Development of HPLC Analysis of α-mangostin in BB Cream Containing *Garcinia mangostana* Fruit Rind Extract

การพัฒนาวิธีวิเคราะห์แอลฟาแมงโกสตินในบีบีครีมที่ผสมสารสกัดเปลือกผลมังคุดโดยวิธีเฮช พี แอล ซี

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ABSTRACT

The ethanol extract of pericarp of mangosteen (*Garcinia mangostana* Linn.) consists of α-mangostin that shows many biological properties including antioxidant, anti-inflammatory, anti-bacterial activities and was prepared as BB cream. The objective of this study was to develop HPLC analysis method for determination of α-mangostin in BB cream. For HPLC method validation, linearity was studied by preparing standard stock solution of α-mangostin at 5 different concentration levels. A good linear relationship (r = 0.9999, y = 25.51x - 5.65) was observed in the range of 13.0 - 65.0 µg/mL. For the accuracy of the method, recovery studies were carried out in triplicate at three concentration levels. The recovery of α-mangostin was found to be in the range of 98.47-99.10 %. The intra-day precision and inter-day precision of the assay method were evaluated and the percentages of RSD for six assay values were found at 0.48 and 0.75%, respectively. LOD and LOQ were also evaluated which were 10 and 30 ng/mL, respectively.

บทคัดย่อ

สารสกัดเอทานอลของเปลือกผลมังคุดที่มีสารแอลฟาแมงโกสตินซึ่งเป็นสารที่มีฤทธิ์ต้านอนุมูลอิสระ ต้านการอักเสบ และต้านเชื้อแบคทีเรีย ได้ถูกนำมาเตรียมให้อยู่ในรูปแบบบีบีครีม วัตถุประสงค์ของการวิจัยนี้คือการพัฒนาวิธีวิเคราะห์สารแอลฟาแมงโกสตินในบีบีครีมโดยวิธีโครมาโตกราฟีของเหลวสมรรถนะสูง จากผลการทดลองพบว่าความเป็นเส้นตรงของการวิเคราะห์มีค่าสัมประสิทธิ์สหสัมพันธ์เท่ากับ 0.9999 และมีสมการเส้นตรง y = 25.51x - 5.65 ในช่วงความเข้มข้น 13.0 - 65.0 ไมโครกรัมต่อมิลลิลิตร ความถูกต้องของการวิเคราะห์หาโดยการคำณวนเปอร์เซนต์การคืนกลับของสารแอลฟาแมงโกสตินในระดับสามความเข้มข้น ซึ่งพบอยู่ในช่วง 98.47-99.10 เปอร์เซ็นต์ ค่าความเที่ยงตรงของการวิเคราะห์ในวันเดียวกันและระหว่างวันมีค่าเท่ากับ 0.48 และ 0.75 เปอร์เซนต์ ค่าความเข้มข้นต่ำที่สุดที่ตรวจพบได้และค่าความเข้มข้นต่ำที่สุดที่จะวิเคราะห์ได้มีค่าเท่ากับ 10 และ 30 นาโนกรัมต่อมิลลิลิตร

Key Words: *Garcinia mangostana*, BB cream, α-mangostin, HPLC

คำสำคัญ: มังคุด บีบีครีม แอลฟาแมงโกสติน เฮช พี แอล ซี

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Introduction

Blemish Balm cream or BB cream is a cosmetic product item sold mainly in Asian markets especially Korea. It was originally formulated in the 1960s in Germany by dermatologist Dr. Christine Schrammek to protect her patients' skin after facial peels and surgery (Chang, 2012). The trend of this kind of cream makes the majority of larger beauty brands have introduced BB creams to Western markets. BB cream composes of 4 major components as base, foundation, sun block and skincare (Latimer, 2012). BB cream incorporate a variety of ingredients such as skin conditioning agents, chelating agents, emulsifying agents, emollients, anti-caking agents, preservative etc. Especially, sunscreen agents like titanium dioxide, micronized zinc oxide and benzophenone 3 (Leelapornpisit, 1991). Also, these sunscreen agents have been increasingly reported for allergic and contact dermatitis

Mangosteen (*Garcinia mangostana* Linn.) belongs to the family Guttiferae. Mangosteen is one of the most famous fruits in Thailand (Priya et al., 2010) and the pericarps of *G. mangostana* have been widely used as a traditional medicine for the treatment of diarrhea, skin infection and chronic wounds in South East Asia for many years. Previous studies have been demonstrated that the best pharmacological of organic compound isolated from pericarp extract of mangosteen is α-mangostin. Alpha-mangostin is a natural xanthonoid, which is a yellow crystalline solid with a xanthone core structure. It shows many biological properties including antioxidant, anti-inflammatory, anticancer, anti-allergy, increasing immunity and anti-bacterial activities (Pedraza-Chaverri et al., 2008). This pericarb extract were included in the formulation in our research as an ingredient in BB cream for anti-anti-oxidant and anti-bacterial properties.

Various analytical methods for quantitative analysis of α-mangostin have been reported such as gas chromatography (GC) (Jefferson et al., 1971) and high performance liquid chromatography (HPLC) (Pothitirat et al., 2009; Yodhnu, 2009; Walker, 2007). However, there is no reported on analysis of α-mangostin in BB cream by HPLC method.

Objective of the study

Our objective was to develop a HPLC method for analysis of α-mangostin in Blemish Balm cream that have never been reported before.

Methodology

Instrument and reagents

Deionized Water, 2Na-EDTA, Glycerin, Nikkomulese WO, Silicone STV-5, Silblend-91, Lexfeel D5, Ultrafine TiO2-A212SA, Color of Iron Oxides, Plastic Powder D400 and DMDM Hydrantoin were purchased from Namsiang, Bangkok, Thailand.

All other reagents and solvents were reagent grade and used without further purification. TLC was performed on silica gel GF_{254} (Merck). For column chromatography, silica gel (Merck 230-400 mesh) was used. The concentration of crude extract was performed on Buchi Rotavapor R215. The HPLC analysis was performed on Agilent 1260 infinity system with UV detector at 320 nm. NMR spectra were recorded with a Bruker Avance (^{1}H, 300 MHz) spectrometer. Chemical shifts are reported in ppm, and coupling constants are reported in Hz. All NMR spectra were obtained in deuterated chloroform ($CDCl_3$) and referenced to the residual solvent peak.

Mass spectra were obtained from Agilent LC 1200 coupled with Bruker microTOF.

Plant materials

Mangosteen was purchased from local market, PathumThani, Thailand on 16 June 2014 and collected only pericarp of mangosteen. The material was identified by Dr.Prasan Tangyuenyongwatana, Faculty of Oriental Medicine, Rangsit University, Thailand. The voucher specimen of *G. mangostana* (SRU 051) was deposited at Faculty of Oriental Medicine, Rangsit University, Pathumthani, Thailand. Pericarps were dried by hot air oven set at 50 °C for 48 hrs. Dried mangosteen pericarp was ground and screened through a 40-mesh sieve.

Preparation of crude and partial-purified extracts

Crude extracts were prepared from mangosteen pericarp. Dried powder material of mangosteen pericarp (500 g.) was macerated in 1.5 L of ethanol for 7 days. Then, ethanol extract was filtrated with Watmans No.1 and evaporated by rotary evaporator to obtain 42 g of finally crude dark brown extract. The crude extract (2 g) was dissolved in CH_2Cl_2 : MeOH (7 : 3) (10 ml). Then the mixture was subjected to silica gel column chromatography and eluted with CH_2Cl_2 : MeOH (7 : 3) to obtain dark brown extract (1.1 g).

Isolation of α-mangostin

The crude extract (1.1 g) was dissolved in CH_2Cl_2 : MeOH (7 : 3) (5 ml). Then the mixture was subjected to silica gel column chromatography and eluted with mobile phase CH_2Cl_2 : MeOH (7 : 3). After that, fractions 11-18 were collected and evaporated to obtain a yellow crystalline solid 198 mg with melting point 180-182 ºC and 95% purity. UV (Abdalrahim et al, 2012) : λ_{max} 244, 343 nm, IR (Madihah et al, 2013): (KBr disc) 3256, 2925, 1639, 1460 cm^{-1} ^1H NMR (Ly et al, 2009) : (300 MHz, $CDCl_3$) δ[ppm] 1.69 (s, 4H), 1.76 (s, 3H), 1.83(s, 6H), 3.45 (d, J = 7.15 Hz, 2H), 3.81(s, 3H), 4.09 (d, J = 6.26 Hz, 2H), 5.26 (m, 2H), 6.29 (s, 1H), 6.82 (s, 1H), 13.77 (s, 1H). ^{13}C NMR : (75.47 MHz, $CDCl_3$) δ[ppm] 182.0, 161.6, 160.6, 155.8, 155.0, 154.5, 142.5, 137.0, 135.7, 132.2, 123.1, 121.4, 112.2, 108.5, 103.6, 101.6, 93.3, 62.0, 26.5, 25.8, 25.8, 21.4, 18.2, 17.9 and MS (GC/MS): M^+ = 410.

Formulation of BB cream containing mangosteen pericarb extracts

The BB cream containing mangosteen extracts was prepared by following procedure.

Step 1 –Aqueous phase preparation: 2Na-EDTA (0.1%w/w) was dissolved in deionized water, then glycerin (7%w/w), 1, 3-butylene glycol (3 %w/w), DMDM Hydrantoin (1%w/w) and mangosteen extract (1, 2, 3%w/w) were added in the water phase. The mixture was then heated up to 80 °C.

Step 2 - Oil phase preparation: Nikkomulese WO (8%w/w), Silicone STV-5(12 %w/w), Silblend-91 (10%w/w), Lexfeel D5 (5 %w/w), Ultrafine Tio2-A212sA (6, 8, 10%w/w), Iron Oxides (0.268%w/w), Benzophenone3 (5, 7, 9%w/w) and plastic powder D400 (5%w/w) were added together and heated at 80 °C.

Step 3 - Mixing aqueous phase and oil phase: Oil phase was added to aqueous phase with continuous stirring for 20-25 min and then the mixture was homogenized to obtain uniform emulsion. The emulsion was then introduced to the container and stored at room temperature not exceeding 30°C for further experiment.

Total nine formulations of BB cream were prepared using two different sunscreen agent and mangosteen extract in three concentration which were Ultrafine Tio2-A212sA (6, 8, 10%w/w), Benzophenone3 (5, 7, 9%w/w) and mangosteen extract (1, 2, 3%w/w), respectively. All the prepared BB cream was then subjected to various evaluate test in order to select the best formulation.

Determination of in vitro SPF in BB cream

In vitro SPF was determined by measure of BB cream containing mangosteen extracts using Optometrics Model SPF-290S (Optometric copration, USA) . The condition for measurement the sun protection factor (SPF) of the BB cream was operated from 290-400 nm. Approximately 110 mg of sample was spread on 56 cm^2 area of Transpore tape to obtain a sample film thickness of 2 mg/cm^2. After that the SPF values were evaluated by WIN SPF software.

Extraction and HPLC analysis.

BB cream (0.50 g) was accurately weighed and transferred to a 50 mL beaker and isopropanol (5 mL) was added. The mixture was stirred with magnetic stirrer until BB cream was dissolved. Then the mixture was added to 10 mL volumetric flask and the flask was placed in an ultrasonic bath and sonicated for 15 min. After that the isopropanol was added to volume and mixed well. Five milliliters of the mixture was transfer to a test tube and centrifuges at 3,000 rpm for 15 min. The upper layer was collected and and 20 µL of the filtrate was injected into the HPLC system, an Agilent 1260 series with UV detector at 320 nm. The separation was performed on ACE 5 C18 V12-7111 (5 µm, 150 x 4.6 mm) column; flow rate was 0.8 mL/min and the solvent system was a gradient elution of 1% acetic acid in water and CH_3CN ranging from 90:10, 100:0, 100:0, 90:10 at 0, 10, 15, and 20 min, respectively.

Method validation

For HPLC validation method, stock standard solution was prepared by dissolving 1.3 mg of α-mangostin in methanol in a 10 mL volumetric flask and adjusted to volume with methanol. Various amounts of the standard solution were prepared by transferring 1, 2, 3, 4, and 5 mL of stock solution into 10 mL volumetric flasks and adjusted to volume with methanol. Linearity relationship between peak area and concentration of the standard solutions was evaluated at 5 concentration levels over the range of 13.0 - 65.0 µg/mL. Each concentration was done in triplicate. Repeatability and intermediate precision of the developed method were expressed in term of relative standard deviation (RSD) of peak area. Intraday precision was studied by repeat analyzing on the same day of mangosteen fruit rind BB cream extract (n=6). Interday precision was determined on three different days at the same concentration by the proposed method. The accuracy of the method was evaluated by performing recovery study by adding known amount of the reference compound at three levels to the isopropanol extract sample. Then the solutions were analysis by the proposed method. Three determinations were performed for each level of concentration and the recovery results were calculated. The limit of detection (LOD) and limit of

quantitation (LOQ) were performed by determination a series of concentrations of α-mangostin standard solution (10-200 ng/mL). Signal-to-noise ratios of 3:1 and 10: 1 were considered as LOD and LOQ, respectively.

Results

The BB cream formulation of mangosteen extracted was studied for physical properties and *in vitro* SPF determination.

All the prepared BB cream was glitter, brown in color and homogeneous. When applied BB cream on the skin, the result was shown a good spreadability. The pH of BB cream was found in range of 5.60 - 5.74. Viscosity of the preparations demonstrated between 3125.32 - 3620.20 cps. The property of BB cream complied with official acceptance criteria and SPF of this cream was found in range of 9.09 - 52.47.

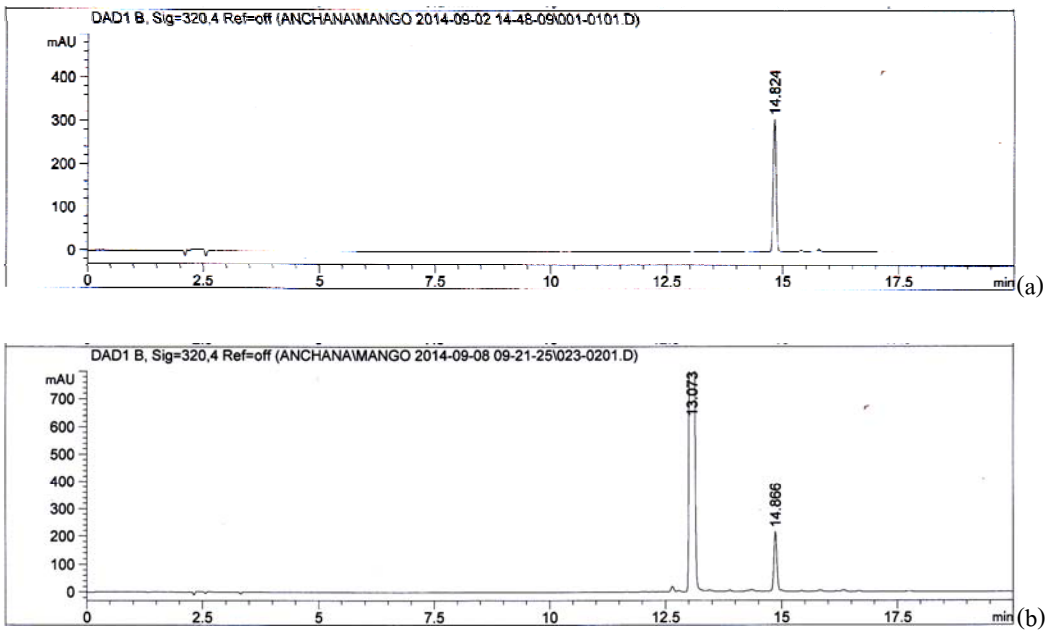

Figure 1 HPLC chromatograms (a) standard α-mangostin (b) sample BB cream

For HPLC method validation, the HPLC chromatograms of standard α-mangostin and BB cream sample were demonstrated in Figure 1. The linear relationship (r = 0.9999, y = 25.51x - 5.65) between peak area and concentration was observed in the range of 13.0 - 65.0 µg/mL (Figure 2). For the accuracy of the method, recovery studies were carried out in triplicate at three concentration levels. The recovery of α-mangostin was found to be in the range of 98.47-99.10 %. The intra-day precision and inter-day precision of the assay method were evaluated and the percentages of RSD of six assay values were found at 0.48 and 0.75, respectively. LOD and LOQ were also evaluated which were 10 and 30 ng/mL, respectively. For sample analysis, the concentrations of α-mangostin ranged from 5.86 – 6.81 %W/W.

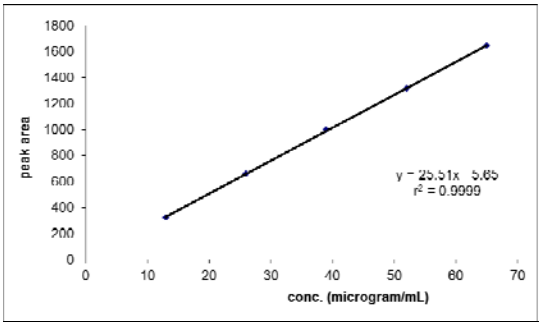

Figure 2 Calibration curve of α-mangostin

Discussion and Conclusions

The proposed HPLC method showed specificity and moderate analysis time. The extraction processes can get rid of insoluble materials in BB cream by using isopropanaol which dissolves fewer amounts of ingredients in the formulation. The ultrasonic extraction made the preparation of sample more easy and accurate. The detector wavelength at 320 nm that was used in this analysis showed specificity for α-mangostin and improved the chromatogram clear and clean. Alpha-mangostin retention time (t_R) showed at 14.8 min. The overall analysis time for one injection was 20 min. The validation of the assay for linearity gave a good linear range of 13.0 - 65.0 µg/mL with correlation coefficient (r) = 0.9999 for α-mangostin. The intraday precision of injection results demonstrated the exceptional relative standard deviation (RSD) of precision of less than 0.5% (n=6) and the interday precision of less than 0.75% (n=6). The recovery study was performed by spiking the standard compound in three different concentrations to a control BB cream extract. After the HPLC analysis, the recovery results of α-mangostin at three different concentrations were 101.1, 97.97 and 99.03% (n=3), with an average of 99.37 ± 1.59%. The LOD and LOQ were 10 and 30 ng/mL, respectively.

This proposed method has more interesting point than other reported methods such as Pothitirat and co-worker use gradient RP-HPLC with 70-80 % acetonitrile in 0.1 %v/v ortho phosphoric acid which give higher run time about 30 min per injection. This method is suitable for mangosteen pericarp control (Pothitirat et al., 2009). Yodhnu and co-worker use isocratic RP-HPLC to control α-mangostin in mangosteen peel extract using 0.2% formic acid–acetonitrile (30:70, v/v) as mobile phase. The method is a good one; however the analysis method still uses to control the mangosteen peel extract (Yodhnu et al., 2009). These two methods are not sure for applying to BB cream of analysis of α-mangostin because there is no report on this finish product.

For our method when applied to sample analysis, the HPLC analysis for BB cream preparation has a convenient procedure for sample preparation of finish products and the concentrations of α-mangostin range from 5.86 – 6.81%W/W. This method shows a good linear relationship, high precision, accuracy and can be used as a method for analysis of α-mangostin in high complex matrix of finish product such as cream or lotion.

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