Journal of Applied Pharmaceutical Science Vol. 5 (01), pp. 037-041, January, 2015
Available online at http://www.japsonline.com
DOI: 10.7324/JAPS.2015.50107
ISSN 2231-3354

Validated UV-Visible Spectrophotometric method for simultaneous estimation of Cefixime and Moxifloxacin in Pharmaceutical Dosage Form

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ARTICLE INFO

Article history:
Received on: 12/10/2014
Revised on: 14/11/2014
Accepted on: 03/12/2014
Available online: 30/01/2015

Key words:
Simultaneous estimation, Cefixime, Moxifloxacin, UV spectrophotometric method, Validation.

ABSTRACT

A simple, accurate, sensitive, economical and reliable first order derivative spectrophotometric method was developed and validated for the estimation of cefixime and moxifloxacin in pharmaceutical dosage form. The optimum conditions for the analysis of the drugs were established. First order derivative method was developed for quantification of cefixime and moxifloxacin. Spectrum was obtained by dissolving cefixime and moxifloxacin in methanol and water (60:40 v/v); wavelength selected was 260 nm for cefixime and 316 nm for moxifloxacin. The Beer's law was obeyed in the concentration range of 2-12 µg/ml. Results of tablet analysis showed percent relative standard deviation (% RSD) in the range of 0.1576 to 0.2183 for cefixime and moxifloxacin which indicate repeatability of the method respectively. Recoveries do not differ significantly from 100% which show there was no interference from the common excipient used in tablet formulation indicating accuracy and reliability of the method. The method was validated as per ICH guideline and found to be accurate, precise and rugged. It was also validated in terms of linearity, accuracy, precision, and specificity, limit of detection and limit of quantitation.

INTRODUCTION

Cefixime is official in British Pharmacopoeia . Chemically cefixime is (6R, 7R)-7- [[(Z)-2-(2-aminothiazol-4-yl)-2 [(carboxymethoxyimino) acetyl] amino]-3-ethenyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid (British Pharmacopoeia, 2009). It is an antibacterial agent and used to treat urinary tract infections, otitis, bronchitis, pneumonia, prostatitis, syphilis and infections of reproductive organs (Ashok Kumar et al, 2011; McMillan and Young, 2007; Adam et al, 1995). Moxifloxacin is official in British Pharmacopoeia. Chemically moxifloxacin is a 1-Cyclopropyl-6-fluoro-8-methoxy-7-[(4aS,7aS)-octahydro-6H- pyrrolo [3,4-B]pyridin- 6-yl]-4- oxo-1,4- dihydroquinoline-3 carboxylic acid hydrochloride (British Pharmacopoeia, 2009). It is a fourth generation fluoroquinolone broad spectrum antibiotic agent used in conjunctivitis (Merck index, 2006). Literature survey reveals that number of methods such as spectrophotometric (Magar et al, 2012; Mahesh and Anroop, 2011; Shahnaz Gauhar et al, 2009), HPTLC (Eric-Jovanovic et al,1998), HPLC (Vijay et al, 2012), colorimetric (Kumar et al., 2011), spectroflurometric (Shah et al., 2011) are reported for the estimation of cefixime from its formulation or biological fluids.

Similarly number of methods such as spectrophotometric (Shirkhedkar et al., 2011; Dewani et al, 2011), spectrofluorometric (Jasmin Shah et al, 2011), RP-HPLC (Arun K Sanapala et al, 2010; Mahwish et al, 2010), voltametric (Ferreira et al, 2005) are reported for the estimation of moxifloxacin from its formulation or biological fluids. This paper is in continuation with our work, (Kalyankar et al, 2013; Wadher et al, 2013; Lokhande et al, 2012; Mohan Raj *et al.*, 2007) where we studied spectrophotometric method for single or multicomponent drugs. There was no first order derivative sectrophotometric method reported for the simultaneous estimation of cefixime and moxifloxacin from their combined dosage form.

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So, present study was aimed to develop and validate spectrophotometric method for simultaneous estimation of cefixime and moxifloxacin from combined dosage form which would be simple, cost effective and easily adopted by small laboratories. The structure of cefixime and moxifloxacin are presented in (Figure 1).

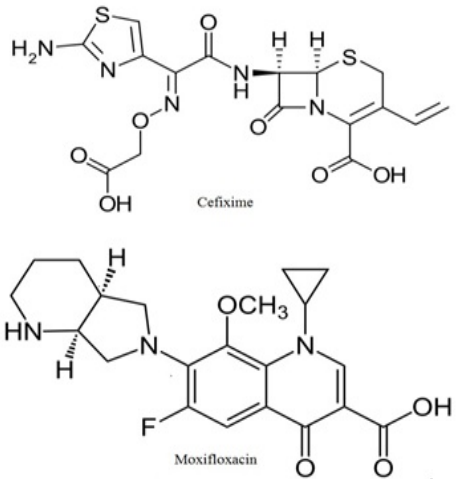

Fig. 1: Structure of Cefixime and Moxifloxacin.

MATERIALS AND METHODS

Reagents and Materials

Working standard of cefixime was pursued as a gift sample from FDC Pharma Ltd., Mumbai, India and moxifloacine were pursued as a gift sample from Dr. Reddy's Lab, Hyderabad, India. All chemicals and solvents of AR grade were purchased from Rankem Ltd, Mumbai, India.

Instrument:

UV- spectrophotometer UV-1800 (Shimadzu) with spectral bandwidth of 2 nm and 10 mm matched quartz shells was used for development analytical method over the range of 200-400 nm.

Preparation of Standard Stock Solutions:

An accurately weighed quantity of both cefixime and moxifloxacin equivalent to 10 mg was taken in two different 100 ml volumetric flasks and it was dissolved by using water and methanol (40:60 v/v) and volume was made to mark with the same (100 µg/ml).

The aliquot portion of standard stock solution of cefixime and moxifloxacin were diluted with water and methanol (40:60 v/v) to obtain concentration 10 µg/ml. The solutions were scanned in range of 400 - 200 nm against blank.

Selection of Analytical Wavelengths:

Appropriate dilutions were done for each drug from the standard stock solution and scanned in the spectrum mode from 400 nm to 200 nm. cefixime and moxifloxacin showed absorbance maxima at 260 nm and 316 nm in water : methanol (40:60 v/v) (Figure 2 and 3) respectively and there first derivative overlain spectrum shown in (Figure 4).

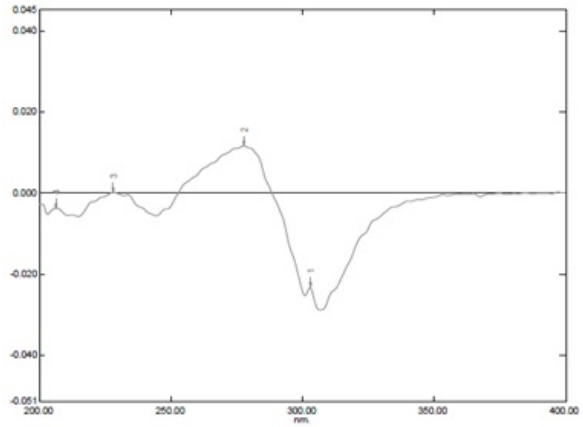

Fig. 2: First derivative spectrum of CEF.

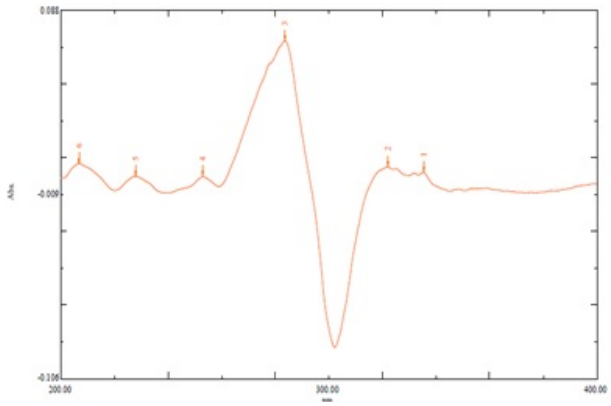

Fig. 3: First derivative spectrum of MOX.

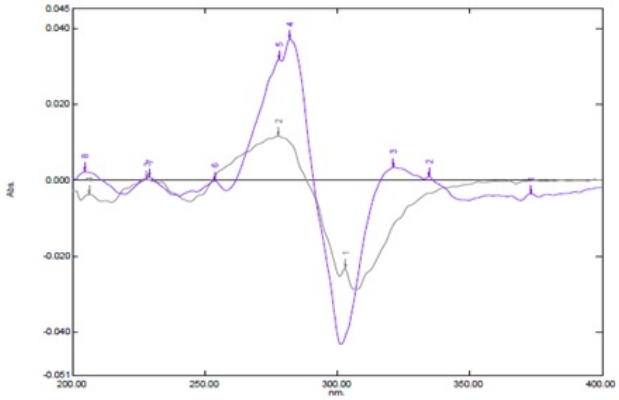

Fig. 4: First derivative overlain spectrum of CEF and MOX.

Selection of Analytical Concentration Ranges:

From the standard stock solution of cefixime, appropriate aliquots were pipetted out into 10 ml volumetric flasks and dilutions were made with water : methanol (40:60 v/v) to obtain working standard solutions of concentrations 2-12 µg/ml. Absorbance for these solutions were measured at 260 nm for cefixime (Table 1). The calibration curve of difference in absorbance against concentration was plotted (Figure 5).

Table 1: Observation for standard calibration curve of CEF.

Sr.No.	Conc. of CEF (µg/ml)	Abs. of CEF at 260 (nm)
1	2	0.099
2	4	0.210
3	6	0.312
4	8	0.410
5	10	0.510
6	12	0.614

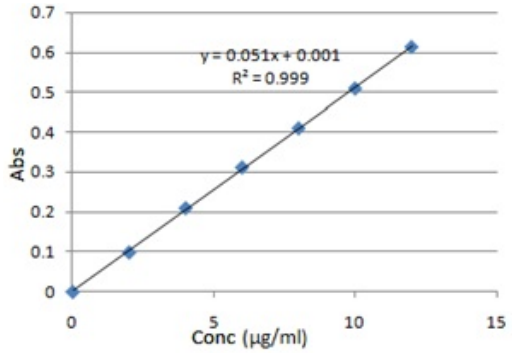

Fig. 5: Plot of Beer –Lambert's law of Absorbance for CEF at 260.

Similarly, the standard stock solution of moxifloxacin, appropriate aliquots were pipetted out into 10 ml volumetric flasks and dilutions were made with water: methanol (40:60 v/v) to obtain working standard solutions of concentrations 2-12 µg/ml. Absorbance for these solutions were measured at 316 nm (Table 2). The calibration curve of difference in absorbance against concentration was plotted (Figure 6).

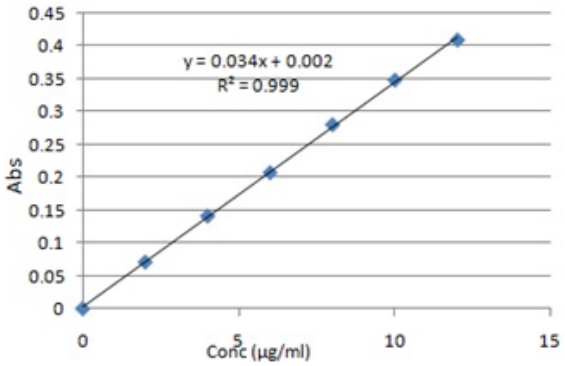

Fig. 6: Plot of Beer –Lambert's law of absorbance for MOX at 316nm.

Table 2: Observation for standard calibration curve of MOX.

Sr. No.	Conc. of MOX	Abs. of MOX at 316 (nm)
1	2	0.071
2	4	0.141
3	6	0.207
4	8	0.280
5	10	0.348
6	12	0.409

Analysis of Tablet Formulation

For analysis of tablet formulation; first twenty tablets were crushed and finely powdered. An accurately weighed quantity of tablet powder equivalent to 4 mg of cefixime and moxifloxacin was transferred in methanol and water (60:40 v/v) in 100 ml volumetric flasks and sonicated for 15 minute, finally volume was made to the mark with same and the solutions were further filtered through Whatman filter paper and the required dilutions were made to get the final concentration containing 2 µg/ml for cefixime and 2 µg/ml for moxifloxacin with water and methanol. The absorbances of the resulting solution of cefixime and moxifloxacin were measured at 260 nm & 316 nm against blank respectively. The analysis procedure was repeated five times. The results of marketed tablet formulation are given in (Table 3).

Table 3: Results of marketed tablet formulation.

Drug	Mean*	SD	% RSD
CEF	99.88	0.1576	0.1580
MOX	99.83	0.2183	0.2186

*Each value is a mean of six observations.

Method Validation

Linearity

Both drugs followed the Beer-Lamberts law in the range of 2-12 µg/ml for both CEF and MOX. Caliberation curve were shown in (Figure 5 & 6); regression coefficient (R^2) are 0.999 and 0.999 for cefixime and moxifloxacin respectively.

Recovery study

Recovery study was done by standard addition method. These studies were carried out at three levels i.e. multiple level recovery studies. i.e. 80, 100 and 120 % of the label claim of the tablet formulation as per ICH guidelines (Table 4)

Table 4: Results of Recovery Study.

Level of recovery	% Mean recovery*		S.D.		% R.S.D.	
	CEF	MOX	CEF	MOX	CEF	MOX
80%	99.97	100	0.0433	0.1028	0.0433	0.1028
100%	100.01	100.03	0.0675	0.1069	0.0674	0.1068
120%	99.95	99.85	0.0938	0.0971	0.0938	0.0972

*Each value is a mean of three observations.

Precision

Precision of the method was verified by using stock solutions in concentration containing 2 µg/ml of cefixime and moxifloxacin. System repeatability was done by repeating the assay three times of six replicate dilutions of the same concentration after every two hours on the same day for intra-day precision. Inter-day precision was carried out by performing the assay of six sample sets after 24 hours and 48 hours. The results of precision study are given in (Table 5).

Table 5: Results of Precision.

Formulation	Parameter	Intra-day precision*	Inter-day precision*
CEF	Mean	99.62	99.90
	SD	0.168	0.147
	% RSD	0.1686	0.1471
MOX	Mean	99.58	99.89
	SD	0.2246	0.1981
	% RSD	0.2255	0.1983

*Each value is a mean of six observations.

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

The LOD is the smallest concentration of the analyte that gives a measurable response (signal to noise ratio of 3). The LOQ is the smallest concentration of the analyte, which gives response that can be accurately quantified (signal to noise ratio of 10). Limit of detection (LOD) and limit of quantification (LOQ) was calculated by using following formula. The results are shown in (Table 6).

$$LOD = \frac{3.3\sigma}{S}$$

$$LOQ = \frac{10\ \sigma}{S}$$

Where σ is the standard deviation of the response, taken as a measure of noise, and S is the slope of the calibration curve of analyte.

Table 6: Optical characteristics and other parameters.

Parameters	CEF	MOX
Working wavelength (nm)	260	316
Linearity range (µg/ml)	2-12	2-12
Limit of detection (µg/ml)	0.0587	0.0881
Limit of quantitation (µg/ml)	0.1944	0.2924
Slope	0.0510	0.0340
Intercept	0.0018	0.0022
Regression Coefficient	0.999	0.999

RESULT AND DISCUSSION

The novel method for simultaneous estimation of cefixime and moxifloxacin was developed using water and methanol (40:60 v/v) as solvent. cefixime and moxifloxacin follow Beer-Lambert's law in range of 2-12 µg/ml and can be estimated in water and methanol. Commercial formulation containing CEF and MOX were analyzed by proposed method. Mean assay values in tablet were found to be 99.88 and 99.83 for CEF and MOX respectively. The accuracy of method was determined by recovery studies. Pure CEF and MOX were added to the preanalyzed tablet powder at three different levels i.e. 80, 100 & 120% of label claims as per the ICH guidelines. Three replicate analyses were carried out at each level. The mean recovery was found to be 99.97%, 100.01%, 99.95% and 100.00%, 100.03%, 99.85% for CEF and MOX in tablet samples respectively. It indicating that the method has required accuracy and there was no interference with API by excipients present in tablets. The RSD value is below 2% indicated that the method has required precision. The LOD and LOQ values of CEF and MOX at 260 nm and 316 nm were found to be 0.0587 and 0.0.1944 µg/ml and 0.0881 and 0.2924 µg/ml respectively.

CONCLUSION

The developed method was suitable for simultaneous estimation of cefixime and moxifloxacin in tablet formulation. The developed method is economic, specific and sensitive. Recovery studies showed that there is no interference of excipients. Hence this method can be used in quality control and routine analysis of the finished product.

ACKNOWLEDGEMENT

Authors are thankful to Dr. Reddy's lab Ltd. Hyderabad, India and FDC Pharma Ltd. Mumbai, India for providing us the gift sample of the pure drug.

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How to cite this article:

S S Pekamwar, T M Kalyankar, B V Tambe, S J Wadher. Validated UV-Visible Spectrophotometric method for simultaneous estimation of Cefixime and Moxifloxacin in Pharmaceutical Dosage Form. J App Pharm Sci, 2015; 5 (01): 037-041.