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Abstract
A novel, simple, sensitive and rapid spectrophotometric method has been developed for simultaneous estimation of Serratiopeptidase and Diclofenac sodium by absorption ration method (Q-Method). The method involved solving simultaneous equations based on measurement of absorbance at two wavelengths 255 nm and 276 nm, the isosbestic point and at λmax of Diclofenac sodium respectively. Beer’s law was obeyed in the concentration range 140-240 μg/ml and 14-24 μg/ml for Serratiopeptidase and Diclofenac sodium respectively. Results of the method were validated statistically and by recovery studies.

Keywords: UV spectrophotometric method, Serratiopeptidase, Diclofenac sodium, simultaneous determination.

1. Introduction
Serratiopeptidase

Serratiopeptidase was administered for the purpose of suppressing the eliminative post-operative bleeding and resultant formation of haematoma which are most serious problem in field of orthopedics. It was also administered for enhancing action of antibiotics.

Diclofenac Sodium
Structure:

Diclofenac Sodium, 2-(2-(2,6-dichlorophenylamino)phenyl)acetic acid is taken to reduce inflammation and as an analgesic reducing pain in conditions such as arthritis or acute injury. It can also be used to reduce menstrual pain, dysmenorrhea. Diclofenac works by inhibiting prostaglandin synthesis by inhibition of cyclooxygenase (COX), inhibiting DNA synthesis.

2. Materials and Methods
Instrumentation
For the present study LABINDIA® Double beam U.V./Visible Spectrophotometer (model 3000°) was used with Path length 10 mm,
equipped with spectra manager software (version: U.V. Win 5.2) A pair of 1-cm matched quartz cells were used to measure absorbance of solution. The samples were weighed on electronic analytical balance. (Shimadzu Model: AY 220)

2.1. Materials
Gift samples of Diclofenac sodium and Serratiopeptidase were provided by Kirti Pharma, Nasik, India.

2.2. Solvent
0.1 N NaOH, Ethanol

2.3. Preparation of stock solutions
Standard stock solutions of both Diclofenac sodium and Serratiopeptidase were prepared by dissolving 10 mg of DICLO and 100 mg of SERRA separately in 20ml of ethanol in 100ml volumetric flasks. Final volume was made up to 100ml with 0.1N NaOH to get working standard solution of each containing100μg/ml of DICLO and 1000ug/mL of SERRA.

2.4. Determination of Absorption Maxima
By appropriate dilution of standard stock solutions of DICLO and SERRA with 0.1N NaOH, solutions containing 20 μg/ml of DICLO and 200μg/ml of SERRA were scanned separately in the range of 200- 400 nm. Wavelength of maximum absorption was determined for both the Drugs. DICLO showed maximum absorbance at 276nm and isosbestic point selected at 255nm.

2.5. Method
2.5.1. Simultaneous Equation method (Q-Method)
From the stock solution, working standard solution of drugs was prepared by appropriate dilution and was scanned from 400nm to 200nm. Two wavelengths were selected for this method i.e. 255 nm and 276 nm that are isosbestic point and λmax of DICLO respectively in 0.1N NaOH. Series of dilution were prepared from standard solutions of SERRA and DICLO. The linearity was observed in the concentration range of 140 to 240 μg/ml and 14 to 24μg/ml. The concentrations of drugs in sample solution were determined by using the following formula,

Amount of each drug was calculated using following formulae,

\[
Q_m - Q_y = A_1
\]

\[
Q_x - Q_y = A_1
\]

Where,

\( C_x \) and \( C_y \): - Concentration of Serratiopeptidase and Diclofenac sodium (g/100ml) respectively.

\( Q_m \): - Absorbance ratio of mixture at 255 nm and 276nm.

\( Q_x \): Absorptivity ratio of Serratiopeptidase at 255nm and 276 nm

\( Q_y \): - Absorptivity ratio of Diclofenac sodium at 255nm and 276nm

By solving the two simultaneous equations the concentration of Serratiopeptidase and Diclofenac sodium in sample solution and laboratory mixture sample were obtained.

2.6. Analysis of Laboratory Mixture Formulation
For estimation of drug in laboratory mixture formulation was prepared, which contains 100mg of Serratiopeptidase and 10mg of Diclofenac Sodium and 100mg of Lactose.

For analysis of drugs serratiopeptidase equivalent to 100 mg of laboratory mixture formulation was taken and transferred to 100ml volumetric flask and 10 ml ethanol was added to dissolve the sample and 50 ml 0.1 N NaOH was added, filter the solution and volume was made up to 100ml with 0.1N NaOH that gives stock solution that contains 1000ppm of Serratiopeptidase and 100 ppm of Diclofenac sodium. Further dilutions were made from this stock solution to get required concentration. In this method concentration of
both Serratiopeptidase and Diclofenac sodium was determined by measuring absorbance of sample solution at 255nm and 276 nm. Results of laboratory mixture formulation are shown in Table No. 1.
The assay procedure was repeated six times (n=6).

2.7. Validation
The method was validated according to ICH guidelines to study linearity, accuracy and precision.

2.7.1. Linearity
The linearity of measurement was evaluated by analyzing different concentration of standard solution of Serratiopeptidase and Diclofenac sodium. For both the methods Beer’s law was obeyed in concentration range 140 to 240 µg/mL for Serratiopeptidase and 14-24 µg/mL for Diclofenac sodium. The correlation coefficient was found to be 0.9838 at 255 for Serratiopeptidase and 0.9954 at 276 for Diclofenac sodium.

2.7.2. Accuracy (Recovery studies)
To ascertain the accuracy of proposed methods recovery studies were carried out by standard addition method at three different levels (80% 100%, 120%) percent recovery was found in the range of 99 to 101% for Serratiopeptidase and 97 to 101% for Diclofenac sodium.

2.7.3. Precision
The reproducibility of the proposed methods was determined by performing laboratory mixture formulation assay at different intervals on same day (intra day precision) and on three different days (inter day precision)

Results and Discussion
The methods discussed in the present work provide a convenient and accurate way for simultaneous analysis of Serratiopeptidase and Diclofenac sodium. In simultaneous equation method, wavelengths selected for analysis were 255nm, isosbestic point and 274nm for Diclofenac sodium.

In this method linearity were observed in the concentration range of140-240µg/mL for both Serratiopeptidase and 14-24 µg/mL for Diclofenac sodium. In this method concentration of the individual drug present in the Laboratory mixture formulation was determined by solving the simultaneous equation at 255nm and 274 nm. The absorptivities of the two drugs were used for the calculations.

Assay values for Serratiopeptidase and Diclofenac sodium for laboratory mixture formulation analysis, by the method, were found in the range of 99 to 101% .S.D. and R.S.D. for six determinations of laboratory mixture formulation sample by the simultaneous method was found to be less than ± 2.0indicating the precision of the method. Accuracy of proposed method was ascertained by recovery studies and the results are expressed as % recovery. Percent recovery for Serratiopeptidase and Diclofenac sodium, by the simultaneous method, was found in the range of 97.4 to 101.54%. The results of validation parameters shown in table no.2 are satisfactory, indicates the accuracy of proposed methods for estimation of Serratiopeptidase and Diclofenac sodium. This method can be employed for routine analysis of the two drugs in combined laboratory mixture formulation as well as tablet dosage form.

Conclusion
Simultaneous method was developed and validated for the simultaneous determination of Serratiopeptidase and Diclofenac Sodium in laboratory mixture formulation. The proposed UV-Spectrophotometric method was found to be simple and economic. Simultaneous method was found to be accurate, precise and reproducible.

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References
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**Fig. 1.** Overlay spectra of Serratiopeptidase and Diclofenac sodium (140 - 240 ug/ml and 14-24 ug/ml).

**Table 1.** Result of laboratory mixture formulation analysis.

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Sample</th>
<th>Label Claim</th>
<th>% Label Claim (Mean ± S.D.)</th>
<th>% R.S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Serratiopeptidase</td>
<td>200ug/mL</td>
<td>199.72±0.2481</td>
<td>0.1242</td>
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<tr>
<td>2</td>
<td>Diclofenac sodium</td>
<td>20ug/mL</td>
<td>19.81±0.0463</td>
<td>0.2397</td>
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</table>

* Mean of six estimations. SERRA= Serratiopeptidase, DICLO: Diclofenac sodium.
Table 2. Result of Recovery Studies.

<table>
<thead>
<tr>
<th>Level of % Recovery</th>
<th>Drug</th>
<th>Conc. of drug in µg/ml</th>
<th>Methods*</th>
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<tr>
<td></td>
<td>Drug Taken</td>
<td>Std. Drug added</td>
<td>Recovery</td>
</tr>
<tr>
<td>80</td>
<td>Diclofenac sodium</td>
<td>10</td>
<td>8</td>
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<tr>
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<td>10</td>
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<td>120</td>
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<td>99.39</td>
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*Average of three determinations.

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