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Determination of Cefixime Using Batch, Cloud Point Extraction and Flow Injection as New Spectrophotometric Methods

Nisreen Kais Abood[1*], Mohammed Jasim M Hassan[1], Muneer A. AL-Da'amy[2]
[1] Department of Chemistry, College of Science, Mustansiriyah University, IRAQ
[2] Department of Chemistry, College of Education for pure Science, Kerbala University, Kerbala, IRAQ
*Correspondent author email: nisreenkais82@gmail.com

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Abstract

Three simple, sensitive, selective, accurate and efficient spectrophotometric methods for determining cefixime in bulk drug and pharmaceutical formulations have described. The first method involved conversion of NH_2 in cefixime to diazonium salt, which has coupled with Bisphenol A in an alkaline medium. The orange colored product showed λ_{max} at 490 nm and followed Beer's law over a concentration range of 1-50 µg mL^{-1}, with molar absorptivity of 0.866×10^4 $L.mol^{-1}.cm^{-1}$ and the detection limit was 0.157 µg.mL^{-1}. The second method involved pre-concentration of a trace amount of cefixime-azo dyes using cloud point extraction (CPE). The extracted drug-dye was spectrophotometrically measured at λ_{max} 500. The constructed calibration curve to determine cefixime followed Beer's law in a range of 0.25-6 µg.mL^{-1}, with a correlation coefficient of 0.9998, molar absorptivity of 0.961×10^5 $L.mol^{-1}.cm^{-1}$ and the detection limit was equal to 0.031 µg.mL^{-1}. The pre-concentration factor was 25 and distribution coefficient (D) was 314.03.

A diazotization of the studied drug (cefixime) and its coupling with Bisphenol A was studied using a developed flow injection analysis method, based on the detection of the absorption of the diazotization product. Chemical and physical properties [of what??] were studied to develop the suggested method and to determine the stability of the colored of product. A flow rate of 2.5mL.min^{-1}, 50cm reaction coil and 100µL sample volume were used to operate the system and the orange colored product was detected at 490nm. The proposed three methods were successfully applied to determine cefixime in pharmaceutical formulation, where results were satisfactory

Keywords: Cefixime, Batch method, Cloud point extraction, Bis phenol A, Flow Injection.

الخلاصة

تم في هذا البحث تطوير ثلاث طرق طيفية بسيطة ذات حساسية وانتقائية لتقدير السفكسيم (CFX) بصورته النقية والمستخدمة في المستحضرات الصيدلانيه. تضمنت الطريقة الاولى تحويل المجموعة الفعالة NH_2 الموجوده في السفكسيم الى ملح الدايازونيوم ومفاعلته مع الكاشف Bisphenol في وسط قاعدي. تم تحديد المركب الناتج ذو اللون برتقالي عند طول موجي ٤٩٠nm، اذ انطبق عليه قانون بير عند التراكيز (١-٥٠ ميكروغرام/مل، وكانت الامتصاصية المولارية0.866×10^4 لتر.مول$^{-1}$.سم$^{-1}$ وحد الكشف 0.157 ميكروغرام / مل. تضمنت الطريقة الثانية استخلاص التراكيز النزره لصبغة السفكسيم بطريقة الاستخلاص بنقطة الغيمة (CPE) وتقدير السفكسيم تحت الظروف المثلى عند طول موجي ٥٠٠nm، اذ انطبق عليه قانون بير عند التراكيز (0.25-6) ميكروغرام/م، وكانت الامتصاصية المولارية 0.961×10^5 لتر.مول$^{-1}$.سم$^{-1}$, وعامل التركيز ٢٥، ونسبة التوزيع 314.03 . اما الطريقة الثالثة فقد تضمنت الحقن الجرياني وهي طريقة سهلة لتقدير السفكسيم، اذ تم قياس اشاره الامتصاص الناتجة من عملة الازوتة وكانت اهم العوامل المعتمدة في الحقن الجرياني عوامل كيميائية وفيزيائية والتي درست لتطوير واستقرار لون الصبغة الناتجة عند طول موجي 490 nm . تم تطبيق الطرق الثلاث انفة الذكر لتقدير السفكسيم في المستحضرات الصيدلانية، اذ امتازت هذه الطرق بالبساطة والسرعة والدقة والتكلفة الواطئة.

Introduction

Cefixime trihydrate is chemically known as 7-{[2-(2-amino-1,3-thiazol-4-yl) 2(carboxy methoxyimino) acetyl] amino}-3-ethenyl-8-oxo5thia1-azabicyclo oct-2-ene-2carboxylic acid [1]. As shown in Figure 1, CFX is a third-generation of some antibiotics such as ceftriaxone and cefotaxime [2]. CFX is more stable when beta-lactamase enzymes buried, that produced by positive gram-negative bacteria [3][4]. Various methods have been used to analyze cefixime such as HPLC [5], spectrofluorometry [6], capillary electrophoresis [7], voltammetry [8], flow injection technique [9], mass spectroscopy, and spectrophotometric methods. These techniques are generally based on the formation of a complex between the drug and the reagent which can be determined using visible spectrophotometer [10]. A coupling reaction between the drug and the reagent is either an ion-pair type or potentiometric titration. Cefixime was also determined in pharmaceuticals preparations, urine [13][14] and human serum [15]. The proposed method here was based on the formation of azo dye of cefixime trihydrate with Bisphenol A. Bisphenol A has used for the first time with significantly low detection limit, high sensitivity, and wider dynamic range. This method required no extraction step or a specific temperature [16][17]. This method could be applied to analyze some pharmaceutical formulations.

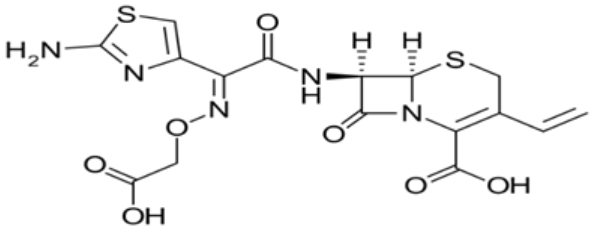

Figure 1: Structure of Cefixime.

Experimental

Instruments

Data was collected using a single beam Shimadzu UV-Visible spectrophotometer 160-A equipped with 1cm and 0.5cm quartz cells. An ultrasonic and thermostatic water bath (Elma Hans Schmidbauer Gmbh and Co.KG) was used for coupling with the extraction of samples. An automated three channel manifold flow injection configuration (Figure 2) was employed for (FIA). The manifold comprises a multichannel peristaltic pump (ALITEA, C4, made in Sweden) with polyvinyl chloride tubing (0.8) mm internal diameter.

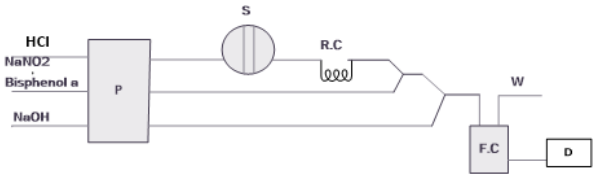

Figure 2: Scheme of the employed flow system, P: peristaltic pump, R.C: reaction coil, S: sample injection, W: waste, FC: flow cell.

Chemicals

All chemicals were of analytical quality and were purchased from Merck Ltd. (Jordan). Cefixime was obtained from The Quality Control Laboratory (The General Company for the Manufacture of Medicines and Medical Supplies-Samarra).

Standard solution

Reagents

Stock solutions of cefixime (1000 $\mu g.mL^{-1}$), Bisphenol A (1000μg mL^{-1}) were freshly prepared.

Solutions of 25% NaOH (6.25 M),1%$NaNO_2$ (0.144M), 4% Urea ,10% Triton X-114,0.01M of HTBA (0.3644g in 100 ml in distilled water) and 5% w/v Na_2SO_4 were prepared as required.

The standard solutions of pharmaceutical formulation

Cefixime Capsules: The content of 10 capsules (400mg/product DAR AL DAWA –Jordan and capsules 400 mg /product Pharma International co. Amman – Jordan) were separately accurately weighed, and the mean weight of the capsule was extracted. A required amount of the formulation was dissolved in distilled water

containing (0.6 ml) of 1M NaOH and the final volume was made up to 100 mL. The resulted solution was filtered off to remove insoluble materials.

The calibration curve for the diazotization method

A method for the preparation of diazotized Cefixime was developed to by mixing 1mL of 1000 $\mu g.ml^{-1}$ of cefixime solution in 20 ml volumetric flask immersed in an ice bath (0-5 ºC). After the addition of 0.5mL of (1:1) HCl, 0.5 mL of 1% $NaNO_2$ was gradually added, and the mixture was allowed to settle for 10 min. Finally, 1mL of (1000 $\mu g.ml^{-1}$) of Bisphenol A solution was added followed by 1.5 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 490 nm against blank solution.

The cloud point extraction (CPE)

The optimal conditions were detected and the calibration curve of the resulted CFX-azo dye was determined using a series of concentrations (0.25-6.00 $\mu g.mL^{-1)}$. A volume of 1 mL 10% v/v Triton X-114 solution was added to a specific amount of CFX-azo dye, followed by 2 mL of 0.01 M (CTAB) solution, 2mL of 5% w/v Na_2SO_4 solution, and the volume was made up to 12.5mL by distilled water. The mixture was sonicated for 2 min. in an ultrasonic bath at room temperature and further sonicated for 50 min. at 60 ° C. The resulted solution was centrifuged at 4000 rpm for 5 min and cooled in an ice bath for 10 min. The supernatant was removed and 0.5 mL of ethanol was added to dissolve the micelle layer. Absorbance at λmax 500 nm against blank was detected using 1 cm quartz cell. A shift of the absorption peak was observed due to different solvents.

The flow injection of cefixime

A volume of 100 µL of CFX solution was injected into the carrier stream that produced by mixing the flow of three channels. The first channel used 5.99×10^{-3} M solution of phenylhydrazine, the second and the third channels used carry. Nitric oxide was prepared by mixing the acid and sodium nitrite solutions using T-shaped connector. Nitrous acid was formed after mixing both reagents in 50 cm reaction coil before passing through the injector. The resulted product was reacted with a stream of 1.5 M NaOH solution and the absorbance of the orange product was measured at λ_{max} 490nm.

Results and Discussion

Diazotization method

The method based on a quantitative conversion of CFX to an azo dye after diazotization and coupling with Bisphenol A in alkaline medium. An orange color product was detected at λ_{max} 490 nm. According to Beer's law, the absorbance of the azo dye at λ_{max} was in linear proportion with CFX concentration. Figure 2.

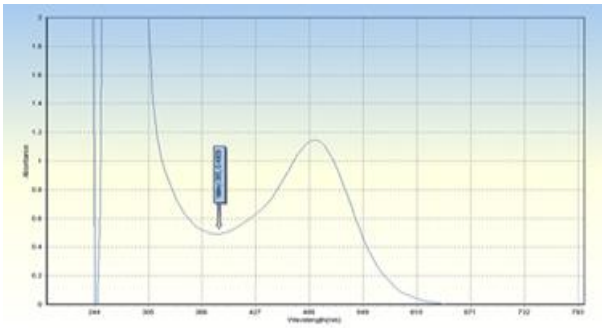

Figure 3: Absorption spectrum for 50 μgmL^{-1} CFX with the reagent against the reagent blank under optimum conditions.

Optimization of the diazotization coupling reaction

The effect of the different variables on the absorbance of the resulted product has studied to determine the optimum conditions for the diazotization and coupling of CFX to form the azo-dye, quantitatively. The concentration of the CFX was 50 $\mu g.mL^{-1}$ (1 mL of stock CFX solution was used). The effect of different types of acids was studied using (1:1) solutions of different acids (viz; HCl, H_2SO_4, HNO_3, CH_3CO_2H). Results indicated that the highest absorbance was observed with HCl, Table 1.

Table 1: Effect of the type of the acids.

Type of acid	Absorbance
HCl	0.976
H_2SO_4	0.721
HNO_3	0.572
CH_3COOH	0.302

The optimum volume of HCl was studied using a series of volumes (0.25-2.5 mL) of (1:1) HCl solution. Absorbance results showed that 0.5 mL in alkaline medium recorded the highest absorption. Increase the HCl volume over 0.5 mL reduced the absorbance as shown in Figure 3. The optimum $NaNO_2$ (%w/v) was studied using a series of volumes (0.5-2.5mL). Absorbance results showed that 0.5 mL recorded the highest absorption, Figure 4.

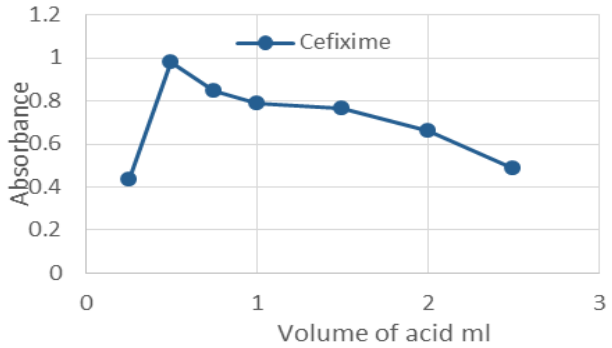

Figure 4: Effect of the volume of (1:1) HCl solution (mL).

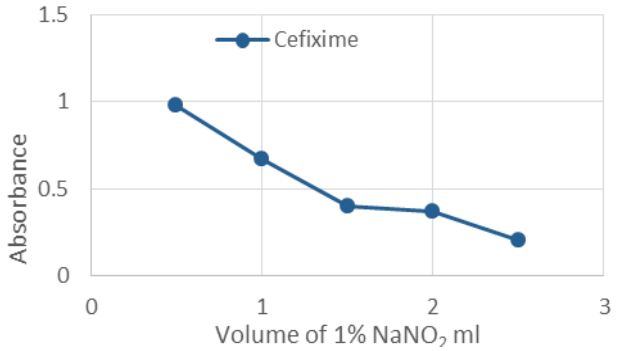

Figure 5: Effect of the volume of 1% (w/v) $NaNO_2$ solution.

The optimum time required to diazotize CFX was found to be 10min as it is illustrated in figure 6. Figure 7 shows that 2mL of 4% urea solution was found to be enough to remove any excess of the formed nitrous acid from diazonium solution. On the other hand, different types of bases (viz; KOH, NaOH, Na_2CO_3, and NH_4OH) in different amounts were tried to provide the alkaline medium necessary for the formation of the azo-dye. The results depicted in Figure 8 suggest that 1.5 mL of 25% NaOH solution was the most effective.

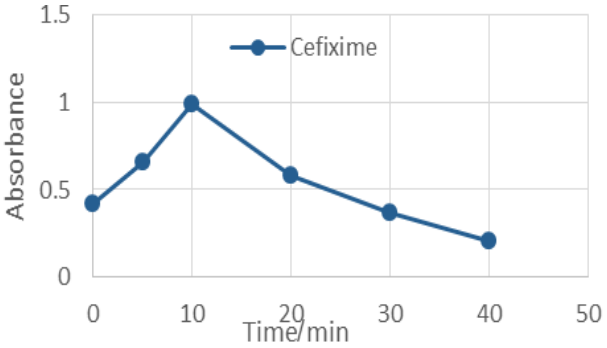

Figure 6: Effect time/min.

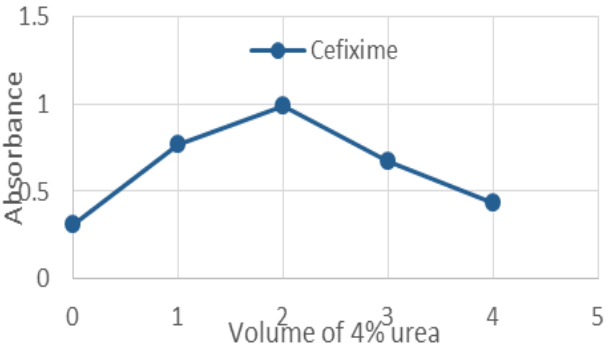

Figure 7: Effect volume of 4%.

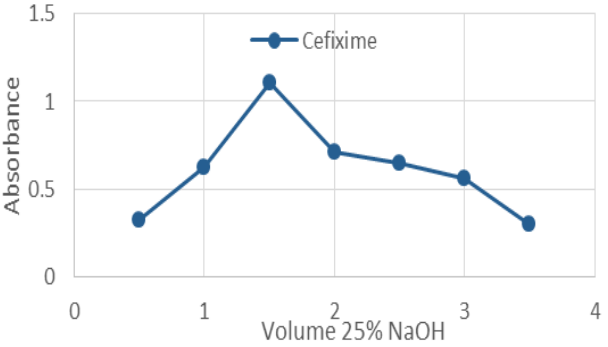

Figure 8: Effect volume of 25% NaOH.

Study the effect of the reagent volume and the nature of the colored dye

The Bisphenol A reagent solution was prepared at a concentration equal to the CFX concentration. So to ensure the same molar ratio is used between the drug and the reagent. Several volumes (0.2-1.4 mL) of 0.252×10^{-2} M Bisphenol A were studied. The best absorption of 1 mL (50 µg/mL) of the drug was at 1 mL of the reagent. That is, the ratio in the resulting colored dye is (1:1) as shown in Figure 9.

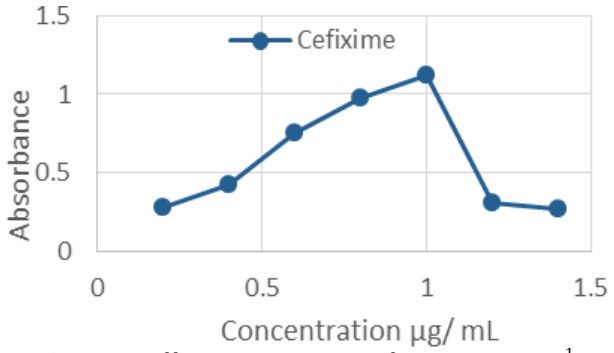

Figure 9: Effect Concentration of reagent µg.mL^{-1}.

The possible reaction path may be written as figure:

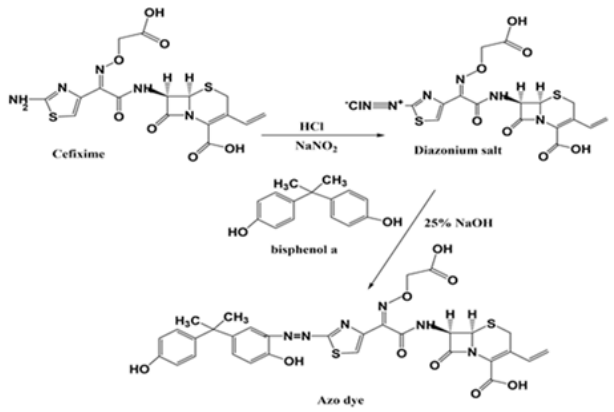

Figure 10: The suggest mechanism of reaction Cefixime drug with bisphenol A colored step.

Analytical characteristics

Under the optimized experimental conditions a calibration graph was constructed by plotting the values of the measure absorbance against the cefixime concentration, Figure 11. Beer's Law was obeyed in the CFX concentration range of (1.0-50.0) µg.mL^{-1} with correlation coefficient (r) of 0.9998. Other analytical parameters are given in Table 2.

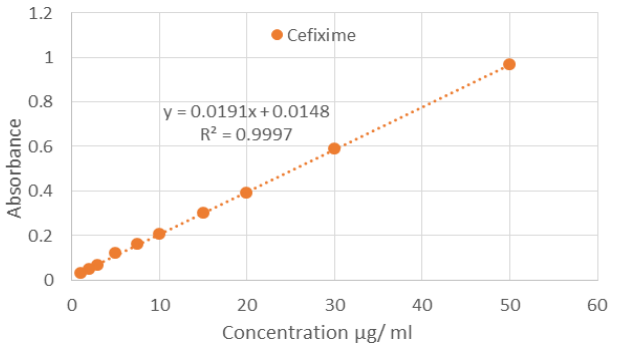

Figure 11: Calibration graph of Cefixime in diazotization method.

Table 2: Characteristic parameter for the regression equation of the proposed diazotization method for Cefixime.

Parameter	Cefixime
λ max(nm)	490
color	Orange
linearity range µg.mL^{-1}	1-50
Molar absorptivity	0.866×104
(L.mol^{-1} cm^{-1})	0.052
Sandell's sensitivity µg/cm^2	0.9998
Correlation coefficient (R)	Y=0.0191x+
Regression equation	0.0148
Slope(b)	0.0191
Intercept(a)	0.0148
Analytical sensitivity µg.mL^{-1}	0.064
Limit of detection µg.mL^{-1}	0.157
Limit quantification µg.mL^{-1}	0.518
C.L. for the slope (b±tsb)at 95%	0.0191±0.000952
C.L. for the intercept (a±tsa at 95%	0.0148±0.01952

Table 3: The accuracy and precision of the proposed method for the determination of pure CFX samples.

Amount of drugs µg.mL^{-1}		Recovery %	RSD% (n=5)
Taken	Found		
10	10.21	102.1	0.10
20	19.88	99.4	0.04
30	29.98	99.93	0.21

Type of Drugs	Amount of drugs µg.mL^{-1}		Recovery %	RSD% (n=5)
	Taken	Taken		
Cefixime (400 mg/capsule) (DAR AL DAWA- Jordan)	400	399.88	99.97	0.16
		396.77	99.19	0.08
		401.88	100.47	0.92
Cefixime (400 mg/capsule) (Pharma International co. Amman- Jordan)	400	403.33	100.83	0.08
		389.88	97.47	0.21
		398.77	99.69	0.13

Interference from excipients

The effect of interference from the presence of the probable excipients in the CFX dosages has been studied under the optimum experimental conditions. This was done by carrying out the determination CFX in the presence of 10 fold excess of each of the studied excipient (viz; [Lactose, Starch, Maltose, Sucrose Fructose, Sodium benzoate). The result shown in Table 5 indicates that the presence of the studied excipients has no significant interference in the spectrophotometric determination of CFX by the recommended procedure.

Table 5: Effect of interference compound on the pure drug.

Interference	Recovery % of Cefixime
compound	100.11
Sucrose	100.43
Lactose	99.89
Maltose	99.97
Fructose	100.89
Sodium benzoate	100.22

Study Optimization of Cloud Point Extraction for CFX

Different experimental parameters that affect the value of the absorbance of the (viz; Triton X-114, cationic surfactant (CTBA), electrolyte salt, the quantity of salt, equilibrium temperature and Incubation time) were investigated.

The study shows that using 1 mL of 10% Triton X-114 solution results in an efficient cloud point extraction, Figure 12. The addition of 2mL of 0.01M of CTBA solution, as a cationic surfactant, improves the extraction efficiency since it increases the hydrophilic characteristic of the micellar [18] phase hence, the added ionic surfactant molecules are shared into non-ionic micelles by changing the surface charge that effects of the repulsion among micelles figure13.

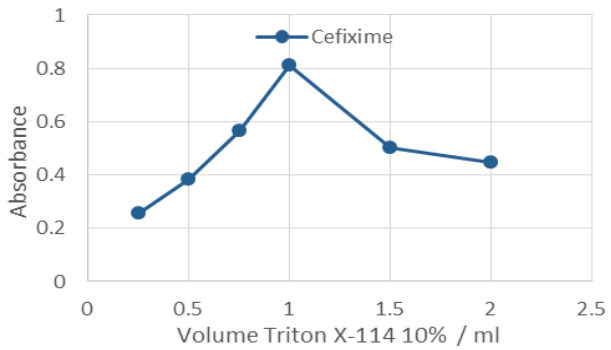

Figure12: Effect volume of Triton X-114/mL.

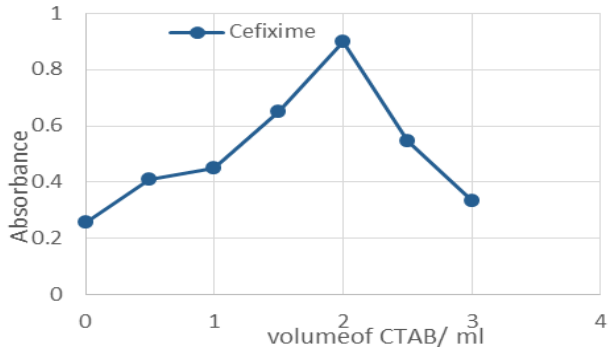

Figure 13: Effect volume of CTAB/mL.

The addition of an electrolyte with a suitable concentration into an aqueous solution of the surfactant micellar system accelerates phase separation and enhances micellar concentration in the surfactant-rich phase due to the salting-out phenomenon. The volume of the surfactant-rich phase will decrease due to the addition of salt, leading to increasing the factor of pre-concentration, however, the surfactant-rich phase will become extra viscous For the selection of an appropriate electrolyte with suitable concentration, the effect of using 5% (w /v) solution of various electrolytes (namely; KCl, HCl, Na_2SO_4, and CH_3COOH) on the extraction efficiency were studied. It was found that 2mL of Na_2SO_4 solution was the best electrolyte and 2 mL the optimum volume required to obtain since it results in the highest extraction efficiency and highest distribution ratio as shown in Figure14.

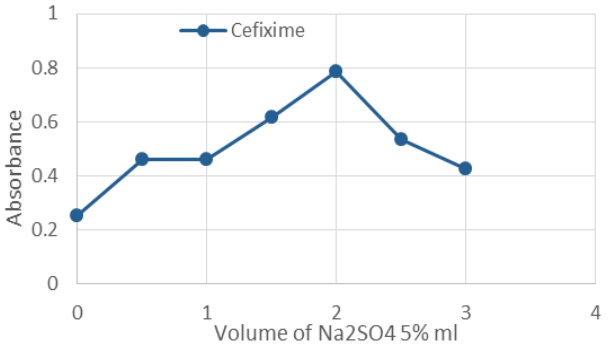

Figure14: Effect volume of Na_2SO_4 5% mL.

Finally, the temperature of the equilibrium and the incubation time that both have a principal role in determining the efficacy of separation and completion of the process were studied. Doing the extraction at 60 °C for 50 min. results in the highest extraction efficiency and absorption signal as shown in Figure15.

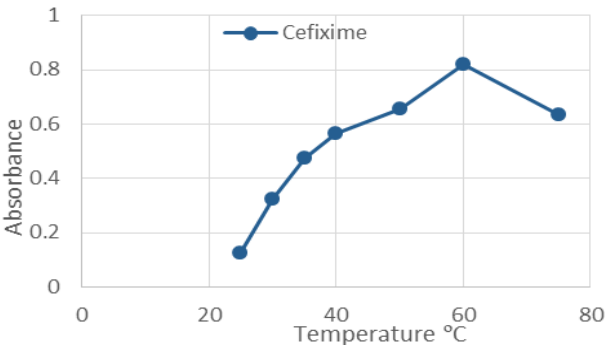

Figure15: Effect of Temperature °C.

Calibration graph of the Cloud Point Extraction

Under the optimized experimental conditions of CPE, calibration curve for CFX determination was built and it shows that a linear relationship was established by plotting the values of CFX concentration in the range of (0.25-6 µg.mL^{-1}) against the measured absorbance, with a correlation coefficient (r) of 0.9998, Table 6. Figure 16 presents the other statistical parameters of the calibration.

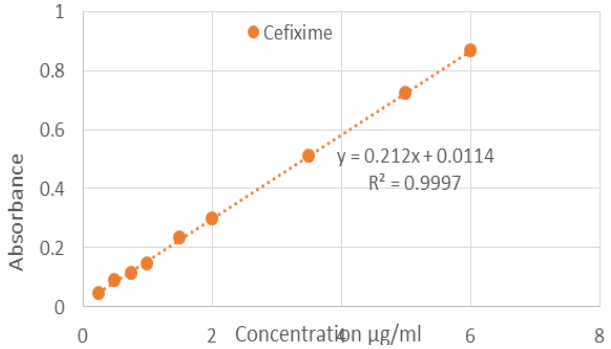

Figure16: Calibration graph of Cefixime by cloud point extraction.

Table 6: Characteristic parameter for the regression equation of the proposed diazotization method for CFX.

Parameter	Cefixime
λ max(nm)	500
color	Purple
linearity range (µg.mL^{-1})	(0.25-6.00)
Molar absorptivity (L.mol^{-1}.cm^{-1})	0.961×10^{5}
Sandell's sensitivity (µg/cm^{2})	0.0047
Correlation coefficient(R)	0.9998
Regression equation	Y=0.212x+0.0114
Slope(b)	0.212
Intercept(a)	0.0114
Analytical sensitivity (µg.mL^{-1})	0.715
Limit of detection (µg.mL^{-1})	0.031
Limit quantification (µg.mL^{-1})	0.094
Enrichment Factor(EF)	11.09
Pre-concentration factor(PF)	25
Distribution coefficient(D)	314.03
C.L. for the slope(b±tsb)at 95%	0.212±0.006678
C.L. for the intercept(a±tsa) at 95%	0.0114±0.020076
Standard error for regression line (Sy/x)	0.005616

Accuracy and Precision

The accuracy and precision of the proposed method were checked by using five replicates of three different CFX concentrations. The results show that the proposed method has reliable accuracy and excellent precision for the determination of CFX in its pure form and in its pharmaceutical preparations. Table 7 & 8.

Table 7: The accuracy and precision of the proposed method for the determination of CFX in of pure samples.

Amount of drugs µg.mL^{-1}		Recovery %	RSD % (n=5)
Taken	Found		
2	1.97	98.5	1.09
5	4.98	99.6	0.83
6	6.12	102.0	0.65

Table 8: The accuracy and precision of the proposed method for CFX determination in commercial pharmaceuticals.

Type of Drugs	Amount of drugs µg.mL^{-1}		Recovery %	RSD% (n=5)
	Taken	Taken		
Cefixime (400 mg/capsule) (DAR AL DAWA - Jordan)	400	400.9	100.23	1.20
		399.9	99.97	0.97
		402.8	100.7	0.73
Cefixime (400 mg/capsule) (Pharma International co. Amman- Jordan)	400	398.9	99.73	0.27
		401.4	100.35	0.69
		404.7	101.17	1.12

FIA-Spectrophotometric determination

A batch method for spectrophotometric determination of CFX was adopted as a basis to develop an FIA procedure. The manifold used for the estimation of CFX was designed to enable the control of different reaction conditions for magnifying the absorbance signal generated by the diazotization of CFX and coupling with Bis phenol A in sodium hydroxide medium.

Optimization of chemical parameters

The optimal experimental conditions of the chemical parameters including the concentration of the reagent, the acid, the sodium nitrate, and the sodium hydroxide solutions were investigated When the total flow rate of the FIA system is 2.5 mL.mim^{-1} the optimum concentrations of the channels streams were 5.99×10^{-3}M, 0.8 M, 1% (wt/v), and 1.5 M for Bisphenol A, HCl, $NaNO_2$, and NaOH solutions respectively, Figures 17-19.

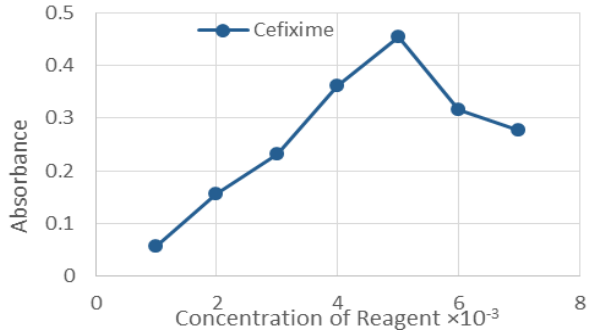

Figure 17: Effect concentration of reagent.

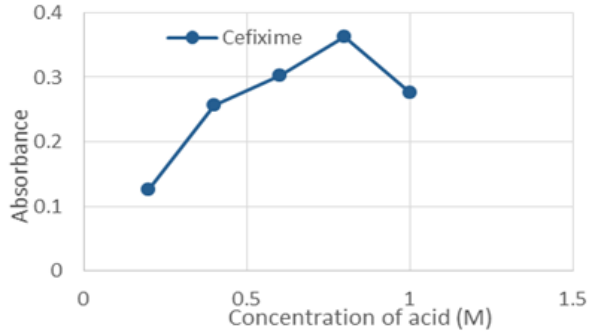

Figure18: Effect concentration of acid.

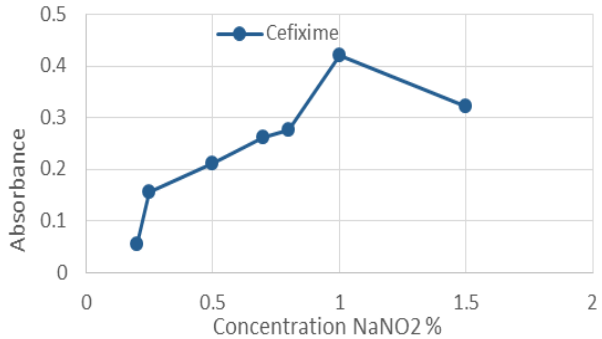

Figure 19: Effect concentration of $NaNO_2$.

Study Optimization of manifold Parameters

Various physical parameters (i.e. coil length, total flow rate, injection volume) affecting the results were studied. The study shows that the best length of the reaction coil was 50 cm when the total flow rate was 2.5 mL.min^{-1}. While, when 100 µl of the sample was injected the highest sensitivity was obtained, Figures 20-22.

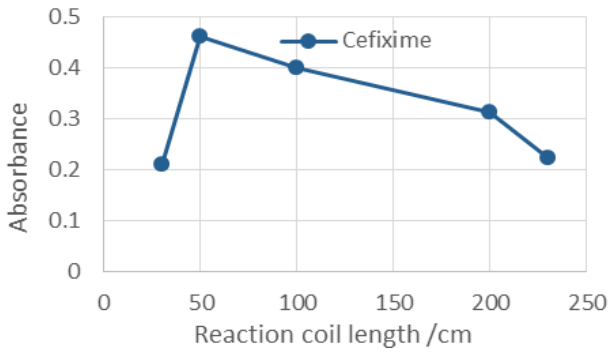

Figure 20: Effect of Length Reaction Coil/cm.

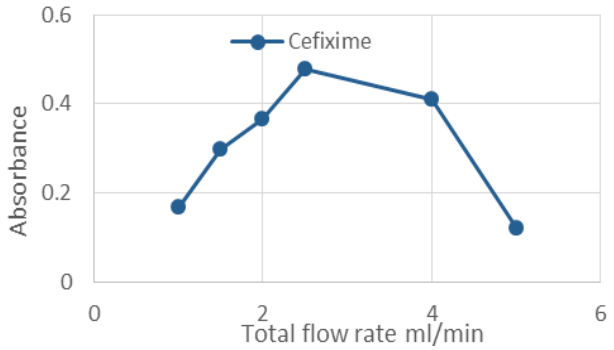

Figure 21: Effect of Total rate mL/min.

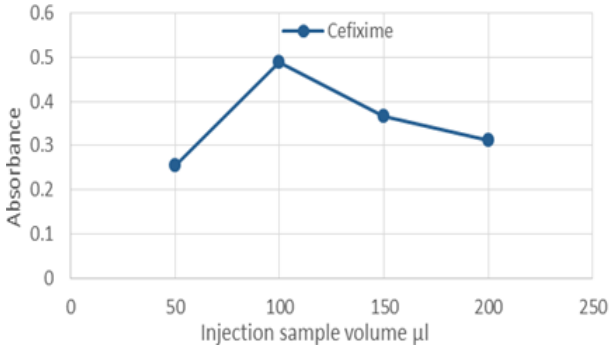

Figure 22: Effect Injection Sample Volume µL.

Analytical characteristics

Under the optimum experimental conditions of the recommended flow injection procedure, a calibration curve was prepared by a plotting the values of the measured absorbance against their respective concentrations of CFX (1-150) µg.mL^{-1}., Figure 23 and Table 9 show plotted graph and the characteristic parameter of the obtained regression equation.

Table 9: data for the regression equation of the Flow.

Parameter	Cefixime
λ max(nm)	490
color	Orange
linearity range (µg.mL^{-1})	1-150
Molar absorptivity (L.mol^{-1}.cm^{-1})	0.304×10^4
Sandell's sensitivity (µg/cm^2)	0.149
Correlation coefficient(R)	0.9997
Regression equation	Y=0.0067x-0.0153
Slope(b)	0.0067
Intercept(a)	-0.0153
Analytical sensitivity (µg.mL^{-1})	0.017
Limit of detection (µg.mL^{-1})	0.045
Limit quantification (µg.mL^{-1})	0.148
C.L. for the slope(b±tsb)at 95%	0.0067±0.000343
C.L. for the intercept(a±tsa) at 95%	-0.0153±0.027069
Standard error for regression line (Sy/x)	0.008081

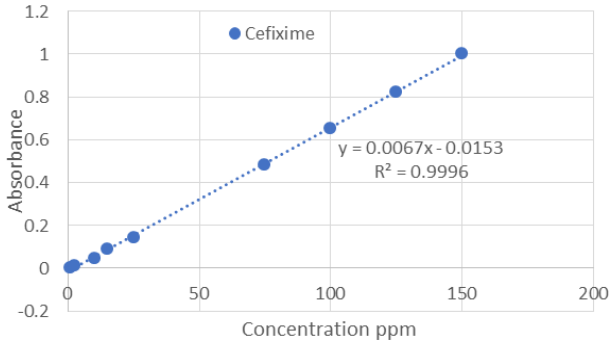

Figure 23: Calbration graph for Cefixime by Flow Injection Analysis.

Accuracy and Precision

The accuracy and precision for the FIA-method were studied, when the recommended procedure was applied to determine the five replicates of the concentration of CFX at three concentration levels of the drug in its pure form and it present in pharmaceutical preparations. Tables 10 and 11 show the method is accurate and precise and could be applied successfully for this purpose.

Table 7: The accuracy and precision of the proposed method for the estimation of CFX in pure samples.

Amount of drugs µg.mL^{-1}		Recovery %	RSD % (n=5)
Taken	Found		
10	9.89	98.90	0.76
30	29.94	99.60	0.89
50	50.33	100.66	0.55

Table 8: The accuracy and precision of the proposed method for CFX determination in commercial pharmaceuticals.

Type of Drugs	Amount of drugs µg.mL^{-1}		Relative Error %	Recovery %	RSD% (n=5)
	Taken	Taken			
Cefixime (400 mg/capsule) (DAR AL DAWA - Jordan)	400	399.68	-0.32	99.68	0.65
		401.60	0.40	100.40	0.94
		398.89	-0.27	99.72	0.88
Cefixime (400 mg/capsule) (Pharma International co. Amman-Jordan)	400	397.88	-0.53	99.47	0.44
		394.99	-1.25	98.75	0.69
		402.55	0.64	100.64	0.89

Table 12: Comparison the proposed method with stander.

Pharmaceutical preparation	Proposed methods						Standard method[19]
	Rec% Batch method	Value		Rec% FIA method	Value		Standard method[19]
		t	F		t	F	
Pure Cefixime	100.32	1.22 (2.131)	2.01 (19.00)	99.72	1.550 (2.131)	4.878 (19.00)	99.12
Cefixime (400 mg/capsule) (DAR AL DAWA- Jordan)	99.87			99.93			99.32
Cefixime (400 mg/capsule) (Pharma International co. Amman- Jordan)	99.33			99.62			99.80

method using t and F- Statistical test at 95% confidence level

Conclusions

Three new simple, sensitive and inexpensive methods for spectrophotometric determination of CFX were developed. In the first, the drug was diazotized and coupled to produce an orange azo-dye product which could easily be determined by measuring its absorbance at 500nm. Could point extraction procedure was developed for the extraction of the formed azo-dye in the second method. An FIA technique was used to semi-automate the batch spectrophotometric method for the determination of the cited drug. The three proposed methods were successfully applied for the determination of pure CFX and in pharmaceutical dosage. Table 12 shows a comparison between the suggested method with a standard method.

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