Journal of Associated Medical Sciences 2019; 52 (3): 170-174

JAMS
Journal of Associated Medical Sciences

Thai-Journal Citation Index Centre (TCI) & ASEAN Citation Index (ACI)

Journal of Associated Medical Sciences

Journal homepage: https://www.tci-thaijo.org/index.php/bulletinAMS/index

Qualitative and quantitative assessment of hydroquinone in skin whitening cosmetics in Pathum Thani Province, Thailand

Pataweekorn Ketkomol[1*] Watchara Chongsa[2] Tadsanee Punjanon[1]

[1]*Pharmacology and toxicology Unit*
[2]*Physiology Unit, Department of Medical Science, Faculty of Science, Rangsit University, Pathum Thani Province, Thailand*

ARTICLE INFO

Article history:
Received 1 March 2019
Accepted as revised 25 June 2019
Available online 15 August 2019

Keywords:
Hydroquinone, skin whitening cosmetic, hydroquinone test kit-2, TLC, HPLC

ABSTRACT

Background: Hydroquinone has been used for decades as a skin bleaching agent, but it has unfavorable effects including contact dermatitis and ochronosis. Therefore, it got prohibited by the Ministry of Public Health of Thailand. However, some cosmetics companies are still illegally using hydroquinone as a cosmetic ingredient that can affect on consumer health.

Objectives: To assess the hydroquinone contamination in skin whitening cosmetic products in Pathum Thani Province, Thailand.

Materials and methods: Fifty skin whitening cosmetic samples were collected from local markets in Lak Hok Subdistrict, Muang District, Pathum Thani Province, Thailand. Screening test for hydroquinone contamination was performed by using hydroquinone test kit-2 from Department of Medical Science, Ministry of Public Health. All samples were confirmed by using Thin-layer Chromatography (TLC) technique. The hydroquinone concentration was estimated by using High-Performance Liquid Chromatography (HPLC) technique.

Results: Nine of the 50 samples showed a positive result in hydroquinone test kit-2, but only 3 of the 9 positive samples found hydroquinone contamination based on TLC and HPLC analysis. The concentration of hydroquinone was 0.0005, 0.0009, and 0.0016%.

Conclusion: Six percent of skin whitening cosmetics in local markets of Lak Hok Subdistrict, Muang District, Pathum Thani Province found hydroquinone contamination. Therefore, consumer should concern about their safety and checked the manufacture's information prior making a purchase decision.

Introduction

White skin is considered to be the highest beauty standard in Thailand. This attitude was influenced by commercial advertisements which divides and excludes dark skin people as the group of "Marginal ones".[1] This perception has encouraged most women to engage in skin bleaching. Skin whitening cosmetic products became a priority for women to bleach their skins. Most of these cosmetic products contain different kinds of depigmentation agents such as retinoid, alpha-and beta-hydroxy acids, ascorbic acid, arbutin, hydroquinone, and derivative mercury. Some agents (especially hydroquinone) are harmful chemicals and can affect on health.[2]

Hydroquinone (HQ) is one of most effective skin bleaching agent by inhibits tyrosinase (key enzyme responsible for melanin production) synthesis and tyrosinase activity, as well as destructs melanocytes.[3,4] In the past, hydroquinone was allowed to use as an ingredient of the blemish cream at a concentration of less than 2%.[5] Later on, it was found that it has numerous unfavorable effects after long-term application, including irritative dermatitis, contact dermatitis, and ochronosis.[6,7] Therefore, its use in cosmetics got prohibited

* **Corresponding author.**
Author's Address: *Pharmacology and toxicology Unit*

** **E-mail address:** : pataweekorn.k@rsu.ac.th
doi: 10.14456/jams.2019.28
E-ISSN: 2539-6056

and been so since 1996.[8] However, the hydroquinone contamination in skin whitening cosmetics is still being reported, indicating that unsafe product is remain distributed in a several areas of Thailand.[9-12]

There are several techniques to assess hydroquinone contamination in a cosmetic product including using hydroquinone test kit, thin layer chromatography (TLC), and high-performance liquid chromatography (HPLC).[11,13,14] These techniques have different characteristics for hydroquinone assessment such as the hydroquinone test kit and TLC provide only qualitative results, as well as hydroquinone test kit can give a false positive when presence of some agents in cosmetic product such as sodium sulfite, sodium metabisulfite, alpha-todopherol, and ascorbic acid.[10] Whereas HPLC technique can provide qualitative result, but it takes a long time with high cost for the analysis. Therefore, concerning to the accuracy of hydroquinone assessment as well as saving time and cost, the qualitative and quantitative analysis of hydroquinone contamination in skin whitening cosmetics in this study will be started with screening test by using hydroquinone test kit-2, and then confirmed by using TLC method. Hydroquinone concentration in a positive sample was estimated by using HPLC method.

Materials and methods

Sample collection

Fifty skin whitening cosmetic samples consisting 45 creams and 5 lotions were collected from local markets in Lak Hok Subdistrict, Muang District, Pathum Thani Province, Thailand. The main intention of the present study was focused on a cosmetic product that advertised "skin whitening effect, depigmentation, and anti-melasma" on the label in combination with giving false labeling such as not mentioning applicant (name and addressee of manufacturer) and/or not mentioning the registration number.

Screening test for hydroquinone by hydroquinone test kit-2

The hydroquinone test kit-2 was obtained from Department of Medical Science, Ministry of Public Health, Thailand. The detection limit was 0.006% w/w in cream, and 0.014% w/w in lotion. The reagent for hydroquinone assessment was freshly prepared before the test started. About 0.2 mg of each skin whitening cream or 2 mL of skin whitening lotion were placed into a palette, and 2 mL of the reagent was added and mixed. The color of mixture was observed within 30 ms, if the color of the mixture turned green to dark blue color (positive result) indicating that hydroquinone may be contaminated in this sample.

Preparation of sample solution for TLC and HPLC analysis

The skin whitening cosmetic sample was prepared as described previously.[13] Briefly, two grams of each sample was accurately weighed in a 25 mL beaker. Fifteen mL of 96% (v/v) ethanol was added and mixed on the water bath at 60°C for 10 min. A mixture was cooled in an ice bath until the separation of fats occurred, and then filtered by using Whatman Filter Paper No.1. The filtrate was collected for TLC and HPLC analysis. The same procedure was repeated for all samples.

TLC analysis

Preparation of standard solution

The standard solution was prepared by dissolving 0.5 gm of hydroquinone in small volume of 96% (v/v) ethanol in 25 mL volumetric flask and the resulting volume was made up to 25 mL.[13]

Chromatographic method

TLC plates (8x4 cm) were made on 0.25 mm thick silica gel 90G (Merck, Germany). A mixture of chloroform:ethyl acetate (3:1) was used as mobile phase. The standard and duplicate of each sample solution were spotted onto the start line of the TLC plate followed by placed into the separating jar containing mobile phase. The plate was developed at room temperature to the height of approximate 8 cm from the start line. After drying or spraying with 0.2% ethanolic dichlorofluorescein, the plate was visually examined under UV light at 254 nm and 366 nm, respectively.[13] The retention factor (Rf) value were calculated by the equation:

$$R_f = \frac{\text{Distance traveled by the compound}}{\text{Distance traveled by the solvent}}$$

Identification of the hydroquinone in each sample was done by comparison of its Rf value with those of the standard

HPLC analysis

Preparation of standard solutions

Stock solutions of concentration 1, 10, 20, 30, 40, and 50 mg/L were prepared by dissolving 0.5 mL HPLC-grade methanol and the resulting volume was made up to 1 mL with the solvent for the mobile phase (MtOH:H_2O; 45:55 v/v).[13]

Chromatographic method

HPLC analysis was performed on a modular Shimadzu LC-10 system (Shimadzu, Japan) equipped with a LC-10AD pump, a CTO-10A column oven, SPD-M20A UV-DAD detector, a CBM-10A interface and a LC-10 Workstation. Reverse phase chromatography analysis was achieved by an innersil-ODS-3 column (5 µm particle size, 250x4.6 mm i.d.) with a mobile phase consisting of a mixture of methanol and water, and employing gradient elution (from 45:55 to 85:15, v/v), volume ejection was 20 µL, and UV detection was at 295 nm.[13] Each sample was identified by comparison of its retention time and UV absorption spectrum with standards under the same conditions. Quantification of hydroquinone in each sample was done by the measurement of integrated peak area and the content was calculated using the calibration curve by plotting peak area against concentration of the respective standard sample. Each quantification was carried out in triplicate.

Results and Discussion

Hydroquinone was considered to be as one of the most effective skin bleaching agent, but it has numerous unfavorable effects after long-term application. Therefore, it is prohibited to be added in cosmetic products.[8] However, some cosmetic companies are still illegally using hydroquinone as a cosmetic ingredient that can affect on consumer health.

Therefore, in the present study focuses on the assessment of hydroquinone contamination in a skin whitening cosmetic products in local markets of Lak Hok Subdistrict, Muang District, Pathum Thani Province, Thailand by using a combination of hydroquinone test kit-2, TLC, and HPLC technique. A screening test of hydroquinone contamination by using hydroquinone test kit-2 showed a positive result in 9 of 50 (18%) skin whitening cosmetic samples, including sample no. 6, 14, 22, 28, 32, 42, 45, 49, and 50 (Table 1). However, hydroquinone test kit-2 can give a false negative when the concentration of hydroquinone was lower than the detection limit (0.06% w/w in cream and 0.014 % w/w in lotion), and give a false positive when presence of some agents such as sodium sulfite, sodium metabisulfite, alpha-todopherol, and ascorbic acid. Therefore, the hydroquinone contamination in all samples was confirmed by using TLC technique. The R_f value of sample spots was compared with the R_f value of the standard solution of hydroquinone (0.05). Based on TLC results, a negative sample in hydroquinone test kit-2 also showed negative result in TLC analysis. Whereas, only 3 of the 9 (33%) positive samples showed a R_f value of 0.50 like the standard solution, including sample no. 6, 28, and 50 (Table 1, Figure 1). The hydroquinone concentration of the positive samples in TLC analysis was estimated by using HPLC technique. The calibration curve of the standard solution of hydroquinone showed the linearity of the detector over the tested range (1-50 mg/L). The linear regression equation was y =37842x-16537 (R^2 =0.9992). The retention time and UV spectrum of hydroquinone in standard solution and sample also coincided (Figure 2). The concentration of hydroquinone in sample No. 6, 28, and 50 was 0.0016, 0.0005, and 0.0009%, respectively (Table 1). This finding showed that 3 of 50 (6%) whitening cosmetic samples were contaminated with hydroquinone. This result was similar reported by Klinsamut and coworkers in 2013.[10] It is indicated that nowadays unsafe skin whitening cosmetic products are remain distributed in the local markets, and did not get approved yet. In addition, the false positive of hydroquinone test kit-2 found about 67% indicating that although some cosmetic products give a positive result, it didn't truly mean hydroquinone contamination. Therefore, the sample with a positive result in hydroquinone test kit-2 needed to be confirmed by other methods

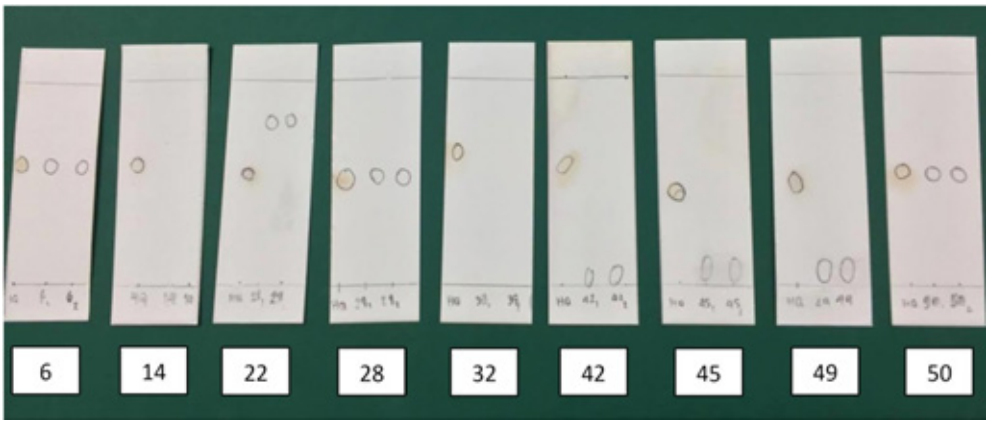

Figure 1*. TLC analysis results of nine positive samples in hydroquinone test kit-2.*

Table 1 Evaluation of hydroquinone by using hydroquinone test kit-2, TLC analysis, and HPLC analysis.

Sample No.	Type	HQ test kit-2	TLC analysis Rf value	HPLC analysis Retention time	HPLC analysis HQ conc. (mg/L)	HQ concentration in sample (% w/w)
6	Cream	+	0.50	4.507	2.14±0.013	0.0016
14	Cream	+	N.D.	-	-	-
22	Cream	+	0.73	-	-	-
28	Lotion	+	0.50	4.536	0.71±0.009	0.0005
32	Lotion	+	N.D.	-	-	-
42	Cream	+	0.96	-	-	-
45	Cream	+	0.91	-	-	-
49	Lotion	+	0.92	-	-	-
50	Cream	+	0.50	4.515	1.18±0.088	0.0009

Notes: HQ; hydroquinone, +; positive result, N.D.; Not detected.

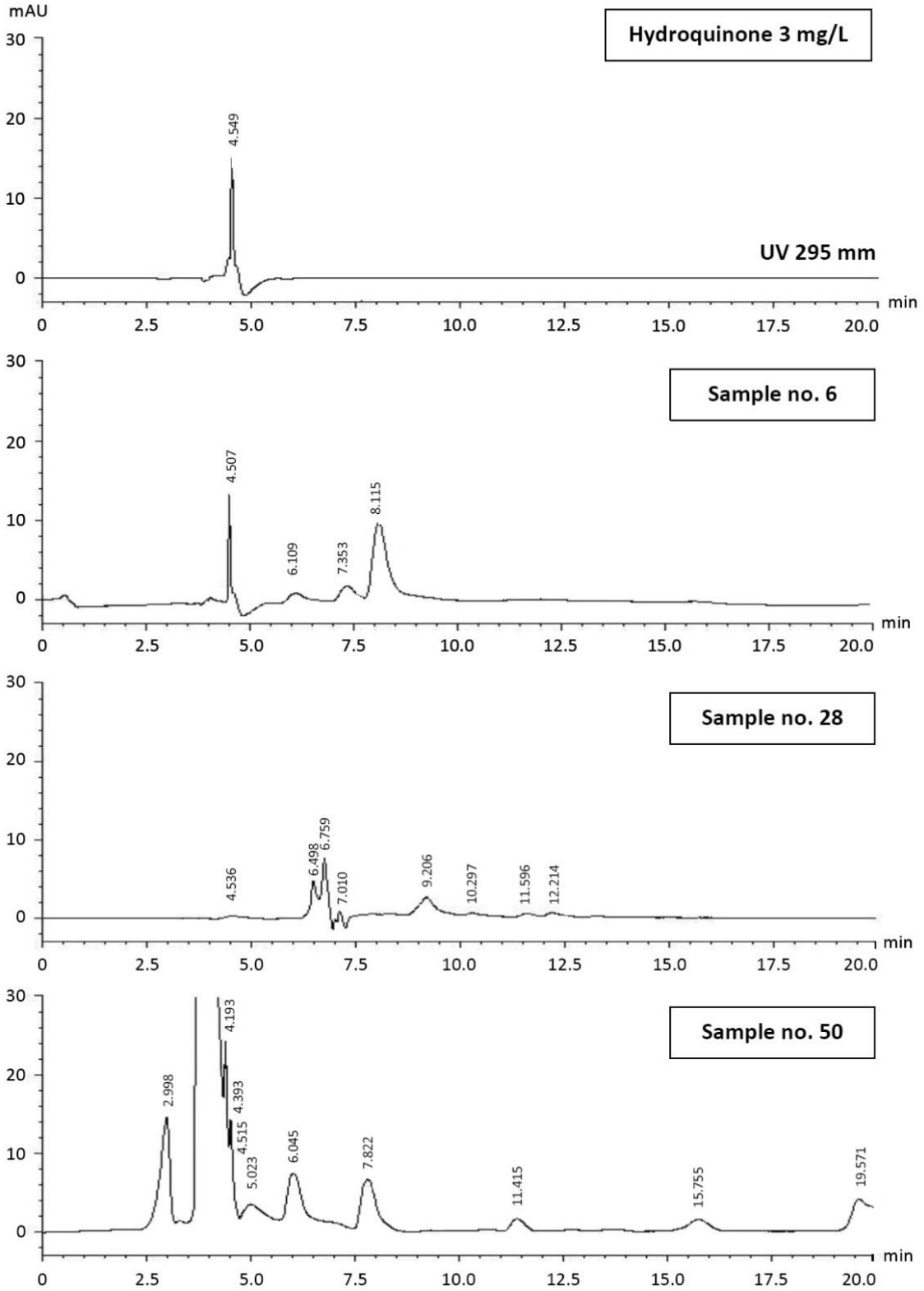

Figure 2. *HPLC chromatogram of the standard of hydroquinone and skin whitening cosmetic samples.*

Conclusion

Nine of the 50 skin whitening cosmetic samples showed a positive result in hydroquinone test kit-2, but only 3 of the 9 positive samples showed hydroquinone contamination when TLC and HPLC analysis were used. We can conclude that about 6% of skin whitening cosmetics in local markets of Lak Hok Subdistrict, Muang District, Pathum Thani Province found hydroquinone contamination. Therefore, the consumer should be concerned about their safety and checked the manufacture's information prior making a purchase decision.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

The authors are grateful to the Pharmacology and toxicology Unit, Faculty of Science, Rangsit University. The authors also extend thanks to Rujira Podee and Suvanan Saentaweesuk for their technical support. This work was funded by Rangsit University.

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