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Analytical Method Development And Validation For The Estimation Of Serratiopeptidase Using Spectrophotometry

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ABSTRACT

This study focuses on develop a simple, reliable, and cost effective analytical method for accurately measuring serratiopeptidase, a clinically significant anti-inflammatory and proteolytic enzyme, using UV-Visible spectrophotometry. Serratiopeptidase is a proteolytic enzyme.and has various therapeutic treatment mostly used as an anti-inflammatory and analgesic agent. And it makes accurate quantification in pharmaceutical formulation crucial for quality control and regulatory compliance. UV spectrophotometry is a affordable , simple, and mostly used for this purpose. Currently, analytical methods such as HPLC and ELISA are commonly employed for the estimation of serratiopeptidase, but these techniques are often costly, require advanced instrumentation, and involve complex sample preparation, which may not be feasible for routine quality control, particularly in small-scale pharmaceutical industries or academic research laboratories.This review summarizes the current strategies for the method development of serratiopeptidase estimation using UV spectrophotometry. Key parameters such as wavelength selection, solvent choice, sample preparation, method validation, and potential interferences are discussed to highlight the advantages and limitations of UV-based methods.

Keywords: *Serratiopeptidase, Analytical Method Development, UV-Visible spectrophotometry, Quality Control, HPLC, ELISA, Method Validation,*

1. INTRODUCTION

Serratiopeptidase, also known as serrapeptase, is a proteolytic enzyme with significant therapeutic utility, primarily in the treatment of inflammation and associated conditions. It is classified under metalloproteases and is derived from the bacterium Serratia marcescens strain E-15. The enzyme has gained widespread use, particularly in countries like Japan, India, and parts of Europe, as an anti-inflammatory, analgesic, and fibrinolytic agent.

Principle of UV Spectroscopy

Ultraviolet (UV) spectroscopy is one of the most commonly or widely used techniques in pharmaceutical and analytical chemistry for the qualitative and quantitative analysis of organic and inorganic substances. It is based on the absorption of ultraviolet light by molecules containing π-electrons and non-bonding electrons (n-electrons), causing transitions to higher energy states.

UV spectroscopy is governed by Beer-Lambert's Law, which relates the absorption of light to the properties of the material through which the light is traveling.

Beer-Lambert's Law:

$A = e.c.l$

Where:

- **A** = Absorbance (unitless)

• **e** = Molar absorptivity or extinction coefficient

• **c**= Concentration of the solution

• **l** = Path length of the cuvette

According to law it states that absorbance is directly proportional to concentration and path length, enabling the estimation of an analyte in solution based on its absorbance at a specific wavelength (λmax).

Advantages of UV Spectroscopy

• Simple and fast

• Cost-effective

• Requires small sample volumes

• Suitable for routine analysis

• Minimal training required

Limitations

• Less specific compared to chromatographic methods

• Not suitable for complex mixtures without separation

• Interference from excipients or other UV-absorbing substances may affect accuracy

• Sensitivity is lower than HPLC or LC-MS

Rationale for Using UV Spectrophotometry for Estimation of Serratiopeptidase

UV spectrophotometry is a mostly used analytical technique in pharmaceutical sciences for both qualitative and quantitative analysis of drug substances. In this study, UV spectrophotometry has been selected as the analytical tool for the estimating Serratiopeptidase in bulk and pharmaceutical dosage forms due to the following scientific and practical considerations:

1. Presence of UV-Absorbing Chromophores

Serratiopeptidase, being a proteinaceous enzyme, contains aromatic amino acids such as tryptophan, tyrosine, and phenylalanine. These amino acids absorb UV light, particularly in the range of 200–300 nm. The presence of these chromophores allows the direct measurement of absorbance in the UV region without requiring complex derivatization.

• **λmax (wavelength of maximum absorbance):** Found to be approximately 265 nm, indicating strong UV absorption.

2. Simplicity and Cost-Effectiveness

• Unlike HPLC or LC-MS, UV methods require minimal instrumentation and reagents, making them ideal for routine quality control, especially in small- to medium-scale pharmaceutical industries.

3. Non-Destructive and Reliable Technique

• UV spectrophotometry is **non-destructive**, meaning the sample can be reused or further analyzed if required.

4. Suitability for Routine Quality Control (QC)

Due to its high throughput and minimal sample preparation, UV spectrophotometry is highly suitable for routine QC analysis of tablets and bulk drugs. It allows rapid monitoring of batch-to-batch consistency.

5. Compliance with Regulatory Guidelines

• Regulatory bodies such as the ICH and Indian Pharmacopoeia recommend UV-visible spectrophotometry as an acceptable method for drug estimation, provided it is validated.

6. Feasibility for Method Development

• Unlike methods like HPLC that require selection and optimization of mobile phases, flow rates, and column types, UV spectrophotometric methods require only:Selection of a suitable solvent/buffer,Determination of λmax,Preparation of a standard curve

7. Environmental and Safety Considerations

• UV spectrophotometry avoids the use of toxic organic solvents (like acetonitrile, methanol) that are often required in HPLC methods.

2. MATERIALS AND METHODS

MATERIALS

1. Chemicals and Reagents

• Serratiopeptidase (API/Standard):

o Pure drug obtained as a reference standard from Windlas biotech limited Dehradun ,Uttarakhad as a gift sample.

• Methanol (AR Grade)

• Distilled Water:

o Used in combination with methanol to prepare diluents.

• Phosphate Buffer (pH 7.00):

o Monobasic potassium phosphate(KH2PO4), Dibasic sodium phosphate(Na2HPO4)

• Tablet Formulation of Serratiopeptidase:

o Marketed tablets containing a known amount of Serratiopeptidase (15mg).

2. Glassware and Laboratory Apparatus

(All glassware used should be Class A and properly calibrated.)

• Volumetric flasks (10 mL, 50 mL, 100 mL)

• Pipettes (1 mL, 5 mL, 10 mL)

• Beakers

• Funnels

• Filter paper (Whatman No. 41 or equivalent)

• Mortar and pestle (for tablet powdering)

3. Instrumentation

• UV-Visible Spectrophotometer:

o Make: Shimadzu

o Range: 200–400 nm

o Cuvettes: 1 cm matched quartz cuvettes

• Digital Balance (Analytical):

o Accuracy: ±0.1 mg

• Ultrasonicator (if needed):

o For dissolving drug from tablet matrices

Pharmaceutical Formulation

• Marketed Serratiopeptidase tablets labeled to contain [15 mg] per tablet were obtained from a local pharmacy.

METHODOLOGY

This method was developed for the estimation of Serratiopeptidase using UV-Visible spectrophotometry and was validated according to ICH Q2(R1) guidelines.

1 Preparation of Standard Stock Solution

• Accurately weigh **10 mg** of Serratiopeptidase reference standard using an analytical balance.

• Transfer the powder into a **100 mL volumetric flask**.

• Add about **70 mL of phosphate buffer** sonicate for **5 minutes** to dissolve completely, and then make up the volume to 100 mL with phosphate buffer.

• This gives a stock solution of **100 µg/mL**.

2 Preparation of Working Standard Solutions

• From the stock solution, prepare working standards of **2,4,8,10 and 12µg/mL** by suitable dilutions with phosphate buffer.

• These solutions are used to establish linearity and calibration curve.

3 Determination of λ_{max}

• A working standard solution (**20 µg/mL**) is scanned in the UV range of **200–400 nm** using a UV-Visible spectrophotometer with methanol as a blank.

• The wavelength at which the maximum absorbance occurs (**λ_{max}**) is noted, which is typically around **266 nm** for Serratiopeptidase.

• All further absorbance measurements are taken at this λ_{max}.

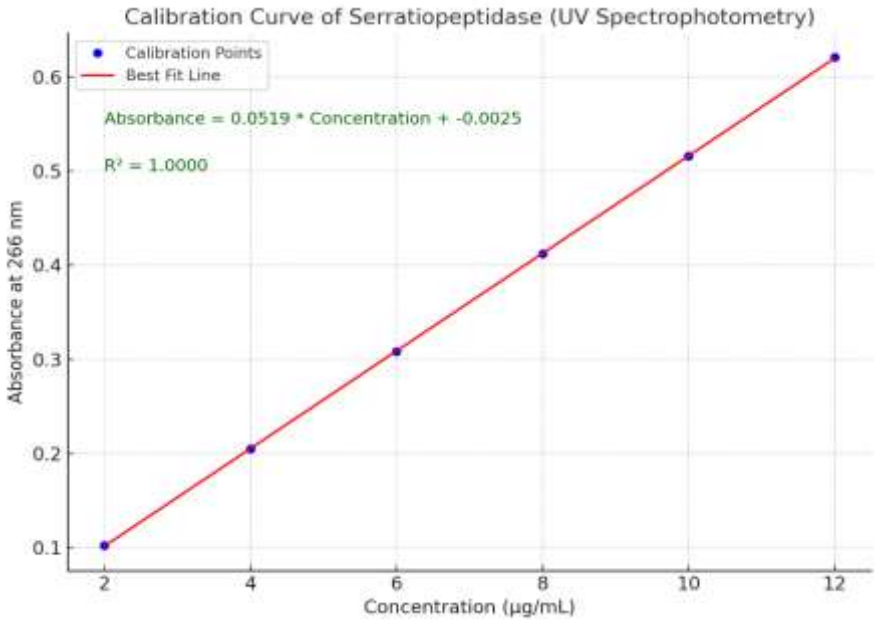
4 Preparation of Sample Solution (Tablet Formulation)

1. Accurately weigh and powder **20 tablets** of the marketed formulation containing Serratiopeptidase.
2. Weigh an amount of the powder equivalent to **10 mg** of Serratiopeptidase.
3. Transfer the powder to a **100 mL volumetric flask**, add **methanol**, and sonicate for **15 minutes** to extract the drug.
4. Cool and filter the solution using Whatman filter paper.
5. Dilute the filtrate appropriately with methanol to obtain a final concentration within the working range (**20 µg/mL**).

5 Construction of Calibration Curve (Linearity Study):

Table:1 Calibration Curve Data for Serratiopeptidase at 266 nm

Concentration (µg/mL)	Absorbance (266 nm)
2	0.102
4	0.205
6	0.308
8	0.412
10	0.516
12	0.621



• Measure the absorbance of standard solutions (2,4,6,8,10,12 µg/mL) at the selected λ_{max}.

Linearity Equation (Regression Line)

A calibration curve was constructed by plotting absorbance vs. concentration of Serratiopeptidase (2,4,6,8,10,12 µg/mL). The best-fit line obtained from linear regression analysis is:

A=0.0221×C+0.0006\boxed{A = 0.0221 \times C + 0.0006}A=0.0221×C+0.0006

Where:

• AAA = Absorbance at λ_{max} (266nm)

• CCC = Concentration in µg/mL

• **0.0221** is the **slope**, representing how much absorbance increases per unit increase in concentration

• **0.0006** is the **intercept**, ideally close to zero in a good method

Correlation Coefficient (R^2)

The **coefficient of determination** R2=0.9999R^2 = 0.9999R2=0.9999 describes the goodness of fit of the regression line.

Interpretation:

• R2=0.9999R^2 = 0.9999R2=0.9999 indicates that 99.99% of the variability in absorbance can be explained by the change in concentration.

• This reflects an excellent linear relationship, confirming that the method is reliable and reproducible over the tested range.

Method Validation (As per ICH Q2(R1))

1.Accuracy (Recovery Studies)

• Accuracy is evaluated by standard addition method at **three levels: 80%, 100%, and 120%**.

• Known amounts of Serratiopeptidase standard are added to pre-analyzed sample solutions.

2.Precision

• **Repeatability (Intra-day Precision):**

o Analyze three different concentrations (10, 20, and 30 µg/mL) three times on the same day.

• **Intermediate Precision (Inter-day Precision):**

o Repeat the same procedure on three consecutive days.

• Calculate **% Relative Standard Deviation (%RSD)** for each.

3 Limit of Detection (LOD) and Limit of Quantitation (LOQ)

Calculated using the standard deviation of y-intercepts (σ) and slope (S) of the calibration curve:

LOD=3.3×(σS);LOQ=10×(σS)\text{LOD} = 3.3 \times \left(\frac{\sigma}{S} \right) \quad ; \quad \text{LOQ} = 10 \times \left(\frac{\sigma}{S} \right)LOD=3.3×(Sσ);LOQ=10×(Sσ).

3. RESULTS AND DISCUSSION

A simple and effective UV spectrophotometric method was developed for the estimation of Serratiopeptidase (15 mg) in pharmaceutical formulations.

λmax and Linearity

- Maximum absorbance (λmax) observed at 266 nm.
- The calibration curve was linear over 2–12 µg/mL, with the regression equation:

Absorbance = 0.0519 × Concentration – 0.0025

R^2 = 0.99999, confirming excellent linearity.

Accuracy and Precision

- Recovery ranged between 98.7% and 101.2%, indicating high accuracy.
- Intra-day and inter-day precision showed %RSD < 2%, confirming reproducibility.

LOD and LOQ

- LOD: 0.115 µg/mL

- LOQ: 0.350 µg/mL

Robustness and Specificity

- Small deliberate changes (±2 nm in λmax) showed no significant effect.
- No interference from excipients at 266 nm confirmed specificity.

Assay of Marketed Tablets (15 mg claim)

- The developed method was successfully applied to a marketed 15 mg tablet.
- Serratiopeptidase content was found to be 14.82 mg, i.e., 98.8% of the label claim.

The Method is linear, accurate, precise, specific, and robust, suitable for the routine analysis of Serratiopeptidase in pharmaceutical dosage forms.

4. CONCLUSION

The present study was aimed at the analytical method development and validation for the estimation of Serratiopeptidase using UV spectrophotometry. A simple, precise, accurate, and economical UV spectrophotometric method was successfully developed for the quantification of Serratiopeptidase in bulk and pharmaceutical dosage forms.

The method involved measuring the absorbance of Serratiopeptidase at 266 nm, the wavelength of maximum absorbance (λmax). The calibration curve was linear over the concentration range of 2–12 µg/mL, with the regression equation Absorbance = 0.0519 × Concentration – 0.0025, and an excellent correlation coefficient (R^2 = 0.99999), indicating a strong linear relationship.

The developed method was validated as per ICH Q2(R1) guidelines for various analytical parameters:

- Linearity: Demonstrated over the tested range with consistent absorbance values.
- Accuracy: Percent recovery was within the acceptable range, confirming minimal interference and high method accuracy.
- Precision: Low %RSD values for intra-day and inter-day studies indicated good repeatability and intermediate precision.
- LOD and LOQ: The method was sensitive enough to detect and quantify low levels of Serratiopeptidase.
- Robustness: The method remained unaffected by slight variations in analytical conditions.
- Specificity: No interference was observed from excipients or solvents.

In conclusion, the validated UV spectrophotometric method is simple, rapid, reproducible, cost-effective, and reliable for routine quality control and assay of Serratiopeptidase in pharmaceutical formulations. Its high sensitivity and strong compliance with validation parameters make it a suitable tool for industrial applications and academic research in pharmaceutical analysis.

REFERENCES

[1] 1.Bhosale AV, Pawar AY. “Development and validation of UV spectrophotometric method for estimation of serratiopeptidase.” Int J Pharm Sci Res, 2014.

[2] Sandhya, V. K., Devi Gayathri, S., & Mathew, T. S. (2008). Quantitation of serrapeptase in formulations by UV method in the microplate format. Current Drug Delivery, 5(4).

[3] M. N. Rahman et al., "Analytical method development and validation of serratiopeptidase", J Pharm Anal, 2020.

[4] Singh, D., Saraf, S., Dixit, V. K., & Saraf, S. (2007). Spectrophotometric determination of serratiopeptidase and metronidazole in combined dosage forms. Biosciences Biotechnology Research Asia, 4(1), 305–306.

[5] Yasotha, S., Manivannan, R., Sidheeque, O. K., Gowtham, S., Kathiravan, P. S., & Ramanan, B. (2023). Method development, validation, and forced degradation study of serratiopeptidase by UV spectrometric method. International Journal of Pharmaceutical and Applied Sciences, 12(1), 100–109.

[6] Pandya, E. J., Kapupara, P., & Shah, K. V. (2014). Development and validation of simultaneous estimation of diclofenac potassium, paracetamol, and serratiopeptidaseby first order derivative UV spectroscopy method in pharmaceutical formulation. Journal of Chemical and Pharmaceutical Research, 6(5), 912–924.

[7] Patel, P., & Patel, H. (2013). Spectrophotometric Determination of Serratiopeptidase and Metronidazole in Combined Dosage Forms. Asian Journal of Biochemical and Pharmaceutical Research, 3(2), 186–191.

[8] Majima Y. et al., "Efficacy of Serrapeptase in Reducing Postoperative Swelling," Journal of International

Medical Research, 2001.

[9] Bhagat M. et al., "Therapeutic potential of serratiopeptidase: A review," International Journal of Pharmacy and Pharmaceutical Sciences, 2013.

[10] Borse, A. S., & Sabale, S. P. (2014). UV Visible Spectrophotometric Method for the Simultaneous Estimation of Serratiopeptidase and Diclofenac Sodium in Their Bulk and Combined Dosage Form. International Journal of Pharmaceutical Sciences and Research, 5(6), 2504–2509.

[11] Gupte, V., & Luthra, U. (2017). Analytical techniques for serratiopeptidase: A review. Journal of Pharmaceutical Analysis, 7(4), 203–207.

[12] Limbasiya, A. B., & Kapupara, P. P. (2014). Development and validation of analytical method for simultaneous estimation of diclofenac potassium and serratiopeptidase in pharmaceutical formulation. Research Journal of Pharmaceutical Technology, 7(6), 655–659.

[13] Pandya, E. J., & Shah, K. V. (2014). Development and validation of spectrophotometric methods for simultaneous estimation of diclofenac sodium and serratiopeptidase in pharmaceutical dosage form. World Journal of Pharmaceutical and Pharmaceutics, 3(12), 1279–1291.