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Determination of benzocaine, dextromethorphan and cetylpyridinium ion by high-performance liquid chromatography with UV detection

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ABSTRACT

A high-performance liquid chromatographic method for the determination of benzocaine, cetylpyridinium ion and dextromethorphan based on the use of an end-capped Ultrabase C_{18} column and an ion-pair formation reaction in a water–chloroform–methanol (10:50:40, v/v/v) mobile phase containing dioctyl sulphosuccinate is proposed. The use of a conventional or diode-array spectrophotometer resulted in calibration graphs with very different features. The determination ranges for benzocaine, cetylpyridinium and dextromethorphan provided by the diode-array detector were 10–100, 250–2000 and 250–2000 μg/ml, respectively, with relative standard deviations smaller than 3.0% for peak-height measurements and less than 1.40% for peak-area measurements. The conventional detector provided poorer results as a compromise wavelength must be chosen for measurements. The method was applied to the resolution of mixtures of the three analytes in pharmaceutical tablets. No interferences from other components of the tablets such as sorbitol, mint flavour and magnesium stearate were observed.

INTRODUCTION

There are several methods for the determination of benzocaine, cetylpyridinium ion and dextromethorphan, both alone and in mixtures with other drugs, some of which involve a prior separation of the analytes. Thus, dextromethorphan was determined individually after high-performance liquid chromatographic (HPLC) separation in syrup [1] and blood [2] samples. However, it is more frequently determined in mixtures with other pharmaceuticals on the basis of different principles depending on the nature of the remainder of the analytes involved and the sample matrix itself. Thus, pseudoephedrine hydrochloride, chlorpheniramine malate and dextromethorphan hydrobromide were determined simultaneously by second-derivative photodiode-array spectroscopy [3] and guaiphenesin, dextromethorphan and diphenhydramine by capillary gas chromatography [4]; however, most of these simultaneous determinations involve HPLC [5–8]. Conversely, methods for benzocaine normally rely on potentiometric (ion-selective electrode) [9] or photometric [10] measurements and on the use of HPLC for mixtures [11]. There are relatively few methods for the determination of cetylpyridinium ion. One of them involves its

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separation using ion-interaction chromatography with methanol–water mobile phases containing an ion-pairing agent [12].

A Spanish pharmaceutical manufacturer (Calmante Vitaminado) is soon to release a new pharmaceutical that includes dextromethorphan as cough supressant, benzocaine as local anaesthetic and cetylpyridinium ion as disinfectant. A method for the determination of these three drugs in mixtures will therefore be needed.

The proposed method relies on the separation of the analytes by HPLC using an end-capped Ultrabase C_{18} column and on measuring their intrinsic absorbances. Two types of detectors (conventional and diode-array) were used to monitor the eluate from the column. The sensitivity achieved depended on whether a single wavelength was used for measurement of all three analytes (conventional spectrophotometer) or whether each was monitored at its maximum absorption wavelength (diode-array spectrophotometer).

EXPERIMENTAL

Standards

Aqueous solutions of benzocaine (1.000 g/l), cetylpyridinium chloride (5.000 g/l) and dextromethorphan hydrobromide (5.000 g/l) solutions were prepared in the medium used as mobile phase. All the standards were supplied by Calmante Vitaminado.

Columns

Nucleosil C_{18} (5-μm) and end-capped Ultrabase C_{18} (5-μm) columns, both 25 cm × 4.6 mm I.D., were supplied by Scharlau (Barcelona, Spain).

Apparatus

An HP 1040A diode-array spectrophotometer equipped with an HP1040 DAD flow-cell (inner volume 2 μl) coupled to an HP 9000 Chem Station (all from Hewlett-Packard) or a Pye Unicam SP500 spectrophotometer furnished with a Hellma 178.2QS flow cell (inner volum 18 μl) was connected to an HP 1050 system (high-pressure quaternary gradient pump and a Rheodyne Model 7125 manual injection system).

RESULTS AND DISCUSSION

The absorption spectra of the analytes are shown in Fig. 1. The method was implemented in two ways: (a) by selecting a compromise wavelength for measurements and using a conventional spectrophotometer; and (b) by connecting the chromatograph to a diode-array detection (DAD) system to monitor each component at its maximum absorption wavelength. The results obtained in each instance are compared below.

Study of variables

The experimental variables optimized to accomplish adequate separation in eluting the analytes were the composition of the mobile phase, the ion-pairing reagent, the flow-rate, the injection volume and the type of column.

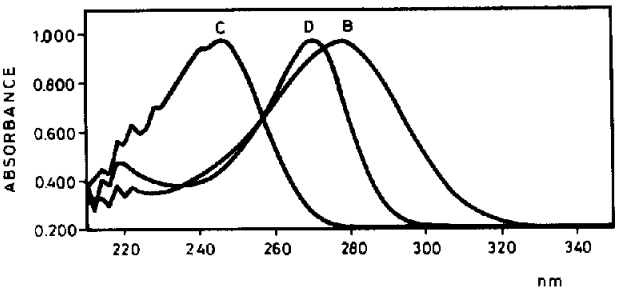

Fig. 1. Absorption spectra of (B) benzocaine, (C) cetylpyridinium ion and (D) dextromethorphan. Absorption maxima: 278, 244 and 270 nm, respectively. Concentrations: B, 10; C, 500; D, 250 μg/ml.

Among the different mobile phase tried, 0.03 *M* KH_2PO_4–acetonitrile–methanol (50:10:40, v/v/v) at different pH values (3.7, 4.0 and 5.3) was found to elute benzocaine alone, as also did 10 m*M* ammonium nitrate–acetonitrile–0.05 *M* dioctyl sulphosuccinate (25:50:25, v/v/v). The presence of chloroform was found to be essential for the three components to be eluted reasonably fast. The different water–chloroform–methanol ratios tried eluted benzocaine very fast, while dextromethorphan and cetylpyridinium were eluted slowly and with very long tails. Addition of an ion-pairing reagent to this mobile phase in the optimal proportions (10:50:40) was also examined. Sodium lauryl sulphate at different concentrations from 0.05 to 0.1 *M* ensured elution of the three analytes, but benzocaine and dextromethorphan overlapped, whereas 0.01 *M* sodium dioctyl sulphosuccinate provided acceptable resolution for all three compounds.

Of the different flow-rates studied over the range 0.6–1.2 ml/min, the best resolution of the mixture was achieved at 0.9 ml/min.

An injection volume of 20 μl resulted in adequate sensitivity.

The above experiments were performed by using a 25-cm Nucleosil C_{18} column (5-μm, pore diameter 120 Å). Despite the absence of overlapping peaks in the chromatogram obtained under the optimum working conditions, the tailing of dextromethorphan and especially cetylpyridinium resulted in long analysis times (about 15 min). To expedite the analyses and to improve the peak shapes we used an end-capped Ultrabase C_{18} column. Undesirable solute–stationary phase interactions were eliminated hy this column, in which surface groups were first capped and then deactivated [13]. The time required to obtain a chromatogram was reduced to less than 6 min. Fig. 2 shows a typical chromatogram of an equimolar mixture of the analytes, obtained by using DAD and monitoring at 240, 270 and 278 nm for cetylpyridinium, dextromethorphan and benzocaine, respectively, and Fig. 3 shows a three-dimensional recording.

A sampling frequency of up to 10 h^{-1} was achieved under the optimum working conditions.

Features of the proposed method

Calibration graphs were obtained by using both the conventional and the diode-array spectrophotometer. Measurements were performed at 254 nm with

TABLE I

FEATURES OF THE CALIBRATION GRAPHS

Detection	Parameter measured	Wavelength (nm)	Analyte[a]	Intercept (absorbance)	Slope [absorbance/ (μg/ml)]	Linear range (μg/ml)	r^2	R.S.D.[b] (%)
Conventional	Peak height	254	B	0.015	$5.8 \cdot 10^{-3}$	15–110	0.999	5.02
			C	−0.114	$6.5 \cdot 10^{-4}$	250–2100	0.995	4.10
			D	−0.003	$9.6 \cdot 10^{-5}$	600–2500	0.998	4.82
DAD	Peak height	278	B	0.054	$14.8 \cdot 10^{-3}$	10–75	0.995	2.84
		244	C	0.016	$6.2 \cdot 10^{-4}$	250–1500	0.998	1.70
		270	D	0.063	$2.8 \cdot 10^{-4}$	250–2500	0.989	1.45
DAD	Peak area	278	B	616.713	149.610	10–100	0.997	1.33
		244	C	1372.219	12.770	250–2500	0.996	1.37
		270	D	544.632	5.965	250–2500	0.997	1.05

[a] B = Benzocaine; C = cetylpyridinium ion; D = dextromethorphan.

[b] n = 11. Concentrations for R.S.D. determination: benzocaine = 50, cetylpyridinium ion = 1000 and dextromethorphan = 1000 μg/ml.

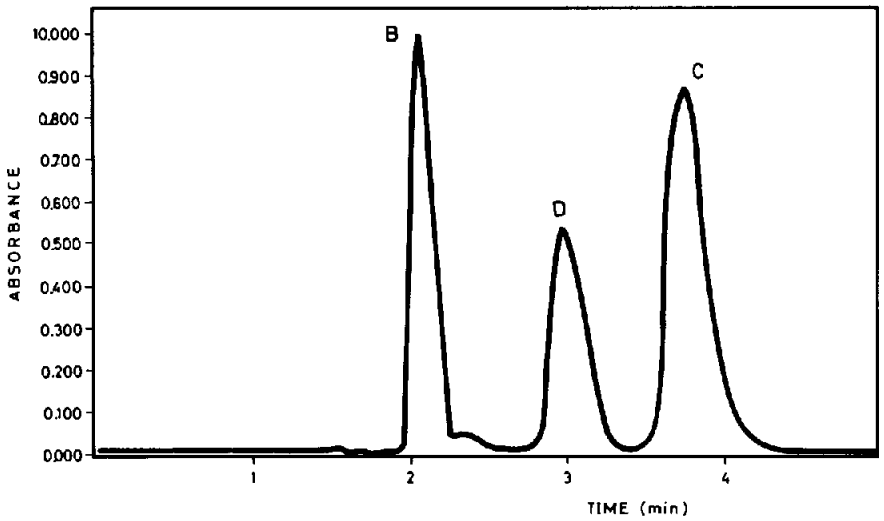

Fig. 2. Chromatogram of (B) benzocaine (25 μg/ml), (D) dextromethorphan (1000 μg/ml) and (C) cetylpyridinium ion (500 μg/ml). Mobile phase, water–chloroform–methanol (10:50:40, v/v/v) containing 0.1 *M* sodium lauryl sulphate; flow-rate, 0.1 ml/min; volume injected, 20 μl; column, end-capped Ultrabase C_{18}.

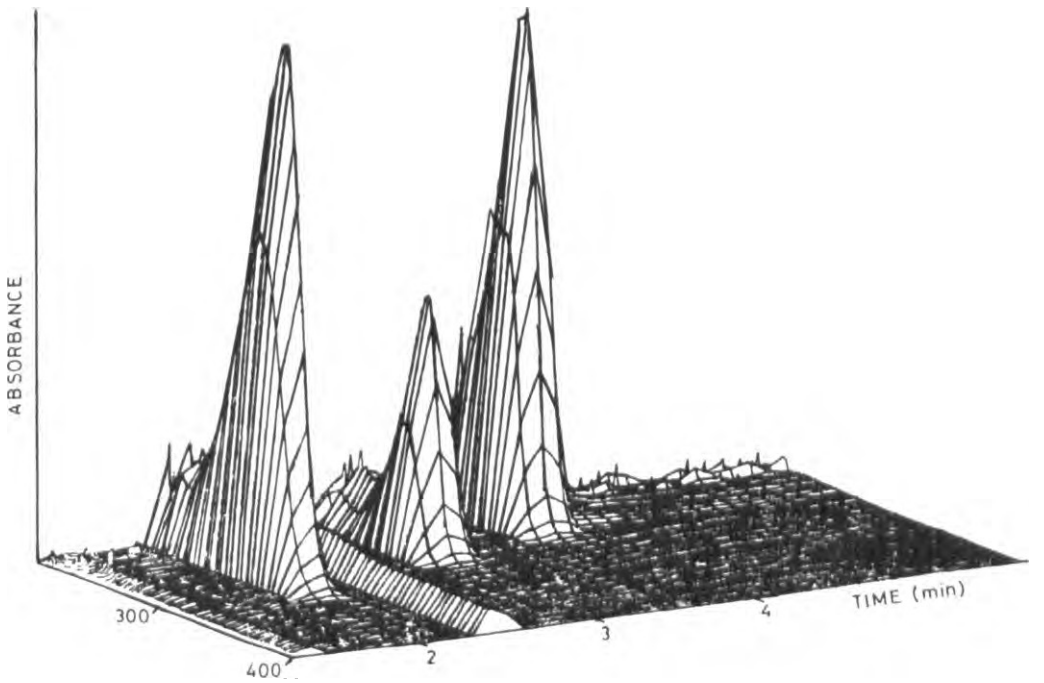

Fig. 3. Three-dimensional chromatogram of benzocaine, dextromethorphan and cetylpyridinium ion obtained using the conditions specified in Fig. 2.

conventional detection and at 278, 244 and 270 nm for benzocaine, cetylpyridinium and dextromethorphan, respectively, with DAD. Both peak-height and peak-area measurements were made in the latter instance. Table I summarizes the results obtained. The detection limits calculated as the ratio of the peak height to three times the baseline noise were 3.5, 80 and 175 μg/ml for benzocaine, dextromethorphan and cetylpyridinium, respectively.

On comparing the calibration graphs based on peak-height measurements obtained by using the two detectors, it is seen that the sensitivity achieved with DAD was higher than that provided by the conventional detector. The limit of determination for benzocaine was slightly improved by using DAD, but the gains were more significant for cetylpyridinium and dextromethorphan. The reproducibility of the method was studied by using eleven different samples containing concentrations of the analytes in the middle of the linear range (50, 1000 and 100 μg/ml for benzocaine, cetylpyridinium and dextromethorphan, respectively). Excellent relative standard deviation (R.S.D.) values were obtained with DAD (less than 3.0% in all instances), but they were higher than 4.0% with conventional detection. The explanation of this behaviour could be the data collection procedure (by a data station and manually, respectively).

Of the two types of measurements made on the data from the chromatogram obtained with DAD, those based on peak areas provided better sensitivity (slopes at least ten times greater), a similar linear range (except for cetylpyridinium, for which the range was wider) and better reproducibility (R.S.D. less than 1.40%).

Application of the proposed method to real samples

Applicability of the proposed method to the analysis of pharmaceutical tablets was tested in two ways. The manufacturer provided the composition of the tablets. We analysed synthetic samples containing the analytes plus additional compounds present in the tablets (sorbitol, mint flavour and magnesium stearate) in several proportions. Then, the tablets supplied by the manufacturer were dissolved in the mobile phase and injected directly into the chromatograph. The chromatogram thus obtained showed no interference from any of the components of the sample matrix in the synthetic or the real samples.

CONCLUSIONS

The proposed method for the chromatographic separation and determination of benzocaine, cetylpyridinium ion and dextromethorphan allows the determination of these drugs in pharmaceuticals. This is the first reported method for the resolution of this type of mixture, which only occurs in a still commercially unavailable pharmaceutical that will require a procedure for monitoring tablet production and for quality control.

As the concentration of the active substances in the tablets is high enough, a conventional spectrophotometer can be used for the routine control method. Future determination of these drugs at lower concentrations in biological fluids will require DAD in order to obtain better sensitivity and precision.

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