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Journal of Chromatography A, 1046 (2004) 159–166
JOURNAL OF CHROMATOGRAPHY A
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Analysis and mass spectrometric characterization of the insect repellent Bayrepel and its main metabolite Bayrepel-acid☆

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Received 8 March 2004; received in revised form 1 June 2004; accepted 23 June 2004

Abstract

Insect repellents such as *N*,*N*-diethyl-*m*-toluamide (DEET) which are used as protection against mosquitoes or ticks were detected in all wastewater and anthropogenically influenced surface waters analyzed. In Germany, the concentrations of DEET have constantly decreased since 1999, when DEET was substituted by Bayrepel (1-piperidinecarboxylic acid, 2-(2-hydroxyethyl), 1-methylpropyl ester; KBR 3023) in commercial insect repellent formulations. A sensitive quantitative method was developed in order to study the occurrence and fate of Bayrepel in the aquatic environment. It was thus determined that Bayrepel undergoes rapid primary aerobic biodegradation, yielding a more stable metabolite, Bayrepel-acid (1-piperidinecarboxylic acid, 1-methylpropyl ester, 2-acetic acid). In order to study the biodegradation and investigate the fate of this metabolite, Bayrepel-acid was synthesized and characterized. Various chromatographic and mass spectrometric techniques, such as gas chromatography–mass spectrometry (MS) after derivatization, liquid chromatography (LC)–electrospray ionization (ESI) MS and LC–ESI time-of-flight MS were applied.

Keywords: Water analysis; Emerging pollutants; Bayrepel; Diethyltoluamide; Insect repellents

1. Introduction

The wastewater in industrialized countries transports contaminants from activities of civilization which can be harmful for the ecosystem. Polar organic chemicals emitted by wastewater discharges have been recognized only over more recent years, and as such there is still a lack of knowledge concerning this kind of pollution [1,2]. In current literature these substances, including household chemicals, such as surfactants, pharmaceuticals, insect repellents, agricultural chemicals, such as e.g. pesticides, and industrial chemicals including for example by-products from chemical synthesis, are often described as "emerging pollutants". The fate of anthropogenic organic pollutants, even present at low concentrations is an established challenge in the production of drinking water out of surface water. It is thus of crucial importance to gain knowledge regarding the biodegradation of such compounds and their metabolite formation.

An example of such an "emerging pollutant" is the insect repellent *N*,*N*-diethyl-*m*-toluamide (DEET). A literature search demonstrated that DEET has been detected at low $\mu g\,L^{-1}$ levels in many different water bodies, e.g. in the river Tama in Japan [3], in several surface waters in the US [4] and in the river Rhine in Germany [5]. More recently DEET was also quantified in samples from the North Sea [6]. An investigation of the Rhine river at Wiesbaden, Germany (sampling point km 507) as well as the Main river at Bischofheim, Germany since 1994 resulted in peak concentrations of DEET in the summer and autumn months until the year 2000 [7]. Additional monitoring of weekly mixed samples taken over a period of more than three years at the wastewater treatment

☆ Presented at the 3rd Meeting of the Spanish Association of Chromatography and Related Techniques and the European Workshop: 3rd Wastewater Cluster, Aguadulce (Almeria), 19–21 November 2003.

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doi:10.1016/j.chroma.2004.06.067

Table 1
Physico-chemical properties of 1-piperidinecarboxylic acid, 2-(2-hydroxyethyl)-, 1-methylpropyl ester (Bayrepel)

Sum formula	$C_{12}H_{23}NO_3$
CAS RN	119515-38-7
Molecular mass	229.3 g mol^{-1}
Boiling point	280 °C/1013 hPa
Water solubility (20 °C)	Soluble in water < 100 mg L^{-1}
Physical properties:	Colourless liquid

plant (WWTP) in Wiesbaden, Germany resulted in a clarification of the entry and behavior of DEET in the aquatic environment. It could be shown, that the entry of DEET into the aquatic environment was mainly after its use as a topically applied repellent via wastewater, and that the degradation only occurred after an ambient adaptation time and at concentration levels exceeding a threshold value of approximately 1 μg L^{-1} [7,8].

The Autan manufacturer Bayer, Leverkusen, Germany produced DEET for more than 40 years as the active substance for their insect repellent formulation. In the year 1986, the company commenced development of a new active ingredient for the repellent based on computer-assisted structural analyses. Out of over 800 different proposed compounds, the new active substance Bayrepel (1-piperidinecarboxylic acid, 2-(2-hydroxyethyl), 1-methylpropyl ester; KBR 3023), described and pictured in Table 1 and Fig. 1, was created [9]. Since 1998, at least in the products produced by Bayer, Bayrepel has gradually been introduced to all markets world-wide. In close co-operation with the USA registration authority, the US Environmental Protection Agency (EPA), an extensive test routine was established, whereby Bayrepel was determined as toxicologically harmless with a minor dermal absorption tendency [10,11].

However, as with any new xenobiotic introduced into the environment, in order to truly determine the toxicological and environmental risk, the persistence and fate of it, and any resulting metabolites, is also of extreme importance.

The purpose of the research performed and described here was thus to fully characterize the analyte and observed degradation products, and to develop reliable methods for their quantitation in environmental matrices.

In Germany, the DEET concentrations in water bodies have decreased constantly since 1999, when DEET was substituted by Bayrepel [7,8]. At this time, we also commenced the development of an analytical method for the sensitive analysis of Bayrepel in wastewater and surface water, in addition to investigation of its fate and metabolite formation. Traditionally the analytical method for the determination of more polar pollutants has been gas chromatography (GC)–mass spectrometry (MS), after several preparation, enrichment and derivatization steps. More recently, liquid chromatography (LC)–electrospray ionization (ESI) MS and LC–ESI-MS–MS methods have developed into the most powerful techniques for the detection of polar water-soluble compounds in aquatic matrices [12–15]. The use of LC–ESI time-of-flight (TOF) MS is one such method that has been shown to give unequivocal information regarding the identity of the investigated compound and further insight into the chemical structure itself [14,15].

In this work, we describe both quantitative methods for the determination of polar target pollutants in waste and surface waters, as well as the identification of so far unknown pollutants using several different LC–MS approaches.

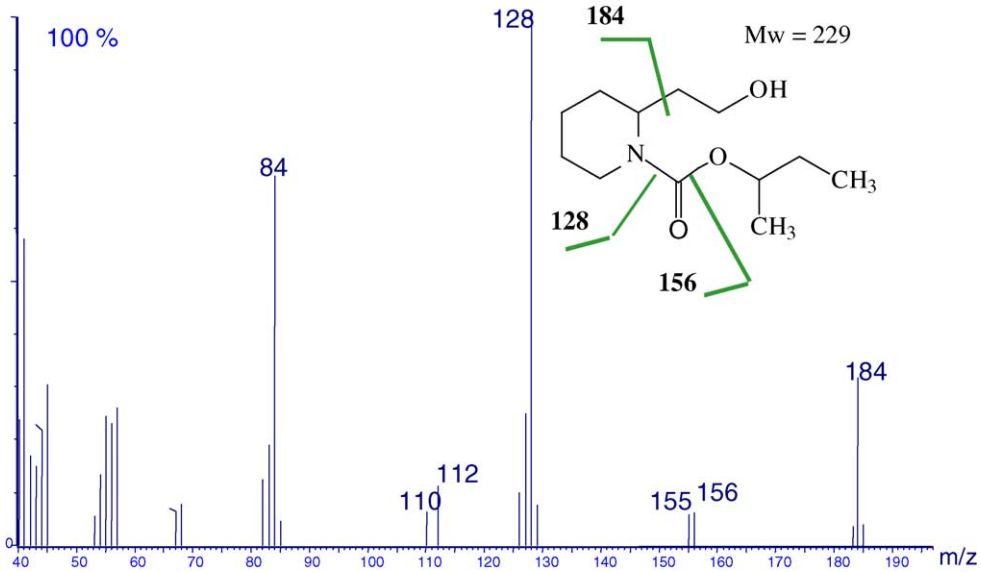

Fig. 1. Electron impact (EI) GC–MS-spectrum of Bayrepel [1-piperidinecarboxylic acid, 2-(2-hydroxyethyl), 1-methylpropyl ester]; assignments given in Table 2.

2. Experimental

2.1. Chemicals

All chemicals used were of analytical grade. Milli-Q water was used in all the experiments. The reference compound Bayrepel (1-piperidinecarboxylic acid, 2-(2-hydroxyethyl), 1-methylpropyl ester; KBR 3023) was provided by Bayer, Leverkusen, Germany. The purity was greater than 99%.

2.2. Sampling and sample preparation

Wastewater was collected as 1 day mixed samples from the influent and effluent of the wastewater treatment plant (WWTP) in Wiesbaden, Germany. Surface water samples were collected randomly from the river Rhine at Wiesbaden, Germany.

All samples were filtered through glass fiber filters (0.45 μm), prewashed with methanol and Milli-Q water. Solid-phase extraction (SPE) was performed on 1 L surface water and 0.5 L WWTP-effluent samples. The SPE of Bayrepel and possible neutral metabolites was performed in the neutral pH-range, whilst when analyzing for possible acidic metabolites, samples were adjusted to pH 2 by adding 3.5 M sulphuric acid prior to enrichment. The samples were filtered under vacuum (20 mL min^{-1}) through glass cartridges filled with 0.1 g LiChrolute EN (*Merck*) and 0.25 g Isolute C_{18}ec (endcapped C_{18}, *IST*). Prior to extraction, the cartridges were conditioned with 6 mL *n*-hexane, 6 mL methanol and 10 mL ground water, respectively for Bayrepel analyses, and with 10 mL ground water adjusted to pH 2 for the screening for acidic metabolites. After enrichment and drying under a gentle stream of nitrogen gas for 60 min, the enriched compounds were eluted and prepared for analysis by the following methods.

2.2.1. Bayrepel

After eluting with 3 × 1.5 mL acetone–ethyl acetate (1:1, v/v), the extracts were evaporated under gentle nitrogen flow to 100 μL, internal standard was added (the certified pesticide standard fluazifop-butyl, Ehrensdorfer, Germany; final concentration: 0.7 μg mL^{-1}) and the extract made up to 200 μL final volume.

2.2.2. Acidic metabolites

After eluting with 2 × 1.5 mL methanol, the extracts were evaporated under nitrogen to dryness. The samples were then either (i) derivatized and analyzed by GC–MS or (ii) redissolved in the HPLC eluent and analyzed by HPLC–ESI-MS. GC–MS derivatization was performed using 700 μL *n*-hexane and 150 μL diazomethane in diethyl ether (in excess) at 20 °C, with the reaction terminated after 60 min by addition of two droplets of acetic acid in acetone (10%, v/v). Internal standard (heptadecanoic nitrilo acid, final concentration: 1 μg mL^{-1}) was added and the extract made up to a final volume of 1 mL with *n*-hexane.

2.3. Synthesis and purification of the Bayrepel-acid

Bayrepel (5 g) was oxidized with potassium permanganate in a sodium carbonate solution according to [16]. The crude chemical reaction mixture was further purified with water and *n*-hexane, which yielded a yellowish oil. This oil was reconstituted in 10 mL of an acetonitrile–water (50:50) mixture. This mixture was further cleaned-up via semi-preparative HPLC (Bischoff Lambda 1010) at a flow-rate of 2 mL min^{-1} of acetonitrile–water (50:50) run under isocratic conditions on a Hypersil ODS 3 μm, 100 mm × 4.6 mm C_{18} column. The progress of separation and clean-up was monitored online by UV detection with a ^2H lamp run at a wavelength of 220 nm. Several fractions were collected (1 mL each) and subsequently analyzed with LC–ESI-MS (see below) screening for the Bayrepel-acid. After identification of the compound, separation parameters were optimized and several runs performed with injection aliquots of 250 μL of the reaction mixture. The total 10 mL sample was further cleaned-up by collecting the fraction between 8 and 10 min on the semi-preparative column (Fig. 4). The organic solvent component of the collected and pooled fractions was removed by rotary evaporation at 60 °C and 58 Torr (1 Torr = 133.322 Pa). The remaining extract was frozen at −23 °C in a round-bottom flask, lyophilized for 12 h, redissolved in acetone for transferal into a 10 mL vial, and evaporated under nitrogen flow to dryness for weighing. The identity and purity of the isolated Bayrepel-acid was proven by GC–MS and LC–MS analyses. Five different stock solutions of the Bayrepel-acid in acetone (2 mg/10 mL acetone) were stored at −23 °C for further use.

2.4. High-performance liquid chromatography separations

2.4.1. Online HPLC analysis with ESI-MS detection of acidic metabolites

It was performed using an LC 200 binary pump (Perkin-Elmer, Norwalk, CT, USA) equipped with an 100-μL injection loop. To assure a flow of 0.25 mL min^{-1} into the ESI-interface, the LC effluent flow (0.5 mL min^{-1}) was split (1:1) by means of a zero dead volume T-piece. The HPLC separation was achieved on a 5-μm, 250 mm × 4.6 mm i.d., C_{18} reversed-phase column (Inertsil ODS-2, MZ-Analysentechnik, Mainz, Germany). The column temperature was held at 35 °C.

Eluent A consisted of 10 mM ammonium acetate in water, adjusted to pH 4.1 with acetic acid; eluent B was acetonitrile. The initial conditions of the gradient program were 100% A, held for 10 min. From 10 to 20 min the eluent A was reduced down to 10%, and this held for 5 min, the solvent composition was then brought back to 100% A over 5 min.

2.4.2. Online HPLC analysis with ESI-TOF–MS detection of Bayrepel and Bayrepel-acid

It was performed using an G1312A binary pump (Agilent Technologies, Waldbronn, Germany) configured with

Table 2
Retention times (t_R), characteristic ions used for GC–MS quantification and LC–MS identification, limits of quantification (LOQ) and detection (LOD) in surface water (sw) and wastewater (ww) and recoveries obtained from Bayrepel and its metabolite Bayrepel-acid

Compound	t_R (min)	Ion 1 (*m*/*z*)	Ion 2 (*m*/*z*)	LOQ (sw) ($\mu g\,L^{-1}$)	LOD (sw) ($\mu g\,L^{-1}$)	LOQ (ww) ($\mu g\,L^{-1}$)	LOD (ww) ($\mu g\,L^{-1}$)	Recovery (sw) (%)
Bayrepel[a]	14.707	128	184	0.03	0.01	0.10	0.05	98
Bayrepel-acid-methyl ester[a]	15.206	128	156	0.03	0.01	0.10	0.05	97
Bayrepel-acid[b]	9.1	168	242	n.d.	n.d.	n.d.	n.d.	n.d.

n.d.: not determined.
[a] GC–MS detection (for conditions see experimental section).
[b] LC–MS detection (for conditions see experimental section).

a G1367A Wellplate sampler for sample introduction. The samples (1 μL each) were injected at a concentration of 5 ppm in methanol. The HPLC separation was achieved on a Zorbax SB-C_{18} 75 mm × 4.6 mm i.d. column (Agilent, Little Falls, DE, USA). Eluent A was 5 mM ammonium acetate in water, adjusted to pH 3.5 with acetic acid; eluent B was acetonitrile. The gradient solvent program used was: 5% B increasing to 80% over 7 min., 80% B held for 1 min further, then return to the initial conditions over the next minute.

2.5. Mass spectrometric analysis

2.5.1. GC–MS

Samples were analyzed with a GC–MS (Fisons) utilizing an AS 800 autosampler, a gas chromatograph 800 and an MD 800 mass selective detector. An 30 m XTI-5 (Restek, Bellefonte, PA, USA) column (film thickness 0.25 μm, 0.25 mm i.d.) was used for separation with helium as the carrier gas. Injections (2 μL) were made in the splitless mode at 50 °C oven temperature. This temperature was held for 1 min, followed by a 12 °C min^{-1} ramp to 300 °C and this temperature held for 10 min. The injector temperature was 230 °C, the transfer line 250 °C and the ion source temperature 200 °C.

2.5.2. LC–ESI-MS

The analyses were performed on an atmospheric pressure ionization (API) 150 single quadrupole mass spectrometer (Perkin-Elmer Sciex API 150, Thornhill, Canada) equipped with an API source, via a turbo ionspray interface. The instrument was run in the negative ion mode at an ionspray voltage applied to the electrospray emitter tip of −3 kV and an orifice voltage of −30 V.

The interface temperature was held at 400 °C. Nitrogen grade 5.0 at a flow rate of 7 L min^{-1} was used as turbo ion spray and curtain gas in the API source, and nitrogen (99%) at a flow rate of 1.48 L min^{-1} as the nebulizing gas.

For the qualitative analysis of Bayrepel-acid, the deprotonated molecular ion at *m*/*z* 242, scanning in the range of 240–244 was used.

2.6. ESI-TOF-MS

The analyses were performed on an LC–ESI-*oa*-TOF mass spectrometer (Agilent, Santa Clara, CA, USA) equipped with a dual sprayer ESI source for automatic calibrant and reference solution introduction. The instrument was run in the positive ion mode at an ionspray voltage applied to the capillary of 3.5 kV, a fragmentor voltage of 120 V and a skimmer voltage of 60 V. The gas temperature of the drying gas (12 L min^{-1}) was held at 350 °C. For this analysis the ions at 121.050873 and 922.009798, simultaneously introduced via the dual sprayer ESI interface, were used as the calibrant masses.

2.7. Quantitative calibration

2.7.1. Bayrepel and Bayrepel-acid

An eight-point calibration was performed in the range of 0.03–2 $\mu g\,L^{-1}$ in ground water for each compound. The values obtained in surface water were checked by three standard additions for which recovery rates of 97% were achieved (Table 2). The calculated limit of quantitation (LOQ) was determined from the calibration curve. The LOQ in surface water with enrichment was 0.03 $\mu g\,L^{-1}$ for both analytes. The limit of detection (LOD) was defined as 2/1 signal/noise (*s*/*n*).

3. Results and discussion

3.1. Analysis of Bayrepel

Using GC–MS the unequivocal characterization of Bayrepel after ionization with electron impact (EI) was possible. A molecular ion could not be obtained, but rather the ion observed with the highest mass was the fragment at *m*/*z* 184 due to the initial loss of *m*/*z* 45, resulting from cleavage of the –CH_2–CH_2–OH group (see Fig. 1). The most intense ion in the EI-spectrum (*m*/*z* 128) was assigned to the piperidine moiety resulting from additional cleavage of the *N*-carboxylgroup.

Cleavage of the oxy-1-methylpropyl moiety from Bayrepel leads to the fragment causing the ion observed at *m*/*z* 156; additional cleavage of the –CH_2–CH_2–OH– group results in the formation of the *m*/*z* 112 ion. The ion observed at *m*/*z* 84 is attributed to the radical cation of [piperidine–H].

Utilizing electrospray ionization and time-of-flight detection for high accuracy mass spectral data, the most intense

Table 3
Assignment of characteristic ions obtained during (+) LC–ESI-TOF–MS characterization of Bayrepel (B)

	$[B+H^+]^+$	$[B\text{-}(1\text{-methyl propyl})+H+H^+]^+$	$[B\text{-O-}1\text{-methyl propyl}]^+$	$[B\text{-}(\text{carboxy-}1\text{-methyl propyl})+H+H^+]^+$
Sum formula	$C_{12}H_{24}O_3N$	$C_8H_{16}O_3N$	$C_8H_{14}O_2N$	$C_7H_{16}ON$
Measured *m/z*	230.17516	174.11244	156.10203	130.12272
Error (ppm)	0.39	−0.17	0.80	0.6

ion in the MS spectrum could be assigned to the protonated molecular ion of Bayrepel. The calculated target mass of the protonated Bayrepel was 230.17507 a.m.u., whereas the measured mass was 230.17516, a difference of 0.39 ppm. Further, ions observed in the mass spectrum are listed in Table 3.

Bayrepel can be enriched from aqueous matrices with recovery rates above 97% with solid phase extraction as described in the experimental section. LODs of 0.03 and 0.1 μg L^{-1} were obtained for surface water and wastewater respectively (Table 2). The calibration curve was linear in the tested range from 0.03 μg L^{-1} up to 2 μg L^{-1} in ground water.

3.2. Quantification of Bayrepel

Until 1999, the insect repellent DEET, which was the main ingredient of the commercial products Autan and Off could be detected in the effluents of WWTP at concentrations up to 2.5 μg L^{-1} during the main months of use, i.e. in summer [7]. After establishing an analytical method for Bayrepel which replaced DEET as the active ingredient in Autan from 1999, we were also capable to detect this insect repellent in the first WWTP influents analyzed during two sampling campaigns. Bayrepel was present in all analyzed daily mixed samples taken and firstly investigated upon Bayrepel during June and August 2000. In the first seven samples taken from 3 to 9 June, the Bayrepel concentrations were all in the range between 0.6 and 1.1 μg L^{-1}. In further daily mixed samples investigated from 5 to 11 August, the Bayrepel concentrations were in the range between 0.7 and 1.4 μg L^{-1}. In all corresponding WWTP effluents Bayrepel was not present at all (<LOD).

It could be shown in laboratory microbial degradation experiments that the primary aerobic biodegradation of Bayrepel is very rapid [8]. This was assumed to be also the case during the WWT at the WWTP in Wiesbaden, Germany, where the processes used include primary settling, activated sludge and nitrification steps.

Further quantitative data regarding the detection and transformation of Bayrepel in environmental samples is discussed elsewhere [8].

3.3. Synthesis, purification and characterization of Bayrepel-acid

Due to this rapid transformation, potential metabolites of Bayrepel were investigated. Enrichment of WWTP effluent and analysis by GC–MS in neutral mode and after derivatization with diazomethane yielded a peak in the chromatogram eluting at t_R = 15.2 min and showing a similar EI-spectrum as Bayrepel itself (Fig. 2). Interpretation of the spectrum led to the proposal, that oxidation of the hydroxy-group had occurred yielding the carboxylic acid derivative (Bayrepel-acid; (1-piperidinecarboxylic acid, 1-methylpropyl ester, 2-acetic acid). The ion with the highest mass in the spectrum could be assigned to the molecular ion of the methylester of Bayrepel-acid with *m/z* 257.

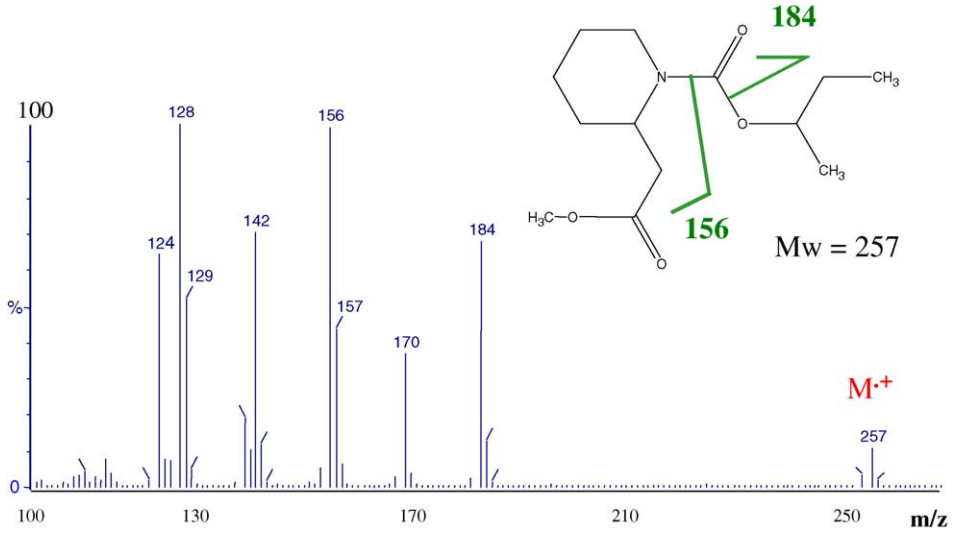

Fig. 2. Electron impact (EI) GC–MS spectrum of Bayrepel-acid (1-piperidinecarboxylic acid, 1-methylpropyl ester, 2-acetic acid) after derivatization to the methyl ester; assignment of masses see Table 2.

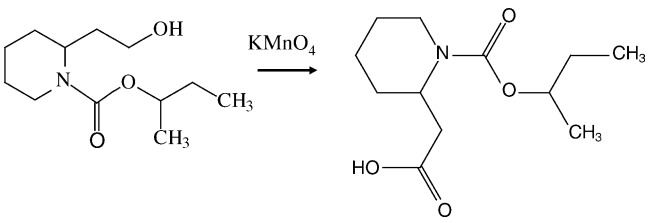

Fig. 3. Simplified scheme of the synthesis of Bayrepel-acid from Bayrepel.

For the fragment ion observed at *m/z* 184, two different possible structures can be proposed: (i) that resulting from cleavage of the $–CH_2–CH_2–OH$ group (see Fig. 1) and (ii) that resulting from cleavage of the oxy-1-methylpropyl-moiety. The latter could subsequently loose a carbonyl group leading to the fragments at *m/z* 156 and 157, varying by protonation at the N atom (Fig. 2). This ion can further fragment to form the ions observed at *m*/z 128 and 129, by loss of an additional carbonyl group, again being either protonated or deprotonated at the N atom. Alternatively cleavage from the methoxy group would result in a ketene with *m/z* 124.

Due to the detection of Bayrepel-acid in effluents it could also be inferred that this product exhibits relatively high stability. Thus there was a need for sourcing of the reference compounds in order to confirm the proposed structure as well as to develop a quantitative analytical method [17,18]. A selective oxidation with Rayney –Ni did not lead to any oxidation of Bayrepel, thus a more general oxidation method using $KMnO_4$ was applied (Fig. 3). The reaction mixture subsequently obtained was purified by semipreparative C_{18} HPLC as described in the experimental section. The obtained fractions were analyzed by negative LC–ESI-MS in order to screen for the desired product (Fig. 4) [19]. In addition, analysis by LC–ESI-MS further confirmed the postulated structure of Bayrepel-acid. GC–MS, after derivatization, of the purified product gave a purity of the Bayrepel-acid obtained of approx. 95% (Fig. 5).

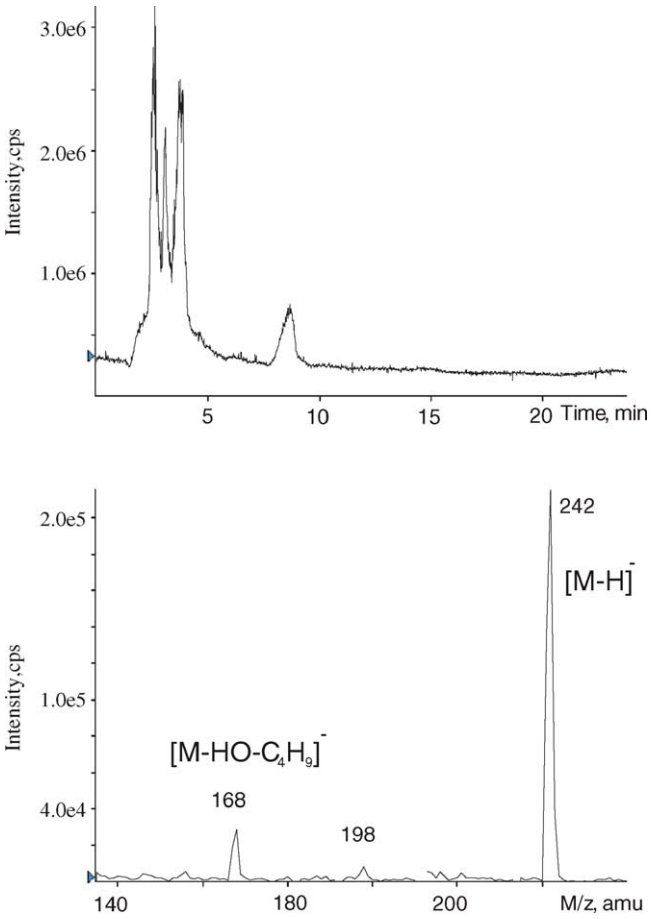

Fig. 4. (−) LC–ESI-MS-chromatogram of the reaction mixture obtained after oxidation of Bayrepel with $KMnO_4$ and spectrum of the desired metabolite Bayrepel-acid (R_t = 8.9 min).

The unequivocal structural characterization of the metabolite Bayrepel-acid was achieved by LC–ESI-TOF–MS analysis (Fig. 6). The molecular ion adducts observed were both extremely conclusive, with the hydrogen and sodium adduct

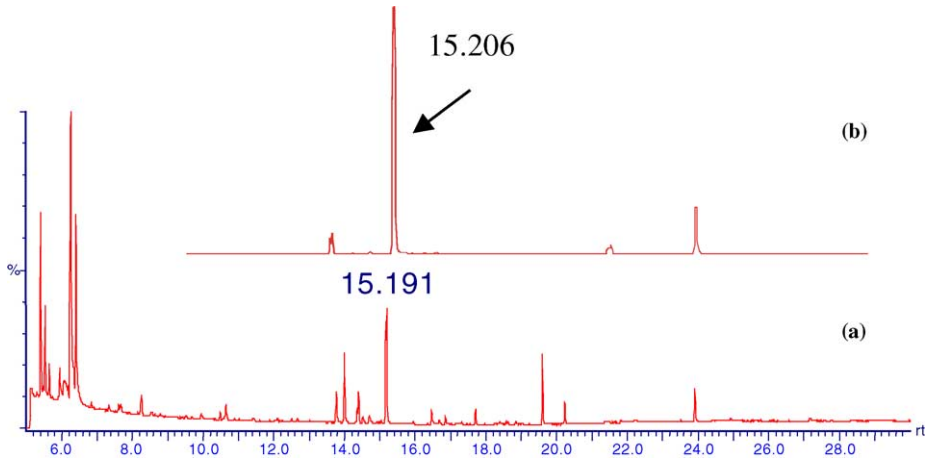

Fig. 5. Comparison of EI-GC–EI–MS spectra of the oxidation mixture of Bayrepel after derivatization with diazomethane (a) before and (b) after LC purification; t_R of Bayrepel-acid = 15.206 min.

Table 4
Assignment of characteristic ions obtained during (+) ESI-TOF–MS characterization of Bayrepel-acid (BA) (see also Fig. 6)

	$[BA + H^+]^+$	$[BA + Na^+]^+$	$[2BA + Na^+]^+$	[BA-(1-methyl propyl) + H + H^+]$^+$	[BA-(carboxy-1-methyl propyl) + H + H^+]$^+$
Sum formula	$C_{12}H_{22}O_4N$	$C_{12}H_{21}O_4NNa$	$C_{24}H_{42}O_8N_2Na$	$C_8H_{14}O_4N$	$C_7H_{14}O_2N$
Measured *m/z*	244.15449	266.13628	509.28328	188.0914	144.10185
Error (ppm)	0.64	0.002	0.11	−1.56	−0.38

ions at *m/z* 244.15449 and *m/z* 266.13628 being in deviation of only 0.64 and 0.002 ppm, respectively from the exact mass calculations for $C_{12}H_{22}O_4N$ and $C_{12}H_{21}O_4NNa$ (Table 4). Additional to the high mass accuracy, the isotope ratio of $^{12}C/^{13}C$ and the fragmentation pattern obtained also fitted perfectly to the proposed structure.

Similar to Bayrepel, the metabolite Bayrepel-acid could also be enriched from aqueous matrices after acidification, with recovery rates above 97% with solid phase extraction, as described in the experimental section. LODs of 0.03 and 0.1 μg L^{-1} were obtained for surface water and wastewater respectively (Table 2). The calibration curve was linear in the tested range from 0.03 μg L^{-1} up to 2 μg L^{-1} in ground water.

Further quantitative data regarding the detection and transformation of Bayrepel-acid in environmental samples is given elsewhere [8]. Thus, as a result of the synthesis, characterization and development of methodology for the quantification of Bayrepel-acid described here, monitoring its further fate during biodegradation, as necessary for xenobiotic compounds introduced into the environment, has been achieved [8].

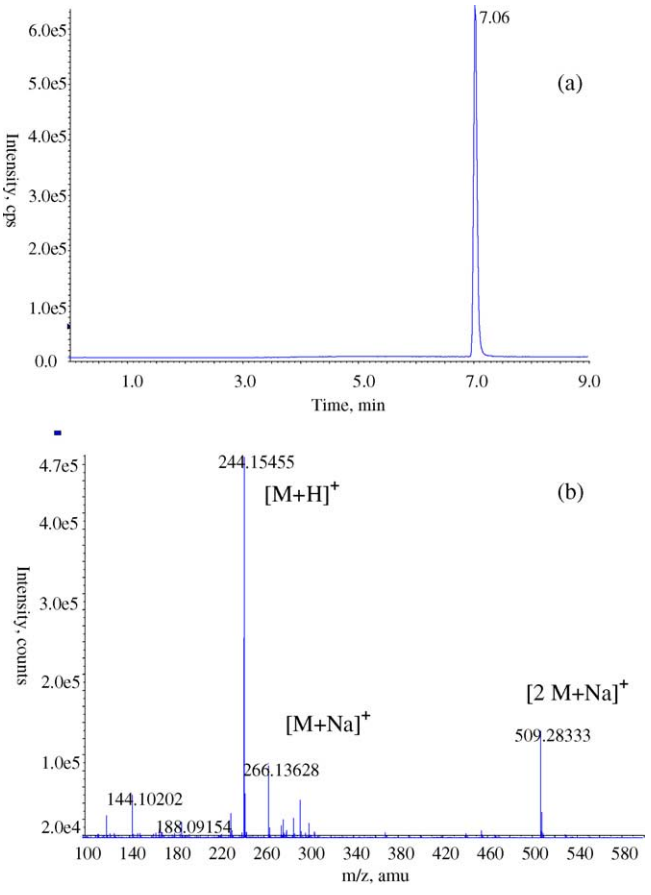

Fig. 6. (a) HPLC-chromatogram and (b) (+) LC–ESI-TOF-MS spectrum of Bayrepel-acid after purification.

4. Conclusion

In order to assess the fate of a product in the environment it is by no means sufficient to only monitor for the parent compound, but is also essential to analyze for stable metabolites being formed. Thus there is a need for analytical methods that enable both the quantification of the parent compound as well as the potential to check for metabolites. Mass spectrometry coupled to either GC or LC has gained increasing use for this purpose. As demonstrated here, the use of complementary methods increases confidence in structural assignments, and the high mass accuracy of ESI-TOF, in particular, can provide extremely useful information. Recent evolutions in ESI-TOF technologies has resulted in significant improvements to mass accuracy over wide concentration ranges (10^3), enabling reduced sample preparation and manipulation requirements for high confidence accurate mass data. This is extremely valuable in the analysis of unknowns and mixtures, and thus holds much potential for environmental degradation studies. Furthermore improvements enabling low-femtomole level sensitivity, up to 10,000 resolving power, and the better than 3 ppm mass accuracy over an extremely wide concentration range for the analytes and automatically-introduced reference compounds indicate the future potential of this detection method.

However, even with such powerful tools, correct quantification, and thereby environmental and toxicological risk assessment, can only be achieved with the availability of reference compounds. Thus, monitoring for metabolites should always go hand-in-hand with the synthesis of such compounds.

Acknowledgements

The experimental help of A. Maes, ESWE Institute during the mass spectrometric analyses, is very much appreciated as well as the helpful discussions with Dr. S. Schüle and Dr. R. Kanne, Bayer AG. The suport of Dr. G. Krummel during the oxidation of the Bayrepel-acid is also acknowledged. Special thanks is given to Mr. B. Wüst and Dr. L. Bonnington from

Agilent Technologies for ESI-TOF–MS analyses. Financial support provided by the European Commission (5th Framework programme, Project P-THREE EVK1-CT-2002-00116) is gratefully acknowledged.

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