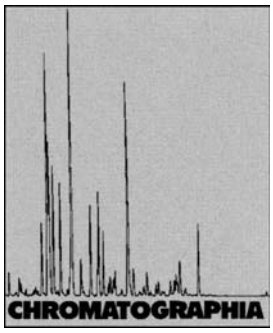
Development and Validation of an HPLC Method for the Determination of Dexamethasone, Dexamethasone Sodium Phosphate and Chloramphenicol in Presence of Each Other in Pharmaceutical Preparations



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Abstract

An HPLC method for the determination of dexamethasone, dexamethasone sodium phosphate and chloramphenicol in presence of each other in pharmaceutical preparations has been developed using a Shim-Pack CLC-ODS column (6.0 × 150 mm^2). These analytes were separated under isocratic conditions. Various chromatographic parameters including linearity, precision and accuracy have been evaluated. The method was found to be suitable for analysis of these drug substances in presence of each other. The run time was less than 15 min. This method is suitable for application to various dosage forms.

Keywords

Column liquid chromatography
Corticosteroids
Chloramphenicol

Introduction

Several preparations contain dexamethasone or its derivatives and chloramphenicol in combination with each other. These include: Spersadexoline and Dispersadron-C (Novartis), Dexol (Belco Pharma, India), Dexacol and Dexachlor (Birzeit–Palestine Pharmaceutical Company, Palestine), Aurocol-DM (Aurolab, India), and Dexachlor (Ethical Labs, Pakistan). Currently separate assay methods are being used for determination of dexamethasone and chloramphenicol [1] in these preparations. In addition to these pharmacopoeal procedures, several other methods have been reported for separate determination of the components. Only a couple of relevant examples are available. Dexamethasone has been separated from its phosphate salt and inactive ingredients present in various formulations [2]. Chloramphenicol has been separated from hydrocortisone in various pharmaceutical preparations [3, 4]. But no method has yet been reported which could be used to determine dexamethasone, dexamethasone sodium phosphate and chloramphenicol in presence of each other in a preparation. Therefore, an assay method needs to be developed for simultaneous determination of all these components that may be present in such preparations. In these preparations dexamethasone is added in the form of dexamethasone or dexamethasone sodium phosphate. In all the cases dexamethasone is the main pharmacologically active component. Dexamethasone sodium phosphate may disproportionate to dexamethasone and/or dexamethasone phosphate in solution. Although these disproportionation products are pharmacologically equivalent, the compendial methods deal them as impurities [1]. This practice is leading to exposure of patients to higher than the intended doses of the pharmacologically active component. In principle, the disintegration products of dexamethasone sodium phosphate, vis-à-v dexamethasone and/or dexamethasone phosphate must be accounted for while determining the assay. In order to assay all the forms of dexamethasone and chloramphenicol in presence of each other in pharmaceutical preparations a suitable method is, therefore, desirable. In the present study we have developed and validated an HPLC method for such determinations.

Experimental

Materials

The following chemicals were used without further purification: sodium dihydrogenphosphate (Merck, Germany), acetonitrile, HPLC grade (Sigma-Aldrich, Germany), methanol, HPLC grade (Merck, Germany), potassium hydroxide (Merck, Germany).

The standards, dexamethasone RS, dexamethasone phosphate RS and

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Table 1. Performance of various mobile phases (with flow rate of 1 cm^3 min^{-1} or as stated)

Compositions	Resolution	Theoretical plates (m^{-1})
Buffer:Acetonitrile:Methanol (3.9:1.15:1) Temperature: 30 °C	Did not resolve	–
Buffer:Acetonitrile:Methanol (1.2:1:1.13); Temperature: 30 °C	Did not resolve	–
Buffer:Acetonitrile:Methanol (1.73:1.16:1) Temperature: 30 °C	Between DSP and CHL: 0.727; between CHL and DEX: 6.5	DSP = 746 CHL = 1,296 DEX = 2,036
Buffer:Acetonitrile:Methanol (1.73:1.16:1) Temperature: 40 °C	Between DSP and CHL: 0.923; between CHL and DEX: 8.7	DSP = 991 CHL = 2,020 DEX = 6,315
Buffer:Acetonitrile:Methanol (1.73:1.16:1) Temperature: 50 °C Flow Rate: 0.5 cm^3 min^{-1}	Between DSP and CHL: 1.50; between CHL and DEX: 11.56	DSP = 10,582 CHL = 11,731 DEX = 14,288
Diethylamine soln.:Methanol (2.85:1) Temperature: 40 °C	The t_R for the peaks of dexamethasone, dexamethasone sodium phosphate and chloramphenicol were very large and peak widths for these peaks were also broad.	–

DEX Dexamethasone, *DSP* dexamethasone sodium phosphate, *CHL* chloramphenicol

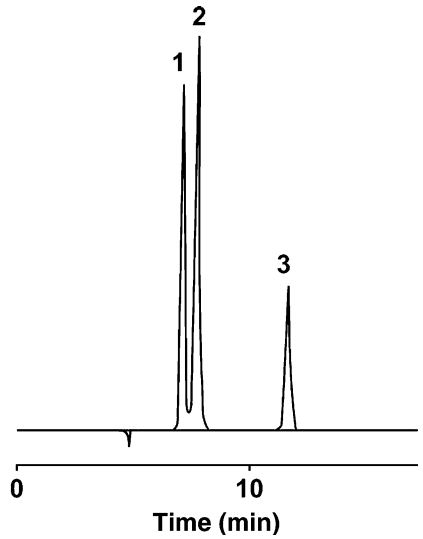

Fig. 1. A typical chromatogram of mixture: standard dexamethasone sodium phosphate (1), chloramphenicol (2) and dexamethasone (3)

chloramphenicol RS were obtained from US Pharmacopeial Convention, Inc., USA. The standard dexamethasone sodium phosphate CRS was obtained from European Pharmacopoeia Commission.

The preparations used were: Ophthdex (dexamethasone, Ophth-Pharma, Karachi), Decadron (dexamethasone sodium phosphate, MSD), Econochlor (chloramphenicol, Alcon), Dexachlor (dexamethasone sodium phosphate + chloramphenicol, Ethical Labs., Lahore), Methachlor (dexamethasone sodium phosphate + chloramphenicol, Remington Pharmaceuticals, Lahore), Dexoptic-C (dexamethasone + chloramphenicol, Sante, Karachi).

Preparation of Solutions

Buffer Solution

0.048 M Sodium dihydrogenphosphate in distilled water, pH adjusted to 5.4 with 0.5 M potassium hydroxide solution.

Mobile Phase

Filtered degassed mixture of the buffer solution, acetonitrile and methanol mixed in the ratio of 1.73:1.16:1.

Standard Solution

Accurately weighed quantities, about 12 mg of dexamethasone RS in 100 mL of the mobile phase, about 8 mg of dexamethasone phosphate RS in 100 mL of the mobile phase, about 10 mg of chloramphenicol RS in 100 mL of the mobile phase.

Assay Preparation

1. An accurately measured volume of dexamethasone ophthalmic suspension (Ophthdex) equivalent to about 3 mg of dexamethasone was transferred into an amount of the mobile phase. After mixing well it was diluted to 25 mL with the mobile phase.
2. An accurately measured volume of dexamethasone sodium phosphate injection (Decadron) equivalent to about 50 mg of dexamethasone sodium phosphate was transferred into an amount of the mobile phase. After mixing well it was diluted to 100 mL with the mobile phase.
3. An accurately measured volume of chloramphenicol ophthalmic solution (Econoclor) equivalent to about 50 mg of chloramphenicol was transferred into an amount of the mobile phase. After mixing well it was diluted to 100 mL with the mobile phase. Five milliliter of this solution was diluted to 25 mL with the mobile phase.

Chromatographic Conditions

The column used was stainless steel, 6.0×150 mm^2, packed with 5 μm CLC-ODS (Shim-Pack, Shimadzu). The chromatographic conditions were: detector wavelength 254 nm; injection volume 10 μL; column temperature 50 °C, and flow rate 0.5 mL min^{-1}.

Procedure

Precision

Ten replicate measurements were made by using the following solutions in the mobile phase: (i) 2 mg of dexamethasone per 100 mL, (ii) 3 mg of dexamethasone sodium phosphate per 100 mL, and (iii) 2.5 mg of chloramphenicol per 100 mL.

Linearity

Six different concentrations of dexamethasone, dexamethasone sodium phosphate, and chloramphenicol were prepared in the mobile phase; 10 μL of each concentration was injected.

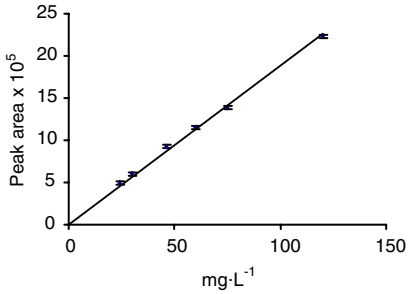

Fig. 2. Linearity graph of dexamethasone. The error bars refer to ± SD of mean of four measurements

Limit of Detection

The solutions of dexamethasone, dexamethason sodium phosphate, and chloramphenicol in the mobile phase were diluted to known concentration to a final response equal to twice the signal-to-noise ratio and chromatographed.

Results and Discussion

Selection of Mobile Phase

Phosphate buffer solution–acetonitrile–methanol mixture and diethylamine solution–methanol mobile phases were studied with a view to select a suitable mobile phase. The suitability criterion was: a good compromise of resolution, peak width and run time. The trial runs indicated that the phosphate buffer solution–acetonitrile–methanol mixture was more appropriate. Therefore, optimization of conditions was carried out by varying the composition of the mobile phase, temperature of the column and flow rate of the mobile phase. The resolution obtained from the three compositions of phosphate buffer solution-acetonitrile-methanol and one composition of diethylamine solution–methanol at 30, 40 and 50 °C with flow rates of the mobile phase from 1 to 0.5 $cm^3\ min^{-1}$ are given in Table 1.

From this study, the mobile phase composition of 1.73:1.16:1 and a temperature of 50 °C with a 0.5 $cm^3\ min^{-1}$ flow rate of the mobile phase were found to be the most suitable because they afforded better resolution and shorter run time. These conditions were used for the subsequent study.

All the substances under investigation were separated under the chromatographic conditions used. A typical chromatogram of the standard mixture is shown in Fig. 1. The analysis was performed with external standard and a good precision was obtained (Table 3). Therefore, the validation of the method was carried out using the external standard method and the optimum conditions as described above. Various chromatographic parameters thus obtained are given in Table 3.

In case of closely eluting components (dexamethasone sodium phosphate and chloramphenicol) an experiment was performed to check the validity of the method if one compound is present in large excess compared to the other. No significant change in validation data was observed when the concentration of either of the components was in a 1:3 ratio within the concentration range under study.

A graph of peak area versus concentration of dexamethasone was a straight line with a correlation coefficient (r^2) of 0.9962. A typical plot of peak area versus concentration of dexamethasone is shown in Fig. 2. This shows an excellent detector response in the range under study. The method appears to be highly sensitive as indicated by very low value of LOD.

The USP method [1] for the assay of dexamethasone uses water–acetonitrile mixture as the mobile phase. A tailing is observed with this method. This requires flushing of the system after use. By the use of the buffer in the newly developed method, the tailing is minimized.

Precision

The within-day and between-days precision of the method was determined for both peak area and retention time of dexamethasone (20 μg mL^{-1}), dexamethasone sodium phosphate (30 μg mL^{-1}) and chloramphenicol (25 μg mL^{-1}) by measuring the response of replicate injections. The results are given in Table 3.

Table 2. Linearity parameters

Substance	r^2	Slope	Concentration range (μg mL^{-1})
Dexamethasone	0.9962	967.68	24–120
Dexamethasone sodium phosphate	0.9971	965.79	16–80
Chloramphenicol	0.9998	921.49	20–100

Linearity

The detector response was measured at 254 nm from six solutions containing the analyte and a graph of peak area versus concentration was plotted. A straight line was obtained. The statistical parameters including correlation coefficient, slope and intercept are given in Table 2.

Limit of Detection and Quantification

Limit of detection (LOD) and limit of quantification (LOQ) are given in Table 3. The very low levels indicate that the method is very sensitive for the determination of the substances under investigation and suitable for the determination of dexamethasone, dexamethasone sodium phosphate and chloramphenicol in the presence of each other.

Accuracy

The accuracy of the proposed method was determined by measuring the response for solutions of analytes of known concentration in triplicate. The concentration of analyte was calculated and a linear regression was performed of the mean concentration.

There was no method available for the simultaneous determination of dexamethasone, dexamethasone sodium phosphate and chloramphenicol by HPLC in the British Pharmacopoeia, European Pharmacopoeia, and United State Pharmacopoeia or in the literature. The methods given in the compendia or in literature are for the individual determination of the drug substances. The USP methods for the individual determination of dexamethasone, dexamethasone sodium phosphate and chloramphenicol use different solvent systems and thus cost much in terms of

Table 3. Chromatographic parameters

Substance	Precision (RSD%) within-day (between-days)		Resolution	Theoretical plates (m^{-1})	LOD (ng mL^{-1})	LOQ (ng mL^{-1})
	Area	R_t				
Dexamethasone	3.35 (5.01)	0.23 (0.42)	Between CHL and DEX: 11.56; between DSP and CHL: 1.5	14,288	10	20
Dexamethasone sodium phosphate	2.89 (5.61)	0.14 (0.33)		10,582	100	200
Chloramphenicol	2.65 (4.83)	0.06 (0.17)		11,731	1.5	3.0

Table 4. Analysis of some commercial formulations

Formulation	%(± RSD) Label claim		
	Dexamethasone	Dexamethasone sodium phosphate	Chloramphenicol
Ophth-Dex	98.35 ± 3.33	–	–
Decadron	–	99.25 ± 2.88	–
Econochlor	–	–	65.69 ± 2.61
Dexachlor	–	95.76 ± 2.79	68.28 ± 2.64
Methachlor	–	94.12 ± 2.83	79.73 ± 2.59
Dexoptic-C	65.27 ± 3.11	–	60.75 ± 2.55

materials, time and labour for the combination products. The newly developed HPLC method appears to be better than the pharmacopeial methods and has the advantage of being the most convenient and cost-effective one for simultaneous determination of the drug substances under investigation in presence of each other. The tailing observed in this method is less than 1.15 for dexamethasone and chloramphenicol and no tailing observed for dexamethasone sodium phosphate.

The newly developed method was validated by comparing the results with those obtained from the use of relevant USP methods. The method is applicable to ophthalmics and injections as such and to other dosage forms after modifying the sample preparation procedure. The analysis of real-life samples of ophthalmic preparations including the combination products was achieved without any interference of adjuvants present in these formulations. The results are listed in Table 4.

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