International Journal of Pharmacy and Pharmaceutical Sciences

Print ISSN: 2656-0097 | Online ISSN: 0975-1491 Vol 16, Issue 1, 2024

Original Article

A SIMPLE RP-HPLC METHOD DEVELOPMENT AND VERIFICATION OF THE DISSOLUTION OF BROMELAIN-A COMPLEX MIXTURE OF PROTEOLYTIC ENZYMES, IN DELAYED-RELEASE TABLETS

BEMBADI MUKUND REDDY, BHEEMI REDDY ASHOK REDDY, UPPULURI TRIVIKRAM NAIDU, AKUTHOTA ASHOK KUMAR*, RAJESH VOOTURI

Formulation AR and D, Aurigene Pharmaceutical Services Limited, Hyderabad, Telangana, India
***Corresponding author: Akuthota Ashok Kumar; *Email: ashokkumara@aurigeneservices.com**

Received: 08 Sep 2023, Revised and Accepted: 23 Nov 2023

ABSTRACT

Objective: To develop a simple, accurate, precise and linear Reverse Phase High-Performance Liquid Chromatographic (RP-HPLC) method and verify for the quantitative estimation (Dissolution) of Bromelain in delayed-release tablets.

Methods: The optimized RP-HPLC method for both acid and buffer stage dissolutions of delayed-release tablets uses Zorbax 300 SB-C8 column (150 mm X 4.6 mm; 3.5µ), a mobile phase-A of 0.1% trifluoroacetic acid in water and a mobile phase-B of 0.1% Trifluoroacetic acid in Acetonitrile in the gradient proportion, flow rate of 1.0 ml/min, injection volume of 25 µl, detection wavelength of 280 nm using a UV/PDA detector, column temperature of 40 °C, sample tray/compartment temperature of 5 °C and a run time of 20 min.

Results: The developed method gave Bromelain eluting at about 6 min. Bromelain exhibited linearity in the range 53.4-800.6 µg/ml (r^2=0.99992). The precision is exemplified by relative standard deviation of 1.3 and 2.3% for acid and buffer stages, respectively. Percentage recovery of the drug was found to be between 90.0 and 110.0 during accuracy studies.

Conclusion: A simple, accurate, precise, and linear RP-HPLC method was developed and verified for the quantitative estimation (Dissolution) of Bromelain in tablets and hence this method can be explored for the analysis of Bromelain in tablets in various pharmaceutical industries.

Keywords: RP-HPLC, Bromelain, Dissolution, Acid and buffer stages, Delayed release tablets, Analytical method development

DOI: https://dx.doi.org/10.22159/ijpps.2024v16i1.49569. Journal homepage: https://innovareacademics.in/journals/index.php/ijpps

INTRODUCTION

Bromelain is a complex natural mixture of proteolytic enzymes derived from pineapple (*Ananas cosmosus*), which has good therapeutic properties. Bromelain can be found in all tissues of pineapple plants, including the stem, fruit, and leaves. Bromelain plays an important role in the treatment of many diseases such as soft tissue inflammation and edema, deep derma, full thickness burns, healing wounds, inflammation and pain of the muscles, angina pectoris, bronchitis, sinusitis, surgical trauma, thrombophlebitis, osteoarthritis, and diarrhea [1-4].

Several chromatographic techniques, such as ion-exchange chromatography, high-speed counter-current chromatography, affinity chromatography, gel filtration chromatography and capillary electrochromatography have been employed for the purification of bromelain [2, 3, 5-9].

A comparative study of the prepared Bromelain gel formulations along with their evaluations by Reverse Phase HPLC were determined by Kahtan Jasim Hasson, where in the Bromelain peak eluted at the retention time of about 7.3 min [2]. Determination of Bromelain in pineapple fruit was done by using Reverse Phase HPLC technique, wherein Bromelain eluted at about 2 min retention time [3]. An HPLC method for Bromelain determination was reported to study the stability of the Bromelain juice by using TSK-gel column which is a complicated mode of separation and lacks efficiency [5]. Gradient elution of high-performance liquid chromatography (HPLC) has been reported to determine of bromelain in stem bromelain [7]. Bromelain has been quantified by visible spectrophotometry in tablets [8]. Determination of bromelain content in pineapple water by isocratic HPLC method using specific column and UV detection has been reported [9].

Though few analytical methods are reported in literature for the quantification (assay) of Bromelain in various samples, no researcher have explored for the determination of dissolution (% drug release) of Bromelain in delayed-release tablets. Accordingly, the objective of this study is to develop a novel, simple, precise, accurate and linear RP-HPLC technique to understand the dissolution (% drug release) of Bromelain from delayed release tablets (acid and buffer dissolution stages).

MATERIALS AND METHODS

RP-HPLC analytical method development

Quantification of Bromelain in tablets by dissolution was explored by using reverse phase HPLC method, as the drug substance present in tablets is Bromelain, a polar drug in comparison to the column of C8 stationary phase, which is non-polar. RP-HPLC analytical method development, specifically chromatographic conditions has been initiated based on the chromatographic conditions reported in Agilent technologies literature entitled "HPLC column choices for the analysis of proteins and peptides" [10]. The literature reported working on various 300A° Zorbax stable bond columns such as SB-C18, SB-C8, SB-C3 and SB-CN for the analysis of proteins and peptides such as Insulin, Lysozyme, Cytochrome, Neurotensin, Rnase, *etc* [10]. However, we employed use of 300A° Zorbax SB-C8 column in this study to have a better recovery of Bromelain from the column by binding less to C8 column in comparative to C18 column. Also, modified other chromatographic conditions such as mobile phase, gradient programme, flow rate, injection colume, diluent, column temperature to an extent as of reported in the Agilent literaure to ensure the repeatability of the peak shape and chromatographic pattern of Bromealin in system suitability. Diluent and extraction time were optimized for ensuring acceptable recovery of Bromelain from the tablets.

Dissolution method development

Acid and buffer stages of dissolution have been considered to meet the objective of developing delayed release tablets. Dissolution

media for Bromelain at acidic stage and buffer stages were initiated with 0.1N hydrochloric acid and various buffers, *viz.* potassium hydrogen phosphate buffer, mixed sodium phosphate buffer and trisodium phosphate buffer respectively. 0.1N hydrochloric acid was considered as dissolution media for acid stage dissolution as per the method B procedure reported for delayed release dosage forms in USP general chapter 711, titled dissolution [11]. Various buffers were explored as dissolution media for the buffer stage dissolution and finalized with the trisodium phosphate buffer, which is reported in the method B procedure of delayed release dosage forms in USP general chapter 711, titled dissolution as % recoveries were higher and meeting the pre-determined acceptance criteria in comparson to the other buffers, whose results are presented in the results section (Tables 2-4).

Chemicals and reagents

Analytically pure sample of Bromelain was received from one of our customer and delayed release (DR) tablet formulation was prepared in our Formulation R and D laboratory, with a label claim of 480 mg of Bromelain. Acetonitrile (HPLC grade of Standard make or equivalent), Trisodium phosphate dodecahydrate (ACS grade of Sigma Aldrich make), Sodium Hydroxide (Extra pure of Merck make or equivalent), Hydrochloric acid (AR grade of Rankem make or equivalent), Trifluoroacetic acid (Merck make of Spectroscopy grade) and water (Milli-Q) were used for the analysis.

Instruments

HPLC analysis was performed on Water's make HPLC's and Agilent 1100/1200 make HPLC's having UV detector capable of setting detection wavelength of 280 nm. A reverse phase C8 column, Zorbax SB (150 X 4.6 mm; 3.5µ), part number 863973-906 was used. The HPLC system was controlled with "EMPOWER" software. An electronic analytical weighing balance (0.1 mg sensitivity, Sartorius make, CPA225D model), a Sonicator (Hwashin Make, Powersonic 420 model), a Dissolution Apparatus equipped with USP Apparatus-II and Auto sampler (Electrolab Make, Trust E14 model) and a digital pH meter (Thermo scientific Make, Orion Star A211 model) were used for the analysis.

Chromatographic conditions

Chromatographic conditions remain the same for both acid and buffer dissolution stages, the details of the method are provided below. The developed RP-HPLC method uses a reverse phase C8 column of Zorbax make, SB C8 (150 X 4.6 mm; 3.5µ) bearing a Part number of 863973-906, mobile phase-A of 0.1% Trifluoroacetic acid in water and mobile phase-B of 0.1% Trifluoroacetic acid in Acetonitrile in the gradient proportion setting a flow rate of 1.0 ml/min, diluent and blank as sodium phosphate pH 6.8 buffer, injection volume as 25µl, detection wavelength as 280 nm, setting column temperature and sample compartment temperature of 40 °C and 5 °C respectively and run time as 20 min. Gradient elution program is summarised in table 1.

Table 1: Gradient program

Time	% Mobile phase A	% Mobile phase B
0	75	25
10	30	70
14	30	70
14.1	75	25
20	75	25

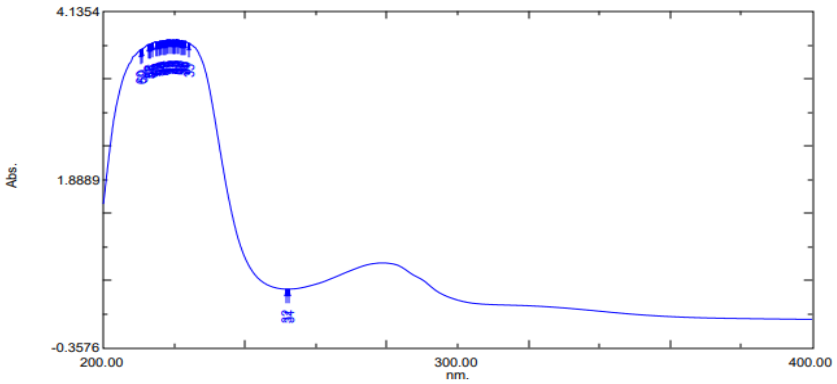

Fig. 1: UV spectrum of bromelain

Selection of wavelength

Suitable wavelength for the HPLC analysis for Bromelain was determined by recording UV spectrum in the range of 200-400 nm. Suitable wavelength selected was 280 nm, considering the maximum absorbance at this wavelength (fig. 1).

Preparation of pH 6.8 sodium phosphate buffer as diluent

Dissolve 76 g of Trisodium phosphate dodecahydrate ($Na_3PO_4.12H_2O$) in water by sonication and dilute to 1000 ml with water. Mix 0.1N Hydrochloric acid and the buffer in the ratio of 3:1, respectively. Adjust the pH to 6.8 with dilute hydrochloric acid or dilute sodium hydroxide solution.

Dissolution (Acid stage)

Preparation of standard solution

Weighed accurately and transferred about 53 mg of Bromelain standard into a clean and dried 100 ml volumetric flask. Added 70 ml of diluent vortexed to dissolve. Added about 25 ml more amount of the diluent and mixed well. Added few drops of Methanol to remove surface foam and then diluted to volume with diluent and mixed well.

Preparation of sample solution for bromelain content (batch analysis in duplicate)

Weighed and transferred 1 dose equivalent of tablets into a cleaned and dried mortar and pestle and then crushed into a powder. Immediately transferred the complete powder into a cleaned and dried 1000 ml volumetric flask. Rinsed the mortar and pestle with about 500 ml diluent (multiple washings of the pestle and mortar with the diluent, maximum diluent to be used is 500 ml) and ensured to completely transfer the contents into the 1000 ml volumetric flask to avoid losses. Added about 200 ml of diluent into the above volumetric flask and then stirred on a magnetic stirrer at 1000 rpm for 5 min exactly. Later, removed the magnetic bead carefully with the magnetic retriever and then rinsed the magnetic bead with about 10 ml of the diluent into the volumetric flask to

avoid losses sticking to the magnetic bead. Then, diluted to volume with diluent and mixed well. Centrifuged a portion of the sample solution at 3000 rpm for 10 min. taken an aliquot of the supernatant for analysis.

Dissolution parameters for acid stage

The finalized dissolution procedure for determination of Bromelain in tablets at acid stage is by using USP Apparatus-II (Paddles), dissolution temperature of 37±0.5 °C, 75 RPM, dissolution time point as 1 h (60 min), dissolution media of 0.1 N HCl and dissolution media of 1000 ml.

Preparation of sample solution for acid stage (Residual assay)

Transferred 1000 ml of 0.1 N Hydrochloric acid into each of the six dissolution vessels and equilibrated at 37±0.5 °C. Transferred one dose for the analysis in one dissolution vessel. Followed the same procedure of dropping one dose into each of the remaining five dissolution vessels and started the apparatus immediately. After completion of the Dissolution run for acid stage (1 hour), drained out the media carefully without losing the tablet contents. Transferred the tablets from each dissolution vessel into a separate clean petri plate of suitable size and proceeded for immediate content determination of Bromelain in these retained tablets. Transferred 1 dose of tablets collected after acidic stage into a cleaned and dried mortar and pestle. Added about 10 ml of the diluent into mortar and pestle and then grinded, making use of about 700 ml of the diluent (multiple washings of the pestle and mortar with the diluent, maximum diluent to use is 700 ml), transferred the complete contents present in the mortar and pestle into a cleaned and dried 1000 ml volumetric flask, without any loss. Later, stirred on a magnetic stirrer at about 1000 rpm for 5 min. Then, removed the magnetic bead carefully with the magnetic retriever and rinsed the magnetic bead with about 10 ml of the diluent into the volumetric flask to avoid losses sticking to the magnetic bead. Then, diluted to volume with diluent and mixed well. Centrifuged a portion of the sample solution at 3000 rpm for 10 min and taken a clear supernatant for injecting into HPLC.

Procedure

Inject Blank, standard solution (6), batch analysis samples (2) and acid stage samples (6) followed by bracketing standard.

System suitability

(i) No peak shall be detected in the blank at>0.2% of the average standard area response of Standard solution at the retention time of Bromelain.

(ii) The percent relative standard deviation of peak area response of Bromelain from 6 Replicate injections of Standard solution, obtained under system suitability, shall be NMT 5.0%.

(iii) Inject Standard solution as a bracketing standard after every six sample injections and at the end of the analysis. %RSD of 6 injections average area of Bromelain obtained under system suitability and the area response of each bracketing standard shall be NMT 5.0%.

Calculation

Calculated the quantity of Bromelain present in the tablets for the batch analysis samples and for the retained/recovered/collected after acid stage dissolution as % of labelled amount using the following equation:

(i) Bromelain content (Batch analysis), Percent Label claim

$$\text{and} = \frac{Au}{A_S} \times \frac{W_S}{200} \times \frac{1000}{1} \times \frac{P}{100} \times \frac{100}{L}$$

and

(ii) Bromelain in Acid stage sample (retained), Percent Label Claim

$$\text{and} = \frac{A_T}{A_S} \times \frac{W_S}{200} \times \frac{1000}{1} \times \frac{P}{100} \times \frac{100}{L}$$

and

Where,

A_u is the peak area of Bromelain in the Batch analysis sample solution.

A_T is the peak area of Bromelain in the Acid stage (retained) sample solution.

A_S is the average peak area of Bromelain from six replicate injections of Standard solution injected under system suitability

W_S is the weight of Bromelain standard taken, in mg for the preparation of Standard solution

P is the potency of Bromelain standard or reference standard on as is basis.

L is the label claim of Bromelain mg, 480

% Bromelain released in the acidic stage: (Average of n=2) Bromelain Content in tablets minus % Bromelain retained in the tablets after acid stage exposure. Calculate the results for individually for all the 6 units and report the average result.

Dissolution (Buffer stage)

Preparation of standard solution

Weighed accurately and transferred about 53 mg of Bromelain standard into a clean and dried 100 ml volumetric flask. Added 70 ml of diluent vortexed to dissolve. Added about 25 ml more amount of the diluent and mixed well. Added few drops of Methanol to remove surface foam and then diluted to volume with diluent and mixed well.

Dissolution parameters for buffer stage

The finalized dissolution procedure for determination of Bromelain in tablets at buffer stage is by using USP Apparatus-II (Paddles); dissolution media as trisodium phosphate buffer, pH 6.8, dissolution media volume is 1000 ml, 75 RPM, dissolution temperature at 37±0.5 °C and dissolution time point as 5 h (300 min).

Preparation of sample solution for buffer stage dissolution

After the Acid stage is completed, drain out 0.1N Hydrochloric acid carefully without losing any tablets. Transfer 1000 ml of preheated pH 6.8 Sodium Phosphate Buffer that has been equilibrated to 37±0.5 °C to each of the six-dissolution vessel and start the apparatus immediately. Examine the vessels and note observations during the run wherever necessary. Run the buffer stage dissolution for 5 h. Later, collect the dissolution sample either through Auto sampling or Manual sampling procedure and inject it into HPLC.

Procedure

Inject blank, standard solution (6), batch analysis samples and acid stage samples followed by bracketing standard.

System suitability

(i) No peak shall be detected in the blank at>0.2% of the average standard area response of Standard solution at the retention time of Bromelain.

(ii) The percent relative standard deviation of peak area response of Bromelain from 6 Replicate injections of Standard solution, obtained under system suitability, shall be NMT 5.0%.

(iii) Inject Standard solution as a bracketing standard after every six sample injections and at the end of the analysis. %RSD of 6 injections average area of Bromelain obtained under system suitability and the area response of each bracketing standard shall be NMT 5.0%.

Calculations

Calculate the quantity of Bromelain dissolved in nth time interval (Dn) as % of labelled amount using the following equation:

$$Dn = \frac{A_T}{A_S} \times \frac{W_S}{100} \times \frac{1000}{1} \times \frac{P}{100} \times \frac{100}{L}$$

Where,

A_T is the peak area of Bromelain in the sample solution.

A_S is the average peak area of Bromelain from six replicate injections of Standard

Solution injected under system suitability

W_S is the weight of Bromelain working or reference standard taken, in mg for the preparation of Standard solution

P is the potency of Bromelain standard or reference standard on as is basis

L is the label claim of Bromelain in mg, 480

RESULTS

Dissolution method conditions for Bromelain at acidic stage and buffer stages were initiated with 0.1 N HCl as dissolution media at acidic stage and various buffers at buffer stage, *viz.* Potassium hydrogen phosphate buffer (Trial 1), Mixed sodium phosphate buffer (Trial 2) and Trisodium phosphate buffer (Trial 3). Results of the trails 1-3 are given in the below tables 2-4.

Trial 1: Dissolution Parameters for buffer Stage: Medium is pH 6.8 Potassium phosphate buffer; Media volume is 1000 ml; Apparatus is USP Type II (Paddle); RPM is 75; Temperature is 37±0.5 °C

Trail 2: Dissolution parameters for buffer Stage: Medium is pH 6.8 Mixed Sodium Phosphate Buffer; Media volume is 1000 ml; Apparatus is USP Type II (Paddle); RPM is 75; Temperature is 37±0.5 °C

Table 2: % Recoveries using potassium phosphate buffer as dissolution media

Level	% Recovery	^% Mean recovery±SD
100%-1	83.3	
100%-2	83.8	84.83±2.24
100%-3	87.4	

^ Data is expressed as mean±SD, n=3. SD = Standard Deviation

Table 3: % Recoveries using mixed sodium phosphate buffer as dissolution media

Level	% Recovery	#% Mean recovery±SD
100%-1	86	
100%-2	87	86.33±0.578
100%-3	86	

#Data is expressed as mean±SD, n=3. SD = Standard Deviation

Trial 3: Dissolution parameters for buffer stage: Medium is pH 6.8 sodium phosphate buffer; Media volumeis 1000 ml; Apparatus is USP Type II (Paddle); RPM is 75; Temperature is 37±0.5 °C.

Table 4: % Recoveries using Trisodium phosphate buffer as dissolution media

Level	% Recovery	@% Mean recovery±SD
100%-1	96	
100%-2	96	95.67±0.58
100%-3	95	

@Data is expressed as mean±SD, n=3. SD = Standard Deviation

The finalized dissolution procedure for determination of Bromelain in tablets is by USP Apparatus-II (Paddles) using Acid stage [75 RPM, 1 hour (60 min)], Dissolution media of 0.1 N HCl, 1000 ml) followed by Buffer stage [75 RPM, 5 h (300 min), 1000 ml] and Dissolution media as Trisodium phosphate buffer, pH 6.8.

A Reverse phase HPLC method chromatographic conditions were developed keeping in mind the system suitability parameters *i.e.,* % RSD from six replicate injections of standard and Blank interference and run time. The optimized method developed resulted in the elution of Bromelain at about 6.0 min. Fig. **2-6** represent specimen chromatograms of blank, placebo, standard and sample solutions. The total run time is 20 min. System suitability tests are an integral part of method development and are used to ensure adequate performance of the chromatographic system, whose details are summarized in Tables 5a and 5b.

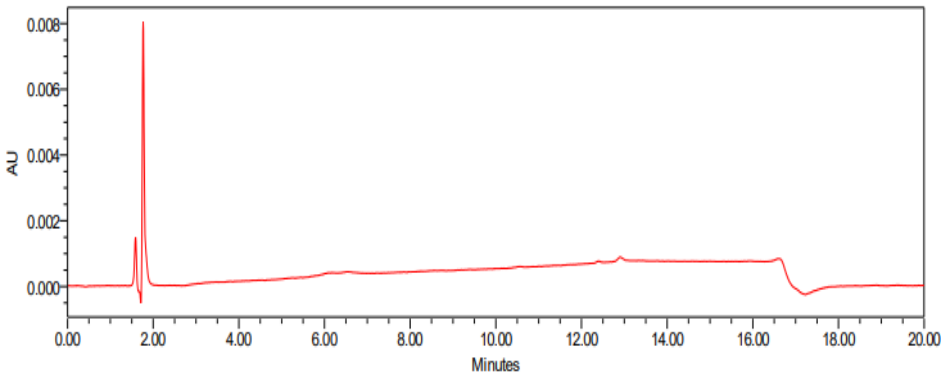

Fig. 2: Typical chromatogram of blank solution

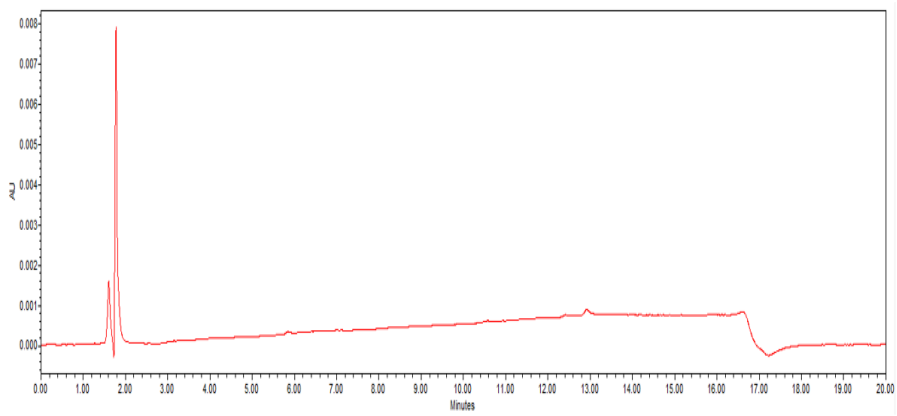

Fig. 3: Typical chromatogram of placebo solution

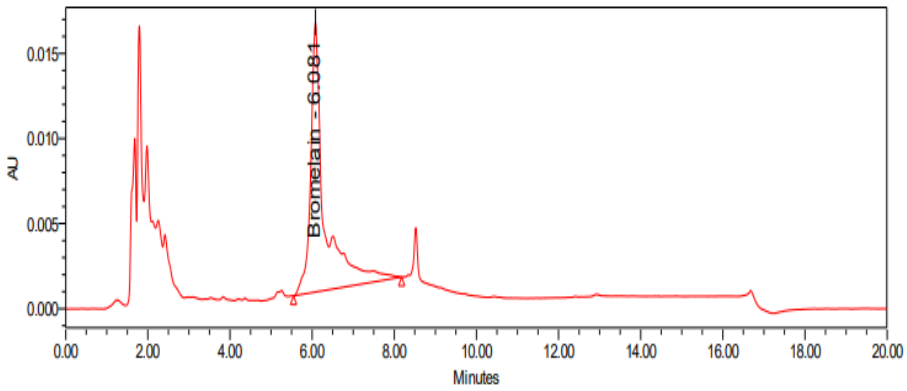

Fig. 4: Typical chromatogram of the standard solution

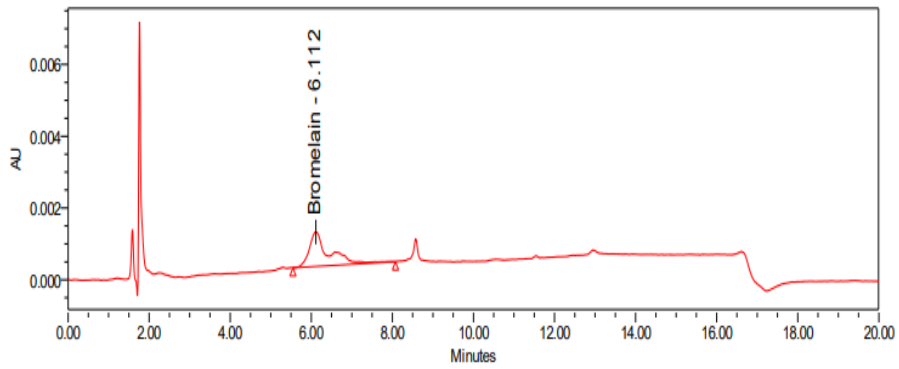

Fig. 5: Typical chromatogram of the sample solution-acid stage-retained assay

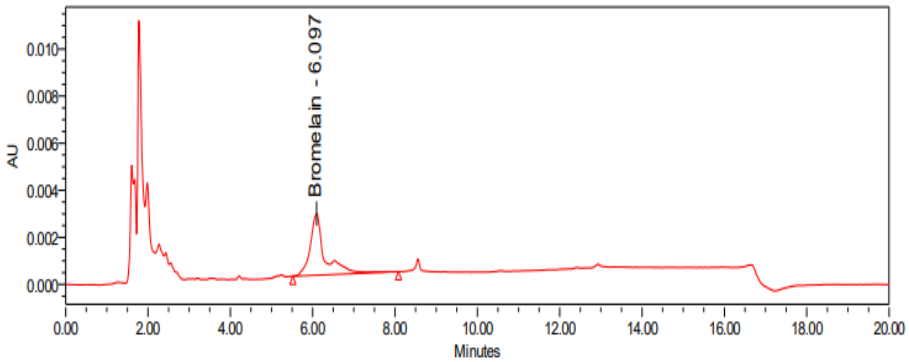

Fig. 6: Typical chromatogram of the sample solution-buffer stage

Table 5a: System suitability acceptance criteria

Parameters	Acceptance criteria
Blank interference	No peak shall be detected in the blank at>0.2% of the average standard area response of Standard solution at the retention time of Bromelain.
% RSD (Relative standard deviation)	The percent relative standard deviation of the average peak area response from 6 injections of Bromelain shall be NMT 5.0%.

Table 5b: System suitability results

Parameters	Bromelain
Blank interference	Nil
^% RSD (Relative standard deviation)	1.1

^Data is expressed as % RSD, n=6.

In order to evaluate the applicability of the developed method to a formulation, Bromelain tablets at acid and buffer stages were chromatographed, and it is shown in fig. 5 and 6. The sample peak was identified by comparing the retention time with the standard drug fig. 4. To ensure the method developed meets the requirements of verification parameters, verification was employed, whose details are mentioned in the below section.

Method verification

Dissolution method verification of the analytical method was done for parameters such as system suitability, specificity, linearity, accuracy and precision [12].

Specificity

To establish non-interference, blank and placebo solutions were prepared and injected into HPLC along with standard and sample solutions of both acid and buffer stages as per the finalized method (Refer fig. 3-6). Fig. reveal that the peaks obtained in the standards solution and sample solution are only because of the drug as blank and placebo have no peak at the retention time of Bromelain standard. Accordingly, it can be concluded that the method developed is said to be specific.

Method precision

Method precision was determined by performing the dissolution of samples (acid stage and buffer stage) on six test preparations. The % RSD (Relative Standard Deviation) of the Bromelain (Retained assay) at acid and buffer stages of six units was not more than 5.0 and hence, can be concluded that the method is precise by the test of repeatability and hence can be understood that the method gives consistently reproducible results (table 6).

Accuracy

Accuracy was determined by means of recovery experiments, by the determination of % individual and % mean recovery of the sample at 100% and 150% levels. At each level, three determinations were performed. Individual and percent mean recoveries were found to be in the range of 90.0% to 110.0% (table 7), which indicates good recovery values and, hence the accuracy of the method developed.

Table 6: Method precision results

Sample no. (n)	% Bromelain (Retained) after exposure to acid stage	% Dissolution (Acid stage)	% Dissolution (Buffer stage)	% Dissolution at buffer stage, cumulative to acidic stage time point
1	96.5	7	84	91
2	94.1	10	86	96
3	94.6	9	84	93
4	94.2	10	85	95
5	93.4	10	85	95
6	92.8	11	86	97
*Average±SD	94.3±1.27	9.5±1.38	85±0.89	94.5±2.17
%RSD	1.34	NA	1.05	2.29

*Data is expressed as mean±SD, n=6. SD = Standard Deviation, NA = Not Applicable

Table 7: Results of accuracy studies

% Level	% Individual recovery	#% Mean recovery±SD
100	96.0	95.67±0.58
	96.0	
	95.0	
150	93.6	94.3±0.7
	94.3	
	95.0	

#Data is expressed as mean±SD, n=3. SD = Standard Deviation

Linearity

Standard solutions of Bromelain at different concentration levels (10%, 25%, 50%, 75%, 100%, 125%, and 150%) were prepared. Calibration curve was constructed by plotting the concentration level of drug versus corresponding peak area. The results show an excellent correlation between peak area and concentration level of drug within the concentration range (53.4-800.6 µg/ml) for the drug and the results are given in table 8 and fig. 7. The correlation coefficient of Bromelain is 0.99992. The results reveal that the method is linear for quantification of Bromelain in the proposed range.

Table 8: Linearity data

% Level	Concentration (µg/ml)	Peak Area
10	53.370	48018
25	128.088	113040
50	256.176	220564
75	373.590	316739
100	533.700	445777
125	640.440	535473
150	800.550	660257
Regression/Correlation coefficient		0.99992
Bias		1.82
Regression equation		y = 819.48x-8121.97

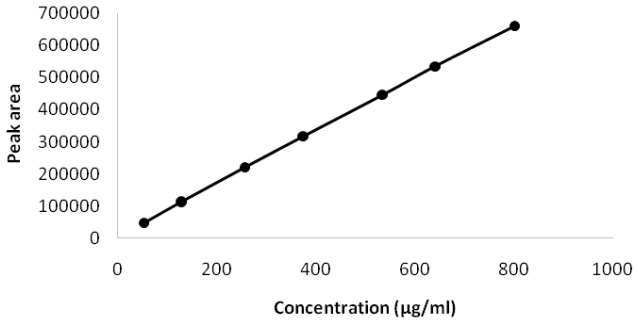

Fig. 7: Linearity graph

DISCUSSION

Reverse phase HPLC method chromatographic conditions were finalized keeping in mind the system suitability parameters *i.e.,* % RSD from six replicate injections of standard and Blank interference and run time. The optimized method developed resulted in the elution of Bromelain at about 6.0 min with % RSD of 1.1, non-interference of blank with the bromelain peak and a run time of 20 min (tables 5a-5b, fig. 3-6). Researchers have reported analytical methods where Bromelain was found to elute at about 7.3 min and 2 min retention time, respectively, while the method developed and verified in this study is about 6 min retention time [2, 3]. It is not advisable to develop methods whose retention times are less than 3 min as void peaks due to mobile phase and diluent elute. Hence, advantages of this study against reported [2]. Also, the reduced retention time of 6 min in this study over 7.3 min reported increases the number of samples analysis in a specified time [3].

There are hardly any articles published/available in the literature on the dissolution study of Bromelain from tablets. Hence, the comparison could not be done with the reported ones. In this study, we observe that as the % recoveries for bromelain from the tablets were meeting the acceptance criterion of 90.0-110.0% in Trisodium phosphate buffer (Trial 3, table 4) in comparison to trials 1 and 2, Trisodium phosphate buffer (Trial 3) was finalized as the dissolution media for the Buffer stage, which is in line with the buffer mentioned in the Method B procedure of USP Chapter on Dissolution<711>under Delayed release dosage forms (tables 2-4). Accordingly, proceeded forward for the method verification activities using the finalized RP-HPLC chromatographic conditions and the trials 3 dissolution media, Trisodium phosphate buffer. Method verification activities include specificity, precision, accuracy and linearity, discussed below.

Fig. 3-6 reveal that the peaks obtained in the standards solution and sample solution are only because of the drug as blank and placebo have no peak at the retention time of Bromelain standard. Accordingly, it can be concluded that, the method developed is said to be specific. The precision of the analytical method is exemplified by relative standard deviation of 1.3 and 2.3% for the dissolution of acid and buffer (cumulative) stages, respectively (table 6) against the acceptance criteria of not more than 10.0%. Dissolution specification for the acid stage was set as not more than 25% for the dissolution of Bromelain from tablets at 60 min time point and for the buffer stage (cumulative), specification is set as not less than 55% of the Bromelain from tablets is dissolved at 5 h time point. Accordingly, observed from table 6 method precision that Bromelain tablets met the specifications at both acid and buffer (cumulative) stages, where the individual and the average values for the acid stage dissolution is not more than 11% against 25%, and the individual and average values for the buffer stage (cumulative) is not less than 91%, against 55% (table 6). During accuracy studies table 7, it was observed that individual and mean percentage recoveries of the drug were found to be between 93.6% and 96.0% against the acceptance criteria of between 90.0% and 110.0and and hence, can be concluded that method is accurate. From table 8, it can be concluded that the developed method exhibited linearity in the range 53.4-800.6 µg/ml as the regression/correlation coefficient is greater than 0.99, observed value being r^2=0.99992. Overall, it can be concluded that developed method is specific, precise, accurate and linear.

CONCLUSION

A reverse phase HPLC method developed has been verified in terms of specificity, accuracy, precision, and linearity for the quantitative estimation (Dissolution) of Bromelain in delayed-release tablets. Accordingly, it can be concluded that the developed reverse phase HPLC method is specific, accurate, precise and linear and therefore, the method can be explored for the routine analysis of Bromelain in tablets.

ACKNOWLEDGEMENT

The authors would thank the management of Aurigene Pharmaceutical Services Limited, Hyderabad for providing the necessary facilities to conduct this analytical research work and encouraging for publication. APSL Clearance No: "APSL_P42_14/09/2023". The author Akuthota Ashok kumar thank all the co-authors for their valuable contribution in getting this research work completed successfully.

FUNDING

The complete infrastructural facilities were provided by our organization, Aurigene pharmaceutical services limited, hyderabad.

Also, the funding was provided by our organization and the customer for whom this project was done.

AUTHORS CONTRIBUTIONS

Bembadi Mukund reddy, Bheemi reddy Ashok reddy, Uppluluri Trivikram naidu have carried out the method development and method verification activities. Dr. Rajesh vooturi has reviewed the manuscript and provided valuable suggestions in drafting the manuscript.

CONFLICTS OF INTERESTS

Declared none

REFERENCES

1. Vidhya R, Noorjahan BA, Subramaniam S, Samikannu K, Rajendran R. Potential role of Bromelain in clinical and therapeutic applications. Bio Med Rep. 2016;5:283-8.
2. Kahtan JH. Comparative study of prepared Bromelain gel formulations and them evaluations by HPLC determination. AJPS. 2016;16(2):77-81.
3. Yantih N, Methananda A, Harahap Y, Sumaryono W, Rahayu L. Validation of high-performance liquid chromatography for determination of bromelain in pineapple (Ananas comosus (L) Merr) water. Pharmacogn J. 2019;11(5):901-6. doi: 10.5530/pj.2019.11.144.
4. Btul D, Betul C, Serkan Y, Neslihan Y, Ismail B. Gastroprotective effects of bromelain on indomethacin-induced gastric ulcer in rats. GSC Bio Pharm Sci. 2023;23(1):277-86.
5. Fadzilah AM, Madihah AG, Sitizalita T, Khadijah KA. Stability of bromelain-polyphenol complex in pineapple juice. J Teknol. 2008;49(F):27-38.
6. Pavan R, Jain S, Shraddha A, Kumar A. Properties and therapeutic application of bromelain: a review. Biotechnol Res Int. 2012;2012:976203. doi: 10.1155/2012/976203, PMID 23304525.
7. Baez R, Lopes MTP, Salas CE, Hernandez M. In vivo antitumoral activity of stem pineapple (Ananas comosus) bromelain. Planta Med. 2007;73(13):1377-83. doi: 10.1055/s-2007-990221, PMID 17893836.
8. Wani SS, Mashru RC. Sensitive and selective methods for determination of proteolytic activity of formulation containing bromelain and trypsin as proteolytic enzymes. Int J Pharm Sci Res. 2014;5(11):4838-45.
9. Majid FAA, Gani AM, Talib SZ, Hasyim KK. Stability of bromelain-polyphenol complex in pineapple juice. J Teknol 2008;49(F):27-38.
10. Agilent technologies. HPLC column choices for the analysis of proteins and peptides. Available from: https://www.agilent.com/cs/library/eseminars/Public/Column%20Choices%20for%20proteins_037214.pdf.
11. Brown WE. Dissolution<711>. Available from: https://online.uspnf.com/uspnf/document/GUID-AC788D41-90A2-4F36-A6E7-769954A9ED09_2_en-US. doi: 10.31003/USPNF.
12. International conference on harmonization of technical requirements for registration of pharmaceuticals for human use. Validation of analytical procedures: text and methodology ICH. Vol. Q2(R1); 2005.