INTRODUCTION

A method for simultaneously extracting and analyzing DPX-M6316 (Harmony®, Figure 1) Herbicide in soil has been developed. The quantitation limit is 100 parts per trillion (ppt) on a 400 gram soil sample. The method involves a methylene chloride and acetonitrile extraction of the soil, requiring vigorous agitation for 2 minutes, a concentration and clean-up step followed by reversed phase HPLC analysis and UV detection. Column switching, between a phenyl and an ODS column, was coupled with eluent switching between an acidic and basic aqueous mobile phase (Reference 1). This provided a background adequately low as to allow UV detection of M6316 at the 100 ppt level in several soil types.

A validation method using thermospray LC/MS with the selected ion monitoring can be incorporated for both detection and confirmation for samples yielding positive results. The same LC separation is applied, replacing the UV detection with the mass spectrometer for confirmation of positive results.

Recoveries of M6316 in three different soil types were determined using the above method. The addition of H_2O to the sample enhances recovery of M6316. Optimum recovery of M6316 from each soil type was achieved at specific % moisture ranges.

Two questions routinely arise in trace level analyses: confirming the identity of the analyte and contamination of the sample. The method discusses both the extraction and chromatographic procedure as well as the possible sources of contamination involved in doing this analysis at the levels of detection required.

Contamination is an issue which requires the utmost in analytical technique and care to avoid. Again, at the levels in question, contamination is best avoided by conducting the extractions and sample preparation in a "clean lab". Also, weighing and fortifying the soil samples should take place in separate areas.

EXTRACTION AND SAMPLE PREPARATION

The extraction procedure is as follows:

1. weigh out 400 grams of soil.
2. divide into 50 gram portions into each of 8 - 200 mL glass centrifuge jars.
3. add a known aliquot of water to the soil to ensure a moisture content appropriate for the soil type (see Table 1).
4. add 50 mL of acetonitrile and 50 mL of methylene chloride to each 50 gram portion of soil.
5. homogenize with Tissumizer® for 2 minutes.
6. centrifuge for 5 minutes at 2000 RPMs.
7. decant and combine solvent from each jar into a 500 mL round bottom flask.
8. rotary evaporate to near dryness.
9. rinse the sides of the round bottom flask with methylene chloride thoroughly to concentrate extract at the bottom.
10. concentrate extract in the round bottom flask to a volume of 2 mL under a stream of nitrogen.
11. quantitatively transfer the extract to a 125 glass separatory funnel using two 5 mL portions of carbon tetrachloride to rinse the round bottom.
12. add 10 mL of 10 mM KH_2PO_4 buffer, pH adjusted to 7 with 80% NaOH, and shake vigorously for 2 minutes.
13. discard the organic layer.
14. collect the aqueous layer into a 50 mL centrifuge tube.
15. centrifuge the aqueous layer for 5 minutes at 2000 rpms.
16. inject the entire 10 mL into a 5 mL sample loop to adequately fill the loop. The sample is introduced via a glass syringe; a 0.45 μm PTFE filter is attached to the syringe to remove any particulates.

Total sample preparation time is approximately 2 hours, allowing daily sample throughput of 4 per technician.

LIQUID CHROMATOGRAPH AND ANALYSIS

The liquid chromatograph used in this method was a Hewlett Packard 1090M with photo-diode array detection. The ternary pumping system allowed the eluent switching and gradient elution programming required in this method. In addition, a separate pump was required for the eluent switching, as described in AMR-1241-88. The pumping system of the 1090M provides pulse free mobile phase delivery necessary for the low detection levels.

The chromatographic mobile phases used include 10 mM KH_2PO_4 aqueous buffer, pH adjusted to 7.5 with 80% sodium hydroxide, water, adjusted to pH 3 with 85% phosphoric acid, and methanol. The aqueous mobile phase pH adjustments are +/- 0.1 pH units.

The eluent switching provided a much cleaner sample; when coupled with the column switching, UV detection at 230 nm easily detects 100 ppt M6316 extracted from 400 grams of soil. For a complete discussion of the theory and background of the eluent switching methodology, see Reference 1, Appendix 2, p.42. In essence, during the chromatography on this first column, the pH of the mobile phase is switched from the initial acidic aqueous phase to a basic aqueous buffer. Subsequently, the amount of organic modifier required for elution of the now ionic sulfonylurea is significantly reduced.

Three sequential injections of M6316 are made on the phenyl column using the eluent switching program to establish a stable retention time. The column switching valve is then timed to accurately introduce the compound of interest onto the second column, now in series with the first column. The valve is switched just prior to and after elution of each peak. See Figures 2-3 for a diagram of the valves, pumps and columns, as well as an example chromatogram demonstrating the times for switching the valve.

Since the sulfonylurea is eluting from the phenyl column with a basic aqueous mobile phase, addition of water pH adjusted to 1.5 (+/- 0.2 pH units with 85% phosphoric acid) is added at a rate of approximately 0.6 mL/min post phenyl column such that the resultant aqueous phase is approximately 2.2 pH units. This is well below the pKa of the sulfonylurea (pKa $\cong$ 3.5). The now acidified aqueous phase is introduced onto the ODS column. The protonated sulfonylurea will not elute through the ODS column under these conditions.

After introduction of M6316 from the phenyl column onto the ODS column, the column switching valve is positioned such that the columns are no longer in series. Mobile phase strength is increased to remove any remaining impurities from the phenyl column. A second switching valve is then activated, diverting the flow of mobile phase only to the ODS column for analysis and UV detection.

Chromatographic conditions can be optimized to accommodate different background profiles found in various soil samples. Retention times on the phenyl and ODS columns are lengthened by decreasing the organic content of the mobile phase. Also, the retention time is related to the mobile phase pH; small changes in mobile phase pH result in small retention time differences and may help in resolution between M6316 and a closely eluting impurity.

SOIL SAMPLES AND STANDARDIZATION

Soil samples representing three different soil types were obtained from Sweden and analyzed with this method. The soils varied in pH, organic content and ionic content. Characterization data for each soil type can be found in Table 3. The chromatographic conditions which allowed separation and detection of M6316 in all three soil types are given in Table 4.

An example chromatogram of M6316 standard is given in Figure 4. Sand, clay and organogenic soil samples fortified at 100 ppt are given in Figures 5-7 with superimposed non-fortified samples. The UV profile of the non-fortified sample was clean in the area where M6316 eluted.

Extraction efficiencies were determined by extracted C-14 radiolabeled M6316 from the three soil types at several fortification levels. Liquid scintillation counting was used to determine the efficiencies. Results are given in Table 1.

Standard solutions of M6316 were made by introducing different aliquots of a 10 μg/mL stock solution containing M6316 into 100 mL volumetric flasks and diluting to volume with 10% methanol/90% water. These solutions were used to construct an example calibration curve (see Figure 8). The dotted assymptotic line represents the 95% confidence values.

Reproducibility of the M6316 retention must be monitored by injecting a standard solution after every two sample injections. Due to the complexibility of this separation, small changes in pump performance, pH of mobile phases or matrix effects will affect the sulfonylurea retention.

CLEAN LABORATORY PRACTICES

At the low levels detected in this method, cross contamination from various sources in the laboratory is a real problem. In order to minimize contamination and insure the integrity of the results, the following points are required:

1. DO NOT weigh M6316 in the laboratory where the sample preparation is to take place.
2. wash all glassware with reagent grade nitric acid, followed by rinses with hot water, distilled water, methanol and methylene chloride.
3. keep all glassware covered when possible.
4. use a separate Tissuemizer® probe to agitate the fortified samples and non-fortified samples.
5. use a very clean syringe to introduce the samples and standards into the LC; preferably, use a separate syringe for each type of sample.
6. make sure the 5 mL sample loop is very clean before introduction of next sample. Sulfonylureas have been known to adhere to stainless steel, thereby affecting the method reproducibility.

APPARATUS AND REAGENTS

Liquid Chromatograph: a liquid chromatographic system with dual pumps, column oven and a 5 mL injection loop is needed. The system used for method development was a Hewlett Packard 1090M.

Detector:	a variable wavelength UV detector is needed. The Hewlett Packard 1040A photo-diode array detector was used in this method.
Switching Valves:	6 port Rheodyne switching valves were installed and controlled via contact switches.
Columns:	a Zorbax® phenyl column, 25 cm x 4.6 mm was the first column in the series. The second column was a Zorbax® ODS column, 15 cm x 4.6 mm.
Integrator/Recorder:	the chromatographic data was collected and integrated on the 1090M data system. Chromatograms were recorded on a Hewlett Packard Think Jet printer.
ph meter:	a Beckman Model 44 pH meter was used to measure pH of the aqueous mobile phase.
Tekmar® Tissumizer® and Shaft:	Soil samples were homogenized with Tissumizer® and SDT182EN shaft.
Mobile Phase Water:	Milli-Q® deionized, distilled water.
Phosphoric Acid:	85% solution, HPLC grade, Fisher Scientific, King of Prussia, PA.
Methanol:	HPLC grade, EM Science, Cherry Hill, NJ.
Rotary Evaporator:	Buchi Model 011 Rotavapor.
Filters:	0.45 µm Teflon filters, SM-256 for organic solvents, Bodman Chemical, Aston, PA.
Representative Soils:	Organogenic, Clay and Sand obtained from Sweden.
Methylene Chloride:	HPLC grade, EM Science, Cherry Hill, NJ.

Harmony®:	DPX-M6316, analytical standard grade, Du Pont Agricultural Products Department, Experimental Station, Wilmington, DE.
Carbon Tetrachloride:	Reagent grade, EM Science, Cherry Hill, NJ.
Potassium Phosphate:	Monobasic, KH_2PO_4, J. T. Baker Scientific, Philipsburg, NJ.
Sodium Hydroxide:	50% solution (w/w), "Baker Analytized" Reagent Grade, J. T. Baker Scientific, Philipsburg, NJ.
Acetonitrile:	HPLC grade, EM Science, Cherry Hill, N.J.

TABLE 4
LIQUID CHROMATOGRAPH INITIAL PARAMETERS

Flow:	1.400 mL/min.	Stop Time:	65.00 min.
Solvent A:	95.0% pH 3. H_2O	Post Time:	0.00 min.
B:	0.0% pH 7.5 10mMKH_2PO_4	Injection Volume:	0.0 µL
C:	5.0% MEOH	Min. Pressure:	off
Oven Temperature:	40.0°C	Column Switch :	0
Max Pressure:	400 bar	Contacts:	0000
		Slowdown:	2

LIQUID CHROMATOGRAPH TIMETABLE

Time (min.)							
0.01	Solvent	A:	95.0	B:	0.0%	C:	5.0%
0.02	Contact	1:	on				
7.00	Contact	1:	off				
7.01	Solvent	A:	95.0%	B:	0.0%	C:	5.0%
7.02	Solvent	A:	45.0%	B:	0.0%	C:	55.0%
16.90	Solvent	A:	45.0%	B:	0.0%	C:	55.0%
17.00	Solvent	A:	0.0%	B:	80.0%	C:	20.0%
23.50	Flow		1.400 mL/min.				
23.60	Flow		1.000 mL/min.				
24.00	Column		1				
29.00	Column		0				
29.10	Flow		1.000 mL/min.				
29.20	Flow		1.400 mL/min.				
29.20	Solvent	A:	0.0%	B:	80.0%	C:	20.0%
30.00	Solvent	A:	20.0%	B:	0.0%	C:	80.0%
35.00	Solvent	A:	20.0%	B:	0.0%	C:	40.0%
40.00	Solvent	A:	60.0%	B:	0.0%	C:	40.0%
42.00	Contact	2:	on				

TABLE 4 (Cont'd.)

LIQUID CHROMATOGRAPH INITIAL PARAMETERS

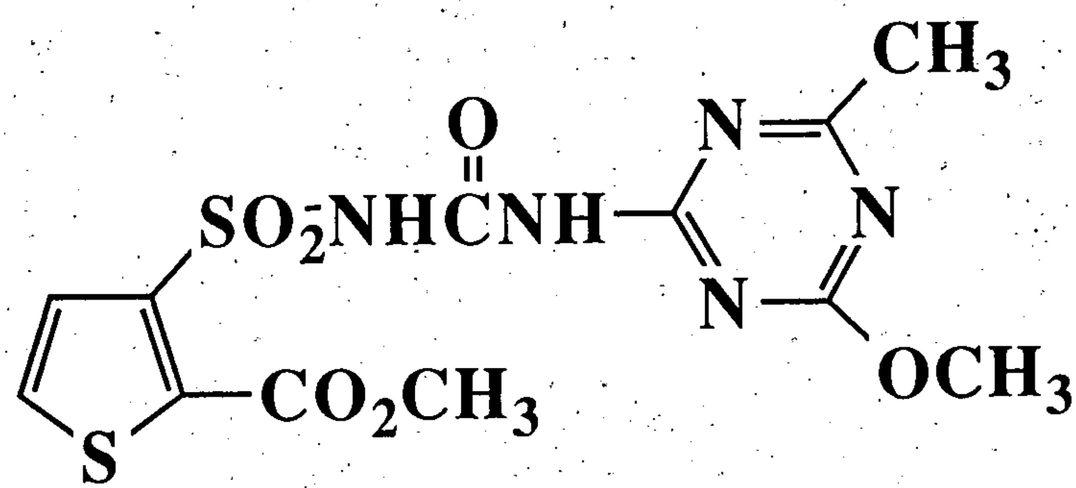
Flow:	1.400 mL/min.	Stop Time:	65.00 min.
Solvent A:	95.0% pH 3. H_2O	Post Time:	0.00 min.
B:	0.0% pH 7.5 10mMKH_2PO_4	Injection Volume:	0.0 µL
C:	5.0% MEOH	Min. Pressure:	off
Oven Temperature:	40.0°C	Column Switch :	0
Max Pressure:	400 bar	Contacts:	0000
		Slowdown:	2

LIQUID CHROMATOGRAPH TIMETABLE

65.00	Contact	2:	off				
65.10	Solvent	A:	60.0%	B:	0.0%	C:	40.0%
70.20	Solvent	A:	95.0%	B:	0.0%	C:	5.0%

Figure 1

Structure of Harmony



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