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Validated HPTLC Method For Simultaneous Estimation Of Ciprofloxacin Hydrochloride And Dexamethasone In Bulk Drug And Formulation.

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Abstract: This paper describes a new, simple, precise, and accurate HPTLC method for simultaneous estimation of ciprofloxacin hydrochloride and dexamethasone as the bulk drug and in eye drops. Chromatographic separation was achieved on aluminum foil plates precoated with silica gel $60F_{254}$, with toluene: chloroform: methanol: ammonia 2: 7: 3: 0.2 (v/v/v/v) as mobile phase. Detection was performed densitometrically at 262 nm. The R_f of ciprofloxacin hydrochloride and dexamethasone were 0.26 and 0.46, respectively. The reliability of the method was assessed by evaluation of linearity (80-280 ng/spot for ciprofloxacin hydrochloride and 50-175 ng/spot for dexamethasone), intra-day and inter-day precision were found to be (%RSD) 0.82–0.94 % and 0.68–0.76 for ciprofloxacin hydrochloride and (%RSD) 1.07–1.21 % and 1.01–1.15 for dexamethasone, accuracy (99.23 % for ciprofloxacin hydrochloride and 99.55 % for dexamethasone), and specificity, in accordance with ICH guidelines. The method can be used for routine simultaneous analysis of ciprofloxacin hydrochloride and dexamethasone in pharmaceutical formulations.
Keywords: Thin layer chromatography-densitometry,Validation, ciprofloxacin hydrochloride and dexamethasone.

Introduction

Ciprofloxacin hydrochloride (CIPRO) is chemically 1-cyclopropyl- 6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinolinecarboxylic acid hydrochloride monohydrate[1] (Figure 1a). CIPRO is a synthetic chemotherapeutic antibiotic of the fluoroquinolone drug class. It is a second-generation fluoroquinolone antibacterial. It kills bacteria by interfering with the enzymes that cause DNA to rewind after being copied, which stops synthesis of DNA and of protein[2]. Dexamethasone is (9œ-Fluoro-11ß, 17œ, 21-trihydroxy-16œ –methyl-1, 4-pregnadiene-3, 20-dione[3] (Figure 1b). Dexamethasone is a potent synthetic member of the glucocorticoid class of steroid drugs. It acts as an anti-inflammatory and immunosuppressant[4].

A recent literature survey revealed that few methods were available for the determination of CIPRO in pure and pharmaceutical dosage forms; it includes high-performance thin-layer chromatographic (HPTLC) [5-6]. Numerous different analytical methods have been developed for quantitative determination of dexamethasone in pure and pharmaceutical dosage forms. These methods include HPTLC and HPLC[7-9]. However, there is no method for the simultaneous determination of these two drugs by high performance thin layer chromatography. HPTLC is a more effective technique for the simultaneous determination in single samples in routine

analysis. The aim of the proposed work was to develop an HPTLC method for the simultaneous determination of CIPRO and dexamethasone. Quantitative estimation was accomplished by densitometric scanning with UV detector at 262 nm wavelength. The assay was validated in accordance with the requirements of ICH guidelines Q2 (R1)[10]. The method was confirmed by application on authentic dosage forms.

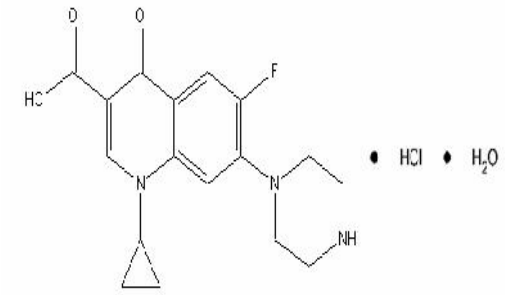

Figure 1a Structure of Ciprofloxacin hydrochloride

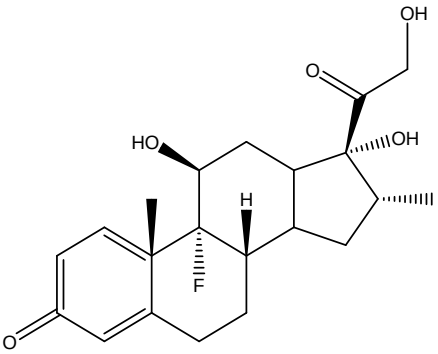

Figure 1b Structure of Dexamethasone

Experimental

Materials

Working standards of pharmaceutical grade CIPRO (Batch No.: WS/03/10) and dexamethasone (Batch No.: WS/06/10) were obtained as generous gifts from Bal Pharma Limited (Maharashtra, India). They were used without further purification and certified to contain 93.53 % and 98.80 % (w/w) on dry weight basis for CIPRO and dexamethasone respectively. Fixed dose combination Eye drop (Brand Name: Ciplox-D) containing 0.3 % w/v of CIPRO and 0.1 % w/v of dexamethasone were procured from Okasa Pvt. Ltd. India. All chemicals and reagents of analytical grade were purchased from Merck Chemicals, Mumbai, India. .

Instrumentation

Camag HPTLC System (with TLC Scanner), WinCATS Softwar V 4.0 and Linomat 5 (as application device) used for the analysis. Precoated silica gel $60F_{254}$ on aluminium sheets (200µm thick) of E-Merck, Germany were used as stationary phase. Pre-washing of plate was done with methanol and then it was activated by keeping in an oven at 115^0C for 10 minutes. The samples were spotted in the form of bands of width 6 mm with a Camag 100 microlitre sample (Hamilton, Bonded, Switzerland) syringe. A constant application rate of 0.1 µl/s was used and the space between two bands was 5 mm. The slit dimension was kept at 5 mm × 0.45 mm and the scanning speed was 10 mm/s. Linear ascending development was carried out in a 20 cm × 10 cm twin trough glass chamber (Camag, Muttenz, Switzerland) saturated with the mobile phase. Each chromatogram was developed over a distance of 8 cm. The source of radiation used was deuterium lamp emitting a continuous UV spectrum between 190 and 400 nm.

Preparation of Standard Stock Solutions

A standard mixed stock solution of CIPRO and dexamethasone was prepared by accurately weighing CIPRO (40 mg) and dexamethasone (25 mg) into a 100 ml volumetric flask. The drugs were dissolved in methanol and the solution was diluted to volume. From the standard stock solutions, diluted mixed standard solutions were prepared containing 40 ng/spot of CIPRO and 25 ng/spot of dexamethasone respectively

Optimization of the HPTLC method

The TLC procedure was optimized with a view to develop a simultaneous assay method for paracetamol and dexketoprofen trometamol. Both the drug solutions were spotted on to TLC plates and run in different solvent systems. Finally the mobile phase consisting of toluene: chloroform: methanol: ammonia (2: 7: 3: 0.2 v/v/v/v) was found to be optimum, resulting in an R_f 0.26 and 0.46 for CIPRO and dexamethasone, respectively (Figure 2). The mobile phase was run up to a distance of 8 cm; which takes approximately 30 min for complete development of the TLC plate.

Validation of the method

Validation of the optimized TLC method was carried out with respect to the following parameters.

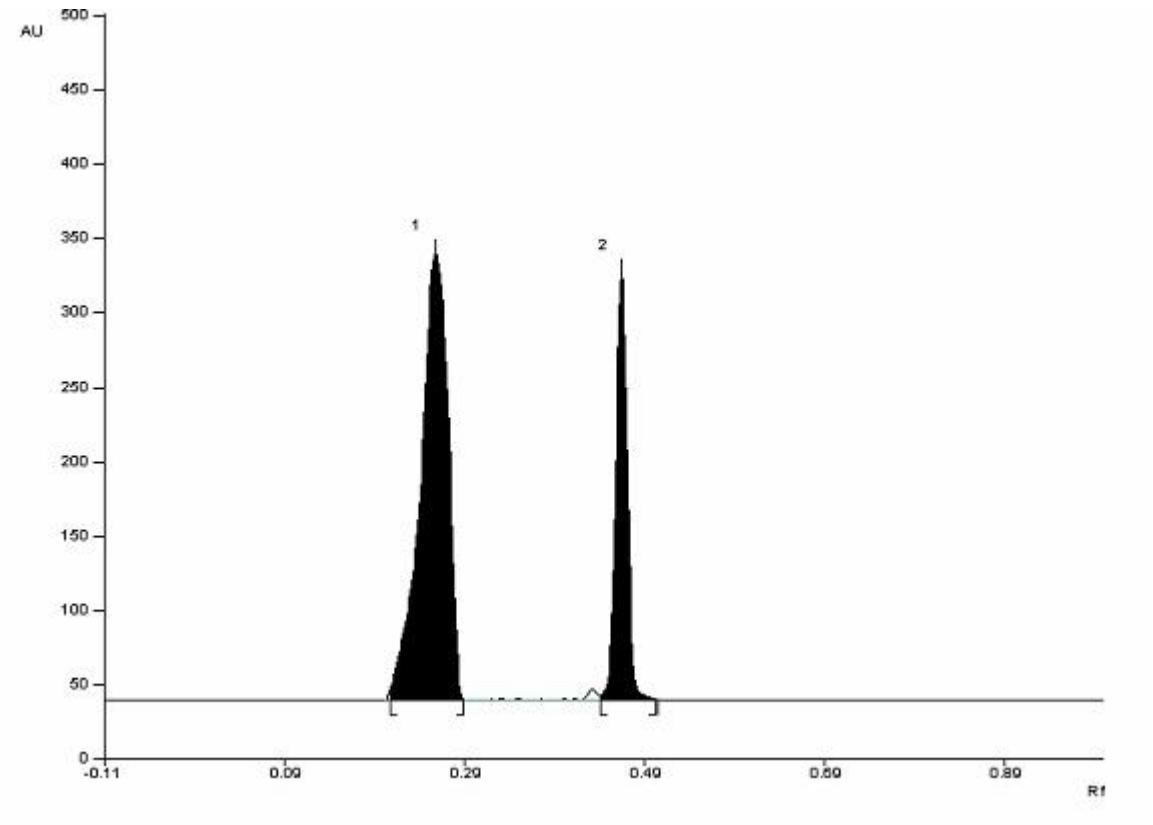

Figure 2 Chromatogram of Ciprofloxacin hydrochloride and Dexamethasone.

Linearity and range

A stock solution containing 40 µg/ml for CIPRO and 25 µg/ml for dexamethasone were prepared in methanol. Different volumes of this solution were applied to the plate resulting in application of 80-280 ng/spot for CIPRO and 50-175 ng/spot for dexamethasone. Each concentration was applied six times to the plate and the plate was developed as described above. Peak areas were plotted against corresponding concentrations to furnish the calibration plot.

Precision

The precision of the method was verified by repeatability and intermediate precision studies. Repeatability studies were performed by analysis of three different concentrations (80 ng/spot, 160 ng/spot and 240 ng/spot for CIPRO and 50 ng/spot, 100 ng/spot and 150 ng/spot for dexamethasone) of the drugs six times on the same day. The intermediate precision of the method was checked by repeating studies on three different days.

Limit of detection and limit of quantitaiton

To determine the limits of detection (LOD) and quantitation (LOQ), solutions of concentration in the lower part of the linear range of the calibration plot were used. LOD and LOQ were calculated using the equations LOD = 3.3 × N/B and LOQ = 10 × N/B, where N is the standard deviation of the peak areas of the drugs (n = 3), taken as a measure of noise, and B is the slope of the corresponding calibration plot.

Robustness of the method

Robustness was assessed by deliberately changing the chromatographic conditions and studying the effects on the results obtained.

Specificity

The specificity of the method was determined by analyzing standard drug and test samples. The spot for CIPRO and dexamethasone in the samples was confirmed by comparing the R_f and spectrum of the spot with that of a standard. The peak purity of CIPRO and dexamethasone was determined by comparing the spectrum at three different regions of the spot i.e. peak start (S), peak apex (M) and peak end (E).

Accuracy

Accuracy of the method was carried out by applying the method to drug sample (CIPRO and dexamethasone combination eye drop) to which known amount of CIPRO and dexamethasone

standard powder corresponding to 80, 100 and 120% of label claim had been added (standard addition method), mixed and the powder was extracted and analyzed by running chromatogram in optimized mobile phase.

Analysis of a marketed formulation

To determine the content of CIPRO and dexamethasone in eye drop (Brand name: Ciplox-D, label claim: 3 mg CIPRO and 1 mg of dexamethasone per ml of eye drop solution), volume equivalent to 9 mg of CIPRO and 3 mg of dexamethasone was taken and transferred into a 10 ml volumetric flask containing 6 ml methanol, sonicated for 15 min with occasional shaking and diluted to 10 ml with methanol and drug content was determined 900 µg/ml for CIPRO and 300 µg/ml for dexamethasone from which 1 µl of the spot was applied which gave final concentration of 900 ng/spot for CIPRO and 300 ng/spot for dexamethasone which was used without dilution and the plate was developed in optimized mobile phase. The analysis was repeated in triplicate.

Results And Discussion

The results of validation studies on simultaneous estimation of CIPRO and dexamethasone was carried out by using toluene:chloroform: methanol: ammonia (2: 7: 3: 0.2 v/v/v/v) as mobile phase are given below

Linearity

The drug response was linear (r^2 = 0.9939 for CIPRO and 0.9951 for dexamethasone) over the concentration range between 80-280 ng/spot for CIPRO and 50-75 ng/spot for dexamethasone.

Precision

The precision of the method in terms of intra-day variation (% RSD) was determined by analyzing CIPRO and dexamethasone standard drug solutions in the calibration range, three times on the same day. Inter-day precision (% RSD) was assessed by analyzing CIPRO and dexamethasone the standard drug solutions within the calibration range on three different days over a period of one week. The results of the precision studies are as shown in (Table 1).

LOD and LOQ

Signal-to-noise ratios of 3:1 and 10:1 were obtained for LOD and LOQ respectively. The LOD and LOQ were found to be 60 ng/spot and 80 ng/spot for CIPRO, 30 ng/spot and 50 ng/spot for dexamethasone respectively.

Robustness of the method

The standard deviation of peak areas was calculated for each parameter and the % RSD was found to be less than 2. The low values of the % RSD, as shown in Table 2 indicated the robustness of the method.

Table 1 Precision Studies

Concentration (ng/spot)	Repeatability (n=6)			Intermediate precision (n=6)		
	Measured conc. ± SD	(%) RSD	Recovery (%)	Measured conc. ± SD	(%) RSD	Recovery (%)
Ciprofloxacin						
80	79.62 ± 3.5	0.82	99.52	79.69 ± 3.2	0.76	99.61
160	159.08 ± 3.6	0.94	99.42	159.11 ± 4.7	0.68	99.44
240	238.31 ± 8.8	0.89	99.29	239.51 ± 10.4	0.71	99.79
Dexamethasone						
50	49.63 ± 10.7	1.21	99.26	49.56 ± 11.0	1.08	99.12
100	99.96 ± 16.0	1.07	99.96	98.56 ± 11.7	1.01	98.56
150	149.98±17.3	1.12	99.98	149.62 ± 1.5	1.15	99.74

Table 2 Robustness testing

Parameter	SD of Peak Area for Ciprofloxacin	% RSD	SD of Peak Area for Dexamethasone	% RSD
Mobile phase composition (±0.1 ml)	4.07	0.73	3.74	0.48
Amount of mobile phase (±5%)	21.09	0.95	8.72	0.15
Time from spotting to chromatography (+ 10 min.)	5.22	0.89	3.31	0.85
Time from chromatography to scanning (+ 10 min.)	6.02	0.49	2.63	0.59

Table 3 Recovery studies (n=3)

Drug	Label claim (mg/ml)	Amount Added (%)	Total amount (mg/ml)	Amount recovered (mg) ± RSD	Recovery (%)
Ciprofloxacin	3	80	5.4	5.34 ± 1.21	98.88
		100	6	5.92 ± 1.78	98.66
		120	6.6	6.61 ± 1.56	100.15
Dexamethasone	1	80	1.8	1.82 ± 1.13	101.11
		100	2.0	1.96 ± 1.43	98.00
		120	2.2	2.19 ± 1.12	99.54

Table 4 Analysis of commercial formulation

Drug	Label claim (mg/ml)	Amount found* (mg)	% of drug content*
Ciprofloxacin	3	2.99	99.66
Dexamethasone	1	0.99	99.00

* Each value is a mean of six determinations.

Specificity

The peak purity of CIPRO and dexamethasone was assessed by comparing their respective spectra at the peak start, apex and peak end positions of the spot i.e., r^2 (S, M) = 0.9989 and r^2 (M, E) = 0.9987. A good correlation (r^2 = 0.9992) was also obtained between the standard and sample spectra of CIPRO and dexamethasone respectively.

Recovery studies

As shown from the data in Table 3 good recoveries of the CIPRO and dexamethasone in the range from 98 to 101 % were obtained at various added concentrations.

Analysis of a formulation

The amount of CIPRO and dexamethasone were found by number of replicates of pharmaceutical preparation (n = 6). The assay results are reported in (Table 4).

Conclusion

The HPTLC method was developed to estimate the drugs in formulation, in order to analyse more samples at a time. This method described for the determination of CIPRO and dexamethasone in pharmaceutical dosage forms is very simple, rapid and provides accurate and precise results.

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