B95-76
METHOD

ATTACHMENT 2

FMC CORPORATION
AGRICULTURAL CHEMICAL GROUP
Princeton, New Jersey

STUDY TITLE: Analytical Methodology for the Determination of Sulfentrazone and Its Metabolites in/on Winter Wheat

TEST SUBSTANCES: Sulfentrazone, 3-desmethyl sulfentrazone, 3-hydroxymethyl sulfentrazone and 3-desmethyl-4-desdifluoromethyl sulfentrazone

DATA REQUIREMENT: Pesticide Assessment Guidelines, Subdivision O, 171-4: Residue Analytical Method

AUTHOR: In-Young Kim, Ph. D.

STUDY DATES:

Study Initiated:	September 10, 1993
Experiment Terminated:	February 6, 1995
Study Completed:	April 7, 1995

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STUDY NUMBER: 162WHW93R1

Non Propriety Information

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

FMC CORPORATION

STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS

No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of FIFRA §10(d)(1)(A), (B), OR (C).

Company: FMC Corporation

John M. Becker
Manager, Residue Chemistry
COMPANY AGENT

04/10/95
Date

GOOD LABORATORY PRACTICES STATEMENT

The study in which these analytical methods were developed and applied (Study Number: 162WHW93R1 "Analytical Methodology for the Determination of Sulfentrazone and Its Metabolites in/on Winter Wheat", FMC Corporation, Agricultural Chemical Group, Report P-2982, April 07, 1995) was conducted and reported in compliance with the Good Laboratory Practice Standards set forth in Title 40, Part 160 of the Code of Federal Regulations of the United States of America.

In-Young Kim
Residue Chemistry
STUDY DIRECTOR

4-7-95
Date

John M. Becker
Manager, Residue Chemistry
SPONSOR

04/10/95
Date

Callista O. Chukwunenye
Manager, Product Registration
SUBMITTER

4/12/95
Date

QUALITY ASSURANCE STATEMENT

It is the intent of FMC Corporation that all studies sponsored by or conducted by our facility shall be of the highest quality and meet or exceed the criteria promulgated by the EPA to assure the quality and integrity of the data generated. Study 162WHW93R1, "Analytical Methodology for the Determination of Sulfentrazone and Its Metabolites in/on Winter Wheat", reported herein, was inspected and the findings signed by the Study Director and management of FMC Corporation on the following dates:

Inspection Date	Signed by Study Director	Signed by Management	Signed by Director
06/14/94	06/22/94	06/23/94	06/28/94
11/22/94	11/23/94	11/29/94	11/30/94

This report and all records and raw data were audited and the report was found to be an accurate reflection of the study. All raw data will be maintained by FMC Corporation, PO Box 8, Princeton, NJ 08543 in the Quality Assurance Unit Archives.

Katherine A. Boyler
Quality Assurance Specialist

4/7/95
Date

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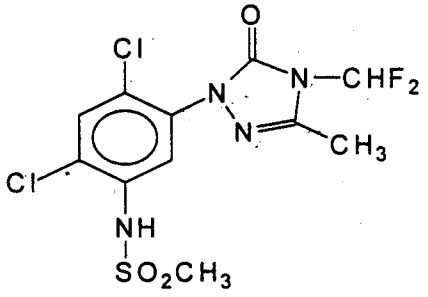
I. INTRODUCTION

Authority® (F6285) 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 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 crop metabolites of sulfentrazone have been identified as 3-desmethyl sulfentrazone, 3-hydroxymethyl sulfentrazone, and 3-desmethyl-4-desdifluoromethyl sulfentrazone. The structure of sulfentrazone is as follows:



Sulfentrazone

A winter wheat crop rotation study (Section XI, Reference 1) has been conducted following the harvest of soybeans which were treated with Authority 75DF. In the winter wheat crop rotation study, residue analytical methods were developed based on previous methodology on soybeans. On the wheat matrices, analyses were performed for sulfentrazone, 3-desmethyl sulfentrazone, 3-hydroxymethyl sulfentrazone, and 3-desmethyl-4-desdifluoromethyl sulfentrazone (forage only). The purpose of this report is to describe the residue analytical methods for sulfentrazone and its metabolites in/on winter wheat matrices.

II. SUMMARY

The residue analytical methods were developed based on previous methodology on soybeans for sulfentrazone and its metabolites (3-desmethyl sulfentrazone, 3-hydroxymethyl sulfentrazone, and 3-desmethyl-4-desdifluoromethyl sulfentrazone) in/on winter wheat matrices (grain, forage and straw).

The analytical methods for sulfentrazone and its metabolites included a solvent/acid reflux, filtration, extraction, clean-up and analysis by a gas chromatograph (GC) equipped with an electron capture detector (ECD). The analyses for 3-hydroxymethyl sulfentrazone and 3-desmethyl-4-desdifluoromethyl sulfentrazone also included a derivatization step. The limits of quantitation (LOQ) were validated at 0.025 ppm and the limits of detection (LOD) were set at 0.005 ppm (for 3-desmethyl-4-desdifluoromethyl sulfentrazone only, the LOQ was validated at 0.05 ppm and the LOD was set at 0.01 ppm). The average method recoveries were 87% ±11% (n=16) for sulfentrazone, 88% ±14% (n=16) for 3-desmethyl sulfentrazone, 74% ±7% (n=14) for 3-hydroxymethyl sulfentrazone and 109% ±17% (n=6) for 3-desmethyl-4-desdifluoromethyl sulfentrazone.

III. SUMMARY TABLES AND GRAPHICS

A. Method Recoveries

TABLE 1

METHOD RECOVERY VALUES FOR SULFENTRAZONE AND ITS METABOLITES FROM LABORATORY FORTIFIED WINTER WHEAT MATRICES

Matrix/ Analyte	Fortification Level (ppm)	Number of Analyses	Recovery Range (%)	Average Recovery (%) ± Standard Deviation (%)
Forage				
Sulfentrazone	0.025	1	88	88
	0.05	4	81 - 112	95 ±14
	0.10	1	89	89
3-Desmethyl sulfentrazone	0.025	1	75	75
	0.05	4	77 - 109	92 ±14
	0.10	1	98	98
3-Hydroxymethyl sulfentrazone	0.025	2	74 - 87	81
	0.05	1	65	65
	0.10	1	72	72
3-Desmethyl-4-desdifluoromethyl sulfentrazone	0.05	5	76 - 122	110 ±19
	0.10	1	104	104
Grain				
Sulfentrazone	0.025	1	82	82
	0.05	4	83 - 101	90 ±8
	0.10	1	91	91
3-Desmethyl sulfentrazone	0.025	1	73	73
	0.05	4	80 - 106	97 ±11
	0.10	1	102	102
3-Hydroxymethyl sulfentrazone	0.025	2	69 - 84	77
	0.05	3	70 - 78	74 ±4
	0.10	1	67	67
Straw				
Sulfentrazone	0.025	2	64 - 81	73
	0.05	2	72 - 86	79
3-Desmethyl sulfentrazone	0.025	2	79 - 87	83
	0.05	2	67 - 75	71
3-Hydroxymethyl sulfentrazone	0.025	3	63 - 82	75 ±10
	0.05	1	73	73

	Fortification Levels (ppm)	Number of Analyses	Overall Range (%)	Overall Average (%) Standard Deviation (%)
Sulfentrazone	0.025 - 0.10	16	64 - 112	87 ±11
3-Desmethyl sulfentrazone	0.025 - 0.10	16	67-109	88 ±14
3-Hydroxymethyl sulfentrazone	0.025 - 0.10	14	63 - 87	74 ±7
3-Desmethyl-4-desdifluoromethyl sulfentrazone	0.05 - 0.10	6	76 - 122	109 ±17

B. Method Flow Schemes

FIGURE 1

FLOW SCHEME FOR SULFENTRAZONE AND 3-DESMETHYL SULFENTRAZONE IN/ON WINTER WHEAT FORAGE, GRAIN AND STRAW

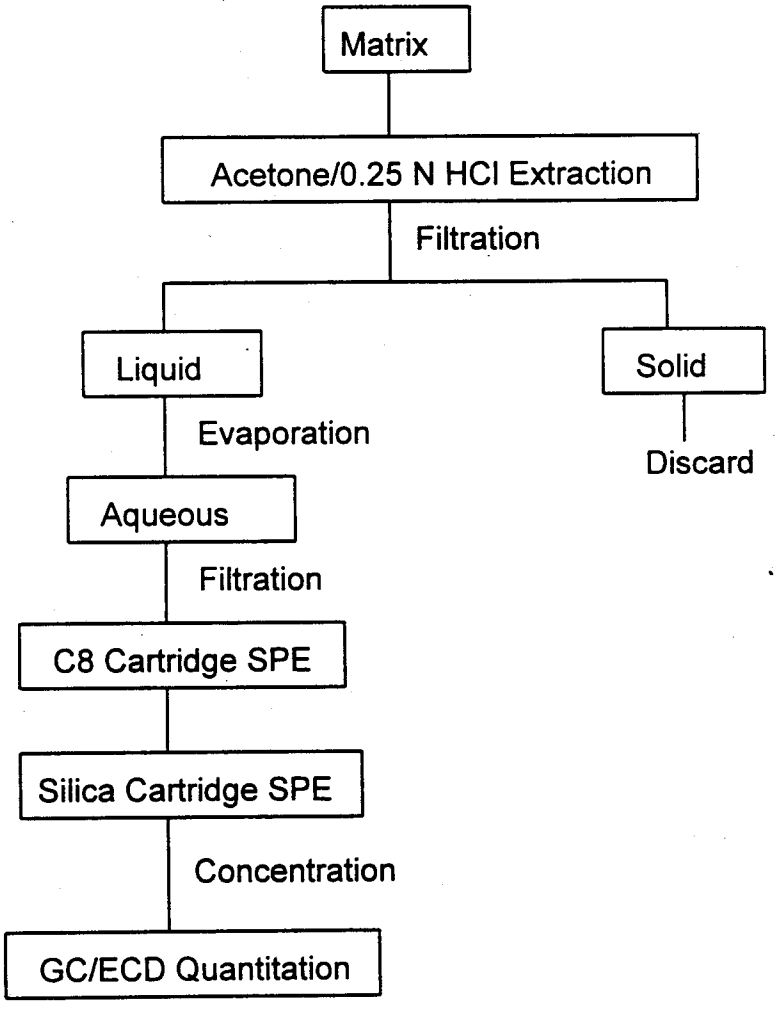


FIGURE 2

FLOW SCHEME FOR 3-HYDROXYMETHYL SULFENTRAZONE
IN/ON WINTER WHEAT FORAGE, GRAIN, AND STRAW

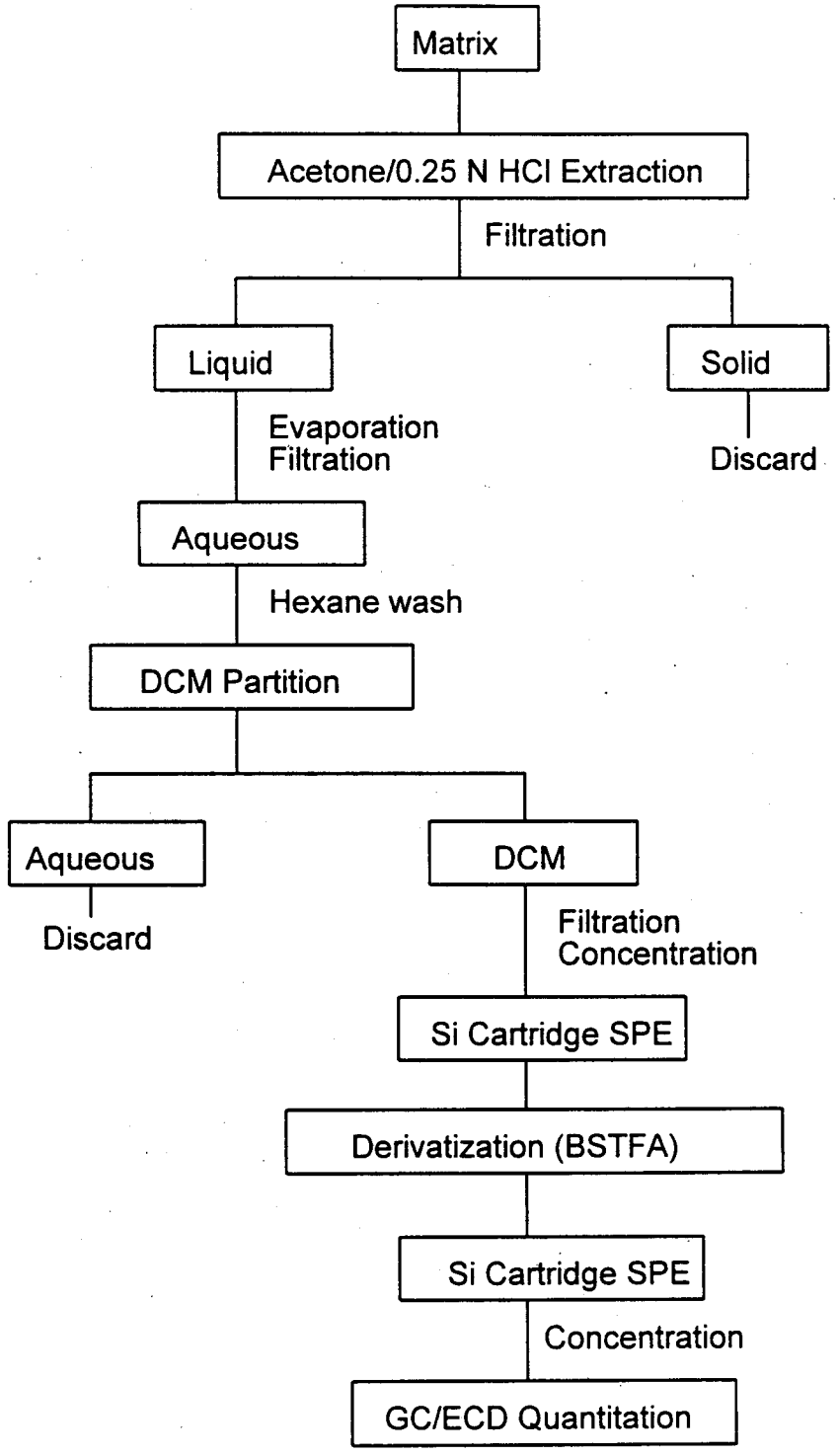
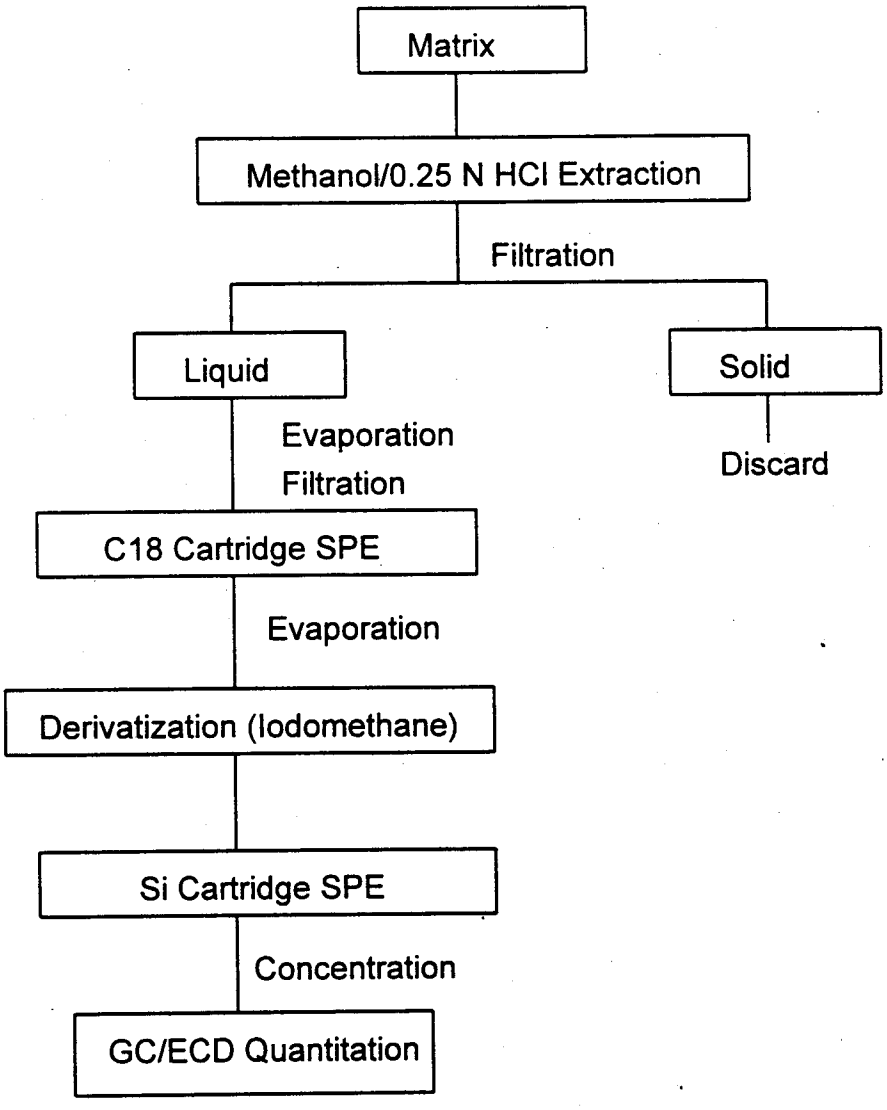


FIGURE 3

FLOW SCHEME FOR 3-DESMETHYL-4-DESDIFLUOROMETHYL SULFENTRAZONE IN/ON WINTER WHEAT FORAGE



IV. MATERIALS AND STUDY DESIGN

A. Test Substances

The test substances used in the winter wheat analytical methods were sulfentrazone, 3-desmethyl sulfentrazone, 3-hydroxymethyl sulfentrazone and 3-desmethyl-4-desdifluoromethyl sulfentrazone. The chemical names, chemical abstract service numbers, residue inventory numbers, and purities of the analytical standards are listed in Section X, Table 2.

B. Test Commodities

Winter wheat is part of the cereal grain crop group. The test commodities analyzed were wheat forage, grain, and straw.

C. Study Design and Procedures

The residue methods were validated with acceptable and reproducible recoveries. Method recoveries used in this report were generated concurrently with the residue data of a winter wheat rotational crop study (Section XI, Reference 1). Control samples of winter wheat were fortified by adding known amounts of sulfentrazone and/or its metabolites. Standard solutions of the analytes were prepared and the solution was added by syringe directly onto the matrix. The solvent was allowed to evaporate and the fortified sample was analyzed as part of an assay set with a minimum of one control sample to determine the method recovery. Generally, samples were analyzed within two days of extraction. No additional laboratory work was conducted for this report.

D. Analytical Standards

The structures and purities of the analytical standards are shown in Section X, Table 2. Stock solutions of 1000 ng/µL of sulfentrazone and its metabolites were prepared individually by dissolving appropriate amounts of the analytical standards in acetonitrile (methanol for 3-desmethyl-4-desdifluoromethyl sulfentrazone) using volumetric flasks. Working solutions of 3-hydroxymethyl sulfentrazone and 3-desmethyl-4-desdifluoromethyl sulfentrazone, individually, were prepared monthly from the stock solutions. Combined working standard solutions containing sulfentrazone and 3-desmethyl sulfentrazone were prepared monthly from the stock solutions. The working standard solutions were used for fortification, injection standards, and instrument linearity calibrations. Stock and working solutions were stored in volumetric containers in a refrigerator/freezer unit to

insure maintenance of proper concentrations. Information on the reference solutions is shown in Section X, Table 3.

E. Equipment

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

Access*Chrom Data Acquisition software running on MicroVax
Balance, Analytical PM 2000, Mettler
Balance, Top Loading, Mettler
Buchner filter funnels, Porcelain, 10.5 cm i.d., Coors
Capillary column, DB-5, 15 m x 0.25 mm id, 0.25 µm, J & W Scientific
Capillary column, DB-5, 30 m x 0.53 mm id, 1.5 µm, J & W Scientific
Capillary column, DB-17, 30 m x 0.53 mm id, 1.0 µm, J & W Scientific
Centrifuge Tubes, 13 mL graduated, 0.1 mL, Pyrex®
Condensers, Pyrex, Graham coil, 41 mm x 500 mm with T 24/40 joint
Cyclo-uniliner®, Restek
Cylinders, Graduated, 50 mL, 100 mL, 250 mL
Dishwasher, BetterBuilt Turbomatic Jr. (Model 7000)
Flasks, Erlenmeyer, Pyrex, 250 mL
Flasks, Filter flasks, 250 mL
Flasks, Round Bottom Boiling, 250 mL, 24/40 joint
Flasks, Volumetric, 100 mL
Gas Chromatograph (Hewlett-Packard 5890 equipped with a HP 7673A Autosampler and an ECD or HP 5970 MSD)
Heating Mantles, 250 mL, Glas-Col®)
Magnetic Stirrers, VWR Scientific, Model 200
Micro Syringes, (25 µL, 50 µL, 100 µL, 250 µL, 500 µL), Hamilton
Multi - Tube Vortexer, VWR Scientific
N-EVAP® Evaporator
Pipets, Disposable
Pipets, Volumetric
Reducing Adapters
Reservoirs, Plastic, 75 mL
Separatory funnels, 250 mL
Single Taper Liner, Hewlett Packard
Solid Phase Extraction Cartridge, Silica gel (1 g), JT Baker
Solid Phase Extraction Cartridge, C_8 (1 g), Varian
Solid Phase Extraction Cartridge, C_{18} (2 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
Whatman Glass MicroFibre No. 934-AH, 7 cm
Whatman Glass MicroFibre GFF, 11 cm

F. 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
Iodomethane, Aldrich Chemical (99.5%)
Methanol, Resi-Analyzed, JT Baker
Methylene Chloride (Resi-analyzed), JT Baker
pH Indicator Strips (EM Science)
Sodium Chloride, Reagent Grade, JT Baker
Sodium Hydroxide, VWR scientific
Toluene, Resi-Analyzed, JT Baker

V. ANALYTICAL PROCEDURE

A. Residue Method

Methodology was developed for the determination of sulfentrazone and its metabolites (3-desmethyl sulfentrazone, 3-hydroxymethyl sulfentrazone, and 3-desmethyl-4-desdifluoromethyl sulfentrazone) in/on winter wheat grain, forage and straw (Section XI, Reference 2 and 3). Clean-up procedures varied for the various matrices. These analytical methods were improved and used in a second year residue winter wheat rotational crop program (Section XI, Reference 1). These methods are similar to the method for sulfentrazone and 3-hydroxymethyl sulfentrazone in/on soybeans which was submitted to the EPA and validated by an independent laboratory (Section XI, Reference 4).

1. Sulfentrazone and 3-desmethyl sulfentrazone (Grain, Forage, and Straw) (Section III, B, Figure 1 for flow chart)

   a. Extraction

   Weigh 2.5 g of the winter wheat sample into a 250 mL round bottom boiling flask. Fortify the method recovery control sample by adding an appropriate volume of standard solution (containing sulfentrazone and 3-desmethyl sulfentrazone) by syringe. Add 100 mL acetone/0.25N HCl (3/1, v/v) and a teflon stirring bar. Gently boil the solution with stirring under reflux for one hour (Variac at ~55). Cool the solution to room temperature and filter through a Whatman glass microfibre filter (No. 934-AH) using a Buchner funnel and vacuum. Pre-rinse the filter paper with ~5 mL of acetone and discard the rinsate. Rinse the boiling flask and post-reflux solid with ~70 mL acetone and pass the rinsate through the filter. Transfer the solution to a 200 mL TurboVap vessel. Rinse the filter flask with ~30 mL acetone and add the rinsate to the TurboVap vessel. Concentrate the sample solution to less than 20 mL using a TurboVap Evaporator. **It is important to remove all traces of acetone.** Filter the concentrated aqueous solution through a Whatman glass microfibre filter (No. 934-AH) using a Buchner funnel and vacuum. Rinse the TurboVap vessel with ~50 mL 0.25N HCl and pass the rinsate through the filter.

   b. C_8 Cartridge Solid Phase Extraction

   Condition a C_8 cartridge (1 g) with 12 mL methanol followed by 12 mL 0.25N HCl. **Do not allow the cartridge to go dry.** Add 6 mL 0.25N HCl to the cartridge barrel. Attach a 75 mL reservoir with a reducing adaptor. Transfer the aqueous sample solution to the reservoir. Rinse the filter flask with ~10 mL 0.25N HCl and add the rinsate to the reservoir. Pass the entire solution through the C_8 cartridge. Maintain the flow rate at ~5 mL/min by regulating the vacuum pump. Blow the cartridge completely dry with nitrogen using a manifold drying attachment (~20 psi for 30 minutes). Rinse the manifold spigots with acetone (Do not allow the solvent to come in contact with the cartridges!). **It is important that the cartridge and manifold are completely dry,** increase nitrogen pressure or extend the drying time if necessary. Elute the analytes with 12 mL 30% ethyl acetate/hexane into a 13 mL glass centrifuge tube. Evaporate the eluate under nitrogen stream to ~0.5 mL and add 6 mL 20% ethyl acetate/hexane.

c. Silica Gel Cartridge Solid Phase Extraction

Condition a silica gel cartridge (1 g) with 6 mL 40% ethyl acetate/ hexane followed by 6 mL hexane. **Do not allow the cartridge to go dry.** Load the sample into the cartridge barrel. Rinse the centrifuge tube with 1 mL 20% ethyl acetate/hexane and add the rinsate to the cartridge barrel. Drain the cartridge until the sample solution reaches the top of the cartridge packing. Maintain the flow rate at ~5 mL/min during the elution by regulating the vacuum pump. Elute the analytes with 12 mL 40% ethyl acetate/hexane into a clean 13 mL glass centrifuge tube. Evaporate the sample solution under nitrogen stream to dryness and add exactly 1.0 mL acetonitrile. Analyze the acetonitrile extract by GC-ECD.

2. 3-Hydroxymethyl sulfentrazone (Grain, Forage, and Straw)
(Section III, B, Figure 2 for flow chart)

a. Extraction:

Weigh 2.5 g of the winter wheat sample into a 250 mL round bottom boiling flask. Fortify the method recovery control sample by adding an appropriate volume of standard solution (containing 3-hydroxymethyl sulfentrazone) by syringe. Add 100 mL acetone/0.25N HCl (3/1, v/v) and a teflon stirring bar. Gently boil the solution under reflux for one hour (Variac at ~55). Cool the solution to room temperature and filter through a Whatman glass microfibre filter (No. 934-AH) using a Buchner funnel and vacuum. Pre-rinse the filter paper with ~5 mL of acetone and discard the rinsate. Rinse the boiling flask and post-reflux solid with ~30 mL acetone and pass the rinsate through the filter. Transfer the solution to a 200 mL TurboVap vessel. Rinse the filter flask with ~10 mL acetone and add the rinsate to the TurboVap vessel. Concentrate the sample solution to less than 20 mL using a TurboVap Evaporator. **It is important to remove all traces of acetone.** Filter the concentrated aqueous solution through a Whatman glass microfibre filter (No. 934-AH) using a Buchner funnel and vacuum. Pre-rinse the filter paper with 0.25 N HCl and discard the rinsate. Rinse the TurboVap vessel with ~20 mL 0.25N HCl and pass the rinsate through the filter.

b. Hexane Wash

Transfer the concentrated aqueous filtrate to a 250 mL separatory funnel. Add ~1 g sodium chloride and 50 mL hexane. Stopper the separatory funnel and hand shake for ~1 minute (be sure to vent the separatory funnel

several times). Collect the lower aqueous phase in a 250 mL Erlenmeyer flask and discard the upper hexane phase. Return the aqueous portion to the separatory funnel and add 50 mL hexane. Repeat the partition, collect the lower aqueous phase in the Erlenmeyer flask and discard the hexane phase. Return the aqueous portion to the separatory funnel.

c. Dichloromethane Partition

Use 50 mL dichloromethane (DCM) to rinse the Erlenmeyer flask. Transfer the DCM to the separatory funnel, stopper, and hand shake for ~1 minute. Collect the lower DCM phase in a 200 mL TurboVap vessel. Add an additional 50 mL DCM to the separatory funnel and repeat the partition. Drain the lower DCM phase into the TurboVap vessel and discard the upper aqueous phase. Concentrate the sample solution to ~3 mL using a TurboVap Evaporator. Transfer the solution to a 15 mL centrifuge tube. Rinse the TurboVap vessel with ~3 mL DCM and add the rinsate to the centrifuge tube. The sample volume should be 6 mL, add DCM if necessary.

d. Silica Gel Cartridge Solid Phase Extraction

Condition a silica gel (1 g) cartridge with 6 mL acetonitrile followed by 6 mL DCM. **Do not allow the cartridge to go dry.** Load the sample into the cartridge barrel. Drain the cartridge until the sample solution reaches the top of the cartridge packing. Maintain the flow rate at ~5 mL/min by regulating the vacuum pump. Rinse the centrifuge tube with 6 mL DCM and pass the rinsate through the cartridge. Wash the cartridge with 12 mL 10% acetonitrile/DCM. Elute with 15 mL 25% acetonitrile/DCM into a clean 13 mL glass centrifuge tube. Evaporate the sample solution under nitrogen stream to less than 0.5 mL and add 1 mL acetonitrile.

e. Derivatization

Add 100 µL BSTFA, stopper and vortex for ~15 seconds. Heat in a water bath at 70°C for one hour. Evaporate the solution under nitrogen stream to ~0.1 mL, an oily film will form. **Do not dry completely!** (If the oily film hardens and gets dark brown, it is overdried.) Add 6 mL 20% ethyl acetate/hexane and vortex for ~15 seconds to resuspend the sample.

Prepare injection standards simultaneously by accurately measuring with a volumetric pipet the appropriate volume of 3-hydroxymethyl sulfentrazone standard (the concentration should be equal to the injection concentration

of the fortified sample) and placing it in a 13 mL glass centrifuge tube. Use 1 mL of standard solution for each standard required (example: if 5 injection standards are required, use 5 mLs of standard solution). Add 100 µL BSTFA per milliliter of standard solution measured. Stopper and vortex for ~15 seconds. Heat in a water bath at 70°C for one hour. Remove from the water bath. Evaporate to **complete** dryness. Traces of BSTFA will cause the GC peaks to broaden and tail. Add an exact volume of acetonitrile equal to the volume of standard solution measured before derivatization to restore the original standard concentration.

f. Silica Gel Cartridge Solid Phase Extraction

Condition a silica gel (1 g) cartridge with 6 mL 45% ethyl acetate/hexane followed by 6 mL hexane. **Do not let the cartridge to go dry.** Load the sample into the cartridge barrel. Drain the cartridge until the sample solution reaches the top of the cartridge packing. Maintain the flow rate at ~5 mL/min by regulating the vacuum pump. Rinse the centrifuge tube with 6 mL 20% ethyl acetate/hexane and pass the rinsate through the cartridge. Wash with 6 mL 30% ethyl acetate/hexane. Elute with 12 mL 45% ethyl acetate/hexane into a 15 mL centrifuge tube. Evaporate the sample solution under nitrogen stream to dryness. Add exactly 1.0 mL acetonitrile. Analyze the acetonitrile extract by GC-ECD.

3. 3-Desmethyl-4-desdifluoromethyl sulfentrazone (Forage only)
(Section III, B, Figure 3 for flow chart)

a. Extraction

Weigh 5 g of the winter wheat sample into a 250 mL round bottom boiling flask. Fortify the method recovery control sample by adding an appropriate volume of standard solution (containing 3-desmethyl-4-desdifluoromethyl sulfentrazone) by syringe. Add 100 mL methanol/0.25N HCl (3/1, v/v) and a teflon stirring bar. Gently boil the solution under reflux for one hour (Variac at ~55). Cool the solution to room temperature and filter through a Whatman glass microfibre filter (No. 934-AH) using a Buchner funnel and vacuum. Pre-rinse the filter paper with ~5 mL methanol/0.25 N HCl and discard the rinsate. Rinse the boiling flask and post-reflux solid with ~10 mL methanol/0.25 N HCl and pass the rinsate through the filter. Transfer the filtrate to a 200 mL TurboVap vessel. Rinse the filter flask with ~10 mL of methanol/0.25 N HCl and add the rinsate to the TurboVap vessel. Concentrate the solution to ~25 mL using a TurboVap evaporator. **It is important to remove all traces of methanol.** Filter the concentrated

aqueous solution through a Whatman fine filter paper (0.8 micron pore size) using a Buchner funnel and vacuum. Pre-rinse the filter paper with ~5 mL 0.25 N HCl. Rinse the TurboVap vessel twice each with ~10 mL 0.25 N HCl and pass the rinsate through the filter paper.

b. C_{18} Cartridge Solid Phase Extraction

Condition a C_{18} SPE cartridge (2 g) with 12 mL methanol followed by 12 mL 0.25N HCl. **Do not allow the cartridge to go dry.** Add 6 mL of 0.25N HCl to the cartridge barrel. Attach a 75 mL reservoir with a reducing adapter. Transfer the concentrated aqueous solution to the reservoir. Rinse the filter flask with ~15 mL 0.25N HCl and add the rinsate to the reservoir. Pass the entire solution through the C_{18} SPE cartridge. Maintain the flow rate at ~5 mL/min by regulating a pump. Blow the cartridge completely dry with nitrogen using a manifold drying attachment (~20 psi for 30 minutes). Rinse the manifold with solvent. **It is important that the cartridge and manifold are completely dry;** increase nitrogen pressure or extend the drying time if necessary. Elute the analyte with 10 mL ethyl acetate into a 15 mL glass centrifuge tube. Evaporate the sample solution under nitrogen stream to ~0.1 mL.

c. Derivatization

Add 500 µL methanol, 500 µL 1 N NaOH, 2 mL acetonitrile, and 100 µL iodomethane to the 13 mL centrifuge tube. Stopper and vortex for ~15 seconds. Heat the solution in a water bath at ~80°C for one hour. Cool to room temperature. Evaporate the contents under nitrogen stream to ~0.5 mL. Add 2 mL toluene to the centrifuge tube and vortex for ~15 seconds.

Prepare injection standards by adding 250 µL by syringe of 5 ng/µL standard solution (containing 3-desmethyl-4-desdifluoromethyl sulfentrazone) to a 13 mL glass centrifuge tube containing 500 µL methanol. Add 500 µL 1 N NaOH, 2 mL acetonitrile, and 100 µL iodomethane to the centrifuge tube. Stopper, vortex for ~15 seconds, and heat the solution in a water bath at ~80°C for one hour. Cool to room temperature. Evaporate the contents under nitrogen stream to ~0.5 mL. Add 2 mL toluene to the centrifuge tube and vortex for ~15 seconds. Allow the phases to separate. Transfer the upper toluene layer into a clean 13 mL centrifuge tube. Add an additional 2 mL toluene to the sample solution and vortex for ~15 seconds. Allow the phases to separate. Transfer the upper toluene layer to the centrifuge tube containing the first toluene partition. Evaporate under nitrogen stream to dryness. Add 5.0 mL acetonitrile.

d. Silica Gel Cartridge Solid Phase Extraction

Condition the silica gel (1 g) cartridge with 6 mL hexane. Transfer the toluene phase from the derivatization step into the cartridge barrel. Add 2 mL toluene to the centrifuge tube and vortex the solution. Add the toluene portion (upper layer) to the cartridge barrel and drain the combined toluene layers. Wash the cartridge with 6 mL 40% ethyl acetate/hexane followed by 6 mL 60% ethyl acetate/hexane. Elute the analyte with 10 mL acetonitrile into a clean centrifuge tube. Evaporate the sample solution under nitrogen stream to less than 1 mL. Make the final volume exactly 1.0 mL with acetonitrile and analyze the acetonitrile extract by GC-ECD.

B. Instrumentation

A Hewlett-Packard (HP) 5890 gas chromatograph equipped with an autosampler, an Electron Capture Detector ^{63}Ni and a J&W Scientific DB-17 Megabore capillary column was used for the analyses of sulfentrazone, 3-desmethyl sulfentrazone and 3-hydroxymethyl sulfentrazone. A Hewlett-Packard (HP) 5890 gas chromatograph equipped with an autosampler, an Electron Capture Detector ^{63}Ni and a J&W Scientific DB-5 Megabore capillary column was used for the analyses of 3-desmethyl-4-desdifluoromethyl sulfentrazone. Detailed instrument parameters are listed in Section XII, A.

A HP 5890 gas chromatograph equipped with an autosampler, HP 5970 Mass Selective Detector (MSD) with selective ion mode and a J&W Scientific DB-5 capillary column was used for spectral confirmation of the compounds.

C. Method Validation and Quality Control

1. Experimental Design

All methods were validated at the limit of quantitation by satisfactory recoveries of the respective analytes from control samples that were laboratory fortified prior to initial extraction. Each analysis set consisted of a minimum of one control sample and one fortified control sample.

2. Preparation of Standards

The chemicals used as reference standards are found in Section X, Table 2. Stock solutions of sulfentrazone and its metabolites were prepared at 1 mg/mL (Section X, Table 3). Working solutions were prepared monthly in acetonitrile (for sulfentrazone, 3-desmethyl sulfentrazone and 3-hydroxymethyl sulfentrazone) or methanol (for 3-desmethyl-4-desdifluoromethyl sulfentrazone only). The working solutions were used for fortification and instrument calibration. All standard solutions were stored in volumetric flasks in a freezer at approximately -18°C. Injection standards of the derivatized analytes were prepared from working solutions of the underivatized analytes concurrently with each analysis set including derivitization.

3. Fortification Procedure

The control winter wheat samples were accurately weighed into round bottom boiling flasks. The samples were fortified by applying the appropriate amount of standard solution to the matrix by syringe. Reference solutions of 2.5 ng/µL and 5.0 ng/µL were used for fortification. The fortification levels ranged from 0.025 ppm to 0.10 ppm for sulfentrazone, 3-desmethyl sulfentrazone and 3-hydroxymethyl sulfentrazone and from 0.05 ppm to 0.1 ppm for 3-desmethyl-4-desdifluoromethyl sulfentrazone.

D. Method of Calculation

Quantitation was carried out using a single point calibration method. The nanogram value of the analytes in each sample was calculated by comparing the area units of the unknown sample to that of the average area units of the run standards using the following formula:

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

If there were detectable residues in control samples, the peak area of the control sample was subtracted from the fortified control samples.

The 2.5 g sample of the winter wheat matrices (5 g for 3-desmethyl-4-desdifluoromethyl sulfentrazone) resulted in a final sample extract volume of 1.0 mL. A 2 µL volume of the final sample extract was injected into the GC/ECD yielding a 5 mg sample injection (10 mg for 3-desmethyl-4-desdifluoromethyl sulfentrazone). The following formula was used to obtain the mg of sample injected:

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

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:

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

No correction for molecular weights was necessary for the derivatized compounds since the injection standards were derivatized simultaneously with the analytes.

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 sulfentrazone in a forage fortified sample (Section XII, Appendix B, Figure 6) is given below:

ng (standard)	=	2.0 µl	x	0.0625 ng/µL	=	0.125 ng
average area units of standard	=	254301				
area units of control sample	=	123785				
area units of fortified control sample	=	348377				
ng of sulfentrazone in fortified control sample	=	$\frac{348377 - 123785}{254301}$ x 0.125 ng			=	0.11 ng
mg of sample injected	=	$\frac{2500 \text{ mg}}{1000 \text{ µL}}$	x	2 µL	=	5 mg
uncorrected ppm (ng/mg)	=	$\frac{0.11 \text{ ng}}{5 \text{ mg}}$	=	0.022 ppm		
method recovery (%)	=	$\frac{0.022 \text{ ppm}}{0.025 \text{ ppm}}$	x	100	=	88%

E. Interferences

In some control forage and straw samples, there were interferences or contaminants eluting at similar retention times as sulfentrazone, 3-desmethyl sulfentrazone, or 3-desmethyl-4-desdifluoromethyl sulfentrazone. Detectable interferences or contaminants found in the control sample were subtracted from the corresponding fortified control samples in calculation. There were no interferences or contaminants in grain control samples.

F. Confirmatory Techniques

A gas chromatograph equipped with a mass selective detector with selective ion mode was used to confirm the sulfentrazone and its 3 metabolites. For sulfentrazone, the molecular ion 386 was used for confirmation. For 3-desmethyl sulfentrazone, molecular ion 372 was used for confirmation. For the derivatized 3-hydroxymethyl sulfentrazone, the molecular ion 459 was used for verification on the mass spectrum. For the derivatized 3-desmethyl-4-desdifluoromethyl sulfentrazone analyte, the molecular ion 350 and the methyl sulfonyl cleaved molecular fragment 271 were used for verification on the mass spectrum. Mass spectra and total ion gas chromatograms of the 4 compounds are included (Section XII, Figures 13-15).

G. Time Required for Analysis

For a set of eight samples, each of the analytical methods can be completed from the time of sample weighing to GC injection within twelve laboratory hours.

H. Modification or Potential Problems

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

2. It is important to carefully adhere to the elution and wash solvent composition, volume and flow rate through the cartridge. The solid phase extraction steps are critical to the clean-up of the sample extract, however, slight variations can lead to analyte loss.

3. After passing the sample solution through the C_8 or C_{18} cartridge, the cartridge and manifold must be completely dry (especially for the analysis of 3-hydroxymethyl sulfentrazone). Extend the drying time if necessary. It is a good practice to rinse the manifold with acetone prior to elution.

Sodium sulfate should **not be used** to remove the trace amounts of water since sodium sulfate can cause loss of the compound.

4. After derivatization in the 3-hydroxymethyl sulfentrazone method, evaporate the sample solution to ~0.1 mL. It is crucial not to dry the sample solutions completely. In the presence of matrix, the analyte is lost when overdried (matrix components are also volatized or baked to the glassware). However, the standard solution can be evaporated to dryness after derivatization.

5. For complete derivatization of 3-desmethyl-4-desdifluoromethyl sulfentrazone, the solution must be basic (pH > 11).

6. An optimized instrument is crucial for the quantitation of sulfentrazone and all its metabolites. Before actual analysis, the GC instrument should be optimized for temperatures, gas flow rates and the glass insert liner. The standard peaks should be sharp and well resolved for compounds. The injection standards must have a low coefficient of variation (<12.5%) and the linearity standards must have a correlation coefficient of at least 0.99. It may be necessary to extend the bake out time to 10 minutes for the analysis of the straw samples. Just before the first injection standard, a few condition injections of matrix sample extract are needed.

VI. STORAGE STABILITY

The analytical reference standards were assayed on a regular basis for percent purity and structural integrity (Section X, Table 2). Standard stock solutions (1 mg/mL) were prepared from these standards in organic solvents and were stored in a freezer (~18°C) for up to one year. Standard working solutions were stored in a freezer (~18°C) for up to one month (Section X, Table 3).

VII. RESULTS AND DISCUSSION

A. Accuracy

The accuracy was determined by the average method recovery of the individual results of the fortified control samples (Section X, Table 4). The average method recoveries were 87% ±11 for sulfentrazone, 88% ±14 for 3-desmethyl sulfentrazone, 74% ±7 for 3-hydroxymethyl sulfentrazone and 109% ±17 for 3-desmethyl-4-desdifluoromethyl sulfentrazone.

B. Precision

The precision of the analytical method was determined by the standard deviation of the individual results of the fortified control samples (Section X, Table 4). The standard deviations were ±11% for sulfentrazone, ±14% for 3-desmethyl sulfentrazone, ±7% for 3-hydroxymethyl sulfentrazone and ±17% for 3-desmethyl-4-desdifluoromethyl sulfentrazone.

C. Limits of Detection and Quantification

The method limit of quantitation for sulfentrazone, 3-desmethyl sulfentrazone and 3-hydroxymethyl sulfentrazone in/on grain, forage, and straw was validated at 0.025 ppm and the limit of detection was set at 0.005 ppm. The method limit of quantitation for the 3-desmethyl-4-desdifluoromethyl sulfentrazone in/on forage was validated at 0.05 ppm and the limit of detection was set at 0.01 ppm.

D. Ruggedness

The average method recoveries and the standard deviations for the analytical methods developed for the winter wheat matrices indicate that these methods are reliable and accurate. Each step in the methods and instruments are routine residue techniques. Careful attention to the detailed method and potential problems (Section V.H.) will ensure the reliable performance of the method.

E. Limitations

At limit of quantitation levels, it was essential to eliminate any potential problems to obtain satisfactory results. The analytical methods described in this report were developed and used for winter wheat matrices only.

VIII.CONCLUSION

The residue analytical methods were developed and successfully employed for the extraction and detection of sulfentrazone, 3-desmethyl sulfentrazone, 3-hydroxymethyl sulfentrazone and 3-desmethyl-4-desdifluoromethyl sulfentrazone residues in/on winter wheat forage, grain, and straw. The average method recoveries were 87% ±11% (n=16) for sulfentrazone, 88% ±14% (n=16) for 3-desmethyl sulfentrazone, 74% ±7% (n=14) for 3-hydroxymethyl sulfentrazone, and 109% ±17% (n=6) for 3-desmethyl-4-desdifluoromethyl sulfentrazone. The method limits of quantitation were 0.025 ppm for sulfentrazone, 3-desmethyl sulfentrazone and

3-hydroxymethyl sulfentrazone, and 0.05 ppm for 3-desmethyl-4-desdifluoromethyl sulfentrazone. The method limits of detection were set at 0.005 ppm for sulfentrazone, 3-desmethyl sulfentrazone, and 3-hydroxymethyl sulfentrazone, and 0.01 ppm for 3-desmethyl-4-desdifluoromethyl sulfentrazone.

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 and its metabolites in/on winter 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.

In-Young Kim
Research Chemist
STUDY DIRECTOR

4-7-95
Date

John M. Becker
Manager
SPONSOR

04/10/95
Date

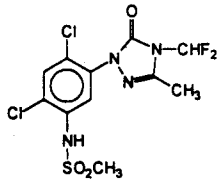
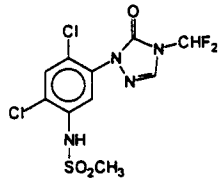
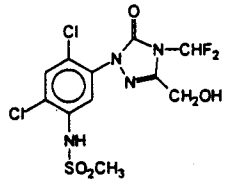
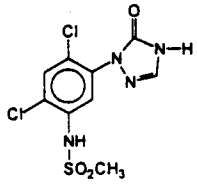
ADDITIONAL STUDY PERSONNEL

Natalie Shevchuk, Senior Chemist
Michael Reel, Senior Research Technician
David Baffuto, Research Technician

X. TABLES AND FIGURES

TABLE 2

TEST AND REFERENCE STANDARDS

Common Name	Chemical Name	CAS Number	Residue 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	243	99.5
3-Desmethyl Sulfentrazone	N-[2,4-dichloro-5-[4-(difluoromethyl)-4,5-dihydro-5-oxo-1H- 1,2,4-triazol-1-yl]phenyl] methanesulfonamide 	134391-02-9	236	95.6
3-Hydroxymethyl Sulfentrazone	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	246	97.2
3-Desmethyl-4-desdifluoromethyl Sulfentrazone	N-[2,4-dichloro-5(4,5-dihydro-5-oxo-1H-1,2,4-triazol-1-yl) phenyl]methanesulfonamide 	NA[a]	250	99.9

[a] NA= Not Available.

TABLE 3

REFERENCE SOLUTIONS

Compound	Solution Solvent	Solution Concentration (ng/µL)	Standard Solution Index Number	Date Prepared
Sulfentrazone	Acetonitrile	1000	707	02/28/94
3-Desmethyl Sulfentrazone	Acetonitrile	1000	676	12/07/93
		1000	785	12/05/94
3-Hydroxymethyl Sulfentrazone	Acetonitrile	1000	708	2/28/94
		2.5	708-33	11/01/94
		2.5	708-40	12/02/94
		2.5	708-46	01/31/95
3-Desmethyl-4-desdifluoromethyl Sulfentrazone	Methanol	1000	723	04/25/94
		5	723-16	10/17/94
Sulfentrazone + 3-Desmethyl Sulfentrazone	Acetonitrile	2.5	779-1	11/01/94
		2.5	779-8	12/05/94
		2.5	794-1	01/31/95

Dilute reference solutions were prepared monthly from the above reference solutions.

TABLE 4A

METHOD RECOVERIES OF SULFENTRAZONE AND 3-DESMETHYL SULFENTRAZONEFROM LABORATORY FORTIFIED WINTER WHEAT SAMPLES

Matrix	Set Number	Sample ID	Fortification Level (ppm)	Sulfentrazone Control (ppm)	Sulfentrazone Recovery %	3-Desmethyl Sulfentrazone Control (ppm)	3-Desmethyl Sulfentrazone Recovery %
Forage	MV05	93SJS141C	0.025	(0.012)[a]	88	ND	75
	MV05	93SJS141C	0.10	(0.012)	89	ND	98
	06	93HLG06C	0.05	ND[b]	81	ND	84
	06	93HGH10C	0.05	ND	87	ND	77
	07	93HGH09C	0.05	ND	98	ND	99
	07	93SJS141C	0.05	ND	112	ND	109
			Average (n=6)		93		90
			Standard Deviation		±11		±14
Straw	08	94HLG02C	0.05	(0.012)	72	ND	75
	08	93HGH10C2	0.05	ND	86	(0.010)	67
	22	94HLG02C	0.025	(0.006)	81	ND	79
	22	94HLG02C	0.025	(0.012)	64	(0.005)	87
			Average (n=4)		76		77
			Standard Deviation		±10		±8
Grain	MV02	93HGH03C	0.025	ND	82	ND	73
	MV02	93HGH03C	0.05	ND	83	ND	80
	04	94HLG19C	0.05	ND	89	ND	101
	04	93HGH10C1	0.10	ND	91	ND	102
	05	93HGH03C	0.05	ND	88	ND	100
	05	93SJS142C	0.05	ND	101	ND	106
			Average (n=6)		89		94
			Standard Deviation		±7		±14
			Overall Average (n=16)		87		88
			Standard Deviation		±11		±14

[a] Values in parenthesis are estimates since they are less than the limit of quantitation (0.025 ppm).
[b] ND = Not detected (< 0.005 ppm).

TABLE 4B

METHOD RECOVERIES OF 3-HYROXY SULFENTRAZONE FROM LABORATORY FORTIFIED WINTER WHEAT SAMPLES

Matrix	Set Number	Sample ID	Fortification Level (ppm)	3-Hydroxy Sulfentrazone Control (ppm)	Recovery %
Forage	14	93HLG06C	0.05	ND[a]	65
	14	93HGH10C	0.1	ND	72
	15	93HGH09C	0.025	ND	74
	15	93SJS141C	0.025	ND	87
			Average (n=4)		75
			Standard Deviation		±9
Straw	19	94HLG02C	0.025	ND	80
	19	93HGH10C2	0.025	ND	82
	20	93HGH03C	0.025	ND	63
	20	93SJS143C	0.05	ND	73
			Average (n=4)		75
			Standard Deviation		±9
Grain	MV04	93SJS142C	0.025	ND	84
	MV04	93SJS142C	0.025	ND	69
	13	94HLG19C	0.05	ND	73
	13	93HGH10C1	0.05	ND	70
	11	93HGH03C	0.05	ND	78
	11	93SJS142C	0.1	ND	67
			Average (n=6)		74
			Standard Deviation		±6
			Overall Average (n=14)		74
			Standard Deviation		±7

[a] ND = Not detected (< 0.005 ppm).

TABLE 4C

METHOD RECOVERIES OF 3-DESMETHYL-4-DESDIFLUOROMETHYL SULFENTRAZONE FROM LABORATORY FORTIFIED WINTER WHEAT SAMPLES

Matrix	Set Number	Sample ID	Fortification Level (ppm)	Desdes[b] Sulfentrazone Control (ppm)	Recovery %
Forage	MV01	93HLG06C	0.05	ND	122
	MV01	93HLG06C	0.1	ND	104
	01R	93HLG06C	0.05	ND	115
	01R	93HGH10C	0.05	(0.01)[c]	76
	03	93HGH09C	0.05	(0.01)	119
	03	93SJS141C	0.05	0.06	117
				Average(n=6)	109
				Standard Deviation	±17

a ND = Not detected (< 0.01 ppm).
b Desdes = 3-desmethyl-4-desdifluoromethyl sulfentrazone.
c Values in parenthesis are estimates since they are less than the limit of quantitation (<0.05 ppm).

XI. REFERENCES

1. Shevchuk, N.A., "Magnitude of the Residue of Sulfentrazone and its Metabolites in/on Winter Wheat as a Rotated Crop following Soybeans which were Treated with F6285 75DF at 0.375 Pound Active Ingredient per Acre", FMC Corporation, Agricultural Chemical Group, P-2982, April 07, 1995.

2. Kim, I.Y., "Determination of the Residue of Sulfentrazone and its Metabolites in/on Winter Wheat as a Rotated Crop following Harvest of F6285/Treflan WDG Treated Soybeans", FMC Corporation, Agricultural Chemical Group, Princeton, NJ. P-2944, July 26, 1994 (MRID 43345429).

3. Kim, I.Y., "Determination of the Residue of Sulfentrazone and its Metabolites in/on Winter Wheat as a Rotated Crop following Harvest of F6285 WDG Treated Soybeans", FMC Corporation, Agricultural Chemical Group, Princeton, NJ. P-2945, July 28, 1994 (MRID 43345430).

4. Chen, A.W., "Residue Analytical Method for the Determination of FMC 97285 and FMC 106091 in/on Soybeans Treated with F6285 4F", FMC Corporation, Agricultural Chemical Group, Princeton, NJ. P-2811M, July 1993 (MRID 42932109).

XII. APPENDIX

A. Instrument Parameters

1. Sulfentrazone, 3-Desmethyl sulfentrazone and 3-Hydroxymethyl sulfentrazone

INSTRUMENT	: HP 5890 GC
COLUMNS	: DB-17, Diphenyl/Dimethyl (50/50) silicone gum, 30 m x 0.53 mm, 1.0 um film thickness
INLET	: Splitless injection : Cyclo-gooseneck uniliner
DETECTOR	: ^{63}Ni electron capture
TEMPERATURE PROGRAM:	
Injection Port	: 250°C
Oven	: 180°C/1 minute (initial) : 20°C/minute (ramp) : 280°C/8 minutes (final)
Detector	: 300°C
GAS FLOW	: He, carrier, 20.5 mL/minute : Ar/methane, make-up, 40 mL/minute
INJECTION VOLUME	: 2 uL
RUN TIME	: 14 minutes
RETENTION TIME	:~ 7.3 minutes (3-desmethyl sulfentrazone) :~ 7.7 minutes (sulfentrazone) :~ 9.6 minutes (3-hydroxymethyl sulfentrazone)

2. 3-Desmethyl-4-desdifluoromethyl sulfentrazone

INSTRUMENT	: HP 5890 GC
COLUMNS	: DB-5, Diphenyl/Dimethyl (5/95) silicone gum, 30 m x 0.53 mm, 1.5 µm film thickness
INLET	: Splitless injection : Single taper liner
DETECTOR	: ^{63}Ni electron capture
TEMPERATURES:	
Injection Port	: 250 °C
Oven	: 240 °C/4 minutes (initial) : 10 °C/minute (ramp) : 280 °C/12 minutes (final)
Detector	: 300 °C
GAS FLOW	: He, carrier, 15 mL/minute : Ar/methane, make-up, 60 mL/minute
INJECTION VOLUME	: 2 uL
RUN TIME	: 20 minutes
RETENTION TIME	: 8-9 minutes (3-desmethyl-4-desdifluoromethyl sulfentrazone)

B. Chromatograms and Spectra

FIGURE INDEX

FIGURE 6
SULFENTRAZONE AND 3-DESMETHYL SULFENTRAZONE
IN/ON WINTER WHEAT FORAGE, SET MV05, SAMPLE INJECTED 5 MG

Chromatogram/ Identification	Sample Type	Peak Area Residue (ppm)/Recovery (%) 3-desmethyl/Sulfentrazone		3-desmethyl/Sulfentrazone	
A/N0201001	Standard, 0.125 ng	228015	240156[a]		
B/N0201002	Control	0	123785	ND[b]	(0.012)[c]
C/N0201003	Fortified at 0.025 ppm	178870	348377	75	88

[a] Average area was 237381for 3-desmethyl sulfentrazone and 254301 for sulfentrazone.
[b] ND = Not Detected (< 0.005 ppm).
[c] Values in parenthesis are estimated numbers since they are less than the LOQ (0.025 ppm).

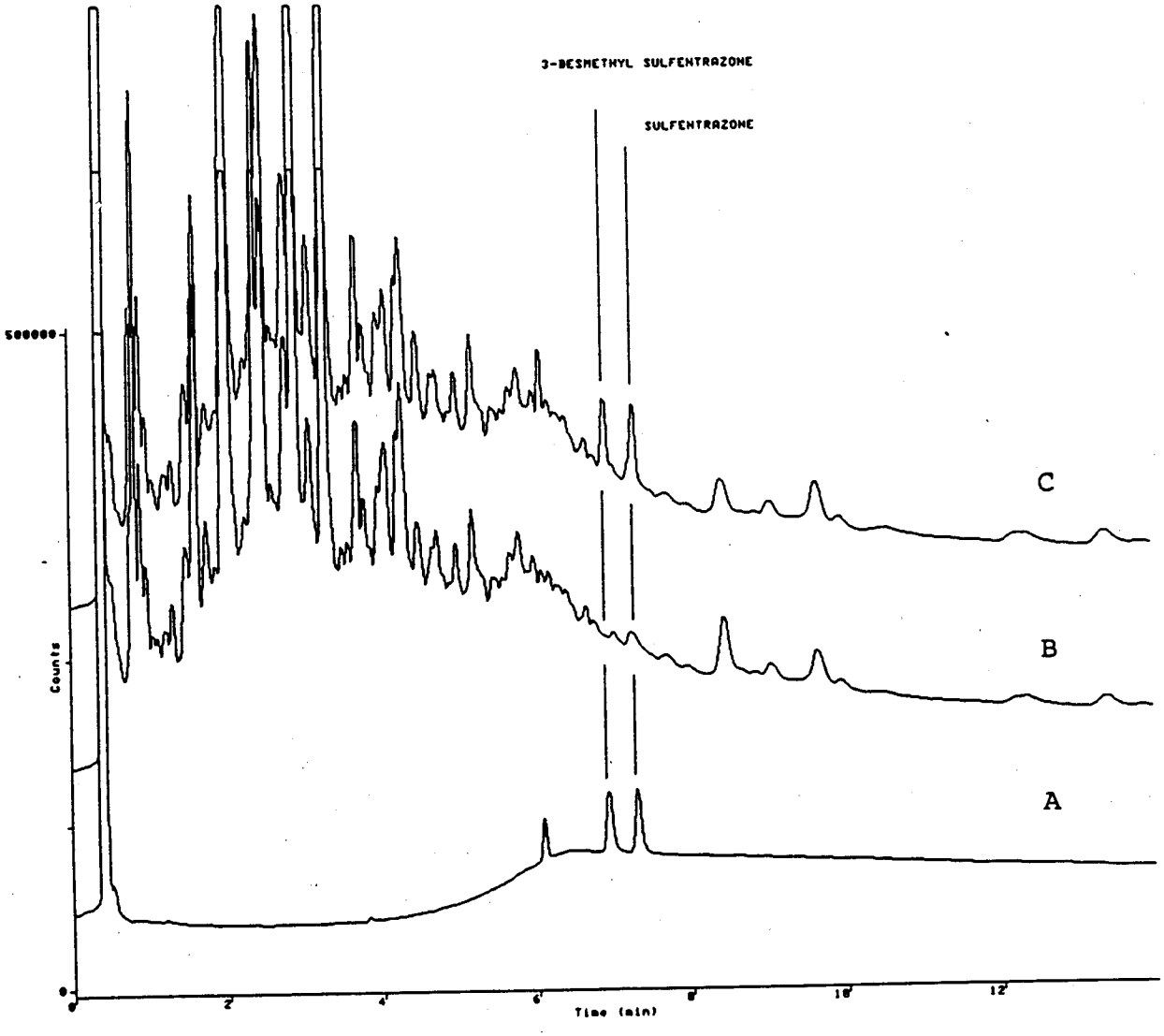


FIGURE 7
3-HYDROXYMETHYL SULFENTRAZONE IN/ON WINTER WHEAT FORAGE,
SET 15, SAMPLE INJECTED 5 MG

Chromatogram	Identification	Sample Type	Peak Area	Residue (ppm)/Recovery (%)
A	R1206007	Standard, 0.5 ng	246797[a]	
B	R1206008	Control	0	ND[b]
C	R1206009	Fortified at 0.025 ppm	220533	87

[a] Average peak area was 254302 for 3-hydroxymethyl sulfentrazone.
[b] ND = Not Detected (< 0.005 ppm).

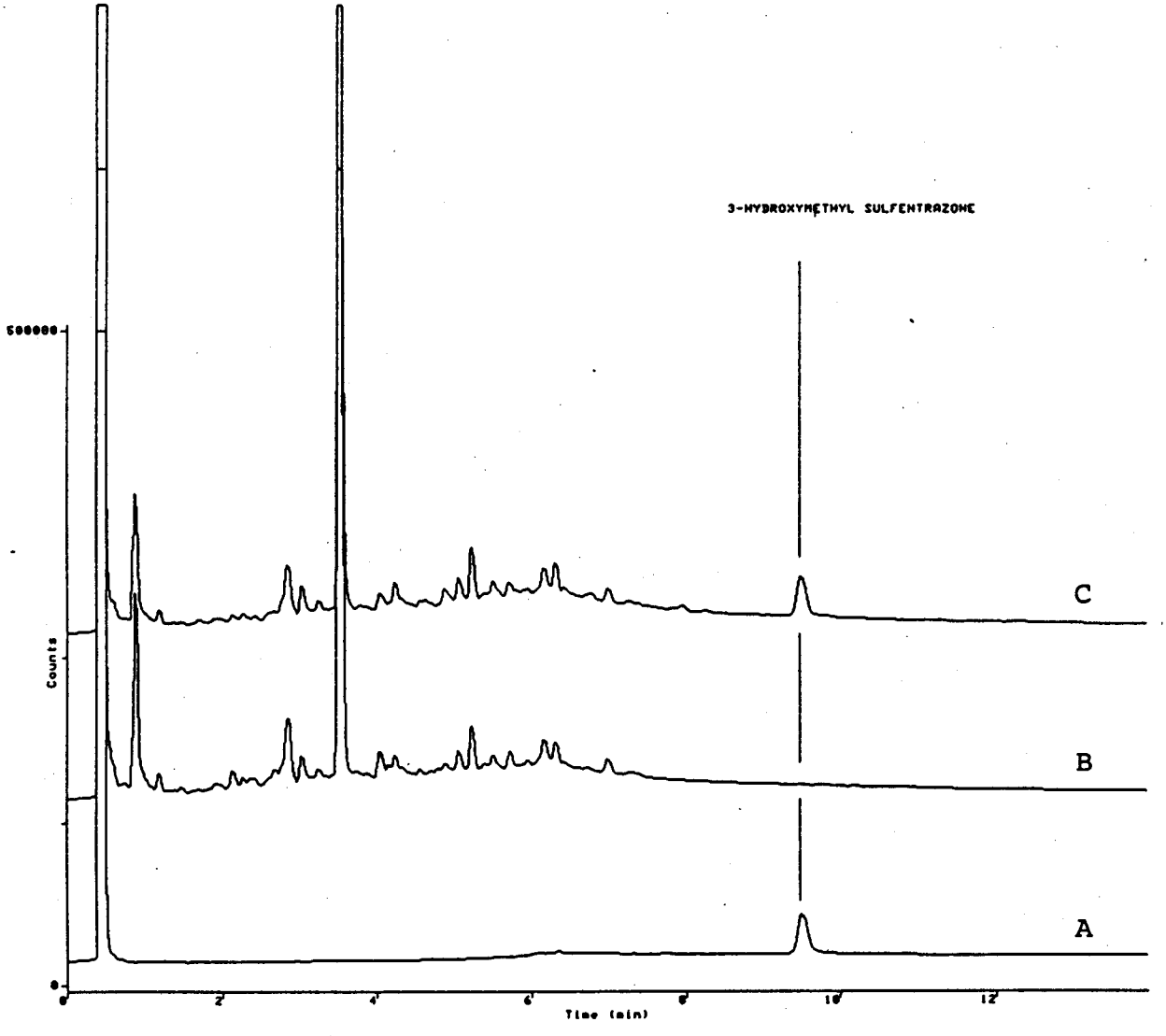


FIGURE 8
3-DESMETHYL-4-DESDIFLUOROMETHYL SULFENTRAZONE
IN/ON WINTER WHEAT FORAGE, SET 01R, SAMPLE INJECTED 10 MG

Chromatogram	Identification	Sample Type	Peak Area	Residue (ppm)/Recovery (%)
A	N1109002	Standard, 0.500 ng	1247267[a]	
B	N1109003	Control	151688	ND[b]
C	N1109004	Fortified at 0.05 ppm	1242769	115

[a] Average area was 1078956 for 3-desmethyl-4-desdifluoromethyl sulfentrazone.
[b] ND = Not Detected (< 0.01 ppm).

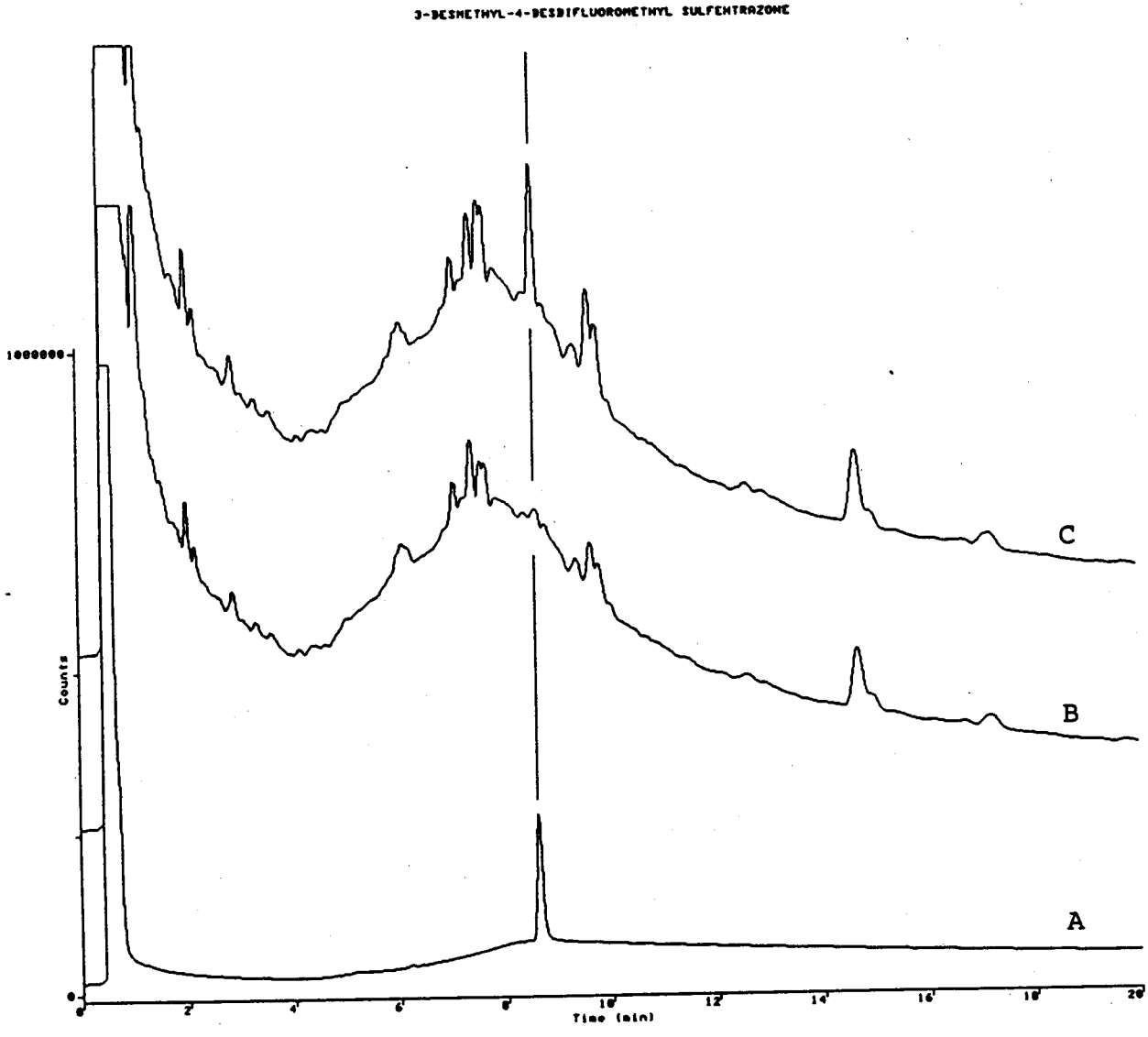


FIGURE 9
SULFENTRAZONE AND 3-DESMETHYL SULFENTRAZONE
IN/ON WINTER WHEAT GRAIN, SET 5, SAMPLE INJECTED 5 MG

Chromatogram/ Identification	Sample Type	Peak Area 3-desmethyl/Sulfentrazone		Residue (ppm)/Recovery (%) 3-desmethyl/Sulfentrazone	
A/R1116007	Standard, 0.250 ng	467848	528463[a]		
B/R1116008	Control	0	14688	ND[b]	ND
C/R1116009	Fortified at 0.05 ppm	511316	532528	106	101

[a] Average area was 483196 for 3-desmethyl sulfentrazone and 526441 for sulfentrazone.
[b] ND = Not Detected (< 0.005 ppm).

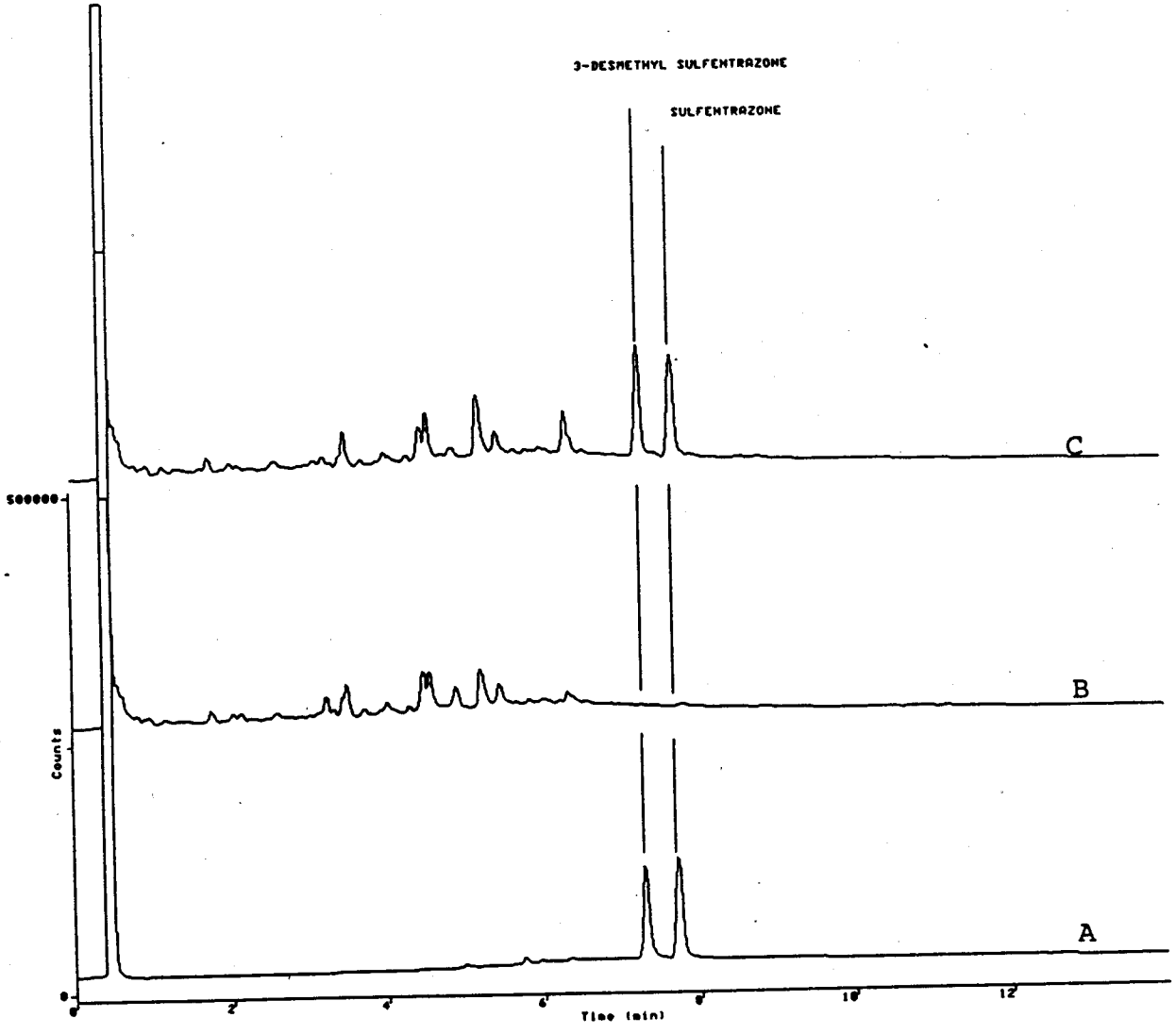


FIGURE 10
3-HYDROXYMETHYL SULFENTRAZONE IN/ON WINTER WHEAT GRAIN,
SET MV04R, SAMPLE INJECTED 5 MG

Chromatogram	Identification	Sample Type	Peak Area	Residue (ppm)/Recovery (%)
A	N0206001	Standard, 0.125 ng	169065[a]	
B	N0206002	Control	0	ND[b]
C	N0206003	Fortified at 0.025 ppm	140444	84

[a] Average peak area was 166781 for 3-hydroxymethyl sulfentrazone.
[b] ND = Not Detected (< 0.005 ppm).

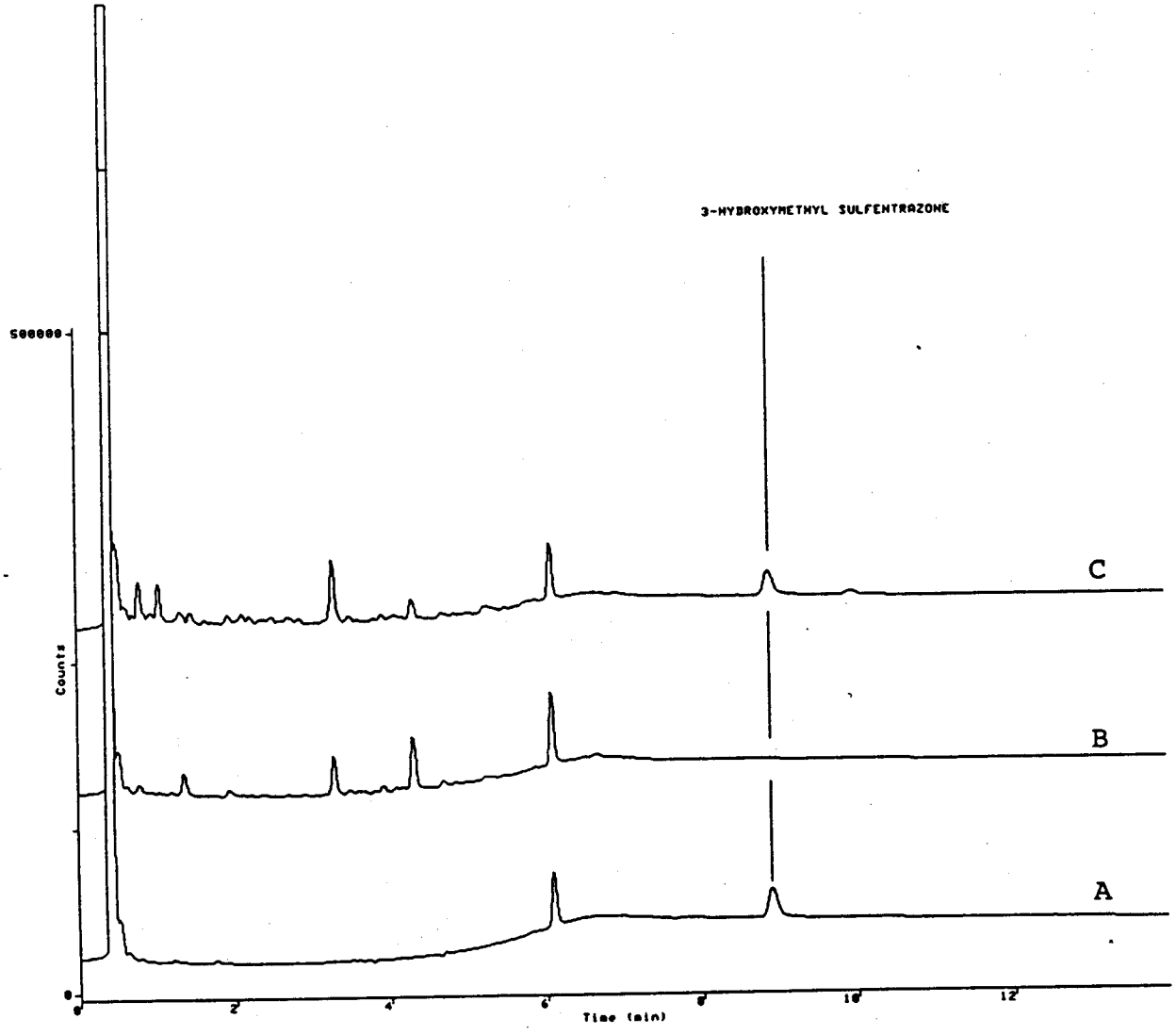


FIGURE 11
SULFENTRAZONE AND 3-DESMETHYL SULFENTRAZONE
IN/ON WINTER WHEAT STRAW, SET 22, SAMPLE INJECTED 5 MG

Chromatogram/ Identification	Sample Type	Peak Area 3-desmethyl/Sulfentrazone		Residue (ppm)/Recovery (%) 3-desmethyl/Sulfentrazone	
A/R1221001	Standard, 0.125 ng	238486	236968[a]		
B/R1221002	Control	0	65615	ND[b]	(0.006)[c]
C/R1221003	Fortified at 0.025 ppm	196051	282590	79	81

[a] Average area was 246907 for 3-desmethyl sulfentrazone and 267526 for sulfentrazone.
[b] ND = Not Detected (< 0.005 ppm).
[c] Values in parenthesis are estimated numbers since they are less than the LOQ (0.025 ppm).

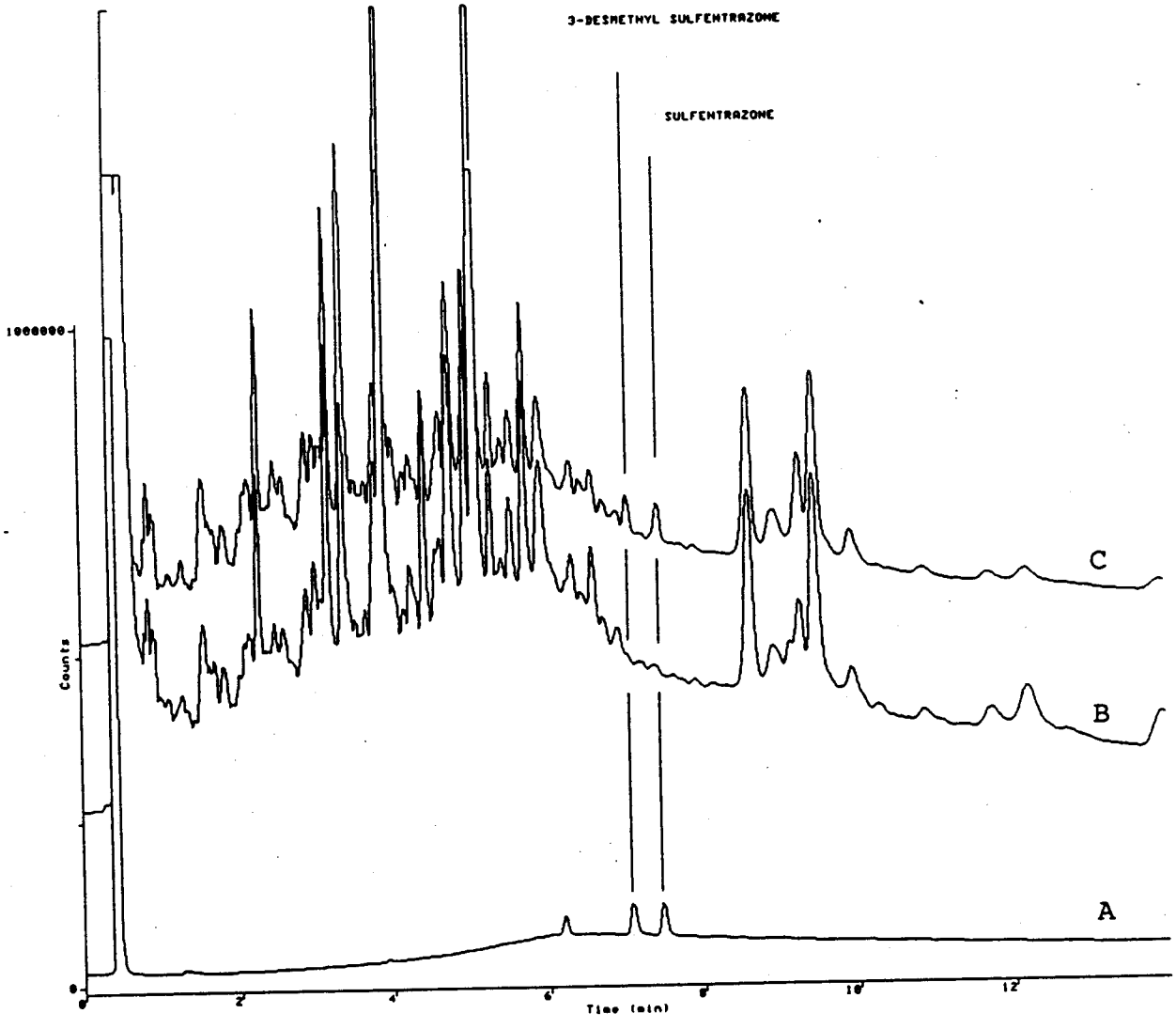


FIGURE 12
3-HYDROXYMETHYL SULFENTRAZONE IN/ON WINTER WHEAT STRAW,
SET 19, SAMPLE INJECTED 5 MG

Chromatogram	Identification	Sample Type	Peak Area	Residue (ppm)/Recovery (%)
A	R1219004(front)[a]	Standard, 0.125 ng	236450[b]	
B	R1219004(end)	Control	11319	ND[c]
C	R1219005(front)	Fortified at 0.025 ppm	194812	82

[a] The Access*Chrom run time was incorrrectly set, two chromatograms were collected in each data file. (front) - first data file; (end) - second data file.
[b] Average peak area was 238928 for 3-hydroxymethyl sulfentrazone.
[c] ND = Not Detected (< 0.005 ppm).

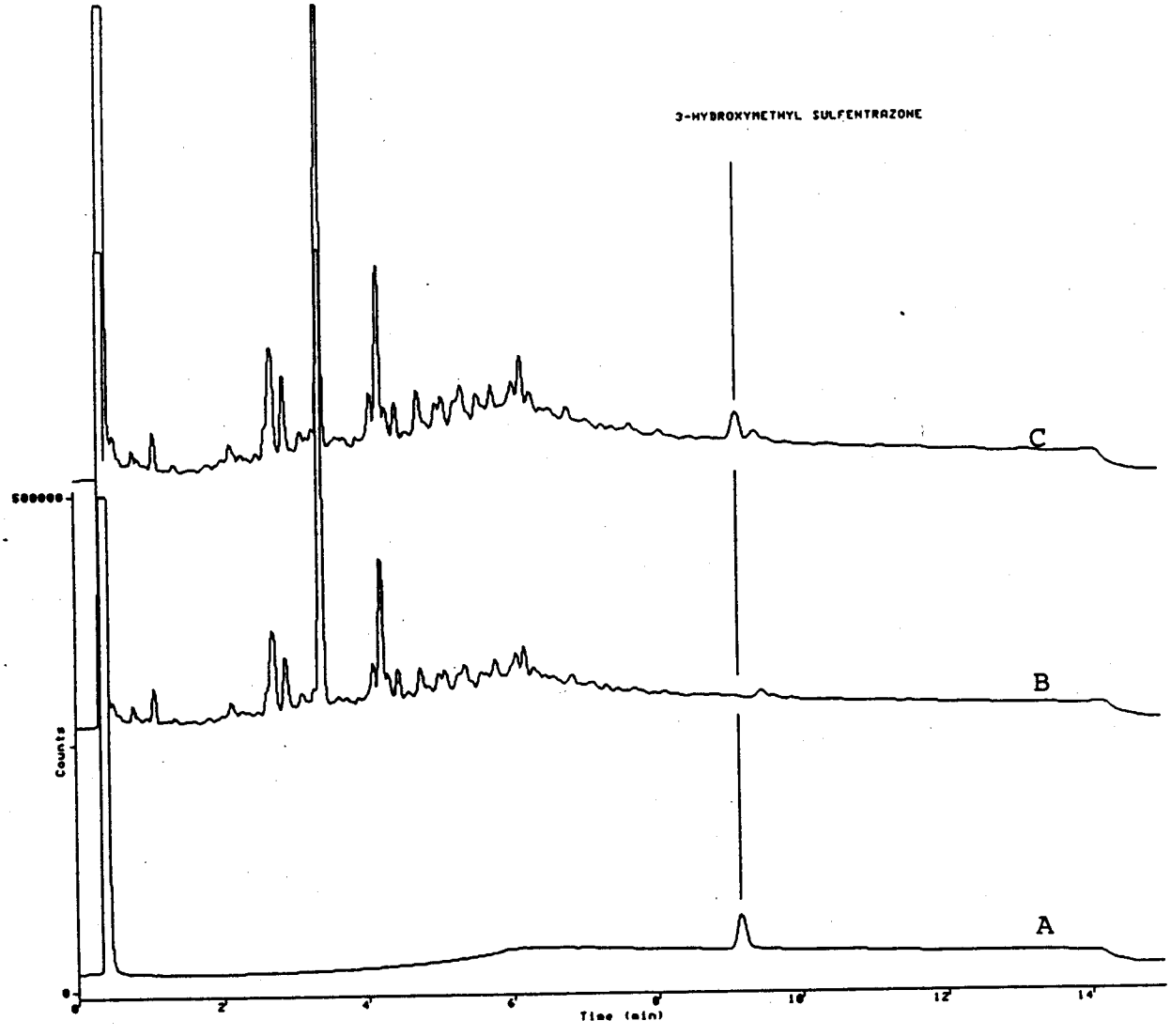
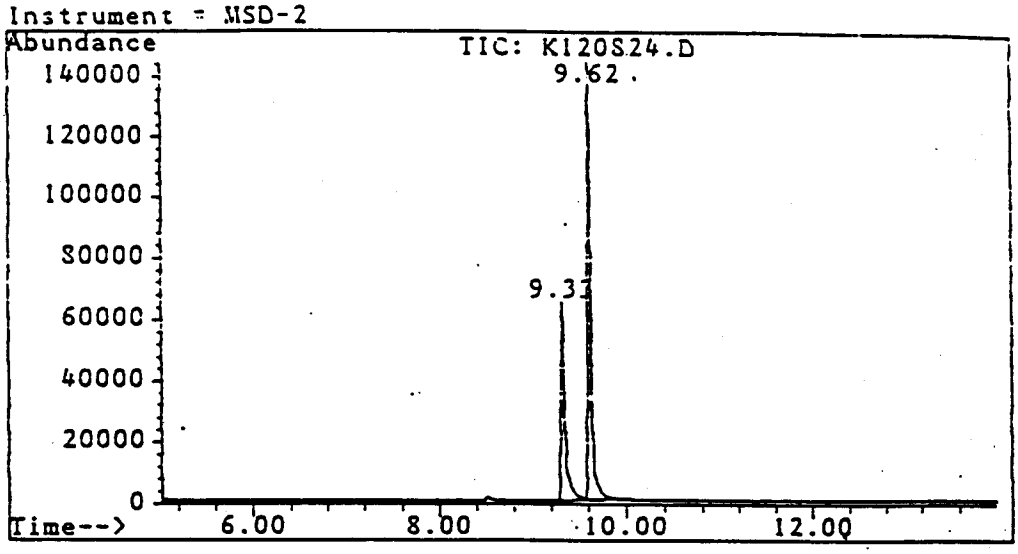
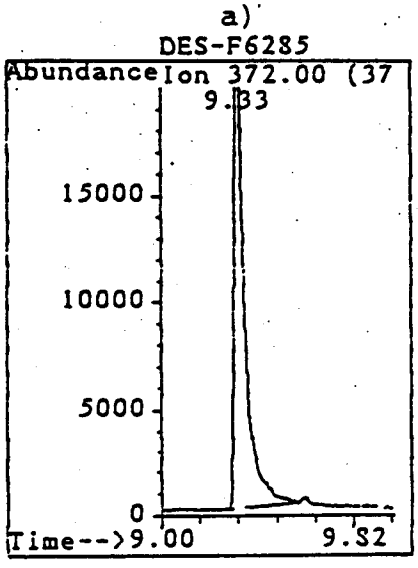


FIGURE 13

GAS CHROMATOGRAMS OF SULFENTRAZONE AND
3-DESMETHYL SULFENTRAZONE MIXTURE; #779-8, 2.5 NG/UL

a) 3-desmethyl sulfentrazone: retention time 9.33, molecular ion 372
b) sulfentraone: retention time 9.62, molecular ion 386





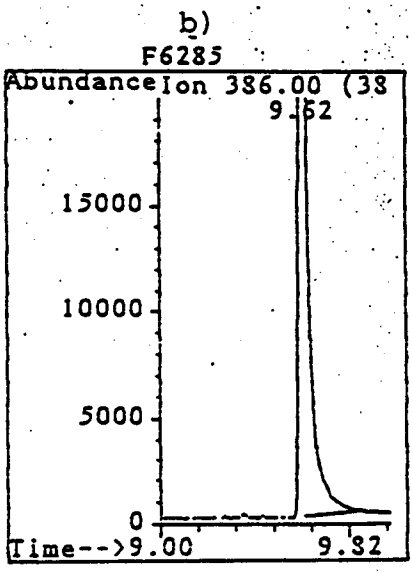
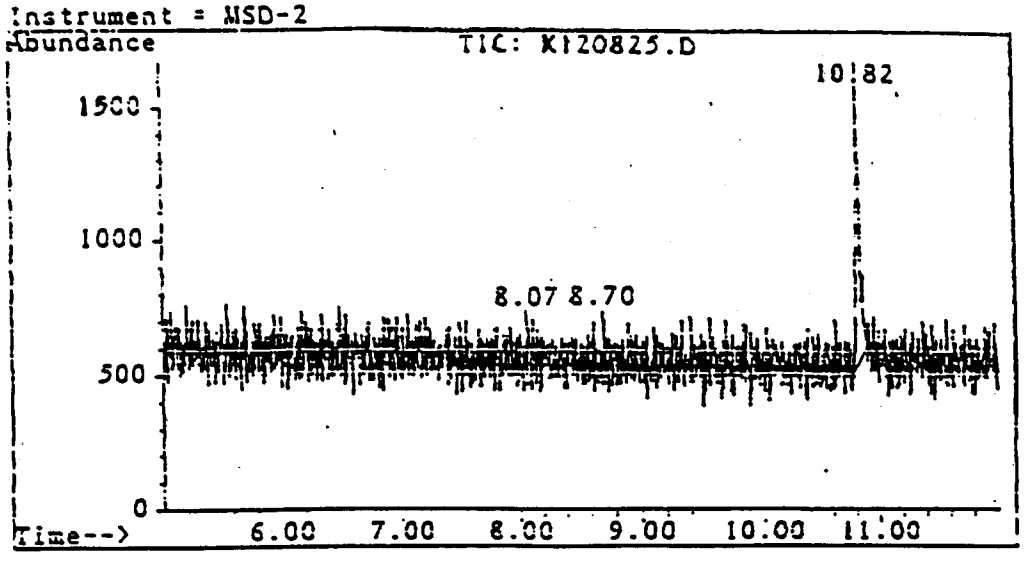


FIGURE 14

GAS CHROMATOGRAMS OF DERIVATIZED 3-HYDROXYMETHYL SULFENTRAZONE
#708-36, 0.125 NG/UL

3-hydroxymethyl sulfentrazone: retention time 10.82, molecular ion 459



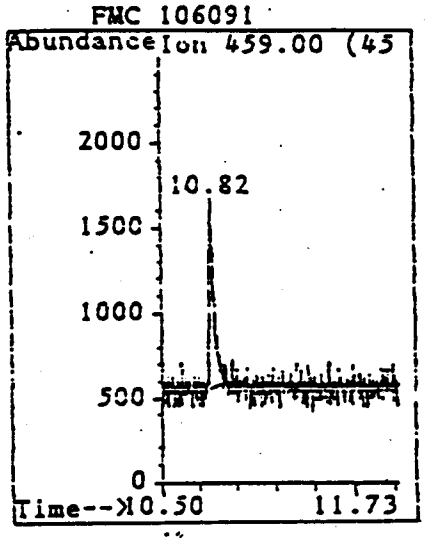


FIGURE 15

GAS CHROMATOGRAMS OF DERIVATIZED
3-DESMETHYL-4-DESDIFLUOROMETHYL SULFENTRAZONE; #723-16, 0.25 NG/UL (CONC.)

**3-desmethyl-4-desdifluoromethyl sulfentrazone:
retention time 11.00,
molecular ions 271 and 350**