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Rapid determination of total benzalkonium chloride content in ophthalmic formulation

Louis-Philippe Labranche, Suzanne N. Dumont, Suzanne Levesque, Alain Carrier *

Sandoz Canada Inc., 145 Jules-Léger Boucherville, Québec J4B 7K8, Canada

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Abstract

A simple and rapid reversed-phase HPLC method was developed for routine analysis of total benzalkonium chloride in ophthalmic formulations. The analysis involves simple sample preparation using the mobile phase as the diluent. The method uses a Waters SymmetryShield RP-18 (75 mm × 4.6 mm, 3.5 μm particle size) column and a mobile phase consisting of a mixture of methanol–potassium phosphate (pH 3.0; 7.5 mM) (68:32, v/v). Using these conditions, three major homologs of the benzalkonium chloride (C_{12}, C_{14} and C_{16}) were separated in less then 7 min. Furthermore, recoveries ranging from 97% to 99% at three levels of the label claim of total benzalkonium chloride content were obtained for different ophthalmic formulations. Data supporting the development and validation of this method are presented.

Keywords: Benzalkonium chloride; Ophthalmic formulation; Reversed-phase chromatography; Validation; BKC homologs; HPLC; Excipient

1. Introduction

One of the typical preservatives used in the ophthalmic form is benzalkonium chloride [1–5], a bacteriostatic agent. Benzalkonium chloride (BKC), a quaternary ammonium compound, is a mixture of alkylbenzyldimethylammonium chloride of the formula $[C_6H_5CH_2N(CH_3)_2R]Cl$, where R is an alkyl group varying from C_8H_{17} to $C_{18}H_{37}$ [6] (Fig. 1). In ophthalmic formulations, it was reported that homologs C_{12} and C_{14} are the most common components of these solutions [7]. Over the past years, toxicities of BKC involving the damage of human nose epithelia and exacerbation of rhinitis have been reported [8]. Due to the antimicrobial potency of the BKC and its influence on human health, quantitative determination of the amount of total BKC homologs present in the formulation has become necessary. For the purpose of this study, the BKC homologs were reported as per USP [9]; results reported correspond to the sum of the individual homologs present to give the total benzalkonium chloride content.

Due to its multicomponent nature, benzalkonium chloride can cause difficulty for analytical measurement. When the sample determination of BKC involved few excipients in the ophthalmic formulation, HPLC [7,10,11] and electrophoresis [12–14] methods were used for qualitative and quantitative determination of BKC homologs.

Although several HPLC methods developed for the determination of BKC were used in the past, these methods involving quantification of BKC in ophthalmic solutions were not suitable for the BKC determination in our products of interest. These methods involved (1) a high wavelength utilization in the range of 240–270 nm, resulting in low detection sensitivity, (2) long run-time sequence in the range of 20–30 min and/or (3) utilization of reversed-phase CN column; this type of column packing can be problematic with certain types of ophthalmic formulations. As our ophthalmic formulations contained sulfamide and sulfamide/morpholine as active ingredient and multiple excipients, injection at high concentration was not suitable with the CN stationary phase. The ideal method should require maximum detection sensitivity, low concentration sample preparation to maintain column robustness and optimized low run-time analysis.

The objectives of this work for the determination of the content of total benzalkonium chloride in different ophthalmic formulations were the following: (1) develop a simple and rapid reversed-phase HPLC method for routine determination of the total content of BKC, (2) validate the method for ophthalmic

* Corresponding author. Tel.: +1 450 641 4903; fax: +1 514 641 0459.
E-mail address: alain.carrier@sandoz.com (A. Carrier).

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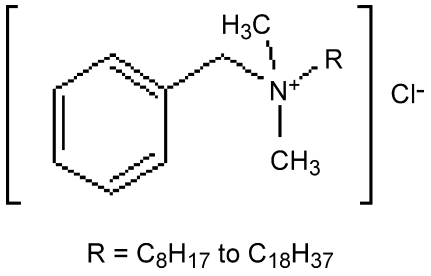

Fig. 1. Representation of benzalkonium chloride.

formulation containing sulfamide and sulfamide/morpholine active drugs, and (3) test the versatility of the method for other types of ophthalmic formulations such as fluoroquinolone and imidazole derivative.

2. Experimental

2.1. Chemicals and reagents

HPLC-grade methanol, acetonitrile and potassium phosphate monobasic were purchased from Anachemia Canada Inc. (Montréal, PQ, Canada). HPLC-grade o-phosphoric acid was purchased from American Chemicals Ltd. (Montréal, PQ, Canada). Distilled water available in the laboratory was filtered prior to use through a 0.2 μm filter. Benzalkonium chloride was supplied as a 50% (p/v) aqueous solution from UNIVAR Canada Ltd. (Montréal, PQ, Canada).

2.2. Equipment

The HPLC system used for method development and method validation was the Agilent 1100 series (manufactured by Agilent Technologies, Waldbronn, Germany) LC system with a diode array detector. The signal was monitored and processed using chemstation software (Agilent Technologies, Waldbronn, Germany) on Pentium computer.

2.3. Method

The utilization of high performance liquid chromatography (HPLC) combined with UV detection was the key analytical tool for the benzalkonium chloride homolog determination in the ophthalmic formulation solutions of interest. The chromatographic response and the sample preparation were tested in a set of experiments where parameters such as column selection (stationary phase, column length and diameter), mobile phases (pH, percentage organic content) and various diluents were analyzed.

As a result of the experimental tests, the chromatographic column chosen was a Waters SymmetryShield RP-18 (75 mm × 4.6 mm, 3.5 μm particle size). The mobile phase consists of a mixture of methanol–potassium phosphate (pH 3.0; 7.5 mM) (68:32, v/v). The flow rate of the mobile phase was kept at 1.0 ml/min. The column temperature was maintained at 50 °C and the optimum wavelength was monitored at a value of 208 nm. The injection volume was 20 μl for the assay determination. The mobile phase was used as diluent for both the standards and the samples preparation.

2.4. Preparation of standard and sample solutions

A working standard solution of 0.016 mg/ml of BKC water solution was prepared for assay determination analysis. Tested samples: sulfamide and sulfamide/morpholine preparations, in the form of ophthalmic solutions, were prepared as follows: 2 ml of solution was diluted in 10 ml of mobile phase to obtain a 0.016 mg/ml solution. Fluoroquinolone and imidazole derivative preparation, in the form of ophthalmic solutions, were prepared as follows: 3 ml of solution was diluted in 10 ml of mobile phase to obtain a 0.016 mg/ml solution.

2.5. Method validation

Method validation was performed by determining the following parameters: specificity, linearity, precision, robustness and accuracy.

2.5.1. Specificity

In order to determine the specificity of the method, the following solutions were injected: Mobile phase, synthetic ophthalmic solution (ophthalmic solution without benzalkonium chloride), standard solution of benzalkonium chloride and ophthalmic solution sample preparation.

2.5.2. Linearity

In order to verify the linearity of the detector, a minimum of seven concentration levels equivalent to 0.0016, 0.0039, 0.0081, 0.0129, 0.0162, 0.0242 and 0.0323 mg/ml of total benzalkonium chloride, corresponding to 10–200% of the standard solution were prepared and injected.

2.5.3. Accuracy of the method

The validation was performed over the range of 50%, 100% and 150% of the label claim, representing 0.042, 0.074 and 0.119 mg/ml of total benzalkonium chloride before sample preparation. A minimum of three samples at each concentration level were prepared and injected in duplicate.

2.5.4. Precision

2.5.4.1. Repeatability. The repeatability of the method was evaluated by the analysis of total benzalkonium chloride in the 100% ophthalmic formulation containing sulfamide and sulfamide/morpholine active drugs. Six sample preparations were performed and each one was injected once.

2.5.4.2. Intermediate precision. In order to evaluate the intermediate precision of the HPLC method, the assay of total benzalkonium chloride in ophthalmic formulation containing sulfamide and sulfamide/morpholine active drugs, was analysed on a different day, using a different batch of mobile phase, a different HPLC system, a different column as well as a different analyst performing the analysis. The same lot of finished

product was used for the repeatability study for a comparison purpose.

2.5.4.3. System precision. System Precision was determined from five replicate injections of the benzalkonium chloride standard solution (benzalkonium chloride at 0.016 mg/ml). The reproducibility of sum of the homologs peak areas, the tailing factor (*T*) for each of the three homolog peaks and the resolution between each adjacent homolog peak was evaluated.

2.5.5. Robustness

2.5.5.1. Stability of solutions. A Standard Solution containing benzalkonium chloride was kept at room temperature for 159 h and compared to a freshly prepared solution. In the same manner, the finished product preparation was kept at room temperature and re-analysed after at least 159 h.

2.5.5.2. Variations in method parameters. The effects of small changes of operating parameters such as mobile phase composition, pH of the buffer and column temperature were tested in order to optimize the method and test its sensitivity to minor changes in operating conditions. These parameters were changed only one at a time. The benzalkonium chloride standard solution and the finished product preparation were injected for each condition tested.

The following chromatographic parameters were varied to verify sufficient robustness of the chromatographic separation: column temperature (±2 °C), pH of phosphate buffer (pH ± 0.2) and mobile phase composition (±1%).

3. Results and discussion

3.1. Optimization of chromatographic conditions

In the literature, different HPLC methods have been reported for the analysis of benzalkonium chloride [7,10–12]. Of these methods, the columns used are CN columns [10,12] or more conventional reversed-phase columns, C-8 and C-18 [7,11]. With the CN column, typical chromatographic conditions used for mobile phase consists of a buffer with a pH of 2.5 to 7.0 with acetonitrile and/or methanol as organic solvent. Initial work was done using CN columns; two different columns were tested: (1) LiChrospher 100 CN (250 mm × 4.0 mm, 5 μm particle size) and (2) Phenomenex Luna CN (50 mm × 4.6 mm, 3 μm particle size). The following chromatographic conditions were used with the LiChrospher 100 CN: (1) a mobile phase consisting of a mixture of methanol–potassium phosphate (pH 3.0; 7.5 mM) (65:35, v/v) and (2) a mobile phase consisting of a mixture of acetonitrile–potassium phosphate (pH 3.0; 7.5 mM) (65:35, v/v).

The LiChrospher column did not provide enough robustness of the method because the ophthalmic formulation used contains excipients that significantly affected peak shape after few injections (less than 30 injections). The second column (Luna CN) tested demonstrated similar behaviour to the LiChrospher column. However, the shorter length of the column showed interesting potential for the development of a rapid chromatographic method. Comparing the retention times of the BKC homologs using the LiChrospher column and the Luna CN column; a drastic change of retention time was observed for the three homologs, LiChrospher: 8–10 min and Luna 1–2 min.

Following the results obtained with the CN column, new experiments were conducted using a more conventional reversed phase C-18 column. In light of the positive results obtained with the shorter column with reduced particle size, the SymmetryShield® RP-18 (75 mm × 4.6 mm, 3.5 μm particle size) was chosen. Several experiments were conducted to optimize both the mobile phase and the column operating temperature. Using a mobile phase of methanol–potassium phosphate (pH 3.0; 7.5 mM) (68:32, v/v) and a column temperature of 50 °C, the SymmetryShield® column provided an acceptable separation for the three benzalkonium chloride homologs (retention time: 1.9, 2.8 and 6.0 min corresponding, respectively to homologs C_{12}, C_{14} and C_{16}). Using these conditions, the resolution and the tailing factor of these three homolog peaks conformed to the method requirements (resolution ≥2 and tailing ≤2). Sequences were run using both ophthalmic formulations containing sulfamide and sulfamide/morpholine active drugs in order to evaluate the column and the method robustness. Good results for more than 100 injections were obtained and no peak shape deterioration was observed.

3.2. Optimization of samples preparation

3.2.1. Recovery studies of benzalkonium chloride

Sample preparation optimization work was challenging due to the complex composition of both ophthalmic formulations tested (sulfamide and sulfamide/morpholine). For example, preparation in aqueous diluting solution created foaming and organic diluting solution induced precipitation of some of the excipients. From the different diluting solutions tested, the mobile phase was found to be the best option for obtaining good recovery of the total BKC. Using this sample preparation, recoveries results obtained were between 96.9% and 99.1%. Typical chromatograms obtained for a mobile phase solution, a standard solution and for a formulation containing sulfamide active drug are presented in Figs. 2–4. As shown in Fig. 2, two peaks related to the mobile phase are present. These peaks are present also in the chromatograms obtained for both the standard solution and the formulation sample (Figs. 3 and 4).

Similar studies were carried out using the same approach in order to determine recoveries of samples containing benzalkonium chloride in fluoroquinolone ophthalmic solution and in

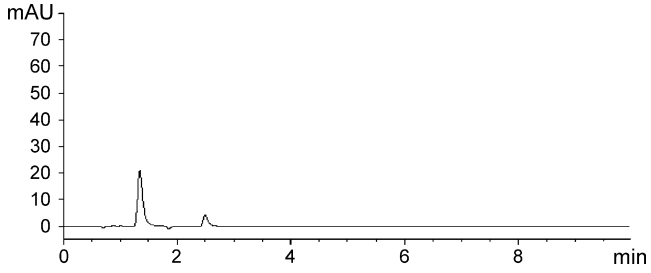

Fig. 2. Mobile phase blank solution.

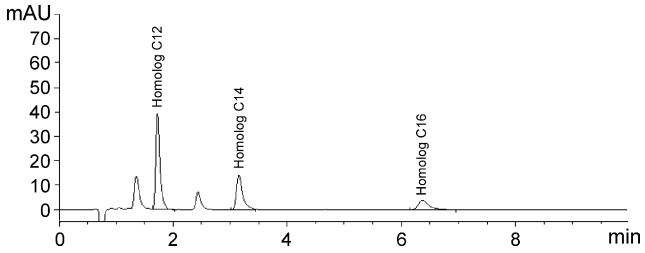

Fig. 3. Standard solution containing benzalkonium chloride equivalent to approximately 0.016 mg/ml.

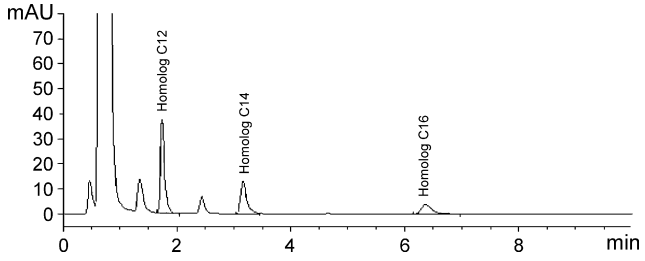

Fig. 4. Sample of ophthalmic formulation (sulfamide active), diluted 2 ml/10 ml with mobile phase (final concentration ~0.016 mg/ml benzalkonium chloride).

imidazole derivative ophthalmic solution. Recoveries obtained for each of the ophthalmic formulations were approximately 100%. Fig. 5A and B are showing examples of chromatograms obtained for these types of ophthalmic formulations.

3.3. Validation

The specificity of the method was tested and no interfering peaks were observed at the retention times of the homolog peaks. The method was proven to be specific for all the ophthalmic formulations tested.

Seven standard solutions containing benzalkonium chloride representing 10–200% of the standard solution were prepared

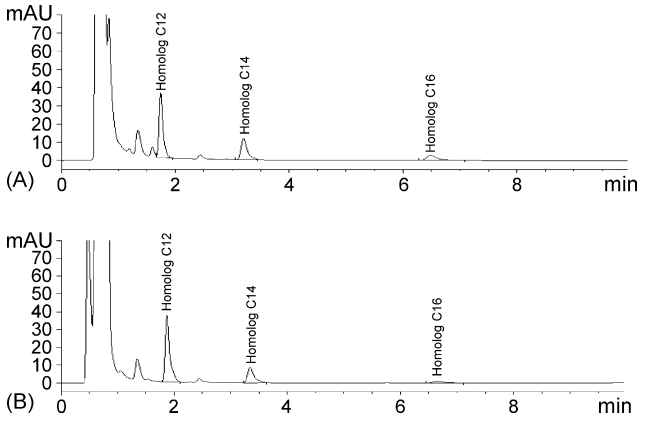

Fig. 5. Sample of ophthalmic formulations diluted 3 ml/10 ml with mobile phase (final concentration ~0.016 mg/ml benzalkonium chloride). (A) fluoroquinolone active (B) imidazole active.

and injected. The linearity curve was generated plotting the experimental concentration of the BKC standard versus the BKC response. A slope of 23809 and a intercept of −1.368 was found. A coefficient of determination of 0.9999 was obtained.

The precision of the system was evaluated from six replicate injections of the benzalkonium chloride standard solution (equivalent to approximately 0.016 mg/ml benzalkonium chloride). Typical results obtained were as follows: Tailing factor ($T \leq 2$) = 1.6 (C_{12}), 1.7 (C_{14}) and 1.9 (C_{16}) with a R.S.D. of 0.24%.

The synthetic finished product was prepared at 50%, 100%, and 150% of the total benzalkonium chloride label claim, representing approximately 0.0422, 0.0739, and 0.1196 mg/ml. Results are reported in Tables 1 and 2. The overall average recovery for total benzalkonium chloride in ophthalmic formulation containing sulfamide active drug is 97.9% with values ranging from 97.2% to 99.1%; the average recovery obtained for ophthalmic formulation containing sulfamide/morpholine

Table 1
Synthetic ophthalmic formulation (sulfamide active drug) containing benzalkonium chloride at 50%, 100% and 150% of the label claim

Accuracy	Theoretical concentration (mg/ml)	Experimental concentration (mg/ml)	Recovery (%)	Average (%)	R.S.D. (%)
50%	0.0422	0.0413	97.9	97.5	0.27
		0.0410	97.2		
		0.0411	97.4		
		0.0412	97.6		
		0.0410	97.2		
		0.0411	97.4		
100%	0.0739	0.0726	98.2	97.5	0.36
		0.0719	97.3		
		0.0719	97.3		
		0.0719	97.3		
		0.0720	97.4		
		0.0720	97.4		
150%	0.1196	0.1185	99.1	99.0	0.15
		0.1180	98.7		
		0.1183	98.9		
		0.1183	98.9		
		0.1185	99.1		
		0.1184	99.0		

Table 2
Synthetic ophthalmic formulation (sulfamide and morpholine active drugs) containing benzalkonium chloride at 50%, 100% and 150% of the label claim

Accuracy	Theoretical concentration (mg/ml)	Experimental concentrataion (mg/ml)	Recovery (%)	Average (%)	R.S.D. (%)
50%	0.0422	0.0416	98.6	98.2	0.27
		0.0413	97.9		
		0.0414	98.1		
		0.0415	98.3		
		0.0414	98.1		
		0.0413	97.9		
100%	0.0739	0.0725	98.1	97.3	0.47
		0.0716	96.9		
		0.0720	97.4		
		0.0718	97.2		
		0.0717	97.0		
		0.0716	96.9		
150%	0.1196	0.1176	98.3	97.9	0.27
		0.1173	98.1		
		0.1170	97.8		
		0.1167	97.6		
		0.1169	97.7		
		0.1172	98.0		

active drugs is 97.8% with a range going from 96.9% to 98.3%. The intermediate precision was evaluated and the results showed a recovery of 97.7% with a coefficient of variation of 0.6%.

The stability of solution was evaluated both for a standard solution of BKC and a finished product preparation containing sulfamide and sulfamide/morpholine active ingredients. The results showed no significant decrease of benzalkonium chloride after 159 h when stored at room temperature both for the standard preparation (98.4% recovery) and for the finished product, sulfamide (100.8% recovery) and sulfamide/morpholine (98.7% recovery).

Results obtained for the robustness tests have showed that the retention time and the peak shape were not affected by any of the changes tested. Therefore, the proposed method was found to be robust.

4. Conclusion

In conclusion, a simple and rapid reversed-phase HPLC method was described for the separation of benzalkonium chloride homolog and for the total benzalkonium chloride determination in different ophthalmic formulations. Different types of ophthalmic formulations containing different active drugs such as sulfamide, sulfamide and morpholine, fluoroquinolone and imidazole were analyzed with success using this analytical method. The performed validation confirmed the usefulness and the robustness of the method. The method was found to be precise, accurate and linear at concentrations ranging from 0.04 mg/ml to 0.11 mg/ml for total benzalkonium chloride.

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