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Method development, validation and forced degradation study of serratiopeptidase by uv spectrometric method

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ABSTRACT

The pharmaceutical analysis is a branch of chemistry, which involves the series of process for the identification, determination, quantitation, and purification. The sample solution of 10 µg/mL of std stock solution of serratiopeptidase in distilled water prepared and the solution was scanned in UV region in the wavelength range from 200-400 nm by using distilled water as a solvent. The overlay spectra of serratiopeptidase was recorded. From the spectra, serrattiopeptidase shows maximum absorbance at 263 nm. Percentage of RSD for intraday and inter day precision studies for the drug was well within the acceptable range (<2%) indicating that the method have excellent repeatability and reproducibility. The percentage relative standard deviation for precision and accuracy was found to be low, which indicates that the method have considerable accuracy and precision. Percent recovery for serrattiopeptidase was found in the range of 99.87% to 100.71% with standard deviation well below two indicating accuracy of the method. Recovery greater than 98% with low standard deviation justifies the accuracy of the method. Intraday and inter day precision studies were carried out by analyzing tablet formulation by this method. The system suitability was found to meet the pre-established criteria at all the conditions and the degradation shows in acid (1.39%), alkaline (1.88%) peroxide (2.88%), thermal (1.14%), water (0.15%).

Keywords: Method development, Validation, serratiopeptidase, Analysis, UV spectrometric.

INTRODUCTION

The purpose of pharmaceutical analysis is to identify substances, purify them, separate them, quantify them, determine the molecular structures of chemical compounds that make up pharmaceuticals, and determine how these compounds are combined to make up a pharmaceutical product. Method validation is a critical activity in the pharmaceutical industry. Validation data are used to confirm that the analytical procedure employed for a specific test is suitable for its intended purposes. These results demonstrate the performance, consistency, and reliability of the analytical method. Analytical method development and validation can be understood as the process of showing that analytical procedures are adequate for the purpose of assessing drugs, and particularly the active pharmaceutical ingredient (API).[6] Pharmaceutical analysis is traditionally defined as analytical chemistry dealing with drugs both as bulk drug substances and as pharmaceutical products (formulations). However, in academia, as well as in the pharmaceutical industry, other branches of analytical chemistry are also involved, viz. bioanalytical chemistry, drug metabolism studies, and analytical biotechnology. The development of drugs in the pharmaceutical industry is a long-term process, often taking more than a decade from the start of the research project to appearance of a drug on the market. That process involves several decision points, such as the choice of the candidate drug after the discovery phase, the application to the

authorities before testing the compound for the first time in humans, and finally the new drug application for marketing, which summarizes the data obtained from all the studies needed for approval of the drug as a medicine. In all these steps the amount of data generated is enormous. Analytical chemists are involved in many of the studies that constitute this documentation. Substance quality and its specifications are based on substance analysis, and that knowledge is later used for quality control (QC) of the substance during full-scale production. Product analysis involves dealing with the various formulations used for toxicological studies, clinical studies, and marketing. The results from such work lead to specifications that form the basis for the QC of the product. For both substances and formulations there is an increasing interest in the introduction of process analytical chemistry.

Biomolecules, i.e., macromolecules such as proteins or hormones, either produced by isolation from biological sources or by means of biotechnology, must also be subjected to careful analytical control. Thus, while the analytical tasks required for biomolecules are somewhat different from those of ordinary pharmaceuticals, when it comes to regulation and documentation of their quality and properties they definitely belong to the same group.

There are a number of regulations that have to be followed in the development of pharmaceuticals as well as in their production. Regulatory approval is required prior to each clinical trial and before marketing is licensed.

An important part of the development process is safety evaluation, primarily the toxicology tests, which run from 1 to 24 months in different species. During this time bioanalytical studies are performed as well as control of the formulations used in the tests.

After approval for marketing the authorities exercise control of products on the market and require postproduction stability data. Public interest in the quality of drugs is also reflected in the compilation of substance monographs in compendia that are known as pharmacopoeias.

In addition to collections of substance monographs, these pharmacopoeias contain general analytical methods and some also contain monographic requirements on the formulation of the substances.

This article provides an overview of mainly substance and product analysis (traditional pharmaceutical analysis), as used in the pharmaceutical industry. The support of other branches of analytical chemistry will be mentioned.[3]

Pharmaceutical analysis plays a major role today, and it can be considered as an interdisciplinary subject. Pharmaceutical analysis derives its principles from various branches of science like chemistry, physics, microbiology, nuclear science, electronics, etc. pharmaceutical analytical techniques are applied mainly in two areas, viz Quantitative Analysis and Qualitative Analysis, although there are several other applications.

The determination of the multi- component sample can be done by using the following methods

i. Simultaneous equations method.
ii. Absorbance ratio method (Q-Analysis).
iii. Geometric correction method
iv. Orthogonal polynomial method
v. Difference spectrophotometry
vi. Derivative spectrophotometry
vii. Chemical derivation[1]

Forced degradation

Pharmaceutical analysis is branch of practical chemistry that involves a series of process for identification, determination, qualification and separation of components of any solution or type of mixtures or it can determine structural chemical compounds. The substances may be a single compound or a mixture of compounds and it may be in any of dosa forms. For example, substances that are used in pharmaceuticals are animal, plants, microorganism various synthetic products.[7] The sample to be analyzed is called 'analyze and it based on size of sample i.e., as macro, semi micro, sub micro, ultra-micro, trace analysis. Forced degradation studies is a type of mechanism which is utilized in pharmaceutical development so to create or perform stability studies of different types of drug substances and drug products. This me can express various forced degradation pathways which can be help to learn stability studies of various substances and drug products.

Forced degradation studies are carried out to bring off following purposes:

1. To create degradation pathways of drug substances and drug products,
2. To elucidate structure of degradation products.
3. To establish stability of drug substances in various formulations.
4. To affirm degradation mechanism such as hydrolysis, oxidation, photolytic, thermolytic of substances and drug products.
5. To solve stability related problems.
6. To understand chemical properties of developed method.
7. To produce more stable formulation.
8. To improve packaging system, storage conditions and shelf life.

The FDA and ICH guidance's state the requirement ofstability testing data to understand how a drug substance and drug product changes with time under the influence various environmental factors.

Degradation

Hydrolysis

Over a wide range of pH most common degradation, chemical reactions are Hydrolysis The decomposition of a chemical or drugs or drug substances by reaction with water is called Hydrolysis. There are two types of degradation pathways of hydrolysis such as, in acidic and basic hydrolysis the catalysis of ionizable functional groups present in the molecule occurs. Forced degradation of a drug substance occurs when the drug interacts with acid and base. It produces primary degradants in the desirable range. Depending on the stability of the drug substance the class and concentrations of acid or base taken should be decided. For acid hydrolysis hydrochloric acid or sulphuric acid (0.1-1 M) considered to be most suitable whereas sodium hydroxide or potassium hydroxides (0.1-1M) for base hydrolysis are suggested. [14]

Oxidation

For oxidative forced degradation, hydrogen peroxide is broadly used. Apart from this as metal ions, oxygen. Drug structure provide guidance to select concentration and different condition of oxidizing agents. An electron transfer

mechanism occurs in the degradation of drug substance. [14]

Photolytic

The light exposure does not affect the drug substance for this purpose photo stability is conducted. Photo stability studies are performed to produce primary degradants of drug substance by exposure to UV or fluorescent conditions: In ICH guidelines some recommended conditions for photo stability testing are described. Samples of drug substance and solid/liquid drug product should be exposed to a minimum of 1.2million I x h and 200 W / m2 light, 300-800 nm is the most commonly accepted wavelength of light to cause the photolytic degradation, million's h is the maximum illumination recommended. Photo oxidation can be caused by light stress conditions by the free radical mechanism. [15]

Thermal

Thermal degradation (e g, dry heat and wet heat) should be carried out at more strenuous conditions than recommended ICH QIA accelerated testing conditions. Samples of solid-state drug substances and drug products should be exposed to dry and wet heat. Liquid drug products should be exposed to dry heat. For a shorter period of studies may be conducted at higher temperatures. Through the Arrhenius equation the effect of temperature on thermal degradation of a substance can be studied. [14,16]

Table 1: Protocol for forced degradation pathways

DEGRADATION	EXPERIMENTAL	STORAGE	SAMPLING
HYDROLYSIS	0.1M HCl	40°C, 60°C	1,3,5
OXIDATION	Hydrogen peroxide	25°C, 60°C	1,3,5
PHOTOLYTIC	Exposure to UV or	NA	1,3,5
THERMAL	Heat chamber	40°C, 100°C	1,3,5

MATERIALS AND METHODS

Materials

Drug

Serratiopeptidase was obtained as a gifted sample from Spectrum lab, Hyderabad.

Instruments

- UV 1700 Pharma Spectrophotometer (SHIMADZU)
- Digital balance

Reagents used

- Methanol
- Distilled water

Methods

Selection of solvent

Solubility of the drug was checked in solvents like distilled water, ethanol, methanol, chloroform, acetone, acetyl nitrate.

Preparation of stock solution

Weigh accurately about 10 mg of sample transfer in to a 100 mL volumetric flask. Add 30 mL of diluent and sonicate to dissolve. Dilute up to mark with diluent. Transfer 1 mL of this solution in to100 mL volumetric flask and dilute up to mark with diluent and mix. Filter through 0.45 µm nylon membrane filter.

Selection of wavelength

10 µg/mL Serratiopeptidase was prepared and ƛ max of the drug was scanned in the range of 200 – 400 nm to determine the maximum wavelength of the drug. Wavelength was selected, 263 nm for serratiopeptidase.

Analysis of tablet formulation

Ten tablets were weighed and average weight was calculated. The tablets were crushed to obtain fine powder. The powder equivalent to 7.8 mg was transferred to 100 mL volumetric flask, distilled water added to dissolve and volumes was made upto mark with distilled water.The solution was then filtered through a whatmann's filter paper. Further dilute the final solution was 10 µg/mL of Serratiopeptidase. The concentration of Serratiopeptidase was determined by measuring the absorbance of sample at 263 nm as single point UV method.

Method validation

Linearity and range

Linearity of serratiopeptidase was determined at 6 levels with concentrations of 2.5-12.5 µg/mL of serratiopeptidase prepared from stock solution. The absorbances of the solution was measured at 263 nm. The calibration curves of absorbance vs concentration were plotted for drug. Regression analysis was performed using least square method to generate the equations for the calibration line. Linearity was established from the correlation coefficient obtained for each calibration line.

Accuracy

The accuracy of the proposed method was assessed by recovery studies which were carried out at three different levels i.e., 50%, 100% and 150% [Pradeep et al, 2015]. A known amount of standard drug solution was added to the pre-analyzed sample solution at three different levels, absorbance was recorded. The % recovery was then calculated.

Precision

Intra-day precision

Standard stock solutions were taken in a 10 mL volumetric flasks and final volume was made up to the mark with buffer. The absorbances of these solutions were individually

measured thrice within a day and recorded.

Inter-day precision

Standard stock solutions were taken in 10 mL volumetric flasks and volume were madeup to the mark with buffer. The absorbances of these solutions were individually measured thrice in three days and recorded.

Limit of detection (lod) and limit of quantitation (loq)

The limit of detection (LOD) and limit of quantitation (LOQ) of serratiopeptidase for single point method were determined by using standard deviation of the response and slope approach as defined in ICH guidelines.

LOD=3.3xσ/S

LOQ=10xσ/S

Robustness and ruggedness

Prepare two test solutions of the same lot of Serratiopeptidase in tablet as per analytical method. Measure the absorbance of this solution along with diluent blank solution and system suitability solution.

Degradation study

Acid Degradation Studies

To 1 mL of stock solution Serratiopeptidase, 1 mL of 2N Hydrochloric acid was added and refluxed for 30mins at 60º c. The resultant solution was diluted to obtain solution and solutions were adsorbed into the system and the chromatograms were recorded.

Alkali Degradation Studies

To 1 mL of stock solution Serratiopeptidase, 1 mL of 2N sodium hydroxide was addedand refluxed for 30mins at 60 ºc. The resultant solution was diluted to obtain 10µg/ml solution and were adsorbed into the system and the chromatograms were recorded to assess the stability of sample.

Oxidation

To 1 ml of stock solution of Serratiopeptidase, 1 ml of 20% hydrogen peroxide (H_2O_2) was added separately. The solutions were kept for 30 min at 60^0c. For UV-VIS study, the resultant solution was diluted to obtain 10µg/ml solution and was adsorbed into the system and the chromatograms were recorded to assess the stability of sample.

Dry Heat Degradation Studies

The standard drug solution was placed in oven at 105°C for 1 h to study dry heat degradation. For UV-VIS study, the resultant solution was diluted to 10 µg/ml solution and were adsorbed into the system and the chromatograms were recorded to assess the stability of the sample.

Photo Stability studies

The photochemical stability of the drug was also studied by exposing the Standard Stock Solution to UV Light by keeping the beaker in UV Chamber for 1days or 200 Watt hours/m^2 in photo stability chamber. For UV-VIS study, the resultant solution was diluted to obtain 10 µg/mL solutions and were adsorbed into the system and the chromatograms were recorded to assess the stability of sample.

Neutral Degradation Studies

Stress testing under neutral conditions was studied by refluxing the drug in water for 1hrs at a temperature of 60º. For UV-VIS study, the resultant solution was diluted to 10 µg/mL solution and were adsorbed into the system and the chromatograms were recorded.

RESULTS AND DISCUSSION

Selction of solvent

Serratiopeptidase showed maximum solubility in distilled water hence it was selected as the solvent (diluents) for further studies.

Selection of wavelength

Standard solution of 10 µg/mL of serratiopeptidase exhibited maximum absorbance at 263 nm showed in fig.no:1

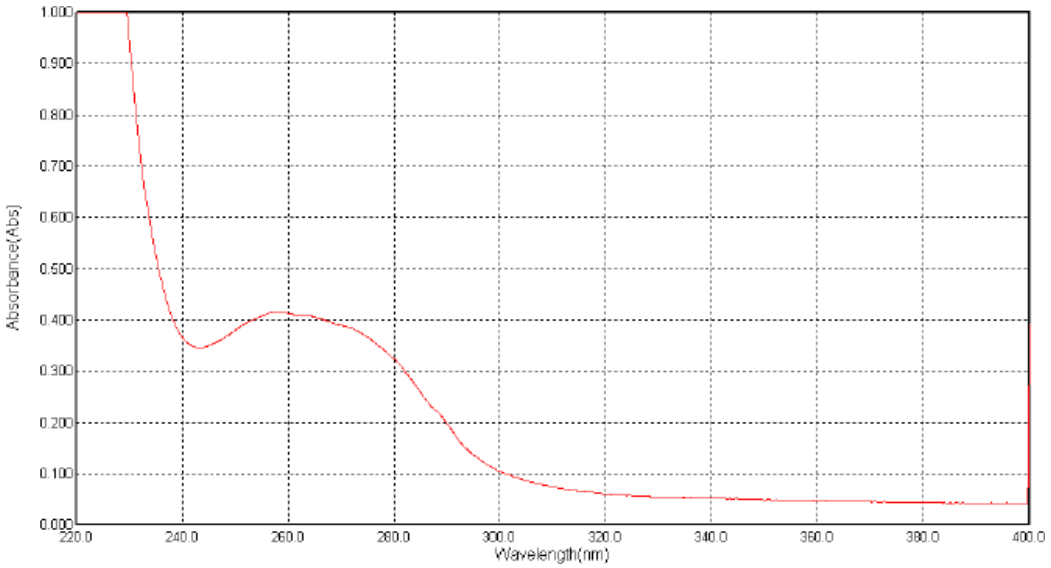

Fig 1: UV spectrum of serratiopeptidase at 263nm

Linearity and range

The linearity data for serratiopeptidase is given in table; the linearity plots of serratiopeptidase at 263 nm is shown in below figure 2. The regression equation and correlation coefficient for the calibration cure of serratiopeptidase at 263 nm were found linear in the concentration range of 2.5 - 15 µg/mL at wavelength. The calibration curve was established by plotting the absorbance vs concentration. Linear concentration were found and described by the regression equations.

For nanometer 263 nm: $y = 0.0402x + 0.003$, $r^2 = 0.999$

Where y is the absorbance of serratiopeptidase and x is the concentration in µg/mL, serratiopeptidase, r^2 is the correlation coefficient. The beers law is obeyed in the concentration range of 2.5-15 µg/mL also calibration curve was reputed by plotting the absorbance vs concentration. Linear concentration was found and described by the regression equation.

Table 2: Results of linearity of standard

S.NO	CONCENTRATION	ABSORBANCE
1.	2.5	0.103
2.	5	0.203
3.	7.5	0.305
4.	10	0.405
5.	12.5	0.506
6.	15	0.603

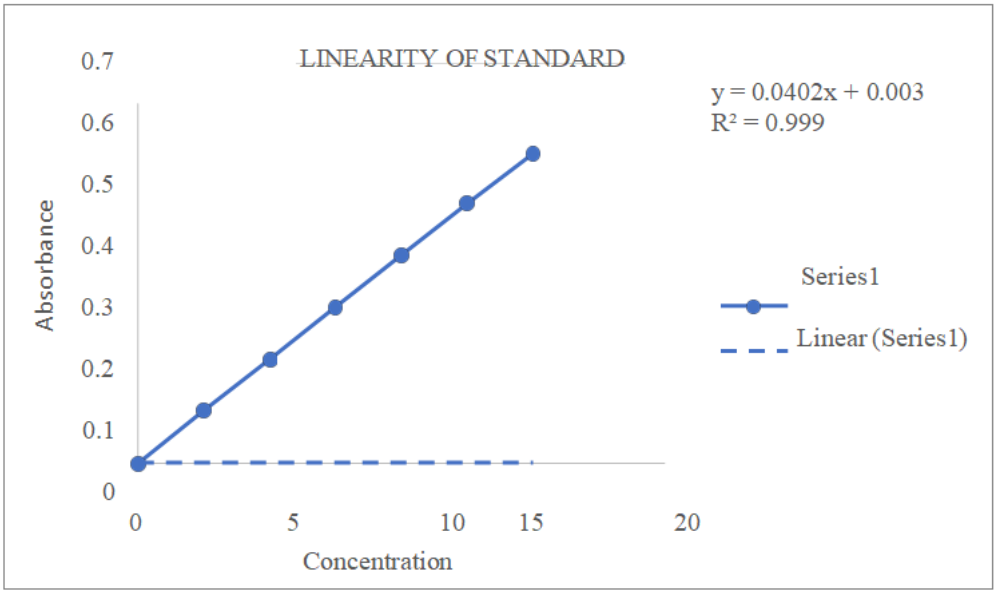

Fig 2: Linearity graph of serratiopeptidase standard

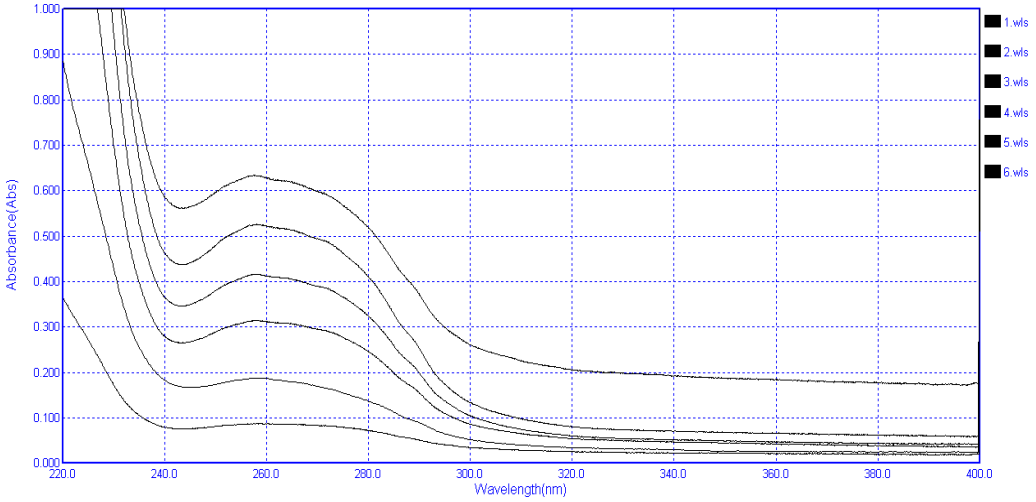

Fig 3: Linearity overlay spectrum

Accuracy

The % recoveries for serratiopeptidase in single point UV method absorption ratio methods are tabulated. The spiked drugs were recovered at concentration range indicating that the developedmethod was accurate.

Table 3: Recovery data of serratiopeptidase

CONCENTRATION µg/mL	SINGLE POINT METHOD		
	AMOUNT	%RECOVERY	%RSD
5 µg/mL	5.10	99.40	
10 µg/mL	10.20	99.15	0.0018
15 µg/Ml	15.40	99.50	

Precision

The developed method was found to be precise as the %RSD values for the intra-day and inter-day precision studies were within acceptable criteria less than 2%.

Table 4: Precision data of serratiopeptidase

	SINGLE POINT METHOD SERRATIOPEPTIDASE		
	MEAN	STANDARD	% RSD
Interday	99.70	0.11	0.11
Intraday	99.91	0.22	0.22

Robustness

Table 5:Robustness data of serratiopeptidase

SAMPLE	WAVELENGTH	MEAN	STANDARD	%RSD
Serratiopeptidase	261nm	0.41	0.00	0.35
	265nm	0.40	0.00	0.52

Ruggedness

Table 6: Ruggedness data of serratiopeptidase

ABSORBANCE OF SERRATIOPEPTIDASE	MEAN	STANDARD DEVIATION	%RSD
261nm	0.41	0.00	0.17
265nm	0.40	0.00	0.35

Table 7: Overall Results for validation

PARAMETERS		SERRATIOPEPTIDASE	LIMIT
Assay (% mean assay)		99.87%	95-105%
Specificity		Specific	No interference of any
Intraday precision %RSD		0.11	NMT 2.0%
Interday precision %RSD		0.22	NMT 2.0%
Accuracy %recovery		99.35%	98-102%
LOD		0.24	NMT 3
LOQ		0.71	NMT 10
Robustness	**Wavelength**	0.35	
	Wavelength	0.52	
Ruggedness	**Analyst 1**	0.17	
	Analyst 2	0.35	

Forced degradation studies

Degradation studies were performed with the formulation and the degraded samples were scanned. Assay of the scanned samples was calculated and all the samples passed the limits of degradation.

Assay: Sample, bearing the label claim seranopeptidase10mg. Assay was performed with the above formulation. Average % Assay for Bempedoic Acid andEzetimibe obtained was 100.15% and 99.95% respectively.

Table 8: Analysis Degradation data of serratiopeptidase

S.no	Standard Area	Sample area	% Assay
1	0.404	0.405	99.95
2	0.403	0.406	100.19
3	0.405	0.406	100.19
4	0.406	0.403	99.45
5	0.404	0.405	99.95
6	0.404	0.403	99.45
Avg	**0.001**	**0.405**	**99.95**
Standard deviation	**0.3**	**0.001**	**0.34**
%RSD	**0.404**	**0.3**	**0.34**

% Assay must be 95% to 105% and % RSD between results obtained with changed condition and average result of method precision is not more than 2.0%.all parameters are within in limit.

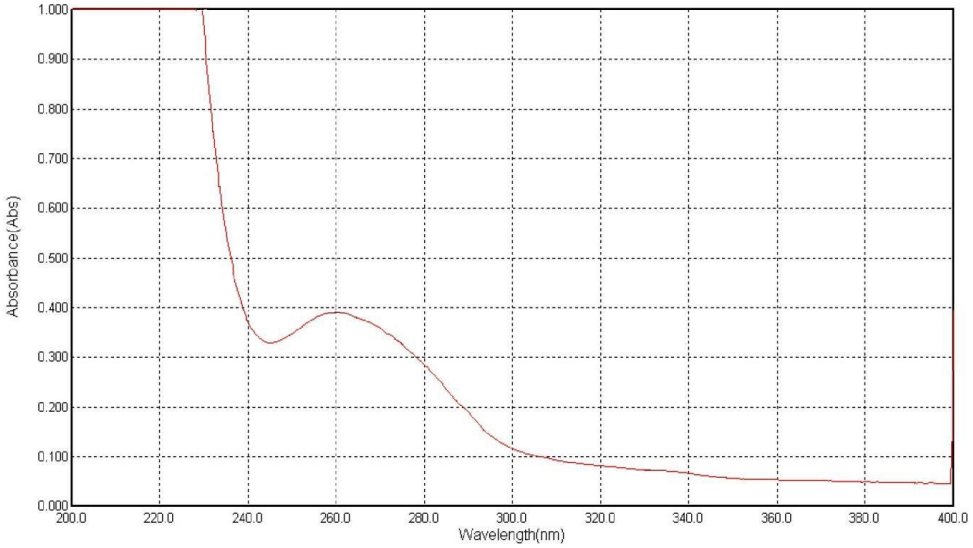

Fig 4: Acid spectrum (0.398 Abs)

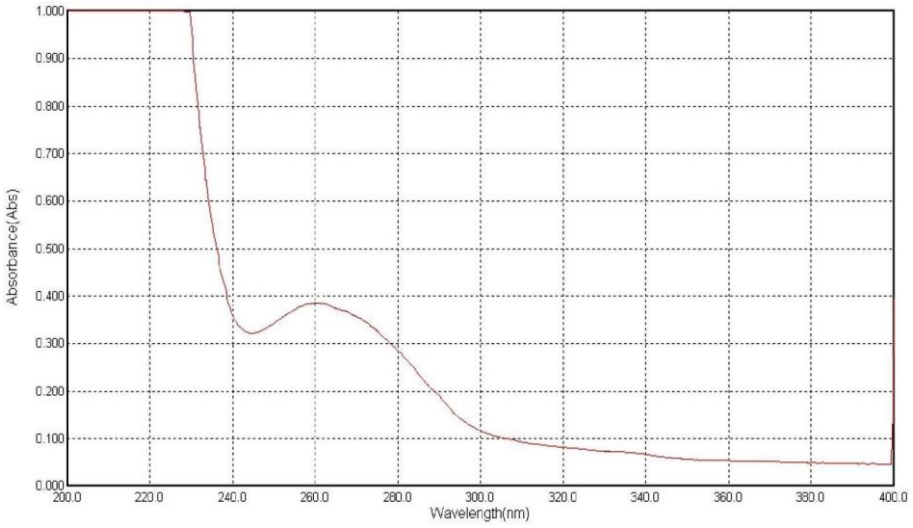

Fig 5: Base spectrum (0.396 Abs)

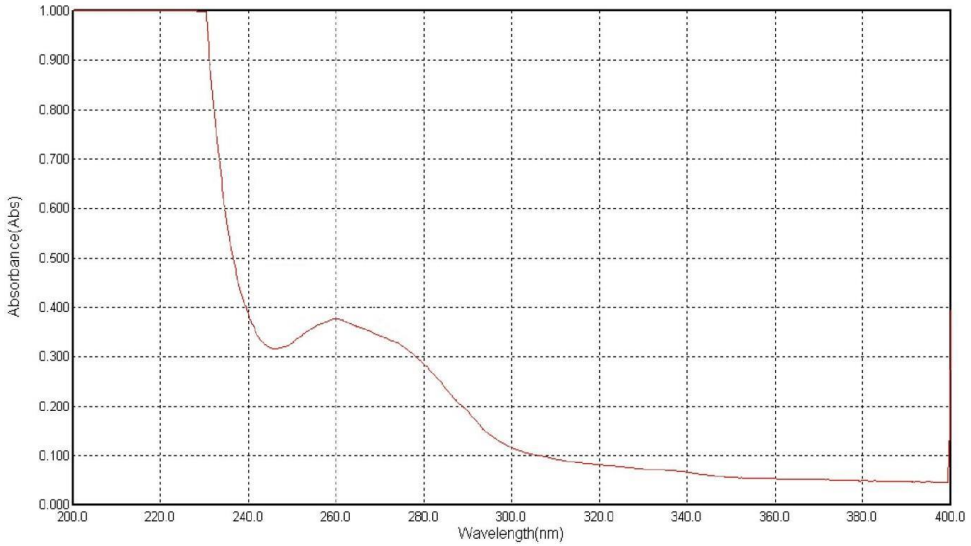

Fig 6: Peroxide spectrum (0.392Abs)

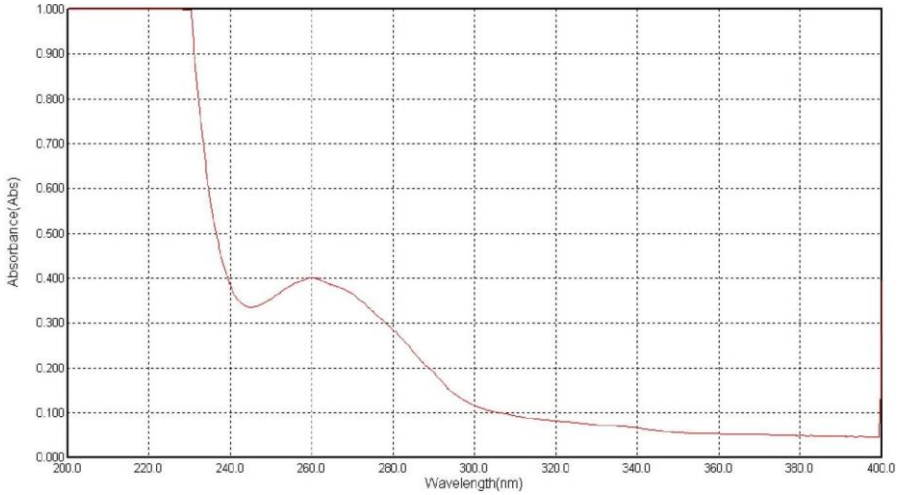

Fig 7: Thermal spectrum (0.399Abs)

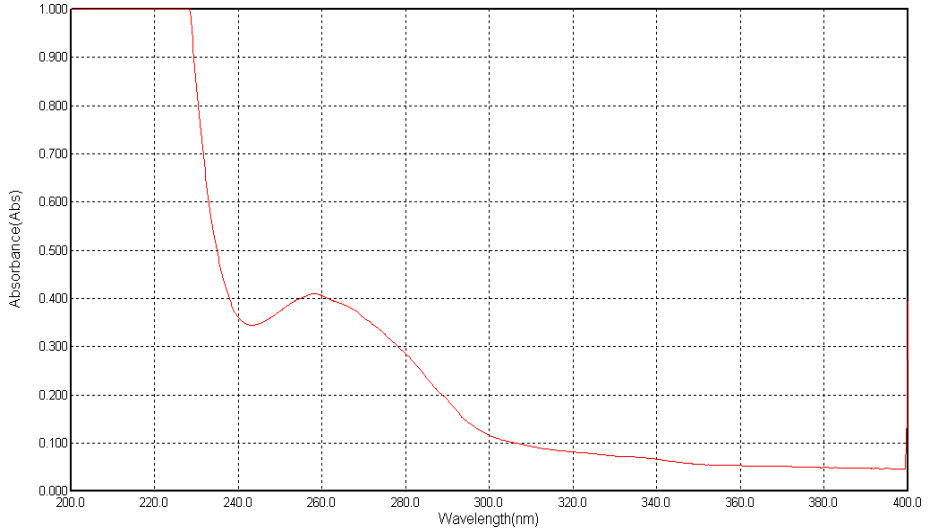

Fig 8: UV spectrum (0.401Abs)

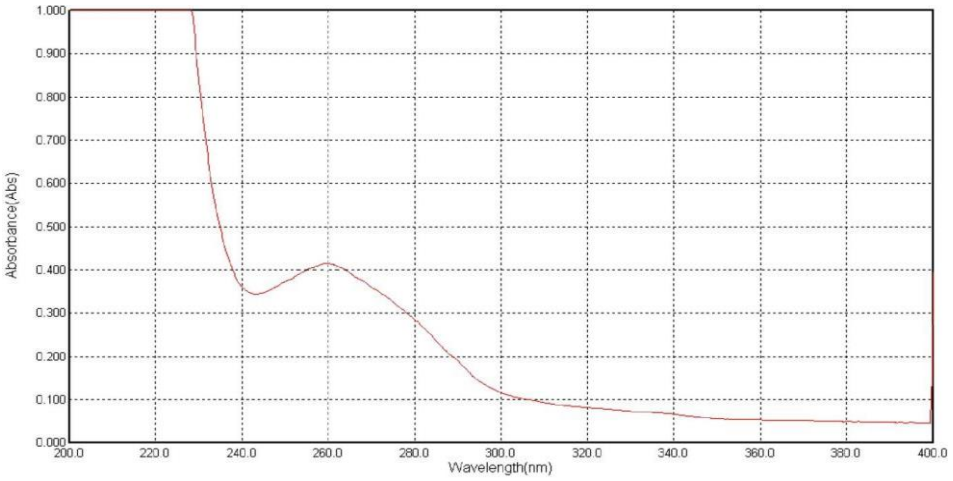

Fig 9: Water spectrum (0.403Abs)

Table 9: Degradation Data of Serratiopeptidase

S.NO	Degradation	% Undegraded	%Drug Degraded
1	Acid	98.61	1.39
2	Alkali	98.12	1.88
3	Peroxide	97.12	2.88
4	Thermal	98.86	1.14
5	UV	99.35	0.65
6	Water	99.85	0.15

The analysis of the same lot of Serratiopeptidase Tablet 10mg was carried out at different conditions of wavelength. The system suitability was found to meet the pre- established criteria at all the conditions and the degradation shows in acid, base and peroxide. The analytical method meets the pre-established acceptance criteria for robustness study as per Method.

SUMMARY AND CONCLUSION

Simple, precise and accurate UV spectrophotometric method were developed and validated as per ICH guidelines for the estimation of serratiopeptidase in tablet dosage form. From the solubility profile, distilled water was chosen as a common solvent for the estimation of serratiopeptidase. The sample solution of 10 µg/mL of std stock solution of serratiopeptidase in distilled water prepared and the solution was scanned in UV region in the wavelength range from 200-400 nm by using distilled water as a solvent. The overlay spectra of serratiopeptidase was recorded. From the spectra, serrattiopeptidase shows maximum absorbance at 263 nm. Percentage of RSD for intraday and inter day precision studies for the drug was well within the acceptable range (<2%) indicating that the method have excellent repeatability and reproducibility. The percentage relative standard deviation for precision and accuracy was found to be low, which indicates that the method have considerable accuracy and precision. Percent recovery for serrattiopeptidase was found in the range of 99.87% to 100.71% with standard deviation well below two indicating accuracy of the method. Recovery greater than 98% with low standard deviation justifies the accuracy of the method. Intraday and interday precision studies were carried out by analyzing tablet formulation by this method. The proposed method is found to be simple, precise, accurate and sensitive. Therefore, can be uses as a quality control tool for the estimation of drug from their dosage form in quality control laboratory. The system suitability was found to meet the pre-established criteria at all the conditions and the degradation shows in acid (1.39%), alkaline (1.88%) peroxide (2.88%), thermal (1.14%), water (0.15%).

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