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Research Article

Development and Validation of a Spectrophotometric method using Vierordt's Method for Simultaneous Estimation of Moxifloxacin and Cefixime in tablet formulation

[1]P.Kathiravan*,[1]S.venkatesan, [2]Rameshwar singh, [1]P.Giriraj

[1]Research scholar, Department of Pharmacy, Annamalai University, Tamilnadu.-608002.
[2]Assistant Professor, A.N.D. College of Pharmacy, Babnan, Gonda,Uttar Pradesh.
Email:kathirpceutics@gmail.com

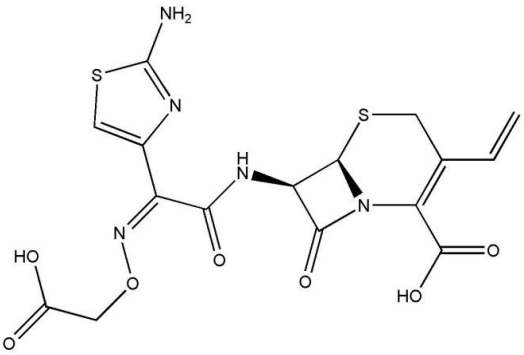
ABSTRACT

Objective: The objective of the current study was to develop rapid, accurate, reproducible, validated and economical Vierordt's Method for the simultaneous determination of CEF and MOX in tablet dosage forms. Method: This method of analysis was based upon the absorption of drugs at wavelength maximum of each other. Two wavelengths of 289.10 and 295.10 nm were selected which are the λ_{max} of two drugs for the development of the simultaneous equations. The absorbance of CEF and MOX were measured and the absorptivity values were determined at all the two selected wavelengths. Result & Discussion: The linearity was found to be 3-9µg/ml for CEF & MOX respectively. Recovery was in the range of 98 –102%; the values of standard deviation and% R.S.D. were found to be <2% shows the high accuracy of the method. The Limit of Detection and Limit of Quantitation were theoretically calculated which were found to be 0.0224 and 0.0678 for CEF and 0.0070 and 0.0214 for MOX respectively. Robustness and Ruggedness were also carried out and percentage RSD was found to be less than 2.0 %. The assay of Cefixime and Moxifloxacin was found to be 99.36% and 98.75%.The proposed method has been validated as per ICH guidelines and successfully applied to the estimation of CEF and MOX in their combined Tablet dosage form.

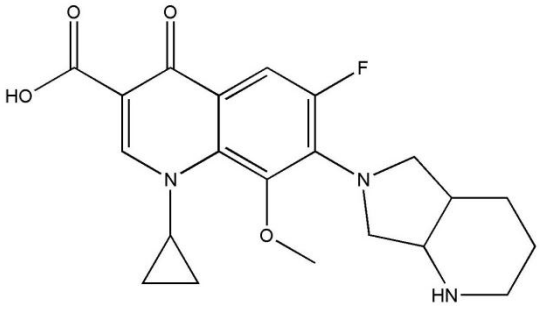
Key words: Cefixime; Moxifloxacin; Simultaneous equation method; ICH guidelines; UV-Spectroscopy.

Introduction

Cefixime trihydrate [(6R,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, 72-(Z)-[O-(carboxymethyl) oxime]trihydrate, (Figure 1) is semi synthetic, oral, third-generation cephalosporin antibiotic. Cefixime is active against a very wide spectrum of bacteria, act by inhibiting cell wall formation [1]. Literature reports many analytical methods for the determination of CEF in single and in combination with cited drug or other drugs, using UV spectroscopy [2,3] spectrofluorometry[4] HPLC[5-12] and HPTLC [13]. Moxifloxacin (1-cyclopropyl-7-(S, S)- S)-2, 8-diazabicyclo (4.3.0)-non-8-yl-6-fluoro-8-methoxy-1,4-dihydro-4-oxo-3-quinoline carboxylic acid hydrochloride, MOX, (Figure 1) is antimicrobial agent, it is fourth generation fluoroquinolone antibiotic. The mechanism of action involve inhibition of an enzyme topoisomerase II (DNA gyrase), which is essential for bacterial DNA replication. Several analytical methods have been reported for the determination of MOX in single or combination with other drugs or cited drugs in formulations and biological fluids, such as UV spectroscopic methods[14,15], Spectrofluorometry [16], RP-HPLC [17-20] and capillary electrophoresis[21,22]. The new combination of CEF and MOX is approved by Central Drugs Standard Control Organization (CDSCO) India, for the treatment of lower respiratory tract infections in adults. Simultaneous determination of these drugs is essential in each step of initial formulation development and screening stage of any solid dosage form. A fast and reliable method for the dissolution and release testing of CEF and MOX were highly desirable. However, there are no simple and rapid analytical methods to estimate the drug content in the combined forms. Hence there is an urgent demand to develop a simple and rapid method such as spectroscopic method to assess the drug content in this combination. The objective of the current study was to develop rapid, accurate, reproducible, validated and economical Vierordt's Method [23] for the simultaneous determination of CEF and MOX in tablet dosage forms.



Cefixime trihydrate



Moxifloxacin

FIGURE 1: Chemical structure of the analytes

EXPERIMENTAL

Apparatus

A double beam UV-visible spectrophotometer (Shimadzu, 1700), attached to a computer software UV probe 2.0, with a spectral width of 2nm and pair of 1cm matched quartz cell.

Materials and reagents

Authentic samples of Cefixime (CEF) was kindly provided by Cipla (Goa, India.) while Moxifloxacin (MOX) was kindly gifted from Dr. Reddy's laboratory, India. Double distilled de-ionized water was used throughout these experiments. Commercially available tablet dosage forms assayed in the study. Moxicip tablets containing 400 mg of moxifloxacin and suprax tablets containing 400 mg of cefixime were purchased from pharmacy.

Study of spectra and selection of wavelength

10 µg/ml solution of all three drugs were scanned over the range of 200-400 nm in 1cm cell against blank and the overlain spectra was observed (Figure 2) While studying the overlay spectra it was observed that Cefixime shows maximum absorbance at 289.10 nm, and Moxifloxacin shows peaks at 295.10 nm respectively. It was observed that there is no interference for each other at absorbance maxima and spectral characteristics are such that all two drugs can be simultaneously estimated by simultaneous equation method.

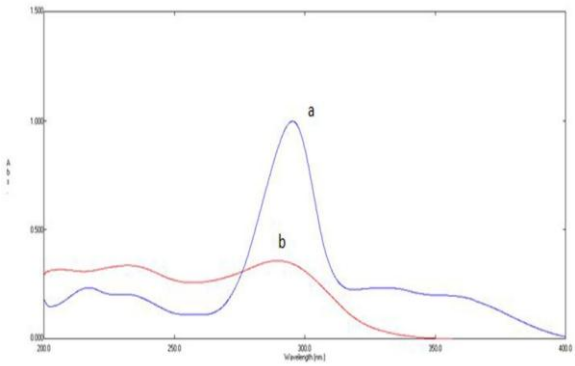


FIGURE 2 :UV Spectrum of cefixime and moxifloxacin

(a) Absorption spectra of 10µg/ml of MOX in distilled water (b) Absorption spectra of 10µg/ml of CEF in distilled water

Procedure

Simultaneous equation method

Standard stock solutions 0.1mg/ml of CEF and MOX were prepared by dissolving 10mg of each in 100ml volumetric flask separately and diluted to 100 ml with double distilled de-ionized water to get 6µg/ml of CEF and MOX.The absorption spectra of all the solutions were recorded between 200 to 400nm.The absorbance were measured for CEF and MOX at 289.10nm (λ_1)(maximum absorbance of CEF), 295.10 nm (λ_2)(maximum absorbance of MOX).The wavelengths 289.10 and 295.10 were selected for the formation of simultaneous equation. The absorbances were measured at the selected wavelengths. The molar absorptivity values were 407.62 at $\lambda_{1\ and}$ 390.73 at λ_2 for cefixime and 849.8 at λ_1 and 996.4 at λ_2 for moxifloxacin. The absorbance and absorptivity values were substituted in the following equation to obtain the concentrations.

$$CX = A2ay1 - A2\ ay1 - ay2/ax2\ ay1 - ax1\ ay2$$

$$Cy = A1\ ax2 - A2ax1/ay1\ ax2 - ay2$$

Where Cx and CY are the concentration of CEF and MOX, ax_1 and ax_2 are absorptivities of CEF at 289.10nm and 295.10nm, ay_1 and ay_2 are absorptivities of MOX at 289.10nm and 295.10nm, respectively. The absorptivity of each solution was calculated by using the following formula

Absorptivity = Absorbance/conc (gm/100 ml).

VALIDATION

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

Specificity

Specificity was studied by measuring the absorbance of CEF and MOX individually at 289.10 nm and 295.10 nm against the blank and comparing the absorbance of drug solutions to the blank. No interference was observed.

Accuracy

Accuracy was calculated as the percentage recoveries of blind samples of pure CEF and MOX and it indicated the agreement between obtained results and those accepted as true, detailed results are presented in Table 1. To ascertain the accuracy of the suggested methods, recovery studies were carried out by at three different levels (50%, 75%,100%,125% and 150% level).The results are presented in Table 1.

TABLE 1: Recovery studies for CEF & MOX

Concentration	Added amount		Amount Recovered		Amount Recovered (%)	
	CEF	MOX	CEF	MOX	CEF	MOX
50	5.0	5.0	4.96	4.99	99.20	99.80
75	7.5	7.5	7.48	7.47	99.73	99.60
100	10.0	10.0	10.20	9.80	102.00	98.00
125	12.5	12.5	12.20	12.60	97.60	100.80
150	15.0	15.0	14.60	14.80	97.33	98.66

Linearity

The calibration curves were plotted over a concentration range of 3- 9µg/ml for CEF and MOX respectively Table 2,3. Accurately measured standard solutions of each CEF and MOX (0.3,0.45,0.6,0.75,9 ml) were transferred to a series of 100 ml of volumetric flasks and diluted to the mark with distilled water. The absorbance of the solutions were measured at 289.10 and 295.10 nm against distilled water as blank. The calibration curves were constructed by plotting absorbance versus concentrations and the regression equations were calculated Figure 3,4.

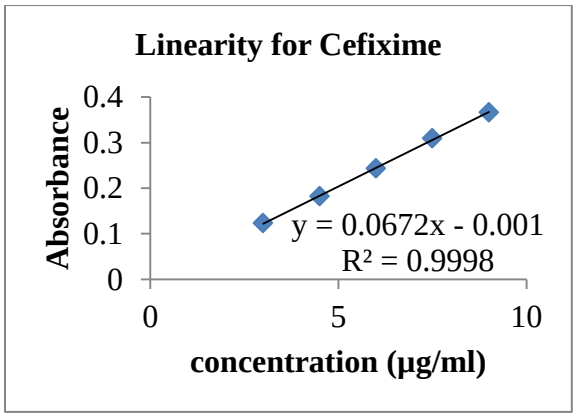


FIGURE 3: Linearity for Cefixime

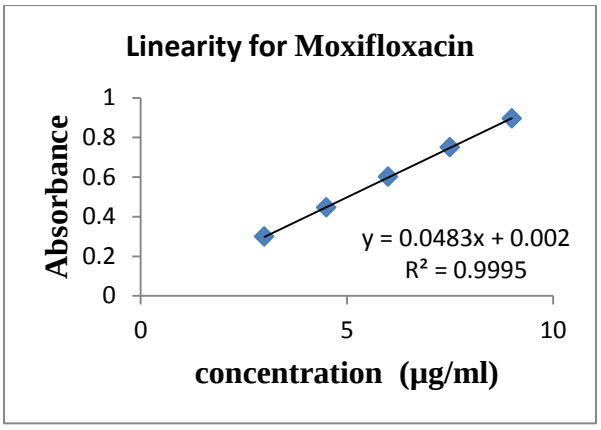


FIGURE 4: Linearity for Moxifloxacin

Precision

Inter and intraday precision studies were done by repeated measurements of the absorbance of standard mixed solution in triplicate in same day and single time for three days respectively by the proposed assay method without changing the method procedure. Percentage RSD was calculated and results are presented in Table 4.

TABLE 2 :Absorptivity value for Cefixime

Concentration	Absorbance	Absorptivity	Absorbance	Absorptivity
	λ_1-289.10	**λ_1-289.10**	**λ_2-295.10**	**λ_2-295.10**
3.0	0.123	410.00	0.115	383.33
4.5	0.182	404.44	0.173	384.44
6.0	0.243	405.00	0.237	395.00
7.5	0.309	412.00	0.297	392.00
9.0	0.366	406.66	0.359	398.88
Absorptivity for λ_1	407.62	Absorptivity for λ_2	390.73	

TABLE 3: Absorptivity value for Moxifloxacin

Concentration	Absorbance	Absorptivity	Absorbance	Absorptivity
	λ_1-289.10	**λ_1-289.10**	**λ_2-295.10**	**λ_2-295.10**
3.0	0.255	850.00	0.299	996.66
4.5	0.381	846.66	0.446	991.11
6.0	0.513	855.00	0.597	995.00
7.5	0.638	850.66	0.747	996.00
9.0	0.762	846.66	0.895	994.44
Absorptivity for λ_1	849.80	Absorptivity for λ_1	994.64	

TABLE 4:Precision results for CEF & MOX

Parameter	Sampling interval	CEF			MOX		
		Amount present (mg)	Amount present (%)	% RSD	Amount present (mg)	Amount present (%)	%RSD
Within day	0 hrs	395.6	98.9	1.05	396.8	99.2	1.31
	8 hrs	396.4	99.1	1.01	396.8	99.2	1.29
	16 hrs	397.6	99.4	1.14	395.2	98.8	0.40
Between day	Day1	399.2	99.8	0.90	394.4	98.6	0.55
	Day2	400.8	100.2	1.40	396.4	99.1	0.16
	Day3	408.0	102.0	0.75	398.0	99.5	0.45

Ruggedness

A study was conducted to determine the effect of variation in analyst to analyst, lab to lab and instrument to instrument in triplicate measurement as per the assay method.% RSD was calculated for each condition and results are presented in Table 5.

TABLE 5: Ruggedness results for CEF & MOX

Parameter	CEF			MOX		
	Amount present			Amount present		
	(mg)	(%)	(RSD)	(mg)	(%)	(RSD)
Analyst 1	396.8	99.2	0.99	394.0	98.5	0.54
Analyst 2	402.0	100.5	1.51	393.6	98.4	0.58
Instrument 1	400.0	100.0	0.68	394.4	98.6	0.98
Instrument 2	400.8	100.2	1.36	394.4	98.6	1.43
Lab1	404.8	101.2	0.60	402.4	100.6	0.89
Lab 2	401.6	100.4	1.59	404.0	101.0	0.84

Robustness Robustness study was carried out by changing the wavelength in ±1 nm from 289.10 nm to 295.10 nm and the results are presented in Table 6.

TABLE 6: Robustness studies (By changing the wavelength)

Analyte	Wavelength (±nm)	Amount present (mg)	Amount present (%)	%RSD
CEF	288.10	402.4	100.68	0.87
	290.10	407.2	101.87	1.11
MOX	294.10	397.6	99.42	0.43
	296.10	398.0	99.56	0.40

Stability

The stability of CEF and MOX standard and sample working solutions in distilled water during handling was verified by keeping them at room temperature for 3 days. No significant degradation was observed. The results are presented in Table 7.

TABLE 7: Stability data of stock solution

Day	CEF		MOX	
	Amount present (mg)	Amount present (%)	Amount present (mg)	Amount present (%)
1	405.6	101.4	406	101.5
2	397.6	99.4	410	102.5
3	401.6	100.4	405.6	101.4

Analysis of Commercial Formulation

Twenty tablets were weighed and powdered. The powder equivalent to 10 mg of each CEF and MOX were transferred into a 100 ml volumetric flask. Distilled water (10 ml) was added to it and sonicated for 15min. The solution was filtered through Whattmann filter paper No. 41, and the volume was adjusted up to the mark with distilled water. The above solution was suitably diluted with distilled water to get a final concentration of 6µg/ml of CEF and MOX. The absorbance of the tablet sample solution, i.e. A1 and A2 were recorded at 289.10 nm and 295.10 nm and ratios of absorbance were calculated, i.e. A2/A1. Relative concentration of two drugs in the sample solution was calculated using respective simultaneous equations generated by using absorptivity coefficients and absorbance values of CEF and MOX at these wavelengths.The results are presented in Table 8.

TABLE 8: Assay results for commercial formulation

Amount present (mg)	Amount Present (%)	Amount Present (mg)	Amount Present (%)
CEF		**MOX**	
397.2	99.3	396.0	99.0
398.4	99.6	396.0	99.0
393.2	98.3	395.6	98.9
404.8	101.2	392.4	98.1
394.8	98.7	395.6	98.9
396.4	99.1	394.4	98.6

DISSCUSSION

The proposed method was validated for precision, accuracy, specificity, linearity and range, limit of detection (LOD) and limit of quantitation (LOQ), robustness and ruggedness. Validation of the proposed method was carried out in accordance with the International Conference on Harmonization (ICH, 2005) guidelines. The linearity of the calibration plots was confirmed by the high value of the correlation coefficients (r^2 = 0.9998 for CEF and 0.9995 for MOX). Recovery was in the range of 98 – 102%; the values of standard deviation and% R.S.D. were found to be <2% shows the high accuracy of the method. The Limit of Detection and Limit of Quantitation were theoretically calculated which were found to be 0.0224 and 0.0678 for CEF and 0.0070 and 0.0214 for MOX respectively. Robustness and Ruggedness were also carried out and percentage RSD was found to be less than 2.0 %. The assay of Cefixime and Moxifloxacin was found to be 99.36% and 98.75%. Stability of CEF and MOX in distilled water was found to be stable up to 3 days at room temperature.

CONCLUSION

The Vierordt's Method has been successfully applied for simultaneous determination of CEF & MOX in combined sample solution, they were found to be accurate, simple, rapid and precise. Once the equations were constructed, analysis required only

measuring the absorbance values of the sample solution at the selected wavelengths followed by few simple calculations. The Proposed method was completely validated showing satisfactory data for all the method validation parameters tested. SE method comparably noted to be very efficient in every aspect. Unlike HPLC, with simultaneous equation method datas can be generated applying simple calculations so these methods can be easily and conveniently adopted for routine quality control analysis of these cited drugs.

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