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# 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 from. 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. HPCL[1] and steric exclusion chromatography[2] have been reported for the estimation of Serratiopeptidase. Metronidazole is a 2-Methyl-5-nitroimidazole-1-ethanol used in anaerobic infection. Liquids chromatography[3] and HPLC[4] 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 metromidazole 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) Aliqouts 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.

A= - 0.0004C + 0.0003 r=0.9950 (1)
A= - 0.0013C + 0.0005 r=0.9981 (2)

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 erratiopeptidase and metronidazole in the laboratory prepared mixtures**

| Sample | Conc.(µg $mL^{-1}$) | | Recovery* (%) | |
|---|---|---|---|---|
| | Serra | Metro | Serra | Metro |
| 1 | 25 | 0 | 99.10 | - |
| 2 | 20 | 5 | 98.94 | 98-96 |
| 3 | 15 | 10 | 99.82 | 99.19 |
| 4 | 10 | 15 | 99.96 | 99.97 |
| 5 | 5 | 20 | 99.19 | 98.99 |
| 6 | 0 | 25 | - | 99.54 |
| Mean | - | - | 99.40 | 99.33 |
| Standard Devitation | - | - | 0.134 | 0.198 |

% of recovery = 100 X recorded amount / added amount.
*Each reading is an average of six replicates.
Serra : Serratiopeptidase, Metro : Metronidazole.
Calculatons of statistical analysis were carried out by SPSS (offical software, version 12.0 )

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

**Procedure for analysis of laboratory mixtures.**

Different aliquots of standard solutions were taken and analyzed by using the procedure described earlier. The peak amplitude of the first-derivative spectra was measured at 229 and 346.5 nm for serratiopeptidase and metronidazole, respectively. The amount of the two drugs were calculated from the computed regression equations (official software of statistical analysis SPSS, version 12.0) or from the suggested simultaneous equation.

**Recovery studies**

Accuracy of the method was checked by recovery studies, where in sample was spiked with known quantity of standard drug of Serratiopeptidase and metronidazole at different levels. The percentage recovery was found to be 99.14 ± 0.89 and 99.19 ± 0.54 by this method. Standard addition technique was applied for assessing the validity of the suggested methods.

**Conclusion**

The main advantage of the proposed method is its suitability for routine determination of Serratiopeptidase and metronidazole from their formulations. The proposed method is economic, simple, sensitive, precise and reproducible and do not require any expensive or sophisticated apparatus, in contrast with the reported chromatographic methods.

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