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Development of a method based on liquid chromatography–electrospray mass spectrometry for analyzing imidazolinone herbicides in environmental water at part-per-trillion levels

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Abstract

An evaluation was made of the feasibility of using reversed-phase liquid chromatography–mass spectrometry with an electrospray interface (LC–ESI-MS) to measure traces of imidazolinone herbicides in different natural water samples. The imidazolinones are a significant new class of low-use-rate, reduced-environmental-risk herbicides for the protection of a wide variety of agricultural commodities. The procedure used involved passing 0.5, 1, 2 l of river, ground and drinking water samples, respectively, through a 0.5 g graphitized carbon black (GCB) extraction cartridge. Analytes were eluted from the GCB surface by 8 ml of a methylene chloride–methanol (80:20, v/v) solution acidified with formic acid, 25 m*M*. Recovery was higher than 89% irrespective of the aqueous matrix in which the analytes were dissolved. A conventional 4.6 mm I.D. 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 50 μl/min of the column effluent was diverted to the ESI source. The effects of acid concentration on ESI-MS detector response in the mobile phase were investigated. The effects on the production of diagnostic fragments produced by varying the orifice plate voltage and the response of the MS detector were also evaluated. For the analyte considered, the response of the mass detector was linearly related to the amount of analyte injected between 1 and 50 ng. The limit of detection (signal-to-noise ratio=3) of the method for the pesticides considered in drinking water samples was estimated to be about 2–5 ng/l. © 1998 Elsevier Science B.V.

Keywords: Imidazolinone herbicides

1. Introduction

The imidazolinone herbicides consist of a group of four products that were all discovered and introduced by American Cyanamid. Several aspects of imidazolinone chemistry have been reported in a recent review [1]. The imidazolinones are a new class of low-use-rate, reduced-environmental-risk herbicides for the protection of a wide variety of agricultural commodities [2]. As shown in Fig. 1, the members of this class of herbicides have similar structural features centered around the imidazolinone ring and an attached aromatic system bearing a carboxylic acid moiety. Weed control in soybean, alfalfa, wheat and barley crops, and non-crop situations is the most important use of these compounds. In general, the imidazolinones display excellent activity against annual and perennial grasses and broad-leaved weeds when applied either pre- or post-emergence. They function by inhibiting acetohydroxy acid synthase, the feedback enzyme in the biosynthesis of the

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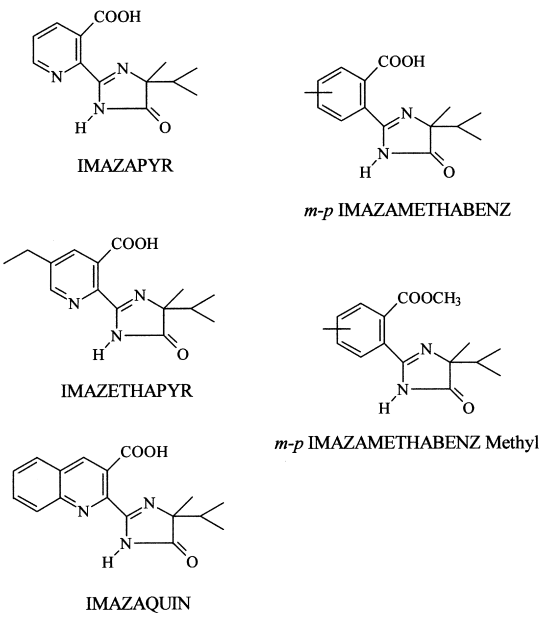

Fig. 1. Structure and common name of imidazolinone herbicides.

branched-chain essential amino acids [3,4]. This enzyme is not present in animals. The useful range of a particular imidazolinone depends upon the susceptibilities of both the selected crop and its associated spectrum of weeds. For instance, imazethapyr is a selective imidazolinone herbicide used to control a wide spectrum of broad-leaved weeds and grasses in soybean and several other leguminous crops. Imazethapyr is a uniquely flexible herbicide which can be applied pre- or post-emergence (70 g/ha). Imazapyr is also a broad-spectrum herbicide effective for weed control in forestry and highway rights-of-way when applied either pre- or post-emergence. Imazapyr at 0.56–1.68 kg/ha is used for selective weed control in forestry management and for total vegetation control in industrial sites [5]. Current analytical methods for the determination of imidazolinones in water are targeted at individual members of the group. Typically, several hundred milliliters of water are processed through a series of solid-phase extraction (SPE) cartridges and solvent-partitioning steps with final analysis by liquid chromatography (LC) with UV detection [2,6]. Alternatively, the final extract can be methylated and analyzed by gas chromatography (GC) using nitrogen–phosphorus detection [7]. Obviously, in these clean-up and concentration steps the loading and eluting of SPE cartridges and subsequent solvent removal involve an outlay in terms of both time and organic solvents. An attempt to directly determine imazapyr in water using LC–UV gave a detection limit of 10 μg/l [8]. Recently Stout et al. [9] developed a method for determining all imidazolinones simultaneously in water at a level of 1 μg/l (1 ppb), without any concentration or clean-up of the sample and based on liquid chromatography–mass spectrometry with an electrospray interface (LC–ESI-MS).

This method is simple and rapid but recent legislation enacted in many European countries (members of the European Community, EC) states that pesticides must not exceed 100 ng/l in water intended for human consumption. In order to judge with sufficient confidence whether a water sample complies with this EC Directive, analytical methods able to detect pesticides at levels of 20–30 ng/l are needed. In the recent past, Di Corcia et al. [10] developed a very sensitive and rapid LC–ESI-MS method for analyzing acid pesticides in aqueous environmental samples at the ng/l level. This method involves the use of a graphitized carbon black (GCB) reversible cartridge for extracting pesticides from water samples. 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–ESI-MS system in order to unambiguously monitor imidazolinone pesticides in drinking, ground and surface water samples at the few ng/l level. The imidazolinones, ester and acid compounds, were simultaneously extracted from water samples using a Carbograph cartridge without any sample pretreatment.

Passing sequentially through two suitable solvent systems in the cartridge, the acidic pesticides were successfully isolated from non-acidic ones by differential elution [11,12]. Class fractionation was possible because the GCB surface framework was contaminated by some positively charged adsorption sites [13] that enabled this material to behave as both a nonspecific sorbent and an anion exchanger [14]. In this way extraction, preconcentration and class fractionation of analytes could be performed using a single cartridge.

This feature of the GCB was not exploited in the present work. In order to make the method as simple as possible we gathered all our analytes, the neutral ester and the other four acid compounds into a single fraction.

2. Experimental

2.1. Reagents and chemicals

Authentic imidazolinone herbicides were purchased from LabService (Bologna, Italy). Their structures and common names are shown in Fig. 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 solution and diluting to 50 ml with acetonitrile (2 ng/μl). All standard solutions were stored at 4°C prior to use.

For LC, distilled water was further purified by passing it through the Milli-Q Plus apparatus (Millipore, Bedford, MA, USA). Methanol ''plus'' and acetonitrile ''plus'' of gradient grade were obtained from Carlo Erba (Milan, Italy). Other solvents were of analytical grade (Carlo Erba), and were used as supplied.

2.2. Apparatus

Extraction cartridges filled with 0.5 g of Carbograph 1 (120–400 mesh size, Carbochimica Romana, Rome, Italy), while the other materials for preparing extraction cartridges were from Supelco, Bellefonte, PA, USA. The preparation and pretreatment of the reversible extraction cartridge were carried out as previously reported [15]. The trap was fitted into a side-arm filtering flask, and liquids were forced to pass through the cartridge by means of a vacuum produced by a water pump.

2.3. Sampling

Grabbed samples of a river (Tiber) water and ground water were collected in brown bottles and kept at 4°C in the dark until analysed. Unless they contained suspended materials liable to plug the SPE cartridge, such as algae and debris, river water samples were extracted unfiltered (although at limited flow-rates).

2.4. Procedure

For recovery studies, aqueous samples were fortified with known amounts of the composite standard solution. Hypochlorite in drinking water samples was eliminated by the addition of 0.5 g/l $Na_2S_2O_3$ before spiking with analyte. The water samples were then stirred vigorously for about 1 min and, after a further 2 min, poured into a glass reservoir connected to the sorbent cartridge. Water was forced through the cartridge at flow-rates of ca. 100 ml/min by reducing the pressure in the vacuum apparatus to a minimum. After the sample had passed through the column, the pump was disconnected and the cartridge filled with 7 ml of distilled water, which was allowed to pass through the cartridge at flow-rates of 5–7 ml/min. Any voids created by shrinking of the sorbent bed during the passage of the sample were eliminated by pushing the upper frit against the sorbent bed. Most of the water was removed from the cartridge by forcing room air through it for 1 min. The pump was disconnected and 1 ml of methanol poured into the cartridge and allowed to pass slowly through the sorbent bed to eliminate part of the residual water. Following the passage of methanol, the pressure was reduced to a minimum for 1 min. Thereafter, a suitably drilled cylindrical PTFE piston with one conically indented base and a Luer tip was forced into the cartridge as far as the upper frit [16]. The trap was turned upside down, a 1.4 cm I.D. glass vial with a conical bottom was placed below it, and the analytes back-eluted by passing first 1 ml of methanol and then 8 ml of a methylene chloride–methanol (80:20, v/v) solution acidified with formic acid, 25 m*M*, through the trap.

The flow-rate at which the eluent phase was percolated through the cartridge was ca. 6 ml/min. The last few drops of this mixture were collected by further decreasing the pressure inside the flask. The eluate was dried in a water bath at 40°C under a gentle stream of nitrogen. The residue was reconstituted with 250 μl of a water–acetonitrile solution (75:25, v/v) acidified with 10 m*M* HCOOH and 20 μl of the final extract was injected into the LC column.

2.5. LC–ESI-MS analysis

LC was carried out using a Perkin-Elmer series 200 binary pump (Perkin-Elmer, Norwalk, CT, USA) equipped with a Rheodyne 7125 injector with a 20 μl loop and a Perkin-Elmer Series 200 Vacuum Degasser. The analytes were chromatographed on an Alltima 25 cm×4.6 mm I.D. column filled with 5 μm C_{18} reversed-phase packing (Alltech, Deerfield, IL, USA). For the purpose of fractionating the analytes, acetonitrile was selected as phase A and water as phase B. Both solvents contained 100 m*M* HCOOH. Gradient elution was performed by linearly increasing the percentage of organic modifier from 10 to 60% in 20 min. The flow-rate of the mobile phase was 1 ml/min. A 50 μl portion of column effluent was diverted to the ES source. ESI-MS was performed on a Perkin-Elmer/Sciex API I single-stage quadruple instrument equipped with an Ion-spray interface (Sciex, Thornton, Canada). 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. (1 p.s.i.= 6894.76 Pa). Mass spectra collected in full-scan mode were obtained by scanning over the range 145–330 m/z in 2.1 s. For recovery studies, the concentrations of the analytes were calculated by measuring peak areas from extracted-ion current (XIC) profiles and comparing them with those obtained from standard solutions. For any given analyte, the selected XIC was that from the most abundant ion. Standard solutions were prepared by dissolving suitable known volumes of the working standard solution in the eluent phase used for eluting analytes from the Carbograph 1 cartridge and then following the rest of the procedure described above. Peak area ratio for selected ions was determined automatically using the PE Sciex package MacQuan 1.3.

3. Results and discussion

3.1. Ion signal optimization

By LC–ESI-MS each imidazolinone generated essentially only one $[M+H]^+$ ion. The effect on the signal strength of varying proton concentration in the LC mobile phase was investigated for some selected pesticides. A total of 5 ng of each pesticide was flow injected into acetonitrile–water (50:50, v/v) mixtures containing increasing proton concentrations. For any given analyte, the signal intensity was estimated by XIC relative to both fragment and parent ions. Measurements were made in triplicate; the results are shown in Fig. 2. As can be seen, a general enhancement of the ion signal was achieved by increasing the HCOOH concentration from 1 to 25 m*M*. Further additions of HCOOH resulted in a gradual weakening of the ion signal. Our results agree with previous work by Zhou and Hamburger [17], who observed a steady increase of the $[M+H]^+$ ion signal for quercetin on increasing the formic acid concentration up to 25 m*M*.

In spite of a slight sensitivity loss caused by increasing the HCOOH concentration in the LC mobile phase from 25 to l00 m*M*, the latter concentration was preferred by us, as it ensured more reproducible spectra and signal.

3.2. Accuracy

As mentioned above, a limit of 0.1 μg/l of individual pesticides in drinking water has been set by a European Union directive [18], which means that methods used to monitor drinking water should preferably exhibit detection limits of about one tenth of the limit or less. This goal can in part be reached by using large water volumes for the analysis in order to achieve sufficiently high enrichment factors. In this respect, the ability of the Carbograph 1 extraction cartridge to retain quantitatively even very highly water soluble pesticides, such as imidazolinones (~11 g/l for imazapyr), was evaluated. This experiment was performed by passing through it 2, 1 and 0.5 l of drinking water, ground water and river water samples, respectively.

Results are shown in Table 1.

These figures were the result of averaging recovery data obtained by analyzing each water sample six times. Recovery exceeded 89% for all analytes investigated, with relative standard deviations (R.S.D.s) ranging from 4 to 7%.

For recovery data we considered a sum of *meta-*

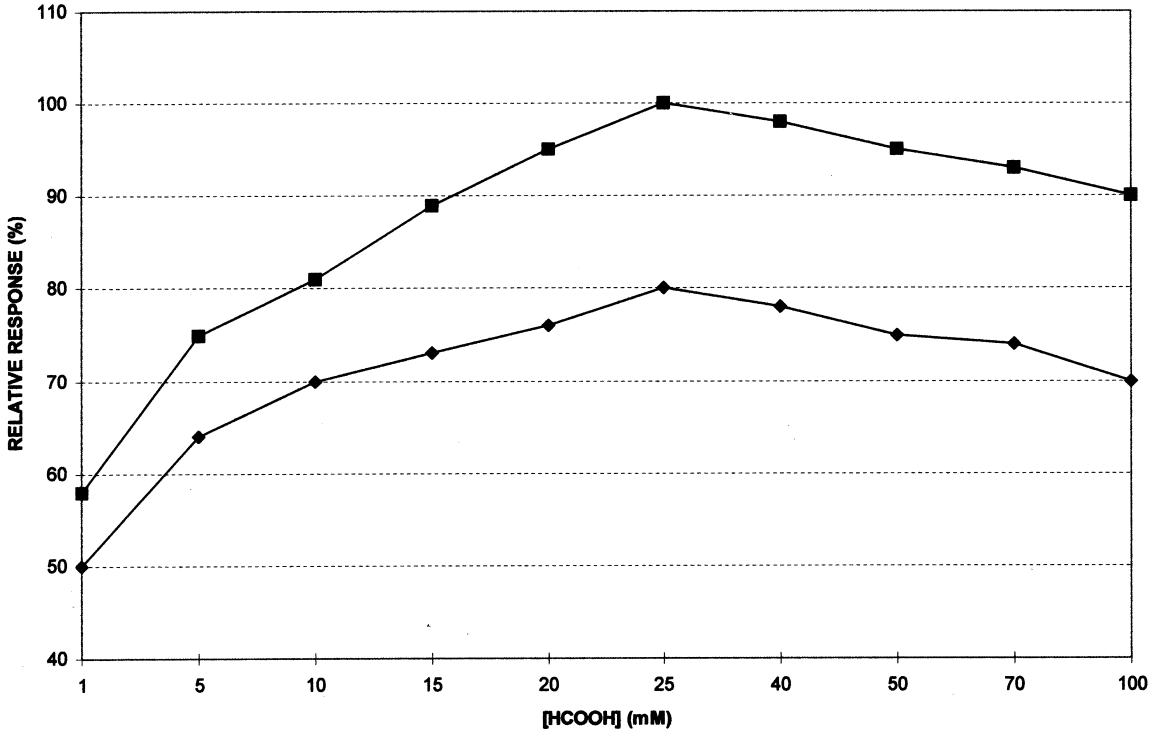

Fig. 2. Ion signal vs. the concentration of HCOOH in mobile phase. (■) Imazamethabenz methyl and (♦) imazaquin.

and *para*-imazamethabenz and imazamethabenz methyl because under the chromatographic condition selected these isomers, present at the same time in commercial formulations and in standard samples, were not separated. Conversely, the separation between imazethapyr and imazamethabenz methyl is very important because they differ by only 1 unit in molecular mass.

3.3. Specificity and sensitivity

By the LC–ESI-MS technique, the correspondence of retention time and molecular mass could provide sufficient specificity for identifying a target compound in a complex matrix, yet legal criteria for testing the presence of contaminants in various matrices usually accept spectra displaying the molec-

Table 1
Recovery and R.S.D. of imidazolinone herbicides added to 2, 1 and 0.5 l of drinking water, ground water and river water

Herbicide	Recovery[a] (%)±R.S.D.		
	Drinking water 50 ng/l[b]	Ground water 200 ng/l[b]	River water 500 ng/l[b]
Imazapyr	92±4	90±6	92±5
Imazamethabenz	93±5	96±6	89±4
Imazamethabenz methyl	94±4	95±4	94±5
Imazethapyr	95±4	95±7	96±5
Imazaquin	94±6	93±6	90±5

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

ular ion species plus characteristic ion fragments. The ESI-MS system provides for fragment ions to be obtained by collision ion induced (CID) reactions [19]. With our instrumentation, molecular ion decomposition can be achieved by increasing the voltage between interface plate and orifice plate (OR) in the desolvation chamber. The effects of varying the OR voltage on both the response of the MS detector and the production of diagnostic ions were investigated. For the pesticides considered, the effects of increasing the potential difference in the desolvation chamber by increasing the OR voltage from 70 (minimum value for fragmentation of analytes) to 110 V on both the response of the ESI-MS detector and the production of fragment ions were evaluated. This experiment was conducted by injecting 5 ng of each pesticide considered onto the LC column. At all the OR voltages selected, background-subtracted spectra were taken from the average of the chromatographic peaks. Ion signal intensities were calculated by measuring the peak areas for each analyte obtained from the total ion current (TIC) chromatograms. Results are reported in Table 2. For the sake of conciseness, in this table only the relative abundance of the molecular ions plus those of the two most intense related fragments have been reported. The product ion spectrum of the $[M+H]^+$ ion of imazethapyr is shown in Fig. 3. The data in Table 2 show that increasing OR voltage did not have any great effect on signal value.

In terms of specificity, abundant fragmentation of the $[M+H]^+$ ion of analyte was obtained in the 90–110 V range. OR=90 V was selected as the operating condition.

3.4. Precision

For the selected pesticides, the repeatability and reproducibility of the signal intensities were assessed, together with those of the CID spectra. The intra-day precision was estimated by injecting 5 ng of each pesticide drawn from a standard solution into the LC column six times during a working day. The inter-day precision was evaluated by analyzing the standard solution four times over one working week. The instrumental conditions were the same as those reported in Section 2. For each analyte, the ion signal intensity was assessed by measuring the peak area from XIC of the most abundant ion. The variation of the abundance of the ion in the CID process at an OR of 90 V ranged from 2.5 to 6.0%. The intra- and the inter-day variations of the signal intensities for the analytes lay within the ranges 4–5% and 8–10%, respectively. These data show that both ion signals and the CID process are stable enough to allow

Table 2
Effects of increasing orifice plate voltage on both signal intensities and production of fragment ions for imidazolinone herbicides

Herbicide	Orifice plate voltage (V)[a]		
	70	90	110
Imazapyr	91 149(24), 202(34), **262**(100)	110 149(74), 202(62), **262**(100)	105 149(68), 202(100), **262**(98)
Imazamethabenz	163 162(–), 215(10), **275**(100)	165 162(81), 215(46), **275**(100)	149 162(100), 215(76), **275**(64)
Imazamethabenz methyl	189 162(–), 229(17), **289**(100)	212 162(74), 229(82), **289**(100)	201 162(100), 229(61), **289**(34)
Imazethapyr	115 177(25), 230(12), **290**(100)	131 177(55), 230(42), **290**(100)	130 177(88), 230(32), **290**(100)
Imazaquin	130 199(–), 252(17), **312**(100)	150 199(38), 252(25), **312**(100)	148 199(68), 252(47), **312**(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.

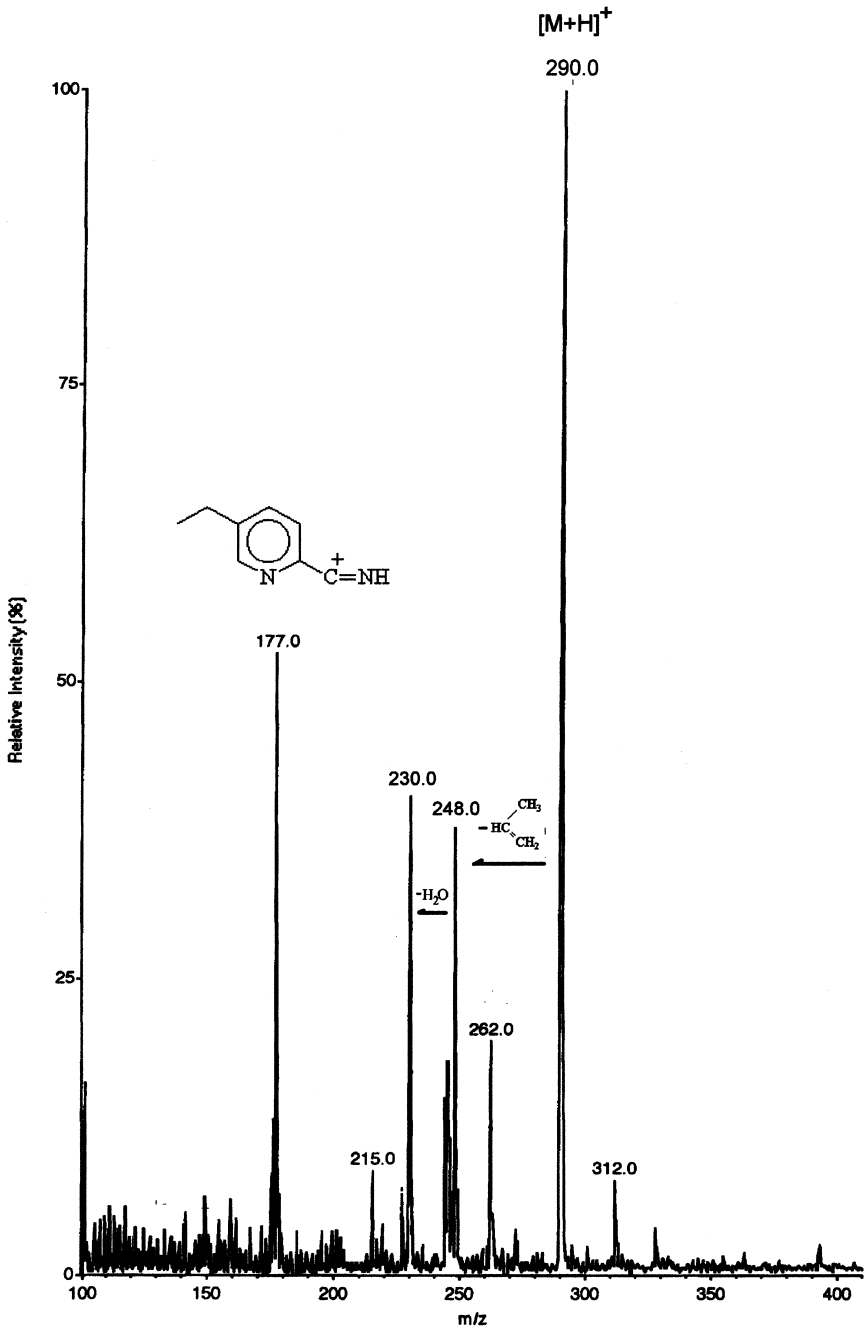

Fig. 3. Product ion spectrum from collision induced dissociation of the $[M+H]^+$ ion of imazethapyr (OR=90 V) and tentative identification of the most abundant fragments.

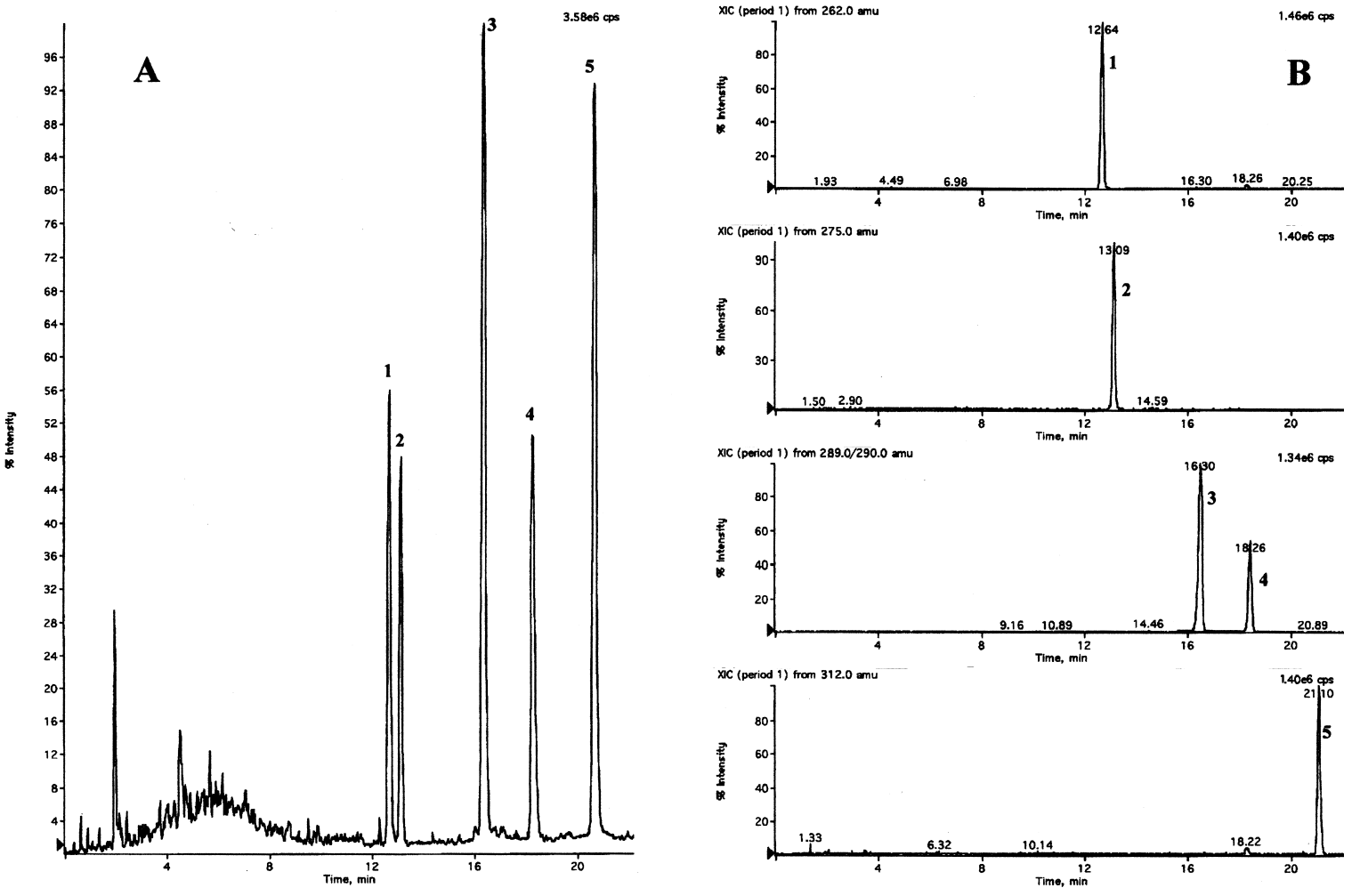

Fig. 4. (A) TIC chromatogram and (B) extract ion current (XIC) profile of the imidazolinones obtained by injecting 20/250 of a final extract relative to 2 l of drinking water spiked with the herbicides at a level of 50 ng/l each. (1) Imazapyr, (2) imazamethabenz, (3) imazamethabenz methyl, (4) imazethapyr, (5) imazaquin. For each pesticide, the selected XIC was the $[M+H]^+$ ion.

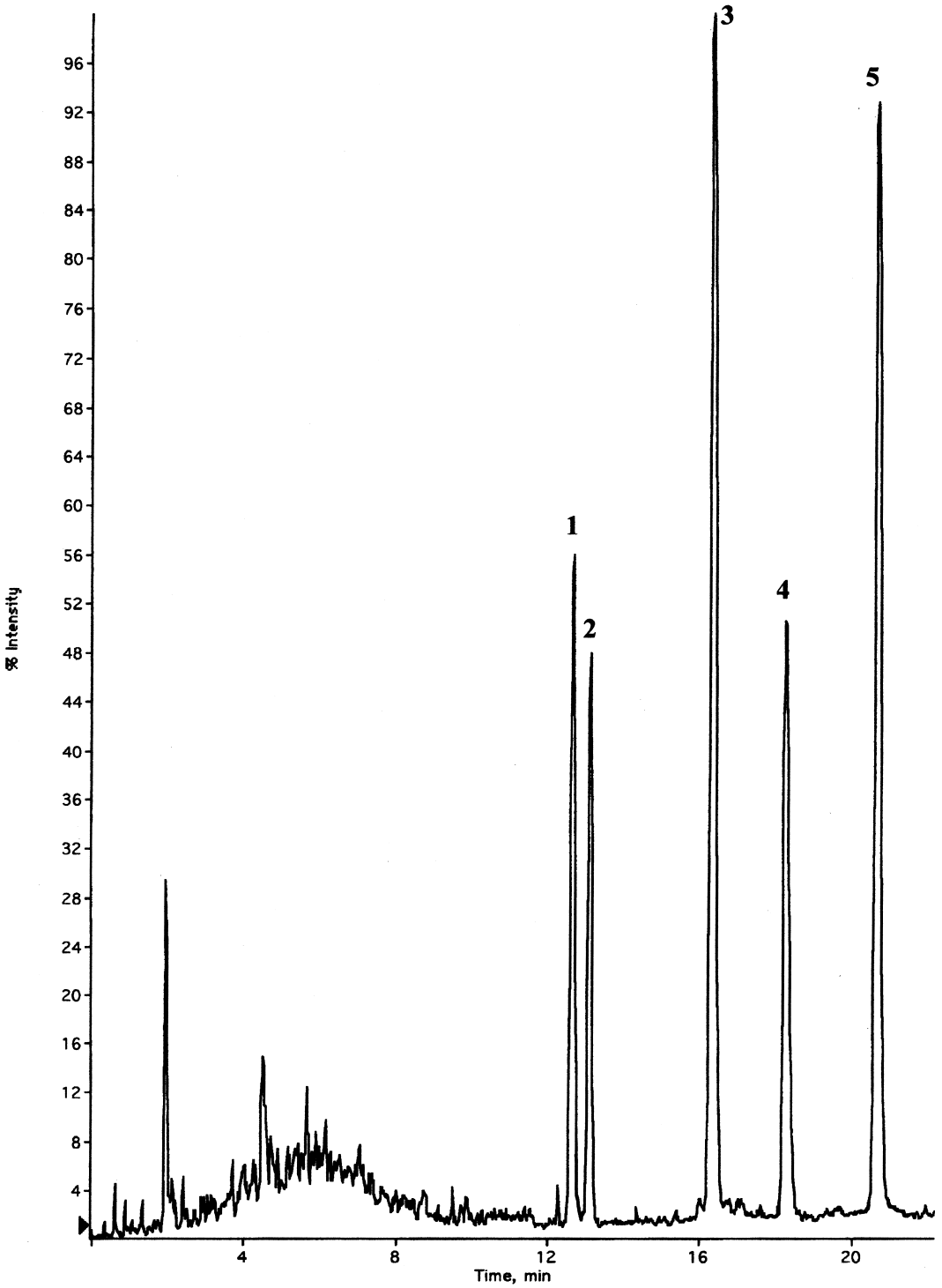

Fig. 5. TIC chromatogram obtained by injecting 20/250 of a final extract relative to 0.5 l of river water spiked with the herbicides at a level of 500 ng/l each. (1) Imazapyr, (2) imazamethabenz, (3) imazamethabenz methyl, (4) imazethapyr, (5) imazaquin.

reliable analysis of imidazolinone herbicides to be performed by this method.

3.5. Limits of detection (LODs)

The LODs given in Table 3 were calculated using a signal-to-noise ratio of 3 (the ratio of the peak intensity and the intensity of the noise was used). These data were obtained by measuring peak heights for any analyte against the averaged background noise from the TIC chromatogram referring to analysis of 2 l of a drinking water sample spiked with the analytes at the individual level of 50 ng/l. In the same table, LODs calculated from selected XICs are also presented. For each pesticide, the selected XIC was the $[M+H]^+$ ion, i.e., the one yielding the best S/N ratio. In Fig. 4 a TIC and XIC chromatogram is shown for this sample. Examination of Table 3 shows that this method has the potential for analyzing pesticides present in drinking water at a few ng/l without appealing to the selected ion-monitoring acquisition mode.

The ruggedness of the ES-MS arrangement was evaluated by analyzing four times per working day an extract obtained by treating 0.5 l of a river water sample spiked with the analytes at the individual level of 500 ng/l (Fig. 5). Although large amounts of organic matter originally contained in the aqueous matrix were introduced into the ESI source, the repeatability of the ESI-MS detector response was less than 10% for all the analytes.

Table 3
LODs for imidazolinone herbicides extracted from 2 l of drinking water

Herbicide	LOD (ng/l)		
	TIC[a]	XIC[b]	m/z[c]
Imazapyr	3.8	0.8	262
Imazamethabenz	4.3	0.7	275
Imazamethabenz methyl	2.3	0.5	289
Imazethapyr	4.7	1.1	290
Imazaquin	2.8	0.5	312

[a] TIC: Total ion current.
[b] XIC: Extracted ion current.
[c] m/z Relative to the ion selected.

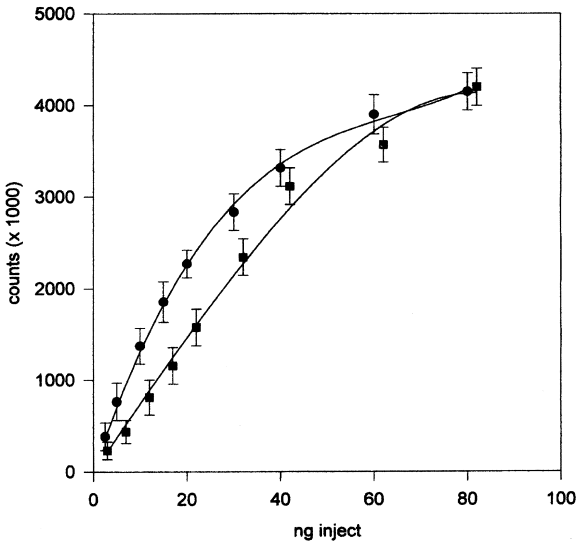

Fig. 6. Ion signal vs. amount of two selected analytes injected into the LC column. (●) Imazamethabenz methyl and (■) imazaquin.

3.6. Linear dynamic range

Under the conditions reported in Section 2, the linear dynamic range of the ESI-MS detector for the two selected imidazolinones was estimated. This set of measurements was performed by injecting into the LC column known variable amounts of imazaquin and imazamethabenz methyl. Measurements were made in triplicate for each amount injected. The average peak area of each set of injections was plotted against the amount injected, and the resulting plot (Fig. 6) indicated that a fairly good linear response could be obtained for two model compounds from 2 to 50 ng/l.

4. Conclusions

We have shown that the combination of a Carbograph 1 SPE cartridge with LC–ESI-MS can be advantageously used for rapid, unequivocal and accurate determination of pesticide traces in aqueous matrices. The proposed method allowed imidazolinones to be determined in environmental water at concentrations below 0.1 μg/l. This repre-

sented an improvement, since no analytical method was previously available for determination at such low concentration levels. Moreover, this method could be used in the near future for routine monitoring of imidazolinone pesticide residue in natural and drinking water.

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