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Short communication

Combining poly(dimethyldiphenylsiloxane) and nitrile phases for improving the separation and quantitation of benzalkonium chloride homologues: In-tube solid phase microextraction–capillary liquid chromatography–diode array detection-mass spectrometry for analyzing industrial samples

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ABSTRACT

The retention and separation of four homologues of benzalkonium chloride (alkyl (C_{12}, C_{14}, C_{16}, C_{18}) dimethylbenzylammonium chloride) have been studied in poly(dimethyldiphenylsiloxane) (TRB) and nitrile capillary phases, respectively. Under the optimized conditions (50% acetonitrile in processed samples, 35% of diphenyl content of the TRB, capillary length 43 cm and water:methanol 60:40 as replacing solvent), the extraction efficiency was similar for all the homologues with satisfactory reproducibility and independently of the amount and proportion of homologues. Industrial samples with high viscosity or with complex composition and washes waters have been analyzed without previous treatment. The coupling of IT-SPME–CapLC–DAD to MS detection allowed the determination of the minority homologues (C_{16} and C_{18}) in the industrial samples and washes waters. No matrix effect was found.

1. Introduction

Benzalkonium chloride (BAK) is a mixture of alkylbenzyldimethylammonium chlorides [$C_6H_5CH_2N(CH_3)_2RCl$] with alkyl groups (R) having a chain length up to C_{18}. The different proportion of the homologues in the mixture and their concentration determine its efficiency as biocide and also as surfactant. In general, the C_{12} homologue is the most abundant and most effective against yeast and fungi, the C_{14} against Gram-positive bacteria and the C_{16} against Gram-negative bacteria. BAK belongs to the group of compounds with high production volume and great annual consumption [1]. BAK is used for cleaning [2–5], preservation of wood [6], in ophthalmic formulations [7] and even as food preservative although it is not permitted [8,9]. In European Union, its usage is regulated by REACH legislation [10]. Toxicity studies have been reported [11,12]. There is an increasing need to determine BAK in several matrices such as waters [2,3,13–16,22,23], sludge [17], sediments [18–21,24], industrial and pharmaceutical products [25–27].

For liquid samples, pre-treatments are mainly carried out by liquid–liquid extraction (LLE) [19,20], solid-phase extraction (SPE) [5,14,15], on-fiber solid-phase extraction [22] or stir bar sorptive extraction (SBSE) [23]. Some attempts have been made for integrating sample pre-treatment and chromatographic separation [15,16,18,21].

van de Voorde et al. [5] reported low recoveries for C_{16} and C_{18}-BAK in storm water samples, using SPE, evaporation and redissolution. Li and Brownawell [24] found different extraction efficiencies depending on the concentration and type of sediment for the determination of C_{12}, C_{14}, C_{16} and C_{18}-BAK. Other authors [6,13–15,18] quantified only C_{12} and C_{14}-BAK or C_{12}-BAK [16]. Suitable recoveries for the four homologues were obtained by direct injection using LC–MS–MS but for spiked water samples or ophthalmic solutions [26]. Nevertheless, the on-line screening of the BAK homologues when the sample matrix does not allow the direct injection, due to high viscosity or when samples present complex mixture composition, has not been reported. For industrial samples capillary electrophoresis has been also employed [27] but using

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off-line pre-treatment and applying the method only to standards and ophthalmic products.

Herein, the objective of this work has been to study the retention and separation of homologues for establishing the conditions in which both, retention and separation, do not depend on the homologe and/or its amount or proportion. This knowledge has been used for developing an on-line IT-SPME–CapLC–DAD-MS method for analyzing industrial and washes water samples.

2. Experimental

2.1. Reagents and solutions

See Appendix A; Supplementary data Section S2.2.

Industrial samples were supplied by a local factory. Sample A has a high viscosity (the content of BAK homologues was around 80%) and B and C have complex mixture composition (several surfactants, EDTA, isopropanol; the contents of BAK were 6 and 12%, respectively). Stock solutions 5 mg mL^{-1} of samples A, B and C and standards were prepared by weighing (0.12 g in 25 mL) and dissolved in water: acetonitrile (50:50). Formulations A, C and B were diluted at 60, 550 and 1000 μg mL^{-1} respectively, in order to obtain a total BAK concentration introduced into capillary around 50 μg mL^{-1} if the diode array detector was used. For MS detection, the samples were diluted in the range 39–140 ng mL^{-1} (total BAK concentration between 5 and 13 ng mL^{-1}). For recovery studies see Appendix A, Section S2.2. Ten washes waters were obtained by a feed industry employing B and C products. The required volume for analysis was prepared in 50% acetonitrile.

2.2. IT-SPME procedure

A piece of TRB-35 GC capillary column of 43 cm in length and coated with 35% diphenyl–65% polydimethylsiloxane was connected in a six-port injection valve replacing the injection loop. Capillary connections were facilitated by the use of a 2.5 cm sleeve of 1/16 in. polyether ether ketone (PEEK) tubing at each end of the capillary. In load position, 2 mL or 100 μL of sample is manually passed through the capillary column and, 34 μL of a mixture methanol:water (40:60) is passed for replacing the solution into capillary. Finally, the valve is rotated to the injection position and dynamic desorption is carried out. Before each injection, the TRB-35 capillary column was conditioned with 1 mL acetonitrile, 1 mL water and finally 50 μL of 50 mM acetate buffer. A configuration similar to that reported in [28] has been used. See also Appendix A, Sections S2.1, S2.3 and S2.4.

3. Results and discussion

3.1. Optimization of the separation of BAK homologues in nitrile capillary analytical column

Three phases for IT-SPME were assayed: 5, 20 and 35% diphenyl-95, 80, 65% polydimethylsiloxane, respectively. The TRB35 phase provided better recoveries for the homologues interfaced to the nitrile column. Isocratic elution mode at 7 μL min^{-1} using the optimized mobile phase shown in Table 1 was used.

The retention times of homologues showed a linear relationship with the carbon number of the alkyl chain of BAK: $\mathbf{t_r} = 0.9861\ nC + 4.1876$, $R^2 = 0.99$; being $\mathbf{t_r}$ retention time of homologues and nC is the number of carbon of the alkyl chain. Fig. S1 shows the chromatogram for a mixture of the four homologues.

3.2. Evaluation of extractive efficiency of BAK homologues in TRB 35

The extraction efficiency of the homologues depended mainly on the sample solvent, diphenyl percentage of the TRB phase and length of the capillary, replacing solvent in IT-SPME and the concentration of BAK in the sample for some conditions. We chose a higher length of capillary than that used in [16], 43 instead 25 cm, for optimizing amount extracted. Higher lengths were not considered in order to save analysis time. In [16] the replacing solvent was a mixture of water:methanol (60:40) which improved the C_{12}-BAK desorption kinetics. We studied here the behavior for the other homologues and the same mixture resulted optimal.

Several sample solvents were studied: water, water:acetonitrile (95:5) and (50:50). For water, the analytical response for C_{14}, C_{16} and C_{18}-BAK increased as function of the processed volume up to 5 mL and for C_{12}-BAK up to 3 mL by breakthrough. The use of volumes higher than 2 mL reduced the interday precision of the longer alkyl chain homologues. The BAK determination should be performed with dissolved surfactant monomers. Note that the adsorption is increased with the length of the alkyl chain of the homologue and the CMC is decreased [29] in the same order. The working concentrations for C_{12}–C_{16} BAK are in the range 1.5–60 μM and CMC values in water are 8–0.49 mM [29]. If the absorption of homologues was close to 100%, the concentration into internal volume of the TRB35 capillary, at the moment when desorption begins, would be around 0.2 mM and then below CMC. Therefore, low reproducibility is probably due to losses by adsorption in the glass wall vial. This adsorption can be minimized using an organic modifier as acetonitrile [23].

Using as sample solvent a mixture of water:acetonitrile (95:5), two effects were observed: differences in the peak area respect the total area for each homologue and variation of the shape of the chromatogram with the concentration. At ng mL^{-1} level, the analytical responses for C_{14} and C_{16}-BAK were higher than those obtained for the other analogues and at μg mL^{-1}, C_{16} and C_{18}-BAK showed the higher response. This behavior was probably due to a competitive extraction between the homologues and the stationary phase of the TRB 35 capillary.

Finally, the amount of acetonitrile in the sample solvent was increased from 5% to 50%. Intermediate percentages provided similar results than those reported for 5% content. The use of 50% acetonitrile resulted in lower analytical signals but similar extraction efficiency was observed for all the homologues independently of the level assayed. Fig. S1 compared the analytical signal (1 μg for each homologue) obtained under, 5 and 50% of acetonitrile as sample solvent and supported the discussion carried out. A volume of 100 μL was optimal as taken sample volume bearing in mind a compromise between all variables affecting the analytical response. The percentages of the area obtained are also independent of the amounts of homologues present in the sample.

The optimized conditions for the determination of the homologues were: (i) sample solvent water:acetonitrile 50:50, (ii) sample volume, 100 μL and (iii) replacing solvent, water:methanol 60:40.

3.3. Analytical performance

Variations in the retention time as function of the concentration have been described for cationic surfactants in conventional chromatography [30]. Suitable results for precision of the retention times were achieved in this work (Table 1). The RSD values were slightly better for the nitrile than for the titania column previously proposed [16]. In addition, as shown in Table 1, the procedure allowed the screening analysis of C_{12}, C_{14}, C_{16} and C_{18}-BAK. That