IJBPAS, October, 2015, 4(10): 6211-6219

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

SIMULTANEOUS QUANTIFICATION OF CEFTOLOZANE AND TAZOBACTAM BY REVERSED PHASE – HPLC IN PURE AND DOSAGE FORMS

SHERIF ABDEL-NABY ABDEL-GAWAD[*1], ABDULLAH ALYAL-KAHTANI[2]ANDELSADIG HASSAN KHAMIS[3]

[1, 2&3]Pharmaceutical Chemistry Department, Collage of Pharmacy, Prince Sattam Bin Abdul-Aziz University, Al-Kharj, Kingdom of Saudi Arabia

[1]Analytical Chemistry Department, Faculty of Pharmacy, Cairo University, Egypt

[2]Chemistry Department, Collage of Science, King Saud University, Riyadh, Kingdom of Saudi Arabia

[*]**Corresponding author: sagawad@yahoo.com; Tel.: +966 540586921 Fax: +966 115886001**

ABSTRACT

A simple, accurate, precise and selective high-performance liquidchromatographic (HPLC) method was developed for the simultaneous quantification of ceftolozane and tazobactam, either in pure form or intheir dosage form (Zerbaxa™ vial).The mobile phase consisted of methanol: acetonitrile (70:30, v/v). The final pH of the mobile phase was adjusted to 4.5 ± 0.1 using *O*-phosphoric acid. The flow rate was 1mL/min, isocratically with UV-detection at 220 nm. The linear regression analysis data for the calibration curves showed a good linear relationship in the range of 1–80 µg/mL for ceftolozane and 2-40 µg/mL for tazobactamwith a regression coefficient of 0.9994 and 0.9998 for ceftolozane and tazobactam, respectively. The developed method was validated according to ICH-guidelines with respect to accuracy, precision, robustnessin addition todetection and quantification limits. The proposed method was successfully applied for the determination of the studied drugs in pure form, their mixtures and in pharmaceutical formulation containing them.

Keywords: Ceftolozane, Tazobactam, Quantification, RP-HPLC

INTRODUCTION

Ceftolozane (CFZ) is (6R,7R)-3- ([3-Amino-4- (2-aminoethyl carbamoyl amino)-2-methylpyrazol-1-ium-1-yl] methyl)-7- ([(2Z)-2- (5-amino-1,2,4-thiadiazol-3-yl)-2-(2-carboxypropan-2-yloxyimino) acetyl] amino)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate [1].

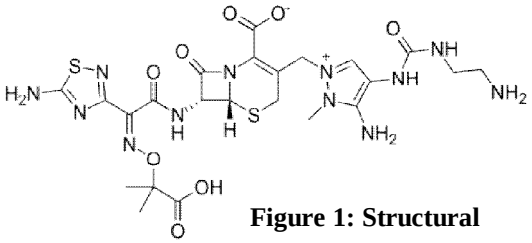

Figure 1: Structural formula of ceftolozane (CFZ)

M.W. **[**$C_{23}H_{30}N_{12}O_8S_2$ = 666.69 g/mol]

It is a fifth generation cephalosporin antibiotic, used for the treatment of infections related to gram-negative bacteria that have become resistant to conventional antibiotics. It was used for the treatment ofintra-abdominal infections, urinary tract infections and ventilator-associated bacterial pneumonia. CFZ works by inhibiting biosynthesis of the bacterial cell wall and so stops bacterial replication. It is combined with the tazobactam (TZB) which is aβ-lactamase inhibitor toavoid its degradation [2].

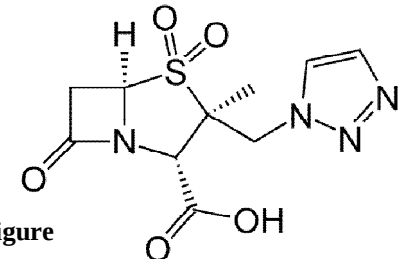

Figure 2.Structural formula of tazobactam (TZB)

There are many papers dealing with the pharmacological, pharmacokinetic and microbiological aspects of CFZ and TZB combination in order to evaluate its efficacy and safety [3-6]. From the analytical point of view, there are no published papers dealing with the simultaneous determination of CFZ and TZB because the proposed combination is recently approved by food and drug administration (FDA) so there is a good chance to develop new simple analytical methods for their co-quantification either in pure or in their dosage forms.

In modern analytical laboratory, there is always a need for simple and rapid methods of analysis. The present work aimed to develop sensitive and rapid HPLC method for the routine analysis and selective quantification of both CFZ and TZB in their pure forms or even in their pharmaceutical formulation.

MATERIALS AND METHODS

Instrument

The chromatographic apparatus (Merck Hitachi interface D-7000, an isocratic

pump (model L-7110), UV-visible detector (Model L-7420) and a Rheodyne injector (model 7161) equipped with 20-µL injector loop, La Chrom Merck Hitachi. Stationary phase consisted ofXterra^{TM} (250 x 4.6 mm, 5µm) C_{18}column. The samples were injected by the aid of a 50 µL Hamilton® analytical syringe.

Materials and reagents

(A) Materials

Pure CFZ and TZB were kindly supplied by Cubist Pharmaceuticals, USA.

(B) Reagents

Methanol, acetonitrile and *O*-phosphoric acid, HiPerSolv.®, HPLC-grade, E. Merck (Darmstadt, Germany). De-ionized bi distilled water obtained from Aquatron Automatic Water Still A4000, Bibby Sterillin Ltd. (Staffordshire, UK).

(C) Pharmaceutical formulations

Zerbaxa^{TM} vial NDC 67919-030-01 claimed to contain 1.147 g CFZ sulfate equivalent to 1 g of CFZ and 0.537 g TZB sodium equivalent to 0.5 g of TZB per each vial, manufactured by Cubist Pharmaceuticals, USA.

(D) Standard solutions

Standard stock solutions were prepared by dissolving CFZ and TZB, separately, in de-ionized bidistilled water into 50-mL volumetric flasks to obtain a final concentration of 0.1 mg/mL.

2.3. Method validation

The developed analytical method was fully validated according to ICH-guidelines.

Linearity

Aliquots (0.1-8mL) from CFZ and (0.2-4 mL) from TZB standard stock solution (0.1 mg/ml) were accurately and separately transferred into a series of 10-mLvolumetric flasks, the volume was then completed with the mobile phase to obtain a concentration range of 1-80µg/mL for CFZ and 2-40 µg/mL for TZB.

The samples were filtered through a 0.45-µm membrane filter, and were injected by the aid of a 50-µL Hamilton®analytical syringe. To reach good equilibrium, analysis was usually performed after passing ~ 50-60mL of the mobile phase, just for conditioning and pre-washing of the stationary phase.

Samples were then chromatographed usingXterra$^{TM}C_{18}$ column (250 x 4.6 mm, 5 µm) as a stationary phase. The mobile phase was formed of methanol: acetonitrile (70:30, v/v). The final pH of the mobile phase was adjusted to 4.5 ±0.1 using phosphoric acid. The flow

rate was 1mL/min, isocratically with UV detection at 220 nm.

Peak area ratios were plotted against concentration to obtain calibration graphs then the regression equations were computed. Regression equations were constructed and used for estimating the concentration of both drugs.

Accuracy

The previously mentioned procedure under linearity was repeated for the determination of different concentrations of CFZ and TZB then the concentrations were calculated from the corresponding regression equation.

Precision

A. Intraday precision (Repeatability)

Three concentrations of each drug were analyzed three times within the same day using the previously mentioned procedure under linearity.

B- Intermediate (interday) precision

Three concentrations of each drug were analyzed on three successive days using the procedure stated under linearity.

Robustness

A slight variation in the method parameters as variation of the composition & flow rate of the mobile phase was done to assess their effects on the suggested method.

2.4. Analysis of laboratory prepared mixtures of CFZ and TZB

Aliquots of CFZ and TZB were mixed to prepare different mixtures containing 10/2, 10/5 and 10/8 (w/w) of CFZ and TZB, respectively, and proceed as mentioned under linearity. The concentration of each drug was calculated from the corresponding regression equation.

2.5. Analysis of pharmaceutical formulation

The contents of one Zerbaxa^{TM} vial was dissolved in small quantity ofde-ionized bidistilled water and transferred to 50-mL volumetric flask then complete to the mark with same solvent. The necessary dilutions were then carried out using the mobile phase to get concentration levels within the linearity range of each drug. The same procedure under linearity was followed to estimate the concentration of CFZ and TZB in Zerbaxa^{TM} vial. All determinations were done in triplicate.

RESULTSAND DISCUSSION

A simple isocratic high-performance liquid chromatographic method was developed for the determination of CFZ and TZB in pure form and in their dosage form. The mobile phase was chosen after several trials. The most suitable one was consisted of methanol:

acetonitrile (70:30, v/v). The final pH of the mobile phase was adjusted to 4.5 ± 0.1 using phosphoric acid at ambient temperature. The flow rate was 1mL/min. By using the described chromatographic conditions, TZB and CFZ were well separated with average retention times of 3.35 ± 0.05 min. and 8.87 ± 0.07 min., for TZB and CFZ, respectively. The linearity of the detector response for both drugs was determined by plotting peak area ratios to the external standard versus concentration. The regression equations were calculated and used for estimation of the concentration of the **studied drugs in unknown samples.**

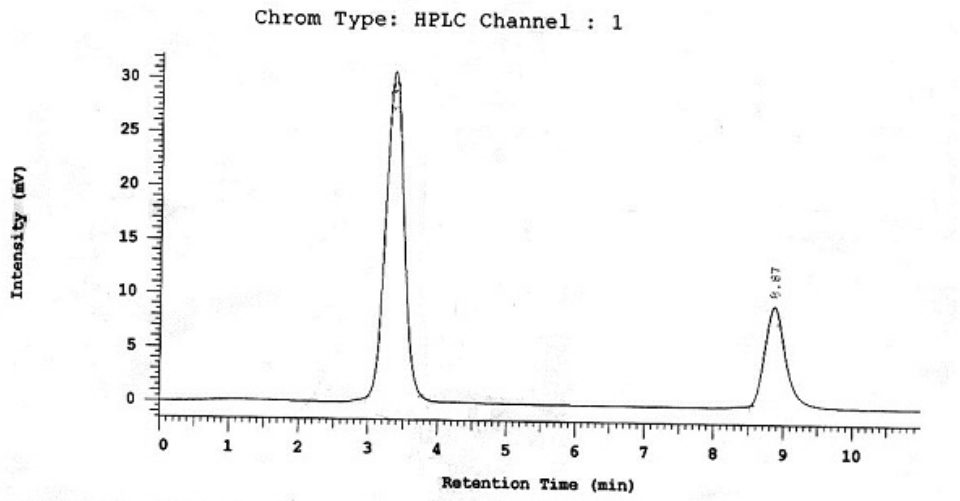

Figure 3: Atypical HPL chromatogram showing the separation of TZB (3.35min.) and CFZ (8.87min.)

The linearity of the detector response for both drugs was determined by plotting peak area ratios to the external standard versus concentration. The regression equations were calculated and used for estimating the concentration of the studied drugs in unknown samples.

Method validation

All of the analytical validation parameters for this proposed method were determined according to ICH-guidelines as follows:

Linearity

Calibration standards at five levels were prepared by appropriately mixing and further diluting stock standard solutions in the concentration range of 1-80 µg/mL for CFZ and 2-40 µg/mL for TZB. Samples in triplicates were injected for each concentration, and peak areas ratios were plotted against the corresponding concentrations to obtain the calibration graph then the regression equations were derived to be.

Y= 0.0512X +0.0037 (r= 0.9994) (for CFZ)

Y= 0.0321 X + 0.0021 (r= 0.9998) (for TZB)

Accuracy

The accuracy of the method was validated by analyzing different concentrations of pure CFZ and TZB by the proposed method then calculating the recovery and percent of relative standard deviation (% RSD) which is considered satisfactory. The % RSD value was <1% for each drug.

Precision

The intraday and interday precisions were checked by analyzing three different concentrations of each drug either in the same day or during three successive days and found to be satisfactory.

Robustness

It was checked by carrying out a slight variation in the method parameters as variation of the composition & flow rate of the mobile phase to study the effect of slight changes in chromatographic parameters on the method. The results were considered good as the % RSD value was <1% for each drug.

Also, limit of detection (LOD) and limit of quantification were calculated for each drug in the proposed method.

All assay parameters and full validation sheet are represented in Table 1.

Table 1: Assay parameters and validation sheet for determination of CFZ and TZB by the proposed method

Parameter	CFZ	TZB
Linearity		
Range	**1-80 µg/mL**	**2-40 µg/mL**
Slope	**0.0512**	**0.0321**
Intercept	**0.0037**	**0.0021**
r	**0.9994**	**0.9998**
Accuracy		
Mean± S.D.*	**101.02 ± 0.751**	**99.72 ± 0.817**
Variance	**0.564**	**0.667**
RSD%	**0.743**	**0.819**
Precision		
Intraday precision	**100.69 ± 0.514**	**101.14 ± 0.843**
Interday precision	**98.61 ± 0.941**	**99.39 ± 0.778**
Robustness	**102.01 ± 0.988**	**101.94 ± 0.995**
LOD	**0.65 µg/mL**	**1.5 µg/mL**
LOQ	**1 µg/mL**	**2 µg/mL**

***Standard deviation *Average of three determinations**

Analysis of laboratory prepared mixtures

Different mixtures containing 10/2, 10/5 and 10/8 (w/w) of CFZ and TZB, respectively, were analyzed by the proposed method and the results are shown in Table 2.

Analysis of pharmaceutical formulation

Assay of CFZ and TZBin Zerbaxa™ vial was done and satisfactory results were obtained, Table 3.

Table 2: Determination of CFZ and TZB in laboratory prepared mixtures by the proposed method

Lab. mixture ratio (CFZ/TZB)	Drug determined	
10/2	CFZ (Mean± S.D.)*	100.84 ± 0.784
	TZB (Mean± S.D.)*	99.57 ± 0.479
10/5	CFZ (Mean± S.D.)*	102.35 ± 0.991
	TZB (Mean± S.D.)*	98.64 ± 0.943
10/8	CFZ (Mean± S.D.)*	98.79 ± 0.822
	TZB(Mean± S.D.)*	100.34 ± 0.667

*Average of three determinations

Table 3: Determination of CFZ and TZB in their pharmaceutical formulation by the proposed method

Preparation Zerbaxa™ vial NDC 67919-030-01	
CFZ (Mean ± S.D.)*	102.68 ± 0.861
TZB (Mean ± S.D.)*	99.85 ± 0.697

*Average of three determinations

CONCLUSION

The developed RP-HPLC method can be efficiently applied for thesimultaneous quantification of CFZ and TZB with excellent accuracy,precision and selectivity. The proposed method produces symmetric peakshape, good resolution and reasonable retention time for both drugs (lower than 10 minutes).So this method can be applied for the simultaneous determination ofCFZ and TZB in quality control laboratories.

ACKNOWLEDGEMENT

This project was supported by the Deanship of Scientific Research at Prince Sattam Bin Abdulaziz University under the research project #2015/01/4078.

REFRENCES

[1] https://en.wikipedia.org/wiki/Ceftolozane

[2] Takeda S,Nakai T,Wakai Y, Ikeda F,Hatano K,In vitro and in vivo activities of a new cephalosporin, FR264205, against *Pseudomonas aeruginosa*, Antimicrobial Agents and Chemotherapy, 51 (3), 2007, 826–830.

[3] MillerB, Hershberger E, BenzigerD, Trinh M,FriedlandI, Pharmacokinetics and safety of intravenous ceftolozane - tazobactam in healthy adult subjects following single and multiple ascending doses, Journal of Antimicrobial Agents and Chemotherapy, 6 (6), 2012, 3086-3091.

[4] LucastiC, HershbergerE, MillerB, YankelevS, SteenbergenJ, FriedlandI,Solomkin j,

Multicenter, double-blind, randomized, phase II trial to assess the safety and efficacy of ceftolozane – tazobactam plus metronidazole compared with meropenem in adult patients with complicated intra-abdominal infections, Journal of Antimicrobial Agents and Chemotherapy, 58 (9), 2014, 5350-5357.

[5] Craig WA, AndesDR,In vivo activities of ceftolozane, a new cephalosporin, with and without tazobactam against Pseudomonas aeruginosa and Enterobacteriaceae, including strains with extended-spectrum - lactamases, in the thighs of neutropenic mice, Journal of Antimicrobial Agents and Chemotherapy, 57(4), 2013, 1577-1582.

[6] EstabrookM, BussellB, ClugstonSL, BushK, In vitro activity of ceftolozane - tazobactam as determined by broth dilution and agar diffusion assays against recent U.S. Escherichia coli isolates from 2010 to 2011 carrying CTX-M-type extended - spectrum – lactamases, Journal of Clinical Microbiology, 52 (11), 2014, 4049-4052.