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Rapid analysis of benzalkonium chloride using paper spray mass spectrometry

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ABSTRACT

A paper spray mass spectrometry (PS-MS) method for rapid and reliable analysis of benzalkonium chloride (BAC) in compound eye drops and body surface disinfectant was developed. The sample was dropped onto triangular filter paper, and high voltage (3.5 kV) was applied to form an electrospray. This method can provide the composition of benzalkonium chloride in samples without pretreatment, solvent or chromatographic separation, and the analysis time is only 10 s. The primary homologues C_{12}-BAC, C_{14}-BAC and C_{16}-BAC of benzalkonium chloride were quantitatively analyzed using PS-MS. Samples were subjected to simple dilution and quantified using the internal standard method. Ion trap mass spectrometry was scanned using SIM mode. The linear ranges of C_{12}-BAC, C_{14}-BAC and C_{16}-BAC were 1–100 $\mu g\, mL^{-1}$; the linear regression coefficients were 0.998–0.999; the detection limits (LODs) were 0.1 $\mu g\, mL^{-1}$; the limit of quantifications (LOQs) was <1 $\mu g\, mL^{-1}$, and the method validation indicated that the method precision and accuracy were good. Compared with HPLC-UV methods, there was no significant difference in the quantitative determination of the actual samples, but the analysis time for PS-MS is shorter (2 min). In addition, reagent consumption in PS-MS is small, and no chromatographic separation is needed, suggesting that PS-MS is especially suitable for high-throughput analysis.

1. Introduction

Biocides are mass-produced chemicals that inhibit harmful microorganisms in many household and industrial applications. Benzalkonium chloride (BAC) is a class of quaternary ammonium compound (QAC) that is widely used as a cationic surfactant, with both hydrophobic and hydrophilic natures [1–4]. It is commonly added as a biocide in personal care products (eye drops, external cleaning solution), cosmetics and skin disinfection products. BAC is classified as a "Category III antiseptic active constituent" by the United States Food and Drug Administration [1]. When available data are insufficient to classify as safe and effective, and further testing is required, the ingredients should be classed as "Category III antiseptic active constituent". It is generally accepted that BAC toxicity is low, but its improper use has serious consequences. For example, BAC was used in eye drops as a biocide from 1950. However, the BAC is not selectively toxic to bacteria, and there is increasing evidence that long-term use of this agent can cause or exacerbate ocular surface disease [5,6]. The Ministry of Health of the People's Republic of China stipulates the content of BAC in disinfectant products with various uses. The highest quality content of skin and mucous disinfectant is not more than 2 $mg\, mL^{-1}$. The limits of BAC content in different types of products are clearly defined in the Chinese cosmetics health standards and the cosmetics regulations of the European Union (Council Directive 76/768/EEC).

BAC is a mixture of alkylbenzyldimethylammonium chlorides [$C_6H_5CH_2N(CH_3)_2RCl$], in which C_{12}, C_{14}, C_{16}-BAC are the main compounds (Fig. 1). Because their antibacterial effects come from the structure of alkyl chains and quaternary ammonium groups, the difference in alkyl chain length results in different antibacterial capacities and irritation [7,8]. The three homologues have different antimicrobial activity: C_{12}-BAC inhibits yeast and fungi, and C_{14}-, C_{16}-BAC inhibit gram-positive or negative bacteria. Therefore, benzalkonium chloride products are often used in a compound form to improve the scope of application of the disinfectant. The United States Pharmacopoeia (USP 34) indicates that the C_{12}-BAC content in the BAC formula is not less than 40%, C_{14}-BAC content is not less than 20%, and the combined amount of the two is not less than 70%.

To date, many analysis methods for BAC have been reported, such as chemical titration [9], ultraviolet spectrophotometry [10],

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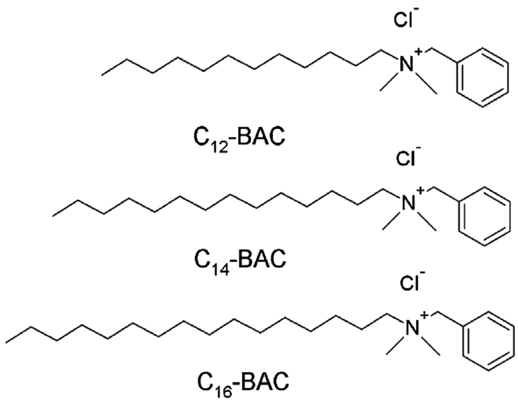

Fig. 1. Structures of quaternary ammonium compounds.

capillary electrophoresis [11], differential spectrophotometry [12], immunoassay [13], liquid chromatography [1,14,15] and liquid chromatography-mass spectrometry [4,16]. Chemical titration, ultraviolet spectrophotometry and differential spectrophotometry can only determine total amounts, and cannot obtain the composition of the major homologous compounds of BAC. Immunoassays can only measure one component. HPLC and HPLC–MS can separate and determine many of these components. However, benzalkonium chloride is a cationic surfactant, and ion pair reagents must be added to the mobile phase. The ion pair reagent often affects the column life and is not conducive to the ionization of mass spectrometry due to its nonvolatility. Therefore, the development of new, highly selective analysis methods is particularly important.

Paper spray mass spectrometry (PS-MS) is a well-developed ambient mass spectrometry (AMS) method [17,18]. It has been used for the fast, qualitative and quantitative analysis of complex mixtures such as blood, urine, saliva, tissues, cell culture, water and bacteria [19–27]. In terms of mechanism, paper spray ionization belongs to electrospray ionization (ESI) processes. It is very simple, i.e., the sample solution was ejected and ionized when a high voltage was applied to a paper triangle. Many analytes could be determined without preparation or less direct preparation.

In our paper, a new and reliable method for the rapid qualitative and quantitative analysis of BACs in compound eye drops and body surface disinfectants was developed using PS-MS. The merits of this method are as follows: 1) the preparation is simpler than other reported methods; 2) the method has low solvent cost and saves labor; 3) the method is highly sensitive. A single PS-MS analysis can be completed in 2 min. Using the deuterium compound as an internal standard, this method can accurately determine the composition and content of BAC. Compared with the HPLC-UV method, there was no significant difference between the two methods in the quantitative determination of actual samples.

2. Materials and methods

2.1. Chemicals and reagents

Benzyldimethyldodecylammonium chloride (98%), benzyldimethyltetradecylammonium chloride (99%), benzyldimethylhexadecylammonium chloride (98%), sodium acetate (99%) and chromatography paper were purchased from J & K Scientific Ltd. (Beijing, China). Deuterated internal standards were prepared by our lab (The preparation processes of D_2—C_{12}-BAC, D_2—C_{14}-BAC and D_2—C_{16}-BAC are shown in S.I.). Methanol and acetic acid were chromatography grade, and other organic solvents were analytical grade and purchased from Tianjin Chemical Reagent Manufacturing Co. (Tianjin, China). Water was purified using a Milli-Q purification system (Millipore Corp., Bedford, MA, USA).

2.2. Instruments and methods

Paper spray was performed using an LCQ Advantage Max spectrometer (Finnigan Corp., Silicon Valley, CA, USA) using the following instrumental parameters. Capillary voltage, tube lens offset and capillary temperature were set at 10 V, 0 V and 200 °C, respectively. Helium was used as a collision gas (no other pneumatic assistance, such as sheath or auxiliary gas was used), and the positive ion mode was employed. Paper triangles were cut from chromatography paper using a CUTOK DC craft cutting plotter (Hefei CNC Equipment Co. Hefei, China). The paper triangles had an angle of 38° and an area of ~60 mm^2 (base width = 9 mm, height = 13.2 mm). An XYZR (three-dimensional and angle regulator) moving platform (Figure S-1, Supporting Information) was constructed in-house and utilized to accurately locate the spray paper triangle tip with the MS cone. The paper triangle was held approximately 5–10 mm from the MS inlet. After directly adding 15 μL of the spray solution to the substrate, high voltage (+3.5 kV) was applied to the paper using an alligator clamp to generate electrospray, and mass spectra were recorded in SCAN or SIM mode.

HPLC-UV was accomplished using an LC-20A high-performance liquid chromatograph (Shimadzu, Japanese) coupled with a UV detection and LC solution workstation and a Lichrospher CN column (4.6 × 300 mm, 5 μm, Hanbon Corp., Jiangsu, China) applied with the following instrumental parameters. Column temperature, flow rate, detection wavelength and injection volume were 35 °C, 1.0 mL min^{-1}, 254 nm and 20 μL. The ratio of mobile A (0.1 mol L^{-1} sodium acetate solution, pH = 1) to mobile B (acetonitrile) was 48:52.

2.3. Preparation of solution and sample

The stock solutions of C_{12}-BAC, C_{14}-BAC and C_{16}-BAC were prepared by dissolving each compound in methanol at a concentration of 3000 mg L^{-1}. A mixed internal standards solution of D_2—C_{12}-BAC, D_2—C_{14}-BAC and D_2—C_{16}-BAC was prepared in methanol at

Table 1
Names and abbreviations of nine samples purchased in local pharmacies.

Sample	Name	Abbreviation
Eye drops-1	Compound Taurine Eye drops	CT-ED
Eye drops-2	Compound Aspartate, Vitamin B_6, and Dipotassium Glycyrrhetate Eye drops	CAVDC-ED
Eye drops-3	Compound Allantoin Vitamin B_6-E and Aminoethylsulfonic Acid Eye Drops	CAVAA-ED
Eye drops-4	Bendazac Lysine Eye drops	BL-ED
Eye drops-5	Naphazoline Hydrochloride, Chlorphenamine Maleate and Vitamin B_{12} Eye drops	NHCMV-ED
Eye drops-6	Brinzolamide Eye drops	B-ED
Eye drops-7	Naphazoline Hydrochloride, Chlorphenamine Maleate and Vitamin B_{12} Eye drops	NHCMV-ED (without BAC)
Sanitizer-1	Hand-foot sterilization spray	HS-S
Sanitizer-2	Sterilization spray	S-S