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

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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 وتقييم هذه الصيغ بالتحليل بواسطة الكروموتوغرافيا السائلة ذات الضغط العالي

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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 الانتشار 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 لأجراج التحليل الكمي خلال هذه الدراسة وتطبيق الحالة العكسية الكروموتوغرافيا السائلة باستعمال عمود الفصل نوع (C18, 5µm) وطول 25 سم، أما الطبقة المتحركة فتكون من 70% ميثانول مع 30% محلول غليان 0.1 مول لمادة فوسفات البوتاسيوم ثنائية القاعدة، وكان وقت الانتظار لمادة البروملين باستعمال هذه الطرقيات هو 7.3 دقيقة. لقد أثبتت هذه الطريق دقتها في التحليل من خلال أنشاء خطي مستقيمين من علاقة مساحات المنحنيات مع كمي تركيز
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 Bromelain from 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 37°C, the soluble Casein is then measured spektrophotometrically 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 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
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.**

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

* 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 °C±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%.
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.

<table>
<thead>
<tr>
<th>Formula</th>
<th>Appearance</th>
<th>pH</th>
<th>Homogeneity</th>
<th>Viscosity</th>
<th>Diffusion %</th>
</tr>
</thead>
<tbody>
<tr>
<td>FA1</td>
<td>Clear and Light green</td>
<td>7.1</td>
<td>good</td>
<td>4200</td>
<td>76</td>
</tr>
<tr>
<td>FA2</td>
<td>Clear and Light green</td>
<td>7.2</td>
<td>good</td>
<td>4500</td>
<td>74</td>
</tr>
<tr>
<td>FB1</td>
<td>Clear and Light green</td>
<td>6.8</td>
<td>good</td>
<td>3000</td>
<td>92</td>
</tr>
<tr>
<td>FB2</td>
<td>Clear and Light green</td>
<td>7.1</td>
<td>good</td>
<td>3100</td>
<td>80</td>
</tr>
<tr>
<td>FB3</td>
<td>Clear and Light green</td>
<td>7.0</td>
<td>good</td>
<td>3400</td>
<td>78</td>
</tr>
</tbody>
</table>

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


