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Sponsor

CORNING Company

Diphenylamine Task Force
 5 Victory Lane, Suite 201
 Liberty, Missouri 64068

FINAL REPORT

431635-01

Study Title:

Determination of Diphenylamine Residues in Apples
 and Processed Apple Fractions by Gas Chromatography (GC) with
 Mass-Selective Detection (MSD)

Data Requirement:

40 CFR 158.240
 EPA Assessment Guidelines
 Subdivision O - Residue Chemistry
 Series 171-4 (c), Residue Analytical Method

Author:

Mandla A. Tshabalala, PhD

Study Completion Date:

March 10, 1994

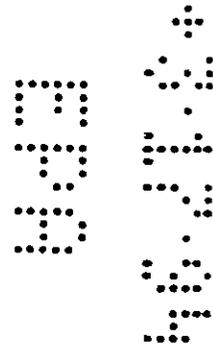
Performing Laboratory:

Hazleton Wisconsin, Inc.
 3301 Kinsman Boulevard
 Madison, Wisconsin 53704

Laboratory Project Identification:

HWI 6524-100

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01/11/94
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STATEMENT OF NO DATA CONFIDENTIALITY CLAIM

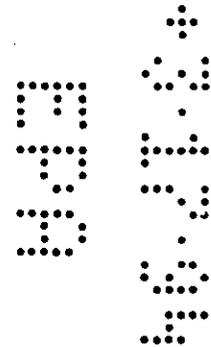
Determination of Diphenylamine Residues in Apples
and Processed Apple Fractions by Gas Chromatography (GC) with
Mass-Selective Detection (MSD)

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

Company: Diphenylamine Task Force
Company Agent: John M. Wise
Title: Chairman, Diphenylamine Task Force

Signature John M. Wise Date 3-15-94 *John M. Wise*

All data are the property of the Diphenylamine Task Force and, as such, are considered to be confidential for all purposes other than compliance with FIFRA § 10. Submission of these data in compliance with FIFRA does not constitute a waiver of any right to confidentiality that may exist under any other statute or in any other country.



COMPLIANCE STATEMENT

Determination of Diphenylamine Residues in Apples
and Processed Apple Fractions by Gas Chromatography (GC) with
Mass-Selective Detection (MSD)

This study was a method development study and therefore, was not required to be conducted in accordance with the Environmental Protection Agency Good Laboratory Practice Standards (40 CFR 160, effective October 16, 1989).

M. D. Theobald

Study Director

03-10-94

Date

John M. Wise

Sponsor's Representative

3-15-94

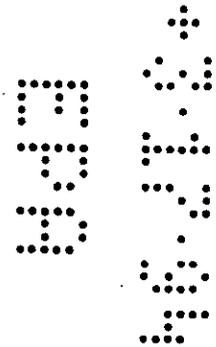
Date

John M. Wise

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John M. Wise, Chairman
Diphenylamine Task Force

3-15-94

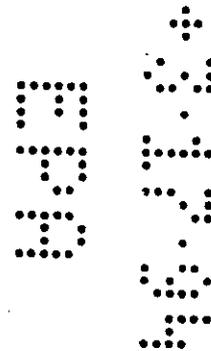
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STUDY IDENTIFICATION

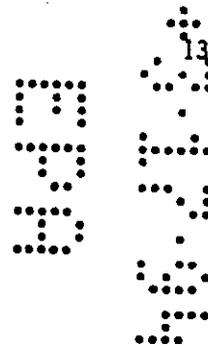
Determination of Diphenylamine Residues in Apples
and Processed Apple Fractions by Gas Chromatography (GC) with
Mass-Selective Detection (MSD)

Test Substances	Diphenylamine
Sponsor	Diphenylamine Task Force 5 Victory Lane, Suite 201 Liberty, Missouri 64068
Sponsor's Representative	Joseph Geronimo, PhD John Wise and Associates, Ltd. 5 Victory Lane, Suite 201 Liberty, Missouri 64068
Study Director	Mandla A. Tshabalala, PhD Hazleton Wisconsin, Inc. P.O. Box 7545 Madison, Wisconsin 53707 (608) 241-4471
Testing Facility	Hazleton Wisconsin, Inc. 3301 Kinsman Boulevard Madison, Wisconsin 53704
Experimental Start Date:	September 20, 1993
Experimental Termination Date:	February 24, 1994



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ABSTRACT

An analytical procedure for the determination of residues of diphenylamine in apples and processed apple fractions by gas chromatography (GC) with mass selective detection (MSD) has been developed. In this procedure a homogenized sample of whole apple, apple juice, or pomace (wet or dry) is extracted with acetone in a blender cup and filtered. The filtrate is mixed with water, and partitioned against hexane. The hexane extract is filtered through a bed of sodium sulfate to remove emulsions and traces of moisture. After exchange with dichloromethane (DCM) and concentration, the DCM solution of diphenylamine is derivatized with trifluoroacetic anhydride. The acetylated diphenylamine (DPA) is determined by GC-MSD in the selected ion monitoring mode.

Evaluation of the method indicated that the average recoveries in the range of concentration tested (0.2 to 2.8 ppm) are 90% \pm 9.7% for whole Red Delicious apples, 83% \pm 7.6% for whole Granny Smith apples, 97% \pm 15% for Red Delicious juice, 96% \pm 9.3% for Granny Smith juice, 75% \pm 3.7% for Red Delicious wet pomace, 89% \pm 12% for Granny Smith wet pomace, 77% \pm 4.1% for Red Delicious dry pomace, and 79% \pm 8.1% for Granny Smith dry pomace.

The limit of detection, defined as the limit of quantitation, is equal to 0.08 ppm for whole apple, apple juice, and wet pomace, and 0.4 ppm for dry pomace.

INTRODUCTION

Hazleton Wisconsin (HWI) was contracted by the Diphenylamine Task Force (Sponsor) to develop a gas chromatography-mass selective detection (GC-MSD) method for the determination of diphenylamine and 4-hydroxy diphenylamine residues in/on fresh and processed apples. This request was subsequently modified by the Sponsor to focus on the development of a GC-MSD method for the determination of diphenylamine residues only. The method described in this report is a modification of the method of Gutenmann et al. It consists of the trifluoroacetylation of the purified acetone-hexane extract of the apple matrix to yield the N-trifluoroacetylated derivative of diphenylamine. The N-trifluoroacetyl diphenylamine is chromatographed on a narrow-bore DB-17 GC capillary column, and determined by GC-MSD in the selected ion monitoring mode. The quantification ion is 265 am.

OBJECTIVE

The objective of this study was to develop a GC-MSD method for the determination of diphenylamine and 4-hydroxy-diphenylamine residues in/on fresh and processed apples. At the direction of the Sponsor, this objective was subsequently modified to the development of a GC-MSD method for the determination of diphenylamine residues in/on fresh and processed apples.

MATERIALS AND METHODS

A detailed description of the method procedure is given in APPENDIX A.

RESULTS AND DISCUSSION

Linearity of Detector Response

A typical calibration curve is shown in Figure 1. The detector response was linear over the concentration range of 0.5 $\mu\text{g/mL}$ to 10 $\mu\text{g/mL}$ of DPA in DCM.

Limit of Detection

The limit of detection, defined as the number of parts per million of diphenylamine residue that is equivalent to the lowest calibration standard tested, was set at 0.08 ppm for whole apple, apple juice, and wet pomace, and 0.4 ppm for dry pomace.

Limit of Quantitation

The limit of quantitation is equivalent to the limit of detection.

Recoveries

The percent recoveries are reported to two significant figures. The percent recoveries are corrected for the average ppm found in the controls. This value, rounded off to three significant figures, was approximately equal to 0 ppm, except for the Red Delicious and Granny Smith whole apple, and for the Granny Smith wet pomace. The average percent recoveries for the individual matrices tested ranged from 75% to 90% and are summarized in Table I.

Calculations

The ppm diphenylamine (DPA) found are calculated from the following relationship.

$$\text{ppm DPA found} = (C \times V \times d \times F) / W$$

- where C = Concentration in $\mu\text{g/mL}$ calculated from the linear calibration curve of the computer area counts versus the concentration of the calibration standards
 V = Final volume in mL of the extract
 d = Dilution factor, determined from the fraction of the crude acetone extract taken through the method
 F = Method factor, determined from the fraction of the final purified extract diluted with dichloromethane
 W = Weight of sample in grams, taken for extraction

$$\text{Percent recovery} = (\text{ppm Found} / \text{ppm Added}) \times 100$$

Percent recovery corrected for positive control values is:
 $\{(\text{ppm Found in the recovery sample} - \text{ppm Found in the control}) / \text{ppm Added}\} \times 100$

Example Calculations

When the method is followed as written, $V = 2$, $d = 2$, and $F = 1$.
 For whole apples, $W = 25$ g.

For the Granny Smith whole apple control:

$$\text{Average concentration found, } C_{(\text{avg})} = 0.5483 \mu\text{g/mL}$$

Hence, the average ppm found in the control samples is:

$$\{(0.5483 \times 2 \times 2 \times 1) / 25\} = 0.0877 \text{ ppm (reported to three significant figures)}$$

For the Granny Smith whole apple 0.2 ppm recovery sample:

$$\text{Average concentration found, } C_{(\text{avg})} = 1.548 \mu\text{g/mL}$$

Hence, the average ppm found in the 0.2 ppm recovery samples is:

$$\{(1.548 \times 2 \times 2 \times 1)/25\} = 0.248 \text{ ppm (reported to three significant figures)}$$

Percent recovery, corrected for the ppm found in the control equals:

$$\{(0.248 - 0.0877)/0.200\} \times 100 = 80\% \text{ (reported to two significant figures)}$$

CONCLUSION

The analytical procedure described in this report is suitable for the determination of residue levels of diphenylamine in a 25-g sample of whole apple, apple juice, or wet pomace, and for a 5-g sample of dry pomace. The limit of detection is estimated at 0.08 ppm for whole apple, apple juice, and wet pomace, and 0.4 ppm for dry pomace.

The sensitivity of the method, defined as the concentration of the lowest calibration standard tested is equal to 0.5 $\mu\text{g/mL}$.

REFERENCES

Gutenmann, Walter H., and Lisk, Donald J., Agricultural and Food Chemistry, Vol. II, No. 6, 1963.

Table I

Summary of Percent Recoveries of Diphenylamine Residues in Apple Matrices

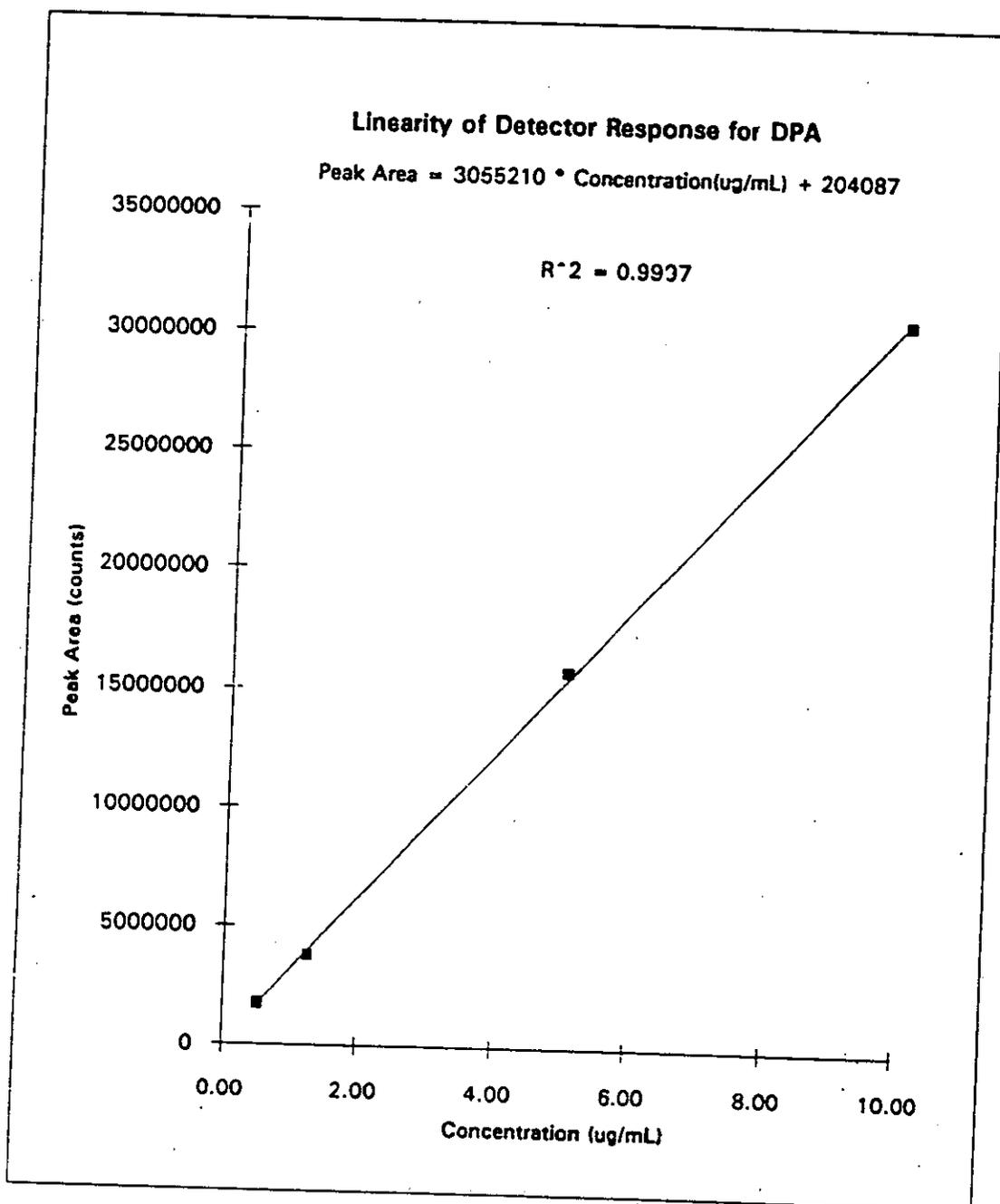
<u>Variety</u>	<u>Matrix</u>	<u>ppm Controls</u>	<u>ppm Fort.</u>	<u>% Recoveries</u>	<u>Average Percent Recovery</u>
Red Delicious	Whole apple	<0.08	0.2	93	
			2.0	110, 84, 88	94
			2.8	81, 92	87
	Apple juice	<0.08	0.4	100, 86	93
			1.0	78, 80, 89	82
			2.0	110, 120, 110	110
	Wet pomace	<0.08	1.0	73, 70, 73	72
			2.0	80, 78, 76	78
	Dry pomace	<0.4	1.0	76, 70, 77	74
			2.0	82, 79, 79	80
Granny Smith	Whole apple	<0.08	0.2	86, 79, 75	80
			2.0	75, 78, 85, 96, 90	85
			0.4	93, 90, 88	91
	Apple juice	<0.08	1.0	91, 84, 99	91
			2.0	100, 110, 110	110
			0.4	94, 110, 94	99
	Wet pomace	<0.08	1.0	91, 76, 90	86
			2.0	86, 73	80
			1.0	93, 78, 79	80
	Dry pomace	<0.4	1.0	93, 78, 79	80
2.0			75, 74, 83	78	

Fort. = Fortification.

Figure 1

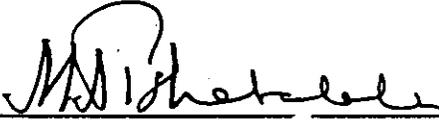
Example of a Typical Calibration Curve for DPA in Apple Matrices

Conc ($\mu\text{g/mL}$)	Peak Area (counts)		
0.50	1766289	1731692	
1.25	3800256	4023099	Slope = 3055210
5.00	15804376	15480136	Intercept = 204086.6
10.00	30620191	30756185	R ² = 0.99967



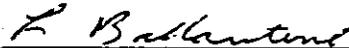
CERTIFICATE OF AUTHENTICITY

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


 Mendla A. Tshabalala, PhD
 Study Director
 Hazleton Wisconsin, Inc.

03-10-94
 Date

Approved by:


 Larry G. Ballantine, PhD
 Director
 Agricultural Chemistry

3/10/94
 Date

SPONSOR CERTIFICATION

The Diphenylamine Task Force certifies that this report for Hazleton Wisconsin, Inc. Study No. 6524-100 is a complete and unaltered copy of the report as provided by the testing facility.

 John M. Wise
 Chairman
 Diphenylamine Task Force

 Date

by and for Hazleton Wisconsin.

APPENDIX A

Method Procedure

ASSAY TITLE: Determination of Diphenylamine Residues in Apples and Processed Apple Fractions by Gas Chromatography (GC) with Mass-Selective Detection (MSD)

AREA OF APPLICABILITY: Hazleton Wisconsin, Inc.
Agricultural Chemistry
Methods Development

SCOPE:

This analytical procedure describes the determination of residue levels of diphenylamine in a 25-g sample of whole apple, apple juice, or wet apple pomace, and for a 5-g sample of dry pomace. The limit of detection is estimated at 0.08 ppm, for whole apple, apple juice, or wet pomace, and 0.400 ppm for dry pomace.

PRINCIPLE:

A homogenized sample of whole apple, apple juice, or pomace (wet or dry) is extracted with acetone in a blender cup and filtered. The filtrate is mixed with water, and partitioned against hexane. The hexane extract is filtered through a bed of sodium sulfate (Na_2SO_4) to remove emulsions and traces of moisture. After exchange with dichloromethane (DCM) and concentration, the DCM solution of diphenylamine is derivatized with trifluoroacetic anhydride (TFAA). The acetylated diphenylamine is determined by GC-MSD.

SENSITIVITY, PRECISION, AND ACCURACY

The accuracy and precision of the method as reflected by the overall average of the recoveries in the concentration range 0.2 to 2.8 ppm is 87% \pm 12% (n=59).

The sensitivity of the method is defined as the concentration of the lowest calibration standard, and is equal to 0.5 $\mu\text{g}/\text{mL}$.

The limit of detection is defined as the limit of quantitation, and is equal to 0.08 ppm for whole apple, apple juice or wet pomace, and 0.4 ppm for dry pomace.

RECOVERY:

The average percent recovery in the concentration range of 0.200 to 2.80 ppm is 87% \pm 12% (n=59).

REFERENCES:

- HWI study binder for HWI Study No. 6524-100
- Facsimile communication from Dr. Joe Geronimo, August 24, 1993.
- Facsimile communication from Dr. Heasook Kim-Kang, October 13, 1993.
- Gutenmann, Walter H., and Lisk, Donald J., Agricultural & Food Chemistry, Vol. II, No. 6, 1963.
- Method attachments. HWI study binder for HWI Study No. 6524-100.

APPROVED BY: L. Ballantine DATE: 2/24/94
Larry G. Ballantine, PhD
Director
Agricultural Chemistry

REVIEWED BY: Sherry R. W. Petsel for DATE: 2/24/94
Sherry R. W. Petsel
Manager
Quality Assurance Unit

SAFETY PRECAUTIONS:

Observe all standard laboratory safety procedures as outlined in the Health and Safety section of the Hazleton Wisconsin Policies and Procedures Manual.

INTERFERENCES:

- Some apple matrices may show a peak at the retention time of diphenylamine, which is believed to be endogenous diphenylamine in apples.
- Detailed interference studies of other pesticides have not been done.
- No significant interferences have been observed when high-performance liquid chromatography (HPLC), pesticide grade solvents, or analytical grade reagents are used.
- No significant interferences have been observed when the glassware is washed in a dishwasher with hot water and detergent and rinsed with deionized water and acetone.

QUALITY ASSURANCE:

In addition to the sample extracts, each analytical set should include at least four calibration standards, a reagent blank, an extract of an unfortified control matrix, and an extract of a fortified control matrix. The recovery of diphenylamine in the fortified control matrix should be between 70% and 120%.

CONFIRMATORY TECHNIQUES:

The presence of diphenylamine residues in the sample may be confirmed by the ratio of the qualifier ion to the quantitation ion. This ratio should be within $\pm 20\%$ of the average ion ratio found in the calibration standards.

APPARATUS:

- Blender cups, small size, glass, Eberbach
- Food processor, Robot Coupe
- Glass jar, 32-oz, equipped with an aluminum-lined screw-cap
- Tissuemizer, Ultra turrax T-25
- Beakers, various sizes
- Round-bottom boiling flasks, 500 and 1,000 mL
- Flat-bottom boiling flasks, 100, 250, and 1,000 mL
- Filter membrane, nylon, 0.2- μm Acrodisc, LC13PVDF

- Separatory funnels, 250 and 500 mL ✓
- Filter paper, No. 541 and 934-AH, Whatman ✓
- Reacti-vials, 10-mL graduated conical, Pierce ✓
- Pierce Teflon silicon discs (No. 12722), ✓
- Rotary evaporator, Buchii, RE III, Type URVrID ✓
- Vials, 2 mL, crimp-top, Hewlett-Packard, Part No. 5181-3375 ✓
- Crimp tops, Teflon/rubber, National Scientific Co., Catalogue No. 4011-1A) ✓
- Beakers, various sizes ✓
- Pasteur pipettes, 5 and 9 in. ✓
- Heating block, Reacti-Therm III, heating/stirring module, Pierce ✓
- Funnels, glass, various sizes ✓
- Graduated cylinders, various sizes ✓
- Volumetric pipettes, various sizes ✓
- Wooden applicators, 12 in. x 1/12 in., Puritan ✓
- Luerlock syringes, micro-mate, 2 cc ✓
- Vortex Genie, VWR Science ✓
- Vacuum filtration apparatus with scintered glass frit and 24/40 glass joint, 150 mL, Pyrex No. 36060 ✓
- Double-Taper Liner deactivated, P/N 5181-3315, Hewlett Packard ✓
- Capillary column, DB-17, Length 30 m, ID 0.25 mm, Film 0.25 μ , P/N 122-1732, J & W ✓
- Autosampler, HP 7673A, Hewlett Packard ✓
- Gas chromatograph, HP 5890, Hewlett Packard ✓
- Mass selective detector, HP 5970, Hewlett Packard ✓
- Data System, HP ChemSystem 400, Hewlett Packard ✓

Note: Equivalent equipment may be substituted.

REAGENTS/CHEMICAL SUPPLIES:

- Acetone, high-purity solvent, Burdick & Jackson
- Water deionized, from tap
- Sodium sulfate, anhydrous granular, E & M Science
- Acetonitrile, high-purity solvent, Burdick & Jackson
- Perfluorotributylamine (PFTBA), Hewlett Packard
- Trifluoroacetic anhydride, Supelco Catalog No. 3-3165 ✓
- DCM, pesticide quality grade, Burdick & Jackson
- Hexane, pesticide quality grade, E & M Science
- Dry ice (for some rotary evaporators)

- Liquid nitrogen (for homogenization of whole apples)
- 2M sodium hydroxide (NaOH). Weigh 80 g of NaOH pellets into a 1-L plastic container. Add 1 L of Milli-Q water, to dissolve pellets.

Note: Equivalent reagents may be substituted.

TEST MATERIAL:

- Diphenylamine

PROCEDURE:

1. Preparation of Diphenylamine Stock Solution (Nominal Concentration of 1.00 mg/mL)

Weigh approximately 0.100 g of diphenylamine standard to three significant figures. Transfer to a 100-mL volumetric flask. Dilute to the mark with acetonitrile.

2. Preparation of Standard Solutions A through H

Prepare the following dilutions of the stock solution in DCM.

<u>Solution</u>	<u>Source Identification</u>	<u>Volume (mL)</u>		<u>Nominal Concentration ($\mu\text{g/mL}$)</u>
		<u>Aliquot</u>	<u>Final</u>	
A	Stock solution	1.0	10	100
B	Stock solution	0.5	10	50
C	Solution A	2.0	10	20
D	Solution B	2.0	10	10
E	Solution B	1.0	10	5
F	Solution B	0.5	10	2.5
G	Solution C	1.0	10	2
H	Solution C	0.5	10	1

3. Preparation of Calibration Standards

3.1 Using a 1-mL Class A volumetric pipet, add 1.0 mL of each standard solution (B through H) to separate 10-mL Reacti-vials.

3.2 Add approximately 1 mL of the TFAA derivatization reagent, and cap tightly.

3.3 Place the Reacti-vials in a heating block at approximately 50°C for approximately 1 hour.

Note: The vials should be examined occasionally for any possible leaks. If a vial is leaking, it must be replaced immediately.

3.4 Let the vials cool to room temperature before adjusting the volume of the reaction mixture to the 2-mL mark with DCM.

3.5 Mix the reaction solution by vortexing before transferring approximately 1 mL to the autosampler vial.

3.6 The nominal concentrations of the calibration standards are:

- | | | | |
|--------------------------|------------------------|-------------------------|-------------------------|
| a) 25 $\mu\text{g/mL}$ | b) 10 $\mu\text{g/mL}$ | c) 5 $\mu\text{g/mL}$ | d) 2.5 $\mu\text{g/mL}$ |
| e) 1.25 $\mu\text{g/mL}$ | f) 1 $\mu\text{g/mL}$ | g) 0.5 $\mu\text{g/mL}$ | |

4. Sample Preparation

4.1 Whole apples:

4.1.1 Remove two apples each from the top, middle, and bottom of the sample box in the freezer.

4.1.2 Remove the stem if present, and cut the apples into small pieces and place them in a stainless steel bowl.

4.1.3 Pour liquid nitrogen over the apple pieces.

4.1.4 Transfer the frozen pieces to a food processor and homogenize. (Refer to Note 9.1.)

4.1.5 Place the homogenized frozen powder in a labeled glass jar equipped with an aluminum foil-lined screw-cap.

4.1.6 Cap the jar tightly and store it in the freezer.

4.1.7 Proceed further as outlined in Sections 5 or 6.

4.2 Wet pomace:

4.2.1 Remove a bag of the wet pomace sample from the freezer.

4.2.2 Take an approximate 1000-g subsample, and proceed as outlined in Steps 4.1.1 to Step 4.1.7.

4.3 Apple juice:

4.3.1 Remove the apple juice container from the freezer, and let the juice thaw to room temperature.

4.3.2 Mix the juice thoroughly by shaking the container.

4.3.3 Proceed as outlined in Sections 5 or 6.

4.4 Dry pomace:

4.4.1 Remove a bag of dry pomace sample from the freezer.

4.4.2 Weigh out approximate 5-g aliquots

4.4.3 Proceed as outlined in Section 5 or 6.

5. Preparation of Blank and Fortified Controls

5.1 Blank Controls. Weigh out approximately 25 g of the untreated apple matrix into a blender cup and proceed with the extraction procedure described below. (Refer to Note 9.2.)

5.2 Fortified Controls.

5.2.1 Weigh out approximately 25 g of the untreated apple matrix into a blender cup. (Refer to Note 9.2.)

5.2.2 Add approximately 100 mL of acetone.

5.2.3 Add the appropriate volumes of the standard solutions to achieve the desired fortification levels.

5.2.4 A typical fortification scheme is outlined below.

5.2.5 Go to Step 6.3.

<u>Solution Identification</u>	<u>Volume Added (mL)</u>	<u>Fortification Level (ppm)</u>
A	0.7	2.8
B	1	2
E	1	0.2
H	0.5	0.02

6. Sample Extraction and Derivatization

- 6.1 Place approximately 25 g of the subsample of the homogenized apple matrix into an Eberbach small size glass blender cup equipped with a metal screw cover. (Refer to Note 9.2.)
- 6.2 Add approximately 100 mL of acetone and cover the blender cup tightly.
- 6.3 Blend at moderate speed for approximately 1 minute.
- 6.4 Pour the mixture into a 250-mL graduated cylinder equipped with a 150-mL scintered glass funnel upon which a Whatman 934-AH glass microfibre filter (5.5-cm circle) has been placed.
- 6.5 Filter by suction into the 250-mL graduated cylinder.
- 6.6 Rinse the blender cup and the filter cake with several portions of acetone, until approximately 190 mL of the filtrate has been collected in the 250-mL graduated cylinder.
- 6.7 Adjust the filtrate to the 200-mL mark with acetone.
- 6.8 Stopper the graduated cylinder and mix the contents thoroughly by inverting the graduated cylinder up and down.
- 6.9 Place 100 mL of the acetone solution in a 1,000-mL separatory funnel.
- 6.10 Add approximately 100 mL of hexane to the separatory funnel.
- 6.11 Add approximately 500 mL of deionized tap water to the separatory funnel.

- 6.12 Place a stopper on the separatory funnel and mix gently by inversion in order to partition the diphenylamine (DPA) into the hexane layer without formation of severe emulsions.
- 6.13 Break up any emulsions with a 12-in. x 1/12-in. Puritan applicator.
- 6.14 Wait for the layers to separate.
- 6.15 Transfer the bottom aqueous layer to a 1,000-mL flat-bottom flask.
- 6.16 Filter the upper hexane layer through a 40-mL anhydrous sodium sulfate bed supported on a Whatman 541 filter placed in a glass funnel, into a 250-mL round-bottom flask.
- 6.17 Transfer the aqueous layer back to the separatory funnel.
- 6.18 Rinse the 1,000-mL flat-bottom flask with approximately 100 mL of hexane.
- 6.19 Transfer the hexane rinsate into the separatory funnel.
- 6.20 Repeat Steps 6.12 through 6.16.
- 6.21 Combine the hexane filtrates in the same 250-mL round-bottom flask.
- 6.22 Evaporate the solvent to near dryness with a Rotary Evaporator at approximately 35 °C.
- 6.23 Add approximately 5 mL of DCM and evaporate it to near dryness.
- 6.24 Repeat Step 6.23.
- 6.25 Add approximately 1 mL of DCM to the flask, and quantitatively transfer the solution to a 10-mL Reacti-Vial.
- 6.26 Repeat Step 6.25 several times, each time combining the rinsing solutions in the same Reacti-vial.
- 6.27 Evaporate the solvent to just below the 1-mL mark under a gentle nitrogen stream.
- 6.28 Add approximately 1 mL of the TFAA reagent to the Reacti-vial.

- 6.29 Close the Reacti-vial tightly with a hole cap equipped with a Teflon-face/Silicone disk.
- 6.30 Mix briefly on a Vortex Genie.
- 6.31 Place the Reacti-vial in a heating block, and heat at approximately 50°C for approximately 1 hour.
- 6.32 Let the mixture cool to room temperature.
- 6.33 Evaporate the solvent to near dryness under a gentle nitrogen stream.
- 6.34 Reconstitute the residue in 2 mL of DCM.
- 6.35 Mix briefly on a Vortex Genie.
- 6.36 Transfer the DCM solution to a labeled 2-mL crimp-top autosampler vial.
- 6.37 Load the vials on the autosampler for analysis by GC-MSD.

7. Gas Chromatography

- 7.1 Ensure that the proper capillary column and double-tapered splitless liner are installed in the GC-MSD as described in the instrument manual.
- 7.2 Set up the following operation conditions.

Note: Unless otherwise indicated, the operating conditions described below may be modified to optimize chromatography.

- Typical Operating Conditions

- a. Injection port temperature: 200°C
- b. Transfer line temperature: 260°C
- c. Oven temperature program:
 - Initial temperature: 80°C
 - Initial time: 1 min.
 - Temperature ramp:

<u>Level</u>	<u>Rate</u> (°C/min.)	<u>Final Value</u> (°C)	<u>Final Time</u> (min.)
1	5.0	220	0.00
2	45.0	250	0.00

Equilibration time: 1 min.
 Maximum oven temperature: 300°C

d. Carrier gas: Helium, 1 mL/min.
 e. Head pressure: 5 psi
 f. Injection mode: Splitless
 g. Purge delay: 2.50 min.
 h. Injection volume: 2 µL
 i. Electron multiplier voltage: 1,400 to 1,800
 j. Dwell time: 200 milli-second
 k. Tune mode: Autotune with PFTBA

- 7.3 Conduct analysis in the selected ion monitoring mode. The quantitation ion is 265 amu, and the qualifier ion is 167 amu. Typical ion ratio for the qualifier/quantitative ion ratio is 70%.

8. Determination of Diphenylamine

- 8.1 Prepare calibration standards to span the expected levels of DPA residues, unfortified and fortified controls, and samples as outlined above.
- 8.2 Inject the calibration standards to determine the calibration curve linearity.
- 8.3 Inject a calibration standard for every two controls or sample extracts.
- 8.4 Calculate the concentration of diphenylamine in the injected solution from the calibration curve.

8.5 Calculate the ppm of diphenylamine in the sample by using the following relationship:

$$\text{ppm DPA} = [C \times V \times F \times d] / W$$

where C = concentration from the calibration curve ($\mu\text{g/mL}$)
V = final volume of residue solution (mL)
d = dilution factor
W = weight of subsample (g)
F = (refer to Step 9.3)

- If the method is followed exactly as written, V=2, F=1, and d=2. If the final volume of the residue solution is diluted 1:4 with DCM, then F=4.
- Percent Recovery = (ppm found/ppm fortified) x 100
- Corrected Percent Recovery = [(ppm found in the fortified sample - average ppm found in the control sample)/ppm added] x 100

9. Notes

- 9.1 Untreated samples should be processed first, and the processing utensils should be thoroughly cleaned between preparations.
- 9.2 For the dry pomace, weigh out approximately 5 g of the sample, and rehydrate with approximately 75 mL of water for approximately 20 minutes.
- 9.3 Some derivatized sample extracts may need to be diluted with DCM. This is the method factor F.

10. Results and Discussion

- 10.1 The limit of detection, defined as the number of parts per million of diphenylamine residue that is equivalent to the lowest calibration standard, was set at 0.0800 ppm for whole apple, apple juice, or wet pomace, and 0.400 ppm for dry pomace.
- 10.2 The percent recoveries are reported to two significant figures. The corrected recoveries were obtained by subtracting the average ppm found in the controls. This value, rounded off to three significant figures, was approximately equal to zero ppm, except for Tables 1, 2, and 6, where the average control values found were 0.0686, 0.0877, and 0.0710 ppm, respectively.

The recoveries for the individual matrices were determined to be 90% \pm 9.7% for whole red delicious apples (Table 1); 83% \pm 7.6% for whole granny smith apples (Table 2); 97% \pm 15% for red delicious juice; 96% \pm 9.3% for granny smith juice (Table 4); 75% \pm 3.7% for red delicious wet pomace (Table 6); 77% \pm 4.1% for red delicious dry pomace (Table 7); 79% \pm 8.1% for granny smith dry pomace (Table 8). The overall average percent recovery was determined to be 87% \pm 12% (n=59).

- 10.3 Representative ion chromatograms for a standard solution, a control extract, and a fortified extract are shown in Figures 1 through 3.

Table 1

Recovery of Diphenylamine (DPA) in Whole Red Delicious Apples

<u>Analysis Set No.</u>	<u>Sample Number</u>	<u>Extraction Date</u>	<u>ppm Added</u>	<u>ppm Found</u>	<u>% Recovery (Corrected)^c</u>
A	101-003-3a	12/21/93	0	0.0686	NA
A	101-003-3a	12/21/93	0.0200	0.0953	130 ^a
A	101-003-3a	12/21/93	0.0200	0.0878	96 ^a
A	101-003-3a	12/21/93	0.0200	0.0986	150 ^a
A	101-003-3a	12/21/93	2.00	1.67	80
A	101-003-3a	12/21/93	2.00	1.96	95
D	101-003-3a	12/26/93	3.00	1.74	58 ^b
D	101-003-3a	12/26/93	2.80	2.27	81
D	101-003-3a	12/26/93	2.80	2.58	92
2W	101-001-03	01/04/94	0.200	0.186	93
2W	101-001-03	01/04/94	2.00	2.16	110
3	101-003-3a1MR1	01/27/94	2.00	1.74	84
3	101-003-3a1MR2	01/27/94	2.00	1.82	88

Average recovery = 90% (n=8)

NA Not applicable.

- a Samples fortified at 0.0200 ppm give variable results.
 b Value low due to spiking problem with pipet.
 c These recoveries were corrected for the 0.0686 ppm found in the control.

Table 2

Recovery of Diphenylamine (DPA) in Whole Granny Smith Apples

<u>Analysis Set No.</u>	<u>Sample Number</u>	<u>Extraction Date</u>	<u>ppm Added</u>	<u>ppm Found</u>	<u>% Recovery (Corrected)</u>
GSV	101-001-20	01/24/94	0	0.0937	NA
GSV	101-001-20	01/24/94	0	0.0803	NA
GSV	101-001-20	01/24/94	0	0.0892	NA
GSV	101-001-20	01/24/94	0.0200	0.100	62 ^a
GSV	101-001-20	01/24/94	0.0200	0.101	67 ^a
GSV	101-001-20	01/24/94	0.0200	0.0986	55 ^a
GSV	101-001-20	01/24/94	0.200	0.259	86
GSV	101-001-20	01/24/94	0.200	0.246	79
GSV	101-001-20	01/24/94	0.200	0.238	75
GSV	101-001-20	01/24/94	2.00	1.59	75
GSV	101-001-20	01/24/94	2.00	1.65	78
GSV	101-001-03	01/24/94	2.00	1.78	85
3	101-003-7a1MR1	01/27/94	2.00	1.99	96
3	101-003-7a1MR2	01/27/94	2.00	1.88	90

Average recovery = 83% (n=8)

NA Not applicable.

- a Samples fortified at 0.0200 ppm give variable results.
 b These recoveries were corrected for the average 0.0877 ppm found in the control.

Table 3

Recovery of Diphenylamine (DPA) in Red Delicious Apple Juice

<u>Analysis Set No.</u>	<u>Sample Number</u>	<u>Extraction Date</u>	<u>ppm Added</u>	<u>ppm Found</u>	<u>% Recovery</u>
1V	101-002-01-J1	01/29/94	0	<0.0800	NA
1V	101-002-01-J2	01/29/94	0	<0.0800	NA
1V	101-002-01-J3	01/29/94	0	<0.0800	NA
1V	101-002-01-JR1	01/29/94	0.400	0.248	62 ^a
1V	101-002-01-JR2	01/29/94	0.400	0.409	100
1V	101-002-01-JR3	01/29/94	0.400	0.342	86
1V	101-002-01-JR4	01/29/94	1.00	0.777	78
1V	101-002-01-JR5	01/29/94	1.00	0.798	80
1V	101-002-01-JR6	01/29/94	1.00	0.894	89
1V	101-002-01-JR7	01/29/94	2.00	2.23	110
1V	101-002-01-JR8	01/29/94	2.00	2.32	120
1V	101-002-01-JR9	01/29/94	2.00	2.16	110

Average recovery = 97% (n=8)

NA Not applicable.

a Sample 101-002-01-JR1 plugged filter in initial filtration step, causing low recovery.

Table 4

Recovery of Diphenylamine (DPA) in Granny Smith Apple Juice

<u>Analysis Set No.</u>	<u>Sample Number</u>	<u>Extraction Date</u>	<u>ppm Added</u>	<u>ppm Found</u>	<u>% Recovery</u>
2V	101-002-09-J1	02/01/94	0	<0.0800	NA
2V	101-002-09-J2	02/01/94	0	<0.0800	NA
2V	101-002-09-J3	02/01/94	0	<0.0800	NA
2V	101-002-09-JR1	02/01/94	0.400	0.372	93
2V	101-002-09-JR2	02/01/94	0.400	0.358	90
2V	101-002-09-JR3	02/01/94	0.400	0.351	88
2V	101-002-09-JR4	02/01/94	1.00	0.912	91
2V	101-002-09-JR5	02/01/94	1.00	0.835	84
2V	101-002-09-JR6	02/01/94	1.00	0.986	99
2V	101-002-09-JR7	02/01/94	2.00	2.07	100
2V	101-002-09-JR8	02/01/94	2.00	2.17	110
2V	101-002-09-JR9	02/01/94	2.00	2.28	110

Average recovery = 96% (n=9)

NA Not applicable.

Table 5

Recovery of Diphenylamine (DPA) in Red Delicious Wet Pomace

<u>Analysis Set No.</u>	<u>Sample Number</u>	<u>Extraction Date</u>	<u>ppm Added</u>	<u>ppm Found</u>	<u>% Recovery</u>
3VW	101-003-04-WP1	02/02/94	0	<0.0800	NA
3VW	101-003-04-WP2	02/02/94	0	<0.0800	NA
3VW	101-003-04-WP3	02/02/94	0	<0.0800	NA
3VW	101-003-04-WPR4	02/02/94	1.00	0.725	73
3VW	101-003-04-WPR5	02/02/94	1.00	0.700	70
3VW	101-003-04-WPR6	02/02/94	1.00	0.734	73
3VW	101-003-04-WPR7	02/02/94	2.00	1.59	80
3VW	101-003-04-WPR8	02/02/94	2.00	1.55	78
3VW	101-003-04-WPR9	02/02/94	2.00	1.52	76

Average recovery = 75% (n=6)

NA Not applicable.

Table 6

Recovery of Diphenylamine (DPA) in Granny Smith Wet Pomace

<u>Analysis Set No.</u>	<u>Sample Number</u>	<u>Extraction Date</u>	<u>ppm Added</u>	<u>ppm Found</u>	<u>% Recovery (Corrected)^b</u>
4VM	101-002-09-WP1	02/04/94	0	<0.0800	NA
4VM	101-002-09-WP2	02/04/94	0	0.0989	NA
4VM	101-002-09-WP3	02/04/94	0	<0.0800	NA
4VM	101-002-09-WPR1	02/04/94	0.400	0.447	94
4VM	101-002-09-WPR2	02/04/94	0.400	0.497	110
4VM	101-002-09-WPR3	02/04/94	0.400	0.447	94
4VM	101-002-09-WPR4	02/04/94	1.00	0.985	91
4VM	101-002-09-WPR5	02/04/94	1.00	0.831	76
4VM	101-002-09-WPR6	02/04/94	1.00	0.975	90
4VM	101-002-09-WPR7	02/04/94	2.00	1.10	51 ^a
4VM	101-002-09-WPR8	02/04/94	2.00	1.80	86
4VM	101-002-09-WPR9	02/04/94	2.00	1.53	73

Average recovery = 89% (n=8)

NA Not applicable.

- a Insufficient volume of extract to make dilution, resulting in the low recovery.
- b These recoveries were corrected for the average 0.0710 ppm found in the controls.

Table 7

Recovery of Diphenylamine (DPA) in Red Delicious Dry Pomace

<u>Analysis Set No.</u>	<u>Sample Number</u>	<u>Extraction Date</u>	<u>ppm Added</u>	<u>ppm Found</u>	<u>% Recovery</u>
SVD	101-003-04-DP1	02/07/94	0	<0.400	NA
SVD	101-003-04-DP2	02/07/94	0	<0.400	NA
SVD	101-003-04-DP3	02/07/94	0	<0.400	NA
SVD	101-003-04-DPR4	02/07/94	1.00	0.759	76
SVD	101-003-04-DPR5	02/07/94	1.00	0.704	70
SVD	101-003-04-DPR6	02/07/94	1.00	0.772	77
SVD	101-003-04-DPR7	02/07/94	2.00	1.64	82
SVD	101-003-04-DPR8	02/07/94	2.00	1.57	79
SVD	101-003-04-DPR9	02/07/94	2.00	1.57	79

Average recovery = 77% (n=6)

NA Not applicable.

Table 8

Recovery of Diphenlyamine (DPA) in Granny Smith Dry Pomace

<u>Analysis Set No.</u>	<u>Sample Number</u>	<u>Extraction Date</u>	<u>ppm Added</u>	<u>ppm Found</u>	<u>% Recovery</u>
6VD	101-002-10-DP1	02/09/94	0	<0.0800	NA
6VD	101-002-10-DP2	02/09/94	0	<0.0800	NA
6VD	101-002-10-DP3	02/09/94	0	<0.0800	NA
6VD	101-002-10-DPR1	02/09/94	0.400	<0.0800	NA ^a
6VD	101-002-10-DPR2	02/09/94	0.400	<0.0800	NA ^a
6VD	101-002-10-DPR3	02/09/94	0.400	<0.0800	NA ^a
6VD	101-002-10-DPR4	02/09/94	1.00	0.929	93
6VD	101-002-10-DPR5	02/09/94	1.00	0.784	78
6VD	101-002-10-DPR6	02/09/94	1.00	0.698	70
6VD	101-002-10-DPR7	02/09/94	2.00	1.52	76
6VD	101-002-10-DPR8	02/09/94	2.00	1.47	74
6VD	101-002-10-DPR9	02/09/94	2.00	1.66	83

Average recovery = 79% (n=6)

NA Not applicable.

a It appears 0.400 ppm samples were spiked incorrectly.

Figure 1
Representative Ion Chromatogram of Acetylated Diphenylamine
Standard Solution (0.500 $\mu\text{g}/\text{mL}$)

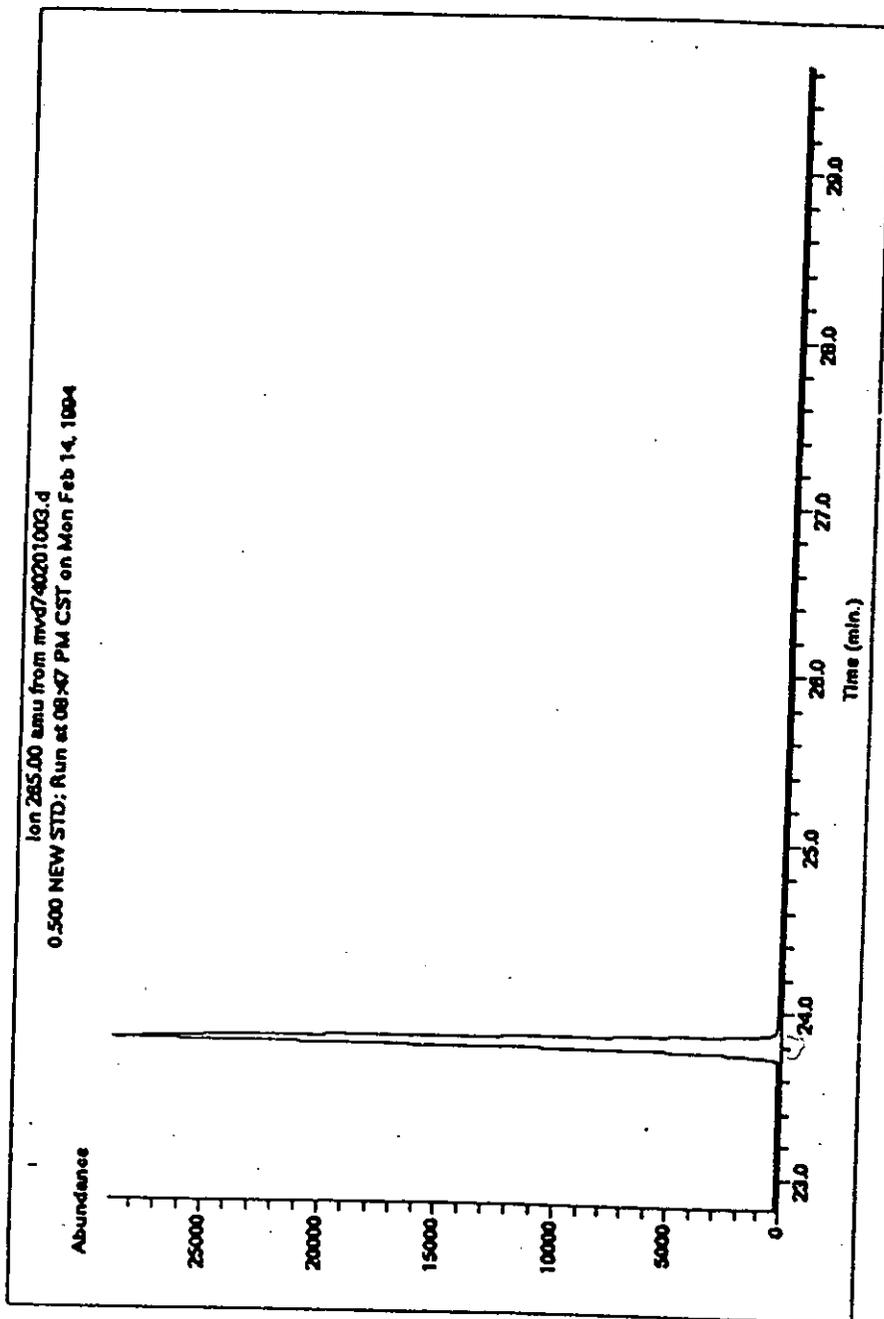


Figure 2
Representative Chromatogram of an Acetylated Extract of
Untreated Red Delicious Control Wet Pomace

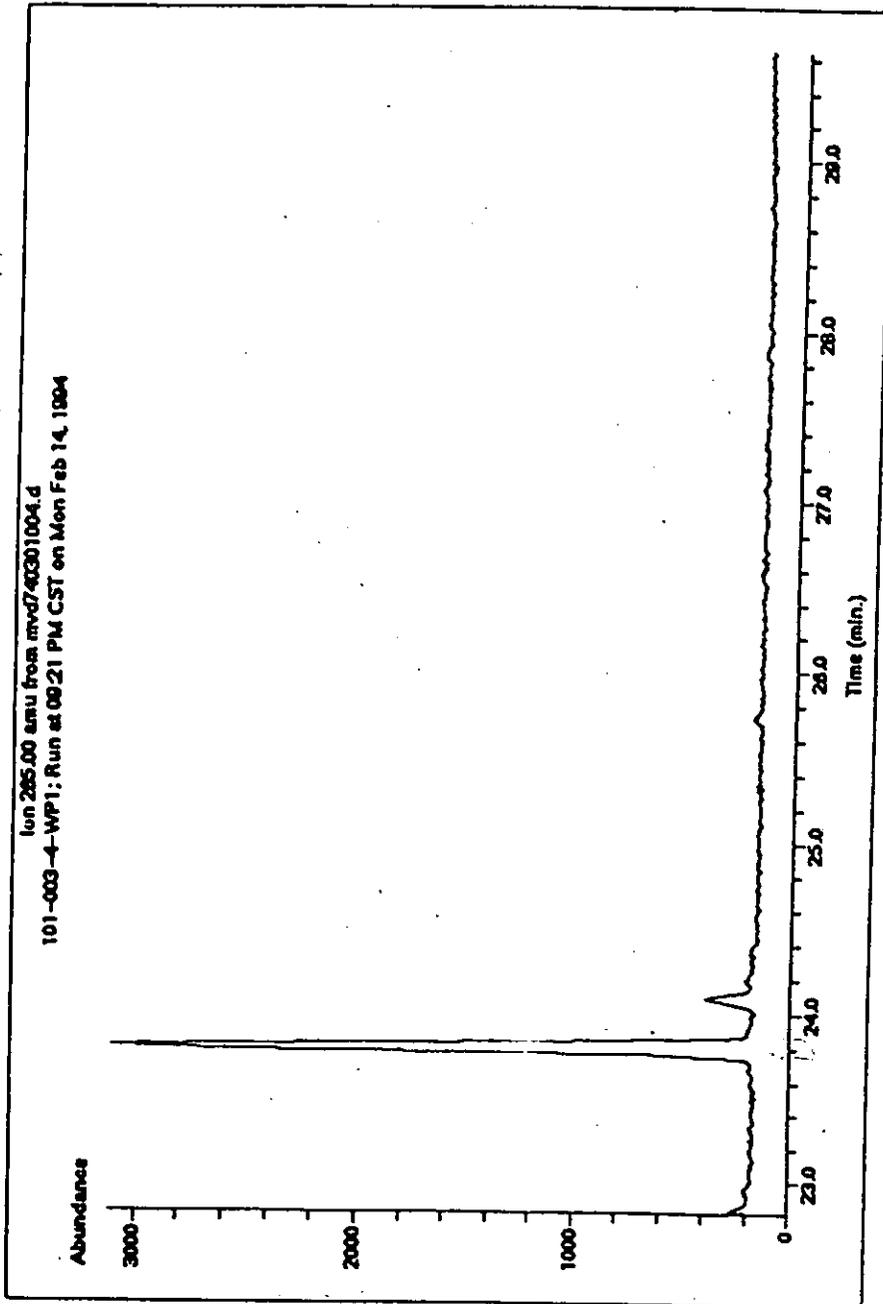
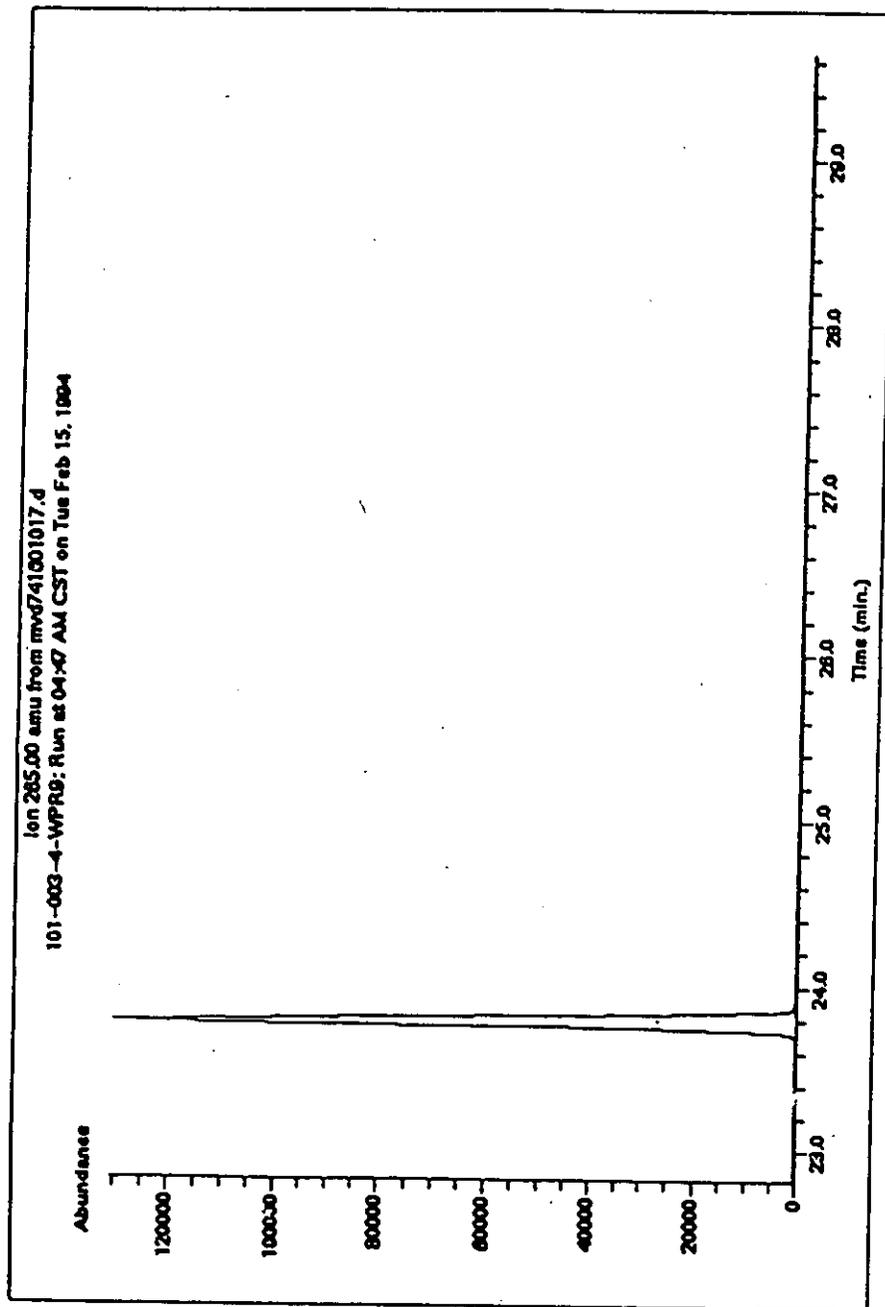


Figure 3
Representative Chromatogram of an Acetylated Extract of
Untreated Red Delicious Control Wet Pomace Fortified at 2.00 ppm



EPA ADDENDUM

1. ACLB used a Hewlett-Packard 5988 GC/MS system equipped with a Hewlett-Packard 5890 GC. A 3uL injection volume was used for all analyses. The transfer line temperature was 280°C instead of 260°C that was used by the task force. All other GC/MS conditions were the same as described in the method.

2. DPA standard response was found to be linear over the range of 1.55 - 15.5 ng injected. All sample analyses were conducted in this range.

3. The method calls for using controls to correct for recoveries. Enforcement laboratories are discouraged from using control values to correct for recoveries as the Pesticide Assessment Guidelines, 860.1340 do not allow for this use.