



Determination of Hydroquinone in Skin- Lightening Creams Sold in Sudan-by Using High-Performance Liquid Chromatography

Abdalla M. Abdalla Mustafa* - Mohamed El Mukhtar A. Aziz

Sudan University of Science and Technology –Department of Chemistry
halawani2003@hotmail.com

ABSTRACT

The use of hydroquinone is forbidden as it is potentially carcinogenic and skin and respiratory irritant, nevertheless, it is still the most conventional and widely used in skin- lightening creams. In this study, thirty samples of Skin-lightening creams, local and imported, were analyzed for total hydroquinone by using high-performance liquid chromatography (HPLC). The concentration of hydroquinone ranged from 0.00% to 5.75 % and from 0.00% to 3.21 % for local and imported creams, respectively. However, Fair and Lovely's cream ranged from 0.47% to 4.76% respectively. The use of such creams may lead to health hazards. Therefore, it is recommended that all skin-lightening creams should be checked for hydroquinone levels before marketing.

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1. Introduction:

Skin bleaching is a phenomenon that can be traced back to the ancient times amongst nations such as Japan. Skin bleaching was also practiced in ancient and medieval times in Asia, Egypt, Europe and China; it has gained real momentum in Sudan only recently (Hamed et al., 2004).

Most skin bleaching products contain one of the two active ingredients; hydroquinone and mercury. Other agents with skin lightening properties include alpha arbutin, beta-arbutin, licorice extract, niacinamide, mulberry extract, glycolic acid, lactic acid, lemon juice extract, Emblica, vitamin C, potato, and Tumeric. Potato is a natural bleaching agent. When the face is massaged with a slice of raw potato as often as possible, the skin can be lightened in color. The slice must not be washed before massaging the face, as it will lose its natural properties (Adebajo, 2002).

Hydroquinone have acute and chronic side effect in humans. The FDA & WHO Standards allows a maximum of two percent of hydroquinone in skin care products. European Bureau of Standards banned some hydroquinone containing skin-lightening creams (Siddique et al., 2012). Despite the side effects of hydroquinone, skin lightening creams containing these harmful chemicals are still found in the Sudanese market and are sold to the public. Considering the toxic effect of hydroquinone, it is important to control, its exposure to humans. This can

have achieved if their levels in skin-lightening creams were known.

In Sudan, a little work has been undertaken to determine the levels of heavy metals and hydroquinone in toning creams even though concern have been expressed about the widespread use of skin lightening cream (Lee et al., 2007).

Many Sudanese women love to keep their skin toned, and thus use skin care products that bleach the skin. However, most of these bleaching products contain different kinds of chemicals that may be harmful to human health (Schaffer & Bologna, 2001). Examples of such chemicals include hydroquinone (most common), mercury, kojic acid, kojic acid dipalmitate, azleic acid, arbutin, bearberry, vitamin C, magnesium ascorbyl phosphate, calcium ascorbate, and L-ascorbic acid.

From ancient history, humans have constantly labeled and stereotyped each other based on skin color (Raper, 1928). In most African and Asian communities, fairness was branded as beauty, grace, and high social status. Those with darker skin are seen as being of lowest social value, whereas, those with lighter skin are regarded as being of highest social value (Tai et al., 2009) This perception encourages most women to indulge in skin care products that lighten the skin. Hydroquinone is potentially carcinogenic and known to be a skin and respiratory irritant. It is also considered a primary topical ingredient for inhibiting the production of melanin,



the amount of which determines skin color. Because hydroquinone is carcinogenic, it has been banned in some countries because of fears of a cancer risk, (Costin & Hearing, 2007). Some concerns about hydroquinone's safety on the skin have been expressed, but research has shown that when it comes to topical application, it has negative reactions which are minor but major as a result of using extremely high concentrations. This is particularly true in Sudan where adulterated skin lightening products are common.

The main objective is to determine levels of hydroquinone in some skin-lightening creams sold in the Sudanese market.

Moreover, to compare levels with standards recommended by the International Standards and to determine if consumers in Sudan are at risk.

2. Materials and Methods:

2.1 Reagents:

All reagents were of analytical reagent grade (BDH Chemicals Ltd, Poole, England) unless otherwise stated. The methanol used for the hydroquinone analysis was HPLC grade.

Standard solution of hydroquinone was prepared by dissolving 1.0g hydroquinone in methanol in a 100 cm³ volumetric flask and made up to volume using methanol. Various concentrations (0.08, 0.12, 0.16 and 0.2 g/dm³) were prepared by diluting aliquots of the stock hydroquinone standard solution with methanol.

2.2 Sampling:

Thirty samples of skin- lightening creams were obtained randomly from cosmetic shops in Khartoum, Khartoum- North and Omdurman Markets, local and imported samples, (Table-3).

2.3 Sample preparation:

About 0.10 g of each cream was weighed accurately into a 10 cm³ flask and 8.0 cm³ of methanol was added and heated at 40 °C in a water bath with occasionally shaking until it dissolved. It was allowed to cool and made up to the mark with methanol. The solution was filtered using a membrane filter before analysis.

2.4 Preparation of Reference Solution:

An accurate weight of 0.05g of hydroquinone standard was transferred to a 50 cm³ volumetric flask, dissolved in small amount of mobile phase and volume was made up to the mark. 5 ml of this solution was pipetted into a 50 cm³ volumetric flask. It was diluted and volume was made up to the mark by mobile phase.

2.5. Method Validation

2.5.1. Linearity:

In this study, 5 concentration levels were used to study the linear dynamic range of the method. Five different concentrations of standard working solutions, prepared from the stock solutions were analyzed

2.5.2. Accuracy

A lotion and cream samples containing no whitening agents were used as blanks in the recovery study. Recovery of the five whitening components was obtained at both low and high concentrations.

2.5.3. Precision:

The precision of the proposed method expressed as a relative standard deviation (RSD) percentage, was determined by analysis of each compound (Hardwick et al., 1989).

2.6. Instrument used for determination of hydroquinone

Determination of hydroquinone was carried out by high-performance liquid chromatograph (HPLC), Agilent 1100 series with DAD.

2.6.1. HPLC-Instrument conditions

- Column: (Hypersil C8 150*4.6mm*5µm)
- Stabilized pressure: 67 bar
- Column temperature: 30°C
- Flow rate: 1 cm³/min
- Elution. Gradient

Table 1 shows HPLC instrument operation conditions.

Table 1: *Instrument operation conditions*

Time (min)	Methanol	Water Milli-Q
0	5	95
13	5	95
17	70	30
20	70	30
25	5	95

2.7. Recovery

Recovery of hydroquinone was determined by adding increasing amounts of standard hydroquinone solution to known weights of two different cream samples. The first flask contained only 0.1 g of each sample and the second flask contained 0.1 g of each sample and 0.08 g of hydroquinone.

The third flask contained 0.1g of each sample and 0.12 g of hydroquinone. The resulting solutions after extraction were analyzed for hydroquinone concentrations and the results obtained were reported in (Table 2), which shows the percentage of recovery of hydroquinone in different skin- lightening creams

collected from the local market in Sudan which is ranged from 99.5 % to 102.4%.

Table 2: Recovery results of hydroquinone for skin – lightening cream samples.

Sample ID	HQ Percent (%)	HQ Added (mg)	HQ Found (mg)	HQ Recovery (mg)	Percentage of Recovery (%)
Sample -1	0.0	80.0	81.9	81.9	102.4 %
Sample -2	0.0	120.0	119.6	119.6	99.7 %
Sample -3	0.2	80.0	78.2	78.0	99.5 %
Sample -4	0.2	120	121.4	121.2	101.0 %
Sample -5	0.1	80.0	81.1	81.2	101.5 %
Sample -6	0.0	80.0	78.4	80.7	100.9 %

Where, HQ; hydroquinone.

In the same conditions as above, a solution independent from that used for the range was injected of test concentrations 0.5mg/dm³ and 5mg/dm³, a blank with the dilution solvent.

The recovery rate relative to the target concentration was calculated. The accepted recover rate had to be between +/- 3% for the low test 100 +/- 5% for the high test, and LOD.

2.8. Calculations

Note: HPLC instrument software can be programmed to perform all necessary calculations. Analytic concentrations in digested samples (CS) were calculated using:

$$C \text{ (ppb)} = \frac{CE \times V \times D}{W}$$

Where: -

CE = Analytic concentration in final extract, in µg/dm³

V = Final sample extract volume in mill- decimeters

D = Dilution factor (Diluted volume/aliquot volume) if secondary dilution was made

W = Sample Weight in grams.

All results were reported in µg/dm³, ppb or mg/dm³, ppm for liquid samples and µg/kg, (ppb) or mg/Kg, ppm for solid samples.

3. Results and discussion:

Hydroquinone was determined by recovery studies (Table-2). Analytical and matrix recovery studies for hydroquinone yielded results between 99.5% and 102.4% with a coefficient of variation between 4% and 9%. Hydroquinone results, for LC- ranged from 0.0 to 5.75%, F&L, 0.47 to 4.76% and for C- ranged from 0.0 to 3.21%. lightening - creams gave 4.76 % for the sample number (F&L 63), imported from Dubai, UAE, and the minimum concentration of HQ in the results of hydroquinone

percentage contained in local and imported creams showed that out of the thirty samples analyzed, one cream contained up to 5.75% of hydroquinone. (Moreover, one skin-lightening cream of Fair & Lovely gave up to 4.76%).

The highest concentration was 5.75% and 3.05% recorded for local preparation sold in Omdurman market. F&L cream imported from India recorded 2.03% of hydroquinone. France cream recorded 1.38% of hydroquinone. Dubai skin lightening cream recorded the highest hydroquinone concentration of 4.76% followed by China cream (2.81%), which was also above the recommended value. In total, 92% of the creams analyzed recorded levels below 2% hydroquinone, which is the threshold limit, and 8% of the creams analyzed contained more than 2% hydroquinone, which is above the threshold limit.

The concentrations of hydroquinone in skin-lightening creams have also been the subject of study in the United Kingdom (European Foundation for the Improvement of Living, 2000). Eight out of forty-one cream samples analyzed were found to contain more than two (2%) percent hydroquinone which is the maximum concentration permitted by the United Kingdom cosmetic product regulations. (FDA, 2006).

Table 3: Percentage of hydroquinone result in skin-lightening cream samples (local & imported).

Sample ID	Type of Sample	Country of Origin	Lot No.	Color	Item No	HQ Results %
LC 51	Skin-lightening Cream	Local/K.N	-	White	-	0.11
LC 52	Skin-lightening Cream	Local/K.N	-	Off white	1	0.51
LC 53	Skin-lightening Cream	Local/K.N	-	White	-	1.63
LC 54	Skin-lightening Cream	Local/OM	-	Off white	2	0.10
LC 55	Skin-lightening Cream	Local/OM	-	White	03	5.75
LC 56	Skin-lightening Cream	Local/OM	-	Off white	-	1.64
LC 57	Skin-lightening Cream	Local/OM	-	Off white	2	ND
LC 58	Skin-lightening Cream	Local/K.N	-	Off white	-	0.89
LC 59	Skin-lightening Cream	Local/OM	-	White	-	3.05
F&L 60	Skin-lightening Cream	India	-	White	01	2.03
F&L 61	Skin-lightening Cream	France	-	Off white	01	1.38
F&L 62	Skin-lightening Cream	UAE Dubai	-	Trans parent Gel	01	0.47
F&L 63	Skin-lightening Cream	UAE Dubai	-	Off white	01	4.76
F&L 64	Skin-lightening Cream	China	-	White	2	2.81

F&L 65	Skin-lightening Cream	China	-	White	-	0.52
C 66	Skin-lightening Cream	Italy	7702- k	Off white	-	0.92
C 67	Skin-lightening Cream	Egypt	-	White	-	3.21
C 68	Skin-lightening Cream	Italy	-	Trans parent Gel	-	0.70
C 69	Skin-lightening Cream	Egypt	512	Trans parent Gel	1	ND
C 70	Skin-lightening Cream	India	-	Off white	2	ND
C 71	Skin-lightening Cream	China	09	White	03	0.66
C 72	Skin-lightening Cream	France	154 LO	Trans parent Gel	-	1.08
C 73	Skin-lightening Cream	UAE Dubai	561	White	22	0.69
C 74	Skin-lightening Cream	Egypt	443	Off white	-	ND
C 75	Skin-lightening Cream	Egypt	-	White	-	2.72
C 76	Skin-lightening Cream	Egypt	-	Off-white	01	ND
C 77	Skin-lightening Cream	Thailand	218Z	Trans parent Gel	01	0.39
C 78	Skin-lightening Cream	China	-	Trans parent Gel	01	1.02
C 79	Skin-lightening Cream	Thailand	92-Y	White	01	ND
C 80	Skin-lightening Cream	China	-	White	-	0.07

Where; ND, not detected. LC; local cream, F & L fair and lovely, and C; imported cream.

4. Conclusion

Most of the cream samples, analyzed for hydroquinone, had concentrations more than the US Food and Drug Administration's acceptable limit of 1µg/g, reaching a percentage of up to 57% of all samples. The low concentrations of hydroquinone were detected in the imported cream samples. Therefore the percentage levels which were higher than the recommended WHO limit of two (2%) was only 23%. Hydroquinone concentration ranged from below detection to 5.75 %. Twenty-three percent of the cream samples analyzed had hydroquinone concentration above the WHO threshold limit of 2 %. This is very alarming and consumers who apply any of these creams are at risk.

5. Recommendations

The routine analysis should be conducted to ascertain the levels of hydroquinone in creams sold in Sudan. Manufacturers should be strongly encouraged to state the exact amount of bleaching agents in

creams. Moreover, 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 to check the quality of cosmetics available at Sudanese market.

Corresponding Author:

Abdalla. M. Abdalla Mustafa,
Department of Chemistry, University of Science and Technology, Sudan.
E-mail: halawani2003@hotmail.com

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