US EPA ARCHIVE DOCUMENT

B. Analytical Methodology for the Determination of Sulfentrazone, 3-Desmethyl Sulfentrazone, and 3-Hydroxymethyl Sulfentrazone in/on Various Matrices

TITLE:	Analytical Methodology for the Determination of Sulfentrazone, 3-Desmethyl Sulfentrazone, and 3-Hydroxymethyl Sulfentrazone in/on Various Matrices
TEST AND REFERENCE SUBSTANCES:	Sulfentrazone, Sulfentrazone-3-Carboxylic Acid, 3-Desmethyl Sulfentrazone and 3-Hydroxymethyl Sulfentrazone
DATA REQUIREMENT:	Pesticide Assessment Guidelines, Subdivision O, 171-4: Residue Analytical Method
AUTHOR:	Natalie A. Shevchuk
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PERFORMING LABORATORY:	FMC Corporation Agricultural Products Group Residue Chemistry P.O. Box 8 Princeton, NJ 08543 (609) 951-3000
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Non Propriety Information

FMC Corporation Authorizes the Release or Use of This Method by Federal and State Agencies

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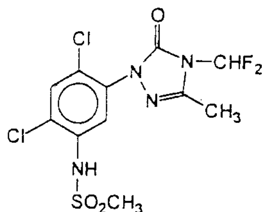
I. INTRODUCTION

Authority® is a soil applied herbicide currently under development by FMC Corporation for use on broadleaf and grass weed species. The common name of the active ingredient in Authority is sulfentrazone and the chemical name of sulfentrazone is N-[2,4-dichloro-5-[4-(difluoromethyl)-4,5-dihydro-3-methyl-5-oxo-1H-1,2,4- triazol-1-yl]phenyl] methanesulfonamide. The major plant metabolites of sulfentrazone were identified as sulfentrazone-3-carboxylic acid and 3-hydroxymethyl sulfentrazone. The structure of sulfentrazone is as follows:



Sulfentrazone

The previously reported residue analytical method (Section XI, Reference 1) for sulfentrazone and its major plant metabolites included a single hydrolysis step and analysis by a gas chromatograph (GC) equipped an electron capture detector (ECD). During a radiovalidation study (Section XI, Reference 2), it became apparent that the method did not account for all of the conjugated 3-hydroxymethyl sulfentrazone (HMS) and that the sulfentrazone-3-carboxylic acid metabolite (SCA) was not completely converted to 3-desmethyl sulfentrazone (DMS). Consequently, the previous method was modified to include a more stringent hydrolysis step, to free all conjugated analytes of concern and to completely convert SCA to DMS. Additionally, a more specific detector was required to discriminate between the residues of sulfentrazone and its metabolites, and the matrix components released during the stringent hydrolysis step. A GC equipped with an electrolytic conductivity detector (ELCD) was utilized.

The revised method has been validated on 13 different matrices. Control samples of soybean, corn, rice, sorghum and wheat matrices (Section XI, References 3, 4, 5, 6 and 7) were analyzed for sulfentrazone, DMS/SCA (not analyzed for in soybean seed) and HMS (both conjugated and free). The purpose of this report is to describe the revised residue analytical method.

Authority® is a registered trademark of FMC Corporation.

II. SUMMARY

The previously reported residue analytical method for sulfentrazone, DMS/SCA (analyzed as DMS) and HMS was modified to include a more stringent hydrolysis step to free all conjugated analytes of concern and to completely decarboxylate SCA (analyzed as DMS). Additionally, a more specific detector was used (ELCD). The ELCD is more specific for halogenated compounds and was essential for discriminating between the residues of sulfentrazone and its metabolites, and matrix components.

The revised method began with an acetone/0.25 N HCl reflux (1hr), filtration, and concentration. The aqueous concentrate was acidified to 1 N, boiled under reflux (2hr), and filtered. The sample was then passed through a C_8 solid phase extraction (SPE) cartridge and a silica gel SPE cartridge for clean-up. The eluate was concentrated and the HMS analyte was derivatized with N,O-bis [trimethylsilyl] trifluoroacetamide (BSTFA). The derivatized solution was passed through a second silica gel SPE cartridge for additional clean-up. The eluate was concentrated and brought to a final volume with acetonitrile. Analysis was performed by a GC equipped with a 35% phenyl methyl silicone megabore column and an ELCD.

This method has been validated on the following matrices: soybean seed; corn forage, fodder and grain; rice straw and grain; sorghum forage, fodder and grain; and wheat forage, hay, straw and grain. For hay and straw matrices, the method limit of quantitation (LOQ) was validated at 0.05 ppm and the method limit of detection (LOD) was set at 0.01 ppm. For all other matrices, the LOQ was validated at 0.025 ppm and the LOD was set at 0.005 ppm. The average method recoveries (n=28, 25 for SCA) were 90 ± 9% for sulfentrazone, 92 ± 18% for SCA, and 84 ± 14% for HMS.

III. SUMMARY TABLES AND GRAPHICS

A. Summary of Method Recovery Data

TABLE 1

METHOD RECOVERY VALUES FOR SULFENTRAZONE
FROM VARIOUS LABORATORY FORTIFIED CONTROL MATRICES

Analyte Matrix	Fortification Levels (ppm)	Number of Analyses	Individual Method Recoveries (%)	Recovery Average(%) ± Standard Deviation
Sulfentrazone				
Soybean Seed	0.025	3	79, 84, 85	83 ± 3
Corn Forage	0.025	2	80, 90	85
Corn Grain	0.025	2	88, 103	96
Corn Fodder	0.025, 0.05	3	80, 84, 97	87 ± 9
Rice Grain	0.025, 0.05	2	94, 114	104
Rice Straw	0.05, 0.5	2	94, 102	98
Sorghum Forage	0.025, 0.05	2	82, 96	89
Sorghum Grain	0.025	2	84, 105	95
Sorghum Fodder	0.025, 0.1	2	82, 85	84
Wheat Forage	0.025, 0.1	2	88, 93	91
Wheat Hay	0.05, 0.2	2	85, 90	88
Wheat Grain	0.025	2	93, 98	96
Wheat Straw	0.05, 0.5	2	82, 91	87
Sulfentrazone Overall	0.025 - 0.5	28	79 - 114	90 ± 9

TABLE 2

METHOD RECOVERY VALUES FOR SCA (ANALYZED AS DMS) FROM VARIOUS LABORATORY FORTIFIED CONTROL MATRICES

Analyte Matrix[a]	Fortification Levels (ppm)	Number of Analyses	Individual Method Recoveries (%)	Recovery Average(%) ± Standard Deviation
SCA				
Corn Forage	0.025	2	76, 88	82
Corn Grain	0.025	2	86, 88	87
Corn Fodder	0.025, 0.05	3	68, 76, 80	75 ± 6
Rice Grain	0.025, 0.05	2	78, 99	89
Rice Straw	0.05, 0.5	2	120, 129	125
Sorghum Forage	0.025, 0.05	2	85, 86	86
Sorghum Grain	0.025	2	72, 92	82
Sorghum Fodder	0.025, 0.1	2	68, 84	76
Wheat Forage	0.025, 0.1	2	80, 97	89
Wheat Hay	0.05, 0.2	2	84, 93	89
Wheat Grain	0.025	2	117, 122	120
Wheat Straw	0.05, 0.5	2	106, 121	114
SCA Overall	**0.025 - 0.5**	**25**	**68 - 129**	**92 ± 18**

a Soybean seed was not analyzed for SCA.

TABLE 3

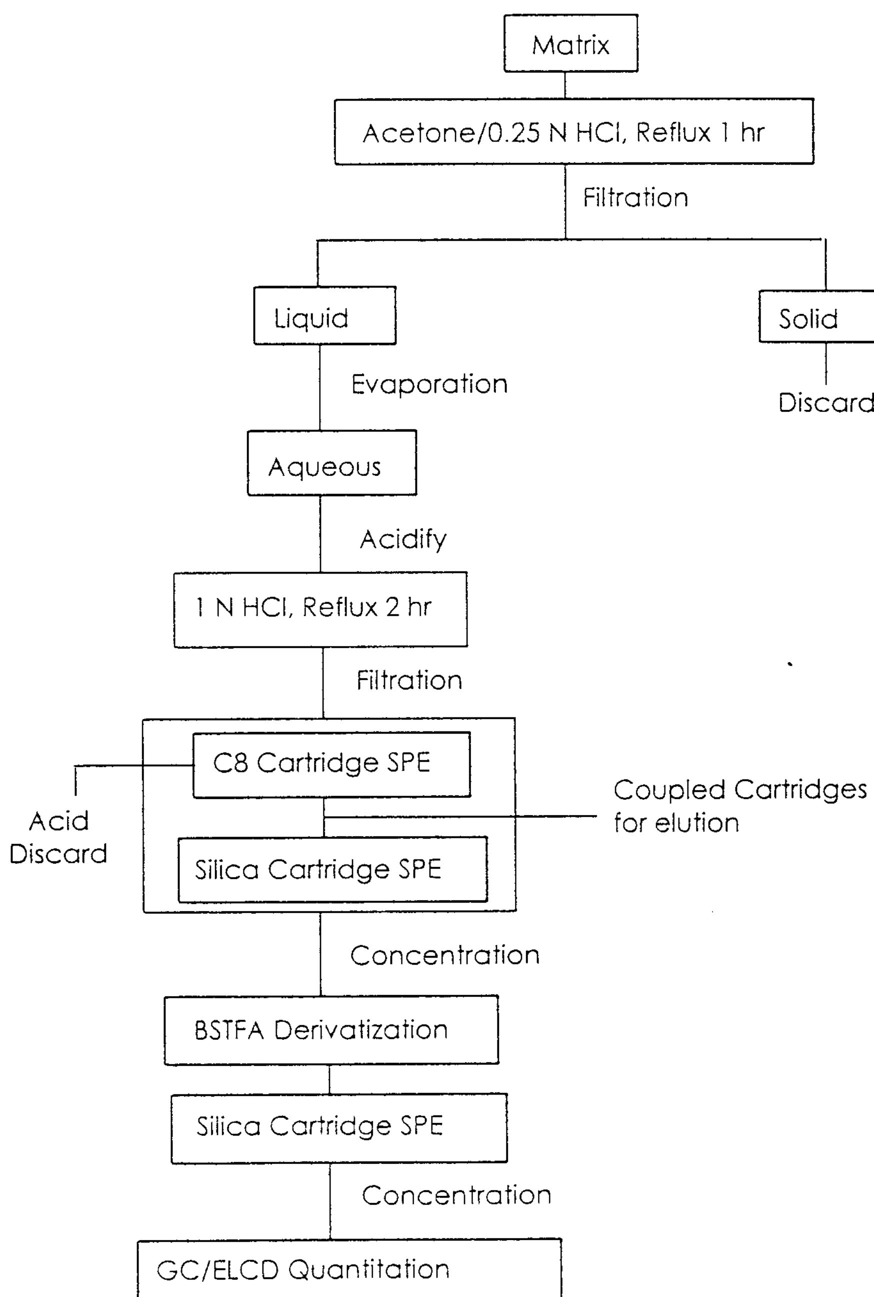
METHOD RECOVERY VALUES FOR HMS
FROM VARIOUS LABORATORY FORTIFIED CONTROL MATRICES

Analyte Matrix	Fortification Levels (ppm)	Number of Analyses	Individual Method Recoveries (%)	Recovery Average(%) ± Standard Deviation
HMS				
Soybean Seed	0.025	3	65, 80, 100	82 ± 18
Corn Forage	0.025	2	72, 79	76
Corn Grain	0.025	2	76, 80	78
Corn Fodder	0.025, 0.05	3	72, 78, 78	76 ± 3
Rice Grain	0.025, 0.05	2	86, 88	87
Rice Straw	0.05, 0.5	2	77, 94	86
Sorghum Forage	0.025, 0.05	2	84, 107	96
Sorghum Grain	0.025	2	79, 110	95
Sorghum Fodder	0.025, 0.1	2	70, 75	73
Wheat Forage	0.025, 0.1	2	80, 86	83
Wheat Hay	0.05, 0.2	2	85, 85	85
Wheat Grain	0.025	2	68, 98	83
Wheat Straw	0.05, 0.5	2	79, 125	102
HMS Overall	**0.025 - 0.5**	**28**	**65 - 125**	**84 ± 14**

B. Method Flow Scheme

FIGURE 1

ANALYTICAL METHOD FOR SULFENTRAZONE,
SCA (ANALYZED AS DMS) AND HMS IN/ON VARIOUS MATRICES



IV. MATERIALS AND STUDY DESIGN

A. Test and Reference Substances

The test and reference substances used in the revised analytical method were sulfentrazone, SCA, HMS and DMS. The chemical names, chemical abstract service numbers, residue inventory numbers, and purity of the analytical standards are listed in Section X, Table 4.

B. Test Commodities

This method has been validated on the following matrices: soybean seed; corn forage, fodder and grain; rice straw and grain; sorghum forage, fodder and grain; and wheat forage, hay, straw and grain.

C. Equipment and Supplies

All glassware was routinely washed in a BetterBuilt® Turbomatic Jr. dishwasher (Model 7000) or a Lancer (Model 910VP) dishwasher using a non-phosphorous detergent, two tap water rinses and three distilled water rinses. The clean glassware was hand-rinsed with acetone prior to use.

Access*Chrom Data Acquisition software running on MicroVax
Adapters, Neoprene
Adapters, Reducing
Balance, Analytical PM 2000, Mettler
Balance, Top Loading, Mettler
Boiling Stones, Hengar granules
Buchner Filter Funnels, Porcelain, 10.5 cm i.d., Coors
Capillary Column, DB-5MS, 15 m x 0.25 mm id, 0.25 µm, J & W Scientific
Capillary Column, DB-35, 30m x 0.54 mm, 1 µm, J & W Scientific
Centrifuge Tubes, 13 mL graduated, 0.1 mL, Pyrex®
Condensers, Pyrex, Graham coil, 41 mm x 500 mm with ₮ 24/40 joint
Cylinders, Graduated, 50 mL, 100 mL (mixing), 250 mL
Filter Paper, Whatman # 1, 11 cm diameter
Filter Paper, Whatman GF/F (0.8 micron), 11 cm diameter
Flasks, Filter, 250 mL
Flasks, Round Bottom Boiling, 500 mL, ₮ 24/40 joint
Flasks, Volumetric, 100 mL

Gas Chromatograph (Hewlett-Packard 6890 GC with HP 6890 series injector and O-I Analytical model 5200 ELCD; an HP 5890 equipped with a HP 7673A Autosampler and an HP 5970 MSD)
Heating Mantles, Glas-Col®
Injection port insert, Cyclo-double gooseneck, Restek
Magnetic Stirrers, VWR Scientific, Model 200
Micro Syringes, Hamilton
Mill, Hobart®
Mill, Wiley®
Multi - Tube Vortexer, VWR Scientific
N-EVAP® Evaporator, Organomation
Pipets, Disposable and volumetric
Reservoirs, Plastic, 75 mL
Solid Phase Extraction Cartridge, Silica gel (1 g), JT Baker
Solid Phase Extraction Cartridge, C_8 (1 g), Varian
Teflon® stirring bars, VWR Scientific
TurboVap® Evaporator, Zymark
TurboVap Vessels, 200 mL, Zymark
TurboVap Vessel Support Rack, Zymark
Visiprep® manifold, Supelco
Visidry® vacuum manifold drying attachment, Supelco

D. Reagents

Acetone, Resi-Analyzed, JT Baker
Acetonitrile, Resi-Analyzed, JT Baker
BSTFA (N,O-bis[trimethylsilyl]trifluoroacetamide), Pierce
Ethyl Acetate, Resi-Analyzed, JT Baker
Hexane, Resi-Analyzed, JT Baker
Hydrochloric acid (HCl, 36.5 - 38.0%), JT Baker
Methanol, Resi-Analyzed, JT Baker

Equivalent equipment and reagents may be substituted as appropriate, unless otherwise specified in the method report.

V. ANALYTICAL PROCEDURE

A. Residue Method

The previously reported analytical method (Section XI, Reference 1) has been modified to include a more stringent hydrolysis step to completely free all conjugated analytes of concern and completely decarboxylate SCA. Additionally, a more specific detector was utilized. With the current method, sulfentrazone, DMS/SCA (analyzed as DMS), and HMS (both conjugated and free) are analyzed simultaneously. Prior to analysis, the samples are chopped and finely ground with liquid nitrogen using a large Hobart® (forage, hay, fodder and straw samples) or a Wiley® mill (grain and seed samples). See Section XII, Appendix A for a step-by-step procedure. The detailed analytical procedure follows:

1. Acetone/0.25 N HCl (3/1,v/v) Reflux

Weigh 10.0 grams of the matrix into a 500 mL round bottom boiling flask. For control samples to be fortified, add an accurately measured volume of a standard solution containing sulfentrazone, SCA, and HMS uniformly to the matrix by syringe. Allow the solvent to evaporate (~1 min). Add 150 mL of acetone/0.25 N HCl (3/1, v/v) and a teflon stirring bar or boiling stones. Place the round bottom flask in a heating mantle and attach it to a cooling condenser. Gently boil the solution under reflux with stirring (if using a stirring bar) for one hour (voltage variac at about 55).

2. Filtration and Concentration

Cool the sample extract to room temperature and filter through a Whatman #1 (11 cm) filter paper (pre-rinsed with ~5 mL of acetone) into a filter flask using a Buchner funnel and vacuum (~15″ Hg). Rinse the boiling flask with 2 x ~25 mL of acetone and pass the rinsate through the post-reflux solid and filter paper. Transfer the filtrate to a 200 mL TurboVap vessel. Rinse the filter flask with ~5 mL of acetone and add the rinsate to the TurboVap vessel. Concentrate the filtrate to less than 25 mL (not to dryness) using a TurboVap Evaporator (water bath at ~50°C, increase pressure up to 30 psi as volume decreases). **It is important to remove all traces of acetone.**

3. 1 N HCl Reflux (*Conversion of SCA to DMS & release of conjugated HMS*)

   Transfer the aqueous concentrate to a 500 mL round bottom boiling flask. Rinse the TurboVap vessel with 2 x ~5 mL of distilled water and add the rinsate to the round bottom boiling flask. Add 3.5 mL of concentrated HCl to the aqueous concentrate to make ~ 1 N. Add a teflon stirring bar or boiling stones. Place the round bottom flask in a heating mantle and attach it to a cooling condenser. Gently boil the solution under reflux with stirring (if using a stirring bar) for two hours (voltage variac at about 80).

4. Filtration

   Cool the sample extract to room temperature and filter through a Whatman GF/F (11 cm) fine filter paper (pre-rinsed with ~5 mL of distilled water) into a filter flask using a Buchner funnel and vacuum (~15″ Hg). Rinse the round bottom boiling flask with 2 x ~10 mL distilled water, and pass the rinsate through the post-reflux solid and filter paper. Transfer the filtrate to a 100 mL graduated mixing cylinder. Rinse the filter flask with 2 x ~10 mL of distilled water, and add the rinsate to the mixing cylinder. Bring the volume up to 100 mL with distilled water. Shake the sample and take a 50 mL (5g) aliquot. (Store the remaining aliquot for possible reanalysis; up to one week under refrigeration.)

5. C_8 Cartridge Solid Phase Extraction (SPE)

   Place a C_8 cartridge (1 g, Varian) on a vacuum manifold and condition it with 6 mL of methanol followed by 6 mL of 0.25 N HCl. When conditioning SPE cartridges, allow the first conditioning solvent to reach the top of the cartridge packing before adding the second solvent. **Maintain the flow rate through the C_8 cartridge at ~5 mL/min by regulating the vacuum pump (~5″ Hg). The flow rate is more important than the vacuum pressure.** Close the cartridge and add an additional 3 mL of 0.25 N HCl to the cartridge barrel. Attach a 75 mL plastic reservoir with an adapter to the top of the C_8 cartridge. Transfer the 50 mL aqueous sample aliquot to the reservoir. Pass the sample through the C_8 cartridge. Once the entire sample has passed through the C_8 SPE cartridge, use full vacuum briefly (~2 min). Blow the cartridge completely dry under nitrogen using a manifold drying attachment (30 psi for at least 30 minutes). Return the C_8 SPE cartridge to the manifold and wash with

6 mL of 5% ethyl acetate/hexane. Remove the C_8 cartridge and prepare the first silica gel cartridge. (See step 6.)

6. C_8 Cartridge SPE/First Silica Gel Cartridge SPE

   Place a silica gel cartridge (1 g, JT Baker) on the vacuum manifold and condition with 6 mL of ethyl acetate followed by 6 mL of hexane. **Do not allow the silica gel cartridge to go dry at anytime during this SPE step. Maintain the flow rate through the silica gel cartridge at ~2 mL/min by regulating the vacuum pump (< 5″ Hg, the flow rate is more important than the vacuum pressure).** Close the cartridge and add 1 mL of 30% ethyl acetate/hexane. Attach the C_8 cartridge to the top of the silica gel cartridge with a reducing adapter. Add 3 mL of 30% ethyl acetate/hexane to the C_8 cartridge. Open the connected cartridges and allow a few drops to drip from the C_8 cartridge into the silica gel cartridge before applying vacuum. This will help prevent the silica gel cartridge from going dry. When the first 3 mL has reached the top of the C_8 cartridge packing, add an additional 6 mL of 30% ethyl acetate/hexane. Allow the C_8 eluant to reach the top of the silica gel cartridge packing. Remove the C_8 cartridge and discard. Wash the silica gel cartridge with 3 mL of 30% ethyl acetate/hexane. Elute and collect the analytes from the silica gel cartridge with 6 mL of ethyl acetate into a 13 mL glass centrifuge tube. Discard the silica gel cartridge. Evaporate the eluate under low nitrogen stream (just enough to produce a ripple on the surface) in a water bath (~45°C) to near dryness **(until a thin oily film remains, do not overdry!).**

7. Derivatization (*silylation of 3-hydroxymethyl sulfentrazone*).

   Add 0.5 mL of acetonitrile and 100 µL of fresh BSTFA **(Precaution: once the ampule containing BSTFA is opened, it should be used within ~10 minutes, since BSTFA will absorb moisture)** to the centrifuge tube containing the sample extract, stopper the tube, and vortex for ~15 seconds. Add 9.5 mL of 10% ethyl acetate/hexane to make 10 mL. Cap the centrifuge tube and vortex until the contents are mixed (there should be no phase separation). If there is a phase separation, gently warm the samples in a water bath (~45°C) for ~1 minute. Vortex again. If phase separation persists, continue warming and vortexing until phases mix.

Injection Standards

Prepare injection standards following the same derivatization procedure noted above. Place an accurately measured volume of standard solution containing sulfentrazone, DMS and HMS into a 13 mL glass centrifuge tube. Add acetonitrile to make 0.5 mL and 100 µL of fresh BSTFA to the centrifuge tube, stopper, and vortex for approximately 15 seconds. Evaporate under nitrogen stream in a water bath (~45°C) to near dryness (~0.1 mL), dilute to the appropriate final volume with acetonitrile and vortex for about 15 seconds. No silica gel SPE clean-up step is required for preparing the injection standards.

8. Second (post-derivatization) Silica Gel Cartridge SPE

   Place a silica gel cartridge (1 g, JT Baker) on a vacuum manifold and condition with 6 mL of ethyl acetate followed by 6 mL of hexane. **Do not allow the cartridge to go dry at anytime during this SPE step. Maintain the flow rate through the silica gel cartridge at about 2 mL/min by regulating the vacuum pump (< 5″ Hg, the flow rate is more important than the vacuum pressure).** Load the derivatized sample extract onto the cartridge. Rinse the centrifuge tube twice, each with 3 mL of 10% ethyl acetate/hexane and add the rinsate to the cartridge. Drain the rinsate to the top of the silica gel packing. Elute the analytes with 9 mL of 50% ethyl acetate/hexane into a 13 mL glass centrifuge tube. Discard the silica gel cartridge. Add 1.0 mL of acetonitrile to the eluate. Evaporate the eluate under low nitrogen stream (just enough to produce a ripple on the surface) in a water bath (~45°C) to near dryness **(until a thin oily film remains, do not overdry).** Dilute to the appropriate final volume with acetonitrile.

9. Analysis

   Analyze the final sample solution with a gas chromatograph equipped with an ELCD.

B. Instrumentation

Gas chromatography was used to analyze the sample extracts. Two detector systems were used, one for quantitation and one for analyte confirmation.

An HP 6890 GC equipped with an HP 6890 autosampler, an OI Analytical 5220 Electrolytic Conductivity Detector (ELCD), a J&W Scientific DB-35 (phenyl/methyl, 35/65) Megabore capillary column and Perkin Elmer Nelson Access*Chrom computer software were used for routine analyses. The ELCD is specific for halogenated compounds and was effective for discriminating between the residues of sulfentrazone and its metabolites, and matrix components. Detailed instrument parameters are listed in Section XII, Appendix B.

An HP 5890 GC equipped with an HP 7673A autosampler, an HP 5970 Mass Selective Detector (MSD) and a J&W Scientific DB-5MS (phenyl/methyl, 5/95) capillary column were used for spectral analyte confirmation. Detailed instrument parameters are listed in Section XII, Appendix B.

C. Method Validation and Quality Control

1. Experimental Design

Control samples of 13 different matrices were fortified with a known amount of sulfentrazone, SCA and HMS prior to the initial extraction. The fortified-control samples were then analyzed to determine the method recovery. The LOQ was validated by acceptable and reproducible recoveries of the respective analytes from laboratory fortified control samples. For hay and straw, the LOQ was validated at 0.05 ppm and the LOD was set at 0.01 ppm. For all other matrices, the LOQ was validated at 0.025 ppm and the LOD was set at 0.005 ppm. Each analysis set contained a minimum of one control sample and one fortified control sample.

A linearity curve ranging from ½ LOQ to 16 times LOQ was generated for each analyte at the initiation of the analytical phase of the study. Standard solutions for injection contained sulfentrazone, DMS and/or derivatized HMS. Standard solutions were injected at the beginning of each set of assays and after every two samples thereafter to gauge the instrument response.

2. Preparation of Standards

   The structure and purity of the analytical standards are shown in Section X, Table 4. Stock solutions of approximately 1000 µg/mL were prepared by dissolving the appropriate amounts of the analytical standards in acetonitrile. Working standard solutions were prepared in volumetric flasks by appropriate dilutions of stock solutions for each analyte or combination of analytes. The standard solution concentrations ranged from 1-10 µg/mL. These solutions were used for fortification.

   During analysis SCA is converted to DMS and HMS is derivatized, therefore, the analytical standard solutions for injection contained sulfentrazone, DMS and derivatized HMS. A measured volume of a standard solution containing sulfentrazone, DMS and HMS (prepared from stock solutions) was derivatized simultaneously with the samples (excluding the silica gel SPE clean-up step). [For example, 100 µl of a 10 ng/µl standard was derivatized and diluted to a final volume of 4 mL to yield a 0.25 ng/µl injection standard.] All standard solutions were stored in a refrigerator/freezer unit (ca. -18°C) when not in use. Information on the analytical standards and reference solutions is shown in Section X, Tables 4 and 5.

3. Fortification Procedure

   The control samples were accurately weighed into round bottom boiling flasks. The samples were fortified by applying a measured volume of standard solution containing sulfentrazone, SCA and HMS to the matrix using a syringe and allowing the solvent to evaporate. Standard solutions of approximately 1 or 10 ng/µL were used for fortification. The fortification levels ranged from 0.025 ppm - 0.5 ppm. The fortified-control samples were analyzed concurrently with the control samples. The individual method recoveries can be found in Section X, Table 6.

D. Method of Calculation

The amounts of sulfentrazone, SCA (for method recovery only), DMS and HMS were quantitated by an external standard calibration method. A computer spreadsheet program (Microsoft Excel®) was used for calculation and reporting.

The nanogram amount of analyte in a sample was calculated by comparing the area units of the analyte in the sample to that of the average area units of the corresponding injection standard using the following formula:

$$\text{ng of analyte in sample} = \frac{\text{area units (sample)}}{\text{average area units (standard)}} \times \text{ng (standard)}$$

To determine method recoveries, the peak area units of any interference found in a control sample above the limit of detection were subtracted from the corresponding peak area units in the fortified-control sample. For enforcement purposes, the subtraction of control sample interferences typically would not be necessary, especially when using ELCD or MSD to minimize interferences.

No correction for molecular weights was necessary for derivatized HMS, since the injection standards were derivatized simultaneously with the samples. However, a correction factor was needed for calculating the recovered amount of SCA since it was quantitated as DMS. The correction factor (molecular weight ratio) between SCA and DMS was 1.12 (417/373, 417 = molecular weight of SCA and 373 = molecular weight of DMS).

The mg of sample injected was determined by the following formula:

$$\text{mg of sample} = \frac{\text{initial sample weight (mg)}}{\text{final sample extract volume } (\mu L)} \times \text{sample extract volume injected } (\mu L)$$

The ng of analyte in the sample and the mg of sample injected were used to calculate the uncorrected ppm (ng/mg) by the following formula:

$$\text{uncorrected ppm (ng/mg)} = \frac{\text{ng of analyte in sample}}{\text{mg of sample injected}}$$

The uncorrected ppm of the fortified-control samples was divided by the fortification level and multiplied by 100 to calculate the method recovery (%). The following formula was used:

$$\text{method recovery (\%)} = \frac{\text{uncorrected ppm}}{\text{fortification level (ppm)}} \times 100\%$$

An example of how to calculate the method recovery of HMS in a rice straw fortified sample (Section XII, Appendix C, Figure 4) is given below:

$$\text{ng (standard)} = 2.0\ \mu l \times 0.25\ \text{ng}/\mu L = 0.5\ \text{ng}$$

$$\text{average area units of standard} = 8505$$

area units of fortified sample	=	6588				
ng of HMS in sample	=	6588 / 8505	x	0.5 ng	=	0.387 ng
mg of sample injected	=	5000 mg / 1000 µL	x	2 µL	=	10 mg
uncorrected ppm (ng/mg)	=	0.387 ng / 10 mg			=	0.0387 ppm
method recovery (%)	=	0.0387 ppm / 0.05 ppm	x	100%	=	77%

E. Interferences

A small number of low-level interference peaks were detected in the control samples during GC/ELCD analysis. Peak areas of interferences above the method LOD were subtracted from the peak areas of the fortified samples. Typically for enforcement purposes, the subtraction of interferences would not be necessary, especially when using an ELCD or MSD to minimize interferences.

F. Confirmatory Technique

A GC equipped with an MSD with a selective ion mode was used for analyte confirmation. The selected molecular ions used were 307 for sulfentrazone, 293 for 3-desmethyl sulfentrazone, and 459 for 3-hydroxymethyl sulfentrazone.

G. Time Required for Analysis

For a set of ten samples, the analytical method can be completed from the time of sample weighing to GC injection within 18 laboratory hours. Sample extracts can be stored after step #1, 3, 4, or 6 (prior to evaporation) in the procedure (Section V, A.) if the entire method can not be completed. Samples should be stored in full-volume solvent and refrigerated.

H. Potential Problems

1. After the initial extraction with acetone/0.25 N HCl, it is important to remove all traces of acetone using a TurboVap Evaporator. Traces of solvent can lead to analyte loss through the SPE cartridge(s).

2. It is important to determine the proper elution and wash solvent composition, volume and flow rate through the cartridges (SPE calibration). The solid phase extraction steps are critical to the separation and clean-up of the sample extract. Listed brands for C_8 and silica gel cartridges should be used, if possible.

3. After passing the sample solution through the C_8 cartridge, the cartridge and manifold must be completely dry. Extend the drying time if necessary. It is a good practice to rinse the manifold with acetone prior to elution. Traces of aqueous solution may interfere with subsequent derivatization.

4. BSTFA should be used within 10 minutes of opening the ampule for complete derivatization. BSTFA readily absorbs moisture, which will interfere with derivatization.

5. If final sample solutions will be stored several days, the derivatization of the HMS metabolite may reverse. If the derivatization has reversed, the HMS method recovery would be low and an additional broad peak (underivatized HMS) would be visible after the derivatized HMS peak. In this case, add 10 µl of fresh BSTFA to the final sample solution in the GC vial, vortex several seconds and reinject.

6. Optimizing the GC instrument is crucial for the quantitation of sulfentrazone and its metabolites. Before actual analysis, the temperatures, gas flow rates, and the glass insert liner should be optimized. The injection standards must have a low coefficient of variation (<15%) and the linearity standards must have a correlation coefficient of at least 0.99. Before injection of the analysis set, it is important to condition the column with sample matrix. This can be done by injecting a matrix sample extract several times before the standard, repeating this "conditioning" until the injection standard gives a reproducible response and provides adequate sensitivity.

7. Operation of the ELCD must be optimized for greatest sensitivity. Operating the ELCD in tandem with another detector may cause a loss in sensitivity.

VI. STORAGE STABILITY

The analytical reference standards were assayed on a regular basis for percent purity and structural integrity (Section X, Table 4). Standard stock solutions (~1000 ng/uL) were prepared from the analytical standards in acetonitrile and were stored in a refrigerator/freezer (ca. -18°C) for up to one year. Standard working solutions were stored in a refrigerator/freezer (ca. -18°C) for up to one month (Section X, Table 5). Under these storage conditions, these analytical standards have shown a pattern of stability.

VII. RESULTS AND DISCUSSION

A. Accuracy

The accuracy was determined by the average method recovery of the individual results of the fortified-control samples of 13 different matrices (Section X, Table 6). The average method recovery (n=28, 25 for SCA) was 90% for sulfentrazone, 92% for SCA and 84% for HMS.

B. Precision

The precision of the analytical method was determined by the standard deviation of the individual results of the fortified-control samples of 13 different matrices (Section X, Table 6). The standard deviation (n=28, 25 for SCA) was ± 9% for sulfentrazone, ± 18% for SCA and ± 14% for HMS.

C. Limit of Detection and Quantification

The method limit of quantitation (LOQ) for all matrices with the exception of hay and straw was established at 0.025 ppm and the method limit of detection (LOD) was set at 0.005 ppm. Due to analytical difficulties and the level of residues with the dried plant matrices, the LOQ for hay and straw was established at 0.05 ppm and the LOD was set at 0.01 ppm.

D. Ruggedness

The acceptable and reproducible method recoveries for the method on 13 different matrices indicate that the method is reliable and accurate. Considering the potential problems noted above and details within the procedure, analyses for sulfentrazone, DMS/SCA (analzyed as DMS) and HMS (both conjugated and free) should be possible on the various matrices included in this report.

VIII. CONCLUSION

The previously reported residue analytical method for sulfentrazone, DMS/SCA (analyzed as DMS) and HMS was modified to include a more stringent hydrolysis step to free all conjugated analytes of concern and to completely decarboxylate SCA (analyzed as DMS). Additionally, a more specific detector was used (ELCD). This current method has been successfully validated on 13 various matrices (Section XI, References 3, 4, 5, 6 and 7). For hay and straw, the LOQ was established at 0.05 ppm and the LOD was set at 0.01 ppm. For all other matrices, the LOQ was established at 0.025 ppm and the LOD was set at 0.005 ppm. The average method recoveries on all matrices analyzed (n=28, 25 for SCA) were 90 ± 9% for sulfentrazone, 92 ± 18% for SCA and 84 ± 14% for HMS.

All equipment needed to perform the analyses is readily available in most residue analytical laboratories. An experienced residue analyst, following the procedure exactly as written and being aware of the potential problems, can obtain adequate recoveries of sulfentrazone, DMS/SCA (analyzed as DMS), and HMS (both conjugated and free) in/on soybean, corn, rice, sorghum and wheat matrices.

IX. CERTIFICATION

We, the undersigned, hereby declare that this study was performed under our supervision according to the procedures herein described, and that this report provides a true and accurate record of the results obtained.

Natalie A. Shevchuk — Date: 9-13-96
Senior Chemist
AUTHOR

Audrey W. Chen — Date: 9/13/96
Research Associate
STUDY DIRECTOR

John M. Becker — Date: 09/13/96
Manager
SPONSOR

ADDITIONAL STUDY PERSONNEL

J. Michael Fink, Chemist
John R. Arabinick, Associate Chemist
David Baffuto, Senior Research Technician
David J. Letinski, Senior Research Technician
Michael C. Reel, Senior Research Technician
C. Maria Suero, Senior Research Technician
Kristen M. Sakala, Research Technician (Summer Intern)

X. TABLES

TABLE 4

TEST AND REFERENCE SUBSTANCES

Common Name Chemical Name	Structure	CAS Number	Inventory Number	Percent Purity
Sulfentrazone N-[2,4-dichloro-5-[4-(difluoromethyl)-4,5-dihydro-3-methyl-5-oxo-1H-1,2,4-triazol-1-yl]phenyl]-methanesulfonamide		122836-35-5	RIN243	99.5
Sulfentrazone-3-carboxylic acid (SCA) 1-[2,4-dichloro-5-(N-(methyl-sulfonyl)amino)phenyl]-4-difluoromethyl-4,5-dihydro-5-oxo-1H-1,2,4-triazole-3-carboxylic acid		134391-01-8	SU-4, M441:60	92.5
3-Desmethyl sulfentrazone (DMS) N-[2,4-dichloro-5-[4-(difluoromethyl)-4,5-dihydro-5-oxo-1H- 1,2,4-triazol-1-yl]phenyl] methanesulfonamide		134391-02-9	RIN236	95.6
3-Hydroxymethyl sulfentrazone (HMS) N-[2,4-dichloro-5-[4-(difluoromethyl)-4,5-dihydro-3-hydroxymethyl-5-oxo-1H-1,2,4-triazol-1-yl]phenyl]-methanesulfonamide		134390-99-1	RIN246	97.2

TABLE 5

REFERENCE SOLUTIONS

Compound	Solution Solvent	Solution Concentration (ng/µL)	Standard Solution Index Number	Date Prepared
Sulfentrazone	Acetonitrile	1000	896	03/22/96
SCA	Acetonitrile	917.6	930	06/26/96
		9.176	940-1	07/31/96
DMS	Acetonitrile	1002	875	01/09/96
		10	941-1	07/31/96
HMS		999	897	03/22/96
Sulfentrazone + HMS	Acetonitrile	10	932-1	07/08/96
Sulfentrazone + SCA + HMS	Acetonitrile	1.0	930-1	06/26/96
		1.0	938-1	07/24/96
		10	938-2	07/24/96
Sulfentrazone + DMS + HMS	Acetonitrile	10	929-1	06/24/96
		10	937-1	07/24/96
		10	944-1	08/14/96
		10	945	08/26/96

TABLE 6

METHOD RECOVERIES OF SULFENTRAZONE, SCA, AND HMS FROM VARIOUS LABORATORY FORTIFIED CONTROL MATRICES

Matrix Set #	Sample ID	Fortification Level (ppm)	Method Recovery (%) Sulfentrazone	SCA	HMS
Soybeans					
S1R	92GJZ1C	0.025	85	NA[a]	100
S2R	92HGH02C	0.025	79	NA	65
S3R	93JES20C	0.025	84	NA	80
		Average/n=3	**83**	**NA**	**82**
		Std. Dev. (±)	**3**	**NA**	**18**
Corn Forage					
C3	94JES25C	0.025	90	88	72
C4	94SJS022C	0.025	80	76	79
		Average/n=2	**85**	**82**	**76**
Corn Grain					
C1	94JES26C	0.025	103	88	76
C2	94SJS023C	0.025	88	86	80
		Average/n=2	**96**	**87**	**78**
Corn Fodder					
C5	94JES27C	0.025	80	68	72 (0.005)
C6	94SJS024C	0.05	84	76	78
C7	94LKF704C	0.05	97 (0.005)[b]	80 (0.008)	78
		Average/n=3	**87**	**75**	**76**
		Std. Dev. (±)	**9**	**6**	**3**
Rice Grain					
R1	94SJS041C	0.025	114	78	88
R2, R2R[c]	95SJS01C	0.05	94	99	86
		Average/n=2	**104**	**89**	**87**
Rice Straw					
R3R2	94SJS042C	0.05	102	120	94
R4R2	95SJS04C	0.5	94	129	77
		Average/n=2	**98**	**125**	**86**

a NA - Not applicable. Soybean seed samples were not analyzed for SCA.

b The values in parenthesis are interferences (ppm) that were found in the control samples and subtracted from the corresponding fortified control samples.

c Set R2 was reanalyzed for SCA only. Therefore, data for sulfentrazone and HMS are from set R2 and data for SCA are from set R2R.

TABLE 6 (*cont.*)

METHOD RECOVERIES OF SULFENTRAZONE, SCA, AND HMS
FROM LABORATORY FORTIFIED CONTROL SAMPLES

Matrix Set #	Sample ID	Fortification Level (ppm)	Method Recovery (%) Sulfentrazone	 SCA	 HMS
Sorghum Forage					
SG3R	95LVE13C	0.025	82	86	84
SG4	95TWM1001C	0.05	96	85	107
		Average/n=2	**89**	**86**	**96**
Sorghum Grain					
SG1	95WDM503C	0.025	84	72	79
SG2	95TWM1003C	0.025	105	92	110
		Average/n=2	**95**	**82**	**95**
Sorghum Fodder					
SG5	95WDM502C	0.025	82	84	70
SG6, SG6R[d]	95TWM1002C	0.1	85	68	75
		Average/n=2	**84**	**76**	**73**
Wheat Forage					
W3R	95SJS0201C	0.025	93	80	80
W4	95TWM501C	0.1	88	97	86
		Average/n=2	**91**	**89**	**83**
Wheat Hay					
W5	95SJS0202C	0.05	85	93	85
W6	95GGS06C	0.2	90	84	85
		Average/n=2	**88**	**89**	**85**
Wheat Grain					
W1, W1R[d]	93HGH03C	0.025	93	117	98
W2	95TWM504C	0.025	98	122	68
		Average/n=2	**96**	**120**	**83**
Wheat Straw					
W7R2	93HGH03C	0.05	91	106	125
W8	95TWM503C	0.5	82	121	79
		Average/n=2	**87**	**114**	**102**
		Overall Average/n=28 (25 for SCA)	90	92	84
		Overall Std. Dev. (±)	9	18	14

d Sets SG6 and W1 were reanalyzed for SCA only. Therefore, data for sulfentrazone and HMS are from set SG6 and W1 and data for SCA are from sets SG6R and W1R.

XI. REFERENCES

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5. Shevchuk, N.A., "Magnitude of the Residue of Sulfentrazone and its Major Metabolites in/on Rice as a Rotated Crop Following Soybeans Treated with Authority Herbicide: Re-Analysis of Selected Trials using Revised Methodology," FMC Corporation, Agricultural Products Group, Princeton, NJ, Report P-3170, September 1996.

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7. Culligan, J.F., "Magnitude of the Residue of Sulfentrazone and its Major Metabolites in/on Winter Wheat as a Rotated Crop Following Soybeans Treated with Authority Herbicide: Re-Analysis of Selected Trials using Revised Methodology," FMC Corporation, Agricultural Products Group, Princeton, NJ, Report P-3176, September 1996.

XII. APPENDIX

A. <u>Analytical Method</u> (See Section V. Analytical Procedure for full details.)

Sulfentrazone, SCA (analyzed as DMS), and HMS in/on Various Matrices

1. <u>Initial Reflux</u>
   a. Weigh 10.0g of sample into a 500 mL boiling flask.
   b. Fortify as appropriate. Allow ~1 minute for the solvent to evaporate.
   c. Add stir bar or boiling stones.
   d. Add 150 mL of 3/1 (v/v) acetone/0.25 N HCl.
   e. Place in heating mantle on stir plate and gently reflux for 1 hour (voltage variac ~55).
2. <u>Filtration #1</u>
   a. Remove from heating mantle when cool enough to handle.
   b. Filter through Whatman #1 (11 cm) filter paper. Rinse boiling flask with ~ 2 x 25 mL of acetone and add the rinsate to the Buchner funnel.
   c. Transfer sample from filter flask to a 200 mL TurboVap vessel. Rinse the flask with ~5 mL acetone and add the rinsate to the TurboVap vessel.
   d. Concentrate the filtrate to less than 25 mL (not to dryness) using a Turbovap Evaporator (water bath at 50°C, increase pressure up to 30 psi as volume decreases). **It is important to remove all traces of acetone!**
3. <u>Second Reflux</u>
   a. Transfer sample to a 500 mL boiling flask.
   b. Rinse TurboVap vessel with 2 x 5 mL of DI H_2O and add the rinsate to the boiling flask.
   c. Add 3.5 mL of concentrated HCl to make 1 N.
   d. Add stir bar or boiling stones.
   e. Place in heating mantle on stir plate and gently reflux for 2 hours.
4. <u>Filtration #2</u>
   a. Remove from heating mantle when cool enough to handle.
   b. Filter through Whatman GF/F (11cm) filter paper. Rinse boiling flask with 2 x 10 mL DI H_2O and add to the Buchner funnel.
   c. Transfer sample to a 100 mL graduated mixing cylinder. Rinse with 2 x 10 mL DI H_2O, and add the rinsate to the graduated mixing cylinder and bring the volume up to 100 mL.
   d. Shake the sample and take a 50 mL (5g) aliquot. Store one aliquot under refrigeration and continue on through the method with the other.
5. <u>SPE #1 - C_8 1g (Varian)</u>
   a. Condition: (cv = column volume, 6 mL)
      1cv MeOH
      1cv 0.25 N HCl
      Note: Maintain the flow rate through the C_8 cartridge at ~5 mL/min.
   b Close the cartridge and add ½ cv 0.25 N HCl.
   c. Attach a 75 mL reservoir with an adapter to the cartridge.
   d. Load the 50 mL sample.
   e. When the entire sample has passed through, use full vacuum briefly (~2 minutes).
   f. Dry the cartridge w/nitrogen, ~30 min @ 30 psi.
   g. Wash with 6 mL of 5% EtOAc/Hex.
   h. Remove C_8 cartridge and prepare SI cartridge (see below).

6. SPE #2 - SI 1g (Baker)
   a. Condition:
      1cv EtOAc
      1cv Hex
      Note: Maintain the flow rate through the SI cartridge at ~2 mL/min. Do not allow the cartridge to go dry.
   b. Close the cartridge and add 1 mL of 30% EtOAc/Hex.
   c. Attach C_8 cartridge to SI cartridge with an adapter.
   d. Elute with total 9 mL of 30% EtOAc/Hex from C_8 onto the SI cartridge as follows:
      1. Add 3 mL of 30% EtOAc/Hex to the C_8 cartridge.
      2. Open the cartridge and allow a few drops to drip from the C_8 into the SI before applying vacuum. This will help prevent the SI from going dry.
      3. When the first 3 mL has reached the top of the C_8 packing add 6 mL of 30% EtOAc/Hex.
   e. Remove the C_8 cartridge from the adapter on top of the SI when the elution solvent has reached the top of the C_8 cartridge packing.
   f. Allow the remaining C_8 cartridge eluant to drain to the top of the SI packing, then remove the adapter.
   g. Wash the SI cartridge with 3 mL of 30% EtOAc/Hex.
   h. Elute and collect with 6 mL of EtOAc into a 13 mL centrifuge tube.
   i. N_2-evap samples to near dryness in a water bath at ~45°C (**until a thin oily film remains, do not overdry!**).
7. Derivatization
   a. Add 0.5 mL of ACN.
   b. Add 100 uL of BSTFA (use within 10 minutes of opening ampule).
   c. Vortex for 15 sec.
   d. Add 9.5 mL of 10% EtOAc/Hex to make 10 mL total.
   e. Cap and vortex until mixed. **If phase separation occurs, gently warm in a water bath (~45°C) for ~1 minute. Vortex again. Repeat until mixed.**
8. SPE #3 - SI 1g (Baker)
   a. Condition:
      1cv EtOAc
      1cv Hex
      Note: Maintain the flow rate through the SI cartridge at ~2 mL/min. Do not allow the cartridge to go dry.
   b. Load 10 mL sample.
   c. Wash centrifuge tube with 2 x 3 mL of 10% EtOAc/Hex and add it to the cartridge.
   d. Elute and collect with 9 mL of 50% EtOAc/Hex into a 13 mL centrifuge tube.
   e. Add 1.0 mL of ACN.
   f. N_2-evap samples to near dryness in a water bath at ~45°C (**until a thin oily film remains, do not overdry!**).
   g. Dilute to the appropriate final volume with ACN.
9. Inject GC/ELCD

B. Instrument Parameters

1. GC/ELCD

INSTRUMENT	: HP 6890 GC
COLUMN	: J&W DB-35, 35% phenyl methyl silicone, 30 m x 0.54 mm, 1.0 μm film thickness
INLET	: Splitless Injection Mode : Cyclo-double gooseneck insert
DETECTOR	: OI Analytical 5220 Electrolytic Conductivity, Halogen Mode
TEMPERATURE PROGRAM:	
Injection Port	: 250°C
Oven	: 180°C/1 minute (initial) : 20°C/minute (ramp) : 260°C/2 minutes (hold) : 5°C/minute (ramp) : 280°C/4 minutes (final)
Reactor	: 900°C
COLUMN GAS FLOW	: He, Carrier, ~16 mL/minute
% 1-PROPANOL FLOW	: 37%
ELCD GAS FLOW:	
H_2 + Carrier, unvented	: ~135 mL/minute
H_2 + Carrier, vented	: ~85 mL /minute

ELCD CONTROLLER SETTINGS:

Configure Menu:

SIG: Linearize: OFF
Smoothing: ON
Calibrate: OFF

VLV: Energized = ON

TEMP: STD

DET: Sensitivity = 4x
Mode = H

Valve Time:
Vent on: 0.01 min
Vent off: 2.5 min

INJECTION VOLUME	: 2 μL
RUN TIME	: 15 minutes
RETENTION TIMES	:~9.1 minutes (DMS) :~9.9 minutes (sulfentrazone) :~12.7 minutes (derivatized HMS)

2. GC/MSD (Analyte confirmation)

INSTRUMENT	: HP 5890 GC
COLUMN	: J&W DB-5MS, Crosslinked 5% phenyl methyl silicone 15 m x 0.25 mm, 0.25 µm film thickness
INLET	: Splitless Injection Mode : Cyclo-double gooseneck insert
DETECTOR	: HP 5970 Mass Selective Detector
TEMPERATURES:	
Injection Port	: 260°C
Oven	: 120°C/2 minutes (initial) : 20°C/minute (ramp) : 280°C/6 minutes (final)
Detector	: 280°C
GAS FLOW	: He, Carrier, 1 mL/minute
INJECTION VOLUME	: 2 µL
RUN TIME	: 16 minutes
RETENTION TIMES	:~7.8 minutes (DMS, m/z = 293) :~8.5 minutes (sulfentrazone, m/z = 307) :~9.5 minutes (derivatized HMS, m/z = 459)

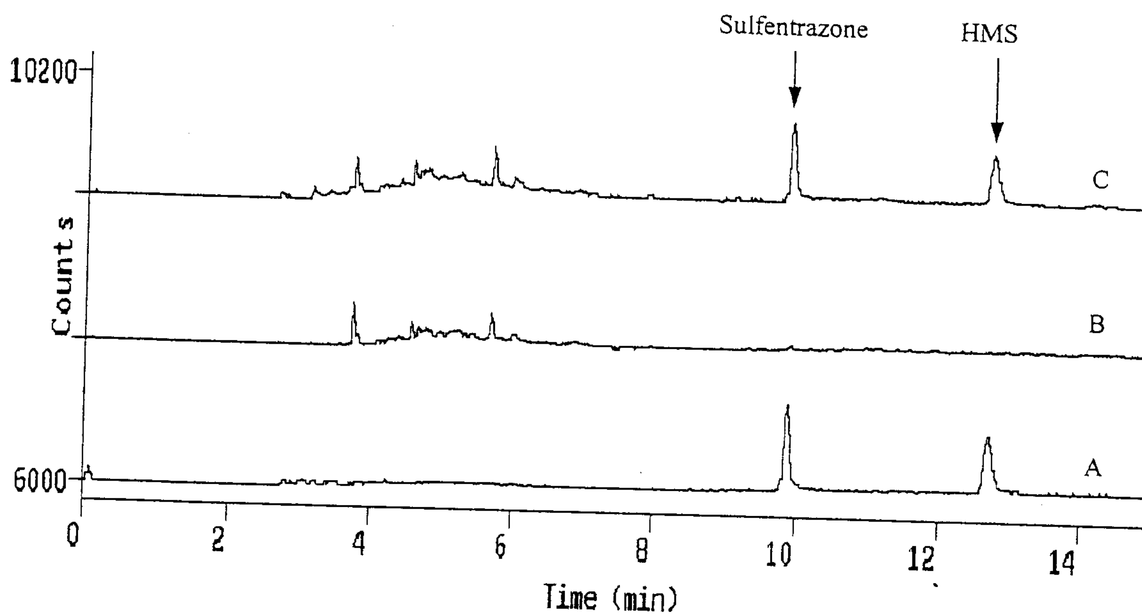
C. Chromatograms

CHROMATOGRAM INDEX

Figure Number	Description
2	Soybeans, Set: S3R, GC/ELCD Standard 932-1 (diluted), 0.125 ng/μL Assay #S3R-1, Control Assay #S3R-2, Fortified 0.025 ppm
3	Corn Forage, Set: C4, GC/ELCD Standard 929-1 (diluted), 0.125 ng/μL Assay #C4-1, Control Assay #C4-2, Fortified 0.025 ppm
4	Rice Straw, Set: R4R2, GC/ELCD Standard 937-1 (diluted), 0.25 ng/μL Assay #R4R2-1, Control Assay #R4R2-2, Fortified 0.5 ppm
5	Sorghum Fodder, Set: SG5, GC/ELCD Standard 929-1 (diluted), 0.125 ng/μL Assay #SG5-5, Control Assay #SG5-2, Fortified 0.025 ppm
6	Winter Wheat Hay, Set: W5, GC/ELCD Standard 937-1 (diluted), 0.125 ng/μL Assay #W5-1, Control Assay #W5-2, Fortified 0.05 ppm

FIGURE 2

SULFENTRAZONE AND HMS IN/ON SOYBEAN SEED
SET NUMBER: S3R, GC/ELCD

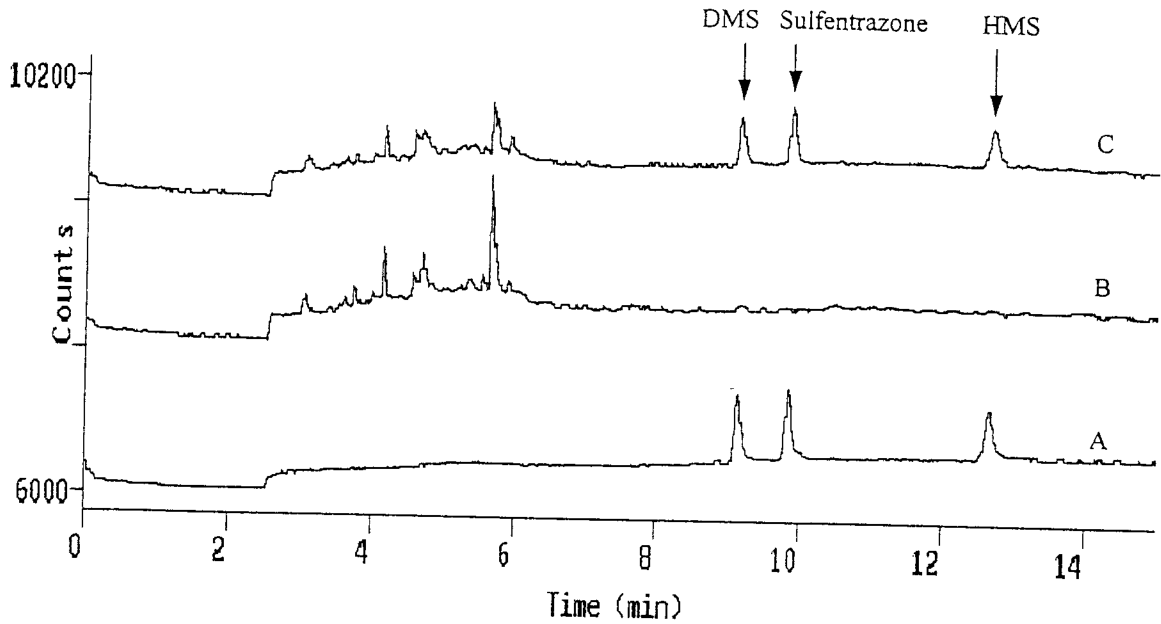


	Assay No.	Sample Type	Amount Injected	sulfentrazone Peak Area	sulfentrazone Amount Detected	HMS Peak Area	HMS Amount Detected
A	932-1(dil)[a]	Standard	0.25 ng	5402[b]	0.24 ng	4606	0.23 ng
B	S3R-1	Control	10 mg	0	ND[c]	0	ND
C	S3R-2	Fort @ 0.025 ppm	10 mg	4702	84%	4014	80%

a A measured volume of Standard #932-1 was derivatized and diluted to produce the run standard.
b The average standard peak area was 5570 for sulfentrazone and 5033 for HMS.
c ND = Not detected (< 0.005 ppm).

FIGURE 3

SULFENTRAZONE, DMS/SCA AND HMS IN/ON FIELD CORN FORAGE
SET NUMBER: C4, GC/ELCD

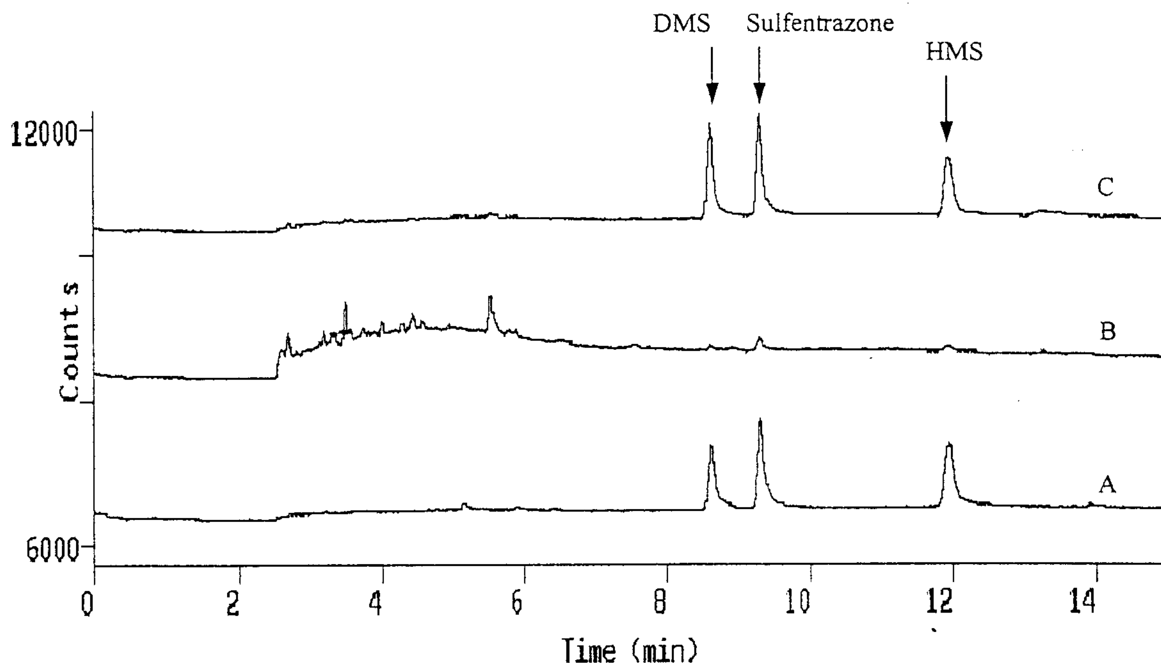


	Assay No.	Sample Type	Amount Injected	sulfentrazone Peak Area	sulfentrazone Amount Detected	DMS/SCA Peak Area	DMS/SCA Amount Detected	HMS Peak Area	HMS Amount Detected
A	929-1 (dil)[a]	Standard	0.25 ng	4811[b]	0.26	4546	0.24	4233	0.25
B	C4-1	Control	10 mg	0	ND[c]	0	ND	211	ND
C	C4-2	Fort @ 0.025 ppm	10 mg	3762	80%	2909	76%[d]	3333	79%

a A measured volume of Standard #929-1 was derivatized and diluted to produce the run standard.
b The average standard peak area was 4671 for sulfentrazone, 4663 for DMS and 4222 for HMS.
c ND = Not detected (< 0.005 ppm).
d A correction factor was included in the calculation for the method recovery of SCA to compensate for the molecular weight ratio of DMS to SCA and to compensate for the acid standard concentration (417/373 x 1/0.918).

FIGURE 4

SULFENTRAZONE, DMS/SCA AND HMS IN/ON RICE STRAW
SET NUMBER: R4R2, GC/ELCD

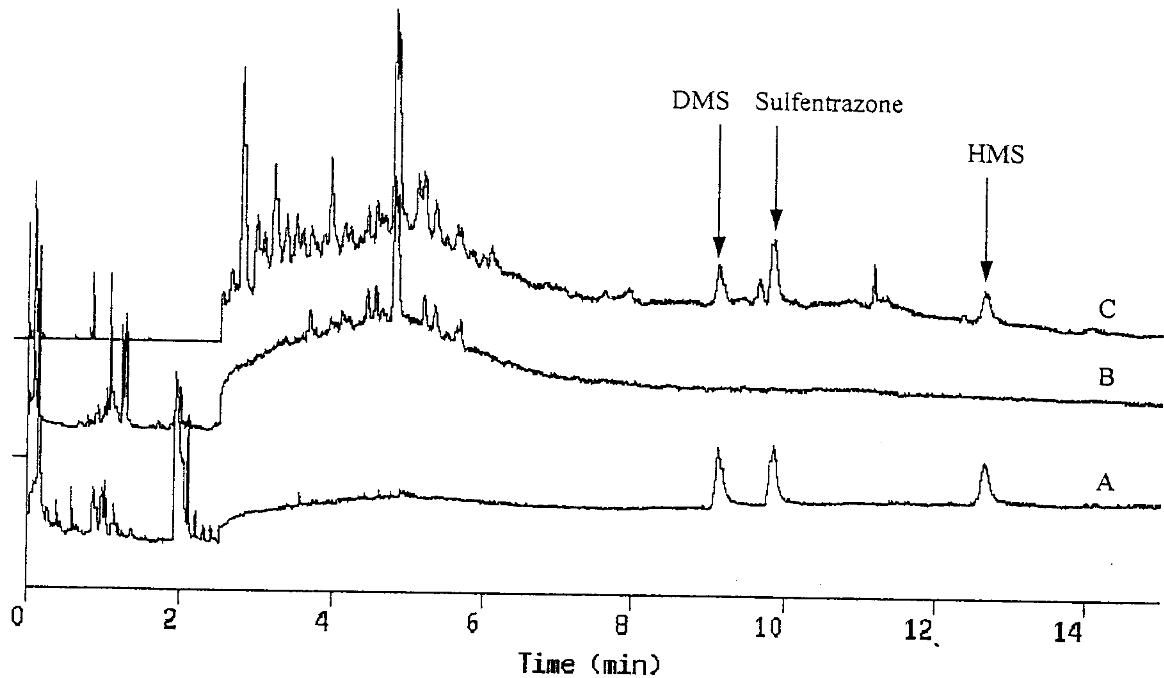


	Assay No.	Sample Type	Amount Injected	sulfentrazone Peak Area	sulfentrazone Amount Detected	DMS/SCA Peak Area	DMS/SCA Amount Detected	HMS Peak Area	HMS Amount Detected
A	937-1 (dil)[a]	Standard	0.5 ng	9001[b]	0.45	6557	0.43	7125	0.42
B	R4R2-1	Control	10 mg	1121	ND[c]	568	ND	0	ND
C	R4R2-2	Fort @ 0.5 ppm	1 mg	9307	94%	8095	129%[d]	6588	77%

a A measured volume of Standard #937-1 was derivatized and diluted to produce the run standard.
b The average standard peak area was 9949 for sulfentrazone, 7620 for DMS and 8505 for HMS.
c ND = Not detected (< 0.005 ppm).
d A correction factor was included in the calculation for the method recovery of SCA to compensate for the molecular weight ratio of DMS to SCA and to compensate for the acid standard concentration (417/373 x 1/0.918).

FIGURE 5

SULFENTRAZONE, DMS/SCA AND HMS ON SORGHUM FODDER
SET NUMBER: SG5, GC/ELCD

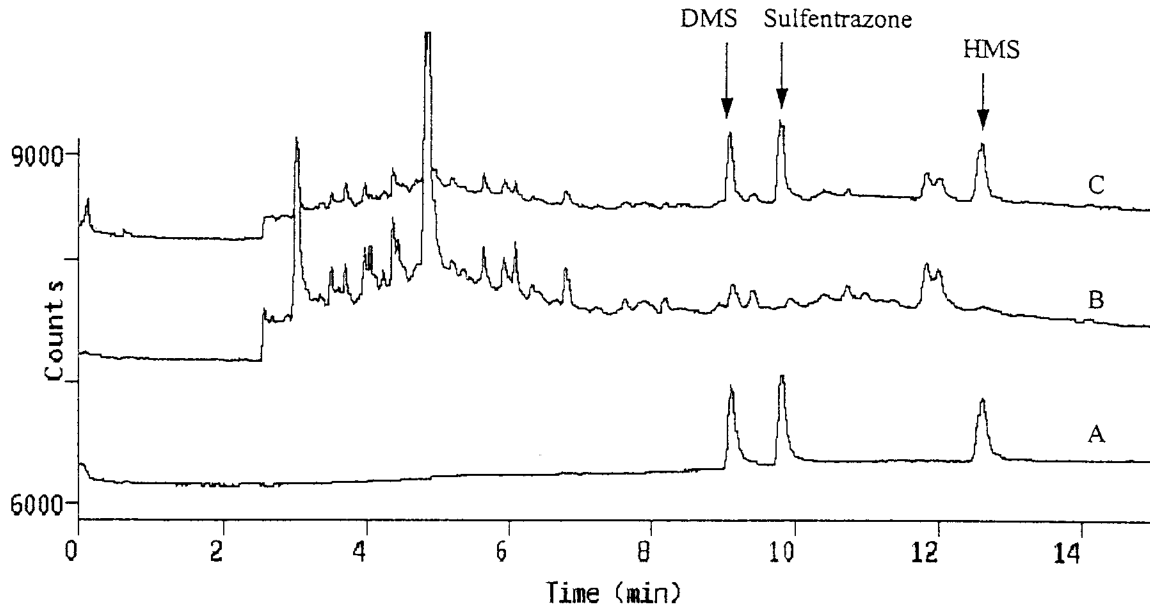


	Assay No.	Sample Type	Amount Injected	sulfentrazone Peak Area	sulfentrazone Amount Detected	DMS/SCA Peak Area	DMS/SCA Amount Detected	HMS Peak Area	HMS Amount Detected
A	929-1 (dil)[a]	Standard	0.25 ng	5205[b]	0.25 ng	5104	0.24 ng	4481	0.24 ng
B	SG5-5	Control	10 mg	899	ND[c]	0	ND	521	ND
C	SG5-2	Fort @ 0.025 ppm	10 mg	4321	82%	3694	84%[d]	3297	70%

a A measured volume of Standard #929-1 was derivatized and diluted to produce the run standard.
b The average standard peak area was 5266 for sulfentrazone, 5375 for DMS and 4706 for HMS.
c ND = Not detected (< 0.005 ppm).
d A correction factor was included in the calculation for the method recovery of SCA to compensate for the molecular weight ratio of DMS to SCA and to compensate for the acid standard concentration (417/373 x 1/0.918).

FIGURE 6

SULFENTRAZONE, DMS/SCA AND HMS ON WINTER WHEAT HAY
SET NUMBER: W5, GC/ELCD



	Assay No.	Sample Type	Amount Injected	sulfentrazone Peak Area	sulfentrazone Amount Detected	DMS/SCA Peak Area	DMS/SCA Amount Detected	HMS Peak Area	HMS Amount Detected
A	937-1 (dil)[a]	Standard	0.25 ng	5847[b]	0.27 ng	5709	0.26 ng	5455	0.27 ng
B	W5-1	Control	10 mg	535	ND[c]	1664	ND	0	ND
C	W5-2	Fort @ 0.05 ppm	5 mg	4684	85%	4172	93%[d]	4280	85%

a A measured volume of Standard #937-1 was derivatized and diluted to produce the run standard.
b The average standard peak area was 5508 for sulfentrazone, 5459 for DMS and 5034 for HMS.
c ND = Not detected (< 0.01 ppm).
d A correction factor was included in the calculation for the method recovery of SCA to compensate for the molecular weight ratio of DMS to SCA and to compensate for the acid standard concentration (417/373 x 1/0.918).