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Development and Validation of Q–Absorbance Ratio Method for Simultaneous Estimation of Cefixime and Moxifloxacin In Synthetic Mixture

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ABSTRACT

A simple and economical Q-Absorbance spectrophotometric method has been developed for the simultaneous estimation of cefixime and moxifloxacin in their synthetic mixture. The method is based on Q-absorption Ratio method using two wavelengths, 293.6 nm (λmax of Moxifloxacin) and 276 nm (Isoabsorptive point). Methanol was used as a solvent in the method. Both drugs showed linearity in the range of 4-14 µg/mL in the methods. The method was validated statistically and recovery studies were carried out. The proposed method was found to be simple, economical, accurate, and reproducible. It can be applied for routine analysis and quality control of both drugs in their combination drug products.

Keywords: Cefixime, Moxifloxacin, Spectrophotometric, Q-absorption Ratio Method

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INTRODUCTION

Cefixime (CEF), an antibiotic, is a third-generation cephalosporin like ceftriaxone and cefotaxime. The antibacterial effect of cefixime results from inhibition of mucopeptide synthesis in the bacterial cell wall. Chemically, it is [6R, 7R] – 7- [[(2Z)- 2- (2-amino thiazole- 4-yl)- [(carboxy methoxy) imino] aetyl] amino]-3-ethenyl -8-oxo 5-thia 1-aza bicyclo [4.2.0] oct-2-ene-2 carboxylic trihydrate[1]. Moxifloxacin (MOXI) is a synthetic fluoroquinolone antibiotic agent. Chemically, it is 1-cyclopropyl-7-[(1S,6S)-2,8-diazabicyclo[4.3.0]non-8-yl]-6-fluoro-8-methoxy-4-oxo- quinoline-3-carboxylic acid[2]. Cefixime is official in IP' 2010[3], BP' 2010[4], USP' 2009[5], JP' 2006[6] while Moxifloxacin is official in BP' 2010[7]. Literature survey reveals that number of methods such as spectrophotometric[8,9], HPLC[10-17], HPTLC[17,18], LC-MS[19], HPCPE[20], Voltametric[21] are reported for the estimation of cefixime from its formulation or biological fluids. Similarly number of methods such as spectrophotometric[22], HPLC[23,24], HPTLC[25], TLC[26], Fluorescence spectroscopy[27], Voltametric[28] are reported for the estimation of diltiazem from its formulation or biological fluids. There was no any method reported for the simultaneous estimation of cefixime and moxifloxacin from their combined dosage form. So, present study was aimed to develop and validate[29] Q-absorption Ratio spectrophotometric method for simultaneous estimation of Cefixime and Moxifloxacin from their synthetic mixture which would be simple, cost effective and easily adopted by small laboratories.

MATERIAL AND METHOD

Instruments and Apparatus

A double beam UV-visible Spectrophotometer (Shimadzu, UV-1700, Japan), attached to a computer software UV probe 2.0, with a spectral width of 2 nm, wavelength accuracy of 0.2 nm and pair of 1 cm matched quartz cells, Analytical Balance (CP224S, Sartorius, Germany), Ultrasonic Cleaner (Frontline FS 4, Mumbai, India), Corning volumetric flasks, pipettes of borosilicate glass were used in the study, and Water Purification System (Millipore Bioscience Division Pvt. Ltd, India) was used during study.

Chemicals and Reagents

Kindly gifted reference standards of Cefixime (Acme Pharmaceuticals, Gujarat, India), and Moxifloxacin (Astron Research Centre, Ahmedabad) was used without further purification. Synthetic mixture containing 40 mg cefixime and 40 mg moxifloxacin was prepared in the laboratory. AR grade methanol (S.D. Fine Chemical Ltd., Mumbai, India) and Whatman filter paper no. 41 (Whatman International Ltd., England) was used for the study.

Preparation of Reagents and Solutions

Preparation of standard solutions

To Prepare standard solution of CEF (100 µg/ml) and MOXI (100 µg/ml), 10 mg of each drugs were transferred in two different 100 ml volumetric flask. Dissolve and diluted up to mark with methanol, from these stock solution, 0.8 ml aliquots were transferred in two different 10 ml volumetric flask and were diluted up to mark with methanol to get working standard solution having concentration of CEF and MOXI of 8 µg/ml each.

Preparation of synthetic mixture

Cefixime (40 mg) and Moxifloxacin (40 mg) were taken and then mixed with starch, Lactose, Magnesium stearate, S.S.G. and Talc. Total 400 mg of mixture was prepared and it was used in further study.

Preparation of sample solution

From synthetic mixture, powder equivalent to 100 mg of drugs was taken and diluted up to 100 ml with methanol. Suitable aliquots were taken and taking absorbances at 293.6 nm and 276 nm. The content was mixed with methanol (50 ml), sonicated for 20 min. to dissolve the drug as completely as possible. The solution was then filtered through a whatman filter paper no. 41.

Determination of the analytical wavelengths

The standard solutions of CEF (8 µg/ml) and MOXI (8 µg/ml) were scanned separately in the UV range of 200-400 nm. Data were recorded at an interval of 1 nm. Overlain spectra shows 276 nm as isoabsorptive point, 288 nm as the λ_{max} of CEF and 293.6 nm as the λ_{max} of MOXI.

Calibration curve for CEF and MOXI

To check linearity of the method, working standard solution having concentration in range of 4-14 µg/ml were prepared from the standard stock solutions of both drugs. For this prepare aliquots of 0.4, 0.6, 0.8, 1.0, 1.2, 1.4 ml of standard stock solutions of both drug were transferred separately to a series of 10 ml volumetric flasks and diluted to mark with methanol and the absorbance was measured at 293.6 nm (λ_{max} of MOXI) and at 276nm (isoabsorptive point). The calibration curves were constructed by plotting absorbances Vs concentrations.

Analysis of CEF and MOXI in synthetic mixture:-

The absorbances of the final sample were measured at 293.6 nm and at 276 nm. The conc. of the both the drugs was calculated by using regression equation 1 & 2 for respective drugs.

RESULTS AND DISCUSSION

Method development

Q-Absorbance method[30] depends on the property that, for a substance which obeys Beer's law at all wavelength, the ratio of absorbances at any two wavelengths is a constant value independent of concentration or pathlength. In this method, the absorbance was measured at two wavelengths, one being the isoabsorptive point of the two components and other being the wavelength of maximum absorption of one of the two components. For this measurement, the solutions of CEF and MOXI were prepared separately in methanol at a concentration of 8 µg /ml they were scanned in the wavelength range of 200-400 nm. Data were recorded at an interval of 1 nm. From the overlain spectra of the two drugs (Figure 1) absorbances were measured at selected wavelength i.e. 276 nm isoabsorptive point and 293.6 nm, λ_{max} of MOXI. The absorbance and absorptivity values at the particular wavelengths were calculated and substituted in the following equation; to obtain the concentration

$C_X = (Q_m - Q_y) \times A / (Q_X - Q_Y) \times ax_1$ ---------- (1)

$C_Y = (A/ax_1) - C_X$ ---------- (2)

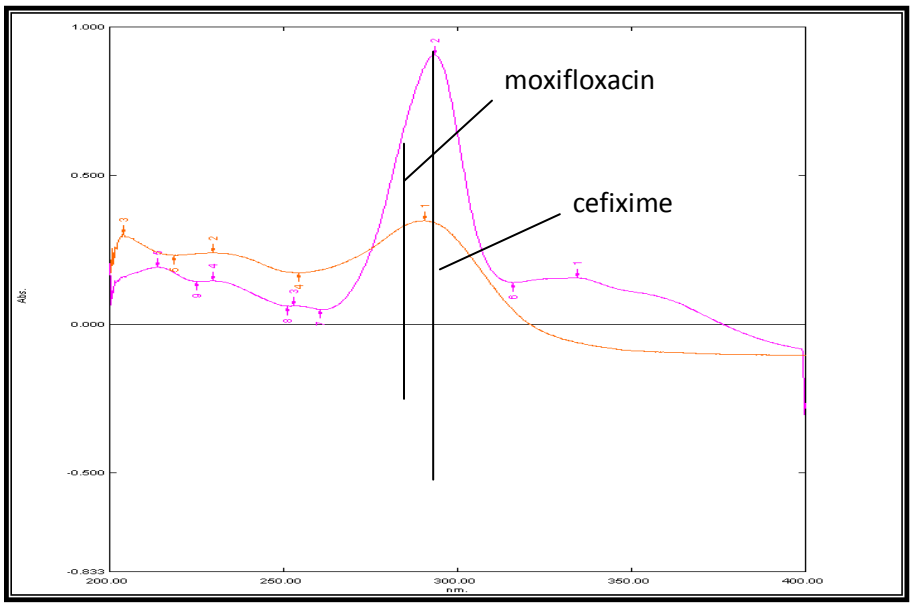

Figure 1: Overlain zero order absorption spectra of standard solutions of CEF and MOXI in methanol (12 µg/ml).

Validation of the proposed method

Linearity

Linear correlation was obtained between absorbance versus concentrations of CEF and MOXI in the concentration ranges of 4-14 µg/ml. Regression parameters are mentioned in Table 1 and the calibration curves of these two drugs at 293.6 nm and 276 nm are shown in Figure 2, 3 and, 4. Calibration curve data of CEF and MOXI shown in Table 2.

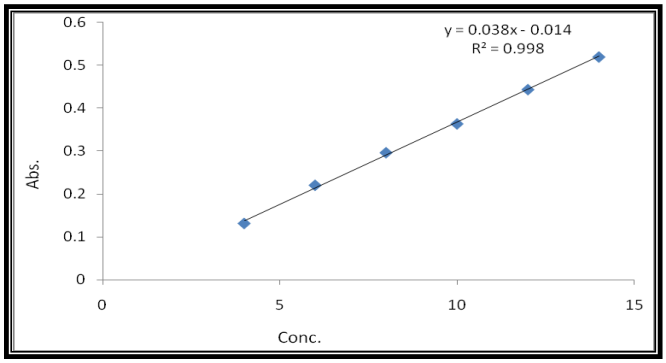

Figure 2: Calibration curve of CEF at 293.6nm

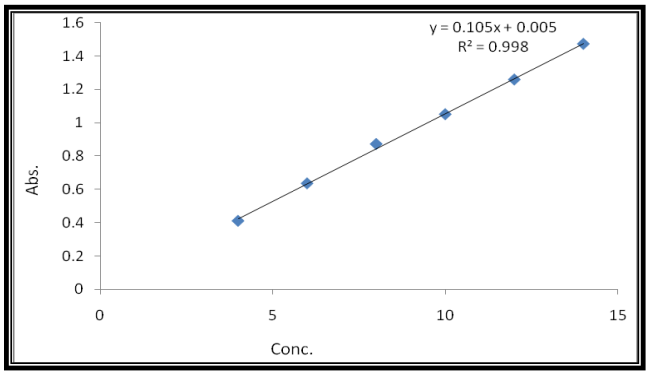

Figure 3: Calibration curve of MOXI at 293.6nm

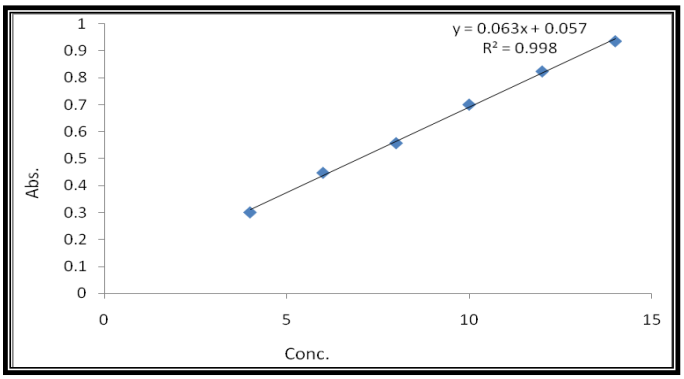

Figure 4: Calibration curve at isoabsorptive point at 276nm

Accuracy

The accuracy of the method was determined by calculating recovery of CEF and MOXI by the standard addition method. Known amounts of standard solutions of CEF and MOXI (4, 8, 12 µg/ml for both drugs) were added to prequantified separate sample solutions of CEF 8 µg/ml and MOXI 8 µg/ml. The amounts of CEF and MOXI were estimated by applying obtained values (n=6) to the equation 1 & 2 given in section 3.1. The mean recoveries were 99.1 ± 1.0 and 100.03 ± 1.05 % for CEF and MOXI, respectively (Table 4.). The low value of standard deviation indicates that the proposed method is accurate. Results of recovery studies are shown in Table 3.

Table 2: Calibration curve data of CEF and MOXI by Q – Absorption ratio method

Sr no.	Concentration (µg/ml)	Absorption of CEF at λmax 293.6 nm	Absorption of MOXI at λmax 293.6 nm	Absorption of CEF and MOXI at Isoabsorptive point 276 nm
1	4	0.132	0.440	0.301
2	6	0.229	0.681	0.450
3	8	0.310	0.906	0.559
4	10	0.386	1.135	0.689
5	12	0.467	1.352	0.831
6	14	0.543	1.455	0.938

Table 3: Recovery data for the proposed method

Drug	Level	Amount of sample taken (µg/ml)	Amount of standard spiked (%)	Mean % Recovery ± SD*
CEF	I	8	50 %	99.7 ± 1.38
	II	8	100 %	98.8 ± 0.78
	III	8	150 %	98.9 ± 0.84
MOXI	I	8	50 %	100.6 ± 1.44
	II	8	100 %	99.5 ± 0.46
	III	8	150 %	100.0 ± 1.25

Table 4: Precision data for CEF and MOXI

Sr. No.	Absorbance of CEF at 293.6nm	Absorbance of MOXI at 293.6 nm	Absorbance at isoabsorptive point (276 nm)
1	0.295	0.843	0.559
2	0.293	0.844	0.558
3	0.300	0.869	0.554
4	0.302	0.839	0.559
5	0.300	0.843	0.557
6	0.300	0.842	0.558
Mean	0.298	0.846	0.557
S.D.	0.003	0.011	0.001
RSD	1.17	1.30	0.17

Method precision (Repeatability)

The precision of the instrument was checked by repeated scanning and measurement of the absorbance of solutions (n = 6) of CEF and MOXI (8 µg/ml) without changing the parameters of the proposed method. The RSD values for CEF and MOXI were found to be 1.17 and 1.30 %, respectively at 293.6 nm while 0.17 % at Isoabsorptive point (Table- 1 and 4). Low relative standard deviation indicates that the proposed method is repeatable.

Intermediate precision (Reproducibility)

The intraday and interday precisions of the proposed method was determined by analyzing corresponding responses in triplicate on the same day and on 3 different days over a period of 1

week for 6 different concentrations of standard solutions of CEF and MOXI (4, 6, 8, 10, 12 and 14 µg/ml).Results were reported in terms of RSD. The low RSD values of interday (0.43 – 1.27 and 0.41-1.38) and intraday (0.23 – 1.34 and 0.36-1.28) variations for CEF and MOXI, respectively at 293.6 nm and interday (0.24 – 1.44 %) and intraday (0.20 –1.43 %) variations at 276 nm, reveal that the proposed method was precise (Table 1).

LOD and LOQ

LOD and LOQ of the drug were calculated as per ICH guideline. LOD values for CEF and MOXI were found to be 0.122 and 0.124 µg/ml, respectively 0.12µg/ml at 276 nm and LOQ values for CEF and MOXI were found to be 0.371 and 0.376 µg/ml, respectively 0.38 µg/ml at 276nm (Table 1). these data show that proposed method is sensitive for the determination of CEF and MOXI.

Table: 1 Regression analysis data& summary of validation parameter for proposed method

Parameters	**Q-Absorbance ratio method**		
	CEF at 293.6 nm	**MOXI at 293.6 nm**	**CEF and MOXI at 276 nm**
Linearity (µg/ml)	4 –14	4 – 14	4–14
Slope	0.038	0.105	0.063
Intercept	0.014	0.005	0.057
Correlation coefficient	0.998	0.998	0.998
LOD (µg/ml)	0.124	0.122	0.120
LOQ (µg/ml)	0.371	0.376	0.365
Repeatability (RSD, n = 6) %	1.17	1.30	0.17
Precision (RSD), %			
Interday (n = 6)	0.43 – 1.27	0.41-1.38	0.24 – 1.44
Intraday (n = 6)	0.23 – 1.34	0.36-1.28	0.20 –1.43

Assay of the synthetic mixture

The result obtained for CEF and MOXI were comparable with the corresponding dose amount (Table 5). No interference of the excipients with the absorbance of interest appeared.

Table 5: Analysis of synthetic mixture of CEF and MOXI by proposed method (n = 6)

Sample No.	**Amount Taken**		**Amount Found**		**% Assay**	
	CEF (mg)	**MOXI (mg)**	**CEF (mg)**	**MOXI (mg)**	**CEF (mg)**	**MOXI (mg)**
1	400	400	399.6	400.2	99.84	100.2
2	400	400	404.2	399.5	101.6	99.5
3	400	400	401.3	398.3	100.5	98.3
4	400	400	400.5	399.7	100.2	99.7
5	400	400	398.9	401.5	99.5	101.5
6	400	400	402.3	399.6	100.9	99.6
Mean			401.1	399.8	100.4	99.8
S.D.			1.92	1.04	0.75	1.04

CONCLUSION

In this proposed method the linearity was observed in the concentration range of 4-14 µg/ml with co-efficient of correlation, r^2 = 0.998 and r^2 = 0.998 for CEF and MOXI, respectively at 293.6 nm and r^2 = 0.9998 at 276 nm. The result of the analysis of synthetic mixture by the proposed method was found to be highly reproducible and reliable and was in good agreement with dose of the drugs. The additive usually present in the synthetic mixture of the assayed samples did not interfere with determination of CEF and MOXI. The method can be used for the routine analysis of the CEF and MOXI in synthetic mixture as well as combination drug products.

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