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Determination of chlorophenoxy acids using high-performance liquid chromatography–particle beam mass spectrometry

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ABSTRACT

An isocratic mobile phase of methanol containing phenoxyacetic acid–dilute acetic acid (70:30) achieved a good high-performance liquid chromatographic (HPLC) separation of 2,4-dichlorophenoxyacetic acid (2,4-D), 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) and 2-(2,4,5-trichlorophenoxy)propionic acid (Silvex). The HPLC eluate was introduced into a mass spectrometer operated under methane-enhanced electron-capture negative ionization conditions through the particle beam interface. With the mass spectrometer operated in selected-ion monitoring mode, detection limits in the low μg/l range were attained for all three acids. This is one of the first reports of liquid chromatography coupled with mass spectrometry through the particle beam interface for the detection of chlorophenoxy acid herbicides.

INTRODUCTION

The widespread use of chlorophenoxy acid herbicides has resulted in their detection as residues in surface water and ground water [1]. For this reason, there is a need for the development of sensitive methods for the analysis of water samples for the presence of these herbicides. The original methods for the detection of the chlorophenoxy acids in water utilized liquid–liquid extraction followed by esterification to permit analysis by gas chromatography (GC) with electron-capture detection [2–6]. Recently, it has been demonstrated that the liquid–liquid extraction step may be replaced by solid-phase extraction (SPE) of the water sample using SPE cartridges. SPE and elution are followed either by high-performance liquid chromatography (HPLC) with UV detection of the intact acids [7] or by methylation of the acids followed by GC–mass spectrometric (MS) analysis of the methyl esters [8].

Because of its specificity, MS detection is preferable to the use of other detectors, such as the variable-wavelength UV detector used by Hoke *et al.* [7]. On the other hand, the derivatization required in the GC–MS approach of Infante and Peréz [8] prolongs the analysis. Obviously, the coupling of LC with MS for the detection of the intact acids can achieve both the specificity and the speed of analysis desirable for non-volatile analytes such as chlorophenoxy acids.

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The particle beam interface permits LC–MS to be attained relatively simply [9]. Among the additional advantages of this interface is its ability to generate classical electron impact (EI) and chemical ionization (CI) mass spectra. This is an asset over other types of LC–MS interfaces such as thermospray (TSP), which yield spectra with little or no fragmentation [10]. The relatively poor sensitivity of the particle beam interface, however, has been a drawback in its application to environmental analyses [11]. The disappointing sensitivity has been attributed to reduced transport efficiency of the analytes through the desolvation chamber and the momentum separator. Improved transport efficiency, as evidenced by MS signal enhancement, has been attained through the addition of a "carrier" to the mobile phase. For example, ammonium acetate has been used as a carrier for several different classes of compounds, such as phenylureas and carbamates [12]. Alternatively, isotopically labelled analogues of each analyte of interest have also been reported as carriers [13]. One analysis has been reported using a carrier, malic acid, specific for one target analyte, alar, which has a structure similar to that of the carrier [14].

The method reported here combines the particle beam interface for LC–MS analysis together with the time-saving use of SPE for three intact chlorophenoxy acids: 2,4-dichlorophenoxyacetic acid (2,4-D), 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) and 2-(2,4,5-trichlorophenoxy)propionic acid (Silvex). Picogram amounts of the acids can be detected by using the carrier, phenoxyacetic acid, as a component in the mobile phase. The detection limit was also improved by operating the mass spectrometer in the selected ion monitoring (SIM) mode under methane-enhanced electron-capture negative ionization (ECNI) conditions. Recovery data for each analyte spiked into distilled water ranged from 89% to 109%. Linear calibration graphs were obtained with approximately 8–60 ng of each analyte injected on-column.

EXPERIMENTAL

Reagents

The chlorophenoxy acids at $\geqslant 99\%$ purity were obtained from Dow Chemical (Midland, MI, U.S.A.). Phenoxyacetic acid (PAA) was purchased from Aldrich (Milwaukee, WI, U.S.A.). Solvents were of HPLC grade or Resi-Analyzed grade (J. T. Baker, Phillipsburg, NJ, U.S.A.). Water was distilled and passed through a Barnstead (Newton, MA, U.S.A.) NANOpure II system followed by 0.2-μm filtration. Acidified water was prepared by adding 0.2 ml of concentrated HCl to 250 ml of distilled, dionized water. SPE cartridges were C_{18} high-capacity 6-ml cartridges from J. T. Baker.

Helium for the nebulizer on the particle beam interface was of ultra-high purity grade (Union Carbide–Linde Division, Danbury, CT, U.S.A.) and was filtered through an Oxyclear disposable gas purifier (Labclear, Oakland, CA, U.S.A.), followed by a Supelco (Bellefonte, PA, U.S.A.) OMI-1 filter. Methane for chemical ionization was of ultra-high purity grade (Union Carbide–Linde Division) and was filtered through an OMI-1 filter.

Apparatus

A Hewlett-Packard Model 1090 liquid chromatograph fitted with a Rheodyne (Cotati, CA, U.S.A.) Model 7010 injector equipped with a Rheodyne Model 7012

loop filler port and a 20-μl loop was coupled to a Hewlett-Packard Model 5988A quadrupole mass spectrometer through the HP 59980A particle beam interface. Data acquisition and processing were under the control of the HP 59970C MS Pascal ChemStation (Rev. 3.2). A Waters Assoc. (Milford, MA, U.S.A.) 300 mm × 2.1 mm I.D. stainless-steel μBondapak (10 μm) C_{18} column was protected by a Supelco LC 18 guard column.

Acrodisc polytetrafluoroethylene (PTFE) 0.2-μm filters (Gelman, Ann Arbor, MI, U.S.A.) were washed with HPLC-grade methanol prior to use.

Calibration graphs

A 1000 μg/ml stock solution of each herbicide was prepared in methanol, and diluted to give a 10.00 ng/μl methanolic solution of each herbicide. Appropriate volumes of the 10.00 ng/μl solutions were diluted with methanol to prepare 2.00 ml of four calibration solutions, each of which contained a mixture of the three herbicides. The concentrations of each herbicide in the calibration solutions were as follows: solution 1, 0.500 ng/μl; solution 2, 1.00 ng/μl; solution 3, 2.00 ng/μl; and solution 4, 3.00 ng/μl. Each calibration solution was well mixed and filtered through a 0.2-μm PTFE filter. To each filtered solution 300 μl of acidified water were added.

Solid-phase extraction

A sample of water was spiked with the 10.00 ng/μl methanolic solution of each herbicide to achieve a 20.00 μg/l concentration level of each herbicide when the final volume of the spiked solution was 100.00 ml. To the spiked water was added 0.08 ml of concentrated HCl and the volume was adjusted to 100.00 ml. The 6-ml C_{18} high-capacity SPE cartridge was conditioned with 5 ml of ethyl acetate, followed by 5 ml of methanol, then 20 ml of distilled, dionized water and finally 5 ml of acidified water. The solvents were drawn through the cartridge under gentle vacuum (5 in. Hg) and the cartridge was not permitted to run dry after addition of the acidified water. A 75-ml reservoir was fitted to the top of the cartridge and the water spiked with the herbicides was drained through the cartridge under gentle vacuum at the rate of *ca.* 5 ml/min. After the solution had drained completely through the cartridge, the cartridge was washed with 5 ml of acidified water. The cartridge was then air dried for 5 min. The herbicides were eluted with 2.00 ml of methanol under very gentle vacuum. To the methanolic eluate 300 μl of acidified water were added.

Liquid chromatography

An isocratic mobile phase consisting of a 70:30 mixture of methanol, which contained PAA at a concentration of 1.7 ng/μl, and 1% acetic acid eluted 2,4-D, 2,4,5-T and Silvex in under 10 min. The injection size was 20 μl and the flow-rate was 0.4 ml/min.

RESULTS AND DISCUSSION

Fig. 1 shows a typical particle beam–methane-enhanced ECNI chromatogram acquired in the SIM mode under the LC conditions specified above for 52 ng of each herbicide injected on-column. 2,4-D was eluted at 5.3 min, 2,4,5-T at 6.7 min and Silvex at 8.6 min. These retention times were steady to within 6 s for all injections

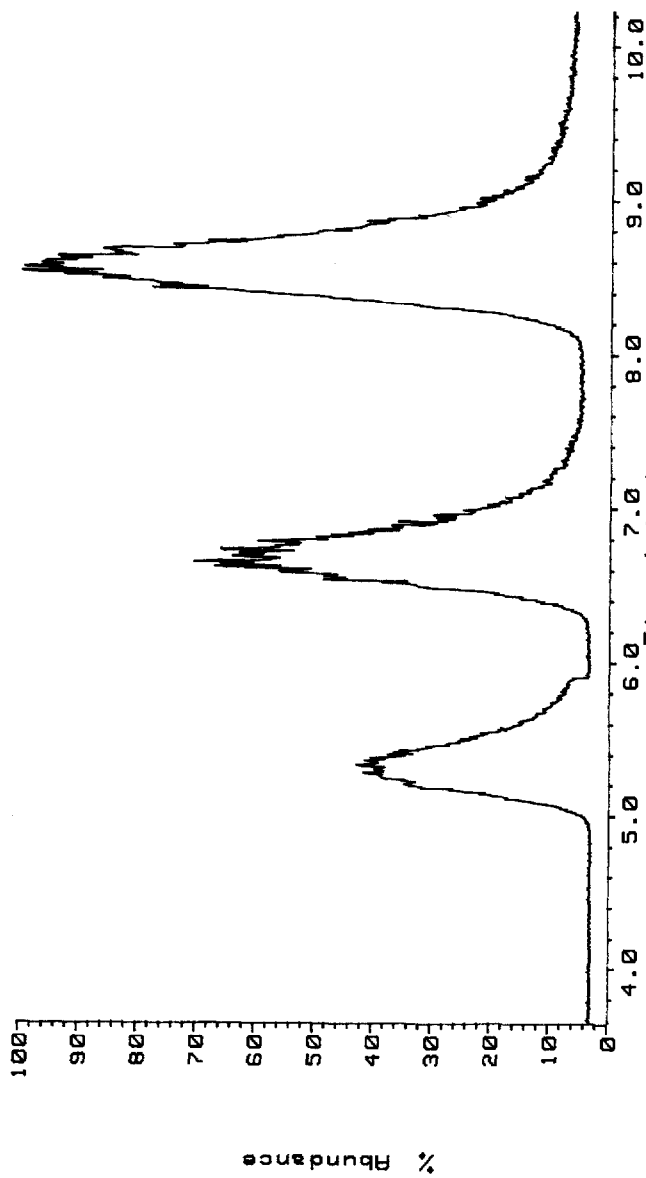

Fig. 1. Chromatogram obtained from HPLC–particle beam MS of 2,4-D, 2,4,5-T and Silvex in the methane-enhanced ECNI mode under SIM conditions.

performed over the course of 5 h. The isocratic elution of 2,4-D, 2,4,5-T and Silvex (in that order) from a C_{18} LC column by means of a mobile phase composed of methanol containing 1.7 ng/μl phenoxyacetic acid–1% acetic acid (70:30) is similar to earlier observations using methanol–1% acetic acid (68:32) as the mobile phase [7]. The addition of phenoxyacetic acid to the mobile phase has no apparent adverse effect on the chromatography.

The initial attempt to use the carrier effect for the improvement of detection limits in the analysis of 2,4-D using HPLC–particle beam mass spectrometry in our laboratory focused on the addition of ring [$^{13}C_6$]-2,4-D to water samples previously spiked with native 2,4-D. The results of this experiment indicated that the native 2,4-D, present in the isotopically labelled material at a 1% impurity level interfered with the 2,4-D concentrations added to the water sample.

It was determined subsequently that PAA can serve as a generic carrier for all three herbicides examined. Another advantage of using PAA as the carrier for the chlorophenoxy acid herbicides derives from the smaller electron-capture cross-section of PAA relative to that of the chlorophenoxy acid herbicides. This means that in the MS source PAA has a lower tendency to capture thermal electrons during the ECNI process than the chlorinated phenoxy acid herbicides [15]. Hence there is no reduction in the ionization efficiency of the analytes or the sensitivity of the analytes to ECNI in the presence of PAA.

PAA was added to the methanol component of the mobile phase at a concentration of 1.7 ng/μl. Using flow-injection analysis (FIA) it was determined that higher concentrations of PAA did not improve the detection limits and lower concentrations did not maximize the signal enhancement. For example, without the PAA carrier in the mobile phase, 20 μl of a 100 ppb solution of 2,4-D (2000 pg) injected in FIA was undetectable by LC–particle beam MS with methane ECNI in the SIM mode. With the phenoxyacetic acid carrier at 1.7 μg/ml in the methanol component of the mobile phase, it was possible to detect 20 μl of a 25 ppb solution of 2,4-D (500 pg) in FIA. With the LC column in place, 20 μl of a 50 ppb solution of each herbicide (1000 pg) injected on-column was detectable at a signal-to-noise ratio of 3:1 when PAA was present in the mobile phase. Beginning with an original sample size of 100 ml and adhering to the procedure outlined above, this detection limit translates into a concentration of 1.1 μg/l (1.1 ppb) for each herbicide in the original sample.

The MS conditions employed are summarized in Table I. Although LC–particle beam MS for 2,4-D and Silvex in the EI mode has been reported [16, 17], the molar response factors for the chlorophenoxy acids, determined by FIA, are approximately ten times larger in the methane ECNI mode than in the EI mode (unpublished results). A similar observation has been made for other chlorinated herbicides [18]. Although the mass spectrometer is tuned at a source temperature of 200°C using perfluorotributylamine (PFTBA)–perfluorobenzonitrile (PFBN) (1000:1), the optimum sensitivity for the chlorophenoxy acid herbicides is achieved with the source at 300°C. The full-scan methane-enhanced ECNI spectra for 200 ng of each herbicide injected on-column are shown in Fig. 2–4. The ions monitored in the SIM analysis correspond to the $[M-HOCl]^{-\cdot}$ fragment for each herbicide.

The settings for the particle beam parameters required for optimum sensitivity, such as the helium nebulizer pressure and the nebulizer position, were determined by means of FIA of the chlorophenoxy acids.

TABLE I

PARTICLE BEAM AND MASS SPECTROMETER CONDITIONS FOR THE ANALYSIS OF CHLOROPHENOXY ACIDS

Parameter	Conditions
Desolvation chamber temperature	47°C
He nebulizer pressure	26 p.s.i.
Ionization mode	Methane ECNI
Source temperature	300°C
Source pressure	0.5 Torr[a]
Acquisition mode	SIM
	m/z 168[b], 170; 4.0–5.9 min
	m/z 202[b], 204; 5.9–7.8 min
	m/z 216[b], 218; 7.8–10.2 min

[a] Pressure as measured with the thermocouple gauge at the GC–MS interface.
[b] The quantification ion for each herbicide is designated.

The well-defined peak shape for all three herbicides in the particle beam–methane-enhanced ECNI chromatogram shown in Fig. 1 was achieved with the addition of acidified water to the calibration solutions. Without this addition the peaks for all three herbicides were broad and poorly resolved. It should be noted that the pH of the mobile phase employed was 3.5. When acidified water was added to the LC mobile phase in the proportion specified under Experimental, the pH dropped to 2.8. As the pK_a values of the three chlorophenoxy acids are in the range 2.8–3.0 [19], the acidified water is necessary to assure that the compounds are in the acid form in the calibration solutions. Under the experimental conditions employed, conversion of the free chlorophenoxy acids to the corresponding methyl esters is unlikely. A preliminary study of the LC–particle beam MS of the methyl esters of 2,4-D, 2,4,5-T and Silvex showed that they do not interfere with the LC or the MS of the free acids.

The calibration graphs for the three herbicides, constructed by performing duplicate injections of each calibration solution in random order and plotting the peak height of the calibration ion *versus* the amount injected, are shown in Fig. 5.
The correlation coefficients were calculated to be 0.994 for 2,4-D, 0.990 for 2,4,5-T and 0.996 for Silvex. In contrast to the linear calibration graphs in Fig. 5, calibration graphs for LC–particle beam MS determined using the methane ECNI mode for chlorinated phenylurea herbicides without the use of a carrier in the mobile phase were non-linear [20]. The reports of other workers confirm that without a carrier in the mobile phase, the calibration graphs obtained with particle beam MS in both the EI [13] and CI [14] modes tend to be non-linear.

The SPE recovery data are summarized in Table II. The spiking level was chosen to be within the range of the maximum contaminant levels set by the EPA National Primary Drinking Water Regulations, *i.e.*, 100 μg/l for 2,4-D and 10 μg/l for Silvex [21]. The data were obtained by making duplicate injections in random order of each recovery solution, three solutions prepared with tap water and three solutions prepared with distilled, deionized water. The recovery results were similar when either 2 μg were added to a 100-ml water sample or 1 μg was added a 50-ml water sample. Whereas the recovery data from the distilled, deionized water samples are acceptable,

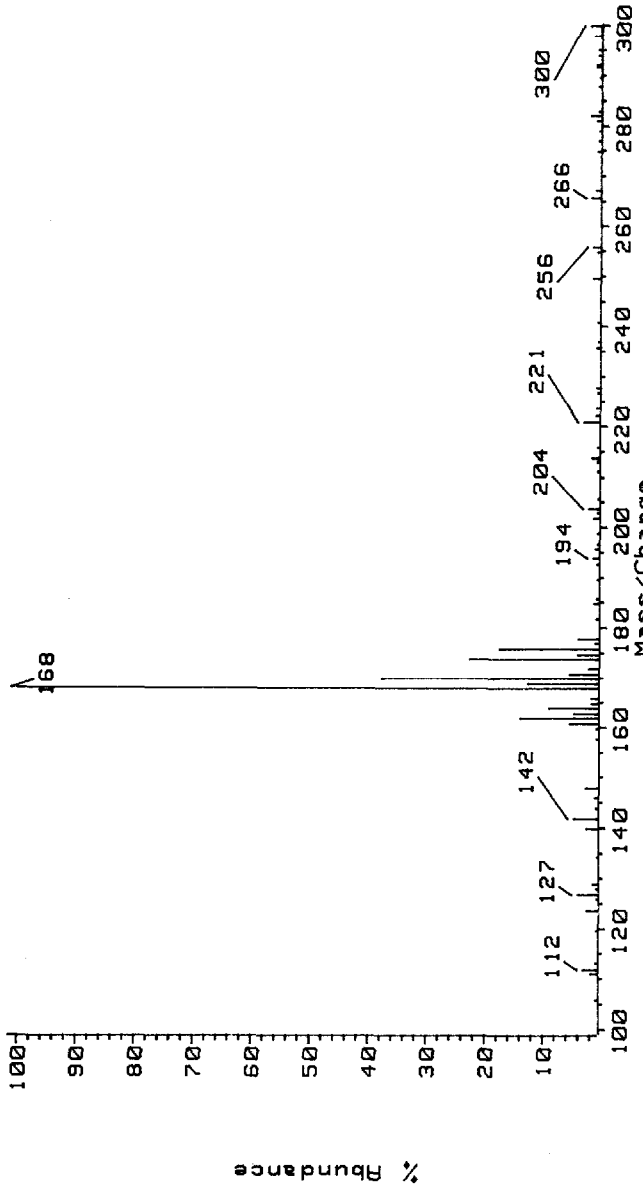

Fig. 2. Full-scan HPLC–particle beam methane-enhanced ECNI mass spectrum for 2,4-D.

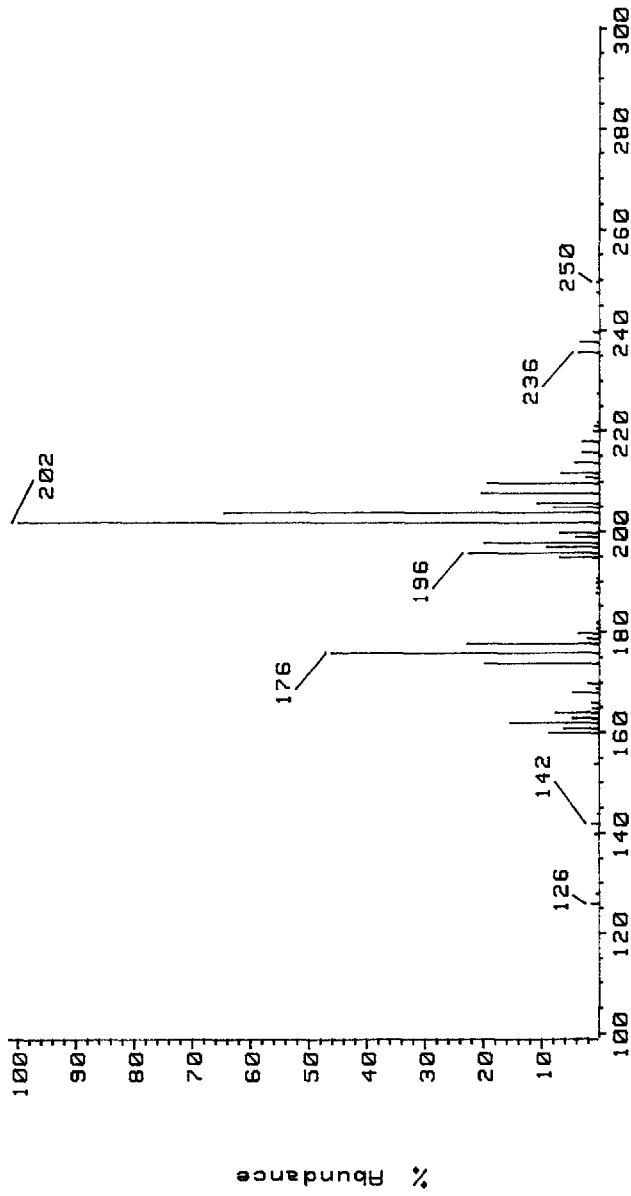

Fig. 3. Full-scan HPLC–particle beam methane-enhanced ECNI mass spectrum for 2,4,5-T.

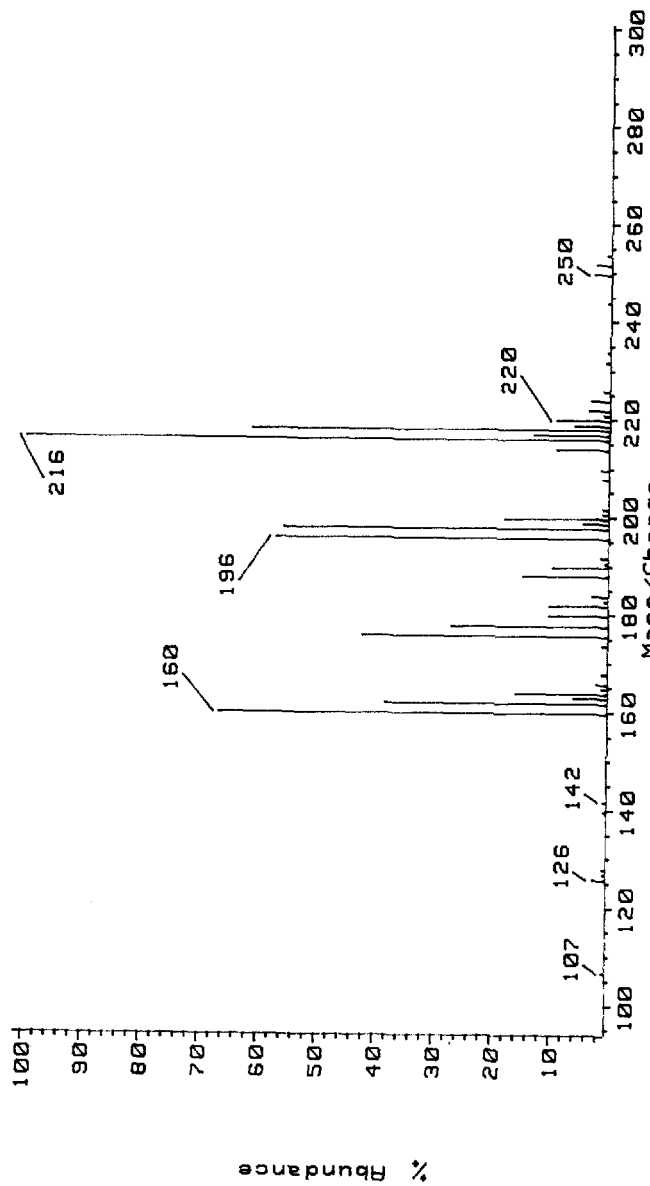

Fig. 4. Full-scan HPLC–particle beam methane-enhanced ECNI mass spectrum for Silvex.

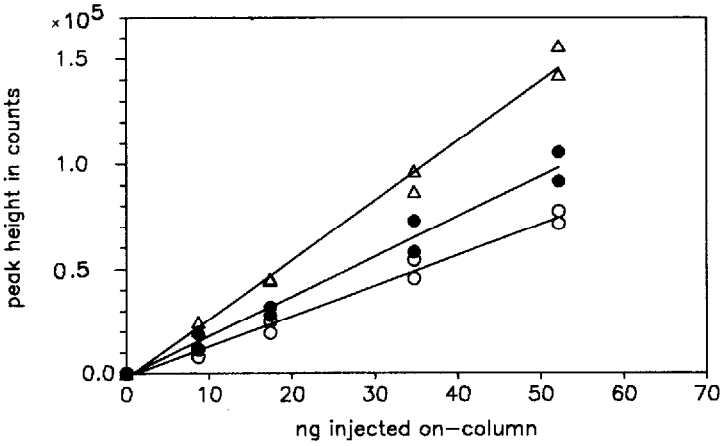

Fig. 5. Calibration graphs for (○) 2,4-D, (●) 2,4,5-T and (△) Silvex using HPLC–particle beam methane-enhanced ECNI in the SIM mode.

those from the tap water samples are consistently high. SPE recovery data reported by other investigators for chlorophenoxy acids in environmental waters range from 29% to 76% [7] and in drinking water recoveries of 31% and 156% have been reported [8].

In summary, HPLC–particle beam mass spectrometry in the methane-enhanced ECNI mode has been shown to provide the sensitivity, accuracy and precision necessary for the determination of three chlorophenoxy acid herbicides in water. The method can be coupled with SPE to provide the rapid analysis of water samples for these herbicides. Further studies are in progress to extend the method to additional compounds in this class and to investigate the higher recoveries observed for environmental water samples.

TABLE II
SPE RECOVERY FOR CHLOROPHENOXY ACIDS FROM WATER AS DETERMINED BY HPLC–PARTICLE BEAM MS IN THE METHANE-ENHANCED ECNI MODE

Spiking level (μg/l)	Recovery (%)			Water
	2,4-D	2,4,5-T	Silvex	
20 (n = 6)	89.3 ± 10.5	109.1 ± 15.4	99.5 ± 8.7	DDI[a]
20 (n = 6)	109.2 ± 6.9	134.8 ± 25.0	123.8 ± 12.9	Tap
0 (n = 3)	0	0	0	DDI[a]
0 (n = 3)	0	0	0	Tap

[a] Distilled, deionized water.

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