Residue Method For Extraction and HPLC Analysis of Harmony® Herbicide from Soil Matrices

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INTRODUCTION AND SUMMARY

Scope

An analytical method is described for extraction and analysis of DPX-M6316, the active ingredient in Harmony® Herbicide, in soil with a quantitation limit of 100 parts per trillion (ppt). Both Harmony® Herbicide and DPX-M6316 will be referred to in the text, the term DPX-M6316 referring to the active ingredient in Harmony® Herbicide. The method involves a methylene chloride/acetonitrile extraction, sample concentration and clean-up, followed by reversed-phase HPLC analysis with UV detection at 230 nm. The separation was achieved by column-switching between a phenyl and ODS column and eluent switching between an acidic and basic aqueous mobile phase. This provided an adequately low background for 100-ppt detection of DPX-M6316 in several soil types. The soils were classified as organogenic, sand, and clay.

A validation method using thermospray LC/MS with selective ion monitoring must be incorporated for detection and confirmation of positive results. The same separation approach is applied, however, the UV detection is replaced with the mass spectrometer. The method validation provides adequate qualitative data to minimize possible misinterpretation of the data.

Principles of the Method

The method is an adaptation of the previously submitted Du Pont residue method AMR-1241-88 (EPA MRID# 410826-32, Reference 1). The analysis involves separation using both eluent switching and column switching. Three sequential injections of DPX-M6316 are made on the phenyl column to establish a retention time window for column switching. The column-switching valve is timed to introduce DPX-M6316 onto the ODS column, which is in series with the first column. The valve is switched prior to and after elution of the DPX-M6316 peak.

The DPX-M6316 first elutes from the phenyl column in a basic aqueous mobile phase. Acidified water is then added at a rate of approximately 0.6 mL/min to acidify the aqueous mobile phase containing the DPX-M6316 to a pH of approximately 2.2, well below the pK_a of the sulfonylurea ($pK_a \simeq 3.5$). This weak mobile phase introduces the DPX-M6316 onto the ODS column. The low solvent strength causes the DPX-M6316 to concentrate at the head of the column.

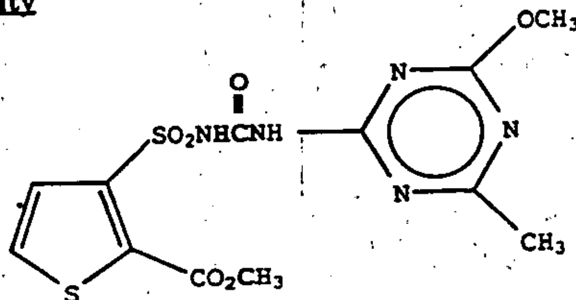
The column-switching valve is then positioned such that the columns are no longer in series. The mobile phase strength is increased to remove any remaining impurities from the phenyl column, and a second switching valve is actuated to divert the flow of mobile phase only to the ODS column for separation and UV detection.

Reproducibility of the DPX-M6316 retention must be monitored by injecting a standard solution after every two sample injections. Because of the complexity of this separation, matrix effects, pH of mobile phase, or even slight changes in pump performance will affect the sulfonylurea retention.

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; slight changes in mobile phase pH can result in relatively large retention time differences and may help resolve DPX-M6316 from a closely eluting impurity.

MATERIALS

Compound Identity



DPX-M6316

Methyl 3-[[[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)amino]-carbonyl]amino]sulfonyl]-2-thiophene carboxylate

Equipment

Chromatograph:	Hewlett Packard 1090M, or other liquid chromatographic system with a ternary pumping system, column oven and a 5-mL injection loop
Metering Pump:	Bodine Electric Company Type NYC-13D3
Detector:	Hewlett Packard 1040A photo-diode array detector, or other variable wavelength UV detector
Switching Valves:	six-port Rheodyne
Columns:	*first column*: Zorbax® phenyl column, 25 cm x 4.6 mm *second column*: Zorbax® ODS column, 15 cm x 4.6 mm
Integrator:	HP 1090M data system
Chromatographic Recorder:	Hewlett Packard Think Jet printer
pH meter:	Beckman Model 44 pH meter
Homogenizer:	Tekmar® Tissumizer® with SDT182EN shaft

Equipment (cont'd)

Rotary Evaporator:	Büchi Model 011 Rotavapor
Filters:	0.45 µm Teflon filters, SM-256 for organic solvents (Bodman Chemical, Aston, PA)

Reagents

Mobile Phase:	Milli-Q® deionized, distilled water, adjusted to pH 3.0 with 85% phosphoric acid (±0.1 pH)
	Milli-Q® deionized, distilled water, adjusted to pH 1.5 with 85% phosphoric acid (±0.1 pH)
	10 mM KH_2PO_4 aqueous buffer (adjusted to pH 7.5 with 80% NaOH (±0.1 pH)
	methanol
Phosphoric Acid:	85% solution, HPLC grade (Fisher Scientific, King of Prussia, PA)
Methanol:	HPLC grade (EM Science, Cherry Hill, NJ)
Methylene Chloride:	HPLC grade (EM Science, Cherry Hill, NJ)
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 Analyzed" Reagent Grade (J. T. Baker Scientific, Philipsburg, NJ)
Acetonitrile:	HPLC grade (EM Science, Cherry Hill, NJ)

Standards

DPX-M6316-53	98.3% pure analytical standard (E. I. du Pont de Nemours and Co., Du Pont Agricultural Products, Wilmington, Delaware, 19880-0402.

ANALYTICAL METHOD

Sample Preparation and Extraction

The extraction procedure is as follows:

1. Weigh out 400 g of soil.
2. Divide into 50-g portions into 200-mL glass centrifuge jars.
3. Add a known aliquot of water to the soil to ensure a moisture content of 18, 36, and 99%, for sand, clay, and organic soils, respectively. Soil moisture % can be calculated using the equation in Table I.
4. Add 50 mL of acetonitrile and 50 mL of methylene chloride to each 50-g portion of soil.
5. Homogenize with Tissumizer® or a similar homogenizer for two minutes.
6. Centrifuge at 2000 RPM for five minutes.
7. Decant and combine solvent from each jar into a 1000-mL round-bottom flask.
8. Place the round-bottom flask onto a rotary evaporator and partially submerge the flask in a room temperature water bath. Connect the evaporator to a house vacuum to facilitate solvent removal. Evaporate the solvent to a volume of ~1 mL.
9. Rinse the sides of the round-bottom flask thoroughly with 10 mL of methylene chloride to concentrate the extract at the bottom of the flask.
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-mL glass separatory funnel using two 5-mL portions of carbon tetrachloride to rinse the round bottom flask.
12. Add 10 mL of 10 mM KH_2PO_4 buffer to the separatory funnel. Place the stopper into the separatory funnel and shake vigorously for two minutes. Place the separatory funnel onto a stand and allow the phases to separate.
13. Discard the organic layer.
14. Collect the aqueous layer into a 50-mL centrifuge tube.
15. Centrifuge the aqueous layer at 2000 rpms for five minutes to break any emulsions that form. There may be CCl_4 at the bottom of the tube if an emulsion was present. If so, do not load CCl_4 into the sample loop; load only the aqueous phase. The samples are now ready for HPLC analysis. Note that the analysis can be paused at this point if the samples are kept refrigerated. The HPLC analysis should be performed within 24 hours.

QUALITY CONTROL

At the low levels detected in this method, cross-contamination from various sources in the laboratory will be a problem. To minimize contamination and ensure the integrity of the results, note the following sampling and laboratory techniques:

Sampling Techniques

- Use a decontaminated soil probe with a known inside diameter. The probe must be capable of sampling to the required depth.
- Sample the untreated control soil before the treated soil.
- When sampling the treated soil, sample in order from the soil treated at the lowest rates to the soil treated at the highest rates; or, sample in order from the highest PHI to the lowest.
- Clean trash from the soil surface. Hold the probe perpendicular to the soil surface and insert it to the proper depth. Carefully remove the probe so that soil does not transfer from one depth to another.
- Distinguish the top from the bottom of each tube. Identify each sample with a unique name or number.
- Bag and freeze samples as soon as possible after colle[illegible] [illegible]ose sample bags and probes securely.

Laboratory Techniques

- Conduct the extractions and sample preparation in a laboratory that does not contain DPX-M6316 or any other sulfonylurea.
- Wash all glassware with reagent grade nitric acid and rinse sequentially with hot water, distilled water, methanol, and methylene chloride.
- Keep all glassware covered when possible.
- Do not store glassware in any area where sulfonylurea may be present.
- If fortified samples are to be extracted, weigh and fortify the soil samples with DPX-M6316 in an area other than the area used for the extractions.
- Use a separate Tissumizer® probe to agitate the fortified and non-fortified samples.
- Use clean, properly washed syringes to introduce the samples and standards into the LC; preferably, use a separate syringe for each type of sample.
- Clean the 5-mL sample loop thoroughly before introducing each sample by flushing the loop with 30-mL of the 10 mM KH_2PO_4 buffer. (Sulfonylureas have been known to adhere to stainless steel, thereby affecting the method reproducibility.)

TABLE IV

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 10 mM KH_2PO_4	*Injection Volume*:	0.0 µL
C:	5.0% Methanol	*Min. Pressure*:	off
Oven Temp.:	40.0°C	*Column Switch*:	0
Max Pressure:	400 bar	*Contacts*:	0000
		Slowdown:	2

LIQUID CHROMATOGRAPH TIMETABLE

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

TABLE IV (Cont'd.)

LIQUID CHROMATOGRAPH TIMETABLE

Time (min.)		*A, 1, 2*	*B*	*C*
30.00	Solvent(%)	20.0	0.0	80.0
35.00	Solvent(%)	20.0	0.0	40.0
40.00	Solvent(%)	60.0	0.0	40.0
42.00	Contact	on		
65.00	Contact	off		
65.10	Solvent (%)	60.0	0.0	40.0
70.20	Solvent (%)	95.0	0.0	5.0