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Research Article

SIMULTANEOUS DETERMINATION OF PIPERACILLIN AND TAZOBACTUM IN BULK AND PHARMACEUTICAL DOSAGE FORMS BY RP-HPLC

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ABSTRACT

A simple, rapid, accurate and precise reverse phase high performance liquid chromatographic method has been developed for the simultaneous determination of Piperacillin and Tazobactum in pharmaceutical dosage forms. Chromatography was carried out on a C_{18} column using a mixture of ammonium acetate and methanol in the ratio of 65:35 v/v as the mobile phase at a flow rate of 1.0 ml/min. and eluents are monitored at 225 nm. The calibration curves were linear over the range of 0.2-80 µg/ml for Piperacillin and 0.3-30 µg/ml for Tazobactum. The retention times of Piperacillin and Tazobactum was found to be 4.8 and 3.2 min., respectively. The intra and inter day variation was found to be less than 1% showing high precision of assay method. Due to its simplicity, rapidness and high precision, the proposed HPLC method may be used for simultaneous determination of these two drugs in pharmaceutical dosage forms.

Keywords: Piperacillin, Tazobactum, HPLC, Method Development.

INTRODUCTION

Piperacillin[1]; [2S-[2α,5α,6β(S*)]]-6-[[[[(4-Ethyl-2,3-dioxo-1-piperazinyl)carbonyl]amino] phenyl-acetyl]amino-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo-[3.2.0]heptane-2-carboxylicacid, is a semi-synthetic broad-spectrum antibacterial agent and is indicated for the treatment of serious infections caused by susceptible strains of microorganisms. Tazobactum[2]; (2S,3S,5R)-3-methyl-7-oxo-3-(1*H*-1,2,3-triazol-1-ylmethyl)-4-thia-1-azabicyclo-[3.2.0]heptanes-2-carboxylic acid-4,4-dioxide, is a beta-lactamase antibiotic and is used in combination with beta-lactamase antibiotic as antibacterial. The combination of Piperacillin and Tazobactum is used to reduce the development of drug-resistant bacteria[3].

Literature survey reveals that various HPLC[4-9] methods were reported for the determination of Piperacillin and Tazobactum in pharmaceutical dosage forms. The present work describes a simple, precise and accurate HPLC method for the simultaneous estimation of Piperacillin and Tazobactum in injection dosage forms.

MATERIALS AND METHODS

All chemicals were of analytical grade, and HPLC grade methanol and ammonium acetate (E.Merck Ltd., Mumbai) were used. Double distilled water filtered through 0.45µm filter was used to prepare solutions; Pharmaceutical grade Piperacillin and Tazobactum were procured from Aurobindo Chemicals and Drugs Ltd. Hyderabad, which was certified to be 98.5% and 99.7% respectively.

Equipments

Chromatographic separation was performed on Agilent 1100 series liquid chromatographic system equipped with binary pump, Agilent variable UV/Vis detector SPD-20A and auto injector. Chemi station software was employed for data collecting and processing. Weighing was done on Dutt balance (AY-120).

Chromatographic conditions

Chromatographic Separation was achieved on water Nova-Pak HR C_{18} (300X3.9mm, 6µ) column. The mobile phase consisting of methanol and 10mM ammonium acetate (35:65) and adjusted to pH 4.5 with phosphate buffer at rate of 1.0 mL/minute. The mobile phase was filtered through 0.45 µm membrane filter (Millipore) and degassed prior to use. Separation was performed at ambient temperature i.e. 25ºC and detection was made at 225 nm. The injection volume was 25 µL with a run time of 15 minutes.

Preparation of standard solution

To obtain standard calibration curve, the following procedure was adopted. About 10 mg each of Piperacillin and Tazobactum were weighed into separate 10 mL volumetric flask, dissolved in the mobile phase and volume was made up with the same solvent to obtain standard stock solution. By dilution, working standard solution of Piperacillin and Tazobactum were prepared.

Preparation of test solution

Sample solution was prepared from different batches of marketed formulation Tazocin powder for injection, 12.6 mg equivalent to 10 mg of Piperacillin and 1.2 mg of Tazobactum was weighed in 10 mL volumetric flask. The powder was dissolved in mobile phase and volume was 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.

Asaay

From the above sample solution 25 µL solution was injected into the chromatographic system along with same concentration of standard solution and chromatogram was recorded. The peak area values of Piperacillin and Tazobactum were calculated. The amount of Piperacillin and Tazobactum solution were estimated using calibration curve method. Results of analysis are tabulated.

RESULTS AND DISCUSSION

Method development and validation

Taking in consideration the instability of Piperacillin and Tazobactum in strong alkaline and strong acidic condition, the pH value of the mobile phase should be limited within the range of 3- 7. Since mild acidic pH favours the retention and separation of two drugs on C-18 column. After some trials potassium dihydrogen orthophosphate with pH 4.5 was finally selected. Binary mixture of methanol and ammonium acetate (35:65 % v/v) was optimized as mobile phase which produced symmetric peak shape, good resolution and reasonable retention time for both the drugs.

The retention times of Piperacillin and Tazobactum for six repetitions were found to be 4.80±0.006 and 3.200±0.02 min respectively. A typical chromatogram of a standard and sample solution is shown in Fig. 1. Since both Piperacillin and Tazobactum in the mobile phase have no significant UV maximum but end absorption, to ensure the sensitivity of the method, the wavelength of 225 nm was employed for the detection.

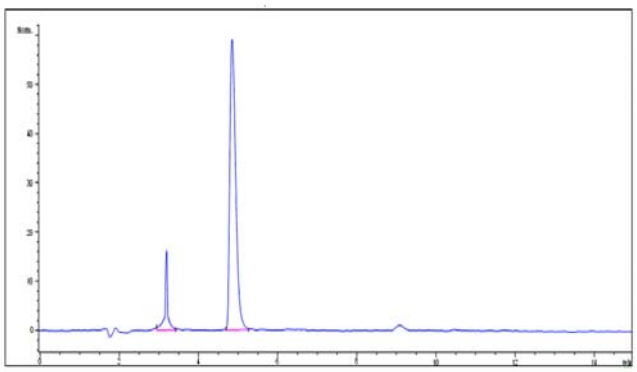

Fig. 1: Typical chromatogram of Piperacillin and Tazobactum

Table 1: System suitability parameters

S. No.	Parameters	Values	
		Piperacillin	Tazobactum
1	Resolution	2.3	5.019
2	Capacity factor	0.467	0.494
3	Theoretical plates	2849	2410
4	Tailing factor	0.985	1.212
5	HETP	0.0052	0.0062
6	Symmetry factor	1.075	1.21

System suitability

System performance parameters of the developed HPLC method were determined by analyzing standard working solutions. Chromatographic parameters, such as number of theoretical plates (N), resolution (Rs), capacity factor (k) and selectivity factor (α) were determined. The results are shown in Table 1, indicating the good performance of the system.

Linearity

Under the experimental conditions described above, linear calibration curves for both Piperacillin and Tazobactum were obtained with five concentration level each. Peak area (A) and concentration (C) of each drug substance was subjected to regression analysis to calculate the regression equation and the correlation coefficients. The regression equation obtained were y=27.318x–12.083 for Piperacillin and y=1.4536x+0.3211 for Tazobactum (r=0.99995, n=5) and (r=0.99996, n=5) for Piperacillin and Tazobactum respectively. The linearity range of Piperacillin was 0.2-80µg/mL and 0.3-30µg/mL for Tazobactum.

Accuracy

The accuracy of an analytical method is the closeness of test results obtained by method to the assay value. Accuracy should be established across the specified range of the analytical procedure. The accuracy was then calculated as the percentage of analytes recovered by the assay. Mean recoveries (mean±S.D.) for Piperacillin and Tazobactum from the combination formulation are shown in Table 2 indicating good accuracy of the method.

Precision

System precision is the measure of the method variability that can be expected for a given analyst performing the analysis. Precision of the method was determined with the product. An amount of the product powder equivalent to 75, 100 and 125% of label of claim was weighed accurately and assayed in six replicate determinations for each of the three weighing amounts. The results for precision are shown in Table 3, indicating that acceptable precision was achieved for Piperacillin and Tazobactum, as revealed by relative standard deviation data (RSD<2.0% in all of the levels of the two drugs).

Table 2: Accuracy of Piperacillin and Tazobactum

Drug Name	Amount added, µg/mL	Amount recovered, µg/mL	% Recovery	Average recovery	% RSD
	5	4.99	99.8		
	5	4.98	99.6	99.866	0.0499
	5	5.01	100.2		
	10	9.99	99.9		
Piperacillin	10	9.98	99.8	99.93	0.099
	10	10.01	100.1		
	15	14.99	99.93		
	15	14.98	99.86	99.95	0.149
	15	15.01	100.06		
	5	4.99	99.8		
	5	4.98	99.6	99.866	0.075
	5	5.01	100.2		
	10	9.99	99.9		
Tazobactum	10	9.98	99.8	99.93	0.148
	10	10.01	100.1		
	15	14.99	99.93		
	15	14.98	99.86	99.95	0.22
	15	15.01	100.06		

Limit of detection and limit of quantitation

The LOD was calculated to be 0.066 µg/mL for Piperacillin and 0.1 µg/mL for Tazobactum. And the LOQ of Piperacillin and Tazobactum were found to be 0.2 µg/mL and 0.3 µg/mL, respectively.

Robustness

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 altered to 3.5%, percent RSD was found to be 0.15% for Piperacillin and 0.6% for Tazobactum. On slight variation in the mobile phase ratio of upto ±10%, the percent RSD was 0.2% for Piperacillin and 0.1% for Tazobactum which indicated that the method is robust, also indicating lack of influence on the test results by operational variable for the proposed method.

Ruggedness

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 results were found to provide percentage content of 96.83-104.37% for Piperacillin and 97.75-104.06% for Tazobactum. When the analysis was carried out by two different analysts on two different days the test results were found to be highly reproducible.

Table 3: Precision of Piperacillin and Tazobactum

Drug Name	Concentration, µg/mL*	Intra-day		Inter-day	
		Mean	% RSD	Mean	% RSD
Piperacillin	5	4.97	0.049	4.95	0.049
	10	10.01	0.099	9.99	0.12
	15	14.99	0.149	14.98	0.149
Tazobactum	5	7.579	0.075	7.574	0.075
	10	14.847	0.148	14.852	0.147
	15	22.115	0.22	22.124	0.22

*Six replicates

CONCLUSION

The developed RP-HPLC method with UV-Visible detection for the estimation of Piperacillin and Tazobactum, offers simplicity, selectivity, precision and accuracy. It produces symmetric peak shape, good resolution and reasonable retention time for both drugs. So this method can be applicable for the simultaneous estimation of Piperacillin and Tazobactum in quality control studies for routine analysis.

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