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Rapid determination of benzalkonium chloride in pharmaceutical preparations with flow injection liquid–liquid extraction*

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Abstract: Benzalkonium chloride was assayed by on-line extraction of the benzalkonium ion with picrate to chloroform. The absorbance of picrate was measured. The extractions were performed with a home-made flow injection extraction unit. Calibration curves ($1.5–180 \times 10^{-4}$% w/v) were straight lines ($r = 0.9993$) and the relative standard deviation of a series of injections was ≤2%. Pharmaceutical benzalkonium preparations, containing xylometazoline, timolol, phenylephrine or carbachol could also be assayed. The method was compared with a modified HPLC assay.

Keywords: *Benzalkonium chloride assay; ion-pair extraction; pharmaceutical preparations; flow injection extraction; HPLC method.*

Introduction

Benzalkonium chloride (BAC) is a widely applied anti-bacterial agent. It is used as a preservative in pharmaceutical preparations such as eyedrops, nosedrops and contact lens solutions. Bleau and Desaulniers [1] also report its usage as an algicide in swimming pools and claim that it is extremely potent in killing the AIDS virus *in vitro*. BAC is a mixture of quaternary ammonium compounds with the general formula $[C_6H_5CH_2N\ (CH_3)_2R]Cl$ in which R represents the alkyl homologue from n-C_8H_{17} to n-$C_{18}H_{37}$. According to USP [2] specifications, a BAC lot should contain not less than 40% of the $C_{12}H_{25}$ compound, not less than 20% of the $C_{14}H_{29}$ compound, and not less than 70% of these two compounds. The C_{14} homologue has the largest antibacterial activity [3].

BAC is determined both by batch and flow methods. Batch methods comprise direct titrations [4, 5], two-phase titrations [6], extractions [7] and pyrolysis followed by GLC [8]. Flow methods are HPLC [9–11] and direct GC [12] techniques. The chromatographic methods [8–12] give the composition of the BAC mixture, whereas the other methods only yield the total amount of BAC. A disadvantage of these assay methods is the relatively long time needed for an analysis. This is a major drawback when large amounts of samples have to be analysed (e.g. in quality control laboratories).

Therefore, a novel flow injection extraction analysis, based upon the ion-pair extraction of BAC with picrate, was developed. Extractions in Flow Injection Analysis (FIA) systems have the advantage of short analysis times (and therefore a large sample throughput) and a low consumption of the organic extractant used. These systems have been described extensively in the literature [13, 14 and references cited therein]. The extractions were performed in a home-made flow extraction apparatus. The applicability of the method for analysis of several pharmaceutical BAC preparations will be demonstrated. It appeared that the adsorption of BAC on the walls of the extraction unit had an influence on the reproducibility of the proposed method. An HPLC assay, slightly modified from the ones reported in literature and applied in routine analysis, was used to further investigate this phenomenon. The qualities of both the FIA and the HPLC method will be compared.

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Experimental

Chemicals and solutions

FIA method. Picric acid solutions were prepared from a moist solid, containing approximately 35% water (Gold Label, Aldrich, USA). The concentration of these solutions was measured photometrically at 355 nm, using an extinction coefficient of 1.45×10^4 l M^{-1} cm^{-1} [15]. Chloroform was of 'Reinst' quality (Merck, Darmstadt, FRG). Phenylephrine, carbachol and BAC were obtained from OPG (Utrecht, The Netherlands). Timolol maleate and xylometazoline were obtained from Pharmachemie (Haarlem, The Netherlands). All chemicals were used as such. All solutions were prepared with demineralized water. Carrier solutions and chloroform were filtered before use. Table 1 gives a summary of some frequently used solutions.

HPLC method. The mobile phase was a 0.1% (v/v) triethylamine solution in acetonitrile–methanol–0.04 M phosphate buffer (52:5:43, v/v/v). The pH of this solution was adjusted to 6.0 with phosphoric acid. The rinsing liquid for the injection needle of the autosampler was acetonitrile–methanol–water (52:5:43%, v/v/v).

Apparatus and methods

FIA method. The home-made extraction apparatus is shown in Fig. 1. The aqueous and organic phases were pumped by pressurized helium gas (1). The pressure was regulated by two precision reducing valves (2) (Doedijns, Rijswijk, The Netherlands) and led to two gas-tight displacement bottles (3). In this way pulse free flows were obtained. Typical flow rates were 1 ml min^{-1}, both for the organic and the aqueous phase.

Table 1
Used solutions and their abbreviations (percentages given are w/v)

Buffers
Borate, 0.01 M, pH 8.5
Phosphate, 0.05 M, pHs of 6.5 and 12.
Standard BAC solutions
All buffers have been used to prepare BAC solutions; the most commonly used BAC concentration was 0.01%.
FNA* solutions
Solutio benzalkonii (SB): 0.01% BAC and 0.1% NaEDTA in demineralized water.
Solutio benzalkonii et acidi borici (SB & AB): 0.01% BAC, 2% boric acid and 0.1% NaEDTA in demineralized water.
Solutio benzalkonii and Solutio benzalkonii et acidi borici (SB+SB&AB)
A mixture of equal amounts of SB and SB & AB.
Pharmaceutical preparations
Oculoguttae carbacholi 1.5% (Oc. Carb); prepared according to FNA specifications: 1.5% carbachol in a mixture of 50 ml SB & AB and 50 ml SB; this solution is 0.01% in BAC.
Oculoguttae phenylephrini 0.125% (Oc. Phen); prepared according to FNA specifications: 0.125% phenylephrine in SB & AB.
Timolol maleate eyedrops† (Tim. dr), as obtained from Pharmachemie. Specifications were not available except for the contents of BAC and timolol, which were 0.01 and 0.5%, respectively. The measured pH was 6.7.
Timolol maleate solution (Tim. sol). A 0.5% timolol solution made in phosphate buffer pH 6.5 and containing 0.01% BAC.
Xylometazoline nosedrops† (Xyl. dr), as obtained from Pharmachemie. Specifications were not available except for the contents of BAC and xylometazoline, which were 0.02 and 0.1%, respectively. The measured pH was 6.7.
Xylometazoline solution (Xyl. sol). A 0.1% xylometazoline solution in phosphate buffer pH 6.5 and containing 0.02% BAC.

*FNA: The Formulary of the Dutch Pharmacists.
†These samples were used as obtained; all other BAC samples were prepared in our laboratory.

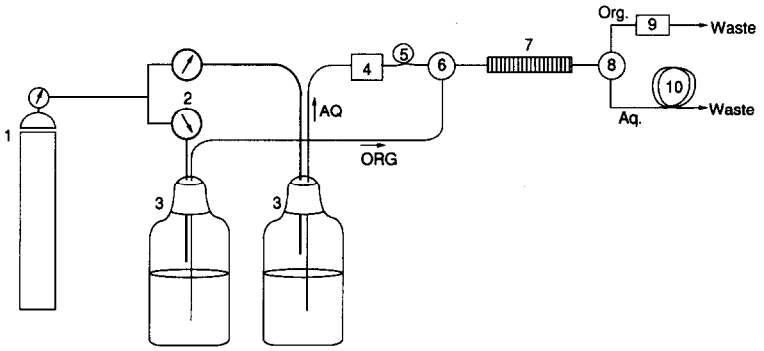

Figure 1
Schematic diagram of the extraction apparatus. For explanations, see text.

The sample was injected in the aqueous carrier by a 4-way teflon rotary valve (4) (Rheodyne model 5041, Cotati, CA, USA), equipped with a 30-μl loop and operated by a pneumatic actuator (Rheodyne model 5701). The carrier was a buffer, containing approximately 4×10^{-4} M picrate. The sample was continuously fed to the injector by a peristaltic pump (Ismatec, Zürich, Switzerland).

After injection the sample was allowed to mix with the carrier in a 10-cm mixing coil (5), whereafter the carrier was segmented with the organic phase (chloroform) in the phase segmentor (6), a stainless-steel Tee (Valco Instruments, Houston, TX, USA). The length of the resulting segments was about 6 mm. The extraction of the picrate-BAC ion-pair took place in the extraction coil (7), a length of 3 m, 0.5 mm i.d. coiled teflon tubing. The phases were separated by a Tecator (Höganäs, Sweden) coiled groove membrane separator (8), mounted in a home-built unit consisting of two stainless-steel disks equipped with Omnifit (Cambridge, UK) fittings; the disks were fastened together by three nuts and bolts. The properties and dimensions of the separator have been described by Sahlestrom and Karlberg [16]. After separation the organic phase was measured spectrophotometrically at 370 nm with a Pye-Unicam (Cambridge, UK) PU 4020 UV detector (9), modified for visible light detection.

The efficiency of the separator, E_s, (i.e. the fraction of the organic phase led to the detector) was controlled by a piece of narrow-bore HPLC capillary (10), connected to the aqueous outlet of the phase separator. By using capillaries of different lengths (and thereby applying different back pressures) it was possible to vary the efficiency of the separator. In most experiments E_s was kept at 95%, but in some cases had to be reduced to 65%. Flow rates were measured by filling measuring cylinders with the effluents during a specified time. In this way the flow rates, the efficiency of the separator and the phase-volume ratio were determined. All tubing (0.5 mm i.d., teflon) was connected by Omnifit fittings, valves and Tee's.

HPLC method. The mobile phase was pumped at a rate of 2 ml min^{-1} by an SP 8800 pump (Spectra Physics, San José, CA, USA); the samples were injected with a SP 8780 XR autosampler and detected at 214 nm with an SP 8490 UV detector. Peak areas were measured with an SP 4270 peak integrator (all Spectra Physics). A Supelco CN, 25 cm × 4.6 mm i.d. (5 μm) column (Supelco SA, Gland, Switzerland) was used.

Results and Discussion

FIA method

Influence of flow rates. A frequently used parameter in flow injection systems is the dispersion, defined as the quotient between the concentration of the analyte in the injected sample solution and the maximum concentration of the analyte in the sample plug that reaches the detector. It reflects the dilution of the injected sample plug in the carrier stream, and, in flow extraction systems, also in the organic stream. In these latter systems the dispersion may also be caused by incomplete extraction of the injected sample. The larger the dispersion, the lower the recorded peaks. In flow extraction systems it appears that the dispersion is also a function of the separator efficiency E_s; the higher E_s, the higher the

peaks [17]. This efficiency should therefore be kept constant. Furthermore, the peak height is a function of the phase–volume ratio, r, which in flow systems is the ratio of the organic and aqueous phase flow rate. The higher r, the lower the concentration of extracted ion-pair in the organic phase. For these reasons it is important that all flows are constant during analysis.

Extraction behaviour of BAC-picrate ion-pair

The extraction behaviour of the BAC-picrate ion-pair was investigated by injecting various concentrations of BAC (standard solutions) in the picrate containing carrier. This procedure was repeated for different carriers (i.e. various picrate concentrations). Typical results are given in Fig. 2. The plateau of the curve with the lowest BAC concentration is caused by the fact that BAC is quantitatively extracted by picrate. The sudden drop of the curves for picrate concentrations higher than approximately 10^{-3} M is probably due to formation of micellar structures consisting of both picrate and benzalkonium ions. Batch solutions in this concentration range indeed are turbid. Several studies [18–20] have been dedicated to the viscoelasticity of solutions where both quaternary ammonium compounds and substituted aromatic compounds, such as benzoic acids and benzene sulphonic acids, are present. This viscoelasticity has been attributed to the formation of large micellar structures. The nature and position of the substituents on the aromatic moiety are crucial to the occurrence of these micellar structures.

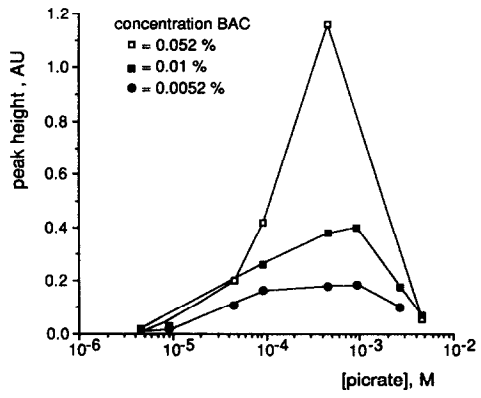

Figure 2
Extraction of BAC with picrate to chloroform. Percentages shown are w/v. E_s = 95%; r = 1; organic and aqueous flow rate: 1 ml/min. All solutions were prepared with a 0.01 M borate buffer, pH 8.5.

Picric acid is not amongst the reported aromatic compounds regarding this subject, but a high increase of viscoelasticity upon adding 2,4-dinitrophenol to a cetyltrimethylammonium solution has been reported [21]. Picric acid may therefore show a similar behaviour; this phenomenon was not further investigated but it was decided not to use picric acid concentrations above 5×10^{-4} M.

The apparent extraction constant of the BAC-picrate ion-pair was determined with a method which will be described in a future paper. The value found was 9×10^6 l M^{-1}. This value serves only as an indication because BAC is a mixture of homologues. A BAC lot with a relatively high amount of the lower homologues will have a lower extraction 'constant' than a lot which contains more higher homologues. But the found value indicates that quantitative extraction of the ion-pair will take place and that the amount extracted will be limited by the ion-pairing reagent present in the lowest concentration.

Adsorption of BAC on the tubing walls of the apparatus

When the injector loop was rinsed with a buffer solution between two series of injections, it was frequently observed that the first injection of a new series yielded a lower (approximately 10%) peak than the subsequent injections. This was also the case when the loop was not rinsed and the series studied was of a higher BAC concentration than the previous one. Furthermore, it appeared that the amount of sample led through the loop prior to injection, V_{load}, had an influence on the recorded peak heights: the larger V_{load}, the higher the peaks. Of course care was taken that V_{load} was always larger than the injected volume, V_{inj}. Finally, the time that the injector was kept in the inject position (t_{inj}) determined the shapes of the peaks; when t_{inj} was made longer, the peaks became somewhat higher and showed considerable tailing.

BAC is known to adsorb on various materials [6, 22]; these phenomena were thus attributed to the adsorption of BAC on the tubing walls of the apparatus and of the injector loop in particular. When the concentration of BAC in the studied sample is higher than in the previous sample, the first injections yield lower peaks because the adsorption equilibrium of BAC is not yet attained. If V_{load} increases, more BAC can adsorb on the walls so that

more BAC can be dissolved by the carrier and subsequently extracted to the organic phase. When longer injection times are employed, the carrier solution washes the loop more thoroughly, i.e. dissolving more BAC. Because the sample was continuously fed to the injector, a shorter t_{inj} implied a larger V_{load}. Therefore, a reproducible injection scheme was applied to minimize these effects: Injection at $t = 0$; return to the load position at $t = 10$ s; next injection at $t = 85$ s. When a new sample was introduced, the first (two) peak(s) was (were) discarded. The nature of the BAC solution (e.g. SB, SB & AB or standard BAC solutions) did not have an influence on the peak height; the presence of EDTA therefore did not interfere with the assay.

Calibration graph of BAC

A plot of the peak heights against the concentration of BAC injected (1.5–180 × 10^{-4}% w/v) yielded a straight line with a correlation coefficient of 0.9993 and an intercept of −0.020 AU. Maximum peak height was reached at approximately 45 s after injection ($E_s = 95\%$); this implies that 40 injections can be performed per hour, which equals 10 samples when four injections per sample are employed; Fig. 3 shows a typical FIA recording.

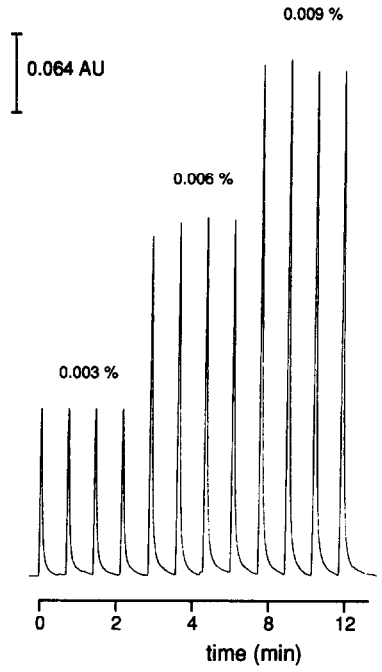

Figure 3
Typical FIA recordings of samples with different BAC concentrations. Percentages are w/v. Carrier: 4.5 × 10^{-4} M picrate. All solutions were prepared with a 0.01 M borate buffer, pH 8.5. $E_s = 95\%$; $r = 1$; organic and aqueous flow rate: 1 ml/min.

Interference of other drugs present

A disadvantage of flow injection methods is the low selectivity, because no physical separation of the components of a sample takes place. In the case of pharmaceutical preparations, other extractable components besides BAC may be present. If these are extracted as an ion-pair with picrate they will disturb the proposed BAC analysis, since the absorbance of picrate is measured. If they are extracted 'on their own' and do not absorb at the used wavelength, they should not cause any problem. Several compounds of pharmaceutical interest were investigated in this regard, i.e. timolol (Tim), phenylephrine (Phen), carbachol (Carb) and xylometazoline (Xyl). The concentration of these compounds in the studied pharmaceutical samples is much higher (10–50 times) than the BAC concentration; even if they were extracted in relatively small amounts their presence might therefore cause significant deviations.

Injections of these compounds were performed to determine the extent of their extractability to chloroform as an ion-pair with picrate. The compounds were dissolved in buffers similar to the ones used in the pharmaceutical preparations, without adding BAC. The results of these 'blank' measurements are given in Table 2. This table also gives values of standard BAC solution injections; the resulting peak heights were taken as 100% and were compared with the peak heights of the investigated drugs.

Because timolol and xylometazoline are amines, their extractability as an ion-pair with picrate should show a pH dependence; this was indeed the case: the observed peaks were lowered when the pH of the carrier was raised. When the pH of the carrier was 12, the efficiency of the phase separator had to be reduced from 95 to 65%, because at higher efficiences breakthrough of the aqueous phase to the detector sometimes occurred, causing severe noise on the base line. The pH of the injected sample before it reaches the extraction coil is governed by the buffer capacity of the carrier and of its own buffer. Thus the pH after mixing may be different from the pH of the carrier. Therefore, some samples were diluted with the carrier buffer (without picrate) prior

Table 2
Extraction of several drugs with picrate

Analyte	Solvent	Carrier	Concentration of analyte injected (%, w/v)	Peak height (AU)	% of BAC-peak
			A		
BAC	Various	Borate	0.01	0.509*	100
Phen	Borate	Borate	0.125	0.0020	0.4
Carb	Borate	Borate	1.5	0.0016	0.3
Tim	Borate	Borate	0.5	0.552	109
Xylo	Borate	Borate	0.1	Signal out of scale	
			B		
BAC	Phosphate (pH 6.5)	Phosphate (pH 12)	0.01	0.460	100
Tim	Phosphate (pH 6.5)	Phosphate (pH 12)	0.5	0.123	27
BAC (1:1)	Phosphate (pH 6.5)	Phosphate (pH 12)	0.005	0.236	100
Tim (1:1)	Phosphate (pH 6.5)	Phosphate (pH 12)	0.25	0.0032	1.4
BAC (1:1)	Phosphate (pH 6.5)	Phosphate (pH 12)	0.005	0.233	100
Xylo (1:3)	Phosphate (pH 6.5)	Phosphate (pH 12)	0.025	0.00286	1.2

*Average peak height, measured over several weeks, with a relative standard deviation of 5.5%.
Conditions: A: $r = 1$, $E_s = 95\%$, carrier buffer: 0.05 M borate, pH = 8.5. B: $r = 1$, $E_s = 65\%$, carrier buffer: 0.01 M phosphate, pH = 12.0. The concentration of picrate in the carriers was 4×10^{-4} M; 1:X denotes that the sample was diluted 1:X with the carrier buffer (without picrate) prior to injection.

to injection. This is designated in the table by (1:X).

Both phenylephrine and carbachol were not extracted in significant amounts (<0.5%); but in order to minimize the extraction of timolol and xylometazoline, the pH of the carrier had to be raised and the samples had to be diluted prior to injection. The resulting peaks were less than 1.5% of the peaks of standard BAC solutions that were diluted in the same way.

Assay of BAC in pharmaceutical samples

The presence of pharmaceutical compounds in the samples had some influence upon the peak heights compared to those of standard BAC solutions. In the case of carbachol and phenylephrine this was surprising, because the 'blank' experiments proved that there was no significant ion-pair extraction of these compounds with picrate. The results of the assays are given in Table 3. This table compares measurements of BAC standard solutions with measurements of the same solutions to which the relevant drug was added. Diluting the samples 1:1 with the carrier buffer usually improved the reproducibility of the assay.

The presence of carbachol caused a rise in peak heights; this rise could be reduced by diluting the sample (1:1) with the carrier buffer. A dilution by a factor of 10 caused a relative lowering of approximately 10% of the peaks. The effect of adding carbachol was not always the same; in undiluted samples the increase of the peaks varied between 4 and 10%. The reason for this anomalous behaviour might lie in a change of the adsorbing equilibrium of BAC on the tubing walls, caused by carbachol. The 'history' of the system, i.e. the surface condition of the tubing walls, may determine the extent that carbachol influences the measurements. When methanol was added to the carrier (30% w/w) as a possible rinsing agent, no significant improvement was observed. Phenylephrine had no significant influence on the peak heights.

The results of the 'blank' experiments of both xylometazoline and timolol indicated that a raise of peak heights was to be expected; in the case of timolol the observed changes of the peaks did not correspond to the expected values: 0.43% compared to 1.4% in the 'blank' experiments. Here also an alteration of the adsorption equilibrium of BAC to the tubing walls might be the underlying mechanism of this phenomenon. The influence of xylometazoline was the same as in the 'blank' experiments (see Table 2).

To eliminate the irreproducibility that some-

Table 3
Mean influence on peak heights (MIOPH) of several pharmaceutical compounds

Sample	Solvent	Carrier	n*	MIOPH† (%)	MRSD‡ (%)
		A			
Oc. Carb	SB + SB & AB	Borate	5	+6.5	2.5
Oc. Carb (1:1)	SB + SB & AB	Borate	4	+2.8	0.80
Oc. Phen	SB & AB	Borate	6	−0.29	0.90
Oc. Phen (1:1)	SB & AB	Borate	2	−0.59	1.4
		B			
Tim. dr	Unknown	Phosphate (pH 12)	1	+0.43	0.50
Xyl. sol (1:1)	Phosphate (pH 6.5)	Phosphate (pH 12)	1	+9.5	1.2
Xyl. sol (1:2)	Phosphate (pH 6.5)	Phosphate (pH 12)	2	+6.0	2.4
Xyl. sol (1:3)	Phosphate (pH 6.5)	Phosphate (pH 12)	1	+2.0	0.90

*The number of series compared with a standard BAC solution; each series consisted of at least three injections.
†The averaged proportional influence of the pharmaceutical compounds on the peak heights of standard BAC solutions.
‡The mean relative standard deviation of the peak heights of all the series containing the relevant drug.
Conditions: see Table 2.

times occurred due to the drugs present in the samples, it was decided to add the drug of interest to the reference BAC solutions. The BAC concentration in the studied preparations was 0.01% (0.02% in the case of xylometazoline nosedrops), which equals approximately 3×10^{-4} M, if the molecular weight of the C_{12} homologue (340) is used. Because the concentration of picrate cannot be raised much higher than 5×10^{-4} M because of micelle formation, it was decided to dilute the samples 1:1 (1:3 in the case of xylometazoline nosedrops) with the carrier buffer (without picrate) as a standard routine. In this way an excess of picrate was ensured and the procedure had the additional advantage of improving the reproducibility of the method. The proposed assay procedure therefore is: (1) dilute the sample with the carrier buffer; (2) prepare a calibration series similar to the diluted sample, including the pharmaceutical components, but with varying BAC concentrations; (3) compare the sample with the calibration graph of this series.

The samples were injected in-between the calibration series. Each solution was injected at least three times, the relative standard deviation within these series varying from 0.2 to 2%. Table 4 gives some properties of typical calibration curves.

All curves had negative intercepts of approximately −0.020 AU. This negative intercept might cause errors when samples with low BAC levels have to be assayed. To minimize these errors, the calibration graphs were constructed with BAC levels lying in an interval of +40 and −40% of the expected value in the sample. Two commercial samples were compared with the appropriate calibration curves: timolol eyedrops and xylometazoline nosedrops; the measured BAC content (after diluting) in these samples was 0.00533 and 0.00540%, respectively.

HPLC method

Reversed-phase HPLC experiments were performed to investigate the adsorption properties of the different components of a BAC sample. Because the C_{12} and the C_{14} homologues should form the major constituent part of BAC samples (according to USP specifications), attention was focused on these two compounds. The influence of the matrix on the peak areas was also determined; Fig. 4 shows a typical chromatogram. It appeared that both the peak areas of the two components as well as the ratio of the peak areas (A_{14}/A_{12}) increased with an increasing V_{load}. This latter effect was not noticed when the sample was diluted 1:1 with the used eluent, but the peak areas of the separate components still increased slightly with increasing V_{load}. These effects are shown in Fig. 5. Table 5 shows the influence of the matrix upon the peak areas.

Table 4
Calibration curves for pharmaceutical preparations

Drug added to standard solution	Concentration* of added drug, % (w/v)	Range* of [BAC], 10^{-3} % (w/v)	Number of calibration points	Correlation coefficient, r
		A		
Carbachol (SB + SB & AB)	0.75	2.5–6.25	4	0.996
Phenylephrine (SB & AB)	0.0625	3.0–7.0	5	0.9990
		B		
Timol (Tim. sol)	0.25	3.0–7.0	5	0.9997
Xylometazoline (Xyl. sol)	0.025	3.0–7.0	5	0.9996

* Concentrations given are the concentrations after diluting with the carrier buffer; all samples were diluted 1:1 with the exception of xylometazoline, which was diluted 1:3.
Conditions: See Table 2.

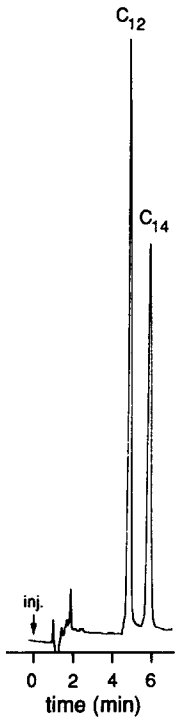

Figure 4
Chromatogram of BAC. Conditions: V_{inj} = 20 µl, V_{load} = 120 µl, flow rate = 2 ml/min. BAC was dissolved in the eluent.

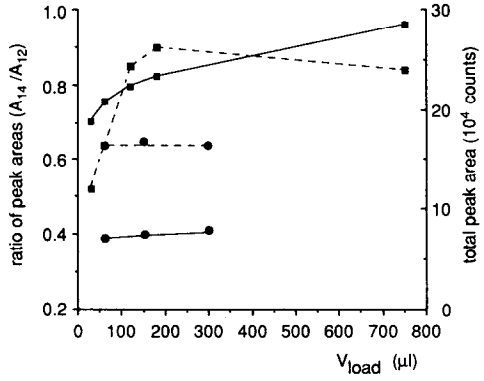

Figure 5
Influence of V_{load} on the peak areas in the HPLC experiments. BAC was dissolved in demineralized water. Conditions: V_{inj} = 20 µl; flow rate = 2 ml/min. Solid lines: total peak area ($A_{12} + A_{14}$). Broken lines: peak area ratio (A_{14}/A_{12}). ■: undiluted samples; ●: samples diluted 1:1 with the mobile phase.

The presence of timolol in undiluted samples lowered the total peak area of the BAC components, but in diluted samples this effect was not observed. Furthermore, the total peak area of the undiluted sample containing timolol is twice as high as in the case of the diluted samples, regardless of their timolol content; 1:1 dilution of the sample without timolol lowered the total peak area with a factor of 2.5.

These results indicate that the BAC components show retention behaviour in the part of the system prior to the column and that diluting with the mobile phase and/or altering the matrix changes this behaviour. The retention seems to be dependent on the chain length of the alkyl group; the nature of the interaction between the BAC components and the tubing walls is of course different from the interaction with the stationary phase of the analytical column. The composition of BAC lots may differ considerably [3]; the influence of V_{load} and the matrix composition on the total peak areas may therefore differ from lot to lot.

It should be kept in mind that for the HPLC experiments stainless steel capillaries were used, as opposed to the FIA experiments,

Table 5
Matrix influence on peak areas in the HPLC system

Sample composition (% w/v)	Peak area, 10^3 counts A		B		C	
	A_{tot}	A_{14}/A_{12}	A_{tot}	A_{14}/A_{12}	A_{tot}	A_{14}/A_{12}
BAC (0.01%)	596.1	0.585	237.3	0.691	243.1	0.695
BAC (0.01%), Tim (0.25%)	465.8	0.623	235.7	0.692	244.8	0.680

A_{tot} = Sum of the C_{14} and C_{12} peak areas.
A_{14}/A_{12} = Ratio of the C_{14} and C_{12} peak areas.
A: Undiluted samples, V_{inj} = 100 µl, V_{load} = 150 µl.
B: Samples diluted 1:1 with eluent, V_{inj} = 100 µl, V_{load} = 150 µl.
C: Samples diluted 1:1 with eluent, V_{inj} = 100 µl, V_{load} = 450 µl.
The samples were prepared with a phosphate buffer, pH 6.5.

Table 6
Properties of the HPLC system

Sample	Concentration drug,* % (w/v)	Concentration BAC,* % (w/v)	Correlation coefficient† r	RSD‡ (%)
Tim. dr	0.25	0.01	0.9996	1.1
Xyl. dr	0.10	0.02	0.997	0.9

*A series of 80, 90, 100, 110 and 120% (of both BAC and the pharmaceutical component) was used to construct the calibration graphs; in the case of timolol eye drops this series was diluted 1:1 with the eluent.
†The total peak area (A_{12} + A_{14}) was used.
‡n = 6.
Conditions: flow rate = 2 ml/min; timolol: V_{inj} = 100 µl, V_{load} = 180 µl; xylometazoline: V_{inj} = 20 µl, V_{load} = 30 µl.

where teflon capillaries were used. Therefore, the adsorption of BAC to the tubing walls might not be the same in both cases. Nevertheless, the results of the FIA experiments showed a dependence of peak size on V_{load} similar to the HPLC experiments; furthermore, the discrepancy between recorded peak heights of samples containing only BAC and of samples also containing other components occurred in both the FIA and the HPLC system. It was therefore concluded that retention of the BAC components in the injector loop (and, to a lesser extent, in the rest of the system) was the cause of these phenomena.

Comparison of the HPLC and the FIA methods

Typical properties of the HPLC assay are given in Table 6. The correlation coefficient of the calibration curves is essentially the same as in the FIA method, whereas the relative standard deviation is somewhat lower. One assay with the HPLC method takes approximately 7 min which is about five times slower than the FIA method. In the HPLC method the pharmaceutical component was also added to the calibration samples; this improved the reproducibility of the method. Because physical separation of the different components takes place, the relative amount of these components was not a critical factor. Therefore, the drugs of interest were added to the standard BAC solution and this solution was used to make the calibration series.

Conclusions

Standard solutions of BAC can easily be assayed with the proposed method, having the advantage of very short analysis times. The presence of other (extractable) components in the samples might make optimization steps necessary. The compounds tested in this work had minor influences on the recorded peak heights after optimization of the experimental conditions, so that the required precision of the analysis determines the feasibility of the method. When samples, with other components present than the ones tested in this study are to be assayed, the influence of these components on the extraction behaviour of BAC has to be investigated as well as the extent to which they are extracted as an ion-pair with picrate. This work shows, that by optimizing the experimental conditions, the influence of other drugs present can in some cases be minimized.

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