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Carrier effect in the analysis of phenylurea herbicides using high-performance liquid chromatography–particle beam-mass spectrometry

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ABSTRACT

The structural similarity between phenylurea and the herbicides diuron, linuron, and monuron is exploited to enhance the analysis for the chlorophenylureas using high-performance liquid chromatography–particle beam-mass spectrometry. Phenylurea functions as the analyte "carrier" through the particle beam interface, improving the detection limits and the linearity of the calibration curves for the cited compounds of the herbicide class. Combined with solid phase extraction the method provides a rapid and reliable analysis for the chlorophenylureas in water matrices.

INTRODUCTION

The phenylurea herbicides, diuron, linuron, and monuron, which function by inhibiting photosynthesis [1], are used in field applications for pre- and post-emergence weed control in a wide variety of crops. Their widespread agricultural use, toxicity, and possible carcinogenicity [2] have stimulated the development of methods for the detection of the phenylureas at low concentration levels in water, soil, and food. Because these compounds are thermally labile, determination based on gas chromatography (GC) is usually preceded by derivatization [3,4]. The derivatization step may be circumvented by the use of liquid chromatography (LC) coupled with electrochemical detection [5], UV detection [6], photoconductivity detection [7], or thermospray mass spectrometry [8–10].

All of the LC detectors enumerated above lack either specificity and/or sensitivity. The particle beam (PB) interface for coupling high-performance liquid chromatography (HPLC) with mass spectrometry (MS) [11] provides the opportunity to develop methods featuring classical electron impact (EI) and chemical ionization (CI) MS with their inherent specificity. However, the detection limits frequently associated with this interface fall short of ideal values [12].

The method presented here couples several techniques to achieve a rapid, sensi-

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tive, and specific analysis for diuron, linuron, and monuron. Solid-phase extraction (SPE) is used to extract and concentrate the analytes from a water matrix. Reversed-phase HPLC with acetonitrile–water as the eluent separates the compounds in under 6 min on a C_{18} column. The PB interface introduces the LC eluate into a quadrupole mass spectrometer operated in selected ion monitoring (SIM) mode under methane enhanced electron capture negative ion (ECNI) conditions. The addition of phenylurea (PHU) to the mobile phase as the analyte carrier significantly improves the detection limits to the low ppb (ng/ml) level for each of the compounds in the original water solution. For one of the target analytes, diuron, the absolute sensitivity achievable is 160 pg injected on-column in the presence of PHU. Additionally, PHU in the mobile phase results in linear calibration curves over a greater-than-ten-fold concentration range.

EXPERIMENTAL

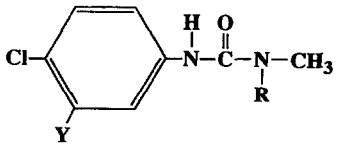
Reagents

Diuron, linuron and monuron (see Table I) at 99% purity were obtained from the US Environmental Protection Agency's (EPA) Pesticides & Industrial Chemicals Repository (Research Triangle Park, NC USA) or from the EPA Pesticide Reference Standards Laboratory (Beltsville, MD, USA) and were used without further purification. Phenylurea was purchased from American Tokyo Kasei (Portland, OR, USA). Solvents were Baker HPLC grade or Resi-analyzed grade (J. T. Baker, Phillipsburg, NJ, USA). Water was distilled prior to being passed through a Barnstead (Newton, MA, USA) NANOpure II system followed by 0.2 μm filtration. SPE cartridges were C_{18} high-capacity 6-ml cartridges from J. T. Baker, catalogue No. 7020-07.

Helium gas for the nebulizer on the PB interface was ultra-high-purity grade (Union Carbide–Linde Division, Danbury, CT, USA) and was filtered through an Oxyclear Disposable Gas Purifier (Labclear, Oakland, CA, USA), followed by a Supelco OMI-1 filter (Bellefonte, PA, USA). The methane enhancement gas was also Linde ultra-high-purity grade and was filtered through both an Oxyclear purifier and an OMI-1 filter.

TABLE I

PHENYLUREA HERBICIDES



Herbicide	Mol. wt.	Y	R
Monuron	198	H	CH_3
Diuron	232	Cl	CH_3
Linuron	248	Cl	OCH_3

Apparatus

The LC column was a Waters (Milford, MA, USA) 300 mm × 2.1 mm stainless-steel μBondapak 10 μm C_{18} column, protected by a Supelco LC18 guard column. A Hewlett-Packard (Palo Alto, CA, USA) 1090 liquid chromatograph, fitted with a Rheodyne (Cotati, CA, USA) 7010 injector equipped with a Rheodyne 7012 loop-filler port and a 20-μl loop, was coupled to a Hewlett-Packard 5988A quadrupole mass spectrometer through the HP 59980A PB interface. Data acquisition and processing were under the control of the HP 59970C MS Pascal ChemStation (Rev. 3.2).

Procedure

Calibration. Separate stock solutions of diuron, linuron and monuron in methanol were prepared at 10 ng/μl concentration, from which working standards of each herbicide were prepared by serial dilution. Five methanolic calibration solutions were then prepared, each of which contained a mixture of the three herbicides, at increasing concentration from lowest concentration to highest concentration for each herbicide. The concentration of the herbicides in the calibration solutions ranged from 0.020 to 2.5 ng/μl for diuron, 0.100 to 2.5 ng/μl for linuron and 1.00 to 3.5 ng/μl for monuron. To generate the calibration curves duplicate injections of each calibration solution were made in random order onto the LC column.

SPE Recovery. Each of three 100-ml samples of tap water and three 100-ml samples of distilled, deionized (DI/DI) water were spiked with a mixture of 1000 ng of diuron (10 μg/l), 4000 ng of linuron (40 μg/l) and 6000 ng of monuron (60 μg/l). Two unspiked tap water samples and two unspiked DI/DI water samples were carried through the SPE procedure as blanks. Each water sample was extracted using an SPE cartridge, which had been conditioned with ethyl acetate, methanol, and DI/DI water under a gentle vacuum and was not permitted to run dry before the sample was applied. After the sample had completely drained through the cartridge, the cartridge was washed with approximately 5 ml of DI/DI water and air dried for 5 min. The herbicides were then eluted from the cartridge with 2 ml of methanol under a very gentle vacuum.

Liquid chromatography. An isocratic mobile phase composed of acetonitrile–water (68:32) at a flow-rate of 0.4 ml/min was used. The acetonitrile contained phenylurea at a concentration of 2.90 ng/μl.

Particle beam. The desolvation chamber temperature was set to 50°C and the helium nebulizer pressure was set at 35 p.s.i.

Mass spectrometer. The mass spectrometer was operated under methane enhanced ECNI conditions. The source temperature was 250°C; the methane pressure was 0.5 Torr, as read on the GC–MS interface thermocouple gauge. For quantitation SIM mode was used with simultaneous monitoring of the *m/z* 198, 232 and 218 ions for monuron, diuron and linuron, respectively.

RESULTS AND DISCUSSION

Fig. 1 shows the typical SIM chromatograms for a calibration curve solution (diuron at 2.50 ng/μl; linuron at 2.50 ng/μl; monuron at 3.50 ng/μl) recorded using the LC and PB settings described above with the mass spectrometer operated under

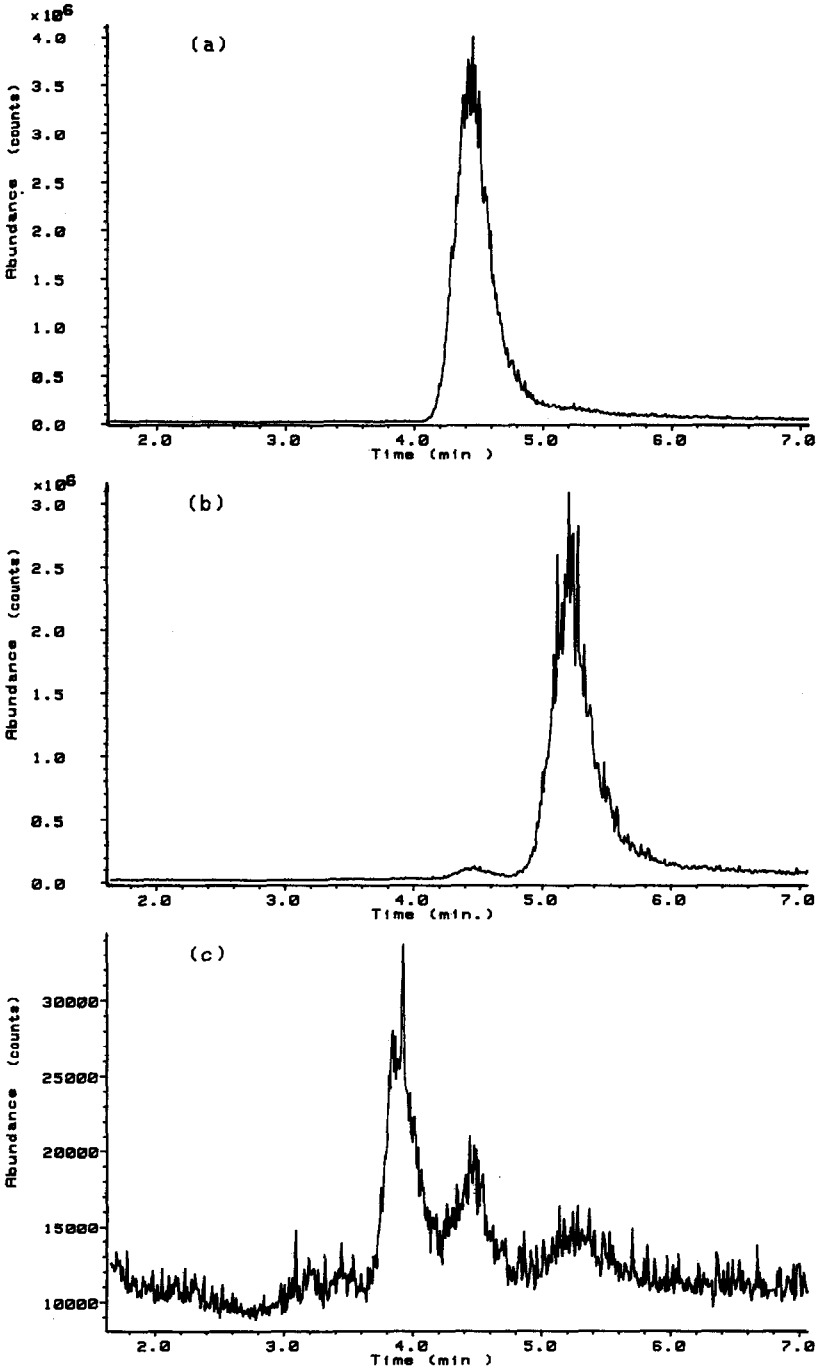


Fig. 1. LC–PB-MS chromatograms for: (a) 50 ng of diuron; (b) 50 ng of linuron; and (c) 70 ng of monuron under SIM methane-enhanced ECNI conditions.

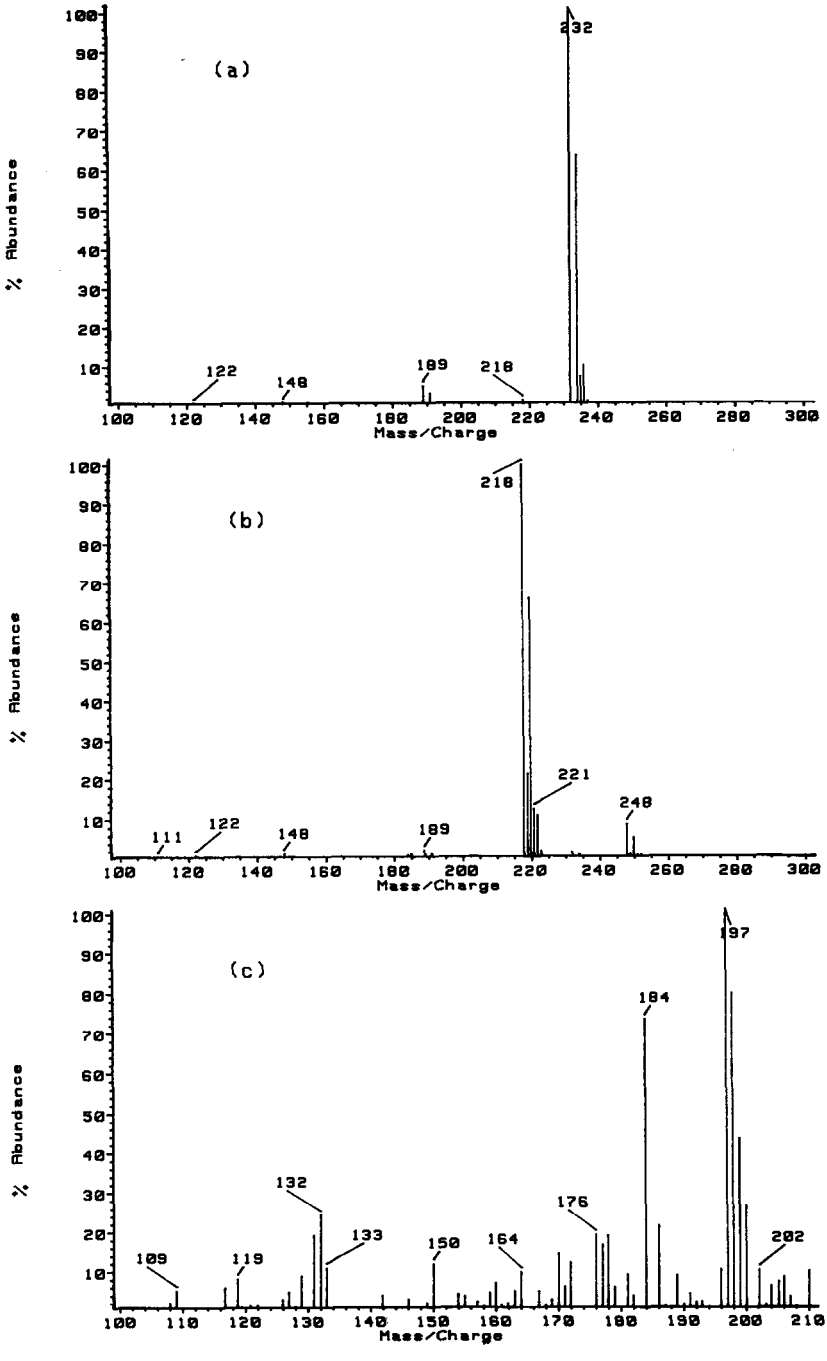


Fig. 2. Full-scan mass spectra acquired under methane-enhanced ECNI conditions for (a) diuron, (b) linuron and (c) monuron.

methane-enhanced ECNI conditions. It has been demonstrated that the molar response factors for diuron and linuron are larger under methane-enhanced ECNI conditions than under EI conditions [13]. The retention times observed for monuron, diuron and linuron are 3.9, 4.5 and 5.3 min, respectively. Under full-scan conditions the particle beam methane enhanced ECNI mass spectra for 160 ng of diuron, 160 ng of linuron, and 200 ng of monuron injected on-column are shown in Fig. 2a, b and c, respectively. The quantitation ion for diuron and monuron corresponds to the $M^{-\cdot}$ molecular ion and for linuron to the $[M-OCH_2]^{-\cdot}$ fragment ion. The base peaks recorded in the HPLC–PB-methane enhanced ECNI mass spectra for diuron and linuron correspond to the base peaks for both these compounds observed under HPLC–MS negative ion conditions using a direct liquid introduction (DLI) interface [14].

The addition of a carrier to the LC mobile phase either pre- or post-column to improve the operation of the particle beam interface has been demonstrated [15–19]. Carriers such as ammonium acetate [16], malic acid [17], or the isotopically labelled analogue of the analyte of interest [18] serve to enhance transport efficiency of the analyte through the PB interface. Improved transport efficiency results in lower detection limits for the analytes as well as linear calibration curves. The carrier, phenylurea, selected in the current experiments for the LC–PB-MS analysis of diuron, linu-

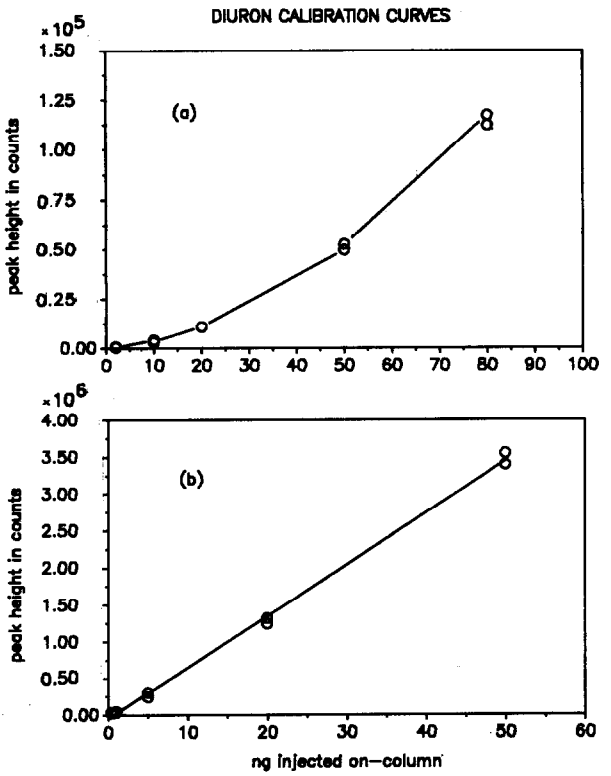


Fig. 3. Diuron calibration curves: (a) without phenylurea and (b) with phenylurea in the mobile phase.

ron and monuron, is structurally similar to the target analytes. Employing the structural similarity between a carrier and the analytes of interest to enhance transport efficiency has been demonstrated previously for the analysis of chlorophenoxy acid herbicides [19]. The improvement in both sensitivity and calibration curve linearity produced by the addition of 2.90 ng/μl PHU to the acetonitrile of the mobile phase is illustrated for diuron in Fig. 3a and b. If no PHU is present in the mobile phase or if 0.01 *M* ammonium acetate is present, the calibration curve shown in Fig. 3a is typically generated and the detection limit for diuron under methane-enhanced ECNI SIM conditions is 2 ng injected on-column. With the PHU carrier in the mobile phase the diuron calibration curve shown in Fig. 3b is generated having a correlation coefficient of 0.999. The detection limit improves to 160 pg injected on-column with a signal-to-noise ratio of 3:1.

Similar results are obtained for linuron. For the linuron calibration curve shown in Fig. 4a, generated with PHU in the mobile phase, the correlation coefficient is 0.980 and a detection limit of 500 pg injected on-column is achieved. These optimum detection limits cannot be achieved if the concentration of the phenylurea in the mobile phases drops below the 2.90 ng/μl specified above.

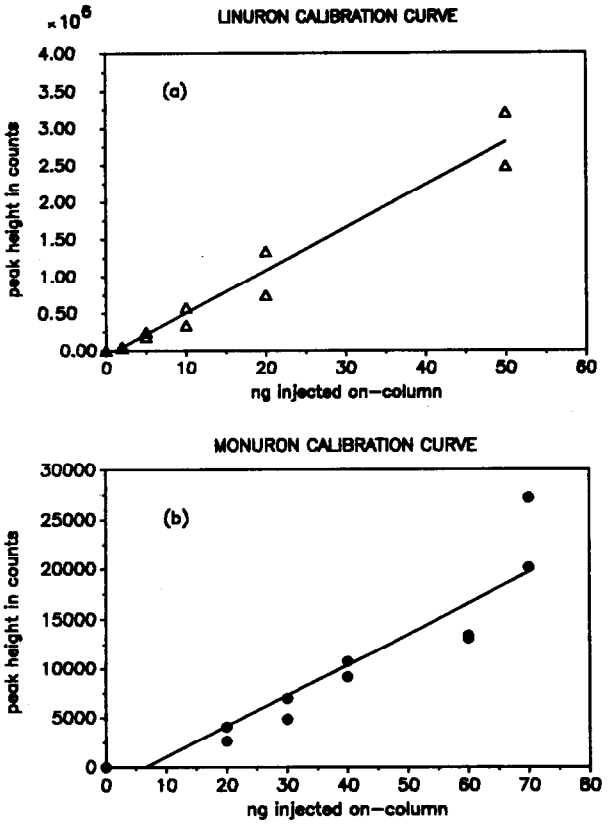


Fig. 4. Calibration curves with phenylurea present in the mobile phase for (a) linuron and (b) monuron.

TABLE II

SPE RECOVERY OF PHENYLUREA HERBICIDES FROM WATER DETERMINED BY HPLC–PB-METHANE-ENHANCED ECNI-MS

Herbicide	Spiking level	Recovery (%)	Matrix
Diuron	10 μg/l	136 ± 13 (n=6)	Tap
	10 μg/l	126 ± 8 (n=6)	DI/DI[a]
	0 μg/l	0 (n=4)	Tap
	0 μg/l	0 (n=4)	DI/DI
Linuron	40 μl/l	104 ± 11 (n=6)	Tap
	40 μg/l	102 ± 16 (n=6)	DI/DI
	0 μg/l	0 (n=4)	Tap
	0 μg/l	0 (n=4)	DI/DI
Monuron	60 μg/l	151 ± 18 (n=6)	Tap
	60 μg/l	109 ± 14 (n=6)	DI/DI
	0 μg/l	0 (n=4)	Tap
	0 μg/l	0 (n=4)	DI/DI

[a] Distilled, deionized water as described in the text.

The calibration curve for monuron generated with PHU in the mobile phase is shown in Fig. 4b. The correlation coefficient for this curve is 0.932.

Table II summarizes the SPE recovery data. For each sample duplicate injections of the SPE eluate onto the LC column were performed in random order. There are two possible explanations for the poor recovery data for monuron from the tap water samples. First, the molar response factor for monuron under methane-enhanced ECNI conditions is much smaller than that for diuron and linuron (compare Fig. 1a and b with Fig. 1c). Monuron sensitivity improves under EI conditions [20]. Second, for the tap water samples an unidentified substance(s) elutes from the C_{18} column at retention time 3.4 min, which interferes with the monuron peak at 3.9 min.

The detection limits for diuron (160 pg on-column) and for linuron (500 pg on-column) translate into optimum detectable concentrations of 160 ng/l (0.160 ppb) and 500 ng/l (0.500 ppb), respectively, for an original water sample size of 100 ml with SPE concentration to 2 ml.

The experimental results reported here support our earlier observation that the use of an appropriate generic carrier for a class of analyte compounds can render LC–PB-MS a sensitive technique for quantitation of target analytes in water matrices. When SPE is employed to extract and concentrate the analytes, a rapid, reliable, and relatively simple method is available for the determination of two classes of polar, thermally labile compounds, the chlorophenoxy acid herbicides and the phenylurea herbicides. Experiments are in progress to improve the LC resolution of the phenylurea herbicides and to extend the method to additional compounds in this class.

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