

เทคนิคโครมาโตกราฟีของเหลวสมรรถนะสูงสำหรับการวิเคราะห์ การปนเปื้อนไฮโดรควิโนนในเครื่องสำอางสมุนไพรชนิดครีม HPLC Analysis for the Contamination of Hydroquinone in Skin-whitening Herbal Cosmetic Cream.

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บทคัดย่อ

การศึกษาในครั้งนี้มีเป้าหมายเพื่อตรวจสอบการปนเปื้อนของไฮโดรควิโนนในเครื่องสำอางสมุนไพรชนิดครีมที่วางจำหน่ายในท้องตลาดในเขตจังหวัดขอนแก่น โดยใช้วิธีโครมาโตกราฟีของเหลวสมรรถนะสูงร่วมกับเครื่องตรวจวัดชนิดไดโอดแอเรย์ในการวิเคราะห์ สำหรับการเตรียมตัวอย่างนั้นได้มีการใช้เมธานอลในการสกัดไฮโดรควิโนนออกจากตัวอย่างชนิดครีม สภาวะในการวิเคราะห์จะใช้คอลัมน์ชนิด Hypersil® ODS C18 และใช้สารละลายผสมระหว่างเมธานอลกับน้ำในอัตราส่วน 40:60 เป็นสถานะเคลื่อนที่ พร้อมกับใช้เครื่องตรวจวัดชนิด UV ที่ความยาวคลื่น 289 นาโนเมตร จากการสร้างกราฟมาตรฐานพบว่าให้ค่าสัมประสิทธิ์การตัดสีนใจเท่ากับ 0.9999 ค่าการเบี่ยงเบนสัมพัทธ์ของความเที่ยงภายในวันเดียวกันและระหว่างวันมีค่าน้อยกว่า 2 ซัดจำกัดในการตรวจวัดและหาปริมาณมีค่าเท่ากับ 0.01 และ 0.1 ไมโครกรัมต่อมิลลิลิตร ตามลำดับจากการวิเคราะห์ตัวอย่างทั้งหมด 23 ตัวอย่าง พบว่ามีการตรวจพบไฮโดรควิโนนปนเปื้อนใน 4 ตัวอย่าง โดยมีปริมาณตั้งแต่ 2.06-3.29 เปอร์เซ็นต์ น้ำหนักต่อน้ำหนัก ดังนั้นจึงควรจะต้องมีการคำนึงถึงการปนเปื้อนของไฮโดรควิโนน ซึ่งก็จะนำไปสู่การควบคุมคุณภาพของผลิตภัณฑ์เหล่านี้ให้ดียิ่งขึ้นต่อไป

Abstract

The purpose of this study was to investigate the contamination of hydroquinone in skin-whitening herbal cosmetic cream sold commercially in the area of Khon Kaen city. A high performance liquid chromatographic method equipped with diode array detector was used for the analysis of hydroquinone in these products. Sample preparation was performed by using methanol for extracting hydroquinone from herbal cosmetic cream. The analysis was carried out using Hypersil® ODS C18 column and a mixture of methanol:water in the ratio of 40:60 was used as the mobile phase. The UV detector was Set at 289 nm for the detection of hydroquinone.

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The coefficient of determination (R^2) for the regression equation of the standard curve was 0.9999. The relative standard deviations for within-day and between-day were less than 2%. The limit of detection and limit of quantitation were 0.01 and 0.1 $\mu\text{g/ml}$, respectively. From the analysis of twenty-three samples, hydroquinone was detected in four samples. The amounts of hydroquinone were in the range of 2.06-3.29 %w/w. Thus, there should be concern about the contamination of hydroquinone, which will lead to the quality control improvement of these products in the future.

คำสำคัญ: ไฮโดรควิโนน, ขจัดสีผิว, เครื่องสำอางสมุนไพรชนิดครีม

Keywords: hydroquinone, skin-whitening, herbal cosmetic cream

Introduction

Hydroquinone is one of the compounds which may be added into skin-whitening herbal cosmetic cream to increase the effectiveness of skin depigmentation. Hydroquinone or 1, 4-benzenediol is a synthetic compound mainly used as antioxidant in the photography industry. Its structure is shown in Figure 1. The synonyms of this compounds are hydroquinol, quinol, *p*-benzenediol, 1,4-benzenediol, *p*-hydroxybenzene, *p*-hydroxyphenol, 1,4-dihydroxybenzene and dihydroquinone. Its appearance is an off-white powder or white needle-like crystals. With respect to organic solvents, the solubility varies from 57 percent in methanol to less than 0.1 percent in benzene (solubility: 1 in 17 of water, 1 in 4 of ethanol, 1 in 51 of chloroform and 1 in 16 of ether). Hydroquinone is combustible when preheated. However it has a depigmentation effect on human skin (Parfitt, 1999). The mechanism of action of hydroquinone is based on the inhibitory effect of enzymatic oxidation of tyrosine, and the hydroxylation of tyrosine is the first step in the formation of the melanin pigment and suppressing other melanocyte metabolic processes which lead to inhibiting melanin formation (Jerrole and Frank, 1998; Kenneth, 2003). Melanin is the black pigment which appears in skin

and hair. The adverse effects on the dermatological system are dermatitis, dry skin, erythema, stinging, ochronosis, inflammatory reaction and skin sensitization. Chronic exposure to hydroquinone on the eye has resulted in injuries, which vary from mild irritation and staining of conjunctiva and cornea to changes in the thickness and curvature, loss of corneal luster and impaired vision. Prolonged exposure can cause severe ocular effects. Due to the adverse effects and toxicological effects of hydroquinone, EU countries have restricted its use in cosmetic products to 2 percent or less. The Food and Drug Administration in the U.S.A. have restricted hydroquinone concentration to 1.5-2 percent in non-prescription skin lighteners (U.S. Environmental Protection Agency, 2007). Thai regulations since 1996 (Minister of Public health, 1996) have not allowed the addition of hydroquinone to cosmetic products.

Thailand is a country in the tropical zone, thus the sunshine is very strong and can cause skin blemishes or severe skin irritation. This is one of the reasons why people use the skin depigmentation products for skin, especially on the face. Some Thai people do not know about the danger of products which have hydroquinone as an additive and some of the companies or manufacturers do not have details in the label of the product about adding hydroquinone

in the skin cosmetic cream. The Thai government encourages people to produce the product in their communities in order to improve the national economy under the self-sufficiency economic guidelines of His Majesty King Bhumibol Adulyadej (Chaipattana Foundation, 2007) and encourages people to use raw materials from herbal plants. At the present, there are many skin-whitening herbal cosmetic products on the market. These products are sold in many local places, shopping centers, department stores, groceries and beauty shops. However, the producers may have an inadequate knowledge of the quality control of their products. They may add some chemical substances into the products which can improve the efficiency of the skin depigmentation. From the headline news of the Bangkok Post newspapers, 12 December 2001 (Bangkok Post, 2001), the Thai FDA examined 25 brands of cosmetics blacklisted by the Foundation for Consumers for containing carcinogenic and other hazardous substances. At that time the survey of commercial cosmetics in the local market, conducted by the Foundation in 11 provinces, found 25 local brands contained prohibited chemical substances. One of the prohibited chemical substances was hydroquinone. The others were mercury and ammoniated mercury. This is a sample situation from which we learn that prohibited chemical substances such as hydroquinone might be added into these herbal cosmetic products.

So, the aim of our study was to determine hydroquinone in the skin-whitening herbal cosmetic cream which is sold commercially in the area of Khon Kaen city. This is one of the standard criteria for the quality control of these products which are important for human health protection and consumer safeguarding. Several analytical methods have been used for the analysis of hydroquinone, e.g. colorimetric methods (Belcher and West, 1951), iodimetric methods

(Sharma et al, 1976), HPLC (Scobbie and Grove, 1999; Lee et al., 1993; Lopez et al., 2005), MEKC (Sakodinska et al., 1992) and capillary electrochromatography (Desiderio et al., 2000). HPLC equipped with a photodiode array detector is a suitable method for the analysis of hydroquinone, because it can give reliable results, enabling the separation of hydroquinone from the other components in the products with a short time for analysis.

Materials and methods

1. Materials

Hydroquinone was purchased from Merck® (Germany). Methanol (AR grade) was purchased from BDH® (England). Blank cream and deionized water (ultrapure for HPLC purpose) were supplied from Faculty of Pharmaceutical Sciences (Khon Kaen University).

2. Instruments

High performance liquid chromatography (HPLC) instrument equipped with photodiode array detector and autosampler system (Hewlett Packard® LC 1100 liquid chromatograph, model G1311A) was used in this study. Ultrasonic bath (Crest®, model 1875TAE) was used for the sample preparation.

3. Skin-whitening herbal cosmetic products

All twenty-three cream products were purchased in the area of Khon Kaen city. The prices varied from 10 to 280 baht. The ingredients were different in each product.

4. Methods

4.1 High performance liquid chromatography conditions

The column used in this study was Hypersil® ODS C18 (4mm x 250mm). Methanol and water in the ratio of 40: 60 were used as the mobile phase for the analysis (Lopez et al., 2005). Hydroquinone was detected at the wavelength of 289 nm. The flow rate was 1.0 ml/min. The volume of each injection in the HPLC system was 20 μ l and the syringe was washed with methanol after each injection. To determine the amount of hydroquinone, the peak area of each sample injection was compared to the peak area of standard hydroquinone solution from the calibration curve.

4.2 Validation method

The analytical method was validated to approve its efficiency. Validation criteria included a study on linearity, accuracy, precision, limit of detection and limit of quantitation. This HPLC method was accurate, reliable and can be used in the determination of hydroquinone since the within-day and between-day precision were lower than 2 %RSD. The assay was linear over the range from 0.1 to 20 μ g/ml ($r^2 > 0.99$). The limits of detection and quantitation were 0.01 and 0.1 μ g/ml, respectively.

4.3 Standard curve preparation

The stock solution of hydroquinone was prepared at a concentration of 1000 μ g/ml. Methanol was used as the solvent. After that, the stock solution was diluted with the mobile phase solution which was the mixture of methanol and water (40:60) to get the series of six different hydroquinone concentrations ranging from 0.1 μ g/ml to 20 μ g/ml. Linear regression equations and coefficient of determination were obtained from plotting the concentration versus the peak area of hydroquinone which appeared in the HPLC chromatogram from

each standard concentration. All standard solutions were analyzed by injecting into the HPLC system. The peak area of each standard solution was obtained. The standard curve was plotted between peak area and standard hydroquinone concentration with the x axis representing concentration while the y axis represented peak area.

4.4 Sample preparation

Sample preparation was applied from the method of Lopez (2005). A portion of each herbal cosmetic cream product was accurately weighted at about 0.3 g in a 25ml volumetric flask. Then methanol in an appropriate volume of about 5ml was added into a volumetric flask and shaken for about 1 min until the homogeneous suspension was obtained. The suspension in the volumetric flask was then placed in an ultrasonic bath in which the temperature was controlled to 50 degree Celsius and sonicated for 30 min so it could be assumed that the hydroquinone was extracted from the sample. After the solution cooled down, the mobile phase was added into the volumetric flask and the volume was adjusted to 25 ml. The suspension was then filtered through a disposable syringe filter (25 mm, 0.45 μ m) before being injected to the HPLC system. To assure the efficiency of extraction, hydroquinone in exact amounts was added into the blank cream and then determined by the HPLC method. Good recoveries of up to 90 % (%RSD < 2, n=5) were obtained from this method.

Results and discussion

This reliable HPLC method was applied to the determination of hydroquinone in skin-whitening herbal cosmetic cream products which were commercially sold in the area of Khon Kaen city. The HPLC chromatogram of standard hydroquinone

and its calibration curve are shown in Figure 2 and Figure 3. From twenty-three different commercial samples, it was found that four samples possibly had hydroquinone contamination in these products (Table 1). The clarification peak at the same position as hydroquinone (retention time, about 2.6) was observed in these four samples (samples No. 3, 10, 15 and 16). The amounts of hydroquinone in these samples were 3.29, 2.78, 2.06 and 2.76 %w/w, respectively. HPLC chromatograms of these four samples are shown in Figure 4. However, further studies to prove these peaks should be done. The HPLC technique could confirm or identify hydroquinone from the retention time, but other components in these cosmetic products could show the same retention time as hydroquinone. Thus false positive results might occur in this case. The LC-MS method is the first choice to use for the confirmation of this peak because the structure or data about its molecular weight could be obtained from this technique.

Conclusion

From the results of this study, the amount of the forbidden chemical substance hydroquinone (followed the Thai regulations for cosmetics, 1996) found in some samples was rather high. The manufacturers of these products must be concerned about the toxicity of this substance. So, the Thai FDA should monitor all these products. In addition, the FDA should provide information about hydroquinone toxicity to all cosmetic manufacturers. These processes will probably protect the people from the dangerous substance in the future.

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Table 1. The amount of hydroquinone from 23 products of skin-whitening herbal cosmetic creams

Sample No.	Amount of hydroquinone (%w/w)
1-3	nd
4	3.29
5-9	nd
10	2.78
11-14	nd
15	2.06
16	2.76
17-23	nd

nd = not detectable



Figure 1. Structure of hydroquinone

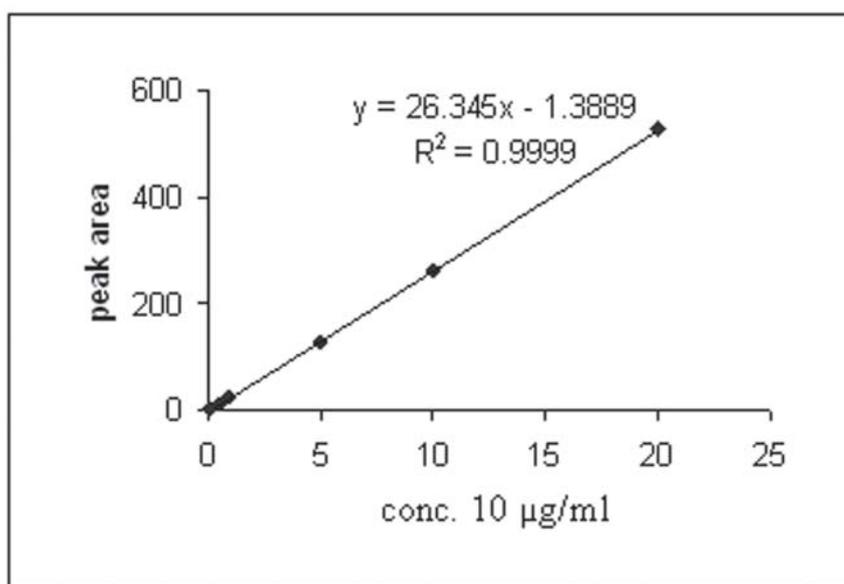


Figure 2. Calibration curve of standard hydroquinone solutions

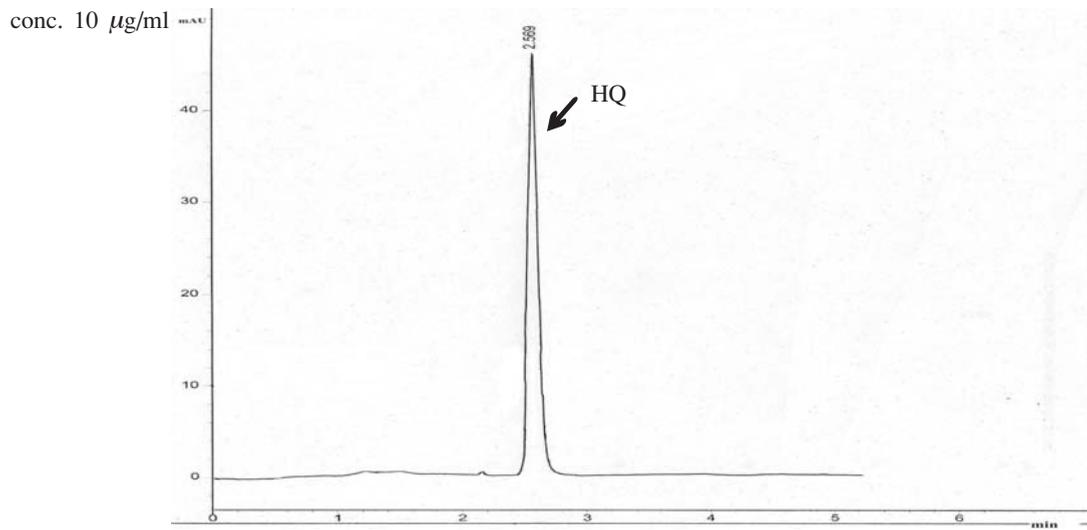


Figure 3. HPLC chromatogram of standard hydroquinone solution (conc. 10 $\mu\text{g/ml}$)

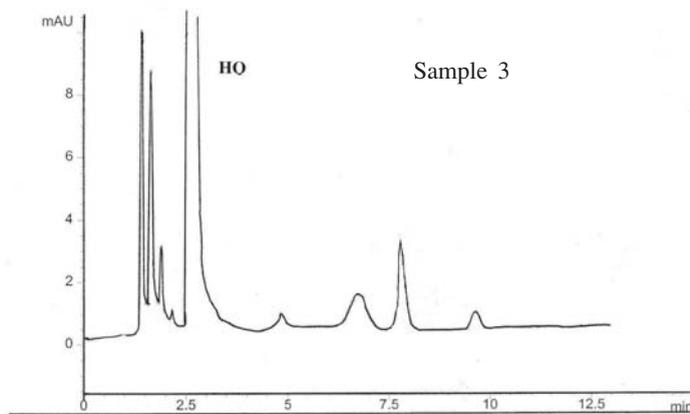


Figure 4. HPLC chromatograms of samples which contain hydroquinone
(Samples No. 3, No. 10, No. 15 and No. 16)

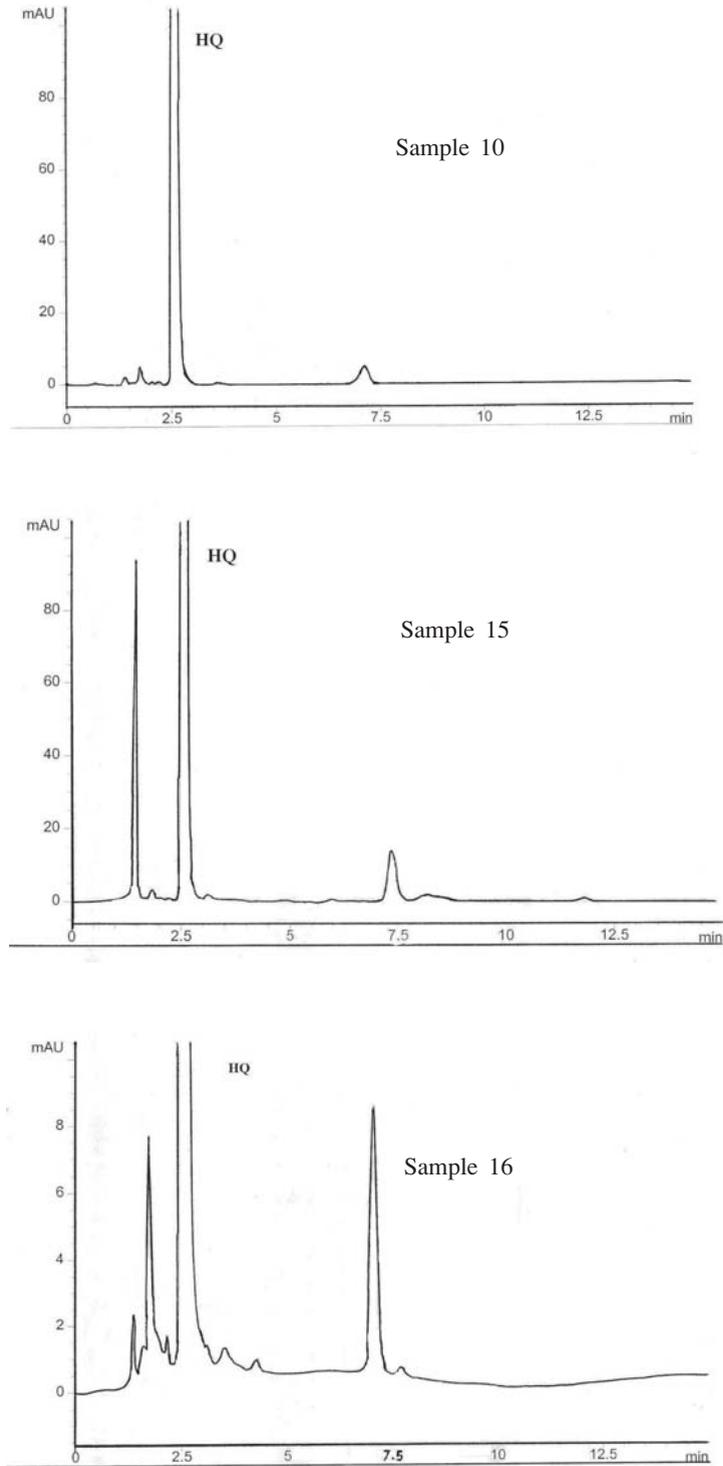


Figure 4. HPLC chromatograms of samples which contain hydroquinone (Samples No. 3, No. 10, No. 15 and No. 16) (cont.)