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Screening for transformation products of pesticides using tandem mass spectrometric scan modes

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Abstract

The applicability of tandem mass spectrometric (MS–MS) scan modes such as constant neutral-loss and precursor-ion scanning to screen for unknown transformation products (TPs) of pesticides at environmentally relevant concentrations (low-μg/l level) is studied. The selection of the MS–MS scan modes is based on the product-ion scan of the parent pesticide, and TPs are detected which are unaltered in the part of the structure concerned. The screening approach is applied to a surface water sample spiked with atrazine and three known TPs at a level of 3 μg/l to study the possibility to extract the TPs from the total ion chromatogram. Next, the approach is used to identify unknown TPs formed after (bio)degradation of two test compounds, fenchlorazole-ethyl (FCE) and furathiocarb (FTC). By using the precursor-ion scan mode, two TPs were detected after biodegradation of FCE, fenchlorazole-methyl and fenchlorazole; in surface water only fenchlorazole was found. The constant neutral-loss scan mode was used to identify carbofuran as TP of FTC. The added value of the proposed procedure is the increased selectivity at the cost of sensitivity. Best results are, therefore, obtained for samples which contain large amounts of matrix constituents.

Keywords: Water analysis; Environmental analysis; Pesticides; Fenchlorazole-ethyl; Furathiocarb; Atrazines

1. Introduction

In the past decades, new pesticides have been developed which show a more specific mode of action and have a higher polarity and lower persistence. Consequently, there is an increased concern regarding the formation of transformation products (TPs) and their presence in the environment. Such concern is even more justified if one considers that over 50% of the TPs of triazines, carbamates and phenoxypropionic acids pose a similar or even higher threat than their parent pesticides [1]. In many cases the identity of the TPs is unknown and laboratory experiments are, therefore, performed to simulate the (bio)degradation of pesticides under environmental conditions. Unfortunately, the field situation is not always properly simulated and concentrations up to the mg/l level are frequently used to ensure detectability of the formed products. In other words, there

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is an urgent need for screening techniques which can detect unknown TPs in real-life samples or in samples obtained from laboratory experiments performed at environmentally relevant, i.e., low-μg/l, concentration levels.

The rather high water solubility and polarity of modern pesticides, characteristics which are in general even more distinctly displayed by their TPs, make liquid chromatography (LC) combined with mass spectrometry (MS) the preferred separation plus identification technique. Identification of unknown TPs can be performed by screening the full-scan LC–MS spectra for specific peaks which are subsequently selected as parent ion in the product-ion scan mode. Typically, a limited m/z range is examined step by step to reduce the time needed for the laborious screening. Unfortunately, the quasi-molecular ion peaks associated with the TPs may be difficult to find when samples contain relatively large amounts of matrix constituents. The selectivity of MS–MS scan modes, such as the constant neutral-loss and precursor-ion modes, may be used to overcome this problem [2–5]. Neutral losses and precursor ions are selected from the product-ion spectrum of the parent compounds, and TPs which are unaltered in the corresponding part of the molecule can then be detected. These procedures are frequently used in, e.g., metabolic studies of drugs but have, so far, found little application in environmental studies. One example is a study performed by Hogenboom et al. [6], who used MS–MS scanning strategies for the identification of TPs after photochemical transformation of diuron, isoproturon and carbofuran.

In this paper the applicability of the constant neutral-loss and precursor-ion scan modes to screen for unknown TPs at environmentally relevant concentration levels is studied. Firstly, the screening approach was evaluated using a surface water sample spiked with atrazine and three of its TPs. Next, the approach was used to identify unknown TPs formed after the (bio)degradation of two test compounds, fenchlorazole-ethyl (FCE) and furathiocarb (FTC). These pesticides were selected because of their low stability in water/sludge systems: their degradation half-lives (DT_{50}) are 5.5 and 4 days, respectively [7].

2. Experimental

2.1. Chemicals

Atrazine, hydroxyatrazine (OH), desethylatrazine (DEA), desisopropylatrazine (DIA), terbutylazine, FCE and FTC were obtained from Riedel-de Haën (Seelze, Germany). Standards were at least 98% pure and stock standard solutions were prepared by dissolving 10 mg in 10 ml of methanol and were stored in the dark at −20°C. In the case of hydroxyatrazine a few drops of concentrated formic acid (J.T. Baker, Deventer, The Netherlands) were added to promote dissolution. The ingredients for the nutrient medium used in the biodegradation experiments (nutrients, trace metals and essential vitamins) were obtained from Sigma–Aldrich (Zwijndrecht, The Netherlands). For a complete listing of ingredients and concentrations used for the medium we refer to Ref. [8]. HPLC-gradient-grade water and methanol were from J.T. Baker. Nitrogen (99.999% purity) and argon (99.9995% purity) were from Praxair (Oevel, Belgium).

2.2. Degradation experiments

Two types of degradation experiment were performed. A biodegradation experiment was performed according to the modified OECD screening test for ready biodegradability [8]. A nutrient medium was inoculated with 0.2% of activated sludge which served as a source of microorganisms. The medium (1 l) was spiked with the pesticide of interest at a concentration level of 10 μg/l. In addition, the solution was spiked with 10 μg/l of terbutylazine which served as an internal standard (DT_{50} of terbutylazine, 580 days [7]). In a second experiment, pesticides were spiked at a level of 10 μg/l to a surface water sample taken from the River Scheldt in July 1998. For both experiments, 100-ml samples were taken after 1, 2 and 4 days and immediately extracted by means of solid-phase extraction (SPE).

2.3. Sample preparation

Water samples (1 l for the atrazine experiments

and 100 ml for the degradation experiments) were filtered though a 0.45-μm ME-25 filter from Schleicher and Schuell (Dassel, Germany) to remove particulate matter. LiChrolut EN cartridges (200 mg, Merck, Darmstadt, Germany) were conditioned with 5 ml of methanol and, next, 5 ml of HPLC-grade water; after conditioning, a sample was added. The cartridge was not allowed to run dry in between conditioning and sample addition. The cartridges were eluted with 3×3 ml of methanol. The extracts were evaporated to approx. 0.5 ml under a gentle stream of nitrogen and 20 μl was injected on the LC column. The resulting concentration factor of 200 is rather low; however, the procedure allowed multiple injections and, consequently, the application of a range of MS–MS scans on the same extract.

2.4. LC–electrospray ionization (ESI) MS–MS analysis

A HP 1090 LC system equipped with a ternary solvent delivery unit (Hewlett-Packard, Waldbronn, Germany) was used. Separations were performed on a 250 mm×4.6 mm I.D. Vydac (Hesperia, CA, USA) column packed with 5 μm C_{18}-bonded silica. Linear gradient LC elution was performed with an aqueous 10 m*M* ammonium acetate buffer, pH 4.5 (A) and methanol (B) with A–B (90:10, v/v) for 1 min to A–B (10:90) in 19 min. The column was operated at a flow-rate of 1 ml/min, 70 μl/min being directed to the ESI interface via a post-column splitter. Tandem MS was performed on a VG Quattro II triple-stage quadrupole equipped with a dual electrospray/atmospheric pressure chemical ionization (ESI/APCI) source (Micromass, Altrincham, UK). The cone voltage (CV) was optimized for optimum sensitivity of the parent pesticide and the collision energy (CE) was optimized to form the desired product ions. In general, higher CEs are required for lower-mass precursor-ion scans and higher-mass constant neutral-loss scans. The source temperature was set at 80°C, the ESI capillary voltage at 3.5 kV and the skimmer lens offset at 5 V. The standard dwell time was 1.5 s. Nitrogen was used as drying and nebulizing gas at flow-rates of 350 and 15 l/h, respectively. The argon pressure in the collision cell was 2.5 μbar.

3. Results and discussion

3.1. Screening approach

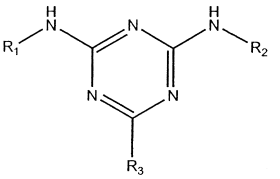
First, product-ion spectra of the pesticides of interest were collected at different CEs. Next, characteristic neutral losses and product ions were selected to set up the constant neutral-loss and precursor-ion scan modes which were subsequently applied to the various types of sample. Spectra taken at the apex of the peaks which appeared in the total ion chromatogram (TIC) revealed the quasi-molecular ions of the compounds which are structurally related to the pesticide. Structural identification of these compounds can then be achieved by applying the product-ion scan mode using the identified quasi-molecular ions as parent ion. ESI was used as it has proven to be a reliable, robust and sensitive ionization technique for relatively small and polar compounds such as TPs of pesticides [9,10]. It should be noted, however, that the relative responses of the various TPs in ESI can be very different; that is, no information is obtained on the concentrations of these TPs.

One important prerequisite is that the rather polar TPs are retained on the sorbent used in the SPE cartridges. Fortunately, the material used in this study, LiChrolut EN, has already been shown to be a good choice to retain relatively polar TPs and even partially ionized compounds (if necessary, after pH adjustment) [9,10]. The approach aims at detecting the TPs which are structurally related to the parent pesticide. This group of TPs is considered the most relevant with regard to the quality criteria (≤0.1 μg/l) of the EU Drinking Water Directive [11].

3.2. Selectivity of constant neutral-loss and precursor-ion scan modes

The applicability of the screening approach was studied by spiking a surface water sample with atrazine and three of its TPs, i.e., OH, DEA and DIA, each at a level of 3 μg/l. Product-ion spectra were collected for 1 μg/l standard solutions of the individual compounds in 10 m*M* aqueous ammonium acetate–methanol (50:50, v/v). The structural information of Table 1 shows that, after transforma-

Table 1
Structure of atrazine and three TPs



	R_1	R_2	R_3
Atrazine	C_2H_5	$CH(CH_3)_2$	Cl
Hydroxyatrazine	C_2H_5	$CH(CH_3)_2$	OH
Desethylatrazine	H	$CH(CH_3)_2$	Cl
Desisopropylatrazine	C_2H_5	H	Cl

tion, the TPs still are, structurally, closely similar to the parent pesticide, with the central ring and the attached –NH– groups intact. This is reflected by the product-ion mass spectral data of Table 2 which summarizes the product ions and neutral losses found for atrazine and at least one of its TPs.

Next, constant neutral-loss and precursor-ion scans were selected based on the product ions of atrazine. These scans were then applied to the spiked surface water extract. From Fig. 1 it is clear that, due to the large amount of matrix constituents, identification of the TPs in the full-scan TIC trace cannot be achieved. On the other hand, the TIC traces of the precursor-ion m/z 68 ($[N{=}C{-}NH{-}C{=}N{+}H]^+$) and constant neutral-loss m/z 42 ($CH_3{-}CH{=}CH_2$) scan modes clearly showed peaks for atrazine and all three TPs. These traces were selected as an illustration; similar results were obtained for the other neutral losses and product ions presented in Table 2. This demonstrates that TPs can be selectively detected without having previous knowledge regarding their identity. The excellent selectivity of the MS–MS scan modes is further demonstrated by the low-noise spectra taken at the apex of the OH and DEA peaks in the neutral-loss trace. The spectra clearly

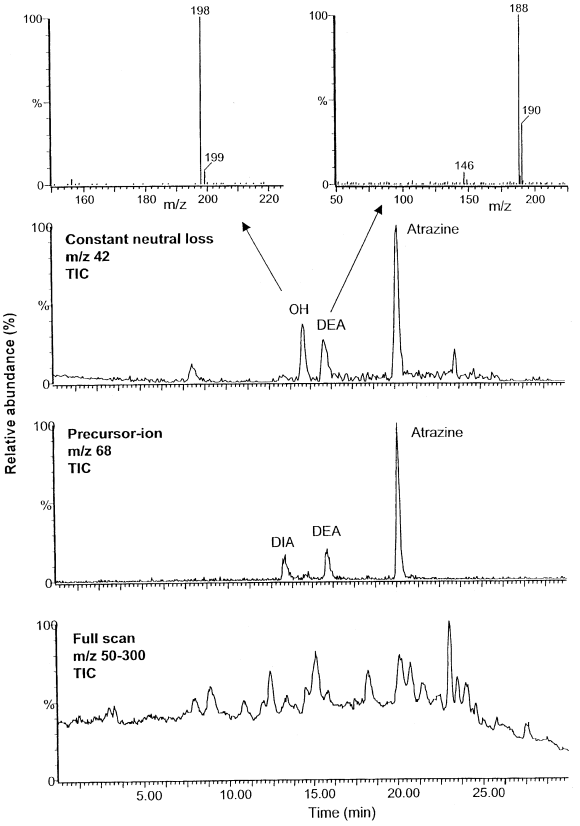


Fig. 1. Full-scan m/z 50–300 (CV=35 V), precursor-ion m/z 68 (CV=35 V; CE=30 V; scanning from m/z 68 to 225) and constant neutral-loss m/z 42 (CV=35 V; CE=20; scanning from m/z 50 to 225) chromatograms and spectra taken at the apex of the indicated peaks of a River Scheldt water sample spiked with 3 μg/l of atrazine and three TPs. For SPE, LC and general ESI-MS(–MS) conditions, see Experimental.

Table 2
Information derived from product-ion spectra of atrazine and three TPs[a,b]

Compound	$[M+H]^+$	Neutral loss (m/z) of										Product ions (m/z)					
		42		70		84		112		120		68		71		79	
Atrazine	216	174	(15)	146	(19)	132	(28)	104	(100)	96	(72)	68	(61)	71	(27)	79	(42)
Hydroxyatrazine	198	156	(10)	128	(5)	114	(51)	86	(100)	–		–		71	(16)		–
Desethylatrazine	188	146	(30)	–		104	(100)	–		68	(58)	68	(58)	–		79	(79)
Desisopropylatrazine	174	–		104	(80)	–		62	(26)	–		68	(100)	71	(26)	79	(27)

[a] Numbers in parentheses give relative abundances of major product ions.
[b] Spectra recorded in positive ion mode with CV=35 V and CE=30 V.

reveal the quasi-molecular ions which can next be used in the product-ion scan mode to elucidate the structure of the TPs. In a subsequent step, the most sensitive transition can then be selected for the single reaction monitoring (SRM) mode. Applying this mode enables analyte monitoring at much lower levels than the low-μg/l concentrations used in this experiment. This was demonstrated in an earlier study on pesticides and TPs in surface and estuarine water samples where microcontaminants such as atrazine, chloridazon, diuron, metolachlor, OH, DEA, DIA, monuron and 3,4-dichlorophenyl-methylurea were detected down to the low-ng/l level [9,10].

The 3 μg/l spiking level of the above experiments should not be considered the lower limit of detection/identification. With the present set-up, only 4% of the final extract was injected: by increasing that fraction according to well-known procedures, a substantial gain in detectability should be achievable.

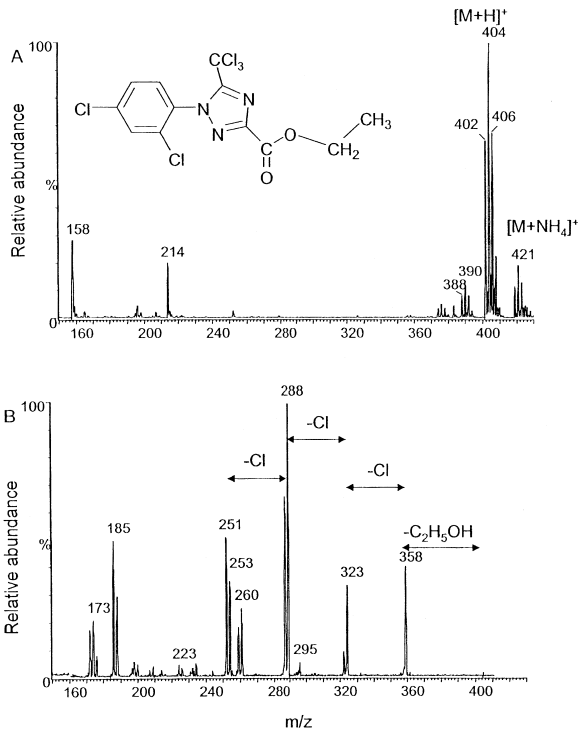


Fig. 2. (A) Full-scan m/z 50–450 (CV=30 V) and (B) product-ion m/z 404 scan (CV=30 V; CE=40 V; scanning from m/z 50 to 410) of a 1 μg/l standard of FCE in 10 m*M* aqueous ammonium acetate–methanol (50:50, v/v). Insert: structure of FCE.

In addition, a modest improvement can be obtained by increasing the MS–MS scan time from the present 1.5 to 2–2.5 s, which will still provide enough data points per peak.

3.3. Screening for transformation products of fenchlorazole-ethyl

Fig. 2 shows the full-scan and product-ion spectra of a 1 μg/ml standard of FCE in 10 m*M* aqueous ammonium acetate–methanol (50:50, v/v). The mono-isotopic molecular mass of FCE is 401. Because of the presence of five chlorine atoms, the base peak of the protonated molecular-ion cluster is at m/z 404. The product-ion scan with m/z 404 as parent ion shows product ions at m/z 358 due to the loss of ethanol and at m/z 323, 288 and 253 due to the sequential loss of chlorine atoms. The product ions at m/z 358 and 288 were selected for a precursor-ion scan applied to a sample taken at the end of day 1 of the biodegradation experiment. Fig. 3 shows the TIC chromatograms for both scans. At the retention time of FCE (indicated by an arrow) no peaks were found. Apparently, FCE has been fully degraded within the first day which is in agreement with its the low stability in water; the DT_{50} for hydrolysis is reported to range between 5.5 days (pH 7) and 1.9 h (pH 9) [7]. The two precursor-ion scans showed two distinct peaks, denoted as products 1 and 2, at retention times of 20.3 and 16.6 min, respectively. The corresponding spectra (right-hand side of Fig. 3) showed base peaks at m/z 390 and 376 for Products 1 and 2, respectively. The precursor-ion scan m/z 288 produced an isotopic pattern which is related to the loss of a chlorine atom. The results of the m/z 358 scan indicate that both products result from a transformation of the ethyl group. The product-ion scans of both base peaks, m/z 376 and 390, provided no additional structural information as the same product ions were found as for m/z 404 of FCE, i.e., m/z 358, 323 and 288. Products 1 and 2 were tentatively identified as fenchlorazole-methyl [methyl-1-(2,4-dichlorophenyl)-5-trichloromethyl-1*H*-1,2,4-triazole-3-carboxylate] and fenchlorazole [1-(2,4-dichlorophenyl)-5-trichloromethyl-1*H*-1,2,4-triazole-3-carboxylic acid], respectively.

The spectra obtained from the precursor-ion scans

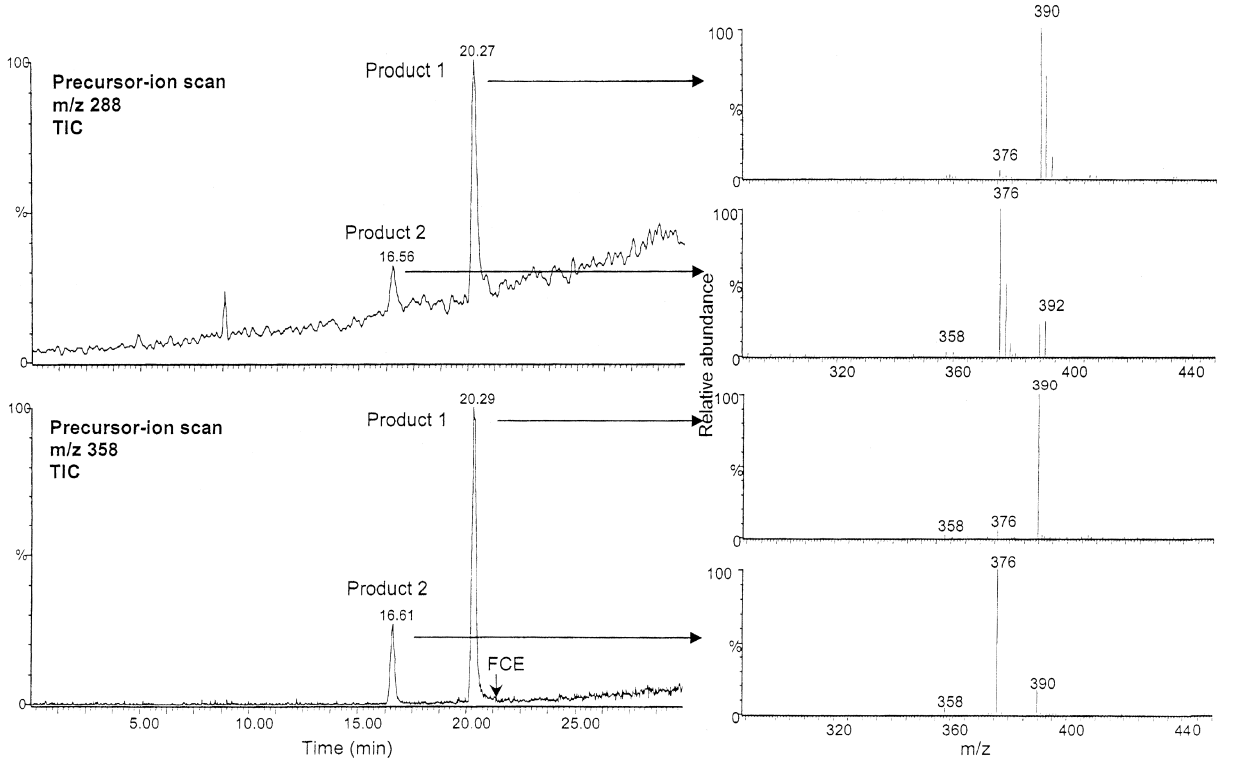


Fig. 3. Precursor-ion m/z 288 (CV=35 V; CE=40 V; scanning from m/z 288 to 450) and precursor-ion m/z 358 (CV=35 V; CE=20 V; scanning from m/z 358 to 450) chromatograms of a sample taken at day 1 of biodegradation experiment of FCE. For details on biodegradation, SPE, LC and general ESI-MS(–MS) conditions, see Experimental.

of fenchlorazole (product 2) also showed a minor peak at m/z 390. The full-scan spectra of Fig. 4 show that this peak most probably represents a methanol cluster of the m/z 358 fragment which is formed by in-source collision-induced dissociation. Further support for the proposed structure was found by the sodium adducts present in the full-scan spectrum. Fenchlorazole is a carboxylic acid and, therefore, partially ionized at the prevailing pH of ca. 4.5. The anion also forms a positively charged double sodium adduct with m/z 420. For fenchlorazole-methyl only the single sodium adduct was found (m/z 412). The retention times of both products are shorter than that of FCE which is in agreement with the higher polarity of the proposed compounds.

In addition to the biodegradation experiment, a River Scheldt surface water sample was spiked with FCE at a level of 10 μg/l. After 1 day, a sample was taken and analyzed in the full-scan and precursor-ion m/z 358 mode. Fig. 5 demonstrates the increased selectivity of the latter mode. Again, FCE was fully degraded in the first day but only one TP was found, fenchlorazole. The absence of fenchlorzole-methyl can be explained by the fact that dealkylation is primarily performed by micro-organisms which were predominant in the biodegradation experiment [12].

3.4. Screening for transformation products of furathiocarb

Fig. 6 shows an example of the use of the constant neutral-loss scan mode which was used for the identification of TPs of FTC. The product-ion spectrum of m/z 383, the $[M+H]^+$ of FTC, showed a product ion at m/z 252 which corresponds to a neutral loss of 131 u. Fig. 6 contains a proposed structure for this fragment which is most likely

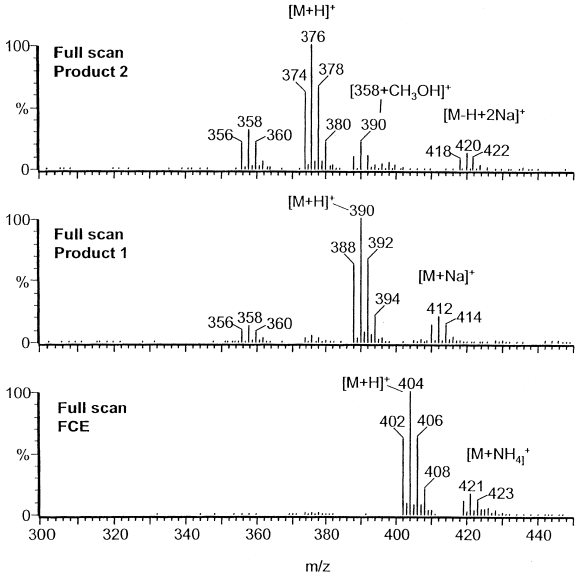


Fig. 4. Full-scan m/z 50–450 spectra of FCE and two TPs identified with precursor-ion scan mode (see Fig. 3).

formed by a rearrangement involving the carbamate moiety and the O-containing ring structure. Since the base peak at m/z 195 cannot be explained by any homolytic cleavage, this fragment is most likely also formed by a rearrangement ($[C_4H_9CO_2NCH_3SNHOH+H]^+$). The TIC trace of the constant neutral-loss m/z 131 scan applied to a sample taken at the end of day 1 of the biodegradation experiment of furathiocarb, showed a distinct peak at a retention time of 15.1 min. Its spectrum featured a peak at m/z 222 which was subsequently selected for the product-ion scan mode for structural identification. Based on the product-ion spectrum and agreement with spectra reported in literature [6] the TP was tentatively identified as carbofuran. Final confirmation was achieved by standard injections of carbofuran.

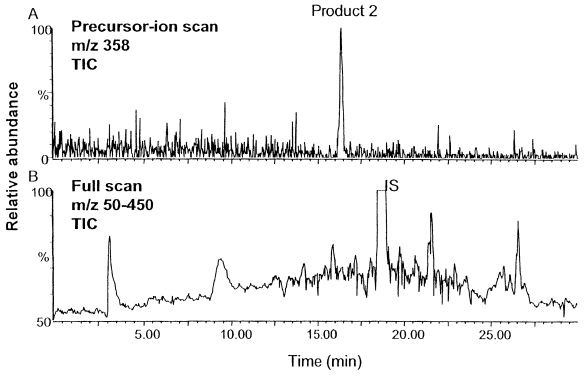


Fig. 5. Full-scan m/z 50–450 (CV=35 V) and precursor-ion scan m/z 358 (CV=35 V; CE=20 V; scanning from m/z 358 to 450) chromatograms of a River Scheldt water sample spiked with 10 μg/l FCE. For details on degradation, SPE, LC and general ESI-MS(–MS) conditions, see Experimental.

3.5. Degradation profiles

Fig. 7 shows the response versus time profiles of FTC and carbofuran, and FCE and its two TPs during the biodegradation experiment. The response is expressed as the peak area of the individual compounds divided by the peak area of the internal standard (terbutylazine) with the initial responses of the parent compounds ($t=0$ days) set to 100%. The rapid transformation of FTC into carbofuran is clearly demonstrated with carbofuran reaching a maximum response after about 1 day. FCE is seen to be much more rapidly degraded than FTC with product 2 (fenchlorazole) showing a distinctly higher stability than the other TP formed, product 1 (fenchlorazole-methyl).

4. Conclusions

The use of MS–MS scan modes such as constant-neutral loss and precursor-ion scanning in LC is a powerful means to selectively screen surface water for the presence of structurally related, but unknown TPs of pesticides at environmentally relevant, i.e., low-μg/l, levels. The approach uses the product-ion scan of the parent pesticide to select MS–MS scan modes to identify TPs which are unaltered in the associated part of the structure. Preferably, neutral losses and product-ions are selected which are associated with characteristic structures of the pesticide. Once the quasi-molecular peaks are identified, the desired ion trace can be extracted from the (more sensitive) full-scan TIC trace for further examinations. Since the procedure typically enhances selectivity at the cost of sensitivity, results will be most rewarding for samples containing relatively large amounts of matrix constituents. The examples dis-

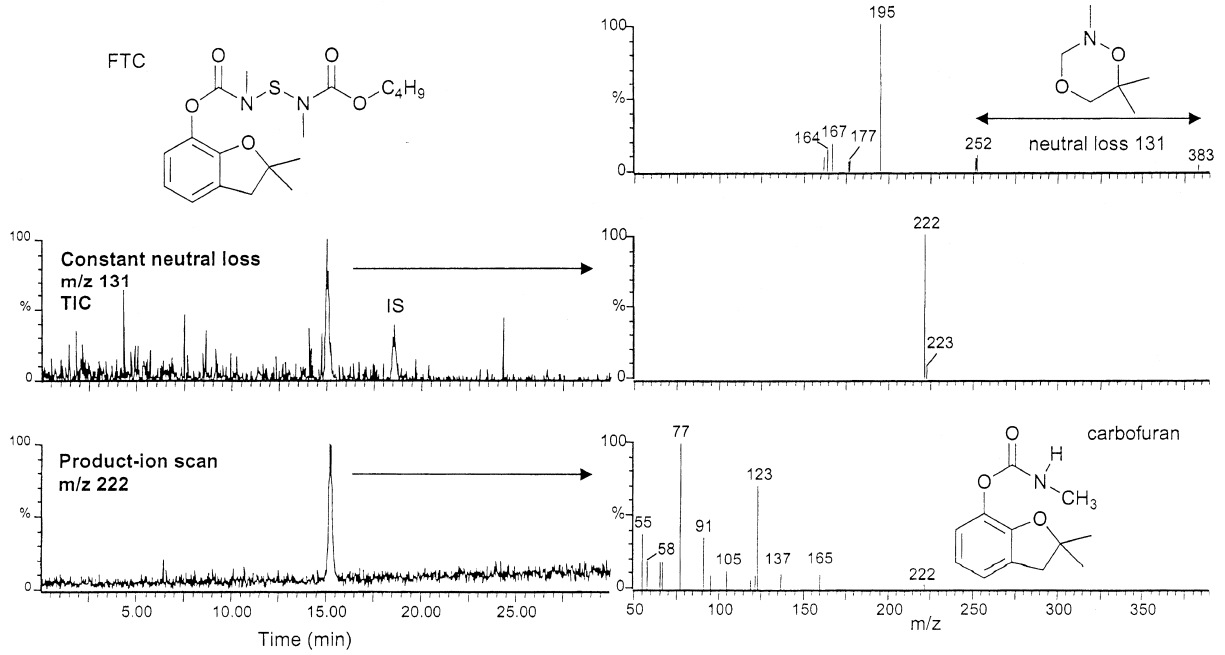


Fig. 6. Product-ion m/z 383 spectrum of furathiocarb (CV=30 V; CE=15 V; scanning from m/z 50 to 390), constant neutral-loss m/z 131 (CE=35 V; CE=30 V; scanning from m/z 132 to 225) and product-ion m/z 222 chromatograms with corresponding spectra of a sample taken at day 1 of biodegradation experiment of furathiocarb. Inserts: structures of furathiocarb and carbofuran. For details on biodegradation, SPE, LC and general ESI-MS(–MS) conditions, see Experimental.

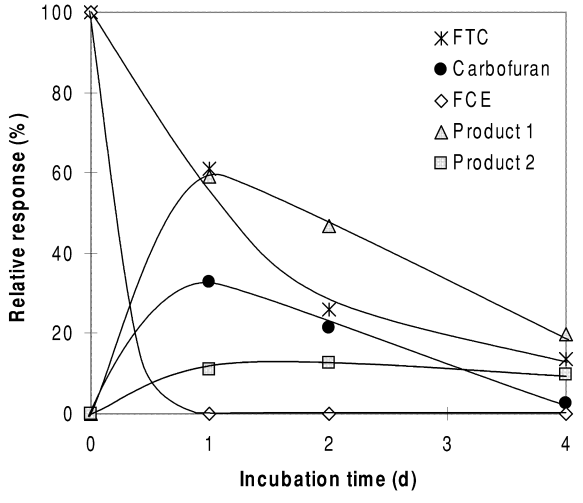


Fig. 7. Concentration versus time plot of the biodegradation of FCE with TPs products 1 and 2 and FTC with TP carbofuran. For details on biodegradation, SPE, LC and general ESI-MS(–MS) conditions, see Experimental.

cussed in this paper illustrate the practicability of the procedure for low-level, real-life (bio)degradation studies using all of the above scan modes, and additional structural information derived from adduct formation. In a subsequent step, the most sensitive transition can be selected for the SRM mode. This mode enables analyte monitoring down to the low ng/l level as was demonstrated in earlier studies on pesticides and TPs in surface and estuarine water samples [9,10].

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