

# SULFENTRAZONE - 02

## STUDY TITLE

Independent Laboratory Validation of  
Analytical Methodology for the Determination of Sulfentrazone, 3-Hydroxymethyl  
Sulfentrazone, and 3-Desmethyl Sulfentrazone in/on Wheat Forage

## TEST SUBSTANCES

Sulfentrazone, Sulfentrazone-3-Carboxylic Acid, 3-Desmethyl Sulfentrazone and  
3-Hydroxymethyl Sulfentrazone

## DATA REQUIREMENT

PR Notice 96-1

## AUTHOR/STUDY DIRECTOR

Julie Burton

## STUDY DATES

Initiation: August 22, 1996  
Experiment Terminated: September 12, 1996  
Study Completed: September 18, 1996

## PERFORMING LABORATORY

Centre Analytical Laboratories, Inc. ("CAL")  
3048 Research Drive  
State College, PA 16801  
(814) 231-8032

## STUDY SPONSOR

FMC Corporation  
P.O. Box 8  
Princeton, NJ 08543  
(609) 951-3000

## PROJECT

Centre Analytical Study Number: 014-04  
FMC Study Number: 162MVL96R2  
FMC Report Number: PC-0274

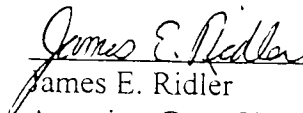
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Non-Proprietary Information  
FMC Corporation Authorizes the Release or Use of this Method by  
Federal and State Agencies  
FMC Corporation

### 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 Section 10 (d) (1) (A), (B), or (C).


Company: FMC Corporation

  
\_\_\_\_\_  
James E. Ridler  
Associate Data Chemist  
Company Agent

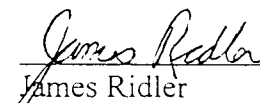
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### GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

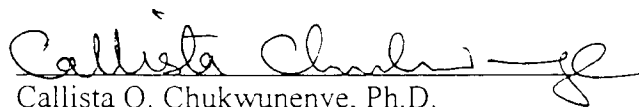
CAL Study Number 014-04, entitled "Independent Laboratory Validation of Analytical Methodology for the Determination of Sulfentrazone, 3-Hydroxymethyl Sulfentrazone, and 3-Desmethyl Sulfentrazone in/on Wheat Forage", conducted for FMC Corporation, FMC Study Number 162MVL96R2, was performed in compliance with US EPA Good Laboratory Practice Standards (40 CFR, Part 160, FR 08/17/89) by Centre Analytical Laboratories, Inc.

  
\_\_\_\_\_  
Julie Burton  
Study Director  
Centre Analytical Laboratories, Inc.

9/18/96  
\_\_\_\_\_  
Date

  
\_\_\_\_\_  
James Ridler  
Sponsor Representative  
FMC Corporation

9/19/96  
\_\_\_\_\_  
Date

  
\_\_\_\_\_  
Callista O. Chukwunenye, Ph.D.  
Manager, Product Registration  
FMC Corporation  
Submitter

9/19/96  
\_\_\_\_\_  
Date

### QUALITY ASSURANCE STATEMENT

CAL Study Number 014-04, was reviewed by Centre Analytical Laboratories' Quality Assurance Unit. All reviewed phases were reviewed for conduct according to Centre Analytical Laboratories' Standard Operating Procedures, Study Protocol, and all applicable Good Laboratory Practice Standards. All findings were reported to the study director and to management.

<u>Phase</u>	<u>Date Inspected</u>	<u>Date Reported to Study Director and CAL Management</u>	<u>Date Reported to Sponsor Representative and Sponsor Management</u>
1. Protocol Review	08/26/96	09/17/96	09/18/96
2. Method Validation	09/06/96	09/18/96	09/18/96
3. Raw Data Review	09/14/96	09/18/96	09/18/96
4. Draft Analytical Phase Report Review	09/14/96	09/18/96	09/18/96
5. Final Analytical Phase Report Review	09/17/96	09/18/96	09/18/96


Gail L. Keller  
Gail Keller  
Quality Assurance Officer

9/18/96  
Date

### CERTIFICATION OF AUTHENTICITY

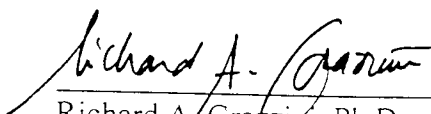
This report is a true and complete representation of the raw data of the study. Further, this is an unaltered version (except for changes required to comply with PR Notice 86-5) of the final report received from the following contract laboratory:

Submitted by: Centre Analytical Laboratories, Inc.  
3048 Research Drive  
State College, PA 16801  
(814) 231-8032

  
\_\_\_\_\_  
Julie Burton  
Study Director

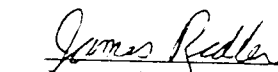
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\_\_\_\_\_  
Date

Facility Management:

  
\_\_\_\_\_  
Richard A. Grazzini, Ph.D.  
Director of Marketing

18-Sep-96  
\_\_\_\_\_  
Date

Sponsor Representative:

  
\_\_\_\_\_  
James Ridler  
FMC Corporation

9/19/96  
\_\_\_\_\_  
Date

### PROJECT PERSONNEL

The Study Director for this project at Centre Analytical Laboratories, Inc., was Julie Burton, Scientist. The Sponsor Representative was James Ridler of FMC Corporation. The following Centre Analytical Laboratories personnel were associated with various phases of the study.

<u>Name</u>	<u>Title</u>
Julie Burton	Study Director
Mitra Arjmand	Technician
Rickey Keller	Sample Custodian

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## SUMMARY

Centre Analytical Laboratories, Inc., (CAL) conducted a successful independent laboratory validation of FMC analytical method, "Analytical Methodology for the Determination of Sulfentrazone, 3-Desmethyl Sulfentrazone, and 3-Hydroxymethyl Sulfentrazone in/on Various Matrices" in wheat forage. This independent laboratory validation study is required by PR Notice 96-1.

A set of samples containing a reagent blank, two control wheat forage samples, two control wheat forage samples fortified at 0.025 ppm, and two control wheat forage samples fortified at 0.2 ppm, were extracted and analyzed. The average recoveries were 87% (SD = 7%), 100% (SD = 18%), and 74% (SD = 3%) for sulfentrazone, 3-desmethyl sulfentrazone as sulfentrazone-3-carboxylic acid, and 3-hydroxymethyl sulfentrazone, respectively.

The samples were fortified by applying a measured volume of standard solution containing sulfentrazone, sulfentrazone-3-carboxylic acid (SCA) and 3-hydroxymethyl sulfentrazone (HMS) to the matrix. During analysis, SCA is converted to 3-desmethyl sulfentrazone (DMS) and HMS is derivatized, therefore, the analytical standard solutions for injection contained sulfentrazone, DMS and derivatized HMS. Measured volumes of a standard solution containing sulfentrazone, DMS and HMS (prepared from stock solutions) were derivatized simultaneously with the samples.

The trial was successful during the first attempt. The extraction time for one set of seven samples was approximately 16 person-hours (2 days). Each GC analytical run required approximately 15 minutes.

## OBJECTIVE

The purpose of this study was to conduct an independent laboratory validation of FMC analytical method, "Analytical Methodology for the Determination of Sulfentrazone, 3-Desmethyl Sulfentrazone, and 3-Hydroxymethyl Sulfentrazone in/on Various Matrices" under the guidelines of PR Notice 96-1, in order to determine the suitability of using this method to determine sulfentrazone, 3-desmethyl sulfentrazone, and 3-hydroxymethyl sulfentrazone residues present in wheat forage.

## INTRODUCTION

US EPA regulations in 40 CFR 158.240, 180.7, and 180.34 require petitioners for pesticide tolerances to furnish adequate residue analytical methods to determine the total toxic residue for pesticides in or on agricultural commodities, and as appropriate, for processed foods/feeds. Subdivision O, 171-4 (b) and PR Notice 96-1 (Tolerance

Enforcement Methods - Independent Laboratory Validation by Petitioner) require petitioners to furnish results of a successful confirmatory trial of the method by an independent laboratory to ensure its suitability as an enforcement method. This report details the results of the confirmatory trial of FMC analytical method, "Analytical Methodology for the Determination of Sulfentrazone, 3-Desmethyl Sulfentrazone, and 3-Hydroxymethyl Sulfentrazone in/on Various Matrices" by Centre Analytical Laboratories, Inc.

In this study, a validation set for wheat forage consisting of a reagent blank, two control samples, two control samples fortified at 0.025 ppm, and two control samples fortified at 0.2 ppm, was extracted and analyzed. The 0.2 ppm fortification level was selected to cover the proposed tolerance level for wheat forage.

The study was initiated on August 22, 1996, when the Study Director signed CAL Protocol 95P-014-04 (Appendix B). The experimental start date was September 6, 1996, and the experimental termination date was September 12, 1996.

### INDEPENDENT LABORATORY

Address:	Centre Analytical Laboratories, Inc. 3048 Research Drive State College, PA 16801
Contact Person:	Julie Burton
Phone:	(814) 231-8032

### TEST SYSTEM

Processed control (untreated) wheat forage sample was received from FMC at Centre Analytical Laboratories on August 20, 1996, frozen on dry ice. Wheat forage is a representative matrix for which the analytical method was developed, has the shortest crop rotation interval, is a major feed item, and has significant detectable residues. The control sample was immediately placed in a freezer (ca.  $\leq -10$  °C) for storage. The sample was removed from the freezer for subsampling, and returned to the freezer for storage. Chain-of-custody information can be found in the raw data associated with this study. Storage records will be kept at Centre Analytical Laboratories.

### REFERENCE MATERIALS

Analytical grade sulfentrazone, sulfentrazone-3-carboxylic acid, 3-desmethyl sulfentrazone and 3-hydroxymethyl sulfentrazone, were received on August 21, 1996

from FMC Corporation and used for preparation of fortification and quantitation standards. FMC performed characterization of the reference materials and maintains the characterization data, stability data, and solubility data for the reference materials. FMC will retain a sample of the test materials.

The available information for the test materials is listed below. The original test materials were stored in a refrigerator ( $+4\text{ }^{\circ}\text{C} \pm 2\text{ }^{\circ}\text{C}$ ) when not in use.

Compound	Lot Number	CAL ID	% Purity	Expiration Date
Sulfentrazone	SU-23	96-014-11	98.8	4/98
Sulfentrazone-3-carboxylic acid (SCA)	SU-4	96-014-13	92.5	6/97
3-hydroxymethyl sulfentrazone (HMS)	SU-2	96-014-14	97.2	12/97
3-desmethyl sulfentrazone (DMS)	SU-24	96-014-12	96.6	7/98

The stock standard solutions used in this study were prepared on August 24, 1996. Serial dilutions for fortification and calibration standards were also prepared on August 24, 1996 as specified in FMC analytical method, "Analytical Methodology for the Determination of Sulfentrazone, 3-Desmethyl Sulfentrazone, and 3-Hydroxymethyl Sulfentrazone in/on Various Matrices" (Reference). All stock standard solutions as well as fortification and calibration standard solutions were stored in a freezer (ca.  $\leq -10^{\circ}\text{C}$ ) when not in use. Documentation of standard preparation can be found in the raw data associated with this report.

## DESCRIPTION OF ANALYTICAL METHOD

FMC analytical method, "Analytical Methodology for the Determination of Sulfentrazone, 3-Desmethyl Sulfentrazone, and 3-Hydroxymethyl Sulfentrazone in/on Various Matrices" (Reference) was used for this study.

### A. Sample Extraction

A ten gram sample of wheat forage was weighed into a round bottom boiling flask, fortified with sulfentrazone, SCA and HMS if necessary, and refluxed for one hour with 150 mL of acetone/0.25N HCl (3/1, v/v). The extract was filtered and concentrated using a rotary evaporator to remove all traces of acetone. Concentrated HCl was added to the aqueous concentrate and refluxed for two hours. The extract was filtered and brought to 100 mL with distilled water before taking a 50 mL aliquot. The aqueous sample aliquot was passed through a  $\text{C}_8$  solid phase extraction (SPE) cartridge. The cartridge was dried for 30 minutes and attached to a silica gel SPE cartridge. The analytes were eluted from the  $\text{C}_8$ /silica gel cartridge and concentrated under nitrogen until a thin oily film remained.

The sample was redissolved in acetonitrile and derivatized with BSTFA (N,O-bis[trimethylsilyl]trifluoroacetamide). After derivatization, the sample was passed through a second silica gel SPE cartridge. The eluate was evaporated under nitrogen in a water bath until a thin oily film remained. The sample was adjusted to the appropriate final volume with acetonitrile and analyzed with a gas chromatograph equipped with a stand-alone electrolytic conductivity detector.

#### B. GC Instrumentation

A Hewlett Packard gas chromatograph, model 5890, equipped with an autosampler and a stand-alone electrolytic conductivity detector (ELCD) was used. Data was acquired and subsequently processed with a Hewlett Packard ChemStation. The GC operating conditions were as follows:

Column:	J&W DB-35, 35% phenyl methyl silicone, 30 m x 0.54 mm, 1.0 $\mu$ m film thickness
Gas Flow:	He, Carrier, ~16.0 mL/min
Inlet:	Splitless Injection Mode, Cyclo-double gooseneck
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
% 1-Propanol Flow:	37%
Injection Volume:	2 $\mu$ L
Run time:	15 minutes
Typical Retention Time:	DMS ~8.3 Sulfentrazone ~9.0 HMS ~11.5

### C. Preparation of Standards and Fortification Solutions

Stock standard solutions of sulfentrazone, 3-desmethyl sulfentrazone, sulfentrazone-3-carboxylic acid, and 3-hydroxymethyl sulfentrazone were prepared at a concentration of 1 mg/mL by dissolving a known amount of the standard in acetonitrile. Fortification solutions of sulfentrazone, sulfentrazone-3-carboxylic acid, and 3-hydroxymethyl sulfentrazone were prepared in acetonitrile at 10 and 1.0 µg/mL. Calibration standards of sulfentrazone, 3-desmethyl sulfentrazone, and 3-hydroxymethyl sulfentrazone were prepared at concentrations of 10 µg/mL and 1.0 µg/mL. Measured volumes of the 10 µg/mL and 1.0 µg/mL calibration standard solutions were derivatized simultaneously with the samples as specified in the method and diluted to appropriate final volumes to prepare linearity standard concentrations of 0.0625 µg/mL, 0.125 µg/mL, and 0.625 µg/mL. The 0.125 µg/mL derivatized standard was used for the working standard.

### D. Quantitation

Two microliters of sample or standard solution was injected into the GC. If necessary, the sample was diluted to give a response within the standard curve of the linearity check. The magnitude of the residue was determined using an external standard single-point calibration method based on the average of the standards in the assay set. The response, as peak area, was transferred to an Excel<sup>®</sup> computer spreadsheet for calculation. The concentration of the residue was determined from the following equations:

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

The amount of sample injected was calculated based on the aliquot sample weight, the final sample solution volume, and the injection volume. No correction for molecular weights was necessary for HMS since the working standard was derivatized simultaneously with the fortified samples. However, a correction factor of 1.118 (417/373, 417 = molecular weight of SCA and 373 = molecular weight of DMS) was needed for calculating the recovered amount of SCA from the quantitation result of DMS.

$$\text{mg of sample injected} = \frac{\text{aliquot sample weight (mg)}}{\text{sample volume } (\mu\text{L})} \times \text{injection volume } (\mu\text{L})$$

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

Method recovery was obtained by comparing the analyte amount recovered from the sample to the initial fortification level.

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

An example of a calculation using an actual sample follows:

Wheat Forage, Spike D (964613), fortified with 0.2 ppm of Sulfentrazone:  
(See Figures 1, 2 and 4)

$$\begin{aligned} \text{ng of analyte in sample} &= \frac{6668 \text{ (area in sample)}}{3827 \text{ (area unit of standard)}} \times 0.25 \text{ ng} \\ &= 0.4356 \text{ ng} \end{aligned}$$

$$\begin{aligned} \text{mg of sample injected} &= \left( \frac{5000 \text{ mg}}{4000 \mu\text{L}} \right) \times 2 \mu\text{L} \\ &= 2.5 \text{ mg} \end{aligned}$$

$$\begin{aligned} \text{ppm residue found} &= \frac{0.4356 \text{ ng}}{2.5 \text{ mg (injected)}} \\ &= 0.1742 \text{ ppm} \end{aligned}$$

$$\begin{aligned} \text{method recovery (\%)} &= \left( \frac{0.1742 \text{ ppm found}}{0.2 \text{ ppm added}} \right) \times 100\% \\ &= 87\% \end{aligned}$$

## RESULTS AND DISCUSSION

FMC analytical method, "Analytical Methodology for the Determination of Sulfentrazone, 3-Desmethyl Sulfentrazone, and 3-Hydroxymethyl Sulfentrazone in/on Various Matrices" was successfully validated during the first attempt using FMC wheat forage sample number 95-TWM-501C (CAL sample number 964613).

The method of quantitation used in this study followed FMC analytical method, "Analytical Methodology for the Determination of Sulfentrazone, 3-Desmethyl Sulfentrazone, and 3-Hydroxymethyl Sulfentrazone in/on Various Matrices". The method specifies that a linearity check should be run, and the magnitude of the residues should be determined using an external standard single-point calibration method based on the average of the standards in the assay set. The response, as peak area, was transferred to an Excel<sup>®</sup> computer spreadsheet for calculation. The protocol, however, states that the concentration of the compound being analyzed will be determined from the standard (linear or polynomial regression) curves. As previously stated, the method of quantitation followed was that described in FMC analytical method, "Analytical

Methodology for the Determination of Sulfentrazone, 3-Desmethyl Sulfentrazone, and 3-Hydroxymethyl Sulfentrazone in/on Various Matrices" instead of that described in the protocol.

Tables III-VI report the results from the wheat forage validation set for each of the three compounds. The average recoveries were 87% (SD = 7%), 100% (SD = 18%), and 74% (SD = 3%) for sulfentrazone, 3-desmethyl sulfentrazone as sulfentrazone-3-carboxylic acid, and 3-hydroxymethyl sulfentrazone, respectively. The limit of quantitation was 0.025 ppm and the limit of detection was 0.005 ppm.

#### A. Problems Encountered

A stand-alone ELCD, versus a tandem PID/ELCD, is necessary for analysis of these compounds. To achieve the required sensitivity, it is necessary to condition the instrument and column with several injections of sample matrix. This may be done periodically to improve the instrument response and peak shape.

#### B. Critical Steps

No critical steps other than those already detailed in the FMC method report were identified while performing the method validation for wheat forage.

#### C. Time Requirement

The extraction time for one set of seven samples was approximately 16 person-hours (2 days). Each GC analytical run required approximately 15 minutes.

#### D. Description of Contact

Audrey Chen and Jim Ridler, of FMC, visited the laboratory on August 22, 1996 to discuss the Sulfentrazone Independent Laboratory Validation project. A list of the items discussed are included in Appendix A.

A conversation was held with Audrey Chen on September 4, 1996 indicating the standard levels to be used for the linearity check.

The sponsor representative was notified of the results after the method validation was successfully completed.

### RETENTION OF DATA AND SAMPLES

When the final report is complete, all original paper data generated by Centre Analytical Laboratories, Inc., will be shipped to the sponsor. This does not include facility-specific raw data such as instrument logs, etc. Exact copies of these records will be sent to the sponsor. Exact copies of all raw data, as well as a signed copy of the final analytical report and all original facility-specific raw data, will be retained in the Centre Analytical Laboratories' archives as specified by the US EPA Good Laboratory Practice Standards.

Samples will be retained by Centre Analytical Laboratories, Inc. for the period of time specified in 40 CFR 160.195 (b). Sample extracts will be disposed of when the results have been accepted by the sponsor representative.

### CONCLUSION

The independent laboratory validation of FMC analytical method, "Analytical Methodology for the Determination of Sulfentrazone, 3-Desmethyl Sulfentrazone, and 3-Hydroxymethyl Sulfentrazone in/on Various Matrices" for sulfentrazone, 3-desmethyl sulfentrazone, and 3-hydroxymethyl sulfentrazone in wheat forage was successful. The results indicate that the method can yield reproducible results and is reliable as written.

### REFERENCE

Shevchuk, Natalie A., "Analytical Methodology for the Determination of Sulfentrazone, 3-Desmethyl Sulfentrazone, and 3-Hydroxymethyl Sulfentrazone in/on Various Matrices" (Draft) FMC Corporation, August 1996.

Table I  
 Test and Reference Substances

Common Name Chemical Name	Structure	CAS Number
<b>Sulfentrazone</b> 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
<b>Sulfentrazone-3-carboxylic acid (SCA)</b> 1-[2,4-dichloro-5-(N-(methylsulfonyl)amino)phenyl]-4-difluoromethyl-4,5-dihydro-5-oxo-1H-1,2,4-triazole-3-carboxylic acid		134391-01-8
<b>3-Desmethyl sulfentrazone (DMS)</b> N-[2,4-dichloro-5-(4-(difluoromethyl)-4,5-dihydro-5-oxo-1H-1,2,4-triazol-1-yl)phenyl]methanesulfonamide		134391-02-9
<b>3-Hydroxymethyl sulfentrazone (HMS)</b> 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

Table II  
Record of Sample Dates

<i>CAL Sample Identification</i>	<i>FMC Sample Identification</i>	<i>Matrix</i>	<i>Date Shipped from FMC</i>	<i>Date Received at CAL</i>	<i>Date Extracted</i>	<i>Date Analyzed</i>
964613	95-TWM-501C	Wheat Forage	8/19/96	8/20/96	9/6-7/96	9/11-12/96

Table III  
 Analytical Data for the Analysis of 3-Desmethyl Sulfentrazone (DMS)

Protocol No.: 95P-014-04  
 Type of Experiment: Independent Laboratory Validation  
 Matrix: Wheat Forage  
 Analyte: 3-Desmethyl Sulfentrazone (DMS)

Limit of Quantitation: 0.025 ppm  
 CAL Study No.: 014-04  
 Analysis Date: 9/11-12/96  
 Analyzed By: Julie Burton  
 Checked By: JB 9/17/96

ng Standard Injected	DMS Response (Area)
0.25	4017
0.25	4087
0.25	3933
0.25	3675
0.25	3198

Average Peak Area: 3782  
 Standard Deviation: 362  
 Coefficient of Variation: 9.6%

Sample ID	Injection No.	Final Volume (mL)	Dilution Factor	Initial Sample Weight (g)	Aliquot Sample Weight (g)	Peak Area	ng Found	mg of Aliquot Sample	ppm Residue Found as DMS	ppm Residue Found as SCA	ppm SCA Added	SCA Percent Recovery
Reagent Blank	2	1.0	1	NA	NA	0	0.0	0.0	0.0	0.0	NA	NA
*Blank A	3	1.0	1	10.0	5.0	0	0.0	0.0	0.0	0.0	NA	NA
*Blank B	5	1.0	1	10.0	5.0	0	0.0	0.0	0.0	0.0	NA	NA
*Spike A	6	1.0	1	10.0	5.0	4071	0.2691	10.0	0.0269	0.0301	0.025	120
*Spike B	8	1.0	1	10.0	5.0	3664	0.2422	10.0	0.0242	0.0271	0.025	108
*Spike C	9	4.0	1	10.0	5.0	6152	0.4067	2.5	0.1627	0.1819	0.2	91
*Spike D	11	4.0	1	10.0	5.0	5447	0.3601	2.5	0.1440	0.1611	0.2	81

\*Sample ID 95-TWM-501C (CAL Sample ID 964613)      \*\*Correction Factor = 417/373 = 1.118

**Table IV**  
**Analytical Data for the Analysis of Sulfentrazone**

**Protocol No.:** 95P-014-04  
**Type of Experiment:** Independent Laboratory Validation  
**Matrix:** Wheat Forage  
**Analyte:** Sulfentrazone

**Limit of Quantitation:** 0.025 ppm  
**CAL Study No.:** 014-04  
**Analysis Date:** 9/11-12/96  
**Analyzed By:** Julie Burton  
**Checked By:** JS 9/17/96

ng Standard Injected	Sulfentrazone Response (Area)
0.25	3862
0.25	4033
0.25	4042
0.25	3765
0.25	3433

**Average Peak Area:** 3827  
**Standard Deviation:** 249  
**Coefficient of Variation:** 6.5%

Sample ID	Injection No.	Final Volume (mL)	Dilution Factor	Initial Sample Weight (g)	Aliquot Sample Weight (g)	Peak Area	ng Found	mg of Aliquot Sample	ppm Residue Found	ppm Added	Percent Recovery
Reagent Blank	2	1.0	1	NA	NA	0	0.0	0.0	0.0	NA	NA
*Blank A	3	1.0	1	10.0	5.0	0	0.0	0.0	0.0	NA	NA
*Blank B	5	1.0	1	10.0	5.0	0	0.0	0.0	0.0	NA	NA
*Spike A	6	1.0	1	10.0	5.0	3157	0.2062	10.0	0.0206	0.025	82
*Spike B	8	1.0	1	10.0	5.0	3114	0.2034	10.0	0.0203	0.025	81
*Spike C	9	4.0	1	10.0	5.0	7446	0.4864	2.5	0.1946	0.2	97
*Spike D	11	4.0	1	10.0	5.0	6668	0.4356	2.5	0.1742	0.2	87

\*Sample ID 95-TWM-501C (CAL Sample ID 964613)

Table V

Analytical Data for the Analysis of 3-Hydroxymethyl Sulflentrazone (HMS)

**Protocol No.:** 95P-014-04  
**Type of Experiment:** Independent Laboratory Validation  
**Matrix:** Wheat Forage  
**Analyte:** 3-Hydroxymethyl Sulflentrazone (HMS)  
**Limit of Quantitation:** 0.025 ppm  
**CAL Study No.:** 014-04  
**Analysis Date:** 9/11-12/96  
**Analyzed By:** Julie Burton  
**Checked By:** JS 9/17/96

ng Standard Injected	HMS Response (Area)
0.25	3878
0.25	4204
0.25	3651
0.25	3500
0.25	3526

**Average Peak Area:** 3752  
**Standard Deviation:** 294  
**Coefficient of Variation:** 7.8%

Sample ID	Injection No.	Final Volume (mL)	Dilution Factor	Initial Sample Weight (g)	Aliquot Sample Weight (g)	Peak Area	ng Found	mg of Aliquot Sample	ppm Residue Found	ppm Added	Percent Recovery
Reagent Blank	2	1.0	1	NA	NA	0	0.0	0.0	0.0	NA	NA
*Blank A	3	1.0	1	10.0	5.0	0	0.0	0.0	0.0	NA	NA
*Blank B	5	1.0	1	10.0	5.0	0	0.0	0.0	0.0	NA	NA
*Spike A	6	1.0	1	10.0	5.0	2663	0.1774	10.0	0.0177	0.025	71
*Spike B	8	1.0	1	10.0	5.0	2779	0.1852	10.0	0.0185	0.025	74
*Spike C	9	4.0	1	10.0	5.0	5768	0.3843	2.5	0.1537	0.2	77
*Spike D	11	4.0	1	10.0	5.0	5414	0.3608	2.5	0.1443	0.2	72

\*Sample ID 95-TWM-501C (CAL Sample ID 964613)

**Table VI<sup>1</sup>**  
**Summary of Fortification Recovery Data**

Fortification Level (ppm)	Percent Recovery		
	DMS as SCA	Sulfentrazone	HMS
0.025	120	82	71
0.025	108	81	74
<b>0.025 ppm Fortification Average:</b>	<b>114</b>	<b>82</b>	<b>73</b>
0.2	91	97	77
0.2	81	87	72
<b>0.2 ppm Fortification Average:</b>	<b>86</b>	<b>92</b>	<b>75</b>
<b>Overall Average:</b>	<b>100</b>	<b>87</b>	<b>74</b>
<b>Overall Standard Deviation:</b> (n = 4)	<b>18</b>	<b>7</b>	<b>3</b>

<sup>1</sup> Unrounded values were used for calculations.

Figure 1

Representative Chromatogram of Standard (0.125  $\mu\text{g}/\text{mL}$ )

Injection No.: 4

Analysis of Sulfentrazone, 3-Hydroxymethyl Sulfentrazone  
and 3-Desmethyl Sulfentrazone in/on Wheat Forage

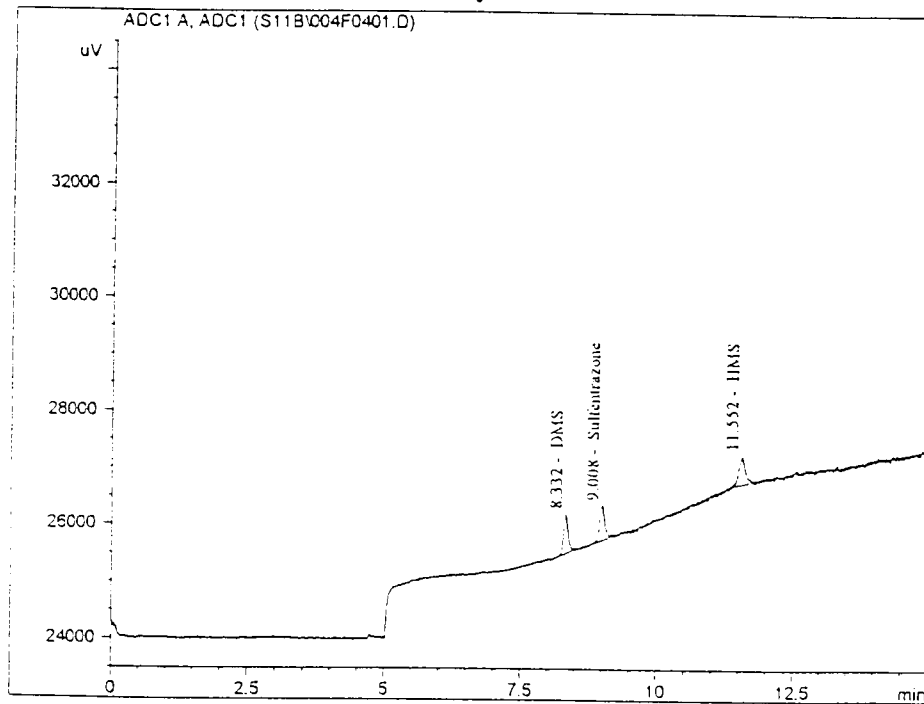
Sample No.: NA

Sample Info: 0.125  $\mu\text{g}/\text{mL}$  standard, C082496-2

Injected on: 9/11/96

11:56:29 PM

Is the chromatogram used? (yes) If not, why?



Compound Name	Measured RT [min]	Height [ $\mu\text{V}$ ]	Area [ $\mu\text{V}\cdot\text{s}$ ]
DMS	8.332	708	4087
Sulfentrazone	9.008	629	4033
HMS	11.552	518	4204

Figure 2

Representative Chromatogram of Control Wheat Forage Sample

Injection No.: 5

Analysis of Sulfentrazone, 3-Hydroxymethyl Sulfentrazone  
and 3-Desmethyl Sulfentrazone in/on Wheat Forage

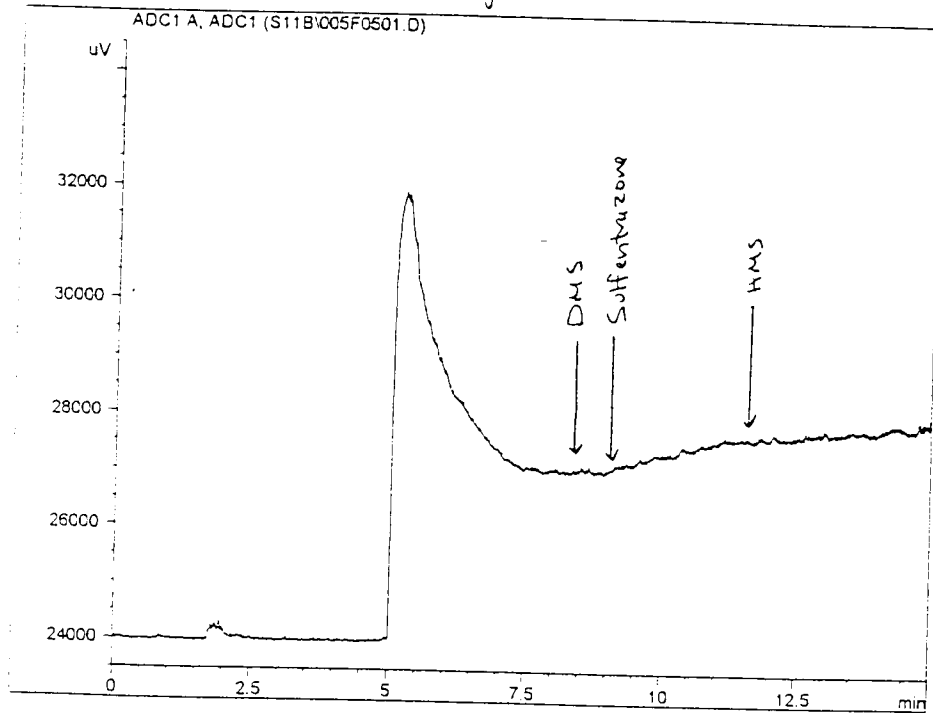
Sample No.: 95-TWM-501C

Sample Info: Control, B1K B

Sample Weight = 10.0 g, Final Volume = 1 mL ->

Injected on: 9/12/96 12:19:11 AM

Is the chromatogram used? (yes) If not, why?



Compound Name	Measured RT [min]	Height [ $\mu$ v]	Area [ $\mu$ v*s]
DMS	0.000	0	0
Sulfentrazone	0.000	0	0
HMS	0.000	0	0

**Figure 3**

**Representative Chromatogram of Fortified Control Wheat Forage Sample  
(0.025 ppm fortification)**

Injection No.: 8

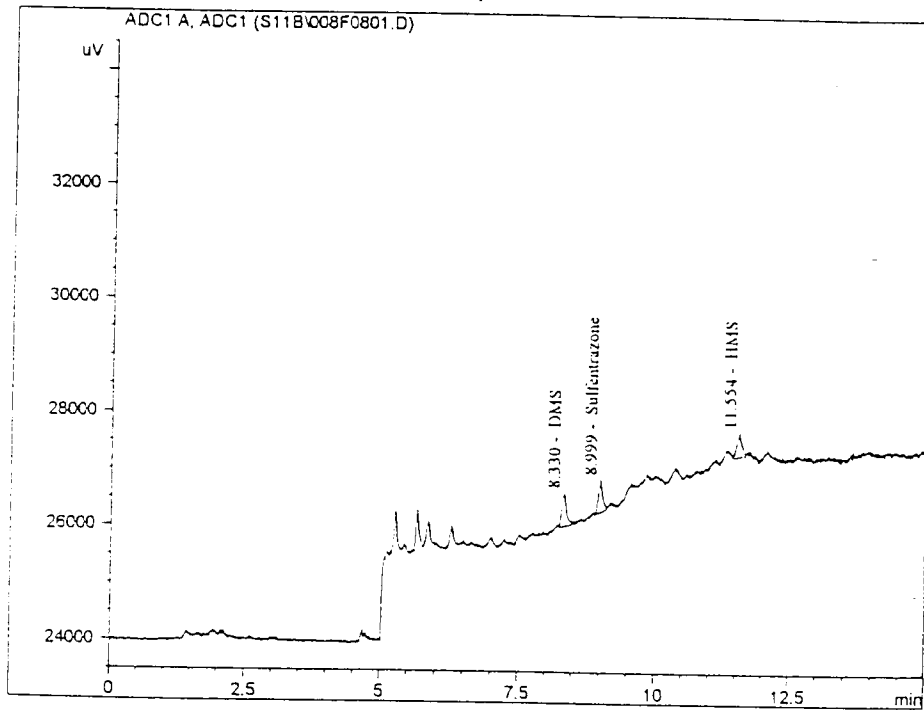
Analysis of Sulfentrazone, 3-Hydroxymethyl Sulfentrazone  
and 3-Desmethyl Sulfentrazone in/on Wheat Forage

Sample No.: 95-TWM-501C

Sample Info: Control Fortified at 0.025 ppm (0.25 ug added)  
Sample Weight = 10.0 g, Final Volume = 1 mL ->  
SPR B

Injected on: 9/12/96 1:27:18 AM

Is the chromatogram used? (yes) If not, why?



Compound Name	Measured RT [min]	Height [ $\mu$ v]	Area [ $\mu$ v*s]
DMS	8.330	594	3664
Sulfentrazone	8.999	580	3114
HMS	11.554	434	2779

**Figure 4**

**Representative Chromatogram of Fortified Control Wheat Forage Sample  
(0.2 ppm fortification)**

Injection No.: 11

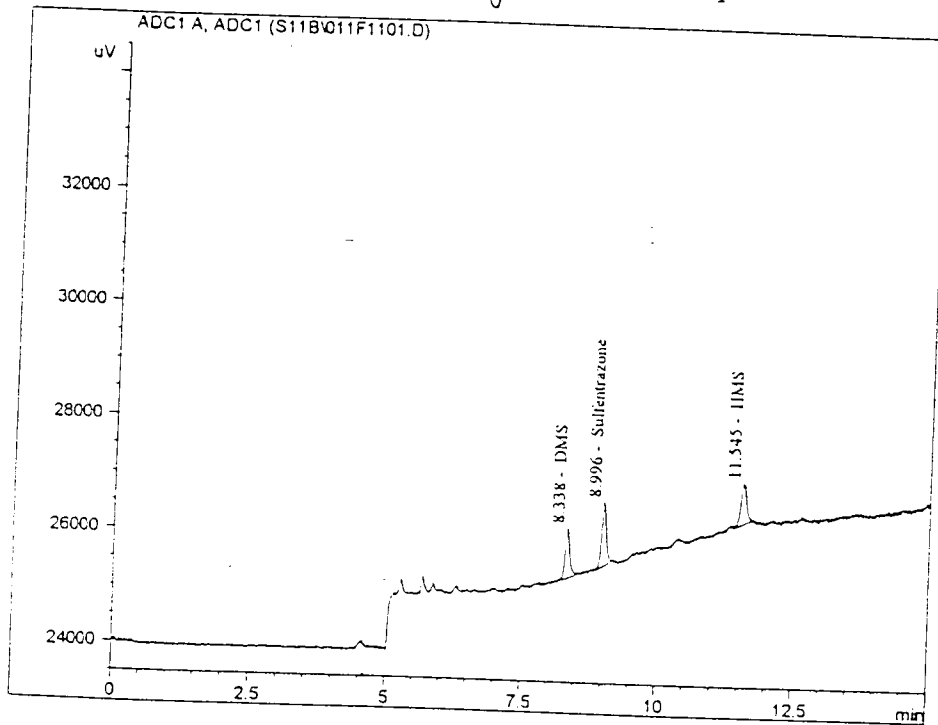
Analysis of Sulfentrazone, 3-Hydroxymethyl Sulfentrazone  
and 3-Desmethyl Sulfentrazone in/on Wheat Forage

Sample No.: 95-TWM-501C

Sample Info: Control Fortified at 0.2 ppm (2.0 ug added)  
Sample Weight = 10.0 g, Final Volume = 4 mL

SpK D

Injected on: 9/12/96 2:35:22 AM  
Is the chromatogram used? (yes) If not, why? ->



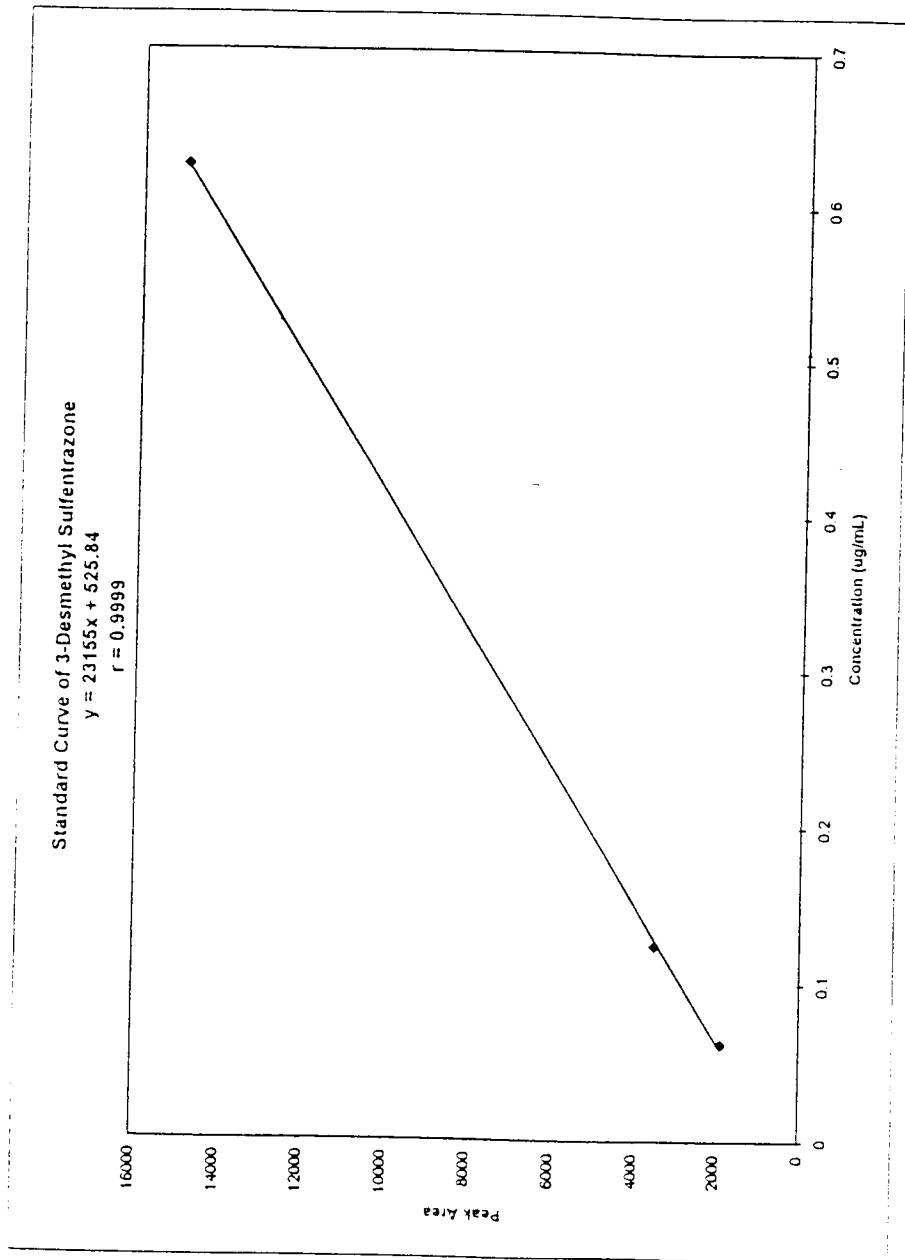
Compound Name	Measured RT [min]	Height [ $\mu$ v]	Area [ $\mu$ v*s]
DMS	8.338	884	5447
Sulfentrazone	8.996	1137	6668
HMS	11.545	735	5414

Figure 5

Representative Standard Calibration Curve of 3-Desmethyl Sulfentrazone

Protocol No.: 95P-014-04

Linear Curve Analysis Date: 9/11/96



CAL Study Number: 014-04  
FMC Study Number: 162MVL96R2

5/13/96

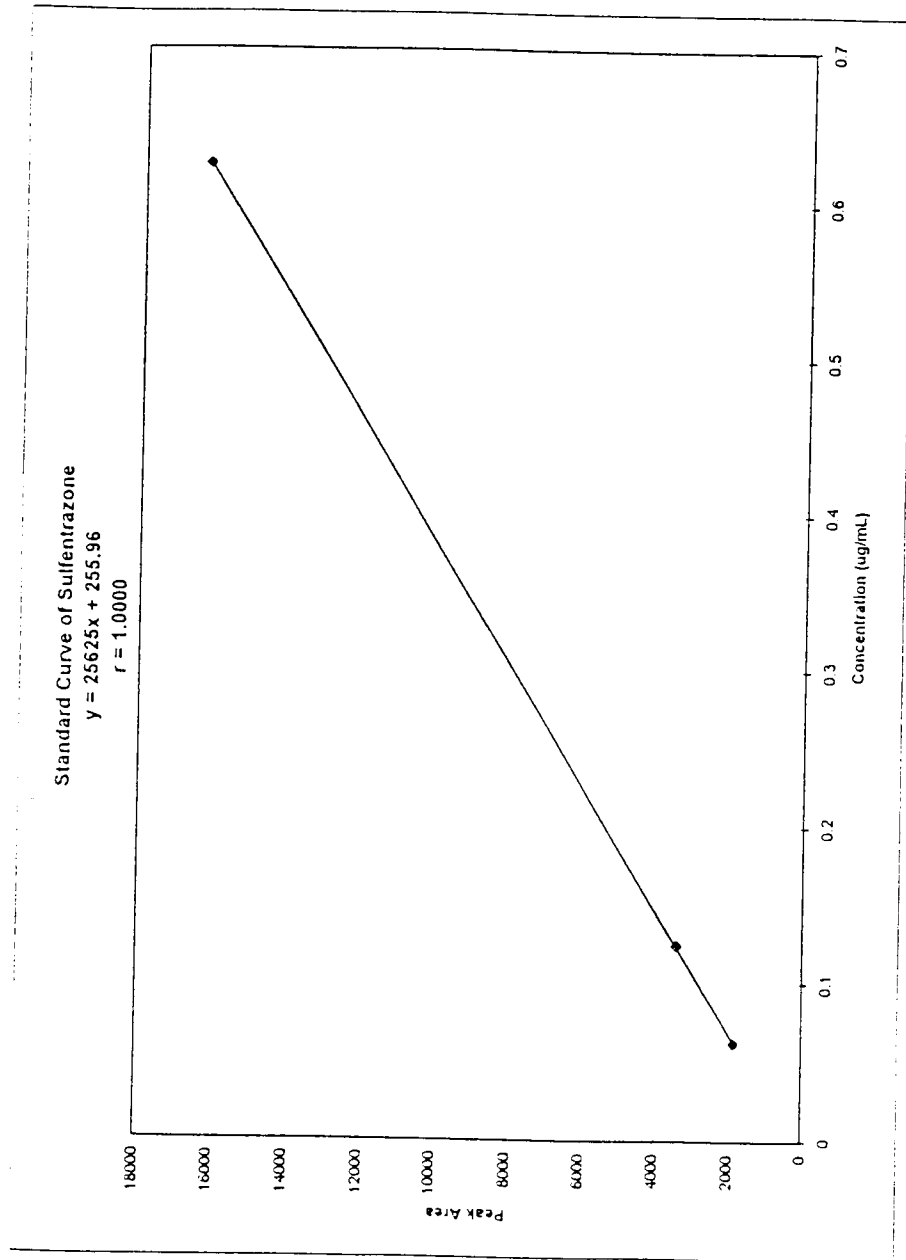
9/13/96 2:37 PM

Figure 6

Representative Standard Calibration Curve of Sulfentrazone

Protocol No.: 95P-014-04

Linear Curve Analysis Date: 9/11/96



9/13/96  
CAL Study Number: 014-04  
FMC Study Number: 162MVL96R2

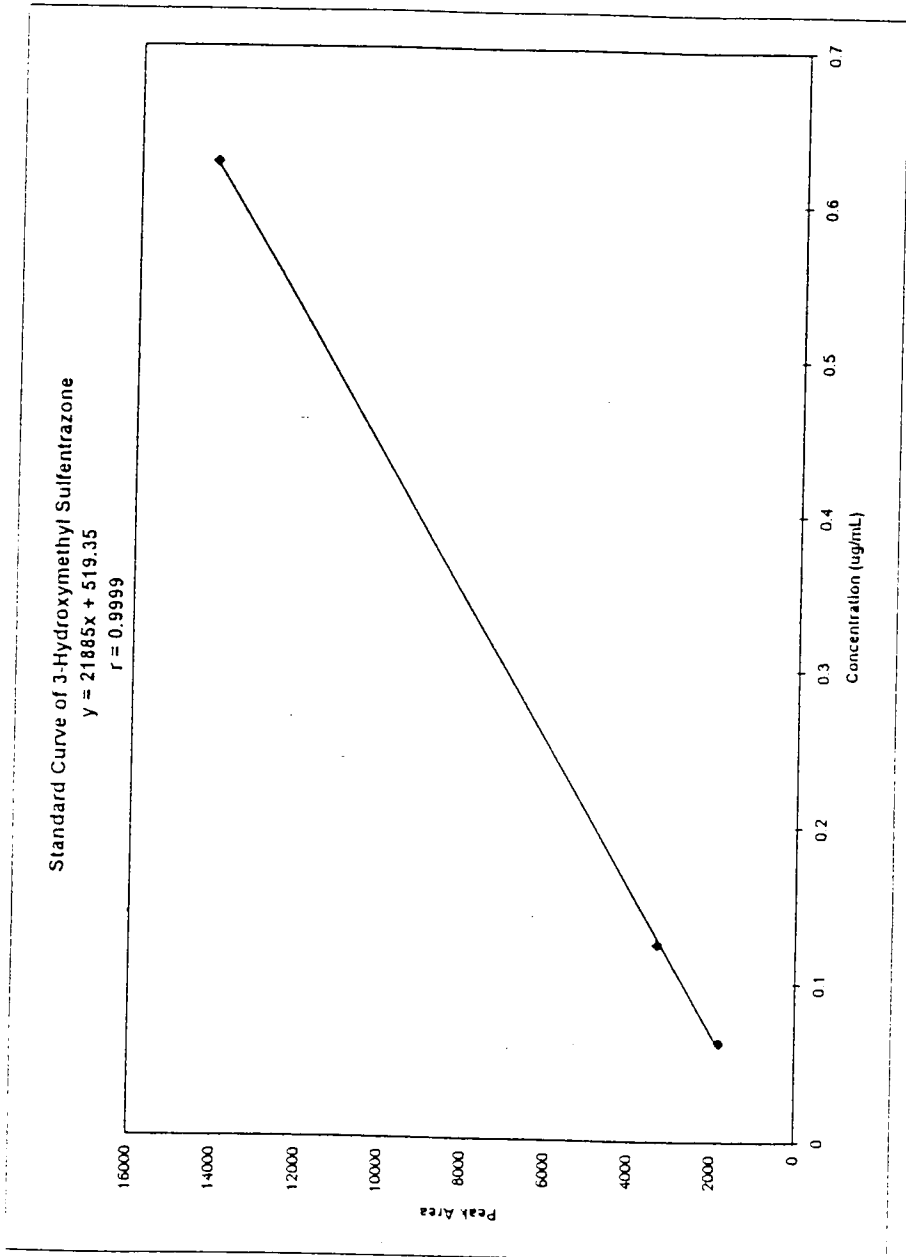
9/11/96 2:21 PM

Figure 7

Representative Standard Calibration Curve of 3-Hydroxymethyl Sulfentrazone

Protocol No.: 95P-014-04

Linear Curve Analysis Date: 9/11/96



9/13/96  
CAL Study Number 014-04  
FMC Study Number 162MVL96R2

9/11/96 2:23 PM

**Appendix A**  
**Correspondence**

Sulfentrazone ILV Project 162 MVL96R2

Centre Analytical Laboratory Visit - August 22, 1996

Audrey Chen and I visited the laboratory to finalize plans for this project. Upon our arrival we met with Julie Burton, the Study Director for the project and Michael Arjmand, President and Laboratory Director for the facility. Rick Grazzini, the Business Director for Centre Analytical joined us later to discuss the cost of the study.

I brought the method recovery table with results for each analyte in wheat forage and four sample chromatograms to supplement the information in the draft method report.

During our visit, the draft study protocol was reviewed and signed by the study personnel, the FMC addendum to the Master Study Agreement was completed and we toured the current laboratory and the new building which was just granted a Certificate of Occupancy. Centre Analytical personnel will begin using the new labs in early September.

In addition, the draft method report, which Centre Analytical will use for the validation project was reviewed. Any changes from the previously validated enforcement method and areas of concern were discussed. These items are listed below:

#### Method

An additional 2 hour reflux step was added to the method to completely hydrolyze the analytes from the sample matrix. Recent Metabolism information indicated that a 1N acid reflux completely releases any conjugated hydroxymethyl sulfentrazone and decarboxylates the sulfentrazone carboxylic acid.

After the initial reflux, all traces of acetone must be removed from the acid solution before the second reflux is initiated.

A fresh 1 mL ampule of BSTFA derivatizing reagent should be used each time since moisture can react with BSTFA.

During the tandem cartridge procedure, the silica cartridge must not be allowed to go dry.

Centre Analytical uses rotoevaporators while FMC used a Turbovap for large volume evaporation. This should not be a problem. Centre will use a low volume Turbovap LV for small volumes.

During the derivatization step if a phase separation occurs, gently warm the samples in a 45°C water bath and vortex for 1 minute. If the phase separation persists, continue warming and vortexing until the phases mix completely.

If the derivatized samples sit for a long period of time before being injected, the derivatization process may reverse. Refer to item #5 in the Potential Problems section of the method report for further information.

Points in the method where the analytical procedure can be stopped at the end of the day were discussed. This information is included in the method report.

The elution pattern of the SPE cartridges should be verified prior to starting the first method attempt.

#### Instrumentation

The ELCD is being used for quantitation since the interference problems from sample matrix associated with ECD are not experienced using ELCD.

The cyclo double gooseneck insert specified in the method report has been ordered by Centre Analytical.

Centre Analytical has the same GC/ELCD set up as FMC.

Centre Analytical has ordered the column (J&W DB-35). Since the column is new, it must be conditioned with plant matrix prior to use.

Sensitivity of the GC/ELCD will be demonstrated prior to starting the first method attempt.

#### Standards

The injection standards are prepared during the derivatization step along with the samples for analysis.

The volume of the injection standard can be diluted or concentrated to the appropriate level for analysis.

A linearity curve (at least 4 points, covering the range of half LOQ to the highest residue level) needs to be run during the study. The linearity curve does not have to be generated during the method validation attempts.

A single point calibration (average area counts for the number of standards injected) will be used for data calculations.

#### General

MSD confirmation does not have to be done for this study.

**The ELCD must be running at the sensitivity levels specified in the method report and maintain stability at that level for successful analysis. If the conditioning starts to decline, the des methyl standard peak will be lower than the sulfentrazone peak.**

## Appendix B

### Protocol and Protocol Revisions



## Centre Analytical Laboratories, Inc.

3048 Research Drive State College, PA 16801  
Phone: (814) 231-8032 Facsimile: (814) 231-1253

# RESIDUE STUDY PROTOCOL

PROTOCOL NUMBER: 95P-014-04  
CAL STUDY NUMBER: 014-04  
FMC STUDY NUMBER: 162MVL96R2

## 1. STUDY TITLE

Independent Laboratory Validation of Analytical Methodology for the Determination of Sulfentrazone, 3-Hydroxymethyl Sulfentrazone and 3-Desmethyl Sulfentrazone in/on Wheat Forage

## 2. PURPOSE

To conduct an Independent Laboratory Validation of the "Analytical Methodology for the Determination of Sulfentrazone, 3-Hydroxymethyl Sulfentrazone and 3-Desmethyl Sulfentrazone in/on Various Matrices" under the guidelines of PR Notice 96-1, in order to determine the suitability of using this method to determine sulfentrazone (STZ), 3-hydroxymethyl sulfentrazone (HMS) and 3-desmethyl sulfentrazone (DMS) residues present in wheat forage.

## 3. SPONSOR

FMC Corporation  
P. O. Box 8  
Princeton, NJ 08543  
phone 609-951-3434  
fax 609-951-3670

Sponsor's Representative: James Ridler, FMC Corporation

## 4. TESTING FACILITY AND PERFORMING LABORATORY

Centre Analytical Laboratories, Inc.  
3048 Research Drive  
State College, PA 16801

## 5. STUDY PERSONNEL

Study Director: Julie Burton, Centre Analytical

## 6. TEST MATERIALS

### REFERENCE MATERIALS

The following analytical standards will be used.

Standard	FMC Reference Number	Purity	Expiration Date
Sulfentrazone (STZ)	MID# SU-23	96.8%	04 / 98
Sulfentrazone-3-carboxylic acid (SCA)	MID# SU-4	92.5%	06 / 97
3-hydroxymethyl sulfentrazone (HMS)	MID# SU-2	97.2%	12 / 97
3-desmethyl sulfentrazone (DMS)	MID# SU-24	96.6%	07 / 98

## 7. PROPOSED EXPERIMENTAL TIMEFRAME

Experimental Start Date	August 26, 1996
Experimental Termination Date	September 15, 1996
Report Issued	September 30, 1996

## 8. DESCRIPTION OF THE TEST SYSTEM

- A. Sample type: Wheat forage
- B. Sources: Untreated wheat forage samples will be received from the sponsor.
- C. Justification: Wheat forage is a representative matrix for which the analytical method was developed, has the shortest crop rotation interval, is a major feed item, and has significant detectable residues.

## 9. HAZARD INFORMATION

A current MSDS for the chemical used in this study will be maintained at the testing facility.

## 10. EXPERIMENTAL DESIGN

### A. Description of study

Control and fortified control samples of wheat forage will be analyzed following the method, "Analytical Methodology for the Determination of Sulfentrazone, 3-Hydroxymethyl Sulfentrazone and 3-Desmethyl Sulfentrazone in/on Various Matrices", and using a calibrated analytical system, with comparison to appropriate controls, reference substances,

Protocol 95P-014-04  
dated August 22, 1996  
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and analytical standards. The Limit of Detection (LOD) for this method is stated as 0.005 ppm from a 10 g sample, with a Limit of Quantitation (LOQ) of 0.025 ppm.

Control wheat forage samples will be fortified with a mixed standard solution of STZ, SCA, and HMS. Fortification levels will be at 0.025 ppm and 0.2 ppm (proposed tolerance level).

During analysis, the SCA metabolite is converted to DMS while the HMS metabolite is silylated. Therefore, the calibration standard solutions will contain STZ, DMS and derivatized (silylated) HMS.

Analytical sample sets will contain calibration standards which bracket the fortification levels, and anticipated residue levels in the sample extracts. Analysis of these calibration standards will be used to generate a standard curve. Fortified samples in which residues are detected will be diluted if necessary so that the residue levels may be quantitated using this standard curve.

A typical analytical sample set will consist of calibration standards, a reagent blank, two control (blank) samples, two fortified control samples at 0.025 ppm, and two fortified control samples at 0.2 ppm. Standards will bracket all treated samples, i.e. the analytical run will begin and end with the analysis of calibration standards.

B. Preparation

The control wheat forage sample will be placed in long-term frozen storage at -5 to -25°C.

C. Fortification solutions

Fortification standard solutions will be prepared from stock standard solutions as described in the FMC method report. Tracking sheets for the preparation of fortification solutions will follow SOP CAL-5H.

D. Fortification

The fortification solution containing the mixed standard solution (STZ, SCA, HMS) will be applied to control wheat forage samples via a Hamilton syringe, or equivalent, as described in the FMC method report.

E. Method of analysis

Samples will be assayed using the method "Analytical Methodology for the Determination of Sulfentrazone, 3-Hydroxymethyl Sulfentrazone and 3-Desmethyl Sulfentrazone in/on Various Matrices". In brief, samples are acid-hydrolyzed, partially purified using SPE cartridges, silylated with BSTFA, purified following derivatization by SPE, and then concentrated and prepared for analysis by gas chromatography (GC). Detection following GC will be by halogen-specific electrolytic conductivity detection (ELCD). The analyte concentration in each sample will be determined by comparison of the peak height or area found for the analyte with a standard curve constructed from calibration standards for each analyte which bracket the target concentration. Fortification recovery values are to be between 70 and 120 percent.

F. Methods to control bias

Methods to control bias will include assay of untreated control samples, fortification of untreated control samples to obtain recovery data, and replicate analysis of fortified samples to provide an indication of reproducibility.

G. Statistical methods to be used

Standard (linear or polynomial regression) curves used to determine the concentration of the compound being analyzed will be constructed from a minimum of three concentrations. Quantification of residues will only occur within the range of the standard curve.

Mean values and standard deviations will be calculated for each fortification level, and across all fortification levels.

No other statistical methods will be used during the course of this study.

11. DATA REPORTING

Records to be maintained include the following (as appropriate):

1. Sample tracking sheet(s)
2. Sample processing records, storage history, and chains of custody
3. History and preparation of standards (stock, fortification, calibration)
4. Any modifications to the method
5. Instrument run sheets, benchsheets or logs
6. Analytical data tables

Protocol 95P-014-04  
dated August 22, 1996  
page 5 of 6

7. All chromatographic and instrumental conditions
8. Sample extraction and analysis dates
9. A complete listing of study personnel, signatures and initials
10. Chronological presentation of all study correspondence
11. Any other data necessary for the reconstruction of the study

**Chromatograms** - All chromatograms will contain the following:

- A. Sample identification, data, final sample volume, sample weight, arrow to indicate the area of interest, and injection number corresponding to the run.
- B. Additionally, fortifications will include the  $\mu\text{g}$  added and the sample number of the sample that was fortified.
- C. Analytical standard chromatograms will additionally include the  $\mu\text{g}/\text{ml}$  concentration.
- D. The first analytical standard chromatogram in a daily run should also include temperatures, flow rates, column parameters, gases, instrument parameters, and instrument type if any of these differ from the method. CAL study number will also be recorded on the first chromatogram of each daily analytical run.

**12. PROTOCOL AMENDMENTS AND DEVIATIONS**

Any deviations from the protocol or from the analytical method as provided will be documented and reported promptly to the Sponsor Representative. Planned changes to this protocol or to the analytical method will be approved by the Study Director and the Sponsor Representative and attached to this protocol as amendments.

**13. FINAL REPORT**

The final report will be written upon completion of analysis. The report will include a description of the materials and methods used, the analytical data generated, and signed and dated Study Compliance and Quality Assurance Statements. The final report and copies of the raw data must be approved by the Sponsor Representative and FMC Quality Assurance prior to signature.

**14. GLP COMPLIANCE STATEMENT**

This analysis will be conducted in compliance with EPA's Good Laboratory Practice Standards (40 CFR 160). All raw data generated in this study will be archived by the Sponsor, or a designated representative of the Sponsor. The Study Director is responsible for this protocol,

Protocol 95P-014-04  
dated August 22, 1996  
page 6 of 6

analytical experimentation, and the final report. The Study Director will review the protocol, procedures, good laboratory practices and safety guidelines with all support personnel prior to their participation in the study. The Study Director will sign the GLP compliance statement.

**15. QUALITY ASSURANCE**

The QA Unit of Centre Analytical Laboratories will inspect the analysis at intervals adequate to assure compliance to GLPs, and will report the findings of the audits to the Study Director, CAL management, and the Sponsor Representative. The QA Unit of the Sponsor will also audit the final report and the study file.

**16. DISTRIBUTION**

Study Director  
Sponsor Representative  
CAL QAU Files

**17. SIGNATURES:**

	<u>Signature</u>	<u>Date</u>
Study Director	<u>Julie Burton</u> Julie Burton Centre Analytical Laboratories	<u>8/22/96</u>
Sponsor Representative	<u>James Ridler</u> James Ridler FMC Corporation	<u>8/22/96</u>
CAL QA Reviewer	<u>Gail Z. Keller</u> Gail Keller Centre Analytical Laboratories	<u>8/26/96</u>



**Centre Analytical Laboratories, Inc.**

3048 Research Drive  
Phone: (814) 231-8032

State College, PA 16801  
Facsimile: (814) 231-1253

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**PROTOCOL/SOP/METHOD AMENDMENT**

Amendment Number: 1

Effective Date: 8/27/96

CAL Study Number: 014-04

Sponsor Study Number: 162MVL96R2

DESCRIPTION OF AMENDED SECTION

1. Protocol Section 6. Test Materials - The purity for Sulfentrazone is 96.8 %.
2. Protocol Section 8. Description of the Test System - The procedure for identifying the test system was not included in the protocol.
3. Protocol Section 10. B. Preparation - The control wheat forage sample will be placed in long-term frozen storage at -5 to -25 °C.

AMENDED TO

1. The purity for Sulfentrazone is 98.8 %.
2. Section 8. D. Identification of the Test System - The test systems are untreated wheat forage samples which will be identified as control and control fortified, with the level of fortification indicated.
3. The control wheat forage sample will be placed in long-term frozen storage at  $\leq -10$  °C.

RATIONALE

1. Typographical error.
2. Section inadvertently omitted from the original protocol.
3. Changed to correspond with our current SOPs.

IMPACT ON THE STUDY

1., 2., 3. No negative impact on the study.

  
Study Director Signature

8/30/96  
Date

  
Sponsor Representative Signature

9/3/96  
Date

CAL QAU Review GW 9/9/96

July 1995/0



**Centre Analytical Laboratories, Inc.**

3048 Research Drive  
Phone: (814) 231-8032

State College, PA 16801  
Facsimile: (814) 231-1253

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**PROTOCOL/SOP/METHOD DEVIATION**

Deviation Number: 1  
Date of Occurrence: 9/12/96

CAL Project Number: 014-04 Sponsor Study Number: 162MVL96R2

**DESCRIPTION OF DEVIATION**

Is this deviation a X PROTOCOL, or      SOP, or      METHOD deviation?

1. The analyte concentration was not determined using a standard curve as specified in the protocol (Section 10E). Instead, the calculation procedure described in Section V.D. of the method was followed.

**ACTIONS TAKEN**

i.e., amendment issued, SOP revision, etc...

Protocol deviation issued.

Recorded By/Date: Julie Bunt 9/16/96

**IMPACT ON THE STUDY**

There are no negative effects on the study.

Julie Bunt  
Study Director Signature

9/16/96  
Date

CAL QAU Review GW 9/16/96