 1%) of soil into a 50-mL plastic centrifuge tubes. Fortify samples if necessary and allow the fortification to dry in a fume hood for approximately 15-minutes. Cap and shake the samples vigorously.
2. Add two 1/4" inch steel balls and 15-mL of 50:50 0.1 M aqueous ammonium carbonate: acetonitrile solution to each sample.
3. Place samples on a genogrinder and homogenize for 3 minutes at a rate of approximately 1000 strokes per minute.
4. Centrifuge the samples for 10 minutes to drive the particulates to the bottom of the tube at a rate of approximately 3000 RPM. Transfer the supernatant into a clean 50-mL centrifuge tubes.
5. Repeat steps 2-4 (without adding additional steel balls) using 15-mL of 50:50 0.1 M aqueous ammonium carbonate: acetonitrile. Combine the two extracts into the same 50-mL centrifuge tubes.
6. Repeat steps 2-4 a third time (without adding additional steel balls) using 15-mL of 50:50 0.1 M aqueous ammonium carbonate: acetonitrile. Combine the three extracts and adjust the volume of the extracts from each sample to 45-mL using 50:50 0.1 M aqueous ammonium carbonate: acetonitrile. Mix the extract using a vortex mixer for approximately 30 seconds.
7. Pipette 10.0-mL of each extract into a clean 14-mL centrifuge tubes. Add 0.25 grams of bondesil bulk SAX material to each extract. Mix using a vortex mixer for approximately 30 seconds.
8. Centrifuge the extracts for 5 minutes to drive the particulates to the bottom of the tube at a rate of approximately 3000 RPM.
9. Attach 6-mL, 0.25-g Supelclean Envi Carb cartridges to an SPE manifold. Using gravity flow condition the cartridges with 5-mL of methanol followed by 5-mL 50:50 0.1 M aqueous ammonium carbonate: acetonitrile. **Do not let the cartridge go to dryness**.
10. Place a clean 14-mL plastic centrifuge tube under each cartridge and load the sample extract from step 8 into the SPE. Take care **not** to transfer the bulk SAX material to the SPE frit. Using gravity flow, pass the extracts through the cartridges collecting them in the clean 14-mL tubes.

11. Rinse the original centrifuge tube and bulk SAX material with 1-mL of 50:50 0.1 M aqueous ammonium carbonate: acetonitrile. Mix using a vortex mixer and centrifuge the extracts for 5 minutes to drive the particulates to the bottom of the tube at a rate of approximately 3000 RPM.
12. Transfer the supernatants to the SPE cartridges and collect the eluate in the 14-mL centrifuge tubes. Using positive pressure or a slight vacuum empty the entire content in the SPE cartridges into the collection centrifuge tubes.
13. Remove the centrifuge tubes from the reservoir and evaporate the extract to less than 4-mL using a flow of nitrogen in an N-Evap at approximately 35°C. Dilute the extract to 4-mL using 0.10 M aqueous ammonium carbonate. Mix using a vortex mixer for 30-seconds.
14. Syringe filter (PTFE) an aliquot of each extract into an auto-sampler vial for LC/MS/MS analysis.

Extracts will be stable for approximately 48 hours if stored at 8°C.

4.3 Instrumentation for the Method

4.3.1 Chromatography

Reversed-phase chromatography was used to separate chlorsulfuron and metabolites from the soil co-extracts. Due to the number on compounds analyzed and the wide range of polarities, two separate injections were required to analyze the extracts. The column selected for the analysis was a Phenomenex Luna C18 (2). The column choice reflected experimental results indicating preferred separation from co-extracts. Alternative chromatographic conditions (See Appendix 4) can be used, provided the analytical method is validated and provides acceptable recoveries as defined by regulatory method guidelines.

The chromatographic condition for the analysis of IN-D5293, IN-A4097, IN-JJ998, IN-M6957 and IN-UND13.

SYSTEM:	Agilent 1290 HPLC			
COLUMN:	2.0 mm i.d. × 15 cm, 3 µm Phenomenex Luna C18 (2)			
COLUMN TEMPERATURE:	40 °C			
SAMPLE TEMPERATURE	6 °C			
INJECTION VOLUME:	0.005 mL			
FLOW RATE:	0.40 mL/min			
CONDITIONS:	A: 0.02 M aqueous Formic Acid			
	B: Methanol			
	Time	%A	%B	Flow (mL/Min.)
	0.0	90	10	0.40
	1.0	90	10	0.40
	2.0	70	30	0.40
	14.0	1	99	0.40
	16.0	1	99	0.40
	16.1	90	10	0.40
	20.0	90	10	0.40
IN-D5293 RETENTION TIME:	5.16 minutes			
IN-A4097 RETENTION TIME:	5.17 minutes			
IN-JJ998 RETENTION TIME:	5.21 minutes			
IN-M6957 RETENTION TIME:	6.83 minutes			
IN-UND13 RETENTION TIME:	8.14 minutes			
TOTAL RUN TIME:	20.0 minutes			

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
0.0-0.10	Waste
0.10-14.0	MS source
14.0-End	Waste

The chromatographic conditions for the analysis of IN-A4098 and chlorsulfuron.

SYSTEM:	Agilent 1290 HPLC			
COLUMN:	2.0 mm i.d. × 15 cm, 3 µm Phenomenex Luna C18 (2)			
COLUMN TEMPERATURE:	40 °C			
SAMPLE TEMPERATURE	6 °C			
INJECTION VOLUME:	0.002 mL			
FLOW RATE:	0.400 mL/min			
CONDITIONS:	A: 0.02 M aqueous Formic Acid			
	B: Methanol			
	Time	%A	%B	Flow (mL/Min.)
	0.0	90	10	0.40
	1.0	90	10	0.40
	14.0	1	99	0.40
	16.0	1	99	0.40
	16.1	90	10	0.40
	20.0	90	10	0.40
IN-A4098 RETENTION TIME:	3.02 minutes			
CHLORSULFURON RETENTION TIME:	10.6 minutes			
TOTAL RUN TIME:	20.0 minutes			

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
0.00-0.10	Waste
0.10-14.0	MS source
14.0-End	Waste

4.3.2 *LC/MS/MS Analysis*

The quantitative analysis of chlorsulfuron and metabolites was performed using an Applied Biosystem API 5000 LC/MS/MS system. Quantitative analysis was based on the integration of a single ion transition. The system parameters were adjusted while a solution of each analyte was infused directly into the ion source. A summary of the experimental conditions for the analysis of IN-D5293, IN-A4097, IN-JJ998, IN-M6957 and IN-UND13 is provided in the following table:

Analytes	Ions Monitored	Declustering Potential (DP)	Collision Energy (CE)	Exit Potential (CXP)
IN-D5293	**233.0→ 78.0 AMU**	**-65**	**-30**	**-13**
	233.0→ 125.9 AMU	-65	-30	-11
IN-A4097	**189.9→ 77.9 AMU**	**-20**	**-36**	**-9**
	189.9→ 125.8 AMU	-20	-22	-11
IN-JJ998	**318.0→ 274.9 AMU**	**-45**	**-16**	**-13**
	318.0→ 189.9 AMU	-45	-30	-19
IN-M6957	**342.0→ 125.0 AMU**	**-55**	**-24**	**-11**
	342.0→ 82.0 AMU	-55	-52	-15
IN-UND13	**319.0→ 189.8 AMU**	**-35**	**-22**	**-19**
	319.0→ 232.7 AMU	-35	-18	-23
Time:	0-20 minutes			
Ion Mode:	Negative			
Turbospray Voltage:	-4500 V			
Source Temperature:	600 C			
CUR:	30			
CAD:	4			
GS1:	40			
GS2:	50			
Dwell	0.150 Seconds			

A summary of the experimental conditions for the analysis of IN-A4098 and chlorsulfuron is provided in the following table:

Analytes	Ions Monitored	Declustering Potential (DP)	Collision Energy (CE)	Exit Potential (CXP)
IN-A4098	**141.0→ 57.0 AMU**	**86**	**27**	**8**
	141.0→ 58.0 AMU	86	35	10
Chlorsulfuron	**358.1→ 141.1 AMU**	**66**	**29**	**24**
	358.1→ 167.0 AMU	66	25	26
Time:	0-20 minutes			
Ion Mode:	Positive			
Turbospray Voltage:	4500 V			
Source Temperature:	600 C			
CUR:	30			
CAD:	4			
GS1:	40			
GS2:	50			
Dwell	0.150 Seconds			

A complete list of the experimental parameters is given in Appendix 3. A typical LC/MS and LC/MS/MS full scan spectrum of each analyte is shown in Figure 1 and Figure 2, respectively.

The instrument was operated in MS/MS-(MRM) positive and negative ion modes for quantitative analysis. Peak area was used for quantitation. **Quantitation was performed using the ion transition displayed in bold face print.** The ion transitions in plan print were used for confirmation analysis.

4.3.3 *Calibration Procedure and Sample Analysis*

A 0.25-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 for the least responsive analyte, 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

4.4.1 *Methods*

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

$$RF_{Ave} = \frac{\begin{array}{c}(\text{Conc. A} \div \text{A Corrected Area A}) + (\text{Conc. B} \div \text{Corrected Area B}) + \\ (\text{Conc. C} \div \text{Corrected Area C}) + (\text{Conc. D} \div \text{Corrected Area D})\end{array}}{\text{Total Number of Standards Injected}}$$

Corrected Area = (Area in the standard – Area on the control)

ng/g (ppb) found was calculated as follows:

$$\text{ng/g Found} = \frac{(\text{Peak Area}) \times (RF_{Ave}) \times (\text{Final Volume}) \times (\text{Aliquot Factor})}{(\text{grams of Sample})}$$

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:

$$\%\ \text{Recovery} = \frac{(\text{ng/g Found})}{(\text{ng/g Fortified})} \text{ x } 100$$

4.4.2 *Example*

For a Hildago soil sample fortified with chlorsulfuron at 1.0 ppb [Date analyzed 24-Oct-14, LOQ 1], the concentration found was calculated as follows:

Average Response Factor was calculated as follows:

$$RF_{Ave} = \frac{\begin{array}{c}(0.25\ \text{ng/mL} \div 22700) + (0.50\ \text{ng/mL} \div 43600) + (0.75\ \text{ng/mL} \div 65800) + \\ (2.5\ \text{ng/mL} \div 218000) + (5.0\ \text{ng/mL} \div 438000) + (7.5\ \text{ng/mL} \div 653000)\end{array}}{6}$$

(AC ≡ Area Counts)

$RF_{Avg} = 1.13747^{-5}$ ng/mL/AC

ng/g (ppb) found was calculated as follows:

$$\text{ng/g Found} = \frac{(32900\ \text{AC}) \times (1.13747\text{e-}5\ \textit{ng}\text{/mL/AC}) \times (4\ \text{mL}) \times (4.5)}{(7.5\ \text{grams})}$$

ng/g Found = 0.898

(ppb values are reported to two significant figures in Table 1 of this report. Rounding was performed using the Microsoft Excel)

The percent recovery found was calculated as follows:

$$\% \text{ Recovery} = \frac{(0.898\ ng/g)}{(1.0\ ng/g)} \text{ x } 100$$

% Recovery = 90%

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

5.2 Time Required for Analysis

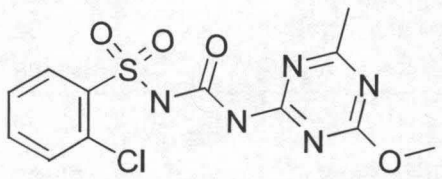
Typically ten to twelve samples can be prepared during the course of an eight-hour day. LC/MS/MS analyses were run unattended overnight.

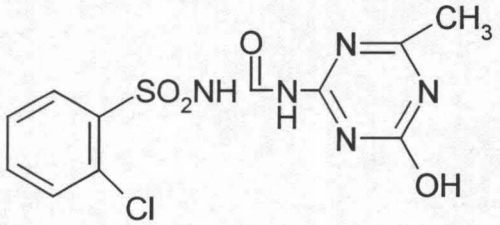
5.3 Modifications or Special Precautions

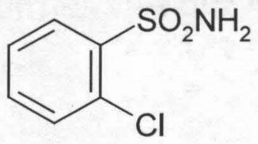
The degradation of chlorsulfuron in solutions has been observed in previous studies. Care must be taken to keep all standards refrigerated when not in use. The stability of the standard should be checked on a routine basis.

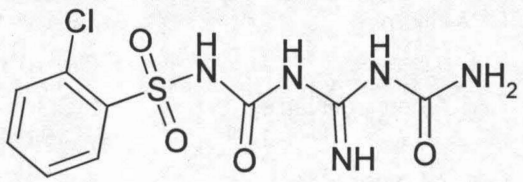
Alternative chromatographic conditions and validated data are presented in Appendix 4. The chromatography presented in the main report is preferred since some column instability was observed when using a basic mobile phase. In the event interference or LC/MS matrix effects are observed the conditions described in Appendix 4 could be used.

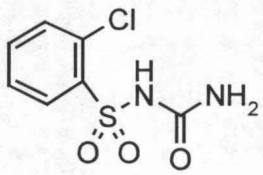
APPENDIX 1 STRUCTURE AND PROPERTIES OF CHLORSULFURON AND METABOLITES

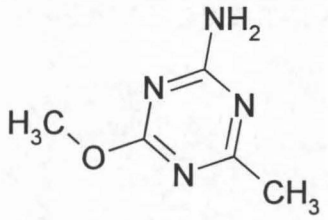
Common Name	Chlorsulfuron
Structure	
DPX- Number	DPX-W4189
Trade Names	Glean, Telar
CAS Chemical Name	2-Chloro-N-[4-methoxy-6-methyl-1,3,5-triazin-2-yl)aminocarbonyl]benzenesulfonamide
CAS Number	64902-72-3
Formula	$C_{12}H_{12}N_5O_4SCl$
Molecular Weight	357.78
Monoisotopic Weight	357.03
pKa	3.6

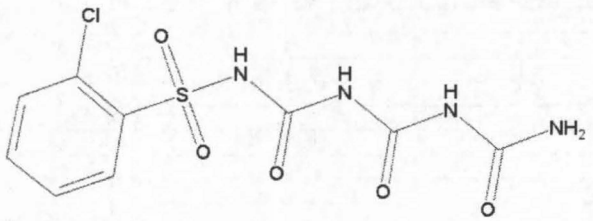
Common Name	None
Structure	
DPX- Number	IN-M6957
Formula	$C_{11}H_{10}N_5O_4SCl$
Molecular Weight	343.75
Monoisotopic Weight	343.01

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	
DPX- Number	IN-D5293
Formula	$C_7H_7ClN_2O_3S$
Molecular Weight	234.6601
Monoisotopic Weight	233.9866

Common Name	None
Structure	
DPX- Number	IN-A4098
Formula	$C_5H_8N_4O$
Molecular Weight	140.15
Monoisotopic Weight	140.07

Common Name	None
Structure	
DPX- Number	IN-UND13
Formula	$C_9H_9ClN_4O_5S$
Molecular Weight	320.7096
Monoisotopic Weight	319.9982

APPENDIX 3 EXPERIMENTAL CONDITIONS

Negative Ion Conditions

File Information for Sample 7 (LOQ 1 Soil) of 10242014W4189andMetsInHildagoSoilVal2SaxNegC18.wiff

File Name:	10242014W4189andMetsInHildagoSoilVal2SaxNegC18.wiff
File Path:	D:\Analyst Data\Projects\DPX-W4189andMetsInSoil05_13_2014\2014_05_13\Data\
Original Name:	10242014W4189andMetsInHildagoSoilVal2SaxNegC18.wiff
Software Version:	Analyst 1.5.2

Log Information from Devices at Start of acquisition:

Pump	Agilent 1290 G4220A	
Firmware Version	B.06.53	
Serial Number	DEBAA03467	
Time from start =0.0000 min	AutoSampler	Agilent 1290 G4226A
Firmware Version	A.06.50	
Serial Number	DEBAP04093	
Linked Pump	G4220A	DEBAA03467
Injection Volume used	5.00 µl	
Time from start =0.0000 min	Column Oven	Agilent 1290 G1316C
Firmware Version	A.06.53	
Serial Number	DEBAC06281	
Switching Valve	CSV	SN# 0030969185
Time from start =0.0000 min	AutoSampler	Agilent 1290 G4226A
Start of Run - Temperature		
Tray Temperature	6.00 C	
Time from start =0.0000 min	Mass Spectrometer	API 5000
Config Table Version	01	
Firmware Version	M401402 B4T0301 M3L1417 B3T0300	
Component Name	Triple Quadrupole LC/MS/MS Mass Spectrometer	
Component ID	API 5000	
Manufacturer	AB Sciex Instruments	
Model	API 5000	
Serial Number	AG13130610	

Time from start =0.0000 min	Mass Spectrometer	API 5000	0
Start of Run - Detailed Status			
Vacuum Status		At Pressure	
Vacuum Gauge (10e-5 Torr)		1.8	
Backing Pump		Ok	
Interface Turbo Pump		Normal	
Analyzer Turbo Pump		Normal	
Sample Introduction Status		Ready	
Source/Ion Path Electronics		On	
Source Type		Turbo Spray	
Source Temperature (at setpoint)		600.0 C	
Source Exhaust Pump		Ok	
Interface Heater		Ready	

Acquisition Info

Acquisition Method:	\8x10222014W4189andMetsC18Neg.dam
Acquisition Path:	D:\Analyst Data\Projects\DPX-W4189andMetsInSoil05_13_2014\2014_05_13\Acquisition Methods\
First Sample Started:	Friday, October 24, 2014 8:59:52 PM
Last Sample Finished:	Saturday, October 25, 2014 4:52:11 AM
Sample Acq Time:	Friday, October 24, 2014 11:03:02 PM
Sample Acq Duration:	20min0sec
Number of Scans:	0
Periods in File:	1
Batch Name:	\10242014W4189andMetsInHildagoSoilVal2SaxNegC18.dab
Batch Path:	D:\Analyst Data\Projects\DPX-W4189andMetsInSoil05_13_2014\2014_05_13\Batch\
Logged-on User:	S3151244LCMS1@dupontnet.net
Synchronization Mode:	LC Sync
Auto-Equilibration:	Off
Software Version:	Analyst 1.5.2
Set Name:	10242014W4189andMetsInHildagoSoilVal2SaxNegC18
Sample Name	LOQ 1 Soil
Autosampler Vial:	12
Rack Code:	10 By 10
Rack Position:	1

Agilent LC Pump Method Properties

Pump Model:	Agilent 1290 Binary Pump
Minimum Pressure (psi):	0.0
Maximum Pressure (psi):	17404.0
Dead Volume (µl):	40.0
Stroke Volume (µl):	-1.0
Maximum Flow Ramp (ml/min²):	100.0
Maximum Pressure Ramp (psi/sec):	290.0
Max Flow Ramp Up (ml/min²):	100.0
Max Flow Ramp Dn (ml/min²):	100.0

Step Table:

Step	Total Time(min)	Flow Rate(µl/min)	A (%)	B (%)
0	0.00	400	90.0	10.0
1	1.00	400	90.0	10.0
2	2.00	400	70.0	30.0
3	14.00	400	1.0	99.0
4	16.00	400	1.0	99.0
5	16.10	400	90.0	10.0
6	20.00	400	90.0	10.0

Left Stroke Volume (µl):	-1.0
Right Stroke Volume (µl):	-1.0
Left Solvent:	A1
Right Solvent:	B1

Agilent Autosampler Properties

Autosampler Model:	Agilent 1290 Infinity Autosampler
Syringe Size (µl):	20
Injection Volume (µl):	5.00
Draw Speed (µl/min):	100.0
Eject Speed (µl/min):	200.0
Needle Level (mm):	0.00
Temperature Control	Enabled
Setpoint (4 - 40 C):	6

Wash is not used

Automatic Delay Volume Reduction	Not Used
Equilibration Time (sec):	2

Enable Vial/Well Bottom Sensing	No
Use Custom Injector Program	Yes

Contents of Custom Injector Program

1: DRAW def. amount from sample	def. speed	def. offset
2: INJECT		
3: WAIT 0.10 min.		
4: CONTACT A CLOSED		
5: WAIT 0.10 min.		
6: CONTACT A OPEN		
7: WAIT 13.90 min.		
8: CONTACT B CLOSED		
9: WAIT 0.10 min.		
10: CONTACT B OPEN		

Agilent Column Oven Properties

Left Temperature (°C):	40.00
Right Temperature (°C):	40.00
Temperature Tolerance +/- (°C):	1.00
Start Acquisition Tolerance +/- (°C):	0.50
Time Table	(Not Used)
Column Switching Valve Installed CSV	SN#: 0030969185
Position for first sample in the batch:	Left
Use same position for all samples in the batch	

Period 1:

Scans in Period:	452
Relative Start Time:	0.00 msec
Experiments in Period:	1

Period 1 Experiment 1:

Scan Type:	MRM (MRM)
Scheduled MRM:	No
Polarity:	Negative
Scan Mode:	N/A
Ion Source:	Turbo Spray
Resolution Q1:	Unit
Resolution Q3:	Unit
Intensity Thres.:	0.00 cps

Settling Time:	0.0000 msec
MR Pause:	5.0070 msec
MCA:	No
Step Size:	0.00 Da

Q1 Mass (Da)	Q3 Mass (Da)	Dwell(msec)	Param	Start	Stop	ID
189.973	77.980	150.00	DP	-20.00	-20.00	IN-A4097
			CE	-36.00	-36.00	
			CXP	-9.00	-9.00	
Q1 Mass (Da)	Q3 Mass (Da)	Dwell(msec)	Param	Start	Stop	ID
189.973	125.878	150.00	DP	-20.00	-20.00	IN-A4097
			CE	-22.00	-22.00	
			CXP	-11.00	-11.00	
Q1 Mass (Da)	Q3 Mass (Da)	Dwell(msec)	Param	Start	Stop	ID
233.007	78.008	150.00	DP	-65.00	-65.00	IN-D5293
			CE	-44.00	-44.00	
			CXP	-9.00	-9.00	
Q1 Mass (Da)	Q3 Mass (Da)	Dwell(msec)	Param	Start	Stop	ID
233.007	125.929	150.00	DP	-65.00	-65.00	IN-D5293
			CE	-30.00	-30.00	
			CXP	-11.00	-11.00	
Q1 Mass (Da)	Q3 Mass (Da)	Dwell(msec)	Param	Start	Stop	ID
318.025	274.994	150.00	DP	-45.00	-45.00	IN-JJ998
			CE	-16.00	-16.00	
			CXP	-13.00	-13.00	
Q1 Mass (Da)	Q3 Mass (Da)	Dwell(msec)	Param	Start	Stop	ID
318.025	189.920	150.00	DP	-45.00	-45.00	IN-JJ998
			CE	-30.00	-30.00	
			CXP	-19.00	-19.00	
Q1 Mass (Da)	Q3 Mass (Da)	Dwell(msec)	Param	Start	Stop	ID
342.046	125.054	150.00	DP	-55.00	-55.00	IN-M6957
			CE	-24.00	-24.00	

			CXP	-11.00	-11.00	
Q1 Mass (Da)	Q3 Mass (Da)	Dwell(msec)	Param	Start	Stop	ID
342.046	82.005	150.00	DP	-55.00	-55.00	IN-M6957
			CE	-52.00	-52.00	
			CXP	-15.00	-15.00	
Q1 Mass (Da)	Q3 Mass (Da)	Dwell(msec)	Param	Start	Stop	ID
319.000	189.857	150.00	DP	-35.00	-35.00	IN-UND13
			CE	-22.00	-22.00	
			CXP	-19.00	-19.00	
Q1 Mass (Da)	Q3 Mass (Da)	Dwell(msec)	Param	Start	Stop	ID
319.000	232.779	150.00	DP	-35.00	-35.00	IN-UND13
			CE	-18.00	-18.00	
			CXP	-23.00	-23.00	
Q1 Mass (Da)	Q3 Mass (Da)	Dwell(msec)	Param	Start	Stop	ID
356.032	139.005	150.00	DP	-50.00	-50.00	DPX-W4189
			CE	-24.00	-24.00	
			CXP	-11.00	-11.00	
Q1 Mass (Da)	Q3 Mass (Da)	Dwell(msec)	Param	Start	Stop	ID
356.032	107.040	150.00	DP	-50.00	-50.00	DPX-W4189
			CE	-60.00	-60.00	
			CXP	-9.00	-9.00	

Parameter Table (Period 1 Experiment 1)

CAD:	4.00
CUR:	30.00
GS1:	40.00
GS2:	50.00
IS:	-4500.00
TEM:	600.00
ihe:	ON
EP	-10.00

Resolution tables

Quad 1	Negative	Unit

Last Modification Date Time: June 09, 2014 10:18:53

Instrument Parameters:
Detector Parameters (Negative):

CEM	2200.0
DF	400.0

Keyed Text:
File was created with the software version: Analyst 1.5.2

Positive Ion Conditions

File Information for Sample 7 (LOQ 1 Soil) of 10242014W4189andMetsInHildagoSoilVal2SaxPosC18.wiff

File Name:	10242014W4189andMetsInHildagoSoilVal2SaxPosC18.wiff
File Path:	D:\Analyst Data\Projects\DPX-W4189andMetsInSoil05_13_2014\2014_05_13\Data\
Original Name:	10242014W4189andMetsInHildagoSoilVal2SaxPosC18.wiff
Software Version:	Analyst 1.5.2

Log Information from Devices at Start of acquisition:

Column Oven	Agilent 1290 G1316C	
Firmware Version	A.06.53	
Serial Number	DEBAC06281	
Switching Valve	CSV	SN# 0030969185
Time from start =0.0000 min	AutoSampler	Agilent 1290 G4226A
Firmware Version	A.06.50	
Serial Number	DEBAP04093	
Linked Pump	G4220A	DEBAA03467
Injection Volume used	2.00 µl	
Time from start =0.0000 min	Pump	Agilent 1290 G4220A
Firmware Version	B.06.53	
Serial Number	DEBAA03467	
Time from start =0.0000 min	AutoSampler	Agilent 1290 G4226A
Start of Run - Temperature		
Tray Temperature	6.01 C	
Time from start =0.0000 min	Mass Spectrometer	API 5000
Config Table Version	01	

Firmware Version	M401402 B4T0301 M3L1417 B3T0300
Component Name	Triple Quadrupole LC/MS/MS Mass Spectrometer
Component ID	API 5000
Manufacturer	AB Sciex Instruments
Model	API 5000
Serial Number	AG13130610

Time from start =0.0000 min	Mass Spectrometer	API 5000
Start of Run -		Detailed Status
Vacuum Status		At Pressure
Vacuum Gauge (10e-5 Torr)		1.8
Backing Pump		Ok
Interface Turbo Pump		Normal
Analyzer Turbo Pump		Normal
Sample Introduction Status		Ready
Source/Ion Path Electronics		On
Source Type		Turbo Spray
Source Temperature (at setpoint)		600.0 C
Source Exhaust Pump		Ok
Interface Heater		Ready

Acquisition Info

Acquisition Method:	\8x10222014W4189andMetsC18Pos.dam
Acquisition Path:	D:\Analyst Data\Projects\DPX-W4189andMetsInSoil05_13_2014\2014_05_13\Acquisition Methods\
First Sample Started:	Saturday, October 25, 2014 5:12:44 AM
Last Sample Finished:	Saturday, October 25, 2014 1:04:03 PM
Sample Acq Time:	Saturday, October 25, 2014 7:15:40 AM
Sample Acq Duration:	20min0sec
Number of Scans:	0
Periods in File:	1
Batch Name:	\10242014W4189andMetsInHildagoSoilVal2SaxPosC18.dab
Batch Path:	D:\Analyst Data\Projects\DPX-W4189andMetsInSoil05_13_2014\2014_05_13\Batch\
Logged-on User:	S3151244LCMS1@dupontnet.net
Synchronization Mode:	LC Sync
Auto-Equilibration:	Off
Comment:	
Software Version:	Analyst 1.5.2
Set Name:	10242014W4189andMetsInHildagoSoilVal2SaxPosC18
Sample Name	LOQ 1 Soil

Autosampler Vial:	12
Rack Code:	10 By 10
Rack Position:	1
Plate Code:	N/A
Plate Position	0

Agilent LC Pump Method Properties

Pump Model:	Agilent 1290 Binary Pump
Minimum Pressure (psi):	0.0
Maximum Pressure (psi):	17404.0
Dead Volume (µl):	40.0
Stroke Volume (µl):	-1.0
Maximum Flow Ramp (ml/min²):	100.0
Maximum Pressure Ramp (psi/sec):	290.0
Max Flow Ramp Up (ml/min²):	100.0
Max Flow Ramp Dn (ml/min²):	100.0

Step Table:

Step	Total Time(min)	Flow Rate(µl/min)	A (%)	B (%)
0	0.00	400	90.0	10.0
1	1.00	400	90.0	10.0
2	14.00	400	1.0	99.0
3	16.00	400	1.0	99.0
4	16.10	400	90.0	10.0
5	20.00	400	90.0	10.0

Left Stroke Volume (µl):	-1.0
Right Stroke Volume (µl):	-1.0
Left Solvent:	A1
Right Solvent:	B1

Agilent Autosampler Properties

Autosampler Model:	Agilent 1290 Infinity Autosampler
Syringe Size (µl):	20
Injection Volume (µl):	2.00
Draw Speed (µl/min):	100.0
Eject Speed (µl/min):	200.0
Needle Level (mm):	0.00
Temperature Control	Enabled

Setpoint (4 - 40 C): 6
Wash is not used

Automatic Delay Volume Reduction Not Used
Equilibration Time (sec): 2
Enable Vial/Well Bottom Sensing No
Use Custom Injector Program Yes

Contents of Custom Injector Program

1: DRAW def. amount from sample	def. speed	def. offset
2: INJECT		
3: WAIT 0.10 min.		
4: CONTACT A CLOSED		
5: WAIT 0.10 min.		
6: CONTACT A OPEN		
7: WAIT 14.00 min.		
8: CONTACT B CLOSED		
9: WAIT 0.10 min.		
10: CONTACT B OPEN		

Agilent Column Oven Properties

Left Temperature (°C):	40.00
Right Temperature (°C):	40.00
Temperature Tolerance +/- (°C):	1.00
Start Acquisition Tolerance +/- (°C):	0.50
Time Table	(Not Used)
Column Switching Valve Installed CSV	SN#: 0030969185
Position for first sample in the batch:	Left

Use same position for all samples in the batch

Period 1:
Scans in Period: 1343
Relative Start Time: 0.00 msec
Experiments in Period: 1

Period 1 Experiment 1:
Scan Type: MRM (MRM)
Scheduled MRM: No
Polarity: Positive

Scan Mode:	N/A
Ion Source:	Turbo Spray
Resolution Q1:	Unit
Resolution Q3:	Unit
Intensity Thres.:	0.00 cps
Settling Time:	700.0000 msec
MR Pause:	5.0070 msec
MCA:	No
Step Size:	0.00 Da

Q1 Mass (Da)	Q3 Mass (Da)	Dwell(msec)	Param	Start	Stop	ID
141.029	57.060	150.00	DP	86.00	86.00	IN-A4098
			CE	27.00	27.00	
			CXP	8.00	8.00	

Q1 Mass (Da)	Q3 Mass (Da)	Dwell(msec)	Param	Start	Stop	ID
141.029	58.030	150.00	DP	86.00	86.00	IN-A4098
			CE	35.00	35.00	
			CXP	10.00	10.00	

Q1 Mass (Da)	Q3 Mass (Da)	Dwell(msec)	Param	Start	Stop	ID
358.115	141.106	150.00	DP	66.00	66.00	DPX-W4189
			CE	29.00	29.00	
			CXP	24.00	24.00	

Q1 Mass (Da)	Q3 Mass (Da)	Dwell(msec)	Param	Start	Stop	ID
358.115	167.067	150.00	DP	66.00	66.00	DPX-W4189
			CE	25.00	25.00	
			CXP	26.00	26.00	

Parameter Table (Period 1 Experiment 1)

CAD:	4.00
CUR:	30.00
GS1:	40.00
GS2:	50.00
IS:	4500.00
TEM:	600.00
ihe:	ON
EP	10.00

Resolution tables
Quad 1 Positive Unit
Last Modification Date Time: June 09, 2014 10:09:21

Instrument Parameters:
Detector Parameters (Positive):
CEM 2500.0
DF -400.0

Keyed Text:
File was created with the software version: Analyst 1.5.2

APPENDIX 4 ALTERNATIVE CHROMATOGRAPHIC CONDITIONS

Alternative reversed-phase chromatography was used to separate chlorsulfuron and metabolites from the soil co-extracts. The column selected for the analysis was an Agilent Extend C18 (Part Number -763750-902). The pH of the water was adjusted to 9.5 by adding ammonia hydroxide (EMD AX1303-13, GR ACS) drop wise using a pH meter and stir bar.

The chromatographic condition for the analysis of IN-D5293, IN-A4097, IN-JJ998, IN-M6957 and IN-UND13.

SYSTEM:	Agilent 1290 HPLC			
COLUMN:	2.1 mm i.d. × 15 cm, 3.5 μm Agilent Extend C18			
COLUMN TEMPERATURE:	30 °C			
SAMPLE TEMPERATURE	6 °C			
INJECTION VOLUME:	0.005 mL			
FLOW RATE:	0.50 mL/min			
CONDITIONS:	A: pH=9.5 Water			
	B: Methanol			
	Time	%A	%B	Flow (mL/Min.)
	0.0	99	1	0.50
	1.3	99	1	0.50
	5.0	90	10	0.50
	12.0	1	99	0.50
	14.0	1	99	0.50
	14.1	99	1	0.50
	18.0	99	1	0.50
IN-D5293 RETENTION TIME:	1.24 minutes			
IN-M6957 RETENTION TIME:	4.06 minutes			
IN-JJ998 RETENTION TIME:	5.36 minutes			
IN-A4097 RETENTION TIME:	5.46 minutes			
IN-UND13 RETENTION TIME:	6.51 minutes			
TOTAL RUN TIME:	18.0 minutes			

The chromatographic conditions for the analysis of IN-A4098 and chlorsulfuron.

SYSTEM:	Agilent 1290 HPLC			
COLUMN:	2.1 mm i.d. × 15 cm, 3.5 µm Agilent Extend C18			
COLUMN TEMPERATURE:	30 °C			
SAMPLE TEMPERATURE	6 °C			
INJECTION VOLUME:	0.005 mL			
FLOW RATE:	0.50 mL/min			
CONDITIONS:	A: pH=9.5 Water B: Methanol			
	Time	%A	%B	Flow (mL/Min.)
	0.0	99	1	0.50
	1.3	99	1	0.50
	5.0	90	10	0.50
	12.0	1	99	0.50
	14.0	1	99	0.50
	14.1	99	1	0.50
	18.0	99	1	0.50
IN-A4098 RETENTION TIME:	4.74 minutes			
CHLORSULFURON RETENTION TIME:	7.59 minutes			
TOTAL RUN TIME:	18.0 minutes			

The soil samples analyzed using these chromatographic conditions were prepared exactly the same as the samples provided above **except** the extracts were passed through the carbon SPE cartridges without the addition of the bulk SAX material. The use of bulk SAX material was not needed when using these chromatographic conditions. The extracts were analyzed by LC/MS/MS using the conditions provided above.

These conditions can be used in the event of a co-eluting peak or if LC/MS matrix effects are observed. Do to instability of the chromatographic column when using a basic mobile phase these conditions may not be as rugged as the condition in the main report.