AJPS, 2016, Vol. 16, No.2

Date of acceptance: 25-7-2016

Comparative Study of Prepared Bromelain Gel Formulations and their Evaluation by HPLC Determination

Kahtan Jassim Hasson
Department of Pharmacy, Al-Rasheed University College.
E-mail : kjhassoun@yahoo.com

Abstract:

Bromelain powder (proteolytic enzyme) was formulated as a gel for topical medical application by using two preparations method; Carbople 940 was used in the first formulation and Lutrol F 127 in the second.

The physical and pharmaceutical properties of the gel formulations including the diffusion rates through the skin in vitro were evaluated.

The best permeability of Bromelain was obtained with the 22% Lutrol F 127 gel formulation. In addition, a simple and rapid reversed-phase HPLC method was developed to monitor the quantitative analysis during the study. The used column was C18, 5µm (25 cm length), the mobile phase consisted of 70% methanol in 0.1 M dibasic potassium phosphate. The retention time of Bromelain was 7.3 minutes and the method proved precision as the straight line relationship of peaks areas and concentrations was with a correlation coefficient 0.998, and the RSD value for five successive determinations for same sample solution was 1.2%.

Key words: *Bromelain Gel, HPLC Determination*

دراسة مقارنة لتحضير البروملين بصيغة الجل وتقيم هذه الصيغ بالتحليل بواسطة الكروموتوغرافيا السائلة ذات الضغط العالي

قحطان جاسم حسون

قسم الصيدلة، كلية الرشيد الجامعة

الخلاصة:

تم تحضير البروملين (أنزيم محل للبروتين) على شكل جل لغرض الاستعمال الطبي الموضعي وبطريقتين تصنيعية : في الصيغة الأولى تم استعمال مادة (كاربوكول 940) أما الصيغة الثانية فكانت باستعمال مادة (اللوترول ف 127) وقد تم دراسة وتقييم الخواص الفيزياوية والصيدلانية للجل المحضر والمتضمنة : قياس درجة النفاذية خلال الجلد بأسلوب مختبري فكان مستحضر الجل الذي يحتوي 22% من مادة (اللوترول ف 127) هي الصيغة الأفضل حيث أعطى أعلى نسبة نفاذية في التجارب المختبرية.

تم وضع طريقة تحليل سهلة وسريعة لأجراء التحليل الكمي خلال هذه الدراسة وبتطبيق الحالة العكسية للكروموتوغرافيا السائلة باستعمال عمود الفصل نوع (C18,5µm) وبطول 25 سم، أما الطبقة المتحركة فتتكون من 70% ميثانول مع 30% محلول عياري 0.1 مول لمادة فوسفات البوتاسيوم ثنائية القاعدة، وكان وقت الاحتفاظ لمادة البروملين باستعمال هذه الطريقة هو 7.3 دقيقة. لقد أثبتت هذه الطريق دقتها في التحليل من خلال أنشاء خط مستقيم من علاقة مساحات المنحنيات مع كمي تركيز

Date of acceptance: 25-7-2016

محلول البروملين الخاضع للتحليل وكان معامل الارتباط لهذه العلاقة 0.998 أما معدل الانحراف المعياري النسبي فكان مقداره 1.2%.

Introduction:

Bromelain (proteolytic enzyme), is extracted from the stem of Pineapple plant, *Ananascomosus* (*A. sativus*) (*Bromeliaceae*), it is used in medicine as a digestive aid, and manufactured commercially as a tablets.

Bromelains have other reported medical uses [1], they are used as an adjunct in the treatment of soft tissue inflammation and edema associated with trauma and surgery, and in the treatment of deep derma, full thickness burns[2].

Several reported studies explained the effect of Bromelain as a debriding agent to be used in treatment of burn [3, 4, 5, 6] and as topical application for healing the wounds[7,8], treatment of muscles sore and control of inflammation and pain [9, 10].

There are new research paper discussing the immunological effect of Bromelain and its benefit in prevention the tumor growth [11, 12].

Recently; Bromelain was prepared as a cream and ointment, the therapeutic compositions of Bromelain were reported between 60 to 300 GDU per gram of product[13].

This represented work; discuss the formulation of Bromelain as a gel by using different concentrations of Bromelain within the therapeutic range stated previously for cream and different types of gelling agents. The physical and pharmaceutical characters of the formulated gel were studied to select the proper properties, particularly the permeability of Bromelainfrom the gel in vitro study, which indicated the need for suitable quantitative method of analysis. The usual biological method in which the proteolytic activity of Bromelain is measured is based on 60 minutes proteolytic hydrolysis of a Casein substrate at pH 6.0 and 37ºC, the soluble Casein is then measured sepctrophotometrically at 280nm. The biological method, however, is specific for measuring the activity and suits the raw material examination, but it cannot be applied for quantitative analysis of Bromelain in dosage form or measuring the amount Bromelain dissolved in diffusion medium, which need highly precise and fast analytical technique. 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 lack efficiency[14]. Therefore, an HPLC method of reversed–phase chromatography was developed in this work to follow the quantitative analysis of the formulated gel of Bromelain and its evaluation tests.

Materials and Methods:

Bromelain powder (2400 GDU/gm); Wisapple Co. (China), Lutrol f 127 (Poloxamer 407); BASF Chem. Co. (Germany). Carbopol 940, Propylene glycol, triethanolamine (TEA), Methyl paraben (M.P) and propyl paraben (P.P) are obtained from Al-Safa Phar. Ind. (Iraq).

High Pressure Chromatography Instrument, Shimadzo C. (Japan). Diffusion Enhancer Cell (Agilent Co.), pH-meter and Brookfield viscometer (Model RVTD II).

Preparation of the gel

Type A: Carbopol 940 (1%) as a gelling agent was used and dissolved in half quantity of water of formulation .In other part of water, the amount of bromelain was dissolved, then the quantities of Methyl paraben (M.P) and Propyl paraben (P.P) were added. Mix both parts and while continuous stirring the amount of Triethanolamine (T.E.A) was added drop wise until homogenous gel was obtained.

Type B: Lutrol F 127 as a gelling agent in two different concentrations was used and these amounts were dissolved in a part of

Date of acceptance: 25-7-2016

water of formulation. In the other part of water the Bromelain quantities were dissolved with addition of Propylene glycol. The two parts were combined and the water was added to volume with continuous stirring until homogenous gel.

The compositions of each ingredient of the gel formulations are recorded in table-1.

Table-1: The compositions of the different gel formulations.

formula	*Bromelain powder	Carbopol 940 %	Lutrol F 127 %	Propylene glycol%	T.E.A %	M.P %	P.P %	Water %
FA1	4	1	-	4	1.5	0.2	0.02	to 100
FA2	8	1	-	4	1.5	0.2	0.02	to 100
FB1	4	-	22	16	-	-	-	58%
FB2	8	-	22	12	-	-	-	58%
FB3	4	-	25	13	-	-	-	58%

***** Bromelain powder contains 2400 GDU/gm

Appearance test:

The clearness, color and homogeneity of the gel are tested by visual observation.

Gel pH: by using pH-meter.

Viscosity: was measured by Brookfield viscometer (Model RVTD II) and spindle No.6, operated at 100 rpm, the test was carried for 100 gm of gel.

Diffusion percent in vitro study:

The permeability of Bromelain from the gel in vitro was studied by using the Diffusion Enhancer Cell (Agilent Co.). A known amount of Bromelain gel was spread in the cavity of the cell on a cellulose membrane and fixed in a closed cell which was immersed in 200 ml of phosphate buffer pH 6.8, carried by a special tube suitable for this volume and adapted on a water bath of the dissolution system which was operated at 37.0 ºC±0.5 and 50 rpm. Samples of diffusion medium were withdrawn at intervals and replaced with the same volume of fresh diffusion medium.

Method of analysis and its validation:

The quantities of bromelain in the different gel formulations were determined by the developed reversed phase chromatography in which the column was C18, 5µm, 25 cm length. The mobile phase was consisted of 70% methanol with 30% of 0.1 M dibasic potassium phosphate and the pH was adjusted to 7.0 with the use of phosphoric acid. Samples of Bromelain solution from standard preparations or from diffusion medium were filtered through membrane filter size 0.45µ,then 25 µl of solution was injected through the column of HPLC. The detection was by U.V detector at 254 nm and the retention time of Bromelain was 7.3 minutes (Figure-1).

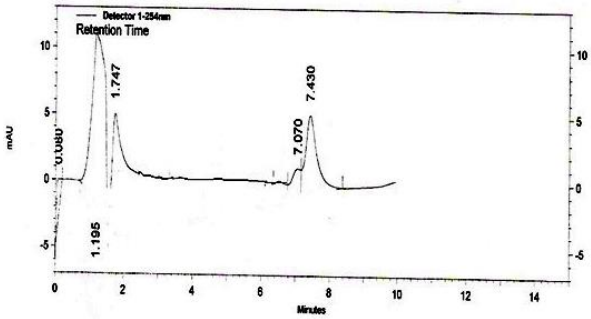

Figure-1: HPLC Chromatogram of Bromelain.

Validation:

Linearity: The precision of the developed HPLC method for quantitative works were proved by analysis of different dilutions of Bromelain standard solution and a straight line relationship between concentrations and peaks areas were constructed as it is shown in (Figure-2). Application of five successive injections of the same sample solution on the chromatograph gave a relative standard deviation (RSD) of 1.2%, the limit requirement RSD of united state pharmacopeia for efficient HPLC method is not more 2.0%.

Date of acceptance: 25-7-2016

The preparation of solution from gel formulation for the assay by HPLC is performed by dilution with 50% Methanol in water then filter through a membrane filter, pore size 0.45µ and doesn't need any farther extraction prior analysis.

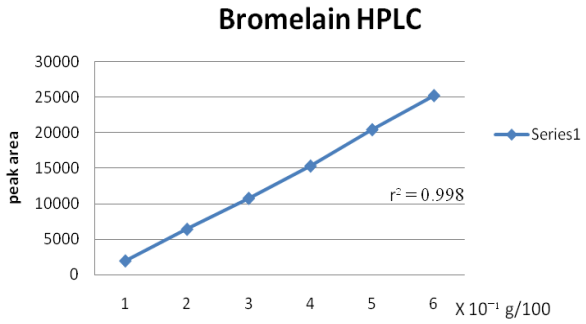

Figure-2: The relationship between concentrations and peaks areas of Bromelain determination by HPLC.

Result and Discussion:

The results of pharmaceutical tests on the different types of prepared Bromelain gel are illustrated in table-2. Formulation with Lutrol F 127 showed good properties, lower viscosity and high percentage of Bromelain released in the diffusion medium after 2 hours of diffusion test.

The diffusion profile of different gel formulations indicated the fast release of the effective dose and high percentage of dissolved bromelain in the diffusion medium of the gel prepared by Lutrol F 127 with relative to other type of gel (Figure-3).

Table-2: Pharmaceutical properties of different gel formulations.

Formula	Appearance	pH	Homogeneity	Viscosity	Diffusion %
FA1	Clear and Light green	7.1	good	4200	76
FA2	Clear and Light green	7.2	good	4500	74
FB1	Clear and Light green	6.8	good	3000	92
FB2	Clear and Light green	7.1	good	3100	80
FB3	Clear and Light green	7.0	good	3400	78

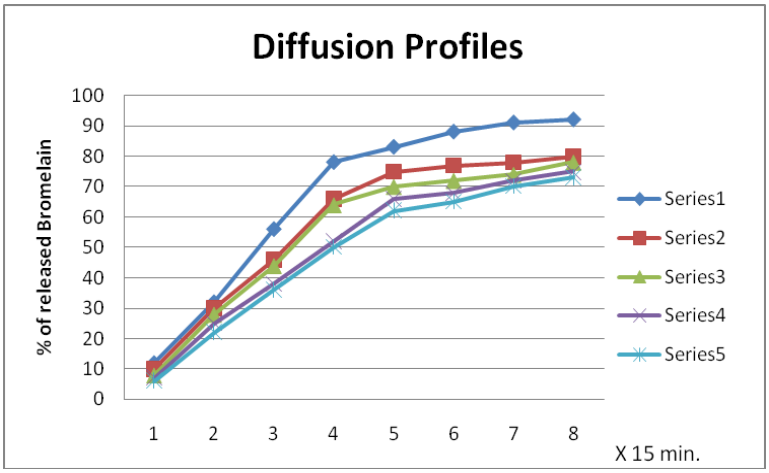

Figure-3: Diffusion profile of different Bromelain gel formulations, according to series; FB1, FB2, FB3, FA1, and FA2.

Date of acceptance: 25-7-2016

References:

1 - Rajendra Pavan, Sapna Jain, Shraddha, and Ajay Kumar. Properties and Therapeutic Application of Bromelain, Biotechnology Research International. 2012; 2012: Article ID 976203, 6 pages.

2 - The Extra Pharmacopea, Martindale, the Complete Drug Reference, Bromelain Monograph. Pharm. Press 36th Edition, 2009. P. 2269.

3 - Houk JC, Chang CM and Klein G, Isolation of an effective debriding agent from the stem of Pineapple plant. Int. J. Tissue React. 1983; 5 (2): 125-34.

4 - Ahle NW, Hamlet MP. Enzymatic frostbite aschar debridement by Bromelain. Ann. Emerg. Med. 1987; 16 (9): 1063–5.

5 - Rosenberg L, Lapid O, Bogdanov-Berezovsky A, Glesinger R, Krieger Y, Silberstein E, *et al.* Saftey and efficacy of a proteolytic enzyme for enzymatic burn debridement, Burn. 2004; 30 (8): 843-50.

6 - Singer AJ, McClain SA, Taira BR, Rooney J, Steinhauff N, Rosenberg L. Rapid and selective enzymatic debridement of Procine comb burn with Bromelain derived-Debrase: acute-phase preservation of non-injured tissue and zone of stasis. J. Burn Care Res. 2010; 31(2): 304–9.

7 - Wu SY, Hu W, Zhang B, Liu S, Wang JM, Wang AM. Bromelain ameliorates the wound microenvironment and improves the healing of firearm wounds. J. Surg. Res. 2012; 176 (2): 503–509.

8 - Ramund J, Gray M. Enzymatic wound debridement. J. Wound Ostomy Continence Nurs. 2008; 35 (3): 273–80.

9 - Stone MB, Merrick MA, Ingersoll CD, Edwards JE. Preliminary comparison of Bromelain and Ibuprofen for delayed Muscle Soreness Management. Clinical Journal of Sport Medicine, 2002; 12 (6): 373–8.

10 - Tassman GC, Zafran JN, Zayon GM. Evaluation of a plate proteolytic enzyme for the control of inflammation and pain. J. Dent. Med. 1964; 19: 73–77.

11 - Barth H, Guseo A, Klein R. In vitro study on the immunological effect of Bromelain and Trypsin on mononuclear cells form. Eur. J. Med. Res. 2005; 10 (8): 325–31.

12 - Tassig SJ, Szekerczes J, Batkin S. Inhibition of tumour growth in vitro by bromelain, an extract of the pineapple plant (Ananas comosus). Planta Med. 1985; 6: 538–9.

13 - Merry Richon. Topical Therapeutic Compositions containing Bromelain. Patent Publication, US, No. 20150238576 A1, dated, 27-8-2015.

14 - Fadzilah AM, Madihah AG, Sitizalita T, Khadijah KA. Stability of Bromelain-polyphenol Complex in Pineapple Juice. J. Teknologi. 2008; 49 (F) Dis.: 27-38.