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Spectrophotometer Determination of Cefixime in pure form and pharmaceutical preparation by Using Cloud point Extraction

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Abstract:

Two simple methods spectrophotometric were suggested for the determination of Cefixime (CFX) in pure form and pharmaceutical preparation. The first method is based without cloud point (CPE) on diazotization of the Cefixime drug by sodium nitrite at 5Cº followed by coupling with ortho nitro phenol in basic medium to form orange colour. The product was stabilized and measured 400 nm. Beer's law was obeyed in the concentration range of (10-160) µg·mL^{-1} Sandell's sensitivity was 0.0888µg·cm^{-1}, the detection limit was 0.07896µg·mL^{-1}, and the limit of Quantitation was 0.085389µg·mL^{-1}.The second method was cloud point extraction (CPE) with using Trtion X-114 as surfactant. Beer's law was obeyed in the concentration range of (10-160) µg·mL$^{-1.}$ Sandell's sensitivity was 0.1470µg·cm^{-1}, the detection limit was 0.06680µg·mL^{-1}, and the limit of quantitation was 0.07293µg·mL$^{-1.}$ All variables including the reagent concentration, reaction time, colour stability period, and mole ratio were studied in order to optimize the reaction conditions. The composition of product (1:1). The methods were effectively useful to the determination of Cefixime in pharmaceutical dose form, and the attained results were in good agreement with the official result and other methods in literature .No interference was observed from the commonly encountered additives and excipients

Key word: Cloud Point Extraction, Cefixime, Diazotization, Orthonitrophenol, Triton X-114.

Introduction:

Sulfa drugs were the first antibiotics used regularly and paved the way for the revolution of antibiotics in medicine. The first sulfonamide, named Prontosil (red dye), was discovered in 1932 by Gerhard Dumagk (1) .Antibiotics are the chemotherapeutic agents to inhibit the microorganisms growth.The chemical agents were used to treatment the disease by destroy pathogenic microorganisms orInhabitation their growth at concentration low enough to shun undesirable damage to the host.Antibiotics are drugs measures which have some chemical substance that are produced by microorganisms and by chemical synthesis. These substances at very low concentrations are well-known to totally raze or partly inhibit microorganisms. Antibiotics include broad spread appli- cation in the cure of bacterial disease (2).

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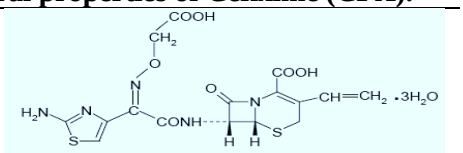
Cefixime :.an antibiotic that belongs to the third generation of cephalosporin and is taken orally to treat bacterial infections, including pharyngitis, middle ear, sore throat and urinary tract infection. It is approved for medical use in 1989. Over-all characterizes of Cefixime are given in Table 1 (3).

Table 1. General properties of Cefixime (CFX).

Structure	
Nomenclature	(6R, 7R)-7-[(Z)-2-(2-aminothiazol-4-yl)-2- (carboxymethoxy) imino] acetyl] amino] 3-ethenyl-8-oxo-5-yhia-1-azabicyclo [4.2.0] oct-2-ene-2-carboxylicacid trihydrates
Formula	$C_{16}H_{15}N_5O_7S_2$
Molecular Weight	453.452gm .mol^{-1}

Materials and Methods:

Instruments:

UV-Vis spectrophotometer: SHIMADZU, Double beam UV-Vis, model UV-1800 /Japan. The range of wavelength (190-1100) nm, cell quartz with path 1cm., Water Bath : A thermostat water bath, Memmert,/ermany, Electric Balance: Sartorius (0.0000), made in Germany, Centrifuge: Triup International corp, TRIU 800 Centrifuge, / Korea & PH meter: HANNA, PH meter, HI83141.

Reagent and Materials:

❖ **Preparation of ortho nitro phenol (1000 µg mL^{-1})** by dissolving 0.1 in volumetric flask 100 mL and complete the mark by water.

❖ **Preparation stock solution of Cefixime (1000 µg mL-1)** by dissolving 1 gm in volumetric flask 100 mL and diluted in water to the mark.

❖ ***Preparation of Sodium nitrite (1% W/ V):***It is prepared by dissolving 1gm in water in volumetric flask 100mL and complete to the mark by water.

❖ ***Preparation of Sulphamic acid (1% W/ V):*** by dissolving 1gm of H3NSO3 in water in a volumetric flask of 100 mL and completed to the mark by water.

❖ ***Preparation of*** **sodium hydroxide NaOH (1M)** was prepared by dissolving (4g) of the solid product in 100 mL of water.

❖ ***Preparation*** **of Triton X-114 (10 %)**: by diluting 10 mL of Triton **X-114** with water in a volumetric flask 100 mL.

General Procedure for CPE:

Uncharacteristic cloud point extraction needs the subsequent step:Taking (10mL) of volumetric flask having the optimal conditions for diazotization and coupling reaction of [CFX] gotten from first batch with [10%(v/v) surfactant] then ending it to the blot by ethanol and the comfortable of volumetric flask transmission to centrifuge test tube . The mix is shuddered for 1min and left in thermos bath at 60 C^{o} for 20 min, then detached by centrifuge at 4000rpm for 20 min. Test tube was set in ice bath to rise thickness micelles coat , at that point the informal detached. The outstanding micellar was softened by 1mL ethanol later that the absorbance is unhurried spectrophotometrically UV-VIS at maximum wavelength.

Sample Preparation of Pharmaceutical Determination Cefixime:

A process on medication Cifixime has been useful, the production company is [Pharma International Co.Amman. Jorden]. Five mL have been taken from drug in volumetric flask 100mL and the volume is completed by distilled water, so it is given (1000 µg mL^{-1}) from CFX.

Result and Discussion:

First methods: Spectrophotometric determination of sulphadimidine sodium (SDMS) by oxidation coupling reactions. Optimization Parameters for Reaction.

All of the factors that affect the absorbance of formation of azo dye product are optimized to improve the sensitivity and detection limit for the determination of the drugs. All optimization work under wavelength at 400 nm is shown in Fig. 1.

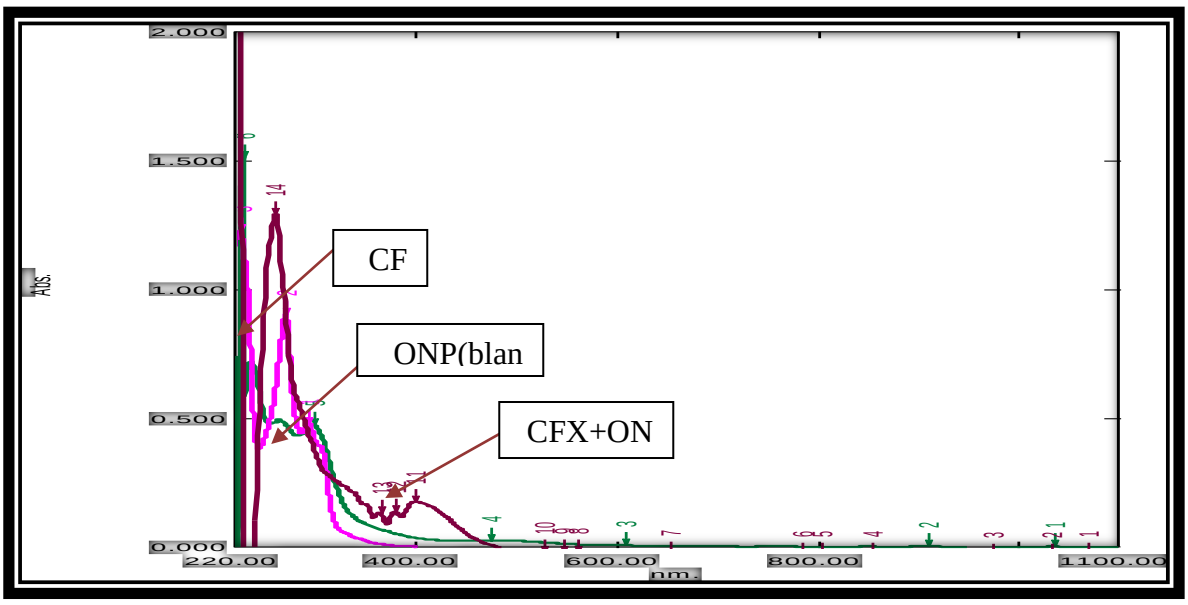


Figure 1. Absorbance spectra of the Resulting Dye CFX

Effect of Acid Type

In this study, using 1mL of (0.5M) from different acids [HCl, H_2SO_4, HNO_3, H_3PO_4 and CH_3COOH] and added [1mL of CFX(100 µg·mL^{-1}), 1 mL of each acid, 1mL of $NaNO_2$, 1mL of H_3NSO_3, 1mL ONP and 1mL of NaOH] in 10 mL of volumetric flask and complete the volume by distilled water to form diazonium salt(azo dye). Then the absorbance was measured at 400 nm, the resulting absorbance is shown in Table 2.

Table 2. Data of absorbance of effect of acid type.

0.5M different acids (1 mL)	HCl	H_2SO_4	HNO_3	H_3PO_4	CH_3COOH
Absorbance at	0.496	0.265	0.439	0.267	0.149

It is clear from this study that the hydrochloric acid gave a higher absorbance, This acid is used in subsequent experiments, as shown in the Tabl 2.

Effect of Optimum Volume of 0.5M of acid.

The same addition is done with CFX[1mL CFX, with varying volumes of 0.5M HCl from (0.1-1) mL ,1 mL $NaNO_2$, 1mL H_3NSO_3, 1mL ONP and 1mL of NaOH] in 10 mL volumetric flask and complete the volume by distilled water. Then, the absorbance and the optimum volume were measured for higher absorbance fixed for sequence experiment. The resultant absorbance is shown in Table 3.

Table 3. Data of Absorbance to Optimum Volume of 0.5M

Volume HCl (1mL)	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1
Abs.	0.264	0.287	0.318	0.337	0.406	0.451	0.464	0.504	0.494	0.496

It is obvious that absorbance increased with increasing the acid volume, suddenly the absorbance decreased because the primary amine became inactive (4). The optimum volume for higher absorbance was fixed in subsequent experiments (for HCl with CFX) which affects the composition of diazonium salt(azo dye).

Base Type.

In this experiment different basics have been used [NaOH, KOH, K_2CO_3, Na_2CO_3, NH_4OH, $NaHCO_3$] and that followed the addition [1mL CFX, **0.8**mL HCl, 1mL $NaNO_2$, 1mL H_3NSO_3, 1mL ONP and 1mL of each base(1M)]in volumetric flask 10 mL and completed to the mark by distilled water .The absorbance was measured and the absorbance results are shown in Fig. 2 .

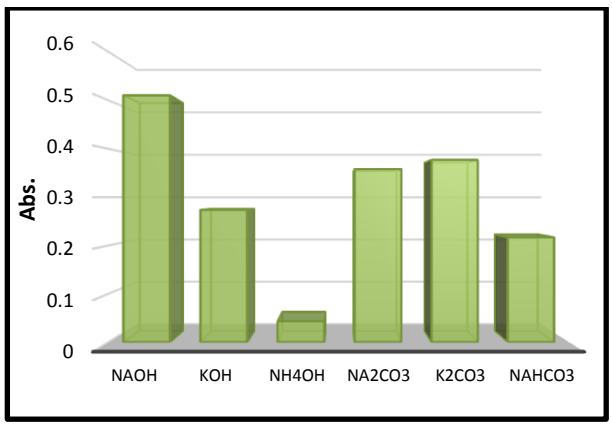


Figure 2. Data of absorbance for different bases with CFX

It is clear that Sodium hydroxide gave the higher absorbance, Therefore, this base was fixed in subsequent (5) show in Fig. 2.

Optimum Volume of 0.5M [NaOH].

The same addition was done for CFX in 10 mL volumetric flask and the volume was completed by distilled water. Then the absorbance and the optimum volume were measured for higher absorbance and fixed for sequence experiment. The absorbance results are shown in Table 4.

Table 4. Data of Absorbance to different volume of 0.5M [NaOH].

Volume of 0.5M bases (mL)	Abs	**Volume of 0.5M bases**	**Abs**
0.1	0.205	0.8	0.476
0.2	0.269	0.9	0.497
0.3	0.293	1	0.532
0.4	0.340	1.1	0.492
0.5	0.399	1.2	0.482
0.6	0.413	1.3	0.411
0.7	0.428		

It is evident that absorbance increased with increasing the volume of NaOH, but suddenly it decreased because the decomposition happened when increasing the volume of NaOH and formation of diazotate ions may form coupling. This shows agreement with previous studies (6). The optimum value of **1** mL for NaOH is with CFX .

Optimum Volume of 1% Sodium Nitrite.

The same additions were [1mL for CFX,**0.8** mL HCl, with varying volume of 1% $NaNO_2$ from (0.1-1) mL, 1mL H_3NSO_3, 1mL ONP and **1** mL NaOH] in volumetric flask 10 mL and complete d to the mark by distilled water. Then the higher absorbance of optimum volume was fixed for sequence experiment as shown in Table 5.

Table 5. Data of Absorbance to Optimum Volume of 1% $NaNO_2$

Volume of 1% Sodium Nitrite(mL)	Absorbance	Volume of 1% Sodium Nitrite	Absorbance
0.1	0.069	0.6	0.432
0.2	0.179	0.7	0.509
0.3	0.243	0.8	0.556
0.4	0.285	0.9	0.497
0.5	0.375	1	0.475

It is clear in from Table 5 that the absorbance increased with increasing the volume of $NaNO_2$, but the absorbance decreased because the increase of nitrate concentration causes the decomposition of diazonium salt (7). The optimum value of Sodium Nitrate 0.8 mL is with CFX.

Effect of Optimum Volume of 1% Sulphamic Acid.

The additions for experimental were [1mL for CFX,**0.8** mL HCl, **0.8** mL1% Na_2NO_2 with varying volume of 1% H_3NSO_3 from (0.1-1) mL,1mL ONP and **1** mL NaOH] in a volumetric flask 10 mL and the volume was completed by distilled water. Then the higher absorbance of optimum volume was fixed for sequence experiment. Table 6 shows the data of the absorbance.

Table 6. Data of the Absorbance od Optimum Volume of 1% Sulphamic Acid.

Volume of 1% Sulphamic Acid(mL)	0.07	0.08	0.09	0.1	0.2	0.3	0.4	0.5
Abs.	0.398	0.456	0.498	0.525	0.557	0.531	0.451	0.420

It clear from this Table that the absorbance increased with increasing the volume of Sulphamic acid, but the signals decreased suddenly because this volume removed nitrite and released nitrogen gas (8).The optimum volume of Sulphamic acid was 0.2 mL.

Effect of Optimum Volume of (100 µg mL^{-1}) Reagent.

The same additions are [1mL for CFX, **0.8** mL HCl, **0.8** mL 1% $NaNO_2$,**0.2**mL H_3NSO_3, with varying volume of (100 µg mL^{-1})ONP from (0.1-1) mL and **1** mL NaOH] in 10 mL of volumetric flask and the volume was completed by distilled water. Then the higher absorbance of optimum volume at maximum wavelength is fixed for sequence experiment as shown in Table 7.

Table 7. Data of Absorbance of Optimum Volume of (100 µg mL^{-1}) Reagent.

Volume of reagent	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8
Abs.	0.298	0.368	0.442	0.501	0.567	0.533	0.478	0.447

The absorbance increased with the reagent volume increase until 0.6(0.5) mL which had a decrease in absorbance. Therefore 0.5 ML was chosen as optimum volume of the regent for drug coupling.

Effect of Reaction Time on Stability Colour Product.

The optimum volumes of parameters were completed [1mL for CFX, 0.8 mL HCl, 0.8 mL 1% NaNO2,0.2 mL H3NSO3, 0.5 mL ONP and 1 mL NaOH]. The time on stability colour of product is one of the most important factors to Cloud Point Extraction and diazotization. So, this factor through time (0-60) min needs to be studied. Then, absorbance was measured and the higher absorbance at maximum wavelength was fixed as show in Table 8.

Table 8. Data of Absorbance of Reaction Time on Stability Colour Product.

Time(min)	**Absorbance**	**Time**(min)	**Absorbance**
0	0.198	35	0.502
5	0.237	40	0.531
10	0.289	45	0.575
15	0.319	50	0.566
20	0.391	55	0.543
25	0.420	60	0.531
30	0.479	65	0.509

This clear the time of product remain stable for CFX was 45 min displayed in table 8.

Effect of Order Addition.

When completing the volume of parameter, the sequence of addition with optimum volume needs to be studied but in a different order . The effect of order addition is shown in Table 9. The optimum order addition is [D+H+N+S+R+B]

Table 9. Effect of Order Addition.

No	Addition	Absorbance
1	**R+H+N+S+D+B**	0.218
2	**D+H+N+S+R+B**	0.589
3	**D+H+N+B+R+S**	0.416
4	**D+B+R+N+H+S**	0.231
5	**R+B+D+H+N+S**	0.389
6	**R+H+N+B+D+S**	0.077

D=Drug(CFX), H=acid(HCl), N=$NaNO_2$, S=H_3NSO_3, B=Base(NaOH), R=Reagent(O-Nitro Phenol).

At the maximum wavelength the absorbance is measured, and the higher absorbance was fixed for the best order addition as displayed in Table 9.

Effect of Solvents.

All additions of diazotization and coupling reaction were done for CFX with optimum condition. This was followed by diluting with polar solvent [water, ethanol, methanol,1- propanol ,acetonitrile & acetone] in volumetric flask 10 mL. At maximum wavelength, the absorbance was measured and recorded for the best solvent. The effect of absorbance is shown in Table 10.

Table 10. Data of Absorbance to Solvents.

Solvent	**Water**	**Ethanol**	**Methanol**	**Acetonitril**	**1-Propanol**	**Acetone**
Abs.	0.539	0.620	0.521	0.157	0.104	0.283

The data shows that ethanol is the best solvent as shown in Table 10 .

Effect Temperature in the Formation of Colour Product and Stabilization.

The conclusion of different temperatures on colour product have been studied from (5-60)C^0. And the rest of addition are optimal settings then dilution done with distilled water except for the CFX dilution which is by ethanol in volumetric flask 10 mL. Then absorbance was measured at the maximum wavelength Table 11.

Table 11. Data of Absorbance to Temperature in the Formation of Colour Product and Stabilization.

Time	5	15	20	30	40	50	60
Abs.	0.350	0.483	0.519	0.493	0.484	0.439	0.419

The best temperature (15C^0& 20 C^0) was the greatest absorbency for CFX , on the other hand when the temperature rises the absorbency , it starts dissociation of product and can be noticed from strength of color. The results are in arrangement with literatures (9), and this temperature is stable in later experiment.

Stoichiometric Determination of Product.

Continuous Variation Method (10)***.***

The conformation of the azo dyes product ratio is supported by using the slope analysis method. In this method,the absorbance is planned against [reagent] / [reagent]+[drug].This test is complete by taking a series of volumetric flasks 10 mL having varying volumes of Drug (0.1-0.9 mL) with concentration [$6X10^{-4}$] M and varying volumes of Reagent (0.9 -0.1 mL) with concentration ($6x10^{-4}$) M and the rest addition is optimum condition then complete the volume by ethanol. That followed the absorbance which is measured at the maximum wavelength λmax 400nm for CFX. The stoichiometric ratio between drug with reagent 1:1 results are displayed in Fig. 3, when [R]=Reagent and [D]=Drug.

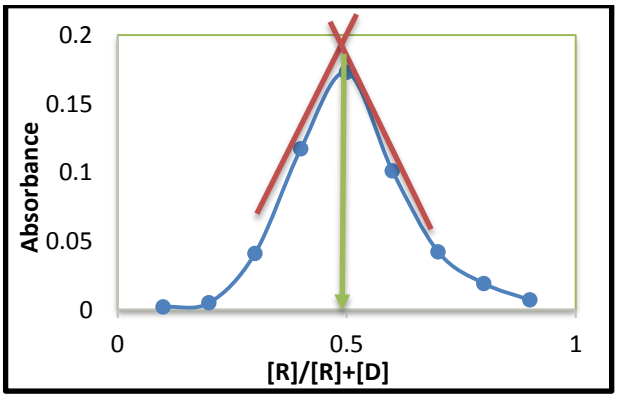


Figure 3. Continuous Variation Method Plot for CFX.

Mole Ratio Method.

Mole Ratio Method is useful to study the coloured of product (11) .In this method the volume of drug is constant in 1 mL with

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concentration ($6x10^{-4}$M) for CFX and concertation of ONP is [$6X10^{-4}$] M in volumetric flask 10 mL, the rest of addition was optimum conditions and the volume was completed by ethanol. That followed the absorbance which is measured at the maximum λmax=400 nm for CFX. The stoichiometric ratio between drug with reagent 1:1 results are displayed in Fig.4 then [R]=Reagent and [D]=Drug.

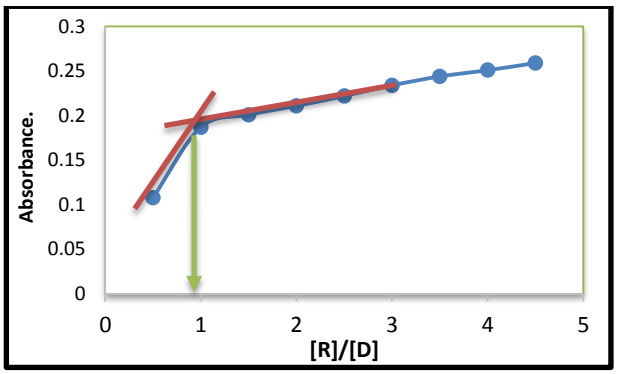

Figure 4. Mole Ratio Plot for CFX

Calibration Curve for CFX-ONP:

Aliquots of 10 mL solution is prepared ,having increasing concentration of CFX [varying volume of CFX (0.1-1.6 mL) with concentration (10-160 µg mL^{-1}), **0.8** mL HCl, **0.8** mL 1% $NaNO_2$,**0.2**mL H_3NSO_3, **0,5** mL ONP and **1** mL NaOH]. The volume was complete by ethanol, then the absorbance was measured at maximum wavelength against a blank solution able under alike condition without drug. Linear calibration graph was plotted by scheming absorbance against concentration of CFX in figure 5 the Concentration (10-160) µg mL^{-1} obeys the Bear law as shown in Fig. 5 .

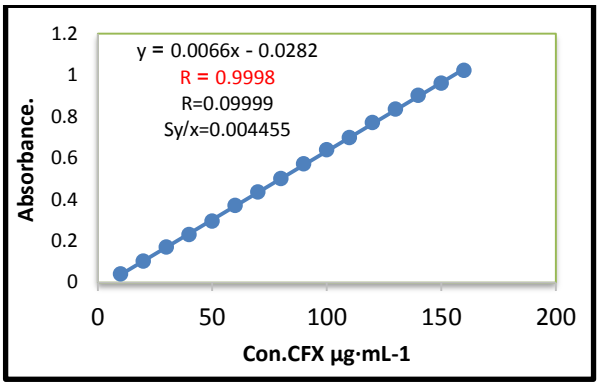

Figure 5. Calibration Graph of CFX.

Effect of Interference.

The effect of interference ordinary was present in [CFX] to identify the method fussiness under learning by addition 1mL (1000 ppm) from interference with 1mL (1000 ppm) from CFX 1mL (1000µg·mL^{-1}). It necessity lies in the size of interference which is lesser for a sample to limit the dilution of sample and use using the maximum concentration probable in the sample (12). The results are shown in Table 12.

Table 12: Data of Absorbance of interference for CFX.

NO.	100ppm interference	Abs.	Recovery %	E_{rel}%
1	Lactose	0.619	98.0606	-1.939
2	Starch	0.622	98.636	-1.363
3	Arabic Gum	0.589	93.515	-6.484
4	Glucose	0.614	97.454	-2.545
5	Talc	0.566	90.181	-9.818
6	Tri methyprine	0.596	94.727	-5.272
7	Without	0.630	99.72	-0.28

The results in this tables that there were displayed +no interference to present with drug in pharmaceuticals.

The Stability Constant of Coloured Product.

Dependent on two conducts, mole ratio and continuous variations methods revealed formerly, the composite product is[D: R] [drug: reagent] in the result is 1:1 as in the following equation.

K= [DR] / [D][R] ⟶ **K= 1- α/ $α^2$ c**

The stability constant K (13)**.** It is clear the stability constant is high, so the dye formed is very stable display in Table 13.

Table 13. Data of The Stability Constant of Colour Product of CFX.

Volume of $4x10^{-4}$M of CFX/ ML	Final con. CFX/M	As*	Am*	α	K ($L.Mol^{-1}$)	Mean of K ($L.Mol^{-1}$)
0.3	1.2×10^{-3}	0.178	0.176	0.01123	3.574×10^{3}	3.0918×10^{3}
0.5	2×10^{-3}	0.299	0.295	0.0133	3.566×10^{3}	
0.7	2.8×10^{-3}	0.435	0.433	4.5977×10^{-3}	2.1355×10^{4}	

[*]= Average of three determinations

It is clear that the stability constant is high, so the dye formed is very stable .

Accuracy and Precision.

Table 14 displays the accuracy and precision of CFX correspondingly. It is obvious that the results from this method have good accuracy and precision (14).

Table 14. Data of Accuracy and Precision of the Proposed Method to Determination of CFX.

Amount of CFX /µg mL^{-1}	*Found	Recovery %	Average Recovery %	E_{rel}%	Average E_{rel}%	RSD%
120	120.9090	100.7575	100.2945	0.7575	0.2945	0.1633
90	90.606	100.6733		0.6733		0.4163
60	60.1515	100.2525		0.2525		0.4506
30	29.8484	99.4947		-0..5053		0.7530

[*]= Average of five determinations

Applications of the Proposed Method on Pharmaceuticals.

The suggested method has been applied on pharmaceutical for CFX. Similar method was applied on Syrup Cefixime, the manufacture company is [Pharma International Co. Amman. Jorden] that contains (200mg) in 100 mL and the sample is prepared in accordance with the preparation of pharmaceutical. The results are good and of great dependability in the analysis of samples in the pharmaceutical preparation. The results are shown in Table 15 for CFX.

Table 15. Data of Determination CFX in the Pharmaceutical Preparation (Cefixime) by the Suggested Method.

Amount of CFX /µg mL^{-1}	*Found	Recovery %	Average Recovery %	E_{rel}%	Average E_{rel}%	RSD%
120	120.5448	100.454	100.0315	0.454	-.0.0595	0.2282
90	90.424	100.4711		0.471		0.4030
60	60.2116	100.3526		0.352		0.0542
30	29.5454	98.8485		-1.515		4.2043

[*]= Average of five determinations

<u>Second methods :Cloud Point Extraction of Cefixime in Aqueous Solution .</u>

Effect of Surfactant: The type of surfactant shows an identical important part in cloud point extraction method wherever each surface keeps ghostly depending on practical centre of Micelles. Aliquots of 10 mL of a solution inclosing [1mL for CFX, **0.8** mL HCl, **0.8** mL 1% $NaNO_2$,**0.2**mL H_3NSO_3, **0,5** mL ONP and **1** mL NaOH] in 10 mL volumetric flask and changed different types of surfactant was used including [Tween 20, Tween 80, Triton X-114, Triton X-100 , CTAP , SDS] , at 60 C^0 for 20 min then centrifuge 4000 rpm for 20 min .The surfactant amusing part is separated, dissolved in 1ML ethanol, at maximum wave length the absorbance was measured for CFX at 400 nm . The results are shown in Table 16.

Table 16. Data of Absorbance to Type of Surfactant with SDMS , SMZ and CFX.

Addition	Tween 20	Triton X-100	Tween 80	SDS	Triton X-114	CTAP
Abs.	0.061	0.122	0.057	0.090	0.275	0.119

It is clear from the results above that surfactant Triton X-114 increases the absorbance and efficiently of cloud point extraction (15).

Effect of Triton X-114 Volume.

Sum of 10 mL solution is primed in 10 mL volumetric flask and custom changing volumes of 10% (v/v) Triton X-114 (0.2-2) mL , then the volume was completed by ethanol , and heated at 60 C^0 for 20 min to practice cloud point then centrifugation at 4000 rpm for 20 min. The surfactant – opulent phase was softened by 1mL ethanol then the absorbance was measured at maximum wavelength at λmax = 400 nm. These results are displayed in Table 17.

Table 17.Data of Absorbance to Triton X-114 Volume with CFX.

Volume of Triton X-114(mL)	0.2	0.4	0.6	0.8	1	1.2	1.4	1.6	1.8	2
Abs	0.095	0.112	0.165	0.189	0.224	0.265	0.278	0.266	0.247	0.201

It can be noticed from the result above that the absorbance rises with the optimum volume of Triton X-114 but unexpectedly drops at higher amount. In conclusion, the volume of surfactant effected on the effectiveness of extraction and the enrichment factor (16). This can be explained due to the effect of surfactant best volume of Triton X-114 (1.4 mL) for CFX individually stable in following experimentations to complete high extraction efficiency.

Effect of Equilibrium Temperature:

Chains of solution are set in volumetric flask 10 mL and the volume was completed by ethanol, the temperature was varied from (35- 65)C^0 and the incubation time ranged from (5-35)min for all drug. At the maximum wavelength the absorbance was measured and recorded. These results are displayed in Table 18.

Table 18. Data of Absorbance to Temperature / ^{0}C with CFX.

Temperature	35	40	45	50	55	60	65
Abs	0.265	0.271	0.278	0.281	0.280	0.278	0.276

It is shown that the maximum absorption pointer of target drug is completed at (50) ºC for the azo dye product because the great number of micelles designed in cloud point layer important to the total transfer of the azo dye product into surfactant-rich phase that makes the most of the sensitivity (17) .

Effect of the Incubation Time: Chains of solution are set in 10 mL volumetric flask and the volume was completed by ethanol, the temperature was 50 ^{0}C for CFX and the incubation time from (5-35) min for all drug. At the maximum wavelength 400 nm, the absorbance was measured and the recorded result is shown in Table 19.

Table 19. Data of Absorbance for the Incubation Time with CFX .

Time /min	5	10	15	20	25	30	35
Abs	0.381	0.392	0.402	0.409	0.400	0.399	0.393

CPE needs enough time to make balance between aqueous phase and surfactant- rich phase by more accumulation the micelles. This time signifies the amount of high temperature accumulated in the solution that lets Micelles drop water molecules in order to give small size hydrophobic with high viscosity easily entrap the product in it. It is perfect that the best incubation time is (20)min is carefully chosen to provide high extraction efficiency and no increases detected for longer time (18) .

Preparation of Calibration Curve in CPE.

Chains of solution are [changing volume of CFX (0.1-1.6mL) with concentration (100-160) g mL^{-1}), **0.8** mL HCl, **0.8** mL 1% $NaNO_2$,**0.2**mL H_3NSO_3, **0,5** mL ONP, **1** mL NaOH and **1.4** mL 10%(v/v) Triton X-114] in 10 mL volumetric flask and full the volume by ethanol , at the best temperature and incubation time are heated in water bath to configuration cloud point and separated by centrifuge at 4000 rpm for 20 min as shown in Fig.6 .

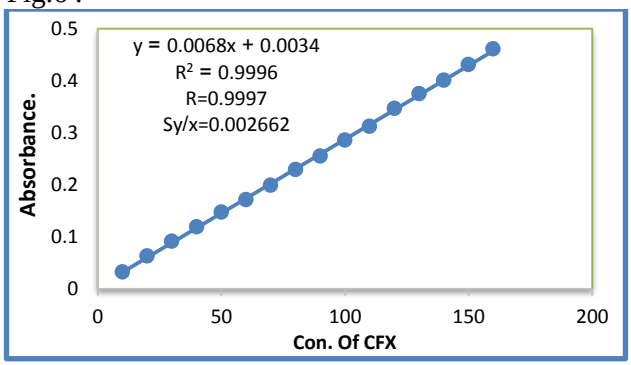

Figure 6. (CFX+CPE) Calibration Curve

Accuracy and Precision: it can be noticed from the results below that the technique has good accuracy and precision as a significance of recovery rate which is 100.8182 % for CFX Table 20.

Table 20. Data for Accuracy and Precision of the CPE to Determination of CFX.

Amount of CFX /µg mL^{-1}	*Found	Recovery %	Average Recovery %	E_{rel}%	Average E_{rel}%	RSD%
120	120.3571	100.2975	100.8182	0.297	0.818	0.3981
90	90.0714	101.190		1.1904		1.0196
60	60.2148	100.357		0.357		0.5136
30	30.4285	101.4283		1.428		1.0735

[*]= Average of five determinations

Applications of the Cloud Point Extraction on Pharmaceticals:

Cefixime (CFX):

A similar method is applied on Syrup Cefixime, the manufacture company is [Pharma International Co.Amman. Jorden] that contains (200mg) in 100 mL. The results are good and of great dependability in the analysis of samples in the pharmaceutical preparation. The results are shown in Table 21 for CFX

Table 21. Data of Determination CFX in the Pharmaceutical Preparation (Cefixime) by the Suggested Method.

Amount of CFX /µg mL^{-1}	*Found	Recovery %	Average Recovery %	E_{rel}%	Average E_{rel}%	RSD%
120	120.1428	100.119	100.5194	0.119	0.5182	0.3875
90	90.2856	100.3173		0.317		0.9020
60	60.5566	100.9276		0.927		2.3892
30	30.2142	100.714		0.71		4.4067

[*]= Average of five determinations.

Conclusion:

Cloud point extraction demeans calm, safe and useful pre-concentration technique to determine Cefixime by UV/VIS. The planned method is a effective, selective and gives good RSD and low limit of detection.

Authors' declaration:

- Conflicts of Interest: None.
- We hereby confirm that all the Figures and Tables in the manuscript are mine ours. Besides, the Figures and images, which are not mine ours, have been given the permission for re-publication attached with the manuscript.
- Ethical Clearance: The project was approved by the local ethical committee in University of Baghdad.

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التقدير الطيفي للسفكسيم في المواد النقية والمستحضرات الصيدلانية باستخدام الاستخلاص بنقطة الغيمة

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الخلاصة :

تم اقتراح طريقتين بسيطتين وسريعتين مفيدتين لتقديرالسفكسيم مع وبدون استخدام تقنية الاستخلاص بنقطة الغيمة في الشكل النقي والمستحضرات الصيدلانية. تعتمد الطريقة الأولى بدون الاستخلاص بنقطة الغيمة **CPE** على ازوتة الدواء بواسطة نتريت الصوديوم في **5C°** متبوعًا بالاقتران مع اورثونايتروفينول لتكوين اللون البرتقالي. تم تثبيت المنتج وقياسه عند 400 نانومتر. يطبق قانون البيرة في نطاق التركيز (10-160) ميكروغرام/مل . كانت حساسية ساندیل هي 0.0888 ملغم/مل، وكان حد الاكتشاف 0.07896 ملغم/مل ، وكان حد الكمي 0.085389 ملغم /مل . الطريقة الثانية هي الاستخلاص بنقطة الغيمة (**CPE**) مع استخدام ترايتون**X-114**. والتي تخضع لقانون بيرلامبرت في حدود التركيز (10-160) ميكروغرام/مل . كانت حساسية ساندیل هي 0.1470 ملغم/مل ، وكان حد الكشف 0.06680 ملغم/مل ، وكان الحد الكمي 0.07293 ملغم/مل. تمت دراسة جميع المتغيرات بما في ذلك تركيز الكاشف ، زمن التفاعل ، فترة استقرار اللون من أجل تحسين ظروف التفاعل. تكوين المنتج (1: 1). كانت الطرق مفيدة بشكل فعال لتقديرالسفكسيم في شكل جرعة صيدلانية ، وكانت النتائج التي تم التوصل إليها متوافقة بشكل جيد مع الطرق الرسمية وغيرها الموجودة في الادبيات. لم يلاحظ أي تداخل من الإضافات بشكل كبير.

الكلمة المفتاحية: الاستخلاص بنقطة الغيمة، السفكسيم، الازوته، اورثونايتروفينول، ترايتون**X-114**.