FMC CORPORATION
AGRICULTURAL PRODUCTS GROUP
Princeton, NJ

B99-116

STUDY TITLE:	Residue Methodology for the Determination of F8426 and Its Acid Metabolites in/on the Small Grain Crops
TEST SUBSTANCE:	F8426, F8426-Chloropropionic Acid, 3-Desmethyl-F8426-Chloropropionic Acid and 3-Hydroxymethyl-F8426-Chloropropionic Acid
DATA REQUIREMENT:	Residue Chemistry Test Guidelines OPPTS 860 1340 Residue Analytical Method
AUTHOR:	Natalie Shevchuk
STUDY DATES:	
Study Initiated:	August 15, 1997
Experiment Terminated:	September 9, 1997
Study Completed	October 6, 1997
Method Study Reported.	October 6, 1997
Method Study Report Reissued	July 30, 1998

NOTE: This report supersedes the previous report issued on October 6, 1997 All changes that were made to this report are noted on page 5a. Also, all pages that were revised are so noted in the upper right hand corner of the page on which the revision was made.

PERFORMING LABORATORY: FMC Corporation
Agricultural Products Group
Residue Chemistry
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STUDY NUMBER: 842MVL97R1

Non-Proprietary Information

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

Linda W. Froelich

Linda W. Froelich,
Manager, Residue Chemistry
COMPANY AGENT

7/30/98
Date

GOOD LABORATORY PRACTICES STATEMENT

The studies (listed below) of the FMC Corporation Agricultural Products Group in which this analytical method was developed and applied were conducted and reported in compliance with the Good Laboratory Practice Standards (GLPS) set forth in Title 40, Part 160 of the Code of Federal Regulations of the United States of America with the following exceptions.

Study Number	Report No		GLPS Exceptions
842MVL97R1	P-3263		None
842COS96R1	P-3231	1	For trial 06, instrument log books for equipment used for the soil characterization were lost in a fire at Agvise Laboratories on 12/29/96
842RIC96R1	P-3237		None
842RIC96R2	P-3243	1	Some field data for Trial 02 was not signed and dated at the time of collection
		2.	The field scale used in Trial 02 for determining field weight of samples was not calibrated and had no SOP at the time it was used
842SOR96R1	P-3254		None

Natalie A Shevchuk — 7/30/98
Natalie A Shevchuk
Associate Research Chemist
STUDY DIRECTOR
Date

John M. Becker — 07/31/98
John M. Becker
Manager, Formulations
SPONSOR
Date

Callista Chukwunenye — 8/3/98
Callista O Chukwunenye, PhD
Manager, Product Registration
SUBMITTER
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 in design and performance Study **842MVL97R1**, "Residue Methodology for the Determination of F8426 and its Major Metabolites in/on the Small Grain Crops" and referenced studies reported herein, were inspected and the findings reviewed and signed by the Study Director and Management of FMC Corporation on the following dates.

Inspection Dates	Signed by Study Director	Signed by Management	Signed by Director
P-3231	**842COS96R1**		
6/17/96	8/15/96	8/19/96	8/30/96
6/20/96	7/17/96	7/29/96	7/30/96
6/28/96	9/19/96	9/23/96	9/23/96
7/19/96	8/29/96	8/30/96	9/3/96
7/23/96	8/28/96	8/30/96	9/3/96
7/29/96	8/5/96	8/7/96	8/8/96
8/1/96	8/15/96	8/16/96	8/16/96
8/2/96	8/29/96	8/30/96	8/30/96
9/3/96	9/19/96	9/23/96	9/23/96
9/3/96	10/10/96	10/11/96	10/11/96
9/7/96	10/4/96	10/4/96	10/7/96
9/10/96	9/20/96	9/23/96	9/23/96
9/18/96	10/8/96	10/9/96	10/11/96
2/20,21/97	2/24/97	2/25/97	3/17/97
P-3237	**842RIC96R1**		
5/20/96	7/11/96	7/12/96	7/16/96
6/10/96	7/11/96	7/12/96	7/15/96
6/14/96	7/11/96	7/12/96	7/15/96
6/14/96	7/11/96	7/12/96	7/15/96
6/17/96	7/17/96	7/29/96	7/30/96
6/27/96	7/11/96	7/12/96	7/15/96
6/28/96	7/11/96	7/12/96	7/15/96
6/28/96	7/17/96	7/29/96	7/30/96
7/9/96	8/21/96	9/3/96	9/4/96
8/27/96	9/6/96	9/10/96	9/12/96

9/2/96	9/20/96	9/25/96	9/25/96
9/9/96	9/20/96	9/25/96	9/25/96
9/9/96	9/20/96	9/25/96	9/25/96
9/23/96	10/4/96	10/7/96	10/7/96
9/24/96	10/4/96	10/7/96	10/7/96
2/4/97	2/12/97	2/14/97	2/24/97
2/6/97	2/12/97	2/13/97	2/13/97
2/27/97	3/3/97	3/4/97	3/5/97
P-3243	**842RIC96R2**		
07/30/96	11/25/96	12/06/96	12/06/96
10/10/96	10/30/96	10/31/96	10/31/96
03/26/97	03/27/97	03/31/97	04/01/97
04/01/97	04/02/97	04/03/97	04/04/97
P-3254	**842SOR96R1**		
6/14/96	7/11/96	7/12/96	7/15/96
4/23/96	6/18/96	6/20/96	7/3/96
4/26/96	5/21/96	5/22/96	5/23/96
5/21/96	7/17/96	7/29/96	7/30/96
7/18/96	8/19/96	8/19/96	8/30/96
8/28/96	9/12/96	9/12/96	9/16/96
10/7/96	10/24/96	10/28/96	10/28/96
10/16/96	10/25/96	10/29/96	10/29/96
10/11/96	11/5/96	11/6/96	11/6/96
11/12/96	11/26/96	11/29/96	12/2/96
4/25/97	4/28/97	4/29/97	5/5/97
P-3263	**842MVL97R1**		
9/4/97	9/4/97	9/5/97	9/5/97

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

Jane W. Brown

Jane W. Brown
Quality Assurance Associate

7/30/98
Date

LIST OF REPORT REVISIONS

This report has been revised to introduce the current methodology in use on small grain crops All revised pages have been indicated with "Revised 7/98" in the upper right hand corner The following pages were revised

Page	Change
1	Revised report date, added notation for page 5a.
2	Updated Linda W Froelich as Manager of Residue, who replaced John M Becker John M Becker is currently Manager of Formulations with FMC Corp
3,5a,29	Updated John M. Becker's job title to Manager of Formulations
3,5a,29	Updated Natalie Shevchuk's job title.
5,5a	Updated Jane Brown's job title
14	Listed the Tekmar Tissuemizer as an alternative to the Omni blender
14	Specified a 20 µm pore size for the 6 mL filtration tubes.
14	Added the Hobart Cutter/Mixer Model# HCM 450 under equipment used.
15	Added the Thomas-Wiley Laboratory Mill Model# ED-5 under equipment used.
15	Specified 40 µm particle size for silica gel, C_{18}, and SCX solid phase extraction cartridges
15	Added Organomation N-Evap as an alternative to the TurboVap
15	Noted that equivalent apparatus to Supelco's Visiprep and Visidry may be used
16	Provided a brief summary of the steps involved with processing the raw agricultural commodities prior to analysis.
23	Removed statement referring to subtraction of peak area from the fortified sample if the magnitude was greater than the Limit of Detection
24	Under "Interferences" removed the statement that interferences were subtracted from appropriate fortified samples.
25	Three ions for confirmation were provided for the acid metabolites.

This page 5a has been added to explain the revisions to the original report. Pagination was affected on pages 14 - 99. These pages have also been indicated as revised.

Natalie A Shevchuk — 7/30/98
Natalie A. Shevchuk, Associate Research Chemist — Date
Study Director

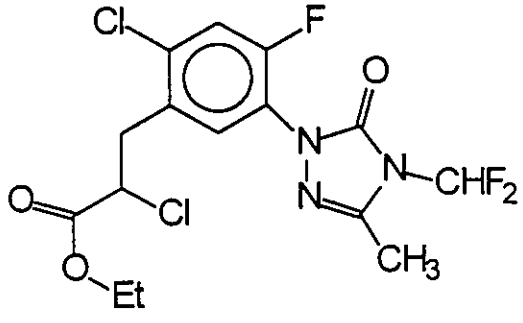
John M. Becker — 07/31/98
John M. Becker, Manager, Formulations — Date
Sponsor

Jane W. Brown — 7/30/98
Jane W. Brown, Quality Assurance Associate — Date
Quality Assurance Unit

TABLE OF CONTENTS

I. INTRODUCTION

F8426 50DF is a herbicide being developed by FMC Corporation for the control of various weeds of wheat and other grain crops F8426 50DF contains 50% active ingredient in a dry flowable base. The active ingredient is F8426 having the proposed common name of carfentrazone-ethyl. The chemical name of F8426 is ethyl α, 2-dichloro-5-[4-(difluoromethyl)-4,5-dihydro-3-methyl-5-oxo-1H-1,2,4-triazol-1-yl]-4-fluorobenzenepropanoate. The CAS Number is 128639-02-1. The chemical structure of F8426 is as follows·



F8426

An analytical method report for F8426 and its major metabolites in/on wheat was previously issued (Section XI, Reference 1) More recently there have been subtle modifications to the method to improve the extraction efficiency of the analytes, and to improve the overall ruggedness of the methodology. This revised residue analytical method for F8426 and its acid metabolites (F8426-chloropropionic acid, 3-desmethyl-F8426-chloropropionic acid and 3-hydroxymethyl-F8426-chloropropionic acid) has been successfully practiced on sorghum, rice and sweet corn in previously reported RAC studies (Section XI, References 2, 3, 4 and 5) and on field corn, sorghum and wheat matrices during this study The purpose of this report is to describe in full detail the revised residue analytical method for F8426 and its acid metabolites in/on the small grain crops and to compile data generated with this method previously and during this study.

II. SUMMARY

The revised analytical method for F8426, F8426-chloropropionic acid (F8426-Cl-PAc), 3-desmethyl-F8426-chloropropionic acid (3-DM-F8426-Cl-PAc) and 3-hydroxymethyl-F8426-chloropropionic acid (3-OH-F8426-Cl-PAc) was successfully used on the following crops (crop parts). field corn (grain, forage and fodder), sweet corn (ears, forage and stover), rice (grain and straw), sorghum (grain, forage and stover), and wheat (grain, forage, hay and straw) Since 3-OH-F8426-Cl-PAc was determined to be a significant metabolite on rice straw and wheat forage, hay and straw only, based on wheat metabolism data (Section XI, Reference 6), analysis for this metabolite was limited to these crop parts.

F8426 and its acid metabolites were determined by a procedure which began with an initial blending of matrix with a binary solvent (acetone water, 4 1) The organic moiety was removed by nitrogen evaporation and the pH of the aqueous fraction was adjusted to 6. The parent F8426 was partitioned from the aqueous media by hexane extraction The hexane containing the parent F8426 was subjected to a silica gel solid phase extraction (SPE) cartridge clean-up, eluted with 7 5 or 10% ethyl acetate in hexane and the solvent was exchanged to acetonitrile for analysis. The determination and quantification of parent F8426 was accomplished using a gas chromatograph equipped with a 30 m DB-17 (50% diphenyl/50% dimethyl silicone) wide-bore capillary column and an electron capture detector. The overall average method recovery for F8426 was 96 ± 9% (n=59) The method limit of quantitation was practiced at 0 05 ppm and the limit of detection was estimated at 0.01 ppm.

The analysis for the acid metabolites began with the remaining aqueous fraction following the hexane partition for parent F8426. Concentrated hydrochloric acid (HCl) was added to the aqueous solution containing the acid metabolites (such that the final acid concentration was 1N or slightly greater) and the mixture was refluxed for one hour to cleave the conjugated residues to free acids Following the reflux, the acids were separated from matrix using a cation exchange SPE cartridge and concentrated on a C_{18} SPE cartridge. The acids were subsequently eluted from the C_{18} SPE cartridge with 5% methanol in dichloromethane and concentrated to about 0.1 - 0.25 mL for derivatization. All three metabolites (F8426-Cl-PAc, 3-DM-F8426-Cl-PAc and 3-OH-F8426-Cl-PAc) were methylated using a solution of 14% boron trifluoride (BF_3) in methanol. Approximately 1 mL of derivatization solution was added to the 0 1 - 0.25 mL extract and allowed to react for 45 minutes at 50°C. The samples were then cooled to room temperature After cooling, the methylated acid metabolites were diluted with 2 mL of water. For samples which did not require analysis of 3-OH-F8426-Cl-PAc, the methylated acids were extracted with hexane. For samples which required analysis of 3-OH-F8426-Cl-PAc, the

methylated acids were extracted with dichloromethane, dried over sodium sulfate, reduced to 0 1 mL volume and acylated with acetic anhydride and pyridine for 45 minutes at 50°C. Following acylation of the methylated hydroxymethyl metabolite, the samples were diluted with water and partitioned into hexane

The hexane fractions from either route were subjected to silica gel SPE clean-up The samples were eluted with 20% ethyl acetate in hexane, concentrated to near dryness and adjusted to a final volume with hexane for analysis The analysis utilized a gas chromatograph equipped with a 15 m DB-35 (35% diphenyl/65% dimethyl silicone) capillary column and a mass selective detector which was set to monitor ions 348, 362, and 413 for 3-DM-F8426-Cl-PAc, F8426-Cl-PAc, and 3-OH-F8426-Cl-PAc, respectively The overall average method recoveries were 99 ± 12% (n=59) for F8426-Cl-PAc, 96 ± 14% (n=59) for 3-DM-F8426-Cl-PAc, and 101 ± 14% (n=16) for 3-OH-F8426-Cl-PAc The method limit of quantitation was practiced at 0 05 ppm. The limit of detection varied from 0 01 to 0 02 ppm depending on the calibration method and matrix

III. SUMMARY TABLES AND GRAPHICS

A Summary of Method Recovery Data

TABLE 1

METHOD RECOVERY VALUES FOR F8426 AND ITS ACID METABOLITES FROM LABORATORY FORTIFIED SMALL GRAIN MATRICES

Analyte	Fortification Range (ppm)	Number of Analyses	Overall Range (%)	Overall Average (%) ± Standard Deviation (%)
F8426	0 05 - 1 0	59	76 - 123	96 ± 9
F8426-Cl-Propionic acid	0 05 - 1 0	59	72 - 125	99 ± 12
3-Desmethyl-F8426-Cl-Propionic-acid	0 05 - 1 0	59	66 - 124	96 ± 14
3-Hydroxymethyl-F8426-Cl-Propionic aci	0 05 - 1 0	16	77 - 125	101 ± 14

Analyte/ Matrix	Fortification Levels (ppm)	Number of Analyses	Recovery Range (%)	Average Recovery (%) ±Standard Deviation (%)
F8426				
Field Corn Grain	0 05	3	88 - 102	97 ± 8
Field Corn Forage	0 05, 0 10	3	84 - 97	92 ± 7
Field Corn Fodder	0 05	3	96 - 109	101 ± 7
Sweet Corn Ears	0 05	5	94 - 104	100 ± 4
Sweet Corn Forage	0 05	5	76 - 87	84 ± 5
Sweet Corn Stover	0 05	6	80 - 98	86 ± 7
Rice Grain	0 05	6	94 - 107	99 ± 5
Rice Straw	0 05, 1 0	5	83 - 108	98 ± 10
Sorghum Grain	0 05	3	85 - 94	90 ± 5
Sorghum Forage	0 05	3	102 - 123	113 ± 11
Sorghum Stover	0 05	4	91 - 103	96 ± 5
Wheat Grain	0 05, 0 25	3	84 - 102	93 ± 9
Wheat Forage	0 05, 0 25, 0 5	4	95 - 101	99 ± 3
Wheat Hay	0 05, 0 25	3	94 - 108	99 ± 8
Wheat Straw	0 05, 0 25	3	91 - 111	100 ± 10
F8426-Cl-Propionic acid				
Field Corn Grain	0 05	3	102 - 104	103 ± 1
Field Corn Forage	0 05, 0 10	3	78 - 103	92 ± 13
Field Corn Fodder	0 05	3	90 - 113	101 ± 12
Sweet Corn Ears	0 05	5	88 - 125	103 ± 14
Sweet Corn Forage	0 05	5	93 - 123	103 ± 12
Sweet Corn Stover	0 05	5	90 - 109	96 ± 8

Continued

TABLE 1 (*Continued*)

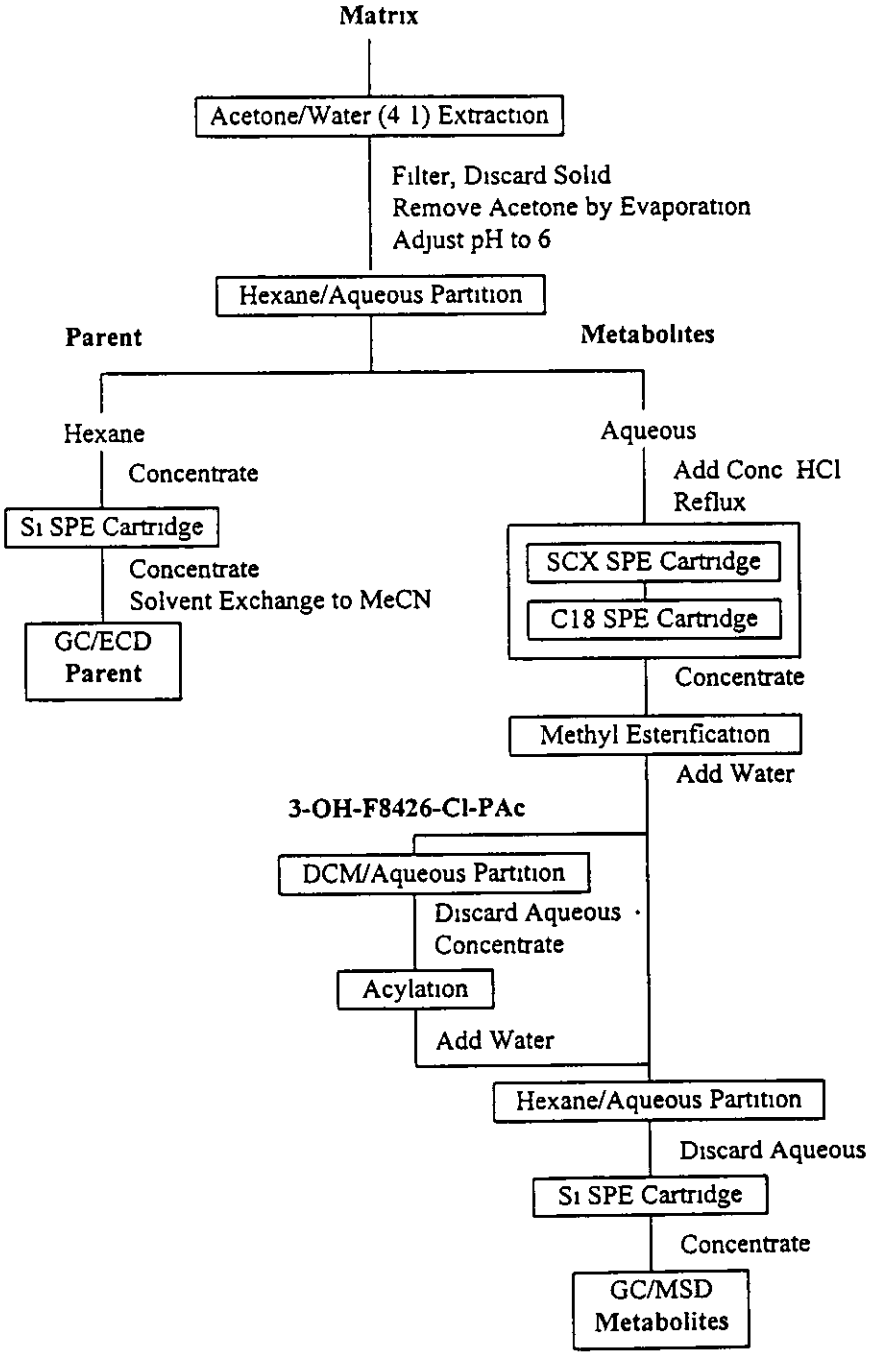
METHOD RECOVERY VALUES FOR F8426 AND ITS ACID METABOLITES FROM LABORATORY FORTIFIED SMALL GRAIN MATRICES

Analyte/ Matrix	Fortification Levels (ppm)	Number of Analyses	Recovery Range (%)	Average Recovery (%) ± Standard Deviation (%)
F8426-Cl-Propionic acid				
Rice Grain	0 05	6	82 - 114	102 ± 12
Rice Straw	0 05, 0 10	6	88 - 110	98 ± 9
Sorghum Grain	0 05	3	86 - 86	86 ± 0
Sorghum Forage	0 05	3	102 - 114	108 ± 6
Sorghum Stover	0 05	4	103 - 114	108 ± 5
Wheat Grain	0 05, 0 25	3	97 - 107	103 ± 6
Wheat Forage	0 05, 0 25, 0 5	4	87 - 121	100 ± 15
Wheat Hay	0 05, 0 25	3	72 - 99	89 ± 15
Wheat Straw	0 05, 0 25	3	77 - 79	78 ± 1
3-Desmethyl-F8426-Cl-Propionic-acid				
Field Corn Grain	0 05	3	93 - 99	97 ± 3
Field Corn Forage	0 05, 0 10	3	66 - 89	80 ± 13
Field Corn Fodder	0 05	3	78 - 114	96 ± 18
Sweet Corn Ears	0 05	5	88 - 121	103 ± 12
Sweet Corn Forage	0 05	5	94 - 120	107 ± 9
Sweet Corn Stover	0 05	5	92 - 122	101 ± 12
Rice Grain	0 05	6	99 - 124	111 ± 9
Rice Straw	0 05, 1 0	6	91 - 117	99 ± 10
Sorghum Grain	0 05	3	71 - 76	73 ± 3
Sorghum Forage	0 05	3	99 - 111	104 ± 6
Sorghum Stover	0 05	4	88 - 113	100 ± 10
Wheat Grain	0 05, 0 25	3	68 - 93	80 ± 13
Wheat Forage	0 05, 0 25, 0 5	4	77 - 95	84 ± 8
Wheat Hay	0 05, 0 25	3	70 - 96	86 ± 14
Wheat Straw	0 05, 0 25	3	80 - 102	88 ± 12
3-Hydroxymethyl-F8426-Cl-Propionic acid				
Rice Straw	0 05, 1 0	6	84 - 116	102 ± 13
Wheat Forage	0 05, 0 25, 0 5	4	90 - 121	103 ± 14
Wheat Hay	0 05, 0 25	3	77 - 114	95 ± 19
Wheat Straw	0 05, 0 25	3	91 - 125	104 ± 18

B Method Flow Scheme

FIGURE 1

ANALYTICAL METHOD FOR F8426 AND
ITS ACID METABOLITES IN/ON SMALL GRAIN CROPS



IV MATERIALS AND STUDY DESIGN

A Test Substance

The test substances used in the small grains analytical method were F8426, F8426-Cl-PAc, 3-DM-F8426-Cl-PAc and/or 3-OH-F8426-Cl-PAc Since 3-OH-F8426-Cl-PAc was determined to be a significant metabolite on rice straw and wheat forage, hay and straw only, based on a wheat metabolism data (Section XI, Reference 6), analysis for this metabolite was limited to these crop parts 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 Commodity

The small grain crops (crop parts) that were analyzed using this method previously and during this study include field corn (grain, forage and fodder), sweet corn (ears, forage and stover), rice (grain and straw), sorghum (grain, forage and stover), and wheat (grain, forage, hay and straw) All crop parts were harvested following normal agricultural procedures.

C. Study Design and Procedures

The revised analytical method for F8426 and its acid metabolites was previously used on rice (grain and straw), sorghum (forage and stover) and sweet corn (ears, forage and stover) (Section XI, References 2, 3, 4, and 5). This study was designed to include other small grain crops that were not analyzed with the revised method. During this study, control field corn (grain, forage and fodder), sorghum (grain) and wheat (grain, forage, hay and straw) samples from previously reported RAC studies were selected for analysis. All samples were collected per EPA guidelines 860 1500. Data generated with this method previously and during this study have been included in this report.

The residue method was validated with acceptable and reproducible recoveries The small grain control samples were fortified by adding known amounts of F8426 and its acid 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. An assay set included a minimum of one control and one fortified control sample to determine the method recovery. At least three fortified control samples for each matrix were analyzed.

D. Equipment

All glassware and sample-handling containers were routinely washed in a Fury® dishwasher (Model 2000) 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.

The following is a list of equipment used to perform this method. Substitutions for equivalent non-critical equipment are possible.

Access*Chrom Data Acquisition Software - running on MicroVax
Balance, Analytical PM 2000, Mettler
Balance, Top Loading, Mettler
Blender, Omni - equipped with a macro generator (20 mm dia. x 145 mm long w/sawteeth part #15401, cat #17105) or equivalent such as a Tekmar tissuemizer
Boiling Stones, Hengar
Buchner Filter Funnels, porcelain, 10.5 cm i d , Coors
Capillary Column, DB-35, 15 m x 0.25 mm id, 0 25 µm, J & W Scientific
Capillary Column, DB-17, 30 m x 0 53 mm id, 1 0 µm, J & W Scientific
Centrifuge Tubes, 15 mL graduated, Pyrex®, 0 1 mL
Centrifuge Tubes, 50 mL graduated, polypropylene, VWR (cat #21008-714)
Condensers, Graham Coil, Pyrex, 41 mm x 500 mm with Ŧ 24/40 joint
Cylinders, Graduated, 10 mL, 50 mL, 100 mL, 250 mL
Cylinders, Mixing, 250 mL graduated
Filtration Tubes (6 mL capacity) containing a (20 µm pore size) polyethylene frit, VWR (cat #JT7121-6)
Flasks, Vacuum Filter, Pyrex, 500 mL
Flasks, Round Bottom Boiling, Kontes, 50 mL, Ŧ 45/50 joint
Gas Chromatograph (GC), Hewlett-Packard (HP) 5890 equipped with a HP 7673A autosampler and an Electron Capture Detector (ECD)
Gas Chromatograph, HP 5890 equipped with a HP 7673A Autosampler and a HP 5972 Mass Selective Detector (MSD)
GC Injector Liner (GC-ECD), Cyclouniliner Insert, Restek (cat #20337)
GC Injector Liner (GC-MSD), Cyclo double Gooseneck Insert, 2 mm, Restek (cat #20907)
Glass Wool
Heating Mantles, 500 mL, Glas-Col®
Hobart® Cutter/Mixer Model# HCM 450
Injection Vials, 2 mL, Wheaton
Injection Vial Crimps, 11 mm, Teflon®/Silicone/Teflon, Sun Brokers
Micro Syringes, (25 µL, 50 µL, 100 µL, 250 µL, 500 µL), Hamilton

Single - Tube Vortexer, VWR Scientific
Pipettes, Disposable (5 75 in and 9 in lengths)
Pipettes, Volumetric Pipette Bulbs
Reducing Adapters (Solid Phase Extraction), plastic, Supelco
Reservoirs, plastic, 75 mL
Screwcap Glass Tubes, 50 mm x 150 mm
Solid Phase Extraction Cartridge, C_{18} (1 g, 40 µm mesh), Bakerbond, VWR (cat#JT7020-07)
Solid Phase Extraction Cartridge, SCX (1 g, 40 µm mesh), Analytichem, Varian (part #1225-6011)
Solid Phase Extraction Cartridge, Silica Gel (1 g, 40 µm mesh, SiOH), J.T Baker, VWR (cat#JT7086-07)
Test Tubes, glass, 25 mm by 150 mm
Stainless Steel Blending Cups, 400 mL capacity, Omni (cat #17079)
Thermometer (°C)
Thomas-Wiley® Laboratory Mill Model# ED-5
TurboVap® Evaporator, Zymark or equivalent such as an Organomation® N-Evap
TurboVap Centrifuge Tube Support Rack, Zymark
TurboVap Vessels, 200 mL, Zymark
TurboVap Vessel Support Rack, Zymark
Visiprep® Vacuum Manifold, Supelco or equivalent
Visidry® Vacuum Manifold Drying Attachment, Supelco or equivalent
Whatman Glass MicroFibre Filters No 934-AH, 7 cm, VWR (cat #28496-955)

E. Reagents

The following is a list of reagents used to perform this method. Substitutions for equivalent reagents may be possible, but may require re-calibration of the method and especially the elution steps.

Acetic Anhydride, ACS Reagent Grade, Sigma Chemical Co. (product No. A6404) or Aldrich (product No 11,004-3)
Acetone, Resi-Analyzed, JT Baker
Acetonitrile, HPLC Grade, JT Baker
Boron Trifluoride (14% in methanol), Sigma Chemical Co (product No. 13-1127)
Ethyl Acetate. Pesticide Grade, JT Baker
Hexane, Resi-Analyzed, JT Baker
Hydrochloric Acid (HCl, 36.5 - 38.0%), JT Baker
Hydrion pH Buffer, VWR (cat #34175-220)
Methanol, Resi-Analyzed, JT Baker

Methylene Chloride (Resi-analyzed), JT Baker
pH Indicator Strips (EM Science), VWR (cat #EM-9590-3)
Pyridine, Fisher (99 9%) or Sigma Chemical Co (product No P-4036)
Sodium Sulfate, Anhydrous, JT Baker
Water, Distilled/D I equivalent

V. ANALYTICAL PROCEDURE

A. Residue Methods

Methodology was developed for the determination of F8426 and its acid metabolites (F8426-Cl-PAc, 3-DM-F8426-Cl-PAc and 3-OH-F8426-Cl-PAc) in/on the small grain crops (Section XI, References 1, 2, 3, 4, and 5)

Prior to analysis, the small grain samples were ground to homogeneity with liquid nitrogen. The grain crop parts were processed in a Thomas-Wiley Laboratory Mill Each grain sample was placed into a mixing bowl, liquid nitrogen was then poured over the sample to maintain its frozen state while processing Each sample was then passed through the Thomas-Wiley Laboratory Mill which processes the grain sample into a fine homogeneous powder-like consistency Forage, hay, stover, straw and ear samples were processed in a Hobart Cutter/Mixer. Each bulk sample was placed into the mixing bowl of the Hobart Cutter/Mixer, liquid nitrogen was poured over the sample to maintain its frozen state during processing. The sample was then processed by the Hobart Cutter/Mixer until a fine homogeneous consistency was achieved. All samples were stored frozen until analysis.

1. INITIAL EXTRACTION OF PARENT F8426 AND ACID METABOLITES FROM SMALL GRAIN MATRICES

1-1 Weigh 2.5 grams of crop into a blending vessel. Fortify samples at this point with the appropriate analytical standards Add ca. 100 mL of acetone.water (4 1, v v) and blend using an Omni mixer equipped with a macro generator (20 mm dia. X 145 mm lg w/sawteeth) for 5 minutes at 6000 - 7000 rpm. Filter the sample through a Whatman 934 AH glass fiber filter paper on a Buchner funnel/vacuum flask setup Rinse the blending cup and filter cake with ca. 100 mL of acetone Transfer the filtrate to a 200 mL TurboVap vessel.

1-2. Concentrate the sample under nitrogen to ca. 20 - 25 mL using a TurboVap (water bath at 50° C). Transfer the sample to a 50 mL

polypropylene centrifuge tube Rinse the TurboVap vessel with ~5 or 10 mL of Hydrion pH 6 buffer The amount of pH 6 buffer required depends on the matrix being analyzed and should be calibrated by the analyst All matrices needed 5 mL of the buffer solution to adjust the sample to pH 6, except for sweet corn (ears, forage, and stover) which required 10 mL Add the rinse buffer to the sample. Rinse the TurboVap vessel with 10 mL of hexane and add the hexane to the sample

1-3 Vigorously mix the aqueous and hexane fraction to partition F8426 into the hexane fraction Centrifugation may be necessary to break any emulsion that occurs. Remove and collect the hexane fraction for analysis of F8426 Partition the aqueous fraction with an additional 10 mL of hexane and add it to the hexane from the first partition step. The aqueous fraction will be used for the analysis of the acid metabolites of F8426 (see Section # 3 below).

2. ANALYSIS OF PARENT F8426

2-1 Concentrate the hexane fraction (~ 20 mL) from the previous hexane/aqueous partition to ~ 3 mL in a TurboVap at ca. 50°C.

2-1a. For grain and forage matrices, condition a 1 gram 6 cc Si SPE cartridge (Baker) with 1 cartridge volume (CV ~ 6 mL) of 10% ethyl acetate in hexane (v v) followed by 1 CV of hexane (vacuum at ~1 in. Hg). Load the 3 mL sample onto the cartridge, but do not drain yet. Rinse the tube with 3 mL of hexane and also load this rinsate onto the cartridge. Drain the ~6 mL of sample solution through the Si cartridge (vacuum at ~1 in. Hg) and discard. Rinse the Si cartridge with 9 mL of 10% ethyl acetate in hexane and discard the rinsate. Elute and collect the sample with an additional 12 mL of 10% ethyl acetate in hexane (vacuum at ~1 in. Hg)

2-1b For fodder, hay, stover or straw matrices condition a 1 gram 6 cc Si SPE cartridge (Baker) with 1 CV of 7 5% ethyl acetate in hexane (v·v) followed by 1 CV of hexane (vacuum at ~1 in. Hg). Load the 3 mL sample onto the cartridge, but do not drain yet. Rinse the tube with 3 mL of hexane and also load this rinsate onto the cartridge Drain the ~ 6 mL of sample solution through the Si cartridge and discard. Rinse the Si cartridge with 9 mL of

10% ethyl acetate in hexane and discard the rinsate Elute and collect the sample with an additional 18 mL of 7 5% ethyl acetate in hexane (vacuum at ~1 in. Hg)

Note: In order to exclude an interference which only occurs in the dry matrices, a slightly less polar elution solvent (7 5% vs 10% ethyl acetate in hexane) and a larger volume is used

2-2. Concentrate the sample to ~ 0 1 mL in a TurboVap at ca. 50°C, and adjust to a final volume of 1 0 mL with acetonitrile. **Note: There is the potential for loss of analytes if the samples go to dryness at this step.**

2-3. Analyze the sample for parent F8426 by GC/ECD. Refer to Section XII, Appendix B for specific instrument parameters

3. ACID METABOLITE ANALYSIS

3-1 Transfer the aqueous fraction from the hexane/aqueous partition (25 - 30 mL) to a 50 mL round bottom flask. Add 3 - 3 5 mL of concentrated HCl (such that the final acid concentration is 1N or greater) and several boiling chips to the round bottom flask and reflux for 1 hour under a water cooled condenser.

3-2. Allow the hydrolyzed sample to cool until it can be safely handled. Assemble tandem SPE cartridges (SCX cartridge on top of the C_{18} cartridge) and install them on the vacuum manifold. Condition both the SCX SPE cartridge (Varian, 1 g), and C_{18} SPE cartridge (Bakerbond, 1 g) in series with methanol (1 CV = 6 mL) and then with 0 25 N HCl (1 CV) using ~5 in. Hg of vacuum After the 0.25 N HCl reaches the top of column packing of the SCX cartridge, turn off the vacuum. Add an additional ~1/2 CV of 0.25 N HCl and attach an SPE filtration cartridge with just a frit installed in the cartridge (no packing material) on top of the SCX cartridge Attach a reducing adapter and a 75 mL reservoir to the top of the SPE cartridge containing the frit. Decant the hydrolyzed sample into the reservoir. Rinse the round bottom flask with ~40 mL of deionized water **but do not add the rinsate to the hydrolyzed sample at this point**. With the cartridge valve opened, apply a vacuum at ~7-10 in. Hg and drain and discard the hydrolyzed sample. When the last of the hydrolyzed sample has passed through the SCX cartridge **now** add the 40 mL of deionized

water rinsate to the reservoir and drain through all three cartridges Discard deionized water rinsate Continue the vacuum of ~7-10 in Hg until all of the filtrate has eluted through all three cartridges

3-3 Remove the reducing adaptor, reservoir, filtration cartridge, and the SCX cartridge and dry the C_{18} SPE cartridge with nitrogen for at least 60 minutes using a drying manifold.

3-4 Elute and collect the analytes from the C_{18} SPE cartridge with 2 CVs (~12 mL) of 5% methanol in dichloromethane (v v). Concentrate the sample under nitrogen using the TurboVap to 0 1 - 0 25 mL (water bath at 50°C). **Note: There is the potential for loss of analytes if the samples go to dryness at this step.**

3-5. Add ~ 1 mL of boron trifluoride in methanol (14% by weight) to the sample, vortex and allow to react for 45 minutes in a water bath at 50°C. After methylation, add ~2 mL of water. **If analysis of 3-OH-F8426-ClPAc is NOT required, extract methylated analytes with 5 mL of hexane and proceed to step 3-9. Otherwise proceed to step 3-6.**

3-6. Partition twice with ~2 mL of dichloromethane (DCM), remove the DCM after each partition step and pass through a 6 mL filtration tube containing a polyethylene frit and packed with ~1 g of anhydrous sodium sulfate. **The use of the anhydrous sodium sulfate can be eliminated if great care is taken when removing the DCM from each partition step so that <u>no water</u> is included with the DCM.** If water droplets are present in the DCM fraction carefully remove them with a small pipet. The DCM is then concentrated in a Turbovap to ~0 1 mL at 50°C.) **Note: There is the potential for loss of analytes if the samples go to dryness at this step.**

3-7. Add 0.5 mL of acetic anhydride and 0.5 mL of pyridine to the sample, vortex and allow to react for 45 minutes at in a water bath at 50° C. This step acylates the hydroxyl group on the 3-OH-F8426-Cl-PAc-methyl ester.

3-8 After acylation, add ~2 mL of water to the sample and partition twice with 2 mL aliquots of hexane. Retain the ~ 4 mL hexane fraction. The aqueous fraction containing excess acetic anhydride and pyridine can be discarded.

3-9 Condition a 1 gram 6 cc Silica gel (Si) SPE cartridge (Baker) with 1 CV of 20% ethyl acetate in hexane (v/v) followed by 1 CV of hexane (vacuum at ~1 in Hg) Load the 4 mL sample onto the cartridge, but do not drain yet Rinse the tube with 2 mL of hexane and also load onto the cartridge Drain the hexane containing the sample through the Si cartridge (vacuum at ~1 in Hg) and discard. Rinse the Si cartridge with 3 mL of 20% ethyl acetate in hexane. Discard the rinsate. Elute and collect the sample with an additional 12 mL of 20% ethyl acetate in hexane (vacuum at ~1 in. Hg). Concentrate the sample under nitrogen to ~ 0 5 mL in a TurboVap (water bath at 50°C), and adjust to a final volume of 1 0 mL with hexane. **Note: There is the potential for loss of analytes if the samples go to dryness at this step.**

3-10 Analyze the sample by GC/MSD monitoring ions 348 for 3-DM-F8426-Cl-PAc, 362 for F8426-Cl-PAc, and 413 for 3-OH-F8426-Cl-PAc. Refer to Section XII, Appendix B for specific instrument parameters.

B Instrumentation

1 F8426

A Hewlett-Packard (HP) 5890 gas chromatograph equipped with a DB-17 (50% diphenyl/50% dimethyl silicone, J&W Scientific) wide-bore (0 53 µm ID) column, an HP 7673A autosampler, a ^{63}Ni Electron Capture Detector and Perkin Elmer Nelson Access*Chrom computer software was used for parent F8426 analyses. Detailed instrument parameters are listed in Section XII, Appendix B.

2 Acid Metabolites

The derivatized acid metabolites were analyzed on a Hewlett Packard Model 5890 gas chromatograph (GC) using a Model 7673A injector and interfaced to a Hewlett Packard Model 5972 Mass Selective Detector (MSD) operated in the single ion mode (SIM). The column was a DB-35 fused silica capillary column, 15 m x 0 25 mm (35% phenyl/65% methyl silicone, J & W Scientific). Detailed instrument parameters are listed in Section XII, Appendix B.

C Method Validation and Quality Control

1 Experimental Design

The analytical method was practiced at the limit of quantitation (0 05 ppm) for each analyte by satisfactory recoveries of the respective analytes from control samples that were laboratory fortified at the initiation of each analysis set The laboratory fortified samples were carried through the procedure with each analysis set. An analysis set consisted of a minimum of one control sample and one laboratory fortified control sample. The individual recovery results are found in Section X, Table 4

2. Preparation of Standards

The structures and purities of the analytical standards used in this study are shown in Section X, Table 2. F8426, F8426-Cl-PAc, 3-DM-F8426-Cl-PAc and 3-OH-F8426-Cl-PAc stock solutions of 1000 µg/mL were prepared by dissolving the appropriate amounts of the analytical standards in acetonitrile. Working solutions were prepared in volumetric flasks by appropriate dilutions of stock solutions for each analyte or combination of analytes. Working solutions containing parent F8426 were prepared only in acetonitrile. Working solutions containing acid metabolites were prepared in hexane or acetonitrile. The combined standard solution concentrations ranged from 0 0625 to 1.0 ng/µL for F8426 and from 0 0625 to 2.0 ng/µL for each acid metabolite Underivatized solutions (containing parent F8426 and/or metabolites, in acetonitrile) were used for fortification. Solutions of derivatized esters (see Section XII, Appendix A for procedure used for derivatizing the acid metabolite standards) or underivatized parent F8426 (in acetonitrile) were used for analyte quantitation and instrument calibration. All working solutions were stored under refrigeration (8°C) when not in use Stock solutions were stored frozen (-18°C) when not in use. Solutions maintained under these conditions have proven patterns of stability. Information on the reference solutions used during this study is shown in Section X, Table 3

3 Fortification Procedure

The validity of the analytical procedures was determined by satisfactory recovery of the analytes from control (check) samples that were laboratory fortified at the initiation of each analysis set. A portion of the control sample was accurately weighed into a boiling flask The sample was

fortified by adding an accurately measured volume of a standard solution, containing F8426 and/or a mixture of the underivatized acid metabolites, with a Hamilton® syringe The solvent was allowed to evaporate before the fortified control sample was analyzed

D Method of Calculation

F8426 and its acid metabolites were quantitated by either a single point or multiple point external standard calibration method. A computer spreadsheet program (Microsoft® Excel) was used for calculation and reporting

1. Single Point Method (previously reported studies)

The single point external standard calibration method is acceptable where expected residues fall within a narrow range (typically between LOD and 5X LOQ) The average area units of an injection standard were obtained by injecting a standard of known concentration throughout the analysis run. The nanogram (ng) 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 injection standards using the following formula:

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

2 Multiple Point Method (this study)

The multiple point external standard calibration method is used for a broader range of quantifiable residues. A multi-point linearity curve was generated by injecting known amounts of various standard concentrations and calculating a response curve from the linear regression analysis of the standard. The ng value of the analytes in each sample was calculated by subtracting the y intercept of the line from the area units of the unknown sample and dividing by the slope of the line using the following formula.

$$\text{ng of analyte in sample} = \frac{\text{area units (sample)} - \text{y intercept}}{\text{slope}} \times \text{sample extract injected (}\mu\text{L)}$$

No correction for molecular weights was necessary for the derivatized compounds since the injection standards were derivatized simultaneously with the analytes and all weights were based on the underivatized acids.

For the single point external calibration method, the LOD was set at 0 01 ppm. For the multiple point external calibration method, the LOD was calculated as the concentration of analyte (ppm equivalent) at 1/5 the area of the LOQ level standard Using this method, the LOD ranged from 0 01 to 0 02 ppm due to the uncertain quantitation in this area

A 2 5 g sample of crop matrix 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 or GC/MSD yielding a 5 mg sample injection The following formula was used to obtain the mg of sample injected:

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

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·

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

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

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

An example of how to calculate the method recovery of F8426-Cl-PAc in a rice grain fortified sample (Section XII, Appendix C, Figure 21) using the single point method is given below:

ng standard = 2 µL x 0 125 ng/µL = 0 25 ng

average area units standard = 56591

area units sample = 60652

final sample extract volume (µL) = 1000 µL

ng of F8426 in sample = $\frac{60652}{56591}$ x 0 25 ng = 0 267 ng

mg of sample injected = $\frac{2500 \text{ mg}}{1000 \ \mu L}$ x 2 µL = 5 mg

uncorrected ppm (ng/mg) = $\frac{0.267 \text{ ng}}{5 \text{ mg}}$ = 0.053 ppm

method recovery (%) = $\frac{0.053 \text{ ppm}}{0.05 \text{ ppm}}$ x 100% = 107%

An example of how to calculate the method recovery of F8426 in a field corn grain fortified sample (Section XII, Appendix C, Figure 2) using the multiple point method is given below:

y intercept = 47310

slope = 1096930

area units (sample) = 187904

final sample extract volume (µL) = 1000 µL

ng of F8426 in sample = $\frac{187904 - 47310}{1096930}$ x 2 µL = 0.256 ng

mg of sample injected = $\frac{2500 \text{ mg}}{1000 \ \mu L}$ x 2 µL = 5 mg

uncorrected ppm (ng/mg) = $\frac{0.256 \text{ ng}}{5 \text{ mg}}$ = 0.051 ppm

method recovery (%) = $\frac{0.051 \text{ ppm}}{0.05 \text{ ppm}}$ x 100% = 102%

E. Interferences

An interference peak of F8426-Cl-PAc was noted at the LOD level during analysis of a wheat straw sample. No contaminants or other interferences were detected for parent or any of the acid metabolites.

F. Confirmatory Techniques

The acid metabolites were detected utilizing mass spectrometry, so no additional confirmatory techniques were required. The parent F8426 can be confirmed using mass spectrometry following the instrument parameters in Section XII, Appendix B, 2 The ions of interest for F8426 are 312, 340, or 411

The acid metabolite F8426-Cl-PAc can be confirmed with ions 290, 326 and 362 The acid metabolite 3-DM-F8426-Cl-PAc can be confirmed with ions 276, 312 and 348 Lastly, 3-OH-F8426-Cl-PAc can be confirmed with ions 360, 384 and 413

G Time Required for Analysis

For a set of four samples, each analytical method (i e. either parent F8426, or acid metabolites) can be completed from the time of sample weighing to GC injection within 8 laboratory hours A total analysis for all analytes requires about 16 laboratory hours. There are no convenient stopping places in the parent analysis scheme. During the metabolite analysis, the samples may be refrigerated overnight immediately following acid reflux or just prior to methylation Derivatization steps through GC analysis should not be interrupted.

H Potential Problems

1. During the initial partition of parent, the solution pH must **not** exceed 8 F8426 is extremely unstable under alkaline conditions and will rapidly degrade

2. The standards of parent F8426 **must** be in acetonitrile. Other solvents (e.g., ethyl acetate) lead to poor chromatography of parent F8426 standard on the GC following injection of matrix samples. This can lead to **apparent enhanced recoveries of F8426 in the fortified samples.**

3. Follow the procedure for derivatizing the injection standards in Appendix A. This procedure greatly reduces the amount of excess derivatizing reagents injected into the GC. Excess derivatizing reagents can be adsorbed onto the insert and column. These deposits can lead to analyte activity in the GC which in turn can lead to **apparent enhanced recoveries of the acid metabolites in the fortified samples.**

4 The use of a **new DB-17 column** for F8426 and a **new DB-35 column** for the acid metabolites is highly recommended Columns demonstrating acceptable chromatographic properties with other compounds may still have active sites present from prior use The analytes in this report tend to be very reactive and these active sites can cause the analytes to degrade in the GC column and lead to **apparent enhanced recoveries of the fortified samples.**

5 The use of a cyclo-uniliner insert from Restek (cat#20337) for the analysis of F8426 and a cyclo double gooseneck (2mm) insert (Restek cat# 20907) for the analysis of the acid metabolites is required for optimal chromatographic properties **Apparent enhanced recoveries** may be eliminated by replacing the injection insert with a new insert.

6. Matrix samples (typically 1 or 2) must be used to condition the GC immediately prior to analysis of an actual sample set to allow the instrument to stabilize.

7. Pyridine and BF_3 in methanol are hazardous and must be used only in a well-ventilated hood. A solvent partition after acylation helps remove residual pyridine from the sample. Material Safety Data Sheets for the derivatizing agents should be reviewed and kept readily available.

VI. STORAGE STABILITY

Underivatized standards of F8426 and its acid metabolites are stable under frozen or refrigerated conditions. Stock solutions should be prepared annually and dilutions should be prepared more frequently to avoid any possible problems. Derivatized acid metabolites should be stored under refrigeration and prepared monthly. Derivatized samples should be analyzed immediately after preparation or stored under refrigeration prior to injection.

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 96% (n=59) with a range of 76 - 123% for F8426, 99% (n=59) with a range of 72 - 125% for F8426-Cl-PAc, 96% (n=59) with a range of 66 - 124% for 3-DM-F8426-Cl-PAc, and 101% (n=16) with a range of 77 - 125% for 3-OH-F8426-Cl-PAc (Section III, Table 1).

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 ± 9% (n=59) for F8426, ± 12% (n=59)

for F8426-Cl-PAc, ± 14% (n=59) for 3-DM-F8426-Cl-PAc, and ± 14% (n=16) for 3-OH-F8426-Cl-PAc.

C Limits of Detection and Quantification

The method limit of quantitation (LOQ) for F8426, F8426-Cl-PAc, 3-DM-F8426-Cl-PAc, and 3-OH-F8426-Cl-PAc was validated at 0.05 ppm The limit of detection (LOD) was set at 0 01 ppm when using the single point external calibration method. For the multiple point external calibration method, the LOD was calculated as the concentration of analyte (ppm equivalent) at 1/5 the area of the LOQ level standard Using this method, the LOD ranged from 0 01 to 0.02 ppm.

D Ruggedness

The steps of the revised analytical method for the small grain matrices are routine residue techniques The average method recoveries and the standard deviations in this report indicate that this method is reliable and accurate. The greatest confirmation of its ruggedness is the large variety of commodities of small grain crops that have been successfully analyzed using this method. Careful attention to the detailed method and the potential problems will ensure the reliable performance of the method.

E. Limitations

No limitations were experienced with this method. Any possible difficulties are described in Section V, Parts E (interferences) and H (potential problems).

VIII. CONCLUSION

A residue analytical method was developed and successfully employed for the extraction and detection of F8426, F8426-chloropropionic acid, 3-desmethyl-F8426-chloropropionic acid, and 3-hydroxymethyl F8426-chloropropionic acid in/on field corn (grain, forage and fodder), sweet corn (ears, forage and stover), rice (grain and straw), sorghum (grain, forage and stover) and wheat (grain, forage, hay and straw). The overall average method recoveries were 96 ± 9% (n=59) for F8426, 99 ± 12% (n=59) for F8426-chloropropionic acid, 96 ± 14% (n=59) for 3-desmethyl-F8426-chloropropionic acid, and 101 ± 14% (n=16) for 3-hydroxymethyl F8426-chloropropionic acid. The method limit of quantitation was validated at 0.05 ppm for each analyte in all matrices The limit of detection ranged from 0 01 to 0 02

ppm. The analytical method presented in this report was the method used to generate all data presented within this report

All equipment needed to perform these 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 F8426 and its acid metabolites in/on small grain crops.

IX. CERTIFICATION

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

Natalie A Shevchuk — 7/30/98

Natalie A. Shevchuk
Associate Research Chemist
STUDY DIRECTOR

Date

John M Becker — 07/31/98

John M. Becker
Manager, Formulations
SPONSOR

Date

ADDITIONAL LABORATORY PERSONNEL
William Nagel, Senior Chemist
Scott Schlenker, Research Technician
Cruz Maria Suero, Senior Research Technician
David Baffuto, Senior Research Technician

X. TABLES

TABLE 2

TEST AND REFERENCE SUBSTANCES

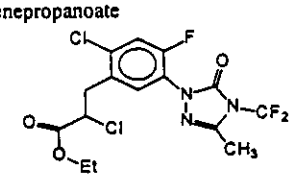
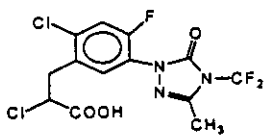
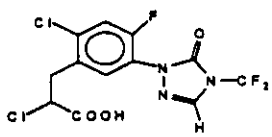
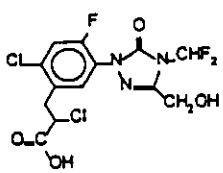
Common Name	Chemical Name / Structure	Reference Number	DCD Inventory Number	Percent Purity
F8426	Ethyl α,2-dichloro-5-[4-(difluoromethyl)-4,5-dihydro-3-methyl-5-oxo-1H-1,2,4-triazol-1-yl]-4-fluorobenzenepropanoate 	E6788 112	CR-52	97 6
F8426-Cl-PAc	α,2-dichloro-5-[4-(difluoromethyl)-4,5-dihydro-3-methyl-5-oxo-1H-1,2,4-triazol-1-yl]-4-fluorobenzenepropanoic acid 	R102-87	CR-47	99 2
3-DM-F8426-Cl-PAc	α,2-dichloro-5-[4-(difluoromethyl)-4,5-dihydro-5-oxo-1H-1,2,4-triazol-1-yl]-4-fluorobenzenepropanoic acid 	M428 43	CR-33	97 4
3-OH-F8426-Cl-PAc	α, 2-dichloro-5-[4-(difluoromethyl)-4,5-dihydro-3-hydroxymethyl-5-oxo-1H-1,2,4-triazol-1-yl]-4-fluorobenzenepropanoic acid 	M451 057	CR-48	92 0

TABLE 3

REFERENCE SOLUTIONS

Compound	Solution Solvent	Solution Concentration (ng/µL)	Standard Solution Index Number	Date Prepared
F8426	Acetonitrile	10	982-7	6/3/97
F8426-Cl-PAc	Acetonitrile	1000	983	4/1/97
3-DM-F8426-Cl-PAc	Acetonitrile	999	961	11/20/96
3-OH-F8426-Cl-PAc	Acetonitrile	1001	959	11/20/96
F8426 + F8426-Cl-PAc + 3-DM-F8426-Cl-PAc + 3-OH-F8426-Cl-PAc	Methanol	10	1012-1	8/14/97
F8426-Cl-PAc + 3-DM-F8426-Cl-PAc + 3-OH-F8426-Cl-PAc	Methanol	100	1011-1	8/14/97

TABLE 4

METHOD RECOVERIES OF F8426 AND ITS MAJOR METABOLITES
FROM LABORATORY FORTIFIED SMALL GRAIN SAMPLES

Matrix Set No	Study No	Sample ID No	Fortification Level (ppm)	Method Recoveries (%) F8426	F8426-Cl-PAc	3-DM-F8426-Cl-PAc
FIELD CORN						
Grain						
CG-1	842MVL97R1	95RSH25C	0 05	102	103	99
CG-1	842MVL97R1	95RSH25C	0 05	100	104	93
CG-1	842MVL97R1	95RSH25C	0 05	88	102	99
Forage						
CFOR-1	842MVL97R1	95RSH23C	0 05	84	95	86
CFOR-1	842MVL97R1	95RSH23C	0 05	95	103	89
CFOR-1	842MVL97R1	95RSH23C	0 10	97	78	66
Fodder						
CFDR-1	842MVL97R1	95RSH24C	0 05	96	113	114
CFDR-1	842MVL97R1	95RSH24C	0 05	109	90	78
CFDR-1	842MVL97R1	95RSH24C	0 05	98	101	97
SWEET CORN						
Ears						
E1	842COS96R1	96JCA370C	0 05	104	125	121
E2	842COS96R1	96JCA376C	0 05	99	96	104
E3	842COS96R1	96JCA382C	0 05	94	106	104
E4	842COS96R1	96JCA436C	0 05	104	88	88
E5	842COS96R1	96JCA388C	0 05	98	98	99
Forage						
F1	842COS96R1	96JCA391C	0 05	87	93	94
F2	842COS96R1	96JCA397C	0 05	76	123	120
F3	842COS96R1	96JCA403C	0 05	86	103	110
F4	842COS96R1	96JCA409C	0 05	87	93	106
F5	842COS96R1	96JCA445C	0 05	86	101	104
Stover						
S1	842COS96R1	96JCA412C	0 05	92	93	95
S2	842COS96R1	96JCA418C	0 05	84	96	103
S3	842COS96R1	96JCA424C	0 05	98	90	92
S4	842COS96R1	96JCA427C	0 05	80	NA[a]	NA
S5	842COS96R1	96JCA430C	0 05	82	92	94
S6	842COS96R1	96JCA454C	0 05	82	109	122

a NA - Not analyzed

Continued

TABLE 4 (*Continued*)

METHOD RECOVERIES OF F8426 AND ITS MAJOR METABOLITES
FROM LABORATORY FORTIFIED SMALL GRAIN SAMPLES

Matrix Set No	Study No	Sample ID No	Fortification Level (ppm)	Method Recoveries (%) F8426	F8426-Cl-PAc	3-DM-F8426-Cl-PAc	3-OH-F8426-Cl-PAc
RICE							
Grain							
RG1, RG1B[a]	842RIC96R1	96JCA300C	0 05	107	82	115	NA[b]
RG2	842RIC96R1	96JCA306C	0 05	99	93	99	NA
RG3	842RIC96R1	96JCA312C	0 05	94	107	115	NA
RG4	842RIC96R1	96JCA321C	0 05	97	114	124	NA
RG1, RG1A	842RIC96R2	96MWB470C	0 05	97	100	102	NA
RG1, RG1A	842RIC96R2	96MWB473C	0 05	102	113	110	NA
Straw							
RS1, RS1A	842RIC96R1	96JCA330C	0 05	99	110	117	99
RS2, RS2A	842RIC96R1	96JCA336C	0 05	83	90	106	114
RS3, RS3A	842RIC96R1	96JCA342C	0 05	97	88	93	108
RS4B	842RIC96R1	96JCA351C	0 05	108	95	91	89
RS1/M/M2	842RIC96R2	96MWB476C	0 05	104	101	91	84
RS3	842RIC96R2	96MWB476C	1 0	NA	106	98	116
SORGHUM							
Grain							
SG-1	842MVL97R1	96JCA141C	0 05	92	86	76	NA
SG-1	842MVL97R1	96JCA141C	0 05	85	86	71	NA
SG-1	842MVL97R1	96JCA141C	0 05	94	86	73	NA
Forage							
SG-05, SG-07RJ	842SOR96R1	96JCA120C	0 05	123	109	99	NA
SG-06, SG-08RJ	842SOR96R1	96JCA126C	0 05	102	114	111	NA
SG-09, SG-09RJ	842SOR96R1	96JCA132C	0 05	115	102	101	NA
Stover							
SG-10	842SOR96R1	96JCA162C	0 05	NA	103	88	NA
SG-11	842SOR96R1	96JCA168C	0 05	93	109	113	NA
SG-12, SG-12RJ	842SOR96R1	96JCA174C	0 05	103	104	102	NA
SG-13	842SOR96R1	96JCA180C	0 05	91	114	98	NA
SG-14	842SOR96R1	96JCA165C	0 05	97	NA	NA	NA

a Metabolites and parent analyzed in two separate sets
b NA - Not analyzed

Continued

TABLE 4 (*Continued*)

METHOD RECOVERIES OF F8426 AND ITS MAJOR METABOLITES
FROM LABORATORY FORTIFIED SMALL GRAIN SAMPLES

Matrix Set No	Study No	Sample ID No	Fortification Level (ppm)	Method Recoveries (%) F8426	F8426-Cl-PAc	3-DM-F8426-Cl-PAc	3-OH-F8426-Cl-PAc
WHEAT							
Grain							
WG-1, WG-2[a]	842MVL97R1	95SJS0103C	0 05	102	97	68	NA[b]
WG-1, WG-2	842MVL97R1	95SJS0103C	0 05	92	106	79	NA
WG-1, WG-2	842MVL97R1	95SJS0103C	0 25	84	107	93	NA
Forage							
WF-1, WF-2	842MVL97R1	95SJS0101C	0 05	101	102	85	105
WF-1, WF-2	842MVL97R1	95SJS0101C	0 05	101	121	95	121
WF-1, WF-2	842MVL97R1	95SJS0101C	0 25	95	87	77	90
WF-1, WF-2	842MVL97R1	95SJS0101C	0 5	99	91	79	95
Hay							
WH-1, WH-2	842MVL97R1	95SJS0102C	0 05	95	96	91	94
WH-1, WH-2	842MVL97R1	95SJS0102C	0 05	108	99	96	114
WH-1, WH-2	842MVL97R1	95SJS0102C	0 25	94	72	70	77
Straw							
WS-1, WS-2	842MVL97R1	95SJS0104C	0 05	91	78[c]	81	97
WS-1, WS-2	842MVL97R1	95SJS0104C	0 05	111	77[c]	102	125
WS-1, WS-2	842MVL97R1	95SJS0104C	0 25	98	79[c]	80	91
		Average		96	99	96	101
		Standard Deviation		±9	±12	±14	±14
		Number of Analyses		59	59	59	16

a Metabolites and parent analyzed in two separate sets

Continued

b NA - Not analyzed

c An F8426 Cl-PAc interference peak was detected in the control sample at the LOD level The peak area was subtracted from the corresponding fortified control samples

XI. REFERENCES

1 Brooks, M , "Analytical Methodology for the Determination of F8426 and Its Acid Metabolites in/on Spring Wheat Forage, Straw and Grain," Report P-3041M, FMC Corporation, Agricultural Products Group, Princeton, NJ, June, 1997.

2 Schlenker, S , "Magnitude of the Residues of F8426 and Significant Metabolites in/on Rice Grain and Straw Samples Following Treatment with F8426 50 DF Herbicide", Report P-3237, FMC Corporation, Agricultural Products Group, Princeton, NJ, May, 1997.

3. Brooks, M., "Magnitude of the Residues of F8426 and Significant Metabolites in/on Rice Grain and Straw Samples Following Treatment with F8426 50 DF Herbicide at 0.3 Pounds Active Ingredient per Acre", Report P-3243, FMC Corporation, Agricultural Products Group, Princeton, NJ, June, 1997.

4. Barros, A., "Magnitude of the Residues of F8426 and Significant Metabolites in/on Sorghum Forage, Stover and Grain Samples Following Treatment with F8426 50 DF Herbicide", Report P-3254, FMC Corporation, Agricultural Products Group, Princeton, NJ, September, 1997

5 Nagel, W., "Magnitude of the Residues of F8426 and Significant Metabolites in/on Sweet Corn Forage, Ears and Stover, Following Treatment with F8426 50 DF Herbicide", Report P-3231, FMC Corporation, Agricultural Products Group, Princeton, NJ, June, 1997

6. ElNaggar, S , "Nature of the Residue Wheat Metabolism of F8426", Report P-2979, FMC Corporation, Agricultural Products Group, Princeton, NJ, January, 1995.

XII. APPENDICES

A Procedure for Preparing Injection Standards for the Acid Metabolites

1. Prepare a 100 ng/µL standard solution of 3-DM-F8426-Cl-PAc, F8426-Cl-PAc, and 3-OH-F8426-Cl-PAc (underivatized) in methanol.

2 Use the following table as a guide for the aliquot volume from the 100 ng/µL standard and for the final volume for the five derivatized injection standards

Concentration of Derivatized Standard (ng/µL)	Aliquot Volume from 100 ng/µL Stock Solution	Final Volume of Derivatized Standard
0 5	50µL	10 mL
0 375	37 5µL	10 mL
0 25	25µL	10 mL
0 125	62 5µL	50 mL
0 0625	31 25µL	50 mL

3 Accurately measure each aliquot and transfer to a 15 mL ground glass centrifuge tube. Add ~ 1 mL of boron trifluride in methanol (14% by weight) to each standard, cap the tube, vortex and allow to react for 45 minutes at 50° C

4 After methylation, add 2 mL of water and partition 3 X 2 mL of DCM. Remove the DCM after each partition **taking great care not** to include any water with the DCM The DCM is then concentrated to ~0 1 mL at 50° C If the standards are allowed to go to dryness **loss of analytes could occur.**

5. Add 0 5 mL of acetic anhydride and 0 5 mL of pyridine to each standard, cap, vortex and allow them to react for 45 minutes at 50° C

6. After acylation, add ~2 mL of water to each standard and partition each standard with 3 X 2 mL of hexane. Remove the hexane fraction after each partition and adjust to final volume using the table as a guide.

B Instrument Parameters

1 F8426

INSTRUMENT	HP 5890 GC
COLUMNS	DB-17, Diphenyl/Dimethyl (50/50) silicone gum, 30 m x 0 53 mm, 1 0 µm film thickness
INLET	Splitless Injection Mode (cyclounilner insert from Restek cat# 20337)
DETECTOR	^{63}Ni Electron Capture
TEMPERATURES:	
Injection Port.	250 °C
Oven	150 °C/1 minute (initial) 20 °C/minute (ramp 1) 200 °C/0 minutes 10 °C/minute (ramp 2) 260 °C/55 minutes (final)
Detector	300°C
GAS FLOW	He, Carrier, 13 mL/minute (column head pressure ~13 psi) Ar/methane, Make-up, 40 mL/minute
INJECTION VOLUME:	2 uL
RETENTION TIME	~ 10 minutes

2 Mass Selective Detector Confirmation of parent F8426 Residues

INSTRUMENT	HP 5890 GC
COLUMNS	DB-35, Diphenyl/Dimethyl (35/65) silicone gum, 15 m x 0 25 mm, 0 25 µm film thickness
INLET	Splitless Injection Mode (cyclo double gooseneck 2mm insert from Restek cat# 20907)
DETECTOR	HP 5972 Mass Selective Detector
TEMPERATURES:	
Injection Port	250 °C
Oven.	150 °C/1 minute (initial) 12 5 °C/minute (ramp) 280 °C/10 minutes (final)
GAS FLOW	He, Carrier, 1 mL/minute (column head pressure ~10 psi)
INJECTION VOLUME:	2 uL
IONS MONITORED:	m/z = 312, 340 and 411
TRANSFER LINE:	280°C
RETENTION TIMES	~8 6 min

3 F8426-Chloropropionic Acid, 3-Desmethyl-F8426-Chloropropionic Acid and 3-Hydroxymethyl-F8426-Chloroproprionic Acid

INSTRUMENT	HP 5890 GC
COLUMNS	DB-35, Diphenyl/Dimethyl (35/65) silicone gum, 15 m x 0 25 mm, 0 25 µm film thickness
INLET	Splitless Injection Mode (cyclo double gooseneck insert 2mm from Restek cat# 20907)
DETECTOR	HP 5972 Mass Selective Detector
TEMPERATURES.	
Injection Port	250 °C
Oven	150 °C/1 minute (initial) 15 0 °C/minute (ramp) 280 °C/18 minutes (final)
GAS FLOW	He, Carrier, 1 mL/minute (column head pressure ~10 psi)
INJECTION VOLUME	2 uL
IONS MONITORED:	m/z = 348 (3-DM-F8426-Cl-PAc) m/z = 362 (F8426-Cl-PAc) m/z = 413 (3-OH-F8426-Cl-PAc)
TRANSFER LINE:	280°C
RETENTION TIMES	3-DM-F8426-Cl-PAc ~7 0 min F8426-Cl-PAc ~7 5 min 3-OH-F8426-Cl-PAc ~9 3 min

C. Chromatograms

CHROMATOGRAM INDEX

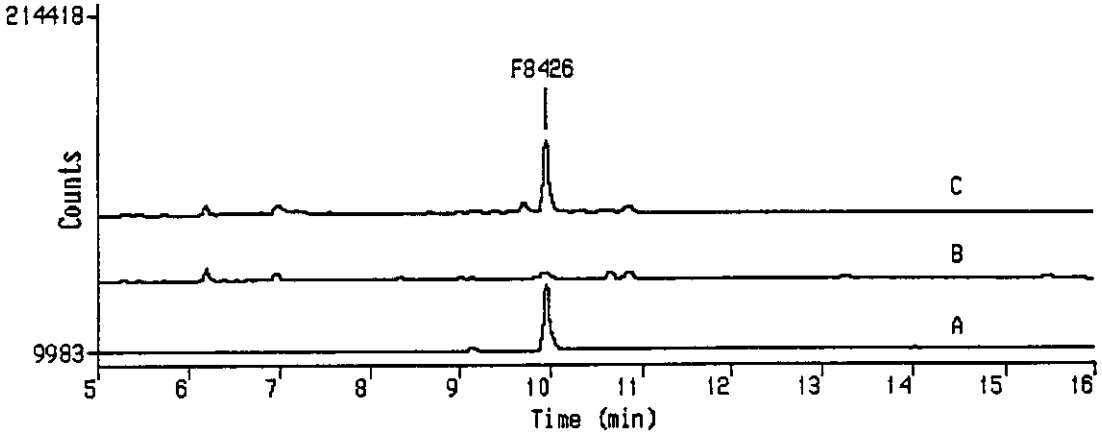
Figure Number	Description
	Field Corn (842MVL97R1, P-3263)
2	Field Corn Grain, F8426
3	Field Corn Grain, F8426-Cl-PAc
4	Field Corn Grain, 3-DM-F8426-Cl-PAc
5	Field Corn Forage, F8426
6	Field Corn Forage, F8426-Cl-PAc
7	Field Corn Forage, 3-DM-F8426-Cl-PAc
8	Field Corn Fodder, F8426
9	Field Corn Fodder, F8426-Cl-PAc
10	Field Corn Fodder, 3-DM-F8426-Cl-PAc
	Sweet Corn (842COS96R1, P-3231)
11	Sweet Corn Ears, F8426
12	Sweet Corn Ears, F8426-Cl-PAc
13	Sweet Corn Ears, 3-DM-F8426-Cl-PAc
14	Sweet Corn Forage, F8426
15	Sweet Corn Forage, F8426-Cl-PAc
16	Sweet Corn Forage, 3-DM-F8426-Cl-PAc
17	Sweet Corn Stover, F8426
18	Sweet Corn Stover, F8426-Cl-PAc
19	Sweet Corn Stover, 3-DM-F8426-Cl-PAc
	Rice (842RIC96R1, P-3237)
20	Rice Grain, F8426
21	Rice Grain, F8426-Cl-PAc
22	Rice Grain, 3-DM-F8426-Cl-PAc
23	Rice Straw, F8426
24	Rice Straw, F8426-Cl-PAc
25	Rice Straw, 3-DM-F8426-Cl-PAc
26	Rice Straw, 3-OH-F8426-Cl-PAc

continued

CHROMATOGRAM INDEX (*Continued*)

FIGURE 2

F8426 IN/ON FIELD CORN GRAIN
SET NUMBER: CG-1, GC/ECD

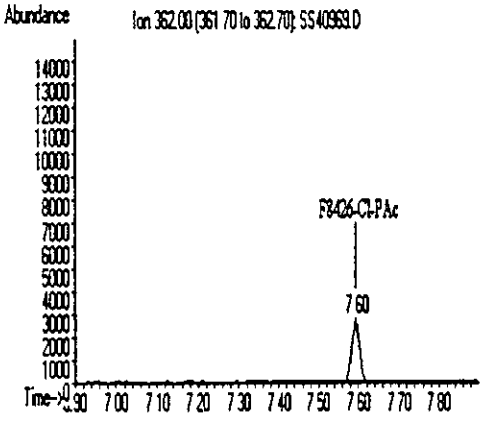


	Assay No	Sample Type	Amount Injected	F8426 Peak Area	F8426 Amount Detected
A	982-11	Standard	0 25 ng	180708[a]	0 24 ng
B	1	Control	5 mg	0	ND[b]
C	2	Fort @ 0 05 ppm	5 mg	187904	102%

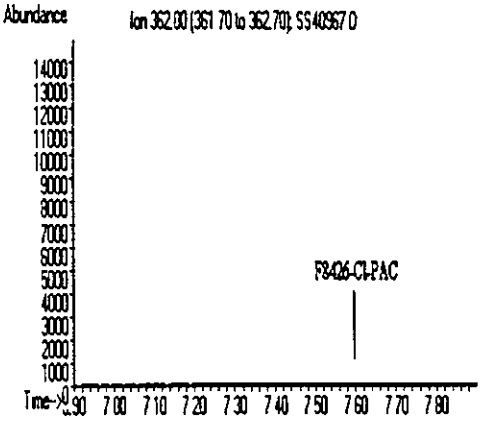
a A multiple point external standard calibration method was used for quantitation, the y intercept was 47310 and the slope was 1096930
b ND = Not detected (< 0 01 ppm)

FIGURE 3

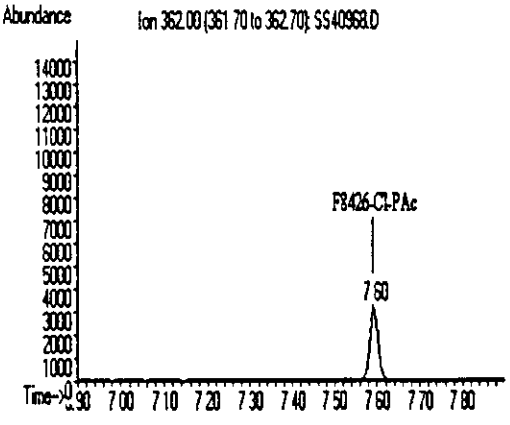
F8426-CHLOROPROPIONIC ACID IN/ON FIELD CORN GRAIN SET NUMBER: CG-1, GC/MSD



Chromatogram A



Chromatogram B



Chromatogram C

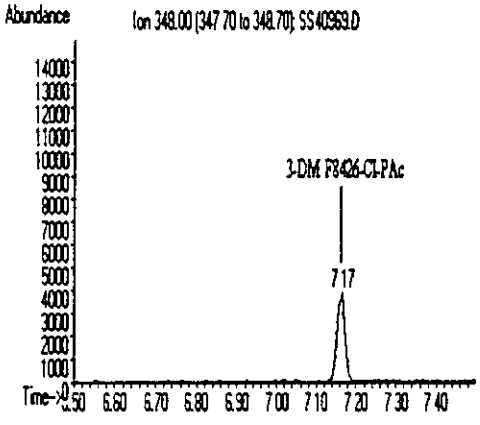
	Assay No	Sample Type	Amount Injected	F8426-Cl-PAc Peak Area	F8426-Cl-PAc Amount Detected
A	1011-5	Standard	0.25 ng	37216[a]	0.23 ng
B	1	Control	5 mg	0	ND[b]
C	2	Fort @ 0.05 ppm	5 mg	41468	103%

a A multiple point external standard calibration method was used for quantitation, the y intercept was 62 and the slope was 321212

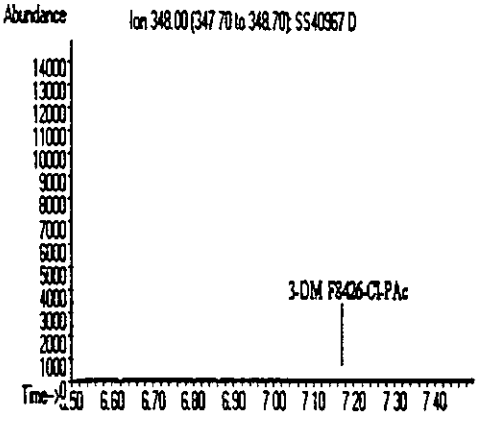
b ND = Not detected (< 0.01 ppm)

FIGURE 4

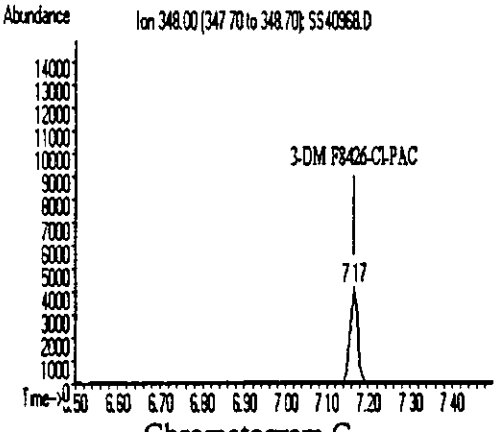
3-DESMETHYL-F8426-CHLOROPROPIONIC ACID IN/ON FIELD CORN GRAIN SET NUMBER: CG-1, GC/MSD



Chromatogram A



Chromatogram B



Chromatogram C

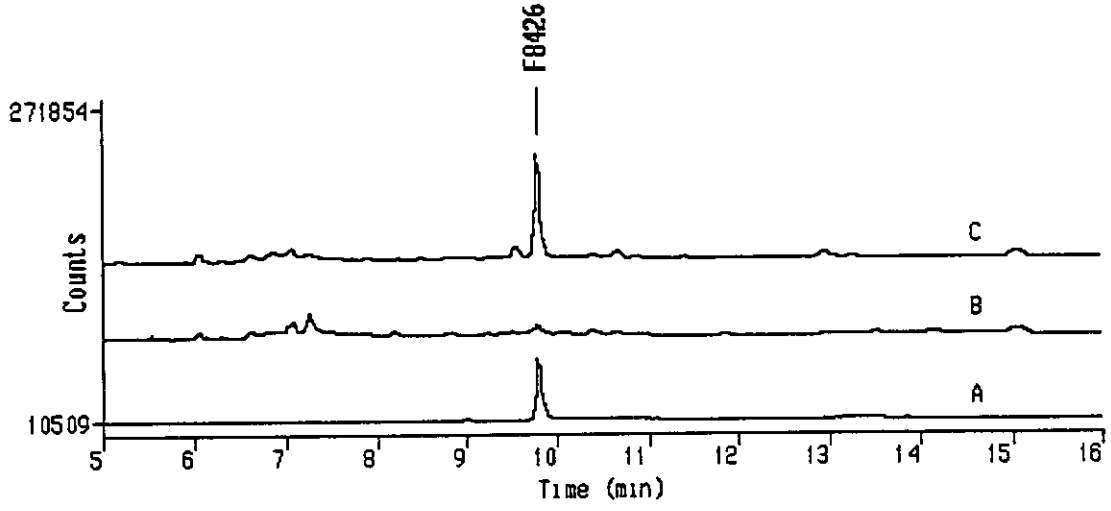
	Assay No	Sample Type	Amount Injected	3-DM-F8426-Cl-PAc Peak Area	3-DM-F8426-Cl-PAc Amount Detected
A	1011-5	Standard	0 25 ng	52956[a]	0 23 ng
B	1	Control	5 mg	0	ND[b]
C	2	Fort @ 0 05 ppm	5 mg	55573	99%

a A multiple point external standard calibration method was used for quantitation, the y intercept was 2340 and the slope was 431566

b ND = Not detected (< 0 01 ppm)

FIGURE 5

F8426 IN/ON FIELD CORN FORAGE
SET NUMBER: CFOR-1, GC/ECD

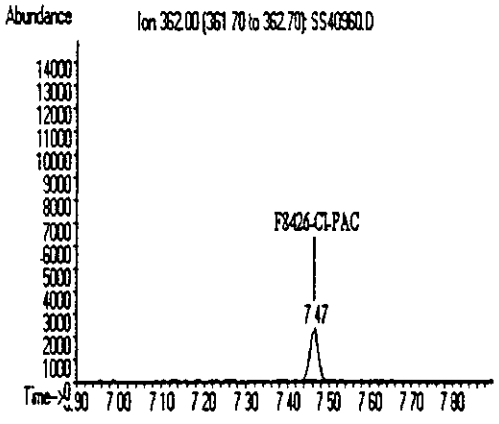


	Assay No	Sample Type	Amount Injected	F8426 Peak Area	F8426 Amount Detected
A	982-11	Standard	0 25 ng	221640[a]	0 27 ng
B	1	Control	5 mg	24126	ND[b]
C	4	Fort @ 0 1 ppm	5 mg	357189	97%

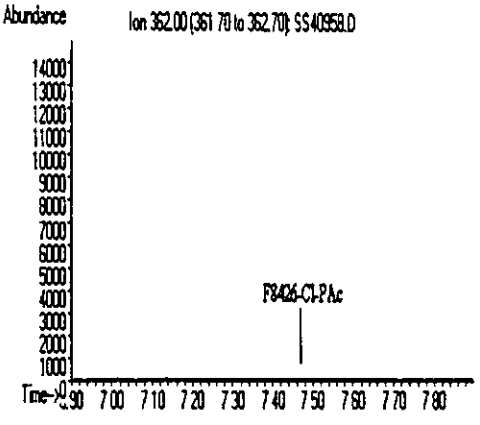
a A multiple point external standard calibration method was used for quantitation, the y intercept was 43581 and the slope was 1299678
b ND = Not detected (< 0 01 ppm)

FIGURE 6

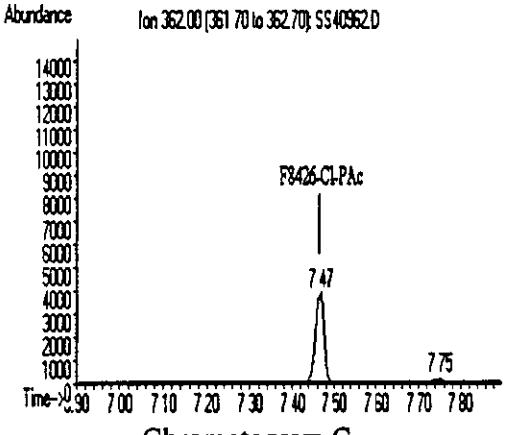
F8426-CHLOROPROPIONIC ACID
IN/ON FIELD CORN FORAGE
SET NUMBER: CFOR-1, GC/MSD



Chromatogram A



Chromatogram B



Chromatogram C

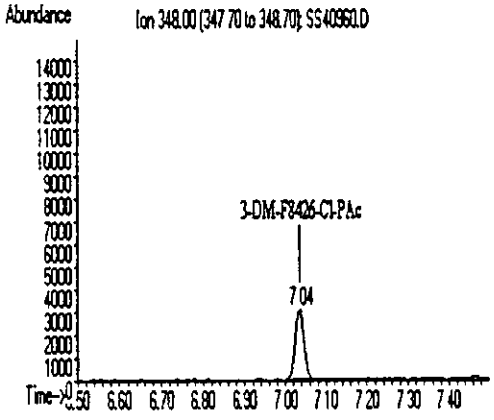
	Assay No	Sample Type	Amount Injected	F8426-Cl-PAc Peak Area	F8426-Cl-PAc Amount Detected
A	1011-5	Standard	0 25 ng	32788[a]	0 24 ng
B	1	Control	5 mg	0	ND[b]
C	4	Fort @ 0 1 ppm	5 mg	55940	78%

a A multiple point external standard calibration method was used for quantitation, the y intercept was -2512 and the slope was 298281

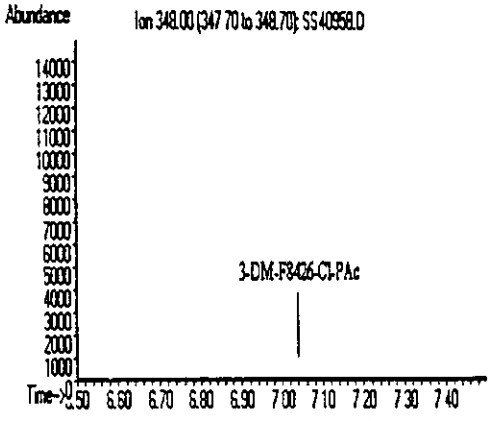
b ND = Not detected (< 0 02 ppm)

FIGURE 7

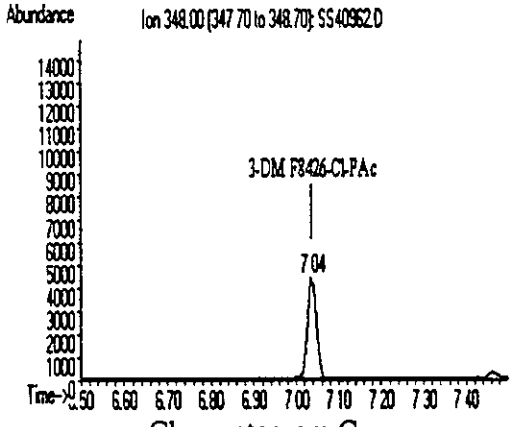
3-DESMETHYL-F8426-CHLOROPROPIONIC ACID IN/ON FIELD CORN FORAGE SET NUMBER: CFOR-1, GC/MSD



Chromatogram A



Chromatogram B



Chromatogram C

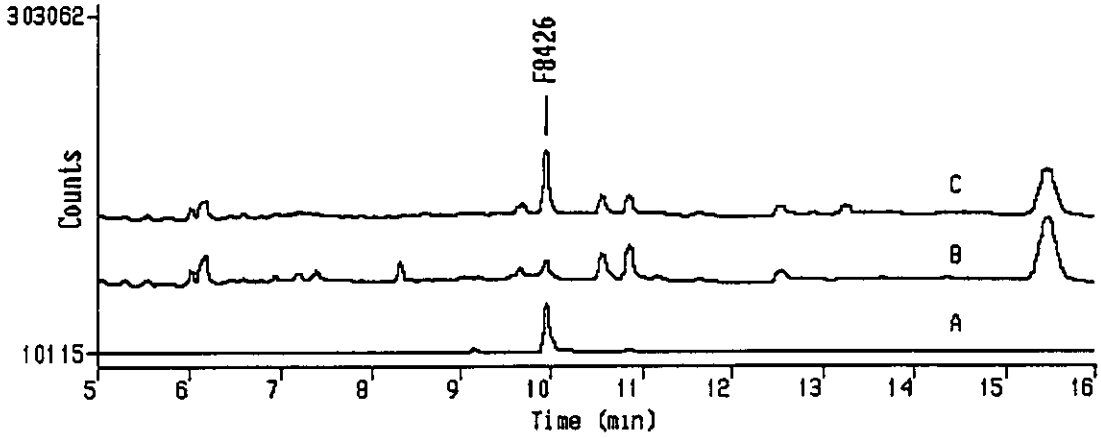
	Assay No	Sample Type	Amount Injected	3-DM-F8426-Cl-PAc Peak Area	3-DM-F8426-Cl-PAc Amount Detected
A	1011-5	Standard	0.25 ng	46336[a]	0.23 ng
B	1	Control	5 mg	0	ND[b]
C	4	Fort @ 0.1 ppm	5 mg	65783	66%

a A multiple point external standard calibration method was used for quantitation, the y intercept was 406 and the slope was 393315

b ND = Not detected (< 0.01 ppm)

FIGURE 8

F8426 IN/ON FIELD CORN FODDER
SET NUMBER: CFDR-1, GC/ECD



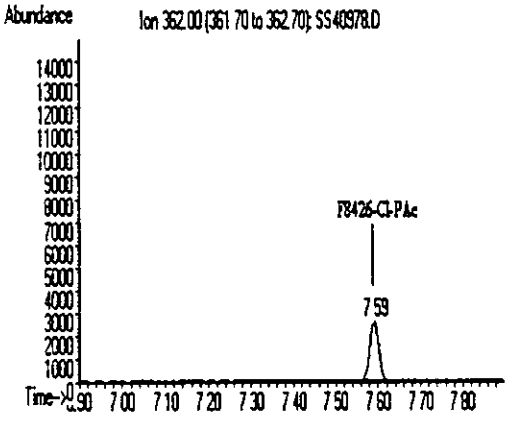
	Assay No	Sample Type	Amount Injected	F8426 Peak Area	F8426 Amount Detected
A	982-11	Standard	0.25 ng	192412[a]	0 25 ng
B	1	Control	5 mg	29605	ND[b]
C	2	Fort @ 0 05 ppm	5 mg	186189	96%

a A multiple point external standard calibration method was used for quantitation; the y intercept was 46753 and the slope was 1160192

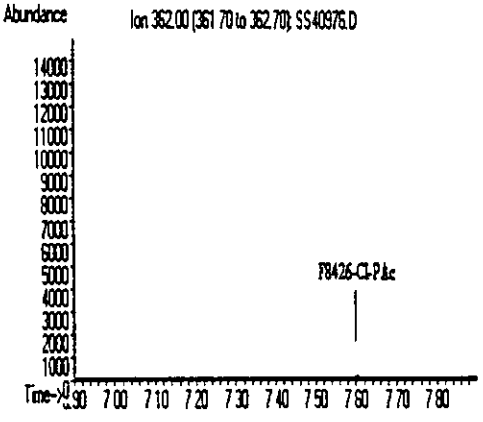
b ND = Not detected (< 0 01 ppm)

FIGURE 9

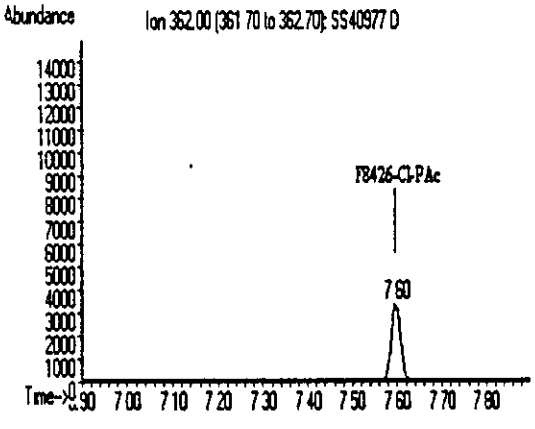
F8426-CHLOROPROPIONIC ACID
IN/ON FIELD CORN FODDER
SET NUMBER. CFDR-1, GC/ECD



Chromatogram A



Chromatogram B



Chromatogram C

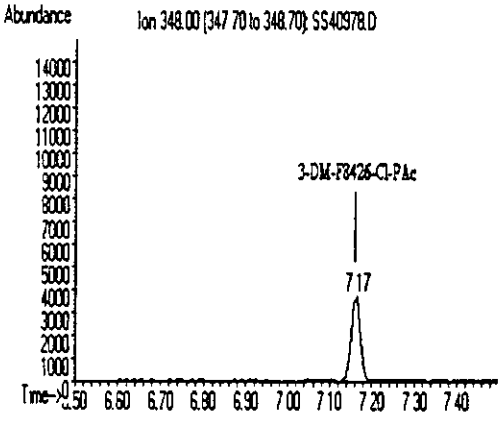
	Assay No	Sample Type	Amount Injected	F8426-Cl-PAc Peak Area	F8426-Cl-PAc Amount Detected
A	1011-5	Standard	0.25 ng	38476[a]	0.24 ng
B	1	Control	5 mg	0	ND[b]
C	2	Fort @ 0.05 ppm	5 mg	46682	113%

a A multiple point external standard calibration method was used for quantitation, the y intercept was -2300 and the slope was 345913

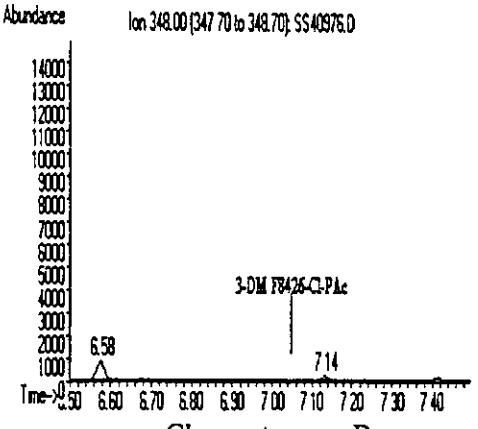
b ND = Not detected (< 0.01 ppm)

FIGURE 10

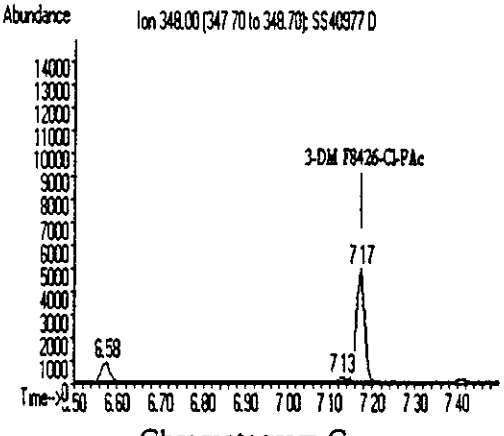
3-DESMETHYL-F8426-CHLOROPROPIONIC ACID
IN/ON FIELD CORN FODDER
SET NUMBER· CFDR-1, GC/MSD



Chromatogram A



Chromatogram B



Chromatogram C

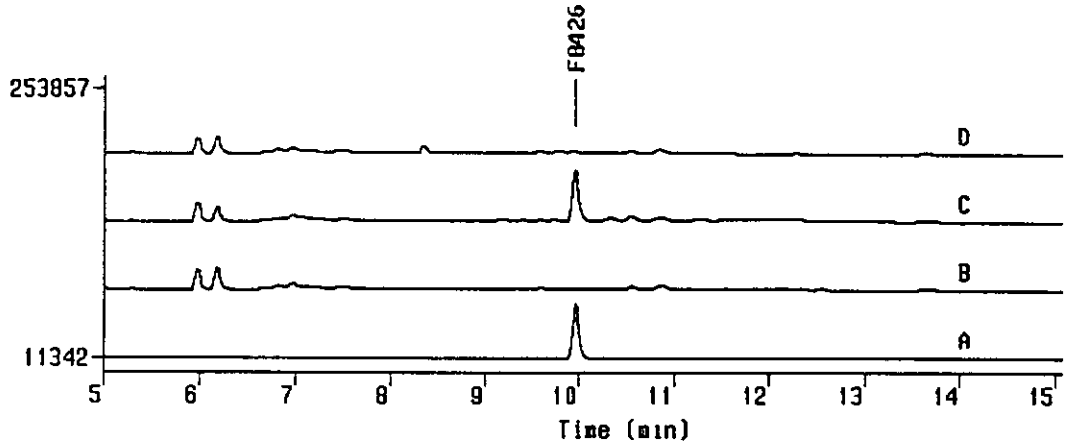
	Assay No	Sample Type	Amount Injected	3-DM-F8426-Cl-PAc Peak Area	3-DM-F8426-Cl-PAc Amount Detected
A	1011-5	Standard	0.25 ng	55013[a]	0.24 ng
B	1	Control	5 mg	0	ND[b]
C	2	Fort @ 0.05 ppm	5 mg	66599	114%

a A multiple point external standard calibration method was used for quantitation, the y intercept was 1831 and the slope was 452187

b ND = Not detected (< 0.01 ppm)

FIGURE 11

F8426 IN/ON SWEET CORN EARS
SET NUMBER. E2, GC/ECD

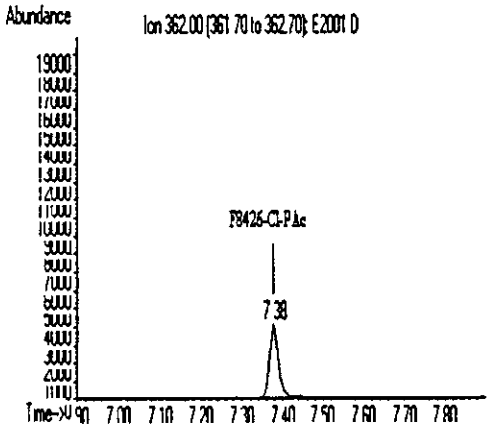


	Assay No	Sample Type	Amount Injected	F8426 Peak Area	F8426 Amount Detected
A	954-11	Standard	0 25 ng	206850[a]	0 23 ng
B	8	Control	5mg	0	ND[b]
C	9	Fort @ 0 05 ppm	5 mg	205111	99%
D	10	Treat	5 mg	0	ND

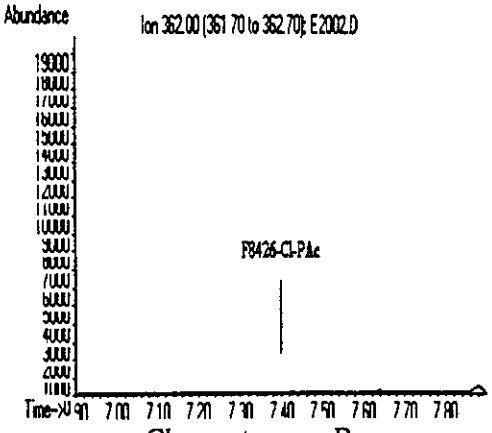
a A single point external standard calibration method was used for quantitation, the average standard peak area was 207871 for F8426
b • ND = Not detected (< 0 01 ppm)

FIGURE 12

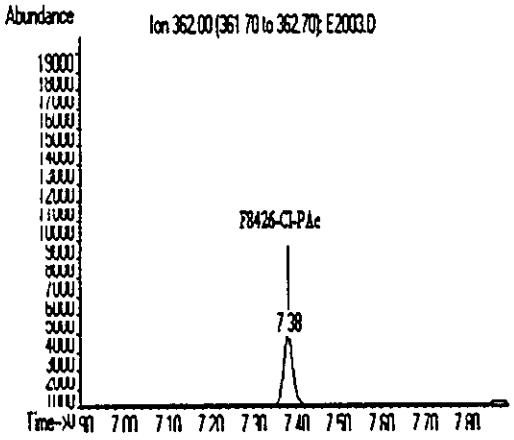
F8426-CHLOROPROPIONIC ACID
IN/ON SWEET CORN EARS
SET NUMBER: E2, GC/MSD



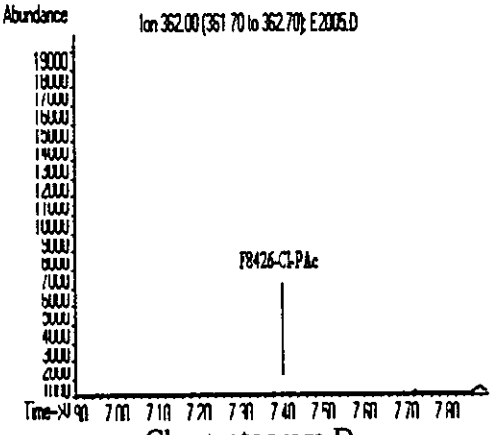
Chromatogram A



Chromatogram B



Chromatogram C



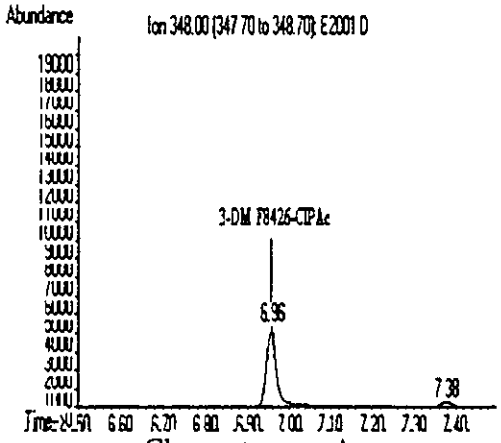
Chromatogram D

	Assay No	Sample Type	Amount Injected	F8426-Cl-PAc Peak Area	F8426-Cl-PAc Amount Detected
A	973-5	Standard	0 25 ng	60389[a]	0 25 ng
B	8	Control	5mg	0	ND[b]
C	9	Fort @ 0 05 ppm	5 mg	56680	96%
D	10	Treat	5 mg	0	ND

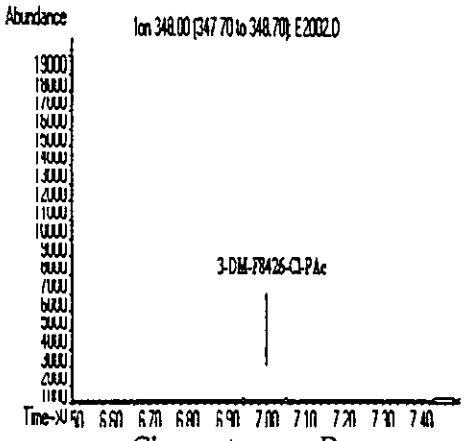
a A single point external standard calibration method was used for quantitation, the average standard peak area was 59347 for F8426-Cl-PAc
b ND = Not detected (< 0 01 ppm)

FIGURE 13

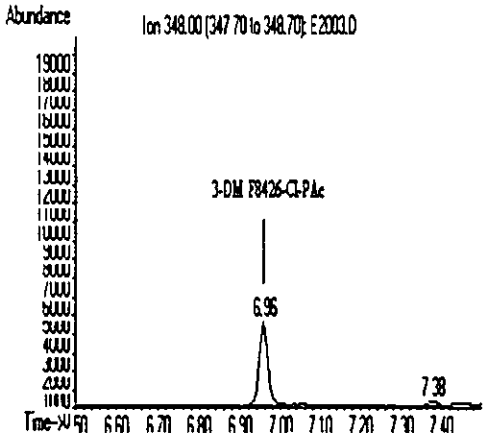
3-DESMETHYL-F8426-CHLOROPROPIONIC ACID
IN/ON SWEET CORN EARS
SET NUMBER: E2, GC/MSD



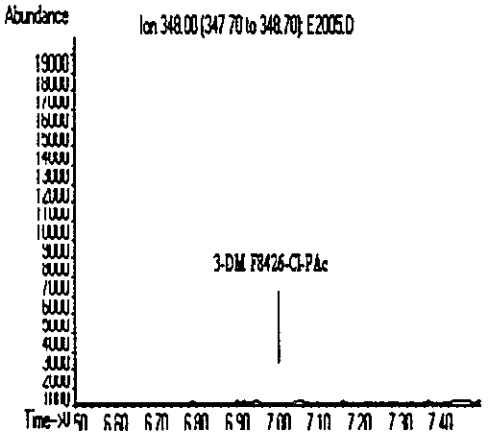
Chromatogram A



Chromatogram B



Chromatogram C



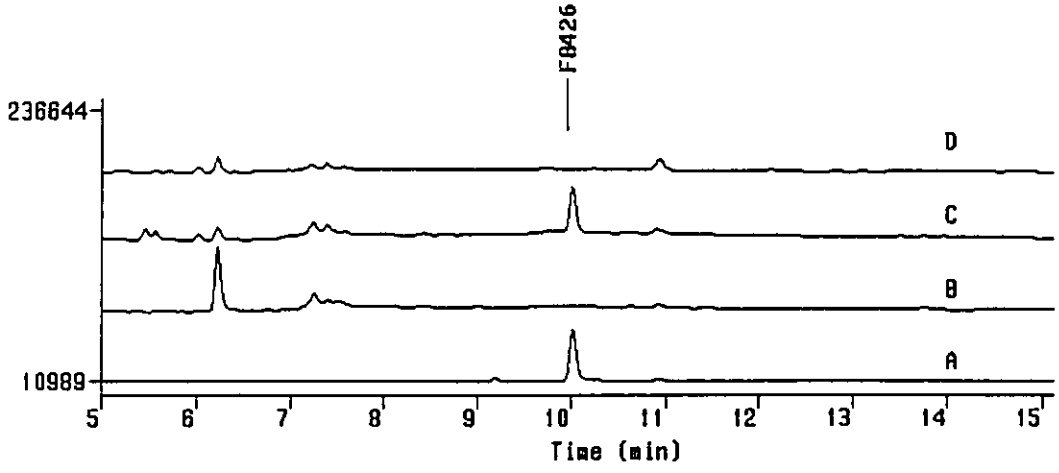
Chromatogram D

	Assay No	Sample Type	Amount Injected	3-DM-F8426-Cl-PAc Peak Area	3-DM-F8426-Cl-PAc Amount Detected
A	973-5	Standard	0 25 ng	69779[a]	0 26 ng
B	8	Control	5mg	0	ND[b]
C	9	Fort @ 0 05 ppm	5 mg	68434	104%
D	10	Treat	5 mg	0	ND

a A single point external standard calibration method was used for quantitation, the average standard peak area was 66098 for 3-DM-F8426-Cl-PAc
b ND = Not detected (< 0 01 ppm)

FIGURE 14

F8426 IN/ON SWEET CORN FORAGE
SET NUMBER: F4, GC/ECD



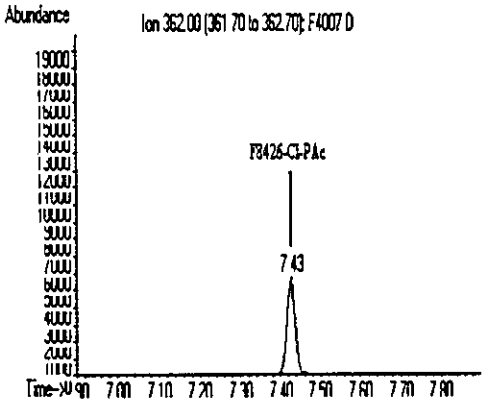
	Assay No	Sample Type	Amount Injected	F8426 Peak Area	F8426 Amount Detected
A	954-11	Standard	0 25 ng	201848[a]	0 25 ng
B	57	Control	5mg	0	ND[b]
C	58	Fort @ 0 05 ppm	5 mg	175568	87%
D	63	Treat	5 mg	0	ND

a A single point external standard calibration method was used for quantitation, the average standard peak area was 201795 for F8426

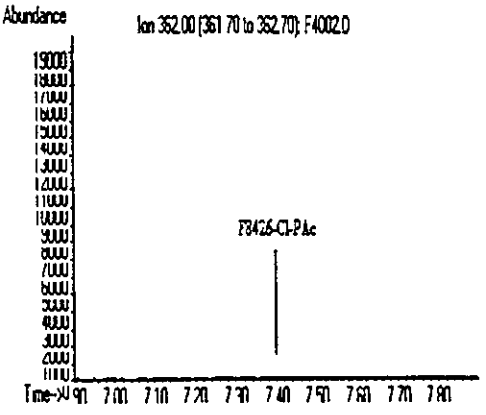
b ND = Not detected (< 0 01 ppm)

FIGURE 15

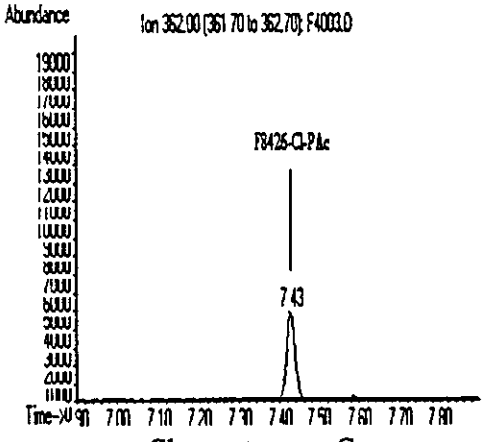
F8426-CHLOROPROPIONIC ACID
IN/ON SWEET CORN FORAGE
SET NUMBER: F4, GC/MSD



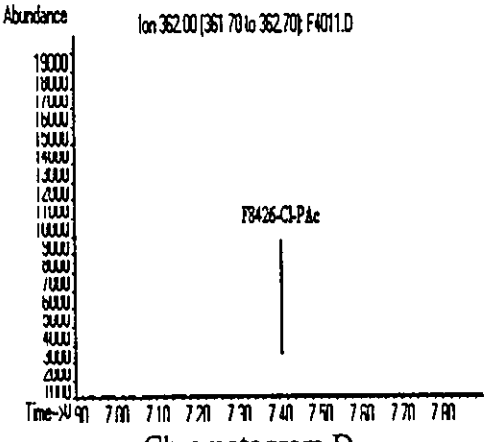
Chromatogram A



Chromatogram B



Chromatogram C



Chromatogram D

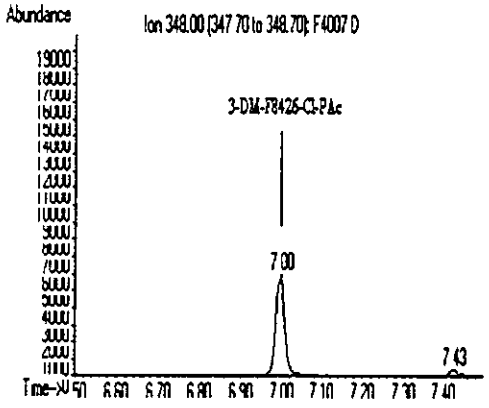
				F8426-Cl-PAc	
•	Assay No	Sample Type	Amount Injected	Peak Area	Amount Detected
A	973-5	Standard	0 25 ng	78782[a]	0 26 ng
B	57	Control	5mg	0	ND[b]
C	58	Fort @ 0 05 ppm	5 mg	70197	93%
D	63	Treat Dup	5 mg	0	ND

a A single point external standard calibration method was used for quantitation, the average standard peak area was 75157 for F8426-Cl-PAc

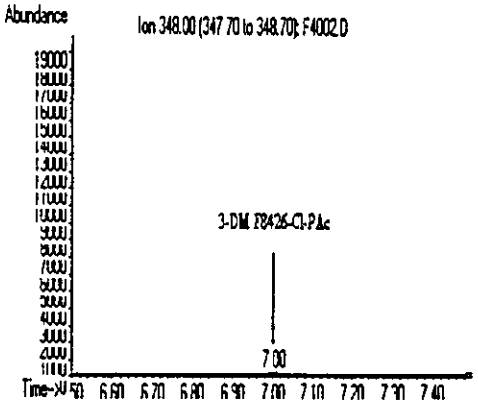
b ND = Not detected (< 0 01 ppm)

FIGURE 16

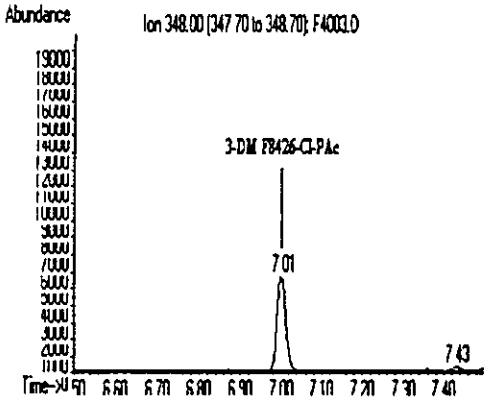
3-DESMETHYL-F8426-CHLOROPROPIONIC ACID
IN/ON SWEET CORN FORAGE
SET NUMBER: F4, GC/MSD



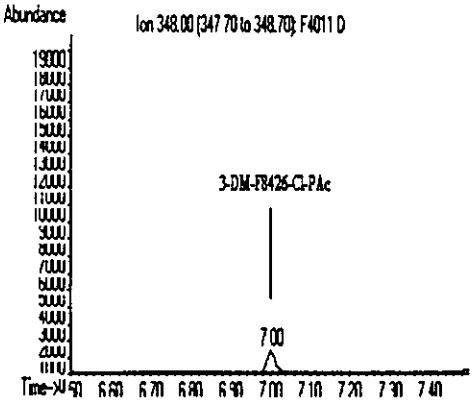
Chromatogram A



Chromatogram B



Chromatgram C



Chromatgram D

				3-DM-F8426-Cl-PAc	
•	Assay No	Sample Type	Amount Injected	Peak Area	Amount Detected
A	973-5	Standard	0.25 ng	87581[a]	0.26 ng
B	57	Control	5mg	3749	ND[b]
C	58	Fort @ 0.05 ppm	5 mg	88197	106%
D	63	Treat Dup	5 mg	19422	(0.01 ppm)[c]

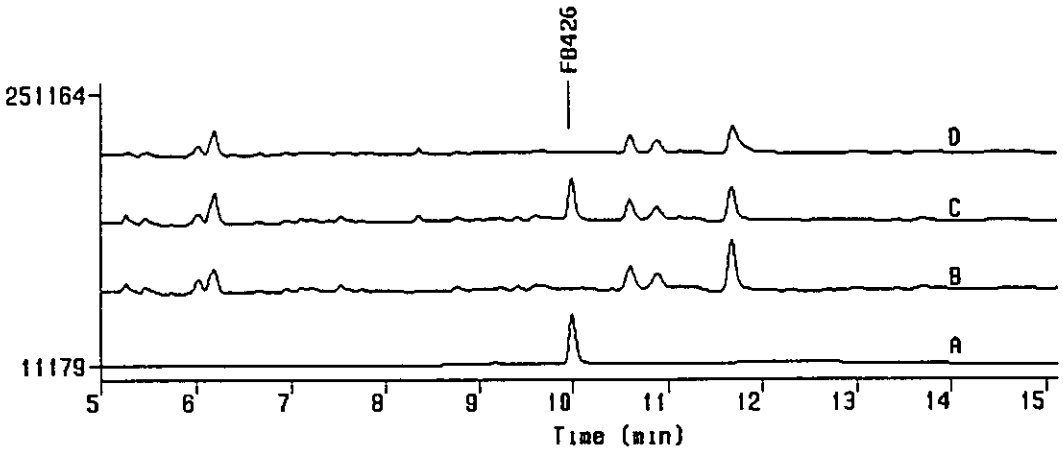
a A single point external standard calibration method was used for quantitation, the average standard peak area was 83098 for 3-DM-F8426-Cl-PAc

b ND = Not detected (< 0.01 ppm)

c Values in parenthesis indicate a value equal to or greater than the LOD (0.01 ppm) but less than the validated LOQ (0.05 ppm)

FIGURE 17

F8426 IN/ON SWEET CORN STOVER
SET NUMBER: S6, GC/ECD

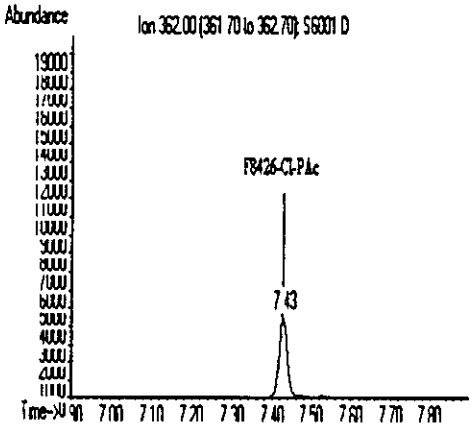


	Assay No	Sample Type	Amount Injected	F8426 Peak Area	F8426 Amount Detected
A	954-17	Standard	0 25 ng	218099[a]	0 25 ng
B	105	Control	5mg	0	ND[b]
C	106	Fort @ 0 05 ppm	5 mg	180168	82%
D	107	Treat	5 mg	0	ND

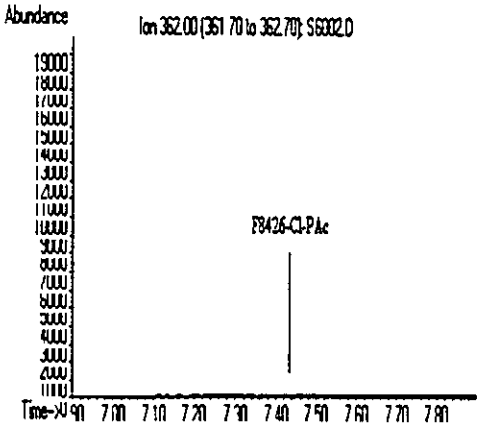
a A single point external standard calibration method was used for quantitation, the average standard peak area was 218736 for F8426
b ND = Not detected (< 0 01 ppm)

FIGURE 18

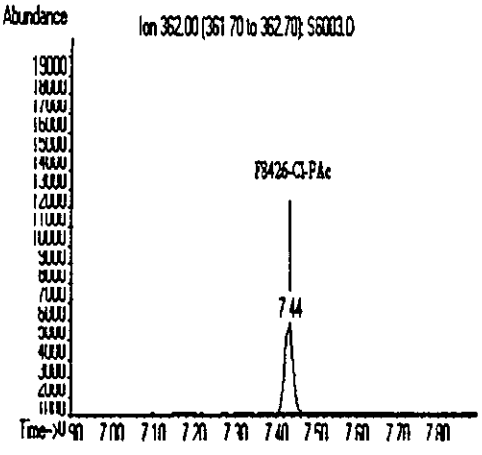
F8426-CHLOROPROPIONIC ACID
IN/ON SWEET CORN STOVER
SET NUMBER: S6, GC/MSD



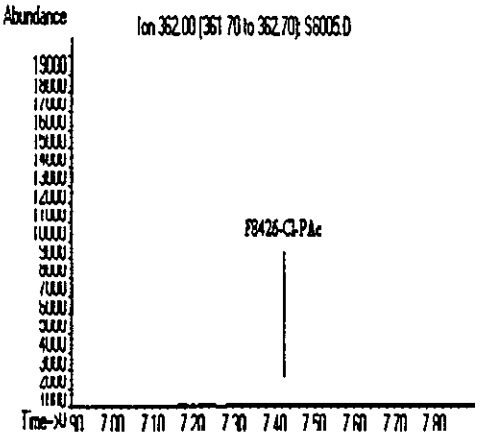
Chromatogram A



Chromatogram B



Chromatogram C



Chromatogram D

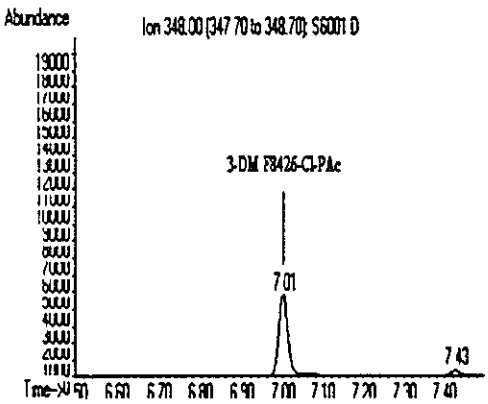
	Assay No	Sample Type	Amount Injected	F8426-Cl-PAc Peak Area	F8426-Cl-PAc Amount Detected
A	978-5	Standard	0 25 ng	63636[a]	0 25 ng
B	105	Control	5mg	0	ND[b]
C	106	Fort @ 0 05 ppm	5 mg	68927	109%
D	107	Treat	5 mg	0	ND

a A single point external standard calibration method was used for quantitation, the average standard peak area was 63523 for F8426-Cl-PAc

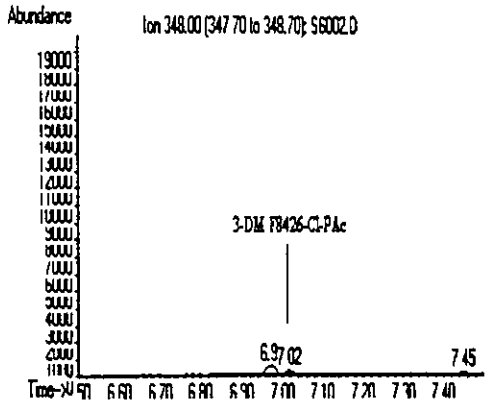
b ND = Not detected (< 0 01 ppm)

FIGURE 19

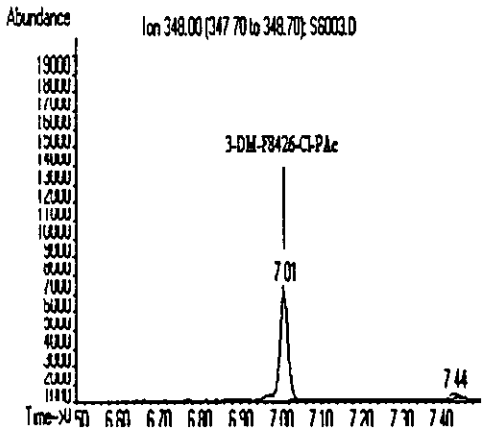
3-DESMETHYL-F8426-CHLOROPROPIONIC ACID
IN/ON SWEET CORN STOVER
SET NUMBER: S6, GC/MSD



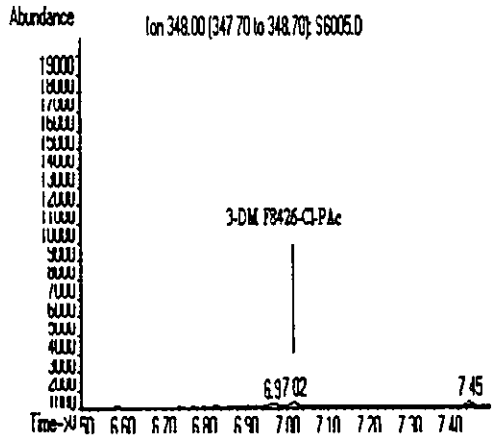
Chromatogram A



Chromatogram B



Chromatogram C



Chromatogram D

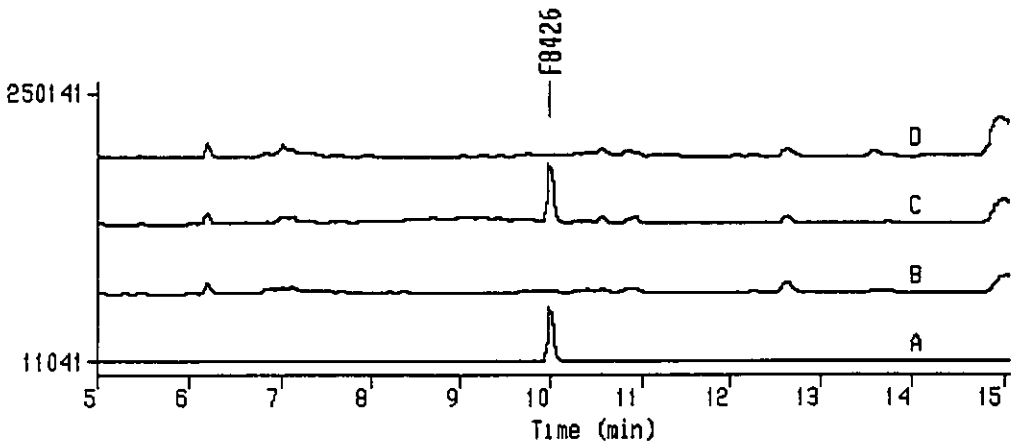
				3-DM-F8426-Cl-PAc	
	Assay No	Sample Type	Amount Injected	Peak Area	Amount Detected
A	978-5	Standard	0 25 ng	74579[a]	0 26 ng
B	105	Control	5mg	0	ND[b]
C	106	Fort @ 0 05 ppm	5 mg	88753	122%
D	107	Treat	5 mg	4025	ND

a A single point external standard calibration method was used for quantitation, the average standard peak area was 73049 for 3-DM-F8426-Cl-PAc

b ND = Not detected (< 0 01 ppm)

FIGURE 20

F8426 IN/ON RICE GRAIN
SET NUMBER: RG1B, GC/ECD

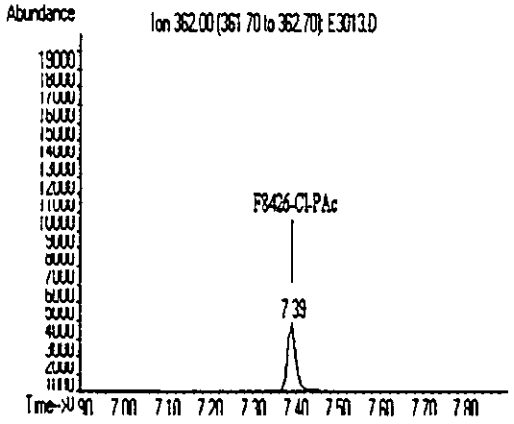


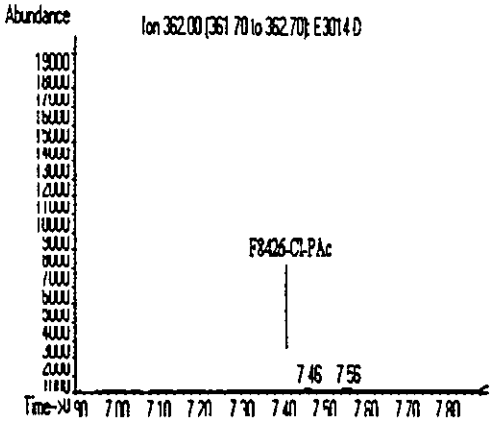
	Assay No	Sample Type	Amount Injected	F8426 Peak Area	F8426 Amount Detected
A	954-11	Standard	0 25 ng	207610[a]	0 25 ng
B	15	Control	5mg	0	ND[b]
C	16	Fort @ 0 05 ppm	5 mg	224639	107%
D	17	Treat	5 mg	0	ND

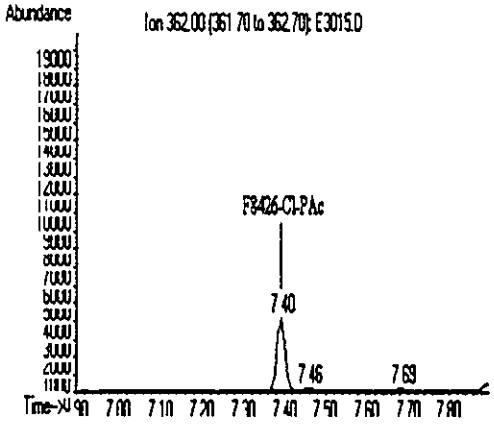
a A single point external standard calibration method was used for quantitation, the average standard peak area was 210725 for F8426
b ND = Not detected (< 0 01 ppm)

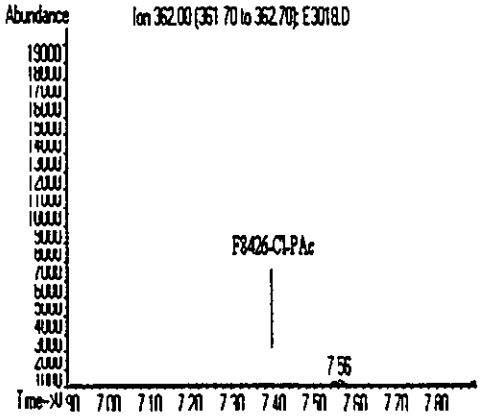
FIGURE 21

F8426-CHLOROPROPIONIC ACID
IN/ON RICE GRAIN
SET NUMBER: RG3, GC/MSD







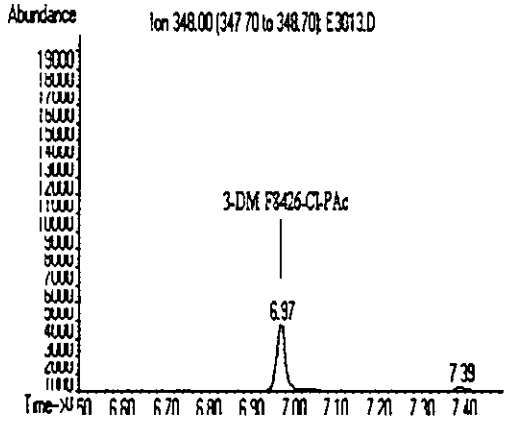


*	Assay No	Sample Type	Amount Injected	F8426-Cl-PAc Peak Area	F8426-Cl-PAc Amount Detected
A	973-5	Standard	0.25 ng	55117[a]	0.24 ng
B	29	Control	5mg	0	ND[b]
C	30	Fort @ 0.05 ppm	5 mg	60652	107%
D	32	Treat Dup	5 mg	0	ND

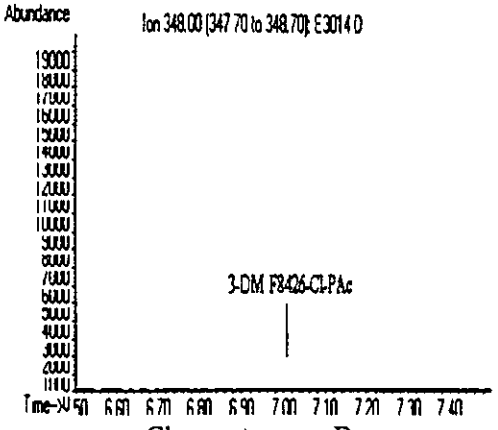
a A single point external standard calibration method was used for quantitation, the average standard peak area was 56591 for F8426-Cl-PAc
b ND = Not detected (< 0.01 ppm)

FIGURE 22

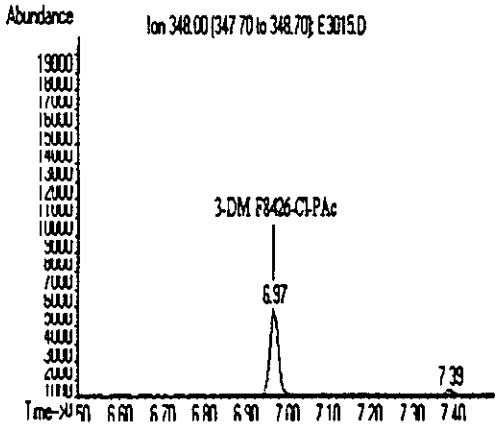
3-DESMETHYL-F8426-CHLOROPROPIONIC ACID
IN/ON RICE GRAIN
SET NUMBER: RG3, GC/MSD



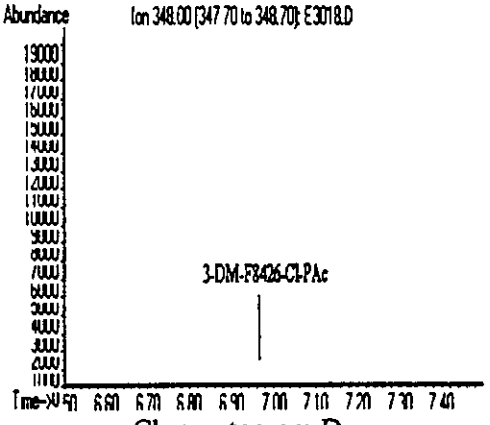
Chromatogram A



Chromatogram B



Chromatogram C



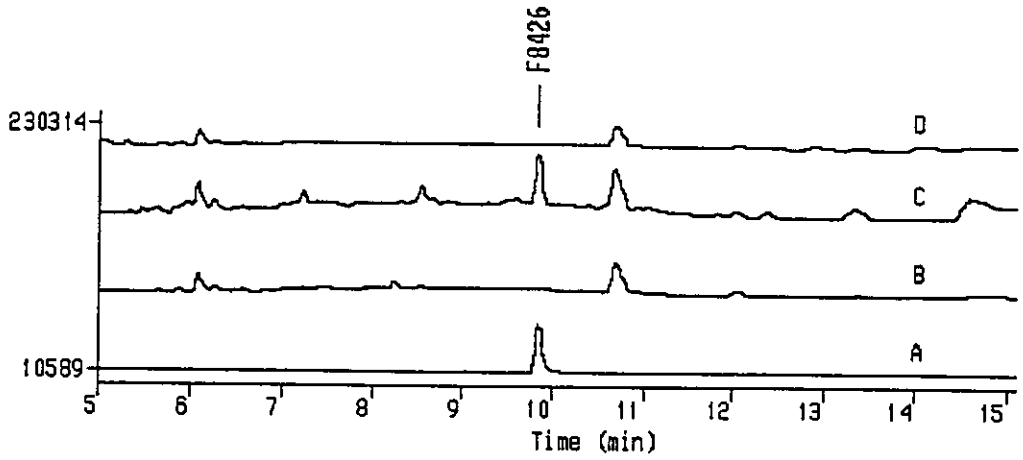
Chromatogram D

	Assay No	Sample Type	Amount Injected	3-DM-F8426-Cl-PAc Peak Area	3-DM-F8426-Cl-PAc Amount Detected
A	973-5	Standard	0 25 ng	59902[a]	0 25 ng
B	29	Control	5mg	0	ND[b]
C	30	Fort @ 0 05 ppm	5 mg	70378	115%
D	32	Treat Dup	5 mg	0	ND

a A single point external standard calibration method was used for quantitation, the average standard peak area was 60988 for 3-DM-F8426-Cl-PAc
b ND = Not detected (< 0 01 ppm)

FIGURE 23

F8426 IN/ON RICE STRAW
SET NUMBER. RS1A, GC/ECD

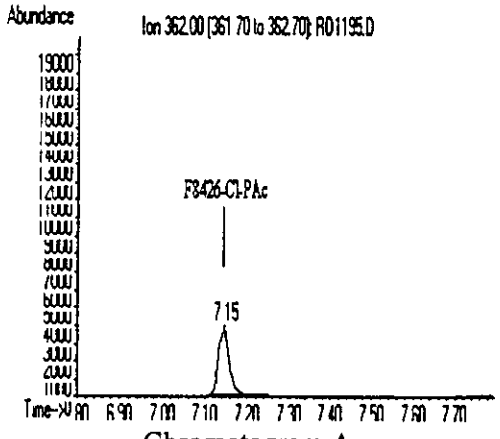


	Assay No	Sample Type	Amount Injected	F8426 Peak Area	F8426 Amount Detected
A	954-17	Standard	0 25 ng	203526[a]	0 27 ng
B	52	Control	5mg	0	ND[b]
C	53	Fort @ 0 05 ppm	5 mg	190072	99%
D	54	Treat	5 mg	0	ND

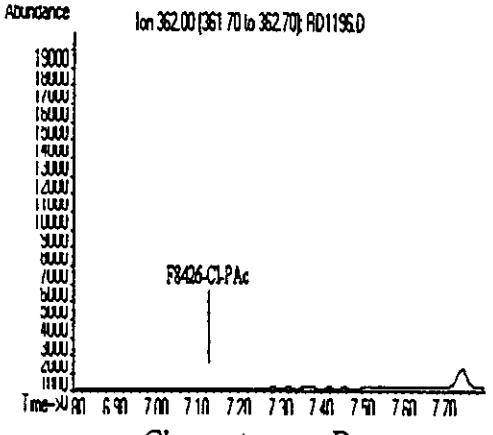
a A single point external standard calibration method was used for quantitation, the average standard peak area was 191933 for F8426
b ND = Not detected (< 0 01 ppm)

FIGURE 24

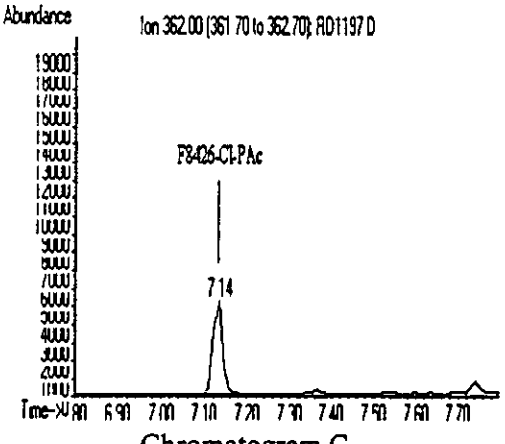
F8426-CHLOROPROPIONIC ACID
IN/ON RICE STRAW
SET NUMBER: RS1, GC/MSD



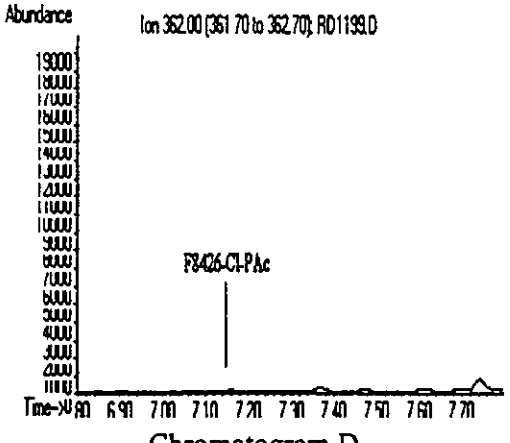
Chromatogram A



Chromatogram B



Chromatogram C



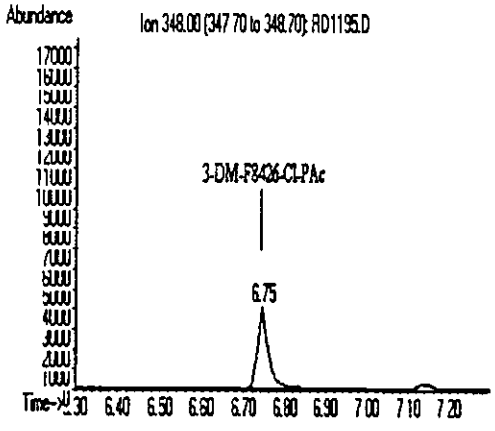
Chromatogram D

				F8426-Cl-PAc	
	Assay No	Sample Type	Amount Injected	Peak Area	Amount Detected
A	975-4	Standard	0 25 ng	67173[a]	0 23 ng
B	1	Control	5mg	0	ND[b]
C	2	Fort @ 0 05 ppm	5 mg	79418	110%
D	3	Treat	5 mg	0	ND

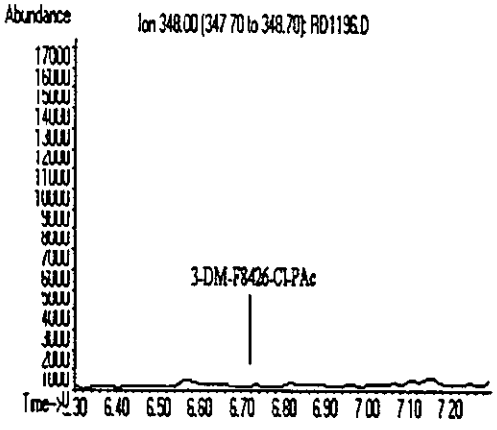
a A single point external standard calibration method was used for quantitation, the average standard peak area was 72184 for F8426-Cl-PAc
b ND = Not detected (< 0 01 ppm)

FIGURE 25

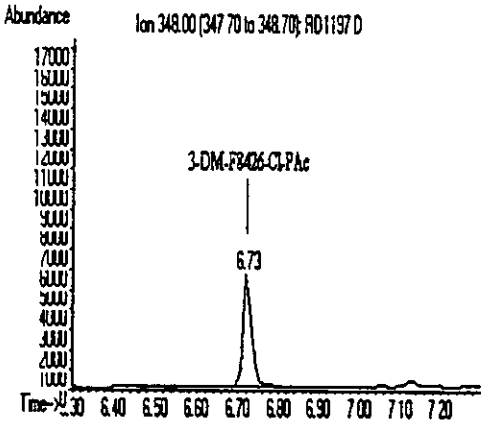
3-DESMETHYL-F8426-CHLOROPROPIONIC ACID
IN/ON RICE STRAW
SET NUMBER: RS1, GC/MSD



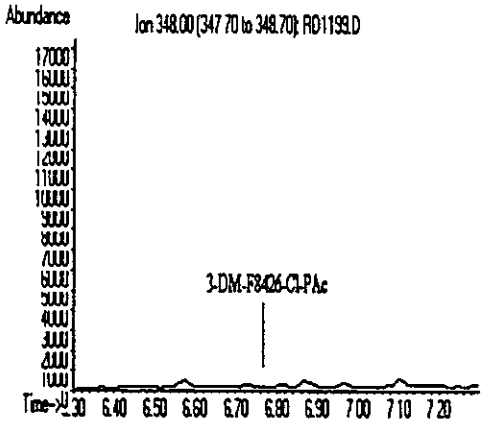
Chromatogram A



Chromatogram B



Chromatogram C



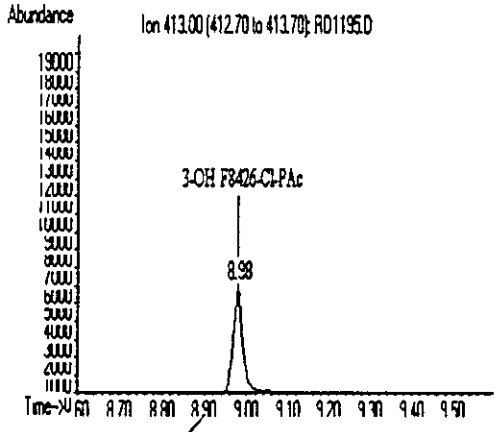
Chromatogram D

	Assay No	Sample Type	Amount Injected	3-DM-F8426-Cl-PAc Peak Area	3-DM-F8426-Cl-PAc Amount Detected
A	975-4	Standard	0 25 ng	71459[a]	0 24 ng
B	1	Control	5mg	0	ND[b]
C	2	Fort @ 0 05 ppm	5 mg	86569	117%
D	3	Treat	5 mg	0	ND

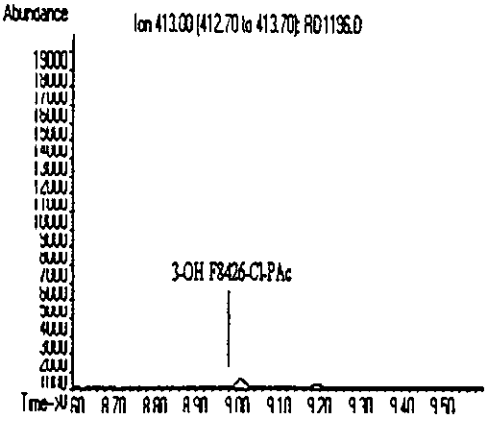
a A single point external standard calibration method was used for quantitation, the average standard peak area was 74041 for 3-DM-F8426-Cl-PAc
b ND = Not detected (< 0 01 ppm)

FIGURE 26

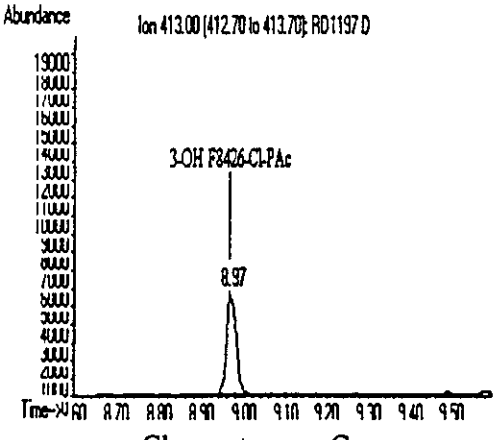
3-HYDROXYMETHYL-F8426-CHLOROPROPIONIC ACID
IN/ON RICE STRAW
SET NUMBER: RS1, GC/MSD



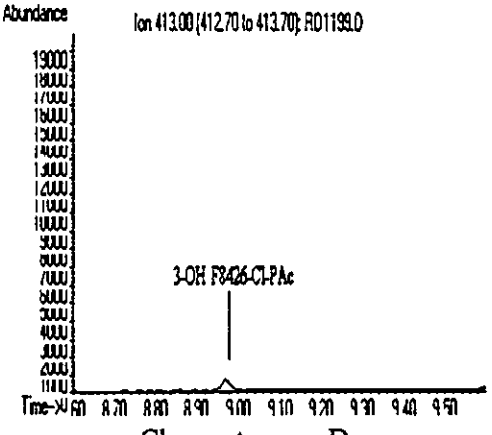
Chromatogram A



Chromatogram B



Chromatogram C



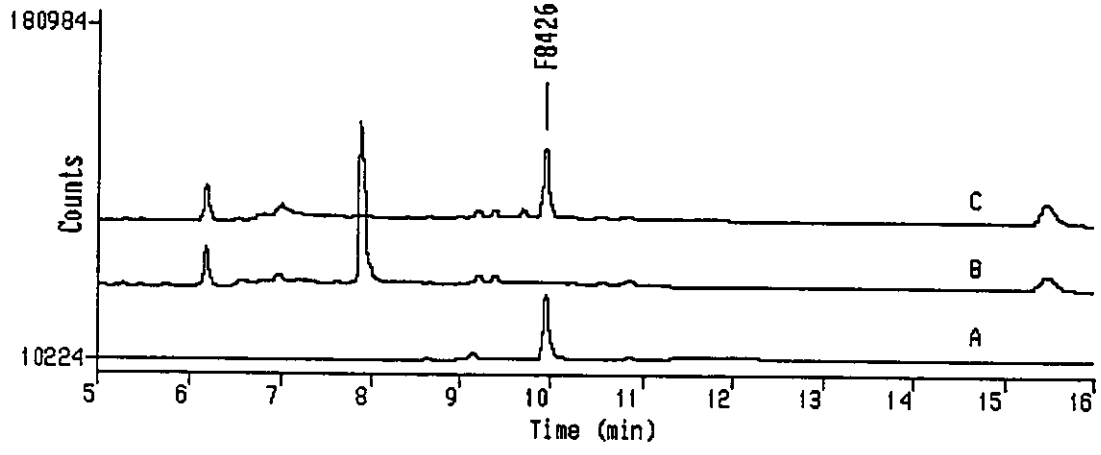
Chromatogram D

	Assay No	Sample Type	Amount Injected	3-OH-F8426-Cl-PAc Peak Area	3-OH-F8426-Cl-PAc Amount Detected
A	975-4	Standard	0 25 ng	84626[a]	0 23 ng
B	1	Control	5mg	0	ND[b]
C	2	Fort @ 0 05 ppm	5 mg	90853	99%
D	3	Treat	5 mg	0	ND

a A single point external standard calibration method was used for quantitation, the average standard peak area was 91783 for 3-OH-F8426-Cl-PAc
b ND = Not detected (< 0 01 ppm)

FIGURE 27

F8426 IN/ON SORGHUM GRAIN
SET NUMBER. SG-1, GC/ECD

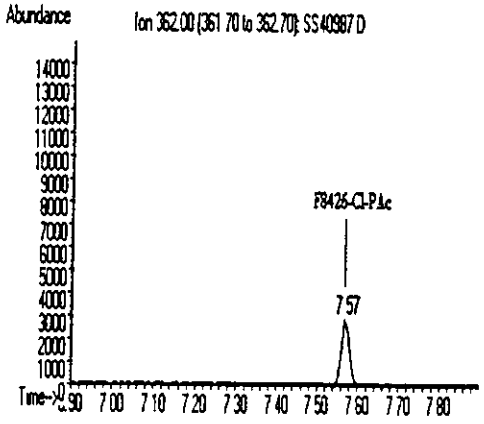


	Assay No	Sample Type	Amount Injected	F8426 Peak Area	F8426 Amount Detected
A	982-11	Standard	0 25 ng	148776[a]	0.23 ng
B	1	Control	5 mg	0	ND[b]
C	2	Fort @ 0 05 ppm	5 mg	149942	92%

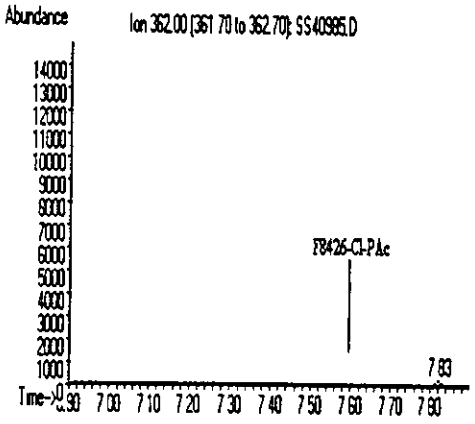
a A multiple point external standard calibration method was used for quantitation, the y intercept was 32736 and the slope was 1016138
b ND = Not detected (< 0 01 ppm)

FIGURE 28

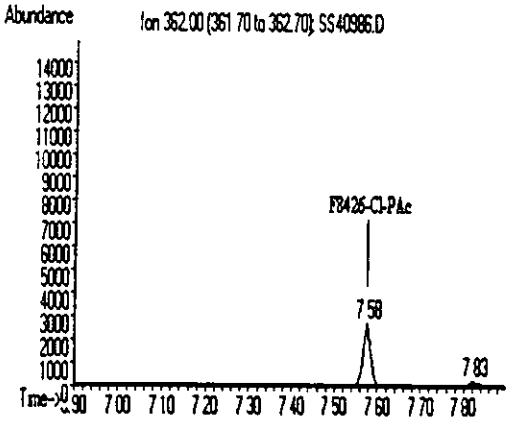
F8426-CHLOROPROPIONIC ACID
IN/ON SORGHUM GRAIN
SET NUMBER: SG-1, GC/MSD



Chromatogram A



Chromatogram B



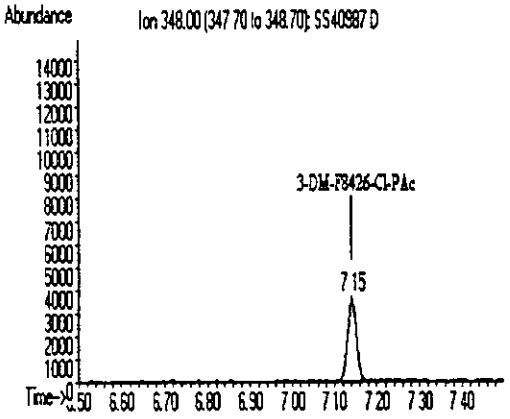
Chromatogram C

	Assay No	Sample Type	Amount Injected	F8426-Cl-PAc Peak Area	F8426-Cl-PAc Amount Detected
A	1011-5	Standard	0.25 ng	39399[a]	0.24 ng
B	1	Control	5 mg	0	ND[b]
C	2	Fort @ 0.05 ppm	5 mg	35346	86%

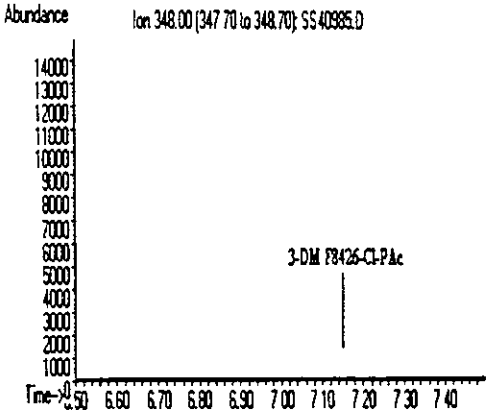
a A multiple point external standard calibration method was used for quantitation, the y intercept was -4064 and the slope was 367756
b ND = Not detected (< 0.01 ppm)

FIGURE 29

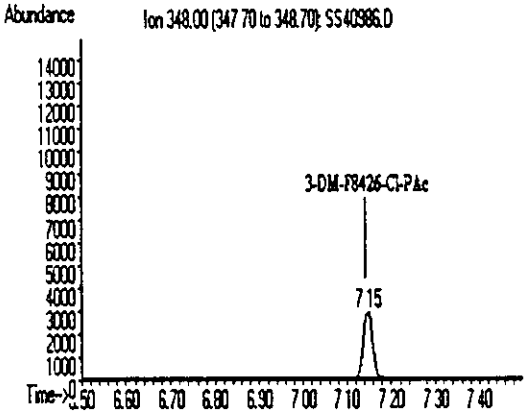
3-DESMETHYL-F8426-CHLOROPROPIONIC ACID
IN/ON SORGHUM GRAIN
SET NUMBER: SG-1; GC/MSD



Chromatogram A



Chromatogram B



Chromatogram C

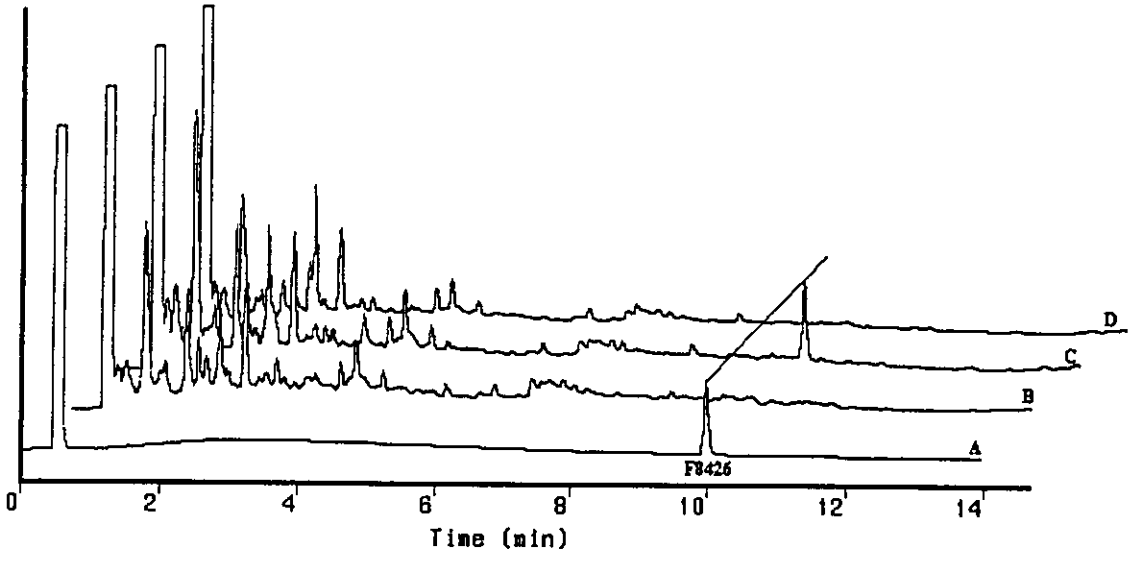
	Assay No	Sample Type	Amount Injected	3-DM-F8426-Cl-PAc Peak Area	3-DM-F8426-Cl-PAc Amount Detected
A	1011-5	Standard	0.25 ng	51880[a]	0.24 ng
B	1	Control	5 mg	0	ND[b]
C	2	Fort @ 0.05 ppm	5 mg	41774	76%

a A multiple point external standard calibration method was used for quantitation, the y intercept was -501 and the slope was 443941

b ND = Not detected (< 0.01 ppm)

FIGURE 30

F8426 IN/ON SORGHUM FORAGE
SET NUMBER: SG-09, GC/ECD

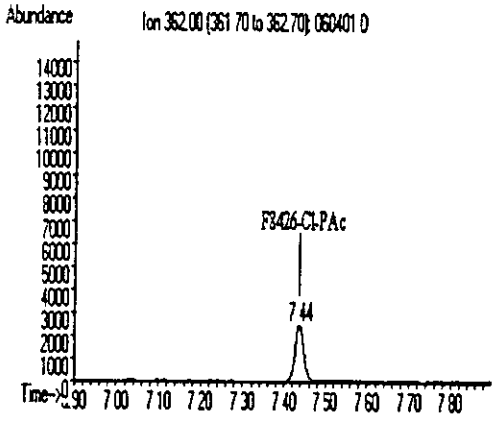


	Assay No	Sample Type	Amount Injected	F8426 Peak Area	F8426 Amount Detected
A	982-11	Standard[a]	0 25 ng	390708	0 28 ng
B	54	Control	5 mg	0	ND[b]
C	55	Fort @ 0 05 ppm	5 mg	407588	115%
D	56	Treat	5 mg	0	ND

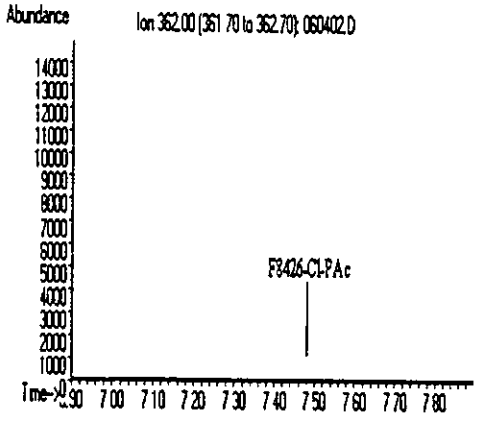
[a] A single point external standard calibration method was used for quantitation, the average standard peak area was 354729 for F8426
[b] ND = Not detected (< 0 01 ppm)

FIGURE 31

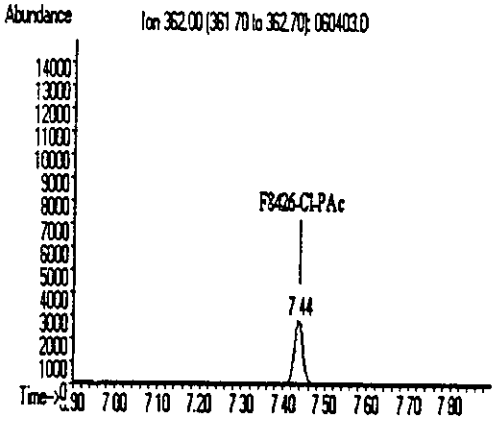
F8426-CHLOROPROPIONIC ACID
IN/ON SORGHUM FORAGE
SET NUMBER: SG09RJ, GC/MSD



Chromatogram A



Chromatogram B



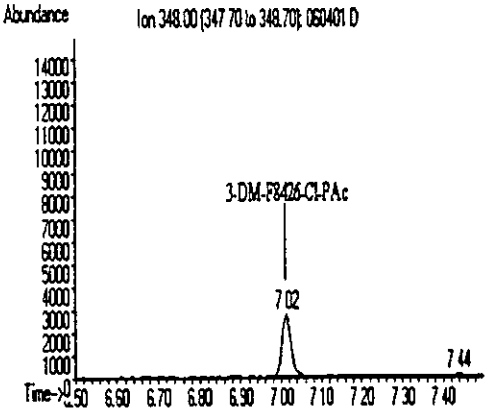
Chromatogram C

	Assay No	Sample Type	Amount Injected	F8426-Cl-PAc Peak Area	F8426-Cl-PAc Amount Detected
A	999-5	Standard	0 25 ng	36113[a]	0 23 ng
B	54	Control	5mg	0	ND[b]
C	55	Fort @ 0 05 ppm	5 mg	39830	102%

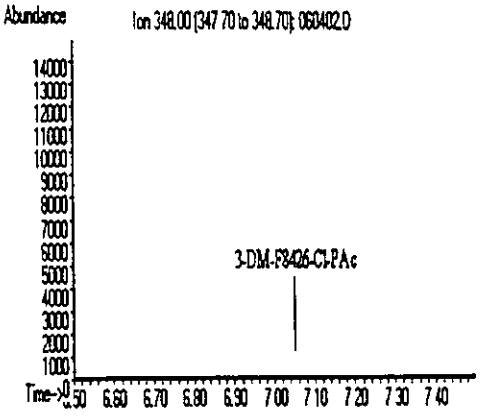
a A single point external standard calibration method was used for quantitation, the average standard peak area was 38841 for F8426-Cl-PAc
b ND = Not detected (< 0 01 ppm)

FIGURE 32

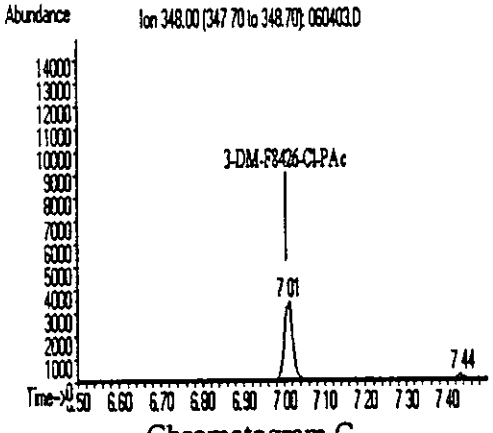
3-DESMETHYL-F8426-CHLOROPROPIONIC ACID
IN/ON SORGHUM FORAGE
SET NUMBER: SG09RJ, GC/MSD



Chromatogram A



Chromatogram B



Chromatogram C

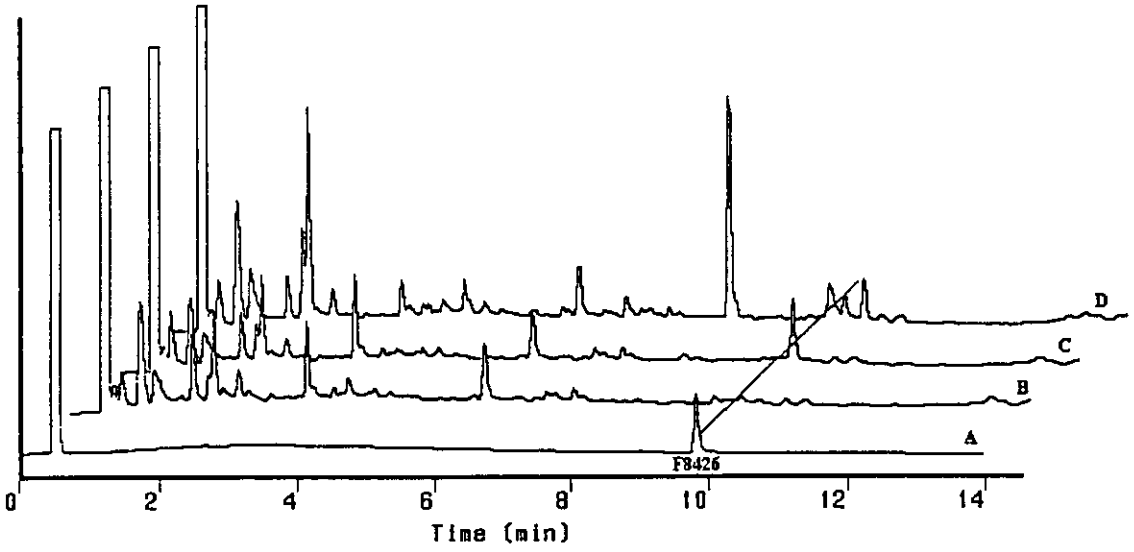
	Assay No	Sample Type	Amount Injected	3-DM-F8426-Cl-PAc Peak Area	3-DM-F8426-Cl-PAc Amount Detected
A	999-5	Standard	0.25 ng	44278[a]	0.23 ng
B	54	Control	5mg	0	ND[b]
C	55	Fort @ 0.05 ppm	5 mg	48662	101%

a A single point external standard calibration method was used for quantitation, the average standard peak area was 48318 for 3-DM-F8426-Cl-PAc

b ND = Not detected (< 0.01 ppm)

FIGURE 33

F8426 IN/ON SORGHUM STOVER
SET NUMBER. SG-11, GC/ECD

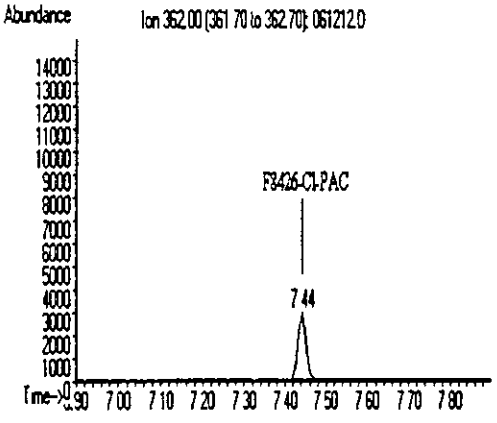


	Assay No	Sample Type	Amount Injected	F8426 Peak Area	F8426 Amount Detected
A	982-11	Standard[a]	0 25 ng	322328	0 25 ng
B	71	Control	5 mg	0	ND[b]
C	72	Fort @ 0 05 ppm	5 mg	306466	93%
D	74	Treat Duplicate	5 mg	108248	(0 02 ppm)[c]

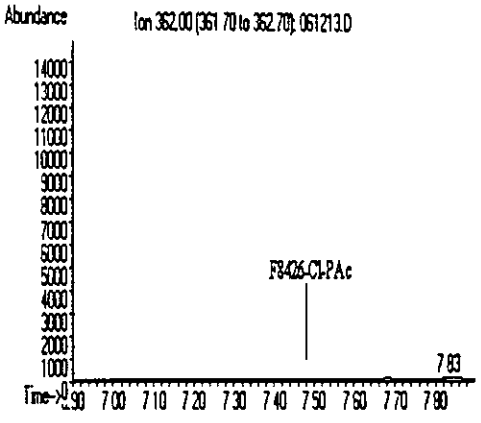
[a] A single point external standard calibration method was used for quantitation, the average standard peak area was 327759 for F8426
[b] ND = Not detected (< 0 01 ppm)
[c] Values in parenthesis indicate a value equal to or greater than the LOD (0 01 ppm) but less than the validated LOQ (0 05 ppm)

FIGURE 34

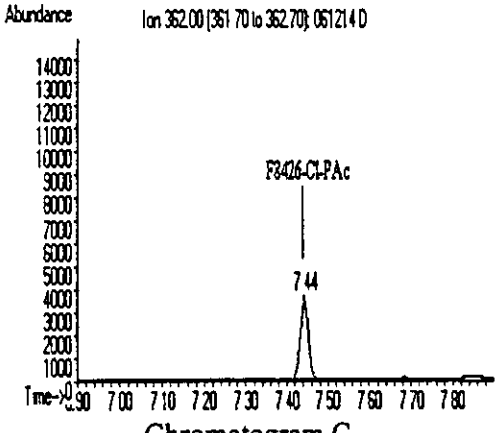
F8426-CHLOROPROPIONIC ACID
IN/ON SORGHUM STOVER
SET NUMBER: SG11, GC/MSD



Chromatogram A



Chromatogram B



Chromatogram C

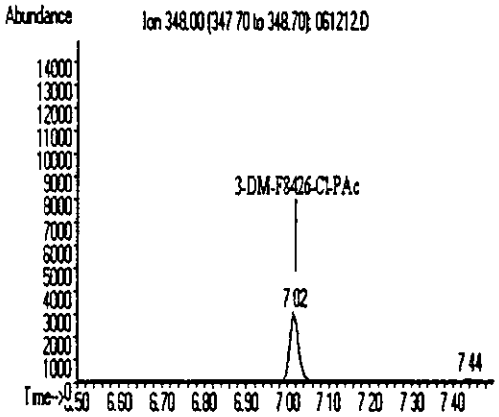
	Assay No	Sample Type	Amount Injected	F8426-Cl-PAc Peak Area	F8426-Cl-PAc Amount Detected
A	999-5	Standard	0.25 ng	40784[a]	0.24 ng
B	71	Control	5mg	0	ND[b]
C	72	Fort @ 0.05 ppm	5 mg	46974	109%

a A single point external standard calibration method was used for quantitation, the average standard peak area was 42892 for F8426-Cl-PAc

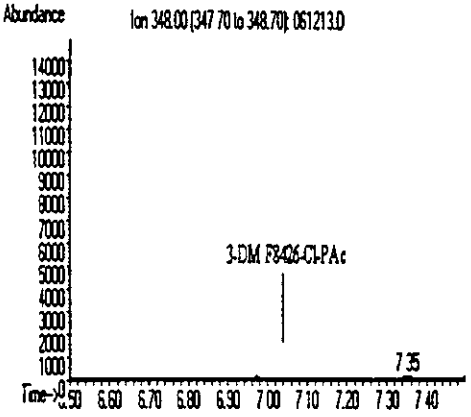
b ND = Not detected (< 0.01 ppm)

FIGURE 35

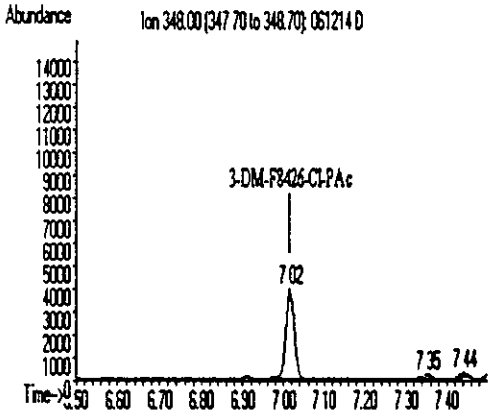
3-DESMETHYL-F8426-CHLOROPROPIONIC ACID
IN/ON SORGHUM STOVER
SET NUMBER: SG11, GC/MSD



Chromatogram A



Chromatogram B



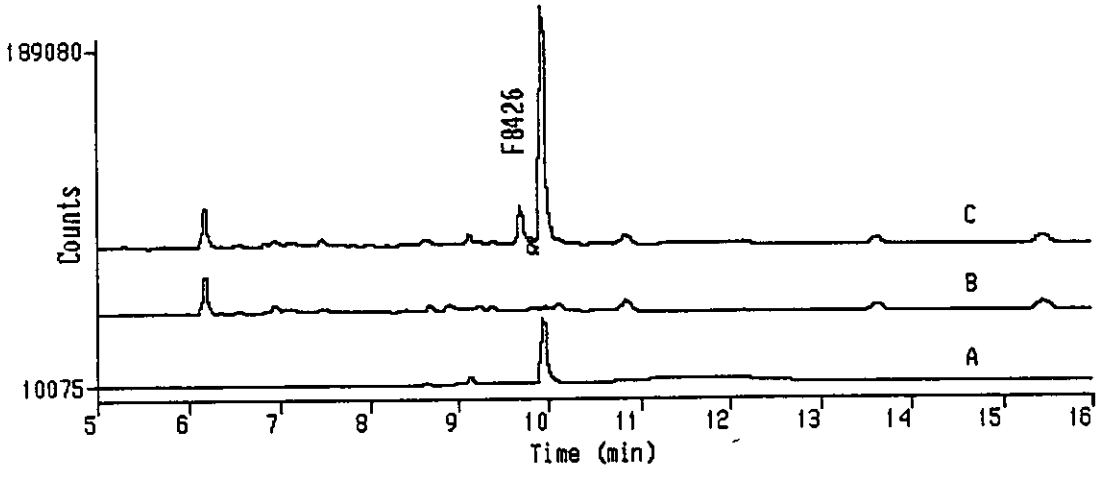
Chromatogram C

	Assay No	Sample Type	Amount Injected	3-DM-F8426-Cl-PAc Peak Area	3-DM-F8426-Cl-PAc Amount Detected
A	999-5	Standard	0.25 ng	44982[a]	0.23 ng
B	71	Control	5mg	0	ND[b]
C	72	Fort @ 0.05 ppm	5 mg	56553	113%

a A single point external standard calibration method was used for quantitation, the average standard peak area was 49875 for 3-DM-F8426-Cl-PAc
b ND = Not detected (< 0.01 ppm)

FIGURE 36

F8426 IN/ON WHEAT GRAIN
SET NUMBER: WG-2, GC/ECD



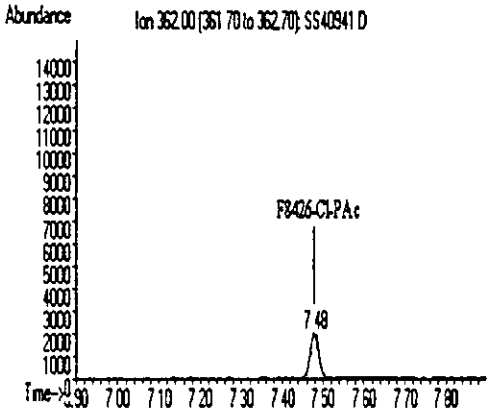
	Assay No	Sample Type	Amount Injected	F8426 Peak Area	F8426 Amount Detected
A	982-11	Standard	0 25 ng	154024[a]	0 25 ng
B	5	Control	5 mg	0	ND[b]
C	8	Fort @ 0 25 ppm	5 mg	556138	84%

a A multiple point external standard calibration method was used for quantitation, the y intercept was 31935 and the slope was 993484

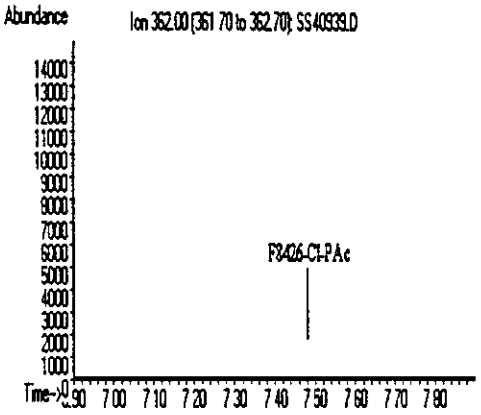
b ND = Not detected (< 0 01 ppm)

FIGURE 37

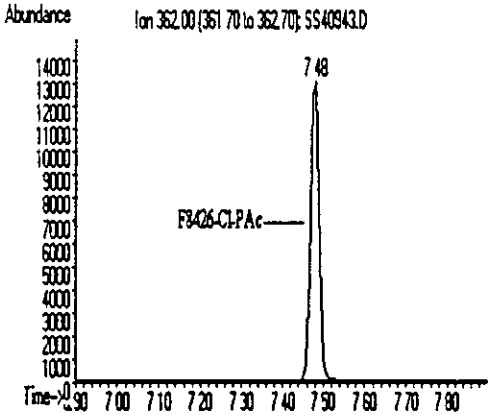
F8426-CHLOROPROPIONIC ACID
IN/ON WHEAT GRAIN
SET NUMBER: WG-2; GC/MSD



Chromatogram A



Chromatogram B



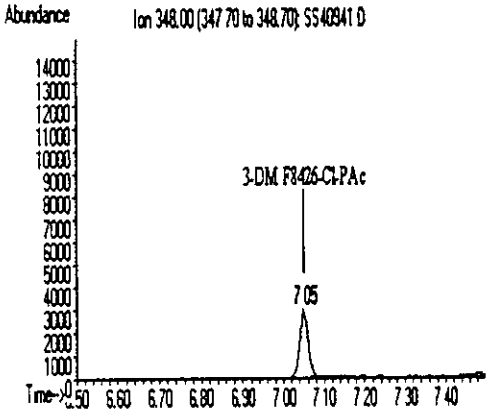
Chromatogram C

	Assay No	Sample Type	Amount Injected	F8426-Cl-PAc Peak Area	F8426-Cl-PAc Amount Detected
A	1011-5	Standard	0.25 ng	29041[a]	0.24 ng
B	1	Control	5 mg	0	ND[b]
C	4	Fort @ 0.25 ppm	5 mg	188541	107%

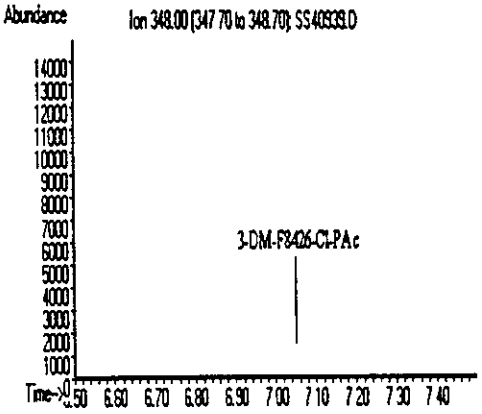
a A multiple point external standard calibration method was used for quantitation, the y intercept was -5617 and the slope was 290448
b ND = Not detected (< 0.02 ppm)

FIGURE 38

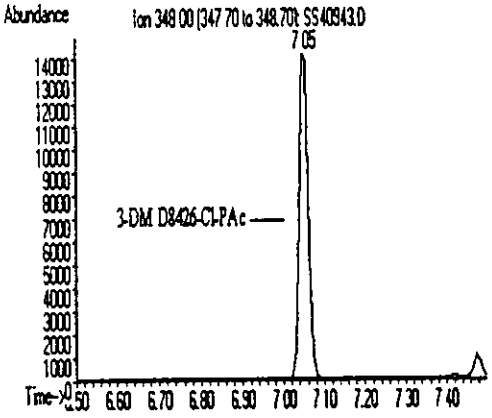
3-DESMETHYL-F8426-CHLOROPROPIONIC ACID
IN/ON WHEAT GRAIN
SET NUMBER. WG-2; GC/MSD



Chromatogram A



Chromatogram B



Chromatogram C

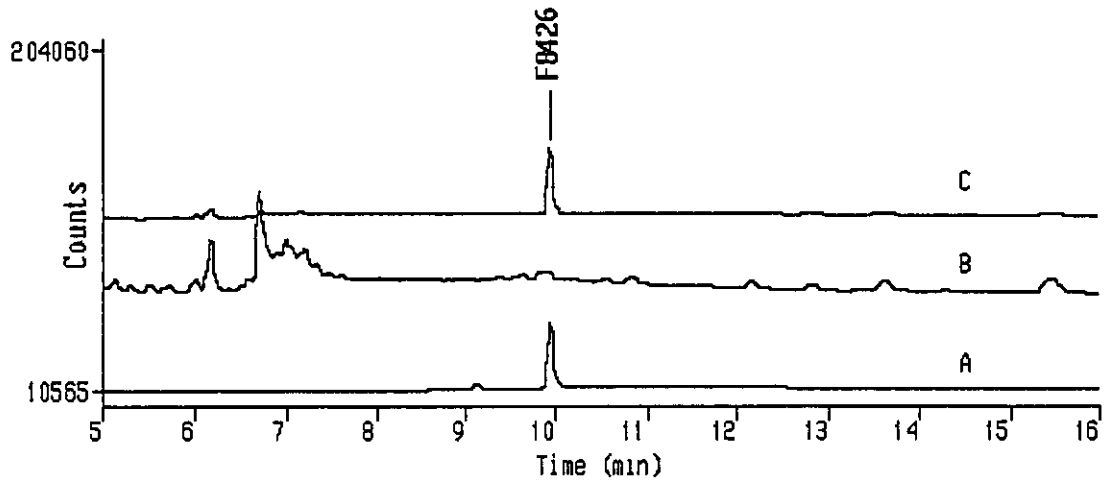
	Assay No	Sample Type	Amount Injected	3-DM-F8426-Cl-PAc Peak Area	3-DM-F8426-Cl-PAc Amount Detected
A	1011-5	Standard	0.25 ng	41551[a]	0.24 ng
B	1	Control	5 mg	0	ND[b]
C	4	Fort @ 0.25 ppm	5 mg	217191	93%

a A multiple point external standard calibration method was used for quantitation, the y intercept was -4393 and the slope was 379259

b ND = Not detected (< 0.01 ppm)

FIGURE 39

F8426 IN/ON WHEAT FORAGE
SET NUMBER: WF-2, GC/ECD

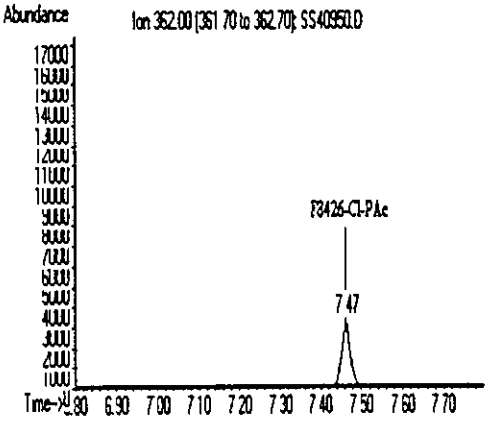


	Assay No	Sample Type	Amount Injected	F8426 Peak Area	F8426 Amount Detected
A	982-11	Standard	0 25 ng	161167[a]	0 25 ng
B	6	Control	5 mg	28802	ND[b]
C	10	Fort @ 0 5 ppm	0 5 mg	161452	99%

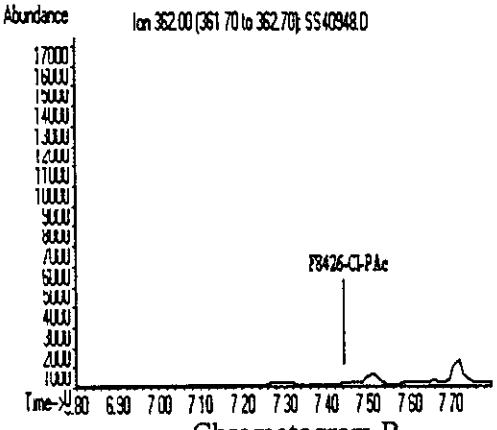
a A multiple-point external standard calibration method was used for quantitation, the y intercept was 21826 and the slope was 1123761
b ND = Not detected (< 0 01 ppm)

FIGURE 40

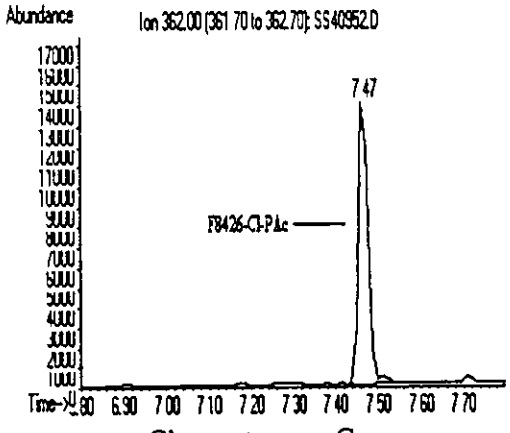
F8426-CHLOROPROPIONIC ACID
IN/ON WHEAT FORAGE
SET NUMBER: WF-1, GC/MSD



Chromatogram A



Chromatogram B



Chromatogram C

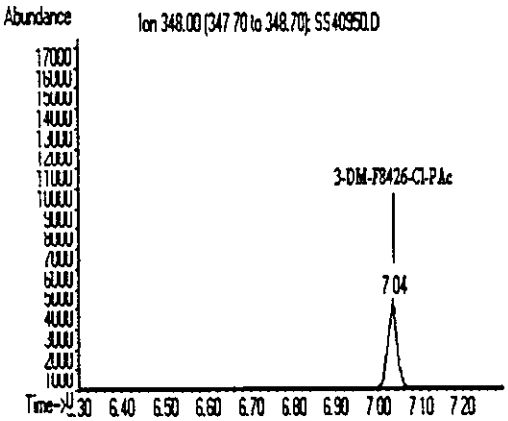
	Assay No	Sample Type	Amount Injected	F8426-Cl-PAc Peak Area	F8426-Cl-PAc Amount Detected
A	1011-5	Standard	0.25 ng	44913[a]	0.24 ng
B	1	Control	5mg	0	ND[b]
C	4	Fort @ 0.25 ppm	5 mg	230992	87%

a A multiple point external standard calibration method was used for quantitation, the y intercept was -7992 and the slope was 438450

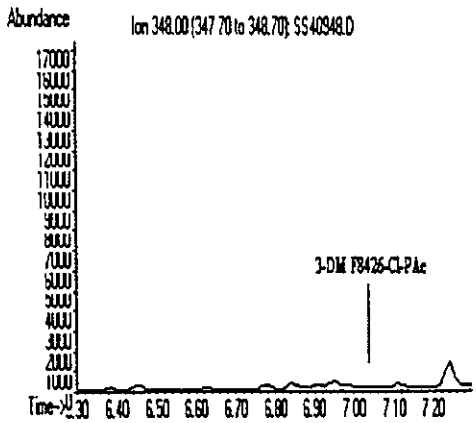
b ND = Not detected (< 0.02 ppm)

FIGURE 41

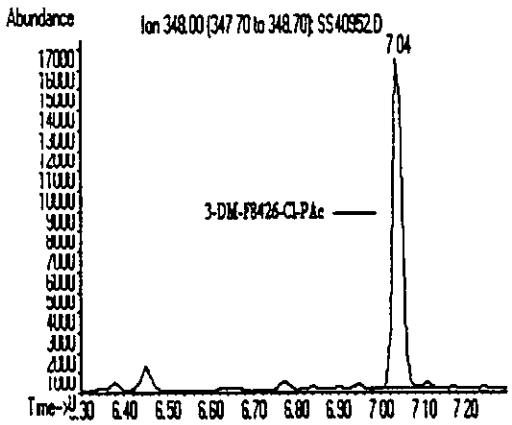
3-DESMETHYL-F8426-CHLOROPROPIONIC ACID
IN/ON WHEAT FORAGE
SET NUMBER: WF-1, GC/MSD



Chromatogram A



Chromatogram B



Chromatogram C

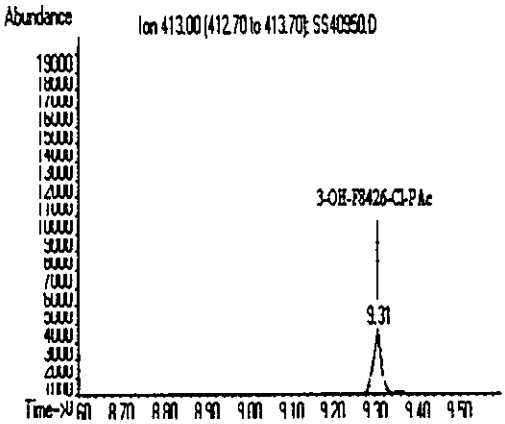
	Assay No	Sample Type	Amount Injected	3-DM-F8426-Cl-PAc Peak Area	3-DM-F8426-Cl-PAc Amount Detected
A	1011-5	Standard	0.25 ng	59800[a]	0.25 ng
B	1	Control	5mg	0	ND[b]
C	4	Fort @ 0.25 ppm	5 mg	262192	77%

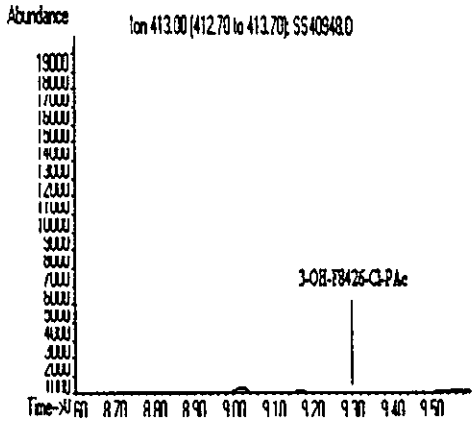
a A multiple point external standard calibration method was used for quantitation, the y intercept was -11213 and the slope was 570662

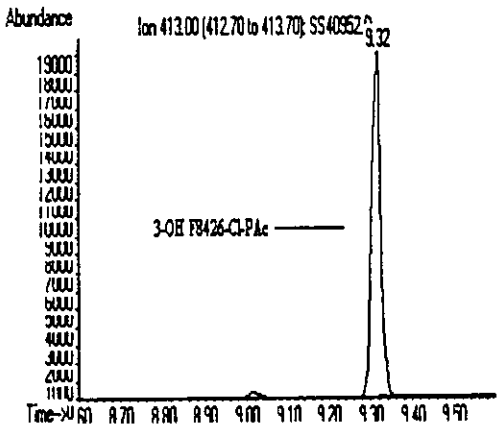
b ND = Not detected (< 0.02 ppm)

FIGURE 42

3-HYDROXYMETHYL-F8426-CHLOROPROPIONIC ACID
IN/ON WHEAT FORAGE
SET NUMBER: WF-1, GC/MSD







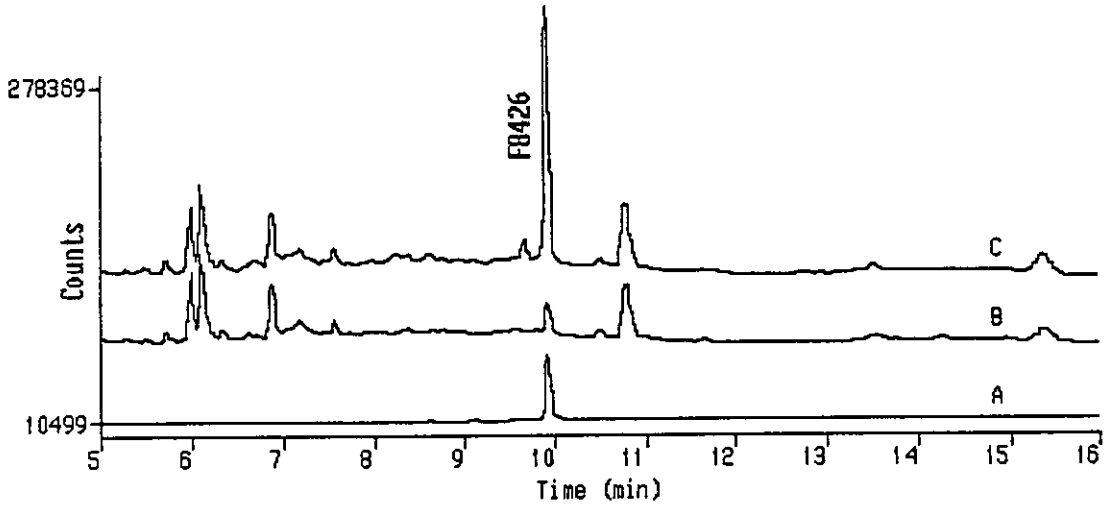
	Assay No	Sample Type	Amount Injected	3-OH-F8426-Cl-PAc Peak Area	3-OH-F8426-Cl-PAc Amount Detected
A	1011-5	Standard	0 25 ng	47110[a]	0 25 ng
B	1	Control	5mg	0	ND[b]
C	4	Fort @ 0 25 ppm	5 mg	297491	90%

a A multiple point external standard calibration method was used for quantitation, the y intercept was -24819 and the slope was 570731

b ND = Not detected (< 0 02 ppm)

FIGURE 43

F8426 IN/ON WHEAT HAY
SET NUMBER. WH-2, GC/ECD

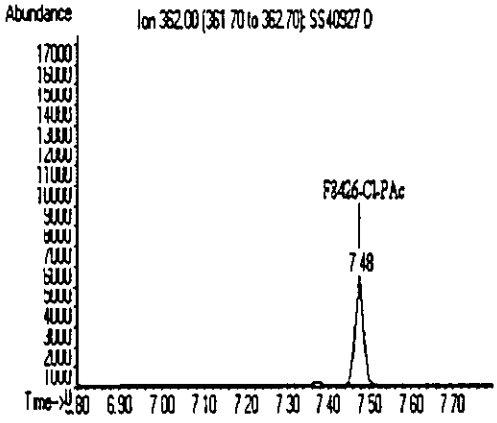


	Assay No	Sample Type	Amount Injected	F8426 Peak Area	F8426 Amount Detected
A	982-11	Standard	0 25 ng	224154[a]	0 24 ng
B	5	Control	5 mg	92893	ND[b]
C	8	Fort @ 0 25 ppm	5 mg	840798	94%

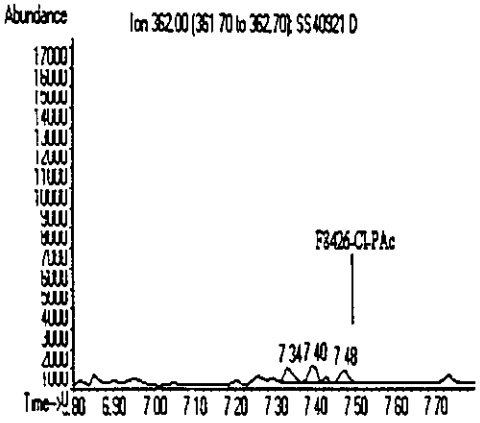
a A multiple point external standard calibration method was used for quantitation, the y intercept was 63489 and the slope was 1324316
b ND = Not detected (< 0 01 ppm)

FIGURE 44

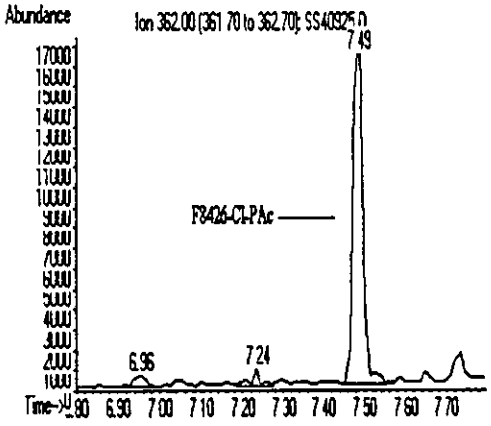
F8426-CHLOROPROPIONIC ACID
IN/ON WHEAT HAY
SET NUMBER: WH-1, GC/MSD



Chromatogram A



Chromatogram B



Chromatogram C

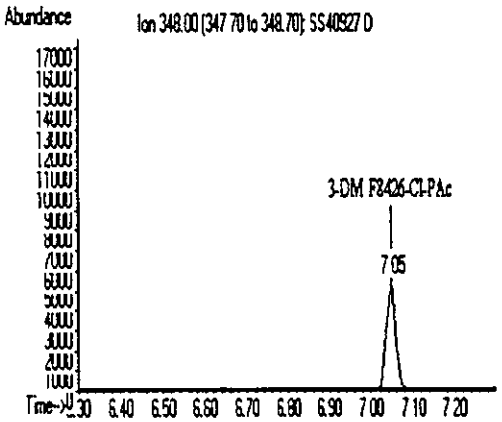
	Assay No	Sample Type	Amount Injected	F8426-Cl-PAc Peak Area	F8426-Cl-PAc Amount Detected
A	1011-5	Standard	0.25 ng	72540[a]	0.26 ng
B	1	Control	5 mg	0	ND[b]
C	4	Fort @ 0.25 ppm	5 mg	283307	72%

a A multiple point external standard calibration method was used for quantitation, the y intercept was -13490 and the slope was 659074

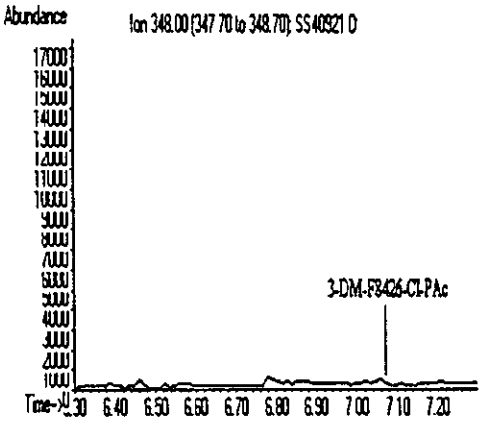
b ND = Not detected (< 0.02 ppm)

FIGURE 45

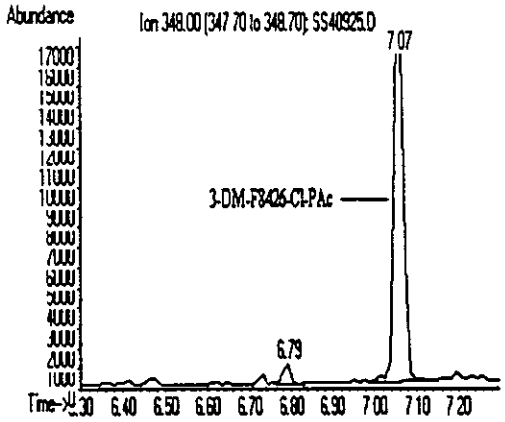
3-DESMETHYL-F8426-CHLOROPROPIONIC ACID
IN/ON WHEAT HAY
SET NUMBER. WH-1, GC/MSD



Chromatogram A



Chromatogram B



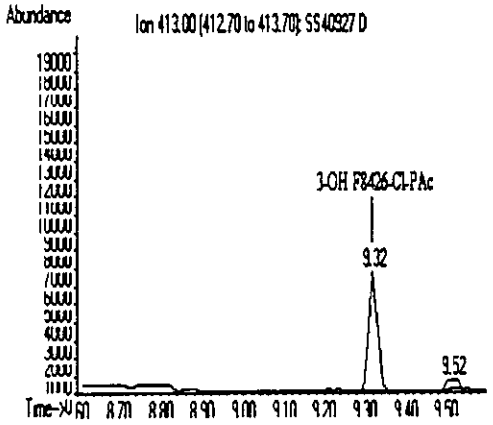
Chromatogram C

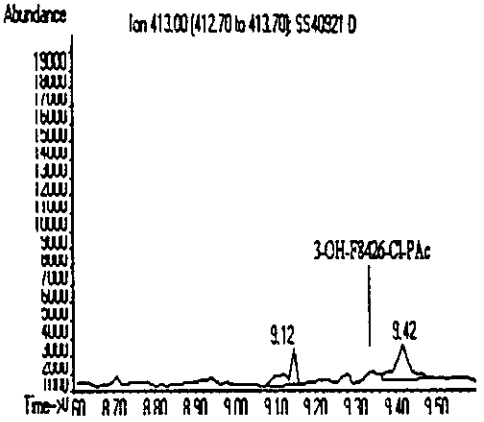
	Assay No	Sample Type	Amount Injected	3-DM-F8426-Cl-PAc Peak Area	3-DM-F8426-Cl-PAc Amount Detected
A	1011-5	Standard	0 25 ng	79626[a]	0 25 ng
B	1	Control	5 mg	0	ND[b]
C	4	Fort @ 0 25 ppm	5 mg	305712	70%

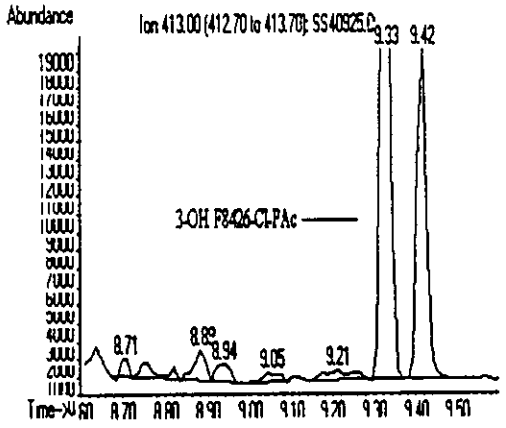
a A multiple point external standard calibration method was used for quantitation, the y intercept was -7502 and the slope was 711025
b ND = Not detected (< 0 01 ppm)

FIGURE 46

3-HYDROXYMETHYL-F8426-CHLOROPROPIONIC ACID
IN/ON WHEAT HAY
SET NUMBER: WH-1; GC/MSD







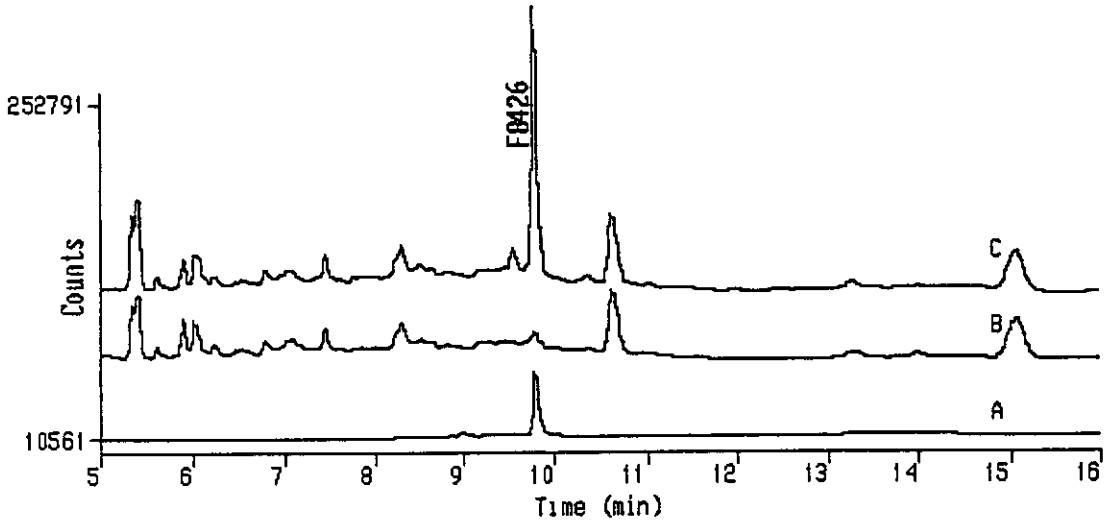
	Assay No	Sample Type	Amount Injected	3-OH-F8426-Cl-PAc Peak Area	3-OH-F8426-Cl-PAc Amount Detected
A	1011-5	Standard	0 25 ng	95952[a]	0 24 ng
B	1	Control	5 mg	0	ND[b]
C	4	Fort @ 0 25 ppm	5 mg	474061	77%

a A multiple point external standard calibration method was used for quantitation, the y intercept was -28344 and the slope was 1040542

b ND = Not detected (< 0 02 ppm)

FIGURE 47

F8426 IN/ON WHEAT STRAW
SET NUMBER: WS-2, GC/ECD

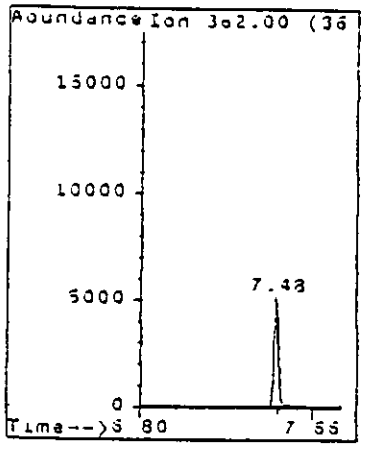


	Assay No	Sample Type	Amount Injected	F8426 Peak Area	F8426 Amount Detected
A	982-11	Standard	0 25 ng	197727[a]	0 25 ng
B	5	Control	5 mg	0	ND[b]
C	8	Fort @ 0 25 ppm	5 mg	782291	98%

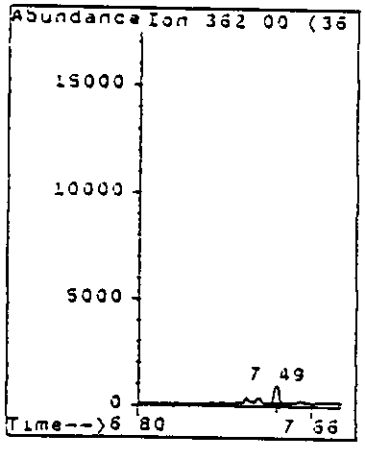
a A multiple point external standard calibration method was used for quantitation, the y intercept was 48025 and the slope was 1203337
b ND = Not detected (< 0 01 ppm)

FIGURE 48

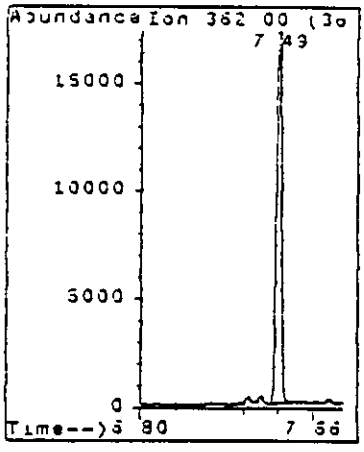
F8426-CHLOROPROPIONIC ACID
IN/ON WHEAT STRAW
SET NUMBER: WS-1, GC/MSD



Chromatogram A



Chromatogram B



Chromatogram C

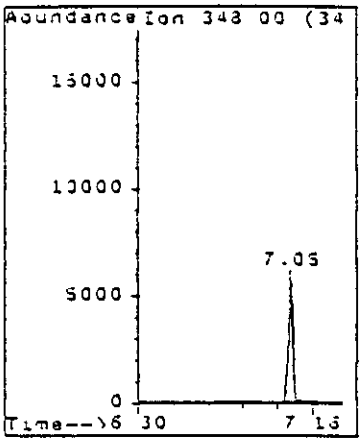
	Assay No	Sample Type	Amount Injected	F8426-Cl-PAc Peak Area	F8426-Cl-PAc Amount Detected
A	1011-5	Standard	0 25 ng	73946[a]	0 26 ng
B	1	Control	5 mg	14356	(0 016)[b]
C	4	Fort @ 0 25 ppm	5 mg	336900	79%

a A multiple point external standard calibration method was used for quantitation, the y intercept was -11751 and the slope was 654597

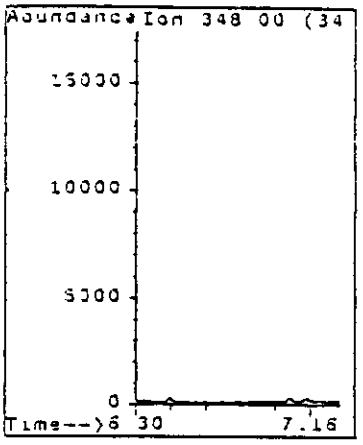
b Values in parenthesis indicate a value equal to or greater than the LOD (0 02 ppm) but less than the validated LOQ (0 05 ppm)

FIGURE 49

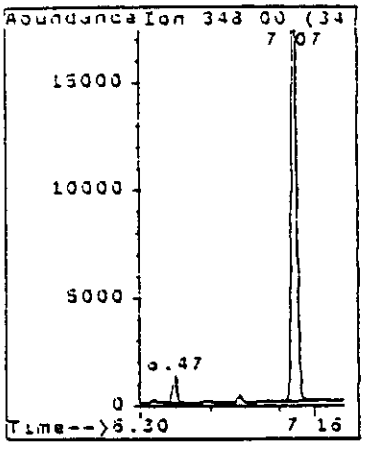
3-DESMETHYL-F8426-CHLOROPROPIONIC ACID
IN/ON WHEAT STRAW
SET NUMBER. WS-1, GC/MSD



Chromatogram A



Chromatogram B



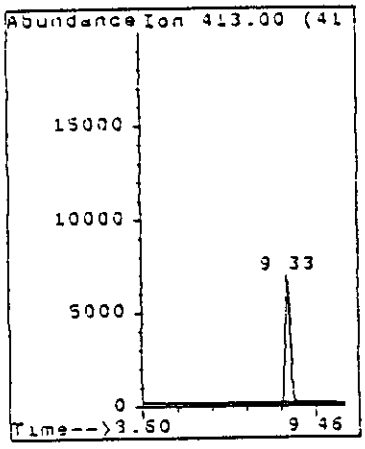
Chromatogram C

	Assay No	Sample Type	Amount Injected	3-DM-F8426-Cl-PAc Peak Area	3-DM-F8426-Cl-PAc Amount Detected
A	1011-5	Standard	0 25 ng	83639[a]	0 25 ng
B	1	Control	5 mg	0	ND[b]
C	4	Fort @ 0 25 ppm	5 mg	355159	80%

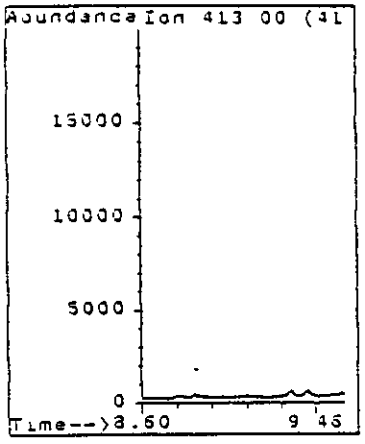
a A multiple point external standard calibration method was used for quantitation, the y intercept was -7664 and the slope was 725365
b ND = Not detected (< 0 01 ppm)

FIGURE 50

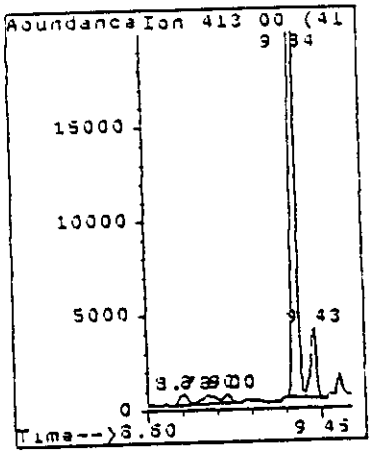
3-HYDROXYMETHYL-F8426-CHLOROPROPIONIC ACID
IN/ON WHEAT STRAW
SET NUMBER: WS-1, GC/MSD



Chromatogram A



Chromatogram B



Chromatogram C

	Assay No	Sample Type	Amount Injected	3-OH-F8426-Cl-PAc Peak Area	3-OH-F8426-Cl-PAc Amount Detected
A	1011-5	Standard	0 25 ng	101174[a]	0 25 ng
B	1	Control	5 mg	0	ND[b]
C	4	Fort @ 0 25 ppm	5 mg	568387	91%

a A multiple point external standard calibration method was used for quantitation, the y intercept was -29820 and the slope was 1051335

b ND = Not detected (< 0 02 ppm)