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RESEARCH ARTICLE

DEVELOPMENT OF UV-SPECTROSCOPY METHOD FOR DETERMINATION OF HYDROQUINONE & CLOBETASOL IN SKIN -LIGHTENING CREAMS IN ADEN, YEMEN

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ABSTRACT

Skin whitening creams & products (SL) contain certain unsafe chemicals such as mercury, steroids such as; Clobetasol propionate, and hydroquinone (HQ), which have negative effects on health if used excessively. The present study aimed to determine the presence of the HQ and CP in the SL creams that are sold in the Aden Market and measure the pH of these creams as well. The determination of the HQ & CP in the SL creams was carried out by using UV-Spectroscopy. This study indicated the presence of HQ in all creams in the range between 3.00 ± 1.08- 6.72 ± 0.02 except in Cream I. The lower % was found in Cream V and the higher % was in Cream II. The CP was detected in five creams out of seven creams. The range of the CP was between 0.0085 ± 0.46 - and 0.164 ± 0.15. The pH of all formulations was found to be between 3.63 ± 0.006 and 7.50 ± 0.02. The SL creams or products may contain HQ and CP even if the label doesn't indicate the presence of these compounds. There should be more control, restriction of the use, and distribution of such products to prevent possible long-term adverse effects.

KEYWORDS

Skin whitening creams, hydroquinone, clobetasol propionate.

1. INTRODUCTION

There is a growing drift toward using SL products worldwide especially in hot tropical countries due to the effect of the weather on the skin (Yousif et al., 2014). Obtaining white skin has become one of the most important requirements for different groups of girls and women, as a result of the role of the media and society, which promotes the idea of the confinement of beauty to white skin (Del Giudice and Yves, 2002). So, getting white skin has become an obsession for many women and a kind of addiction, as many girls indicated that they could not stop using these products. However, the use of these products is not without risk and is usually associated with adverse reactions (Kuffour et al., 2014). A recent meta-analysis study stated there is a global pooled lifetime prevalence for using SL products of 27.7%. The authors warned that these outcomes represent a serious worldwide community health problem and emphasized the necessity for epidemiologic studies in other regions not involved in the study (Lewis et al., 2011).

Most of these products especially creams contain HQ, CP, and mercury as the main active ingredients (Yousif et al., 2014). These compounds might have harmful and permanent effects on the skin. Many countries banned the use of HQ and mercury in cosmetic products. However, these products are still sold in Yemen's local market, as well as, there are locally mixed SL products that have no label. Many traders resort to these mixtures as a result of the increasing demand for them. Many local-made creams are made by mixing several SL creams without considering the ingredient percentages from each mixed cream which may lead to the presence of a high concentration of some contents in the final products. They may mix different products with the same active ingredient to make their creams more effective in skin lightening. These products have been considered a cause of serious health-related consequences among users, particularly those who subjected their bodies to creams formulated without proper safety precautions regarding chemical contents used in their production. The use of skin bleaching has also been reported to be responsible for skin cancers, skin staining, and depression among users leading to negative consequences and suicide (Sagoe et al.,2019).

HQ acts as an SL agent by preventing melanin synthesis (Sofen et al., 2016). It is one of the utmost recurrent categories of preparations prescribed by dermatologists for reducing the hyperpigmentation of the skin (Tehranchinia et al.,2018). Different strengths of HQ are used in pharmaceutical products up to 10% (w/w), though the most common percentages range between 4–5% (w/w) (Ghanbarzadeh et al., 2015; Smiles et al., 2007). It is chemically unstable and leads to the formation of p-benzoquinone (pBQ) on degradation. This compound is carcinogenic that may cause dark chromatic aberrations (Matsubayashi et al., 2003). Its effect is not only localized on the skin, but could be absorbed systematically leading to side effects such as; hepatotoxicity, nephrotoxicity, and neurobehavioral alterations (Bahadar et al., 2015).

CP is the propionate salt form of clobetasol, a topical synthetic corticosteroid with anti-inflammatory, anti-pruritic, and vasoconstrictive properties. CP is applied to treat skin diseases such as eczema, contact dermatitis, seborrheic dermatitis, and psoriasis. It is used in several pharmaceutical dosage forms such as cream, ointment, or shampoo. It should be applied for the short-term and solely if other common corticosteroids are not effective (Abraham, 2008). The most common or very common skin reactions are telangiectasia, burning sensation of the skin, dry skin, flushing or redness of the skin, and rare or very rare adrenal suppression. hypertrichosis, skin depigmentation (may be reversible) (Joint Formulary Committee, 2020). The literature review revealed that there are several studies carried out worldwide for the determination of HQ and CL in SL products. Two studies in Nigeria indicated the presence of the HQ in SL cream by UV-spectroscopy, even if their label didn't

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indicate its presence among the contents (Odumosu and Ekwe, 2010; Mahmoud, 2015). Another study in Nigeria, on the detection of HQ and heavy metals, revealed that the % of the HQ was within the permitted limit (Oyedeji et al., 2011).

Also, two studies carried out in Pakistan determine HQ in SL cosmetics by TLC and HPLC, the results revealed the presence of HQ (Siddique et al., 2012; Arshad et al., 2021). A study in Ghana determined HQ and mercury in SL creams and soaps by Atomic Absorption Spectrophotometry also the results indicated their presence (Agorku et al., 2016). In addition, a study in South Africa stated the presence of HQ and mercury in the SL products as well a study in West Africa and Canada indicated the presence of HQ, mercury, and CP in percentages that exceed the permitted limits of US FDA standards (Maneli et al., 2016; Gbetoh and Amyot, 2016). A study in Korea monitored the presence of CP and other corticosteroids in SL products (Nam et al., 2011).

The studies relating to the analysis of these locally made creams are scary. There was a previous study conducted on the manufactured SL cream and the study revealed a high percentage of hydroquinone and mercury in some products (Saleh et al., 2015). however, there was no study carried out to determine the contents and percentage of the CP. The previous study focused on ready-made or manufactured skin creams but the recent study will concern locally mixed creams and imported ones. This work aimed to analyze the presence of HQ & CP in some imported and local-made or mixed SL creams. The measurement of the pH was also involved. The study will provide information about the presence or absence of the potentially toxic ingredients and their percentages (HQ & CP) in locally mixed skin-lightening creams. There is no official department that evaluates the quality and the contents of them in Aden, Yemen.

2. Material and Methods

The information related to the instruments used in this study is listed in Table 1.

Table 1: List of Instruments.

No.	Instrument	Made In
1	Electronic Balance	A&D company LTD, Japan, HR-250
2	pH Meter	Inolab WTW, Germany
3	Electronic Shaker	Patterson Scientific LTD, Germany, KS130
4	Water Bath	Clifton, England, NE2-56D
5	UV-Visible Spectroscopy	(Lasany® advanced microprocessor UV-VIS-L1-295)
6	Centrifuge	P-selecta, Mixtasel
7	Vortex	Heidolph Reax Top, Germany

All chemicals were of analytical grads. The Most common creams were collected from community pharmacies and cosmetics stores. Some of these creams were imported and some of them were locally mixed. These creams are coded from I to VII. The information related to the creams is illustrated in Table 2.

Table 2: Different Brands of SL Creams.

Brand Code	Made In	Contents	Manufacture Date & Expire Date	Batch No
Cream-I	Local Mixed	No label	-	-
Cream-II	Al-Haitham-Aden-Yemen	Milk, turmeric, vitamin (C), Chamomile herbs	Mfg: Dec-2020 Exp: Dec-2024	0033
Cream-III	Designed by U.A.E / Al-Haitham-Aden	Arbutin, vitamin (C), licorice, ginseng, herbal materials	Mfg: Aug-2020 Exp: Aug-2024	402
Cream-IV	-	No label	Mfg: Jun-2021 Exp: Jun-2024	-
Cream-V	Local Mixed	No label	-	-
Cream-VI	Gandour-Cote Ivory	Water (aqua), mineral oil, petrolatum (paraffin), lanolin, stearic acid, cetyl-stearylic alcohol, isopropyl myristate, BHT, methylparaben, propylparaben, sodium lauryl sulfate, sodium sulfate, citric acid, glycerin, tocopheryl acetate (Vit. E), Acid Kojic, collagen, carrot oil, fragrance	Mfp: Mar-2021 Exp: Mar-2024	1041124
Cream-VII	-	No label	-	19364

The study was carried out by detecting the presence of the HQ and CP in the SL creams. Modified UV- spectroscopy method was used for simultaneous determination of the HQ and CP. HQ and CP were analyzed according to the method of Odumosu, P. O for HQ and Devi, et.al 2016 for CP with a little modification to adapt to the condition of our laboratory feasibilities and material. To determine the wavelength of absorption maxima (λ max) of HQ and CP an aliquot of 20 ppm of HQ and 0.65 ppm of the solution was prepared and scanned by a UV-Visible spectrophotometer in the wavelength range of 400-200 nm against ethanol as a blank. The wavelength at which maximum absorption for HQ is 294 nm and 260 nm for CP was determined. Then both compounds were mixed and examined using ethanol as blank. There was no interference between the two compounds' peaks. Accurately 0.1 -0.7 mL from HQ stock solution (400 ppm) was mixed with 0.015-0.095 mL from CP stock solution (100 ppm) and completed to 10 mL. These diluted solutions were checked for linearity, precision, accuracy, Limit of Quantification (LOQ), and Limit of Detection (LOD).

Method validation was performed as per the International Conference on Harmonization (ICH) guidelines Q2 (R1) (ICH, 2005). The linearity of this method was checked for HQ at concentrations (4 -28 ppm) and for CP (0.15-0.95 ppm). The investigated concentrations followed Beer's Lambert law. The precision of the UV method was performed by intermediate precision (inter-day) and repeatability (intra-day). To assess the intra-day variation, the % RSD was calculated from absorbance as obtained. Accuracy is defined as closeness between the actual (true) value and the value obtained by repeating the test method several times. Accuracy may be expressed as % Recovery by the assay of known analyte which is added. It gives the exact measure of the analytical method. The accuracy of the method was tested via the standard addition method; a known amount of HQ & CP were added to pre-analyzed cream samples. The pre-analyzed samples were spiked with an extra 50, 100, and 150% of the HQ and CP, and the mixtures were analyzed using a UV visible spectrophotometer. The experiment was performed in triplicate. One of the SL creams was selected, exactly 200 ppm of the cream was weighted and spiked with different concentrations of HQ and CP. The Limit of Detection (LOD) is the lowest concentration of analyte present in a sample, which can be analyzed but not necessarily quantitated. The Limit of Quantitation (LOQ) is the lowest concentration of analyte present in a sample, which can be quantitatively analyzed with acceptable precision and accuracy. The LOD and LOQ were assessed based on the technique of signal-to-noise ratio10 using Equations (1) and (2).

$$LOQ = \frac{10\,x\,\sigma}{S} \text{----------} \quad (1)$$

$$LOD = \frac{3.3\,x\,\sigma}{S} \text{----------} \quad (2)$$

Where σ is the standard deviation of the intercept of the calibration plot and S is the slope of the calibration curve. The pH of the creams samples was measured using a pH meter, having an Automatic Temperature

Compensation (ATC) probe. The pH meter was calibrated using standard buffer solutions of pH 4.01, 7.00 & 10.01, before the measurements. Approximately, 1 gm of creams in 10 mL of purified water (10 %w/v) was heated in a water bath at 50°C, then the pH was measured. Measurements were made in triplicate and the averages of three readings were noted.

3. Results and Discussion

The literature review revealed that there is no study about the simultaneous determination of HQ and CP. So, according to our laboratory facilities, a new method for simultaneous detection of both drugs was developed. Since both drugs are soluble in ethanol and it was available at our laboratory. It was selected as a solvent. Each drug was examined alone to determine the maximum absorbance. The ʎmax of drugs were found to be 294 nm and 259 nm respectively. Then both drugs were mixed and examined by UV-Spectroscopy, there was no interference between them as shown in Figure 1.

Method validation was performed as per (ICH) guidelines Q2 (R1). The linearity of this method was checked for HQ at concentrations (4, 8, 12, 16, 20, 24, 28 ppm) and for CP was (0.15, 0.25, 0.5, 0.65, 0.75, 0.85, 0.95 ppm). The investigated concentrations followed Beer's Lambert law. Three sets were evaluated for linearity. A linear relation was found between absorbance at λ max and the concentration of HQ and CP. The calibration is described by the equation:

$$Y = a + b X \quad (3)$$

(Where Y = absorbance, a = intercept, b = slope and X = concentration in µg ml^{-1}) obtained by the method of least squares.

The straight-line equation for the HQ was as indicated by the following equation:

$Y=0.0265\ X+0.0118$ $R^2= 0.9998$

The straight-line equation for the CP was as indicated by following equation:

$Y= 0.7313\ X+ 0.0259$ $R^2= 0.9995$

The LOD and LOQ were calculated as LOD (k = 3.3) and LOQ (k = 10) and these were found to be 0.49 ppm and 1.49 ppm respectively for the HQ and 0.026 ppm and 0.078 ppm for CP. The precision (measurements of intra-day and inter-day) results are demonstrated in Table 2, significant reproducibility with % RSD below 2.0 was observed. This showed that the method is highly precise. The percent recovery value in Table 3, was observed within the range which indicates the accuracy of the method. All validation parameters are illustrated in Table 4.

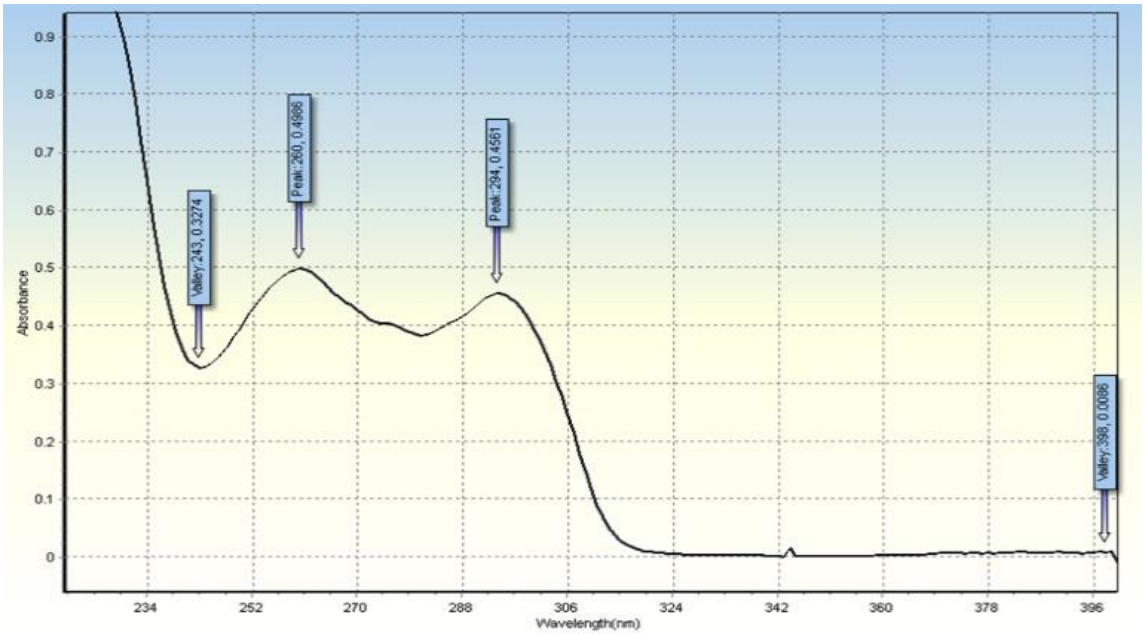

Figure 1: The UV spectrum of HQ and CP in ethanol ($ʎ_{max}$ at 294 nm and 259 nm).

Table 3: Statistical Evaluation of Precision Studies for HQ and CP.

	Parameters	Lower Conc. (8ppm) (n=9)	Medium Conc. (16 ppm) (n=9)	High Conc. (24 ppm) (n=9)
HQ	Average	0.2258	0.4338	0.6666
	SD	0.0045	0.0084	0.0070
	RSD%	2.01	1.94	1.06
CP	**Parameters**	**Lower Conc. (0.15 ppm) (n=9)**	**Medium Conc. (0.65ppm) (n=9)**	**High Conc. (0.95ppm) (n=9)**
	Average	0.1356	0.4528	0.8307
	SD	0.0029	0.0048	0.0072
	RSD%	2.15	1.05	0.87

Table 4: Recovery Data for the Accuracy Analysis of HQ and CP.

Excess of HQ Added (%)	Con. of the Sample (ppm)	Theoretical conc. of the Spiked Sample (ppm)	Conc. of Spiked sample ± SD (ppm) (n=3)	Recovery± SD (%)	%RSD
50 %	200	4	3.92 ± 0.081	98.21 ± 2.03	2.07
100%	200	16	15.90±0.149	99.35 ±0.93	0.94
150%	200	24	23.65 ±0.17	98.55±0.70	0.71
Excess of CP Added (%)	**Conc. of a Sample (ppm)**	**Theoretical conc. of the Spiked Sample (ppm)**	**Conc. of Spiked sample ± SD (ppm) (n=3)**	**Recovery± SD (%)**	**%RSD**
50 %	200	0.15	0.15 ±0.0016	103.63 ±1.05	1.02
100%	200	0.65	0.68 ±0.0056	104.04 ±0.87	0.83
150%	200	0.85	0.88±0.016	103.67±1.92	1.86

Table 5: Validation Parameters for the Developed Method.			
HQ		**CP**	
Validation Parameters	**Data (Mean ± SD)**	**Validation Parameters**	**Data (Mean ± SD)**
ʎmax (nm)	294 nm	**ʎmax (nm)**	259 nm
Range (ppm)	2.0-28 ppm	**Range (ppm)**	0.15-0.95 ppm
Correlation Coefficient	0.9998 ± 0.00139	**Correlation Coefficient**	0.9995 ± 0.0014
Intercept	0.0265 ± 0.0059	**Intercept**	0.0259 ± 0.0025
Slope	0.0118± 0.0012	**Slope**	0.7313 ± 0.0045
LOD (ppm)	0.49 ppm	**LOD (ppm)**	0.026 ppm
LOQ (ppm)	1.49 ppm	**LOQ (ppm)**	0.078 ppm
Precision (%RSD) for 3 Concentrations	1.06-2.01%	**Precision (%RSD) for 3 Concentrations**	0.87-2.15%
Accuracy	0.71-2.07	**Accuracy**	0.83-1.86

SL products are types of cosmetics (creams, gels, lotions, and soaps) applied voluntarily on the skin. Several of these products contain a variety of active ingredients that are highly toxic. Among those toxic agents, the present study focuses on mercury, HQ, and CP. The two ingredients were selected amongst others because they are among the most toxic and most used agents in lightening products. A previous study was conducted in Aden- Yemen for the assessment of HQ (Saleh et al., 2015). However, the current study used other SL creams that were not investigated before. Most of these products are imported from countries that have weak regulatory inspection and screening as well as no standard conditions for manufacturing or are made locally by mixing several SL creams that have no regulation on their quality. Several creams are mixed to get a more efficient and rapid effect of whiting.

The pH of the skincare product that is used daily can influence skin properties. The pH of all formulations was found to be between 3.48 ± 0.01and 7.50 ± 0.02, the results are presented in Table 6. There is a consensus that topical products should have pH values in the range of 4 to 6 (Lukić and Savić, 2021). The pH of all formulations lies more or less in the normal pH range of the skin 4.0 to 7.0. Two products were slightly acidic and below the normal range of skin pH. Some manufacturers make facial creams slightly acidic to help cell turnover gently. However, long-term application to the skin may lead to irritation of the skin. Three products have a pH above 7 which means slightly basic. A study indicated that skin products with pH 8 implied that long-term application of alkaline skincare products can alter the stratum corneum barrier function more than acidic cosmetic products (Lambers et al., 2006). It can be stated that the pH of the daily used skincare product is very important for the skin barrier, homeostasis, and sensitivity. The current results are near a previous study for SL creams in Aden-Yemen which indicated a pH range between 3.09 - 7.70. The pH ranges of these products were higher than to of products available in Nigeria (4.99 – 6.73) (Oyedeji et al., 2011).

The creams were analyzed for total HQ and CP by UV Spectrophotometry. The results of this investigation studies are shown in Table 6. The graphs of creams that contain the max amount of HQ and CP are presented in Figure 2,3. The presence of the HQ and CP was verified by spiking of HQ and CP creams that were used as standard. This study indicated the presence of HQ in all creams in the range between 3.00 ± 1.08- 6.72 ± 0.02 except in Cream I. The lower % was found in Cream V and the higher % was in Cream II. The CP was detected in five creams out of seven creams. The range of the CP was between 0.0085 ± 0.46 - and 0.164 ± 0.15. The previous study carried out in Aden-Yemen for the SL creams exported to the local market indicated that HQ in the creams ranged from below 0.076 to 4.533% (Saleh et al., 2015).

The results of the current study are similar to the previous study carried out in Nigeria to detect the presence of the HQ in some cosmetic creams that the labels on the packages strikingly did not specify the levels of HQ. The percentage of HQ ranged from below 2 to above 5 % (Odumosu and Ekwe, 2010). As well as, the result is comparable with the study in Pakistan. The HQ in SLC ranged from 0 to 7.14 ± 0.18% with a median value of 0.33%. In 25% of the studied samples, HQ was not detected, 70% of the samples showed values within the limit and 5% of the samples (1 sample) had an HQ concentration above the permissible limit defined by Pakistan (5%) (Arshad et al., 2021). Another study in Pakistan indicated concentration of HQ ranged from 0.002% to 0.092% in eleven out of 22 cosmetic samples (Siddique et al., 2012).

Also, an estimation of the active ingredients of 29 SL products in South Africa was performed. The result indicated the presence of the steroids, Hg, and HQ. About 11 (37.9%) of the products contained HQ below 2% (0.69 -46.52 µg/mL) and CP in nine products in the range between (1.15-19.43µg/mL) (Maneli et al., 2016). Similarly analyzed the presence of HQ, CP, and Hg in 93 lightening soaps and 98 creams in West Africa and Canada. Among the 98 SL creams, levels of HQ in creams ranged from 0 to 6% (w/w). CP was detected in 39% (38/98) of creams with a mean of 0.0265% (w/w). However, 20 % (20/98) of creams exceeded US FDA standards limits of 0.05%, (w/w) (Gbetoh and Amyot, 2016).

A study in Korea monitored the presence of CP and Betamethasone in cosmetic products. The results showed 32–96.4 µg /g levels of CP in five different cosmetic products (Nam et al.,2016). The result from a study carried out in Nigeria for 35 SL formulation, indicated the presence of HQ in 15 samples in concentrations ranging from 0.017 to 7.096%, and 8 samples were found to contain CP 0.007% (Mahmoud, 2016). The HQ is very commonly used in skincare products as a whitening agent to reduce skin color, it is the most toxic substance used in cosmetics. The results indicated the presence of HQ % which is higher than 2% which is recommended by WHO,2014 & the U.S. Food & Drug Administration (USFDA). HQ is prohibited in cosmetics products, as it is listed in Annex II (entry 1339) of the European Cosmetic Regulation No 1223/2009.

Table 6: The % of HQ and CP and pH for the Evaluated SL creams.				
No.	**Name of Cream**	**% of HQ ± SD**	**% of CP ± SD**	**pH value ± SD**
1	*Cream I*	0	**0.0085 ± 0.46**	5.5 ± 0.11
2	*Cream II*	**6.72 ± 0.02**	0	**7.50 ± 0.02**
3	*Cream III*	5.479 ± 1.27	0	7.40 ± 0.07
4	*Cream IV*	4.07 ± 0.20	**0.164 ± 0.15**	6.13 ± 0.01
5	*Cream V*	**3.00 ± 1.08**	0.067± 0.67	6.62 ± 0.05
6	*Cream VI*	3.11 ± 1.5	0.063 ± 0.97	**3.63 ± 0.006**
12	*Cream VII*	4.196±1.80	0.051 ± 0.828	7.35±0.017

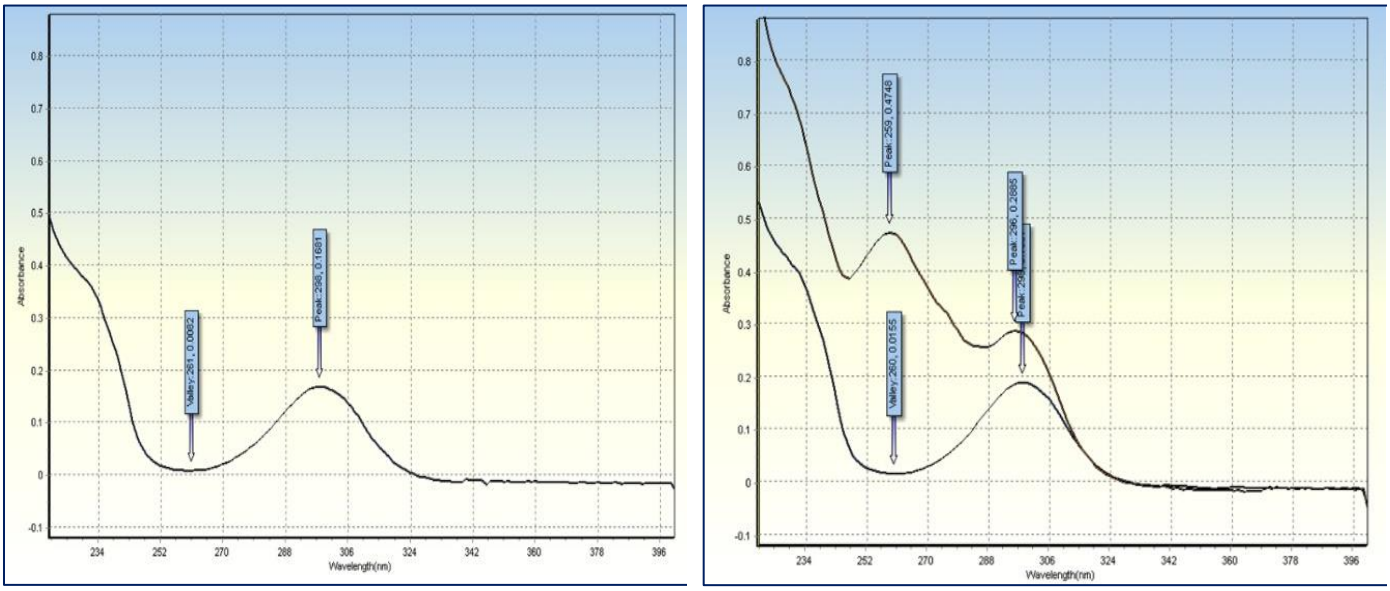

Figure 2: The UV spectrum of (a) Cream II in ethanol, and (b) Cream II in ethanol was spiked by HQ and CP.

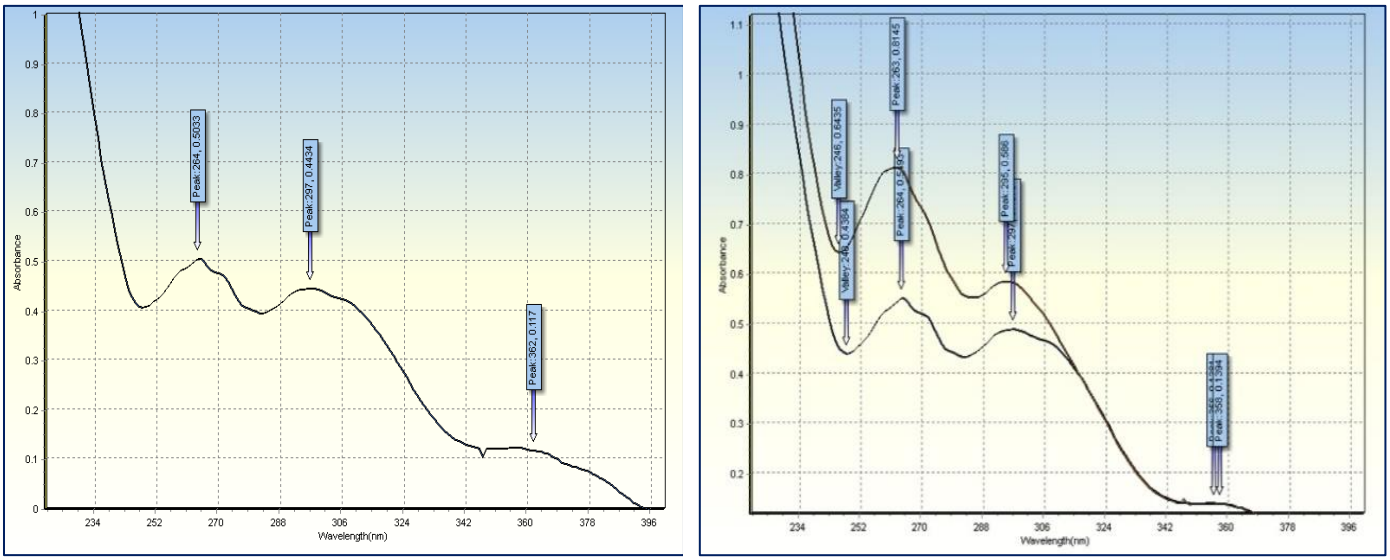

Figure 3: The UV spectrum of (a) Cream VI in ethanol and (b) Cream VI in ethanol was spiked by HQ and CP.

4. Conclusion

The SL creams or products may contain HQ and CP even if the label doesn't specify the presence of these compounds. Most of the examined products contain HQ & CP in varying percentages and some of them exceed the recommended limit by some official regulations over the world. As well as the pH of some evaluated creams, the results revealed that some of the products were too acidic and some of them were basic which may harm the skin. There should be more stringent control of the use and distribution of such products to prevent possible long-term adverse effects. So, it further highlights the importance of managing the problem of getting these products without a medical prescription. The continuous use of these products without control will lead to complicated side effects not only on the skin but overall body systems.

Recommendations

1. Other quality specification tests should be carried out such as; sterility, isotonicity, pyrogen, viscosity, and spreadability of the creams.
2. To analyze the cited drugs by using more precise equipment such as LC-Mass to be sure about the presence of these drugs in the analyzed creams.
3. Analysis of mercury and other heavy metals in these products by using ICP-MASS.
4. Increase the awareness, and knowledge of females and even males about the health risks of continuous use without medical supervision.
5. The sales and marketing of SL products must be controlled, which could be done by imposing fines and penalties on the marketers of such products.
6. This is quite worrisome, and the challenges require key health policymakers and officials to prevent the dispensing of these medicines without a prescription.

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