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Short Communication

Simultaneous determination of chloramphenicol and benzocaine in topical formulations by high-performance liquid chromatography

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ABSTRACT

A reversed-phase high-performance liquid chromatographic method was developed for the simultaneous determination of chloramphenicol and benzocaine in topical solutions and suppositories. The method was statistically evaluated for its accuracy and precision in the assay of chloramphenicol and benzocaine in commercial pharmaceutical preparations. Excipients and impurities normally present did not interfere, and the mean recoveries were 99.68% (R.S.D. = 1.20%) for benzocaine and 99.96% (R.S.D. = 0.66%) for chloramphenicol. A Waters Assoc. Guard-Pak precolumn module with a μBondapak C_{18} precolumn was utilized to protect the analytical column from contamination. The ratio-plotting technique was used to confirm the non-interference of excipients and other impurities with the peak of interest from formulations, and for validation of the method.

INTRODUCTION

Topical solutions and suppositories of chloramphenicol are widely used clinically in bacterial infections of the ear and eyes. The topical solutions are formulated with a local anaesthetic to avoid local irritation produced by chloramphenicol [1]. Benzocaine is commonly used in combination with chloramphenicol in formulations of topical solutions and suppositories.

Topical solutions and suppositories usually consist of large amounts of a non-polar, lipophilic material such as petrolatum, mineral oil or waxes. Prior to analysis, the active ingredients from pharmaceutical preparations normally have to be separated from the complex formulation matrices and analysed individually by conventional pharmacopoeial or literature procedures.

There are various methods for the individual determination of chloramphenicol [2–7] and benzocaine [8–12] in pharmaceutical preparations and biological fluids, but the only procedure reported for the simultaneous determination of these drugs is that

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by Kannan *et al.* [13], using differential spectrophotometry. This procedure involves reduction of the nitro group of chloramphenicol with zinc and hydrochloric acid. Benzocaine and unreacted chloramphenicol were determined by use of differential spectrophotometry. The method is laborious and non-specific.

Hence there is a need for a faster, specific and accurate method for the simultaneous determination of benzocaine and chloramphenicol in topical solutions in a single run, and high-performance liquid chromatography (HPLC) is the method of choice.

EXPERIMENTAL

Chemicals

Chloramphenicol and benzocaine (reference compounds) were donated by Parke-Davis Pharmaceuticals (Bombay, India). The internal standard, sulphamethoxazole, was obtained from Ana-Lab (Bombay, India) and acetonitrile (HPLC grade) from Glaxo Chemicals (Bombay, India). Formulations were obtained from commercially available sources.

Apparatus

The liquid chromatograph consisted of a dual-piston constant-flow pump (Model 510A solvent-delivery system), a programmable multi-wavelength detector with a four-channel monitoring system (Model 490), an autosampler (Model 712 WISP) and a data station (Model 840 with a Model 350 computer) with a Quickset HPLC programme, all from Waters Assoc. (Milford, MA, U.S.A.)

Chromatographic conditions

A reversed-phase 10-μm μBondapak C_{18} column (30 cm $\times$ 3.9 mm I.D.) was used together with a Guard-Pak precolumn module equipped with a disposable μBondapak C_{18} precolumn (Waters Assoc.) at ambient temperature (24 $\pm$ 2°C). The mobile phase was acetonitrile–water (35:65, v/v), filtered and deaerated before use. The flow-rate was 1.4 ml/min at a back-pressure of 1800 MPa. The detector was set at 280 nm (0.05 a.u.f.s.).

Ratio plotting

The other channel of the programmable multi-wavelength detector was set with a ratio-plotting programme with 280 nm as the observation wavelength and 240 nm as the master wavelength. The ratio observation maxima were set at 10% full scale of the recorder response with 0.05 a.u.f.s. detector sensitivity.

Stock solutions

Stock solutions in methanol of chloramphenicol (2.5 mg/ml) (stock solution I), benzocaine (1.0 mg/ml) (stock solution II) and sulphamethoxazole (2.0 mg/ml) (internal standard solution) were prepared.

A 2.0-ml volume of chloramphenicol stock solution I and 1.0 ml of benzocaine stock solution II were pipetted into a 25-ml volumetric flask, followed by addition of 2 ml of internal standard solution and dilution to volume with the mobile phase. The solution was mixed well and 20-μl aliquot of this standard preparation were used in the HPLC assay.

Sample preparations

Ear and eye drops. After shaking thoroughly, the topical solution equivalent to 5 mg of benzocaine or 25 mg of chloramphenicol was accurately pipetted into a 25-ml volumetric flask and diluted to volume with methanol, the vortex mixed for 5 min. A 2.0-ml volume of this solution ws transferred into a 25-ml volumetric flask, followed by addition of 2.0 ml of internal standard solution, and diluted to volume with the mobile phase. Aliquots of 20 μl were used in the HPLC assay.

Ointment. A cream equivalent to 25 mg of chloramphenicol was weighed into a 25-ml volumetric flask, dissolved in methanol, vortex mixed for 15 min and the insoluble excipients were allowed to settle. A 2.0-ml volume of the supernatant was transferred into a 25-ml volumetric flask, followed by addition of 2.0 ml of internal standard solution, and diluted to volume with the mobile phase. It was mixed well and 20-μl aliquots were used directly in the HPLC assay.

Assay procedure

The column was flushed with the mobile phase under the HPLC operating conditions until a stable baseline was obtained. Volumes of 20 μl each of the standard preparation and sample preparations were injected in triplicate at intervals of 15 min. The peak-area ratios of chloramphenicol and benzocaine with respect to the internal standard were calculated. By comparing the peak-area ratios for the standard and sample preparations the amount of each drug could be calculated.

Linearity

Linearity of detection was determined from the calibration graph. Triplicate samples of each of five concentrations of chloramphenicol and benzocaine containing the internal standard were injected onto the HPLC column. A linear correlation was observed between the peak-area ratios of the drug to the internal standard and the concentration of each active ingredient. The method found to be linear over the concentration range 0.04 –0.2 mg/ml for benzocaine and 0.12–0.6 mg/ml for chloramphenicol. The straight lines passed through the origin with a correlation coefficient of 0.9978 for chloramphenicol and 0.9997 for benzocaine.

Recovery

To study the accuracy, precision and reproducibility of the method, a recovery experiment was carried out. To a preanalysed sample, a known amount of standard drug was added at four different levels and each level was analysed at least seven times. From the amount of drug found by the proposed method, the percentage recovery (R) was calculated using the equation $R = 1/N \Sigma [100(Y - Z/X]$, where X is the amount of drug added, Y the amount of drug found, Z the amount of drug in the preanalysed sample and N the number of observation.

RESULTS AND DISCUSSION

The applicability of the proposed method was tested by determining chloramphenicol and benzocaine simultaneously in commercially available combined pharmaceutical preparations, or individually in combination with other ingredients, and the results are presented in Table I. Table II shows the recovery for added drug

TABLE I

HPLC ASSAY OF CHLORAMPHENICOL AND BENZOCAINE IN FORMULATIONS

Sample No.[a]	Formulation	Ingredients	Claimed (mg/ml)	Found (mg)	S.D.[b] (mg)	Recovery (%)
1	Ear drops	Chloramphenicol	50.0	50.06	0.38	100.12
		Benzocaine	10.0	9.82	0.45	98.20
2	Ear drops	Chloramphenicol	50.0	48.93	0.27	97.86
		Benzocaine	10.0	10.14	0.51	101.40
3	Ear drops	Chloramphenicol	50.0	49.18	0.48	98.36
		Prednisolone	5.0	–	–	–
		Lignocaine · HCl	20.0	–	–	–
4	Eye drops	Chloramphenicol	10.0	9.96	0.33	99.60
		Dexamethasone	1.0	–	–	–
5	Eye drops	Chloramphenicol	4.0	4.02	0.56	100.50

[a] Formulation 1 is from Parke-Davis Pharmaceuticals (Bombay, India), 2 from Juggat Pharma (Bangalore, India) and 3–5 are from FDC Pharmaceuticals (Bombay, India). Formulations 1, 2, 3 and 5 contain propylene glycol as vehicle and 4 contains water as vehicle.

[b] $n = 3$.

standards in preanalysed samples of suppositories. A 99.96% recovery (R.S.D. = 0.66%) for chloramphenicol and 99.68% (R.S.D. = 1.20%) for benzocaine indicate non-interference from excipients and high precision of the method. The method is easy to apply as it does not involve the use of any solid buffer for preparation of the mobile phase. Sample preparation is also simple and does not involve labour-intensive extraction for separation of the ingredients or any chemical modification prior to analysis. The proposed method is efficient and fast, as elution of drugs and internal standard is completed within 12 min and the peaks obtained are symmetrical with good resolution between the drugs and the internal standard (Fig. 1a).

Compared with other techniques, the proposed method is highly selective. The use of this method for the simultaneous determination of these drugs is advantageous as both the components have the same absorption maxima and the response of ben-

TABLE II

RECOVERY STUDIES AND STATISTICAL EVALUATION OF THE HPLC METHOD

Level No.	Benzocaine					Chloramphenicol				
	Amount added (mg)	Amount found (mg)	S.D. (mg) ($n=7$)	R.S.D. (%)	Recovery (%)	Amount added (mg)	Amount found (mg)	S.D. (mg) ($n=7$)	R.S.D. (%)	Recovery (%)
1[a]	0	9.86	–	–	–	0	49.31	–	–	–
2	1.12	10.96	0.16	1.46	99.81	4.21	53.45	0.31	0.58	99.86
3	2.24	12.14	0.11	0,99	100.36	8.42	57.59	0.46	0.81	99.76
4	3.36	13.15	0.19	1.44	99.47	12.63	62.08	0.38	0.61	100.23
5	4.48	14.21	0.13	0.91	99.09	16.84	66.11	0.43	0.65	99.94

[a] Preanalysis sample of a topical solution of chloramphenicol and benzocaine in combination.

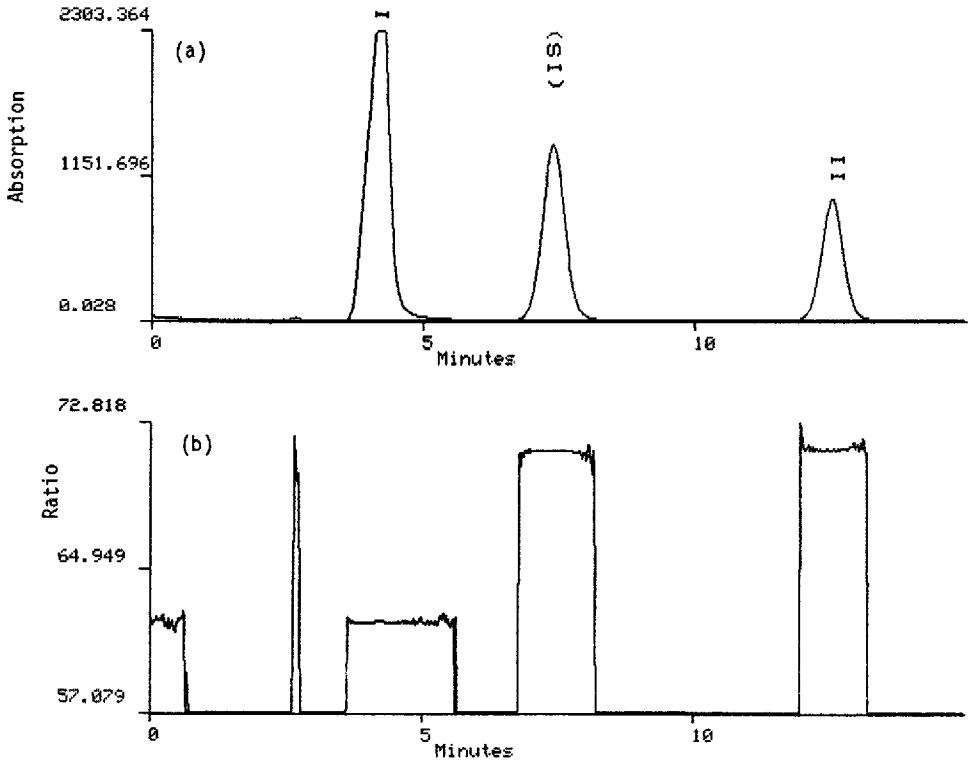

Fig. 1. (a) Typical HPLC trace for chloramphenicol (I, 0.8 mg/ml) and benzocaine (II, 0.18 mg/ml) in pharmaceutical preparations with sulphamethoxazole (IS, 0.4 mg/ml) as an internal standard. Detection, UV, absorption at 280 nm. (b) Ratiogram at observation wavelength 280 nm and master wavelength 240 nm.

zocaine at the observation wavelength, which is approximately five times greater than that of chloramphenicol, compensates for the concentration difference in commercially available combined formulations of these two ingredients. The flat top on the peaks for the drugs and internal standard in the ratiogram of the formulation (Fig. 1b) indicates non-interference from excipients or other impurities from pharmaceutical preparations [14].

Two lots of different formulations containing 0.5% of chloramphenicol and 0.1% of benzocaine were assayed with and without a μBondapak C_{18} precolumn. The retention time, resolution factors and tailing factors were not affected by incorporating the precolumn as an on-line column clean-up system [15]. In our study, we observed that one precolumn can efficiently protect the column during 70–75 sample injections of ointment and 100–110 sample injections of topical solutions. Hence the proposed method can be used for routine quality control analysis of these drug formulations, either for individual drug formulations or in combined dosage forms.

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