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Simultaneous estimation of cefixime trihydrate and moxifloxacin hydrochloride in their combined tablet dosage form by RP-HPLC

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ABSTRACT

A simple, accurate, specific and sensitive reverse phase high performance liquid chromatographic method has been developed and validated for the simultaneous estimation of cefixime trihydrate and moxifloxacin hydrochloride in tablet dosage form. The separation was achieved on a NeoSphere C18, 250 mm × 4.6 mm, 5 µm column with detection at 290 nm. A mobile phase comprising of 0.025M phosphate buffer and methanol (60:40 v/v), adjusted to pH 3.5 with 5% o-phosphoric acid was used at a flow rate 1.2 ml/min. The retention times for cefixime trihydrate and moxifloxacin hydrochloride were found to be 3.8 min and 16.5 min respectively. The calibration curves for the two drugs were linear in the concentration range 2-10 µg/ml. The developed method, validated as per ICH guidelines. It can be used for the simultaneous estimation of cefixime and moxifloxacin in tablet dosage forms.

Keywords: Cefixime Trihydrate, Moxifloxacin Hydrochloride, Simultaneous Estimation, RP-HPLC

INTRODUCTION

Cefixime trihydrate (CEF), (6R,7R)-7-{[2-(2-amino-1,3-thiazol-4-yl)-2-(carboxy methoxyimino) acetyl] amino}-3-ethenyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid trihydrate is a third generation cephalosporin antibiotic[1] (Fig 1). Moxifloxacin (MOX), 1-cyclopropyl-7-[(1S,6S)-2,8-diazabicyclo [4.3.0] non-8-yl]-6 fluoro -8-methoxy-4-oxo-quinoline-3-carboxylic acid hydrochloride is a fluoroquinolone antibiotic[2] (Fig 2). This combination is used for treatment of lower respiratory tract infections in adults[3]. Various analytical methods like UV spectrophotometry, HPLC, HPTLC have been reported for estimation of cefixime[4-6] and moxifloxacin[7-9] individually and combination with other drugs[10-12]. Only one UV-Visible spectrophotometric method has been reported for simultaneous estimation of CEF and MOX in combined tablet dosage form[13]. No reported RP-HPLC method is available for their simultaneous estimation in their combined dosage forms. The present work describes a simple, sensitive and accurate RP-HPLC method for simultaneous estimation of the two drugs in their combined tablet dosage form.

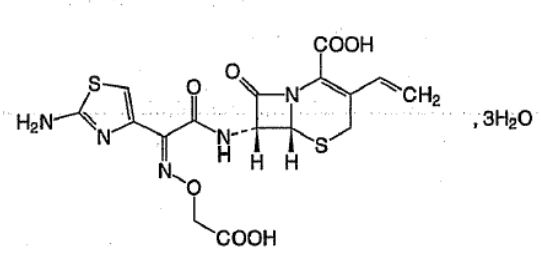

Fig 1. Chemical structure of Cefixime trihydrate

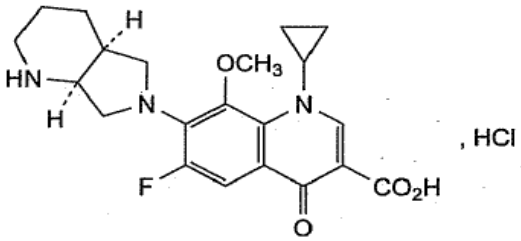

Fig 2. Chemical structure of Moxifloxacin hydrochloride

MATERIALS AND METHODS

Chemicals and Reagents

Cefixime trihydrate and moxifloxacin hydrochloride were received as gift samples from Cadila Pharmaceuticals, Ahmedabad and Yash Laboratories, Surat, India, respectively. HPLC grade methanol and analytical grade potassium di-hydrogen phosphate and o-phosphoric acid were purchased from Finar Ltd., Ahmedabad. Double distilled water was used for analysis.

Chromatographic conditions

Chromatographic analysis was performed on HPLC system (Shimadzu, LC-2010C_{HT}) consisting of autosampler, column thermostat and UV detector. Separation was achieved on a NeoSphere, C18, 250 mm × 4.6 mm, 5 µm column. The optimized mobile phase used was 0.025M phosphate buffer solution in water and methanol in a volume ratio 60:40 and pH was adjusted to 3.5 with 5% o-phosphoric acid. The mobile phase was delivered at flow rate 1.2 ml/min. UV detection was performed at 290 nm.

Preparation of standard solutions

Standard stock solution

Standard stock solutions were prepared by dissolving separately 25 mg of each drug in 25 ml of methanol (1000 µg/ml).

Working standard solution

A 5 ml aliquot of stock solution was diluted to 50 ml with methanol to prepare 100 µg/ml solution of each drug.

Working standard solution of binary mixture of CEF and MOX

Accurately measured 10 ml CEF stock solution and 10 ml MOX stock solution were transferred to a 100 ml volumetric flask and diluted to mark with methanol to give concentration 100 µg/ml of CEF and 100 µg/ml of MOX. Aliquots 1, 2, 3, 4 and 5 ml were transferred to a series of 50 ml volumetric flasks and diluted up to mark with mobile phase to get concentration range 2,4,6,8 and 10 µg/ml of CEF and MOX.

Assay of CEF and MOX in synthetic mixture

Accurately weighed 400 mg CEF and 400 mg MOX were mixed with 200 mg of immediate release placebo. An accurately weighed quantity of synthetic mixture equivalent to 25 mg CEF or 25 mg MOX was transferred to a 25 ml volumetric flask and sonicated with 10 ml methanol for 5 min. The volume was made up to 25 ml using methanol and mixed. The solution was filtered through Whatman filter paper no. 41 and 1 ml of the filtrate was diluted to 10 ml with methanol to obtain a solution having concentration of 100 µg/ml of CEF or 100 µg/ml of MOX.

Validation of the method [14]

System suitability

Five replicate injections of working standard solution of binary mixture were injected to determine the reproducibility of the chromatographic system and expressed in terms of percent relative standard deviation (%RSD). Theoretical plates per meter, asymmetry factor and resolution were also determined.

Specificity

Interference from solvents and endogenous matrix components was investigated by analyzing blank samples as well as placebo by the proposed method.

Linearity range

The linearity was expressed in terms of correlation co-efficient of linear regression analysis. The linearity for CEF and MOX was assessed by analysis of five independent levels of calibration curve in range of 2-10 µg/ml for both drugs.

Precision

Repeatability was determined by analyzing six replicates of standard solution containing 6 µg/ml of each drug. Intraday and Interday precision were evaluated by determining the corresponding responses three times on the same day and on three different days respectively for CEF and MOX (4, 6, 8 µg/ml). Results were expressed as (% RSD).

Accuracy
The accuracy of the method was evaluated by standard addition method. A previously analyzed test solution was spiked with drug standard solutions at 80%, 100% and 120% concentration levels and percent recovery was determined.

LOD and LOQ
Calibration curve was repeated five times and the standard deviation of the intercepts of regression equations was calculated. The LOD and LOQ were calculated using equation:

LOD = 3.3 * SD/ S and LOQ = 10 * SD/ S

Where; SD = standard deviation of intercepts
S = mean slope of calibration curve

RESULTS AND DISCUSSION

System suitability
Theoretical plates, asymmetry factor and resolution between CEF and MOX were determined for each drug. The results were within acceptable limits and are summarized in Table 1.

Table 1: System Suitability parameters

Parameters	CEF	MOX
Retention time (minutes ± SD)	3.8 ± 0.01	16.5 ± 0.06
Repeatability (% RSD)	0.18	0.08
Theoretical plates per meter	32000	8600
Asymmetry factor	1.03	1.10
Resolution	25.5	

The developed method was validated as per ICH guidelines in terms of specificity, linearity, precision, accuracy, limit of detection (LOD) and limit of quantification (LOQ).

Specificity
Chromatograms of blank and placebo showed that there is no interference from solvents and excipients at retention times of CEF and MOX (Fig 3 and 4).

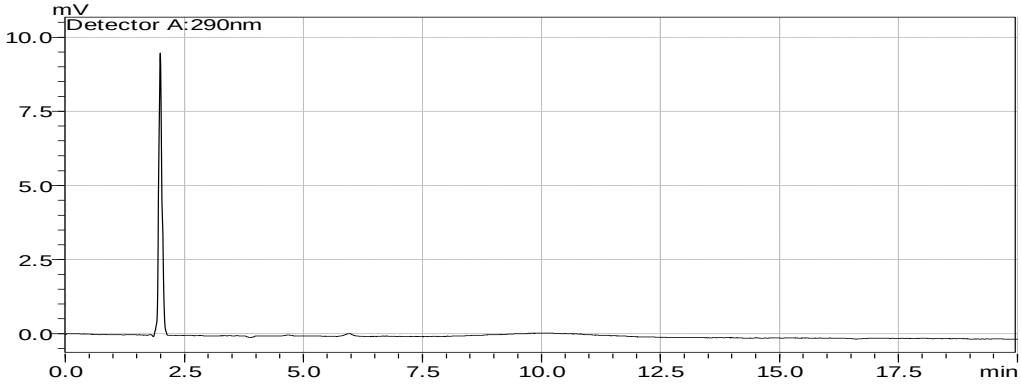

Fig 3. Chromatogram of blank

Linearity range
Calibration curves for peak areas of CEF and MOX versus corresponding concentrations were linear over the range 2 -10 µg/ml. Correlation coefficient for CEF and MOX was found to be 0.9995 and 0.9999 respectively (Table 2).

Table 2: Results for linearity

Parameters	CEF (n=5)	MOX (n=5)
Linearity range	2-10 µg/ml	2-10 µg/ml
Regression equations	y = 46011x – 14257	y = 94009x – 4822
Slope	46011	94009
Intercept	14257	4822
Correlation coefficient (R^2)	0.9995	0.9999

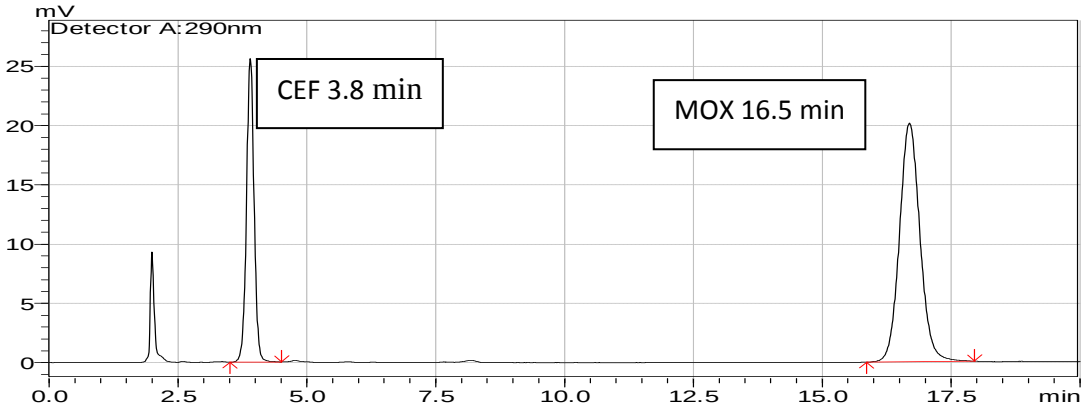

Fig 4. Chromatogram of CEF(6 µg/ml) and MOX(6 µg/ml) mixture

Precision

The repeatability was expressed in terms of % RSD and found to be 0.18 and 0.08 for CEF and MOX respectively. Percent RSD for intraday and interday variation at three different concentration levels were less than 1 % as shown in Table 3.

Table 3: Intraday and interday precision of proposed method

Name of Drug	Concentration (µg/ml)	Intraday precision		Interday precision	
		Area Mean ± S.D. (n=3)	% RSD	Area Mean ± S.D. (n=3)	% RSD
CEF	4	161130 ± 747	0.46	160797 ± 828	0.51
	6	248438 ± 662	0.27	247104 ± 947	0.38
	8	328457 ± 1190	0.36	328124 ± 1447	0.44
MOX	4	370928 ± 365	0.09	370728 ± 373	0.10
	6	557116 ± 447	0.08	556782 ± 594	0.11
	8	756780 ± 420	0.06	753114 ± 3280	0.44

LOD and LOQ

LOD and LOQ for CEF were found to be 0.19 µg/ml and 0.58 µg/ml respectively. LOD and LOQ for MOX were found to be 0.13 µg/ml and 0.39 µg/ml respectively. The data of LOD and LOQ for CEF and MOX are given in Table 4.

Table 4: LOD and LOQ results

Parameter	**CEF**	**MOX**
Standard deviation of the Y- intercepts of the 5 calibration curves	2673.9	3682.6
Mean Slope of the 5 calibration curves	46011	94269
LOD = 3.3 × (SD/Slope) (µg/ml)	0.19	0.13
LOQ = 10 × (SD/Slope) (µg/ml)	0.58	0.39

Accuracy

Standard addition was performed at three concentration levels. Percent recovery was found to be between 99.76 - 100.33 for CEF and 99.55-100.12 for MOX. The proposed method enables accurate simultaneous estimation of CEF and MOX. Results of accuracy study are shown in Table 5.

Table 5: Accuracy study

Drug	Concentration in sample (µg)	Amount of drug added (µg)	Total drug amount (µg)	Mean drug recovered ± SD (µg) (n=3)	% Recovery (n=3)
CEF	4	3.2	7.2	3.19±0.025	99.76
	4	4.0	8.0	4.01±0.015	100.33
	4	4.8	8.8	4.79±0.025	99.99
MOX	4	3.2	7.2	3.20±0.012	100.07
	4	4.0	8.0	3.97±0.015	99.55
	4	4.8	8.8	4.81±0.008	100.12

Assay of synthetic mixture

Applicability of the proposed method was tested by analyzing synthetic mixture of CEF and MOX. The results are shown in Table 6.

Table 6: Assay of synthetic mixture

Synthetic mixture	mg/gm (equivalent to label claim)		Assay ± S.D. (% of label claim) (n=6)	
	CEF	MOX	CEF	MOX
CEF & MOX	400	400	99.40±0.42	99.88±0.29

Solution stability

Stability of standard solution was determined by placing the solution at room temperature for a period of 6 hours and analyzing at each hour. No significant change in peak areas of the two drugs was observed at the end of 6 hours. The solutions were found to be stable up to 6 hours at ambient temperature.

The results of all validation parameters are summarized in Table 7.

Table 7: Summary of validation parameters

Parameters	Result for CEF	Result for MOX
Linearity Range (µg /ml)	2-10	2-10
Correlation coefficient (R^2)	0.9995	0.9999
Precision (% RSD) Repeatability (n=6) Intraday precision (n=3) Interday precision (n=3)	 0.18 0.27-0.46 0.38-0.51	 0.08 0.06-0.09 0.10-0.44
Accuracy (% recovery)	99.76-100.33	99.55-100.12
LOD (µg/ml)	0.19	0.13
LOQ (µg/ml)	0.58	0.39

CONCLUSION

A RP-HPLC method which is simple, sensitive, accurate and precise has been developed and validated for simultaneous estimation of CEF and MOX. The method can be applied to determine CEF and MOX content in commercial combined tablet dosage forms.

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REFERENCES

[1] Indian Pharmacopoeia 2010, The Indian Pharmacopoeia Commission, Govt. of India. Ministry of Health and Family Welfare, Ghaziabad, **2010**, 1012.
[2] British Pharmacopoeia 2010, HMSO Publication, London., **2010**, 1459.
[3] Patents: Knezevic, Igor, (Bayer Healthcare AG, Knezevic, Igor), EP and WO2007098868 (**2006**).
[4] A.J. Falkowski, Z.M. Look, *Journal of Chromatography*, **1987**, 422, 145-152.
[5] P.B. Shah, K. Pundarikakshudu, *American Journal of Analytic Chemica*, **2006**, 89, 987-94.
[6] J.D. Smet, K. Boussery, K. Colpaert, P.D. Sutter, *Journal of Chromatography B*, **2009**, 877, 961-967.
[7] A.L. Djurdjevic, M.J. Stankov, P. Djurdjevic, *Journal of Chromatography B*, **2006**, 844, 104-111.
[8] M.K. Motwani, S. Chopra, *Spectrochimica Acta Part A*, **2007**, 68, 250-256.
[9] M.V. Baldaniya, S.A. Shah, B.N. Suhagia, I.S. Rathod, *Indian Journal of Pharmaceutical Science*, **2005,** 67**,** 112-115.
[10] A. Khan, I. Zafar, M.I. Khan, J. Khalid, *Journal of Chromatography B*, **2011**, 879, 2423-2429.
[11] S.E. Jovanovic, D. Agbaba, D. Zivanov-Stakic, *Journal of Pharmaceutical and Biomedical Analysis*, **1998**, 18, 893-898.
[12] J.D. Smet, K. Boussery, K. Colpaert, P.D. Sutter, *Journal of Chromatography B*, **2009**, 877, 961-967.
[13] R.K. Patel, R.R. Parmar, V.M. Patel, A. Dushyant, *International Journal of Pharmaceutical Research and Bio-Science*, **2012**, 2, 81-93.
[14] International Conference on Harmonisation (ICH) of technical requirements for registration of pharmaceuticals for human use: Validation of analytical procedures: Text and methodology Q2(R1), **2005**.