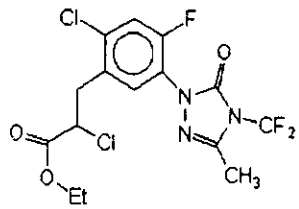
I. INTRODUCTION

F8426 50DF Herbicide is currently being developed by FMC Corporation for use in the control of broadleaf weeds, grasses and sedges in cereal grains and soybeans. The common name of the active ingredient is carfentrazone-ethyl. The chemical name of carfentrazone-ethyl is Ethyl α, 2-dichloro-5-[4-(difluoromethyl)-4,5-dihydro-3-methyl-5-oxo-1H-1,2,4-triazol-1-yl]-4-fluorobenzene propanoate. Its Chemical Abstract Service (CAS) number is 128639-02-1, and its Environmental Protection Agency (EPA) Registry number is 128712. Carfentrazone-ethyl was originally developed by FMC Corporation with the code name F8426. The chemical structure of F8426 is shown below:



Study Number 842E4195E1 was conducted to address the EPA Pesticide Assessment Guidelines (Subdivision N) requirement for a Terrestrial Field Dissipation study (164-1). Carfentrazone-ethyl has been shown to rapidly hydrolyze to F8426-chloropropionic acid (F8426-Cl-PAc) which further degrades to F8426-cinnamic acid (F8426-CAc), F8426-propionic acid (F8426-PAc), F8426-benzoic acid (F8426-BAc) and 3-hydroxymethyl-F8426-benzoic acid (3-OH-F8426-BAc) via soil metabolism. Therefore these suspected major soil degradates were analyzed in the field dissipation study. The purpose of this report is to describe the residue analytical method for carfentrazone-ethyl and its five metabolites in soil. The basic methodology for the analysis of carfentrazone-ethyl and its soil metabolites in this report is a modification of the method described in FMC Corporation report RAN-0270M (Reference 1). Modifications were made to allow for the simultaneous analysis of a fifth metabolite, 3-OH-F8426-BAc.

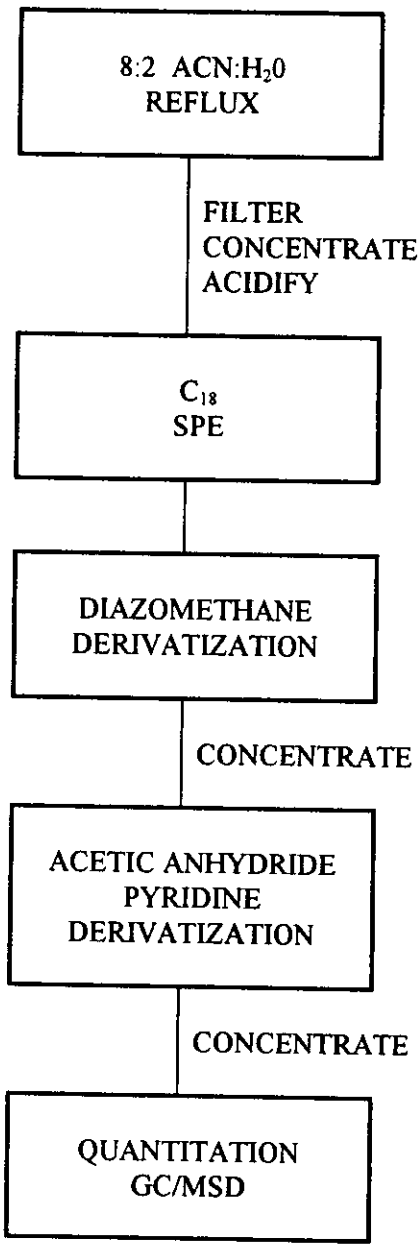
II. SUMMARY

An analytical method has been developed for the analysis of carfentrazone-ethyl and its five soil metabolites. This general method has been employed on field soils from four dissipation sites representing soil types of Kansas, Minnesota, North Carolina, and Iowa, the major use regions. Data presented here are from the North Carolina and Iowa sites. For carfentrazone-ethyl and its acid metabolite residue determinations, soil samples were subjected to an initial acetonitrile/water reflux extraction. The extract was concentrated by rotary evaporation to remove the organic solvent, and the remaining aqueous sample was acidified and then cleaned with C_{18} Solid Phase Extraction (SPE). The extract was concentrated and derivatized with diazomethane/ether followed by pyridine and acetic anhydride to methylate the acid metabolites. The derivatized sample was concentrated prior to analysis. Quantitation was achieved using capillary gas chromatography coupled with a mass selective detector (GC/MSD).

Method validation and recovery experiments were conducted for each of the six compounds. The analytical method was validated by fortifying and analyzing soil samples and monitoring the recoveries. A typical analysis set had nine to twelve samples which included treated samples, at least one untreated control sample, and a minimum of one fortified control sample. Fortification levels ranged from 5 ppb to 50 ppb for each of the six compounds. The limit of quantitation (LOQ), or lowest fortification level, for each compound was established to be 5 parts per billion (ppb). The limit of detection (LOD) for each compound was estimated to be 1 ppb. Signal response less than LOD was considered non-detectable (ND).

B. Analytical Method Flow Scheme

Figure 1.
Analytical Method Flow Scheme for the Analysis of
Carfentrazone-ethyl and its Five Metabolites on Soil.



IV. MATERIALS AND STUDY DESIGN

A. Test Substance

The common name of the active ingredient in F8426 50DF herbicide is carfentrazone-ethyl. The chemical name of the active ingredient F8426 is Ethyl α, 2-dichloro-5-[4-(difluoromethyl)-4,5-dihydro-3-methyl-5-oxo-1H-1,2,4-triazol-1-yl]-4-fluorobenzene propanoate. Its FMC number is 116426, its Chemical Abstract Service (CAS) number is 128639-02-1, and its EPA Registration number is 128712.

Technical standards of carfentrazone-ethyl, F8426-chloropropionic acid (F8426-Cl-PAc), F8426-cinnamic acid (F8426-CAc), F8426-propionic acid (F8426-PAc), F8426-benzoic acid (F8426-BAc) and 3-hydroxymethyl-F8426-benzoic acid (3-OH-F8426-BAc) were used for fortification and quantitation. Refer to Section X, Table 3 for compound structures.

B. Test System

The two soil types utilized in Study Number 842E4195E1, and used for these recovery experiments were classified according to USDA standards as "Sandy Loam" and "Clay Loam". The pH, cation exchange capacity (meq/ 100 g), and percentage of organic matter of the top 30 cm of the Sandy Loam soil was 6.0, 8.8, and 1.0 respectively. The pH, cation exchange capacity (meq/ 100 g), and percentage of organic matter of the top 30 cm of the Clay Loam soil was 8.0, 44.3, and 6.3 respectively.

C. Study Design and Procedures

The analytical method was validated by fortifying and analyzing soil samples from control plots and monitoring the recoveries. Untreated soil samples were fortified by adding known amounts of F8426 and its metabolites prior to the addition of reflux solvent. All fortifications were made using a microliter syringe (typical spiking volume should be less than 1000 µL). Fortification levels ranged from 5 ppb to 50 ppb for each of the six compounds. The Limit of Quantitation (LOQ) for each compound was established at 5 ppb. The Limit of Detection (LOD) for each compound was estimated to be 1 ppb. Signal response less than LOD was considered non-detectable (ND).

A typical set of samples had nine samples which included treated samples, at least one untreated control sample, and a minimum of one fortified control sample.

Generally, a set was considered valid when method recoveries were between 60% and 120%. In some cases when only one analyte in a fortification of six compounds failed this criteria, the set was still considered acceptable. The acceptance of these recovery values produced larger relative standard deviations than would normally have been seen in a single component analysis. However, to minimize analytical effort and streamline the method all six analytes were determined together simultaneously. Individual method recovery data can be found in Section X, Table 2.

D. Dates

Samples from Trial 01 were collected between April 1995 and November 1996. The Samples were shipped on dry ice using an overnight delivery service and were received at the laboratory the following day. For Trial 02, samples were collected from July 1995 through April 1997. These samples were shipped on dry ice within three days of collecting, using an overnight delivery service, and were received at the laboratory the following day. Analyses were conducted between October 1995 and May 1997. The maximum storage interval for any sample was 18 months.

E. Equipment

The following equipment was utilized to perform the methodology. Where a manufacturer is not listed this is an indication that equipment from various manufacturers have been or can be used interchangeably. When a manufacturer is listed, products of other manufacturers may be utilized if equivalent results will be produced. However, this method was not tested using alternate manufacturers.

Analytical Balance
Diazomethane generation apparatus, Macro Diazald® kit, Z10,851-0, Aldrich
Evaporator, N-Evap®, Organomation
Evaporator, rotary
Filter paper, GF/A, glass microfibre filters, Whatman
Heating Block, Pierce
Hot Plate / Stirrers
Nitrogen manifold
Refrigerated constant temperature circulator 1174, VWR
Solid Phase Extraction (SPE) Processor/vacuum box J.T. Baker
Syringes, various volumes, Hamilton
Standard laboratory glassware
Water Deionizer, Barnstead PCS System

F. Reagents

The following reagents were used in this study. All solvents were pesticide analysis grade and/or free of contaminants. Where a manufacturer is not listed this is an indication that reagents from various manufacturers have been or can be used interchangeably. When a manufacturer is listed, products of other manufacturers may be utilized if equivalent results will be produced. However, this method was not tested using alternate manufacturers.

Acetone, JT Baker
Acetic Anhydride, JT Baker
Acetonitrile, Omnisolv, EM Science
Carbitol® (Di(ethylene glycol) ethyl ether), Aldrich
Diazald®, Aldrich

Dichloromethane, Omnisolv, EM Science
Ether, Anhydrous, JT Baker
Ethyl Acetate, Omnisolv, EM Science
Glass Wool
Hexane, Omnisolv EM Science
Hydrochloric Acid, 0.25 N, JT Baker
Methanol, Omnisolv, EM Science
Potasium Hydroxide, pellets, JT Baker
Pyridine, Anhydrous, Aldrich
Solid Phase Extraction (SPE) columns, C_{18}, 1g/6 mL, Varian
Sulfuric Acid, concentrated, JT Baker
Sylon-CT
Toluene, Omnisolv, EM Science
Water, deionized, House prepared

V. ANALYTICAL PROCEDURE

A. Residue Method

The basic methodology for the analysis of carfentrazone-ethyl and its soil metabolites in this report is a modification of the method described in FMC Corporation report RAN-0270M (Reference 1). Modifications were made to allow for the simultaneous analysis of a fifth metabolite, 3-OH-F8426-BAc. Following these modifications the method was independently validated (Reference 2). The method was successfully validated on the first try. Suggestions for clarification were made in the Independent Method Validation report and incorporated into this report.

Sample Preparation:

Samples may be prepared for analysis by processing with liquid nitrogen in a Hobart® Food Chopper to thoroughly mix the various soil cores. However, it has been determined that the better method of preparation is for the soils to be thawed and mixed by hand by kneading the closed sample bag. This report contains results obtained from samples which had been mixed by hand kneading. Prior to sample work-up, soil cores were thoroughly mixed before taking a subsample. All soil samples were analyzed as received from the field without drying to remove moisture prior to analysis.

Glassware Preparation:

Glassware must be cleaned with detergent and water prior to use. Just before utilizing the glassware, generously pre-rinse with 0.25 N HCl, followed by acetone to remove acid. This is considered a critical method step.

Diazomethane/Ether Preparation:

Diazomethane is not commercially available but can be generated in the laboratory. The procedure utilized to prepare diazomethane/ether is detailed in the Journal of Organic

Chemistry, 1980 (Reference 3). Tests at FMC and a contract lab have shown that diazomethane is the only derivatization agent that can be used for all metabolites without affecting the parent metabolite (Reference 4).

CAUTION: Diazomethane is an experimental tumorigen and carcinogen. It is a poison and irritant when inhaled. It is also a strong allergen. Diazomethane can cause pulmonary edemas and can bring about a hypersensitivity characterized by asthma-like symptoms. Diazomethane, in either its liquid or gaseous state, is highly explosive when shocked or exposed to heat, high intensity light, rough surfaces, or incompatible chemicals (References 5 and 6).

Sample Analysis:

REFLUX & FILTRATION

Weigh 80 g of soil into a 500 mL flat-bottom boiling flask.

Spike the laboratory fortification sample with the fortification standard directly onto the soil prior to adding reflux solvent.

Add 200 mL of acetonitrile/water 8/2 (v/v) to the boiling flask. Place on a hotplate under a cold water condenser. Manually swirl the flask several times throughout the reflux period, or use magnetic stir bars to ensure adequate extraction. Reflux the sample for one hour, and allow to cool for fifteen minutes. Before removing the sample, rinse the condenser with ca. 5-10 mL of reflux solvent.

Pre-wet the vacuum filtering apparatus, including filter paper with reflux solvent. After refluxing, vacuum filter the sample through Whatman GF/A filter paper in a Buchner funnel. Rinse the 500 mL flat-bottom boiling flask and filter cake with ca. 2 x 25 mL of reflux solvent. Transfer the filtrate to a clean acid-rinsed 1000 mL flat-bottom boiling flask.

CONCENTRATION (ROTOVAP)

Concentrate the sample on a rotatory evaporator (water bath 45± 10°C) to ~30 mL to remove the acetonitrile. Use caution during concentration to prevent bumping.

SPE COLUMN PREPARATION

After concentration, prepare a 1 g C_{18} SPE column by placing about 0.1 g of glass wool into the center of the column. Rinse the column with ~10 mL of methanol followed by ~10 mL of 0.2% H_2SO_4. Do not allow the liquid level to go below the top of the packing at any time. Attach a ~50 mL reservoir to the top of the column.
CLEANUP

Bring the sample volume to ~100 mL using deionized water. Add 1 mL of 10% H_2SO_4 to the sample and transfer the acidified sample to the SPE reservoir. Allow the sample to pass

through the column at a rate of about 5 mL per minute. After the sample has passed through the column rinse the flat-bottom boiling flask with ~5 mL of deionized water and pass the rinse through the column. Remove the glass wool plug.

Dry the column with a strong vacuum (~20" Hg) for 5 minutes followed by a positive N_2 or air flow of ~5 psi for 60 minutes. Elute the compounds of interest with 5 mL of ethyl ether into a 15 mL centrifuge tube. The centrifuge tubes should be rinsed with Sylon-CT, followed be toluene then methanol and thoroughly dried before use.

DERIVATIZATION

Add 1 mL diazomethane/ether solution to the sample and swirl the mixture. Immediately place the sample on a nitrogen evaporator at ~45°C and concentrate the sample to <0.1 mL. Do not concentrate to dryness. In a fume hood add 0.5 mL of pyridine and 0.5 mL of acetic anhydride to the sample and swirl to mix. Heat the sample for 30 minutes at 50°C±2°C. Concentrate the sample to <0.1 mL with a N-Evap with a water bath temperature of ~ 45°C. Do not concentrate to dryness. Dilute the sample with 10 mL of hexane and concentrate to 1 mL for GC/MSD analysis.

QUANTITATION STANDARDS

The quantitation standard is prepared by taking a control sample through the extraction and cleanup steps of the procedure and then spiking the sample prior to derivatization. The C_{18} eluate is spiked with 400 uL or less of the fortification standard and the method is continued with the derivitization step described above. Following the final concentration, this standard should be serially diluted with hexane to produce standards of various concentrations for quantitation purposes.

B. Instrumentation

Analyses for F8426, F8426-Cl-PAc, F8426-CAc, F8426-PAc, F8426-BAc, and 3-OH-F8426-BAc were performed on either a Hewlett-Packard 5890 Series II gas chromatograph equipped with a HP 7673 Auto Sampler and a HP 5972 mass selective detector set in SIM (Selected Ion Monitoring) mode or a Hewlett-Packard 6890 gas chromatograph equipped with a HP 6890 series autosampler and a HP 6890 mass selective detector set in SIM. A HP-5 capillary column (5% phenyl methyl silicone) was used. Typical GC operating conditions are shown in Appendix A.

C. Method Validation and Quality Control

Experimental Design:

Method validation and recovery experiments were conducted with every set of samples. The analytical method was validated by fortifying and analyzing soil samples from control plots and monitoring the recoveries. A typical set consisted of nine samples which included treated samples, at least one untreated control sample, and a minimum of one fortified control sample. Fortification levels ranged from 5 ppb to 50 ppb for each of the

six compounds. The limit of quantitation (LOQ), or lowest fortification level, for each compound was established at 5 ppb. The limit of detection (LOD) for each compound was estimated to be 1 ppb. A summary of the method recovery is in Section III, Table 1. Individual method recovery data can be found in Section X, Table 2.

Preparation of Standards:

Technical standards of carfentrazone-ethyl and its metabolites were certified every two years for purity. Stock standard solutions were prepared from these technical standards by weighing a known amount of the technical material and diluting it into a volumetric flask with acetonitrile. The resulting stock standard concentration was 1 µg/µL. A 10 ng/µL mixed dilution was made from the stock solutions of the six compounds for the purpose of fortification and preparation of quantitation standards.

Quantitation standards were usually prepared with each set of samples by spiking a control sample just prior to derivatization and carrying it through the rest of the procedure. Subsequent dilutions were made with hexane for GC analysis.

A complete inventory of the technical standards used during this study and pertinent information can be found in Section X, Table 3. An inventory of the standard solutions utilized can be found in Section X, Table 4.

Fortification Procedure:

Untreated soil samples were fortified prior to the addition of reflux solvent with the appropriate standard. All fortifications were made using a microliter syringe. Typical spiking volume was less than 1000 µL.

D. Method of Calculation

Quantitation of residues was based on a best-fit line equation generated from external standard injections at not less than four different levels.

Using a computer spreadsheet, linear regression is determined in the form of the standard equation for a straight line: $y = mx + b$ where y is the response in area counts, x is the amount of analyte (pg), m is the slope, and b is the y-intercept.

Rearranging the equation gives:

$$\frac{y - b}{m} = x$$ where x is the amount of analyte (pg).

The pesticide residue in the soil sample can be calculated using:

amount of sample injected = volume injected (µL) x sample concentration (mg/µL)

$$\text{Sample Residue Amount (ppb)} = \frac{\text{amount of analyte (pg)}}{\text{amount of sample injected (mg)}}$$

The following example was derived from the recovery sample 02-B-20C, fortified at 25 ppb and analyzed on 11/18/95. Compound: 3-OH-F8426-BAc. See Figure 8 for the corresponding chromatogram.

SAMPLE ID	WT/VOL	AMT INJ	AREA COUNTS
standard	800 pg/µL	1600 pg	1679539
standard	400 pg/µL	800 pg	851088
standard	200 pg/µL	400 pg	410408
standard	100 pg/µL	200 pg	199268
standard	50 pg/ µL	100 pg	85966
fortified: 02-B-20C + 25 ppb	80g/ 10 mL	16 mg	355380
control: 02-B-20C	80g/ 10 mL	16 mg	0

From the above data, a best-fit linear regression line was generated, where the independent variable (x) was the amount of standard injected, and the dependent variable (y) was the area count. The equation to the line was: $y = 1062x - 13271$

$$\frac{y + 13271}{1062} = x$$

Inserting 355380 for y, and solving for x gives

$$\frac{355380 + 13271}{1062} = 347 \text{ pg}$$

Amount of sample injected = $(2\mu L)(8 \text{ mg}/\mu L) = 16$ mg

Therefore, sample residue = $\frac{347 \text{ pg}}{16 \text{ mg}} = 21.69$ ppb

To determine the percent recovery for a fortified sample, residues greater than the LOD found in the control sample were subtracted from the fortified sample. This value was then divided by the nominal fortification level.

$$\% \text{ Recovery} = \frac{21.69 - 0.00}{25.0} \times 100\% = 87\%$$

E. Interferences

Depending upon chromatographic conditions and the ions monitored, interference may be found at the appropriate retention time for individual compounds. These interferences may

be easily negated by switching the ion monitored. For example, when utilizing the HP 6890 chromatographic system described in Appendix A, the parent compound F8426 has significant detector responses at 290 amu, 312 amu, and 330 amu. In several instances there was a background response for 312 amu but none for 290 amu. Therefore, quantitation was performed with the peak produced at 290 amu.

F. Confirmatory Techniques

Prior to the initiation of the study, mass spectrums were obtained for the six analytes. All analyses were performed using a gas chromatograph equipped with a mass selective detector in selected ion mode. For the study, analytes were quantitated using an ion selected from the mass spectrums.

G. Time Required for Analysis

The time required for an experienced analyst to work up a set of nine samples was about 10 hours. It took about five additional hours for the samples to be analyzed by gas chromatograph. It took about five hours in advance to prepare diazomethane/ether. A practical place for stopping the sample work up is after the C_{18} SPE cleanup step.

H. Modifications or Potential Problems

Laboratory-incurred contamination and low F8426 recoveries due to the compound's adherence to glassware can occur if glassware is not properly pre-rinsed. All glassware should be pre-rinsed with acid, followed by acetone before use. Similarly, the centrifuge tubes used for derivatization should be treated with Sylon-CT, and rinsed with toluene and then methanol and thoroughly dried before use.

Reduction in the linearity of the analytical response of the standards should be viewed as an indication that instrument maintenance must be performed.

Failure to remove the organic solvent from the samples prior to SPE cleanup can result in poor recoveries.

VI. STORAGE STABILITY

Technical standards of Carfentrazone-ethyl and its metabolites are certified every two years for purity. Stock standard solutions were prepared from these technical standards annually. Qualitative comparisons of freshly made standard solutions and older standard solutions showed no signs of degradation of carfentrazone-ethyl or its five metabolites.

Table 3. REFERENCE SUBSTANCES.

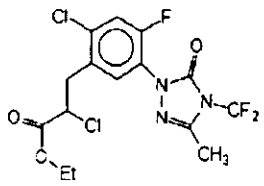
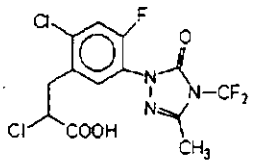
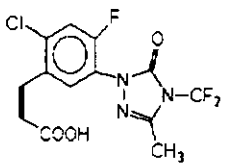
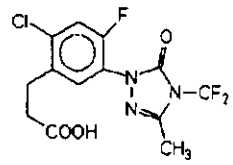
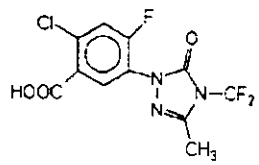
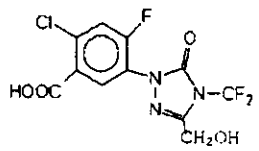
Common Name	Chemical Name/Structure	Code Number	Richmond Inv. No.	% Purity
Carfentrazone-ethyl	Ethyl α, 2-dichloro-5-[4-(difluormethyl)-4,5-dihydro-3-methyl-5-oxo-1H-1,2,4-triazol-1-yl]-4-fluorobenzene-propanoate 	FMC 116426	281 A-296 A-314	97.6 97.6 97.6
F8426-chloroproprionic acid	α, 2-Dichloro-5-[4-(difluoromethyl)-4,5-dihydro-3-methyl-5-oxo-1H-1,2,4-triazol-1-yl]-4-fluorobenzenepropanoic acid 	FMC 124161	282	99
F8426-cinnamic acid	2-Chloro-5-[4-(difluoromethyl)-4,5-dihydro-3-methyl-5-oxo-1H-1,2,4-triazol-1-yl]-4-fluorobenzenepropenoic acid 	FMC 125151	269 A-311	96.9 96.9

Table 3. REFERENCE SUBSTANCES (continued)

Common Name	Chemical Name/Structure	Code Number	Inv. No.	% Purity
F8426-proprionic acid	2-Chloro-5-[4-9difluoromethyl0-4,5-dihydro-3-methyl-5-oxo-1H-1,2,4-triazol-1-yl]-4-fluorobenzenepropanoic acid 	FMC 125165	263 A-268 A-312	98.5 98.8 98.8
F8426-benzoic acid	2-Chloro-5-[4-difluoromethyl)]-4,5-dihydro-3-methyl-5-oxo-1H-1,2,4-triazol-1-yl]-4-fluorobenzoic acid 	FMC 97083	280 A-290	97 99
3-hydroxymethyl-F8426-benzoic acid	2-Chloro-5-[4-difluoromethyl)]-4,5-dihydro-3-hydroxymethyl-5-oxo-1H-1,2,4-triazol-1-yl]-4-fluorobenzoic acid 	FMC 125171	274 A-298	91 92

XII. APPENDICES

A. List of Instrument Parameters

For F8426 and its Metabolites Analysis:

GC System: Hewlett-Packard 5890 Series II
Hewlett-Packard 5972 Mass Selective Detector
Hewlett-Packard 7673A Automatic Liquid Sampler

Column: Hewlett-Packard HP-5, 5% Phenyl Methyl Crosslinked Silicone; ~22 meter x 0.32 mm id x 0.52 um film thickness

Carrier Gas: Helium

Carrier Flow: ~1.8 mL/min

Temperatures:

Injector: 250°C
Oven: 150°C hold 1 minute,
then increased at 25°C/min. to 250°C,
then increased at 5°C/min to 260°C, holding for 3 min,
then increased at 30°C/min to 295°C, holding for 4 min.

Ions Monitored:

compound	retention time	ion monitored
F8426	5.3	330
F8426-Cl-PAc	5.1	326
F8426-CAc	5.2	361
F8426-PAc	4.8	303
F8426-BAc	4.2	335
3-OH-F8426-BAc	5.3	351

These are typical parameters and can be adjusted to improve chromotographic conditions.

A. List of Instrument Parameters (continued)

For F8426 and its Metabolites Analysis:

GC System: Hewlett-Packard 6890 Series
Hewlett-Packard 6890 Mass Selective Detector
Hewlett-Packard 7673A Automatic Liquid Sampler

Column: Hewlett-Packard HP-5 MS, 5% Phenyl Methyl Crosslinked Silicone; ~30 meter x 0.25 id x 0.25 um film thickness

Carrier Gas: Helium

Carrier Flow: ~1.8 mL/min

Temperatures:

Injector: 200°C
Oven: 60 °C hold 1.5 minute,
then increased at 25 °C/min to 300°C,
then increased at 70°C/min to 320°C, holding for 5 min

Ions Monitored:

compound	retention time	ion monitored
F8426	10.8	312 or 290
F8426-Cl-PAc	10.6	290
F8426-CAc	10.7	326
F8426-PAc	10.2	303
F8426-BAc	9.6	335
3-OH-F8426-BAc	10.8	351

These are typical parameters and can be adjusted to improve chromotographic conditions.