Analysis of Chlorotriazines and Their Degradation Products in Environmental Samples by Selecting Various Operating Modes in Thermospray HPLC/MS/MS[†]

Joaquín Abián,[*,‡] Gaël Durand,[§] and Damià Barceló[§]

Departments of Neurochemistry and Environmental Chemistry, CID, CSIC, Jordi Girona 18-23,
08034 Barcelona, Spain

Positive ion (PI) and negative ion (NI) modes in thermospray HPLC/MS as well as the daughter ion and neutral loss modes in thermospray HPLC/MS/MS were applied for the characterization of atrazine, simazine, cyanazine, deethylatrazine, deisopropylatrazine, hydroxyatrazine, and chlorodiamino-s-triazine. PI mode gave always the $[M + H]^+$ ion as base peak. Filament-on NI mode, which was 2–3 orders of magnitude less sensitive, gave $[M + 2H]^{·-}$ ions except for cyanazine and hydroxyatrazine, which showed the $[M - H]^-$ ion. In the filament-off NI mode only cyanazine and hydroxyatrazine could be detected, both giving the $[M - H]^-$ signal. Detection limits by selected ion monitoring were below 400 pg injected on column. The collision-activated dissociation of the $[M + H]^+$ ions was used to obtain very selective modes of operation with detection limits in the 0.4–4-ng range. Application to the characterization of photolysis and transformation products of several chlorotriazines is presented.

INTRODUCTION

Chlorotriazines (Chart I) are broad-spectrum residual herbicides widely used for pre- and post-emergence weed control in corn, wheat, barley, and sorghum, as well as in railways, roadside verges, and golf courses (Barceló, 1991). After application, three different types of degradation affect pesticides: physical, e.g., photolysis and temperature; chemical, e.g., hydrolysis; and biological, e.g., microbial (Coats, 1991). These transformation processes control pesticide persistence in soil and yield different dealkylated metabolites (Durand et al., 1989; Pereira et al., 1990; Durand and Barceló, 1991). With regard to photodegradation studies, it is of environmental interest to establish the aquatic photolysis processes under field conditions, and as a consequence, laboratory experiments have been conducted showing mechanisms of dealkylation, hydroxylation, dehalogenation, and deamination (Durand and Barceló, 1990; Durand et al., 1991a). These studies on the fate of chlorotriazine pesticides in the environment have prompted the need for sensitive, specific methods for their determination.

The GC/MS analysis of chlorotriazine pesticides using a variety of ionization techniques has been approached in a number of ways. Applications reported so far include GC/MS with electron impact (EI) (Durand et al., 1989; Pereira et al., 1990; Durand and Barceló, 1991) and with positive and negative chemical ionization (PCI and NCI, respectively) (Durand and Barceló, 1991; Huang and Mattina, 1989). Confirmation of pesticide residues is accomplished by using two or three diagnostic ions in the selected ion monitoring (SIM) mode. When higher selectivity is needed, e.g., to avoid false-positive identifications in environmental samples, GC/tandem mass spectrometry (GC/MS/MS) is highly recommended.

Chart I. Trivial Names, Chemical Structures, and Retention Times (C_8 Column, HPLC Conditions As Indicated under Experimental Procedures) of the s-Triazine Standards Tested in This Work
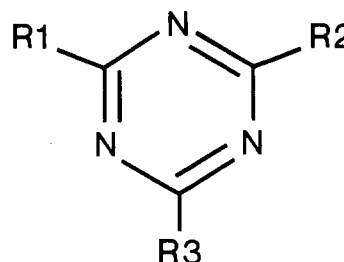
Compound name	Abrev.	RT	R1	R2	R3
Chlorodiamino-s-triazine	CAAT	2.6	NH_2	NH_2	Cl
Deisopropilatrazine	DIA	5.0	NH_2	NHEt	Cl
Hydroxyatrazine	HYAT	5.7	NHiPr	NHEt	OH
Deethylatrazine	DEA	7.1	NHiPr	NH_2	Cl
Cyanazine	CYAN	9.3	$NHC(CH_3)_2CN$	NHEt	Cl
Symazine	SIM	10.4	NHEt	NHEt	Cl
Atrazine	ATZ	12.2	NHiPr	NHEt	Cl

Collision-activated dissociation (CAD) MS/MS has scarcely been applied to pesticide analysis. Most work reported in this respect involves the use of triple-quadrupole MS in combination or not with GC and with electron impact (Hunt et al., 1983; Levsen, 1988; Johnson and Yost, 1985) or chemical ionization (Rostad et al., 1989; Roach and Carson, 1987; Hummel and Yost, 1986). This approach was used to confirm a variety of organic (Hunt et al., 1983; Levsen, 1988; Johnson and Yost, 1985), chlorotriazine (Rostad et al., 1989), organophosphorus (Roach and Carson, 1987; Hummel and Yost, 1986), and carbamate (Hummel and Yost, 1986) insecticides. By

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* Author to whom correspondence should be addressed.

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‡ Department of Neurochemistry.

§ Department of Environmental Chemistry.

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using this approach, but with a hybrid instrument, we have noticed an enhancement in selectivity vs the use of high-resolution MS resolving power of 10 000 in the determination of chlorotriazines in soil samples (Durand et al., 1992).

In the past few years, liquid chromatography/MS (HPLC/MS) has been applied to the determination of a variety of polar pesticides, such as organophosphorus, chlorotriazines, and phenylurea, and different metabolites of polar nature corresponding to photodegradation and microbial degradation processes (Durand and Barceló, 1990; Durand et al., 1991a). The use of thermospray HPLC/MS has the advantage of the direct analysis of polar hydroxy metabolites and direct injection of photodegraded water solution into the HPLC/MS system, whereas GC/MS needs several intermediate steps such as evaporation of water and derivatization prior the characterization of these very polar metabolites. However, in thermospray HPLC/MS there is a lack of structural information due to the scarce fragmentation. Current research efforts have been directed toward the use of different methods to enhance structural information in HPLC/MS. This will be very valuable since the identification of unknown pesticide metabolites formed during degradation processes can be unequivocally identified. These difficulties have prompted the need of using HPLC in combination with tandem MS which has been applied to the determination of chlorotriazine pesticides (Voyksner, 1987; Voyksner et al., 1990) and organophosphorus (Betowski and Jones, 1988) and carbamate insecticides (Chiu et al., 1989), all of which testify to an increasing use of tandem MS for screening different groups of pesticides in environmental matrices.

The lack of literature reports on the application of various modes of thermospray HPLC/MS/MS instruments to pesticide analysis and their corresponding metabolites in various matrices and in photodegradation studies has prompted us to carry out a study of this nature. Thus, the aims of this work were (1) to optimize a thermospray HPLC/MS/MS instrument using CAD for the characterization of the chorotriazine pesticides atrazine, simazine, cyanazine, their dealkylated degradation products deethylatrazine, deisopropylatrazine, and chlorodiamino-*s*-triazine, and hydroxyatrazine; (2) to compare the selectivity and sensitivity of HPLC/MS/MS with the use of different operating modes such as daughter ion and neutral loss; and (3) to assess the performance of different MS methods for the determination of various chlorotriazines and their metabolites in polluted soil samples and in aquatic photodegradation studies.

EXPERIMENTAL PROCEDURES

Chemicals. Pesticide grade solvents ethyl acetate, *n*-hexane, ethyl ether, and dichloromethane were supplied by Mallinckrodt (Paris, KY). Florisil (100–200 mesh) was purchased from Merck (Darmstadt, Germany). Cyanazine was supplied by Riedel-de-Haën (Seelze-Hannover, Germany), and atrazine and simazine were supplied by Polyscience (Niles, IL). Deethylatrazine and deisopropylatrazine were provided by Ciba-Geigy (Basel, Switzerland).

Ammonium acetate was obtained from Fluka (Ronkonkoma, NY). Water was purified through a Milli-Q system from Millipore-Waters (Bedford, MA). Other reagents and solvents were of analytical or chromatographic grade.

Soil Sample Preparation. Soil samples from the Ebro Delta (Tarragona, Spain) were used for the analysis. The average soil composition was 8% clays, 28% silt, 64% sand, and 2.5% organic matter, pH of 7.5. The concentrations of ATZ and DEA in these samples were calculated previously by GC (nitrogen–phosphorus detector) as 25 and 5 ng/g, respectively (Durand and Barceló,

1992). The soil samples were pretreated by using a modification of a procedure commonly used at our laboratory for the residue analysis of chlorotriazine pesticides (Durand et al., 1989; Durand and Barceló, 1990). Thus, 5 g of soil sample was freeze-dried and sieved through 120-μm mesh and Soxhlet-extracted with methanol for 12 h. The extracts were concentrated in a rotary evaporator to ca. 20–25 mL, evaporated to dryness, and redissolved in 400 μL of *n*-hexane.

Cleanup was carried out in glass columns (150 mm × 5 mm i.d.) filled with ca. 2 g of Florisil previously activated at 300 °C overnight, cooled, and deactivated with 2% water. The packing material was mixed with *n*-hexane and placed onto the glass column. The soil extracts in *n*-hexane (400 μL) were placed on top of the column and eluted with a 1:1 mixture of ethyl ether and *n*-hexane (20 mL) according to cleanup procedures reported elsewhere (Durand et al., 1989; Durand and Barceló, 1990). The fractions were evaporated to near dryness, and the remaining residue was dissolved in 500 μL of water/methanol (1:1 v/v). The volume injected into the LC/MS/MS was generally 20 μL.

Photolysis Experiments. Photodegradation studies were performed as described (Durand and Barceló, 1990; Durand et al., 1991a) in a suntest apparatus (Heraeus, Hanau, Germany) equipped with a xenon lamp. Distilled water samples, previously spiked at 10 μg/mL level with the pesticide in methanolic solution, were introduced into the suntest, and the temperature was set at 44 °C. At different periods of time, 20 μL of the solution was directly analyzed by thermospray HPLC/MS.

Liquid Chromatography/Mass Spectrometry. Chromatography was carried out using a Model SP8700XR Spectra Physics (San Jose, CA) pump and gradient programmer, a Model 7125 Rheodyne (Cotati, CA) injector with a 20-μL sample loop, and a 5-μm (20 × 0.46 cm) Spherisorb C$_8$ column (Phase Separations Inc., Norwalk, CT). For comparative purposes, a 5-μm (15 × 0.46) Spherisorb ODS column was also tested. The exit of the column was connected to the thermospray interface through a 0.5-μm in-line HPLC filter. Gradient elution was carried out using two different solvents: (A) a 0.05 M ammonium acetate solution in water (1% formic acid) and (B) a 0.05 M ammonium acetate solution in water/methanol (1:1 v/v, 1% formic acid). Chromatography was carried out at a flow rate of 1 mL/min using a solvent program starting at 40% solvent B. After injection, a linear gradient up to 55% B in 1 min and then to 100% B in 9 min was initiated. For chromatography of the standards the first gradient step was eliminated.

A Finnigan (San Jose, CA) TSQ 70 triple-quadrupole mass spectrometer equipped with a Finnigan thermospray source and interface was used. The interface temperature was set around 85–95 °C. The source and manifold temperatures were kept at 250 and 70 °C, respectively.

Acquisition was made at a rate of 0.5 scan/s in the scan mode and at 1 scan/s in the SIM or selected reaction monitoring (SRM) modes. Electron multiplier was set at 1400 (scan) or 1700 V (SIM mode and MS/MS). For CAD, argon gas was used as collision gas.

Optimization of CAD Parameters. Optimum collision energy and collision gas pressure were determined by monitorization of the abundance of selected daughter ions using different values of these parameters. For this purpose, consecutive injections of the tested pesticide were made using a 3-cm (10-μm) RP-18 cartridge and MPLC holder (Brownlee Labs, Santa Clara, CA). The isocratic conditions (MeOH concentration in the range 50–70% depending on the pesticide) were selected to obtain short elution times (10–30 s). The use of a column was necessary to obtain reliable results. When no column is used (thus working in the so-called flow injection mode), peak intensity and spectra appearance are usually unstable and influenced by the flow and eluent composition disturbances that the injection process originates. Collision energy was varied between injections in the range 0–120 eV with a constant collision gas pressure of 0.5 mTorr. Gas pressure was varied in the range 0–1 mTorr using a collision energy of 30 eV.

In addition, the effect of the voltage offset applied to Q3 (parameter MSMSC in the software of Finnigan) was also studied. Daughter ion spectra were acquired as indicated above using MSMSC values in the range 0–100 and at fixed 30-eV collision energy and 0.5-mTorr collision gas pressure.
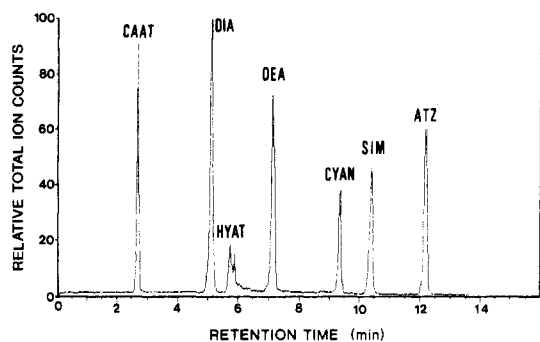
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Figure 1. Total ion chromatogram obtained by thermospray HPLC/MS (PI mode, filament-on mode) of a 200 ng (20 ng for HYAT) mixture of the seven pesticides. HPLC conditions were as indicated under Experimental Procedures.
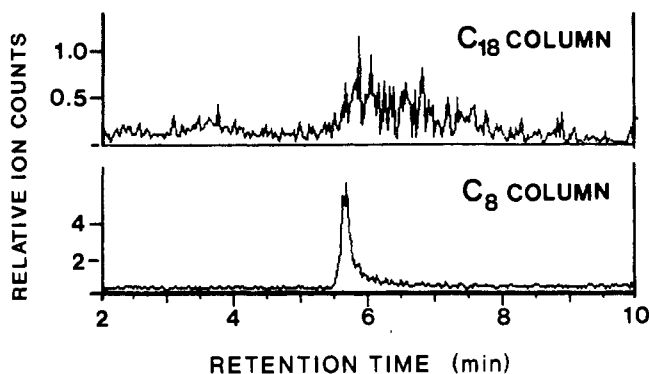

Figure 2. Ion chromatogram of a 20-ng injection of HYAT obtained using (a) C_{18} or (b) C_8 Spherisorb columns. HPLC conditions were as indicated under Experimental Procedures.

RESULTS AND DISCUSSION

HPLC/MS with PI and NI. Two different columns were tested for chromatography: Spherisorb C_{18} and Spherisorb C_8 columns. A typical thermospray chromatogram obtained in the PI mode using the C_8 column is shown in Figure 1. Although the C_{18} column allowed the chromatography of the chlorotriazines, the hydroxylated triazine HYAT showed poor comportment in this column (Figure 2), so the C_8 column was preferred for the chromatography of the pesticides.

The thermospray chromatogram in Figure 1 was obtained using the electron emitter filament (filament-on mode). There were no differences in the peak intensity between the filament-on and filament-off ionization modes. In the filament-on NI mode, all of the pesticides tested showed a response between 2 (HYAT) and 3 (the other triazines) orders of magnitude lower than in PI mode. With the filament off, only HYAT and CYAN could be observed with chromatographic peak areas that were the same (CYAN) or around 2 times higher (HYAT) than those obtained with the filament on.

Spectra in the PI mode (not shown) were characterized by the presence of a [M + H]+ base peak without other significative signals except for CYAN, which showed a [M + H – HCN]+ ion [m/z 214; relative abundance (RA) 8%]. No differences were observed in the spectra whether the filament was used or not. The loss of chlorine, which generates the base peak in the methane PCI of the triazines DIA, DEA, SIM, and ATZ (Rostad et al., 1989), was not observed in the thermospray spectra of these compounds.

In the case of CYAN, the thermospray spectra and the methane PCI spectra are very similar, although, in thermospray, the loss of HCN is less favored than in PCI. A signal at [M + 59]+ (tentatively the [M + CH₃COOH

Table I. Negative Ion Mode Thermospray Spectra of Triazines

M_r	compounds and ions (m/z; tentative identification)		filament on	filament off
145	CAAT			
	147	[M + 2H]	100	
	193	[M + 2H + FoH[a]]	4	
173	DIA			
	175	[M + 2H]	100	
	221	[M + 2H + FoH]	4	
197	HYAT			
	196	[M – H]	11	13
	242	[M + Fo]	100	100
187	DEA			
	189	[M + 2H]	100	
240	CYAN			
	174	[M – 66]	50	
	221	[M + 17 – HCl]	8	24
	239	[M – H]	100	100
	242	[M + 2H]	25	
	285	[M + Fo]	7	11
201	SIM			
	203	[M + 2H]	100	
	249	[M + 2H + FoH]	3	
173	DIA			
	175	[M + 2H]	100	
	221	[M + 2H + FoH]	4	
215	ATZ			
	217	[M + 2H]	100	
	263	[M + 2H + FoH]	5	

[a] FoH, formic acid.

+ NH₃ – H₂O + NH₄]+ ion) was previously observed as the base peak in the thermospray spectra of CYAN (Barceló, 1988) or with a 10% RA in the spectra of DIA, hydroxysimazine (HYSIM), and CYAN (Durand et al., 1991a). In our conditions, which used similar experimental conditions but a different instrument (a Finnigan TSQ instead of a HP 5988 A single quadrupole), these signals show abundances always under the 1% RA.

In the negative ion mode (Table I), the [M + Fo]⁻ (Fo being formate) and [M – H]⁻ ions were observed as the base peaks for HYAT and CYAN, respectively, regardless of the use of the electron emitter filament. The ion at m/z 221 in CYAN showed a isotopic pattern indicative of the loss of the chlorine atom and was observed in both filament modes. The structure of this ion could be tentatively explained as [M + OH – HCl]⁻. In the filament-on mode, CYAN showed some additional signals that could arise from reduction and electron capture processes: ions at m/z 242 (M_r + 2) and at m/z 174 (M_r – 66). All of the other chlorotriazines tested also showed, in the filament-on mode, the signal at m/z M_r + 2 that could correspond to the [M + 2H]·⁻ ion. This signal was the base peak in the spectra of ATZ, CAAT, SIM, DIA, and DEA. The ion at m/z 174 could be explained by reduction and loss of the cyanopropyl lateral chain (m/z at M_r + 2 – 68).

In previous works that used a mobile phase consisting of water/acetonitrile mixtures containing 0.05–0.1 M ammonium acetate and a Hewlett-Packard thermospray source, a [M – H]⁻ ion was observed as the base peak in the thermospray spectra of ATZ, DIA, and DEA (Barceló, 1989) and the [M]·⁻ and [M + Cl]⁻ ions in the spectra of CYAN (Durand et al., 1991b). These ions were not observed in our conditions. It is interesting to note that for some chlorophenols some differences in the type of negative ion spectra have been observed depending on the source and interface used for thermospray (Vreeken et al., 1990). In the case of a Hewlett-Packard instrument the expected [M – H]⁻ ion was the base peak. When a Finnigan MAT interface and source were used, then the [M + H]⁻ ion was the base peak. These observations and
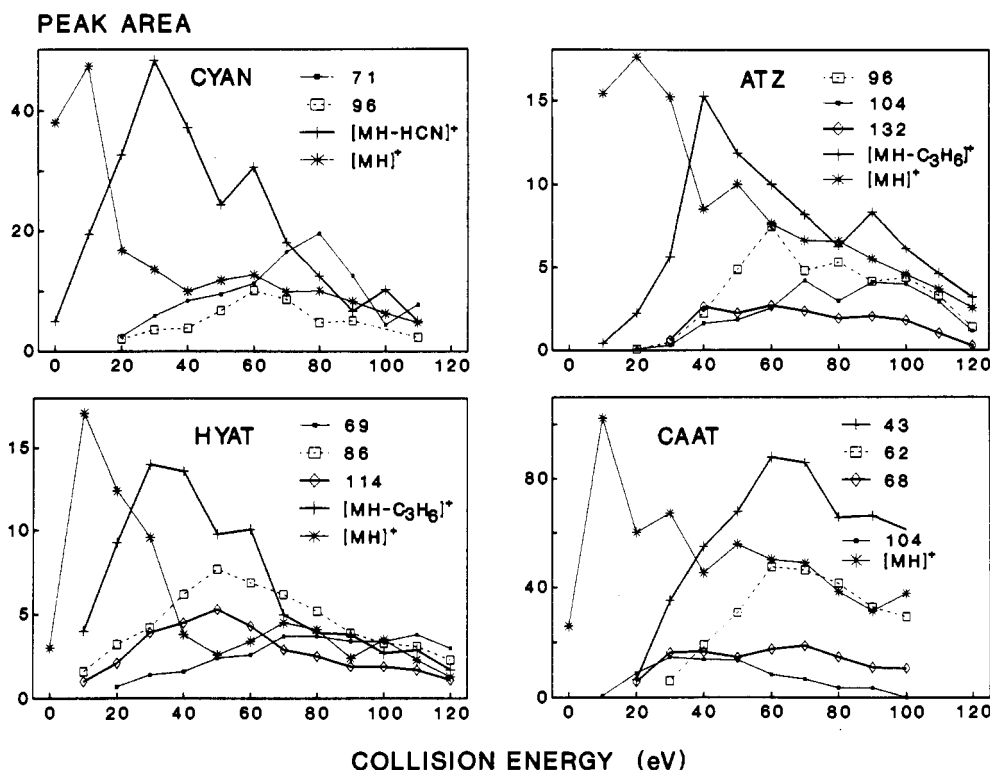
Figure 3. Effect of the collision energy on the intensity of the parent and some daughter ions in the CAD spectra of CYAN, ATZ, HYAT, and CAAT.

our present results suggest the existence of a reduction path for these compounds generating M + 2H species that could ionize, depending on the particular structure, by proton abstraction yielding the [M + H]⁻ ion or by electron capture yielding the [M + 2H]·⁻ ion radical. The chloride attachment reported for CYAN and attributed to chloride availability from pyrolysis of the analyte (Durand et al., 1991b) was not observed for any of the triazines tested in our system.

HPLC/MS/MS. *Daughter Ion (DAU) Scan Mode.* The spectra corresponding to the CAD of the [M + H]⁺ ion of the seven pesticides are show in Table II.

The pesticides bearing the isopropyl group showed abundant ions derived from the loss of this moiety (ions at m/z 104, 174, 146, and 156 for CAAT, ATZ, DEA, and HYAT, respectively). The loss of the ethyl group was less favored, and only DIA and SIM showed low abundance ions due to this process. Elimination of HCN in the case of CYAN affords an intense daughter ion at m/z 214. A signal at m/z 174 formed from the elimination of the lateral chain ([M + H − HCN − CH₂CCH₂]) was also observed for CYAN.

Other daughter ions at low m/z correspond to ring-opening processes. The ion at m/z 71 was common to all compounds containing the ethyl group and could correspond to the [EtNHCN + H]⁺ ion. In the case of ATZ, DEA, and HYAT the equivalent [iProNHCN + H]⁺ ion at m/z 85 was not observed, probably due to the more favored initial elimination of the isopropyl group. The ion at m/z 132 was common to all of the chlorinated pesticides with an ethyl substituent and could correspond to the [EtNHC(NH)NCCl]⁺ moiety. Loss of HCl from this ion generates the signal at m/z 96, and loss of the CH₂CH₂ group generates the signal at m/z 104. DEA and CAAT, originally without the ethyl substituent, also showed the ion at m/z 104. These spectra are qualitatively similar to those previously reported using thermospray HPLC/MS/MS (Voyksner, 1987) or PCI GC/MS/MS (Rostad et al., 1989).

Table II. Relative Intensities Observed in the Triazine TSP MS/MS Daughter Ion Spectra

m/z	DIA	ATZ	SIM	CYAN	DEA	CAAT	HYAT
43	35					100	
62	15		11			57	
68	17			6	15	30	
69							23
71	28	18	100	18			17
79	34	15		6	24	22	
86							100
96	56	35	30	17			
97							36
104	40	17	15	12	30	25	
110					10[a,c]	9[c]	
114							34
124			49				
128							6[a,b]
132	100	21	50	7			
138	16[c]	7[a,c]					
144						14	
145						26	
146	24[b]	12[a,b]			100[a]	PAR[d]	
156							94[a]
166			10[c]				
172	10						
173	37						
174	PAR	100[a]	7[b]	11			
188					PAR		
198							PAR
200			7				
202			PAR				
214				100[e]			
215		12					
216		PAR					
241				PAR			

[a] Loss of the isopropyl group. [b] Loss of the ethyl group. [c] Loss of HCl. [d] PAR, [M + H]⁺ parent ion. [e] Loss of HCN.

The DAU spectra of HYAT showed characteristic ions due to the presence of the oxygen instead of the chlorine as the signals at m/z 114 and at m/z 86 (114 − CH₂CH₂)
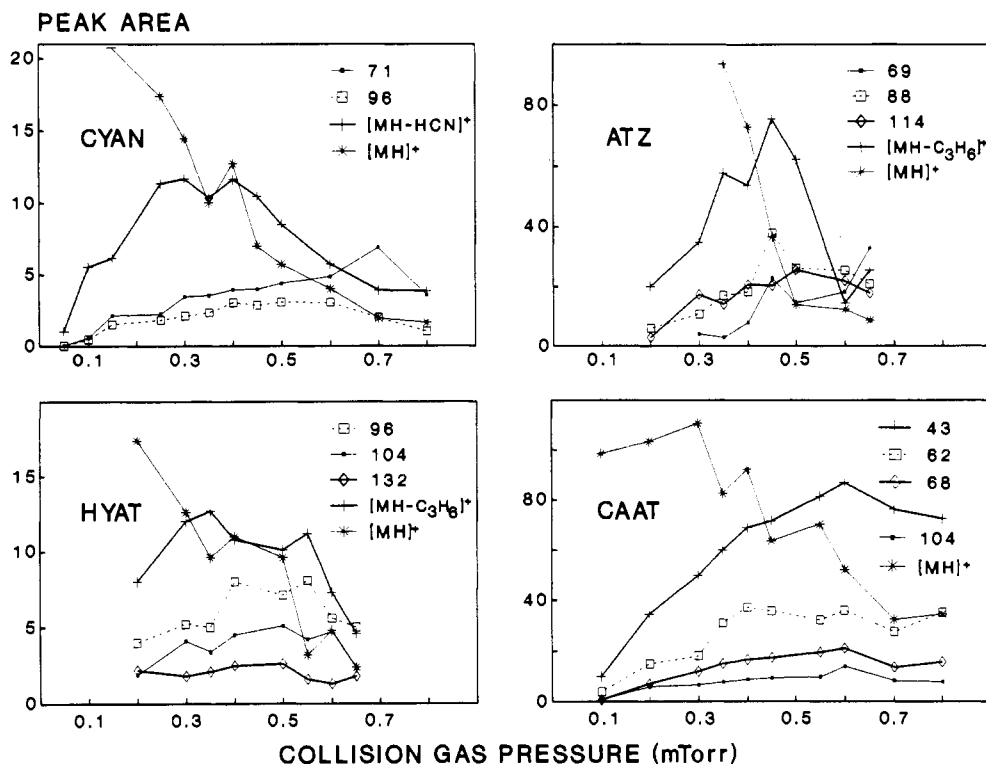
PEAK AREA

Figure 4. Effect of the collision gas pressure on the intensity of the parent and some daughter ions in the CAD spectra of CYAN, ATZ, HYAT, and CAAT.

that could be equivalent to the ions at m/z 132 and at m/z 104, respectively, in the spectra of the chlorinated compounds. The ions at m/z 97 and 69 were also characteristic of HYAT. Both were 17 amu below the ions at m/z 114 and 86, respectively, suggesting a similar structure but with a NH_3 moiety left.

Optimization of HPLC/MS/MS. The effect of collision energy and collision gas pressure on the CAD mass spectra of the parent [M + H]$^+$ ion is shown in Figures 3 and 4 for CYAN, ATZ, HYAT, and CAAT.

Optimum collision energy depends on the compound and the selected transition. Loss of HCN, which affords the dominant daughter ion in the spectra of CYAN, optimized at 30 eV; elimination of the isopropyl group from the parent ions of ATZ and HYAT optimized around 40 eV. Ring-breakdown processes take place at higher energy as can be seen in the case of the ions at m/z 71 in CYAN (maximum at 80 eV) or at m/z 69 in HYAT (maximum above 70 eV). In the case of CAAT, which does not have any lateral chain, the major daughter ion in the spectra (m/z 43) optimized at 60 eV. These results indicate that, for maximum sensitivity in the thermospray HPLC/MS/MS of mixtures of these triazines, a suitable collision energy program during the analysis time is required.

All of the parent ions in Figure 3 showed a lower intensity at 0 eV in comparison with the intensity at 10 eV. This unexpected result is due to the method used for instrument tuning that optimizes the lens voltages at a fixed collision cell offset of 10 V. When the 0-eV collision energy is selected, the ions entering the collision cell are halted.

Optimum collision gas pressure was also dependent on the compound and the selected fragmentation (Figure 4). In most cases a collision pressure around 0.4–0.5 mTorr afforded intense daughter ions that originate from HCN or lateral chain losses. When fragmentation implies ring opening, a higher pressure is needed as for the ion at m/z 43 of CAAT, which showed at maximum at 0.7 mTorr.
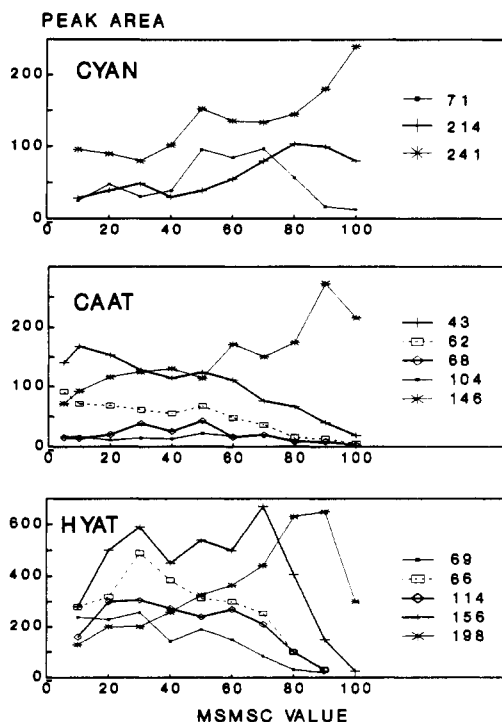
PEAK AREA

Figure 5. Effect of the quadrupole offset correction parameter MSMSC on the intensity of the parent and some daughter ions in the CAD spectra of CYAN, CAAT, and HYAT.

The effect of the parameter MSMSC in the abundance of some selected daughter ions is presented in Figure 5 for CYAN, CAAT, and HYAT. The MSMSC parameter is a correction factor of the offset voltage applied to the third quadrupole (Q3). The parameter MSMSC controls the kinetic energy of the ions entering Q3. When a parent collides on Q2, the fragments formed in the process show energies that are functions of the parent initial energy and the parent–daughter mass ratio. For optimum

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Table III. Detection Limits for the Triazine Standards in the Different Acquisition Modes

	CAAT, ng (S/N)[a] (trnstn)[b]	DIA	HYAT	DEA	CYAN	SIM	ATZ
SIM[c]	0.4 (11)	0.4 (20)	2 (13)	0.4 (16)	0.4 (9)	0.4 (22)	0.4 (29)
DAU (SRM)[d]	0.4 (11)	4 (15)	4 (9)	0.4 (10)	4 (26)	4 (20)	0.4 (5)
	(146–43)	(174–132)	(198–156)	(188–146)	(241–214)	(202–132)	(216–174)
NL 42[e]	4 (8)	4 (10)	4 (6)	4 (30)	ND	ND	4 (12)
NL 28[e]	ND	40 (5)	ND	ND	ND	40 (4)	ND
NL 42 (SRM)[f]	4 (13)	4 (22)	4 (8)	0.4 (5)	ND	ND	0.4 (5)

[a] Calculated from the corresponding ion chromatogram. [b] Transition for selected reaction monitoring. [c] Alternate and continuous monitorization of the seven [M + H]⁺ ions. [d] Alternate and continuous monitorization of theseven indicated transitions. [e] Scan range (Q1) 100–250 amu, 1 scan/s. [f] Q1 focuses alternatively and continuously on the seven [M + H]⁺ ions.

resolution and transmission on Q3 these fragment ions must be accelerated to acquire the optimum kinetic energy. As can be seen in Figure 5, this factor affects total and relative abundance of the parent and daughter ions and thus has an important influence in the final aspect of the daughter ion spectra.

Neutral Loss (NL) Scan Mode. The NL mode constitutes a very selective technique for the analysis of compound classes that show common neutral losses by CAD. In the NL mode, both analyzers (Q1 and Q3) scan the selected mass range. The scan of Q3 is delayed over Q1, which results in a constant mass difference between the ions reaching the detector and the corresponding parents. This method could be more sensitive than the DAU mode because it spent all of the acquisition time scanning a constant neutral loss instead of swiching between different parent ions as in the first case. The pesticides tested showed two major neutral losses by elimination of the alkyl substituents: loss of C_3H_6 (42 amu) and loss of C_2H_4 (28 amu). The loss of HCN (27 amu) was specific for CYAN and was not used here. Acquisition using the 42 amu NL allowed the detection of the pesticides bearing the isopropyl group (DEA, ATZ, and HYAT) as well as the triazines CAAT and DIA. The loss of 42 amu from the [M + H]⁺ ion of CAAT and DIA could be due to the elimination of a CN_2H_2 group corresponding to a moiety containing the nonsubstituted primary amine. As can be seen in Table II, the loss of CH_2CH_2 was less favored than the elimination of the isopropyl group and only DIA and SIM showed some response using the 28 amu NL (Table III). The NL spectra of these compounds showed only signals due to the [M + H]⁺ ion. In the case of chlorotriazines, these ions exhibited a pattern indicative of the presence of the chlorine atom (Figure 6), adding specificity to the method in comparison with the DAU scan mode.

Limits of Detection. The detection limits obtained by thermospray HPLC/MS and HPLC/MS/MS of the seven pesticides in the different acquisition modes are depicted in Table III. Detection limits for some chlorotriazines have been reported to be in the 5–20-ng range for full-scan data and in the 1–10-ng range for the DAU full-scan data (Voyksner, 1987). In this work, the best responses were obtained working in the selected ion monitoring (SIM) mode (continuous monitorization of the seven [M + H]⁺ ions), where detection limits were below 400 pg for all of the chlorotriazines. MS/MS methods give always higher detection limits due to the ion intensity losses that take place during the collision process. Despite this, the also higher specificity of MS/MS could overcome the loss of sensitivity, especially when complex real samples are analyzed. These detection limits are, except for the 28 amu NL mode, lower than those reported previously by HPLC/UV (Beilstein et al., 1981; Vermeulen et al., 1982; Pacakova et al., 1988). More recently, and using a diode array UV detector, Wenheng reported detection limits
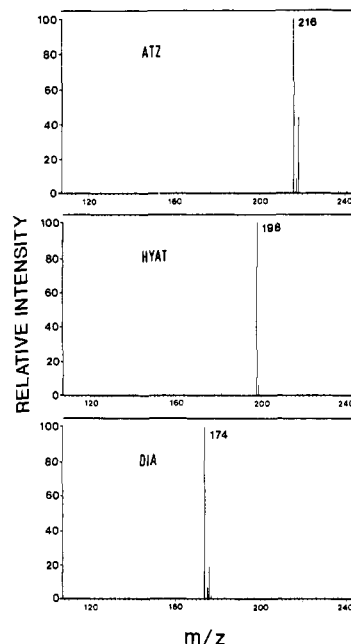

Figure 6. Neutral loss (42 amu) spectra of HYAT, DIA, and ATZ. Collision energy was 50 eV and collision gas pressure 0.5 mTorr.

that can be estimated in the same order (0.1–0.5 ng of ATZ injected on column) as those reported here for the SIM mode (Wenheng et al., 1991). Rostad et al. (1989), using PCI GC/MS/MS, reported detection limits (S/N = 3) of 5 (DIA) and 0.1 ng (DEA, SIM, ATZ) when working in the 28 amu (DIA), 42 amu (DEA and ATZ), or 78 amu (SIM) NL mode. In a recent GC/MS/MS work, we reported (Durand et al., 1992) much lower detection limits using a multiple reaction monitoring procedure on a hybrid instrument (5–24 pg).

ENVIRONMENTAL APPLICATIONS

The behavior of ATZ, DEA, and DIA in solutions submitted to UV radiation and of ATZ in soil samples was studied as indicated under Experimental Procedures. Possible degradation processes to be considered were dealkylation, dechlorination, deamination, and dechlorination and hydroxylation (Durand et al., 1989, 1992; Pereira et al., 1990; Durand and Barceló, 1990). A tentative photodegradation pathway of various chlorotriazines has been suggested in a previous paper (Durand and Barceló, 1990). Since thermospray HPLC/MS was used, the presence of many of the putative degradation intermediates could not be proved due to the lack of structural information.

Photodegraded Solutions. Characteristic thermospray chromatograms corresponding to photodegraded DEA and DIA solutions are shown in Figure 7. The CAD DAU spectra of the compounds eluting at 3.4 and 7.9 min
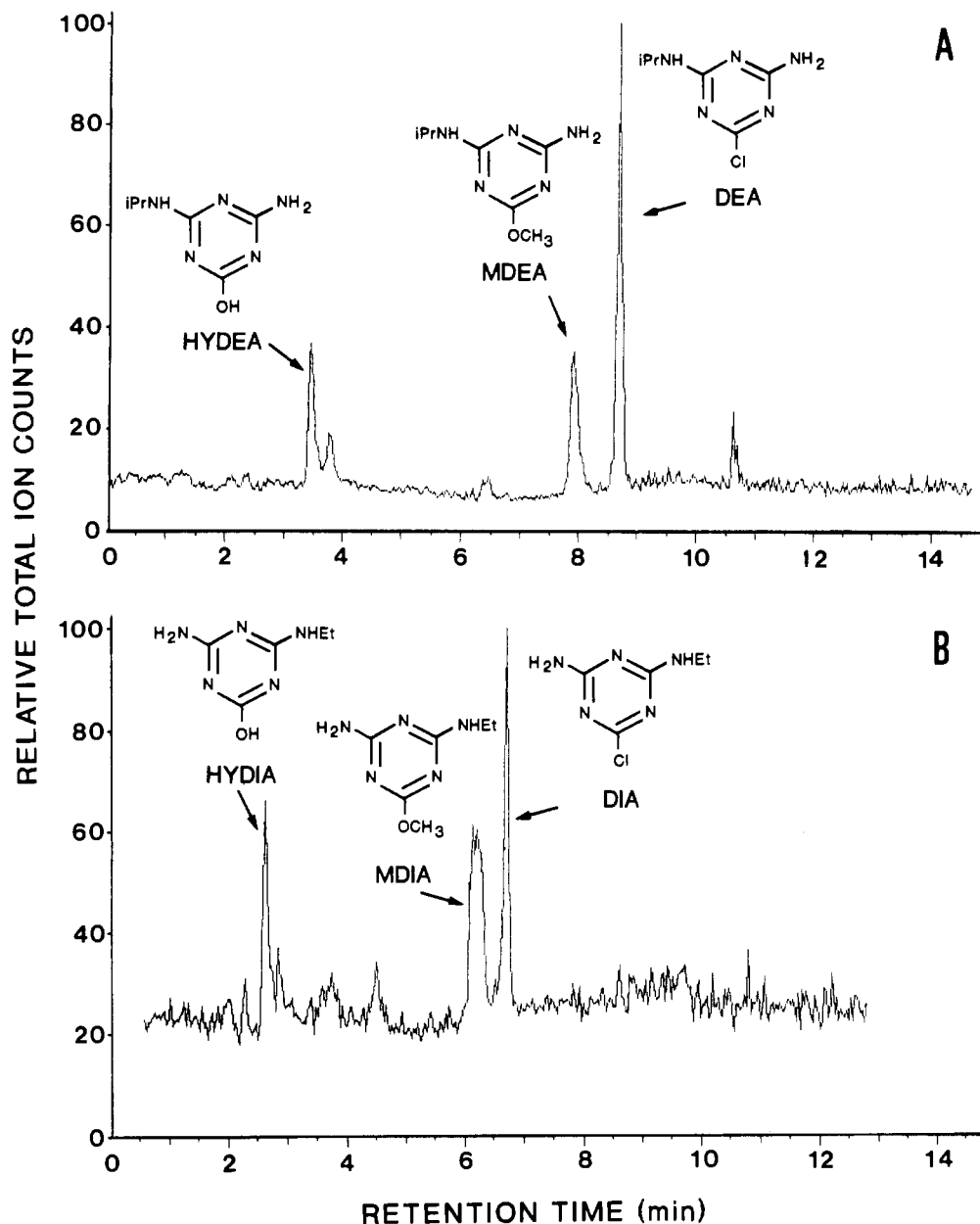
Figure 7. Thermospray HPLC/MS chromatogram (SIM mode, positive ions) of DEA and DIA solutions submitted to photodegradation. HPLC conditions were as described under Experimental Procedures.

(DEA sample) and at 2.2 and 5.6 min (DIA sample) are shown in Figure 8. These compounds were tentatively characterized as the hydroxylated (HYDEA and HYDIA; $[M + H]^+$ ion at m/z 170 and 156 amu, respectively) and methoxylated (MDEA and MDIA; $[M + H]^+$ ion at m/z 184 and 170 amu, respectively) derivatives of these compounds (Table IV). Methoxylated derivatives originate from methylation of the hydroxy compounds in the samples due to the presence of methanol as a solvent additive. The CAD DAU spectra of the putative $[M + H]^+$ confirmed the assigned structures. The expected 42-amu losses indicative of the isopropyl group in the DEA derivatives were observed at m/z 128 (HYDEA) and 142 (MDEA). The ion at m/z 86 that was shown to be characteristic of the HYAT structure (Table II) was also present in the spectra of HYDEA and HYDIA.

The MS/MS analysis of the DEA samples using the 42 amu NL is shown in Figure 8. Although some sensitivity is lost, a very selective detection of HDEA, HYDEA, MDEA, and DEA could be obtained. The method was

not useful in the case of DIA metabolites due to its lower response to the 42 or 28 amu NL.

The ion traces and HPLC data of the compounds detected in the ATZ samples submitted to the suntest method are shown in Figure 9 and Table IV. As in the other cases, the major photolysis pathway is dechlorination, affording either hydroxylated, methoxylated, or hydrogenated compounds. Dealkylation processes are responsible for the presence of DIA and DEA in the ATZ sample and for CAAT in the DEA and DIA samples. Other minor compounds detected in the samples such as the dechlorinated CAAT (HCAAT) in the DEA and DIA samples and the dechlorinated DIA (HDIA) in the ATZ samples are the result of both dealkylation and dechlorination processes.

Soil Samples. The thermospray HPLC/MS ion traces of some ATZ metabolites from an extracted soil sample are shown in Figure 10. The chromatogram was obtained in the SIM mode focusing continuously on eight different $[M + H]^+$ ions (only three are shown). In this case, where a high number of ions on a narrow mass range must be

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Table IV. Compounds Detected by Thermospray HPLC/MS in the Solutions Spiked with DIA, DEA, and ATZ and Submitted to Photodegradation and in the Soil Samples

compound[a]						photodegradation			
R1	R2	R3	abbrev	RT[b]	M_r	DIA	DEA	ATZ	soil
NH₂	NH₂	H	HCAAT	2.3	112	×	×		
NH₂	EtNH	H	HDIA	3.6	140	××		×	
NH₂	NH₂	Cl	CAAT	3.2	146	×	×		
iPrNH	NH₂	H	HDEA	5.1	154		××	××	
NH₂	EtNH	OH	HYDIA	2.6	156	×××			
iPrNH	NH₂	OH	HYDEA	3.4	170		×××		
NH₂	EtNH	OMe	MDIA	6.2	170	×××			
NH₂	EtNH	Cl	DIA	6.7	174	×××		×	×
iPrNH	EtNH	H	HAT	9.9	182		××		
iPrNH	NH₂	OMe	MDEA	7.9	184		×××		
iPrNH	NH₂	Cl	DEA	8.7	188		×××	×	××
iPrNH	EtNH	OH	HYAT	7.5	198			×××	
iPrNH	EtNH	Cl	ATZ	13.6	216			××	×××

[a] See Chart I. [b] Retention time in the HPLC conditions described under Experimental Procedures. The number of crosses is indicative of the relative abundance range of the compound in the chromatograms: ×××, 30–100%; ××, 3–30%; ×, <3%.
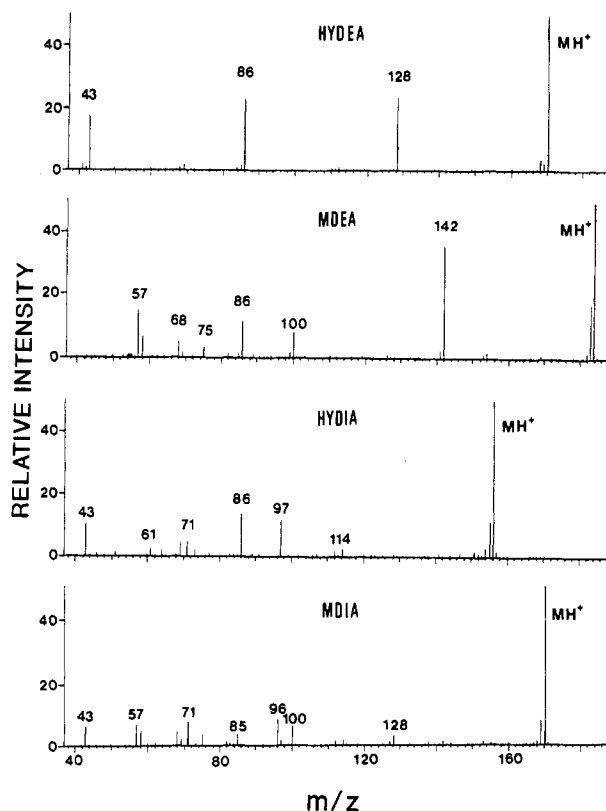

Figure 8. Daughter ion spectra of the corresponding [M + H]⁺ ion from HYDEA, MDEA, HYDIA, and MDIA.

Figure 9. Thermospray HPLC/MS ion chromatograms of ATZ derivatives from a solution submitted to photodegradation. HPLC conditions were as described under Experimental Procedures.

Figure 10. Thermospray HPLC/MS total ion chromatogram and ion chromatograms (SIM mode) of the ATZ degradation products from an extracted soil sample. HPLC conditions were as described under Experimental Procedures.

monitored, there is no special sensitivity advantage using SIM over a short-range scan mode (not shown) and, in fact, the latter technique should be preferred because of the greater information obtained. Whether the scan or SIM technique was used, only the dealkylated derivatives of ATZ, DEA, and DIA could be detected. A previous analysis of these samples using GC with a nitrogen-phosphorus detector (Durand and Barceló, 1992) indicated the presence of ATZ and DEA in a ratio ca. 5:1 but the method failed to detect the presence of DIA. Using TSP, DIA was clearly detected and the ratio ATZ:DEA:DIA could be calculated from the ion traces as ca. 50:10:1. Chromatograms obtained in the 42 amu NL scan mode and in the DAU SRM mode are also shown in Figure 11. DIA could not be detected using the 42 amu NL scan mode and is near the detection limit of the DAU SRM mode. Despite this, both methods allowed a very specific characterization of ATZ and DEA.
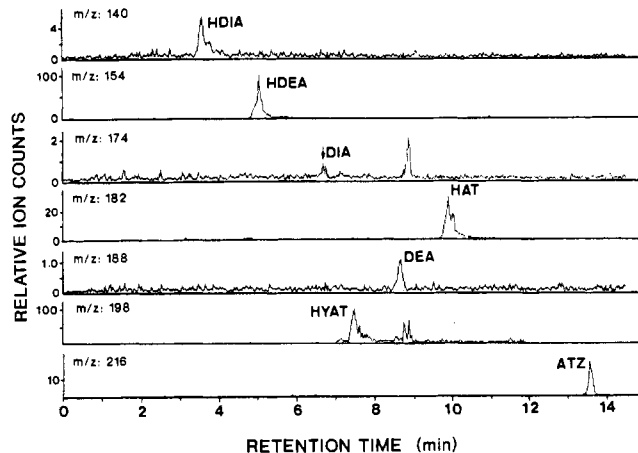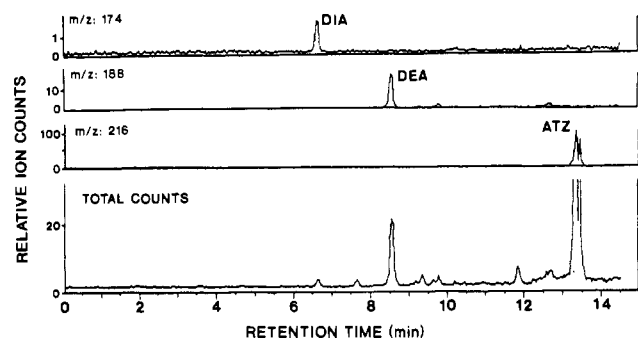
CONCLUSIONS

The use of thermospray HPLC/MS/MS offers valuable structural data and overcomes the lack of information in the spectra obtained under thermospray HPLC/MS conditions. The proper use of this technique with collision-activated dissociation has required the optimization of the collision energy, collision gas pressure, and quadrupole offset correction parameter for each of the studied chlorotriazines.

Thermospray HPLC/MS/MS with SRM, using either the daughter ion mode or the neutral loss of 42 (C_3H_6) amu, was demonstrated to be a useful technique for the characterization of chlorotriazines in aquatic photodeg-
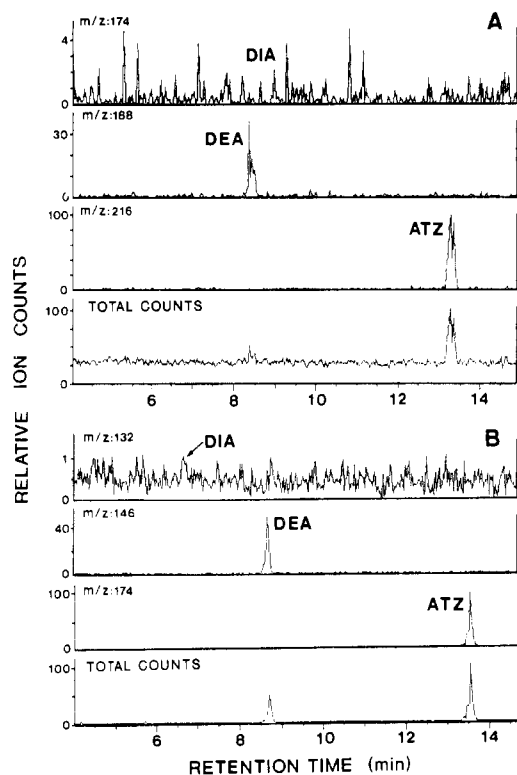
Figure 11. Thermospray HPLC/MS/MS ion chromatograms of ATZ degradation products from an extracted soil sample: (A) 42 amu neutral loss mode (100–250 amu scan range); (B) daughter ion mode (selected reaction monitoring as in Table III).

radation studies carried out under laboratory conditions and in real polluted soil samples. Both modes of operation can easily detect the different pesticides and the various transformation products at the low nanogram level, with limits of detection between 0.4 and 4 ng. Many of the matrix interferences present in the soil were avoided by the use of these modes.

By the use of thermospray HPLC/MS/MS in the daughter ion mode it was possible to identify various photoproducts of irradiated deethylatrazine and deisopropylatrazine. Such an identification proves a previous hypothesis of the authors of this paper that tentatively identified some of these compounds with thermospray HPLC/MS, but we cannot prove completely their presence due to the lack of structural information (Durand and Barceló, 1990).

Future experiments will include the use of on-line precolumn techniques in combination with thermospray HPLC/MS/MS, thus allowing preconcentration and determination of the different polar photodegradation products. In this way, the limits of detection will be improved and it will be possible to unequivocally identify more photoproducts by the information obtained by tandem MS. This approach could be a useful tool for a better understanding of the transformation processes of pesticides in real word environmental samples.

ACKNOWLEDGMENT

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