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Short Communication

Determination of benzalkonium chloride in eye care products by high-performance liquid chromatography and solid-phase extraction or on-line column switching

Lee Elrod, Jr., Timothy G. Golich and James A. Morley

PPD Physical Analytical Chemistry Department, Abbott Laboratories, 1401 Sheridan Road, North Chicago, IL 60064 (USA)

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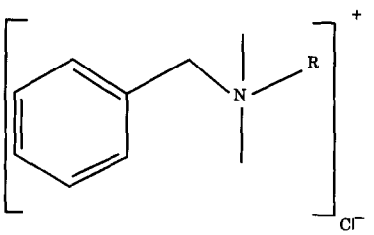
ABSTRACT

Benzalkonium chloride (BAK) is a mixture of alkylbenzyldimethylammonium chlorides, which is commonly used as a bacteriostat. In this work, the three major homologues of BAK are quantitated in the over-the-counter eye care products Murine and Murine Plus using high-performance liquid chromatography (HPLC). The analytes are separated from various product excipients and concentrated by either solid-phase extraction onto Sep-Pak C_{18} cartridges or by an on-line column-switching technique using 1-cm reversed-phase precolumns. Absolute recoveries of BAK homologues by the solid-phase extraction technique ranged from 97.2 to 98.7% for standards and from 98.0 to 98.4% for samples. Absolute recovery of the BAK homologues by the column-switching technique was 101.3% for standards and ranged from 99.9 to 103.7% for samples. Relative recoveries were quantitative by both techniques. Assay precision (R.S.D. values) were ±2.2% to ±2.6% and ±0.4% to ±0.8% by solid-phase extraction and column-switching techniques, respectively. The method provides advantages of high sample throughput, excellent column life and automation.

INTRODUCTION

Benzalkonium chloride (BAK) is a bacteriostat, which is commonly used in a wide variety of health care and cosmetic preparations. Chemically, BAK is a mixture of alkylbenzyldimethylammonium chlorides consisting of three major homologues having straight chain alkyl substituents of C_{12}, C_{14} and C_{16} at the quaternary ammonium salt (Fig. 1). In order to control the effectiveness of the bacteriostat, reliable quantitation of BAK is necessary. High-performance liquid chromatography (HPLC) has been used extensively for this purpose using bonded phases of C_{18} [1,2] and CN [3–5], or by using ion-exchange phases [6,7].



Benzalkonium Chloride ($R=C_{10}H_{21}$ to $C_{16}H_{33}$)

Fig. 1. Structure of BAK.

Correspondence to: L. Elrod, Jr., PPD Physical Analytical Chemistry Department, Abbott Laboratories, 1401 Sheridan Road, North Chicago, IL 60064, USA.

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In this work, BAK is determined in the over-the-counter eye care products Murine and Murine Plus (Abbott Labs., North Chicago, IL, USA) using HPLC and low-wavelength UV detection. Previously reported procedures for determining BAK in ophthalmic preparations [8,9] have used direct injection of the sample. While simple, this approach did not provide enough sensitivity for routine determination of all three major BAK homologues using normal chromatographic equipment. Concentration techniques for BAK have been reported using liquid–liquid extraction [1,10] to improve the quantitation by HPLC. Solid-phase extraction of BAK from cosmetics has been reported using silica gel columns [11], while the C_{12} and C_{14} homologues of BAK have been quantitated in plasma using C_{18} extraction columns in combination with extensive additional sample treatment [12]. To improve the detectability of all three major homologues of BAK, we developed (1) a solid-phase extraction procedure and (2) an automated column-switching technique for use with HPLC. The solid-phase extraction procedure uses C_{18} reversed-phase cartridges, while the column-switching procedure uses 1-cm commercially packed precolumns plumbed into a 10-port high-pressure valve which is electrically actuated. For the chromatographic finish, a cyanopropyl bonded phase (Zorbax SB-CN) was chosen over older, conventional cyano bonded phases, which typically suffer from instability over prolonged use.

EXPERIMENTAL

Apparatus

The HPLC system consisted of a Model SP-8800 ternary pump and a Model Chromjet data handling system (Spectra-Physics, Santa Clara, CA, USA). A Model 757 variable-wavelength UV detector (ABI Analytical Kratos Division, Ramsey, NJ, USA) was used. A Model WISP-710B autosampler and Model 6000 HPLC pump (Waters, Milford, MA, USA) was used. A Model 7940 HPLC column heater (Jones Chromatography, Lakewood, CO, USA) was used. Chromatographic separations described in the method were made using Zorbax Stablebond CN columns (5 μm) measuring 15 cm × 4.6 mm I.D. (Mac-Mod Analytical, Chadds Ford, PA, USA). Precolumns employed in the column-switching system were LiChrosorb RP-8 (5 μm) 1 cm × 4.6 mm I.D. (Alltech, Deerfield, IL, USA; cat. No. 1542). Other precolumns evaluated included Adsorbosphere C_{18}, Hypersil C_{18}, Partisil ODS-2 and LiChrosorb RP-18. These precolumns all measured 1 cm × 4.6 mm I.D. and were also obtained from Alltech. For the column-switching system, a 10-port high-pressure valve and electric actuator was used (Valco, Houston, TX, USA, cat. No. ECIOU). Prior to use, the components of the eluent were filtered through 0.45-μm nylon membranes (Alltech).

Reagents

A commercial USP grade BAK solution containing 169.8 mg/ml of BAK was used as a standard throughout this work. The BAK content is expressed as the sum of the three major homologues characterized *versus* the USP standard. Tetrahydrofuran (THF) was UV grade, distilled in glass, from Fisher Scientific (Fair Lawn, NJ, USA). Triethylamine was reagent grade (99%) from Aldrich (Milwaukee, WI, USA). Phosphoric acid was reagent grade (85%) from J.T. Baker (Phillipsburg, NJ, USA).

The chromatographic eluent used was distilled water–THF–triethylamine (2500:1500:20), which was adjusted to an apparent pH of 3.0 ± 0.1 with phosphoric acid. The solid-phase extraction solution was a mixture of 70% THF and 30% chromatographic eluent (v/v). The column-switching wash solvent was distilled water–THF–phosphoric acid (1600:400:3). Murine and Murine Plus were formulated at Abbott Laboratories.

Chromatographic conditions

The following conditions were used: flow-rate, 2.0 ml/min; pressure, approximately 2600 p.s.i. (1 p.s.i. = 6894.76 Pa); detector, 215 nm at 0.10 a.u.f.s.; attenuation at 128 (solid-phase extraction) or 256 (column switching); and injection volume, 100 μl (solid-phase extraction) or 200 μl (column switching). The analytical column was maintained at 40°C.

Analytical procedure

The BAK standard solution was prepared by serially diluting the 169.8 mg/ml BAK solution in distilled water to a concentration of 51.0 μg/ml total

BAK. Replicate injections of the standard and sample preparations were made to obtained summed integrated areas of the C_{12}, C_{14} and C_{16} homologues with typical agreement of $\leqslant 2\%$. The sample was quantitated using the external standard method by ratioing the total peak areas of sample to standard and multiplying by the BAK concentration (μg/ml).

For the solid-phase extraction procedure Sep-Pak cartridges were pre-conditioned with the extraction solution followed by distilled water. In a batch process, 10 ml of sample, standard or blank (distilled water) were eluted through the cartridge. Each cartridge was washed with distilled water and the BAK was eluted with 3.0 ml of extraction solution followed by a 3.0-ml aliquot of eluent. The two extracts were combined and mixed.

For the column-switching procedure, the arrangement used to preconcentrate the sample and standard is shown in Fig. 2. A 0.20-ml aliquot of the sample or standard preparation was injected into the system with the valve in the load position. The wash pump was pumping the wash solvent at 3.0 ml/min. After 2 min, the valve was switched to the inject position to back-flush the BAK onto the analytical column. After the C_{16} homologue was detected, the valve was switched to the load position and the precolumn was re-equilibrated for 5 min before the next injection.

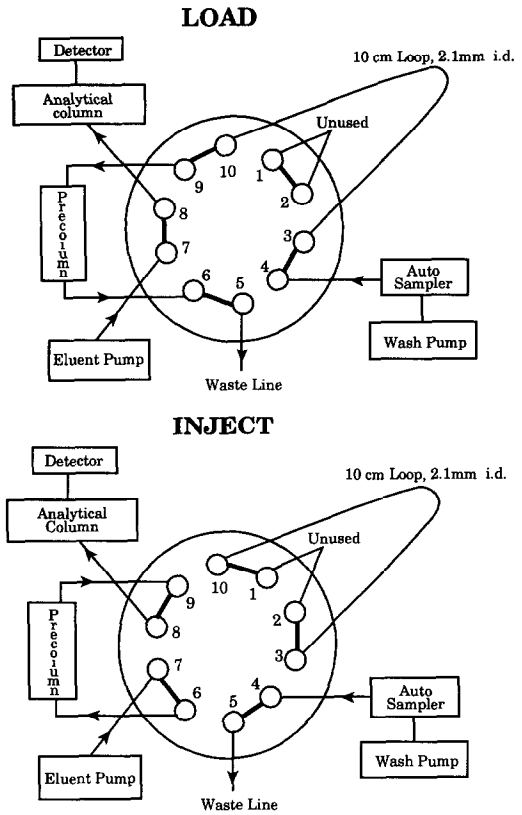

Fig. 2 Schematic diagram of HPLC system for column-switching procedure.

RESULTS AND DISCUSSION

In this work, our goal was to develop a rugged and reliable determination of BAK at a target concentration of 50 μg/ml in Murine and Murine Plus. Initial attempts at routine quantitation of BAK by direct injection using a previously reported method [9] did not provide acceptable sensitivity or precision at the target concentration. This approach failed because the chromatographic separation must resolve the C_{12} BAK homologue from relatively high concentrations of excipients present in the formulations while eluting the C_{16} BAK homologue in a reasonable time and with enough sensitivity to obtain reproducible peak areas. To overcome these difficulties, we originally developed the solid-phase extraction technique to remove the majority of the detectable product excipients and preconcentrate the analytes of interest. Shown in Fig. 3 are typical chromatograms for the BAK determination by solid-phase extraction. Detector linearity of the chromatographic finish was demonstrated by chromatographing standard solutions containing 40.8 to 204% of the analyte present after preconcentration. A plot of concentration (range = 34.0 to 169.9 μg/ml) *versus* total integrated peak area of BAK (range = $2.59 \cdot 10^6$ to $13.0 \cdot 10^6$ counts) gave a linear regression line having correlation coefficient > 0.9999, slope = $7.69 \cdot 10^4$, y-intercept = $-4.06 \cdot 10^4$. Standard errors in slope and y-intercept were $\pm 0.05 \cdot 10^4$ and $\pm 4.96 \cdot 10^4$, respectively.

The routine described in the solid-phase extraction was a compromise of conditions necessary for removing the majority of the formulation excipients while obtaining quantitative recovery of BAK. Absolute recoveries of individual BAK homologues and total BAK were determined from standards

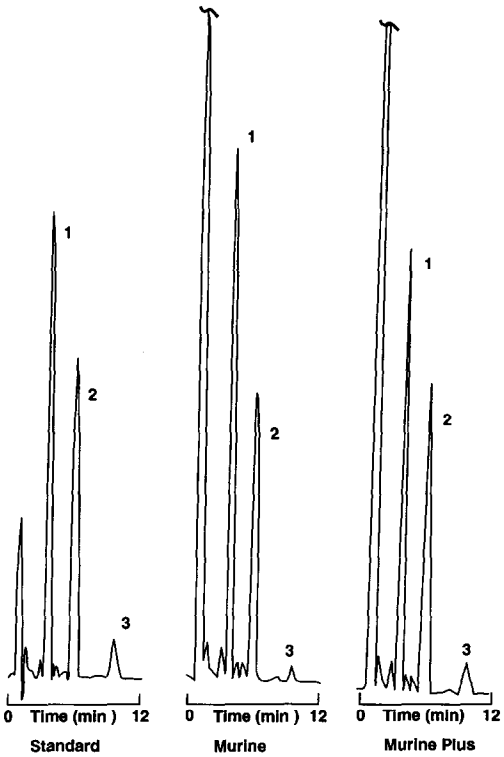

Fig. 3. Typical chromatograms by solid-phase extraction procedure. Peaks: 1 = C_{12} homologue; 2 = C_{14} homologue; 3 = C_{16} homologue.

and placebos using the procedure and a non-preconcentrated standard at appropriate concentrations. The recoveries were comparable between standards and the synthetic preparations containing the formulation excipients (Table I). Total recoveries ranged from 97.2 to 98.7%, with a slight, but consistent, loss of the C_{16} homologue. Also included in Table I are data from standard addition and recovery experiments in which both standard and synthetic mixtures were taken through the assay procedure to give relative recoveries. As shown, relative total recoveries of BAK ranged from 99.2 to 101.7% at 67.9 to 169.8% of the target concentration.

The assay precision for the solid-phase extraction technique was evaluated by determining BAK in Murine and Murine Plus on separate days using different analysts, chromatographic equipment and analytical columns. In Murine, a mean BAK concentration of 47.7 μg/ml was obtained with a relative standard deviation (R.S.D.) of ± 2.6%. In Murine Plus, a mean BAK concentration of 47.4 μg/ml was obtained with a R.S.D. of ± 2.2%.

To fully automate the preconcentration step, the column-switching procedure was designed. We chose to investigate this approach using 1 cm cartridges for the following reasons: (1) these columns are conveniently plumbed directly into the switch-

TABLE I

STANDARD ADDITION AND RECOVERY OF BAK BY SOLID-PHASE EXTRACTION

Homologue	Absolute recovery (%)					
	Standard 1	Standard 2	Standard 3	Average	Murine Plus	Murine
C_{12}	99.8	100.5	102.4	100.9	101.8	100.7
C_{14}	95.5	95.8	96.4	96.0	95.9	97.1
C_{16}	91.3	92.2	91.7	91.7	90.1	93.8
Total	97.2	97.6	98.7	97.8	98.0	98.4

Addition level (%)	Added (μg/ml)	Relative recovery			
		Murine		Murine Plus	
		Recovery (μg/ml)	Recovery (%)	Recovery (μg/ml)	Recovery (%)
67.9	34.0	34.5	101.7	34.2	100.6
101.9	51.0	51.8	101.6	51.2	100.4
169.8	84.9	85.7	100.9	84.2	99.2

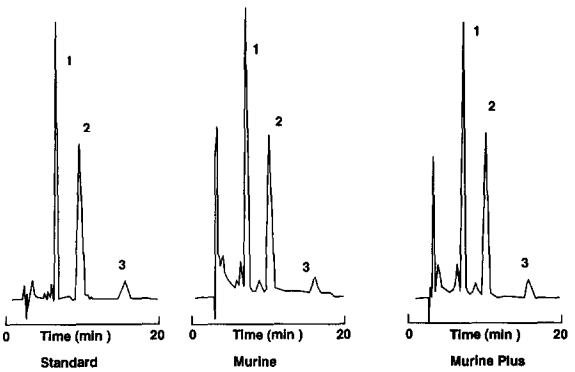

Fig. 4. Typical chromatograms by column-switching procedure. Peaks: 1 = C_{12} homologue; 2 = C_{14} homologue; 3 = C_{16} homologue.

ing valve, minimizing the dead volume of the system, and (2) this format is now commercially available in a variety of different packings. In order to obtain comparable results as shown for the solid-phase extraction, we investigated packings of Partisil ODS-2, Adsorbosphere C_{18}, LiChrosorb RP-18 and RP-8 and Hypersil ODS. The behavior of BAK on the LiChrosorb RP-8 phase was most acceptable in our application. This packing material retained BAK strongly enough to elute the formulation excipients prior to the back-flush while allowing the analytes of interest to be quantitatively removed by the HPLC eluent. Fig. 4 shows typical chromatograms for BAK using the column-switching procedure. Both the LiChrosorb RP-18 and Hypersil ODS materials show extremely strong retention of the analytes and would require eluents containing prohibitively large amounts of organic modifier in the back-flush step.

Detector linearity for the column-switching procedure was demonstrated using aqueous standards with the switching valve functioning as described in the procedure. A plot of BAK concentration (range = 17.0 to 169.8 μg/ml) *versus* total integrated area (range = $2.96 \cdot 10^6$ to $28.2 \cdot 10^6$ counts) gave a linear regression line having correlation coefficient > 0.9999, slope = $1.65 \cdot 10^5$ and *y*-intercept = $1.63 \cdot 10^5$). Standard errors in slope and *y*-intercept were $\pm 0.003 \cdot 10^5$ and $\pm 2.87 \cdot 10^5$, respectively.

The absolute recovery of BAK was assessed in a similar manner as described previously for the solid-phase extraction experiment. A standard solution of appropriate concentration was chromatographed directly without precolumn concentration.

TABLE II

STANDARD ADDITION AND RECOVERY OF BAK BY COLUMN SWITCHING

Homologue	Absolute recovery (%)						
	Standards, average (± R.S.D., %; n = 5)	Synthetic mixtures					
		At 68% level		At 102% level		At 170% level	
		Murine Plus	Murine	Murine Plus	Murine	Murine Plus	Murine
C_{12}	100.6 (± 0.98)	100.1	99.5	98.8	99.6	99.4	98.8
C_{14}	103.0 (± 0.66)	104.6	103.9	102.6	104.4	100.1	100.0
C_{16}	95.7 (± 1.2)	118.5	103.3	115.7	123.3	103.8	105.1
Total	101.3 (± 0.55)	103.6	101.8	101.9	103.7	100.0	99.9

Addition level (%)	Added (μg/ml)	Relative recovery (%)			
		Murine		Murine Plus	
		Recovery (μg/ml)	Recovery (%)	Recovery (μg/ml)	Recovery (%)
67.9	34.0	34.2	100.5	34.8	102.2
101.9	51.0	52.1	102.2	51.2	100.4
169.8	84.9	83.7	98.5	83.8	98.7

Absolute recoveries of total BAK from the standard averaged 101.3% (Table II). For the synthetic placebo mixtures, at concentrations of 68, 102 and 170% of the target value, total recoveries ranged from 99.9 to 103.7%. The variability of the recovery for the C_{16} homologue is attributable to the lower relative concentration of this component and to a slight peak broadening in the chromatography. Also shown in Table II are relative recoveries when the stated procedure is performed on the standard preparation and on synthetic mixtures of BAK added to placebos. Relative recoveries ranged from 98.5 to 102.2%.

The assay precision by column-switching was evaluated by determining BAK in Murine and Murine Plus on separate days using different analysts, chromatographic columns and precolumns. In Murine, a mean BAK concentration of 50.1 μg/ml was obtained with R.S.D. of ± 0.4%. In Murine Plus, a mean BAK concentration of 50.9 μg/ml was obtained with R.S.D. of ± 0.8%. The lots used in this study were different than those described previously in the solid-phase extraction experiments.

Throughout this work, the chromatographic finish appeared quite rugged. More than 200 injections of samples were made on columns packed from separate batches of packing with little loss in resolution. This probably results from the choice of the analytical column used and from eliminating the majority of the formulation excipients from the sample preparations. The two methods described provide comparable relative and absolute recoveries of the major BAK homologues and total BAK. Using the column-switching procedure, a slight amount of peak-broadening was observed in the chromatography. Both methods of pretreatment provide advantages of high sample throughput and excellent precision. The column-switching procedure allows total automation of the determination, eliminating the cost of the disposable cartridges and the time expended in manipulating the samples.

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