Detection of Stability and Degradation of Piperacillin and Tazobactam in Injectables from In-Patient Wards and Pharmacy by RP-HPLC Method

Ramalingam P[1*], Rajendran K

[1]College of Pharmacy, Gulf Medical University, Ajman, UAE

*Presenting Author

ABSTRACT

New RP-HPLC method for the detection of degradants and quantification piperacillin and tazobactam in injectables stored in inpatient wards and pharmacy has been developed on C_8 column (250 x 4.6, 5 micron) using methanol and water (55:45% *v/v)* as mobile phase and Diode array detection at 215 nm. Linearity regression coefficients were more than 0.999 and % RSD for intra- and inter-assay precision and accuracy were less than 2. Selectivity of the method for all possible degradants and analytes were established by mild acidic and alkaline stress degradation using 0.001M HCl and 0.001M NaOH. Method was applied on various samples of injectables collected from inpatients wards and pharmacy. Results revealed that few minor degradants were observed in samples collected from refrigerator and 9 degradants were found in samples collected from trays of inpatient ward. Formed degradants were identical with acid/base hydrolytic products of stress studies. This RP-HPLC method is highly reliable in hospitals and clinical analysis of Piperacillin and Tazobactam in Injectables to preserve potency, to prevent resistance and to ensure efficacy. This study educates the paramedical staff in handling and storage of Piperacillin and Tazobactam in injectables in wards and pharmacy stores.

Key Words: RP-HPLC, Stabilty, Piperacillin, Tazobactam, in-patients wards, degradation

INTRODUCTION

Piperacillin (PIP) is a derivative of α-acylureido-substituted penicillin, (2S,5R,6R)-6-[[(2R)-2-[[(4-ethyl-2,3-dioxopiperazin-1-yl)carbonyl] amino]-2- phenyl-acetyl]amino]-3,3-dimethyl-7-oxo-4-thia-1- azabicyclo[3.2.0.] heptane-2-carboxylic acid[1] That has been shown effective in the treatment of many serious infections associated with Gram-positive and Gram-negative organisms, including *Pseudomonas aeruginosa, Proteus, Klebsiella pneumoniae* and *Serratia marcescens*. It is a β-lactam antibiotic, susceptible to hydrolysis by a range of β-lactamases, including the plasmid-mediated enzymes. These enzymes inactivate β-lactam antibiotics by opening the β-lactam ring. Tazobactam (TAZ), [2S-(2a,3b,5a)]-3-methyl-7-oxo- 3-(1H- 1,2,3 - triazol -1-ylmethyl)-4- thia -1-azabicyclo[3.2.0] heptane-2-carboxylic acid 4,4-dioxide, is a potent and novel β-lactamase inhibitor belonging to a class of penicillanic acid sulfones. The combined use of tazobactam and piperacillin has been more effective against various β-lactamase-producing bacteria. Tazobactam has been shown to act synergistically with piperacillin and many other β-lactam antibiotics against a broad spectrum of bacterial pathogens. Piperacillin has been determined by many analytical methods, such as capillary zone electrophoresis[2], cyclic voltammetry[3], spectrophotometry[4], potentiometric titration[5] and especially by high performance liquid chromatography (HPLC)[6-13]. Tazobactam has been determined by HPLC[14-21]. Some reports available for simultaneous determination of piperacillin and tazobactam in biological fluids and few works for assay in dosage forms such as gradient elution HPLC, isocratic elution HPLC, ion-pair HPLC. But those works have demonstrated on C_{18} column and showed high retention time for piperacillin with more than 12 minutes and do lack selectivity due to void elution or poor retention of tazobactam, thus none of work is suitable for detecting degradation products. In connection with the importance of stability of beta-lactam antibiotics and emerging resistance in antibiotic therapy, the present work demonstrates a suitable validated HPLC method on C_8 column in reverse mode for the detection of possible degradants and quantitation of piperacillin and tazobactam in injectables collected from inpatients wards and pharmacy, so as to prove the significance of storage condition in preserving potency of piperacillin and tazobactam dosage form in efficacious treatment regimen.

EXPERIMENTAL

Chemicals

HPLC grade methanol, HPLC grade water and orthophosphoric acid were obtained from Merck (Mumbai, India). Piperacillin sodium standard and Tazobactam sodium standard were obtained as gift sample from Orchid Chemicals and Pharmaceuticals (Chennai, India). Samples were collected from inpatients wards of local hospitals and pharmacy and stored in laboratory as directed in label. The work was carried out at analytical research laboratory, Raghavendra Institute of Pharmaceutical Education and Research (RIPER), Andhra pradesh and the study was conducted during Jan-June 2013.

Instruments

HPLC system used was Agilent LC 1200 with Diode array detector (DAD) and manual rheodyne injector with a fixed loop of 20 µL volume. EZ chrom elite software was employed. The chromatographic analysis was performed on Qualisil gold C_8 column 250 mm x 4.6 mm i.d., 5µm particle size.

Standard solutions

Standard stock solutions were prepared by dissolving a quantity of 10 mg of tazobactam and 80 mg of piperacillin in a 50 mL standard flask using water to obtain a concentration of 400 µg mL^{-1} of piperacillin and 50 µg mL^{-1} of tazobactam. Quality control standards were prepared by diluting the stock solution in the range of 8-96 µg mL^{-1} for Piperacillin and 1-12 µg mL^{-1} for Tazobactam estimation. Regression equation was obtained for both drugs for quantification of analytes in samples.

Sample Analysis

Quality control sample was prepared by dissolving injection powders of various samples equivalent to 40 mg of Piperacillin and 5 mg of Tazobactam with diluent in 100 mL flask. Concentration obtained was 400 µg mL^{-1} of piperacillin and 50 µg mL^{-1} of tazobactam and the stock was diluted to 40 µg mL^{-1} and 5 µg mL^{-1} of piperacillin and tazobactam, respectively. All the standards and samples were injected into the optimized chromatographic condition by manual injections. Eluents were detected at 210, 215, 220, 225 nm using UV-DAD detection. Among, 215 nm was optimized for quantification of drugs and their degradants. The percentage degradation was calculated based on control and whereas number of degradants assessed by additional peaks. Peak area of PIP and TAZ were used for quantification. Peak purity was determined by purity plot.

RESULTS AND DISCUSSION

Method development

Different trails were performed on C_8 reversed-phase column using several mobile phase combinations for the separation of Piperacillin and Tazobactam with better chromatographic parameters like improved capacity factor, optimum resolution, theoretical plates, minimized tailing factor. The method was optimized on C_8 column (5 µm, 250 mm x 4.6 mm i.d.) with a mobile phase of methanol/water pH 3.0 (55:45, v/v) at a flow rate of 1 mL min-1 and a detection wavelength of 215 nm. The optimized chromatogram is shown in Figure 1.

Method Validation

The validation of developed method was performed as per ICH Q2 (R2) guidelines which include accuracy, precision, specificity, detection limit (LOD), quantitation limit (LOQ), linearity and range.

System suitability

The developed method was evaluated for system suitability testing by injecting a solution containing two drugs of 10 µg mL^{-1}concentrations in replicate (n = 10). The parameters analyzed were USP plate count, tailing factor, capacity factor, HETP and Retention time and results were shown in Table 1.

Table 1: System suitability parameters for Piperacillin and Tazobactam

Parameter	Values obtained (n = 6)		Acceptance Criteria
	PIP	TAZ	
Plate Count	9035±62	9954±91	> 4000
Tailing Factor	1.05	0.95	≤ 2.0
Capacity factor	2.12	1.27	> 2
HETP	0.028	0.025	--
Rt	8.064	5.783	--
Resolution	7.8	--	>2

Table 2: Linearity, LOD and LOQ Data

Parameter	Piperacillin	Tazobactam
Regressions equation	y = 513041x + 844133	y = 551453x + 19639
Correlation coefficient (R^2)	0.9992	0.9995
LOD[a]	0.250 µg/mL	0.132 µg/mL
LOQ[a]	1.003 µg/mL	0.530 µg/mL
Concentration Range	8-96 µg/mL	1-12 µg/mL

[a] *LOD and LOQ are not required for assay method validation.*

Table 3: Recovery Report of Piperacillin and Tazobactam

Drug	Amount taken (µg/ml)	Recovery Level	Amount of Drug Added	Amount of Drug Found (µg/ml) Mean± S.D	% RSD	% Recovery
PIP	40	80%	32	71.36±0.652	0.9136	99.92
		100%	40	78.95±0.145	0.6599	98.23
		120%	48	88.84±0.055	0.7181	100.26
TAZ	5	80%	4	8.82±0.105	1.1904	98.13
		100%	5	9.89±0.096	0.9706	98.92
		120%	6	11.16±0.055	0.4928	101.48

Table 4: Report of Precision for Piperacillin and Tazobactam

Precision	%RSD		Acceptance Limit
	PIP	TAZ	
Precision - Repeatability	0.8276	0.4921	NMT 2%
Intraday Precision	0.8730	0.6909	NMT 2%
Interday Precision	1.4200	1.2021	NMT 2%

Linearity and range

A plot of mean peak area (n = 3) versus concentration was linear in the concentration ranges of about 1-12 µg mL^{-1} for tazobacam and 10-96 µg mL^{-1} for piperacillin. The regression equation for piperacillin is y = 513041x + 844133 (r^2 = 0.9992) and for tazobactam is y = 551453x + 19639 (r^2 = 0.9995). The results were calculated and shown in Table 2.

Limit of detection (LOD) and limit of quantification (LOQ)

LOD and LOQ were calculated from the formula 3.3 x (σ/S) and 10 x (σ/S), respectively where, σ is standard deviation of intercept and S is the mean of slope. The obtained concentrations were prepared and injected to prove S/N value of 3-5 for LOD whilst 10-15 for LOQ. The same results were shown in Table 2.

Accuracy and percentage recovery

To the assay concentration of formulation, the reference standards were added at three levels viz. 80%, 100%, 120%, respectively 32, 40, 48 µg $mL^{-1}$for piperacillin and 4, 5, 6 µg mL^{-1} for tazobactam. The recovery studies were carried out in three replicate and the percentage recovery and percentage relative standard deviation of the recovery were calculated and shown in Table 3.

Precision – Repeatability

The precision of the method was evaluated by calculating the RSD of peak areas of three replicate injections for three different standard concentrations. The average RSD of piperacillin was found to be 0.87% and for tazobactam it was found to be 0.69%. These results are shown Table 4.

Precision - Intermediate precision (Intra and Interday)

The precision expressed within the same laboratory on different days variability of analytical results. The RSD of peak areas of three replicate injections for three different standard concentrations was calculated. The average RSD of piperacillin was found to be 1.42% and for tazobactam it was found to be 1.20%. These results are shown Table 4.

Specificity and Selectivity

Specificity of the method was shown by quantifying the analyte of interest in the presence of matrix and other components. Blank injections have shown no peaks at retention time of 8.02 min and 5.7 min, the proposed method was specific for the detection of Piperacillin and Tazobactam respectively. The selectivity of the method was performed by injecting the solution after the degradation in 0.001M HCl and 0.001M NaOH. The degradents formed during solution stability study were well separated from the analyte peak after 20 h of sample preparations.

Robustness

Robustness of the method was demonstrated by analyzing three different standard concentrations using the same optimized chromatographic conditions to give unaffected results for small deliberate changes in system parameters and method parameters. The changes were (a) using flow rate 0.9 and 1.1 mL min^{-1}; (b) change in volume fraction of methanol is 53% and 57% instead of 55%; (c) change in the pH of mobile phase from 3.0 to 2.9 and 3.1.

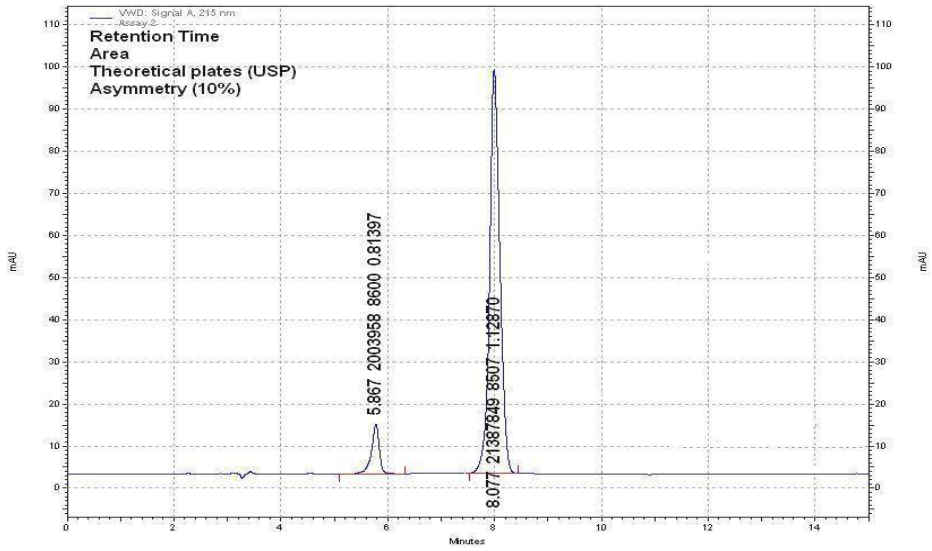

Figure 1. Chromatogram on C8 Column for piperacillin and tazobactam in injectables

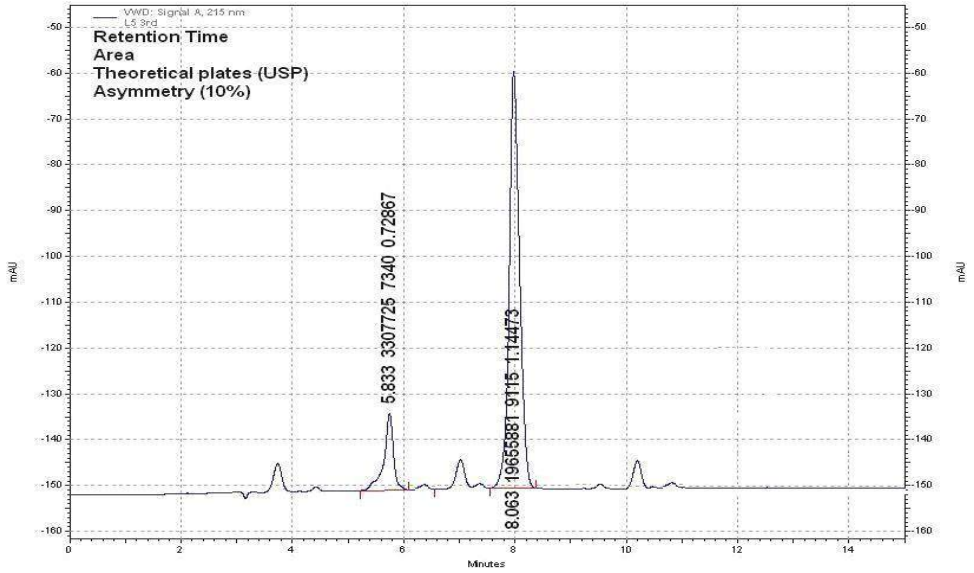

Figure 2. Detection degradants formed in injectables of Piperacillin and Tazobactam

(stored at 25-30° C)

RESULTS OF SAMPLE ANALYSIS

A total of six injection samples were selected from different storage condition. Mass ratio of piperacillin to tazobactam in injection vials was 8:1. After reconstitution of injection powder with 10 mL of diluent, the concentration of piperacillin to tazobactam was finally made up to 40 µg mL^{-1} and 5 µg mL^{-1}, respectively and analyzed. Results revealed that few minor degradants were observed in samples collected from refrigerator and 9 degradants were found in samples collected from trays of inpatient ward. Formed degradants were identical with acid/base hydrolytic products of stress studies. The % purity was calculated by regression analysis.

Number of degradation products and assay values were shown in the Table 5 and Figure 2.

Table 5: Analysis report of injectables samples obtained from inpatient wards and pharmacy

Sample No	Sample origin (Storage)	Content	% Assay (n = 5)	% Degradation	Total no. Degradants
1	Pharmacy	Piperacillin	99.86 ± 0.76	0	0
	(Refrigerator)	Tazobactam	100.81 ± 0.85	0	
2	Pharmacy	Piperacillin	95.16 ± 0.75	4.1	02
	(20-25 °C)	Tazobactam	97.92 ± 0.96	3.6	
3	Pharmacy	Piperacillin	92.66 ± 0.54	7.5	07
	(25-30 °C)	Tazobactam	94.07 ± 0.85	5.9	
4	Inwards	Piperacillin	98.44 ± 0.56	1.3	01
	(Refrigerator)	Tazobactam	99.05 ± 0.15	0	
5	Inwards	Piperacillin	94.66 ± 0.33	5.6	03
	(20-25° C)	Tazobactam	97.86 ± 0.85	2.1	
6	Inwards	Piperacillin	89.22 ± 0.98	10.1	09
	(25-30° C)	Tazobactam	91.03 ± 0.47	8.5	

IMPORTANCE OF THIS STUDY

There is a necessity for reliable simultaneous stability assay method for determination piperacillin and Tazobactam in injectable as they are available as powder form and highly unstable if appropriate storage condition is not maintained. This may result in loss of potency and develop resistance especially in antimicrobial therapy. This study was designed to evaluate the stability of piperacillin and tazobactam in Injectables in hospitals and pharmacy at various environments. The method revealed a total of 9 degradation products for products collected from trays at

room temperature in between 25-30°C. Formed degradation products were identical with those formed in acid/base hydrolysis. The developed RP-HPLC method is highly reliable in hospitals and clinical analysis of piperacillin and tazobactam in injectables formulations to preserve potency and prevent resistance and also to ensure efficacy. This study educates the paramedical staff in handling and storage of piperacillin and tazobactam in Injectables in wards and pharmacies.

CONCLUSION

The present study represents an accurate, precise and specific HPLC method for routine analysis of piperacillin and tazobactam combination in parenteral dosage form. In addition to assay it may be used to detect related substance or other impurities which are formed during storage conditions and the analyte of interest could be estimated without any interferences. The use of C_8 column in the present work has shown better elution of analytes with good resolution, improved plate count, capacity factor, reduced tailing than reported methods which were performed on C_{18}. This RP-HPLC method is highly reliable in hospitals and clinical kinetics of Piperacillin and Tazobactam in Injectables to correlate potency, resistance and storage conditions. This study indicates and educates the paramedical staff in handling and storage of Piperacillin and Tazobactam in Injectables in wards and pharmacy.

ACKNOWLEDGMENT

We would like to thank Hetero labs, Hyderabad, India and for providing reference standards of piperacillin and tazobactam.

REFERENCES

1. Sean CS. Martindale's The Complete Drug Reference, 35th edition, London: The Pharmacpoeial Press. 2007: p.307-08 2007.
2. Taniguchi S, Hamase K, Kinoshita A, et al. Simple and rapid analytical method for carbapenems using capillary zone electrophoresis. J Chromatogr B. 1999; 727:219–25.
3. Abo-EL-Maali N, Ghandour MA, Khodari M. Electroreduction and determination of Pipril (Piperacillin) in both aqueous and biological samples. Talanta. 1993;40: 1833–38.
4. Zhang A, Bai X, Tong, L. Determination of piperacillin in compound injection by double wavelength ultraviolet spectrophotometry. Shanxi Med Univer. 2004;.35(6): 572-73, 2004.
5. Pospisilová B, Simková M, Kubes J. Conditions for the mercurimetric determination of the drug content of penicillins. Die Pharmazie. 1986;41(10):705-08.
6. Garcia-Glez JC, Mendez R, Mart-In-Villacorta J. Determination of piperacillin and mezlocillin in human serum and urine by high-performance liquid chromatography after derivatisation with 1,2,4-triazole. J Chromatogr A. 19980; 812:213–20.
7. Martin J, Mender R, Negro A. Effect of Temperature on HPLC Separations of Penicillins. J Liq Chromatogr. 1998;11: 1707–16.
8. Riegel MA, ELLI, PP. High-performance liquid chromatographic assay for piperacillin in aqueous humor of the eye. J Chromatogr B. 1988;424:177–81.
9. Annesley T, Wilkerson K, Matz K, Giacherio, D. Simultaneous determination of penicillin and cephalosporin antibiotics in serum by gradient liquid chromatography. Clin Chem.1984;30:908–10.

10. Aravind MK, Miceli JN, Kauffman, RE. Determination of moxalactam by high- performance liquid chromatography. J Chromatogr B. 1982; 227: 418–22.
11. Jung D, Mahajan NK. An improved micro-scale liquid-chromatographic assay for piperacillin in plasma and urine. Clin Chem. 1984;30: 122–24.
12. Raphael D, Corinne C. Simultaneous determination of five β-lactam antibiotics (cefepim, ceftazidim, cefuroxim, meropenem and piperacillin) in human plasma by high-performance liquid chromatography with ultraviolet detection. J Chromatogr B.2008; 864:161–67.
13. Tsukamoto T, Ushio T. Determination of (2S, 3S, 5R)-3-methyl-7-oxo-3-(1H-1,2,3-triazol-1-ylmethyl)-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid 4,4-dioxide (YTR-830H) and piperacillin in pharmaceutical preparations by high-performance liquid chromatography. J Chromatogr A. 1994;678:69–76.
14. Rama KVP, N. Sharmila N, Narayana KJP, et al. Simultaneous determination of piperacillin and tazobactam in pharmaceutical formulations by RP-HPLC method. Journal of Pharmacy Research. 2013;7(1):127-131.
15. Peyrin E, Guillaume Y, Guinchard C. High-performance liquid chromatographic determination of tazobactam by precolumn derivatization. J Chromatogr B. 1995;672: 160-64.
16. Matsushima E, Yoshida KI, Azuma R, et al. Degradation of β-Lactamase Inhibitor (2S, 3R, 5S)-3-Methyl-7-oxo-3-(1H-1, 2, 3-triazol-1-yl-methyl)-4-thia-1-azabicyclo [3.2.0]-heptane-2-carboxylic Acid 4, 4-Dioxide (YTR-830H) in the Solid State: Structural Elucidation. Chem Pharm Bull. 1988;36:4593–96.
17. Augey V, Grosse PY, Albert G, et al. High-performance liquid chromatographic determination of tazobactam and piperacillin in human plasma and urine. J Chromatogr B. 1996;682:125-36.
18. Ocampo AP, Hoyt KD, Wadgaonkar N, et al. Determination of tazobactam and piperacillin in human plasma, serum, bile and urine by gradient elution reversed-phase high-performance liquid chromatography. J Chromatogr B. 1989;496:167-79.
19. Marselos SC, Archontaki HA. Development and optimization of a reversed-phase high-performance liquid chromatographic method for the determination of piperacillin and tazobactam in tazocin injectable powder. J Pharm Biomed Anal. 2002;29:787–9
20. Zhiping Li and Qin Li. Determination of Free and Total Piperacillin–Tazobactam in Plasma by HPLC–MS–MS: An Adapted Method for Neonates**,** Chromatographia. 2012;75(9)533-539.
21. Marunaka T, Matsushima E, Minami Y, et al. Degradation of β-Lactamase Inhibitor, (2S, 3R, 5S)-3-Methyl-7-oxo-3-(1H-1, 2, 3-triazol-1-yl-methyl)-4-thia-l-azabicyclo [3.2.0] heptane-2-carboxylic Acid 4, 4-Dioxide (YTR-830H), in Aqueous Solutions and Alkaline Methanol Solution; Pathway and Structural Elucidation of Products. Chem Pharm Bull. 1998;36:4478–87.