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Sensative Spectrophotometric method for determination of hydroquinone in some common cosmetics in Najran region in K.S.A

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Abstract

A highly sensitive, simpler, economical and faster visible spectrophotometric method has been established for determination of hydroquinone in some common cosmetics in Najran region. The method is based on using ammonium molybdate in acidic medium as an oxidizing agent for conversion of hydroquinone to p-benzoquinone. As a result of higher absorption of visible light by (Mo^{V}) producing from reduction of (Mo^{VI}). The effect of various parameters such as amount of oxidizing agent, temperature, and solvents has been studied upon the absorption of (Mo^{V}). Under optimum conditions, Beer's law was obeyed in the range of 10-100ug/ml at 580nm using methanol as solvent with linear regression coefficient of 0.9999. The newly developed method has been successfully applied to diluted samples of various skin lightening creams for hydroquinone determination.

Keywords: Lightening and whitening creams, Hy.

1. Introduction

A cosmetic agent is a preparation used for the purpose of increasing beauty and hiding the defects of something especially on the face. Cosmetic preparations include skin care preparations (creams, lotions, emollients and de-pigmentation agents such as hydroquinone, hair preparations, perfumes and fragrances[9]. Visible pigmentation in mammals results from the synthesis and distribution of melanin pigment in the skin and hair bulbs[25,24]. 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[19]. The hyperpigmentary disorders of the skin are caused by the over production of melanin,

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either by a normal or increased number of melanocytes, or by hormonal disorders[5,26]. However, the dark skin caused by melanin accumulations is not considered cosmetically pleasing to many people[2,6,27] the hydroquinone (HQ) has been used for decades in creams, gels and lotion for the treatment of the hyperpigmentary disorders of the skin.It is most frequently used compound in skin-toning preparations[14,15,17].

Some studies indicate that hydroquinone darkens certain area of the skin permanently, and has cancer causing potential making it potentially hazardous[28].

The effects of HQ are transitory and Concentrations below 3.0%do not cause skin injuries, however Concentration above 5.0% could provoke local irritation being this the main reason for the validation of a simple method[3,12,32].

Several analytical methods for the determination of HQ in skin –preparations are described, including high performance liquid chromatography[4,10,11,22]; Capillary electro-chromatography[8], micellar chromatography[23]; Adsorptive Stripping Voltammetry[1]; Flowinje-ctionwith electrochemical detection[21] and other spectrophotometric Techniques[7,13,16,18,29,31].

The aim of the present study is to determine the concentration of hydroquinone in some popular kinds of whitening creams in Nigran markets.

2. Material and Methods

2.1 Apparatus

The absorbance measurements were performed using shimadzu UV-VIS spectro-photometer model UV-1601 (shimadzu-Japan) with 1-cm quartz cells were used for all measurements.

MLW type thermostatically controlled water bath (Memmert GmbH, Co. Germany) was used.

2.2 *Chemicals and Reagents:*

All the chemicals used were of analytical reagent grade (Merck). Double distilled water was used for the preparation of all solution.

- Reference standard hydroquinone (Sigma chemical Co., USA) 0.1% (W/V) was prepared in methanol (Aldrich).

- Ammonium molybdate (Merck) was 4 %(W/V) in 10% (V/V) Sulphuric acid. 98 % (Aldrich).

2.3 *Sample preparation:*

Withdrawal of the test portion either:

- Narrow – necked containers, expel at least the first centimeter of the product. Extrude the test portion and reseal the container immediately.

- Wide - necked containers; scrape the surface evenly to remove the top layer. Take out the test portion and reseal the container immediately.

2.4 Recommended procedure for spectropho-tometric determination of hydroquinone.

Table 1. Cream samples

Cream samples	Production date	Expire date	Production country
OLAY (Day)	5/2010	5/2013	Thailand by Procter and gamble
FADE OUT (Day).	5/2012	5/2015	UK
RICOWith Hydroquinone	10/2011	10/2014	Rico skin care LTD. Egham UK
REO	2/2012	2/2016	General health care LTD. England
FACIAL SCRUB With lemon and honey extract	6/2012	6/2015	Thailand
WHITE PERFECT Transparent rosy whitening (Toner)	2/2012	2/2015	L /OREAL PARIS Indonesia
WHITE PERFECT Transparent rosy whitening (Night)	7/2012	7/2015	L /OREAL PARIS Indonesia
FAIREVER	11/2012	10/2014	India
WHITE PERFECT Double eye-zone brightener	2/2012	2/2015	Indonesia
HIMALAYA (day)	11/2012	10/2015	India
TRITOSPOT 3%Hydroquinone	11/2012	11/2015	Eva Pharm. Egypt
STRENEX	8/2012	8/2015	Elmasria for cosmetics company Egypt
GARNIER Skin naturals (Night)	2/2012	2/2015	India
MAGICAL Beauty Facial whitening	12/2011	12/2015	Turkey
KELLY PEARL	4/2010	4/2015	Thailand
EXTRADERM Plus Exfoliant 2%Hydroquinone	4/2011	4/2013	Philippines
FAIRNESS	2/2011	2/2014	KSA Under licence of J.Cassanova
WHITENING 1.9% Hydroquinone	7/2012	7/2015	Avalon pharm. KSA
MENA	4/2011	4/2016	Thailand
BEANNE	10/2012	10/2017	Thailand
REVITALLIFT	3/2012	3/2015	Germany
REVITALLIFT (Double Lift)	2/2012	2/2015	Germany
COLLGEN RE-PLUMPER (Day)	5/2011	5/2014	Germany

Into a series of boiling test tubes, different volumes (0.1- 1ml) of 1% hydroquinone were pipetted. To each test tube 2ml of 4% ammonium molybdate in 10%Sulphuric acid was added, mixed well and heated in a water bath at 100 ± 1°C for 20 min. After heating, the solutions were cooled at room temperature and transferred to 10 ml standard volumetric flasks and diluted to volume with methanol. The absorbance was measured within the stability time period of 4 h at 580nm against the reagent blank treated similarly. The amount of the hydroquinone in a given sample can be calculated from a calibration graph or regression equation.

2.5 Procedures for the assay of hydroquinone in cosmetics creams.

Two grams of samples were weighed in a 25 ml beaker and 15 ml of 96 % (V/V) methanol was added. The mixture was homogenized on water bath at 60 °C for 10 min and then cooled in an ice bath till separation of fats occurred. Finally it was filtered using whatmann No.42 filter paper. The filtrate was diluted to 50ml with methanol and the assay was completed following the recommended procedures for determination of hydroquinone.

The same procedure was repeated for all samples Table1.

3. Results

Variations in the skin color 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[20].

Hydroquinone was considered as one of the most effective skin lightening agent as it decreases tyrosinase activity by 90%. 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[30].

The present study focuses on the determination of hydroquinone in various skin lightening cosmetics flooding the Najran market.

In this study spectrophotometry was used for quantitative determination of hydroquinone from skin whitening cosmetics. Ammonium molybdate (Mo^{VI}) behaves as an oxidizing agent in acidic medium and is reduced to molybdenum (V) on treated with reducing substances. This property is exploited for the spectrophotometric determination of hydroquinone which reduces Mo^{VI} to Mo^{V} showing maximum absorbance at 580 nm [20].

3.1. *Optimization of variables and method development :*

The concentration of different reagents used for method development was optimizing by performing a series of experiments.

3.1.1. *Effect of heating:*

To study the effect of heating time for

the development of maximum color, 0.5 ml of 0.1% hydroquinone was mixed with 2ml of 4% Ammonium molybdate in 10% H_2SO_4. The contents of the mixture were heated for up to 20 min. at 100 ± 1°C. The intensity of the color developed was measured at room temperature (25 ± 1°C), after dilution to 10.0 ml with methanol. It is apparent from this investigation that the maximum intensity of color was obtained after 15 min of heating and remained constant up to 25min. Therefore the optimum heating time was fixed at 20 min.

3.1.2. *Effect of the concentration of ammonium molybdate :*

The effect of the volume of 4% ammonium molybdate on the color development was investigated by adding different volumes (0.5 →3 ml) of ammonium molybdate to 80 µg of hydroquinone. It was found that the maximum absorbance of color was reached with 1.6 ml of the reagent, and remains constant with higher volumes. Therefore, 2ml of the reagent was used throughout the experimental investigations.

3.1.3 *Effect of solvent :*

The effect of dilution solvent was investigated by adding different types of solvent (ethanol, methanol and acetone). It was found that the higher absorbance of color was reached with methanol as diluting solvent.

3.2. *Analytical data :*

Under the optimized experimental conditions calibration graphs were constructed by plotting the absorbance against the concentration range10-100 μgml^{-1} with molar absorption coefficient 1.856×10^4 $1mol^{-1}cm^{-1}$. Table 2 summarizes the results of statistical analysis of the experimental data.

Table 2. Optical and regression characteristics of the proposed method.

Parameters	Proposed method
λmax (nm)	580
Beer's law limit µg/ ml	10-100
Molar absorption coefficient ($1mol^{-1}cm^{-1}$)	1.856×10^4
Linear regression equation*	$A= 0.57 + 6 \times 10^{-3}c$
Correlation coefficient	0.9999

*With respect to A =a + b c where c is the concentration ($\mu g\ ml^{-1}$) and A is absorbance.

3.3. *Spectrophotometric analysis of hydroquinone skin whitening cosmetics:*

Comparative study of contents of hydroquinone by Spectrophotometry was carried out in 23 different skin whitening cosmetics collected randomly from Najran markets. The content of hydroquinone in analysed samples is presented in Table 3.

Among the studied samples the highest content of hydroquinone was found in Tritospot and Whitening cream (3.9 and 3.3 %) respectively, although the labeled content in first 3% and in second 1.9%. Hydroquinone was not present in labeled contents of many samples cream but on analysis hydroquinone detected in twelve samples in varying percentage range from 0.06 to 2.4%. On other hand there are nine samples of cream from different production countries are free from hydroquinone Table 3.

Table 3. Percentage concentration of hydroquinone

Sample cream	% of hydroquinone	Sample cream	% of hydroquinone	Sample cream	% of hydroquinone
OLAY (Day)	0.42	WHITE PERFECT Double eye-zone brightener	2.4	FAIRNESS	ND
FADE OUT (Day).	0.06	HIMALAYA (day)	N D	WHITENING 1.9% Hydroquinone	3.3
RICOWith Hydroquinone	ND	TRITOSPOT 3%Hydroquinone	3.9	MENA	ND
REO	0.59	STRENEX	2.17	BEANNE	1.74
FACIAL SCRUB With lemon and honey extract	ND	GARNIER Skin naturals (Night)	0.58	REVITALLIFT	0.33
WHITE PERFECT Transparent rosy whitening (Toner)	ND	MAGICAL Beauty Facial whitening	0.67	REVITALLIFT (Double Lift)	ND
WHITE PERFECT Transparent rosy whitening (Night)	ND	KELLY PEARL	0.5	COLLGEN RE-PLUMPER(Day)	ND
FAIREVER	0.09	EXTRADERM Plus Exfoliant 2%Hydroquinone	0.25		

ND: Not detected

4. Conclusion

From the method validation, we can conclude that the method is suitable to be used for the simultaneous determination of hydroquinone in skin whitening formulations. Since hydroquinone is detected in some formulation with higher percentage more than 2% but other formulation free from any amount of hydroquinone in its content. On other hand, many samples were containing different percentage of hydroquinone below 3% although their composition not labeled it.

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