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New Spectrophotometric Estimation and Cloud Point Extraction of Cefdinir

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Abstract:

A sensitive spectrophotometric method was developed for the estimation of cefdinir (CFD), a cephalosporin species. This study involves two methods, and the first method includes the preparing of azo dye by the reaction of CFD diazonium salt with 4-Tert-Butylphenol (4-TBP) and 2-Naphthol (2-NPT) in alkaline medium, which shows colored dyes measured at λ_{max} 490 and 535 nm, respectively. Beer's law was obeyed along the concentration range of (3-100) μg.ml^{-1}. The limits of detection were 0.246, 0.447 μg.ml^{-1} and molar absorptivities were 0.6129×10^4, 0.3361×10^4 L.mol^{-1}cm^{-1} for (CFD-4-TBP) and (CFD-2-NPT), respectively. The second method includes preconcentration for cefdinir dyes by using cloud point extraction in the presence of Triton X-114 (10% v/v) and recording measurements using the UV-Visible technique. Cloud point extraction enables the drug to be precisely estimated under the optimal experimental conditions. The concentrations were ranged between (0.1-6.0) and (0.2-6.0) μg.ml^{-1}. The limits of detection were 0.032, 0.054 μg.ml^{-1} and molar absorptivities were 0.4733×10^5, 0.2788×10^5 L.mol^{-1}cm^{-1}, respectively. Enrichment factors were 24.61, 24.58, and distribution coefficients were 1526, 1393 for (CFD-4-TBP), (CFD-2-NPT), respectively. The proposed methods have been applied for the determination of CFD in commercial formulation with no interference. The results appear to be no significant difference between the two methods.

Keywords: Cefdinir, Cloud point, Diazotization, Spectrophotometric, Triton X-114.

Introduction:

Chemically, cefdinir (CFD) is [6R- [6α, 7β (Z)]]-7- [[(2- amino-4- thiazolyl) (hydroxyimino) acetyl] amino]-3-ethenyl-8-oxo-5-thia-1- azabicyclo [4.2.0] oct-2-ene-2-carboxylic acid (Fig. 1). It is a broad-spectrum, semi-synthetic, and third-generation cephalosporin (1). The molecular formula of CFD is $C_{14}H_{13}N_5O_5S_2$, with a molecular weight of 395.42 (2). It has a broad spectrum of activity with excellent therapeutic action, specifically antimicrobial activity, against susceptible Gram-positive bacteria and Gram-negative bacteria. It also has excellent efficacy, convenient dosing and favourable tolerability compared with other antimicrobial agents. CFD was studied the United States Pharmacopoeia (USP) and Japanese Pharmacopoeia (JP) (3). Both USP and JP use a high-performance liquid chromatography (HPLC) method when assaying the raw material, capsules and oral powder for suspension.

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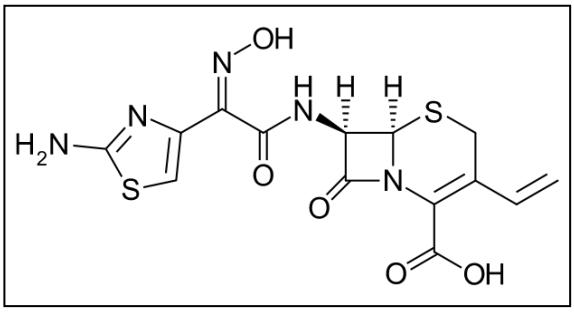

Figure 1. Structural formula for cefdinir

Many studies have been performed which estimate CFD in pharmaceutical preparations, involving HPLC (4), spectrofluorometric (5), spectrophotometry (5,6), HPLC-MS/MS (2) and electrochemical methods (7). The extraction of Cloud point is a process of separation, pre-concentration, fast, selective and sensitive method that has been widely applied to micro quantities of inorganic and organic species (8). CPE is an analytical method that has the ability to improve the detection limit and other analytical parameters, which studies the separation and pre-concentration of micro amounts of generally hydrophobic organic compounds and elements (9). Several papers were published using these methods to estimate active ingredients in industrial drugs (10,11). Cloud point

extraction has several advantages, such as inexpensive, high pre-concentration efficiency, reduced toxicity, simple procedure and green chemistry. Using these techniques, a hydrophobic analyte can be concentrated to a small volume of the surfactant-rich phase (12). The separated surfactant-rich phase can be directly subjected to HPLC or flow-injection analysis (13,14). In this study, a new analytical method was developed for estimation of cefdinir, which is based on the diazotization-coupling reaction and cloud point extraction (CPE). In this article, the estimate and detection of trace concentration of CFD in the form of an azo dye and CPE-spectrophotometry. Statistical calculations show that this study could be applied to sample small batches of pharmaceutical drugs, including individual pills and bottles on the shelves of local pharmacies. This study is a new method for the estimation of cefdinir, where the diazotization-coupling and cloud point extraction methods are novel for this compound.

Materials and Methods:

Apparatus

Spectral measurements were performed by ADVANCED MICROPROCESSOR UV-VIS SPECTROPHTOMETER SINGLE BEAM LI-295, Lasany®- (India), with a 1.0 cm quartz cell. An ultrasonic and thermostatic water bath, from Elma Hans Schmidbauer GmbH &Co. KG, was used for the extraction of samples and the study temperature effects on cloud point extraction (CPE). A centrifuge (HERMLE LABORTECHNIK Z 200 A, Germany) was used to complete phase separation. An Electronic Balance Mettle Adventurer pro AV264 (Switzerland) and pH meter, type inoLab7110, WTW® (Germany) were also used.

Reagents

All chemicals were of analytical grade and were purchased from Merck KGaA (Darmstadt, Germany). Cefdinir was obtained from the Quality Control Lab (The General Company for the Pharmaceutical Industry - Samarra).

Preparations for the standard solutions

Preparations for the drug stock solution

A Stock solution (1000 μg/ml, 0.252×10^{-2} M) of cefdinir was prepared by dissolving 0.1 g of the drug in 100 mL of double distilled water and A few drops of 1 M NaOH were added to ensure complete dissolution.

Preparation for the sample solutions

Capsules: Ten capsules containing cefdinir were carefully weighed from the commercial products (Sefarin® and Azord®). Each capsule was weighted separately, and then the average weight of each capsule was extracted. The mean capsule weights were 0.35512 g, 0.36672g, respectively. An aliquot of the target drug was dissolved in double distilled water with few drops of 1M NaOH and filled to a volume of 100 ml.

Preparation of 4-Tert-Butylphenol (4-TBP) solution

A 0.252×10^{-2} M solution of 4-TBP was prepared by dissolving 0.0378 g of it in 100 ml of double distilled water with a small amount of 1M NaOH.

Preparation of 2-Naphthol (2-NPT) solution

A 0.252×10^{-2} M solution of 2-NPT was prepared by dissolving 0.0363 g of it in 100 ml of double distilled water with a small amount of 1M NaOH.

Other solutions

A 50% w/v NaOH (12.5M), 1% w/v $NaNO_2$ (0.144M), 4% w/v urea, 10% v/v Triton X-114, 0.01M (0.3644g in 100ml of double distilled water) hexadecyltrimethylammonium bromide (CTAB), 5% w/v Na_2SO_4 solutions were prepared in double distilled water and 6.2M HCl (1:1 ratio) were prepared for the following procedures.

General procedure of Diazotization-coupling Reaction:

A series of differing concentrations (3-100 μg/ml) were prepared from a standard solution of cefdinir, by adding different volumes of the stock solution (1000 μg / ml, 0.252×10^{-2} M) in two series of volumetric flasks (20 ml) which was This was placed in an ice bath for 2 min. After that, (1.75, 1.5 mL) of 6.2M HCl and (1, 0.75 mL) of 1% $NaNO_2$ were added in two series of volumetric flasks. After 10 min, 2 mL of 4-TBP and 2-NPT (0.252×10^{-2}M) were added to the flasks respectively. Finally, 1.25 ml of NaOH (50% w/v) was added to both of the flasks with gentle mixing and the volume was completed with a distilled water. The absorbance of coloured products was measured at λ_{max} (490, 535 nm) for CFD-4-TBP, CFD-2-NPT, respectively against their reagent blank, which was prepared with the same steps.

General procedure of CPE-spectrophotometry method

Different concentrations, ranging from 0.1-6.0 μg/ml and 0.2-6.0 μg/ml from the two coloured dyes formed in the previous method (CFD-4-TBP and CFD-2-NPT, respectively) were transferred to a two series of 15 ml centrifuge tubes. Using these tubes, 0.75, 1.5 ml of Triton X-114 (10% v/v), (2, 1 ml of 0.01M) cationic surfactant (CTAB) and 2.5ml of (5% w/v) Na_2SO_4 were added to that series. After that, the volume was completed up to 12.5 ml, and

the tubes were put in an ultrasonic-thermostatic water bath device. The samples were placed under ultrasonic effect for 2 minutes to mix the components carefully at 70, 60 °C for 75, 45 min for CFD-4-TBP, CFD-2-NPT, respectively until the formation of a cloudy solution and separation of the mixture into two phases. Then the tubes were transferred to the centrifuge for 5 min at 4000-rpm speed to complete the separation. The centrifuge tubes were placed in an ice bath until the micellar phase settles at the bottom of the tube and the aqueous phase was removed. At this point, 0.5 ml of ethanol was added to dilute the micellar phase (dye) and measured at λ_{max} 505, 545 nm for each of the CFD-4-TBP and CFD-2-NPT, respectively. There was a displacement of the maximum wavelength due to changing in the solvent type (ethanol). The capacity of cell used 1ml (L: 1cm). A blank solution was prepared under the same conditions.

Result and Discussion:

Part-I (the diazotization-coupling method)

Absorption spectra

Figures 2 and 3 show the spectral readout of a 100 µg/ml solution of CFD-4-TBP and CFD-2-NPT against their blank solutions recorded under the optimal conditions. The spectra show that the λ_{max} for the cited drug were 490 nm and 535 nm for CFD-4-TBP and CFD-2-NPT, respectively.

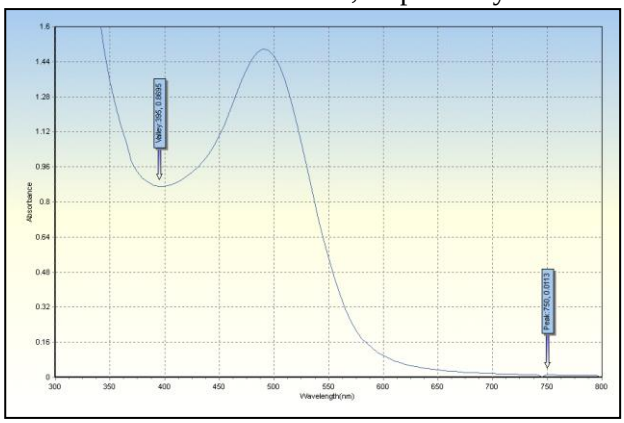

Figure 2. Absorption spectrum for 100 µg/ml of CFD-4-TBP against the reagent blank

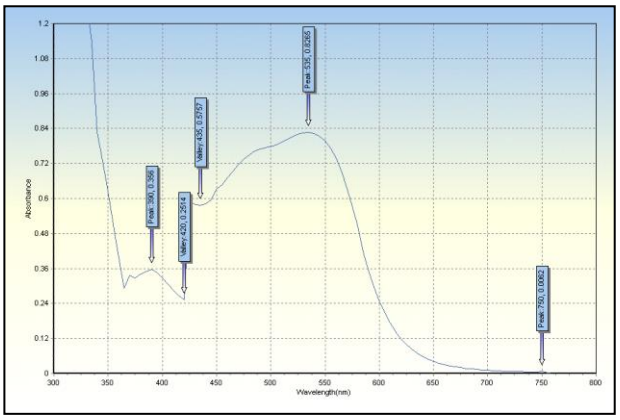

Figure 3. Absorption spectrum for 100 µg/ml of CFD-2-NPT against the reagent blank

Optimization of experimental conditions

Factors influencing diazotization-coupling reaction were studied to reach the maximum analytical signal. All these studies were performed with 100 µg/ml of cefdinir standard solution in 20 ml volumetric flasks.

The effect of the acid type on the diazotization-coupling was studied. Several acids have been used: H_2SO_4, HCl, HNO_3, and CH_3COOH diluted (1:1). The obtained results indicate that HCl (1:1) was the optimum acid used in this method because it gives the highest absorbance signal for both dyes, as it is shown in Table 1.

Table1 . Effect of acid type on absorbance signal of CFD (100µg/ml)

Type of acid (1:1)	Abs of CFD-4-TBP at 490nm	Abs of CFD-2-NPT at 535nm
HCl	1.260	**0.583**
H_2SO_4	0.703	**0.531**
HNO_3	1.255	**0.545**
CH_3COOH	0.571	**0.220**

The effect of (1:1) HCl volume on the diazotization-coupling reaction was studied. Different volumes (0.25-2.00 ml) of (1:1) HCl were used to obtain the optimum absorbance signal. The optimum volume of acid for the determination of CFD-4-TBP and CFD-2-NPT was 1.75 ml (0.543 M) and 1.5 ml (0.465M) respectively, in a final volume of 20 ml, as it is shown in Figure 4.

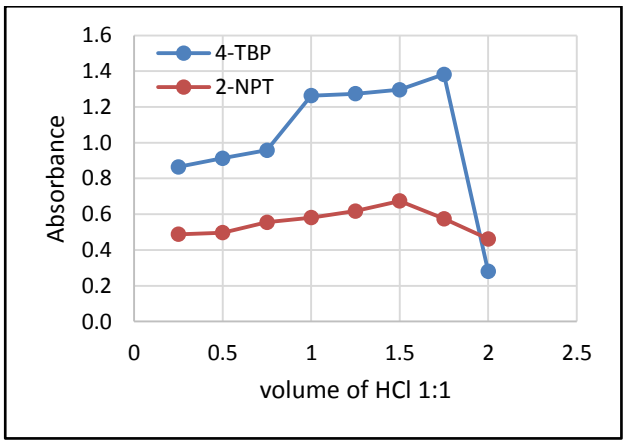

Figure 4. Effect of (6.2M) HCl volume

The effect of the volume of 1% $NaNO_2$ on the diazotization reaction was studied. The amount of sodium nitrite has a significant role in this reaction as the use of the appropriate concentration that leads to the rapidity and completeness of the reaction (10). Different volumes ranging from 0.25-2.00 mL of 1% NaNO2 were used, and the optimum volumes of the $NaNO_2$ solution for CFD-4-TBP and CFD-2 -NPT were 1.00, 0.75 ml respectively, as it is shown in Figure 5.

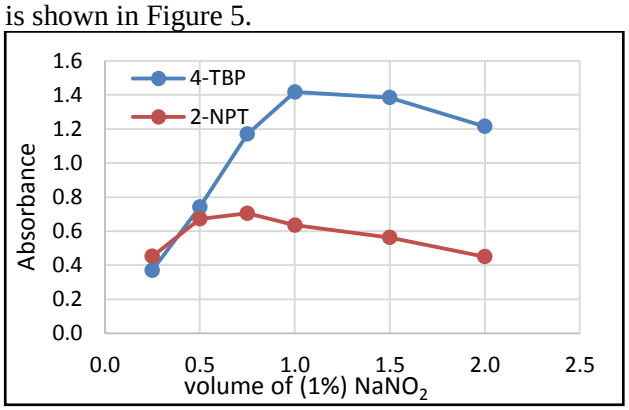

Figure 5. Effect of 1% sodium nitrite volume

The reaction time after the addition of sodium nitrite has an important effect on the value of absorbance signal because the diazonium salts are generally unstable (15). Several intervals after the addition of nitrite (5-30 min) were tried and the optimum reaction time for CFD-4-TBP and CFD-2-NPT was found at 10 minutes, as it gave the highest absorbance signal for both dyes.

The residual nitrite, in the form of nitrous acid, is undesirable as it leads to side reactions. Therefore, it must be eliminated by the addition of urea solution 4% w/v, according to the reaction equation below (16):

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

However, through testing different volumes of urea 4% (0-4 ml), we have determined that there is no need for the addition of this solution since diazotization reaction was without urea, which lead to the highest intensity of absorption in both dyes.

The effect of NaOH, KOH and NH_3 were studied. The highest absorbance signal of both dyes was obtained when using (50%) NaOH. Different volumes, ranging from 0.25-2.00 ml of 50% NaOH were studied to obtain the highest absorbance signal. The optimum absorbance signal of the two dyes was attained with 1.25 ml, as it is shown in Figure 6.

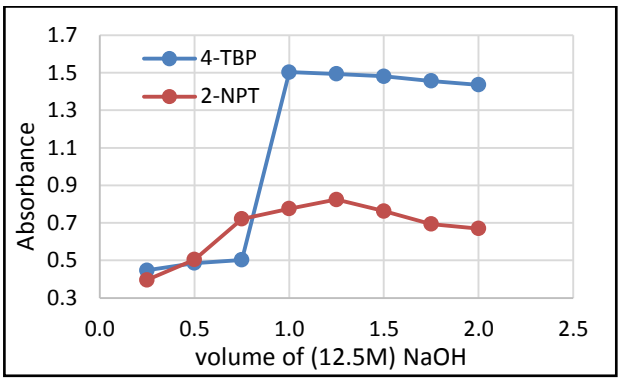

Figure 6. Effect of (12.5M) NaOH volume

Effects of the sequence of additions

The sequence of the additions of reagents was studied with its effect on the absorption intensity of the formed dyes. The results show that the sequence of (diazonium salt + reagent + base) is optimal since it gives the highest absorbance signal in both cases, as it is shown in Table 2.

Table 2.Effect of additions sequence on absorbance signal

Order Additions	**Absorbance for CFD (100 µg/ml) with**	
	4-TBP at 490nm	**2-NPT at 535nm**
Salt +reagent+ base	1.503	**0.824**
Salt +base+ reagent	0.571	**0.597**
Salt+(reagent + base)	1.075	**0.473**

The Effect of reagent volume and the nature of colored dye product

Different volumes, ranging from 0.25-3.00ml, of the 0.252×10^{-2} M reagents 4-TBP and 2-NPT were studied with 2 ml of 0.252×10^{-2} M for cefdinir solution. The optimum volume of the reagents in both cases was 2.00 ml. After that, the absorbance volume signal was almost constant. The results were used to determine the ratio of drug: reagent according to mole ratio method. The results indicate that the dyes have a combination of 1:1 ratio of diazotized CFD to both reagents, as it is shown in Figure 7.

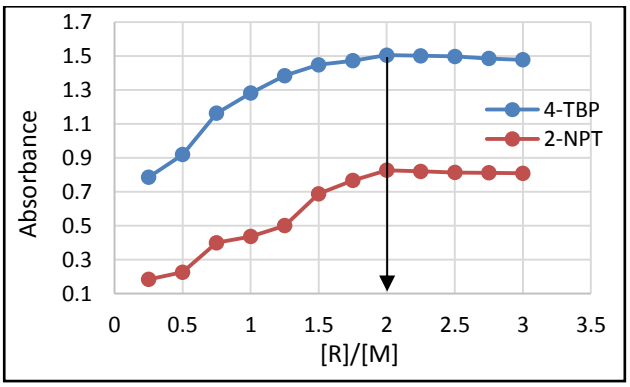

Figure 7. The mole-ratio plot for diazotized cefdinir to 4-TBP and 2-NPT

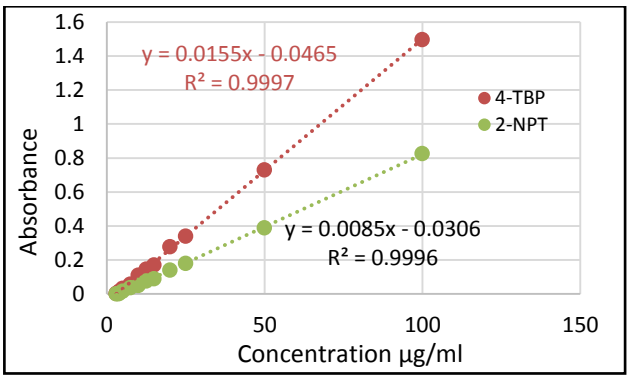

Figure 9. Calibration curves of CFD-4-TBP & CFD-2-NPT

The possible reaction mechanism can be written as in the Figure 8:

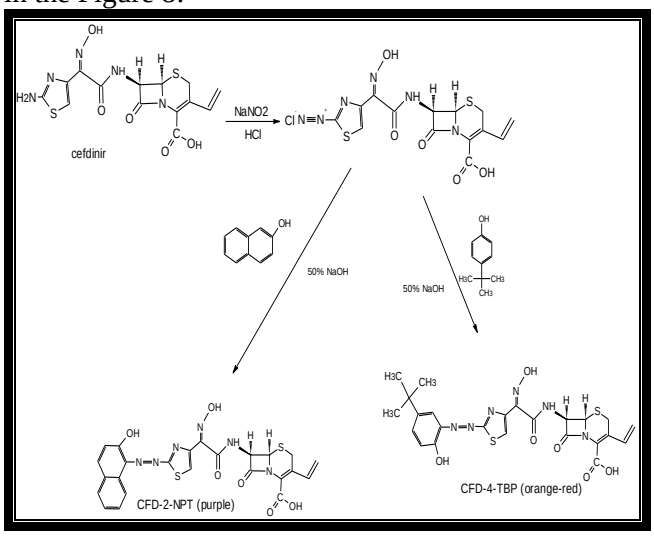

Figure 8. The suggested mechanism for the diazotization-coupling reaction

Calibration curves and analytical data

Under the optimal experimental conditions, the calibration curves were constructed. The optical characteristics such as Beer's law limits, molar absorptivity, Sandell's sensitivity, LOD and LOQ in each methods were calculated. In addition, the regression characteristics slope (b), intercept (a), and correlation coefficient (r) were derived using Microsoft Excel Data Analysis were calculated and are presented in Table 3, and Figure 9 shows the calibration curves. Specifically, excellent linearity within the range of concentrations utilized. The limit of detection (LOD) was calculated based on $LOD = 3\times (S_B / b)$ and limits of quantification (LOQ) based on $LOQ = 10\times (S_B / b)$, where S_B and b are the standard deviation of 10 blank signals, and slope or sensitivity of the calibration curves, respectively (17).

Table 3. Characteristic parameters of the proposed diazotization-coupling methods

Parameter	CFD-4-TBP	CFD-2-NPT
Color of product	Orange-red	purple
λ_{max} (nm)	490	535
Dynamic range ($\mu g.ml^{-1}$)	(3-100)	(3-100)
Molar absorptivity, Ɛ ($L.mol^{-1}.cm^{-1}$)	0.6129×10^4	0.3361×10^4
Regression equation	y = 0.0155x - 0.0465	y = 0.0085x - 0.0306
Sandell sensitivity, S ($\mu g .cm^{-2}$)/0.001A.U	0.0645	0.1176
Intercept (a)	-0.0465	-0.0306
Slope (b) ($L.mg^{-1}.cm^{-1}$)	0.0155	0.0085
Coefficient of determination % R^2	99.97	99.96
Correlation coefficient (r)	0.9998	0.9998
Limit of detection ($\mu g.mL^{-1}$)	0.246	0.447
Limit of quantification ($\mu g.mL^{-1}$)	0.820	1.489
C.L. for the slope (b±tSb) at 95%	0.0155±0.0003	0.0085±0.0001
C.L. for the intercept (a±tSa) at 95%	-0.0465±0.0101	-0.0306±0.0050
Standard error for regression line ($S_{y/x}$)	0.0096	0.0053

Effect of Interferences

The effect of some foreign excipients on the determination of pure drug was studied, which are often added to the commercial pharmaceutical. 500 µg/ml of these compounds were added individually to 100 µg/ml of pure drug before determination.

Table 4. Effect of foreign compounds on pure drug

Foreign Compound	% Recovery of 100 µg/ml CFD per 500 µg/ml Foreign compound added	
	CFD-TBP	CFD-2-NPT
Sucrose	99.34	99.68
Fructose	99.38	99.67
Lactose	99.40	99.66
Maltose	99.34	99.49
Sodium benzoate	99.32	99.50
Starch	99.29	99.37

The results in Table 4 show that the presence of these compounds has no significant effect on the determination of 100 µg/ml of the cited drug since the recovery percentage was ranged from 99.29-99.40 and from 99.37-99.68 for both methods, respectively.

Accuracy and Precision

The accuracy and precision of the proposed methods were tested by analyzing five replicates of pure samples and commercial pharmaceuticals for three different concentrations from calibration curve. The values of the T-test and F-test were calculated and compared with the reported method (18) and shown in Tables 5 and 6. These results show that the suggested methods gave acceptable results in the estimation of the CFD with the comparison of the reported method (18). The excipients present in the pharmaceutical dosage forms are not interfered in the valuation, when it is analyzed by these methods, so it can be adopted in the estimation of the CFD.

Table 5. Accuracy and precision of the proposed methods for the estimation of pure samples and their comparison with the reported method

Type Of Reagent	amount of CFD (µg/ml)		E_{rel} %	t-value	F-value	RSD% (n=5)
	Taken	Found*				
4-TBP	10	10.03±0.14	0.32	1.53	12.06	1.44
	25	24.96±0.22	-0.15			0.87
	50	50.10±0.10	0.19			0.20
2-NPT	10	10.14±0.18	1.41	0.53	1.03	1.76
	25	25.13±0.26	0.52			1.05
	50	49.84±0.30	-0.33			0.60

Table 6. accuracy and precision of the methods proposed in the estimation of commercial pharmaceuticals

sefarin® capsules300mg/product by pharma international Co. Amman-Jordan	amount of CFD (µg/ml)		% Recovery	Average Recovery**	RSD% (n=5)
	Taken	Found*			
4-TBP	10	9.77±0.10	97.74	98.45	1.04
	25	24.68±0.19	98.71		0.76
	50	49.45±0.14	98.90		0.29
2-NPT	10	9.72±0.19	97.18	98.10	1.91
	25	24.60±0.13	98.40		0.53
	50	49.36±0.26	98.73		0.53
Azord® capsules300mg/product by DAR AL DAWA DEVELOPMENT& INVESTMENT CO.LTD (Na'ur-Jordan)	amount of CFD (µg/ml)		% Recovery	Average Recovery**	RSD% (n=5)
	Taken	Found*			
4-TBP	10	9.71±0.20	97.10	97.94	2.10
	25	24.55±0.10	98.19		0.42
	50	49.26±0.16	98.52		0.33
2-NPT	10	9.71±0.20	97.06	97.83	2.03
	25	24.54±0.19	98.16		0.76
	50	49.13±0.26	98.26		0.54

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

Part-II (the CPE-spectrophotometry method)
Optimization of CPE to estimate CFD

The optimal conditions of the separation and extraction method help to obtain the accurate concentration of the drug in the micellar phase and the highest absorption signal. These conditions are the amount of Triton X-114, the amount of cationic surfactant (CTAB), salt type and amount of salt, temperature, equilibration time and pH effect. The concentration of CFD in this study is 4µg / ml, and the pH value in the preliminary study is 12.

The preconcentration factor theoretically depends on the surfactant concentration (19). The effect of 10% v/v Triton X-114 was studied by using different volumes ranging from 0.25-2.00 ml. The optimum efficiency of extraction was achieved at (0.75, 1.50 ml) for CFD-4-TBP and CFD-2-NPT, respectively as it is shown in Figure 10.

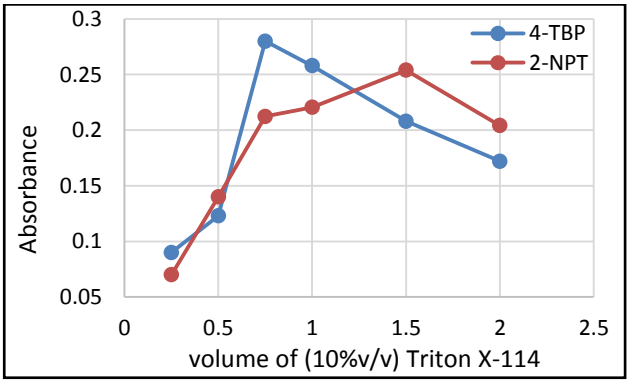

Figure 10. Effect of (10% v/v) Triton X-114 volume

Mixed micelle formation depends on the nonionic and cationic surfactant concentrations and on the balance between these factors (17). The addition of CTAB increases the cloud point temperature because of the increase of the hydrophilic characteristic of the micellar phase. This can be explained recalling that the ionic surfactant molecules added are combined into non-ionic micelles, changing the surface charge and increasing the repulsion among micelles, which makes them more hydrophilic (20). The effect of the volume of (0.01M) CTAB on the extraction efficiency was studied by using different volumes (0-3 ml). The optimum volume was (2, 1 ml) for CFD-4-TBP and CFD-2-NPT, respectively. This is because it increases the efficiency of extraction and the concentration of the surfactant-rich phase and increases the pre-concentration factor, which results in an increase in the absorbance signal as it is shown in Figure 11.

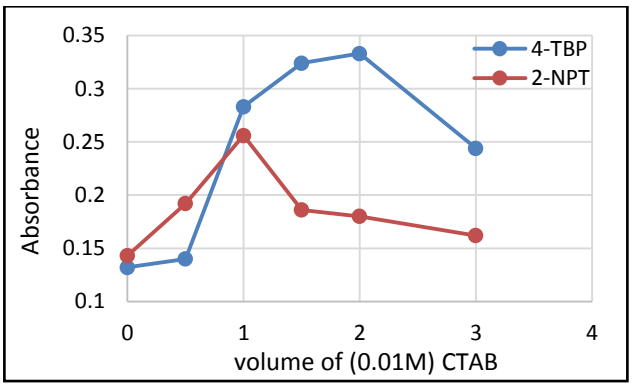

Figure 11. Effect of cationic surfactant CTAB volume

It was reported that the cloud point (CP) of mixed nonionic and ionic surfactants were reduced with the addition of a small quantity of inorganic salts (21). The CP depended on the nature and concentration of the salt added and the concentration of the surfactant used (22). The salting-in and salting-out effects could be used to interpret the electrolyte effects on the cloud points of a non-ionic surfactant (23). Various salts (sodium chloride, potassium chloride, sodium sulphate, sodium acetate) were studied by using 5% w / v solution to obtain the optimum extraction efficiency. In this study, 2.5ml of the Na_2SO_4 solution was found to give the results and to increase pre-concentration for both dyes.

It is most desirable to employ the lowest possible equilibration temperature and shortest equilibration time as a compromise between completion of extraction and the efficiency of phase separation (24). In the present work, the thermostatic water bath was kept in the range of 40-80 °C and the equilibration time was studied for a time span of 30-90 min to examine its dependence upon the extraction efficiency of the proposed method. The absorbance of the surfactant-rich phase reached a maximum value above 70, 60 ˚C at 75, 45 min for CFD-4-TBP and CFD-2-NPT, respectively.

Organic dyes can be affected by pH, since they change color in different pHs. Therefore, pH plays an important role in the extraction of them (25). The effect of the pH on both dyes extraction was assessed by varying the pH from 4 to 14. The results indicates that optimal extraction efficiency is verified in the pH =12.

Calibration Curves and Analytical Data

The measured absorbance at 505 nm and 545 nm versus different standard concentrations of CEF-4-TBP and CFD-2-NPT respectively, were plotted to construct calibration curves. The calibration curves were obtained by preconcentration of 12.5 ml of both dyes in presence of 10% v/v Triton X-114 at pH 12 under

the optimum conditions, as it is shown in Figure 12. The analytical figures of merit of the suggested CPE–spectrophotometry method was evaluated with the recommended procedure under the optimum conditions for the target analyte are shown in Table 7.

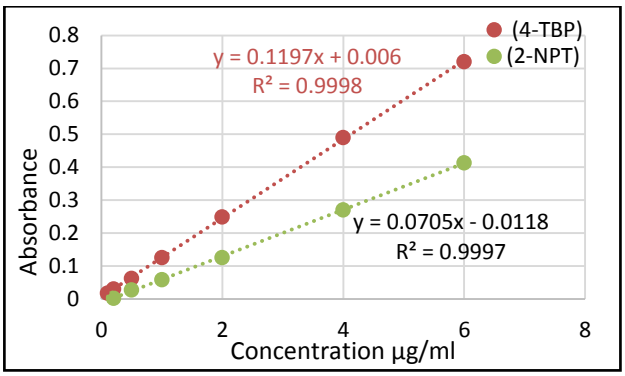

Figure 12. The calibration curve of the CPE method.

Table 7. Characteristic parameters for the regression equation of the proposed CPE method

Parameters	CFD-4-TBP	CFD-2-NPT
Color of product	purple	Purple
λ_{max} (nm)	505	545
Dynamic range (µg.ml^{-1})	(0.1-6.0)	(0.2-6.0)
Molar absorptivity, Ɛ (L.mol^{-1}.cm^{-1})	0.4733×10^5	0.2788×10^5
Regression equation	y = 0.1197x + 0.006	y = 0.0705x - 0.0118
Sandell sensitivity, S (µg .cm^{-2})/0.001A.U	0.0084	0.0142
Intercept (a)	0.006	-0.0118
Slope (b)	0.1197	0.0705
Coefficient of determination % R^2	99.98	99.97
Correlation coefficient (r)	0.9999	0.9999
Limit of detection (µg.mL^{-1})	0.032	0.054
Limit of quantification (µg.mL^{-1})	0.106	0.180
C.L. for the slope (b±tSb) at 95%	0.1197±0.0018	0.0705±0.0017
C.L. for the intercept (a±tSa) at 95%	0.006±0.0051	-0.0118±0.0052
Standard error for regression line ($S_{y/x}$)	0.0038	0.0031
Enrichment (EF) factor	24.61	24.58
Preconcentration factor (PF)	25	25
Distribution coefficient (D)	1526	1393

LOD and LOQ are determined from the slope (b) of calibration curves and the standard deviation of 10 blank signals (S_B) as LOD = 3 × (S_B / b) and LOQ = 10 × (S_B / b) (17). The distribution coefficient is calculated as D = $[M]_S/[M]_W$, where $[M]_S$ and $[M]_W$,which are the final analyte concentrations in the surfactant-rich phase (SRP) and in the aqueous phase, respectively (26). The enrichment factor (EF) is calculated as EF = C_S/C_O, where C_S and C_O are the analyte concentration in SRP and the analyte concentration in initial aqueous solution, respectively. The preconcentration factor is calculated as the ratio of a volume of the initial solution (V_O) to that of the final solution (V_S) after the preconcentration (PF = V_O/V_S) (27).

Accuracy and Precision

The accuracy and precision of the proposed methods were tested by analyzing five replicates of pure samples and commercial pharmaceuticals for three different concentrations. The value of the T-test and F-test was calculated by comparing it to the reported method (18), as shown in the Tables 8 and 9. The statistical results show that the method is accepted in the estimation of the CFD compared to the reported method (18), so it can be adopted in the estimation of the CFD.

Table 8. The accuracy and precision of the methods proposed in the estimation of pure samples and their comparison with the reported method

Type Of Reagent	amount of CFD (µg/ml)		E_{rel}%	t-value	F-value	RSD% (n=5)
	Taken	Found*				
4-TBP	2	1.99±0.02	-0.42	1.02	1.73	1.21
	4	4.04±0.01	0.88			0.33
	6	6.02±0.04	0.36			0.69
2-NPT	2	1.997±0.03	-0.14	0.75	2.51	1.59
	4	4.03±0.02	0.64			0.56
	6	6.05±0.04	0.90			0.60

Table 9. Accuracy and precision of the methods proposed in the estimation of commercial pharmaceuticals

sefarin® capsules300mg/product by pharma international Co. Amman-Jordan	amount of CFD (µg/ml)		% Recovery	Average Recovery**	RSD% (n=5)
	Taken	Found*			
4-TBP	2	1.95±0.03	97.74	98.41	1.51
	4	3.96±0.02	98.91		0.63
	6	5.91±0.01	98.58		0.22
2-NPT	2	1.940±0.04	97.02	98.27	1.86
	4	3.95±0.04	98.87		0.91
	6	5.93±0.03	98.91		0.52
Azord® capsules300mg/product by DAR AL DAWA DEVELOPMENT& INVESTMENT CO.LTD (Na'ur-Jordan)	amount of CFD(µg/ml) Taken	Found*	% Recovery	Average Recovery**	RSD% (n=5)
4-TBP	2	1.95±0.02	97.33	98.07	0.96
	4	3.94±0.01	98.58		0.33
	6	5.90±0.03	98.30		0.45
2-NPT	2	1.95±0.02	97.73	98.06	1.15
	4	3.93±0.04	98.16		0.92
	6	5.90±0.02	98.30		0.38

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

Comparison of the methods

Table 10, shows the comparison between the suggested methods and that of another literature spectrophotometric methods of some measured analytical parameters. The results show that the methods have reasonable accuracy compared with the other methods.

Table 10. Some suggested methods for estimating cefdinir by spectrophotometry

Coupling Reagent Used/ Reaction Type	λ_{max} (nm)	Linearity µg/ml	LOD µg/ml	ε (l mol^{-1} cm^{-1})	Ref
Catechol-IO4/ oxidative coupling	460	50-250	-	4.5×10^3	(28)
MBTH-FeCl3/ oxidative coupling	660	0.5-6.0	0.04	6.20×10^4	(3)
1,10-PTL-FeCl3/ oxidation	512	2-8	0.5176	0.2991×10^4	(29)
acac-CH_2O/ condensation	403	10-100	1.2734	2523.41	(5)
K3Fe(CN)6-FeCl3/ Charge transfer	700	4-12	0.5176	1.423×10^3	(29)
NBD-Cl/ hydrolysis	390	5-30	0.280	0.618×10^4	(30)
$Fe(NH4)_2(SO4)_2$/ complexation	550	8-160	0.56	3720	(31)
4-tert-butylphenol/ diazotization	490	3-100	0.246	0.6129×10^4	Present study
4-tert-butylphenol/ CPE	505	0.1-6.0	0.032	0.4733×10^5	
2-Naphthol/ diazotization	535	3-100	0.447	0.3361×10^4	
2-Naphthol/ CPE	545	0.2-6.0	0.054	0.2788×10^5	

Conclusion:

The research includes two simple, sensitive, fast and inexpensive methods for estimating cefdinir, as the 4-TBP and 2-NPT reagents are available and cheap. The first method (diazotization-coupling) involves the conversion of the cefdinir into a colored dye measured by a UV-Vis spectrophotometer. The second method involves the pre-concentration of colored dye by the cloud-point extraction method to obtain the maximum possible analytical information and to eliminate the interferences that may exist during the measurement. This method is the first method to extract the CFD at the cloud point. These methods have been successfully applied in the estimation of cefdinir in pharmaceuticals.

Conflicts of Interest: None.

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طريقة طيفية جديدة لتقدير السيفدينير بالاستخلاص بنقطة الغيمة

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الخلاصة:

تم تطوير طريقة طيفية حساسة لتقدير عقار السيفدينير(CFD)، هذا البحث يتضمن على طريقتين. تتضمن الطريقة الأولى تحضير صبغة الآزو وذلك من خلال تفاعل ملح الديازونيوم للسيفدينير مع الكواشف 4-Tert-Butylphenol (4-TBP) و2-Naphthol (2-NPT) في وسط قلوي، والذي يُعطي صبغات ملونة لها اعلى امتصاصية عند الطول الموجي 490 و535 نانومتر، على التوالي. اطاع قانون بير في مدى التراكيز (3-100) ميكروغرام/مل، وكانت حدود الكشف 0.246 و0.447 ميكروغرام/ مل والممتصية المولارية 0.6129×10^4 و0.3361×10^4 لتر. مول$^{-1}$. سم$^{-1}$ لكل من (CFD-4-TBP) و (CFD-2-NPT)، على التوالي. الطريقة الثانية كانت استخلاص التراكيز النزرة للسيفدينير في الصبغات بواسطة الاستخلاص بنقطة الغيمة بوجود Triton X-114 (10% v/v). حيث اطاع قانون بير مدى التراكيز (0.1-6.0) و(0.2-6.0) ميكروغرام/مل، وكان حد الكشف 0.032، 0.054 ميكروغرام/مل والممتصية المولارية 0.4733×10^5 و$\times10^5$ 0.2788 لتر. مول $^{-1}$. سم $^{-1}$، على التوالي. وكانت عوامل التركز تساوي 25، وعوامل الاغناء 24.61، 24.58، ومعامل التوزيع 1526، 1393 لكل من (CFD-4-TBP) و (CFD-2-NPT) على التوالي.

تم تطبيق الطرق المقترحة لتحديد المركب الفعال في المستحضرات التجارية وأثبتت نجاحها دون أي تداخل وتمت مقارنة الطرق المقترحة مع طريقة منشورة في تقدير السيفدينير النقي وتبين انه لا يوجد اختلاف واضح بين الطرق.

الكلمات المفتاحية: سيفدينير، الأزوتة، نقطة الغيمة، طيفية، ترايتون اكس-114