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Cloud Point Extraction for the Spectrophotometric Determination of Cefdinir

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Abstract

In this study, two spectrophotometric methods were developed for the estimation of cefdinir (CFD). The first method depends on the conversion of cefdinir to diazonium saltof cefdinir, and then coupled with the 2, 5-Dimethylphenol (2, 5-DMP) reagent in the alkaline medium. The formed azo dye has a purple color with absorption intensity at λ_{max} 510 nm. Concentration range was obeyed Beer's law at (1-50 µg/ml), the correlation coefficient was 0.9998, molar absorptivity was 1.554×10^4 $L.mol^{-1}.cm^{-1}$and the detection limit was 0.097 $\mu g.ml^{-1}$.The second method involves cloud point extraction (CPE) of a trace amount of the formed azo dye in the first method followed by measuring with a UV-visible spectrophotometer. Concentration range that obeyed the Beer's law was (0.1-6.0 µg/ml). The correlation coefficient was 0.9998. Molar absorptivity was $1.52156\times10^5 L.mol^{-1}.cm^{-1}$. The detection limit was 0.010 $\mu g.ml^{-1}$, the Pre-concentration factor was 25 and the Distribution coefficient was 3906. The proposed methods were applied and it proved their compatibility for estimating of ingredient compound in pure samples and pharmaceuticals by comparing them with previous studies.

Keywords: Cefdinir, Diazotization, Cloud Point Extraction, 2, 5-Dimethylphenol.

الخلاصة

في هذه الدراسة، تم تطوير طرق طيفية جديدة لتقدير السيفديني(CFD) ، يتكون البحث من طريقتين. تعتمد الطريقة الأولى على تحويل السيفديني إلى ملح الديازونيوم للسيفديني ثم اقترانهُ مع الكاشف 2,5-Dimethylphenol (2,5-DMP)في الوسط القلوي. صبغةالآزو المتكونة ذات اللون الأرجواني مع اعلى شدة امتصاص عند λ_{max}510 نانومتر. اطاع قانون بير مدى التراكيز (١-٥٠ ميكروغرام/مل)، وكان معامل الارتباط ٠,٩٩٩٨، والممتصية المولارية 1.554×10^4 لتر.مول$^{-1}$. سم$^{-1}$ وكان حد الكشف ٠,٠٩٧ ميكروغرام / مل. أما الطريقة الثانية فتشمل الاستخلاص بنقطة الغيمة (CPE) لكمية ضئيلة من السيفديني في صبغةالآزوالمتكونة في الطريقة الأولى متبوعة بالقياس باستخدام مطيافالأشعة فوق البنفسجية-مرئية. مدى التراكيز الذي اطاع قانونبير(0.1-6.0 ميكروغرام/مل)، وكان معامل الارتباط ٠,٩٩٩٨، والممتصية المولارية 1.52156×10^5 لتر.مول$^{-1}$.سم$^{-1}$، وحد الكشف كان 0.010 ميكروغرام. مل$^{-1}$،وعامل التركيز ٢٥، ومعامل التوزيع ٣٩٠٦.تم تطبيق الطرق المقترحة وأثبتت توافقها لتقدير المركب الفعّال في العينات النقية والمستحضرات الصيدلانية بمقارنتها مع الدراسات السابقة.

Introduction

Cefdinir (CFD) is an antibiotic belonging to the third-generation broad spectrum of the Cephalosporin family, which belongs to the beta-lactam class that has the molecular formula $C_{14}H_{13}N_5O_5S_2$ and its molecular weight 395.416 g / mol, a semi-artificial antibiotic, its scientific name under IUPAC is:(6R, 7R)-7-[[(2Z)-(2-Amino-4-thiazolyl) (hydroxyimino) acetyl] amino]-3-ethenyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid[1] (Figure 1).

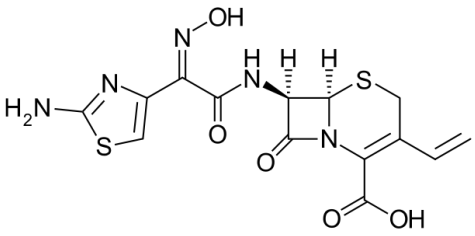

Figure 1: Chemical structure of cefdinir.

As with other cephalosporin's, bactericidal activity of cefdinir results from the inhibition of cell wall synthesis [2]. Cefdinir is highly effective against many gram positive and gram

negative bacteria and proven effective for common bacterial infections of the ear, sinus, throat and skin and it is utilized to treat otitis media, delicate tissue diseases, and respiratory tract contaminations, including sinusitis[3,4].

In the literature, CFD has been analyzed in several methods, including liquid chromatographic-tandem mass spectrometric LC–MS/MS [2]**,** spectrophotometric[5,6], RP-HPLC[7,8], colorimetric[9], voltammetry[10], and spectrofluorometric[9].

The analysis of trace amounts of organic compounds in different environmental and biological samples may be considered as a difficult analytical mission, mostly due to their low concentration and complex sample matrix, which needs advanced instrumental techniques or a pre-concentration method like cloud point extraction (CPE). The cloud point extraction method has generated widespread interest as an alternative to conventional extraction[11]. In aqueous solutions for the surfactant micellar systems, the temperature at which the solution becomes turbid before separation into two phases (a surfactant-richphase and an aqueous phase) known as the cloud point[12].

This research involves two methods, the first method depends on Diazotization of cefdinir with nitrous acid, to form diazotized cefdinir, followed by its coupling with 2,5 - Dimethylphenol (2,5-DMP) to form a purple colored product (azo dye) with the absorption maximum at 510 nm. The second method, aimed to develop a new CPE procedure for selective separation and pre-concentration of cefdinir dye from the commonly encountered matrix components prior to its determination using visible spectrophotometry.

These methods have several advantages including low cost, sensitivity, accuracy, rapidity, Low toxicity, the simplicity of procedure and environmentally friendly and it was applied to pharmaceuticals and it was proved successful in estimating the effective ingredient.

Experimental

Instrumentation

The advanced microprocessor UV-Vis spectrophtometer single beam LI-295 (Lasany®-India) was connected to a computer fitted was used for spectral measurements with a 1.0 cm quartz cell. A thermostatic water bath and ultrasonic, from Elma Hans Schmidbauer GmbH &Co. KG, was used to mix samples with non-ionic and cationic surfactant and to study the effect of temperature on cloud point extraction. Electronic Balance Sensitive Adventurer pro AV264, Switzerland, was used for precise weight. A centrifuge (HERMLE LABORTECHNIK Z 200 A, Germany) was used to complete the separation of the two phases. pH meter used for acidity measurement, type inoLab7110.

Chemicals and Reagents

All chemicals were analytical quality and were bought from Merck Ltd. (Darmstadt, Germany). Cefdinir wasgained from the quality control laboratory (The General Company for the manufacture of medicines and medical supplies - Samarra).

Preparation of standard solution

The standard stock solution of cefdinir

The standard solution of pure CFD (1000µg/ml, 0.252×10^{-2}M) was prepared by dissolving 0.1 g in distilled water with a small dropsof NaOH (1 M) and then the volume was completedto 100 ml in a volumetric flask. This solution should be prepared weekly because it oxidizes when it stay long.

The standard solutions of pharmaceutical

Capsules: the contents ten cefdinir capsules were weighed for the commercial drugs (sefarin®) and (Azord®) each capsule separately, and then the mean weight of the capsule was extracted. The sum weights were 3.5512 g, 3.6672g, while the average capsule was 0.35512 g, 0.36672g, respectively. Then take aliquot amount for both drugs(Azord®) and(sefarin®), respectively and dissolve in distilled water with drops of(1M) NaOH and then complete the volumein a volumetric flask to 100 ml, after which the solutions were filtered to get cleared of the insoluble remains.

Preparation of 2, 5-DMP solution

A 2,5-Dimethylphenol (2,5-DMP) solution was prepared at a concentration of 0.252×10^{-2}M by dissolving 0.0308 g in the distilled water with a small drops of NaOH (1M) and then completing the volume with distilled water to 100 ml in a flask Volumetric.

Other solutions

(8.8 M) (1:1) H_2SO_4, (1% w/v, 0.144M) NaNO2, 4% w/v urea, 50% w/v (8.93M) KOH, 5% w/v Na_2SO_4, 0.01M (0.3644g in 100ml in distilled water) hexadecyltrimethylammonium bromide (CTAB) and 10% v/v Triton X-114, solutions were prepared in distilled water.

General procedure of calibration curve for the diazotization-coupling method

After selecting optimal conditions, the calibration curve was prepared by transferring several concentrations (1-50 µg / ml) from the standard solution (1000 µg / ml) to a series of volumetric flasks (20 ml). Then, 0.25 ml and 0.5 ml of (1:1) H_2SO_4 and (0.144 M) $NaNO_2$were added respectively, and all these volumetric flasks into the ice bath (≤4°C) and it wait for 15 minutes. After that, 1ml of the reagent 2, 5 –DMP was added. Thereafter, 1 ml of (8.93M) KOH was added and shake the flasks. Finally, the azo dye formed (CFD-2, 5-DMP)was measured at the highest absorption which is λ_{max} 510 nm against the reagent blank.

General procedure of the (CPE)

the calibration curve was constructed through a series of different concentrations (0.1-6.0 µg/ml) of the azo dye (CFD-2, 5-DMP) in centrifuge tubes (15 ml), and mixed it with 1 ml, 1.5 ml and 2.5ml of Triton X- 114 10% v/v, 0.01 M (CTAB) and 5% w/v Na_2SO_4 respectively. After that, the volume was completed with distilled water up to 12.5 ml and the tubes were transferred to the ultrasonic-thermostatic water bath device.The samples were placed under the ultrasonic effect for 2 minutes and then in the water bath at 50 ° C for 45 minutes. Then, the tubes were transferred to the centrifuge for 5 minutes at 4000 rpm. Thereafter, the tubes were transferred to the ice bath for 10 minutes to stabilize the micelle layer at the bottom of the tube. Then the aqueous phase was poured. Finally,0.5 ml of ethanol was added for dissolve the micelle layer and absorption measurement of the dye at λ_{max} 525 nm against the reagent blank in UV-Vis spectrophotometer using a quartz cell (1 cm, 1 ml).In the same steps,the blank solution was prepared and measured.

Calculations

The limit of detection was calculated as three times the ratio between the standard deviation of 10 blank signals and slope of the calibration curve ($3S_B/m$). The limit of quantification was calculated as ten times the ratio between the standard deviation of 10 blank signals and slope of the calibration curve ($10S_B/m$)[13]. The enrichment factor wascalculated as the ratio between the analyte concentration in the Surfactant-rich phase and the analyte concentration in the initial aqueous solution.The preconcentration factor was calculated as the ratio of the volume of the initial solution to that of the final solution after preconcentration. The distribution coefficient was calculated as the ratio between the analyte concentration in the surfactant-rich phase and the analyte concentration in the aqueous phase[14].

Results and Discussion

Part I. the diazotization-coupling method

Figure 2 shows spectra of (100 µg/ml) solution of CFD-2, 5-DMP against the reagent blank solution recorded under the optimal conditions.

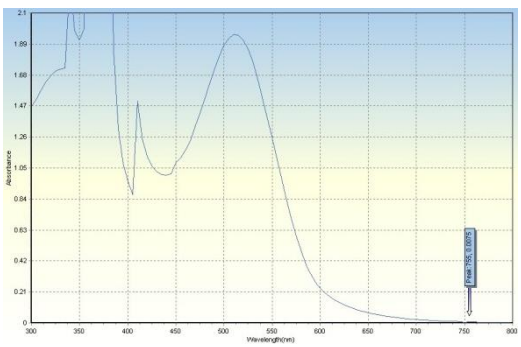

Figure 2: Absorption spectra of 50 µg/ml of CFD-2,5-DMP against the reagent blank at 510 nm.

Optimization experimental conditions

The effects of the different variables on the absorption intensity were studied to determine the optimum conditions in the CFD estimate. The concentration of the CFD studied was 50 µg / ml in 20 ml volumetric flask.

The effect of type acid was studied, several acids (HCl, H_2SO_4, HNO_3, and CH_3COOH) diluted (1:1) that were tested in the reaction of diazotization-coupling, and the highest absorption was obtained when sulfuric acid was used**.**

Various volumes (0.25-2.00 ml) of (1:1) H_2SO_4 were studied in the diazotization-coupling reaction and the highest absorption was reached when using 0.25 ml because the diazotization reaction was done in alkaline medium and an increase of acidity leads to reduced absorption, as it is shown in Figure 3.

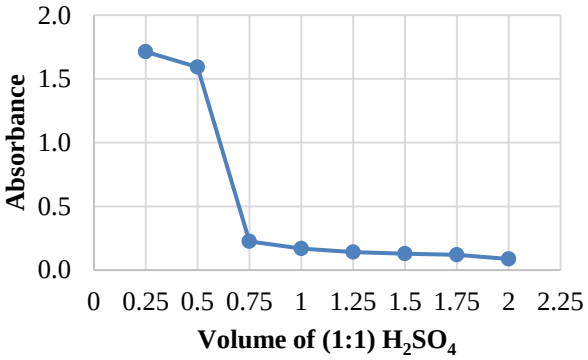

Figure 3: Effect of (1:1) H_2SO_4 volume.

The effect of volume $NaNO_2$ was studied by various volumes (0.25-2.00ml) of 0.144M (1% w/v $NaNO_2$) were tested in the diazotization process and it was found that 0.5 ml gave the optimum absorption intensity, as it is shown in Figure 4.

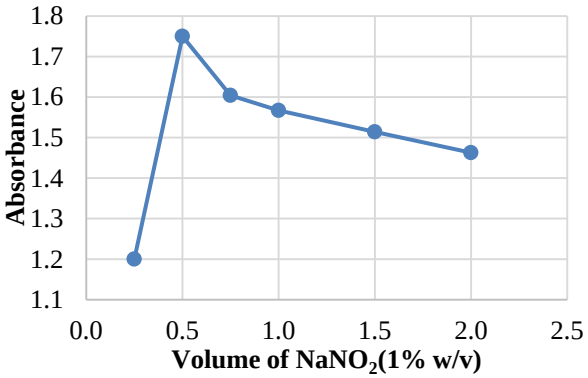

Figure 4: Effect of (1% w/v $NaNO_2$) volume.

After adding nitrite to the mixture (CFD + acid) in the ice bath, different waiting intervals were tried at (5-30 minutes). It was found that the waiting time of 15 minutes was the optimum time to get the highest absorption intensity, as it is shown in Figure 5.

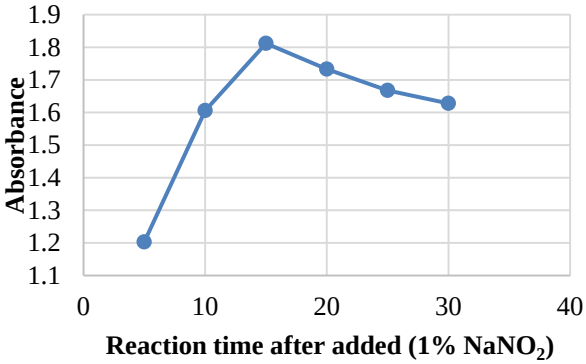

Figure 5: Effect of reaction time.

Nitrite acid is the result of an increase in sodium nitrite that leads to side reactions[15]. Therefore, it should be removed by urea and according to the following equation[16,17]:

$$H_2NCONH_2 + HNO_2 \rightarrow CO_2\uparrow + 2N_2\uparrow + 3H_2O$$

The effect of different volumes of 4% urea solution (0-4 ml) was studied. The highest absorption was obtained when urea was not added, indicating no increase of sodium nitrite.

The effect of three types of base (KOH, NaOH, and NH3) was studied on the diazotization-coupling reaction. It was found that KOH gives the highest absorption intensity in this reaction. Therefore, the effect of different volumes of 8.93 M KOH (0.25-2.00 ml) was studied on absorption. 1 ml was the optimum volume to obtain the highest absorption as it is shown in Figure 6.

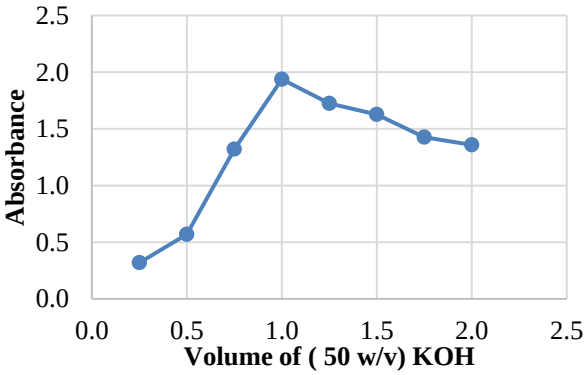

Figure 6: Effect Of (50 W/V) KOH Volume.

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

2, 5-DMP reagent solution was prepared at a concentration equal to the CFD concentration. Therefore, this study is the same method of mole ratio, through which it is possible to know the ratio between the drug and the reagent. Several volumes (0.25-2.00 ml) of (0.252×10^{-2}M) 2, 5- DMP were studiedwith1 ml (0.252×10^{-2}M) of drug. The optimum absorption was obtained at 1 ml of the reagent. Then, the absorption was almost stabilized. That is, the ratio in the resulting colored dye is (1:1), as it is shown in Figure 7.

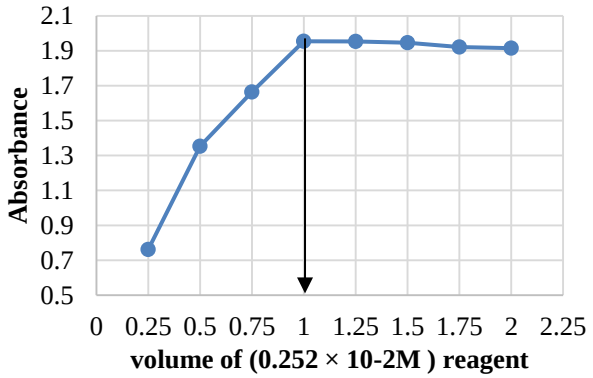

Figure 7: Effect of (0.252×10^{-2}M) reagent volume.

The possible reaction mechanism can be illustrated in the following Figure 8:

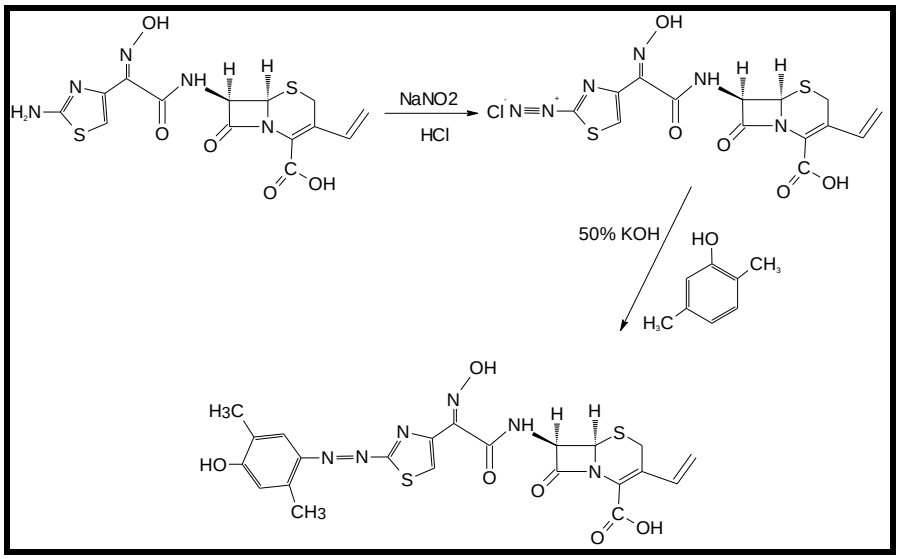

Figure 8: Expected reaction mechanism.

Effect of Sequence of Additions

The effect of the sequence of additives on the absorption intensity has been tested. Sequencing (diazo salt + reagent + base) was preferable to obtain highest absorption, as it is shown in Table 1.

Table 1: Effect of Sequence of Additions.

Sequence of Additions	Abs at λ_{max} 510 nm (50 µg/ml)
Salt+ reagent+ base	1.941
Salt+ base + reagent	0.726
Salt+ (reagent+ base)	1.657

Effect of Interferences

The several types effect of common additives to commercial pharmaceuticals has been studied; 250µg/ml of foreign excipients was added to 50 µg/ml of the drug, where the foreign excipients quantity is five times larger than the amount of drug. Results of the recovery in Table 2 show that the effect of the interference is no significant.

Table 2: The effect of adding foreign compounds.

Foreign Compound	% Recovery of 50µg/ml CFD per 250 µg/ml Foreign compound added
Sucrose	99.59
Fructose	99.59
Lactose	99.58
Maltose	99.59
Sodium benzoate	99.54
Starch	99.59

Analytical data for diazotization method

After determining the optimal experimental conditions, the calibration curve was prepared as it is shown in Figure 9 and Table 3 calibration curve and analytical parameters, respectively.

Table٣: Analytical data for diazotization method.

Parameter	CDN-2,5-DMP
Color of product	purple
λ max (nm)	٥١٠
Dynamic range (µg.ml^{-1})	(1-50)
Molar absorptivity, Ɛ (L.mol^{-1}.cm^{-1})	1.554×10^4
Regression equation	y = 0.0393x - 0.0167
Sandell sensitivity S (µg.cm^{-2})/0.001A.U	0.0254
Intercept (a)	-0.0167
Slope (b)	0.0393
Coefficient of determination % R^2	99.97
Correlation coefficient (r)	0.9998
Limit of detection (µg.ml^{-1})	0.097
Limit of quantification (µg.ml^{-1})	0.323
C.L. for the slope (b±tsb) at 95%	0.0393 ± 0.0006
C.L. for intercept (a±tsa) at 95%	- 0.0167 ± 0.0124
Std. error for regression line ($S_{y/x}$)	0.0118

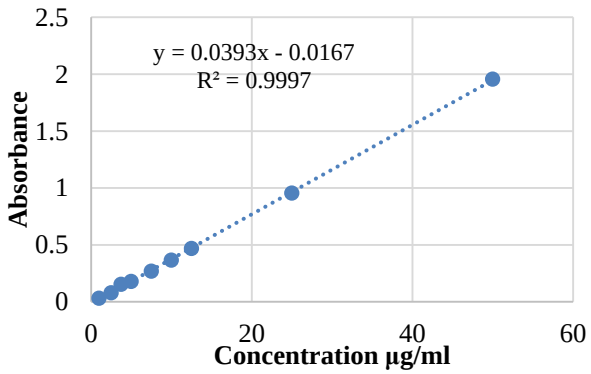

Figure 9: Calibration curve of CFD-2, 5-DMP.

Precision and accuracy

Three different concentrations and five replicates for each concentration were selected to determining the precision and accuracy of the pure drug and pharmaceuticals as it is shown in Table 4 and 5. the t-test and F-test value were obtained and compared with the published method[18]. Results show that the proposed method is acceptable, accurate and it can be used in drug evaluation in pharmaceuticals.

Table 3: Accuracy and precision of the diazotization proposed method for drug pure.

Amount of CFD (µg/ml)		E_{rel} %	t-value	F-value	RSD% (n=5)
Taken	Found*				
10	-0.08	9.99±0.1			0.97
25	-0.18	24.95±0.1	1.69	9.59	0.30
50	0.34	50.17±0.2			0.40

Table 4: The accuracy and precision of the proposed method of drug evaluation in commercial pharmaceuticals.

Type of pharmaceutical product	Amount of CFD (µg/ml)		% Recovery	Average% Recovery**	RSD% (n=5)
	Taken	Found*			
sefarin® capsules 300 mg/product by pharma international Co. Amman-Jordan	10	9.84±0.1	98.40	98.64	0.98
	25	24.65±0.1	98.60		0.36
	50	49.46±0.06	98.92		0.13
Azord® capsules 300 mg/product by DAR AL DAWA	10	9.79±0.06	97.89	98.32	0.66
	25	24.58±0.05	98.31		0.20
	50	49.39±0.07	98.77		0.08

* Mean ± SD of five replicates. **Mean of three concentrations. Critical values at 95% confidence limits, t=2.78, F=19

Part II. Cloud point extraction method

The trace concentration of the CFD in the azo dye is estimated by the UV-visible spectrophotometer to be inaccurate due to several factors: detector sensitivity, amplitude efficiency, interference. Therefore, the pre-concentration of trace concentrations by cloud-point extraction (CPE) will increase the enrichment factor and remove the interference effect, thus improve the detection limit and accuracy in the estimation. In the preliminary study, 4 µg / ml of the CFD-2, 5-DMP dye was used. Its pH is 12 and the maximum wavelength is 525 nm (the peak of absorption was shifted due to the solvent changing from water to ethanol).

Optimization experimental conditions

Surfactant concentration is important in determining the value of the pre-concentration factor, so the appropriate surfactant concentration should be selected until the

analyte is fully extracted [12,19]. Several volumes (0.25-2.00ml) were tested from 10% w/v Triton X-114, and it found that 1 ml gave the optimum extraction efficiency as shown in Figure 10.

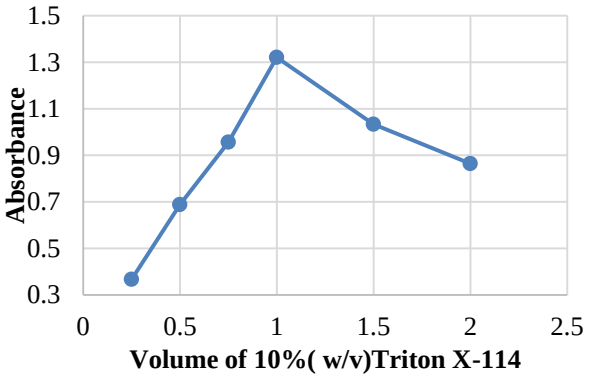

Figure 10: Effect of (10% w/v) Triton X-114 volume.

To increase the hydrophilic characteristic of the micellar phase, cationic surfactant molecules (CTAB) are added that are incorporated into non-ionic micelles and lead to changing the surface charge to increasing repulsion between micelles, thus increasing the cloud point[20].Therefore, the effect of a different volumes (0-3 ml) of (0.01M) CTAB on the extraction efficiency were studied. It found that 1.5 ml gave the highest distribution ratio and highest absorption as shown in Figure 11.

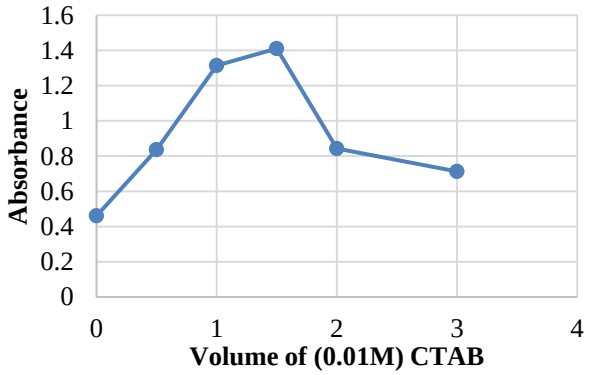

Figure 11: Effect of (0.01M) CTAB volume.

The addition of electrolyte with a suitable concentration in 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. Therefore, the volume of surfactant-rich phase will decrease due to the addition of salt, leading to an increase in the pre-concentration factor, but the surfactant-rich phase will become more viscous[12]. For the selection of salt and the appropriate concentration from it, several electrolytes (KCl, NaCl, Na_2SO_4, and CH3COONa) were studied at a concentration of 5% w / v of each salt. It was shown that, 2.5 ml of Na_2SO_4 was the optimum of type and quantity of salt to obtain the highest extraction efficiency and distribution ratio (D).

The temperature of the equilibrium and the equilibration time has an important role in the efficiency of separation and completion of the reaction. Therefore, different temperatures (40-80 °C) and several equilibration times (30-90 min) were studied. The highest extraction efficiency and absorption signal were obtained at 50 °C and 45 minutes.

pH was an important factor in the selection of the resulting color pigment and the pre-concentration factor[21]. A pH range (4-14) was tested and pH 12 was the optimum in the extraction of CFD-2, 5-DMP, and was therefore adopted in the optimal experimental conditions.

Analytical data of proposed method (CPE)

The calibration curve was constructed by measuring the difference between the absorbance signals of the sample and blank as a function of the standard cefdinir concentration. The calibration curve and analytical figures of merit of proposed method are shown in Figure 12 and Table 6.

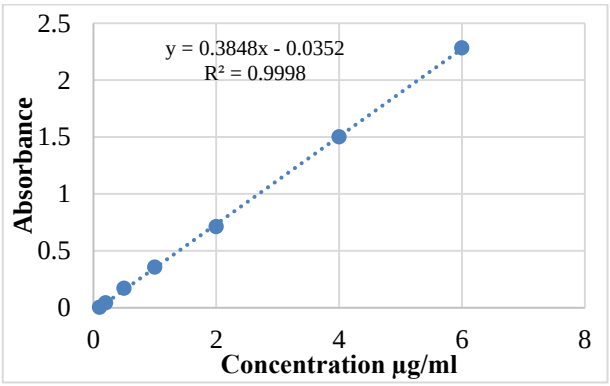

Figure 12: Calibration curve of CPE method.

Table 5: Analytical data of CPE method.

Parameter	CFD-2,5-DMP
Color of product	Purple
λ max (nm)	٥٢٥
Dynamic range (µg.ml^{-1})	(0.1-6.0)
Molar absorptivity, Ɛ (L.mol^{-1}.cm^{-1})	1.52156×10^{5}
Regression equation	y = 0.3848x-0.0352
Sandell sensitivity S (µg .cm^{-2})/0.001A.U	0.0026
Intercept (a)	-0.0352
Slope (b)	0.3848
Coefficient of determination % R^2	99.98
Correlation coefficient (r)	0.9999
Limit of detection (µg.ml^{-1})	0.010
Limit of quantification (µg.ml^{-1})	0.033
C.L. for the slope (b±tsb) at 95%	0.3848±0.0061
C.L. for intercept (a±tsa) at 95%	-0.0352±0.0174
Std. error for regression line ($S_{y/x}$)	0.0129
Enrichment (EF)factor	24.85
Preconcentration factor (PF)	25
Distribution coefficient (D)	3906

Accuracy and precision of the CPE for CFD

To check the possibility of using the method in the estimation of CFD, the study of precision and accuracy were performed. Three different concentrations of five replicates were tested for both pure medicine and pharmaceuticals. The results in Tables 7 and 8 indicate that the proposed method is accurate and acceptable through t-test and F-test values. Which were obtained by comparing the proposed method with the reported method[18].

Table 6: Precision and accuracy of CPE method for pure CFD.

Amount of CFD (µg/ml)		E_{rel} %	t-value	F-value	RSD% (n=5)
Taken	Found*				
2	1.99±0.01	-0.52			0.48
4	3.99±0.01	-0.26	1.87	3.57	0.15
6	6.02±0.01	0.36			0.11

Table 7: Precision and accuracy of CPE method of CFD in pharmaceuticals.

Type of pharmaceutical product	Amount of CFD (µg/ml)		% Recovery	Average% Recovery**	RSD% (n=5)
	Taken	Found*			
sefarin® capsules300mg/product by pharma international Co. Amman-Jordan	2	1.98±0.01	99.01	98.92	0.58
	4	3.98±0.02	99.42		0.52
	6	5.90±0.01	98.33		0.18
Azord® capsules 300mg/product by DAR AL DAWA Development& Investment CO. LTD (Na'ur-Jordan)	2	1.97±0.01	98.73	98.53	0.63
	4	3.95±0.01	98.74		0.23
	6	5.89±0.01	98.11		0.17

* Mean ± SD of five replicates. **Mean of three concentrations. Critical values at 95% confidence limits, t=2.78, F=19

Conclusions

The current study introduced cloud point extraction for the estimation of cefdinir for the first time. The proposed spectrophotometric method is based on the chromatic intensity of the resulting dye in the determination of different CFD concentrations as well as the proposed spectrophotometric-CPE to estimate the CFD trace concentrations in dye produced by the diazotization-coupling method. This method is a qualitative detection of the CFD at the maximum wavelength of 510 nm. This method proved its accuracy and acceptance is by measuring several different concentrations of several replicates and comparing them with the method reported.

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