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Study Title:

**Method for Determination of Diflufenzopyr and Phthalazinone (M1)
Residues in Corn by GC-MS**

Method No. D9709

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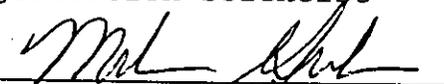
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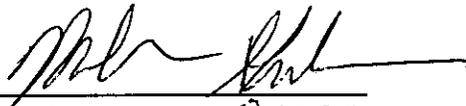
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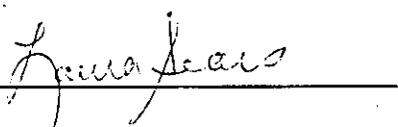
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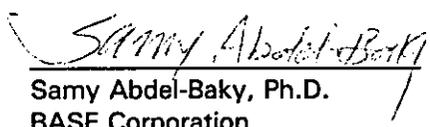
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STATEMENT OF GLP COMPLIANCE

This study meets the requirements for 40 F Part 160 Good Laboratory Practices.

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**Method for Determination of Difufenzopyr and Phthalazinone (M1)
Residues in Corn by GC-MS**

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BASF Method No. D9709

Report Date: February 13, 1998

ABSTRACT:

Analytical Method Number D9709 was developed to determine the residues of Diflufenopyr (BAS 654H) and its metabolite M1 in corn RAC (fodder, forage and grain) and Corn Process Fractions (starch and refined oil), by GC-MS. Method development and validation were carried out at BASF Corporation, Research Triangle Park, NC, using representative control corn. Parent BAS 654 H and its metabolite M1 are extracted from the corn matrices by shaking with dilute aqueous NaHCO₃ and ammoniated acetone. Aliquots of the extracts are acidified with conc. HCl and evaporated to dryness. The residue is dissolved in EtOAc/MeOH solution and refluxed for 2 hours to convert BAS 654 H to M1. The purification step is achieved by mini-column chromatography with Oasis HLB™ sorbent. A gas chromatography system with a selective mass detector is used for the final determination. Analytical Method Number D9709 is suitable for measuring residues of BAS 654 H in corn and its process fractions down to a quantitation limit of 0.05 ppm. Validation of this method was conducted through analyses of the control fortification samples. The average recoveries of BAS 654 H and M1 for all matrices were: 80 ± 9.3% (n=46) and 92 ± 8.5% (n=43) respectively. Method D9709 has also been shown to give equivalent results for the quantitation of weathered residues in corn in comparison to an earlier method AM-0966-0955-0. Method Number D9709 was used to analyze selected treated corn samples from the studies previously analyzed by AM-0966-09550 method (Reference 1-3). The residue level from the repeat analysis is consistent with what was reported previously. BASF Method D9709 is recommended as a replacement for AM-0966-0955-0 for enforcement purposes, because of its brevity and ease of use. Analytical Method Number D9709 has successfully passed an Independent Lab Validation (Reference 4).

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Experimental Dates:

Start: December 31, 1997

Termination: January 5, 1998

QAU STATEMENT

Study Initiation Date: December 30, 1997

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>
12-30-97	12-30-97
12-31-97	12-31-97
1-2-98	1-2-98
2-4-98	2-4-98

Signature of QAU 

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1. Introduction and Summary

1.1 Scope and Source of the Method

1.1.1 Scope

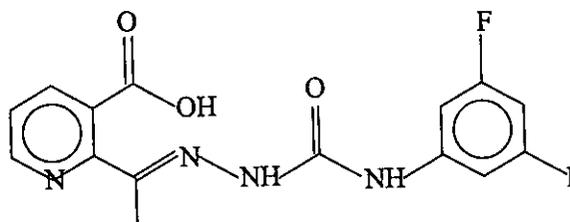
BAS 654 H (diflufenzopyr) is an auxin transport inhibitor that, in concert with dicamba, controls dicotyledonous weeds in corn. This report describes the analytical method developed by BASF to determine residues of BAS 654 H and its metabolite 8-methyl-5-(6H)-pyrido[2,3-d]pyridazinone (phthalazinone, M1) in corn RAC (grain, forage, and fodder) and its process fractions (refined oil and starch). The principal of the method, based on conversion of the parent compound to the metabolite M1, is similar to a previous method used for the analysis of the treated samples (Method AM 0966-0995-0, Reference 5). This new method is shorter and quite rugged, making it more suitable for enforcement purpose.

1.1.2 Source

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

1.2 Test Substance

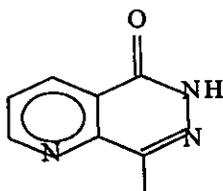
Common Name:	Diflufenzopyr
BAS Number:	BAS 654 H
Chemical Name:	2-[Methyl[[(3,5-fluorophenylamino)carbonyl]hydrazono]3-pyridinecarboxylic acid
Empirical Formula:	C ₁₅ H ₁₂ F ₂ N ₄ O ₃
Molecular Weight:	334.28 g/mole



Fortification Compound

Phthalazinone (M1)

BAS Number:	M1
Chemical Name:	8-Methyl-5(6H)pyrido[2,3-d]pyridazinone
Empirical Formula:	C ₉ H ₇ N ₃ O
Molecular Weight:	161.16 g/mole



1.3 Principle of the Method

The metabolism of diflufenzopyr has been studied in corn RAC (Reference 6). Diflufenzopyr has been found to rapidly degrade to metabolites M1 and M10. Parent is considered the major residue of interest, and can be determined analytically as M1. Method D9709 has been developed to measure parent (by conversion to M1) and M1 in corn matrices.

Residues of BAS 654 H and M1 are extracted from corn matrices by shaking with dilute aqueous NaHCO₃ and ammoniated acetone. The extract is filtered, and an aliquot is acidified with Conc. HCl and evaporated to dryness. The residue is dissolved in methanol/ethyl acetate and refluxed for 2 hours in presence of concentrated HCl. The solution is evaporated to dryness and purified by mini-Oasis HLB™ chromatography column; the eluant is evaporated to dryness and the residue is dissolved in MeOH and analyzed by GC-MS.

2. MATERIALS

2.1 Equipment-Suggested Sizes/Manufacturer

Flat-bottom flask, 24/40	125, 250 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
Glass Jars (wide mouth)	VWR, 480 mL (part # 16189-154)
Filter flask	500 mL
Glass SPE column 8 mL	J.T. Baker, Item No. 7328-06 (or Prism Research Glass telephone: 919-571-0078)
Glass Reservoir for SPE, 80 mL	Burdick & Jackson
Polyethylene frit	Supelco 6 mL, part # 5-7181
Filter paper	Whatman 90 mm GF/F and No. 1, 2, and 4
Filter paper frits for glass columns	VWR, Grade 417 cat. No.28313-057
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
Shaker	HS 501 Digital IKA Labor Technik or equivalent
Ultrasonic bath	Branson 1200 or equivalent
Nitrogen stream evaporator	N-Evap Organomation Associates, Inc. or equivalent
Balance (with at least one-tenth of a gram capability)	Mettler or equivalent
Balance (with 0.0001 g)	Mettler 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
Vacuum system for rotavap

Buchi or equivalent
Elnik Systems IPM Inc. or
equivalent

Note: Equivalent equipment may be used

2.2 Reagents and Chemicals - Source/Preparation

Reagents and Chemicals
Acetone, Methanol, Ethyl Acetate

Source/Preparation

Distilled, high purity
(Burdick& Jackson)

Ultra pure water
(18 Megohm·cm resistivity)

Millipore water purification
system or equivalent

Sodium bicarbonate
Oasis HLB™ sorbent

Distilled, high purity

J.T. Baker

Waters Corporation

Tel.(800)252-4752, part# WAT
086529

Dimethyl Sulfoxide
Ammonium Hydroxide

High Purity (Burdick& Jackson)

JT Baker

Note: Do not use other types or suppliers of HLB™ sorbent materials.

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 1mg/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.

2.2.1.1 Diflufenzpyro (BAS 654 H)

- a) Prepare 1.0 mg/mL stock solution of BAS 654 H by weighing an appropriate amount of standard into a volumetric flask. Dissolve with dimethyl sulfoxide (20% of total volume) and dilute to the mark with acetone. For example, to prepare 10 mL stock solutions, dissolve 10.0 mg of BAS 654 H in 2 mL dimethyl sulfoxide and complete the volume in a 10 mL volumetric flask to the mark with acetone.
- b) Prepare a 100 µg/mL standard solution by transferring an appropriate amount of the 1.0 mg/mL stock solution from step 2.2.1.1a with a volumetric pipette to a volumetric flask (typically 5 mL of each of the 1.0 mg/mL stock solution into a 50 mL volumetric flask). Dilute to the mark with acetone. Other serial dilutions can be made in a similar manner.

2.2.1.2 Phthalazinone, M1

- a) Prepare 1.0 mg/mL stock solution of M1 by weighing an appropriate amount of standard into a volumetric flask. Dissolve and dilute to the mark with methanol. For example, to prepare 10 mL stock solutions, dissolve 10.0 mg of BAS 654 H in a 10 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 2.2.1.2a with a volumetric pipette 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: 2.5, 7.5, 20, and 37.5 ng/mL for M1 (phthalazinone). Other concentrations may be used as appropriate.

Note: Do not inject high concentration (>300 ng/mL) of M1 because a carry over may be observed in the subsequent injection(s).

For the preparation of the GC-MS standards see section 2.2.1.2. All dilutions are in methanol.

3 Analytical Procedure

The following procedure is for corn (grain, fodder and forage) and its process fractions (starch 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 Corn grain, forage, fodder and starch

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

1. Weigh 10 g (± 0.2 g) of the homogenized grain or forage or fodder samples into a ≈ 500 mL wide mouth glass Jar. Fortify controls to be used as procedural recovery samples with appropriate concentrations of the standards of BAS 654 H and M1. For example, for 0.05 ppm add 1 mL of 0.5 µg/mL BAS 654 H standard (0.5 µg/mL in case of M1) into 10 grams grain.

Note: BAS 654H and M1 can not be fortified into the same control sample, as they both are quantitated as M1.

2. Add 25 mL of 0.5% aqueous sodium bicarbonate solution and 100 mL 0.03% (V/V) ammonia/acetone solution to the glass container. Shake the sample for 30 minutes (speed ~ 300 cycle/minute). Filter into a Buchner funnel containing a sheet of Whatman GF/F filter paper into a 500 mL filter flask.

3. Rinse the sample jar and the marc with 0.03% ammonia/acetone solution (about 50 mL).
4. Transfer the filtrate to a 200 mL volumetric flask. Adjust to the mark by adding 0.03% ammonia/acetone solution.

Note: For filtration, use one sheet of filter paper (GF/F, 90 mm) to cover the base of the Buchner funnel. Prewet the filter paper with some acetone.

3.2.2 Refined Oil

1. Weigh 10 g (± 0.2 g) of the refined oil into a ~250 mL separatory funnel. Fortify controls to be used as procedural recovery samples with appropriate concentrations of the standards of BAS 654 H and M1. For example, for 0.05 ppm add 1.0 mL of 0.5 $\mu\text{g/mL}$ BAS 654 H standard (0.5 $\mu\text{g/mL}$ for M1) into 10 grams oil.
2. Add 10 mL hexane, 25 mL of 0.5% aqueous sodium bicarbonate solution and 100 mL 0.03% ammonia/acetone solution to the separatory funnel. Shake the sample vigorously for ~60 seconds. Allow enough time for complete separation. Drain the aqueous layer (bottom layer) into a 200 mL volumetric flask. Discard the organic layer (at top).
3. Adjust to the mark by adding 0.03% ammonia/acetone solution.

3.3 Hydrolysis

The hydrolysis step converts BAS 654 H into M1.

Note: If the analysis is required for M1 only (not for BAS 654 H), the hydrolysis step could be eliminated. Proceed to step 3.4.

Notes

1. Use rotary evaporator traps for all evaporation to eliminate possible sample losses from bumping.
2. Use N-evaporation after every rotary evaporation step to ensure complete dryness.

1. Transfer a 6% aliquot from the extract (step 3.2.1.4 or 3.2.2.3) to a 250 mL flat bottom flask. Add 100 μL concentrated HCl. Evaporate at 60°C with a rotary evaporation to complete dryness. Estimated time for complete evaporation is 4-8 minutes.
2. Add 5 mL methanol to the reaction flask from the previous step, sonicate for ~30 second. Add 100 mL of ethyl acetate and 50 μL concentrated HCl to the 250 mL flat bottom flask, attach a reflux condenser and reflux for 2 hours. Boiling stones may be used.

Note: Sonicate the MeOH solution (5 mL) until the residue is completely dissolved. Precipitation occurs upon addition of ethyl acetate and adheres to the flask during the hydrolysis.

3. Evaporate to dryness by roto-vap at 60°C. Estimated time for complete evaporation is 5-10 minutes

Notes:

- a) It is not recommended to use a stirring bar because the analyte may stick to it.
- b) Start vacuum at a point where bumping is minimal.

3.4 Mini-Oasis™HLB Column Chromatography

Note: Oasis™HLB sorbent is a polymer based sorbent that is designed to separate polar compounds such as carboxylic acids.

A mini-Oasis™HLB column will be used as a cleanup step.:

3.4.1 Column Preparation

- a) Weigh out 500 ± 10 mg Oasis™HLB sorbent. Transfer the sorbent into an appropriately sized column with polyethylene frit (Supleco, 6 mL) or 2 filter paper frits placed on the bottom (grade 417 filter paper from VWR, Cat. No. 28313-057). Place a glass wool plug or a filter paper frit at the top of the Oasis HLB sorbent. If a glass wool plug is to be used, the proper amount is about 0.05 g; the weight is not crucial, but also should not be excessive.

Note: For the columns, use two filter paper frits (or polyethylene frit) that fit the bottom of the columns. A frit of the correct size can be made with an appropriate hole punch or with the tool used to cut holes through rubber stoppers. We used glass columns (8 mL column) and reservoirs (80 mL reservoir). However, plastic columns and reservoirs may be substituted.

- b) Use an SPE vacuum manifold (aspirator) to perform all the steps for Oasis™HLB column. A solvent flow rate between 6-10 mL/min is usually adequate.

Note: The vacuum may change depending on the type of matrix. In this case keep the solvent flow as close as possible to 8 mL/min. Manifold should be able to hold four 125 mL flat-bottom flasks for highest efficiency in sample processing.

- c) Condition the Oasis™HLB column with methanol, followed by water (10 mL each), without allowing the column to go dry.

3.4.2 Sample Load

Add methanol (2 mL) to the reaction flask from step 3.3.2, and sonicate for ~30 seconds. Add water (20 mL) to the solution and sonicate for ~30 seconds.

Note: Particulate matter may remain undissolved after the addition of MeOH (2 mL) and water (20 mL), ignore the particles and continue with the column.

Pour this solution (may contain particles) to the conditioned Oasis™HLB mini-column equipped with the reservoir. Collect the eluant in a waste container (for example, 500 mL beaker) without allowing the column to go dry.

3.4.3 Column Wash

Note: All washes are rinsed through the reaction flask from step 3.4.2. Wash the column with 10 mL of deionized water. Then wash the column with 5 mL 20% methanol/water. Collect both eluants in the waste container.

Note: Do not dry the column during this step.

3.4.4 Analyte Elution

Notes: 1. Use rotary evaporator traps for all evaporation steps to eliminate possible sample losses from bumping.

2. Use N-evaporation after every rotary evaporation step to ensure complete dryness.

Elute M1 with 40 mL of 40% methanol/water into a 125 mL flat bottom flask. Evaporate to dryness by roto-vap at 60°C. Estimated time for complete evaporation is 10-20 minutes.

Dilute with methanol for GC quantitation and sonicate for about 30 seconds. Typically 2 mL is appropriate for the quantitation at the LOQ.

Note: The composition and the volume of the 40% methanol/water may change depending on the lot number of the Oasis™HLB sorbent. Elution profiles must be established for each lot of Oasis™HLB sorbent. Collect the elution solvent in an appropriately sized container.

Note: Elution profiles for Oasis™HLB sorbent material should be done in the presence of matrix to match the conditions of use as closely as possible. It would also help to use a high concentration of M1. Run the method with 2 aliquots from control samples, spike the controls with 300 ng/mL solution of M1 (5 ppm, corrected for aliquot weight) immediately before Oasis HLB column, the final volume should be 20 mL. This should result in no problems with detection or interference from matrix.

3.4.5 Preparation for Final Determination by Gas Chromatography/Mass Spectrometry

The solutions from the last step 3.4.4 are ready for injection into the Gas Chromatograph/Mass Spectrometer (GC-MS). The 2 mL dilution is adequate for samples ranging from LOQ (0.05 ppm) to 0.25 ppm. Samples with residues exceeding 0.25 ppm will require appropriate dilution, if all other sample weight, dilution and aliquot volumes were observed.

3.5 GC-MSD Instrumentation

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

3.5.1 Description of Equipment

Instrument: A Model 5970 (or 5972) Mass Selective Defector from Hewlett Packard. The instrument is automatically and manually tuned for maximum sensitivity (for ion m/z 219) using perfluorotributylamine. Detection is by selected ion monitoring (SIM) at m/z 161 (M^+). The dwell time is 500 msec. The gas chromatography (Model 5890 Series II or 6890 from Hewlett Packard) is connected to the MSD with a capillary interface kept at 250°C. The GC column is DA-Stabilwax from Restek (30 m, 0.25 mm ID, 0.25 μ m film thickness, part # 11023), 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 DA-Stabilwax were the most efficient columns for separation with good peak shape.

3.5.2 Operating Conditions

The operating parameters are in the following table:

Column Parameters	Injection Parameters	Detector Parameters
<u>Carrier Gas</u> Ultra-high purity He (99.999)	<u>Glass Insert Type</u> Restek, 4mm cyclo splitter part number 20707	<u>Detection Mode</u> SIM mode to monitor the molecular ion at m/z 161, (M ⁺ of M1).
<u>Head Pressure*</u> 15 psi, flow rate is 1.5 mL/min and velocity is 45 cm/Sec.	<u>Injection Type</u> Splitless injection, solenoid valve opens after 1 minute, septum purge is 2-3 mL/min.	<u>Dwell Time</u> 500 msec
<u>Oven Program</u> 120°C hold 0.5 minute, program to 250°C at 70°C/minute, hold for 11.6 minutes.	<u>Injection Temperature</u> 250°C	
<u>Interface</u> 250 °C	<u>Injection Volume</u> Typical injection volume is 1 to 5 µL. The commended volume is 4 µL.	

* 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.

- Notes:**
1. The GC parameters may be varied depending on required peak resolution or specific separation problems.
 2. Condition the GC system by injecting a sample and two standards
 3. New GC column may need to be conditioned by injecting more samples and standards until the calibration curve achieves acceptable correlation coefficient (>0.98).

3.6 METHODS OF CALCULATION

3.6.1 Calibration Procedures

Calculation of results is based on peak area (or height) measurements using a calibration curve. To obtain a standard curve, 4 µL of at least three different standard concentrations, for example 2.5, 20 and 37.5 ng/mL of M1 are injected. These correspond to 30, 80 and 150 pg/4µL, respectively. The peak area or height (signal counts) is plotted versus the amount of injected standard (pg).

3.6.2 Analyte in Sample

3.6.2.1 Principle

Calculation of results is based on peak area measurements. The amount of M1 for injected samples is determined from the calibration curve, and the equation described in 3.6.2.2 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 M1 for fortification trials should be on the order of magnitude of the expected residue. The recovery is determined from the fortification experiments (see 3.6.2.2).

3.6.2.2 Calculation of Recoveries

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

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

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)

$$\text{MWCF} = 2.074 \text{ for M1 to BAS 654 H (used only for fortifications of BAS 654 H)}$$

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

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

3.6.2.3 Calculation of Residues

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

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

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	= 2.074 to convert M1 to BAS 654 H

Note: Treated samples should not be corrected for control contributions.

3.7 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.

3.7.1 Sample Matrices

None observed to date.

3.7.2 Other Sources

Solvents: None observed to date.

Lab Ware: None observed to date.

Other Pesticides: Not tested. GC-MS is used as a confirmatory technique, see section 3.9.

3.8 Confirmatory Techniques

GC-MS is used as a confirmatory technique to confirm the residue of M1 by monitoring three ions at m/z 161 (base peak, M⁺), 132 and 104. The relative abundance observed for these ions were: 100%, 23% and 29% for m/z 161, 132 and 104 respectively (Figure 22, Appendix B). 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 M1 residue analysis.

3.9 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 hydrolysis step, and keep the reaction flasks in the freezer (~-20°C).

3.10 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 at least one standard before starting to inject samples. A new GC column may need to be conditioned by injecting more samples and standards until the calibration curve achieves acceptable correlation coefficient (>0.98).

b) Make column cuts for mini Oasis-HLB™ column for each new lot number received.

c) Before hydrolysis step make sure the flask is dry (use N-evaporate). The presence of water may affect the yield of the hydrolysis step.

4 RESULTS AND DISCUSSION

4.1 Accuracy and Precision of Validation Results

Grain samples were homogenized with a Urschel food cutter. Fodder and forage samples were homogenized with a Stephan vertical cutter/mixer with dry ice. Samples were stored in suitable containers with labels containing the same information as the original label.

Subsamples of control grain, fodder, forage, starch and refined oil were fortified at levels of 0.05 ppm and 0.1 ppm with BAS 654 H. Other control samples were fortified at levels of 0.05 ppm and 0.1 ppm with M1. The samples were analyzed by Method D9709. The mean recoveries for M1 were: 98±5.1% (n=9) for forage, 95±4.7% (n=9) fodder, 96±5.5% (n=9) for grain, 88±6.0% (n=8) for starch and 81±8.1% (n=8) for refined oil. The mean recoveries for BAS 654 were: 83±10.2% (n=9) for forage, 81±8.0% (n=9) fodder, 85±3.9% (n=9) for grain, 73±8.5% (n=11) for starch and 79±10.9% (n=8) refined oil. The average recoveries of all matrices at all levels were 80±9.3 (n=46) for BAS 654 H and 92±8.5 (n=43) for M1. A summary of the results is given in Table 1a. Individual recovery data is shown in Table 2, and standard responses are shown in Table 3. Example chromatograms are shown in the Appendix B.

Standard materials used in the validation were:

Standard	Lot Number	Purity
Diflufenzopyr	RS-835-101096 (AR-031078)	98.8%
M1	RS-M1-051895	99.8%

4.2 Quantitation Limit

The quantitation limit for BAS 654 H residues in corn matrices using Method D9709 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 0.017 ppm, based on the lowest standard to which response could be detected.

4.3 Ruggedness Testing

Four analysts executed 14 analysis sets of corn RAC and its process fractions. Two sample sets will be run for each matrix. Each contained one control, treated sample(s), duplicate analyses of the control fortified with the BAS 654 H at 0.05 ppm and 0.1 ppm level, and duplicate analyses of the control fortified with M1 at 0.05 ppm and 0.1 ppm level.

This method also has been demonstrated to measure weathered residues by comparison to analyses by a different method (AM-0966-0955-0, Reference 5). Selected treated samples were reanalyzed using this procedure. Residue results were consistent between the two methods. A comparison of the treated data from both methods is shown in Table 1b. Individual raw data results are shown in Table 4. An Independent lab validation (Reference 4) of this method has been done successfully. The new method D9709 is faster and more rugged than the previous method ((AM-0966-0955-0). It also requires less solvent and can be completed in one working day (8 hours/set).

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 (MSDS) for all solvents and reagents used in this method.

6 CONCLUSIONS

This study has shown that Analytical Method Number D9709 is suitable for measuring residues of BAS 654 H in corn RAC (grain, fodder and forage) and corn process fractions (starch and refined oil) down to a quantitative limit of 0.05 ppm. The average recoveries in all matrices were $80 \pm 9.3\%$ ($n = 46$) for BAS 654 and $92 \pm 8.5\%$ ($n = 43$) for M1. The analytical method number D9709 is rugged, environmentally safe and fast.

This method has been demonstrated to give good agreement for residue results from the reanalysis of the corn samples from Report numbers 97/5312, 97/5293, and 97/5302.

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 0.1 ppm levels.

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

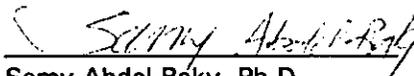
7 REFERENCES

1. Abdel-Baky, S.; Riley, M.; Smith, K. "Magnitude of Diflufenzopyr in Field Corn Resulting from Treatments with BAS 662 H." BASF Registration Number 97/5312, August 1997.
2. Abdel-Baky, S.; Riley, M.; Smith, K. "Residue Decline Study in Field Corn Resulting from Treatments with BAS 662 H." BASF Registration Number 97/5293. August 1997.
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4. Binski, C.; Perez, R. " Independent Method Validation of BASF Analytical Method D9709 for Determination of BAS 654 H and Phthalazinone Residue in Corn by GC-MS." BASF Registration Number 98/5024. February 1998.
5. Salam, M.; Schoester, M.; Wei, L.; Bade, T. "Determination of 8-Methyl 5(6H)-pyrido[2,3-d]pyridazinoone and all Materials Convertible to 8-Methyl-5(6H)-pyrido[2,3-d]pyridazinone in Corn RAC's by Gas Chromatography." Sandoz Agro Inc Laboratory. October 1996.
This method was submitted as an appendix to References 1, 2 and 3.
6. Su, L.Y.; AbdelNour, B.; Van Cott, A. "Corn Metabolism of ¹⁴C-SAN 836 H with and without Dicamba Present." Sandoz Agro, Inc. Laboratory Report # 414215-3 MRID # 44170156. November 1996.

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.

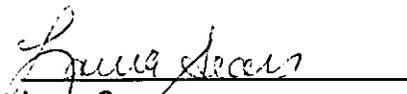
Author/Study
Director:



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

Date: 2-13-98

Approved By:



Laura Sears
Technical Center Leader

Date: 2/13/98

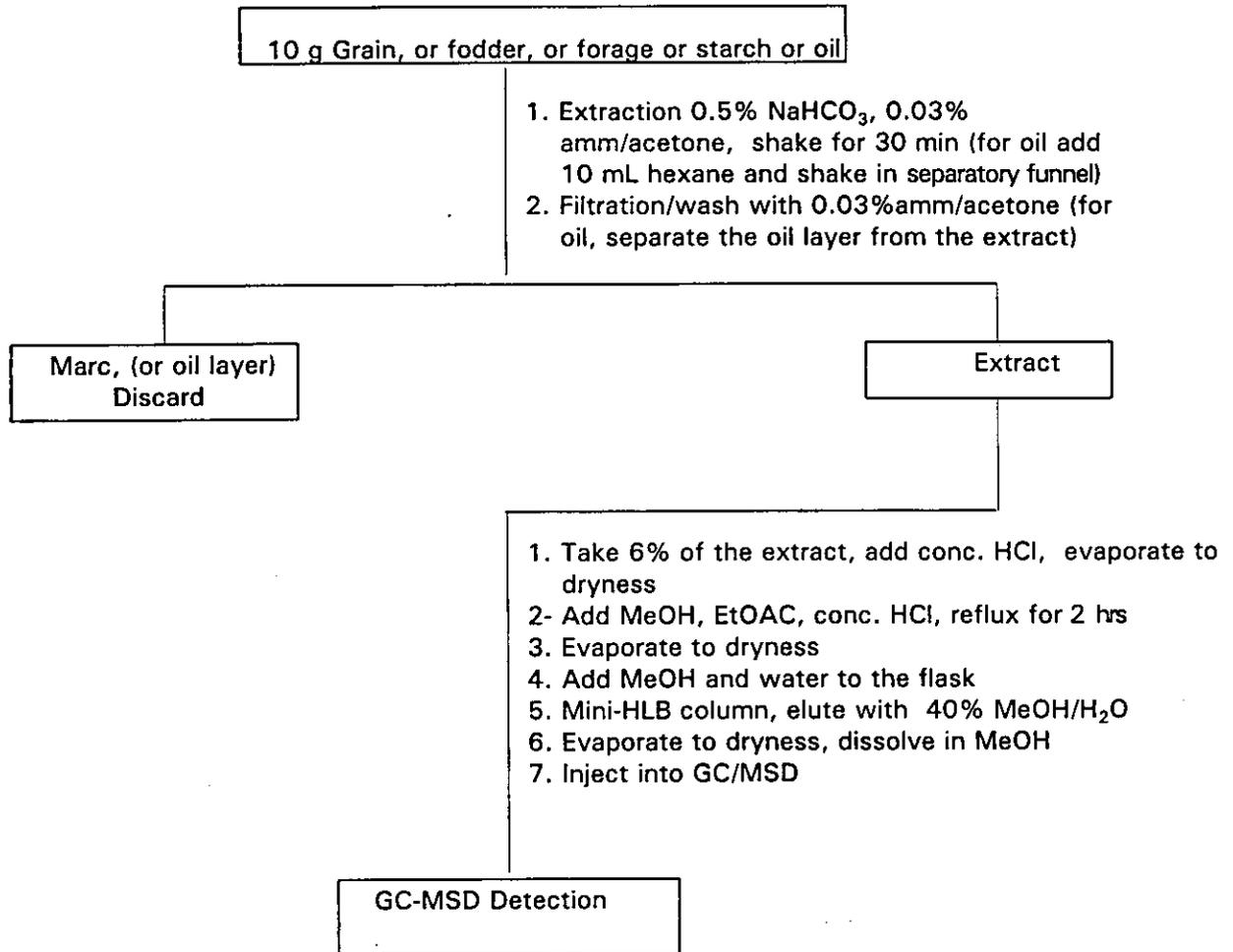


Figure 1: Flow Chart for BAS 654 W Method for Corn and Corn PF

Sample Number 350-95-50-660-01-04-003-1, Vial Number 14 of Control Corn Fodder Fortified with 0.05 ppm of BAS 654 from Master Sheet 97223-04.

M1 (pg) interpolated from standard curve:

$$\text{Standard curve: pg (M1)} = \frac{\text{Peak Area} - \text{Intercept}}{\text{Slope}}$$

$$\begin{aligned} \text{Peak_Area:} &= 324902 \\ \text{Slope:} &= 16000 \\ \text{Intercept:} &= 26800 \end{aligned}$$

$$\begin{aligned} \text{pg (M1)} &= \frac{324902 - 26800}{16000} = 18.63 \text{ pg (M1)} \\ &= 0.01863 \text{ ng (M1)} \end{aligned}$$

$$\text{Residue (ppm)} = \frac{\text{M1 from Curve (ng)}}{\text{Sample Weight (g)}} \times \frac{\text{Final Dilution Vol (mL)} \times 100}{\text{Injection Vol (mL)} \times \text{Aliquot}} \times \text{MWCF}$$

$$\text{MWCF} = 2.074 \text{ from M1 to BAS 654 H}$$

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

$$\begin{aligned} \text{M1 from Curve} &= 18.63 \text{ pg} \\ \text{Sample Weight} &= 10 \text{ g} \\ \text{Final Dilution Volume} &= 2 \text{ mL} \\ \text{Injection Volume} &= 4 \mu\text{L} \\ \text{Aliquot (\%)} &= 6\% \end{aligned}$$

$$\text{M1 Residue (ppm)} = \frac{0.01863 \text{ ng} \times 2 \text{ mL} \times 100}{10 \text{ g} \times 4 \mu\text{L} \times 6} \times 2.074 = 0.032 \text{ ppm}$$

Figure 2. Typical Residue Calculation for GC-MS Quantitation

Sample Number 350-95-50-671-01-01-003-2, Vial Number 20 of Control Corn Fodder Fortified with 0.05 ppm of BAS 654 from Master Sheet 97223-03.

M1 (pg) interpolated from standard curve:

$$\text{Standard curve: pg (M1)} = \frac{\text{Peak Area} - \text{Intercept}}{\text{Slope}}$$

$$\text{Peak Area:} = 114247$$

$$\text{Slope:} = 4320$$

$$\text{Intercept:} = 6590$$

$$\text{pg (M1)} = \frac{114247 - 6590}{4320} = 24.92 \text{ pg (M1)}$$

$$\equiv 0.02492 \text{ ng (M1)}$$

$$\text{Residue (ppm)} = \frac{\text{M1 from Curve (ng)}}{\text{Sample Weight (g)}} \times \frac{\text{Final Dilution Vol (mL)} \times 100}{\text{Injection Vol (mL)} \times \text{Aliquot}} \times \text{MWCF}$$

$$\text{MWCF} = 2.074 \text{ from M1 to BAS 654 H}$$

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

$$\text{M1 from Curve} = 24.92$$

$$\text{Sample Weight} = 10 \text{ g}$$

$$\text{Final Dilution Volume} = 2 \text{ mL}$$

$$\text{Injection Volume} = 4 \mu\text{L}$$

$$\text{Aliquot (\%)} = 6\%$$

$$\text{Amount Fortified} = 0.05$$

$$\text{Residue in Control} = 0.00$$

$$\text{M1 Residue (ppm)} = \frac{0.02492 \text{ ng} \times 2 \text{ mL} \times 100}{10 \text{ g} \times 4 \mu\text{L} \times 6} \times 2.074 = 0.0431 \text{ ppm}$$

$$\text{Recovery \%} = \frac{0.0431 \text{ ppm} - 0.00}{0.05 \text{ ppm}} \times 100 = 86\%$$

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

Figure 3. Typical Recovery Calculation for GC-MS Quantitation

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

Matrix	Fort. ppm	M1 %Rec	M1 Ave± SD	BAS 654H %Rec	BAS 654H Ave± SD
Forage	0.05	89, 96, 99, 107, 94	97±6.7 n=5	84, 75, 87, 96, 97	88±8.8 n=5
	0.1	97, 100, 102, 101	100±2.2 n=4	69, 70, 90, 82	78±10.1 n=4
Fodder	0.05	95, 102, 91, 94, 89	94±5.0 n=5	86, 88, 78, 72, 84	82±6.5 n=5
	0.1	100, 100, 95, 90,	96±4.8 n=4	84, 94, 73, 71	81±10.7 n=4
Grain	0.05	89, 97, 97, 98, 87	94±5.2 n=5	89, 88, 83, 90, 80	86±4.3 n=5
	0.1	97, 93, 104, 102	99±5.0 n=4	83, 81, 89, 82	84±3.6 n=4
Starch	0.05	79, 91, 97, 92	90±7.6 n=4	73, 78, 80, 54, 70	71±10.3 n=5
	0.1	83, 83, 91, 90	87±4.3 n=4	68, 72, 74, 83, 85, 69	75±7.2 n=6
Refined Oil	0.05	90, 75, 91, 73	82±9.6 n=4	75, 78, 67, 103	81±15.5 n=4
	0.1	79, 70, 88, 80	79±6.7 n=4	77, 71, 83, 76	77±4.9 n=4
Average M1			92±8.5 n=43	Average BAS 654H	80±9.3 n=46
Overall Recoveries				86±10.7 n=89	

Table 1b Summary of Total Residues of BAS 654H H in Corn

Site Number	Report Number	Previous Residue (ppm) ¹	Residues of M1 (ppm) ²		Average Residue (ppm)
			Analysis A ³	Analysis B ³	
350-95-50-606-01-04-001-1 (Forage)	97/5312	0.012	0.024	<0.017 (0.007) ⁴	0.020 ⁵
350-95-50-607-01-04-001-1 (Forage)	97/5293	0.017	<0.017	NA	<0.017
350-95-50-662-01-04-001-1 (Forage)	97/5312	0.012	<0.017	NA	<0.017
350-95-50-660-01-04-003-1 (Fodder)	97/5312	0.018	0.032	0.032	0.032 ⁵
350-95-50-606-01-04-003-2 (Fodder)	97/5312	0.017	<0.017 (0.004) ⁴	NA	<0.017 (0.004) ⁴
350-95-50-624-01-04-003-2 (Fodder)	97/5312	0.017	<0.017 (0.013) ⁴	NA	<0.017 (0.013) ⁴
350-95-50-664-01-04-003-1 (Fodder)	97/5312	0.014	<0.017	NA	<0.017
97375-17 (Grain)	97/5302	<0.01	<0.017	<0.017	<0.017 ⁵
97375-0021 (Starch)	97/5302	<0.01	<0.017	<0.017	<0.017 ⁵
97375-0023 (Refined Oil)	97/5302	<0.01	<0.017	<0.017	<0.017 ⁵

¹These residue values are from different report numbers (Reference 1-3)

²Value is an individual analysis, unless otherwise footnoted. See Table IV for individual data for residue samples.

³Analysis A and B are duplicate analysis. NA = not analyzed

⁴ Values in parentheses are below the limit of Detection (LOD) of 0.017 ppm, extrapolated for comparison purpose. If no signal was detected "ND" is listed in the table

⁵Value is the average of duplicate analyses. See Tables 4 for individual data for residue samples.

Table 2. Individual Recovery Data of the Fortified BAS 654H and M1 in Corn matrices using GC-MS.

Fortified Level ppm, Analyte, Matrix (Vial Number) ¹	Master Sheet # 97223- ²	Extract. Date	Injection Date	Final Volume (mL) ³	Peak Area (Count X10 ⁴) ⁴	Residue ppm ⁵	Rec %
Control, Forage (1)	1	12-31-97	12-31-97	2	NA	NA	--
Control, Forage (2)	2	12-31-97	12-31-97	2	NA	NA	--
Control, Forage (13)	13	1-5-98	1-5-98	2	NA	NA	--
0.05 M1 Forage (1)	1	12-31-97	12-31-97	2	88.4	0.0446	89
0.05 M1 Forage (1)	1	12-31-97	12-31-97	2	95.0	0.0482	96
0.05 M1 Forage (2)	2	12-31-97	12-31-97	2	26.4	0.0494	99
0.05 M1 Forage (2)	2	12-31-97	12-31-97	2	28.6	0.0536	107
0.05 M1 Forage (13)	13	1-5-98	1-5-98	2	23.9	0.0471	94
0.1 M1 Forage (1)	1	12-31-97	12-31-97	2	186.1	0.0974	97
0.1 M1 Forage (1)	1	12-31-97	12-31-97	2	190.3	0.0996	100
0.1 M1 Forage (2)	2	12-31-97	12-31-97	2	53.7	0.1021	102
0.1 M1 Forage (2)	2	12-31-97	12-31-97	2	53.4	0.1014	101
0.05 BAS 654H Forage (1)	1	12-31-97	12-31-97	2	43.5	0.0422	84
0.05 BAS 654H Forage (1)	1	12-31-97	12-31-97	2	39.2	0.0375	75
0.05 BAS 654H Forage (2)	2	12-31-97	12-31-97	2	11.6	0.0434	87
0.05 BAS 654H Forage (2)	2	12-31-97	12-31-97	2	12.8	0.0479	96
0.05 BAS 654H Forage (13)	13	1-5-98	1-5-98	2	12.0	0.0486	97
0.1 BAS 654H Forage (1)	1	12-31-97	12-31-97	2	67.3	0.0689	69
0.1 BAS 654H Forage (1)	1	12-31-97	12-31-97	2	68.2	0.0699	70
0.1 BAS 654H Forage (2)	2	12-31-97	12-31-97	2	23.2	0.0896	90
0.1 BAS 654H Forage (2)	2	12-31-97	12-31-97	2	21.2	0.0816	82
Control, Fodder (3)	3	12-31-97	12-31-97	2	NA	NA	--
Control, Fodder (4)	4	12-31-97	12-31-97	2	NA	NA	--
Control, Fodder (14)	14	1-5-98	1-5-98	2	NA	NA	--
0.05 M1 Fodder (3)	3	12-31-97	12-31-97	2	25.4	0.0477	95
0.05 M1 Fodder (3)	3	12-31-97	12-31-97	2	27.0	0.0508	102
0.05 M1 Fodder (4)	4	12-31-97	12-31-97	2	89.5	0.0453	91
0.05 M1 Fodder (4)	4	12-31-97	12-31-97	2	93.2	0.0472	94
0.05 M1 Fodder (14)	14	1-5-98	1-5-98	2	23.3	0.0443	89
0.1 M1 Fodder (3)	3	12-31-97	12-31-97	2	52.7	0.1004	100
0.1 M1 Fodder (3)	3	12-31-97	12-31-97	2	52.5	0.1000	100
0.1 M1 Fodder (4)	4	12-31-97	12-31-97	2	183.8	0.0945	95
0.1 M1 Fodder (4)	4	12-31-97	12-31-97	2	174.5	0.0897	90
0.05 BAS 654H Fodder (3)	3	12-31-97	12-31-97	2	11.4	0.0431	86
0.05 BAS 654H Fodder (3)	3	12-31-97	12-31-97	2	11.6	0.0439	88
0.05 BAS 654H Fodder (4)	4	12-31-97	12-31-97	2	38.6	0.0389	78
0.05 BAS 654H Fodder (4)	4	12-31-97	12-31-97	2	36.0	0.0361	72
0.05 BAS 654H Fodder (14)	14	1-5-98	1-5-98	2	12.1	0.0419	84
0.1 BAS 654H Fodder (3)	3	12-31-97	12-31-97	2	21.7	0.0843	84
0.1 BAS 654H Fodder (3)	3	12-31-97	12-31-97	2	24.2	0.0940	94
0.1 BAS 654H Fodder (4)	4	12-31-97	12-31-97	2	70.2	0.0731	73
0.1 BAS 654H Fodder (4)	4	12-31-97	12-31-97	2	68.2	0.0709	71

Table 2. Individual Recovery Data of the Fortified BAS 654H and M1 in Corn matrices using GC-MS (continued)

Fortified Level ppm, Analyte, Matrix (Vial Number) ¹	Master Sheet # 97223- ²	Extract. Date	Injection Date	Final Volume (mL) ³	Peak Area (Count X10 ⁴) ⁴	Residue ppm ⁵	Rec %
Control, Grain (5)	5	1-2-98	1-2-98	2	NA	NA	--
Control, Grain (6)	6	1-2-98	1-2-98	2	NA	NA	--
Control, Grain (11)	11	1-5-98	1-5-98	2	NA	NA	--
0.05 M1 Grain (5)	5	1-2-98	1-2-98	2	104.9	0.0444	89
0.05 M1 Grain (5)	5	1-2-98	1-2-98	2	114.7	0.0486	97
0.05 M1 Grain (6)	6	1-2-98	1-2-98	2	23.8	0.0483	97
0.05 M1 Grain (6)	6	1-2-98	1-2-98	2	24.1	0.0489	98
0.05 M1 Grain (11)	11	1-5-98	1-5-98	2	75.6	0.0433	87
0.1 M1 Grain (5)	5	1-2-98	1-2-98	2	226.7	0.0973	97
0.1 M1 Grain (5)	5	1-2-98	1-2-98	2	217.0	0.0931	93
0.1 M1 Grain (6)	6	1-2-98	1-2-98	2	49.7	0.1039	104
0.1 M1 Grain (6)	6	1-2-98	1-2-98	2	48.6	0.1015	102
0.05 BAS 654H Grain (5)	5	1-2-98	1-2-98	2	52.2	0.0444	89
0.05 BAS 654H Grain (5)	5	1-2-98	1-2-98	2	52.0	0.0442	88
0.05 BAS 654H Grain (6)	6	1-2-98	1-2-98	2	10.6	0.0415	83
0.05 BAS 654H Grain (6)	6	1-2-98	1-2-98	2	11.4	0.0449	90
0.05 BAS 654H Grain (11)	11	1-5-98	1-5-98	2	36.2	0.0400	80
0.1 BAS 654H Grain (5)	5	1-2-98	1-2-98	2	95.4	0.0833	83
0.1 BAS 654H Grain (5)	5	1-2-98	1-2-98	2	93.1	0.0813	81
0.1 BAS 654H Grain (6)	6	1-2-98	1-2-98	2	21.4	0.0894	89
0.1 BAS 654H Grain (6)	6	1-2-98	1-2-98	2	19.6	0.0818	82
Control, Starch (7)	7	1-2-98	1-2-98	2	NA	NA	--
Control, Starch (8)	8	1-2-98	1-3-98	2	NA	NA	--
Control, Starch (12)	12	1-5-98	1-5-98	2	NA	NA	--
0.05 M1 Starch (7)	7	1-2-98	1-2-98	2	90.0	0.0394	79
0.05 M1 Starch (7)	7	1-2-98	1-2-98	2	103.6	0.0456	91
0.05 M1 Starch (8)	8	1-2-98	1-3-98	2	96.8	0.0485	97
0.05 M1 Starch (8)	8	1-2-98	1-3-98	2	92.2	0.0460	92
0.1 M1 Starch (7)	7	1-2-98	1-2-98	2	185.6	0.0831	83
0.1 M1 Starch (7)	7	1-2-98	1-2-98	2	184.9	0.0828	83
0.1 M1 Starch (8)	8	1-2-98	1-6-98	2	175.6	0.0912	91
0.1 M1 Starch (8)	8	1-2-98	1-3-98	2	173.6	0.0901	90
0.05 BAS 654H Starch (7)	7	1-2-98	1-2-98	2	42.4	0.0366	73
0.05 BAS 654H Starch (7)	7	1-2-98	1-2-98	2	45.2	0.0392	78
0.05 BAS 654H Starch (8)	8	1-2-98	1-3-98	2	43.0	0.0402	80
0.05 BAS 654H Starch (8)	8	1-2-98	1-3-98	2	31.1	0.0269	54
0.05 BAS 654H Starch (12)	12	1-5-98	1-5-98	2	31.1	0.0351	70
0.1 BAS 654H Starch (7)	7	1-2-98	1-2-98	2	75.8	0.0682	68
0.1 BAS 654H Starch (7)	7	1-2-98	1-2-98	2	79.3	0.0715	72
0.1 BAS 654H Starch (8)	8	1-2-98	1-3-98	2	73.0	0.0739	74
0.1 BAS 654H Starch (8)	8	1-2-98	1-3-98	2	81.0	0.0829	83
0.1 BAS 654H Starch (12)	12	1-5-98	1-5-98	2	64.3	0.0850	85
0.1 BAS 654H Starch (12)	12	1-5-98	1-5-98	2	53.9	0.0694	69

Table 2. Individual Recovery Data of the Fortified BAS 654H and M1 in Corn matrices using GC-MS (continued)

Fortified Level ppm, Analyte, Matrix (Vial Number) ¹	Master Sheet # 97223- ²	Extract. Date	Injection Date	Final Volume (mL) ³	Peak Area (Count X10 ⁴) ⁴	Residue ppm ⁵	Rec %
Control, Oil (9)	9	1-5-98	1-5-98	2	NA	NA	--
Control, Oil (10)	10	1-5-98	1-7-98	2	NA	NA	--
0.05 M1 Oil (9)	9	1-5-98	1-5-98	2	65.7	0.0450	90
0.05 M1 Oil (9)	9	1-5-98	1-5-98	2	55.9	0.0375	75
0.05 M1 Oil (10)	10	1-5-98	1-7-98	2	64.2	0.045	91
0.05 M1 Oil (10)	10	1-5-98	1-7-98	2	53.7	0.037	73
0.1 M1 Oil (9)	9	1-5-98	1-5-98	2	109.3	0.0785	79
0.1 M1 Oil (9)	9	1-5-98	1-5-98	2	97.7	0.0696	70
0.1 M1 Oil (10)	10	1-5-98	1-7-98	2	116.1	0.088	88
0.1 M1 Oil (10)	10	1-5-98	1-7-98	2	105.8	0.080	80
0.05 BAS 654H Oil (9)	9	1-5-98	1-5-98	2	30.7	0.0377	75
0.05 BAS 654H Oil (9)	9	1-5-98	1-5-98	2	31.4	0.0388	78
0.05 BAS 654H Oil (10)	10	1-5-98	1-7-98	2	28.6	0.034	67
0.05 BAS 654H Oil (10)	10	1-5-98	1-7-98	2	39.1	0.051	103
0.1 BAS 654H Oil (9)	9	1-5-98	1-5-98	2	55.2	0.0767	77
0.1 BAS 654H Oil (9)	9	1-5-98	1-5-98	2	51.8	0.0712	71
0.1 BAS 654H Oil (10)	10	1-5-98	1-7-98	2	57.8	0.083	83
0.1 BAS 654H Oil (10)	10	1-5-98	1-7-98	2	53.6	0.076	76

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 BASF study (97223) 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 0.017 ppm. If no signal was detected "ND" is listed in the table.

⁵See Figure 2 for an example calculation of the residue.

The following values were constant for all analyses:

- a) Sample size = 10.0 g for all matrices
- b) Injection volume = 4 μ L
- c) Aliquot = 6% for all matrices

Table 3. Summary of the Standard Data for M1 in Corn Matrices Using GC-MS.

Master ¹ Sheet No. 97223	Peak Area (Count x 10 ⁴)/Injection				Calibration Curve Data ²	
	10 pg	30 pg	80 pg	150 pg	Slope x 10 ⁴	Intercept x 10 ⁴
1	19.7, 21.0,18.5	51.3, 53.4, 58.7	128.1, 130.9,125.4	241.3, 241.5, 229.8	1.54	5.79
2	4.4, 5.5, 5.3	12.6, 14.1, 14.4	33.4, 37.3, 35.7	67.4, 64.8, 64.5	0.432	0.765
3	6.1, 4.6, 4.8	13.4, 14.1, 13.2	36.2, 35.2, 33.4	67.8, 67.2, 61.8	0.432	0.659
4	17.3, 19.4, 19.8	51.2, 51.6, 50.3	129.9, 129.0, 128.9	251.5, 239.6, 236.8	1.60	2.68
5	21.5, 22.5, 22.5	59.3, 64.5, 61.3	149.4, 156.0, 156.8	290.1, 290.8, 292.8	1.92	2.99
6	6.4, 5.4, 5.5	11.4, 12.8, 12.7	31.5, 32.6, 32.7	59.9, 59.9, 59.2	0.388	1.27
7	23.8, 22.2, 22.7	56.6, 60.2, 60.2	143.4, 147.5, 150.8	280.9, 272.2, 282.0	1.82	3.87
8	20.7, 26.5, 19.1	56.6, 54.8, 48.4	140.8, 128.9, 124.4	246.0, 238.0, 228.9	1.54	7.15
9	16.0, 18.4, 15.1	43.1, 40.3, 37.0	101.4, 92.4, 93.6	157.9, 178.7	1.09	7.05
10	16.4, 18.6, 19.7	40.8, 38.5, 37.1	96.1, 93.3, 89.8	153.9, 165.2, 160.8	1.02	8.96
11	23.5, 16.2	37.4, 47.6	117.4, 114.2	206.9, 210.9	1.37	4.59
12	22.1, 17.3	43.8, 39.0	98.3, 102.4	173.6, 185.9	1.15	7.86
13	4.9, 4.6	12.1, 12.9	33.8, 33.5	64.2, 62.1	0.419	0.238
14	10.6, 5.5	11.8, 12.7	32.7, 34.6	61.8, 61.8	0.393	2.41

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:

$$(\text{pg}) \text{ M1} = \frac{\text{Peak Area} - \text{Intercept}}{\text{Slope}}$$

TABLE 4. Individual Data for Residue Samples in Corn Matrices

Site Number	Vial Number ¹	Master Sheet Number (97223--) ²	Final Volume (mL) ³	Peak Area (Count) ⁴	Net Residue Equivalent (ppm) ⁵
Forage (350-95-50-)					
606-01-04-001-1	24	1	2	273627	0.024
606-01-04-001-1	14	2	2	25322	<0.017 (0.0071)
607-01-04-001-1	24	13	2	ND	<0.017
662-01-04-001-1	25	13	2	ND	<0.017
Fodder (350-95-50-)					
660-01-04-003-1	24	3	2	86520	0.032
660-01-04-003-1	14	4	2	324902	0.032
606-01-04-003-2	18	14	2	33436	<0.017 (0.004)
624-01-04-003-2	19	14	2	52808	<0.017 (0.013)
664-01-04-003-1	20	14	2	ND	<0.017
Grain					
97375-17	14	5	2	ND	<0.017
97375-17	14	6	2	140954	0.057 ⁶
97375-17	23	11	2	ND	<0.017
97375-17	24	11	2	ND	<0.017
Starch					
97375-0021	24	7	2	ND	<0.017
97375-0021	24	8	2	ND	<0.017
Refined Oil					
97375-0023	14	9	2	ND	<0.017
97375-0023	14	10	2	ND	<0.017

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 BASF study (97223) 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 0.017 ppm. If no signal was detected "ND" is listed in the table.

⁵See Figure 2 for an example calculation of the residue.

⁶The residue value is indicated a contaminated sample

The following values were constant for all analyses:

- a) Sample size = 10.0 g for all matrices
- b) Injection volume = 4 μ L
- c) Aliquot = 6% for all matrices

APPENDIX A

Changes to Protocol Number 95164

APPENDIX A
changes to Protocol Number 97233

During the course of the study, one change to the protocol was documented.

Amendments

- a) Correction of fodder control sample number.
- b) Sample set may contain one control, treated sample(s), control fortified with BAS 654H at 0.05 and or 0.1 ppm and (or) control fortified with M1 at 0.05 and or 0.1 ppm level

Reason:

- a) Misprint in the original protocol
- b) More sets with different spiking scheme was needed for the validation of the method

APPENDIX B

Typical Raw Data and Chromatograms

Description

- Figure 1 Typical GC-MS parameters from master sheet number 97223.
- Figure 2 Typical chromatogram of a 10 pg standard of M1 from master sheet number 97223. Data from this standard can be found in Table 3.
- Figure 3 Typical chromatogram of a 30 pg standard of M1 from master sheet number 97223-4. Data from this standard can be found in Table 3.
- Figure 4 Typical chromatogram of a 80 pg standard of M1 from master sheet number 97223-4. Data from this standard can be found in Table 3.
- Figure 5 Typical chromatogram of a 150 pg standard of M1 from master sheet number 97223-4. Data from this standard can be found in Table 3.
- Figure 6 Typical standard Curve for 10, 30, 80 and 150 pg amounts of M1 from master sheet number 97223-4. Data from these standards can be found in Table 3
- Figure 7 Typical chromatogram of a control corn fodder sample. Vial number 5 from master sheet number 97223-4. Data for this sample can be found in Table 2.
- Figure 8 Typical chromatogram of a control corn fodder sample fortified with 0.05 ppm of M1. Vial number 6 from master sheet number 97223-4. Data for this sample can be found in Table 2. Recovery 91%.
- Figure 9 Typical chromatogram of a control corn fodder sample fortified with 0.1 ppm of BAS 654H. Vial number 12 from master sheet number 97223-4. Data for this sample can be found in Table 2. Recovery 73%.
- Figure 10 Typical chromatogram of a control corn forage sample. Vial number 5 from master sheet number 97223-2. Data for this sample can be found in Table 2.
- Figure 11 Typical chromatogram of a control corn forage sample fortified with 0.05 ppm of BAS 654H. Vial number 10 from master sheet number 97223-2. Data for this sample can be found in Table 2. Recovery 87%.
- Figure 12 Typical chromatogram of a control corn forage sample fortified with 0.1 ppm of M1. Vial number 8 from master sheet number 97223-2. Data for this sample can be found in Table 2. Recovery 102%.
- Figure 13 Typical chromatogram of a control corn grain sample. Vial number 5 from master sheet number 97223-6. Data for this sample can be found in Table 2.
- Figure 14 Typical chromatogram of a control corn grain sample fortified with 0.05 ppm of M1. Vial number 6 from master sheet number 97223-6. Data for this sample can be found in Table 2. Recovery 97%.
- Figure 15 Typical chromatogram of a control corn grain sample fortified with 0.1 ppm of BAS 654H. Vial number 12 from master sheet number 97223-6. Data for this sample can be found in Table 2. Recovery 89%.
- Figure 16 Typical chromatogram of a control corn starch sample. Vial number 15 from master sheet number 97223-7. Data for this sample can be found in Table 2
- Figure 17 Typical chromatogram of a control corn starch sample fortified with 0.05 ppm of M1. Vial number 16 from master sheet number 97223-7. Data for this sample can be found in Table 2. Recovery 79%.

- Figure 18 Typical chromatogram of a control corn starch sample fortified with 0.1 ppm of BAS 654H. Vial number 22 from master sheet number 97223-7. Data for this sample can be found in Table 2. Recovery 68%.
- Figure 19 Typical chromatogram of a control corn refined oil sample. Vial number 5 from master sheet number 97223-10. Data for this sample can be found in Table 2
- Figure 20 Typical chromatogram of a control corn refined oil sample fortified with 0.1 ppm of M1. Vial number 8 from master sheet number 97223-10. Data for this sample can be found in Table 2. Recovery 88%.
- Figure 21 Typical chromatogram of a control corn refined oil sample fortified with 0.05 ppm of BAS 654H. Vial number 10 from master sheet number 97223-10. Data for this sample can be found in Table 2. Recovery 67%.
- Figure 22 Typical GC-MS (scan) chromatogram/mass spectrum of 5 ng standard of M1.

Figure 1. Typical GC-MS parameters from master sheet number 97223.

TOPLEVEL PARAMETERS

Method Information For: C:\HPCHEM\1\METHODS\1231M1FD.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 SAN654H M1; GCMSD 1238; RESTEK
STABILWAX DA COL.; CAT# 11023; SN 129537

END OF TOPLEVEL PARAMETERS

ACQUISITION PARAMETERS

General Information

Inlet : GC
Tune File : 112697.U
Acquisition Mode : Sim

MS Information

Solvent Delay : 6.50 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 : No
Group Start Time : 6.50
Int 1 Ion : 161.00
Ions In Group : 161.00

Method: 1231M1FD.M

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1.6.98
EK

Figure 1. Typical GC-MS parameters from master sheet number 97223.

[Real Time Plot Parameters]

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

1-6-98
EK

GC Inlet Information

[Inlet A Temperature Program Information]

Oven Track : Off
Initial Temp. : 250 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 : Off
Inlet B Off

[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	25.0	18.00
2	99.00	10.0	5.00
3	0		

Total Program Time: 23.90 min
Pressure Units : psi

[Inlet A Flow Settings]

Column length : 30.00 m
Column diameter : 0.250 mm
Gas : He
Vacuum compensation : On
Pressure : 0.0 psi
Flow : 0.0 ml/min
Linear velocity : 0.0 cm/sec

[Inlet B Pressure Program Information]

Constant Flow : Off

Figure 1. Typical GC-MS parameters from master sheet number 97223.

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
Pressure Units : psi

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EK

[Inlet B Flow Settings]

Column length : 30.00 m
Column diameter : 0.530 mm
Gas : He
Vacuum compensation : On
Pressure : 0.0 psi
Flow : 0.0 ml/min
Linear velocity : 0.0 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 97223.

[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

1.6.98
EX

GC Temperature Information

[GC Zone Temperatures]

Inj. A : 250 C
Inj. B : 250 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. : 120 C
Initial Time : 0.50 min

Level	Rate (C/min)	Final Temp. (C)	Final Time (min)
1	70.00	250	11.64
2	0.00		

Next Run Time : 14.00 min

Injector Information

Injection Source : Auto
Injection Location : Front

Sample Washes : 1

Figure 1. Typical GC-MS parameters from master sheet number 97223.

Sample Pumps : 2
Sample Volume : 4 stop(s)
Viscosity Delay : 0 sec
Solvent A Washes : 3
Solvent B Washes : 2
C Column : No

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EK

[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)
6.50	On
15.00	Off

END OF ACQUISITION PARAMETERS

DATA ANALYSIS PARAMETERS

Method Name: C:\HPCHEM\1\METHODS\1231M1FD.M

Percent Report Settings

Sort By: Signal

Output Destination

Screen: No
Printer: Yes
File: No

Integration Events: 654.E

Generate Report During Run Method: No

Signal Correlation Window: 0.020

Qualitative Report Settings

Method: 1231M1FD.M

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Figure 1. Typical GC-MS parameters from master sheet number 97223.

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

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EK

Quantitative Report Settings

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

bas 654h
Calibration Last Updated: Thu Jan 01 07:44:36 1998

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) BAS654 ()

Ret. Time 9.32 min., Extract & Integrate from 8.82 to 9.82 min.

Signal Rel Resp. Pct. Unc.(rel) Integration
Tgt 161.00 654.E

ID	Conc (pg)	Response
.	10.000	172670
1B	10.000	193796
1C	10.000	198087

Figure 1. Typical GC-MS parameters from master sheet number 97223.

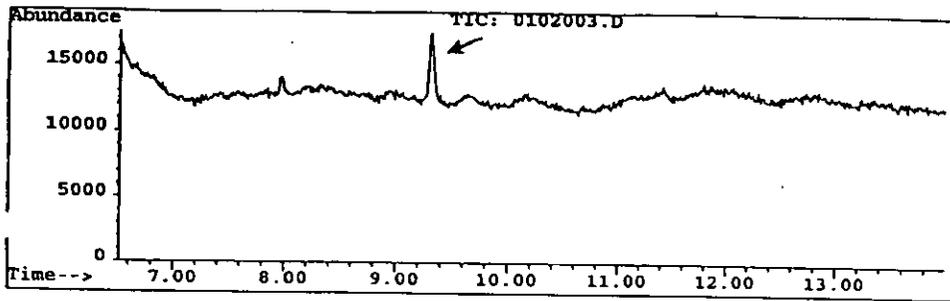
2A	30.000	511911
2B	30.000	516259
2C	30.000	502999
3A	80.000	1298606
3B	80.000	1290118
3C	80.000	1289006
4	150.000	2515269
4B	150.000	2396555
4C	150.000	2368592

1.6.98
EK

Qualifier Peak Analysis ON
Curve Fit: Linear

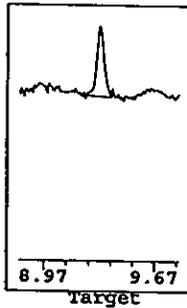
END OF DATA ANALYSIS PARAMETERS

Figure 2. Typical chromatogram of a 10 pg standard of M1 from master sheet number 97223. Data from this standard can be found in Table 3.



File : C:\HPCHEM\1\DATA\GC1238\BAS654H\123197\0102003.D
 Operator :
 Acquired : 31 Dec 97 4:30 pm using AcqMethod 1231M1FD
 Sample Name: 10PG/4UL M1 STD
 Misc Info :
 Vial Number: 1
 CurrentMeth: C:\HPCHEM\1\METHODS\1231M1FD.M

Compound: BAS654
 Ret Time: 9.32
 Concentration: 9.14 pg
 Pk # and Type: 1



	Signal	Ratio	Limits		RT	Limits	Resp	Integ Type
Tgt	161.00	100.0%			9.32	9.09	172669	654
Q1	0.00	0.0	0.0-	0.0	0.00	to	0	654
Q2	0.00	0.0	0.0-	0.0	0.00	9.55	0	654
Q3	0.00	0.0	0.0-	0.0	0.00		0	654

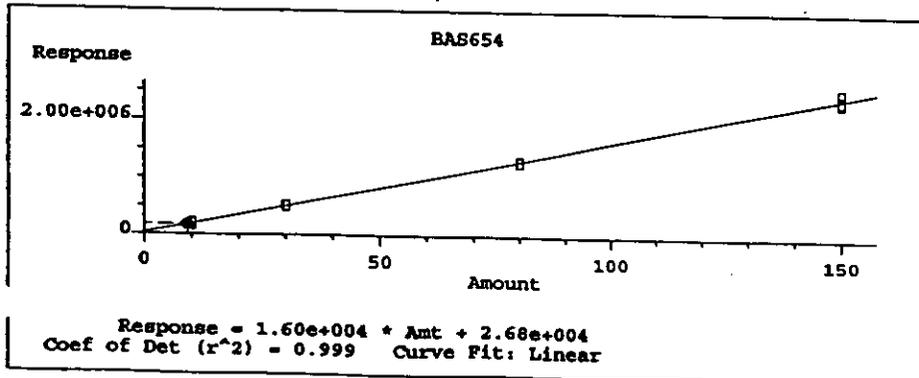
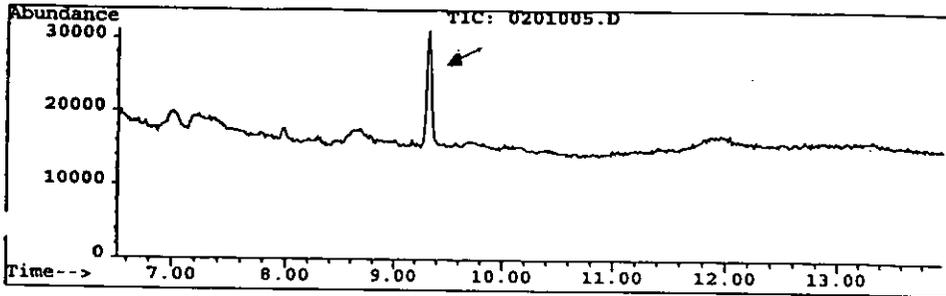
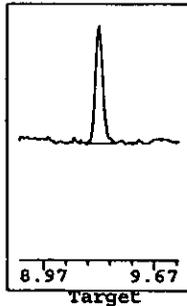


Figure 3. Typical chromatogram of a 30 pg standard of M1 from master sheet number 97223-4. Data from this standard can be found in Table 3.



File : C:\HPCHEM\1\DATA\GC1238\BAS654H\123197\0201005.D
 Operator :
 Acquired : 31 Dec 97 5:04 pm using AcqMethod 1231M1FD
 Sample Name: 30PG/4UL M1 STD
 Misc Info :
 Vial Number: 2
 CurrentMeth: C:\HPCHEM\1\METHODS\1231M1FD.M

Compound: BAS654
 Ret Time: 9.33
 Concentration: 30.39 pg
 Pk # and Type: 1



	Signal	Ratio	Limits	RT	Limits	Resp	Integ Type
Tgt	161.00	100.0%		9.33	9.09	511911	654
Q1	0.00	0.0	0.0- 0.0	0.00	to	0	654
Q2	0.00	0.0	0.0- 0.0	0.00	9.55	0	654
Q3	0.00	0.0	0.0- 0.0	0.00		0	654

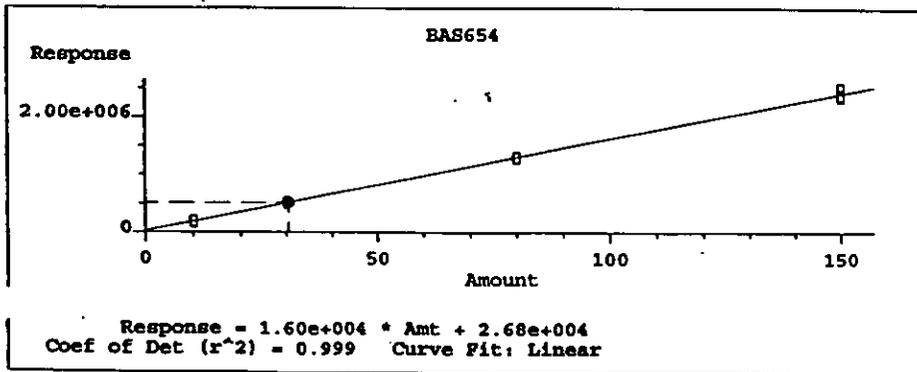
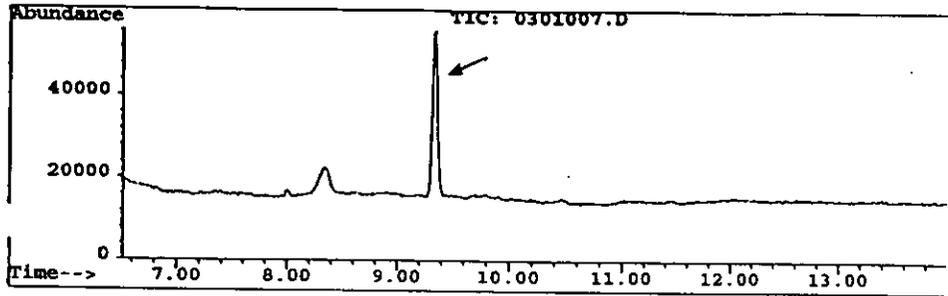
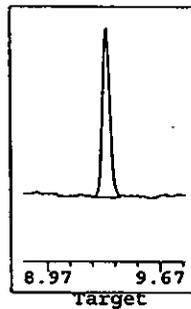


Figure 4. Typical chromatogram of a 80 pg standard of M1 from master sheet number 97223-4. Data from this standard can be found in Table 3.



File : C:\HPCHEM\1\DATA\GC1238\BAS654H\123197\0301007.D
 Operator :
 Acquired : 31 Dec 97 5:38 pm using AcqMethod 1231M1FD
 Sample Name: 80PG/4UL M1 STD
 Misc Info :
 Vial Number: 3
 CurrentMeth: C:\HPCHEM\1\METHODS\1231M1FD.M

Compound: BAS654
 Ret Time: 9.34
 Concentration: 79.66 pg
 Pk # and Type: 1



	Signal	Ratios	Limits	RT	Limits	Resp	Integ Type
Tgt	161.00	100.0%		9.34	9.09	1298605	654
Q1	0.00	0.0	0.0- 0.0	0.00	to	0	654
Q2	0.00	0.0	0.0- 0.0	0.00	9.55	0	654
Q3	0.00	0.0	0.0- 0.0	0.00		0	654

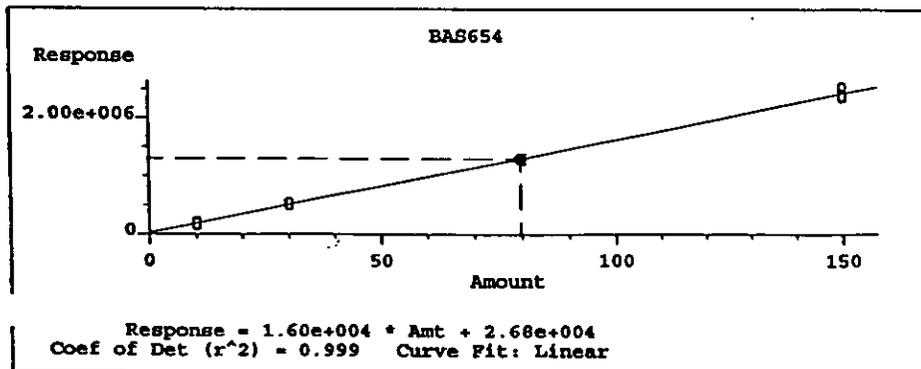
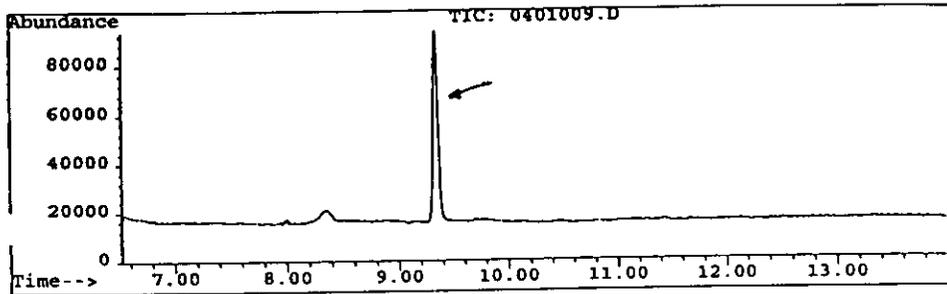
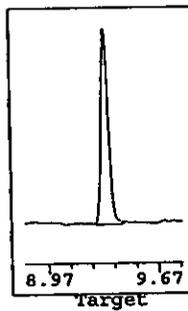


Figure 5. Typical chromatogram of a 150 pg standard of M1 from master sheet number 972234. Data from this standard can be found in Table 3.



File : C:\HPCHEM\1\DATA\GC1238\BAS654H\123197\0401009.D
 Operator :
 Acquired : 31 Dec 97 6:12 pm using AcqMethod 1231M1FD
 Sample Name: 150PG/4UL M1 STD
 Misc Info :
 Vial Number: 4
 CurrentMeth: C:\HPCHEM\1\METHODS\1231M1FD.M

Compound: BAS654
 Ret Time: 9.34
 Concentration: 155.86 pg
 Pk # and Type: 1



	Signal	Ratio	Limits		RT	Limits	Resp	Integ	Type
Tgt	161.00	100.0%			9.34	9.09	2515268	654	
Q1	0.00	0.0	0.0-	0.0	0.00	to	0	654	
Q2	0.00	0.0	0.0-	0.0	0.00	9.55	0	654	
Q3	0.00	0.0	0.0-	0.0	0.00		0	654	

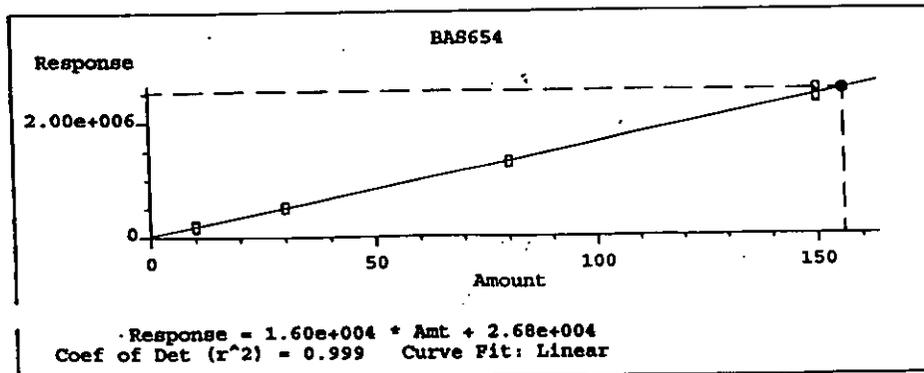
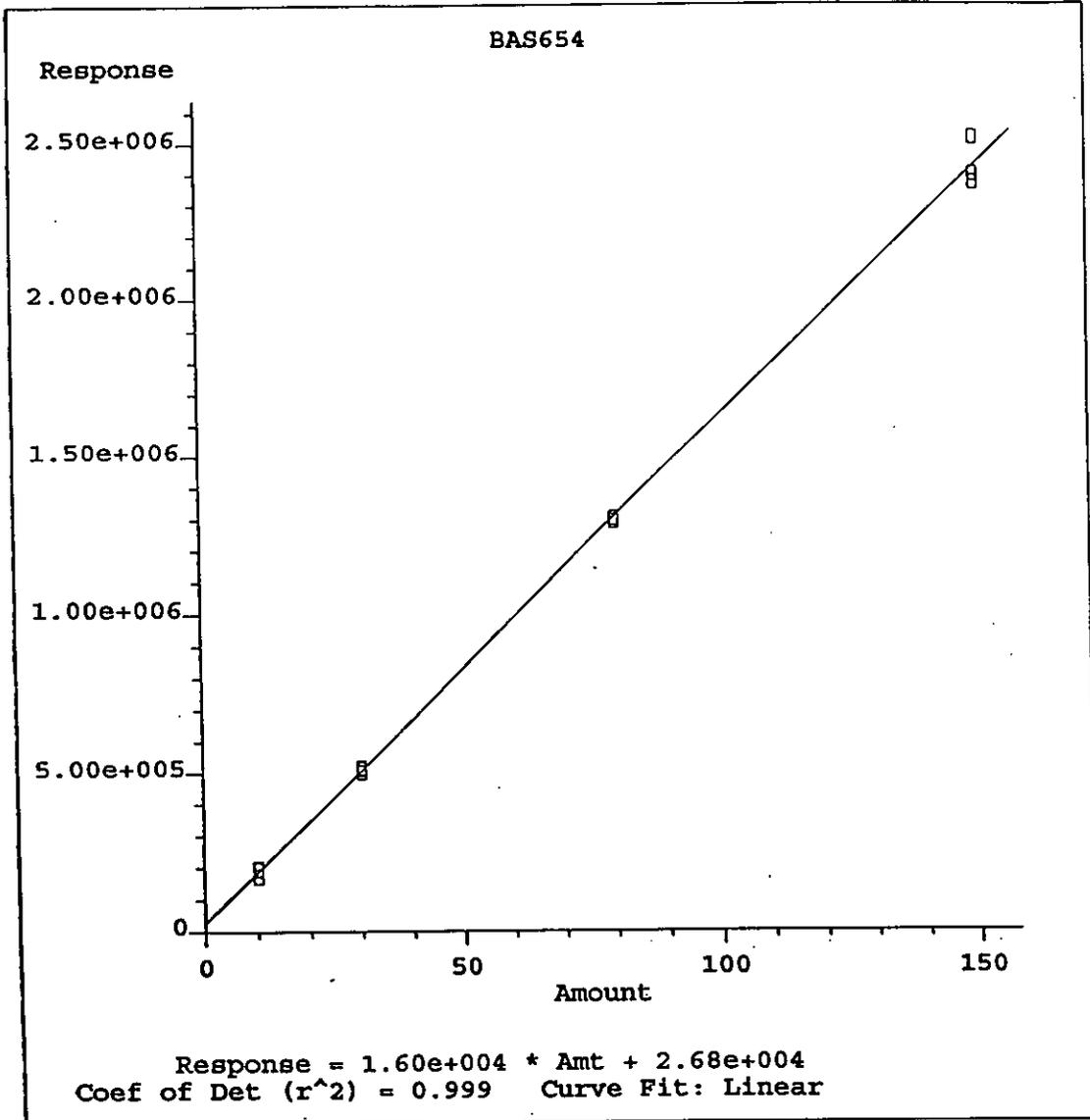


Figure 6. Typical standard Curve for 10, 30, 80 and 150 pg amounts of M1 from master sheet number 97223-4. Data from these standards can be found in Table 3.

1.6.98
EK



Method Name: C:\HPCHEM\1\METHODS\1231M1FD.M
Calibration Table Last Updated: Thu Jan 01 07:44:36 1998

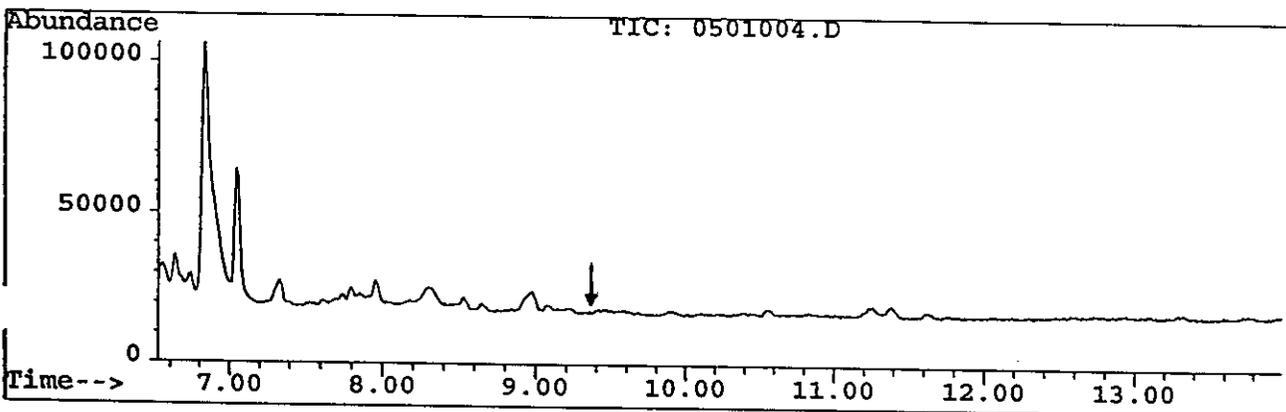
Figure 7. Typical chromatogram of a control corn fodder sample. Vial number 5 from master sheet number 97223-4. Data for this sample can be found in Table 2.

Area Percent Report -- Sorted by Signal

1-6-98
EK

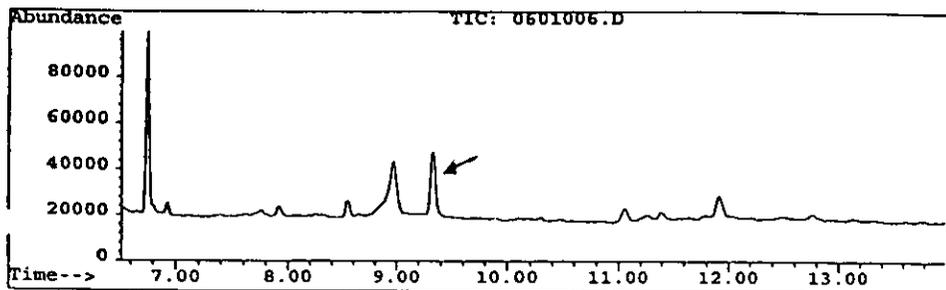
Information from Data File:

File : C:\HPCHEM\1\DATA\GC1238\BAS654H\123197\0501004.D
Operator :
Acquired : 31 Dec 97 4:47 pm using AcqMethod 1231M1FD
Sample Name: EK, CONTROL FODDER
Misc Info :
Vial Number: 5
CurrentMeth: C:\HPCHEM\1\METHODS\1231M1FD.M



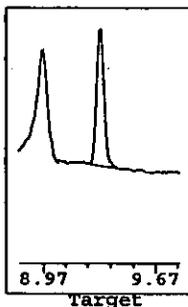
Retention Time	Area	Area %	Ratio %
Total Ion Chromatogram			
9.215	42404	5.590	32.139
9.451	56962	7.509	43.172
9.583	51345	6.769	38.915
9.888	33012	4.352	25.020
9.914	42225	5.566	32.002
10.115	30155	3.975	22.855
10.398	59088	7.789	44.783
10.466	41228	5.435	31.247
10.554	131942	17.393	100.000
10.717	84628	11.156	64.140
10.832	72621	9.573	55.040
10.886	57362	7.562	43.475
10.956	55618	7.332	42.154

Figure 8. Typical chromatogram of a control corn fodder sample fortified with 0.05 ppm of M1. Vial number 6 from master sheet number 97223-4. Data for this sample can be found in Table 2. Recovery 91%.



File : C:\HPCHEM\1\DATA\GC1238\BAS654H\123197\0601006.D
 Operator :
 Acquired : 31 Dec 97 5:21 pm using AcqMethod 1231M1FD
 Sample Name: EK, CONTROL FODDER + 0.05 A M1
 Misc Info :
 Vial Number: 6
 CurrentMeth: C:\HPCHEM\1\METHODS\1231M1FD.M

Compound: BAS654
 Ret Time: 9.33
 Concentration: 54.41 pg
 Pk # and Type: 1



	Signal	Ratios	Limits	RT	Limits	Resp	Integ Type
Tgt	161.00	100.0%		9.33	9.09	895445	654
Q1	0.00	0.0	0.0- 0.0	0.00	to	0	654
Q2	0.00	0.0	0.0- 0.0	0.00	9.55	0	654
Q3	0.00	0.0	0.0- 0.0	0.00		0	654

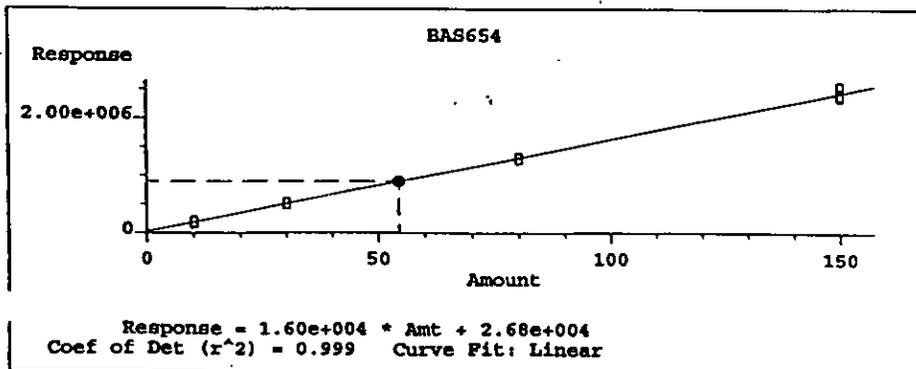
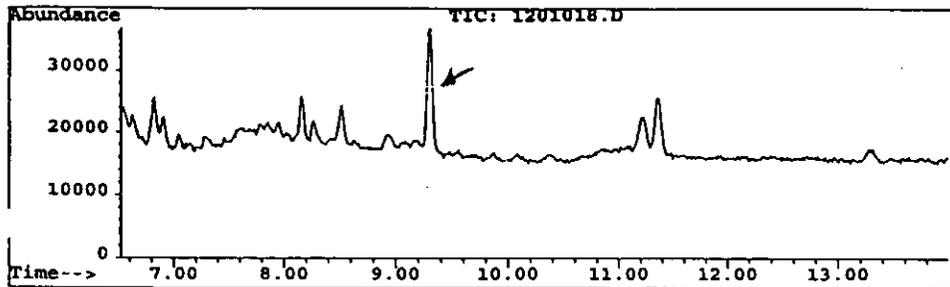
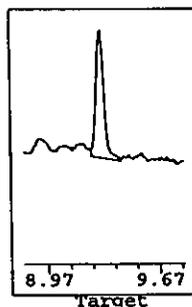


Figure 9. Typical chromatogram of a control corn fodder sample fortified with 0.1 ppm of BAS 654H. Vial number 12 from master sheet number 97223-4. Data for this sample can be found in Table 2. Recovery 73%.



File : C:\HPCHEM\1\DATA\GC1238\BAS654H\123197\1201018.D
 Operator :
 Acquired : 31 Dec 97 8:45 pm using AcqMethod 1231M1FD
 Sample Name: EK, CONTROL FODDER + 0.1 A PT
 Misc Info :
 Vial Number: 12
 CurrentMeth: C:\HPCHEM\1\METHODS\1231M1FD.M

Compound: BAS654
 Ret Time: 9.31
 Concentration: 42.30 pg
 Pk # and Type: 1



	Signal	Ratios	Limits	RT	Limits	Resp	Integ Type
Tgt	161.00	100.0%		9.31	9.09	702189	654
Q1	0.00	0.0	0.0- 0.0	0.00	to 0.00		654
Q2	0.00	0.0	0.0- 0.0	0.00	9.55		654
Q3	0.00	0.0	0.0- 0.0	0.00			654

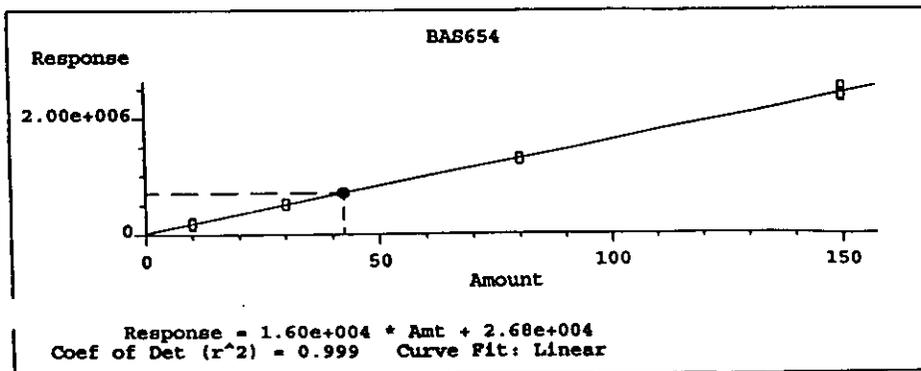


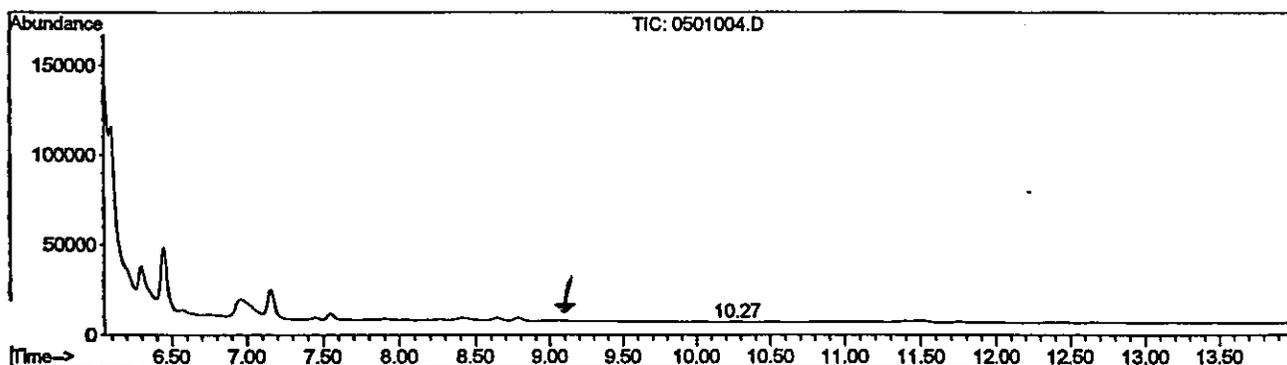
Figure 10. Typical chromatogram of a control corn forage sample. Vial number 5 from master sheet number 97223-2. Data for this sample can be found in Table 2

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1/6/98

Area Percent Report -- Sorted by Signal

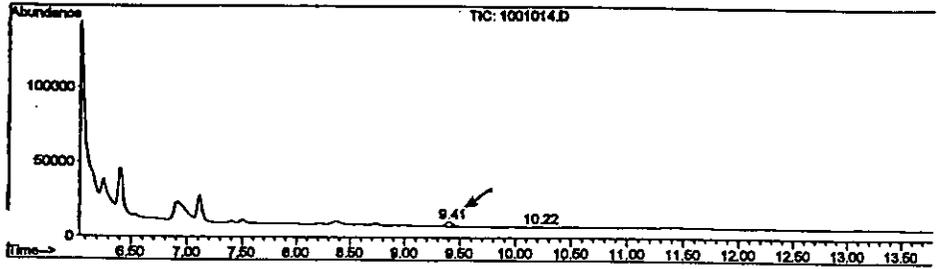
Information from Data File:

File : C:\HPCHEM\1\DATA\BAS654\GC1244\123197\0501004.D
Operator :
Acquired : 31 Dec 97 6:03 pm using AcqMethod 1231M1FG
Sample Name: RT, CONTROL FORGE
Misc Info :
Vial Number: 5
CurrentMeth: C:\HPCHEM\1\METHODS\1231M1FG.M



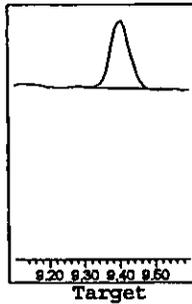
Retention Time	Area	Area %	Ratio %
Total Ion Chromatogram			
10.266	25308	100.000	100.000

Figure 11. Typical chromatogram of a control corn forage sample fortified with 0.05 ppm of BAS 654H. Vial number 10 from master sheet number 97223-2. Data for this sample can be found in Table 2. Recovery 87%.



File : C:\HPCHEM\1\DATA\BAS654\GC1244\123197\1001014.D
 Operator :
 Acquired : 31 Dec 97 8:55 pm using AcqMethod 1231M1PG
 Sample Name: RT, CONTROL FORGE + 0.05 A PT
 Misc Info :
 Vial Number: 10
 CurrentMeth: C:\HPCHEM\1\METHODS\1231M1PG.M

Compound: BAS-654H M1
 Ret Time: 9.41
 Concentration: 25.12
 Pk # and Type: 1



	Signal	Ratios	Limits	RT	Limits	Resp	Integ Type
Tgt	161.00	100.0%		9.41	9.16	116261	654
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	9.64	0	auto
Q3	0.00	0.0	0.0- 0.0	0.00		0	auto

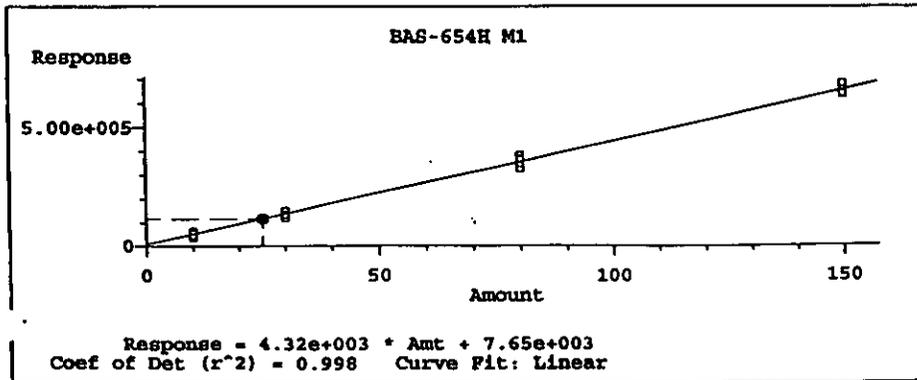
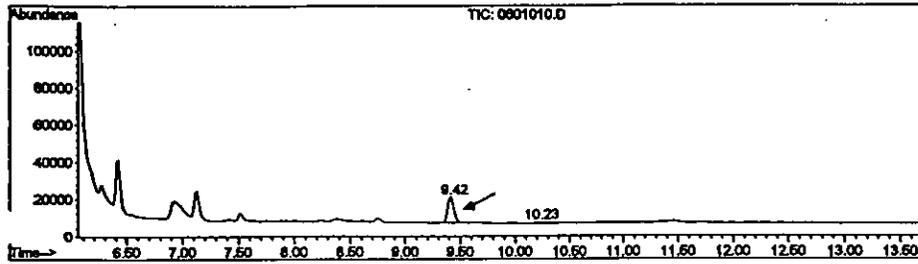


Figure 12. Typical chromatogram of a control corn forage sample fortified with 0.1 ppm of M1. Vial number 8 from master sheet number 97223-2. Data for this sample can be found in Table 2. Recovery 102%.



File : C:\HPCHEM\1\DATA\BAS654\GC1244\123197\0801010.D
 Operator :
 Acquired : 31 Dec 97 7:46 pm using AcqMethod 1231M1PG
 Sample Name: RT, CONTROL FORGE + 0.1 A M1
 Misc Info :
 Vial Number: 8
 CurrentMeth: C:\HPCHEM\1\METHODS\1231M1PG.M

Compound: BAS-654H M1
 Ret Time: 9.42
 Concentration: 122.49
 Pk # and Type: 1



	Signal	Ratios	Limits	RT	Limits	Resp	Integ Type
Tgt	161.00	100.0%		9.42	9.16	537285	654
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	9.64	0	auto
Q3	0.00	0.0	0.0- 0.0	0.00		0	auto

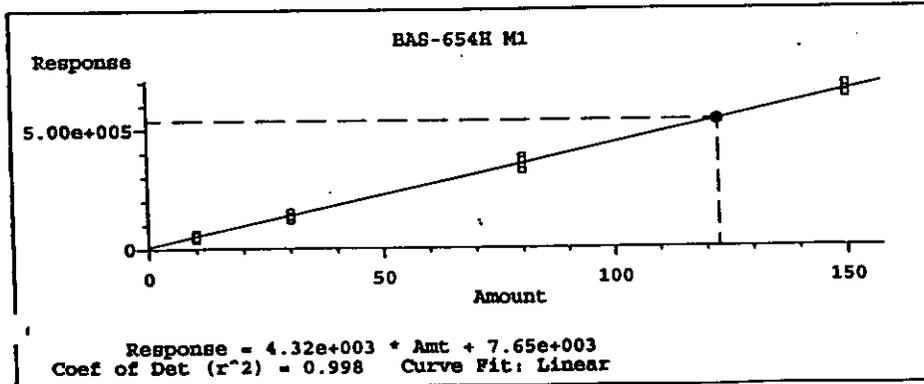


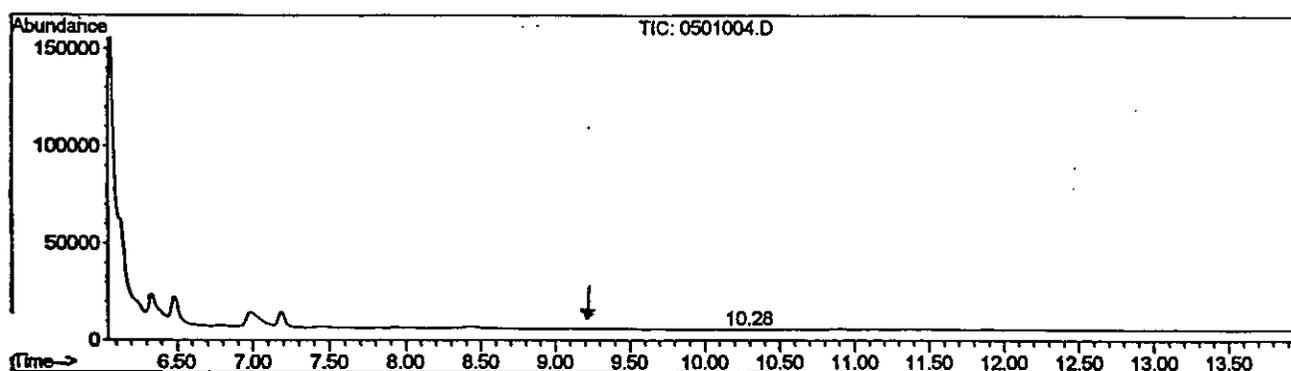
Figure 13. Typical chromatogram of a control corn grain sample. Vial number 5 from master sheet number 97223-6. Data for this sample can be found in Table 2.

DEP
1/6/9

Area Percent Report -- Sorted by Signal

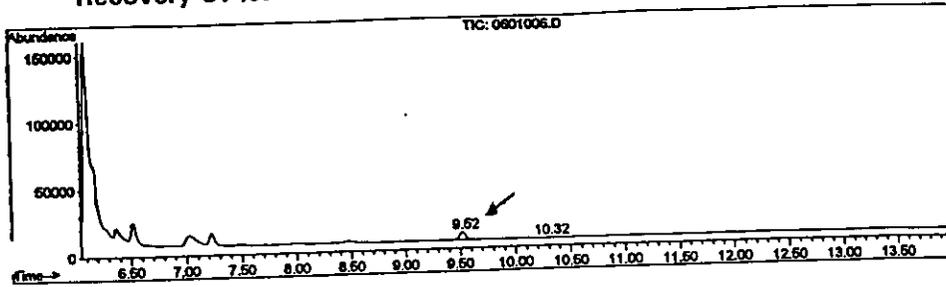
Information from Data File:

File : C:\HPCHEM\1\DATA\BAS654\GC1244\010298\0501004.D
Operator :
Acquired : 2 Jan 98 5:34 pm using AcqMethod 0102M1GN
Sample Name: DEP; CNTRL GRAIN
Misc Info :
Vial Number: 5
CurrentMeth: C:\HPCHEM\1\METHODS\0102M1GN.M



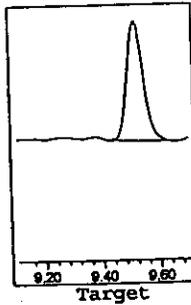
Retention Time	Area	Area %	Ratio %
Total Ion Chromatogram			
10.285	25102	100.000	100.000

Figure 14. Typical chromatogram of a control corn grain fortified with 0.05 of M1. Vial number 6 from master sheet number 97223-6. Data for this sample can be found in Table 2. Recovery 97%.



File : C:\HPCHEM\1\DATA\BAS654\GC1244\010298\0601006.D
 Operator :
 Acquired : 2 Jan 98 6:09 pm using AcqMethod 0102M1GN
 Sample Name: DEP; GRAIN; 0.05PPM M1 A
 Misc Info :
 Vial Number: 6
 CurrentMeth: C:\HPCHEM\1\METHODS\0102M1GN.M

Compound: BAS-654H M1
 Ret Time: 9.52
 Concentration: 57.93
 Pk # and Type: 1



	Signal	Ratios	Limits	RT	Limits	Resp	Integ Type
Tgt	161.00	100.0%		9.52	9.16	237774	654
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	9.64	0	auto
Q3	0.00	0.0	0.0- 0.0	0.00		0	auto

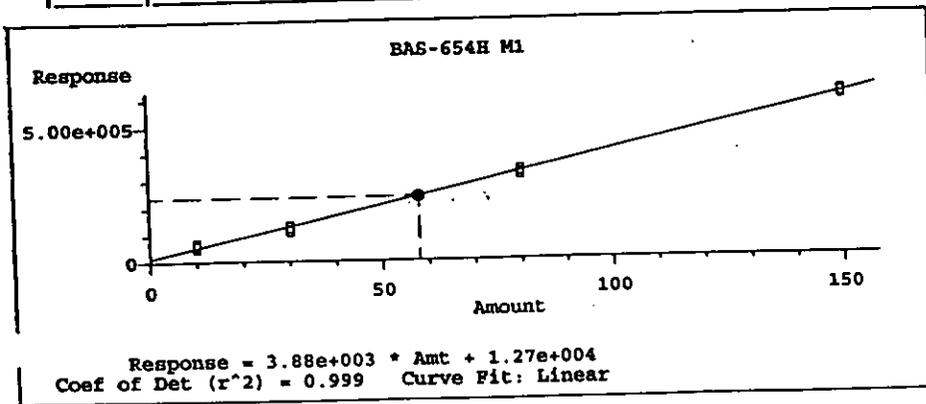
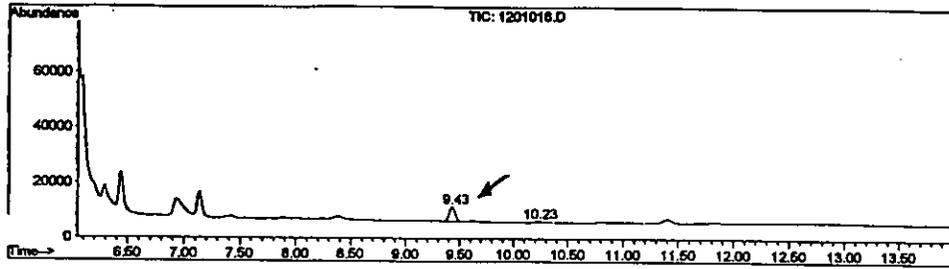
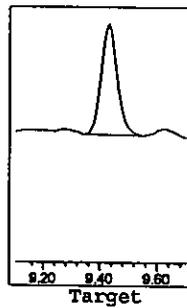


Figure 15. Typical chromatogram of a control corn grain sample fortified with 0.1 ppm of BAS 654H. Vial number 12 from master sheet number 97223-6. Data for this sample can be found in Table 2. Recovery 89%.



File : C:\HPCHEM\1\DATA\BAS654\GC1244\010298\1201018.D
 Operator :
 Acquired : 2 Jan 98 9:35 pm using AcqMethod 0102M1GN
 Sample Name: DEP; GRAIN; 0.1PPM PT A
 Misc Info :
 Vial Number: 12
 CurrentMeth: C:\HPCHEM\1\METHODS\0102M1GN.M

Compound: BAS-654H M1
 Ret Time: 9.44
 Concentration: 51.70
 Pk # and Type: 1



	Signal	Ratios	Limits	RT	Limits	Resp	Integ Type
Tgt	161.00	100.0%		9.44	9.16	213580	654
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	9.64	0	auto
Q3	0.00	0.0	0.0- 0.0	0.00		0	auto

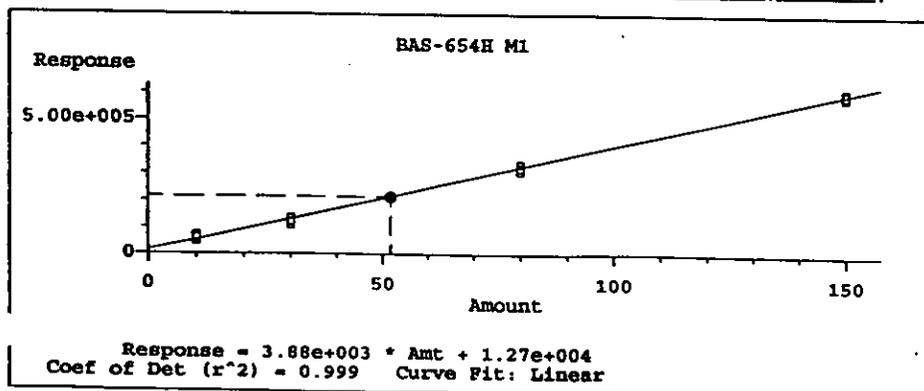


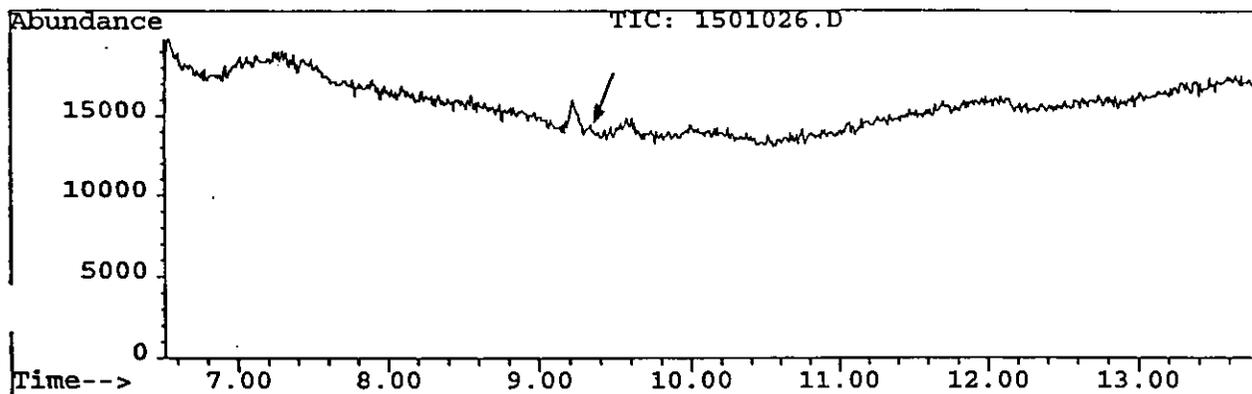
Figure 16. Typical chromatogram of a control corn starch sample. Vial number 15 from master sheet number 97223-7. Data for this sample can be found in Table 2.

1.6.98

Area Percent Report -- Sorted by Signal

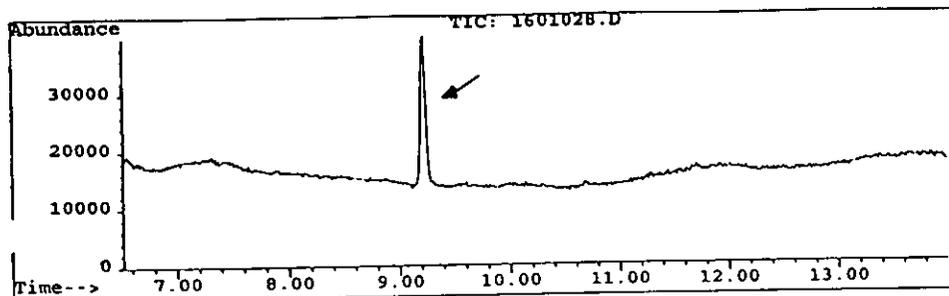
Information from Data File:

File : C:\HPCHEM\1\DATA\GC1238\BAS654H\010298\1501026.D
Operator :
Acquired : 2 Jan 98 9:37 pm using AcqMethod 0102M1ST
Sample Name: EK, CONTROL STARCH
Misc Info :
Vial Number: 15
CurrentMeth: C:\HPCHEM\1\METHODS\0102M1ST.M



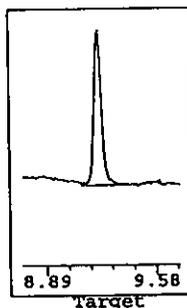
Retention Time	Area	Area %	Ratio %
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Figure 17 Typical chromatogram of a control corn starch sample fortified with 0.05 ppm of M1. Vial number 16 from master sheet number 97223-7. Data for this sample can be found in Table 2. Recovery 79%.



File : C:\HPCHEM\1\DATA\GC1238\BAS654H\010298\1601028.D
 Operator :
 Acquired : 2 Jan 98 10:11 pm using AcqMethod 0102M1ST
 Sample Name: EK, CONTROL STARCH + 0.05 A M1
 Misc Info :
 Vial Number: 16
 CurrentMeth: C:\HPCHEM\1\METHODS\0102M1ST.M

Compound: BAS654
 Ret Time: 9.23
 Concentration: 47.28 pg
 Pk # and Type: 1



	Signal	Ratios	Limits	RT	Limits	Resp	Integ	Type
Tot	161.00	100.0%		9.23	9.01	900228	654	
Q1	0.00	0.0	0.0- 0.0	0.00	to	0	654	
Q2	0.00	0.0	0.0- 0.0	0.00	9.47	0	654	
Q3	0.00	0.0	0.0- 0.0	0.00		0	654	

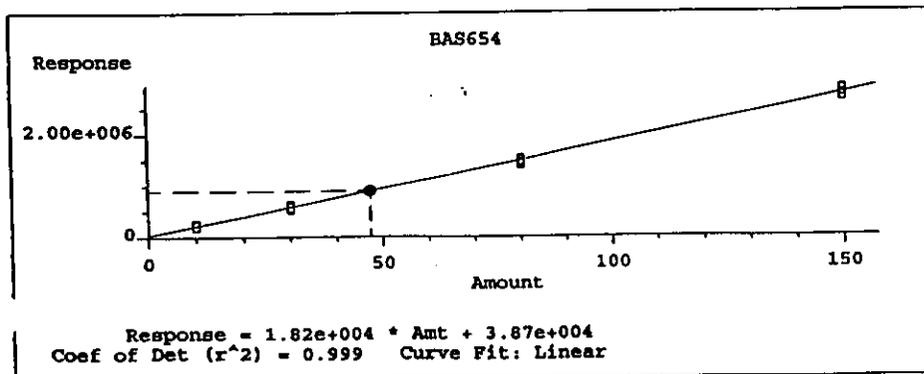
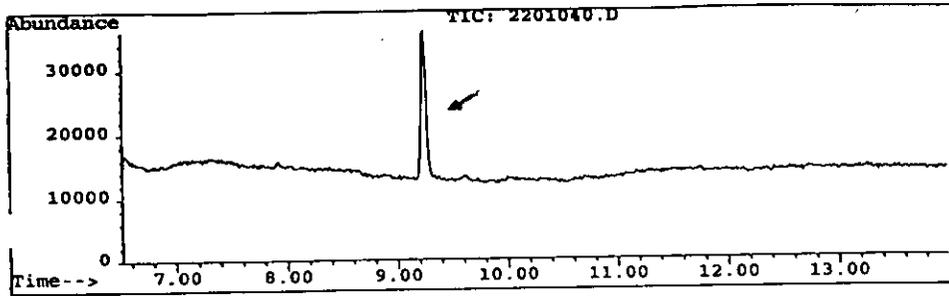


Figure 18. Typical chromatogram of a control corn starch sample fortified with 0.1 ppm of BAS 654H. Vial number 22 from master sheet number 97223-7. Data for this sample can be found in Table 2. Recovery 68%.



File : C:\HPCHEM\1\DATA\GC1238\BAS654H\010298\2201040.D
 Operator :
 Acquired : 3 Jan 98 1:36 am using AcqMethod 0102M1ST
 Sample Name: EK, CONTROL STARCH + 0.1 A PT
 Misc Info :
 Vial Number: 22
 CurrentMeth: C:\HPCHEM\1\METHODS\0102M1ST.M

Compound: BAS654
 Ret Time: 9.25
 Concentration: 39.47 pg
 Pk # and Type: 1



	Signal	Ratios	Limits	RT	Limits	Resp	Integ	Type
Tgt	161.00	100.0%		9.25	9.01	758016	654	
Q1	0.00	0.0	0.0- 0.0	0.00	to	0	654	
Q2	0.00	0.0	0.0- 0.0	0.00	9.47	0	654	
Q3	0.00	0.0	0.0- 0.0	0.00		0	654	

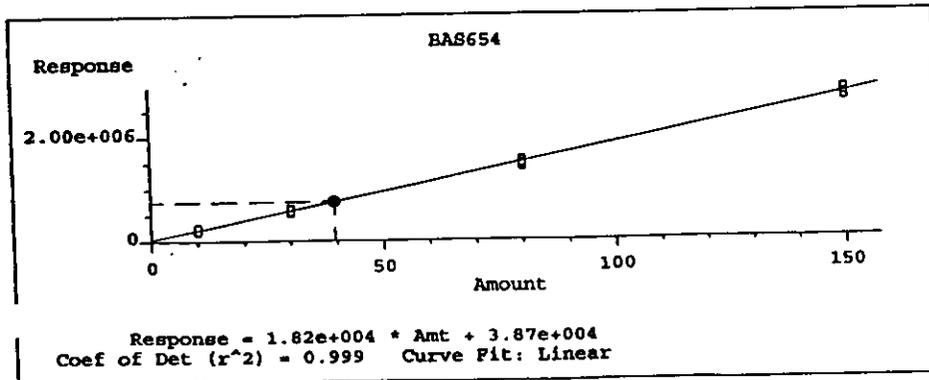


Figure 19. Typical chromatogram of a control corn refined oil sample. Vial number 5 from master sheet number 97223-10. Data for this sample can be found in Table 2.

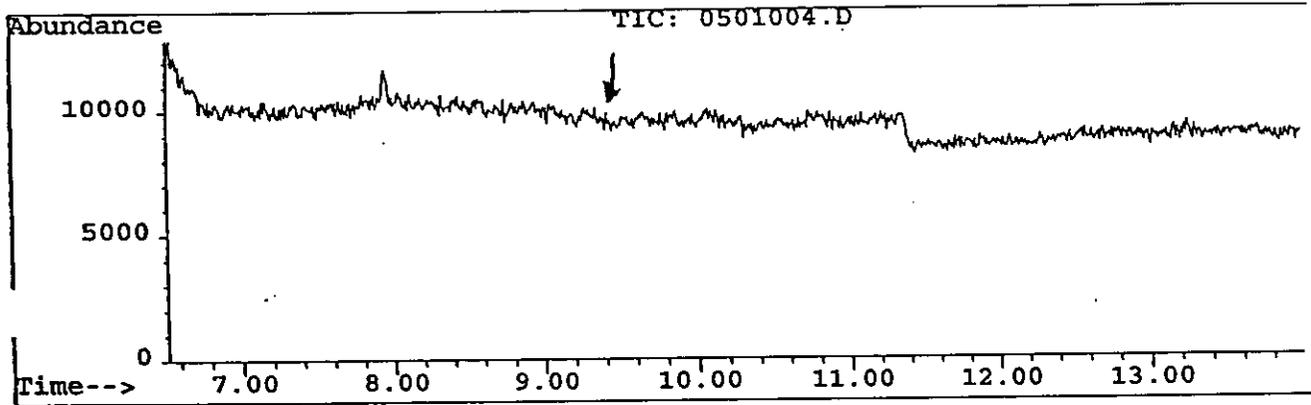
SECOND INJECTION

Area Percent Report -- Sorted by Signal

RT 1.8.98

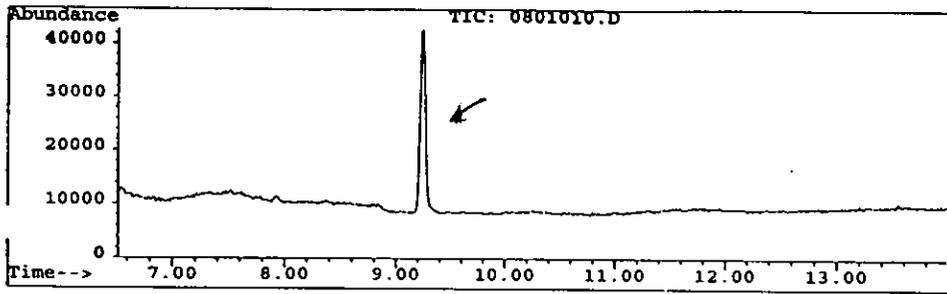
Information from Data File:

File : C:\HPCHEM\1\DATA\GC1238\BAS654H\010798\0501004.D
Operator :
Acquired : 7 Jan 98 10:22 am using AcqMethod 0107M1OL
Sample Name: RT, CONTROL R.OIL
Misc Info :
Vial Number: 5
CurrentMeth: C:\HPCHEM\1\METHODS\0107M1OL.M



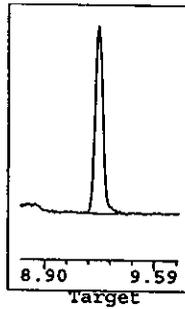
Retention Time	Area	Area %	Ratio %
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Figure 20. Typical chromatogram of a control corn refined oil sample fortified with 0.1 ppm of M1. Vial number 8 from master sheet number 97223-10. Data for this sample can be found in Table 2. Recovery 88%.



File : C:\HPCHEM\1\DATA\GC1238\BAS654H\010798\0801010.D
 Operator :
 Acquired : 7 Jan 98 12:04 pm using AcqMethod 0107M1OL
 Sample Name: RT CONTROL R.OIL + 0.1 A M1
 Misc Info :
 Vial Number: 8
 CurrentMeth: C:\HPCHEM\1\METHODS\0107M1OL.M

Compound: BAS654
 Ret Time: 9.25
 Concentration: 105.53 pg
 Pk # and Type: 1



	Signal	Ratios	Limits	RT	Limits	Resp	Integ Type
Tgt	161.00	100.0%		9.25	9.02	1161491	654
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	9.48	0	auto
Q3	0.00	0.0	0.0- 0.0	0.00		0	auto

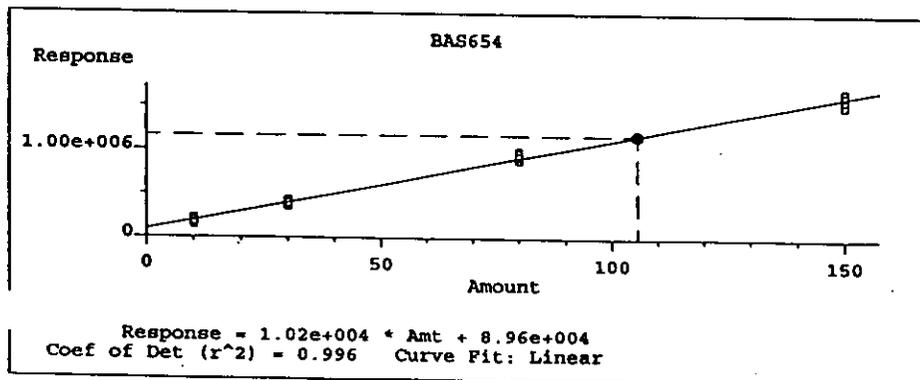


Figure 22. Typical GC-MS (scan) chromatogram/mass spectrum of 5 ng standard of M1.

File : C:\HPCHEM\1\DATA\835\M1SCAN1.D
Operator :
Acquired : 29 Jan 98 9:28 am using AcqMethod M1SCAN
Instrument : BASF 6890
Sample Name: 1NG/1UL M1 SCAN
Misc Info :
Vial Number: 6

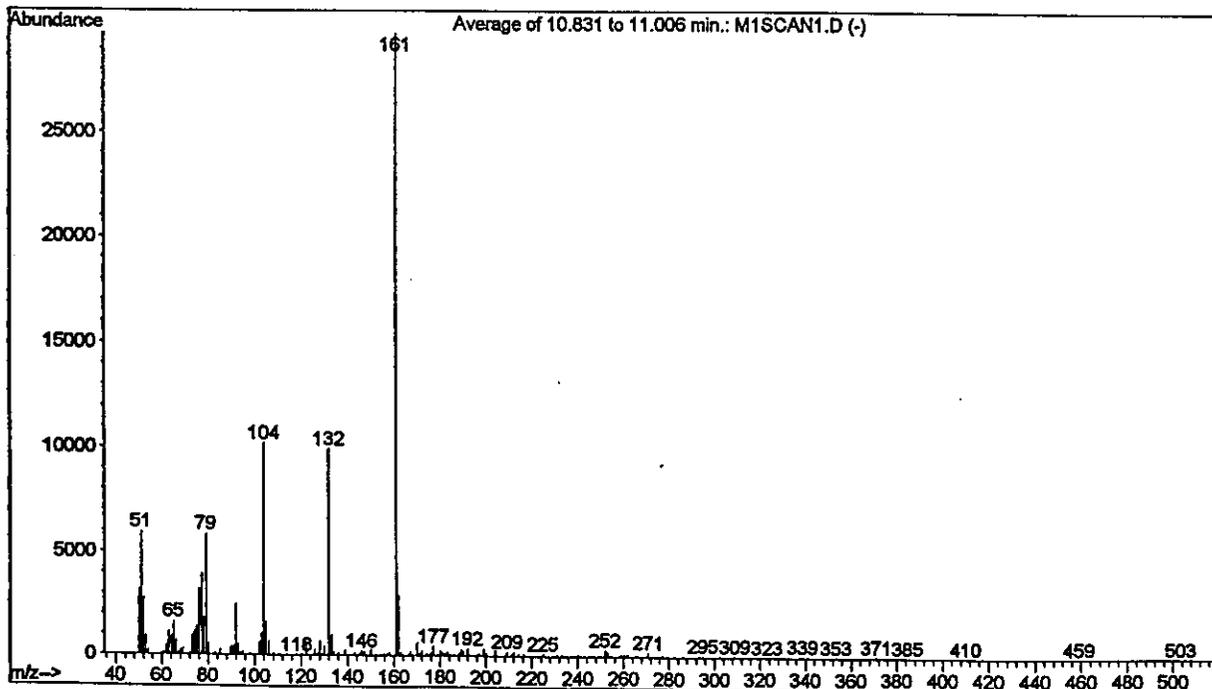
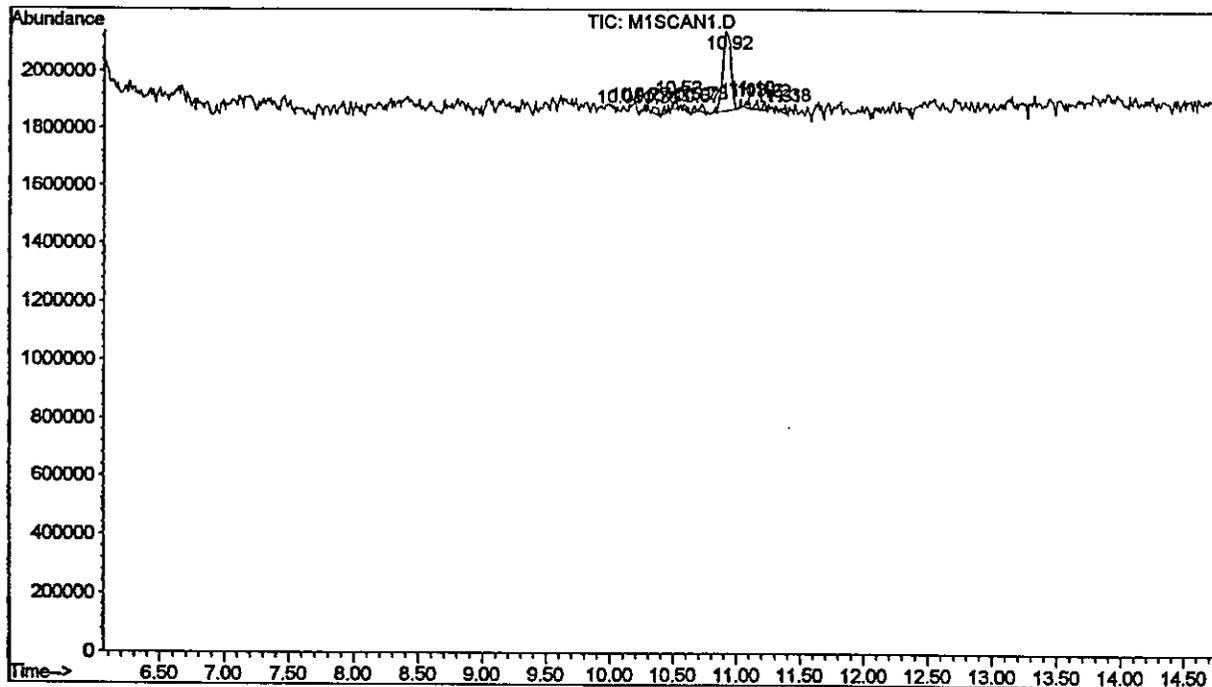
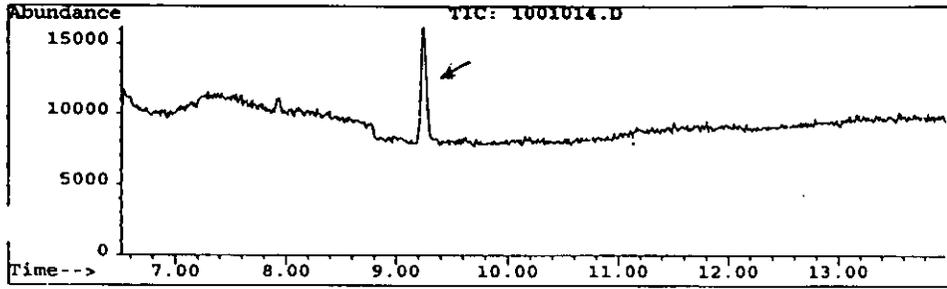
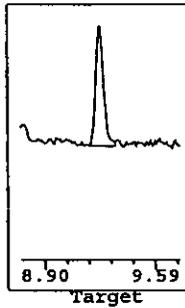


Figure 21. Typical chromatogram of a control corn refined oil sample fortified with 0.05 ppm of BAS 654H. Vial number 10 from master sheet number 97223-10. Data for this sample can be found in Table 2. Recovery 67%.

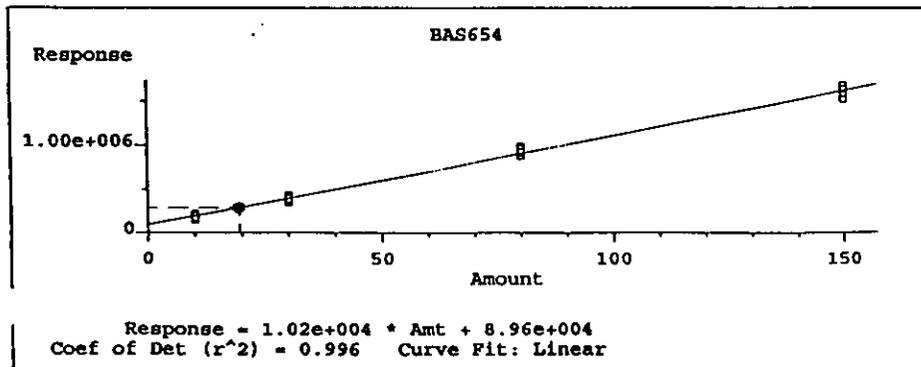


File : C:\HPCHEM\1\DATA\GC1238\BAS654H\010798\1001014.D
 Operator :
 Acquired : 7 Jan 98 1:13 pm using AcqMethod 0107M1OL
 Sample Name: RT, CONTROL R.OL + 0.05 A PT
 Misc Info :
 Vial Number: 10
 CurrentMeth: C:\HPCHEM\1\METHODS\0107M1OL.M

Compound: BAS654
 Ret Time: 9.25
 Concentration: 19.38 pg
 Pk # and Type: 1



	Signal	Ratios	Limits	RT	Limits	Resp	Integ Type
Tgt	161.00	100.0%		9.25	9.02	286388	654
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	9.48	0	auto
Q3	0.00	0.0	0.0- 0.0	0.00		0	auto



Diflufenzopyr in Corn Commodities

ADDENDUM

- 1) ACB made only slight modifications to the GC/MSD instrument parameters.

The parameters for the GC/MSD were:

Gas Chromatograph: HP 5972 Mass Selective Detector.
Column: Restek Stabilwax^R-DA 30m x 0.25 mm x 0.25 um film thickness
Carrier gas: Helium, 0.78/min.
Oven Temperature: Initial 120°C for 0.50 min.
70°C/min to 250°C hold for 27 min.
Injector temperature: 250°C
MS mode: SIM total ion current of m/z 161.
Transfer line temperature: 280°C
Injection volume: 4 uL

- 2) The petitioner used control values to correct for recoveries. This practice is not permitted in our guidelines.

3) ACB found that additional reflux time was needed to convert the Diflufenzopyr to Phthalazinone. When using a neat Diflufenzopyr standard ACB found that the 2 hour reflux time (As written in registrant's procedure) only yielded a 67% conversion of Diflufenzopyr to Pthalazinone. However, a 4 hour reflux time yielded an 85% conversion. In this method trial ACB used the 4 hour reflux time.

4) ACB found that **sonicating the samples and thoroughly rinsing the vessels, when transferring the sample extracts, was a critical step** as stated in the method. The occasional low recoveries, as shown in the recovery data, was attributed to the analyte adhering to the walls of the glassware.

5) The part number for the HLB solid phase extraction (SPE) material should be corrected to 106068. The specifications for the HLB SPE material are:

Average pore diameter(A ⁰)	= 82
Specific surface area (m ² /g)	= 832
Mean particle diameter (μm)	= 31.4
Mesh(μm)	= 30