Sudan University of Science and Technology
College of Graduate Studies

Determination of Heavy Metals and Hydroquinone Contents in Cosmetic Products Sold in Sudan

تعيين محتويات الفلزات الثقيلة والهيدروكينون الموجودة في منتجات التجميل التي تباع في السودان

A thesis submitted in fulfillment for the degree of Doctor of philosophy in Chemistry

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DEDICATION

To my family for their patience, love and care

List of Publications

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ABSTRACT

Nowadays, the use of facial makeup among most **Sudanese** ladies becomes very popular without being aware about the potential dangers that can threaten their health while using these cosmetics. Several facial cosmetics available in Sudanese markets are unsafe. A questionnaire survey and statistical analysis were employed in this investigation. Actual participants size (500) were females, university students (70%), employees ladies (20%), house wives (7.5%) and others (2.5%). It was found that 80% of the participants are used to apply makeup, 32% apply local cosmetic preparations; 40%, share or borrow makeup; 14%, do not store makeup properly; 40%, have never read the ingredients list; the majority, do not use natural and homemade cosmetics; several, suffer from harmful effects due to the use of cosmetics.

In this study, thirty samples of Skin-lightening creams (local and imported) were analyzed for total hydroquinone by using high performance liquid chromatography (HPLC). Most of the creams samples analyzed for hydroquinone had concentrations more than the US Food and Drug Administration's acceptable limit of 2.0% the detected percentage was 5.7% in most of the samples. However, the percentage of local preparation cream having hydroquinone content higher than the recommended WHO limit of 2% was only 23%.

Ninety six samples of different facial makeup cosmetic products, were collected randomly from the local Sudanese markets. A microwave- assisted digestion with

concentrated nitric acid, hydrochloric acid was applied for sample preparation. High levels of all analyzed elements in the imported eye shadow in all brown, golden, blue and pink colors at wide range of concentrations were obtained, but low levels in wide range of concentrations, in locally prepared lipstick and eye shadow samples were detected for Pb, Hg, and Cd. Although Pb was present in low concentrations in all samples, locally prepared whitening creams, lipstick and eye shadow showed highest concentration levels of 33.63 µg /g, 42.42 µg /g, and 52.02 µg /g, respectively. The highest concentration levels for Hg in locally prepared whitening creams and eye shadow samples were found to be 42.09 µg /g, and 21.30 µg /g, respectively. The highest concentration levels for Cd, however, were found to be5.21µg /g, 22.53µg /g, and 7.06µg /g, in imported lipstick, eye shadow and local whitening creams, respectively. High concentration levels of Zn were not obtained in all analyzed samples except in the imported eye shadow, (72.46µg /g).

المستخلص

في هذه الأيام ،نجد أن استخدام ماكياج الوجهأصبح شائعاً جداً بين معظم السيدات السودانيات دون وعي حول الأخطار المحتملة التي يمكن أن تهدد صحتهم أثناء استخدام هذه المستحضرات . معظم مستحضرات تجميل الوجه المتاحة في الأسواق السودانية غير آمنة . من خلال الاستبيان والتحليل الإحصائي في هذا البحث كان الحجم الفعلي للمشاركين (500) إناث ، طلاب الجامعات (70 ٪) ، والموظفات السيدات (20 ٪) ، ربات البيوت (7.5 ٪) وغيرهم (2.5 ٪) . وقد وجد أن 80 ٪ من المشاركات يتستخدمن الماكياج ، منهم 32 ٪ يفضلن مستحضرات التجميل المحلية؛ 40 ٪ يشاركن الأخريات في استخدام الماكياج ، 14 ٪ منهم لا تقم بتخزين الماكياج بالطرق الصحيحة . 40 ٪ ، لا يقومون بقراءة قائمة المكونات . الأغلبية لايستخدمن مستحضرات التجميل الطبيعية أو محلية الصنع (المصنعة من المواد الطبيعية) . و كنتيجة لذلك وجد أن الغالبية منهن تعاني من الآثار الضارة الناجمة عن استخدام مستحضرات التجميل .

في هذه الدراسة، تم تحليل **ثلاثين** عينة من كريمات تفتيح البشرة (المحلية والمستوردة) بغرض الكشف عن الهيدروكينون باستخدام جهاز الكروماتوجرافيا السائلة (HPLC). وكان معظم عينات الكريمات التي تم تحليلها لمعرفة تركيزات الهيدروكينون أكثر من الحد المسموح به من إدارة الغذاء والدواء الأمريكية (2.0٪) كانت أعلي نسبة الكشف 5.7٪ في معظم العينات. ومع ذلك، فإن النسبة المئوية للهيدروكينون في عينات الكريمات المحلية الصنع أعلى بكثير من الحد الموصى به منظمة الصحة العالمية من 2٪ وكانت النسبة 23٪ .

هناك **ستة وتسعون** عينة من مختلف أنواع ماكياج الوجه و منتجات التجميل، قد جمعت عشوائيا من الأسواق السودانية المحلية. وقد تم هضمها بمساعدة جهاز Microwave - بأستخدام حمض النتريك وحمض الهيدروكلوريك لإعداد العينات للتحليل . وجد أن هناك مستويات عالية من العناصر كما تم تحليل العناصر في عينات ظلال العيون المستوردة و المحلية في جميع الألوان البني والذهبي والأزرق والوردي وأيضاً في مجموعة واسعة من تركيزات تم الحصول عليها، ولكن مستويات منخفضة في مجموعة واسعة من التركيزات، في عينات أحمر الشفاه وظلال العيونالمصنعة محلياُ و تم الكشف عن الرصاص والزئبق، والكادميوم. على الرغم من أن الرصاص كانت نسبته منخفضة في جميع العينات، التي تم تحضيرها محليا من كريمات التبييض. وقد أظهرت الدراسة أن أحمر الشفاه وظلال العيون أعلى مستويات تركيز لها كانت 33.63 ميكروغرام / غرام، 42.42 ميكروغرام / غرام، و52.02 ميكروغرام / غرام على التوالي.

وقد وجدت أعلى مستويات التركيز للزئبق في كريمات التبييض التي تم تحضيرها محليا وعينات ظلال العيون وكانت كالاتي : 42.09 ميكروغرام / غرام، و21.30 ميكروغرام / غرام على التوالي. أعلى مستويات التركيز للكادميوم 5.21 ميكروغرام / غرام ، 22.53 ميكروغرام / غرام ، 7.06 ميكروغرام / غرام في أحمر الشفاه المستوردة، ظلال العيون، وكريمات التبييض المحلية، على التوالي. لم يتم الحصول على مستويات تركيز عالية من الزنك في جميع العينات التي تم تحليلها إلا في ظلال العيون المستوردة، 72.46 ميكروغرام / غرام.

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LIST OF ABBREVIATIONS

AAS ……. …Atomic Absorption Spectrometer

AD Rome……Timeline of the history of the Roman Empire.

Ag ………… Average

ATSDR……… **A**gency for **T**oxic **S**ubstances and **D**isease **R**egistry

AWWA……American So
ASE……… …..Accelerated Solvent Extraction

ATSDR………Agency for Toxic Substances and Disease Registry

amu…………...atomic mass unit.

BC……………*S*tands for Before Christ, and it means the number of years before the time of Jesus Christ. That was about 2000 years ago

Cm^3…………..(SI; unit) **c**ubic centimeters (or cubic centimeters in US/ English) = 1ml

CIR……………Cosmetic Ingredient Review.

CFR……………Code of Federal Regulations.

CRM…………..Certified Reference Material

D & C…………..Drug and Cosmetic

DAD……………Diode-Array Detector

DHICA…………5,6-dihydroxyindole-2-carboxylic acid

DRC-e mode……Dynamic Reaction Cell technology

dm^3..............decimeter^3 to liter (**dm^3**—l) measurement **units** conversion. ... A liter (L or l) is a non-**SI** metric **unit** of volume equal to **one** cubic decimeter (**dm^3**), 1,000 **cm3** (cubic-centimeter) ·

1 dm3 (cubic-decimeter) · **0.001 m3** (**cubic-meter**) OR (1 m3 = 1000 dm3; 1 dm3 = 0.001 m3).

DW ……………….Deionized Water.

ES…………………Eye Shadow.

EPA……………….Environmental Protection Agency.

F&L………………Fare &Lovely.

FD & C…………..Food, Drug, and Cosmetic

FDA……… ……..U.S Food and Drug Administration

GC……………….Gas Chromatograph .

GC-MS-MS………Gas Chromatography Mass Spectrometry- Mass Spectrometry.

GC–MS…………Gas Chromatograph – Mass Spectrometry.

GC–FT–IR…… ...Gas Chromatograph-Fourier Transformer Infrared.

H …………………Hair Dye (imported).

HPLC……………High performance liquid chromatography

HS–SPME………Headspace Solid Phase Micro Extraction.

HSSE………………Headspace Sportive Extraction.

HS–SPDE…………Headspace solid phase dynamic extraction

HQ ………………...Hydroquinone.

IARC ………………International Agency for Research on Cancer.

IC …………………..Ion Chromatograph.

ICH ………………. International Conference on Harmonization

ICP-OES…. ………..Inductively Coupled Plasma-Optical Emission Spectrometry.

JAPA ………..Journal of the American Pharmaceutical Association

IRM ………..An In-house Reference Material

KH …………Khartoum

K.N…………Khartoum North

LC-ESI-HRMS ……………High Performance Liquid Chromatography coupled to Electrospray Ionization with High Resolution Mass Spectrometry.

L……………Lipstick

LL…………Local Lipstick

LC…………Local (whitening – preparation) Cream

LC-MS-MS ……...Liquid Chromatography Mass Spectrometry- Mass Spectrometry.

LDI-MS……Laser Desorption Ionization-Mass Spectrometry

LH…………..Local Hair Dye.

LMC………..The Quantification Limit

LOD…………Minimum Detection Limit .

MAE…………Microwave-Assisted Extraction

MDGC………Multidimensional Gas Chromatographic Technique.

MBKs………Method Blanks

NIOSH…….Occupational Safety and Health

OM…………Omdurman.

ORS………..Octapole Reaction System.

O/W………..Oil-in-Water
OED ……….The Oxford English Dictionary, definition
P……………..Face Powder

ppm………..Part Per Million
ppt…………..part per trillion.

PT …………Proficiency Test Sample.

RSD……… ..Relative Standard Deviation

SD…………..Standard Deviation

SPSS……….(Statistical Package for Social Science), program

Sp. gr………Specific Gravity.

SF1 …………Standard Flask 1,……

SFE…………Supercritical Fluid Extraction
Si……………Silica gel columns

THF…………TETRAHYDROFURAN | C4H8O |.

UV……………Ultraviolet .

UAE…………..United Arab Emirates
UPD-Pb………using its Under-Potential Deposition process.
USA, NGS………National Geographic Society
W/O……………Water-in-Oil
XRF ……………X-Ray Fluorescence.

CHAPTER ONE

1- General Introduction and Literature Review

1.1. Introduction

A cosmetic product is any substance or preparation intended to be placed in contact with the various external parts of the human body (epidermis, hair system, nails, lips and external genital organs) or applied to the teeth and the mucous membranes of the oral cavity,*(Reed 2007), (Oyedeji 2011).* Most of cosmetics are mixtures of some surfactants, oils and other ingredients, which are required to be effective, long-lasting, stable and safe to human use *(Rao 1998).* It is hard, however, to keep track of the safety of every product, and some of them may carry carcinogenic contaminant. *(Peter 2005).* Acceptable limits for heavy metals vary according to the subpopulation of interest g(for example, children are more susceptible to heavy metal toxicity than adults are). Assessment of dermal absorption by a single component in a cosmetic product is complex and depends on factors such as its concentration in the product, the amount of product applied, the length of time left on the skin and the presence of emollients and penetration enhancers in the cosmetic product. Considering this complexity beside the lack of well-conducted dermal absorption studies incorporating these factors, setting units for heavy metals in cosmetics, based on human health risk alone, is a challenge. *(Peter 2005).*

Heavy metals are found naturally in the environment in rocks, soil and water; therefore, they exist in the manufacture of pigments and other raw materials in all industries including the cosmetics industry. Some of these metals have been used as cosmetic ingredients in the past. Examples include the preservative thiomersal (mercury), the progressive hair dye lead acetate and a number of tattoo pigments such as red cinnabar (mercuric sulfide), *(Oyedeji 2011).*

Since the issue of heavy metals, as deliberate cosmetic ingredients have been addressed, attention is turned to the presence of these substances as impurities.

The metals of primary toxicological concern in cosmetics are lead, arsenic, cadmium, mercury and antimony *(Sainio 2000).*

Dermal exposure is expected to be the most significant route for cosmetic products since the majority of cosmetics are applied to the skin. Dermal absorption of heavy metals is minimal, with absorption of individual elements influenced by a number of factors including physical & chemical properties of the mixtures. Oral exposure can occur for cosmetics used in and around the mouth as well as from hand-to-mouth contact after exposure to cosmetics containing heavy metal impurities. However, inhalation exposure is typically considered negligible, *(Sainio 2000).*At higher concentrations, heavy metals have been shown to have negative effects. Cancerous breast biopsies show higher accumulations of nickel, chromium, cadmium, mercury and lead than non-cancerous biopsies and several metals act like estrogen in the presence of some breast cancer cells. Lead, which may be an impurity, is a proven neurotoxin linked to learning, language and behavioral problems. It has also been linked to miscarriage, reduced fertility in men and women, hormonal changes, menstrual irregularities and delays in puberty onset in girls. At puberty, boys developing testes may be particularly vulnerable to lead. Pregnant women and young children are also vulnerable because lead crosses the placenta and may enter the fetal brain. *(Horowitz 2002).* Mercury is linked to nervous system toxicity, as well as to reproductive, immune and respiratory toxicity. Mercury is also, found in thiomersal, which is mercury – based preservative. Neither mercury nor thiomersal are highly common as direct ingredients or impurities, but the high toxicity of this metal means that the presence of mercury in any cosmetic is a concern. Other heavy metals show also a similar tendency to be toxic *(Horowitz 2002).*

Cosmetics are definitely more widely used by females than males; however, there are numerous useful cosmetic applications that men can also use , *(Al-Saleh 1999).*

Unnecessary packaging of cosmetic products, like most other similar commodities, nowadays, have harmful effects on the environment as they usually end up fastening in a landfill for several hundred years. Furthermore, washed cosmetic product residues of human hair and body, which are flushed down the drain flow into the wastewater and can disrupt all sorts of micro-ecosystems out there,(*Adepoju 2012).* The usage of cosmetics is an important experience in life and leadership fashion for modern females as students, employees (larger consumer) as well as housewives. Whoever, scientists are, now raising concerns about the damage that chemicals in these cosmetics can cause to their bodies, *(Ami 2010).* Several ways, were mentioned by which makeup can be contaminate human bodies, (*B JD 2003):-*

1- In sharing makeup (during use)
2- Low quality makeup (use of cheap, impure raw materials)
3- Poor handling procedures during manufacturing, especially for those, which are not following good laboratory practices, (GLP)
4- Inadequate storage conditions (more likely for bacteria to grow).

1.2 History of body painting, Nubba tribe in Sudan

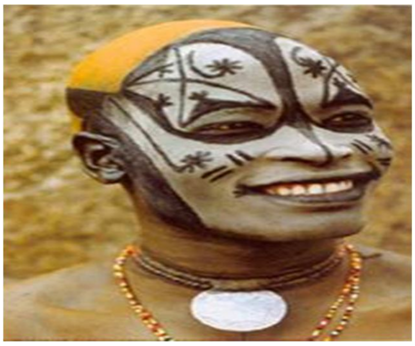

Figure 1-1 **Figure 1-2**

The colors of body painting and special hairstyles showed a person's age group. The use of colors is strictly controlled, red and white are the first colors that an older boy allowed to use from about the age of eight. Black decorations were not normally permitted until he was affiliated with an older group, *(Titherington 1925).* These ways strictly monitored and if a younger man used a color that he was not entitled to, the elders punished him. The young men had particularly elaborate hairstyles, divided up into sections. The younger boys had smaller styles. Often the painting on the body and face represented animals, with the shapes adapted to human shape. *(Yusuf 1976).*

1.2.1 - Process of face painting in Nubba tribes

Although it is impossible to know for certain, it seems likely that ancient peoples were painting their faces and bodies far before the first tattoos or piercings were innovated, sometime, body painting is considered the earliest form of art,(*Yusuf 2003).*

The question of how many hundreds of thousands of years our ancestors might have decorated their bodies with simple paints over countless generations of hunters and gatherers is lost to the ages. Body painting with natural pigments such as clay existed in most tribal communities. *(Ḍayf Allah 1971).*In India and the Middle East, body painting known as Mehndi. Mehndi is a form of body painting that uses dyes made from Henna. It is typically applied on brides. Beauty products are now widely available from dedicated internet-only retailers, *(Kerosuo 1996),* who have more recently been joined online by established outlets, including the major department stores and traditional bricks and mortar beauty retailers. Although modern make-up traditionally used mainly by women, an increasing number of males are gradually using cosmetics usually associated to women to enhance or cover their own facial features. *(Hamilton 2009).*

1.2.2 Sudanese perfumes and cosmetics

Sudanese women have unique local perfumes and cosmetic rituals, such as; *Khumra*, *dilka*, *Karkar*, *Dukhan*, and henna decoration. These cosmetics can only be used by women who were married or about to get married. The traditional Sudanese perfume is called *Khumra* and the word is said to be derived from the word *khumra* "to cover" and could also be derived from the verb "*khaamara*", which means mix up (whether confused or literally mix up). The origins of the word is a lovely description of Sudanese "potpourri" which forms the basis of the Sudanese perfumes."*Mahlab*" is an essential ingredient in both *Khumra* and *Dilka*. *Mahleb* was widely used by Arabian and Persian perfumers who also used it as an ingredient for incenses, and although no mention is made of its use in ancient Egypt, no reason why they would not also have used it, as the spice is widely used in Egypt, *(UNICEF 2001). Dilka* is used by women as an exfoliating scrub that leaves the skin soft and perfumed. Like *Khumra* and *dukhan*, *dilka* is only allowed to be used by married or about to get married women. Unmarried girls are only allowed to use dilkat-burtuqal (orange paste). *Dilka* is well known as scented massage among Sudanese ladies. It has been noted that women who use *dilka* frequently, have a supple, clean, fragrant, and healthy skin.

1.2.3 – Sudanese marriage traditions

The Sudanese women esteem their marriage traditions including usage of *Dukhan, Henna* and incense (*Bakhour)*. The making of perfumes is an important part of Sudanese wedding rituals.

As the preparations for the wedding perfumes start about a month before the wedding by the women of the bride's relatives and friends and it is a skill that is passed on by through family members. They make their local perfumes from various aromatic materials like Acacia Seyal wood, Sandal wood white

Musk and Al Dufra, (*Holt 2000)*.

The perfume preparation processes including, crushing, extracting, simmering and mixing is performed in ceremonial manner in a special day known as the “Perfume making day” during which professional women sing marriage songs praising the bride. At least a month prior to the wedding, the bride is barricaded in her house or a relative's house. She is scrubbed, massaged, pampered and transformed into a completely different person. It is not uncommon for the groom to be utterly confused and shocked at the sight of his bride on their wedding day. Almost every day for a whole month, the bride goes through a process called "*Dokhan*" in which she wraps her entire body in a blanket and sits on a hole in the ground. The hole contains burning "*Talih*" or ember-wood, an aromatic wood that gives the skin a breath-taking glow. During this time, the bride only bathes with water until the layer formed by the "*Dokhan*" is removed by home-made scrubs made out of lentils, fresh cream, oranges among others and for the more affluent, and a Moroccan bath, *(Petterson 1999).*The Sudanese value their traditions from Dokhan to incense (Bakhour) and making their own perfume. It was the perfume-making day, at that day Women are hired to sing while making Henna in all different shapes as well as perfume, in order to incense the bride in her special day.

1.2.4 - How does henna work on normal hair Lawsonia, is known to bond to protein, which is what hair is made of Figure (1-3) Once applied to the hair henna will bind itself to the keratin present in hair and provide a permanent coating to the cuticle of the hair Figure (1-3), it does not penetrate the hair, and hence it is a better alternative to manufactured dyes, (*Santa 2004)* .

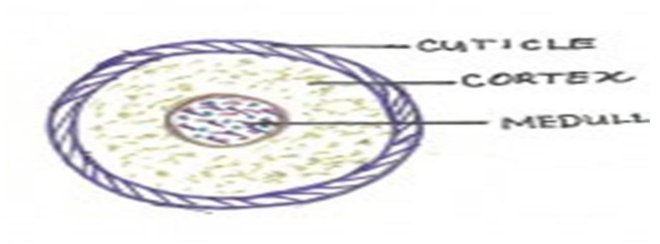

Figure 1-3 Cross section of hair without henna application

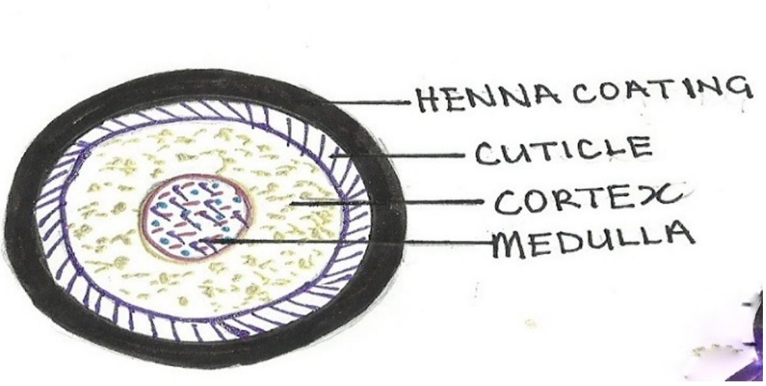

Figure 1-4 Cross section of hair with a single coating of henna

Each time henna is applied to the hair, another coat of color is added to the hair strands Figure (1-4), and which is what will result in the change of color or covering of grays that may be desired.

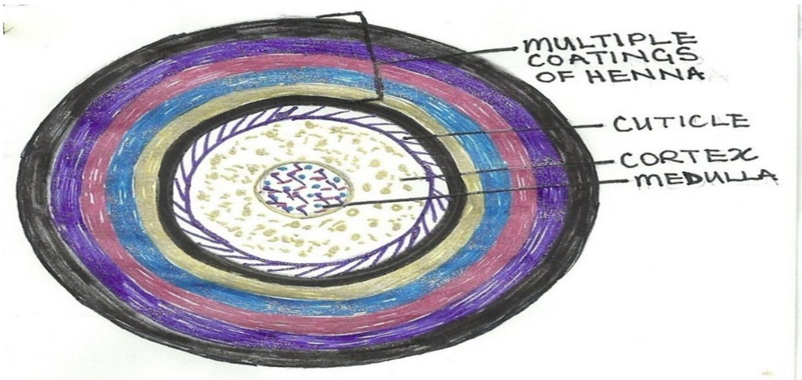

Figure 1-5 Cross section of hair with multiple coatings of henna

The color deposited by henna can only be removed by growing out the hair or eventually cutting off the hennaed parts, definitely this is not recommend, but, some people apply a dye over it.

1.2.5 --How Does Henna Dye Gray Hair

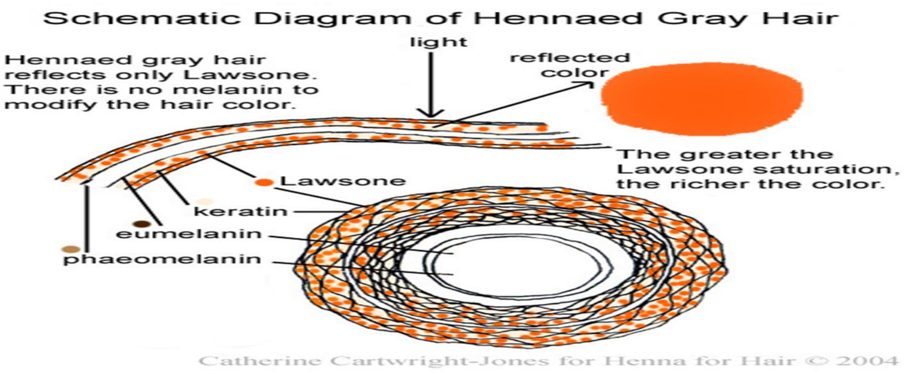

Figure 1-6 Schematic Diagram of Hennaed Gray Hair

Figure (1.6) Shows that if henna hair that has no melanin, the only color could be seen from henna, the Lawsone red-orange color. If the lawsone content is low, the color will be weak red-orange. If the lawsone content is high, the color will be dark red-orange, oxidizing to deep auburn.

1.3 - Disadvantages of makeup

a). Makeup made the skin oilier.

b). Extra makeup makes some women look worse.

c). It can create skin cancer.

d). Sometimes, extra makeup can damage eyes.

e). If not choose the proper band then it could also cause skin irritations.

f). Putting makeup takes valuable time.

1.4- Methods cited in literature for the assay of heavy metals and hydroquinone in cosmetic products

1.4.1 - Heavy metals toxicity of cosmetics

The toxicity of metals and its cure is well documented, *(Palpandi 2010)*.The metals of primary toxically concern in cosmetics are : Pb, Hg, Cd, As, Ni and Zn. Dermal exposure is expected to be the most significant route for cosmetic products since the majority of cosmetics are applied to the skin. Dermal absorption of heavy metals is minimal, with absorption of individual elements influenced by a number of factors including physical and chemical properties of the mixture *(Reed 2007).* In Sudanese market there are plenty of cosmetic preparations available which are prepared locally without any control; the most alarming issue in Sudan is the large percentage of people practicing skin-lightening techniques. Globally however, black women in all over the world employ similar practices. Even the cosmetic industry*,* (*Adebajo 2002*).

1.4.2 - Analysis of ancient Greco-Roman cosmetic materials using laser desorption ionization and electro spray ionization mass-spectrometry,

Micro samples of pink cosmetic powders from the Greco-Roman period were analyzed using two complementary analytical approaches for identification of the coloring agents (lake pigments originally manufactured from madder plants with an inert binder, usually a metallic salt) present in the samples. (*Bocca 2007*). The first technique was a methanolic acidic extraction of the archaeological samples with an additional Ethyl acetate extraction of the anthraquinone-type coloring agents which were identified using high performance liquid chromatography coupled to electrospray ionization with high resolution mass spectrometry and the second was direct analysis of a micro sample by laser desorption ionization-mass spectrometry. The latter technique is well suited when the quantity of samples is very low. This soft ionization technique enables the detection of very small quantities of compounds using the combination of positive and negative ion modes. It was also successfully applied for the direct analysis of some laboratories made reference compounds.

However, the presence of lead in one of these ancient samples induced a spectral suppression phenomenon,*(Kuligowski 2008).*

1.4.3 Trace metal contents of facial (make-up) cosmetics commonly used in Nigeria.

Several facial cosmetics available in Nigeria were analyzed for their contents of the heavy metals; lead, cadmium, chromium, nickel, zinc and iron. The levels of chromium, iron and zinc were much higher in the samples than those of the non-essential toxic metals; lead, cadmium and nickel, *(Cotte, M 2005).*

1.4.4 Korean research team analyze an extremely small amount of several heavy metals had been detected in cosmetic products as impurities, which can cause skin allergies through the skin. We present here a fast, accurate, and highly sensitive method for simultaneous termination of P b2 +,F e2 +, Cu2 +,N i 2+,Z n2 +,C o2 +,C d2 + and M n2 + in metals are well separated through a bi -functional ion-exchange column (Ion Pac CS 5A) and detected by post-column spectrophotometer detection, *(SO-MI LEE 2008).*
The calibration graphs were linear (r2 > 0.999), in the range 0.1-1000 pg/ml. Detection limits for a 200 sample solution were at the pg/l level, which is sufficient prejudging whether the product is safe or not. The relative standard deviations (RSDs) of the retention time and the peak area were less than 0.21% and 1.24%, respectively. The recovery rates are 97-104%. The result shows that the propose determination method is more sensitive, more accurate and faster than current methods such as HPLC, ICP-MS and Flame-AAS. The new method was applied to analyze the amount of heavy metals contained in 22 cosmetic products and 11 coloring agents, *(SO-MI LEE 2008).*

1.4.5 Thin Layer Chromatography (TLC) for HQ analysis The hydroquinone was extracted from samples by using 96% ethanol and was subjected to TLC analysis. Eleven out of 22 samples were found to contain hydroquinone. The HPLC analysis showed the concentration of hydroquinone ranged from 0.002% to 0.092% in the cosmetic samples, *(Siddique 2012).*

1.4.6 At **Ghana** institute of scientific research, fifty (50) samples of Skin-lightening creams were analyzed for total mercury by Cold Vapor Atomic Absorption Spectrophotometry using an automatic mercury analyzer and for total hydroquinone by High Performance Liquid Chromatography. The concentration of mercury in the creams ranged from below 0.001 to 0.549 µg/g and that of hydroquinone ranged from below 0.001 to 3.45 %. All the creams sampled for mercury had concentrations less than the US Food and Drug Administration's acceptable limit of 1µg/g, *(Odumoso P. O 2010)* and (*Kooyers T. J 2005).*

1.4.7 A study of the chemical composition of traditional eye cosmetics "kohls" used in **Qatar** and **Yemen.**

The study concentrated on the chem. compn. of traditional eye cosmetics "kohls" used in Qatar and Yemen. Of especial interest was how many samples in each country contain the toxic element lead. In Qatar 19 observably different kohl samples were obtained, and in Yemen ten such samples obtained. The anal. techniques of SEM and x-ray powder diffraction (XRPD) were used to study the samples. For the samples from Qatar, six of the 19 (32%) contained galena (lead sulfide, PbS) - all as the main component. However, for the samples from Yemen, five of the ten (50%) samples contained galena as the main component, with another three having it present as a minor component, *(Journal of Cosmetic Science 2008).*

1.4.8 Availability and chemical composition of traditional eye cosmetics "kohls" used in the United Arab Emirates of **Dubai**, Sharjah, Ajman, Umm Al-Quwain, Ras Al-Khaimah, and Fujairah, *(Hardy 2006).*

The study was undertaken in order to det. the availability and chem. compn. of potentially lead-toxic traditional eye cosmetics "kohls" in six of the seven emirates of the United Arab Emirates (UAE). Thus of especial interest was the percentage of the purchased samples that contained the toxic element lead. *(Hardy 2006).*

A total of 53 observably different kohl samples were found to be available overall in the six emirates: Dubai, Sharjah, Ajman, Umm Al-Quwain, Ras Al-

Khaimah, and Fujairah. It was found that 19 of these samples were previously analyzed in studies covering Oman, Abu Dhabi (city), and Egypt (Cairo). The techniques of x-ray powder diffraction (XRPD) and SEM were used to analyze the remaining 34 samples. Overall, for the 53 kohl samples, it was found that 20 (38%) contained a lead compd. (galena, PbS) as the main component, *(Hardy 2006) and (Journal of Cosmetic Science 2006).*

1.7 Objectives

- To determine toxic heavy metal and hydroquinone contents in some cosmetic products commonly sold in Sudan.
- To compare the obtained results with those reported in the literature.
- To raise the awareness about the hazards of topical cosmetics that contain heavy metals and hydroquinone.
- To set up key fundamental testing capabilities for chemical testing of cosmetic and perfume products
- To encourage research on daily-use products that may contain heavy metals or hydroquinone of potential harmful effect to human.
- The objectives of this study were to evaluate purchase habits, asses' application methods and raise awareness of health hazards of cosmetic products purchased from local markets by Sudanese ladies.

CHPTER TWO

2- Statistical Evaluation of Health Hazards and Practices of Cosmetic Products Marketed in Sudan

2.1 Introduction

Cosmetics are definitely more widely used by females than males; however, there are numerous useful cosmetic applications that men can also use. Cosmetics have to have ingredient labels that indicate precisely what is included in the product. This is not specific with regards to fragrances, though, the manufacturer needs to list out the exact composition of his fragrance, *(Al - Saleh 1999).* Cosmetic markets are growing rapidly on a daily basis. Normally, if the practices are legal, there will add value to the business, but unfortunately there are illegal practices in some cosmetic manufacturers as they work without license from legal authorities*, (British Journal of Dermatology · 2015).* Allergic eyelid dermatitis might more often be due to cosmetics put on the fingernails, face or hair rather than that applied right to the eyelids. Historically, women have risked their health for beauty by using cosmetics laden with poisons, ***(WHO 1995*)**, (*Feingold1992*).

2.2 Methodology

A questionnaire survey and statistical analysis were employed in this investigation:

The self- structured questionnaire was used to measure knowledge, and practice of Sudanese women on cosmetic products usage. The survey was chosen as a simple means of data collection to assess the awareness of Sudanese women for potential cosmetic health hazards, the types of makeup they used, the places from where they purchased them and the bases upon which selection is made. This cross section study was based on data collected from 500 copies of the questionnaire surveillance that was distributed to volunteers to answer the questions raised. Volunteers were divided into four categories:

1- Female university students, 70%,
2- Employee ladies of different jobs), 20%,
3- House wives (without jobs), 7.5%, and
4- Males, only 2.5%.

2.3 Results and discussion

The data collected through the questionnaire were statistically analyzed using SPSS program, (Statistical Package for Social Science) Version 12.0. In total, approximately 500 copies of the questionnaire were distributed to some volunteer participants to answer the questions raised in the questionnaire. The number of the participants who admitted that are using makeup was 251 (79.9 %) and only 63, (20.1%) declared that they are not using any type of cosmetics, Table (2-1).

2.4 The Questionnaire

The questionnaire (Appendix-II), was distributed to 500participants,82**%** of them were between (20 and 25) years of age (Table 2-1).The vast majority of the participants (84.6%) were single (Table 2-2) because the questionnaire was done at the universities and the percent of married women is low (majority of females prefer to marry after completing the university study). It is not real to reflect that married women do not use cosmetic products. Regarding the participant's study level, the majority (37.6%) was in the fourth class level Table (2-3): 44% of them were rural and 56% were urban Table (2-4). Ninety-six samples were classified into several groups as showed in Table (4-1).

2.5 The Statistical analysis of collected samples

The questionnaire also clarified the personal motives underlying cosmetic products usage by the participants. On this respect, most of the participants

(68%) revealed that, they use cosmetics under the influence of the community, while 27.5% of the participants were found to use cosmetics in response to an advice from a consultant doctor, and only 4.5% use cosmetics because of their cheap price Fig.(2-1).Regarding the participant's previous knowledge and/or interest to know the contents of the cosmetic product they used, it was found that most of the participants (60%) were interested to know the contents of their cosmetic product, while 40% paid no attention to such issue Fig.(2-2). These results justified the importance of informing the consumers of cosmetic product about their sources, ingredients, safety or the hazards behind the blind use of such products. At least a leaflet containing this mentioned information should be available for each cosmetic product, Table (2-1) shows that the majority of the participants (82%) were between 20 and 25 years. Table (2-2), shows that the majority of the participants (84.6%) were single. Table 2-3, shows that the majority of the participant students (37.6%) were studying in fourth year. The majority of the participants (56%) were urban as is shown in Table (2-4).

Table 2-1 Distribution of the participants according to their age

Age group	Number of Participants	Percent (%)
20- 25 years	410	82.0
26- 31 years	90	18.0
Total	**500**	**100.0**

Table 2-2 Distribution of the participants according to their Social status

Social status	Number of Participants	Percent (%)
Single	423	84.6
Married	77	15.4
Total	**500**	**100**

Table 2-3 Distribution of the participants according to their present study level in the university

Level of Study	Number of Participants	Percent (%)
First year	54	10.8
Second year	48	09.6
Third year	115	23.0
Fourth year	188	**37.6**
Not Student (others)	95	19.0
Total	**500**	**100 .00**

Table 2-4 Distribution of the participants according to their residence.

Residence	Number of Participants	Percent (%)
Rural	220	44.0
Urban	280	**56.0**
Total	**500**	**100.0**

Figure (2-1) shows that the majority of participants (68%) used cosmetics according to community factor. Figure (2-2), shows that the majority of the participants (60%) interested in knowing the constituents of the topical cosmetic before use, (by reading the label). Distribution of the participants according to their motivation for the use of topical cosmetic.

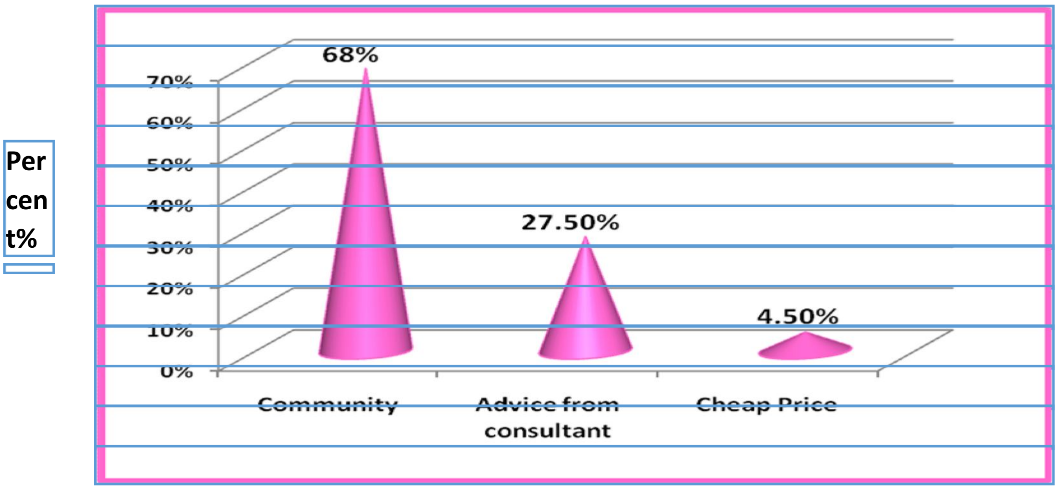

Figure 2-1 Motive for using of Topical Cosmetics

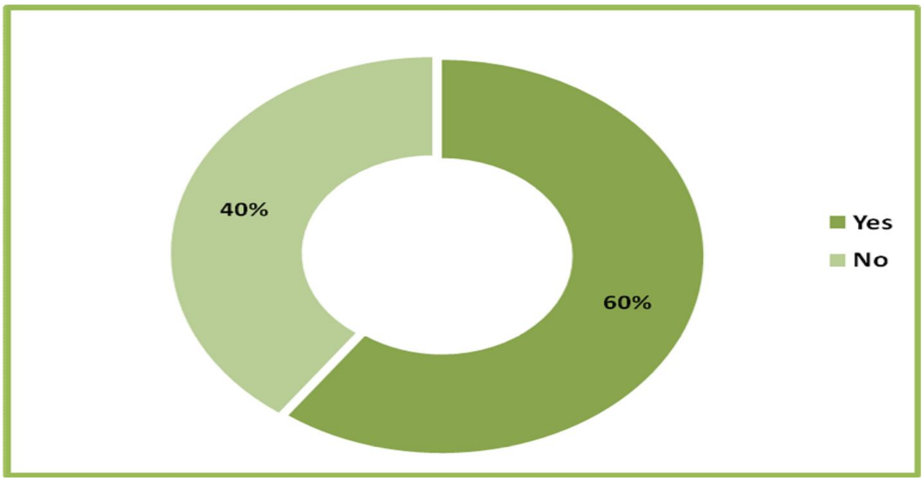

Figure 2-2 Distribution of the participants according to their interest of knowing cosmetics before use.

2.5.1 Lipsticks

Lipsticks that are available in local market in Sudan with different brands (Table 2-6). In this study, 67% of the 500 participants were used to apply lipsticks and 165, (33%) participants were not using any lipsticks (Table 2-5) and Table (2-6), shows the distribution of the percentage of different brands of lipsticks preferred by participants. Ninety- one (18.7%) of participants used Tazol brand lipstick 'L2' in Table (2-6) of the 487 participants who use lipstick frequently. One brand of the collected lipstick samples 'L9' in Table (4-7), were found to be contaminated with Pb, compared with FDA acceptable Pb limit (20 ppm). Eighty – one (16.6% out of 487 participants of lipstick users) frequently used Pop lipstick brand (L12 in Table (2 -6).

The third consumed favorable lipstick was Holly Table (2-6) representing 14.8% out of 487 participants of lipstick users and containing 29.00 ppm Table(4-8), sample, LL25). Due to the cumulative nature of lead (most of heavy metals are not biodegradable element), it can be postulated that, the greater the frequency of application of lipsticks containing lead, the greater would be the likelihood of lead toxicity. In this study, the majority of lipstick users (42.20 % out of 436) were shown to prefer pink colored lipsticks followed by cupric colored lipsticks (14.44% out of 436); while 11.70% out of 436 participant reported the preference of the chocolate colored lipstick Table (2-7). Most of the lipstick users understudy confirmed no complaints from the lipstick they used (67.4%); while the remainders (32.6%) who suffered after using lipsticks reported cracking of the lips (57.4% out of 333 users or dryness of the lips 42.6% out of 333 users Tables (2-8) and (2-9).

This indicates that some lipsticks might contain substances that might be responsible for these complaints; however, the cracking and dryness of the lips are not known to be symptoms of lead toxicity. The reasons behind changing from one brand of lipstick to another was another question raised to the participants, while 34.6% out of 434 lipstick users stated the usage of the same brand all over the time, the remaining65.4% reported, that they are always changing from one brand to another brand of lipstick as indicated in Table (2-10). The justification behind this behavior could be due to complaints or changing to a more efficient one.

Table 2-5 percentage of the participants using lipsticks

Usage	Number of Participants	Percent (%)
Non-users	165	33.0
Users	335	**67.0**
Total	**500**	**100.0**

Table 2.6 Distribution of the percentage of the different brands of lipsticks used by the participants

Brand of lipsticks	Number of Participants	Percent (%)
1- OLAY	19	3.9
2 -TAZOL	**91**	**18.7**
3 -POND'S	11	2.3

4 -CASABELLA	15	3.1
5- GARNIER	18	3.7
6- HOLLY	72	**14.8**
7- LAURENT	22	4.5
8- LANCÔME	70	14.4
9- AVON	19	3.9
10-Pala	27	5.5
11- Global	42	8.6
12- POP	81	16.6
Total	**487**	**100.0**

Table 2-7 Distribution of the number of the participants who used lipstick according to the color which they preferred

The Color	**Number of Participants**	**Percent (%)**
Pink	**184**	**42.20**
Red	19	4.36
Chocolate	51	11.70
Brown	11	2.51
Dark Brown	18	4.13
Light Blue	10	2.30
Dark Violet	09	2.05
Light Orange	13	3.00
Colorless	44	10.10
Cupper	63	14.44
Dark red	14	3.21
Total	436	100.0

Table 2-8 Distribution of the participants according to whether they have complaints or not, during using lipsticks

Appearance of complaints	Number of Participants	Percent (%)
Yes	143	32.6
No	295	67.4

Complaints	Number of Participants	Percent (%)
Cracking of lips	191	57.4
Dryness of lips	142	42.6
Total	**333**	**100**

Table 2-9 Distribution of the frequency of the participants suffered from cracking of lips

Table 2-10 Distribution of the frequency of the participants who change from one brand of lipsticks to another

Changing	Number of Participants	Percent (%)
Yes	284	65.4
No	150	34.6
Total	**434**	**100.0**

2.5.2 Face Powders

Face powder is a cosmetic powder applied to the face to set a foundation after application. It can also be reapplied throughout the day to minimize shininess caused by oily skin. There is translucent sheer powder, and there is pigmented powder. Certain types of pigmented facial powders are meant to be applied alone with no base foundation. Powder tones the face and gives an even appearance. Besides toning the face, some powders with sunscreen can also reduce skin damage from sunlight and environmental stress.

Because of the wide variation among human skin tones, there is a corresponding variety of colors of face powder. There are also several types of powder. A common powder used in beauty products is talc (or baby powder), which is an absorbent and provides toning to the skin, Table (4-11) shows that 247 participants (73.5%) out of the 400 participants questioned reported that they use face powder. Among the twelve brands of face powder available in the Sudanese market, 23.0% of the number of the face powder users (330) preferred the use of Dove brand no. 9 in Table (2-12). However, Table (2-13), shows that more than one third (37.02%) of number of face powder users (470) did suffer from using it. Accordingly, the percentage of applications of face powder per day, 43.50 % out of 434 users, reported **once** daily application, 30.60 %, twice daily and 25.90% of the participants applied face powder three times a day, as is shown in Figure (2-3).

Table (2-14) shows that 65.24% of the number of 351 participants suffered from allergy; whereas 34.76 % of them suffered from dark spots on their faces. Table (2-15), shows that 64.6%; almost two thirds of the face powder users did not stick to one brand, but they change from one brand to another

Table 2 -11 Distribution of the participants according to frequency of use of face powder

The usage	Number of Participants	Percent (%)
Non- user	153	26.5
User	247	73.5
Total	**400**	**100.0**

Table 2-12 Distribution of the participants use of the different brands of face powder.

Brand of lipsticks	Number of Participants	Percent (%)
1- LLOREAL	69	20.91
2- AVEDA	41	12.42
3 –Christian Dior	10	3.01
4 -CASABELLA	05	1.50
5- GARNIER	16	4.85
6- HOLLY	22	6.70
7- LAURENT	0.0	0.0
8- LANCÔME	06	1.82
9- Dove	**76**	**23.0**
10-Pala (skin care)	30	9.10
11- Global	24	7.28
12- P. TAZOL	31	9.41
Total	**330**	**100.00**

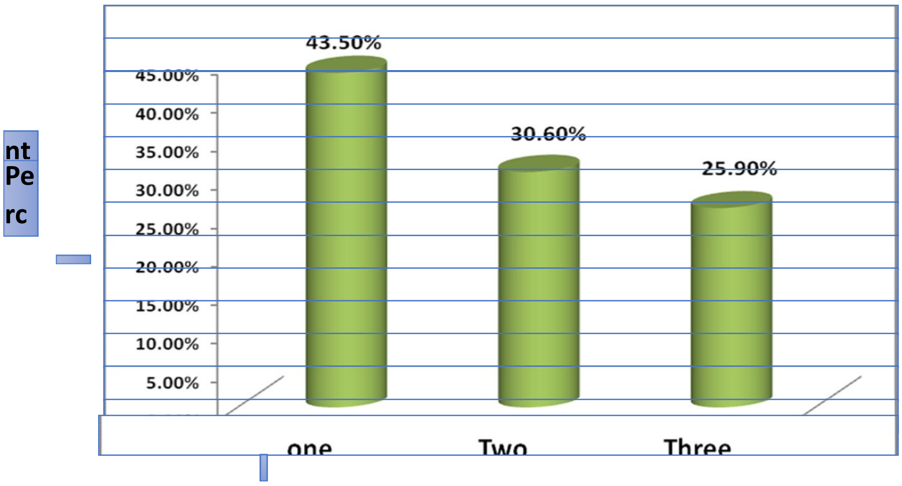

Figure 2-3 Number of Applications of Face Powders per day.

Table 2-13 Percentage of participants suffering from face powder use

Appearance of complaints	Number of Participants	Percent (%)
Yes	174	37.02
No	296	62.98
Total	**470**	**100.00**

Table 2-14 Percentage of distribution according to the types of complaint during the use of face powders.

Type of complaints	Number of Participants	Percent (%)
Allergy	229	65.24
Darkening of skin color	122	34.76
Total	**351**	**100.00**

Table 2-15 Distribution of participants percentage who Change from one brand of face powder to another.

Changing	Number of Participants	Percent (%)
Yes	95	64.6
No	52	35.4
Total	**147**	**100.0**

2.5.3 Eye shadows

Eye shadow is a cosmetic that is applied on the eyelids and under the eyebrows. It is commonly used to make the wearer's eyes stand out or look more attractive. Eye shadow can add depth and dimension to one's eyes complement the eye color or simply draw attention to the eyes. Eye shadow comes in many different colors and textures. It is usually made from a powder and mica, but can also be found in liquid, pencil, or mousse form. Civilizations across the world use eye shadow predominantly on females, but also occasionally on males. In Western society, it is seen as a feminine cosmetic, even when used by men. Depending on skin tone and experience, the effect of eye shadow usually brings out glamor and gains attention. The use of eye shadow attempts to replicate the natural eye shadow that some women exhibit due to a natural contrasting pigmentation on their eyelids.

Common ingredients in eye shadows consist of talc, mica sericite, magnesium stearate, colorants, and preservatives.

Fillers in eye shadows are primarily talc. The liquid binders are typically a silicone and the dry binders are typically magnesium stearate. In order to make an eye shadow, there has to be a balance between the fillers, dry binders and liquid binders.

Natural eye shadow can range anywhere from a glossy shine to one's eyelids, to a pinkish tone, or even a silver look. Table (4-16) shows that three–fifths (60.2%) of the participants (497) did not use eye shadows.

Table (2-17) shows that two thirds (67.81%) of the participants 320 did suffer from eye itching; whereas the rest (34.76 %) did suffer from allergy. Nevertheless, more than one –third (36.49%) of the participants did not stick to one type of eye shadow Table (2-18). Golden yellow, Silver or dark brown colored eye shadow and other types of eye makeup are popular amongst all genders, as per the participant's selection Table (2-19). However, almost one-third (31.76%) of the participants (381) did prefer dark brown eye shadow color.

Table 2-16 Distribution of the participants using eye shadow

The usage	**Number of participants**	**Percent (%)**
Non-users	299	60.2
users	198	39.8
Total	**497**	**100.0**

Table 2-17 Distribution of the number of the participants according to the type of complaint during the use of eye shadow

Type of complaints	Number of participants	Percent (%)
Allergy	103	32.19
Eye Itch	217	67.81
Total	**320**	**100.00**

Table 2-18 Percentage of the participants who change from one brand of eye shadow to another.

Changing	Number of participants	Percent (%)
Yes	268	63.51
No	154	36.49
Total	**422**	**100.00**

Table 2-19 Distribution of the participants percentage and different eye shadow colors preference

Color of Eye shadow	Number of participants	Percent (%)
Silver	62	16.27
Green	39	10.24
Dark green	15	3.94
Light Brown	23	6.05
Golden yellow	81	21.26
Orange	22	5.77
Purple	18	4.72
Dark brown	**121**	**31.76**
Total	**381**	**100.00 %**

Whereas only two- thirds (67.60%) of the participants applied eye shadow once per day, almost one - fourth (23%) of them, twice per day and almost one-tenth (9.5%) of them, three times per day Figure (2-4). However, Table (2-20), shows that 71.43% (260) of the participants (364) had no complaints of using eye shadow.

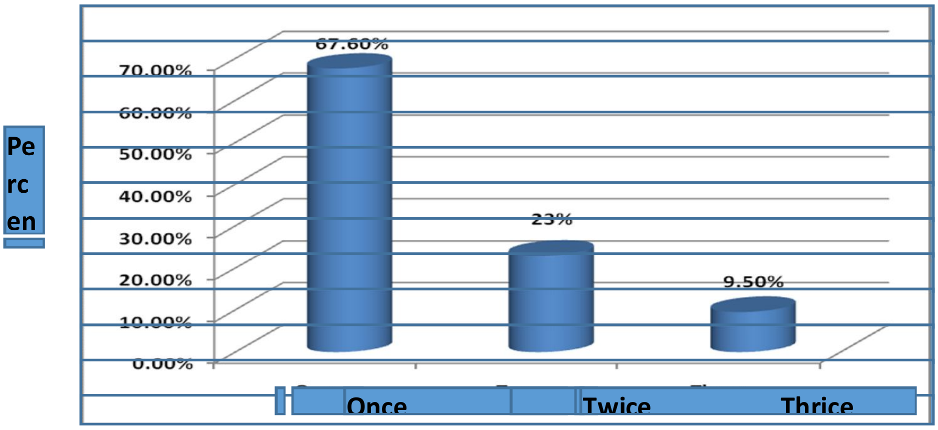

Figure 2-4 Distribution of the participants according to the number of applications of eye shadow per day.

Table 2-20 Distribution of the participants according to their complaint during the use of eye shadow.

Appearance of complaints	**Number of participants**	**Percent (%)**
Yes	104	28.57
No	260	71.43
Total	**364**	**100.00**

2.5.4 Skin - lightening Creams

Skin whitening, skin lightening, and skin bleaching refer to the practice of using chemical substances in an attempt to lighten skin tone or provide an even skin complexion by lessening the concentration of melanin. Adding to the controversy surrounding their use and impacts on certain ethnic groups, several chemicals have been shown to be effective in skin whitening, and some have proven to be toxic or have questionable safety profiles. Skin-whitening products have become increasingly in demand in the past few years. The main purpose for skin-lightening products is to lighten the skin as well as to even out skin tone or to treat pigmentation disorder such as freckles, melasma, pregnancy marks, and age spots (*Adebajo 2002*). The most successful recent and natural skin-whitening agents are arbutin, vitamin C, kojic acid, licorice extract, burnet root extract, scutellaria extract, and mulberry. These agents are all tyrosinase inhibitors, which inactivate tyrosinase (the enzyme responsible for skin pigmentation) by chelating with its vital copper ion and suppressing tautomerization from dopachrome to DHICA, *(Odumoso 2010).* Normal skin color is formed by melanin, a natural pigment that also determines hair and eye color. In the skin, the enzyme tyrosinase biochemically converts the amino acid tyrosine into melanin. Hyper pigmentation occurs when too much melanin is produced and forms deposits in the skin. Hyper pigmentation is not a medically harmful condition. However, it is always advisable to have new brown spots checked by a dermatologist to make sure that they are not skin cancers, *(Lodén 1999).* Table (2-21) shows that almost one – third (31.50%) of the number of the participants (480) frequently used lightening creams. The survey questionnaire reveals that, among the dozen brands of skin- lightening creams collected from the Sudanese market, one quarter (100) of the participant users (399) preferred the application of local preparation, (no. 8 brand) listed in Table 2-22. Table 4-23shows that 59.60% of the participants suffer from allergy and the rest (40.40%) of the users suffer from darkening of their skin after removal of the cream. Table (2-24) shows that 57.55% (202) of the participants (351) changed from one brand to another in response to their colleagues recommendation; however, they did not specify whether this change from local to imported whitening cream or vice versa. Nevertheless, the survey questionnaire, Table (2-25) reveals that 60.19 %

(257) of the participants (427) confessed that they used the local skin-lightening cream instead of the imported brands.

Table 2-21 Distribution of the participants according to frequency of the use of skin- lightening creams.

Complaints	**Number of participants**	**Percent (%)**
Users	151	31.50
Non- users	329	68.50
Total	**480**	**100.00**

Table 2-22 Distribution of the frequency of the different brands of Skin-lightening creams selected by the participants

Brand of lipsticks	**Number of participants**	**Percent (%)**
1- DOVE	44	11.02
2 -TAZOL	29	**7.27**
3 -POND'S	10	2.51
4 -NIVEA	55	13.78
5- GARNIER	08	2.00
6- HOLLY	32	8.03
7- LAURENT	12	3.00
8- Local Preparation	**100**	**25.07**
9- AVON	19	4.76
10-Fare & Lovely	27	6.77
11- White Face	42	10.53
12- TOP Beauty	21	5.26
Total	**399**	**100.00**

Table 2-23 Distribution of the frequency of the participants according to the type of complaint during the use of skin–lightening creams.

Complaints	**Number of participants**	**Percent (%)**
Allergy	90	59.60
Darkening of skin	61	40.40
Total	**151**	**100.00**

Table 2-24 Distribution of the frequency of the participants change from one brand to another while using skin –lightening creams.

Changing Brands	**Number of participants**	**Percent (%)**
Yes	202	57.55
No	149	42.45
Total	**351**	**100.00**

Table 2-25 Distribution of the frequency of the participants according to the preferable brands.

Brands	**Number of participants**	Percent (%)
Local	257	60.19
Imported	170	39.81
Total	**427**	**100.00**

2.5.5 Hair Dyes

Hair dyes are used by people all over the world. Commonly used hair dyes contain modified aromatic aniline and phenolic compounds that may cause allergic reactions or cancer, *(Harling 2010)*.Due to these potentially harmful effects, the amounts of these compounds are restricted in many countries. Methods for the quantitative measurement of these compounds in hair dyes include GC, GC/MS, LC and LC/MS. HPLC methods are popular because they can analyze compounds that are not thermally stable, but are strongly polar with low volatility, *(Bolt 2007)*. Twenty–two components were determined in hair dyes by high performance liquid chromatography, (*Brigs 1982*). Table(**2**-26) shows that more than one-third (177) of the participants (500) were using hair dyes of different brands and different colors. Among the various colors of hair dyes shown in Table (2-27), more than half (56.57 %) of the number of participants (327) reported that they prefer the black color, but they did not specify the origin of the hair dye whether local or imported.

Table 2-26 Percentage of the participants using of hair dyes.

Usage	Number of participants	Percent (%)
Non-users	323	64.5
Users	177	36.5
Total	**500**	**100.00**

Table (2-28) shows that 29.6% (127) of the participants (430) reported that they suffer from the use of different hair dyes. Table 2-29 shows that half (51.0 %) of the number of the participants (410) who reported harmful effects from the use of hair dyes complained from allergy, and the other half (49.0 %) suffered from eye itching. Table (2-30) shows that (73.5%) of the participants change from one brand to another. Table (2-31) summarizes the important information, gathered from some of the answers of the participants to the questions raised in the survey questionnaire, about the practice and usage of cosmetics by Sudanese ladies, which induced the analytical approach study of the harmful constituents of selected cosmetic samples in Sudanese market.

Table 2-27 The distribution of the participants using of hair dyes according to their colors

The Color	**Number of participants**	Percent (%)
Golden yellow	10	3.05
Chocolate	18	5.50
Brown	13	3.98
Dark Brown	51	15.60
Black	**185**	**56.57**
Dark Violet	12	3.67
Light Orange	8	2.45
Cupper	26	7.95
Dark red	4	1.22
Total	**327**	**100.00**

Table 2-28 Percentage of the participants according to their Complain from use of hair dyes

Appearance of complaints	**Number of participants**	Percent (%)
Yes	127	29.6
No	303	70.4
Total	**430**	**100.00**

Table 2-29 Distribution of the number of the participants according to the type of complaint during the use of hair dyes

Type of Complaints	**Number of participants**	**Percent (%)**
Allergy	209	51.0
Eye itching	201	49.0
Total	**410**	**100.0**

Table 2-30 Distribution of the number of the participants using hair dye change from one brand to another.

Changing	**Number of participants**	**Percent (%)**
Yes	291	73.5
No	105	26.5
Total	**396**	**100.0**

Table 2 -31 Summary of important information of Cosmetic Frequency Percentage.

Cosmetics Frequency Percentage (%)					
Matrix	Usage (%)	Preferred Color	Preferred Brands	Complaints of using	Changing Brands
Lipstick	67.00 %	Pink – 42.20 %	No.2 (18.70 %)	67.00 %	65.40 %
Face powder	73.50 %	Rose	No.9 (23.0 %)	64.00 %	64.60 %
Skin- lightening creams	39.80 %	-	No.8 (25.07 %)	38.90 %	42.63 %
Eye shadow	52.90 %	Dark Brown	No.6 (27.16 %)	36.50 %	54.20 %
Hair dye	61.00 %	Black	No.2 (18.7 %)	22.50 %	73.5%

CHPTER THREE

3- Determination of Hydroquinone in Skin-Lightening Cream sold in Sudan

3.1 Introduction

Many Sudanese women love to keep their skin toned and beautiful but unfortunately most of them end up indulging in skin care products that bleach the skin and eventually pose potential risk to their health. Most of these bleaching products contain different kinds of chemicals that may be harmful and affect the health of women. Examples of chemicals in these products include hydroquinone, mercury, Kojic acid, Kojic acid dipalmitate, Azleic acid, Arbutin, Bearberry, Vitamin C, Magnesium ascorbyl phosphate, Calcium ascorbate, and L-ascorbic acid, *(Olumide, 2010).*The most commonly used lightening agents were known to contain hydroquinone. The skin color was determined by the amount of melanin produced. Looking at the five hundred years of human history, humans have constantly labeled and stereotyped each other based on skin color, *(JAPA 2003).* In most African and Asian communities, fairness was branded as beauty, grace and high social status. The one with darker skin seeing as being of lowest social value, whereas, that with lighter skin is regarded as being of highest social value. This perception encourages most women to indulge in skin- care products that lighten the skin. Hydroquinone is potentially carcinogenic and known to be a skin and respiratory irritant. It is also considered a primary topical ingredient for inhibiting melanin production. Hydroquinone is a strong inhibitor of melanin production, meaning that it prevents skin from making the substance responsible for skin color*, (Rendonet 2006).* Because hydroquinone is carcinogenic, it has been banned in some countries because of fears of a cancer risk. Some concerns about hydroquinone’s safety on skin have been expressed, but research has shown that when it comes to topical application, it has negative reactions which are minor but major as a result of using extremely high concentrations. This is particularly true in Africa where adulterated skin lightening products are common. Figure (3 -1), shows an effective product used for whitening dark and black skin. It is mostly cherished by many African American women due to its effectiveness in the removal of dark spots while providing an even skin tone on the body. Acute animal test on rats, mice and rabbits have demonstrated hydroquinone to have high acute toxicity from oral exposure,*(Leslie 2006).*

Figure 3-1 – Skin- lightening Cream Effects

Hydroquinone can cause various deadly diseases such as thyroid disorder, leukemia and liver damage. Chronic occupational exposure to hydroquinone dust has resulted in eye injuries, which varied from mild irritation and staining of cornea, conjunctivae and cornea to changes in the thickness and curvature of the loss of corneal luster and impaired vision, *(Health Canada 2007).*Prolonged exposure is required for the development of severe ocular effects. Side effects of hydroquinone are mild when used in low concentrations. Higher concentrations frequently irritate the skin and, if used for prolonged periods, cause disfiguring effects including epidermal thickening. Brown discoloration of nails has been reported occasionally when application of two percent hydroquinone is used on the back of the hand, *(Gattrell 1987)*. If very low dose of hydroquinone was ingested, it seldom produces systematic toxicity. However, oral ingestion of between 5g and 15g doses has produced convulsions and hemolytic anemia *(Guy 1999)*. Intraperitoneal injection of hydroquinone caused chromosomal aberrations in magnitude as in mouse bone marrow cells. Cells of intoxication have been reported after ingestion of hydroquinone alone or of photographic developing agents containing hydroquinone. Deaths have been reported after ingestion of photographic developing agents containing hydroquinone, *(Veralla 1981)*. Hydroquinone is an important phenolic compound used in a wide variety of biological and industrial processes. Hydroquinone is used as an intermediate in the manufacturing of antioxidants for rubber, dyestuffs and food products. The major use of hydroquinone is as a reducing agent in photographic developing solution, which reduces silver halides to elemental silver in black-and-white photography and lithography. Besides its importance, it is also very much toxic and creates serious water pollution problems in many localities, (*Merck Index 1989*). Most of the skins lightening creams on the Sudanese market were imported from China, Thailand, Italy, Saudi Arabia, India, Egypt, UAE, Europe, and USA. A few of these countries have not restricted the use of hydroquinone to **2%** or less, *(Yourick 2004), (Yoshimura 2001)*. Hydroquinone have acute and chronic side effect in humans. FDA &WHO Standards allows a maximum of **two** percent of hydroquinone in skin care products. European Bureau of Standards banned some hydroquinone containing skin-lightening creams, despite the side effects of hydroquinone; skin lightening creams containing these harmful chemicals are still found on the Sudanese market and are sold to the public. Considering the toxic effect of hydroquinone, it is important to control, its exposure to humans. This can be achieved if their levels in skin lightening creams were known. In Sudan, a little work has been undertaken to determine the levels of heavy metals and hydroquinone in toning creams even though concern has been expressed about the wide spread use of skin lightening cream. *(Yetunde 2008).*

Today, skin whitening products are available in the form of creams, pills, soaps or lotions. The mechanism of permanent whitening is usually by the breakdown of melanin by enzymes, such as that contained in the droppings of the Japanese bush warbler (a drab-colored and secretive bird normally seen in spring before there is foliage in the trees) or reducing agents such as hydroquinone

3.2- Chemistry of Hydroquinone's - Structure

Hydroquinone, also benzene-1, 4-diol or quinol, is an aromatic organic compound which is a type of phenol, having the chemical formula $C_6H_4(OH)_2$.

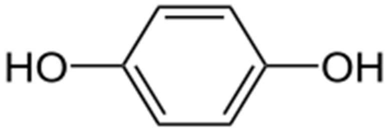

Figure 3.2 The structure of **HQ,** (benzene-1, 4-diol)

HQ- described as having two hydroxyl groups bonded to a benzene ring in a para -position.

3.3- Physical Properties of HQ :

(It is a white granular solid at room temperature and pressure)

- Molecular formula - $C_6H_4(OH)_2$
- Molar mass - 110.1 g/mol
- Appearance - white solid
- Density - 1.3 g/cm3, solid
- Melting point - 172 °C
- Boiling point - 287 °C
- Solubility in water - 5.9 g/100 ml (15 °C)

3.4- Materials and Methods

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

A) Hydroquinone **99%** (Sigma Aldrich) Certified standard reference material.

B) Methanol gradient plus for HPLC (Carlo Erba)

C) L- Ascorbic Acid (Prolabo) – Batch No. L129.

D) Two types of **blanks** were required for the analysis.

3.4.2 - Equipment

3.4.2.1 Instrumentation- HPLC

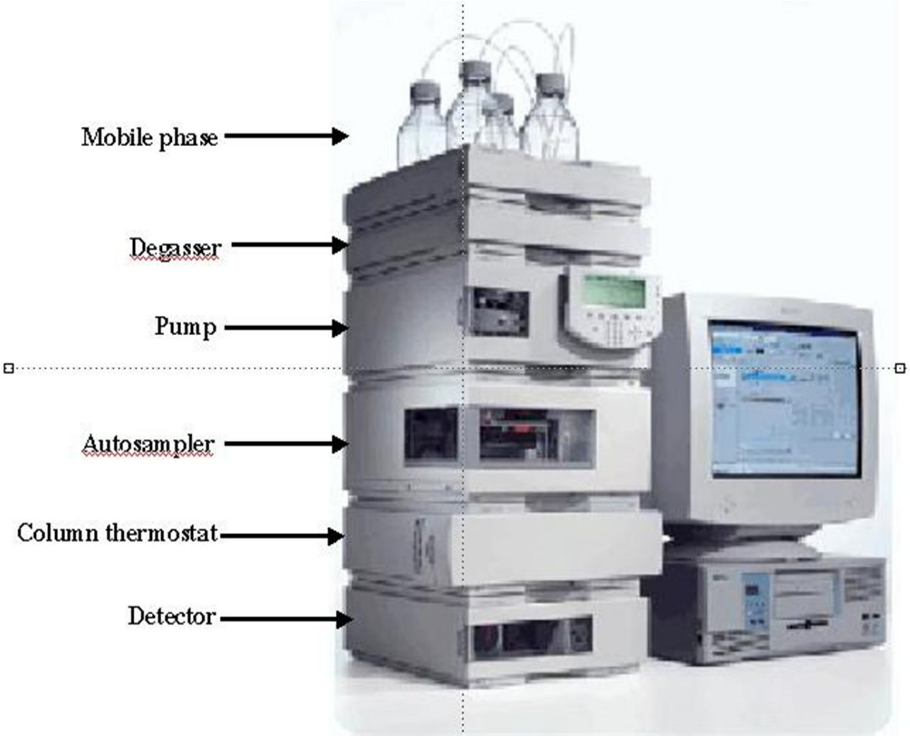

Figure 3.3 HPLC instrument (Agilent 1100 Series with DAD)

HPLC, formerly referred to as **high-pressure liquid chromatography**, is a technique in analytical chemistry used to separate, identify, and quantify each component in a mixture. It relies on pumps to pass a pressurized liquid solvent containing the sample mixture through a column filled with a solid adsorbent material. Each component in the sample interacts slightly differently with the adsorbent material, causing different flow rates for the different components and leading to the separation of the components as they flow out the column. *(Gerber 2004)*.

HPLC has been used for manufacturing (e.g. during the production process of pharmaceutical and biological products), legal (e.g. detecting performance enhancement drugs in urine), research (e.g. separating the components of a complex biological sample, or of similar synthetic chemicals from each other*),(Morgan 2015).*

Chromatography can be described as a mass transfer process involving adsorption. HPLC relies on pumps to pass a pressurized liquid and a sample mixture through a column filled with adsorbent, leading to the separation of the sample components. The active component of the column, the adsorbent, is typically a granular material made of solid particles (e.g. silica, polymers, etc.), 2–50 micrometers in size. The components of the sample mixture are separated from each other due to their different degrees of interaction with the absorbent particles. The pressurized liquid is typically a mixture of solvents (e.g. water, acetonitrile and/or methanol) and is referred to as a "mobile phase". Its composition and temperature play a major role in the separation process by influencing the interactions taking place between sample components and adsorbent. These interactions are physical in nature, such as hydrophobic (dispersive), dipole–dipole and ionic, most often a combination.

HPLC is distinguished from traditional ("low pressure") liquid chromatography because operational pressures are significantly higher (50–350 bar), while ordinary liquid chromatography typically relies on the force of gravity to pass the mobile phase through the column. Due to the small sample amount separated in analytical HPLC, typical column dimensions are 2.1–4.6 mm diameter, and 30–250 mm length. Also HPLC columns are made with smaller sorbent particles (2–50 micrometer in average particle size).

This gives HPLC superior resolving power (the ability to distinguish between compounds) when separating mixtures, which makes it a popular chromatographic technique, (*Lindsay 1987).*

The schematic of an HPLC instrument typically includes a sampler, pumps, and a detector. The sampler brings the sample mixture into the mobile phase stream which carries it into the column. The pumps deliver the desired flow and composition of the mobile phase through the column. The detector generates a signal proportional to the amount of sample component emerging from the column, hence allowing for quantitative analysis of the sample components.

A digital microprocessor and user software control the HPLC instrument and provide data analysis. Some models of mechanical pumps in a HPLC instrument can mix multiple solvents together in ratios changing in time, generating a composition gradient in the mobile phase. Various detectors are in common use, such as UV/Vis, photodiode array (PDA) or based on mass spectrometry. Most HPLC instruments also have a column oven that allows for adjusting the temperature at which the separation is performed.

3.4.2.2 Reversed-phase chromatography (RPC)

Reversed phase HPLC (RP-HPLC) has a non-polar stationary phase and an aqueous, moderately polar mobile phase. One common stationary phase is a silica which has been surface-modified with RMe_2SiCl, where R is a straight chain alkyl group such as $C_{18}H_{37}$ or C_8H_{17}. With such stationary phases, retention time is longer for molecules which are less polar, while polar molecules elute more readily (early in the analysis). An investigator can increase retention times by adding more water to the mobile phase; thereby making the affinity of the hydrophobic analyte for the hydrophobic stationary phase stronger relative to the now more hydrophilic mobile phase. Similarly, an investigator can decrease retention time by adding more organic solvent to the eluent. RP-HPLC is so commonly used that it is often incorrectly referred to as "HPLC" without further specification. The pharmaceutical industry regularly employs RP-HPLC to qualify drugs before their release, *(Iler 1979).*

RP-HPLC operates on the principle of hydrophobic interactions, which originates from the high symmetry in the dipolar water structure and plays the most important role in all processes in life science. RP-HPLC allows the measurement of these interactive forces. The binding of the analyte to the stationary phase is proportional to the contact surface area around the non-polar segment of the analyte molecule upon association with the ligand on the stationary phase. This solvophobic effect is dominated by the force of water for "cavity-reduction" around the analyte and the C_{18}-chain versus the complex of both. The energy released in this process is proportional to the surface tension of the eluent (water: 7.3×10^{-6} J/cm², methanol: 2.2×10^{-6} J/cm²) and to the hydrophobic surface of the analyte and the ligand respectively. The retention can be decreased by adding a less polar solvent (methanol, acetonitrile) into the mobile phase to reduce the surface tension of water. Gradient elution uses this effect by automatically reducing the polarity and the surface tension of the aqueous mobile phase during the course of the analysis.

Structural properties of the analyte molecule play an important role in its retention characteristics. In general, an analyte with a larger hydrophobic surface area (C–H, C–C, and generally non-polar atomic bonds, such as S-S and others) is retained longer because it is non-interacting with the water structure. On the other hand, analytes with higher polar surface area (conferred by the presence of polar groups, such as -OH, $-NH_2$, COO^- or $-NH_3^+$ in their structure) are less retained as they are better integrated into water. Such interactions are subject to steric effects in that very large molecules may have only restricted access to the pores of the stationary phase, where the interactions with surface ligands (alkyl chains) take place. Such surface hindrance typically results in less retention, *(Giddings 1965).*

Retention time increases with hydrophobic (non-polar) surface area. Branched chain compounds elute more rapidly than their corresponding linear isomers because the overall surface area is decreased. Similarly organic compounds with single C–C bonds elute later than those with a C=C or C–C triple bond, as the double or triple bond is shorter than a single C–C bond.

Determination of hydroquinone was carried out by a chromatographic method using a high performance liquid chromatography (HPLC), Agilent 1100 series with DAD (Appendix I). This was equipped with an analytical column, (Hypersil C8 150*4.6mm*5µm), *(Huang 1984).* A syringe with a capacity of 50µl was used to inject the sample into the sample loop. After loading the sample, the injector was turned to the inject position. The HPLC detector (DAD) Figure (3.4) based on spectroscopic measurements, *(Levin 1988).*The resulting chromatogram was a plot of absorbance as a function of elution time. The signals were obtained on a computer. The schematic diagram of HPLC is shown in Fig (3-3). In addition, the schematic diagram of DAD is shown in Figure (3-4).

3.4.2.3 Check of instrument before analysis The instrument condition checked properly, the temperature displayed was stabilized at 30°C (if not the oven door was checked). The pressure was stable at 67 bar. (if not seals, connections were checked that there were not blockage or leaks).

3.4.2.4 HPLC-Instrument conditions

- Column: (Hypersil C8 150*4.6mm*5µm)
- Stabilized pressure: 67 bar
- Column temperature: 30°C
- Flow rate: 1cm^3/min
- Elution. Gradient
- Mobile phase : 80:20 (methanol – water)
- Stop time : 25min, post time 5min
- Injection volume : 10µdm^3
- Signal 1(B): 290nm , 4nm ref =550.100
- Retention time of hydroquinone 4.1min.

Table 3-1 Instrument operation conditions

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

Elution gradient

Stop time: 25 min; post time: 5min – injection volume: 10µl

3.5 Methodology

3.5.1 Sampling

Thirty samples of local and imported skin- lightening creams were obtained randomly from cosmetic shops in Khartoum, Khartoum North and Omdurman Markets local and imported samples, Table(4-1).

3.5.1.1 Sample preparation

Accurate weight of 0.5g and 1g of lightening cream were taken directly in the 20-cm^3 volumetric flask then filled up to the mark with 1g/dm^3 ascorbic acid solution. If the sample did not dissolve in the ascorbic acid solution, it could be dissolved in a (50/50) mixture of methanol/ ascorbic acid solution. The sample was filtered through 0.45 µm. If the sample was not dissolved, it was added directly to the 8 cm^3 test portion of THF; then stir for 5 minutes. Then it was filled up to the mark with the ascorbic acid.

3.5.1.2 Preparation of the standard solutions for HQ

Standard solution of Hydroquinone was prepared by dissolving 1.0g hydroquinone in methanol in a 100 cm^3 volumetric flask and made up to volume mark using methanol. Various concentrations (0.08, 0.12, 0.16 and 0.2g/dm^3) were prepared by diluting aliquots of the stock hydroquinone standard solution with methanol.

Table 3-2 Concentration of hydroquinone standards

Volume (ml)	SF1	SF2	SF3	SF4	SF5
SM	1	1	1	1	1
Qs (cm^3) Ascorbic acid solution	2000	1000	200	100	50
Concentration of hydroquinone (mg/dm^3)	0.5	1	5	10	20

The standards SF1 to SF5 as described in Table (3-2), using calibrated volumetric flasks . The volume of stock solution was taken using 1cm^3 pipette filled to the mark.

3.5.1.3 Calibration curve of HQ

The calibration curve was prepared by using the standard solutions SF1, SF2, SF3, SF4 and SF5 concentrations calculated exactly during preparation of the stock solutions, taking the purity into the consideration.

3.6 Method Validation

Method validation was executed by linearity, precision and accuracy test on the basis of ICH guidelines. Linearity was confirmed by the correlation coefficient (R^2). To calculate the regression equation, five different concentrations of the standard solution were used to draw up a calibration curve. In addition to the clear guidelines regarding the validation study records, same records should be retained to demonstrate the validity of the method, *(Saunders2004).*

3.6.1 Linearity, Five different concentrations of standard working solutions Table (3-2) were prepared from the stock solutions and analyzed to study the linear dynamic range of the method.

3.6.2 Accuracy, It is the proximity of measurement results to the true value; precision, the repeatability, or reproducibility of the measurement.

3.6.3 Precision

Precision, The precision of the proposed method, expressed as relative standard deviation (RSD) percentage, was determined by analysis of each compound, *(Miller1984)*.

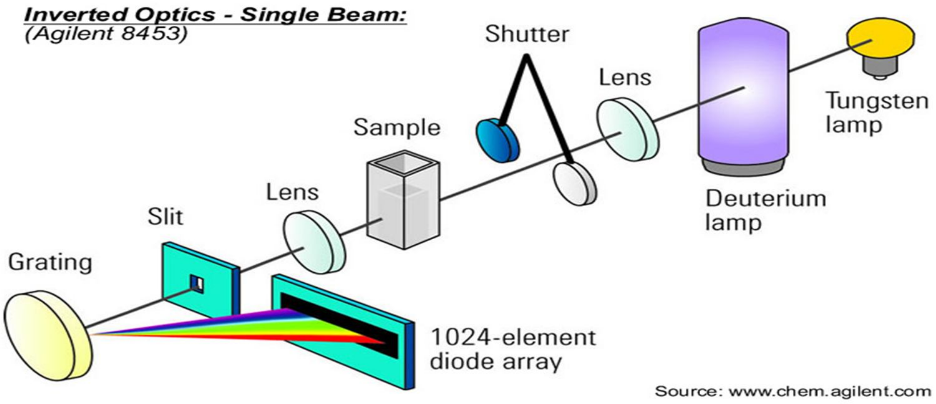

Figure 3-4 -Schematic diagram of Diode Array Detector (DAD)

3. 6.4 Recovery Study of HQ

Recovery of hydroquinone was determined by adding increasing amount of standard hydroquinone solution to known weights of two different cream samples. The first flask contained only 0.1g of each sample and the second flask contained 0.1 g of each sample and 0.08g of HQ. The third flask contained 0.1g of each sample and 0.12g HQ. The resulting solutions after extraction were analyzed for hydroquinone concentrations and the results obtained are reported in Table (3.3) & Table (3-4) shows the percentage of recovery of hydroquinone in different skin- lightening creams collected from the local market in Sudan which is ranged from 99.5 % to 102.4% .

Table 3 -3 Recovery results of HQ for skin-lightening cream samples.

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

(*Where, HQ ; hydroquinone)*

In the same conditions as above, a solution independent from that used for the range was injected of test concentrations 0.5mg/dm^3 and5mg/dm^3, a blank with the dilution solvent. The recovery rate relative to the target concentration was calculated. The accepted recover rate had to be between +/- 3% for the low test 100+/-5% for the high test, and an LOD. For HQ identification, determine the purity of the measured peak in the sample.

3.6.5 Checking the Results

The concentration of analyte in the two preparations must be the same ± 5percentage. If there is no breakdown of the sample, a preparation is made with extra THF. If the analyte concentration in this preparation is the same as that of the preparations with Me OH/ascorbic acid, then the value is accepted. If the analyte concentration is higher, then this means that the sample had not been sufficiently dissolved to allow the product to be completely absorbed in the solvent.

3.6.6 Calculations method

Note: HPLC instrument software can be programmed to perform all necessary calculations.

Analyte concentrations in digested samples (CS) were calculated using:

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

Where:

CE = Analyte concentration in final extract, in μg/dm^3

V = Final sample extract volume in milli -decimeters

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

W = Sample Weight in grams.

All results were reported in μg/dm^3, ppb or mg/dm^3, ppm for liquid samples and μg/kg, (ppb) or mg/Kg, ppm for solid samples.

3.7 Results and Discussion

Hydroquinone was determined by recovery studies Table (3-3). The recovery studies for hydroquinone yielded recoveries, which ranged from 97 to 102 %. The results for recovery studies were presented in Table (3-3). Table 3-4 shows the percentage of hydroquinone result in skin- lightening cream samples (local & imported), collected from different shops in Sudan, (Appendix I).

Table 3-4 Hydroquinone concentration in the collected samples (30 samples).

Sample ID	Type of Sample	Country of Origin	Lot No.	Color	Item No	HQ – Results(percentage)
LC 51	Skin-lightening Cream	Local/K.N	-	White	-	**4.11**
LC 52	Skin-lightening Cream	Local/K.N	-	Off –white	1	**0.51**
LC 53	Skin-lightening Cream	Local/K.N		White		**5.63**
LC 54	Skin-lightening Cream	Local/OM	-	Off –white	2	**0.10**
LC 55	Skin-lightening Cream	Local/OM	-	White	03	**5.75**
LC 56	Skin-lightening Cream	Local/OM	-	Off –white	-	**1.64**
LC 57	Skin-lightening Cream	Local/OM	-	Off –white	2	**ND**
LC 58	Skin-lightening Cream	Local/K.N	-	Off –white	-	**3.89**
LC 59	Skin-lightening Cream	Local/OM	-	White	-	**3.05**

(Continued)

F&L 60	Skin-lightening Cream	India	-	White	01	**2.03**
F&L 61	Skin-lightening Cream	France	-	Off –white	01	**1.38**
F&L 62	Skin-lightening Cream	UAE Dubai	-	Transparent Gel	01	**0.47**
F&L 63	Skin-lightening Cream	UAE Dubai	-	Off –white	01	**4.76**
F&L 64	Skin-lightening Cream	China	-	White	2	**2.81**
F&L 65	Skin-lightening Cream	China	-	White	-	**0.52**
C 66	Skin-lightening Cream	Italy	-7702-MK	Off –white	-	**0.92**
C 67	Skin-lightening Cream	Egypt	-	White	-	**3.21**
C 68	Skin-lightening Cream	Italy	-	Transparent Gel	-	**0.70**
C 69	Skin-lightening Cream	Egypt	512	Transparent Gel	1	**ND**
C 70	Skin-lightening Cream	India	-	Off – white	2	**ND**
C 71	Skin-lightening Cream	China	09	White	03	**0.66**
C 72	Skin-lightening Cream	France	154 LO	Transparent Gel	-	**1.08**
C 73	Skin-lightening Cream	UAE Dubai	561	White	22	**0.69**
C 74	Skin-lightening Cream	Egypt	443	Off –white	-	**ND**

(Continued)

C 75	Skin-lightening Cream	Egypt	-	White	-	**2.72**
C 76	Skin-lightening Cream	Egypt	-	Off-white	01	**ND**
C 77	Skin-lightening Cream	Thailand	218Z	Transparent Gel	01	**0.39**
C 78	Skin-lightening Cream	China	-	Transparent Gel	01	**1.02**
C 79	Skin-lightening Cream	Thailand	92-Y	White	01	**ND**
C 80	Skin-lightening Cream	China	-	White	-	**5.07**

Table (3-4) Indicates the hydroquinone concentration in the collected samples of skin – lightening creams. Hydroquinone results, for LC- ranged from 0.0 to 5.75%, F&L, 0.47 to 4.76% and for C- ranged from 0.0 to 5.07%, respectively. Highest concentration of HQ was 5.75% and 3.05% recorded for local preparation sold in Omdurman market. F&L cream from India recorded 2.03% of hydroquinone. France cream recorded 1.38% of hydroquinone. Dubai skin lightening cream recorded the highest hydroquinone concentration of 4.76% for imported creams, followed by China cream 2.81%, which was also above the recommended value (2.0%). In total, 92% of the creams analyzed recorded levels below 2% hydroquinone, which is the threshold limit, and 8% of the creams analyzed contained more than 2% hydroquinone, which are above the threshold limit. The concentrations of hydroquinone in skin-lightening creams have also been the subject of study in the United Kingdom *(Boyle 2006)*. Eight out of forty one cream samples analyzed were found to contain more than two (2%) percent hydroquinone which is the maximum concentration permitted by the United Kingdom cosmetic product regulations. In a similar study of creams obtained from the open market in Plateau state, Nigeria, ten cosmetic creams containing hydroquinone were subjected to chromatographic test for identification. All ten creams sampled gave positive results to the test for hydroquinone. The level of hydroquinone was below two (2%) percent for seven of the creams, between 2 and 5 % for two and above 5 % for one (*Odumoso 2010).*High levels of hydroquinone detected in the cream analyzed samples pose a potential health risk to the consumer. Exogenous ochronosis is a paradoxical hyper pigmentation of the skin caused by long-term use of hydroquinone-containing bleaching creams. Ochronosis is an uncommon condition characterized by yellow-brown pigmented deposits in the dermis. Two cases of exogenous ochronosis in two female patients of the sub-Saharan African population were reported, *(Bongiono 2005).* In one case the lesions were characterized by an asymptomatic hyper-pigmentation of the face with gradually progressive blue-black macular patches, and in the other case in addition to dyschromic lesions, *striae- atrophicae* were present. This phenomenon was the outcome of the use of skin care products containing high concentrations of hydroquinone and glucocorticoid-based products, and, in addition, certain modalities in the use of bleaching products are likely to facilitate complications (*Bongiono 2005*). Hydroquinone has been used for decades as a skin lightening agent. As a result of concerns about mid-term effects like leukomelanoderma, its use in cosmetics has been banned in the Netherlands since January 2001, (*Kooyers 2005*). Until recently no attention was paid to potential long-term side-effects, despite the fact that there are indications that these may exist. It was decided that a clearer picture of these potential long term effects is needed. It appeared that since 1996 an enormous amount of articles have been published on the carcinogenicity of hydroquinone, benzene and related molecules, (*Kooyers 2005*). The literature search on hydroquinone as a skin- lightening agent suggests that possible long-term effects like carcinogenesis may be expected. The risks of long-term effects (cancer) of topically applied hydroquinone may no longer be ignored. A 23-year-old left-handed white male was referred with a complaint of brown discoloration of the fingernails of his left hand. He had been applying 4% hydroquinone cream to facial melasma. The discoloration occurred within 2 months of cessation of hydroquinone (*Parlak 2004).*

CHAPTER FOUR

4- Determination of Heavy Metals Impurities in Cosmetic Products in Sudanese Markets

4.1---Introduction

In **Sudan** women every day absorb through their skin, a lot of chemicals from beauty products and for that they spend a lot of money. Many older women feel that their confidence would decrease if they allow themselves to go gray .They feel that they look older, and thus they would be at a disadvantage in their personal and professional lives if they have gray hair. The usage of cosmetics has become a prevailing fashionable practice for modern females such as students, employees (larger consumers) and house wives. Cosmetic markets are growing rapidly on a daily basis. Normally, if the practices are legal, they will add value to the business, but unfortunately there are illegal practices by some cosmetic manufacturers as they work without license from legal authorities, (*Chukwuma 1997*). As a daily routine, cleansing, toning and moisturizing are thought to be essential for a healthy and youth complexion, but scientists are now raising concerns about the damage caused by chemicals in cosmetics to our bodies. Moreover, there are several factors which can contaminate makeup cosmetic products: Sharing makeup, poor handling procedures during manufacturing, bad storage conditions (more likely for bacteria to grow, in addition to poor quality of raw materials of makeup, (*Health Canada 2011*). Cosmetics can be defined as many preparations to be applied to the human body for the purpose of cleansing, beautifying, promoting, making attractive or altering the appearance without affecting the body's structure and function .They can also be used to repair or hide skin imperfections, to cleanse, adorn, protect and treat the human body, (*Reed 2007*).

In Sudanese markets there are plenty of cosmetic preparations available which are prepared locally without any control. The most alarming issue in Sudan is the large number of people practicing skin- lightening techniques. Globally, however, similar practices are employed by black ladies in all over the world, (*Dwivedi 1996*). The challenging problem of the cosmetic industry is to design safe products that help dark- skin woman looks "Lighter". It is also reported, for instance, that facial attractiveness is associated with positive evaluation by others and that many Sudanese ladies believe that with lighter skin they become more competent. Interestingly, level of education, social class, marital and employment status seem to have less influence on the skin lightening practice. The toxicity of metals and its curie is well documented (*Nnorom 2005*). Since the issue of heavy metals, as deliberate cosmetic ingredients, has been addressed, attention is turned to the presence of these substances as impurities, (*Al Trabulsy 2013*).

Heavy metals are not added intentionally to cosmetics as constituent ingredients, they are simply trace impurities in the product and are thus not required to be listed on the labels (*Nnorom 2005*). Minute traces may be carried into the product during preparation. The metals of primary toxically concern in cosmetics are : Pb, Hg, Cd, As, Ni and Zn**.** Dermal exposure is expected to be the most significant route for cosmetic products since the majority of cosmetics are applied on the skin. Dermal absorption of heavy metals is fairly minimal, with absorption of individual elements influenced by a number of factors including physical and chemical properties of the mixture, (*Volpe 2012*). Heavy metals are individual metals and metal compounds that can impact human health. Six common heavy metals arsenic, cadmium, lead, mercury, nickel, and zinc, were discussed, *(Palpandi 2010).* These are all naturally occurring substances, which are often present in the environment at low levels. In larger amounts, they can be dangerous. Generally, humans are exposed to these metals by ingestion (drinking or eating) or inhalation (breathing). Working in or living near an industrial site, which utilizes these metals and their compounds increases ones risk of exposure, as does living near a site where these metals have been improperly disposed. Subsistence lifestyles can also impose higher risks of exposure and health impacts because of hunting and gathering activities, *(Tremblay 2007).* In cosmetic products by analyzing a solution (obtained after acid digestion of the samples) by calibration using two internal standards; Rhodium (Rh) and Indium (In). This procedure applies to all cosmetic products with the exception of samples containing alcohol (Perfumes or fragrance base), in this case refer to method ICP-OES, (*Palpandi 2010*).

4.2- Materials and Methods

4.2.1 Materials

All standard solutions were stored in polypropylene or other inert containers. If glassware was used, it should be cleaned with nitric acid and dedicated for trace metals analyses. Standards prepared in glassware were used immediately or transferred to suitable containers for storage. Maximum care was taken to ensure reagent purity, clean working conditions to minimize sample contamination through handling, cleaning, atmosphere of sample preparation and cleanliness of materials and equipment.

4.2.1.1 Regents: All chemicals used were of analytical reagent grades unless otherwise stated. Acids used in the preparation of standards and for sample processing were ultra-high purity grade or equivalent.

Aquarequia, 3 volume conc. HCl to 1 volume conc. HNO_3

Hydrochloric acid, conc. (sp gr 1.19), Fluka Analytical**,** Switzerland**.**

Hydrochloric acid, (1+1): Add 500 cm^3 conc. HCl, Sigma-Aldrish USA.

(sp gr 1.19), to 400 cm^3 deionized, distilled water and diluted to 1dm^3

Nitric acid, conc. (sp. gr 1.41), Sigma-Aldrish USA.

Nitric acid, (1+1): 500 cm^3 conc. HNO3 (sp. gr 1.41) were added to 400 cm^3deionized, distilled water and diluted to 1 dm^3.

Deionized, distilled water: High purity 0.5 µs/cm or better.

Standard stock solutions: Such solutions were purchased as certified reference standard solutions (ICP-MS Grade), purchased from Merck Germany.

Element stock solutions = 1000 mg/dm^3 each of, As, Cd, Hg,

Pb, Sb, Zn, and other elements. These solutions that were prepared in 5% acid were considered to be stable for more than one year.

Dilute single element calibration standards were prepared in 5% Nitric acid. All standards were stored at room temperature.

Multi-element standard solutions: Such solutions were purchase in ICP-MS grade, from Fluka Analytical**,** Switzerland, certified standard solution mixtures or prepared from ultra-high purity elemental reference standards of ICP-MS grade, (in-house reference standard). Dilute multi-element calibration standards were prepared in 5% Nitric acid. All standards were stored at room temperature, from Merck Germany.

The calibration blank was used to correct for

possible contamination resulting from varying amounts of the acids used in the sample processing.

The calibration blank was prepared by diluting 2 cm^{3}of (1+1) HNO_3 and 10 cm^3 with deionized distilled water.

A sufficient quality to be used to flush the system between standards and samples were prepared.

The reagent blank contained all the reagents and in the same volumes as used in the processing of the samples.

The reagent blank was carried through the complete procedure and contained the same acid concentration in the final solution as the sample solution used for analysis.

The instrument (calibration) check standard was prepared by the analyst by combining compatible elements at a concentration equivalent to the midpoint of their respective calibration curves but different from those used in any calibration standard. Prepare in same manner calibration standards were prepared in same manner, diluted with 2%HNO_3.

The quality control sample was prepared in the same acid matrix as the calibration standards at a concentration near 1 mg/dm^3 and in accordance with the instruction provided by the supplier.

4.3—Equipment

4.3.1 Instrumentation- Agilent, ICP-MS

Figure (4-1) Shows the Agilent Technologies 7700 Series ICP- MS in details.

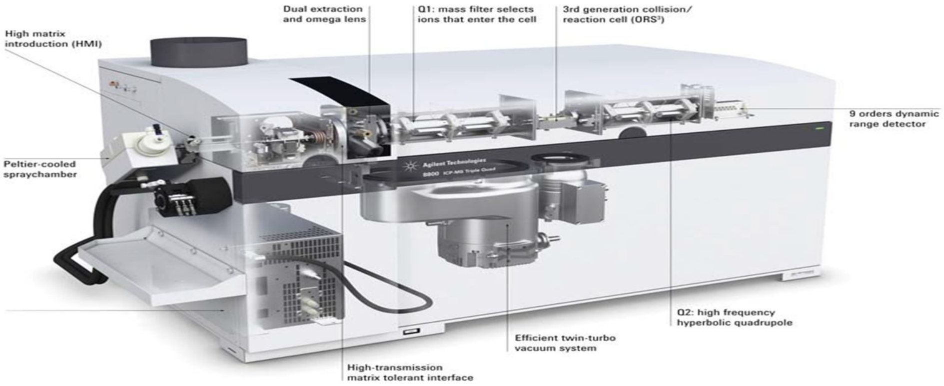

Figure 4.1 Agilent 7700 Series ICP-MS Hardware

4.3.2 BASIC OVERVIEW OF ICP

Inductively Coupled Plasma-Optical Emission Spectrometry is an elemental analytical technique used for the measurement of trace and minor components in liquid samples or solid samples after acid digestion. A liquid is nebulized into an Inductively Coupled Plasma at 5500 K which excites and/or ionizes the atoms in the sample. The intensity of the light emitted is measured using optical detection at the wavelengths characteristic of the elements of interest. These measurements can be compared to a standard to quantify the concentration of the elements in the sample.
ICP is capable of measuring 78 elements from sub-ppb to % level. While the technique is less sensitive than ICP-MS, it has several advantages:

ICP-AES is an emission spectrophotometric technique, exploiting the fact that excited electrons emit energy at a given wavelength as they return to ground state. The fundamental characteristic of this process is that each element emits energy at specific wavelengths peculiar to its chemical character. Although each element emits energy at multiple wavelengths, in the ICP-AES technique it is most common to select a single wavelength (or a very few) for a given element. The intensity of the energy emitted at the chosen wavelength is proportional to the amount (concentration) of that element in the analyzed sample. Thus, by determining which wavelengths are emitted by a sample and by determining their intensities, the analyst can quantify the elemental composition of the given sample relative to a reference standard, (*Ahrends 2007).*

ICP-AES analysis requires a sample to be in solution. Thus, interstitial waters can be analyzed simply, requiring only dilution in most cases. Igneous rocks, sedimentary rocks, and sediments, however, must be dissolved. This can be achieved either by a combined acid attack employing HF, HNO_3, and HCl acids, or by a $LiBO_2$ flux-fusion technique similar to that used for XRF preparation. In addition to being somewhat dangerous for routine shipboard work (because of the highly reactive nature of HF), the acid attack is not able to generate consistent and reliable data for Si because it volatilizes in the presence of HF, *(Klotz 2013).* The acid digestion procedure also often results in incomplete analysis of refractory elements such as Ti, Cr, and Zr because their host minerals are often difficult to dissolve. The flux-fusion approach is employed on board the *Resolution* for several reasons:

(1) It is safer because HF is not involved;

(2) It is a complete dissolution technique, allowing determination of all elements, including Si and the refractory elements;

(3) The resultant solutions are similar in composition (or matrix) because they are dominated by the presence of the $LiBO_2$ flux; and

(4) The solutions are stable in dilute HNO_3 acid and can be transported safely back to the scientist's laboratory for further shore-based study.

All ICP-AES systems consist of several components. The three main aspects: the sample introduction system, the torch assembly, and the spectrometer. The sample introduction system on the ICP-AES consists of a peristaltic pump, Teflon tubing, a nebulizer, and a spray chamber. The fluid sample is pumped into the nebulizer via the peristaltic pump. The nebulizer generates an aerosol mist and injects humidified Ar gas into the chamber along with the sample. This mist accumulates in the spray chamber, where the largest mist particles settle out as waste and the finest particles are subsequently swept into the torch assembly. Approximately 1% of the total solution eventually enters the torch as a mist, whereas the remainder is pumped away as waste, *(Wang 2008).*

4.3.2.1 Plasma torch

The plasma used in an ICP-MS is made by partially ionizing argon gas ($Ar \rightarrow Ar^+ + e^-$). The energy required for this reaction is obtained by pulsing an alternating electric current in wires that surround the argon gas.

After the sample is injected, the plasma's extreme temperature causes the sample to separate into individual atoms (atomization). Next, the plasma ionizes these atoms ($M \rightarrow M^{+} + e^{-}$) so that they can be detected by the mass spectrometer.

Humidification of the Ar gas injected into the nebulizer is important when analyzing samples with high dissolved solids, as is often the case with analysis of ODP rocks, sediments, and interstitial waters. Humidification takes place in the Ar humidifier, where Ar is bubbled through deionized water prior to its expulsion in the nebulizer, *(Tanner 1999).* The fine aerosol mist containing Ar gas and sample is injected vertically up the length of the torch assembly into the plasma. There are several recommended Ar flow rates used in the torch, as described in detail in the owner's manual and in the various publications provided. The radio frequency-generated and maintained Ar plasma, portions of which are as hot as 10,000 K, excites the electrons. When the electrons return to ground state at a certain spatial position in the plasma, they emit energy at the specific wavelengths peculiar to the sample's elemental composition. The plasma is viewed horizontally by an optical channel. Light emitted from the plasma is focused through a lens and passed through an entrance slit into the spectrometer. There are two types of spectrometers used in ICP-AES analysis: sequential (monochromator) and simultaneous (polychromator). The JY2000 has a sequential spectrometer. This means that the diffraction grating in the spectrometer is analogous to a prism that refracts visible light into its component colors. The detector (photomultiplier tube) is fixed in space at the far end of the spectrometer. Rotation of the diffraction grating sequentially moves each wavelength into the detector. The computer control ensures that the detector is synchronized with the grating so that the intensity at the detector at any given time is correlated with the wavelength being diffracted by the grating. The operator enters the wavelengths that he or she wishes to detect into the computer, the grating sequentially moves to the specified wavelengths, and the energy intensity at each wavelength is measured to provide a quantitative result that can be compared to a reference standard. Using standard spectroscopic techniques (e.g., background corrections), sequential ICP-AES can provide extremely flexible and rapid analysis of a number of chemical elements. The spectrometer is flushed with N_2 gas to improve the detection limits of elements with emission wavelengths that are severely compromised by interference with air (e.g., P). This N_2 flush, which is constantly maintained in the instrument regardless of whether such elements are being analyzed, also protects the optics from the corrosive aspects of the atmosphere, which are particularly acute at sea.

ICP mass spectrometers (ICP-MS) opens up whole new worlds of efficiency for environmental, food, biomonitoring, geochemical, and semiconductor laboratories. Measure more in less time, accurately characterize nanoparticles, and choose from a wide range of interference-removal techniques, including those that are ideal for: High-throughput samples with few interferences Samples that require the removal of unknown interferences, *(Tanner 1999).* Applications requiring the best performance with the lowest detection limits. Inductively coupled plasma mass spectrometry (ICP-MS) is a type of mass spectrometry which is capable of detecting metals and several non-metals at concentrations as low as one part in 10^{15} (part per quadrillion, ppq) on non-interfered low-background isotopes. This is achieved by ionizing the sample with inductively coupled plasma and then using a mass spectrometer to separate and quantify those ions. Compared to atomic absorption spectroscopy, ICP-MS has greater speed, precision, and sensitivity.

However, ICP-MS introduces many interfering species: argon from the plasma, component gases of air that leak through the cone orifices, and contamination from glassware and the cones. *(Long1983)*.

4.3.3 Microwave digestion system

Figure 4-2 Closed microwave digestion/extraction workstation.

In sample digestion and Heavy metals analysis

Microwave. Equipped with a 40-vessel integrated digestion rotor, it was developed into cosmetic products for high throughput capability. Although, cosmetics contain a variety of components that can be challenging to digest, including fats and silica-based compounds, microwaves with the addition of HF to digest siliceous materials may be necessary. Bioavailability information, may influence also the sample preparation method and types of acids added. Microwave digestion also provides a clean digestion and retains mercury so that a full group of elements may be easily examined. ICP – MS is a good choice for determination of low concentration of analytes, allowing evaluation of toxic and potentially toxic compounds in cosmetics.

4.3.3.1 The Digestion goals could be summarized as follows:

a) Complete solution of the element
b) Complete decomposition of the mixture
c) Avoiding loss and contamination
d) Reduction of handling and process times

4.3.3.2 Analytical balance An electronic damping circuit enables the balance to follow very rapid changes in weight without oscillating and with an accuracy better than 0.5 percent of full scale for ranges of 100 mg and over, (capacity 200 gram - sensitivity 0.0001 gram). The analytical balance calibrated annually by an external authority and checked every day prior to the use with the aid of standard masses.

4.3.3.3 Plastic and glassware All glassware and plastic were cleaned with liquid soap 1:1 nitric acid –water then rinsed with deionized water and oven dried at 60°C.

Teflon vessels 100 cm^3 for microwave digestion
Volumetric flasks glass, 10 - 1000 cm^3, as needed for preparation of standards, reagents.
Micropipettes - fixed or variable, covering ranges 10 - 5000 µdm^3
Bottles – polypropylene 250 cm^3.
Auto sampler plastic tubes.

4.4 – Samples

Generally, the cosmetic products marketed in Sudan could be listed according to the following categories :

Creams, emulsions, lotions, gels and oils for the skin (hands, face, feet, etc.).

Face masks (with the exception of chemical peeling products).

Tinted bases (liquids. pastes, powders).

Make-up powders, after-bath powders, hygienic powders, etc.

Toilet soaps, deodorant soaps, etc.

Perfumes, toilet waters and eau de Cologne.

Bath and shower preparations (salts, foams, oils. gels, etc.).

Depilatories.

Deodorants and anti-perspirants.

Hair care products. *(Nohynek 2010)*

hair tints and bleaches.

products for waving, straightening and fixing,

setting products,

cleansing products (lotions, powders, shampoos),

conditioning products (lotions, creams, oils),

hairdressing products (lotions, lacquers, brilliantines).

Shaving products (creams, foams, lotions, etc.).

Products for making-up and removing make-up from the face and the eyes, *(DIRECTIVE 2003).*

Products intended for application to the lips.

Products for care of the teeth and the mouth.

Products for nail care and make-up.

Products for external intimate hygiene.

Sunbathing products.

Products for tanning without sun.

Skin-whitening products.

Anti-wrinkle products.

Selected samples of the most commonly used brands of total number of (**96**) Samples were collected from local markets (Khartoum, Omdurman and Khartoum North) in Sudan at the time of study, as follows:-

A) Lipstick : **25** , (**20** imported samples), and (**5**-Local)
B) Face Powder : **25** samples
C) Creams : **30** , (**20** imported samples), and (**10** Local)
D) Hair Dyes : **10** samples (5 local & 5 imported)
E) Eye Shadow : **6** samples

Table (4-1); shows a full details of all samples (**96** different cosmetic samples), collected randomly from Sudanese market, mainly from Khartoum State.

Table 4.1 Details of collected Cosmetic products samples from Sudanese markets

Sample No	Type	Country of origin	Lot No	Color	No	Item No
L 1	Lipstick	China	-	Pink	-	9902-GI
L 2	Lipstick	China	-	Chocolate	-	150
L 3	Lipstick	India	-	Dark violet	-	-
L 4	Lipstick	UAE-Dubai	154 MK	Green	-	-
L 5	Lipstick	UAE-Dubai	120F	Pink	-	A8218
L 6	Lipstick	Egypt	016071/A	Red	-	-
L 7	Lipstick	China	-	Cupper	-	-
L 8	Lipstick	India	-	Cupper	-	-

L 9	Lipstick	Thailand	324/005	Dark violet	-	-
L 10	Lipstick	Indian	-	Light brown	-	814706
L 11	Lipstick	Egypt	991	Pink	-	-
L 12	Lipstick	Italy	MK250	Light brown	-	-
L 13	Lipstick	Thailand	-	Brown	-	-
L 14	Lipstick	Saudi	-	Purple	-	-
L 15	Lipstick	Saudi	-	Light orange	-	-
L 16	Lipstick	Thailand	KJ/0420	Brown	-	932-GI
L 17	Lipstick	Saudi	65463	Cupper	-	-
L 18	Lipstick	Egypt	-	Violet	-	-
L 19	Lipstick	Saudi	-	Colorless	-	670-J
L 20	Lipstick	UAE	-	Orange	-	-
L L 21	Lipstick	Local/K.N	-	Dark violet	-	-
L L 22	Lipstick	Local/K.N	-	Cupper	-	-
L L 23	Lipstick	Local/OM	-	Brown	-	-
L L 24	Lipstick	Local/OM	-	Dark violet	-	-
L L 25	Lipstick	Local/KH	-	Cupper	-	-
P 26	Face Powder	Syria	18-1029	Yellow – Based, F.P	-	-
P 27	Face Powder	China	M-1120	Off White	-	3602-08
P 28	Face Powder	China	Z1190	Yellow – Based, F.P	2	NK-2003
P 29	Face Powder	China	7301-282M	Pink- Based F. Powder	3	
P 30	Face Powder	Thailand	37801	Pink Based F.P	-	-
P 31	Face Powder	China	-	Dark Orange	3	-
P 32	Face powder	Egypt	-	Yellow – Based, F.P	02	-
P 33	Face Powder	China	323	Pink- Based F. Powder	5	-
P 34	Face Powder	China	-	Bronze coloring	3	-
P 35	Face Powder	China	-	Yellow – Based, F.P	1	155-DO
P 36	Face Powder	China	-	Yellow – Based, FP	01	-

P 37	Face Powder	China	-	Bronze coloring	2	-
P 38	Face powder	China	-	Pink- Based F. Powder	03	-
P 39	Face Powder	France	NA8700	Bronze coloring	-	112
P 40	Face Powder	Egypt	-	Yellow – Based, F.P	2	18j-DO
P 41	Face Powder	Egypt	-	Bronze coloring	-	983-5
P 42	Face Powder	China	-	Pink- Based F. Powder	-	155-DO
P 43	Face Powder	USA	ZX78	Yellow – Based, F.P	01	3602-08
P 44	Face Powder	USA	5501	Bronze coloring	01	483-38
P 45	Face Powder	China	-	Yellow – Based, F.P	01	155-JA
P 46	Face Powder	France	-	Bronze coloring	01	932-GI
P 47	Face Powder	China	B185	Pink- Based F. Powder	2	A8218
P 48	Face Powder	France	-	Copper Tinted F.P	-	814906
P 49	Face Powder	USA	-	Pink- Based F. Powder	-	3602-08
P 50	Face Powder	France	ZV114	Yellow – Based, F.P	-	222-uy
LC 51	Whitening Cream	Local/K.N	-	White	-	-
LC 52	Whitening Cream	Local/K.N	-	Off -White	1	-
LC 53	Whitening Cream	Local/K.N		White		
LC 54	Whitening Cream	Local/OM	-	White	2	-
LC 55	Whitening Cream	Local/OM	-	Off -White	03	-
LC 56	Whitening Cream	Local/OM	-	White	-	-
LC 57	Whitening Cream	Local/OM	-	White	2	-
LC 58	Whitening	Local/K.N	-	Off -White	-	-

	Cream					
LC 59	Whitening Cream	Local/OM	-	White	-	-
LC 60	Whitening Cream(local)	Local/K.N	-	White	01	-
LC 61	Whitening Cream(local)	Local/KH	-	White	01	-
LC 62	Whitening Cream(local)	Local/KH	-	Off -White	01	-
LC 63	Whitening Cream(local)	Local/KH	-	White	01	-
LC 64	Whitening Cream (local)	Local/KH	-	Off -White	2	-
LC 65	Whitening Cream (local)	Local/KH	-	Off -White	-	-
C 66	Whitening Cream	India	-7702-MK	Off -White	-	PL/8510
C 67	Whitening Cream	France	-	Off -White	-	222
C 68	Whitening Cream	UAE Dubai	-	White	-	155-JA
C 69	Whitening Cream	UAE Dubai	512	White	1	983-5
C 70	Whitening Cream	China	AV-214	White	2	423A-38
C 71	Whitening Cream	China	09	Off -White	03	-
C 72	Whitening Cream	France	154 LO	Off -White	-	483-30
C 73	Whitening Cream	UAE Dubai	561	Off -White	22	-
C 74	Whitening Cream	Egypt	443	White	-	-
C 75	Whitening Cream(**F&L**)	Egypt	-	Off -White	-	155-Do
C 76	Whitening Cream(**F&L**)	Egypt	-	Off -White	01	3602-08
C 77	Whitening Cream(**F&L**)	Thailand	218Z	White	01	483-38
C 78	Whitening Cream(**F&L**)	China	4521/60	Off -White	01	-
C 79	Whitening Cream(**F&L**)	Thailand	92-Y	White	01	932-GI

C 80	Whitening Cream (**F&L**)	China	-	Off -White	2	A8218
H 81	Hair Dye	France	143R	Black	-	F620706
H 82	Hair Dye	India	016	Dark Brown	-	-
H 83	Hair Dye	France	-	Black	-	222
H 84	Hair Dye	China	12	Black	-	155-JA
H 85	Hair Dye	China	OV540	Black	1	983-5
LH 86	Hair Dye	Local/OM	-	Brown	-	-
LH 87	Hair Dye	Local/OM	-	Black	-	-
LH 88	Hair Dye	Local/KH	-	Black	-	-
LH 89	Hair Dye	Local/KH	-	Dark Brown	-	-
LH 90	Hair Dye	Local/KH	-	Black	-	-
ES 91	Eye Shadow	UAE – Dubai	154 MK	Golden	-	425-J
ES 92	Eye Shadow	India	-	Blue	-	-
ES 93	Eye Shadow	China	-	Light Green	-	3351-OC
ES 94	Eye Shadow	Italy	176-H	Dark Brown	-	-
ES 95	Eye Shadow	Egypt	-	Dark Blue	-	-
ES 96	Eye Shadow	Thailand	85-J	Dark Brown		1010E

Where :- **L**, lipstick – **LL**, local lipstick – **P**, powder – **LC**, local preparation cream – **C**, imported cream, for fare &lovely (**F&L**) – **H,** hair dye – **LH**, local hair dye – **ES**, eye shadow, and all local samples indicated with colors. Local samples showed in colors. {**K.N**; Khartoum North, **OM**; Omdurman, **KH**; Khartoum}.

4.5 Methods

4.5.1 Sample Preparation

The following conditions were required for pretreatment of sample:

1- All analytical solutions were stabilized by the addition of nitric acid (5% v/v) and hydrochloric acid (5% v/v), as dilute mercury solutions, tend to be unstable.

2- Acid digestion was used, if digestion was necessary, simple dry ashing is not recommended as the mercury will be lost.

3- Sample was weighed in teflon vessels.

4- 10cm^3 conc. HNO3 were added, if sample was organic.

5- 10cm^{3}of aqua regia were added if sample was inorganic or mixture of organic and inorganic.

6- The sample solution was digested in programmed microwave.

7- The digested solution was diluted to a suitable volume (25cm^3) with distilled deionized water.

8- Reagent blank was prepared same as sample preparation without adding sample.

9- Digestion should be safe, reproducible and simple, that it can be performed without excessive manual effort. Also the process time is very short and with economic significance, *(Al- Dayel 2011).*

Sample or required number of sub samples was homogenized using appropriate laboratory grinders/mills. Analytical portion (0.4 - 5.0 g) of sample was taken. Generally, use the equivalent of about 0.5 dry materials was used. The maximum mass would depend on the specific microwave digestion vessel. For most cosmetic samples this would be between 0.5 and 2.0 g less than the maximum mass should be used for samples of high salt content to avoid matrix interference. A maximum analytical portion of 5 g should not be exceeded to avoid excessive dilution of the nitric acid. Use one gram reagent water for method blanks (MBKs) was used. Adding 1 g of reagent water first could help control exothermic reactions during the acid addition and digestion for dry samples and dry reference materials. Eight cm^3 (11.3 g) HNO3 were added. Acid was added drop -wise for the first few cm^3 until it could be established that the sample would not react violently. Always acid was added as a drop- wise to dry, finely ground samples until it could be sure that the reaction would not proceed too rapidly. If foaming or reaction with the acid is observed, the vessels were let to sit uncovered in a class 100 clean hood for 20 minutes or until reaction subsided. If a clean hood was unavailable, place caps were placed on vessels without pressing down fully or, so equipped, cap vessels but loosen the pressure relief nut was loosen (with the safety membrane) to allow pressure to escape,*(Larsen1994)*. Teflon® FEP wash bottle was used to add 1 cm^3 high purity H_2O_2. The vessel was caped and placed in microwave system and digested. The power was ramped for 15 minutes until 200 °C was reached. This temperature was maintained for 15 minutes, cooled to < 50 °C and the carousel was removed from oven. Its contents were diluted to approximately 100 cm^3 with reagent water double distilled and 1 cm^3(1.2 g) HCl was added. More reagent water was added for a final volume of 200 cm^3. Gravimetric dilution was recommended into an acid washed 250 cm^3 polyethylene bottle or other suitable plastic container. Mass of 200 cm^3 2% HNO3 – 0.5% HCl solution was approximately 202 g. Acid concentration in sample digests will be approximately 2% HNO3 and 0.5% HCl. The HCl would help to stabilize Hg. Glass should be avoided for dilution or storage of solutions because of possible contamination. Total dilution factor would be approximately 100 to 400 depending on the analytical portion. This dilution was used mainly to eliminate matrix interference. Dilution factor of less than this was not recommended unless it can be demonstrated that matrix interference had been adequately compensated for. CRM included at least once for each analyte with each batch. Fortification is required so that the resulting analytical solution would contain an additional 0.2 - 0.5 ng/cm^3 Hg and 0.5 - 1.0 ng/cm^3 As, Cd, Ni, Pb and Zn, *(US.EPA 1987).*

4.5.2 Instrument calibration

4.5.2.1 Linear regression analysis

4.5.2.1.1 A calibration blank

Standards covering the range of interest was immediately analyzed after the calibration curve set. The response of the blank was similar to that observed when initially analyzed.

4.5.2.1.2 Using instrument software, linear regression analysis was performed, by plotting response vs. concentration in µg/dm^3 and slope (m), intercept (b), and correlation coefficient of the calibration curve were determined. Correlation coefficient had to be $\geq$0.995, Figures (4.3), (4.4), (4.5), (4.6), (4.7) and (4.8) shows the calibration curves of Cd, Ni, Zn, Hg, As, and Pb respectively.

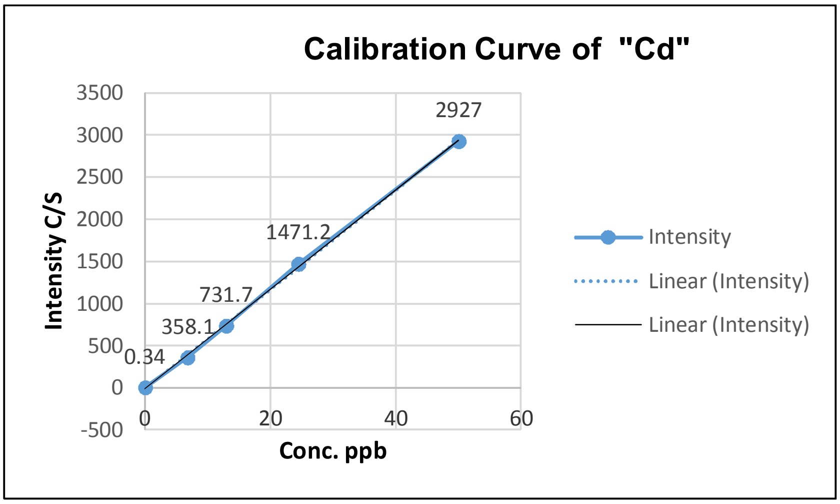

Figure 4.3 Calibration curve of Cadmium measured at 214.439 nm ($R^2 = 0.9998$)

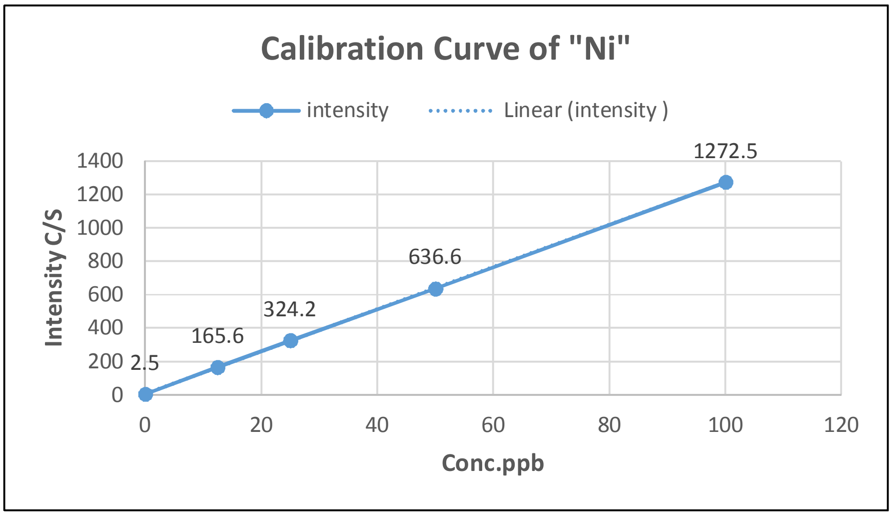

Figure 4.4 Calibration curve of Nickel measured at 231.604 nm ($R^2 = 0.9999$)

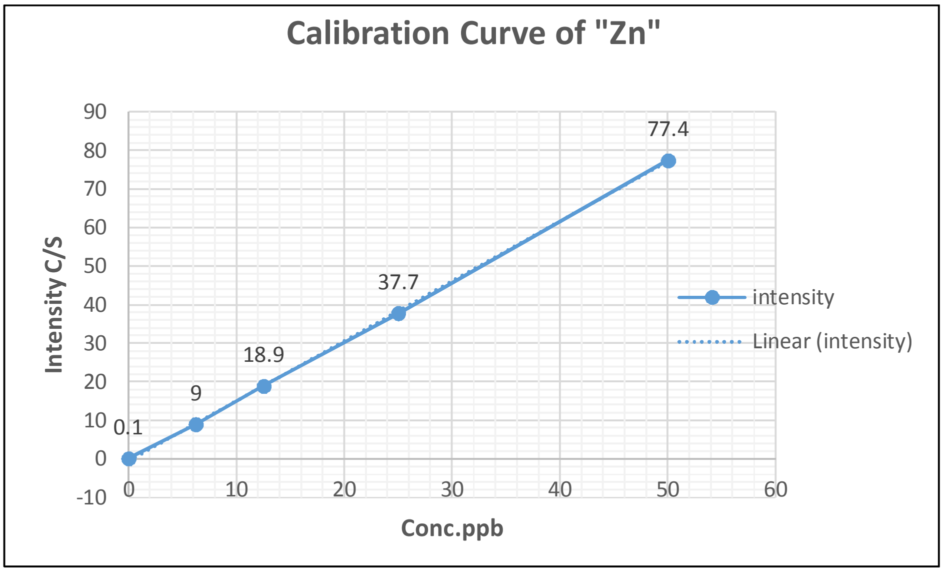

Figure 4.5 Calibration curve of Zink measured at 206.920 nm ($R^2 = 0.9996$).

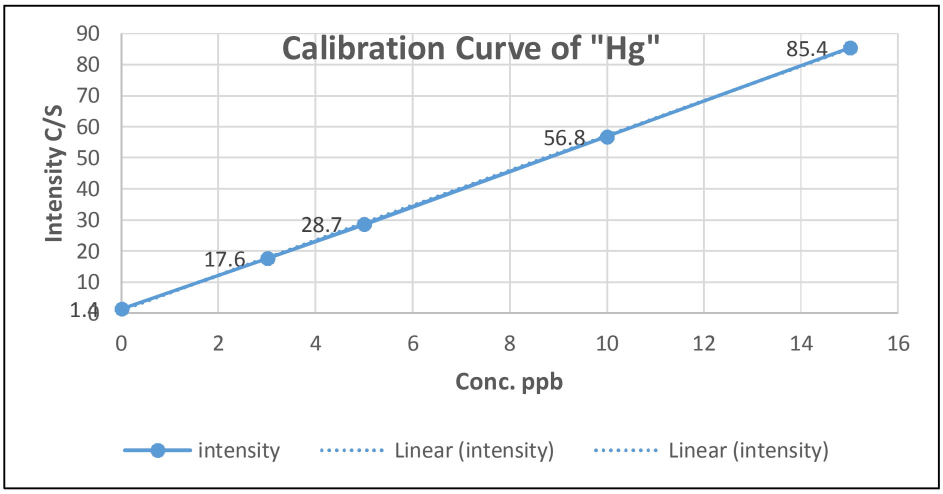

Figure 4.6 Calibration curve of Mercury measured at 194.164 nm ($R^2 = 0.9998$).

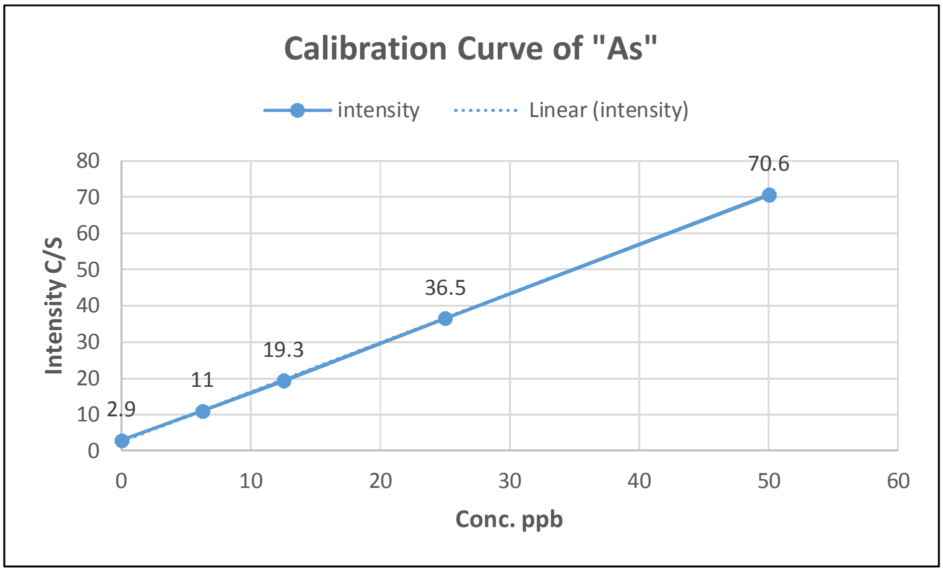

Figure 4.7 Calibration curve of Arsenic measured at 188.980 nm ($R^2 = 0.9997$).

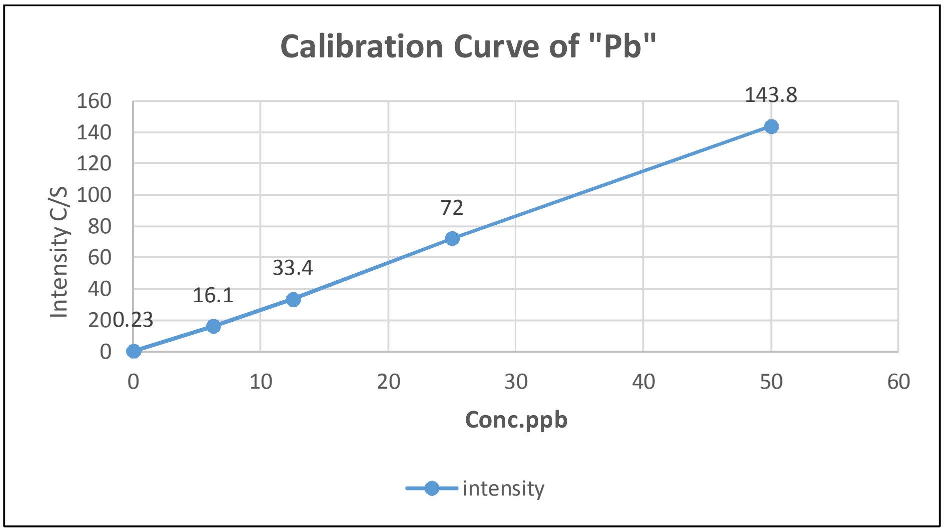

Figure 4.8 Calibration curve of Lead measured at 220.353 nm ($R^2 = 0.9995$).

The coefficient of determination, denoted (R^2) is a number that indicates how well data fit a statistical model – sometimes simply a line or a curve. An R^2 of 1 indicates that the regression line perfectly fits the data, while an R^2 of 0 indicates that the line does not fit the data at all. This latter can be because the data is utterly non-linear, or because it is random.

4.5.2.1.3 Instrument (calibration) check standard was immediately analyzed after the calibration blank. The calculated concentrations of parameters in the calibration check standard had to be within ± 10% of their accepted value. If these conditions were not met, the calibration sequence had to be repeated until results were acceptable.

4.5.3 Measurements Once instrument met calibration requirements, test samples were analyzed, taking care to meet conditions listed below;

4.5.3.1 Calibration check standard had to be included in the sample analysis sequence after at least every 15 consecutive samples analyzed.

4.5.3.2 Quality control sample also had to be analyzed at the end of the sample sequence to verify instrument performance, (Appendix I).

4.5.3.3 Response if any sample exceeded highest standard in the calibration curve, an appropriate dilution was made in 2% HNO_3 and then re-analyzed.

The following operations were Performed after preparing of the samples: The cone clearance, torch, nebulizer, spray chamber were cleaned. Once heating of the plasma was completed, the inlet pipe of the internal standard (ISTD) was connected, and the volumetric flask containing the internal standard solution was positioned in such a way that the other end of the pipe was immersed in it. The method was loaded to implement the desired method; then the sequence that would be run in the following manner (including two or three blanks between each sample) was created.

4.6 Method validation

The objective of method validation was to demonstrate that the method is suitable for its intended purpose as it is stated in ICH guidelines, (*ICH)*

The method was validated for *linearity*, *precision* (*repeatability*, inter-day and intra-day *precision*), *accuracy*, *specificity* and *robustness*.

4.6.1 Accuracy was assessed by measuring recovery at three different levels.
4.6.2 Precision was assessed by measurement of inter and intraday precision. In the intraday study the concentrations of all the drugs were calculated six times on the same day at different time intervals. In the inter day study the concentration of the drugs were calculated on six different days, *(Eurachem 1998*), (Appendix I).

4.6.3 Specificity of the method was assessed by injecting solutions containing all the drugs; after chromatography three sharp peaks were obtained for all drugs.

4.6.4 LOD and LOQ were measured to evaluate the detection and quantization limits of the method and to determine whether these were affected by the presence of impurities. They were calculated by using equations LOD = 3.3 × SD/slop of calibration curve and LOQ = 10 × SD/slop of calibration curve, (*Funk 1995***).**

Then it is necessary to prepare a solution of As, Cd, Hg, Pb and Ni &Zn at the concentration estimated by the calculation. The background noise N is measured by an injection of blank.

4.6.5 Results of estimation One blank was prepared and injected 10 times

The results are shown below:

Table 4.2 – Final estimation of the LOD of As, Cd, Hg, Pb, Ni and Zn

<table>
<tr><th colspan="7">Results of the estimation of the LOD: Concentration (µg/dm^3)</th></tr>
<tr><th>Elements</th><th>As</th><th>Cd</th><th>Hg</th><th>Pb</th><th>Ni</th><th>Zn</th></tr>
<tr><td>LOD</td><td>0.0828</td><td>0.0379</td><td>0.011</td><td>0.0062</td><td>0.0181</td><td>0.0587</td></tr>
</table>

Moreover, in method validation the process used to confirm that the analytical procedure employed for a specific test is suitable for its intended use. Results from method validation can be used to judge the quality, reliability and consistency of analytical results; it is an integral part of any good analytical practice. It is the process of defining an analytical requirement, and confirms that the method under consideration has performance capabilities consistent with what the application requires.

4.6.6 Acceptance criteria; the signal-to-noise ratio SNR must be greater than 3 for the 10 successive injections and the RSD of the 10 injections must be less than 10%.

4.6.7 - Performance characteristics

The following validation data apply for Agilent 7700x ICP-MS

Table 4.3; indicates the method performance of ICP-MS for the following elements; “As” concentration 3.12 ppm at 188.98 nm, “Hg” conc.1.0 ppm at 194.164nm ,“Cd” conc. 3.125ppm at 226.502 nm, “Ni” conc. 6.25 ppm at 231.604 nm, “Pb” 3.125 ppm at 220.353 nm and “Zn” 0.3125 ppm at 206.200 nm. Use of equipment that is within specification, working correctly and adequately calibrated is fundamental to the method validation process, *(ISO/IEC 17025 2005),* **(**Appendix I).

Table (4-2), shows Results of the estimation of the LOD: Concentration (µg/dm^3) by analyzing ten replicates of the spiked cosmetic samples.

The percent recovery for each analyte corrected for background concentration measured in the unfortified sample was calculated together with the standard deviation and uncertainty**.**

0000Table 4.3 Method Performance for ICP- MS (two days readings)

Test Number	Date	As 188.98 (3.12 ppb)	Hg 194.164 (1 ppb)	Cd 226.502 (3.125 ppb)	Ni 231.604 (6.25 ppb)	Pb 220.35 (3.125 ppb)	Zn 206.200 (0.3125 ppm)
1	19-May-14	2.7086	0.1850	3.1920	6.4890	3.1500	0.3073
2	19-May-14	3.4702	0.7303	3.1643	6.2414	3.4500	0.3089
3	19-May-14	2.8604	1.2126	3.1567	6.5015	3.6689	0.3091
4	19-May-14	3.2222	1.1833	3.2327	6.6473	3.6875	0.3071
5	19-May-14	2.7272	1.0535	3.1752	6.2519	3.4942	0.3099
6	19-May-14	3.2818	1.2651	3.1812	6.4866	3.3720	0.3089
7	19-May-14	2.8918	1.0196	3.1688	6.3463	3.2254	0.3090
8	19-May-14	3.3244	0.7861	3.2000	6.4378	3.3981	0.3088
9	19-May-14	2.1888	1.0391	3.1553	6.6168	3.1200	0.3086
10	19-May-14	2.9133	0.9418	3.1783	6.3959	3.4972	0.3074
11	19-May-14	2.5601	0.6574	3.1933	6.3451	3.5021	0.3085
12	19-May-14	2.9109	0.9824	3.2508	6.2468	3.3649	0.3067
13	19-May-14	3.3219	0.8493	3.1461	6.5885	3.2200	0.3078
14	20-May-14	2.7035	0.9720	3.1832	6.4392	3.1408	0.3069
15	20-May-14	3.4368	0.8240	2.9842	6.8394	3.6952	0.2608
16	20-May-14	3.5673	1.0662	2.9830	6.8815	3.5839	0.2594
17	20-May-14	3.1926	0.9798	3.0016	6.8259	3.3756	0.2585
18	20-May-14	3.0677	1.0937	3.3909	7.0738	4.0459	0.2959
19	20-May-14	3.6632	0.9858	3.4062	6.7935	3.7959	0.2990
20	20-May-14	2.9942	1.2156	3.4064	6.6479	3.6814	0.2992
21	20-May-14	3.0786	1.4247	3.4052	7.1000	3.2300	0.3007
22	20-May-14	3.5587	1.1958	3.4552	7.3279	3.5738	0.3001
23	20-May-14	3.2383	1.0411	3.3983	7.2450	3.4785	0.3016
Mean		3.0818	0.9871	3.2178	6.6421	3.4675	0.2996
Stand div		0.36427369	0.251270366	0.136236808	0.31923368	0.233007269	0.01636
Accuracy (Recovery)		98.61883826	98.71279565	102.9689043	106.274073	110.9584139	95.8617
Precision		0.118200116	0.254546904	0.042338781	0.048061947	0.067198443	0.05461
Uncertainty		0.72854738	0.502540731	0.272473616	0.638467359	0.466014538	0.03272

4.6.8 Determination of limit of detection

The limit of detection (LOD) is the smallest quantity of a substance to be examined in a sample that can be detected but not quantified as an exact value. The limit of detection is equivalent to 3 times the signal to noise ratio (SNR). The values obtained were shown in Table (4-4).

Table 4 -4 The limit of detection of toxic heavy metals, As, Hg, Ni, Zn, Pb, Cd – (Rb & In as internal standards).

Element	*I blank*	RSD_{blank}	SD_{blank}	I_{net}	LOD (ng/dm³)	LOD mg/kg	LMC (ng/dm³)	LMC mg/kg
As	34.28	25.92	8.88	16100.87	16.60	0.0083	165.62	**0.0828**
Hg	9.42	4.28	0.40	54582.57	0.22	0.0001	2.22	**0.0011**
Ni	25.42	11.12	2.82	23374.07	3.63	0.0010	36.29	**0.0181**
Zn	99.14	9.37	9.29	23722.02	11.80	0.0058	117.53	**0.0587**
Pb	0.85	141.42	1.21	29396.88	1.24	0.0006	12.36	**0.0062**
Cd	10.57	34.40	3.63	14359.92	7.60	0.0037	75.96	**0.0379**
Rh	26.28	21.52	5.65	101952.52	1.66	0.0008	16.64	**0.00832**
In	24.57	3.28	0.80	11036.72	2.20	0.0011	21.96	**0.01098**

Table (4.4) shows the lowest concentration that can be quantitatively determined with an acceptable level of repeatability. The quantification limit (LMC) is generally considered to be approximately ten times the minimum detection limit (LOD). The maximum measurement limit is conditioned by the dynamics of the spectrometer detectors.

4.7- Quality control procedures

The working calibration curve was verified before each analytical run as described before performing analysis of samples.

4.7.1 Proficiency test sample (PT), Certified Reference Material (CRM) were used or an in-house quality control sample was prepared either by spiking cosmetic or water samples with representative concentrations for each analyte. Proficiency testing determines the performance of individual laboratories for specific tests or measurements and is used to monitor laboratories' continuing performance, (*ISO Guide 1997).* Proficiency testing is also called inter - laboratory comparison. As this term implies, proficiency testing compares the measuring results obtained by different laboratories. In a proficiency test one or more artifacts are sent around between a number of participating laboratories. Each laboratory measures the artifacts (e.g. a ring gage or a set of gage blocks) according to a given set of instructions and reports its results to the administrator*, (ISO IEC 170252005).* The two most common ways are to use a reference laboratory or use the average of the values reported by the participants*, (ISO IEC 17025 2005).*Table (4-5) shows the validation data of heavy metals measured in DRC.

Table 4 -5 Validation Data of heavy metals (As, Cd, Hg, Pb, Ni & Zn).

Element	*Mass*	**Units**	**Assigned Conc.**	**Standard deviation**	*Recove-ry (%)*	*RSD %*	*Uncertainty(U) %*	*Detection Limit µg/dm³*
As	75	ppb	10	0.20	98.6	2.03	4.05	0.01
Cd	111	ppb	10	0.20	98.5	2.02	4.04	0.01
Hg*	201	ppb	191	7.43	97.3	3.89	7.78	0.01
Pb	207	ppb	50	0.38	100	0.76	1.52	0.001
Ni	59	ppb	10	0.31	98.3	1.13	6.26	0.001
Zn	66	ppb	10	0.08	99.7	0.78	1.56	0.01

measured in DRCe Mode** (Dual Response Choice Experiments***).

4.8 - Uncertainty of measurement

Spiked determinations of drinking water sample were performed and Standard deviation (SD) and Relative standard deviation (RSD) were calculated for the results. The measurement uncertainty at the 95% confidence level was calculated as follows:

$$U = k \times RSD$$

Where: U = uncertainty

k = coverage factor

(for 95% confidence a factor of 2 is used)

Table (4-6) shown the calculated expanded measurement Uncertainty for different elements.

4.8.1 Uncertainty evaluation and calculation

It is important that customers for analytical data release that analytical data are not exact; the above values indicate the extent of the uncertainty that could be expected. In practice, when considering a maximum value in legislation, the analyst will determine the analytical level and estimate the measurement uncertainty at that level, *(Stephanie 1999)*. The value obtained by subtracting the uncertainty from the reported concentration, is used to assess compliance.

Table 4-6 Values of the measurement uncertainty

Concentration	Expanded uncertainty	Range of acceptable concentrations*
100g/100g	4%	96 to 104g/100g
10g/100g	5%	5% 9.5 to 105g/100g
1g/100g	8%	92 to 108g/100g
1g/kg	11%	89 to 111g/kg
100mg/kg	16%	84 to 116mg/kg
10mg/kg	22%	7.8 to 12.2mg/kg
1mg/kg	32%	0.68 to 1.32mg/kg
< 100µg/kg	44%	56 to 144µg/kg

Best Estimate ± Uncertainty: Example; a measurement of **5.07 g** ± 0.02 g means that the experimenter is confident that the actual value for the quantity being measured lies between **5.05 g** and **5.09g**. The uncertainty is the experimenter's best estimate of how far an experimental quantity might be from the "true value*, (Stephanie 1999)*.

4.9- Results and Discussion

Principle, Organic samples were digested by wet digestion or dry digestion or high pressure microwave digestion, and the amount of heavy metals Pb, Hg, Cd, As, Ni, and Zn were determined by means of ICP-MS . The accuracy was best checked with certified standard reference materials. The comparison with data gained with the instrument currently used, might agree with the “true “results. At least one sample should contain a spike of the most important analytes. **(** *Lee 2008).*

Table (4-7) shows the concentration of heavy metals in imported lipstick samples collected from Sudanese market. The concentration of Pb, Cd and As ranged from 4.18 µg/g to 42.42 µg/g, 0.0 µg/g to 12.0 µg/g and from 0.0 µg/g to 10.21 µg/g respectively.

However, Table (4-8) shows the results of concentration of heavy metals in, five local samples of lipstick collected from Sudanese markets. The concentration of heavy metals, Pb, Hg, Ni and Zn ranged from 11.45 µg/g to 29.0 µg/g, 2.35 µg/g to 9.24 µg/g, 0.16 µg/g to 9.41 µg/g and 1.0 µg/g to 10.23 µg/g respectively.

Table (4-9) shows that the concentration of heavy metals, Pb and Zn in twenty five samples face powder, collected from Sudanese market were ranged from 0.05 µg/g to 20.02 µg/g and from 2.47 µg/g to 9.52 µg/g, respectively.

Table (4-10) shows the concentration of heavy metal impurities of six fair & lovely samples, and nine samples of local skin lightening cream, which were purchased from different shops in Khartoum State. The results were expressed as an average of replicate analysis. Concentration of Pb and Zn ranged from 2.94µg/g to 7.27 µg/g and 1.09 µg/g to 4.15 µg/g, respectively. Table (4-11) shows that the concentration of heavy metals in a different skin-lightening cream, fifteen samples were collected from Sudanese market. Concentration of Pb, Hg and Zn ranged from 0.09µg/g to 4.49µg/g, 0.08µg/g to 6.64 µg/g and1.19 µg/g to 10.35 µg/g respectively.

Table (4-12) shows that the concentration of heavy metals, Pb, Hg, Cd, Ni and Zn, in imported hair dye, five samples, ranged from 0.25 µg/g to 3.40µg/g, 0.12 to 4.04 µg/g, 1.16 µg/g to 4.24 µg/g, 1.88 µg/g to 4.00 µg/g, and from 1.05µg/g to 4.80 µg/g respectively.

The concentration of heavy metals Cd and Zn, in local hair dye five samples collected from Sudanese market, ranged from 0.00 µg/g to 7.35 µg/g and 2.80 µg/g to 14.30 µg/g respectively, indicated in (Table 4-13). Table (4-14) shows the concentration of heavy metals Pb, Hg, Cd, Ni and Zn, in eye Shadow six samples collected from Sudanese market, ranged from 17.22 µg/g to 52.02 µg/g, 8.10 µg/g to 21.30 µg/g, 10.52 µg/g to 22.53 µg/g, 12.25 µg/g to 73.94 µg/g and 9.9 µg/g to 72.46 µg/g respectively.

Table 4 -7 Concentration of heavy metals in imported lipstick samples collected from Sudanese market

Sample ID	Avg. conc. of Pb (µg/g	Avg. conc. of Hg (µg/g	Avg. conc. of Cd(µg/g)	Avg. conc. of As(µg/g)	Avg. conc. of Ni (µg/g	Avg. conc. of Zn(µg/g)
L1	**9.83**	**0.43**	**0.11**	**ND**	**ND**	**2.09**
L2	**12.53**	**ND**	**2.05**	**ND**	**0.95**	**1.32**
L3	**14.28**	**0.06**	**5.21**	**0.08**	**ND**	**2.17**
L4	**11.04**	**ND**	**0.74**	**ND**	**ND**	**1.04**
L5	**21.04**	**ND**	**0.69**	**ND**	**ND**	**5.06**
L6	**10.03**	**ND**	**1.82**	**ND**	**1.95**	**0.04**
L7	**16.93**	**0.22**	**1.77**	**5.14**	**ND**	**0.86**
L8	**8.47**	**1.33**	**2.11**	**4.40**	**3.16**	**ND**
L9	**42.42**	**0.25**	**0.43**	**2.71**	**ND**	**1.07**
L10	**12.34**	**0.61**	**ND**	**10.21**	**ND**	**2.35**
L11	**15.25**	**1.44**	**ND**	**5.01**	**ND**	**ND**
L12	**4.97**	**2.04**	**7.26**	**ND**	**0.20**	**0.69**
L13	**16.18**	**6.02**	**ND**	**3.00**	**ND**	**0.23**
L14	**22.10**	**ND**	**4.05**	**0.53**	**ND**	**0.84**
L15	**4.1 8**	**ND**	**12.00**	**0.08**	**ND**	**ND**
L16	**12.67**	**1.09**	**5.62**	**ND**	**ND**	**ND**
L17	**19.11**	**0.88**	**4.33**	**ND**	**ND**	**0.94**
L18	**11.75**	**ND**	**ND**	**ND**	**0.58**	**0.51**
L19	**6.08**	**ND**	**ND**	**ND**	**0.24**	**ND**
L20	**10.18**	**0.25**	**0.57**	**0.79**	**ND**	**ND**

*(where, **ND**, not detected, and **L**, for lipstick).*

Table 4-8 Concentration of heavy metals in local lipstick samples collected from Sudanese market

Sample ID	Avg. conc. of Pb (µg/g	Avg. conc. of Hg (µg/g	Avg. conc. of Cd(µg/g)	Avg. conc. of As(µg/g)	Avg. conc. of Ni (µg/g	Avg. conc. of Zn (µg/g)
L L21	23.29	3.07	4.02	ND	2.35	10.23
L L22	28.59	8.96	ND	ND	9.41	5.20
L L23	11.45	8.01	0.66	0.24	5.80	1.00
L L24	17.41	2.35	1.50	ND	0.59	3.84
L L25	29.00	9.24	6.43	ND	0.16	7.47

*(where, **ND**, not detected, and **LL**, local lipstick).*

Table 4-9 Concentration of heavy metals in face powder samples collected from local Sudanese market

Sample ID	Avg. conc. of Pb (µg/g	Avg. conc. of Hg (µg/g	Avg. conc. of Cd(µg/g)	Avg. conc. of As(µg/g)	Avg. conc. of Ni (µg/g	Avg. conc. of Zn (µg/g)
P26	2.15	1.73	0.52	ND	0.84	4.30
P27	0.04	0.30	1.50	ND	1.32	6.23
P28	16.20	ND	0.37	ND	0.42	2.47
P29	8.64	2.10	0.80	0.51	0.08	9.52
P30	3.25	ND	0.53	0.09	ND	5.81
P31	14.07	1.08	2.06	1.21	ND	7.04
P32	16.31	ND	0.73	ND	1.68	2.47
P33	8.12	ND	3.11	0.84	0.92	5.88
P34	2.48	1.06	0.64	ND	1.30	3.30
P35	6.07	0.94	0.39	ND	0.37	2.56
P36	3.37	ND	0.87	0.24	ND	7.11
P37	9.51	ND	1.25	ND	0.89	4.38
P38	0.73	0.05	ND	ND	1.24	6.01
P39	2.04	1.06	ND	1.07	0.51	2.67
P40	20.02	2.11	0.56	1.25	ND	7.81
P41	5.47	ND	0.43	0.06	ND	3.22
P42	6.13	ND	1.72	ND	2.50	6.30
P43	10.48	ND	0.84	ND	0.77	9.21
P44	8.09	ND	ND	ND	1.80	8.63
P45	1.55	0.77	ND	ND	0.08	5.00
P46	2.93	2.01	ND	ND	0.85	9.52
P47	0.05	ND	1.20	0.54	1.35	3.30
P48	7.15	ND	0.61	ND	0.87	2.61
P49	4.28	1.09	0.72	ND	0.99	6.82
P50	6.54	ND	1.46	ND	2.01	7.43

*(where, **ND** ; not detected, and **P**, face powder)*

Table 4- 10 Concentration of heavy metals in local skin-lightening cream samples (Preparation) collected from Sudanese market

Sample ID	Avg. conc. of Pb (μg/g)	Avg. conc. of Hg (μg/g	Avg. conc. of Cd (μg/g)	Avg. conc. of As (μg/g)	Avg. conc. of Ni (μg/g)	Avg. conc. of Zn (μg/g)
LC51	**28.90**	**14.62**	**7.06**	**0.04**	**ND**	**2.41**
LC52	**14.49**	**14.10**	**ND**	**0.19**	**2.27**	**11.04**
LC53	**33.63**	**20.08**	**ND**	**0.05**	**0.54**	**2.92**
LC54	**17.13**	**42.09**	**1.09**	**0.96**	**ND**	**6.86**
LC55	**19.30**	**24.50**	**0.22**	**ND**	**0.88**	**1.18**
LC56	**30.63**	**16.05**	**ND**	**ND**	**1.36**	**3.20**
LC57	**23.68**	**21.63**	**2.79**	**0.29**	**1.26**	**6.27**
LC58	**17.20**	**13.02**	**ND**	**1.02**	**2.01**	**8.21**
LC59	**28.36**	**13.98**	**2.66**	**ND**	**ND**	**6.01**
F&L 60	**3.10**	**2.93**	**0.06**	**0.01**	**1.82**	**3.76**
F&L 61	**2.71**	**4.01**	**0.08**	**ND**	**0.65**	**7.63**
F&L 62	**3.80**	**2.51**	**ND**	**ND**	**0.27**	**2.40**
F&L 63	**7.27**	**1.67**	**0.03**	**0.05**	**0.51**	**5.16**
F&L 64	**6.51**	**2.04**	**0.09**	**ND**	**1.44**	**1.19**
F&L 65	**2.94**	**3.98**	**0.61**	**0.03**	**2.04**	**4.91**

*(**where, ND** not, detected, **F&L** fare & lovely and **LC,** local skin- lightening creams).*

Table 4 – 11 Concentration of heavy metals in imported skin-lightening cream samples collected from Sudanese market

Sample ID	Avg. conc. of Pb(µg/g)	Avg. conc. of Hg(µg/g)	Avg. conc. of Cd(µg/g)	Avg. conc. of As(µg/g)	Avg. conc. of Ni (µg/g	Avg. conc. of Zn (µg/g)
C66	1.04	2.93	0.06	0.01	1.82	3.67
C67	0.53	4.01	0.08	ND	0.65	7.63
C68	0.09	2.51	ND	ND	0.27	2.40
C69	0.41	1.67	0.03	ND	0.51	5.16
C70	2.37	2.04	0.09	0.05	1.44	1.19
C71	0.34	3.98	0.61	ND	2.04	4.91
C72	2.73	2.86	0.14	0.03	1.12	4.14
C73	3.44	0.08	ND	0.15	ND	10.35
C74	0.65	2.21	ND	ND	3.09	4.40
C75	2.47	1.08	0.77	ND	2.23	3.11
C76	1.07	6.64	0.80	ND	0.91	6.20
C77	0.18	3.21	ND	ND	1.55	8.62
C78	4.49	1.84	ND	ND	8.21	1.36
C79	1.55	0.96	0.21	ND	7.35	6.24
C80	2.53	0.88	1.50	ND	7.12	4.58

*(**where, ND**, not detected, and **C**, for skin lightening creams)*

Table 4 -12 Concentration of heavy metals in imported hair dye samples collected from Sudanese market

Sample ID	Avg. conc. of Pb(µg/g)	Avg. conc.o Hg(µg/g)	Avg. conc. of Cd(µg/g)	Avg. conc. of As(µg/g)	Avg. conc. of Ni (µg/g)	Avg. conc. of Zn(µg/g)
H 81	**1.93**	**0.12**	**2.08**	**ND**	**ND**	**4.80**
H 82	**3.40**	**4.04**	**1.50**	**ND**	**2.10**	**2.23**
H 83	**0.25**	**0.90**	**4.24**	**0.15**	**1.88**	**3.01**
H 84	**2.19**	**ND**	**1.90**	**0.18**	**1.97**	**4.31**
H 85	**0.27**	**1.53**	**1.16**	**ND**	**4.00**	**1.05**

*(**where, ND** not detected, H ; hair dye)*

Table 4 - 13 Concentration of heavy metals in local hair dye samples collected from Sudanese market.

Sample ID	Avg. conc. of Pb(µg/g)	Avg. conc.o Hg(µg/g)	Avg. conc. of Cd(µg/g)	Avg. conc. of As(µg/g)	Avg. conc. of Ni (µg/g)	Avg. conc. of Zn(µg/g)
L H 86	**0.73**	**2.12**	**1.08**	**ND**	**ND**	**14.30**
L H 87	**1.43**	**1.48**	**ND**	**0.99**	**ND**	**2.80**
L H 88	**0.62**	**ND**	**7.35**	**0.05**	**2.81**	**3.23**
L H 89	**0.09**	**ND**	**1.97**	**0.78**	**3.00**	**4.56**
L H 90	**0.19**	**ND**	**1.15**	**1.94**	**4.52**	**9.51**

*(**where, (w(Where, ND** not detected, **LH**; local hair dye).*

Table 4 – 14 Concentration of heavy metals of different brands of eye shadow samples collected from Sudanese market.

Sample ID	Avg. conc. of Pb(µg/g)	Avg. conc.of Hg(µg/g)	Avg. conc. of Cd(µg/g)	Avg. conc. of As(µg/g)	Avg. conc. of Ni (µg/g)	Avg. conc. of Zn(µg/g
ES 91	24.48	12.73	10.52	0.07	73.94	14.30
ES 92	33.06	21.30	11.37	0.17	54.37	61.23
ES 93	40.07	19.21	18.72	ND	68.42	72.46
ES 94	17.22	8.10	22.53	ND	12.81	9.90
ES 95	52.02	9.74	12.13	ND	17.66	19.85
ES 96	29.95	11.12	10.56	ND	12.25	32.8

*(**where, ND**: not detected, **ES**; eye shadow).*

Moreover, many methods have been developed and validated for the detection and quantification of heavy metals including those in cosmetic products. Inductively Coupled Plasma- Mass Spectrometer, (ICP-MS) is one of these methods. It has many advantages being feasible, accurate and quick method that make a valuable tool for detection and quantification of various products including cosmetics.

Statistically, the number of samples included in such type of research should be represented by a large number of samples; however, this study was confined to 96 samples only due to the high expenses for the assay of a single sample.

CHAPTER FIVE

5- GENERAL CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

From the outcome of this research work it can be concluded that: Evaluation of data obtained from the questionnaire strongly indicates that Sudanese ladies are unaware of cosmetic products health risks. They do not follow the right procedures when purchasing them from local markets, storing them at home and applying cosmetic products. However, it is important to point out that toxic heavy metals can cause chronic toxicities as it can accumulate in the body. Therefore, cautious use of these preparations should be considered. It is strongly advised that females should refrain from sharing, borrowing makeup cosmetics to avoid spread of skin, hair, and eye bacterial and viral infections.

From the collected data it evident that it will lead to studies to determine the toxicological and microbiological hazards and developing of analytical methods for the identification and determination of harmful of ingredients and microbial contaminants.

Most of the cream samples, analyzed for hydroquinone, had concentrations more than the US Food and Drug. Administration's acceptable limit of

1µg/g, reaching a percentage of up to 5.75% of most of the samples.

The low concentrations of hydroquinone were detected in the imported cream samples. Therefore the percentage levels which were higher than the recommended WHO limit of two (2%) was only 23%. Hydroquinone concentration ranged from below detection to 5.75%. Twenty-three percent of the cream samples analyzed had hydroquinone concentration above the WHO threshold limit of 2 %. This is very alarming and consumers who apply any of these creams are at risk.

Almost all imported and locally prepared facial makeup cosmetic samples were found to be contaminated with heavy metals. Results of ICP –MS analysis showed that the concentration ranged from 2.70µg/g to 52.02 µg/g and from 1.09 µg/g to 72.46 µg/g for lead and zinc respectively, however, the concentration ranged from 11.04 µg/g to 22.53 µg/g and 73.94 µg/g for Cd, As, and Ni, respectively. Moreover, the concentration of Hg, determined by using ICP-MS technique as well, ranged from 0.00 µg/g to 22.53 µg/g.

5.2 Regulatory limits

In most countries, it is legally prohibited to use lead, arsenic, and mercury in skin cosmetic products. For example, lead is prohibited as part of cosmetic composition in Korea, the European Union, and China. The maximum allowable level of lead is 20 ppm in those jurisdictions. There are no specific rules on other heavy metals, such as cobalt, nickel, and copper .

Table 5-1 Recommendations levels for As, Cd, Hg and Pb as per (HBP/GS).

Metal	**Recommended highest level (mg/kg)**
Arsenic (As)	3
Cadmium (Cd)	3
Mercury (Hg)	1
Lead (Pb)	10 for ingested products 20 for products applied dermally

Health Canada's recommended impurity limits for cosmetic products are listed in Heavy metal impurity concentrations in cosmetic products are seen to be technically avoidable when they exceed the following limits:

- Lead: 10 ppm
- Arsenic: 3 ppm
- Cadmium: 3 ppm
- Mercury: 1 ppm
- Antimony: 5 ppm

Table 5-2 Summary of recommended highest level (pp)

Metal	Recommended highest level (mg/kg) ppm			
	EPA	**OSHA**	**FDA**	**WHO**
Arsenic (As)	1ppm	(10 µg/ m3) for 8 hour shifts and 40 hour work weeks.	-	3ppm
Cadmium (Cd)	5 ppm	(5 µg/ m3) for 8-hour workday, 40-hour work week	5ppm	3ppm
Mercury (Hg)		0.01 µg/ m3	1ppm	1ppm
Lead (Pb)	15ppb	-	10ppm	10ppm

5.3 - Recommendations

- Screening of other topical cosmetic preparations like body creams, lotions, kohl…etc. is recommended and it should be mandatory.
- The government should care about FDA, EPA, and WHO regulations and /or any other relevant regulation to cosmetics selling and distribution to prevent the sale of products with harmful ingredients that jeopardizes consumer health.
- Further, research should be encouraged to investigate the presence of other heavy metals (Cu, Fe, Sb and Cr), allergens, and other toxic materials in daily-use product.
- Analysis of heavy metal contents in any brand of cosmetics

should be one of the guidelines of regulation of the registration.

- Users should be aware about the accumulative behavior of heavy metals and other constituents so that they can take care in consuming cosmetics.

- Manufacturers should be encouraged to state the exact amount of bleaching agents in creams.
- All concerned authorities should be aware of all types of cosmetics prepared locally without license in small shops, mainly for selling clothes and perfumes and also even in some pharmacies.
- Regular strict inspection for behind the doors of local cosmetic preparation and for under the table of illegal cosmetic products trade, becomes necessary.
- Development of screening and field methods of analysis for toxic ingredients and bio-contamination should be attempted.
- Strict laws and rules for cosmetic products similar to those of drugs and poisons should be set.

CHAPTER SIX

6 –REFERENCES

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APPINDEXES

Appendix I

ARSINIC Measured at 188.98 nm

Date	Elements	As (2 ppb)	As (3ppb)	As (4ppb)	As (5ppb)	As (6ppb)	As (7ppb)	As (7ppb)
15/04/2014	1	1.79	2.3465	4.0300	4.7981	5.9000	6.6000	6.6000
15/04/2014	2	1.93	3.3586	4.0353	5.1863	5.6500	7.2000	7.2000
15/04/2014	3	1.626	3.1083	3.2889	5.6868	5.6800	7.0000	7.0000
22/04/2014	4	1.9	3.2000	4.0300	5.0200	5.7000	6.6500	6.6500
22/04/2014	5	1.83	3.2700	3.7860	4.8000	6.0700	6.9700	6.9700
22/04/2014	6	1.99	2.9400	4.0400	4.5000	6.3000	6.8700	6.8700
22/04/2014	7	1.96	3.1500	3.3200	4.9000			
06/05/2014	8	1.52	2.9860	3.8000	5.0000			
06/05/2014	9	1.4	3.1100	4.0100	4.6000			
06/05/2014	10	1.2	2.8750	3.5000	4.5000		6.6000	6.6000
Mean		1.7146	3.0344	3.7840	4.8991	5.8833	6.8414	6.8414
Accuracy(Recovery%)		85.730	101.148	94.601	97.983	98.056	97.735	97.735
Standard Deviation (SD)		0.268	0.283	0.306	0.358	0.260	0.232	0.232
MDL (SD*t. Student)		0.803	0.850	0.918	1.075	0.780	0.697	0.697
LOQ (10*SD)		2.678	2.833	3.059	3.584	2.601	2.325	2.325
LOQ (6*SD)		1.607	1.700	1.835	2.150	1.560	1.395	1.395
LOQ (5*SD)		1.339	1.417	1.529	1.792	1.300	1.162	1.162

Cadmium measured at (214.439nm)

Date	Elements	Cd (1 ppb)	Cd (2 ppb)	Cd (3 ppb)	Cd (4 ppb)	Cd (5 ppb)
14/5 &22/4	1	0.9695	1.8800	2.9802	4.0040	5.1433
14/5 &22/4	2	1.0158	1.8990	2.9740	4.0422	5.1084
14/5 &22/4	3	0.9861	1.9000	3.0437	4.0446	5.1452
14/5 &22/4	4	1.0069	1.9400	2.9000	4.0700	5.2000
14/5 &22/4	5	1.0129	1.8700	2.9400	4.2000	5.2000
14/5 &22/4	6	1.0058	1.7600	2.9500	4.0700	5.1000
5/6/2014& 14/5	7	0.975038	1.7070	2.9600		
5/6/2014& 14/5	8	0.963325	1.7680	3.2000		
5/6/2014& 14/5	9	0.99954	1.7860	3.1000		
5/6/2014& 14/5	10	0.975038	1.8040	3.1700		
Mean		0.9910	1.8314	3.0218	4.0718	5.1495
Accuracy(Recovery%)		99.100	91.570	100.726	101.795	102.989
Standard Deviation (SD)		0.019	0.076	0.103	0.067	0.043
MDL (SD*t. Student)		0.058	0.229	0.308	0.202	0.129
LOQ (10*SD)		0.194	0.762	1.027	0.673	0.431
LOQ (6*SD)		0.117	0.457	0.616	0.404	0.259

Nickel measured at (231.604 nm)

Date	Elements	Ni (2 ppb)	Ni (3 ppb)	Ni (4 ppb)	Ni (5 ppb)	Ni (6 ppb)	Ni (7 ppb)
09/04/2014	1	1.2	2.7094	4.1018	4.8911	5.8400	7.0700
09/04/2014	2	1.14	2.8022	3.8225	4.7444	6.1000	7.0800
09/04/2014	3	1.06	2.8875	3.8099	4.7991	5.8000	7.0700
22/04/2014	4	1.168	2.6000	3.7000	4.7400	5.5000	6.5000
22/04/2014	5	1.24	2.7000	3.8000	4.6000	5.4000	6.5300
22/04/2014	6	1.04	2.6300	3.6000	4.8000	5.4000	6.6700
24/04/2014	7	2.58127	2.7400	3.7300	4.7000	5.6000	6.6700
24/04/2014	8	2.64774	2.5100	4.9846			
24/04/2014	9	2.59881	2.6290	5.1241			
24/04/2014	10	2.41103	2.6260	4.9000			
Mean		1.7087	2.6834	4.1573	4.7535	5.6629	6.7986
Accuracy(Recovery%		85.434	89.447	103.932	95.071	94.381	97.122
Standard Deviation (SD)		0.737	0.109	0.600	0.091	0.261	0.265
MDL (SD*t. Student)		2.212	0.326	1.799	0.274	0.784	0.795
LOQ (10*SD)		7.372	1.086	5.997	0.912	2.614	2.649
LOQ (6*SD)		4.423	0.651	3.598	0.547	1.568	1.589
LOQ (5*SD)		3.686	0.543	2.998	0.456	1.307	1.324

Lead measured at (220.353 nm)

Date	Elements		Pb (2 ppb)	Pb (3 ppb)	Pb (4 ppb)	Pb (5 ppb)
15/04/2014	1		1.508	2.6600	4.0018	5.0156
15/04/2014	2		1.426	2.7000	3.9791	4.4777
15/04/2014	3		1.12	2.7815	3.8417	5.3000
22/04/2014	4		1.19	2.8000	3.9000	4.7000
22/04/2014	5		1.487	2.8000	3.9500	4.7000
22/04/2014	6		1.74	2.8000	3.8499	5.0100
06/05/2014	7		1.615	3.1000	3.9443	4.8300
06/05/2014	8		1.588	2.9900	4.2419	4.2559
06/05/2014	9		1.0269	3.2000	4.0718	4.5228
06/05/2014	10		1.0715	2.9900	4.2556	4.1617
Mean			1.3772	2.8822	4.0036	4.6974
Accuracy(Recovery%)			68.862	96.072	100.090	93.947
Standard Deviation (SD)			0.254	0.178	0.146	0.356
MDL (SD*t.Student)			0.763	0.534	0.439	1.068
LOQ (10*SD)			2.542	1.778	1.462	3.560
LOQ (6*SD)			1.525	1.067	0.877	2.136
LOQ (5*SD)			1.271	0.889	0.731	1.780

Mercury

measured at (194.164 nm)

Date	Elements	Hg (0.1 ppb)	Hg (0.4ppb)	Hg (0.5 ppb)	
15-Apr-14	1		0.6637	0.5300	
15-Apr-14	2		0.6691	0.5200	
15-Apr-14	3		0.7031	0.5100	
22-Apr-14	4		0.3849	0.4700	
22-Apr-14	5		0.3687	0.5300	
22-Apr-14	6		0.1499	0.4200	
22-Apr-14	7		0.1325	0.5000	
29/04/2014	8		0.2170	0.5630	
29/04/2014	9		0.3337	0.4000	
29/05/2014	10		0.3723	0.4600	
Mean			0.3995	0.4903	
Accuracy(Recovery%)			99.871	98.060	
Standard Deviation (SD)			0.213	0.052	
MDL (SD*t.Student)			0.639	0.156	
LOQ (10*SD)			2.129	0.519	
LOQ (6*SD)			1.277	0.311	
LOQ (5*SD)			1.064	0.259	

Zinc measured at (206.200 nm)

Date	Elements	Zn (3 ppb)	Zn (4 ppb)	Zn (5 ppb)	Zn (6 ppb)	Zn (7 ppb)	Zn (8 ppb)
11/5 &12/5/2014	1	1.7000	2.8000	4.7000	5.4000	6.5063	
11/5 &12/5/2014	2	1.8000	2.7000	4.6000	5.2000	6.5786	6.7081
11/5 &12/5/2014	3	1.8000	2.9000	4.7400	5.6000	6.6211	6.5489
11/5 &12/5/2014	4		5.3625	5.4000	6.0100	6.5063	7.6996
11/5 &12/5/2014	5		5.3603	5.2000	6.2000	6.5786	7.7277
11/5 &12/5/2014	6		5.3613	4.2000	6.0600	6.6211	7.6015
11/5 &12/5/2014	7		5.3227	4.1000	6.5500	8.3000	
11/5 &12/5/2014	8		6.0000	4.5000	6.5600	8.2700	
11/5 &12/5/2014	9		6.8000	4.9000	6.5900	8.2000	
8/5 &12/52014	10		6.7000	4.7000	6.6400	8.3000	
Mean		1.7667	4.9307	4.7040	6.0810	7.2482	7.2572
Accuracy(Recovery%)		58.889	123.267	94.080	101.350	103.546	90.714
Standard Deviation (SD)		0.058	1.568	0.400	0.528	0.879	0.579
MDL (SD*t.Student)		0.173	4.703	1.201	1.585	2.636	1.736
LOQ (10*SD)		0.577	15.677	4.002	5.283	8.786	5.786
LOQ (6*SD)		0.346	9.406	2.401	3.170	5.271	3.471
LOQ (5*SD)		0.289	7.838	2.001	2.641	4.393	2.893

Appendix II

Questionnaire of Cosmetics Survey
For Ph.D research work

Age	**Status**	Comments
Gender	M. ☐ F. ☐	
Student	Level : ☐	
Other	Employee: ☐ House Wife ☐	
Q / A	**Question**	**Remarks**
Q-1	Do you think the use of cosmetics can change the general appearance of a person?	
A-1	Definitely ☐ May be ☐ Not at all ☐ Don't know ☐	
Q-2	What do you prefer ?	
A-2	Local preparations ☐ Imported ready made ☐	
Q-3	Whom do you prefer for beauty advice ?	

A-3	Beautician ☐ Friends ☐ Magazines ☐ Dermatologist ☐	
Q-4	Who among the following influences you the most in selecting cosmetics ?	
A-4	Friends ☐ Singer ☐ Cinema actors ☐	
Q-5	Do you think that the use of cosmetics increase your self – confidence ?	
A-5	Yes ☐ No ☐	
Q-6	Have you ever developed allergy to any cosmetic products ?	
A-6	Yes ☐ No ☐	
Q- 7	If yes it is ,	
A-7	Rash ☐ Itching ☐ Pigmentation ☐ Others ☐	
Q- 8	How much money do you spend per month?	
A-8		
A-9	Weekly ☐ Twice a month ☐ Once a month ☐ Not at all ☐	

Q-10	Which among the following do you use regularly ?	
A-11	Eyeliner ☐ Powder ☐ Foundation ☐ Lip stick ☐ Sunscreen ☐ Shampoo ☐ Hair conditioner ☐ Hair Gel ☐ Hair Dyes ☐ Nail Polish ☐ Depilators ☐ Perfumes ☐ Deodorants ☐	

استبيان بغرض البحث العلمي

- الأخت الكريمة نقوم بدراسة هدفها تحديد كمية العناصر الثقيلة و مادة الهيدروكينون المحتمل وجودها في بعض مواد التجميل التي تستخدمها النساء .

- وذلك بغرض البحث العلمي فقط لذا نرجو تعاونك معنا بالإجابة علي جميع الأسئلة بصدق ونعدكم بعدم نشر الأسماء (أي السرية التامة لهذه المعلومات).

أ- العمر ☐

ب-الحالة الاجتماعية:

1- عازبه ☐ 2– متزوجة ☐ 3- مطلقة ☐

4- أرملة ☐

ج- السنة الدراسية:

1- الأولي ☐ 2- الثانية ☐ 3- الثالثة ☐

4- الرابعة ☐ 5- الخامسة ☐ 6- موظفة ☐

د-المنحدر (الأصل) : -

1- هل انتي من المدينة ☐ 2- أم من الريف ☐

هـ - هل تستخدمين مواد التجميل ؟؟

1- نعم ☐ 2- لا ☐

و- أي مواد التجميل الآتية تستعملين ؟(يمكنك اعطاء أكثر من إجابة) :

1- الروج ☐ 2- بدره الوجه ☐

3- كريم تفتيح البشرة ☐ (مستورد – محلى) ☐

4- صبغة الشعر ☐ (مستورد – محلى) ☐

5- مظلل العيون ☐ (مستورد – محلى) ☐

ز- ما الذي يدفعك لاستعمالنوع معين من مواد التجميل السابقة ؟

1- هل رواجهافي المجتمع ☐ 2- هل بعد إستشاره خبيره تجميل ☐

3- هل سعره أرخص ☐ 4- أي أسباب أخري ☐

ع -هل حاولت معرفة مكونات مواد التجميل قبل إستعمالها لضمان سلامتك :

1- نعم ☐ 2- لا ☐

أولا : **الأرواج إذا كان إستعمالك يوميا فكم عدد المرات؟**

ب- أي الأنواع الآتية تستعملين؟

1- Golden Rose 2-Moisterising lipsticks

2- Luoys 4-Cmyk

5-AQUA Platinum

7- Baby Fact 8-VOV

9-Oriflame 10-Dov

11-Doez New 12-Lid Anxu

13-Sweet Heart 14-Black Opal

15- Xxl 16-Esabel

18-روجسحرى 19- روج فيينا

20– Local Preparations(From where you purchase ??) .

ج-إذا كنت تستعملين نوعا آخر ما هو

د- ما هو لون / ألوان الروج المفضلة لديك

هـ- هل شكوت من أي أعراض عن إستعمالك للروج

و- هل غيرت من إستعمال نوع لآخر وما السبب

ثانيا :بدره الوجه: أ-هل تستعملينها يوميا

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كم مره في اليوم............................

ب - أي الأنواع الآتية تستعملين:-

1- Dove 2- Vitamin C	☐	☐	
3- LG 4- Mac	☐	☐	
5- POP 6- Seven girl	☐		☐
7- MF-8-Chenal	☐		☐
9-Julander10-Rose leaf	☐	☐	

11- Global	☐	**-Sweet heart**	☐	
13- Montana	☐			☐
15- Mulndo16- X	☐		☐	

17- L,oreal18- Fashion lady	☐		☐
19- ADS	☐	**20 - Others**	☐

ج- إذا كنت تستعملين نوعا آخر ☐ ما هو ☐

د-هل شكوت من أي أعراض عند إستعمالك لبدره الوجه - ☐ وما هي ☐

هـ- هل غيرت من إستعمال نوع لآخر - ☐ ما السبب ☐

أ- إذا كنت تستعملينه يوميا فكم مره في اليوم ☐

ب- أي الأنواع الآتية تستعملين؟

1- Sweet Heart	☐	2- Dove	☐
3- Vitamin c4- Fair	☐		☐
5- SevenGirl	☐	6-NideFound	☐
7- Mac- On8- Lancome	☐		☐
9- Fair and lady	☐	10- 3XL	☐
11- Ads 12- -Jadore	☐		☐
13- Global	☐		☐

ج- إذاكنتت ستعملين نوعا آخر ماهو

د- هل شكوت من أي أعراض عندإستعمالك لأى كريم ☐ وماهو

ه- هل غيرت من إستعمال أي نوع لآخر ماالسبب

1- لم أغير ☐ **2** - نعم غيرت ☐

ما السبب ☐

هل توصين بشيءله علاقة باختيارموادالتجميل أوإستعمالها

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ختاما نشكر لك تعاونك معنا وجزاك الله خيرا