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New spectrophotometric method for determination of cefepime in pure and pharmaceutical formulation by cloud point extraction in trace

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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 3-aminophenol in alkaline medium to obtain a stable orange colored dye at λ_{max} 495 nm. The concentration ranges 2–50 μg / mL, Beer's law is obeyed, correlation coefficient was 0.9997, molar absorptivity was $0.704 \times 10^4 L.mol^{-1}.cm^{-1}$ and the detection limit was 0.259 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} 495 nm. The concentration range obeyed the Beer's law was 0.25–10 μg / mL, correlation coefficient was 0.9996, molar absorptivity was $1.362 \times 10^5 L.mol^{-1}.cm^{-1}$, detection limit was 0.013 μg/mL, Pre-concentration factor was 25 and Distribution coefficient(D) was 333.35.

Introduction

Cefepime chemically show in Fig. 1,referred to as 7- -(2-aminothiazol-4-yl)- (z)methoxyiminoacetamido]-3-(1-methylpyrrolidino)-methyl-3-cephem-4-carboxylate [1] is an overseas 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. The authors have already conducted a comprehensive analysis of the analytical approaches available to analyze these drugs [11,12]. Azo dyes are essential organic compounds with at least one conjugated azo group as well (-N = N-). Chromophore [13]. Two or more aromatic or heterocyclic rings can be connected to this chromophore moiety. Color enhancement. In the dye molecules, there may be more than one azo group present and therefore categorized into depending on the amount of azo moieties present in the molecule [14,15]. 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 [16,17]. The key assets of flow injection are a well-defined gradient of concentration when an analyte reaches the reagent strom (which provides an infinite number of well-reproduced analyte/reagent ratios) as well as an exact period of fluid manipulations [18]. The aim of the present work is to provide an optimized spectrophotometric method using the diazotization, cloud point extraction and flow injection methods with distinguish between them that give the best optimization that can be determined.

Materials and methods

Instrumentation

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.

Chemicals and Reagents: All chemicals of analytical quality obtained from Merck. Pure CFM drug sample was kindly provided from state company for Drug Industries and Medical Appliance, SDI, Samara, Iraq.

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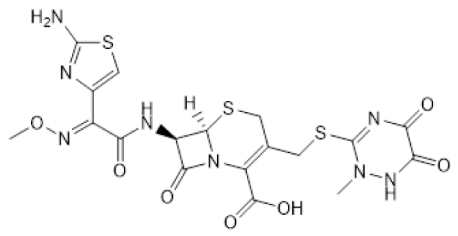


Fig. 1. Structure of Cefepime.

Preparation of standard solution

Reagents: All Chemicals were analytical grade.

Stock 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 3-amino phenol A (1000 μg/mL) by dissolving 0.1 mg of 3-aminophenol in distilled water and dilution to the mark in 100 mL volumetric flask.

Preparation 25 % NaOH,1%$NaNO_2$, 4 % Urea.

The standard solutions of pharmaceutical formulation

Preparation 1000 ppm 0.1 g from Cefepime provided 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.

General process for the preparation of the calibration curve for the diazotization system

The basic method was developed to prepare Azo-Coupling by precisely adding (1 mL 1000 μg/mL) cefepime in a 20 mL volumetric flask submerged in an ice bath of 0–5 °C, adding 0.75mLof (1:1) HCl, gradually adding 0.5 mL of 1 percent $NaNO_2$, waiting 10 mint for CFM Then add 1 mL of (1000) μg/mL of 3-aminophenol, then add 2 mL of 25 % NaOH for CFM to 20 mL of distilled water. The Azo-dye developed the absorbance Orange, colored, which gave a wavelength absorbance of 495 nm against a CFM blank reagent.

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 min, 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 495 nm. via centrifugation for 5 min at 4000 rpm.

Results

The fundamental research is the diazotization reaction of cefepime with nitrous acid, coupled with 3-aminophenol as a reagent, orange colored at a wavelength 495 nm. The absorption spectra of the product against the blank as shown in Fig. 2.

The diazotization coupling reaction optimization

Different variables effect on the absorption intensity has been studied 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 was tested, and the highest absorption was observed when HCl was used, as shown in Table 1.

Various volumes (0.25–2 mL) of HCl were studied in the diazotization process and the highest absorption intensity was reached when using 0.75 mL for CFM because the diazotization process was done in alkaline medium the absorbance increased with increase acid volume but the absorptivity suddenly decreases because of the protonation of

Table 1
Effect type of acids on absorbance.

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

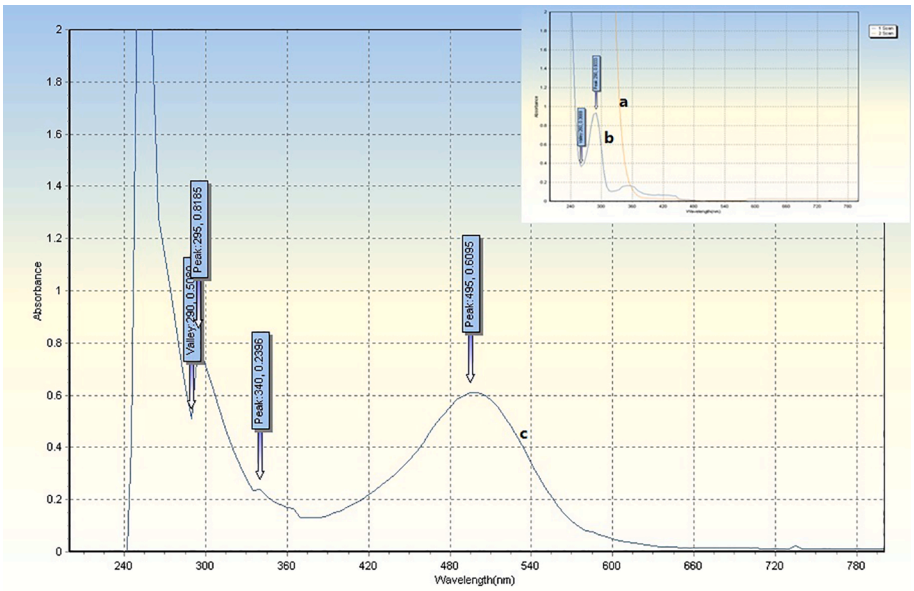


Fig. 2. Absorption spectrum of cefepime 50 μg.mL-^{1} a) CFM solution b) blank solution c) Azo dye of CFM + 3-aminophenol.

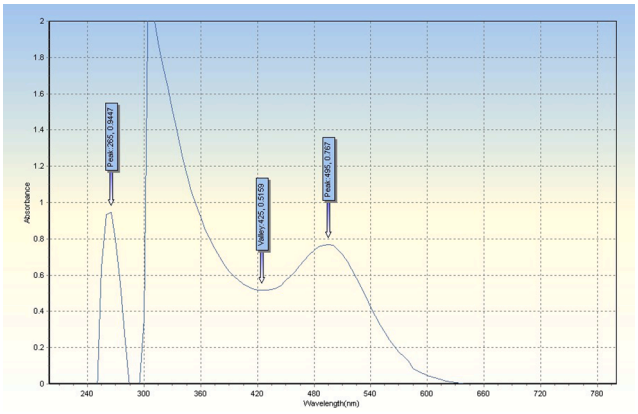


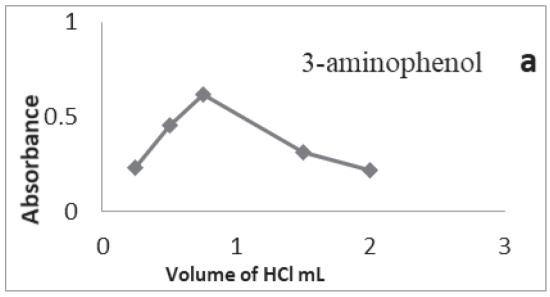
Fig. 3. Absorption spectrum of cefepime 50 μg.mL^{-1}.

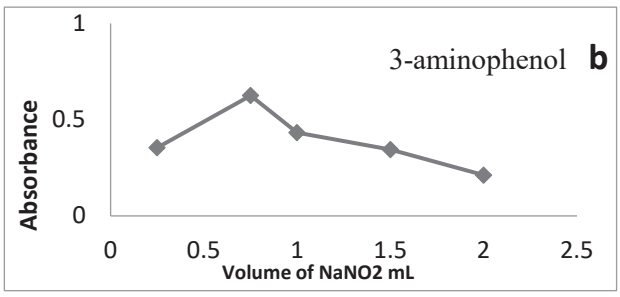
primary amine became inactive does not couple [19] and obtained parting process to the primary amine The results shown in Fig. 4a. The effect of the amount of $NaNO_2$ was studied by varying the volumes of (0.144 M (1 % w/v) $NaNO_2$) used from 0.25 to 2 mL in the diazotization process, the absorption 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[19].The results as shown in Fig. 4b. 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 10 min for the CFM. Best waiting time that gives the highest absorbance intensity at the λ_{max} 495 nm. Show in Fig. 4C, 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), show in Fig. 4d. The alkali medium type has an important effect on the absorption intensity. See (Fig. 3).

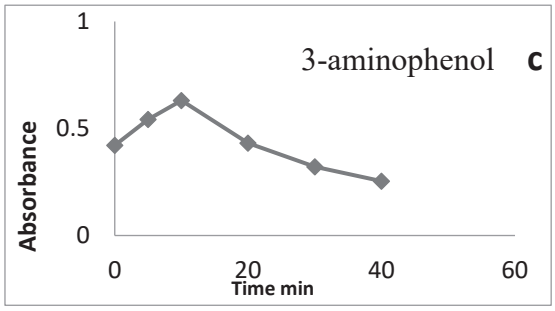
Four types of bases (KOH, NaOH, Na_2CO_3 and NH_4OH) was studied. It was found that NaOH gives the highest absorption intensity in this reaction. Therefore, the effect of various volumes of 6.25 M NaOH (0.5–2.5 mL) was studied. The addition of 1 mL was the best volume to obtain the highest absorption intensity as shown in Fig. 4e.

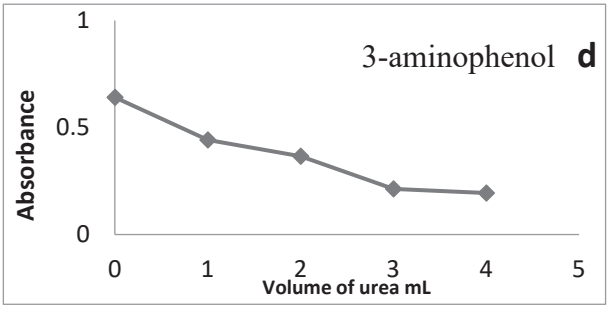
Effect of interferences

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









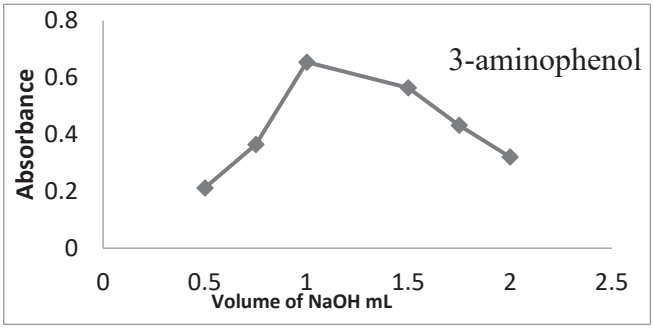


Fig. 4. The effect of experimental conditions a: effect of acid b: effect of sodium nitrate c: time d: urea and e: base.

Table 2
Effect on pure drug of an interference compound.

	Recovery %of CFM
Sucrose	99.68
Lactose	100.12
Maltose	99.64
Fructose	99.45
Glucose	98.75
Starch	99.89

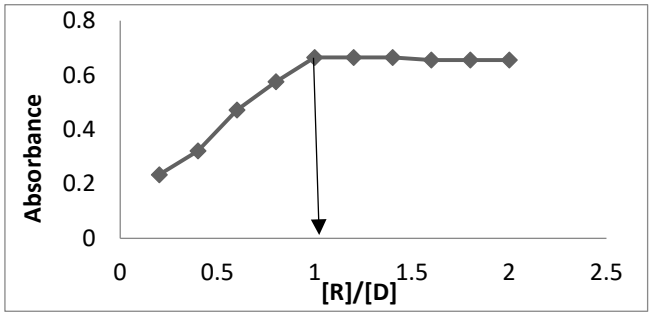


Fig. 5. Mole ratio for diazotized the cefepime coupled with 3-aminophenol.

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.

Stoichiometric ratio determination

The stoichiometry of the diazotization reaction between Cefepime and reagent Bisphenol A as investigated using mole ratio method. (Fig. 5) shows that the orange azo dye is formed in the ratio1:1 (3-aminophenol reagent[R]: Cefepime[D]).

From the mole ratio method shown, the ratio between the drugs and reagents were 1:1, the proposed formula for the resulting dye produce are therefore as follows [20] show in Fig. 6.

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 Fig. 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

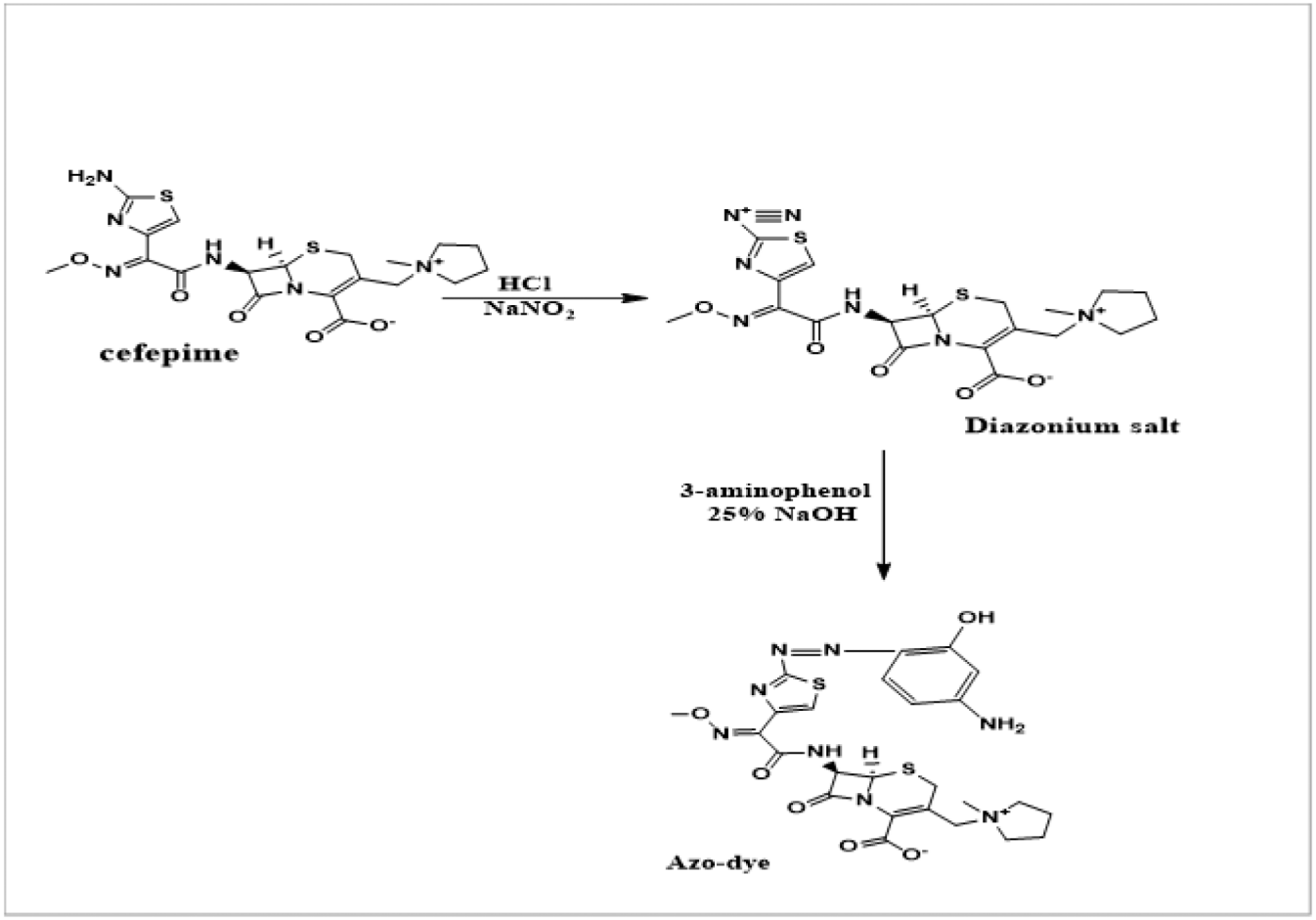


Fig. 6. The suggest mechanism of Cefepime reaction with 3-aminophenol.

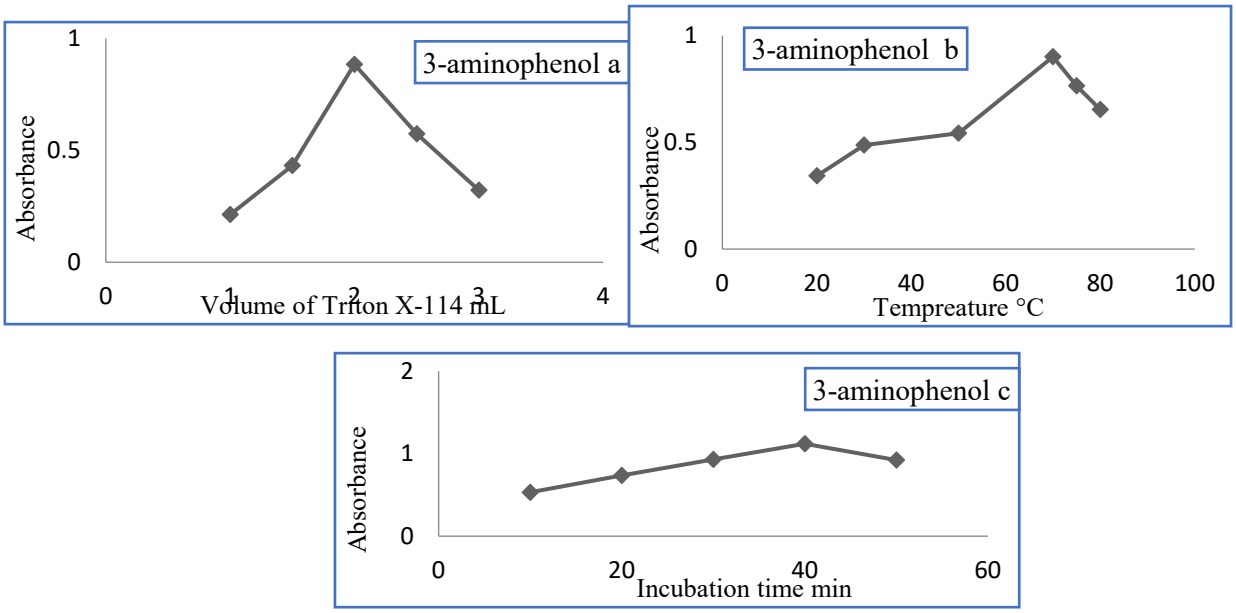


Fig. 7. Effect of experimental conditions a: TritonX-114b: temperature c: incubation time.

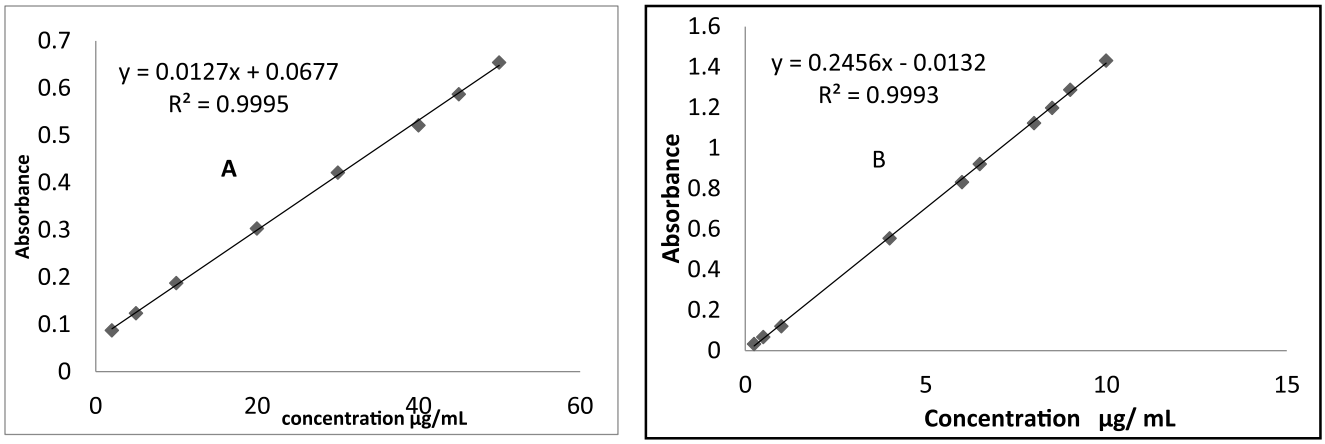


Fig. 8. Calibration curve of CFM A) Diazotization B) cloud point extraction.

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

Parameters	diazotization	Cloud point extraction
λ_{max} nm		495
Color	Orange	Orange
Regression equation	Y = 0.0127X + 0.0677	Y = 0.2456X-0.0132
Linearity range(µg/mL)	0.25–50	0.25–10
Correlation Coefficient (r)	0.9997	0.9996
ε(L.mol^{-1}.cm^{-1})	0.704 × 10^4	1.362 × 10^5
Sandal' sensitivity (µg. cm^{-2})	0.078	0.004
Slope (b)	0.0127	0.2456
Intercept(a)	0.0677	−0.0132
Limit of detection(µg/mL)	0.259	0.013
Limit quantification(µg/mL)	0.787	0.041
Enrichment Factor (EF)	———	19.34
Pre-concentration factor (PF)	———	25
Distribution coefficient(D)	———	333.35

LOD = 3.3 × SDb/S, SDb = the standard deviation of intercepts of regression lines [21].

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	9.89	−1.1	98.9		
	20	20.05	0.25	100.25	99.63	0.972
	30	29.92	−0.26	99.73		
Cloud point method						
Cefepime	2	1.98	−0.1	99.0		
	4	4.03	0.75	100.75	99.75	0.761
	8	7.96	−0.5	99.5		

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	10	10.02	0.6	100.6		
1.0 g Pharma Roth	20	19.98	−0.1	99.9	100.2	0.811
Germany	30	29.98	−0.06	99.93		
Cefepime injection	10	10.01	0.1	100.1		
1.0 g Nevzat Turkey	20	19.89	−0.55	99.45	99.75	0.341
	30	29.91	−0.3	99.7		
Cloud point method						
Cefepime injection	2	1.99	−0.5	99.5		
1.0 g Pharma Roth	4	3.95	−1.25	98.75	99.66	0.511
Germany	8	8.06	0.75	100.75		
Cefepime injection	2	2.03	1.5	101.5		
1.0 g Nevzat Turkey	4	3.94	−1.5	98.5	99.70	0.390
	8	7.93	−0.8	99.12		

Table 6
s

Pharmaceutical preparation	Rec% Batch method	Value		Rec% Cloud point method	Value		Standard method[22]
		T	F		T	F	Rec %
Cefepime pure	98.63			99.87			100.01
Cefepime injection 1.0 g Pharma Roth Germany	100.2	0.933 (2.131)	0.021 (0.052)	99.95	1.257 (2.131)	1.083 (19.00)	99.89
Cefepime injection 1.0 g Nevzat Turkey	99.75			99.73			99.91

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 °C and 40 min was chosen. Show in Fig. 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.

Analytical characteristics: After optimization experimental conditions, prepared of the diazotization and flow injection curve by a plotting absorbance different concentration CFM 2–50 and 0.25–10 μg / mL respectively show in Fig. 8 and the Table 3 show the parameter Characteristic for the regression equation of Diazotization and CPE.

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 Tables 4 and 5.

The statistical analysis results exhibited in Table 6 proved that the calculated t-values and F-values for Cefepime estimation in different pharmaceuticals are less than t-tabulated and F-tabulated at 95 % confidence interval and (n-1) degrees of freedom.

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. We used Method to determine the best method can be used depend on the recovery and limit of detection for this procedure. The first method conversion primary amine to azo-dye by react cefepime with sodium nitrite and hydrochloric acid followed by coupling with 3-aminophenol in alkaline medium to obtain a stable orange colored dye, Cloud point extraction (CPE) second method to estimation a trace amount in aqueous solution product from diazotization and measuring with a UV–visible spectrophotometer.

CRediT authorship contribution statement

Dalia M. Jamil: Writing – review & editing, Writing – original draft, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Dalia M. Jamil reports financial support, article publishing charges, statistical analysis, and writing assistance were provided by AL_Nahrain University. Dalia M. Jamil reports a relationship with AL_Nahrain University that includes: employment and speaking and lecture fees. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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