THERMOSPRAY LC/MS ANALYTICAL METHOD FOR THE QUANTITATION OF DPX-79406 AND METABOLITES IN SOIL

Lamaat M. Shalaby and Fred Q. Bramble

ABSTRACT

A method has been developed and validated to extract and quantitate DPX-E9636, DPX-V9360, IN-70941, and IN-V9367 simultaneously from soil using thermospray LC/MS. DPX-E9636 and DPX-V9360 are sulfonylurea corn herbicides which degrade in soil to IN-70941 and IN-V9367, respectively. The compounds were extracted from Canadian soil with acetonitrile/water solution and extracts were concentrated prior to thermospray LC/MS analysis. The four compounds are separated within 20 minutes with a liquid chromatograph and introduced on-line to the mass spectrometer. The mass spectrometer was used in the selected ion monitoring mode to detect and quantitate the four compounds simultaneously.

This LC/MS multi-residue method is fast and specific for DPX-E9636, DPX-V9360, IN-V9367, and IN-70941 in soil. Total analysis time for the four compounds is less than 30 minutes. The limit of quantitation is 0.02 ppm for each of the parent compounds and metabolites.

INTRODUCTION

DPX-E9636 and DPX-V9360 are the active ingredients of two separate sulfonylurea corn herbicides, and a premix candidate (DPX-79406) for registration in US and Canada. IN-70941 and IN-V9367 are soil degradation products of DPX-E9636 and DPX-V9360, respectively. Figure 1 provides the Chemical Abstract names and structures for each compound. This report describes and provides validation data for an LC/MS residue method for simultaneous extraction and quantitation of these herbicides and degradates in soil. A similar LC/MS method (AMR-1184-88) has been used successfully in determining DPX-E9636 and IN-70941.

Thermospray LC/MS is especially applicable to the analysis of low application rate herbicides such as sulfonylureas. The high sensitivity and selectivity offered by this technique permit minimal sample processing and clean-up prior to analysis. Sample preparation can be 12-20 samples/day and LC/MS analysis takes less than 30 minutes/sample.

The combined LC/MS technique has additional value over conventional HPLC that it offers structure confirmation. While conventional HPLC analysis provides retention time confirmation, the use of mass spectrometer as a detector further confirms the structure of the compounds based on the specific ions and their relative intensities.

MATERIALS

Chemicals

DPX-E9636-14 (99% pure), DPX-V9360-35 (99% pure), IN-V9367-2 (99% pure) and IN-70941-001 (100% pure) analytical standards used to fortify samples and prepare calibration standards were synthesized at the Experimental Station (Ag Products Department, E. I. du Pont de Nemours and Co., Inc., Wilmington, Del.). [Pyrimidine-2-^{14}C]DPX-E9636 (NEN Lot# 2512-013, 51.7 mCi/mg specific activity) and [pyridine-2-^{14}C]DPX-V9360 (NEN Lot# 2385-050, 62.9 mCi/mg specific activity) used to determine the extraction efficiencies were synthesized by Du Pont NEN Products (Boston, Mass.)

The solvents were HPLC grade acetonitrile, EM OmniSolv® solvent, (EM Science, Gibbstown, N.J.) and distilled, deionized water obtained from a Milli-Q® water Purification System (Millipore Corp., Milford, Mass.). Ammonium acetate used to prepare the 0.5 M solution added postcolumn for thermospray ionization was 'Baker Analyzed' Reagent (J. T. Baker, Phillipsburg, N.J.). Acetic acid, glacial, used to prepare the 0.1 M acetic acid mobile phase was ULTREX 'Baker Analyzed' Ultrapure Reagent (J. T. Baker, Phillipsburg, N.J.).

The radioactivity in sample extracts was determined by liquid scintillation counting (LSC) in Tru-Count scintillation cocktail (IN/US Service Corp., Fairfield, N.J.).

Soil

Canadian soil from a corn growing area near London, Ontario was used to prepare the soil samples for this study.

Equipment

The HPLC system consisted of a Varian® model 5560 liquid chromatograph equipped with a constant-flow pump, a variable wavelength detector (Varian®, Instrument Group, Walnut Creek, Calif.), a Rheodyne injector valve

(Rheodyne, Inc., Cotati, Calif.) and a Whatman® Partisil C8 column, 4.6 mm i. d. x 25 cm (Whatman Lab Sales, Inc., Hillsboro, Oreg.).

The mass spectrometer was a Finnigan model 4600 quadrupole instrument with an INCOS Data System (Finnigan MAT, San Jose, Calif.). The LC/MS interface was a Vestec thermospray with discharge electrode and filament ionization (Vestec Corporation, Houston, Tex.).

A Kratos model Spectroflow 400 dual piston pulseless HPLC pump (ABI Analytical Kratos Division) was used for post-column addition of the 0.5M ammonium acetate solution. A pulseless HPLC pump is necessary with thermospray LC/MS to maintain a stable ion signal.

The LC/MS system is equipped with 2 µm on-line Kel-F A-101X ring filters (Thomson Instrument Co., Newark, Del. 19711) located before the injector valve and on the Vestec interface line prior to the mass spectrometer to prevent clogging of the capillary LC/MS interface line.

Samples were extracted using a Thermolyne® Maxi-mix Model M16715 vortex mixer (Thermolyne Corporation, Dubuque, Iowa) and a Branson Model B-22-4 Ultrasonic Cleaner (Branson Cleaning Equipment Co., Shelton, Conn.). The soil extracts were centrifuged on an International Clinical Centrifuge Model CL (International Equipment Co., Needham Hts., Mass.). Extracts were filtered with Gelman® 0.45 µm Acrodisc-CR filters (Gelman Sciences, Ann Arbor, Mich.). Soil extract aliquots were evaporated on an N-Evap® Model 111 Analytical Evaporator (Organomation Association, South Berlin, Mass.) in Falcon® 2087 15 mL polypropylene centrifuge tubes (Becton Dickinson Labware, Lincoln Park, N.J.). Extract concentrates were filtered with 0.45 µm ACRO™ LC13 filters (Gelman Sciences, Ann Arbor, Mich.).

The liquid scintillation counter used for measuring the radioactivity was a TM Analytic Mark 3 model 6881 (Elk Grove Village, Ill.).

TEST METHOD

Preparation of Standards

Separate 100 µg/mL standard stock solutions of DPX-E9636, DPX-V9360, IN-V9367, and IN-70941 were prepared in HPLC grade acetonitrile. A 1.0 µg/mL fortification standard mixture of the four test compounds was prepared by diluting the 100 µg/mL stock solution 1:100 in HPLC grade acetonitrile into a common volumetric flask. All standard solutions were refrigerated. The stock standard solutions are stable for at least two weeks in the refrigerator.

Calibration solutions were prepared fresh daily from dilutions of the fortification standard to minimize decomposition (Reference 1). Standard concentrations used in LC/MS analyses were 0.03, 0.2, and 0.4 µg/mL. Calibration solutions were made to contain less than 10% acetonitrile in an aqueous solution to maintain consistent chromatography, particularly for the early-eluting IN-V9367.

Sample Preparation and Fortification

Soil samples were prepared by weighing 10.0 g of soil into tared 50 mL graduated, centrifuge tubes on a top-loading analytical balance.

Soil samples were fortified with the four compounds at levels of 0.02, 0.05, 0.1, and 0.2 ppm as outlined in Table I for three separate validation sets. The solvent was evaporated from fortified samples under a stream of nitrogen for 5 minutes. An untreated control sample was prepared for each validation set.

EXTRACTION PROCEDURE

1. Add 10 mL of extraction solvent (80% HPLC grade acetonitrile/20% Milli-Q® water) to each 10 g sample.

2. Vortex mix each sample for few seconds then ultrasonicate for 10 minutes, vortex mix, ultrasonicate for 5 minutes, vortex mix, centrifuge for 15 minutes at ~1000 rpm, and decant into separate 50 mL graduated cylinders.
3. Repeat steps 1 and 2 twice.
4. Record the total extract volume recovered for each sample.
5. Filter (0.45 µm syringe filter) each sample extract into glass bottles. The bottles should be labeled appropriately.
6. Transfer a 5 mL aliquot from each sample extract to a 15 mL centrifuge tube for later concentration.

SAMPLE EXTRACT CONCENTRATION

The 5 mL extract aliquots (Step 6, EXTRACTION PROCEDURE) were reduced to ~0.5 mL in a stream of nitrogen on an N-EVAP® analytical evaporator at ambient temperature. Water was added to each concentrate to adjust the volume to 1 mL. The concentrates were ultrasonicated for a minute to homogenize the solutions. The final volumes of the concentrated extracts were determined with 2 mL pipets (0.01 mL graduation) as they were transferred to 1.5 mL autosampler vials through 0.45 µm syringe filters. Extract concentrate volumes were recorded. Extracts and concentrates were stored in a freezer.

EXTRACTION EFFICIENCY DETERMINATION

Soil samples were fortified with radiolabeled DPX-E9636 and DPX-V9360 at 0.1 ppm level. Separate soil fortifications were made for each compound in duplicate and extracted at 0 day. Additional soil fortifications were made, 4 samples for each compound, and then aged for a 2-week period under refrigeration. Duplicate aliquots equal to the application volume of radiolabeled DPX-E9636 and DPX-V9360, were transferred to scintillation vials for liquid scintillation counting at the time of application. The amount applied to each sample (1 µg/10 g) was determined from the liquid scintillation counter results.

Samples were extracted using the same procedure described in this method for samples fortified with non-radiolabeled material. The recoveries were determined from duplicate 5 mL aliquots removed from each sample extract and measured by the liquid scintillation counter. The extraction efficiencies were determined by comparing the total recovered radioactivity with the amount originally applied. The recoveries for DPX-E9636 averaged 95% for the 0 day and the 2-week aged soil extractions. The recoveries for DPX-V9360 averaged 85% for the 0 day and 86% for the 2-week aged soil extractions. Table II presents individual and average recoveries for the 0-day and aged extractions.

THERMOSPRAY IONIZATION MASS SPECTRAL ANALYSIS

Figures 2 - 5 show the thermospray positive ion mass spectra for DPX-E9636, DPX-V9360, IN-V9367, and IN-70941 generated by LC/MS full scan (143-650 amu) analyses of the individual test compounds. We selected the most intense ions to quantitate each compound; these were m/z 156, 199, and 325 for DPX-E9636, m/z 156, 199, 230, and 247 for DPX-V9360, m/z 230 and 247 for IN-V9367 and m/z 325 for IN-70941 (see Table III). Peak integrations were done automatically by the INCOS data system algorithim after defining each peak using the data system.

Soil extract concentrates and calibration standards were analyzed by LC/MS to determine recovery. The conditions for LC/MS analysis for this test procedure are given in the experimental procedure section. The calibration was done by bracketing a pair of sample extract concentrates with calibration standards at levels lower and higher than expected in the sample.

The back pressure (35-45 bar) generated from the thermospray evaporation process in the capillary interface line is monitored at the Spectroflow pump used for the post-column addition of the ammonium acetate solution. The

pressure should be stable (+/- 1 bar) to insure good reproducibility of the ion signal. An increase in the back pressure would indicate partial clogging of either the in-line filter prior to the mass spectrometer or the thermospray probe tip. The blockage must be eliminated before proceeding with the analysis. Instability of the high vacuum or excessive noise in the background signal could also indicate a problem with thermospray probe performance. The problem can be easily treated by cleaning the probe tip or replacing the probe insert. Rinsing the interface daily first with water then methanol will minimize the clogging problem. Frequency of interface clogging may vary from a few weeks to a few months, depending on how well the system is maintained.

An ammonium acetate solution (0.5 M introduced to the mass spectrometer) is required for thermospray ionization. Addition of this buffer on-column could affect the LC retention time, especially for sulfonylureas (Reference 2) where an acidic mobile phase is needed for retention on the LC column. In this work, we added solution post-column at 0.3 mL/minute to prevent effects of ammonium acetate on retention times.

EXPERIMENTAL CONDITIONS

Column: Whatman® Partisil C8 column, 4.6 mm i.d. x 25 cm

LC Flow Rate: 1.0 mL/min on-column

Method:

Time (min)	%ACN	%0.1M acetic acid
0	0	100
5	30	70
12	45	55

Post-column Addition: 0.5 M ammonium acetate at 0.3 mL/minute

Injection Volume: 200 µL loop

UV Detector: 254 nm

Retention Times:	IN-V9367	=	9 minutes
	IN-70941	=	14 minutes
	DPX-V9360	=	15 minutes
	DPX-E9636	=	18 minutes
Selected ions monitored:	m/z (156, 199, 230, 247, 325)		
	IN-V9367	: m/z	(230, 247)
	IN-70941	: m/z	(325)
	DPX-V9360	: m/z	(156, 199, 230, 247)
	DPX-E9636	: m/z	(156, 199, 325)
Thermospray Probe Control Temperature (T1):	153°C (specific for each probe)		
Thermospray Probe Tip Temperature (T2):	200-210°C		
Thermospray Mass Spec Source Temperature:	325°C		
Ionization Mode:	Thermospray positive ion		
Mass Calibration:	Polypropylene glycol (PPG) with the thermospray LC/MS source		
Electron Multiplier Voltage:	1050 V		

Finnigan Incos Multiple Ion Detection (MID) Descriptor

INT #	BEGIN Mass	END Mass	TIME (SECS) Request	Actual	MPW	MFW	MA	TH	BL	ION
1	155.547	156.547	0.200	0.229	1	300	20	1	0	POS
2	198.560	199.560	0.200	0.236	1	300	20	1	0	POS
3	229.569	230.569	0.200	0.236	1	300	20	1	0	POS
4	246.574	247.574	0.200	0.229	1	300	20	1	0	POS
5	324.597	325.597	0.200	0.230	1	300	20	1	0	POS

CALCULATIONS

Extraction Efficiency Determination

$$\text{Recovery (\% of Applied)} = \frac{\left(A + B - (2 * C)\right)(E)}{(2 * F) * G} * 100$$

where,

A = dpm in Aliquot 1
B = dpm in Aliquot 2
C = Background dpm
E = Extract Volume (mL)
F = Aliquot Volume (mL)
G = Total dpm applied to Sample

Example Calculation: DPX-E9636, 0 Day, Sample 1

$$\frac{\left(3757 \text{ dpm} + 3787 \text{ dpm} - (2 * 16 \text{ dpm})\right)\left(29 \text{ mL}\right)}{(2 * 1 \text{ mL}) * 111467 \text{ dpm}} * 100 = 98\%$$

LC/MS Sample Analyses

$$\text{Recovery (\% of Applied)} = \frac{(A)\ (TV)}{(RF)\ (CF)\ (P)} * 100$$

where,

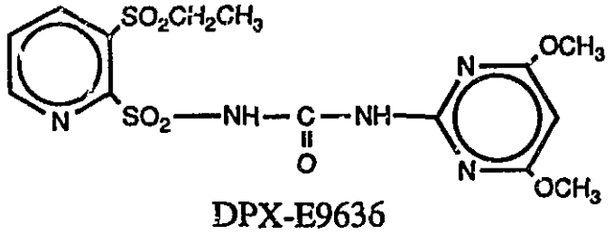
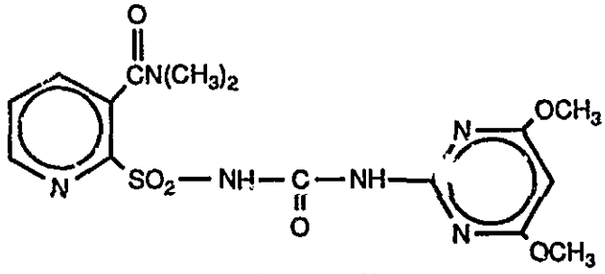
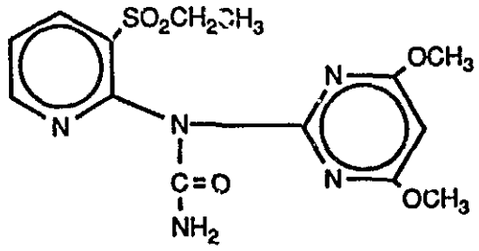
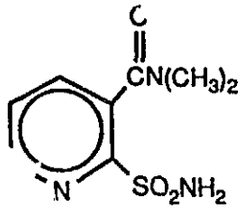
A = Test Compound Peak Area
TV = Extract Total Volume (mL)
RF = Average Standard Peak Area divided by Concentration (Ion Counts/µg/mL)
CF = Concentration Factor: Volume before concentration / Volume after concentration
P = Test Compound Applied (µg)

Example Calculation: V9367 in 50 ppb Sample, Validation Set 3

$$\frac{(109700)\ (28 \text{ mL})}{\left(((465665/.4\ \mu g/mL) + (41224/.03\ \mu g/mL))/2\right)\left(5 \text{ mL}/0.97 \text{ mL}\right)\left(0.5\ \mu g\right)} * 100 = 94\%$$

FIGURE 1

CHEMICAL ABSTRACT NAMES AND STRUCTURES OF DPX-E9636, DPX-V9360, IN-70941, IN-V9367

Structure / Du Pont IN	Chemical Name
 DPX-E9636	N-((4,6-dimethoxypyrimidin-2-yl)-aminocarbonyl)-3-(ethylsulfonyl)-2-pyridinesulfonamide
 DPX-V9360	N-(4,6-dimethoxy-2-pyrimidinyl)-N-((3-(ethylsulfonyl)-2-pyridinyl)) urea
 IN-70941	N,N-dimethyl 2-[[[[(4,6-dimethoxy-pyrimidin-2-yl)-amino]carbonyl]amino] sulfonyl]-3-pyridinecarboxamide
 IN-V9367	2-(Aminosulfonyl)-N,N-dimethyl-3-pyridine-carboxamide