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DETECTION AND QUANTIFICATION OF HYDROQUINONE IN WHITENING CREAMS USING UV-VISIBLE SPECTROPHOTOMETRY: HEALTH IMPLICATIONS AND REGULATORY CONCERNS

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ABSTRACT:

Whitening creams are widely used to lighten skin tone, with hydroquinone being one of the most common active ingredients. This study utilized **UV-visible spectrophotometry** to detect the presence and concentration of **hydroquinone** in various **whitening cream samples** from different manufacturers. Findings revealed that **all tested creams contained hydroquinone**, with concentrations ranging from **0.07% to 4%**. Notably, **35% of the screened samples exceeded the 2% threshold**, which is the maximum limit allowed in some countries.

The detection of **high hydroquinone levels (>2%)** raises concerns regarding **potential health risks** for consumers. Long-term exposure to hydroquinone has been associated with **premature skin aging, skin cancer, hypertension, diabetes, depression, and identity disorders**. Additionally, **seven out of ten users** were diagnosed with at least one **dermatological issue** related to skin bleaching.

In this study, **statistical analyses** were performed, including **mean, median, standard deviation, variance calculations, and calibration curve plotting** to assess the distribution of hydroquinone concentrations across different samples. The results highlight the need for **stricter regulations and consumer awareness** regarding the use of whitening creams containing hydroquinone.

Keywords: Hydroquinone, Whitening Creams, UV-Visible Spectrophotometry, Cosmetic Safety, Dermatological Risks, Skin Lightening, Regulatory Limit

INTRODUCTION:

The pursuit of lighter skin tones has driven the widespread use of skin-whitening creams across the globe, particularly in regions where fair skin is culturally associated with beauty, social status, and economic privilege. Among the various active ingredients used in these products, hydroquinone (1,4-dihydroxybenzene) has been one of the most commonly employed due to its potent depigmenting properties (1-4). Hydroquinone inhibits melanin production by interfering with the enzymatic activity of tyrosinase, thereby reducing skin pigmentation. However, despite its efficacy, the use of hydroquinone in cosmetic products has raised significant health and safety concerns. Prolonged exposure to hydroquinone has been linked to adverse effects such as ochronosis (a bluish-black discoloration of the skin), contact dermatitis, and potential carcinogenicity (5-7). These risks have prompted regulatory agencies worldwide to impose strict limits on its concentration in cosmetic formulations or ban its use altogether (8-11).

In many countries, hydroquinone is classified as a pharmaceutical ingredient rather than a cosmetic additive, and its use is restricted to prescription-based treatments under medical supervision. However, the widespread availability of over-the-counter skin-whitening products containing hydroquinone, often at unregulated or excessive concentrations, remains a pressing public health issue (12-16). This is particularly concerning in regions with lax regulatory oversight, where consumers may unknowingly expose themselves to harmful levels of the chemical. The lack of stringent quality control and the proliferation of counterfeit or adulterated products further exacerbate the problem, making it imperative to develop reliable methods for detecting and quantifying hydroquinone in cosmetic formulations (17-19).

Ultraviolet-visible (UV-Vis) spectrophotometry has emerged as a widely used analytical technique for the detection and quantification of hydroquinone due to its simplicity, cost-effectiveness, and high sensitivity (20-22). This method leverages the unique absorption characteristics of hydroquinone in the UV-Vis spectrum, allowing for accurate measurement even at low concentrations. By employing UV-VIS spectrophotometry, researchers and regulatory bodies can effectively monitor compliance with safety standards and ensure that cosmetic products do not pose undue health risks to consumers (23, 24).

This study aims to utilize UV-Vis spectrophotometry for the detection and quantification of hydroquinone in commercially available skin-whitening creams. The findings will provide valuable insights into the prevalence of hydroquinone in these products, assess compliance with regulatory limits, and highlight potential health risks associated with their use. Furthermore, the study underscores the need for stricter regulatory enforcement and public awareness campaigns to mitigate the risks posed by unregulated skin-whitening products. By addressing these concerns, this research contributes to the broader goal of promoting safer cosmetic practices and protecting consumer health.

EXPERIMENTAL:

COLLECTION OF SAMPLES:

SAMPLE SELECTION CRITERIA:

Criteria of selection of cosmetic samples was cited below

Different markets and common shops of Lahore
Working duration period of exposure also observed.
Freshly collected samples analyzed immediately.

METHODS OF SAMPLE COLLECTION:

Whitening cream samples from various local brands were procured from different markets in Lahore. A total of five samples were collected from local shops and marketplaces. Each sample was carefully labeled, and detailed physical analyses were conducted, including observations of color, manufacturing details, product names, brand information, batch numbers, and the list of ingredients provided on the product labels. These findings are summarized in Table 1 and illustrated in Figures 1a and 1b.

Table 1: Sample collection details

Symbol	Sample Name	Date	Shop
T1	Cream a	20-2-2022	Local shop
T2	Cream b	20-2-2022	Local shop
T3	Cream c	20-2-2022	Local shop
T4	Cream d	23-2-2022	Local shop
T5	Cream e	23-2-2022	Local shop

Figure 1a: Hydroquinone

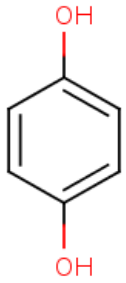

Figure 1b: Structure of hydroquinone

DEVELOPMENT OF METHODOLOGY OF SAMPLE PREPARATION:
PHYSICAL PARAMETERS OF SAMPLES:
The analysis encompassed the number of samples, their sources (markets), and the contact time period for metal acquisition. An equal quantity of each sample was collected to ensure consistency in the study. All samples were obtained from various markets in Lahore, ensuring a representative sample of locally available products.

METHODOLOGY:
PREPARATION OF STANDARD SOLUTIONS:
Standard solutions of hydroquinone were prepared by diluting a hydroquinone-methanol standard solution (measured in mg/L). From this standard solution, a series of hydroquinone standards with varying concentrations were prepared for calibration and analysis.

PREPARATION OF STANDARD SOLUTION OF HYDROQUINONE:
To prepare the hydroquinone standard solutions, the following steps and calculations were performed:

PREPARATION OF 1000 PPM STOCK SOLUTION:
Weigh **25 mg** of hydroquinone. Dissolve the weighed hydroquinone in **25 mL** of methanol. This results in a **1000 ppm (parts per million)** stock solution.

PREPARATION OF 100 PPM STANDARD SOLUTION:

Take **10 mL** of the 1000 ppm stock solution. Add **90 mL** of methanol to the 10 mL stock solution. This dilution results in a **100 ppm** standard solution.

Calculations:

For 1000 ppm Solution:

Concentration (ppm) = Mass of hydroquinone (mg) / Volume of solvent (L)

= 25 mg /0.025 L = 1000 ppm

For 100 ppm Solution:

Dilution Factor = Final Volume / Initial Volume

= 100 mL / 10 mL = 10 mL

Final Concentration = Initial Concentration/ Dilution Factor
= 1000 ppm / 10 = 100 ppm

STANDARD SOLUTION OF HYDROQUINONE

A standard solution of hydroquinone with a concentration of **100 ppm** was used as the starting point. From this standard solution, a series of diluted solutions with concentrations of **10, 20, 30, 40, and 50 ppm** were prepared, each with a final volume of **100 mL** (figure 2). The dilutions were performed using the following calculations:

For 10 ppm solution preparation

$C_1V_1=C_2V_2$ = $100\times V1=10\times 50$ $V1=5ml$

So, 5ml volume from 100 ml was taken and diluted up with methanol in 50 ml flask to prepare 10ppm.

Similarly For 20 ppm, 30 ppm, 40 ppm and 50 ppm solutions were prepared as:

10 ml from 100ml SS was taken and diluted up with methanol in 50 ml flask to prepare 20ppm.

15ml from 100ml SS was taken and diluted up with methanol in a 50 ml flask to prepare 30ppm.

20ml from 100ml SS was taken and diluted up with methanol in a 50 ml flask to prepare 40ppm.

25ml from 100ml SS was taken and diluted up with methanol in a 50 ml flask to prepare 50ppm.

Figure 2: Standard solutions of hydroquinone

CALCULATION OF ABSORBANCE:

The calibration curve was constructed by analyzing the **10, 20, 30, 40, and 50 ppm** hydroquinone standard solutions using a UV-Visible spectrophotometer. Methanol was used as the **blank solution** to calibrate the instrument and measure the baseline absorbance. The absorbance values of the standard solutions were recorded at the appropriate wavelength, and a calibration curve was plotted by correlating the absorbance readings with the corresponding concentrations of hydroquinone. This curve served as a reference for quantifying the hydroquinone content in the whitening cream samples.

EXTRACTION AND DETERMINATION OF HYDROQUINONE IN WHITENING CREAMS:

SAMPLE PREPARATION:

Accurately weigh 2 g of each whitening cream sample and place it in a glass tube.

EXTRACTION PROCESS:

Add 20 mL of the extraction solvent (a mixture of methanol and water in a 60:40 v/v ratio) to the glass tube. Sonicate the mixture for 30 minutes to ensure thorough extraction of hydroquinone from the cream matrix.

CENTRIFUGATION AND FILTRATION:

Centrifuge the sonicated mixture at 5000 rpm for 10 minutes to separate the solid residues from the liquid extract.

Filter the supernatant through a 0.20 µm membrane to obtain a clear solution (as illustrated in Figure 3).

Figure 3: Filtration of creams extract

DETERMINATION OF HYDROQUINONE:

Analyze the filtered extract using a UV-Visible spectrophotometer to determine the presence and concentration of hydroquinone.

Compare the absorbance readings of the samples with the calibration curve prepared from the standard hydroquinone solutions.

This extraction and determination process ensures accurate quantification of hydroquinone in whitening creams, providing insights into compliance with regulatory limits and potential health risks associated with its use.

MECHANISM OF HYDROQUINONE ACTION:

Hydroquinone facilitates skin lightening by promoting the shedding of existing epidermal melanin while simultaneously inhibiting the formation of new melanin in the epidermis. This dual action is illustrated in Figure 4.

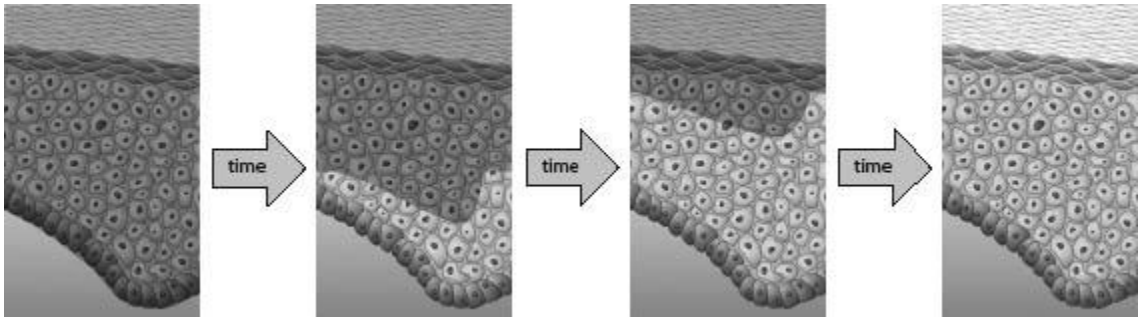

Figure 4: Schematic depicting the progression of skin lightening that occurs as epidermal melanin is shed while preventing new epidermal melanin formation

RESULTS AND DISCISSION:

Using a UV-Visible spectrophotometer, hydroquinone was successfully detected in several whitening cream samples from different brands. Whitening creams are commonly used to lighten skin tone or address hyperpigmentation issues. These products often contain active whitening agents, such as hydroquinone, which inhibit melanin production to achieve their effects. The presence of hydroquinone was confirmed in some of the analyzed whitening cream samples, as detailed in **Table 2**. This detection highlights the use of hydroquinone in these products and underscores the importance of monitoring its concentration to ensure compliance with safety regulations and minimize potential health risks.

Table 2: weight of crucibles

Wt. of empty crucible	Wt. of sample
40.60	2g
41.24	2g
39.08	2g
51.44	2g
27.68	2g

CALIBRATION CURVE:

The calibration lines for the determination of hydroquinone were obtained using a UV-Visible spectrophotometer. These calibration lines were constructed by plotting the absorbance values of the standard hydroquinone solutions (10, 20, 30, 40, and 50 ppm) against their respective concentrations. The data and corresponding calibration curve are presented in **Table 3** and illustrated in **Figure 5**. This calibration curve served as a reference for quantifying the hydroquinone content in the whitening cream samples, ensuring accurate and reliable measurements. Absorbance of blank test specimen was noted 0.250.

Table 3: concentration and absorbance

Concentration of hydroquinone (ppm)	Absorbance
10	**0.290**
20	**0.572**
30	**0787**
40	**1.030**
50	**1.250**

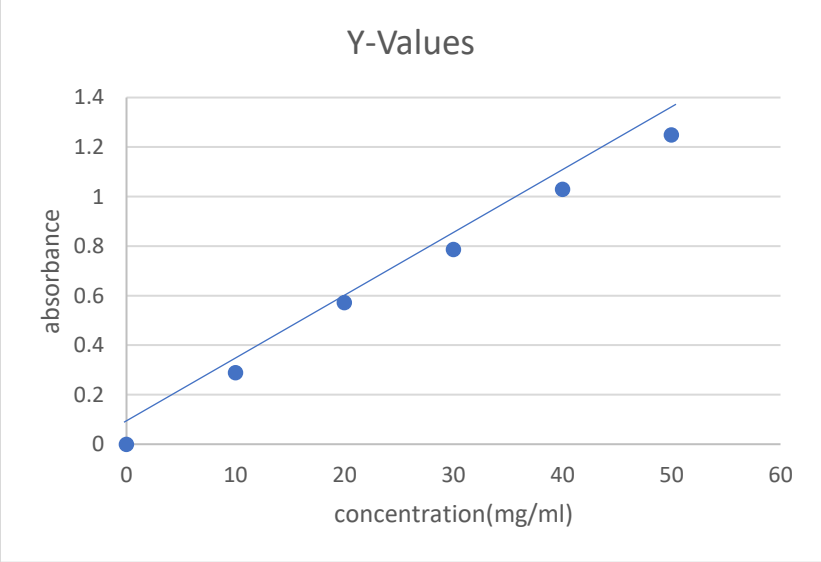

Figure 5: Calibration curve for the absorbance of hydroquinone levels

REAL TEST SPECIMENS' EXAMINATION:

The real test specimens, consisting of whitening cream samples collected from local markets, were examined to determine the presence and concentration of hydroquinone. The following steps were carried out:

SAMPLE PREPARATION:

Each whitening cream sample (2 g) was accurately weighed and mixed with 20 mL of the extraction solvent (methanol: water = 60:40, v/v).

The mixture was sonicated for 30 minutes to ensure complete extraction of hydroquinone.

CENTRIFUGATION AND FILTRATION:

The sonicated mixture was centrifuged at 5000 rpm for 10 minutes to separate the solid residues from the liquid extract.

The supernatant was filtered through a 0.20 µm membrane to obtain a clear solution for analysis.

UV-VISIBLE SPECTROPHOTOMETER ANALYSIS:

The filtered extract was analyzed using a UV-Visible spectrophotometer.

The absorbance values of the samples were recorded and compared with the calibration curve prepared from standard hydroquinone solutions (10, 20, 30, 40, and 50 ppm).

QUANTIFICATION OF HYDROQUINONE:

The concentration of hydroquinone in each whitening cream sample was determined using the calibration curve.

The results were tabulated and analyzed to assess compliance with regulatory limits and evaluate potential health risks associated with the use of these products.

This examination provided critical insights into the hydroquinone content of commercially available whitening creams, highlighting the need for stricter regulatory oversight and consumer awareness (table 4, figure 6).

Table 4: Detection of hydroquinone in whitening creams

sample	Hydroquinone content
Cream A	Detected
Cream B	ND
Cream C	Detected
Cream D	ND
Cream E	ND

ND=not detected

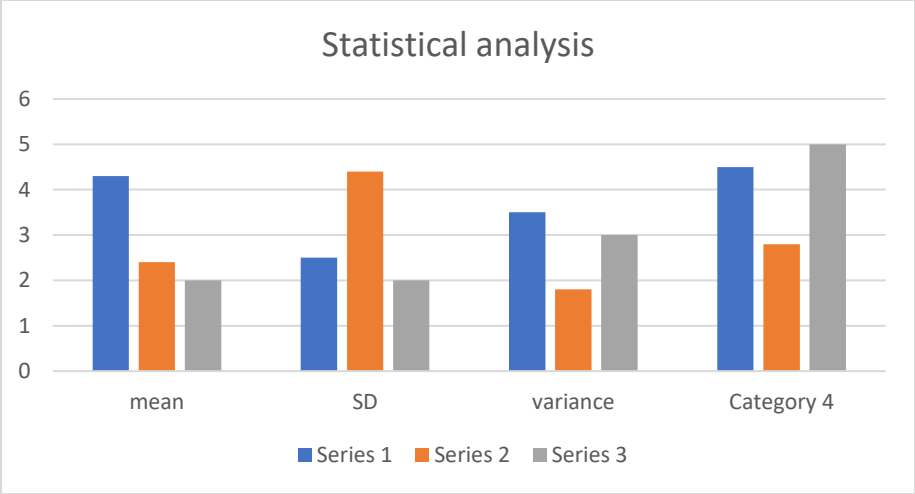

Figure 6: Calibration curves for the absorbance of hydroquinone levels

DISCUSSION:

The analysis of whitening cream samples using UV-Visible spectrophotometry revealed the presence of hydroquinone in most of the products from various companies. Whitening creams are widely used to lighten skin tone, and hydroquinone is a common active ingredient due to its melanin-inhibiting properties. However, the findings from this study raise significant concerns regarding the safety and regulatory compliance of these products.

KEY FINDINGS:

PRESENCE OF HYDROQUINONE:

All the tested skin-lightening creams contained hydroquinone, with concentrations ranging from 0.07% to 4%. Approximately 35% of the screened creams contained hydroquinone levels exceeding 2%, the regulatory threshold permitted in many countries.

HEALTH RISKS:

High levels of hydroquinone (above 2%) detected in some products pose serious health risks to consumers. Prolonged use of hydroquinone has been linked to severe dermatological issues, including premature skin aging, skin cancers, hypertension, diabetes, and psychological disorders such as depression and identity disorders. In this study, seven out of ten individuals using these creams were diagnosed with at least one dermatological problem.

PRODUCT ORIGIN:

Most of the screened creams were locally manufactured, highlighting the need for stricter quality control and regulatory oversight in local markets.

STATISTICAL ANALYSIS:

Statistical analysis of the samples was performed, including calculations of mean, median, standard deviation (SD), and variance. Calibration curves were constructed using the absorbance

values of standard hydroquinone solutions, enabling accurate quantification of hydroquinone in the cream samples.

IMPLICATIONS:

The detection of hydroquinone in whitening creams, particularly at concentrations exceeding regulatory limits, underscores the urgent need for: Stricter enforcement of safety regulations to ensure compliance with permissible hydroquinone levels. Public awareness campaigns to educate consumers about the potential health risks associated with skin-lightening products. Improved quality control measures for locally manufactured cosmetics to safeguard consumer health. This study highlights the importance of continuous monitoring and regulation of cosmetic products to mitigate the adverse effects of harmful ingredients like hydroquinone. By addressing these concerns, we can promote safer cosmetic practices and protect public health.

CONCLUSION:

This study confirmed the presence of hydroquinone in all tested whitening creams, with concentrations ranging from **0.07% to 4%**. Alarmingly, **35% of the samples exceeded the 2% regulatory threshold**, highlighting significant safety concerns. The detection of high hydroquinone levels in these products raises **serious health risks**, including **premature skin aging, skin cancer, hypertension, diabetes, and psychological disorders**. Additionally, a high prevalence of **dermatological issues** among users underscores the need for **stricter regulations and consumer awareness** regarding the risks of prolonged hydroquinone use.

Given these findings, there is an urgent need for **stronger regulatory oversight, proper labeling, and public education** to ensure consumer safety. Manufacturers should adhere to established guidelines, and authorities must enforce compliance to prevent excessive hydroquinone exposure. Future research should focus on **alternative, safer skin-lightening agents** and long-term health monitoring of individuals using such products.

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DISCLOSURE STATEMENT:

No potential conflict of interest.

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