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Qualitative and Quantitative Estimation of Hydroquinone in Skin Whitening Cosmetics

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ABSTRACT

Hydroquinone has been used for decades as a skin lightening agent. Its use in cosmetics has been banned as a result of skin problems including contact dermatitis and ochronosis. A total of 22 samples of different skin whitening cosmetics were collected from local market. They were analyzed by using thin layer chromatography and HPLC for qualitative and quantitative determination of their hydroquinone contents. The hydroquinone was extracted from samples by using 96% ethanol and was subjected to TLC analysis. Eleven out of 22 samples were found to contain hydroquinone. The HPLC analysis showed the concentration of hydroquinone ranged from 0.002% to 0.092% in the cosmetic samples.

Keywords: Hydroquinone; HPLC; Whitening Cosmetics; Thin Layer Chromatography

1. Introduction

Visible pigmentation in mammals results from the synthesis and distribution of melanin pigment in the skin and hair bulbs [1,2]. Melanin also plays a crucial role in the absorption of free radicals generated within cytoplasm and in shielding of the host from various types of ionizing radiations including UV [3]. However, the dark skin caused by melanin accumulation is not considered cosmetically pleasing to many people [4-6]. The increased levels of melanin are also characteristic of a great number of skin diseases including melasma, solar lentigines, and post-inflammatory hyperpigmentation. Thus, there is an increasing desire for the development of skin whitening agents for both beauty and therapeutic purposes [7,8]. Tyrosine is the precursor for the synthesis of melanin. Tyrosinase is the key and rate-limiting enzyme responsible for the conversion of tyrosine into melanin by melanocytes in human skin [9,10]. Inhibition of the enzymatic activity of tyrosinase by competitive inhibitors results in decreased or absent melanin synthesis by the melanocytes in human skin [11,12].

Many compounds that bind to the tyrosinase active site and inhibit melanin synthesis have been developed as agents to lighten skin color, including hydroquinone [13]. Hydroquinone is the most conventional skin whitening agent. However, it has numerous unfavorable effects with long-term application, including irritative dermatitis, melanocyte destruction, contact dermatitis and ochronosis [14,15]. Its use has been recommended to ban in cosmetics [16-18]; however, it is still being used in developing countries in skin lightening cosmetics. The aim of the present study is to analyze different skin whitening cosmetics present in the market for the determination of their hydroquinone contents.

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2. Experimental

Different samples of skin whitening creams were collected randomly from the local market of Lahore District, Punjab, Pakistan.

3. TLC Analysis

3.1. Sample Preparation

Two grams of sample was weighed in a 25 ml beaker and 15 ml of 96% (V/V) ethanol was added. The mixture was homogenized on water bath at 60˚C for 10 min and then cooled in an ice bath till the separation of fats occurred. Finally it was filtered and filtrate was used for TLC analysis. The same procedure was repeated for all samples.

3.2. Preparation of Reference Solution

It was prepared by dissolving 0.05 g of hydroquinone in small amount of ethanol 96% (V/V) in 25 ml volumetric flask and finally making up the volume up to the mark.

Note: The standard solution should be freshly prepared as it is stable for less than one day at room temperature.

3.3. TLC Procedure

TLC plates (20 × 20 cm) of 0.25 mm thickness were prepared by using silica gel (90 g) and water (180 ml) [16]. The plates were air dried and activated by heating in an oven at 105˚C for an hour. The solvent system; n-Hexane/Acetone, 3:2 was used [19]. Twenty micro litres of each sample and standard solution were deposited on plates and they were developed at room temperature in a vertical separating chamber to the height of approximately 16 cm from the start. The chamber was previously saturated with the appropriate mobile phase (saturation time was 1 hour). After drying, visualization was performed in two ways:

1) In short UV light (254 nm);

2) Spraying with 0.2% ethanolic dichlorofluorescein; chromatograms were interpreted in long wave UV light (366 nm).

The R_f values were calculated for each spot and their results are given in **Table 1**.

4. HPLC Analysis

4.1. Sample Preparation

2 ± 0.1 g of a sample was weighed accurately into a beaker and 25 ml of mobile phase (Water: Methanol mixture 45:55) was added and mixed. It was homogenized in a water bath at 60˚C for 10 min and then cooled in an ice bath until the separation of fats and waxes occurred. The mixture was filtered and transferred to 100 ml volumetric flask. The volume was made up to the mark by mobile phase.

4.2. Preparation of Reference Solution

0.05 g of hydroquinone was weighed and transferred to a 50 ml volumetric flask. It was dissolved in small amount of mobile phase and volume was made upto the mark. 5 ml of this solution was pipetted into a 50 ml volumetric flask. It was diluted and volume was made up to the mark by mobile phase.

Table 1. R_f Values of analyzed samples.

Sample No.	Sample Name	Manufacturers	R_f value
1	WT Whitens Cream	Anonymous	0.61
2	Face Fresh Beauty Cream	Shaheen Cosnetics Company (Pvt.) Ltd., Pakistan	0.61
3	Barbie Whitening Cream		0.50
4	Faiza Beauty Cream	Poonia Brothers, Pakistan	0.50
5	Blesso Cream	Blesso Cosmetics	0.91
6	Care Cream	Coslab (Pvt.) Ltd.	0.86
7	English Fairness Cream	English Laboratories (Pvt.) Ltd., Pakistan	0.86
8	Virgo Acne Cream	Virgo Cosmetics, Pakistan	0.50
9	White Gold Cream	Trade Masters, Pakistan	0.50
10	19-Herbs Cream	Allied Medical & Chemical Research Co., Pakistan	0.50
11	Pearl Whitening Cream	Cosmo Care, Pakistan	0.50
12	Chun Chehra		0.81
13	Sanober Beauty Cream	Future Sky Marketing Ltd., London	0.50
14	Kamal Face Cream	Anonymous	0.86
15	X-Cream	Anonymous	0.81
16	Farzana Beauty Cream	Farzana Cosmetics, Pakistan	0.50
17	Fair & Lovely	Unilever (Pvt.) Ltd., Pakistan	0.88
18	Bio Nikhar	Forvil Cosmetics, Pakistan	0.50
19	Roop Nikhar	Singh Herbals, India	0.50
20	Gipsy Cream	Gipsy Cosmetics, Pakistan	0.88
21	Dabar Cream	H. & Sons Enterprises, Pakistan	0.88
22	Ever Green Cream	Anonymous	0.50

R_f values of Reference Hydroquinone Solution = 0.50.

5. HPLC Procedure

A Schimadzu LC-9 was used with UV detector set at 295 nm having ODS column (25 cm × 4.6 mm). Twenty was adjusted at 35˚C ± 1˚C. The mobile phase was a mixture of water and ethanol (45:55) with a flow rate of 1.5 ml/min.

20 µl of each sample solution was injected and chromatogram was recoded. Peak area for each sample was measured and comparison was made between reference and sample solutions peaks. The amount of hydroquinone was calculated as percentage by mass using the formula A

$$\%\text{age of Hydroquinone} = \mathrm{bi}/\mathrm{pi} \times \mathrm{w}_{\mathrm{ref}}/\mathrm{w}_{\mathrm{spl}} \times \mathrm{d} \times 100$$

where

bi = Peak area of hydroquinone in sample solution;
pi = Peak area of hydroquinone in reference solution;
d (dilution factor) = 0.1;
w_{ref} = Weight of hydroqinone in reference solution;
w_{spl} = Weight of hydroqinone in sample solution.

6. Discussion

The concept of skin whitening is very old. Variations in the skin colour are caused by different levels of melanin pigment in the skin. Melanin is synthesized in organelles called melanosomes, in melanocytes cell, by the action of an enzyme called tyrosinase. Most skin lightening products aim at tyrosinase production inhibition as it is the one of the first steps in the pigment formation and can therefore block all pigment producing pathways.

Hydroquinone was considered as one of the most effective skin lightening agent as it decreases tyrosinase activity by 90% [20]. However its side effects include skin irritation or contact dermatitis, development of exogenous ochronosis; an adverse effect that is characterized by darkening of the skin area where hydroquinone containing cream is applied [21]. The present study focuses on the determination of hydroquinone in various skin lightening cosmetics flooding the Pakistani market. A review of literature showed use of different techniques for the determination of hydroquinone in cosmetics mainly GC-MS and HPLC [22-24].

In the present study a combination of TLC and HPLC was used for the qualitative and quantitative determination of hydroquinone from skin whitening cosmetics respectively. On spraying developed plates with 0.2% ethanolic dichlorofluorescein, they showed several spots some of which coincided with that of the reference solution. The presence of more than one spot in a sample revealed the presence of more than one ingredient in the sample. A further confirmation was made by comparing the R_f value of the reference spot (0.50) with the R_f values of samples spots. Based on TLC results 11 out of 22 samples were found to contain hydroquinone. The R_f values for different samples are given in **Table 1**. HPLC was used for the quantitative estimation of hydroquinone concentration. The sample and reference solutions showed UV absorption at λ_{max} 295 nm. The retention time (5.480) of reference and sample also coincided (**Figures 1** and **2**). The peak areas for both reference and sample solutions were also measured and the quantity of hydroquinone was also calculated by using formula A. The results are given in **Table 2**. The concentration of hydroquinone in the samples ranged from 0.002% to 0.092%.

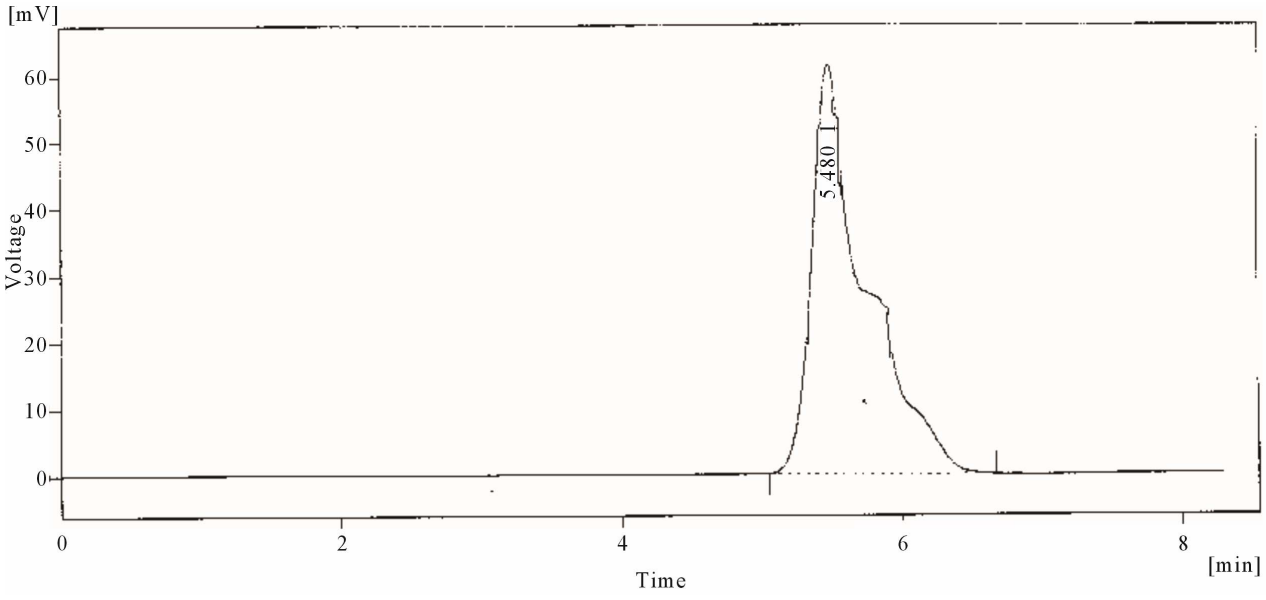

Figure 1. Chromatogram of reference solution of hydroquinone.

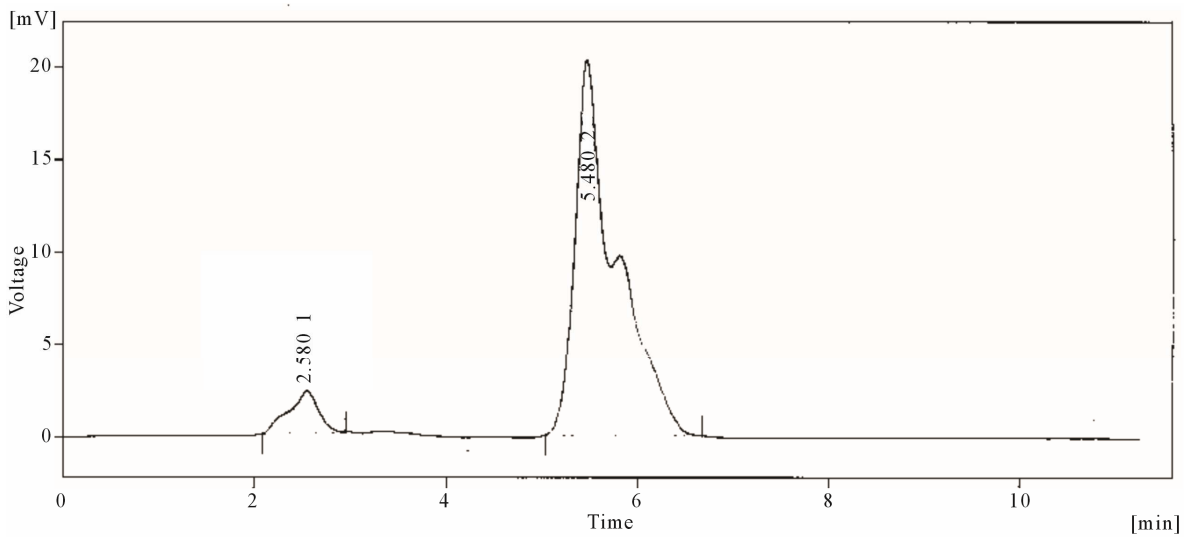

Figure 2. Chromatogram of representative sample (3) solution of hydroquinone.

Table 2. Percentage concentration of hydroquinone.

Sample No.	Sample Name	Sample Concentration (Y)	Percentage Concentration of Hydroquinone $= bi/pi \times w_{ref}/w_{spl} \times d \times 100$
3	Barbie Whitening Cream	35.071	0.005
4	Faiza Beauty Cream	40.817	0.006
8	Virgo Acne Cream	10.00	0.002
9	White Gold Cream	220.855	0.034
10	19-Herbs Cream	607.071	0.092
11	Pearl Whitening Cream	25.318	0.004
13	Sanober Beauty Cream	549.325	0.08
18	Bio Nikhar	30.574	0.005
19	Roop Nikhar	21.652	0.003
16	Farzana Beauty Cream	230.76	0.035
22	Ever Green Cream	25.512	0.004

Peak area of reference solution of hydroquinone(pi) = 1643.

7. Recommendations

Based on the above findings and keeping in view the harmful effects caused by hydroquinone as cited in this publication it is highly recommended that there should be a regulatory body appointed by government to check the quality of cosmetics available at market.

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