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Determination of the herbicide amitrole in water with pre-column derivatization, liquid chromatography and tandem mass spectrometry

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Abstract

Amitrole is a widely used polar herbicide, difficult to isolate from water. Due to its persistence, it can easily pollute ground and surface waters used in drinking water production. A fully automated on-line SPE–HPLC (solid-phase extraction–high-performance liquid chromatography) method with atmospheric pressure chemical ionisation–tandem mass spectrometry detection is described for the determination of amitrole. A pre-column derivatization with 9-fluorenylmethoxycarbonyl chloride directly in the native aqueous sample allows an enrichment step by SPE and HPLC separation. Due to the sensitivity of tandem mass spectrometric detection, a limit of detection and quantification as low as 0.025 μg/l was achieved in drinking water and ground and surface water. Based on the constant ratio of two selected product ions together with the retention time, the identification is very selective and quantitation is very reliable. The performance characteristics of the described method fully meet the requirements set by the EU Drinking Water Directive: recoveries of >95% in drinking water and >75% in surface water were achieved, as well as RSD values for repeatability of <9% in drinking water and <12% in surface water (determined at a spiking level of 0.1 μg/l). The method was successfully applied to real samples of ground and surface water with actual concentration up to 1.1 μg/l.

Keywords: Derivatization, LC; Water analysis; Environmental analysis; Pesticides; Amitrole

1. Introduction

Amitrole, also known as aminotriazole (Fig. 1), is a non-selective herbicide, which is widely used, quite often as a replacement for other, banned herbicides, such as diuron. This herbicide, although potentially carcinogenic, is of low toxicity to mammals. Because of the good solubility of amitrole in water, leaching may occur and can lead to polluted ground and surface water and consequently may lead to drinking water contamination.

Due to its very polar, and in most cases ionic character, ion chromatography is often the method of choice in amitrole determination [1–3]. Pichon et al. [1] compared ion-exchange with ion-pair extraction, but found a competition between amitrole and inorganic cations, obstructing the analysis of amitrole at low concentrations in real life samples. As long as there is no efficient way to remove a large amount of the competitive cations in order to prevent breakthrough, the 0.1 μg/l limit is very difficult to achieve. Dugay et al. [2] experienced the same difficulties using the ion-exchange and the ion-pair extraction. Pachinger et al. [3] seemed to have no such problems and they achieved a detection limit of 0.1 μg/l, however, their electrochemical detection

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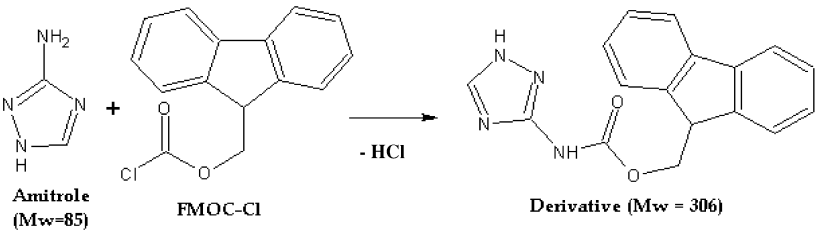

Fig. 1. Proposed mechanism of the derivatization of amitrole with 9-fluorenylmethoxycarbonyl chloride (FMOC-Cl). M_w = molecular mass.

cell needs to be cleaned after each run and the detector needs to equilibrate, which makes this method unfit for routine analysis.

As a consequence of its polar character, chromatographic separation of amitrole with GC [4] or LC [2,5] is only possible after derivatization. Van der Poll et al. [4] used an acetylation of amitrole in the concentrated (50×) water sample followed by an extraction step with ethyl acetate. The extract was examined by gas chromatography with alkali flame ionisation detection and a detection limit of 0.1 μg/l was achieved.

Because of its suitability for aqueous samples, HPLC is the analytical technique of choice for polar compounds. Several RP-HPLC methods for amitrole analysis were developed using pre-column derivatization and either fluorescence or UV detection [2,5]. Dugay et al. [2] diazotated amitrole in the native aqueous sample and were able to separate the derivative with HPLC, reaching a detection limit of 100 μg/l with UV detection. García Sánchez et al. [5] derivatized amitrole with fluorescamine and analysed the samples with RP-HPLC using fluorescence detection. The best achieved detection limit was 0.75 μg/l.

From 2001, the new European Union (EU) Drinking Water Directive is valid, setting a requirement for the limit of detection (LOD) of analytical methods used for the determination of pesticides in drinking water as low as 0.025 μg/l [6]. Conclusively, it can be said, that none of the methods described above, achieve the detection limit required by the EU.

In our laboratory, for compounds with similar chemical properties, glyphosate, glufosinate and aminophosphonic acid (AMPA), an approach was developed using derivatization of the amine groups in the native aqueous sample [7]. This allows on-line solid-phase extraction (SPE) and RP-HPLC separation with subsequent fluorescence [7] or tandem MS detection [8]. This paper describes the development of a new method for amitrole, based on this approach. The performance characteristics of the developed method were determined according to the requirements set by the new EU Drinking Water Directive 98/83 [6].

2. Experimental

2.1. Chemicals

All chemicals are of analytical reagent grade and used without further purification, except for the methanol, which is distilled. Amitrole is obtained from Dr Ehrenstorfer (Augsburg, Germany) with a 97.4% certified purity. Methanol is obtained from Chemproha (Zwijndrecht, Netherlands). Ammonium acetate is obtained from Baker (Deventer, Netherlands). Borate buffer is prepared by dissolving 5 g of disodium tetraborate decahydrate (Baker) in 100 ml of ultrapure water. Concentrated phosphoric acid (reagent grade) to stop the derivatization is obtained from Baker. The 9-fluorenylmethoxycarbonyl chloride (FMOC-Cl) reagent solution is prepared by dissolving 100 mg of FMOC-Cl from Fluka (a Sigma–Aldrich company, Buchs, Switzerland) in 50 ml of acetonitrile. Acetonitrile (Chromasolv quality) is obtained from Riedel–de Haen (Seelze, Germany).

2.2. Instrumentation

2.2.1. HPLC analysis

The robotic autosampler is a Gilson 233XL/402 sample delivery unit (Gilson, Villiers le Bel, France) with the sample loop of the injection valve replaced by two 20×3 mm (I.D.) pre-columns packed with

PLRP-s sorbent (15–25 μm). The HPLC system, a Perkin-Elmer (Norwalk, CT, USA) model 250 gradient pump, introduces an aqueous ammonium acetate (5 m*M*)–methanol mobile phase with a flow-rate of 1 ml/min. The analytical column is a 250×4.6 mm (5 μm particles) Inertsil ODS-2 column (GL-Science, Tokyo, Japan). Preliminary experiments were performed with different gradients of acetonitrile and water as the mobile phase. During the course of the study, methanol was found to be more suited as the organic modifier. A linear gradient from 10 to 35% (in 5 min), from 35 to 80% (in 15 min) and from 80 to 100% of methanol (in 8 min) is used for the elution of the derivatized amitrole. Hereafter, the mobile phase composition is maintained at 100% of methanol for 5 min, in order to elute the excess of derivatization reagent from the column. Before the next injection, the composition of the mobile phase is brought back to 10% methanol, with a linear gradient (in 10 min).

2.2.2. Mass spectrometry

The mass spectra were recorded on a Finnigan MAT (San José, CA, USA) TSQ 7000 mass spectrometer using the standard atmospheric pressure chemical ionization (APCI) interface of Finnigan MAT. The pure FMOC derivative of amitrole is commercially not available, therefore the instrument is tuned in the positive ion mode by infusing several μl of a 10 mg/l solution of poly(ethylene glycol) (PEG, Baker) in methanol–water (1:1, v/v) with 0.01 *M* of ammonium acetate.

The optimised atmospheric pressure ionization (API) interface settings are: heated capillary 215°C, vaporizer temperature 500°C, corona needle current 5.00 μA, sheath gas (nitrogen) pressure 75 p.s.i. (1 p.s.i.=6894.76 Pa), the auxiliary gas is not used. In full-scan analysis, mass spectra were acquired from m/z 50 up to m/z 500 during each second. MS–MS experiments are performed using argon as the collision gas at a pressure of 2.0 mTorr (1 Torr=1.33.322 Pa). Optimal collision energy for the amitrole–FMOC was determined to be −20 eV. Detection is achieved with a multiplier setting of 1300 V in full-scan analysis and 1800 V during selective reaction monitoring in the MS–MS experiments. In order to achieve sufficient sensitivity, resolution lower than nominal is used for the first quadrupole during MS–MS experiments. No source fragmentation is used in any of the described experiments.

2.3. Sample preparation

All samples are collected in glass bottles. Prior to derivatization, the samples are subjected to a filtration step by passing them through a 0.45-μm regenerated cellulose filter (Schleicher & Schuell, Dassel, Germany). The derivatization is performed in duplicate and one sample is stored in the fridge (+4°C).

2.4. Analytical procedure

Samples are derivatized upon receipt in the laboratory by adding FMOC-Cl solution to the sample together with borate buffer, and allowing the reaction to take place overnight at 37°C [8]. The reaction is stopped by adding phosphoric acid, i.e. lowering the pH to 2. The sample flasks containing the derivatized samples, standards and quality control samples are placed in the autosampler. A 9 ml volume is passed over the pre-concentration column and after rinsing with water, the six-port valve of the Gilson autosampler is switched. A start signal is sent to (I) the LC pump to start the gradient programme and (II) the MS system to start data acquisition, and the pre-column is eluted with the HPLC eluent [9] in the back flush mode. The compounds are separated on the HPLC column and subsequently analysed by MS.

For quality assurance purposes, blanks, standard solutions, performance standards and spiked control samples are included in each sample series.

3. Results and discussion

3.1. General

Because FMOC-Cl can react with both primary and secondary amino groups and amitrole contains both, the possibility of three different amitrole–FMOC derivatives exists. However, only one peak, corresponding to m/z 307 (one derivatized group) is observed. No peaks with m/z 485 (two derivatized groups) are present in the chromatogram, suggesting that a single reaction takes place. Which of the two amino groups reacts has not been further examined,

but because of the stability of the cyclic structure of amitrole, the secondary amino group is not likely to react with FMOC-Cl, whereas the primary amino group is, Fig. 1. All compounds containing amino functional groups (also amino acids) present in the sample are also readily derivatized with FMOC-Cl and can interfere with both the reaction and the detection.

The derivatization reduces the polar character of amitrole and enables conventional SPE isolation with PLRP material and consequently, also RP-HPLC separation. The fluorescence properties of the FMOC can also be utilized during the detection of the derivatives by fluorescence. Even though this approach has proved useful for glyphosate [7], in the case of amitrole, the target analyte co-elutes with a broad peak, caused by the excess of the derivatization reagent (see Fig. 2a). This does not improve even after extensive optimization of the chromatographic conditions. If, for the same standard, MS–MS is used for detection, the selectivity improves significantly (Fig. 2b).

The samples are always derivatized in the presence of excess of derivatization reagent, which can cause contamination of the MS system and reduce the performance of the method. To prevent this, the LC effluent is discarded during the first (t_R<20 min) and the last part (t_R>30 min) of the chromatogram (the amitrole–FMOC derivative has a t_R of approximately 26.20 min) using a divert-valve. The separation conditions are optimized in order to minimize the interference of other derivatized amino compounds, present in real-life water samples (see applications) and derivatization by-products formed due to the excess of reagent.

The relatively high proton affinity of the amitrole–FMOC derivative is used during the ionisation process in the APCI ionisation interface. Full-scan APCI–MS spectra and product ion MS–MS spectra are recorded in the positive ion mode. If acetonitrile (with 1% ammonium acetate) is used as the organic modifier, in addition to the protonated amitrole–FMOC (m/z 307), an ammonium adduct (m/z 348) is observed as one of the highest ions in the spectrum. This effect is quite common in the analysis of nitrogen-containing compounds [10,11] and reduces the sensitivity of the multiple reaction monitoring (MRM) experiments. If, instead of acetonitrile, methanol is used as the organic modifier, the formation of adducts, other than protonated molecules, is reduced and the sensitivity improves significantly. Under the optimized conditions, very little fragmentation occurs (m/z 179). Upon the selection of the protonated molecule, i.e. the ion at m/z 307,

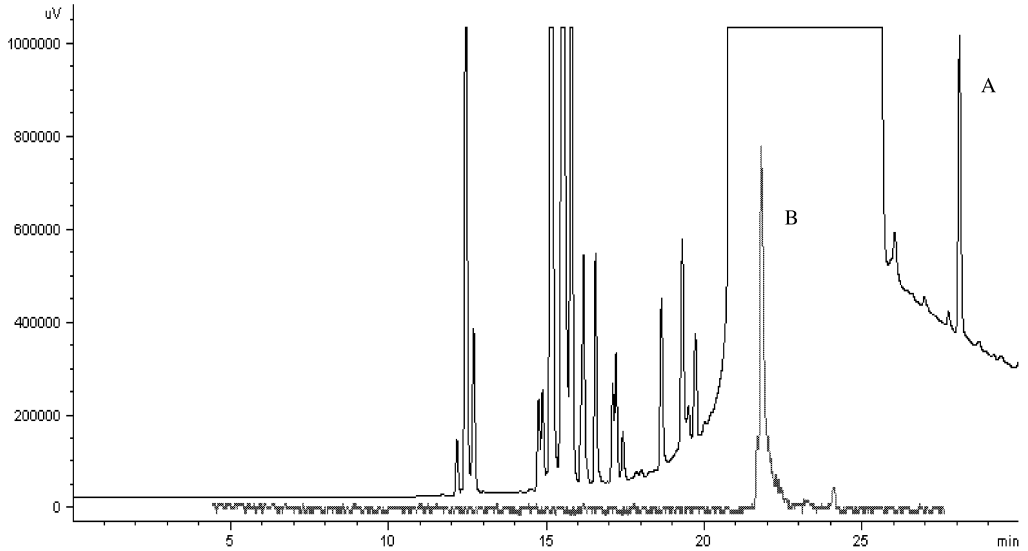

Fig. 2. Detection of amitrole derivatized with FMOC-Cl. On-line SPE–HPLC and detection with fluorescence (A) and MS–MS (B). In these preliminary experiments, acetonitrile and water were used for elution.

as pre-cursor ion for collision induced dissociation (CID) measurements (2.0 mTorr Ar, collision energy −5 to −40 eV) two product ions are obtained, at m/z 179 and m/z 129 (Fig. 3). The first ion originates in the FMOC derivatized part of the molecule, the second ion is specific for amitrole.

The summed intensities of these product ions maximise around −20 eV of collision energy. The formation of the two product ions is ''scanned'' in a consecutive way, in the MRM mode. The relative intensity of m/z 129 with respect to m/z 179 is calculated based on the peak area of the individual chromatograms at the correct retention time. The ratio of these two signals appeared (more or less) constant (reproducibility <15% within a series of 50 samples, including standards and quality control standards). Due to lack of independent identification criteria for environmental LC–MS–MS applications, criteria described for the identification of banned substances in veterinary residues were applied [12]. The compound is regarded as positively identified when (I) the retention time is within a time window of ±0.5% of the last analysed performance standard (a standard solution containing 0.15 μg/l, which is analysed after every 5 samples) and (II) the relative intensity of m/z 129 lies between $(x \pm 0.25x)$%, x being the relative intensity of this ion determined in the last performance standard (calculated from the peak areas of the two product ions). In the cases that the deviation is higher, e.g. both monitored reactions are observed at the correct retention time, the sample is reanalysed (using the duplicate derivatized sample which is stored in the fridge at +4°C). Standard addition can be used in cases where extra confirmation of the retention time is needed.

3.2. Performance characteristics

The determination of the performance characteristics of the method was in agreement with the EU 98/83 directive [6,13]. The linearity and the LODs were determined for the three signals: (I) m/z 129, (II) m/z 179 and (III) the sum of these two.

The calibration curves were calculated by un-

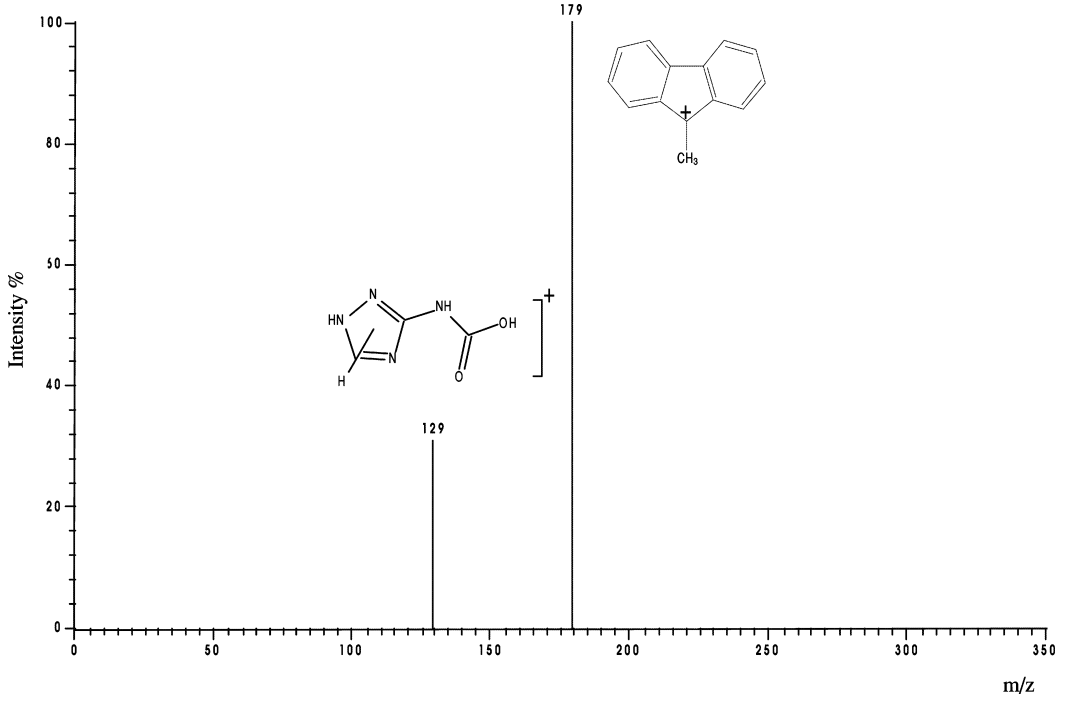

Fig. 3. Full scan product ion spectra of pre-cursor m/z 307, protonated amitrole–FMOC derivative. Proposed structures of the main product ions.

weighed linear regression. For concentrations between 0.025 and 0.5 μg/l the response was taken as a linear function of the concentration and the coefficient of regression was better than 0.998 for all three signals.

LODs were calculated on the basis of the standard deviation of seven samples (Ultrapure water fortified at 0.025 μg/l, LOD = 3.14·RSD). The calculated detection limits were <0.017 μg/l for all individual signals. The calculated LODs were verified in drinking, surface and ground water fortified with 0.025 μg/l. In all cases, signal-to-noise ratio >3:1 was obtained for the individual signals.

For a 9-ml sample volume, recoveries of 75% (surface and ground water) and 95% (drinking water) were obtained for all tested matrices (spiking levels 0.1 μg/l in drinking water and 0.1 and 0.5 μg/l in ground and surface water).

The relative standard deviation for the repeatability was <2% ($n=5$) and reproducibility was <9% ($n=6$) in spiked drinking water samples (0.1 μg/l). These data were all below 12.5%, required by the EU Drinking Water Directive [6,13]. In the case of spiked surface and ground waters the relative standard deviation for both the repeatability and reproducibility was <12%.

3.3. Applications

At present, the method described above is successfully used for the determination of amitrole in large batches of samples. In the example in Fig. 4, the specificity of this method, using the combined information of the two selected product ions and the retention time is demonstrated. Local drinking water, fortified with 0.1 μg/l of amitrole was analysed with the developed method. In addition to amitrole (t_R 26.24 min), two additional peaks with approximately the same intensity were observed for the m/z 307 to m/z 129 reaction (t_R 22.92 and 27.09 min) and one

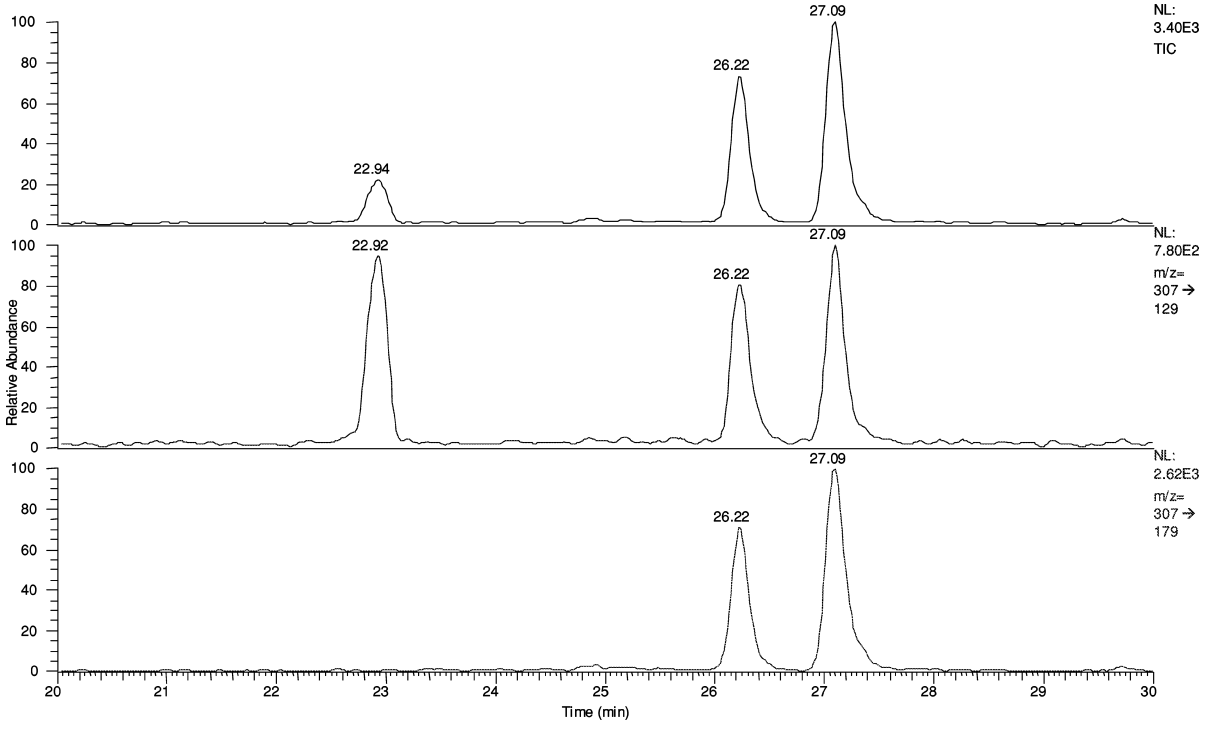

Fig. 4. Drinking water fortified with 0.1 μg/l of amitrole and analysed with the developed method. The presence of amitrole is indicated by the peak at the correct retention time (26.24 min) and the correct ratio of the two signals. Peaks eluting before and after amitrole are caused by natural organic matter present in the local drinking water and some surface waters and do not interfere with the determination of the target analyte.

for the m/z 307 to m/z 179 reaction (t_R 27.09 min) (Fig. 5). Both unknown compounds were also present in the derivatized drinking water blank. Further experiments indicated that both peaks were caused by natural organic matter which was present in drinking and some surface waters. The peaks were not observed in the chromatograms if during the MS–MS experiments, the resolution of the first quadrupole was set to nominal (not shown). However, even with lower resolution, both unknown peaks do not interfere with the determination of the target analyte, as they were resolved from amitrole by both a difference in retention time (more than the allowed 0.5%) and by the ratio of the two traces. In the earlier eluting peak, m/z 179 was absent and in the compound eluting after amitrole, the ratio of the two ions did not fulfill the identification criteria [12]. For the applications described here, this selectivity is considered sufficient, however, if needed, enhanced selectivity can be obtained at the expense of sensitivity.

Fig. 5 shows the total ion current (TIC) and the extracted ion chromatograms in a real ground water sample. The presence of amitrole (1.1 μg/l) was indicated by the elution of the two selected ions in one peak, having the proper ratios and within the corresponding retention time window.

4. Conclusions

A method for the analysis of amitrole in different types of water matrices was developed. The performance characteristics meet the requirements of the EU Drinking Water Directive: linearity 0.998, LOD 0.025 μg/l, recoveries 75–95% and the variation coefficients of repeatability and reproducibility both better than 9% in all matrices. The developed method is very robust and shows good performance over a long period of time. It has been successfully applied to drinking water, ground and surface water. A complete automation of the method, including

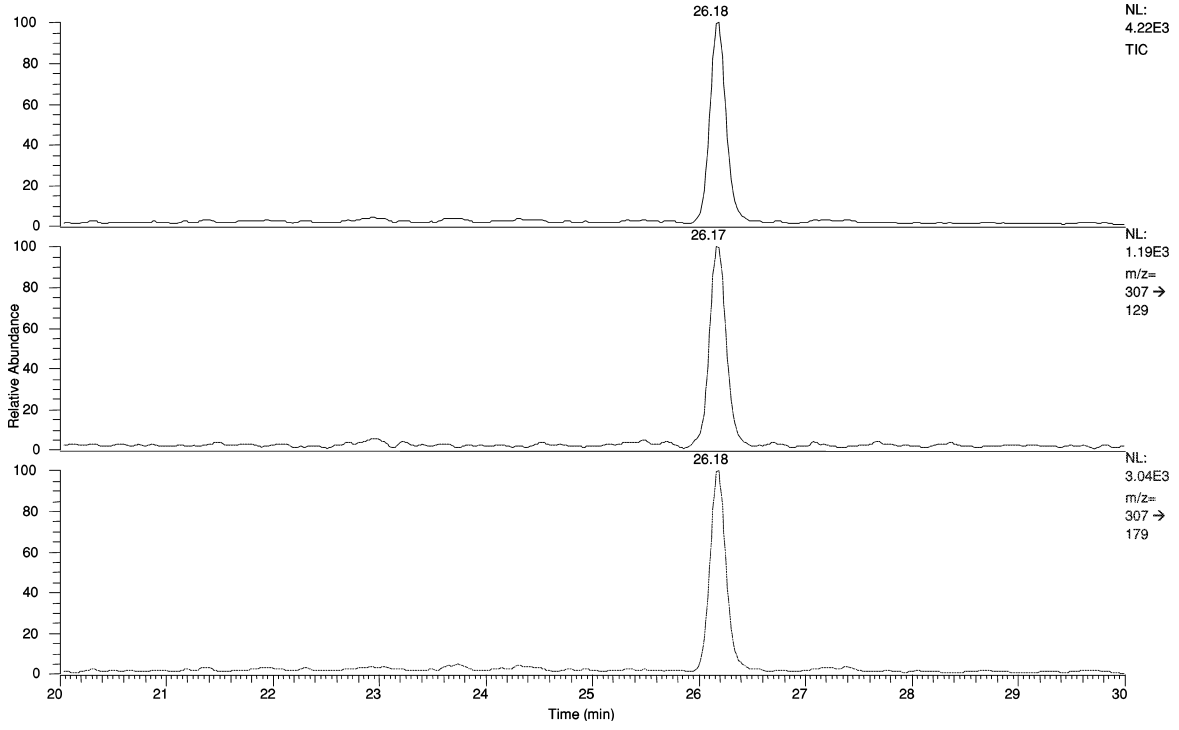

Fig. 5. Ground water sample. The presence of amitrole (1.1 μg/l) is indicated by the peak at the correct retention time (26.24 min) and the correct ratio of the two signals. This concentration is out of the linear range of the method the sample was therefore diluted and re-analysed.

on-line SPE–HPLC was achieved. In this way unattended LC–MS–MS analysis of more than 100 samples in one sequence is possible.

As a proper internal standard, i.e. a deuterated compound, is not commercially available, identification is based on the elution of the target compound within a retention time window of ±0.5% of that of the last analysed performance standard and the proper ratio between the two product ion signals. Quantification is based on the summed intensity of the monitored product ions.

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