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 µm nylon syringe filters.
0.2 µm 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 µm 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 µm 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 µL
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).

D. INSTRUMENTATION

Instrument	Varian 9010 ternary gradient pump and an ESA Coulochem II electrochemical detector.
Column	25cm x 4.6mm i.d. Inertsil 5µm 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 µL
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 µg/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 µg/L by the following equation:

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

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 1mL)
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:

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

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 thrsoughout this range with a correlation coefficient of 0.9995 (*see Appendix II*).