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High Performance Thin Layer Chromatographic determination of Cefixime and Ofloxacin in combined tablet dosage form

K. S. Khandagle, S. V. Gandhi *, P. B. Deshpande, A. N. Kale, P. R. Deshmukh,

Department of Pharmaceutical Analysis, A.I.S.S.M.S. College of Pharmacy, Pune, India

ABSTRACT

A new simple High Performance Thin Layer Chromatographic (HPTLC) method for determination of Cefixime and Ofloxacin in combined tablet dosage form has been developed and validated. The mobile phase selected was Methanol: Ethyl acetate: Ammonia (3.5: 3.5: 1.5 v/v/v) with UV detection at 295 nm. The retention factor for Cefixime and Ofloxacin were found to be 0.78 ± 0.10 and 0.61 ± 0.12. Results fo und to be linear in the concentration range of 50-500 ng/band for both Cefixime and Ofloxacin. The method has been successfully applied for the analysis of drugs in pharmaceutical formulation. The % assay (Mean ± S.D.) was found to be 99.89 % ± 0.14 for Cefixime and 102 .2 % ± 0.11 for Ofloxacin. The method was validated with respect to linearity, accuracy, precision and robustness as per the International Conference on Harmonisation (ICH) guidelines.

Key words: Cefixime, Ofloxacin, High Performance Thin Layer chromatography.

INTRODUCTION

Cefixime (CEFI) (6*R*, 7R)-7-[2-(2-amino-4-thiazolyl)glyoxylamido]-8-oxo-3-vinyl-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid,7-(*Z*)-[*o*-(carboxymethyl)-oxime] trihydrate is third-generation cephalosporin antibiotic [1]. Ofloxacin (OFLOX) chemically 9-fluoro-2, 3-dihydro-3-methyl-10-(4-methyl-1piperazinyl)-7-oxo-7H-pyrido (1, 2, 3-di)-1, 4-benzoxazine carboxylic acid used as an antibacterial [2].

Literature survey reveals High performance liquid chromatographic (HPLC) [3, 4], High performance thin layer chromatographic method [5] for determination of CEFI in tablet in combination with others drugs. Spectrophotometric method for simultaneous estimation of CEFI with other drugs also reported [6]. HPLC methods have been reported for the determination of OFLOX either in single or in combination with other drugs [7-10]. HPTLC method has been reported for determination of OFLOX in combination with other drugs [11].

Spectrophotometric methods for simultaneous estimation of OFLOX with other drugs also reported [12-14].

No work has been reported for the determination of the CEFI and OFLOX in combined tablet dosage form by HPTLC method. This paper presents HPTLC method for determination of Cefixime and Ofloxacin in combined tablet dosage form. The proposed method is optimized and validated as per the International Conference on Harmonization (ICH) guidelines [15].

EXPERIMENTAL SECTION

Chemicals and reagents

Analytically pure samples of CEFI and OFLOX were kindly supplied by Cipla Ltd. (Solan, H.P.). Methanol, Ammonia and Ethyl acetate (all AR grade) were used for the method development. The pharmaceutical dosage form used in this study was CEFI-O 200 Tablets (Accent Pharma, Puducherry, India) labeled to contain 200 mg of Cefixime and 200 mg of ofloxacin per tablet were procured from local market.

Instrumentation and chromatographic conditions

The samples were spotted in the form of bands of width of 6 mm with space between bands of 5 mm, with a 100 µL sample syringe (Hamilton, Bonaduz, Switzerland) on precoated silica gel aluminium plate 60 F_{254} (10 cm ×10 cm) with 250 µm thickness (E. MERCK, Darmstadt, Germany) using a CAMAG Linomat 5 sample applicator (Switzerland). The slit dimensions 5 mm × 0.45 mm and scanning speed of 20 mm/sec was employed.

The linear ascending development was carried out in 10 cm × 10 cm twin trough glass chamber (CAMAG, Muttenz, Switzerland) using Methanol: Ethyl acetate: Ammonia (3.5: 3.5: 1.5 v/v/v) as mobile phase. The optimized chamber saturation time for mobile phase was 25 min. The length of chromatogram run was 9 cm and development time was approximately 20 min. TLC plates were dried in a current of air with the help of a hair drier. Densitometric scanning was performed on CAMAG thin layer chromatography scanner at 295 nm for all developments operated by WINCATS software version 1.4.2. The source of radiation utilized was deuterium lamp emitting a continuous UV spectrum between 200 to 400 nm.

Preparation of standard stock solutions

Standard stock solution of CEFI and OFLOX was prepared by dissolving 5 mg of each drug in 10 mL of methanol separately to get concentration of 0.5 mg/mL from which 1 mL was further diluted to 10 mL to get stock solution of 50 ng/µL of each drug.

Selection of Detection Wavelength

After chromatographic development bands were scanned over the range of 200-400 nm and the spectra were overlain. It was observed that both drugs showed considerable absorbance at 295 nm. So, 295 nm was selected as the wavelength for detection as shown in Fig. 1.

Preparation of Calibration Curve

The standard stock solutions of CEFI and OFLOX (50 ng/µL each) were applied by overspotting on TLC plate in range of 1, 2, 4, 6, 8 and 10 µL with the help of CAMAG 100 µL sample syringe, using Linomat 5 sample applicator. The plate was developed and scanned under above established chromatographic conditions. Each standard in five replicates was

analyzed and peak areas were recorded. Calibration curves of CEFI and OFLOX were plotted separately of peak area vs respective concentration of CEFI and OFLOX.

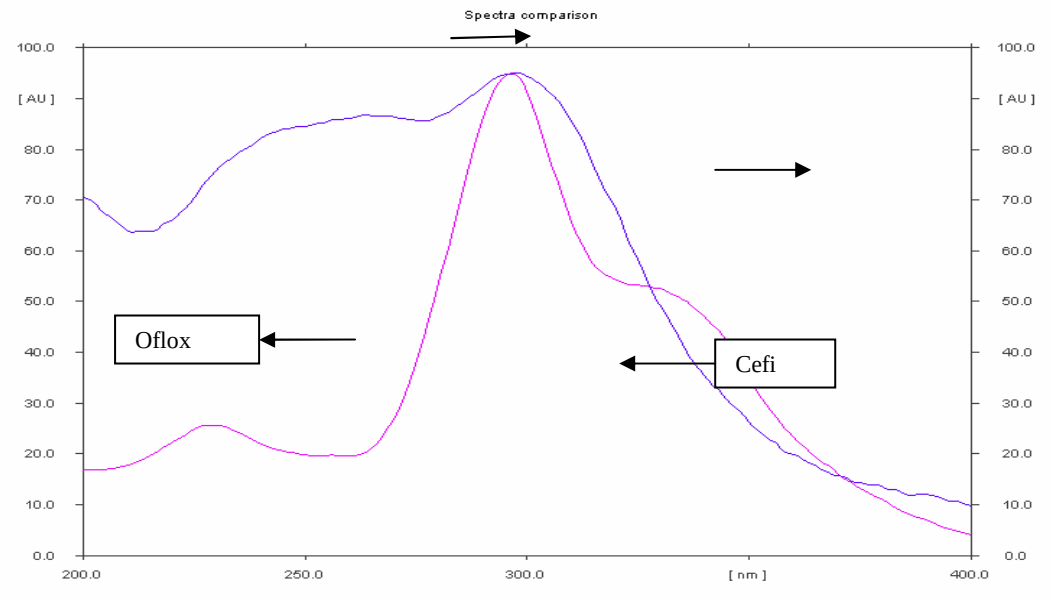

Fig. 1: Overlain spectra of CEFI and OFLOX

Analysis of Tablet Formulation

Twenty tablets were weighed accurately and finely powdered. A quantity of powder equivalent to 5 mg of OFLOX was weighed and dissolved in 10 mL of methanol. The solution was filtered using Whatman paper No. 41 and 1 mL of filtrate was further diluted to 10 mL. Two µL volume of this solution was applied on TLC plate. After chromatographic development peak areas of the bands were measured at 295 nm and the amount of each drug present in sample was estimated from the respective calibration curves. Procedure was repeated six times for the analysis of homogenous sample.

Robustness Studies

In the robustness study, the influence of small, deliberate variations of the analytical parameters on peak area of the drugs was examined. Factors varied were development distance (± 5 %), time from application to development (0, 10, 20, and 30 min) and from development to scanning (0, 30, 60, and 90 min). One factor at a time was changed to estimate the effect. Robustness of the method was checked at a concentration level of 400 ng/ band for both the drugs. The results of robustness data obtained are given in Table1.

Table 1: Robustness Data in Terms of Peak Area (% RSD)

Sr. No.	Parameter Varied	CEFI	OFLOX
1	Development distance	0.98	1.03
2	Time from application to development (Mins.)	0.79	0.64
3	Time from development to scanning (Mins.)	1.23	1.07

Recovery Studies

To check the accuracy of the method, recovery studies were carried out by addition of standard drug solution to pre-analyzed sample solution at three different levels 50, 100 and 150 %. The percentages of recoveries were calculated, results of which are represented in Table 2.

Table 2: Recovery Studies of CEFI and OFLOX

Drug	Amount taken (ng/band)	Amount added (ng/band)	Total amount found (ng/band)	% Recovery	% RSD[a]
CEFI	100	50	148.98	99.32	0.50
	100	100	204.41	102.20	0.87
	100	150	249.53	99.81	1.06
OFLOX	100	50	147.51	98.34	0.46
	100	100	206.92	103.46	1.58
	100	150	254.19	101.67	0.86

[a] *Average of three determinations*

RESULTS AND DISCUSSION

Different mobile phases containing various ratios of Methanol, Toluene, Triethylamine, Chloroform, Ethyl acetate, Ammonia were examined (data not shown). Finally the mobile phase containing Methanol: Ethyl acetate: Ammonia (3.5: 3.5: 1.5, v/v/v) was selected as optimal for obtaining well defined and resolved peaks. The optimum wavelength for detection and quantitation used was 295 nm. The retention factors for CEFI and OFLOX were found to be 0.78 ± 0.10 and 0.61 ± 0.12 respectively. Representative densitogram of mixed standard solution of CEFI and OFLOX is shown in Fig. 2.

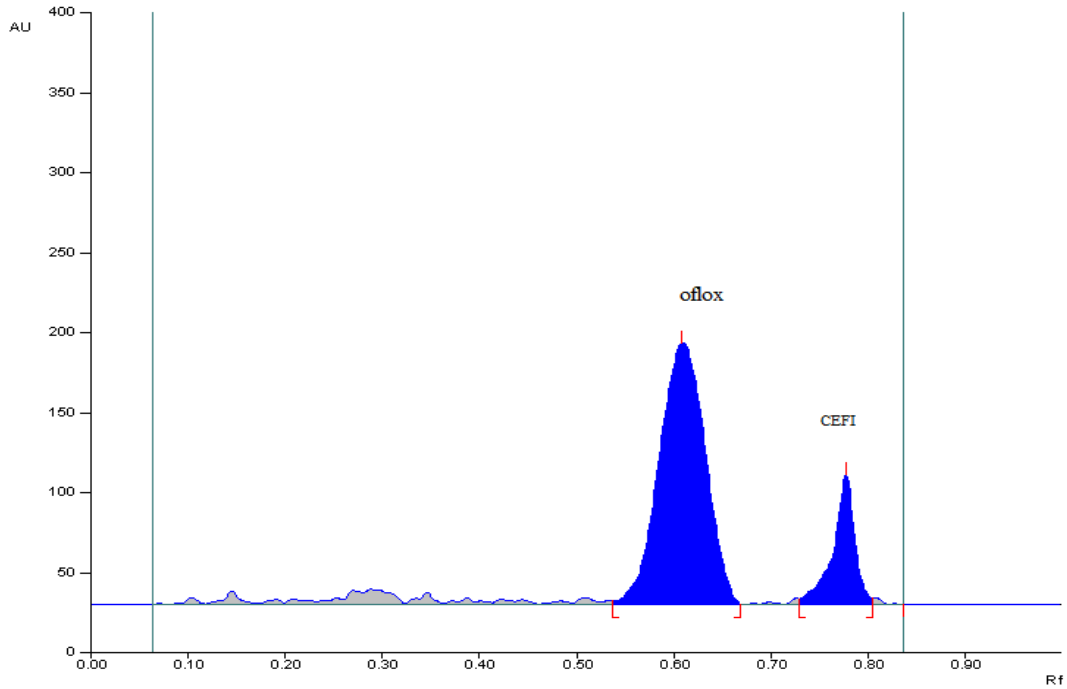

Fig. 2. Representative densitogramof mixed standard solution of OFLOX (200 ng/band, R_f = 0.61 ± 0.10) and CEFI (200 ng/band, R_f = 0.78 ± 12).

Straight-line calibration graphs were obtained for CEFI and OFLOX in the concentration range 50-500 ng/band for both the drugs with high correlation coefficient > 0.982. The proposed method was also evaluated by the assay of commercially available tablets containing CEFI and OFLOX. The % assay (Mean ± S.D.) was found to be 99.89 ± 0.14 for CEFI and 102.2 ± 0.11 for OFLOX. Robustness of the method checked after deliberate alterations of the analytical parameters showed that areas of peaks of interest remained unaffected by small changes of the operational parameters (% RSD < 2), which demonstrated

that the RP-HPLC method developed is robust. . For CEFI, the recovery study results ranged from 99.32 to 102.2 % with % RSD values ranging from 0.50 to 1.06. For OFLOX, the recovery results ranged from 98.34 to 103.46 % with % RSD values ranging from 0.46 to 1.58. The method was found to be accurate and precise, as indicated by recovery studies and % RSD not more than 2.

CONCLUSION

The validated HPTLC method employed here proved to be simple, fast, accurate, precise and robust, thus can be used for routine analysis of CEFI and OFLOX in combined tablet dosage form.

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