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Identification of a new degradation product of the antifouling agent Irgarol 1051 in natural samples

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Abstract

A main degradation product of Irgarol [2-(methylthio)-4-(*tert*-butylamino)-6-(cyclopropylamino)-*s*-triazine], one of the most widely used compounds in antifouling paints, was detected at trace levels in seawater and sediment samples collected from several marinas on the Mediterranean coast. This degradation product was identified as 2-methylthio-4-*tert*-butylamino-*s*-triazine. The unequivocal identification of this compound in seawater samples was carried out by solid-phase extraction (SPE) coupled on-line with liquid chromatography–atmospheric pressure chemical ionization-mass spectrometry (LC–APCI-MS). SPE was carried out by passing 150 ml of seawater sample through a cartridge containing a polymeric phase (PLRP-s), with recoveries ranging from 92 to 108% ($n=5$). Using LC–MS detection in positive ion mode, useful structural information was obtained by increasing the fragmentor voltage, thus permitting the unequivocal identification of this compound in natural samples. Method detection limits were in the range of 0.002 to 0.005 μg/l. Overall, the combination of on-line SPE and LC–APCI-MS represents an important advance in environmental analysis of herbicide degradation products in seawater, since it demonstrates that trace amounts of new polar metabolites may be determined rapidly. This paper reports the LC–MS identification of the main degradation product of Irgarol in seawater and sediment samples.

Keywords: Water analysis; Environmental analysis; Irgarol; Antifouling compounds

1. Introduction

The herbicide 2-(methylthio)-4-(*tert*-butylamino)-6-(cyclopropylamino)-*s*-triazine (trade name Irgarol 1051) is used in antifouling paints as a biocide agent in substitution to the tributyltin (TBT) and copper-based agents. This compound is used in tin-free antifouling paint formulations that are mainly based on copper and zinc metal oxides. The herbicide is added in order to inhibit the primary growth of copper-resistant fouling organisms such as algal slimes and the growth of seaweeds. Few data concerning Irgarol 1051 contamination of the aquatic environment are available. For example, important coastal concentrations of Irgarol have been found in areas of high yachting activity, particularly in marinas and sport harbors [1–4]. Recently, concentrations of Irgarol and diuron in the ppt level were detected in a pilot monitoring study carried out in the coastal Mediterranean area of Catalonia during 1996–1997 [5,6].

The degradation of contaminants in water is an area of research interest and, in this sense, sunlight photoalteration processes are known to play an important role. The study of the photochemical behavior of a contaminant is a key issue in environmental studies in order to assess its degradation

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and the formation of toxic transformation products. Irgarol degradation studies have been reported in the literature, such as the biodegradation work of Liu et al. [7]. Photodegradation studies of this compound have been recently reported by Okamura et al. [8]. Since degradation studies are difficult to carry out under real conditions, natural sunlight photodegradation processes are usually compared with those obtained under controlled conditions, generally using xenon arc lamp [8–11]. In these studies [7,8] the formation of the 2-methylthio-4-*tert*-butylamino-*s*-triazine degradation product was observed and the environmental detection of this compound in seawater samples was reported [8]. Information on degradation products is necessary to understand the environmental fate of pesticides and to establish important degradation pathways, which will allow us to get a better knowledge of the transformation of target compounds in the environment. For these reasons, continued development of reliable and sensitive methods of analysis for metabolites are important for studies of water quality. Moreover, the toxicity of Irgarol and its degradation products should be taken into account. One study from Okamura et al. reported the phytoxicity of Irgarol and its degradation product [12].

The objectives of this work were: (i) to develop a sensitive methodology for the detection of Irgarol and its main degradation product in seawater samples; (ii) to carry out a monitoring study evaluating the presence of the degradation products of Irgarol in environmental seawater samples from the Mediterranean coast; and (iii) to analyze sediments from a sport marina to assess the fate of Irgarol and degradation products. To our knowledge this work represents the first identification of the degradation product of the antifouling agent Irgarol in natural samples by liquid chromatography–atmospheric pressure chemical ionization-mass spectrometry (LC–APCI-MS).

2. Experimental section

2.1. Chemicals

Irgarol 1051 (99%) and the byproduct (2-methylthio-4-*tert*-butylamino-*s*-triazine) were obtained from Ciba-Geigy (Barcelona, Spain). HPLC-grade solvents acetonitrile, methanol, and water were purchased from Merck (Darmstadt, Germany).

The solid–phase extraction (SPE) cartridges used consisted of C_{18} (6 ml containing 500 mg of octadecylsilica) from Merck (Darmstadt, Germany) and PLRP-s (10×2 mm I.D. disposable precolumns containing 20 mg of polymeric material) from Spark Holland (Netherlands).

2.2. Sampling

Sampling of seawater was carried out at a depth of 1 m from the surface layer of the Masnou marina (Barcelona, Spain). The samples were collected in 2.5-l precleaned amber glass bottles and kept at 4°C in the dark until analysis. Water sample pH varied from 7.9 to 8.3. Before analysis, seawater samples were filtered through a glass fiber filter (0.45-μm pore size) in order to remove the suspended particles. Blanks of seawater samples were also taken from the open sea in order to spike them with known amounts of Irgarol and construct the calibration curves. Two sediment samples were collected at two different points in the Masnou marina from the top layer and stored at −20°C in glass containers.

2.2.1. Sample preparation

2.2.1.1. Seawater samples

Preconcentration of the seawater samples was performed with an automated SPE system (Prospekt, Spark Holland, Netherlands) coupled on-line with a liquid chromatograph. The SPE system consists of a cartridge exchange module, a solvent delivery unit (SDU), and a low-pressure six-port valve, which is connected directly to the gradient pumps of the HPLC system. Water samples were preconcentrated on PLRP-s polymeric cartridges. The cartridges were conditioned sequentially with 6 ml acetonitrile and 4 ml LC-grade water. Afterwards, a 150-ml aliquot of seawater sample was percolated through the cartridge at a flow-rate of 3 ml/min. Finally, the compounds trapped on the sorbent were eluted with the chromatographic mobile phase by switching the valve into the elute position.

2.2.1.2. Sediment samples

A 10-g amount of sediment was extracted by sonication twice with a methanol (15 ml)–water (5 ml) mixture at 75°C for 30 min. The sediment extracts were combined and evaporated to 10 ml under nitrogen removing most, if not all of the methanol. The aqueous extracts obtained were passed through a C_{18} cartridge to perform the clean-up. Methanol was added to the cartridge to elute the compounds and the final extracts were injected into the LC–APCI-MS system. The recoveries of Irgarol and its metabolite from sediment samples were also studied. The spiking level was 100 μg/kg and the recoveries were 90 and 80% for Irgarol and its metabolite, respectively.

2.2.2. LC–diode array detection (DAD)

The analyses were performed with a Waters 600-MS solvent delivery unit with a 20-μl injection loop and a Waters 996 DAD system (Waters, Millipore, MA). The analytical column used was 25 cm×4.6 mm I.D. packed with 5 μm of octylsilica gel from Shandon (Cheshire, UK). The gradient elution was performed as follows: from 30% A (acetonitrile) and 70% B (HPLC-grade water) to 100% A and 0% B in 25 min. Quantification was carried out with UV detection at 230 nm for Irgarol and its metabolite.

2.2.3. LC–APCI-MS

LC–APCI-MS, in positive ion mode of operation, was used for the identification of Irgarol and its degradation product. The eluent was delivered by a liquid chromatograph model HP 1090 (Hewlett-Packard, CA, USA). The mobile phases used for the elution of the analytes consisted of acetonitrile and water at a flow-rate of 0.8 ml/min. The gradient elution and the analytical column were the same as those used in the LC–DAD analysis. This HPLC system was connected to a Hewlett-Packard mass spectrometer, model HP 1100, system equipped with an APCI probe. The different operating parameters included a drying gas (N_2) flow-rate of 4 l/min and a nebulizer pressure of 40 p.s.i., a capillary voltage of 2000 V, a corona voltage of 8 μA and a fragmentor voltage of 70 V (1 p.s.i.=6894.76 Pa). The drying gas temperature was set at 350°C and the nebulizer temperature was 400°C. Chromatograms were recorded under selected ion monitoring (SIM) and full scan (m/z 120–300) conditions.

3. Results and discussion

3.1. Analytical performance

The retention of Irgarol and its metabolite (Fig. 1) was investigated on polymeric cartridges. The recoveries of extraction obtained after the preconcentration of 150 ml of seawater sample, spiked at 1 μg/l with Irgarol and its metabolite, were studied. The recovery for Irgarol was 95% and the recovery for the Irgarol degradation product was 92%. Thus, high recoveries of extraction for both compounds from seawater matrices are obtained as can be concluded from these results. This method has been proven to be reliable for the analysis of seawater samples containing trace levels of antifouling compounds [5,13]. However, in these previous studies, concentrations for the metabolite of Irgarol were not reported. The method developed in this work shows the capability of detecting low levels of concentration for these compounds in seawater samples.

The relative standard deviation of the method was calculated from five independent extractions of the compounds from seawater samples by PLRP-s cartridges. The relative standard deviation ranged from 5 to 9%, indicating good performance of the method developed in this work. One advantage of automation in an on-line preconcentration is that more reproducible results can be expected, provided that the manipulation of the samples is avoided as compared with an off-line methodology.

Calibration curves were constructed by passing 150 ml of seawater sample, fortified with a solution containing Irgarol and its metabolite, through a

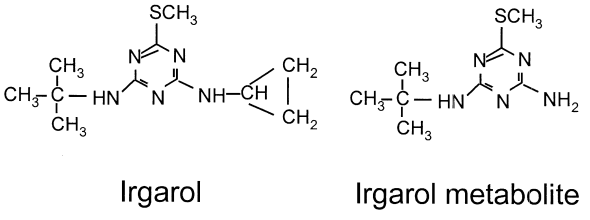

Fig. 1. Chemical structures of Irgarol and its degradation product.

PLRP-s cartridge. The curves were linear in the concentration range studied, from 0.01 to 1.5 μg/l, and the correlation coefficients were higher than 0.98 for both compounds, thus indicating good performance of the on-line methodology developed in this work.

The limits of detection were calculated using a signal-to-noise ratio of 3 (the ratio between the peak intensity under SIM conditions and the noise). Limits of detection were 2 and 5 ng/l for Irgarol and its degradation product, respectively. Low detection limits at the ppt level can be obtained due to the high selectivity and sensitivity encountered by the APCI-MS system since few if any interferences are encountered under SIM conditions.

Table 1
Typical fragment ions and relative abundances (RAs) of the compounds studied by LC–MS using an APCI interface in the positive ion (PI) mode of operation

Compound	M_r	APCI in PI			
		70 V		120 V	
		m/z	RA	m/z	RA
Irgarol	253	254	100	254	70
				198	100
Irgarol degradation product	213	214	100	214	30
				158	100

Fragmentor voltage set at 70 and 120 V. LC mobile phase: acetonitrile–water (40:60) at a flow-rate of 0.8 ml/min. M_r, nominal molecular mass.

3.2. Structural information (LC–APCI-MS)

The different operational parameters of the APCI interface were optimized for the compounds under study and those presenting the maximum sensitivity were chosen for further analysis. It was observed that both compounds, Irgarol and its metabolite, showed a higher response under positive ion mode as a result of the easy protonation of the amine nitrogen. This result has been reported for many triazines [14].

The fragmentor voltage is one of the most important parameters in LC–APCI-MS. This voltage affects the transmission and fragmentation of sample ions. In general, the higher the fragmentor voltage, the more fragmentation will occur. In compounds that do not fragment readily, higher fragmentor voltages often result in better ion transmission. The fragmentor voltage gives the ions a ''push'' that helps them traverse the relatively high pressure region between the exit of the capillary and the skimmer. Thus, at higher voltages, maximum structural information is obtained. Table 1 reports the typical fragment ions of the two compounds studied in this work in positive ion mode. Fragmentor voltages of 70 and 120 V were examined in order to study the main fragment ions formed under LC–APCI-MS. At the low fragmentor value of 70 V, both compounds studied gave only the molecular ion plus a proton as a base peak. So this fragmentor value does not provide useful structural information. However, it was observed that the compounds presented major fragmentation at 120 V, which provided both good structural information and enough sensitivity for the compounds studied. Fig. 2 shows the mass spectrum of the metabolite of Irgarol at a fragmentor voltage of 120 V. As can be seen in this figure the compound suffers the loss of the *tert*-butyl group, one of the typical fragmentations of the triazine compounds.

3.3. A new degradation product in natural samples

3.3.1. Seawater samples

Seawater samples from a marina (Masnou, Catalonia) were analyzed by the methodology developed in this work in order to assess the presence of the possible degradation products formed by natural photolysis. Samples were taken over a period of 1 year and they were analyzed by on-line SPE–LC–APCI-MS since this was the most sensitive technique for the analysis of Irgarol and its degradation product. The structural confirmation of these compounds in groundwater was possible by using APCI–MS and the main fragmentation ions. Concentrations of Irgarol and its degradation product in the ppt level were found in all the seawater samples collected (Table 2). It is important to mention that the degradation product of Irgarol had not been detected in seawater by LC–MS in any previous studies and this is the first unequivocal confirmation of the presence of this compound by LC–MS in seawater samples. The presence of this metabolite in environmental seawater samples was totally confirmed by identical match of retention time and

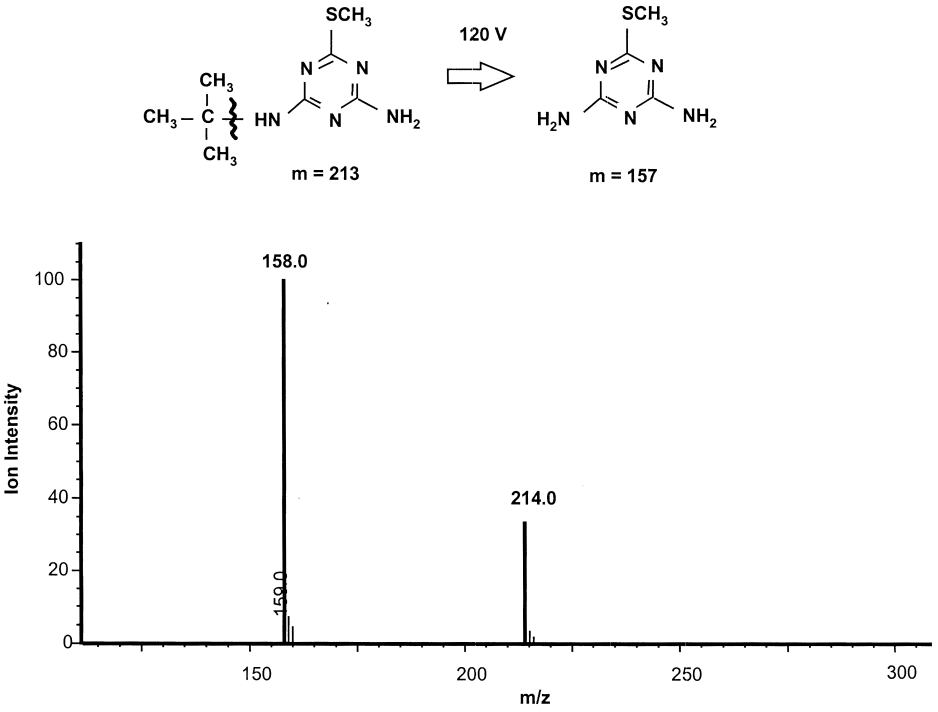

Fig. 2. Mass spectrum and proposed fragmentation ion for Irgarol metabolite at a fragmentor value of 120 V.

spectrum of a standard. Fig. 3 shows the chromatograms of a seawater sample taken in the Masnou marina in October 1998 and the corresponding spectra of the identified metabolite and that of the standard.

In a previous study by our group [11], Irgarol photodegradation experiments were carried out under controlled conditions. In these studies, it was observed that the only degradation product of Irgarol, formed under environmental conditions, was 2-methylthio-4-*tert*-butylamino-*s*-triazine. This degradation product has also been detected in other degradation experiments, such as in a biodegradation study of Irgarol by *Phanerochaete chrysosporium* carried out by Liu et al. [7]. This compound is formed from Irgarol by losing easily the cyclopropyl group both in photodegradation and biodegradation processes. Thus, this study indicates a similar behavior for Irgarol under environmental conditions as in the photolysis experiments conducted under controlled conditions.

Table 2
Concentrations of Irgarol and its metabolite in seawater samples, after their analysis by on-line SPE–LC–APCI-MS under the positive ion mode of operation

Samples	Concentration (μg/l)	
	Irgarol	Metabolite
February, 1997	0.012	0.02
July, 1997	b.d.l.[a]	0.023
October, 1997	0.034	0.004
November, 1997	0.037	0.012
February, 1998	0.028	0.013
March, 1998	0.003	0.07
April, 1998	0.009	b.d.l.
May, 1998	0.091	0.02
June, 1998	0.119	0.005

[a] Volume percolated: 150 ml. Relative standard deviation ($n=3$) varied between 5 and 10%. b.d.l., below detection limit.

3.3.2. Sediment samples

Two sediment samples, taken at two different locations in the Masnou marina, were analyzed by the methodology developed in this work. High concentrations of Irgarol were found in these samples in comparison to the concentrations found in seawater samples (from 3 up to 57 μg/kg). This

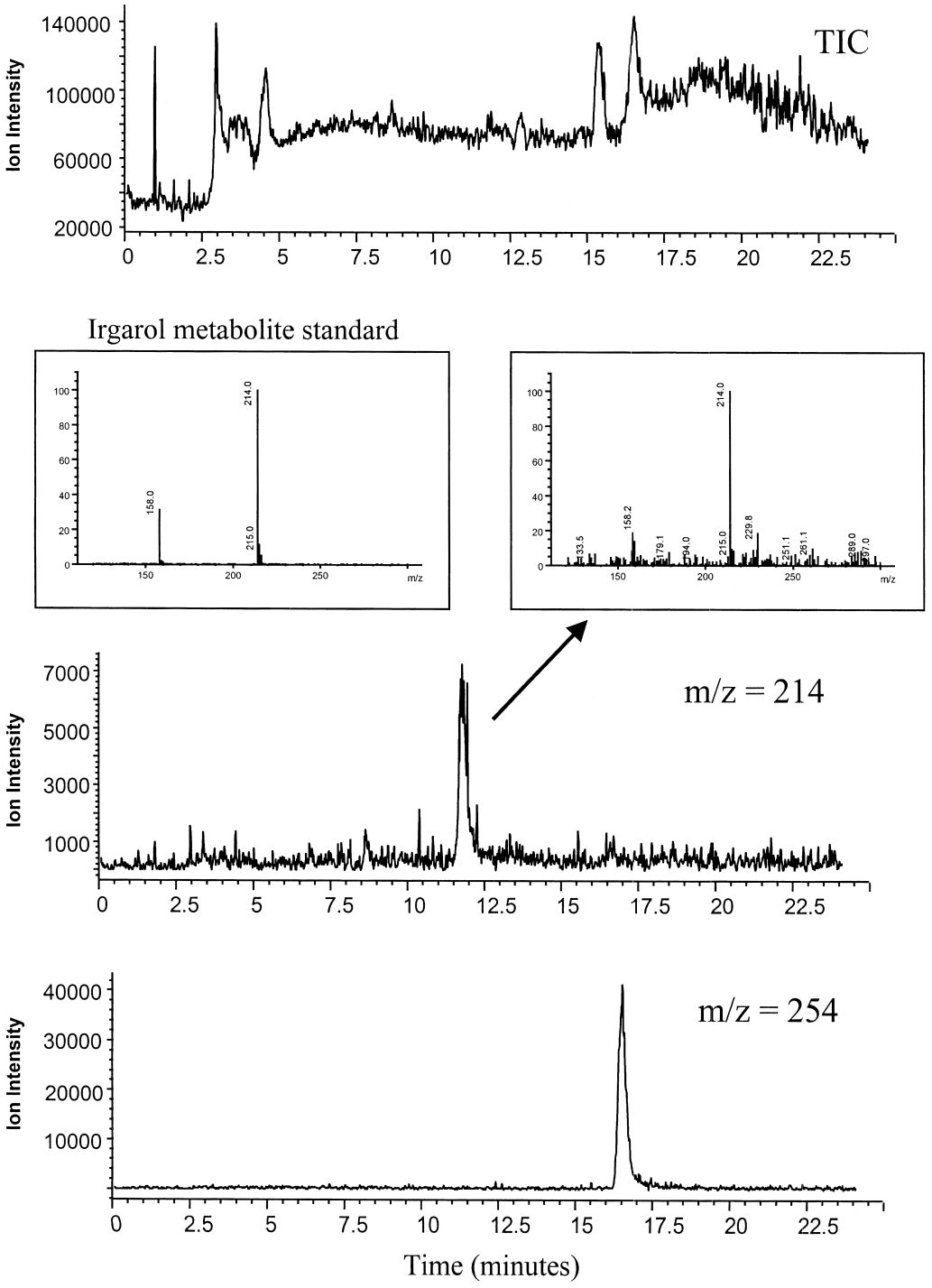

Fig. 3. Total ion current (TIC) and extracted ion chromatograms (Irgarol metabolite: m/z 214, Irgarol: m/z 254) from a seawater sample from the Masnou marina after its preconcentration through polymeric cartridges by LC–APCI-MS in the positive ion mode of operation under scan conditions at a fragmentor voltage of 90 V. The spectra of the Irgarol metabolite standard and that corresponding to the seawater sample are also shown.

result is a consequence of the high K_{oc} value of this compound. Irgarol is the most hydrophobic compound of the family of the triazines due to the presence of both the *tert*-butyl group and the cyclopropyl group. Thus, it is expected that this compound is detected at higher concentrations in sediment samples than in the aqueous phase. Moreover, concentrations for the degradation product of Irgarol were found in the same range (from 0.2 to 3.3 μg/kg). This result can be explained by a mixed-mode sorption of this compound in the sediment matrix. Fig. 4 shows the chromatogram obtained after the analysis of a sediment sample. Irgarol and its degradation product could be easily identified by monitoring both the molecular and the fragmentation ions.

Increased knowledge about the degradation of herbicides and the dissipation of their metabolites in the environment could include consideration of herbicide metabolites as part of the basis for the establishment of health guidelines and water-quality regulations. Therefore, further research is needed to identify major degradation pathways for all pesticides and to develop analytical methods to determine their concentrations in water and other matrices.

The methodology described in this paper can be a useful approach to carry out the monitoring of the degradation product of one of the most important antifouling compounds, Irgarol 1051, in natural samples. The high sensitivity achieved, the capability to analyze complex sediment matrixes and the selectivity of the method provide a useful tool in the metabolite monitoring of natural samples.

This area will continue to expand since efforts for pesticide detection in the environment will be directed towards the development of methods for very polar pesticide analytes. The use of this technique opens up new possibilities for the trace determination of polar pesticide metabolites in natural matrices and will probably facilitate a better understanding of the fate of pesticides in the aquatic environment.

Overall, the present study reported that a main degradation product of Irgarol was detected in Mediterranean coastal waters and sediments at ppb concentrations. This degradation product was identified as 2-methylthio-4-*tert*-butylamino-*s*-triazine [4].

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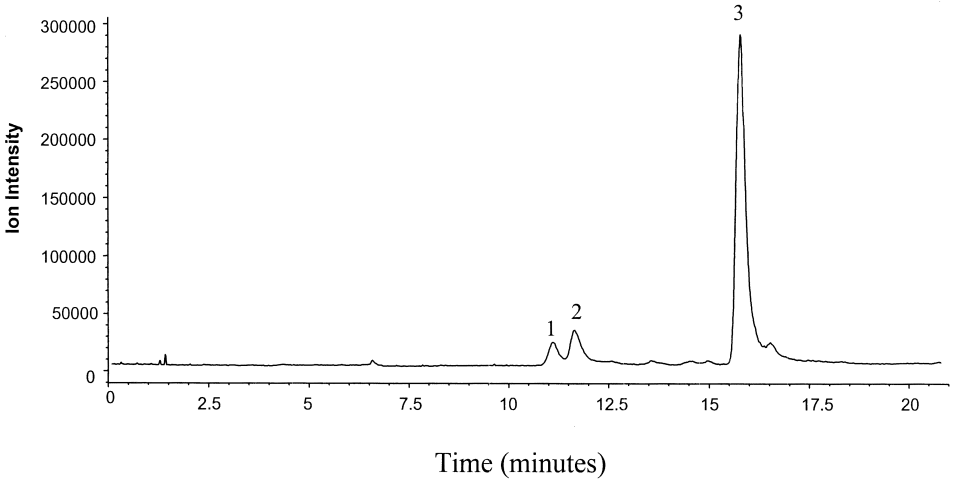

Fig. 4. Analysis of a sediment extract by LC–APCI-MS in the positive ion mode of operation under SIM conditions. Peak numbers: (1) Irgarol metabolite, (2) deuterated atrazine, (3) Irgarol. LC conditions as described in the Experimental section.

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