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Note

High-performance liquid chromatographic determination of chloramphenicol and 2-amino-1-(*p*-nitrophenyl)-1,3-propanediol in pharmaceutical formulations

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The recent report of Margosis[1] demonstrated the presence of a degradation product, 2-amino-1-(*p*-nitrophenyl)1,3-propanediol (AMPD) in chloramphenicol (CP) ophthalmic solutions and its interference in the Code of Federal Regulations (CFR) spectrophotometric method[2] used for assaying these formulations.

The British Pharmaceutical Codex (BPC)[3] specifies presently a limit of 5% AMPD in CP ophthalmic solutions and describes extraction procedures for the determination of CP in these and ophthalmic ointment preparations. However, we have found that the parabens, commonly used excipients in ophthalmic solutions, interfere in this assay.

A number of colorimetric methods[4–7] have been reported for the assay of CP and its precursors or degradation products. However, these methods either fail to distinguish between CP and AMPD or require a prior chromatographic separation. A fluorimetric method of analysis of CP and its metabolites employing quantitation of the reduction product, 1-(*p*-aminophenyl)-2-amino-1,3-propanediol has been reported[8]. This method is unable to distinguish between CP and AMPD.

Gas–liquid chromatographic methods of analysis of CP[9–14] involving derivatization with various silyl reagents have been reported. However, problems with the method of Bentley *et al.*[9] have been demonstrated[15] and Janssen and Vanderhaeghe[16] have shown that mixtures of silyl compounds are formed during derivatization. Only one method[12] reported the separation of CP from AMPD.

High-performance liquid chromatographic (HPLC) methods have been reported for the separation of CP from its precursors and congeners[17,18] and the determination of CP in serum by HPLC has been described[19]. Recently, Ali[20] described the separation of CP from its hydrolysis products including AMPD. No reference was made to interference by excipients, nor was the CP quantitated.

The present paper describes an isocratic HPLC method requiring neither extraction nor derivatization for the determination of both CP and AMPD in capsule, ophthalmic solution and ophthalmic ointment formulations.

EXPERIMENTAL

Equipment

A liquid chromatograph consisting of a Model 6000A pulseless pump (Waters Assoc., Milford, Mass., U.S.A.), a Model 155-40 variable wavelength detector operating at 254 nm (Altex Scientific, Berkeley, Calif., U.S.A.) and a U6K septumless injector (Waters Assoc.) was used. The range of the detector was set at 0.05 a.u.f.s. A 4.6 mm × 25 cm reversed-phase column (RP-2, 10 μm, Brownlee Laboratories, Santa Clara, Calif., U.S.A.) at ambient temperature and a flow-rate of 1.5 ml/min were employed. A SP4000 chromatography data system (Spectra-Physics, Santa Clara, Calif., U.S.A.) was used for integration of peak areas. Injections (5 μl) were made of all solutions for analysis.

Mobile phase

The mobile phase consisted of 0.01 *M* monobasic potassium phosphate buffer–methanol (58:42). The methanol was HPLC grade (Fisher Scientific, Fair Lawn, N.J., U.S.A.) and the buffer was made up volumetrically with distilled water. The mobile phase was filtered through a millipore system using Reeve Angel glass fiber filters 934 AH (Whatman, Clifton, N.J., U.S.A.). The filtrate was then degassed under vacuum for 10–15 min.

Solutions

Stock solutions. Chloramphenicol standard (House Standard 101.1% *vs.* USP standard) stock solution was prepared with a final concentration of 10 mg/ml in methanol. A solution of AMPD (Aldrich, Milwaukee, Wisc., U.S.A.) was prepared in distilled water to a concentration of 1 mg/ml. The internal standard, 1,3,5-trimethoxybenzene (TMB) (Aldrich) was dissolved in methanol (solution A) or absolute ethanol (solution B) to give a concentration of 10 mg/ml.

Standard solutions. Solutions of CP (0.2–3.0 mg/ml) and AMPD (0.02–0.40 mg/ml) containing 4 mg/ml of internal standard solution A were prepared from the stock solutions and made to volume with methanol.

Sample preparation

Bulk drug substance. To an accurately weighed amount of bulk drug (about 25 mg) was added 10 ml of internal standard solution A and the volume made to 25.0 ml with methanol.

Capsules. To an accurately weighed quantity of capsule contents equivalent to about 25 mg CP was added 10 ml of internal standard solution A and the volume made to 25.0 ml with methanol.

Ophthalmic solutions. To 2.0 ml of ophthalmic solution was added 2.0 ml of internal standard solution A and the solution made to 5.0 ml with methanol.

Ophthalmic ointments. To 2 g of ointment containing 1% CP was added 10 ml of hexane. The mixture was swirled for a few minutes to disperse the ointment, 10 ml of internal standard solution B was added, the solution was shaken for 2 min and the volume was made to 25.0 ml. A portion of the solution was centrifuged at 500 *g* for 10–15 min and the supernatant injected.

RESULTS AND DISCUSSION

The retention time of AMPD was dependent on the pH of the mobile phase. Optimum separation of AMPD from CP and excipients was obtained at the pH (5.0) used in this study. Baseline separation of the peaks resulting from AMPD, CP and the internal standard, TMB, was achieved. Common excipients such as the parabens did not interfere.

Fig. 1 shows a chromatogram of a standard mixture of AMPD (1 μg), CP (2.5 μg) and TMB (20 μg).

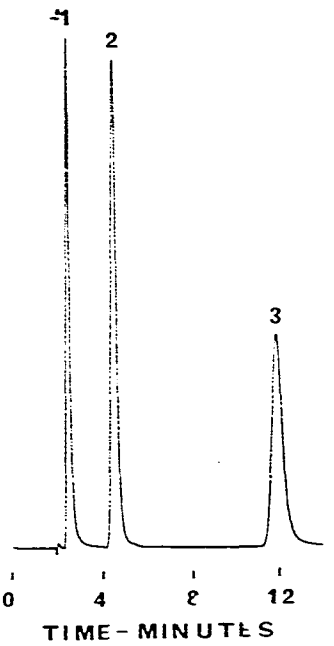

Fig. 1. Chromatogram of AMPD (1), CP (2) and TMB (3) on an RP-2 reversed-phase column with a mobile phase of 0.01 *M* monobasic potassium phosphate buffer–methanol (58:42).

The minimum detectable amount of injected AMPD was 5 ng which at a level of 1 mg/ml chloramphenicol corresponds to a sensitivity of 0.1%.

The linearity of the chromatography system was verified by injection of five solutions containing CP (0.2–3.0 mg/ml), AMPD (0.02–0.40 mg/ml) and internal standard. For chloramphenicol a coefficient of correlation of 0.9999 ($y = 2.279x + 0.056$) and for AMPD a coefficient of correlation of 0.9998 ($y = 4.178x + 0.116$) was obtained when the ratios of the areas of the peaks for CP and AMPD to the area of internal standard were plotted versus concentration (mg/ml).

Six consecutive injections of a solution of CP (1 mg/ml) and AMPD (0.1 mg/ml) resulted in relative standard deviations (RSD) of the area ratios of CP and AMPD to TMB of 0.48 and 1.00%, respectively.

The determination of the CP content of ten aliquots of a homogeneous capsule formulation composite yielded a mean of 97.3% and RSD, of 0.9%.

All results shown in Tables I–III are an average of duplicate determinations.

TABLE I

DETERMINATION OF CHLORAMPHENICOL OPHTHALMIC SOLUTIONS

Sample	% Label claim			
	HPLC		BPC	
	Chloramphenicol	AMPD	Chloramphenicol	AMPD
1	51.7	26.2	53.4	30.2
2	95.0	10.0	—	—
3	104.7	10.2	102.6	10.7
4	99.9	20.8	Parabens*	
5	102.1	21.3	Parabens*	
6	95.9	12.3	Parabens*	
7	96.1	7.5	97.1	13.0
8	101.0	0.2	100.2	0.1
9	106.5	16.2	107.2	14.2

* These formulations contained parabens which resulted in high assay values.

TABLE II

DETERMINATION OF CHLORAMPHENICOL OPHTHALMIC OINTMENTS

Sample	% Label claim			
	HPLC		BPC	
	Chloramphenicol	AMPD	Chloramphenicol	AMPD
1	105.1	1.5	—	
2	98.0	0.6	—	
3	107.0	0.4	105.1	
4	106.5	0.5	105.3	

Table I shows the results obtained from the analysis of nine ophthalmic solutions. Included are the results for five formulations using the method of the BPC modified according to Margosis[1]. Three formulations contained parabens and gave erroneously high results which are not shown. Insufficient sample of formulation 2 precluded analysis by the BPC method. Good agreement between the HPLC and BPC methods was usually obtained. Formulation 7 may have contained a water soluble expicient which resulted in slightly high results for AMPD content by the BPC method.

TABLE III

DETERMINATION OF CHLORAMPHENICOL CAPSULES

Sample	% Label claim		
	HPLC		CFR
	Chloramphenicol	AMPD	Chloramphenicol
1	104.7	0.4	104.9
2	97.9	0.1	100.2
3	103.6	0.2	101.8

Common excipients present in ophthalmic solutions, for example, the parabens and chlorobutanol, did not interfere in the HPLC assay. Methyl, ethyl and propyl parabens had retention times of 6.5, 9.7 and 16.6 min. Quantitation of the parabens could be carried out simultaneously with the determination of AMPD and chloramphenicol. Chlorobutanol was not detected at 254 nm.

Similarly, Table II shows the results of the HPLC determination of four chloramphenicol ointments and the results from the BPC method for ointments for two formulations. The other two formulations consisted of a hydrophilic base which yielded erratic results when determined by the BPC method. Good agreement was obtained between the two methods. The BPC results may be slightly low due to incomplete extraction of the CP from the ointment base. Centrifugation of the final solution before injection into the liquid chromatograph to prevent sampling a small amount of suspended ointment base is recommended. As no pre-injection extraction is applied to the ointment preparations, a column wash cycle must be employed after each sample. After the internal standard is eluted, the column is flushed with methanol for 20 min to remove the non-polar fractions of the ointment preparations. No interfering peaks were present in the chromatograms of these formulations.

Good agreement between the values obtained from the determination of CP and AMPD in capsules by HPLC and the CFR spectrophotometric method[21] for CP is demonstrated in Table III.

Confirmation that the peak quantitated as AMPD was, in fact, the hydrolysis product of CP was obtained by proton magnetic resonance (PMR). The compound corresponding to the AMPD peak in ophthalmic solution formulation 1 was collected from the HPLC using 42% methanol in water acidified to pH 5 with 1 *M* hydrochloric acid as the mobile phase and the PMR spectrum (Bruker WP-80) recorded in deuterium oxide. The spectrum was identical to that obtained under the same conditions for the authentic AMPD used as a standard in the HPLC analysis.

The data presented in Tables I–III indicate that the presence of AMPD is most pronounced in ophthalmic solutions suggesting post-formulation degradation as previously reported[1].

This HPLC method is a rapid, precise and accurate method for the determination of chloramphenicol and its degradation product 1-amino-2-(*p*-nitrophenyl)-1,3-propanediol in ophthalmic solution, ointment and capsule formulations. It is an improvement on present methods in that direct analysis without extraction or derivatization is performed.

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