November 1993
Volume 82, Number 11

JOURNAL OF PHARMACEUTICAL SCIENCES

A publication of the American Pharmaceutical Association

Journal of Pharmaceutical Sciences

APhA

The Journal of Pharmaceutical Sciences (ISSN 0022-3549) is published monthly by the American Pharmaceutical Association (APhA) at 2215 Constitution Ave., NW, Washington, DC 20037-2981. Second-class postage paid at Washington, DC, and at additional mailing offices.

Offices—2215 Constitution Ave., NW, Washington, DC 20037-2981; all *Journal* staff may be contacted at this address. Printing: William Byrd Press, Richmond, VA 23261.
Annual Subscriptions—The *Journal of Pharmaceutical Sciences* is the monthly research journal of the American Pharmaceutical Association. Annual membership dues are $150.00, and $50.00 of this amount applies towards a subscription to the *Journal of Pharmaceutical Sciences* or *American Pharmacy*. Nonmember rates are $195.00 for industrial, government, and educational institutions and $85.00 for individuals for personal use only. All foreign subscriptions add $60.00 for postage. Subscription rates are subject to change without notice. Single copy rates are $20.00, domestic and <None>foreign.
Page Charges—A page charge of $45.00 per printed page is levied after the acceptance of the manuscript to cover part of the publication cost. Payment is not a condition for acceptance; articles are accepted or rejected on merit alone.
Claims—Missing numbers will not be supplied if dues or subscriptions are in arrears for more than 60 days, or if claims are received more than 60 days after the date of issue, or if loss was due to failure to give notice of change of address. APhA cannot accept responsibility for foreign delivery when its records indicate shipment was made.
Postmaster—Send address changes to APhA Membership Division, 2215 Constitution Avenue, NW, Washington, DC 20037-2981.
Information for Authors—"Instructions for Authors" appears in the January issue. It is understood that a manuscript submitted to the *Journal* has not been published previously and is not being submitted elsewhere.

Microfilm—Available from University Microfilms International, 300 N. Zeeb Road, Ann Arbor, MI 48106.
Advertising—Advertisers should contact the Administrative Assistant, *Journal of Pharmaceutical Sciences*, 2215 Constitution Avenue, NW, Washington, DC 20037-

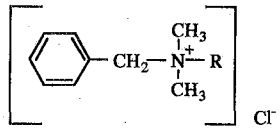
Determination of Benzalkonium Chloride in Ophthalmic Solutions Containing Tyloxapol by Solid-Phase Extraction and Reversed-Phase High-Performance Liquid Chromatography

TONY Y. FAN[x] AND G. MICHAEL WALL

Received September 3, 1992, from *Analytical Chemistry, Alcon Laboratories, Inc., 6201 South Freeway, Fort Worth, TX 76134.* Accepted for publication February 25, 1993.

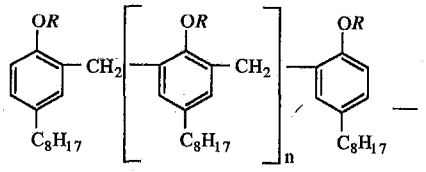
Abstract □ A procedure using solid-phase extraction (Supelcoclean CN) followed by HPLC [Beckman Ultrasphere CN, acetonitrile:phosphate solution (60:40, v/v)] was developed and validated to quantitate the quaternary ammonium preservative benzalkonium chloride in an experimental ophthalmic formulation containing the polymeric material tyloxapol. This procedure makes routine determinations of benzalkonium chloride at concentrations of 0.0035 to 0.01% simpler than the traditional ion-pairing colorimetric methods. This method is quick, specific, and especially useful for drug product stability studies. In addition, because the method distinguishes each homologue, it can be extended to routinely determine the homologue ratio for quality control purposes.

Benzalkonium[1] chloride (BAC; **1**) is widely used as an antimicrobial preservative in aqueous pharmaceutical preparations, especially in ophthalmic solutions. BAC is actually a mixture of *n*-alkylbenzyldimethyl ammonium chlorides with *n*-alkyl chain lengths varying from C_8 to C_{18}.[1] Because the homologues present different bactericidal activity,[2] it is sometimes necessary to determine not only the total amount of BAC but also the ratio of its homologues in the formulations. Among the European,[3] British,[4] and United States[1] pharmacopoeias, only the USP specifies the percentage of individual homologues: (1) the content of the *n*-$C_{12}H_{25}$ homologue is not <40.0%, (2) the content of the *n*-$C_{14}H_{29}$ homologue is not <20.0%, and (3) the total content of the $C_{12}H_{25}$ and $C_{14}H_{29}$ homologues comprise together not <70.0% of the total alkylbenzyldimethylammonium chloride content.[1]



$R = C_8H_{17}$ to $C_{18}H_{37}$

1



[*R* is $CH_2CH_2O(CH_2CH_2O)_mCH_2CH_2OH$; *m* is 6 to 8; *n* is not more than 5]

2

A quick and easy method for the determination of both the total and relative homologue ratio is desirable. HPLC with UV detection is a desirable technique because of its separation capability and suitability for automated routine analysis. For ophthalmic preparations, such determinations are not trivial because BAC is usually present in very low concentrations (0.007–0.01%, w/v), requiring low UV detection wavelengths (210–215 nm) for good sensitivity. Because other excipients are usually present in much higher concentrations, interference at these low wavelengths is a common problem.

Over the years, various specific and nonspecific methods have been developed for the determination of BAC. These have included extraction by complexing BAC with various dyes,[5–9] titration of quarternary ammonium compounds with iodate[10] or tetraphenylboron,[11] pyrolysis and subsequent gas chromatography,[12] chemical ionization mass spectrometry,[13] and HPLC of simple aqueous solutions.[14–16] In addition, the USP monograph describes a reversed-phase HPLC method to determine the homologue ratio of BAC raw material at a relatively high concentration (4 mg/mL) and a titration method to determine the total content of BAC in solution based on potassium iodate equivalents.[1] None of these methods could be directly used for the analysis of BAC in complex ophthalmic solutions because either they do not have the required specificity and sensitivity or they can not completely separate BAC from the matrix. Interferences have been observed by the presence of polymeric material, suspended particles, and active ingredients. These kinds of samples are not suitable for direct HPLC injection and therefore require some kind of sample preparation prior to HPLC.

The purpose of this study was to develop an HPLC method appropriate for measuring BAC in an experimental ophthalmic solution containing BAC (0.007%, w/v) and the polymeric material tyloxapol (0.25%, w/v). Tyloxapol (**2**), a polymeric alkyl aryl polyether alcohol commonly used as an emulsifier or surfactant, presented a problem for HPLC analysis of BAC because it produced a large solvent front that partially masked the BAC peaks. The USP HPLC method for BAC could not be used because of the interference of tyloxapol and the lack of sensitivity at 254 nm. To solve this problem, a combination solid-phase extraction (SPE)/HPLC procedure was developed. The combination of SPE sample clean-up with the resolving capability of HPLC provided a powerful tool for the routine analysis of complex ophthalmic solutions. This paper describes a SPE/HPLC method suitable for the determination of the total BAC content as well as each homologue ratio in an experimental ophthalmic solution containing tyloxapol. This technology should be applicable to other types of complex formulations.

Experimental Section

Apparatus—An HPLC system that consisted of a Hewlett-Packard 1090 quaternary pump (Hewlet-Packard, Fullerton, CA), a Waters Associates (Waters, Milford, MA) WISP 710B autoinjector, 490 programmable multiwavelength detector, and a Spectra-Physics ChromJet Integrator (Spectra-Physics, San Jose, CA) was used. All HPLC separations were performed isocratically on a 5 μm (150 × 4.6 mm, i.d.) Ultrasphere cyano (nitrile-bonded silane, CN) column (Beckman, San Ramon, CA). A Burdick & Jackson, 12-port solid-phase extraction manifold (Burdick & Jackson, Muskegon, MI) was used for the sample extractions. The manifold was connected to an in-house vacuum source, and a control valve was used to regulate the magnitude of vacuum applied. The container itself was large enough to allow 12 10-mL volumetric flasks to be attached to the rack at the same time for sample collection. A stop valve was also provided on each port for individual flow stoppage. All extractions were performed with Supelcoclean (Supelco, Bellefonte, PA) disposable cyano SPE columns with 1-mL capacity.

Reagents and Solutions—All reagents and solvents were reagent or HPLC grade and purchased from J. T. Baker (Phillipsburg, NJ). The phosphate solution was prepared by dissolving 6 mL of concentrated phosphoric acid (reagent grade) in 1950 mL of distilled water. The pH was adjusted to 5.0 by the addition of 50% NaOH solution, and the total volume was adjusted to 2 L with distilled water. The mobile phase was acetonitrile:phosphate solution (60:40, v/v), and the wash solvent was acetonitrile:phosphate solution (30:70, v/v). An experimental ophthalmic formulation was used for this study that contained proprietary drug (0.1%), mannitol (4.7%), sodium citrate (0.04%), citric acid (0.02%), tyloxapol (0.25%), BAC (0.007%), and edetate disodium (0.01%; all w/v). BAC was deleted for validation purposes, making this formulation a BAC vehicle.

Sample Preparation—Test solutions were prepared by the addition of appropriate amounts of BAC to an ophthalmic solution BAC vehicle (an ophthalmic solution containing all ingredients except BAC) that contained tyloxapol (0.025%, w/v) as one of the ingredients. A flow control valve was attached to each SPE column, and the whole unit was placed onto the female luer fitting of the vacuum manifold. Reduced pressure (~10 mmHg) was applied to the manifold with an in-house vacuum line. The SPE columns were conditioned with acetonitrile (2 mL) followed by distilled water (2 mL). When the level of the distilled water had reached ~1 mm above the top of the column packing, slow addition of the test sample (4 mL) was initiated. (Caution was taken not to disturb or dry out the column packing bed). After the sample had passed through, the column was washed with wash solvent (2 mL). The vacuum was disconnected, a 10-mL volumetric flask was placed under each SPE column, then the reduced pressure was applied again. The retained BAC was eluted from the column with mobile phase (5 mL), the vacuum was then disconnected, and the flasks were removed and diluted to volume with distilled water. These samples were directly analyzed by HPLC.

HPLC Assay Procedure—The mobile phase was mixed and filtered before use. The chromatographic system employed a flow rate of 2 mL/min, 100-μL injection volume, 10-min run time, UV detection (210 nm) at 0.01 AUFS, a recorder attenuation of ×8, and a chart speed of 0.5 cm/min. After a stable baseline was established, replicate standards were injected to ensure reproducibility prior to sample analysis. System suitability criteria were established: relative standard deviation of six replicate injections, ≤2.0%; resolution between the C_{12} and C_{14} peaks, ≥2; tailing factor for the C_{12} peak, ≤2; and number of theoretical plates, >3000 plates/column. A standard was inserted between every six samples. The BAC homologues were quantitated by the calculation described in the USP HPLC method,[1] taking into consideration the molecular weight of each homologue. The percentage of each BAC homologue and the percent recovery of total BAC were calculated as follows: % of each homologue = 100 A/B, and % recovery = 100 $B_{sample}/B_{standard}$, where A is the product of the area obtained from each homologue multiplied by its molecular weight and B is the sum of all of these products. The molecular weights of the C_{10}, C_{12}, C_{14}, C_{16}, and C_{18} BAC homologues (most common) are 312, 340, 368, 396, and 424, respectively.

Results and Discussion

A combination SPE/HPLC method was developed for determination of BAC in ophthalmic solutions containing the polymeric material tyloxapol. The low concentration of BAC in this experimental ophthalmic formulation (0.007%, w/v) necessitated using low UV wavelength (210 nm) detection for increased sensitivity. However, this low UV wavelength magnified interference problems encountered with direct HPLC analysis: tyloxapol eluted as a large peak after the solvent front, making quantitation of the BAC C_{12} homologue difficult and the BAC C_{10} homologue impossible (Figure 1). Solid-phase extraction was employed prior to HPLC to remove most of the interference by tyloxapol and, thereby, reduce excessively long run times (Figure 2). Only SPE columns from one manufacturer were used to obtain the data herein because vendor-to-vendor variability in SPE columns has been previously reported for cyano cartridges.[17]

Validation data were generated for this method with the experimental formulation. Linearity was satisfactory (Table I). Three six-point vehicle standard curves (duplicate samples at three different concentrations) were generated for the experimental ophthalmic formulation with concentrations of BAC ranging from 50 to 150% Label [0.007% (w/v) BAC = 100% Label; the concentration range for injected samples was 0.014–0.042 mg/mL]. The curves obtained were linear (r^2 = 0.999) and the y-intercepts, ranging from 1.2 to 3.1%, were small enough to justify the use of a single-point standard (Table I). Total recoveries were acceptable and in the range 97–103% (Table I). The precision was also satisfactory (Table II). The injection of three sets of six vehicle standard replicates [0.007% (w/v) BAC] gave acceptable values for relative standard deviations (ranging from 0.74 to 1.52%).

Though good results were obtained with this method most of the time, spurious results were infrequently observed; occasionally, an unexpected value (low or high by ~2%) was obtained for BAC. A significant amount of effort was expended trying to track down spurious data that might have been the

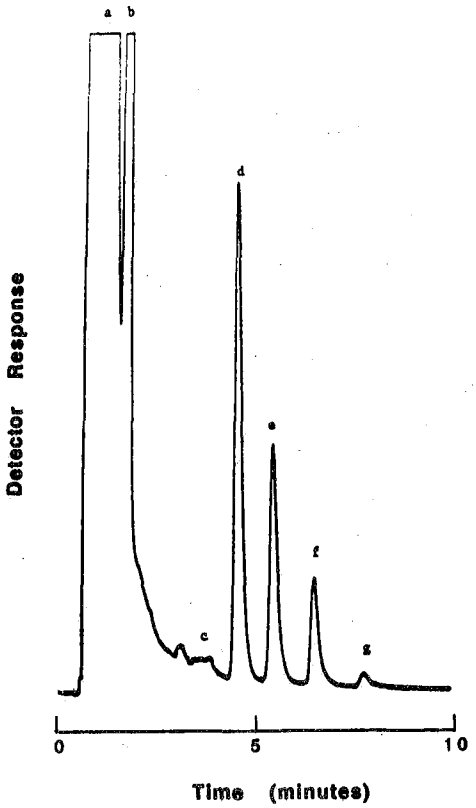


Figure 1—A typical HPLC chromatogram of BAC sample prior to extraction: (**a**) drug; (**b**) tyloxapol; (**c**) BAC C_{10}; (**d**) BAC C_{12}; (**e**) BAC C_{14}; (**f**) BAC C_{16}; (**g**) BAC C_{18}.

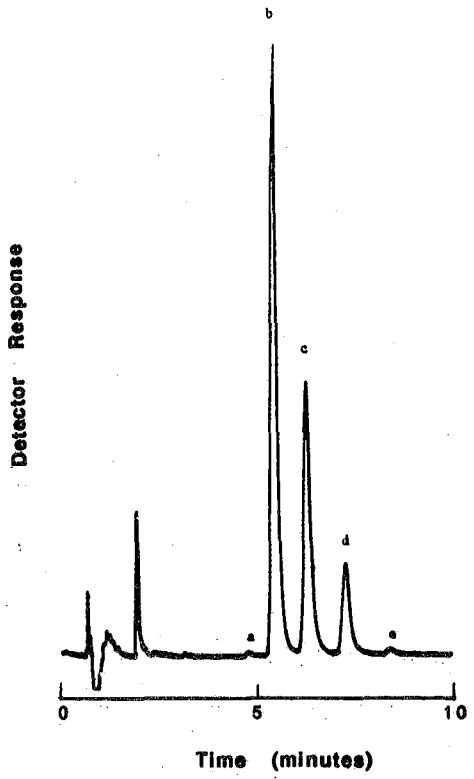


Figure 2—A typical HPLC chromatogram of BAC sample after SPE extraction: (**a**) BAC C_{10}; (**b**) BAC C_{12}; (**c**) BAC C_{14}; (**d**) BAC C_{16}; (**e**) BAC C_{18}.

Table I—BAC Vehicle Standard Curves[a]

Concentration, mg/mL	Area Counts (Recovery %)		
	Standard Curve 1	Standard Curve 2	Standard Curve 3
0.014	128 352 (98)	128 372 (98)	123 153 (98)
0.014	128 641 (98)	125 067 (97)	124 270 (98)
0.028	263 479 (98)	264 238 (101)	258 765 (100)
0.028	263 836 (98)	269 979 (100)	263 762 (102)
0.042	393 348 (97)	394 036 (103)	397 710 (103)
0.042	397 215 (98)	389 692 (100)	392 404 (101)
r^2	0.999	0.998	0.999
Avg. Recovery, %	98	100	100

[a] Samples ranging from 50% to 150% of the target concentration (0.028 mg/mL) were prepared by solid-phase extraction and were analyzed by high-performance liquid chromatography.

result of one or more possibilities; for example, allowing the SPE column to dry after conditioning and SPE column-to-column variability. The drying of a column after conditioning probably resulted in desolvation of the column packing and, hence, variable adsorption characteristics.[18] Also, batch-to-batch variation has been previously reported for disposable SPE columns for basic drugs on cyano (Bakerbond[19]) or C18 (Bakerbond[19] or Polymer Institute[20] brands), and catharanthus alkaloids on diol (Analytichem[21]) cartridges, suggesting the possibility of poor quality control of the SPE column packing process.

In conclusion, this method was proven to be sensitive, specific, precise, and accurate for the SPE/HPLC analysis of BAC in an experimental ophthalmic solution containing tyloxapol. The sample clean-up step (i.e., SPE extraction) and lower wavelength detection represent improvements over existing methods (e.g., the USP HPLC BAC method) that allow for analysis of BAC at low concentrations in tyloxapol-containing formulations. The method should be easily adapted to other solutions and suspensions containing polymeric material. The disadvantage of an infrequent spurious result was easily remedied by reassay of the suspect sample. It is thought that the occasional lack of precision was a result of variability between the SPE columns. None of these problems was deemed significant enough to preclude the use of this method because the magnitude of error for total BAC content was seldom >2%. Improvements in the commercially available SPE columns or alterations in the HPLC conditions may render this technique even more reliable, but until then, it is still an acceptable method for the analysis of BAC in complex ophthalmic solutions.

Table II—BAC Vehicle Standard Replicates[a]

Concentration, mg/mL	Area Counts (Recovery %)		
	Replicate Set 1	Replicate Set 2	Replicate Set 3
0.028	260 543 (101)	259 993 (101)	258 516 (100)
0.028	259 225 (101)	260 055 (101)	256 329 (100)
0.028	256 120 (99)	256 736 (100)	253 007 (98)
0.028	260 979 (101)	260 260 (101)	263 153 (102)
0.028	256 638 (100)	257 514 (100)	263 088 (102)
0.028	257 997 (100)	261 950 (102)	258 469 (100)
Rel. Std. Dev., %	0.78	0.74	1.52
Avg. Recovery, %	100	101	100

[a] Samples of 100% target concentration (0.028 mg/mL) were prepared by SPE and were analyzed by HPLC (see *Experimental Section* for conditions).

References and Notes

1. *United States Pharmacopeia*, 22nd rev.; U.S. Pharmacopeial Convention: Rockville, MD, **1990**; p 1905.
2. Giles, R.; Daoud, N. N.; Gilbert, P.; Dickson, N. A. *J. Pharm. Pharmacol.* **1983**, *34(suppl.)*, 110.
3. *European Pharmacopeia*, 2nd. ed.; Council of European: France, 1985, Part II-9, p 371.
4. *British Pharmacopeia*, vol. 1; British Pharmacopeia Commission: U.K., **1988**; p 63.
5. Auerbach M. E. *Anal. Chem.* **1943**, *15*, 492.
6. Colichman, E. L. *Anal. Chem.* **1947**, *19*, 430.
7. Ballard, C. W.; Isaacs, J.; Scott, P. G. W. *J. Pharm. Pharmacol.* **1954**, *6*, 971.
8. Chatten, L. G.; Okamura, K. O. *J. Pharm. Sci.* **1973**, *62*, 328.
9. Marsh, D. F.; Takahashi, L. T. *J. Pharm. Sci.* **1983**, *72*, 521.
10. Brown, E. R. *J. Pharm. Pharmacol.* **1963**, *15*, 379.
11. Metcalfe, L. D.; Martin, R. J.; Schmitz, A. A. *J. Am. Oil Chem. Soc.* **1966**, *43*, 355.
12. Jennings, E. C.; Mitchner, H. *J. Pharm. Sci.* **1967**, *56*, 590.
13. Daoud, N. N.; Crooks, P. A.; Speak, R.; Gilbert, P. *J. Pharm. Sci.* **1983**, *72*, 290.
14. Meyer, R. C. *J. Pharm. Sci.* **1980**, *69*, 148.
15. Ambrus, G.; Takahashi, L. T.; Marty, P. A. *J. Pharm. Sci.* **1987**, *76*, 174.
16. Comez-Gomar, A.; Gonzalez-Aubert, M. M.; Garces-Torrents, J.; Costa-Segarra, J. *J. Pharm. Biomed. Anal.* **1990**, *8*, 871.
17. Moors, M.; Massart, D. L. *Anal. Chim. Acta* **1992**, *262*, 135.
18. Van Horne, K. C. *Sorbent Extraction Technology*; Analytichem International: Harbor City, CA, 1985; pp 14–16.
19. Moors, M.; Massart, D. L. *J. Pharm. Biomed. Anal.* **1991**, *9*, 129.
20. Marko, V.; Radova, K.; Novak, I. *J. Liq. Chromatogr.* **1991**, *14*, 1659.
21. Vendrig, D. E. M. M.; Holthuis, J. J. M. *J. Chromatogr.* **1987**, *414*, 91.