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Method Development and Validation of Piperacillin and Tazobactum in Pharmaceutical dosage form by using RP-HPLC Method

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ABSTRACT

A simple and selective LC method is described for the determination of Piperacillin and Tazobactum in tablet dosage forms. Chromatographic separation was achieved on a c18 column using mobile phase consisting of a mixture of 50 volumes of Triethylamine and 50 volumes of Acetonitrile with detection of 226 nm. Linearity was observed in the range 5-15 µg /ml for Piperacillin (r^2 =0.996) and 10-30 µg /ml for Tazobactum (r^2 =0.997) for the amount of drugs estimated by the proposed methods was in good agreement with the label claim. The proposed methods were validated. The accuracy of the methods was assessed by recovery studies at three different levels. Recovery experiments indicated the absence of interference from commonly encountered pharmaceutical additives. The method was found to be precise as indicated by the repeatability analysis, showing %RSD less than 2. All statistical data proves validity of the methods and can be used for routine analysis of pharmaceutical dosage form.

1. INTRODUCTION

A new simple**,** precise, accurate and selective HPLC method has been developed and validated for estimation of Pipperacillin and Tazobactum in pharmaceutical formulation. The detection was carried out at 226nm. The retention time for Pipercillin 2.4 minutes and Tazobactum were found to be 4.2 minutes respectively. The method was validated for precision, recovery, robustness, specificity, and detection and quantification limits, in accordance with International Conference on Harmonization guidelines. Linearity was observed in the concentration range from 5-15 µg/ml (r^2=0.996) for Piperacilllin and for Tazobactum 10-30 µg/ml (r^2=0.997). The limit of detection and quantification of Piperacillin were 0.81 µg/ml and 2.46 µg/ml respectively. While for Tazobactum it was 0.53 µg/ml and 1.633µg/ml, respectively. The drug content was found to be 100.12 % for Piperacillin and 98.8% for Tazobactum. The % RSD below 2.0 shows the high precision of proposed method.

Piperacillin is a semisynthetic, broad-spectrum, ampicillin derived ureidopenicillin antibiotic proposed for pseudomonas infections. It is also used in combination with other antibiotics. Tazobactam is an antibacterial penicillin derivative which inhibits the action of bacterial beta-lactamases.

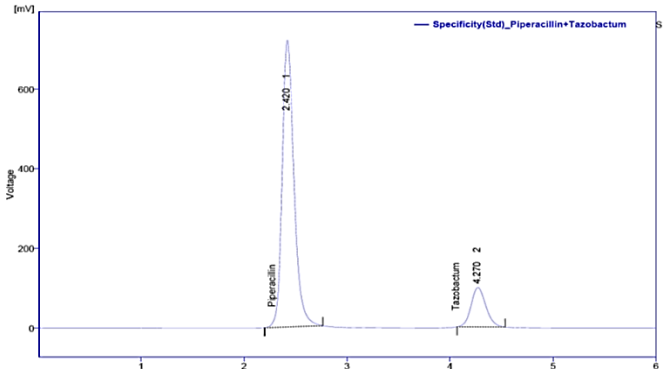

Figure.1.Standard chromatogram of Pipperacillin and Tazobactum

2. MATERIALS AND METHODS

Reagent and chemicals: Pipercillin and Tazobactum were supplied as a gift sample by Chandra labs hyderabad. These drugs were used as working standard. All the chemicals used of HPLC Grade (MERCK. Chem. Ltd., Mumbai) and double distilled water was used for mobile phase preparation.

Selection of chromatographic parameters:

Selection of chromatographic mode: The reverse phase HPLC was selected for separation because it was convenient and rugged than other forms of the liquid chromatography and was more likely to give good resolved peaks at a reasonable retention time at a specific pH.

Selection of stationary phase: On the basis of reversed phase HPLC mode and number of carbon present in molecule (analyte) stationary phase with Inertsil ODS 3V (250x4.6mm) 5µm was selected.

Preparation of standard stock solution: Weigh accurately 10 mg of piperacillin and 10mg of tazobactum in 100 ml of volumetric flask and dissolve in 100ml of mobile phase and make up the volume with mobile phase.From above stock solution 10 µg/ml of piperacillin and 20 µg/ml of tazobactum is prepared by diluting 3ml to 10ml with mobile phase. This solution is used for recording chromatogram.

Preparation of mobile phase: A mixture of 50 volumes of Triethylamine and 5 volumes of Acetonitrile buffer were prepared. The mobile phase was sonicated for 10 min to remove gases.

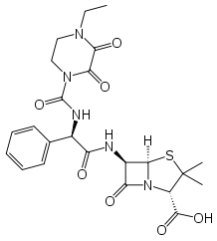

Figure.2.Molecular structure of Piperacillin

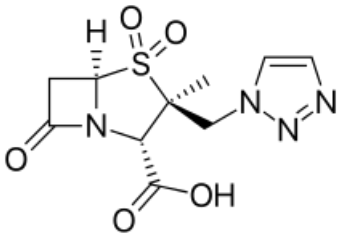

Figure.3.Molecular structure of Tazobactum

3. RESULTS AND DISCUSSION

Method Validation: The proposed method was validated as per ICH guidelines. The solutions of the drugs were prepared as per the earlier adopted procedure given in the experiment.

Linearity studies: Standard stock solutions of piperacillin and tazobactum (microgram/ml) were prepared by dissolving 10 mg of piperacillin and 20 mg of tazobactum dissolved in sufficient mobile phase and dilute to 100 ml with mobile phase.

Table.1.Linearity of Piperacillin

Concentration	Area
5	3769.741
7.5	4743.96
10	5538.159
12.5	6714.107
15	7678.012

Table.2.Linearity of Tazobactum

Concentration	Area
10	609.077
15	774.576
20	1007.518
25	1180.863
30	1417.216

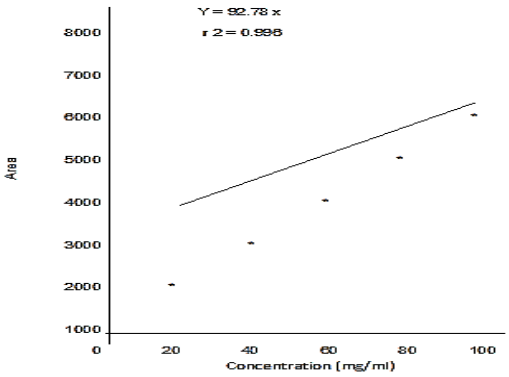

Figure.4.Linearity graph of Piperacillin

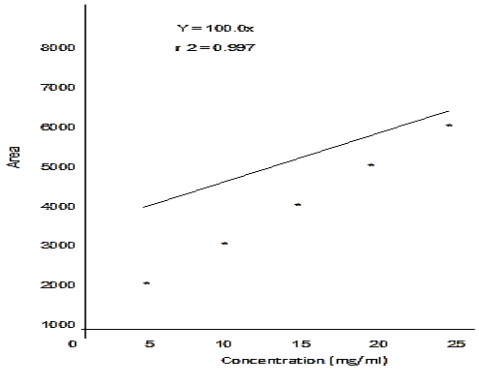

Figure.5.Linearity graph Tazobactum

Accuracy: Accuracy of the method was determined by recovery studies to the formulation (Pre analyzed sample). The reference standards of the drugs were added at the level of 80%, 100%. The recovery studies were carried out three times and the percentage recovery and percentage mean recovery were calculated for drug is shine in table to check the accuracy of method, recovery studies were carried out by addition of standard drug solution to pre-analyzed sample solution at three different levels 80%, 100%,120%.

Acceptance Criteria: The % recovery of Piperacillin and Tazobactum should lie between 98 – 102%

Table.3.Recovery Results for Piperacillin

Recovery level	Accuracy of Piperacillin			Average % Recovery
	Amount taken (mcg/ml)	Area	% Recovery	
50%	60	4879.050	85.95	98.33
	60	4874.809		
	60	4880.624		
100%	72	6715.130	117.03	
	72	6416.924		
	72	6794.146		
150%	84	7889.449		
	84	7976.993		
	84	7876.999		

Table.4.Recovery results far Tazobactum

Recovery Level	Accuracy of Tazobactum			Average % Recovery
	Amount taken (mg/ml)	Area	% Recovery	
50%	10	815.841	83.55	102.45
	10	793.602		
	10	816.547		
100%	12	1211.449	121.69	
	12	1115.466		
	12	1206.480		
150%	14	1483.271	102.11	
	14	1515.624		
	14	1493.453		

Method Precision: Prepared sample preparations of tazobactum and Piperacillin as per test method and injected 6 times in to the column.

Acceptance criteria: The % relative stander deviation of Assay preparations of Tazobactum and piperacillin should be not more than 2.0%

Table.5.Method precision of piperacillin and Tazobactum

Piperacillin			Tazobactum		
S.No.	RT	Area	S.No.	RT	Area
1	2.443	5710.568	1	4.293	991.742
2	2.417	5633.849	2	4.257	954.143
3	2.423	5662.646	3	4.270	948.278
4	2.423	5679.338	4	4.263	955.36
5	2.447	5659.977	5	4.293	951.360
6	2.423	5645.244	6	4.267	968.283
AVG	2.429333	5673.604	AVG	4.273833	961.4977
SD	0.01242	22.86586	SD	0.15471	16.33345
%RSD	0.005113	0.00403	%RSD	0.00362	0.016988

Observation: Test results for Tazobactum and Piperacillin are showing that the % RSD of Assay results are within limits.

Robustness: To evaluate robustness few parameters were deliberately varied. The parameters include variation of flow rate and wave length. The system suitability should pass per the test method at variable conditions.

Table.6.Results of Robustness study

Piperacillin			Tazobactum	
Parameter	**Retention (min)**	**Tailing factor**	**Retention time (min)**	**Tailing factor**
Flow Rate 0.2ml/min	2.562	1.679	5.059	1.263
Wavelength 249nm	3.148	1.678	4.235	1.264
249nm	2.566	1.687	5.0523	1.262
253nm	2.570	1.686	5.65	1.265

Observation: From rate observation it was found that the system suitability were within limit at all variable condition.

Sensitivity: Sensitivity of the proposed method was estimated in terms of Limit of Detection (LOD) and Limit of Quantification (LOQ). LOD = 3.3 SD/S and LOQ = 10 SD/S, where SD is the residual standard deviation and S is the slope of the line. The limit of detection and quantification of Piperacillin were 0.81 µg/ml and 2.46 µg/ml respectively. While for Tazobactum it was 0.53 µg/ml and 1.633µg/ml, respectively.

Specificity and Selectivity: The analytes should have no interference from other extraneous components and be well resolved from them. Specificity is a procedure to detect quantitatively the analyte in presence of component that may be expected to be present in the sample matrix, while selectivity is the procedure to detect qualitatively the analyte in presence of components that may be expected to be present in the sample matrix.

Ruggedness: The ruggedness of the method was studied by the determining the analyst to analyst variation by performing the Assay by two different analysts.

Acceptance criteria: The % Relative standard deviation of Assay values between two analysts should be not more than 2.0%

Table.7.Results for Ruggedness

Piperacillin	% Assay	Tazobactum	% Assay
Analyst 01	100.5	Analyst0.1	98.9
Analyst02	99.5	Analyst 02	100.3

Observation: From the observation the between two analysts Assay values not Greater than 2.0% hence the method was rugged.

System suitability test: System suitability testing is essential for the assurance of the quality performance of the chromatographic system. Earlier prepared solutions for chromatographic conditions were tested for system suitability testing.

4. CONCLUSION

From the above experimental results and parameters it was concluded that, this newly developed method for the simultaneous estimation of Piperacillin and Tazabactum was found to be simple, precise, accurate and high resolution and shorter retention time makes this method more acceptable and cost effective and it can be effectively applied for routine analysis in research institutions, quality control department in meant in industries, approved testing laboratories studies in near future.

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