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Spectroscopic Estimation of Cefepime by using Batch, Cloud Point extraction and Flow Injection Analysis methods

Hawraa Jumaa Hashim, Nisreen Kais Abood* and Olfat A. Nief

Department of Chemistry, College of Science, Mustansiriyah University Baghdad, Iraq

Abstract

New simple sensitive spectrophotometric methods are developed for determination of cefepime (CFM) in pure and Pharmaceutical Formulations. The first method conversion primary amine to azo-dye by react cefepime with sodium nitrite and hydrochloric acid followed by coupling with 2,6-dimethylphenol in alkaline medium to obtain a stable reddish-orange colored dye at λ_{max} 515nm. The concentration ranges 2-50 µg / mL, Beer's law is obeyed, correlation coefficient was 0.9996, molar absorptivity was 0.826×10^{4}L.mol^{-1}.cm^{-1} and the detection limit was 0.192 µg/mL. Cloud point extraction (CPE) second method to estimation a trace amount in aqueous solution product from diazotization and measuring with a UV-visible spectrophotometer at λ_{max} 515 nm. The concentration range obeyed the Beer's law was 0.25-10 µg / mL, correlation coefficient was 0.9997, molar absorptivity was 1.169×10^{5}L.mol^{-1}.cm^{-1}, detection limit was 0.027 µg/mL, Pre-concentration factor was 25 and Distribution coefficient(D) was 332.35. Last methods flow injection analysis it's simple for estimation the cefepime .The concentration ranges 1-150 µg / mL, Beer's law is obeyed, correlation coefficient was 0.9997, molar absorptivity was 0.341×10^{4}L.mol^{-1}.cm^{-1} and the detection limit was 0.464µg/mL. The proposed methods were successfully, applied to the determination cefepime in pharmaceutical formulation.

Keyword: Cefepime, Batch method, Cloud point extraction, 2,6-dimethylphenol, Flow injection analysis.

Introduction

Cefepime chemically 7- -(2-aminothiazol-4-yl)-(z)methoxyiminoacetamido]-3-(1-methylpyrrolidino)-methyl-3-cephem-4-carboxylate [1], as shown in figure 1. CFM is a third-generation cephalosporin bactericidal activity spectrum in vivoandin vitroagainstaerobic gram-negative and gram-positive micro-organisms[2,3], including penicillin resins, For the analysis of Tazo-bactam (TZB) in pharmaceutical preparations[4,5] and plasma[6] either alone or in combination with other products, various analytical methods such as spectrophotometry[7,8]HPLC8 [9] TLC[10] exist for the analysis of cefepime (CFM) and similarly different HPLC methods are available. Diazotization method inexpensive and very simple method, the Azo-dye it is very important families of dye, that contain –N=N- band [11,12]. The various methods found to extraction and determination of azo-dye such as liquid-liquid extraction (LLE)[13-15].The CPE methodology uses the surfactants as extracting media rather than of toxic organic solvents and this idea has been facing a wide acceptance in the scientific community especially for the analytical chemists[16]. In fact, the mechanism of extraction of the CPE process is similar to that of conventional liquid-liquid extraction LLE which separates the desired analyses by their affinity in two immiscible phases. CPE has become an attractive field for trace metal ion separation and pre-concentration. Some insightful reviews [17] have demonstrated the concepts of CPE. Aquatic surfactants form and are turbid to a certain temperature known as "cloud point temperature" in aqueous solutions. Upstream of the temperature, the micellar solution splits into two phases: a small volume and a diluted aqueous phase

*Corresponding author e-mail: nisreenkais82@gmail.com.; (Nisreen Kais Abood).
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with a large surfactant material [18]. FIA is an automatic chemical analysis system that injects a sample into a flowing carrier solution that mixes with reagents before the detector is reached. Automated sample preparation, more repeatability, micro-miniaturization adaptability, chemical containment, waste reduction, and reagent economy are all valuable assets that contribute to the application of flow injection to real-world assays in a system that operates at microliter levels [19,20]. The key assets of flow injection are a well-defined gradient of concentration when an analyte reaches the reagent (which provides an infinite number of well-reproduced analyte /reagent ratios) as well as an exact period of fluid manipulations [21]. The aim of the present work Theses methods could be applied to analyze some pharmaceutical formulations.

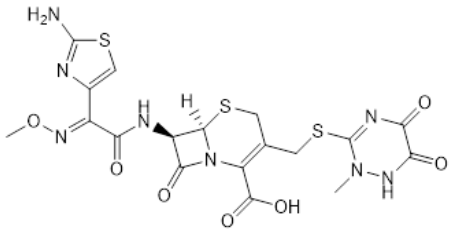


Figure 1: Structure of Cefepime

Experimental

Instruments : Measurements of absorbance were carried out in Spectrophotometric single-beam UV-visible 295 (Lasany- India), fitted with 1 cm and 0.5 cm of quartz cells. An ultrasonic and thermostatic water bath from Elma Hans Schmidbauer Gmbh and Co.KG in combination with sample extraction. Three channel manifold atomic flow injection configuration figure2.

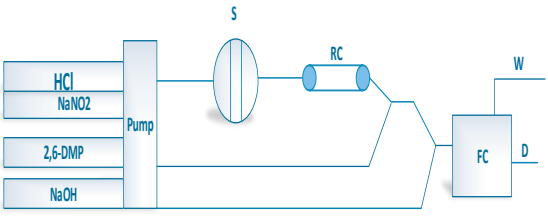


Figure 2: Scheme of the employed flow system, P: peristaltic pump, R.C: reaction coil, S: sample injection, W: waste, FC: flow cell

Chemicals and Reagents: Chemicals were of analytical quality and were obtained from Merck. The quality management laboratory received cefepime (the general company for the manufacture of medicines and medical supplies - Samarra).

Standard solution

Reagents:

Stander solutions (1000 µg.m-1) CFM was prepared by dissolving 0. 1 g of pure drug in water and completed volume to the mark in volumetric flask 100 mL with distilled water. Stock solution of 2,6-dimethyl phenol A (1000 µg/mL) by dissolving 0.1 mg of 2,6-di methyl phenol in distilled water and dilution to the mark in 100ml volumetric flask.

Preparation 25% NaOH,1%NaNO2, 4% Urea ,10% Triton X-114, 5% w/v Na2SO4 and 0.01M of CTAB (0.3644g in 100 ml in distilled water).

The standard solutions of pharmaceutical Formulation

Preparation 1000 ppm 0.1 g from Cefepime from injection 1.0 g. pharma Roth Germany and Nevzat Turkey. The weight was dissolved in distilled water to ensure the complete solubility and then made up to flask 100 mL, filtered solution to avoid un dissolved and any suspended before use.

Preparation the calibration curve for the diazotization method

The method for preparation of diazotized cefepime was developed to by mixing 1mL of 1000 µg.ml^{-1} of CFM solution in 20 ml volumetric flask immersed in an ice bath (0-5 ^{o}C),then addition of 0.75mL of (1:1) HCl, 0.5 mL of 1% NaNO2 was gradually added, and the mixture was allowed to settle for 10 min. Finally, 1mL of (1000 µg.ml^{-1}) of 2,6-dimehylphenol solution was added followed by 2 mL of 25% NaOH solution. The final volume of the mixture was brought to 20 mL by distilled water. The absorbance of the resulted orange azodye was measured at 515 nm against blank solution.

General procedure of technique cloud point extraction (CPE)

Different concentrations ranging 0.25-10 µg/mL of azo-dye CFM to transfer to a centrifuge 15 mL tube then added 1 mL of Triton X-114 10% v/v 0.0 1 mL (CTAB) and 5% w/v Na_2SO_4 . The solutions were put at room temperature under ultrasonic for 2 minutes, mixture of the solution keeping in the water bath (60 ^{0}C) at 55 min. Two phases were separated rich phase and the aqueous phase was easily disposal by decantation. The rich-surfactant phase from this technique was diluted with 0.5 mL of ethanol then transferred into quartz cell to measure absorption intensity at λmax 515 nm.via centrifugation for 5 min at 4000 rpm. Cooled mixture that increases the

viscosity of the surfactant-at wave length of 515 nm general analytical CPE procedure can be carried out with only a few experimental steps.as shown in figure 3.

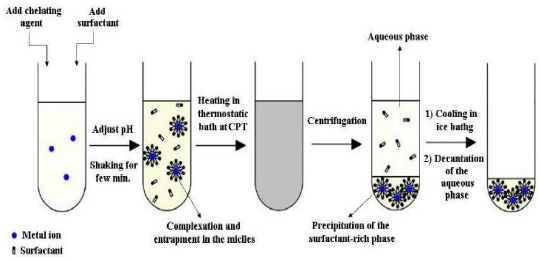


Figure3: Steps in the general experimental protocol CPE in metal separation / process pre-concentration.

The Flow Injection of cefepime

A 100 µl of CFM drugs injected into the carrier stream of that substance by mixing three channels, the first channel used for transporting (0.818×10^{-3}) M 2.6-dimethylphenol, the second channel containing carrier acid and sodium nitrite using T-shaped, this reaction was carried out by mixing well in a 100 cm reaction coil after the mixture was allowed to pass through the injector and the resulting product reacted with a stream.

Results and Discussion

The fundamental research is the diazotization reaction of cefepime with nitrous acid, coupled with 2,6-dimethylphenol as a reagent, orange colored at a wavelength 515 nm. The absorption spectra of the product against the blank as shown in figure 4

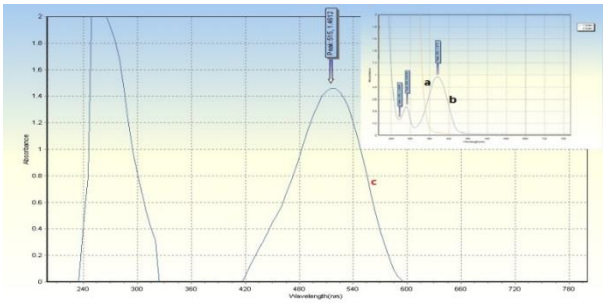


Figure 4: Absorption spectrum of cefepime 50 µg.ml^{-1} a) CFM solution b) blank solution c) Azo dye of CFM+2,6-DMP

The Diazotization Coupling Reaction Optimization

The effect of the different variables on the absorption intensity has been studied to estimation the optimum experimental conditions required to determine the CFM concentration in the samples.The effect type of acids was studied by some (1:1) dilute acids (HCl, H_2SO_4, HNO_3, CH_3CO_2H) the process of diazotization were tested, and the highest absorption was observed when HCl was used, as shown in Table1.

Table 1: Effect type of acids on absorbance

Type of acid	CFM λ_{max} 515nm
HCl	0.816
H_2SO_4	0.641
HNO_3	0.432
CH_3COOH	0.256

Various volumes (0.25-2 mL) of HCl were studied in the diazotization process and the highest absorption intensity was reached when using 0.75mL for CFM because the diazotization process was done in alkaline medium theabsorbance increased with increase acid volume but the absorptivity suddenly decreases because of the protonation of primary amine became inactive does not couple [22] and obtained parting process to the primary amine as in the following equation 1.

$$Ar\text{-}NH_2 \xrightarrow{+H^+} Ar\text{-}NH_3^+ \quad \text{.....equation 1}$$

The results shown in figure 5a. The effect of the amount of $NaNO_2$ was studied by varying the volumes of (0.144M (1% w/v) $NaNO_2$) used from 0.25-2ml in the diazotization process, the absorption was increased with increased in the volume of $NaNO_2$,but the absorptivity suddenly decreased with increased the volume of sodium nitrite in the solution because the excess of $NaNO_2$ causes a rise in pollutants that effect on diazonium salt and the occurrence of other reaction such as nitration ,which occur for the drugs containing the amino group ,which leads to the absence azo-coupling process[22] as in the following equation 2.

$$Ar\text{-}NH_2 \xrightarrow[HCl]{\text{excess from } NaNO_2} Ar\text{-}N{=}NO \quad \text{.....equation 2}$$

The results as shown in figure 5b. The waiting time effect was studied by using different range of times (0-40) min, the time required to complete the interaction diazotization for the drug was 5 min for the CFM. Best waiting time that give the highest absorbance intensity at the λ_{max} 515 nm. Show in

figure **5c**, this waiting time is therefore used in subsequent studies. Nitrite acid is also formed due to excess amount of sodium nitrite which leads to side reactions by different volumes of urea (0-4 mL) as shown in figure **5d**.

The alkali medium type has an important effect on the absorption intensity. Four types of base (KOH, NaOH, Na_2CO_3 and NH_4OH) was studied. It was found that NaOH gives the highest absorption intensity in this reaction. Therefore, the effect of various volumes of 6.25 M NaOH (0.5-2.5 mL) was studied. The addition of 2 mL was the best volume to obtain the highest absorption intensity as shown in figure **5e**.

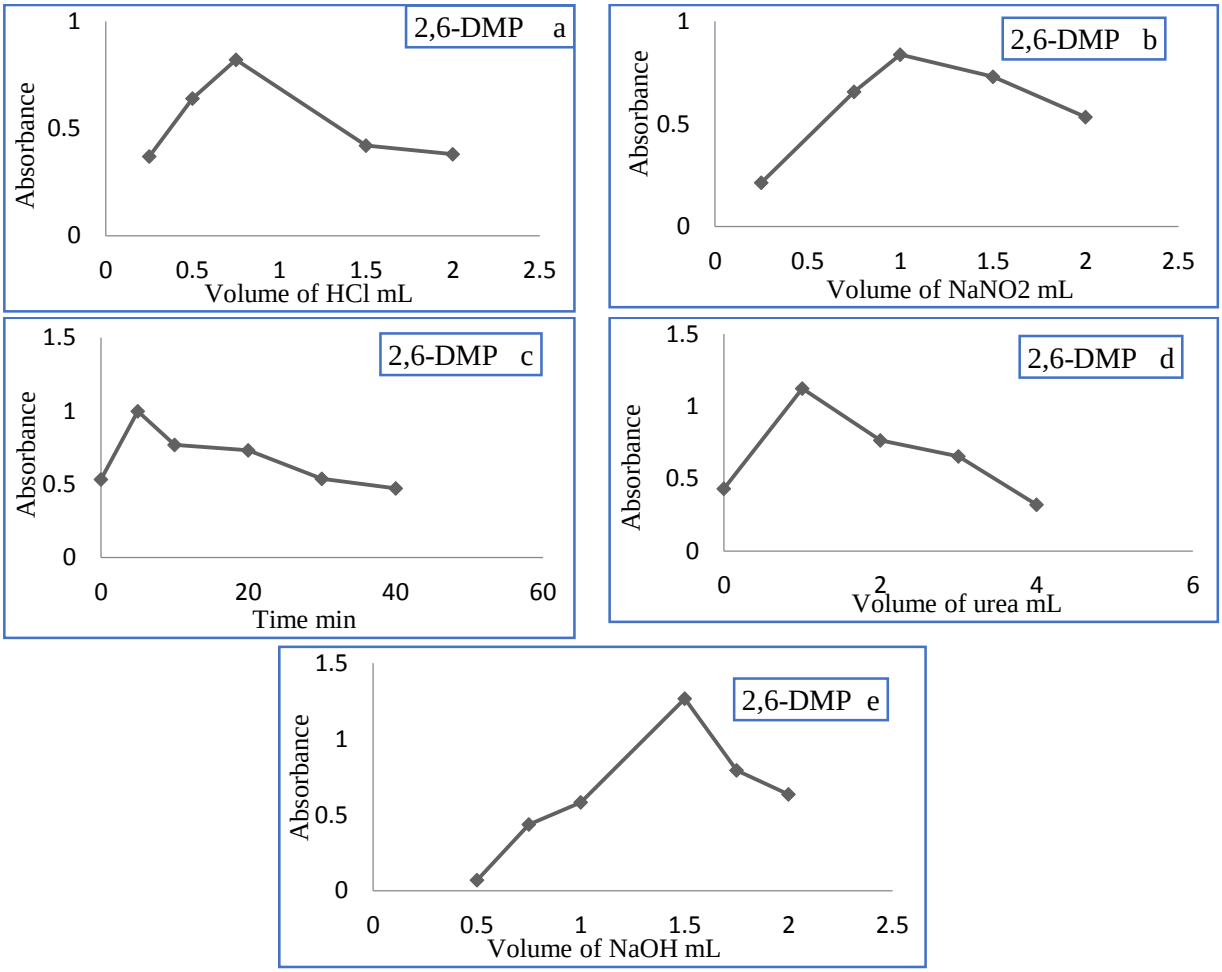


Figure5:The effect of experimental conditions a:effect of acid b: effect of sodium nitrate c: time d: urea and e: base

The possible reaction path may be written as follows as shown if figure 6

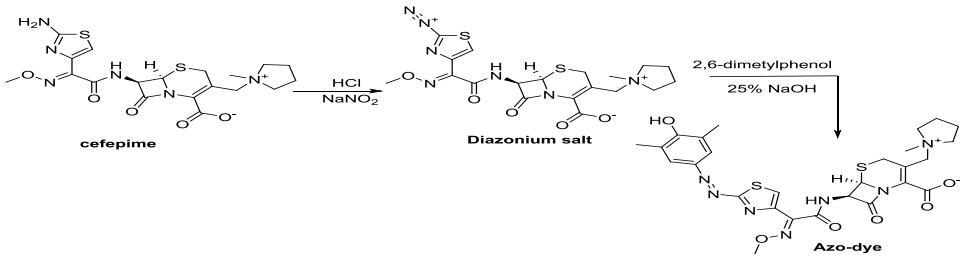


Figure6: The suggest mechanism of reaction CFM-2,6-DMP colored step

Effect of Interferences

The method was selective it was examined in which 1 mL of the sample solution, including CFM and 1 mL (1000 mg. L^{-1}) of maltose, sucrose, glucose, galactose and fructose were isolated and extracted under ideal experimental conditions. The results obtained in Table 2, has no significant interference in the spectrophotometric determination with the various compounds present at a moderate concentration. The findings indicate good selectivity of the proposed procedure and the applicability of the procedure to the correct evaluation of the CFM drug in pharmaceutical formulations.

Tabl 2: Effect on pure drug of an interference compound

Interferences compound	Recovery %of CFM
Sucrose	99.68
Lactose	100.12
Maltose	100.33
Fructose	99.58
Glucose	100.23
Starch	99.78

Cloud point extraction optimization analysis for CFM drug

To estimation the trace concentration of the CFM, used the cloud point extraction. Study effect different volume of Triton X-114 (1-3) mL. When increasing the amount of TritonX-114 up to 2 mL, the absorption of the process increased and the absorption decreased at higher concentrations. In this work, 2 mL TritonX-114 were therefore selected show in figure 7a, effect of temperature on the efficiency of CFM extraction. The nonionic surfactant's CMC decreased with temperature as hydrophobic micelles increased with temperature rise in the Surfactant process due to an increased Triton X-114 spacing and extraction capacity due to the dehydration of the micella external layer [23]. The CFM absorption grew from 40 to 80 ^{0}C, while absorption decreased over 80 ^{0}C due to viscosity increases. Extraction by cloud point requires sufficient time to obtain the equilibrium between the aqueous and the rich phase of the surface effective material by means of greater concentration of the micelles. This period Incubation time (10-50) min represented the amount of heat accumulated in the solution which allows the Micelles to lose the water molecules to give a hydrophobic mass of small size and high viscosity entrap dye easily, the temperature of 70^{0}C and 40 min was chosen. Show in figure 7b and 7c, the aqueous phase was extracted by decantation and ethanol was added to the surfactant-rich phase to decrease the viscosity of the surfactant-rich phase.

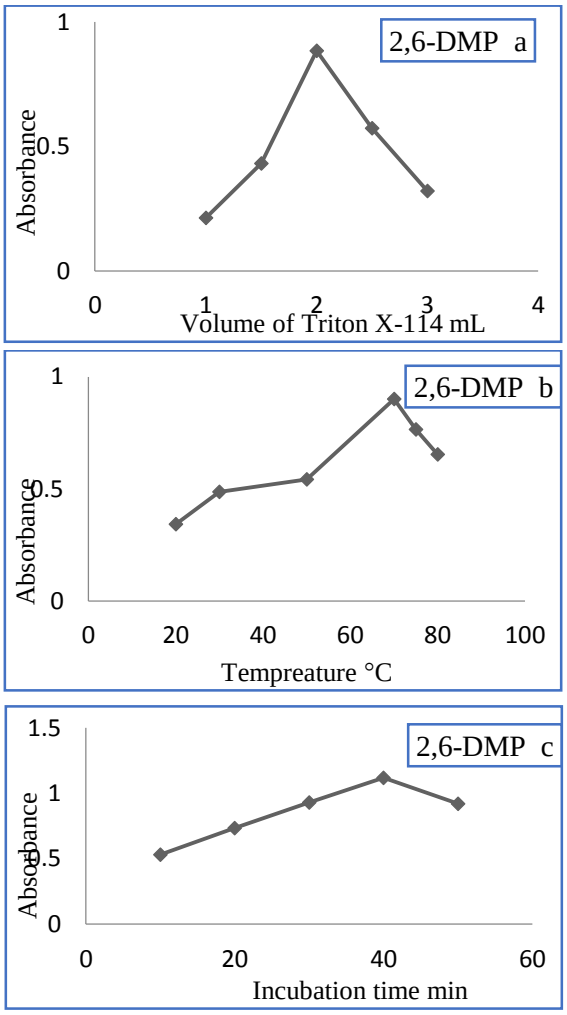


Figure 7: Effect of experimental conditions a: TritonX-114 b: temperature c: incubation time

The optimum reaction conditions of flow injection analysis technique

Study all optimization conditions chemical parameters such as concentration of reagent, acid and sodium nitrate. Different concentrations of 2,6-dimethylphenol (4.09×10^{-5} 2.45×10^{-4}) M µg/mL studied increase the absorbance with increasing the concentration for 2,6-dimethylphenol up to (1.63×10^{-4} M), decreases the absorbance with increasing the concentration. Sundry concentration of acids HCl was used to obtain the highest absorption on the flow injection for CFM, the concentration of Acids was HCl (0.8) M shown figure 8, sodium nitrite has an active role in this reaction use appropriate

concentration lead to the speed and completeness of the reaction, the different concentration of $NaNO_2$ was used to find higher concentration for CFM in flow injection, the results shown in figure 9.

Study manifold Parameters optimization

Different physical parameters have been studied, such as the length of the reaction coil range (25-200) cm, 50 cm was the best reaction coil giving high absorbance at wavelength 515 nm for CFM figure 10,it clears an increase the reaction coil decrease the absorbance because of an increase dispersion. The increasing the reaction coil length leads to decrease the total flow rate and preferred the coil length as short as is practically possible. total flow rate of (1-5) mL/min was studied, with the maximum absorption of 2 mL/min,the absorbance of colored product decreased by increasing a total flow rate because the residence time is not enough for the reaction to be completed The result shows figure 11 by usage fixed in all subsequent experiments. The different volumes (50-200) µL from the injection sample studied, with 100 µl, was the best volume which provided greater absorption.

Analytical characteristics: After optimization experimental conditions, prepared of the diazotization , cloud point extraction and flow injection curve by a plotting absorbance different concentration CFM 2-50, 0.25-10 and 1- 150 µg / mL respectively show in figure 12 and the Table 3 show the parameter Characteristic for the regression equation of Diazotization, CPE and FIA techniques.

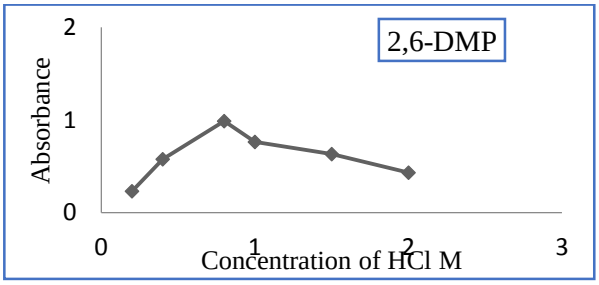


Figure8: Effect concentration of acid

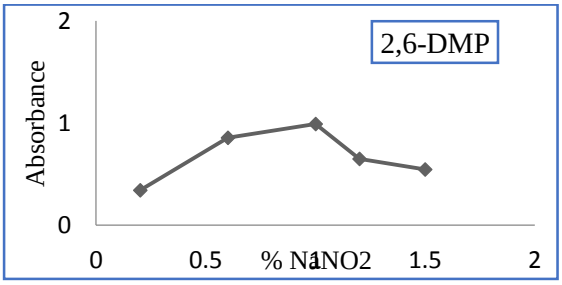


Figure 9: Effect concentration of $NaNO_2$

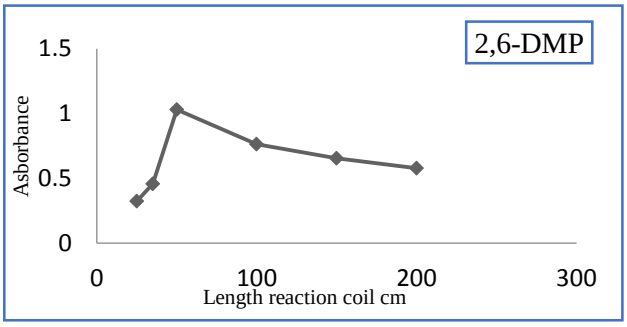


Figure10: Effect of Length Reaction Coil /cm

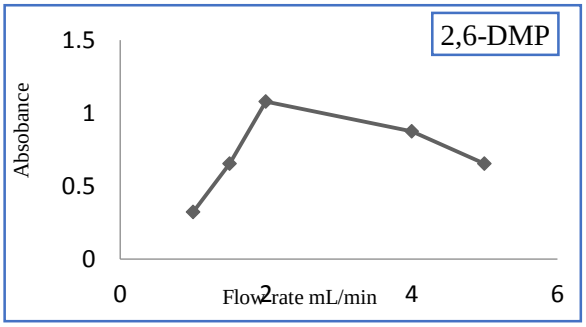
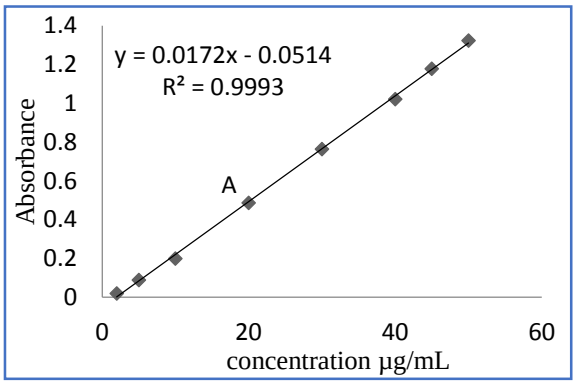
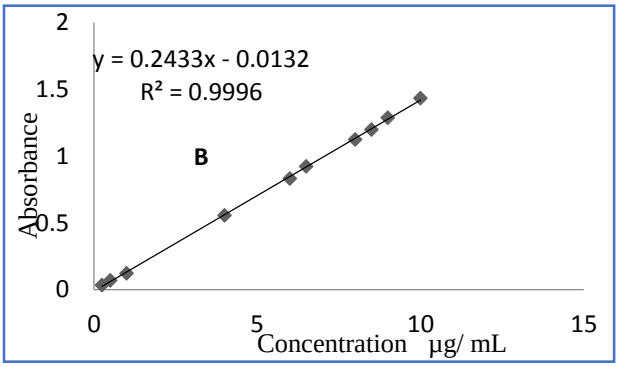


Figure11: Effect of Total rate mL/min





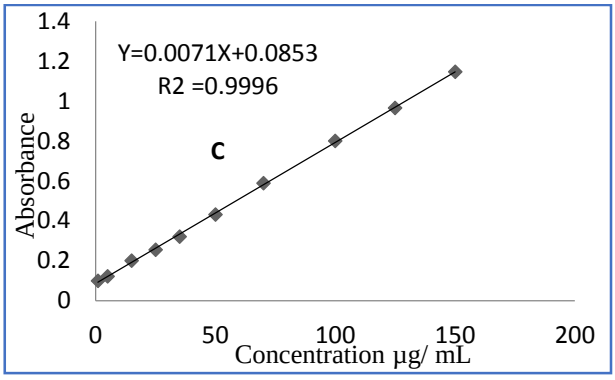


Figure12: Calibration curve of CFM: A) Diazotization B) Cloud point extraction C) Flow Injection Analysis

Table 3: A characteristic parameter for the regression equation of the proposed diazotization cloud point and flow injection of CFM drug

Parameters	diazotization	Cloud point extraction	Flow injection analysis
λ_{max} nm	515		
Color	Reddish-Orange	deep Reddish-Orange	Reddish-Orange
Regression equation	Y=0.0172X-0.0514	Y=0.2433X-0.0132	Y=0.0071X+0.0853
Linearity range(µg/mL)	2-50	0.25-10	1-150
Correlation Coefficient (r)	0.9996	0.9997	0.9997
Ɛ(L.mol^{-1}.cm^{-1})	0.826×10^4	1.169×10^5	0.341×10^4
Sandal' sensitivity (µg . cm^2)	0.058	0.004	0.141
Slope (b)	0.0172	0.2433	0.0071
Intercept(a)	-0.0514	-0.0132	+0.0853
Limit of detection(µg/mL)	0.192	0.027	0.464
Limit quantification(µg/mL)	0.581	0.082	1.408
Enrichment Factor (EF)	------	14.14	-------
Pre-concentration factor (PF)	-------	25	-------
Distribution coefficient(D)	------	332.35	--------

LOD = 3.3×SDb/S, SDb= the standard deviation of intercepts of regression lines [24]

Accuracy and Precision

Study the accuracy and precision for the proposed methods diazotization, cloud point and flow injection, under optimum conditions using different concentrations and measured absorbance at a minimum for five readings per concentration. precision and accuracy determination by RE (%), R(%) and RSD (%), as shown in Table 4 &5.

Table 4: Data the accuracy and precision of proposed methods for estimation of pure samples

Drug	Amount of drugs µg /ml		Relative Error %	Recovery %	Average Recovery%	RSD% (n=5)
	Taken	Found				
Diazotization method						
Cefepime	10	9.93	-0.7	99.3	99.84	0.6
	20	19.98	-0.1	99.9		
	30	30.1	0.33	100.33		
Cloud point method						
Cefepime	2	1.98	-0.1	99.0	99.75	0.87
	4	4.03	0.75	100.75		
	8	7.96	-0.5	99.5		
Flow injection method						
Cefepime	15	14.97	-0.2	99.8	99.99	0.82
	25	25.05	0.2	100.2		
	35	34.99	-0.02	99.97		

Table 5: The accuracy and precision of proposed method for estimation of commercial pharmaceuticals

Diazotization method						
Type of Drugs	**Amount of drugs mg**		**Relative Error %**	**Recovery %**	**Average Recovery %**	**RSD% (n=5)**
	Taken	**Found**				
Cefepime injection 1.0g Pharma Roth Germany	10 20 30	10.06 19.95 29.96	0.6 -0.25 -0.13	100.6 99.75 9986	100.07	0.98
Cefepime injection 1.0 g Nevzat Turkey	10 20 30	9.95 19.96 30.04	-0.5 -0.2 0.13	99.5 99.8 100.13	99.81	0.48
Cloud point method						
Cefepime injection 1.0g Pharma Roth Germany	2 4 8	1.99 3.95 8.06	-0.5 -1.25 0.75	99.5 98.75 100.75	99.66	1.2
Cefepime injection 1.0 g Nevzat Turkey	2 4 8	2.03 3.94 7.93	1.5 -1.5 -0.8	101.5 98.5 99.12	99.70	0.48
Flow injection method						
Cefepime injection 1.0g Pharma Roth Germany	15 25 35	14.96 24.93 35.07	-0.26 -0.28 0.20	99.73 99.71 100.2	99.88	1.3
Cefepime injection 1.0 g Nevzat Turkey	15 25 35	15.01 24.97 34.90	0.06 -0.12 -0.28	100.06 99.88 99.72	99.88	0.48

The comparison of my current work with another method as shown in Table 6, refers to the comparison of success between the proposed procedures with other methods in evaluating CFM of different samples. The proposed approach offers advantages explaining the current work. LOQ, LOD and senstivity values appear to be lower compared to other methods used to quantify Cefepime

Table.6: Compared the values of LOD and LOQ and sensitivity of the present work method with similar methods reported in literature

Methods	**LOD µg/mL**	**LOQ µg/mL**	**Sandal' sensitivity (µg . cm^{-2})**	**Ref.**
Spectrophotometric method	1.198	0.652	0.005	[14]
Spectrophotometric method	0.097	0.323	0.117	[15]
Spectrophotometric analysis of cefepime through Method its Hg(I) complex	1.20	3.65	-------	[25]
Validated Spectrophotometric Method	1.097	3.656	0.007	[26]
Spectrophotometric method Cloud point extraction	0.027	0.082	0.004	Present work

Conclusions

The suggested approach to CFM estimation has the advantages of high sensitivity, low cost, streamlined, recurrent and reproducible CFM drug evaluation techniques in pharmaceutical preparedness that can be applied to actual samples. The surfactant was used in pharmaceutical preparations for the isolation and pre-concentration of the CFM compound. For this procedure, a comparison between the methods already documented using different instrumental techniques appears to be more sensitive and stable, simple, fast, quick and cheap. An FIA method was used to automate the batch spectrophotometric method for the estimation of CFM drug. The proposed methods were successfully applied for the

estimation of pure CFM and in pharmaceutical dosage.

Acknowledgment

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