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Validated RP-HPLC Method for Simultaneous Estimation of Tazobactam and Piperacillin in Combined Dosage Form

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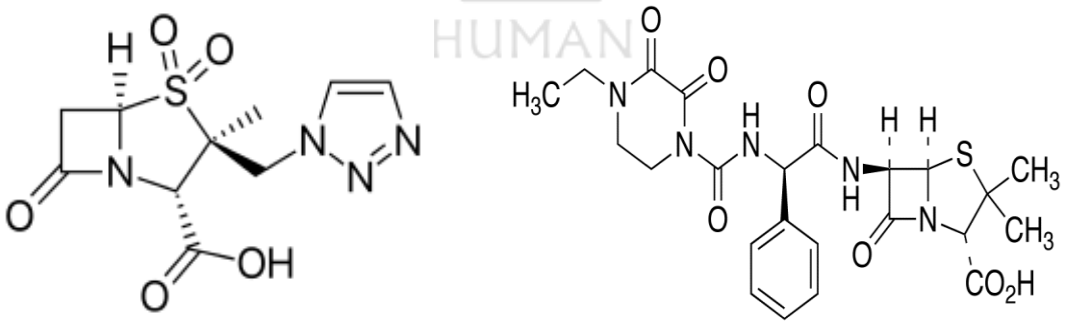
ABSTRACT

A precise, accurate, sensitive and robust RP-HPLC method was developed for the simultaneous estimation of tazobactam and piperacillin in the combined dosage form. Chromatographic separation was achieved on Waters Model NO.2690/5 series Compact System fitted with an Inertsil-C18 ODS column. (4.6 mm i.d. X250mm, 5µm particle size) at ambient temperature. A binary mobile phase consisting of Methanol, 25mM potassium dihydrogen phosphate buffer of pH-6.4 was delivered through the column at a flow rate of 1mL/min. Measurement was carried out at a wavelength 225nm. The method was linear over the concentration range of 2.5-10 μg/mL for tazobactam and 20-80 μg/mL for Piperacillin. The percentage content found for tazobactam was 99.81 -100.76% and 99.4-100.55% for piperacillin in the pharmaceutical formulation. The method was validated for linearity, precision, accuracy, and robustness as per ICH Q2 (R1) guidelines.

1. INTRODUCTION

Tazobactam[1], chemically [2S-(2α,3β,5α)]-3-Methyl-7-oxo-3-(1H,2,3-triazol-1-ylmethyl)-4-thia-1-azabicyclo [3.2.0] heptane -2- carbonsäure-4, 4-dioxide a pharmaceutical drug that inhibits the action of bacterial β-lactamases, especially those belonging to the SHV-1 and TEM groups. It is commonly used as its sodium salt, tazobactam sodium. In simple terms, it is an ingredient that can be added to certain antibiotics to make them less vulnerable to bacteria's antimicrobial resistance. Tazobactam is combined with the extended-spectrum β-lactam antibiotic piperacillin[2] in the drug piperacillin/tazobactam, used in infections due to *Pseudomonas aeruginosa*. By binding to specific penicillin-binding proteins (PBPs) located inside the bacterial cell wall, Piperacillin inhibits the third and last stage of bacterial cell wall synthesis. Cell lysis is then mediated by bacterial cell wall autolytic enzymes such as autolysins, Piperacillin may interfere with an autolysin inhibitor. Tazobactam broadens the spectrum of piperacillin by making it effective against organisms that express β-lactamase and would normally degrade piperacillin.

Literature survey revealed that few chromatographic[3-10] methods were available for the estimation of piperacillin and tazobactam in single and in combination with other drugs.



(a) **(b)**

Figure no 1: Chemical structures of (a) tazobactam and (b) piperacillin.

2. MATERIALS AND METHODS

2.1. Instrumentation

Chromatographic separation was performed using Waters Model NO.2690/5 series Compact HPLC System equipped with a Rheodyne injector, Photo Diode Array Detector G1315D. Winchrome software was employed for data collecting and processing.

2.2. Reagents and Materials

Tazobactum and Piperacillin reference standard (RS) were obtained from Hetero. Pvt. Ltd. India. A fixed-dose combination of tazobactam and piperacillin [Tazocin] was purchased from the local pharmacy. Methanol (HPLC grade) and 25mM potassium dihydrogen phosphate of pH-6.4 were obtained from Fischer Scientific, Mumbai.

2.3. Chromatographic Conditions

Chromatographic separation was performed on Waters Model NO.2690/5 series compact System equipped with an anodyne injector, photodiode Array Detector G1315D. Inertsil-C18 ODS column (4.6 mm i. d. X250mm, 5µm particle size). A binary mobile phase consisting of methanol HPLC grade and 25mM potassium dihydrogen phosphate buffer, pH6.4 (80:20, v/v) was delivered through a column at a flow rate of 1mL/min. The methanol and phosphate buffer, pH 6.4 were filtered separately through a 0.45μm membrane filter paper, mixed. The mobile phase was degassed before use. HPLC analysis was performed at ambient temperature with detection at 225 nm. The injection volume was 20μL.

2.4. Preparation of mixed standard solution

12.5mg of Tazobactum and 100 mg Piperacillin were accurately weighed and transferred into a 100 mL volumetric flask containing 70 mL of the mobile phase. From the above solutions, 10 mL of each were taken into another 100 mL volumetric flasks and volume were made up to mark with the mobile phase. The solution is sonicated for 15 mins and filtered using a 0.45µ millipore filter paper and appropriately diluted for further analysis.

2.5. Preparation of sample solution

A quantity of dry sample powder equivalent to 100.0 mg of Piperacillin and 12.5 mg of Tazobactum was weighed accurately, transferred into a 100.0 mL volumetric flask containing 70 mL mobile phase and dissolved with the aid of ultrasonication for 20 min. From the above solution, 10.0 mL was taken into a 100.0 mL volumetric flask and volume was made up to 100 mL with the mobile phase. This solution was appropriately diluted with mobile phase for further analysis.

2.6. Validation[11-13]

The method was validated for system suitability, linearity, precision, accuracy, the limit of detection (LOD), the limit of quantitation (LOQ), specificity and robustness as per ICH Q2 (R1) guidelines. System suitability parameters of the developed HPLC method were determined by analyzing the standard working solution. Chromatographic parameters such as retention time, asymmetry, theoretical plates, capacity factor, and resolution were determined. Linearity was calculated with six concentration levels of tazobactam and piperacillin. Precision has measured both system and as well as method. In the system precision study, standard working concentrations of tazobactam and piperacillin (5µg/ml and 40µg/ml) was analyzed six times. In method precision study, the sample working concentrations of tazobactam and piperacillin was analyzed six times. Accuracy was studied by the measurement to recovery at three different levels such as 50%, 100% and 150% of the amount expected in the formulation. LOD and LOQ of the method were studied to detect the lowest amount of analyte and quantitative determination of an analyte in a sample, respectively.

3. RESULTS

In the present work, an RP-HPLC method was developed and validated for the simultaneous estimation of tazobactam and piperacillin in the pharmaceutical dosage form. The final optimized chromatographic conditions were Inertsil-C18 ODS column (4.6 mm i.d.X250mm, 5µm particle size) as a stationary phase eluted with a binary mobile phase consisting of methanol HPLC grade and 25mM potassium dihydrogen phosphate buffer, pH6.4 (80:20, v/v) delivered through the column at a flow rate of 1mL/min with detection wavelength 225nm. By using optimized chromatographic conditions, tazobactam and piperacillin were eluted at 2.95 min and 4.19 min, respectively (Figure 2).

3.1. Assay of Marketed Formulation

The assay result of tazobactam and piperacillin in pharmaceutical dosage form was comparable with the value claimed on the vial. The percentage of the content found for tazobactam was 99.74±1% and 100.4±0.5% for piperacillin (Table1). The assay results obtained has shown that the method is suitable for the routine analysis of tazobactam and piperacillin in their combined dosage form.

3.2. Validation

3.2.1. System Suitability

System suitability parameters of the developed HPLC method were determined (Table 2). The parameters like capacity factor, theoretical plates, and asymmetry factor were within the range of the specified limit.

3.2.2. Linearity Study

Linearity graph of working standard solution concentration versus peak area was plotted for tazobactam and piperacillin. The graph was found linear in the range of 2.5– 10 μg/mL for tazobactam and 20-80 μg/mL for piperacillin (Table2).

3.2.3. Precision

The precision of the method was verified by the system and method of precision study. System precision was measured by determining % RSD of multiple injections of a homogenous working standard solution of 5 μg/mL of tazobactam and 40 μg/mL of piperacillin which was found to be 0.56 and 0.36, respectively. Method precision was calculated by determining % RSD of analysis of working sample solution of 5 μg/mL of tazobactam and 40 μg/mL of piperacillin which was found to be 0.41 and 0.32, respectively (Table2). The % RSD value of less than 2 indicates that the developed method was precise.

3.2.4. Accuracy

To study the accuracy of the method recovery study was carried out by standard addition method at 50, 100 and 150% of label claim. At each level, three determinations were carried out. The % RSD value of % recovery less than 2 indicate that the developed method was accurate (Table2).

3.2.5. Specificity

To study the interference of the excipients that are usually present in the formulation specificity study was carried out. The result of specificity study has shown that no interference of impurity or any formulation excipient was found at the Rt of tazobactam and piperacillin (Table 2). Thus, the developed method was found to be specific.

3.2.7. Robustness

Robustness testing was performed to obtain information about critical parameters affecting the selected response (peak area, retention time and found concentration). The investigated range of experimental variables has shown in Table 3. From the experimental response, it was found that a change in mobile phase composition, flow rate, detection wavelength did not affect in case of tazobactam and piperacillin. Thus, the developed method was found to be robust.

4. DISCUSSION

The RP-HPLC method has been developed for the simultaneous determination of tazobactam and piperacillin in the pharmaceutical dosage form. Advantages of proposed HPLC method were as simple, easy mobile phase preparation, runtime < 10min and economical one. A buffer concentration of 25mM phosphate buffer was used in the mobile phase as it is a good compromise. Methanol was the preferred component in the mobile phase as it allows the quantitative measurements below 230nm. It is evident from a system suitability test that the method developed tazobactam and piperacillin combination had passed the standard regulatory requirements.

The method was found to be linear over the wide range and to be useful for bulk and pharmaceutical analysis. The method was successfully applied to bulk material and pharmaceutical formulation assay. From specificity study, it was observed that drug combination is free from excipient interaction and method is suitable for analysis of tazobactam and piperacillin in the presence of their impurity. According to ICH $Q_2(R_1)$ guideline, the robustness of an analytical procedure refers to its capability to remain unaffected by small and deliberate variation in method parameters. Experimental results were computed using twin chrome version 8.0.6 software. From the results of the robustness study, it was observed that the proposed method was found to be robust when deliberate variations were made in the optimized chromatographic conditions.

5. CONCLUSION

The proposed RP-HPLC method is simple, rapid, precise, accurate and robust for the simultaneous estimation of tazobactam and piperacillin in its powder for injection formulation. Hence, it can be conveniently adopted for routine quality control analysis.

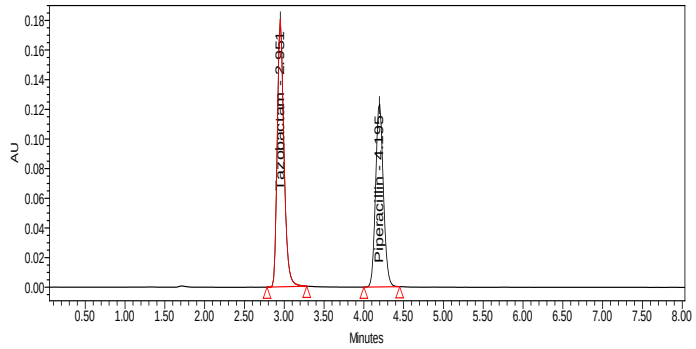


Figure no. 2: Typical chromatogram showing the separation of tazobactam and piperacillin

Table no 1: Analysis of marketed formulation

Parameter	Tazobactum	Piperacillin
Amount took (*µ*g/ml)	5.0	40
Amount found (*µ*g/ml)	5.08	39.26
% Assay ± SD(n=6)	100.08 ±0.17	99.74 ±0.49
%RSD	0.39	0.18

Table no. 2: Results of Validation parameters

Parameter	Tazobactum	Piperacillin
System suitability parameters(n=6)		
Retention time ± %RSD	2.949±0.035	4.1905±0.83
Theoretical plates ± %RSD	8730.389	8958.236
Asymmetric factor ± %RSD	1.02±0.92	0.99±1.24
Linearity study		
Linearity range µg/ml	2.5-10 µg/ml	20-80 µg/ml
Slope	202181	64197
Intercept	201835	310252
Correlation coefficient	0.9998	0.9996
Precision(n=6)		
System precision %RSD	0.569288	0.367864
Method precision %RSD	0.414498	0.328767
Accuracy(n=3)		
50% ± %RSD	0.67	0.38
100% ± %RSD	0.399	0.189
150% ± %RSD	0.72	0.132

Table no 3: an Investigated range of experimental variables during robustness testing.

Variable	Optimized value	Range investigated
Mobile phase flow rate	1.0 ml/min	0.8-1.2ml
Mobile phase composition Methanol: Phosphate buffer(pH-6.4)	80:20	85:15 75:25
Detection wavelength	225nm	230nm 220nm

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