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 5°C 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 0.08538μg∙mL⁻¹. The second method was cloud point extraction (CPE) with using Triton 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 application 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 characteristics of Cefixime are given in Table 1 (3).

Table 1. General properties of Cefixime (CFX).

| Structure | Nomenclature | Formula | Molecular Weight |
|-----------|--------------|---------|-----------------|
| ![Structure](image) | (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 | C_{16}H_{13}N_{2}O_{7} S_{2} | 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, Germany, 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 diazotation 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 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](image-url)
Effect of Acid Type

In this study, using 1mL of (0.5M) from different acids [HCl, H2SO4, HNO3, H3PO4 and CH3COOH] and added [1mL of CFX(100 μg/mL)] , 1 mL of each acid, 1mL of NaNO2, 1mL of H3NSO4, 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. |
|--------------------------------------------------|
| Absorbance at 0.5M different acids (1 mL)         |
| HCl   | H2SO4 | HNO3  | H3PO4 | CH3COOH |
| 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-

| Table 3. Data of Absorbance to Optimum Volume of 0.5M |
|-----------------------------------------------------|
| Volume (1mL) | HCl | H2SO4 | HNO3 | H3PO4 | CH3COOH |
|--------------|-----|-------|------|-------|---------|
| Abs.         | 0.264 | 0.287 | 0.318 | 0.337 | 0.406   |

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, K2CO3, Na2CO3, NH4OH, Na2CO3] and that followed the addition [1mL CFX, 0.8mL HCl, 1mL NaNO2, 1mL H3NSO4, 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 .

| 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% NaNO₂ from (0.1-1) mL, 1mL H₃NSO₃, 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.

| 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 from Table 5 that the absorbance increased with increasing the volume of NaNO₂, 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 mL 1% NaNO₂ with varying volume of 1% H₃NSO₃ 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.

| Volume of 1% Sulphamic Acid | Abs. |
|----------------------------|------|
| 0.07                       | 0.98 |
| 0.08                       | 0.498|
| 0.09                       | 0.525|
| 0.1                        | 0.557|
| 0.2                        | 0.531|
| 0.3                        | 0.451|
| 0.4                        | 0.420|

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% NaNO₂, 0.2 mL H₃NSO₃, 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.

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

| 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].

| No | Addition | Absorbance |
|----|----------|------------|
| 1  | R+H+N+S+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 |

**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). 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 | Abs. |
|------|------|
|      | 0.350 | 0.483 | 0.519 | 0.493 | 0.484 | 0.439 | 0.419 |

The best temperature (15°C & 20°C) 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 (6x10⁻³) 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
concentration (6x10^{-4}M) for CFX and concentration of ONP is [6X10^{-5}] 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% NaNO₂, 0.2 mL H₂NSO₄, 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.

\[ K= \frac{[\text{DR}]}{[\text{D}][\text{R}]}, \quad K= 1 - \frac{a}{a^3 c} \]

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^{-3} M of CFX/ML | Final con. CFX/M | As* | Am* | α  | K (L.Mol^{-1}) | Mean of K (L.Mol^{-1}) |
|---------------------------------|------------------|-----|-----|----|----------------|------------------------|
| 0.3                             | 1.2x10^{-3}      | 0.178| 0.176| 0.01123| 3.574x10^{4} | 3.0918 x10^{4}         |
| 0.5                             | 2x10^{-3}        | 0.299| 0.295| 0.0133  | 3.566 x10^{4}  | 3.145 x10^{4}         |
| 0.7                             | 2.8x10^{-3}      | 0.435| 0.433| 4.5977 x10^{-3} | 2.1355 x10^{4}        |
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).

| 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.

| 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

**Second methods: Cloud Point Extraction of Cefixime in Aqueous Solution.**

**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% NaNO₂, 0.2mL H₂N₂O₃, 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 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.

| Addition | Tween | Triton | Tween | SDS | Triton | 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 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.

| 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 affected 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.

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

It is shown that the maximum absorption pointer of target drug is completed at (50) °C 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 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 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.

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 °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.

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% NaNO₃, 0.2 mL H₂NSO₄, 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.

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 18. Data of Absorbance to Temperature / °C with CFX.

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

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

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

Figure 6. (CFX+CPE) Calibration Curve

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             | 0.9997 | 0.002662        |       |
| 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 Pharmaceuticals:
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 % | Frel % | Average Frel % | RSD % |
|------------------------|--------|------------|---------------------|--------|----------------|-------|
| 120                    | 120.1428 | 100.119 | 100.5194 | 0.119 | 0.5182 | 0.3875 |
| 90                     | 90.2856  | 100.3173 |         | 0.317 |                   |       |
| 60                     | 60.5566  | 100.9276 |         | 0.927 |                   |       |
| 30                     | 30.2142  | 100.714  |         | 0.71  |                   |       |

[*]= 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.

Reference:
1. Gonzalez CA, Usher KM, Chester W, Brooks AE, Majors RE. Determination of Sulfonamides in Milk Using Solid-Phase Extraction and Liquid Chromatography-Tandem Mass. Interchim j. 2011;4(33):1–5.
2. Dhahir SA, Mohammed NJ. Cloud point extraction of Cefixime drug by direct (UV-Vis) spectrophotometer and indirect (Flame Atomic Absorption) technique. J Phys Conf Ser.2019 Jul (Vol. 1234, No. 1, p. 012093). IOP Publishing.
3. Nayon AU, Nesa J-U, Uddin N, Amran S, Bushra U. Development and validation of UV Spectrometric Method for the Determination of Cefixime trihydrate in Bulk and Pharmaceutical Formulation. Asian J Biomed Pharm Sci. 2013;3(22):1–5.
4. Esraa AK. Spectrophotometric Determination of Some Sulfa Drugs in Pure Form and Pharmaceutical Preparation Using Cloud Point Extraction. M. Sc. Thesis, College of Science for Women, University of Baghdad. 2018.
5. Alsamarrai MSA-RKF. Spectrophotometric Determination of Paracetamol by Diazotization and Coupling Reaction with Anthranilic Acid Mohammed. Ibn Al-Haitham Jour. 2016;29(2):409–20.
6. Saadiyah, A. D, Anal, H. M. Spectrophotometric Determination of Sulfamethoxazole and Sulfadiazine in Pure and Pharmaceuticals Preparation. ASIAN J CHEM. 2012; 24: 6.
7. Saadiyah, A. D, Isra A K. Micro-Spectrophotometric Determination and cloud point Extraction of Sulphamethoxazole in Pure form And Pharmaceutical Preparation. RJPBCS.2018; 9(6):480.
8. Saadiyah, A. D, Esraa A K. Micro Spectrophotometric Determination and Cloud Point Extraction of Sulphadimidine Sodium in pure form and Pharmaceutical Drug. Baghdad Sci. J. 2019; 16(2):332-344.
9. Saadiyah, A. D, Saud SM, Nazk M A, Sahar T A. New Diaz Coupling Reactions for Visible Spectrophotometric Determination of Thymol in Pharmaceutical Preparations with phenylendiamine as the coupling reagent. MEJIM. 2012; 2(3):25-30.
10. Domagk G, Prize N. Antimicrobial sulfonamide drugs. Adv Technol. 2017;6(1):58–71
11. Emmanuel AI, Saganuwan SA, Onyezili PA. Effects of piroxicam on pharmacokinetics of sulphadimidine as the coupling reagent. PMR-2012; 2(3):25-30.
12. Shatha M A. Spectrophotometric Determination and Cloud Point Extraction of Some Drugs in Pure Form and Pharmaceutical Preparation. M. Sc. Thesis, College of Science for Women, University of Baghdad. 2017.
13. Saadiyah,A, D, Sana R B. Cloud point extraction spectrophotometric determination of nickel, copper, cobalt and chromium by 4- HBDA1, 5DPHP as reagent in wastewater of Iraq. ESAIJ ;2015; 10(4):150-160.
14. Darweesh SA. Spectrophotometric Determination of Cefixime Following Simple Diazotization and Coupling with α-Naphthol. Iraqi J. Pharm Sci. 2017; 26(2):1–6.
15. Valarcel M. Principles of analytical chemistry .Springer Verlag , Berlin.Germany:2000;65-69.
16. Lal M, Ali A, Memon S, Memon F, Ur U, Mughal R. Optimization of HPLC method for determination of cefixime using 2-thiophenecarboxaldehyde as
التنقيط الطبقي للسفكسيم في المواد النقية والمستحضرات الصيدلانية باستخدام الاستخلاص بنقطة الغيمة

الخلاصة:

تم اقتراح طريقتين سريعتين وسريعتين مفيدتين لتقييم السفكسيم مع وبدون استخدام تقنية الاستخلاص بنقطة الغيمة في المواد النقية والمستحضرات الصيدلانية. استخدمت تقنية الاستخلاص بنقطة الغيمة CPE على كولوريد البوتاسيوم بناءً على تفاعل الأزوتة بليستروفيول، تم تثبيت المنتج وقياسه عند 400 نانومتر. انخفضت قيمت两只 في نطاق 5e10-160 ميكروغرام/مل، وكان حساسية الساذج 0.0888 ملغم/مل، وكان حد الكشف 0.07896 ملغم/مل. تم استخدام ترابرتون X-114 مع التقنيات الأخرى لقياس السفكسيم في نطاق 5e10-160 ميكروغرام/مل. كانت حساسية الساذج 0.1470 ملغم/مل، وكان حد الكشف 0.06680 ملغم/مل. يتم دراسة جميع المتغيرات بما في ذلك تركيز الكشف، زمن التفاعل، فترة استقرار اللون من أجل تحسين وضوح التفاعل. تكون الحساسية من خلال استخدام تلك التقنيات في فحص المواد الصيدلانية وتشمل النتائج التي تم التوصل إليها متوافقة بشكل جيد مع الطرق الرسمية وغيرها الموجودة في الادبيات. تم بلاحظ أي تداخل بين الإضافات بشكل كبير.

الكلمات المفتاحية: الاستخلاص بنقطة الغيمة، السفكسيم، الأزوتة، بليستروفيول، ترابرتون X-114.