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Analysis of benzalkonium chloride in the effluent from European hospitals by solid-phase extraction and high-performance liquid chromatography with post-column ion-pairing and fluorescence detection

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Abstract

A highly reproducible and specific method for the analysis of the quaternary ammonium compound, benzalkonium chloride, in effluents from European hospitals is presented. Benzalkonium chloride was extracted with end-capped RP-18 solid-phase cartridges and was selectively eluted. The resulting solution was analysed by high-performance liquid chromatography (HPLC). After elution from the analytical column of the HPLC system, 9,10-dimethoxyanthracene-2-sulfonate was added continuously as a fluorescence marker, forming a hydrophobic ion-pair with benzalkonium chloride. The ion-pair was analysed by fluorescence detection. The method was applied to highly complex effluent samples from different sized European hospitals. The measured concentrations were between 0.05 and 6.03 mg/l. The amounts emitted per bed and year were 4.5–362 g and did not correlate with the size of the hospital. The total amounts were 2.6–909 kg/year.

Keywords: Hospital effluent; Derivatization, LC; Fluorescence labelling; Benzalkonium chloride; Quaternary ammonium compounds; Surfactants; Disinfectants; Dimethoxyanthracene-2-sulfonate

1. Introduction

Benzalkonium chloride (BzCl) is a widely used cationic surfactant and disinfectant in hospitals [1]. The technical product consists of homologues of different alkyl chain length (Fig. 1). In Germany, approximately 19 000 tons of quaternary ammonium compounds (QACs) were used in 1989 [2]. Their toxicity to fish (LC_0=0.5–4 mg/l, LC_{100} 2–5 mg/l) is high [3], and the toxicity to daphnids is even higher: LC_0=0.1 mg/l, LC_{100}=1 mg/l [3,4]. For toxicity to bacteria, an EC_{50} of 10 mg/l was reported [3]. In our own investigations, we found an EC_0 of 4 mg/l with *Pseudomonas putida* [5] in a growth

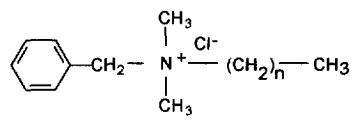

Fig. 1. Structural formula of BzCl (n=11, 13).

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inhibition test, a test for acute toxicity [5,6]. Sanchez Leal et al. [4] reported an EC_{50} of 0.2–18 mg/l in the Microtox Test for QACs. The Freiburg University Hospital is known for using lesser amounts of BzCl-based disinfectants than most other hospitals in Germany [7]. The total QAC concentration in the effluent from the Freiburg University Hospital was calculated to be 0.6 mg/l on average, annually [8]. Because of its poor biodegradation in screening tests (without an adaptation of the inoculum used) and its poor elimination via ion-pairing in other tests, as well as its high toxicity to aquatic organisms, some manufacturers of hospital disinfecting products substituted BzCl by dimethyldidecylammonium chloride (DDMAC) and related compounds. In the meantime, it is well known that DDMAC is poorly biodegradable too [9], like other QACs [10]. Furthermore, the QACs are eliminated in sewage treatment plants with the sewage sludge by forming a neutral ion-pair with anionic surfactants and other anionic compounds and particles present in sewage and sewage sludge. By this ion-pairing, the toxicity of QACs is reduced [5,11], but preliminary investigations showed the biodegradation of linear alkylbenzene sulfonates (LASs) to be worse in the presence of BzCl and DDMAC [12,13]. A final ecotoxicological assessment of the QACs is not possible yet [2].

Despite the fact that the hospital effluent is often diluted by communal sewage, a disturbance of the sewage treatment process by cationic surfactants cannot be excluded.

For most hospitals in Germany and Europe, information on the amounts of disinfectants used is not available. Therefore, the amounts of QACs on the one hand as well as the water usage on the other hand are not known. Furthermore, the manufacturers' declaration of the substances present in a particular disinfectant is only crude. Additionally, most of the QAC-containing disinfectants are used as disinfectants for surfaces and it is unclear what percentage of the disinfectant is found in the effluent. Therefore, the concentration of BzCl in hospital effluents and the associated environmental risk is not assessable and a calculation of the BzCl concentrations in the hospital effluents was not possible.

Because of their ionic character, analysis by gas chromatography (GC) is only possible for dealkylated BzCl and DDMAC, in which case, structural information is lost. Other methods for monitoring cationic surfactants use thin-layer methods or the disulphine blue method [14], which is not suitable for environmental analysis [15]. These methods are not sensitive and not specific enough. Therefore, we used a HPLC method for the analysis of BzCl in hospital effluents. UV–Vis detectors may be used because of the benzene ring present in BzCl [16]. This detection mode is not very specific and selective for BzCl and, therefore, is not suitable for complex samples like sewage from hospitals. Refractive index or electrical conductivity detection was used for the analysis of QACs in surface water, but is not recommended for sewage analysis because of its poor sensitivity, as other components present in hospital effluents may disturb detection. Furthermore, for these detectors, it is absolutely necessary to maintain constant conditions, which are difficult to obtain with complex samples, and their selectivity is low [17]. Therefore, we used a method first introduced by De Ruiter et al. [18] and modified by Versteegh et al. [19] for the analysis of surfactants with a fluorescent counter-ion, which is added to the solution containing the ions to be analysed. The aqueous and the organic phases are separated using a phase separator. The organic phase contains the ion-pair detectable by fluorescence. With this method, selective and sensitive analysis of BzCl in complex samples like hospital effluents is possible.

This paper describes a highly reproducible method for the trace-level analysis of BzCl as a lipophilic and fluorescent ion-pair after solid-phase extraction (RP 18ec) of BzCl and separation by HPLC in hospital effluent at the low mg/l level.

2. Experimental

BzCl was extracted from the sewage with a RP 18ec cartridge, eluted with a calcium chloride solution and injected into a HPLC system. After separation of the sample on the analytical column, an aqueous solution of fluorescent 9,10-dimethoxyanthracene-2-sulfonate (DMAS, Fig. 2) was continuously added to the effluent from the analytical column. DMAS formed a highly lipophilic ion-pair with BzCl. The ion-pair formed in organic phase was

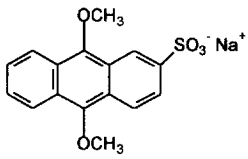

Fig. 2. Structural formula of fluorescent DMAS (9,10-dimethoxy-2-anthracenesulfonate).

separated on-line by a phase separator, and was detected by fluorescence.

2.1. Benzalkonium chloride

BzCl, of technical grade, containing the same mixture of homologues as the BzCl used in most hospital disinfectants, was obtained from Bode Chemie (Hamburg, Germany) as a 50% aqueous solution.

2.2. Sampling, extraction and clean-up

The hospitals under investigation were the Centre Hospitalier New Paul Brien (Brussels, Belgium; 174 hospital beds), Ospedale Maggiore (Bologna, Italy; 791 hospital beds), a large Austrian hospital (2514 hospital beds), Academisch Ziekenhuis (Utrecht, Netherlands; 857 hospital beds) and the University Hospital (Freiburg, Germany; 1356 hospital beds). The hospitals are of different sizes, have different equipment and use different hygiene standards. Therefore, a good overview of the possible concentration span of BzCl should result. A sample (1 l) was taken from the main drain of the hospitals every 2 h for 24 h. In the case of the Austrian hospital, there were five different main drains. From each of the five effluents, a 24-h mixed sample (proportion by volume) was used. The samples were placed in polyethylene bottles and stored at −4°C until analysis. Loss of BzCl by adsorption was accounted for by a standard addition method. Prior to analysis, the samples were allowed to settle. From the supernatant of each sample, an equivalent of 8 ml was taken for analysis and extracted with an automated solid-phase extraction unit, Abimed Aspec XL (Abimed, Heidelberg, Germany) using C18ec SPE cartridges (Machery–Nagel, Düren, Germany). The cartridge was preconditioned with three bed volumes of methanol (Merck, Darmstadt, Germany) followed by three bed volumes of deionised water. The sample was applied and then the cartridge was rinsed with three bed volumes of water and with two bed volumes of ethyl acetate (Merck). BzCl was eluted with two bed volumes of a mixture of methanol–ethyl acetate (1:1, v/v) containing 1% calcium chloride (Merck). For all steps, the flow-rate was set at 3 ml/min. All chemicals used were of analytical grade at least. An equivalent of 15 μl was automatically injected into the HPLC system via a sample loop.

2.3. Chromatography

An LC 10 HPLC system, equipped with two pumps (LC 10 AT), an autosampler, a column oven and a flourescence detector (Shimadzu, Duisburg, Germany) was used. The oven temperature was set at 15°C for the analysis of BzCl. Data acquisition and evaluation were accomplished with the Class LC10 software package (Shimadzu). The analytical column dimensions were 250×4 mm I.D.). The stationary phase was 5 μm CN–NH_2 (Partisil PAC, Latek, Heidelberg, Germany). The mobile phase was chloroform–methanol (80:20, v/v). The mode of operation was isocratic. The total flow was set at 1 ml/min. A 15-μl aliquot was injected via a sample loop.

2.4. Post-column derivatisation and detection

An aqueous solution of DMAS (37 mg/l) was delivered using a third pump (LC 10 AS, Shimadzu) to the effluent from the analytical column at a flow-rate of 0.3 ml/min. The mixed phases were separated on-line with a laboratory-made sandwich phase separator, according to de Ruiter et al. [18] and Versteegh et al. [19]. A needle valve was used to optimise phase separation and the detection limit. The organic phase was analysed and the aqueous phase was discarded. The ion-pair was monitored by fluorescence detection (excitation at 383 nm, emission at 459 nm).

2.5. Calculations

The daily average concentration of BzCl was calculated by using the measured 2 h concentrations

and the measured flow rates during sampling. From this result, the annual averages were calculated. The total emitted amounts of BzCl were calculated on the basis of the measured concentrations and the measured flow-rates. For the Bologna hospital, these data were not available. Therefore, to obtain a rough estimation, the average daily concentration was calculated using the measured concentrations.

3. Results

Standards of BzCl (0.5, 0.7, 1 and 2 mg/l) were injected three times at each concentration. The retention time was 2.49 min, with a relative standard deviation of 0.8%. The precision was 1.6% of the peak area at the tested concentrations. The recovery of BzCl dissolved in deionised water after solid-phase extraction (SPE) was 93–95% and 87–94% when dissolved in sewage (standard addition) in the concentration range of 0.5–2 mg/l. The calibration curve was linear (R^2=0.999 when extracted from deionised water and R^2=0.998 with sewage samples). The detection limit was 0.05 mg/l in sewage samples. Standard addition of DDMAC and BzCl to sewage samples showed that the peaks of the two compounds were base-line separated. The retention times were 2.5 min for BzCl and 3.28 min for DDMAC (Fig. 3). Therefore, the analysis of BzCl was not disturbed by the presence of the structurally related compound, DDMAC.

Table 1
BzCl concentrations measured in the Austrian hospital (24 h mixed samples, volume proportional sampling).

Drain No.	Water usage (m^3)	BzCl concentration (mg/l)	BzCl amount emitted (24 h) (g)
1	1290	1.25	1613
2	212	5.63	1194
3	109	6.03	657
4	135	2.05	277
5	425	0.92	391
Total	2171	1.90	4132

The concentrations measured in the effluent from the Austrian hospital are summarised in Table 1. The measured concentrations varied between 0.05 and 0.69 mg/l in the main drains of the hospitals in

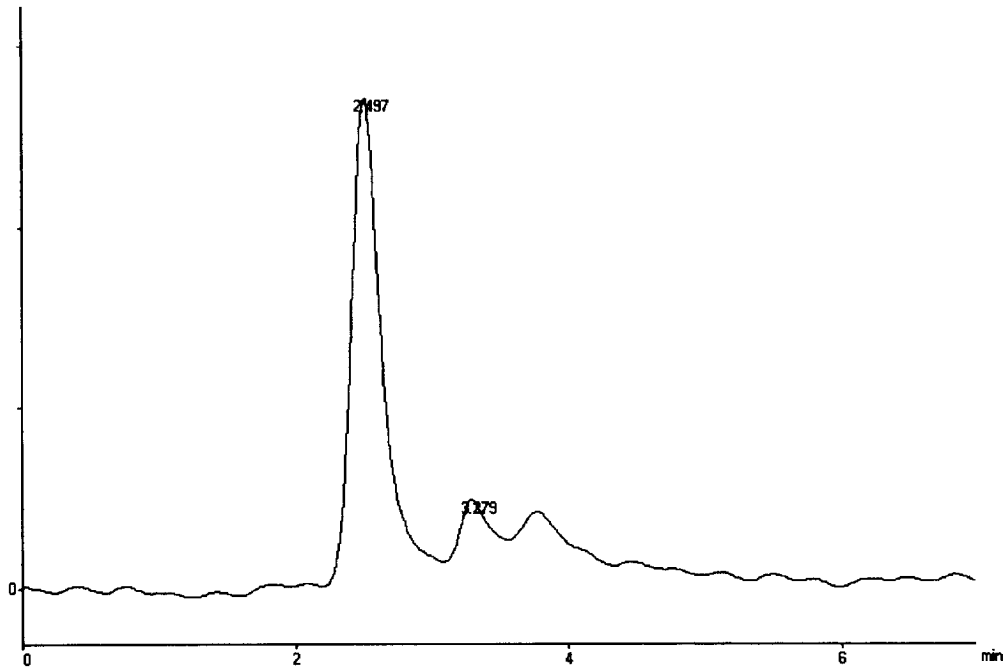

Fig. 3. Chromatogram of a sample from the Austrian hospital (peak at 2.497 min, BzCl; peak at 3.279 min, DDMAC).

Table 2
Amounts of BzCl emitted by each hospital per year as well as per year and hospital bed.

	Brussels	Utrecht[a]	Austria	Bologna[b]	Freiburg
Per year (in kg)	2.6	7.0	909	3.6	29.0
Per bed and year (in g)	15.0	8.2	362	4.5	21.5

[a]24 h sampling only at one of the three main drains possible, so that the real amounts are probably higher.
[b]No data of effluent flow available, therefore calculated with the average daily concentration.

Freiburg, Bologna, Brussels and Utrecht. The concentrations in the effluents from the Austrian hospital were about ten-fold higher. The concentrations varied during the day but were different in each hospital. For the Freiburg University hospital, several minima and maxima were observed, whereas for the Bologna, Brussels and Utrecht hospitals, the concentrations varied only slightly during the 24 h period. The amounts emitted per year as well as per year and hospital bed are summarised in Table 2. The amounts emitted by the Freiburg University hospital are about ten-fold and by the Austrian hospital about threehundred-fold higher than those by the other hospitals. The emissions of BzCl during the 24 h sampling period were 11.9 g for the Brussels Hospital, 16.2 g for the Bologna Hospital, 32.0 g for the Utrecht Hospital, 131.9 g for Freiburg University Hospital and 4132 g for the Austrian hospital.

4. Discussion

The method presented for the extraction and analysis of BzCl in sewage proved to be highly reproducible and effective. The retention time depends slightly on the needle valve adjustment. For this reason and for quality assurance, standards of BzCl were analysed prior to each sample series. The possible loss by adsorption was accounted for by using a standard addition method. Adsorption of BzCl was only observed with new vessels. The loss by adsorption on sewage particles was negligible. The solid-phase extraction worked well because of the interaction between the RP 18ec solid phase and the apolar part of BzCl and, especially, because of the selective elution of BzCl by the highly polar calcium chloride solution. Because of this and because of the specific detection by flourescence after post-column derivatisation with DMAS, further purification of the sample was not necessary. There was no interference with other QACs like DDMAC or other substances present in the effluents. The detection limit was low and the homologues of BzCl or DDMAC were not separated into single peaks. This was not disadvantageous, because it allows an easy quantification of the total BzCl content of a sample.

The concentrations measured during the day did not vary much with the time of sampling or the hospital. The measured concentrations for the Freiburg University Hospital were in the same range as that calculated in an earlier study [8]. The amounts of BzCl emitted with the effluents are of the same order of magnitude for the Bologna, the Brussels and the Utrecht hospitals, and higher for the Freiburg University Hospital. In case of the Freiburg University Hospital the disinfecting activities are reflected by the emitted amounts, exhibiting maxima between 6–8 a.m. and 2–4 p.m. Minima at midnight and 6 p.m. are caused by the lunch-break and the end of normal day-time working hours at 4:30 p.m.

The amounts emitted per year were highest for the two large-sized hospitals in Austria and in Freiburg, with a thirty-fold higher amount in Austrian hospital than in Freiburg. This difference is remarkable as both hospitals have comparable equipment and as the Austrian hospital is only double sized.

A much better comparison is possible by using the specific emission expressed as per bed and year (Table 2). As expected, the specific emission is highest for the Austrian hospital, but, surprisingly, is lowest for the mid-sized Bologna Hospital. This fact results from a very small specific water consumption in Bologna, which is caused by the hospitals' structure (no laundry, use of one-way-dishes). The amount for the Utrecht hospital is probably a little bit higher than shown in Table 2. Full sampling over a 24-h period was possible only for one of the three

main drains; with the other two, the amount of water was sometimes too low to be sampled continuously. It is surprising that the specific emission of the small-sized Brussels hospital is in the same order of magnitude as for the large-sized Freiburg University Hospital. This reflects the fact that in the Freiburg University Hospital, BzCl is only used as a disinfectant when absolutely necessary, and when substitution by other, more environmentally sound substances, is not possible for hygienic reasons [7]. In contrast, the Austrian hospital and the Brussels hospital could still reduce the environmental impact of their effluents by reducing the use of the poorly biodegradable disinfectant, BzCl if this is possible for hygienic reasons.

Acknowledgments

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