

## Spectrophotometric Determination of Cefixime Following Simple Diazotization and Coupling with $\alpha$ -Naphthol

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### Abstract

Cefixime (CFX) was treated with sodium nitrite and hydrochloric acid for diazotization reaction followed by coupling with  $\alpha$ -Naphthol in alkaline medium to form, a yellow colored azo dye compound which exhibits maximum absorption ( $\lambda_{max}$ ) at 412 nm where the concentration of (CFX) was determined spectrophotometrically. The optimum reaction conditions and other analytical parameters were evaluated. Beer's law was obeyed in the concentration range of (1-20)  $\mu\text{g.mL}^{-1}$  with a molar absorptivity of 34870.5  $\text{L.mol}^{-1}.\text{cm}^{-1}$ . The limit of detection was found to be 0.1090  $\mu\text{g.mL}^{-1}$  and the Sandell's sensitivity value was 0.0130  $\mu\text{g.cm}^{-2}$ . The proposed method could be successfully applied to the determination of (CFX) in pharmaceutical formulations.

**Keywords:** Spectrophotometric determination, Cefixime, Diazotization reaction,  $\alpha$ -Naphthol, Coupling reaction.

### التقدير الطيفي للسفكسيم بالأزوتة البسيطة والأقتران مع الفا نافثول

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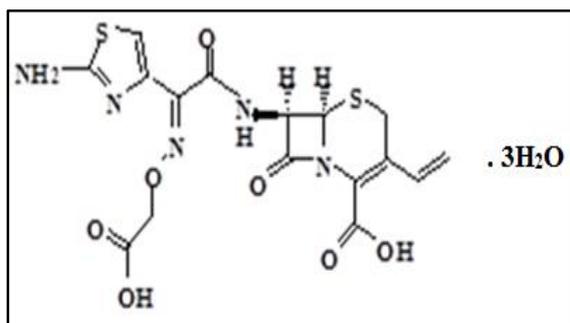
### الخلاصة

عومل عقار السفكسيم (CFX) مع نترتيت الصوديوم وحامض الهيدروكلوريك لأزوتته ثم تبع ذلك اجراء تفاعل اقترانه مع الفا نافثول في وسط قلوي لتكوين صبغة الأزوت ذات اللون الأصفر التي تظهر أعظم امتصاص ( $\lambda_{max}$ ) عند 412 نانوميتر ومن ثم تم تقدير تركيز السفكسيم طيفياً. وقد تم تعيين الظروف الفضلى التي تؤثر على التفاعل والعوامل التحليلية الأخرى. تم التجاوب مع قانون بير على مدى من التراكيز الذي يتراوح بين (1-20) مايكروغرام/مل وكانت قيمة معامل الامتصاص المولي مساوية لـ 34870,5 لتر/مول/سم وكان حد الكشف يساوي 0,1090 مايكروغرام/مل ومعامل ساندل يساوي 0,0130 مايكروغرام/سم<sup>2</sup>. لقد أمكن تطبيق الطريقة المقترحة بنجاح لتقدير السفكسيم في المستحضرات الصيدلانية.

الكلمات المفتاحية: التقدير الطيفي، سفكسيم، تفاعل الأزوتة، الفا نافثول، تفاعل الأقتران.

### Introduction

Cefixime is a semisynthetic, cephalosporin antibacterial for oral administration (Scheme 1).



**Scheme (1):** The chemical structure of cefixime.

Chemically it is (6R,7R)-7-[(2-(2-Amino-1,3-thiazol-4-yl)-2-(Carboxy-methoxyimino)acetyl)amino]-3-ethenyl-8-oxo-5-thia-1-azabicyclo(4.2.0)oct-2-ene-2-carboxylic acid trihydrate. Its chemical formula is  $(\text{C}_{16}\text{H}_{15}\text{N}_5\text{O}_7\text{S}_2 \cdot 3\text{H}_2\text{O})$  and with molecular weight of (507.50) as the trihydrate. Its under the category of  $\beta$ -Lactam Antibiotics/Cell Wall inhibitor. It Acts by inhibiting an enzyme transpeptidase, involved in the building of bacterial cell walls. It is used in lower respiratory tract infections. It is helpful in acute urinary tract infections, biliary tract infections, sinusitis, acute otitis media, peptic ulcer and many more <sup>(1)</sup>. cefixime has been determined by the development of several analytical techniques such as flow injection analysis <sup>(2)</sup>, HPLC <sup>(3-5)</sup>, potentiometric method <sup>(6)</sup>, voltametric method <sup>(7)</sup>, spectrophotometric method <sup>(8-12)</sup>.

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The present study describes the use of  $\alpha$ -naphthol as a chromogenic reagent in the development of simple, sensitive and a rapid spectrophotometric method for the estimation of CFX with reasonable precision, accuracy.

## Experimental

### Instruments

The absorption spectra and all spectrophotometric measurements were carried out on a double - beam (shimadzu 1800) spectrophotometer with 1cm matched quartz cells.

### Materials and reagents

Pharmaceutical grade cefixime trihydrate received as a gift powder sample with a purity of pure form (99.99%) from The State Company for Drug Industries and Medical Appliances Samara-Iraq (SDI). All chemicals and reagents used were of analytical grade. Cefixime (400 mg capsules or 200 mg film coated tablets were purchased under the brand names (Suprax-caps-Al-Hikma-Jordan and Samaxim-S.D.I-Iraq) from the Iraqi pharmaceutical market.

### Preparation of solutions

1- Sodium nitrite (1 % (wt/v)): was prepared by dissolving 1 g of  $\text{NaNO}_2$  in 100 ml distilled water.

2- Hydrochloric acid (1 M): was prepared by taken 8.6 ml of concentrated HCl and diluted to 100 ml with distilled water.

3- Sulfamic acid (0.5 % (wt/v)): was prepared by dissolving 0.5 g of sulfamic acid in 100 ml of distilled water.

4- Sodium hydroxide (6 M): was prepared by dissolving 24 g of NaOH in 100 ml of distilled water.

5-  $\alpha$ -Naphthol (2 % (wt/v)): was prepared by dissolving 2 g of  $\alpha$ -Naphthol in 100 ml of 4 M NaOH.

### Standard drug preparation ( $100 \mu\text{g}\cdot\text{ml}^{-1}$ )

Standard solution of CFX was prepared by dissolving accurately weighted amount of the pure drug equivalent to 10 mg in 1ml of concentrated HCl and further diluted to 100 ml with distilled water.

### Preparation of drug solutions

Ten capsules of cefixime were weighed, and an accurately weighted portion of the powder equivalent to 200 and 400 mg of cefixime were dissolved in a 5 mL of concentrated HCl and mixed well and then filtered by using Whatman filter paper No.41. Then the volume was diluted to 100 ml with distilled water and analyzed as given under the assay procedures for bulk samples.

### Procedure for assembling the calibration curve

To aliquots of the standard solution ( $100 \mu\text{g}\cdot\text{mL}^{-1}$ ) containing (5-150  $\mu\text{g}$ ) of CFX were transferred into a series of 5 mL volumetric flasks and cooled in an ice bath, and then 0.5 mL of 0.5 % (w/v) sodium nitrite solution and 0.5 mL of 0.3 M HCl were added. Each solution was shaken thoroughly and left to stand for 2 min., then 1.0 mL of 0.1 % (w/v) sulfamic acid was added. The solutions were swirled and the resulting diazotized product was coupled with  $\alpha$ -naphthol by the addition of 0.5 mL of 0.8 % (w/v) of this reagent in 1 M sodium hydroxide solution. The mixtures allowed to stand for 2 min., after that they were made up to the mark with distilled water. The absorbance of the yellow colored chromogen was measured at 412 nm against the reagent blank. The constructed calibration curve was used to compute the amount of CFX in the given samples.

## Results and discussion

### Absorption spectra and reaction scheme

In the method developed the presence of the aromatic amino group in cefixime, enable the use of diazotization of the drug with nitrous acid and coupling the resulting diazonium salt with  $\alpha$ -naphthol to form colored azo-dye with a maximum absorption at 412 nm. The absorption spectra of the above dye are presented in Figure 1.

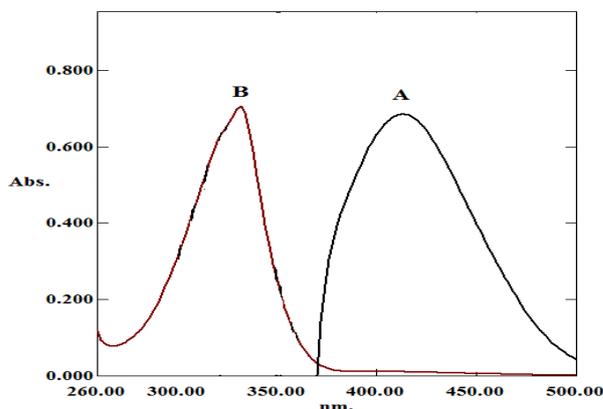
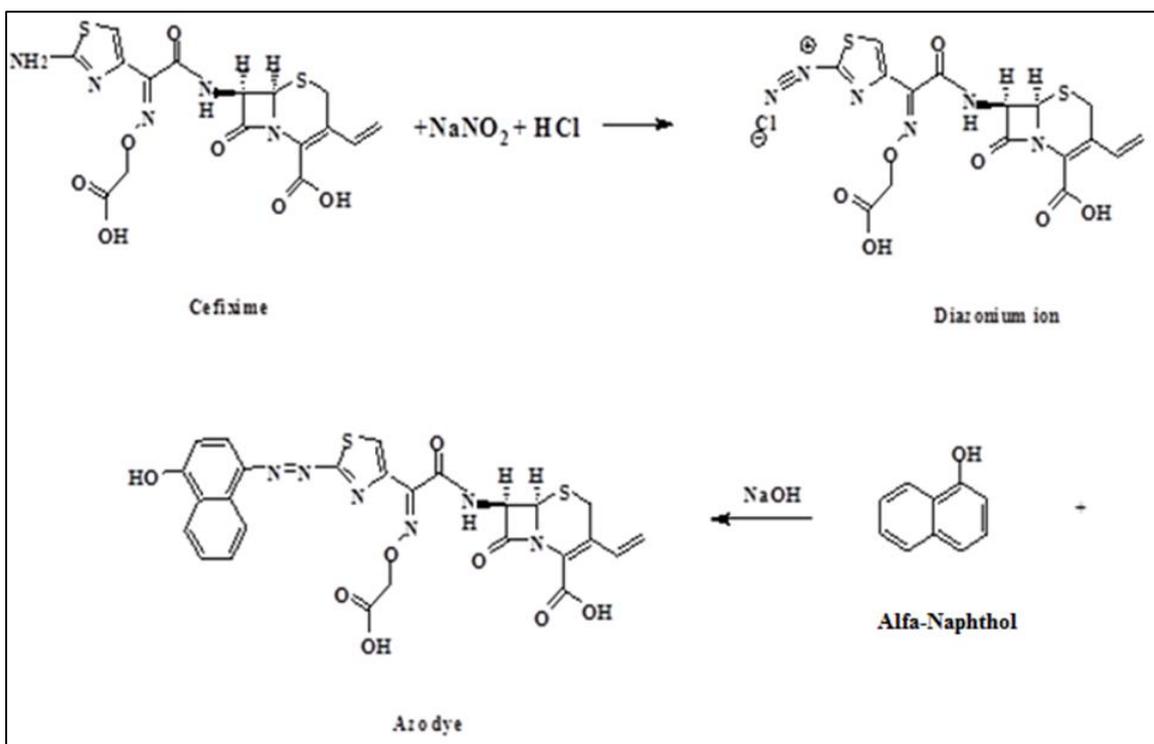


Figure (1): Absorption spectrum of (A) complex against (B) reagent blank solution.

Two steps are involved in the reaction that produces the colored dye. In the first step, cefixime is treated with nitrite solution in hydrochloric acid medium, undergoes diazotization to give diazonium ion. In the second step, the diazonium ion is coupled with the coupling agent  $\alpha$ -naphthol, to form yellow colored azo-dye in an alkaline medium. The proposed chemical reactions are shown in Scheme 2.

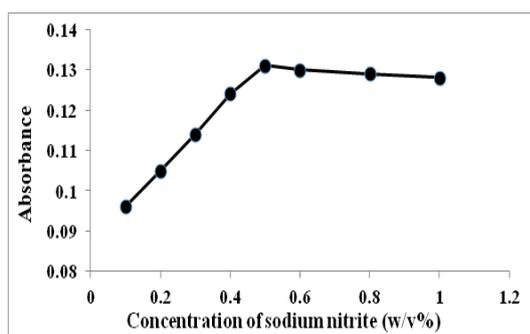


**Scheme (2):** The suggested reaction mechanism for diazotization and reaction between cefixime and  $\alpha$ -naphthol.

#### Optimization of reaction variables

##### Effect of sodium nitrite

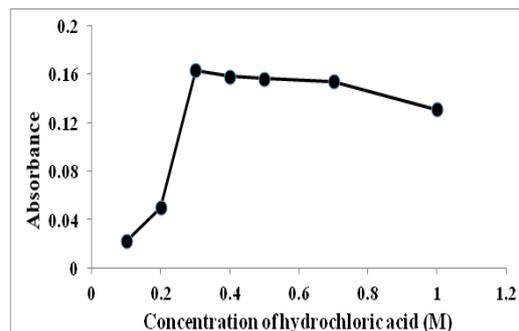
The effect of adding various amounts of sodium nitrite solution on absorbance of 10  $\mu\text{g mL}^{-1}$  cefixime was examined. The concentration of sodium nitrite was varied between 0.5 mL of (0.1-1%) sodium nitrite in water. The results showed that 0.5 mL of 0.5% sodium nitrite gave maximum absorbance (Figure 2). Below and above this concentration the absorbance was slightly decreased.



**Figure (2):** Effect of sodium nitrite concentration.

##### Effect of hydrochloric acid

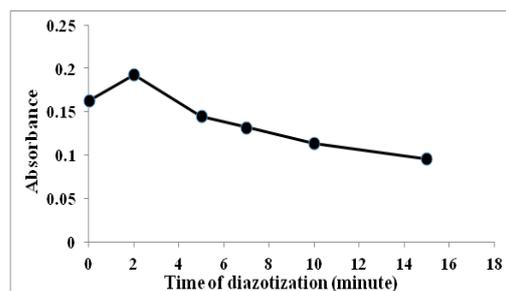
Diazotization was carried out at 0-5 °C. The hydrochloric acid concentration was studied between (0.1-1.0) M and 0.3 M hydrochloric acid concentration was fixed for getting a stable diazonium ion, (Figure 3).



**Figure (3):** Effect of hydrochloric acid concentration.

##### Effect of diazotization reaction time

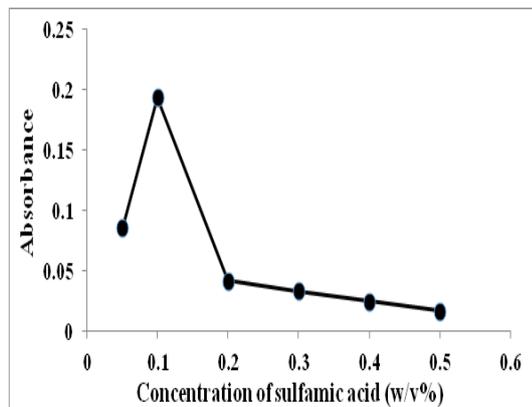
The optimum time for the diazotization of cefixime were established at 0-5 °C and 2 minutes, where longer times led to extremely low absorbance values. This implies that long time destroy the diazotized product as shown in Figure 4.



**Figure (4):** Effect of diazotization time.

**Effect of sulfamic acid concentration**

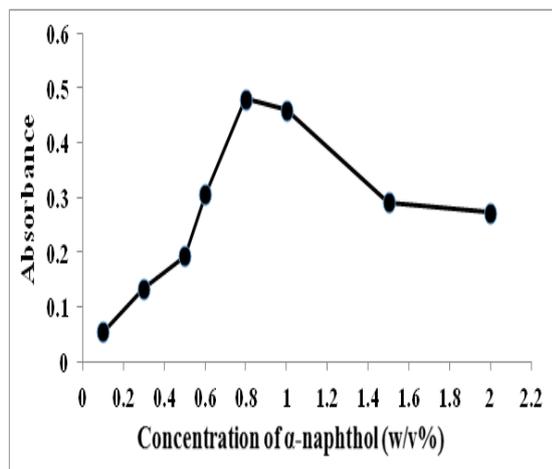
The excess of nitrite could be removed by the addition of 1 mL of 0.1% sulfamic acid. An excess of sulfamic acid decrease the color intensity of product, (Figure 5).



**Figure (5): Effect of sulfamic acid concentration.**

**Effect of  $\alpha$ -naphthol concentration**

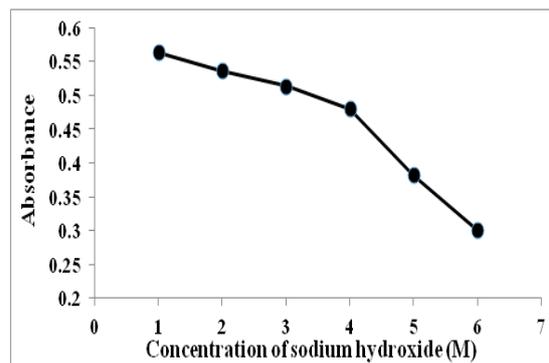
To optimize the concentration of coupling agent, 0.5 mL of different amounts (0.1-2%)  $\alpha$ -naphthol were added to the mixture under study. It was found that 0.8% of  $\alpha$ -naphthol solution was sufficient for maximum and stable color development. There was a decrease in absorbance at higher concentration of 0.8%  $\alpha$ -naphthol, as shown in Figure 6.



**Figure (6): Effect of  $\alpha$ -naphthol concentration.**

**Effect of alkalinity**

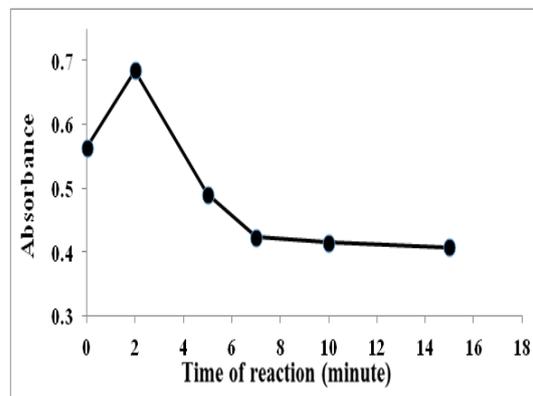
The optimum concentration of sodium hydroxide leading to a maximum intensity of the azo dye was found to be 1 M. Higher concentrations of alkali may lead to partial decolorization of the dye. Figure 7 illustrates the results of the study.



**Figure (7): Effect of sodium hydroxide concentration.**

**Effect of coupling reaction time**

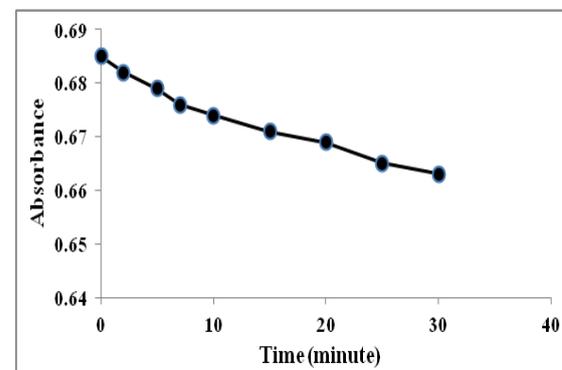
It was found that maximum absorbance is attained after 2 min further increase of the reaction time results in a gradual decrease in the absorption intensity, therefore 2 min was chosen as the optimum reaction time, Figure 8.



**Figure (8): Effect of coupling reaction time.**

**Stability of the colored product**

As shown in Figure 9, a slight decrease is observed upon leaving the colored product, after dilution, for a while of time. Therefore, it is advisable to measure the absorbance immediately after color development.



**Figure (9): Stability of the colored product.**

**Calibration curves and analytical data**

A calibration curve for cefixime drug was constructed and found to be linear over the concentration range of (1-20)  $\mu\text{g.mL}^{-1}$ . Figure 10 shows that the high value of the correlation coefficient for the regression equation, which indicates a good linearity over working concentration range. The statistical treatments of the analytical data are summarized in Table 1.

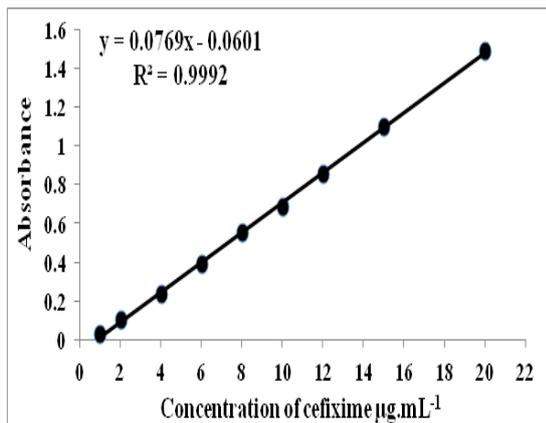


Figure (10): Calibration curve for the determination of cefixime.

Table (1): Optical characteristics and statistical data for the determination of cefixime.

Parameter	Value
$\lambda_{\text{max}}$ (nm)	412
Color	Yellow
Linearity range ( $\mu\text{g.mL}^{-1}$ )	1-20
Regression equation	$A = 0.0769 (\text{CFX. } \mu\text{g.mL}^{-1}) - 0.0601$
Calibration sensitivity	0.0769
Correlation coefficient (R)	0.9996
Correlation of linearity (R2)	0.9992
Molar absorptivity ( $\text{L.mol}^{-1}.\text{cm}^{-1}$ )	34870.5
Sandell's sensitivity ( $\mu\text{g.cm}^{-2}$ )	0.0130
Detection limit ( $\mu\text{g.mL}^{-1}$ )	0.1090

**Precision and accuracy**

Cefixime was determined at four different concentrations in three replicates during the same day. The analytical results found after the investigation are briefed in Table 2 indicated good accuracy with reasonable precision of the proposed method.

Table (2): Accuracy and precision of the proposed method.

Sample	Conc. of CFX ( $\mu\text{g.mL}^{-1}$ )		Relative Error %	standard deviation	Relative standard deviation %
	Taken	Found*			
Cefixime	5.00	5.10	+2.0	1.8470	0.0942
	10.00	9.86	-1.4	1.0375	0.1023
	15.00	15.15	+1.0	0.7551	0.1144

\*Average of three determinations

**Application on pharmaceutical formulations**

The suggested method was used for the quantification of cefixime in commercial tablets

and capsules. The results, which are demonstrated in table 3, were satisfactory.

Table (3): Application of the proposed method to the cefixime concentration measurements in pharmaceutical formulations.

Sample	Labeled amount of CFX (mg)	*Found amount of CFX (mg)	Recovery %	standard deviation	* Relative standard deviation %
Suprax-caps Al-Hikma Jordan	200	204.92	102.46	0.8673	0.4232
	400	397.53	99.39	0.9542	0.2400
Samaxim S.D.I Iraq	200	198.06	99.03	0.7301	0.3686
	400	396.73	99.18	1.1420	0.2879

\*Average of three determinations.

## Conclusions

Diazotization reaction of primary amine group followed by coupling with  $\alpha$ -naphthol in alkaline medium was found to be a simple, sensitive, accurate and economic spectrophotometric method for quantitative determination of cefixime in pure form and pharmaceutical formulations. Different variables affecting the completion of the reaction were optimized. The proposed method offers good linearity and precision.

## Acknowledgement

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