IJSRM
INTERNATIONAL JOURNAL OF SCIENCE AND RESEARCH METHODOLOGY
An Official Publication of Human Journals

Human Journals
Research Article
June 2018 Vol.:9, Issue:4

Validation of Analytical Method to Measure Bromelain Activity in Gel Formulation

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INTERNATIONAL JOURNAL OF SCIENCE AND RESEARCH METHODOLOGY
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Submission: 22 May 2018
Accepted: 29 May 2018
Published: 30 June 2018

HUMAN JOURNALS
www.ijsrm.humanjournals.com

Keywords: Elastase, validation, Bromelain, Spectrometry, *Ananas comosus*

ABSTRACT

Bromelain containing gel developed at the TSMU Iovel Kutateladze Institute of Pharmacochemistry has fibrinolytic, antithrombotic, anti-tumor, anti-edema and anti-inflammatory properties. The active compound of gel formulation is complex of proteolytic enzymes – proteases derived from the stem of Ananas comosus. To determine the proteolytic activity of the enzyme complex - bromelain in medicinal formulations containing compounds absorbing at 280 nm and interfering with the measurement of the optical density of casein hydrolysates is adapted the method in which is used the substrate RBB-elastin, allowing measurements of the substrate hydrolysates at 595 nm. The developed method is linear, precise and sensitive; Intra- and inter-day measurements. All methods were validated as per ICH guidelines and can be adopted for the routine analysis of Bromelain in gel formulations.

INTRODUCTION

The proteolytic enzyme - Bromelain was obtained from the stems of Pineapple *(Ananas comosus* (L.) Merr.) and is used as a major component of the gel formulation.

The use of medicinal products containing enzymes has increased due to their broad therapeutic potential. Currently, such products are being used in the pharmaceutical field for several applications [1-2].

Bromelain belongs to the proteolytic enzymes, a complex of proteases, which reveal fibrinolytic, antidepressant, anti-thrombotic, antibacterial and anti-inflammatory activities. Like as a proteolytic enzyme Papain, Bromelain is used for the treatment of traumatic skull-brain injuries, inflammations and degenerative processes of the spine, tumors, breast cancer, burns, edema, spontaneous trauma, osteoarthritis.

In the gel formulation, there are some compounds absorbing the light at the 280 nm wavelength and interfering with the measurement of the optical density of casein hydrolysates. For solving the problem is adapted the method using the colored substrate - RBB (*Remazol Brilliant Blue*) elastin, which allows measuring the substrate hydrolysates at 595 nm.

Bromelain absorbs in the body without loss of proteolytic activity and does not have side effects.

For the first time, we have developed a method of standardized Bromelin containing gel formulation, which is based on the study of the enzymatic activity of the Bromelain using the RBB Elastin.

The spectrophotometric method developed can be used for the standardization of Bromelain containing gel formulation [3-11].

MATERIALS AND METHODS

Apparatus:

The research was conducted in quartz cuvettes (10 mm), spectrophotometer Jasco V-730, option 1 nm, wavelength accuracy of ± 0.1. The spectra were automatically processed by

UV-Probe system software (version 2.14.02). For weighing of samples were used scales Radwag 3000. pH of the solutions was determined by pH meter Milwaukee - Mi 150.

Chemicals and reagents:

Bromelain; Remazol Brilliant Blue R, RBB; "Sigma; Elastin (from bovine neck ligament) "Sigma", 0,067 M Phosphate Buffer solutions pH 7,0; Cysteine "Sigma"; Ethylenediaminetetraacetic acid (EDTA) "Sigma".

RBB-elastin was used as a substrate to define the elastic activity of Bromelain [12].

Experimental Conditions

The experiments were conducted in a water thermostat at a 40^0C temperature at pH 7.0. The analysis was carried out on a spectrophotometer at λ = 595 nm.

RESULTS AND DISCUSSION

Validation of analytical methodology

The method was validated on the gel formulation of Bromelain according to the ICH guidelines. The linearity of the UV spectrometric method was performed for Bromelain and RBB elastin concentrations. Five different concentrations of Bromelain (0.4-2.4 mg/ml) and RBB elastin (2.0-20 mg/ml) were prepared and analyzed in triplicate for each concentration. Calibration curves were constructed by plotted optical density against concentrations. The linearity was assessed by calculating the slope, y-intercept and coefficient of correlation (r^2) using least squares regression (***Fig. 1*** and ***Fig. 2***).

Calibration curve of elastolytic activity of Bromelain

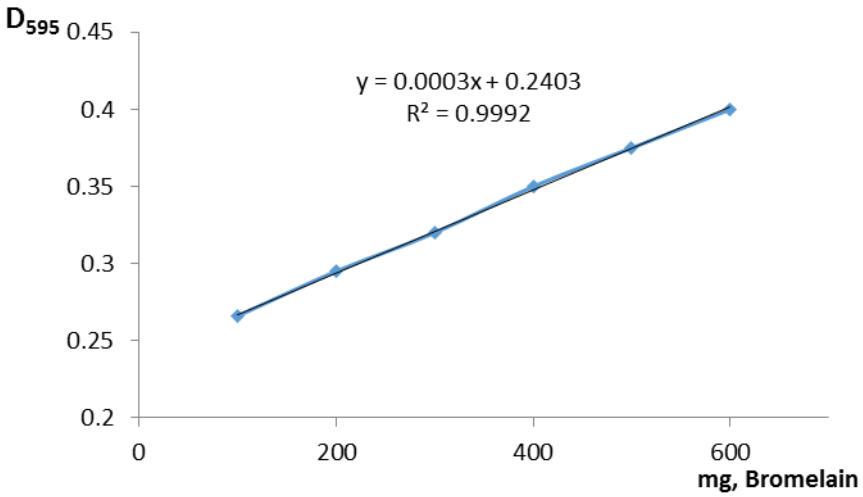

Figure 1.

Calibration curve of the substrate activity

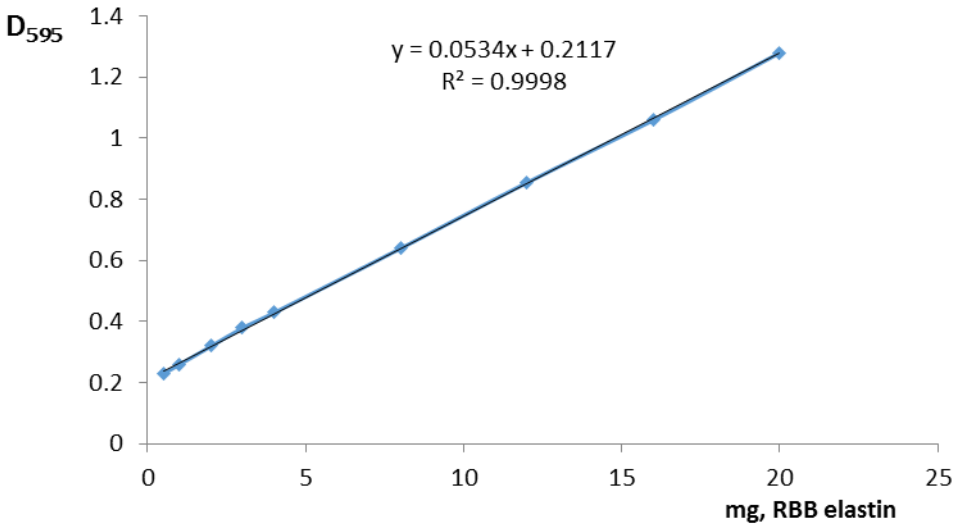

Figure 2.

Were studied the dependence of elastolytic activity of Bromelain on pH and temperature (***Fig. 3*** and ***Fig. 4***).

pH dependence of elastolytic activity of Bromelain

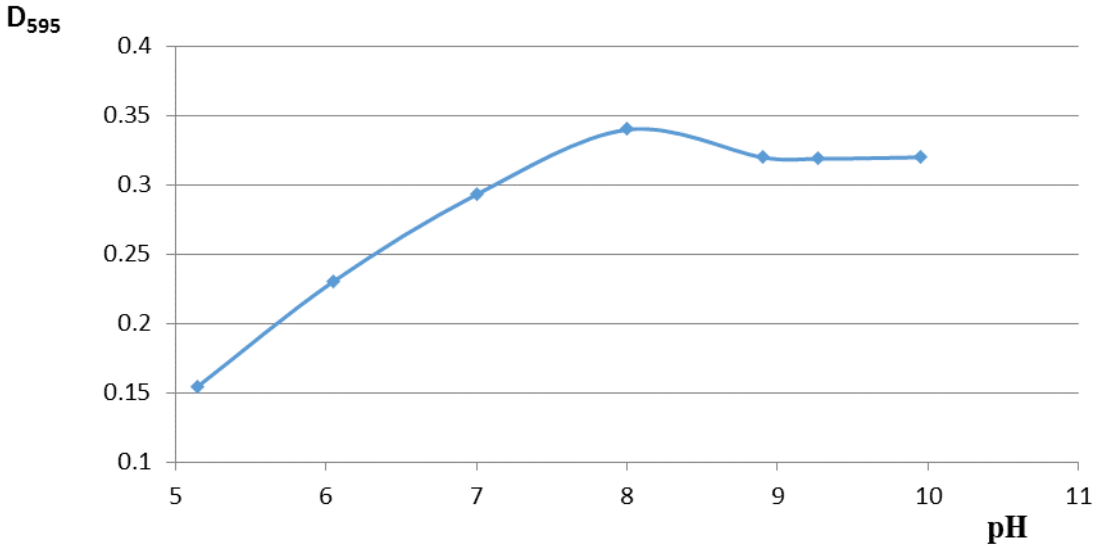

Figure 3.

Temperature dependence of elastolytic activity of Bromelain

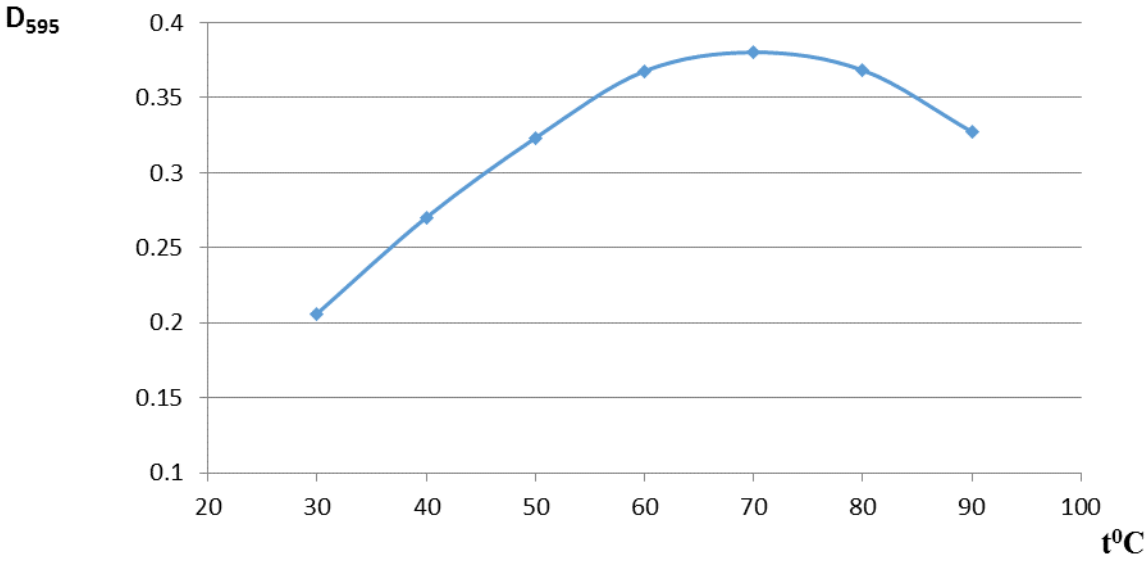

Figure 4.

The data reveal that the optimal elastolytic activity of Bromelain is occurred on pH 8.0 and at the temperature 70^0C.

The recovery test performed to evaluate the accuracy of the technique, corresponded to 96.0% (Table 1) and thus indicated a highly accurate technique. Additionally, such value

provides an evidence of possible deviation between the estimated values and their theoretical concentrations. All parameters combined provided experimental evidence to assure the applicability of the method in further experiments as well as defined limitations and features of the technique.

Table 1.

Results of the recovery test

Theoretical activity	Estimated value	Waste	Accuracy, %
31,125	30,086	1.039	96.0

CONCLUSION

The developed method of quantification of Bromelain in Bromelain containing gel is linear, precise and sensitive; Intra- and inter-day measurements. Are determined optimal pH area and temperature for revealing the optimal elastolytic activity of Bromelain. The method was validated as per ICH guidelines and can be adopted for the routine analysis of Bromelain in gel formulations.

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