Spectrophotometric Determination of Cefotaxime via Diazotization Reaction in Pure and Pharmaceutical Samples

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Abstract

An accurate and sensitive spectrophotometric method has been developed for the determination of cefotaxime (CEF) in pure and pharmaceutical samples. The suggested method depended on the coupling reaction between diazotized cefotaxime and 3,5-dimethyl phenol (3,5-DMPH) in basic medium to form light orange, water soluble dye, that is stable and has a maximum absorbance at 497nm. The calibration graph was liner over the concentration range (1-70) µg.mL^{-1} with LOD of 0.750 µg.mL^{-1} and LOQ of. 2.740 µg. mL^{-1}, sandal sensitivity of 0.0526 µg. cm^{-2} . molar absorptivity 11328 Lmol^{-1} cm^{-1} . The stoichiometry composition was found by Jobs and mole ratio methods to the formation dye (1:1). The suggested method has been applied successfully to the determination of CEF in pure and pharmaceutical synthetic sample.

Key word: Spectrophotometric determination, Diazotized CEF, 3,5-dimethylphenol.

Introduction

Since antibiotics introduction in to medicine ,it's have been centered to modern healthcare. Their role has expanded from treating serious infection to prevent infection in surgical patient ,protecting cancer patient and people with compromised immune system and promoting growth and preventing disease livestock and other food animals[1]Cefotaxime sodium is among cephalosporin antibiotic wildly used in contemporary clinical practice[2]. Some of classified the third generation cephalosporin antibiotic was characterized by a broad antibacterial spectrum and resistance to beta-lactanase –producing organisms in addition to its antimicrobial activity [3].

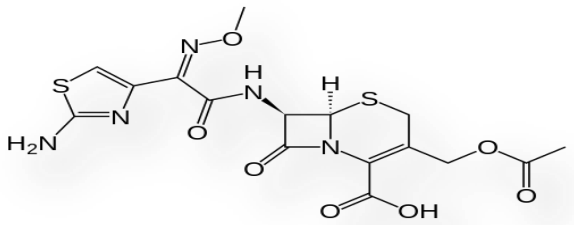

Figure (1)

A literate survey revealed that cefotaxime was determined in many spectrophotometric methods [4-10]

Azo dyes are characterized by the presence of the diazo linkage which brings two rings in to conjugated and thereby extending the ʎmax to the visible reign as a result of such dye formation lower detection limits are obtained while at the same time some measure of selectivity is afforded, also improving sensitivity therefore application of azo dyes on colorimetric determination of some drugs have been studied[11-15].In this work the free amino group of cefotaxime has been diazotization and became ready to enter coupling reaction with 3,5-dimethyl phenol and being used to colorimetric analysis of CEF in pure and pharmaceutical preparation samples. The aim of this work was to develop a validate, simple and economic spectrophotometric procedure for the determination of cefotaxime sodium by diazotization reaction.

Experimental

Apparatus

1- A shimadzu (Model 1601 UV-Visible spectrophotometer from shimadzu , Kyoto , Japan) the UV-VIS spectra of standard and solution were recorded in 1 cm quartz cell at wavelength of 497 nm.

2- Sartorius BL 210 electronic balance was used for weighing the samples.

Material and Reagents

All chemical used were of analytical reagent grade or Chemically pure grade and distilled water was used for all dilution of reagent and samples , expect 3,5-DEPH was dissolved in methanol . Standard powder of cefotaxime was kindly provided by Ibn Al –Bitar Research Center for Pharmaceutical Raw Material.

Standard Cefotaxime Sodium Solution (100µg.mL^{-1})

Standard CEF (100µg.mL^{-1}) was prepared by dissolving 0.05 g of pure drug in 10mL of distilled water and the volume was made up to the mark in 500mL volumetric flask .

Reagent Solutions

1- Sodium nitrite Solution (1%) was prepared by dissolving 1g of sodium nitrite (Merck) in distilled water and diluting to the mark in 100mL volumetric flask.

2- Sulphuric acid solution (1M) was prepared by diluting 1.388mL of (18M) of concentrated acid (BDH) with distilled water in 25 mL volumetric flask.

3- 3,5-Dimethyl phenol (Merck) (5×10^{-3}M): A Solution was prepared by dissolving (0.03)g in methanol and diluting to the mark with 50 mL volumetric flask .

4- Sodium hydroxide solution (1.5M) was prepared by dissolving (6)g in distilled water and diluting to the mark with 100mL volumetric flask .

Calibration curve procedures

A set of different concentration of CEF solution of (1-70) µg.mL^{-1}were prepared using the stock solution . Samples for each test were prepared by adding 0.5 mL of freshly prepared 1% $NaNO_2$ and 0.5mL of 1M $H_2SO_4$and left the solution 5min to complete the diazotization reaction for CEF in ice bath , then 1mL of 3,5- DMPH added to the resulting solution followed by 1mL of 1.5M NaOH to each flask with shaking . The solutions were making up to the mark with distilled water with volumetric flask of 10 mL and mixed well. The absorbance of light orange dye were measured at 497nm against the reagent blank.

Results and discussion

Absorption spectra

The primary test of the present method involved diazotization of CEF with sodium nitrite in acidic medium then reaction with3,5-DMPH in alkaline medium to form a colored product. The absorbance and λ_{max} of the colored product was measured against the reagent blank: Figure (2) shows that the maximum absorption was obtained at a wavelength of 497nm.

Optimization of reaction variables

The various parameters related to the colored product formation has been studied by varying the parameters one at a time and controlling another fixed and optimum conditions have been selected .

1- Effect of 3,5-dimethyl phenol volume

The influence of the different volumes of 3,5-dimethyl phenol on the absorbance of the colored product was investigated in the range between (0.25-3) mL of 5×10^{-3} M Figure (3). It was found that the maximum absorbance of light orange color was achieved with 1mL of the reagent. Above this value a decrease in absorbance was observed. Therefore, 1mL of 5×10^{-3} M 3,5-dimethyl phenol was used during the subsequent work.

2- Effect of sodium nitrite volume

This study involved testing different volumes (0.1-2.5)mL of 1% $NaNO_2$ on the maximum absorbance of the colored product . The high absorbance was attained when the concentration of $NaNO_2$0.5mL of 1% $NaNO_2$. Above this value a decrease in the absorbance reading occurred as in Figure (4).

3- Effect of different acids

The effect of different acids solution with concentration of (1M) on the absorption intensity of the colored dye formed was investigated . Four types of acids namely: nitric acid, sulphuric acid, hydrochloric acid and acetic acid were tested and the results are listed in Table (1) .

As can be seen it was found that sulphuric acid shows the maximum absorption intensity of the colored product, subsequent, the better absorbance was achieved with 0.5mL of(1M) H_2SO_4 .

4-Effect of different bases

The effect of different bases solution with concentration of (1.5M) was studied. Three types of bases : potassium hydroxide , sodium bicarbonate and sodium hydroxide were tested . The result listed in Table (2). It was found that the sodium hydroxide shows a high intensity of the colored product ,then it was selected to finished the work . it appeared that 1mL of (1.5 M) NaOH gave the best result.

5- Effect of diazotization reaction time

The optimum time for the diazotization reaction for CEF was studied at different periods of time, absorbance values recorded at different intervals ranging from immediate to a waiting period of 25 min. The reaction completed in 5 min as shown in Table (3).

6- Effect of reagent mixing order

Effect of two different orders of components addition on colored product formation was studied by changing the order of reactants two times as shown in Table(4). From the result a best maximum intensity was followed in the subsequent experiment.

7- The stability

The stability study was carried out by measuring the absorbance of the colored product at different time . The color of the solution was nearly stable up to 1440 min as shown in Table (5). The absorbance spectra of colored product under optimum conditions was recorded and showed a maximum absorbance at 497nm against reagent blank as shown in Figure (9).

Calibration curves and analytical data

Employing the optimum condition, the measured absorbance at 497nm versus different standard concentration of (CEF) were plotted to construct a calibration curve. The linearity of the obtained plot of the (CEF) was in the concentration range (1-70)µg.mL^{-1} as shown in Figure(5) .The statistical treatment of the analytical data are summarized in Table (7).

Evaluating the linear regression

Evaluating the linear regression of suggested method is done by of plots the standardized concentration residuals vs the predicated concentration of the calibration curve . The residuals for CEF in all points appear to be randomly distributed around zero as show in Figure (6).

Composition of the product

Jobs and mole ratio method have been used in the determination of stoichiometry of the colored product. CEF and 3,5-DMPH solution of 2×10^{-2}M were prepared and mixed in various molar ratio in 10 ml volumetric flasks with suggested procedure, the absorbance was measured at 497 nm. The graph of the results obtained as in Figures (7)(8) gave maximum at mole ratio $X_{max}=0.5$,$X_{max}=1$ in Jobs and mole ratio method respectively , showed that 1:1 (CEF) to 3,5-dimethyl phenol ratio is obtained .The probable structure of the final dye suggested in scheme(1).

Precision and accuracy

The precision and accuracy of the proposed method was tested by analyzing five replicate samples of (CEF) in three different levels (within Beer's law range). The result listed in Table (7) indicates an acceptable accuracy and precision to suggested work.

Interference study

The extent of interfering by some excipients which often accompanied pharmaceutical preparation was studied by measuring the absorbance containing 10µg.mL^{-1} of (CEF) and different volumes (0.1,0.3,0.5)mL of (1000) µg.mL^{-1} of excipient in final volume of 10 mL. The results in Table (8) show that the suited excipients don't interfere in the determination of (CEF) .

Application in pharmaceutical forms

In order to demonstrate the applicability of the proposed method for the determination of (CEF), the method was applied to different manufacturing source containing (CEF):

1-Cefotaxime (injection 1000 mg) Roth (Germany).
2-Bektasime (injection 1000 mg) mn. Pharmaceutical (turkey).
3- Cefotaxime (injection 500 mg) LDP (Spain).

To apply the suggested method on pharmaceutical formulation, an accurately weighed (0.01)g of CEF powder injection dissolved with 10 mL of water then transferred into 100mL volumetric flask, and completed to the mark with distilled water to prepare 100 µg.mL^{-1}of CEF. Further appropriate solutions of pharmaceutical preparation were made up by simple dilution with distilled water in find volume of 10 Ml, the results are shown in Table (9).

Standard Additions

Application in synthetic drug sample by standard addition to increase the insurance, the proposed spectrophotometric method was applied for determination of CFE in synthetic drug sample (prepared by weight 1 g from each : glucose , starch , acacia , magnesium setrate and lactose) mixed well and weight 0.01 g from the mixture dissolved in ethanol and complete the volume to the mark with distilled water to 50mLvolumetric flask). Following the standard addition technique. Good recovery of the drug present in studied sample indicates that no interference from the matrix affect the determination of CEF. Figure (10) shows the standard additions plot and Table (10) shows the result of recovery for the method.

Comparison of the methods

Table (11), shows comparison between the proposed method and that of another literature spectrophotometric methods throughout some measured analytical parameters.

Conclusions

The proposed methods were found to be very simple, rapid, low cost and fairly selective than other method. The proposed method was applied to the analysis of cefotaxime in injection, and can be used for the routine analysis of commercial formulations. The presence of free amino group in cefotaxime makes it possible for diazotization reaction with sodium nitrate in acidic media then coupling with a suitable reagent: 3,5-dimethyl phenol in alkaline media to give a light orange azo dyes. The accuracy and precision of the proposed method were tested by analyzing five replicate of CEF for three different concentrations, the values of RSD% and relative error $E_{rel.}$ % indicated high accuracy and precision.

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Table (1): Effect of different acids on coupling reaction

Acid(1M)	Abs.
HNO_3	0.213
HCl	0.235
H_2SO_4	0.232
CH_3COOH	0.004

Table (2): Effect different bases on coupling reaction

The Base	Abs.
KOH	0.225
Na_2CO_3	0.231
NaOH	0.239

Table (3):Effect of diazotization reaction time on coupling reaction

Time(min.)	Abs.
0	0.181
5	0.245
10	0.219
15	0.210
20	0.196
25	0.173

Table (4):The change of reactant addition order

No.	Squence	Abs.
1	Azo compound. + Base + reagent	0.004
2	Azo compound + reagent + Base	0.246

Table (5): The stability

Time(min.)	Abs.
0	0.181
5	0.245
10	0.219
15	0.210
20	0.196
25	0.173

Table (6): Optical characteristics and statistical data

Parameter	**Value**
λ_{max}(nm)	497
Product color	Light Orange
Regression equation	y = 0.0192x + 0.058
Standard Errors of Regression line	0.0043
Correlation coefficient(r)	0.9999
Coefficient of determination (R^2)	0.9999
Beer s law range (µg mL^{-1})	(1-70)
Limit of detection (µg mL^{-1})	0.750
Limit of quantitation (µg mL^{-1})	2.740
Sandell s sensitivity (µg cm^{-2})	0.0521
Molar absorptivity ($L.mol^{-1}.cm^{-1}$)	11328
Nature of azo dye structure	1:1

Table (7): Evaluation of the accuracy and precision of the proposed method

Compound	Amount taken (µg.mL^{-1})	Amount found (µg.mL^{-1}) *	Relative error%	R.S.D%
Cefotaxime	3.00	3.03	1.06	1.94
	8.00	8.13	1.68	0.72
	10.00	10.04	0.47	0.58
	20.00	20.05	0.25	0.29

***Average of five replicate**

Table (8): The relative error value for 10µg.mL^{-1}of CEF in the presence of different excipients

Foreign Compound 1000µg.mL^{-1}	Amount added (mL)	Relative error%
Glucose	0.1	0.52
	0.3	1.04
	0.5	2.60
Lactose	0.1	1.04
	0.3	2.08
	0.5	3.13
Vanillin	0.1	1.04
	0.3	2.60
	0.5	3.64
Starch	0.1	0.52
	0.3	1.04
	0.5	3.13
Magnesium citrate	0.1	0.52
	0.3	1.56
	0.5	2.60
Acacia	0.1	0.52
	0.3	1.56
	0.5	3.13

Table (9): Recovery data obtained by application of the present method in drug formulation

Pharma. preparation	Amount taken(µg)	Amount found (µg)	Recovery%
Cefotaxime (injection 1000mg)Roth (Germen)	5.00	5.05	101.04
	10.00	10.10	101.04
Cefotaxime (injection 1000mg) mn. Pharmaceuticals (Turkish)	5.00	4.95	99.10
	10.00	9.96	99.64
Cefotaxime (injection 1000 mg) LDP(Spain)	5.00	4.96	99.28
	10.00	10.05	100.52

Table (10): Recovery of data obtained by standard addition method for CEF in synthetic drug samples

Sample CEF. With 200µg.mL-1 of (Aca., Magnesium citrate, Starch, Van., Lac., Gluc.)	Conce. Taken µg.mL^{-1}	Conce. Found*µg.mL^{-1}	Recovery%
	10.00	9.90	99.01

***Average of three replicate**

Table (11): Analytical parameter for the determination of CEF by the proposed method comparing to another methods

Technique used	D.Lµg.mL^{-1}	Liner rangeµg.mL^{-1}	Recovery%	Ref.
Spectrophotometric	3.00	3.80 – 114.60	98.90–101.20	16
Spectrophotometric	0.74	1.00– 20.00	-	17
Spectrophotometric	14.41	10.00– 90.00	100.76-101.08	18
HPLC	0.14	1.00-20.00	-	19
Fluoresces	0.36	1.00– 24.00	103.47	20
Present method	0.75	1.00-150.00	99.00-101.28	-

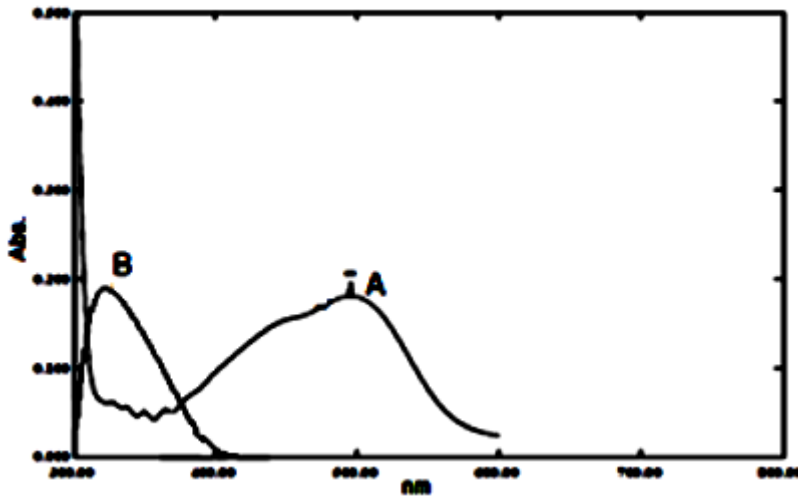


Figure (2) :Absorption spectra of :(A)10µg.mL^{-1} CEF against reagent blank,(B)blank solution

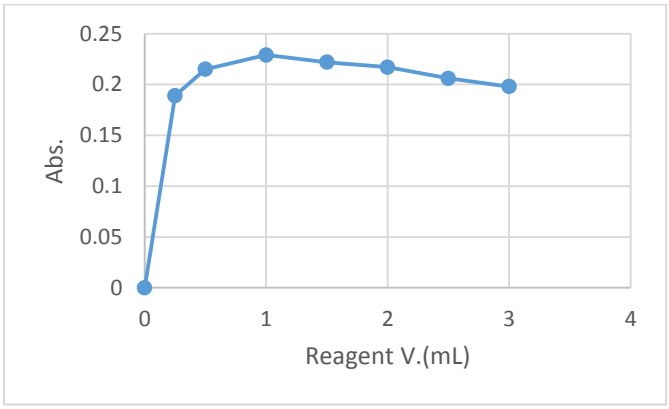


Figure (3): Effect of the volume of 3,5-dimethyl phenol on color development

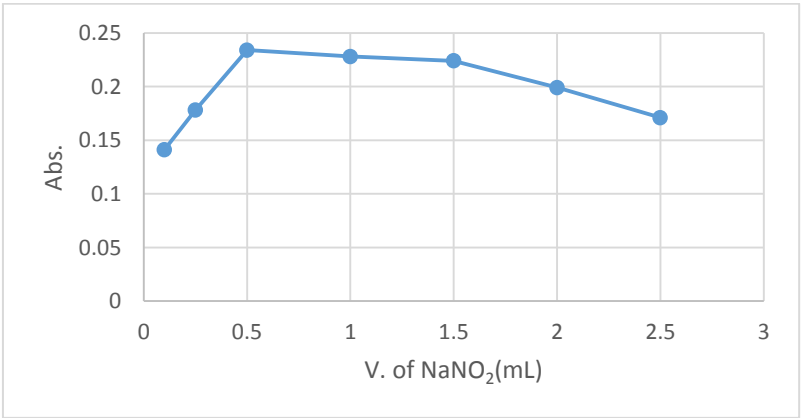


Figure (4): Effect of Sodium nitrite Volume on color development

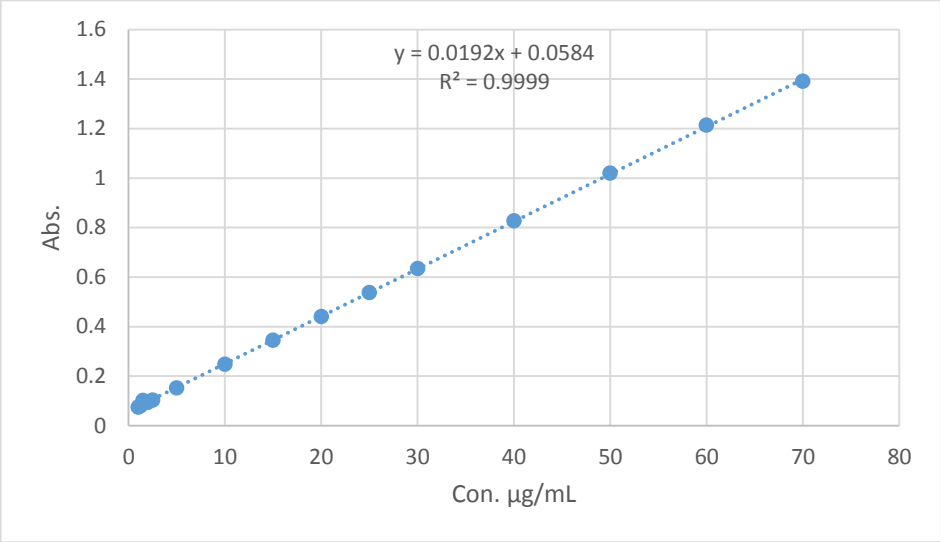


Figure (5): Calibration curve for the determination of CEF under optimum condition

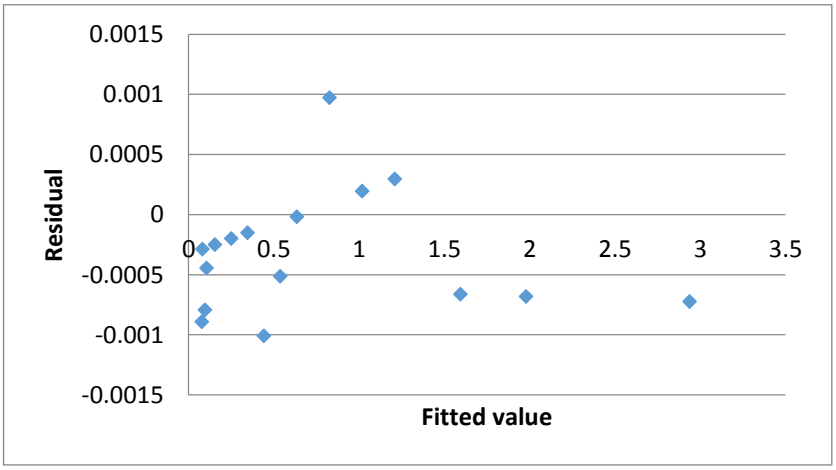


Figure (6): The residual error of liner regression model

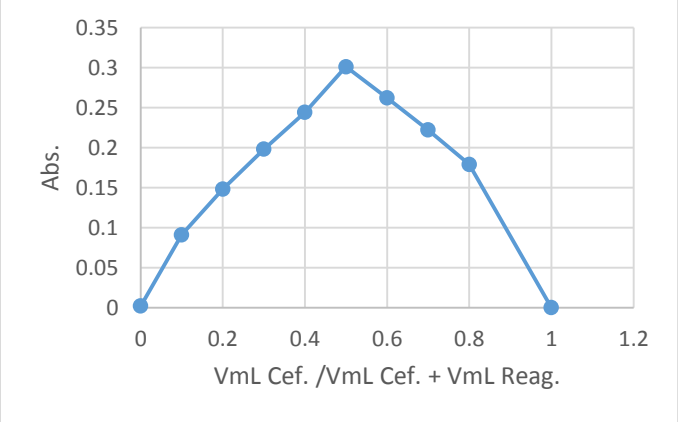


Figure (7): Job's curve of 2×10^{-2}M CEF and 3,5-DMPH

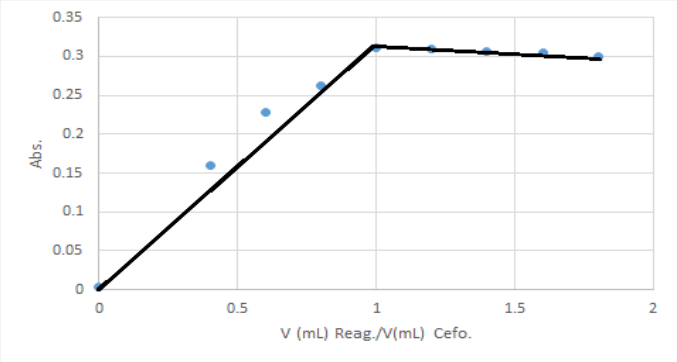


Figure (8) :Mole ratio of 2×10^{-2}M for each CEF and 3,5-DMPH

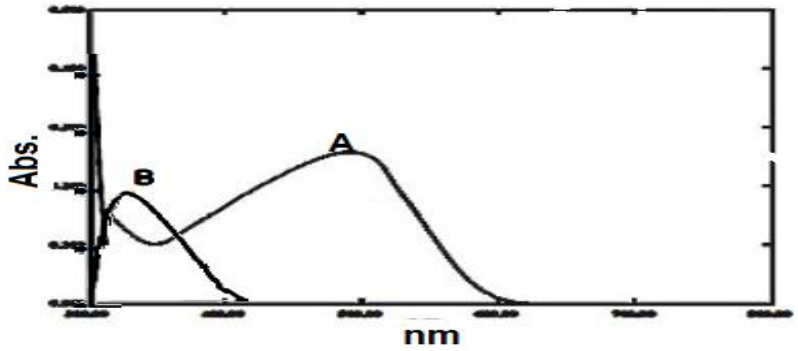


Figure (9): Absorption spectra of,(A) 10µg.mL^{-1}of colored compound under optimum condition, (B) blank solution against solvent

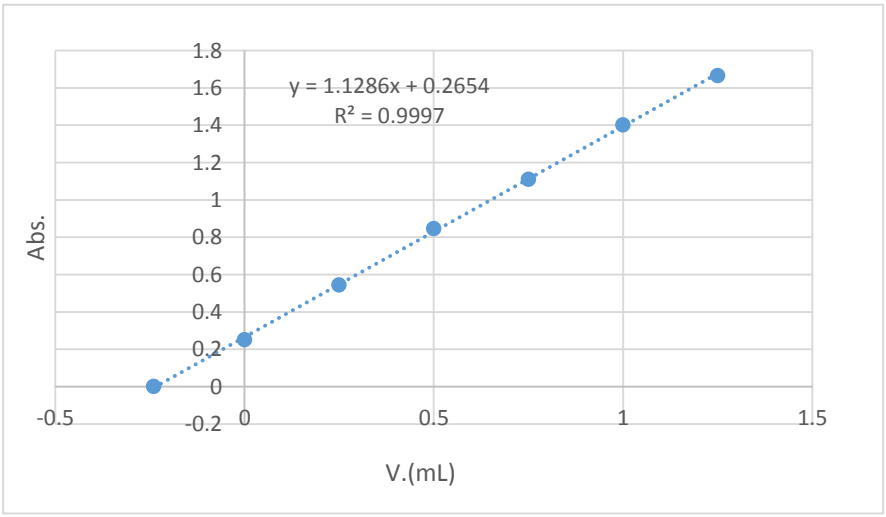
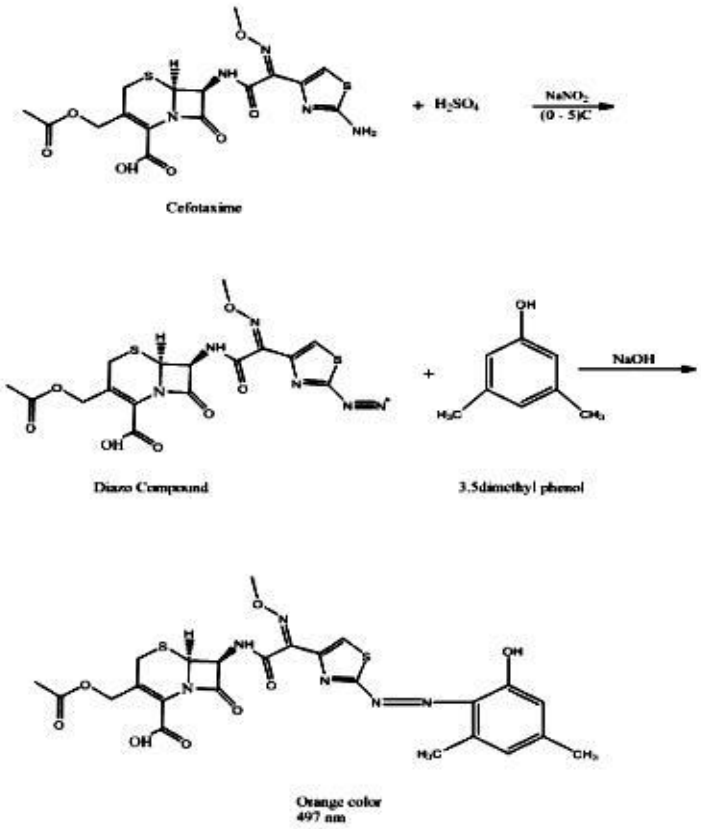


Figure (10):Plot of standard addition method for application suggested study for the determination of CEF



Scheme (1): The suggested reaction mechanism between CEF and 3,5-DMPH