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New Spectrophotometric Method for Determination of Ceftazidime in Pure Form and Pharmaceutical Dosages

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Abstract

A new simple, rapid, inexpensive and nontoxic spectrophotometric method for the determination of Ceftazidime in the pure and local market formulation was proposed. The method is based on the coupling reaction between the diazotized drug and 4-tert-butylphenol (4-TBP) to produce a colored compound with a (λ_{max} 500 nm). The optimal conditions for the factors affecting the formation of this compound were studied. Beer's law was obeyed and a linear calibration curve was obtained over the range of (1–10 µg/mL) with a correlation coefficient (r=0.9890), molar absorptivity ($\varepsilon=6.627\times10^3$ L/mol.cm), the limit of detection 0.314 µg/mL, and the limit of quantification LOQ 1.047 µg/mL. the accuracy and precision of the method were checked and the method was applied successfully for the determination of Ceftazidime in commercial preparation and the values of recovery percent and RSD% ranged between (93.11 - 102.20%) and (0.48 - 9.54%) respectively.

Keywords: Ceftazidime, spectrophotometric.

الخلاصة

الهدف من هذا البحث هو اقتراح طريقة طيفية جديدة بسيطة وسريعة وغير مكلفة وامنة لتقدير عقار السفتازيديم بصورته النقية ومستحضراته الصيدلانية. الطريقة تعتمد على تفاعل اقتران بين العقار المؤزوت (ملح دايزونيوم) مع الكاشف ٤-ثلاثي بيوتايل فينول لتكوين مركب ملون بقمة امتصاص عند طول موجي ٥٠٠ نانوميتر. تم دراسة الظروف المثلى للعوامل المؤثرة على كفاءة الامتصاص للمركب الملون الناتج .الطريقة المقترحة تنسجم مع قانون بيرعلى مدى تركيز (١-١٠ ملغم/لتر) تم رسم منحني المعايرة بمعامل انحدار(r=0.9890) وامتصاصية مولارية ($\varepsilon=6.627\times10^3$) لتر/مول.سم .قيمة حد الكشف ٠,٣١٤ ملغم/لتر وقيمة حد الكمية 1.047 ملغم/لتر .تم التحقق من الضبط والدقة لهذه الطريقة تبين بالامكان تطبيقها بنجاح في تقدير عقار السفتازيديم بصورته النقية ومستحضراته الصيدلانية بقييم نسب مئوية للاسترجاع وللانحراف القياسي بلغت (93.11-102.20%) و(0.48 - 9.54%) على التواليز

Introduction

Ceftazidime is the third generation of Cephalosporin group compounds. It has a chemical structure as follows: (6R,7R)-7-((Z)-2-(2-aminothiazol-4-yl)-2-(((2-carboxypropan-2-yl)oxy)imino)acetamido)-8-oxo-3-(pyridin-1-ium-1-ylmethyl)-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate [1]. It has a molecular weight 546.58 g/mol and has a molecular formula $C_{22}H_{22}N_6O_7S_2$. It acts as an effective, broad-spectrum antibiotic containing beta-lactam, a derivative of cephaloridine, classified as a semi-synthetic compound and used extensively in the treatment of infections caused by gram-negative bacteria and Pseudomonas [4][5]. The Cephalosporin group compounds are divided into the first, second, third and fourth generation depending on the effectiveness and the time of discovery [6]. Ceftazidime is used in the treatment of many arthritis and bone infections, in the treatment of types of skin infections, and in the treatment of inflammation of the nervous system and lower respiratory infections [7]. In the literature survey, HPLC was used to estimate Ceftazidime in the dose and its pure form [8]. There are also other analytical methods in estimating Ceftazidime such as charge transfer complexation [13].

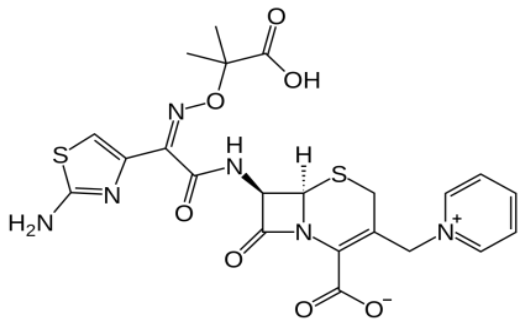

Figure 1: Chemical structure of Ceftazidime.

Other methods based on the spectral property for Ceftazidime within the visible and ultraviolet region using Uv-visible spectroscopy a technique [14], and by plasmon resonance peak of silver nanoparticles (Ag-NPs) $\lambda_{max.}$ = 410-430 nm [19]. The aim of this research is to develop an easy, flexible, sensitive and repetitive analytical method that can be used in routine analysis and quantification of Ceftazidime.

Experimental

Equipment

A Shimadzu UV-visible model 1800 spectrophotometer was used in the absorbance measurements and is equipped with a (1.0 cm path) quartz cells. Electronic sensitive Balance Mettle AE 200, PH-meter WTW, a model HI 83141 water bath with thermostat and shaker were used.

Reagents

The Ceftazidime standard was obtained from Quality Control Laboratory in The State Company for Drugs Industry & Medical Appliances (SDI-IRAQ) 4-tert-butylphenol (4-TBP), concentrated ammonia solution, potassium hydroxide, sodium hydroxide, sulfuric acid, sodium nitrite, hydrochloric acid, and ammonium chloride. All materials and the analytical grade reagents were purchased from Riedel-de Haen, Scharlau, ROMIL, PHILIP HARRIS, J.T. Baker, CHEM-SUPPLY, CDH, and MERCK. Ceftazidime drug samples which include injections and other formulations were purchased from the local market.

Preparation of stock solution

A stock (1000 µg/mL) Ceftazidime solution was prepared by dissolving 100 mg of standard Ceftazidime in 100 mL of distilled water. The calibration curve standard solutions in the range of (1-12 µg/mL) were prepared by subsequent serial dilution of the stock solution by with distilled water.

Preparation of sodium nitrite solution

1% w/v (0.144M) solution of sodium nitrite was prepared by dissolving 0.1 g of the compound in 10 mL distilled water. This solution is used in the diazotization of the Ceftazidime.

Preparation of 4-tert-butylphenol solution and other solutions

A 2.52×10^{-3} M solution of 4-tert-butylphenol was prepared by dissolving 0.378 g of the reagent in 1000 mL of distilled water containing a small volume of one molar sodium hydroxide solution. 25% (w/v) solutions of sodium hydroxide, potassium hydroxide, and sodium carbonate were individually prepared by dissolving appropriate weights of these compounds individually in10 mL of distilled water.

General recommended procedure

To a series of 20 mL volume flasks, varying amounts of stock Ceftazidime solution were added to cover the concentration range of (1-10 µg/mL), and the flasks were maintained in an ice bath (0-5 °C) for 5 min. Then, to each flask, 1 mL of concentrated (12M) hydrochloric acid and 1 mL of (1% w/v) sodium nitrite were added with gentle stirring. After then to each flask, 2 mL solution of (2.52×10^{-3} M) 4-tert-butylphenol and 4 mL of 25% (w/v) sodium hydroxide solutions were successively added and the content of the flasks was mixed gently and left to stand for 5 min. before completion the volumes to the mark with distilled water. The absorbance of the resulting colored solution (red) was measured at (λmax) 500nm against the reagen t blank, which was prepared in the same manner.

Result and Discussion

The spectrum of the standard 10 µg/mL solution of Ceftazidime was recorded in the range of 200-500 nm against the solvent blank,

Figure 2. The spectrum shows a wavelength of maximum absorption at 554 nm. On the other hand, Figure 3 shows the spectrum of 20 µg/mL solution of the formed Ceftazidime-4-tert-butylphenol azo dye versus its reagent blank recorded under the optimal experimental conditions. The spectrum shows that the λ_{max} for the formed coupling product appears at 500nm which confirm the formation of the suggested azo dye. This wavelength was selected to carry out all spectral measurements throughout.

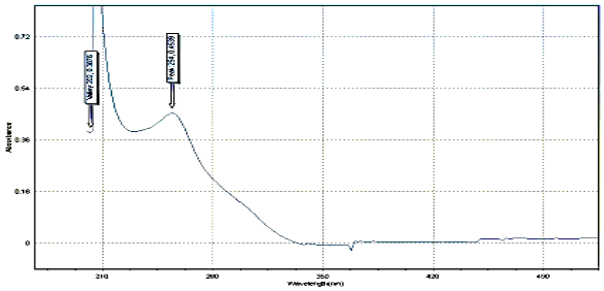

Figure 2: The absorption spectrum of 10 µg/mL of a standard solution of ceftazidime vs solvent blank**.**

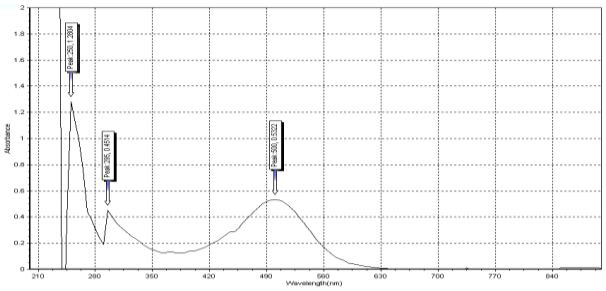

Figure 3: The spectrum of 20 µg/mL solution of Ceftazidime−4-tert-butylphenol versus the reagent blank recorded under the optimal conditions.

Optimization of experimental variables

Various experimental operating parameters it is influencing in the diazo-coupling reaction were optimized to have the maximum value of absorbance at 500nm by varying one parameter at a time. The optimization was accomplished using 400 µg of Ceftazidime in a final volume of 20 mL. Figure 4 shows the effect of the amounts of HCl, $NaNO_2$ and the reagent on the absorbance of the reaction product. It is evident that 1.0 mL (12M) hydrochloric acid solution, 1.0 mL of 1% (w/v) solution of $NaNO_3$, and 2.0 mL of 2.52×10^{-3} M 4-tert-butylphenol gave the best results in terms of speed and complete reaction (ref) and in obtaining the highest value of absorption of the colored reaction product.

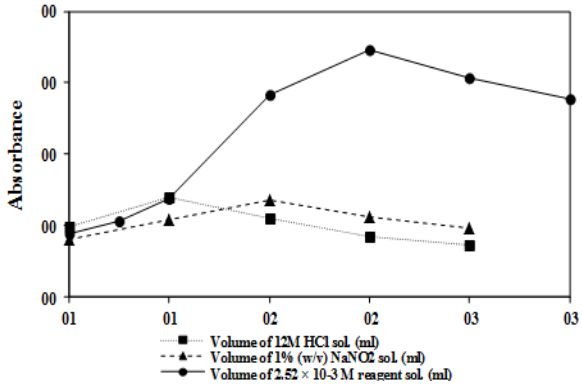

Figure 4: The effect of the amount of HCl, $NaNO_2$ and the reagent on the absorbance of the reaction product.

The effect of the type and the amount of the bases used to increase the reactivity towards the coupling with the cationic diazonium salt was studied. 4 mL of 25% (w/v) solution of different alkalis (namely; KOH, NaOH, NH_4OH, and Na_2CO_3) was used to provide a suitable medium for the coupling of the diazotized drug with 4-tert-butylphenol. NaOH was chosen since it gave the best result, Figure 5. Moreover, results in Figure 6 depict that 4 mL of the NaOH solution gave the highest absorbance.

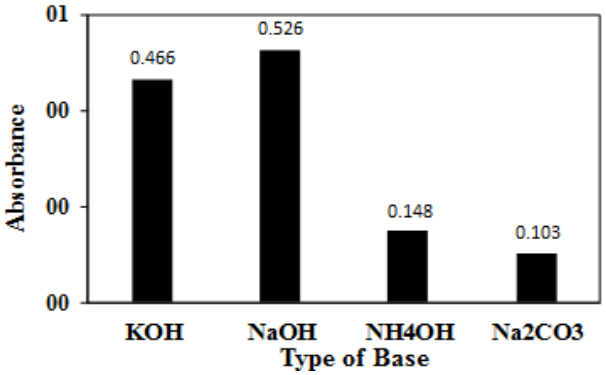

Figure 5: The effect of the type of bases.

Finally, the effect of time on the coupling reaction after the addition of the base was investigated as it is shown in Figure 7. Five minutes was required to get the highest value of the coupling reaction yield. The reason for the change in the absorption value of the reaction product over time is due to side

reactions that break down part of the dye and reduce absorption.

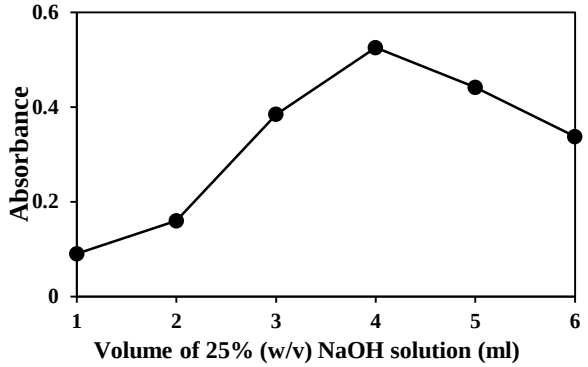

Figure 6: The effect of the volume of the NaOH solution.

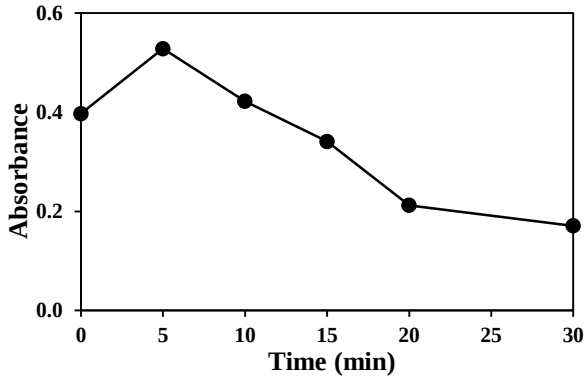

Figure 7: The effect of time on the coupling reaction.

Table 1 shows the established optimum experimental conditions for the diazotization of Ceftazidime and its coupling with 4-tert-butylphenol in alkaline medium to form a colored azo-dye which was used as a spectrophotometric method for the determination of the cited drug. A mechanism for the diazotization of Ceftazidime and its coupling reaction with 4-tert-butylphenol in alkaline medium is suggested as shown in Figure 8.

Table 1: The optimal conditions for the spectrophotometric determination of the 20 µg/mL Ceftazidime in a final volume of 20 mL.

Parameter	value
Wavelength of maximum absorption	500 nm
Volume of concentrated (12M) HCl solution	1.0 mL
Volume of 1%(w/v) sodium nitrite solution	1.0 mL
Volume of 2.52×10^{-3}M 4-tert-butylphenol solution	2.0 mL
Type of Base	NaOH
Volume of 25% (w/v) NaOH solution	4.0 mL
The time required to stand the solution after mixing	5.0 min.
Coupling time	5.0 min.

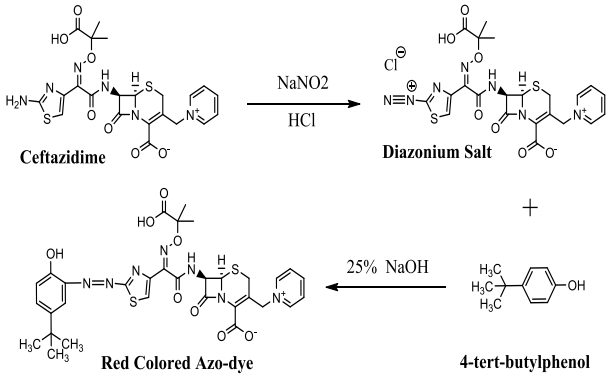

Figure 8: The suggested mechanism of diazotization and coupling reactions [20].

Calibration curve and linearity

Under the optimum experimental conditions, the values of absorbance at 500 nm was found to be a linear proportion with the concentration of the Ceftazidime. Beer's law plot was constructed in the Ceftazidime concentration range of (1-10 µg/mL), this range of concentration was selected to assess the validity of this supposed method because it accommodates the potential analytical applications of this method and this is inconsistent with the use of a higher concentration of this range in studying the optimal conditions of this method. Figure 9, and the value of the molar absorptivity was 6.627×10^3 L/mol.cm. Table 2 shows other quantitative and statistical parameters for the determination of Ceftazidime.

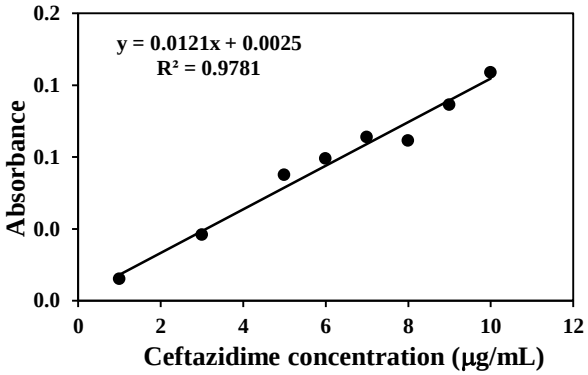

Figure 9: Standard calibration curve of Ceftazidime.

Table 2: Quantitative parameters for the studied reaction of Ceftazidime with 4-tert-butylphenol.

Parameter	value
λmax (nm)	500
Linear range (µg/mL)	1-10
Regression equation	y = 0.0121x + 0.0025
Slop (L/mg.cm)	0.0121
Intercept	0.0025
Molar absorptivity (L/mol.cm)	6.627×10^3
Correlation coefficient	0.9890
Detection Limit (µg/mL′)	0.3140
Quantification limit (µg/mL)	1.0468
Sandell's sensitivity (µg/cm)	0.0837

Accuracy and precision

The accuracy and precision of the described method were examined using replicate standards of three different concentrations within the calibration curve. The accuracy of the proposed method was expressed in terms of the relative error percent (RE%), and the precision in terms of the percent of relative standard deviation (RSD%). Through the obtained data presented in Table 3, it is obvious that the proposed method has yielded acceptable results.

Table 3: The accuracy and precision of the proposed method.

Concentration of pure Ceftazidime (µg/mL)		R.E %	RSD %
Taken	Found		
1	1.09	9.0**	0.0363**
3	3.07	2.3*	0.0153*
5	5.08	1.6**	0.1225**

* n=3, ** n=5.

Analysis of pharmaceutical dosage forms

The method was applied for the determination of Ceftazidime in pharmaceuticals formulations. The results depicted in Table 4 represent replicate analysis of each sample. Acceptable values of average recovery percent (n=3) ranged between 93.11% to 102.20 were obtained, while the values of relative standard deviation percent ranged between 0.48 and 9.54 % for the dosage samples at a concentration of 1 µg/mL and 5 µg/mL respectively. The (RSD %) value at concentrations (5 ppm) is high because one of the three values it was deviated from the rest of the values. These cases can be treated in the future by increasing the number of experiments and excluding deviant values to obtain acceptable values.

Table 6: Accuracy and precision of this method for the determination of pharmacological samples.

Name of company	Concentration samples of Ceftazidime (µg/mL)		Recovery %	RSD% n = 3
	Taken	Found		
Roth	1	1.069	93.11	3.91
	3	3.058	98.07	0.83
	5	5.028	99.45	9.54
So.Se.Pharma	1	1.003	99.72	0.48
	3	2.934	102.20	8.26
	5	5.055	98.90	9.54
LDP	1	0.992	100.83	0.83
	3	2.961	101.29	4.77
	5	4.972	100.55	9.54

Conclusion

In this work, a simple, inexpensive, and reasonably sensitive method was proposed for the determination of Ceftazidime in its pure samples and in pharmaceuticals. This method is based on the conversion of the drug into a colored product diazotization of the drug and coupling with a chromogenic reagent. The suggested method was statistically evaluated and successfully applied for the determination of Ceftazidime in pure form and in pharmaceutical preparation.

References

[1] Indian Pharmacopoeia, Govt. Of India, Ministry of Health and Family Welfare, The Indian Pharmacopeia Commission, Ghaziabad, 2007, 3, 876-878.

[2] The United States Pharmacopoeia; Asian edition, US pharmacopeial convention Inc., Rockville, 2003, 381-383.

[3] British Pharmacopoeia, The Department of Health, British Pharmacopoeia Commission, London, 2010, 2, 419.

[4] S. C. Sweetman, Martindale The Complete Drug Reference, 33rd edition, Pharmaceutical Press, London, 1996, 194.

[5] Rabindra K. Nanda, Ashwini V. Shelke. Development And Validation Of RP-HPLC Method For The Simultaneous Estimation Of Ceftazidime Sodium And Tazobactam Sodium In Marketed Formulation. International Journal of Pharm Tech Research Vol.5, No.3, pp 983-990, July-Sept 2013.

[6] Olajire A. Adegoke, Monsurat O. Quadri. "Novel spectrophotometric determinations of some cephalosporins following azo dye formation with p-dimethylaminobenzaldehyde" Arabian Journal of Chemistry (2016) 9, S1272-S1282.

[7] Cerner Multum "Medically reviewed by Drugs.com ceftazidime-injection" on Sep 11, 2017

[8] P. Jun, R. Yun, S. Yue, "Determination of ceftazidime injection by HPLC" Chinese Journal of Hospital Pharmacy, 2001,10,6
[9] A.H. Moreno, H.N. Salgado. "Development of a New High-Performance Liquid Chromatographic Method for the Determination of Ceftazidime" Journal of AOAC International, 2008, 91(4),739-43.
[10] S.Sanli, N.Sanli, M.Gumustas, "Simultaneous Estimation of Ceftazidime and Ceftizoxime in Pharmaceutical Formulations by HPLC Method" Chromatographia, 2011,74(7-8),549-558.
[11] S.A. Signs, T.M. File, J.S. Tan, "High-pressure liquid chromatographic method for analysis of cephalosporins" Antimicrobial Agents and Chemotherapy, 1984, 26(5),652.
[12] Mohan Gandhi Bonthu, Lakshmana Rao Atmakuri, Venkateswara Rao Jangam" Validated Chromatographic Method for the Estimation of Ceftazidime and Tazobactam in Pure and Tablet Dosage Form" Der Pharma Chemica, 2017, 9(16):61-66.
[13] Indian Pharmacopoeia, Controller of Publications, Delhi 1996, pp. 149–150.
[14] *K.* Arun, C. Saravanan, R. Balachandar, "UV-Spectrophotometric determination of Ceftazidime in pure and pharmaceutical formulation" Journal of Chemical and Pharmaceutical Research. 2010, 2(1), 424-431.
[15] A.H. Moreno, H.R. Salgado. PubMed. "Rapid and selective UV spectrophotometric method for the analysis of ceftazidime" Journal of AOAC International. 2009, May-Jun, 92(3),820-3.
[16] A.B. Devkhile, K.A. Shaikh. Method development and validation for third-generation cephalosporin by UV-vis spectrophotometer" International Research Journal of Pharmacy, 2011, 2(1): 222-229.
[17] H. Basavaraj, H.M. Bennikallu, M. Swamy. "Development and validation of spectrophotometric methods for determination of ceftazidime in pharmaceutical dosage forms" Acta Pharma, 2008, 58, 275-285.
[18] Hiremath B, Mruthyunjaya Swamy BH, " Development and validation of spectrophotometric methods For determination of ceftazidime in pharmaceutical dosage forms" Acta Pharm. 2008 Sep;58(3):275-85.
[19] Hesham Salem, Ebtihal Samir, " Determination of cefotaxime, cefoperazone, ceftazidime, and cefadroxil using surface plasmon resonance band of silver 1 nanoparticles" Braz. J. Pharm. Sci. vol.54 no.3 São Paulo 2018 Epub Nov 29, 2018.
[20] Baliz Merino " Synthesis of azobenzenes: the coloured pieces of molecular materials" Chem. Soc. Rev., 2011, 40, 3835–3853, www.rsc.org/csr.