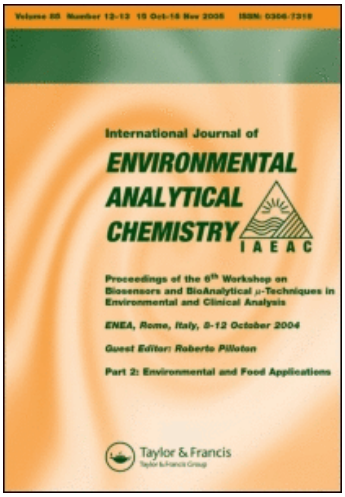

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Multi-Residue Analysis by Liquid Chromatography-Mass Spectrometry Coupling. Application to Drinking and River Waters

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MULTI-RESIDUE ANALYSIS BY LIQUID CHROMATOGRAPHY-MASS SPECTROMETRY COUPLING. APPLICATION TO DRINKING AND RIVER WATERS

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The coupling between liquid chromatography and mass spectrometry with an APCI or ESI interface (in positive or negative mode) is used here for multi-residue analyses in natural waters, covering basic and neutral pesticides as well as acid pesticides. The methods developed are applied to drinking and, river waters after the samples are concentrated by liquid-liquid extraction or solid phase extraction on C18 cartridges. Comparisons are made between UV detection and mass spectrometry and between two chromatographic methods for acid substances. The quantitation limits range from 0.01 to 0.1 µg/l according to the substance.

Keywords: Multi-residue analysis; LC-MS; atmospheric pressure interfaces; acid pesticides; neutral pesticides

INTRODUCTION

Recent years have witnessed the growing use of pesticides, chiefly in agriculture, but also in public works (road and rail maintenance) and home products[1]. This development has undeniable repercussions on our environment, and the quality control of natural waters, both groundwater and surface water, has become a major issue. To address the subject, several national and international regulations now exist, including the European regulation on drinking water quality, which sets a maximum concentration of 0.1 µg/l for an isolated pesticide and 0.5 µg/l

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for total pesticides present in the sample. The variety of substances determined and their degradation products[2] entails the use of wide-spectrum, reliable and sensitive multi-residue methods to produce analyses to meet the regulations.

Conventionally, most environmental analyses are performed by gas chromatography (GC), either associated with various detectors such as the electron capture detector (ECD), the thermoionic nitrogen and phosphorous detector (NPD), and the flame photometry detector (FPD), or coupled with mass spectrometry (MS). These methods are ideal for apolar substances[3]. However, higher polarity, heat-sensitive and nonvolatile substances cannot be analysed by this technique without previous derivatization (methylation with diazomethane[4], derivatization with HFBA[5], acetylation, etc.). In this context, liquid chromatography (LC) has gradually gained the environmental field. Originally, combined with UV detection at a preselected wavelength, and then diode array UV detection, LC is now increasingly combined with mass spectrometry, which adds to the wide-spectrum multi-residue character of liquid chromatography the identification capacity and sensitivity required to meet the European standards.

In recent years, among the principal interfaces usable for LC-MS coupling are atmospheric pressure interfaces: APCI (atmospheric pressure chemical ionization) and ESI (electrospray ionization), which allow ionization of the molecules in positive or negative modes[6]. Atmospheric pressure ionization methods are mild ionization methods that mostly yield protonated molecular ions $(M+H)^+$ in positive mode, or deprotonated molecular ions $(M-H)^-$ in negative mode. However, to enhance identification by this method, these molecular ions can be fragmented by applying a collision potential of a few tens of volts to the inlet octapole of the mass spectrometer. The growing number of publications of results obtained with LC-MS and LC-MS-MS coupling using atmospheric pressure interfaces illustrate the considerable potential of this technique[7,11], associated upstream with off-line or on-line liquid-solid preconcentration methods, which have been improved recently by using a short column (10 mm × 2 mm ID) for both SPE and analytical operation[12].

Acid pesticides without derivatization can also be analysed by capillary electrophoresis. For example, CE using cyclodextrins has been used to analyse phenoxy acid herbicides and to isolate enantiomers in water samples[13].

The present paper presents the results obtained in the investigation of surface waters by LC-MS using APCI interface in PI mode for neutral substances and ESI interface in NI mode for acid substances. ESI interface in NI mode was used in combination with two reversed phase separation modes: ion pairing and separation at acidic pH.

EXPERIMENTAL

Apparatus

All the analyses were performed on a Varian liquid chromatography chain (Les Ulis, France) consisting of a 9012 quaternary pump, a 9100 automatic passer and a 9065 diode-array UV detector, with the system run by the Varian LC-Star software. Detection by mass spectrometry was carried out using a Finnigan SSQ7000 type instrument (Thermoquest, Les Ulis, France), equipped as required with an APCI or ESI interface and run by the ICIS software.

Stationary phases

The chromatographic columns used were of the LC-ABZ 250 × 4.6 mm ID type (5 µm) (Supelco, St Quentin Fallavier, France) to analyse neutral pesticides, and Kromasil 250 × 4.6 mm ID (5 µm) (Touzart et Matignon, Courtaboeuf, France) to analyse acid pesticides.

Chemicals

The certified pure products used to prepare the calibration solutions were supplied by CIL Cluzeau Info Labo (France), and the solvents (acetonitrile, methanol and dichloromethane) by Carlo Erba (France) and BDH Laboratory Supplies (UK). The mQ water was obtained using a Seradest S600 deionizer. Stock solutions of pesticides were prepared by weighing and dissolving them in methanol. The standard solutions were stored at 4°C.

Liquid chromatography- mass spectrometry

Basic and neutral pesticides analysis by LC-APCI-MS

Chromatographic separations were carried out by elution gradient of an acetonitrile/water mobile phase, from 15 to 60% acetonitrile in 50 minutes. The mobile phase flowrate was set at 1 ml/min. A volume of 20 µl was injected.

Detection was carried out simultaneously by UV / DAD and APCI-MS in PI mode, with both detectors mounted in series. With the APCI interface using the heated pneumatic nebulizer with Corona discharge ionization allows LC flowrate up to 2 ml/mn. Figure 1 shows an example of chromatographic separation carried out on a calibration solution.

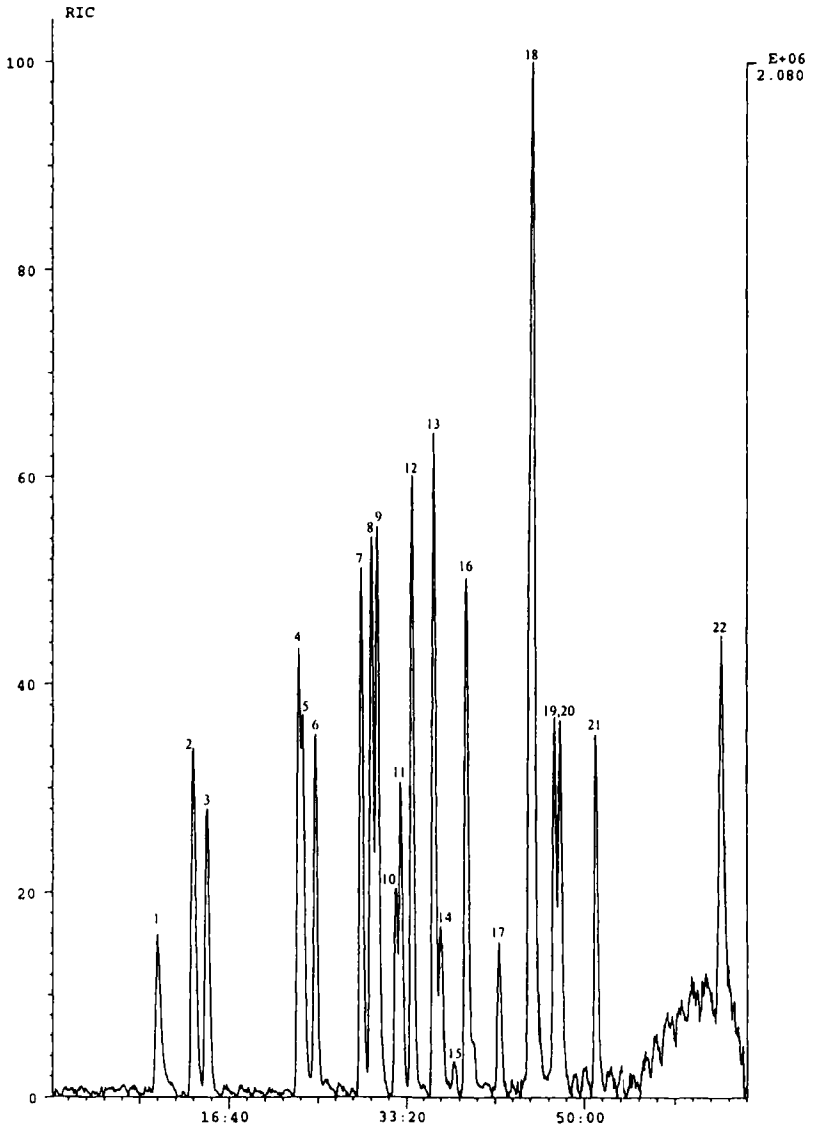

FIGURE 1 LC separation ofa neutral pesticides mixture belonging to the regional list (about 10 µg/ml of each compound in methanol) using APCI-MS in PI mode (acquisition in TIC mode). Injected volume: 20 µl

1. Deisopropylatrazine 2. Metamitron 3. Deethylatrazine 4. Simazine 5. Metribuzin 6. Cyanazine 7. Desmetryn 8. Atrazine 9. Secbumeton 10. Chlorotoluron 11. Isoproturon 12. Terbumeton 13. Ametryn 14. Diuron 15. Triadimenol (isomer 1) 16. Terbuthylazine + Triadimenol (isomer 2) 17. Linuron 18. Penconazole+Flusilazole+Tebuconazole 19. Hexaconazole 20. Prochloraz 21. Neburon 22. Fenpropimorph

Acid pesticides analysis by LC-ESI-MS

The analysis of acid pesticides by LC-ESI-MS in NI mode was carried out using two different RPLC methods: ion pairing chromatography[14] and acid pH chromatography[15]. In both cases, the ionization mode selected was electrospray in NI mode[16]. The effluent from the chromatographic column, containing the analyte, was simultaneously nebulized and subjected to a negative voltage. The ions entering the detector were then primarily quasi-molecular ions, i.e. molecular ions having lost one or more protons $[M\text{-}nH]^{n-}$. This ionization mode offers the advantage of not degrading heat-sensitive molecules, and is suitable for polar and nonvolatile products.

Use of the ion pairing method

Using RPLC according to Ref. 20, the mobile phase was a binary phase consisting of water and a mixture of methanol/acetonitrile 85/15 v/v, containing 0.2 mmol/l TBAF and 0.1 mmol/l K_2HPO_4. Chromatographic separation was carried out with an elution gradient of 20 to 85% of organic phase in 45 minutes, with the mobile phase entering the column at a rate of 0.5 ml/min, and an injected volume of 20 µl. Detection was carried out simultaneously in UV/DAD and ESI-MS in NI mode, with both detectors mounted in parallel.

The presence of salts in the mobile phase generates a background after working some hours. The flow divider system avoids a fast signal decrease with the ESI interface caused by the presence of potassium hydrogenophosphate additive in the mobile phase. Parameters affecting ionization (spray voltage, capillary temperature, sheath gas pressure and auxiliary gas flowrate) were optimized. The final experimental conditions adopted, which helped achieve maximum ionization yield while maintaining a stable spray voltage, were: voltage applied to spray: −5 kV; sampling capillary temperature: 250 °C; sheath gas pressure: 70 psi; auxiliary gas flowrate: 10 to 15 ml/min; flowrate of eluant phase at interface inlet: about 130 µl/min.

Reversed phase chromatographic separation at acidic pH

The mobile phase used for chromatographic separation consisted of a mixture of methanol/water/acetic acid (90/810/1 v/v/v) [phase A] and a mixture of methanol/acetic acid (900/1 v/v) [phase B]. The presence of acetic acid guarantees a sufficiently low pH to ensure that the compounds are mostly in molecular form and permits their retention on C18 grafted silica phase. Acetic acid was picked for its high volatility, allowing a 0.5 ml eluant flowrate, its complete vaporization at the same time as the eluant, and thereby only allowing the solute ions to enter the analyzer.

Separation was carried out by elution gradient from 0 to 100% of phase B in 30 minutes, and return to initial conditions, for a total analytical period of 45 minutes. The flowrate of the mobile phase in the column was 0.5 ml/min. A volume of 40 µl was injected. Detection was carried out by ESI-MS in NI mode.

Electrospray ionization conditions were optimized by a process similar to the one used in experiments with ion pairing chromatography. The final conditions adopted were as follows: voltage applied to spray: − 5 kV; sampling capillary temperature: 250°C; sheath gas pressure: 65 psi; auxiliary gas flowrate: 5 to 10 ml/min; flowrate of eluant phase at interface inlet: 0.5 ml/min; voltage applied to octapole: + 15 V (allowing a higher signal-to-noise ratio for certain compounds); acquisition in TIC mode with extraction of the signal obtained on the quasi-molecular ion.

The chromatograms obtained in mass spectrometry are shown in Figure 2.

Samples handling

Solutes were extracted by liquid-liquid extraction with methylene chloride or liquid-solid extraction (on Envi-18 Supelclean, Supelco cartridges) at neutral pH for neutral substances, and liquid-solid extraction at acid pH for acid substances. Before analysis of neutral substances, the samples were preconcentrated by liquid-liquid extraction with methylene chloride[17], or by solid-phase extraction on cartridges packed with C18 grafted silica at neutral pH[18], particularly for substances such as Deethylatrazine, Deisopropylatrazine and Carbendazim.

For the acid substances the samples (500ml)were extracted and preconcentrated on C18 cartridge at pH 2.0 according to ISO/DIS 15913 method in preparation. The final extract was preserved for ion-pairing method in a saline solution (water/methanol 70/30 v/v + TBAF 0.2 mmol/l + K_2HPO_4 4 mmol/l) in a final volume of 1 ml. The final extract was preserved in methanol in a final volume of 500 µl for RPLC at acidic pH.

RESULTS AND DISCUSSION

Analysis of basic and neutral pesticides by LC-APCI-MS coupling

The application of this coupling, with APCI in PI mode, is the subject of another paper[19]. We shall, therefore, restrict ourselves here to a review of the essential results. Experiments were conducted on 22 substances belonging to different pesticide families included in the French priority list[20]: triazines, amides, phenylureas, triazoles, benzimidazoles and morpholines.

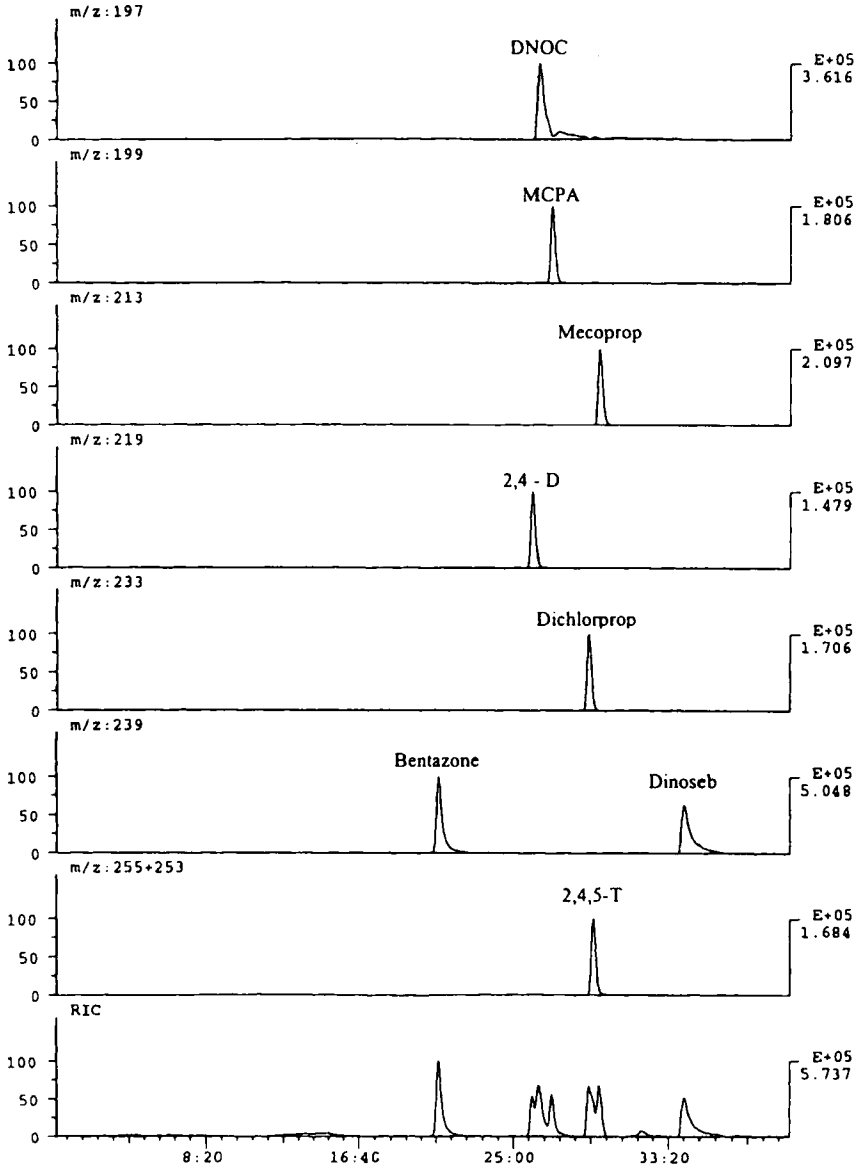

FIGURE 2 LC separation ofan acidic pesticides mixture (about 10 µg/ml for each compound in methanol) in RPLC at acidic pH using ESI-MS in NI mode
Signal was acquired in TIC mode with ion extraction at different m/z values. Injected volume: 40 µl

TABLE I Quantitation limits obtained by LC-APCI-MS in PI mode for neutral pesticides in TIC and SIM acquisition modes, after 1 litre water samples liquid-liquid extraction with methylene chloride or 0,5 liter water samples solid-phase extraction on lg C18 silica cartridges. Injected volume: 20 µl

Substances	*m/z used for quantitation*	*Tr (min)*	*Lowest limit of quantitation in TIC mode (ng/l)*	*Lowest limit of quantitation in SIM mode (ng/l)*
Priority substances of the regional list:				
Deisopropylatrazine	174	10.03	20	5
Metamitron	203	13.18	20	nd
Deethylatrazine	188	14.36	20	5
Simazine	202	23.10	20	5
Metribuzin	215 5	23.30	50	10
Cyanazine	214+241	24.47	20	nd
Desmetryn	214	29.04	10	nd
Atrazine	216	30.00	20	5
Secbumeton	226	30.34	10	nd
Chlorotoluron	213	32.10	50	5
Isoproturon	207	32.47	20	5
Terbumeton	226	33.54	20	nd
Ametryn	228	35.59	10	nd
Diuron	233	36.40	20	5
Triadimenol	296	37.50 (isomer 1)	100	50
		39.02 (isomer 2)		
Terbuthylazine	174+230	39.02	20	5
Linuron	249	42.04	50	nd
Penconazole	284	45.17	20	5
Flusilazole	316	45.17	20	10
Tebuconazole	308	45.17	20	5
Hexaconazole	314	47.17	20	5
Prochloraz	310+376	47.47	50	nd
Neburon	275	51.06	20	nd
Fenpropimorph	304	62.45	20	5

nd: not determined.

Calibration: The calibration curves obtained show very good response linearity on the ionic current signal extracted on the specific m/z ions of each substance, in a broad concentration range (0.025 to 10 µg/ml).

Sensitivity and Quantitation limits: The response sensitivities are influenced by the ionization yield of the substances analysed in the foregoing instrumental conditions. They vary by a factor of 4 from the least sensitive substance (Chlorotoluron) to the most sensitive group including, for example, Terbuthylazine. The practical limits of quantitation, determined by the signal corresponding to 10 times the background on the chromatograms extracted on the specific ions of the substances investigated, range from 0.02 µg/l to 0.1 µg/l in TIC mode. In Selected Ion Monitoring mode, the practical limits of quantitation are improved by a factor of about 5 (see Table I). A fourfold increase of the response sensitivity is observed for Diuron using LC-APCI-MS in NI mode[21].

Mass spectra: The mass spectra primarily contain the protonated molecular ion $(M+H)^+$[22] sometimes accompanied by the adduct with acetonitrile $(M+CH_3CN)^+$ or $(M+41)^+$. Some substances, such as Carbendazim, atrazine and terbuthylazine, undergo fragmentation despite the mild ionization conditions. Others, like Diuron, only contain the protonated molecular ion and the adduct. Fragmentation can also be accentuated by imposing a potential of a few tens of volts on the inlet octapole, thereby generating fragmentations by collision. The repeatability of the mass spectra obtained in identical experimental conditions was confirmed, demonstrating the feasibility of the compilation of a library of reference spectra usable to identify the substances detected.

Analysis of acid pesticides by LC-ESI-MS coupling

Ion-pairing method

UV Detection: In the gradient conditions described above and in UV detection at a wavelength of 220 nm, eight compounds out of eleven were isolated. The remaining three, MCPA, 2,4-D and Ioxynil, were co-eluted, and also featured UV spectra that were too similar to be distinguished simply by wavelength variation. Hence, the advantage of using mass spectrometry which allows clear identification by permitting the extraction of the signal on the specific ions of each substance.

The calibration curves obtained in UV on the eight separate compounds were satisfactory, with correlation factors ranging from 0.9967 (Dichlorprop) to 0.9998 (Bromoxynil), with lows close to 70–80 µg/l, and even approaching 50 µg/l for Bentazone.

TABLE II Calibration data obtained with acid pesticides in standard solutions (concentration range: about 0.01 to 2 µg/ml in saline solution) using ion-pairing LC-ESI-MS in NI mode. Signal was acquired in SIM mode. Injected volume: 20 µl

Substances	*m/z used for quantitation*	*Tr (min)*	*Linear regression*	*Correlation coefficient*	*Lowest limit of calibration curve (µg/l in injected solution)*
Fluoroxypyr	253	19:41	$Y = 577911 \times + 3438$	0.9929	8.3
Bentazone	239	19:58	$Y = 6.10^{E}6 \times + 42312$	0.9976	9.2
Bromoxynil	276	21:41	$Y = 2.10^{E}6 \times - 15191$	0.9987	9.3
MCPA	199	23:30	$Y = 2.10^{E}6 \times - 79595$	0.9925	42.0
2,4-D	219	23:43	$Y=980004 \times - 5178$	0.9985	17.5
Ioxynil	369	23:48	$Y = 4.10^{E}6 \times - 462619$	0.9916	57.2
Dichlorprop	233	25:32	$Y = 2.10^{E}6 \times - 1144$	0.9996	9.3
2,4,5-T	253	26:30	$Y = 1.10^{E}6 \times - 17444$	0.9987	8.2
Dinoseb	239	27:25	$Y = 1.10^{E}7 \times - 350589$	0.9986	10.4
Dinoterb	239	27:59	$Y = 2.10^{E}7 \times -175735$	0.9996	10.8

Mass Spectrometry: Unlike diode-array UV detection, mass spectrometry allows unambiguous identification of all the compounds, even in case of co-elution, by isolating the signal acquired in total ionic current on a specific ion for each substance, here the molecular ion depleted of one or two protons $[M-H]^-$ or $[M-2H]^{2-}$ depending on the solute. It is also possible to work directly on this specific ion by acquisition in SIM mode, which was adopted here.

Linearity: The correlation factors of the different calibration curves ranged from 0.991 to 0.9996 with lows often less than 50 µg/l, and even close to 10 µg/l for some compounds (Dichlorprop, Dinoterb, Dinoseb, 2,4,5-T, Bromoxynil) (see Table II).

Application to real matrices: After having assessed the performance of MS detection on standard solutions, we integrated the solid-phase extraction step into the method, and determined the recoveries in the different matrices. To do this, spikings were carried out at two concentration levels on public supply water and river water (see Tables IIIa and IIIb). The recovery rates obtained were satisfactory because better than 70% in most cases, for both drinking water and river waters.

TABLES IIIA and IIIB Acid pesticides recoveries in two water matrices after solid-phase extraction of 0.5 litre on lg C18 cartridge followed by LC-ESI-MS in NI mode analysis. The two matrices studied were: drinking water and surface water (3 replicates in each case). Injected volume: 20 µl -: undetermined value

Drinking water				
Concentration	*20 µg/l*		*4 µg/l*	
Substances	*Recovery (%)*	*RSD (%)*	*Recovery (%)*	*RSD (%)*
Fluoroxypyr	81	5	91	1
Bentazone	65	5	76	24
Bromoxynil	97	4	114	1
Dichlorprop	81	5	69	5
2,4,5-T	66	1	69	1
Dinoseb	72	1	73	8
Dinoterb	99	4	110	6
MCPA	68	7	74	1
2,4-D	94	2	119	6
Ioxynil	-	-	-	-

Surface water				
Concentration	*20 µg/l*		*4 µg/l*	
Substances	*Recovery (%)*	*RSD (%)*	*Recovery (%)*	*RSD (%)*
Fluoroxypyr	78	3	67	2
Bentazone	84	4	73	4
Bromoxynil	96	3	88	9
Dichlorprop	88	1	93	8
2,4,5-T	61	2	69	6
Dinoseb	78	3	84	17
Dinoterb	98	2	83	23
MCPA	74	3	78	4
2,4-D	106	1	100	8
Ioxynil	-	-	-	-

Reversed phase chromatographic separation at acidic pH

The calibration curves plotted revealed very good linearity of the method for most of the compounds over a broad concentration range (0.05 to 5 µg/ml, except for Dinoterb, Dinoseb and Bromoxynil, for which the range was limited to a maximum of µg/ml), with correlation factors close to 0.9999 for all the substances (see Table IV). The quantitation limits were evaluated from the chromatograms by calculating, for each substance, the concentration equivalent to a signal-to-noise ratio of 10. They ranged from 5 to 250 µg/l depending on the compound in the injected solutions, and less than 100 µg/l in most cases. Taking into account the preconcentration step, this method allows the determination of acid pesticides in concentrations from a few ng/l to a few tens of ng/l, and is in perfect agreement with the requirements of the European legislation.[23]

Multi-residue analysis by LC-MS: Application of LC-APCI-MS in PI mode and LC-ESI-MS in NI mode to real matrices

The LC-MS methods described above were used in connection with two studies, one on some 20 groundwaters on which analyses were performed by LC-APCI-MS in PI mode and by LC-ESI-MS in NI mode at acidic pH according to 2.2, and the second on some 40 surface waters on which analyses were per-

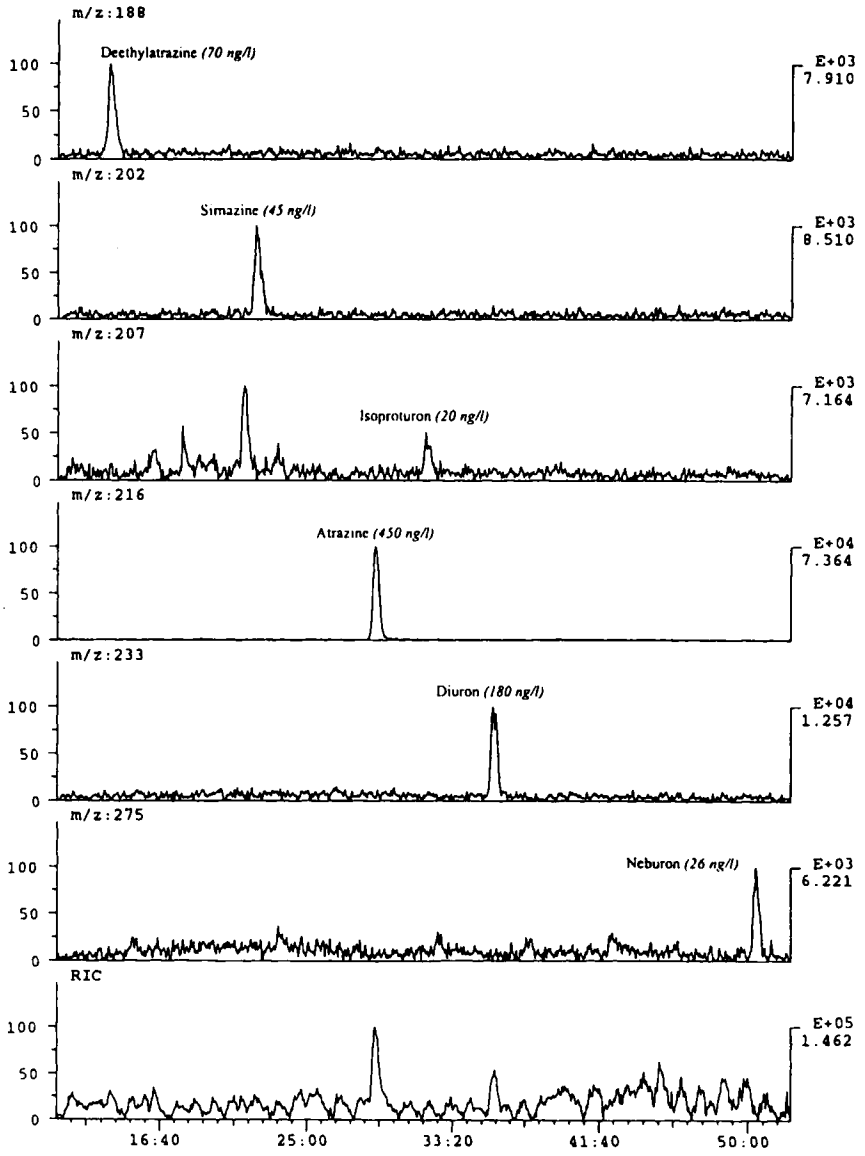

FIGURE 3 An example ofa neutral pesticides analysis in a surface water sample by LC-APCI-MS in PI mode after liquid-liquid extraction with methylene chloride. Injected volume: 20 µl

formed by LC-APCI-MS in PI mode (see Figure 3). As to the results obtained by LC-MS with atmospheric interfaces, a comparison with compound concentrations determined by LC-UV-DAD using the ISO11369 method on real samples

and showing no interferences in their UV spectra reveals excellent correlation. In the case of Diuron, the slope of the equation linking the concentrations in ng/l obtained by LC-APCI-MS with those obtained in ng/l by LC-UV-DAD using the reference ISO 11 369 method is 1.0187 ($R^2 = 0.97$). This comparison, extended to the other compounds detected in the samples with an high purity UV spectrum, allowed validation of the results obtained in LC-API-MS using a standardized method. An illustration of these results is given in Figure 4 for Diuron over some 30 measurements on real samples. In case of coelution and in the presence of many interfering compounds which can hinder the detection of pesticides, the gain in information as compared with diode-array UV detection was crucial, because it is possible to identify unambiguously all the molecules found.

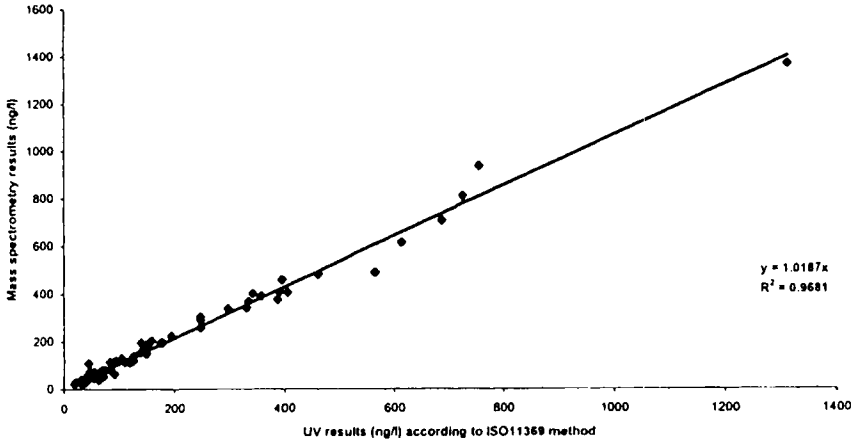

FIGURE 4 Validation of results obtained by LC-APCI-MS in PI mode: LC-APCI-MS results (y axis) versus LC-UV-DAD results according to ISO 11369 method (over 30 surface samples, Example of Diuron

The main substances detected on surface waters, and identified by their mass spectrum with the APCI interface, are Deethylatrazine, Deisopropylatrazine, Atrazine, Simazine, Terbuthylazine, Diuron, Isoproturon, Tebuconazole, Penconazole and Fenpropimorph. The concentration interval measured ranged from a few ng/l to a few µg/l, particularly for Diuron and Atrazine. An example of the chromatogram extracted on the specific ions of the compounds shows the signal-to-noise ratio obtained on various substances (see Figure 5).

The main substances detected on groundwaters by LC-APCI-MS in PI mode and Bentazone by LC-ESI-MS in NI mode, according to method 2.2. (reverse phase chromatographic separation at acidic pH), were Deethylatrazine, Deisopropylatrazine, Atrazine, Simazine, Terbuthylazine and Metolachlor. The chro-

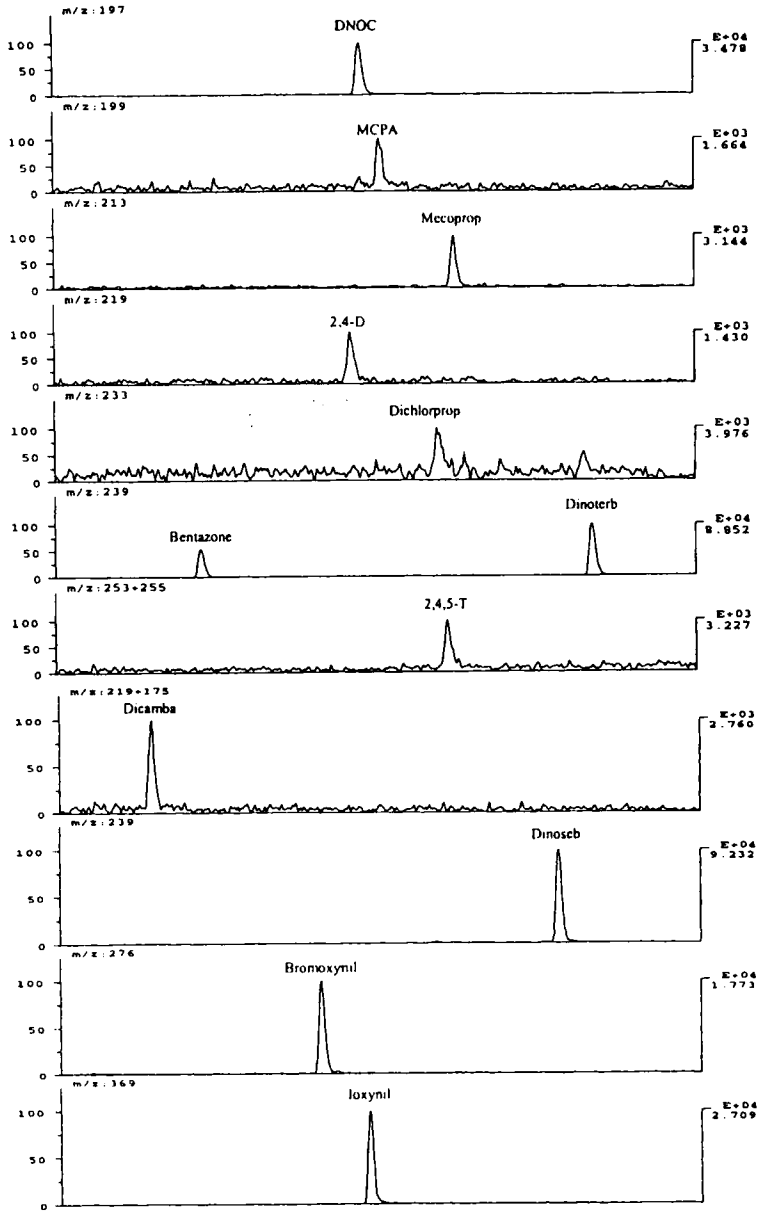

FIGURE 5 Determination of quantitation limits (according to signal-to-noise ratio. S/N ≥ 10) – Chromatograms obtained by LC-ESI-MS in NI mode with an acidic pesticides mixture (0.1 µg/ml for each compound in methanol) (acquisition in TIC mode with ion extraction). Injected volume: 40 µl

matograms obtained for a sample on the specific ions of the substances detected with their concentrations and some of their mass spectra are shown in Figures 6a to 6c. For this sample, the repeatability of the measurements was confirmed throughout the analytical procedure. The concentrations determined on two tests were Bentazone (m/z: 239) 141 and 135 ng/l, Deethylatrazine (m/z: 188) 133 and 156 ng/l, Deisopropylatrazine (m/z: 174) 38 and 46 ng/l, Simazine (m/z: 202) 37 and 46 ng/l, and Atrazine (m/z: 216) 149 and 159 ng/l. Repeatability was less than 5% for values above 100 ng/l and less than 10% for values between 30 and 50 ng/l. For all the samples, Deethylatrazine, a degradation product of Atrazine, was present in concentrations close to or higher than that of Atrazine.

For a number of neutral pesticides analysable by LC-APCI-MS in PI mode, including triazines, phenylureas and metolachlor the present method shows equivalent performances to others recently published although, using small volumes of water samples (4 to 10 ml) with short analysis times (15 min).

The analysis of neutral substances in waters from different sources by APCI in PI mode includes an SPE-GC-MS on-line method[24–25] using an on-column interface applied to the screening of various organic pollutants, including triazines and metolachlor. This method allowed the identification and quantitation of these pollutants in concentrations ranging from 0.01 to 0.1 µg/l, using volumes of 10 ml per sample. Fourteen phenylureas and triazines, including 10 common to the present paper, were also subjected to quantitative analyses on real samples using SPE on-line with APCI-MS-MS[12]. For water volumes of 4 ml, the quantitation limits obtained ranged from 0.01 to 0. 1 µg/l with analysis times of 15 min.

CONCLUSIONS

The work presented here shows that LC-MS coupling using atmospheric pressure interfaces is suitable technique for the multi-residue analysis of pesticides. The APCI interface, used in positive mode, allows the analysis of neutral substances, while acidic substances are ionized by electrospray in the negative mode.

Two chromatographic methods were compared for acidic substances. Ion pairing chromatography yielded excellent results, but with some limitations (Bentazone, Fluoroxypyr, Bromoxynil and Ioxynil). It also required considerable maintenance due to the presence of salts in the mobile phase. Conversely, chromatography at acid pH, in the presence of acetic acid, helped remove all the components of the mobile phase before entering the analyzer, and was less aggressive to the experimental rig.

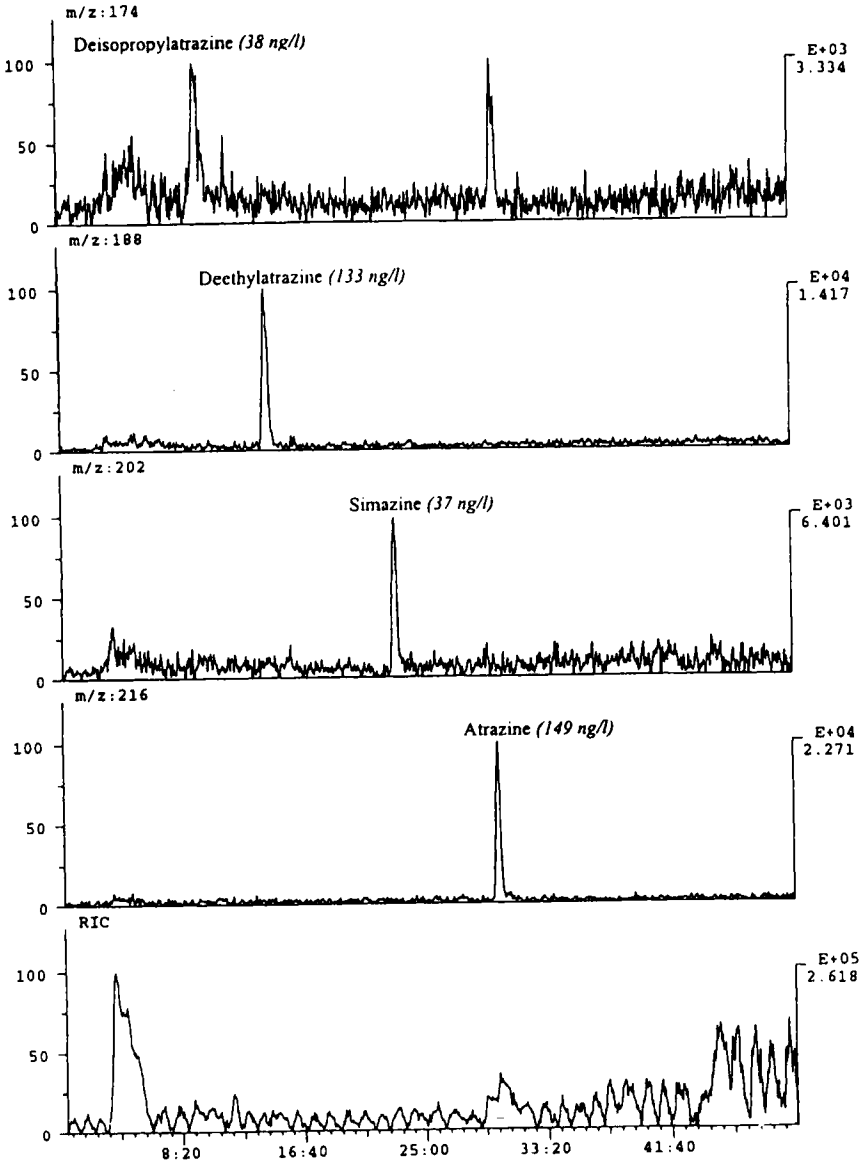

FIGURE 6A An example of neutral pesticides analysis in a groundwater sample by LC-APCI-MS in PI mode (after preconcentration by solid-phase extraction on C18 sorbent). Injected volume: 20 µl

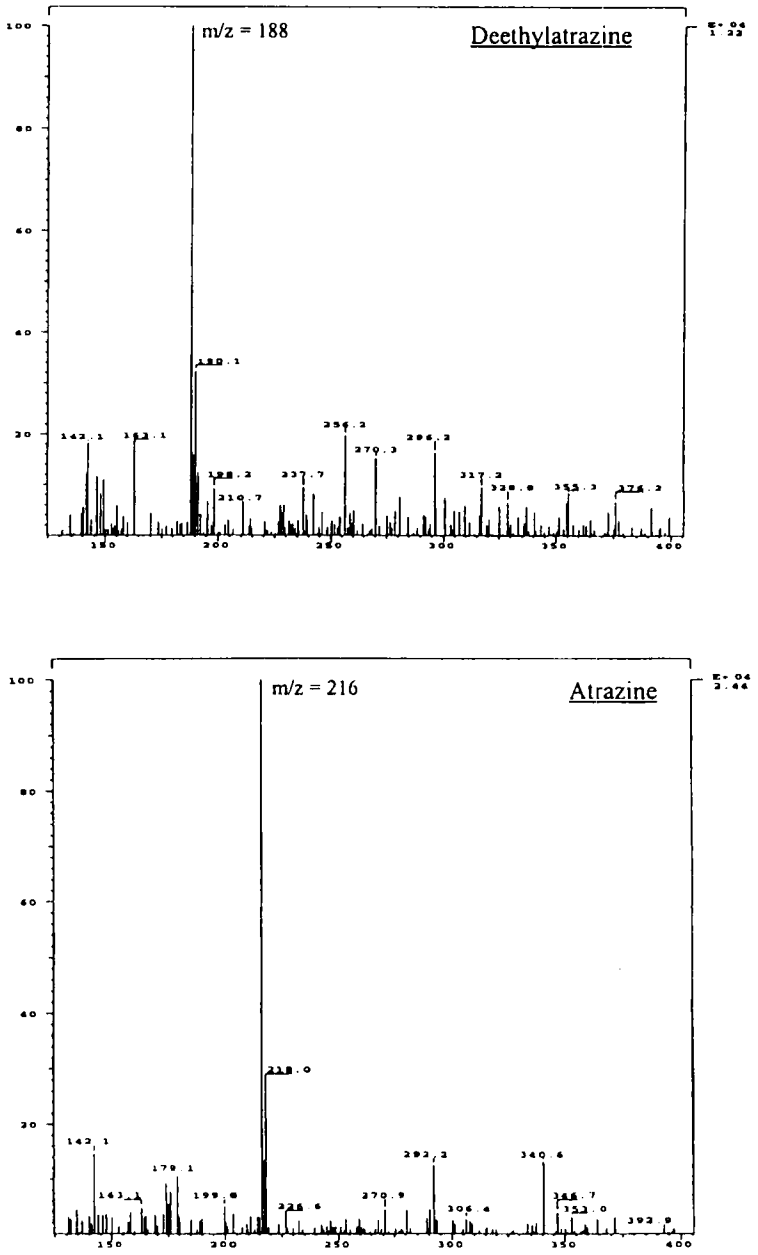

FIGURE 6B Some mass spectra corresponding to compounds detected in a groundwater sample analysis by LC-APCI-MS in PI mode (see figure 6a). Injected volume: 20 µl

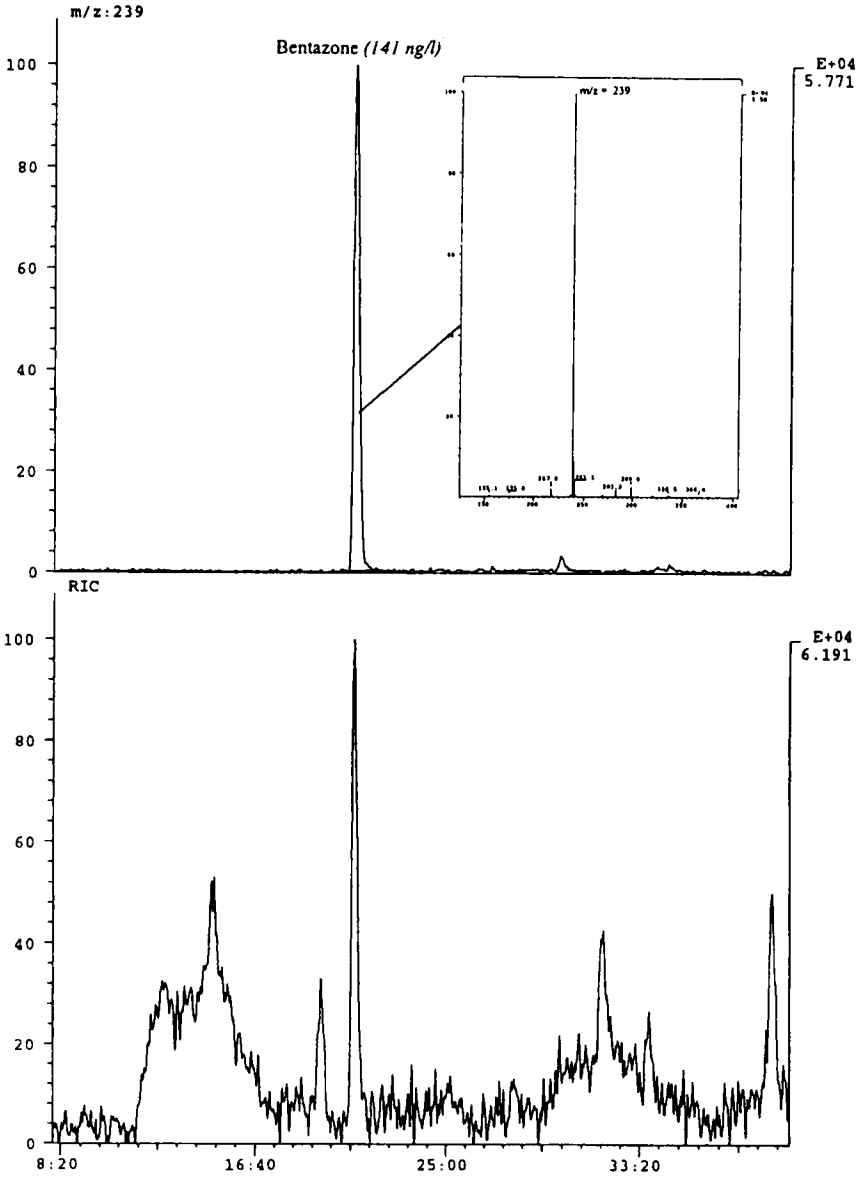

FIGURE 6C An example of acid pesticides analysis (with corresponding mass spectrum) in a groundwater sample by LC-ESI-MS in NI mode, after preconcentration by solid-phase extraction on C18 sorbent. Injected volume: 40 µl

TABLE IV Calibration data obtained on acid pesticides mixtures (concentration range: about 0.05 to 10 µg/ml in methanol) using LC-ESI-MS in NI mode. Signals were acquired in TIC mode combined with ion chromatogram extraction at different m/z values. Injected volume: 40 µl

Substances	*m/z used for quantitation*	*Tr (min)*	*Linear regression*	*Correlation coefficient*	*Practical limit of quantitation* (µg/l) (for S/N ≥ 10)*
Dicamba	219+175	19.30	Y=373.1x – 5160,2	0.9983	70
Fluoroxypyr	253+255	20.40	Y= 168.18x- 157.55	0.9982	150
Bentazone	239	20.56	Y=5695.2x + 54446	0.9954	10
Bromoxynil	276	25.40	Y=1852.6x + 100406	0.9938	15
2,4-D	219	26.02	Y=236.1x + 2344.6	0.9996	70
DNOC	197	26.22	Y=5648.1x – 23479	0.9971	10
MCPA	199	27.03	Y=314.2x + 4487.3	0.9995	100
Ioxynil	369	27.27	Y=3515x + 60696	0.9900	5
Dichlorprop	233	29.03	Y=347.8x + 36877	0.9988	250
2,4,5-T	253+255	29.15	Y=292.1x + 14331	0.9994	80
Mecoprop	213	29.37	Y=474.5x + 11024	0.9948	50
Dinoseb	239	34.11	Y=8693.3x + 296688	0.9901	5
Dinoterb	239	34.41	Y=8180.9x + 473096	0.9943	5

* quantitation limits in injected extract.

In both cases, the gain in information as compared with diode-array UV detection was crucial, because it is now possible to identify unambiguously all the molecules found, even in case of co-elution. The use of SIM mode for acquisition in mass spectrometry further enhances the sensitivity of the method.

Prospects for LC-MS coupling as a method of analysis and identification have advanced further thanks to coupling with the MS-MS tandem[12]. This type of coupling provides complete information, allowing the identification of unknown substances through the structural data supplied by the fragmentation of the molecular ions.

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