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Alkyl methylphosphonic acids, the degradation products of organophosphorous CWA – preparation and direct quantitative GC-FID analysis

Research Article

Iwona Połeć*, Anna Kiełczewska, Leszek Konopski, Grażyna Oleksa, Hanna Nowacka Krukowska and Jan Legocki

Institute of Industrial Organic Chemistry, 03-236 Warsaw, Poland

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Abstract: Seven alkyl methylphosphonic acids, products of hydrolytic degradation of organophosphorus chemical warfare agents, were obtained with a high purity (mostly above 98%), with the aim of being applyed as future certified reference materials. Ethyl (EMPA), isopropyl (IMPA), pinacolyl (PMPA), butyl (BUMPA), isobutyl (IBUMPA), cyclohexyl (CHMPA) and 2-ethylhexyl (EHMPA) monoesters of MPA were synthesized and characterized by MS EI, FTIR and NMR (^{1}H, ^{13}C, ^{31}P), TLC, as well as GC and GC-MS after derivatization. The conditions for a direct quantitative GC FID analysis on CP-FFAP CB column of non-derivatized alkyl methylphosphonic acids were developed. This is the first successful attempt of a directed GC analysis of free alkyl phosphonic acids. Their chemical purity was determined and limit of quantification (LOQ) values for some of them were evaluated for the GC-FID method.

Keywords: Organophosphorus chemical warfare agents • Degradation products • Alkyl methylphosphonic acids • Gas chromatography • Reference materials

1. Introduction

Chemical warfare agents (CWA) are compounds that persistently remain in the environment and decompose with difficulty. However, some of their degradation products – are (fortunately) non toxic, but exists for longer periods in the ground, and may be a proof for the earlier presence of a toxic agent. They may also provide some indication of the type of chemical used, be a useful tool for the estimation of method by which the given CWA was prepared, and help to identify the sources of origin of the precursors for CWA production [1,2]. The group of such compounds form alkyl monoesters of methylphosphonic acid (MPA), Fig. 1. One of the examples endorsing the above described abilities may be the detection of IMPA (isopropyl methylphosphonic acid) in urine and blood serum of the people jeopardized for a sarin toxic action during a terrorist attack in Tokio subway [3,4]. When one considers the estimated theoretical values of LC_{50} and EC_{50} of these monoesters for aquatic organisms (calculated on the basis of the given compound chemical structure) [5], it seems to be clear that their toxicity, at least towards aquatic animals is not really high (Table 1). Although there is a deficiency of published data about their toxicity against mammals, the acute LD_{50} value of IMPA in rats was reported as 7650 mg kg^{-1} (oral, male) and in mice - 5620 mg kg^{-1} (oral, male) [1].

These values confirm that the degradation products of extremely toxic CWA, can be characterized as practically non toxic.

The implementation of the Convention for the Prohibition of Chemical Weapons (Chemical Weapons Convention, CWC), and its requirements to perform the verified and compliant measurements, prompted us to develop reliable and fast analytical methods for identification of CWA and their degradation products in the environment [6].

Certified reference materials, are necessary in any kind of the analytical research work, this is also the case with CWA degradation products. Additionally, a good description of the analytical procedures is needed, since they are utilized for identification, as well as for quantitative determination purposes.

The following synthetic methods have been used for preparation of alkyl alkyl- or arylphosphonic acids: partial

* *E-mail: polec@ipo.waw.pl*

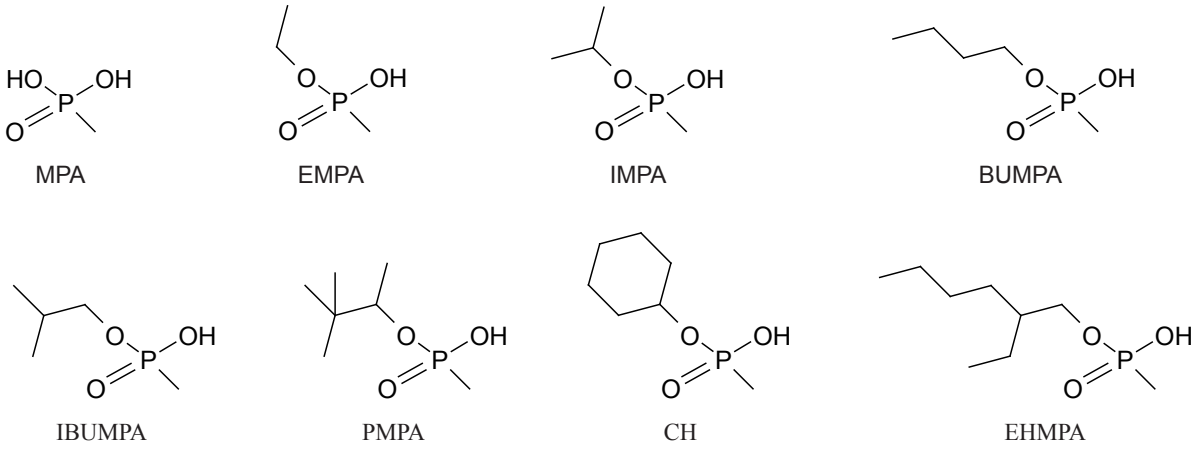


Figure 1. Chemical structures of the synthesized alkyl methylphosphonic acids.

Table 1. Theoretical toxicity values of some alkyl methylphosphonic acids against aquatic organisms (determined on the basis of calculated values of octanol-water coefficient (K_{ow}); according to EPI Suite™ v4.00 programme, EPA [5])

Alkyl MPA	*Predicted (theoretically calculated) values in mg L^{-1} [ppm] for aq. Organisms*		
	Fish LC_{50} (after 96 h)	Dafnes LC_{50} (after 48 h)	Green algae EC_{50} (after 96 h)
MPA			
CAS No [993-13-5]	1.75e+005	66207	9739
EMPA			
CAS No [1832-53-7]	7460	3061	571
IMPA			
CAS No [1832-54-8]	3563	1555	348
BUMPA			
CAS No [1832-55-9]	1250	593	169
IBUMPA			
CAS No [1604-38-2]	1451	681	188
PMPA			
CAS No [616-52-4]	294	156	63
EHMPA			
CAS No [13688-82-9]	37	23	15
CHMPA			
CAS No [1932-60-1]	291	155	63

hydrolysis of the respective diester [7,8], alcoholysis of the anhydride of the appropriate phosphonic acid (the acid was obtained earlier, usually from alkylphosphonic dichloride [9-11] and the direct catalytical mono esterification of the respective phosphonic acid with alcohol [12-13].

Because these compouinds are considerably hydrophilic and have very few chromophors in their structure, the identification and quantitative analysis of them is usually carried out after the derivatization to the respective, less polar compounds. The most frequently used derivatizing agents are N-methyl-

N-(tert-butyldimethylosilyl)trifluoroacetamide, N,O-bis(trimethylsilyl)acetamide and N,O-bis(tri methylsilyl) trifluoroacetamide, all of which are used to produce silyl derivatives [14-20]; and diazomethane, (trimethylosilyl) diazometane, methyl iodide, trimethylphenylammonium hydroxide, and phenyltrimethylammonium fluoride which are used to produce methyl derivatives [12-13,21-23]. The last two compounds work through the formation of an ionic pair with the MPA monoester in methanol; the derivatization takes place instantaneously on introduction to the GC injector, as a result of a thermal decomposition of the reagent [23]. Pentafluorobenzyl bromide may be also used for derivatization [23-25]. In a review concerning derivatization reactions of CWA and their degradation products applicable in chromatographic analysis, Black and Muir [23], Mesekete Seemane [26] and Papouskova *et al*. [27] described in detail the methods for qualitative and quantitative evaluation of such compounds in different matrices.

The most commonly used method for the analysis of derivatized alkyl monoesters of MPA, is gas chromatography coupled to a mass spectrometry (GC-MS) with different ionization types. Ionization types include EI (electron ionization), CI (chemical ionization), EI-MS-MS, CI-MS-MS [14-17,24,28], ICP (inductively coupled plasma ionization), as well as using a TOF (time-of-flight) MS analyser [18-20].

Methods of the selective detection of these compounds using MS with the following types of ionisation were also described; DESI (desorption electrospray ionisation) [29], ESI (electrospray ionization) [30], ESI APIM (electrospray atmospheric pressure ion mobility) and ESI IM TOF (electrospray ion mobility time-of-flight) [31].

HPLC methods for the determination of the alkyl phosphonic acids and their derivatives, by means of different detectors have been also described; they were analyzed on anion-selective ion-exchange column with amperometric or conductometric detection [32], HPLC-FPD (flame photometric detection) [33], HPLC-EI, -ESI, -APCI (atmospheric pressure chemical ionization)-MS-MS [34-37], and HPLC-ESI TOF-MS [38]. For their separation and identification, capillary electrophoresis has been also used, with direct or indirect UV detection [39-41], conductometric detection [40], FPD [41-42], and ESI-MS detection [41,43,44].

The aim of this work was to synthesize certain alkyl methylphosphonic acids: ethyl (EMPA), isopropyl (IMPA), butyl (BUMPA), isobutyl (IBMPA), pinacolyl (PMPA), cyclohexyl (CHMPA), and 2-ethylohexyl (EHMPA), and to apply them as certified reference materials in both the laboratory, and non-laboratory environments. We expected to optimize methods of their synthesis and purification, and to develop some methods of semiquantitative and quantitative analytical control.

2. Experimental Procedure

Methylphosphonic acid (MPA) 98%, methylphosphonic dichloride 98%, phenylarsonic acid (PASA) 97% were obtained from AlfaAesar; pinacolyl alcohol (3,3-dimethylbutan-2-ol) 98%, ethyl methylphosphonic acid (EMPA) 98% (for comparative studies), (trimethylsilyl) diazomethane/2.0 M solution in hexane were obtained from Sigma Aldrich, iodine (crystals) >99.5 %, butanol >99%, isobutanol >99%, cyclohexanol >99%, 2-ethyl-1-hexanol >99% were obtained from Fluka; methanol, ethanol, 2-propanol, tetrachloroethylene, toluene, ethyl acetate, magnesium sulfate, sodium chloride, sodium hydroxide – all p.a., hexane - petroleum fraction were obtained from POCh. Silica gel for column chromatography cat. nr. 1.07734.2500 (Silicagel 60, 0.063-0.200 mm, 70-230 mesh), and TLC plates (20 × 20 cm) cat. nr. 1.05554.0001 (TLC Silicagel 60F_{254}) were obtained from Merck.

GC of non-derivatized alkyl methylphosphonic acids: Varian GC apparatus, with split/splitless injector; column CP-FFAP CB (Varian), 25 m × 0.53 mm, film thickness 1.0 μm; detector: FID, flow gas: helium, flow 22 mL min^{-1}, injector temperature 230°C, detector temperature 270°C, the weights of analyzed esters: 6 mg, each one dissolved in 1 mL of acetone and introduced to column in the quantity of 0.2 μL, temperature program: 50°C (5 min) ↗ 190°C (10°C min^{-1}) ↗ 190°C (15 min for IMPA, 25 min for PMPA, IBUMPA, EMPA, 50 min for EHMPA, CHMPA) ↗ 230°C (10°C min^{-1}).

GC-MS EI (70 eV) of alkyl methylmethylphosphonates: selective mass detector of Agilent Technologies MSD Series 5975B, coupled with GC apparatus of Agilent Technologies Series 6890N Network System, equipped with split/splitless injector; analysis conditions: column DB-5MS (30 m × 0.25 mm), film thickness 0.25 μm. Column temperature 50°C (3 min) ↗ 240°C (10°C min^{-1}), injector temperature 240°C, flow gas – helium, flow speed 1 mL min^{-1}, split – 50:1, 1 μL of the tested solution was introduced to injector, ion source temperature 230°C. Derivatization was performed with (trimethylsilyl) diazomethane, according to [12,13] : to ~ 4 mg of the respective alkyl methylphosphonic acid (IMPA – 5.77 mg, CHMPA – 5.63 mg, EHMPA – 4.98 mg, PMPA – 4.72 mg, IBUMPA – 5.08 mg, BUMPA –5.38 mg, EMPA – 5.87 mg, MPA – 4.41 mg) 1 mL of dry acetone was added. Next, 100 μL of this solution was mixed with 700 μL of dry benzene and 200 μL of dry methanol,

followed by methylation with 20 µL of 2.0 M/hexane solution of (trimethylsilyl)diazomethane. After 30 min at room temperature, the solution of respective methylated ester was injected into the column of gas chromatograph. The blank sample was prepared in the same manner, except for the addition of the ester.

MS EI (70 eV): selective mass detector Agilent Technologies MSD Series 5975B, equipped with device for a direct sample introduction HPP7 of Scientific Instrument Services. MS: system for a direct sample introduction to ion source with a probe (DI-direct injection), temperature of the ion source 250ºC.

GC-MS EI (70 eV) of non-derivatized alkyl methylphosphonic acids and dialkyl methylphosphonates: selective mass detector of Agilent Technologies MSD Series 5975B, coupled with GC apparatus of Agilent Technologies Series 6890N Network System, equipped with split/splitless injector; analysis conditions: column CP-FFAP CB (Varian), 25 m × 0.53 mm, film thickness 1.0 µm. Temperature program 50ºC (2 min) ↗ 190ºC (10ºC min^{-1})-190ºC (15min) ↗ 220ºC (5ºC min^{-1}), injector temperature 240ºC, flow gas: helium, flow speed 12 mL min^{-1}, split 10:1, ion source temperature 250ºC.

FT-IR: Jasco 420 apparatus, film between KBr plates, cm^{-1}.

^{1}H, ^{13}C, ^{31}P NMR: INOVA-500 or Varian 500 apparatus (500 MHz for proton, 202 MHz for phosphorus, 125.9 MHz for carbon), chemical shifts (δ) are given in ppm, analyses were performed in deuterated acetone, TMS was used as the internal standard for ^{1}H and ^{13}C, and H_3PO_4 for ^{31}P NMR.

TLC: aluminium plates covered with Silicagel F_{254}, eluents: benzene-methanol-acetic acid (90:16:8, v/v/v), cyclohexane-chloroform-acetic acid (4:5:1,v/v/v), chloroform-acetic acid (12:1, v/v), detection sprayed with 1% methanol solution of silver nitrate, followed by heating under UV lamp for 15 min.

2.1. General procedure for the preparation of alkyl methylphosphonic acids from methylphosphonic dichloride [9-11]

The reaction was done under the inert atmosphere of nitrogen. Under the well ventilated hood, methylphosphonic dichloride (6.4 g, 48.5 mmol) was weighed directly to the reaction flask, filled with 100 mL of dry toluene. Next, an equimolar amount of water (48.5 mmol, 0.87 mL) was added to the vigorously stirred mixture. The reaction was continued for 2.5-3 h at room temperature, during which time the evolved gaseous hydrogen chloride was removed by the nitrogen stream introduced under the surface. The created methylphosphonic anhydride (a white, sticky solid, visibly different from the starting grey coloured, sticky dichloride) was then treated with an equimolar amount of the respective alcohol (48.5 mmol) and heated at 100°C for 3 h, while anhydride precipitate disappeared and the reaction mixture became a clear, oily, light amber coloured liquid. The solvent was then distilled off on rotavapor (bath temperature 50°C under 30 mm Hg) to give the crude product. All of the obtained MPA monoesters (except EMPA) then underwent the purification procedure, according to the sequence: (1) alkalization with saturated aqueous sodium bicarbonate solution [11], or with 2.5 molar aqueous sodium hydroxide [12], (2) extraction of the alkaline water solution with ethyl acetate (2×50 mL) to remove the diester and other possible organic impurities, (3) acidification of water solution with concentrated hydrochloric acid pH 1-2, and additional saturation of such the solution with 20-30 g of sodium chloride, (4) extraction of the alkyl methylphosphonic acid from the water solution with ethyl acetate (4×50 mL), (5) drying the organic layer over anhydrous magnesium sulfate, filtration, solvent removal using a rotavapor, following by the high vacuum stripping to remove the residual amounts of the solvents, as well as the possible volatile impurities from the product at 35-40°C, under 0.1 mm Hg).

2.2. Ethyl methylphosphonic acid (EMPA)

The quantities of reagents were the same as given above in the general procedure (ethyl alcohol - 48.5 mmol, 2.23 g, 2.83 mL). The crude product (5.8 g) obtained was washed with hexane (100 mL) at room temperature, to remove the non-polar impurities. Extraction of hexane on rotavapor yielded 0.3 g of a light yellow liquid, containing mainly diethyl ester (diethyl methylphosphonate, DEMP), this was confirmed by TLC analysis on silica gel plates, eluent: hexane-ethyl acetate, 7:3, v/v, detection in iodine vapor and also by GC/MS. Finally 5.4 g of the monoester (ethyl methylphosphonic acid, EMPA) was obtained (yield 68.2%). FTIR [cm^{-1}]: 2987, 1653, 1315, 1250, 999; ^{1}H NMR, acetone d_6 [ppm]: 1.26(t, 3H, J=6.8), 1.44(d, 3H, J=17.6 Hz), 4.00(q, 2H, J=7.0 Hz), 10.74(s,1H); ^{13}C NMR: 15.89, 16.00, 61.30, 172.91, 206.59; ^{31}P NMR: 32.104 (s,1P); purity by GC 94.4% – R_t 23.3 min.

2.3. Isopropyl methylphosphonic acid (IMPA)

The reagents were as follows: 80 mmol of methylphosphonic dichloride (10.77 g), 80 mmol of water (1.44 mL), 80 mmol of isopropyl alcohol (6.1 mL). The crude product (11.0 g) obtained was next subjected to the alkalization – acidification procedure, as described above. Saturated aq. sodium bicarbonate solution was used for alkalization (to pH 8.5) and 0.9 g of non-polar

impurities (mainly diisopropyl methylphosphonate, DIMP) was obtained after extraction-concentration sequence. Finally 8.1 g of product (isopropyl methylphosphonic acid, IMPA) was obtained, after stripping under high vacuum at 35°C for 2.5-3 h (yield 73.4%). FT-IR [cm^{-1}]: 2981, 2302, 1681, 1387, 1314, 1179, 1003; ^{1}H NMR, acetone-d_6 [ppm]: 1.28(d, 6H, J=6.2 Hz), 1.40(d, 3H, J=17.8 Hz), 4.53-4.76(m, 1H); ^{13}C NMR: 10.84 (s), 13.74 (s), 24.14(d, J=17.4 Hz), 70.26 (d, J=6.3 Hz, secondary C); ^{31}P NMR: 31.055 (s, 1P); purity by GC >98 %, R_t 20.9 min.

2.4. Pinacolyl methylphosphonic acid (PMPA)

The reagents were as follows: 76 mmol of methylphosphonic dichloride (10.17 g), 76 mmol of water (1.37 mL), 76 mmol of pinacolyl alcohol (7.8 g, 9.6 mL). The crude product (13.3 g) obtained was next subjected to the alkalization – acidification procedure, as described above. Saturated aqueous sodium bicarbonate solution was used for alkalization (to pH 8.5) and 0.7 g of the non polar impurities (mainly dipinacolyl methylphosphonate DPMP) was obtained after extraction-concentration sequence. Finally 9.8 g of product (pinacolyl methylphosphonic acid, PMPA) was obtained, after stripping under high vacuum at 35°C for 2.5-3 h (yield 71.6%). FT-IR [cm^{-1}]: 2964, 2940, 2300, 1680, 1312, 1207, 1015; ^{1}H NMR, acetone d_6 [ppm]: 0.92 (s, 9H), 1.26(d, 3H, J=6.4 Hz), 1.40(d, 3H, J=17.8 Hz), 4.13-4.27(m, 1H), 11.63 (d,1H, J=3.8); ^{13}C NMR: 10.85, 13.79, 17.11, 25.83, 35.32, 35.44, 80.84, 80.99, 206.04; ^{31}P NMR: 32.139 (s,1P); purity by GC 98.6 %, R_t 25.2 min.

2.5. 2-Ethylhexyl methylphosphonic acid (EHMPA)

The reagents were as follows: 38.1 mmol of methylphosphonic dichloride (5.08 g), 38.1 mmol of water (0.69 mL), 38.1 mmol of 2-ethylhexyl alcohol (4.95 g, 5.95 mL). The crude product obtained was next subjected to the alkalization – acidification procedure, as described above. Saturated aq. sodium bicarbonate solution was used for alkalization (to pH 8.5) and 0.2 g of non-polar impurities (mainly bis(2-ethylhexyl) methylphosphonate, BEHMP) was obtained after extraction-concentration sequence. Finally 5.6 g of product (2-ethylhexyl methylphosphonic acid, EHMPA) was obtained, after stripping under high vacuum at 35°C for 2.5-3 h (yield 70.6%). FT-IR [cm^{-1}]: 2960, 2940, 2290, 1680, 1464, 1312, 1203, 1000; ^{1}H NMR, acetone d_6 [ppm]: 0.87(t, 6H, J=7.0 Hz), 1.31-1.59(m, 9H), 1.40(d, 3H, J=17.8 Hz), 3.88(t, 2H, J=5.8 Hz); ^{13}C NMR: 9.95, 11.20, 12.85, 14.31, 23.58(d, J=16.15 Hz), 29.55, 30.61, 40.75(d, J=6.7 Hz), 67.36(d, J= 6.7 Hz); ^{31}P NMR: 32.725 (s,1P), purity by GC 99+ % – R_t 51.2 min.

2.6. General procedure for the preparation of alkyl methylphosphonic acids from methylphosphonic acid [12,13]

Methylphosphonic acid (MPA) (100 mmol, 9.6 g), phenylarsonic acid (PASA) (3% of molar equivalents, 3 mmol, 0.6 g) were suspended in 200 mL of toluene, this was followed by the addition of 2- 4 molar equivalents of the respective alcohol. The mixture was then refluxed for at least 72 h (depending on the alcohol used), and the water-toluene azeotrope formed was continuously removed by means of Dean-Stark apparatus. Next, the solvent was distilled off on rotavapor and the oily residue was worked-up in the same manner as in the method starting from dichloride, described above. After the high vacuum stripping step to remove the residual amounts of solvents and other possible volatiles, the pure product was obtained. Only in the case of EMPA preparation another purification method was used.

2.7. Ethyl methylphosphonic acid (EMPA)

The reagents were as follows: 96 mmol of MPA (9.2 g), 2.9 mmol of PASA (0.57 g), 150 mL of dry toluene, 2 molar equivalents of ethanol (0.192 mol, 11.5 mL). The reaction was continued for 72 h. Toluene was then distilled off on rotavapor and 100 mL of hexane was added to the brown colored residue (12.8 g). This residue was then stirred at 50°C for 0.5 h, then the hexane with an oil dispersed in was separated. The residue was treated four times in this manner. The collected hexane extracts were concentrated using a rotavapor to 150 mL (water bath temperature 50°C, at 400 mm Hg), and cooled to the room temperature. Hexane was separated from the lower oily layer. This way the less polar impurities were removed. Finally, 6.5 g of a slightly yellow liquid was obtained. TLC analysis on silica gel plates, eluent: ethyl acetate, detection in iodine vapors, confirmed the absence of less polar (than EMPA) impurities (DEMP). The brown colored sticky oil, remaining from the crude mixture, was dissolved in ethyl acetate. The solution was mixed with 10 g of silica gel, and the solvent was evaporated using a rotavapor. The obtained dry mass was put into the Soxhlet apparatus tube and extracted with hexane for 12 h. Next, the hot hexane solution was separated from the lower waxy layer (2.8 g). After evaporation of solvent, the obtained 2.8 g of EMPA was collected with previously separated part, giving finally 6.2 g of product, after high vacuum stripping step (yield 52.1%).

2.8. Isopropyl methylphosphonic acid (IMPA)

The reagents were as follows: 100 mmol of MPA (9.6 g), 3 mmol of PASA (0.6 g), 200 mL of dry toluene, 4 molar equivalents of isopropyl alcohol (0.4 mol, 24.4 g, 31.2 mL). The reaction was continued for 76 h. Saturated aqueous sodium bicarbonate solution was used for alkalization (pH 8.5) and 1.4 g of non-polar impurities (mainly DIMP) was obtained after the extraction-concentration sequence. Finally 6.7 g of the product (IMPA) was obtained, after stripping under high vacuum at 35°C for 2.5-3 h (yield 48.5%).

2.9. Butyl methylphosphonic acid (BUMPA)

The reagents were as follows: 63 mmol of MPA (6.1 g), 0.38 g PASA (1.9 mmol), 95 mmol of butanol (1.5 molar equivalent, 7.0 g, 8.6 mL), 150 mL of dry toluene. The reaction was continued for 15 h. After solvent evaporation, 10.2 g of crude residue was obtained, which was next subjected to the alkalization-acidification sequence. 2.5 molar aqueous sodium hydroxide solution was used for alkalization (pH 13-14) and 1.5 g of non-polar residue was obtained (mainly dibutyl methylphosphonate, DBUMP). Finally 4.2 g of the product (BUMPA) was obtained (yield 43.9%), after stripping under high vacuum. FT-IR [cm^{-1}]: 2961, 2300, 1700, 1460, 1313, 1205, 999; ^{1}H NMR, acetone d_6 [ppm]: 0.89(t, 3H, J= 7.6 Hz), 1.32-1.70 (m, 4H), 1.39 (d, 3H, J=17.8 Hz), 3.94 (q, 2H, J=6.6), 11.70 (d, 1H, J=6.8 Hz); ^{13}C NMR: 9.45, 12.35, 13.23, 18.73, 32.45, 32.5964.58, 64.70, 205.51; ^{31}P: 32.031 (s,1P); purity by GC: 98.2% – R_t 31.6 min

2.10. Isobutyl methylphosphonic acid (IBUMPA)

The reagents were as follows: MPA 100 mmol (9.6 g) PASA 0.6 g (3 mmol), isobutyl alcohol 150 mmol (11.1 g, 13.9 mL), 200 mL of toluene. The reaction was continued for 65 h. After solvent evaporation, 18.1 g of crude residue was obtained, which was next subjected to the alkalization-acidification sequence. 2.5 molar aqueous sodium hydroxide solution was used for alkalization (pH 13-14) and 1.2 g of a non-polar residue was obtained (mainly diisobutyl methylphosphonate, DIBUMP). Finally, 12.9 g of the product (IBUMPA) was obtained, after acidification (to pH 1-2) – extraction – concentration step, an stripping under high vacuum (yield 77.7%). FT-IR[cm^{-1}]: 2982, 2290, 1677, 1472, 1313, 1210, 1038; ^{1}H NMR, acetone d_6 [ppm]: 0.92(s, 3H), 0.95(s, 3H), 1.41(d, 3H, J=18 Hz), 2.03-2.08(m, 1H), 3.73 (t, 2H, J=6.8); ^{13}C NMR: 9.95(s), 12.85(s), 18.93(s), 71.53(d, J=3.18 Hz); ^{13}C NMR DEPT: 71.53 (secondary C); ^{31}P NMR: 32.84 (s); purity by GC 99+% – R_t 26.0 min.

2.11. 2-Ethylhexyl methylphosphonic acid (EHMPA)

The reagents were as follows: MPA 78 mmol (8.0 g) PASA 0.44 g (2.34 mmol), 2-ethylhexanol 110 mmol (1.5 molar equivalent, 14.5 g, 17.4 mL), 150 mL of toluene. The reaction was continued for 72 h. After solvent evaporation 19.4 g of crude residue was obtained. After alkalization with 2.5 molar aqueous sodium hydroxide solution (to pH 13-14), it was separated 4.1 g of non-polar liquid (mainly bis(2-ethylhexyl) methylphosphonate, BEHMP). After acidification, saturation of water solution with sodium chloride, finally 10.2 g of product was obtained (yield 62.9%), when stripped for some time under high vacuum.

2.12. Cyclohexyl methylphosphonic acid (CHMPA)

The reagents were as follows: 33 mmol of MPA (3.2 g), 0.2 g of PASA, 49 mmol of cyclohexanol (5.0 g, 1.5 molar equivalent), 50 mL of toluene. The reaction was continued for 40 h. After solvent evaporation, 7.3 g of the crude residue was obtained, which was subjected to the alkalization-acidification procedure with saturated aqueous sodium bicarbonate (to pH 8.5) and 36% hydrochloric acid (to pH 1-2) and additional saturation of acidic solution with 30 g of sodium chloride gave 1.6 g of a non-polar residue of dicyclohexyl methylphosphonate (DCHMP) and 5.0 g of the pure product (CHMPA), after stripping under high vacuum (yield 85.1%). FT-IR [cm^{-1}]: 2932, 2850, 2287, 16860 1318, 1182, 10003; ^{1}H NMR, acetone d_6 [ppm]: 1.37(d, 3H, J=17.4 Hz), 1.16-1.94(m, 10H), 4.26-4.4.42(m, 1H); ^{13}C NMR: 4.61, 15.52, 18.42, 25.32, 28.76, 30.40, 38.83, 38.9179.71, 79.84, 177.38, 210.71; ^{31} P: 31.328 (s, 1P); purity by GC 96.8% – R_t 52.8 min.

3. Results and Discussion

We synthesized seven alkyl monoesters of methylphosphonic acid: ethyl, isopropyl, butyl, isobutyl, pinacolyl, cyclohexyl, and 2-ethylhexyl using two different methods. In the first method, the starting material was methylphosphonic dichloride, converted by reaction with one equivalent of water to methylphosphonic anhydride, which was next esterified with one equivalent of the respective alcohol [9-11]. In the alternative synthesis, the starting material was MPA – this method was described by Crenshaw *et al.* [12,13]. It requires the use of catalytic amounts of phenylarsonic acid (PASA) to perform a direct mono esterification. Although the use of PASA as acidic catalyst can be supposed as an analogy to the esterification of carboxylic acids *e.g.* by sulfuric acid

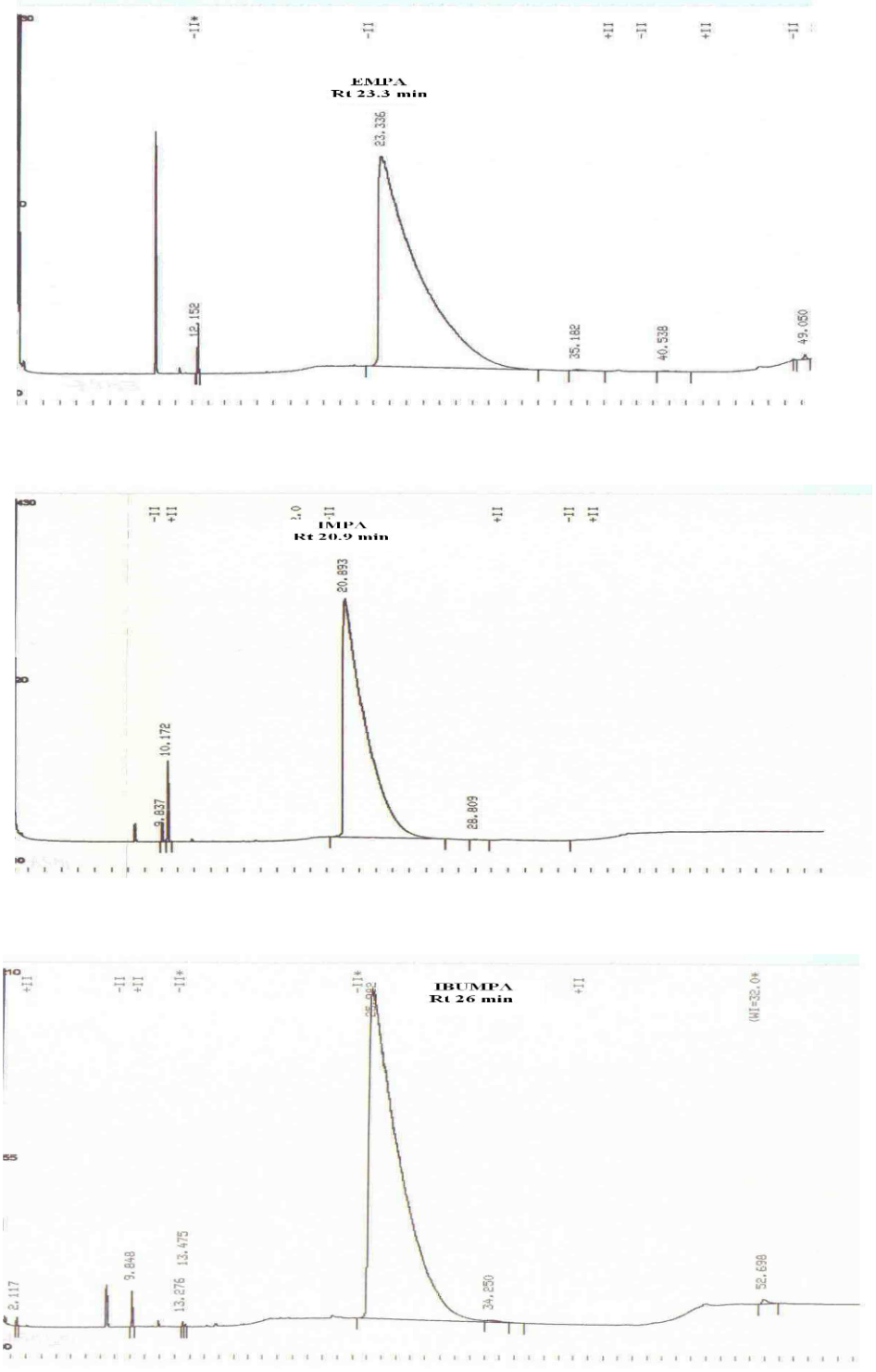
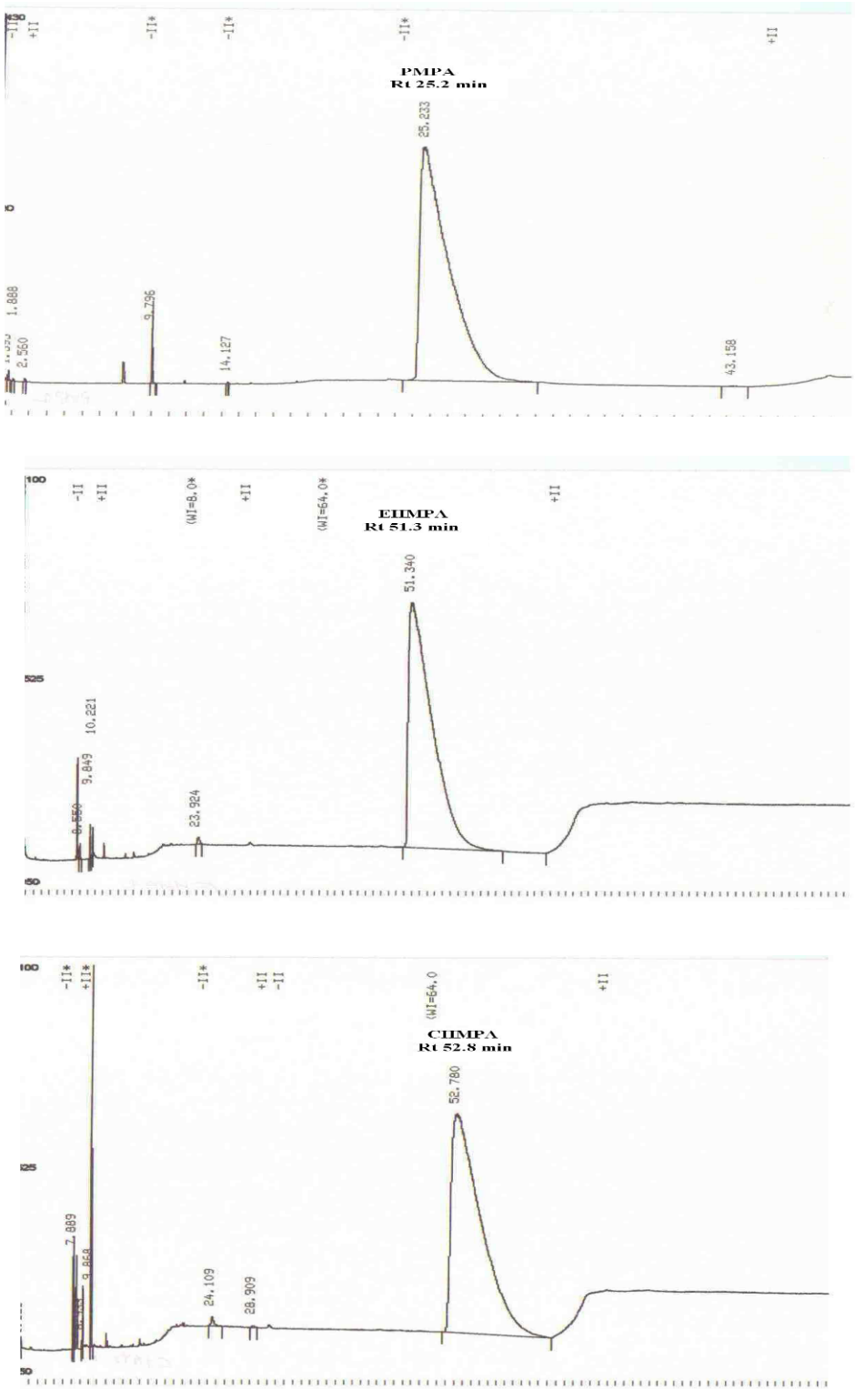
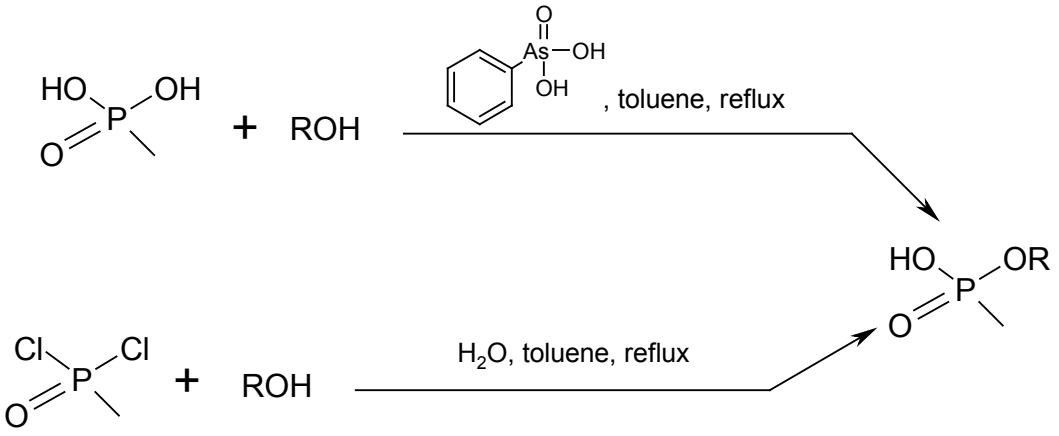


Figure 2. GC FID chromatograms of alkyl methylphosphonic acids: 1. EMPA, 2. IMPA, 3. IBUMPA, 4.PMPA, 5. EHMPA, 6. CHMPA; GC performed on column CP-FFAP CB, temperature program: 50°C (5 min) ↗ 190°C(10°C min^{-1}) ↗ 190°C (15 min for IMPA, 25 min for EMPA, PMPA and IBUMPA, 50 min for EHMPA and CHMPA) ↗ 230°C (10°C min^{-1}), helium flow 22 mL min^{-1}, injector temperature 230°C, detector (FID) temperature 270°C.



Continued **Figure 2.** GC FID chromatograms of alkyl methylphosphonic acids: 1. EMPA, 2. IMPA, 3. IBUMPA, 4.PMPA, 5. EHMPA, 6. CHMPA; GC performed on column CP-FFAP CB, temperature program: 50°C (5 min) ↗ 190°C(10°C min^{-1}) ↗ 190°C (15 min for IMPA, 25 min for EMPA, PMPA and IBUMPA, 50 min for EHMPA and CHMPA) ↗ 230°C (10°C min^{-1}), helium flow 22 mL min^{-1}, injector temperature 230°C, detector (FID) temperature 270°C.



R = ethyl, isopropyl, butyl, isobutyl, pinacolyl, 2-ethylhexyl, cyclohexyl

Scheme 1. Synthetic pathways to alkyl methylphosphonic acids [7-13]

or p-toluenosulfonic acid, Crenshaw *et al.* [12] underlined the differences between these reactions, as PASA is involved to the reaction in two manners – the first relies on PASA esterification, and then its transesterification with MPA, what results in the respective alkylated MPA and free PASA, the second one incudes formation of a mixed MPA-PASA anhydride, which decomposes into alkylated MPA and free PASA after the addition of one equivalent of the respective alcohol.

Since our aim was to synthesize the declared alkyl methylphosphonic acids with the highest possible yield and purity, we applied both methods.

In the method starting from MPA [12], originally the toxic tetrachloroethylene was used as a solvent, what gave in some cases an advantage of better yields (*e.g.* IMPA was obtained in 64% yield, after 64 h reaction course, with six fold molar excess of alcohol) [12-13]. Our attempts for such a high yield preparation of targeted compounds by reaction in tetrachloroethylene were not successful. Esterification of MPA with isopropyl or cyclohexyl alcohol yielded, after solvent evaporation, a dark residue, from which products separation was difficult. Finally, on the basis of the preliminary experiments carried out, we decided to conduct both types of esterification in our modification in the less toxic toluene, because of safety reasons. Our results in the production of alkyl methylphosphonic acids (in both primary and secondary alcohols), agrees well with the results of Crenshaw *et al.* [12], in their synthesis of alkyl phosphonic acids. Usually, the reaction was carried out for 72 hours. Although it takes significantly more time compared to the experiment with methylphosphonic dichloride use, the products achieved by both methods were obtained with similar yields. In fact, the relevant impact on the final efficiency of pure products has a method of separation the product from the reaction mixture. All of the compounds discussed here are highly polar; but polarity decreases with increasing of the alcohol alkyl chain length. Nonetheless, to find the best way to separate product from the crude residue, it was necessary – particularly for EMPA, to determine a method of to first separate it from the non-polar diester and next, from strongly polar unreacted MPA. Ethyl acetate was used by many authors for extraction of such compounds from water solutions [7,8,10,11]. The use of standard procedures, worked well up for the other alkyl methylphosphonic acids, *i.e.*, alkalization/extraction of non polar impurities – acidification/ extraction of product, in the case of EMPA led to a significant loss of product, due to its good solubility in water. Although the small amounts of the formed diester were easily extracted from the crude alkaline residue, it was practically impossible to get out EMPA from acidified solution, even after its strong over-saturation with sodium chloride and multifold extraction with ethyl acetate. Consequently, we managed to obtain only 20-30% of the expected quantity, however it was still contaminated with unreacted MPA. Therefore, we washed out EMPA from the crude mixture with hot hexane. Even if it is practically insoluble in cold hexane, EMPA still formed a dispersion in this solvent, when hot. Multifold repetition of the washing-out procedure gave product, free from the polar impurities (mainly MPA), however containing DEMP. The last was removed by a single washing with hexane of the concentrated, preliminary purified EMPA, at ambient temperature.

The alternative way to purify EMPA, which we also successfully tried, is the preparative chromatography on silica gel. When order of solvents was kept, as follows: hexane – ethyl acetate, ethyl acetate – methanol, in the v/v gradient of solvents, pure EMPA was easy separated.

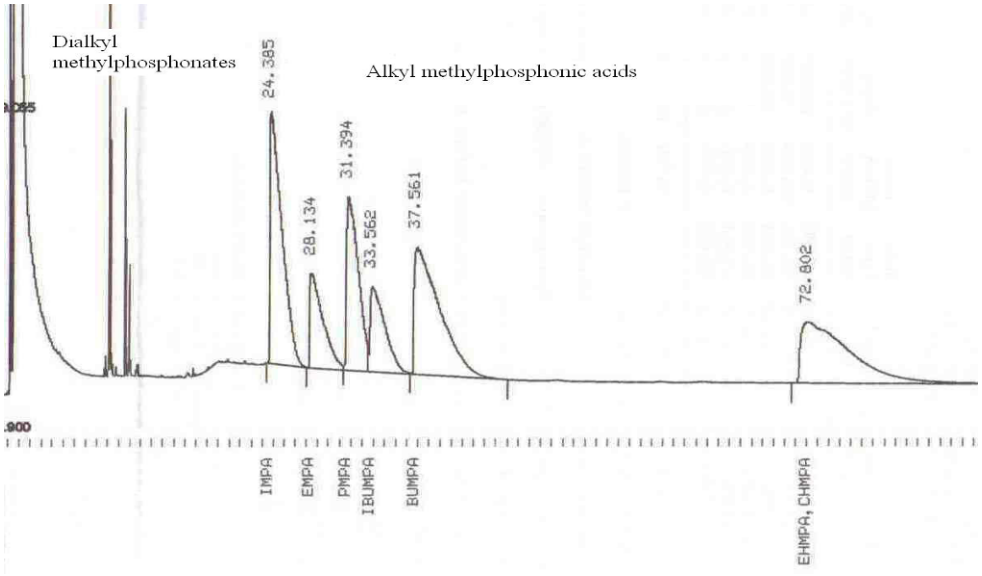


Figure 3. GC FID chromatogram of a mixture of alkyl methylphosphonic acids (samples injected in acetone (1 μL), column CP-FFAP CB, temperature program: 50°C (5 min) ↗ 190°C (10°C min^{-1}) ↗ 190°C; helium flow 7.2 mL min^{-1}, injector temperature 230°C, detector (FID) temperature 270°C; concentration of analyzed compounds [mg mL^{-1}]: IMPA – 0.91, EMPA – 0.44, PMPA – 0.58, IBUMPA – 0.36, BUMPA – 0.98, EHMPA – 0.92, CHMPA – 0.59.

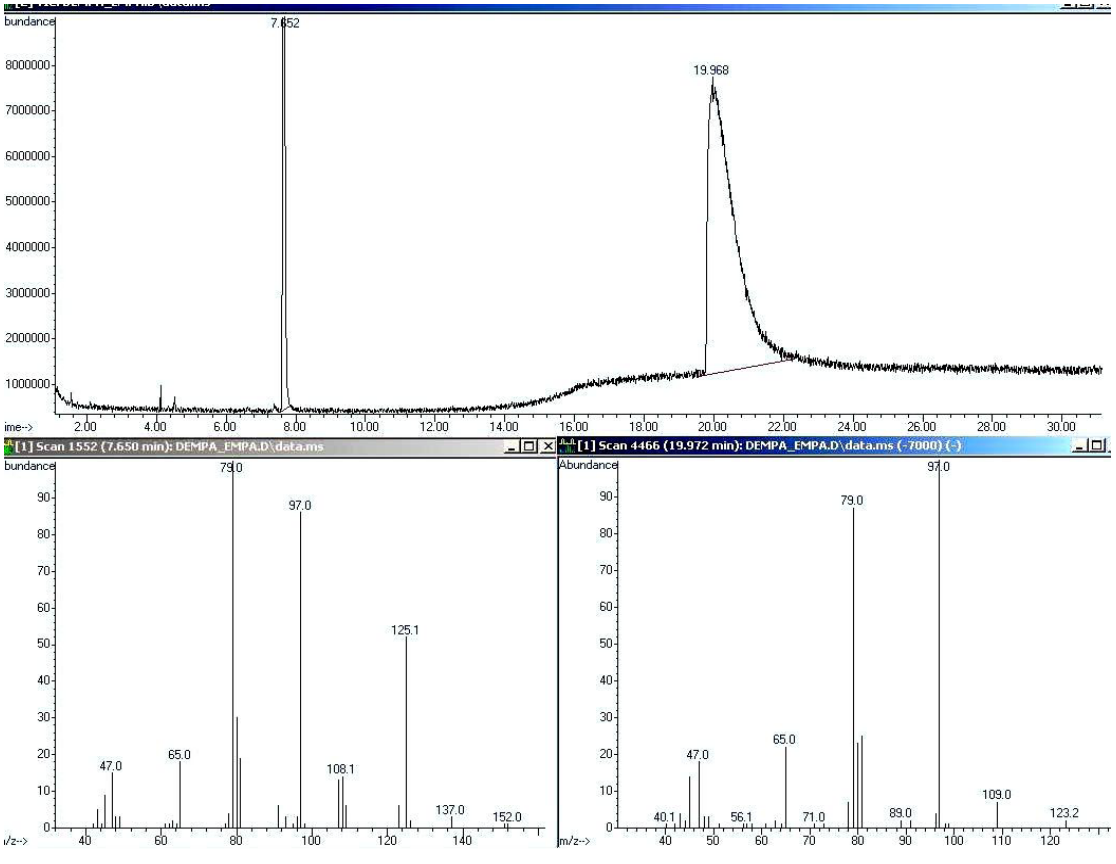


Figure 4. Direct GC-MS EI (70 eV) of EMPA/DEMP on CP FFAP CB column - chromatogram and mass spectra; temperature program: 50°C (2 min) ↗ 190°C (10°C min^{-1}) ↗ 190°C (15 min) ↗ 220°C (5°C min^{-1}), injector temperature 240°C, ion source temperature 250°C, split 10:1; helium flow 12 mL min^{-1}.

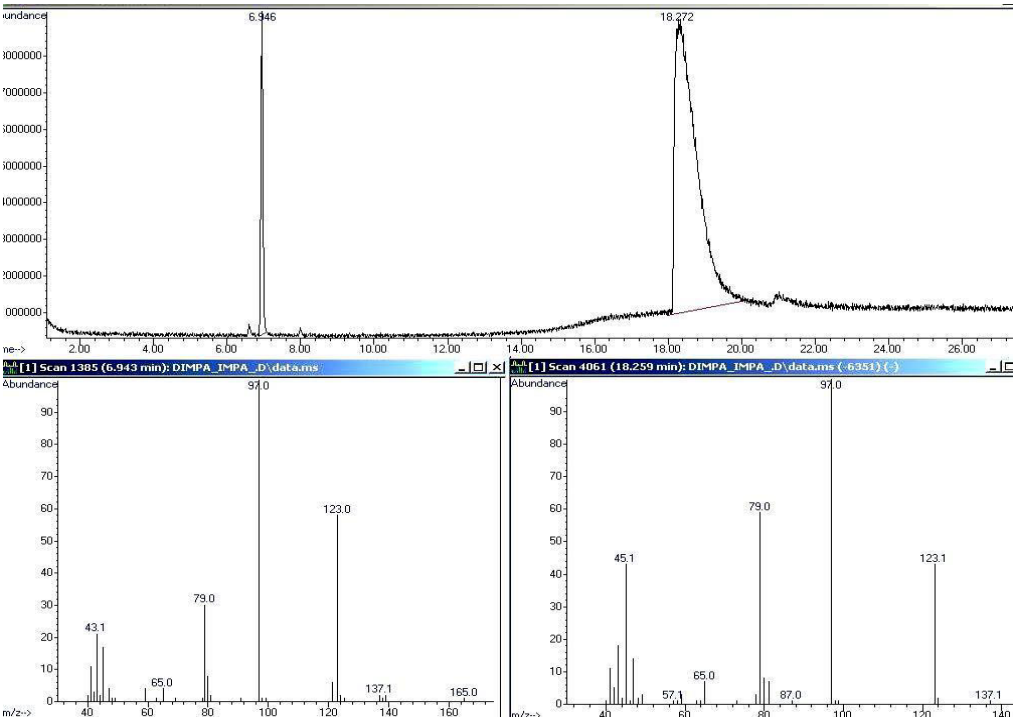


Figure 5. Direct GC-MS EI (70 eV) of IMPA/DIMP on CP FFAP CB column - chromatogram and mass spectra (temperature program as in Fig. 4).

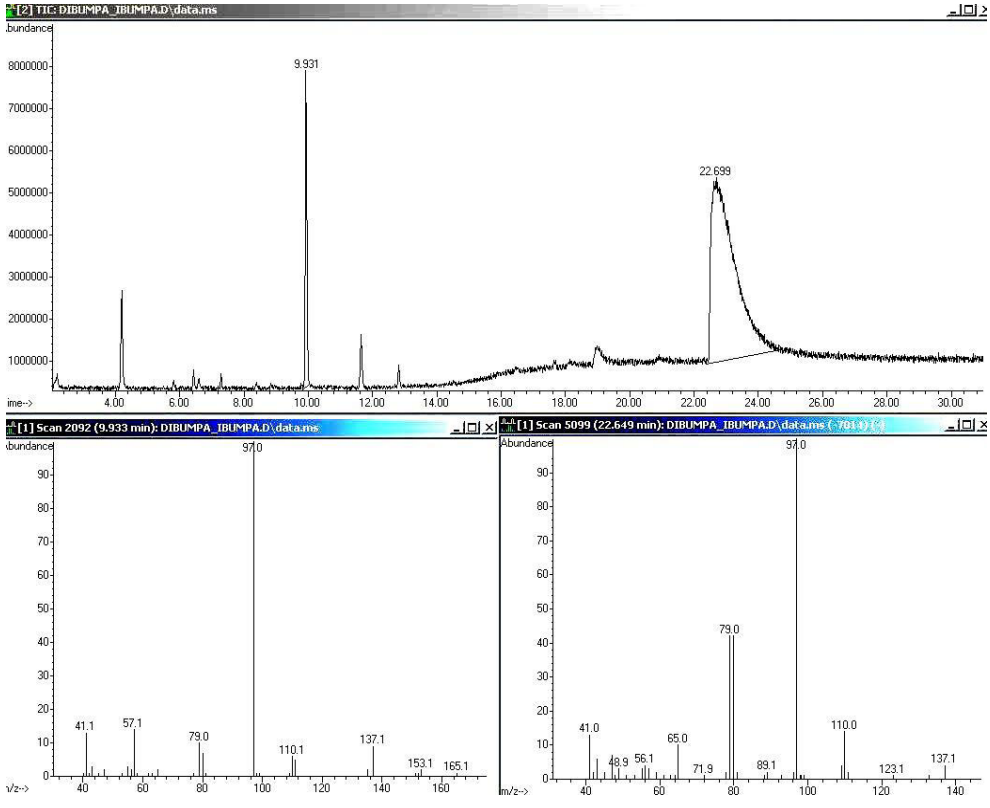


Figure 6. Direct GC-MS EI (70 eV) of IBUMPA/DIBUMP on CP FFAP CB column - chromatogram and mass spectra (temperature program as in Fig. 4).

Table 2. MS EI of methylphosphonic acid, its alkyl derivatives RO(HO)P(=O)CH_3 , and GC-MS EI of alkyl methyl methylphosphonates RO(CH_3O)P(=O)CH_3

Compound	Selected fragment ions of alkyl methylphosphonic acids *m/z* (relative intensity, %)	Selected fragment ions of alkyl methyl methylphosphonates *m/z* (relative intensity, %) R_t in GC-MS (min)
MPA*	$M^{+\cdot}$ 96 (55); 81 (100); 78 (34); 47 (14).	$M^{+\cdot}$ 124 (14);109 (41); 123 (3); 94 (100); 93 (31); 79 (99); 63 (18); 47 (23); R_t 5.9.
EMPA	$M^{+\cdot}$ 124 (3); $[M+H]^+$ 125 (24); 109 (31); 97(100); 81(50); 80 (59); 79 (98); 65 (35); 47 (25); 45 (15).	$M^{+\cdot}$ 138 (missing); $[M-H]^+$ 137 (2); 111 (100); 95 (27); 94 (22); 93 (67); 80 (15); 79 (38); 63 (10); 47 (10); R_t 7.1.
IMPA	$M^{+\cdot}$ 138 (<1); $[M+H]^+$ 139 (3); 123 (43); 97 (100); 79 (39); 47 (6); 45 (45).	$M^{+\cdot}$ 152(missing); $[M-H]^+$ 151 (2); 137 (42); 111 (100); 94 (11); 93 (96); 79 (19); 47 (8); R_t 7.7.
BUMPA	$M^{+\cdot}$ 152 (<1); $[M+H]^+$ 153 (6); 123 (15); 109 (14); 97 (100); 80 (14); 79 (68); 47 (7).	$M^{+\cdot}$ 166 (missing); $[M-H]^+$ 165 (<1); 137 (7); 123 (5); 111 (100); 93 (55); 79 (10); 47 (3); R_t 10.4.
IBUMPA	$M^{+\cdot}$ 152 (<1); $[M+H]^+$ 153 (12); 137 (5); 110 (19); 97 (100); 80 (29); 79 (22); 47 (3).	$M^{+\cdot}$ 166 (missing); $[M-H]^+$ 165 (<1); 151 (5); 124 (7); 111 (100); 94 (22); 93 (48); 79 (16); 47 (5); R_t 9.7.
PMPA	$M^{+\cdot}$ 180 (<1); $[M+H]^+$ 181 (4), 124 (66); 123(14); 97 (100); 80 (45); 47 (2); 45 (6).	$M^{+\cdot}$ 194 (missing); 179 (1); 138 (49); 137 (35); 111 (100); 94 (34); 93 (37); 84 (13); 79 (14); 69 (41); 47 (2); R_t 11.2 (diastereoisomer 1), 11.3 (diastereoisomer 2).
EHMPA	$M^{+\cdot}$ 208 (missing); MH^+ 209 (5); 179 (1); 151(12); 110 (37) 97 (100); 80 (21); 79 (20); 70 (20); 57 (14); 55 (21); 47 (2).	$M^{+\cdot}$ 222 (missing); 193 (<1); 165 (1); 124 (12); 111 (100); 94 (11); 93 (14); 70 (14); 55 (17); R_t 14.8.
CHMPA	$M^{+\cdot}$ 178(<1); $[M+H]^+$ 179 (2); 97 (100); 79 (13); 67 (8); 47 (2).	$M^{+\cdot}$ 19 (missing); $[M-H]^+$ 191 (<1); 111 (100); 93 (24); 79 (10); 67 (23); 54 (14); R_t 13.7 .

Preparative chromatography was earlier applied by Kataoka *et al.* [14] for IMPA purification.

The synthesized compounds were analyzed for their purity and identity by a number of methods, including MS-EI, FTIR, ^{1}H, ^{13}C, and ^{31}P NMR spectroscopy. Moreover, for identification, and – (indirectly) for their quantitative estimation, we derivatized them with (trimethylsilyl) diazomethane, affording the respective methyl alkyl methylphosphonates, accordingly to the method described previously in the papers of other authors, and then analyzed them by GC-MS [12,13]. In Table 2 there are presented the results we obtained, together with the results from a direct MS EI of the respective alkyl methylphosphonic acids (comparatively). Both types of compounds are characterized by the most characteristic fragment ions.

The spectral data of FTIR, MS EI and GC-MS EI for the majority of synthesized compounds, as well as for alkyl methyl methylphosphonates have already been described (for instance [8,10,12,13]). However, in our work we present reliable results, come out from the use of two alternative synthetic methods of alkyl methylphosphonic acids, confirmed by the wide spectrum of comparative and alternative analytical methods, in order to prove their identity, as well as to determine their purity. The breakdown of the conditions and the results obtained, for the two synthetic methods performed to obtain alkyl methylphoshonic acids, is presented in Table 3. We developed novel conditions for a direct GC FID quantitative determination of non-derivatized alkyl monoesters of MPA, with the use of a selective GC column, type CP-FFAP CB (Varian), deployed by the producer to analyze i.a. the polar compounds, such as free fatty acids. The estimated purities of our compounds (counted by internal normalization), and the retention times of them, are also given in Table 3.

GC chromatograms of every analyzed alkyl methylphosphonic acid, and a GC chromatogram of a mixture of them are shown in Figs. 2 and 3, respectively.

The limit of quantification (LOQ), defined as the lowest concentration of the analyzed compound taken

Table 3. The synthesis of alkyl methylphosphonic acids: a) – esterification of MPA catalyzed by PASA (following the procedures of [12-13]), b) – esterification of methylphosphonic dichloride (following the procedures of [9-11]), and their purities and retention times obtained in direct GC FID analysis on CP FFAP CB column.

Compound	Alcohol excess [mol]	Reaction time [h] *	Yield [%]	Retention time [min]	Purity [%]
EMPA	a) 2.0; b) 1.0	72	a) 78.2; b) 89.7	23.3	99.2
IMPA	a) 4.0; b) 1.0	76	a) 48.5; b) 85.1	20.9	98.1
BUMPA	a) 1.5	15	52.0	26.0	99.3
IBUMPA	a) 1.5	45	90.0	25.2	98.6
PMPA	b) 1.0	–	82.6	52.8	96.8
CHMPA	a) 1.5	40	92.8	52.1	99.1
EHMPA	a) 1.5; b) 1.0	72	a) 70.3; b) 82.0	51.3	99.2

**only for reaction a)*

Table 4. Direct GC-MS EI analysis of alkyl methylphosphonic acid/dialkyl methylphosphonate pairs on CP FFAP CB column (temperature program described in Experimental part).

Compound	Selected fragment ions m/z (relative intensity,%)	R_t [min]
EMPA	$M^{+\cdot}$ 124 (missing); $(M-H)^+$ 123 (2); $(M+H)^+$ 125 (missing); 109 (7); 97 (100); 79 (81); 65 (22); 47 (18); 45 (22); conformity with the data in the NIST* base: NIST#273563, ID#54530. Difference: MF 900, RMF 938.	19.9
DEMPA	$M^{+\cdot}$ 152 (2); 137 (4);125 (56); 108 (15); 97 (91); 79 (100); 65 (19); 47 (17);conformity with the data in the NIST base: NIST#133639, ID#38787. Difference: MF 960, RMF 976.	7.6
IMPA	$M^{+\cdot}$ 138 (missing); $(M-H)^+$ 137 (1); $(M+H)^+$ 139 (missing); 123(43); 97 (100); 79 (59); 47 (13); 45 (42); there is no MS data of IMPA in the NIST base.	18.3
DIMPA	$M^{+\cdot}$ 180 (missing); 165 (1); 139 (3); 123 (54); 97 (100); 79 (30); 65 (4); 47 (5); 45 (17); conformity with the data in the NIST base: NIST#226289, ID#12689. Difference: MF 953, RMF 953.	6.9
IBUMPA	$M^{+\cdot}$ 152 (missing); $(M-H)^+$ 151 (<1); $(M+H)^+$ 153 (missing); 137(4); 110 (14); 97 (100); 80 (42); 79 (44); 47 (8); there is no MS data of IBUMPA in the NIST base.	22.7
DIBUMPA	$M^{+\cdot}$ 208 (missing); 165 (1); 153 (3); 137 (7); 110 (6); 97 (100); 79 (10); 57 (16); 47 (2); conformity with the data in the NIST base: NIST#273173, ID#54221. Difference: MF 949, RMF 956.	9.9

** NIST Database (05,2006).*

for analysis (on column), was estimated, in the GC method defined above, for EMPA at 12 ng, for IMPA at 6 ng, for IBUMPA at 30 ng, and for PMPA at 28 ng level.

We applied CP FFAP CB column to perform the likely novel simultaneous analysis of the pairs of respective alkyl methylphosphonic acid and its diester. We described conditions and made analyses for three of such pairs, i.e., EMPA/DEMP, IMPA/DIMP and IBUMPA/DIBUMP, with the goal to present the possibilities given by this column. The connection of CP FFAP CB column (with the internal diameter of 0.53 mm) to Agilent GC-MS, created a necessity to increase the flow of an inert gas (helium) in the system from 1ml min^{-1}(usual helium flow speed, when the internal diameter of column is 0.25 mm, as for instance in typical DB-5MS) to

12 mL min^{-1}, what significantly increased the pressure in the MS ion source and in all the system. In Figs. 4, 5 and 6 there are depicted GC chromatograms and mass spectra obtained for these three pairs of alkyl/dialkyl MPA derivatives. In Table 4 we presented the selected fragment ions from MS EI and retention times from GC of the discussed compounds. On the basis of presented results we can confirm that the simultaneous and direct GC-MS identification of alkyl methylphosphonic acids and their dialkyl derivatives is possible.

In our search to find a credible, effective and convenient analytical method to determine the purity of alkyl methylphosphonic acids, we also made some attempts using the thin layer chromatography (TLC). The analyses were done on the alumina Merck plates covered with silica gel, with the use of different mobile phases: benzene-methanol-acetic acid (90:16:8, v/v/v), cyclohexane-chloroform-acetic acid (4:5:1,v/v/v), chloroform-acetic acid (12:1, v/v). The retardation factor (R_F) of analyzed alkyl methylphosphonic acids was rather low in such conditions, and they developed on plates in a shape of elongated spots. However, we could semi-quantitatively estimate the level of impurities, by comparison spots of the analyzed compound dropped, in acetone, on a TLC plate in a quantity of 10, 100, 500 and 1000 µg. The spots of examined substances were detected by spraying TLC plates with 1% silver nitrate solution in methanol, followed by 15 min heating under UV lamp. In most cases three impurities were detected. Only for EMPA and CHMPA could we observe two impurities. The determined quantities of impurities were about 1% in EMPA, IMPA and IBUMPA, 1.5% in PMPA, 2% in BUMPA and EHMPA, and 4% in CHMPA, what was in a quite good agreement with their purity calculated from a direct GC FID method.

4. Conclusions

We synthesized alkyl methylphosphonic acids, which are the degradation products of some organophosphorus chemical warfare agents. The chemical purity and identity of all of the acids were determined by different analytical methods (^{1}H, ^{13}C, ^{31}P NMR, FTIR, MS EI, GC-MS EI on DB-5MS column of their methyl derivatives, semi quantitative TLC, quantitative direct GC FID on CP-FFAP CB column). To the best of our knowledge, this was the first time non-derivatized alkyl methylphosphonic acids were analyzed using GC FID. Their purity in the above method was estimated by internal integration, and in the most of cases exceed 98%.

For some of the obtained compounds we successfully directly determined and characterized them in GC-MS EI on CP FFAP CB column, together with their respective dialkyl derivatives. Three pairs were analyzed in this way: EMPA/DEMP, IMPA/DIMP and IBUMPA/DIBUMP. The results provide a new possibility of a simultaneous and direct analysis of these two groups of MPA derivatives. The obtained substances can be used as reference materials in the studies on detection and identification of chemical contamination, and also in the routine examination of the specialized analytical equipment applied to such investigations.

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