REVIEW ON ESTIMATION OF FRAGRANCE ALLERGENS AND HEAVY METALS IN COSMETICS BY VARIOUS ANALYTICAL METHODS

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***Abstract*- The fragrance substances are naturally or synthetically derived organic compounds which are present in cosmetics. Heavy metals are used as Preservative in many cosmetic products. Presence of these fragrance allergens and heavy metals in cosmetics can cause many skin reactions. The present review focuses on the recent developments in different analytical methods for estimation of Fragrance allergens and heavy metals in cosmetics. This review was critically examine the separation methods such as Reverse phase high performance liquid chromatography (RP-HPLC), Micro emulsion electrokinetic chromatography (MEEKC), Size exclusion chromatography (SEC), Matrix solid phase dispersion (MSPD), Solid phase microextraction (SPME), Flame atomic absorption spectroscopy (FAAS), Inductively coupled plasma – mass spectroscopy (ICP-MS), Flow injection mercury system (FIMS), Direct mercury analyzer (DMA), Gas chromatography and mass spectroscopy(GC-MS). This review has many applications in different areas like academics, industries, etc.**

***Keywords*- Fragrance allergens, Heavy metals, Analytical methods, RP-HPLC, MEEKC, SEC, MSPD, SPME, FAAS, ICP-MS, FIMS, DMA, GC-MS.**

I.INTRODUCTION

❖ ***FRAGRANCE ALLERGENS***

Cosmetic is defined as "Any article intended to be rubbed, poured, sprinkled or sprayed on, or introduced into, or otherwise applied to, the human body or any part thereof for cleansing, beautifying, promoting attractiveness, or altering the appearance, and includes any article intended for use as a component of cosmetic".

Fragrance substances are naturally or synthetically derived organic compounds with a characteristic, usually pleasant odours. Cosmetic products are regulated for health and safety. There are concerns regarding the presence of harmful chemicals, including heavy metals and fragrances in these products.

Cosmetic products (such as soaps, lotions, face and eye make-up, perfumes, etc.) can provoke allergic reactions in some people. Fragrances are the most common chemicals in cosmetics to which people expose every day. Many people suffer from allergies and anyone at any age can develop allergies. Allergic reactions are the immune system's overreaction to substances that may otherwise be harmless. An allergen can trigger the immune system to release chemical substances such as antibodies that result in allergy symptoms. Allergic reactions to cosmetics most often appear as itchy, red rashes on the skin or contact dermatitis. [2]

The List of **26 fragrance substances were introduced into annex III of the Cosmetics Directive** by the 7thamendment (2003/15/EC) on the basis of the SCCNFP draft opinion (SCCNFP/0017/98) published on 30 September 1999 for public consultation and the final opinion adopted by the SCCNFP during the plenary session of 8 December 1999.

1. Alpha isomethylionone
2. Amyl cinnamal
3. Amyl cinnamyl alcohol
4. Anisyl alcohol
5. Benzyl alcohol
6. Benzyl benzoate
7. Benzyl cinnamate
8. Benzyl salicylate
9. Butylphenylmethylpropional (Lilial)
10. Cinnamal
11. Cinnamyl alcohol
12. Citral
13. Citronellol
14. Coumarin
15. Eugenol
16. Farnesol
17. Geraniol
18. Hexyl cinnamaladehyde
19. Hydroxycitronellal
20. Hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC, Lyral)
21. Isoeugenol

22. d-Limonene
23. Linalool
24. Methyl 2-octynoate
25. Everniafurfuracea (Treemoss) extract
26. Everniaprunastri (Oakmoss) extract [3]

The aforementioned Cosmetic Regulation states that any of the 26 allergens mentioned in Annex III should be mentioned in the ingredients, but only if:

- Leave-on products (e.g. body lotion, make-up), exceed 0.001% (10ppm)of the total concentration
- Rinse-off products (e.g. shower gel, shampoo) exceed 0.01% (100ppm)of the total concentration

A list of 26 substances that have to be identified on products to inform consumers was established in 1999 by the European Commission. Their usage and limit are regulated by IFRA. [1]

➢ **Effects Of Fragrance Allergens**:

Fragrance allergens can cause skin reactions that usually take the form of redness, blisters, vesicles, etc. Skin reactions are very common and can have a significant impact on the quality of life.

A distinction is made between different types of skin reactions:

- Irritations
- Urticaria
- Contact dermatitis
- Photosensitization
- Eczema

Fragrances are volatile substances and therefore, in addition to the skin, the eyes, nose and respiratory tract are also exposed. Allergy requires prior sensitisation to the fragrance chemical. Subsequent skin contact with the chemical causes a delayed hypersensitivity reaction (type IV) in the hours to days after exposure.

➢ **Clinical Features Of Fragrance Allergy:**

Fragrance allergy presents as a dermatitis which is often in a streaky pattern where there has been direct contact with the fragrance allergen.

- In women, the hands, face, and neck are most commonly affected.
- In men, the hands, face, and lower legs are most often affected.
- The fragrance chemical can be transferred to an unexpected site, for example, via the hands onto the face.
- Involvement of the armpits is common in both sexes.
- Other locations affected include perianal skin if perfumed toilet paper wet wipes, or haemorrhoid preparations are used.

Fragrance allergy may affect the mouth resulting in gingivitis, blisters and erosions. A diagnosis of fragrance allergy will typically require a detailed patient history and is confirmed by Patch testing. Around 10% of those undergoing patch testing are found to have a fragrance allergy.

- The relevance of a positive patch test should be determined by the distribution of the rash and the products used.
- A weakly positive patch test can be due to irritation by the fragrance chemical rather than a true contact allergy. [4]

1) Allergic contact dermatitis:

Allergic contact dermatitis is a form of dermatitis/eczema caused by an allergic reaction to a material, called an allergen, in contact with the skin. The allergen is harmless to people that are not allergic to it. Allergic contact dermatitis is also called contact allergy.

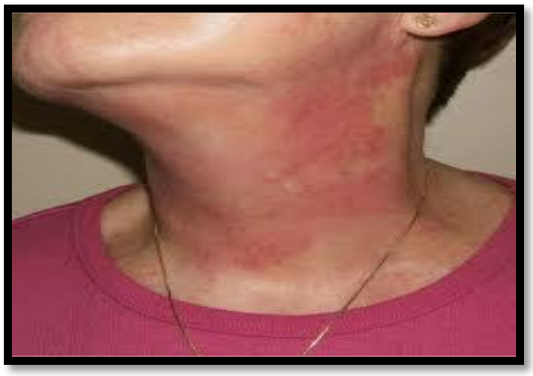

Fig.1: Allergic contact dermatitis

Figure no.1: Structures of fragrance allergens (10)

Allergic contact dermatitis is a type 4 or delayed hypersensitivity reaction and occurs 48–72 hours after exposure to the allergen. The mechanism involves CD4+ T-lymphocytes, which recognise an antigen on the skin surface, releasing cytokines that activate the immune system and cause the dermatitis.

- Contact allergy occurs predominantly from an allergen on the skin rather than from internal sources or food.
- Only a small number of people react to the specific allergen, which is harmless to those who are not allergic to it.
- They may have been in contact with the allergen for years without it causing dermatitis.

- Contact with tiny quantities of an allergen can induce dermatitis.
- Patients with impaired barrier function of the skin are more prone to allergic contact dermatitis, eg.Patients with legulcers, perianal dermatitis, or chronic irritant contact dermatitis.
- Patients with atopic dermatitis associated with defective filaggrin (a structural protein in the stratum corneum) have a high risk of also developing allergic contact dermatitis. [5]

2) Urticaria:

Urticariais characterised by very itchy weals (hives), with or without surrounding erythematous flares. The name urticaria is derived from the common European stinging nettle Urticadioica. Urticaria can be acute or chronic, spontaneous or inducible.

Fig.2: Urticaria

- A **weal** (or **wheal**) is a superficial skin-coloured or pale skin swelling, usually surrounded by **erythema** that lasts anything from a few minutes to 24 hours.
- Urticaria can co-exist with angioedema which is a deeper swelling within the skin or mucous membranes. [4]

3) Asthma:

Asthma is an inflammatory disease of airways to the lungs. It is a long-term condition affecting children and adults. The air passages in the lungs become narrow due to inflammation and tightening of the muscles around the small airways. It makes breathing difficult and can make some physical activities challenging or even impossible.

This causes asthma symptoms such as cough, wheeze, and shortness of breath and chest tightness. These symptoms are intermittent and are often worse at night or during exercise. Other common triggers can make asthma symptoms worse. Triggers vary from person to person, but can include viral infections (colds), dust, smoke, fumes, changes in the weather, grass and tree pollen, animal fur and feathers, strong soaps and perfume. [6]

❖ ***HEAVY METALS***

Heavy metal impurities in cosmetic products are unavoidable due to the ubiquitous nature of these elements, but should be removed wherever technically feasible. Although human external contact with a substance rarely results in its penetration through the skin and significant systemic exposure, cosmetic produce local (skin, eye) exposure and are used in the oral cavity, on the face, lips, eyes and mucosa. Therefore, human systemic exposure to their ingredients can rarely be completely excluded.

Heavy metals like Arsenic, Cadmium, Chromium, Cobalt, Lead, Mercury, Nickel, Aluminium, Zinc and iron are found in wide variety of cosmetics. Health Impact of Heavy Metals in Cosmetics A variety of chemicals are used in cosmetics as ingredients and some are used as preservatives.

A number of them are known to exhibit different chronic health effects, such as cancer, contact dermatitis, developmental, neurological and reproductive disorders, brittle hair and hair loss. Some metals are potent endocrine disruptors and respiratory toxins. Moreover, some metals, such as Cd, As, Pb, Hg and Sb, are exceptionally toxic with a wide variety of chronic health effects, whereas Cr, Ni and Co are well known skin sensitizers. Since the issue of heavy metals as deliberate cosmetic ingredients has been addressed, attention is turned to the presence of these substances as impurities. [7]

➢ **Effects Of Heavy Metals:**

- **Chromium** (Cr+6) is corrosive and allergic to the skin. Cr+6 compounds are enlisted as carcinogens by the International Agency for Research on Cancer (IARC). Adverse effects of the Cr+6 on the skin may include ulcerations, dermatitis, and allergic skin reactions. Inhalation of Cr+6 compounds can result in ulceration and perforation of the mucous membranes of the nasal septum, irritation of the pharynx and larynx, asthmatic bronchitis, bronchospasms and edema. Respiratory symptoms may include coughing and wheezing, shortness of breath, and nasal itch. [2]
- **Nickel** can cause allergic reaction when it comes in contact with the skin. Studies on animals show that if consumed in high amounts, it affects kidneys, stomach and liver. Nickel compound exposure can lead to nephrotoxicity, skin irritation and hypersensitivity.
- **Mercury** is used in cosmetic products such as skin whitening creams. Mercury is a neurotoxin. Mercury has been used by many names such as “mercurous chloride”, calomel, mercuric, mercurio or mercury. The prolonged use of products containing mercury can lead to inflammation of the liver, kidneys and urinary tract. Presence of mercury in Skin Creams has become a global public health problem. Mercury compounds are readily absorbed through the skin on topical application and have the tendency to accumulate in the body. Distribution of mercury-containing creams and soaps is banned in the European Union. A European Union Directive specifies that mercury and mercury compounds are not allowed as ingredients in cosmetics (including soaps, lotions, shampoos and skin bleaching products).

However, phenyl mercuric salts for use as a preservative in eye makeup and eye makeup removal products are allowed at concentrations equal to or less than 0.007% by weight. The cream contained very high levels of mercury: 56,000 parts per million (ppm) or 5.6%.

- **Zinc** has been reported to cause the same signs of illness as does lead, and can easily be mistakenly diagnosed as lead poisoning. Excess zinc exposure may induce toxic effects on the hematopoietic system, biochemistry and endocrine system function.
- **Arsenic** is a ubiquitous, naturally occurring metalloid that may be a significant risk factor for cancer. It is used in cosmetics as colour additive and preservative. Once arsenic compounds are absorbed, they are generally processed via the liver's metabolic pathway, and then converted into many different types of inorganic and organic species including arsenite (As^{3+}), arsenate (As^{5+}), dimethylarsinate (DMA), and monomethylarsonate (MMA). Inorganic arsenic and organic arsenic are absorbed quickly into the blood and circulated to the human gastrointestinal tract. Arsenic metal limit in cosmetics is not more than 3 ppm. [8]

II. ANALYTICAL METHODS FOR ESTIMATION OF FRAGRANCE ALLERGENS IN COSMETICS:

Various analytical techniques have been employed for the estimation of fragrance allergens in cosmetics.

A. Reverse phase high performance liquid chromatography (RP-HPLC)
B. Micro emulsion electrokinetic chromatography (MEEKC)
C. Size exclusion chromatography - gas chromatography and mass spectroscopy (SEC-GC-MS)
D. Matrix solid phase dispersion - gas chromatography–mass spectroscopy (MSPD-GC-MS)
E. Solid phase microextraction – gas chromatography (SPME-GC)
F.

A. REVERSE PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (RP-HPLC):

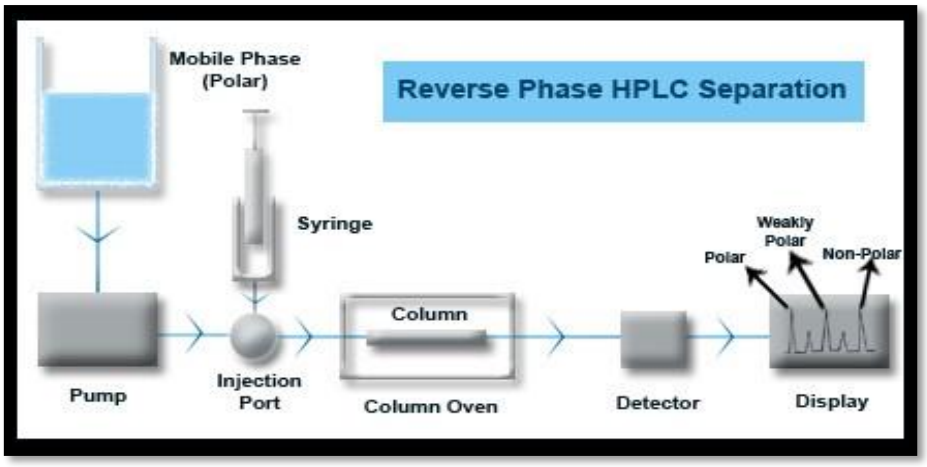

Fig.3: Reverse Phase High Performance Liquid Chromatography

In this study a reverse-phase liquid chromatographic method with gradient elution and UV detection was developed to simultaneously identify and quantify all the 24 fragrance allergens mentioned. The intrinsic selectivity of the HPLC method was enhanced by the use of a diode array detector which provided UV spectra for each chromatographic peak, allowed to optimize wavelength of detection, to set spectral references during method development and to confirm peak identity and peak purity. Quantification was carried out by using the internal standard method (*p*-anisaldehyde as the standard). [9]

The method developed was then applied to the detection of the potential allergens in two commercially available scented products: an all-purpose moisturizing cream ("leave-on" cosmetic product) and a hair conditioner ("rinse-off" cosmetic product).

Experimental:

➢ **Reagents and materials:**

Acetonitrile (MeCN, HPLC gradient grade), Allergen standards, Internal standard (*p*-anisaldehyde), Deionized water was distilled and filtered by cellulose filters (0.45m pore size), Millipore nylon membrane filters (0.2m pore size), commercial scented products (moisturizing cream and hair conditioner).

➢ **Apparatus and chromatographic conditions:**

Chromatographic experiments were performed with a HPLC system Hewlett-Packard HP1100 (PaloAlto,CA) consisting of a quaternary pump, continuous vacuum degasser, equipped with a Rheodyne7125 manual sample injector (20linjectionvolume) and a Hewlett-Packard HP UV–Vis diode array detector (DAD).

Chromatographic separations were achieved by a LiChroCARTPurospher Star RP18-e column (250mm×4.6mm) combined with a Merck LiChroCART 4-4 LiChrospher 100 RP18 (5m) guard column. [10]

A gradient elution was carried out with a mobile phase of acetonitrile (MeCN) and water (H_2O). The best chromatographic assays were performed at room temperature at the following conditions:

Table No.1: Chromatographic assay conditions

Time (min)	Flow (ml/min)	MeCN (%)	H_2O (%)
0	0.7	50	50
5	0.7	50	50
15	1.0	60	40

24	1.0	60	40
40	1.0	90	10

The diode array detector (DAD) was scanned from 190 to 500 nm, and the chromatographic acquisitions were set at three different wavelengths (210, 254 and 280 nm), close to the λ max of all the allergens studied, using the multisignal capability of DAD.

- **Sample preparation:**

Samples A and B. An accurately weighted amount of each product (about 1.0g) was treated with 6 ml of acetonitrile under ultra-sonication for 15min. The suspension was filtered through a 0.2m nylon membrane, added of an appropriate volume of internal standard stock solution (100l) and diluted to 10 ml with acetonitrile in a volumetric flask. The resulting sample was then subjected to HPLC analysis. [11]

B. MICRO EMULSION ELECTROKINETIC CHROMATOGRAPHY (MEEKC) :

Capillary electrophoresis (CE) has gained attention due to its ability for rapid separation, high efficiency, low sample consumption, on-line detection and easy automation. An apparent drawback of CE is the lack of standardized systems suitable to routinely solve different analytical challenges.

In this method, a microemulsion system previously optimised and used by the authors as starting point for further optimisation studies was directly applied to the determination of 18 fragrance allergens in scented products. The novelty of this study consists in the use of Microemulsion electrokinetic chromatography (MEEKC) for the quantitative analysis of 18 compounds included in the original group of 24 SAs. These 18 ingredients, have been selected as target of this work due to their property of being UV-active compounds and thus detectable by CE on-column diode-array detector (DAD). The use of a pseudo stationary phase was necessary due to the neutral characteristics of the 18 analytes, and this opened the way to direct application of the standard microemulsion system previously cited and thus to evaluate its potential for the separation of another complex sample. A suitable cyclodextrin (CD) was also added to the background electrolyte (BGE), improving the separation and giving rise to CDMEEKC. The separation of the compounds was obtained in about 20 min and the analytical performance of the method was tested in terms of selectivity, robustness, linearity, accuracy and precision. The developed procedure was then applied to the analysis of allergens in real rinse-off scented products (i.e. a shampoo and a bath gel). [12, 13]

***Experimental*:**

- **Reagents and materials:**

The reference standards, internal standard p-anisaldehyde (AN), Sodium tetraboratedecahydrate (borax), n-butanol, Acetonitrile (ACN) (HPLC grade), n-heptane, sodium dodecyl sulphate (SDS), methyl-cyclodextrin (MCD), heptakis(2,6-di-O-methyl)--cyclodextrin (DMCD), heptakis(2,3,6-tri-O-methyl)--cyclodextrin (TMCD), (2-hydroxypropyl)--cyclodextrin (HPCD), (2-hydroxypropyl)-cyclodextrin (HPCD), (2-hydroxyethyl)—cyclodextrin (HECD) were used in to the experiment.

- **Sample preparation:**

The considered real samples were commercially available rinse-off products, namely a shampoo and a bath gel. For sample preparation, an accurately weighed portion of the cosmetic product, corresponding to about 1 g, was transferred into a beaker to which 2 mL of water were added. This mixture was gently stirred for 2min. A 300 L aliquot of this mixture together with 25 L of internal standard stock solution and 175 L of water were added to a 500 L vial. In this way, if the concentration of the fragrance allergens present in the original sample was 0.01%, the final test concentration for the CE analysis would be 0.020 $mgmL^{-1}$.

- **Instrumentation and capillary electrophoresis analysis:**

A multiple magnetic stirrer Multipoint HP15 (Ney Company, Bloomfield, USA) was used to stir microemulsion. All electropherograms were obtained on an Agilent Technologies ^{3D}CE system (Agilent Technologies, Waldbronn, Germany) equipped with a UV–Vis DAD. The capillary was thermo stated by air. Instrument control and data acquisition and analysis were performed by ^{3D}CE ChemStation software (Rev. A.09.01, Agilent Technologies). The length of the capillary to the detector was 56.0cm (total length, 64.5cm).

CE separation conditions were: voltage 25kV; temperature 20◦C; detection wavelength 195nm; hydrodynamic sample injection for 5s at 50mbar. A generated current of 45A was observed. Each new capillary was initially conditioned with 1M NaOH and water for 5min each. Between the electrophoretic runs, the capillary was rinsed for 2min with methanol, 2min with 0.1M NaOH, 1min with water and 3min with the BGE. [14]

C. SIZE EXCLUSION CHROMATOGRAPHY - GAS CHROMATOGRAPHY–MASS SPECTROSCOPY (SEC) - GC -MS:

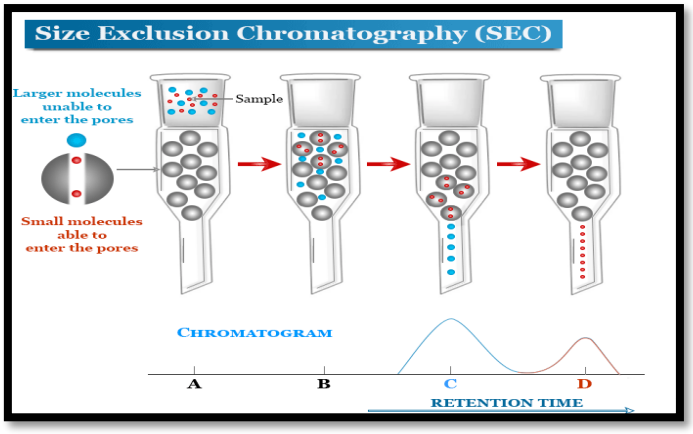

Fig.4: Size-exclusion chromatography

A method using size-exclusion chromatography (SEC) combined with gas chromatography–mass spectrometry (GC–MS) was developed for the quantitation of 24 restricted allergenic fragrance compounds in cosmetic samples. Size-exclusion chromatography (SEC) is also called as gel permeation chromatography. SEC was successfully applied to the isolation of small molecules of interest (<1000Da) from larger matrix compounds in the case of migrants from food packaging materials.

In this study, a clean-up method using SEC was developed for the sample preparation of cosmetics by GC–MS analysis. Determination of 24 of the 26 restricted fragrance compounds was possible, 2 are natural extracts (oak moss and tree moss) unsuitable for GC–MS. [15]

***Experimental*:**

➢ **Reagents and materials:**

The organic solvents acetone and propyl acetate (analytical grade), pigment Foron RubinS-2GFL (disperse red 167.1, CAS 61968-52-3, MW=505.9) was used as internal marker for SEC. Purities of the reference compounds are at least 98%. $^{2}H_{8}$ naphthalene (Nap-d8, 99.0%) and hexachlorobenzene (HCB, 99.5 %) were used as internal standards.

➢ **Instrumentation:**

Analytical balance, ultra sonic bath, vortex, Teflon syringe filters of 0.45m was used in this method. SEC system consisting of a low pressure mixing gradient pump, an autosampler, photodiode array detector, a sampling injector and Millenium software. Semi preparative column: Phenogel.

SEC conditions: acetone isocratic 100% (0–30min); flow rate 0.8 ml/min, UV 515nm for SECM and UV 350nm for matrix; fractions: routinely 10.5–16.9 min for all 24 compounds.

GC–MS system (PolarisQ, Thermo Finnigan, Austin, TX, USA) with a PAL autosampler, Analytical column: DB-35 fused silica column.

GC–MS conditions: carrier gas (helium) flow 1.5 ml/min constant with vacuum compensation; split less injection.

➢ **Sample preparation:**

1 gm weight of samples were weighed into a 10 ml volumetric flask. Approximately 5ml of acetone containing 10 mg/L of dye stuff was added. The capped flask was intensely shaken until a homogenous suspension or solution was achieved. The volume of the suspension/solution was adjusted to 10 ml with the SECM solution and then sonicated for 15 mini nan ultrasonic bath at room temperature. After sedimentation of the matrix at 5◦C for 30min the organic supernatant (raw extract) was filtered with a syringe filter and used for SEC.

A stock solution of the 24 analytes was prepared in acetone (10 mg/L of each compound). From this stock solution, intermediate calibration solutions were prepared in propyl acetate in the range of 0.63–10.00 mg/L. These solutions were diluted with matrix solution and spiked with the internal standards (final concentration 0.25 mg/L each) to a final concentration of 0.06–1.00 mg/L for each compound.

Identification and determination of target compounds were performed by GC–MS with a 60m DB-35 column under the conditions. [16]

D. MATRIX SOLID PHASE DISPERSION - GAS CHROMATOGRAPHY –MASS SPECTROSCOPY (MSPD-GC-MS):

MSPD involves blending a viscous, solid or semisolid sample with a solid support. The shearing forces of blending with a mortar and pestle disrupt the gross architecture of the sample, breaking the material in smaller pieces. At the same time, sample components dissolve and disperse into the bound organic phase on the surface of the particle, leading to complete disruption of the sample and its dispersion over the surface. The possibility of performing extraction and clean-up at the same time is one of the main advantages of this technique, which reduces sample contamination during the procedure and decreases the amount of solvent required.

The aim of this work is to develop a method based on MSPD followed by gas chromatography–mass spectrometry (GC–MS) to simultaneously identify and quantify 25 fragrances in multi-matrix cosmetic samples. [17,18]

Experimental:

➢ **Reagents and materials:**

Internal standard PCB-30 (2, 4, 6-trichlorobiphenyl), Acetone, ethyl acetate, and n-hexane, Florisil(60–100 mesh), Neutral alumina, C_{18}, and sand (50–70 mesh), Silica gel 60 (230–240 mesh). Individual stock solutions of each compound were prepared in acetone.

Further dilutions and mixtures were prepared in acetone, hexane/acetone (1:1, v/v), and ethyl acetate. All solutions were stored in amber glass vials at −20◦C. All solvents and reagents were of analytical grade.

➢ **MSPD procedure:**

0.5 gm of cosmetic sample were exactly weighted into a 10 mL glass vial. When it was necessary, the sample was spiked with 50 L of the corresponding acetone solution of the target compounds to get the desired final concentration. The sample was gently blended with 1 g of a drying agent (anhydrous Na_2SO_4) and 2 g of the dispersive sorbent into a glass mortar using a glass pestle, until a homogeneous mixture was obtained (5 min). Then, the mixture was transferred into a column with a polypropylene frit at the bottom containing 0.5 g of Florisil. A second frit was placed on top of the sample before compression with a syringe plunger. Elution was made by gravity flow with ethyl acetate or hexane/acetone (1:1, v/v), depending on the experiment. 5 mL of eluents were collected into a graduated conical tube and 50 L of PCB 30 solution (200gmL^{-1}) were finally added. The MSPD extracts diluted when it's necessary (dilution factors of 1:10 to 1:1000), were directly analysed by GC–MS.

➢ **GC–MS analysis:**

Analyses were performed on an Agilent 7890A (GC)-Agilent 5975C inert MSD with triple axis detector and an Agilent 7693 autosampler from Agilent Technologies (Palo Alto, CA, USA). The temperatures of the transfer line, the quadrupole and the ion source were set at 290, 150 and 230◦C, respectively. Separation was carried out on a HP5 capillary column (30m × 0.25mm, 0.25 m film thickness) from Agilent Technologies. Helium (purity 99.999%) was employed as carrier gas at a constant column flow of 1.0 mLmin^{-1}.

The mass spectra detector (MSD) was operated in the scan mode and the mass range was varied from 40 to 300 m/z, starting at 4 min and ending at 25 min. The electron multiplier was set at a nominal value of 1300 V. [19]

E. SOLID PHASE MICROEXTRACTION – GAS CHROMATOGRAPHY (SPME-GC):

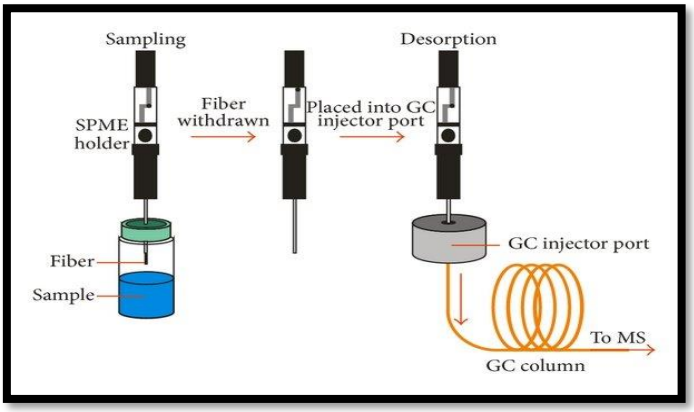

Fig.5: Solid-phase micro extraction - gas chromatography

The aim of this study was to optimize and validate an analytical method for simultaneous determination of fragrance allergens present in cosmetic products using solid-phase micro extraction method (SPME) and gas chromatography coupled with flame ionization detector (GC–FID).

The SPME method became very popular in recent years because it is simple, low-cost, solvent-free, and sensitive. In comparison with GC–MS, GC– FID is widely available in many laboratories and it is used for routine analysis of organic compounds. Only a little work has been done before in the field of simultaneous determination of all fragrance allergens using SPME–GC–FID, and this method is often used only as complementary technique.

Experimental:

➢ **Reagents and materials:**

Standards: 2-(phenyl methylene)heptanal, 97% (amyl cinnamaladehyde); benzene methanol, 99% (benzyl alcohol); 3-phenyl-2-propen-1-ol, 98% (cinnamyl alcohol); 3,7-dimethylocta2,6-dienal, 95% (Citral, cis/trans); 2-methoxy-4-prop-2-enyl phenol, 99% (eugenol); 7-hydroxy-3,7-dimethyloctanal, ≥95% (hydroxycitronellal); 2methoxy-4-(1-propenyl) phenol, 98% (isoeugenol, cis/trans); 2-(phenylmethylene)-1-heptanol, ≥ 85% (amyl cinnamyl alcohol); 2-hydroxy-phenylmethyl ester benzoic acid, 99% (benzyl salicylate); 3-phenyl-2-propenal, ≥ 93% (cinnamaldehyde); 2H-1-benzopyran-2-one, 98% (coumarin); 3,7dimethyl-2,6-octadien-1-ol, 97% (geraniol); 4-(4hydroxy-4-methylpentyl)cyclohex-3-ene-1-carbaldehyde, 97% (lyral); 4-methoxybenzyl alcohol, 98% (anis alcohol); 3-phenyl-phenylmethyl ester-2-propenoic acid, 99% (benzyl cinnamate); 3,7,11-Trimethyl-2,6,10-dodecatrien-1-ol, 95% (farnesol); 2-(4tert-butylphenyl)-2-methylpropanal, 95% (lilial); 3,7-dimethyl-1,6-octadien3-ol, 97% (linalool); phenylmethyl benzoate, 99% (benzyl benzoate); 3,7dimethyl-1,6-octadien-3-ol, 96% (citronellol); 2-(phenylmethylene) octanal, 95% (hexylcinnamaldehyde); 1-methyl-4-prop-1-en-2-yl-cyclohexene, 97% ((R)-(+)-limonene); methyl ester 2-octynoic acid, 99% (methyl 2-octynoate); 3-methyl-4-(2,6,6-trimethyl-2-cyclohexen-1-yl)-3-buten-2-one, ≥ 85% (ionone).

➢ **Sample preparation:**

Fragrance extraction was performed in the headspace above 1mL of the sample introduced in 4 mL vials. After equilibrium, the fragrance compounds were adsorbed onto an SPME fiber that was introduced into headspace vial through septum and persisted for a specified time.

➢ **SPME–GC–FID Conditions:**

The various SPME fibers were used in this study: polydimethylsiloxane– divinylbenzene, divinylbenzene–carboxen–polydimethylsiloxane, 50/30 µm (DVB–CAR–PDMS); carboxen–polydimethylsiloxane, 85 µm (CAR–PDMS); polyacrylate, 85 µm (PA); polydimethylsiloxane, 100 µm (PDMS).
The oven temperature was programmed from 40 °C (held for 1 min) to 220 °C at 5 °C min^{-1} (held for 28 min). Total analysis time was 65 min. The split less mode (maintained for 5 min) was used for injection, and injector temperature was kept at 250 °C. [20]

III. ANALYTICAL METHODS FOR ESTIMATION OF HEAVY METALS IN COSMETICS:

Various analytical techniques have been employed for the estimation of heavy metals in cosmetics.

a. Flame atomic absorption spectroscopy (FAAS)
b. Inductively coupled plasma – mass spectroscopy (ICP-MS)
c. Flow injection mercury system (FIMS)
d. Direct mercury analyzer (DMA)
e. Gas chromatography – mass spectroscopy (GC-MS)
f.

a. FLAME ATOMIC ABSORPTION SPECTROSCOPY (FAAS):

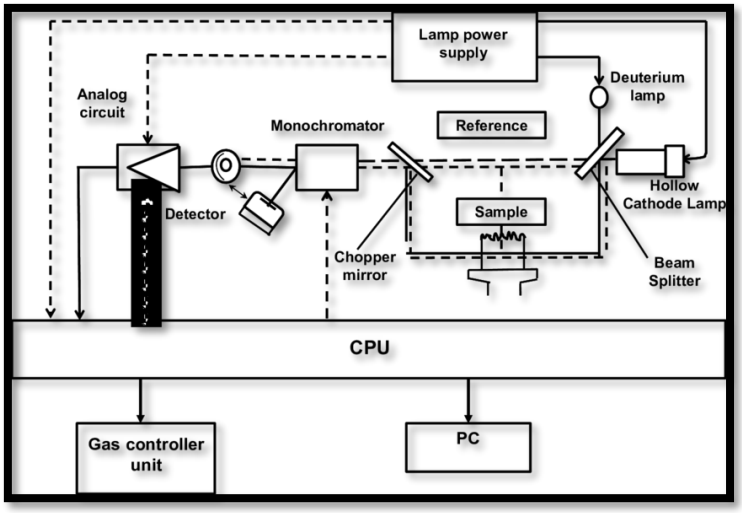

Fig.6: Flame atomic absorption spectroscopy

The flame atomic absorption spectroscopy method is a fast, practical and economical method that is widely used to determine heavy metals. However, it is insufficient to determine the ppb and sub-ppb concentrations directly. In addition, the complex matrices of the analysed samples also cause difficulties in the analysis. For these reasons, separation and/or preconcentration step is needed to increase sensitivity before analysis by FAAS. In this, ultrasound assisted-deep eutectic solvent based-dispersive liquid-phase micro extraction (DES-UA-LPME) was developed, and the determination of trace elements was carried out by flame atomic absorption spectrometry. [21]

Experimental:

➢ **Reagents and materials:**

Concentrated nitric acid and perchloric acid were used for digestion while de-ionized water was used throughout. All reagents were of analytical grade. Also Atomic Absorption Spectrophotometer (model: AA320N, Wincom Company Ltd. China) was used for metal ions estimation.

➢ **Sample preparation:**

Samples were digested by the wet digestion method. 10 mL of concentrated nitric acid and perchloric acid mixture (3:1 ratio) was added to 2 g of each sample in a 100 mL beaker and allowed to stand in a water bath for 2-3 h at 60 ºC. The digested samples were allowed to cool to room temperature, filtered with Whatman no. 1 filter paper and then made up to 20 mL with de-ionized water. The lipstick samples were allowed to stand for 24 h in the refrigerator. [22]

b. INDUCTIVELY COUPLED PLASMA – MASS SPECTROSCOPY (ICP-MS):

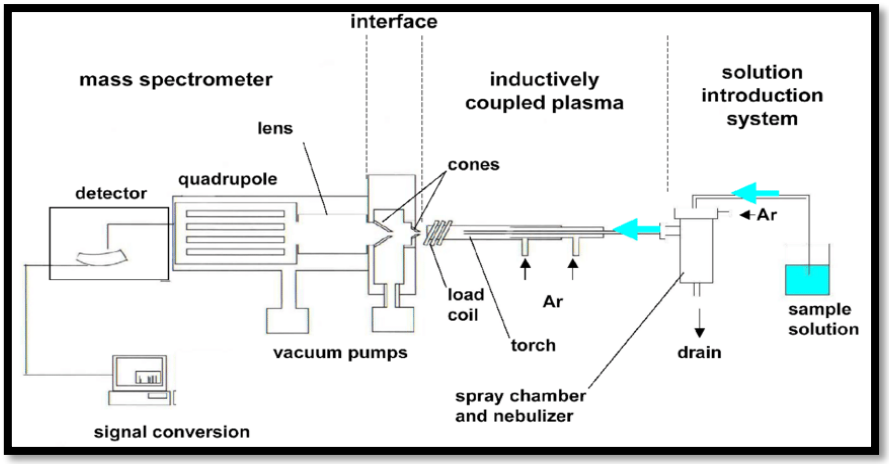

Fig.7: Inductively Coupled Plasma – Mass spectroscopy

To identify the heavy metals in cosmetics powder using the Inductively Coupled Plasma – Mass spectroscopy. This method gives a clear picture of the powder ingredients of cosmetics. The emission spectra were analysed using the Atomic Spectrometer Database, published by the National Institute of Standards and Technology (NIST). [24]

In this technique, liquid samples are injected into the argon plasma using one of the sampling techniques and the sample spray that quickly reaches the plasma is quickly dried and activated. The emission from the plasma is then seen and collected by a lens or mirror and photographed to the input of the wavelength selection device. A total of nineteen cosmetic samples marketed under different brand names and widely used by Sudanese women for cosmetic purposes (ICPOES) were analysed. [25]

***Experimental*:**

➢ **Reagents and materials:**

Standard solutions were prepared daily from 1000 mg L^{1} Cd, Co, Cr, Cu, Ni and Pb stock solutions. Deionized water (18.2 Ω M cm^{1} resistivity) was generated using a Milli-Q^{s} plus Total Water System (Millipore Corp., Bedford, MA, USA) and was used to prepare all solutions. Prior to use, all glassware and polypropylene flasks were washed with soap, soaked in 10% v v^{1} HNO_3 for 24 h, rinsed with deionized water and dried to ensure that no contamination had occurred. For mineralization of the samples, a mixture of H_2O_2 (30% w w^{1}), HNO_3 (Synth) and Triton X-100 (5% and 25% v m^{1}) was used. HNO_3 was previously purified by sub-boiling distillation using Distillacid™ BSB-939-IR.

➢ **Instrumentation:**

An ICP OES instrument (iCAP 6000, Thermo Scientific, Waltham, MA, USA) was used for Cd, Co, Cr, Cu and Ni determination. This instrument allows sequential analytical signal collection using axial and radial viewings.

➢ **Sample preparation:**

For the sample mineralization employing a hot block with PFA tubes, 250 mg of the lipstick sample was weighed using an analytical balance. 5 mL of HNO_3 (7 mol L^{1}), 2 mL of H_2O_2 (30% w w^{1}) and 1 mL of Triton X-100 (25% w v^{1}) were added to the lipstick sample. The tubes were closed with PFA lids and the mixture was heated at 100 °C for 180 min, and the solutions were quantitatively transferred to polypropylene flasks and diluted with water to 25 mL, resulting in a final acidity of 1.4 mol L^{1}. The mineralization was performed in triplicate with and without the addition of a standard to verify the accuracy of the analytical method and to detect possible loss of analytes during sample preparation. The final concentrations added for the standards were 24, 24, 420, 420, 120 and 48 mg L^{1} for Cd, Co, Cr, Cu, Ni and Pb, respectively. [26]

c. FLOW INJECTION MERCURY SYSTEM (FIMS):

***Experimental*:**

➢ **Reagents and materials:**

High purity water (DDW) (Specific resistivity 18 $M\Omega.cm^{-1}$) obtained from E-pure water purification system was used throughout the work. HNO_3, HF and HCl used for sample digestion were of Suprapure® grade with certified impurity contents and were purchased from Merck, Germany. A multi-element standard containing 27 elements were prepared from PerkinElmer single-element ICP standards (1000 or 10000 ppm). The Standard Reference Material (SRM), IAEA-SOIL-7 was purchased from the International Atomic Energy Agency, Vienna. [27]

➢ **Sample preparation:**

Accurately weighed portion (0.1 – 0.2 g) of Mascara or Eye Shade sample was transferred to a TEFLON digestion tube (120 mL) and 7.0 mL of the acid mixture (HNO_3/HF/HCl, 4.5:2:0.5) was introduced. The tube was sealed and the sample was digested inside a microwave oven (Milestone ETHOS 1600).

After being cooled to ambient temperature, the tube was opened; the inside of the lid was rinsed with distilled and de-ionized water (DDW) and the mixture heated on a hotplate (120°C) for 30 min. to drive off the residual HF and HCl. The resulting digest was filtered in a polypropylene flask using 1% HNO_3 and made up to 50ml volume. For ICPMS measurement the clear digest obtained were diluted 10 times incorporating 10 μgL^{-1} solution of ^{103}Rh. In general, samples and standard reference materials (SRM) were prepared in a batch of six including a blank (HNO_3/HF/HCl) digest. [28]

d. DIRECT MERCURY ANALYZER (DMA):

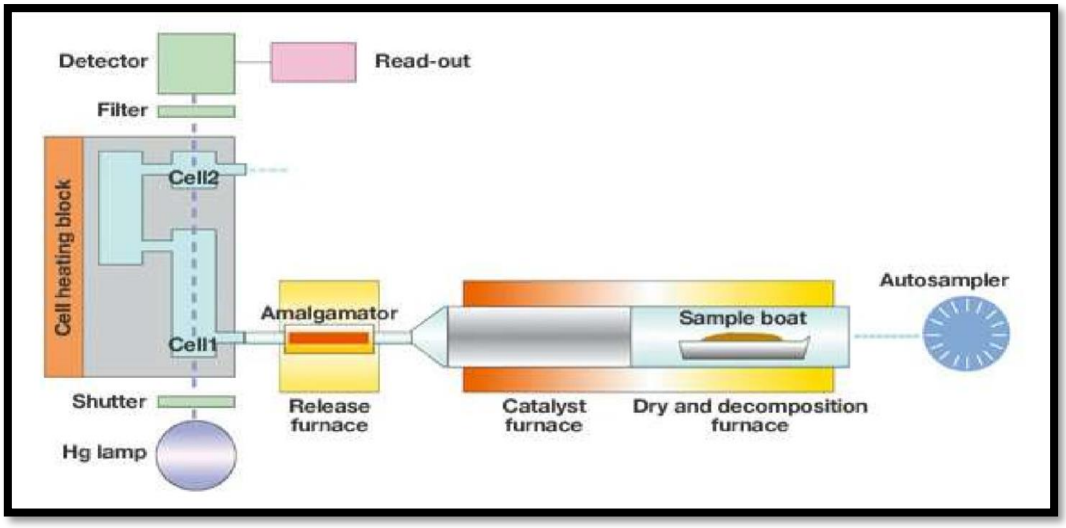

Fig.8: Direct mercury analyzer

***Experimental*:**

➢ **Reagents and materials:**

Mercury stock standard solution concentration of 1000µg/Kg. purchased from Fisher Scientific, UK. HCl (37%) Scharlau, Spain, Highly purified water (interference free). [29]

➢ **Sample preparation:**

A series of dilution covering a wide range of mercury concentration was made from the stock solution and conc HCl (37%) according to the following protocol shown in Table No.2.

Table No.2: Preparation of the standard mercury solutions

No.	conc of the stock	Volume taken ml	Volume made up	Concentration (ppm)
1	1000µg/Kg	10	100	100
2	100ppm	1		1
3		2		2
4		5		5
5		7		7
6		10		10

e. GAS CHROMATOGRAPHY – MASS SPECTROSCOPY (GC-MS):

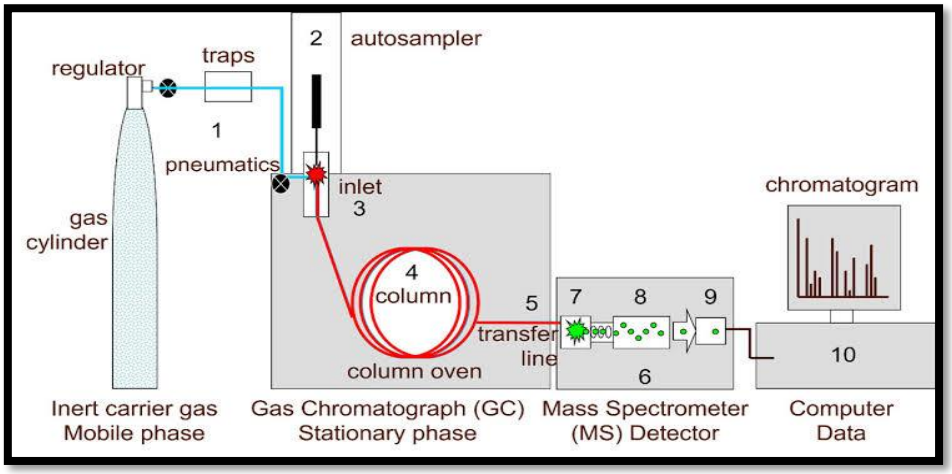

Fig.9: Gas chromatography - Mass spectrometry

***Experimental*:**

➢ **Reagents and materials:**

Commercially accessible and widely used hair colors in Aswan as Black hair dye, also known as “Stone Hair Dye” (SHD), Tancho dye powder and Bigen dye powder were obtained from hair supplies markets.

➢ **Instrumentation:**

Gas chromatography/ mass spectrometry (GC/MS) analysis was carried out in Analytical Chemistry Unit (ACAL) - Faculty of Science- Assuit University using a GC/MS system (7890A-5975B) made in USA, equipped with capillary Column DB 5ms (30m*0.250mm*0.250 µl).

Energy dispersive x-ray (E.D.X.) microanalysis was done in Electron Microscope Unit- Assuit University for analysis of elemental components of different hair dyes using Scanning Electron Microscope (JSM5400LV), made in England.

- **Sample preparation:**

Half gm of sample was added to 2 ml of ethyl acetate then sonicated for 15 minutes and centrifuged at 8000 rpm / 40°C for 15 minutes. The GC/MS analysis was performed in a spit less mode using helium as the carrier gas at a low rate of 0.5 ml/min for 10.9 minutes, then 1 ml/min per minute for 30 minutes. The injection port was set to 250°C, and the oven was preheated to 60°C for 2 minutes before being programmed at 14°C/min to a nil temperature of 280°C for 20 minutes. [31]

IV. RESULTS:

Result obtained from various analytical methods listed below:-

A. Estimation of fragrance allergens by RP-HPLC:

Reversed-phase chromatographic conditions were found suitable to modulate the retention of all the selected compounds. Due to the different polarity of the analytes a gradient elution ($MeCN/H_2O$) was adopted; *p*-anisaldehyde was chosen as internal standard (ISTD) because no interference was obtained at the same Rt and UV spectra showed absorption bands close to the maximal UV absorbance of all the compounds of interest. The 24 analytes were appropriately separated over a running time of 40min.

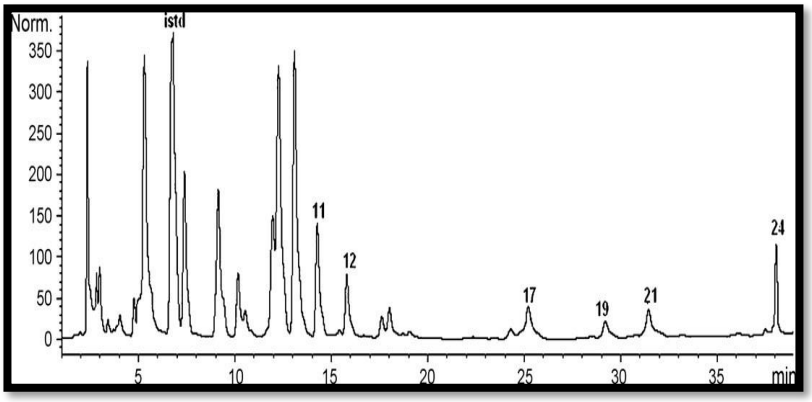

Fig.10: Typical chromatographic acquisition of sample A (all-purpose moisturizing cream) at 210nm.

Table No.3 Quantitative assay in commercial scented products: sample A- all-purpose moisturizing cream

Compound	Peak	Content (%)±S.D.	R.S.D. (%)
Linalool	11	$5.55\times10^{-2} \pm 1.81\times10^{-3}$	3.26
Citronellol	12	$8.50\times10^{-2} \pm 0.61\times10^{-3}$	0.71
Benzyl salicylate	17	$1.12\times10^{-2} \pm 0.12\times10^{-3}$	1.03
Lilial	19	$9.59\times10^{-2} \pm 0.32\times10^{-3}$	3.33
Alpha-isomethyl ionone	21	$1.99\times10^{-2} \pm 0.25\times10^{-3}$	1.26
Limonene	24	$3.27\times10^{-2} \pm 0.40\times10^{-3}$	1.23

All compounds displayed a good linearity (r^2 >0.99) in a relative wide range concentrations. LODs ranged from 0.01 to 0.74g/ml for all compounds except for three synthetic compounds: Lyral® (1.89g/ml), Lilial® (1.68g/ml) and hydroxy-citronellal (10.88g/ml). The intra-day precision expressed as R.S.D. % ranged from 0.6% (eugenol) to 3.5% (geraniol) with an accuracy ranging from 90.9% to 104.6 %.

The inter-day precision and accuracy were determined by analysing in triplicate a standard mixture at the same concentrations on three consecutive days; accuracy ranged from 90.0% and 105.3% with a R.S.D.% from 0.9% to 3.5%.

B. Estimation of fragrance allergens by MEEKC:-

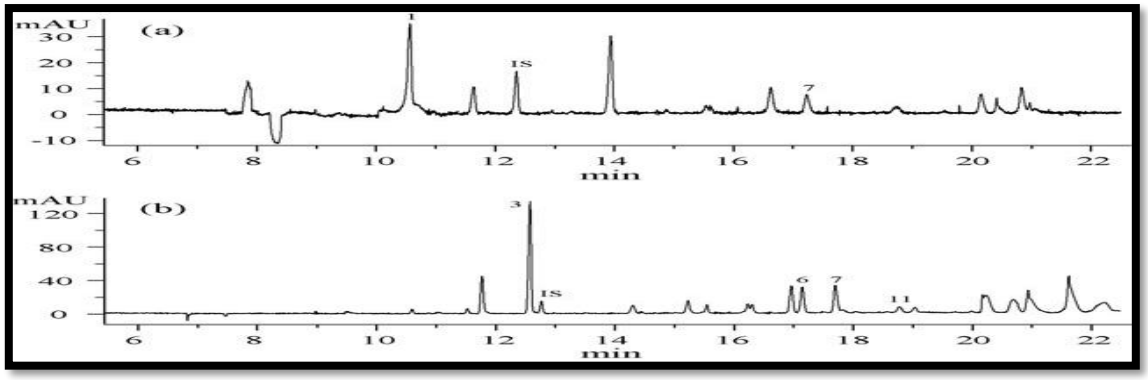

Fig.11: Electropherograms of the real samples: (a) shampoo; (b) bath gel

Selectivity of the method was assessed by measuring the values of resolution between peaks at the higher level of the calibration curve (0.200mgmL^{-1}). Robustness of the method was assessed at 0.125mgmL^{-1} (near the middle of the linearity range) by means of a multivariate strategy using a 4-run Plackett–Burman matrix.
Linearity was evaluated in the range0.010–0.200mgmL^{-1} apart from cetone alpha and hexyl cinnamal for which it was evaluated in the range 0.020–0.200mgmL^{-1} due to the low absorbance of these two compounds.
Accuracy and precision of the method were verified at three concentration levels (0.020–0.125–0.175mgmL^{-1}), each with three replicates, evaluating the recovery values together with their confidence interval and the RSD. For cetone alpha and hexyl cinnamal the lower concentration level was set at 0.030mgmL^{-1}.
For these compounds the determined percentages (n =3, ¸2=0.025) were: benzyl alcohol, 0.031±0.003%, RSD 3.4%; linalool, 0.013±0.001%, RSD 4.2%. In the bath gel, four labelled allergens were detected, namely coumarin, eugenol, linalool, and citronellol (peaks 3, 6, 7 and 11, respectively), and the related electropherograms is shown in Figure no. 7(b). The percentages of these compounds were (n =3, ¸2=0.025): coumarin, 0.126±0.007%, RSD 2.1%; eugenol, 0.025±0.002%, RSD 3.2%; linalool, 0.055±0.007%, RSD 5.3%; citronellol, 0.027±0.003%, RSD 4.9%. [14]

C. Estimation of fragrance allergens by SEC-GC-MS:-

Acetone is a very strong solvent, known to be able to dissolve column materials which can disrupt chromatography and interfere with MS-detection. However, because of acetone having an UV-cut-off of 330 nm, UV detection of fractions containing allergens is not feasible. Therefore, to visualise the chromatographic process, the internal marker Foron Rubin (SECM) was chosen. This pigment has a higher absorption maximum (515nm) than acetone and has a molecular size and a retention time in a similar range as the analytes.

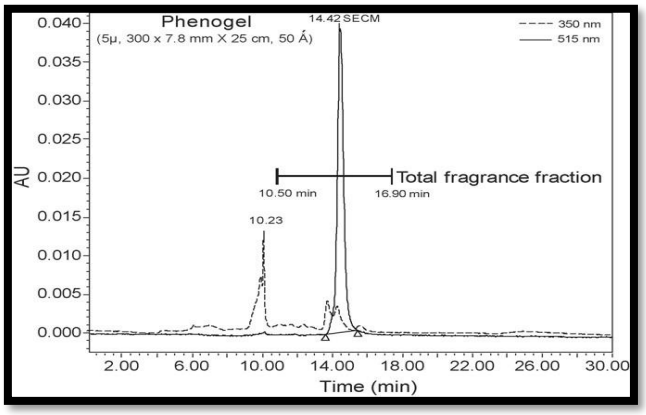

Fig.12: SEC of a hand cream reference sample (350nm) with pigment Foron Rubin (515nm) as internal marker (SECM).

The high reproducibility, a new column must be tested only once With this routinely applied clean up, in about 70% of the samples, all 24 compounds could be detected by GC–MS. For this purpose the total fraction of interest of a spiked hand cream was divided into 12 small sub fractions. Each of them was analysed separately by GC–MS in order to determine the elution time of individual fragrance compounds. The highly concentrated matrix compounds of molecular weights greater than 250Da such as methicone, isopropyl myristate and fatty materials are not eluted earlier than the allergens demonstrate, that interactions between compounds and the surface of the packing material may occur.
The mean accuracy (relative error) was 1±10% for all 24 compounds in five spiked creams (10mg/kg per allergen) and 8±34% in a reference sample (4–15mg/kg).

D. Estimation of fragrance allergens by MSPD-GC-MS:-

The results obtained for the first fraction were analyzed by ANOVA. For most compounds, the sorbent used was statistically significant. Analysing the multiple range tests, we could realize that, in many cases, the results with the different sorbents were

equivalent excluding sand, which gave lower general results**.** We maintained all 5 sorbents (factor A). The second factor considered was the elution solvent (factor B), that it was studied at two levels: hexane/acetone (1:1, v/v), and ethyl acetate. Both solvents have intermediate polarity, which should favour the simultaneous extraction of all the analytes. In both cases, the solvent volume was 5mL. The instrumental linearity was proved at a concentration range between 0.05 and 10gmL^{-1}.

E. Estimation of fragrance allergens by SPME-GC:-

The obtained chromatograms showed that DVB–PDMS fiber has a higher affinity for amylcinnamal, farnesol, hexylcinnamal, coumarin, benzyl benzoate, benzyl salicylate, and benzyl cinnamate in comparison to other compounds, while CAR–PDMS fiber for highly volatile compounds specifically for limonene, linalool, Citral, methyl 2-octynoate, citronellol, geraniol, benzyl alcohol, hydroxycitronellal, eugenol, ionone, and cinnamaldehyde.

All calibration curves were linear in the tested concentration range. The R^2 values were above 0.999 for all compounds. The repeatability and inter day precision of proposed method were very good. The results were satisfactory, and the recovery values were in general over 80%.

Table No.4: Linearity range, LOD, LOQ, Repeatability and Precision

Fragrance compound	Linearity range (µg mL^{-1})	LOD (µg mL^{-1})	LOQ (µg mL^{-1})	Repeatability (RSD %)	Inter-day precision (RSD %)
α-Amylcinnamaldehyde	2.4–970	0.7	2.4	0.5	7.2
Amylcinnamyl alcohol	3–1000	0.8	2.5	1.4	6.3
Anisalcohol	4.18–1113	1.25	4.17	2.8	5.3
Benzyl alcohol	0.05–104	0.015	0.05	3.5	6.6
Benzyl benzoate	1.12–560	0.3	1.09	5.3	7.6
Benzyl cinnamate	4.9–949	1.5	4.9	5.8	7.1
Benzyl salicylate	1.18–590	0.4	1.16	1.1	4.3
Cinnamaldehyde	0.12–1153	0.04	0.13	1.2	4.1
Cinnamyl alcohol	5–1000	1.4	4.1	2.6	6.4
Citral	8.9–1340	2.7	8.9	1.7	6.1
Citronellol	8.6–1659	2.6	8.5	1.5	6.5
Coumarin	1–1000	0.28	0.95	5.8	7.4
Eugenol	6.6–1166	1.9	6.4	2.1	6.6
Farnesol	8.9–1331	2.6	8.7	1.8	5.7
Geraniol	2.22–1334	0.7	2.2	2.1	6.9
Hexylcinnamaldehyde	2.14–1048	0.6	2.09	1.7	6.1
Hydroxycitronellal	2.5–1104	0.7	2.18	1.4	5.3
Isoeugenol	6.8–1080	2	6.7	0.9	3.9
Isomethyl ionone	5.8 –1116	1.7	5.8	2.6	4.7
Lilial	0.23–930	0.06	0.21	1.4	4.4
Limonene	4.2–1680	1.26	4.2	0.9	5.7

a. Estimation of heavy metals by FAAS:-

The concentrations of Pb in the cosmetic samples analyzed were fairly constant in the white powder, brown powder and pink lipstick at 0.10 ppm. It increased to 2.10 ppm sin black eye pencil while in brown eye pencil and red lipstick Pb was not detected. Cadmium was found to be 0.03 ppm in red lipstick and black eye pencil. It was lowest in white powder at 0.02 ppm and then increased in brown eye pencil and pink lipstick respectively at 0.05 ppm and 0.07 ppm. Cobalt was found in its highest concentration in black eye pencil at 0.182 ppm and its lowest in brown powder at 0.085 ppm, Red lipstick was next highest in concentration at 0.174 ppm followed by brown eye pencil at 0.123 ppm. White powder contained 0.121 ppm while pink lipstick had a cobalt concentration of 0.096 ppm. [23]

Table No.5: Concentrations of the Heavy Metals (ppm)

Sample	Pb	Cd	Fe	Co
White Powder	0.10 ± 0.023	0.02 ± 0.012	150.00 ± 0.025	0.121 ± 0.016

Brown Powder	0.10 ± 0.015	-	255.00 ± 0.030	0.085 ± 0.019
Red Lipstick	-	0.03 ± 0.014	5.50 ± 0.026	0.174 ± 0.030
Pink Lipstick	0.10 ± 0.017	0.07 ± 0.015	4.80 ± 0.023	0.096 ± 0.021
Black Eyepencil	2.10 ± 0.022	0.03 ± 0.011	371.25 ± 0.030	0.182 ± 0.025
Brown Eyepencil	-	0.05 ± 0.010	144.50 ± 0.021	0.123 ± 0.020

b. Estimation of heavy metals by ICP-MS:-

Analytical parameters were obtained for the Cd, Co, Cr, Cu, Ni and Pb determinations. The determinations were performed using ICP OES for Cd, Co, Cr, Cu and Ni, and GFAAS was used for Pb because it was not possible to select an ICP OES emission line without spectral interferences.

The limits of detection (LOD) and quantification (LOQ) of the method were calculated using the concept of background equivalent concentration (BEC), and the LOD and LOQ are adequate for the quality control of lipsticks.

Different volumes of standard were added to study the accuracy, and the final concentrations were 24, 24, 420, 420, 120 and 48 mg L^1 for Cd, Co, Cr, Cu, Ni and Pb, respectively, in the sample and in the blank before the sample preparation. The recovery rates obtained were between 80% and 111%. According to Taverniers metal these values are considered adequate for concentrations in the range of 10 mg L^1.

c. Estimation of heavy metals by FIMS:-

Analysis of the results of heavy metal testing conducted in the Health Canada Product Safety Laboratory on a number of cosmetics sold in Canada lead to the determination of limits. Furthermore, comparison of conservative are seen to be technically avoidable when they exceed the following limits: Lead: 10 ppm, Arsenic: 3 ppm, Cadmium: 3 ppm, Mercury: 3 ppm, Antimony: 5 ppm.

The concentration of twenty eight elements on the Mascara and Eye Shade samples from the Saudi market. Comparing the results with the literature it is clear that lead, arsenic, cadmium, mercury and antimony level in the samples under investigation are within the normal level. The nickel concentration reach 46.8 ppm in sample C40. Aluminium concentration reach 5E+4 ppm in two samples C30 and C5. Concentration is high. In literature aluminium reach 5570 ppm in kohl sample.

d. Estimation of heavy metals by DMA:-

Based on the calibration curve, the results of the mercury measurements in the whitening creams are shown in table 3.

Table No.6: The concentration of Hg in the whitening creams samples

Sample	Hg concentration (ppm)
Beauty gel	1.715
Avalon	2.308
Melano free	3.373
Amaluco	3.198
Lucocid	1.883
Fair and lovely	0.035

The highest concentration of mercury 3.373 ppm was found in Melano free followed by Amaluco cream (3.198 ppm). The least mercury concentration was found to be 0.035 ppm in Fair and Lovely cream.

This interface of the three instruments does suit speciation analysis and provides a detection limit in the range of 1-10 ppt (ng/L) but its disadvantage is the need for careful and tedious optimization of the chromatographic conditions. Another point to highlight in trace metal analysis is the importance of the detection limit of a particular instrument that is used for such analysis. For instance, mercury and other toxic metals might exist in trace amounts beyond the detection limit and therefore quantification is not attainable. However, the use of direct mercury analyser offers the advantage of extremely low detection limit (0.001ppb). [30]

e. Estimation of heavy metals by GC-MS:-

Paraphenylene diamine (PPD) in Stone hair dye (SHD) had a retention time of 9.286 min, while the retention time of PPD in Tancho HD was 9.470 min, in Bigen HD powder was 10.37 min and nally in Bigen HD cream was 10.52 min, as illustrated.

At concentration of 500 mg/ml, the concentration of PPD in SHD was 99.7% while the concentration of PPD in Tancho hair dye was 99.8%, the concentration of PPD in Bigen HD powder was 0.492% and the concentration of PPD in Bigen HD cream was 5.563%.

Sample powder had highest concentration percentage of lead (Pb) at (74.49%) whereas cream and SHD contain least amount of Pb (8.995%) and (6.265%) respectively and absent in Tancho HD.

CONCLUSION:

The different analytical methods are used in estimation of fragrance allergens from cosmetics such as RP-HPLC, MEEKC, SEC-GC-MS, MSPD and SPME-GC-MS; and for estimation of heavy metals FAAS, ICP-MS, FIMS, DMA, & GC-MS methods used. The RP-HPLC technique is simple, fast, economic and efficient which can be used for quality assessment of complex matrices. This method could overcome some problems related to sample preparation, critical point of analytical procedure, analytes loss or degradation. The MEEKC technique has main advantage of inexpensive instrumentation and small amount of solvents usage in separation of fragrances. SEC has biggest benefit compare to other methods was the flexible clean up with SEC which allows the determination of large range of compounds in difficult matrices with GC-MS. MSPD is the fast, efficient and cheap technique followed by GC-MS for simultaneous determination of fragrance allergens in cosmetics. SPME technique effective with advantage of low cost and less laborious sample preparation than other techniques. FAAS is rapid and simple technique of determining heavy metals in liquid and powder type of cosmetics with high confidence level. It is economical to maintain and easy to control. The DMA technique is having advantages of low detection limit and no sample preparation. The GC-MS method is reproducible, sensitive and accurate analytical method.

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