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ORIGINAL ARTICLE

Identification and Characterization of Counterfeit Kohl Samples using Sophisticated Analytical Techniques

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ABSTRACT

CONTEXT: This research paper is divided into three parts: (a) packaging analysis (b) chemical analysis to determine the composition (c) contamination analysis of questioned kohl cosmetics.

AIM: The present work deals with the comparative analysis of authentic and questioned cosmetic products using different analytical techniques.

SETTINGS AND DESIGN: The study examined 24 samples of two different kohl brands named B1 and B2. Four genuine samples were used as a benchmark to check the variability and relevancy of the obtained results. 20 questioned samples were named C1-C10 of first brand (B1) and C11-C20 of second brand (B2). The references were named GA1-GA2 (authentic samples of brand 1) obtained from the original website of the first brand and as GB1-GB2 (authentic samples of brand 2) purchased from the original website of the second brand. Packaging, chemical and contamination analysis were done on questioned, and authentic samples of two dissimilar brands of kohl cosmetics. **MATERIALS & METHODS:** Chemical analysis was done by utilizing sophisticated techniques such as FTIR, 1HNMR, and EDX. Additionally, contamination analysis was performed on the questioned samples by employing optical microscopy.

STATISTICAL ANALYSIS: The SPSS version 23.0 was used to determine the difference between the mean elemental composition of authentic and suspected counterfeit samples. **RESULTS:** Through the first-line investigation of the hologram on the samples, it was detected that out of 20 samples, 17 samples contained damaged or scrambled holograms (B1) or just a silver tag (B2). The micro-text was not detected in these samples compared to genuine samples (n=4). In terms of chemical analysis using EDX, the presence of palladium, cadmium, and mercury were detected in all samples. NMR delta values for both the authentic and questioned samples were different which concluded that the molecular structure and composition of both samples were dissimilar and consisted of different elements. Optical microscopy affirmed the presence of E. coli in two samples.

CONCLUSION: From the analysis, it was observed that the visual comparison with authentic sample is the first step to detect counterfeit packaging but due to the adaptation of new printing technology by the counterfeiters, they can easily replicate authentic product packaging including security features such as barcodes. Therefore, it is essential to analyze the sample through chemical investigation to check the product in detail. The study was performed on a limited number of samples and therefore encourages chemical and packaging profiling of counterfeits on a bigger scale.

KEYWORDS | counterfeits, cosmetic products, analytic strategy, composition analysis

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INTRODUCTION

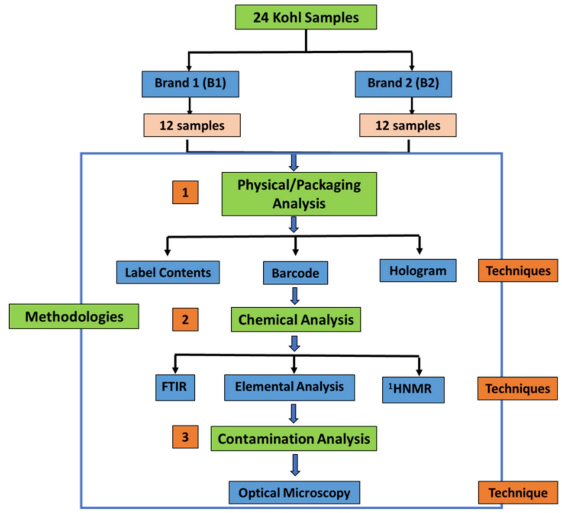
Counterfeiting is a serious problem that has magnified over the past decade as the cosmetics we use on our skin if not made legitimately can hurt our wellbeing.[1] With no valid verification of vendors by the organizations and proficient-looking sites, the counterfeit cosmetic business has amplified in recent years.[2-4] Packaging is usually accompanied by security features as a visible indicator which if absent or altered, indicates as evidence that the product has been tempered or completely a substandard product.[5] Counterfeiters are focused on getting maximum profit from minimum investment and as replicating packaging is potentially expensive to do, therefore, it's much harder to replicate the exact quality, weight, and feel of a paper or contents on the label including security features such as holograms and barcodes.[6-8] Holograms and barcodes frequently give clear first-line authentication and have turned out to be extremely difficult to duplicate precisely. [9-11] Unlike medicines that include active substances with pharmacological importance, and are distributed and controlled by pharmacies, cosmetics do not have any such type of pharmacological use and can be sold without any restraints.

The current market for cosmetics in India is US$6.5 billion and is expected to reach US$ 20 billion with a Compound Annual Growth Rate (CAGR) of 25% by 2025. On the other hand, the global cosmetic market is expected to reach US$450 billion with a CAGR of 4.3% by 2025.[12] In India, under the Drugs and Cosmetics Act, 1940 and Rules, 1945 and labeling declarations by the Bureau of Indian Standards (BIS), the cosmetics products are regulated. Under Schedule 'S' of the Drugs and Cosmetics Rules 1945, BIS sets the standards of cosmetics for the products listed. BIS has provided the specification for skin creams and lipsticks in the Indian Standards (IS) 9875:1990 and 6608:2004, respectively. [13]

Kohl or eye pencils are popular eye cosmetics being widely used across Asia, Africa, and the Middle East. Since ancient times, kohl has been believed to protect the eye from different eye diseases. Kohl is described as an ultra-fine powder consisting of various ingredients such as herbs, pearls, gemstones, galena, etc., for the prophylaxis and cure of eye ailments.[14,15] Today, the ingredients have changed considerably and different eye cosmetic brands employ various components to make their products more consumer-friendly by giving diverse features to it such as by making it water-proof, various color options, etc. But counterfeit manufacturers rip off these brands to manufacture copycats and produce the duplicate version of the eye cosmetics, thus creating problems to consumers as well as to authentic cosmetic companies. Counterfeit cosmetics are sold at huge discounts, thus harming the image of authentic proudcts and causing huge loss for original cosmetics companies.

One may need to investigate the counterfeit products in great detail to differentiate them from genuine products. The investigation should be carried out by sophisticated techniques in a dedicated laboratory. Numerous analytical techniques are available for the detection of counterfeit cosmetics. Some of these methods are non-invasive while others involve chemical analysis that is capable of examining the active ingredients and impurities in counterfeit cosmetic products. The presence of heavy metals like zinc and lead found in the vast majority of cosmetic products are of insignificant amount, but if the amount increases even a little above the approved level, they can harm the entire body.[16-17] According to a few reports, counterfeit cosmetic products contain heavy metals in lipsticks, kohl, eyeliner, etc, and the presence of heavy metals in cosmetics like lipsticks and kohl, heavy metals get absorbed or enter the human body through the mouth and eye. There have been some studies on contaminated eye cosmetics like kohl which have shown ingestion of lead up to 15% in adults and 41% in children.[18,19] Currently, counterfeiters use a small percentage of active ingredients in their illegal preparations therefore, detection should not only be qualitative but also quantitative.

In the present study, an investigation has been done on questioned kohl samples which were bought from third-party sellers. Two different brands of kohl/eye pencils (n=12/brand), which were bought from different genuine sources, and third-party sellers (total 24 samples) were analyzed. Analysis was done in three steps: in the first step, packaging analysis was performed by checking the seal, holograms, and barcodes. Physical analysis revealed that out of 20 questioned samples bought from third-party sellers, 7 samples from brand 1 and 8 samples from brand 2 were found to be suspect. In the second step, chemical analysis was done by utilizing sophisticated analytical techniques such as Fourier-transform infrared spectroscopy (FTIR), Proton nuclear magnetic resonance (1HNMR), and Energy Dispersive X-Ray Analysis (EDX). In the third or final step, the analysis was done by employing optical microscopy which confirmed the presence of E. coli. The schematic representation of the work carried out is given in Figure 1. From the chemical and contamination analysis of kohl samples selected from two different brands, it was found that counterfeit samples contained heavy metals which are harmful to the human body, and in addition, microbial contamination was also detected that could cause different diseases.



MATERIALS AND METHOD

Materials:

Nutrient broth, Nutrient agar, and Safranine dye were obtained from HiMedia Laboratories Pvt. Limited, (Mumbai, India). Ethanol (99.9% purity) was obtained from Changshu Yangyuan Chemical Co., Ltd, (Suzhou, China). E. coli (MTCC 40) was obtained from IMTECH Chandigarh, India. All the chemicals were used as such without purifying any further.

Samples:

(a) *Authentic/genuine products:* Four genuine samples were utilized as a control for the investigation. These products were ordered online from original websites and these were employed as a control to compare the results to other samples obtained from suspected third-party sellers.

These four samples were from the two different brands named as B1 and B2. Two samples of brand B1 and B2, separately utilized as control, were employed to check the variability of the genuine products and also to assure the relevancy of the obtained results. The references were named as GA1-GA2 obtained from the original website of the first brand and as GB1-GB2 purchased from the original website of the second brand. The naming of the brands was done as such because of confidentiality reasons. The kohl product was selected for the examination because of its frequency to be counterfeited.

(b) *Counterfeits:* Twenty suspected counterfeits of the kohl product were examined in the study which was bought from third-party sellers, local vendors, local and street shops. The counterfeit samples were named as C1-C10 for the first brand and C11-C20 for the second brand.

Methodologies:

Packaging Analysis: The authentic and questioned cosmetic products have been purchased online from genuine e-commerce websites and suspected third-party sellers, respectively. The questioned cosmetic products were bought after reading critics' reviews in which the quality of the product has been questioned because of the torn packaging, smell from the cosmetics, irritation after application, and finally having massive discounts up to 79% which seemed too good to be true. The visual comparison was performed between genuine and suspect kohl cosmetic packings.

The packaging was analyzed considering the keypoint i.e. authentication of the hologram analyzing concealed features, for example, scrambled pictures, micro-text, and UV-sensitive inks, the barcode using the mobile app (QR & Barcode Scanner by Gamma Play), content on the label, spelling mistakes or misprint, presence/absence of words on the product body, repeated letters usually at the end of the word, tags, modifications that appear to be typos, and poor printing. All the samples that were bought online were scanned first using a barcode scanner mobile app.

Chemical analysis: The determination of elemental composition in counterfeits is important since it enabled us to determine the level of toxicity of the counterfeits. The chemical composition of the questioned and authentic product was confirmed by EDX using Phillips XL 30 EDX unit. The phase transition temperature for both questioned and authentic products was estimated using Differential Scanning Calorimetry (DSC) on DSC-Q20 (TA Instrument, USA) using an aluminum cup with T zero hermetic lids. Measurements were performed in the dry phase. Perkin Elmer- FTIR Spectrophotometer-100 was used to obtain the FTIR spectra; samples were prepared as pellets operated in the spectral domain of 50-600 and 400-4000 cm-1 at a resolution of 4 cm-1. Bruker Avance 400 MHz was utilized to obtain the 1HNMR spectra. Tetramethylsilane was utilized as an internal standard and the solvent used was deuterated chloroform.

Contamination analysis: Leica DM 3000 optical microscope was utilized to check the microbial contamination in questioned samples.

Culture preparation to assess the contamination in questioned products:

(a) *Bacterial culture preparation:* Nutrient Broth (NB) is a basic medium, used for the general cultivation of bacteria. NB comprises 5.0 g/L peptic digest of animal tissue, 1.50 g/L yeast extract, 5.0 g/L sodium chloride, and 1.50 g/L beef extract. The nutrient broth was weighed and autoclaved at 121oC and 15 psi. The questioned sample was used as an inoculum in the broth and incubation was given for 24 h at 37oC.

(b) *Microbial contamination analysis in questioned products:* To check the microorganism contamination or growth on questioned kohl samples, the tip of questioned kohl was cut using a sterile cutter and dipped in autoclaved water for 10 minutes. Further, this water was used as an inoculum in nutrient broth to grow the contaminants and which additionally employed to prepare slides. Staining was done using safranine dye 0.5% w/v.[20]

RESULTS

Packaging Analysis:

Most of the cosmetic products available in the open market are easily replicated. Packaging is one of the key components that different companies use to show the reliability of their products. To analyze the packaging of different samples, various parameters were taken into consideration such as contents on the label, hologram investigation, and barcode reading.

Label Content:

Spelling mistakes, misprints, presence or absence of words on packaging, repeated letters usually at the end of the word, tags, modifications that appear to be typos, and poor printing were found on the packaging of most samples bought from street vendors.

Whereas in the case of samples bought from genuine websites (n=4), quality inks were used for printing of the packaging. It was noted that the packaging of questioned products had poor quality of inks that were fading off.

Holograms:

The hologram is the most reliable sign of authenticity in which a 3-D image is seen and is readable on the product surface. The visualization is done by keeping the hologram at a particular angle/space to maintain the white light on it and situated on the watcher's side of the 3D image. Through the first-line investigation of the hologram on the samples, it was found that out of 20 samples, 17 samples contained damaged or fake holograms (B1) or just a silver tag (B2). The absence of micro-text was also detected in these samples when compared to genuine samples (n=4).

In conclusion, it was observed that the advancement in optical technology and a combination of opaque and translucent inks has not only enhanced the appeal of holographic effects but also made it difficult to replicate and produce the exact copy of hologram by the counterfeiters.

Barcode:

Barcodes are divided into 1D, 2D, and 3D categories. The 1D barcodes consist of a series of bars, 2D barcodes comprise of dots and squares, and 3D barcodes use the same basic principle as 1D (linear) and 2D barcodes but are engraved on the product. Examined samples contained only 1D barcodes, to authenticate the cosmetic samples, a barcode reader mobile app was utilized i.e. QR & Barcode Scanner by Gamma Play. All the samples bought online were scanned using a barcode scanner app on mobile. Few of the questioned cosmetic products having 1D linear barcodes that did not scan or had no online data about its origin were red-flagged but on most items, counterfeiters did a good job duplicating the 1D linear barcode on the packaging thus the scanning app showed results of that product from the web to original barcodes which are manufactured and fed information regarding its SKU (Stock Keeping Unit) and it was concluded that one positively can't know a counterfeit item from a 1D barcode scanner. More chemical analysis was performed to investigate all the questioned samples (n=20).

Chemical analysis: The determination of elemental composition in counterfeits is important since it enables the determination of the level of toxicity of the counterfeit. Many technologies are available for that purpose; for example, Energy Dispersive X-Ray Spectroscopy (EDX), Proton Nuclear Magnetic Resonance (1HNMR) technologies, etc.

EDX:

Energy Dispersive X-ray analysis (EDX) is a widely used non-destructive elemental analysis method. The elemental analysis of all samples was confirmed by EDX. The examples were then exposed to morphological perception and basic investigation through filtering electron microscopy/vitality dispersive X-beam examination (SEM/EDX) with no coatings.

Figure 1 represents the elemental composition (EDS) of the authentic kohl samples i.e. GA1 and GA2 of first brand(B1), confirming the presence of carbon, iron, oxygen, silicon, and nitrogen with an average weight percentage of 53.44%, 24.67%, 17.91%, 2.39%, and 1.58%, respectively (Fig. 1a and Table I). Whereas elemental analysis of 10 questioned kohl samples indicated the presence of carbon, silicon, iron, and oxygen with an average weight percentage of 88.07%, 0.82%, 0.69%, and 10.03%, respectively (Fig. 1b and Table II). The presence of palladium, cadmium, and mercury was also detected in all the samples with an average weight percentage of 0.01%, 0.10%, and 0.26% in trace amount indicating the harmfulness of counterfeit kohl samples C1-C10 of first brand (B1).

The elemental analysis of the authentic kohl sample GB1 and GB2 of the second brand (B2), confirmed the presence of carbon, iron, oxygen, silicon, and nitrogen with a weight percentage of 65.39%, 14.42%, 14.86%, 1.80%, and 3.54%, respectively (Fig. 2a and Table III). Whereas, elemental analysis of questioned counterfeit kohl samples confirmed the presence of carbon, silicon, iron, and oxygen with the average weight percentage of 74.30%, 1.70%, 1.44%, and 22.35%, respectively(Fig. 1b and Table IV). The presence of palladium, cadmium, and mercury was also detected in all questioned counterfeit samples with an average weight percentage of 0.02%, 0.07%, and 0.11% respectively in trace amount indicating harmfulness of questioned kohl sample B2. This comparison study confirmed that all the questioned kohl cosmetics of both brands i.e., B1 and B2 which were purchased from suspected third party sellers on 70% discount contained very harmful heavy metals that could cause harm.

FTIR

Fourier transforms infrared (FTIR) spectroscopy is a rapid analytic technique that can identify

Element	Series	unn. C [Wt%]	norm. C [Wt%]	Atom. C [at%]
Carbon K	K-series	62.73	53.44 ± 2.00	71.66
Iron K	K-series	28.96	24.67 ± 1.00	7.11
Oxygen K	K-series	21.03	17.91 ± 0.05	18.03
Silicon K	K-series	2.81	2.39 ± 0.42	1.37
Nitrogen K	K-series	1.86	1.58 ± 2.00	1.82
	Total	117.39	100.00	100.00

Table 1: Average elemental composition of authentic kohl sample GA1 and GA2 of first brand (B1) (mean= ± S.D., n = 2).

Element	Series	unn. C [Wt%]	norm. C [Wt%]	Atom. C [at%]
Carbon K	K-series	88.07	88.07 ± 4	91.60
Silicon K	K-Series	0.82	0.82 ± 1	0.36
Iron K	K-series	0.69	0.69 ± 2	0.15
Oxygen K	K-series	10.03	10.03 ± 2	7.83
Palladium L	L-series	0.01	0.01 ± 0.23	0.00
Cadmium L	L-series	0.10	0.10 ± 0.48	0.03
Mercury M	M-series	0.26	0.26 ± 0.39	0.02
	Total	100.00	100.00	100.00

Table 2: Average elemental composition of questioned counterfeit kohl samples C1-C10 of first brand i.e. B1 (mean= ± S.D., n = 10).

Element	Series	unn. C [Wt%]	norm. C [Wt%]	Atom. C [at%]
Carbon K	K-series	73.15	65.39±4	78.36
Iron K	K-series	16.12	14.42±3	3.72
Oxygen K	K-series	16.62	14.86±3	13.37
Silicon K	K-series	2.01	1.80±1	0.92
Nitrogen K	K-series	3.96	3.54±3	3.63
	Total	111.86	100.00	100.00

Table 3: Elemental analysis of authentic kohl sample GB1 and GB2 of second brand (B2) (mean= ± S.D., n = 2).

Element	Series	unn. C [Wt%]	norm. C [Wt%]	Atom. C [at%]
Carbon K	K-series	74.30	74.30 ± 4	80.60
Silicon K	K-Series	1.70	1.70 ± 1	0.79
Iron K	K-series	1.44	1.44 ± 2	0.34
Oxygen K	K-series	22.35	22.35 ± 5	18.19
Palladium L	L-series	0.02	0.02 ± 0.6	0.00
Cadmium L	L-series	0.07	0.07 ± 0.4	0.01
Mercury M	M-series	0.11	0.11 ± 0.1	0.07
	Total	100.00	100.00	100.00

Table 4: Average elemental composition of questioned counterfeit kohl samples C1-C10 of first brand i.e. B1 (mean= ± S.D., n = 10).

Sl. No.	Functional Groups	Authentic Sample of GA1 & GA2	Questioned Sample C1-C10 of first brand
1.	Alkane (C-H)	2916, 2958 stretching (strong)	2915, 2958 (strong)
2.	Alkene (C=C)	1634 weak stretching	-
3.	Alkyne	2165 medium stretching	-
4.	Aromatics	1733 bending Weak	-
5.	Aldehyde	-	1738
6.	Nitrosamine	1462	1462
7.	Halogen Group	556	543, 378 and 718
8.	Alkyl Amine	1049	-
9.	Alkyle Ketone	1258	-

Table 5: FTIR spectra values of authentic samplesGA1 and GA2 and Questioned samples i.e.C1-C10 of first brand B1.

Sl. No.	Functional	Authentic Sample B2	Questioned Sample B2
1.	Alkane (C-H)	2915 (strong), 2962 stretching	2915 (stretching prominent) 2966 stretching
2.	Aromatics	1466	1462 strong
3.	Aldehyde	1748 stretching	1738 strong stretching
4.	Alkyl amine	1060 prominent	1017 (for amines)
5.	Alkyl ketone	1258 prominent	-
6.	Aromatic Compound	2915 (strong), 802 prominet bending	-
7.	Halogen Compound	534 prominent, 706	722
8.	Nitrosamine	1466 weak	1462 prominent
9.	carboxylic acid	2855 stretching	2848 stretching
10.	Ester carbonyl	-	1172

Table 6: FTIR spectra values of Authentic samples(GB1 and GB2) and Questioned samples (C11-C20)of second brand i.e. B2

Value	Authentic Samples (GA-1 & GA2)	Value	Questioned Sample (C1-10)
1.12, 1.17 1.24	Amino, Alpha-methylene	0.9	Primary aliphatic RCH3 Protons of $CH_2CH_2N(CH_3)_3$
7.20	Aromatic	1.28	Methylene protons
		2.31	Benzylic 2.33 2.36
		4.00 7.29	Fluorides $R\text{-}CH_3\text{-}X$ Aromatic

Table 7: 1HNMR δ value of authentic sample (GA1-GA2) and questioned samples (C1-C10)of first brand (B1).

Value	Authentic Samples (GA-1 & GA2)	Value	Questioned Sample (C1-10)
0.13-1.6 1.6 hydrocarbon chains	Methylene proton, terminal methyl groups of 2	0.88 0.98 1.10	Primary and secondary aliphatic chain
7.1-7.4	Aromatic	2.29	Benzylic
-	-	3.97-3.98	RCH-X, Alpha to halogen (C is attached to chlorine) alkyl halyde
-	-	7.2	Aromatic

Table 8: 1HNMR δ value of authentic sample (GA1-GA2) and questioned samples (C1-C10)of first brand (B1).

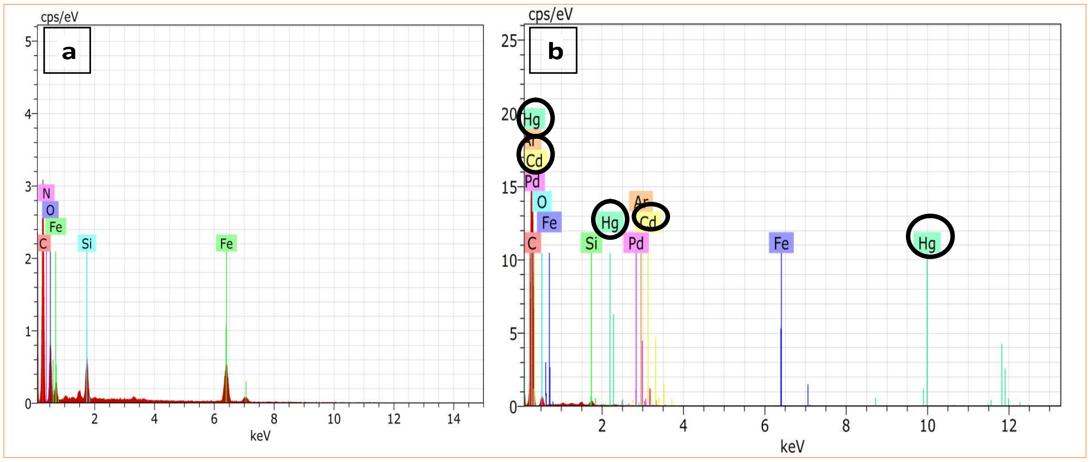


Figure 1: Combined results of elemental analysis of (a) authentic kohl sample (GA1 and GA2) of B1 (b) Questioned kohl samples (C1-C10).

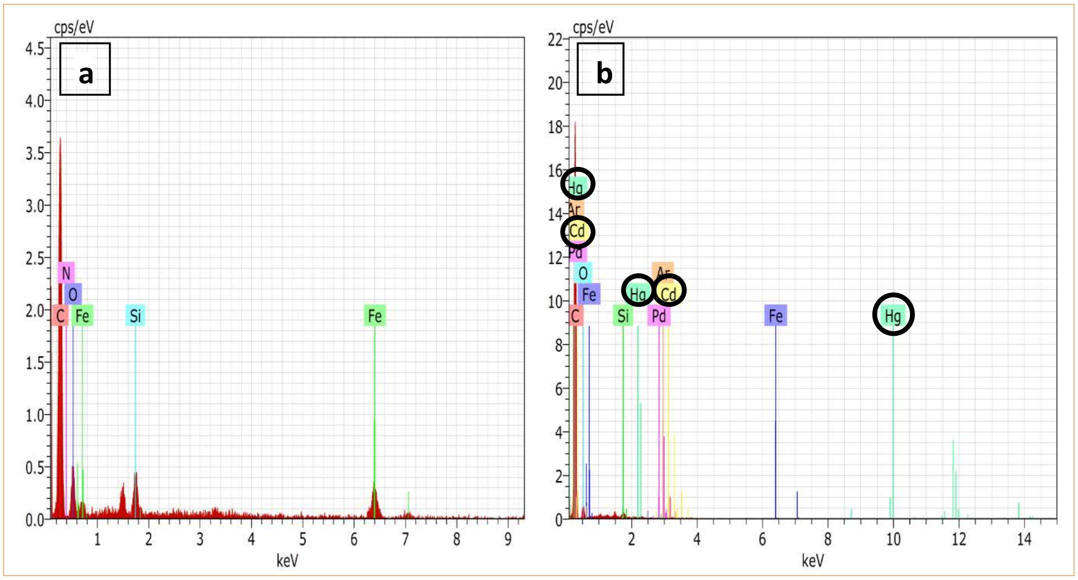


Figure 2: Combined result of elemental analysis of (a) authentic kohl sample and (b) questioned kohl sample C11-C20 of second brand

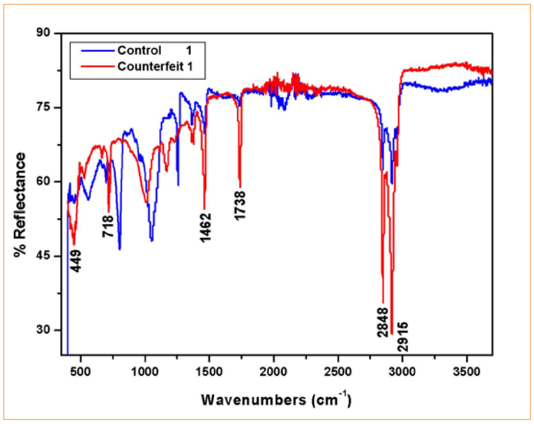


Figure 3: FTIR spectra of Authentic sample (GA1-GA2) B1 and Questioned samples (C1-C10) of first brand B1.

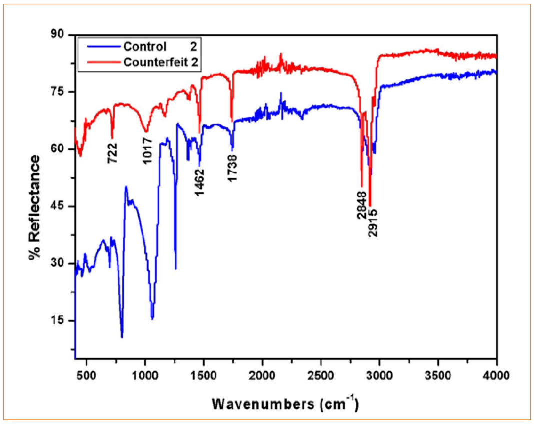


Figure 4: FTIR spectra of authentic sample (GB1 and GB2) and counterfeit samples (C11-C20) of second brand

METALS	BRAND B1 (ppm)	BRAND B2 (ppm)
Pd	100±3	200±2
Cd	1000±3	700±1
Hg	2600±2	1100±2

Table 9: Concentration of heavy metals found in the kohl samples (i.e.B1 and B2) (mean= ± S.D., n = 10)

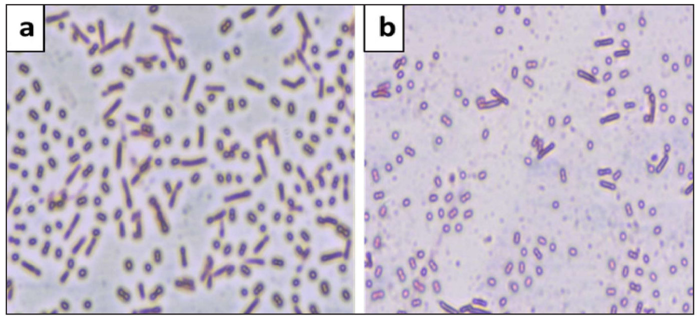


Figure 7: Contamination analysis of two different questioned kohl samples (a) Presence of E. coli observed in C3 sample of first brand (b) Presence of E. coli observed in counterfeit sample C14 of second brand.

organic and inorganic materials in a short time. FTIR offers a simple, rapid, and non-destructive technique for the identification of counterfeit cosmetics. The composition of the authentic and all the counterfeits showed high similarity with the chemical groups. However, counterfeit cosmetic samples showed lower absorbance intensity.

The elemental analysis of the authentic kohl samples from GB1 and GB2 of the second brand (B2), confirmed the presence of carbon, iron, oxygen, silicon, and nitrogen with a weight percentage of 65.39%, 14.42%, 14.86%, 1.80%, and 3.54%, respectively (Fig. 2a and Table III). Whereas, the elemental analysis of questioned kohl samples confirmed the presence of carbon, silicon, iron, and oxygen with the average weight percentage of 74.30%, 1.70%, 1.44%, and 22.35%, respectively (Fig. 1b and Table IV). The presence of palladium, cadmium, and mercury was also detected in all counterfeit samples with an average weight percentage of 0.02%, 0.07%, and 0.11% in trace amount indicating harmfulness of questioned kohl sample B2. This comparison study confirmed that all the questioned kohl cosmetics of B1 and B2 which were purchased from suspected third party sellers on 70% discount, contained very harmful heavy metals that could cause harm to the human body.

FTIR analysis was employed to check the profile of the samples and also to compare the authentic and questioned samples. FTIR assisted in identifying the organic as well as inorganic components present in the questioned sample. FTIR spectra of the authentic samples (GA1 and GA2) gave a peak at 2916 and 2958 cm-1 which corresponds to CH2 stretching vibration. The peak at 1634 and 2165 cm-1 indicated stretching of the alkene and alkyne group, respectively. The peak at 1733 cm-1 corresponds to the aromatic group. The peak at 1049, 1258, 802, 556, and 1462 cm-1 confirmed the presence of alkyl amine, alkyl ketone, aromatic compound, halogen compound, and nitrosamine, respectively (Fig. 3).

In comparison, the questioned samples confirmed many prominent peaks and also indicated the absence of many functional groups such as alkene, alkyne, alkylamine, and alkyl ketone. FTIR spectra of the questioned sample gave peaks at 1738 and 1462 cm-1 which correspond to the aldehyde and nitrosamine functional group, respectively. The peaks at 543, 678, and 718 cm-1 indicate the presence of the halogen group (Fig. 3).

FTIR spectra of the authentic samples (GB1 and GB2) gave the peaks at 2915 and 2962 cm-1 which corresponds to alkane chains present in the sample. The peak at 1748, 1060, and 1258 cm-1 correspond to aldehyde, alkylamine, and alkyl ketone, respectively. The peak at 1466 and 802 cm-1 confirmed the bending of the aromatic group. The peak at 534 and 706 indicates the presence of a halogen compound. A weak peak was also found in 1466 cm-1

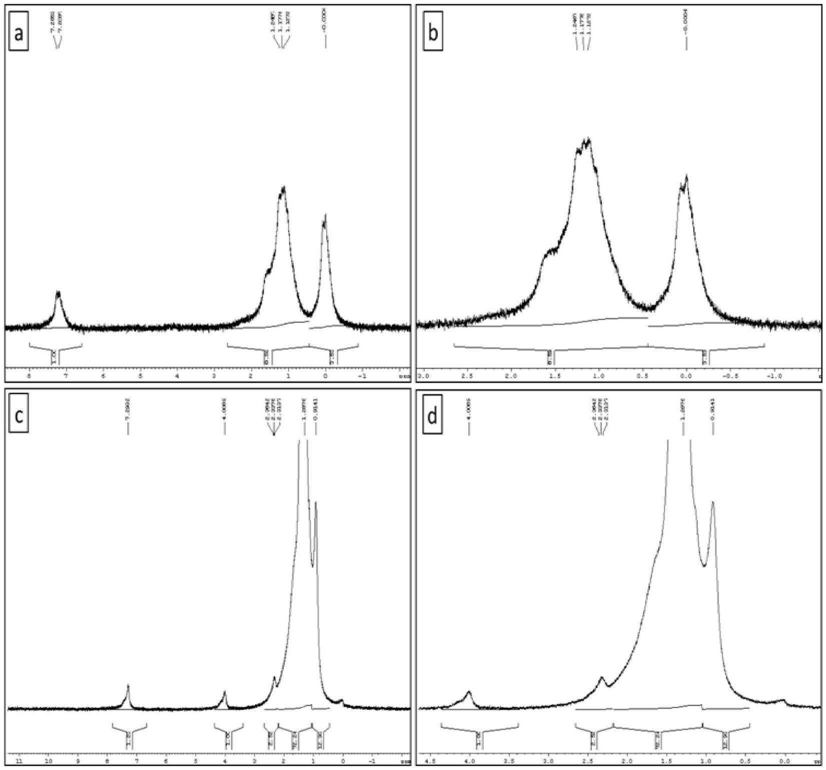


Figure 5: Combined results of elemental analysis of (a) authentic kohl sample (GA1 and GA2) of B1 (b) Questioned kohl samples (C1-C10).

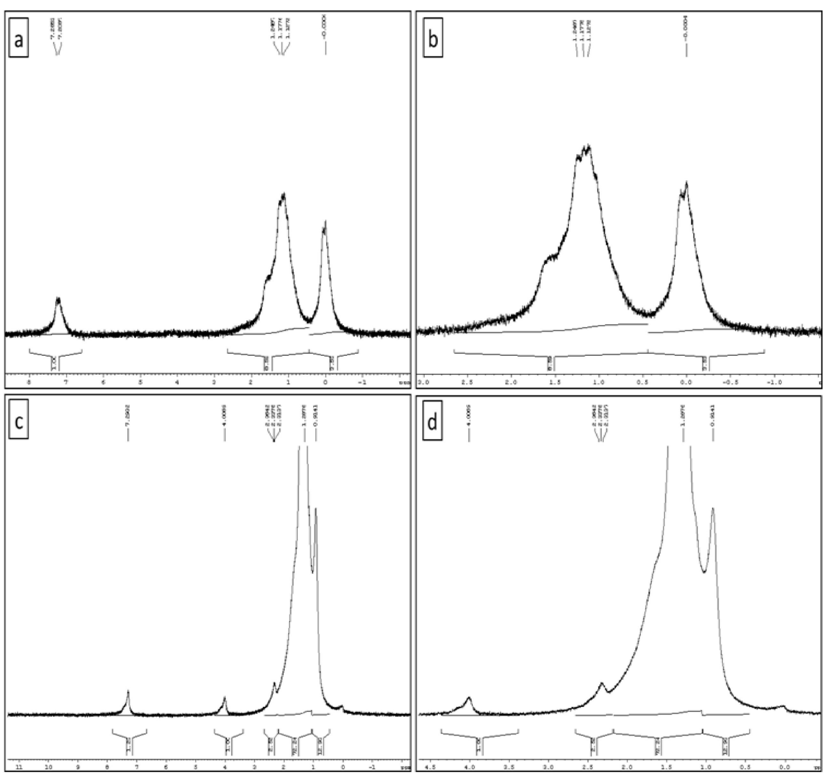


Figure 6: Combined results of elemental analysis of (a) authentic kohl sample (GA1 and GA2) of B1 (b) Questioned kohl samples (C10-C20).

relates to a nitrosamine. The peak at 2855 cm-1 corresponds to a carboxylic acid (Fig. 4).

In comparison, the FTIR spectra of all the questioned samples (C11-C20) confirmed the absence of the alkyl ketone group. The peak at 2915, 1462, and 1738 cm-1 correspond to the alkane chain, aromatic, and aldehyde group, respectively. The peak at 722, 1462, and 2848 cm-1 indicate the presence of halogen compound, nitrosamine, and carboxylic acid, respectively. A peak at 1172 cm-1 corresponds to ester carbonyl which was not detected in the FTIR spectra of the authentic sample (Fig. 4).

HNMR

Nuclear magnetic resonance spectroscopy or (NMR spectroscopy) determines the physical and chemical properties of atoms or the molecules in which they are contained. 1HNMR provides useful information for resolving the structures of closely related compounds.

The 1HNMR spectra obtained for authentic samples (GA1 and GA2) of B1 reports signal at δ=1.12, 1.17, and 1.24 ppm which was assigned to alkyl-methylene protons. The signals obtained at δ=7.20 and 7.26 ppm were assigned to the aromatic proton present in the sample (Fig. 5a & b).

In comparison, 1HNMR spectra obtained for all the questioned samples (C1-C10) confirmed a peak at δ=0.9 ppm which was assigned to primary aliphatic RCH3. A peak at δ=1.28 corresponds to methylene protons. A peak at δ=4 ppm was obtained which was allocated to R-CH3-X (X=halogen, O). The peak at δ=2.31, 2.33, and 2.36 ppm were assigned to benzylic protons, and a peak at 7.29 ppm was assigned to aromatic, a proton is on the phenyl ring (Fig. 5c & d). From 1HNMR, it was found that both the samples (authentic and questioned) have different molecular structures and protons are allocated in different positions as confirmed from the spectra. It was also confirmed from 1HNMR spectra of first brand that questioned samples contained many elements which were noted to be absent in the authentic samples. All the peaks obtained in the 1HNMR spectra of authentic and questioned samples are provided in Table VII.

In authentic samples of B2, 1HNMR spectra confirmed the peak at δ=0.13 & 1.6 ppm which were assigned to methylene protons. A peak at 7.29 ppm was assigned to an aromatic compound, a proton is on the phenyl ring (Fig. 6a & b).

In comparison, 1HNMR spectra of questioned samples of second brand (B2) obtained peaks at δ= 0.88 and 0.98 ppm which was assigned to primary aliphatic RCH3. The peaks at δ= 1.10, 1.25, 1.41, and 1.61 were assigned to peak secondary aliphatic R2CH2. A peak at δ=2.29 ppm was assigned to benzylic protons. The peaks at δ=3.97 & 3.98 ppm were obtained which was allocated to R-CH3-X (X=halogen, O). A peak at 7.2 ppm was assigned to an aromatic compound, a proton is on the phenyl ring (Fig. 6c & d). All the peaks obtained in the 1HNMR spectra of authentic (GB1-GB2) and questioned samples (C11-C20) are provided in Table VIII. NMR delta values for both the authentic and questioned samples B2 are different therefore it is concluded that the molecular structure and composition of both samples are dissimilar and consist of impure elements.

Microbiological contamination analysis: Different questioned samples were investigated to observe the presence or absence of microorganisms. Optical microscopy was used to detect microbial contamination. Two questioned samples were found to be contaminated with E. coli. E. coli and budding E. coli were found in the questioned samples (Fig. 7a & b). Figure 7a depicts the contamination of questioned samples C3 of the first brand (B1) and figure 7b represents the contamination of questioned samples C14 of the second brand (B2).

Toxicity assessment of heavy metals:

From the EDS data, the mean concentration obtained for the toxic heavy metals i.e. Pd, Cadmium, and Mercury were 100, 1000, and 2600 ppm, respectively for the case of brand B1 (mean of 10 samples), whereas for the case of brand B2, the mean concentration obtained for Pd, Cd, and Hg were 200, 700, and 1100 ppm, respectively (mean of 10 samples, Table IX). In all the confirmed counterfeit products, the maximum concentration observed was of mercury (in both kohl sets of B1 &B2). And the decreasing concentration trend found in both sets was Hg>Cd>Pd.

DISCUSSION

Packaging analysis:

It was observed from the hologram analysis that the advancement in optical technology and a combination of opaque and translucent inks has not only enhanced the appeal of holographic effects but also made it difficult to replicate and produce the exact copy of hologram by the counterfeiters.

Through the investigation of barcodes, it was found that counterfeiters did a good job of 1D linear barcode duplication on the packaging and it was concluded that counterfeit items can't be detected from a 1D barcode scanner. So a chemical analysis is needed to investigate the questioned samples.

Chemical Analysis:

The result of questioned and authentic samples was confirmed through EDX test and it was discovered that all the questioned kohl cosmetics of both B1 and B2, which were purchased from third party sellers on 70% discount, contained the very harmful heavy metals that could cause harm to the human body. Fourier transforms infrared (FTIR) spectroscopy identified organic and inorganic materials. NMR delta values for both the authentic and questioned samples B2 are different therefore it is concluded that the molecular structure and composition of both samples are dissimilar and consist of different elements. Through microbial study, two questioned samples were found to be contaminated with E. coli.

Toxicity assessment of heavy metals:

The most common route of exposure is the dermal layer as cosmetics are applied directly on the skin. The ions released from the heavy metals form complexes after getting absorbed into the skin and makes a bond with amine (-NH2), carboxylic acid (-COOH), and thiol (-SH) of proteins that lead to the distribution in the working of cells and also causes cell death. This bond formation causes various diseases, however, the information related to the exposure of metal toxins through skin is very limited. [21]

In all the confirmed counterfeit products, the maximum concentration observed was that of mercury (in both kohl sets i.e. B1 & B2). And the decreasing concentration trend found in both sets was Hg>Cd>Pd (Table IX).

The concentration obtained in the kohl samples indicated the high level of toxicity, as according to the guidelines of US food and drug administration 'standard level of mercury concentration should not exceed 1 ppm and the use should be restricted if the utilization is inevitable, FDA has restricted the use of mercury and other heavy metals in cosmetics.[22]

Contradictory to the FDA regulations, the concentration attained in the kohl sample was in excess amount i.e. 2600 ppm for B1 samples and 1100 ppm for B2 samples, signifying the high toxicity level of mercury.

Many investigations reported the toxic behavior of cadmium in the biological system, according to various research studies, heavy metals such as cadmium,[23] mercury[24,25] etc release the reactive oxygen species (ROS) and also causes oxidative stress in the cells leading to cytotoxicity, genotoxicity, and carcinogenicity. These heavy metals are known to cause a high degree of toxicity and induce organ damage even at a lower level.[26] United States Environmental Protection Agency (U.S. EPA), and the International Agency for Research on Cancer (IARC), also mentioned these metals as 'known' or 'probable' carcinogen in humans.

CONCLUSION

Counterfeit cosmetics business is a hazard not only at the economic level but also at the consumer level. Consumers around the world have been victims of counterfeits. Therefore, consumers need to be informed and be vigilant. The strategy presented here is based on packaging analysis to recognize security traits such as overt and covert features to distinguish between genuine and counterfeit cosmetics. The analysis of the chemical composition of questioned products in a dedicated lab can help law enforcement. The advantage of this combined set-up is to facilitate the screening of counterfeits in the market to speed up the investigation to determine the danger to which the consumers are being exposed.

Investigation unravel the huge nexus among the manufacturers of counterfeit products. Important information collected during the packaging examination of the greater part of the counterfeit and the chemical examination can be recorded to a database and then compared with the data acquired from former counterfeits. By promoting authentication, security features, tracking mechanisms, and investigative services, these standards will bring confidence to consumers, administration, and commerce. IJFMP

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Conflict of Interest:

The authors declare that there is no commercial or financial links that could be construed as conflict of interests.

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