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Research Article

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VALIDATED HPTLC METHOD FOR SIMULTANEOUS QUANTITATION OF CEFIXIME AND OFLOXACIN IN BULK DRUG AND IN PHARMACEUTICAL FORMULATION

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ABSTRACT

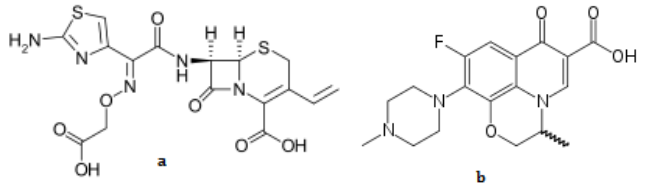
This work is concerned with the simultaneous determination of cefixime and ofloxacin in a bulk drug and pharmaceutical formulations by high performance thin layer chromatographic (HPTLC) method. Chromatographic separation was achieved on aluminum foil plates precoated with silica gel 60GF-254, with n-butanol: ammonia: water: DMSO (8:3:1:2, v/v/v/v) as mobile phase. Detection was performed densitometrically at 297 nm. The R_F of cefixime and ofloxacin were 0.55 and 0.65, respectively. The reliability of the method was assessed by evaluation of linearity (30-180 ng/spot for both cefixime and ofloxacin respectively. Accuracy (99.82 % for cefixime and 99.84 % for ofloxacin), and specificity, in accordance with ICH guidelines. The method is simple, accurate, and rapid and can therefore be used for routine analysis of both drugs in quality control laboratories.

Keywords: Cefixime, Ofloxacin, High performance thin layer chromatography, validation.

INTRODUCTION

Cefixime (CEF) is an oral third generation cephalosporin antibiotic. Chemically , it is (6R, 7R)-7- { [2-(2-amino -1, 3-thiazole–4-yl)-2- (carboxymethoxyimino)acetyl]amino}-3-ethenyl-8-oxo-5-thia-1-azabicyclo- [4.2.0]oct-2-ene-2-carboxylic acid (Figure **1a**) is used to treat gonorrhoea, tonsillitis and pharyngitis. Ofloxacin (OFL) is a fluoroquinolone derivative.Chemically it is(+/-)-9-fluoro-2,3-dihydro-3-methyl-10- (4-methyl-1-piperazinyl)-7h-oxo-7H-pyrido- [1,2,3-di]-1,4benzoxazine-6-carboxylic acid (Figure **1b**) is mainly used as antibacterial for the treatment of urinary tract infection and sexually transmitted diseases. Literature survey revealed that a number of methods are available for quantitative estimation of cefixime and ofloxacin in combination with other drugs like Spectrophotometric[1-4], HPLC[5-9] and HPTLC[10-11]. So far no simultaneous HPTLC method has been reported for estimation of CEF and OFL. The aim of present work is to develop a simple, rapid, precise and selective HPTLC method for the estimation of CEF and OFL.

Figure 1. Chemical structure of Cefixime and ofloxacin



MATERIALS AND METHODS

Chemicals and Reagents

Pure drugs of CEF and OFL were obtained from Lupin Limited, Pune as a gift sample. The commercial formulation of CEF and OFL are available in the ratio of 1:1 (Mahacef-plus) (200/200 mg) as tablets. All chemicals and reagents used were of analytical grade and were purchased from Merck Chemicals, India.

Instrumentation

The samples were spotted in the form of bands of 6 mm width with a Camag 100 microlitre sample (Hamilton, Bonaduz, Switzerland) syringe on silica gel precoated aluminum plate 60 $GF_{254,}$ (20 × 10cm) with 250 µm thickness; E. Merck, Darmstadt, Germany (supplied by Anchrom Technologists, Mumbai), using a Camag Linomat V (Switzerland).

The plates were prewashed by methanol and activated at 110˚C for 5 min prior to chromatography. A constant application rate of 0.5 µL/s was employed and the space between two bands was 6 mm. The slit dimension was kept at 5 mm × 0.45 mm and 10 mm/s scanning speed was employed.

The monochromator bandwidth was set at 20 nm, each track was scanned thrice and the baseline correction was used. Linear ascending development was carried out in a 20 cm × 10 cm twin trough glass chamber (Camag,Muttenz, Switzerland) saturated with the mobile phase. The optimized chamber saturation time for mobile phase was 30 min at room temperature (25˚C±2) at relative humidity of 60±5%. The length of chromatogram run was 8 cm. Subsequent to the development, HPTLC plates were dried in current of air with the help of air dryer in a wooden chamber with adequate ventilation. Densitometric scanning was performed on a Camag HPTLC scanner III in the reflectance absorbance mode at 297 nm and operated by CATS software (V 3.15, Camag). The source of radiation utilized was deuterium lamp emitting continuous UV spectrum between 190 and 400 nm.

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Preparation of standard solutions

Standard stock solutions of a concentration of 1 mg/ml of CEF and 1 mg/ml of OFL were freshly prepared separately using methanol. From the standard stock solution, the mixed standard solution was prepared using the methanol to contain 0.1 mg/ml of CEF and 0.1 mg/ml of OFL.

Validation of Method

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

Linearity and range: From the mixed standard stock solution (0.03 mg/ml of CEF and 0.03 mg/ml of OFL), 1 to 6 µl was applied. Solution was spotted on the TLC plate to obtain the final concentration 30-180 ng/spot for both CEF and OFL. Each concentration was applied three times to the TLC plate. The plate was then developed using the previously described mobile phase and the peak areas were plotted against the corresponding concentrations to obtain the calibration curves.

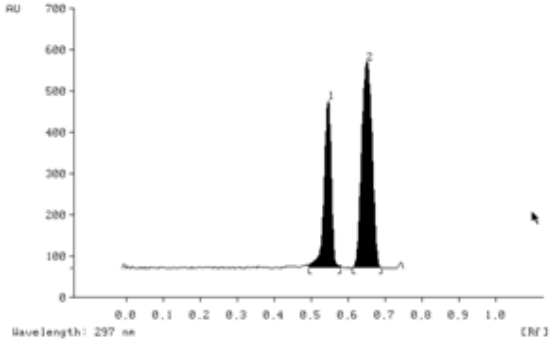
Precision: The precision of the method was verified by repeatability and intermediate precision studies. Repeatability studies were performed by analysis of three different concentrations by HPTLC. 60, 120, 180 ng/spot for both CEF and OFL, from the drug solution, six times, to a TLC plate followed by development of the plate. The intra-day precision (RSD, %) was assessed by analyzing standard drug solutions within the calibration range, three times on the same day. Inter- day precision (RSD %) was assessed by analyzing drug solutions within the calibration range on three different days over a period of a week.

Limit of detection and limit of quantification: Limits of detection (LOD) and quantification (LOQ) represent the concentration of the analyte that would yield signal-to-noise ratio of 3 for LOD and 10 for LOQ, respectively. The LOD and LOQ were determined by measuring the magnitude of analytical background by spotting a blank and calculating the signal-to-noise ratio for CEF and OFL by spotting a series of solutions until the S/N ratio 3 was obtained for the LOD and 10 for the LOQ. To determine the LOD and LOQ, serial dilutions of mixed standard solution of CEF and OFL were made from the standard stock solution in the range of 10-70 ng/spot.

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 ascertained by analysis of drug standards and samples. The mobile phase resolved both the drugs very efficiently, as shown in Figure **2**.

Figure 2. Densitogram obtained from Cefixime (1) and Ofloxacin (2)



The identities of the bands for CEF and OFL were confirmed by comparing the R_F and spectra of the bands with those of standards. Typical overlain absorption spectra of CEF and OFL are shown in Figure 3; 297 nm was selected for densitometric scanning. Peak purity for CEF and OFL was assessed by comparing the spectra of standards with those acquired at three different points on peaks obtained from the sample, i.e. the peak start (S), peak apex (M), and peak end (E) positions.

Recovery: Accuracy of the method was carried out by applying the method to the drug sample (CEF and OFL combination tablet) to which a known amount of CEF and OFL 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 an optimized mobile phase. This was done to check for the recovery of the drug at different levels in the formulation.

Analysis of a marketed formulation: To determine the content of CEF and OFL in a conventional tablet (Brand name: MAHACEF-PLUS, Label claim: 200 mg cefixime and 200 mg ofloxacin per tablet), twenty tablets were weighed, their mean weight determined and finely powdered. The weight of the tablet triturate equivalent to 200 mg of CEF and 200 mg of OFL was transferred into a 100 ml volumetric flask containing 60 ml of methanol, sonicated for 45 mins and diluted to 100 ml with methanol. The resulting solution was centrifuged at 3000 rpm for 5 min and the drug content of the supernatant was determined (2 mg/ml for both CEF and OFL). Then 1 ml of the after mentioned filtered solution was diluted to produce a concentration of 0.2 mg/ml or 200 µg/ml for both CEF and OFL and 2 µl was applied. The final concentration obtained was 400 ng/spot for both CEF and OFL which was developed in an optimized mobile phase. The analysis was repeated in triplicate. The possibility of excipient interference with the analysis was examined.

RESULTS AND DISCUSSION

Method Development: The TLC method was optimized for simultaneous determination of CEF and OFL. The mobile phase n-butanol: ammonia: water: DMSO 8:3:1:2, (v/v/v/v) resulted in good resolution and sharp and symmetrical peaks of R_F 0.55 for CEF and 0.65 for OFL. It was observed that prewashing of TLC plates with methanol (followed by drying and activation) and pre-saturation of TLC chamber with mobile phase for 30 min (optimum chamber saturation time) ensured good repeatability and peak shape of both drugs.

Linearity: Linear regression data for the calibration plots revealed good linear relationships between response and concentration over the ranges 30–180 ng/spot for both CEF and OFL. The linear regression equations were $Y = 0.7596X + 1147.32$ ($r^2 = 0.998$) for CEF and $Y = 3.0345X + 1380.9$ ($r^2 = 0.998$) for and OFL.

Precision: The precision of the method was expressed as relative standard deviation (RSD, %). The results listed in Table **1** reveal the high precision of the method.

Table 1 Precision of the method

Drug	Concentration (ng/spot)	Inter-day % RSD*	Intra-day % RSD*
Cefixime	60	1.040	1.326
	120	0.317	0.269
	180	0.865	1.141
Ofloxacin	60	1.904	1.708
	120	0.671	0.618
	180	0.627	0.635

** mean from six analyses*

LOD and LOQ: Signal-to-noise ratios of 3: 1 and 10: 1 were obtained for the LOD and LOQ, respectively. The LOD and LOQ were found to be 10 ng/spot and 20 ng/spot for both CEF and OFL. This indicates the method is sufficiently sensitive.

Recovery: When the method was used for extraction and subsequent analysis of both drugs from the pharmaceutical dosage forms, and the extract was over applied with 80, 100, and 120% of additional drug, the recovery was 99–100 %, as listed in Table **2**.

Table 2. Results from recovery studies

Drug	Label claim (mg/tablet)	Amount Added (%)	Total amount (mg)	Amount* recovered (mg)	Recovery (%)	RSD* (%)
Cefixime	200	80	360	358.90	99.78	0.518
		100	400	399.26	99.87	0.279
		120	440	438.85	99.83	1.24
Ofloxacin	200	80	360	359.76	99.95	0.866
		100	400	398.49	99.74	0.905
		120	440	438.98	99.85	0.838

Table 3. Robustness of the method

Experimental Condition	Cefixime		Ofloxacin	
	SD of peak area	RSD (%)	SD of peak area	RSD (%)
Mobile phase composition				
n-butanol–Ammonia–water-DMSO 8.1:3:1:2 (*v/v*)	34.06	0.81	43.15	1.13
n-butanol–Ammonia–water-DMSO 7.9:3.:1:2 (*v/v*)	37.11	0.89	35.97	0.94
Mobile phase volume				
14 .1ml	41.13	0.98	40.48	1.06
13.9ml	43.51	1.03	38.15	1.0
Development distance				
7 cm	35.02	0.83	39.45	1.03
7.5cm	34.44	0.81	32.68	0.86
8cm	41.52	0.99	44.35	1.16
Duration of Saturation				
20min	41.00	0.98	34.46	0.91
25min	34.15	0.81	40.09	1.05
30min	44.40	1.06	43.59	1.14
Activation of prewashed TLC plates				
8min	47.81	1.14	32.05	0.84
10min	38.15	0.91	37.88	0.99
12min	44.58	1.06	40.43	1.06
Time from application to chromatography	35.56	0.85	43.83	1.15
Time from chromatography to scanning	38.40	0.92	34.64	0.91

Robustness of the method: The relative standard deviation of peak areas was less than 2%. The % RSD shown in Table **3** indicates the robustness of the method.

Specificity: The peak purity of CEF and OFL was assessed by comparing their respective spectra at the peak start, apex, and peak end positions of the spot, i.e., r (S, M) =0.9983 and r (M, E) =0.9996. A good correlation (r=0.9997) was also obtained between the standard and sample spectra of CEF and OFL, respectively. Also, excipients from formulation were not interfering with the assay.

Analysis of marketed formulation: Experimental results of the amount of CEF and OFL in tablets, expressed as a percentage of label claims were in good agreement with the label claims thereby suggesting that there is no interference from any of the excipients which are normally present in tablets. The drug content was found to be 99.10 % for CEF and 99.69 % for OFL.

CONCLUSION

Introducing HPTLC into pharmaceutical analysis represents a major step in terms of quality assurance. Today, HPTLC is rapidly becoming a routine analytical technique due to its advantages of low operating costs, high sample throughput and the need for minimum sample preparation. The major advantage of HPTLC is that several samples can be run simultaneously using a small quantity of mobile phase-unlike HPLC; thus reducing the analysis time and cost per analysis.

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