

METALDEHYDE



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EN-CAS Method No. ENC-3/99

**Analytical Method for the Determination
of Metaldehyde in Lettuce by GC/MS**

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Issue Date: 2/18/00

Total Pages = 25

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EN-CAS Method No. ENC-3/99	Author: Wayne Barker	Date Issued: 2/18/00 Revision:
TITLE: Analytical Method for the Determination of Metaldehyde in Lettuce by GC/MS	QA APPROVAL <i>Kathleen Taltynski 2/18/00</i> MGMT APPROVAL <i>Ben Clifton 2/18/00</i>	

1.0 INTRODUCTION

1.1 Scope

This analytical method is used for the determination of Metaldehyde residues in/on lettuce. A method from the Research and Consulting Company AG, entitled Determination of Metaldehyde Residues in Plant Material (reference 1) was used by EN-CAS to develop a method for the determination of Metaldehyde in soil. Subsequently, the EN-CAS soil method (EN-CAS Method No. ENC-2/91, entitled Analytical Method for the Determination of Metaldehyde in Soil, reference 2) and EN-CAS Method No. ENC-1/97, entitled Analytical Method for the Determination of Metaldehyde in Crops (reference 3) were used to develop the extraction portion of this method. The gas chromatographic mass spectrometry (GC/MS) analysis portion of this method is based on procedures used by the FDA to monitor residues of multiple pesticides, including Metaldehyde, in crop samples (see reference 4). The limit of quantitation (LOQ) is 0.05 ppm ($\mu\text{g/g}$) Metaldehyde. Method validation results are included as Table I. See Figure 1 for a flowchart of the analytical procedure.

1.2 Principle

Metaldehyde is extracted from well-mixed, homogenized lettuce by shaking with dichloromethane (DCM) and sodium sulfate. An aliquot of the extracted analyte is then filtered through a small pad of sodium sulfate and reduced to incipient dryness. The sample extract is adjusted to an appropriate final volume in DCM, and injected on a GC/MS.

2.0 APPARATUS

All apparatus listed may be replaced by equivalent apparatus from alternate sources if experimental verification supports such substitutions.

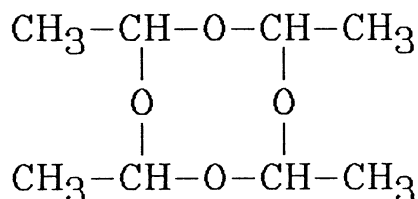
- 2.1 French square bottles, 16-oz, with Teflon-lined caps.
- 2.2 Erlenmeyer flasks, 250-mL, with 24/40 ground glass fittings.
- 2.3 Stoppers, ground glass, 24/40.
- 2.4 Volumetric flasks, 100-mL, for preparing analytical standards.
- 2.5 Powder funnels, glass or aluminum, 4-inch diameter.
- 2.6 Disposable Pasteur pipettes, 23-cm.
- 2.7 Scintillation vials, 20-mL.
- 2.8 GC vials, 2-mL, with crimp caps, Hewlett Packard, Catalog No. 5181-3375.
- 2.9 Mechanical shaker, G10 Gyrotory, New Brunswick Scientific.
- 2.10 Graduated cylinders, 50- or 100-mL and 250-mL.
- 2.11 Hobart Food Processor.
- 2.12 Sonicating bath, Branson model 5200.
- 2.13 Rotary evaporator (Brinkman Rotovapor, Model #RE111).
- 2.14 Top loading balance (Fisher Scientific, Model XT-3KD).
- 2.15 Mettler analytical balance capable of 5 decimal accuracy, for weighing analytical standards.

3.0 REAGENTS

All reagents listed may be replaced by equivalent reagents from alternate sources if experimental verification supports such substitutions.

- 3.1 DCM, pesticide grade.
- 3.2 Methanol, Optima grade (Fisher Scientific).
- 3.3 Glass wool, Pyrex Fiberglass, 8-mm.
- 3.4 Sodium sulfate (Na_2SO_4), anhydrous, ACS certified, heated in a muffle furnace at 600°F for 2 hours and cooled overnight in a desiccator.

4.0 REFERENCE STANDARD IDENTIFICATION AND STRUCTURE



Metaldehyde
(C₈H₁₆O₄)
MW = 176.2

5.0 PREPARATION OF ANALYTICAL STANDARDS

5.1 Fortification Standards

Weigh 100 mg (active ingredient) of the test substance, Metaldehyde, using an analytical balance into a 100-mL volumetric flask. Dissolve and dilute to volume with methanol to prepare a 1000 µg/mL stock solution.

Serially dilute the 1000-µg/mL stock solution in methanol to prepare 100-µg/mL and 10-µg/mL standard solutions. Use these solutions to fortify lettuce control samples in order to monitor procedural recovery. The 1000 µg/mL and 100 µg/mL stock standard solutions are presumed stable for approximately 1 year unless standard comparison data indicates otherwise. The 10 µg/mL stock standard solution is presumed stable for approximately 6 months.

NOTE: Store all Metaldehyde standard and stock solutions in a freezer at a temperature of less than -10°C.

5.2 GC/MS Standards

Dilute the 10-µg/mL Metaldehyde standard solution with the appropriate amounts of DCM to obtain Metaldehyde GC/MS calibration standards ranging from 0.01 µg/mL to 0.50 µg/mL. The GC/MS calibration standards are presumed stable for approximately 6 months.

6.0 ANALYTICAL PROCEDURE

6.1 Homogenization

Chop the lettuce sample appropriately by breaking, tearing or cutting. Homogenize the chopped sample using a Hobart food processor (or equivalent) and dry ice to obtain a homogeneous sample. Allow the dry ice to sublime prior to taking a subsample.

NOTE: Chop and homogenize control samples first and thoroughly clean the food processor between samples. Homogenization times may vary depending on the matrix.

6.2 Extraction

6.2.1 Weigh a 15-g homogeneous sample into a 16-oz French square bottle. If appropriate, make appropriate laboratory fortifications with the Metaldehyde stock or standard solutions prepared in Section 5.1.

NOTE: Allow solvent from the fortification to evaporate for approximately 15 minutes in a fume hood.

6.2.2 Add 150 mL of pesticide grade DCM to the French square bottle.

6.2.3 Add 50 g of anhydrous sodium sulfate to the DCM/lettuce mixture. Cap the bottle, give the sample a quick shake and then release the pressure by opening the cap slightly. Recap the bottle.

6.2.4 Place the bottle on its side in the mechanical shaker and shake vigorously (approximately 200 rpm) for 60 minutes.

6.2.5 Remove the bottle from the shaker, place it upright and let the sample settle for at least 15 minutes.

6.3 Filtration and Evaporation

6.3.1 Filter a 50-mL aliquot of the sample through a pad of anhydrous sodium sulfate (approximately 50 g) on top of a glass wool plug, into a 250-mL Erlenmeyer flask. Rinse the sodium sulfate pad with 25 mL of DCM and combine the rinse with the filtrate.

6.3 Filtration and Evaporation (continued)

6.3.2 Evaporate the sample just to incipient dryness under vacuum rotary evaporation, using a bath temperature of approximately 30°C - 35°C.

NOTE: Rotary evaporation for longer than absolutely necessary may result in loss of recovery.

6.3.3 Remove the sample from the evaporation device and allow any remaining DCM to evaporate in a fume hood for ~15-30 minutes.

6.3.4 Dissolve the sample extract to an appropriate final volume (usually 10 mL) with DCM. Sonicate for at least one minute prior to transferring the sample from the sample flask into the GC vial. Proceed to GC/MS analysis.

6.4 GC/MS Determination

Use a 12-m x 0.2-mm, 0.33- μ m film thickness, capillary DB-1 column to achieve separation on the GC/MS. Use a Hewlett-Packard Model 6890 (or equivalent) with a Hewlett Packard model 5973 GC/MSD to provide adequate sensitivity and selectivity. GC/MS conditions are listed in Section 7.0 of this method.

6.5 Safety Precautions

Use normal safety precautions, including the wearing of gloves and safety glasses. The use of a fume hood is necessary to minimize exposure to the analytes, fumes and organic solvents used in this procedure.

6.6 Limit of Quantitation

For lettuce samples validated herein, this method was successful to a LOQ of 0.05 ppm Metaldehyde. Adjust the instrument sensitivity, GC/MS calibration standards and final sample volumes to allow detection of Metaldehyde at 50% of the LOQ.

6.7 Time Required for Analysis

An experienced analyst can process a set of approximately 12 samples (including controls and recoveries), and prepare them for injection on the GC/MS in approximately one 8-hour day.

7.0 GC/MS ANALYSIS

7.1 Description and Typical Operating Conditions

Instrument: Hewlett-Packard Model 5973 GC/MSD. Data will be collected and processed with a Hewlett-Packard G1701BA Chemstation

Column: Fused silica DB-1 capillary column, 12-m x 0.2-mm (J & W Scientific), 0.33- μ m film thickness

Injection Port: 175°C splitless

Injection Volume: 1- μ L, splitless

Temperatures: Injector: 175°C
Detector: 200°C

Temperature Program:

Initial Temperature:	= 45°C
Initial Time:	= 4.0 min
Ramp A:	= 20°C/min
Temperature A	= 70°C
Final Time A:	= 3.0 min
Ramp B:	= 35°C/min
Temperature B:	= 200°C
Final Time B:	= 7.0 min

Typical Metaldehyde Retention Time: 5.38 minutes

Ions: m/z = 89 (Quantitation)
m/z = 45 (Confirmatory)

7.2 Calibration

Use the Metaldehyde GC/MS calibration standards that were prepared in Section 5.2 in concentrations typically ranging from 0.01 μ g/mL to 0.50 μ g/mL to calibrate the instrument. Inject appropriate standards at the beginning of the run, after approximately every 2 or 3 samples

7.2 Calibration (continued)

throughout the run, and at the end of the run. A linear regression function is generated using the peak height vs. nanograms injected (see Section 8.0). The correlation coefficient for the line should be equal to, or greater than, 0.990. The sample nanograms found are determined by inserting the peak count values into appropriate standard curve linear regression equation.

7.3 Representative Chromatograms

Typical chromatograms illustrating GC/MS calibration standards, as well as a reagent blank, control lettuce and control lettuce fortified with Metaldehyde, are shown in Figures 2 to 10. A typical calibration curve is shown in Figure 11.

8.0 CALCULATIONS

8.1 Calculation of ng Found

The nanograms found is obtained from a standard curve constructed using linear regression analysis results of the GC/MS calibration standards (ng injected vs. peak height).

$$\text{ng Found} = \frac{\text{Peak height (counts)} - \text{standard curve y-intercept (counts)}}{\text{standard curve slope (counts/ng)}}$$

8.2 Calculation of mg-Equivalent Injected

$$\text{mg-equiv. injected} = \frac{\text{g sample extracted} \times \text{aliquot (mL)} \times \mu\text{L injected} \times 1000 \text{ mg/g}}{\text{mL blended} \times \text{final volume (mL)} \times \text{dilution factor} \times 1000 \mu\text{L/mL}}$$

8.3 Calculation of ppm Found

$$\text{ppm found} = \frac{\text{ng found}}{\text{mg-equivalent injected}}$$

8.0 CALCULATIONS (continued)**8.4 Calculation of ppm Found (Corrected for Control Contribution)**

Only laboratory fortification (procedural recovery) samples, not residue samples, are corrected for corresponding control contribution.

$$\begin{array}{l} \text{ppm found} \\ \text{(corrected)} = \text{raw ppm found (recovery samples)} - \text{ppm found (control)} \end{array}$$

8.5 Calculation of Percent Procedural Recovery

$$\% \text{ Recovery} = \frac{\text{ppm found}}{\text{fortification level (ppm)}} \times 100$$

8.0 CALCULATIONS (continued)

8.6 Example Calculation for a Laboratory Fortification

Lettuce Sample EEQ6350-S10, set # 1-01-MVR, GC/MS run # 71938 see also Figure # 7

Sample Wt	= 15 g
Extraction volume	= 150 mL
Aliquot volume	= 50 mL
Final volume	= 10 mL
Dilution factor	= 1
Injection volume	= 1.0 μ L
Peak height (sample)	= 13532 counts
y-intercept	= -304.062 counts
Slope	= 547792.3 counts/ng
Fortification level (ppm)	= 0.05 ppm
Control Contribution	= 0 ppm

$$\text{ng found} = \frac{13532 \text{ counts} - (-304.062 \text{ counts})}{547792.3 \text{ counts/ng}} = 0.0253 \text{ ng}$$

$$\text{mg-equiv. injected} = \frac{15 \text{ g} \times 50 \text{ mL} \times 1 \mu\text{L} \times 1000 \text{ mg/g}}{150 \text{ mL} \times 10 \text{ mL} \times 1 \times 1000 \mu\text{L/mL}} = 0.50 \text{ mg}$$

$$\text{ppm found} = \frac{0.0253 \text{ ng}}{0.50 \text{ mg}} = 0.0505 \text{ ppm}$$

$$\text{ppm found} = 0.0505 \text{ ppm (sample)} - 0 \text{ ppm (control)} = 0.0505 \text{ ppm (corrected)}$$

$$\% \text{ Recovery} = \frac{0.0505 \text{ ppm}}{0.05 \text{ ppm}} \times 100 = 101\%$$

9.0 VALIDATION RESULTS

See Table I in this method.

9.1 Statistical Method

The mean recoveries and standard deviations are calculated from the validation data and appear in the validation tables included in this method. The standard deviation is calculated using the following equation:

When n is < 30:

$$\sigma_{(n-1)} = \sqrt{\frac{\sum_{i=1}^n (x_i - \bar{x})^2}{n-1}}$$

where the sum of the squares of the individual deviations from the mean ($x_i - \bar{x}$) is divided by one less than the total number of measurements in the set, n-1 (when the total number of measurements is less than 30; in the case of $n \geq 30$, the sum of the squares is divided by n).

9.2 Discussion of Validation Results

Recovery means and standard deviations calculated for the method validation in lettuce indicate a reliable method for the determination of Metaldehyde by GC/MS. The method validation raw data can be found in EN-CAS Project # 99-0055.

The following table summarizes the validation results obtained using this method.

9.2 Discussion of Validation Results (continued)

TABLE I: Summary of Method Validation Results in Lettuce

Fortification Level	% Recovery
0.05 ppm	90, 101
0.10 ppm	100, 103
0.20 ppm	93, 97
1.0 ppm	89, 85
Recovery Range (%):	
85 - 103	
Mean and Standard Deviation:	
95% ± 6.5 (n=8)	

10.0 SUGGESTED QUALITY CONTROL PROCEDURES FOR APPLICATION OF THE METHOD TO RESIDUE ANALYSIS

10.1 Laboratory Fortifications

When analyzing residue samples, laboratory fortified control samples should be included in each analysis set to demonstrate acceptable procedural recovery. Within each analytical set, laboratory fortifications should correspond to a minimum of 10% of the analyzed samples. Procedural recovery results should typically be within 70-120%.

10.2 Sample Storage

All residue samples should be stored frozen until analyzed. After subsampling for analysis, the remaining bulk sample should be re-frozen and stored until appropriate authorization for sample disposal is received.

11.0 REFERENCES

1. Research and Consulting Company AG Method, entitled Determination of Metaldehyde Residues in Plant Material, issued April 24, 1985.
2. EN-CAS Method No. ENC-2/91, entitled Analytical Method for the Determination of Metaldehyde in Soil, issued April 7, 1992.
3. EN-CAS Method No. ENC-1/97, entitled Analytical Method for the Determination of Metaldehyde in Crops, issued February 28, 1997.
4. FDA Laboratory Information Bulletin entitled The Use of Mass Spectrometric Selected Ion Monitoring to Screen for Pesticides which Contain Combinations of Only Nitrogen, Sulfur and/or Oxygen Heteroatoms, written by Gregory E. Mercer; personal communication from Gregory Mercer (FDA) to Sari Weston (TRS) dated July 17, 1998.

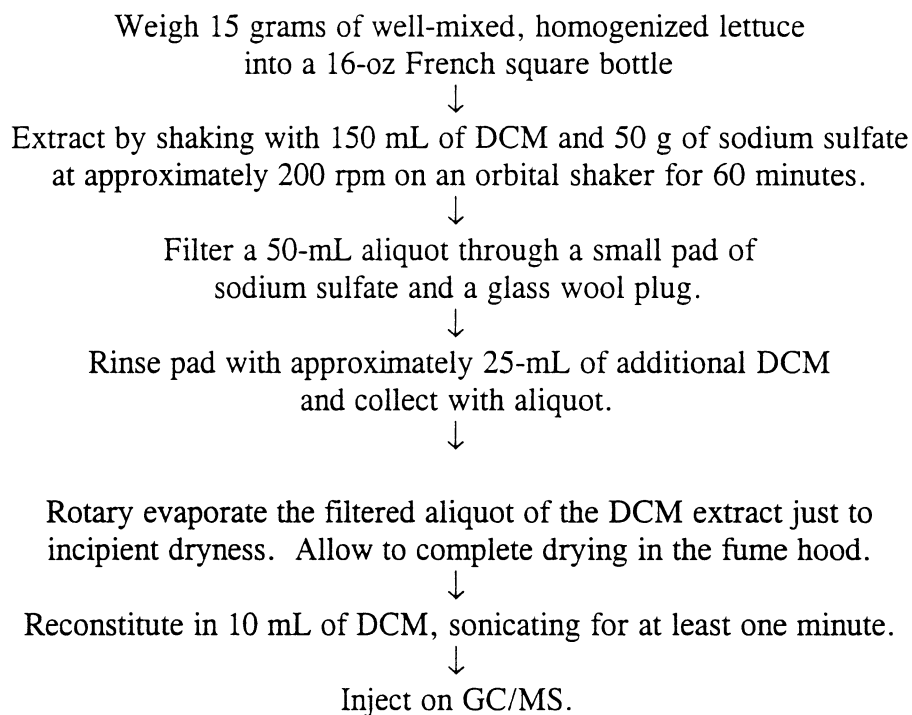
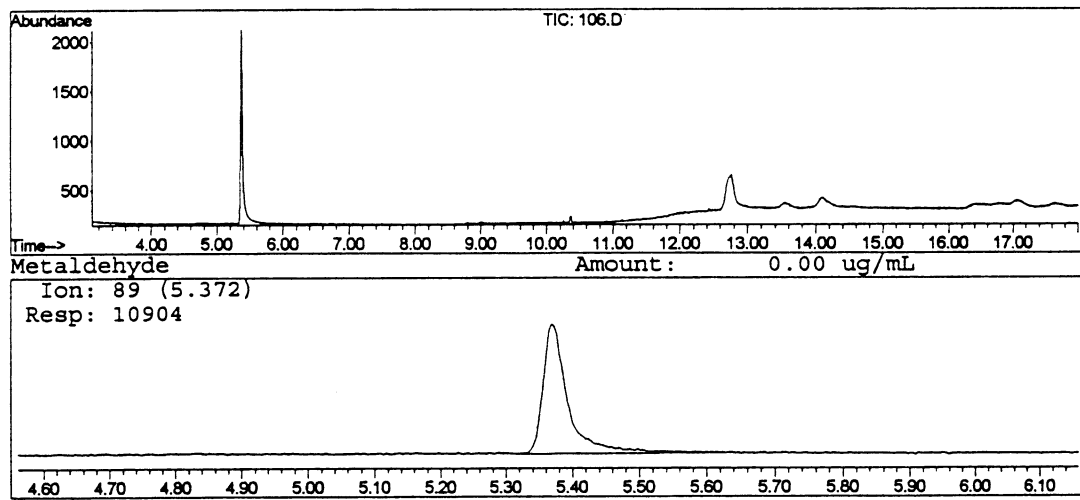
FIGURE 1**Flowchart of Analytical Method ENC-3/99****Analytical Method for the Determination
of Metaldehyde in Lettuce by GC/MS**

FIGURE 2

0.02 $\mu\text{g/mL}$ Standard

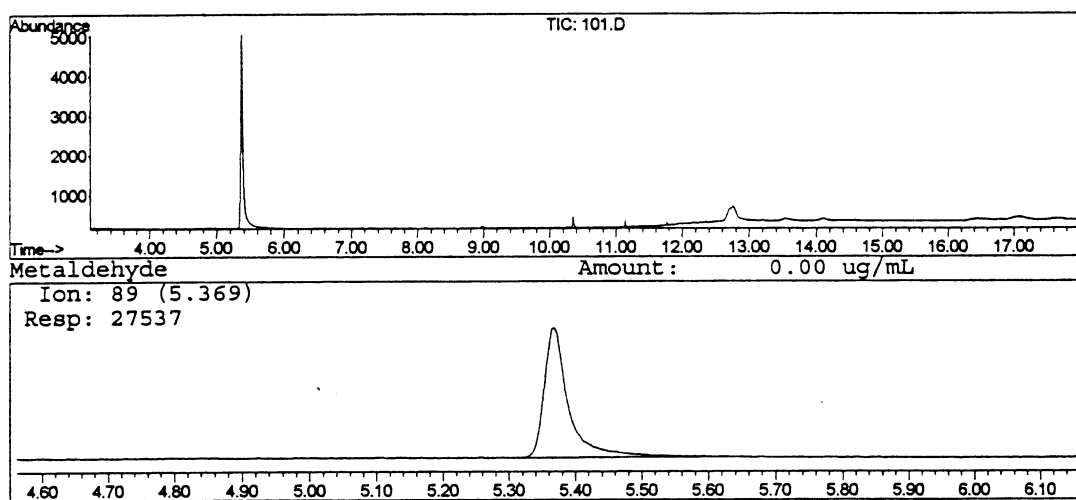


0.02 ng injected

GC/MS Run # 71938, Set # 1-01-MVR, Dated 12/2/99

FIGURE 3

0.05 $\mu\text{g/mL}$ Standard

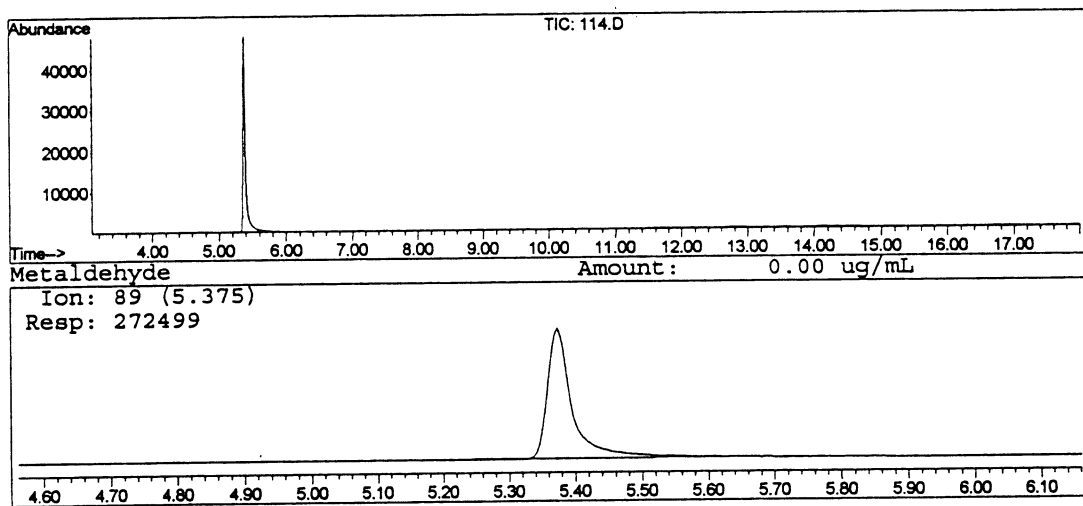


0.05 ng injected

GC/MS Run # 71938, Set # 1-01-MVR, Dated 12/2/99

FIGURE 4

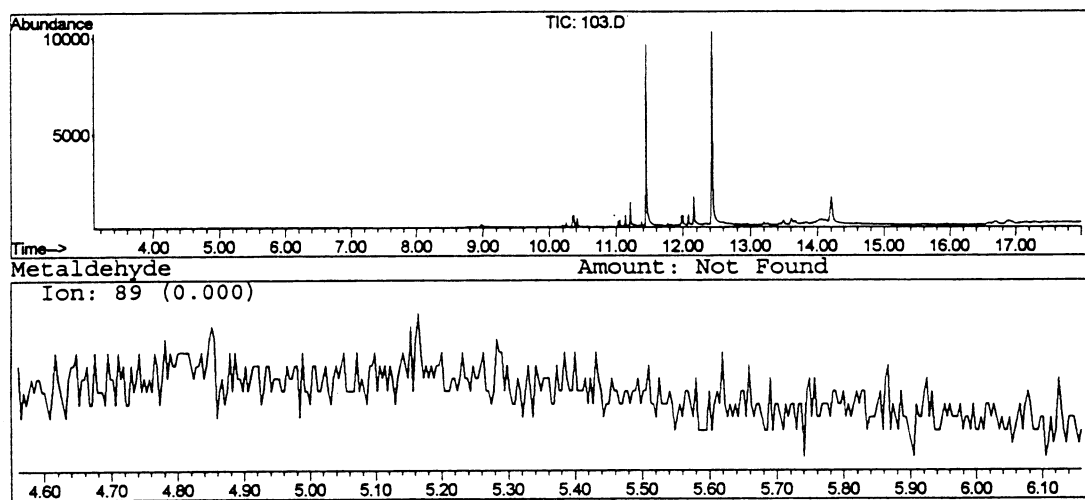
0.50 $\mu\text{g/mL}$ Standard



0.50 ng injected
GC/MS Run # 71938, Set # 1-01-MVR, Dated 12/3/99

FIGURE 5

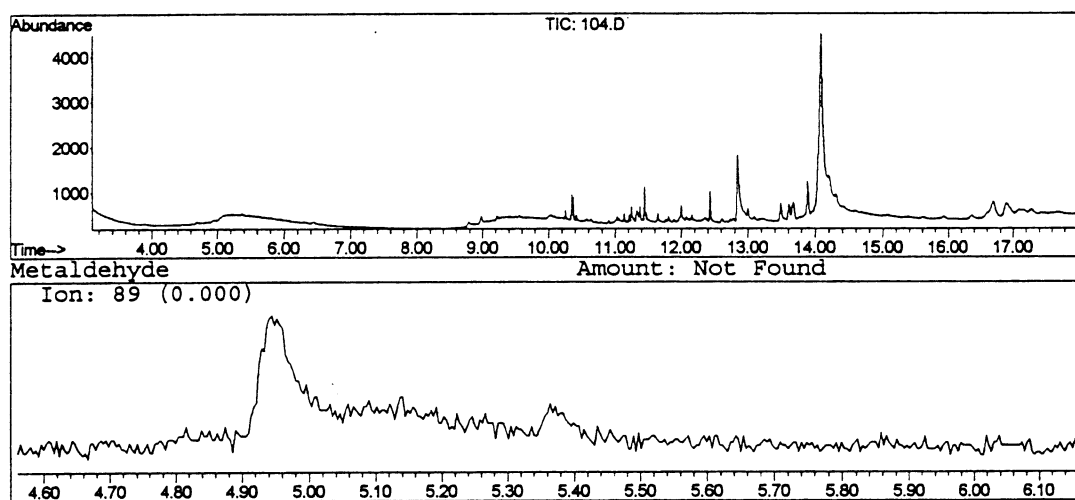
Reagent Blank



EN-CAS Sample # ID: RGT BLK
Metaldehyde ppm Found: <0.05 ppm
GC/MS Run # 71938, Set # 1-01-MVR, Dated 12/2/99
Final Volume = 10 mL

FIGURE 6

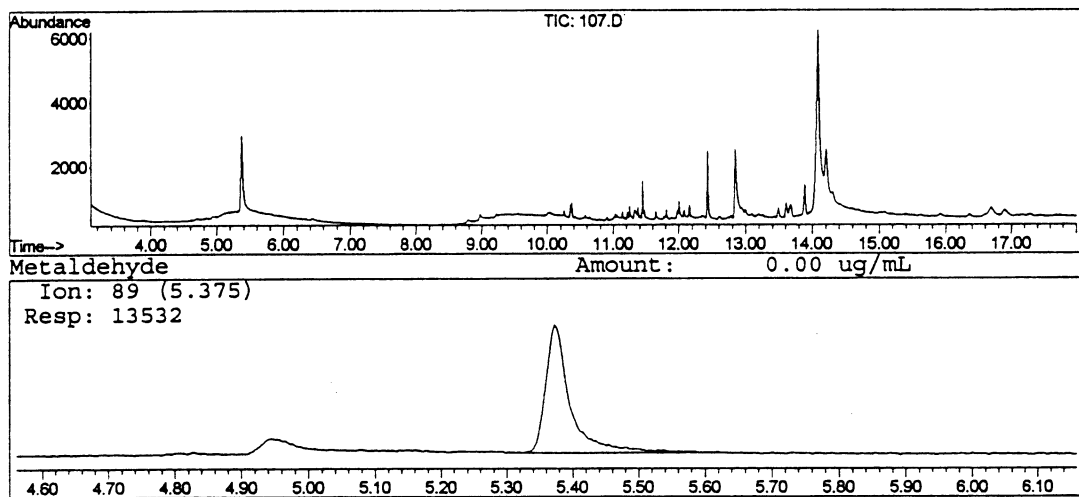
Control Lettuce



EN-CAS Sample # ID: EQ6350-C2
Metaldehyde ppm Found: <0.05 ppm
GC/MS Run # 71938, Set # 1-01-MVR, Dated 12/2/99
Final Volume = 10 mL

FIGURE 7

Control Lettuce + 0.05 ppm Metaldehyde



EN-CAS Sample # ID: EQ6350-S10

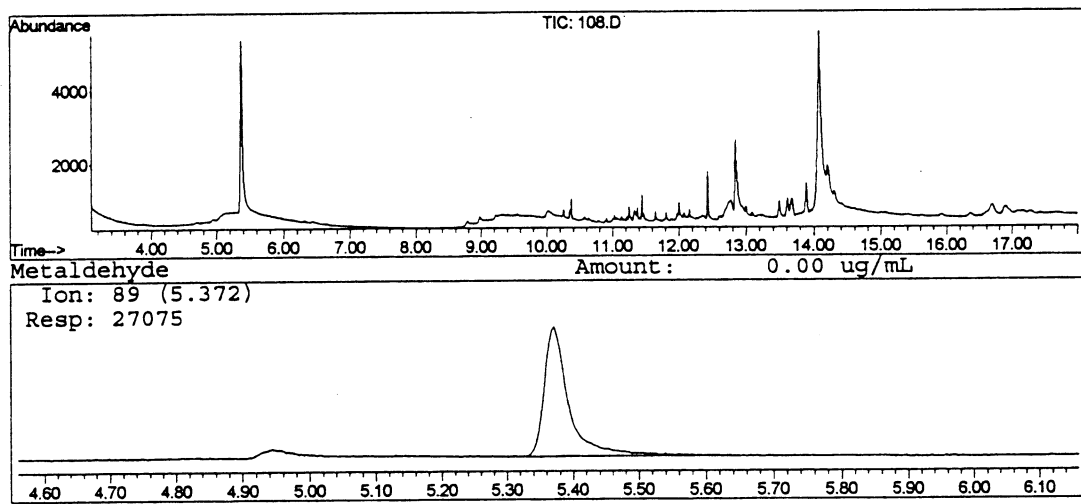
Metaldehyde Recovered: 101%

GC/MS Run # 71938, Set # 1-01-MVR, Dated 12/2/99

Final Volume = 10 mL

FIGURE 8

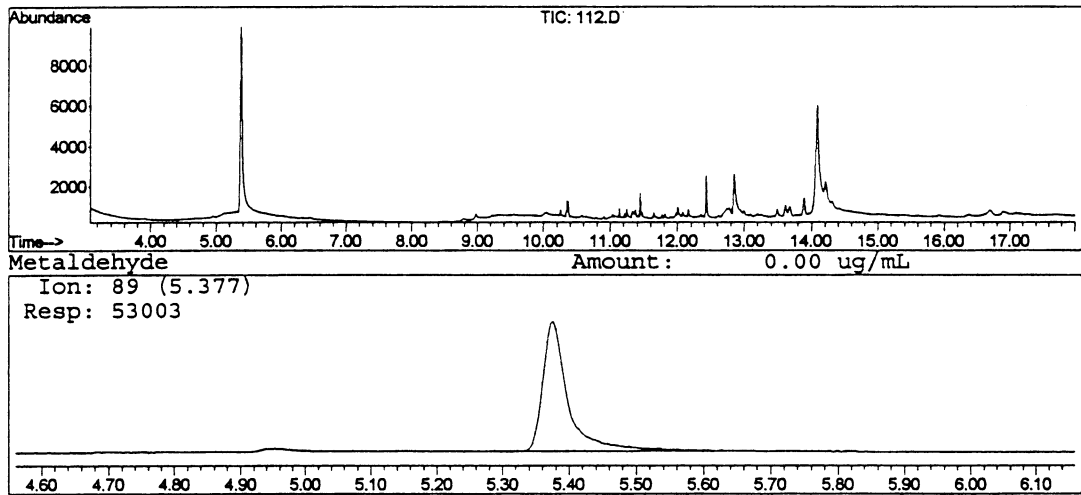
Control Lettuce + 0.10 ppm Metaldehyde



EN-CAS Sample # ID: EQ6350-S11
Metaldehyde Recovered: 100%
GC/MS Run # 71938, Set # 1-01-MVR, Dated 12/2/99
Final Volume = 10 mL

FIGURE 9

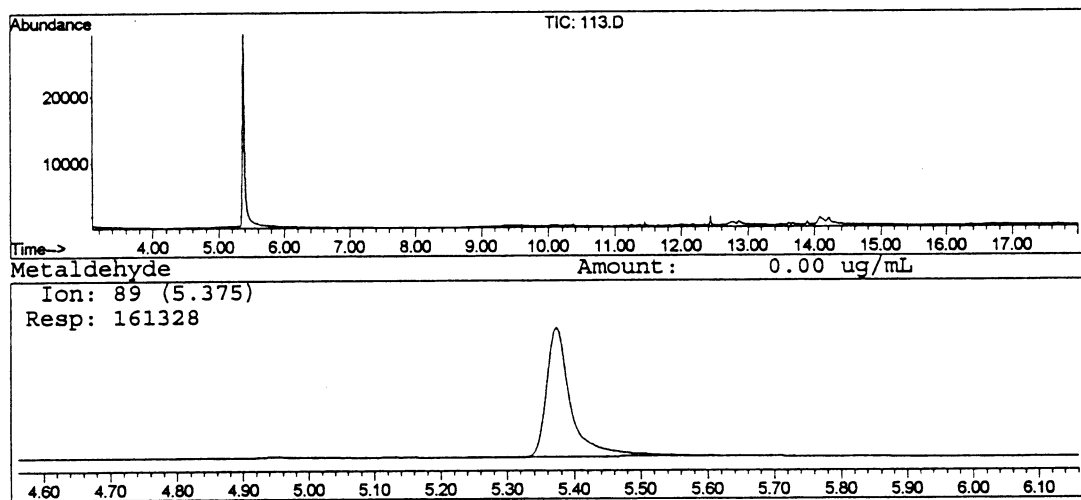
Control Lettuce + 0.20 ppm Metaldehyde



EN-CAS Sample # ID: EQ6350-S14
Metaldehyde Recovered: 97%
GC/MS Run # 71938, Set # 1-01-MVR, Dated 12/2/99
Final Volume = 10 mL

FIGURE 10

Control Lettuce + 1.0 ppm Metaldehyde



EN-CAS Sample # ID: EQ6350-S15

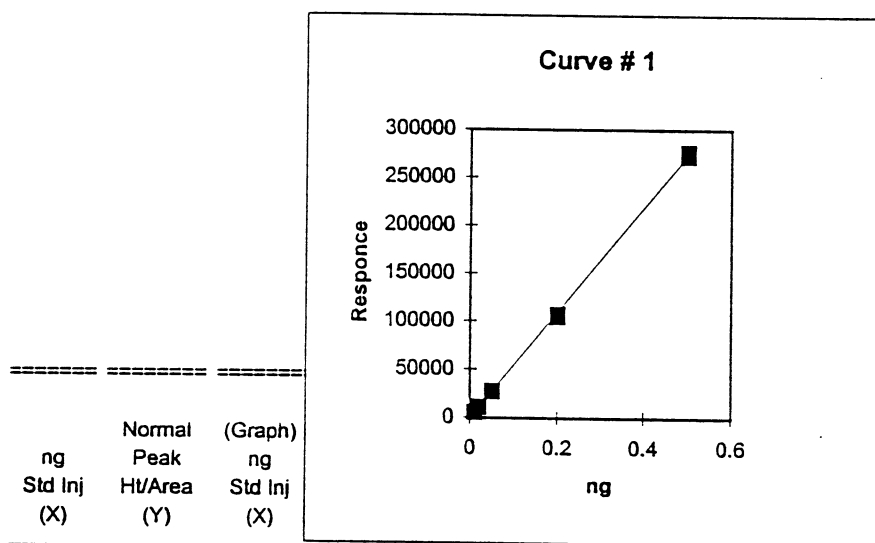
Metaldehyde Recovered: 89%

GC/MS Run # 71938, Set # 1-01-MVR, Dated 12/2/99

Final Volume = 15 mL

FIGURE 11

GC/MS Calibration Curve



ng Std Inj (X)	Normal Peak Ht/Area (Y)	(Graph) ng Std Inj (X)		
0.05	27537	0.05	Intercept	-304.06
0.01	5625	0.01	Slope	547792
		#N/A	R Squared	0.99969
		#N/A	Corr. Coeff.	0.99984
		#N/A		
0.02	10904	0.02		
		#N/A		
		#N/A		
		#N/A		
0.2	107421	0.2		
		#N/A		
		#N/A		
		#N/A		
0.5	272499	0.5		
		#N/A		
0.01	5837	0.01		
0.02	11190	0.02		
0.05	27287	0.05		
0.2	105080	0.2		
0.5	276930	0.5		
		#N/A		
0.01	5630	0.01		
0.02	11401	0.02		