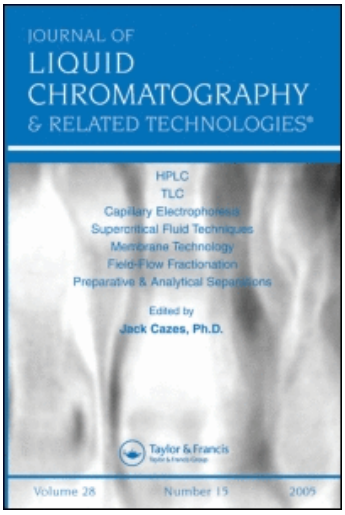

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R. Brent Miller[a]; Chuan Chen[a]; Charles H. Sherwood[a]
[a] Materials Characterization Department, IOLAB Corporation (a Johnson & Johnson Company), Claremont, California

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HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC DETERMINATION OF BENZALKONIUM CHLORIDE IN VASOCIDIN® OPHTHALMIC SOLUTION

R. BRENT MILLER*, CHUAN CHEN, AND CHARLES H. SHERWOOD
IOLAB Corporation (a Johnson & Johnson Company)
Materials Characterization Department
500 Iolab Drive
Claremont, California 91711

ABSTRACT

A high-performance liquid chromatographic (HPLC) procedure employing UV detection for the analysis of benzalkonium chloride (BAK) in Vasocidin® ophthalmic solution is reported. The method requires no sample pretreatment and is sensitive, accurate, and reproducible. The peak area versus BAK concentration is linear over the range of 50-150% of its label claim in Vasocidin®. The mean absolute recovery of BAK using the described method is 101.3 ± 2.16%, (mean ± SD, n = 10). A stress study with heat, acid, base and UV radiation indicates that the method is stability-indicating with no interference from drugs, excipients or their degradation products.

INTRODUCTION

Vasocidin® is a sterile topical ophthalmic solution possessing both anti-infective and anti-inflammatory properties. The anti-infective properties are obtained from sulfacetamide sodium (100 mg/mL), which impedes the synthesis of folic acid by competition with p-amino-

benzoic acid. The anti-inflammatory properties are gained from the corticoid steroid, prednisolone sodium phosphate (2.5 mg/mL). Vasocidin® is preserved with either thimerosal (0.1 mg/mL) or benzalkonium chloride (0.025 mg/mL).

Benzalkonium chloride, a preservative, is a mixture of alkylbenzyldimethylammonium chlorides of the general formula $[C_6H_5CH_2N(CH_3)_2R]Cl$. "R" represents a mixture of alkyls with the $n\text{-}C_{12}H_{25}$, $n\text{-}C_{14}H_{29}$, and $n\text{-}C_{16}H_{31}$ homologues comprising the major portion.

In Vasocidin®, a separation problem exists because sulfacetamide is 4000 times more concentrated than BAK. Attempts to separate the 3 major homologues of BAK from sulfacetamide with ODS, cyano, and amino bonded phases proved unsuccessful. Although HPLC methods exist for the determination of BAK in ophthalmic solutions [1-6], none of these formulations contained sulfacetamide. Consequently, this prompted the development of a method for quantitating BAK in an ophthalmic solution containing sulfacetamide, which is sensitive, accurate, and reproducible. In this manuscript, BAK is determined by ion-pair reversed-phase HPLC using a phenyl column with UV detection at 215 nm. Furthermore, Vasocidin® is injected with no sample pretreatment.

According to the USP XXII quidelines, analytical methods for the quantitation of major components of bulk drug substance or preservatives in finished pharmaceutical products fall under Assay Category I [7]. Data elements required for Assay Category I include precision, accuracy, selectivity, range, linearity, and ruggedness. The method for BAK in Vasocidin® ophthalmic solution satisifes all of these requirements. Moreover, this method was determined to be stability-indicating.

EXPERIMENTAL

Chemicals and Reagents

Vasocidin® ophthalmic solution was obtained from IOLAB Corporation (Claremont, CA, USA). Sodium sulfacetamide, prednisolone sodium phosphate, benzalkonium chloride, edetate disodium, and pluronic F127 were USP Reference Standards, while boric acid was NF. HPLC grade acetonitrile, 1 **N** sodium hydroxide, hydrochloric acid, and ACS reagent grade potassium phosphate, monobasic, monohydrate were purchased from J.T. Baker (Phillipsburg, NJ, USA). Hexane sulfonate, sodium salt, HPLC grade, was purchased from Eastman Kodak (Rochester, NY, USA). The water was deionized and distilled. All reagents were used without further purification.

Apparatus

The chromatographic system consisted of a Waters model 600E system controller and pump, a WISP 712D autosampler, and a Waters 486 variable-wavelength UV detector set at 215 nm (Waters Associates, Milford, MA, USA). A stainless-steel μBondapak™ phenyl column (30 cm x 3.9 mm, 10 μm, Waters Associates, Milford, MA, USA) was maintained at ambient temperature.

Mobile Phase

The mobile phase consisted of acetonitrile - buffer (65:35 V/V), where the buffer was comprised of 50 **m**M potassium phosphate, monobasic, monohydrate and 57 **m**M hexane sulfonate, sodium salt, adjusted to pH 6.3 with 1**N** NaOH. The mobile phase was filtered through a 0.45 μm filter and degassed for 30 minutes. The flow rate was 1.8 mL/minute with a typical operating pressure of **ca.** 1200 psi. Under these conditions, the retention times of the C_{12}, C_{14}, and C_{16} homologues of BAK were 7.4, 9.5, and 12.4 minutes, respectively.

Preparation of BAK Solutions

A BAK Stock Solution was prepared by accurately weighing a 125 mg equivalent (W_s) of BAK into a tared 50 mL volumetric flask and diluting to volume with water.

A BAK Standard Solution was prepared by pipeting 600 μL of the BAK Stock Solution into a 50 mL volumetric flask and diluting to volume with water.

System Suitability

The system suitability results were calculated according to Chromatography <621> of the USP XXII from typical chromatograms [7]. The instrument precision as determined by six successive injections of a BAK Standard Solution should provide a relative standard deviation (RSD) not greater than 1.0%. The column efficiency, when calculated using the C_{14} peak should be greater than 4500 theoretical plates. The tailing factor for any homologue should not exceed 2.0 at 5% peak height. Finally, the resolution between peaks should be greater than 1.5.

The Test Solution (Vasocidin® ophthalmic solution with no sample work-up) is used to verify that the method meets all of the suitability limits. If the system suitability limits are not met, adjust the parameters, change the analytical column or calibrate the system until system suitability is achieved.

Stress Study

The specificity of the method was studied through the analysis of stressed Test Solutions containing 100% label claim of BAK and stressed Placebo Solutions (Test Solution without BAK). The stressed samples were subjected to heat, acidic, basic and UV light environments.

Five mL aliquots of the Test and Placebo Solutions were sealed in transparent glass containers and exposed to a UV radiation source (200-400 nm, 40 mWatt/cm^2) for

15 hours. Other 5.0 mL aliquots were adjusted to either pH 2 with concentrated HCl or pH 12 with 50% NaOH and sealed in glass containers with equal head space and stored at 120°C for 15 hours.

Data Acquisition

The peak areas of the C_{12}, C_{14}, and C_{16} homologues of BAK were measured using a PE Nelson 900 series interface and down-loaded to PE Nelson Turbochrom II workstation (Perkin-Elmer Corporation, Cupertine, CA, USA). The chromatographic data was automatically processed for peak area followed by an unweighted linear regression analysis.

Calculations

The BAK content of the Test Solution was calculated according to the individual BAK homologue peaks. The total BAK content was obtained by combining the BAK homologue concentrations of the Test Solution. The response factor, RF_i, for each BAK homologue peak is:

$$RF_i = \frac{W_S \times F \times P_i \times V_1}{50.0\ mL \times 50.0\ mL \times PA_{Si} \times 100}$$

where W_S is the quantity (mg) of the BAK standard used, F is the purity factor (mg/mg) of the BAK standard, P_i is the composition (%) of any homologue in the BAK standard, V_1 is the volume (≈0.600 mL) of the BAK Stock Solution, and PA_{Si} is the peak area of the corresponding homologue of the BAK Standard Solution.

The concentration of any BAK homologue, C_{Ti}, is:

$$C_{Ti}\ (mg/mL) = RF_i \times PA_{Ti}$$

where RF_i is the response factor of any homologue peak and PA_{Ti} is the peak area of the corresponding homologue

of the Test Solution. The total BAK content, C, is:

$$C\ (mg/mL) = \Sigma C_{Ti}$$

where ΣC_{Ti} is the sum of all the BAK homologue concentrations calculated in the above section.

RESULTS AND DISCUSSION

Chromatography

Typical chromatograms obtained from a 100 μL injection of a Standard, Placebo and Test Solution are illustrated in Figure 1 (a-c), respectively. The retention times of the C_{12}, C_{14}, and C_{16} homologues of BAK were 7.4, 9.5, and 12.4 minutes, respectively. The overall chromatographic run time was 16 minutes.

The mobile phase was fine-tuned by examining the effect of k' over the pH range of 4.5 to 7.5 (Figure 2). The mobile phase buffer pH of 6.3 was selected because it met all system suitability parameters and furnished the best overall chromatograms.

System Suitability

The column efficiency for the C_{14} homologue of BAK was 5600 theoretical plates. The tailing factors of the C_{12}, C_{14}, and C_{16} homologues were 1.3, 1.3, and 1.2, respectively. The resolution was 3.2 between the C_{12} and C_{14} peaks and 3.1 between the C_{14} and C_{16} peaks. The instrument precision, determined by 6 replicate injections of the BAK Standard Solution, exhibited a RSD of 0.3%.

Precision and Accuracy

The precision (RSD) and accuracy (relative error, RE) were determined by analyzing Placebo Solutions spiked with BAK, in replicates of six, ranging from 50-150%

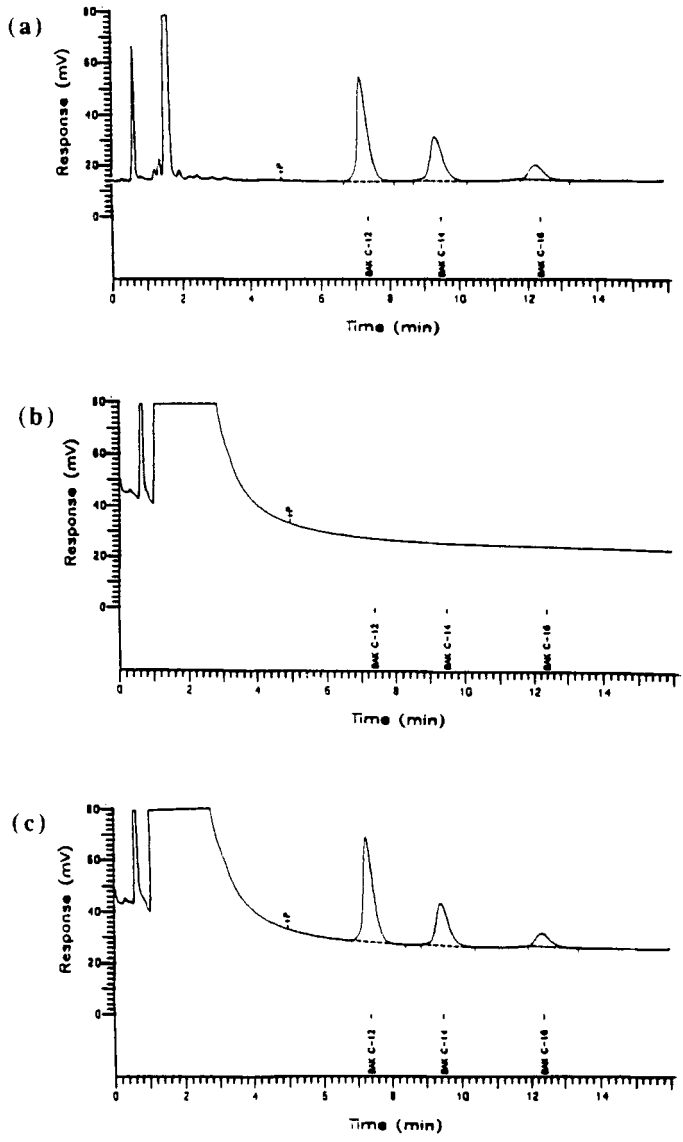

FIGURE 1. Typical chromatograms of (a) a Standard Solution of BAK, (b) Vasocidin® ophthalmic solution not containing BAK, and (c) Vasocidin® ophthalmic solution containing BAK.

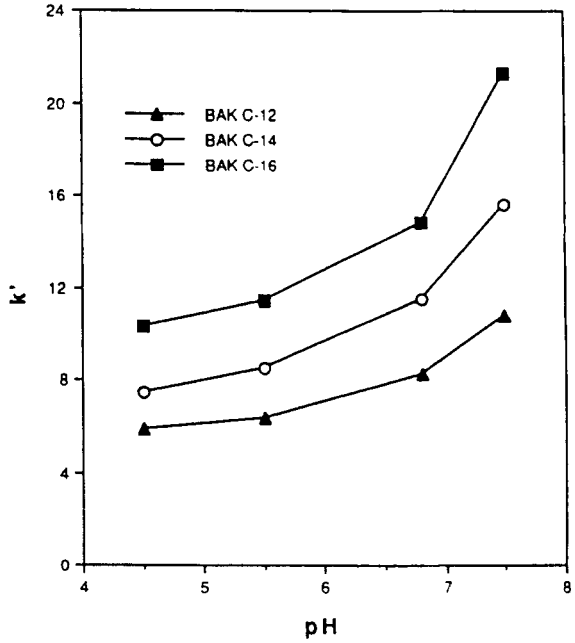

FIGURE 2. Influence of mobile phase pH on capacity factor (k') for the C_{12}, C_{14}, and C_{16} homologues of BAK.

(12.5-37.5 μg/mL) of its label claim in Vasocidin® (Table 1).

Linearity

A linear response in peak area for BAK over the range of 50-150% of its label claim in Vasocidin® was observed. The correlation coefficients were 0.998 or better (n=6).

Recovery

The recovery of BAK was determined by comparing the concentration found in Vasocidin® to that of the BAK

TABLE 1

Accuracy and Precision of BAK in Vasocidin®

Nominal Conc. (μg/mL)	n	Mean Found Conc. (μg/mL)	%RSD	%RE
12.50	6	12.19	1.6	-2.5
18.75	6	19.13	1.5	2.0
25.00	6	25.40	2.0	1.6
31.25	6	32.09	1.9	2.7
37.50	6	38.57	0.9	2.9

TABLE 2

% Recovery of BAK in Vasocidin®

% Label Claim of BAK in Vasocidin®	% Recovery
50	97.5
50	97.4
75	101.5
75	102.9
100	100.6
100	102.0
125	102.6
125	102.8
150	103.1
150	102.6

Standard Solution. The mean found recovery was 101.3 ± 2.16% (mean ± SD, n=10, Table 2).

Stress Study

All solutions were analyzed by the method described herein. Vasocidin® was stressed with heat, acid, base, and UV radiation to produce approximately 10% degradation of sulfacetamide. The acid stressed samples were adjusted to pH 2 with concentrated HCl and heated at

120°C for 15 hours. Due to precipitation, the pH 2 samples were neutralized with 1 N NaOH prior to analysis. The base stressed samples were adjusted to pH 12 with 50% NaOH and heated at 120°C for 15 hours. Ultraviolet light stressed samples were placed in the path of a UV lamp at 40 mWatt/cm^2 for 15 hours. No interfering peaks at the retention times for the BAK homologues were observed in any of the stressed sample or diluent solutions.

Conclusion

Various bonded phases including ODS, cyano, amino, and phenyl were evaluated in an attempt to satisfactorily resolve the three major homologues of BAK from a solution containing sulfacetamide. From these bonded phases, the phenyl provided the best overall chromatographic properties due to its increased polarity.

The wavelength selection of 215 nm for the quantitation of BAK was based on the UV characteristics of both BAK and sulfacetamide. At 215 nm, BAK exhibits a maxima whereas sulfacetamide exhibits a minima. Due to the high concentration of sulfacetamide in Vasocidin®, the selection of 215 nm was very favorable.

The described assay for the analysis of BAK in an ophthalmic solution containing sulfacetamide is sensitive, accurate, and reproducible. Furthermore, the method is stability-indicating with no interference from sulfacetamide or excipients or their degradation products under the described stress conditions.

REFERENCES

1. L. Elrod, Jr., T.G. Golich and J.A. Morley, J. Chromatogr., 625: 362-367 (1992).

2. R.C. Meyer, J. Pharm. Sci., 69: 1148-1150 (1980).

3. M.R. Euerby, J. Clin. Hosp. Pharm., 10: 73-77 (1985).

4. G. Ambrus, L.T. Takahashi and P.A. Marty, J. Pharm. Sci., 76: 174-176 (1987).

5. A. Gomez-Gomar, M.M. Gonzalez-Aubert, J. Garces-Torrents and J. Costa-Segarra, J. Pharm. Biomed. Anal., 8: 871-876 (1990).

6. D.F. Marsh and L.T. Takahaski, J. Pharm. Sci., 72: 521-525 (1983).

7. The United States Pharmacopeia XXII, pp. 1558-1567, U.S. Pharmacopeial Convention, Rockville, MD (1990).

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