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Solid-phase extraction followed by high-performance liquid chromatography–ionspray interface–mass spectrometry for monitoring of herbicides in environmental water

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Abstract

In this work we developed a sensitive and specific multiresidue method, based on reversed-phase liquid chromatography–mass spectrometry, with an ionspray interface (LC–ISI-MS), for determining 52 of most representative compounds of herbicides in water samples. The procedure used involved passing 0.5 l of surface water, 2 l of ground water and 4 l of drinking water samples, respectively, through a 0.5 g graphitized carbon black (GCB) extraction cartridge. Base–neutral and acid herbicides were differential eluted from GCB cartridge and follow analyzed by HPLC–ISI-MS apparatus. A conventional 4.6-mm-ID reversed-phase LC C_{18} column, operating with a mobile phase flow-rate of 1 ml/min, was used to chromatograph the analytes. A flow of 100 μl/min of the column effluent was diverted to the ISI source. The study demonstrates the sensitivity of the technique, with detection limit under 10 ng/l in drinking water samples. Performance data for the method such as recovery and precision are also reported.

Keywords: Herbicides; Water samples

1. Introduction

Considering the global consumption of herbicides (about 3 million metric tons per year) [1] it is not surprising that many of these compounds have been detected in natural waters and, therefore, have raised considerable concern both from a human health and from an environmental point of view. In order to assess possible impacts of herbicides on aquatic ecosystems and drinking water supplies, analytical methods for the routine simultaneous determination of a large number of such compounds at trace concentrations in water samples are required.

Although capillary column gas chromatography (GC) remains the major determinative techniques of organic compounds present in water [2–4], the number of publication describing reversed-phase high-performance liquid chromatography (HPLC) coupled to both off- and on-line Solid-Phase Extraction (SPE) is steadily increased over the last years [5–11].

However, because of the legal implication of many environmental data, coupling HPLC with mass spectrometry (MS) by a atmospheric pressure ionization (API) interface is a key element for the future of HPLC procedures.

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In a recent paper [12] we have developed a multiresidue method for monitoring 15 post-emergence herbicides in drinking water, at few ng/l level, below the 100 ng/l limit needed for pesticide compliance with the Commission of the European Communities–Drinking Water Directive (CEC–DWD). Base–neutral and acid herbicides, were simultaneously extracted by a Carbograph 4 cartridge from water samples without any sample pretreatment.

The aim of the present work was to evaluate the potentiality of coupling our sample preparation procedure with the high confirmational power of the LC–ISI-MS system in order to unambiguously monitor selected herbicides in environmental water samples.

Passing sequentially through the cartridge two suitable solvent systems, isolation of acidic pesticides from non acidic ones was successfully achieved by differential elution [13–15]. Class fractionation was made possible because the GCB surface is contaminated by some positively charged adsorption sites that enable this material to behave as both a nonspecific sorbent and an anion-exchanger [16].

In this way, extraction, preconcentration and class fractionation of analytes can be performed with a single cartridge. Neutral and acid fraction was analyzed by HPLC–MS equipped with TurboIonspray interface, a new high flow IonSpray Interface (ISI) developed by Sciex.

Among the enormous number of existing herbicides, 52 herbicides (from twelve different chemical class) were considered in this study. This selection was made with the criteria of including many of those pesticides that are widely used in both European [17] and American countries (Table 1).

2. Experimental

2.1. Reagents and chemicals

All herbicides were purchased from LabService (Bologna, Italy) and were listed in Table 1.

Individual standard solutions were prepared by dissolving 10 mg of each in 10 ml of acetonitrile.

Composite working standard solutions were prepared by mixing 0.1 ml of each standard solutions and diluting to 50 ml with acetonitrile (2 ng/μl). When unused, all standard solutions were stored at 4°C.

For HPLC, distilled water was further purified by passing it through the Milli-Q RG apparatus (Millipore, Bedford, MA, USA). Acetonitrile ''plus'' and methanol ''plus'' of LC gradient grade were from Carlo Erba (Milan, Italy). Formic acid was purchased from Merk (Darmstadt, Germany). All other solvents were of reagent grade (Carlo Erba) and were used as received.

LiChrolut EN and ENVI-Chrom P are two PS-DVB materials. LiChrolut EN has a surface area of about 1200 m^2/g and a particle size range between 40 and 120 μm, and was kindly supplied by Merck (Darmstadt, Germany). Envi-Chrom P has a surface area of about 900 m^2/g, a particle size range between 80 and 160 μm, and was supplied by Supelco (Bellefonte, PA, USA). Humic acids was supplied by Aldrich Chemical (Milwaukee, WI, USA).

2.2. Apparatus

Extraction cartridges were filled with 0.5 g of Carbograph 4 (120–400 mesh size, Carbochimica Romana, Rome, Italy), while the other materials for preparing extraction cartridges were from Supelco, Bellefonte, PA. The preparation and pretreatment of the reversible extraction cartridge were carried out as previously reported [18].

The trap was fitted into a side-arm filtering flask, and liquids were forced to pass through the cartridge by vacuum from a water pump.

Those containing LiChrolut EN and ENVI-Chrom P were pretreated with 10 ml of a methanol–acetonitrile (50:50, v/v) mixture. Introducing the sample from a reservoir, to ensure that the SPE packing does not dry between conditioning and sample, 2 ml of conditioning mixture were added above the top of the frit.

2.3. Sampling

Grabbed samples of a surface water and ground waters (various sources near Rome) were collected in brown bottles and kept at 4°C in the dark until

Table 1
Time-scheduled SIM condition for monitoring selected herbicides in water samples

		Class	Channel mass (m/z)	Retention windows (min)
Base–neutral analytes				
1	Cyanazine	Triazine	104+214+241	0–11.0
2	Imazamethabenz methyl	Imidazolinone	229+257+289	
3	Simazine	Triazine	68+104+202	
4	Metribuzine	Triazine	185+215	
5	Sethoxydim	Cicloexanedione	282+328	
6	Atrazine	Triazine	68+174+216	11.0–15.0
7	Isoproturon	Phenilureas	46+72+207	
8	Diuron	Phenilureas	46+72+233	
9	Propazine	Triazine	146+188+230	
10	Linuron	Phenilureas	160+182+249	15.0–19.0
11	Ametrine	Triazine	104+174+230	
12	Terbutilazine	Triazine	71+186+228	
13	Alachlor	Acetanilide	162+238+270	19.0–30.0
14	Metolachlor	Acetanilide	176+251+289	
15	Terbutrine	Triazine	71+186+242	
16	Fluazifop butyl	APPEs	254+281+383	
17	Oxyfluorfen	Diphenilether	317+362+379	
18	Fluorglycofen ethyl	Diphenilether	317+447+464	
19	Haloxyfop ethyl	APPEs	289+317+391	
20	Fenoxaprop ethyl	APPEs	259+289+363	
21	Lactofen	Diphenilether	318+462+479	
22	Quizalofop ethyl	APPEs	269+299+373	
23	Diclofop methyl	APPEs	255+283+343	
Acid analytes				
24	Imazapyr	Imidazolinone	177+262	0–18.0
25	Imazethapyr	Imidazolinone	246+288	
26	Imazamethabenz	Imidazolinone	229+275	
27	Triasulfuron	Sulphonylurea	139+400	
28	Imazaquin	Imidazolinone	265+310	
29	Dicamba	Carboxilic acid	176+220	
30	Metsulfuron	Sulphonylurea	139+380	
31	Bentazone	Tiadiazina	197+239	
32	Clorsulfuron	Sulphonylurea	139+356	
33	Rimsulfuron	Sulphonylurea	179+430	
34	Bromoxinil	Benzonitrile	81+276	18.0–25.0
35	Tribenuron	Sulphonylurea	153+394	
36	2,4 D	Phenoxy acid	161+220	
37	MCPA	Phenoxy acid	141+199	
38	Ioxinil	Benzonitrile	127+370	
39	Bensulfuron	Sulphonylurea	254+409	
40	Chlorimuron	Sulphonylurea	157+412	
41	Primisulfuron	Sulphonylurea	256+467	
42	Fluazifop	APPAs	253+325	
43	Flamprop	APPAs	248+320	
44	Diclorprop	Phenoxy acid	161+233	
45	Mecoprop	Phenoxy acid	141+213	
46	Acifluorfen	Diphenilether	314+360	
47	2,4 DB	Phenoxy acid	161+247	25.0–30.0
48	MCPB	Phenoxy acid	141+229	
49	Fenoxaprop	APPAs	260+332	
50	Quizalofop	APPAs	270+342	
51	Haloxyfop	APPAs	288+360	
52	Diclofop	APPAs	254+326	

analysis. Unless they contained large amount of suspended sediments, water samples were extracted unfiltered. When necessary, Whatman GF/C glass-fiber pads (pore size 10 μm) were used. Rome municipal drinking water samples were taken after the water had been kept flowing for 30 min.

2.4. Procedure

2.4.1. With GCB cartridges

For recovery studies, aqueous samples were fortified with known amounts of the composite standard solution. Water samples were then agitated firmly for about 1 min and poured in a glass reservoir connected to the sorbent cartridge. Water was forced to pass through the cartridge at flow-rates of ca. 100 ml/min by reducing the pressure in the vacuum apparatus to the minimum. Alter the sample was passed through the column, the pump was disconnected, and the cartridge was filled with 7 ml of distilled water, which was allowed to pass through the cartridge at flow-rates of 5–7 ml/min. Most water was removed from the cartridge by forcing room air through it for 1 min. and 1 ml of methanol was poured into the cartridge, which was slowly passed through the sorbent bed to eliminate part of the residual water. Following the passage of methanol, the pressure was reduced to the minimum for 1 min.

Thereafter, a suitably drilled cylindrical teflon piston with one conically indented base and a Luer tip was forced to enter the cartridge until it reached the upper frit. The trap was turned upside down, a 1.4 cm ID glass vial with a conical bottom was placed below the trap.

Base–neutral herbicides were eluted by passing through the cartridge 2 ml of methanol followed by 8 ml of a methylene chloride–methanol (80:20, v/v) solution, at a flow-rate of ca. 8 ml/min obtained by suitably regulating the vacuum. Acid herbicides were eluted by passing through the trap 8 ml of a methylene chloride–methanol (80:20, v/v) solution acidified with formic acid, 50 mmol/l. The flow-rate at the which the eluent phase was percolated through the cartridge was about 6 ml/min. The last drops of this mixture were collected by a further decrease of the pressure inside the flask.

To the acid fraction was partial neutralized with 50 μl of concentrated ammonia and was dried in water bath at 40°C under a gentle nitrogen stream. The residue was reconstituted with 200 μl of a water–methanol solution (50:50, v/v). 50 μl of the final extract was injected into the LC column. If NH_3 was not added to the solvent mixture, severe losses of the APPAs acids [19] and some SUs were observed on drying the extract. The neutral fraction was concentrated down to about 200 μl in a water bath at 30°C under a nitrogen stream to remove the solvent. In these conditions, no trace of methylene chloride was present in the final extract [20]. The final extract was measured using a 500 μl syringe, and 50 μl were injected into the HPLC apparatus.

2.4.2. With LiChrolut EN and ENVI-Chrom P cartridges

With both materials, after the passage of the water sample, the last part of a previously reported procedure involving the use of a LiChrolut EN cartridge was appropriately modified to shorten the analysis time. After partial water removal by air-drying cartridges for 5 min, analytes were eluted from both LiChrolut EN and ENVI-Chrom cartridges by 2×5 ml of a methanol–acetonitrile (50:50, v/v) mixture. To save time, eluates were concentrated down to about 0.5 ml by the procedure reported above. After measuring the exact volumes of partially concentrated extracts, suitable amounts were injected into the LC apparatus.

2.5. HPLC–ISI-MS analysis

Liquid chromatography was carried out with a Perkin-Elmer series 200 binary pump (Perkin-Elmer, Norwalk, CT) equipped with Rheodyne 7125 injector having a 50 μl loop and with Perkin-Elmer Series 200 Vacuum Degasser. The analytes were chromatographed on an Alltima 25 cm×4.6 mm ID column filled with 5 μm C-18 reversed-phase packing (Alltech, Deerfield, IL). Electrospray mass spectrometry was performed on a Perkin-Elmer/Sciex API *I* single-stage quadruple instrument equipped with an TurboIonspray interface (Sciex. Thornton, Canada).

2.5.1. Neutral fraction analysis

For separating neutral analytes, phase A was methanol and phase B was water. Both solvents

contained 1 mmol/l CH_3COONH_4 (pH 7.5). Gradient elution was performed by linearly increasing the percentage of the organic modifier from 60 to 90% in 25 min. The flow-rate of mobile phase was 1 ml/min. A 100 μl portion of column effluent was diverted to ISI source. The mass spectrometer was operated in positive ion mode by applying to the capillary a voltage of 5000 V. The orifice voltage was set at 90 V and the interface temperature at 62°C. Nitrogen was used as curtain gas with a flow-rate of 1.1 l/min and as nebulizer gas with a pressure setting of 46 p.s.i. Mass spectra collected in full-scan mode were obtained by scanning over the range 45–500 *m/z* in 2 s.

2.5.2. Acid fraction analysis

For separating acid analytes, in neutral and acid fraction, phase A was acetonitrile and phase B was water. Both solvents contained 25 mmol/l HCOOH.

Gradient elution was performed by linearly increasing the percentage of the organic modifier from 40 to 62% in 25 min and to 90% in 5 min. The flow-rate of mobile phase was 1 ml/min. A 100 μl portion of column effluent was diverted to IS source.

Postcolumn addition (after splitting) of 10 μl/min of CH_3COONH_4 (100 mmol/l acetonitrile–water 85:15 v/v, pH 8) was carried out using a Harvard model 11 syringe pump (Harvard Apparatus, South Natick, MA). The mass spectrometer was operated in negative ion (NI) mode by applying to the capillary a voltage of 4000 V. The orifice voltage was set at 70 V and the interface temperature at 62°C. Nitrogen was used as curtain gas with a flow-rate of 1.1 l/min and air as nebulizer gas with a pressure setting of 42 p.s.i. Mass spectra collected in full-scan mode were obtained by scanning over the range 80–500 *m/z* in 2 s.

Time-scheduled, selected ion monitoring (SIM) LC–MS was performed by following the procedure reported in Table 1.

For recovery studies, the concentrations of the analytes were calculated by measuring peak areas from extracted-ion current profiles (XIC) and comparing them with those obtained from standard solutions. For any analyte, the selected XIC was that from the most abundant ion. Standard solutions were prepared by dissolving known and appropriate volumes of the working standard solution in the eluent phase used for eluting analytes from the Carbograph 4 cartridge and then following the rest of the procedure reported above. Peak area ratio for selected ions were determined using the PE Sciex package Multiview 1.3.

3. Results and discussion

3.1. Recovery studies

The ability of the Carbograph 4 extraction cartridge to retain quantitatively even very highly water soluble pesticides, was evaluated. For the herbicide considered the extraction efficiency of the Carbograph-4 cartridge was calculated by spiking 4 l of municipal drinking water, 2 l of ground water and 0.5 l of lake or river water with, respectively, 50 ng/l, 100 ng/l and 500 ng/l of each herbicides and then analyzing six times each aqueous matrices.

Unspiked water samples were used as controls.

In Fig. 1 was shown a TIC chromatogram for neutral and acid fraction obtained from 2 l of ground water. Results are shown in Table 2.

These figures were the result of averaging recovery data obtained by analyzing each water sample six times. Recovery exceeded 83% for all the analytes investigated and was unaffected by the nature of the aqueous matrix in which the analytes were dissolved.

A partial recovery of imazapyr and imazethapyr in ground and surface water can be explained with a saturation of ISI. This effect could be due to the reason that these analyte has a retention time rather low and they come elute from the chromatographic column then together to best part of the hydrophilic interference. A similar effect has been observed from other authors [21], using the ISI-MS detector, a substance, present in elevated quantity, coeluted with a analyte could interfere in their ionization process with reduction of analyte signal.

This loss were evident when a Dissolved Organic Carbon (DOC) of sample is up of 10 mg/l.

Roughly, measured average DOC contents in ground water samples were in the few milligram per liter range, and are typically around 1 mg/l in lake water.

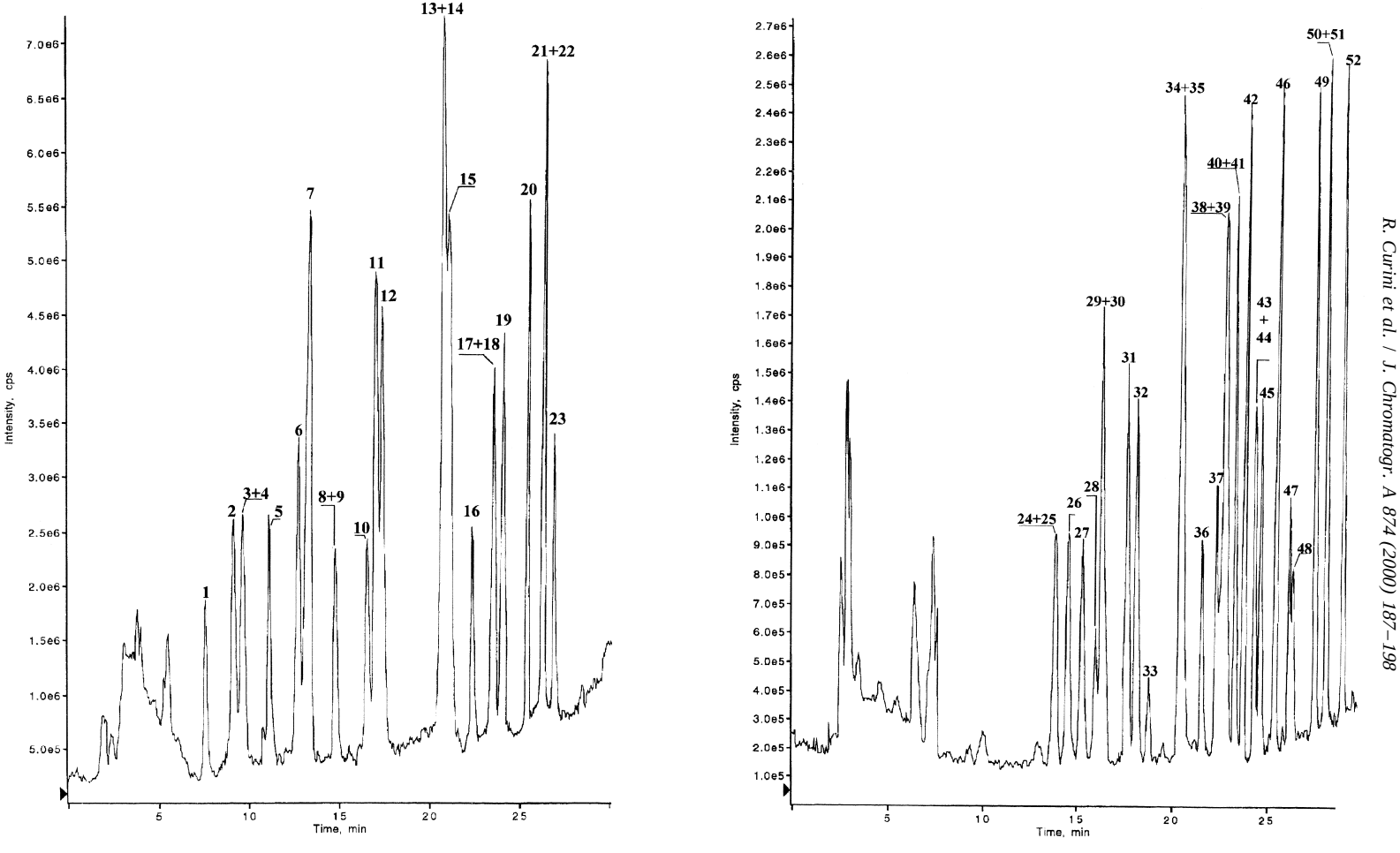

Fig. 1. TIC chromatogram for (A) neutral and (B) acid fraction obtained by injecting 50/200 of a final extract relative to 4 l of drinking water spiked with the herbicides at level of 50 ng/l each. Peak numbering is reported in Table 1.

Table 2
Recovery and relative standard deviation (RSD) of herbicides added to 4 l of drinking water and 2 l of ground water and 0.5 l of surface water

		Recovery[a] (%)±RSD		
		Drinking water 50 ng/l[b]	Ground water 100 ng/l[b]	Surface water 500 ng/l[b]
Base–neutral analytes				
1	Cyanazine	97±5	107±6	88±7
2	Imazamethabenz methyl	96±4	102±4	95±6
3	Simazine	93±5	94±7	90±8
4	Metribuzine	90±6	92±6	91±10
5	Sethoxydim	90±6	92±6	93±9
6	Atrazine	102±6	92±5	100±5
7	Isoproturon	93±5	91±5	93±6
8	Diuron	91±8	91±8	98±5
9	Propazine	92±6	94±6	89±8
10	Linuron	92±5	94±5	92±6
11	Ametrine	90±8	90±8	92±6
12	Terbutilazine	92±6	90±6	94±6
13	Alachlor	94±6	94±6	92±5
14	Metolachlor	92±5	92±5	93±4
15	Terbutrine	93±4	97±5	91±4
16	Fluazifop butyl	91±4	94±4	98±6
17	Oxyfluorfen	92±7	92±5	97±5
18	Fluorglycofen ethyl	98±4	102±5	89±5
19	Haloxyfop ethyl	103±4	93±5	99±7
20	Fenoxaprop ethyl	104±5	94±5	91±7
21	Lactofen	92±7	92±7	95±9
22	Quizalofop ethyl	108±5	110±7	91±10
23	Diclofop methyl	92±5	96±5	95±8
Acid analytes				
24	Imazapyr	90±7	88±7	83±8
25	Imazethapyr	94±7	86±6	85±6
26	Imazamethabenz	91±5	90±7	94±6
27	Triasulfuron	90±5	92±6	94±6
28	Imazaquin	91±6	93±5	97±5
29	Dicamba	100±5	98±6	108±7
30	Metsulfuron	93±5	91±5	93±6
31	Bentazone	99±4	101±4	98±5
32	Clorsulfuron	91±6	92±8	89±8
33	Rimsulfuron	93±6	94±6	92±6
34	Bromoxinil	98±5	94±6	93±6
35	Tribenuron	89±8	90±7	97±5
36	2,4 D	92±6	90±6	93±4
37	MCPA	93±6	96±6	111±4
38	Ioxinil	97±5	97±5	90±6
39	Bensulfuron	93±4	87±5	106±6
40	Chlorimuron	91±4	89±6	97±5
41	Primisulfuron	93±6	92±5	85±5
42	Fluazifop	98±4	102±5	89±7
43	Flamprop	101±4	93±5	92±5
44	Diclorprop	90±5	92±5	93±4
45	Mecoprop	92±7	92±7	91±4
46	Acifluorfen	106±5	100±5	93±6
47	2,4 DB	91±4	94±4	98±4
48	MCPB	92±7	92±5	95±5
49	Fenoxaprop	93±5	90±5	106±5
50	Quizalofop	96±6	93±6	94±4
51	Haloxyfop	99±4	102±4	92±7
52	Diclofop	93±4	97±5	97±5

[a] Mean values from six determination.
[b] Spike level for each pesticide.

3.2. Ion signal optimization

With a view to optimizing the sensitivity of the ISI-MS detector, the dependence of the ion signal intensities for analytes upon the composition of the LC mobile phase was evaluated.

3.2.1. Neutral fraction

Ion spectra for major part of base–neutral compounds displayed major peaks for MNa^+MK^+ and MH^+ ions, the latter generally being more abundant than the former ones.

We observed that, in good agreement with the results of Pleasance et al. [22], the addition of HCOOH to the LC mobile phase suppressed production of Na^+ and K^+ adduct ions.

The only exception to this behavior has represented from the diphenyl ethers.

These compounds form preferably adducts with the NH_4^+ and they show an insufficient affinity toward the H^+. From the analysis of the fragmentation spectra the relative abundance of the MH^+ don't overcome the 14% in every case.

This behavior does not represent a trouble because for obtain a good chromatographic separation of the basic compounds present in this fraction (which ametrine and alachlor) is necessary add a buffer to mobile phase. The best solution is addition from the assistant of ammonium acetate 2 m*M* (pH=7.1). In these conditions have an excellent chromatographic separation of our compounds, a good chord and also a good suppression of K^+ and Na^+ adducts.

Interesting triazines and phenilureas also in presence of ammonium buffer continues to give preferably MH^+ adducts.

3.2.2. Acid fraction

An improvement in sensitivity in the determination of acidic herbicides by HPLC–ISI-MS in the NI mode can be achieved by postcolumn addition of a base or buffer.

In our recent paper we [16] develops a method for the determination of arylphenoxypropionic acid herbicides in water based on HPLC–ISI-NI-MS with a neutralization of the formic acid mobile phase by a CH_3COONH_4 buffer added postcolumn. For this method a partial neutralization of mobile phase by a 10 μl/min. of 50 m*M* CH_3COONH_4 was utilized, because a further increase of buffer did not produce any improvement in sensitivity and because we were thus able to improve the ruggedness of the method.

Our results agree with previous work by Wang and Cole [23], who observed a loss of signal intensity for phenoplphtalein diphosphate (free acid form) when increasing ionic strength of mobile phase.

3.3. Collision induced dissociation spectra

For the analytes considered, the effect of increasing the orifice (OR) plate voltage on the extent of fragmentation of the $[MH]^+/[MNH_4]^+$ and $[M-H]^-$ ions was investigated. The ISI-MS system provides for fragment ions to be obtained by collision ion induced (in source CID) reactions [24]. With our instrumentation, molecular ion decomposition can be achieved by increasing the voltage between OR plate and skimmer cone in the desolvation chamber.

This experiment was conducted by injecting 30 ng each of the analytes from a standard solution into the LC column and separating them by following the conditions reported in the Experimental Section. At any OR plate voltage increasing from 30 to 100 V, background-subtracted spectra were taken from the average of chromatographic peaks. Relative abundance of parent and product ions were calculated by averaging six determinations.

In terms of specificity, abundant fragmentation of the $[M+H]^+$ and $[M+NH_4]^+$ ion of neutral analyte was obtained in the 70–100 V range. OR=90 V was selected as the operating condition. For ions of acid analytes a best compromise between various tendency to fragmentation of compound was obtained at OR=70 V. The relative abundance of ions, in the experimental conditions selected for some herbicides studied are reported in Table 3 (detailed data available upon request).

Interestingly, as the collision energy was increased by increasing the OR plate voltage, the production of fragment ions for the analytes considered was regularly accompanied by an enhancement of ion signal intensity. This effect may be traced to a more efficient adduct ion sampling occurring in a sample cone region.

Table 3
Effects of increasing orifice plate voltage on both signal intensities and production of fragments ions for some selected herbicides

Base–neutral analytes	Orifice plate voltage (OR)		
	60 V	80 V	100 V
Cyanazine	7499[a]	7700	8009
	104(40)214(78)**241**(100)	104(62)214(100)**241**(68)	104(80)214(100)**241**(47)
Imazamethabenz methyl	5558	6001	6151
	229(16)257(19)**289**(100)	229(37)257(22)**289**(100)	229(50)257(39)**289**(100)
Sethoxydim	5124	5754	6275
	282(35)**328**(100)	282(46)**328**(100)	282(67)**328**(100)
Atrazine	7682	8256	9752
	68(84)174(100)**216**(74)	68(100)174(75)**216**(66)	68(100)174(71)**216**(41)
Diuron	6935	7521	6935
	46(78)72(100)**233**(75)	46(65)72(100)**233**(66)	46(58)72(100)**233**(29)
Linuron	5847	6582	7025
	160(96)182(100)**249**(70)	160(100)182(95)**249**(36)	160(87)182(100)**249**(20)
Ametrine	6845	7220	7314
	104(38)174(75)**230**(100)	104(57)174(98)**230**(100)	104(77)174(100)**230**(71)
Alachlor	5984	6254	6541
	162(–)238(15)**270**(100)	162(26)238(40)**270**(100)	162(35)238(55)**270**(100)
Metolachlor	4582	4758	4985
	176(–)251(21)**289**(100)	176(25)251(56)**289**(100)	176(35)251(64)**289**(100)
Fluazifop butyl	9520	9750	9680
	254(21)281(38)**383**(100)	254(55)281(65)**383**(100)	254(31)281(90)**383**(100)
Oxyfluorfen	3587	4012	4101
	317(35)**362**(15)379(100)	317(45)**362**(17)379(100)	317(67)**362**(15)379(100)
Lactofen	4521	4658	4789
	318(31)**462**(14)479(100)	318(54)**462**(10)479(100)	318(77)**462**(–)479(100)
Acid analytes	60 V	70 V	90 V
Imazaquin	11258	12369	13525
	265(23)**310**(100)	265(33)**310**(100	265(46)**310**(100
Dicamba	1937	2084	2037
	176(80)**220**(100)	176(100)**220**(33)	176(100)**220**(–)
Bentazone	3684	3980	5480
	197(–)**239**(100)	197(20)**239**(100)	197(70)**239**(100)
Clorsulfuron	2554	4058	4807
	139(40)**356**(100)	139(61)**356**(100)	139(89)**356**(100)
Bromoxinil	4988	4988	4988
	81(–)**276**(100)	81(–)**276**(100)	81(–)**276**(100)
Tribenuron	3244	3784	4258
	153(81)**394**(100)	153(100)**394**(100)	153(100)**394**(50)
2,4 D	984	1550	2554
	161(75)**220**(100)	161(90)**220**(100)	161(100)**220**(35)
Acifluorfen	5514	5745	5874
	314(52)**360**(100)	314(78)**360**(100)	314(100)**360**(85)
2,4 DB	333	950	1200
	161(100)**247**(45)	161(100)**247**(19)	161(100)**247**(–)
Haloxyfop	8952	9312	9758
	288(15)**360**(100)	288(28)**360**(100)	288(37)**360**(100)
Diclofop	7584	7794	8121
	254(13)**326**(100)	254(25)**326**(100)	254(34)**326**(100)

[a] The signal (arbitrary units) is given in the first line. The relative abundance of ions (%) is given in second line. Protonated molecular ions are reported in bold. Fragment ions having relative abundance less than 10% were not considered. For conciseness only relative abundance of molecular ions and most intense fragment ions are reported.

3.4. Precision

The day-to-day precision of this method at very low analyte concentrations in water was estimated. A drinking water sample was spiked with the analytes at 50 ng/l levels, and analyzed five times over 8 days by according to the conditions reported in Experimental. In all cases, the reproducibility of peak areas obtained from the mass spectrometric data was found to be very good; peak areas varied by less than 10% in each case for the same amount of analyte injected. The variation of the abundance of the ion in the CID process ranged from 4 to 10%. These data show that both ion signals and the CID process are stable enough to allow reliable analysis of selected herbicides to be performed using this method.

3.5. Limits of detection (LODs)

Recent legislation enacted in many European countries (members of the European Community EC) states that pesticides must not exceed the 100 ng/l level in waters intended for human consumption. In order to judge with sufficient confidence whether a water sample is in compliance with this EC Directive, analytical methods able to detect pesticides at 20–30 ng/l levels are needed.

Under two different acquisition modes and based on the peak to peak noise measured on the base line close to the analyte peak, LODs ($S/N = 3$) were calculated for the selected analytes by eluting them as reported in the Experimental Section and measuring peak heights against average background noise. When operating in the full-scan mode, LODs were estimated from the TIC chromatogram referring to analysis of 4 l of a drinking water sample spiked with the analytes at the individual level of 25 ng/l and ranged between 3 and 10 ng/l.

The limits of quantification (LOQs, defined as 3 times the limits of detection) by full-scan LC–MS-CID analysis for the compounds of interest in drinking water ranged from about 5 to 30 ng/l. For ground water, the above limits have to be increased by a factor 2.

Acquisition in time-scheduled SIM mode affords the highest sensitivity. Related LODs were calculated in the same way as reported above and in all cases are less than 3 ng/l.

This results shows that this method has the potential for analyzing pesticides present in drinking water at a few nanograms per liter in the selected ion-monitoring acquisition mode.

3.6. Linear dynamic range

The linear dynamic range of the ISI-MS detector for selected herbicides was estimated under the conditions reported in the Experimental. This set of measurements was performed by injecting into the LC column different known amounts of analytes (5, 10, 50, 100, 150, 200, 250 ng for each herbicide). For each amount injected, measurements were made in triplicate. The average peak areas of each set of injections were plotted against the amount injected, and the resulting plot indicate that a good linear response can be obtained from 5 to 250 ng for all analytes.

3.7. Comparison with new PS-DVB-type sorbents

The abilities of Carbographs 4 adsorbing media in extracting very polar compounds from the two aqueous matrices taken as models, drinking water and Aldrich humic acid-spiked drinking water, were compared with those of two recently introduced PS-DVB sorbent material commercially referred to as LiChrolut EN and ENVI-Chrom P. For the purpose of comparison, very polar model compounds to be extracted from the two types of aqueous matrices considered were selected from different classes of pesticides.

For comparative purposes, we selected 9 high- and medium-polar pesticides, some acidic in nature. This selection was made with the purpose of simulating a multi-component analysis of pesticides in aqueous samples involving SPE cartridges. When extracting with cartridges filled with both Carbograph 4 and 5 materials, recovery experiments were conducted by spiking 4 l of drinking water and 1 l of the simulated river water sample (DOC of sample is 1 mg/l) with the pesticides at the respective concentration levels of 0.25 and 2 μg/l (see Table 4).

The necessity to acidify the sample in order to be able to keep acid pesticides on polymeric surface,

Table 4
Recovery (%) of selected pesticides with their adsorbent media at two spiking levels[a]

Analyte	Sorbent material					
	ENVI-Chrom P		LiChrolut EN		Carbograph 4	
	0.25 μg/l	2 μg/l	0.25 μg/l	2 μg/l	0.25 μg/l	2 μg/l
Simazine	80	72	85	82	88	93
Sethoxydim	96	98	98	96	99	101
Propazine	97	97	98	94	98	96
Diuron	98	97	97	98	96	96
Alachlor	97	96	97	96	95	96
Dicamba	80	80	91	90	102	93
Tribenuron	65	56	77	87	98	99
Bentazone	87	85	93	95	98	96
Quizalofop	48	55	53	55	98	96

[a] Mean recovery values obtained from three measurements.

like Imazethapyr ($pK_a = 2.2$) or Haloxyfop ($pK_a = 2.6$), leads to loss of compounds that are barely stable in an acidic environment. Especially Quizalofop and Fenoxaprop are quickly degraded by the presence of dilute mineral acid.

In this situation the capacity of Carbograph to extract acid analytes from water without any sample pretreatment is a substantial advantage over the PS-DVB-type cartridge.

4. Conclusion

In this article a trace analysis method for detecting herbicides has been developed. We have shown that the combination of a Carbograph 4 SPE cartridge with LC–ISI-MS can be advantageously used for rapid, unequivocal, and accurate determination of a large number of herbicides in aqueous matrices. As a result, the method can be used as a routine screening tool for the assessment of some of the most widely used herbicides but also have proved rugged and sensitive enough to study their fate and behavior in various water samples.

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