an initial 2-ml sample. The day-to-day coefficients of variation (n = 24) of the procedure over a 3-week period, during which ~1000 plasma samples were analyzed, were 7% at 20 ng/ml and 6% at 75 ng/ml. The day-to-day coefficients of variation (n = 5) for the urine analyses were 6% at 20 ng/ml and 4% at 158 ng/ml. Within-day reproducibility for the plasma assay with n = 5 at concentrations of 2 and 50 ng/ml was CV = 8.7 and 1.3%, respectively.

Bioavailability Study Results—The assay procedure was used to measure the plasma and urine concentrations of phendimetrazine in 20 subjects in a two-way crossover bioavailability study (Fig. 2). The peak concentration of 70 ng/ml observed after one 35-mg dose of phendimetrazine tartrate and the elimination half-life of 2 hr observed for the immediate-release product were similar to those previously reported (5, 7). The average total recovery in urine over 48 hr after a 105-mg dose amounted to 5.72 ± 3.01 mg for the controlled-release formulation and 4.72 ± 2.89 mg for the immediate-release formulation. The assay procedure had adequate sensitivity to measure phendimetrazine in plasma at 24 hr, even for the subjects who received the immediate-release formulation. No predose plasma or urine samples had interferences at the phendimetrazine or internal standard retention times.

REFERENCES

(1) *Fed. Reg.*, **44**, 47618 (1979).

(2) R. T. Macnab, *Can. J. Med. Technol.*, **25**, 43 (1965).

(3) A. H. Beckett, *J. Pharm. Pharmacol.*, **19**, 273 (1967).

(4) D. V. Canfield, P. Lorimer, and R. L. Epstein, *J. Forensic Sci.*, **22**, 429 (1977).

(5) H. D. L. Hundt, E. C. Clark, and F. O. Muller, *J. Pharm. Sci.*, **64**, 1041 (1975).

(6) S. M. Walters, *ibid.*, **69**, 1206 (1980).

(7) A. H. Beckett and A. Raisi, *J. Pharm. Pharmacol.*, **28**, Suppl: 40P (1976).

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Determination of Benzalkonium Chloride in the Presence of Interfering Alkaloids and Polymeric Substrates by Reverse-Phase High-Performance Liquid Chromatography

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Abstract □ A specific assay for the analysis of benzalkonium chloride in the presence of interfering substances was conducted. The approach involved complexing benzalkonium chloride in an ophthalmic system with methyl orange, extraction of the complex into 1,2-dichloroethane, and subsequent analysis by reverse-phase high-performance liquid chromatography. Since the method separates each homologue of benz*alkonium chloride*, homologues not resident in the ophthalmic system were added as internal standards to improve both recovery and precision in the method.

Keyphrases □ High-performance liquid chromatography—determination of benzalkonium chloride, ophthalmic systems, complex with methyl orange □ Benzalkonium chloride—high-performance liquid chromatography, ophthalmic systems, complex with methyl orange

Various nonspecific dye extraction methods (1–3) have been developed for the determination of benzalkonium chloride. Recently, a specific analysis for benzalkonium chloride in aqueous solution by high-performance liquid chromatography (HPLC) was developed (4). This method involves direct injection of the aqueous formulation onto the chromatographic column. However, this method of analysis proved unsatisfactory for ophthalmic systems containing polymeric material. Polyvinyl alcohol, for example, precipitates under assay conditions, thus plugging the HPLC column. Suspended particulate matter in the formulation, such as steroid suspensions, also precludes the use of the method for the same reason.

Another difficulty encountered with the direct injection procedure is the interference of active alkaloids with the benzalkonium chloride during chromatography. Benzalkonium chloride is generally present in ophthalmic systems at the antimicrobial level of 0.004%, while active ingredients are present in considerably greater concentration. Also, active ingredients have considerably higher extinction coefficients in the UV than does the benzalkonium chloride preservative. These factors make benzalkonium chloride difficult to detect by HPLC if its retention time and that of the active ingredients are at all similar.

The purpose of this paper is to describe an extraction procedure to determine benzalkonium chloride in problem systems which preclude direct injection of the samples.

EXPERIMENTAL

Apparatus—The HPLC consisted of a pump[1], an automatic sampler[2], a reverse-phase microcyano column[3], a 254-nm detector[4], and a recorder[5]. Peak integrations were performed with a laboratory data system[6].

Reagents and Solvents—The mobile phase was 58% acetonitrile[7] (UV grade) and 42% 0.161 *M* sodium propionate at pH 5.35. Sodium carbonate[8] (7.5 g) was mixed with distilled water in a 2000-ml volumetric flask. Propionic acid[9] (12 ml) was added, and the solution was brought to volume (2000 ml) with distilled, deionized water. This solution was mixed with 2800 ml of acetonitrile.

Preparation of the C_{10} and C_{18} Homologues of Benzalkonium Chloride (as the Methyl Orange Complex)—In a 1000-ml round-bottom flask 22.1 g (0.1 mole) of 1 bromodecane[10], 13.5 g (0.1 mole) of *N,N*-dimethylbenzylamine[9], 500 ml of acetonitrile, and 30 ml of dimethylformamide[9] were added. This solution was allowed to reflux (86°)

[1] Model 6000A, Waters Associates, Milford, Mass.
[2] Wisp 710B, Waters Associates, Milford, Mass.
[3] μBondapak CN (10-μm particle size, 30-cm long × 4-mm i.d. column), Waters Associates, Milford, Mass.
[4] Model 440, Waters Associates, Milford, Mass.
[5] Omniscribe, Houston Instruments, Austin, Tex.
[6] Model 3352B, Hewlett-Packard, Fullerton, Calif.
[7] Burdick & Jackson Laboratories, Muskegon, Mich.
[8] Mallinckrodt, Inc., St. Louis, Mo.
[9] J. T. Baker Chemical Co., Phillipsburg, N.J.

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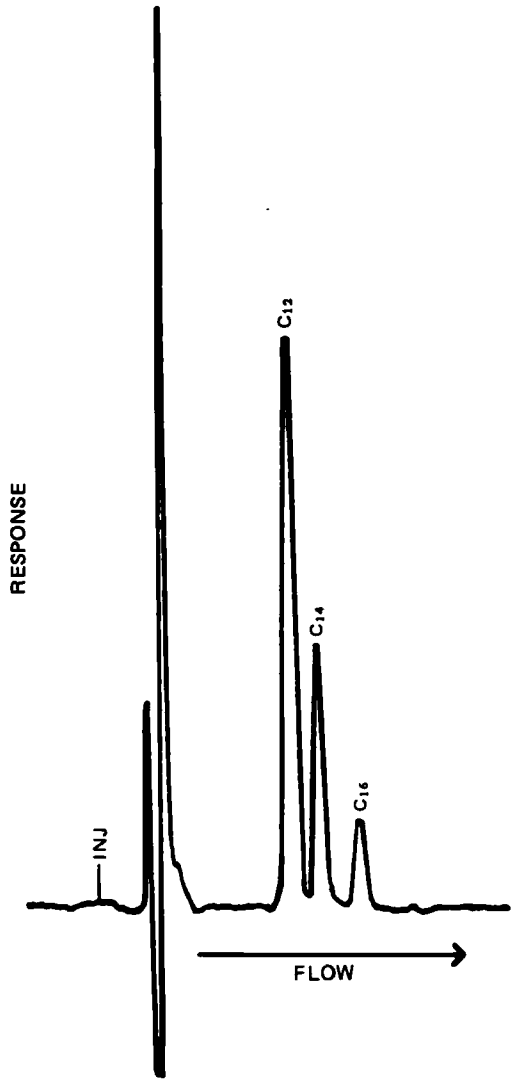

Figure 1—*Chromatogram of benzalkonium chloride without internal standards.*

for 3 days. To the hot, well-stirred solution was added 20 g of methyl orange[9]. The resulting solution was filtered hot. After cooling, the filtrate was refiltered. The orange crystalline needles thus obtained were analyzed by HPLC. The C_{10} homologue was the only peak observed. This peak had the same retention time as a USP standard benzalkonium chloride sample containing the C_{10} homologue. The C_{18} homologue was prepared in an analogous manner from 0.1 mole each of 1-chlorooctadecane[10] and *N,N*-dimethylbenzylamine in 500 ml of acetonitrile and 200 ml of dimethylformamide (heated at reflux for 5 days).

Preparation of the Internal Standard Mixture—Three grams of the C_{10} homologue of benzalkonium chloride was combined with 2 g of the C_{18} homologue. This mixture was finely ground with a mortar and pestle. A 0.06-g portion of this mixture was placed in a 1000-ml volumetric flask and 250 ml of dimethylformamide was added. A 0.250-g sample of methyl orange was dissolved in 500 ml of water. The aqueous solution of methyl orange was combined with the dimethylformamide solution of the benzalkonium chloride complex and 25 ml of acetic acid[9] was added. The solution was brought to volume with dimethylformamide.

Procedure—Samples and standards can be prepared and analyzed by HPLC both with and without the use of internal standards (Methods 1 and 2, respectively). Samples containing suspended steroids have been assayed by either method.

Standard Preparation—Raw material (50% w/v)[11] to be used as a secondary reference standard was diluted to 0.1% with water and assayed using a USP primary reference standard (10% w/v)[12] diluted to 0.100%.

[10] Matheson Coleman & Bell, Norwood, Ohio.
[11] Ruger Chemical Co., Inc. Irvington, N.J.
[12] U.S. Pharmacopeial Convention, Inc., Rockville, Md.

Table I—Recoveries of Benzalkonium Chloride in Nonextracted *versus* Extracted Samples

	UV Detector Responses of Homologues, mV/min			
	C_{12}	C_{14}	C_{16}	Total
	(A) Standard—Direct Injection			
	693	304	112	1,109
	673	302	119	1,094
	679	294	112	1,085
	687	319	119	1,117
	660	321	119	1,127
	660	309	112	1,081
	666	301	116	1,083
Mean	673.9	307.1	115.6	1,099
SD	14.6	9.9	3.5	18.3
RSD (±%)	2.2	3.2	3.0	1.7
	(B) Standard—Extraction Procedure			
	662	277	100	1,039
	659	285	121	1,065
	669	262	105	1,066
	660	283	117	1,060
	639	291	109	1,000
	670	280	109	1,069
Mean	659.8	280.4	109.0	1,049
SD	11.2	9.1	7.7	26.3
RSD, ± %	1.7	3.2	7.0	2.5
	(C) In Spiked Placebo—Extraction Procedure			
	639	280	102	1,021
	652	282	99	1,033
	670	290	99	1,059
	644	278	81	1,003
	649	266	84	1,000
	669	249	98	1,016
Mean	653.8	274	93.8	1,022
SD	12.9	14.6	8.9	21.8
RSD, ± %	2.0	5.3	9.5	2.1

Since no interfering substances are present in these aqueous solutions, these standards were not extracted and were injected directly without use of internal standards. For the assay of benzalkonium chloride in samples, the secondary reference standard was further diluted to the same concentration as the samples, usually 0.004% w/v.

Method 1 (Using Both Internal Standards)—The solution of sample or working standard (1.00 ml) was placed into a 12 ml plastic centrifuge tube[13], and 2.00 ml of the working internal standard solution was added. The solution was agitated briefly and allowed to equilibrate for 10 min. Then, 1.00 ml of 1,2-dichloroethane[9] was added, the mixture was vortexed for 1 min, allowed to stand for a few moments, and vortexed for an additional minute. The mixture was then centrifuged for 20 min at 8000 rpm. The phases were separated, and the organic phase was dried (anhydrous sodium sulfate) to give a yellow solution which was assayed by HPLC.

Method 2 (Using No Internal Standard)—The solution (1.00 ml) was added to a 10 ml plastic centrifuge tube, and 1.00 ml of a 0.05% aqueous solution of methyl orange, followed by 1.00 ml of a 10% aqueous solution of acetic acid were added. The solution was agitated briefly and allowed to equilibrate for 10 min. 1,2-Dichloroethane (1.0 ml) was added and the procedure in Method 1 was followed.

Assay—The mobile phase was filtered (1 μm) and deaerated. The system had the following parameters: a flow rate of 2.0 ml/min, giving a pressure of 1000–1500 psi, a 180-μl injector volume, an analysis time of 13 min, 254 nm detection at 0.01 AUFS, and a chart speed of 0.25 cm/min. After a stable baseline was achieved, replicate standards were run to ensure reproducibility, followed by the samples. A standard was run after every third sample.

The laboratory data system[6] was used to monitor the internal standard and the benzalkonium chloride peak areas. Since the concentration of the homologues other than C_{12}, C_{14}, and C_{16} in a benzalkonium chloride sample typically comprised <2% of the total, only the C_{12}, C_{14}, and C_{16} peaks were monitored. Chromatograms of benzalkonium chloride with and without internal standards are shown in Figs. 1 and 2.

Calculations for samples run without the use of internal standards are straightforward, relating the peak areas of the sample to the standard and adjusting this ratio for the standard concentration. When both in-

[13] Nalge Co., Division of Sybron Co., Rochester, N.Y.

Table II—Recovery and Precision of Benzalkonium Chloride in Spiked Placebo Containing Naphazoline Hydrochloride, Polyvinyl Alcohol, and Disodium Edetate [a]

	Weight/Volume, %		
	Operator 1—Day 1	Operator 1—Day 2	Operator 2
	0.00419	0.00421	0.00435
	0.00417	0.00423	0.00434
	0.00418	0.00417	0.00430
	0.00410	0.00421	0.00424
	0.00422	0.00420	0.00432
Mean	0.00417	0.00420	0.00431
SD	0.000044	0.000022	0.000044
RSD, ± %	1.1	0.52	1.0
Recovery, %	99.8	100.5	103.1

[a] No internal standard used; Method 2; theoretical value 0.00418% (w/v).

Table III—Recovery and Precision of Benzalkonium Chloride in Spiked Placebo Containing Phenylephrine Hydrochloride, Disodium Edetate, Pyrilamine Maleate, and Polyvinyl Alcohol [a]

	Weight/Volume, %		
	Operator 1—Day 1	Operator 1—Day 2	Operator 2
	0.00397	0.00404	0.00389
	0.00414	0.00409	0.00403
	0.00404	0.00404	0.00389
	0.00406	0.00405	0.00420
	0.00417	0.00404	0.00401
Mean	0.00408	0.00405	0.00400
SD	0.000080	0.000022	0.00013
RSD, ± %	2.0	0.54	3.2
Recovery, %	97.6	95.7	95.7

[a] No internal standard used; Method 2; theoretical value 0.00418% (w/v).

Table IV—Recovery and Precision of Benzalkonium Chloride in Spiked Placebo Containing Fluorometholone (as Suspension) and Polyvinyl Alcohol [a]

	Weight/Volume, %		
	Operator 1—Day 1	Operator 1—Day 2	Operator 2
	0.00404	0.00402	0.00401
	0.00398	0.00408	0.00400
	0.00408	0.00407	0.00389
	0.00401	0.00405	0.00406
	0.00406	0.00405	0.00402
Mean	0.00403	0.00405	0.00400
SD	0.000040	0.000023	0.000064
RSD, ± %	0.99	0.57	1.6
Recovery, %	96.5	97.0	95.8

[a] No internal standard used; Method 1; theoretical value 0.00418% (w/v).

ternal standards are used (Method 1), the calculations are more involved.

The weighting system described below is used to adjust for the varying partition coefficients of each homologue of benzalkonium chloride in the systems studied.

In many formulations, the extractability of each homologue follows the chain length. Recoveries of the individual homologues diminish as the chain length increases (see Table IB and C). For this reason, two internal standards, the C_{10}–C_{18} homologues are used to bracket the C_{12}–C_{16} benzalkonium chloride homologue resident in the formulation. If the C_{10} homologue alone is used as the internal standard, recoveries of total benzalkonium chloride are low due to the disproportionately high percentage of longer length homologues retained by the polymeric formulations. Conversely, inordinately high recoveries are obtained if the C_{18} homologue is used as the internal standard.

Thus, it is necessary to use both internal standards in such a way as to correct for the retention of each individual homologue. While both internal standards are used in these corrections, the internal standard with a chain length nearest the homologue in question is given the heaviest weight. All weighting factors (Method 1) in the equations that follow are a function of the chain-length differences of the homologues involved.

$$\% \text{ Benzalkonium Chloride} = \frac{C^*[F_1{}^*P_2 + F_2{}^*P_3 + F_3{}^*P_4]}{[PS_2 + PS_3 + PS_4]}$$

Table V—Recovery and Precision of Benzalkonium Chloride in Spiked Placebo Containing Polyvinyl Alcohol and Prednisolone Acetate (as Suspension) [a]

	Weight/Volume, %		
	Operator 1—Day 1	Operator 1—Day 2	Operator 2
	0.00420		0.00395
	0.00412	0.00415	0.00417
	0.00409	0.00440	0.00413
	0.00425	0.00419	0.00408
	0.00428	0.00420	0.00404
Mean	0.00419	0.00423	0.00407
SD	0.000082	0.00011	0.000085
RSD, ± %	1.9	2.6	2.1
Recovery, %	100.3	101.4	97.5

[a] No internal standard used; Method 2; theoretical value 0.00418% (w/v).

Table VI—Effectiveness of the Internal Standard in the Recovery of Benzalkonium Chloride [a]

	% Recovery Without Internal Standards	% Recovery With Internal Standards
	82.9	99.9
	93.4	97.4
	88.5	99.7
	88.0	101.9
	90.2	102.9
	87.8	95.5
		96.9
		102.4
Mean	88.5	99.6
SD	3.4	2.7
RSD, ± %	3.9	2.8

[a] Sample contains polyvinyl alcohol and prednisolone acetate.

Table VII—Recovery and Precision of Benzalkonium Chloride in Spiked Placebo Containing Polyvinyl Alcohol and Disodium Edetate [a]

	Weight/Volume, %		
	Operator 1—Day 1	Operator 1—Day 2	Operator 2
	0.00395	0.00378	0.00404
	0.00385	0.00383	0.00396
	0.00394	0.00385	0.00400
	0.00391	0.00379	0.00412
	0.00393	0.00384	0.00415
	0.00395	0.00380	0.00392
Mean	0.00392	0.00381	0.00403
SD	0.00004	0.00003	0.00009
RSD, ± %	0.97	0.76	2.2
Recovery, %	99.5	96.8	102.4

[a] Using both internal standards; Method 1; theoretical value 0.00394% (w/v).

where,

C = Concentration of benzalkonium chloride standard in % (w/v)

$F_1 = [3^*R_1 + R_2]/4$

$F_2 = [R_1 + R_2]/2$

$F_3 = [R_1 + 3^*R_2]/4$

and,

$R_1 = PS_1/P_1$

$R_2 = PS_5/P_5$

and,

P_1 = Peak area of the C_{10} homologue in the sample

P_2 = Peak area of the C_{12} homologue in the sample

P_3 = Peak area of the C_{14} homologue in the sample

P_4 = Peak area of the C_{16} homologue in the sample

P_5 = Peak area of the C_{18} homologue in the sample

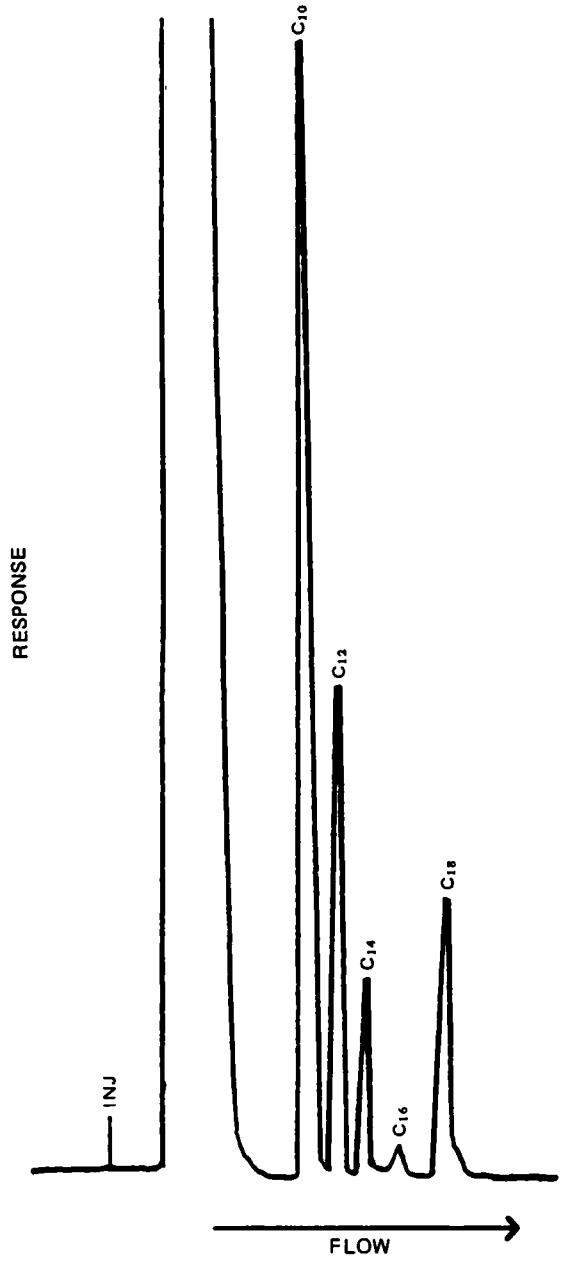

Figure 2—*Chromatogram of benzalkonium chloride with internal standards.*

and,

PS_1 = Average peak area of the C_{10} homologue in the standard

PS_2 = Average peak area of the C_{12} homologue in the standard

PS_3 = Average peak area of the C_{14} homologue in the standard

PS_4 = Average peak area of the C_{16} homologue in the standard

PS_5 = Average peak area of the C_{18} homologue in the standard

The PS_{1-5} values represent an average of the standard peaks that precede and follow the sample being calculated.

RESULTS AND DISCUSSION

Since benzalkonium chloride is now widely used in a variety of ophthalmic formulations, several problems have surfaced which have made direct sample injection impossible. These problems, which have been mentioned earlier, have been circumvented by preliminary treatment of the benzalkonium chloride with methyl orange. This procedure takes advantage of the lipophilic properties of the benzalkonium chloride–methyl orange ion pair. The dye complex is removed from interfering polymers and alkaloids by extraction into 1,2-dichloroethane. It is this isolated dye complex and the subsequent chromatography that gives the assay procedure its specificity. Experiments in validating this procedure are described.

Table VIII—Recovery and Precision of Benzalkonium Chloride in Spiked Placebo Containing Fluorometholone, Gentamicin, and Polyvinyl Alcohol [a]

	Weight/Volume, %		
	Operator 1—Day 1	Operator 1—Day 2	Operator 2
	0.00429	0.00430	
	0.00431	0.00424	0.00410
	0.00390	0.00430	0.00422
	0.00425	0.00428	0.00418
	0.00422	0.00426	0.00434
	0.00435	0.00437	0.00424
	0.00422	0.00441	0.00401
	0.00425	0.00425	0.00415
	0.00416	0.00426	0.00376
Mean	0.00422	0.00430	0.00413
SD	0.00013	0.00006	0.00018
RSD, ± %	3.1	1.3	4.3
Recovery, %	100.9	102.8	98.7

[a] Using both internal standards; Method 1; theoretical value 0.004176% (w/v).

An aqueous benzalkonium chloride standard (0.004%) was injected several times onto the chromatograph. The results, shown in UV-detector response for the three major homologues and the total, are shown in Table IA. This same aqueous standard was extracted into an equal volume of 1,2-dichloroethane as the methyl orange complex. The results are shown in Table IB. Also, a typical ophthalmic formulation containing polyvinyl alcohol was spiked to give 0.004% benzalkonium chloride and extracted in the same manner as the standard into an equal volume of 1,2-dichloroethane. The results are shown in Table IC. Analysis of the mean values for total response in the three experiments indicates that, while benzalkonium chloride is readily extractable into dichloroethane as the methyl orange complex, the extraction is not complete. The Student's *t* values obtained by comparing the mean total response value in Table IA *versus* those in Table IB and C demonstrate that significant differences exist between the mean values involved. Comparison of the mean values for total response for the extracted aqueous standard in Table IB and the extracted spiked formulation in Table IC show equivalence of means and variances using Student's *t* and F tests. The mean total response for the extracted placebo in Table IC represents a 97.4% recovery when compared with the mean total response for the extracted standard in Table IB. If standards and samples are treated in the same manner, recoveries of 95–100% are found for most ophthalmic systems. Shown in Tables II–V are the results obtained in four representative studies.

While in the majority of cases acceptable recoveries are obtained by the simple extraction method (Method 2), low recovery values are sometimes observed. To improve the recovery, two benzalkonium chloride homologues not present in the ophthalmic formulation were added as internal standards (Method 1). Observations of the data shown in Table IA, B, and C demonstrate that not only does the extractability of benzalkonium chloride vary in ophthalmic systems but also within the homologous series itself. The longer length homologues are clearly more

Table IX—Recovery and Precision of Benzalkonium Chloride in Spiked Placebo Containing Polyvinyl Alcohol (3%) and Disodium Edetate [a]

	Weight/Volume, %		
	Operator 1—Day 1	Operator 1—Day 2	Operator 2
	0.00414	0.00429	0.00420
	0.00447	0.00433	0.00423
	0.00423	0.00444	0.00405
	0.00430	0.00417	0.00449
	0.00438	0.00444	0.00456
	0.00450	0.00430	0.00456
	0.00408	0.00410	0.00456
	0.00389	0.00407	0.00439
	0.00400	0.00405	0.00435
Mean	0.00422	0.00424	0.00438
SD	0.00021	0.00015	0.00019
RSD, ± %	5.0	3.6	4.3
Recovery, %	101.0	101.5	104.8

[a] Using both internal standards; Method 1; theoretical value 0.004176% (w/v).

Table X—Recovery and Precision of Benzalkonium Chloride in Spiked Placebo Containing Oxymetazoline and Polyvinyl Alcohol [a]

	Weight/Volume, %		
	Operator 1—Day 1	Operator 1—Day 2	Operator 2
	0.00434	0.00409	0.00410
	0.00402	0.00419	0.00446
	0.00418	0.00389	0.00414
	0.00419	0.00399	0.00452
	0.00418	0.00410	0.00429
	0.00415	0.00424	0.00425
	0.00431	0.00426	0.00434
	0.00421	0.00434	0.00426
	0.00446	0.00409	0.00423
Mean	0.00423	0.00413	0.00430
SD	0.00013	0.00014	0.00014
RSD, ± %	3.0	3.4	3.2
Recovery, %	101.1	98.9	102.8

[a] Using both internal standards; Method 1; theoretical value 0.004176% (w/v).

difficult to extract from aqueous solutions containing polymers. Therefore, both internal standards must be used to achieve consistently good recoveries for benzalkonium chloride in the many different types of ophthalmic solutions. For instance, shown in Table VI is an example of a formulation containing polyvinyl alcohol and prednisolone acetate in which poor recoveries were obtained without internal standards, but recoveries were acceptable with the internal standards. In Tables VII–X are four representative studies using Method 1 to determine benzalkonium chloride.

The methyl orange-benzalkonium chloride complex proves surprisingly stable in both acid and basic environments. Several aliquots of the 1,2-dichloroethane solution of the methyl orange-benzalkonium chloride complex were washed with 1*N* sodium hydroxide and analyzed. Several aliquots of the complex were washed with 10% acetic acid and analyzed. No significant differences were observed (Student's *t*) when the two studies were compared. This permits an acid or base wash of the extracted methyl orange-benzalkonium chloride complex in further clean-up of the sample.

Table XI shows the assay results obtained by a modification of Method 1 and Method 2 on a formulation containing a 1000:1 ratio of antazoline phosphate to benzalkonium chloride. The dichloroethane solution of methyl orange–benzalkonium chloride complex, obtained by the extraction of the aqueous formulation, also contains significant amounts of antazoline. The presence of the antazoline makes analysis of benzalkonium chloride impossible, as retention times for each substance are similar. The antazoline can be removed, however, by two extractions with 10% acetic acid. As the results in Table XI clearly indicate, internal standards must be used when additional extractions are necessary.

Table XI—Recoveries After Double-Acid Wash of Extracted Sample (% w/v)

Method 2—No Internal Standard [a]	Method 1 with Internal Standard [b]
0.00300	0.00387
0.00325	0.00392
0.00303	0.00399
0.00303	0.00388
0.00305	0.00398
	0.00420
	0.00402
	0.00415
Mean 0.00307	0.00400
SD 0.000101	0.00012
RSD, ± % 3.3	3.0
Recovery, % 72.7	98.9

[a] Theoretical value 0.00418% (w/v). [b] Theoretical value 0.00404% (w/v).

System suitability requirements for multiple standard injections are that the relative standard deviation be <±3%. A column should have at least 2000 plates per column for the C_{12} homologue calculated by $N = 16\text{-}(w/v)^2$.

The versatility of this method has been shown in its ability to be used for various types of formulations including suspensions of steroids, polymeric compounds, and other ingredients which would interfere without prior sample workup. Because of the capability of using either (or both) acid and base washing of the extracted benzalkonium–dye complex, the method is very flexible in removing these interferences. Whatever modifications to the assay are required, it is essential that the standards and samples be treated in exactly the same way throughout the assay.

REFERENCES

(1) C. W. Ballard, J. Isaacs, and P. G. W. Scott, *J. Pharm. Pharmacol.*, **6**, 971 (1954).

(2) E. L. Colichman, *Anal. Chem.*, **19**, 430 (1947).

(3) L. G. Chatten and K. O. Okamura, *J. Pharm. Sci.*, **62**, 1328 (1973).

(4) R. C. Meyer, *ibid.*, **69**, 1148 (1980).

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