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Ion-pair liquid–liquid–liquid microextraction of nerve agent degradation products followed by capillary electrophoresis with contactless conductivity detection

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ABSTRACT

The four nerve agent degradation products methylphosphonic acid (MPA), ethyl methylphosphonic acid (EMPA), isopropyl methylphosphonic acid (IMPA) and cyclohexyl methylphosphonic acid (CMPA) have been successfully extracted from aqueous sample solution by ion-pair liquid–liquid–liquid microextraction. In this procedure, the target analytes in the sample solution were converted into their ion-pair complexes with tri-*n*-butyl amine and then extracted by an organic solvent (1-octanol) layer on top of the sample solution. Simultaneously, the analytes were back-extracted into a drop of an aqueous acceptor solution which was suspended in the organic phase at a microsyringe needle tip. The factors influential to extraction: type of organic solvent, type of ion-pair reagent and its concentration, pH values of sample solution and acceptor aqueous phase, stirring rate and extraction time were investigated in detail. After extraction, the drop of the acceptor solution was withdrawn into the syringe and injected into a capillary electrophoresis system for analysis. Using contactless conductivity detection, direct quantification of these compounds is possible. Moreover, large-volume sample injection was employed for further preconcentration. Improvements in the limits of detection between 2.5 and 4 orders of magnitude could be achieved and concentrations at the ng/mL level can be determined. This newly established approach was successfully applied to a spiked river water sample.

1. Introduction

Chemical warfare agents are not likely to be used by major powers, but there is an increased risk that these highly toxic substances may be employed by terrorists. Simple methods for detecting such nerve agents in the environment are thus desired. As the compounds are generally unstable, confirmation of their use may be carried out via the determination of the degradation products. The hydrolysis of G- and V-type nerve agents leads to alkyl alkylphosphonic acids which can then be quantified. An overview of methods for their determination in environmental matrices is available [1].

In order to achieve high sensitivity and selectivity, sample preparation procedures for the extraction of chemical warfare agent degradation products are generally needed. Reported techniques include pressurised liquid extraction [2], solid-phase extraction (SPE) [3,4], SPE using molecularly imprinted polymers [5,6], ion-pair (IP) SPE [7,8], solid-phase microextraction (SPME) [9], on-matrix derivatisation extraction [10], and a new technique known as microemulsion-mediated *in situ* derivatisation and extraction [11]. Recently, Lee et al. developed a liquid-phase microextraction (LPME) approach [12,13] for such compounds. Zirconia hollow fibre-based microextraction was also proposed for these compounds by the same research group [14].

IP extraction is a method for partitioning of ionic compounds with the aid of lipophilic counter ions [15]. Due to the high polarity and acidity of alkyl alkylphosphonic acids, IP reagents facilitate their extraction. IP-SPE, for example, has been applied to determine the nerve agent degradation products in aqueous matrices using cetyltrimethyl ammonium bromide (CTAB) or tetrabutylammonium bromide (TBAB) [7], and phenyltrimethylammonium hydroxide as IP reagents [8]. Apart from IP-SPE, IP liquid–liquid extraction (LLE) is also a possible approach to realize the extraction of polar compounds from an aqueous phase directly to an organic phase. This has been successfully used, for example for fatty acids [16–19]. LLE has also been miniaturized in the mid-1990s, and has been termed liquid-phase microextraction [20–22]. As its name

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suggests, LPME makes use of only a small amount of solvent for concentrating analytes from aqueous samples. It overcomes many disadvantages of conventional LLE as well as some of those of SPME (e.g. fragility of the extraction fibre and sample carryover); it is simple, fast and is characterized by its affordability, and reliance on widely available apparatus.

For quantification by capillary electrophoresis (CE), or for that matter by high-performance liquid chromatography (HPLC), the sample should be contained in a phase which is miscible with water. Thus it is necessary to follow the extraction from an aqueous sample into an organic phase with a back-extraction into an aqueous solution. This step may also be miniaturized [23,24], and the miniaturized simultaneous overall procedure has been termed liquid–liquid–liquid microextraction (LLLME) [25]. As only small sample volumes are injected, LLLME is particularly well suited for CE [25–27].

CE has previously been investigated for the determination of nerve agent degradation products. However, the compounds cannot be determined by direct UV-absorbance [5], as these possess neither chromophores nor fluorophores. Indirect UV-detection is relatively insensitive and has a limited linear range [28–30]. Laser-induced fluorescence allows sensitive determination, but derivatisation is necessary to afford fluorescence [31–33]. CE–mass spectrometry is a favorable technique to detect nerve agent degradation products with good limits of detection (LODs) [34]. However, it is costly and not available in many laboratories.

In recent years, capacitively coupled contactless conductivity detection (C^4D) has emerged as a new detection method for CE (for a recent review see [35]). In contrast to the optical methods of absorption and fluorescence, conductivity measurements can be considered universal in CE as all ionic species can be detected directly, and this eliminates the need for derivatisation or indirect approaches. Herein, for the first time, the study of the combination of ion-pair liquid–liquid–liquid microextraction (IP-LLLME) for sample pretreatment with CE–C^4D for separation and detection of four phosphonic acid nerve agent degradation products is reported.

2. Experimental

2.1. Chemicals and reagents

HPLC-grade methanol, 1-octanol, sodium hydroxide and hydrochloric acid were purchased from Merck (Darmstadt, Germany). Methylphosphonic acid (MPA), ethyl methylphosphonic acid (EMPA), isopropyl methylphosphonic acid (IMPA) and cyclohexyl methylphosphonic acid (CMPA), which had been prepared in methanol separately as standards of 1000 μg/mL, were purchased from LGC Promochem (Wesel, Germany). Cetyltrimethylammonium bromide (CTAB) was obtained from Acros Organic (Geel, Belgium). Tri-*n*-butyl amine (TrBA), triethylamine (TEA), tetrabutylammonium bromide (TBAB), phenyltrimethylammonium hydroxide (PTAH), L-histidine (L-His) and 2-(*N*-morpholino) ethanesulfonic acid (MES) were purchased from Fluka (Buchs, Switzerland). Water used throughout the work was obtained from a NANOpure water purification system (Barnstead, Dubuque, IA, USA).

2.2. Instrumental details

An instrument constructed in-house from a 30 kV high-voltage power supply with dual polarity (CZE 2000R) from Spellman (Pulborough, UK) was used for the determinations. The detector is based on two tubular electrodes of 4 mm length which are separated by a gap of 1 mm and a Faradaic shield. For cell excitation a sinusoidal voltage with a peak-to-peak amplitude of 400 V and a frequency of 200 kHz was employed. The detector current was amplified, rectified, and low-pass filtered with a 2-pole Butterworth filter with a 3-dB frequency of 10 Hz [36,37], and the signal was acquired with a MacLab/4e system (AD Instruments, Castle Hill, Australia).

A 54-cm long, 50-μm i.d. bare fused-silica capillary (Polymicro Technologies; Phoenix, AZ, USA) with an effective length of 46 cm was used for the separations. The capillary was conditioned with 1 M sodium hydroxide for 10 min, water for 10 min, 1 M hydrochloric acid for 10 min, and then water for 10 min in that order. The pretreated capillary was then rinsed with the running buffer for 30 min. LODs were determined as the concentrations corresponding to peak heights for signal-to-noise ratios of 3.

The running buffer consisted of 12 mM L-His, 8 mM MES and 25 μM CTAB and had a pH value of 6.3. Before use, the buffer solution was filtered through a membrane of 0.25 μm pore size and degassed in an ultrasonic bath for 5 min. Normal injection was performed by siphoning at 15 cm height for 10 s. When large-volume stacking injection (LVSI) was employed, injection was performed by siphoning at a height of 15 cm for 100 s. The separation voltage was −25 kV.

2.3. Sample preparation

All standards were diluted with water to 0.5 μg/mL for optimization of the IP-LLLME conditions unless otherwise stated. A water sample was collected from a local river. It was first filtered through a membrane of 0.25 μm pore size. Afterwards, the filtrate was spiked with the four target analytes and treated with the IP-LLLME procedure.

2.4. Microextraction procedure

Extraction was carried out in the setup shown in Fig. 1. A 4-mL sample solution was placed in a 5-mL vial. A certain volume of 1-octanol was dispensed onto it. Then a 2-μL aliquot of acceptor phase was drawn into a microsyringe with a cone needle tip. The needle was pushed through the septum of the vial and its tip carefully placed in the centre of the octanol phase. Then a single drop of the acceptor phase was pushed out and suspended in the octanol phase. After extraction for a prescribed time under agitation with the magnetic stirrer, the analyte-enriched drop was withdrawn back into

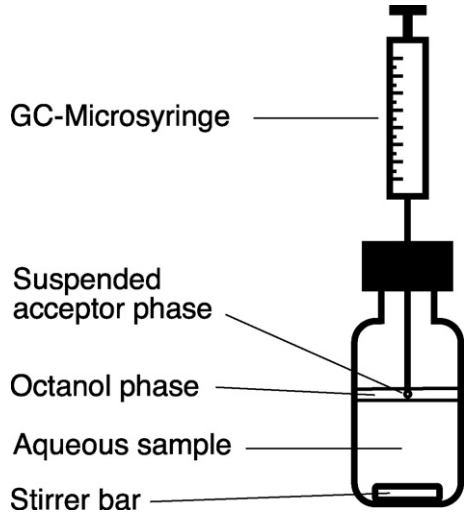

Fig. 1. Schematic drawing of the extraction setup (not to scale).