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Journal of Chromatography A, 1043 (2004) 275–284
JOURNAL OF CHROMATOGRAPHY A
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Determination of pentafluorobenzyl derivatives of phosphonic and phosphonothioic acids by gas chromatography–mass spectrometry

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Received 4 September 2003; received in revised form 10 May 2004; accepted 14 May 2004

Abstract

A method based on gas chromatography–mass spectrometry (GC/MS) has been evaluated and standardized for the analysis of pentafluorobenzyl (PFB) derivatives of alkylphosphonic, *O*-alkyl alkylphosphonic and phosphonothioic acids. The pentafluorobenzyl (PFB) derivatives are much more stable as compared to the conventionally used trimethylsilyl derivatives. The conditions for the derivatization and analysis have been optimized to achieve the best detection limits in negative chemical ionization (NCI) mode.

Keywords: Derivatization, GC; Warfare agents; Phosphonic acid; Phosphonothioic acid

1. Introduction

The Chemical Weapons Convention (CWC) calls for the inspection and verification of various provisions of the Treaty, such as production, storage, destruction, etc., as well as of alleged use or alleged production of CW agents [1]. Military establishments and the chemical industries are subjected to systematic routine inspections under the CWC. There is also a possibility of unannounced challenge inspection in cases of alleged violation of the CWC provisions. On-site and off-site chemical analysis is a key issue in these procedures. The recent incidents of chemical and biological terrorism further illustrate the risk of widespread proliferation of these compounds [2]. These facts emphasize the need to develop better analytical methods for the detection and analysis of CW agents, which can be adapted to the requirements of the CWC, military and law enforcing agencies.

The CWC-related chemicals are listed in three Schedules, in accordance with their toxicity and potential use. Amongst the lethal CW agents, the nerve agents have had an entirely dominant role since the Second World War. Nerve agents acquired their name because they affect the transmission of nerve impulses in the nervous system. All nerve agents belong chemically to the group of organophosphorus compounds. Most of the organophosphorus warfare agents are unstable compounds in both environmental and physiological conditions and degrade to yield their corresponding *O*-alkyl alkylphosphonic acids and ultimately to the respective alkylphosphonic acids (Fig. 1). Organophosphorus compounds containing the C–P bond have only limited industrial use and hence, the detection of *O*-alkyl alkylphosphonic and alkylphosphonic acids provides a possible indication of the presence of nerve agents.

The detection of phosphonic acids in order to verify the CWC compliance is complicated. The difficulties encountered in detecting phosphonic acids in complex matrices like soil and biological matrices has been well documented [3–6]. These phosphonic acids are polar and non-amenable to gas chromatography–mass spectrometry (GC/MS) analysis due to low volatility and require derivatization prior to analysis. The most commonly used derivatization methods are methylation [7,8], benzylation [9] trimethylsilylation [10], *tert*–butyldimethylsilylation [11], or pentafluorobenzylation [4,12,13]. Diazomethane, which is the most common reagent for effecting methylation, involves carcinogenic and explosive reagents. Furthermore, methylation may be incomplete at least in case of *O*-alkyl methylphosphonic acids

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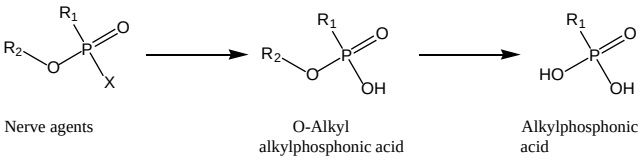
doi:10.1016/j.chroma.2004.05.038



R_1 = $C_1 – C_3$ alkyl groups
R_2 = $C_1 – C_{10}$ alkyl groups including cycloalkyl
X = -F, $-SCH_2CH_2N(R_1)_2$, CN

Fig. 1. General structure and hydrolysis pathway for the organophosphorus nerve agents.

[12]. Trimethylsilyl derivatives are unstable and sensitive to moisture.

These methylated and silylated derivatives can only be analysed in positive mode by GC/MS. The universal nature of EI is also a drawback, especially for the analysis of environmental contaminants, which occur in complex matrices. Ionization of the sample matrix together with trace levels of analytes of interest often makes analysis difficult by masking it unless extensive sample preparation is used. Negative ion chemical ionization (NICI) can be used to alleviate this problem, since it is applicable only to the electrophilic molecules. This makes the NICI more sensitive and selective for the determination of very small amounts of selected environmental contaminants.

Because of the foregoing limitations with derivatizing methods, we tried pentafluorobenzyl derivatives and found them to be the most suitable derivatives for analyzing these phosphonic acids in electron ionization (EI), positive ion chemical ionization (PICI) and our optimized NICI mode. The reported method for effecting pentafluorobenzylation [14] involved the use of sodium hydride and 18-Crown-6 at 45 °C for several hours to ensure complete conversion. At higher temperature this method yielded several degradation products. In a recent report, pentafluorobenzylation [15] has been carried out by using potassium carbonate. This report clearly stated that a low and variable recovery was noted for methylphosphonic acid, and no further investigation had been carried out by these authors. They had further stated that their method will have limited applications.

We report here a better method of pentafluorobenzylation of phosphonic acids and the optimization of pentafluorobenzylation of alkylphosphonic acids taking methylphosphonic acid as a model compound and application of this method for different *O*-alkyl alkylphosphonic acids and alkylphosphonic acids. We are also reporting here the NICI conditions optimized by us where we can achieve higher sensitivity with the pseudomolecular weight information, which was lacking in the earlier reported NICI methods [4,15]. We have also compared the hydrolytic stability of pentafluorobenzyl and trimethylsilyl derivative of *O*-alkyl methylphosphonic and methylphosphonic acids. We have also extended the work to the analysis of pentafluorobenzyl derivatives of phosphonothioic acids, the degradation products of highly toxic VX.

2. Experimental

2.1. Materials

Pentafluorobenzyl bromide (PFBBr) and *N*,*O*-bis(trimethylsilyl) trifluoroacetamide (BSTFA) were obtained from Lancaster. Tri-*n*-propylphosphate (TPP) and 18-crown-6 ether were obtained from Fluka. Methylphosphonic acid (MPA), ethylphosphonic acid (EPA), propylphosphonic acid (PPA), isopropylphosphonic acid (IPA), *n*-butylphosphonic acid (BPA), ethylthiophosphonic acid (ETPA), *n*-propylthiophosphonic acid (PTPA), *O*-methyl methylphosphonic acid (MMPA), *O*-ethyl methylphosphonic acid (EMPA), *O*-propyl methylphosphonic acid (PMPA), *O*-*i*-propyl methylphosphonic acid (i-PMPA), *O*-pinacolyl methylphosphonic acid (PinMPA), *O*-pentyl methylphosphonic acid (PenMPA), *O*-isoamyl methylphosphonic acid (i-AMPA), *O*-octyl methylphosphonic acid (OMPA) and *O*-methyl ethylphosphonic acid (MEPA) were synthesized and characterized by NMR and MS.

2.2. Instrumentation

Gas chromatography–mass spectrometry (GC/MS) analysis was performed on a Finnigan Mat TSQ 7000 mass spectrometer coupled to a Varian 3400 gas chromatograph. The GC was fitted with 30 m × 0.32 mm i.d. BPX-5 bonded phase column, film thickness 0.25 μm (SGE, Australia). The oven temperature was held initially at 80 °C for 2 min, programmed from 80 to 280 °C at 20 °C min^{-1}, held at 280 °C for 5 min. The injection port temperature and transfer line temperature were set at 250 and 280 °C, respectively.

2.3. Derivatization procedure

Generally, derivatization involved combining carefully measured quantities of the materials in the vials, heating for definite time at a definite temperature and allowing the reaction mixture to cool followed by concentration and analysis. Volumetric measurements were made by using Qualigens qualipette, derivatization reaction, and storage were carried out in screw cap glass vials with PTFE silicone septa (Supelco). Heating and temperature control were carried out in heating blocks. Derivatizations were carried out at normal conditions, and no extra precautions were taken. The derivatization did not include any extraction or clean-up procedures. The stability experiments involved storage at 0 and 40 °C with and without water.

3. Results and discussion

3.1. Derivatization reaction

The main advantages associated with the PFB derivatives of phosphonic acids over the other commonly used derivatives are their good chromatographic profile and that

the peaks in the RIC appear in the less occupied region of the chromatogram due to lower volatility and increased molecular weights. Our main aim was to optimize the reaction conditions for the pentafluorobenzylation of dibasic methylphosphonic acid, which is the final degradation product of all the organophosphorus nerve agents. The earlier investigators had observed low or variable recovery of pentafluorobenzyl derivative of this dibasic acid. Several parameters have been studied in order to optimize the reaction conditions for the pentafluorobenzylation of methylphosphonic acid. The reaction yields were optimized by using different bases at 70 °C. A series of aprotic organic solvents of different polarity were tried with the best base at 70 °C. To optimize the reaction condition and time, the reaction was carried out by taking the best solvent and the best base between temperature range of 50–110 °C. During the optimization of reaction temperature, any degradation or side product formation was also studied. While optimizing these derivatization parameters, the concentration of methylphosphonic acid was kept constant at 50 ng μL^{-1} and the peak area response of the derivatized product was used for the comparison purposes.

In pentafluorobenzylation of *O*-alkyl methylphosphonic acid, K_2CO_3 has been used as base and acetonitrile as solvent [15]. Here, again we tested different inorganic and organic bases like K_2CO_3, Na_2CO_3, triethylamine, diethylamine, di-*n*-propylamine, diisopropylamine and pyridine. For initial optimization, we had selected acetonitrile since higher recovery of pentafluorobenzylation for pinacolyl methylphosphonic had been reported [4]. We observed best yields with diisopropylamine as a base (Table 1). Furthermore, we compared the relative yields of pentafluorobenzylation of pinacolyl methylphosphonic acid between K_2CO_3 and diisopropylamine and found that the yield in case of K_2CO_3 was only 73% as compared to 100% in diisopropylamine.

Amongst the various aprotic solvents tested, the derivatization yields were greater when the polarity index [16] is over three, which is in accordance with the earlier report [4] on the pentafluorobenzylation of monobasic phosphonic acid (Table 2). Acetonitrile was found as the best solvent on the basis of derivatization yield. For all further evaluations and derivatization, acetonitrile was used as solvent.

Table 1
Derivatization yields using different bases

Sl. no.	Bases	Relative yields of methylphosphonic acid	Relative yields of pinacolyl methylphosphonic acid
1	K_2CO_3	3	73
2	Na_2CO_3	1	–
3	Triethylamine	0	–
4	Diethylamine	1	–
5	Diisopropylamine	100	100
6	Pyridine	1	–

Table 2
Derivatization yields in different solvents

Sl. no.	Solvents	Polarity index [16]	Relative yields of methylphosphonic acid
1	Hexane	0.1	0
2	CCl_4	1.6	0
3	Ethyl ether	2.8	5
4	CH_2Cl_2	3.1	30
5	Acetone	5.1	60
6	Acetonitrile	5.8	100

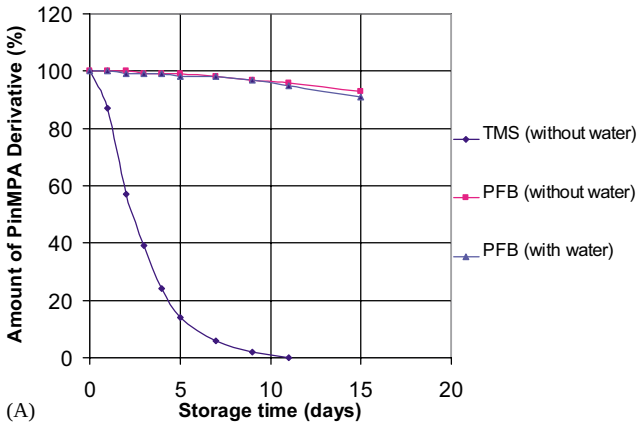
In order to optimize the reaction temperature of PFB derivatization, the reactions were carried out at 50, 60, 70, 90 and 110 °C overnight (∼17 h); the yields are summarized in Table 3. The quantities produced at 70 °C are taken as representing 100% conversion for the basis of comparison. The results are within the margin of analytical error, checked by placing simultaneously three sets of same experiment. It is evident from Table 3 that a maximum of 100% conversion has been achieved at 70 °C, while at 50 °C, the yields were poor. We observed that while increasing the reaction temperature, there was no increase in side products or any degradation of PFB derivative of both the phosphonic acids, whereas in other reported method of PFB derivatization [14], there was increase in the degradation product on increasing the reaction temperature. The PFB derivatization time can be reduced by using 18-crown-6 ether.

3.2. Stability of derivatives

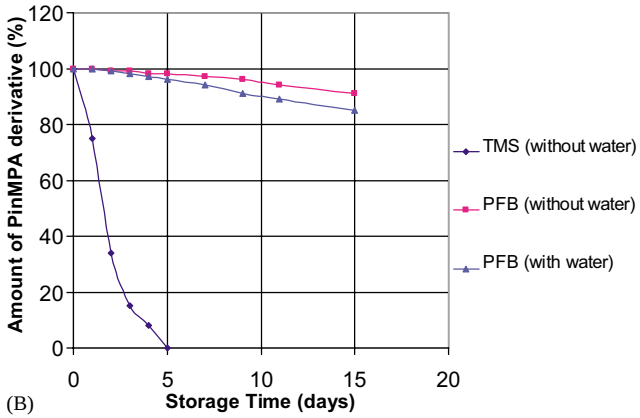
Mixtures of methylphosphonic acid (50 ng/μL) and pinacolyl methylphosphonic acid (50 ng/μL) were silylated and pentafluorobenzylated. The resulting solution of silyl derivative was stored in a refrigerator (∼4 °C) and at 40 °C in digitally controlled oven after the addition of an internal standard, TPP (25 ng/μL). Similarly, the PFB derivatives were also stored under the same environmental conditions with and without water. The GC/MS analyses were carried out over a period of 15 days, and the results are graphically summarized in Figs. 2 and 3. For checking the stability of PFB derivative further, the solution kept at 4 °C containing water

Table 3
Derivatization yields at different derivatizing temperatures

Sl. no.	Derivatizing temperature (°C)	Relative yields of methylphosphonic acid
1	50	30
2	60	80
3	70	100
4	90	99
5	110	102

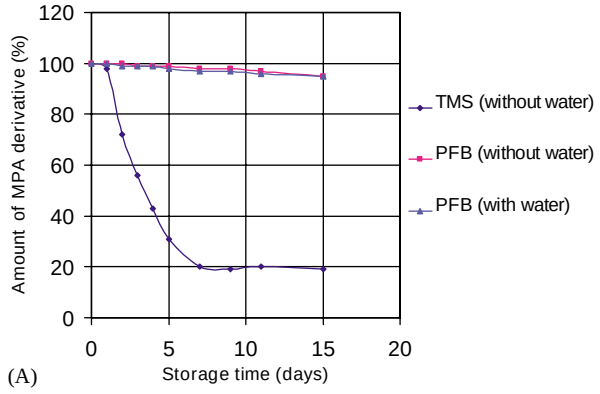


(A)

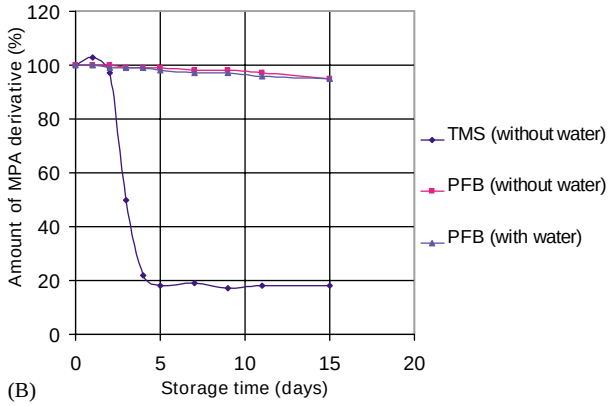


(B)

Fig. 2. Storage stability of PFB and TMS derivatives of pinacolyl methylphosphonic acid at: (A) 4 °C and (B) 40 °C.



(A)



(B)

Fig. 3. Storage stability of PFB and TMS derivatives of methylphosphonic acid at: (A) 4 °C and (B) 40 °C.

was analysed by GC/MS from time-to-time over a period of 6 months.

The behavior observed with the PFB derivative of PinMPA and MPA indicated the long-term stability in the presence of water whereas the silyl derivatives hydrolyse very fast even in traces of moisture. Over a storage period of 15 days at 4 and 40 °C with and without water, PFB derivatives of both the phosphonic acids have displayed less than 10% degradation (Figs. 2 and 3). The TMS derivative, on the other hand, degraded completely within 5–7 days. In Fig. 3B, an increase in the response for trimethylsilyl derivative of MPA is observed on first day, which might have been due to further completion of derivatization on keeping it at 40 °C. From Fig. 3A and B, it seems that trimethylsilyl derivative of MPA has not been completely degraded, but it is not true since this response is due to memory effect. It has been confirmed when a peak corresponding to trimethylsilyl derivative of MPA was observed even when BSTFA from new bottle had been injected. Thus, this amount can be taken as baseline, i.e. all the trimethylsilyl derivatives of MPA has been completely hydrolysed with in 5 days at 40 °C.

3.3. EI mass spectral analysis of pentafluorobenzylated phosphonic acids

In order to validate and check the applicability of this derivatizing method, we derivatized few other phosphonic acids to their corresponding pentafluorobenzyl derivatives. All these pentafluorobenzyl derivative of phosphonic acids showed a regular trend in both retention time and mass spectral data. The retention time of pentafluorobenzyl derivative of phosphonic acids increased with the increase in the molecular mass of the derivative within a class. The EI mass spectra of all the pentafluorobenzyl derivative of phosphonic acids except that of pinacolyl methylphosphonic acid showed significant molecular ion peaks and the base peak at *m/z* 181 corresponding to pentafluorobenzyl $[(C_6F_5CH_2)^+]$ group. The mass spectra of pentafluorobenzyl derivative of dibasic acids (both phosphonic and thiophosphonic acids) are shown in the Fig. 4(see also Table 4). The genesis for the formation of various fragment ions in PFB derivative of ethylphosphonic acid (Scheme 1) and ethylthiophosphonic acid (Scheme 2) are described as representative examples.

3.4. Negative ion chemical ionization (NICI)

We have optimized the NICI conditions, which enabled us to get higher sensitivity and pseudomolecular weight infor-

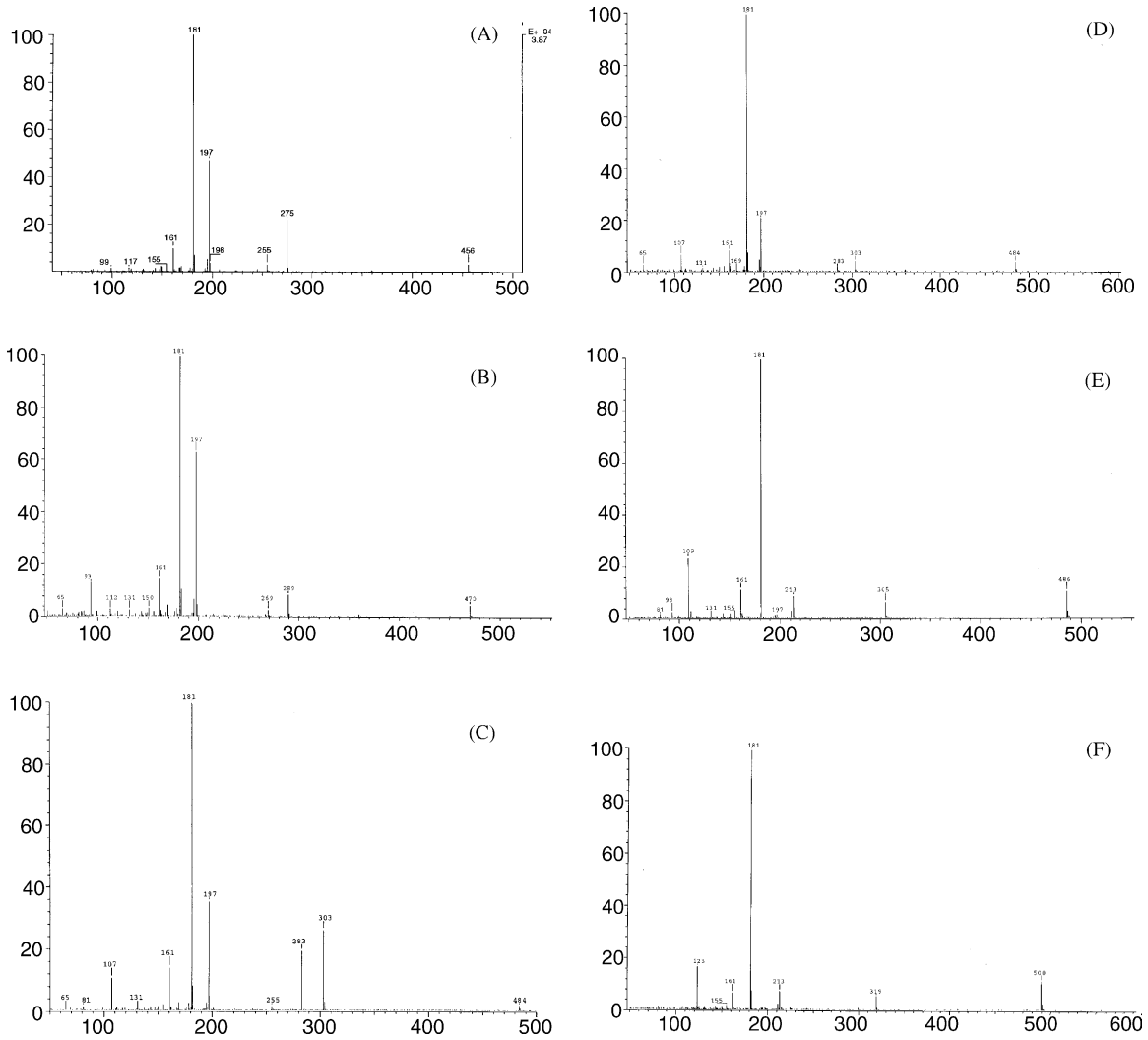


Fig. 4. Mass spectra of pentafluorobenzyl derivative of alkylphosphonic acids: (A) *O*,*O*-dipentafluorobenzyl methylphosphonate; (B) *O*,*O*-dipentafluorobenzyl ethylphosphonate; (C) *O*,*O*-dipentafluorobenzyl propylphosphonate; (D) *O*,*O*-dipentafluorobenzyl isopropylphosphonate; (E) *O*,*O*-dipentafluorobenzyl ethylthiophosphonate; (F) *O*,*O*-dipentafluorobenzyl isopropylthiophosphonate.

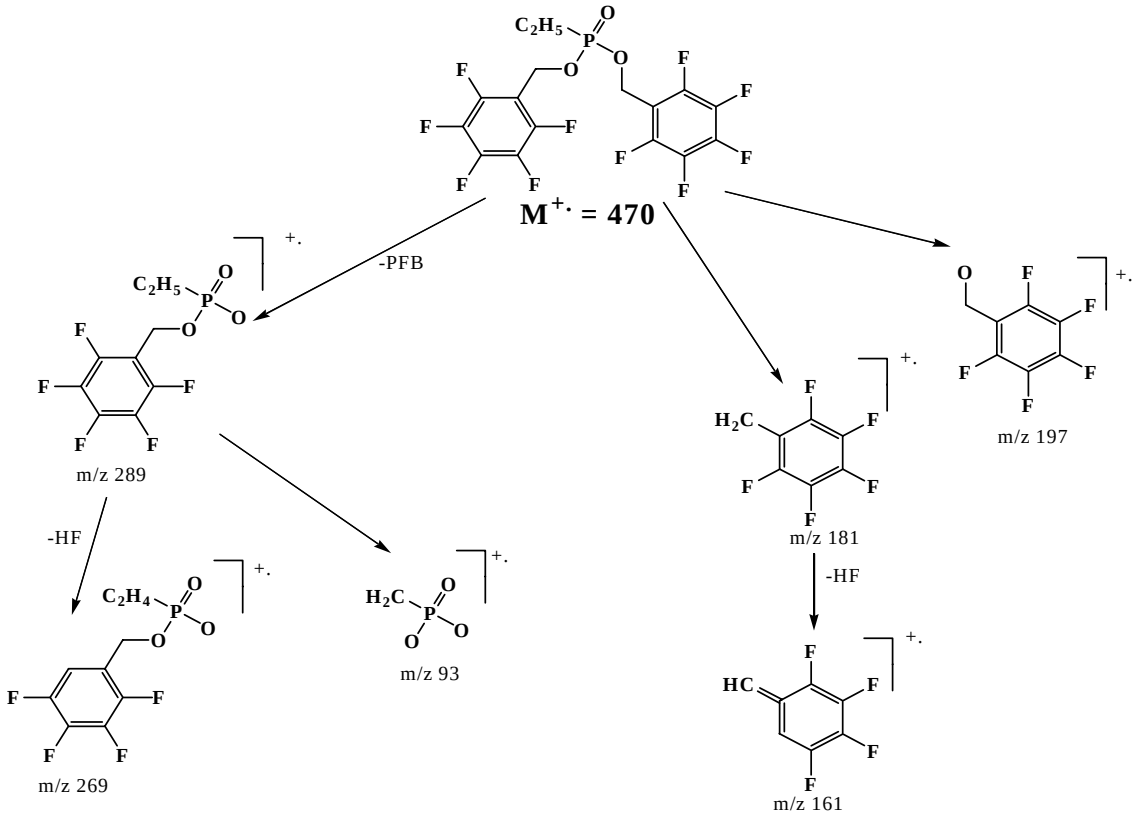
mation. The optimization was achieved by observing the signal intensity of 100 ppm pentafluorobenzylated methylphosphonic acid and simultaneously observing any adduct that can give molecular weight information. For this we had tried methane, ammonia and isobutane as moderating gases at different pressures and at different temperatures. The effect of ion source temperature on the response was very minimal when the temperature had been decreased from 250 to 180 °C, and furthermore, no change was observed even when it decreased from 180 to 100 °C. With moderating gas methane and ammonia at the same pressure, the signal intensities are comparable, slightly better with ammonia, but it was lower with isobutane, as shown in Table 5.

With methane as the moderating gas, when the ion source pressure reached 8200 m Torr, we observed a small peak at *m*/*z* 731 in the mass spectrum. The intensity of this peak increased further with increasing pressure, and at 8800 m Torr and 100 °C, the peak at *m*/*z* 731 became the base peak. This phenomenon of adduct formation was not observed with ammonia or isobutane.

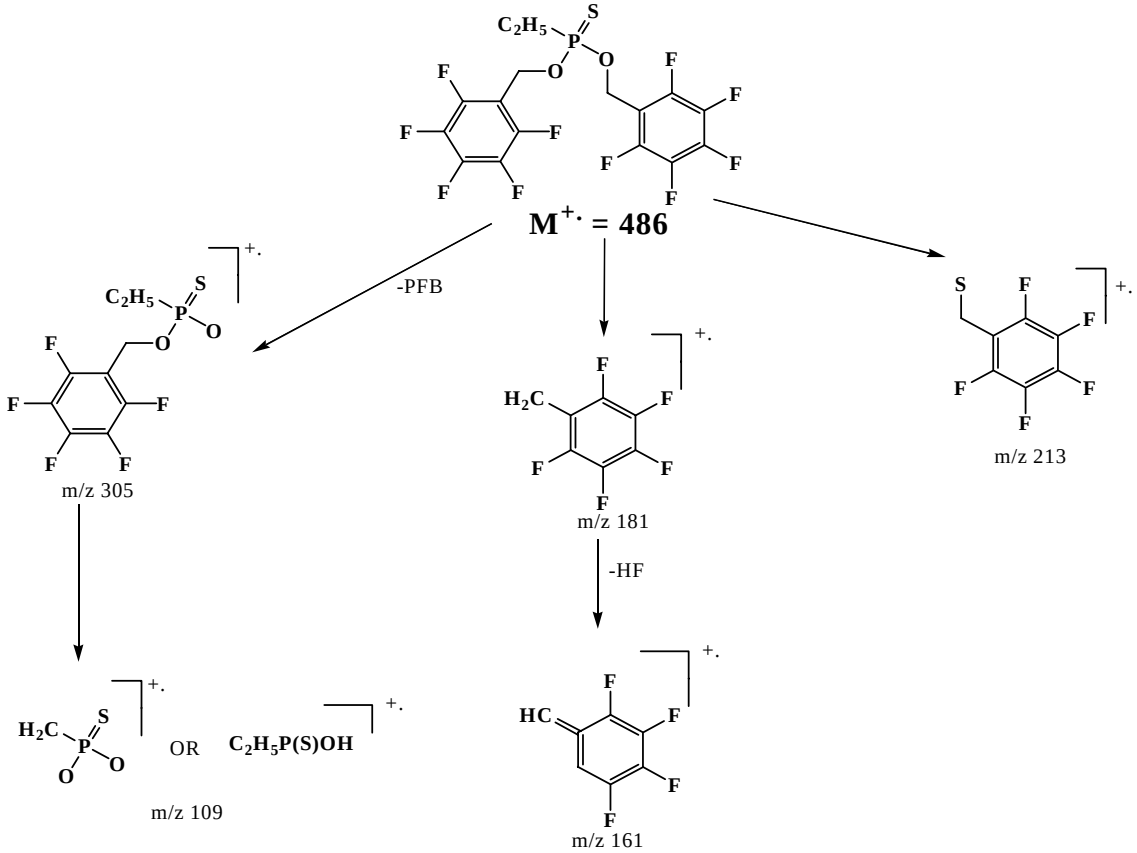
3.5. NICI mass spectrum

In NICI mode, we studied dibasic phosphonic acids, the mass spectrum of these dibasic phosphonic acids are shown in the Fig. 5. All these phosphonic acids were showing phosphonate anion $[M\text{-}181]^-$ and $[M + (M\text{-}181)]^-$. We observed that the adduct formation was concentration dependent, as adduct ion was constituting the base peak at 100 ppm, but on decreasing the concentration of phosphonic acid, the intensity decreased till 10 ppm. No adduct ions were observed below 10 ppm, only phosphonate anion was present.

Since the ultimate motive for developing this analytical technique is to use it in Official Proficiency Tests conducted by the OPCW, where the test compounds are spiked at 1 to 10 ppm levels and after extraction, concentration and deriva-



Scheme 1. Genesis of major fragment ions in PFB derivative of ethylphosphonic acid.



Scheme 2. Genesis of major fragment ion in PFB derivative of ethylthiophosphonic acid.

Table 4
Mass spectral data of pentafluorobenzyl derivatives of phosphonic acids

Sl. no	Compound	EI spectrum *m/z* (relative abundances)	CI spectrum $(M + H)^+$
1	Bis-(pentafluorobenzyl) methylphosphonate	456 (4), 360 (5), 275 (8), 255 (2), 197 (30), 181 (100), 161 (11), 155 (4), 150 (4), 130 (2), 131 (2), 99 (1), 79 (1)	457
2	Bis-(pentafluorobenzyl) ethylphosphonate	470 (5), 289 (9), 269 (3), 197 (62), 181 (100), 169 (5), 161 (14), 150 (4), 131 (4), 112 (4), 93 (13), 65 (3)	471
3	Bis-(pentafluorobenzyl) propylphosphonate	484 (2), 360 (3), 303 (26), 283 (20), 197 (36), 181 (100), 161 (14), 131 (4), 107 (11), 81 (2), 65 (4)	485
4	Bis-(pentafluorobenzyl) isopropylylphosphonate	484 (5), 303 (5), 283 (4), 197 (21), 181 (100), 169 (3), 161 (10), 131 (1), 107 (8), 65 (4)	485
5	Bis-(pentafluorobenzyl) butylphosphonate	498 (7), 317 (18), 197 (15), 181 (100), 161 (7), 121 (14), 86 (8), 84 (16), 51 (8)	499
6	Bis-(pentafluorobenzyl) ethylthiophosphonate	486 (10), 305 (7), 213 (9), 211 (3), 197 (1), 181 (100), 161 (12), 155 (3), 150 (2), 143 (2), 131 (4), 111 (3), 109 (24), 93 (3), 81 (2)	487
7	Bis-(pentafluorobenzyl) propylthiophosphonate	500 (11), 319 (6), 299 (1), 213 (8), 211 (2), 181 (100), 161 (6), 155 (2), 150 (1), 123 (18)	501
8	*O*-methyl *O*-pentafluorobenzyl methylphosphonate	290 (41), 194 (3), 181 (100), 161 (6), 109 (7), 94 (71), 79 (58)	291
9	*O*-ethyl *O*-pentafluorobenzyl methylphosphonate	304 (6), 276 (4), 256 (21), 197 (8), 181 (100), 161 (8), 123 (22), 108 (6), 93 (6), 80 (76)	305
10	*O*-propyl *O*-pentafluorobenzyl methylphosphonate	318 (6), 289 (1), 276 (21), 256 (25), 197 (2), 181 (100), 161 (4), 137 (3), 97 (4), 80 (43)	319
11	*O*-*i*-propyl *O*-pentafluorobenzyl methylphosphonate	318 (1), 303 (7), 276 (6), 256 (46), 197 (1), 181 (100), 161 (2), 137 (1), 97 (7), 80 (44), 79 (6), 65 (6), 55 (2)	319
12	*O*-pentyl *O*-pentafluorobenzyl methylphosphonate	346 (3), 277 (11), 276 (6), 256 (23), 195 (2), 181 (100), 161 (10), 131 (1), 97 (3), 80 (8), 69 (2), 65 (1), 55 (7)	347
13	*O*-*i*-amyl *O*-pentafluorobenzyl methylphosphonate	346 (2), 277 (13), 276 (9), 256 (22), 194 (3), 181 (100), 161 (11), 131 (4), 109 (3), 97 (7), 80 (19), 79 (8), 71 (6), 55 (20)	347
14	*O*-pinacolyl *O*-pentafluorobenzyl methylphosphonate	303 (8), 277 (10), 256 (55), 207 (5), 181 (100), 161 (34), 123 (40), 97 (7), 79 (8), 69 (20)	361
15	*O*-methyl *O*-pentafluorobenzyl ethylphosphonate	304 (14), 264 (5), 256 (5), 206 (3), 195 (8), 181 (100), 161 (8), 155 (2), 131 (4), 123 (4), 108 (20), 95 (8), 93 (8), 79 (20)	305

tization the concentration lies somewhere between 50 and 200 ppm; this method will provide valuable information with respect to the molecular weight of the derivatized acid and that too with higher sensitivity.

3.6. Limits of detection

The limit of detection (LOD) is an important parameter of any developed analytical technique. One of the major advantages of pentafluorobenzyl derivatization is the increase in the sensitivity than the other conventional derivatives. The LOD of MPA pentafluorobenzyl derivative had been determined in the different modes of mass spectrometry. The LOD of pentafluorobenzylated MPA in EI with full scan mode was found to be 1 ng, which is comparable to the LOD obtained for trimethylsilylated MPA. The LOD of pentafluorobenzylated MPA in EI under selected ion monitoring (SIM) mode was found to be 10 pg having signal to noise ratio of 10:1. There was, however, drastic increase in LOD with negative chemical ionization (NICI) where it had gone down to 100 ag with signal to noise ratio of 10:1 as shown in Fig. 5. The NICI-based method developed by us resulted in extremely high sensitivity (Fig. 6).

Table 5
Response (Peak Area) of 100 ppm MPA PFB derivative in different buffer gases at different pressure

Sl. no.	Pressure at Ion Sourcee (mTorr)	Buffer gas		
		Methane	Ammonia	Isobutane
1	1100	8.8×10^3	7.3×10^3	3.6×10^2
2	2200	8.1×10^6	8.4×10^6	1.4×10^6
3	4200	2.6×10^7	2.9×10^7	2.0×10^7
4	7200	4.9×10^7	5.5×10^7	3.5×10^7
5	8800	1.17×10^8	1.38×10^8	–

3.7. Application of developed analytical method

In order to see the practical applicability of the derivatizing technique discussed in this paper, we spiked methylphosphonic acid at 0.1 ppm to the water sample of 14th Official Proficiency Test, which contained lots of background but no convention-related chemical. In sample preparation, we had

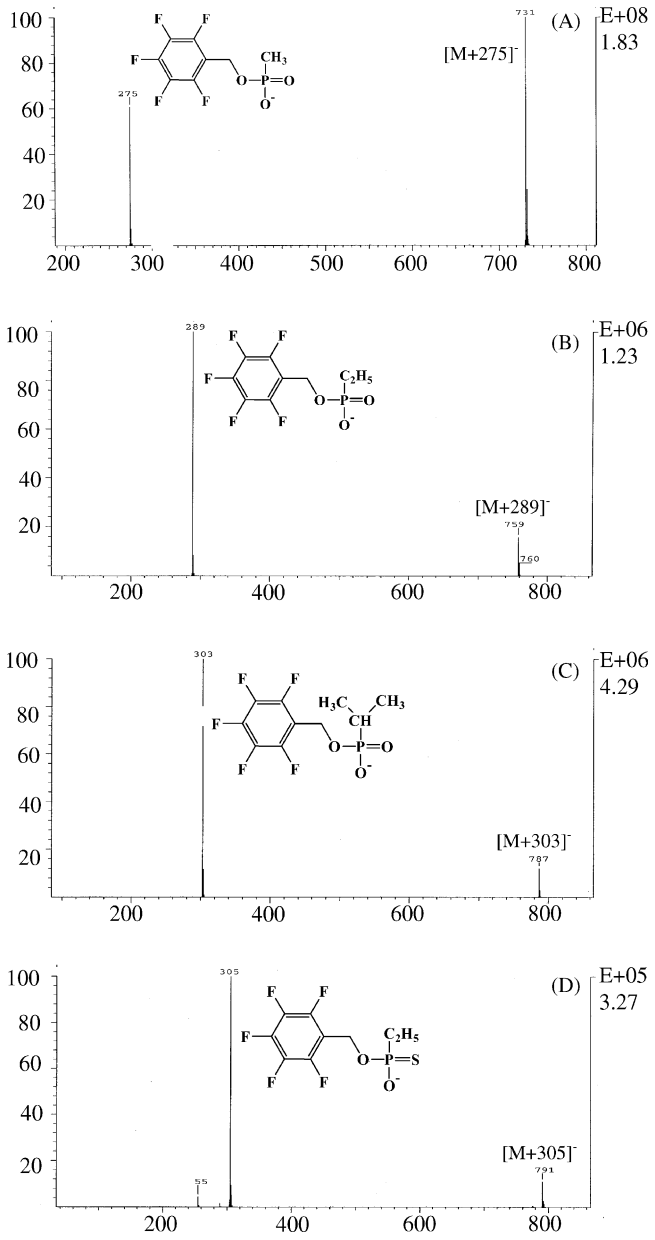


Fig. 5. NICI mass spectra of pentafluorobenzyl derivative of alkylphosphonic acids. (A) *O*,*O*-dipentafluorobenzyl methylphosphonate; (B) *O*,*O*-dipentafluorobenzyl ethylphosphonate; (C) *O*,*O*-dipentafluorobenzyl isopropylphosphonate; (D) *O*,*O*-dipentafluorobenzyl ethylthiophosphonate.

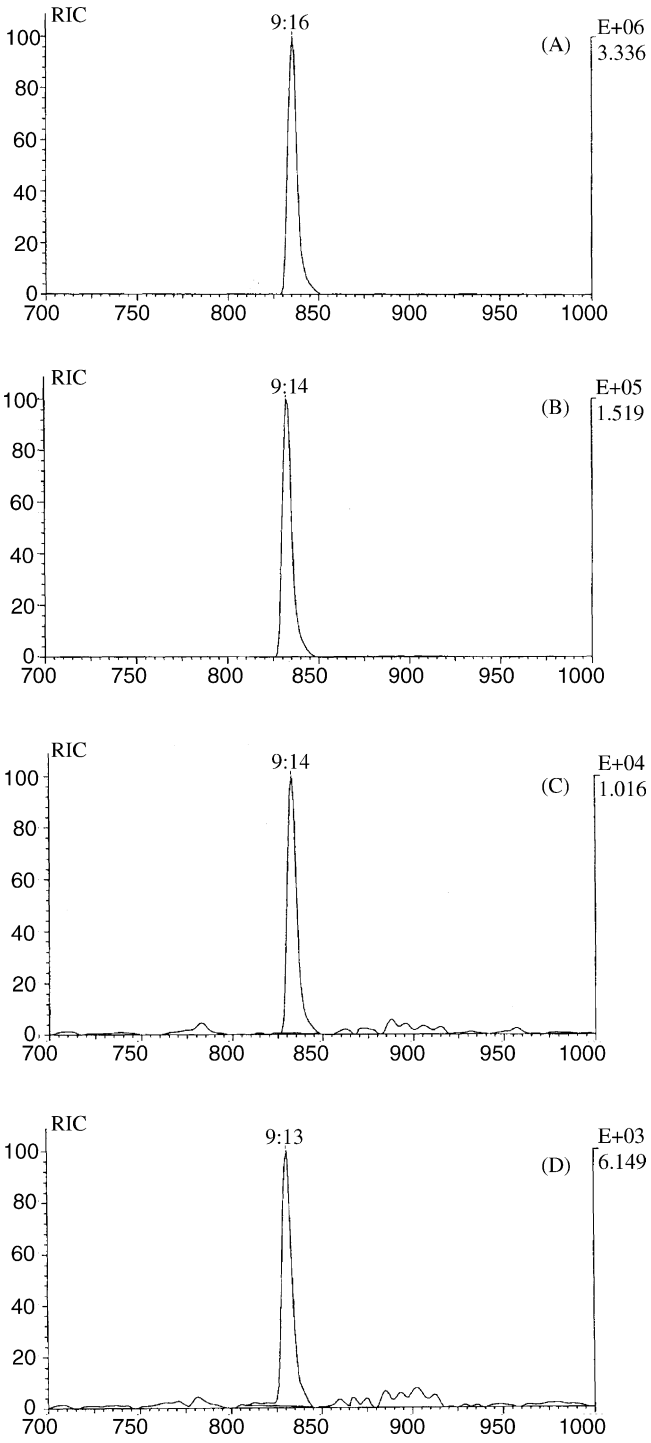


Fig. 6. Limit of detection of pentafluorobenzyl derivative of methylphosphonic acid. (A) 100 pg, (B) 1 pg, (C) 1 fg, (D) 100 ag.

just removed the water on rotavapour, and it was silylated as well as pentafluorobenzylated. Silylated and pentafluorobenzylated samples were analysed in EI mode on Agilents GC/MSD, and pentafluorobenzylated sample was analysed in NICI mode on TSQ 7000. The Fig. 7 clearly shows the detection of MPA PFB derivative in only NICI mode, whereas no signal of silylated MPA or pentafluorobenzylated MPA was observed even in the extracted ion chromatograms in EI mode. In EI, both the derivatives are showing lots of background whereas NICI TIC was quite clean. Hence, such type of background can mask the trace analyte, whereas in NICI due to selectivity cleaner, TICs were obtained, which helped in detecting analyte of our interest at trace level. This clearly shows the supremacy of the developed analytical method, which can be applied to the trace analysis of phosphonic acids in complex matrices.

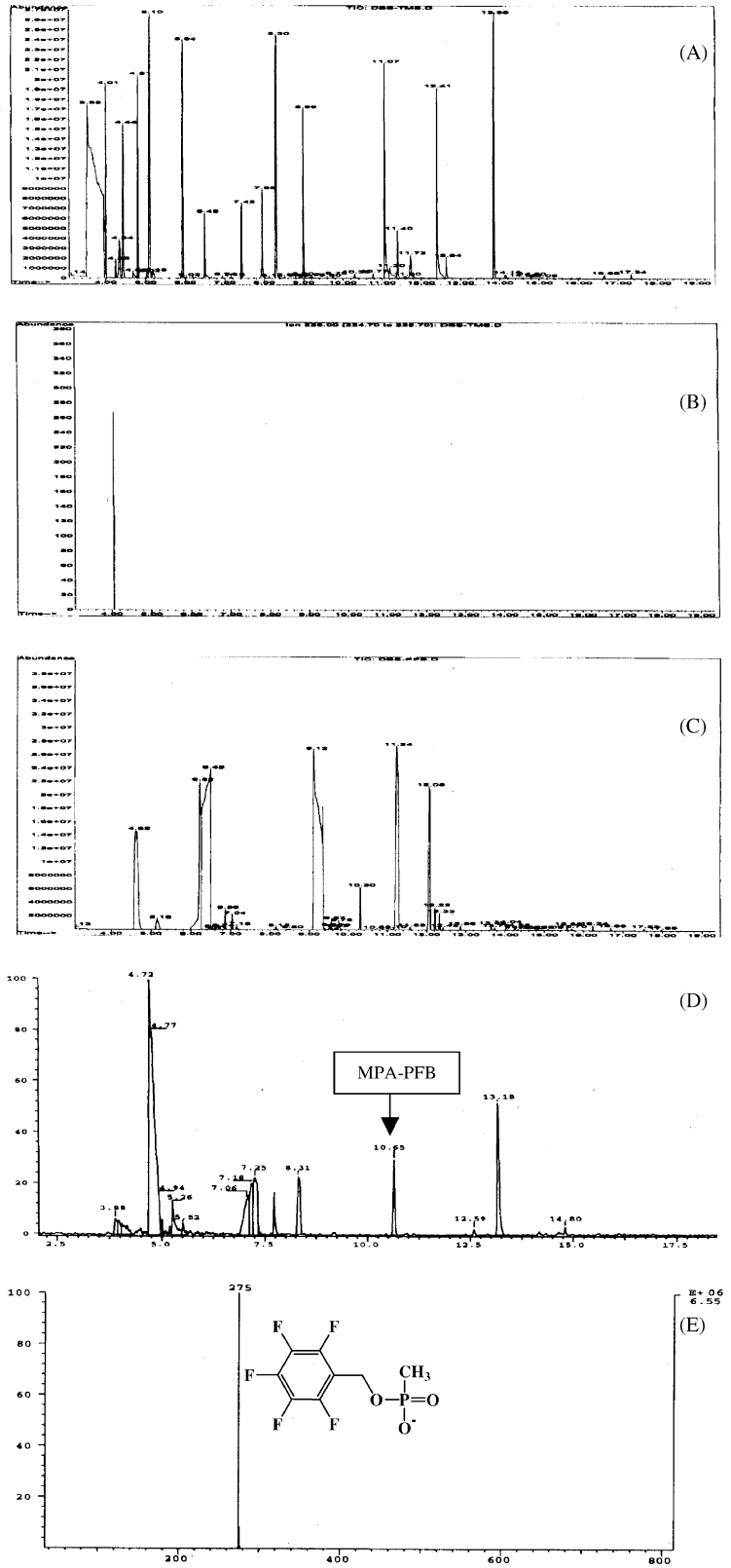


Fig. 7. Real world application of developed pentafluorobenzylation and NICI. (A) GC/MS EI Full TIC of water matrix spiked with 0.1 ppm MPA as its silyl derivative. (B) GC/MS EI extracted ion chromatogram (*m/z* 225) of water matrix spiked with 0.1 ppm MPA as its silyl derivative. (No peak observed for MPA–TMS.) (C) GC/MS EI Full TIC of water matrix spiked with 0.1 ppm MPA as its PFB derivative. (No peak observed for MPA–PFB.) (D) GC/MS NICI Full TIC of water matrix spiked with 0.1 ppm MPA as its PFB derivative. (E) NICI mass spectra of MPA–PFB derivative from water matrix spiked with 0.1 ppm MPA.

4. Conclusion

The pentafluorobenzyl (PFB) derivatives of alkylphosphonic, *O*-alkyl alkylphosphonic and phosphonothioic acids have been found to be quite useful markers for unequivocal verification of organophosphorus nerve agents because of their ease of preparation, high stability and greater sensitivity particularly in negative ion chemical ionization mass spectrometry.

Acknowledgements

The authors are thankful to Mr. K. Sekhar for providing necessary facilities and encouragement.

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