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On-line solid-phase extraction–short-column liquid chromatography combined with various tandem mass spectrometric scanning strategies for the rapid study of transformation of pesticides in surface water

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Abstract

The applicability of solid-phase extraction–short-column liquid chromatography using two short columns (i.e., 10 and 20 mm long) coupled on-line with tandem mass spectrometric detection is demonstrated for the rapid degradation study of pesticides and their transformation products in water at the low-μg/l level. Photolysis was used as a means to transform the parent compounds into their degradation products and the experiments were carried out at environmentally relevant concentrations. The use of on-line sample enrichment/separation in photodegradation studies allows the rapid analysis of aqueous samples directly after irradiation without further transformation of the compounds of interest. The versatility of MS allows various selective screening strategies to be employed, i.e., full-scan mode, neutral loss, precursor-ion and product-ion scan modes. This allows the identification of possible degradation products and the calculation of the rates of disappearance of the parent compound and appearance of transformation products.

Keywords: Water analysis; Environmental analysis; Pesticides

1. Introduction

The applicability of single-short-column liquid chromatography (SSC) on-line combined with diode array UV detection (DAD UV) and/or (tandem) mass spectrometry (MS–MS) for the rapid screening and target analysis of a wide number of microcontaminants in aqueous samples at the low-μg/l level has been demonstrated in a number of communications [1–4]. In this approach both trace enrichment and separation are performed on one short column, and smaller sample volumes can consequently be used without sacrificing too much selectivity (chromatographic resolution) while fully maintaining analyte detectability. A considerable gain in total analysis time and, therefore, an increase in sample throughput can be achieved which is especially useful when highly selective detection techniques such as tandem MS and nuclear magnetic resonance (NMR) are used [4,5] The new analytical procedure

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can rapidly provide information in studies dealing with the degradation of pesticides and other organic contaminants in the aquatic environment. In a recent study, the potential of the SSC approach and/or solid-phase extraction–short column liquid chromatography (SPE–LC) using two short columns coupled on-line with tandem MS was demonstrated for this goal [6]. Photolysis was used as a rapid means to transform the parent compounds into degradation products [7,8].

The primary focus of this paper is to describe the development of analytical methods with the ability to rapidly identify potential degradation products. In real-life samples, parent compounds and certainly their degradation products are present at trace concentration levels. Consequently, analytical methods should be able to detect these environmentally relevant, i.e., low-μg/l, concentration levels. This is necessary, because, in the literature, photodegradation studies are usually carried out at high concentration (0.2–1 mg/l) in order to be able to identify as many breakdown products as possible. However, the nature of the (photo)degradation products and the rates of degradation are not necessarily the same at low and high concentration levels. In addition, DAD UV is sometimes used in combination with LC for specific identification purposes. However, information obtained from a UV spectrum is insufficient to enable structural elucidation of an analyte. For the successful identification of unknown transformation products, LC in combination with MS and tandem MS is necessary.

In our previous study [6], emphasis was on the general practicality and reliability of the use of SPE–LC (with two short columns) and/or SSC–MS–MS in pesticide degradation studies. In this paper, SPE–LC–MS–MS (with two short columns) is applied to the on-line study of the transformation of a few selected pesticides from different compound classes. Information can be obtained rapidly on the fate of the parent compound and on the approximate rate of its degradation. In addition, the use of different (tandem) MS scanning modes, i.e., the full-scan, neutral loss, precursor-ion and product-ion scan modes, can provide detailed structural information which enables transformation products to be identified.

2. Experimental

2.1. Chemicals, reagents and samples

HPLC gradient-grade methanol, acetonitrile and water, sodium dihydrogenphosphate and phosphoric acid were from J.T. Baker (Deventer, The Netherlands). Acetic acid and ammonium acetate were purchased from Riedel-de Haën (Seelze, Germany). Buffer solutions (10 m*M*) were prepared by dissolving 1.779 g of sodium dihydrogenphosphate or 0.771 g of ammonium acetate in 1 l of water and adding an appropriate volume of a 10 m*M* acid solution (phosphoric or acetic acid, respectively) to obtain a pH of ca. 4.5.

The triazine herbicides, atrazine and hydroxyatrazine, and the phenylurea herbicides, diuron and isoproturon were from Riedel-de Haën. Carbofuran and alachlor were purchased from Dr. Ehrenstorfer (Augsburg, Germany).

Stock standard solutions were prepared by dissolving 10 mg of each compound in 10 ml of methanol and were stored in the dark at −20°C. Standard solutions were prepared by dilution of the stock solutions with HPLC-grade water to give concentrations of 10 and 100 μg/ml. The standard solutions were used for loop injections and spiking of the water samples.

Milli-Q water, generated by purifying demineralised water in a Milli-Q filtration system (Millipore, Bedford, MA, USA), was used in the photolysis experiments. Surface water was collected from the River Rhine (Lobith, German/Dutch border, September/December 1997). The samples were pretreated by filtering 1 l over a 0.45-μm membrane filter (Schleicher and Schuell, Dassel, Germany). Spiking was done by adding an appropriate amount of a compound of interest to 1-l water samples.

Nitrogen (99.999% purity; for MS experiments) used for drying and as nebulising gas, and argon (99.9995% purity; for MS–MS experiments) were from Praxair (Oevel, Belgium).

2.2. Instrumentation and columns

2.2.1. Photolysis

Irradiation was done using a high-pressure mer-

cury (Hg) lamp with its main output at 254 nm. The irradiation experiments were carried out at room temperature over a period of ca. 15 h under constant stirring of the water sample in a glass reservoir. The distance from the lamp to the surface of the sample was ca. 15 cm. Prior to the photolysis experiment a 1-l water sample was spiked with the compound of interest dissolved in maximum 50 μl of methanol (maximum methanol percentage, 0.005%). The first 10-ml water sample taken from the reservoir was analysed prior to irradiation ($t=0$ min). During irradiation, every 30-min 10-ml water samples were automatically taken from the 1-l reservoir and analysed by on-line SPE–LC–MS–MS.

2.2.2. SPE–short-column-LC

Automated sample handling, including conditioning and washing of the 10-mm column and loading of the water sample onto the SPE column was done by a Prospekt (Spark Holland, Emmen, The Netherlands) sample handling module equipped with three six-port switching valves and a solvent delivery unit (SDU). Trace enrichment was performed on 10 mm×2 mm I.D. columns packed with experimental batches of (10–15) μm PLRP-S copolymer (Polymer Labs., Church Stretton, UK). A single 20 mm×4.6 mm I.D. short LC column high-pressure packed with 5 μm C_{18}-bonded silica (LC-18-DB, Supelco, Bornem, Belgium) was used for separation. The total analytical set-up used was similar to those used in recently published papers on on-line sample enrichment and separation/detection [2,4,6].

2.2.3. LC–DAD UV

The LC system consisted of an HP 1090 LC system equipped with a ternary solvent delivery unit (Hewlett-Packard, Waldbronn, Germany) to generate gradient elution conditions, and an HP1040 diode array detector. The eluents were helium-degassed. DAD UV detection was performed at 210, 225 and 254 nm.

2.2.4. MS–MS

Tandem MS was performed on a Micromass Quattro II triple-stage quadrupole equipped with an electrospray (ESI) source (Micromass, Altrincham, UK). The Masslynx software (version 2.22), installed on a Digital Pentium personal computer, was used for control of the system and data acquisition.

2.3. Analytical conditions and procedures

2.3.1. SPE–LC

For all analyses, the same trace-enrichment procedure was used. First, the pre-column was conditioned with 4 ml of methanol (2 ml/min for 2 min) to wet the packing material and remove hydrophobic compounds left from the previous run and, next, with 4 ml of water (2 ml/min for 2 min) to obtain suitable conditions for analyte sorption. Then, after flushing the solvent delivery lines with 8 ml (4 ml/min for 2 min), a 10-ml water sample (2 ml/min for 5 min) was passed through the pre-column. These 10-ml water samples were taken from a reservoir filled with 1-l of water. Finally, the analytes trapped on the top of the pre-column were on-line transferred to the 20-mm LC column and subsequently detected by DAD UV and/or ESI-MS(–MS). Total sample preparation time was ca. 10 min. Total analysis time was about 15 min.

2.3.2. LC analysis

Gradient LC elution in UV experiments was performed with an aqueous 10 m*M* phosphate buffer of pH ca. 4 (bottle A) and acetonitrile (bottle B). In MS experiments, 10 m*M* aqueous ammonium acetate of pH ca. 4.5 (bottle A) and methanol (bottle B) were used for gradient LC elution. Separation of the compounds of interest and their degradation product(s) from the 20 mm C_{18} column to the DAD UV was performed with a gradient starting at an eluent composition of A–B (90:10, v/v) which was changed linearly to A–B (25:75, v/v) in 4 min. Elution to the MS–MS was performed with a gradient starting at an eluent composition of A–B (90:10, v/v) which was changed linearly to A–B (10:90, v/v) in 3 min and kept at that eluent composition for 2 min at a flow-rate of 1.0 ml/min.

2.3.3. MS–MS

To maintain the LC flow-rate of 1 ml/min of the eluent entering the ESI interface at 70 μl/min, a post-column splitter was inserted. The source temperature was maintained at 100°C. Nitrogen was

used as drying and nebulising gas at flow-rates of 350 l/h and 15 l/h, respectively. Mass spectra were collected in the full-scan mode (m/z 100–300; scan time, 2 s; interscan time, 0.1 s). The sampling cone voltage was optimised for each of the compounds selected and was set at 32 V for atrazine and diuron, at 30 V for isoproturon and carbofuran and at 25 V for alachlor, with a skimmer lens offset of 5 V. The electrospray capillary voltage was set at 3.5 kV.

MS–MS experiments were carried out with an argon pressure of approx. $2.5 \cdot 10^{-3}$ mbar in the collision cell. The collision energy was optimised for each compound and was set at 17 eV for atrazine, 18 eV for diuron, and 15 eV for isoproturon and carbofuran. Product-ion spectra were collected with the first quadrupole (Q1) set at a fixed m/z value representing $[M+H]^+$ and with the third quadrupole (Q3) scanning from m/z 40 up to 10 u above the molecular mass of the compound studied (scan time, 2 s; interscan time, 0.1 s). For selected reaction monitoring (SRM) experiments, both Q1 and Q3 were set at fixed m/z values, viz. $[M+H]^+$ for Q1 and two of the most intense product ions for Q3 (dwell time, 0.2 s; interchannel delay, 0.01 s). For precursor-ion scan experiments, Q3 was set at a fixed m/z value, viz. that of the most intense product ion of the analyte and Q1 was scanning from 5 u above this product ion up to 300 u (scan time, 2 s; interscan time, 0.1 s). For neutral-loss scan experiments, Q1–Q3 was set at a fixed m/z value, viz. neutral loss of the compound of interest and Q1 was scanning from 5 u above the neutral loss mass up to 300 u (scan time, 2 s; interscan, time 0.1 s).

2.3.4. Automated on-line analysis

Automated and unattended data acquisition was performed in most cases. During trace enrichment, the LC and MS are in the ''waiting'' position. After the enrichment procedure has been completed, a contact closure signal is given to the liquid chromatograph to start the LC analysis. The liquid chromatograph is connected to the MS through an electronic contact closure switch enabling the automated start of MS data acquisition at the pre-programmed time of the LC gradient time table. At the end of a run, a signal is given to the tandem MS by the liquid chromatograph, and the former returns to the ''waiting'' position for the next analysis. In all the photolysis experiments, a 10-ml water sample was taken every 30 min from the reservoir and analysed by LC–MS. With this fully automated set-up, it is possible, if necessary, to analyse a fresh sample every 15 min.

3. Results and discussion

3.1. General remarks

UV absorbance is still the most commonly used detection mode in LC for environmental analysis. However, a major limitation of this technique is its inability to identify compounds. MS, on the other hand, and in particular MS–MS with its range of concomitant scan functions is a powerful technique to use in the screening and characterisation of (un)knowns [9]. The advent of atmospheric-pressure ionization has given great impetus to LC–MS hyphenation although it should be noted that, while a wide range of compounds can be observed, the relative responses in ESI-MS can be widely different. This should be recognised in all multi-residue screening procedures.

In the current study, LC–MS and LC–MS–MS were used to detect pesticide degradation products in irradiated spiked surface water. With known degradation products, a multi-residue screening method based on SRM, can be applied. However, when the degradation products are not known, the procedure is substantially more complex, especially when such a study is performed at environmentally relevant concentration levels, i.e., at or below ca. 10 μg/l in surface water. First, the samples must be screened for possible degradation products. This can be done in the full-scan mode, although the relatively low response of the compounds of interest, compared to background response can easily cause problems. A more powerful method is based on the use of MS–MS strategies like the constant neutral-loss and the precursor-ion scan modes [10,11]. These allow the screening for products with structural characteristics that are also present in the parent compound. After finding a particular peak of interest, the product-ion scan mode must be applied to achieve identification of the degradation product.

3.2. Monitoring rapid transformation of compounds

3.2.1. detection by DAD UV and identification/confirmation by MS

The practicality and reliability of the approach was shown for a well-known pesticide, atrazine, as model compound [6]. Both detection by DAD UV and selected reaction monitoring of precursor-ion/two most abundant product-ion transitions (see Table 1) were performed to rapidly monitor the disappearance of atrazine and the formation of one major transformation product. Comparison of the UV spectrum of this product with a laboratory-built UV spectral library allowed its tentative identification as hydroxyatrazine. This was confirmed by checking the retention time and product-ion MS–MS spectrum of the degradation product with those of a standard hydroxyatrazine solution.

When the previous rapid monitoring experiments were performed in the full-scan MS mode, however, several additional peaks showed up, some of which were generated upon irradiation of the demineralized and river water samples. Six such, potentially interfering, major peaks were observed: A (t_R 0.6 min; m/z 214, 236), B (t_R 0.9 min; m/z 152, 135), C (t_R 1.2 min; m/z 149, 297), D (t_R 1.7 min; m/z 205, 119), E (t_R 2.0 min; m/z 180, 163) and F (t_R 3.3 min; m/z 149, 205, 279). The responses of peaks B, C, E and F increased with the time of irradiation, while peaks A and D were present at a constant level during the whole experiment. A rapid attempt was made to identify these compounds. Product-ion mass spectra of all six compounds were recorded. Peak A was identified as a *N*-butylbenzenesulphonamide, a well-known contaminant frequently observed during ESI-MS [12]. Peaks C and F were identified as phthalate esters which are widely used as plasticisers. These compounds may have been present in the matrix, but are also reported to show up in full-scan GC–MS and LC–ESI-MS chromatograms if SPE cartridges are utilised for trace enrichment [13]. Peaks B, D and E have not been identified yet.

The degradation of diuron (t_R, 3.5 min) in demineralized and River Rhine water was studied under similar conditions. Two major peaks were detected at t_R 1.2 and 2.0 min, but these could not be matched with UV spectra in the library. However, one has to keep in mind that the library contains only the spectra of a limited number of pesticides and other microcontaminants (ca. 250 spectra). As regards these two non-identified peaks, LC–full-scan MS showed that they matched peaks C and E discussed above, and were no diuron degradation products at all. This was confirmed by product-ion MS–MS.

The above experiences show that strategies more sensitive and selective than UV detection and even

Table 1
Retention times, ions found in precursor-ion and neutral-loss MS–MS experiments, and product ions using SPE–short-column-LC–PA[c]-ESI–MS–MS of irradiated solution of pesticides in Milli-Q water and River Rhine water

No.	Parent compound and degradation products	t_R (min)	$[M+H]^+$ [a]	Product ions in MS–MS[b]				
1	Atrazine	3.2	216	174 (100)	96 (20)	132 (15)	146 (15)	104 (10)
	Hydroxyatrazine	2.0	198	156 (100)	86 (40)	114 (30)	97 (15)	
2	Diuron	3.5	233	72 (100)	46 (40)	160 (6)	188 (5)	
	[*N*-(4-Chloro-3-hydroxyphenyl)-*N′*,*N′*-dimethylurea]	2.3	215	72 (100)	46 (50)	142 (12)	170 (5)	
3	Isoproturon	3.3	207	72 (100)	46 (50)	164 (35)	134 (5)	
	({*N*-[4-(Isopropyl)-*x*-nitro-phenyl]-*N′*,*N′*-dimethylurea})	3.8	252	72 (100)	46 (2)	164 (2)		
	({*N*-[4-(*x*-Hydroxyisopropyl)phenyl]-*N′*,*N′*-dimethylurea})	2.4	223	72 (100)	165 (82)	46 (50)	160 (40)	59 (35)
	({*N*-[4-(Isopropyl)-*x*-hydroxyphenyl]-*N′*,*N′*-dimethylurea})	3.2	223	72 (100)	136 (25)	46 (20)	178 (10)	181 (5)
4	Carbofuran	3.0	222	165 (100)	123 (70)	137 (8)		
	3-Hydroxycarbofuran	2.3	220/238	163 (100)	135 (50)	107 (15)		

[a] Ions found in precursor-ion and neutral-loss MS–MS experiments.

[b] Numbers in parentheses give relative abundances of major product ions.

[c] PA=pneumatically-assisted.

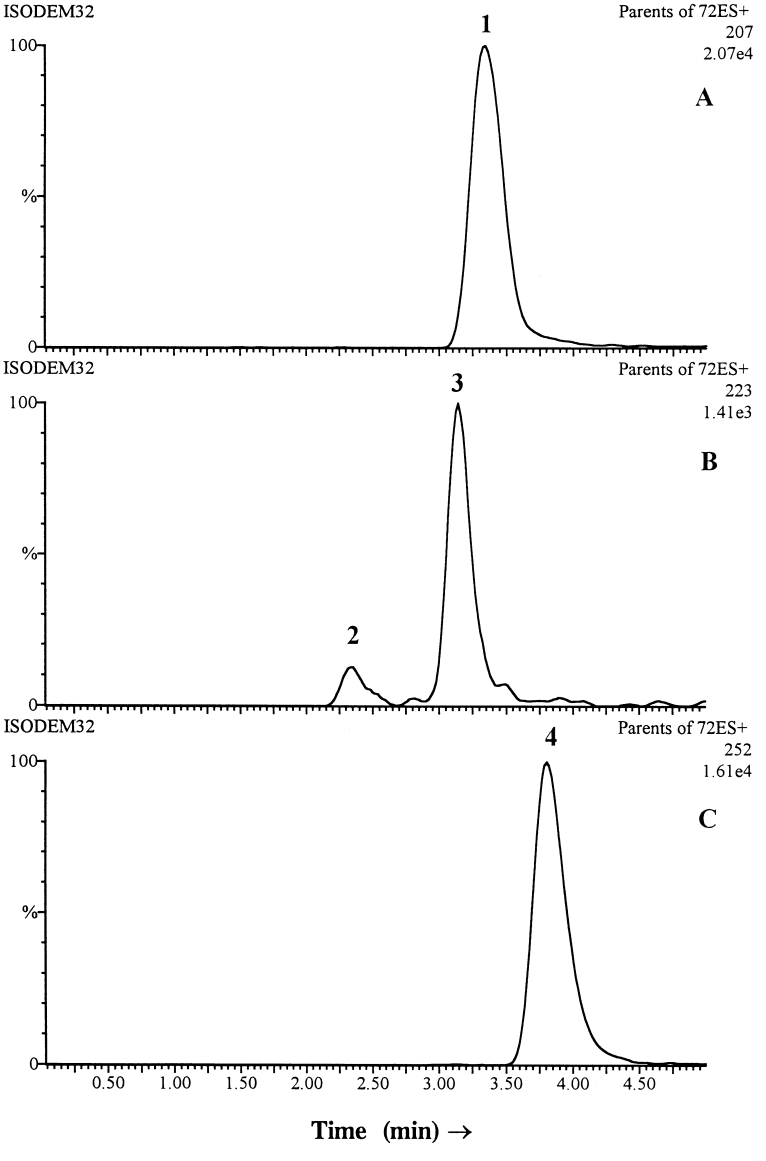

Fig. 1. Precursor-ion m/z 72 chromatograms obtained by SPE–LC–MS–MS of 10 ml of river water spiked with 50 μg/l of isoproturon, after 150 min of irradiation. Peak assignment: (1) m/z 207, (2) m/z 223, (3) m/z 223, (4) m/z 252. Peak 1 (m/z 207) is due to isoproturon. For SPE conditions, see Experimental. LC conditions: 20 mm×4.6 mm LC-18-DB Supelco column. Linear gradient from A–B (90:10, v/v) to (10:90, v/v) in 3 min, (10:90, v/v) from 3 to 5 min at flow-rate of 1 ml/min (flow into ESI interface: 70 μl/min). MS conditions: capillary, 3.5 kV; cone voltage, 30 V; collision energy, 15 eV; precursor-ion scan mode m/z 72: Q3 set at m/z 72 and Q1 scanning from m/z 77 to 300 in 2 s/scan.

full-scan MS are generally necessary for the identification of unknown degradation products, because the ''atrazine situation'' with one dominating and large additional peak is, in real practice, an exception. If the degradation products are sufficiently structurally related to the parent compound, MS–MS scanning strategies using constant neutral-loss and precursor-ion scans can be applied to screen for degradation products. Such strategies are frequently applied for e.g., drug monitoring but, until now they have not

found much application in environmental studies. Because of the rapidity of our analytical procedure, several different on-line SPE–LC–MS–MS experiments could be performed within 1 h [6]. This enabled the monitoring of relatively fast transformation processes in a number of modes, viz. the full-scan mode, precursor-ion scan or neutral-loss scan mode for screening, and product-ion scan mode for structure elucidation.

3.2.2. MS–MS scanning strategies: precursor-ion mode

The precursor-ion scan mode using m/z 72 corresponding to $[(CH_3)_2NCO]^+$ as the common product ion can be used to detect all degradation products unaltered in this part of the molecule and, thus, still producing this fragment, which is specific for *N′*,*N′*-dimethylphenylureas.

The repetitive rapid sampling of the water sample proved important, because a peak with m/z 215 was found to both appear and disappear within the first few hours of the 15-h experiment. Next, a series of experiments was performed in which a sample was taken from the reservoir every 15 min and analysed, first in the full-scan mode, then in the precursor-ion m/z 72 mode, and finally in the product-ion mode, using m/z 215 as the precursor ion. MS and MS–MS characteristics are included in Table 1. The product-ion spectra of both diuron and its degradation product are dominated by the $[(CH_3)_2NCO]^+$ and $[(CH_3)_2NH_2]^+$ ions at m/z 72 and 46, respectively. The less abundant fragments in the spectra, i.e., fragments m/z 142 and 170 indicate the substitution of one chlorine atom at the phenyl ring by a hydroxy group. From the MS–MS spectrum and the retention time, the compound could be identified as [*N*-(4-chloro-3-hydroxyphenyl)-*N′*,*N′*-dimethylurea]. Final proof was obtained by purchasing a pure standard and checking its retention time and MS characteristics. It has previously been reported as a major photolysis product under similar experimental conditions (Hg lamp) in aqueous solution [14].

The above studies were performed with 50 μg/l diuron samples. Such a relatively high concentration is needed to enable a reliable structure elucidation of the unknown degradation products. However, as soon as their nature is known, SRM strategies can be applied to monitor them in environmental samples at much lower levels, e.g., below 0.1 μg/l, as was demonstrated earlier [4].

The transformation of a structure-related herbicide, isoproturon, was also monitored every 30 min during a 15-h period and analysed in both the full-scan and precursor-ion m/z 72 scan modes. Fig. 1 shows a precursor-ion m/z 72 chromatogram obtained by SPE–LC–MS–MS after 150 min of irradiation. Three degradation products are detected, i.e., two with m/z 223 and one with m/z 252 (Fig. 1B and C).

The m/z 252 degradation product (t_R, 3.8 min) was found in both demineralized water and surface water (see Table 1). To confirm that this compound is related to isoproturon, a neutral-loss 180 scan was performed. The observed molecular mass of 251 u is 45 u higher than that of isoproturon. The odd mass indicates the incorporation of an additional nitrogen, most likely as a nitro group, in the isopropyl/phenyl part of the molecule. The compound has been tentatively identified as a nitro-isoproturon ({*N*-[4-(isopropyl)-*x*-nitrophenyl]-*N′*,*N′*-dimethylurea}).

Fig. 2 shows product-ion spectra of the two m/z 223 degradation products (t_R, 2.4 and 3.2 min), which were formed in demineralized water, and their proposed structures and tentative structure elucidation. The transformation occurs at the isopropylphenyl side of the molecule, most likely by the incorporation of a hydroxy group. The fragment at m/z 165 in the product-ion mass spectrum of the compound with t_R 2.4 min (Fig. 2 top) can be attributed to the loss of propenol (also detected as protonated molecule at m/z 59). This indicates that the hydroxy group is in the isopropyl group in this compound. The exact position of the hydroxy group cannot be derived from the MS–MS data. That is, the compound is hydroxyisoproturon ({*N*-[4-(*x*-hydroxyisopropyl)phenyl]-*N′*,*N′*-dimethyl urea}) with $x=1$ or 2. Both compounds have been reported in the literature as major transformation products of isoproturon in soil after heavy rainfall [15].

The product-ion spectrum of the compound at t_R 3.2 min shows a peak at m/z 181, corresponding to the loss of 42 (propene), and a peak at m/z 136 due to an additional loss of dimethylamine. This indicates that the hydroxy group is probably incorporated in the phenyl ring. This product can, therefore, tentatively be identified as a ({*N*-[4-(isopropyl)-*x*-

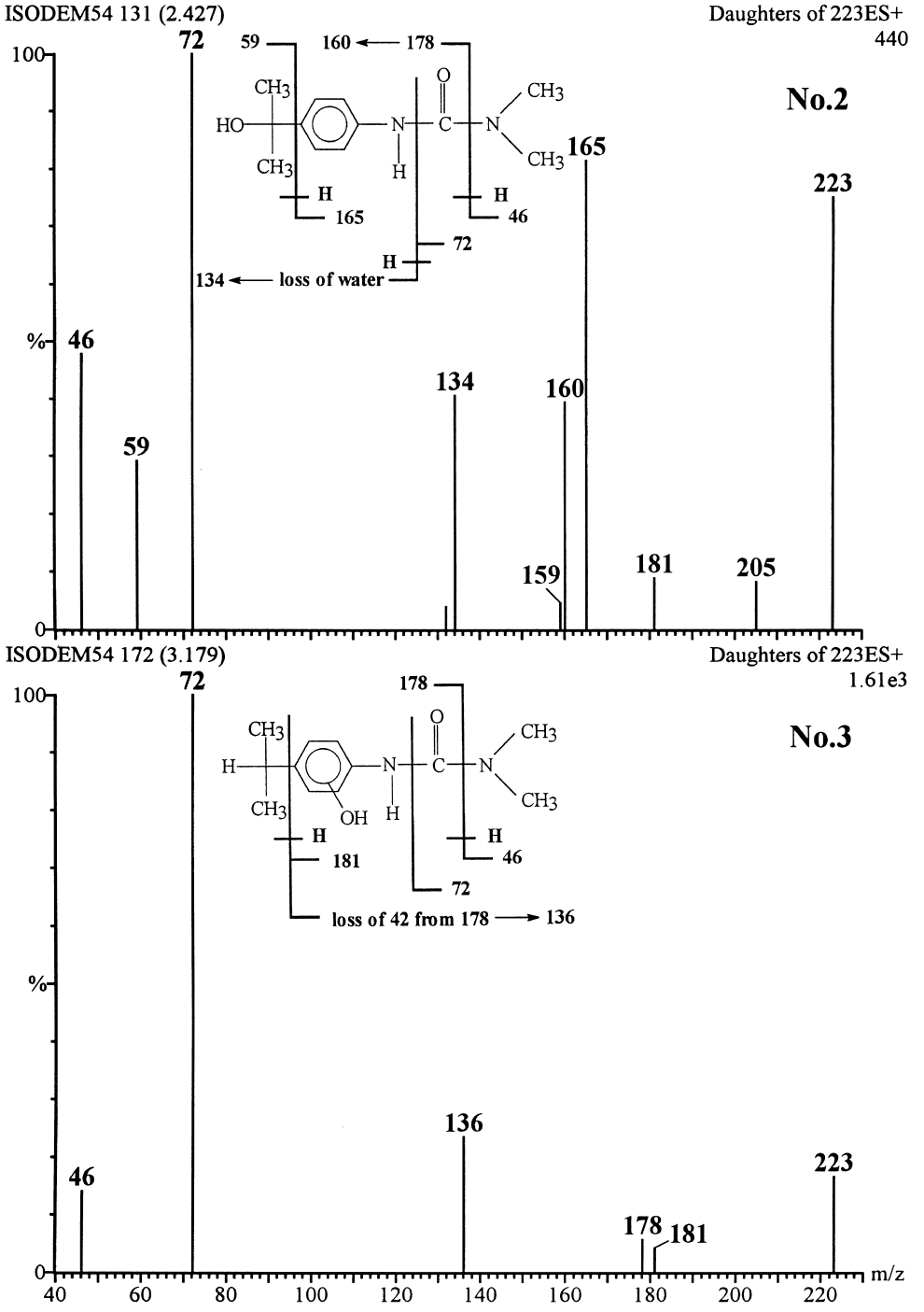

Fig. 2. Product-ion spectra of mass spectra of the two m/z 223 degradation products observed upon irradiation of demineralized water spiked with isoproturon at the 50 μg/l level (t_R, 2.4 and 3.2 min; peaks 2 and 3 in Fig. 1). Inserts: proposed structures of degradation products of isoproturon. For SPE, LC and MS conditions, see Fig. 1 and Experimental.

hydroxyphenyl]*N′*,*N′*-dimethylurea}) (Fig. 2, bottom).

The results of the monitoring of isoproturon and its degradation products by the precursor-ion *m*/*z* 72 scan are summarised in Fig. 3. The figure clearly demonstrates the advantages of the rapid measurement strategy: two data points are obtained every hour, which allows an accurate description of the degradation time curves. Under the applied conditions the degradation of isoproturon had an estimated half-life of 300–330 min in demineralized water and 80–110 min in River Rhine water, respectively.

3.2.3. MS–MS scanning strategy: neutral-loss mode

The neutral-loss scan mode was already used as an additional means to confirm the identity of the nitro-degradation product of isoproturon. A more typical example of its use is with *N*-methylcarbamates, which in MS–MS show the characteristic loss of O=C=N–CH_3, which corresponds to a neutral loss of 57 [11]. For carbofuran, which was selected as test compound, one peak was detected in both demineralized and River Rhine water, when applying the constant neutral-loss 57 scan mode, viz., at t_R 2.3 min. The mass spectrum contains peaks at *m*/*z* 220 and *m*/*z* 238 which can probably be assigned to a protonated molecule and a fragment due to loss of water. The product-ion mass spectrum of this degradation compound (see Table 1) is similar to that reported by Chiu et al. [16] for 3-hydroxycarbofuran. Final confirmation was obtained by purchasing pure 3-hydroxycarbofuran and repeating the above mentioned MS and MS–MS experiments. Identical results were obtained.

In a study on photodegradation of carbofuran in aqueous samples, Chiron et al. [17] did not report the formation of 3-hydroxycarbofuran, but two other degradation products which were identified by LC–ESI-full-scan MS as 2-hydroxy-3-(2-methyl-prop-1-enyl)-phenyl-*N*-methylcarbamate and 3-hydroxy-7-carbofuranphenol. These products were not found by us. Careful inspection of our data shows the appearance of a second degradation product (t_R 2.7 min) with a peak in the mass spectrum at *m*/*z*

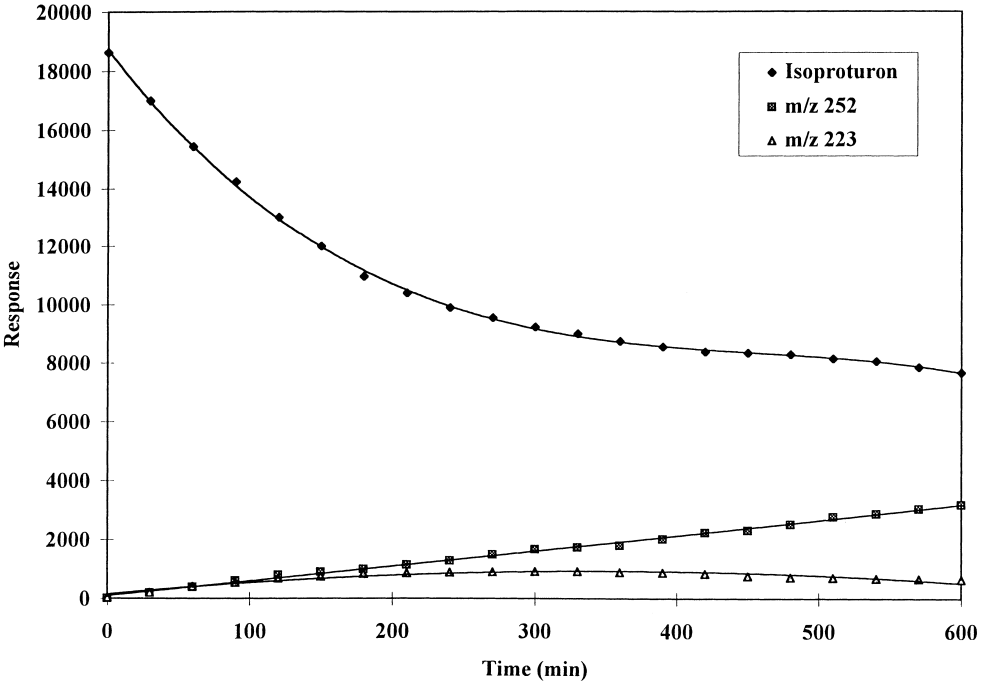

Fig. 3. Response versus time plots of photodegradation of isoproturon in demineralized water. The response obtained in the precursor-ion *m*/*z* 72 scan mode was used to construct the curves. For SPE, LC and MS conditions, see Fig. 1 and Experimental.

220, but without a clear peak at m/z 238. The response in ESI-MS was not high enough to obtain a useful product-ion mass spectrum.

The response vs. time profile of carbofuran (Fig. 4) is of some interest. The responses of the two m/z 220 degradation products (which show a mutual different behaviour) are much lower than that of the parent compound. However, because nothing is known about the relative ESI-MS responses of the analytes (and the neutral-loss approach only monitors degradation products not altered in the *N*-methylcarbamoyl group), little can be said about the concentration levels of the two m/z 220 compounds. This aspect is not at all discussed in the literature quoted above.

3.2.4. A more complicated degradation study: an example

With the model compounds studied above, only a few degradation products showed up. It is, of course, of some interest to investigate to which degree the present approach will be useful when the compound of interest shows a much more complicated degradation pattern. As an example, we briefly discuss the photodegradation of alachlor, a compound known to be photodegraded to up to 15–20 transformation products [18,19]. Fig. 5 shows the full-scan total ion chromatogram obtained by SPE–LC–MS of a 10-ml River Rhine sample spiked with 50 μg/l of alachlor, after 4 h of irradiation. Comparison with the $t=0$ chromatogram (which featured a single large peak due to alachlor; t_R, 3.6 min) immediately revealed that, during the 4-h irradiation, over 50% of the parent compound was lost and that some 10–20 new peaks show up. Several peaks show up in each of the recorded mass chromatograms (m/z 252, 234 and 220), which will further complicate identification; the general picture agrees with what was found in the quoted literature. In other words, the restricted resolution provided by the short column indeed is a limiting factor, and all studies beyond the first screening stage should be conducted with a conventional, i.e., 25-cm long, LC column. Actually, ongoing work with such a column shows that the

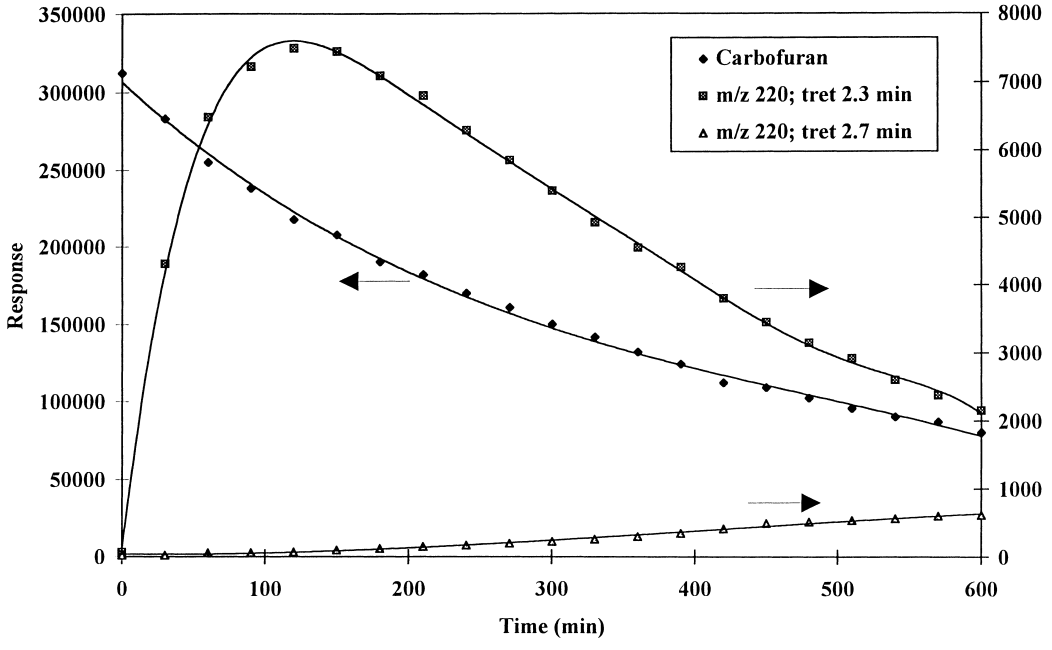

Fig. 4. Response versus time plots of photodegradation of carbofuran in River Rhine water (50 μg/l spiking level). The responses obtained in full-scan MS were used to construct the curves. Note: responses of the m/z 220 with t_R, 2.3 min and m/z 220 with t_R, 2.7 min curves on the right-hand *y* axis. For SPE and LC conditions, see Experimental. MS conditions: capillary, 3.5 kV; cone voltage, 30 V; collision energy, 15 eV; neutral-loss scan mode 57: Q3 set at mass 57, and Q1 scanning from 62 to 300 in 2 s/scan.

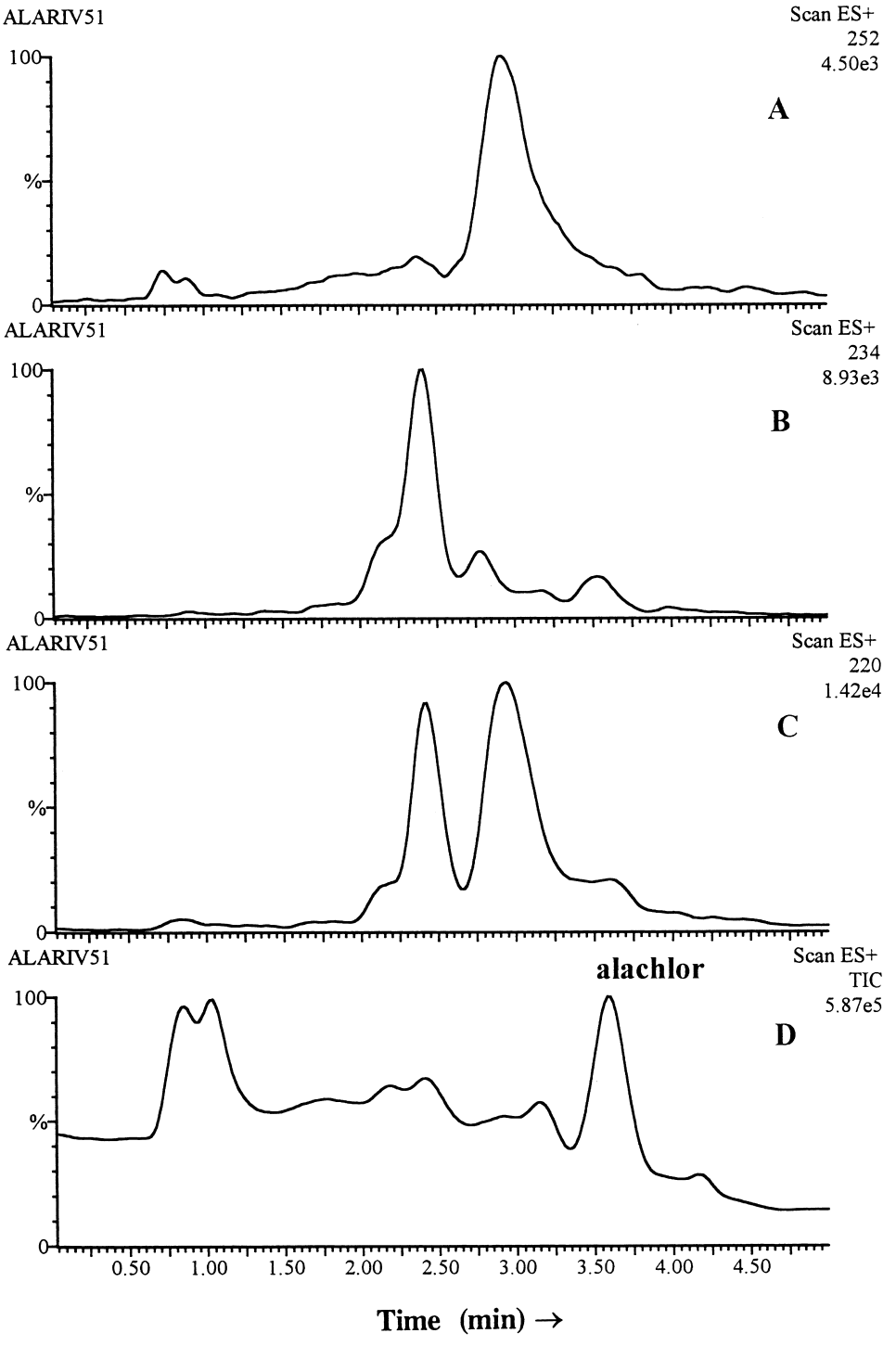

Fig. 5. SPE–LC–MS total ion and mass chromatograms of 10 ml of River Rhine water spiked with 50 μg/l of alachlor, obtained after a 4-h irradiation. Mass chromatograms of (A) *m/z* 252, (B) *m/z* 234, (C) *m/z* 220 and (D) total ion chromatogram (TIC). For SPE and LC conditions, see Experimental. MS conditions: capillary, 3.5 kV; cone voltage, 25 V, *m/z* 100–300; 2 s/scan.

situation is even more complicated, with the longer column contributing less to better chromatographic resolution of the many partly isobaric and/or isomeric, compounds than was initially expected. The complete study will be presented in a separate paper [20].

4. Conclusions

The data discussed indicate that the analytical strategy based on combined SPE–short-column-LC and MS–MS with its varying scan modes is successful with regard to the rapid screening for degradation products and, in several cases, of also identifying them. The strategy essentially allows the ''real-time'' monitoring of the pertinent decay and formation curves, even when the half-life is on the order of 30–60 min. With a total analysis time per sample of 10–15 min and a chromatographic run time of 3–4 min, sample throughput is high. Because of the trace enrichment achieved by SPE, the concentration of the parent compound can be in the lower-μg/l range, i.e., 20–50 μg/l for the identification of unknowns, and 0.1–10 μg/l for the monitoring of known degradation products, which is rather low compared with many degradation studies. In addition, the on-line set-up allows the analysis of aqueous samples directly after irradiation without further transformation of compound(s) of interest. The application of different MS and MS–MS scanning strategies enables detailed information on the identity of the degradation products to be obtained.

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