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Determination of antibacterial quaternary ammonium compounds in lozenges by capillary electrophoresis

R.B. Taylor*, S. Toasaksiri, R.G. Reid

School of Pharmacy, The Robert Gordon University, Schoolhill, Aberdeen AB10 1FR, UK

Abstract

A method for the specific determination of three quaternary ammonium compounds, benzalkonium chloride, cetylpyridinium chloride and dequalinium chloride, used as antibacterial agents in candy-based lozenges, is described based on capillary zone electrophoresis. It is shown that, following optimisation of buffer composition with respect to organic modifier concentration, pH and buffer concentration together with the inclusion of sodium dodecylsulphate as an ion-pairing agent in the case of dequalinium chloride, these analytes migrate in less than 5 min. The resultant electrophoretic peaks are sharp and readily quantified. The individual alkyl components of benzalkonium chloride can be resolved as can related impurities in dequalinium chloride lozenges. The quantitative characteristics of the assay method, based on peak areas normalised with respect to migration times, are reported and the method is compared with a previously published method based on liquid chromatography. © 1998 Published by Elsevier Science B.V.

Keywords: Pharmaceutical analysis; Quaternary ammonium compounds; Benzalkonium chloride; Cetylpyridinium chloride; Dequalinium chloride; Antibacterial agents

1. Introduction

The quaternary ammonium compounds benzalkonium chloride (BZK), cetylpyridinium chloride (CPC) and dequalinium chloride (DQC) are widely used as antimicrobial agents in the treatment of common infections of the mouth and throat. It has been shown that candy-based lozenge formulations are more effective in this respect than tablet preparations [1]. Classical methods of analysis of these compounds such as non-aqueous titration [2,3] and ion-pairing extraction followed by spectrophotometric estimation [4–8] are unsuitable for the general determination of these compounds in single lozenges due to a lack of both selectivity with respect to excipients and also sensitivity. Assay methods for certain of these compounds have appeared in the literature based on various chromatographic techniques. Several methods including TLC [9], GLC [10] and HPLC [11–19] have been published for BZK. Fewer methods have appeared in the literature for CPC or DQC [7,20–25]. These include a method for identification of CPC and DQC in mouthwash by TLC and a method in which CPC and DQC were estimated by a volumetric procedure [24]. Methods have been reported for the determination of DQC in drug preparations (lozenge, ointment and tablet) based on ultraviolet [7] and infrared spectrophotometry [25]. LC methods for these compounds, because of their hydrophobicity, have required the use of cyanopropyl stationary phases or C_8 and C_{18} with a very non-polar mobile phase. Of the LC methods in the literature only one has evaluated the technique for the determination of these three compounds in

*Corresponding author.

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candy-based lozenges and it was found that the cyanopropyl stationary phase used was not adequately selective for the quantitative determination of BZK in the lozenges [26].

Capillary electrophoresis offers possible advantages over LC in terms of selectivity and also peak capacity as a result of the increased efficiency attainable with this technique. This potential for resolution has been demonstrated in several reports in the literature on the separation of diverse quaternary ammonium compounds [12,27–31]. In these reports the emphasis has been on using the resolving power of CE to separate individual compounds on the basis of their differing alkyl chains. A major difficulty in obtaining optimum peak shape and controlling migration times of such quaternary ammonium compounds has been attributed to their propensity for adsorption on the silica capillary and in the case of long alkyl chains for forming micelles, since the initial concentrations introduced into the capillary is often in excess of the critical micelle concentration. Both of these effects have been overcome by the inclusion of appreciable concentrations of tetrahydrofuran in the running buffer [27,28]. Subsequently the effect of adding other organic liquids on the separation of alkylbenzyl quaternary ammonium compounds was shown to be general in improving separation [29,30]. The application of both direct and indirect UV absorption for the determination of alkylbenzyl and alkyl quaternary ammonium compounds has been demonstrated and a comparison made between CE and LC for the determination of various important anionic, cationic and non-ionic surfactants [12].

Relatively few reports have appeared concerning the CE behaviour of the quaternary ammonium compounds used as antibacterial agents in lozenges. There has been one report on CPC in mouthwash [12] but no CE methods have been located for DQC. It is the purpose of the present work to describe a capillary electrophoresis method for the determination of BZK, CPC and DQC in commercial candy-based lozenges. It is intended to show the capability of capillary electrophoresis in resolving the active ingredient from the various other lozenge components and to compare the results obtained both in terms of resolution and quantitation characteristics with those obtained by the previously published method based on LC [26]. This is felt to be of some interest since CE is seen currently as the analytical method capable of providing an alternative to liquid chromatography.

2. Experimental

2.1. Equipment

A Hewlett-Packard HP3D capillary electrophoresis system was used, fitted with an unmodified silica capillary 50 μm internal diameter of total length 275 mm and effective length 205 mm. Detection was by diode-array UV spectrophotometry. Detection wavelengths used were optimised for the individual compounds and were 210, 254 and 230 nm for BZK, CPC and DQC, respectively. The hydrodynamic injection mode was used for all measurements at a pressure of 50 mbar with an injection time of 10 s. The applied voltage was 15 kV. Before each daily series of measurements the capillary was flushed successively for 5 min each with an aqueous 0.1 *M* NaOH solution, water purified by a Milli-Q system (Millipore, Watford, UK) and with the running buffer. Between each separation the capillary was conditioned by flushing with 0.1 *M* NaOH (2 min), water (1 min) and running buffer (3 min). After each daily series of measurements the capillary was flushed with 0.1 *M* NaOH aqueous solution and water each for 5 min.

2.2. Materials

Benzyldimethyldodecyl ammonium bromide (C_{12} benzyl), benzyldimethyltetradecyl ammonium chloride (C_{14} benzyl), benzyldimethylhexadecyl ammonium chloride (C_{16} benzyl), cetylpyridinium chloride, dequalinium chloride and diphenhydramine hydrochloride were obtained from Sigma–Aldrich (Poole, UK). The purity of these compounds used as standards were certified by the supplier as C_{14} benzyl—100%, C_{12} benzyl and CPC—greater than 99% and for C_{16} benzyl and DQC greater than 98.5% with respect to dried mass.

For quantitative measurements DQC was dried to constant mass at 100°C. Residual water was determined by coulometric Karl Fischer reaction using a Mitsubishi CA-20 moisture meter standardised by adding known amounts of water directly into the

reaction vessel. The C_{12}, C_{14}, C_{16} benzyls were stored in the refrigerator and CPC was stored at ambient temperature in a desiccator. Water contents were determined as above. In preparing the standard solutions the purity of the material and the residual water content was taken into account in calculating the concentration of the solutions. Samples of candy-based lozenges containing the different quarternary ammonium compounds were obtained commercially through retail outlets. Acetonitrile and methanol (HPLC grade) were obtained from Rathburn (Walkerburn, UK). Phosphate salt was purchased from Merck (Poole, UK). Sodium dodecyl sulphate was obtained from Fisons (Loughborough, UK).

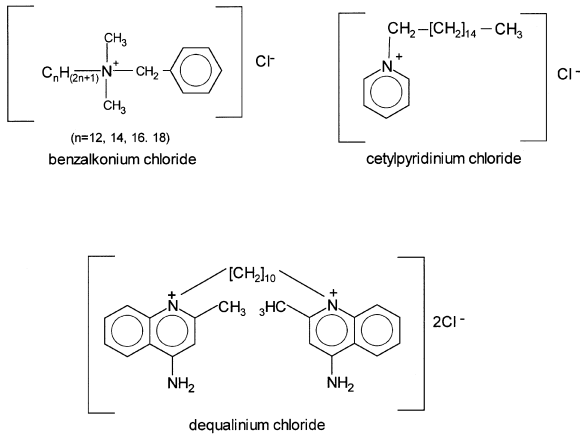

Fig. 1. Structures of the quarternary ammonium compounds studied.

2.3. Methods

Stock solutions of CPC, DQC and diphenhydramine hydrochloride as the internal standard were prepared in water at a concentration of 500 μg cm^{-3}. Standard solutions containing C_{12} and C_{14} benzyls at concentrations of 1000 and 500 μg cm^{-3}, respectively, were prepared in water. These stock solutions were diluted appropriately to prepare calibration lines with methanol–water (60:40). The C_{16} benzyl compound was omitted from the calibration since examination of the electropherogram of the BZK lozenges showed no evidence of the presence of this compound.

Lozenge sample preparation was by dissolving a single lozenge in the minimum volume of methanol–water (60:40), placing in an ultrasonic bath for 30 min, adding diphenhydramine hydrochloride stock solution and making up to 25 cm^3 for CPC and DQC and to 10 cm^3 for BZK. The resulting solutions were filtered through a 0.45 μm membrane filter. These solutions were divided into aliquots. One of these was used for the determination of quaternary ammonium content by comparison with an external calibration line. Each of the other aliquots was spiked with appropriate concentrations of the active ingredient to allow recovery of the analyte from the matrix to be determined. Buffer solutions were filtered through a 0.45 μm membrane filter and degassed before use.

2.4. Electrophoretic optimisation

The structures of the three quarternary ammonium compounds are shown in Fig. 1. This shows the differences among the three compounds both in hydrophobicity (alkyl chain) and in ionic charge.

Preliminary work on the electrophoretic behaviour of the three analytes using purely aqueous buffer with both unmodified and hydrophillic polymer coated capillary (Hewlett-Packard CEP coated capillary) produced excessively tailed peaks and long electromigration times for all analytes. The inclusion

Table 1
Running buffer, solvent for sample and standard preparation and the detection wavelength for the compounds analyzed

Drug	Running buffer	Wavelength (nm)
BZK	50% ACN in 50 m*M* NaH_2PO_4, pH=3	210
CPC	50% ACN in 20 m*M* NaH_2PO_4, pH=5	254
DQC	50% ACN in 20 m*M* NaH_2PO_4 and 5 m*M* SDS, pH=2.5	230

Hydrodynamic injection at 50 mbar pressure for 10 s duration was employed. The capillary was fused silica of effective length 205 mm×50 μm I.D. Diphenhydramine HCl was added in the sample solution as an internal standard. Samples and standards were dissolved in methanol–water (60:40). The voltage applied was 15 kV.

Table 2
Details of the regression parameters, within-day and day-to-day precision, the percentage recovery of spiked lozenge samples and the LOQ for all compounds analyzed

Drug (calibration levels)	Regression equation	Corr. Coefficient r^2	Precision (%R.S.D.)		Recovery (%) ±R.S.D.	LOQ (μg/ml)
			Within-day	Day-to-day		
C_{12} benzyl (10–50μg/ml)	$y=-0.012+0.104$conc	0.9979	1.10 ($n=5$)	1.96 ($n=3$)	99.5 ±1.6	5
C_{14} benzyl (5–25μg/ml)	$y=0.045+0.105$conc	0.9989	1.58 ($n=5$)	3.30 ($n=3$)	100.6 ±1.2	5
CPC (10–50μg/ml)	$y=-0.013+0.076$conc	0.9988	1.69 ($n=5$)	3.36 ($n=4$)	100.6 ±2.9	10
DQC (2.5–12.5μg/ml)	$y=-0.033+0.589$conc	0.9995	0.98 ($n=5$)	1.54 ($n=3$)	101.8 ±3.5	2

of acetonitrile in the aqueous buffer with an unmodified silica capillary was found to improve peak shape and reduce migration times [29,30] but it was found necessary to optimise acetonitrile concentration, pH and buffer concentration individually for each analyte in order to obtain optimum peak shape for CPC and adequate resolution among the three alkyl components of BZK. Adequate resolution of DQC from its minor impurities could only be obtained with the addition of sodium dodecyl sulphate to the aqueous-organic buffer. This was found to reduce the mobility of DQC and both impurities.

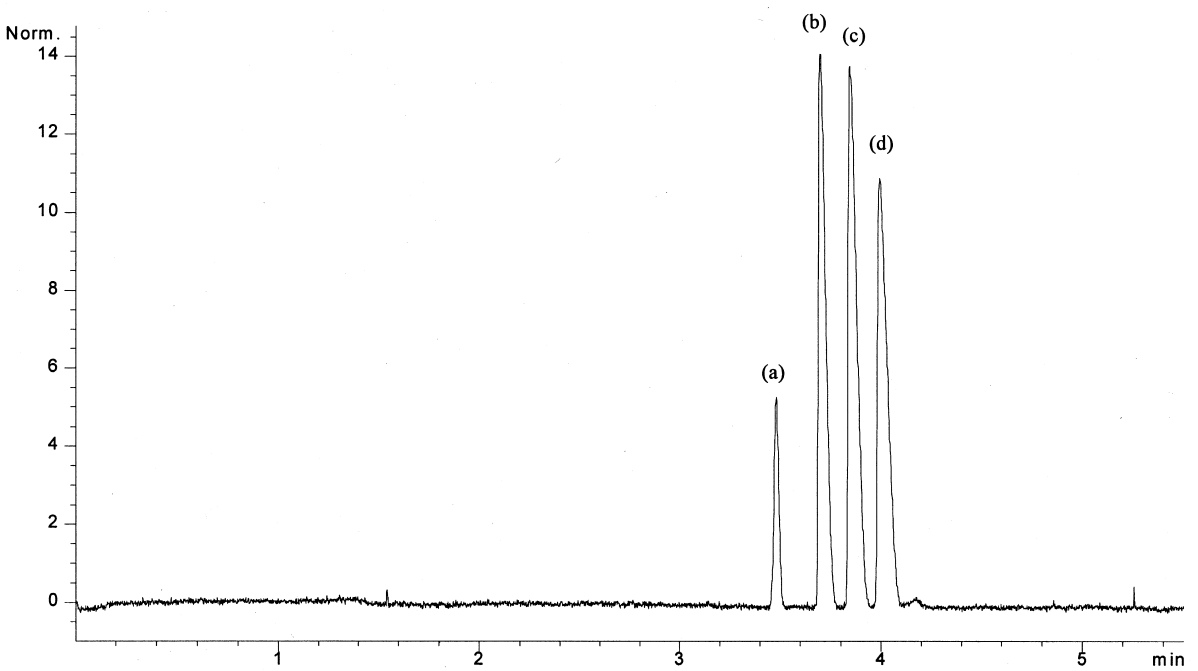

Fig. 2. An electropherogram of diphenhydramine·HCl (a), C_{12} benzyl (b), C_{14} benzyl (c), and C_{16} benzyl (d). Capillary, unmodified silica 50 μm internal diameter of total length 275 mm and effective length 205 mm. Running buffer: 50% acetonitrile in 50 m*M* phosphate buffer pH 3.0. Hydrodynamic injection at 50 mbar for 10 s. Detection at 210 nm.

The optimised buffers used for the determination of the individual quaternary ammonium compounds are listed in Table 1.

2.5. Quantitative measurements

To maximise precision [32] and accuracy [33] normalised peak areas were determined by normalising with respect to migration time and the ratio of corrected peak area of analyte to that of the diphenhydramine hydrochloride internal standard was used as the quantitative response. Linear calibration lines for C_{12} benzyl, C_{14} benzyl, CPC and DQC were established by preparing five standard solutions of each compound over appropriate concentration ranges. The concentration ranges used are shown in Table 2. Internal standard was included at concentrations of 2.6 $\mu g\,cm^{-3}$ for each of the BZK components, 60 $\mu g\,cm^{-3}$ for CPC and 5 $\mu g\,cm^{-3}$ for DQC. Each concentration was injected five times. Triplicate injections of the lozenge samples, prepared as detailed above were made and the concentrations calculated from the regression equation of normalised peak area ratio on concentration. For a single lozenge five replicates of the aliquots of the lozenge samples spiked with increasing known amounts of the appropriate quaternary ammonium compound were also injected. Within-day and day-to-day precision were determined by injecting replicates of standard solutions of each analyte at concentrations corresponding to the mid-value of the calibration range. Limits of quantification were established by injecting successively decreasing concentration of each analyte until a signal-to-noise ratio of 8 was reached. Concentration of analyte in a lozenge sample was determined by direct comparison of the sample response with the regression equation. In the BZK containing lozenges the content was calculated as the sum of the C_{12} and C_{14} components.

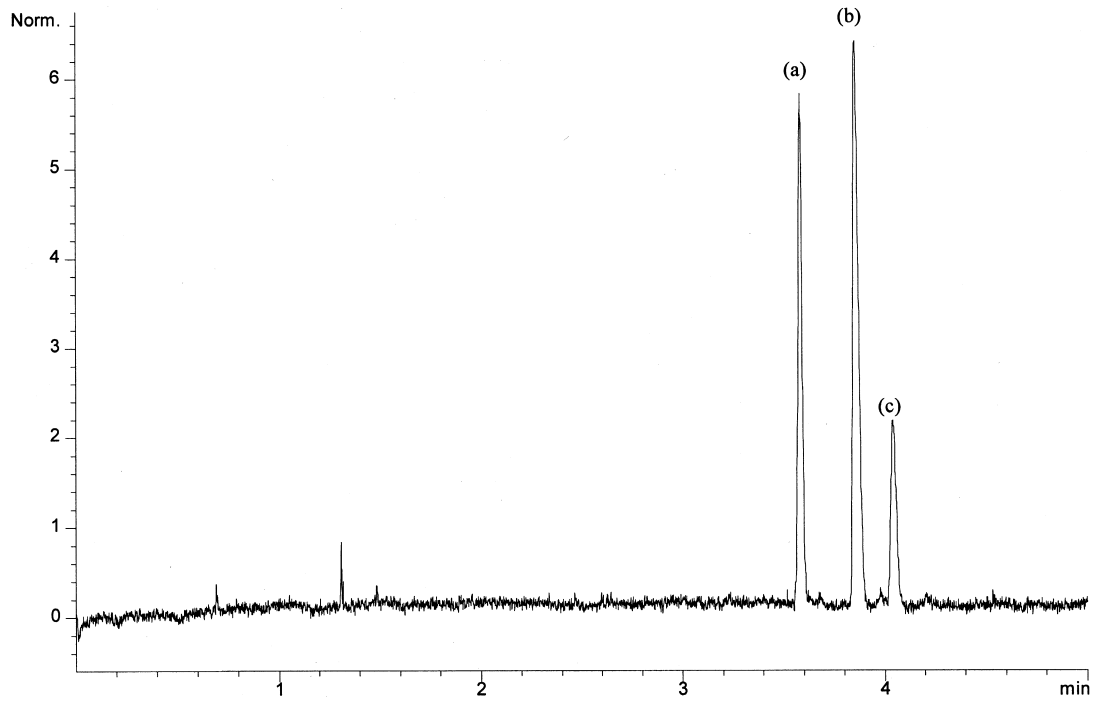

Fig. 3. An electropherogram of BZK lozenge with diphenhydramine·HCl as internal standard. Capillary, unmodified silica 50 μm internal diameter of total length 275 mm and effective length 205 mm. Running buffer: 50% acetonitrile in 50 m*M* phosphate buffer pH 3.0. Hydrodynamic injection at 50 mbar for 10 s. Detection at 210 nm. Compound identification: (a) diphenhydramine·HCl, (b) C_{12} benzyl, and (c) C_{14} benzyl.

3. Results and discussion

Fig. 2 shows the separation among the three alkyl benzyl components C_{12}, C_{14} and C_{16} of BZK and the internal standard diphenhydramine hydrochloride. Fig. 3 shows a representative electropherogram of a typical BZK lozenge sample and internal standard. Only the C_{12} and the C_{14} benzyls are seen to be present. This is consistent with other work concerning BZK in eye drops by HPLC [19]. The identities of the peaks were established by spiking with the appropriate species. Resolution between all components is baseline and all compounds migrate in less than 5 min. Figs. 4 and 5a show representative lozenge samples containing CPC and DQC, respectively, each containing the internal standard. Fig. 4 shows a single peak corresponding to CPC while that of DQC shows the presence of two minor impurities. These are assumed to be associated with the dequalinium since they were also observed in the dequalinium standard. Fig. 5b is an electropherogram in the absence of SDS showing the reduced migration time and the altered separation. Diode-array detection was used to confirm peak purity for all analytes. To obtain these separations it was found necessary to incorporate higher concentrations of acetonitrile than previous workers [29,30]. This was attributed to the higher concentrations of quaternary ammonium compounds being injected in the present study. In addition, in the case of DQC, in order to achieve adequate separation between the main DQC peak and the associated impurities it was necessary to include a low concentration of sodium dodecyl sulphate (SDS) as ion-pairing agent [12]. Table 2 shows the main quantitative validation parameters of the proposed method. Good linearity is shown by the high values of the correlation coefficient and the intercept, at less than 2% of the highest calibration response for each analyte, was not significantly different from zero. The values of the relative standard deviations

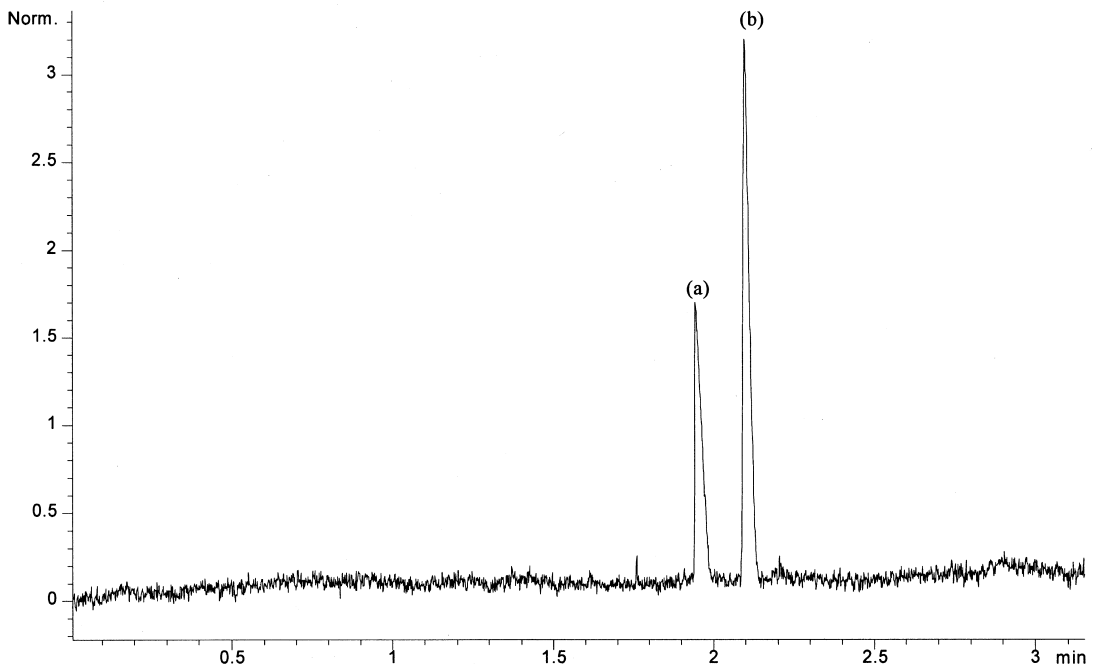

Fig. 4. An electropherogram of CPC lozenge with diphenhydramine·HCl as internal standard. Capillary, unmodified silica 50 μm internal diameter of total length 275 mm and effective length 205 mm. Running buffer: 50% acetonitrile in 50 m*M* phosphate buffer pH 3.0. Hydrodynamic injection at 50 mbar for 10 s. Detection at 254 nm. Compound identification: (a) diphenhydramine HCl, and (b) CPC.

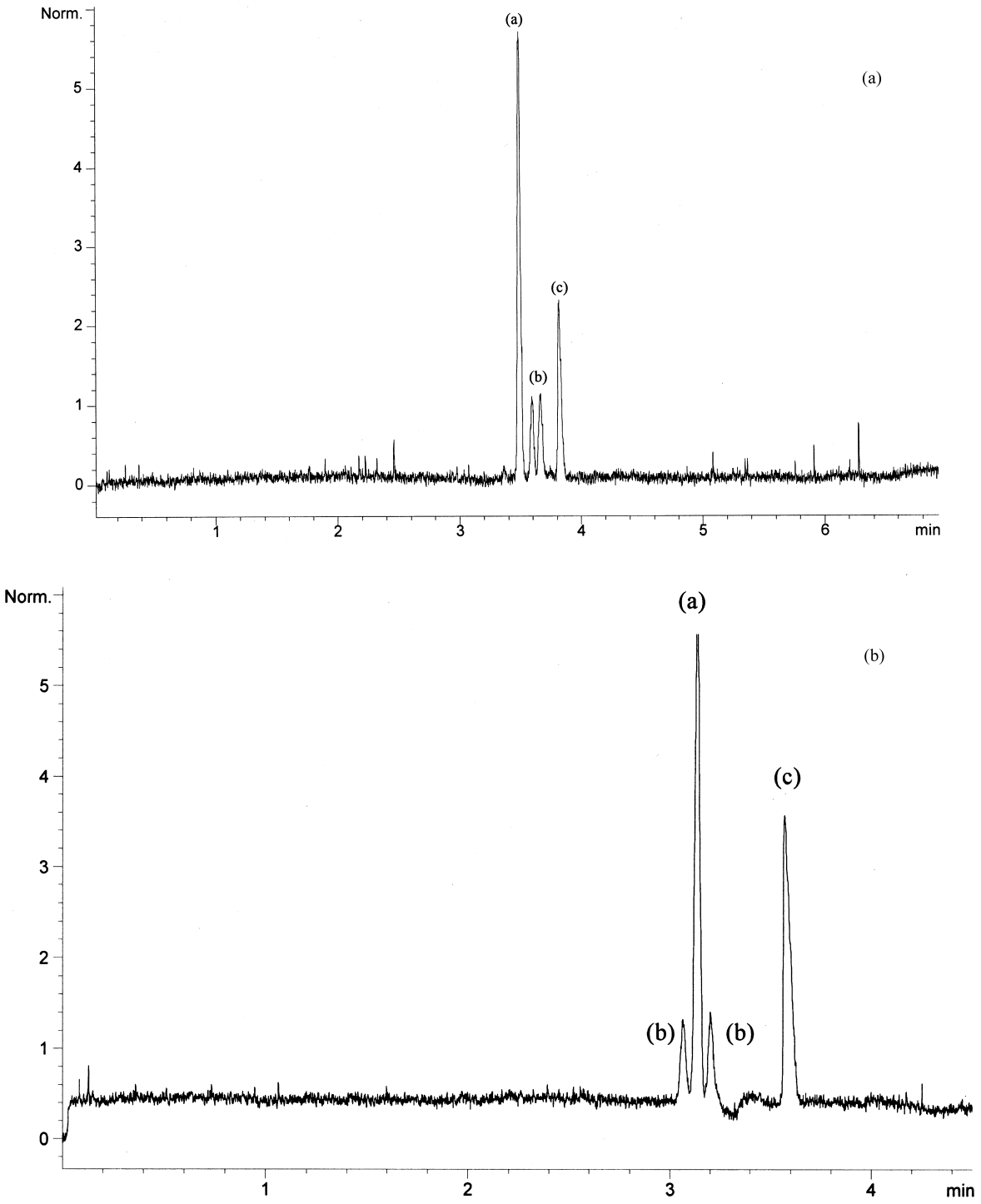

Fig. 5. (a) An electropherogram of DQC lozenge with diphenhydramine·HCl as internal standard. Capillary, unmodified silica 50 μm internal diameter of total length 275 mm and effective length 205 mm. Running buffer: 50% acetonitrile in 20 m*M* phosphate buffer pH 2.5 and 5 m*M* SDS. Hydrodynamic injection at 50 mbar for 10 s. Detection at 230 nm. Compound identification: (a) DQC, (b) DQC impurities, and (c) diphenhydramine HCl. (b) As for part (a), without added SDS.

quoted are relatively small compared with other reported CE methods as a result of using area response normalised by the electromigration time. The recovery quoted is the mean value of the values determined by measuring the same lozenge sample before and after spiking with known amounts of the appropriate quaternary ammonium compound. These values determined are not significantly different from 100%. This indicates that there were no matrix effects from other components in lozenge preparations. The limits of detection under the conditions used are adequate for the determination of the analytes in the lozenges determined.

Table 3 shows the results obtained following the analysis of commercial lozenges of different types containing these quaternary ammonium compounds. Individual lozenges of each type from various manufacturers were assayed by the above method. Each lozenge was assayed in triplicate and the results shown represent the mean and standard deviation for five lozenges. The analytical variation among the triplicate determinations for individual lozenges was in the region of 2% R.S.D. Content determined for the BZK containing lozenges Types 1 and 2 were 128% and 115% of the stated dose, respectively. For the three CPC lozenges these values were 101%, 96% and 96%, respectively, for Type 3, 4 and 5. The limits of content which could be located in official compendia are 90–125% for CPC in such preparations [2]. The DQC content of the two lozenges tested were found to be 116% and 118% of the stated dose. Other determinations of DQC in lozenges using spectrophotometry found values of up to 112% of the stated dose [7]. Table 3 also shows other compounds stated to be present in the individual lozenges.

A comparison of this capillary electrophoresis method with the previously published LC method based on a cyanopropyl stationary phase [26] shows that this proposed CE method has considerable advantages in selectivity. No peaks due to excipients were observed using CE while with the LC method the system had to be carefully optimised to allow specific determination of CPC and DQC and several matrix peaks were evident on the chromatograms. It was not possible to estimate BZK using the cyanopropyl stationary phase due to lack of adequate resolution of matrix components from the BZK species, while with the present CE method it was possible to resolve and quantitate the various alkyl components of BZK at resolutions comparable with separations on more selective LC stationary phases [16]. Also the time required to complete the separation in CE is considerably reduced and even allowing for the specified between-run conditioning the CE method affords a considerable saving in analysis time. To obtain adequate precision in the CE method it was necessary to use an internal standard and to normalise peak areas with respect to migration time. The quantification limits established in this work are approximately twice those obtained for CPC and DQC in LC and the mean value of content found for these compounds is not significantly different. It appears from this direct comparison that CE offers considerable advantages over LC for the assay of such charged species in such matrices where

Table 3
Determination of quarternary ammonium compounds in various commercial lozenge formulations

Lozenges	Stated mass	Mean mass found (mg±S.D.; n=5)	Adjuvents and concentrations
Type 1	0.5 mg BZK	0.639±0.026	Isomalt, citric acid, menthol, eucalyptus oil, acesulfame K. quinoline yellow and indigo carmine
Type 2	0.5 mg BZK	0.576±0.023	Isomalt, citric acid, menthol, eucalyptus oil, acesulfame K. quinoline yellow and indigo carmine
Type 3	1.4 mg CPC	1.42±0.11	Benzocaine 10 mg
Type 4	1.4 mg CPC	1.35±0.026	
Type 5	1.4 mg CPC	1.34±0.026	Menthol 5 mg, eucalytol 3 mg
Type 6	0.25 mg DQC	0.289±0.0040	Benzocaine 10 mg
Type 7	0.25 mg DQC	0.296±0.033	

the mass per sample is in the μg region. It appears adequately rugged and affords considerable savings in solvents and column materials.

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