

## SUMMARY

Maleic Hydrazide (MH) is extracted from soil using methanol:water (50:50 v/v) as extractant. The extract is analyzed by HPLC using a reverse phase column and electrochemical detection. The analytical method compiled here is taken mainly from Uniroyal Chemical report 9366.

### A) MATERIALS

#### A.1 Equipment

HPLC vials with teflon lined caps.

Centrifuge

Solvent filtration apparatus with 0.2  $\mu\text{m}$  filters to filter and degass mobile phase.

Screw capped glass jars (ca 100 ml capacity).

Sieve for soil preparation (2-3 mm)

Analytical balances, both top pan and 5-figure precision.

Solvent filters (0.45  $\mu\text{m}$ ) for extract filtration prior to chromatography.

Orbital shaker capable of shaking the screw capped glass jars.

#### A.2 Reagents/Supplies

1. Maleic Hydrazide (3,6-Dihydroxypyridazine; 1,2-Dihydro-3,6-Pyridazinedione).

2. Deionised water
3. Methanol ('HPLC' grade)
4. Potassium Hydroxide (AnalaR grade)
5. Formic Acid (AnalaR grade; 90%)
6. Ammonia Solution (AnalaR grade; 35%. 0.88 g.ml<sup>-1</sup>)

Reagents (5) and (6) are used to prepare buffer:

Mobile Phase Buffer (0.05 M Ammonium Formate)

2.2 g formic acid (90%) is dissolved in ca 900 ml of deionised water. The pH is adjusted to 3.2 with ammonium hydroxide solution and the volume diluted to 1 litre.

**A.3 Analytical Standards**

Maleic hydrazide standard can be obtained from Uniroyal Chemical Inc. division of Crompton & Knowles. A typical COA for maleic hydrazide and its MSDS sheet is found in appendix 1.

**B. SAFETY AND HEALTH**

This method should be performed by trained chemical personnel. Hazards associated with the use of maleic hydrazide are shown in the MSDS sheet in Appendix 1. Analysts should refer to the MSDS sheets for the other reagents listed in section A.2.

## **C. ANALYTICAL METHOD**

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

Soil samples are sieved and then extracted with methanol/water (50:50 v/v) for at least 15 hours. The solvent phase is analyzed by HPLC using an electrochemical detector.

### **C.2 Types of Soils**

This method is predicted to be applicable to most soil types. In Uniroyal Chemical Inc. project 9366 the soil was from a potato growing area in Washington state USA. Samples of this soil obtained from various depths 0-30 cm, 30-60 cm, 60-90 cm and 90-120 cm were all classified as sandy loam. In Uniroyal Chemical Inc. project 9354 the soil was from a tobacco growing area in North Carolina USA. Samples of the soil obtained from depths of 0-30 cm, 30-60 cm and 60-90 cm were all classified as loam. Soil from the 90-120 cm depth was classified as sandy clay loam. In Uniroyal Chemical Inc project 9367 the soil was from a turf growing area in California USA. Samples of soil obtained from 0-30 cm and 30-60 cm depths were classified as sandy loam while those obtained from 60-90 cm and 90-120 cm depths were classified as sand. The analytical method described here worked equally well on all these soil types.

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

Soil samples obtained from a typical study (like Uniroyal study 9366) were prepared before being sent to the analytical laboratory. The cores were normally frozen in dry ice chests immediately after being taken. Upon being

received at the preparation facility the samples were stored in a freezer at  $-50^{\circ}\text{C}$  to  $-25^{\circ}\text{C}$ . The samples were prepared by allowing the soil to warm only until it was warm enough to break up the frozen soil. They were then sieved through a No. 3 ½ sieve ( $5.6\text{ mm} \pm 0.2\text{ mm}$  opening) to remove pebbles and organic debris such as twigs and leaves. Following this, the sieved soil was mixed homogeneously in a Hobart brand food chopper. The sample was then frozen and sent to the analytical laboratory. At the analytical laboratory the frozen sample was thawed and sieved once more before being analyzed.

#### **C.4 Extraction Method**

Soil samples (25g) are extracted by adding 25 ml of methanol:water (50:50 v/v) and shaking for a minimum of 15 h (ie overnight). Following extraction, the samples are allowed to settle, a portion of the supernatant is centrifuged at an appropriate speed and time to separate the extraction solvent from the soil particles, and filtered ( $0.45\text{ }\mu\text{m}$  filter) prior to injection onto the HPLC.

During Method Establishment 25 ml extraction solvent was added to each standard, blank or Q.C. sample. Subsequent analysis during method validation and routine analysis should ensure that the final volume in each blank, standard, QC and test sample during extraction is identical.

#### **C.5 Chromatography Method**

##### **C.5.1 HPLC Method**

The chromatographic system used in this method consisted of a Waters Model 712 W1SP autosampler, Waters Model 600E quaternary solvent

delivery system and an ESA Coulochem 5100A Electrochemical Detector. The chromatographic conditions employed were as detailed below. These conditions may be altered when using alternative instrumentation or columns to provide adequate resolution and sensitivity. In particular voltammograms must be produced for each new analytical cell employed and also the voltammograms currently being employed with the analytical cell should be confirmed periodically. Instrumentation employed must be shown to the precise with respect to injection of sample onto the column since no internal standard is employed in the assay. An ESA Coulochem II electrochemical detector is also satisfactory for the analysis. If this instrument is employed then appropriate range settings must be established to relate to the quantity of test material being injected on column.

Analytical column: Partisil ODS Cartridge, 5  $\mu\text{m}$  particle size, 250 x 4.6 mm i.d.

Guard column: Partisil ODS Cartridge, 5 or 10  $\mu\text{m}$  particle size, 10 x 4.6 mm i.d.

Filter: A 0.45  $\mu\text{m}$  inlet filter should be employed between the injector and guard column.

Mobile phase: 0.05M ammonium formate, pH 3.2 (2.2 g formic acid (90%) prepared in deionised water, pH adjusted to 3.2 with ammonium hydroxide and volume diluted to 1 litre)

Flow rate: 1.3 ml.min<sup>-1</sup>

**Injection volume:** 20  $\mu$ l

**Column temperature:** Ambient

**Detection:** Coulochem 5100A Electrochemical Detector (see note above) equipped with a Model 5020 guard cell and a Model 5011 analytical cell. (Model 5010 also suitable)

**Detector Potentials:** Guard Cell: +1.0 V  
Screen Cell: +0.60 V  
Analytical Cell: +0.85 V

Note that potentials employed during this study were for a Model 5011 analytical cell (Serial No. 3253HL).

Potentials must be established for each analytical cell.

**Data handling:** Trivector Trilab 3000 or a Trivector Trio data station coupled to a thermal printer.

**Run time:** ca 15-20 minutes for retained peak to be eluted during subsequent sample

### **C.5.2 Selection of Conditions for Electrochemical Detection**

The oxidation potential applied to the electrochemical analytical cell must be selected by reference to a voltammogram relating to the conditions of the analytical cell at the time of analysis and using the

chromatographic conditions described in section C.5.1.

A representative voltammogram for Maleic Hydrazide is shown in Figure 1. The cell conditions producing this voltammogram would suggest that an oxidation potential of +0.85 V would be appropriate to quantify Maleic Hydrazide and the screening electrochemical cell would be set at +0.6 V. The guard cell being set at a potential slightly above the voltage applied to the analytical cell, say +1.0 V.

### C.5.3 Chromatographic Conditions

The chromatographic conditions are as described under C.5.1. Representative chromatograms are shown in Figures 2, 3 and 4. It should be noted that the upper limit of linearity of detector response should be known prior to chromatographing the extracted samples. An appropriate detection range and injection volume should be selected to chromatograph the samples in order to inject a suitable volume of extract for each sample to elicit a response within the linear working range of the detector (under the detector range setting used). This linear range may also vary from day to day due to inherent properties of the electrochemical detector.

A representative linear range of Detection can be seen in Figure 5. Under these conditions the limit of detection was ca 70 picograms and the upper standard injected was ca 110,000 picograms (cell Model 5011, Serial No. 3253HL).

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

### **C.6.1 Preparation of Standard Solutions and Soil Samples**

Procedural standardization methodology is employed. That is, a series of soil samples are spiked with standard amounts of MH and are then extracted as per the method shown in C.4.

Accurately weigh by difference ca 20-50 mg Maleic Hydrazide into a 100.0 ml flask, add one pellet of potassium hydroxide and ca 80 ml deionised water. Sonicate for 5-10 min, if necessary, allow to cool and make to volume with deionised water. A series of dilutions of this stock standard are prepared in methanol:water (50:50 v/v) in order to produce appropriate concentrations for spiking soil samples with ca 1-2 ml of standard solution. The spiked soil standard curve should have a limit of reliable determination ie lowest standard of 10 p.p.b. (10 ng.g.<sup>-1</sup> soil) up to the highest standard concentration appropriate for the assay (ca 1000-5000 p.p.b).

### **C.6.2 Preparation of Quality Control Solutions and Soil Samples**

Accurately weigh by difference ca 20-50 mg Maleic Hydrazide into a 100.0 ml flask, add one pellet of potassium hydroxide and ca 80 ml deionised water. Sonicate for 5-10 minutes, allow to cool and make to volume with deionised water. A series of dilutions of this stock quality control solution are prepared in methanol:water (50:50, v/v) in order to produce appropriate concentrations for spiking soil samples with ca 1-2 ml of quality control solution. Quality control soil spike concentrations should be selected with reference to the standard curve range.

## C.7 Extraction Efficiency

### C.7.1 Determination of Optimum Period of Extraction

A series of 6 sieved soil samples weighing 25.0 g were weighed into screw-capped glass jars. The soil which was a bulk sample from the turf site of IRI Project No. 352866 (Uniroyal Chemical Project 9367) had previously been sterilised by autoclaving for 2 h. Each soil sample was spiked with 1 ml of a solution of [ $^{14}\text{C}$ ]-Maleic Hydrazide containing ca  $2.0 \times 10^8$  d.p.m. and 179.5  $\mu\text{g}$  Maleic Hydrazide resulting in a soil concentration of 7.18 p.p.m. Each soil sample was thoroughly shaken to disperse the sample as evenly as possible.

The soil samples were then stored for 2 days at ambient room temperature in the dark. After 2 days storage the samples were extracted by orbital shaking for variable periods of time using 25 ml methanol: water (50:50, v/v) as solvent. Two samples were shaken for 1 h, 2 for 3 h and the last 2 for 6 h. Following extraction each sample was centrifuged at 3000 r.p.m. for 10 min and 1 ml of the supernatant was counted in a liquid scintillation counter preset to count [ $^{14}\text{C}$ ] using 10 ml of Quickszint 1 (Zinsser Analytic, Maidenhead) as scintillant. 2 ml of the spiking solution was diluted to 50 ml and a 1 ml portion of the resultant dilution was counted for radioactivity to represent 100% recovery of test material.

Table 1 tabulates the data obtained after extracting spiked soil extracts for Maleic Hydrazide during a varying period of time. The results indicate that the extraction must be performed for a period of between 3 and 6 h in order to obtain satisfactory recovery.

The method described here recommends 15 hrs of extraction because it is convenient to prepare samples during the day and let them extract overnight

### **C.7.2 Recovery from Soil After Storage**

The recovery of Maleic Hydrazide from soil after storage was assessed by spiking a series of sieved soil samples, with [ $^{14}\text{C}$ ] labelled test compound. Four soil samples weighing 25.0 g were weighed into screw capped glass jars. The soil which was a bulk sample from the turf site of IRI Project No. 352866 (Uniroyal Project 9367) had previously been sterilised by autoclaving for 2 h.

Each soil sample was spiked with 1 ml of a solution of [ $^{14}\text{C}$ ] Maleic Hydrazide containing ca  $1.7 \times 10^8$  d.p.m. and 154  $\mu\text{g}$  Maleic Hydrazide, resulting in a soil concentration of 6.16 p.p.m. Each soil sample was thoroughly shaken to disperse the sample as evenly as possible. Two spiked soil samples were sealed and stored at ambient room temperature in the dark to be analysed after 8 days storage. The remaining 2 jars were extracted immediately as follows:

Following the addition of 25 ml water:methanol (50:50, v/v) each jar was shaken for 90 minutes at room temperature on an orbital shaker. The samples were then centrifuged at 300 r.p.m. for 10 min and 1 ml of the supernatant counted in a liquid scintillation counter preset to count [ $^{14}\text{C}$ ] using 10 ml of Quickszint 1 (Zinsser Analytic, Maidenhead) as scintillant. 2 ml of the spiking solution was diluted to 50 ml and a 1 ml portion of

the resultant dilution was counted for radioactivity to represent 100% recovery of test material. The 2 samples stored at room temperature in the dark were extracted and analysed for Maleic Hydrazide after 8 days storage in an identical manner to that described above, except that the extraction period was lengthened to 6 h.

#### **C.8 Fortifications**

Soil samples from the untreated control plot, spiked in the field, accompanied each set of field samples analyzed in Uniroyal Chemical report 9366. These spiked samples were transported and stored along with (and hence under the same conditions as) the field samples. These QC spikes were then analyzed along with the field samples to ensure that the methodology provided reliable results during the course of the study. The QC spikes recovery data at various spike levels is summarized in Table X of Unroyal Chemical report 9366 and this table has also been included in this report as Table 3.

#### **D. INSTRUMENTATION**

The instrumentation used is described below:

Liquid Chromatograph with a passivated pump (passivated with nitric acid)

ESA Coulochem Model 5100A or Coulochem II Electrochemical Detector fitted with a Model 5020 guard cell and a Model 5010 or Model 5011 Analytical cell.

Analytical Column: Partisil ODS cartridge, 5  $\mu\text{m}$  particle size 250 x 4.6 mm i.d.

Guard column: Partisil ODS cartridge, 5 or 10  $\mu\text{m}$  particle size, 10 x 4.6 mm i.d.

HPLC Inlet Filter: A 0.45  $\mu\text{m}$  inlet filter to be placed between the injector and guard column.

#### **E. SAMPLE BRACKETING**

The calibration was done for each set of samples by standard bracketing. A typical run involved running the standard curve, a control containing no MH, 6 spikes to check recovery (two each at 2040 ppb, 204 ppb and 51.0 ppb), and finally the actual soil samples. Data from a typical run done for Uniroyal Report 9366 is shown in appendix 2 of this report (Appendix 7 of Uniroyal report 9366). Typical chromatograms generated from a standard curve and for some soil samples are also shown in Appendix 3 (appendix 9 of Uniroyal report 9366).

#### **F. POTENTIAL INTERFERENCES**

This method could have interferences from other oxidizable compounds which chromatograph with similar retention times. The potentials applied to the guard column, the screening cell, and the analytical cell were chosen to give good detector response for MH. These potentials were determined as follows:

Solutions containing ca 8, 16 and 79 ng Maleic Hydrazide per 20  $\mu$ l to be injected onto the HPLC were prepared in deionised water which had been made alkaline with potassium hydroxide (see Appendix 1, Section VI). These solutions were injected onto the HPLC repeatedly with the potential applied across the working electrode surface of the electrochemical cell being varied between injections. The Maleic Hydrazide response at the electrode was recorded during each injection. The following potentials were applied to the ESA guard and analytical cells:

Guard cell: +1.0 V

Cell 2: 0 V

Cell 1: 0.55 V  $\rightarrow$  0.90 V altering by 0.05 V increments; the output from this cell was recorded.

The background current at each applied potential was monitored.

Table 4 tabulates the data showing the relationship between potential applied to the electrochemical analytical cell and the Maleic Hydrazide response. The responses, shown graphically in Figure 6, demonstrate that a potential of +0.85 V is appropriate for the analysis of Maleic Hydrazide using the analytical cell under investigation and employing the mobile phase described in Section C.5.1. An appropriate potential at which to set the screen cell of the electrochemical detector is +0.6 V. The guard cell should then be set at a potential slightly greater than that applied at the analytical cell, say +1.0 V.

It should be noted that these selected potentials relate to this specific cell under the described conditions at that point in time. The conditions of the analytical

cell will change and may result in alterations being observed in the voltammogram.

Blank soil types should be run under the conditions for maximum detection of MH to ensure that no interferences are present.

**G. CONFIRMATORY TECHNIQUES**

No confirmatory techniques were used in this study.

**H. TIME REQUIRED FOR ANALYSIS**

In Uniroyal report 9366 one run involved preparing 8 standards for linearity, 6 spikes and 35 soil samples. This total of 49 samples could be prepared in one eight hour day and extracted overnight (15 hours). In the second 8 hour day the extracts could be worked up to prepare for HPLC analysis which could be run overnight. Hence the total time for analysis would be two days.

**I. MODIFICATION OR POTENTIAL PROBLEMS**

None.

**J. CALCULATIONS**

Weighted linear regression analysis is performed on a plot of peak height versus Maleic Hydrazide concentration in each standard. The concentration of Maleic Hydrazide in each quality control sample and test sample is then computed by linear interpolation from this line. A representative calibration line can be seen in Appendix 2.