Spectrophotometric determination of serratiopeptidase and metronidazole in combined dosage forms

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ABSTRACT

Serratiopeptidase and metronidazole are the leading prescribing drugs by the physicians for various symptoms. Literature revealed that there is no combination formulation present in the market, which provokes the preparation of dual drug delivery system containing serratiopeptidase and metronidazole in a single dosage form. The estimation of Serratiopeptidase and metronidazole in a combined dosage form was developed, without prior separation. The method is the application of the derivative spectrophotometric method at zero crossing wavelengths. Serratiopeptidase is determined at 229 nm and metronidazole at 346.5 nm respectively. This method is simple, accurate and rapid and they require no preliminary separation and can therefore be used for routine analysis of both drugs in quality control laboratories.

Key words: Serratiopeptidase, metronidazole, derivative spectrophotometry

INTRODUCTION

Serratiopeptidase is a metalloenzyme derived from bacteria belonging to genus Serratia. Controlled fermentation of enterobacteria, Serratia secretes serratiopeptidase in medium. HPCL¹ and steric exclusion chromatography² have been reported for the estimation of Serratiopeptidase. Metronidazole is a 2-Methyl-5-nitroimidazole-1-ethanol used in anaerobic infection. Liquids chromatography³ and HPLC⁴ methods are reported for the estimation of metronidazole. Since there is no simultaneous method reported for the estimation of these drugs in combined dosage forms, we present a simple, economical and accurate simultaneous estimation.

MATERIALS AND METHODS

Instrumentation

Shimadzu 1700 Pharmaspec UV-visible spectrophotometer with a matched pair of 10mm quartz cells were used.

Reagents and Chemicals

Serratiopeptidase, received as gift sample from Advance enzyme technologies Ltd. Mumbai and metronidazole from Ranbaxy Labs were used as such without further purification. Chemicals, AR Grade (Merck, India Limited, Mumbai) were used in the study.

Preparation of standard stock solution

Serratiopeptidase and metronidazole (100mg) were accurately weighed and dissolved separately in 100mL of phosphate buffer pH 7.4 to give stock solution (1000µg/mL). Aliquots of 100µg/mL solution were suitably diluted with buffer to give final concentration. The peak amplitude of the obtained first-derivative spectra was measured at 229 nm and 346.5 nm for serratiopeptidase and metronidazole respectively.

RESULTS AND DISCUSSION

Employing First derivative method

Upon examining the first-derivative spectra of the two drugs. It can be noticed that serratiopeptidase is determined at 229nm where metronidazole has no contribution and metronidazole is determined at 346.5 nm where serratiopeptidase shows a zero crossing.
where C is the concentration in µg/mL, A is the peak amplitude of the first-derivative curves at 229 and 346.5 nm for serratiopeptidase and metronidazole respectively and r is the correlation coefficient.

The selectivity of the proposed procedure was examined by determining the recovery of the two drugs in laboratory-prepared mixtures containing different ratios of the two drugs and satisfactory results were obtained (Table-1).

### Table 1: Results of analysis of serratiopeptidase and metronidazole in the laboratory prepared mixtures

<table>
<thead>
<tr>
<th>Sample</th>
<th>Conc.(µg mL⁻¹)</th>
<th>Recovery* (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Serra</td>
<td>Metro</td>
</tr>
<tr>
<td>1</td>
<td>25</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>20</td>
<td>5</td>
</tr>
<tr>
<td>3</td>
<td>15</td>
<td>10</td>
</tr>
<tr>
<td>4</td>
<td>10</td>
<td>15</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>20</td>
</tr>
<tr>
<td>6</td>
<td>0</td>
<td>25</td>
</tr>
<tr>
<td>Mean</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Standard Deviation</td>
<td>0.134</td>
<td>0.198</td>
</tr>
</tbody>
</table>

*Each reading is an average of six replicates.

% of recovery = 100 X recorded amount / added amount.

The validity of the Method was further assessed by applying the standard addition technique.

### References