Journal of Chromatography A, 1218 (2011) 5393–5400

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Dispersive derivatization liquid–liquid extraction of degradation products/precursors of mustards and V-agents from aqueous samples

Meehir Palit*,[1], Gary Mallard[1]

Organisation for the Prohibition of Chemical Weapons (OPCW) Laboratory, Heulweg 28-30, 2288GN Rijswijk, The Netherlands

ARTICLE INFO

Article history:
Received 14 December 2010
Received in revised form 18 April 2011
Accepted 5 June 2011
Available online 12 June 2011

Keywords:
Sulfur mustard
Nitrogen mustards
V-agents
Amino alcohols
Derivatization
Heptafluorobutyrylation

ABSTRACT

A new derivatization and extraction technique termed as dispersive derivatization liquid–liquid extraction (DDLLE) speeds up the analysis process by removing the requirement for drying of the sample. The derivatization process takes place at the interface between the analyte containing aqueous phase and derivatization agent laden organic phase. The organic phase is highly dispersed using disperser solvent so that the total surface area is large. The derivatizing agent used is 1-(heptafluorobutyryl)imidazole and the resulting heptafluorobutyryl (HFB) derivatized analytes are partitioned into the organic phase. In addition to reduced sample preparation time, for some of the analytes, the HFB derivatives provide better spectral differentiation between isomers than conventional trimethylsilyl (TMS) derivatives. Method parameters for the DDLLE, such as extraction, and disperser solvent and their volume, type and amount of base, amount of heptafluorobutyrylimidazole and extraction time were optimized on diisopropylaminoethanol (DiPAE), ethyldiethanolamine (EDEA), triethanolamine (TEA) and thiodiglycol (TDG). The DDLLE was also used on various real world samples, which also includes few OPCW organized proficiency test and a spiked urine sample. The observed limit of detection (LOD) with 1 mL of sample for DDLLE in full scan with AMDIS was 10 ng/mL and with methane chemical ionization, multiple reaction monitoring (MRM) was 100 pg/mL, i.e., 100 fg on-column.

1. Introduction

The Chemical Weapons Convention (CWC) [1] covers not only the production of chemicals used as weapons, but also the production of a number of chemicals that are common precursors of the chemicals used in the weapons. These chemicals such as the ethanolamines. *N*-Ethyldiethanolamine (EDEA), *N*-methyldiethanolamine and triethanolamine (TEA), thiodiglycol (TDG) and some of the *N*,*N*-dialkylaminotethanols are both precursors to chemical weapons (mustards and V-agents) and common industrial chemicals. The verification of the proper use of such chemicals is an important part of inspections carried out by the Organisation for the Prohibition of Chemical Weapons (OPCW). In inspecting some of the sites that manufacture or use such chemicals, the OPCW undertakes to analyze for these chemicals at on-site using gas chromatograph–mass spectrometer (GC–MS) instruments that are transported to the site. For on-site analysis, GC–MS is operated in electron ionization (EI) mode and the data were analyzed by automated mass deconvolution and identification system (AMDIS) [2,3]. AMDIS searches these data against the very specific reference database, the OPCW central analytical database (OCAD).

The time frame in which these inspections can be carried out is limited and thus methods for speeding up the analysis are critical to these inspections. The analytes noted above are especially difficult when they are in aqueous matrices, which is common in industrial settings. These compounds cannot readily be analyzed by GC–MS due to their polarity and nonvolatility. They are typically derivatized prior to their analysis. Black and Muir [4] have reviewed derivatization reactions of chemical warfare agents (CWAs) and their degradation products. These reactions include methylation, trimethylsilylation, tert-butyldimethylsilylation, pentafluorobenzylation and pentafluorobenzylation. Most such derivatization methods require that the water to be evaporated prior to the reaction [4–7], which is typically slow. In contrast to conventional sample preparation methods [4,8–18], derivatization that do not require the evaporation of the water can substantially increase the number of samples that can be analyzed in the inspection period.

The removal of the water is essential because the derivatizing agents typically react faster with water than with the analyte. If the derivatizing agent is sufficient hydrophobic it will be retained in an organic phase and it will be protected from the hydrolysis. On partition of analytes from aqueous phase to organic phase, analytes will react with the derivatizing agent and will get derivatized. The

* Corresponding author.
E-mail address: meehirpalit@rediffmail.com (M. Palit).
[1] The views and recommendations are those of authors and do not represent official OPCW policy.

0021-9673/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2011.06.008

use of a highly dispersed derivatizing agent loaded organic phase could provide a high surface area to increase contact between the analyte and the derivatizing agent. To accomplish this, it is essential that the derivatizing agent and derivatives should be somewhat stable to hydrolysis. Dispersing solvent is needed to be used to allow the dispersion of organic phase in the aqueous samples.

Here we explore a new method—dispersive derivatization liquid–liquid extraction (DDLLE), where derivatization and extraction is accomplished in a single step with the dispersion of derivatizing reagent in the organic solvent that is immiscible with water using a dispersing solvent added to the aqueous samples. In this study, for the analysis of CWC related alcohols derivatization by heptafluorobutyrylation has been used. To our best of knowledge, this is the first report of heptafluorobutyrylation of these alcohols directly in the water. The parameters associated with the DDLLE were optimized and it was applied for some real world samples including spiked urine sample.

2. Experimental

2.1. Materials

The model analytes used for this study are diisopropylaminoethanol (DiPAE), ethyldiethanolamine (EDEA), triethanolamine (TEA) and thiodiglycol (TDG) were procured from Aldrich (Germany) with purity higher than 95%. The analytical or HPLC grade solvents dichloromethane (DCM), trichloroethylene (TCE), cyclopentyl methylether (CPME), trifluorotoluene (TFT), sodium carbonate, sodium hydroxide and methyl-tert-butyl ether (MTBE) were from Sigma–Aldrich, USA. 1-(Heptafluorobutyryl)imidazole (HFBI) was procured from Sigma, USA and acetonitrile (ACN) was procured from Merck, Germany. Acetone, chloroform, carbon tetrachloride (CCl_4) and ethyl acetate (EA) were from J.T. Baker, Deventer, Holland. Hexachlorobenzene (HCB), trifluoroacetylimidazole, tetrahydrofuran, heptane, toluene, triethylamine, and pyridine were from Aldrich, Germany. Dimethyl formamide (DMF), pentafluoropropionylimidazole and hexane were from Fluka, USA. The MilliQ water (18 MΩ cm) was used for preparation of aqueous solution for the optimization of DDLLE. The stock solution of agents was prepared in acetonitrile and stored at 4 °C, and these stock solutions were used for spiking various water samples.

2.2. GC–MS analysis

The GC–MS analyses were performed in electron ionization (EI) at 70 eV in full scan (40–800 amu) with an Agilent 6890 GC equipped with a model 5973 mass selective detector (Agilent Technologies, USA). The capillary column was Rxi-5MS (Restek, USA) 30 m length × 0.25 mm internal diameter × 0.25 μm film thickness used with temperature program of 40 °C (2 min)–10 °C/min–280 °C (5 min). Helium was used as a carrier gas with a constant flow rate of 0.9 mL/min. The samples were analyzed in splitless mode at injection temperature of 250 °C, transfer line temperature of 280 °C, EI source temperature was 230 °C and quadrupole analyzer at 150 °C. In this study for optimization, normalized peak area was used; normalized peak area is the ratio of peak area of analyte with the peak area of internal standard [hexachlorobenzene (HCB)] obtained from AMDIS.

The GC–MS/MS analyses were performed in EI (70 eV) or chemical ionization (CI) at 240 eV with an Agilent 7890 GC equipped with Agilent 7693 autosampler and Agilent 7000 triple quadrupole mass spectrometer (Agilent Technologies, USA). The capillary column was HP-5MS (Agilent, USA) with 30 m length × 0.25 mm internal diameter × 0.25 μm film thickness was used at temperature program of 40 °C (2 min)–10 °C/min–280 °C (5 min). Helium was used as a carrier gas with a constant flow rate of 1.0 mL/min. The samples were analyzed in splitless mode at injection temperature of 250 °C, transfer line temperature of 280 °C. With EI, ion source temperature was 230 °C and with CI ion source temperature was 250 °C, quadrupole analyzer temperature was set at 150 °C. For CI, methane was used as a reagent gas. For MS/MS, helium was used as quenching gas and nitrogen was used in the collision cell.

2.3. Dispersive derivatization liquid–liquid extraction procedure for optimization

A 3.0 mL aliquot of MilliQ water was placed in a 4 mL screw cap glass vial. DiPAE, EDEA, TDG and TEA were spiked in the sample at a level of 40 μg/mL for initial screening and 10 μg/mL for final optimization. This spiked sample was split into three samples of 1.0 mL each for triplicate analysis of each parameter. For all samples except where the effect of the base was investigated, 120 μL of 2.4 M sodium carbonate was added. Subsequently 1.0 mL of disperser solvent was added in each vial. For derivatization and extraction of each vial, 1.0 mL of extraction solvent containing 10 μL of HFBI was added into each sample in five aliquots of 0.2 mL and shaken for few seconds after each addition. An emulsion was formed in the vial. Finally, the sample was centrifuged for phase separation. The organic layer was removed and evaporated to almost dryness with gentle nitrogen flow and the sample reconstituted with heptane containing hexachlorobenzene (HCB) as internal standard (8 μg/mL) and 2 μL of HFBI (to derivatize underivatized alcohols extracted into the organic layer and any hydrolyzed esters. This heptane layer was analyzed by GC–MS in triplicate.

2.4. Dispersive derivatization liquid–liquid extraction procedure for practical applications

In 1.0 mL of sample, 120 μL of 2.4 M sodium carbonate and 100 μL of acetonitrile was added in the vial. For DDLLE 1.0 mL of DCM with 50 μL of HFBI was added into the sample in five aliquot's with shaking the mixture for dispersing the DCM layer. At each step, an emulsion was formed that was stable until the next addition of DCM. After the final addition of the DCM mixture, the sample was centrifuged for phase separation and the organic layer was removed and evaporated to near dryness by nitrogen purging followed by the addition of heptane (250 μL) containing hexachlorobenzene (HCB) as internal standard (8 μg/mL) and 2 μL of HFBI. The heptane solution was analyzed by GC–MS.

3. Results and discussion

To our best of knowledge, the mass spectral data for the heptafluorobutyryl (HFB) derivatives for all these compounds have not been reported. Hence, all the HFB derivatives were synthesized and their retention indices (RI) and mass spectral data were measured prior to the method development. As Garg et al. [7] had reported enhanced detectability of amino alcohols on heptafluorobutyrylation with Fourier transform infrared spectroscopy, similarly we had also observed the advantage of using heptafluorobutyrylation with respect to mass spectrometry, which can reduce the false positive identification (Fig. S-1; provided in supplementary material). The electron ionization (EI) mass spectra of diethylaminoethanol, methylpropylaminoethanol and isopropylmethylaminoethanol as their trimethylsilyl (TMS) and HFB derivatives were shown in Fig. S-1 (provided in supplementary material). The spectra of TMS derivatives were very similar with only minor differences in intensities which are not sufficient for unambiguous identification in trace analysis of complex matrices. In contrast, the spectra of HFB

derivatives are significantly different and easy to distinguish even during trace analysis of complex matrices.

The fragmentation mechanism of these HFB derivatives is examined since methods that are even more sensitive can be developed using methods based on multiple reactions monitoring (MRM). The MRM based highly selective and sensitive method can be used for low concentrations in very complex matrices; such biomedical samples. The probable mass spectral fragmentation mechanism for representatives of each type of chemicals were worked out and shown in Figs. S-2 to S-4 (provided in supplementary material).

3.1. Probable mass spectral fragmentation mechanism

Electron ionization mass spectra of HFB derivatives of scheduled model alcohols (Fig. S-5; provided in supplementary material) and mass spectral data of HFB derivatives of 22 CWC related alcohols (Table S-1; provided in supplementary material) are provided as supplements. The geneses for the formation of various fragment ions are typically illustrated in Figs. S-2 to S-4 (provided in supplementary material) based on product and precursor ion analysis. The fragmentation mode is illustrated by taking three examples of each categories namely dialkylaminoethanol with one hydroxyl functionality and triethanolamine having more than one hydroxyl functionality, and thiodiglycol. By same analogy the mass spectra of all other compounds can also be explained. Straight forward fragmentation routes can justify formation of most of the ions.

Molecular ion was observed in all the HFB derivatives of dialkylaminoethanols. The ions of m/z value 69 ($[CF_3]^+$), 169 ($[C_3F_7]^+$) and 241 ($[C_3F_7COOC_2H_4]^+$) were present in all the derivatives with medium to high relative abundances. Loss of heptafluorobutyryl group from molecular ion itself $[M-213]^+$ was characteristic fragmentation observed in all the derivatives. The characteristic (beta-cleavage from molecular ion produces either $[M-CH_3]^+$ or $[M-C_2H_5]^+$ preferably in accordance with the alkyl group attached to the nitrogen or $[M-C_3F_7OCOCH_2]^+$ ion with 100% relative abundance.

The molecular ion for the HFB derivatives of aminoalcohols with more than one hydroxyl functionality is always very weak. The ions of m/z value 69 ($[CF_3]^+$), 169 ($[C_3F_7]^+$) and 241 ($[C_3F_7COOC_2H_4]^+$) were present in all the derivatives with medium to high relative abundances. Loss of heptafluorobutyryl group from molecular ion itself $[M-213]^+$ was characteristic fragmentation observed in all the derivatives. The characteristic (beta-cleavage from molecular ion produced $[M-C_3F_7OCOCH_2]^+$ ion with 100% relative abundance.

Molecular ion for the HFB derivatives of TDG is very weak and the pseudo-molecular ion is only observed in chemical ionization (CI) using isobutane as reagent gas. The ions of m/z value 69 ($[CF_3]^+$), 169 ($[C_3F_7]^+$) and 241 ($[C_3F_7COOC_2H_4]^+$) were present in significantly higher abundances, whereas m/z 241 is the base peak. Loss of heptafluorobutyric acid and heptafluorobutyryl group from molecular ion $[M-214]^+$ and $[M-213]^+$ were the characteristic fragmentations observed.

3.2. Optimization of DDLLE

To optimize the method a number of different derivatizing agents, base, extraction solvents, and disperser solvents were examined. In addition for the optimal case, the amount of derivatizing agent, disperser solvent, base and extraction time were optimized. The optimization was done in two stages, the first stage of optimization (screening) was carried out with the analyte concentration of 40 μg/mL and in second stage for final selection was carried out at the concentration of 10 μg/mL. All the data presented in this study are from the analyte concentration of 10 μg/mL unless otherwise stated. Four scheduled alcohols: diisopropylaminoethanol (DiPAE), ethyldiethanolamine (EDEA), thiodiglycol (TDG) & triethanolamine (TEA) were used for optimization (details of its selection are given as scheme S-1 in supplementary material).

3.2.1. Selection of derivatizing agents

Since the analytes are alcohols, derivatization reactions involving esterification were selected. In all the reports, esterification can be achieved either with acidic anhydride or with the corresponding imidazole in non-aqueous media. On using imidazole form produces the corresponding derivatives with significantly cleaner chromatogram and there is no need to use an acid scavenger during derivatization.

The initial study of derivatization in aqueous medium showed that with heptafluorobutyric anhydride only DiPAE was derivatized whereas with heptfluorobutyrylimidazole (HFBI) all the analytes were derivatized except TEA. Using an inorganic base, TEA could also be derivatized. Heptfluorobutyrylimidazole was thus adopted for further study.

3.2.2. Selection of extraction solvent

Organic solvents were selected based on their immiscibility with the water and extraction capability of interested compounds as well as their gas chromatographic behavior. The preliminary screening of extraction solvents included chloroform, CCl_4, CPME, DCM, EA, heptane, hexane, MTBE, toluene, TCE and TFT. A series of samples were studied (in triplicate) by using 1 mL of each extraction solvent containing 10 μL of HFBI with 1 mL of acetone (as disperser solvent). After initial screening of organic solvents using averaged (triplicate) normalized peak area, the most promising extraction solvents were screened with all the most promising disperser solvent to select best combination of extraction and disperser solvent (Fig. S-6; provided in supplementary material).

3.2.3. Selection of disperser solvent

Miscibility of disperser solvent in organic phase (extraction solvent) and aqueous phase is the main point for selection of disperser solvent. The disperser solvent is responsible for the proper dispersion of extraction solvent into the aqueous sample and providing adequate stability and size to the organic droplets. Acetone, ACN, DMF and THF were selected for this purpose. A series of samples were studied in triplicate by using 1 mL of each disperser solvent with 1 mL of DCM (as extraction solvent) containing 10 μL of HFBI. Since DMF had shown poor response with all the analytes, it was not used for the final selection. In the final selection the remaining three disperser solvents were screened against the best five extraction solvents (Fig. S-6; provided in supplementary material). The best combination is ACN as disperser solvent and DCM as extraction solvent.

3.2.4. Optimization of base

As noted above, the use of an inorganic base was essential to detect TEA as its HFB derivative. At high pH, the aminoalcohols in their unionized form (free base) and thus more soluble in the organic phase. However, at higher pH, created by the use of strong bases such as KOH or NaOH hydrolysis of the HFBI and the final product (esters) occurred. A number of bases were screened including both organic amines and weaker inorganic bases. The best results were obtained with potassium and sodium carbonate and bicarbonate. The sodium carbonate was selected because of its availability as primary standard. Its amount was optimized by varying the concentration from 0.007 to 0.7 M in the initial set of experiments with the higher concentration of analytes and in second set where the analyte concentration were 10 μg/mL, it was varied between 0.09 and 0.5 M (Fig. S-7; provided in supplementary material) keeping other variables constant. The maximum

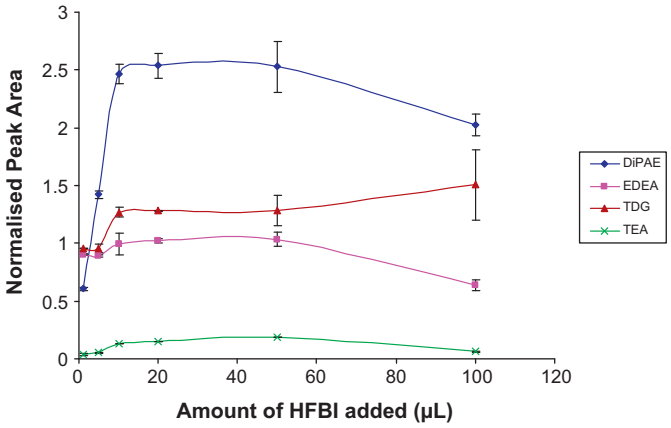

Fig. 1. Optimization of amount of heptafluorobutyrylimidazole (HFBI) required for HFB-DDLLE keeping other experimental conditions constant. Here the amount of HFBI in the DCM was varied from 1 to 100 µL.

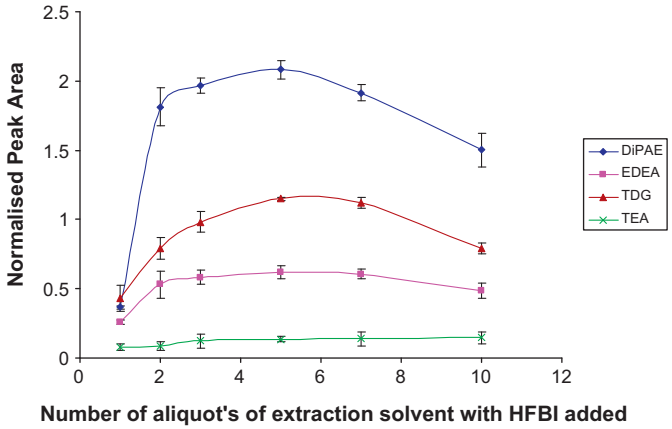

Fig. 2. Optimization of number of addition of fractions of DCM with HFBI. Here 1 mL of the DCM with 50 µL of HFBI was added once, in two, three, five, seven and ten fractions.

recoveries were obtained at 0.3 M and beyond this, decreases in the recoveries were observed.

3.2.5. Effect of DDLLE time

DDLLE time could be one of the important factors as in this extraction procedures derivatization is also included. DDLLE time is defined as an interval time between final addition of mixture of extraction solvent (DCM) containing derivatization agent (HFBI), and before starting to centrifuge. The time was varied as 0, 5, 15 and 30 min keeping other experimental conditions constant (see Fig. S-8; provided in supplementary material). The effect of the extraction time was minimal, which revealed that the derivatization and extraction is almost instantaneous. In addition to this, the effect of heating and of sonication was also studied—both reduced the total signal recovered (see Fig. S-8 in supplementary material).

3.2.6. Effect of amount of HFBI

In DDLLE, the extraction efficiency is directly related to the degree of derivatization and hence, the recoveries by DDLLE can be affected by the amount the derivatizing agent, here heptafluorobutyrylimidazole (HFBI) is used as derivatizing agent. The effective amount of HFBI for DDLLE was studied by varying the amount of HFBI (1 µL, 5 µL, 10 µL, 20 µL, 50 µL and 100 µL) in 1 mL of DCM and the data were plotted in Fig. 1. From Fig. 1, it is quite evident that with the increase of HFBI, there was increase in the efficiency of DDLLE and finally it reaches a plateau from 10 µL to 50 µL of HFBI. A standard of 50 µL of HFBI in 1 mL of DCM was used in later studies.

3.2.7. Optimization of number of addition of aliquots of extraction solvent with HFBI

In the initial stage of this study all of the extraction solvent with HFBI was added in a single step resulting in only DiPAE being detected as an HFB derivative. However, when the extraction solvent and HFBI were added in multiple steps, all the analytes were detected. In this step of optimization, the total volume of extraction solvent was kept constant at 1 mL but the solvent was added in multiple aliquots; for example, 0.2 mL was added 5 times and for 10 times 0.1 mL was used for addition. Fig. 2 shows the normalized response obtained for the HFB derivatives when the 1 mL of DCM with HFBI was added as 1–10 aliquots. There is increase in the recoveries with the increase in the number of aliquots of addition of DCM with HFBI. It reaches its plateau for most of these analytes with three aliquots, but to ensure repeatability and maximum recovery, the use of 5 aliquots of 0.2 mL was used for all further experiments.

3.2.8. Amount of ACN (disperser solvent)

Another factor which influences the DDLLE, is the amount of disperser solvent, i.e., ACN. ACN is essential for dispersing the organic solvent in the aqueous sample. It enhances the contact time and in result enhancement in recoveries. The recoveries get double in comparison to that scenario where ACN was not used at all. On further increase in the amount of ACN, recoveries decreases and finally reaches an equilibrium where any further changes does not affect the DDLLE process, as shown in Fig. 3. The maximum recovery was observed at 10% of disperser solvent with respect to sample (i.e., for 1 mL of sample 0.1 mL of disperser solvent), hence in all later studies 100 µL of ACN was used for 1 mL of sample.

3.2.9. Salting out effect on DDLLE

Increases in recoveries for liquid-liquid extractions by "salting out" are caused by the increased ionic strength of the aqueous phase aiding the partition of the analyte to the organic phase. To determine if this was a major effect on the HFB-DDLLE the ionic strength of the aqueous phase was modified by the addition of sodium sulfate (Na_2SO_4). Up to 30% Na_2SO_4 (saturation of the salt in water) the recovery showed increases for all analytes (see Fig. S-9; provided in supplementary material).

3.3. Limit of detection, linearity, recovery and repeatability

Limits of detection (LOD) were determined by spiking the tap water with the analytes at different concentrations and perform-

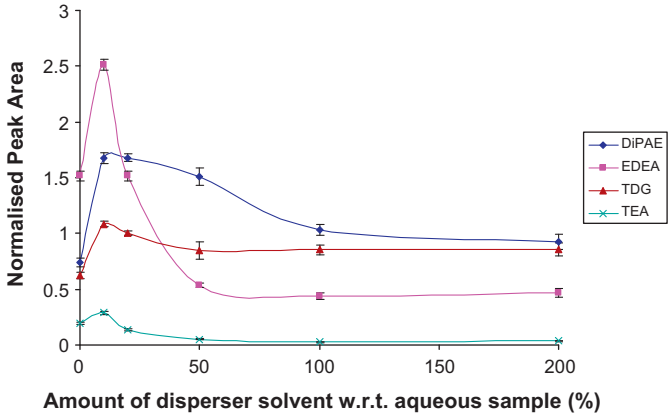

Fig. 3. Effect of volume of acetonitrile on HFB-DDLLE keeping other experimental conditions constant.

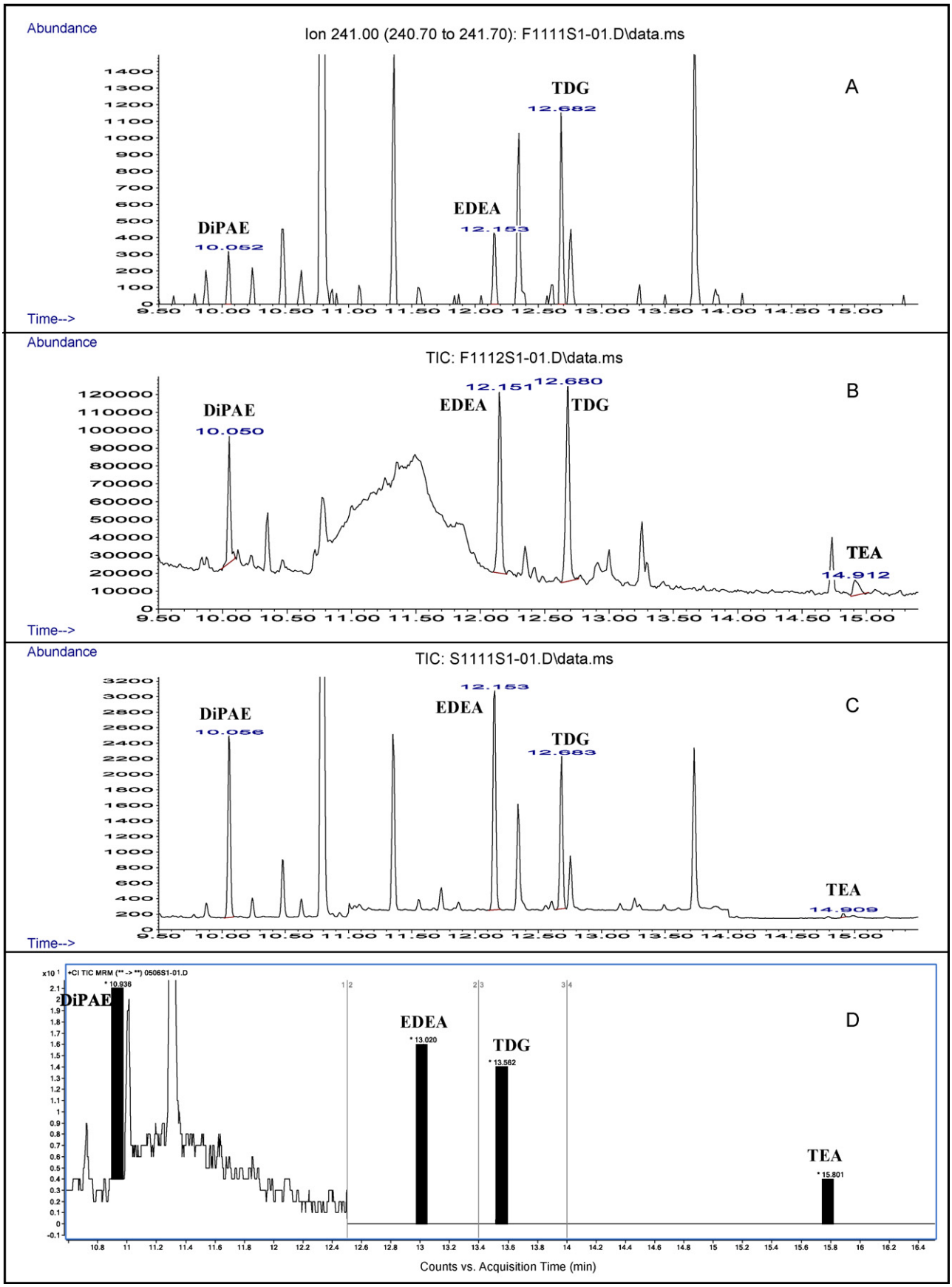

Fig. 4. Limit of detection for heptafluorobutyryl derivative of DiPAE, EDEA, TDG and TEA (A) with full scan at 10 ng/mL (extracted ion chromatogram, *m*/*z* 241), (B) with full scan at 100 ng/mL, (C) with SIM at 10 ng/mL and (D) with MRM at 100 pg/mL.

Table 1
Limit of detection (ng/mL), linearity and recovery data for HFB-DDLLE of chemical weapon convention related alcohols in aqueous sample.

S. no.	Analyte	Ion monitored in SIM (m/z)	LOD (ng/mL)			Linearity (μg/mL)	r^2	Transitions monitored in MRM	Recovery (%)
			SIM (S/N)	Full scan (AMDIS S/N)	MRM				
1	DiPAE	241, 284, 326	10 (780)	10 (34)	0.1	0.1–40	0.9981	128 → 44 128 → 86	56–76
2	EDEA	241, 298, 312	10 (157)	10 (30)	0.1	0.1–40	0.9974	312 → 69 312 → 169	55–71
3	TDG	241, 300, 301	10 (141)	10 (29)	0.1	0.1–40	0.9987	301 → 169 301 → 241	78–104
4	TEA	241, 510, 524	10 (22)	100 (39)	0.1	0.1–40	0.9945	524 → 169 524 → 241	16–98

ing the HFB-DDLLE as described in Section 2.4. The DCM layer was evaporated and reconstituted in 100 μL of heptane containing 2 μL of HFBI and analyzed by GC–MS in full scan, selected ion monitoring (SIM) and multiple reaction monitoring (MRM) mode. The results of LOD were presented in Table 1 and respective chromatograms are shown in Fig. 4. It is evident from these results that, in the GC–MS full scan mode with AMDIS the scheduled alcohols could be detected at the concentration of 10 ng/mL except TEA (100 ng/mL). The LOD with SIM was studied up to 10 ng/mL and from the result shown in Table 1, clearly indicates that their was a possibility to go further lower for all these analytes except TEA. The LOD of DDLLE with MRM was specifically studied for its application to the more complex matrices, such as biomedical samples. The LOD in MRM with both the electron ionization (EI) and chemical ionization (CI) mode for all the model agents were 100 pg/mL, but with the methane CI, S/N was better than EI.

The repeatability of the DDLLE at each step of optimization was assessed by performing it in triplicates. The values shown in figures and tables are average of triplicate runs with RSD of 2–17%.

Recoveries of DDLLE were calculated by preparing a separate calibration curve by appropriately diluting the stock solution of HFB derivatives of model agents. This stock solution of HFB derivatives was prepared by direct heptafluorobutyrylation of these model agents at 100 μg/mL with excess HFBI. Statistical figures of recoveries of analytes as their Heptafluorobutyryl derivatives by DDLLE are shown in Table 1. This range of recoveries shown in Table 1, were obtained when it was determined and calculated at different concentrations of analytes (0.1, 0.2, 0.5, 1, 5, 10 and 40 μg/mL). It was observed that the recovery for TEA increases gradually with the decrease in the concentration of TEA and it reaches around 100% when its concentration was 0.1 μg/mL.

The linearity of DDLLE was studied in the range of 0.01–40 μg/mL for all the analytes under full scan mode of GC–MS analysis (except for TEA 0.1–40 μg/mL) and it was linear in the 0.1–40 μg/mL range. Squared correlation coefficients (r^2) were varied from 0.9945 to 0.9987 (shown in Table 1).

3.4. Applications

DDLLE was specifically developed for the aqueous samples containing CWC related alcohol and hence it was applied on the various aqueous samples. Some of the aqueous samples were prepared by taking water from specific sources followed by spiking with CWC related alcohols and some aqueous samples from the earlier OPCW organized proficiency tests (PTs).

DDLLE had been applied for aqueous sample containing DiPAE, EDEA, TDG, TEA, 3-quinuclidinol (3-Q) and pinacolyl alcohol (PinOH) at the concentration of 10 μg/mL at two different pH of 0.5 by appropriate amount of hydrochloric acid (HCl) and 12 by sodium hydroxide in the tap water. DDLLE was applied on theses samples after neutralization. In both the samples all the spiking chemicals were successfully identified.

An aqueous samples was prepared by spiking water collected from a canal in front of OPCW Laboratory at Rijswijk in the Netherlands with DiPAE, EDEA, TDG, TEA, 3-Q and PinOH at the concentration of 10 μg/mL. In this sample DDLLE is applied directly as per the procedure described in Section 2.4. All the spiking chemicals were identified successfully. Another water sample was prepared by spiking the water collected from the north sea at Scheveningen, the Hague with DiPAE, EDEA, TDG, TEA, 3-Q and PinOH at the concentration of 10 μg/mL. In this sample also DDLLE is applied directly as per the procedure described in Section 2.4. All the spiking chemicals were identified successfully as shown in Fig. 5(A).

One of the important advantages of HFB-DDLLE is the analysis of pinacolyl alcohol from aqueous samples, since underivatized PinOH is early eluting compound and it elutes on the tailing of solvent peak. There is always possibility of losing PinOH on concentrating the extract. In HFB-DDLLE, HFB derivative of PinOH elutes at higher retention time and well separated from the solvent. Loss of HFB derivative of PinOH on concentrating the extract was specially tested by drying the extractant completely. No significant difference was observed between completely drying and leaving little bit of DCM.

Table 2
Sample composition of OPCW organized proficiency tests (PTs) samples where HFB-DDLLE has been applied.

S. no.	Sample name	Analytes	Analytes concentration	Matrix composition
1	215 (25th PT)	1,2-Bis(2-hydroxy ethylthio)ethane, i.e., sesquidiol	20 μg/mL	0.5% polyethylene glycol 200 1% DCM in deionised water
2	226 (25th PT)	Thiodiglycol Ethyldiethanolamine	20 μg/mL 20 μg/mL	Calcium chloride and sodium sulfate at 1000 μg/mL in the 50% acetonitrile aqueous solution
3	154 (24th PT)	Triethanolamine Pinacolyl methylphosphonic acid	14.84 μg/mL 9.8 μg/mL	Magnesium sulfate (120 μg/mL), sodium carbonate (106 μg/mL), sodium sulfate (284 μg/mL), calcium chloride (222 μg/mL), 3,5-dihydroxy-toluene (10 μg/mL), nafronyl oxalate (10 μg/mL), methyl-propyl succinimide (10 μg/mL), polyethylene glycol 200 (505 μg/mL) and polyethylene glycol 400 (505 μg/mL) in deionised water

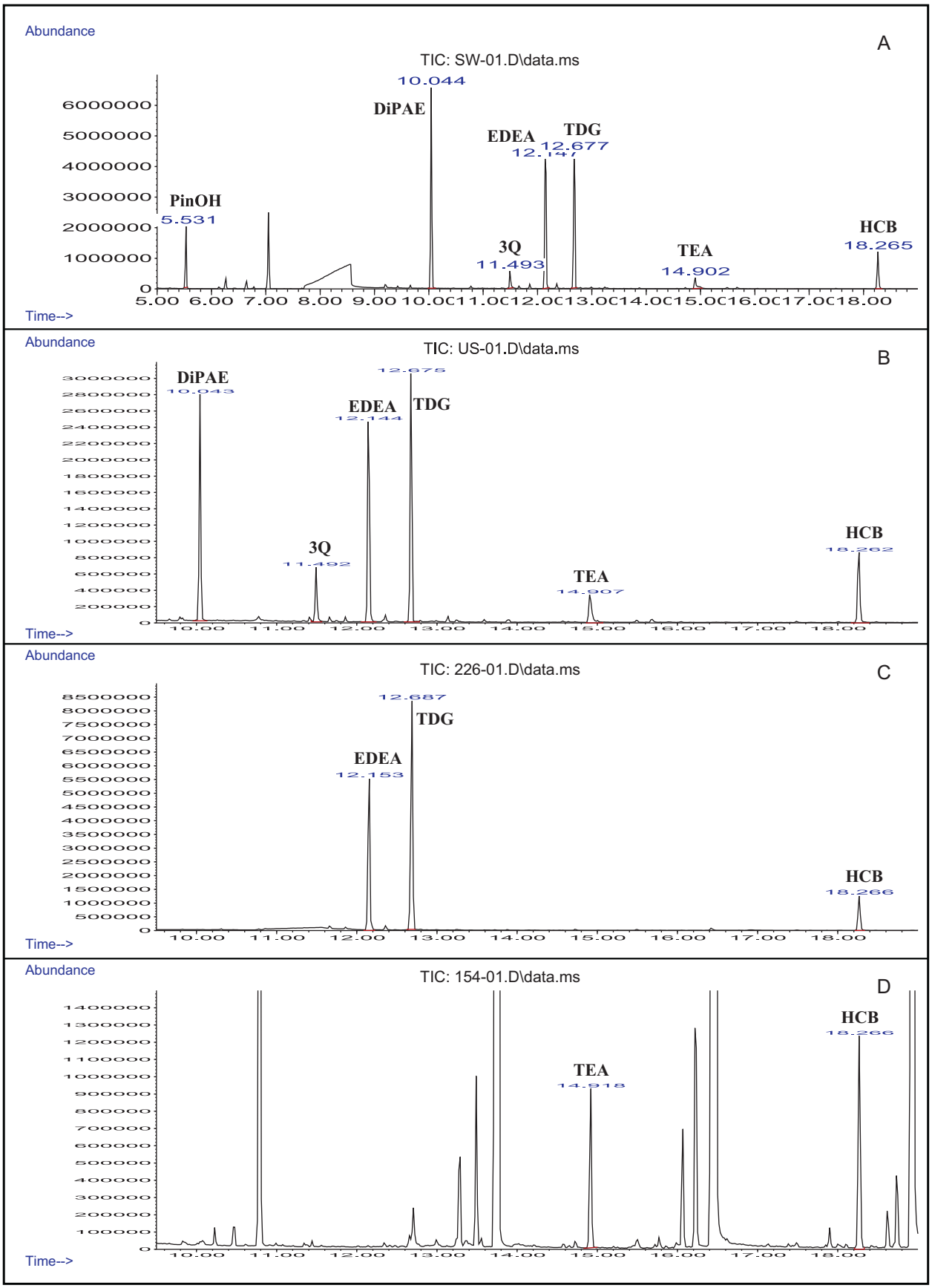

Fig. 5. Real world application of HFB-DDLLE shown as total ion chromatogram from GC_MS analysis of (A) the spiked north sea water, (B) the spiked urine sample, (C) the sample number 226 from 25th proficiency test and (D) the sample number 154 from 24th proficiency test.

To see the matrix affect on HFB-DDLLE and its further possibility of application for biomedical samples, urine sample was spiked with DiPAE, EDEA, TDG, TEA and 3-Q at the concentration of 10 μg/mL. In this sample also DDLLE is applied directly as per the procedure described in Section 2.4. All the spiking chemicals were identified successfully and shown in Fig. 5(B).

Finally, HFB-DDLLE was applied on the three OPCW organized proficiency tests (PTs) samples, two samples were from 25th PT (215 and 226) and one was from 24th PT (154). The sample composition is tabulated in Table 2. In all the samples DDLLE is applied directly as per the procedure described in Section 2.4. Sesquidiol as HFB derivative was identified in 215, thiodiglycol and ethyldiethanolamine were identified in 226, the result of 226 is shown in Fig. 5(C). Triethanolamine was identified in 154, the result is shown in Fig. 5(D) and the out of scale peaks are for the HFB derivatives of polyethylene glycol.

4. Conclusion

Dispersive derivatization liquid–liquid extraction (DDLLE) is a fast and efficient method of sample preparation of aqueous samples for GC–MS analysis by combining extraction and derivatization in a single step, without drying the aqueous samples. Heptafluorobutyryl-dispersive derivatization liquid–liquid extraction (HFB-DDLLE) is a very simple and rapid method for heptafluorobutyrylation and extraction of convention related alcohols directly from water samples. Heptafluorobutyryl derivatives of CWC related alcohols provide better spectral differentiation between the resulting derivatives. The optimal conditions for HFB-DDLLE are heptafluorobutyrylimidazole as derivatizing agent, DCM as extraction, and acetonitrile as disperser solvent in presence of 0.3 M of sodium carbonate. The applicability of DDLLE was shown by applying on various real samples, which also includes OPCW organized proficiency test samples. With HFB-DDLLE limit of detection (LOD) is achieved in full scan with AMDIS at 10 ng/mL and LOD can be extended to 100 pg/mL with methane chemical ionization, multiple reaction monitoring (MRM).

The future prospective of HFB-DDLLE is its application for the analysis of CWA's in biomedical sample and also for other target compounds with alcoholic functionality.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.chroma.2011.06.008.

References

[1] Convention on the Prohibition of the Development, Production, Stockpiling and use of Chemical Weapons and their Destruction, Technical Secretariat of the Organisation for Prohibition of Chemical Weapons, The Hague, 1997, Accessible through internet http://www.opcw.org.
[2] S. Dagan, J. Chromatogr. A 868 (2) (2000) 229.
[3] V.I. Babushok, P.J. Linstrom, J.J. Reed, I.G. Zenkevich, R.L. Brown, W.G. Mallard, S.E. Stein, J. Chromatogr. A 1157 (1–2) (2007) 414.
[4] R.M. Black, B. Muir, J. Chromatogr. A 1000 (2003) 253.
[5] OPCW Internal Procedures: Work Instruction for the Preparation of Samples On-site for GC/MS Analysis QDOC/LAB/WI/SP2, Issue 2 Rev 0, 29 April 2009.
[6] P.K. Kanaujia, V. Tak, D. Pardasani, A.K. Gupta, D.K. Dubey, J. Chromatogr. A 1185 (2) (2008) 167.
[7] P. Garg, A. Purohit, V.K. Tak, D.K. Dubey, J. Chromatogr. A 1216 (2009) 7906.
[8] M. Palit, D. Pardasani, A.K. Gupta, D.K. Dubey, Anal. Chem. 77 (2005) 711.
[9] D. Pardasani, M. Palit, A.K. Gupta, P.K. Kanaujia, D.K. Dubey, J. Chromatogr. A 1059 (2004) 157.
[10] B.A. Tomkins, G.A. Sega, J. Chromatogr. A 911 (1) (2001) 85.
[11] D.K. Dubey, D. Pardasani, M. Palit, A.K. Gupta, R. Jain, J. Chromatogr. A 1076 (2005) 27.
[12] M. Kuitunen, in: R.A. Meyers (Ed.), Encyclopedia of Analytical Chemistry, Wiley, Chichester, 2000.
[13] P.A. D'Agostino, L.R. Provost, J. Chromatogr. A 645 (2) (1993) 283.
[14] D.J. Harvey, M.G. Horning, J. Chromatogr. 79 (1973) 65.
[15] J.G. Purdon, J.G. Pagotto, R.K. Miller, J. Chromatogr. 475 (2) (1989) 261.
[16] I. Ohsawa, Y. Seto, J. Chromatogr. A 1122 (2006) 242.
[17] R.M. Black, R.W. Read, J. Chromatogr. B 665 (1995) 97.
[18] E.M. Jakubowski, C.L. Woodard, M.M. Mershon, T.W. Dolzine, J. Chromatogr. 528 (1990) 184.