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Simultaneous determination of cationic surfactants in disinfectants

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Objectives. *Cationic surfactants are one of the classes of substances most commonly used in disinfectants. The trend in recent years has been the use of mixtures of several biocides, which poses new challenges for analytical chemistry. In this study, we describe a method for simultaneous determination in the disinfectants alkyldimethylbenzylammonium chloride (ADBAC), alkyldimethyl(ethylbenzyl)ammonium chloride (ADEBAC), chlorhexidine bigluconate (CHG), and polyhexamethylene biguanide hydrochloride (PHMB).*

Methods. *The proposed method is based on the use of reverse-phase and hydrophilic high-performance liquid chromatography with diode-array detection.*

Results. *The best separation of ADBAC, ADEBAC, and CHG was achieved using a column filled with modified spherical silica gel (5 μm, 4.6 × 250 mm) in gradient elution mode. Acetonitrile and acetate buffer with a pH of 5.4 were used as eluents at a flow rate of 1 ml/min. For the determination of PHMB in the presence of the substances under consideration, hydrophilic high performance liquid chromatography was used. The best separation was achieved on an amine phase column (5 μm, 4.6 × 250 mm) using the same eluents. To determine all the substances under consideration, a diode array detector was used. 3D chromatograms were recorded in the wavelength range from 190 to 400 nm.*

Conclusions. *We have shown that the result of the analysis does not depend on the ratio of cationic surfactants in disinfectants. There is also no influence of N,N-bis-(3-aminopropyl)-dodecylamine (Triamine, TA) and the components most commonly used for the manufacture of disinfectants, which was confirmed by testing the method for analyzing real objects. The linearity range for ADBAC was from 0.0062 to 0.97%, for ADEBAC from 0.000726 to 0.201%, for CHG from 0.0128 to 0.111%, and for PHMB from 0.00311 to 0.0205%. The calculated relative error for all determined substances was about 4%.*

Keywords: *alkyldimethylbenzylammonium chloride, alkyldimethyl(ethylbenzyl)ammonium chloride, chlorhexidine digluconate, polyhexamethylene biguanide hydrochloride, cationic surfactants, disinfectants, high performance liquid chromatography.*

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Определение катионных ПАВ в дезинфицирующих средствах при совместном присутствии

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Цели. *Катионные поверхностно-активные вещества являются одним из классов веществ, наиболее часто использующихся в качестве активнодействующих в дезинфицирующих средствах. Тенденцией последних лет является использование смесей нескольких биоцидов, что ставит новые задачи перед аналитической химией. В этой работе описан метод для определения при совместном присутствии в дезинфицирующих средствах алкилдиметилбензиламмоний хлорида (АДБАХ), алкилдиметил(этилбензил)аммоний хлорида (АДЭБАХ), хлоргексидина биглюконата (ХГБГ) и полигексаметиленбигуанид гидрохлорида (ПГМБ).*

Методы. *Предложенный метод основан на применении обращенно-фазовой и гидрофильной высокоэффективной жидкостной хроматографии с диодно-матричным детектированием.*

Результаты. *Наилучшее разделение АДБАХ, АДЭБАХ и ХГБГ было достигнуто при использовании колонки, заполненной модифицированным сферическим силикагелем (5 мкм, 4.6×250 мм) в режиме градиентного элюирования. В качестве элюентов использовали ацетонитрил и ацетатный буфер с pH 5.4 при скорости потока 1 мл/мин. Для определения ПГМБ в присутствии рассматриваемых веществ была использована гидрофильная высокоэффективная жидкостная хроматография. Наилучшее разделение было достигнуто на аминофазной колонке (5 мкм, 4.6 × 250 мм) при использовании тех же элюентов. Для определения всех рассматриваемых веществ использовали диодно-матричный детектор. 3D хроматограммы регистрировали в диапазоне длин волн от 190 до 400 нм.*

Выводы. *Проведенные исследования показали, что результат анализа не зависит от соотношения катионных поверхностно-активных веществ в дезинфицирующих средствах. Также отсутствует влияние N,N-бис(3-аминопропил)додециламина (Триамин, ТА) и наиболее часто используемых для изготовления дезинфицирующих средств компонентов, что было подтверждено при апробации метода для анализа реальных объектов. Диапазон линейности для АДБАХ составил от 0.0062 до 0.97 %, для АДЭБАХ – от 0.000726 до 0.201 %, для ХГБГ – от 0.0128 до 0.111 %, для ПГМБ – от 0.00311 до 0.0205 %. Рассчитанная относительная погрешность для всех определяемых веществ составила около 4 %.*

Ключевые слова: *алкилдиметилбензиламмоний хлорид, алкилдиметил(этилбензил)-аммоний хлорид, хлоргексидина биглюконат, полигексаметиленбигуанид гидрохлорид, катионные ПАВ, дезинфицирующие средства, высокоэффективная жидкостная хроматография.*

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Introduction

Cationic surfactants are used as active components in disinfectants. They are represented by quaternary ammonium compounds (QACs), guanidine derivatives and tertiary amines. The most common cationic surfactants are alkyldimethylbenzylammonium chloride (ADBAC), didecyldimethylammonium chloride, polyhexamethylene guanidine hydrochloride, polyhexamethylene biguanide hydrochloride (PHMB), chlorhexidine bigluconate (CHG), and *N,N*-bis-(3-aminopropyl)-dodecylamine.

Disinfectants with surfactants are commonly used in medical organizations where compounds containing chlorine have been replaced for the purposes of prophylactic disinfection in the presence of patients [1]. A large spectrum of antimicrobial activity, as well as detergency, allow such compounds to be used

in pre-sterilization cleaning. Cationic surfactants are less common in other types of disinfectants.

The substances mentioned above have different toxicological parameters that may change upon mixing with other compounds [2]. Thus, it is important to be able to quantify their concentrations, especially at the test stage prior to disinfectant registration; this is when efficacy and safety are evaluated.

Traditionally, quantification of quaternary ammonium compounds employs titrimetry methods based on formation of ion pairs with anionic surfactants. Various indicators, as well as ion-selective electrodes, are used to determine the titration endpoint. These methods do not require special equipment; however, they cannot be used for simultaneous quantification of several QACs. Analysis of CHG and PHMB may be performed by titrimetry [3–5] and chromatography [6–10]. However, these methods do not work for quantification of cationic surfactant mixtures. The results of the analyses may be affected by other active ingredients, such as *N,N*-bis-(3-aminopropyl)-dodecylamine and some functional components of disinfectants.

Several methods have been described where surfactant separation is achieved through high performance liquid chromatography (HPLC). Detection can be performed by mass spectrometry and spectrophotometry (UV). For mixtures of cationic and anionic surfactants, a charged aerosol detector (CAD) and evaporative light scattering detector (ELSD) have been suggested [11], whereas a diode array detector (DAD) can be used for quantification of ADBAC and trimethyltetradecylammonium chloride in disinfectants [12]. A large number of papers is dedicated to quantification of residual QAC mixtures in food [for example, see 13–15]. Capillary electrophoresis has been suggested for simultaneous quantification of PHMB and CHG [16]. A conductometric detector was used; separation was performed in a capillary filled with silica gel, with the length of 40 cm and inner diameter of 0.375 μm. The sensitivity of this method was 4 mg/L for PHMB and 0.4 mg/L for CHG.

HPLC with UV and DAD detection has been proposed for simultaneous quantification of ADBAC, CHG, and triclosan [17]. Component separation was performed on columns grafted with C8 and CN, with isocratic elution in the solvent mixture of acetonitrile–acetate buffer at pH 5.0. Better sensitivity was observed with C8 columns.

[1] MVI-2-2007-05-3. *Metodika vypolneniya izmerenii soderzhaniya khlorgeksidina biglyukonata v probakh dezinfitsiruyushchikh sredstv titrimetricheskim metodom* (MVI-2-2007-05-3. The method of quantification of chlorhexidine digluconate in samples of disinfectants by a titrimetric method) (in Russ.).

[2] The United States Pharmacopeia. USP 31. NF 26. 2008. 1732 p.

Studies [2–17] suggest quantification methods for individual surfactants as well as their mixtures. However, there are still no techniques for simultaneous determination of mixtures of several cationic surfactants in disinfectants, especially for ADBAC, didecyldimethylammonium chloride, polyhexamethylene guanidine hydrochloride, PHMB, CHG, *N,N*-bis-(3-aminopropyl)-dodecylamine. In the present work, we use HPLC with DAD to separate mixtures of the surfactants mentioned above.

Materials and Methods

Reagents

We used a 20% aqueous solution of chlorhexidine bigluconate (CHG); alkyldimethylbenzylammonium chloride (ADBAC; >95%); ammonium acetate for HPLC (*Sigma-Aldrich*, Germany); a 20% solution of polyhexamethylene biguanide hydrochloride (PHMB; *Vantocil TG, Lonza,* Switzerland); a 25% solution of alkyldimethyl(ethylbenzyl)ammonium chloride (ADEBAC; *Wuhan Dachu Hexing Technology Co., Ltd.,* China); a 30% solution of *N,N*-bis-(3-aminopropyl)-dodecylamine (*Lonza,* Switzerland); acetonitrile for HPLC (*Merck,* Germany); deionized water with resistivity not less than 18.2 MΩ×cm; distilled water according to GOST 6709-72. Other reagents were of *Pro Analysi* or higher grade. Commercial reagents were used without additional purification. Disinfectant samples were supplied by various Russian manufacturers.

Equipment

Chromatographic separation of the components was performed on the Thermo Ultimate 3000 HPLC system (*Thermo Scientific,* USA) with a built-in degasser, automatic sample injection, a column thermostat with the possibility of maintaining temperatures between 15 °C and 50 °C, and a diode array detector (DAD). Absorption spectra were registered in the wavelength range between 190 and 400 nm.

Chromatographic conditions for mixtures of ADBAC, ADEBAC, and CHG (method 1)

We achieved the best separation using the Thermo Acclaim Surfactant 5 μm column (4.6×250 mm) with the following eluents: acetonitrile (A) and a 0.1 M aqueous solution of ammonium acetate (B) (pH 5.4 with glacial acetic acid). Eluent ratios are shown in Table 1. The column thermostat was set at 30 °C. The flow rate was 1 mL/min. The sample volume was 10 μL. The chromatograms were recorded in the wavelength range between 190 and 400 nm. For calculations, we selected the wavelength of 264 nm.

Table 1. Gradient elution for ADBAC, ADEBAC, and CHG separation

Time, min	Eluent A, %	Eluent B, %
0	0	100
8.0	0	100
18.0	80	20
24.0	30	70

Chromatographic conditions for PHMB (method 2)

We achieved the best separation using the Phenomenex Luna NH_2 5 µm column (4.6×250 mm) with the following eluents: acetonitrile (eluent A) and a 0.1 M aqueous solution of ammonium acetate (pH 5.4, eluent B). The gradients were the same as for ADBAC, ADEBAC, and CHG (Table 1). The chromatograms were recorded in the wavelength range between 190 and 400 nm. For calculations, we selected the wavelength of 240 nm. The column thermostat was set at 30 °C. The flow rate was 0.5 mL/min. The sample volume was 10 µL.

Data processing

Surfactants were identified according to their retention times and absorption spectra. After analysis of any five samples, we performed a run with pure acetonitrile to clean the column.

Calibration curves were based on six measurements. The linearity range was determined by peak area concentration graphs. We used the following criteria to determine the linearity range: linear regression with a correlation coefficient exceeding 0.99; deviation from the trendline not exceeding 15% for all points.

We collected and processed the chromatography data using Chromeleon 6 and Chromeleon 7 software (*Thermo Fischer Scientific Inc.*, USA). Statistical analysis was performed in accordance with RMG 61-2010, "Parameters of accuracy, correctness, and precision for methods of quantitative chemical analysis. Methods of assessment" and "Guideline I.C.H.H.T. Validation of analytical procedures: text and methodology Q2 (R1)." We used Excel 2016 (*Microsoft Inc.*, USA) and OriginPro (*OriginLab Corp.*, USA) software.

Results and Discussion

Alkyldimethylbenzylammonium chloride (ADBAC) and alkyldimethyl(ethylbenzyl)ammonium chloride (ADEBAC) consist of several homologs differing in the length of the alkyl chain. Due to the structural similarity of these substances, their chromatographic separation is challenging.

In attempting to find optimal conditions for chromatography, we took into account the resolution R_s whose value should be no lower than 1.5; and the peak asymmetry factor whose value should be between 0.8 and 1.5.

According to the specification sheet of the ADBAC used in our work, the substance consisted of two components: dodecyldimethylbenzylammonium chloride and tetradecyldimethylbenzylammonium chloride. Thus, the chromatogram of a 0.2% solution of ADBAC had two peaks at approximately 7.9 and 9.0 min. They are shown in Fig. 1 as C12 and C14, respectively.

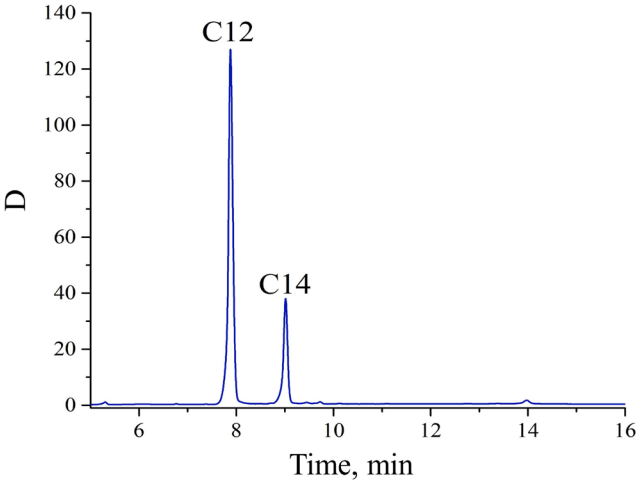

Fig. 1. Chromatogram of a 0.2% ADBAC solution in deionized water (method 1, Surfactant column).

For ADEBAC, we did not have information about the homologs comprising it. The chromatogram of a 0.2% ADEBAC solution in deionized water had four peaks, with retention times of approximately 8.4, 8.7, 9.5, and 9.7 min. We identified the components as dodecyldimethyl(ethylbenzyl)ammonium chloride, tetradecyldimethyl(ethylbenzyl)ammonium chloride, hexadecyldimethyl(ethylbenzyl)ammonium chloride, and octadecyldimethyl(ethylbenzyl)ammonium chloride. These peaks are shown in Fig. 2 as C12, C14, C16, and C18.

As expected, the mixture of ADBAC and ADEBAC had a chromatogram with 6 major peaks that, according to their retention times, corresponded to the peaks in the samples of ADBAC and ADEBAC (Fig. 3).

When we added CHG to the mixture of ADBAC and ADEBAC, it did not affect our analysis, since the retention time of CHG in our conditions was approximately 6.5 min (Table 2). Figure 4 shows that PHMB was not retained on the Surfactant column, whereas *N,N*-bis-(3-aminopropyl)-dodecylamine

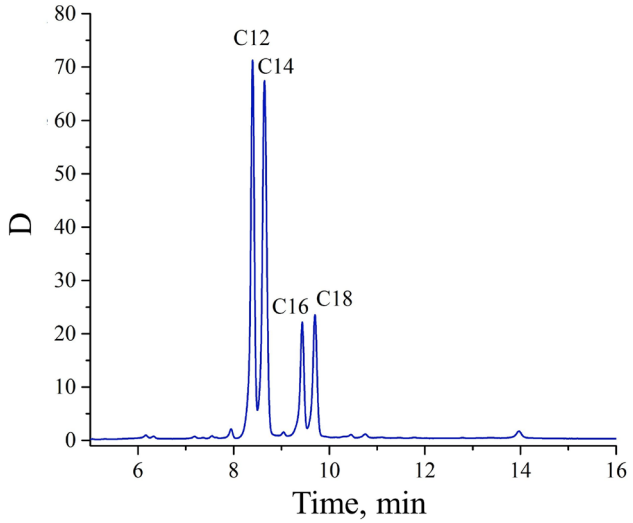

Fig. 2. Chromatogram of a 0.2% ADEBAC solution in deionized water (method 1, Surfactant column).

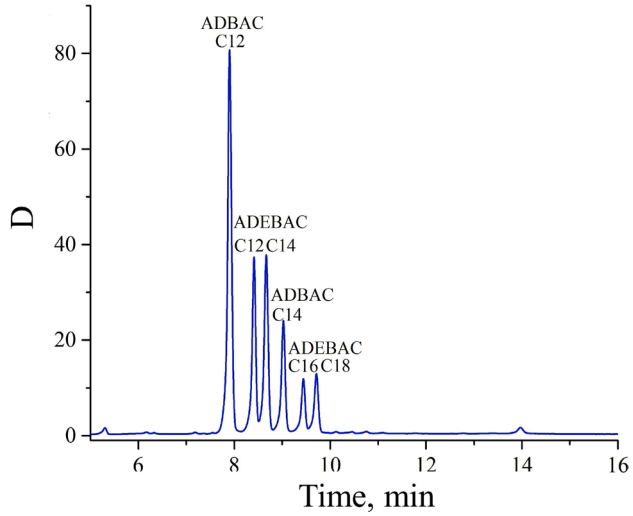

Fig. 3. Chromatogram of a mixture of ADBAC and ADEBAC in deionized water (method 1, Surfactant column).

(Triamine, TA) does not absorb light in the UV range of the spectrum, and so it also did not affect the analysis.

The peak asymmetry factors and the distances between the peaks were within the acceptable range (Table 2). Thus, our conditions are suitable for separation of CHG, ADBAC, and ADEBAC.

In our conditions (method 1, Surfactant column), the peak area concentration graph for ADBAC is linear in the range between 0.005 and 1.000%, for ADEBAC between 0.06 and 0.33%, and for CHG between 0.012 and 0.111%.

Table 2. Chromatography parameters (method 1, Surfactant column) for a mixture of ADBAC, ADEBAC, and CHG*

Substance		Retention time, min	R_s
CHG		6.5	2.96
ADBAC	C12	7.9	3.32
	C14	9.0	2.58
ADEBAC	C12	8.4	1.64
	C14	8.7	2.22
	C16	9.5	1.67
	C18	9.7	2.71

* *Note:* concentration of each component is 0.2%; average parameter value of five measurements is calculated.

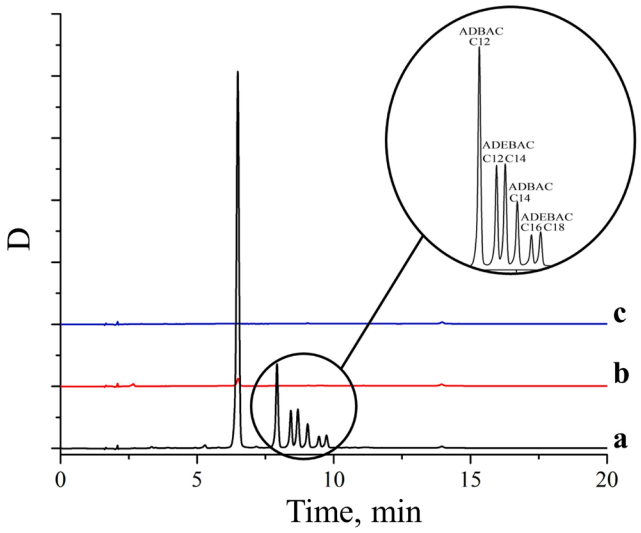

Fig. 4. Method 1, Surfactant column: **a** – chromatogram of a mixture of CHG, ADBAC, and ADEBAC; **b** – chromatogram of a 0.2% PHMB solution in deionized water; **c** – chromatogram of a 0.2% solution of *N,N*-bis-(3-aminopropyl)-dodecylamine in deionized water.

As we have shown, the Surfactant column is not suitable for PHMB quantification. Since this substance has an absorption peak at 240 nm, we could use HPLC with DAD. We were able to separate PHMB from other cationic surfactants only when we performed hydrophilic chromatography on a column with grafted amino groups. The retention time for PHMB in these conditions was approximately 6.5 min. The peak area–concentration graph for PHMB was linear in the range between 0.003 and 0.02%. The determination factor was 0.9961.

Quantification of PHMB in disinfectants may be affected only by ADBAC, ADEBAC, and CHG. When we performed chromatography of these substances and PHMB in deionized water, we determined that in these conditions the retention time for PHMB was approximately 6.5 min, for ADBAC approximately 9.2

min, for CHG approximately 9.8 min, and for ADEBAC approximately 11 min (Fig. 5). We could clearly identify only the peaks of dodecyldimethylbenzylammonium chloride and dodecyldimethyl(ethylbenzyl)ammonium chloride. The other homologs comprising ADBAC and ADEBAC also have peaks on the chromatogram, but they cannot be used for quantitative analysis. Thus, we did not see that these components could affect the analysis.

We used the methods described above for quantification of cationic surfactants in disinfectants. We then compared our results with those obtained by other methods, such as: two-phase titration of individual substances with methylene blue in alkaline conditions (for ADBAC)[3]; two-phase titration with bromophenol blue (for PHMB); and acid–base titration with hydrochloric acid in a water–ketone solution (for CHG)[4]. Model disinfectant formulations are presented in Table 3. A comparison of quantification methods for the cationic surfactants in these samples is shown in Table 4.

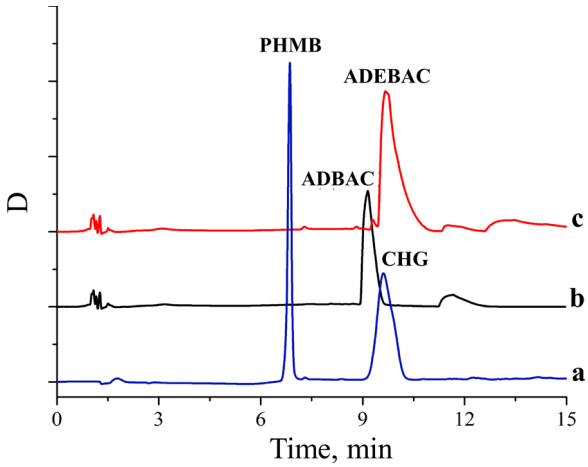

Fig. 5. Chromatography by method 2, Luna NH_2 column: **a** – chromatogram of a mixture of PHMB (0.02%) and CHG (0.05%) in deionized water; **b** – chromatogram of a 0.2% ADBAC solution in deionized water; and **c** – chromatogram of a 0.2% ADEBAC solution in deionized water.

Table 3. Model disinfectants formulations

Component	Contains, % (w/w)
Sample 1	
ADBAC	2.0
N,N-Bis-(3-aminopropyl)-dodecylamine	13.0
PHMB	0.4
Cocamidopropyl betaine	3.0
Nonoxynol AF 9-10	4.0
Potassium citrate	1.5
Water	To 100
Sample 2	
PHMB	0.25
CHG	0.25
2-Propanol	35.0
1-Propanol	30.0
Pentadecanol	0.3
Lactic acid	0.5
Citric acid	0.3
Water	To 100
Sample 3	
ADBAC	2.5
ADEBAC	2.5
N,N-Bis-(3-aminopropyl)-dodecylamine	10.0
Nonoxynol AF 9-10	5.0
Citric acid	0.8
Dye	0.001
Water	To 100

[3] GOST R 57474-2017. *Dezinfektologiya i dezinfektsionnaya deyatel'nost'. Khimicheskie dezinfitsiruyushchie sredstva i antiseptiki. Metody opredeleniya chetvertichnykh ammonievykh soedinenii* (Disinfectology and disinfection activities. Chemical disinfectants and antiseptics. Methods for determination of quaternary ammonium compounds) (in Russ.).

[4] MVI 01.00282-2008 / 0184.23.12.13. *Opredelenie khlorgeksidina biglyukonata v vodnykh i vodno-spirtovykh rastvorakh* (Determination of chlorhexidine digluconate in aqueous and aqueous-alcoholic solutions) (in Russ.).

Table 4. Comparison of supposed and literature described methods for real disinfectants analysis

Surfactant	Amount introduced, %	Found by the proposed method, %	Found by titration method , %
Sample 1			
ADBAC	2.0	2.1±0.1	Separate quantification of the substances could not be performed*
PHMB	0.4	0.39±0.02	
Sample 2			
PHMB	0.25	0.24±0.01	0.22±0.02*
CHG	0.25	0.26±0.02	0.28±0.01**
Sample 3			
ADBAC	2.5	2.5±0.1	5.2±0.1* (sum of both QACs)
ADEBAC	2.5	2.4±0.1	

* Found by two-phase titration, %.
** Found by acid–base titration, %.

Table 5. Metrological parameters of the proposed methods*

Substance	S_r, %	S_R, %	r, %	R, %	±δ, %
ADBAC	1.21	1.69	3.98	5.57	3.94
ADEBAC	1.17	1.64	3.24	4.54	3.87
CHG	1.03	1.44	3.39	4,75	3.84
PHMB	1.23	1.73	4.07	5.70	3.68

* S_r – repeatability; S_R – reproducibility; r – repeatability limit; R – reproducibility limit; ±δ – relative error at P = 0.95.

In some cases, such as in Sample 2, titration methods can provide quantification of active compounds in disinfectants; however, they are often useless for a mixture of even two cationic surfactants, not to mention a greater number of analytes in a mixture. This statement is supported by our results for Samples 2 and 3. Additionally, the data shown in Table 3 suggest that chromatographic analysis is not affected by the presence of nonionic surfactants and other functional additives in disinfectants, thus making such chromatographic methods valuable.

The metrological characteristics of the proposed methods are shown in Table 5.

Conclusions

We have described the application of HPLC for quantification of cationic surfactants in disinfectants and have proposed methods for quantification of ADBAC, ADEBAC, PHMB, and CHG.

The proposed methods allow for the first time to quantify these four substances in disinfectants when they are present simultaneously. Titrimetry methods that are traditionally used for this purpose are not reliable, because their results may be affected by other components of disinfectants.

The proposed methods are quite simple and do not require complex equipment. This makes them highly desirable for quality control purposes and for tests prior to disinfectant registration. We have demonstrated the reliability of these chromatographic methods. They are not affected by other biologically active surfactants that may be found in disinfectants; additionally, we have successfully applied them for analysis of commercial disinfectants.

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The authors declare no conflicts of interest.

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