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Development and validation of UV spectrophotometry area under curve method for estimation of Cefixime trihydrate in pure and tablet dosage form

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Abstract
The aim of present work was to develop a simple, accurate and precise area under curve (AUC) spectrophotometric method for estimation of cefixime trihydrate and validation according to ICH Q2 (R1) guideline. The area selected for estimation of cefixime trihydrate was between 262.80 to 303.60 nm. The method represented correlation coefficient (R^2= 0.999) at concentration rang 5-25 µg/ml. The Satiafactory value of percent relative standard deviation for the intraday and inter-day precision indicates that method was precise. The recovery of the cefixime trihydrate was found upto 100.12 %. The developed methods can be successfully applied in routine work for the estimation of cefixime trihydrate in its pharmaceutical dosage form.

Keywords: cefixime trihydrate, area under curve (AUC), validation, ICH Q2 (R1) guideline

Introduction [1-5]

Cefixime Trihydrate is a third generation cephalosporin antibiotic. Chemically it is a 5-Thia-1-azabicyclo [4.2.0] oct-2-ene-2-carboxylic acid, 7-[(2 amino-4thiazolyl) {(carboxymethoxy) imino] acetyle} amino] 3ethynyl-8-oxo-trihydrate. Cefixime Trihydrate clinically used in the treatment of susceptible infections including gonorrhea, otitis media, pharyngitis, lower respiratory-tract infections such as bronchitis and complicated and uncomplicated Urinary Tract Infection. It is soluble in methanol and 0.1M NaOH, insoluble water and 0.1M HCl. Cefixime Trihydrate is effective against a wide spectrum of sensitive Gram–Ve, Gram+Ve and anaerobic bacteria pathogens including β-lactamase producing strains. The most commonly reported adverse reactions include coma,confusion, cough,dark urine, decreased urine output, depression, difficulty with swallowing , dizziness, fast heartbeat, feeling of discomfort, fever, general tiredness and weakness. The antibacterial effect of cefixime results from inhibition of mucopeptide synthesis in the bacterial cell wall.

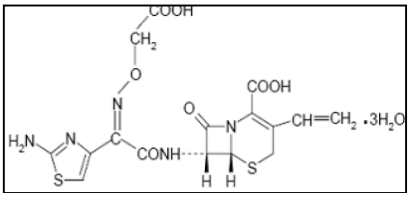

Fig 1: The Chemical structure of Cefixime trihydrate.

Literature survey reveals no AUC methods have been reported for the estimation of cefixime trihydrate in pure and tablet dosage forms. The proposed method was validated according to ICH guidelines.

Material and Method

Materials

Cefixime trihydrate (API) was obtained as gift sample from Dalas biotech India. A Tablet formulation 200 mg cefixime trihydrate (fixime 200) was procured from local market (Health care medical, boiser, Tarapur). Manufactured by wockhardt. Limited, Mumbai, Methanol (S.D. Fine Chemicals, Mumbai, India) was used as solvant.

Instruments Used

A double beam UV-VIS spectrophotometer (UV-1800, Shimadzu) connected to computer loaded with spectra manager software UV Probe 2.21 with 10 mm quartz cells was used. The spectra were obtained with the instrumental parameters as follows: wavelength range: 200-400 nm; scan speed: medium; sampling interval: 1.0 nm; band width (Δλ):10.0 nm; spectral slit width: 1 nm. All weights were taken on electronic balance (Model Shimadzu AUX 120).

Preparation of standard Stock solution

Accurately weighed 15 mg of cefixime trihydrate was transferred into 100ml volumetric flask and dissolve in methanol and diluted up to the mark with water to get a stock solution containing of 100 µg /ml.

Selection of wavelength range

The standard solution of 15 µg /ml was scanned between 400nm to 200nm in UV spectrophotometer against methanol as a blank after baseline correction. The wavelength range was selected around wavelength maxima (283nm). The final wavelength range selected between 262.80-303.60nm was selected on the basis of linear relationship between area and corresponding concentration.

Area under curve [6]

The area under curve method is applicable where there is no sharp peak or when broad spectra are obtained. This method involves the calculation of integrated value of absorbance with respect to the wavelength between the two selected wavelengths λ1 and λ2. Area calculation processing item

calculates the area bound by the curve and the horizontal axis. The horizontal axis is selected by entering the wavelength range over which area has to be calculated. This wavelength range is selected on the basis of repeated observation so as to get the linearity between area under curve and concentration. The above mentioned spectrums were used to calculate AUC. This wavelength range is selected on the basis of repeated observation so as to get the linearity between area under curve and concentration.

Preparation of calibration curve

Working solution were prepared from standard stock solution by further dilution with water to obtain the concentration of 5, 10,15,20,25 µg /ml respectively. These solution were scanned from 400 to 200nm and area under curve (AUC) was integrated in the range of 262.80-303.60 nm. The calibration curve was plotted between areas under curve against concentration. (Figure 2)

Assay of Tablet

Twenty tablets (cefixime trihydrate 200mg equivalent to 15 mg) containing 15 mg of cefixime trihydrate weighed, average weight calculated and triturated to fine powder and then weight equivalent 15 mg of cefixime trihydrate transferred into 100ml of volumetric flask and dissolve in methanol and diluted up to the mark with water to get a solution containing of 100 µg /ml from the 5ml was transferred to 50 ml volumetric flask and diluted up to the mark with water to get cefixime trihydrate solution containing 15 µg /ml of cefixime trihydrate.

Validation [7-11].

The developed method was validated as per ICH guidelines.

1. Linearity

The linearity was determine by using working standard solution between 5-25µg/ml. The spectrum of these solution were recorded and area under curve was integrated in wavelength range 262.80-303.60nm. Calibration curve of area under curve verces concentration was plotted after suitable calculation and simple linear regression was performed. (Show Table no.6 and Fig no.3)

2. Precision (Reproducibility)

The intr-day and inter-day precision of the proposed method was determined by analyzing the corresponding response 3 times on the same day and 3 different days.the concentration of standared solution of cefixime trihydrate 15 µg/ml. Area under curve of each of these solution was measured in the range of 262.80- 303.60nm. The result were reported interms of relative standard deviation (RSD) was calculated. (Table.7, 8, and 9).

3. Accuracy

The accuracy for the analytical procedure was determined at 80% 100% and 120% level of standard solution. Area under curve was measured in the range 262.80-303.60 nm and result were expressed in terms% recoveries. Three determination at each level were performed and %RSD was calculated. The results were tabulated in Table 10, 11, 12.

Result & Discussion

The calibration curve of cefixime trihydrate was performed and graph plotted concentration vs. area under curve. Show in fig.no.2

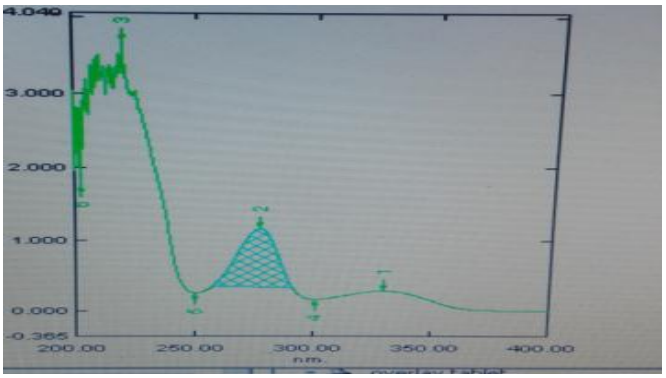

Fig 2: Spectrum of cefixime trihydrate for Area under curve (15 µg/ml)

Table 1: Calibration data of cefixime trihydrate for area under curve (5-25 µg/ml).

Concentration (µg/ml)	Area under curve
5	3.123
10	6.342
15	9.76
20	12.87
25	15.89

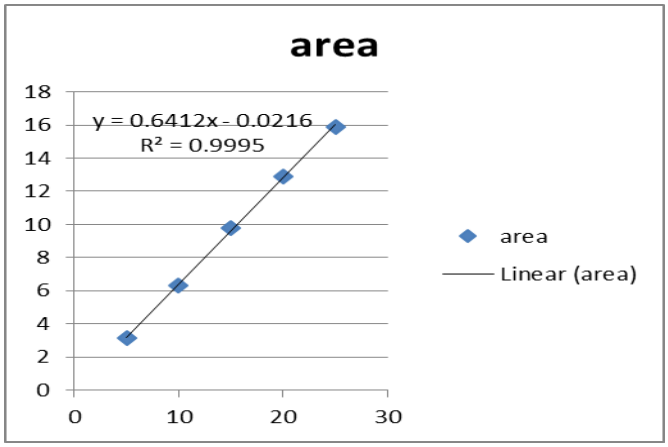

Figure 2: Calibration curve of cefixime trihydrate for AUC (5-25 µg /ml).

Analysis of Pure Drug

The standard solution of 15 µg /ml was scanned between 400nm to 200nm in UV spectrophotometer against methanol as a blank after baseline correction. The area under curve of solution was measured as shown in Table. 2

Table 2: analysis of pure drug

Sr. No	Amount Taken(Mg/Ml)	Auc	Amount Of Drug Found (Mg/Ml)	% Amount Found
1	15	3.1412	10.11	101
2	15	3.1101	10.00	100
3	15	3.901	9.980	99.80
4	15	3.123	10.10	101
5	15	3.897	9.87	98.87
6	15	3.1567	10.12	101

Table 3: Statistical evaluation of pure drug

% Mean*	±S.D.*	%RSD*	±S.E.*
100.46	0.01	0.018	0.006

*average of six determinations.

Analysis of Marketed Formulation

Twenty tablets (cefixime trihydrate200mg equivalent to 15mg) containing 15 mg of cefixime trihydrate weighed, average weight calculated and triturated to fine powder and then weight equivalent 15 mg of cefixime trihydrate transferred into 100ml of volumetric flask and dissolve in methanol and diluted up to the mark with water to get a solution containing of 100 µg /ml from the 5 ml was transferred to 50ml volumetric flask and diluted up to the mark with water to get cefixime trihydrate solution containing 15 µg /ml of cefixime trihydrate and result shown in Table. 4.

Table 4: Analysis of marketed formulation.

S. No	Amount Taken(Mg/Ml)	AUC	Amount of Drug Found (Mg/Ml)	% Amount Found
1	15	3.1876	10.24	104
2	15	3.15	10.14	101
3	15	3.1234	10.02	100
4	15	3.09	9.99	99.9
5	15	3.897	9.987	99.87
6	15	3.124	10.021	100

Table 5: Statistical evaluation of pure drug.

% Mean*	±S.D.*	%RSD*	±S.E.*
100.86	0.01	0.01	0.006

*average of five determinations

1. Linearity

Calibration curve of area under curve versus concentration was plotted after suitable calculation and simple linear regression was performed and results were shown in Table 6 and Figure 3.

Table 6: Calibration curve data of linearity cefixime trihydrate for area under curve (5-25 µg/ml)

Concentration (µg/ml)	Area under curve
5	3.123
10	6.342
15	9.76
20	12.87
25	15.89

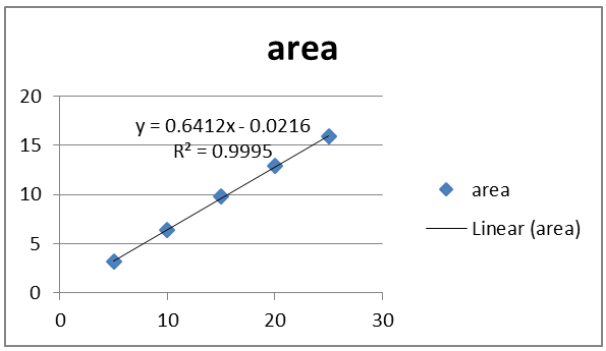

Fig 3: Calibration curve for cefixime trihydrate for AUC

2. Precision

Percent relative standard deviation (RSD) was calculated and results are shown in Table 7-9.

Table 7: Intra-day precision data of cefixime trihydrate *(n=6)

Sr. No	Amount taken (µg/ml)	Amount of drug found (µg/ml)	% Amount found
1	15	10.0700	100.70
2	15	9.9345	99.34
3	15	10.2088	102.08
4	15	10.04216	100.42
5	15	9.9317	99.31
6	15	9.9750	99.75

Table 8: Inter-day precision data of cefixime trihydarte *(n=6)

Sr. No	Amount taken (µg/ml)	Amount of drug found (µg/ml)	% Amount found
1	15	10.02	100.70
2	15	9.890	98.34
3	15	10.00	100.08
4	15	9.97	99.42
5	15	10.020	100
6	15	10.0040	100

Table 9: Statistical evaluation of inter-day and intra-day precision studies

Parameter	% Mean*	± S.D. *	%RSD *	±S.E.*
Interday	100.26	1.0526	1.0526	0.4297
Intraday	99.84	0.4978	0.490	0.2032

*average six determinations.

3. Accuracy

The results of accuracy are tabulated in Table 10-12

Table 10: Data of recovery study.

Level of Recovery (%)	Amt taken(µg/ml)	Amount of std. Added (µg/ml)	Area under curve	Total amt recovered (µg/ml)	% Recovery
80	10	8	5.523	17.34	100.88
80	10	8	5.545	17.35	100.46
80	10	8	5.589	17.38	98.76
100	10	10	6.2358	17.	100.56
100	10	10	6.234	18.23	100.90
100	10	10	6.298	19.45	100.90
120	10	12	6.734	20.23	98
120	10	12	6.785	20.09	100
120	10	12	6.7098	20.08	99.45

Table 11: Data of Statistical evaluation of recovery studies

Level recovery	%mean	±S.D.*	%RSD*	±S.E.*
80	100.54	1.00	0.011	0.578
100	100.83	0.05	0.56	0.0345
120	99	0.75	0.633	0.43

Summary Data of Validation Parameter

Table 12: summery data of validation parameter.

Sr. No.	Parameter	Area Under Curve Method
1.	Linearity range	5-25(µg/ml)
2.	Regression equation	Y= 0.642x-0.021
3.	Correlation co-efficient	R2=0.999
4.	Precision	
	Inter-day	1.0526%RSD
	Intra- day	0.490%RSD
5.	% recovery	0.011-0.6352%RSD

Discussion

The attempt was made to develop a simple and specific AUC spectrophotometric method for the determination of cefixime trihydrate in tablet dosage form. The correlation coefficient equation was y=0.641x-0.021 (R^2=0.999). The area under curve between 262.80-303.60 nm, x is concentration and R is correlation coefficient. The R^2 value as 0.999 as indicates that developed method was linear. The proposed method was found to be precise %RSD value for inter day and intraday precision were satisfactory. The drug at each of the good recoveries (100%). Hence, it can be said that this method was accurate. The result of the analysis of pharmaceutical formulation by the developed method was consistent with the label claim, highly reproducible and reliable.

Conclusion

The developed UV spectroscopic area under curve method provide simple ,precise and accurate ,quantitative analysis for estimation of cefixime trihydrate in pharmaceutical dosage form. No any spectrophotometric methods have been described for AUC estimation of cefixime trihydrate. Therefore, simple, fast and reliable area under curve spectrophotometric method was developed for the routine analysis of cefixime trihydrate.

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