B96-41

432564-04

Study Title

ANALYTICAL METHOD FOR THE DETERMINATION OF BENSULFURON METHYL RESIDUES IN CRAYFISH BY HPLC USING A COLUMN AND ELUENT SWITCHING SYSTEM

Data Requirement

U.S. EPA Pesticide Assessment Guidelines
Subdivision O, 171-4 (c) & (d)

Authors

Min Zhou
Timothy J. Devine

Date Study Completed

May 18, 1994

Performing Laboratory

E. I. du Pont de Nemours and Company
DuPont Agricultural Products
Global Technology Division
Experimental Station
Wilmington, Delaware 19880-0402

Laboratory Project ID

AMR 2981-94

Statement of No Data Confidentiality Claims

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

Company: E. I. du Pont de Nemours and Company, Inc

Company Agent: John H. Cain, Jr. (Typed Name) Date: 5/23/94

Product RegistrationManager (Title) (Signature)

GOOD LABORATORY PRACTICE STATEMENT

Good Laboratory Practice (GLP) requirements as specified in 40 CFR Part 160 are not applicable to the method development.

Sponsor:

E. I. du Pont de Nemours and Company

Submitter:

E. I. du Pont de Nemours and Company

Study Director

Min Zhou
Group Chemist

18-May-94
Date

Company Representative

DuPont Registration Representative

5/23/94
Date

TABLE OF CONTENTS

Analytical Method for the Determination of Bensulfuron Methyl Residues in Crayfish by HPLC Using a Column and Eluent Switching System

Min Zhou and Timothy J. Devine

Summary

A method is described for the extraction and quantitation of bensulfuron methyl residues in crayfish. Samples are extracted with methylene chloride, centrifuged, and the methylene chloride removed by evaporation under a stream of air. Sample residues are reconstituted in methylene chloride. An aliquot of the extract is then cleaned up and concentrated by solid-phase extraction using a disposable Silica cartridge. The analysis is done by HPLC with ultraviolet detection at 254 nm, using a column-and eluent-switching technique.

A simple extraction and cleanup is sufficient for the HPLC analysis, therefore considerable time is saved in the sample preparation, compared to reported methods (References 2 and 3).

The limit of quantitation for this method was determined to be 0.025 ppm, and the limit of detection was determined to be 0.005 ppm for crayfish.

I. INTRODUCTION

Bensulfuron methyl (structure shown in Figure 1) is the active ingredient in Londax® Herbicide registered for use on rice to control weeds (Reference 1). This method is intended to establish a method for the determination of residues of bensulfuron methyl in crayfish, rice grain, and its processed fractions (in some states, crayfish are also cultivated in rice paddies for human consumption). In the earlier reported methods (for rice grain and rice straw only), samples are taken through a series of conventional extraction processes, cleaned up by solid-phase extraction, and bensulfuron methyl is then quantitated by normal-phase HPLC with photoconductivity detection (Reference 2 and 3). These methods require two different approaches in sample preparation, requiring more than one day for rice grain sample preparation, and at least two to three days for rice straw sample preparation. The limits of detection in these methods are 0.02 ppm for rice grain, and 0.05 ppm for rice straw.

The method described in this report uses an extraction and cleanup procedure for crayfish (with one exception: using centrifugation, instead of filtration), similar to the procedures for rice grain, rice straw, or its processed fraction(s) to recover bensulfuron methyl from the matrix (Reference 4). Ultraviolet detection at 254 nm, with an HPLC column and eluent-switching system is employed. Considerable time is saved in sample preparation. In comparison to previous methods, sample preparation (six samples/set) time for crayfish, rice grain, rice straw, or rice processed fractions is approximately five hours.

With the combination of the multidimensional approach used in the method (normal-phase solid-phase extraction for cleanup and reversed-phase HPLC for further cleanup and quantitation), this method also provides the necessary selectivity and sensitivity for residue analysis in the samples with a quantitation limit of 0.025 ppm for crayfish.

The samples are extracted by maceration in methylene chloride. After centrifugation, the methylene chloride is evaporated under a stream of air. An aliquot of extract is cleaned up by passing it through a disposable Silica cartridge, trapping the bensulfuron methyl while other potential interferents are eluted out by washing the cartridge with solutions of [9%/91% (v/v) iso-propanol/hexane and followed

by 10%/5%/85% (v/v/v) iso-propanol/methanol /hexane]. The bensulfuron methyl is then selectively eluted from the Silica cartridge with 10%/14%/76% (v/v/v) iso-propanol/methanol/hexane, leaving behind more strongly retained potential interferents.

Quantitation was done by high-performance liquid chromatography on a column and eluent-switching system. For HPLC analysis, the following procedure was used. A 50-µL sample was injected on a Zorbax® SB-Phenyl column. At a selected time window relative to the average retention time of DPX-F5384 on the SB-Phenyl column, a switching valve was positioned to allow DPX-F5384 to be loaded and concentrated onto the second column, a Zorbax® RX-C8 analytical column. After the transfer of DPX-F5384 to the second column, further separation was performed. Detection was accomplished by an ultra-violet (UV) detector with an option of diode-array detection (DAD) for confirmation. The first column was then cleaned out with a mobile phase containing a higher percentage of acetonitrile, and reequilibrated to the mobile phase composition of the first column for the next analysis.

II. MATERIALS

A. Equipment

Equivalent equipment or apparatus may be substituted.

Balances

Mettler Model PM400 and Model AE100 (Mettler Instrument Corporation, Hightstown, N.J.).

Centrifuge Bottles

250-mL polypropylene, IEC Maxiforce® #2050, or 250-mL polypropylene, Nalge™ #21020-028 (VWR Scientific, Bridgeport, N.J.).

Glass Fiber

Pyrex® Brand Fiber (Corning 3950), # 32848-003 (VWR Scientific, Bridgeport, N.J.)

Centrifuge

DuPont Sorvall® Model RC-5C refrigerated centrifuge (DuPont Instruments, Wilmington, Del.).

Filters

0.45-µm, 47-mm filter, Rainin® 38-114 (Rainin Instrument, Inc., Woburn, Mass.).

5.0-µm, 47-mm SV filter, Millipore® SVLP 047 00 (Millipore, Inc., Bedford, Mass.).

Solid-Phase Extraction Apparatus

Visiprep SPE Manifold, Catalog No. 5-7030M (Supelco, Inc., Bellefonte, Pa.).

Silica SPE Cartridges

Silica Bond-Elut, 2000 mg/12 mL, Catalog No. 1225-6018 (Varian, Sugarland, Tex.).

Evaporator

TurboVap® Model 500 with 1-mL concentrator tubes, Catalog No. ZA 7527, (Zymark Corporation, Hopkinton, Mass.).

Nitrogen Evaporator

N-EVAP® Model 111 (Organomation Assoc., Berlin, Mass.).

Vortex Mixer

Vortex Model G-560 (Scientific Industries, Inc. Bohemia, N.Y.)

HPLC System

HP1090 M Liquid Chromatograph with UV DAD Detector, and equipped with Autoinjector (Hewlett-Packard, Avondale, Pa.).

Electronic Switching System

Two electronic switching valves are used to conduct column and eluent switching (Hewlett-Packard).

LC Columns

Column 1: Zorbax® SB-Phenyl 4.6 mm ID x 150 mm, 5 µm (Mac-Mod Analytical, Chadds Ford, Pa.)

Column 2: Zorbax® RX-C8 4.6 mm ID x 250 mm, 5 µm (Mac-Mod Analytical)

An equivalent column can be substituted (i.e., Phenyl can be substituted for SB-Phenyl, or RX-C18 can be substituted for RX-C8).

Macerator

Tissumizer® EDT 1810 motor with SDT-182EN probe (Tekmar Co., Cincinnati, Ohio).

Ultrasonic Bath

Branson Model 2200 ultrasonic bath (VWR Scientific Co., Bridgeport, N.J.).

B. Reagents and Standards

Equivalent reagents may be substituted.

Water

Deionized water passed through a Milli-Q® Water Purification System (Millipore Corp.).

Acetonitrile

EM OmniSolv® #AX0142-1 (EM Science).

Methanol

EM OmniSolv® #MX0488-1 (EM Science).

Iso-propanol

EM OmniSolv® #PX1834-1 (EM Science).

Hexane

EM OmniSolv® #HX0296-1 (EM Science).

Methylene Chloride

EM OmniSolv® #DX0831-1 (EM Science).

Potassium Monohydrogen Phosphate

ACS grade (Fisher Scientific).

Potassium Phosphate Monobasic

ACS grade (Fisher Scientific).

Phosphoric Acid

ACS grade (J. T. Baker)

Bensulfuron Methyl

Reference Standard: bensulfuron methyl (DPX-F5384-83) 99.5% pure (DuPont Agricultural Products, E. I. du Pont de Nemours and Company, Wilmington, Del.).

C. *Preparation of Solutions*

Extraction Solution

methylene chloride.

SPE Washing Solutions:

Solution A

iso-propanol/hexane (9/91 v/v). Mix 9-mL iso-propanol with 91-mL hexane (in a graduate cylinder).

Solution B

iso-propanol/methanol/hexane (10/5/85 v/v/v). Mix 10-mL iso-propanol and 5-mL methanol with 85-mL hexane (in a graduate cylinder).

SPE Eluting Solution

iso-propanol/methanol/hexane (10/14/76 v/v/v). Mix 10-mL iso-propanol and 14-mL methanol with 76-mL hexane (in a graduate cylinder).

HPLC Eluents:

Stock Solution A

1M potassium monohydrogen phosphate. Weigh 17.4 g potassium monohydrogen phosphate in a beaker, dissolve into 100 mL of deionized water, and filter it through 0.45-µm filter disk.

Stock Solution B

1M potassium phosphate monobasic. Weigh 13.6 g potassium phosphate monobasic in a beaker, dissolve into 100 mL of deionized water, and filter it through 0.45-µm filter disk.

Eluent A

Four mM potassium monohydrogen phosphate in water at pH 7.6. Mix 4-mL 1M potassium monohydrogen phosphate (stock solution A) into a 1.0 L graduate cylinder, and bring up to 1.0 L with deionized water. Adjust pH to 7.6 with diluted phosphoric acid (10% conc. phosphoric acid), and filter it through 0.45-µm filter disk.

Eluent B

Four mM potassium phosphate monobasic in water at pH 3.2. Transfer 4-mL 1M potassium phosphate monobasic (stock solution B) into a 1.0 L graduate cylinder, and bring up to 1.0 L with deionized water. Adjust pH to 3.2 with phosphoric acid, and filter it through 0.45-µm filter disk.

Eluent C

Acetonitrile.

Standards:

Stock Standard Solution

To prepare 500 µg/mL of bensulfuron methyl standard, approximately 12.5 mg of bensulfuron methyl standard is accurately weighed and dissolved into 25-mL volume with acetonitrile. Intermediate dilutions from the stock standard to 10.0 and 1.0 µg/mL in acetonitrile are made and used for fortification of samples and preparation of chromatographic standards. The stock standard solutions are kept in the freezer for storage.

Chromatographic Standard Solutions

Prepare chromatographic standards ranging from 0.050 to 0.40 µg/mL in 1:1 water: acetonitrile from the 1.0-µg/mL standard solution above (i.e., pipet 50, 100, 200 and 400 µL of 1.0 µg/mL standard into each individual vials (autosampler injections vials), and bring them up to 1.0 mL with 1:1 acetonitrile/water, respectively). Keep all chromatographic standards at or below 4°C following preparation. These standards are stable for about a week.

III. METHODS

A. Homogenization of Samples

Crayfish samples (whole crayfish) are chopped and homogenized with dry ice in a Hobart Chopper.

B. Preparation of Samples

1. Weigh out 2 g of crayfish sample, into a 250-mL polypropylene centrifuge bottle. Record weight to nearest 0.1 g. If sample is to be fortified, add appropriate volume of fortifying solution to the sample, and allow the acetonitrile to evaporate before proceeding.
2. Add 100 mL of extracting solution and soak for a few minutes.
3. Homogenize with the Tissumizer® for approximately 2 min.
4. Let it stand for a few minutes.

5. Centrifuge extract at 7000 rpm for 20 minutes.
6. Pass supernatant through glasswool bed and collect it into the Turbovap® concentrator tube.
7. Rinse the glasswool bed with an additional 10 mL of methylene chloride, collecting in Turbovap® concentrator tube.
8. Evaporate the extract to a volume of approximately 1 mL using the Turbovap® system with the water bath set at 40°C and a fan speed of 6000 rpm.
9. Transfer sample into a 13 or 15-mL graduated centrifuge tube. Rinse the Turbovap® concentrator tube with about 2 to 3 mL of methylene chloride twice, and collect into the graduated centrifuge tube.
10. Evaporate down to 5-mL with nitrogen evaporator.
11. Pass through 0.45-µm Acrodisc® filter into graduated centrifuge tube.
12. Transfer 2.5-mL an aliquot of sample extract to a 13-mL or 15-mL graduated centrifuge tube.
13. Place onto the nitrogen evaporator with the water bath set at 40°C, and apply a gentle stream of nitrogen for about 15 min, to evaporate down to dryness.
14. Reconstitute residue into ca. 1-mL iso-propanol and sonicate for 5 minutes.
15. Add 9-mL hexane into the 1-mL extract (Step 14) and mix well.
16. Connect Silica Bond-Elut® cartridge to the SPE vacuum manifold.
17. Pass approximately 5 mL of hexane, followed by 6 mL of 9/91 v/v iso-propanol/hexane (washing solution A), through the cartridges at a flow rate of about 1-3 mL/min. Do not allow the cartridges to go dry at any time during these steps.
18. Pass the sample through the cartridges at a flow rate of about 1-3 mL/min. Rinse the centrifuge tubes with 10 mL of washing solution A, and pass the rinsate through the cartridges. Allow cartridge to go dry only after all the rinsate has passed through.
19. Wash the Silica cartridge with an additional 10 mL of washing solution A, then 8 mL of washing solution B.

Allow the cartridge to go dry after each wash. The wash solvents may be discarded.

20. Collect the elute in a 40-mL to 50-mL graduated centrifuge tube from the cartridge by eluting with 25 mL of eluting solution (10/14/76 v/v/v iso-propanol/methanol/hexane).

21. Evaporate to dryness under a stream of nitrogen with the water bath set at approximately 40°C. Reconstitute with 0.5 mL of 1:1 water/acetonitrile for crayfish sample (or up to 10 mL of 1:1 water/acetonitrile, depending on the level of residues anticipated), and ultrasonicate and mix on a Vortex mixer to dissolve the residue.

22. Filter sample through 0.45-µm Acrodisc®. Sample is now ready for LC analysis.

Note that calibration is necessary for any new batch of Silica Bond-Elut® columns, since washing and eluting profiles may be different resulting from various lots of Bond-Elut® columns (i.e., one may add more methanol in the eluting solution, if one finds that 14% methanol is not strong enough to elute bensulfuron methyl, or vise versa.)

C. Chromatographic Analysis

Principle of the Liquid Chromatography Using Eluent and Column-Switching Technique

Eluent switching in combination with column switching provides the basis for the determination of DPX-F5384 in crayfish. The HPLC system is described in the "Equipment" section and suggested operating conditions are listed below:

Columns:

Column 1:	SB-Phenyl 4.6 mm x 150 mm, 5 µm.
Column 2:	RX-C8 4.6 mm x 250 mm, 5 µm.

Eluents:

Eluent A:	4 mM potassium monhydrogen phosphate in water at pH 7.6 (hipH).

Eluent B: 4 mM potassium phosphate monobasic in water at pH 3.2 (lopH).

Eluent C: Acetonitrile (ACN)

Oven Temperature:

Ambient to 40°C (column compartment).

Injection Volume:

50 µL

UV Detector:

254 nm

Four different switching valve positions are employed in chromatographic run and are fully illustrated in Figure 2.

These configurations are explained below:

Position A - Flow is from the pump, through Column 1, and to the detector.

Position B - This configuration places Columns 1 and 2 in series. Flow is from the pump, through Column 1, through Column 2, and to the detector.

Position C - This configuration takes Column 1 off line. Flow is from the pump, through Column 2, and to the detector.

Position D - This configuration takes both Column 1 and 2 off line, resulting in No Columns at the end of sequence run to flush the system overnight. Flow is from the pump to the detector.

Chromatography begins by injecting the prepared samples onto the phenyl column with an acidic mobile phase containing 35% acetonitrile at pH 3.2, and switching valves set to position A. At this pH, DPX-F5384 (in its acid [neutral] form) is uncharged and relatively nonpolar. It migrates through the phenyl column slowly relative to most of the matrix compounds, which elute soon after injection under these conditions. As the DPX-F5384 peak elutes from Column 1, it is transferred to Column 2 (RX C8 column, valve

Position B)within a 2- to 3-minute time window which corresponds to approximately 0.5 to 1 minute before and after the DPX-F5384 elution time off Column 1. Under this condition (lower pH), DPX-F5384 will concentrate onto the head of the second column to minimize peak broadening due to a longer retention time. After a few minutes, the DPX-F5384 peak is eluted from Column 2 (Valve Position C) relatively free of interferences, using a gradient from 15 to 25% acetonitrile at pH 7.6. At this high pH, DPX-F5384 is negatively charged and consequently more polar and will elute rapidly from the second column, and is detected by UV absorbance at 254 nm. After elution of the DPX-F5384, 60% acetonitrile at pH 7.6 is used to clean the Column 1 (Valve Position A), and reequilibrated with 35% acetonitrile at pH 3.2 in preparation for the next injection.

Flow rate was held constant at 1.0 mL per minute throughout the entire chromatographic run. The following table is an example of the timed events of a column- and eluent-switching program in this analysis:

Eluent and Column Programming:

Time (min)	Eluent A % hipH	Eluent B % lopH	Eluent C % ACN	Valve Position	Column 1-Phenyl; 2-C8
0	0	65	35	A	1
12.5	0	65	35	B	1+2
15.5	0	65	35	B	1+2
19.99	0	65	35	A	1
20.0	85	0	15	C	2
45	75	0	25	C	2
45.01	40	0	60	A	1
53.0	40	0	60	A	1
53.01	0	65	35	A	1
60.0	0	65	35	A	1

Above programming is an example. It may be altered to meet specific needs.

Establishing Switching Times

Prior to chromatographing each analysis set, appropriate switching times must be established. The procedures are outlined below:

Establishing the DPX-F5384 Column Switching Time Window

1. Pump Eluent B (65%) and C (35%) through Column 1 for about ten minutes, to equilibrate Column 1.
2. To determine the column switching time (time window during which transfer of analyte from Column 1 to Column 2 will take place), inject the 0.4-µg/mL DPX-F5384 standard onto the phenyl column with the HPLC programmed to start at zero time (see above time table). Note the DPX-F5384 retention time.
3. Set the time window: the start of the time window will correspond to approximately 0.5 minute before the start of the peak, and the end of the time window will correspond to 0.5 minute after the end of the peak. Usually, this yields a time window of 3 to 3.5 minutes wide but may vary from day to day or column to column depending on the peak width.

Establishing the Eluent Switching Time

1. Allow the phenyl column (Column 1) to equilibrate for 10 minutes with Eluent B (65%) and C (35%). Flow rate is maintained at a constant 1.0 mL per minute during all column equilibrations.
2. Inject a 0.4-µg/mL standard of DPX-F5384. Note the retention time of the DPX-F5384. Eluent switching will correspond to the start time of second column elution (usually a few minutes after transfer of the analyte onto the second column, i.e., 20 minutes).
3. Pump Eluent A (85%) and Eluent C (15%) while eluting DPX-F5384 from the second column using a gredient from 15% acetonitrile to 25% acetonitrile within 20-25 minutes (be sure that analyte is eluted from the second column).
4. Pump Eluent A (40%) and Eluent C (60%) to clean up Column 1 for about 10 minutes.
5. Reequilibrate Column 1 with Eluent B (65%) and Eluent C (35%) for about 10 minutes.

Establishing Column Wash Start Time

1. Pump Eluent A (40%) and Eluent C (60%), after elution of DPX-F5384 from Column 2, and Switch the valve to Position A (Column 1 only).

2. Reequilibrate Column 1 for 10 minutes with its initial eluent condition (Eluent B 65% with Eluent C 35%) after washing.

Note: Column-switching times are not critical and may be altered, except for the time window of transferring the analyte from Column 1 onto Column 2. This must be consistent to obtain a comparable chromatogram throughout a set of analysis.

Eluent-switching times are not critical and may be altered, except for the time of eluting analyte from the second column. This must be started as soon as column switching valve turns to Position C for eluting the analyte.

Analysis

Inject the control, treated, and recovery samples to be analyzed. Inject a standard after every 2-3 samples.

Calculations

A Response Factor is calculated for each standard using the peak areas (or heights) from the analysis of standards. A plot of peak area versus concentration of bensulfuron methyl in the standards can be used to construct a standard curve via a least-squares fit program. This curve should be linear over the range of interest with a near-zero intercept. Therefore, the Response Factor can be obtained simply by calculating:

$$\text{Response Factor} = \frac{\text{peak height or area}}{\text{bensulfuron methyl } (\mu g/mL)}$$

The Average Response Factor used to calculate a sample concentration is the average of Response Factors for the standards analyzed the same day.

If a peak is detected for bensulfuron methyl in a treated or recovery sample, the ppm bensulfuron methyl is calculated using:

$$\text{ppm bensulfuron methyl} = \frac{\text{height or area} \times 2.0^{*} \times \text{mL final volume (mL)}}{\text{response factor} \times \text{g weighed (g)}}$$

* Aliquot Factor of 2.0 results from the taking of a 1/2 aliquot.

Recoveries can be calculated using the following expression:

$$\% \text{ recovery} = \frac{\text{ppm bensulfuron methyl found} \times 100\%}{\text{ppm bensulfuron methyl spiked}}$$

As an example, one of the crayfish samples fortified at 0.025 ppm yielded a peak area of 11.04 (area counts). The average response factor for the analysis was 226 (area units). The final volume of the sample was 0.5 mL and a 2-g sample was used. Therefore:

$$\text{ppm bensulfuron methyl} = \frac{11.04 \text{ counts} \times 2.0 \text{ aliquot factor} \times 0.5 \text{ mL}}{226 \text{ average response factor} \times 2 \text{ g}} = 0.024$$

$$\% \text{ recovery} = \frac{0.024 \text{ ppm} \times 100\%}{0.025 \text{ ppm}} = 96\%$$

Time Required for Analysis

Typically, six samples can be prepared during the course of a normal five to six-hour day. LC analysis can be run unattended overnight with a fully automated system.

IV. Results and Discussion

Figure 2 displays a schematic diagram for the column switching system used for this method, showing different configurations. A standard curve of peak area (counts) versus concentration in µg/mL for bensulfuron methyl chromatography standards is given in Figure 3. The relationship is shown to be linear over the tested range of 0.05 to 0.40 µg/mL and was approximated by a least-squares fit line with the equation:

area (counts) = 226 x µg/mL bensulfuron methyl + intercept

where the slope is the Response Factor. The correlation coefficient for this fit was 0.999. The standard curves were very consistent throughout the method development and validation. The representative chromatograms were shown

in Figure 4 through 6, reflected an excellent linearity within the range from 0.05 to 0.20 µg/mL used for quantitation.

The responses obtained for unspiked samples of crayfish were negligible compared to the responses obtained for the lowest fortifications evaluated, as shown in Figures 7-9.

Through eluent and column-switching, one chromatograph is able to accomplish both sample cleanup and analysis for the quantitation of DPX-F5384 in crayfish. A normal phase solid phase extraction was used to selectively resolve the analyte from coextractives, and give a multidimensional technique in conjunction with the reversed-phase HPLC column and eluent-switching system. As a result, samples were relatively free from interferences upon injection into the HPLC. It is therefore not a drawback in terms of column life, as reported in the other column-switching methods developed in the past. After more than 50-sample injections on the column, there was no deterioration observed. In this method, it is not required to load a large volume on the column to achieve method detection limit(s) as used in most column-switching methods. Band-broadening due to large volume injection was eliminated. Fifty µL of sample was introduced on the column, and adequate sensitivity as well as good chromatography were obtained.

HPLC separation and quantitation on the second column can be achieved using isocratic elution. However, a gradient elution is used to optimize the resolution and chromatography for quantitation purposes.

The recovery of bensulfuron methyl from freshly spiked crayfish was determined to be 90 ± 9%. The range of recoveries was 76 to 108%. Fortifications were made at 0, 0.025, 0.04, 0.05, 0.10, and 0.20 ppm, and the individual results obtained are summarized in Table I. Untreated crayfish, supplied by Morse Laboratories, Inc., were fortified and used for recovery measurements. Representative chromatograms of a standard, an unfortified crayfish sample, and a fortified crayfish sample are given in Figures 4, 7, and 8. Based on signal/noise ratio, the data would support a detection limit of 0.003 ppm and a quantitation limit of 0.009 ppm (three times the detection limit), within a confidence interval (CI) of 95%. However, since the lowest level fortified was 0.025 ppm, this method can only be certified to quantitate residues at or above that level.

Several sulfonylureas have been tested using the similar technique, and found to be very specific and relatively free of

significant interference. The acid/base character of bensulfuron methyl may be used for confirmation. Changing the pH of mobile phase A or B by 0.2 to 0.5 pH units will selectively change the retention times of the analyte's peak. The presence of bensulfuron methyl may also be confirmed by substituting an Rx-C18 or Zorbax® Phenyl analytical column for the Zorbax® Rx-C8 column and slightly increasing the acetonitrile concentration in mobile phase A to maintain the retention time of bensulfuron methyl.

This method uses reversed-phase liquid chromatography with UV detection; both are well understood and known to be stable and reliable. The eluent-collection time window is large (3.0 minutes) relative to the baseline peak width of the analyte (2.0 minutes). This allows for dead volume in the tubing of the chromatograph, as well as variability in retention time of bensulfuron methyl and non-ideal analyte peak shape on the first column (band broadening or peak tailing). This method has shown to be applicable to the determination of bensulfuron methyl in other matrices (Reference 4).

Validation of the extraction with radiolabeled bensulfuron methyl residues in whole crayfish was conducted using crayfish samples that were stored for almost 5.5 years (from AMR 965-87 study) (Reference 5). Extraction efficiency was determined to be 74%.

CONCLUSION

This method is suitable for the quantitation of bensulfuron methyl in crayfish at 0.025 ppm with the limit of detection at 0.005 ppm. The established tolerances for residues of bensulfuron methyl are 0.05 ppm for crayfish. The quantitation limit and recovery efficiencies are therefore more than adequate for the analysis of bensulfuron methyl in crayfish.

CERTIFICATION

ANALYTICAL METHOD FOR THE DETERMINATION OF BENSULFURON METHYL RESIDUES IN CRAYFISH BY HPLC USING A COLUMN AND ELUENT SWITCHING SYSTEM

We, the undersigned, declare that the work described in this report was performed under our supervision, and that this report provides an accurate record of the procedures and results.

Report by:

Min Zhou
Study Director

18-May-94
Date

Approved by:

Sidney S. Goldberg, Ph.D.
Research Supervisor

18-May-94
Date

Date Study Initiated:
January 10, 1994

Date Study Completed:
May 18, 1994

Storage Location of Records and Final Report:
E. I. du Pont de Nemours and Company
DuPont Agricultural Products
Experimental Station
Wilmington, Delaware 19880-0402
and/or
DuPont Records Management Center
200 Todds Lane
Wilmington, Delaware 19880

Sponsor:

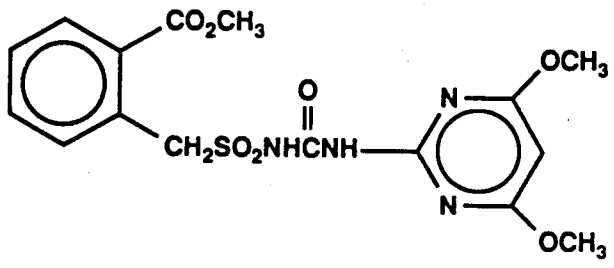
E. I. du Pont de Nemours and Company
DuPont Agricultural Products
Global Technology Division
Experimental Station
Wilmington, Delaware 19880-0402

TABLE I
METHOD VALIDATION RECOVERIES

Crayfish

Fortification Level (ppm)	Final Volume (mL)	Peak Area (Counts)	Measured ppm	% Recovery
0	0.5	0.0	0.000	-
0	0.5	0.0	0.000	-
0	1.0	0.0	0.000	-
0	1.0	0.0	0.000	-
0.025	0.5	10.51	0.044	88
0.025	0.5	11.00	0.046	92
0.025	0.5	8.57	0.038	76
0.025	0.5	11.04	0.048	96
0.04	1.0	9.09	0.034	85
0.05	1.0	8.54	0.046	92
0.100	1.0	23.35	0.098	98
0.100	1.0	22.02	0.092	92
0.100	1.0	21.28	0.086	86
0.100	1.0	19.99	0.081	81
0.200	1.0	31.76	0.215	108
			Average	90
			Std. Dev.	9

FIGURE 1
STRUCTURE AND CHEMICAL NAME FOR BENSULFURON METHYL



Chemical Name

Methyl 2-[[[[[(4,6 dimethoxypyrimidin-2-yl)amino]carbonyl]amino]sulfonyl]methyl]benzoate

CAS Registry Number

83055-99-6

Molecular Formula

$C_{16}H_{18}N_4O_7S$

FIGURE 2
SCHEMATIC OF COLUMN SWITCHING SYSTEM

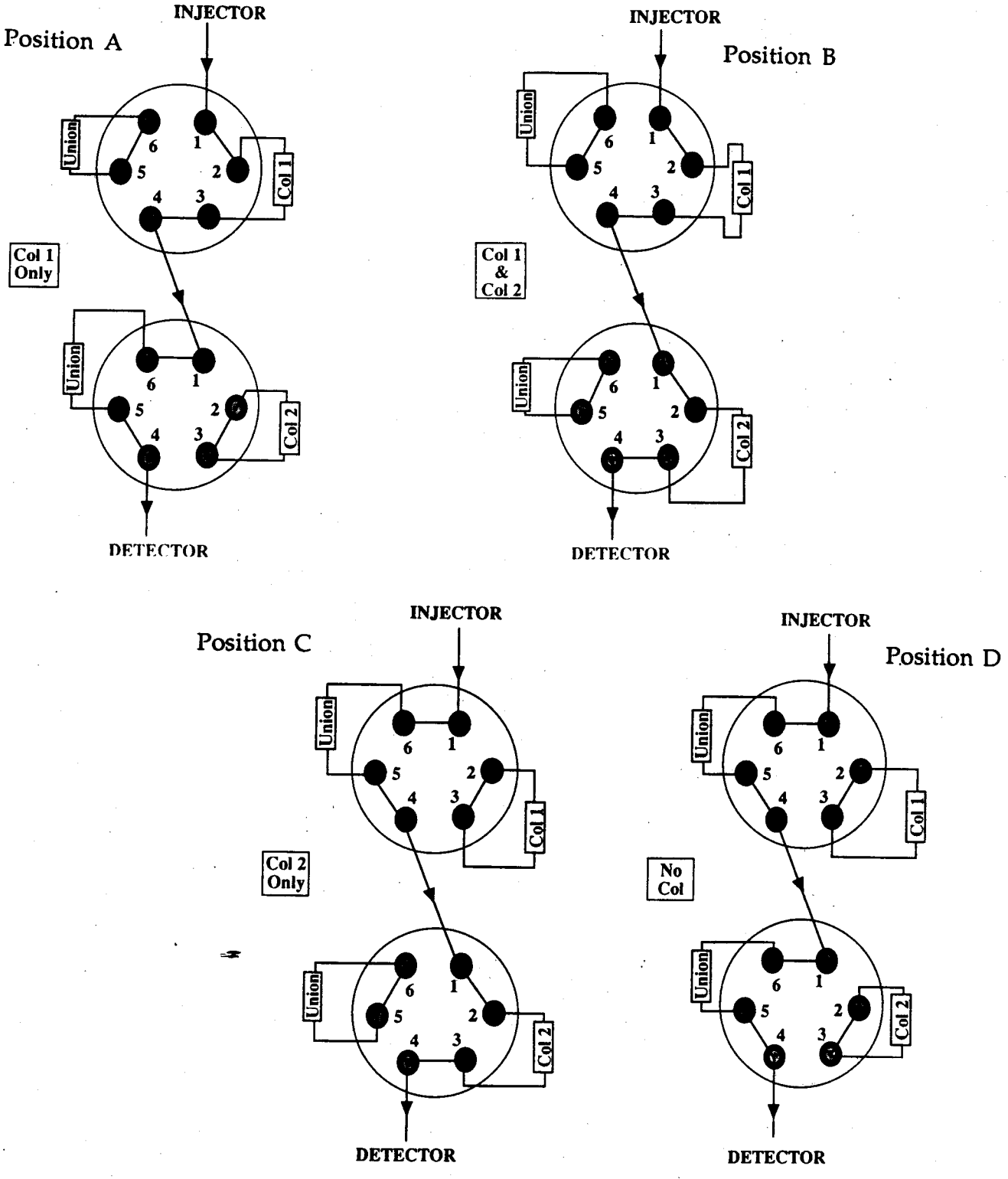
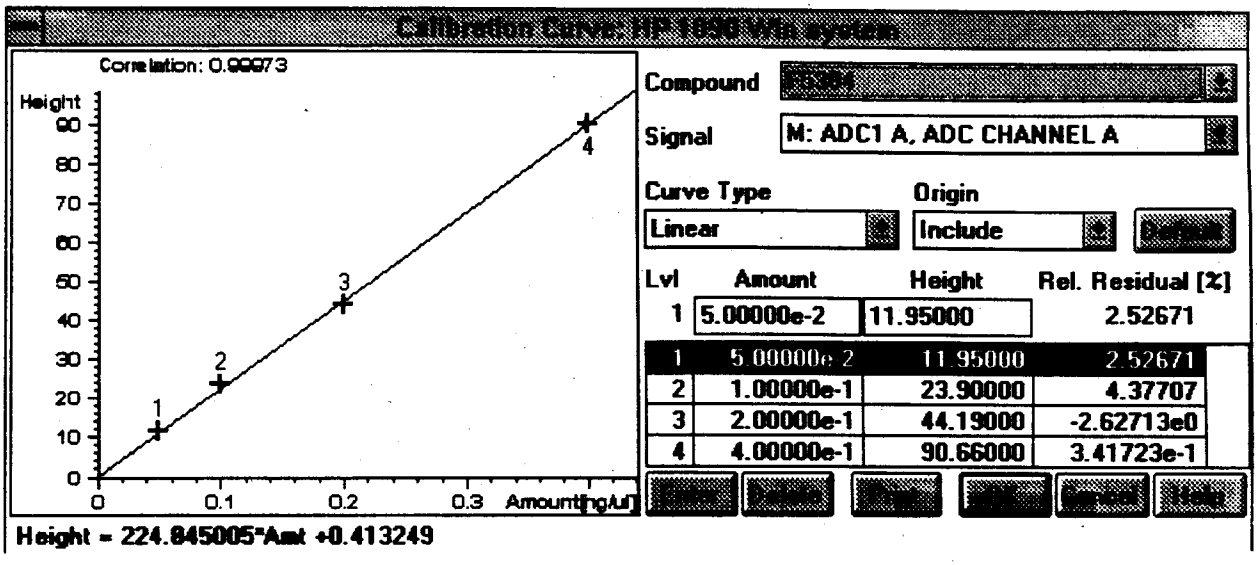


FIGURE 3
STANDARD CURVE FOR AMR 2981-94 METHOD VALIDATION




```
F5384 at exp. RT: 31.300
ADC1 A, ADC CHANNEL A
Correlation:              0.99973
Residual Std. Dev.:       0.96243
Formula: y = mx + b
      m:     224.84500
      b:       4.13249e-1
      x: Amount
      y: Height
```

FIGURE 4
BENSULFURON METHYL 0.050-µG/ML STANDARD

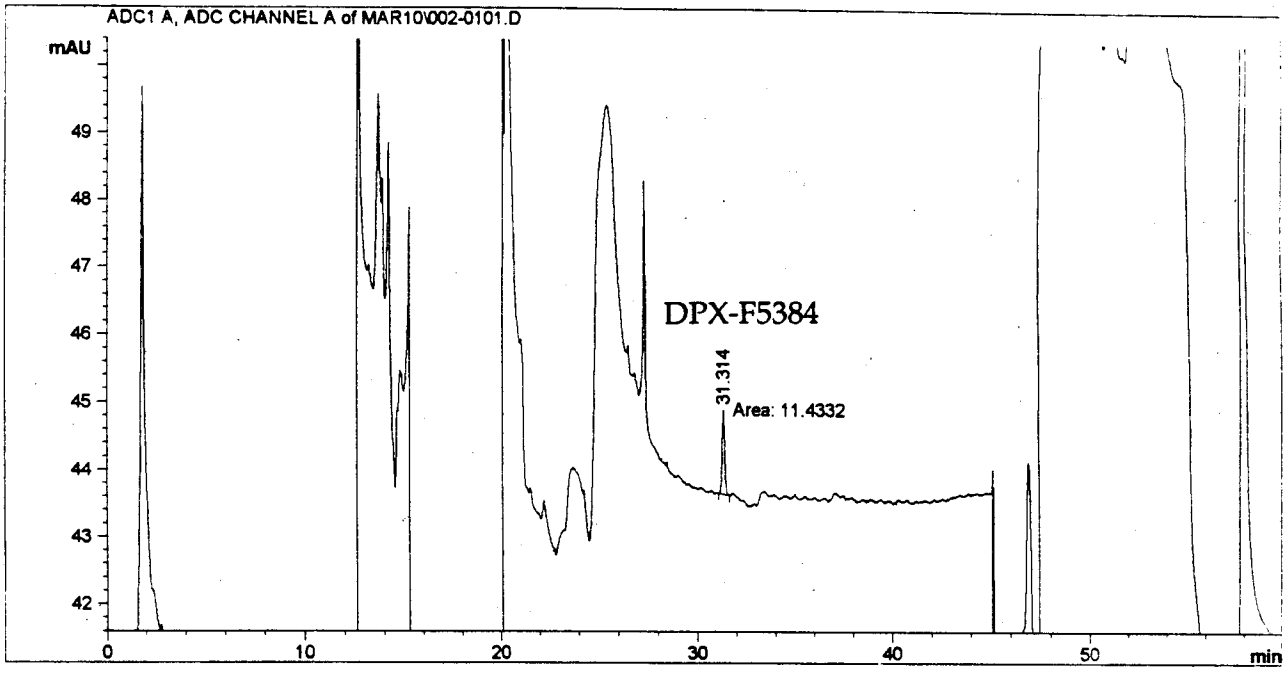


FIGURE 5
BENSULFURON METHYL 0.10-µG/ML STANDARD

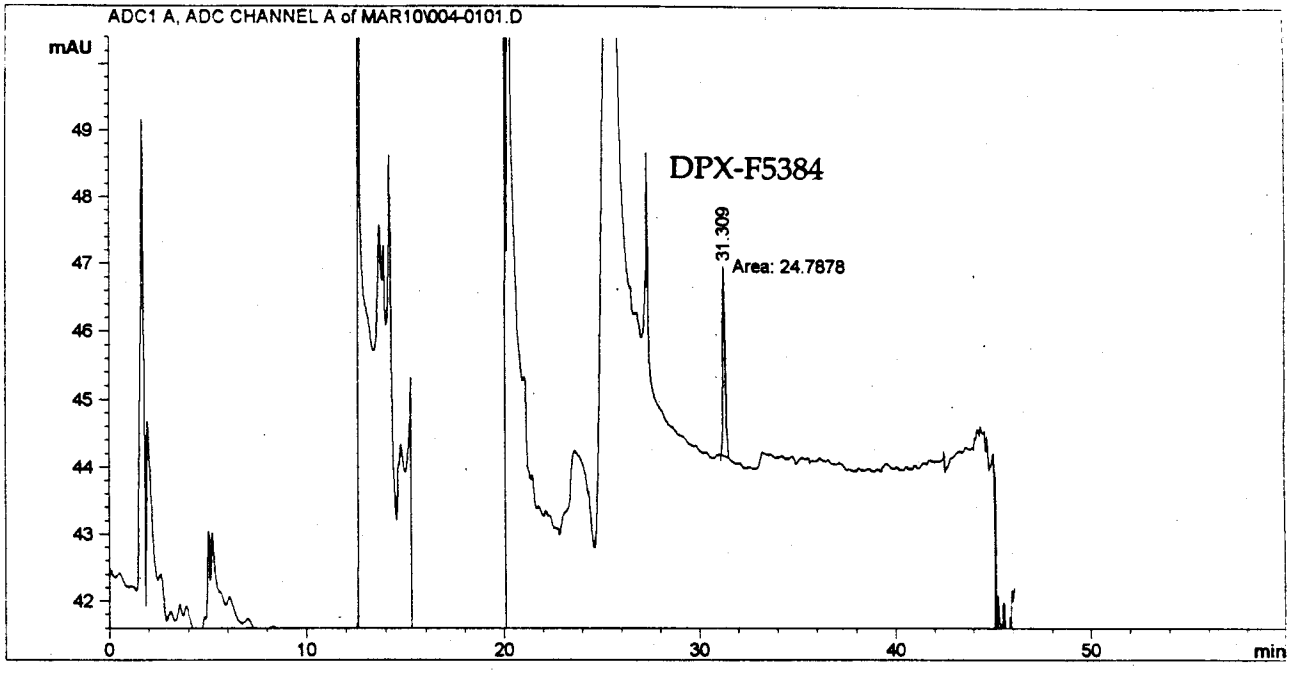


FIGURE 6
BENSULFURON METHYL 0.20-µG/ML STANDARD

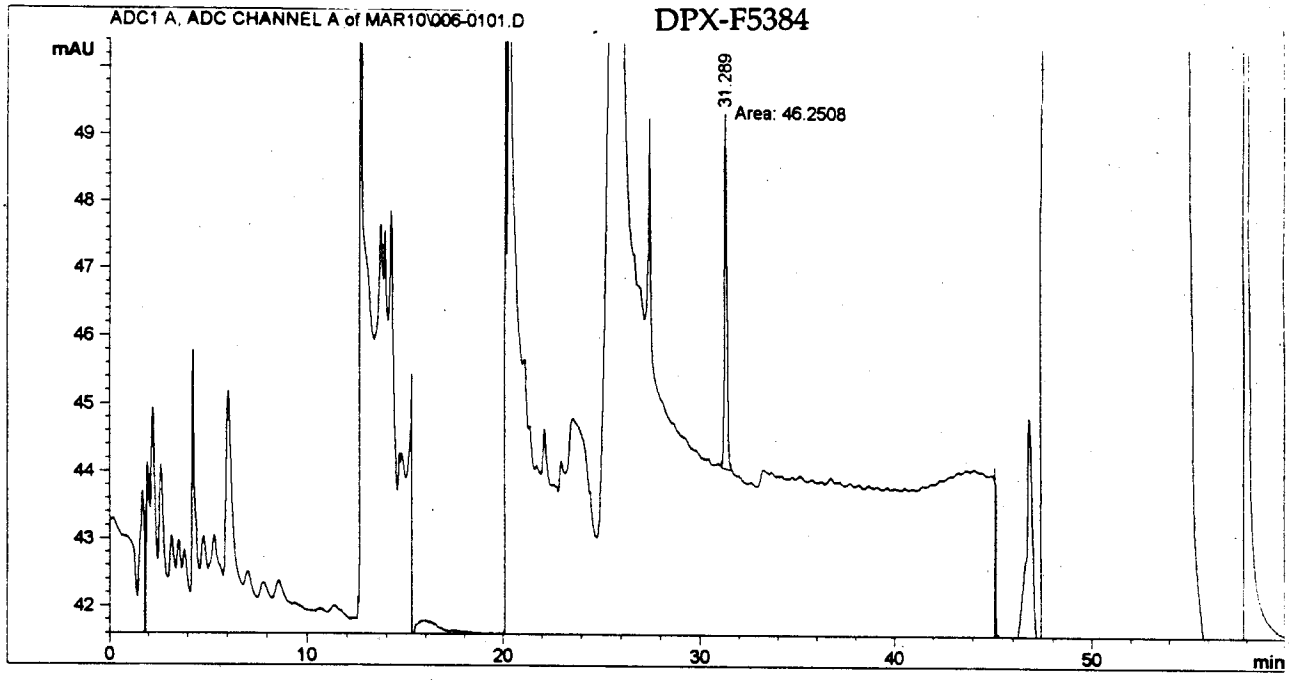


FIGURE 7
UNTREATED CRAYFISH SAMPLE - NO FORTIFICATION

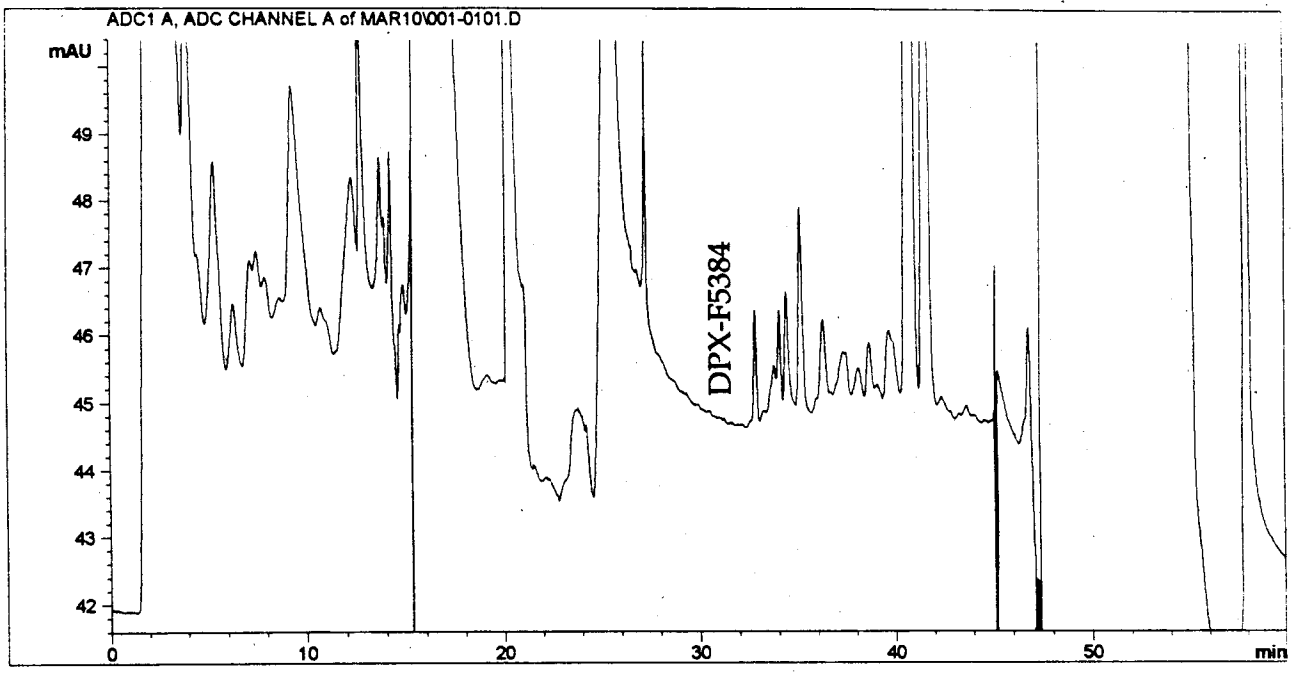


FIGURE 8
UNTREATED CRAYFISH SAMPLE - 0.025-PPM FORTIFICATION

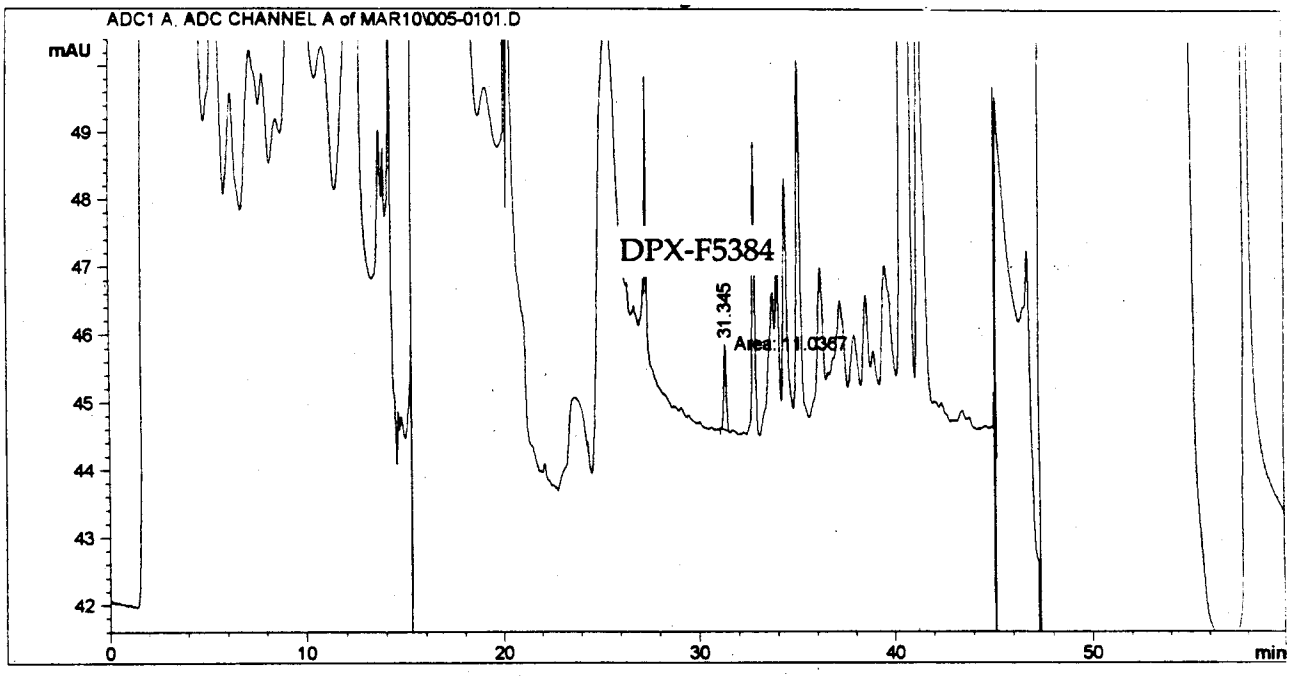
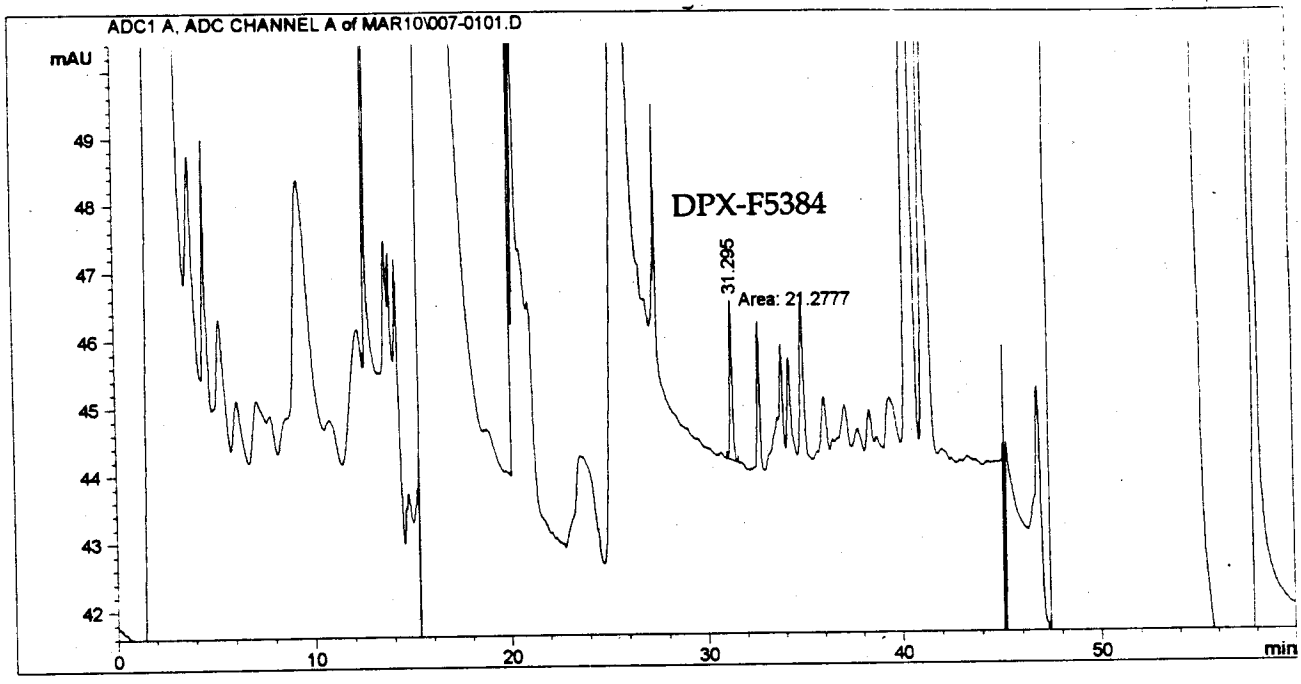


FIGURE 9
UNTREATED CRAYFISH SAMPLE - 0.10-PPM FORTIFICATION



REFERENCES

1. 1985. "DuPont Technical Bulletin for Londax® Herbicide," No. E-62943, Agricultural Chemicals Department, E. I. du Pont de Nemours and Company, Inc., Wilmington, Del.

2. Slates, R. V. 1983. "Determination of Rice Herbicide Candidate DPX-F5384 in Rice Grain," DuPont Report No. AMR 132-83, Revised October 31, 1983, Agricultural Chemicals Department, E. I. du Pont de Nemours and Company, Inc., Wilmington, Del. [MRID No. 40312001]

3. Slates, R. V. 1983. "Determination of Rice Herbicide Candidate DPX-F5384 in Rice Straw," DuPont Report No. AMR 163-83, revised October 31, 1983, Agricultural Chemicals Department, E. I. du Pont de Nemours and Company, Inc., Wilmington, Del. [MRID No. 40767014]

4. Zhou, M. 1993. "Analytical Method for the Determination of Bensulfuron Methyl Residues in Rice Grain and Rice Straw by HPLC Using a Column and Eluent Switching System," DuPont Report No. AMR 2722-93, Agricultural Products Department, E. I. du Pont de Nemours and Company, Inc., Wilmington, Del.

5. Priester, T. 1989. "Flow-through Crayfish Bioconcentration Study with ^{14}C-Bensulfuron Methyl," DuPont Report No. AMR-965-87, Agricultural Products Department, E. I. du Pont de Nemours and Company, Inc., Wilmington, Del.