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Spectrophotometric determination for the analysis of cefuroxime axetil in pharmaceutical dosage forms

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ABSTRACT

Three simple and accurate spectrophotometric methods have been developed for the estimation of Cefuroxime Axetil in bulk drug and its solid dosage forms. Method A was described about UV spectrophotometric measurement at maximum absorption of 277 nm while Method B related to formation of green colored chromogen by utilizing the oxidative coupling reaction between MBTH and Cefuroxime Axetil in presence of ferric chloride. It was measured at 624 nm against reagent blank. Method C was based on the formation of pink colored chromogen with PDAC and it was measured at 537 nm. The conditions of reaction pathway affecting the varied parameters were studied thoroughly and optimized. Under optimized conditions, the beer's law obeyed at the concentration of 5-25 µg/ml with correlation coefficient of 0.999 (n=5), 1-5 µg/ml with correlation coefficient of 0.9992 (n=5) and 2-10 µg/ml with correlation coefficient of 0.998 (n=5) for Method A, B and C respectively. The results of validated parameters like linearity, precision, accuracy, robustness and ruggedness compiled with those of official methods. There is no interference found from tablet excipients at the selected wavelength (Method B & C). The developed analysis could be considered successfully for the determination of Cefuroxime Axetil in pharmaceutical formulation.

KEYWORDS

Cefuroxime Axetil;
Spectrophotometry;
Validation.

INTRODUCTION

Cefuroxime Axetil (Figure 1), a semi-synthetic, second generative broad spectrum bactericidal antibiotic used to treat infections of gram positive and negative of aerobic, anaerobic and spirochetes[1]. Chemically, it was found to be (6*R*, 7*R*)-3-{[(aminocarbonyl)-oxy]-methyl}-7-{[(2*Z*)-2-(2-furyl)-2-(methoxyimino)acetyl]-amino}-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid[2]. It has been used as an attractive medicine for the conditions of Pharyngitis/Tonsillitis, Otitis media, Sinusitis, Acute and Chronic bronchitis, Uncomplicated skin and skin-structure infections, Uncomplicated urinary tract infections (UTI), Gonorrhea and Early Lyme disease (erythema migrans)[1,3]. The number of peer reviewed literatures was surveyed for the estimation of Cefuroxime Axetil in bulk dosage forms. It includes the methods of spectrophotometry[4-6], stability indicating by means of long term, intermediate and accelerated method[7], electrophoretic[8], HPLC[9-12],

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HPTLC[13], spectrofluorimetry[14], mercurimetric[15] and HPLC-MLC[16]. In the present study, we are aimed to develop a simple and sensitive UV and colorimetric method for the application in pharmaceutical dosage forms.

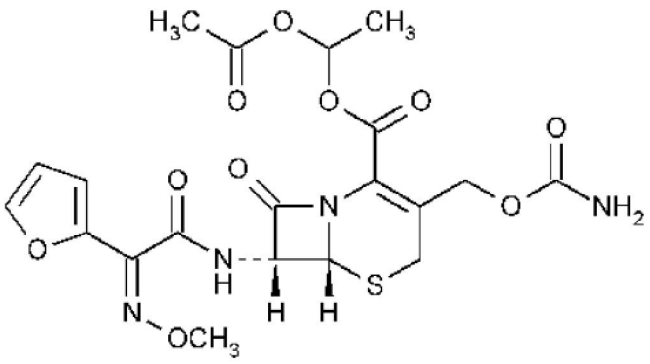

Figure 1 : Structure of Cefuroxime Axetil

EXPERIMENTAL PART

Materials and instruments used

The reference standard of Cefuroxime Axetil was provided as a gift sample by GlaxoSmithKline, Mumbai, India. Methanol, Hydochloric acid and Sulphuric acid was obtained from S.D Fine-Chem Ltd (Mumbai, India). 3-Methyl 2-benzothiozolidine hydrazone (MBTH), Para dimethyl amino cinnamaldehyde (PDAC) and ferric chloride was procured from Loba Cheme Pvt Ltd (Mumbai, India). For the whole experimental part, double distilled water and calibrated glass wares was used for the analysis. A Shimadzu UV 1800 spectrophotometer (Japan) with 1 cm used quartz cell was used for analysis. The data processing was monitored by using software of UV probe with a version of 2.32.

Preparation of standard stock solution

The standard stock solution of Cefuroxime Axetil (1000 µg/ml) was prepared by transferring 100 mg of drug into100 ml volumetric flask. To the flask add an amount of 40 ml of methanol and made to dissolve it completely and then the volume was made up to the mark using methanol. The working standard solution of (100 µg/ml) was prepared by transferring the 10 ml of above stock solution into 100 ml volumetric flask and then diluted up to the volume adjusted with double distilled water.

Method B

Preparation of 0.2 % w/v MBTH reagent

The amount of 0.2 gm of 3-methyl-2-benzothiazolinone hydrazone reagent (MBTH) or Besthorn's reagent was accurately weighed and transferred into the 100 ml volumetric flask. To this add 50 ml of methanol and dissolved it completely and then the remaining volume was adjusted up to the mark using the same.

Preparation of 0.5 % w/v $FeCl_3$ solution

The 0.5 % of FeCl3 was prepared by the weighed quantity of 0.5 gm of $FeCl_3$ was added to 100 ml volumetric flask and dissolved it using 50 ml of double distilled water. The remaining volume was adjusted using the same solvent.

Method C

Preparation of 0.5 % w/v PDAC reagent

Dissolved 0.5 gm of 4-dimethyl amino cinnamaldehyde (PDAC) in 50 ml of 5 M hydrochloric acid in an 100 ml volumetric flak and the remaining volume was made up to the mark by using methanol.

General procedure for the analysis of cefuroxime axetil

Method A

The volume of 0.5-2.5 ml was transferred into the series of 10 ml volumetric flasks from the working standard of 100 µg/ml and then diluted up to the mark using double distilled water. The resulted solutions were scanned under the range of 400-200 nm. The spectrum of Cefuroxime Axetil was observed at 277 nm against the blank.

Method B

To a series of 10 ml volumetric flaks, volume of 0.1 to 0.5 ml (1-5 µg) of drug solution was added by transferring the working standard of 100 µg/ml of Cefuroxime Axetil. To each flask, add an amount of 2.0 ml MBTH (0.2 % w/v) and 2.0 ml of ferric chloride (0.5 % w/v) and kept aside for 20 min for complete color development. The contents in each flask was made up to volume 10 ml with double distilled water and mixed well. The absorbance of green color chromogen was measured at 624 nm against the reagent blank.

Method C

The standard stock solution of Cefuroxime Axetil containing 100 µg/ml ranged from 0.2 to1.0 ml (2-10 µg) was transferred to a series of 10 ml volumetric flasks. To each volumetric flask, 2.0 ml of PDAC (0.5 % w/v) was added and heated on a heating mantle or water bath at the temperature of 40±5 ºC for 1 h and then kept aside for 20 min. for complete color development. The volume in each flask was made up to 10 ml with distilled water. The absorbance of pink colored chromogen was measured at 537 nm against the reagent blank.

Assay of tablet formulation

Twenty tablets of Cefuroxime Axetil were weighed accurately and ground into a fine powder. An accurately weighed amount of the finely powdered tablets equivalent to 100 mg of Cefuroxime Axetil was transferred into the 100 ml volumetric flask and then the volume was adjusted with methanol to get the concentration of 1000 µg/ml. By using, the above solution transfer 10 ml to 100 ml volumetric flask and diluted up to 100 ml using water to obtain the concentration of 100 µg/ml. From this, 1 ml was pipette out to 10 ml volumetric flask and then make up with using water. The resulted solution was scanned in the range of 400-200 nm (Method A). By utilizing the above concentration of 100 µg/ml transfer 1 ml to 10 ml volumetric flask added 2.0 ml MBTH (0.2 % w/v) and 2.0 ml of ferric chloride (0.5 % w/v) and then kept aside for 20 min for complete color improvement (Method B). Transfer 1 ml of 100 µg/ml to 10 ml volumetric flask add 2.0 ml of PDAC (0.5%) and boiled it for 1 h using heating mantle at 40±5 ºC then keep it for 20 min for complete color improvement (Method C). The resulted concentrations were calculated using linear regression equation.

RESULTS AND DISCUSSION

Method B

Reaction mechanism of oxidative coupling

In general, the colored chromogen formation occurred by the reaction of oxidative coupling. Cefuroxime Axetil forms a green colored chromogen by reacted with electrophilic intermediate of MBTH in presence of oxidizing agent ferric chloride[17,18]. The intense green colored chromogen that absorbed at 624 nm and it was stable for 4 h. The expected reaction mechanism was shown in Scheme-I

Optimized conditions for the chromogen formation

The preliminary studies were carefully studied to determine the optimized conditions of the reagent for the estimation of Cefuroxime Axetil. The reagent concentration, volume of the oxidizing agent used and the time interval greatly influences on the reaction procedure. Finally, the optimized conditions were maintained throughout the analysis.

Effect of concentration and volume of MBTH reagent

To assess the effect of reagent concentration, different concentrations of reagent varying from 0.1 to 0.5 % w/v was prepared and evaluated. The effects of reagent concentrations on drug were obtained by plotting concentration versus absorbance and from the results, it has been found that 0.2 % w/v of MBTH showed maximum absorbance. The influence of on the effect of volume was investigated using 1-5 ml of reagent. It was found to be maximum absorbance showed using 2 ml of reagent. Hence, 0.2 % w/v concentration of 2 ml MBTH was selected for the further studies and it was showed in Figure 2.

Effect of oxidant and its volume

The preliminary trails conducted using the oxidants of ferric chloride, potassium ferri cyanide and ceric ammonium sulfate. The oxidant of ferric chloride gave good intensity compared to others. The concentration of ferric chloride was selected by observing varied concentrations of 0.1-1.0 % w/v. The color intensity was investigated by adding varied volumes of 1-5 ml of ferric chloride. It was found that maximum absorbance of the green colored chromogen obtained with 2 ml of 0.5 % of ferric chloride. Therefore, it was used for further measurements.

Effect of reaction time, diluting solvent and order of addition of reagents

To ensure the completion of the reaction, the effect of reaction time was monitored up to 30 min. A reaction time of 20 min. at 25±5 °C was found to be the optimum to produce maximum absorption intensity of

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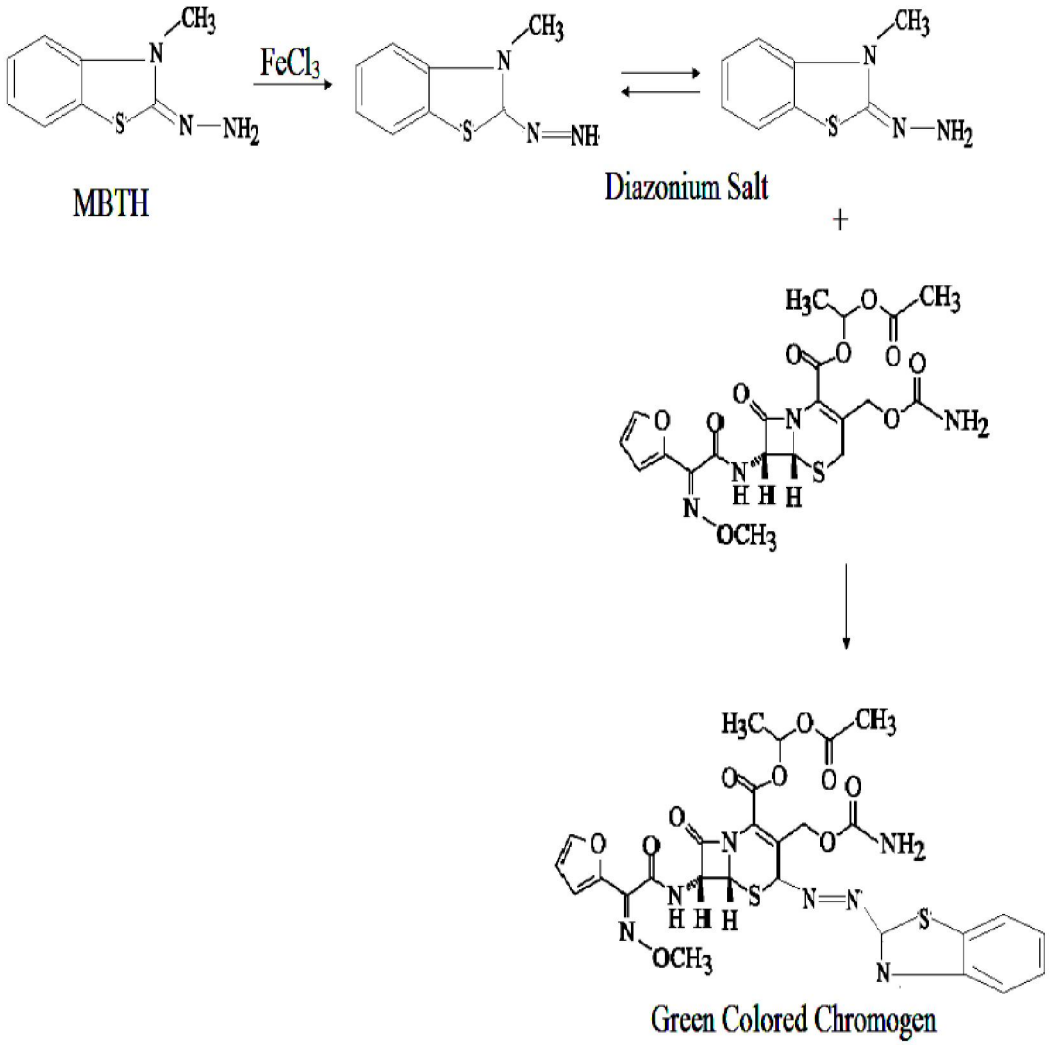


Scheme I : Oxidative coupling reaction of Cefuroxime Axetil with MBTH

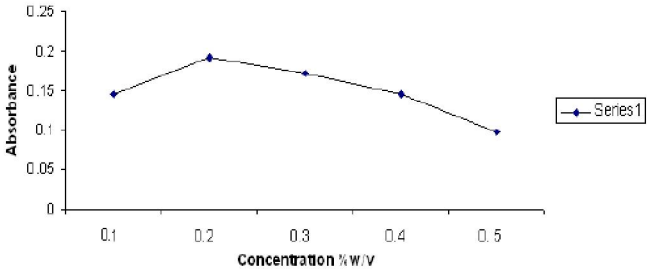


Figure 2 : Effect of concentration of MBTH on Cefuroxime Axetil

the colored species. The effect of dilution of the chromogen was studied using solvents *viz.* water and methanol. It was found that water was the best solvent as it gave the highest absorbance. The drug solution, reagents were added in different order and absorbance was observed.

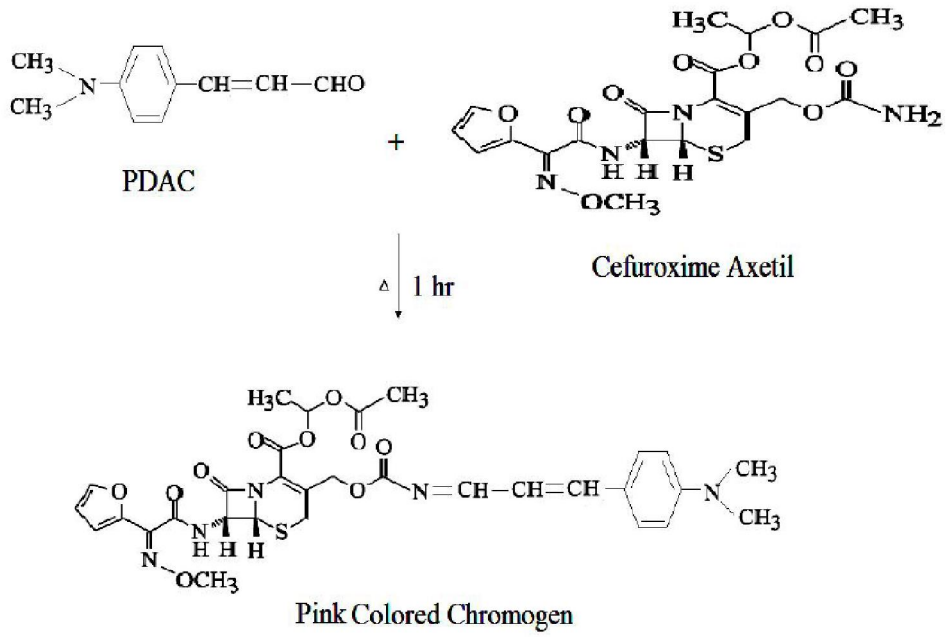
Method C

Reaction mechanism of Schiff's base formation

In thermal conditions the Cefuroxime Axetil reacts with PDAC reagent resulting to form a colored species. The colored product was based upon the formation of schiff's base. The drug may reduce the aldehyde portion of PDAC reagent produced the species which have a characteristic pink color with maximum wavelength of 537 nm. The stability of colored product was found it for 6 h. The mechanism of reaction was shown in Scheme-II.

Optimized conditions for the chromogen formation

Many trails are conducted to confirm the optimized procedure of PDAC reagent for determination of Cefuroxime Axetil. The levels of reagent concentration and its volume, effect on temperature and time interval effectively influence on the reaction procedure.



Scheme II : Schiff's base formation between Cefuroxime Axetil and PDAC

At last, the optimized conditions are used throughout the analysis.

Effect of concentration and volume of PDAC reagent

To assess the effect by adding the various concentrations of PDAC on absorbance of 6 µg/ml drug solution and it was examined. The volume of the reagent was ranged between 0.5-2.5 ml was noted. The results found to be 2 ml of PDAC reagent with 0.5 % concentration gave maximum absorbance (Figure 3). Therefore, the results are used throughout the analysis.

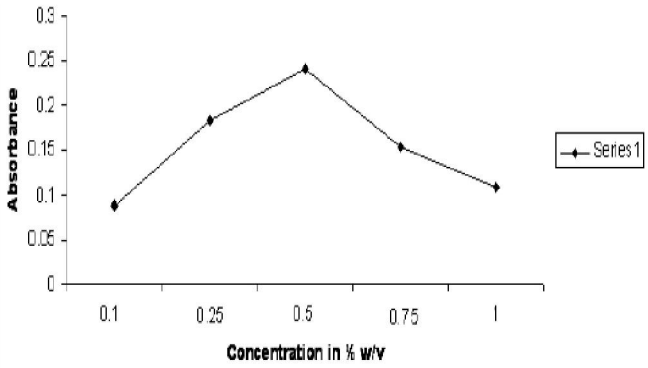


Figure 3 : Effect of concentration of PDAC on Cefuroxime Axetil

Method validation

The validated parameters conducted according to ICH guidelines i.e., of linearity, specificity, precision, accuracy and recovery studies[19,20]. The optical characters were calculated for the proposed methods and the results are showed in TABLE 1.

TABLE 1 : Analytical optical characters for Method A, B and C

Parameter	Method A	Method B	Method C
λ_{max} (nm)	277	624	537
Beer's law range (µg/ml)	5-25	1-5	2-10
Molar absorbitivity (L mole^{-1} cm^{-1})	5.24×10^{3}	3.126×10^{3}	4.234×10^{3}
Correlation coefficient, r	0.999	0.999	0.998
SD	1.60×10^{-3}	8.36×10^{-5}	2.51×10^{-5}
Slope (b)	0.178	0.193	0.185
Intercept	0.050	0.003	0.037
Sandell's sensitivity (µg/ml^{-1}/0.001A)	2.56×10^{-3}	5.05×10^{-3}	1.03×10^{-3}
Precision (% RSD)	< 2	< 2	< 2
Stability of chromogen	-	3 h	6 h
Reaction time	-	20 min	1 h
LOD (µg/ml)	0.02	0.05	0.07
LOQ (µg/ml)	0.07	0.17	0.21

Linearity

The linear regression analysis was conducted and the resulted wavelength showed a good correlation. The overlay spectrum and the linearity graph of Method A

were showed in Figure 4 and 5 while as Method B in Figure 6 and Method C in Figure 7 respectively.

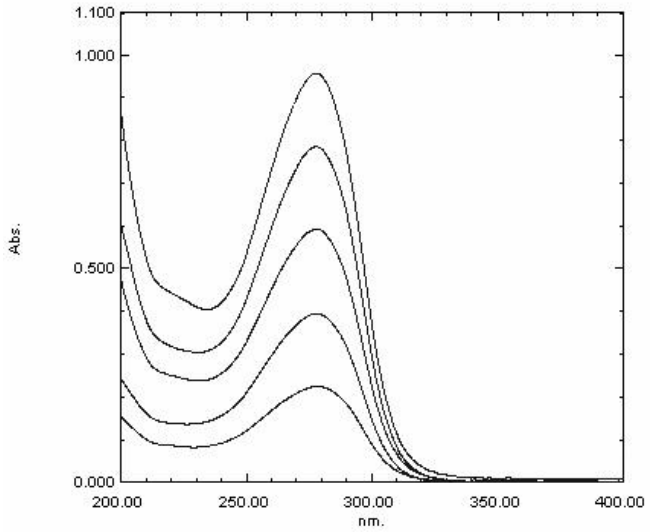


Figure 4 : Overlay spectrum of Cefuroxime Axetil (5-25 µg/ml)

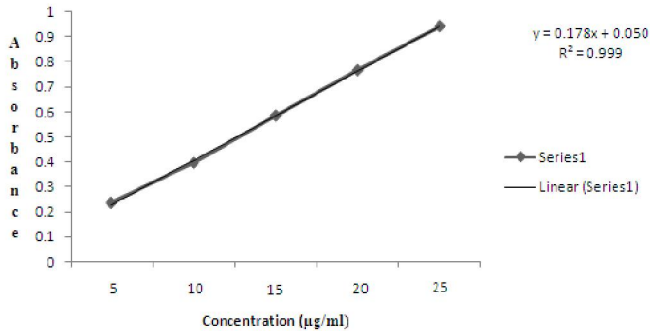


Figure 5 : Linearity graph of Cefuroxime Axetil (5-25 µg/ml)

Precision

The intra-day and inter-day precision was performed by carrying out 6 replicate injections of labeled concentrations of Cefuroxime Axetil. The % RSD showed that the proposed precision was good and it is summarized in TABLE 2.

TABLE 2 : Summary of precision data for Method A, B and C

Method	Precision	Concentration (µg/ml)	Average	SD	% RSD
A	Intra-day	15	0.5228	0.001602	0.30
	Inter-day	15	0.5180	0.001789	0.34
B	Intra-day	3	0.5818	0.00343	0.58
	Inter-day	3	0.5911	0.007083	1.1
C	Intra-day	6	0.5801	0.00405	0.69
	Inter-day	6	0.5961	0.008519	1.4

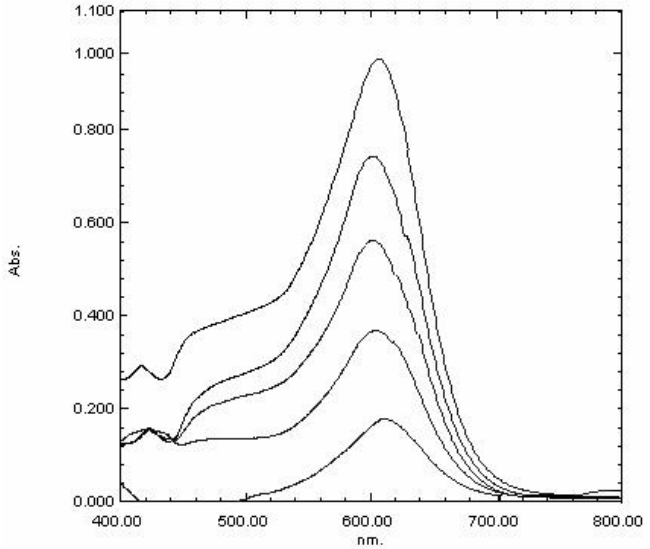


Figure 6 : Overlay spectrum of Cefuroxime Axetil with MBTH (1-5 µg/ml)

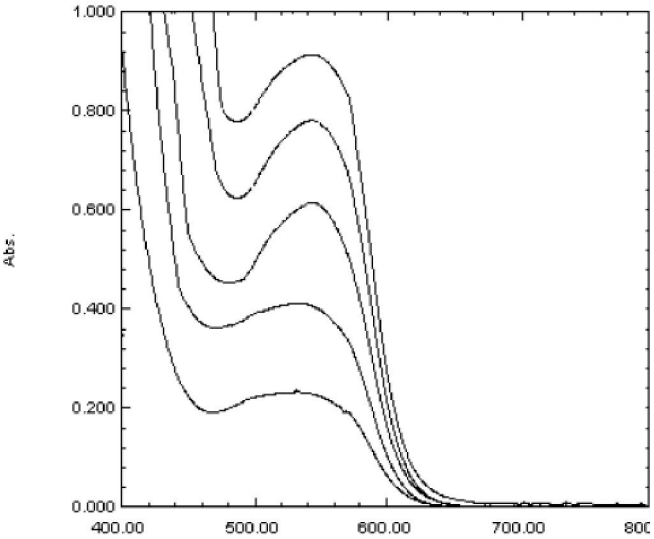


Figure 7 : Overlay spectrum of Cefuroxime Axetil with PDAC (2-10 µg/ml)

Accuracy

Accuracy study was carried out, by the standard addition technique in which a fixed quantity homogenous formulation was added with standard Cefuroxime Axetil at a level of 75 %, 100 % and 125 % and reanalyzed by the proposed method. The proposed method compiled with the reference and it was tabulated in TABLE 3.

Limit of detection (LOD) and limit of quantification (LOQ)

The LOD and LOQ were carried out by 3 replicate determination values of blank and slope (s) of the calibration. The calculated values coincide with the stan-

TABLE 3 : Recovery test of analysis for the proposed methods of A and B

Method	Level (%)	Amount added (mg)	Amount found (mg)	% Recovery	% RSD
A	75	75	74.88	99.84	0.11
	100	100	101.17	101.17	0.82
	125	125	124.76	99.80	0.41
B	75	75	74.21	98.94	0.74
	100	100	99.34	99.34	0.46
	125	125	123.78	99.02	0.69
C	75	75	75.17	100.22	0.16
	100	100	99.59	99.59	0.29
	125	125	124.63	99.70	0.20

dard and it was found to be within limits. The data showed in TABLE 1.

Ruggedness and robustness

The ruggedness of the method relative to each operational parameter was determined. By changing the parameters of varying the concentration of reagents (±0.02 %w/v), volume of reagents (±0.2 ml) and reaction time (0.5 min) was noted. The ruggedness of the proposed method relative to each operational parameter was examined with standard Cefuroxime Axetil. The results showed a mean % RSD of 0.53, which indicated that the developed colorimetric methods are rugged. A second analyst evaluated the Cefuroxime Axetil, by using freshly prepared standard and sample solutions. The results obtained were compared and found to have % RSD less than 2.

Specificity

The specificity of the proposed method was investigated by comparing the spectrum of reagent blank and chromogens formed by Cefuroxime Axetil. It was found that placebo did not possess any color and due that it does not have any absorbance in visible region. Hence, it could be concluded that there was no interference with the reagent. (Method B & C)

Application of proposed method to tablet formulation

The proposed method was applied to commercially available tablet formulation and the results are compared statistically by applying linear regression analysis. The calculated values showed that there is no significant difference between the proposed method and the reference method (TABLE 4).

TABLE 4 : Determination of Cefuroxime Axetil in tablet formulation

Method		Amount found (mg/tab)*	% Label claim*
A	Labeled amount (250 mg/tab)	250.12 ± 0.14	99.4 ±0.12
	% RSD	0.0938	0.1214
B	Labeled amount (250 mg/tab)	250.22 ± 0.11	99.6 ±0.09
	% RSD	0.0998	0.1624
C	Labeled amount (250 mg/tab)	250.12 ± 0.14	99.4 ±0.12
	% RSD	0.0938	0.1214

***Average of 3 determinations**

CONCLUSION

By the above considerations, proposed method was found to be simple, economical, selective and accurate. The present performed procedure can be considered as a general method for spectrophotometric determination of Cefuroxime Axetil in bulk and its solid dosage forms of quality control laboratories.

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