

A Quantitative Estimation of Piperacillin and Tazobactam in Generic and Branded Pharmaceutical Formulations by Reverse Phase High Performance Liquid Chromatography

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ABSTRACT

Background: In India, the incidence of sub-standard drugs has risen from 0.04% to 2.3% in 2012 as per Drug Controller General of India (DCGI). There are 66 substandard drugs found in Jammu and Kashmir (India), as per Drugs and food control organization, Jammu and Kashmir 2013. Looking at the rising incidences of sub-standard drugs in the local market of our state, we have chosen piperacillin and tazobactam, used extensively in tertiary care hospital, to verify quality and quantity by employing High Performance Liquid Chromatography (HPLC) based analytical technique.

Methods: Chromatographic separation of the two drugs was achieved using analytical column C18 250/4.6 mm, 5 μ m particle size, 20 μ L sample loop size. The mobile phase consisted of a mixture of acetonitrile, methanol and 1% orthophosphoric acid (50:30:20). The drugs were analyzed using UV detector (Diode array detector, DAD) at wavelength 226 nm. The flow rate was maintained at 0.5 ml/min. Column temperature was at 25°C by in built column thermostat. The injection volume was 20 μ L, with a run time of 8 minutes.

Results and conclusion: Amongst the six formulations (Tazar, Tazomac, Zosyn, Durataz, Jan Aushadhi and Jammu and Kashmir Medical Supplies Corporation Limited, i.e, JKMSCL), all the formulations contained piperacillin within the standards specified by the Indian Pharmacopoeia (94-115%), except JKMSCL, which had slightly higher concentration. Further, tazobactam was present in all the formulations. However, the standards for comparison of tazobactam have not been specified within the Indian Pharmacopoeia.

Keywords: Branded, Generic, Piperacillin, Tazobactam, HPLC.

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INTRODUCTION

The term 'generic' word in India denotes the medicines which are marketed under a generic name. Another term called 'Branded generics' is used to connote medicines which are now off patent and sold under a brand name by companies, representing almost all the drugs in the Indian Pharmaceutical Market.¹ Piperacillin is a broad spectrum β -lactam antibiotic of the ureidopenicillin class of drugs.² Tazobactam is a pharmaceutical drug that inhibits the action of β -lactamases, especially those belonging to the sulfhydryl reagent variable (SHV-1)

group. It is commonly used as its sodium salt-tazobactam sodium. Tazobactam contains a beta-lactam ring and irreversibly binds to beta-lactamase at or near its active site. This protects other beta-lactam antibiotics from beta-lactamase catalysis. Piperacillin- tazobactam is recommended as a part of a three drug regimen for the treatment of hospital-acquired pneumonias suspected as being due to infection by multi-drug-resistant pathogens.³ In India, the incidence of sub-standard drugs has risen from 0.04% to 2.3% in 2012 as per Drug Controller General of India (DCGI). Various state drug controllers, between 2003 and 2008, concluded that 6 to 7.5 % of drug samples failed quality standard tests annually.⁴ Additionally, there are 66 substandard drugs found in Jammu and Kashmir (India), as per Drugs and food control organization, Jammu and Kashmir 2013.⁵ Looking at the rising incidences of sub-standard drugs in the local market of Jammu and Kashmir,

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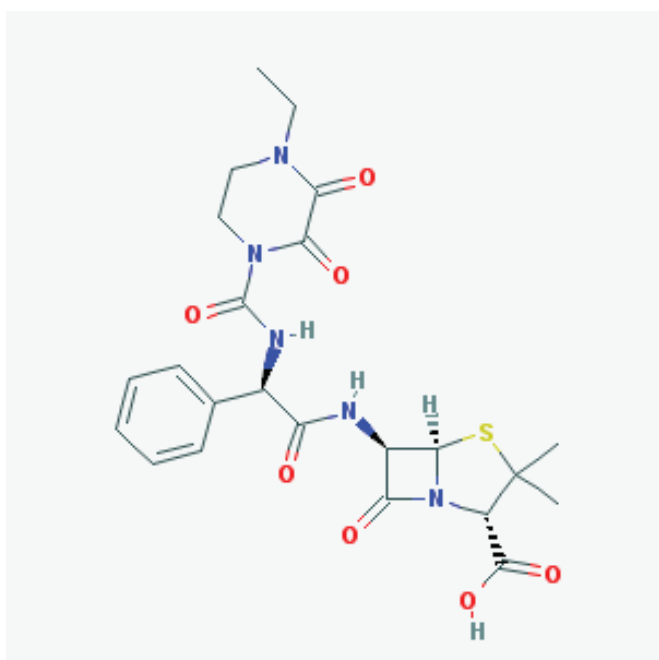


Figure 1: Chemical structure of piperacillin.

we have chosen piperacillin and tazobactam (four branded-generics namely TAZAR, TAZOMAC, DURATAZ, ZOSYN, and two generic formulations procured from Jan Aushadhi and Jammu and Kashmir Medical Supplies Corporation Limited-JKMSCL), which are used extensively in tertiary care hospital, to verify quality and quantity by employing HPLC based analytical technique.

MATERIALS AND METHODS

Chemicals and reagents

The reference sample of piperacillin/tazobactam and other HPLC grade chemicals like acetonitrile, methanol and orthophosphoric acid were procured from Sigma-Aldrich and Merck Specialties Private Limited, Mumbai, India, respectively.

Instruments

For the analysis of the pharmaceutical formulations, we used HPLC 1260 infinity model from Agilent Technologies USA, and the column was analytical column C18 250/4.6 mm, 5 μ m particle size, 20 μ L sample loop size. Electronic balance-Mettler Toledo, was used for weighing the samples. The software which is routinely utilized in our laboratory for HPLC data processing is EZChrom S1.

Chromatographic conditions

Various combinations of eluent like methanol, acetonitrile

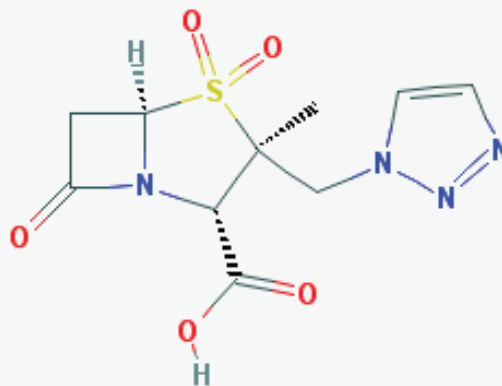


Figure 2: Chemical structure of tazobactam.

and 1% orthophosphoric were tested by making slight modifications in reported methods to improve resolution and analysis time of the samples under study.⁶ The drugs were analyzed using UV detector (Diode array detector-DAD) at wavelength 226 nm. The flow rate was maintained at 0.5 ml/min. Column temperature was at 25°C by in built column thermostat. The injection volume was 20 μ L, with a run time of 8 minutes.

Preparation of standard solution and plotting of calibration curves

Pure standards of piperacillin and tazobactam were used as external standards in the analysis. Different concentrations of the both standards (piperacillin and tazobactam) were used to plot a suitable 6-point calibration curve (25 μ g/ml to 150 μ g/ml).

20 mg each of piperacillin and tazobactam were weighed separately using a digital weighing electronic balance. Twenty (20) mg of piperacillin was dissolved in a 20 ml capacity volumetric flask using acetonitrile and the final volume was made to 20 ml. Similarly, 20 mg of tazobactam was dissolved in 20 ml of volumetric flask using 1:1 acetonitrile and methanol and final volume was made to 20 ml. For the dilution of stock solution, 100 ml of solvent system was prepared in a ratio of 50:30:20 of Acetonitrile, methanol and 1% orthophosphoric acid respectively. Dilution of 25, 50, 75, 100, 125 and 150 μ g/ml were made for piperacillin and

tazobactam separately. Each of the dilutions was injected in triplicates. Chromatographs were obtained for each of the dilutions. 6-Point calibration curves were finally plotted for piperacillin and tazobactam.

Preparation and assay of sample solution

Different branded and generic formulations of piperacillin and tazobactam (each vial of 4.5 g - contains 4 g of piperacillin and 0.5 g of tazobactam) were reconstituted as per manufacturer's instructions. 0.22-micron (μ) syringe filters were used to filter the reconstituted vial. Then stock solution for piperacillin and tazobactam were prepared in triplicates separately containing 1000 μ g/ml of piperacillin and tazobactam. From the stock solutions, dilutions of 50 μ g/ml and 100 μ g/ml were prepared in duplicates for piperacillin and tazobactam. Firstly, dilutions of piperacillin were injected followed by tazobactam to prevent any carry over. The peak area values of piperacillin and tazobactam were fit in linear equation ($y = mx + C$) and the concentration was calculated, where y is peak area, x is unknown concentration, c in intercept and m is slope of the curve.

Statistical Analysis

Data was entered in a Microsoft Excel spreadsheet. Continuous variables were summarized as mean and standard deviation. Categorical variables were

summarized as percentages. Data analysis was done using SPSS version 23.

RESULTS & DISCUSSION

The study was conducted in the Department of Pharmacology, Government Medical College, Srinagar, after getting approval from the Institutional Ethics Committee. In this study, concentrations of antibiotics-piperacillin and tazobactam were estimated. The studies conducted by Pai *et al.* (2006);⁷ Rao *et al.* (2010);⁸ Singh *et al.* (2017);⁹ Navle *et al.* (2017),¹⁰ also estimated concentrations of antibiotics piperacillin and tazobactam.

In our study, we have used RP-HPLC method to estimate the concentrations of piperacillin and tazobactam. The same method was also used for estimating concentrations of piperacillin and tazobactam in studies conducted by Pai *et al.* (2006);⁷ Rao *et al.* (2010);⁸ Singh *et al.* (2017);⁹ Navle *et al.* (2017).¹⁰ A study conducted by Sangeetha *et al.* (2017)¹¹ used UV spectrophotometric method to estimate concentrations of piperacillin and tazobactam.

In this study, six formulations were tested to estimate concentrations of piperacillin and tazobactam. The studies conducted by Pai *et al.* (2006);⁷ Rao *et al.* (2010);⁸ Singh *et al.* (2017);⁹ Navle *et al.* (2017)¹⁰ tested only a single formulation for quantitative estimation of

Table 1: Illustrates that all the formulations contain piperacillin within the standards specified by the Indian pharmacopoeia.

Expected concentration (PPCN)		N	Average percent recovery	Std. Deviation	Minimum	Maximum	%RSD
50	Durataz	6	104.4	3.57	99.2	109.5	3.4
	Jan Aushadhi	6	104.1	6.82	97.6	114.0	6.5
	JKMSCL	6	115.6	18.13	93.4	141.8	15.7
	Tazar	6	108.2	6.86	95.8	115.9	6.3
	Tazomac	6	112.7	6.02	106.9	122.4	5.3
	Zosyn	6	110.1	14.60	91.5	126.8	13.3
	Total	36	109.2	10.76	91.5	141.8	9.9
100	Durataz	6	104.5	4.30	100.5	110.4	4.1
	Jan Aushadhi	6	104.0	3.73	100.1	109.6	3.6
	JKMSCL	6	120.9	8.13	117.9	139.9	6.2
	Tazar	6	110.2	6.73	97.8	116.0	6.1
	Tazomac	6	114.2	9.60	107.8	133.4	8.1
	Zosyn	6	108.7	14.55	90.9	125.9	13.4
	Total	36	112.8	12.43	90.9	139.9	11.0

PPCN = Piperacillin, RSD = Relative Standard Deviation

Table 2: Tazobactam is present in all the formulations.

Expected concentration (TZB)		N	Average percent recovery	Std. Deviation	Minimum	Maximum	%RSD
50	Jan Aushadhi	6	67.2	2.91	64.3	71.1	4.3
	JKMSCL	6	84.4	9.05	76.0	95.4	10.7
	Durataz	6	77.0	3.06	73.3	80.8	4.0
	Tazomac	6	97.4	15.24	73.9	117.9	15.6
	Zosyn	6	85.8	4.56	81.5	91.3	5.3
	Tazar	6	82.3	3.95	77.6	87.0	4.8
	Total	36	82.3	11.76	64.3	117.9	14.3
100	Jan Aushadhi	6	62.7	2.54	60.0	65.4	4.1
	JKMSCL	6	76.2	8.15	67.8	84.6	10.7
	Durataz	6	70.3	3.27	66.1	74.0	4.6
	Tazomac	6	83.9	17.74	69.2	113.5	21.1
	Zosyn	6	79.6	4.25	75.1	84.4	5.3
	Tazar	6	76.1	4.99	70.9	81.4	6.6
	Total	36	74.8	10.52	60.0	113.5	14.1

TZB = Tazobactam, RSD = Relative Standard Deviation.

piperacillin and tazobactam. This is the first study of its kind where multiple formulations were tested to estimate piperacillin and tazobactam quantitatively.

Table 1 illustrates that all the formulations contain piperacillin within the standards specified by the Indian pharmacopoeia (94-115%), except JKMSCL which has slightly higher concentration. The studies conducted by Rao AL *et al.* (2011)⁸; Pai PNS *et al.* (2006)⁷; Singh H *et al.* (2017)⁹ also contained the concentrations of piperacillin within the standards specified by the Indian pharmacopoeia (94-115%).

From Table 2, it is clear that tazobactam is present in all the formulations. However, the Indian pharmacopoeia standards for tazobactam has not been specified.

CONCLUSION

It can be concluded that all the formulations contain piperacillin within the limits specified by the Indian pharmacopoeia, except JKMSCL which contains slightly higher concentration, which may be due to dilution or instrumental errors. Further, tazobactam is present in all the formulations. However, the Indian pharmacopoeia standard for tazobactam has not been specified.

REFERENCES

1. Jain A. The conflict between Generic vs. Branded Medicines in India. ETHealthWorld Updated: June 04, 2019
2. Tan JS, File TM. Antipseudomonal penicillins. Medical Clinics of North America. 1995; 79 (4): 679-93
3. Mandell LA, Wunderink R, in Harrison's Principles of Internal Medicine 18th Ed., Chapter 257, pp, 2139-2141
4. Palmer E. Indian regulator finds number of substandard drugs on the rise. [homepage on internet.] 2014. [cited 2014 May 20]. Available from: <http://fiercepharmamanufacturing.com/story/indian-regulator-finds-number-substandard-drugs-rise/2014-03-20>
5. http://dfcojk.org/images/sub_2013.pdf
6. Krishna R, Veni P, Sharmila N, Narayana KJP, Hari Babu B, Satyanarayana PVV. Simultaneous determination of piperacillin and tazobactam in pharmaceutical formulations by RP-HPLC method. Journal of Pharmacy Research. 2013;7:127-131.
7. Pai PNS, Rao GK, Murthy MS, Prathibha H. Simultaneous estimation of piperacillin and tazobactam in injection formulations. Ind J Pharma Sci. 2006;68:799e801.

8. Rao AL, Krishna KS, Kumar CK and T Raja. *Simultaneous determination of piperacillin and tazobactam in bulk and pharmaceutical dosage forms by RPHPLC. International Journal of Pharmacy and Pharmaceutical Sciences. 2011; 3(2): 134-136.*
9. Singh H, Devilal. A RP-HPLC Method Development and Validation for Simultaneous Estimation of Piperacillin and Tazobactam in injection formulation. *IJIPSR 2017; 5 (07): 72-82.*
10. Navle K, Prasanna BL, Dometti HS and Moodu S. Stability indicating RP-HPLC method for the determination of piperacillin and tazobactam and their related substances in bulk and pharmaceutical formulations. *World Journal of Pharmacy and Pharmaceutical Sciences, 2017.*
11. Sangeetha S, Kumar M, Kumudhavalli MV, Alexander S and Jaykar B. Development and validation of UV Spectrophotometric Area under Curve Method for Quantitative Estimation of Piperacillin and Tazobactam. *Journal of ChemTech Research, 2017; 10(2): 988-994*