ELSEVIER

Journal of Chromatography A, 794 (1998) 187–199

JOURNAL OF CHROMATOGRAPHY A

Selective analysis of the herbicides glyphosate and aminomethylphosphonic acid in water by on-line solid-phase extraction–high-performance liquid chromatography–electrospray ionization mass spectrometry

R.J. Vreeken[1], P. Speksnijder, I. Bobeldijk-Pastorova*, Th.H.M. Noij

Kiwa N.V. Research and Consultancy, P.O. Box 1072, 3430 BB Nieuwegein, Netherlands

Abstract

A fully automated on-line solid-phase extraction–high-performance liquid chromatography–electrospray ionization mass spectrometry (SPE-HPLC–ESI-MS–MS) method was developed for the determination of glyphosate and its degradation product aminomethylphosphonic acid (AMPA), in the concentration range of 0.05 to 3 μg/l in various types of water. Derivatisation of glyphosate and AMPA directly in the native aqueous sample with 9-fluorenyl methoxycarbonyl chloride allowed the preconcentration by SPE and HPLC separation. Identification and quantification based on the constant ratio of three selected ions for each compound (precursor ion and two product-ions) together with the retention time proved to be a very selective procedure for the reliable determination of these pesticides. Although developed for drinking water and surface water, the selectivity of this method also allows their determination in waste water. Moreover, the structurally similar herbicide glufosinate could also be determined using the same derivatisation procedure and analytical method. Validation of the HPLC–MS method for glyphosate and AMPA showed detection limits as low as 0.03 μg/l. Recoveries of 96% and relative standard deviations for repeatability <8.4% were determined at 0.2 concentration.

Keywords: Water analysis; Derivatisation, LC; Mass spectrometry; Electrospray ionization; Pesticides; Glyphosphate; Glufosinate

1. Introduction

One of the important fields of environmental analytical chemistry is the development of analytical methods for the determination of pesticides and their degradation products. A substantial number of polar pesticides presently in use is still very difficult to analyse in water at the 0.1 μg/l level, the maximum allowable concentration in drinking water set by the European Community (EC) [9]. Amongst them are the two broad spectrum nonselective herbicides, glyphosate [N-(phosphonomethyl)glycine] and glufosinate [DL-homoalanine-4-yl(methyl)phosphinic acid]. These herbicides are of low toxicity to mammals and are widely used even though their effects on nontarget organisms and overall environmental fate are still not fully known. It is known that glyphosate is rapidly degraded into AMPA (aminomethylphosphonic acid), its major degradation prod-

*Corresponding author.

[1]Present adress: TNO Pharma, Utrechtseweg 48, P.O. Box 360, 3700 AJ Zeist, Netherlands.

0021-9673/98/$19.00
PII S0021-9673(97)01129-1

uct. The use of glufosinate, a structurally related pesticide with a higher degradation rate, is increasing and reliable methods are needed for its determination in aqueous samples.

Glyphosate, glufosinate and AMPA are well soluble in water. Due to their very polar, and in most cases ionic character, derivatisation is often needed to enable chromatographic separation with gas chromatography (GC) [1] or liquid chromatography (LC) [2,3]. Because of its suitability for aqueous samples, high-performance liquid chromatography (HPLC) is the analytical technique of choice for polar compounds and several HPLC methods were developed using pre- or postcolumn derivatisation and fluorescence detection (FD) [2–4]. As no concentration step is included, the sensitivity of these methods is generally not sufficient for drinking water control. Therefore, we developed a HPLC method based on the derivatisation of glyphosate and AMPA in the native water sample with 9-fluorenyl methoxycarbonyl chloride (FMOC-Cl), Fig. 1, prior to solid-phase extraction (SPE). By this approach the extraction efficiency was improved and it allowed efficient HPLC separation and sensitive FD as well [5]. During the past 3 years, hundreds of samples were analysed routinely by this method, using an on-line SPE–HPLC–FD set up. However, being nonspecific, FD often requires MS confirmation to ascertain the identity of the glyphosate and AMPA peaks, especially since for real-life samples many derivatisation products can be observed in the chromatogram.

Therefore, a selective and sensitive method for the determination of glyphosate and AMPA by means of LC–MS–MS was developed. This method was designed for a Quattro II MS–MS system, which allowed the use of a special scan routine, i.e., alternate scanning between LC–MS (selected ion monitoring mode) and LC–MS–MS (selective reaction monitoring mode) [8]. This scan routine, resulted in three (in)dependent ion signals, viz., the $[M\text{-}H]^-$ ion signal from the derivative during the LC–MS scan and two product-ion signals formed upon collision induced dissociation (CID). Quantification and identification based on the ratios of these three signals and the retention time appears to be very specific, thus reducing the number of false positives.

This paper presents the method as it was finally implemented as a fully automated procedure for

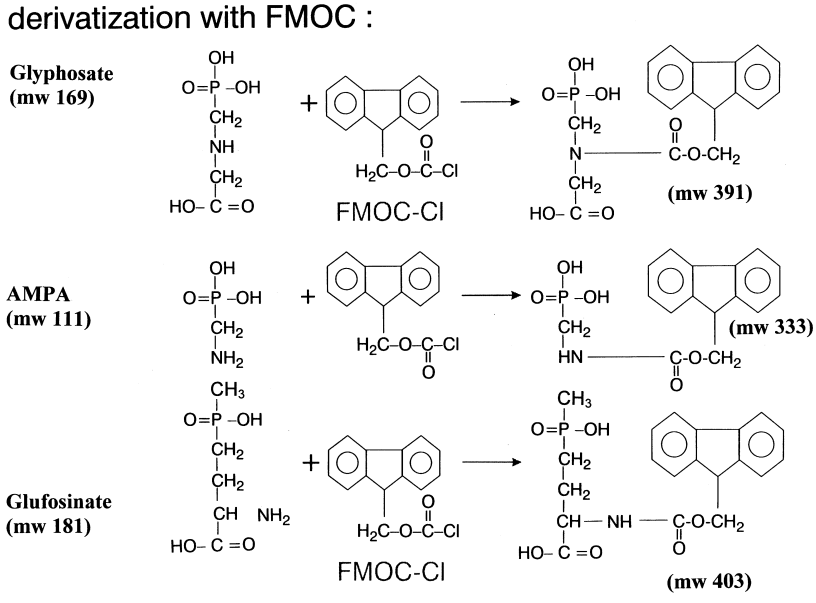

Fig. 1. Derivatisation of glyphosate and AMPA with 9-fluorenyl methoxycarbonyl chloride (FMOC-Cl)

routine analysis on a TSQ 7000 mass spectrometer, i.e., a system unable to switch between LC–MS and LC–MS–MS in a single scan routine. Performance data for the method as well as several practical applications are presented.

2. Experimental

2.1. Chemicals

All chemicals were of analytical reagent grade and used without further purification. Glyphosate was obtained from Schmidt (Amsterdam, Netherlands) with a 98% certified purity. AMPA was obtained from Acros (Geel, Belgium) with a >99% purity. Glufosinate and acetonitrile (Chromosolv quality) were obtained from Riedel de Haen (Seelze, Germany). Ammonium acetate was obtained from Baker (Deventer, The Netherlands). Borate buffer was prepared by dissolving 5 g of disodium tetraborate decahydrate (borate, Baker) in 100 ml of ultra pure water. Concentrated phosphoric acid (reagent grade) to stop the derivatisation was obtained from Baker. The FMOC-Cl reagent solution was prepared by dissolving 100 mg of FMOC-Cl from Fluka (Buchs, Switzerland) in 50 ml of acetonitrile.

2.2. Instrumentation

2.2.1. HPLC analysis

The HPLC system used in this work consisted of a Perkin–Elmer (Norwalk, CO, USA) model 250 gradient pump, which delivered an aqueous ammonium acetate (5 m*M*)–acetonitrile mobile phase to a (250×4.6 mm; 5 μm particles) Inertsil ODS-2 column (GL Science, Tokyo, Japan) with a flow-rate of 1 ml/min. A linear gradient from 10 to 54% of acetonitrile, within 20 min, was used for the elution of the test compounds. Hereafter, the mobile phase composition was maintained at 90% of acetonitrile to elute the excess of derivatisation reagent from the column.

2.2.2. Mass spectrometry

Electrospray ionization (ESI) full-scan mass spectra (m/z 50–500/s) were recorded on a Finnigan MAT (San José, CA, USA) TSQ 7000 mass spectrometer using the standard electrospray interface of Finnigan MAT. The instrument was tuned in the negative ion mode by infusing several μl of a 10 mg/l solution of polyethyleneglycol (PEG, Baker) in methanol–water (1:1, v/v) with 0.01 *M* of ammonium acetate.

To assure a flow of 200 μl/min into the ESI interface during LC–MS and LC–MS–MS, the LC effluent (1 ml/min) was split (200:800) by means of a zero dead volume T-piece. This flow was found to be the optimum under these conditions. Optimised values for the atmospheric pressure ionization (API) interface parameters are: heated capillary 250°C; ESI voltage −3.6 kV; sheath flow pressure 75 psi; auxilliary gas flow 50 units (on the flowmeter). The mass spectrometer was used either in single MS mode or in product-ion mode with a optimum collision offset for each compound (5–25 eV). In full-scan analysis mass spectra were acquired from m/z 50 up to m/z 500 during each second. MS–MS experiments were performed using argon (Ar) as the collision gas at a pressure of 2.0 mTorr. Detection was achieved with a multiplier setting of 1700 V in full-scan analysis and 1900 V during selective reaction monitoring of the MS–MS experiments. No source CID was used in any of the described experiments.

2.3. Sample preparation and preconcentration

The samples were collected in full polypropylene bottles and care was taken that the samples did not come into contact with glass prior to derivatisation, because of the possible adsorption of glyphosate or AMPA to glass. Prior to derivatisation, which is performed in duplo (one sample being stored in the freezer at −20°), the samples were subjected to a filtration step by passing it through a 0.45 μm regenerated cellulose filter (Schleicher and Schuell, Dassel, Germany).

The robotic autosampler was a Gilson 233XL/402 sample delivery unit (Gilson, Villiers le Bel, France) with the sample loop of the injection valve replaced by a 20×3 mm (l×I.D.) precolumn packed with PLRP-s sorbent (15–25 μm).

2.4. Analytical procedure

Samples were derivatised 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 [5]. The reaction is stopped by adding phosphoric acid, i.e., lowering the pH. Up to a maximum of 50 sample flasks containing the derivatised sample are placed in the autosampler. A 4 ml volume is passed over the PLRP-s preconcentration column and after rinsing with water the six-port valve of the Gilson autosampler is switched, a start signal is send to: (i) the LC pump to start the gradient programme and (ii) the MS to start data acquisition, and the precolumn is eluted with the HPLC eluent [6]. 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 were included in each sample series.

In the scope of automation is noteworthy to state that the Gilson autosampler is in charge of the sequential analysis, i.e., the Gilson is the ''master''. The LC pump is connected to the master via a start–stop link and the MS is connected via a contact-closure loop. Therefore, a malfunction in either the LC pump or the autosampler will hold the analysis queue as in the case of an error on the side of the MS the queue will continue. Fortunately, the divert valve which is used on the TSQ to direct the LC effluent to waste or the MS, is in default mode connected to the waste outlet.

3. Results and discussion

An LC–ESI-MS–MS method for determination of glyphosate and AMPA in water as their FMOC derivatives was initially developed on a Quattro II LC–MS–MS instrument in close cooperation with the Laboratory for Special Research of the Water-Transport Company Rijn-Kennemerland (WRK). A full description of the method development and optimisation of the Quattro II instrument will be given elsewhere [8]. In order to: (i) enable a large sample throughput; (ii) extend the applicability and (iii) to implement the method in house, the analytical procedure was automated, adapted for another MS and subjected to the analysis of various sample types. Also the applicability of the method to analyse another structurally related pesticide, i.e., glufosinate ammonium, was investigated and quantitative performance data were established.

Compared to the on-line SPE–HPLC–FD method [5] the sampling, sample derivatisation and preconcentration remained unchanged. This also holds for the HPLC conditions, except for the buffers: ammonium acetate is used as the buffer for the LC–MS measurements as opposed to a mixture of potassium hydroxide and potassium dihydrogenphosphate for FD. In order to discard (early eluting) peaks corresponding to the excess of reagent and by-products of the derivatisation procedure, the first (t_R<11 min) and the last part (t_R>20 min) of the chromatogram were diverted to waste by means of the divert valve. This feature is very important for automation of the method as it prevents contamination of the mass spectrometer with buffers and reagents and hence ensures prolonged optimum performance even for large numbers of analyses.

Because pure FMOC derivatives of glyphosate and AMPA are not commercially available, the mass spectrometer was tuned in the negative ion mode using a solution of PEG 400 (10 mg/l in methanol–water (1:1, v/v) with 0.01 *M* ammonium acetate) instead.

Full-scan ESI-MS spectra and product-ion MS–MS spectra of the FMOC derivatives of glyphosate and AMPA were recorded in the negative ion mode. Despite differences in the interface and instrumentation as compared to the original method [8], similar results were obtained for the standards. Both compounds hardly showed any fragmentation. During LC–MS analysis only deprotonated molecules of the FMOC derivatives were observed for both compounds, i.e., an ion at m/z 390 in the case of glyphosate and an ion at m/z 332 in the case of AMPA. Upon selecting these ions as precursor ions for CID measurements (2 mTorr Ar, collision energy 20 eV) several product-ions were obtained. In the case of glyphosate, product-ions at m/z 168 and 150 were observed, corresponding to the $[(\text{glyphosate–FMOC})\text{–FMOC}]^-$ ion and the $[(\text{glyphosate–FMOC})\text{–FMOC–H}_2\text{O}]^-$ ion, respectively. In the

case of AMPA next to the product-ion at m/z 136, corresponding to the $[(AMPA–FMOC)–FMOC]^-$ ion, also an ion at m/z 110 is quite abundant. The proposed structure of this ion is a very stable 5-membered ring structure, see Fig. 2.

The summed intensities of these product-ions maximised around 25 eV of collision energy (range tested 5–40 eV). It is interesting to note the fact that at 5 eV hardly any fragmentation was observed.

As a result, upon selection of the precursor ion (the $[M-H]^-$ ion of the derivative) and switching between low (5 eV) and higher (20–25 eV, depending on the compound) collision energy, three (in)-dependent signals were produced: the $[M-H]^-$ ion (of the derivative) at low collision offset and two product-ions at high collision offset, which were ''scanned'' in a consecutive way. The ratio of these three signals appeared (more or less) constant (reproducibility <10% within a series of 50 samples, including standards and quality control standards) if a single compound enters the ion source. This fact together with the compound's retention time can be used for positive identification, especially when deuterated standards are not commercially available.

Determination of the calibration curves is based on the peak areas in the measurements of five standard solutions at concentrations within the working range of the method, i.e., 0.05 up to 3 μg/l. For quantification purposes the signals of the three (in)dependent product-ions described above are used as well as the sum of those three. Processing of the sample data thus yields four calculated concentrations per compound: three calculated from the individual precursor to product-ion signals and one from the summed signals.

The compound is regarded as positively identified when: (i) the retention time is within a time window of ±6 s of the last analysed performance standard (a standard solution containing 0.2 μg/l, which is analysed after every five samples) and (ii) concentrations calculated from the individual selected ion signals deviate less than 20% (based on daily practice and levels set by the EPA and EC for confirmation in GC–MS) from the concentration calculated from the sum of the three signals. A higher apparent concentration for one or two of the ions indicates a false positive. In the cases that the deviation was between 20 and 30% the sample was reanalysed (using the duplo derivatised sample which was stored in the freezer at −20°C).

As said before, deuterated glyphosate and AMPA are not commercially available, i.e., there is no proper internal standard available. Therefore next to the procedure described above, standard addition was used in cases where extra confirmation of the retention time was needed.

3.1. Performance characteristics

The calibration curves were calculated by linear regression without any weighing. For concentrations between 0.05 and 3 μg/l the response is taken as a linear function of the concentration when the coefficient of regression is better than 0.998.

Detection limits (LOD's) were determined by diluting spiked water samples until a signal-to-noise ratio of 3 was obtained. For a 4 ml sample volume LOD's of 0.03 μg/l can be obtained for both glyphosate and AMPA which is sufficient to control drinking water quality according to the EC directive. The limit of quantification of the method was determined to be 0.05 μg/l in drinking, surface and waste water.

The relative standard deviation for the repeatability and reproducibility in spiked drinking water samples (0.2 μg/l) are, respectively <6% ($n=6$) and <9% ($n=16$) in the case of glyphosate and in the case of AMPA they were slightly higher, i.e., <9% ($n=6$) and <10% ($n=16$). These data are quite satisfactory, i.e., all below 10%. The recoveries of glyphosate and AMPA in drinking as well as surface waters are all above the 94% ($n=16$). In the case of spiked surface and even waste waters performance characteristics hardly changed.

These data clearly show that a reliable method is successfully implemented and that data acquired by means of this method can be used without hesitation for drinking water control in accordance with the EC criteria.

3.2. Applications

At present, the above described method is successfully used for determination of glyphosate and AMPA in large batches of samples. The following example demonstrates the specificity of this method,

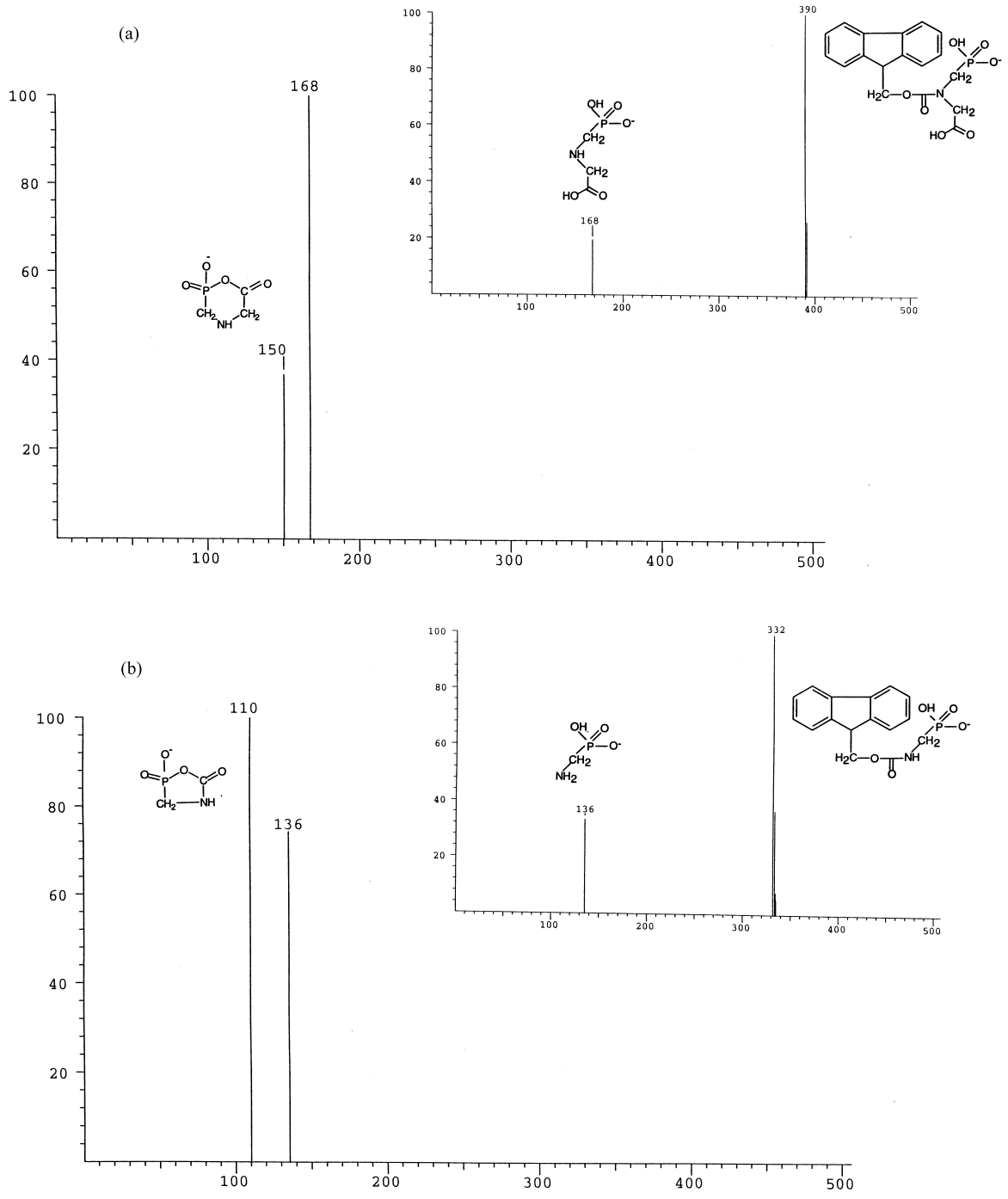

Fig. 2. Full-scan MS–MS spectra of glyphosate–FMOC (a; precursor ion m/z 390) and AMPA–FMOC (b; precursor ion m/z 332) derivatives with a low (5 eV) and high (25 eV) collision energy (2.0 mTorr Ar) at concentrations of 0.2 μg/l. Included in the spectra are the proposed structures of the observed ions. Conditions, see Section 2.

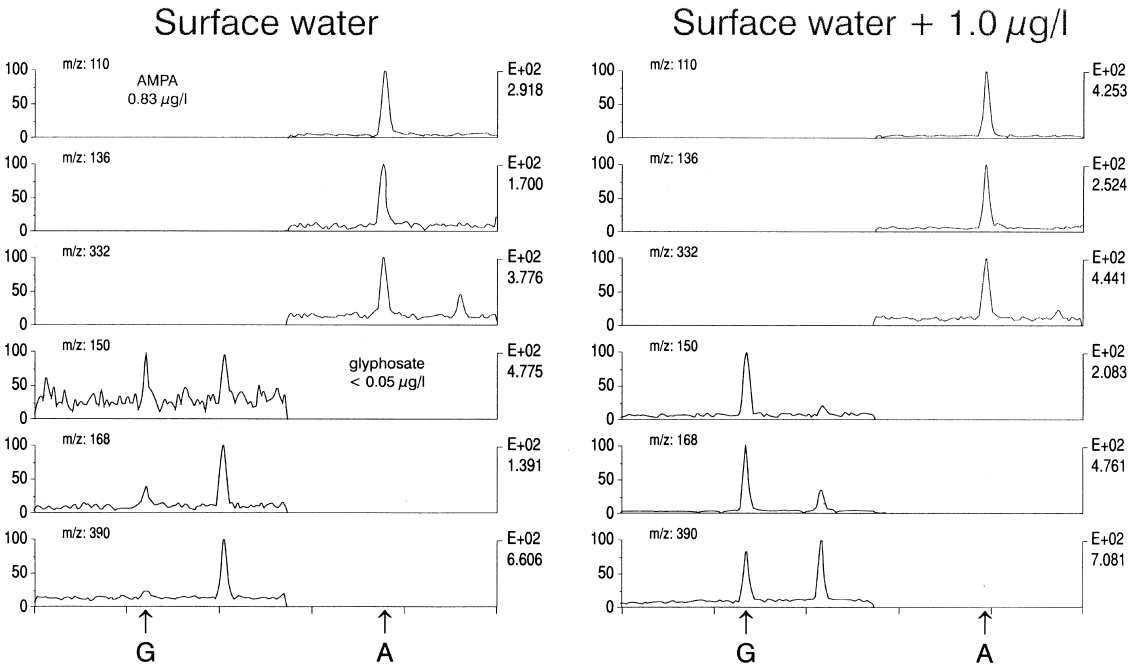

Fig. 3. Reconstructed ion chromatograms of product-ions of glyphosate (glyphosate–FMOC derivative) and AMPA (AMPA–FMOC derivative) in a water sample from the river Rhine without (a) and with (b) standard addition (at the level of 1.0 μg/l of glyphosate).

using the combined information of three selected ions per compound and the retention time.

Fig. 3a shows chromatograms of the ions reported above in a surface water sample (river Rhine). The presence of AMPA is indicated by the elution of all three selected ions in one sharp peak, having the proper ratio's and within the corresponding retention time window.

The individual ion chromatograms for m/z 390, 168 and 150 (glyphosate) indicate elution of two well separated compounds which yield ions with the same m/z. Although it is difficult to distinguish which peak corresponds to glyphosate without comparing the retention times with those in the closest performance standard, the ratio of the intensities of the selected ions in the earlier eluting peak suggests the elution of glyphosate at this retention time. The retention time can easily be confirmed by spiking glyphosate to the sample in a second run (here: 0.1 μg/l), as is shown in Fig. 3b. The increase of the ion-peaks is evident, confirming the presence of glyphosate as the first peak.

Encouraged by the performance and robustness of this method for drinking water and various surface waters, its applicability was tested for waste water as well.

Fig. 4 shows the selected ion chromatograms of glyphosate and AMPA in two different types of municipal waste water. In the first sample (Fig. 4a) only a very low concentration of glyphosate was detected (<0.05 μg/l) in the presence of 2.6 μg/l of AMPA. In Fig. 4b, a chromatogram of a waste water is shown, where a relatively high concentration of glyphosate was determined (0.54 μg/l) as well as 1.7 μg/l of AMPA.

3.3. Glufosinate

Glufosinate shows structural similarities to glyphosate with respect to the derivatisation site for FMOC and it can be derivatised by FMOC-Cl in the same procedure as glyphosate and AMPA. However, in HPLC-FD the glufosinate–FMOC derivative co-eluted with remnants of the FMOC-reagent and therefore the high selectivity of LC–ESI-MS–MS

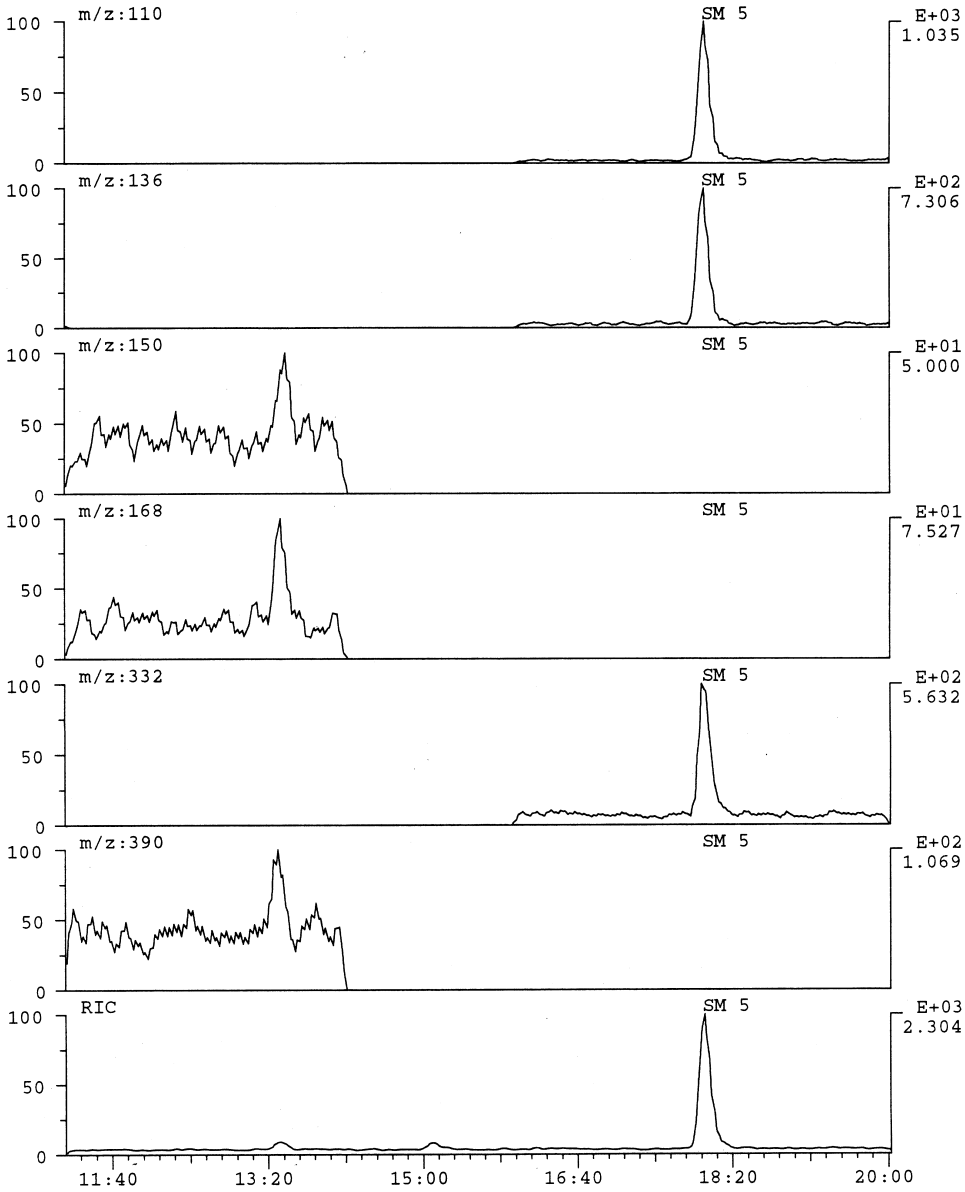

Fig. 4. Reconstructed ion chromatograms of product-ions of glyphosate (glyphosate–FMOC derivative) and AMPA (AMPA–FMOC derivative) in a municipal waste water sample containing glyphosate on a low (a; 0.05 μg/l) and high (b; 0.5 μg/l) concentration level.

was required to evaluate the presence of glufosinate. Glufosinate was analysed under the same LC–MS–MS conditions as described for glyphosate and AMPA. The full-scan ESI mass spectrum contained only the deprotonated molecular ion of the glufosinate–FMOC derivative. Performing MS–MS on this ion, product-ions at m/z values 180 and 206 were observed when collision energy was increased. Fig. 5 shows the LC–MS–MS spectra of glufosinate at 5 eV and 20 eV. The structure of the product-ions

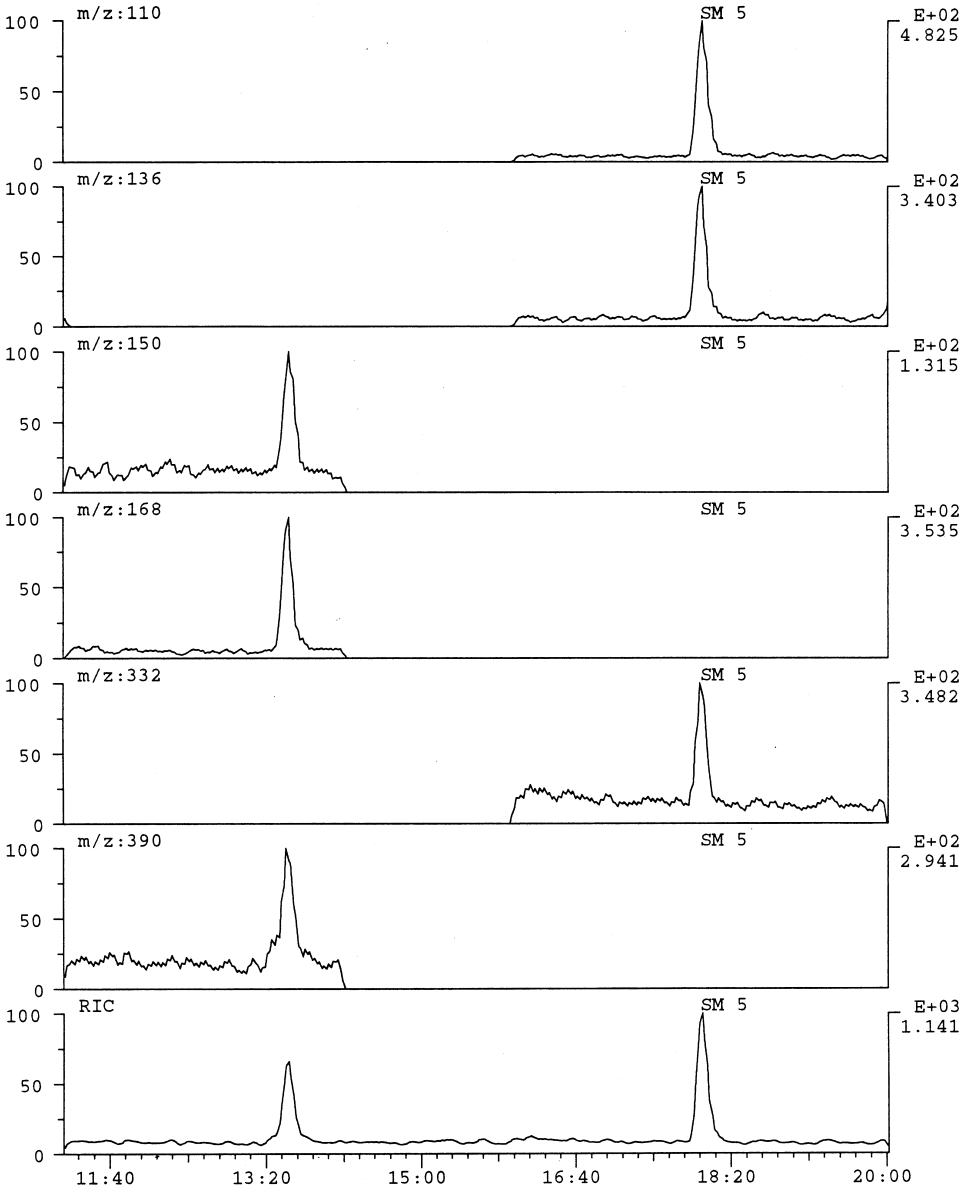

Fig. 4. (*continued*)

resemble those produced by CID of the derivative of AMPA, Fig. 2c. The optimum collision energy was 20 eV, giving the highest intensity of the product-ions. The ratio between the ions was again fairly constant, therefore the above described quantification and identification procedure can also be applied to glufosinate. No extensive validation was performed yet, but preliminary results show that the recovery, repeatability, reproducibility and LOD compare well to those for glyphosate and AMPA.

Fig. 6a shows the LC–ESI-MS–MS chromatograms of the ions at m/z 402, 206 and 180 of a waste

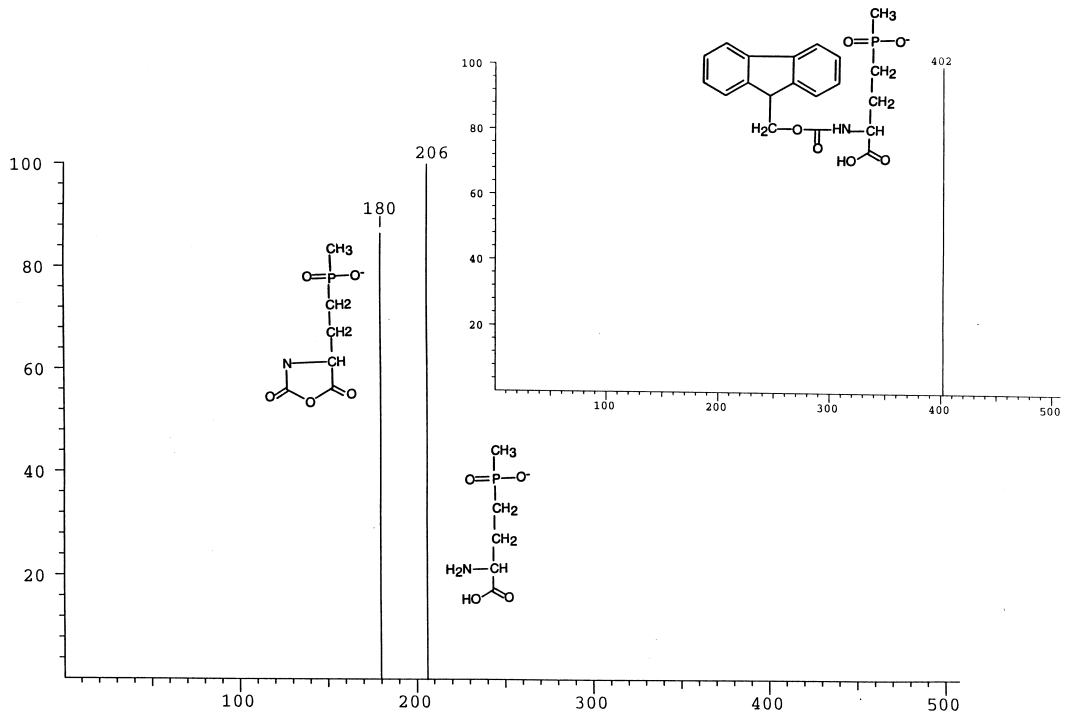

Fig. 5. MS–MS spectra of glufosinate–FMOC derivative (precursor ion m/z 402) with a low (5 eV) and high (25 eV) collision offset (2.0 mTorr Ar), at a concentration of 0.2 μg/l. Included in the spectrum are the proposed structures of the observed ions. Conditions, see Section 2.

water sample. In the proper retention time window the presence of glufosinate is indicated at a concentration level of 0.15 μg/l. Standard addition at a level equal to 0.15 μg/l (Fig. 6b) reveals a final concentration on all four ion traces of approximately 0.3 μg/l, confirming the presence of glufosinate. It must be noted that in this waste water the LOD is less favourable, i.e. approximately 0.1 μg/l.

3.4. Occurrence of AMPA

Recent research revealed that AMPA is not only the major degradation product of glyphosate, but several phosphonates can be regarded as precursors for the environmental occurrence of AMPA as well [7]. These phosphonates are used on a wide scale in corrosion inhibitors, soaps and shampoos and as detergents. As a consequence AMPA might be widely spread, much more than can be accounted for by glyphosate alone.

4. Conclusions

The previously developed method for the determination of glyphosate and AMPA by means of LC–ESI-MS–MS, was successfully implemented on a mass spectrometer of a different producer. The method is very robust and shows good performance characteristics over a long period of time. Although developed for drinking and surface waters, the method can also be applied to waste water monitoring. Furthermore, a complete automation of the method, including on-line SPE–HPLC was achieved. In this way unattended LC–MS–MS analysis of over 50 samples (62 min/run) in a 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 ±6 s of the last analysed performance standard and the proper ratio between the (in)dependent ion signals. For quantification, calculation of four concentrations based on

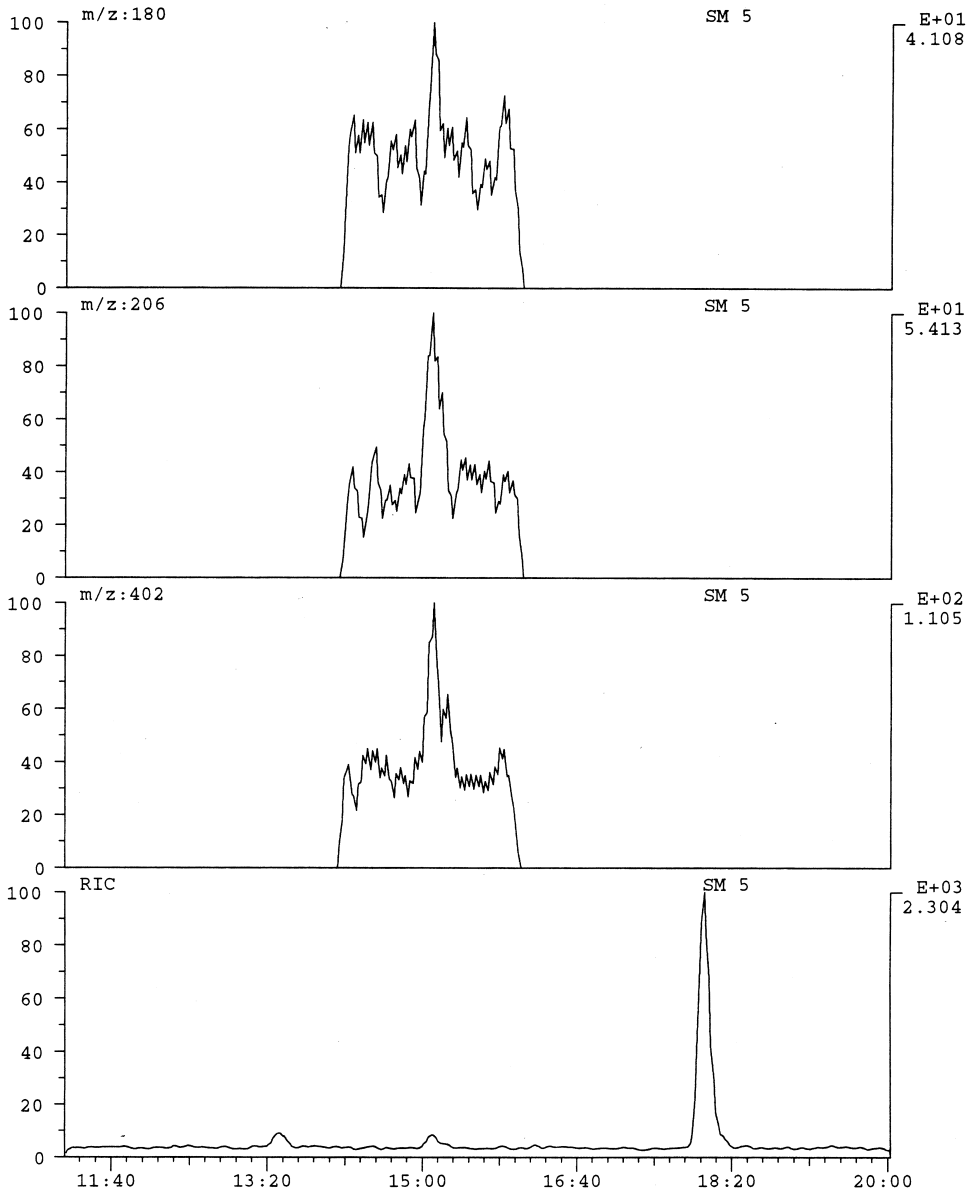

Fig. 6. Reconstructed ion chromatograms of the product-ions of glufosinate (glufosinate–FMOC derivative) in a municipal waste water sample containing glufosinate at ca. 0.15 μg/l without (a) or with the addition of a standard containing 0.15 μg/l (b).

four calibration graphs is performed. A deviation of more than 20% for one or two of the concentrations is not allowed.

The structurally related pesticide glufosinate was included as an additional target compound in the method. Present results indicate that performance characteristics, comparable to those of glyphosate and AMPA can be obtained.

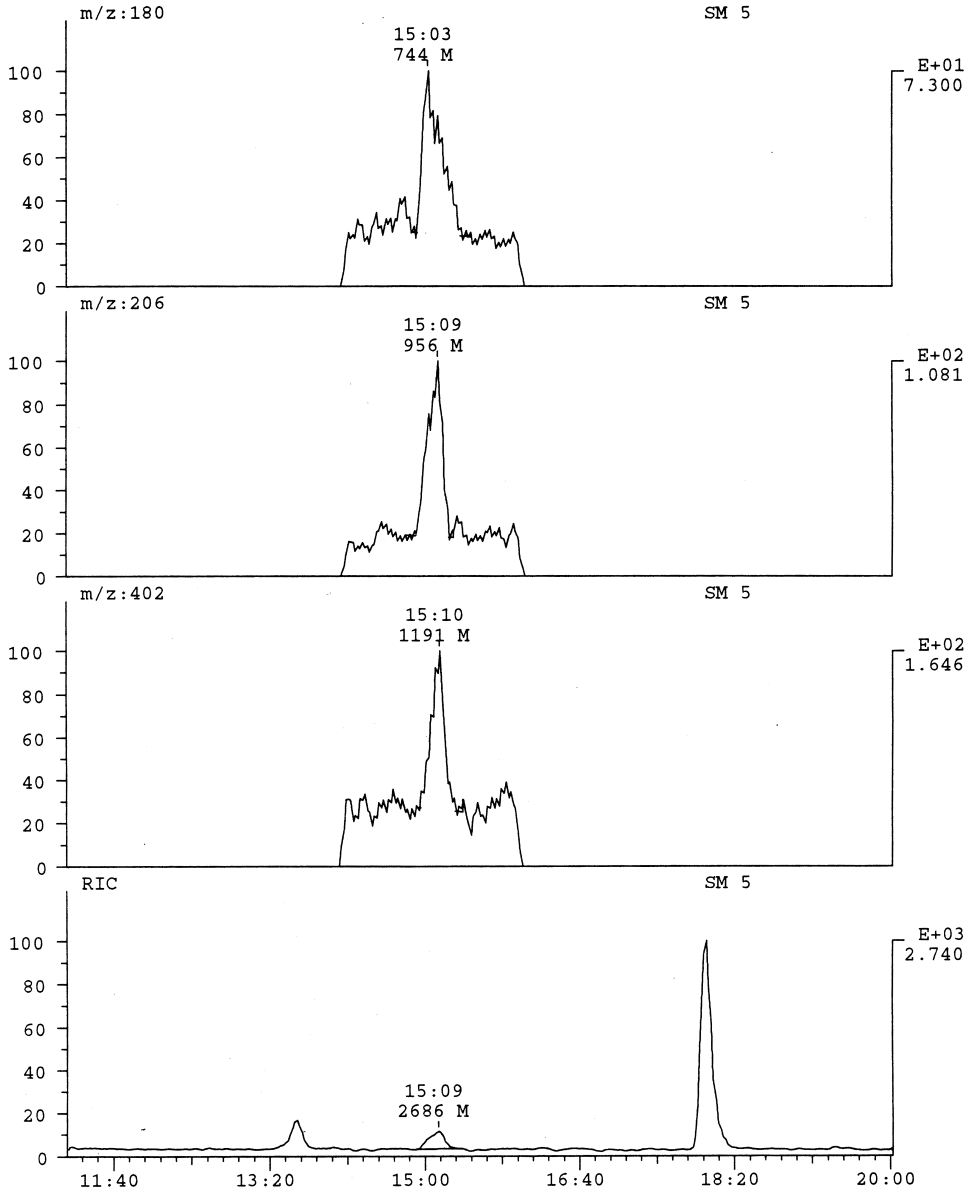

Fig. 6. (*continued*)

Acknowledgements

This study is part of the research program financed by the Netherlands Water Works Association (VEWIN) executed at Kiwa Research and Consultancy. The authors would like to thank I.M. Huitema and R. de Groot from the Water-Transport Company Rijn-Kennermerland (WRK) for the use of their Quattro II instrument and their help during the development of the first LC–MS–MS method [7]. The helpful and stimulating discussions with A. Noordsij are deeply acknowledged.

References

[1] H. Kataoka, S. Ryu, N. Sakiyama, M. Makita, J. Chromatogr. A 726 (1996) 253–258.
[2] J.V. Sancho, F.J. Lopez, F. Hernandez, E.A. Hogendoorn, P. van Zoonen, J. Chromatogr. A 678 (1994) 59–67.
[3] A.J. Cesna, N.P. Cain, Can. J. Plant. Sci. 72 (1992) 1359.
[4] H.A. Powell, N.W. Kerby, P. Rowell, J. Chromatogr. 502 (1990) 201.
[5] Th.H.M. Noij, H_2O 27(24) (1994) 708.
[6] Th.H.M. Noij, A. Brandt, in: H.-J. Stan (Ed.), Analysis of pesticides in ground and surface water II, Chemistry of Plant Protection 12, Springer, Berlin, 1995, pp. 91–108.
[7] R. Hopman, E.F. Beerendonk, L.M. Puiker in: Glyphosate and AMPA in the production of drinking water, Kiwa report, SWE 95.026, 1995.
[8] R.J. Vreeken, Th.H.M. Noij, I.M. Huitema, R. de Groot, in preparation.
[9] Council Directive of 15 July 1980 concerning the quality of water intended for human consumption, No. 80/778/EC.