



RESEARCH ARTICLE

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**METHOD DEVELOPMENT AND VALIDATION OF CEFIXIME AND
MOXIFLOXACIN IN PHARMACEUTICAL DOSAGE FORM BY UV
SPECTROPHOTOMETRIC METHOD**

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Abstract: Versatile, accurate, precise and economic method for determination of cefixime and moxifloxacin in fixed dose combination mixture was developed. The absorbance values at 286.0 nm and 295.0 nm and 279nm (isoabsorptive point) were used for the estimation of cefixime and moxifloxacin, respectively without mutual interference. This method obeyed Beer's law in the concentration range of 2–18 µg /ml for cefixime and 2-12 µg /ml for moxifloxacin. The results of analysis have been validated statistically for linearity, accuracy and precision, LOD and LOQ of the proposed method.

Keywords: Moxifloxacin, Cefixime, Simultaneous equation method, Absorption ratio method, validation

INTRODUCTION

Cefixime (cef) is official in British pharmacopoeia. It is chemically 8-[[2-(2-amino-1, 3-thiazol-4-yl)-2-(carboxymethoxyimino) acetyl] amino]-4-ethynyl-7-oxo-2-thia-6-azabicyclo [4.2.0] oct-4-ene-5-carboxylic acid. It is used as a Cefixime is commonly used in the treatment of otitis media, respiratory tract infections, and urinary tract infections caused by susceptible organisms. Moxifloxacin (Mox) is official in British pharmacopoeia. It is chemically 1-Cyclopropyl-6-fluoro-8-methoxy-7-[(4*a*S, 7*a*S)-octahydro-6*H*-pyrrolo [3,4*B*] pyridin-6-yl]-4-oxo-1, 4-dihydroquinoline-3-carboxylic acid hydrochloride. Use in Ocular infection, acute sinusitis, Lower respiratory tract infections, UTI. A formulation containing 400 mg of CEF and 400 mg of MOX (SR) tablet Approved by CDSCO. Work done on mixture of individual plain tablets. A survey of literature revealed that few chromatographic and Spectrophotometric, HPLC and HPTLC methods are reported for determination of CEF and MOX individually. However there is no method reported so far its determination of CEF and MOX from

combine dosage form. The present work describes a validated, simple, precise and accurate spectrophotometric method for estimation of CEF and MOX from combined synthetic mixture form.

MATERIALS AND METHODS

Chemicals and Reagents

CEFIXIME and MOXIFLOXACIN were obtained as a gift sample from Cadila Healthcare Ltd. Gujarat, India. All other chemicals used were of analytical grade. 0.1N HCL and calibrated glassware were used throughout the work.

Apparatus

A shimadzu model 1700 (Japan) double beam UV-Visible spectrophotometer with spectral width of 2 nm, wavelength accuracy of 0.5 nm and a pair of 10 mm matched quartz cell was used to measure absorbance of all the solutions. A Reptech electronic weighing analytical balance based on EMFC technology was used in the study.

Preparation of standard stock solutions

An accurately weighed quantity of CEF (100 mg) and MOX (100 mg) were transferred to a separate 100 ml volumetric

flask and dissolved and diluted to the mark with 0.1N HCL to obtain standard solution having concentration of CEF (1000 µg/ml) and MOX (1000 µg/ml). Sonicator used for getting clear solution.

Calibration Curve

A calibration curve was plotted over a concentration range of 2-18 µg/ml cefixime (CEF), 2-12µg/ml moxifloxacin (MOX) Accurately measured standard stock solution of cefixime (2, 4, 6, 8, 10, 12, 14, 16, 18 mL) and standard stock solution of Moxifloxacin (2, 4, 6, 8, 10, 12 mL) were transferred to a separate series of 100 mL of volumetric flasks and diluted to the mark with 0.1N HCL . The absorbance of each solution was measured at the wavelengths 286.0 nm 295.0nm and 279.0nm

(isoabsorptive point). Calibration curves were constructed for by plotting absorbance versus concentrations.

Each reading was average of five determinations.

Selection of Analytical Wavelength

For selection of analytical wavelength for the simultaneous estimation. The stock solutions of CEF and MOX were separately diluted in 0.1N HCL to get a concentration of 6 µg/ml of CEF and 6 µg/ml of MOX respectively and scanned in the wavelength range of 200-400 nm. From the overlay spectra of both drugs, wavelengths 279.0 nm (isoabsorptive point), 286.0 nm (λ max of CEF) and 295.0 nm (λ max of MOX) were selected.



Overlain UV spectra of Cefixime and moxifloxacin.

Preparation of sample solution

Individual Cefixime 400 mg and Moxifloxacin 400 mg tablets crush and transferred to 100 ml volumetric flask. 70 ml of diluent and sonicated it for 30 min. The volume was made up to the mark with 0.1 N HCl filter the solution through 0.45 μ whatman filter paper no.42. Transfer 10 ml of filtrate into 100 ml volumetric flask and add diluents up to mark then again take 10ml from above and make up to 100 from that take 10 ml make up to 100 to get final concentration contain CEF 4 μ g/ml and MOX 4 μ g/ml in test sample solution.

METHODS OF ESTIMATION

Method I (Simultaneous equation method)

In simultaneous equation method (vierodt's method) two wavelengths were selected i.e.

286.0 nm and 295.0 nm which were absorbance maxima of cefixime and moxifloxacin respectively. For calibration curves, stock solutions of Cefixime and Moxifloxacin in the concentration of range of 2–18 μ g/ml and 2–12 μ g/ml respectively. The absorbance of Cefixime and

moxifloxacin were measured at 286.0 and 295.0 nm, calibration curves were plotted. The absorptivities of both the drugs at both the wavelengths were determined. The content of both ingredient in the sample were obtained by using following equations:

$$C_x = \frac{A_2 a_{y1} - A_1 a_{y2}}{a_{x2} a_{y1} - a_{x1} a_{y2}}$$

$$C_y = \frac{A_1 a_{x2} - A_2 a_{x1}}{a_{x2} a_{y1} - a_{x1} a_{y2}}$$

Where,

A₁ = Absorbance of the diluted sample at 286 nm

A₂ = Absorbance of the diluted sample at 295 nm

A_{x1} = Absorptivity of cefixime at 286 nm

a_{x2} = Absorptivity of cefixime at 295 nm

a_{y1} = Absorptivity of moxifloxacin at 286 nm

a_{y2} = Absorptivity of moxifloxacin at 295 nm

C_x = Concentration of cefixime in the diluted sample

C_y = Concentration of moxifloxacin in the diluted sample

Method II (Absorbance ratio - Q analysis method)

In this method the ratio of absorbance at any two wavelengths is a constant value independent of concentration or wavelength. In the assay of the drug product under study which contains two active ingredient i.e. cefixime and moxifloxacin the absorbance were measured at two wavelengths, one being the λ_{max} of moxifloxacin (295 nm) and other being the wavelength of equal absorptivity of the two components i.e. an isosbestic point (279.0 nm). The content of both ingredient in the sample were obtained by using following equation:

$$C_x = [(Q_M - Q_Y) / (Q_X - Q_Y)] \times A_1 / a_{x1}$$

$$C_y = (A_1 / a_{x1}) - C_x$$

A₁ = Absorbance of the diluted sample at 279.0 nm

A₂ = Absorbance of the diluted sample at 295.0 nm

a_{x1} = Absorptivity of moxifloxacin at 279.0 nm

a_{x2} = Absorptivity of moxifloxacin at 295.0 nm

a_{y1} = Absorptivity of cefixime at 279.0 nm

a_{y2} = Absorptivity of cefixime at 295.0 nm

$Q_M = A_2/A_1$, $Q_X = a_{x2}/a_{x1}$, $Q_Y = a_{y2}/a_{y1}$

Validation of the Proposed Method

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

Linearity (Calibration curve)

The calibration curves were plotted over a concentration range of 2-18 $\mu\text{g/ml}$ and 2-12 $\mu\text{g/ml}$ for CEF and MOX respectively. Accurately measured standard solutions of CEF (2, 4, 6, 8, 10, 12, 14, 16, 18, ml) and MOX (2, 4, 6, 8, 10, 12 ml) were transferred to a series of 100 ml of volumetric flasks and diluted to the mark with 0.1N HCL. The absorbance of the solutions were measured at 286 nm of CEF, 295 nm of MOX and 279.0 nm (Isoabsorptive point) against 0.1N HCL as blank. The calibration curves were constructed by plotting absorbance versus concentrations and the regression equations were calculated.

Precision

The intraday and interday precision of the proposed method was determined by analyzing the corresponding responses 3 times on the same day and on 3 different days 3 different concentrations of standard solutions of CEF and MOX.

Accuracy (recovery study)

The accuracy of the method was determined by calculating recovery of CEF and MOX by the standard addition method. Known amounts of standard solutions of CEF and MOX were added at 80, 100 and 120 % level to prequantified sample solutions of CEF and MOX (4000 $\mu\text{g/ml}$ for CEF and 4000 $\mu\text{g/ml}$ for MOX). The amounts of CEF and MOX were estimated by applying obtained values to the respective regression line equations. The experiment was repeated for five times.

Limit of detection and Limit of quantification

The limit of detection (LOD) and the limit of quantification (LOQ) of the drug were derived by calculating the signal-to-noise ratio (S/N) using the following equations designated by International Conference on Harmonization (ICH) guidelines.

$$\text{LOD} = 3.3 \times \sigma/S$$

$$LOQ = 10 \times \sigma/S$$

Where, σ = the standard deviation of the response and S = slope of the calibration curve.

RESULTS AND DISCUSSION

The solubility of CEFIXIME (CEF) and MOXIFLOXACIN (MOX) was studied and 0.1N HCL was selected as a choice of solvent. Cefixime and Moxifloxacin showed well defined λ_{max} at 286.0 nm and 295.0 nm respectively. The two drugs also show an isoabsorptive wavelength at 279.0 nm, where both the drugs have same absorptivity value. The wavelengths 286.0 and 295.0 nm was considered for development of Simultaneous Equation Method where as 279. and 295.0 nm for absorbance ratio method (Figure 1). The two drugs

individually and in their mixture were found to follow Beer-Lambert's law over the concentration range of 2-18 $\mu\text{g/ml}$ and 2-12 $\mu\text{g/mL}$ for CEF and MOX respectively. The developed method was validated for parameters like linearity, precision, accuracy, LOD, LOQ. the data for which are presented in the Table 1, 2. Analytical recovery experiments were carried out by standard addition method to check the accuracy of the developed methods and to study the interference of formulation additives (Table 3). The validated method was successfully applied for the determination of in tablets mixture of CEF and MOX the results are given in Table 4 indicate that the amount of drug in tablet samples met with requirements.

TABLE1.
OPTICAL CHARACTERISTICS DATA

Parameters	Method I			
	Simultaneous Equation Method			
	CEF	CEF	MOX	MOX
Wavelength (nm)	286	295	286	295
Beer's law limit (µg /ml)	2-18	2-18	2-12	2-12
Regression equation (y = a + bc)	y = 0.044X-0.001	y = 0.042X-0.010	y = 0.074X-0.008	y = 0.090x - 0.002
Slope (b)	0.044	0.042	0.074	0.090
Intercept (a)	-0.001	-0.010	-0.008	-0.004
Correlation coefficient (r ²)	0.999	0.999	0.999	0.999
LOD (µg/ml)	0.106	0.152	0.052	0.027
LOQ(µg/ml)	0.322	0.460	0.158	0.082
Precision(% RSD, n=3)	0.48	0.55	0.33	0.21
Interday	0.15-0.65	0.17-0.69	0.13-1.4	0.12-0.65
Intraday				

TABLE 2.
OPTICAL CHARACTERISTICS DATA

Parameters	Method II			
	Absorbance ratio - Q analysis method			
	CEF	CEF	MOX	MOX
Wavelength (nm)	279	295	279	295
Beer's law limit($\mu\text{g/ml}$)	2-18	2-18	2-12	2-12
Regression equation	$y = 0.0445X -$	$y = 0.0404X -$	$y = 0.0434X +$	$y = 0.0902X -$
(y = a + bc)	0.0082	0.0013	0.0023	0.0047
Slope (b)	0.0445	0.0404	0.0434	0.0902
Intercept (a)	0.0082	0.0013	0.0023	0.0047
Correlation coefficient (r^2)	0.999	0.999	0.999	0.999
LOD ($\mu\text{g/ml}$)	0.161	0.077	0.047	0.039
LOQ($\mu\text{g/ml}$)	0.490	0.236	0.144	0.11
Precision(% RSD)				
(n=3) Interday	1.05	0.72	0.78	0.47
Intraday	0.1-1.05	0.16-0.72	0.19-0.78	0.09-0.47

TABLE 3.
RECOVERY RESULT OF MOXIFLOXACIN AND CEFIXIME

Method	Recovery Level	% Recovery	SD	% Recovery	SD
		CEFIXIME		MOXIFLOXACIN	
I	80%	101.75	±0.14	99.96	±0.33
	100%	101.40	±0.15	99.30	±0.20
	120%	101.02	±0.21	101.58	±0.30
II	80%	99.37	±0.26	99.13	±0.50
	100%	98.22	±0.36	99.25	±0.65
	120%	101.66	±0.41	101.83	±0.86

*SD = Standard deviation

TABLE 4.
ANALYSIS OF TABLET FORMULATION

Method	TABLET MIXTURE	Label claim (mg/capsule)	% Label claim	SD
I	CEF	400 mg	101.25%	±0.10
	MOX	400 mg	98.75%	±0.35
II	CEF	400 mg	100.75%	±0.45
	MOX	400 mg	99.00%	±0.67

*SD = Standard deviation

CONCLUSION

The developed UV spectrophotometric method for estimation of cefixime and moxifloxacin was found to be rapid, simple, inexpensive, reproducible and applicable over a wide concentration range with high precision and accuracy. The method was validated as per the guidelines laid by ICH. The results of the validated tests were found to be satisfactory and therefore this method can be applied successfully for routine quality control

analysis of cefixime and moxifloxacin in bulk and pharmaceutical formulation.

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