



Spectrophotometric Method for the Estimation of Ceftriaxone in Pure Form and Pharmaceuticals

Aya W. Ahmed

Salam A.H. Al-Ameri

Department of Chemistry, College of Science, University of Mustansiriyah

Ayawaleed47@yahoo.com

Article history: Received 17 June 2019, Accepted 6 August 2019, Published in April 2020.

Doi: 10.30526/33.2.2441

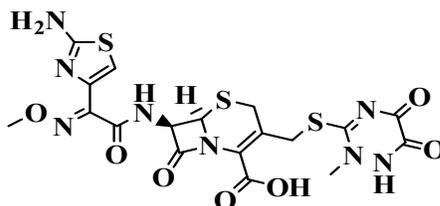
Abstract

Ceftriaxone sodium were one of the widely antibacterial drugs used. Azo dye derivatization of diazonium salt that formed via the reaction between ceftriaxone with hydrochloric acid and sodium nitrite was developed for the on-research drug analysis then coupling with each one 2,5-dimethylphenol (2,5-DMP) and 4-tertbutylphenol (4-TBP) respectively in the alkaline media. The developed diazonium coupling methods include an optimization study. The results show a limit of detection and limit of quantification 0.482, 0.284 $\mu\text{g/mL}$, and 1.607, 0.945 $\mu\text{g/mL}$ using 2,5-DMP and 4-TBP reagents respectively. Moreover, the recovery % obtained was 100.89%, and 103.37% at linear concentration range 3.0 – 50, and 10 – 30 $\mu\text{g/mL}$, with molar absorptivity of 4.377×10^3 , 7.446×10^3 $\text{L. mol}^{-1} \cdot \text{cm}^{-1}$ using 2,5-DMP and 4-TBP reagents respectively. An acceptable accuracy represented by the relative standard deviation (RSD%) was achieved of a 0.46 and 0.37 for 2,5-DMP and 4-TBP respectively. The proposed method was successfully used for the determination of ceftriaxone sodium in pure and in pharmaceuticals.

Keywords: Ceftriaxone; Azo dye; Coupling; Spectrophotometric; 2,5-dimethylphenol; 4-tertbutylphenol.

1. Introduction

Cephalosporins consist of two – incorporated rings system of beta-lactam and dihydrothiazine [1]. Ceftriaxone sodium (CFT) is one of the third generation cephalosporins and one of the most widely used antibiotics to treat both bacterial infections Gram positive and Gram negative [2, 3]. The molecular formula is $\text{C}_{18}\text{H}_{18}\text{N}_8\text{O}_7\text{S}_3$, **Scheme I**. [4].



Scheme 1. Structure of Ceftriaxone Sodium.



Several analytical methods have been reported for the estimation of CFT in pure and pharmaceuticals such as spectrophotometric [5-8]. Spectrofluorimetry [9]. Infrared [10]. HPLC – UV [11-13]. Chromatography [14]. And HPLC- MS [15]. As well as in biological samples HPLC-UV [16-20]. The idea of this work is to develop a simple, sensitive and effective method for the validation of the Ceftriaxone in pure and commercial preparation as a quality control purpose.

2. Materials and Methods

2-1. Chemicals and Equipment:

All chemicals used were of analytical reagent grade with high purity. Sodium nitrite, hydrochloric acid, potassium hydroxide, analytical standard of ceftriaxone sodium, analytical standard of 2,5-DMP and 4-TBP were purchased from Sigma-Aldrich, Al Qiffaf Scientific Company - Baghdad, Iraq. Double distilled water was used throughout the experiments for preparation of the reagents and samples.

For all absorbance detection, a double beam dual chopper, UV-Vis spectrophotometer, Varian, Cary 100, Australia was used pH S-3E Precision acidity Meter, Ray Magnetic Instrument Factory, Shanghai, China. Mettler AE 200 Electronic Balance, Germany and shaker.

2-2. Preparation of a Ceftriaxone Sodium Stock Solution

A 1000 $\mu\text{g. mL}^{-1}$ of standard CFT was prepared by dissolving 0.1g in 100 mL of distilled water then prepared a diluted solution.

2-3. General Procedures:

2-3-1. Coupling the Ceftriaxone with the 2,5-DMP reagent:

In an ice bath at zero to 5 °C; in a 20 mL volumetric flasks take a 0.5 mL of 1000 $\mu\text{g. mL}^{-1}$ of standard CFT solution then add 2.0 mL of 0.57M HCl and 0.5 mL of 0.14M NaNO₂ which lead to forming a diazonium salt after leaving the solution for 10 minutes which coupling with 1.5 mL of 2.0 x 10⁻³ M 2,5-DMP reagent in alkali media by using 1.5 mL of 0.45 M KOH. The Azo dye formed was diluted with distilled water to the mark. The colored product solution was measured at 520 nm.

2-3-2. Coupling the Ceftriaxone with the 4-TBP Reagent:

In an ice bath at zero to 5 °C; in a 20 mL, volumetric flasks a 0.5 mL of 1000 $\mu\text{g. mL}^{-1}$ of standard CFT solution was taken then a 1.5 mL of 0.57M HCl and 1.0 mL of 0.14 M NaNO₂ was added that led to form a diazonium salt after leaving the solution for 10 minutes which coupling with 1.5 mL of 2.5 x 10⁻³ M 4-TBP reagent in alkali media by using 2.0 mL of 0.45 M KOH. The Azo dye formed was diluted with distilled water to the mark. The product colored solution was measured at 500 nm.

3. Result and Discussion

Initial tests were performed via the spectrophotometric method for the estimating of CFT by using 2,5-DMP and 4-TBP reagents and a red color of the azo dye formed was illustrate, **Figure 1 A, B.**

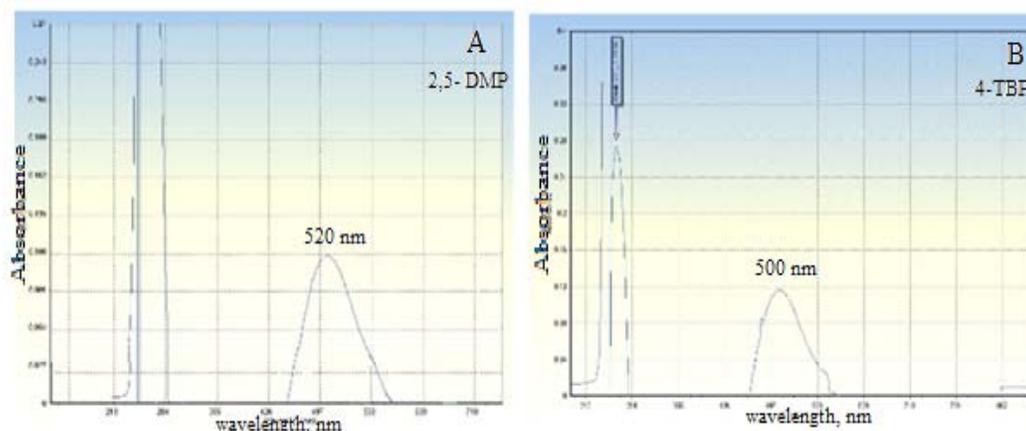


Figure 1. Initial UV-Vis spectra for CFT azo dye with (A) 2,5-DMP and (B) 4-TBP reagents.

3-1. Method Optimization

This spectrophotometric method for CFT estimation including diazonium coupling was optimized. The optimization parameters are; HCl and NaNO_2 concentrations also the alkaline type and concentration.

3-1-1. Hydrochloric acid volume

The volume of hydrochloric acid that is important to the nitrous acid resulting with sodium nitrite which reacts with the amine group of ceftriaxone to form the diazonium salt.

The increase of acid volume was effect on the resulting azo dye absorbance. The optimum volume was 1.0 and 1.5 mL of 0.57 M HCl for 2,5-DMP and 4-TBP respectively, **Figure 2**. The intensity was reduced at higher or additional acid concentration which due to the incompetently converted of the primary amine [21].

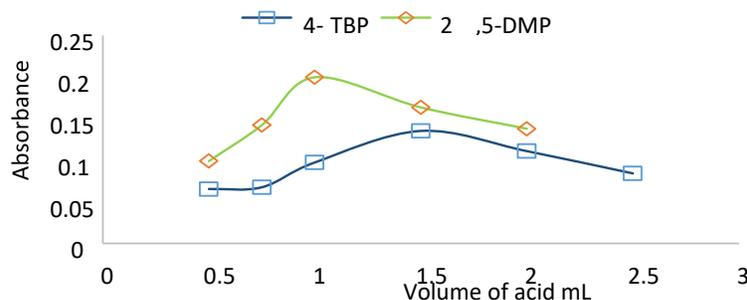


Figure 2. Effect of different HCl concentration (volume) on the CFT azo dye formation via 2,5-DMP and 4-TBP reagents.

3-1-2. Sodium Nitrate Volume

Sodium nitrate with HCl produced nitrous acid, which was responsible for the diazonium salt forming that was administered for the formation of azo dye. Increasing amounts of 0.14 M sodium nitrate were studied in the range of 0.25 - 2.0 mL. The best of the volume of sodium nitrate were 0.5 and 1.0 mL and the product dyes measured at 500 nm and 520 nm for 4-TBP and 2,5-DMP reagents respectively-as in **Figure 3**. The excess of the NaNO_2 volume caused interferences contamination that led to reduce the absorbance [22].

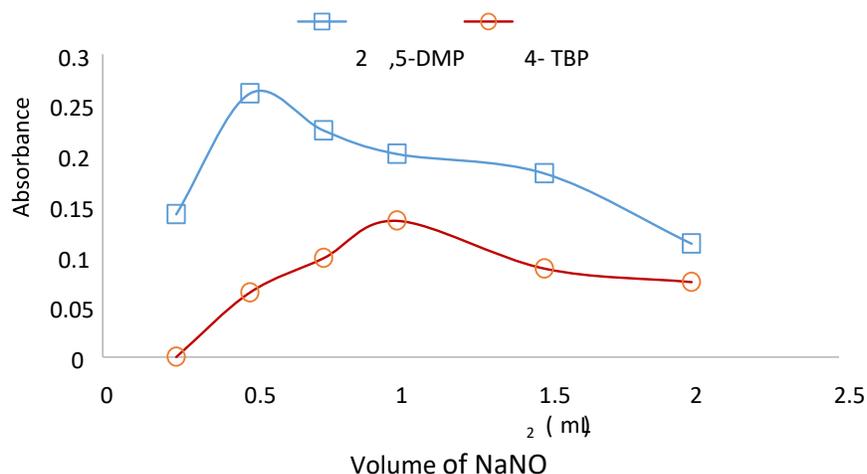


Figure 3. Effect of different NaNO₂ concentration (volume) on the CFT azo dye formation via 2,5-DMP and 4-TBP reagents.

3-1-3. Reaction Time

The reaction time was studied over a period of zero to 30 minutes to discover the ideal time for the diazonium salts and the developed azo dyes affect the absorbance intensity. The results illustrated that the 10 minutes was a sufficient time-as in **Figure 4**.

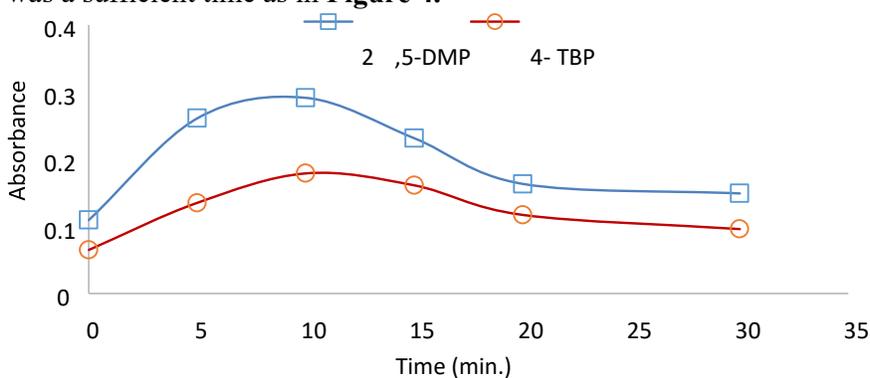


Figure 4. Effect of different reaction time on the CFT azo dye formation via 2,5-DMP and 4-TBP reagents.

3-1-4. Base type and Concentration

The alkaline media was so important to remove hydrogen atom of the phenolic compounds, which changed to very active species called phenoxide ion that coupling with diazonium salts to form azo dyes. A range of bases such as NaOH, KOH, NH₄OH and Na₂CO₃ at the concentrations range of 0.23, 0.35, 0.45 and 0.62 M. Potassium hydroxide was chosen as a best with 2,5-DMP and 4-TBP respectively as in **Figure 5-A**.

Additionally, optimum volume of KOH was studied in a range of 0.5 - 2.5mL which showed that the 1.5 and 2.0 mL with 2,5-DMP and 4-TBP respectively were the appropriate volumes which gave a good dye with higher absorbance as in **Figure 5-B**.

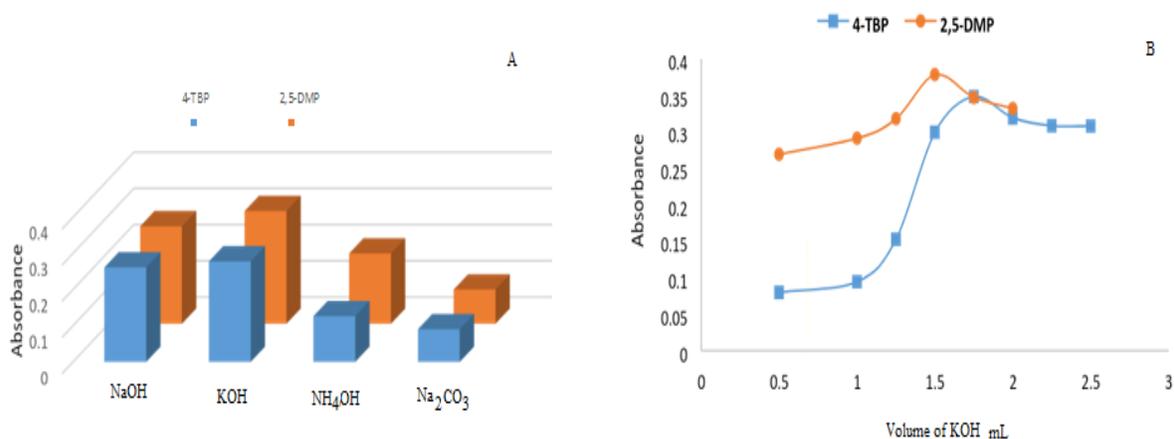


Figure 5. Effect of different alkaline types (A) and concentrations (B) on the CFT azo dye formation via 2,5-DMP and 4-TBP reagents.

3-1-5. Volume of the Reagents 2,5-DMP and 4-TBP

Various volumes of 2.0×10^{-3} M 2,5-DMP and 2.5×10^{-3} M 4-TBP reagents were used to select the appropriate volume, which was combined with CFT diazonium salt to form a dark azo dye that gave a high intensity and higher absorbance. The results showed that the 1.5 mL of 2,5-DMP and 4-TBP is the best volume, as in **Figure 6**.

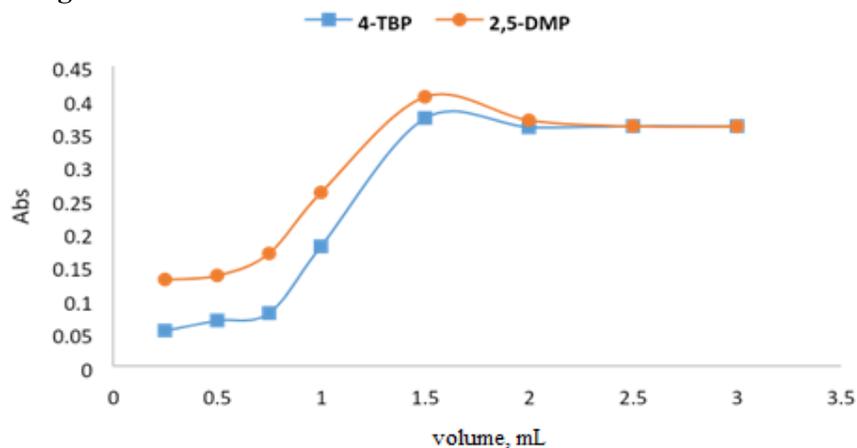


Figure 6. Effect of different 2,5-DMP and 4-TBP reagents on the CFT azo dye formation.

3-2. Calibration Curve and Analytical Merits

Calibration curves was constructed using 2.0×10^{-3} M 2,5-DMP and 2.5×10^{-3} M 4-TBP solutions with a concentration ranges of $3.0 - 50 \mu\text{g. mL}^{-1}$ and $10 - 30 \mu\text{g. mL}^{-1}$ of CFT solution via 2,5-DMP and 4-TBP respectively versus the absorbance resulted, as in **Figure 7**. The absorbance spectra were measured at λ_{max} of 520 nm and 500 nm for CFT with 2,5-DMP and 4-TBP respectively, as in **Figure 8**.

All the analytical data such as limit of detection (LOD), limit of quantification (LOQ) and relative standard deviation (%RSD) were summarized in **Table 1**. [21].

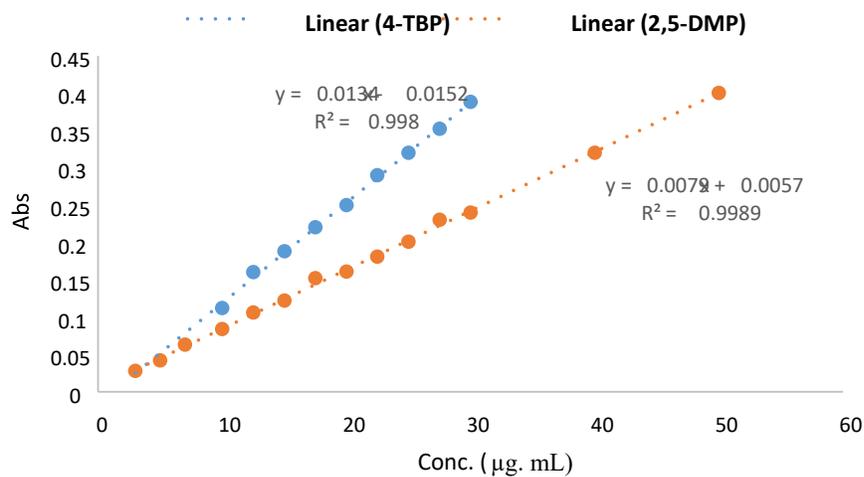


Figure 7. Calibration graph of 10 – 30 and 3.0 – 50 µg. mL⁻¹ of CFT with 2.0 x 10⁻³ M 2,5-DMP and 2.5 x 10⁻³ M 4-TBP respectively.

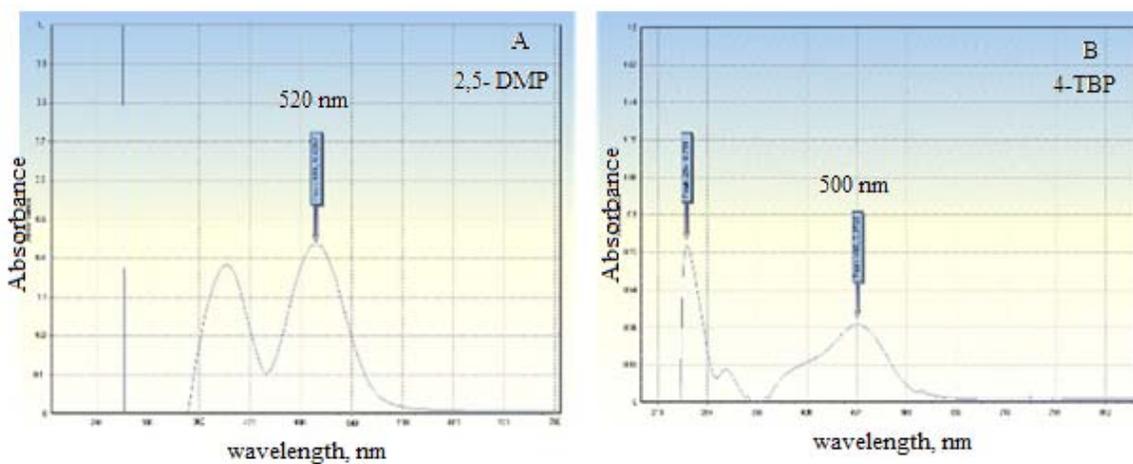


Figure 8. UV-Vis spectra of Calibration graph of 10 – 30 and 3.0 – 50 µg. mL⁻¹ of CFT with 2.0 x 10⁻³ M 2,5-DMP and 2.5 x 10⁻³ M 4-TBP respectively.

Table 1. Analytical data of the proposed diazotization-coupling methods.

Parameters	2,5-DMP	4-TBP
Color	red	Orange
λ_{max} , nm	520	500
Linearity range, $\mu\text{g. mL}^{-1}$	(3-50)	(10-30)
Molar absorptivity, ϵ , $\text{L. mol}^{-1}.\text{cm}^{-1}$	4.377×10^3	7.446×10^3
Slope, b	0.0079	0.0134
Intercept, a	0.0057	-0.0152
Sandal sensitivity, S	0.1267	0.0745
coefficient of determination, % R^2	99.89	99.8
correlation coefficient, r)	0.9994	0.9990
limit of detection, LOD, $\mu\text{g. mL}^{-1}$	0.4820	0.2840
Limit of quantification, LOQ, $\mu\text{g. mL}^{-1}$	1.6080	0.9454

At a linear concentration range between 3.0 -50 and 10 -30 $\mu\text{g. mL}^{-1}$, the results showed a limit of detection and a limit of quantification of 0.482, 0.284 and 1.608, 0.945 $\mu\text{g. mL}^{-1}$ with 2,5-DMP and 4-TBP respectively with recovery of 100.89% and 103.37%, with molar absorptivity of 4.377×10^3 and 7.446×10^3 $\text{L. mol}^{-1}.\text{cm}^{-1}$ for 2,5-DMP and 4-TBP respectively.

3-3. Accuracy and Precision

The precision and accuracy were calculated by the analysis a five replicates for three different CFT concentrations [21]. The precision was estimation by the determine of percent relative error %R.E whereas the accuracy was determination by calculating the percent relative standard deviation %RSD. An reasonable precision with acceptable accuracy was obtained which illustrated a 0.46 and 0.37 for 2,5-DMP and 4-TBP as in **Table 2**.

Table 2. The accuracy and precision of the developed method with 2,5-DMP and 4-TBP reagents.

Type of reagent	Conc. Taken, $\mu\text{g. mL}^{-1}$	Conc. Found $\mu\text{g. mL}^{-1}$	Relative Error%	%RSD	*Recovery, %	**Average Recovery
2,5-DMP	1.0	0.98	-2.0	0.46	98.0	100.89
	3.0	3.08	2.7		102.7	
	5.0	5.1	2.0		102.0	
4-TBP	1.0	1.09	9.0	0.37	109.0	103.378
	3.0	3.1	3.3		103.3	
	5.0	4.89	-2.2		97.8	

* Average for 5 determinations. ** Average for 15 determinations.

3-4. CFT: Reagent Stoichiometry

A continuous variation (Job) method [22]. Was applied for the estimation of CFT: reagent stoichiometry. The solutions were prepared at an equal concentration of 2.0×10^{-3} M for CFT with 2,5-DMP and 2.5×10^{-3} M for CFT and 4-TBP. This method was applied by increasing the volumes from 0.1 to 0.9 mL of CFT and reducing the volumes from 0.9 to 0.1 mL of reagents to a final volume of 1.0 ml. The results showed a conjugated ratio is of 1:1 between CFT with two reagents, **Figure 9, 10**.

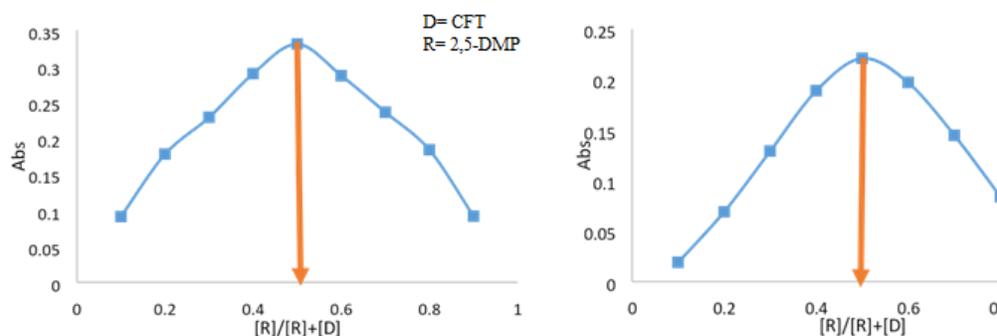


Figure 9. Continuous variation method for CFT: Reagent stoichiometry with 2,5-DMP at 520nm and with 4-TBP at 500nm.

3-5. Application of the developed method for the CFT estimation in pharmaceuticals

This developed method was successfully used for the CFT determine in commercial pharmaceuticals for diverse products from Malaysia, Jordan and United Arab of Emirate at a concentration of 6.0 and 10 $\mu\text{g. mL}^{-1}$. The results were summarized in **Tables 3, 4**.

Table 3. Assay of CFT in pharmaceutical preparations using 2,5-DMP reagent.

pharmaceuticals of CFT	Conc. Taken $\mu\text{g. mL}^{-1}$	Conc. Found $\mu\text{g. mL}^{-1}$	%RE	*Recovery %	**Average Recovery
Malaysia	6.0	6.002	0.03	100.03	100.57
	10	10.11	1.1	101.1	
Jordan	6.0	6.1	1.67	101.67	101.99
	10	10.23	2.3	102.3	
United State Emirate	6.0	5.897	-1.72	98.28	99.14
	10	9.999	-0.01	99.99	

* Average for 5 determinations, ** Average for 10 determinations for two concentration.

Table 4. Assay of CFT in pharmaceutical preparations using 4-TBP reagent.

pharmaceuticals of CFT	Conc. Taken $\mu\text{g. mL}^{-1}$	Conc. Found $\mu\text{g. mL}^{-1}$	%RE	*Recovery %	**Average Recovery
Malaysia	6.0	6.011	0.18	100.18	101.39
	10	10.26	2.6	102.6	
Jordan	6.0	6.32	5.3	105.3	104.7
	10	10.41	4.1	104.1	
United State Emirate	6.0	5.994	-0.1	99.9	100.35
	10	10.08	0.8	100.8	

* Average for 5 determinations. ** Average for 10 determinations for two concentration.

4. Conclusion

This study is successfully employed for the CFT determination in pharmaceuticals, which gave an increase in the analytical sensitivity, selectivity and precision. The procedure in spectral mode was simple, inexpensive and accurate.

Acknowledgement

Authors are so grateful to Dr. Ammer S. Mahdi, College of Science, Mustansiriyah University, Chemistry department for his hard helpful during this work.

References

1. Shahbaz, K.J.P.; Evaluations, B. Cephalosporins: pharmacology and chemistry. *pharmaceutical and biological evaluations*.**2017**, 4, 6, 234-238.
2. Zeng, L.; Hu, D.; Choonara, I.; Mu, D.; Zhang, L.; Li.X.; Zhang, Z.; Hu, Z.; Quan, S. A prospective study of the use of antibiotics in the Emergency Department of a Chinese University Hospital. *International Journal of Pharmacy Practice*.**2017**, 25, 1, 89-92.
3. Ivády, B.; Kenesei, É.; Tóth-Heyn, P.; Kertész, G.; Tárkányi, K.; Kassa, C.; Ujhelyi, E.; Mikos, B.; Sápi, E.; Varga-Heier, K.J.I. Factors influencing antimicrobial resistance and outcome of Gram-negative bloodstream infections in children. *A Journal of Infectious Diseases*.**2016**, 44, 3, 309-321.
4. Okorie, O.; Abayomi, E.O.; Onyinyechi, E. J. J. o. P. R. I. Pharmaceutical Quality Analysis of Ceftriaxone Sodium Brands Marketed in Southern Nigeria. *Journal of Pharmaceutical Research International*.**2016**, 1-8.
5. Sajjad, A.I.; Naresh, M.I.; Jasmin, K.; Rasul Jan, S.M.; Zohra, K.; Muhammad, A. Development of spectrophotometric method for determination of ceftriaxone sodium in different brands of pharmaceutical preparation involving diazotization, *International J. of Pharmaceutical Sciences and Research*.**2015**, 6,12.
6. Rind, F.M.A.; Memon1, M.G.H.L.; Mughal, A.H.; Almani1, U.R.; Memon1, F.; Khuhawar N.; Maheshwari, M.Y. Spectrophotometric Determination of Ceftriaxone Using 4-Dimethylaminobenzaldehyde, *Pak. J. Anal. Environ. Chem*.**2008**, 9, 1, 43 – 48.
7. Lakshmi, K.S.; Nithya Mathi, K.I.N.; Balaji, S.; Kibe Victor Wathugi, D.; Satish, A.V. Visible Spectrophotometric Determination of Ceftriaxone Sodium in Vials , *Asian J. of Chemistry*.**2007**, 19, 4, 2517-2520.
8. Patel*, S.A.N.M.P.a.M. M. P. Spectrophotometric estimation of cefotaxime and ceftriaxone in pharmaceutical dosage forms, *Indian J Pharm Sci*.**2006**, 68, 1, 101-103.
9. Shah, J.; Jan, M.R.; Shah, S.J.L. Development and validation of a spectrofluorimetric method for the quantification of ceftriaxone in pharmaceutical formulations and plasma. *Journal of Biological and Chemical Luminescence*.**2013**, 28, 4, 516-522.
10. Trindade, M.; Salgado, H.J.P.C. Development and validation of a modern and stability-indicating method for the quantification of ceftriaxona sodium in powder for injection by infrared spectroscopy. *Physical Chemistry*.**2017**, 7, 55-62.
11. Naguib, I.A.; Abdelaleem, E.A.; Zaazaa, H.E.; Hussein, E.A.E.J.E.J.o.C. Development and validation of stability indicating spectrophotometric and HPTLC methods for determination of acemetacin. *European Journal of Chemistry*.**2014**, 5, 2, 219-226.
12. El-Adl, S.M.; El-Shanawany, A.A.; Abdel-Aziz, L.M.; Hassan, A.F.J. A.J.o.P.A. HPLC Determination of Three Cephalosporins (Cefepime, Cefotriaxone and Cefotaxime) in Their Bulk and Dosage Forms. *Asian Journal of Pharmaceutical Analysis*.**2014**, 4, 3, 91-97.

13. Panchal, V.J.; Desai, H.T.; Dave, A.G.J.I.J.P.S. R. Development and Validation of Stability Indicating Method for Simultaneous Estimation of Ceftriaxone and Tazobactam Injection Using RP-UPLC Method. *INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH*. **2014**, *5*, 2398-2405.
14. Page-Sharp, M.N.T.; Salman, S.; Moore, B.R.; Batty, K.T.; Davis, T.M.; Manning, L.J. A. a. chemotherapy. Validation and application of a dried blood spot ceftriaxone assay. **2016**, *60*, *1*, 14.
15. Tange, M.Y., M.; Nakai, Y.; Uchida, T.J.C.; Bulletin, P. The role of an impurity in ceftriaxone sodium preparation for injection in determining compatibility with calcium-containing solutions. **2016**, *64*, *3*, 207.
16. Gergs, U.; Clauss, T.; Ihlefeld, D.; Weiss, M.; Pönicke, K.; Hofmann, G.O.; Neumann, J.J.J.o.P. Pharmacology, Pharmacokinetics of ceftriaxone in plasma and bone of patients undergoing hip or knee surgery. *Journal of Pharmacy and Pharmacology*. **2014**, *66*, *11*, 1552-1558.
17. Kratzer, A.; Liebchen, U.; Schleibinger, M.; Kees, M.G.; Kees, F.J.J.o.C.B. Determination of free vancomycin, ceftriaxone, cefazolin and ertapenem in plasma by ultrafiltration: impact of experimental conditions. *Journal of Chromatography B*. **2014**, *961*, 97-102.
18. Shah, J.; Rasul Jan, M.; Shah, S.; Naeem Khan, M.J.J.o.t.M.C.S. Development and validation of HPLC method for simultaneous determination of ceftriaxone and cefaclor in commercial formulations and biological samples. *Journal of the Mexican Chemical Society*. **2013**, *57*, *4*, 314-320.
19. Sun, H.; Wang, H.; Ge, X.J.J.o.c. I.a. Simultaneous determination of the combined drugs of ceftriaxone sodium, metronidazole, and levofloxacin in human urine by high-performance liquid chromatography. *Journal of Clinical Laboratory Analysis*. **2012**, *26*, *6*, 486-492.
20. Briscoe, S.E.; McWhinney, B.C.; Lipman, J.; Roberts, J.A.; Ungerer, J.P.J.J.o.C.B. A method for determining the free (unbound) concentration of ten beta-lactam antibiotics in human plasma using high performance liquid chromatography with ultraviolet detection. *Journal of Chromatography B*. **2012**, *907*, 178-184.
21. James N.M.; Jane, C.M. Statistics and chemometrics for analytical chemistry, P. E. L., Prentice Hall, 5th Ed. **2005**.
22. Renny, J.S.; Tomasevich, L.L.; Tallmadge, E.H.; Collum, D.B.J.A.C.I.E. Method of continuous variations: applications of job plots to the study of molecular associations in organometallic chemistry. *journal of the German Chemical Society*. **2013**, *52*, *46*, 11998-12013.