DEVELOPMENT OF SPECTROPHOTOMETRIC METHOD FOR DETERMINATION OF CEFOTAXIME BASED ON CHARGE TRANSFER COMPLEXATION REACTION

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TÓM TẮT

XÂY DỰNG PHƯƠNG PHÁP QUANG PHỔ XÁC ĐỊNH CEFOTAXIM TRÊN CƠ SỞ PHẢN ỨNG TẠO PHỨC CHUYỂN DỊCH ĐIỆN TÍCH

Bài báo mô tả xây dựng phương pháp quang phổ đơn giản và chọn lọc để xác định cefotaxim trong các mẫu bột pha tiêm. Phương pháp này dựa trên sự hình thành phức chuyển dịch điện tích giữa cefotaxim và thuốc thử DDQ trong dung môi axetonitril tạo thành dung dịch màu đỏ có cực đại hấp thụ tại 583 nm. Khoảng nồng độ tuân theo định luật Bia là 10-100 µg/mL với giới hạn phát hiện (LOD), giới hạn định lượng (LOQ), hệ số hấp thụ mol lần lượt là 0,8 µg/mL, 2,64 µg/mL và 2,983.10^3 L.mol^{-1}.cm^{-1} tương ứng. Phần trăm độ thu hồi (R%) cefotaxim trong ngày và giữa các ngày liên tiếp từ 97,56% đến 107,52%, phần trăm độ lệch chuẩn tương đối (RSD%) nhỏ hơn 2%. Phương pháp đã được áp dụng thành công để xác định cefotaxim trong các mẫu bột pha tiêm với độ chính xác cao.

Keywords: *Cefotaxim, UV-Vis, phức chuyển dịch điện tích, DDQ.*

1. INTRODUCTION

Cefotaxime (CFTX) is a third-generation cephalosporin antibiotic. It has a strong activity against Gram-negative bacteria and is indicated for the treatment of serious infections caused by bacteria sensitive to cefotaxime such as meningitis brain, endocarditis, pneumonia, and sepsis [1]. On the market, the dosage form of Cefotaxime is a powder for injection.

Cefotaxime has shown maximum absorption at 260 nm. The structure of Cefotaxime is shown in Figure 1.

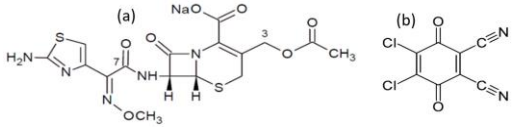


Figure 1. Chemical structure of CFTX(a) and DDQ(b)

Several reports have published different analytical methods for determining cefotaxime in different subjects such as powder for injection, biological fluids, food, and wastewater by liquid chromatography [2-3], voltammetric [4], capillary electrophoresis [5], near-infrared spectroscopy [6], and spectrophotometric [7-8]. In Vietnamese pharmacopeia, HPLC-UV is usually used to determine CFTX in powder for injection [9]. Spectrophotometric methods are the most convenient techniques because of their inherent simplicity, high sensitivity, low cost, and wide applicability in laboratories. Visible region spectroscopy is based on a derivatization reaction with different organic reagents such as diazotization reaction with reagents of 3-aminophenol [10] and p-dimethyl amino benzaldehyde [11], redox reaction with reagents of molybdophotphoric acid [12] and metol-chromium (VI) [7], ion-pair complex formation reaction with reagent of black Eriochrome T [13], and charge-transfer complexation reaction with reagents of p-

benzoquinone reagents [14], iodine, 2,3-Dichloro-5,6-dicyano-1,4-benzoquinone (DDQ), and 7,7,8,8-tetracyanoquinodimethane (TCNQ) [15]. However, these methods have some disadvantages such as low sensitivity, narrow linear range, derivatization reaction occurring at low or high temperatures, and long time for the reaction to complete.

2,3-Dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) (Figure 1b) is an orange-yellow powder that well dissolves in the solvents of methanol, dioxane, acetonitrile. Therefore, it is used as a π-acceptor reagent for the determination of cephalosporin [15] and sulfonamide antibiotics [16] in pharmaceutical preparations based on charge-transfer complexation reaction. In this study, we developed a simple, fast, and inexpensive method to analyze cefotaxime in powder for injection based on the charge transfer reaction with the DDQ reagent in acetonitrile solvent.

2. EXPERIMENTAL

2.1. Instrumentation

A Biochrom Model SP-60 double beam of UV-Vis spectrophotometer (Biochrom Ltd., UK) with 1.0 cm matched quartz cells was used for absorbance measurements.

2.2. Chemical

All chemicals used were of analytical grade. Cefotaxime (Sigma Aldrich, USA, certified to be 99%), 2,3-Dichloro-5,6-dicyano-1,4-benzoquinone (Maya-R, China, certified to be 99%) were used. Other chemicals were of analytical reagent grade.

Injectable preparations containing 1 g of cefotaxime were collected from the market and labeled CFTX1, CFTX2, and CFTX3.

2.3. Preparation of solutions

A stock solution of cefotaxime (1 mg/mL) in methanol was employed. The working standard solution of cefotaxime with a concentration of 500 µg/mL was then prepared by dilution of the stock solution.

DDQ solution (2 mg/L) was prepared by dissolving 0.100 g of DDQ in 25 mL of acetonitrile. After dissolution, the volume was completed to 50 mL in a volumetric flask with acetonitrile.

Excipients (50 mg/mL) such as glucose, starch, lactose, sucrose, and sodium chloride were prepared with double-distilled water and appropriate amounts of chemical powder.

Vials: Mix the powder from each vial. An accurately weighed injection powder equivalent to 100 mg of cefotaxime was dissolved in 60 mL methanol and transferred to a 100-mL volumetric flask. The volume was made up to 100 mL with methanol. The working solution with a drug concentration of 500 µg/mL cefotaxime was prepared by the dilution method. After that, 1.00 mL of working solution of the drug (500 µg/mL) was transferred into a 5-mL volumetric flask. The solution was then added with 1.50 mL of 2 mg/L DDQ solution. The volume was completed with acetonitrile and after 5 min and the absorbance was measured at 583 nm against the reagent blank. The experiment was repeated 6 times and the average results were obtained.

2.4. General procedures and construction of the calibration graphs

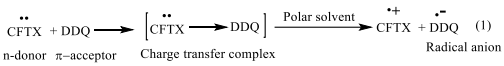
Solutions (100 - 1000 µL) of CFTX working standard solution (500 µg/mL) were transferred into a series of 5-mL volumetric flasks. A volume of 1.50 mL of DDQ solution (2 mg/L) was then added and the volume was then completed with acetonitrile. After 5 minutes, the absorbance was measured at 583 nm against the reagent blank.

3. RESULTS AND DISCUSSION

3.1. Proposed mechanisms of the reactions and spectral characteristics

A charge-transfer complex is a complex formed between an electron donor and an electron acceptor. It is characterized by the electronic transition(s) to an excited state in which there is a partial transfer of electronic charge from the donor to the acceptor moiety. As a result, the excitation energy of this resonance occurs very frequently in the visible region of the electromagnetic spectrum [17].

In the structure of CTFX, a pair of free electrons on the nitrogen atom of a secondary amine is the electron donor agent (n-donor). One electron transfers to the DDQ reagent (π-acceptor) to form a charge-shift complex which subsequently dissociates into radical anions depending on the polarity of the solvent used. In polar solvents such as acetonitrile, the complex dissociate to intensely colored radical anion according to Equation 1 [16-18].



The interaction of CFTX with DDQ at three different concentrations of CFTX (25, 50, and 75 µg/mL) occurred rapidly at room temperature in acetonitrile solvent. It forms a red color solution that is stable for 60 min and exhibits a maximum absorbance at 460 nm, 540 nm, and 583 nm (Fig. 2). These wavelengths are characteristic of the DDQ radical anion formation while the reagent blank has negligible absorption at 583 nm. Thus, in the absence of CFTX, DDQ anion radicals did not form. In other words, the formation of DDQ anion radicals increases the selectivity of the CFTX determination method. Therefore, we choose the wavelength of 583 nm for the next experiments.

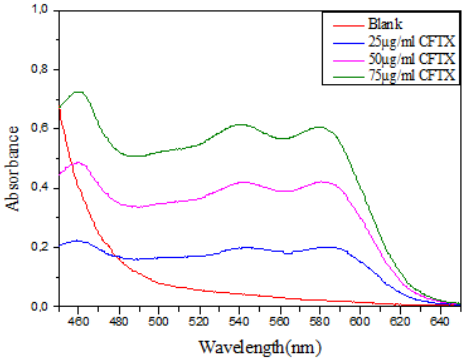


Figure 2. Absorption spectra of the reaction product between CFTX with DDQ in acetonitrile.

3.2. Study of the optimum reaction conditions

Different experimental parameters affecting color development and stability were carefully studied and optimized. Such factors were varied individually while the others were kept constant.

3.2.1. Effect of solvents

Solvents play an important role in the reaction to form charge-transfer complexes between CFTX and DDQ, complex dissociation, and stability of the DDQ anionic radical. Previous studies have shown that polar solvents are highly effective in radical anion formation [18-19]. The effects of 7 different types of polar organic solvents (acetone, acetonitrile, isopropanol, dimethyl sulfoxide, ethanol, methanol, and ethylene glycol) on the formation of charge shift complexes between CFTX and DDQ reagent were investigated. Results showed that the absorbance and color fastness of the solution were highest when using acetonitrile (Fig. 3). Therefore, it was considered an ideal solvent for these reactions.

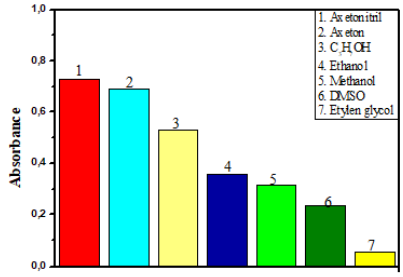


Figure 3. Effect of differnt organic solvents (CFTX 100µg /mL)

3.2.2. Effect of reagent amount

The effect of DDQ amount on the color of the product has been studied in the range of 0.25 - 2.5 mL) (Fig. 4). The greatest absorbance intensity was obtained with 1.5 mL of DDQ reagent. Therefore, we chose 1.50 mL of 2 mg/mL DDQ for the next experiments.

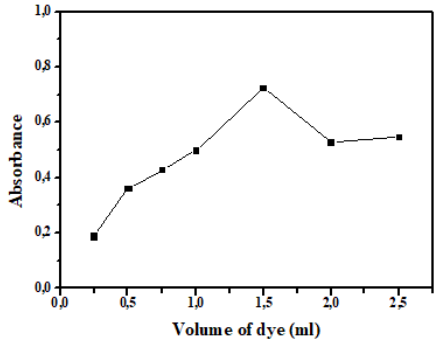


Figure 4. Effect of reagent amount (CFTX 100 µg/mL)

3.2.3. Molar ratio of the reaction and reaction pathway

The effect of the reaction ratio between CFTX and DDQ was investigated by the molar ratio method. The result in Figure 5 shows that the interaction occurs on an equimolar basis via the formation of charge-transfer complexes of 1:1. The possible reaction pathway of CFTX and DDQ is shown in Figure 6.

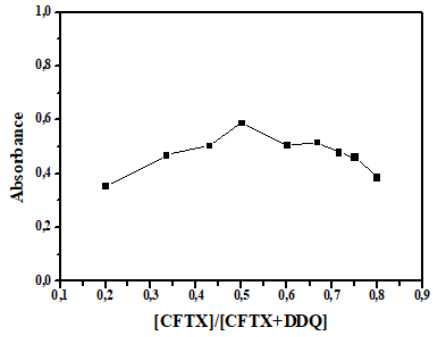


Figure 5. Molar ratio of the reactants by molar ratio method

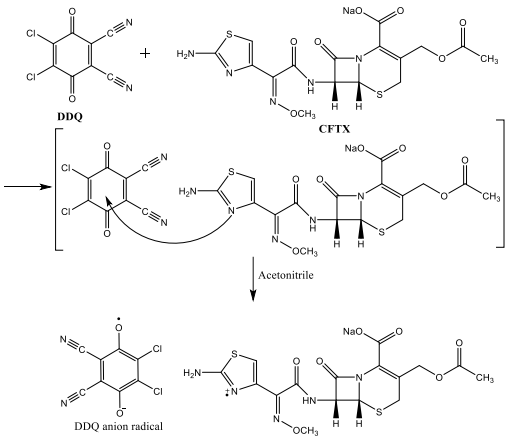


Figure. 6. Possible reaction pathway of a charge-transfer complex between CFTX and DDQ

3.3. Validation of the proposed method

The proposed method was validated according to the International Conference on Harmonization (ICH) guidelines on validation of analytical procedures [20].

3.3.1. Calibration curve, linearity, and sensitivity

The calibration curve was built based on the correlation between the absorbance of a solution with the CFTX concentration. The result in Figure 7 shows a linear range of calibration curve for determination of CFTX from 10 µg/mL to 100 µg/mL, the calibration curve in the form of A = 0.0066C + 0.0485, with a linear correlation coefficient of R^2 = 0.9948.

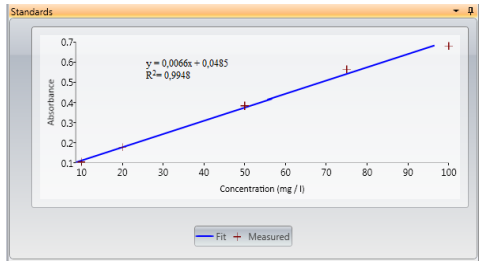


Figure 7. The standard curve for determining CFTX

The limit of detection (LOD) and quantitative (LOQ) were determined by the following formula.

$$LOD = \frac{3.3SD}{b} \qquad LOQ = \frac{10SD}{b}.$$

Where SD is the standard deviation and b is the slope of the calibration curve.

The LOD and LOQ were determined to be 0.8 µg/mL and 2.64 µg/mL, respectively. On the other hand, the molar absorption coefficient (ε) is determined directly from the slope of the calibration curve. (ε = 2.983×10^3 L/(mol.cm)).

3.3.2. Accuracy

The accuracy of the proposed method was evaluated by repeatability and trueness intra-day and inter-day at three different concentrations of CFTX (i.e., 25, 50, 100 µg/mL) with six replicates for each concentration. The repeatability and trueness are evaluated using the percentage of relative standard deviation and recovery, as follows.

able 1. Evaluation of accuracy of the proposed methods (n = 6)

Amount taken (µg/mL)	Amount found (µg/mL)	R (%)	RSD (%)
Intra-day			
25.00	25.78	103.13	0.89
50.00	53.49	106.97	0.56
100.00	97.56	97.56	0.53

$$RSD(\%) = \frac{SD.100}{\bar{x}} \quad R(\%) = \frac{C_f.100}{C_a}.$$

Where $\bar{x}$ is the mean concentration and C_f is the concentration value of the analyte determined from the calibration curve. C_a is the value of the added analyte concentration.

As given in Table 1, the result shows that the percentage recovery intra-day and inter-day of the proposed method from 97.56% to 107.52%. The percentage of relative standard deviation is smaller than 2%, showing that the proposed method is highly accurate.

3.3.3. Selectivity

The selectivity of the proposed method was evaluated via the interfering ability of some common excipients (e.g., lactose, glucose, starch, saccharose, and sodium chloride) to the determination of CFTX with the DDQ reagent. Using a concentration of 50 µg/mL CFTX, the excipients were added one by one to the reaction mixture with concentration from 5×10^3 µg/mL to 50×10^3 µg/mL (Table 2). The recovery (R%) of the proposed method was from 97.15% to 105.56%, showing high accuracy. Moreover, the relative standard deviation of the proposed method (RSD%) was smaller than 2%, indicating a good method repeatability. The results above indicate that there was no potential interference from the excipients, confirming the selectivity of the proposed methods.

Table 2. Evaluation of Selectivity of the (n=6) proposed methods in the presence of excipients

Excipient	Amount of excipient taken (µg/mL)	R(%)	RSD(%)
Lactozo	50.10^3	99.62	1.38
Glucose	5.10^3	102.87	0.80
Starch	50.10^3	105.56	0,57
Saccarozo	10.10^3	99.75	0.80
Sodium chloride	50.10^3	97.15	0.83

3.4. Comparison with other spectrophotometric methods

Table 3. Comparison of the proposed methods with other spectrophotometric methods

N_o	Reagent	λ_{max} (nm)	Range of determination (µg/mL)	Molar absorbitivity ($L.mol^{-1}.cm^{-1}$)	Remarks	Ref.
1	Metol- Crom (VI)	520	0.2 - 22	1.22×10^4	The redox reaction occurs at high temperature (50 °C) and reaction time is long.	[7]
2	3-amino phenol	500	20 - 140	2.88×10^3	The diazotization reaction occurs at low temperature (-2 - 3 °C), through many step and the reaction time is long.	[10]
3	p-dimethyl amino benzaldehyde	400	20 - 45	8.49×10^2	The diazotization reaction occurs at low temperature (0 – 5 °C), through many step and reaction time is long. Less sensitive.	[11]
4	Molybdophot phoric acid	820	0.5 - 4.5	9.05×10^3	The redox reaction occurs at high temperature (100°C), the reaction time is long and narrow linear range.	[12]
5	Black Eriochrome T	510	30 - 120	3.9×10^4	Ion-pair complex extracted by solvent dichloromethane.	[13]
6	Iodine	364	6 - 40	8.47×10^3	Solvent 1,2-dichloroethane is used for iodine reagent, methanol for DDQ and acetonitril for TCNQ. Narrow linear range.	[15]
7	DDQ	460	40 - 200	1.92×10^3		
8	TCNQ	843	6 - 18	19.77×10^3		
9	Fe(III)-hexacyanofer ate (III)	700	2 - 20	3×10^4	Hydrolysis and complexing reactions occurs at high temperature (70 °C) for a long time and narrow linear range.	[21]
10	DDQ	583	10 - 100	2.98×10^3	Charge transfer complexation reaction occur at room temperature, short reaction times, wide linear ranges.	This work

As shown in Table 3, the proposed method can favorably compare with other reported methods in the literature. In fact, the proposed method has higher sensitivity than other methods.

Furthermore, it needs no heating and are free from interference with common excipients.

3.5. Apply the proposed method

The proposed method was successfully applied to determine the presence and concentration of cefotaxime in some injectable powder samples. Six replicate determinations were made. Table 4 shows that the percentage of recovery (R%) from 95.45 to 98.56 % and the percentage of relative standard deviations (RSD%) were less than 2%. The accuracy of the proposed method is satisfactory. The high % recovery value indicates that there was no interference from excipients used in formulations.

4. CONCLUSIONS

The proposed method was found to be simple, sensitive, selective, accurate, precise, and economical, which can be used in the determination of cefotaxime in powder for injection. This method is based on well-characterized charge-transfer complexation reactions utilizing DDQ analytical reagents. Hence, the suggested charge transfer spectrophotometric methods are economical and practical for routine analysis of cefotaxime in quality control laboratories.

Table 4. Results of analysis of CFTX in injectable powder samples using the proposed method

Sample	Labeled amount (g/vial)	Amount found by proposed methods (g)	R(%)	RSD(%)
CFTX1	1	0.9545	95.45	0.51
CFTX2	1	0.9775	97.75	0.50
CFTX3	1	0.9856	98.56	0.36

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