ELSEVIER

Journal of Chromatography A, 805 (1998) 127–135

JOURNAL OF CHROMATOGRAPHY A

Identification of pesticides by liquid chromatography–particle beam mass spectrometry using electron ionization and chemical ionization

C. Aguilar*, F. Borrull, R.M. Marcé

Department of Analytical Chemistry and Organic Chemistry, Universitat Rovira i Virgili, Imperial Tàrraco 1, 43005 Tarragona, Spain

Received 27 March 1997; received in revised form 8 December 1997; accepted 14 January 1998

Abstract

Liquid chromatography–mass spectrometry (LC–MS) with a particle beam (PB) interface is used to separate and identify a group of pesticides. The mass spectra obtained under the different ionization modes, electron ionization (EI) and positive and negative chemical ionization (PCI and NCI) are compared. The operating conditions under each mode, determined by studying the influence on the ion abundance of the ion source temperature of the EI mode, and the gas pressure and ion source temperature in the methane CI were optimized. EI was more sensitive than PCI and NCI and of the latter two modes, NCI gave higher responses, especially for organophosphorus compounds. When on-line solid-phase extraction–LC–PB-MS was applied to real samples, limits of detection in full scan mode were in the range of 0.5 and 10 $\mu g\ l^{-1}$ for EI. The analysis of real samples by on-line solid-phase extraction–LC–PB-MS enabled EI detection of one of the pesticides studied and confirmation by PCI and NCI. The combined EI/CI information also enabled the detection of some non-target compounds.

Keywords: Interfaces, LC–MS; Water analysis; Environmental analysis; Pesticides

1. Introduction

Toxicity and persistence of pesticides in the environment open up the possibility of animal and human exposure to dangerous levels of these compounds. Therefore, analytical chemistry should provide methods for their reliable detection and quantification. Many pesticides are commonly determined by gas chromatography (GC) with selective detectors [1–6] but the more polar pesticides are generally subjected to liquid chromatography (LC) [4–9], diode-array detection (DAD) being the most commonly used detection method [8–10]. If more selectivity is required, mass spectrometric (MS) detection can be used. Among the LC–MS interfaces [11–19], particle beam (PB) takes a special position, because it enables the use of electron impact (EI) MS [20–24]. EI mass spectra often provide sufficient clues for identification, but in some cases chemical ionization (CI) MS is required to get adequate molecular mass information or confirmation. The PB interface allows the use of positive (P) or negative (N) ion CI with a free choice of reagent gas. PB does have some drawbacks such as the carrier effect, non-linearity calibration curves and relatively poor sensitivity [25–30]. In recent years, the poor sensitivity has successfully been overcome, at least in some cases, by using on-line trace enrichment via solid-phase extraction (SPE) [19–22] but non-linearity and the possible carrier-effect still cause problems.

*Corresponding author.

0021-9673/98/$19.00
PII S0021-9673(98)00039-9

In this study, LC–PB-MS with EI, PCI and NCI detection has been applied to the identification of pesticides of different chemical nature in water. The aim of the study was the characterisation of the pesticides and the comparison of the efficiency of the three ionization modes for detection and identification.

2. Experimental

2.1. Chemicals

The structures of the pesticides included in this study, ethyl-parathion, malathion, vamidothion, ametryn, prometryn, terbutryn, bentazone, isoproturon, (4-chloro-2-methylphenoxy)acetic acid (MCPA), mecoprop, dinoseb and molinate, are shown in Fig. 1. All pesticides were of 98–99% purity and were obtained from Riedel-de Häen (Seelze, Germany). Stock solutions of each compound were prepared at the 1000 μg ml^{-1} level in HPLC-grade methanol and stored in the refrigerator at 4°C. Working solutions were prepared by diluting to appropriate concentrations the stock solutions in methanol; they were stored in the same way.

HPLC-grade methanol was obtained from Scharlau (Barcelona, Spain). Ultra-pure water was prepared by ultrafiltration with a Milli-Q water purification system (Millipore, Bedford, MA, USA). Ammonium acetate was from Panreac (Montcada i Reixac, Spain); the solution prepared in Milli-Q purified water was filtered through a 0.45-μm nylon filter (Millipore) prior to use.

Helium used for the PB interface and for degassing the LC solvents and methane for CI were 99.995% pure and supplied by Carburos Metálicos (Barcelona, Spain).

2.2. Instrumentation

An HP1090 liquid chromatograph (Hewlett-Packard, Palo Alto, CA, USA) equipped with a DAD system, a six-port rotary valve and an autosampler was used for LC experiments. The system was controlled by an HP 79994A Workstation. Separations were carried out on a 200×4.6 mm I.D. stainless steel analytical column packed with Spherisorb ODS2, 5 μm (Teknokroma, Barcelona, Spain).

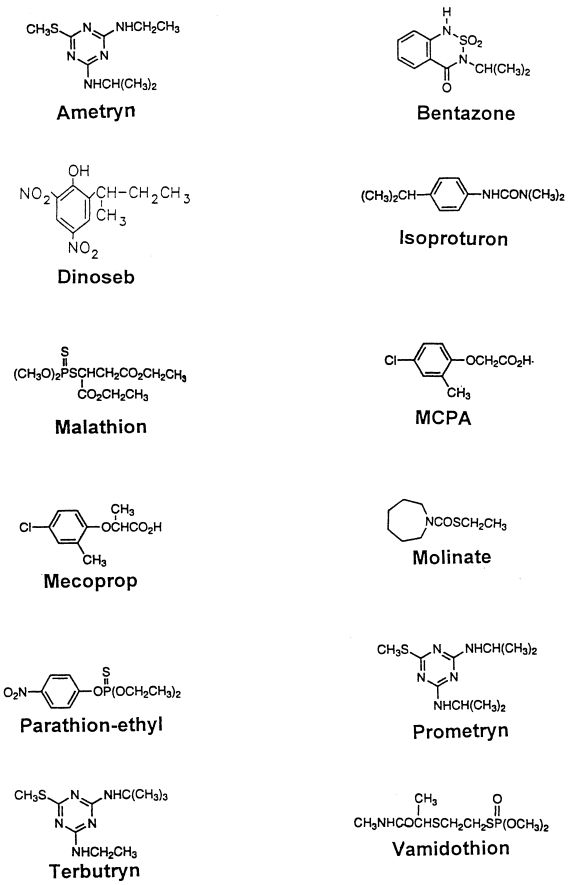

Fig. 1. Chemical structures of the pesticides studied.

An HP 5989 A mass spectrometer (MS Engine), equipped with a dual EI/CI source was connected to the liquid chromatograph outlet via an HP59980B PB interface. The connection was made with a 50 cm×0.12 mm stainless steel capillary. All data were acquired on an HP UX 59944C data system. The interface conditions were as follows: the desolvation chamber temperature was set at 65°C and the helium nebulizer pressure at 60 p.s.i. (1 p.s.i.=6894.76 Pa). The mass spectrometer was calibrated and settings were optimised using the standard tuning facilities.

For electron impact ionization (EI), the ion source block and quadrupole temperatures were set at 250°C and 100°C, respectively. In the full-scan mode, spectra were acquired in the 64–400 u range at a scan rate of 2.32 scans s^{-1}. The ionization energy was 70 eV. In CI, methane was used as reagent gas. The gas pressure was kept at 1.0 Torr for PCI (1 Torr=132.33 Pa, forevacuum pressure) and 1.3 Torr for NCI. The ion source temperatures were set at 250°C and 200°C for PCI and NCI, respectively. Spectra were acquired for 81–400 u for PCI at 2.43 scans s^{-1} and for 64–400 u for NCI at 2.32 scans s^{-1}.

Trace enrichment was performed on a 10×2.0 mm cartridge packed with PLRP-S styrene–divinylbenzene copolymer (Spark Holland, Emmen, Netherlands). An Applied Biosystems (Ramsey, USA) pump was used to deliver the sample and to condition the pre-column.

2.3. Analytical procedures

Flow injection analysis (FIA) was performed by introducing 0.12–1.25 μg solutions of the analytes into a carrier stream of methanol or methanol–0.1 *M* ammonium acetate (50:50, v/v) at a flow-rate of 0.4 ml min^{-1}.

For gradient LC separation, the pH of the 0.1 *M* ammonium acetate solution was adjusted to pH 5 by adding acetic acid [24]. The linear gradient was from 30% to 60% methanol in 20 min, then to 70% at 40 min. After 5 min at 90% methanol, the mobile phase was returned to initial conditions. The flow-rate was 0.4 ml min^{-1} and the column temperature, 40°C.

The pre-column was washed with 5 ml of methanol at 4 ml min^{-1} and, next, 5 ml of methanol–ammonium acetate (30:70, v/v) at 0.4 ml min^{-1}. Subsequently, a 100-ml sample was preconcentrated at 4 ml min^{-1}. The analytes trapped on the pre-column were desorbed in the backflush mode, with methanol–0.1 *M* ammonium acetate (30:70, v/v), and transferred on-line to the analytical column. Prior to analysis river water samples were filtered through a 0.45-μm filter. The pH was adjusted to 2 before the preconcentration step, by adding hydrochloric acid.

3. Results and discussion

3.1. Optimization of the sensitivity of detection

First, the nebulizer position, the desolvation chamber temperature (65°C) and the helium nebulizer pressure (60 p.s.i.) were optimized to achieve the maximum response under FIA conditions by injecting 500 ng of isoproturon.

The optimization of the source temperature is especially important when studying thermally labile substances [23]. In this paper, the responses obtained at temperatures of 200, 225 and 250°C were compared by FIA experiments with 25 μl of a 50 mg l^{-1} methanol solution of ametryn, bentazone, isoproturon, MCPA and malathion. In EI, a significant increase in the responses of ametryn, isoproturon and malathion was observed when the temperature was higher while a modest influence on signal intensity was observed for the other compounds. These results show that the overall best source temperature was 250°C, which was selected for further experiments.

FIA of all pesticides was carried out by injecting solutions with different concentrations of each analyte into a carrier stream of the methanol or methanol–0.1 *M* ammonium acetate (50:50, v/v). No significant differences were observed between the spectra of most of the pesticides with these two carrier streams; only for malathion and parathion-ethyl was there a slight variation in the relative abundances of the ions present in their spectra. The base peak for malathion with the methanol carrier stream was at m/z 127 whereas it was at m/z 173 with methanol–0.1 *M* ammonium acetate. The base peak for parathion-ethyl with methanol was at m/z 97, and at m/z 109 with methanol–0.1 *M* ammonium acetate. The spectra obtained were used to determine the detection limit (S/N 3:1) in PB-EI-MS; these data are included in Table 1.

For methane PCI and NCI, several ion source temperatures (200, 225 and 250°C) and source pressures (0.8, 1.0, 1.3 Torr) were studied using the same analyte test set as above. Bentazone could not be detected under any condition and MCPA did not show up in PCI. There was a little variation for ametryn, isoproturon and MCPA with temperature, yielding 10–15-fold higher responses in NCI. Op-

Table 1
Detection limits (μg) for all pesticides, using FIA with EI and CI

Compound	EI		PCI		NCI	
	Methanol	Methanol–ammonium acetate	Methanol	Methanol–ammonium acetate	Methanol	Methanol–ammonium acetate
Ametryn	0.12	0.12	1.25	1.25	0.25	n.d.
Bentazone	0.25	1.25	n.d.[a]	n.d.	n.d.	n.d.
Dinoseb	1.25	1.25	n.d.	n.d.	1.25	n.d.
Isoproturon	0.25	1.25	1.25	1.25	0.25	1.25
Malathion	0.12	0.12	n.d.	n.d.	0.12	0.25
MCPA	1.25	1.25	n.d.	n.d.	1.25	n.d.
Mecoprop	0.25	1.25	n.d.	n.d.	1.25	n.d.
Molinate	1.25	1.25	n.d.	n.d.	n.d.	n.d.
Parathion-ethyl	0.12	1.25	1.25	1.25	0.12	0.25
Prometryn	0.12	0.12	1.25	n.d.	0.25	1.25
Terbutryn	0.12	0.12	0.25	1.25	0.25	1.25
Vamidothion	0.12	0.12	1.25	1.25	0.25	1.25

[a] n.d.=Not detected.

timization was important for malathion with 250°C and 1.0 Torr, and 200°C and 1.3 Torr being the best settings for PCI and NCI, respectively. Detection limits for all analytes are included in Table 1.

3.2. Information content of mass spectra

As far as the spectra of the various compounds are concerned, considerable differences were observed for the different ionization modes as can be seen in Table 2.

For organophosphorus compounds, the molecular ion in EI is obtained for ethyl-parathion and also for vamidothion, although at very low abundance (2%). The PCI spectra showed the $[M+H]^+$ for malathion and ethyl-parathion. In the NCI mode the base peak for ethyl-parathion was the $[M]^-$ ion because the molecule contains an aromatic ring which stabilizes the negative charge by electron delocalization and there is a nitro group with high electron affinity; the anion is therefore more stable under negative conditions. No molecular ion information was obtained for malathion and vamidothion in NCI. The fragments shown in the table for organophosphorus pesticides are similar to those obtained in other studies, even though these dealt with GC–CI–MS [31,32] or LC–MS with a TSP [33] or APCI [34] interface.

As illustrative examples, Fig. 2a–c show the mass spectra of the ethyl-parathion under EI, PCI and NCI conditions, respectively.

Under EI, the molecular ion is present in the spectra for all the triazines studied. They further decompose with the loss of $-CH_3$, $CH_3CH=CH_2$ or $CH_3CH(CH_3)_2$. The spectra obtained for the triazines under CI show the deprotonated or protonated molecule as the base peak in the negative or positive ion modes, respectively. This is in agreement with previous observations of other authors using GC–MS [35,36].

In EI, the base peak of the phenylures isoproturon was at m/z 72 which corresponds to the typical phenylurea fragment $[(CH_3)_2NCO]^+$ [21]. $[M+H]^+$ and $[M-H]^-$ were the base peaks when methane PCI and methane NCI ionization were used, respectively. For this kind of compounds, chemical ionization gives useful additional information to distinguish phenylureas which have their base peak at m/z 72 in EI.

For the chlorophenoxyacetic acids, MCPA and mecoprop, the base peak was the corresponding phenoxy ion that was assigned to the loss of the corresponding alkanecarboxylic acid, as was also observed by other authors [37]. The presence of the molecular ion is observed in EI and also in NCI spectra, but at very low abundance in the last case. Under EI, the base peak for bentazone at m/z 120 corresponds to the ion $[C_6H_4NH_2CO]^+$.

For dinoseb, a phenolic compound, no peak

Table 2
Mass spectral fragments and relative abundance (R.A.) obtained by FIA–PB-MS

Compound	M_r	EI		PCI		NCI	
		m/z and tentative ions	R.A. (%)	*m/z* and tentative ions	R.A. (%)	*m/z* and tentative ions	R.A. (%)
Ametryn	227	227 $[M]^+$	100	228 $[M+H]^+$	100	226 $[M-H]^-$	100
		212 $[M-CH_3]^+$	55				
		170 $[M-C_3H_8NH]^+$	36				
Bentazone	240	120 $[C_6H_4NH_2CO]^+$	100	n.d.[a]		n.d.	100
Dinoseb	240	211 $[M-CH_2CH_3]^+$	100	n.d.		141 [¿?]	100
						240 $[M]^-$	11
Isoproturon	206	72 $[(CH_3)_2NCO]^+$	100	207 $[M+H]^+$	100	205 $[M-H]^-$	
		206 $[M]^+$	36				100
Malathion	330	173 $[M-(CH_3O)_2PS_2]^+$	100	127 $[M-(CH_3O)_2PS_2)-(C_2H_6O)]^+$	100	172 $[M-(CH_3O)_2PS_2-H]^+$	100
		127 $[M-(CH_3O)_2PS_2)-(C_2H_6O)^+]$	88	173 $[M-(CH_3O)_2PS_2]^+$	82	157 $[(CH_3O)_2PS_2]^-$	75
				331 $[M+H]^+$	31		
MCPA	200	141 $[M-CH_2COOH]^+$	100	n.d.		141 $[M-CH_2COOH]^-$	100
		200 $[M]^+$	63			200 $[M]^-$	6
Mecoprop	214	142 $[M-CH(CH_3)COO]^+$	100	n.d.		141 $[M-CH_3CHCO_2H]^-$	100
		214 $[M]^+$	61			200 $[M]^-$	9
Molinate	187	126 $[M-SC_2H_5]^+$	100	n.d.		n.d.	
		187 $[M]^+$	26				
Parathion-ethyl	291	109 $[C_2H_5OPO_2H]^+$	100	262 $[M-C_2H_5]^+$	100	291 $[M]^-$	100
		291 $[M]^+$	54	292 $[M+H]^+$	27	154 $[(C_2H_5O)_2PSH)]^-$	16
Prometryn	241	226 $[M-CH_3]^+$	100	242 $[M+H]^+$	100	240 $[M-H]^-$	100
		184 $[M-NCH(CH_3)_2]^+$	93				
		241 $[M]^+$	64				
Terbutryn	241	226 $[M-CH_3]^+$	100	242 $[M+H]^+$	100	240 $[M-H]^-$	100
		184 $[M-C_4H_8]^+$	91				
		241 $[M]^+$	64				
Vamidothion	287	87 $[CH_3NHCOC_2H_5]^+$	100	146 $[CH_3NHCOCH_3CHSCH_2CH_2]^+$	100	272 $[M-CH_3]^-$	100
		146 $[CH_3NHCOCH_3CHSCH_2CH_2]^+$	31	88 $[CH_3NHCOC_2H_6]^+$	87		
		109 $[(CH_3O)_2PO]^+$	14				

[a] n.d.=Not detected.

corresponding to the molecular ion was observed in EI, whereas for NCI $[M]^-$ appeared with low abundance. The EI spectrum for molinate shows the molecular ion; this pesticide was not detected in PCI and NCI.

3.3. On-line SPE

The samples were on-line preconcentrated on a styrene–divinylbenzene copolymer. The 100 ml sample was acidified to pH 2 before preconcentration to improve the recovery for some of the pesticides studied, in particular the chlorophenoxyacetic acids [24]. For 100 ml, the analyte recoveries were higher than 80% for all compounds except vamidothion (recovery, about 15%).

Initial tests were made with aqueous standard solution on Milli-Q water. For obvious reasons, EI-MS was selected as the detection mode. At a spiking level of 10 μg l^{-1} all analytes showed up except bentazone and molinate, while at 1 μg l^{-1} only isoptroturon and ametryn could be observed. For real samples, the detection limits were between 0.5 and 10 μg l^{-1}.

Sensitivity could have been improved by using

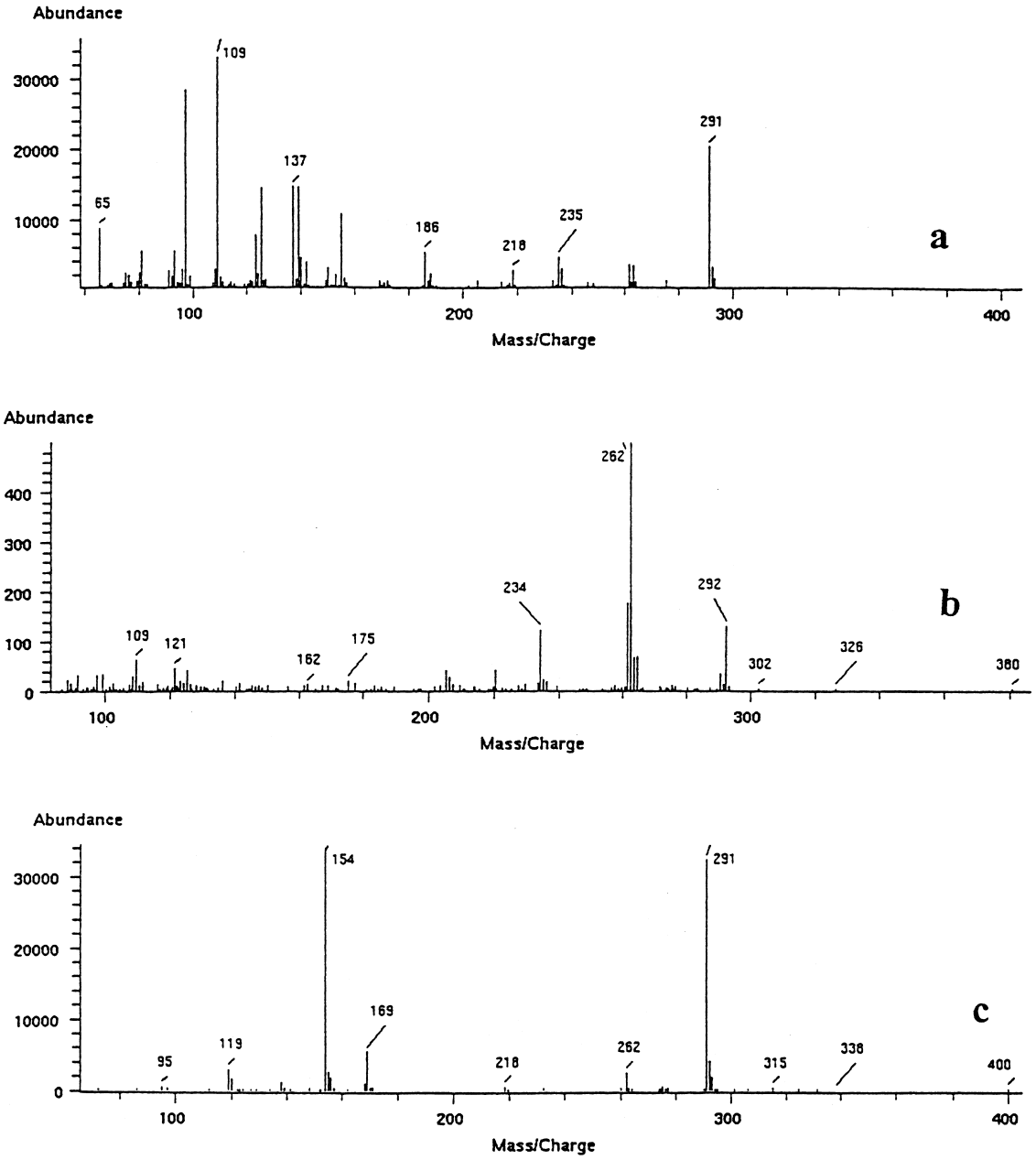

Fig. 2. Spectra of parathion-ethyl using (A) EI, (B) PCI and (C) NCI.

selected ion monitoring (SIM), however, as identification was the main objective of this paper, the full scan mode was used.

3.4. Applications

Although the above results are not too promising

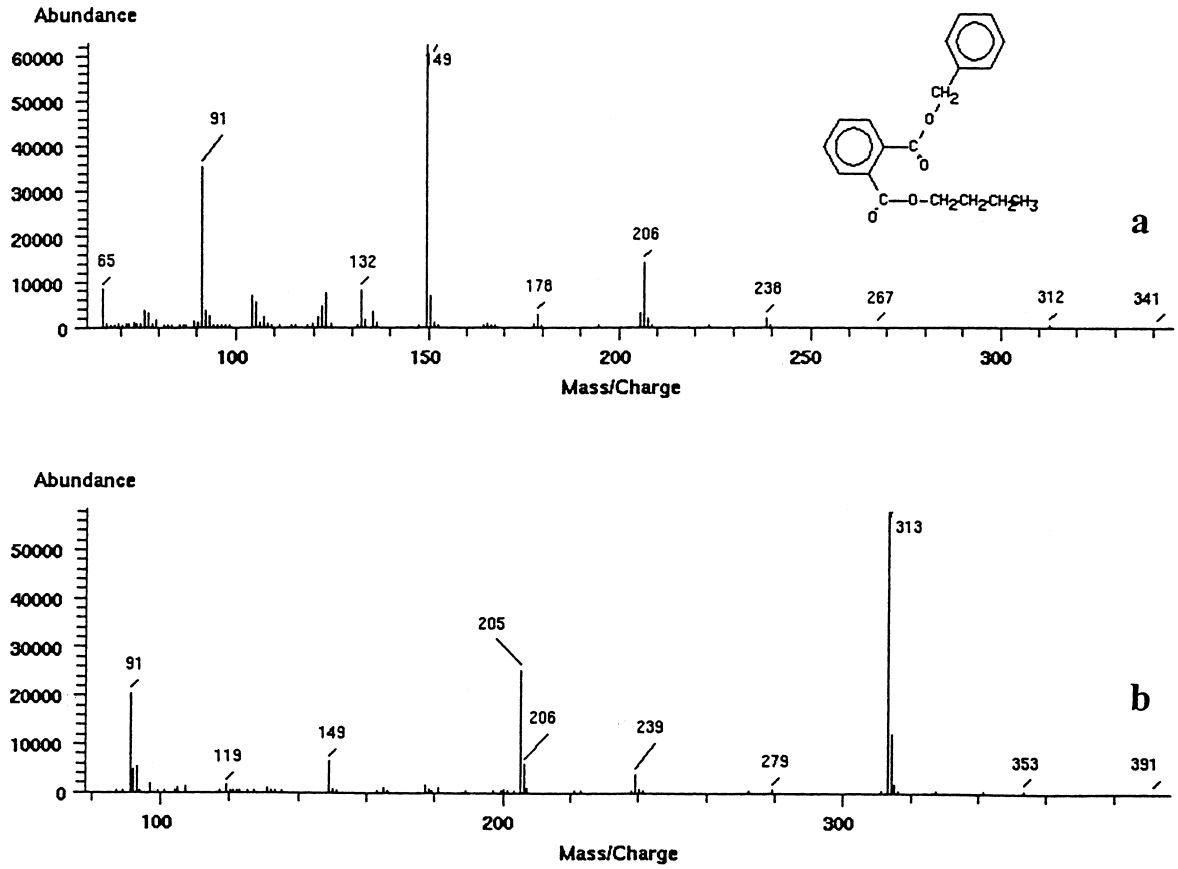

Fig. 3. PB-EI and PB-PCI mass spectra and structure of the peak assigned to 1,2-benzenedicarboxylic acid butyl phenylmethyl ester.

for real-life applications, several river water samples were subjected to analysis. In one sample, several peaks showed up. None of them could be assigned to one of our test analytes. However, comparison of the mass spectra at 51.5 and 51.8 min with library spectra enabled the tentative identification of 1,2-benzenedicarboxylic acid butyl phenylmethyl ester and 1,2-benzenedicarboxylic acid dibutyl ester, respectively. Confirmation of the presence of these two compounds was possible through their PCI spectra which showed ion $[M+H]^+$ as the base peak corresponding to m/z 313 and m/z 278, respectively. No peaks appeared at the pertinent retention times in the NCI-MS chromatogram. Fig. 3 shows the EI and PCI spectrum of the peak at 51.5 min and also the structure assigned by the library search.

In another sample, from the Ebro delta, a peak appeared in the chromatogram at the retention time of malathion. Fig. 4a shows the selected ion chromatogram at m/z 173 obtained by LC–PB-EI-MS and the full spectrum of the peak. When the sample was analyzed by CI (PCI and NCI) the presence of malathion was confirmed. The pertinent results are included in Fig. 4b,c. The corresponding spectra are shown in the inserts.

4. Conclusions

Mass spectra of a group of 12 pesticides were obtained by PB-MS operated in the EI, PCI and NCI modes. After optimization of the experimental con-

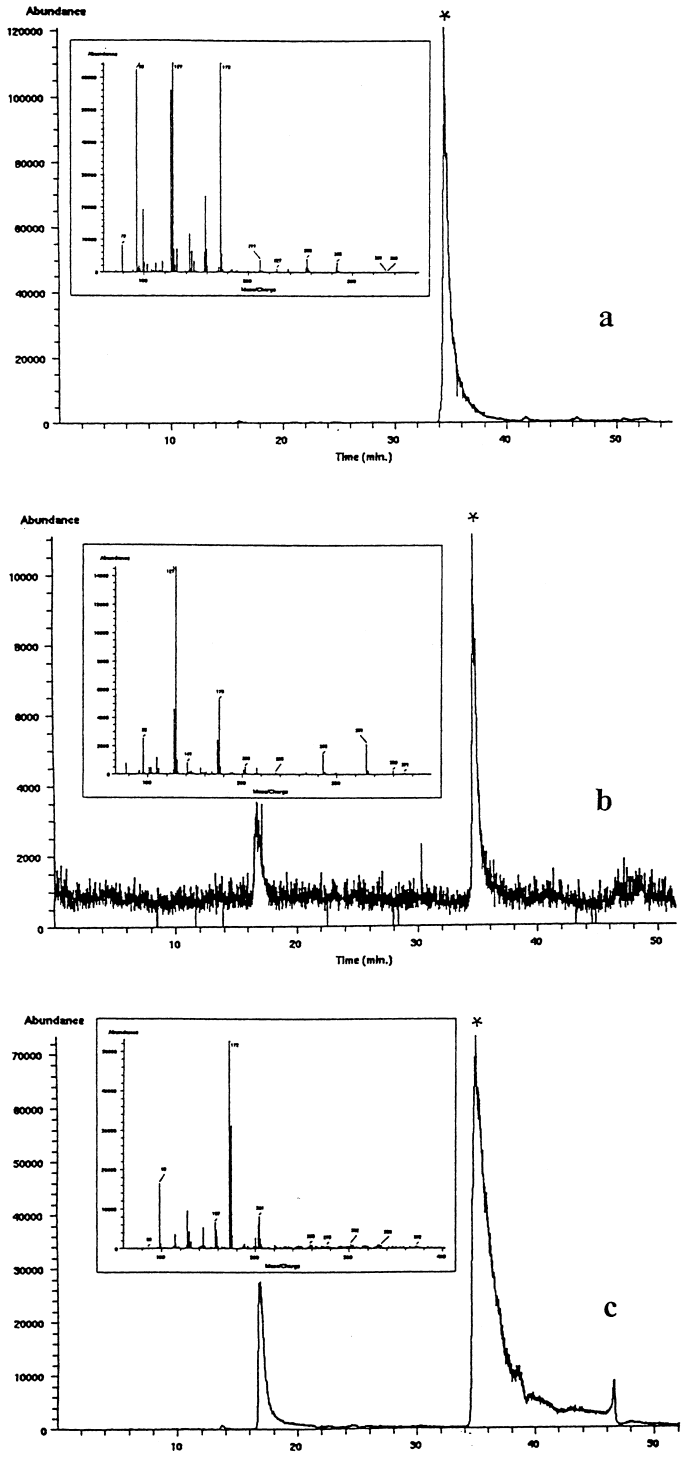

Fig. 4. Selected ion chromatogram for an Ebro delta sample obtained using HPLC–PB-MS with (a) EI by monitoring the m/z 173 and (b) NCI by monitoring the m/z 172 and (c) PCI by monitoring the m/z 127. The inserts show the corresponding spectra. Peaks labelled with an asterisk correspond to malathion.

ditions it was observed that analyte detectability is much better in EI than in the CI modes. Unfortunately, even in the former ionization mode, sensitivity was not very high (which agrees with findings by other workers) and sample volume of about 100 ml are required to obtain (in full scan detection) limits of around 1 $\mu g\ l^{-1}$. If such detection limits can be obtained, PB-MS is an attractive mode of operation, as was demonstrated by the identification of several microcontaminants in surface water samples. In all instances, confirmatory information was provided by CI-mode PB-MS. The present results of poor analyte detectability versus good structural information again emphasise the relevance of studies devoted to improve the particle beam interface.

Acknowledgements

The authors gratefully acknowledge ''Direcció General de Recerca de la Generalitat de Catalunya'' for financial support given and C. Crespo for technical assistance.

References

[1] C. de la Colina, F. Sánchez-Rasero, G. Dios Cancela, A. Peña, Analyst 120 (1995) 1723.
[2] C. Crespo, R.M. Marcé, F. Borrull, J. Chromatogr. A 670 (1994) 135.
[3] C. Aguilar, F. Borrull, R.M. Marcé, LC·GC 14 (1996) 1048.
[4] J. Sherma, Anal. Chem. 67 (1995) 1R.
[5] I. Liska, J. Slobodnik, J. Chromatogr. A 733 (1996) 235.
[6] H.J. Stan (Editor), Analysis of Pesticides in Ground and Surface Water, Vols. I and II, Springer, Berlin, 1995.
[7] R. Carabias Martínez, E. Rodríguez Gonzalo, F. Garay García, J. Hernández Méndez, J. Chromatogr. 644 (1993) 49.
[8] E.R. Brouwer, I. Liska, R.B. Geerdink, P.C.M. Fintrop, W.H. Mulder, H. Lingeman, U.A.Th. Brinkman, Chromatographia 32 (1991) 32.
[9] C. Aguilar, F. Borrull, R.M. Marcé, J. Chromatogr. A 754 (1996) 77.
[10] A. Junker-Buchheit, M. Witzenbacher, J. Chromatogr. A 737 (1996) 67.
[11] A.L. Yergey, C.G. Edmons, I.A.S. Lewis and M.L. Vestal, Liquid Chromatography–Mass Spectrometry – Techniques and Applications, Plenum Press, New York, 1990.
[12] W.M.A. Niessen and J. Van der Greef, Liquid Chromatography–Mass Spectrometry, Marcel Dekker, New York, 1992.
[13] D. Barceló (Editor), Applications of LC–MS in Environmental Chemistry, Elsevier, Amsterdam, 1996.
[14] H. Bagheri, E.R. Brouwer, R.T. Ghijsen, U.A.Th. Brinkman, J. Chromatogr. A 647 (1993) 121.
[15] C. Molina, M. Honing, D. Barceló, Anal. Chem. 66 (1994) 4444.
[16] W.M.A. Niessen, A.P. Tinke, J. Chromatogr. A 703 (1995) 37.
[17] S. Chiron, S. Papilloud, W. Haerdi, D. Barceló, Anal. Chem. 67 (1995) 1637.
[18] M.T. Galcerán, E. Moyano, J. Chromatogr. A 731 (1996) 75.
[19] J. Slobodnik, A.C. Hogenboom, J.J. Vreuls, J.A. Rontree, B.L.M. van Baar, W.M.A. Niessen, U.A.Th. Brinkman, J. Chromatogr. A 741 (1996) 59.
[20] R.M. Marcé, H. Prosen, C. Crespo, M. Calull, F. Borrull, U.A.Th. Brinkman, J. Chromatogr. A 696 (1995) 63.
[21] H. Bagheri, J. Slobodnik, R.M. Marcé, R.T. Ghijsen, U.A.Th. Brinkman, Chromatographia 37 (1993) 159.
[22] H. Prosen, L. Zupancic-Kralj, J. Marcel, J. Chromatogr. A 704 (1996) 121.
[23] J. Slobodnik, M.E. Jager, S.J.F. Hoekstra-Oussoren, M. Honing, B.L.M. van Baar, U.A.Th. Brinkman, J. Mass Spectrom. 32 (1997) 43.
[24] C. Aguilar, F. Borrull, R.M. Marcé, Chromatographia 43 (1996) 592.
[25] T.D. Behymer, Th.A. Bellar, W.L. Budde, Anal. Chem. 62 (1990) 1686.
[26] T.A. Bellar, T.D. Behymer, W.L. Budde, J. Am. Soc. Mass Spectrom. 1 (1990) 92.
[27] M.J.I. Mattina, J. Chromatogr. 549 (1991) 237.
[28] J.S. Ho, T.D. Behymer, W.L. Budde, T.A. Bellar, J. Am. Soc. Mass Spectrom. 3 (1992) 662.
[29] C.S. Creaser, J.W. Stygall, Analyst 118 (1993) 1467.
[30] T.J. Jones, L.D. Betowski, V. López-Avila, Trends Anal. Chem. 13 (1994) 333.
[31] G. Durand, D. Barceló, Anal. Chim. Acta 243 (1991) 259.
[32] S. Lacorte, C. Molina, D. Barceló, Anal. Chim. Acta 281 (1993) 71.
[33] S. Lacorte, D. Barceló, J. Chromatogr. A 712 (1995) 103.
[34] S. Lacorte, D. Barceló, Anal. Chem. 68 (1996) 2464.
[35] H.J. Stan, A. Bockhorn, Fresenius J. Anal. Chem. 339 (1991) 158.
[36] H. Bagheri, J.J. Vreuls, R.T. Ghijsen, U.A.Th. Brinkman, Chromatographia 34 (1992) 5.
[37] I.S. Kim, F.I. Sasinos, R.D. Stephens, J. Wang, M.A. Brown, Anal. Chem. 63 (1991) 819.