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

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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

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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