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Page 1

BASF CORPORATION
AGRICULTURAL PRODUCTS GROUP
P. O. Box 13528
Research Triangle Park, NC 27709-3528

Study Title:

Method for Determination of BAS 125 W (Prohexadione) Residues in Peanut RAC (Nutmeat and Hay), and Peanut Process Fractions (Meal and Refined Oil) by GC-MS.

Study No. 95164

Method No. D9601

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Author:

Samy Abdel-Baky
Stephan Baumann

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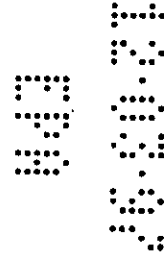
November 12, 1996

Testing/Performing Laboratory:

BASF Corporation
Agricultural Products Group
Agricultural Research Center
P. O. Box 13528
Research Triangle Park, NC 27709-3528

BASF Registration Document No. 96/5223

This report consists of 71 pages



PR 86-5 DATA CONFIDENTIALITY CLAIM

No claim 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 BASF CORPORATION / AGRICULTURAL PRODUCTS GROUP

COMPANY AGENT Edward G. Jordan, Ph.D. DATE January 22, 1997

Registration Scientist Edward H. Jordan
Title Signature

STATEMENT OF GLP COMPLIANCE

This study meets the requirements for 40 F Part 160 Good Laboratory Practices, with the exception that an analyst had not read the updated SOP on the laboratory notebook.

SUBMITTER: Edward G. Jordan January 22, 1997
Edward G. Jordan, Ph.D. Date
Registration Scientist
BASF CORPORATION, Agricultural Products

Sponsor: Greg Lewis

Study Director: Samy Abdel-Benly
Samy Abdel-Baky, Ph.D.
BASF Corporation
Agricultural Products Group
P.O. Box 13528
Research Triangle Park, NC 27709-3528

BASF CORPORATION
Agricultural Products Group
Agricultural Research Center
Post Office Box 13528
Research Triangle Park, NC 27709-3528

Method for Determination of BAS 125 W (Prohexadione) Residues in Peanut RAC (Nutmeat and Hay), and Peanut Process Fractions (Meal and Refined Oil) by GC-MS

STUDY DIRECTOR/AUTHOR: Samy Abdel-Baky (919) 547-2295
SUPERVISORY PERSONNEL: Laura Sears (919) 547-2348
ANALYSES DONE BY: Stephan Baumann, Dan Perry, David Broadwell
and Dan Wilkinson

Method No. D9601

Report Date: November 12, 1998

ABSTRACT:

Analytical Method Number D9601 was developed to determine the residues of BAS 125 W in Peanut RAC (nutmeat and hay) and Peanut Process Fractions (meal and refined oil), by GC-MS. Method development and validation were carried out at BASF Corporation, Research Triangle Park, NC, using representative control peanut. Parent BAS 125 W is extracted from the peanut matrices by using an acetonitrile/1.5 M H₂SO₄ solution (9:1 v/v). Aliquots of the extracts are purified by mini-isolute ENV +TM column, the eluant is methylated with MeOH/H₂SO₄ and refluxed for 30 minutes. The purification step is achieved by mini-isolute ENV +TM column chromatography. A gas chromatography system with a selective mass detector is used for the final determination. This study has shown that the Analytical Methods Number D9601 is suitable for measuring residues of BAS 125 W in peanut and its process fractions down to a quantitation limit of 0.05 ppm. The average recoveries for all matrices were 81 ± 12% (n=32). The Analytical Method Number D9601 showed specificity for determination of BAS 125 W in peanut and its process fractions. This method has also demonstrated to give a good accountability of incurred radioactive residues. Determination of the parent compound (BAS 125 W) by this method of a nutmeat sample was comparable to the residue levels identified in the metabolism study (Reference 1).

Pages of Report: 71

Study Initiation Date: October 24, 1995

Experimental Dates:

Start: June 28, 1998

Termination: October 14, 1998

QAU STATEMENT

Study Initiation Date: October 24, 1995

The quality assurance unit of the testing facility at the APC has audited the protocol, the analytical portion including the raw data, and the report for this study and reported its findings to the study director and to management.

<u>Date of Audit</u>	<u>Report to Study Director and to Management</u>
10-24-95	10-24-95
12-13-95	12-13-95
12-18-95	12-18-95
7-3-96	7-3-96
7-10-96	7-10-96
9-10-96	9-10-96
9-11-96	9-11-96
9-25-96	9-25-96
10-30-96	10-30-96

Signature of QAU



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1. Introduction and Summary

1.1 Scope and Source of the Method

1.1.1 Scope

BAS 125 W (prohexadione) is a plant growth regulator active in a variety of crops. This report describes the analytical method developed by BASF to determine residues of BAS 125 W in peanut. The parent compound has been determined to be the major residue of concern from a peanut metabolism study.

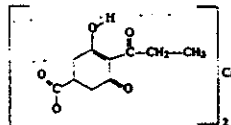
1.1.2 Source

This method was developed at the BASF Agricultural Products Center in Research Triangle Park, North Carolina.

1.2 Test Substance

1.2.1 Active Ingredient

Common Name: Prohexadione calcium
BAS Number: BW 9054-CA or BX-112
Chemical Name: Calcium 3-oxido-4-propionyl-5-oxo-3-cyclohexene carboxylate
CAS Name: 127277-53-6
Empirical Formula: $C_{10}H_{10}O_5Ca$
Molecular Weight: 250.268 g/mole
Melting point: Greater than 300°C



Solubility in organic solvent at 20°C

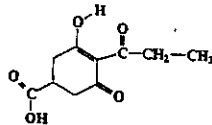
Methanol	1.1 mg/L
Hexane	Less than 0.003 mg/L
Acetone	0.038 mg/L
Toluene	0.004 mg/L

Solubility in water at 20°C

pH = 5	1602 mg/L
pH = 7	785.7 mg/L
pH = 9	665.4 mg/L

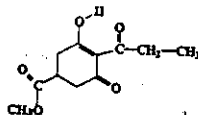
1.2.2 Fortification Compound

Common Name: Prohexadione carboxylic acid
BAS Number: BAS 125 W or BAS 9054 W or KI-2817
Chemical Name: 3-oxido-4-propionyl-5-oxo-3-cyclohexene carboxylic acid
Lot Number: L 43-271
Empirical Formula: $C_{10}H_{12}O_5$
Molecular Weight: 212.2g/mole



1.3 Standard Analytes

Common Name: Methyl prohexadione carboxylate
BAS Number: BW 9054-M7 or BX-112-M7
Chemical Name: Methyl 3,5-dioxo-4-propionyl-1-cyclohexene carboxylate
Lot Number: 4
Empirical Formula: $C_{12}H_{14}O_5$
Molecular Weight: 226.2g/mole



1.4 Principle of the Method

Prohexadione (BAS 125 W) is extracted from the homogenized peanut matrices using an acetonitrile/1.5 M H_2SO_4 solution (9:1 v/v). This solvent mixture was demonstrated to effectively extract weathered residues through radiovalidation work. After filtration to remove solid material, an aliquot is purified by mini-isolute ENV+™ column: the eluant is methylated by MeOH/ H_2SO_4 , mixed with water and applied to a second mini-isolute ENV+™ column. The eluent from the column (acetone-0.01% oxalic acid) is injected directly into GC-MSD for determination of BAS 125 W.

2. MATERIALS

2.1 Equipment-Suggested Sizes/Manufacturer

Flat-bottom flask, 24/40	50, 125, 250, 600 mL
Buchner funnel	11 cm diameter
Funnel, long stem	75 mm diameter, 150 mm stem
Funnel, short stem	75 mm diameter, 75 mm stem
Volumetric flask	10-500 mL
Volumetric pipette	0.5-10, 20 and 50 mL
Erlenmeyer flask	250 and 500 mL
Filter flask	500 mL
Glass SPE column 8 mL	J.T. Baker, Item No. 7328-06
Glass Reservoir for SPE, 80 mL	Burdick & Jackson
Filter paper	Whatman No. 2, 4 and 6
Filter paper frits for glass columns	VWR, Grade 417 cat. No. 28313-067
Pasteur pipets, disposable	23 cm long
Autosampler vials 1.5 mL	Sun Brokers, Inc. or equivalent
Autosampler caps 11 mm	Sun Brokers, Inc. or equivalent (snap caps)
Glass wool	e.g. sterile
Vortex mixer	Fisher Scientific or equivalent
Ultrasonic bath	Branson 1200 or equivalent
Nitrogen stream evaporator	N-Evap Organomation Associates, Inc. or equivalent
Stirring hot plate	Corning or equivalent
Balance (with at least one-tenth of a gram capability)	Mettler or equivalent
Balance (with 0.0001 g)	Mettler or equivalent
Polytron homogenizer	Brinkmann Instruments or equivalent
SPE manifold	Supelco, Inc. or equivalent
Rotary evaporator	Buchi or equivalent
Rotary evaporator traps, 100 mL	Fisher Scientific, part number K520210-0124 or equivalent
Water bath	Buchi or equivalent
Vacuum system for rotavap	Elvik Systems IPM Inc. or equivalent

Note: Equivalent equipment may be used.

2.2 Reagents and Chemicals - Source/Preparation

<u>Reagents and Chemicals</u>	<u>Source/Preparation</u>
Acetone, Toluene, Methanol	Distilled, high purity (Burdick & Jackson)
Acetonitrile	Millipore water purification system or equivalent
Ultra pure water (18 Megohm-cm resistivity)	Distilled, high purity
Isolute ENV +™	Jones Chromatography, Tel.(800)874-6244, part #9915-0100 sorbent materials.

Note: Do not use other types or suppliers of ENV +™

Sulfuric acid, Sodium acetate	Fisher Scientific
Oxalic acid	Aldrich Chemical.
Potassium Hydroxide, 50% (w/v)	Fisher Scientific, Certified ACS

Note: Equivalent reagents and chemicals from other suppliers may be used

2.2.1 Standard Solutions for Fortifications

Note: These standard concentrations are suggested. A different concentration scheme may be used and additional standards may be prepared as needed.

Amber bottles should be used as storage containers for the standard solutions. Any standard stock solutions (made from the solid analyte) with a concentration of 1 mg/mL or greater should be stored for a maximum of three months. Any standard solutions prepared from the stock solution should be stored for a maximum of one month.

BAS 125 W (Parent Acid)

- a) Prepare 1.0 mg/mL stock solution of BAS 125 W by weighing an appropriate amount of standard into a volumetric flask. Dissolve with methanol and dilute to the mark. For example, to prepare 25 mL stock solutions, dissolve 25.0 mg of BAS 125 W in 25 mL volumetric flask. Dilute to the mark with methanol.
- b) Prepare a 100 µg/mL standard solution by transferring an appropriate amount of the 1.0 mg/mL stock solution from step 2.2.1a with a volumetric pipet to a volumetric flask (typically 5 mL of each of the 1.0 mg/mL stock solution into 50 mL volumetric flask). Dilute to the mark with methanol. Other serial dilution can be made in a similar manner.

2.2.2 Standard Solutions for GC Analysis

Note: These standard concentrations are suggested. A different concentration scheme may be used and additional standards may be prepared as needed.

Amber bottles should be used as storage containers for the standard solutions. Any standard stock solution with a concentration of 1 mg/mL or greater can be stored for a maximum of three months. Any standard solution prepared from the stock solution should be stored for a maximum of one month.

The recommended standard solutions for GC-MSD are: 1.875, 3.75, 7.5 and 15 ng/mL for BW 9054-M7 (methyl prohexadone carboxylate). Other concentrations may be used as appropriate.

Note: Do not inject high concentration (>300ng/mL) of BW 9054-M7 because a carry over may be observe in the following injection(s).

- a) Prepare 1.0 mg/mL stock solution of BW 9054-M7 by weighing an appropriate amount of the standard into a volumetric flask. Dissolve with acetone containing 0.01% oxalic acid (w/v) and dilute to the mark. For example, dissolve 25.0 mg of BW 9054-M7 in a 25 mL volumetric flask. Dilute to the mark with acetone containing 0.01% oxalic acid
- b) Prepare a 50 µg/mL standard solution by transferring an appropriate amount of the 1.0 mg/mL stock solution from step 2.2.2.a with a volumetric pipet to a volumetric flask (typically 5 mL of the 1.0 mg/mL stock solutions into 100 mL volumetric flask). Dilute to the mark with acetone containing 0.01% oxalic acid. Other serial dilutions can be made in a similar manner.

Note: The presence of 0.01% oxalic acid as additive to all solutions improve the peak shape in the GC-MS.

3 Analytical Procedure

The following procedure is for peanut (nutmeat and hay) and its process fractions (meal and refined oil). A Flow chart for the analytical method is presented in Figure 1.

3.1 Preparation of Samples

Homogenize the samples thoroughly before subsampling and weighing.

3.2 Extraction

3.2.1 Peanut Nutmeat, Meal And Hay

Note: The glassware should be clean with no inside scratches to minimize the possibilities of sample losses.

1. Weigh 25 g (± 0.2 g) of the homogenized nutmeat or meal samples (10 g hay) into a 500 mL glass beaker or other wide mouth container. Fortify controls to be used as procedural recovery samples with appropriate concentrations of the standards of BAS 125 W (e.g. for 0.05 ppm add 1.25 $\mu\text{g/mL}$ of the standard into 25 grams nutmeat).
2. Add 120 mL of acetonitrile-1.5M H_2SO_4 (9:1, v/v) to the beaker and macerate the sample for 1-2 minutes with a polytron. Decant into a Buchner funnel (leave the solid in the container) containing a sheet of Whatman No. 2 filter paper covered with a thin layer of celite (about 5 g, weight is not crucial, but also should not be excessive) into a 500 mL filter flask.
3. Repeat extraction from step 3.2.1.2
4. Add 70 mL of NaOAc (0.05M) and macerate for 1-2 min (third extraction). Vacuum filter the extract through a Buchner funnel. Wash the marc with water.

Note: Use a forceps to clean the polytron generator from the matrix if necessary. ~~If the celite becomes hard use a spatula carefully to stir the mixture so that the sample will filter.~~

5. Rinse the polytron blade with deionized water (about 50 mL). Collect the rinses in the beaker and filter through the Buchner funnel as before.
6. Transfer the filtrate to a 500 mL volumetric flask. Adjust to the mark by adding deionized water.

Note: For filtration, use one sheet of filter paper (No. 2) to cover the base of the Buchner funnel and a thin layer of celite to prevent the small particles from clogging the filter paper.

3.2.2 Refined Oil

1. Weigh 10 g (± 0.2 g) of the refined oil in a beaker, add 80 mL of hexane to the beaker and transfer the solution from the beaker into a 250 mL separatory funnel. Fortify controls to be used as procedural recovery samples with appropriate concentrations of

the standards of BAS 125 W (for example for 0.05 ppm add 0.5 mg/mL of the standard into 10 g).

2. Add 120 mL of acetonitrile-1.5M H₂SO₄ (9:1, v/v) to separatory funnel, shake for about one minute. Separate and discard the hexane layer (top layer). Wash the acetonitrile-1.5M H₂SO₄ extract with an additional 60 mL of hexane using the separatory funnel, then transfer the acetonitrile-1.5M H₂SO₄ solution into a 200 mL volumetric flask.

Note: More than two layers may be observed during the separation, discard only the top layer (hexane).

3. Adjust to the mark by adding deionized water.

Note: After addition of water, a hexane layer may separate at the top of the volumetric flask. Bring the acetonitrile-1.5M H₂SO₄ layer to the mark and discard the hexane layer by pipeting it out.

3.3 Mini-isolute ENV+™ Column Chromatography No. 1

A mini-isolute ENV+™ column clean-up step will be used before the methylation step. The following procedure is used for the ENV+™ cleanup:

3.3.1 Column Preparation

1. Weigh out 0.50 g ± 0.05 gram ENV+ sorbent. Transfer the sorbent into an 8 mL glass column with 2 filter paper frits, (grade 417 from VWR Cat. No. 28313-057) at the bottom and place a glass wool plug (about 0.05 g; the weight is not crucial, but also should not be excessive) at the top of the ENV+™ sorbent.

Note: For the glass columns, use two filter paper frits (grade 417 from VWR Cat. No. 28313-057) that fit the bottom of the glass columns. The correct size of the filter papers could be made by hole puncher.

2. Use an SPE vacuum manifold (aspirator) to perform all the steps for ENV+™ column. A solvent flow rate between 30- 35 mL/min is usually adequate.

Note: The flow rate may change depending on the type of matrix. In this case keep the solvent flow as close as possible to 30 mL/min. Manifold should be able to hold four 125 mL flat-bottom flasks for highest efficiency in sample processing.

3. Condition the mini- ENV+™ column by eluting 5 mL acetonitrile-1.5 M H₂SO₄ (9:1, v/v), then 5 mL of methanol, followed by 10 mL water, without allowing the column to go dry.

Note: Glass reservoir (80 mL) may be used at the top of the glass column.

3.3.2 Sample Load

Quantitatively remove 3% (e.g. take 15 mL from 500 mL) of the nutmeat (or meal) extract from step 3.2.1 (8% for hay, e.g. 40 mL from 500 mL, and 8% for oil, e.g. 16 mL from 200 mL) and place into an approximately 500 mL flask or container. Add deionized water (200 mL for oil or meat or meal and 350 mL for hay). Date, label and store the remaining extract.

Swirl the flask to dissolve any residues left on the wall of the sample flask. Transfer this solution to the conditioned mini-ENV +™ column. Collect the eluant in a waste container (for example, 500 mL beaker) without allowing the column to go dry.

Note: The stored extract can be stable for at least 48 hours at room temperature.

Note: Depending on the sensitivity of the GC-MSD detector, and the injection volume, different aliquot sizes may be used as needed

3.3.3 Column Wash

Wash the sample container with 35 mL of deionized water, add to the column, and dry for about 1 minute. Then wash the column with 2 mL of methanol and dry it for about 5 minutes. Collect the eluant in the waste container.

Note: It is very important to dry the column well before elution to minimize the water content before methylation. Strong vacuum (~20 mm/Hg) may be used to dry the column.

3.3.4. Analyte Elution

Elute BAS 125 W with 25 mL of methanol. The volume of methanol may change depending on the lot number of the ENV +™ sorbent. Elution profiles must be established for each lot of ENV +™ sorbent. Collect the elution solvent in a 125 mL flat bottom flask.

3.4 Methylation Using MeOH/ Conc. H₂SO₄

Add 100µL concentrated H₂SO₄ to the reaction flask from the previous step 3.3.4, attach a reflex condenser and reflex for 30 minutes. Boiling stones may be used.

Note: It is not recommended to use a stirring bar, because the analyte may stick on it.

3.5 Mini-Isolute ENV +™ Column Chromatography No. 2

A mini-Isolute ENV +™ column clean-up step will be used before the final GC determination. The following procedure is used for the ENV +™ cleanup:

3.5.1 Column Preparation

1. Weigh out 0.50 g ± 0.05 gram ENV +™ sorbent. Transfer the sorbent into an 8 mL glass column with 2 filter paper frits (grade 417 from VWR Cat. No. 28313-057) at the bottom and place a glass wool plug (about 0.05 g; the weight is not crucial, but also should not be excessive) at the top of the ENV +™ sorbent.

Note: For the glass columns, use two filter paper frits (grade 417 from VWR Cat. No. 28313-057) that fit the bottom of the glass columns. The correct size of the filter papers could be made by hole puncher.

2. Use an SPE vacuum manifold (aspirator) to perform all the steps for ENV +™ column. A solvent flow rate between 30- 35 mL/min is adequate.

Note: The flow rate may change depending on the type of matrix. In this case keep the solvent flow close to 30 mL/min. Manifold should be able to hold four 125 mL flat-bottom flasks for highest efficiency in sample processing..

3. Condition the mini-ENV+ column by passing acetone-0.01% oxalic acid (v/w), and methanol (5 mL each) followed by 10 mL water through, without allowing the column to go dry.

Note: Glass reservoir (80 mL) may be used at the top of the glass column.

3.5.2 Sample Load

The sample flask from step 3.4 is taken directly from methylation to this step. Swirl the flask to dissolve any residues left on the wall of the sample flask. Add 100 mL of water to the reaction flask. Transfer this solution to the conditioned mini-ENV+ column. Collect the eluant in a waste container (for example 200 mL beaker).

3.5.3 Column Wash

Wash the sample container with 35 mL of water, add to the column, and dry for about 1 minute. Then wash the column with 2 mL of methanol and dry it for about 5 minutes. Collect the eluant in the waste container.

Note: It is very important to dry the column well before elution. Strong vacuum (~20 mm/Hg) may be used to dry the column

3.5.4 Analyte Elution

Elute BW 9054-M7 with 10 mL of acetone-0.01% oxalic acid (v/w). The volume of the acetone-0.01% oxalic acid solution may change depending on the lot number of the ENV+™ sorbent. Elution profiles must be established for each lot of ENV+™ sorbent. Collect the elution solvent in a 10 mL volumetric flask. Complete the volume to 10 mL by adding acetone-0.01% oxalic acid (v/w).

Note: The ENV+™ sorbent may be used multiple times if properly cleaned and regenerated. An elution profile does not have to be re-established if regenerated material is being used.

3.5.5 ENV+™ Regeneration: Optional Procedure for re-use of ENV+™ sorbent

Place the ENV+ material into a beaker (ENV+ should not fill more than 1/4 of the beaker) and cover with MeOH, stir with magnetic stirrer and heat at about 80°C for 10 minutes. Decant the MeOH solution and add THF to cover the ENV+ material. Heat and stir for 10 minutes. Repeat washing with DCM and filter into a Bucher funnel with a sheet of filter paper (No. 2). Place ENV+ material in a pan to dry thoroughly (in the hood).

Note: ENV+™ materials can be re-generated more than once. Consistent performance of the ENV+™ sorbent has been observed after 5 re-generation cycles.

3.5.6 Preparation for Final Determination by Gas Chromatography/Mass Spectrometry

The solutions from the last step 3.5.4 are ready for injection into the gas chromatograph/Mass Spectrometer (GC-MS). The 10 mL dilution is adequate for samples ranging from LOQ (0.05 ppm) to 0.2 ppm. Samples with residues exceeding 0.2 ppm will require appropriate dilutions, if all other sample weight, dilutions and aliquot volumes were observed.

3.6 GC-MSD Instrumentation

Different equipment and parameters than those listed below may be substituted into the method as long as interpretable chromatography results.

3.6.1 Description of Equipment

Instrument: A model 5970 mass selective detector from Hewlett Packard. The instrument is automatically and manually tuned for maximum sensitivity (for ion m/z 219) using perfluorotributylamine. Detection by selected ion monitoring (SIM) at m/z 226 (M^+). The dwell time is 500 msec. The gas chromatography (model 5890 series II from Hewlett Packard) is connected to the MSD with a capillary interface kept at 250°C. The GC column is Stabilwax from Restek (30 m, 0.25 mm ID, 0.25 μ m film thickness), or J & W DB-FFAP (nitroterephthalic acid modified polyethylene glycol).

Note: Different GC columns have been tried e.g. DB-1, DB-5, DB-17, DB-1701, but DB-FFAP or Stabilwax were the most efficient columns for separation with good peak shape.

3.6.2 Operating Conditions

Column Parameters:

The carrier gas is ultra-high purity He (99.999%), the head pressure is 15 psi, flow rate is 1.5 mL/min and velocity is 45 cm/Sec.

Oven Program: Start at 80°C hold 1.0 minute, program to 220°C at 70°C/minute, hold for 3 minutes. Then heated to 250°C at 70°C/minute, hold for 3 minutes. Interface kept at 250 °C.

Injection Parameter:

A glass insert from Restek (4mm Gooseneck splitless sleeve part number 20799) is used. Splitless injection with solenoid valve opens after 1 minute, septum purge is 2-3 mL/min. Injector temperature is 180°C, flow of 1.5 mL/min. Injection volume depends on the sensitivity of the GC-MS (typical injection volume is 1 to 5 μ L).

Note: Electronic pressure regulator may be used, (suggested conditions are: pulsed splitless, 50 psi for 0.5 min, then 15 psi for 20 min). Other glass inserts and injection conditions with similar performance can also be used.

Detector Parameters:

MSD in the "SIM" mode to monitor the molecular ion at m/z 226, (M^+ of BW 9054-M7).

- Notes:**
1. The GC parameters may be varied depending on required peak resolution or specific separation problems. It is highly recommended that the temperature ramps not be shortened or deleted.
 2. Condition the GC system by injecting a control and two standards

3.7 METHODS OF CALCULATION

3.7.1 Calibration Procedures

Calculation of results is based on peak area measurements using a calibration curve. To obtain a standard curve, 4 μL of at least three different standard concentrations, for example 1.875, 3.75 and 15 ng/mL of a BW 9054-M7 are injected. These correspond to 7.5, 15 and 60 pg/4 μL , respectively. The peak area (signal counts) is plotted versus the amount of injected standard (pg).

3.7.2 Analyte in Sample

3.7.2.1 Principle

Calculation of results is based on peak area measurements. The amount of BW 9054-M7 in injected samples is determined from the calibration curve and the equation described in 3.7.2.2 is utilized for the determination of the residue (R). Calculation can also be made by a suitable computer program.

At least one fortification and one untreated sample (control) are run with each set of samples. The amount of BW 9054-M7 for fortification trials should be on the order of magnitude of the expected residue. The recovery is determined from the fortification experiments (see 3.7.2.2).

3.7.2.2 Calculation of Recoveries

Residue
(ppm) = $\frac{\text{ng Analyte Found} \times \text{Molecular Weight Conversion Factor (MWCF)}}{\text{mg Sample Injected}}$

Sample Weight
Injected (mg) = $\frac{\text{g Sample} \times \mu\text{L Injected} \times \text{Aliquot \%}}{\text{Dilution Volume (mL)} \quad 100}$

ng Analyte found	= Amount of analyte read from calibration curve in ng
g Sample	= Weight in gram of sample extracted
μL Injected	= μL Injected into GC-MS
Aliquot %	= Aliquot in % taken from sample extract through the method
Dilution Volume	= Final volume after all dilution steps (mL)

MWCF = 0.938 for BW 9054-M7 to BAS 125

Recovery % = $\frac{\text{ppm Found in Fortified Sample} - \text{ppm Found in Control}}{\text{ppm Added to Fortified Sample}} \times 100$

Note: Correction of fortification recoveries for control residues is optional. Treated sample are not corrected for control contributions.

3.7.2.3 Calculation of Residues

Residue
(ppm) = $\frac{\text{ng Analyte Found}}{\text{mg Sample Injected}} \times \text{Molecular Weight Conversion Factor (MWCF)}$

Sample Weight
Injected (mg) = $\frac{\text{g Sample} \times \mu\text{L Injected} \times \text{Aliquot \%}}{\text{Dilution Volume (mL)} \times 100}$

ng Analyte found = Amount of analyte read from calibration curve in ng
g Sample = Weight in gram of sample extracted
 $\mu\text{L Injected}$ = $\mu\text{L Injected into GC-MS}$
Aliquot % = Aliquot in % taken from sample extract through the method
Dilution Volume = Final volume after all dilution steps (mL)

MWCF = 0.938 for BW 9054-M7 to BAS 125

Note: Treated samples should not correct for control contributions.

3.8 Interferences

If interfering peak(s) from the matrix occur in the chromatogram, alter the GC oven program or column flow rate. Other types of GC columns may be used.

3.8.1 Sample Matrices

None observed to date.

3.8.2 Other Sources

Solvents: None observed to date.
Lab Ware: None observed to date.

Other Pesticides:

In order to determine the specificity for this analytical method, the compounds registered for use on peanut, apple, meat and milk were examined. Of 199 compounds that have the tolerances established (Table 4), 174 compounds were injected into GC-MS. Standard solutions of the pesticides (as a mixture) were prepared in methanol or other proper solvents at a concentration equal to or higher than the tolerance. Serial dilutions in solvents were made from each standard solution, such that the theoretical amount run through the method, was equivalent to or greater than the tolerance. The pesticides that were not volatile enough for GC analysis (for example metals and inorganic salts) were not analyzed. All the standard mixture solutions were taken through the methylation procedure. Each of the standard mixtures was methylated with $\text{MeOH}/\text{H}_2\text{SO}_4$, then eluted through the mini-ENV +™ column as described in the method. The elution from the ENV +™ column was injected into GC-MS under the conditions used for parent ester (BW 9054-M7) with retention time ± 0.1 min. There were interferences from mixture #1 (20 compounds). This mixture was divided into three mixtures: # 11 (7 compounds), #12 (7 compounds), and #13 (6 compounds). These pesticides mixtures were again run through the methylation and mini-ENV +™ clean up step. After injection into GC-MS, mixture #13 (6 compounds) was found to interfere with BW 9054-M7. Each individual pesticide in mixture #13 (α -naphthalenesacetic acid, dioxathion, dodine, ethion, tetradifon and ethoxyquin) was spiked into 25 grams of apple and run through the method. After injection into GC-MS there were no pesticides that interfered with the determination of parent methyl ester (BW 9054-M7). Running the pesticides through the whole method is the reason for the disappearance of the interference from the pesticides.

Note Some pesticides were not injected into GC-MS (see Table 4) because they were not available (10 compounds) from suppliers (Axact and ChemServ) or they were not GC compatible (15 compounds) e.g. metals, inorganic salt.

3.9 Confirmatory Techniques

GC-MS is used as a confirmatory technique to confirm the residue of BW 9054-M7 by monitoring three ions at m/z 228 (M^+), 195 and 165 (base peak). The relative abundance of these ions are: 100%, 10% and 58% for m/z 165, 195 and 228 respectively (Figure 21). A different column, DB-5 or DB-1 or DB-17 (30 m, 0.32 mm, 0.5 mm), can be used as an alternative column for BAS 125 residue analysis.

3.10 Time Required for Analysis

The time required for a set of 5 samples, 2 recoveries and 1 control is 8 hours, plus GC analysis and calculation times that can be automated and unattended, provided that no special problems arise.

It is recommended that the work-up be completed in one day, without any stopping points. If it is necessary to stop the set, complete the methylation reaction, and keep the reaction flasks in the freezer (-20°C).

3.11 Potential Problems

- a) During large analytical sets, the detector sensitivity can vary due to matrix effects. It is recommended to condition the column by injecting matrix extract followed by two standards before starting to inject samples.
- b) Make column cuts for both mini-ENV + TM column conditions for each new lot number received.
- c) Before analyte elution from the first mini-ENV + TM column, make sure the column is dry. The presence of water may affect the yield of the methylation step.
- d) Before analyte elution from the second mini-ENV + TM column, make sure the column is dry. The presence of water may hydrolyze the ester in the injection port.

3.12 ¹⁴C Accountability

The accountability of this method for incurred residues was demonstrated using a peanut nutmeat sample (BASF Sample No: 828-68-25) from a ¹⁴C peanut metabolism study (Reference 1). The sample was harvested on October 26, 1993. The metabolism study concluded that BAS 125 W was the major residue accounting for 34% of the TRR. The radiovalidation gave very similar results, accounting for 40% of the TRR as BAS 125W by GC-MS. A summary of individual recovery measurements is given in Table 5. A comparison of the radioactive distribution between the accountability and metabolism work is shown in Table 6. The detailed recovery of radioactivity in each method step is shown in Table 7.

Sample Preparation and Extraction

Sample no.: 828-68-25 was homogenized using a polytron with liquid nitrogen as the solvent. After homogenization, the nitrogen was allowed to evaporate in BASF Freezer # 723. The specific activity of this sample was 63,400 dpm/ μg . A 3.5g aliquot of the peanut nutmeat was taken to be analyzed by BASF Draft Method D9601. The extraction procedure consisted of two extractions with 120 mL of

acetonitrile/1.5M H₂SO₄ (9:1, V:V), followed by 70 mL of NaOAC (0.05M). The filtrate was then brought to 500 mL prior to taking two 3% aliquots. In addition to the radioactive peanut nutmeat, control nutmeat and fortified control nutmeat were analyzed. The fortification samples were fortified at the 0.05 and 1.0 ppm levels with unlabeled (cold) BAS 125 W. Extraction and analyses were performed on 10-14-96.

Radioassays for the total radioactive residues (TRR) in the starting material as well as the nonextractable residues (RRR) remaining in the marc after extraction were determined by combustion using an oxidizer and counting the evolved CO₂ with a liquid scintillation counter (LSC). The combustion analysis of the starting material was 281,953 dpm/gram (4.43 ppm). The extraction yielded 82% of TRR. The RRR (marc) incorporated 25% bound radioactivity for the treated sample. In order to determine the distribution of radioactivity, aliquots were taken at various stages of the method. The dpms counted and the percentage TRR at each step are summarized in Figure 2.

Mini-Isoelute ENV+™ Column #1

The samples were applied to 500 mg ENV+ mini-columns that had been washed with Acetonitrile-1.5M H₂SO₄ (9:1, V:V), methanol, and water prior to loading with the samples. After loading the samples, the columns were washed with water and methanol. The columns were eluted with 26 mL of methanol. Most of the radioactivity added to the column was accounted for in either the load/wash, or the eluate. For both samples the main eluate fraction contained 45% of the TRR.

Methylation

The elution from the previous step was refluxed in presence of concentrated H₂SO₄ for 30 minutes. Recoveries from both samples indicated that there is no losses of radioactivity through methylation. The TRR was 46% for both samples after methylation.

Mini-Isoelute ENV+™ Column #2

The samples were applied to 500 mg ENV+ mini-columns that had been washed with acetone, methanol, and water prior to loading with the samples. After loading the samples, the columns were washed with water and a small quantity of methanol. The columns were eluted with acetone-0.01% Oxalic Acid. Most of the radioactivity added to the column was accounted for in the elute. For both samples the elute contained 40% of the TRR. At this stage the samples are ready for GC injection, no additional handling is required.

GC Injection

The samples were injected on a GC-MS using the reported conditions. Due to the increased Mass-to-Charge Ratio of ¹⁴C labeled BW 9054-M7, the treated samples were analyzed at an m/z of 228 while the control and fortified samples were quantitated at the m/z of 226. The residue of ¹⁴C labeled BAS 125 W in peanut nutmeat was 1.46 ppm (average of 2 samples), which is in agreement with the 1.43 ppm concentration reported in the Metabolism Study (Reference 1). The recoveries for the procedural spikes (cold BAS 125 W) were 94% for 0.05 ppm and 91% for 1.0 ppm. Raw data was recorded on Master Sheet No. A95184-02.

4 RESULTS AND DISCUSSION

4.1 Accuracy and Precision of Validation Results

Nutmeat samples were homogenized with a Urschel food cutter. Hay samples were homogenized with a Stephan vertical cutter/mixer with dry ice. Samples were stored in suitable containers with bar coded labels containing the same information as the original label.

Subsamples of control peanut nutmeat, hay, meal and refined oil were fortified at levels of 0.05 ppm and 1.0 ppm with BAS 125 W and were analyzed by Method D9601. The mean recoveries for nutmeat were: $87 \pm 12\%$ (n=8), hay: $87 \pm 17\%$ (n=8), meal: $77 \pm 6\%$ (n=8) and refined oil: $74 \pm 7\%$ (n=8). The average recoveries of all matrices at all levels were $81 \pm 12\%$ (n=32). A summary of the results is given in Table 1. Individual recovery data is shown in Table 2, and standard responses are shown in Table 3. Example chromatograms are shown in the Appendix B.

4.2 Quantitation Limit

The quantitation limit for BAS 125 W residues in peanut matrices using Method D9601 is 0.05 ppm. At this level, control samples are relatively clean and good recoveries are obtainable. This is the lowest level which is proven by recovery data. The limit of detection (LOD) for this method is 0.025 ppm, based on the lowest standard to which response could be detected.

4.3 Ruggedness Testing

Two analysts executed eight analysis sets of peanut and its process fractions. Two sample sets were run for each matrix. Each contained a control and duplicate analyses of control fortified with test substance at 0.05 and 1.0 ppm levels. This method also has been used for determination of residues in peanuts (Reference 2) and peanut process fractions (Reference 3). Independent lab validation (Reference 4) of this method has been done successfully from the first trial.

4.4 Limitations

None known to date.

5 SAFETY AND HEALTH CONSIDERATIONS

5.1 General

Use personal protective equipment such as lab coats, safety glasses and gloves (nitrile/latex gloves are recommended) when performing the operations described in this method. Conduct all filtrations, nitrogen-stream evaporations and SPE procedures in a well-ventilated hood. Guard vacuum equipment, such as rotovaps, to minimize the possibility of injury caused by flying broken glass. Dispose of hazardous wastes in an environmentally acceptable manner, in compliance with applicable laws and regulations.

5.2 Solvents and Reagents

Review the Material Safety Data Sheets (MSDSs) for all solvents and reagents used in this method.

6 CONCLUSIONS

This study has shown that Analytical Method Number D9601 is suitable for measuring residues of prohexadione (BAS 125 W) in peanut RAC (nutmeat and hay) and peanut process fractions (meal and refined oil) down to a quantitative limit of 0.05 ppm. The average recoveries in all matrices were $81 \pm 12\%$ ($n = 32$). The analytical method number D9601 showed specificity for determination of prohexadione (BAS 125 W) in the presence of other pesticide having tolerances in peanuts, apples, animal tissues and milk.

This method has been demonstrated to give good accountability of incurred residues. A nutmeat sample taken from a peanut metabolism study gave comparable results (1.43 vs 1.46 ppm) to the metabolism study when analyzed by Method D9601.

Statistical treatment of the validation data included determination of an average and standard deviation. Generally, good recoveries were obtained for the fortified crop matrices at the 0.05 and 1.0 ppm levels.

The raw data and final method pertaining to this study are maintained in the BASF Corporation Agricultural Products Center Archives.

7 REFERENCES

1. Steginsky, C.; Powell, J.; Winkler, V.; Venkatesh, K.; Wood, N. "Metabolism of ¹⁴C-BAS 125 W (Prohexadione Calcium) in Peanut". BASF Study Number 93148. BASF Registration Number 96/5227. November 1996
 2. Wofford, T.J.; Abdel-Baky, S.; Riley, M. "Magnitude of BAS 125 W Residues in Peanuts". BASF Study Number 94157. BASF Registration Number 96/5206.
 3. Wofford, T.J.; Abdel-Baky, S.; Riley, M. "Magnitude of BAS 125 W Residues in Peanuts Process Fractions". BASF Study Number 94158. BASF Registration Number 96/5207
 4. Bixler, T.A. "Independent Method Validation of BASF Analytical Method D9601, for Prohexadione-Calcium (BAS 125 W) in Peanuts". BASF Study Number 98127. BASF Registration Number 96/5199.
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8. SIGNATURES

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

Author/Analytical
Project Director:

S. A. B.

Date: 11.12.96

Author/Study
Director:

Samy Abdel-Baky
Samy Abdel-Baky, Ph.D.
Senior Research Associate

Date: 11-12-96

Approved By:

Laura Sears
Laura Sears
Technical Center Leader

Date: 11/12/96

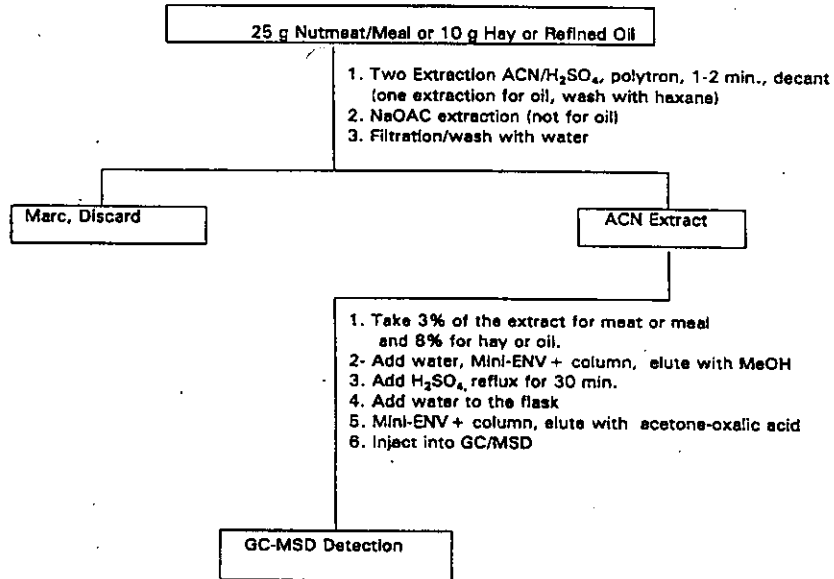


Figure 1: Flow Chart for BAS 125 W Method for Peanut and Peanut PF

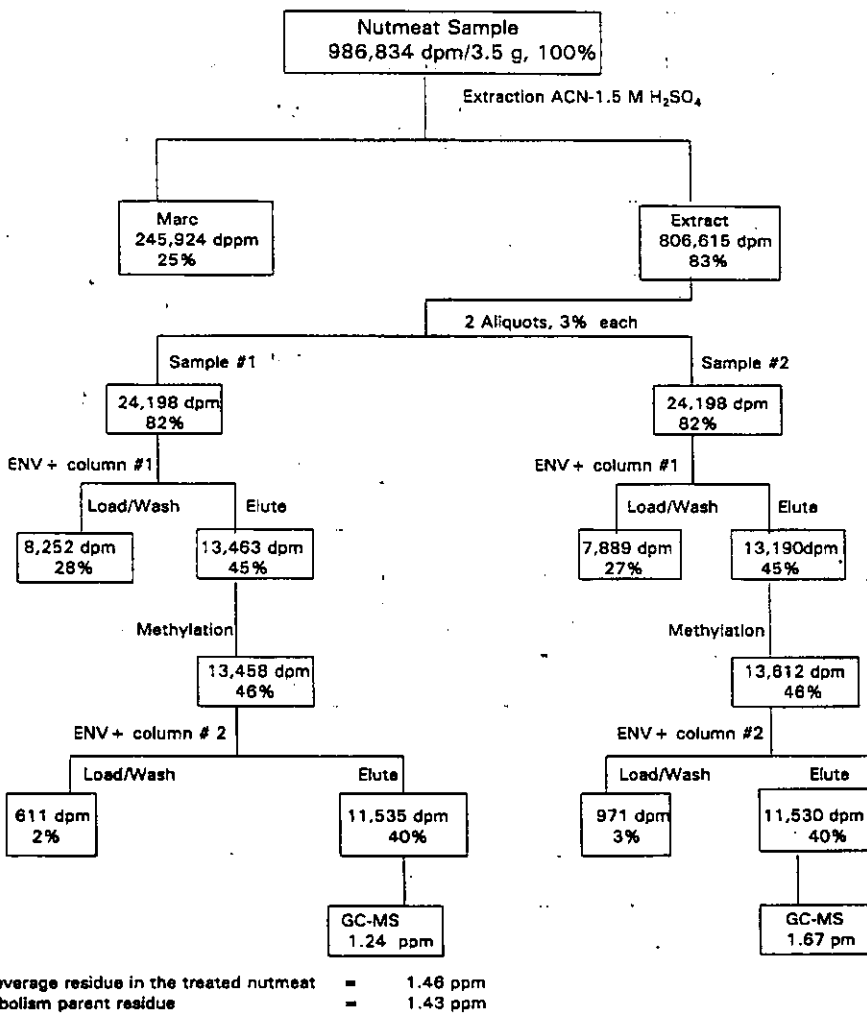


Figure 2. Accountability of 14C-Prohexadione Treated Peanut Nutmeat

Sample Number 95121-02, Vial Number 7 of Control Peanut Nutmeat Fortified with 0.05 ppm
of BAS 125 W from Master Sheet B95164-05.

BW9054-M7 (pg) interpolated from standard curve:

$$\text{Standard curve: pg (BW9054-M7)} = \frac{\text{Peak Height} - \text{Intercept}}{\text{Slope}}$$

Peak height: = 317646
Slope: = 22200
Intercept: = 6530

$$\begin{aligned} \text{pg (BW9054-M7)} &= \frac{317646 - 6530}{22200} = 14.014 \text{ pg (BW9054-M7)} \\ &= 0.01401 \text{ ng (BW9054-M7)} \end{aligned}$$

$$\text{Residue (ppm)} = \frac{\text{BW9054-M7 from Curve (ng)} \times \frac{\text{Final Dilution Vol (mL)} \times 100}{\text{Injection Vol (mL)} \times \text{Aliquot}}}{\text{Sample Weight (g)}} \times \text{MWCF}$$

MWCF = 0.938 from BW9054-M7 to BAS 125 W

$$\text{Recovery \%} = \frac{\text{Residue in Fort Sample (ppm)} - \text{Residue in Control (ppm)}}{\text{Amount Fortified (ppm)}}$$

BW 9054-M7 from Curve = 14.014
Sample Weight = 25 g
Final Dilution Volume = 10 mL
Injection Volume = 4 μ L
Aliquot (%) = 3%
Amount Fortified = 0.05
Residue in Control = 0.00

$$\text{BW 9054-M7 Residue (ppm)} = \frac{0.0140 \text{ ng} \times 10 \text{ mL} \times 100 \times 0.938}{25.0 \text{ g} \times 4 \mu\text{L} \times 3} = 0.0438 \text{ ppm}$$

Figure 3. Typical Residue Calculation for GC-MS Quantitation

Sample Number 92166-15, vial Number 9 of Control Peanut Meal Fortified with 1.0 ppm of
BAS 125 W from Master Sheet B95164-09

BW 9054-M7 (pg) interpolated from standard curve:

$$\text{Standard curve: pg (BW 9054-M7)} = \frac{\text{Peak Area} - \text{Intercept}}{\text{Slope}}$$

Peak Area : = 286251
Slope: = 11200
Intercept: = 1480

$$\text{pg (BW 9054-M7)} = \frac{286251 - 1480}{11200} = 25.43 \text{ pg (BW 9054-M7)}$$
$$= 0.02543 \text{ ng (BW 9054-M7)}$$

$$\text{Residue (ppm)} = \frac{\text{BW 9054-M7 from Curve (ng)} \times \frac{\text{Final Dilution Vol (mL)} \times 100}{\text{Injection Vol (mL)} \times \text{Aliquot}}}{\text{Sample Weight (g)}} \times \text{MWCF}$$

MWCF = 0.938 from BW 9054-M7 to BAS 125 W

$$\text{Recovery \%} = \frac{\text{Residue in Fort Sample (ppm)} - \text{Residue in Control (ppm)}}{\text{Amount Fortified (ppm)}}$$

BW 9054-M7 from Curve = 25.43 pg
Sample Weight = 25 g
Final Dilution Volume = 100 mL
Injection Volume = 4 µL
Aliquot (%) = 3%
Amount Fortified = 0.05
Residue in Control = 0.00

$$\text{BW 9054-M7 Residue (ppm)} = \frac{0.02543 \text{ ng} \times 100 \text{ mL} \times 100}{25.0 \text{ g} \times 4 \text{ µL} \times 3} \times 0.938 = 0.795 \text{ ppm}$$

$$\text{Recovery \%} = \frac{0.795 \text{ ppm} - 0.00}{1.0 \text{ ppm}} \times 100 = 80\%$$

Use full computer/calculator precision in any intermediate calculations. Round only the final value.

Figure 4. Typical Recovery Calculation for GC-MS Quantitation

Table 1. Summary of Recovery Data of Fortified Samples Using GC-MS

Matrix	Fortification Level ppm	Individual Recovery (%)	Average± SD (n=4)
Nutmeat	0.05	78, 100, 88, 106	93±12
	1.0	75, 72, 85, 90	81±8
Hay	0.05	105, 113, 76, 92	97±18
	1.0	88, 90, 61, 76	78±13
Meal	0.05	82, 86, 66, 74	78±6
	1.0	78, 86, 69, 69	77±7
Refined Oil	0.05	66, 74, 78, 78	74±6
	1.0	70, 65, 77, 85	74±9
Overall Recovery			81±12 (n=32)
Nutmeat (Accountability)	0.5	94	
	1.0	91	93±2 (n=2)

Table 2. Individual Recovery Data of the Fortified BAS 125 W in Peanut matrices using GC-MS

Fortified Level ppm, Matrix (Vial Number) ¹	Master Sheet Number B95164- ²	Extract. Date	Injection Date	Final Volume (mL) ³	Peak Area (Count X10 ⁴) ⁴	Residue ppm ⁵	Recovery %
Control, hay, (6)	02	9-10-96	9-14-96	10	33.4	0.042	--
0.05, Hay (7)	02			10	73.1	0.053	105
0.05, Hay (8)	02			10	76.2	0.057	113
1.0, Hay (9)	02			100	66.9	0.863	86
1.0, Hay (10)	02			100	69.9	0.902	90
control nutmeat (11)	03	9-10-96	9-16-96	10	ND	<0.05	--
0.05, Nutmeat (12)	03			10	30.8	0.039	78
0.05, Nutmeat (13)	03			10	39.4	0.050	100
1.0, Nutmeat (14)	03			100	58.9	0.750	75
1.0, Nutmeat (15)	03			100	58.8	0.723	72
control nutmeat (6)	05	9-11-96	9-13-96	10	ND	<0.05	--
0.05, Nutmeat (7)	05			10	31.8	.044	88
0.05, Nutmeat (8)	05			10	38.2	.053	106
1.0, Nutmeat (9)	05			100	60.9	.850	85
1.0, Nutmeat (10)	05			100	64.6	.903	90
control refined oil (11)	07	9-11-96	9-16-96	10	ND	<0.05	--
0.05, refined oil (12)	07			10	12.6	.033	68
0.05, refined oil (13)	07			10	14.1	.037	74
1.0, refined oil (14)	07			100	26.9	.703	70
1.0, refined oil (15)	07			100	24.9	.665	66
Control, hay, (6)	08	9-11-96	9-16-96	10	ND	<0.05	--
0.05, hay (7)	08			10	13.9	.038	76
0.05, hay (8)	08			10	17.0	.046	92
1.0, hay (9)	08			100	22.9	0.614	61
1.0, hay (10)	08			100	28.7	.760	76
Control, meal, (6)	09	9-17-96	9-17-96	10	ND	<0.05	--
0.05, meal (7)	09			10	14.5	.041	82
0.05, meal (8)	09			10	15.2	.043	86
1.0, meal (9)	09			100	28.6	.782	78
1.0, meal (10)	09			100	30.0	.818	82
Control, refined oil, (11)	10	9-17-96	9-17-96	10	ND	<0.05	--
0.05, refined oil (12)	10			10	14.9	.039	78
0.05, refined oil (13)	10			10	14.9	.039	78
1.0, refined oil (14)	10			100	29.2	.787	77
1.0, refined oil (15)	10			100	32.4	.851	85
Control, meal, (11)	11	9-26-96	9-26-96	10	ND	<0.05	--
0.05, meal (7)	11			10	10.0	.033	66
0.05, meal (8)	11			10	11.3	.037	74
1.0, meal (9)	11			100	20.8	.687	69
1.0, meal (10)	11			100	20.8	.685	69
Control, meal, (6) ¹	A95164-02	10-14-96	10-14-96	10	11.1	<0.05 (0.014)	--
0.05, nutmeat (7)	A95164-02			10	41.0	0.047	94
1.0, nutmeat (8)	A95164-02			100	61.2	0.909	91

FOOTNOTES

¹Vial numbers were assigned to distinguish between separate analyses of the same RCN, but different sample number, within the sample set.

²Master sheet number identifies specific analysis sets and consists of Phase number (A or B), BASF study (95164) followed by a sequential analysis set number

³Final dilution volume.

⁴Peak area from GC-MS. Values in parentheses are below the limit of Detection (LOD) of 0.025 ppm. If no signal was detected "ND" is listed in the table.

⁵See Figure 6 for an example calculation of the residue. The value of BAS 125 W is as parent equivalent.

The following values were constant for all analyses:

- a) Sample size = 25.0 g for nutmeat, meal
= 10.0 g for hay and refined oil
 - b) Injection volume = 4µL
 - c) Aliquot = 3% for nutmeat, meal
= 8% for hay and refined oil
-

Table 3. Summary of the Standard Data for BAS 9054-M7 in Peanut Matrices Using GC-MS.

Master Sheet No: B95164-	Peak Area (Count x 10 ⁴)/Injection				Calibration Curve Data ^a	
	7.5 pg	15 pg	30 pg	60 pg	Slope x 10 ⁴	Intercept x 10 ⁴
02	18.2, 18.7	35.4, 39.4	68.0, 57.3	147.6, 124.7	2.22	1.13
03	18.8, 20.8	37.0, 34.9	74.0, 72.3	149.5, 145.3	2.46	0.267
05	17.0, 19.0	32.7, 34.4	66.3, 66.4	134.8, 133.1	2.22	0.653
07	9.4, 8.4	16.4, 16.1	34.2, 33.7	67.9, 66.4	1.12	0.148
08	7.0, 7.9	16.9, 16.3	33.8, 33.0	66.0, 67.2	1.12	0.538
09	9.4, 8.4	16.4, 16.1	34.2, 33.7	67.9, 66.4	1.18	-0.979
10	8.3, 8.4	15.7, 17.0	33.7, 33.2	66.9, 66.8	1.11	0.124
11	8.0, 8.1	15.5, 15.5	32.2, 31.5	63.4, 63.2	0.948	-0.0014
A95184-02	16.7, 16.1	30.2, 31.3	62.3, 65.0	121.1, 122.0	1.99	2.28

¹Master sheet numbers consist of the BASF study number (94157) followed by a sequential analysis set number. Injection dates for each Master Sheet are shown in Table 2.

²The standard curves were constructed using the following equation:

$$(\text{pg}) \text{ BW } 9054\text{-M7} = \frac{\text{Peak Area} - \text{Intercept}}{\text{Slope}}$$

Table 4. Pesticides Used for Specificity Study

40 CFR 180	CHEMICAL	TOLERANCE				Status of Pesticide
		Peanut	Apple	Meat	Milk	
.102	SESONE	8.0	-	-	-	GC
.103	CAPTAN	-	25.0	0.05	-	GC
.106	DIURON	-	1.0	1.0	-	GC
.108	ACEPHATE	0.2	-	0.1	0.1	GC
.109	CHLORBENZILATE	-	-	0.5	-	GC
.110	MANEB	-	2.0	-	-	GC
.111	MALATHION	8.0	8.0	4.0	-	GC
.113	ALLETHRIN	-	4.0	-	-	GC
.114	FERBAM	7.0	7.0	-	-	GC
.115	ZINEB	7.0	2.0	-	-	GC
.116	ZIRAM	7.0	7.0	-	-	NGC
.118	DICHLONE	-	3.0	-	-	GC
.120	METHOXYCHLOR	14.0	14.0	3.0	-	GC
.121	PARATHION	1.0	1.0	-	-	GC
.123	METHYL BROMIDE	-	5.0	-	-	NGC
.124	GLYDIN	-	5.0	-	-	GC
.127	PIPERONYL BUTOXIDE	8.0	8.0	0.1	-	GC
.128	PYRETHRINS	1.0	1.0	0.1	-	GC
.129	O-PHENYLPHENOL	-	25.0	-	-	NA
.130	HYDROGEN CYANIDE	25.0	-	-	-	NGC
.132	THURAM	-	7.0	-	-	NA
.133	LINDANE	-	1.0	7.0	-	GC
.142	2,4-D	-	5.0	2.0	0.1	GC
.143	DIPROPYL ISOCINCHOMERONATE	-	-	0.1	0.004	GC
.144	CYHEXATIN	-	2.0	0.5	-	NGC
.145	CRYOLITE	7.0	7.0	-	-	NGC
.150	DALAPON	-	3.0	0.2	0.1	GC
.163	DIAZINON	10.0	0.5	0.7	-	GC
.154	AZINPHOSMETHYL	-	2.0	0.1	0.04	GC
.155	α -NAPHTHALENEACETIC ACID	-	1.0	-	-	GC
.167	MERINPHOS	-	0.5	-	-	GC
.161	MANGANOUS DIMETHYL DITHIOCARBAMATE	-	7.0	-	-	NGC
.163	DICOLFOL	-	5.0	-	-	GC
.167	NICOTINE COMPOUNDS	-	2.0	-	-	GC
.188	CARBARYL	100	-	1.0	0.3	GC
.171	DIOXATHION	-	5.0	-	-	GC
.172	DODINE	-	5.0	-	-	GC
.173	ETHION	-	2.0	2.5	-	GC
.174	TETRADIFON	-	5.0	-	-	GC
.176	MANCOZEB	0.5	7.0	0.5	-	GC
.176	ETHOXYQUIN	-	3.0	-	-	GC
.181	CIPC	-	-	0.05	.05	GC
.182	ENDOSULFAN	-	2.0	0.2	-	GC
.183	DISULFOTON	5.0	-	-	-	GC
.184	LINURON	-	-	1.0	-	GC
.188	AMS	-	5.0	-	-	GC
.189	COUMAPHOS	-	-	1.0	-	GC
.190	DIPHENYLAMINE	-	10.0	-	-	GC
.191	FOLPET	-	25.0	-	-	GC
.197	DBCP (NEMAGON)	-	-	-	1.8	GC
.198	TRICHLORFON	4.0	-	0.1	0.01	GC
.204	DIMETHOATE	-	2.0	0.02	0.002	GC
.205	PARAQUAT	0.5	0.05	0.05	0.01	GC
.206	PHORATE	0.3	-	0.05	0.02	GC
.207	TRIFURALIN	0.05	-	-	-	GC
.208	BENEFIN	0.05	-	-	-	GC
.209	TERBACIL	-	0.1	0.1	0.1	GC

Table 4. Pesticides Used for Specificity Study (Continued)

40CFR 180	CHEMICAL	TOLERANCE				Status of Pesticide
		Peanut	Apple	Meat	Milk	
.211	PROPACHLOR	-	-	0.02	0.02	GC
.213	SIMAZINE	-	0.25	0.02	0.02	GC
.214	FENTHION	-	-	0.1	0.01	GC
.215	NALED	-	-	0.05	0.05	GC
.217	METIRAM	0.5	2.0	-	-	NGC
.219	TIBA	-	0.05	-	-	GC
.220	ALTRAZINE	-	-	0.02	0.02	GC
.221	FONOFOS	0.1	-	-	-	GC
.224	GIBBERELLINS	-	0.5	-	-	GC
.225	ALUMINUM PHOSPHIDE	0.1	-	0.01	0.01	NA
.226	DIQUAT DIBROMIDE	-	-	0.02	0.02	GC
.227	DICAMBA	-	-	1.5	0.3	GC
.230	DIPHENAMID	2.0	0.1	0.05	0.01	GC
.231	DICHOLOBENIL	-	0.15	-	-	GC
.233	FAMPHUR	-	-	0.1	-	GC
.235	DICHLORVOS	-	-	0.02	0.02	GC
.236	FENTIN HYDROXIDE	0.05	-	0.05	-	NGC
.239	PHOSPHAMIDON	-	1.0	-	-	GC
.240	VERNOLATE	0.1	-	-	-	GC
.242	THIABENDAZOLE	-	10.0	0.1	0.4	GC
.246	DEMINOZIDE	4.0	1.0	-	-	GC
.249	ALACHLOR	3.0	-	0.02	0.02	GC
.252	TERACHLORVINPHOS	-	10.0	1.5	-	GC
.253	METHOMYL	0.1	1.0	-	-	GC
.254	CARBOFURAN	4.0	-	0.05	0.1	GC
.257	CHLORONEB	-	-	0.2	0.05	GC
.259	PROPARGITE	10.0	3.0	0.1	0.08	GC
.261	PHOSMET	-	10.0	0.2	-	GC
.262	ETHOPROP	0.02	-	-	-	GC
.263	PHOSALONE	-	10.0	0.25	-	GC
.266	CHLORAMBEN	0.1	-	-	-	GC
.267	CAPTAFOL	0.05	0.25	-	-	GC
.269	ALDICARB	0.05	-	0.01	0.002	GC
.272	DEF	-	-	0.02	0.002	GC
.274	PROPANIL	-	-	0.1	0.05	GC
.275	CHLOROTHALONIL	0.3	-	-	-	GC
.276	FORMETANATE HYDROCHLORIDE	-	3.0	-	-	GC
.280	CROTOXYPHOS	-	-	0.02	0.02	GC
.287	AMITRAZ	-	-	0.1	0.03	GC
.291	PCNB	1.0	-	-	-	GC
.292	PICLORAM	-	-	8.0	0.05	GC
.294	BENOMYL	15.0	7.0	0.1	0.1	GC
.296	MONOCROTOPHOS	0.05	-	-	-	GC
.297	NAPTALAM	0.1	-	-	-	GC
.298	METHIDATHION	-	-	0.05	0.03	GC
.300	ETHERPHOM	-	5.0	0.1	0.1	GC
.301	CARBOXIN	0.2	-	0.1	0.01	GC
.303	OxAMYL	2.0	2.0	-	-	GC
.309	α-NAPHTHALENE-ACETAMIDE	-	0.1	-	-	GC
.311	CACODYLIC ACID	-	-	1.4	-	NGC
.316	PYRAZON	-	-	-	0.01	GC
.317	PRONAMIDE	-	0.1	0.2	0.02	GC
.319	PROPHAM	-	-	0.05	0.05	GC
.319	PHENOTHIAZINE	-	-	2.0	-	GC
.321	SEC-BUTYLAMINE	-	-	3.0	0.75	GC
.322	CHLORFENVINPHOS	-	-	0.2	-	GC
.324	BROMOXNYL	-	-	0.1	-	GC

Table 4. Pesticides Used for Specificity Study (Continued)

40CF R 180	CHEMICAL	TOLERANCE				Status of Pesticide
		Peanut	Apple	Meat	Milk	
.326	2-(4-CHLOROPHENOXY)PROPIONICACID	-	-	0.5	-	GC
.326	DIALFOR	-	1.5	0.15	-	GC
.327	DINITRAMINE	0.05	-	-	-	GC
.330	OXYDEMENTONMETHYL	-	1.0	0.01	0.01	GC
.331	2,4-DB	0.2	-	-	-	GC
.332	METRIBUZIN	-	-	0.7	0.05	GC
.338	OXYTHIOQUINOX	-	0.05	0.05	0.01	GC
.339	MCPA	-	-	0.1	0.1	GC
.341	DINOCAP	-	0.1	-	-	GC
.342	CHLORPYRIFOS	0.2	1.5	0.3	0.01	GC
.344	DNOC	-	0.02	-	-	GC
.345	ETHOFUMESATE	-	-	0.05	-	GC
.346	OXADIAZON	-	-	0.01	-	GC
.347	TEPP	-	0.01	-	-	GC
.349	FENAMIPHOS	0.02	0.25	0.05	0.1	GC
.350	NITRAPYRIN	-	-	0.05	-	GC
.355	BENTAZON	3.0	-	0.05	0.02	GC
.356	NORFLURAZON	5.5	0.1	0.1	0.1	GC
.359	METHOPRENE	2.0	-	0.1	0.1	GC
.361	PENDIMETHALIN	0.1	-	-	-	GC
.362	HEXAKIS	-	15.0	0.5	-	GC
.363	FLUCHLORALIN	0.05	-	-	-	GC
.364	GLYPHOSATE	0.5	-	0.5	-	GC
.368	METOLACHLOR	30.0	-	0.2	0.02	GC
.369	DIFENZOQUAT	-	-	0.05	-	GC
.370	TERAZOLE (Etridazole)	-	-	0.1	0.05	GC
.371	THIOPHANATE METHYL	15.0	7.0	2.5	1.0	GC
.374	MERDAFOX (Sulprofos)	-	-	0.1	0.01	GC
.375	MAGNESIUM PHOSPHIDE	0.1	-	-	0.01	NGC
.376	6-BENZYLADENINE	-	0.15	-	-	GC
.377	DIFLUBENZURON	-	-	0.05	0.05	GC
.378	PERMETHRIN	-	0.05	3.0	0.25	GC
.378	FENVALERATE	0.02	2.0	1.5	0.3	GC
.381	OXYFLUORFEN	-	-	0.05	0.05	GC
.382	TRIFORINE	-	0.01	-	-	GC
.383	ACIFLUORFEN	0.1	-	0.02	0.02	GC
.384	N,N-DIMETHYLPERIDINUMCHLORIDE (Mepiquat Chloride)	-	-	0.1	0.05	NGC
.387	ISOFENPHOS	-	-	0.1	0.02	GC
.390	TEBUTHIURON	-	-	2.0	0.3	GC
.396	HEXAZINONE	-	-	0.1	0.1	GC
.399	IPRODIONE	150	-	3.0	0.5	GC
.400	FLUCYTHRINATE	-	1.0	0.1	0.1	GC
.401	THIOBENCARB	-	-	0.2	0.05	GC
.403	THIOAZURON	-	-	0.2	0.05	GC
.404	PROFENOFOS	-	-	0.05	0.01	GC
.405	CHLORSULFURON	-	-	0.3	0.1	GC
.406	DMETHIPIN	-	-	0.02	-	GC
.408	METALAXYL	20.0	0.2	0.4	0.02	GC
.409	PRIMIPHOSMETHYL	-	-	2.0	0.1	GC
.410	TRIAZIMEFON	-	1.0	1.0	0.04	GC
.411	FLUAZIFOPBUTYL	-	-	0.05	0.05	GC
.412	SETHOXYSOBM	25.0	-	0.2	0.05	GC
.413	IMAZALE	-	-	0.5	0.01	GC
.416	ETHALFURALIN	0.05	-	0.05	0.05	GC
.417	TRICLOPYR	-	-	0.5	0.01	GC
.419	CLORPYRIFOSMETHYL	-	-	0.5	0.05	GC

Table 4. Pesticides Used for Specificity Study (Continued)

40CF R 180	CHEMICAL	TOLERANCE				Status of Pesticide
		Peanut	Apple	Meat	Milk	
.420	FLURIDONE	-	-	0.1	0.05	GC
.421	FENARIMOL	-	0.1	0.1	0.05	GC
.423	FENRIDAZONE POTASSIUM	-	-	1.0	0.05	NA
.427	FLUVALINATE	-	-	0.01	0.01	GC
.428	METSULFURONMETHYL	-	-	0.5	0.05	GC
.429	CHLORIMURON ETHYL	0.02	-	-	-	GC
.430	FENOXAPROP-ETHYL	0.05	-	0.05	0.02	GC
.431	CLOPYRALID	-	-	12.0	0.1	GC
.434	PROPICONAZOLE	20.0	-	2.0	0.05	GC
.438	CYFLUTHRIN	-	-	0.4	0.01	GC
.438	LAMBDA-CYHALOTHRIN	-	-	0.02	0.01	GC
.441	QUIZALOFOP ETHYL	-	-	0.02	0.01	GC
.442	BIFENTHRIN	-	-	0.5	0.02	GC
.443	MYCLOBUTANIL	-	0.5	0.3	0.05	GC
.446	CLOFENTEZINE	-	-	0.4	0.01	GC
.447	IMAZETHAPYR	0.1	-	-	-	GC
.449	AVERMECTIN	-	-	0.02	0.00	NA
.450	TRIADIMENOL	-	-	0.1	0.01	GC
.452	PRIMISULFURON METHYL	-	-	0.1	0.02	NA
.458	CLETHODIM	-	-	0.2	0.05	NA
.458	TRIASULFURON	-	-	0.2	0.02	GC
.462	PYRIDATE	0.03	-	-	-	GC
.463	QUINCLORAC	-	-	0.05	0.05	GC
.472	IMIDACLOPRID	-	0.5	0.3	0.1	GC
.473	GLUFOSINATE AMMONIUM	-	0.05	-	-	NA
.474	TELRICONAZOLE	0.1	-	-	-	NA
.475	DIFENOCONAZOLE	-	-	0.05	0.01	GC
.476	TRIFLUMIZOLE	-	0.5	0.05	0.05	GC
.479	HALOSULFURON	-	-	0.1	-	NA
Total 189	GC Compatible = 174 compounds	NA, NGC	= 25 compound			

NGC = Pesticide that is not GC compatible
 NA = Pesticides that were not available
 GC = Pesticides that are compatible with GC.

Table 6: Radioactivity Distribution in Accountability and in Metabolism Study

Step	Accountability Results		Step	Metabolism Results	
	ppm	%TRR		ppm	%TRR
Start Material	4.15	100	Start Material	4.15	100
Extraction	3.44	83	Extraction	2.82	63
Marc	1.04	25	Marc	1.73	42
ENV + #1 Methylation	1.68	40	CH ₂ Cl ₂ Partition	1.43	34
ENV + #2			Hexane/ether		
GC-MS Quantitation ^{2,3}	1.46	40	NaOH hydrolysis	1.43	34
			HPLC Quantitation ³		

¹Values from Reference 1

²Average of duplicate samples

³Quantitation of the parent acid (BAS 125) was done by HPLC for the metabolism study, while its methyl ester (BW 9054-M7) was determined in the accountability phase by GC-MS.

Table 7 Percent Extracted at Each Method Step for ¹⁴C-Prohexadione Treated Peanut Nutmeat

Steps of the Method (Date Performed)		% TRR ¹ (dpm)			
Extraction (10-14-96)	Extract	82%	(806,615)		
	Marc	25%	(245,924)		
The Aliquots used for Sample 1 and Sample number 2 are 3% of the Initial Extract					
Steps of the Method (Date Performed)		Sample number 1		Sample number 2	
		% TRR (dpm)	Recovery /Step ²	% TRR (dpm)	Recovery /Step ²
ENV + #1 (10-14-96)	Load /Wash	28%	34%	27%	33%
	Elution	46%	56%	45%	55%
Methylation (10-14-96)	Post Methylation	46%	100%	46%	103%
ENV + #2 (10-14-96)	Load/ Wash	2%	5%	3%	7%
	Elution	40%	86%	40%	85%

¹The starting TRR = 986,834 dpm/3.5 g is based on the combustion analysis of the peanut nutmeat. The dpm values are corrected for the aliquot taken in the previous step.

The %TRR was calculated as follows:

$$\frac{\text{Corrected dpm} \times 100}{\text{Starting TRR}}$$

The correct dpm was calculated as follows:

$$\frac{\text{Measured dpm} \times \text{Volume measured (previous step)}}{\text{Volume Measured} - \text{Volume taken for LSC (previous step)}}$$

²The step recovery was calculated as follow:

$$\frac{\text{Corrected dpm in current step} \times 100}{\text{Measured dpm in previous step}}$$

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APPENDIX A

Changes to Protocol Number 95164

APPENDIX A
changes to Protocol Number 95164

During the course of the study, several changes to the protocol were documented.

Amendments

1. Define the study phases as: Phase A is the accountability, Phase B is the method validation and Phase C is the method specificity.
Reason: To increase the scope of the study.
2. The study Title was changed to include peanut, apple animal tissues and milk.
Reason: To increase the scope of the study.
3. The study Title was changed to method for peanut and its process fractions.
Reason: To increase the efficiency of the analysis by focusing on the analysis of the peanut and its process fractions.

Amendments to Phase A

1. The accountability phase will be done to determine ¹⁴C-prohexadine carboxylic acid in nutmeat and not for animal kidney.
Reason: To increase the efficiency of the analysis by focusing on the analysis of the peanut.
2. The sample size will not be limited to 2 grams and will be mixed with cold control nutmeat, total weight will be 25 grams.
Reason: The treated ¹⁴C nutmeat sample became available to use a larger sample size. Nutmeat cold sample was added to ¹⁴C sample to eliminate the need to adjust aliquot sizes.

Amendment to Phase B

1. Method validation will determine the residue of BAS 125 W in peanut and its process fractions.
Reason: To increase the efficiency of the analysis by focusing on the analysis of the peanut and its process fractions.
 2. Addition of peanut meal sample (RCN 92166)
Reason: The original meal sample was not enough to complete the validation of the method.

These changes had no adverse impact on the study.
-

APPENDIX B

Typical Raw Data and Chromatograms



Description

- Figure 1 Typical GC-MS parameters from master sheet number A95164-02.
- Figure 2 Typical chromatogram of a 7.5 pg standard of BW 9054-M7 from master sheet number A95164-02. Data from this standard can be found in Table 3.
- Figure 3 Typical chromatogram of a 15 pg standard of BW 9054-M7 from master sheet number A95164-02. Data from this standard can be found in Table 3.
- Figure 4 Typical chromatogram of a 30 pg standard of BW 9054-M7 from master sheet number A95164-02. Data from this standard can be found in Table 3.
- Figure 5 Typical chromatogram of a 60 pg standard of BW 9054-M7 from master sheet number A95164-02. Data from this standard can be found in Table 3.
- Figure 6 Typical standard Curve for 7.5, 15, 30 and 60 pg amounts of BW 9054-M7 from master sheet number A95164-02. Data from these standards can be found in Table 3.
- Figure 7 Typical chromatogram of a control nutmeat sample. Sample number 95115-02, vial number 11 from master sheet number B95164-03. Data for this sample can be found in Table 2.
- Figure 8 Typical chromatogram of a control nutmeat sample fortified with 0.05 ppm (quantitation limit) of BAS 125 W. Sample number 95115-02, vial number 12 from master sheet number B95164-03. Data for this sample can be found in Table 2. Recovery 100%.
- Figure 9 Typical chromatogram of a control nutmeat sample fortified with 1.0 ppm of BAS 125 W. Sample number 95115-02, vial number 14 from master sheet number B95164-03. Data for this sample can be found in Table 2. Recovery 75%.
- Figure 10 Typical chromatogram of a control hay sample. Sample number 95119-06, vial number 6 from master sheet number B95164-08. Data for this sample can be found in Table 2.
- Figure 11 Typical chromatogram of a control hay sample fortified with 0.05 ppm (quantitation limit) of BAS 125 W. Sample number 95119-06, vial number 8 from master sheet number B95164-08. Data for this sample can be found in Table 2. Recovery 82%.
- Figure 12 Typical chromatogram of a control hay sample fortified with 1.0 ppm of BAS 125 W. Sample number 95119-06, vial number 10 from master sheet number B95164-08. Data for this sample can be found in Table 2. Recovery 76%.
- Figure 13 Typical chromatogram of a control meal sample. Sample number 92166-15, vial number 6 from master sheet number B95164-09. Data for this sample can be found in Table 2.
- Figure 14 Typical chromatogram of a control meal sample fortified with 0.05 ppm (quantitation limit) of BAS 125 W. Sample number 92166-15, vial number 8 from master sheet number B95164-09. Data for this sample can be found in Table 2. Recovery 88%.
- Figure 15 Typical chromatogram of a control meal sample fortified with 1.0 ppm of BAS 125 W. Sample number 92166-15, vial number 10 from master sheet number B95164-09. Data for this sample can be found in Table 2. Recovery 82%.
-

- Figure 16 Typical chromatogram of a control refined oil sample. Sample number 94208-23, vial number 11 from master sheet number B95164-10. Data for this sample can be found in Table 2.
- Figure 17 Typical chromatogram of a control refined oil sample fortified with 0.05 ppm (quantitation limit) of BAS 125 W. Sample number 94208-23, vial number 12 from master sheet number B95164-10. Data for this sample can be found in Table 2. Recovery 78%.
- Figure 18 Typical chromatogram of a control refined oil sample fortified with 1.0 ppm of BAS 125 W. Sample number 94208-23, vial number 15 from master sheet number B95164-10. Data for this sample can be found in Table 2. Recovery 85%.
- Figure 19 Typical chromatogram of a ¹⁴C-treated nutmeat sample number 1127-35-9, vial number 9 from master sheet number A95164-02. Data for this sample can be found in Tables 5 and 6. Residue of BW 9054-M7 = 1.46 ppm.
- Figure 20 Typical chromatogram of a specificity mixture number 10 (contains 5 compounds), vial number 12 from master sheet number 95164-02. Data for this sample can be found in Table 4.
- Figure 21 Typical GC-MS (scan) chromatogram/mass spectrum of 5 ng standard of BW 9054-M7.
-

Figure 1 Typical GC-MS parameters from master sheet number A95164-02.

TOPLEVEL PARAMETERS

Method Information For: C:\HPCHEM\1\METHODS\P101496.M
Method Sections To Run:
 () Save Copy of Method With Data
 () Pre-Run Cmd/Macro =
 (X) Data Acquisition
 (X) Data Analysis
 () Post-Run Cmd/Macro =
Method Comments:
 SPLITLESS INJECTION FOR PARENT M7; GCMSD # 11

END OF TOPLEVEL PARAMETERS

ACQUISITION PARAMETERS

General Information

Inlet : GC
Tune File : 091296.U
Acquisition Mode : Sim

MS Information

Solvent Delay : 4.00 min
EM Absolute : False
EMV Offset : 400.0
Resulting Voltage : 2400.0

[Sim Parameters]
GROUP 1
Group ID : Group 1
Dwell Per Ion : 500 msec.
Low Resolution : Yes
Group Start Time : 4.00
Ions In Group : 226.00

[Real Time Plot Parameters]

Method: P101496.M

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Figure 1 Typical GC-MS parameters from master sheet number A95164-02 (continue).

Time Window : 15 min
Iconize Real Time Display : False
Plot 1 type : Total ion
Scale minimum : 0
Scale maximum : 200000
Plot 2 type : No plot

GC Inlet Information

[Inlet A Temperature Program Information]

Oven Track : Off
Initial Temp. : 180 C
Initial Time : 480.00 min

Level	Rate (C/min)	Final Temp. (C)	Final Time (min)
1	0		

Total Program Time: 480.00 min

[Inlet B Temperature Program Information]

Oven Track : On

[Inlet A Pressure Program Information]

Constant Flow : Off
Initial Pres. : 50.0 psi
Initial Time : 0.50 min

Level	Rate (psi/min)	Final Pres. (psi)	Final Time (min)
1	99.00	15.0	15.00
2	99.00	10.0	5.00
3	0		

Total Program Time: 20.90 min
Pressure Units : psi

[Inlet A Flow Settings]

Column length : 30.00 m
Column diameter : 0.250 mm
Gas : He
Vacuum compensation : On
Pressure : 50.0 psi
Flow : 3.7 ml/min
Linear velocity : 74.2 cm/sec

[Inlet B Pressure Program Information]

Constant Flow : Off
Initial Pres. : 0.0 psi
Initial Time : 30.00 min

Figure 1 Typical GC-MS parameters from master sheet number A95164-02 (continue).

Level Rate(psi/min) Final Pres.(psi) Final Time (min)
1 0
Total Program Time: 30.00 min
Pressure Units : psi

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10.15.96

[Inlet B Flow Settings]

Column length : 30.00 m
Column diameter : 0.250 mm
Gas : He
Vacuum compensation : On
Pressure : 15.0 psi
Flow : 1.7 ml/min
Linear velocity : 47.1 cm/sec

[Auxiliary Channel C Information]

Comment:

Pressure Program:
Initial Pres. : 0.0 psi
Initial Time : 480.00 min

Level Rate(psi/min) Final Pres.(psi) Final Time (min)
1 0
Total Program Time: 480.00 min

[Auxiliary Channel D Information]

Comment:

Pressure Program:
Initial Pres. : 0.0 psi
Initial Time : 480.00 min

Level Rate(psi/min) Final Pres.(psi) Final Time (min)
1 0
Total Program Time: 480.00 min

[Auxiliary Channel E Information]

Comment:

Pressure Program:
Initial Pres. : 0.0 psi
Initial Time : 480.00 min

Level Rate(psi/min) Final Pres.(psi) Final Time (min)
1 0
Total Program Time: 480.00 min

Figure 1 Typical GC-MS parameters from master sheet number A95184-02 (continue).

[Auxiliary Channel F Information]

Comment:

Pressure Program:
Initial Pres. : 0.0 psi
Initial Time : 480.00 min

Level	Rate (psi/min)	Final Pres. (psi)	Final Time (min)
1	0		

Total Program Time: 480.00 min

GC Temperature Information

[GC Zone Temperatures]

Inj. A : 180 C
Inj. B : 100 C Off
Det. A : 50 C Off
Det. B : 250 C
Aux. : 50 C Off

[Oven Parameters]

Oven Equib Time : 0.50 min
Oven Max : 250 C
Oven : On
Cryo : Off
Ambient : 25 C
Cryo Blast : Off

[Oven Program]

Initial Temp. : 80 C
Initial Time : 0.50 min

Level	Rate (C/min)	Final Temp. (C)	Final Time (min)
1	70.00	220	3.00
2	70.00	250	12.00
3	0.00		

Next Run Time : 17.93 min

Injector Information

Injection Source : Auto
Injection Location : Front

Sample Washes : 1
Sample Pumps : 2
Sample Volume : 4 stop(s)

Method: F101496.M

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Figure 1 Typical GC-MS parameters from master sheet number A95164-02 (continue).

Viscosity Delay : 0 sec
Solvent A Washes : 3
Solvent B Washes : 2
On Column : No

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10/15/96

[Purge Information]

Purge A/B	Init. Value	On Time	Off Time
A	Off	1.00	0.00
B	Off	1.00	0.00

Timed MS Detector Entries

time (min)	State (MS on/off)
5.00	On
14.00	Off

END OF ACQUISITION PARAMETERS

DATA ANALYSIS PARAMETERS

Method Name: C:\HPCHEM\1\METHODS\P101496.M

Percent Report Settings

Sort By: Signal

Output Destination
Screen: No
Printer: Yes
File: No

Integration Events: 090496.E

Generate Report During Run Method: No

Signal Correlation Window: 0.020

Qualitative Report Settings

Method: P101496.M

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Figure 1 Typical GC-MS parameters from master sheet number A95164-02 (continue).

Peak Location of Unknown: Apex
Library to Search Minimum Quality
C:\DATABASE\NBS75K.L 0
Integration Events: AutoIntegrate
Report Type: Summary
Output Destination
Screen: Yes
Printer: Yes
File: No
Generate Report During Run Method: No

Quantitative Report Settings

Report Type: Detailed (single compound 1)
Output Destination
Screen: No
Printer: Yes
File: No
Generate Report During Run Method: Yes

WET POMACE TEST
Calibration Last Updated: Tue Oct 15 06:42:17 1996

Reference Window: 10.00 Percent
Non-Reference Window: 5.00 Percent
Correlation Window: 0.02 minutes
Default Multiplier: 1.00
Default Sample Concentration: 0.00

Compound Information

1) BAS125W M7 ()

Ret. Time 8.00 min., Extract & Integrate from 7.70 to 8.30 min.

Signal Rel Resp. Pct. Unc. (rel) Integration
Tgt 226.00 090496.E

Lvl ID	Conc ()	Response
1A	3.750	115900
1B	3.750	90111
1C	3.750	114436
2A	7.500	156962
2B	7.500	161033

Method: P101496.M

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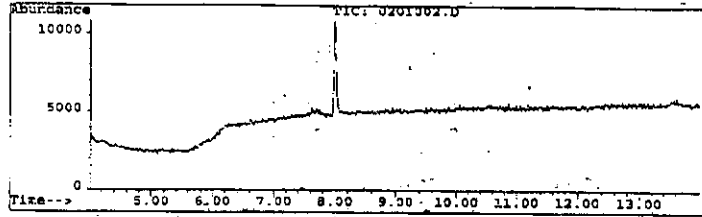
Figure 1 Typical GC-MS parameters from master sheet number A95164-02 (continue).

2C	7.500	176929
3A	15.000	301965
3B	15.000	312975
4A	30.000	622844
4B	30.000	650050
5A	60.000	1211428
5B	60.000	1220140

Qualifier Peak Analysis ON
Curve Fit: Linear

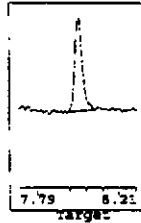
END OF DATA ANALYSIS PARAMETERS

Figure 2 Typical chromatogram of a 7.5 pg standard of BW 9054-M7 from master sheet number A95164-02. Data from this standard can be found in Table 3.



File : C:\NPCHEM\1\DATA\125w\101496\0201002.D
 Operator :
 Acquired : 14 Oct 96 5:31 pm using AcqMethod P101496
 Sample Name: 7.5 PG/ 4 UL M7 STANDARD
 Misc Info :
 Vial Number: 2
 CurrentMeth: C:\NPCHEM\1\METHODS\P101496.M

Compound: BAS125W M7
 Ret Time: 8.01
 Concentration: 5.93
 Pk # and Type: 1



	Signal	Ratio	Limits	RT	Limits	Resp	Integ Type
Tgt	226.00	100.0%		8.01	7.80	161033	090496
Q1	0.00	0.0	0.0- 0.0	0.00	to	0	auto
Q2	0.00	0.0	0.0- 0.0	0.00	8.20	0	auto
Q3	0.00	0.0	0.0- 0.0	0.00		0	auto

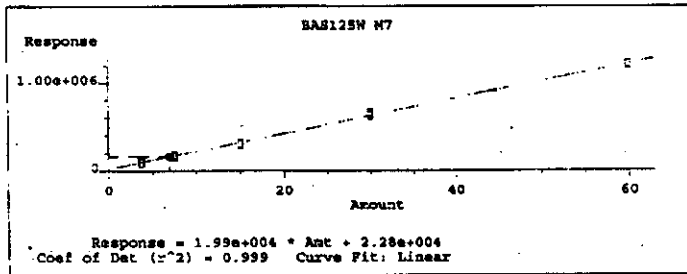
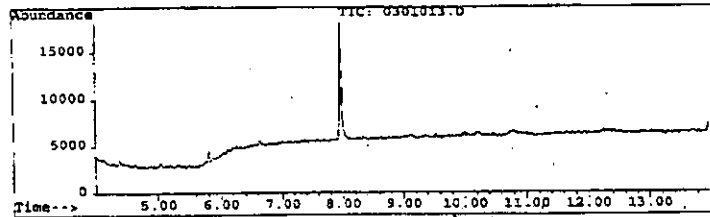


Figure 3 Typical chromatogram of a 15 µg standard of BW 9054-M7 from master sheet number A95164-02. Data from this standard can be found in Table 3



File : C:\HPCHEM\1\DATA\125w\101496\0301013.D
 Operator :
 Acquired : 14 Oct 96 9:35 pm using AcqMethod P101496
 Sample Name: 15 µg/ 4 µL M7 STANDARD
 Misc Info :
 Vial Number: 1
 CurrentMeth: C:\HPCHEM\1\METHODS\P101496.M

Compound: BAS125W M7
 Ret Time: 7.98
 Concentration: 14.55
 Pk # and Type: 1



	Signal	Ratio	Limits	RT	Limits	Resp	Integ Type
Tot	126.00	100.04		7.98	7.80	312975	090496
Q1	0.00	0.0	0.0- 0.0	0.00	to	0	auto
Q2	0.00	0.0	0.0- 0.0	0.00	8.20	0	auto
Q3	0.00	0.0	0.0- 0.0	0.00		0	auto

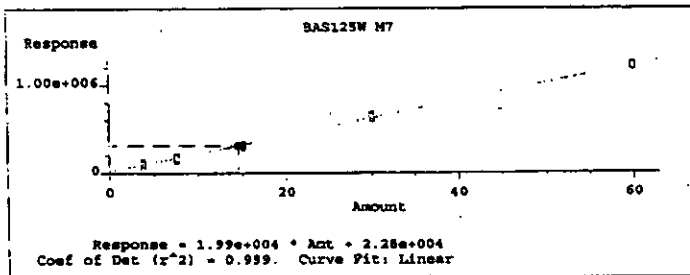
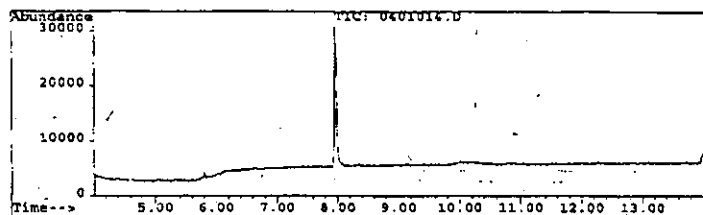
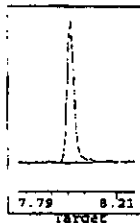


Figure 4 Typical chromatogram of a 30 pg standard of BW 9054-M7 from master sheet number A95164-02. Data from this standard can be found in Table 3



File : C:\NPCHEM\1\DATA\125W\101496\0401014.D
 Operator :
 Acquired : 14 Oct 96 9:57 pm using AcqMethod P101496
 Sample Name: 30 PG/ 4 UL M7 STANDARD
 Misc Info :
 Vial Number: 4
 CurrentMeth: C:\NPCHEM\1\METHODS\P101496.M

Compound: BAS125W M7
 Ret Time: 7.98
 Concentration: 31.45
 Pk # and Type: 1



	Signal	Ratio	Limits	RT	Limits	Resp	Integ Type
Tgt	226.00	100.0%		7.98	7.80	650049	090496
Q1	0.00	0.0	0.0- 0.0	0.00	to	0	auto
Q2	0.00	0.0	0.0- 0.0	0.00	8.20	0	auto
Q3	0.00	0.0	0.0- 0.0	0.00		0	auto

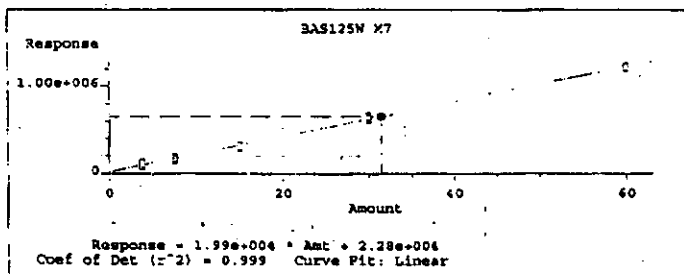
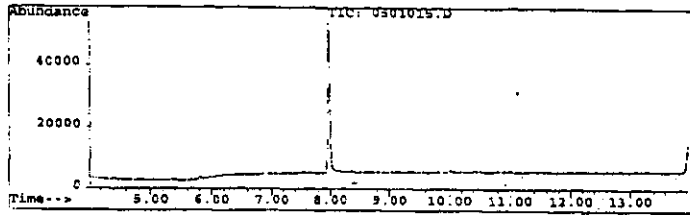
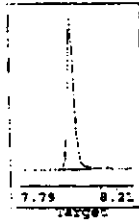


Figure 5 Typical chromatogram of a 60 µg standard of BW 9054-M7 from master sheet number A95164-02. Data from this standard can be found in Table 3



File : C:\HPCHEM\1\DATA\125W\101496\0501015.D
 Operator :
 Acquired : 14 Oct 96 10:19 pm using AcqMethod P101496
 Sample Name: 60 PG/ 4 UL M7 STANDARD
 Misc Info :
 Vial Number: 5
 CurrentMeth: C:\HPCHEM\1\METHODS\P101496.M
 Compound: BAS125W M7
 Ret Time: 7.98
 Concentration: 60.04
 PK # and Type: 1



	Signal	Ratio	Limit	RT	Limit	Resp	Integ	Type
Tot	226.00	100.0%		7.98	7.80	1220140	090496	
Q1	0.00	0.0	0.0- 0.0	0.00	to	0	auto	
Q2	0.00	0.0	0.0- 0.0	0.00	8.20	0	auto	
Q3	0.00	0.0	0.0- 0.0	0.00		0	auto	

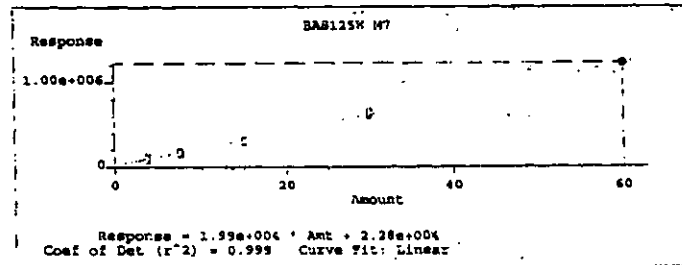
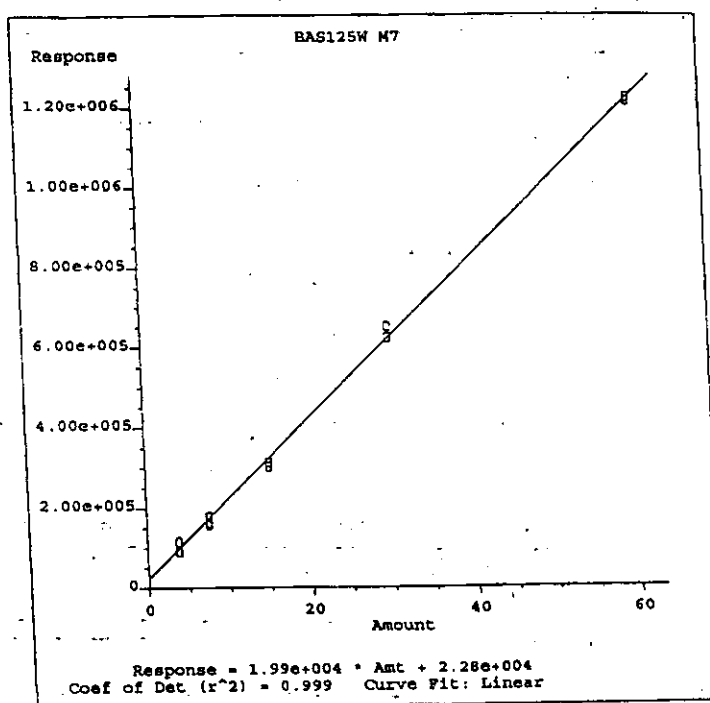


Figure 6 Typical standard Curve for 7.5, 15, 30 and 60 pg amounts of BW 9054-M7 from master sheet number A95164-02. Data from these standards can be found in Table 3

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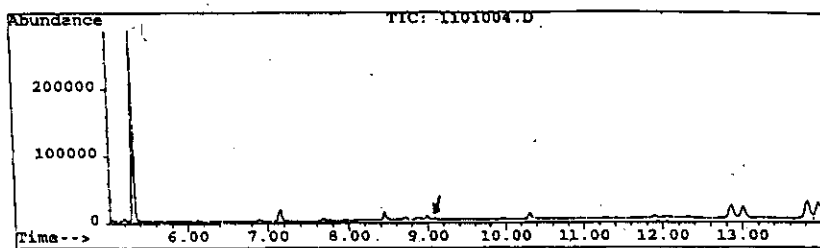
Method Name: C:\HPCHEM\1\METHODS\P101496A.M
Calibration Table Last Updated: Tue Oct 15 06:42:17 1996

Figure 7 Typical chromatogram of a control nutmeat sample. Sample number 95115-02, vial number 11 from master sheet number B95164-03. Data for this sample can be found in Table 2.

9-17-96

Area Percent Report -- Sorted by Signal

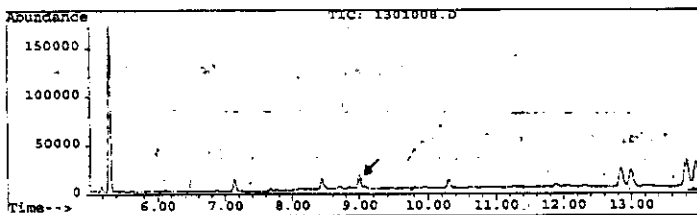
Information from Data File:
File : C:\HPCHEM\1\DATA\125W\091696\1101004.D
Operator :
Acquired : 16 Sep 96 5:58 pm using AcqMethod P0914960
Sample Name: DEP; NUTMEAT; CONTROL
Misc Info : 95115-02
Vial Number: 11
CurrentMeth: C:\HPCHEM\1\METHODS\P0914960.M



Retention Time	Area	Area %	Ratio %
Total Ion Chromatogram			
7.163	545251	12.488	57.748
7.692	109592	2.510	11.607
8.455	342310	7.840	36.255
8.718	152793	3.499	16.183
9.001	166663	3.817	17.652
10.313	281132	6.439	29.775
11.904	176154	4.034	18.657
12.866	856956	19.626	90.761
13.014	791311	18.123	83.809
13.831	944185	21.624	100.000

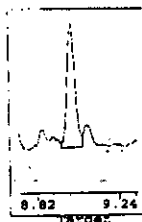
Figure 8

Typical chromatogram of a control nutmeat sample fortified with 0.05 ppm (quantitation limit) of BAS 125 W. Sample number 95115-02, vial number 12 from master sheet number B95164-02. Data for this sample can be found in Table 2. Recovery 100%.



File : C:\HPCHEM\1\DATA\125W\091696\1301008.D
 Operator :
 Acquired : 16 Sep 96 7:34 pm using AcqMethod P0914960
 Sample Name: DEP:NUTMEAT; CONTROL+0.05 B
 Misc Info : 95112-02
 Vial Number: 13
 CurrentMeth: C:\HPCHEM\1\METHODS\P0914960.M

Compound: BAS125W M7
 Ret. Time: 9.01
 Concentration: 16.01
 Pk # and Type: 1



	Signal	Ratio	Limits	RT	Limits	Resp	Integ Type
Tot	226.00	100.0%		9.01	8.80	194420	EVENTS
Q1	0.00	0.0	0.0- 0.0	0.00	to	0	auto
Q2	0.00	0.0	0.0- 0.0	0.00	9.26	0	auto
Q3	0.00	0.0	0.0- 0.0	0.00		0	auto

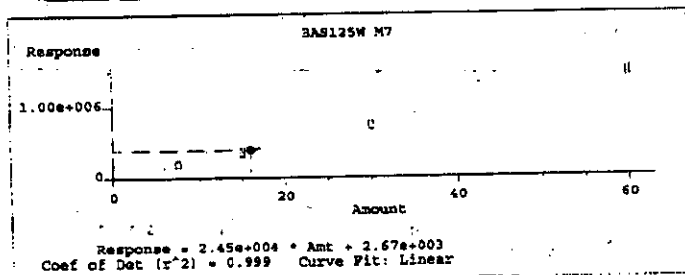
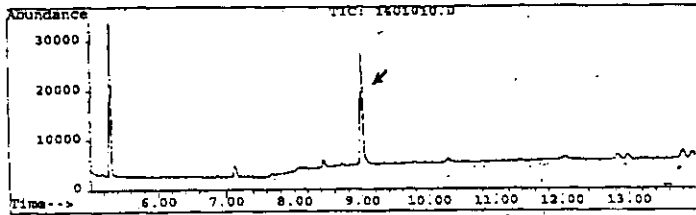
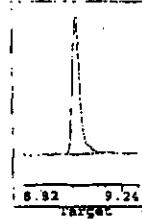


Figure 9 Typical chromatogram of a control nutmeat sample fortified with 1.0 ppm of BAS 125 W. Sample number 95115-Q2, vial number 14 from master sheet number B95164-Q3. Data for this sample can be found in Table 2. Recovery 75%.



File : C:\HPCHEM\1\DATA\125W\091696\1401010.D
 Operator :
 Acquired : 16 Sep 96 8:22 pm using AcqMethod P0514960
 Sample Name: DEP:NUTMEAT; CONTROL-1.0 A
 Misc Info : 95112-02
 Vial Number: 14
 CurrentMeth: C:\HPCHEM\1\METHODS\P0514960.M
 Compound: BAS125W M7
 Ret Time: 9.01
 Concentration: 23.98
 Pk # and Type: 1



	Signal	Ratio	Limits	RT	Limits	Resp	Integ Type
Tgt	226.00	100.0%		9.01	8.80	589344	EVENTS
Q1	0.00	0.0	0.0- 0.0	0.00	to	0	auto
Q2	0.00	0.0	0.0- 0.0	0.00	9.26	0	auto
Q3	0.00	0.0	0.0- 0.0	0.00		0	auto

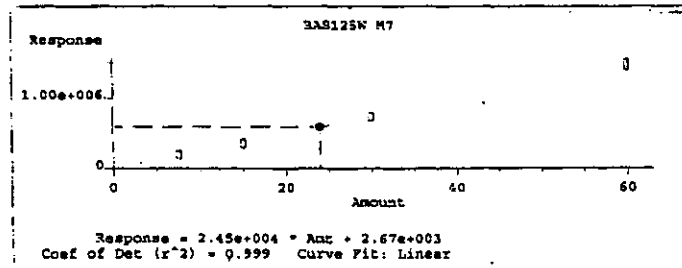
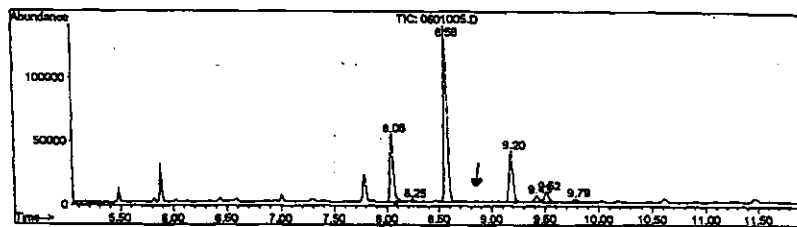


Figure 10 Typical chromatogram of a control hay sample. Sample number 95119-06, vial number 6 from master sheet number B95164-08. Data for this sample can be found in Table 2.

DE
9-17-

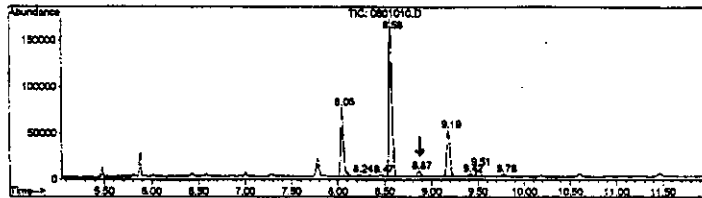
Area Percent Report -- Sorted by Signal

Information from Data File:
File : C:\HPCHEM\1\DATA\125W\091696\0601005.D
Operator :
Acquired : 16 Sep 96 5:04 pm using AcqMethod P091396H
Sample Name: DP; HAY; CONTROL
Misc Info :
Vial Number: 6
CurrentMeth: C:\HPCHEM\1\METHODS\P091396H.M



Retention Time	Area	Area %	Ratio %
Total Ion Chromatogram			
8.059	1170246	20.627	39.410
8.247	44507	0.784	1.499
8.584	2969393	52.339	100.000
9.196	997540	17.583	33.594
9.429	155524	2.741	5.238
9.519	236556	4.170	7.966
9.790	99611	1.756	3.355

Figure 11 Typical chromatogram of a control hay sample fortified with 0.05 ppm (quantitation limit) of BAS 125 W. Sample number 95119-06, vial number 8 from master sheet number B95164-08. Data for this sample can be found in Table 2. Recovery 92%.



File : C:\HPCHEM\1\DATA\125W\091696\0801010.D
 Operator :
 Acquired : 16 Sep 96 6:41 pm using AcqMethod P091396.H
 Sample Name: DP; HAY; 0.05PPM B
 Misc Info :
 Vial Number: 8
 CurrentMeth: C:\HPCHEM\1\METHODS\P091396.H

Compound: 125 W
 Ret Time: 8.87
 Concentration: 15.63 PG
 Pk # and Type: 1



	Signal	Ratio	Limits	RT	Limits	Resp	Integ Type
Tgt	228.00	100.0%	0.0- 0.0	8.87	8.66	149966	091396
Q1	0.00	0.0	0.0- 0.0	0.00	to	0	091396
Q2	0.00	0.0	0.0- 0.0	0.00	to	0	091396
Q3	0.00	0.0	0.0- 0.0	0.00	to	0	091396

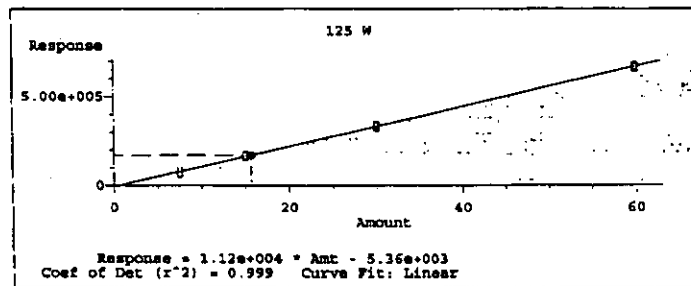
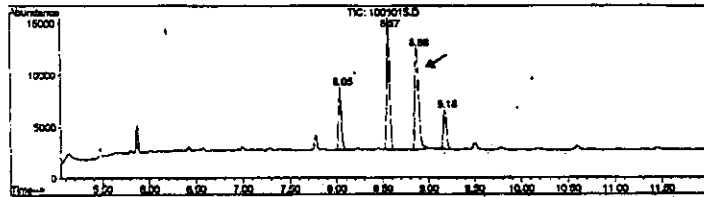
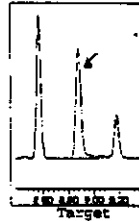


Figure 12 Typical chromatogram of a control hay sample fortified with 1.0 ppm of BAS 125 W. Sample number 95119-06, vial number 10 from master sheet number B95184-08 Data for this sample can be found in Table 2. Recovery 76%.



File : C:\EPCHEM\1\DATA\125W\091696\1001015.D
 Operator :
 Acquired : 16 Sep 96 8:18 pm using AcqMethod P091396H
 Sample Name: DP HAY; 1.0PPM B
 Misc Info :
 Vial Number: 10
 CurrentMeth: C:\EPCHEM\1\METHODS\P091396H.M
 Compound: 125 W
 Ret Time: 8.88
 Concentration: 26.07 PG
 Pk # and Type: 1



	Signal	Ratio	Limits	RT	Limits	Resp	Integ	Type
Tgt	226.00	100.0%		8.88	8.66	287011	091396	
Q1	0.00	0.0	0.0- 0.0	0.00	to	0	091396	
Q2	0.00	0.0	0.0- 0.0	0.00	9.10	0	091396	
Q3	0.00	0.0	0.0- 0.0	0.00		0	091396	

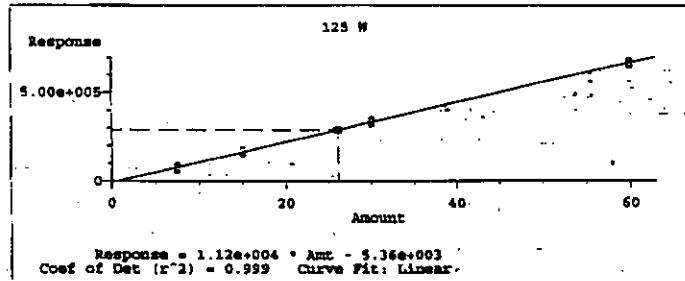
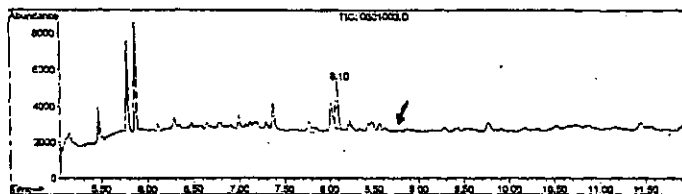


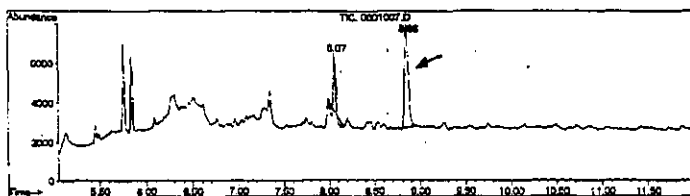
Figure 13 Typical chromatogram of a control meal sample. Sample number 92186-15, vial number 6 from master sheet number B95164-09. Data for this sample can be found in Table 2.



File : C:\HPCHEM\1\DATA\125W\091796\0601003.D
Operator :
Acquired : 17 Sep 96 6:21 pm using AcqMethod P091796L
Sample Name: DM, MEAL, CONTROL
Misc Info :
Vial Number: 6
CurrentMeth: C:\HPCHEM\1\METHODS\P091796L.M

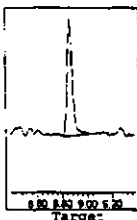
Compound: 125 W
Ret. Time:
Concentration:
Pk # and Type: 1
**** NOT FOUND ****

Figure 14 Typical chromatogram of a control meal sample fortified with 0.05 ppm (quantitation limit) of BAS 125 W. Sample number 92166-15, vial number 8 from master sheet number B95164-09. Data for this sample can be found in Table 2. Recovery 84%.



File : C:\HPCHEM\1\DATA\125W\091796\0801007.D
 Operator :
 Acquired : 17 Sep 96 7:19 pm using AcqMethod P091796L
 Sample Name: DW, MEAL, 0.05PPM B
 Misc Info :
 Vial Number: 8
 CurrentMeth: C:\HPCHEM\1\METHODS\P091796L.M

Compound: 125 W
 Ret Time: 8.86
 Concentration: 13.63 PG
 Pk # and Type: 1



	Signal	Ratio	Limits	RT	Limits	Resp	Integ	Type
Tgt	226.00	100.0%		8.86	8.66	151327	091396	
Q1	0.00	0.0	0.0- 0.0	0.00	to	0	091396	
Q2	0.00	0.0	0.0- 0.0	0.00	9.10	0	091396	
Q3	0.00	0.0	0.0- 0.0	0.00		0	091396	

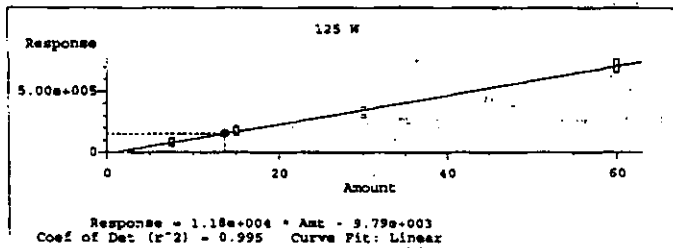
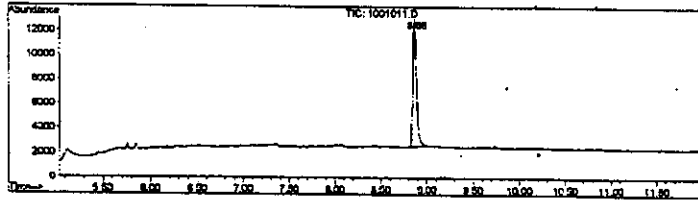


Figure 15 Typical chromatogram of a control meal sample fortified with 1.0 ppm of BAS 125 W. Sample number 92166-15, vial number 10 from master sheet number B95164-09. Data for this sample can be found in Table 2. Recovery 84%.



File : C:\HPCHEM\1\DATA\125W\091796\1001011.D
 Operator :
 Acquired : 17 Sep 96 8:56 pm using AcqMethod P091796L
 Sample Name: DW, MEAL, 1.0PPM B
 Misc Info :
 Vial Number: 10
 CurrentMeth: C:\HPCHEM\1\METHODS\P091796L.M

Compound: 125 W
 Ret Time: 8.88
 Concentration: 26.15 PG
 Pk # and Type: 1



	Signal	Ratio	Limits	RT	Limits	Resp	Integ Type
Tgt	226.00	100.0%		8.88	8.66	259720	091396
Q1	0.00	0.0	0.0- 0.0	0.00	0.00	0	091396
Q2	0.00	0.0	0.0- 0.0	0.00	9.10	0	091396
Q3	0.00	0.0	0.0- 0.0	0.00		0	091396

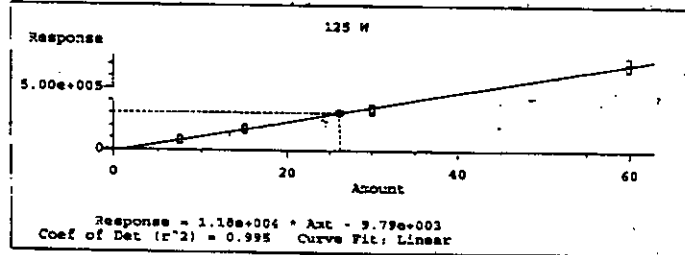
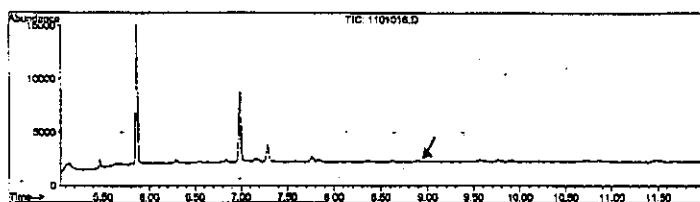


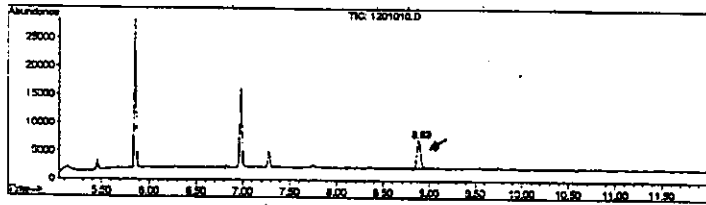
Figure 16 Typical chromatogram of a control refined oil sample. Sample number 94208-23, vial number 11 from master sheet number B95164-10. Data for this sample can be found in Table 2.



File : C:\HPCHEM\1\DATA\125W\091756\1101016.D
Operator :
Acquired : 17 Sep 96 10:33 pm using AcqMethod P0917960
Sample Name: DM, OIL, CONTROL
Misc Info :
Vial Number: 11
CurrentMeth: C:\HPCHEM\1\METHODS\P0917960.M

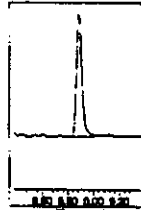
Compound: 125 W
Ret Time:
Concentration:
Pk # and Type: 1
***** NOT FOUND *****

Figure 17 Typical chromatogram of a control refined oil sample fortified with 0.05 ppm (quantitation limit) of BAS 125 W. Sample number 94208-23, vial number 12 from master sheet number B95164-10. Data for this sample can be found in Table 2. Recovery 78%.



File : C:\HPCHEM\1\DATA\125W\091796\1201018.D
 Operator :
 Acquired : 17 Sep 96 11:12 pm using AcqMethod P0917960
 Sample Name: DW, OIL, 0.05PPM A
 Misc Info :
 Vial Number: 12
 CurrentMeth: C:\HPCHEM\1\METHODS\P0917960.M

Compound: 125 W
 Ret Time: 8.89
 Concentration: 13.35 PG
 Pk # and Type: 1



	Signal	Ratio	Limits	RT	Limits	Resp	Integ	Type
Tot	226.00	100.04		8.89	8.66	149333	091396	
Q1	0.00	0.0	0.0- 0.0	0.00	to	0	091396	
Q2	0.00	0.0	0.0- 0.0	0.00	9.10	0	091396	
Q3	0.00	0.0	0.0- 0.0	0.00		0	091396	

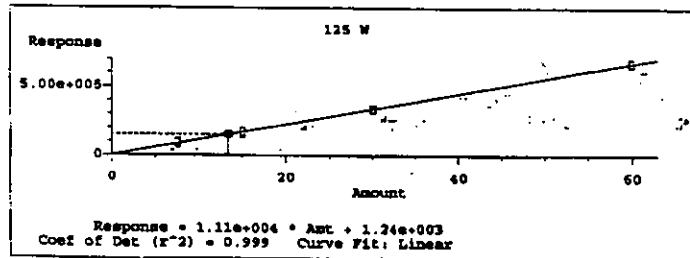
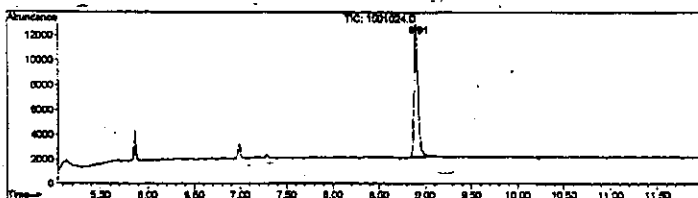


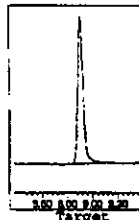
Figure 18

Typical chromatogram of a control refined oil sample fortified with 1.0 ppm of BAS 125 W. Sample number 94208-23, vial number 15 from master sheet number B95164-10. Data for this sample can be found in Table 2. Recovery 85%.



File : C:\HPCHEM\1\DATA\125W\091796\1501024.D
 Operator :
 Acquired : 18 Sep 96 1:09 am using AcqMethod P0917960
 Sample Name: DM, OIL, 1.0PPM B
 Misc Info :
 Vial Number: 15
 CurrentMeth: C:\HPCHEM\1\METHODS\P0917960.M

Compound: 125 W
 Ret Time: 8.91
 Concentration: 29.02 PG
 PK # and Type: 1



	Signal	Ratio	Limits		RT	Limits	Resp	Integ	Type
Tgt	226.00	100.0%			8.91	8.66	323534	091396	
Q1	0.00	0.0	0.0-	0.0	0.00	to	0	091396	
Q2	0.00	0.0	0.0-	0.0	0.00	9.10	0	091396	
Q3	0.00	0.0	0.0-	0.0	0.00		0	091396	

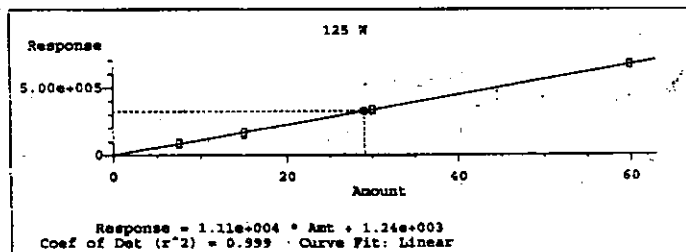
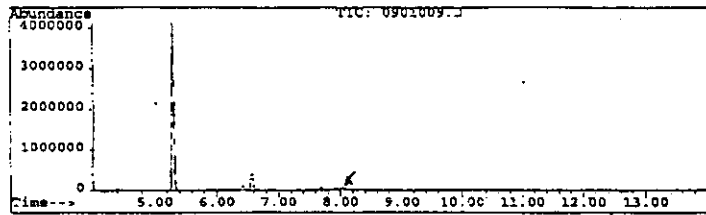
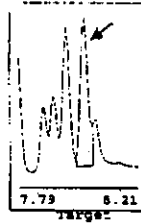


Figure 19 Typical chromatogram of a ¹⁴C-treated nutmeat sample number 1127-35-9, vial number 9 from master sheet number A95164-02. Data for this sample can be found in Tables 5 and 6. BW 9054-M7 residue = 1.46 ppm.



File : C:\HPCHEM\1\DATA\125W\101496\0901009.D
 Operator :
 Acquired : 14 Oct 96 8:06 pm using AcqMethod RD101496
 Sample Name: SAB C14-TRTD NUTMEAT #1
 Misc Info : ACCOUNTABILITY
 Vial Number: 9
 CurrentMeth: C:\HPCHEM\1\METHODS\RD101496.M
 Compound: BAS125W M7
 Ret Time: 8.03
 Concentration: 74.86
 Pk # and Type: 1



	Signal	Ratio	Limits	RT	Limits	Resp	Integ Type
Tgt	228.00	100.00		8.03	7.80	1515628	090496
Q1	0.00	0.0	0.0- 0.0	0.00	to	0	auto
Q2	0.00	0.0	0.0- 0.0	0.00	8.20	0	auto
Q3	0.00	0.0	0.0- 0.0	0.00		0	auto

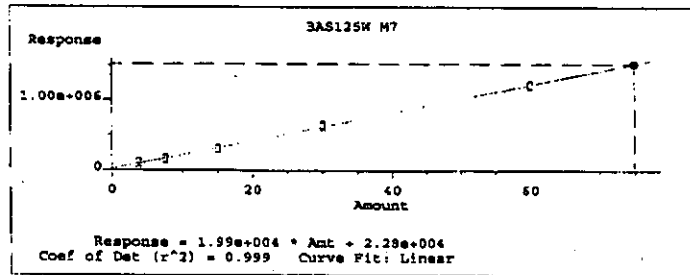
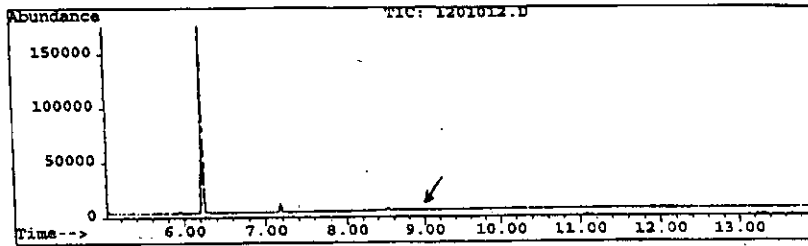


Figure 20 Typical chromatogram of a specificity mixture number 10 (contains 5 compounds), vial number 12 from master sheet number 95164-02. Data for this sample can be found in Table 4.

DW
7-25-96

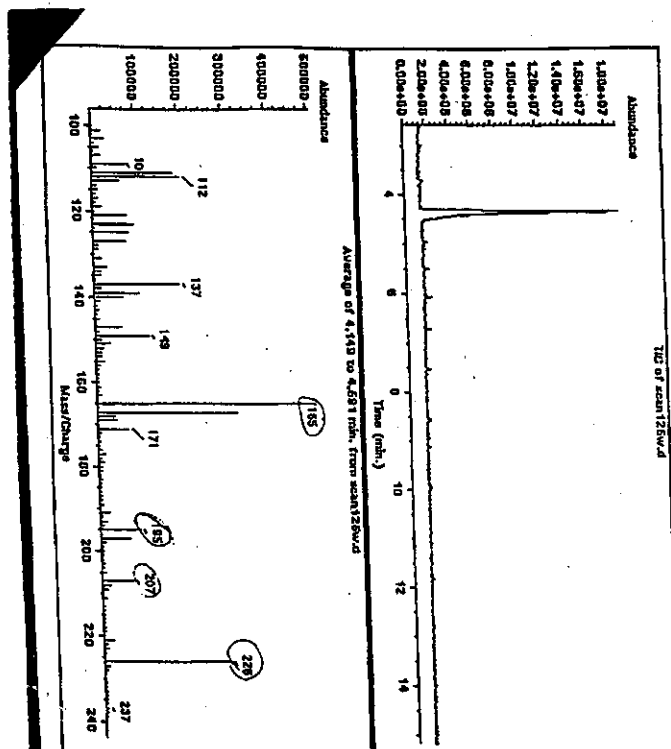
Area Percent Report -- Sorted by Signal

Information from Data File:
File : C:\HPCHEM\1\DATA\062796G\1201012.D
Operator :
Acquired : 19 Jul 96 2:54 pm using AcqMethod P071996
Sample Name: DW MIXTURE #10 SPECIFICITY
Misc Info :
Vial Number: 12
CurrentMeth: C:\HPCHEM\1\METHODS\P071996.M



Retention Time	Area	Area %	Ratio %
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Figure 21 Typical GC-MS (scan) chromatogram/mass spectrum of 5 ng standard of BW 9054-M7.



EPA ADDENDUM

PP#8F04941

BASF Method D9601 on Peanut Nutmeat
for BAS 125 W Residues
and

BASF Method D9608 on Apples
and Kidneys for BAS 125 W Residues

ACB successfully used teflon frits on the bottom and top of the glass columns for mini-isolute ENV + TM column chromatography in place of the 417 grade paper frits and glass wool cited in the analytical methods. If the petitioner wishes, this substitution can be added to the method as an alternate to the paper frits.

ACB used a Restek 4mm cyclo double gooseneck glass insert (cat. # 20896) in the GC inlet for all analyses. The ILV made this same substitution and noted that it increased their sensitivity to the BW 125-M7 analyte. Even with this substitution, ACB found that it was still necessary to use 4 uL injection volumes to achieve adequate sensitivity for the lowest fortification levels while monitoring three ions. ACB also extended the GC run times to prevent late eluting sample co-extractives from being carried over into subsequent injections.

ACB monitored three ions during the MSD analysis of all samples; m/z 226 (M+ of BW 125-M7, quantitation ion), 195 and 165 (base peak). Ion ratios were then calculated (m/z 226 response / m/z 165 response and m/z 195 response / m/z 165 response) for confirmation of BW 125-M7 residues by comparison of calibration standard ion ratios to fortified sample ion ratios. ACB could not "confirm" residues of BW 125-M7 in beef kidney since ion ratios in these samples did not match expected ion ratios from calibration standards due to chromatographic interferences. The unfortified kidney control samples contained similar interferences in the BW 125-M7 retention time window for both m/z 195 and 165 ions. No interferences were detected in the m/z 226 ion, however, which enabled the analytical quantitation of BW 125-M7 residues in beef kidney.