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Developing and Optimising a New Spectrophotometric Method Using Orthogonal Polynomial Method for Simultaneous Estimation of Moxifloxacin and Cefixime in Tablet Formulation

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Abstract: Cefixime trihydrate is semisynthetic, oral, third-generation cephalosporin antibiotic. Cefexime is active against a very wide spectrum of bacteria act by inhibiting cell wall formation.Moxifloxacin is antimicrobial agent, it is fourth generation fluoro quinolone antibiotic.The mechanism of action involve inhibition of an enzyme topoisomerase 11(DNA gyrase),which is essential for bacterial DNA replication. Developing a new spectrophotometric method for simultaneous estimation of cefixime and moxifloxacin in tablet formulation facilitates to validate the method as per ICH guidelines. The orthogonal polynomial method is simple, accurate, economical, less time consuming and no prior separation is required for analysis.

Key words: Orthogonal polynomial function method · Simultaneous estimation · Moxifloxacin · Cefixime

INTRODUCTION

Orthogonal polynomial function method is a mathematical model [1] for the elimination of irrelevant absorption. This method is based upon the difference in the shape of the spectra of the components in a mixture in the selected wavelength range. The absorption spectrum can be represented in terms of orthogonal function and contribution to the coefficient of the given degree of orthogonal polynomial depends upon the shape of the spectrum and concentration [2]. Thus, a quadratic curve will contribute to coefficients of zero degree, first degree and second degree polynomials; as a linear curve will contribute to coefficients of zero degree polynomial and first degree polynomial and not to that of second degree polynomial. Hence, from the coefficient of second degree polynomial value of sample spectrum, calculated from the wavelength range in which the spectra of one component is linear and the other is quadratic or cubic, it is possible to estimate the content of the second component. Though it is a potential method for the analysis of multicomponent samples, the method involves complex calculations to select the right combination of degree of polynomial, number of points in the spectrum, interval between the points and optimization of these parameters [3].

Objective of the Study: The main objective of this study was to develop a new spectrophotometric method using orthogonal polynomial function analysis for simultaneous estimation of Cefixime and Moxifloxacin in tablet formulation and to validate the above method as per the ICH guidelines.

MATERIALS AND METHODS

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, Analytical balance and ultra sonicator were used in this study. Methanol (AR grade) was procured from Fischer scientific, Mumbai. The sample of moxifloxacin was procured from Micro Labs, India and Cefixime trihydrate was procured from Dr. Reddy's laboratory, India. The marketed tablet formulations Suprax®having 100 mg of CEF from Lupin, Mumbai and Moxif®having 100mg MOX from Torrent Pharmaceutical Industries Ltd., Ahmedabad were purchased from the local market.

Preparation of Standandard Stock Solutions: To prepare standard solution of cefixime and moxifloxacin10 mg of each drugs were transferred in two different 100 ml

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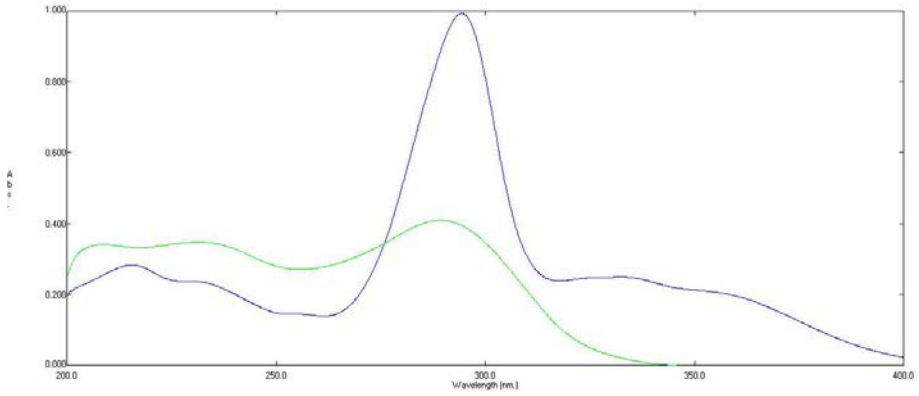

Fig. 1: UV spectrum of cefixime and moxifloxacin

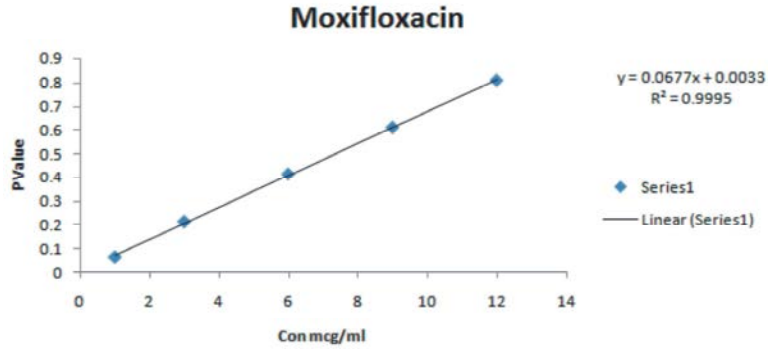

Fig. 2: Linearity study for Moxifloxacin

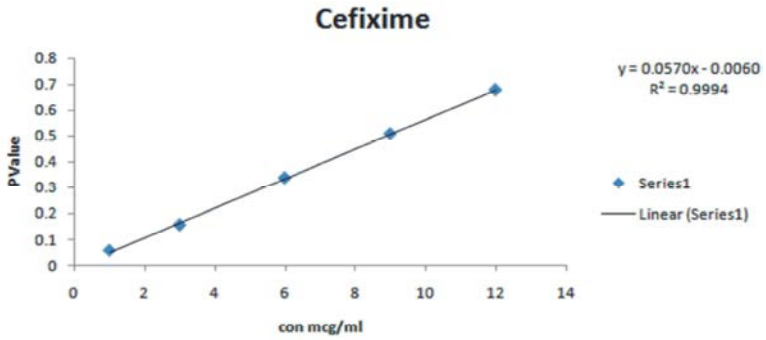

Fig. 3: Linearity study for CefiximeAugust 6, 2013

volumetric flask. Dissolve and dilute up to mark with methanol. From these stock solution, 0.6 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 cefixime (CEF) and moxifloxacin (MOX) of 6µg/ml each. The final concentration (6µg/ml) of CEF and MOX were recorded from 200-400nm (Fig.1.) and stored in ASCII format [4]. Five replicate solutions were prepared by individual weighing.

Calibration Curve: A calibration curve was plotted over a concentration range of 1-12µg/ml cefixime (CEF) and moxifloxacin (MOX). A series of dilutions of CEF and MOX were made with methanol to get concentrations of 1, 3, 6, 9 and 12 µg/mL (Fig 2&3). For each drug, 6 replicates were made by individual weighing. The spectra were recorded between 200 and 400 nm, using its export function of UV PC software and absorbance at respective ëmax (236.80 for cefixime and 306 for moxifloxacin) were noted. The calibration graphs were constructed taking mean polynomial values at ëmax on Y-axis and

concentration on X-axis. The regression coefficient and intercept on Y-axis were calculated. The spectra of the solutions were used for further linearity studies by orthogonal polynomial function method [5].

Analysis of Tablet Formulation: A total number of 20 tablets were weighed and powdered by a mortar and pestle. Quantities of the powder equivalent to 10 mg CEF and MOX were accurately weighed and transferred to 100ml volumetric flask. Weighed tablet powder was dissolved in methanol and ultrasonicated for 5min. Then the volume made upto 100ml of methanol and mixed well. Solution obtained was filtered through Whatmann filter paper no.42. Transfer 0.6 ml of solution to 10ml volumetric flask, made up to mark with methanol. The concentration of CEF and MOX were calculated using simultaneous estimation method. The solution was scanned between 350 and 200 nm in UV visible spectrophotometer.

Recovery Studies: Recovery studies was carried out by adding CEF and MOX to excipients of synthetic mixture at three different levels (50%, 100% and 150%) from the assay concentration. The spectra of resulting solutions were recorded and stored in ASCII format as described in linearity[5]

RESULT AND DISCUSSION

Optimization of Parameters for the Estimation of Cef and Mox: Convoluted graphs were generated, by executing the programme as described in software section of orthogonal polynomial chapter, for various combinations of degree of polynomial (2 or 3, that is quadratic or cubical), number of wavelength points in the spectrum (6 to 12) and interval between the wavelength points (2 nm to 9 nm) using the spectral data of standard CEF and standard MOX in ASCII format recorded as described in spectra of standard section [5]. In total, 112 convoluted graphs each for CEF and MOX were generated. Convoluted graph of CEF were compared with that of corresponding convoluted graph of MOX and the optimum conditions for orthogonal polynomial function method of analysis were selected taking the following points into consideration.

- The coefficient value (P value) was negligible for one drug and as high as possible for the other [6].
- The wavelength range, where there was steep rise in coefficient value of either drug was avoided and
- Whenever comparable results were obtained for more than one set of conditions, the one with less number of wavelengths was selected for further studies.

The details of the wavelengths satisfying the above conditions for each of the 56 convoluted graphs for the estimation of CEF and MOX(fig.4) by quadratic polynomial are given in Table (1).

Calculation of Coefficient of Polynomial: Coefficient of polynomial is directly proportional to the concentration of analyte and it can be calculated by using equation (1) for CEF and equation (2) for MOX, where the factors are those of six point quadratic polynomials obtained from the text of numerical analysis (Fisher and Yates).

Where, P_{CEF} and P_{MOX} are coefficients of polynomial of CEF and MOX, respectively and A is absorbance at respective wavelength. The calculation of coefficient could be carried out either manually from absorbance values at corresponding wavelength or directly from the corresponding output, when the software was executed [7].

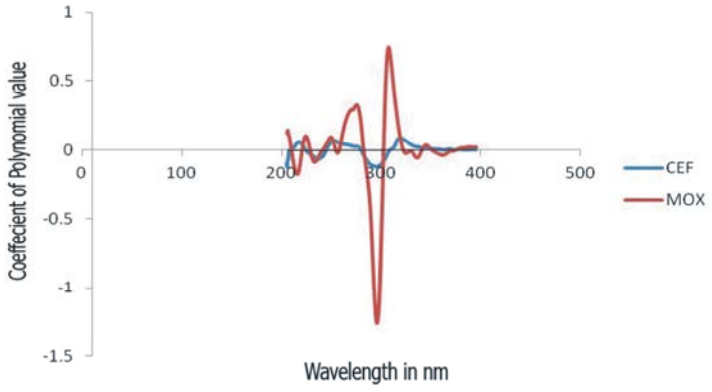

Fig. 4: Convoluted Graph For the estimation of Cefixime And Moxifloxacin

Table 1: Optimised Parameters For Orthogonal Polynomial Function Method Of Analysis

Drug	Degree of polynomial	Number of points	Wavelength (nm)
Cefixime	Quadratic	221.80,227.80,233.80,239.80,245.80 and 251.80	236.80
Moxifloxacin	Quadratic	304,306,308,310,312 and 314	309.00

Table 2: $P_{1\,cm}^{1\,\%}$ For Cefixime and Moxifloxacin

Con of CEF P Value (µg/mL)	P value for CEF	$P_{1\,c\,m}^{1\,\%}$ for CEF	Con of MOX P Value (µg/mL)	P value for MOX	$P_{1\,c\,m}^{1\,\%}$ for MOX
6.00	0.3397	566.16	5.99	0.4063	678.29
5.98	0.3324	555.85	6.01	0.4015	668.05
6.02	0.3358	557.80	5.98	0.4054	677.92
6.01	0.3296	548.41	6.03	0.4060	673.30
6.01	0.3331	554.24	6.02	0.4022	668.10
Mean		556.92			673.132
SD		6.441			5.017
% RSD		1.157			0.745

P value is coefficient of polynomial value

Table 3 : Analysis Of Tablet Formulation

	Cefixime			Moxifloxacin		
S.no	P value	Amt Present	% Amt Present	P value	Amt Present	% Amt Present
1	0.5674	99.23	102.20	0.6221	99.76	101.78
2	0.5621	98.30	101.08	0.6192	99.68	101.14
3	0.5682	98.92	102.50	0.6219	99.72	101.90
4	0.5682	99.16	102.34	0.6209	98.96	101.58
5	0.5679	99.02	101.97	0.6298	99.08	102.71
Mean		100.87				100.98
SD		68.3				54
%RSD		7.80				2.87

Label claim: Each tablet contains 100mg of cefixime and 100mg Moxifloxacin

Table 4: Recovery Study For Moxifloxacin And Cefixime

Level	MOX Added(mg)	Amount found (mg)	%Recovery	Mean ±SD	CEF Added(mg)	Amount found (mg)	% Recovery	Mean ±SD
50%	5	4.920	98.40%	4.955	5	5.023	100.4%	5.008
	5	4.934	98.68%	0.820	5	5.009	100.1%	0.220
	5	5.013	100.26%		5	4.993	99.86%	
100%	10	9.867	98.67%	9.892	10	9.943	99.4%	9.983
	10	9.976	99.76%	0.613	10	10.02	100.2%	0.327
	10	9.834	98.34 %		10	9.986	99.86%	
150%	15	14.94	99.60%	14.96	15	15.07	100.46	14.99
	15	15.03	100.20%	0.320	15	14.97	99.80	0.367
	15	14.92	99.46%		15	14.94	99.60	

Label claim: Each tablet contains 100 mg of Cefixime and 100mg Moxifloxacin

Determination of $P_{1\,cm}^{1\,\%}$: The $P_{1\,cm}^{1\,\%}$ is a constant, which represents the coefficient corresponding to the absorbance of 1% solution kept in 1 cm cell. It can be used for the calculation of concentration of sample similar to the use of $A_{1\,c\,m}^{1\,\%}$ in conventional spectrophotometry. Using the spectral data of standard solutions recorded, the coefficients of polynomials at the optimized conditions were computed for CEF as well as MOX. From the coefficient of polynomial values and the concentration of corresponding solution, $P_{1\,cm}^{1\,\%}$ *P* values were calculated and the results are given in Table (2).

Analysis of Cefixime and Moxifloxacin in Tablet Formulations: P values were calculated by executing the programme. The results are given in Table (3).

Recovery Study: Using the spectral data of solution prepared for recovery studies, P values were calculated by executing the programme [8] The MOX and CEF contents were calculated by recovery study and results were described in the Table (4).

UV spectra of 6 µg/mL solution of CEF in Methanol and 6 µg/mL solution of MOX in Methanol were recorded separately between 200 nm and 400 nm (Fig. 1).

These spectral properties make this an ideal combination for orthogonal polynomial function analysis. Analytical conditions were optimized by the help of the software. A total of 112 convoluted graphs were obtained each from the absorbance spectra of CEF and the absorbance spectra of MOX. Out of the convoluted graphs under 112 different conditions, 6 graphs exhibited considerable coefficient values for CEF and almost negligible coefficient values for MOX. All the five conditions could be used for the estimation of CEF [9] The one having the least number of wavelength points and lesser wavelength range was selected for further studies. Since it will be much easier for routine analysis particularly, when the calculations are done manually or by a calculator. Condition chosen for estimation of CEF was 6 point quadratic polynomial covering the wavelength range from 221.80 to 251.80nm. The UV spectrum of CEF was parabolic whereas the spectrum of MOX in the same wavelength range was a straight line. Due to this property of the spectrum, the coefficient of quadratic polynomial was negligible for MOX. Whereas CEF exhibited considerable coefficient values under the same conditions. Hence, under this condition, CEF can be estimated without interference from MOX [10]. A similar study was carried out to optimize the conditions for estimation of MOX. The optimized condition was 6 points quadratic polynomials covering the wavelength range from 304 to 314 nm. Orthogonal polynomial function method for MOX estimation was attempted since the estimation could be carried out conveniently using the software. Further orthogonal polynomial function method of analysis would estimate interference from formulations excipients, if any. Orthogonal polynomial function method is based upon the measurement of absorbance at many wavelength points whereas conventional spectrophotometric method is based upon measurement of absorbance at single wavelength i.e. at ëmax. Hence, a separate linearity study was carried out to establish the linearity of coefficient value with concentration although absorbance at single wavelength; i.e. respective ëmax of CEF and MOX exhibited linearity. Ideally in spectrophotometric method, the concentration of analyte is determined by comparing the absorbance of analyte with that of standard solution of known concentrations. Similarly, for the orthogonal polynomial function method of analysis, the concentration of analyte can be calculated by comparing P value of sample solution with that of standard solution of known concentration [11]. However, for routine analysis, it will be more convenient to calculate analyte concentration using some constant. In the case of conventional Spectrophotometric method, $1\%A1cm$ is the constant used for the calculation of analyte concentration. Similarly, $P_{1\ cm}^{1\ \%}$ is coefficient of polynomial, when concentration is 1% w/v and the measurement is taken using 1 cm cuvete. To establish $P_{1\ cm}^{1\ \%}$, the P value of 6 replicates standard solutions were determined and the average was calculated as $P_{1\ cm}^{1\ \%}$ separately for CEF and MOX and 556.92 and 613.132 respectively. The method was used for analysis of marketed formulations. Tablet assay result shows that mean percentage were 102.2% with RSD 0.490 for CEF and 101.82% with RSD 0.50 for MOX. The accuracy of the method was established by recovery study, as per ICH guidelines [12], at three different levels viz 50%, 100% and 150%. The recovery was within the limits, in all the three levels, prescribed by ICH guidelines.

Stability of the Analytical Solutions: Deviation from the mean initial absorbance was less than 2.4% for both MOX and CEF, which is well within the acceptable range (not more than ± 3%) indicating the stability of the analytical solutions.

CONCLUSION

Selected tablet formulation containing cefixime and moxifloxacin could be analyzed by orthogonal polynomial function method with required accuracy and precision. The proposed method is simple, accurate, economical, time consuming and no prior separation is required for analysis. Moreover, the present methods were rapid as compared to conventional spectroscopic methods and sophisticated chromatographic techniques; hence the proposed method can be used for the quality control of the cited drugs and can be extended for routine analysis of the drugs in their pharmaceutical preparations.

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