U.P.B. Sci. Bull., Series B, Vol. 85, Iss. 2, 2023 ISSN 1454-2331

DETERMINATION OF BENZALKONIUM CHLORIDES FROM WASTEWATER USING HPLC-DAD METHOD

Iuliana PAUN[1], Cristina Ileana COVALIU-MIERLA[2*]

This article presents the development and validation of a new high performance liquid chromatography (HPLC) method coupled with diode array detector for the simultaneous analysis of benzalkonium chlorides (benzyl dimethyl dodecyl ammonium chloride, C12-BZCL, benzyl dimethyl tetradecyl ammonium chloride, C14-BZCL and benzyl dimethyl hexadecyl ammonium chloride, C16-BZCL) from wastewater samples. The chromatographic analysis was performed on an Acclaim Surfactant Plus (150 x 3.0 mm, 3 µm d.p.) column acquired from Thermo Scientific, kept at 30°C. All experiments were performed in isocratic elution conditions at a flow-rate of 0.5 mL/min. Mobile phase composition was a mixture of acetonitrile (A) and 0.2 M ammonium acetate solution (50:50, v/v). UV –VIS detection was λ = 262 nm for the three BZCL. The limits of detection (LODs) were obtained 15 µg/L (C12- BZCL), 19 µg/L (C14- BZCL) and 25 µg/L (C16- BZCL). The calibration curves were linear between 1 mg/L - 100 mg/L, with R^2 values above 0.999 for all BZCL. Solid phase extraction (SPE) using polymeric (Strata X) cartridges has been applied to extract and concentrate the target analytes from the wastewater. BZCL recoveries after SPE procedure were situated between 86.0÷94.6%. Intra-day and inter-day precision (RSD%) were situated between 1.4 ÷ 2.3% and 6.2 ÷ 6.7%, respectively. Limits of quantification (LOQs) was 4.5 µg/L (C12-BZCL), 5.6 µg/L (C14-BZCL) and 7.6 µg/L (C16- BZCL).

Keywords: benzalkonium chloride, HPLC/DAD, SPE, surfactant, wastewater

1. Introduction

Benzalkonium (alkylbenzyldimethylammonium) chlorides (BZCL), are mixtures of linear alkyl chomologues of dodecyl-(C12-), tetradecyl-(C14-), hexadecyl-(C16-) and octadecyl-(C18-) chlorides) benzyldimethylammonium. BZCLs are cationic surfactants with bactericidal and disinfectant properties used in sanitary products and personal care products and as antistatic agents in the formula of laundry conditioners [1]. Benzalkonium chlorides (BZCL) are used in pharmaceutical industry because there are bacteriostatic agents with preservative properties for aerosol preparations [2]. In table 1 are presented the structures, molecular formulas and molecular mass for the three benzalkonium chlorides. After use, residual amounts of benzalkonium chlorides and their degradation products are

---

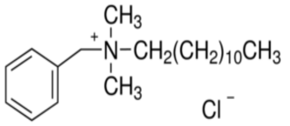
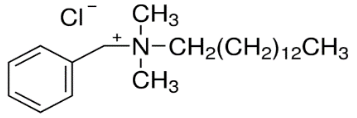
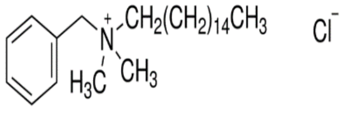
[1] PhD student, University POLITEHNICA of Bucharest, National Institute for Research and Development for Industrial Ecology – INCD ECOIND, Romania, e-mail: iuliana_paunita@yahoo.com

[2] Professor, University POLITEHNICA of Bucharest, Romania, e-mail: cristina_covaliu@yahoo.com

discharged into sewerage, reaching wastewater treatment plants or directly into surface and underground waters. Benzalkonium chlorides belong to the category of emerging pollutants [3]. The presence of these compounds in the environment causes serious environmental problems, including ecological risk and negative effects to human health [4].

Table. 1

Benzalkonium chlorides

Compound	**Structure**	**Molecular formula**	**Molecular Weight**
C12-BZCL		$C_{21}H_{38}ClN$	340 g/mol
C12-BZCL		$C_{23}H_{42}ClN$	368 g/mol
C12-BZCL		$C_{25}H_{46}ClN$	396 g/mol

Benzalkonium chlorides, present in the wastewater, could have negative impact on the efficiency of the treatment plant [5]. Currently, sewage treatment plants reduce the level of pollution mainly through two methods: mechanical and biological through the biodegradation of pollutants with the help of microorganisms [6]. Due their presence, in the treatment of municipal and industrial wastewater plants, large amounts of benzalkonium chlorides could disturb the biological processes of microorganisms, making the biodegradation steep ineffective, and therefore, large amounts of them could reach from the wastewater discharged directly into the rivers [7,8]. For these reasons, it is necessary to find the most efficient methods for removing surfactants from wastewater and to develop precise, sensitive and reproducible methods for the analysis of benzalkonium chlorides in traces from wastewater and in the aquatic environment.

The analysis of benzalkonium chlorides from environmental samples was carried out using several analytical techniques. Spectrophotometry [9], potentiometric titration [10] and potentiometric sensor [11] have been optimized to measure the total content of cationic surfactants although their sensitivity and/or specificity tend to be reduced compared to chromatographic techniques coupled to several detectors.

The separation and identification of cationic surfactants, of which benzalkonium chlorides are also a part, can be difficult, due to their diversity and the complexity of the matrices in which they are found. Although high performance liquid chromatography (HPLC) is the most used technique for surfactant analysis, it is always a challenge to choose the appropriate methods for specific applications depending on the classes of surfactants in the mixture. In general, existing HPLC methods do not provide optimal separation for complex mixtures of anionic surfactants, nonionic surfactants, cationic surfactants and amphoteric surfactants in a single analysis.

For these reasons, a series of pretreatment stages of the samples to be analyzed are necessary before the actual chromatographic analysis.

The methods presented in the scientific literature for the determination of surfactants are based on the HPLC chromatographic technique with various detectors with applications for all classes of surfactants (anionic surfactants, nonionic surfactants, cationic surfactants and amphoteric surfactants), used in various industrial fields (food and beverages, pharmaceutical products, chemical products, semiconductors) and for environment monitorization (water, soil) [12-15].

There are few studies in scientific literature regarding the determination of benzalkonium chlorides from environmental samples, as can be seen in table 2 where the methods for determining cationic surfactants, the chromatographic columns, the chromatographic conditions and the detectors used are presented.

Tabele 2.

Methods used for the determination of benzalkonium chloride in water

Compound	Chromatographic technique	Chromatographic column	Chromatographic conditions
C12-BZCL C14-BZCL C16-BZCL	HPLC - ultraviolet detection	C18 Xterra MS (150 mm x 4.6, 5 µm)	Mobile phase: 70(A):10(B) (A) acetonitrile(B) amonium formiate, 100mM (C) Flow: 1 mL /min Injection volume 200 µL [12]
C12-BZCL C14-BZCL C16-BZCL	IC - conductivity detection	Acclaim Surfactant (2.1x150 mm, 5µm)	Mobile phase: 90(A):10(B) (A) apa (B) formic acid,100mM (C) acetonitrile:water (7:3, v/v) Flow: 250µL/min injection volume 10 µL [13]
C12-BZCL C14-BZCL C16-BZCL	HPLC - ultraviolet detection	Acclaim Surfactant (5µm, 4.6 mm x 250 mm	Mobile phase: 70(A):10(B) (A) formic acid,100mM (B) water (C) acetonitrile:water (70:30, v/v) Flow: 1 mL/min Injection volume 5 µL [14]
C12-BZCL C14-BZCL C16-BZCL	HPLC- ultraviolet detection	Acclaim Surfactant Plus (3µm, 3.0 x 150 mm)	Mobile phase: (A) acetonitrile (B) sodium phosphate75 mM Flow: 0.425mL/min Injection volume:2 µL [15]

The aim of this work was the development and validation of a new High Performance Liquid Chromatography (HPLC) combined with diode array detector method for simultaneous analysis of C12-BZCL, C14-BZCL and C16-BZCL in wastewater samples.

2. Experimental part

2.1. Materials and methods

Benzyl dimethyl dodecyl ammonium chloride (C12-BZCL), benzyl dimethyl tetradecyl ammonium chloride (C14-BZCL) and benzyl dimethyl hexadecyl ammonium chloride (C16-BZCL) were obtained from Sigma-Aldrich. Ammonium acetate, HPLC grade acetonitrile, methanol and acetic acid were purchased from Merck. HPLC grade water was obtained in house using Milli-Q water purification system (Merck-Millipore). The stock solutions were prepared by dissolving the surfactants in methanol and bringing them to a final volume of 10 mL with the same solvent. Stock standard solutions were 1000 mg/L of each surfactant. The standard calibration solutions were made by dilution of the stock standard solutions with mobile phase solutions.

The experiments were carried out on an Agilent 1200 liquid chromatograph composed of: a) quaternary pump with isocratic and gradient elution capable of supplying a mobile phase with up to 4 components, with variable flow rate; b) mobile phase vial holder and membrane degasser; c) autosampler with a capacity of 100 positions and variable injection volume (0.1-100µL); d) thermostat for maintaining a constant temperature in the chromatographic column; e) Acclaim Surfactant Plus chromatographic column, 3µm, 3.6x150mm (Thermo Scientific); f)UV-VIS detector (DAD) with variable wavelength and the ability to simultaneously record up to 8 different wavelengths; g) Agilent ChemStation software for data acquisition, processing, reporting.

The chromatographic column used in the development of the method was Acclaim Surfactant Plus, 3µm, 3.6x150 mm dedicated for the determination of surfactants. The working temperature of the column was the one recommended by the manufacturer of 30ºC.

To optimize the chromatographic parameters, a mixed solution of the three cationic surfactants was used, each having a concentration of 100 mg/L. For the chromatographic separation of the three cationic surfactants, the elution of the mobile phase was used in isocratic mode. The mobile phases used were ammonium acetate in a concentration of 0.2 M and acetonitrile. Different proportions of 0.2 M ammonium acetate and acetonitrile were tested for elution, and the optimal experimentally determined composition was 0.2 M ammonium acetate: acetonitrile = 50%: 50 % (V:V).

For the sensitivity of the method, a range of injection volumes between 5 - 50 µL was tested. The optimal injection volume was 20 µL for the separation with the highest resolution, the best efficiency and the shortest duration. The optimized conditions of the liquid-chromatographic parameters allowed the separation of the three cationic surfactants in an interval of only 5 minutes. The chromatogram of mixture solution in ammonium acetate 0.2M obtained in these conditions is presented in Fig.1.

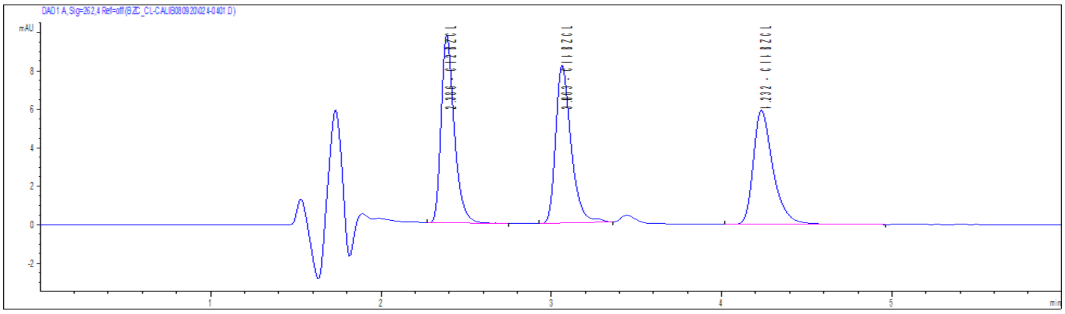


Fig. 1. Chromatogram of surfactant solution and retention time

Solid phase extraction procedure: The samples were treated with Dionex Autotrace 280 automated solid-phase extractor apparatus (Thermo Scientific). The extraction applied to the wastewater sample was applied to isolate the surfactants from sample. The cartridges were conditioned with 5 mL (acetonitrile with 10% acetic acid) and 10 mL (ultrapure water with 10% acetic acid) with a flow rate of 1 mL/min. After passing the water sample (100 mL) through the cartridge with a flow rate of 20 mL/min, the cartridges were washed with 10 mL (ultrapure water with 10% acetic acid) with a flow rate of 5 mL/min. Next, the cartridges were dried under vacuum for 10 minutes. Elution of cationic surfactants was carried out with 2 x 10 mL mixture of 90 ((acetonitrile with 10% acetic acid):10 (ultrapure water with 10% acetic acid)) and with a flow rate of approximately 1 mL/min. The eluted fractions were evaporated, and the residue was resuspended with 1 mL of acetonitrile: ammonium acetate 0.2M=50:50 and analyzed by HPLC/DAD [16].

2.2. Results and discussion

The parameters determined for the validation of the method for the determination of cationic surfactants by HPLC with DAD detector were: selectivity, linearity, detection limits and quantitation limits, intra-day and inter-day precision, accuracy and recovery.

The calibration curve for each surfactant was obtained by analyzing standard solutions at six concentrations, namely 1 mg/L, 5 mg/L, 10 mg/L, 25 mg/L, 50 mg/L and 100 mg/L The correlation coefficient for each individual compounds were 0.9992 for C12 BZCL, 0.9996 for C14 BZCL and 0.9997 for C16 BZCL, which confirms the linear dependence in concentration range of 1 and 100 mg/L solutions. The calibration curve was obtained by plotting the peak area depending of concentration Fig. 2.

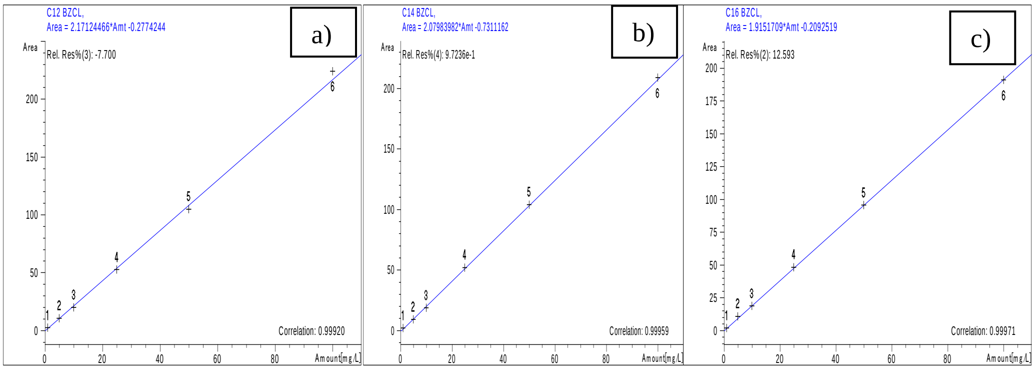


Fig 2. Calibration curve for a) C12-BZCL, b) C14-BZCL,   c) C16-BZCL

By determining the limit of detection (LOD) and the limit of quantification (LOQ) the sensitivity of the method is determined. The LOD is the lowest detectable analyte concentration and the LOQ is the lowest concentration that can be accurately quantified by the proposed HPLC method.The method limits of quantification for (LOQ) for the three cationic surfactants correspond to an S/N ratio of approximately 10. These are presented below. Taking into account the fact that in the pretreatment stage of the samples, they are concentrated up to 100 times, the quantification limit decreases 100 times, obtaining quantification limits of the order of micrograms per liter.

To determine the precision of the intra-day method, the three surfactants were extracted and analyzed on the same day from 10 replicates, and to determine the precision of the inter-day method, the three surfactants were extracted and analyzed from three replicates on four consecutive days.

The individual recoveries for the overall analytical procedure for the extraction of selected compounds using a cartridge SPE were determined by spiking the wastewater with the working standard mixture for concentration of 10 mg/L without each surfactant. Obtained chromatographic validation data are presented in Table 3.

Table 3.

The chromatographic validation data

Surfactant	**R^2**	**Precision (RSD%)**		**Analyte recovery (%)**	**LODs (μg/L)**	**LOQs (μg/L)**
		Intra-day	Inter-day			
C12 BZCL	0.9992	1.36	6.14	93.5	1.50	4.5
C14 BZCL	0.9996	2.27	6.49	90.3	1.87	5.6
C16 BZCL	0.9997	2.20	6.65	86.0	2.53	7.6

Determination of surfactants from wastewater samples: For the determination of the three cationic surfactants from waste water samples, SPE extraction and sample concentration were used. Wastewater from office buildings, hospitals, car washes, as well as gas stations were analyzed. The results of the analysis are presented in table 4.

Table 4.

Concentrations of benzalkonium chlorides determined by different types of wastewater

Sample/ Concentration	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
C12 BZCL (μg/L)	5.8	10.8	44	<4.5	<4.5	26.8	48	2700	<4.5
C14 BZCL (μg/L)	87.6	25.6	9.4	21.2	259	231	<5.6	146	13.4
C16 BZCL (μg/L)	<7.6	<7.6	<7.6	<7.6	9.4	<7.6	135	<7.6	<7.6

Note:1 - purified wastewater; 2 - hospital waste water; 3 - used car wash water; 4,5,6,7,8 - fuel station waste water; 9 - water used office building.

The concentrations of C12-BZCL determined from the wastewater were in the range of < 4.5 µg/L and 2700 µg/L. Concentrations of C14-BZC determined from the samples were in the range <5.6 µg/L - 259 µg/L. It is observed that C14-BZC concentrations are present in 8 of the 9 analyzed samples, while the presence of C16- BZC concentrations is determined only in 2 samples out of the 9 analyzed samples, with concentrations of 9.4 µg/L and 135 µg/L.

6. Conclusions

The separation and identification of surfactants from water is very difficult due to their diversity and the complexity of the matrices in which they are found. The existing HPLC methods do not ensure an optimal separation for cationic surfactants such as benzalkonium chlorides in a single analysis. The sensitivity and selectivity of chromatographic methods are usually insufficient for the direct determination of surfactants at trace levels, in wastewater samples with complex matrices. For these reasons, a series of pretreatment stages of the samples to be analyzed (SPE extraction) are necessary before the actual chromatographic analysis.

In conclusion, the developed method can be used to determine benzalkonium chloride concentrations in wastewater. Also, the developed method will be used to determine the concentrations of benzalkonium chlorides in wastewater for the development of non-conventional wastewater treatment technologies.

REFERENCES

[1]. *W.-H. Ding, Y.-H. Liao, "*Determination of Alkylbenzyldimethylammonium Chlorides in River Water and sewage Effluent by Solid-Phase Extraction and Gas Chromatography/ Mass Spectrometry*",* in Analytical Chemistry, **vol. 73**, *No. 1, 2001, pp.36-40*

[2]. *J. Dudkiewicz-Wilczynska, J. Tautt, I. Roma,* "Application of the HPLC method for benzalkonium chloride determination in aerosol preparations" in Journal of Pharmaceutical and Biomedical Analysis, **vol. 34,** 2004, pp. 909–920

[3]. *B.O. Clarke, S.R. Smith, "*Review of 'emerging'organic contaminants in biosolids and assessment of international research priorities for the agricultural use of biosolids*"* in Environment international, vol. 37, 2011, pp. 226–247

[4]. *K. Jardak, P. Drogui, R. Daghrir, "*Surfactants in aquatic and terrestrial environment: occurrence, behavior, and treatment processes" in Environmental Science and Pollution Research, vol. 23, 2016, pp. 3195-3216

[5]. *A. H. Khan*, "Fate of Benzalkonium Chlorides in Natural Environment and Treatment Processes" Electronic Thesis and Dissertation Repository, 2016, 4249

[6]. *M. Palmer, H. Hatley,*"The role of surfactants in wastewater treatment: Impact, removal and future techniques: A critical review" in Water Research Vol.147, 2018, pp. 60-72

[7]. *A. Hongxue, R. Jianjiao, M. Jun, L. Zaixing, L. Yanfang, L. Xiaoshuai, "*Effect of benzalkonium chloride on microbial activity of activated sludge" in Chinese Journal of Environmental Engineering, **vol. 14(10**), 2020, pp. 2701-2709

[8]. *O. Williams Barber, E. M. Hartmann,* Benzalkonium chloride: A systematic review of its environmental entry through wastewater treatment, potential impact, and mitigation strategies in Critical Reviews in Environmental Science and Technology, **vol. 52**, 2022, pp. 2691-2719

[9]. *J. Idkowiak, A. Zgoła-Grześkowiak, B. Karbowska, R. Plackowski, B. Wyrwas,*"Determination of cationic surfactants in soil samples by the disulphine blue active substance (DBAS) procedure" in Journal of Analytical Chemistry **vol. 72** (7), 2017, pp. 745–750

[10]. *T. Masadome,"*Determination of Cationic Surfactants by a Photometric Titration Method with Crystal Violet as a Color Indicator", Analytical Letters, **vol. 37**, 2004, pp. 499-506

[11]. *D. Madunić-Cacić, M. Sak-Bosnar, O. Galović, N. Sakac, R. Matesić-Puac, "*Determination of cationic surfactants in pharmaceutical disinfectants using a new sensitive potentiometric sensor" in Talanta, 2008, **vol.76(2)**, pp. 259-64

[12]. *T. Miyauchi, M. Mori, K. Ito, "*Quantitative determination of benzalkonium chloride in treated wood by solid-phase extraction followed by liquid chromatography with ultraviolet detection" in Journal of Chromatography, 2005, **vol. 1095** (1-2), pp. 74-80

[13]. *E. Olkowska, Z. Polkowska, J. Namiesnik,* Solid phase extraction- ion chromatography with conductivity detection procedure for determining cationic surfactants in surface water samples in Talanta, **vol. 116**, 2013, pp. 210-216

[14]. Analysis of benzalkonium chloride on the Acclaim Surfactant Column by High -Performance Liquid Chromatography, Dionex, Application Note 237, 2010

[15]. *H. Xiongfeng, X. Qun, J. Rohner* "Rapid determination of benzalkonium chloride in a cosmetic", Thermo Fisher Scientific, Shanghai, People's Republic of China, Thermo Fisher Scientific, Sunnyvale, CA, USA, Aplication Update 190, 2012

[16]. *I. Paun, V. I. Iancu, L. Cruceru, M. Niculescu, F. L. Chiriac,*"Simultaneous Determination of Anionic, Amphoteric and Cationic Surfactants Mixtures in Surface Water", Revista de chimie, 2018**Vol 69**, (1), pp. 27-30