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RP-HPLC Method for Simultaneous Estimation of Ciprofloxacin and Dexamethasone in Eye/Ear Drops

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ABSTRACT

High performance liquid chromatography method was applied to the simultaneous determination of ciprofloxacin and dexamethasone. The chromatographic separation was achieved on reversed-phase C18 column(25 cm × 4.6 mm id, 5 µm) in the isocratic mode using methanol-water-triethylamine (55:45:0.6, v/v/v), pH adjusted to 3.0 ± 0.05 with orthophosphoric acid as the mobile phase at a flow rate 0.8 ml/min. Quantitation was achieved with UV detection at 254 nm. In the HPLC method, quantification was achieved over the concentration range of 3-18 and 1-6µg/ml, with mean recoveries of 99.94 ± 1.51 and 100.28 ± 1.25% for ciprofloxacin and dexamethasone respectively. The proposed methods were successfully applied for the analysis of synthetic mixtures and pharmaceutical formulations of ciprofloxacin and dexamethasone without any interference from common excipients.

Keywords: Ciprofloxacin, Dexamethasone, Liquid Chromatography, Validation.

INTRODUCTION

Ciprofloxacin (1 cyclopropyl- 6-fluoro- 1, 4-dihydro- 4-oxo- 7[1-piperazinyl] - 3-quinolinecaroboxylic acid) is a broad spectrum antibacterial agent, belonging to the group of fluoroquinolones. [1-2] Ciprofloxacin is active against a wide variety of gram-positive and gram-negative organism, use in the treatment of urinary tract infection, conjunctivitis, gonorrhoea and respiratory tract infection. [3] Dexamethasone (9α-fluoro-11β, 17, 21-trihydroxy- 16α-methylpregna- 1, 4-diene-3, 20-dione) is a synthetic glucocorticoid with varied pharmacological activities including anti-inflammatory, anti-rheumatic and immunosuppressant effect. [1-3]

Ciprofloxacin (CIP) was determined individually by non-aqueous titration [4], UV-spectrophotometry [5], colorimetry [6-8], high performance liquid chromatography [9-15], HPLC with mass spectrometry [16], thin-layer chromatography [17-18], gas chromatography [19] and capillary electrophoresis. [20-21] On the other hand dexamethasone (DEX) was determined individually by UV spectroscopy [22], HPLC [23-26], TLC [27], HPLC with mass spectrometry [28-30], gas chromatography with mass spectrometry. [31] Analytical method was reported for Pharmacokinetics of CIPRODEX® otic suspension in pediatric and adolescent patients by LC/MS/MS. [32] Ciprofloxacin and Dexamethasone are formulated together in

the form of eye/ear drops (solution) or otic suspension which is used in the treatment of conjunctivitis, keratitis, acute otitis externa and acute otitis media in pediatric patients with tympanostomy tubes. [33-35] Ciprofloxacin and Dexamethasone eye/ear drops (solution) are not official in any pharmacopoeia while Ciprofloxacin and Dexamethasone otic suspension is official in United States Pharmacopoeia. [26] The main problem of spectrophotometric binary mixtures analysis is the simultaneous determination of two drugs without prior separation. No Isocratic HPLC method and spectrophotometric methods are available for simultaneous determination of ciprofloxacin and dexamethasone, so there was a need to develop accurate method for their determination in combination. The aim of this work is to determine both drugs concurrently by simple, rapid, and selective HPLC method which could be used for quality control and routine analysis.

MATERIAL AND METHODS

Instrumentation

HPLC instrument (Shimadzu, Kyoto, Japan) was equipped with a model series LC-10 AS pump, Rheodyne 7725i injector with a 100µl loop and SPD-10A UV-Visible detector. A Grace smart reversed-phase C18 column (Grace Discovery and Division; 5µm, 25 cm × 4.6 mm id) was used as the stationary phase. Class CR10 software was used for data acquisition.

Chemicals and Reagents

CIP powder was procured from Astron Research (Ahmedabad, India). DEX powder was procured from

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Lincoln Pharmaceutical Ltd. (Ahmedabad, India). Purity of CIP and DEX were 99.69 and 99.28 respectively. All experiments were performed with analytical-reagent grade chemicals. For HPLC work, methanol, phosphoric acid and triethylamine were of HPLC grade. Triple distilled water was used. Ciplox-D Eye/Ear Drops (Batch No. MG-8051) was manufactured by Cipla. Zoxan-D Eye/Ear Drops (Batch No. ZZC-9065) was manufactured by FDC. Each eye/ear drop claimed to contain 0.3 % w/v CIP (as free base) and 0.1% w/v DEX. All were purchased from Indian market.

Chromatographic Conditions
A 250 mm × 4.6 mm i.d Grace smart (5μm particle size) reversed-phase C_{18} column was used for separation and quantitation. The mobile phase consisted of methanol-water-triethylamine (55:45:0.6, v/v/v) pH adjusted to 3.0 ± 0.05 with orthophosphoric acid. The mobile phase was filtered through Millipore filter paper type HV (0.45μm) and degassed by sonication. A flow rate of 0.8 ml/min was maintained. The injection volume was 20μl. The detector was set at 254 nm.

Standard Solutions
Stock standard solutions
Stock standard solutions CIP (as free base, 1 mg/ml) and DEX (1 mg/ml) were prepared separately in methanol.
Working standard solutions
The stock solutions were diluted with mobile phase to prepare final concentrations of 300 and 100μg/ml for CIP and DEX respectively.

Linearity
Aliquot equivalent to 0.1-0.6 ml of CIP working standard solution and 0.1-0.6 mL of DEX working standard solution were transferred separately into 10-mL volumetric flasks from their respective working standard solution and completed to volume with mobile phase. Calibration graphs for both CIP and DEX were obtained by plotting the peak area against concentration, and the corresponding regression equations were calculated.

Preparation of Pharmaceutical Samples
A volume of eye/ear drops (Ciplox-D/Zoxan-D) equivalent to 3 mg and 1 mg of CIP and DEX respectively was diluted up to 10 ml with methanol. Further dilutions were made with mobile phase to reach the calibration range of each drug and the solutions were treated according to the procedure for linearity of HPLC method.

RESULTS AND DISCUSSION
Optimization of Chromatographic Conditions
The developed HPLC method has been applied for simultaneous determination of CIP and DEX. To optimize the chromatographic conditions for the separation of CIP and DEX, mobile phase composition, the effect of pH and wavelength of detection were investigated. During the method development work, Grace smart RP-C_{18} column (250 mm × 4.6 mm i.d.) with particle size of 5μm was used and gave the most suitable resolution. A satisfactory separation was obtained with mobile phase consisting of methanol-water-triethylamine (55:45:0.6, v/v/v); adjustment of pH was performed using drops of orthophosphoric acid to achieve a pH 3.0 ± 0.05 with flow rate of 0.8 ml/min. At lower fractions of organic solvent (<30%), separation occurred but with excessive delay for the DEX chromatographic peak. A very strong mobile phase (organic solvent fraction of >65%) shows low capacity factor. Quantitation based on peak area

was achieved with UV detection at 254 nm. The specificity of HPLC method is illustrated in Fig. 1, which show complete separation of compounds in mixtures. The average retention time ± standard deviation (SD) for CIP and DEX were found to be 4.125 ± 0.014 and 9.688 ± 0.049 respectively, for seven replicates.

External standard calibration method was applied for analysis of CIP and DEX. [36] A linear relation was obtained between peak area and the concentration of the drug in the range of 3-18μg/ml and 1-6μg/ml for CIP and DEX respectively. The regression equations were computed to be:

$Y = 396299.16X + 11465.47$ $r = 0.9998$ (for ciprofloxacin)
$Y = 489368.18X - 53134.28$ $r = 0.9995$ (for dexamethasone)

Where Y is the peak area, X is the concentration in μg/ml and r is the correlation coefficient.

The method was successfully applied for simultaneous determination of CIP and DEX in different laboratory-prepared mixtures, with mean recoveries of 99.87 ± 0.70 and 100.39 ± 0.91, respectively (Table 2). The proposed method has been also applied for the determination of the two drugs in Ciplox-D and Zoxan-D Eye/Ear Drops (Table 3) and validity was further assessed by applying the standard addition technique (Table 4) and HPLC chromatogram of Ciplox-D and Zoxan-D Eye/Ear Drops are shown in Fig. 2 and Fig. 3.

System Suitability
System Suitability parameters such as number of theoretical plates, HETP, Capacity factor, Tailing factor and Resolution were determined. Results of System Suitability parameters are shown in Table 1.

Table 1: System Suitability Parameters

Parameters	Compound	
	CIP	DEX
Retention time (min)	4.123 ± 0.014	9.688 ± 0.049
Capacity factor (K′)	0.52	2.56
Selectivity (α)	4.95	
Resolution (R_S)	14.27	
Tailing factor (T)	1.05	0.96
RSD of Retention time (%)	0.34	0.51
Injection Repeatability (RSD %)	0.67	0.96
Number of theoretical plates (N)	6155.09	4650.86
Height equivalent to theoretical plates (HETP)	4.09×10^{-3} cm	5.38×10^{-3} cm

Validation of the Methods
Linearity and range
The linearity of the HPLC method for the determination of CIP and DEX was evaluated by analyzing a series of different concentrations of each drug. In this study six concentrations were chosen for each drug. Each concentration was repeated six times. The linearity of the calibration graphs was validated by the high value of the correlation coefficient and the intercept value, which was not statistically ($P = 0.05$) different from zero. The calibration range was established through consideration of the practical range necessary, according to each drug concentration present in the pharmaceutical products to give accurate, precise and linear results. The calibration range of the proposed method is given in Table 5.

Precision
Evaluation of the precision estimates repeatability and intermediate precision were performed at three concentration levels for each drug on three different days. The low values of relative standard deviation (RSD) of the intraday and
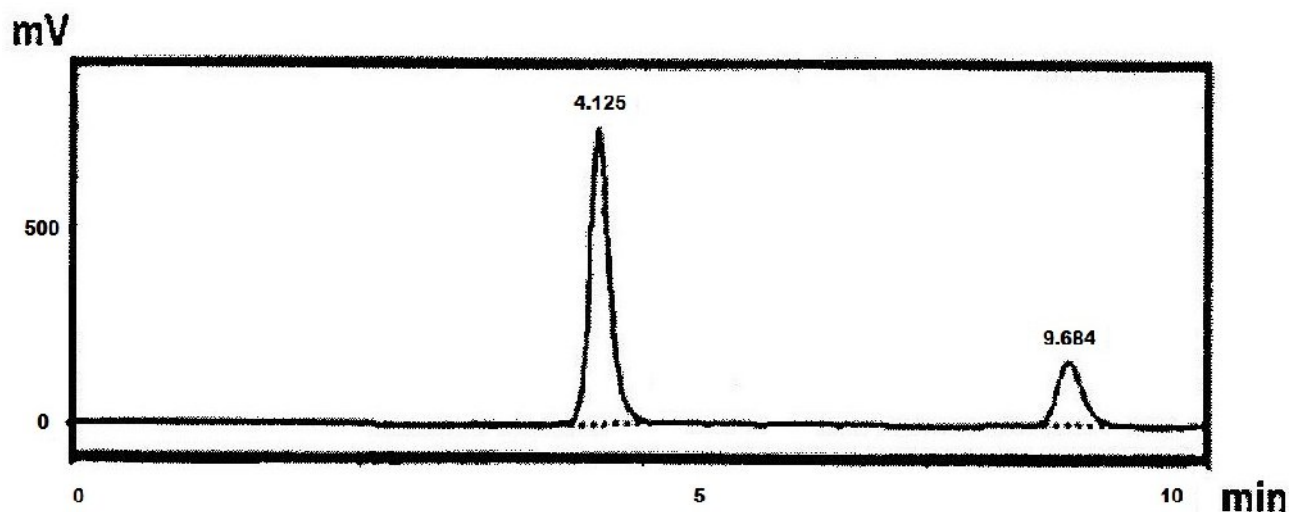
Fig. 1: HPLC Chromatogram of 15μg/ml CIP (RT = 4.125 min) and 5μg/ml DEX (RT = 9.684 min)
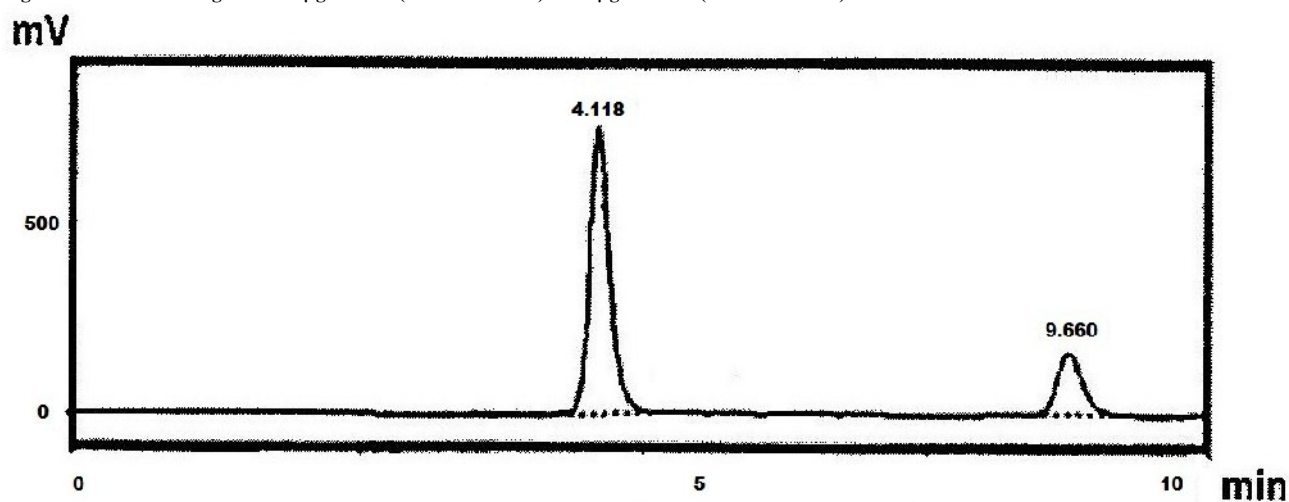

Fig. 2: HPLC Chromatogram of CIP (RT = 4.118 min) and DEX (RT = 9.660 min) in Ciplox-D Eye/Ear Drops
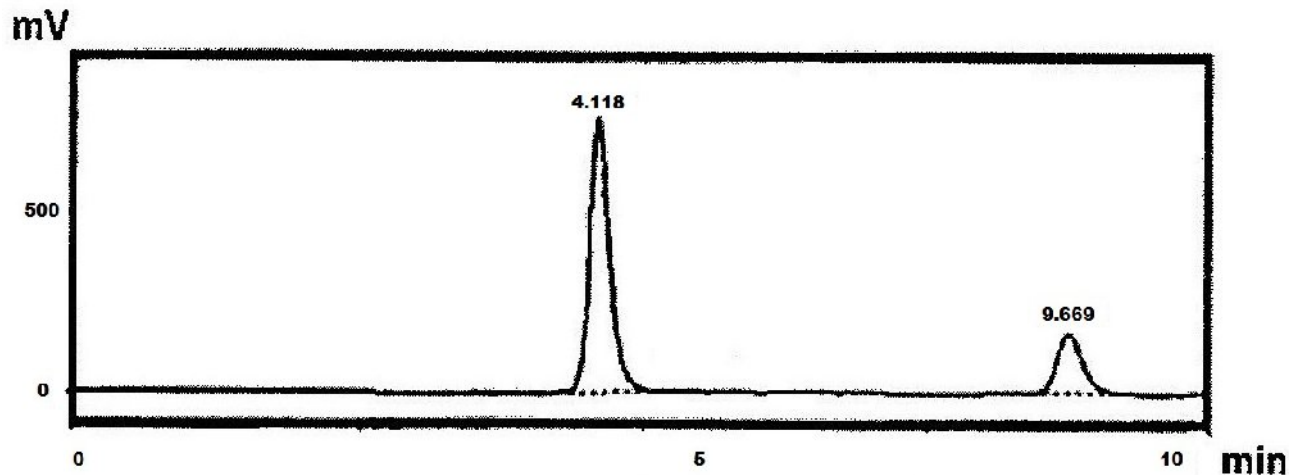

Fig. 3: HPLC Chromatogram of CIP (RT = 4.118 min) and DEX (RT = 9.669 min) in Zoxan-D Eye/Ear Drops

Table 2: Determination of CIP and DEX in laboratory-prepared mixtures by HPLC method

Samples	CIP			DEX		
	Taken (μg/ml)	Found[a] (μg/ml)	Recovery (%)	Taken (μg/ml)	Found[a] (μg/ml)	Recovery (%)
1 (1:1)	3	2.98	99.33	3	3.03	101.00
2 (2:1)	12	12.08	100.67	6	6.05	100.83
3 (3:1)	18	17.93	99.61	6	5.96	99.33
Mean ± S.D.			99.87 ± 0.70			100.39 ± 0.91

[a] Average of three experiments.

Table 3: Determination of CIP and DEX in pharmaceutical formulations by HPLC method

Samples	CIP		DEX	
	Label claim (% w/v)	% Recovery Mean ± S.D.[a]	Label claim (% w/v)	% Recovery Mean ± S.D.[a]
Ciplox-D	0.3	100.43 ± 0.78	0.1	100.07 ± 0.93
Zoxan-D	0.3	97.40 ± 0.98	0.1	96.94 ± 1.67

[a] Average of three experiments.

Table 4: Application of the standard addition technique to analysis of CIP and DEX in Ciplox-D Eye-Ear drops by HPLC method

Product	Found[a] %	Pure added (μg/ml)	Pure Found[a] (μg/ml)	Recovery (%)
Ciprofloxacin in Ciplox-D Eye-Ear Drop	100.43 ± 0.78	3	3.04	101.33
		6	6.01	100.17
		9	8.85	98.33
Mean ± S.D.				99.94 ± 1.51
Dexamethasone in Ciplox-D Eye-Ear Drop	100.07 ± 0.93	1	0.99	99.00
		2	2.03	101.50
		3	3.01	100.33
Mean ± S.D.				100.28 ± 1.25

[a] Average of three experiments.

Table 5: Validation results obtained by applying proposed method for the determination of Ciprofloxacin and Dexamethasone

Parameters	HPLC method	
	CIP	DEX
Linearity Range (μg/ml)	3-18	1-6
Slope	396299.16	489368.18
Intercept	11465.47	53134.28
Correlation coefficient (*r*)	0.9998	0.9995
Repeatability RSD (%)	0.67	0.96
Intraday RSD (%)	0.87-1.38	1.19-1.92
Interday RSD (%)	1.12-2.05	1.51-2.13
Accuracy (% Recovery)	99.94 ± 1.51	100.28 ± 1.25
LOD (μg/ml)	0.29	0.25
LOQ (μg/ml)	0.89	0.77

interday determinations (Table 5) show that there was no statistically significant difference between the mean results obtained from one day to another.

Detection and quantitation limits

According to ICH recommendation [37], the approach based on the standard deviation of the response and slope was used for determining the limit of detection (LOD) and limit of quantitation (LOQ). The theoretical values for HPLC method are given in Table 5.

Selectivity

Method selectivity was achieved by different laboratory-prepared mixtures of the studied drugs at various concentrations within the linearity range. The laboratory-prepared mixtures were analyzed according to the procedure described under the proposed methods. Satisfactory results were obtained (Table 2) indicating the high selectivity of the proposed methods for simultaneous determination of CIP and DEX.

Accuracy

The interference of excipients in the pharmaceutical formulations was studied in detail by proposed methods. For this reason, standard addition method was applied to the pharmaceutical formulation containing these compounds. This study was performed by addition of known amounts of studied drugs to a known concentration of the commercial pharmaceutical product. The excellent recoveries of standard addition method (Table 4) prove the good precision and accuracy of the proposed methods. Consequently, the

excipients in the studied pharmaceutical formulations do not interfere in the analysis of these compounds.

Analysis of Laboratory Mixtures and Pharmaceutical formulations

The proposed HPLC method was applied to the simultaneous determination of CIP and DEX in Laboratory Mixtures (Table 2) and in Ciplox-D and Zoxan-D eye/ear drops (Table 3). Three replicated determinations were made. Satisfactory results were obtained for each compound in good agreement with label claims.

For routine analytical purposes, it is always of interest to establish methods capable of analyzing a large number of samples in a short time period with due accuracy and precision. The proposed HPLC method gives a good resolution between Ciprofloxacin and Dexamethasone within a short time. These methods are rapid, sensitive, selective, accurate and precise and can be used in routine and quality control analysis.

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