

Cover Sheet for

**ENVIRONMENTAL CHEMISTRY METHOD**

***Pesticide Name:*** Maleic Hydrazide Technical

***MRID #:*** 449000-01

***Matrix:*** Water

***Analysis:*** HPLC/ELCD

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**STUDY TITLE**

**Analytical Method for Maleic Hydrazide in Water**

**Data Requirement**

Not Applicable

**Author**

James B. Pierce

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July 20, 1999

**Performing Laboratory**

Uniroyal Chemical Company, Inc.  
Middlebury, CT 06749

**Laboratory Project Identification**

Analytical Method AC7002  
Uniroyal Project No. 99113

**Related Reports**

Uniroyal Research Project	MIRD
99100 (97/FLD/16/UK)	None (never submitted to EPA)

**Key Words:**

Analytical Method, maleic hydrazide, water

## SUMMARY

Water containing maleic hydrazide is made basic with potassium hydroxide and then cleaned up by extraction with dichloromethane. The aqueous phase is then concentrated, made up to a known volume with mobile phase (formic acid/water/ammonium hydroxide, adjusted to pH 3.2) and analyzed by high performance liquid chromatography using an electrochemical detector.

### A. MATERIALS

#### A.1 Equipment

Analytical balance: Capable of weighing to the nearest 0.1mg.

Top pan balance: Capable of weighing to the nearest 0.1mg.

General laboratory glassware:

Volumetric flasks and pipettes, various volumes.

Round bottom flasks, 500 mL and 50 mL.

Separating funnels, 250 mL.

Measuring cylinders, 100 mL.

Ultrasonic water bath.

Rotary evaporator and water bath – Buchi or equivalent.

Disposable 5 mL plastic syringes.

0.2  $\mu$ m nylon syringe filters.

0.2  $\mu$ m nylon filters for preparation of mobile phase.

#### A.2 Reagents/Supplies

Analytical standard: maleic hydrazide – analytical grade.

Solvents and general laboratory reagents: These should be of the highest possible grade.

All solvents should be HPLC grade or equivalent

Methanol

Dichloromethane:

Water

Potassium hydroxide

Formic acid

Ammonium hydroxide

HPLC mobile phase: 2.2g formic acid dissolved in approximately 900 mL of water. The pH is adjusted to 3.2 with ammonium hydroxide and the volume is then adjusted to 1000 mL. Prior to use the mobile phase is filtered, under vacuum, through a 0.2  $\mu$ m nylon filter.

### **A.3 Analytical Standards**

Analytical standards of MH can be obtained from Uniroyal Chemical Company, Inc. The analytical standard should be stored in a cold room or refrigerator at 4°C or less until used. The structure of MH and its current standard sheet are shown in Figure I. The MSDS sheet is found in Appendix I.

## **B. SAFETY AND HEALTH**

This method should be performed by trained chemical personnel. Care must be taken when handling concentrated acids and alkalis. The use of a well ventilated fume hood, protective gloves, and eye protection is advised. This method uses a variety of organic solvents. For details of the hazards associated with handling these solvents refer to MSDS sheets available from their manufacturers.

## **C. ANALYTICAL METHOD**

### **C.1 Principle of the Method**

Water samples (usually 100 ml) are made basic with potassium hydroxide to convert the maleic hydrazide to the very water soluble potassium salt. The samples are then extracted with methylene chloride to remove interfering substances. The aqueous phase is then evaporated to dryness on a rotary evaporator and taken up in the mobile phase. The mobile phase is a mixture of formic acid in water adjusted to pH 3.2 using ammonium hydroxide. Under these conditions the maleic hydrazide potassium salt is converted back to maleic hydrazide. The aqueous solution is analyzed by HPLC using an electrochemical detector.

### **C.2 Types of Water**

This method is expected to be applicable to most water samples. Ground water samples were analyzed using this method in report 99100, but it should be applicable to surface water and drinking water samples.

### **C.3 Sample Processing**

No processing is required. Samples of water are usually received frozen and are kept frozen until analysis.

**C.4 Extraction Method**

Water samples are made basic with potassium hydroxide and extracted with dichloromethane to remove interfering substances. The procedure is outlined as follows:

Place an aliquot (100 mL) of sample in a 250 mL separatory funnel. Recovery samples should be fortified at this stage.

Add dichloromethane (100 mL) to the funnel and shake for one minute. Allow the layers to separate and discard the lower organic layer.

Transfer the aqueous layer to a 500 mL round bottom flask. Rinse the separatory funnel with methanol (100 mL) and transfer the rinsings to the round bottom flask.

Evaporate the sample to a volume of approximately 30 mL on a rotary evaporator with the water bath set at 50°C. A reduced vacuum should be used initially to minimize sample foaming.

Transfer the concentrated sample to a 50mL round bottom flask.

Evaporate the sample to dryness on a rotary evaporator with the water bath set at 50°C.

Add mobile phase (1 mL) to the round bottom flask and sonicate for approximately 30 seconds. Pass the sample through a 0.2  $\mu$ m nylon syringe filter using a 5 mL disposable syringe before injecting onto the HPLC. Samples with residues above the highest linearity standard concentration should be diluted accordingly.

**C.5 Chromatography**

The following conditions were used:

Column Temperature	20°C
Detector Potentials	Guard cell 0.900 V Screening cell 0.600 V Analytical cell 0.850 V
Flow Rate	1.0 mL/minute
Injection Volume	20 $\mu$ L
Mobile Phase	0.05M ammonium formate

Under these conditions the retention time for maleic hydrazide has been found to be approximately 8 minutes.

The linearity of the test system is checked by injecting standards of 5, 10, 25 and 50 ng/mL onto the HPLC. Within a batch of samples the 50 ng/mL standard is used as the working standard and a standard injection is followed by two sample injections.

Occasionally some samples of water have interfering substances which are not removed by the methylene chloride extraction. In such cases, these interfering residues could be resolved from the chromatographic retention time of maleic hydrazide by using modified chromatographic conditions. Changing the flow rate to 0.5 mL/minute and reducing the column temperature to 1°C gave better separation. Under these conditions, the retention time of maleic hydrazide was around 19 minutes compared to the 8 minutes described above.

### **C.6 Preparation of Spiking and Standard Solutions**

The following procedure is used to generate a stock solution of maleic hydrazide in water and by serial dilution spiking and standard solutions.

Weigh out accurately, using an analytical balance, approximately 0.05g maleic hydrazide into a 100 mL volumetric flask. Add one pellet of potassium hydroxide and approximately 80 mL of water. Sonicate for 5-10 minutes, and then allow the flask to cool. Make up to volume with water to give a 500 µg/mL stock solution. Make serial dilutions of this stock to give 50, 5.0, 0.50, and 0.05 µg/mL individual solutions in 50/50 (v/v) methanol/water. These standards are used for fortification purposes. From these dilutions make a further set of dilutions to give 50, 25, 10 and 5 ng/mL in mobile phase. These standards are used for quantitation purposes.

When not in use, the standards should always be stored in a refrigerator to prevent decomposition and/or concentration of the solvent. These solutions are valid for three months from the date of preparation of the stock (500 µg/mL standard solution).

### **C.7 Fortifications**

Spiked water samples should be analyzed along with the test samples. In study number 99100, spikes at 0.1 µg/L and 0.5 µg/L were usually used. Recoveries of maleic hydrazide were in the range of 68 to 120% with an average of 95% (over 14 runs) were obtained at the 0.1 µg/L level. At the 0.5 µg/L level the range was 70 to 94% with an average of 83% (over 13 runs). Typical chromatograms showing blanks, study water samples, and the 0.1 µg/L and 0.5 µg/L spikes are shown in Appendix II.

**D. INSTRUMENTATION**

Instrument	Varian 9010 ternary gradient pump and an ESA Coulochem II electrochemical detector.
Column	25cm x 4.6mm i.d. Inertsil 5 $\mu$ m ODS 2
Column Temperature	20°C
Detector Potentials	Guard cell 0.900 V Screening cell 0.600 V Analytical cell 0.850 V
Flow Rate	1.0 mL/minute
Injection Volume	20 $\mu$ L
Mobile Phase	0.05M ammonium formate

**E. POTENTIAL INTERFERENCES**

Occasionally some water samples will have interfering peaks. This was observed in some samples from study #99100. In some of these cases, these interferences could be eliminated by modifying the chromatographic flow rate and column temperature (see section C.5, chromatography). However, this more than doubled the retention time.

Because this method uses an electrochemical detector, interferences from other oxidizable substances in the water or reagents can occur. Blank water samples and reagent blanks should always be run concurrently with samples to check for such interferences.

**F. CONFIRMATORY TECHNIQUES**

No confirmatory techniques were used in this analytical method. Identification depends solely on having the correct retention time for maleic hydrazide.

**G. TIME REQUIRED FOR ANALYSIS**

Based on the experience gained during study #99100, it is anticipated that extraction and cleanup of a batch of up to 15 determinations could be carried out within a normal working day with the final chromatographic determination of residues taking place overnight.

**H. MODIFICATIONS OR POTENTIAL PROBLEMS**

As mentioned in section E (potential interferences) and section C.5 (chromatography) the analytical procedure was occasionally modified to accommodate water samples that had chromatographic peaks that interfered with the maleic hydrazide peak.

In study #99100, a problem which was also encountered with some of the laboratory blank water samples was an interfering peak at the retention time of maleic hydrazide. This varied in concentration from 0.13-4.8  $\mu\text{g/L}$  and could not be resolved using the alternative chromatographic conditions described above. This meant that these samples could not be used for fortified recovery samples. Fortified recovery samples were, therefore, carried out using deionized water.

It is anticipated that other HPLC instruments and electrochemical detectors could be used for this method.

## I. CALCULATIONS

The residue of maleic hydrazide in samples or blanks is calculated in  $\mu\text{g/L}$  by the following equation:

$$\text{Residue } (\mu\text{g/L}) = A \times B/C$$

A = concentration of maleic hydrazide (ng/mL) calculated by the integrator based on the preceding 50 ng/mL standard

B = final volume of extract (normally 1 mL)

C = initial water volume (normally 100 mL)

Response of the standards and sample are measured as peak height or peak area.

For spikes, the percent recovery is calculated as follows:

$$\% \text{ Recovery} = \frac{\text{Measured Residue } (\mu\text{g/L})}{\text{Amount of MH in Spike } (\mu\text{g/L})} \times 100$$

In study #99100 residue values in the samples were corrected for the mean recovery of the spikes, if this mean recovery was less than 100%. The spikes were also corrected, where necessary, for any apparent residue in the corresponding control samples.

The amount of maleic hydrazide in the samples and sample spikes were calculated based on the response from the preceding 50 ng/mL standard. A linearity check from 0 to 200 ng/mL indicated a linear response throughout this range with a correlation coefficient of 0.9995 (see *Appendix II*).

## J. COPIES OF CHROMATOGRAMS

Typical chromatograms for the sample runs are shown in Appendix II. Chromatograms for control and spiked samples used to validate the method are shown in Appendix III.



**K. METHOD VALIDATION****K.1 Accuracy (USA)/Recovery (EU)**

A formal study of accuracy was done during development of the analytical method for study #99100, the data and chromatograms from which is included in this report as Appendix III. Accuracy was checked at 0.1, 0.5, 1.0, and 2.0 µg/L. The data from study #99100 is reformatted in Table 1 to show the mean recoveries, standard deviations (SD), relative standard deviations (RSD), the range of recoveries, and the ± confidence limits for 95% confidence for the above spiking levels. Additional accuracy data can be obtained from report #99100, where recovery experiments for 14 samples at each of the 0.10 and 0.50 µg/L levels were performed prior to running water samples from this water leaching study. These results are shown in Appendix IV and reformatted in Table 2 to more clearly show the statistical parameters. Results from Tables 1 and 2 show that average recoveries at each level of fortification are between 70 and 110% as required by the EU (70-120% as required by the USA).

In Tables 1 and 2, the RSD was calculated as:

$$\text{RSD} = \frac{\text{SD}}{\text{Average}} \times 100\%$$

The 95% confidence limits (CL) were calculated as:

$$\text{CL} = \frac{t \times \text{SD}}{\sqrt{n}}$$

Where SD = standard deviation

n = the number of observations

t = the value t for n-1 degrees of freedom at 95% confidence as taken from table C.3 page 267 of Quality Assurance of Chemical Measurements, John K. Taylor, Lewis Publishers, Inc. 1987.

**K.2 Precision**

The USA requires a calculation of the relative standard deviation of recoveries (RSDs) at various concentration levels. These RSDs are shown in Tables 1 and 2, and are less than or equal to 20% for all levels of maleic hydrazide.

The EU requires a repeatability study where the same sample is analyzed several times on the same instrument with the same operator within a short time interval. Although no formal repeatability study was carried out in study 99100, there was a set of four standards that were

analyzed using the same stock solution (156/8A/33) over the short period April 16, 1998 to April 20, 1998. All analyses were performed by the same operator (S.H. Kennedy). The data for these experiments is found in Appendix V, and is reformatted in table 3 to more clearly show the statistical parameters at the 0.10 and 0.50 microgram/L levels. Results in table 3 show that the recovery at both levels meets the EU guideline of between 70-110% and the RSDs also meet the requirement of equal or less than 20%. Consequently, the method is considered to have good repeatability and to meet EU requirements for this parameter.

### **K.3 Limit of Quantitation (USA)/Limit of Determination (EU)**

The lowest concentration tested as shown in Tables 1 and 2, was 0.1 µg/L. At this level the mean recovery of maleic hydrazide was between 70 and 110%, and the relative standard deviation was equal or less than 20%. Thus, the limit of quantitation (LOQ) is 0.1 µg/L.

### **K.4 Limit of Detection**

No formal estimate of the limit of detection (LOD) was performed. However, if we assume that the LOD is roughly one-third the LOQ, the LOD would be approximately 0.03 µg/L. In this connection, the chromatographic traces for samples spiked at 0.1 µg/L, and the ground water control sample can be considered (*Appendix III*).

### **K.5 Specificity**

This method uses an electrochemical detector which will detect other compounds with appropriate oxidation potentials. One should be careful to check the water blanks for the presence of such peaks. Because MH is rather easily oxidized, a good degree of specificity is obtained using electrochemical detection. No confirmatory identification was used in this method.

### **K.6 Ruggedness**

No ruggedness testing was done.

### **K.7 Limitations**

No general limitations are known but occasionally water samples have contained interfering peaks (*see Sections E & H*).

Some laboratories may not be equipped with an electrochemical detector. However, these can be purchased at a reasonable cost in most countries.

L. **INDEPENDENT LABORATORY VALIDATION (ILV) (USA)/  
REPRODUCIBILITY (EU)**

Reproducibility (EU) is defined as an independent lab validation. Reproducibility is not required for water samples according to EU directive 91/414/EEC, July 16, 1996. An ILV is suggested by the USA EPA. No independent lab validation of this method has been done.

M. **CONCLUSIONS**

The analytical method AC 7002 described in this report should be applicable to both surface, drinking and ground water. The LOD is about 0.03  $\mu\text{g/L}$  and the LOQ is 0.1  $\mu\text{g/L}$ . Recoveries and the relative standard deviation at the LOQ are both well within the regulatory guidelines of both the EPA and EU.