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

Robert M. Henze and James J. Stry

1.0 SUMMARY

The purpose of this study was to develop an analytical method for the detection, quantitative analysis, and confirmation of tribenuron methyl (DPX-L5300) and potential metabolites IN-A4098, IN-D5119, IN-00581, IN-GN815, IN-GK521, IN-B5528, IN-R9805, and IN-L5296 in soil.

Tribenuron methyl and metabolites were extracted from soil samples using a solution of 4:1 acetone/ 0.1 M ammonium carbonate. Aliquots of the extracts were removed and transferred into a clean centrifuge tubes. The aliquots were evaporated under a stream of nitrogen until the extract composition was approximately 1:1 acetone/ 0.1 M aqueous ammonium carbonate. The extracts were filtered through a carbon solid phase extraction cartridge. The extracts were than evaporated until only the aqueous phase remanded. The volume of the extracts were adjusted to 6-mL using HPLC grade water. Tribenuron methyl and metabolites were separated from co-extracts by reversed phase liquid chromatography (LC) and were detected by turbospray mass spectrometry/mass spectrometry (MS/MS). Due to the number of analytes two LC/MS/MS runs were necessary. The compounds IN-D5119, IN-00581, IN-A4098, IN-GK521, IN-GN815, and IN-R9803 were detected using one set of chromatographic conditions. The compounds IN-B5528, IN-R9805, IN-L5296 and tribenuron methyl were detected using alternative chromatographic conditions.

To compensate for LC/MS matrix effects all standards were prepared in control extracts. 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).

2.0 INTRODUCTION

The structure, CAS name, CAS registry number, and various physical properties of tribenuron methyl (DPX-L5300) and potential metabolites IN-A4098, IN-D5119, IN-00581, IN-GN815, IN-GK521, IN-B5528, IN-R9805, and IN-L5296 can be found in Appendix 1.

Tribenuron methyl and metabolites were extracted from soil samples using a solution of 4:1 acetone/ 0.1 M aqueous ammonium carbonate. The extracts were purified using a solid phase extraction cartridge. The purified extracts were diluted and analyzed using reversed phase liquid chromatography (LC) and were detected turbospray mass spectrometry/mass spectrometry (MS/MS). Due to the number of analytes two LC/MS/MS runs were necessary.

To compensate for LC/MS matrix effects all standards were prepared in control extracts. 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) based on the least responsive analyte.

Quantitative analysis is report for the two ion transition the quantitative ion transitions and a confirmatory ion transition. Confirmation criteria and examples are discussed in this report. The same extraction solvents used for the metabolism study were used for this residue method therefore an extraction efficiency study is not required.

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, HP1200 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 1.4 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)

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.

Extractor

Genogrinder : Spex SamplePrep Model number 2000

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

Solid-Phase Extraction Equipment

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

Supelclean™ Envi™-Carb cartridge, 0.25g/ 6-mL, Lot SP1759D, PN 57092 (Bellefonte, PA). **Do not substitute.**

Chromatographic Supplies

HPLC Column: 150 × 3.0 mm ID, 3.0-µm Mac Mod ACE 3 C18-AR, analytical column Part # ACE-119-1503, Serial #A80610 (Chads Ford, PA)

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 with stopper, Pyrex 15-mL capacity, Cat. No. 21048-027 (VWR Scientific Co., Bridgeport, NJ)

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

Miscellaneous

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

Centrifuge – Sorvall Instruments GLC-4 General Lab Centrifuge (VWR Scientific Co., Bridgeport, NJ)

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)

Acetone - EM Omni Solv®, HPLC-grade acetone, #AX0116-1 (EM Science, Gibbstown, NJ)

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

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)

Tribenuron Methyl – DPX-L5300-226, Purity 98.4%, prepared by DuPont Crop Protection, Global Technology Division, E. I. du Pont de Nemours and Company

IN-D5119-001, Purity 99.5%, prepared by DuPont Crop Protection, Global Technology Division, E. I. du Pont de Nemours and Company

IN-00581-005, Purity 99.9%, prepared by DuPont Crop Protection, Global Technology Division, E. I. du Pont de Nemours and Company

IN-A4098-005, Purity 98.7%, prepared by DuPont Crop Protection, Global Technology Division, E. I. du Pont de Nemours and Company

IN-GK521-000, Purity 69.3%, prepared by DuPont Crop Protection, Global Technology Division, E. I. du Pont de Nemours and Company

IN-GN815-001, Purity 58%, prepared by DuPont Crop Protection, Global Technology Division, E. I. du Pont de Nemours and Company

IN-B5528-005, Purity 99.2%, prepared by DuPont Crop Protection, Global Technology Division, E. I. du Pont de Nemours and Company

IN-R9805-003, Purity 100%, prepared by DuPont Crop Protection, Global Technology Division, E. I. du Pont de Nemours and Company

IN-L5296-006, Purity 99.6%, prepared by 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

Tribenuron methyl and metabolites were extracted from soil samples using a solution of 4:1 acetone/ 0.1 M aqueous ammonium carbonate. An aliquot of the extracts were purified using solid phase extraction and analyzed using HPLC/MS/MS analysis. Two injections of the extracts was required due to the number of analytes in the method. To compensate for LC/MS matrix effects all standards were prepared in control extracts.

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 aqueous ammonium carbonate. 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. Adjust the pH to 7.5 using concentrated acetic acid.

Extraction Solution: 4:1 acetone: 0.1 M aqueous ammonium carbonate - Combine 800-mL of acetone and 200-mL of 0.1 M aqueous ammonium carbonate. Mix the resulting solution to homogeneity.

1:1 acetone: 0.1 M aqueous ammonium carbonate - Combine 500-mL of acetone and 500-mL of 0.1 M aqueous ammonium carbonate. Mix the resulting solution to homogeneity. Prepare this solution daily, it may turn yellow if stored for an extended period.

Mobile Phase A: 0.01 M aqueous formic acid solution - Add 460 µL of formic acid to 1000 mL of water and mix the resulting solution to homogeneity.

4.2.3 *Preparation and Stability of Stock Standard*

Use Class A volumetric flasks when preparing standard solutions.

Tribenuron methyl, IN-00581, IN-GK521, and IN-L5296

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 standard in approximately 50 mL of HPLC-grade acetonitrile. Sonicate the standards for approximately 10 minutes to aid in dissolving the solid material. 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.

IN-A4098, IN-D5119 and IN-R9805

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 standard in approximately 25 mL of HPLC-grade methanol. Sonicate the standards for approximately 10 minutes to aid in dissolving the solid material. 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.

IN-B5528

Prepare standard stock solutions by accurately weighing 10 ± 0.01 mg of IN-B5528 into a 100-mL volumetric flask using an analytical balance. Record the accurate weight of the standard. Dissolve the standard in approximately 50 mL of DMSO. Sonicate the standard for approximately 10 minutes to aid in dissolving the solid material. After dissolving, bring the solution to a volume of 100 mL using DMSO and invert the volumetric flask to mix the solution to homogeneity. The standard solution is stable for approximately 3 months when stored in a refrigerator at approximately 4°C immediately after each use. The concentration of IN-B5528 in solution is 100 µg/mL.

IN-GN815

Prepare standard stock solutions by accurately weighing 10 ± 0.01 mg of IN-GN815 into a 100-mL volumetric flask using an analytical balance. Record the accurate weight of the standard. Dissolve the standard in approximately 50 mL of HPLC-grade water. After dissolving, bring the solution to a volume of 100 mL using HPLC-grade water and invert the volumetric flask to mix the solution to homogeneity. The standard solution is stable for approximately 3 months when stored in a refrigerator at approximately 4°C immediately after each use. The concentration of IN-GN815 in solution is 100 µg/mL.

Standards less than 95% pure will need to be corrected for purity.

4.2.4 *Preparation and Stability of Intermediate and Fortification Standards*

Use Class A volumetric flasks when preparing standard solutions.

Multi-analyte (Tribenuron methyl, IN-A4098, IN-00581, IN-D5119, IN-GK521, IN-B5528, IN-R9805, IN-L5296 and IN-GN815

Prepare a 10.0-µg/mL Tribenuron methyl, IN-A4098, IN-00581, IN-D5119, IN-GK521, IN-B5528, IN-R9805, IN-L5296 and IN-GN815 intermediate standard in acetonitrile by pipetting 1.00 mL of each 100.0-µg/mL stock standard into a 10-mL volumetric flask. Bring to volume using HPLC-grade water and mix to homogeneity. This solution is to be prepared weekly.

Prepare a 0.10-µg/mL Tribenuron methyl, IN-A4098, IN-00581, IN-D5119, IN-GK521, IN-B5528, IN-R9805, IN-L5296 and IN-GN815 fortification standard in HPLC grade water by pipetting 0.200 mL of the 10.0-µg/mL standard into a 20-mL volumetric flask. Bring to volume using HPLC-grade water and mix to homogeneity. This solution is to be prepared daily.

Alternate or additional solutions may be prepared as needed. **Standards less than 95% pure will need to be corrected for purity.**

4.2.5 *Preparation and Stability of Calibration Standards*

All chromatographic standards were prepared in control extract. Prepare the calibration standards as showed in the table below. (Alternative or additional standards may be prepared as needed):

Standard Concentration (ng/mL)	Standard Used	Volume of Standard Added (µL)	Volume of Control Extract (µL)
10.0	0.1 µg/mL	100	900
7.0	0.1 µg/mL	70	930
5.0	0.1 µg/mL	50	950
2.0	0.1 µg/mL	20	980
1.0	10.0 ng/mL	100	900
0.70	7.0 ng/mL	100	900
0.50	5.0 ng/mL	100	900
0.20	2.0 ng/mL	100	900

The 0.1 µg/mL multi-analyte intermediate standards was used to prepare the 10.0, 7.0, 5.0 and 2.0 standards. 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 filling the auto-sampler vials.

4.2.6 *Source of Samples*

Soil control samples were obtained from a field test site located in Lleida, Spain and Speyer from Germany. The soil characteristics are shown in the following table:

Soil Name	Country	Type	% Clay	% Sand	% Silt	% OM	pH_w	Notebook
Lleida	Spain	Silty Clay Loam	32	14	54	3.9	8.0	2004-032A
Speyer	Germany	Loamy Sand	8	84	8	2.6	6.2	2005-029

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.

4.2.8 *Sample Fortification Procedure*

All fortifications were made directly to the 5.0-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	0.050
10.0	0.500

4.2.9 *Analyte Extraction and Purification Procedures*

1. Accurately measure 5.0-g (± 1%) of soil into a 50-mL plastic centrifuge tubes. Fortify samples if necessary and add two 3/8" steel balls. Allow the fortification to dry in a fume hood for approximately 15-minutes. Cap and shake the samples vigorously.
2. Add 15-mL of 4:1 acetone/ 0.1 M aqueous ammonium carbonate to each sample.
3. Place samples on a GenoGrinder shaker set to 1100 strokes per minute. Shake the samples for 3 minutes.
4. Centrifuge the samples for 10 minutes to drive the particulates to the bottom of the bottle at a rate of approximately 3000 RPM. Transfer the supernatants into a clean 50-mL centrifuge tubes.
5. Repeat steps 2-4 combining the extracts.
6. Repeat steps 2-4 a third time combining the extracts. Adjust the volume of the extracts from each sample to 50-mL using the 4:1 acetone/ 0.1 M aqueous ammonium carbonate solution. Mix the extract using a vortex mixer for approximately 30 seconds.
7. Pipette 20-mL of each extract into a clean 50-mL centrifuge tube. Evaporate the extracts to approximately 5 to 7-mL using a flow of nitrogen in an N-Evap at 25-30°C.
8. Attach a 6-mL, 0.25-g Envi™-Carb cartridge to an SPE manifold. Condition the cartridges with 5-mL of methanol followed by 10-mL of 1:1 acetone: 0.1 M aqueous ammonium carbonate. **Do not let the cartridge go to dryness.**
9. Place a 14-mL glass centrifuge tube under the cartridge and load the extract into the SPE. Using gravity flow, allow the extract to pass through the cartridge at a flow rate of 2-5 mL/min. Rinse the centrifuge with 3-mL of 1:1 acetone: 0.1 M aqueous ammonium carbonate and load the rinse into the reservoir just before all of the extract passes through. Repeat the rinse with an additional 3-mL of 1:1 acetone: 0.1 M aqueous ammonium carbonate. Once the rinse completely passes through the cartridge use vacuum to draw the remaining solution into the collection tube.
10. Remove the 14-mL centrifuge tube and evaporate the extract to approximately 5 to 6-mL using a flow of nitrogen in an N-Evap at 25-30°C. Dilute the extract to 6-mL with HPLC grade water. Vortex for 30-seconds and transfer an aliquot of the extract using a disposable pipette into an HPLC vial. Analyze the solution by LC/MS/MS as described in the following section.

Extracts will be stable for approximately 24 hours if stored at 4°C.

4.3 Instrumentation for the Method

4.3.1 Chromatography

Reversed-phase chromatography was used to separate tribenuron methyl and metabolites from co-extracts. 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.

Conditions used for the analysis of IN-A4098, IN-00581, IN-D5119, IN-GN815, and IN-GK521

SYSTEM:	Agilent 1200 HPLC			
COLUMN:	150 × 3.0 mm ID, 3 µm ACE 3 Mac Mod C18-AR			
COLUMN TEMPERATURE:	40°C			
SAMPLE TEMPERATURE	10°C			
INJECTION VOLUME:	0.010 mL			
FLOW RATE:	0.40 mL/min			
CONDITIONS:	A: 0.01 M aqueous Formic Acid			
	B: Methanol			
	Time	%A	%B	Flow (mL/Min.)
	0.0	98	2	0.50
	1.0	98	2	0.50
	4.0	35	65	0.50
	11.0	1	99	0.50
	13.0	1	99	0.50
	14.0	98	2	0.50
	20.0	98	2	0.50
ANALYTE:	Approximate Retention time:			
IN-A4098 RETENTION TIME:	6.38 minutes			
IN-00581 RETENTION TIME:	6.87 minutes			
IN-D5119 RETENTION TIME:	6.99 minutes			
IN-GN815 RETENTION TIME:	8.59 minutes			
IN-GK521 RETENTION TIME:	9.20 minutes			
TOTAL RUN TIME:	20.5 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
0.00-3.0	Waste
3.0-13.0	MS source
13.0-End	Waste

Conditions used for the analysis of IN-B5528, IN-R9805, IN-L5296 and tribenuron methyl:

SYSTEM:	Agilent 1200 HPLC			
COLUMN:	150 x 3.0 mm ID, 3 µm ACE 3 Mac Mod C18-AR			
COLUMN TEMPERATURE:	40°C			
SAMPLE TEMPERATURE	10°C			
INJECTION VOLUME:	0.010 mL			
FLOW RATE:	0.50 mL/min			
CONDITIONS:	A: 0.01 M aqueous Formic Acid			
	B: Methanol			
	Time	%A	%B	Flow (mL/Min.)
	0.0	99	1	0.50
	4.0	99	1	0.50
	5.0	1	99	0.50
	6.0	1	99	0.50
	7.0	99	1	0.50
	14.0	99	1	0.50
ANALYTE:	Approximate Retention time:			
IN-B5528 RETENTION TIME:	2.0 minutes			
IN-R9805 RETENTION TIME:	3.3 minutes			
IN-L5296 RETENTION TIME:	8.8 minutes			
DPX-L5300 RETENTION TIME:	10.0 minutes			
TOTAL RUN TIME:	14.0 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
0.00-1.0	Waste
1.0-11.0	MS source
11.0-End	Waste

4.3.2 *LC/MS/MS Analysis*

The quantitative analysis of tribenuron methyl and metabolites was performed using a API 5000 LC/MS/MS system. The system parameters were adjusted while a solution of each analyte was infused directly into the TSI 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:

Conditions used for the analysis of IN-A4098, IN-D5119, IN-00581, IN-GN815 and IN-GK521:

ANALYTES	IONS MONITORED	DECLUSTERING POTENTIAL (DP)	COLLISION ENERGY (CE)	EXIT POTENTIAL (CXP)
Period 1				
IN-A4098	141.1→ 57.0 AMU	60	27	28
	141.1→ 99.9 AMU	60	27	22
Period 2				
IN-00581	181.9→ 41.8 AMU	-60	-30	-11
	181.9→ 105.8 AMU	-60	-26	-9
IN-D5119	200.1→ 91.9 AMU	-60	-28	-7
	200.1→ 156.1 AMU	-60	-16	-9
Period 3				
IN-GN815	366.1→ 181.9 AMU	-70	-16	-13
	366.1→ 42.0 AMU	-70	-52	-7
IN-GK521	380.1→ 138.9 AMU	-5	-22	-22
	380.1→ 54.9 AMU	-5	-60	-11
Time:	0-20.4 minutes			
Ion Mode:	Period 1: Positive Period 2: Negative Period 3: Negative			
Turbospray Voltage:	-4500 V / +4500 V			
Source Temperatures:	600 C			
CUR:	30			
CAD:	4			
GS1:	40			
GS2:	50			
Dwell	0.15 Seconds			

Conditions used for the analysis of IN-B5528, IN-R9805, IN-L5296 and tribenuron methyl:

PERIOD 1 ANALYTES	IONS MONITORED	DECLUSTERING POTENTIAL (DP)	COLLISION ENERGY (CE)	EXIT POTENTIAL (CXP)
IN-B5528	127.0→ 86.0 AMU	66	21	12
	127.0→ 42.0 AMU	66	53	18
IN-R9805	141.1→ 82.9 AMU	116	23	30
	141.1→ 100.0 AMU	116	21	20
IN-L5296	155.0→ 57.1 AMU	46	35	10
	155.0→ 71.1 AMU	46	31	10
Tribenuron methyl	396.1→ 155.1 AMU	11	21	10
	396.1→ 181.0 AMU	11	27	14
Time:	0-14 minutes			
Ion Mode:	Positive			
Turbospray Voltage:	4500 V			
Source Temperatures:	600 C			
CUR:	30			
CAD:	4			
GS1:	40			
GS2:	50			
Dwell	0.15 Seconds			

A complete list of the experimental parameters is given in Appendix 2. Typical LC/MS and LC/MS/MS full scan spectra 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. Recovery data was calculated form both ion transitions.

4.3.3 *Calibration Procedure and Sample Analysis*

A 0.20-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*

4.4.1 *Methods*

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

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

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:

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

4.4.2 *Example*

For a soil sample fortified with tribenuron methyl at 1.0 ppb [Date analyzed 14-Sept-12, LOQ1 Fortification], the concentration found was calculated as follows:

Average Response Factor was calculated as follows:

$$RF_{Ave} = \frac{\begin{array}{c}(0.20\ \text{ng/mL} \div 142000) + (0.50\ \text{ng/mL} \div 309000) + (0.70\ \text{ng/mL} \div 421000) \\ + (1.0\ \text{ng/mL} \div 645000) + (2.0\ \text{ng/mL} \div 1280000) + (5.0\ \text{ng/mL} \div 3090000)\end{array}}{6}$$

(AC ≡ Area Counts)

$RF_{Avg} = 1.57005e^{-6}$ ng/mL/AC

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

$$\text{ng/g Found} = \frac{(183000\ \text{AC}) \times (1.57003\text{e-}6\ \text{ng/mL/AC}) \times (6.0\ \text{mL}) \times (2.5)}{(5\ \text{grams})}$$

ng/g Found = 0.862

(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:

$$\% \text{ Recovery} = \frac{(0.862 \text{ ng/g})}{(1.00 \text{ ng/g})} \text{ x } 100$$

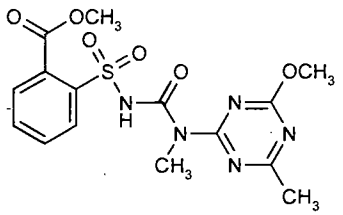
% Recovery = 86.2%

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

APPENDIX 1 STRUCTURE AND PROPERTIES OF TRIBENURON METHYL AND METABOLITES

DPX-L5300 **CAS name:** methyl 2-[[[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl) methylamino]carbonyl]amino]sulfonyl]benzoate

Trivial name: Tribenuron methyl

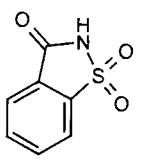


CAS number: 101200-48-0 **Molecular Weight:** 395.40

Structural formula: $C_{15}H_{17}N_5O_6S$

IN-00581 **CAS name:** 1,2-benzisothiazol-3(2H)-one, 1,1-dioxide

Trivial name: Saccharin

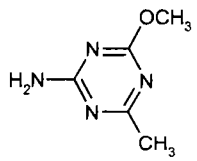


CAS number: 81-07-2 **Molecular Weight:** 183.19

Structural formula: $C_7H_5NO_3S$ **Observed in:** Soil, hydrolysis, water/sediment, wheat, goat and rat

IN-A4098 **CAS name:** 4-Methoxy-6-methyl-1,3,5-triazin-2-amine

Trivial name: N-demethyl-triazine amine

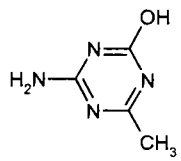


CAS number: 1668-54-8 **Molecular Weight:** 140.14

Structural formula: $C_5H_8N_4O$ **Observed in:** Soil, wheat, rotational crops, goat and rat

IN-B5528 **CAS name:** 4-amino-6-methyl-1,3,5-triazin-2-ol

Trivial name: O-demethyl, N-demethyl triazine amine

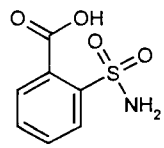


CAS number: Not available **Molecular Weight:** 126.12

Structural formula: $C_4H_6N_4O$ **Observed in:** Wheat, goat

IN-D5119 **CAS name:** 2-(Aminosulfonyl)benzoic acid

Trivial name: Acid sulfonamide

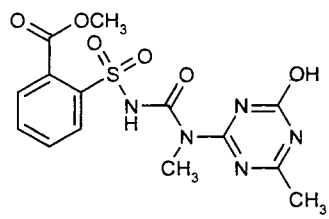


CAS number: 632-24-6 **Molecular Weight:** 201.20

Structural formula: $C_7H_7NO_4S$ **Observed in:** Soil, hydrolysis, water/sediment, wheat, goat and rat

IN-GK521 **CAS name:** methyl 2-[[[[(4-hydroxy-6-methyl-1,3,5-triazin-2-yl) methylamino]carbonyl]amino]sulfonyl]benzoate

Trivial names: O-demethyl Tribenuron methyl, ODM-Tribenuron methyl

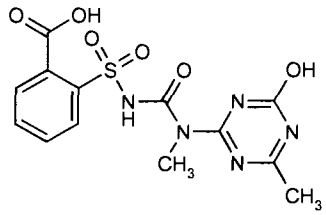


CAS number: Not available **Molecular Weight:** 381.37

Structural formula: $C_{14}H_{15}N_5O_6S$ **Observed in:** Water/sediment

IN-GN815 **CAS name:** 2-[[[[(4-hydroxy-6-methyl-1,3,5-triazin-2-yl)amino]methyl amino]carbonyl]amino]sulfonyl]benzoic acid

Trivial names: *O*-Demethyl-Tribenuron Free Acid, ODM-Tribenuron methyl-FA

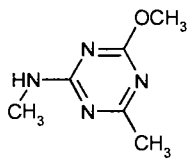


CAS number: Not available **Molecular Weight:** 367.34

Structural formula: $C_8H_9NO_4S$ **Observed in:** Water/sediment

IN-L5296 **CAS name:** 4-methoxy-N,6-dimethyl-1,3,5-triazin-2-amine

Trivial name: Triazine Amine

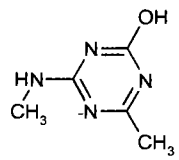


CAS number: 5248-39-5 **Molecular Weight:** 154.17

Structural formula: $C_6H_{10}N_4O$ **Observed in:** Wheat, goat, rat, hydrolysis, soil, water/sediment

IN-R9805 **CAS name:** 4-methyl-6-(methylamino)-1,3,5-triazin-2-ol

Trivial name: *O*-Demethyl-triazine amine



CAS number: Not available **Molecular Weight:** 140.15

Structural formula: $C_5H_8N_4O$ **Observed in:** Rat, wheat, soil, water/sediment, hydrolysis