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DEVELOPMENT AND VALIDATION OF HPTLC METHOD FOR SIMULTANEOUS ESTIMATION OF CIPROFLOXACIN AND ORNIDAZOLE IN TABLET DOSAGE FORMS

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ABSTRACT
A simple, sensitive, precise and accurate HPTLC method has been developed for the simultaneous estimation of the ciprofloxacin and ornidazole in tablets. The stationary phase used was precoated silica gel $60F_{254}$ plate. The mobile phase used was a mixture of n-butanol: ethanol: 8M ammonia (5:0.5:1.5, v/v/v). The densitometric scanning was carried out at 315 nm. This system was found to give compact spots for ciprofloxacin (R_f value of 0.25 0.004) and ornidazole (R_f value of 0.82 □ 0.007). The method was validated in terms of linearity, accuracy, precision, limit of detection, limit of quantification and specificity in accordance with the ICH guidelines. The calibration curve was found to be linear between 40 to 140 ng/spot for each ciprofloxacin and ornidazole with significantly high value of correlation coefficient ($r^2 > 0.99$). The limit of detection and quantitation were found to be 10.01 and 33.03 ng/spot, respectively for ciprofloxacin and the limit of detection and quantification were found to be 7.62 and 25.15 ng/spot, respectively for ornidazole. The results of analysis have been validated statistically and by recovery studies. The proposed HPTLC method can be successfully applied in the routine analysis of commercial pharmaceutical tablets.

Keywords: *Ciprofloxacin; Ornidazole; HPTLC; Validation; Tablet.*

INTRODUCTION
Ciprofloxacin (CPX), 1-Cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinolinecarboxilic acid[1], is a broad spectrum fluoroquinolone antibacterial agent used in the treatment of various bacterial infections caused by gram-positive and gram-negative microorganisms[2]. Its antibacterial spectrum is wider than that of aminoglycosides, third generation cephalosporins and other fluoroquinolones. Ornidazole (ORN), (RS)-1-chloro-3-(2-methyl-5-nitroimidazole-1-yl) propan-2-ol[3], is used as an antiinfective agent. ORN is used in the treatment of susceptible protozoal infections and also in the treatment and prophylaxis of anaerobic bacterial infections[4]. ORN is used in combination with ciprofloxacin in the treatment of intraabdominal infection[5]. Ciprofloxacin is official in IP, USP and BP. The IP[6] and USP[7] describe HPLC method and BP[8] describes non-aqueous titration method for estimation of CPX. Literature survey reveals HPLC[9-11], spectrophotometric[12-18] and spectrofluorimetric[19] methods for its determination in pharmaceutical dosage form as well as in biological fluids. Ornidazole is official in IP. The IP[20] describes non-aqueous titration method for estimation of ORN. Literature survey reveals HPLC[21], chemiluminescence[22] and spectrophotometric[23] methods for its determination in dosage forms and biological fluids. The combination of two drugs is not official in any pharmacopoeia; hence no official method is available for the estimation of CPX and ORN in their combined dosage form. Literature survey reveals spectrophotometric[24] and HPLC[25] methods for simultaneous estimation of CPX and ORN in combined dosage forms. Since no HPTLC method is reported for simultaneous estimation of these drugs in combined dosage form. The present communication describes simple, sensitive, accurate and precise HPTLC method for simultaneous estimation of CPX and ORN in combined tablet dosage form.

MATERIALS AND METHODS
Apparatus
A Camag HPTLC system (Switzerland) comprising of Camag Linomat V semiautomatic sample applicator, Camag TLC Scanner 3, Camag (Muttenz, Switzerland) flat bottom and twin-trough developing chamber (10 × 10 cm), UV cabinet with dual wavelength UV lamp, Camag winCATS software, Hamilton syringe (100 ìl), Sartorius CP224S analytical balance (Germany), Ultrasonic bath (Frontline FS-4, Mumbai, India) were used in the study.

Reagents and Materials
Ciprofloxacin and ornidazole standard were procured as a gift sample from Torrent Research Centre,

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Ahmedabad, Gujarat, India. Silica Gel 60 F_{254} TLC plates (10 × 10 cm, layer thickness 0.2 mm, E. Merck, Darmstadt, Germany) were used as stationary phase. The marketed formulation containing 200 mg of CPX and 500 mg of ORN was procured from the local pharmacy. Ammonia, n-butanol and methanol (AR grade, S. D. Fine Chemicals Ltd., Mumbai) and absolute alcohol (Baroda Chemical Industries, Baroda, Gujarat, India) were used for mobile phase preparation and as solvents.

Preparation of standard and sample solution

CPX (10 mg) and ORN (25 mg) were individually weighed accurately, dissolved and diluted with methanol to obtain the final concentration of 10 µg/ml of CPX and 25 µg/ml of ORN. Twenty tablets (each containing 200 mg CPX and 500 mg ORN) weighed accurately and ground to fine powder. The powdered equivalent to 10 mg of CPX and 25 mg of ORN was accurately weighed and transferred to a volumetric flask and dissolved in 50 ml of methanol. The solution was sonicated for 15 min. The extracts were filtered through Whatman filter paper No. 41 and the residue was washed thoroughly with methanol. The extracts and washing were pooled and transferred to a 100 ml volumetric flask and volume was made up to 100 ml with methanol. One milliliter of above solution was further diluted to 10 ml with methanol to get 10 µg/ml of CPX and 25 µg/ml of ORN.

Chromatographic conditions

The experiment was performed on silica gel 60F_{254} aluminum sheets (10 x 10 cm) as stationary phase, using mobile phase comprised of n-butanol: ethanol: 8M ammonia (5:0.5:1.5, v/v/v). TLC plates were prewashed with methanol and activated in an oven at 50° for 5 min prior to chromatography. The solutions were applied on TLC plate in the form of bands of 6 mm width under a stream of nitrogen gas using a Camag Linomat V automatic sample applicator. A constant application rate of 0.1 µl/s was employed and space between two bands was fixed at 5 mm. Ascending development to 80 mm was performed in 10 cm x 10 cm Camag twin trough glass chamber (Muttenz, Switzerland) saturated with the mobile phase for 30 min at room temperature. The developed TLC plate was air dried and then scanned between 200 to 400 nm using Camag TLC scanner 3 using WinCATS software. Both components show reasonably good response at 315 nm keeping the slit dimension of 5 × 0.45 mm and scanning speed of 10 mm/s. The monochromatic band width was set at 20 nm, each track was scanned thrice and baseline correction was used. Four microlitres of standard and sample solutions of CPX and ORN were spotted and developed.

Chromatographic separation

Four microlitres of standard solution of CPX and ORN was applied on TLC plate under nitrogen stream using semiautomatic spotter. The plate was dried in air and developed up to 80 mm at constant temperature using mixture of n-butanol: ethanol: 8M ammonia (5:0.5:1.5, v/v/v) as mobile phase in Camag twin-trough chamber previously saturated with mobile phase for 30 min. The plate was removed from the chamber and dried in air. Photometric measurements were performed at 315 nm in absorbance/reflectance mode with Camag TLC Scanner 3 using winCATS software incorporating the track optimization option.

Validation of the proposed method

The proposed method was validated according to the International Conference on Harmonization (ICH) guidelines[26].

Linearity (Calibration curve)

Accurately measured standard stock solutions of CPX (4, 6, 8, 10, 12 and 14 µl) and standard stock solutions of ORN (4, 6, 8, 10, 12 and 14 µl) were spotted on precoated TLC plate under nitrogen stream using Linomat 5 semiautomatic spotter. The TLC plate was developed and photometrically analyzed as described under chromatographic separation. The calibration curve was prepared by plotting peak area versus concentration (ng/spot) corresponding to each spot. Each reading was an average of three determinations.

Accuracy (% Recovery)

The accuracy of the method was determined by calculating recoveries of CPX and ORN by the standard addition method. Known amounts of standard solutions of CPX and ORN was added at 50, 100 and 150 % level to prequantified sample solution of CPX and ORN (40 ng/spot). The amount of CPX and ORN was estimated by applying obtained values to the respective regression line equations.

Method Precision (% Repeatability)

The precision of the instrument was checked by repeatedly injecting (n = 6) solutions of CPX and ORN without changing the parameters of the proposed method. The results were reported in terms of relative standard deviation (% RSD).

Intermediate Precision (Reproducibility)

The intraday and interday precisions of the proposed method was determined by estimating the corresponding responses 3 times on the same day and on 3 different days over a period of one week for different concentration of standard solution of CPX and ORN for the proposed method. The results were reported in terms of relative standard deviation (% RSD).

Limit of detection (LOD) and limit of quantification (LOQ)

LOD and the LOQ of the drug were calculated using the following equations as per International Conference on Harmonization (ICH) guidelines[26].

LOD = 3.3 × □/S

LOQ = 10 × □/S

Where □ = Standard deviation of the response

S = Slope of calibration curve

Specificity

The specificity of the method was ascertained by analyzing standard drugs and the sample. The spots for CPX and ORN in the samples were confirmed by comparing the R_f and spectra of the spots with that of the standards.

Analysis of the marketed formulations

Four microlitres of sample solution from formulation was applied separately on TLC plate, developed and scanned as described in chromatographic separation. The amount of CPX and ORN present in the sample solution was determined by fitting area values of peak corresponding to CPX and ORN into the respective calibration curve.

RESULTS AND DISCUSSION

For optimization of method, different mobile phase compositions were employed to achieve good separation. Finally, the mobile phase consisting of n-butanol-ethanol-8M ammonia (5.0: 0.5: 1.5, v/v/v) gave sharp and symmetrical peaks with the R_f values of 0.25 □ 0.004 and 0.82 □ 0.007 for CPX and ORN, respectively (Fig 1). Fig 2 showing three dimensional (3-D) chromatogram of CPX and ORN peaks in different concentrations at 315 nm. The proposed HPTLC method was validated in terms of linearity, precision, accuracy, LOD, LOQ and specificity. The calibration plot was found to be linear over the concentration range 40-140 ng/spot for each CPX and ORN, with a correlation coefficient of 0.9989 and 0.9974 for CPX and ORN, respectively. LOD for CPX and ORN were found to be 10.01 ng/spot and 7.62 ng/spot, respectively. LOQ for CPX and ORN were found to be 33.03 ng/spot and 25.15 ng/spot, respectively indicate the sensitivity of the method. The low % RSD values of intraday (0.39 - 1.83 for CPX and 0.53 - 1.75 for ORN) and interday (0.63 - 1.98 for CPX and 0.42 - 1.64 for ORN) precision reveals that the proposed method is precise. Relative standard deviation for repeatability of measurements is less than 2% (0.93 for CPX and 0.49 for ORN), which indicates that the proposed method is repeatable. To study the accuracy of the method, recovery studies were performed. The percent average recoveries obtained were 99.36 ± 0.95 and 98.96 ± 0.98 for CPX and ORN, respectively indicating that the proposed HPTLC method is highly accurate (Table 1). The proposed validated method was successfully applied to determine CPX and ORN in tablet dosage forms. The percent average assay was found to be 99.11 ± 0.85 and be 99.05 ± 1.28 for CPX and ORN, respectively (Table 2). The low values of standard deviation indicate the suitability of this method for routine analysis of CPX and ORN in pharmaceutical dosage forms. To confirm the specificity of the proposed method, the solution of formulation was spotted on TLC plate, developed and scanned. It was observed that the excipients present in the formulation did not interfere with the sample peak. The method validation parameters are presented in Table 3.

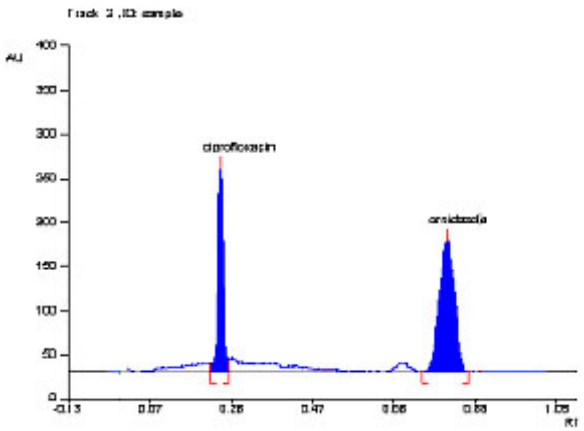

***Fig. 1**: Chromatogram of CPX and ORN with corresponding Rf values at 315 nm*

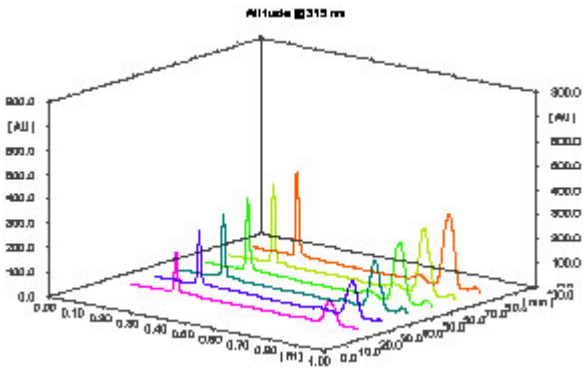

***Fig. 2**: 3-D Chromatogram showing peaks of CPX and ORN in different concentrations at 315 nm*

***Table 1:** Data of recovery study for CPX and ORN by HPTLC method*

Drug	Level	Amount taken (ng/spot)	Amount added (ng/spot)	Amount found (ng/spot)	% Recovery ± S.D.[a] (n[b] = 3)
CPX	I	60	20	80.24	100.3 ± 1.15
	II	60	40	98.54	98.54 ± 1.03
	III	60	60	119.1	99.23 ± 0.68
ORN	I	60	20	79.87	99.84 ± 0.76
	II	60	40	98.34	98.35 ± 1.23
	III	60	60	118.4	98.69 ± 0.96

[a] = standard deviation [b] = number of determinations CPX = ciprofloxacin ORN = ornidazole

Table 2: *Analysis of marketed formulation of CPX and ORN*

Tablet	Labeled amount (mg)		Amount found (mg)		% Assay ± S.D.[a] (n[b] = 5)	
	CPX	ORN	CPX	ORN	CPX	ORN
Tablet 1	200	500	201.8	493.7	100.9 ± 1.38	98.74 ± 1.14
Tablet 2	200	500	199.1	509.0	99.52 ± 0.74	101.8 ± 0.84

[a] = standard deviation [b] = number of determinations CPX = ciprofloxacin ORN = ornidazole

Table 3: *Regression analysis data and summary of validation parameters*

Parameters	HPTLC method	
	CPX	ORN
Concentration range (ng/spot)	40-140	40-140
Slope	25.631	40.531
Intercept	1305.2	114.5
Correlation coefficient (r^2)	0.9989	0.9974
LOD[a] (ng/spot)	10.01	7.62
LOQ[b] (ng/spot)	33.03	25.15
Accuracy (n[c] = 5)	99.36 ± 0.95	98.96 ± 0.98
Repeatability (% RSD[d], n = 6)	0.93	0.49
Precision (% RSD)		
Interday (n = 3)	0.63 - 1.98	0.42 - 1.64
Intraday (n = 3)	0.39 - 1.83	0.53 - 1.75

[a] = Limit of detection [b] = Limit of quantification
[c] = number of determinations
[d] = Relative standard deviation CPX = ciprofloxacin ORN = ornidazole

CONCLUSION

The results of the analysis of pharmaceutical dosage forms by the proposed HPTLC method are highly reproducible, reliable and are in good agreement with the labeled claim of the drug. The percent recoveries obtained was 98.35 to 100.3 indicates none interference from the common excipients in the tablet formulations. The proposed HPTLC method is simple, sensitive, rapid, accurate, precise, specific and economical. It can be used for the routine simultaneous estimation of CPX and ORN in pharmaceutical formulations.

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