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Novel approach for the simultaneous analysis of glyphosate and its metabolites ☆

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Abstract

A novel approach for the simultaneous analysis of glyphosate (PMG), and aminomethylphosphonic (AMPA, Gly^P), *N*-methylaminomethylphosphonic (MAMPA, Sar^P) and methylphosphonic (MPA) acids is presented. This includes a preliminary ^{31}P NMR analysis of mixtures of PMG, MPA, AMPA and MAMPA, their further derivatization to volatile phosphonates by means of the trifluoroacetic acid–trifluoroacetic anhydride–trimethyl orthoacetate reagent and subsequent MS [chemical ionization (CI) MS, GC–CI-MS, GC–electron impact ionization MS] and/or GC–flame ionization detection (FID) analysis of the products of derivatization. The detection limits of PMG, AMPA, MAMPA and MPA by means of GC–CI-MS and GC–FID were determined. The calibration graphs (GC–FID) for these derivatives were in the range 0.1 to 100 nmol linear and sufficiently reproducible for quantitative determinations. The applicability of the method was demonstrated during the analysis of water samples fortified with PMG, AMPA and MAMPA, characterized by recoveries of >95%.

Keywords: Derivatization, GC; Glyphosate; Phosphonic acids; Methylphosphonic acids; Aminophosphonic acids; Pesticides

1. Introduction

Glyphosate [*N*-(phosphonomethyl)glycine; PMG] represents a broad spectrum, non-selective aminophosphonate-type herbicide, which has been accepted worldwide as an environmentally friendly agent for agricultural application. This herbicide exhibits low toxicity to animals, however its long-term influence on non-target organisms and its overall environmental fate have not been fully evaluated [1,2]. Therefore, the availability of reliable and sensitive methods for the determination of PMG and its metabolites [3,4], and/or products of degradation, still presents an important topic for contemporary environmental analytical chemistry [5–7].

Gas chromatography–mass spectrometric (GC–MS) methods present superior analytical potential for such analyses, since they display the advantages of the high resolution of the GC capillary column, high sensitivity and the supreme selectivity of the mass spectrometric detector. However, PMG itself and related compounds are not volatile and their analysis by GC and/or GC–MS methods requires prior derivatization of the compounds to volatile, usually

☆Dedicated to Prof. F. Jordan from Rutgers, the State University of New Jersey, on the occasion of his 60th birthday.

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diester-type phosphonates. Thus, Moye and co-workers [8,9] and Tsunoda [10] applied MTBSTFA for the simultaneous *O*- and *N*-silylation of PMG and aminomethylphosphonic acid (AMPA) and subsequent GC [8,9] and/or GC–ion-trap (IT) MS [10] determination of the formed silyl derivatives. Kataoka and co-workers reported a two-stage procedure for the derivatization of PMG, involving prior *N*-acylation with chloroformates, followed by subsequent *P*- and *C*-esterification of its acidic functions by means of diazomethane [11,12]. The other protocol for the derivatization of PMG and AMPA [13–17] applied simultaneous *N*-acylation/*O*-esterification of these amino acids by treatment with prepared in situ mixtures of perfluorinated anhydrides and halogenated alcohols and/or by treatment with AcOH–trimethyl orthoacetate (TMOA) reagent [18]. For all the procedures presented above, the implicit assumption that the considered conversions of non-volatile aminophosphonic acids into corresponding volatile ester-type phosphonates are quantitative should be made. This requirement, however, does not always seem to be fulfilled.

Recently, we reported the procedure for the derivatization of aminoalkanephosphonic acids into the corresponding volatile *N*-acylaminoalkanephosphonates (Fig. 1), the quantitative course of which was verified in the range from 0.1 mg [19] to 1000 mg [20] by means of ^{31}P NMR.

In this paper we present our approach for the simultaneous analysis of PMG and its metabolites. This includes the ^{31}P NMR pre-analysis of PMG and its potential metabolites **1**–**4**, their derivatization to volatile phosphonates by application of the anhydride–orthoester method [19], and subsequent GC, GC–MS and/or MS analysis of the volatile products of derivatization.

2. Experimental

2.1. Materials

Methanephosphonic acid (**4**, MPA), aminomethylphosphonic acid (**3**, AMPA, Gly^{P}) and *N*-methylaminomethylphosphonic acid (**2**, MAMPA, Sar^{P}) were prepared according to Ref. [21]. Phosphonomethylglycine (**1**, PMG, GLYP) and other reagents were purchased from Aldrich (Milwaukee, WI, USA).

2.2. Solutions

Phosphonic acids **1**–**4** (0.1 mmol of each individual component or their mixtures) were dissolved in 10 ml trifluoroacetic acid. The obtained stock solutions of **1**–**4** (10 m*M*) were diluted with trifluoroacetic acid in the appropriate ratio, affording solutions in the concentrations range 1 to 0.01 m*M*.

2.3. Preparation of samples for analysis

River water was collected from rivers in the neighborhood of the city, filtered if necessary, and used directly for analysis.

Aqueous solutions of mixtures of PMG, Gly^{P} and Sar^{P}, simulating real solutions of these herbicides in surface water (at the concentrations given in Table 4), were obtained by the addition of appropriate volumes of stock solutions of phosphonic acids **1**–**3** to 1 litre of water (distilled, drinking or river water). The fortified water samples (1 litre) were preconcentrated in vacuo (50 °C, 21 Torr, 30 min; 1 Torr = 133.322 Pa) to ca. 2–5 ml, then lyophilized, and the

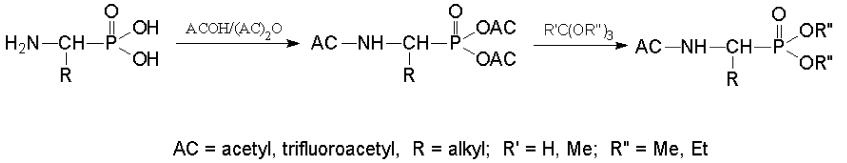

Fig. 1. Scheme for the derivatization of aminoalkanephosphonic acids by means of the carboxylic acid–anhydride–orthoester system.

residues were dissolved in trifluoroacetic acid (0.05 ml) prior to subsequent derivatization.

2.4. Derivatization of phosphonic acids *1–4*

The conversion of phosphonic acids **1**–**4** into volatile derivatives **1A**–**4A** was carried out in a Wheaton 1 ml (or 5 ml) micro product V-vial, equipped with a spin vane, placed in a thermostated oil bath.

In order to achieve this conversion, the samples of phosphonic acids (up to 0.05 mmol) in trifluoroacetic acid (TFA) solution (0.05 ml) were mixed with trifluoroacetic anhydride (TFAA) (0.05 ml) and the mixtures were heated with stirring for 10 min at 30–40 °C. Trimethyl orthoformate (0.40 ml) was then added carefully and the resulting reaction mixtures were stirred at 100 °C for 1.5 h. The derivatization mixtures were analyzed directly using ^{31}P NMR, GC–MS and MS or pre-concentrated in vacuo (20 or 50 °C, 21 Torr, 30 min) prior to analysis. For quantitative analysis, the derivatization mixtures containing **1A**, **2A** and **3A** were pre-concentrated in vacuo (50 °C, 21 Torr, 30 min), the residues were dissolved in benzene (0.1 ml) and the solutions were injected (0.5–2 μl) into the GC–MS or GC–flame ionization detection (FID) system.

2.5. Recoveries

For the determination of recoveries, two independent samples derived from the stock solutions of **1**–**3** and containing the same quantities of amino acids were derivatized directly (affording the reference reaction mixture) and the second, after prior dissolution of the sample in 1 l of water and subsequent concentration in vacuo. The obtained derivatization mixtures were concentrated as described above and analyzed by the GC–FID system.

2.6. Gas chromatography and mass spectrometry

A Finnigan MAT 95 mass spectrometer was used for MS and GC–MS [electron impact ionization (EI) or chemical ionization (CI)] analysis of the derivatization products. Sample introduction (GC–MS) was via a Varian 3400 gas chromatograph equipped with a DB-1 and/or DB-17 capillary column (30 m×0.25 mm I.D.). The injector temperature was maintained at 200 °C and the transfer line temperature was 250 °C. The columns were introduced directly to the ion source of the mass spectrometer. EI-MS mass spectra were recorded at an electron energy of 70 eV. CI-MS spectra were recorded using isobutane as reacting gas.

The GC quantitative analysis was performed on an Ai Cambridge GC 95 gas chromatograph, equipped with a flame ionization detector and a DB-17 capillary column (30 m×0.25 mm I.D.). The injector temperature was maintained at 260 °C and the detector temperature at 280 °C. The conditions applied for separation in both the GC–MS and GC–FID mode are given in the legends of Tables 2 and 3.

Fast atom bombardment (FAB) MS measurements in a positive and negative mode were recorded on a Finnigan MAT 95 mass spectrometer using Cs^+ at 13 keV (matrix: glycerin).

^{31}P NMR spectra were recorded on a Bruker AC 200 spectrometer operating at 81.01 MHz.

3. Results and discussion

In this study, as a continuation of our aminophosphonate research [6,19,20], we concentrated on the analysis of glyphosate (PMG, **1**) and its potential products of degradation and/or biodegradation **2**–**4** (MAMPA, AMPA and MPA), formed according to the equations presented in Fig. 2.

Our approach involved a ^{31}P NMR investigation (pre-analysis) of PMG, MPA, AMPA and MAMPA (alone and/or in mixtures), their derivatization into volatile diester-type phosphonates, and subsequent MS and/or GC–MS analysis of the obtained derivatization products.

3.1. ^{31}P NMR analysis

The results of the ^{31}P NMR investigations [$\delta(^{31}P)=f(pH)$] carried out for phosphonic acids **1**–**4** are illustrated in Fig. 3. The graphs exhibit the characteristic relationship $\delta(^{31}P)=f(pH)$ for all the investigated phosphonic acids, similarly for amino acids **1**–**3** and differing substantially from that for

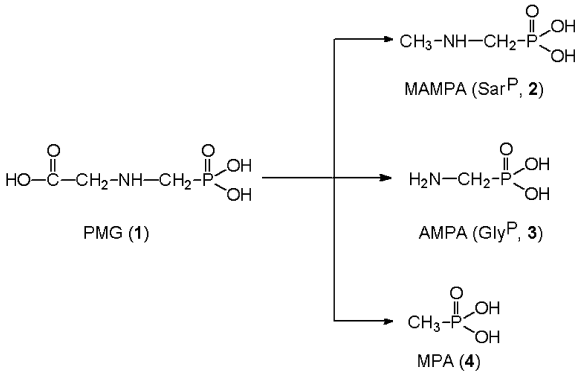

Fig. 2. Scheme of biodegradation of PMG.

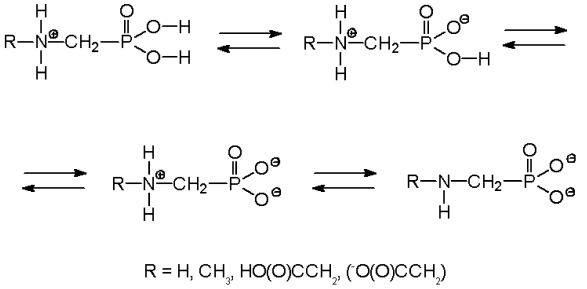

Fig. 4. Scheme of the dissociation/protonation equilibria of AMPA (R=H), MAMPA (R=CH_3) and PMG [R=$CH_2C(O)OH$ or $CH_2C(O)O^-$].

MPA. We reported [22] a similar phenomenon earlier for analogs of Met^P. The graphs reflect the dependence of the chemical shift $\delta(^{31}P)$ of the investigated compounds on their ionization states, resulting from their dissociation/protonation equilibria (Fig. 4) and determined by the corresponding pK values and pH of the applied solution.

These results also suggest the possibility of the simultaneous preliminary ^{31}P NMR analysis (identi-

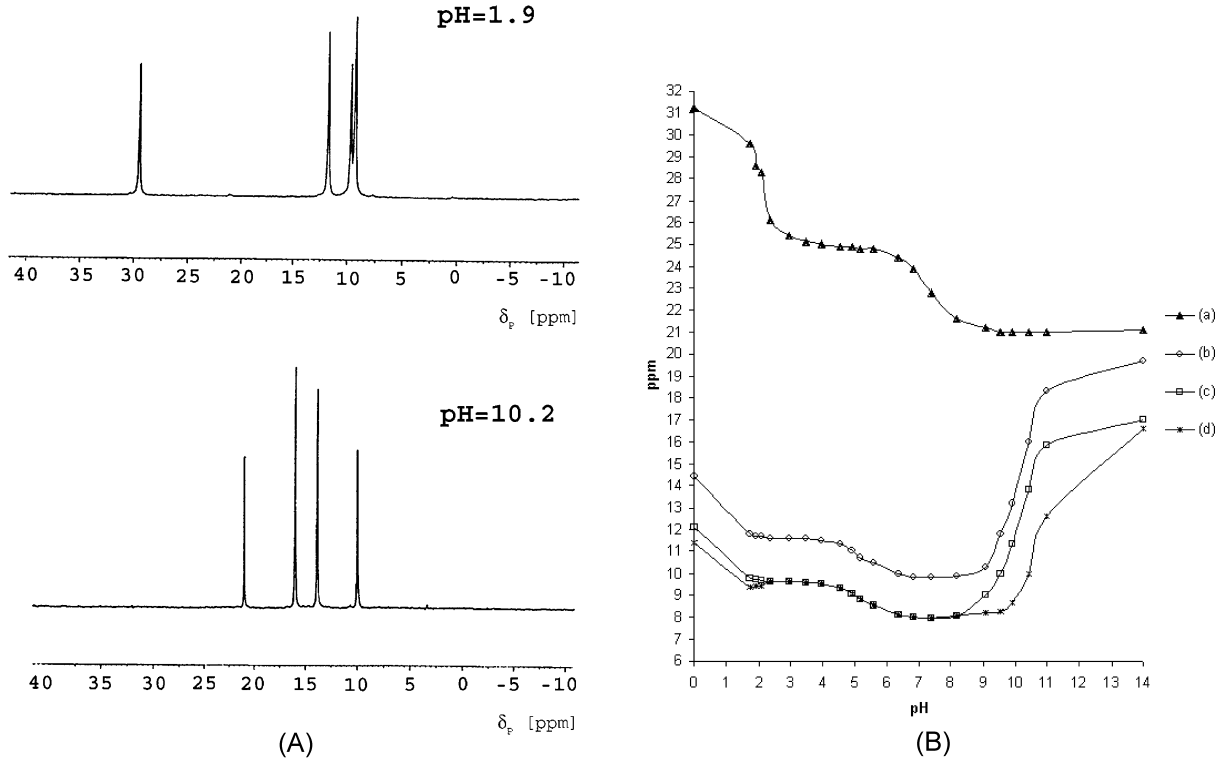

Fig. 3. Simultaneous ^{31}P NMR analysis of PMG and its phosphonic metabolites (MPA, AMPA and MAMPA). (A) Proton-decoupled phosphorus spectra of a mixture of PMG (1.5 mmol), MPA (1 mmol), AMPA (1.5 mmol) and MAMPA (1 mmol) in 15 ml of water (containing 5% ^2H_2O) recorded at pH 1.9 and 10.2, respectively. (B) pH dependence of the phosphorus shifts [$\delta(^{31}P)=f(pH)$] of 0.05 M aqueous solutions (5% ^2H_2O) of mixtures of (a) MPA, (b) AMPA, (c) MAMPA and (d) PMG.

fication and quantification) of mixtures of PMG with MPA, AMPA and MAMPA, with some limitations. Thus, in the range 2.2<pH<9.2, PMG and MAMPA exhibit very similar chemical shifts (Fig. 3A), which hinders their differentiation in this pH region. This problem can become worse during the ^{31}P NMR analysis of real samples (e.g. soil or tissue extracts) due to the usual effect of the broadening of the phosphorus signal occurring in the presence of metal ions and/or colloids [6].

For this reason, the simultaneous ^{31}P NMR analysis of PMG, MPA, AMPA and MAMPA in real samples should be supported by a supplementary method. The superior analytical potential of mass spectrometry suggests the application of MS, particularly GC–MS techniques, as the method of choice [6,7]. However, non-volatile zwitterionic aminophosphonic acids are unsuitable for GC–MS and are only just suitable for MS analysis [6]. Our attempt to apply the FAB technique for the identification of PMG, illustrated by the corresponding spectra in Fig. 5, unambiguously supports this thesis.

3.2. Derivatization reaction

To resolve the problem of the volatilization of phosphonic acids **1**–**4**, we started extensive research on their conversion into volatile derivatives, using ^{31}P NMR spectroscopy to monitor the reaction course. We tested several derivatization reagents, based on the AcOH–Ac_2O–orthoester and/or TFA–TFAA–orthoester system, with trimethyl and triethyl orthoformates and orthoacetates, respectively. As a result we established that derivatization of the individual phosphonic acids **1**–**4** by means of the TFA–TFAA–TMOA reaction system afforded the most selective transformation of derivatized phosphonic acids **1**–**4** into the corresponding stable diesters **1A**–**4A**, according to the equations shown in Fig. 6.

The ^{31}P NMR spectra of the derivatization mixtures of individual phosphonic acids **1**–**4** obtained by means of the TFA–TFAA–TMOA system are shown in Fig. 7 and illustrate the structural influence of the derivatized phosphonic acids on the reaction course. Thus, the ^{31}P NMR spectra of the crude derivatization mixture of MPA (**4**) (Fig. 7D) or AMPA (**3**) (Fig. 7C) exhibited quantitative conversion, whereas the corresponding spectra of derivatized PMG (**1**) (Fig. 7A) and/or MAMPA (**2**) (Fig. 7B) suggest the formation of mixtures containing two major phosphonate-type products.

These unexpected observations show the need for more detailed investigations of the derivatization of PMG (and/or MAMPA). These experiments, involving mass spectrometric investigations (GC–CI-MS, CI-MS, FAB-MS) of the obtained derivatization mixture of PMG by means of the TFA–TFAA–

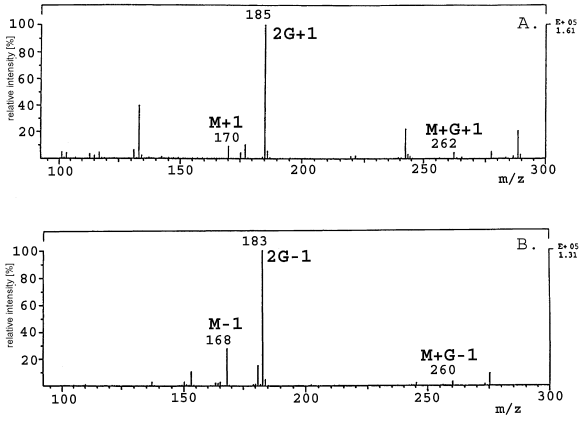

Fig. 5. FAB mass spectra [relative intensity (%) vs. m/z] of PMG in the positive (A) and negative (B) mode (matrix: glycerin). Molecular ions of PMG and glycerin are indicated by M and G, respectively.

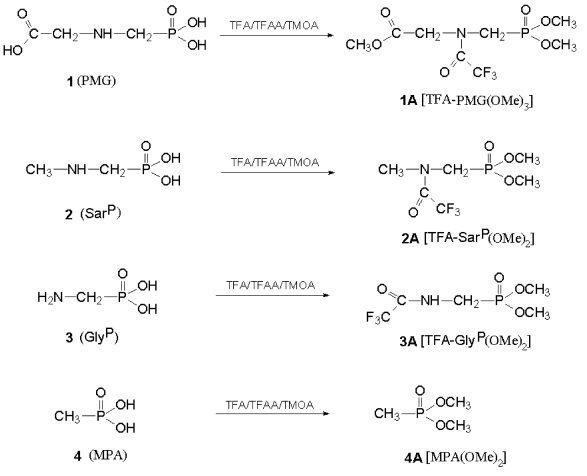

Fig. 6. Scheme of derivatization of PMG, AMPA, MAMPA and MPA by means of the TFA–TFAA–TMOA reagent.

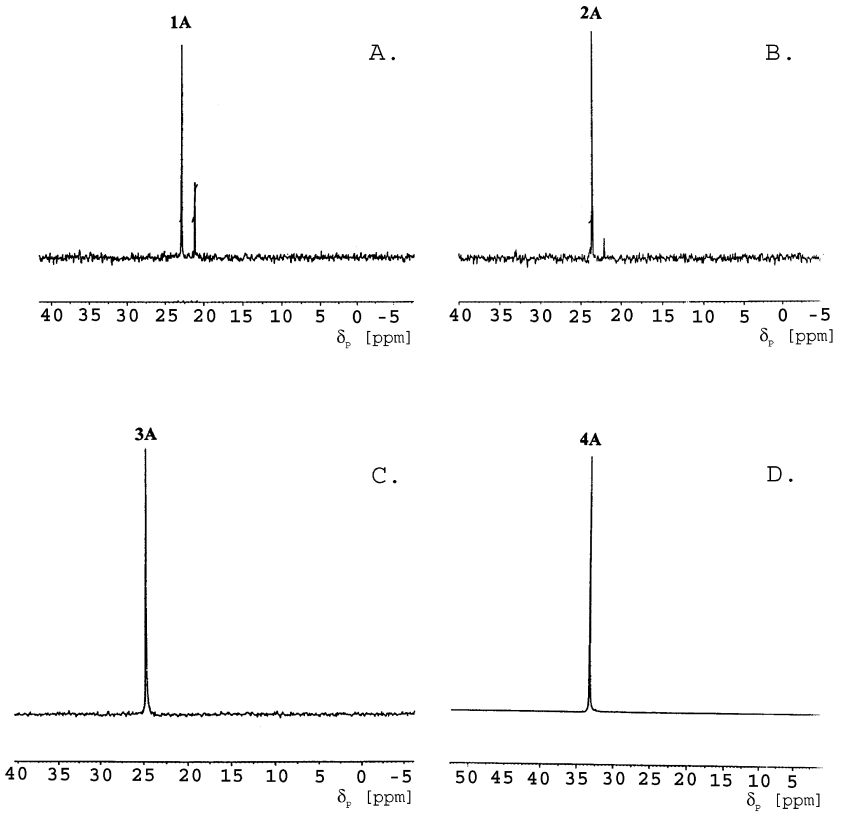

Fig. 7. Proton-decoupled phosphorus spectra of the products of the derivatization of PMG (**1**→**1A**) (A), MPA (**4**→**4A**) (D), AMPA (**3**→**3A**) (C) and MAMPA (**2**→**2A**) (B) obtained by means of the TFA–TFAA–TMOA reagent.

TMOA reagent, suggest the existence of only one product characterized by m/z 308, which presumably corresponds to structure **1A**. Additionally, this derivatization mixture of PMG (in a reaction carried out on the 100 mg PMG scale) was found to be distilled quantitatively (Kugelrohr, 100 °C, 0.01 Torr), affording a distillate fraction identical to, according to the ^{31}P NMR spectrum, that of the starting mixture (Fig. 7A). DCI-MS investigation of the distillate (Fig. 8) confirmed that the derivatization product is homogeneous (Fig. 8A) and its molecular ion $[M+1]^+$ corresponds to that assigned to **1A**. These results suggest the quantitative conversion of PMG into derivative **1A** (Fig. 6), presumably present in two conformational (or tautomeric) forms with different ^{31}P NMR spectra. In a similar way, this can explain the presence of two ^{31}P NMR signals corresponding to **2A** formed during the derivatization of MAMPA.

We also established the conditions under which the derivatizations of all the investigated acids **1**–**4** by means of the TFA–TFAA–TMOA reagent are quantitative in the range of 0.1 to 2 mg of the individual acid. Moreover, this procedure was extended to simultaneous derivatizations of four-component mixtures containing PMG and its metabolites **2**, **3** and **4** (applied as a mixture of four components, each 0.5 mg). The ^{31}P NMR spectrum of such a derivatization mixture is presented in Fig. 9A.

3.3. GC–MS investigation

Chromatographic investigations of this reaction mixture were performed in the GC–FID, GC–CI-MS and GC–EI-MS mode using various chromatographic conditions.

The corresponding GC–CI-MS chromatogram obtained with a DB-17 column, supplemented by CI-

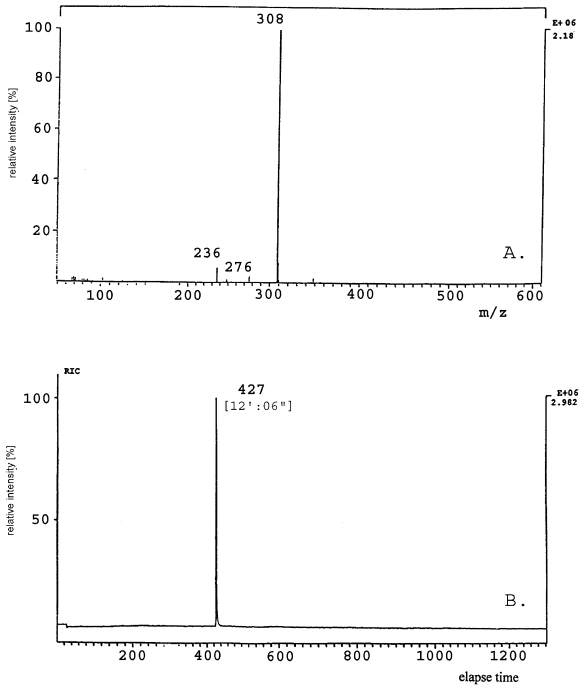

Fig. 8. CI-MS analysis of the derivatization mixture of PMG (**1**→**1A**) obtained by means of the TFA–TFAA–TMOA reagent. (A) Isobutane chemical ionization mass spectrum [relative intensity (%) vs. m/z] of the derivatization mixture of PMG. (B) RIC [relative intensity (%) vs. elapse time] of the derivatization mixture of PMG.

MS spectra of the separated phosphonates **1A**–**4A** are shown in Fig. 9B and C, respectively.

Complementary analysis by GC–EI-MS (DB-17) afforded similar chromatograms, revealing, however, apparent differences in the corresponding relative peak areas of the analyzed derivatives **1A**–**4A** when recorded in the CI-MS and EI-MS detection modes. This can be interpreted as being due to the different ionization susceptibilities exhibited by **1A**–**4A** during electron ionization and chemical ionization. A supplementary EI-MS characterization of **1A**–**4A** is given in Table 1.

The results of GC analyses of the derivatization mixture (Fig. 9) carried out on DB-1 and DB-17 columns are compared in Table 2. These revealed less satisfactory separations on a DB-1 column, especially with respect to the partly separated derivatives **2A** and **3A** exhibiting a retention time of 7 min 24 s. Supplementary CI-MS analysis of this peak exhibits, unambiguously, the presence of compounds **3A** (m/z 236) and **2A** (m/z 250).

A more detailed investigation of all chromatograms revealed that the retention times of the analyzed phosphonates **1A**–**4A** are much longer than those exhibited by the majority of the components derived from the TFA–TFAA–TMOA reagent, which allows subsequent direct GC–CI-MS analysis of the obtained derivatization mixture. The chromatograms also contain peaks with retentions comparable to **4A**, which, in accordance with their molecular ions recorded by CI-MS and EI-MS, can be assigned to condensation products **5** and **6** (or **7**) generated from methyl acetate formed in situ during derivatization (Fig. 10). As a matter of fact, these compounds do not interfere with the analyzed phosphonates **1A**–**4A**, and can even be treated as internal standards in analyzed mixtures.

Careful pre-concentration (20 °C, 21 Torr, 30 min) eliminates the majority of volatile by-products derived from side-reactions of the applied TFA–TFAA–TMOA reagent, without a noticeable decrease (^{31}P NMR) of the methanephosphonate (**4A**) concentration.

Evaporation carried out at a higher temperature (50 °C, 21 Torr, 30 min) also enables the removal of by-products **5**–**7**, accompanied, however, by a simultaneous co-evaporation of methanephosphonate **4A**, and affording a residue consisting only of the less volatile phosphonates **1A**, **2A** and **3A**.

3.4. MS Analysis

Although the direct MS analysis of aminophosphonic acids affords rather ambiguous results [6,23,24], the application of the CI-MS technique to the analysis of volatile derivatization products lead to quite promising results [24]. The adaptation of this technique for the simultaneous analysis of phosphonates **1A**–**4A**, derived from PMG and its metabolites **2**, **3** and **4** by pre-treatment with the TFA–TFAA–TMOA reagent, is illustrated in Fig. 11.

Chemical ionization of the phosphonates **1A**–**4A** by means of isobutane as the reacting gas affords simple CI-MS spectra containing the protonated molecular ions ($m/z = [M + 1]^+/z$) as the dominant ions (100% relative intensity). These are as follows:

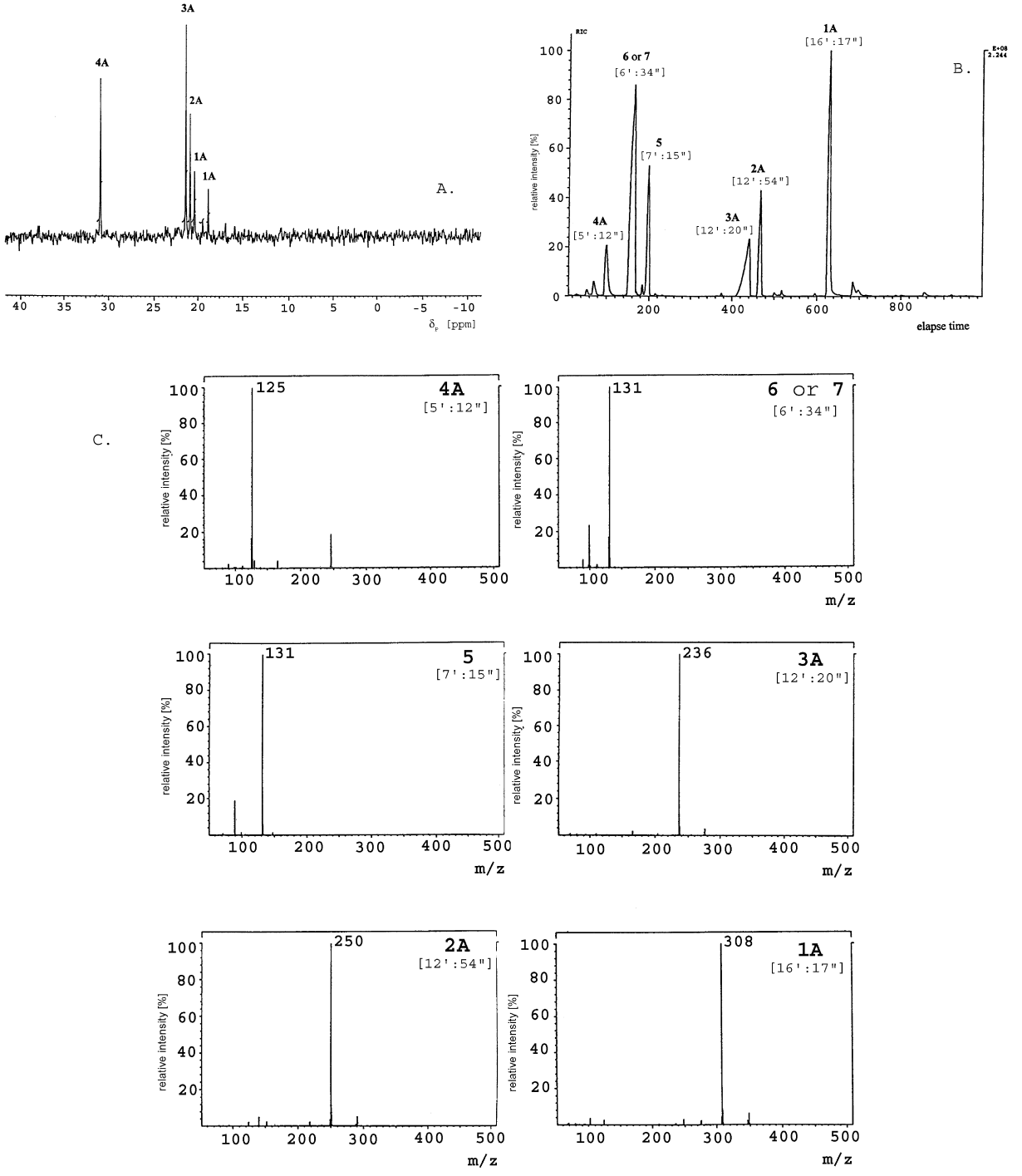

Fig. 9. ^{31}P NMR (A), GC–CI-MS (B) and CI-MS (C) analyses of products of the simultaneous derivatization of a mixture of PMG, MPA, AMPA and MAMPA obtained by means of the TFA–TFAA–TMOA reagent. Conditions as described under Experimental. (A) Proton-decoupled phosphorus spectrum of the derivatization products (mixture of **1A**–**4A**). (B) GC–CI-MS chromatogram [relative intensity (%) vs. elapsed time] (retention time) of the mixture of derivatization products (mixture of **1A**–**4A**). Conditions as described in Table 2, variant A. (C) Isobutane chemical ionization mass spectra [relative intensity (%) vs. *m*/z] of the chromatographically separated products of derivatization.

Table 1
Partial EI-MS spectra of derivatives **1A**–**4A**

Derivative	*m/z* [intensity (%)]							
	$[M]^{+\cdot}$	$[M-31]^+$	$[M-69]^+$	$[M-97]^+$	$[M-P]^+$	$[P]^{+(+\cdot)}$	Base peak	Others
TFA–PMG(OMe)$_3$	307	275	–	210	198	109	124[a]	233
(**1A**)	(24)	(40)		(22)	(2.5)	(7.7)		(12)
TFA–Sar^P(OMe)$_2$	249	218	180	152	140	109	140	124
(**2A**)	(11)	(1.3)	(1.2)	(25)	(100)	(5.2)		(32)
TFA–Gly^P(OMe)$_2$	235	204	166	138	126	110[b]	110	79
(**3A**)	(13)	(3.3)	(72)	(2.4)	(28)	(100)		(18)
MPA(OMe)$_2$	124	94[c]	–	–	–	109[d]	94	79[e]
(**4A**)	(21)	(100)				(46)		(83)

Abbreviations: $[M-31]^+=[M-OMe]^+$; $[M-69]^+=[M-CF_3]^+$; $[M-97]^+=[M-CF_3C(O)]^+$; $[M-P]^+=[M-P(O)(OMe)_2]^+$; $[P]^+=[P(O)(OMe)_2]^+$; $[P]^{+\cdot}=[H-P(O)(OMe)_2]^{+\cdot}$.

[a] $[M-183]^{+\cdot}=[MeP(O)(OMe)_2]^{+\cdot}=[M-MeO(O)C-CH=N-C(O)CF_3]^{+\cdot}$.

[b] $[P]^{+\cdot}=[H-P(O)(OMe)_2]^{+\cdot}$.

[c] $[M-30]^{+\cdot}=[M-2Me]^{+\cdot}$.

[d] $[M-15]^+=[M-Me]^+$.

[e] $[M-45]^+=[M-3Me]^+$.

Table 2
Chromatographic separation of the derivatization products of a mixture of PMG, MPA, AMPA and MAMPA by means of the TFA–TFAA–TMOA reagent

Derivative	Retention time (min:s)		
	A	B	C
6	6:34	12:36	0:15
5	7:15	13:16	0:15
MPA(OMe)$_2$ (**4A**)	5:12	11:32	0:47
TFA–Gly^P(OMe)$_2$ (**3A**)	12:20	17:31	7:24
TFA–Sar^P(OMe)$_2$ (**2A**)	12:54	18:08	7:24
TFA–PMG(OMe)$_3$ (**1A**)	16:17	21:21	11:44

Conditions: (A) DB-17 column, 30 m, 100 °C (5 min)—10 °C/min—250 °C (10 min); (B) DB-17 column, 30 m, 50 °C (5 min)—10 °C/min—250 °C (10 min); (C) DB-1 column, 30 m, 100 °C (5 min)—10 °C/min—250 °C (10 min).

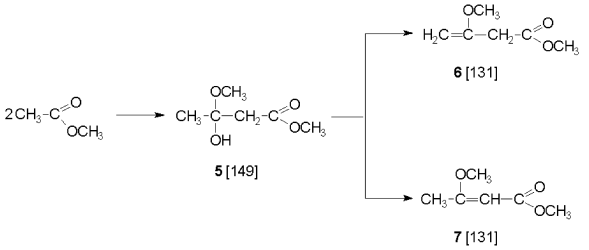

Fig. 10. Scheme of side-reactions occurring during derivatization with the TFA–TFAA–TMOA reagent.

for TFA–PMG(OMe)$_3$ (**1A**) $-[M+1]^+_{(1A)}=308$; TFA–Sar^P(OMe)$_2$ (**2A**) $-[M+1]^+_{(2A)}=250$; TFA–Gly^P(OMe)$_2$ (**3A**) $-[M+1]^+_{(3A)}=236$; and MPA(OMe)$_2$ (**4A**) $-[M+1]^+_{(4A)}=125$. Analysis of Fig. 11A illustrates the evaporation mode of the examined derivatives, namely the recombination ion current (RIC) derived from a mixture of **1A**–**4A** and from the specific molecular ions ($[M+1]^+$: 125, 236, 250 and 308, respectively) as a function of time. This reveals fractionation of the analyzed phosphonates **1A**–**4A** during evaporation in the ionization chamber. Thus, methylphosphate **4A** (125) evaporates very quickly (Fig. 11A) and, after 17 s, only a small amount (8%) was observed in the corresponding CI-MS spectrum (Fig. 11B). This spectrum contains a dominant ion derived from **2A** (250; 100%) and ions derived from **3A** (236; 4%) and **1A** (308; 4%). The spectrum recorded after 1 min 19 s still shows the 250 ion as being dominant (100%), but with an increase in the relative intensity of ions 236 (12%) and 308 (24%). The spectra recorded at longer time periods (2 min 1 s and 2 min 15 s) reveal only small changes in the intensity of ions 308 (**1A**) (94 and 100%) and 250 (**2A**) (46 and 42%), and a rapid decrease for ion 236 (**3A**) (100 and 2%).

These results have diagnostic value, since they indicate the contents of the analyzed mixture, suggesting the application of this technique to a tentative

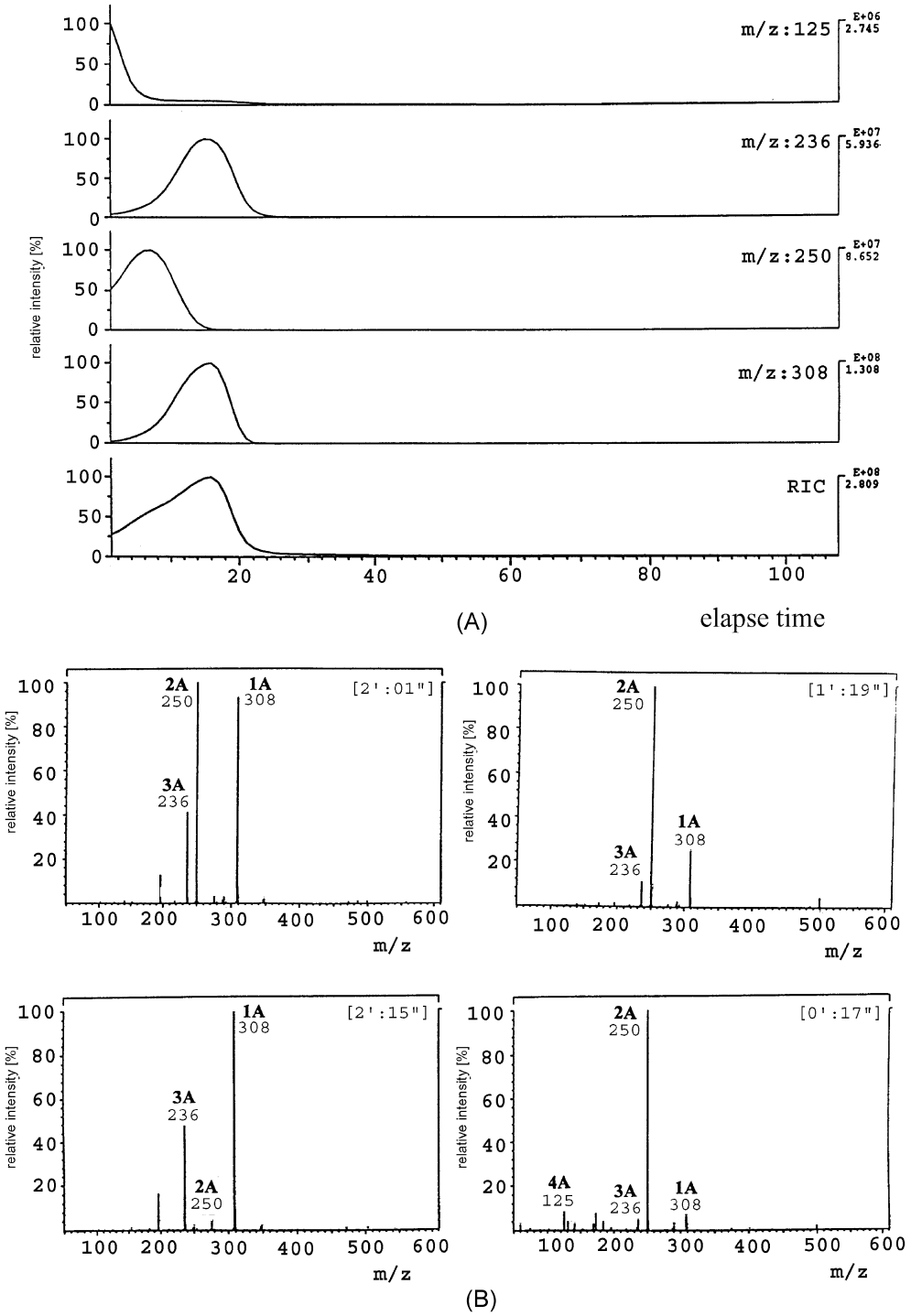

Fig. 11. CI–MS analysis of the products of the simultaneous derivatization of a mixture of PMG, MPA, AMPA and MAMPA obtained by means of the TFA–TFAA–TMOA reagent (the corresponding ^{31}P NMR spectrum of the derivatization mixture is presented in Fig. 9A). (A) RIC [relative intensity (%) vs. elapsed time] of **1A** (*m*/*z* 308), **2A** (*m*/*z* 250), **3A** (*m*/*z* 236), **4A** (*m*/*z* 125) and a mixture of **1A**–**4A**, respectively. (B) Isobutane chemical ionization mass spectra [relative intensity (%) vs. *m*/*z*] recorded in the indicated elapse time.

qualitative/semiquantitative analysis of the examined phosphonate mixtures prior to their further GC–MS analysis.

3.5. Quantitative analysis

The calibration graphs for phosphonates **1A**–**4A** determined by GC–FID were linear in the range from 0.1 to 10 nmol of the analyzed compounds and were sufficiently reproducible for their quantitative determination.

The detection limits (DLs) of **1A**–**4A** determined by GC–FID and GC–CI-MS under the applied experimental conditions are summarized in Table 3. The values demonstrate detection limits at least one order lower for **2A**–**4A** obtained by GC–CI-MS than for the corresponding values determined by GC–FID, with the exception of **1A**, which exhibits comparable DLs for both applied modes of GC analysis. The results also demonstrate a clear dependence of detectability on molecular structure for GC–FID, exhibited by a decrease of the DL values with an increase in the number of structural carbon atoms. Thus, the DL values of phosphonates **1A**–**4A** were found to follow the order TFA–PMG$(OMe)_3$< TFA–Sar$^P(OMe)_2$<TFA–Gly$^P(OMe)_2$< MPA$(OMe)_2$. The GC–CI-MS data on the detectability of **1A**–**4A** exhibit a more complex influence of the structure, illustrated by the following DL value order: TFA–PMG$(OMe)_3$>MPA$(OMe)_2$> TFA–Gly$^P(OMe)_2$>TFA–Sar$^P(OMe)_2$.

In order to demonstrate the applicability of the method to the quantitative analysis of environmental samples, river and drinking water were subjected to the pretreatment described in the Experimental section. Such analyses included prior evaporation of the analyzed solutions of aminophosphonic acids **1**–**3**, subsequent derivatization of the amino acidic residue and, finally, GC–FID analysis of the formed TFA–aminophosphonates **1A**–**3A**.

Since the tested water samples (river and drinking) were not found to contain any traces of PMG or its metabolites **2**–**4** down to their determined DL levels, in further experiments the samples were fortified with known amounts of aminophosphonic acids **1**–**3** (to a concentration of 0.01 to 10 μmol/l) prior to analysis.

Taking into account the quantitative course of the derivatization of **1**–**3** into **1A**–**3A** (established by ^{31}P NMR), their recoveries in analytical samples were calculated in accordance with the equation

$$\text{Recovery} = (S_1/S_2)\cdot 100\%$$

where S_1 is the determined peak area of derivatives **1A**–**3A** obtained by derivatization of a standard quantity (0.01 to 10 μmol) of aminophosphonic acids **1**–**3** contained in 1 l aqueous solution (after prior evaporation to dryness), and S_2 is the determined peak area of derivatives **1A**–**3A** obtained by derivatization of a standard quantity (0.01 to 10 μmol) of aminophosphonic acids **1**–**3** (reference).

The determined recoveries are summarized in Table 4. Recoveries are as high as 96.8–97.5% with the relative standard deviation varying from 1.8 to 3.5% ($n=5$) (Table 4).

Since the derivatization of 1-aminoalkanephosphonic acids has been shown to be quantitative [19], these amino acids can be successfully applied as internal standards (e.g. 1-aminobutanephosphonic acid) and, in this way, allow additional simplification of the simultaneous analysis of PMG and its metabolites in more complex environmental samples (e.g. food samples), in a manner similar to that described earlier by Kataoka et al. [12].

Table 3
Detection limits of phosphonates **1A**–**4A** determined by GC–FID and GC–CI-MS

Phosphonate	Detection limit[a] (DL) (pmol)	
	GC–FID[b]	GC–CI-MS[c]
TFA–PMG$(OMe)_3$ (**1A**)	15	10.0
TFA–Sar$^P(OMe)_2$ (**2A**)	20	0.5
TFA–Gly$^P(OMe)_2$ (**3A**)	30	2.5
MPA$(OMe)_2$ (**4A**)	80	5.0

[a] The detection limit (DL) is defined as the minimum detectable amount of analyzed compound affording a signal three times greater than the noise. DL were determined under chromatographic conditions.
[b] DB-17, 30 m, 80 °C (5 min)—10 °C/min—200 °C (3 min).
[c] DB-17, 30 m, 100 °C (5 min)—10 °C/min—250 °C (10 min).

4. Conclusions

The described analytical procedure presents a novel approach for the simultaneous analyses (identification, semiquantitation and quantitation) of PMG

Table 4
Recoveries of PMG, Sar^P (MAMPA) and Gly^P (AMPA) from water using GC–FID

Sample		Analysis	
Determined	Analyzed as	Fortification (**1–3**)[a]	Recovery[b] (%)
PMG	TFA–PMG$(OMe)_3$	10	96.8
		1	96.9[c]±1.8[d]
		0.1	97.0
		0.01	96.8
Gly^P	TFA–$Gly^P(OMe)_2$	10	97.0
		1	97.2[c]±1.9[d]
		0.1	97.4
		0.01	97.5
Sar^P	TFA–$Sar^P(OMe)_2$	10	97.6
		1	97.7[c]±3.5[d]
		0.1	97.4
		0.01	97.5

[a] Fortification level in μmol/l.
[b] Average results of two or five[c] independent determinations.
[d] Mean±SD ($n=5$).

and its metabolites (MAMPA, AMPA and MPA). This protocol includes a preliminary ^{31}P NMR analysis of the starting mixtures (in the range $0<pH<2$ and/or $8.5<pH<14$; Fig. 3), followed by simultaneous derivatization of acids **1**–**4** to volatile phosphonates **1A**–**4A**, and subsequent GC–MS and/or CI-MS analysis of the derivatives. The derivatization procedure, based on the application of the TFA–TFAA–TMOA reagent, displays superb capability for the simultaneous derivatization of PMG in mixtures with MPA, AMPA and MAMPA, and constitutes the only derivatization procedure for PMG and its metabolites (MAMPA, AMPA and MPA) for which a quantitative course has been verified by ^{31}P NMR measurements.

Simultaneous CI-MS analysis of a mixture of derivatives **1A**–**4A** provides rapid, qualitative–semiquantitative information about its content prior to further GC–MS analysis. Subsequent GC–CI-MS (and/or supplementary GC–EI-MS) analysis of the derivatization products enables, even in the case of an unsatisfactory chromatographic separation (e.g. derivatives **2A** and **3A** on a DB-1 column), unequivocal identification/semiquantification of all the major components of the derivatization mixture, and direct glyphosate residue analysis. The applied derivatization scale (up to 0.02 μmol of derivatized components), preceded by a routine extraction/concentration pre-stage [3,5], provides the possibility for the simultaneous GC–FID analysis of phosphonic acids **1**–**4** in real samples in the range 0.01 to 10 μmol/l. The detection limits of **1A**–**4A**, determined by GC–CI-MS and GC–FID, were at the nmol–pmol level.

Fortification of the analyzed mixtures with selected 1-aminoalkanephosphonic acids [17] as internal standards [14] can further simplify the procedure.

References

[1] J.A. Sikorski, E.W. Logusch, in: R. Engel (Ed.), Handbook of Organophosphorus Chemistry, Marcel Dekker, New York, 1992, p. 736, Chapter 15.
[2] H.R. Hudson, in: H.R. Hudson, V. Kukhar (Eds.), Aminoalkanephosphonic and Aminoalkanephosphinic Acids: Chemistry and Biological Activity, Wiley, Chichester, 2000, p. 443, Chapter 13.
[3] M.L. Rueppel, B.B. Brightwell, J. Schaefer, J.T. Marvel, J. Agric. Food Chem. 25 (1977) 517.
[4] L.Z. Avilla, S.H. Loo, J.W. Frost, J. Am. Chem. Soc. 109 (1987) 6758.
[5] P.C. Bardalaye, W.B. Wheeler, H.A. Moye, in: E. Grossbard, D. Atkinson (Eds.), The Herbicide Glyphosate, Butterworths, London, 1984, p. 263.
[6] Z.H. Kudzin, M. Sochacki, in: H.R. Hudson, V. Kukhar (Eds.), Aminoalkanephosphonic and Aminoalkanephosphinic Acids: Chemistry and Biological Activity, Wiley, Chichester, 2000, p. 358, Chapter 11.
[7] C.D. Stalikas, C.N. Konidari, J. Chromatogr. A 907 (2001) 1.
[8] H.A. Moye, C.L. Deyrup, J. Agric. Food Chem. 32 (1984) 192.
[9] C.L. Deyrup, S.M. Chang, R.A. Weintub, H.A. Moye, J. Agric. Food Chem. 33 (1985) 994.
[10] N. Tsunoda, J. Chromatogr. A 637 (1993) 167.
[11] H. Kataoka, K. Horii, M. Makita, J. Agric. Food Chem. 55 (1991) 195.
[12] H. Kataoka, S. Ryu, N. Sakiyama, M. Masami, J. Chromatogr. A 726 (1996) 253.
[13] R.A. Guinivan, N.P. Thompson, W.B. Wheeler, J. Assoc. Off. Anal. Chem. 65 (1982) 35.
[14] D.N. Roy, S.K. Konar, J. Agric. Food Chem. 37 (1989) 441.
[15] P.L. Eberbach, L.A. Douglas, J. Agric. Food Chem. 19 (1991) 1776.
[16] P.L. Alferness, Y. Iwata, J. Agric. Food Chem. 42 (1994) 2751.
[17] C.D. Stalikas, G.A. Pilidis, M.I. Karayannis, Chromatographia 51 (2000) 741.
[18] C.D. Stalikas, G.A. Pilidis, J. Chromatogr. A 872 (2000) 215.

[19] Z.H. Kudzin, M. Sochacki, J. Drabowicz, J. Chromatogr. A 678 (1994) 299.
[20] Z.H. Kudzin, J. Luczak, Synthesis (1995) 509.
[21] R. Gallenkamp, W. Hoper, B. Kruger, F. Haurer, T. Pfister, in: H. Regitz (Ed.), Methoden der Organischen Chemie (Houben-Weil), Vol. III, Georg Thieme, Stutgart, 1982, p. 300.
[22] Z.H. Kudzin, G. Andrijewski, J. Drabowicz, Heteroatom Chem. 5 (1994) 1.
[23] Z.H. Kudzin, J. Drabowicz, M. Sochacki, W. Wiśniewski, Phosphorus, Sulfur Silicon 92 (1994) 77.
[24] E. Constantin, E. Neuzil, P. Traldi, Org. Mass Spectrom. 21 (1986) 431.