Journal of Chromatography, 502 (1990) 275–286
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 22 103

Determination of chlorotriazines and their photolysis products by liquid chromatography with photodiode-array and thermospray mass spectrometric detection

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(First received June 21st, 1989; revised manuscript received September 6th, 1989)

SUMMARY

Liquid chromatography with diode-array (LC–DA) and positive ion (PI) mode thermospray LC–mass spectrometry (LC–MS) were used for the determination of atrazine, cyanazine, simazine, deethylatrazine and deisopropylatrazine and their photolysis products. The LC analyses were performed on RP-18 columns using methanol–water (70:30) or methanol–water (70:30) + 0.05 *M* ammonium formate for LC–DA or LC–MS, respectively. The main degradation products observed after photolysis experiments with the suntest apparatus at 44°C were the corresponding hydroxytriazines, which could easily be differentiated from their parent compounds by the structured and distinctive UV spectral information given by LC–DA. The degradation rates after 5 h of irradiation varied from atrazine, deethylatrazine and deisopropylatrazine, which rapidily disappeared, to cyanazine and simazine, with 22 and 36%, respectively, remaining. Special emphasis was devoted to the characterization of the different photolysis products. By employing PI mode thermospray LC–MS, the identification of several photodegradation products such as the hydroxy, 2-H and 2-methoxy analogues was feasible, showing the $[M + H]^+$ ion as the base peak and a second abundant ion corresponding to $[M + CH_3OH + H]^+$. The combined approach allows the identification directly after the photolysis experiments of the different solutions containing chlorotriazines, which is a clear advantage over current methods that require isolation of the photolysis products and elimination of water prior to MS characterization.

INTRODUCTION

Chlorotriazine herbicides are of interest owing to their wide use as herbicides for pre- and post-emergence weed control and as algicides[1,2]. The potential for contamination of water and sediment samples with pesticides is high owing to their physico-chemical properties such as water solubility >30 mg/l, adsorptivity K_{oc} (partition coefficient between soil organic carbon and water) <300–500 and hydrolysis

0021-9673/90/$03.50

half-life >25 weeks[2], and residue levels between 0.01 and 30 ppb[a] have been reported[2–6]. The degradation of these pesticides after application depends on several factors such as hydrolysis, photolysis and microbial activity. The influence of photolysis in different aquatic systems has been widely studied under laboratory conditions as one of these main degradation processes of different chlorotriazines[7–9].

It is of environmental interest, in these photodegradation processes, to establish the photodegradation pathways and to determine the different photodegradation products obtained. In this sense, most of the methods reported in the literature have used gas chromatography (GC) in combination with nitrogen–phosphorus (NPD) and mass spectrometric (MS) detection, and these are the most extensively used methods for the analysis of chlorotriazines[1,9–13].

Considering that some of the photodegradation products of the chlorotriazines are the corresponding hydroxy metabolites, it is of interest to directly determine the photodegraded samples by liquid chromatography (LC). Advantages claimed for LC, with UV detection, are its suitability for the analysis of thermally labile compounds such as cyanazine[14] and polar herbicides and their degradation products[15–19]. Other detection methods such as electrochemical[19] and MS[20–22] have also been employed with LC.

In the work described here, LC with diode-array detection (LC–DA) and thermospray LC–MS have been applied for the first time to the characterization of atrazine, cyanazine, deethylatrazine, deisopropylatrazine and simazine breakdown products obtained under photolysis using the suntest. Solutions of the decomposed analytes in distilled water were directly injected into the LC–DA and LC–MS systems, which is a clear advantage over GC methods that require evaporation of water and derivatization of the polar degradation products prior to injection into the GC system. The use of LC–DA and LC–MS offers good selectivity and sensitivity and the possibility of differentiating between the chlorotriazines and their degradation products. In this sense and in previous papers we have demonstrated the utility of LC–DA[23] and thermospray LC–MS[21,22] for characterizing the different chlorotriazines.

EXPERIMENTAL

Chemicals

HPLC-grade water from Riedel-de-Haën (Seelze-Hannover, F.R.G.) and methanol from Merck (Darmstadt, F.R.G.) were passed through a 0.45-μm filter (Scharlau, Barcelona, Spain) before use. Analytical-reagent grade ammonium formate was obtained from Fluka (Buchs, Switzerland), cyanazine (CYAN) from Riedel-de-Haën, atrazine (ATRZ) and symazine (SIM) from Polyscience (Niles, IL, U.S.A.), hydroxyatrazine (HA) and chlorodiamino-*s*-triazine (CAAT) from Promochem (Wesel, F.R.G.). Deethylatrazine (DEA), deisopropylatrazine (DIA), deethylhydroxyatrazine (DEHA) and deisopropylhydroxyatrazine (DIHA) were gifts from Ciba-Geigy (Basle, Switzerland).

Chromatographic conditions

An eluent flow-rate of 1.0 ml/min was delivered by a Model 64 high-pressure

[a] Throughout this article, the American billion (10^9) is meant.

pump from Knauer (Bad-Homburg, F.R.G.) coupled with a Chrom-A-Scope rapid-scanning UV–VIS detector from Barspec (Rehovot, Israel). Samples were injected via a 20-μl loop from Rheodyne (Cotati, CA, U.S.A.). Cartridge columns (22 cm × 4.6 mm I.D.) from BrownLee, Applied Biosystems (Santa Clara, CA, U.S.A.) and LiChroCART cartridge columns (12.5 cm × 4.0 mm I.D.) from Merck (Darmstadt, F.R.G.), were packed with 5-μm Spherisorb ODS and 5-μm LiChrospher 100 RP-18, respectively, from Merck.

The LC–DA and LC–MS experiments were performed separately. For LC–DA analysis the eluent was methanol–water (70:30) with DA detection at 220 nm and for the LC–MS experiments 0.05 *M* ammonium formate was added to the eluent as an ionizing additive.

Mass spectrometric analysis

A Hewlett-Packard (Palo Alto, CA, U.S.A.) Model 5988A thermospray (TSP) LC–MS quadrupole mass spectrometer and a Hewlett-Packard Model 35741B instrument for data acquisition and processing were employed. The TSP temperatures were stem 100°C, tip 178°C, vapour 194°C and ion source 296°C. In all the experiments the "filament-on" mode was used, in which conventional positive ion chemical ionization can be carried out by using the vaporized LC solvent, which is ionized by an electron beam, as the chemical ionization reagent gas.

Photolysis apparatus

In order to obtain stable and reproducible results in the photodegradation studies, a suntest apparatus from Heraeus (Hanau, F.R.G.) equipped with a xenon lamp was used. The wavelength range varies from 300 to 800 nm, which represents radiation very close to natural sunlight, and the temperature was set at 44°C.

Distilled water samples were spiked with 0.1 ppm of the different chlorotriazine pesticides in methanolic solution and kept in a quartz reaction reservoir and subsequently introduced into the suntest apparatus. At different periods of time, an unknown amount of the solution was analysed by LC–DA and thermospray LC–MS. Although most of the experiments were performed using distilled water containing 3–4% of methanol for solubility reasons, additional experiments with artificial sea water containing humic acids were also carried out. These experiments, closer to real environmental situations, were undertaken in order to examine the enhancement of photodegradation by using this matrix, as previously reported by Fukuda *et al.*[24].

RESULTS AND DISCUSSION

Photodegradation studies by LC with diode-array detection

The photolysis studies of the chlorotriazine herbicides in water samples containing 3–4% methanol were performed as described under Experimental by using the suntest apparatus. The degradation of DIA, DEA, CYAN, SIM and ATRZ was followed by LC–DA. The results obtained after degradation of solutions containing between 111 and 232 μg/l of the chlorotriazine herbicides at different times are shown in Table I.

After 180 min, the proportions of pesticides remaining were 3, 15, 18, 22 and 36% for ATRZ, DIA, DEA, CYAN and SIM, respectively, indicating that ATRZ

TABLE I

PHOTODEGRADATION OF ATRAZINE, CYANAZINE, SIMAZINE, DEISOPROPYLATRAZINE AND DEETHYLATRAZINE AS A FUNCTION OF TIME

Suntest apparatus at 44°C. Results given are concentrations of pesticides in distilled water in μg/l.

Pesticide	*Time (min)*						
	0	*30*	*45*	*60*	*120*	*180*	*300*
Atrazine	166	158	117	112	52	5	0
Cyanazine	232	210	188	166	139	51	30
Simazine	134	66	56	52	50	49	49
Deisopropylatrazine	116	114	89	78	40	18	2
Deethylatrazine	111	101	89	86	42	20	0

degrades the faster and SIM the slowest. When the concentrations of the parent compounds were measured at 300 min it was observed that ATRZ, DIA and DEA had completely disappeared and 12 and 36% of CYAN and SIM, respectively, remained. The fact that ATRZ can be easily degraded by UV radiation has been proposed as a safe means of disposal to avoid groundwater contamination[8].

The main degradation products observed for the different chlorotriazines are their corresponding hydroxy metabolites, the concentrations of which increase with time of UV irradiation. This is illustrated in Fig. 1, where the LC–DA chromatogram at 220 nm of degraded solutions of DIA, DEA, CYAN, SIM and ATRZ after 4, 4, 7, 5 and 3 h of UV irradiation are shown and the corresponding hydroxy metabolites are indicated by compounds 1–5, respectively. Fig. 2 shows the UV spectra of these hydroxychlorotriazines. The UV spectra of HA, DEHA and DIHA agree with those of the standard compounds and with previous spectra published by Vermeulen *et al.*[18]. The UV spectra of the hydroxy metabolites of CYAN and SIM (compounds 3 and 4, respectively, in Figs. 1 and 2) are reported here for the first time.

The hydroxy metabolites exhibit completely different spectra to the parent compounds. Thus, whereas the chlorotriazines generally exhibit a λ_{max} at 220 nm and second maximum with lower absorption at 260–270 nm, for the hydroxychlorotriazines a much broader maximum at 220 nm is observed and the second lower absorption maximum has completely disappeared[18,23]. For DEHA and DIHA (compounds 1 and 2 in Fig. 2), their λ_{max} values tend to shift to shorter wavelengths by 5–10 nm in comparison with HA (compound 5 in Fig. 2), which is to be expected because either ethyl or isopropyl groups have been lost.

In order to obtain a better approach to real environmental situations, preliminary experiments with the chlorotriazines added to artificial sea-water samples containing 1 mg per 600 ml of humic acids were carried out. The preliminary results indicated that the photodegradation rates are faster in the presence of salts, as previously shown for alkylated naphthalenes[24] and that the corresponding hydroxy metabolites do not exhibit an increase with time of UV irratiation; in contrast, they are easily degraded and their concentration decreases after 30–45 min of UV irradiation.

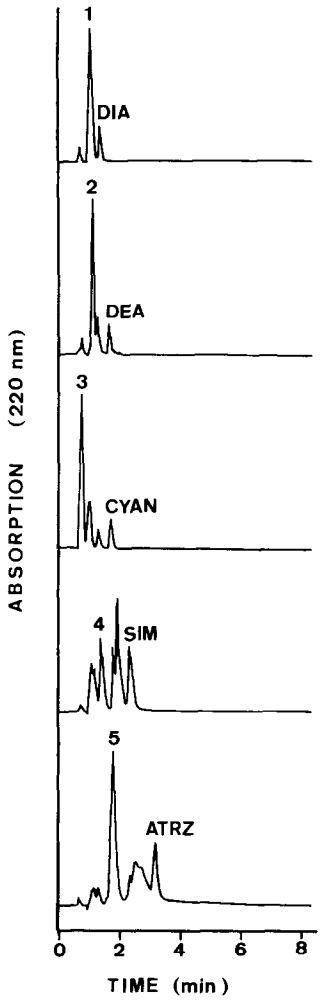

Fig. 1. LC of degraded solutions of deisopropylatrazine (DIA), deethylatrazine (DEA), cyanazine (CYAN), simazine (SIM) and atrazine (ATRZ) after 4, 4, 7, 5 and 3 h of photodegradation with the suntest apparatus. Peaks 1–5 correspond to the different hydroxy metabolites. Mobile phase: methanol–water (70:30) at 1 ml/min; diode-array detection at 220 nm; LC column, LiChroCART; loop volume, 20 μl.

Thermospray LC–MS of chlorotriazines and their photolysis products

By employing the thermospray LC–MS technique, the characterization of the hydroxy metabolites of the chlorotriazines was confirmed and other possible photodegradation products could also be identified.

The characterization of different chlorotriazine herbicides using ammonium formate as ionizing additive in PI mode thermospray LC–MS showed an $[M + H]^+$ ion as the base peak, which can be attributed to a higher proton affinity of the chlorotriazines than ammonia[25]. When methanol–water solutions containing 0.05 *M* ammonium formate were used as the LC eluent, a second abundant ion with a lower

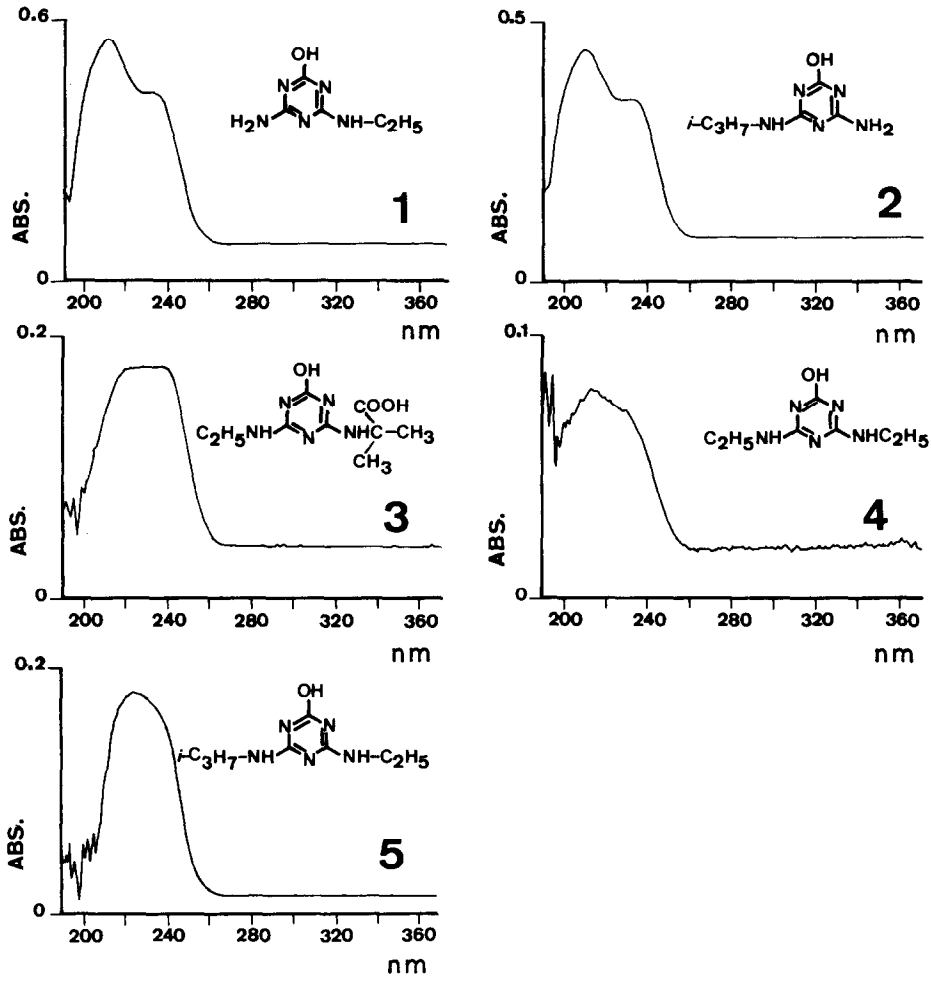

Fig. 2. UV spectra of peaks 1–5 in Fig. 1 corresponding to the hydroxy metabolites of the chlorotriazines. Abs. = Absorption.

relative intensity was formed which corresponds to $[M + CH_3OH + H]^+$, which has previously been explained by the gas-phase equilibrium between $[M + H]^+$ and CH_3OH, strongly dependent on pressure and temperature[22]. The formation of $[M + CH_3OH + H]^+$ is unique to the Hewlett-Packard thermospray system in comparison with the use of the Finnigan thermospray system[20], where the abundance of adduct ions with the LC eluent is generally less than 10%. An extensive comparison of these two thermospray LC–MS interfaces for chlorinated organic pollutants, which will be published elsewhere[26], showed that the Hewlett-Packard interface has higher tendency than the Finnigan interface to form eluent adduct ions.

For the hydroxychlorotriazines, in which a chlorine atom has been replaced with a hydroxy group, the rest of the triazine molecule remaining identical, a similar behaviour to the corresponding parent compounds is to be expected. In Fig. 3, the PI

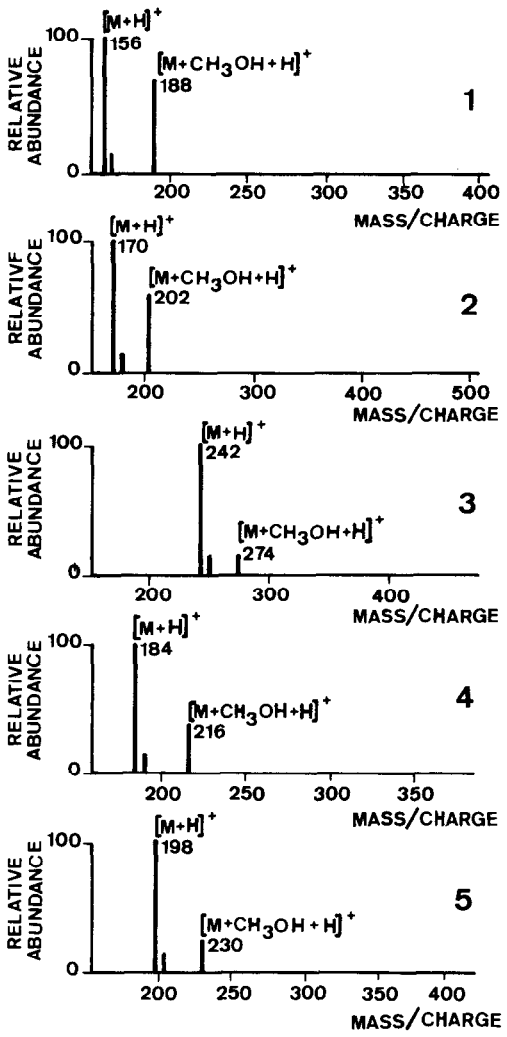

Fig. 3. PI mode thermospray LC–MS spectra of compounds 1–5 in Figs. 1 and 2. Mobile phase, methanol–water (70:30) containing 0.05 *M* ammonium formate at 1 ml/min; loop volume, 20 μl.

mode thermospray LC–MS spectra of the compounds 1–5 in Figs. 1 and 2 are shown. As mentioned, they correspond to the different hydroxychlorotriazines and exhibit the typical behaviour observed with the chlorotriazines, with $[M + H]^+$ as base peak and a second abundant ion corresponding to $[M + CH_3OH + H]^+$. Isotope peaks are virtually unobserved because the thermospray LC–MS spectra, obtained under full scan conditions, correspond to low nanogram levels (*ca.* 1–2 ng) of hydroxytriazines, which are very close to the detection limits of the chlorotriazines[22].

Although the hydroxychlorotriazines were the most abundant degradation products formed, other products have been detected, as can be observed in Fig. 1. A solution of ATRZ degraded after 3 h (Fig. 1) was injected into the thermospray LC–MS system and the total ion current and selected ion chromatograms obtained

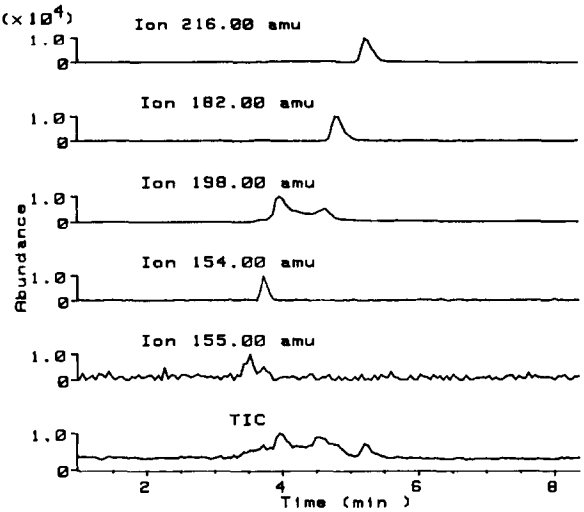

Fig. 4. Total ion current and selected ion chromatograms in PI mode thermospray LC–MS of a 166 μg/l degraded solution of atrazine (ATRZ) after 3 h of photodegradation with the suntest apparatus. The ions identified correspond to $[M + H]^+$ and their structures are given in Fig. 5. LC column, BrownLee. Other experimental conditions as in Fig. 3.

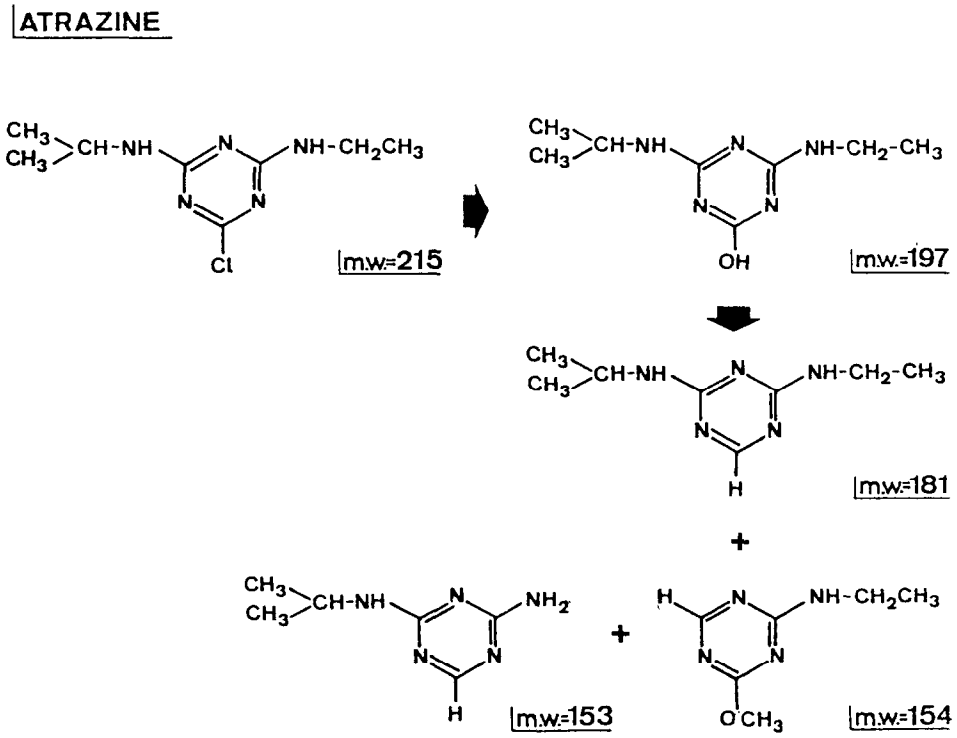

Fig. 5. Tentative photodegradation pathway of atrazine (molecular weight, m.w. = 215) in aqueous solution containing 3–4% of methanol.

are shown in Fig. 4. Here the HA and ATRZ $[M + H]^+$ ions correspond to m/z = 198 and 216, respectively. The other three ions, corresponding to m/z = 154, 155 and 182, can be attributed to three different degradation products of ATRZ of MW 153, 154 and 181, respectively. The tentative identification these photodegration products is shown in Fig. 5. They correspond to the 2-H (MW = 181), 2-H deethyl (MW) = 153) and 2-methoxy deisopropyl (MW = 154) analogues due to the photolysis in the presence of a small amount of methanol.

In the case of SIM, other analogues were detected following a similar photodegradation pathway. In Fig. 6 the total ion current and selected ion chromatograms of SIM after 5 h of photodegradation (see Fig. 1) can be seen with m/z values at 156, 170, 184, 168 and 198 for the different degradation products and at m/z = 202 for SIM. The tentative identification of these photodegradation products is shown in Fig. 7; the structures corresponding to MW of 183, 167, 197, 155 and 169 have been attributed to the hydroxy SIM, 2-H, 2-methoxy, DIHA and 2-methoxy deisopropyl analogues. The identification of these different degradation products agrees with the general scheme of the phtodegradation pathway for SIM in the presence of a methanolic solution, which has been described elsewhere[27].

A similar photodegradation process was observed for the other chlorinated triazines. For CYAN, in addition to the hydroxy metabolite, the 2-methoxy and DIHA analogues at m/z 256 and 156 were also identified. The 2-methoxy analogues were also observed for DEA and DIA at m/z 184 and 170, respectively. The 2-H analogue

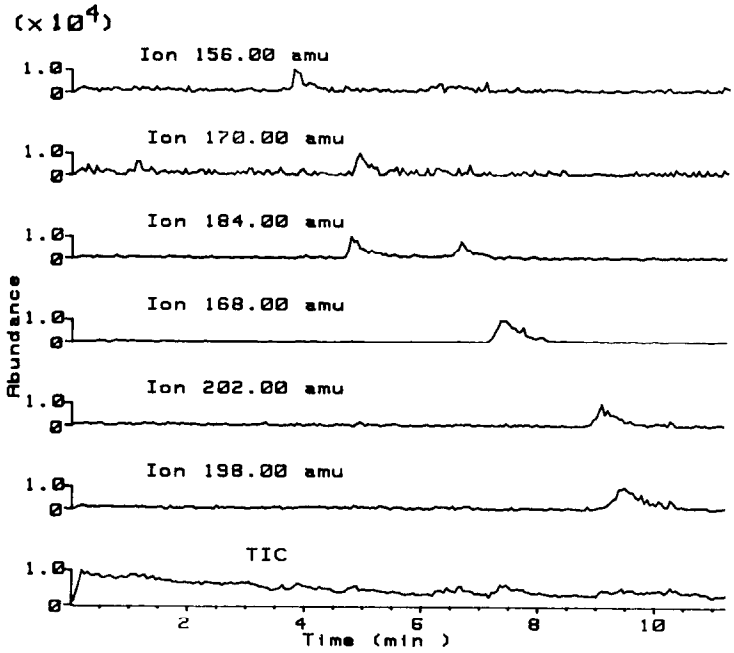

Fig. 6. Total ion current and selected ion chromatograms in PI mode thermospray LC–MS of a 134 μg/l degraded solution of simazine (SIM) after 5 h of photodegradation with the suntest apparatus. The ions idenfitied correspond to $[M + H]^+$ and their structures are given in Fig. 7. LC column, BrownLee. Other experimental conditions as in Fig. 3.

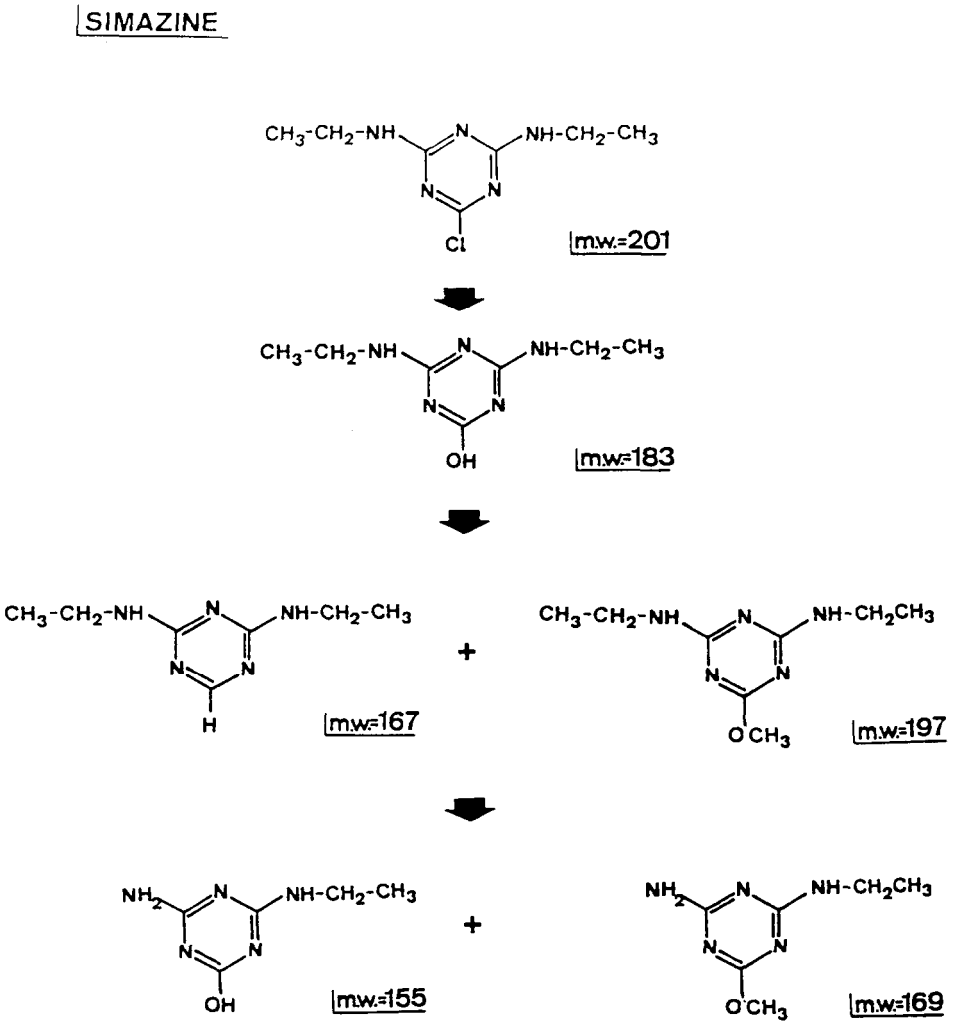

Fig. 7. Tentative photodegradation pathway of simazine (molecular weight, m.w. = 201) in aqueous solution containing 3–4% of methanol.

of DEA at m/z 154 was identified, whereas the 2-H analogue for DIA could not be determined because scanning was restricted to m/z values higher than 150[22].

CONCLUSIONS

The combination of LC–DA and TSP LC–MS has allowed the identification of different photolysis products of chlorotriazine herbicides in water by direct injection of the photodegradation solutions into the LC system. The method is much simpler than these currently used that require isolation of the different photodegradation products and off-line GC–MS characterization. The different polar degradation

products identified correspond to the hydroxy, 2-H and 2-methoxy analogues. Atrazine degraded faster the other chlorotriazines and simazine was the slowest under the experimental conditions of the suntest apparatus. Preliminary experiments using artificial sea-water containing humic acids indicated that less hydroxy metabolites are formed than when distilled water is used, together with a much faster and a different photodegradation pathway.

Future experiments will include the use of on-line precolumn techniques in LC[15], thus allowing the preconcentration and determination of the different polar photodegradation products by increasing the detection limits and consequently the possibility of detecting other degradation products.

ACKNOWLEDGEMENTS

G. D. is the recipient of a fellowship from the Commission of the European Communities (contract No. ST2*-0488). We thank Scharlau (Barcelona, Spain) for the loan of a Chrom-A-Scope rapid scanning UV–VIS detector from Barspec. This work was partly supported by the Community Bureau of Reference (Commission of the European Communities), the Ministerio de Educación y Ciencia and the BMFT through the Spanish-German cooperation programme (Acciones Integradas). Dr. M. Mansour (GSF, Munich, F.R.G.) is thanked for stimulating discussions. Dr. W. D. Hörmann and M. K. Huber (Ciba-Geigy, Basle, Switzerland) are thanked for kindly providing deethylhydroxyatrazine and deisopropylhydroxyatrazine. R. Alonso (CSIC) is thanked for laboratory assistance.

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