Research Article **ISSN: 2349 – 7106**

Asian Journal of Research in Chemistry and Pharmaceutical Sciences

Journal home page: www.ajrcps.com

https://doi.org/10.36673/AJRCPS.2023.v11.i02.A09

DEVELOPMENT OF CHROMATOGRAPHIC METHOD FOR DETERMINATION OF RELEASE OF ENZYME FROM ENTERIC COATED TABLETS BY HPLC

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ABSTRACT

During this study, a reverse phase gel permeation chromatographic method to quantitate the amount of protease enzyme released from Serratiopeptidase enteric coated tablets, was developed. An isocratic mobile phase in combination with gel column was finally optimized for the study. Serratiopeptidase is a Proteolytic enzyme produced by purification from the culture of non-pathogenic enterobacterium Serratia sp. E-15. It hydrolyses the protein Casein into its constituent amino acids at a selected temperature and pH. It is used to reduce pain and swelling related to conditions like back pain, arthritis, tension headaches, migraine headaches etc. the tablet is intended for targeted delivery in intestine and the drug release had been monitored in pH 6.8 phosphate buffer media. The GPC technique had been applied to separate enzyme from excipients. The result showed that the chromatography is suitable to separate and quantitate the amount of drug released from tablet in presence of other excipient and coating materials.

KEYWORDS

Serratiopeptidase, Dissolution, Chromatography and Gel Permeation Chromatography (GPC).

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INTRODUCTON

A solid pharmaceutical dosage form is a complex mixture of drug and other excipients. The absorption of drug from solid composition through oral route is dependent on various factor such as release from solid composition, and dissolution under various physiological compositions. The dissolution is key indicator to predict in vivo performance of a drug.

Various guidelines and stringent specifications imposed by regulative authorities, like the United States Food and Drug administration (FDA), International Conference on Harmonization (ICH),

European Medical agency (EMA), Therapeutic Governance Authority (TGA) and Canadian Drug and Health Agency (CDHA) accentuate on the equivalency of finished pharmaceutical dosages form with reference listed drug. In order to grating the approval these agencies expect the release characteristic of any drug composition.

These tests are also required to assess the batch to batch reproducibility and as a quality parameter mentioned in different pharmacopoeia like USP and BP etc.

The dissolution medium and the analytical methodology used to determine the amount of release depends up on physio-chemical properties of drug. Serratiopeptidase contains protease enzyme as marker component. The determination of Serratiopeptidase from enteric coated tablets is based on measurement of protease enzyme. Traditional methods used to determine the protease activity requires, long analysis time and tedious analytical procedure. The main challenges are:

Instability of marker compound protease.

The complexity of analytical procedure increases the variability of results

Very narrow linear range

Due to long analysis time inability to produce multipoint dissolution profile

And there is lack of specific procedure to overcome from these challenges.

Protease enzyme may be inactive or may loss its activity under gastric conditions (acidic medium). It exhibits the maximum activities under slight alkaline or neutral medium. Orally administered enzymes are generally protected for gastric degradation or inactivation by coating. This coating protects the enzyme in gastric medium during transit towards duodenum.

Unfortunately, no procedure is available for measuring the amount of enzyme released from solid composition with good sensitivity and inter and intra laboratory precision. The present work describes the procedure for determination enzyme released from slid oral drug in dissolution medium.

MATERIAL

Method development

Serratiopeptidase standard, HPLC grade water, Sodium hydroxide, Acetonitrile, Purified water, Hydrochloric acid, Disodium hydrogen phosphate anhydrous, Potassium dihydrogen orthophosphate, 0.45µm nylon syringe filter and Serratiopeptidase tablets formulated in Formulation Research and Development department, Kusum Healthcare Pvt. Ltd, Bhiwadi, Raj, India.

Instrumentation

The dissolution was carried out on Electrolab dissolution apparatus EDT14LX and Lab india dissolution apparatus S 14000+ coupled with auto sampler. The pH was measured and maintained by using Orion star A211 pH meter, Sartorius MSA 225P-100-DI and mettle Toledo model XS204 balances used for weighing the chemicals and standards. The chromatograph consisted of a Waters HPLC system, Alliance model equipped with Waters e2695 Separations Module and Waters 2998 PDA Detector. The analysis, collection and Integration of data were carried out using Empower software. All the weighing in the experiments was done with Mattlertole do electronic balance capable of measuring with an accuracy of 0.01mg. All the volumetric glassware used in the study was Borosil 'A' grade.

Dissolution Parameter

In vitro drug release was performed for the tablets in line with the USP 27 ''Dissolution procedure'' for delayed release dosage forms. A minimum of 6 tablets of each product were tested. The dissolution of Serratiopeptidase from the enteric coated tablets was monitored using an Electrolab automated dissolution and Lab India dissolution tester coupled with automated sample collector. The USP 27 (apparatus 2) paddle method was used at 75rpm. The media used was 0.1N HCl at a pH 1.2 and a volume of 900ml for the first 2 h after which media replaced with 900ml of pH 6.8 phosphate buffer the temperature was maintained at 37 ± 0.5°C. Serratiopeptidase release from each tablet (in the dissolution samples) was determined by HPLC coupled with UV detector.

Chromatographic conditions

The chromatographic separation was performed on a Waters Ultrahydrogel Linear column, 300mm x 7.8mm purchased from Waters India limited. The separation was achieved using pH 9.50 Potassium dihydrogen orthophosphate buffer, with a flow rate of 0.5mL min-1, injection volume of 10µL, column oven temperature maintained at 650C, sample oven temperature maintained at 150C and detection wavelength of 220nm.

Preparation of dilute sodium hydroxide solution

Accurately weigh and dissolve about 10g of sodium hydroxide in 100mL of water.

Preparation of buffer for mobile phase

Accurately weigh and dissolve about 6.8g of Potassium dihydrogen orthophosphate in 1000mL of water. Adjust the pH to 9.50 ± 0.05 with dilute sodium hydroxide solution

Preparation of Standard Solution

Accurately weigh and transfer about Serratiopeptidase working standard equivalent to 55mg of Serratiopeptidase into a 100mL previously dried volumetric flask. Add about 60mL of diluent and sonicate for about 15 minutes with intermittent vigorous shaking. Allow to settle down frothing. Make up the volume up to the mark with diluent, mix well.

Preparation of Sample Solution

Transfer one intact tablet into each of the six dissolution vessels containing 900mL of 0.1N hydrochloric acid which is previously maintained at temperature of 37.0°C + 0.5°C and carry out dissolution as prescribed under acidic stage dissolution parameters. Withdraw 10mL of aliquot at specified time interval. Filter this solution through Whatman GF/C filter paper; discarding about first 2mL of filtrate. Further, filter the above filtrate through 0.45µm nylon syringe filter; (Make: Millipore) discarding about first 5mL of filtrate.

After completion of acidic stage dissolution, replace the acidic stage media with buffer stage media which is previously maintained at temperature of 37.0°C + 0.5°C and carryout dissolution as prescribed under buffer stage dissolution parameters. Withdraw 10mL of aliquot at specified time interval. Filter this solution through Whatman GF/C filter paper; discarding about first 2mL of filtrate. Further, filter the above filtrate through 0.45µm nylon syringe filter; (Make: Millipore Millex-HN, Catalog no.: SLHN033NK) discarding about first 5mL of filtrate

SUMMARY OF METHOD VALIDATION

The method evaluated for Specificity, Precision, Linearity and accuracy, stability of solution also established. The results are summarized in below Table No.2.

RESULTS AND DISCUSSION

For the successful separation of Serratiopeptidase from excipients, different types of column and mobile phase have been tried. But best results were obtained in Waters Ultra hydrogel linear column, 300mm x 7.8mm using phosphate buffer pH 9.50 as mobile phase at flow rate 0.5mL min-1 and detection at 220nm. Also, the method is rapid which separated drug from all the excipient within 25 minutes. The % drug release from Serratiopeptidase enteric coated tablets are presented in below Table No.3.

Table No.1

S.No	Instrument Name	Make/Model
1	HPLC	Waters e2695 Separations Module, Waters 2998 PDA Detector, Waters 2489 UV/Visible Detector
2	Dissolution Apparatus	Electro Lab / EDT14LX
3	Dissolution Apparatus	Lab India / DS 14000+
4	Analytical Balance	Mettler Toledo/XS204
5	Analytical Balance	Sartorius/MSA225P-100-DI
6	pH meter	Orion star A211

Table No.2

S.No	Parameter	Acceptance Criteria	Results
1	Specificity	No interference should be observed at the retention time of Serratiopeptidase peak from blank and placebo solutions and Peak purity should pass	No interference observed Peak purity Pass
2	System Precision	The % relative standard deviation should not be more than 3.0 %.	% RSD 1.5 %.
3	Method precision	The % relative standard deviation should not be more than 5.0 %.	Mean drug release113.0 %. % RSD 3.4%.
4	Accuracy	% Recovery should be within 95.0% to 105.0%.	Recovery Level: % Recovery 55%level: 97.8% 100%level: 98.3% 130% level: 98.8% 150% level: 99.6% Over all % RSD: 1.4%
5	Linearity	The Regression coefficient for Serratiopeptidase peak should not be less than 0.99	Regression coefficient: 0.99923.
6	Stability in Analytical Solution	Solution should be stable	solution is stable up to about 24 hrs at 15°C

Table No.3

S.No	Unit	% Release in acid stage	% Release in buffer stage	Mean	% RSD
1	Unit-1	0	106	109%	3.4%
2	Unit-2	0	112		
3	Unit-3	0	112		
4	Unit-4	0	113		
5	Unit-5	0	106		
6	Unit-6	0	105		

Chromatograms of study

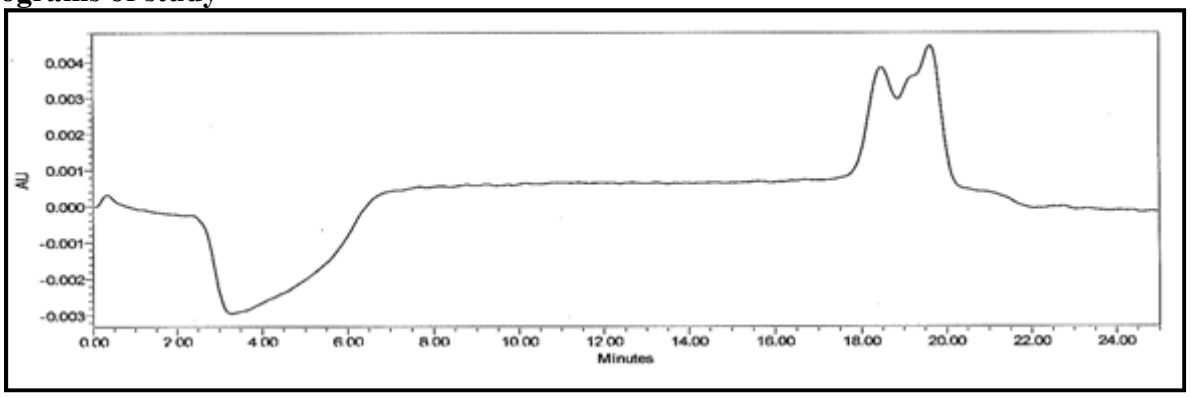

Figure No.1: Blank Chromatogram

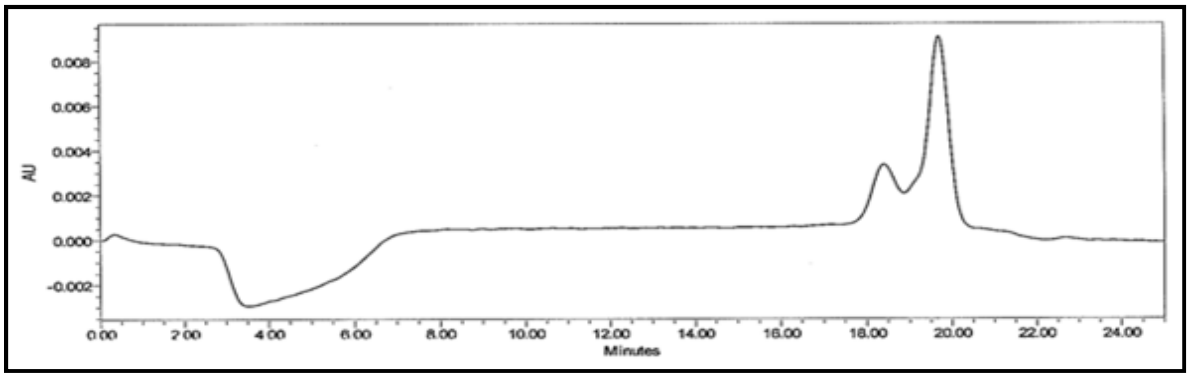

Figure No.2: Placebo Chromatogram

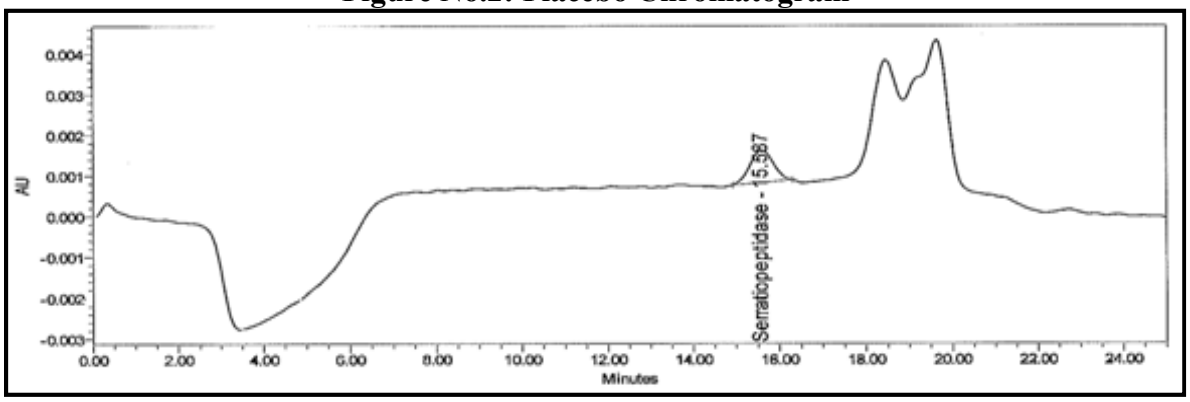

Figure No.3: Standard Chromatogram

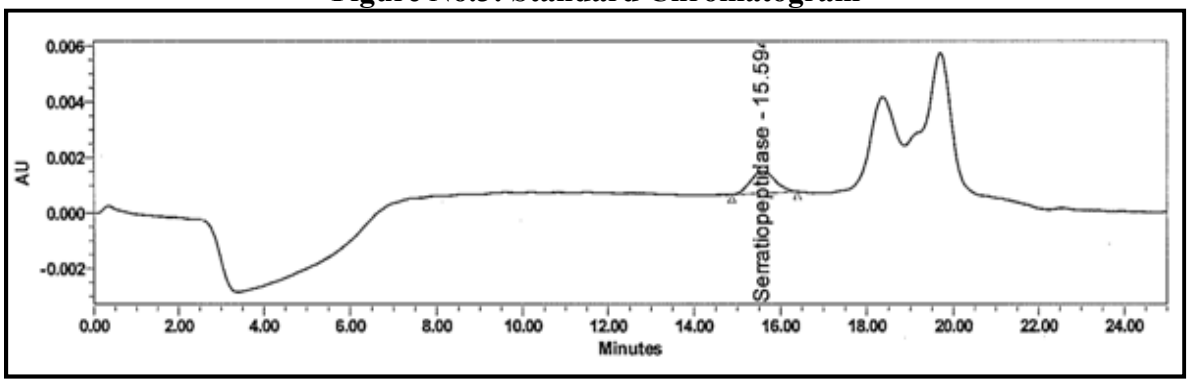

Figure No.4: Sample Chromatogram

CONCLUSION

A simple and specific method for the determination of Serratiopeptidase in Serratiopeptidase enteric coated tabletsby using HPLC was developed, validated and applied for the analysis of Serratiopeptidase enteric coated tablets. The method was validated to ensure the feasibility of the method for its application in routine analysis. The Recovery was achieved through this method between 95.0% to 105.0%.

ACKNOWLEDGEMENT

The author is highly grateful to Dr TG Chandrashekhar, Mr. Sanjeev Gupta, Mr. Rajeev Gupta, Kusum Healthcare Pvt. Ltd, Dr. Rahul Kumar for providing the necessary facilities and their kind support.

CONFLICT OF INTEREST

We declare that we have no conflict of Interest.

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Please cite this article in press as: Rahul Kumar and Shyam Sunder Shrivastava. Development of chromatographic method for determination of release of enzyme from enteric coated tablets by HPLC, *Asian Journal of Research in Chemistry and Pharmaceutical Sciences,* 11(2), 2023, 54-59.