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Note

Simultaneous determination of chlorhexidine, tetracaine and their degradation products by ion-pair liquid chromatography

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Chlorhexidine is a widely used antiseptic. On its own or in combination with other active principles, such as tetracaine, it is incorporated into pharmaceutical preparations of various degrees of complexity. Here we report a method, using ion-pair liquid chromatography, for the simultaneous determination of chlorhexidine and tetracaine in a pastille-type pharmaceutical preparation, together with, so long as the detection wavelength is correctly chosen, the estimation of *p*-chloroaniline, the main degradation product of chlorhexidine, and *n*-butyl-*p*-aminobenzoic acid, a degradation product of tetracaine. Chlorhexidine and tetracaine have already been separately the subjects of various analytical developments, particularly by high-performance liquid chromatography (HPLC). Detailed bibliographies concerning analytical methods applied to these two compounds can be found in various publications[1,2].

Among the possible variations of HPLC on a non-polar phase, that using an eluting phase containing an amphiphilic ion has been the subject of a large number of publications (refs. 3 and 4 and references therein). In general, at the pH of the eluting phase used the amphiphilic ion has an opposite electrical charge to that of the solute being chromatographed; the result is an increase in the capacity factor (k'), at least within a certain range of concentration. Another way of obtaining a selective effect on k' is to use an amphiphilic ion that, at the pH considered, has an electric charge of the same sign as the solute[3,5,6], which produces a decrease in k'. We adopted this latter approach to optimize the separation of chlorhexidine, tetracaine, *p*-chloroaniline and *n*-butyl-*p*-aminobenzoic acid, using tetrabutylammonium hydrogen sulphate as the amphiphilic ion.

EXPERIMENTAL

Apparatus

A liquid chromatograph (Model 6000 A, Waters Assoc., Milford, MA, U.S.A.), equipped with a variable-wavelength detector (Model 450, Waters Assoc.), (with measurement wavelengths specified in each case), an automatic injector (Model 710 B WISP, Waters Assoc.), and an integrating recorder (Model 3390 A, Hewlett-Packard France, Toulouse, France), were used. All calculations were performed using a programmable calculator (Model HP 41 C, Hewlett-Packard, Avondale, PA,

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U.S.A.). The stainless-steel column (30 cm × 3.9 mm I.D.) was packed with silica-bonded C_{18} (μBondapak C_{18}, 10 μm), Waters Assoc.

Preparation of the eluting phase

The acetonitrile [Chromasol-SDS, Peypin (13 124), France] used was of chromatography grade. The pH 3, 4 and 5 buffers were prepared from an aqueous solution of 13.6 g/l potassium dihydrogen phosphate (Normapur 26 926, Prolabo, Paris, France), adjusted to the required pH by addition of either concentrated phosphoric acid (Normapur 20 624, Prolabo), or of potassium hydroxyde (Normapur 26 632, Prolabo). Each eluting phase was prepared by mixing 250 ml of acetonitrile, 750 ml of pH buffer, 5.9 g of sodium chloride (Normapur 27 810, Prolabo) and 0, 1.7, 3.4, 6.4, 10.2, or 13.6 g of tetrabutylammonium hydrogen sulphate (Art. 818-518, Merck-Schuchardt, Hohenbronn, F.R.G.).

For a given pH, each solvent thus prepared corresponds to recorded concentrations of 0, 5, 10, 20, 30 or 40 m*M* tetrabutylammonium hydrogen sulphate. The pH was readjusted as required. Each eluting phase thus obtained (eighteen in all) for the construction of Fig. 1 was filtered through a membrane and degassed under vacuum. The sodium chloride was introduced to buffer the ionic strength of the medium[7]. The column was conditioned by the passage of 300 ml of solvent. Between each conditioning at a given level of tetrabutylammonium hydrogen sulphate, the initial state was regained by the passage of 300 ml of eluting phase containing no tetrabutylammonium hydrogen sulphate. For all the experiments, which were carried out at 21°C, a flow-rate of 2 ml/min was used.

Reagents

Tetracaine hydrochloride (Rhone-Poulenc, Paris, France), and *p*-chloroaniline (Hopkin and Williams, Chadwell Heath, U.K.) were of analytical grade. Chlorhexidine hydrochloride was the British Pharmacopoeia standard. The pharmaceutical formulation tested was a pastille of mean weight 2.5 g, containing 3 mg of chlorhex-

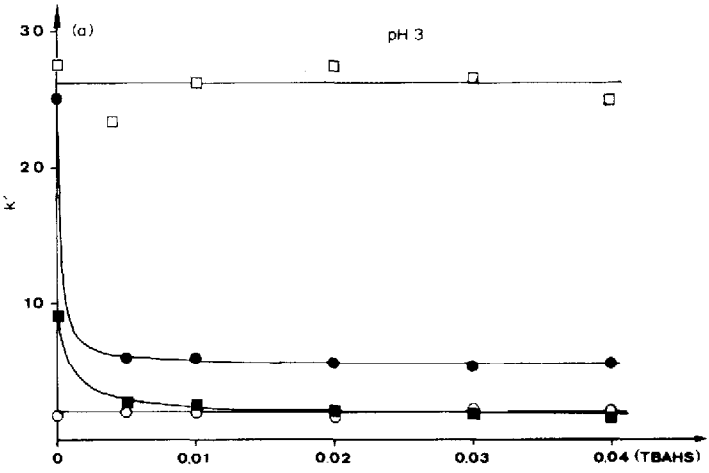


Fig. 1.

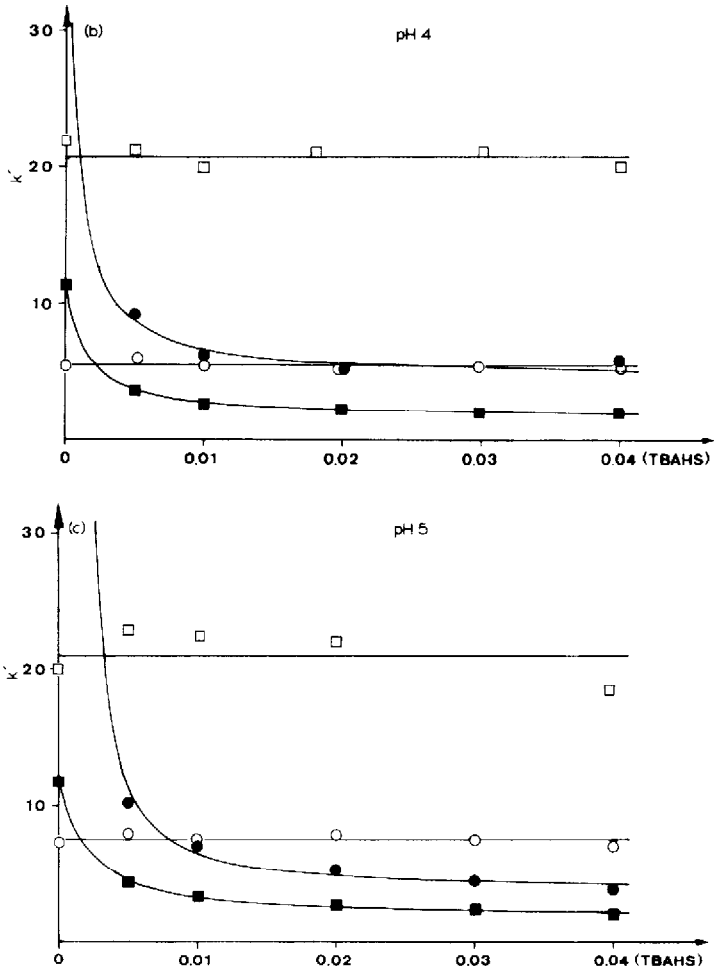


Fig. 1. Variation of k' for chlorhexidine (●), tetracaine (■), *p*-chloroaniline (○) and *n*-butyl-*p*-aminobenzoic acid (□), as a function of tetrabutylammonium hydrogen sulphate (TBAHS) molarity at pH 3, 4 and 5.

idine digluconate, 0.2 mg of tetracaine hydrochloride, ascorbic acid, sugar, flavouring and colouring. We obtained the *n*-butyl-*p*-aminobenzoic acid as a white powder by saponifying 1.5 g of tetracaine in 40 ml of water plus 10 ml of 1.0 *M* sodium hydroxide for 2 h, cooling, acidifying with 1.0 *M* hydrochloric acid, extracting with chloroform, drying over anhydrous sodium sulphate, and evaporating under vacuum. The analysis was as follows. Calculated for $C_{11}H_{15}NO_2$: C, 68.23; H, 7.81; N, 7.29; found: C, 68.21; H, 7.76; N, 7.18. ^{1}H NMR (Model R-24 B, 60 MHz, Hitachi, Tokyo, Japan): δ = 7.8 and 6.5 ppm (4*p*, aromatic); δ = 3.05 ppm (t, 2*p*, CH_3–CH_2–CH_2–CH_2–N); δ = 1.6 ppm (4*p*, CH_3–CH_2–CH_2–CH_2–N); δ = 1 ppm (t, 3*p*, CH_3–CH_2–CH_2–CH_2–N), spectra in C^2HCl_3. IR (Model 957, Perkin-Elmer, Beaconsfield, U.K.): 1650 cm^{-1}, –C_6H_4–COOH, spectra in KBr.

Preparation of solutions for routine estimation of chlorhexidine and tetracaine

Starting with a 0.0644 g/l aqueous solution of chlorhexidine hydrochloride (equivalent to 0.1 g/l of the digluconate) and with a 0.006 g/l aqueous solution of tetracaine, the following range of standard solutions was prepared:

Solution I (ml)	0.5	1	1.5	2
Solution II (ml)	0.5	1	1.5	2
Eluting phase (ml)	←to 50 ml→			

The test solution was prepared from an exactly weighed-out sample of *ca.* 1.25 g of material from five pastilles ground up as finely as possible and dissolved by mechanical stirring in a volume of the relevant eluting phase sufficient to make 50 ml. For each solution the volume injected was 50 μl, the measurement wavelength was 294 nm, and the sensitivity 0.02 a.u.f.s.

Preparation of the solutions for estimation of p-chloroaniline

The standard solution of *p*-chloroaniline contained 0.01 mg in 100 ml of eluting phase, and the test solution was identical with that described above. The volumes injected were 50 μl for the standard solution of *p*-chloroaniline and 100 μl for the test solution, the measurement wavelength was 240 nm and the sensitivity was 0.01 a.u.f.s.

Preparation of the solutions for estimation of n-butyl-p-aminobenzoic acid

The solution of *n*-butyl-*p*-aminobenzoic acid contained 0.008 mg in 100 ml of eluting phase, and the test solution was identical with that described above. The volumes injected were 50 μl for *n*-butyl-*p*-aminobenzoic acid and 100 μl for the test solution, the measurement wavelength was 300 nm, and the sensitivity was 0.01 a.u.f.s.

RESULTS AND DISCUSSION

Fig. 1 shows, at three pH values, the variation in k' as a function of the tetrabutylammonium hydrogen sulphate concentration for chlorhexidine, tetracaine-*p*-chloroaniline and *n*-butyl-*p*-aminobenzoic acid. The theoretical curves for I and II were calculated according to the thermodynamic model of Deming and Stranahan[3]; the principles of the calculation are given in an Appendix. For the problem that concerns us, close examination of the Fig. 1 shows that at pH 5 and with 30 m*M* tetrabutylammonium hydrogen sulphate, the separation of the four products was good enough for them to be estimated. Fig. 2 shows the chromatogram obtained under these conditions with a mixture of the four products.

For other pharmaceutical forms not studied here, other conditions may be selected. In addition, examination of Fig. 2 enables us to measure the chromatographic parameters that make it possible to validate the method. In particular, an asymmetry factor less than 1.3 was found for each product; the number of theoretical plates of the column was calculated to be 2400 for chlorhexidine, 3460 for tetracaine, 6800 for *p*-chloroaniline, and 6300 for *n*-butyl-*p*-aminobenzoic acid; the factor of resolution between chlorhexidine and tetracaine must be at least 2.5 (for the calcu-

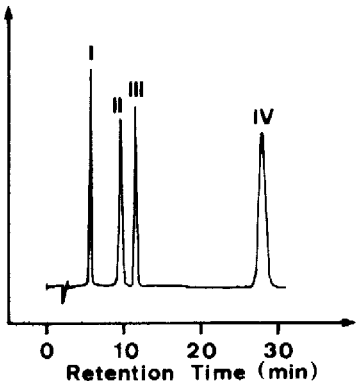


Fig. 2. Chromatogram of a control mixture of tetracaine (I), chlorhexidine (II), *p*-chloroaniline (III) and *n*-butyl-*p*-aminobenzoic acid (IV) obtained at pH 5 with 30 m*M* tetrabutylammonium hydrogen sulphate and detection at 294 nm.

lation of these parameters see ref. 8). We insist on the necessity of checking these parameters because the use of other C_{18} bonded phases did not lead to such good results. The area–concentration least-squares[9] standardization straight lines were studied for amounts injected between 0.64 and 2.56 μg for chlorhexidine and between 0.06 and 0.24 μg for tetracaine (determination at 294 nm). In all cases the correlation coefficient was found to be better than 0.999. To study the repeatability of the analytical method, we prepared and analysed five independent samples from one homogeneous ground-up preparation. A coefficient of variation (C.V.) of 0.95% was found for chlorhexidine of 0.88% for tetracaine. We also studied the degree of recovery of the method by adding known amounts of chlorhexidine and tetracaine to the test solutions. Tables I and II show that the level lies between 98 and 102%.

TABLE I

RECOVERY OF CHLORHEXIDINE

Initial amount injected (μg)	*Amount measured (μg) after addition of 0.6 μg*	*The same (μg) after addition of 1.2 μg*
0.662	1.280	1.880
Recovery (%)	101.4	100.9

TABLE II

RECOVERY OF TETRACAINE

Initial amount injected (μg)	*Amount measured (μg) after addition of 0.036 μg*	*The same (μg) after addition of 0.072 μg*
0.052	0.088	0.123
Recovery (%)	100	99.2

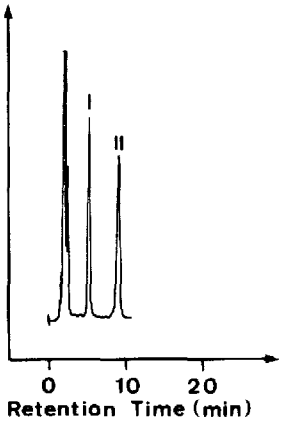
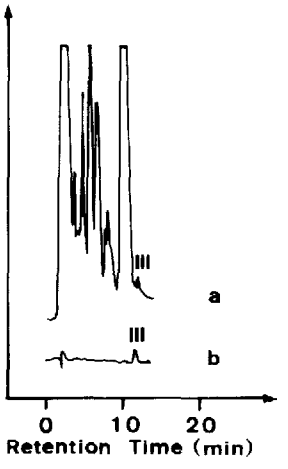


Fig. 3. Chromatogram obtained for the determination of tetracaine (I) and chlorhexidine (II) in a manufacturing batch of pastilles.

In order to automate the determination, we studied the stability over a period of 16 h of a test solution prepared in the eluting phase of pH 5 with 30 m*M* tetrabutylammonium hydrogen sulphate. We measured C.V. value of 1.22% for chlorhexidine and of 0.9% for tetracaine, which is perfectly acceptable. Fig. 3 represents the chromatogram obtained with one manufacturing batch.

Fig. 4a and b represents the chromatograms obtained at 240 nm for detection of *p*-chloroaniline in one manufacturing batch, and of a control solution of *p*-chloroaniline of concentration 400 ppm (here the batch contains *ca.* 300 ppm of *p*-chloroaniline).

Figs. 5a and 5b represent the chromatogram obtained at 300 nm for detection



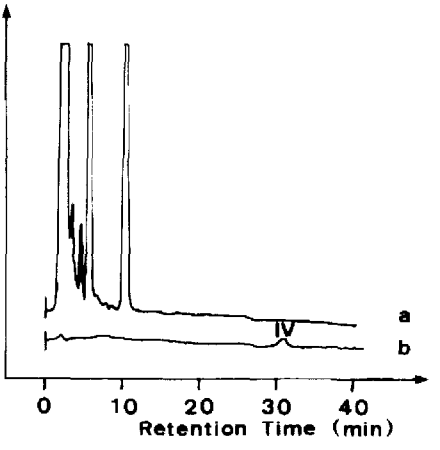


Fig. 4. (a) Detection at 240 nm of *p*-chloroaniline (III) in a manufacturing batch. (b) Control containing 400 ppm *p*-chloroaniline.

Fig. 5. (a) Detection at 300 nm of *n*-butyl-*p*-aminobenzoic acid (IV) in a manufacturing batch. (b) Control containing 4000 ppm *n*-butyl-*p*-aminobenzoic acid.

of *n*-butyl-*p*-aminobenzoic acid in one batch, and that of a control solution of *n*-butyl-*p*-aminobenzoic acid of concentration 4000 ppm (here no *n*-butyl-*p*-aminobenzoic acid was detected).

Finally, we note that the *British Pharmacopoeia*[10] has recently proposed an HPLC method for the estimation of related impurities in the various commercial chlorhexidine salts, while maintaining the colorimetric estimation of *p*-chloroaniline. Without going into details, we point out that with the HPLC method described here one can simultaneously and selectively estimate chlorhexidine, *p*-chloroaniline and related impurities.

APPENDIX

Calculation of the theoretical curves in Fig. 1

To calculate the theoretical curve of k' for chlorhexidine and tetracaine as a function of tetrabutylammonium hydrogen sulphate concentration, C, at the three pH values considered, we have adopted the thermodynamic model of Deming and Stranahan[3], which shows that:

$$\ln k' = a_0 + a_1 C/(a_2 + C)$$

where a_0 is $\ln k'$ of the product when $C = 0$, a_1 is a parameter related to the energy of interaction between the solute and the amphiphilic ion, and a_2 is a parameter related to the energy of absorption of the amphiphilic ion on the bonded C_{18} phase. Fitting of the parameters to the experimental data requires the use of a method of non-linear regression. We chose to fix an initial arbitrary value of a_2, which we call a_2^i, and then to define a new variable $X_j = C/(a_2^i + C)$. Then we found the least-squares best fit to the straight line $\ln k' = a_0 + a_1 X_j$. Then we varied a_2^i step by step until a maximum value was obtained for the correlation coefficient, halting the iteration when the eighth figure after the decimal point no longer altered. For chlorhexidine the figures were:

pH 3: $a_0 = 3.29$; $a_1 = -1.53$; $a_2 = 4.22$
pH 4: $a_0 = 3.81$; $a_1 = -2.71$; $a_2 = 1.82 \cdot 10^{-3}$
pH 5: $a_0 = 349.63$; $a_1 = -348.33$; $a_2 = 1.63 \cdot 10^{-5}$

and for tetracaine the figures were:

pH 3: $a_0 = 2.22$; $a_1 = -1.69$; $a_2 = 2.29 \cdot 10^{-3}$
pH 4: $a_0 = 2.42$; $a_1 = -1.90$; $a_2 = 3.53 \cdot 10^{-3}$
pH 5: $a_0 = 2.46$; $a_1 = -1.93$; $a_2 = 5.13 \cdot 10^{-3}$

For *p*-chloroaniline and *n*-butyl-*p*-aminobenzoic acid, k' was almost independent of C, so we drew straight lines through the mean values of k'.

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REFERENCES

1 L. Miribel, J. L. Brazier, F. Comet and D. LeCompte, *J. Chromatogr.*, 268 (1983) 321.
2 M. Bauer, L. Mailhé, D. Ménard and J. P. Rouanet, *J. Chromatogr.*, 259 (1983) 360.
3 J. J. Stranahan and S. N. Deming, *Anal. Chem.*, 54 (1982) 2251.
4 W. R. Melander and Cs. Horváth, in Cs. Horváth (Editor), *High Performance Liquid Chromatography*, Vol. 2, Academic Press, New York, 1980, p. 240.
5 W. Y. Lin, M. Tang, J. J. Stranahan and S. N. Deming, *Anal. Chem.*, 55 (1983) 1872.
6 M. Dreux and M. Lafosse, *Analusis*, 10 (1982) 87.
7 J. H. Knox and R. A. Hartwick, *J. Chromatogr.*, 204 (1981) 3.
8 L. R. Snyder and J. J. Kirkland, *Introduction to Modern Liquid Chromatography*, Wiley, New York, 1979, p. 218.
9 H. G. Mendelbaum, F. Madaule and M. Desgranges, *Bull. Soc. Chim. Fr.*, 5 (1973) 1619.
10 *British Pharmacopoeia 1980, addendum 1983*, Her Majesty's Stationery Office, London, p. 220.