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

A.LAKSHMANA RAO1, K.SAI KRISHNA1, C.H.KIRAN KUMAR2 AND T.RAJA2

1V.V. Institute of Pharmaceutical Sciences, Gudlavalleru- 521 356, A.P., India, 2Shri Vishnu College of Pharmacy, Bhimavaram- 534 202, A.P., India, 3Vishwa Bharathi College of Pharmaceutical Sciences, Guntur- 522 009, A.P., India. Email: drralrao@gmail.com

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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 C18 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

Piperacillin1; [2S-[2α,5α,6β(S*)]-6-[[[(4-Ethyl-2,3-dioxo-1-piperazinyl)carbonyl]amino] phenylacetyl]amino-3, dimethyl-7-oxo-4-thia-1-azabicyclo-[3.2.0]heptane-2-carboxylic acid, is a semi-synthetic broad-spectrum antibiotic agent and is indicated for the treatment of serious infections caused by susceptible strains of microorganisms. Tazobactum2; (2S,3S,5R)-3-methyl-7-oxo-3-[1H-1,2,3-triazol-1-ylmethyl]-4-thia-1-azabicyclo-[3.2.0]heptanes-2-carboxylic acid, 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.

Literature survey reveals that various HPLC3-9 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 C18 [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/min. 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. 25°C and detection was made at 225 nm. The injection volume was 25 µL with a run time of 15 minutes.
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 up to ±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

<table>
<thead>
<tr>
<th>Drug Name</th>
<th>Concentration, μg/mL*</th>
<th>Intra-day Mean</th>
<th>% RSD</th>
<th>Inter-day Mean</th>
<th>% RSD</th>
</tr>
</thead>
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<td>Piperacillin</td>
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<td>4.97</td>
<td>0.049</td>
<td>4.95</td>
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<tr>
<td></td>
<td>10</td>
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<td>0.099</td>
<td>9.99</td>
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<td>14.99</td>
<td>0.149</td>
<td>14.98</td>
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</tr>
<tr>
<td>Tazobactum</td>
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<td>7.579</td>
<td>0.075</td>
<td>7.574</td>
<td>0.075</td>
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<tr>
<td></td>
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<td>0.22</td>
<td>22.124</td>
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</tr>
</tbody>
</table>

*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.

REFERENCES

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