Sys Rev Pharm 2020; 11(6): 330 – 336
A multifaceted review journal in the field of pharmacy
E-ISSN 0976-2779 P-ISSN 0975-8453

# A New Spectrophotometric Method for the determination of Cefepime in pharmaceutical preparation

Imad Tarek Hanoon
University of Samarra, College of Applied Science, Samarra, Iraq.

*Article History:* *Submitted: 08.04.2020* *Revised: 12.05.2020* *Accepted: 15.06.2020*

ABSTRACT
A simple spectrophotometric method was improved for the validation of cefepime (CE)in the pure and traditional drug forms. Themethodwas characterized inexpensive, rapid, sensitive, selective, and accurate. Itincludestwo reagentsforthe formation of azo dye.The methods involve CE diazonium salt in first reagent4-tetra-butylphenol, and in second reagent2,5-dimethylphenolinalkaline medium. The result obtained a stable red colored dye and gives absorption at 500nmand 515nm respectivelyagainst reagent blank. Law of Beer is obeyed in the concentration range of 1.0 – 50 µg. $mL^{-1}$ with molar absorptivity of $6x10^3$ and $4.5x10^3$ $L.mol^{-1}.cm^{-1}$. Limit of detection was 0.316, 0.631 $\mu g.mL^{-1}$, RSD% was 0.16, 0.23 and Sandell's sensitivity value 0.08, $0.01\mu g.cm^{-1}$respectively.
**Keywords:** Spectrophotometry, cefepime, infections
**Correspondence:**
Imad Tarek Hanoon
University of Samarra, College of Applied Science, Samarra, Iraq
**DOI:** 10.31838/srp.2020.6.52


## INTRODUCTION

Considered the cefepime (CE) one of cephalosporins group is used for the treatment of infections caused by Gram (+) and Gram (–) bacteria. [1]Cephalosporinsare mostly classified based on the time of their detect and their pharmacologicalantibacterial compounds target growth processes or bacterial functions i.e.with the effectiveness of spectral or chemical structure and most of these to generation first, second, third, fourth and fifth. Cefepime is one of fourth generation, is an injection cephalosporin with high stability for broad spectrum β-lactamase. [2]The name of cefepime is 7-[α-(2-aminothiazol-4-yl)-α-(z)methoxyiminoacetamido]-3-(1-methylpyrrolidino)-methyl-3-cephem-4-carboxylate and structure formula as shown in Figure 1. It plays an important role in treatment of lower respiratory tract, intra-abdominal, urinary tract, skin and soft tissue infections and also used for prophylaxis in biliary tract and prostate surgery as used clinically. [3,4]The drug has been determined by a variety of analytical techniques such as Chromatographic, Ultra-high performance liquid chromatography coupled to tandem mass spectrometry (UHPLC-MS/MS) [5], Solid Phase Extraction HPLC [6], High-Performance liquid chromatography (HPLC) [7,8], High-Performance liquid chromatography and Spectro-photometry (HPLC with UV) [9], Liquid chromatography with Mass spectrometry LC-MS [10], Micellarelectrokinetic chromatographywith UV. [11] and spectrophotometric methods.[12] The spectrophotometric method has been applied for the determination of many drugs includes adrenaline [13], Ezetimibe and Cefepime[14] and Tazobactam and Cefepime[15]. The aim of present work was to develop simple, sensitive and selective spectrophotometric method in alkaline medium on based on dizotization-coupling reaction ofcefepimewith 4-tetra-butylphenol, and 2,5-dimethylphenol reagent for the determination of cefepimein pure and pharmaceutical forms.

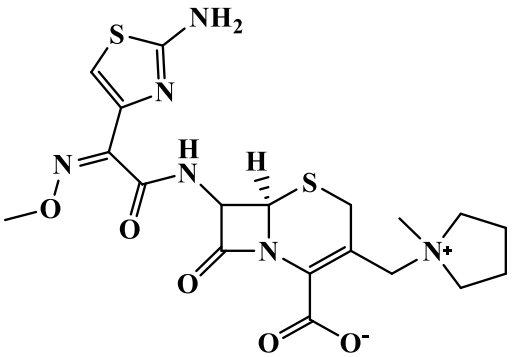


Figure 1: Structure of cefepime

## MATERIALS AND METHODS

The apparition used for the measurements of absorbance in a UV – Vis model (UV-1800) Shimadzu spectrophotometer equipped with a 1.0 cm quartz cell. Thermostated water bath, shaker, a model, HI 83141 , WTW pH meter (Germany), and Electronic Balance Mettle AE 200 (Germany) were used.

Reagents

All chemicals were of analytical grade and werefrom Merck KGaA (Darmstadt, Germany) and used as received. Stock solution (1000 mg $L^{-1}$CE) was prepared by dissolving 0.1 gm of drug in 100 mL of distilled water. 4-tetra-butylphenol, 2,5-dimethylphenol 2.0 x $10^{-3}$mol $L^{-1}$and other chemicals solutions are:

Hydrochloric acid solution (6.0 N). Is prepared by diluting 50.8 mL of 11.8 N concentrated acid to the mark in a 100mL volumetric flask with distilled water.

Sodium nitrite solution (1.0 %). Is prepared by dissolution 1.0 gm of sodium nitrite in distilled water and diluted to the mark in a 100mL volumetric flask with distilled water.

Solution of Sulphamic acid (2.0 %). Is prepared by dissolution 2.0gm of sulphamic acid in distilled water and anddissolved in 100 ml volumetric flask with distilled water.

Sodium hydroxide solution (2.5N). Is prepared by dissolution 10 gm of sodium hydroxide in distilled water and dissolved in 100 ml volumetric flask with distilled water.

Potassium hydroxide solution (3.0 N). Is prepared by dissolution 16.8 g of potassium hydroxide in distilled water and dissolved in 100 ml volumetric flask with distilled water.

General procedure of diazotization reaction for cefepimedrug.

To a series of 20 mL volumetric flask transfer an aliquot of samples solutionscontaining 1.0 mL of 1000mg $L^{-1}$ of CE, put in an ice bath at zero ºC. and added 1.0 mL of 6.0 mol $L^{-1}$HCl, 1.0 ml of 1.0% (w/v)$NaNO_2$and 1.0 mL of 2.0%(w/v)sulphamic acid with shaking. The mixture leave to stand form10 and 15 minutes for 4-tetra-butylphenol andcefepim e with 2,5-dimethylphenolin aalkaline medium respectively. Followed by adding 1.0 mL of $2.0x10^{-3}$mol $L^{-1}$4-tetra-butylphenol and 2,5-dimethylphenolrespectively. After that 2.5 mL of 3.0 mol $L^{-1}$ KOH solution to mixture that containing4-tetra-butylphenol and 3.0 mL of 2.5 mol $L^{-1}$NaOH tomixture that containing 2,5-dimethylphenol and competed the volume to the mark with distilled water. The formed azo dye was measured maximum wavelength of 500 nanometer and 515 nanometer for 4-tetra-butylphenol and 2,5-dimethylphenolrespectively.

## RESULTS AND DISCUSSION

### Absorption characteristics

The azo dye formed through the diazotization and coupling reaction ofcefepime with 4-tetra-butylphenol and 2,5-dimethylphenolin aalkaline medium respectively. The maximum wavelengths obtainedunder optimized conditions at500, and 515 nm, respectively, against the reagent blank solution . The spectra are shown in Figures 2a, 2b.

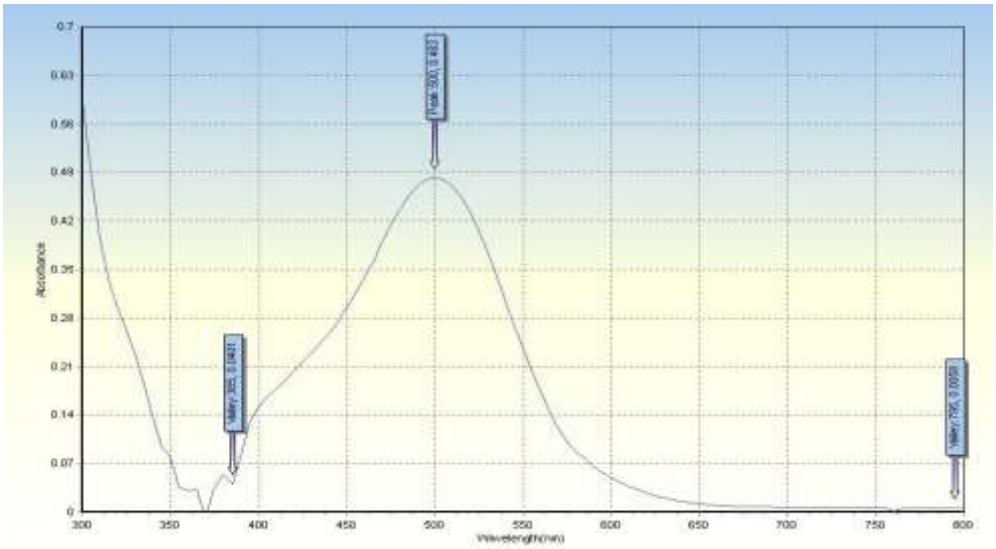



Figure 2a: Spectrum for 50 mg $L^{-1}$ CE with the 4-tetra-butylphenol at optimum conditions.

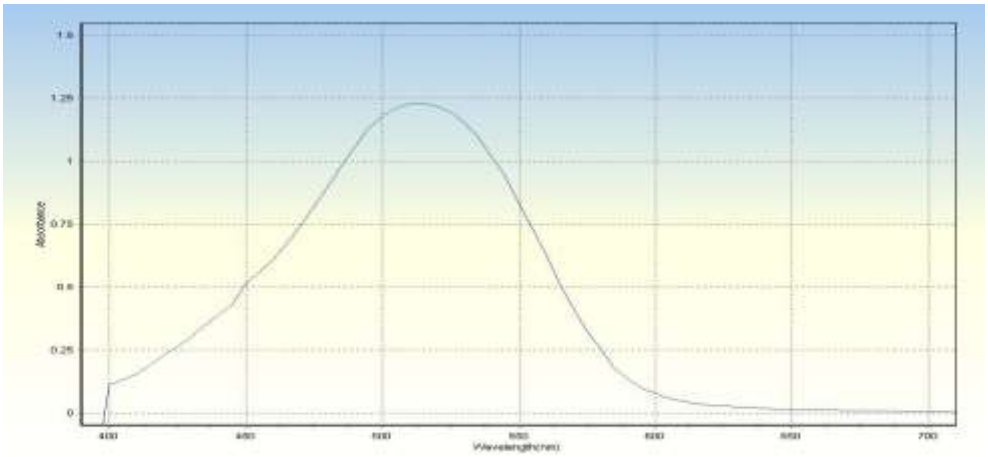


Figure 2b: Spectrum for 100 mg $L^{-1}$ CE with the 2,5-dimethylphenolat optimum conditions.

Investigation of the optimum conditions for the reaction of diazonium salt

The conditions that affect the intensity of the absorption was studied

Theeffect of the acidic medium of acids

The effect a variant of acids($H_2SO_4$ , HCl, $HNO_3$) strong acids , and ($CH_3COOH$ ) of weak acidswith concentrations of (1:1)on the intensity of the colored dye were done,the results indicate that hydrochloric acid gives the best results s that it was selected for the diazotization steps for both reagents . Table 1 shows the results.

Table 1: The acid Effect VS Absorbance with cefepime

| Type of acid | Absorbance 4-tetra-butylphenol | Absorbance 2,5-dimethylphenol |
|---|---|---|
| HCl | 0.251 | 0.783 |
| $H_2SO_4$ | 0.202 | 0.716 |
| $CH_3COOH$ | 0.097 | 0.584 |
| $HNO_3$ | 0.164 | 0.647 |

The effect of hydrochloric acid concentration

Different concentrated of hydrochloric acid that ranged from (0.075 – 0.6) mol $L^{-1}$ was used for this study ,0.3 mol $L^{-1}$ the best concentration because is give maximum absorbance for both reagents

The effect of sodium nitrite volume

Various volume of the $NaNO_2$ was used for studying this effect on formation of diazonium salt the $NaNO_2$ volume were rangedfrom 0.25 to 1.75 mL.Theseinvestigations showed that 1.0 mLof $NaNO_2$was enough for completing the reaction.

Reaction time effect of the adding sodium nitrite

Different reaction time that ranged from5 – 30 min.were used for studying thisparameters the the resultsindicatethat 10 min and 15 min., were enough to obtain the maximum absorbancefor 4-tetra-butylphenol reagent and 2,5-dimethylphenol respectively.

The effect of 2.0% (w/v) sulphamic acid volume

Sulphamic acid solution used for removing the excess $NaNO_2$. The volume of sulphamic acid ranged from (0.25 – 2.0). The results indicated that1.0 mL of 2.0% (w/v)sulphamic acid considered to be the most suitable volumeand used in further studies.

The effect of bases

several bases{ KOH, NaOH, $Na_2CO_3$, and $NH_4OH$}were used in this studyThis effect on the color intensity of formed product The results, asindicator in Table2. The KOH and NaOHsolution gave the highest intensity with 4-tetra-butylphenol and 2,5-dimethylphenol respectively.

Table 2: The base effect VS Absorbance with cefepime

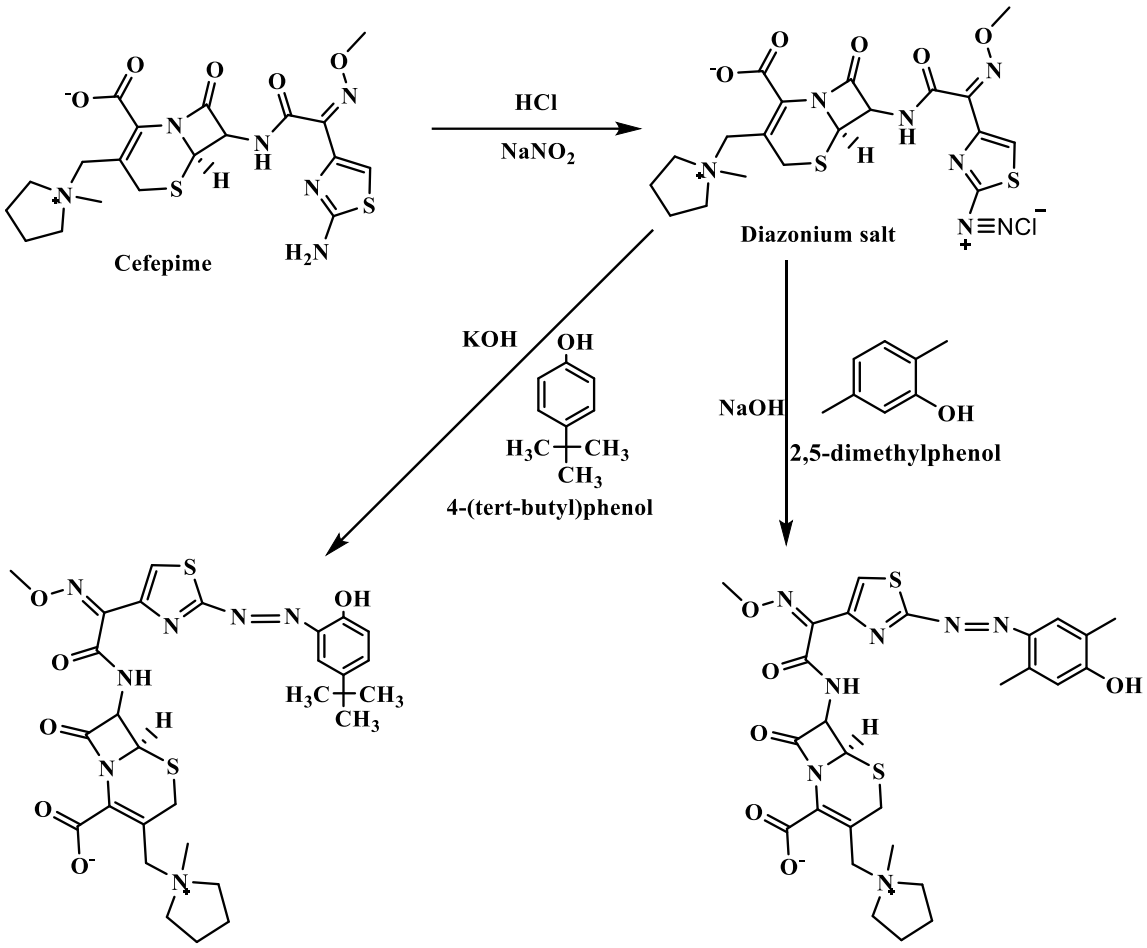
| Type of base | Absorbance 4-tetra-butylphenol | Absorbance 2,5-dimethylphenol |
|---|---|---|
| KOH | | 0.989 |

| | | |
|---|---|---|
| | 0.409 | |
| NaOH | 0.391 | 1.098 |
| $Na_2CO_3$ | 0.301 | 0.761 |
| $NH_4OH$ | 0.374 | 0.853 |

The effect ofKOH and NaOHconcentration

The effect of different KOH and NaOHconcentrations with 4-tetra-butylphenol and 2,5-dimethylphenol respectivelyfor studying the effect of KOH and NaOHconcentrationon absorbance ofazo dye formed. different concentrationsof base ranged from 0.2 – 0.55 mol $L^{-1}$ were used .the results shows0.3 and 0.45mol $L^{-1}$of potassium hydroxide and sodium hydroxide respectively were enough to obtain the maximum absorbance and it is enough to formation Azo dye.

The probuble reaction mechanism is as follows




Scheme 1: The proposed mechanism for the formation of azo dye from cefepime with 4-tetra-butylphenol and 2,5-dimethylphenol respectively.

The effect of reagent concentration

The effect of 4-tetra-butylphenol and 2,5-dimethylphenol reagent concentrations that ranged from $0.5x10^{-3}$ – $4.5x10^{-3}$mol $L^{-1}$ on the maximum formation of the colored product was investigated. The results in Figure 3 indicated that $2.0x10^{-3}$mol $L^{-1}$ of 4-tetra-butylphenol and 2,5-dimethylphenol respectively is the optimum concentration due to the higher color intensity.

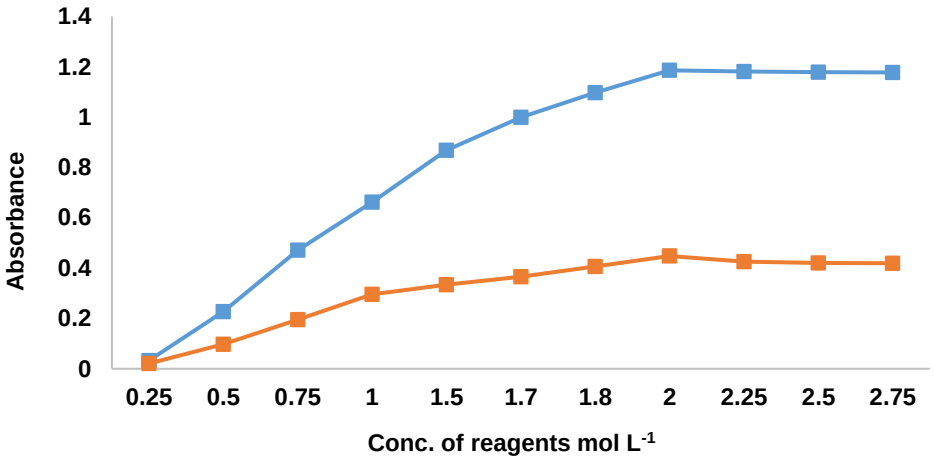



Figure 3: The reagent effect concentration VS Absorbance with cefepime.

Calibrationgraph obtained using the coupling of diazotizedcefepime with 4-tetra-butylphenol and 2,5-dimethylphenol respectively.

The absorbance of cefepime increases linearly as the concentration of cefepimeincreases when coupling with 4-tetra-butylphenol and 2,5-dimethylphenol respectively.At the optimum experimental conditions .

The calibration graph was gained by the series of standard solution (1.0 – 50 mg $L^{-1}$) for cefepime as shown in Figure 4.

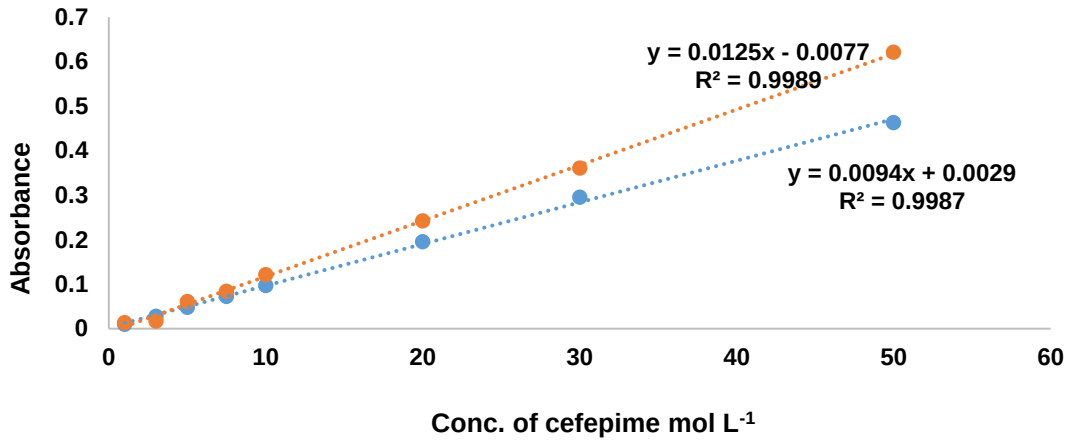



Figure 4: Calibration graphs getting by using the diazotization method the cefepime with two reagents 4-tetra-butylphenol and 2,5-dimethylphenol respectively.

Table 3: The analytical parameters getting by using the diazotization method the cefepime with two reagents 4-tetra-butylphenol and 2,5-dimethylphenol respectively.

| $\lambda_{max}$ (nm) | 500 | 515 |
|---|---|---|
| Linear range (mg $L^{-1}$) | 1.0 –50 | 1.0 –50 |
| Molar absorption coefficient (ε L $mol^{-1}$ $cm^{-1}$) | $6x10^3$ | $4.5x10^3$ |
| Regression equation | y = 0.0125x - 0.0077 | y = 0.0094x + 0.0029 |
| Sandellindex ($S_{gen}^2$) | 0.08 | 0.01 |
| Correlation coefficient ($R^2$) | 0.9989 | 0.9987 |
| Limit of quantification (mg $L^{-1}$) | 0.316 | 0.631 |
| Limit of detection (mg $L^{-1}$) | 0.096 | 0.191 |
| (RSD, n = 5) % | 0.16 | 0.23 |
| Recovery (n = 5) % | 100.51 | 100.5 |

Accuracy and precision

Accuracy was evaluated by the determination while the precisionwas determined by the (RSD%),(for five replicates of three different concentrations), the results shown in Table 10 indicated that an acceptable accuracy and precision for using the present method.

Table 4: Accuracy and precision to the determination the cefepimewith 4-tetra-butylphenol and 2,5-dimethylphenol respectively.

| Standard(cefepime) | Amount of Drug mg $L^{-1}$ | | Relative | Recovery % | RSD%* | RSD%** |
|---|---|---|---|---|---|---|
| | Taken | Found | | | | |
| Cefepime with 4-tetra-butylphenol | 1.5 | 1.52 | 1.33 | 101.33 | 0.284 | |
| | 20 | 20.05 | 0.25 | 100.25 | 0.081 | 0.16 |
| | 40 | 39.98 | -0.05 | 99.95 | 0.117 | |
| Cefepime with 2,5-dimethylphenol | 1.5 | 1.51 | 0.67 | 100.67 | 0.067 | |
| | 20 | 19.96 | -0.2 | 99.8 | 0.221 | 0.23 |
| | 40 | 40.41 | 1.03 | 101.03 | 0.407 | |

* average of fivedeterminations.
** average of fifteendeterminations.

Table 5: Determination of cefepimewith 4-tetra-butylphenol

| Drug | Amount of Drug mg $L^{-1}$ | | Relative | Recovery % | RSD%* | RSD%** |
|---|---|---|---|---|---|---|
| | Taken 1gm | Found 1gm | | | | |
| KON – Cefepime (China) 1 gm | 5 | 4.91 | -1.8 | 101.33 | 0.342 | |
| | 15 | 14.88 | -0.8 | 100.25 | 0.193 | 0.21 |
| | 30 | 30.09 | 0.3 | 99.95 | 0.104 | |
| Ceftazidime (Roth) 1gm | 5 | 4.94 | -1.2 | 98.8 | 0.081 | |
| | 15 | 14.78 | -1.47 | 98.53 | 0.37 | 0.29 |
| | 30 | 29.76 | -0.8 | 99.2 | 0.441 | |

* average of fivedeterminations.
** average of fifteen determinations.

Table 6: Determination of cefepimewith2,5-dimethylphenol respectively.

| Drug | Amount of Drug mg $L^{-1}$ | | Relative | Recovery % | RSD%* | RSD%** |
|---|---|---|---|---|---|---|
| | Taken 1gm | Found 1gm | | | | |
| KON – Cefepime (China) 1 gm | 8 | 7.88 | -1.5 | 100.67 | 0.115 | |
| | 25 | 25.1 | 0.4 | 99.8 | 0.049 | 0.11 |
| | 5 | 4.91 | -1.8 | 101.33 | 0.342 | |
| Ceftazidime (Roth) 1gm | 8 | 8.09 | 1.13 | 101.13 | 0.033 | |
| | 25 | 25.41 | 1.64 | 101.64 | 0.145 | 0.24 |
| | 35 | 34.88 | -0.34 | 99.66 | 0.542 | |

* average of fivedeterminations.
** average of fifteen determinations.

## CONCLUSION

The suggested method was simple, inexpensive, rapid, sensitive, selective, and accurateIt based on determination of cefepime by azo-coupling reaction of cefepime with two reagents first was 4-tetra-butylphenol the second was 2,5-dimethylphenol respectively in alkaline medium. The azo dye product was measured at $\lambda_{max}$ 500, and 515 nm, for first and second reagents respectively Therefore.This method is successfully applied for the determination of these drugs in pure and dosage forms.

## REFERENCES


1. Aymen Abdul RasoolJawadand Kasim Hassan Kadhim "2,5-dimethoxyaniline as a New Coupling Agent for the Spectrophotometric Determination of Sulfamethoxazole by Diazotization-Coupling Reaction" Journal of Applicable Chemistry, 2013, 2 (4):817-824.
2. Jing Fan,Yahong CHEN, Suling FENG, Gunling YE and Jianji WANG "Flow-injection spectrophotometric determination of sulfadiazine and sulfamethoxazol in pharmaceutical and urine" analytical science march 2003, vol 19.
3. Mysore, Manasagangothri Mysore, CharakPharma and Silvassa "A sensitive spectrophotometric method for the determinationof sulfonamides in pharmaceutical preparations" Acta Pharm. 57 (2007) 333–342.
4. British Pharmacopoeia 2009 British Pharmacopoeia Volume I & II Monographs: Medicinal and Pharmaceutical.
5. Cazorla-Reyes R, Romero-Gonzalez R, GarridoFrenich A, Rodriguez Maresca MA and Martinez Vidal JL: Simultaneous analysis of antibiotics in biological samples by ultra-high performance liquid chromatography-tandem mass

spectrometry. Journal of pharmaceutical and biomedical analysis 2014; 89:203-212.
6. Chen Y-n and Zhang Y: Determination of cefepime in serum by solid phase extraction HPLC. Chinese Journal of Hospital Pharmacy 2006; 26(4):420.
7. Isla A, Arzuaga A, Maynar J, Gascón A, Solinis M, Corral E and Pedraz J: Determination of ceftazidime and cefepime in plasma and dialysate-ultrafiltrate from patients undergoing continuous veno-venous hemodiafiltration by HPLC. Journal of pharmaceutical and biomedical analysis 2005; 39(5):996-1005.
8. Nemutlu E, Kir S, Katlan D and Beksac MS: Simultaneous multiresponse optimization of an HPLC method to separate seven cephalosporins in plasma and amniotic fluid: Application to validation and quantification of cefepime, cefixime and cefoperazone. Talanta 2009; 80(1):117-126.
9. López K, Bertoluci D, Vicente K, Dell'Aquilla A and Santos S: Simultaneous determination of cefepime, vancomycin and imipenem in human plasma of burn patients by high-performance liquid chromatography. Journal of Chromatography B. 2007; 860(2):241-245.
10. Ohmori T, Suzuki A, Niwa T, Ushikoshi H, Shirai K, Yoshida S, Ogura S and Itoh Y: Simultaneous determination of eight beta-lactam antibiotics in human serum by liquid chromatography-tandem mass spectrometry. J Chromatogr B AnalytTechnol Biomed Life Sci. 2011; 879(15-16):1038-1042.
11. Yang Y, Wu W, Yeh H and Chen S: Simultaneous determination of cefepime and vancomycin in plasma and cerebrospinal fluid by MEKC with direct sample injection and application for bacterial meningitis. Electrophoresis 2007; 28(11):1788-1797.
12. Abdel-Aziz O, Abdel-Ghany MF, Nagi R and Abdel-Fattah L: Application of Savitzky–Golay differentiation filters and Fourier functions to simultaneous determination of cefepime and the co-administered drug, levofloxacin, in spiked human plasma. SpectrochimicaActa Part A: Molecular and Biomolecular Spectroscopy 2015; 139:449-455.
13. Mohammed Jasim M. Hassan and ThulfiqarJabbar Al-hraishawi "Batch and Cloud Point Extraction Spectrophotometric Methods for theDetermination of Two Types Catecholamine Drugs" International Journal of PharmTech Research, ISSN: 0974-4304 19 August 2017.
14. D. GOWRI SANKAR*, B. DURVASA RAO, P.V. MADHAVI LATHA and M. VAMSI KRISHNA "Spectrophotometric Determination of Ezetimibe and Cefepime" Asian Journal of Chemistry Vol. 19, No. 2 (2007), 1613-1615.
15. Ravi Kant, Ramesh Bodla, RubinaBhutani, GarimaKapoor and Sonia Goswami "Spectrophotometric absorbance correction method for the estimation of Tazobactam and Cefepime in combined tablet dosage forms" J. Chem. Pharm. Res., 2015, 7(6):648-656.