

Simultaneous Estimation of Piperacillin and Tazobactam in Injection Formulations

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An high performance liquid chromatography method for simultaneous estimation of piperacillin and tazobactam was developed using Wakosil H, C18, 250 × 4.6 mm, 5 μm column, with mobile phase composition of methanol, phosphate buffer-pH 4 and acetonitrile in the ratio of 1:2:1 v/v/v with the flow rate of 1 ml/min and UV detection at 220 nm. The retention time for piperacillin and tazobactam was found to be 6.4 and 3.1 min respectively. Linearity was observed over the concentration range of 10-80 μg/ml for piperacillin and 2-10 μg/ml for tazobactam. Recovery was found to be 100.7-104.7% for piperacillin and 103.6-105.7% for tazobactam.

Piperacillin, chemically (2S,5R,6R)-6-[(R)-2-(4-ethyl-2,3-dioxo-1-piperazine carboxamido)-2-phenylacetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo-[3.2.0]-heptane-2-carboxylic acid-monohydrate, is a broad spectrum, semisynthetic, ureido-penicillin¹. Tazobactam, chemically [2S,3S,5R]-3-methyl-7-oxo-3-(1H-1,2,3-triazol-1-yl methyl)-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate-4,4-dioxide, is penicillanic acid residue and a β-lactamase inhibitor². The

use of β-lactamase inhibitors along with β-lactam antibiotics augments the activity of β-lactam antibiotics against β-lactamase-producing organisms. The combination of piperacillin and tazobactam is administered intravenously for management of serious infections associated with gram-positive and gram-negative organisms, including *Pseudomonas aeruginosa*, *Proteus* species and *Klebsiella pneumoniae*³. Literature survey revealed separate high performance liquid chromatography (HPLC) methods for determination of piperacillin⁴⁻⁶ and tazobactam⁷. Simultaneous estimation of piperacillin and tazobactam has

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been reported in biological samples⁸. In a method reported for estimation of piperacillin and tazobactam in injection formulation⁹, the net retention time was found to be around 15 min. The objective of the proposed method was to select a new system of simple mobile phase that provides rapid separation with low retention time and good resolution of peaks.

An HPLC system from Shimadzu (LC 10AT-VP) fitted with a Rheodyne injector, UV detector (SPD 10A-VP) and Winchrome software for processing the data generated was used. Analysis was carried out using Wakosil C18 column (SGE), 5 μm , 4.6 \times 250 mm, with a flow rate of 1 ml/min. UV detection was carried out at 220 nm. Acetonitrile HPLC grade, methanol HPLC grade, sodium dihydrogen orthophosphate AR grade and orthophosphoric acid were used for the analysis. Millipore water was used in the process. The phosphate buffer pH 4 was prepared by the following method. Sodium dihydrogen orthophosphate dihydrate, 71.63 g, was dissolved and volume made up to 1000 ml with water. The pH was adjusted to 4 with orthophosphoric acid. Volume of 250 ml each of methanol and acetonitrile was mixed with 500 ml of phosphate buffer, sonicated for 5 min and used as mobile phase. Injection formulation of combined piperacillin and tazobactam, Tazact, Cipla Ltd., Batch No ZB4015 and ZB5030, was purchased from a local pharmacy.

To obtain standard calibration curve, the following procedure was adopted. About 10 mg each of piperacillin and tazobactam were weighed into separate 10 ml volumetric flasks, dissolved with the mobile phase and volume made up with the same solvent to obtain standard stock solution. By dilution, working standard solutions of piperacillin and tazobactam of concentration 50 $\mu\text{g/ml}$ were prepared from the standard stock solution. The working standard solutions were injected into the chromatograph. The retention time for piperacillin and tazobactam was found to be 6.4 and 3.1 min, respectively. Calibration curves were obtained by plotting mean peak areas against the corresponding drug concentration. The

detector response was found to be linear over the concentration range of 10-80 $\mu\text{g/ml}$ and 2-10 $\mu\text{g/ml}$ for piperacillin and tazobactam respectively. The method was validated for statistical parameters like precision, accuracy, specificity, linearity, range, ruggedness and robustness. Results of the method validation experiments and precision were determined by three replicate solutions of the two independent samples.

Sample solution was prepared from different batches of marketed formulation Tazact. Powder for injection, 12.6 mg, equivalent to 10 mg of piperacillin and 1.2 mg of tazobactam was weighed into 10 ml volumetric flask. The powder was dissolved in the mobile phase and volume made up to 10 ml to obtain sample stock solution. Further, 0.5 ml of the sample stock solution was diluted to 10 ml with mobile phase to obtain working sample solution of 50 $\mu\text{g/ml}$. The working sample solution was injected into the chromatograph, and the compounds piperacillin and tazobactam were found to show satisfactory separation with retention time of 6.4 min and 3.1 min respectively.

The accuracy of the method was determined by adding known amounts of standard to those of sample at four levels, keeping the sample concentration constant. The recovery study data is presented in Table 1. The precision of the method was determined by replicate injections of standard solution. The percent RSD of the assay was found to be 0.68% for piperacillin and 0.78% for tazobactam, indicating high degree of precision and reproducibility. The precision of the system was determined for replicate samples by multiple injections of a set of solutions of the same concentration of piperacillin and tazobactam. The instrument response was found to be reproducible as found from percent RSD of 0.71% for piperacillin and 0.70% for tazobactam.

The robustness was determined by carrying out the assay during which the mobile phase ratio and pH of the mobile phase was altered slightly. When the pH was

TABLE 1: RECOVERY STUDY DATA OF PIPERACILLIN AND TAZOBACTAM

Levels	Standard piperacillin ($\mu\text{g/ml}$)	Sample piperacillin ($\mu\text{g/ml}$)	% recovery of piperacillin* (%)	Standard tazobactam ($\mu\text{g/ml}$)	Sample tazobactam ($\mu\text{g/ml}$)	% recovery of tazobactam* (%)
1	20	20	100.7	2	2	105.7
2	30	20	104.5	3	2	104.6
3	40	20	104.7	4	2	105.1
4	50	20	102.5	5	2	103.6

*Average of six determinations on standard addition of each drug separately at four levels in marketed formulation of Tazact powder for injection, marketed by Cipla Ltd., Mumbai

altered to 4.5, percent RSD was found to be 0.15% for piperacillin and 0.6% for tazobactam. On slight variation in the mobile phase ratio of up to $\pm 10\%$, the percent RSD was 0.2% for piperacillin and 0.1% for tazobactam, which indicated that the method is robust, also indicating lack of influence on the test results by operational variables for the proposed method.

The ruggedness of the method was determined by performing the same assay by different analysts and performing the assay on different days to check the reproducibility. The test result was found to provide percentage content of 96.83-104.37% for piperacillin and 97.75-104.06% for tazobactam, respectively, when the analysis was carried out by two different analysts on two different days. Thus the results were found to be highly reproducible despite variations in the conditions which could be normally expected because of the analysis carried out by different analysts and the analysis being carried out on different days.

The system suitability parameters for the proposed method were calculated. The number of theoretical plates per column was found to be 3630 for piperacillin and 985 for tazobactam. The symmetry factor and tailing factor were calculated statistically and found to be 1.05 for piperacillin and 1 for tazobactam. The resolution of the method was found to be 0.88, indicating good and complete separation of the two components from each other with a well-defined baseline.

Estimation of piperacillin and tazobactam in the marketed formulation gave assay results of 99.9-100.7% and 99.7-102.8%, respectively. The proposed method is thus precise, accurate, rugged, robust and can be conveniently used for the estimation of piperacillin and tazobactam in their injection formulation.

REFERENCES

1. The United States Pharmacopoeia and National Formulary, Asian Edn., XXIV, U.S. Pharmacopoeial convention, Rockville, MD 2000, 1338.
2. Bryson, H.M. and Brogden, R.N., **Drugs**, 1994, 47, 506.
3. Hardman, J.G., Limbird, L.E. and Gilman, A.G., Eds., In; Goodman and Gilman's The Pharmacological Basis of Therapeutics, 10th Edn., McGraw Hill, USA, 2001, 1203.
4. Brisson, A.M. and Fourtillan, J.B., **Antimicrob. Agents Chemother.**, 1982, 21, 664.
5. Zarzuelo, A., Lopez, F.G., Santos, M. and Lanao, J.M., **J. Liq. Chromatogr. Relat. Technol.**, 1996, 19, 601.
6. Garcia, G.J.C., Mendez, R. and Martin, V. J., **J. Chromatogr.**, 1998, 812, 213.
7. Peyrin, E., Guillaume, Y. and Guinchard, C., **J. Chromatogr. Biomed. Appl.**, 1995, 672, 160.
8. Ocampo, A.P., Hoyt, K.D., Wadgaonkar, N., Carver, A.H. and Puglisi, C.V., **J. Chromatogr. Biomed. Appl.**, 1989, 496, 167.
9. Marselos, S.C. and Arahontaki, H.A., **J. Pharm. Biomed. Anal.**, 2002, 5, 78.

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