# ESTIMATION OF N-NITROSODIETHANOLAMINE IN COSMETICS BY DIFFERENT ANALYTICAL TECHNIQUES

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***Abstract*- N-nitrosodiethanolamine (NDELA), a type of nitrosamine, is a possible human carcinogen that may form in cosmetic products. The aim of this study was to examine the NDELA by different analytical techniques. A highly toxic contaminant in cosmetic raw materials and products was developed and validated. This review focuses on the recent developments in analytical techniques for estimation of N-nitrosodiethanolamine (NDELA) in cosmetic products. This review will examine the a) sample pre-treatment methods such as solid phase extraction (SPE), b) separation methods such as Liquid chromatography–electrospray ionization tandem mass spectrometry (LC–ESI-MS/MS), Ion-Exchange Chromatographic Separation, Open tubular capillary electrochromatography, Gas-Liquid Chromatographic-Thermal Energy Analyzer Method, Gas chromatography with electron capture detection methods.**

***Key Words*- Analytical Techniques, N-nitrosodiethanolamine (NDELA), DEA-Diethanolamine, TEA-Triethanolamine, HPLC**

## INTRODUCTION:

- N-Nitrosamines have long been recognized as group of hazardous compounds, about 80% of these tested have been found to be carcinogenic to test animals. Their carcinogenicity, mutagenicity, health effects, environmental presence, and occurrence in commercial products have been reviewed. In 1997, it was reported that N-nitrosodiethanolamine (NDELA) had been found in cutting fluids, tobacco, cosmetics, lotions, and shampoos. Subsequently, NDELA was shown to be carcinogenic in the rat. Since these reports, scientists in industry and government have been working to develop and improve upon analytical methods for detecting NDELA.[1]
- The unintended by-products and toxic compounds such as carcinogens, dioxin pesticides, polycyclic aromatic hydrocarbons, or metals present in consumer products including cosmetics through manufacturing process or environmental release are of concern and might adversely affect the skin and body.[1]
- NDELA is classified as Group 2B (possible human carcinogen) by IARC (International Agency for Research on Cancer), and under a wide variety of conditions is generated through the reaction of secondary amines such as mono-ethanolamine (MEA), di-ethanolamine (DEA), or tri-ethanolamine (TEA) with the nitrosating agents such as nitrate and nitrite found in cosmetics and foods. Therefore, the formation of nitrosamine in cosmetics may result from the presence of certain ingredients in cosmetics and/or through nitrosation of the precursors detected in the finished cosmetic products.
- Under certain conditions, nitrosation of DEA and TEA produces NDELA. The production of NDELA was increased in aqueous solutions of ethanolamines in a pH-, temperature-, and time-dependant manner.[2]
- Strategies for reducing levels of nitrosamine and NDELA in cosmetics include the minimization or elimination of unintended nitrite sources in the formulation. The cosmetics directive has imposed specific restriction on use of sodium nitrite with secondary and/or tertiary amines or other substances that generate nitrosamines. The cosmetic directive and the Cosmetic ingredient Review (CIR) Expert Panel also recommended banning all (secondary) dialkyl- and dialkanolamines and their salts, DEA, diisopropanolamine bis (hydroxyalkyl) amines, cocamide MEA, and cocamide DEA. In addition, the use of monoalkanolamines, trialkanolamines, trialkylamines, their salts, fatty acid dialkylamides, and dialkanolamides was limited.
- Cocamidopropyl betaine (CAPB), sodium lauryl sulphate (SLS), and ammonium lauryl sulphate (ALS) are surfactants employed in scrubs, shampoo, body wash, dish soap, and bubble bath. These ingredients may produce contact dermatitis, eye irritation, and other allergic reactions. Imidazolidinyl urea (imidurea) is an antimicrobial preservative that releases formaldehyde and nitrosamines in cosmetic products at temperature exceeding 10°C.

- **What are ethanolamines?** [4][5]

- Diethanolamine (DEA) and triethanolamine (TEA) are key examples of ethanolamines-a chemical group comprised of amino acids (the building blocks of proteins) and alcohols. They are used in a wide range of applications including cosmetics and personal care products.
- DEA is used as an emulsifier in shampoos, cleaners, and detergents.
- TEA is used as fragrance, pH adjuster and emulsifying agent.
- When ethanolamines are used in the same product as certain preservatives that break down into nitrogen, they can form nitrosamines. Nitrosamines are a class of more than a dozen different chemicals, which the International Agency for Research on Cancer (IARC) lists individually as possible and known carcinogens.

- **Effects of N-Nitrosodiethanolamine in Humans:** [3]

➢ The Working Group was not aware of any studies that specifically examined the risk of cancer among persons exposed to N-nitrosodiethanolamine. However, ethanolamines and sodium nitrite have been used as additives for metalworking fluids since the 1950s and together these can lead to the formation of N-nitrosodiethanolamine.

➢ There are three major types of metalworking fluid; straight (generally mineral oils), soluble (straight oils diluted with water and additives), and synthetic (water and additives). Ethanolamines and nitrites are additives used in both soluble and synthetic metalworking fluids. Several studies have examined the risk of cancer among workers exposed to metalworking fluids.

**Health Concerns:**

➢ **Cancer:** Nitrosodiethanolamine (NDEA) is listed as a carcinogen in the National Toxicology Program's Report on Carcinogens. Experimental studies show that NDEA causes liver cancer and kidney tumors in rats and cancer of nasal cavity in hamsters. TEA and DEA have found to be hepatocarcinogenic (producing or tending to produce cancer in the liver) in female mice. [6][7]

➢ **Bioaccumulation:** Studies show that 52 to 68 percent of DEA in hair dyes, body lotions and shampoos remain on the upper layers of the skin after exposure. [8][9]

➢ **Organ system toxicity:** Studies have found that DEA affects human male reproductive health. DEA alters sperm structure, causing abnormalities that affect the sperm's ability to swim and fertilize the egg. DEA accumulates in the liver and kidney, causing organ toxicity and also possible neurotoxic effects such as tremors. Another study suggests that memory function and brain development in offspring could be permanently affected by mothers' exposure to DEA. [10][11]

## MATERIAL AND METHODS

**Analytical Methods:**

➢ Analytical method development and validation plays an important role in the discovery, development, and pharmaceuticals. The official test methods that result from these processes are used by quality control laboratories to ensure the identity, purity, potency, and performance of drug products.

➢ The most common methods used for estimation of NDELA are:

**1)Reverse Phase Dispersive Liquid-Liquid Microextraction (RP-DLLME):**[12]

- **Apparatus and materials:**

➢ The LC system comprised of a degasser, a quaternary pump, an autosampler, a thermostated column oven and a UV/Vis detector.

- **Reagents and samples:**

➢ N-Nitrosodietanolamine (NDELA) used as standard.

➢ LC-grade ethanol and acetonitrile, ultrapure acetone, toluene > 99.5%, analytical reagent-grade chloroform, ethyl acetate and dichloromethane.

➢ N-decane, n-hexane, Sulfanilamide, phosphoric acid and N-(1-naphthyl) ethylenediamine (NED) dihydrochloride, ammonium acetate

➢ Extra pure anhydrous sodium sulphate powder used as drying agent for the samples.

➢ Deionized water

➢ **Proposed method:**

➢ A stock solution containing 400 μg $mL^{-1}$ of the target analyte was freshly prepared in deionized water. Then, an aliquot of this solution was properly diluted with acetonitrile to prepare a 1 μg mL−1 stock solution. This last solution was used to prepare the organic working solutions (5−25 ng $mL^{-1}$) in toluene.

➢ An aliquot of 5 mL of each of these working standard solutions was transferred to a 7.5 mL glass centrifuge tube. Then, a mixture of 750 μL of acetonitrile and 125 μL of water were quickly injected into the solution. Once the cloudy solution was formed, the tube was centrifuged at 6000 rpm during 5 min. After phase separation, the sedimented phase (ca. 100 μL) was collected using a 100 μL Hamilton 1705 RNR syringe and transferred into 200 μL inserts placed inside 1.5 mL injection vials for LC-UV/Vis analysis.

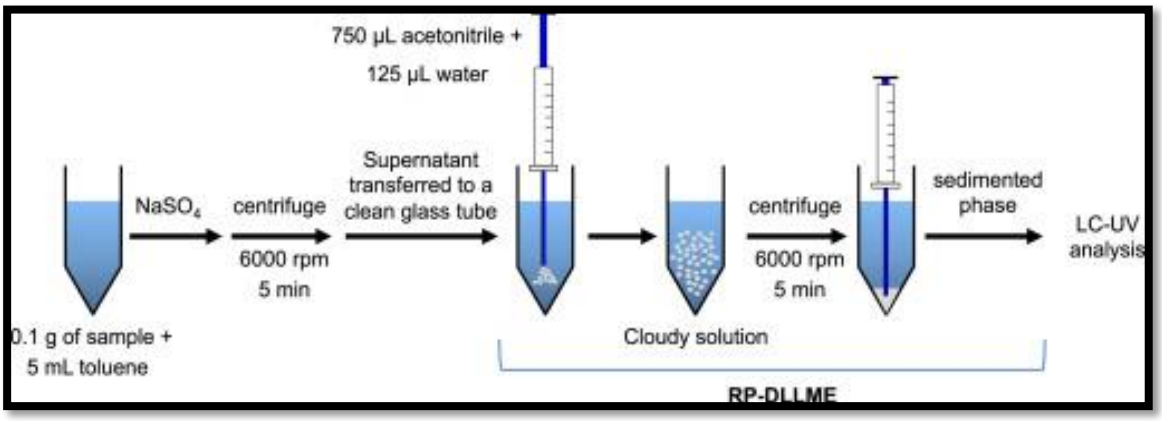


**Figure 5:** Schematic diagram of the proposed RP-DLLME procedure followed for the determination of NDELA in cosmetic samples.

- Regarding sample preparation (either cream or gel), in triplicate, a 0.1 g amount was weighted into 7.5 ml glass centrifuge tube and 5 mL of toluene were added. Then, sample was solved/ dispersed by means of ultrasounds or vortex agitation. Then, a proper amount of anhydrous sodium sulphate (ca. 0.1–0.2 g) was added to remove any possible water residue from the sample. After 5 min, the sample was centrifuged (6000 rpm, 5 min) and the whole supernatant (5 ml) was transferred to another 7.5 ml glass centrifuge tube.
- Then, it was subjected to the RP-DLLME procedure as previously described for standards preparation. Fig. 1 shows a schematic diagram of the experimental procedure.
- Aliquots of 80 µL of all the above-mentioned extracts were injected into the LC system. As expected, lower injection volumes provided lower signals, whereas higher volumes were avoided to not surpass the collected sedimented phase. The mobile phase consisted of a solution of ammonium acetate 0.02 M pumped at 1ml $min^{-1}$.
- The eluate passed throughout the photolysis unit, and then it was merged with a flow stream of Griess Reagent (i.e., 0.02% phosphoric acid aqueous solution containing NED at 0.5 mg $mL^{-1}$ and sulfanilamide at 8 mg $ml^{-1}$) propelled by the auxiliary pump at 0.5 mL $min^{-1}$. Finally, the reaction took place in the post-column reactor at 50 °C, and the formed azo-dye was measured spectrophotometrically at 540 nm.

**2) Liquid chromatography-electrospray ionization tandem mass spectrometry(LC-ESI-MS/MS:**[13]

- **Chemicals:**

- NDELA, DEA (diethanolamine), TEA (triethanolamine), Sodium nitrite, N-nitrosobis-(2-hydroxyethyl)-d8-amine (NDELA-d8, internal standards [IS]), HPLC-grade methanol, acetonitrile, ethanol, chloroform, and dichloromethane, formic acid, nitri3 powder packs, ultrapure water.
- **Sample preparation:**
- To prepare the cosmetic samples (n = 195), 1-g aliquots of each sample were placed in flasks and IS added (100 ng/ml NDELA-d8, 1 ml). Subsequently, 10 ml water was added, and the tube capped tightly.
- The mixture was placed in a water bath for sonication and then mixed for approximately 10 min until a homogeneous sample was obtained. The 5 ml sample was applied to a 20-ml Chem-Elut cartridge and left for 5 min. The analytes were eluted from the cartridge in two stages in 10 ml and 8 ml ethyl acetate. The ethyl acetate was evaporated to dryness under a nitrogen stream using a heating block at 45°C. The dry residue was dissolved in acetonitrile/water (90:10, v/v, 250 µl) and filtered through a 0.2-µm polyvinylidene fluoride filter into an amber vial. An aliquot of 20 µl was injected into the LC-ESI-MS-MS system.

**3) High Pressure Liquid Chromatographic-Thermal Energy:**[14]

- **Apparatus:**
- Nitrosamine detector, HPLC pump, Injection valve, HPLC guard column, HPLC analytical column, strip chart recorder, Freeze dryer, Chromatographic tubes, HPLC with uv detector, Gas chromatography-mass spectrometer, test tubes, uv lamp.
- **Reagents:**
- Solvents: Ethyl acetate, hexane, acetone, methylene chloride, methanol, and 2,2,4-trimethylpentane.
- NDELA:
- Stock solution 1: Accurately weigh 25 mg NDELA and transfer, with aid of acetone, to 100 mL volumetric flask. Dilute to volume with acetone and mix.
- Stock solution 2: Pipette out 5.0 ml stock solution 1 into 100 ml volumetric flask, dilute to volume with acetone, and mix.
- Working solution 1: Pipette out 3.0 ml stock solution 2 into 25 ml volumetric flask, dilute to volume with acetone, and mix.
- Working solution 2: Pipette out 1.0 ml stock solution 2 into 25 ml volumetric flask, dilute to volume with acetone, and mix. Cover containers of stock and working solutions with aluminium foil to protect contents from light. Store solutions in refrigerator. Prepare working solutions fresh weekly.
- N, O-bis-(Trimethylsilyl)-trifluoroacetamide (BSTFA), Trichloromethyl silane
- Dichlorodimethylsilane
- Silanizing solution: Toluene-trichloromethyl silane-Dichlorodimethylsilane (69:2:2).

- **Preparation of Sample**
- Accurately weigh Ca 5 g sample into tared 150 ml beaker. Add 250 mg ammonium sulfamate, 20 g Na2S04, and ca 10 g Celite, and stir until mixture is uniform. Let stand >4 h to let Na ^ remove water. Place small plug of glass wool in bottom of chromatographic tube and add sample mixture. Pack lightly with tamper.
- Add 80 ml hexane to column. Discard collected eluate. Add 125 ml ethyl acetate to column, collecting eluate in 250 m l beaker. Add 2 drops of propylene glycol to ethyl acetate extract and concentrate to 3-4 mL under slow stream of nitrogen. Transfer, with aid of several small portions of ethyl acetate, to tapered 50 ml centrifuge tube.
- Add 1 ml water, shake well, and, after phases have separated, remove aqueous layer with small pipet or syringe. Repeat extraction twice more, if emulsion forms because of presence of surfactants, evaporate ethyl acetate to dryness in 50 ml beaker.
- Extract residue with 3-4 small portions (ca 1 ml) of water. Place combined aqueous extracts in silanized test tube and freeze-dry. Extract residue with 2-3 small portions (ca 1 mL) of acetone, transferring extracts to tared screwcap vial. Evaporate to dryness under nitrogen, and weigh. Cap tightly and refrigerate residue. Before HPLC-TEA analysis, prepare analytical sample by adding 1.0 mL acetone to dissolve residue.

**4) Ion-Exchange Chromatographic Separation:**

- **Cosmetic Ingredients:**

- Commercial cosmetic ingredients, sodium dodecyl sulphate and dodecanoic acid diethanolamide, polyoxyethylene tetradecyl ether, and polyethylene glycol (PEG) were extracted with ethanol to remove inorganic salts and used after being dried.

- Triethanolamine was used without purification.

- **Apparatus:**
- HPLC
- The mobile phase was a mixture of ethanol and 2,2,4-trimethylpentane (20:80) and its flow rate was 2 ml/min.
- A Model TEA-502 Thermal Energy was used for NDELA determination.

- **Ion-Exchange Chromatographic Isolation of NDELA:**
- Each 2 g of samples, whether spiked with a known quantity of NDELA or not, was weighed into a 100-mL beaker and then dissolved with 80% ethanol (40 ml). If necessary, it was dispersed by ultra-sonification and then filtered.
- This solution or filtrate (test solution) was introduced into an anion exchange column or a pair of columns consisting of a cation and an anion exchange column in series. The beaker and filter were rinsed with 10 ml of 80% ethanol, and the rinsing were added to the test solution in the column. The solution was passed through the column at a flow rate of 2-3 mL/min. The nonadsorptionable material were washed out with 150 ml of 80% ethanol.
- NDELA was eluted from the anion exchange column with 10% acetic acid/ethanol (150 ml). The eluent was evaporated to almost dryness by a rotary evaporator at 35 ℃. The remaining acetic acid was removed with a jet of nitrogen. A 2 ml portion of ethanol:2,2,4- trimethylpentane (10:90) was added to dissolve the residue. A 100-µL portion of this solution was injected into the HPLC-TEA.

5**) Headspace solid phase microextraction using a novel aluminium hydroxide grafted fused silica fibre followed by gas chromatography-mass spectrometry:**[15]

- **Reagents and materials:**
- N-Nitrosodietanolamine and sodium chloride, Aluminium tri-tert-butoxide and other chemicals including toluene and NaOH.

- **Apparatus:**
- The GC–MS analyses were performed on a Shimadzu Qp-2010 plus. Helium was used as the carrier gas with a flow rate of 1 mL/min . The separation of extracted compounds was performed on an RTX-5 MS column (30 m length, 0.025 mm I.D., 0.25 mm df, crossbond, 5% diphenyl-95% dimethyl polysiloxane).
- Column: The initial column temperature was set at 80 1℃. Then, the temperature was increased to 100℃ at a rate of 4℃ $min^{-1}$ and maintained for 6 min. Finally, the column temperature was raised up to 250 ℃ at a rate of 40 ℃ $min^{-1}$ and held for 2 min. The transfer line temperature was 260℃.
- Electron ionization mass spectra were recorded at 70 eV ionization energy. Thermal gravimetric analysis (TGA) was carried out on a Bahr STA-503 instrument under air atmosphere. A Heidolph heater/stirrer was used for heating and stirring the samples.

- **The SPME GC–MS analysis:**
- For the headspace solid phase microextraction (HS-SPME) procedure, a 10 ml of water diluted sample (with sample: water ratio of 3 g:5 g) and 1 g of NaCl with a magnetic stirring bar were placed in a 20 ml sample vial.
- After sealing the vial by polytetrafluoroethylene (PTFE) septum, the vial was placed on a stirrer and was stirred at a rate of 250 rpm for 1 min to homogenize the mixture. The fiber was then placed in the inner needle of a syringe and withdrawn into the outer needle of the syringe. The outer needle of the syringe was passed through the PTFE septum and the fiber was exposed in the headspace above the aqueous phase and the solution was stirred at a rate of 150 rpm, at 70℃ for 15 min.
- Then the fiber was introduced into the GC injection port at a temperature of 260℃ for 5 min for the desorption of the analyte. Initially, selected experiments were performed to optimize the main parameters affecting the GC–MS response of the extracted NDELA from water-diluted sample (containing 2000 µg $Kg^{-1}$ NDELA and 1 g of NaCl).

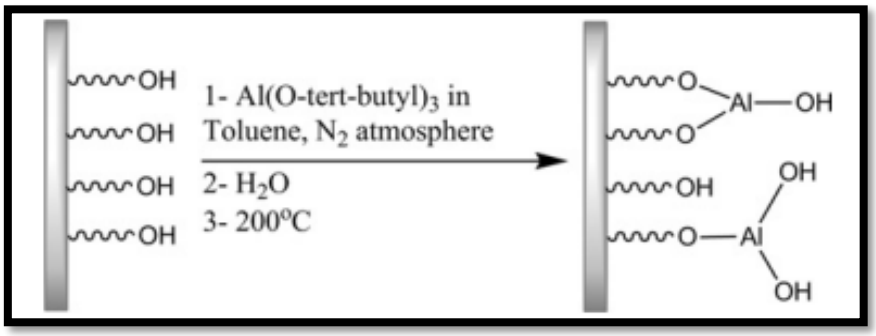


**Figure 6:** Proposed scheme of possible covalent attachment of aluminium to the substrate.

- These parameters were the percentage of added sodium chloride (%NaCl), stirring rate, sample volume (Vs/Vt), extraction temperature ($T_{ext}$), extraction time ($t_{ext}$), desorption temperature ($T_{des}$) and desorption time ($T_{des}$). The parameters were optimized by varying one of them at a time, keeping the other parameters constant. The extractions were repeated three times for each condition. A fiber blank was run between each sample to avoid carry over effects.

**6) Open tubular capillary electrochromatography:**[16]

- **Reagents:**
- All solvents used were HPLC grade.
- Deionized water, Triethoxysilane (TES), Hexachloroplatinic acid, DEA, and TEA, NDELA.
- The buffer composition used: pH 4.41,0.3 M acetic acid+0.375 M γ-aminobutyric acid.

- **Extraction procedure:**
- A 1-g amount of the cosmetic product was dispersed in 10 ml of warm buffer, pH 4.41, and extracted in a separatory funnel with three 20-ml portions of methylene chloride. The aqueous (upper) layer was then separated from the methylene chloride (lower) layer. The final volume of buffer extract was brought up to 10 ml.

**7) Gas-Liquid Chromatographic-Thermal Energy Analyzer Method:**[17]

- **Apparatus:**
- Gas-liquid Chromatograph-thermal energy analyzer (GLC-TEA), Rotatory evaporator, Centrifuge.
- **Reagents:**
- Methyl ethyl ketone, ethylene dichloride, Acetonitrile, Sodium-L-ascorbate, 1-Butaneboronic acid, Silylating agent- N, O-Bis(trimethylsilyl)-acetamide (BSA).
- **Procedure:**
- Accurately weigh ca 2 g cosmetic product and ca 50 mg sodium ascorbate into 25 mL screw-cap tube (125 × 20 mm). Pipette out3.0 ml of water (deionized and residue-free) into tube. Add ca 1 g NaCl and 0.1 ml of glacial acetic acid to aqueous mixture and shake vigorously by hand for 3 min with 15 ml ethylene dichloride.
- Centrifuge mixture for 10 min. If upper aqueous layer is not clear or not well separated, add additional 0.2 ml acetic acid, shake, and re-centrifuge. With Pasteur pipette, carefully transfer entire upper aqueous layer to Clin Elut column and let soak in 5 min. Elute NDELA from column by passing five 20 ml portions of methyl ethyl ketone through column, waiting at least 4 min between additions. Slowly pass combined eluates through silica Sep-Pak cartridge, fitted to 50 mL Luer-Lok glass syringe, into round-bottom flask. Pass additional 10 mL methyl ethyl ketone through Sep-Pak into round-bottom flask.
- Evaporate methyl ethyl ketone solution to ca 20 ml on rotary evaporator (water bath maintained at 40°C). Add ca 25 mg 1-butaneboronic acid, let mixture react 15 min at 40°C and evaporate solution to dryness on rotary evaporator. Dissolve residue in 50 mL ethylene dichloride with vigorous shaking and pass solution through fresh silica Sep-Pak cartridge; discard eluate. Elute trapped NDELA from cartridge with 15 ml anhydrous ethanol into 50 ml round-bottom flask and remove solvent on rotary evaporator at 40°C.
- React residue in flask with 0.3 mL BSA and 1.00 ml internal standard solution at room temperature for 15 min. Inject 8 µl calibration solution and silylated sample solution into GLC-TEA system. Compute NDELA level in ng/g (ppb) in cosmetic with reference to calibration solution.

**RESULTS AND DISCUSSION:**

**1.Reverse Phase Dispersive Liquid-Liquid Microextraction (RP-DLLME):**

After optimization of the whole procedure, quality parameters such as linearity, enrichment factor, limits of detection (LOD) and quantification (LOQ) and repeatability were evaluated to validate the proposed method. The results are summarized in Table 4.

| EF ± s | LOD (ng $ml^{-1}$ ) | LOQ (ng $ml^{-1}$ ) |
|---|---|---|
| 31.5±0.9 | 1.1 | 3.6 |

**Table 4:** Analytical parameter of the proposed RP-DLLME-LC-UV/Vis method

- EF: Enrichment factor, as the mean of three replicates, s: standard deviation
- LOD: Limit of detection, calculated as 3Sy/x/b criteria, where Sy/x is the residual standard deviation and b is the slope of the calibration curve.
- LOQ: Limit of quantification, calculated as 10Sy/x/b criteria, where Sy/x is the residual standard deviation and b is the slope of the calibration curve.

| Repeatability (%RSD) | | | |
|---|---|---|---|
| Intra-Day | | Inter-Day | |
| 5 ng/ml | 20 ng/ml | 5 ng/ml | 20 ng/ml |
| 2.0 | 2.8 | 7.4 | 4.2 |

**Table 5:** Relative standard deviation (RSD), five replicate analysis of an aqueous standard solution containing 5 and 20 mg $ml^{-1}$ of the target analyte

**2.Liquid chromatography–electrospray ionization tandem mass spectrometry (LC–ESI-S/MS):**
A serial dilution of standard samples was used to check the linearity of the application. The calibration range using the deuterated analogue d8-NDELA was the IS. The resulting calibration curve showed good linearity ($R^2 > 0.995$) in the concentration range of 10–1000 ppb for NDELA.

**3.High Pressure Liquid Chromatographic-Thermal Energy:**

- An aim in the development of the sample preparation procedure were only to concentrate the NDELA fraction and to remove ingredients, such as pigments and ionic compounds, that would be deleterious to the analytical HPLC column.
- Results are shown in Table 7.

**Table 6:** LC-ESI-MS/MS Parameters and Performance Characteristics for detection of NDELA[20]

| PARAMETER | NDELA |
|---|---|
| Column | Waters $C_{18}$ column (Waters, Milford, MA, 3 µm, 2.1 × 100 mm i.d.) |
| Mobile Phase | 0.1% Formic acid/acetonitrile = 10:90 (v/v) |
| DP(V) | 46/46 |
| EP(V) | 10/10 |
| CE(V) | 11/7 |
| CXP(V) | 6/8 |
| Linearity | $R^2$= 0.997 |
| LOD (µg/ml) | 5 |
| LOQ (µg/ml) | 10 |
| Repeatability(%CV) | 11.5, 9.5, and 4.5 |
| Reproducibility | 11.5, 10.5, and 7.5 |
| Recovery | 93.3% |
| Mean(%) | 89-120% |

**Table 7:** Recovery of NDELA from an emulsion cream and hair grooming gel

| SAMPLE | ADDED ng/g (ppb) | FOUND ng/g (ppb) | RECOVERY (%) |
|---|---|---|---|
| Emulsion Cream | 362 | 290 | 80 |
| | 362 | 338 | 93 |
| | 362 | 331 | 91 |
| | 290 | 261 | 90 |
| | 290 | 258 | 89 |
| | 290 | 206 | 71 |
| | 120 | 115 | 96 |
| | 120 | 94 | 78 |
| | 120 | 101 | 84 |
| Average Rec. (N=9) | | | 86 |
| SD | | | ± 6.4 |
| Hair Grooming Gel | 362 | 278 | 77 |
| | 362 | 311 | 86 |
| | 362 | 284 | 78 |
| | 290 | 267 | 92 |
| | 290 | 299 | 103 |
| | 290 | 249 | 86 |
| | 120 | 96 | 80 |
| | 120 | 104 | 87 |
| | 120 | 115 | 96 |
| | 97 | 86 | 89 |
| | 97 | 90 | 93 |
| | 97 | 96 | 99 |
| Average Rec. (N=12) | | | 89 |
| SD | | | ±8.2 |

**4. Ion-Exchange Chromatographic Separation:**

➢ TEA Response. As for the peak response to the concentration of NDELA working solutions (10-250 mg/ml), good linearity was obtained. Relative error of peak height was less than 3% in the range from 50 to 250 mg/ml concentration.

- **Recovery from Cosmetic Products and Ingredients:**

➢ The levels of 100 and 500 ppb of NDELA spiked to triethanolamine and LS-Na were determined by repeating five times. The recoveries were 90-100% with less than 5% relative standard deviation.

➢ The recoveries of NDELA spiked to eight cosmetic products (two creams, two lotions, two foundations, and two shampoos) at the 500-ppb level were confirmed to be more than 79% (Table 8), with an overall average recovery of 87% for these products. In addition, good recoveries were obtained at the 50 and 250 ppb levels.

| PRODUCT | NDELA SPIKED (ng/g) | RECOVERY (%) |
|---|---|---|
| Cream (A) | 500 | 90.03 |
| | 250 | 93.00 |
| | 50 | 81.00 |
| Cream (B) | 500 | 80.80 |
| Cream (C) | 500 | 83.90 |
| | 250 | 85.00 |
| | 50 | 82.00 |
| Lotion (D) | 500 | 78.6 |
| Foundation (E) | 500 | 89.30 |
| Foundation (F) | 500 | 87.70 |
| Shampoo (G) | 500 | 89.50 |
| Shampoo (H) | 500 | 95.20 |
| Mean Value (n=3) | | |

**Table 8:** Recoveries from Cosmetic Products.

**5.Headspace solid phase microextraction using novel aluminium hydroxide grafted fused silica fibre followed by gas chromatography-mass spectrometry:**

➢ After setting the optimized conditions, the determination of NDELA in the real samples was carried out in five replicates by the standard addition method.

➢ Samples of cosmetics were examined for NDELA as shown in Table 9.

| SAMPLES | NDELA CONTENT± SD (µg $Kg^{-1}$) | RECOVERY ± SD (%) |
|---|---|---|
| Shampoo 1 | 83±5 | 96±0.5 |
| Shampoo 2 | 160±10 | 97±5 |
| Body Shampoo | 50±3 | 99±2 |
| Dishwashing liquid | 4800±100 | 95±4 |
| Hand washing liquid | 6800±110 | 98±2 |

**Table 9:** Determination of NDELA in real samples.

**6.Open tubular capillary electrochromatography:**

➢ On a real cosmetic - a sample of fresh Charlie White Musk body lotion was analyzed.

| PEAK AREA | AREA AVERAGE | SD | RSD(%) | CALCULATED CONCENTRARTION (ppm) |
|---|---|---|---|---|
| White Musk (5 years old sample) | | | | |
| 276.9 | 274.7 | 2.32 | 0.84 | 14.00 |
| 272.3 | | | | |
| 274.9 | | | | |

**Table 10:** Determination of NDELA in cosmetic (White Musk)[23]

**7) Gas-Liquid Chromatographic-Thermal Energy Analyzer Method:**

| NDELA Added (ppb) | NDELA Recd (ppb) | REC. (%) | NDELA Added (ppb) | NDELA Recd (ppb) | REC. (%) |
|---|---|---|---|---|---|
| 237 | 196 | 82.7 | 1064 | 826 | 77.6 |
| 242 | 177 | 73.1 | 1047 | 843 | 80.5 |
| 244 | 189 | 76.8 | 1010 | 946 | 93.7 |
| 243 | 189 | 77.8 | 1047 | 863 | 82.4 |
| | MEAN 77.6 | | | MEAN 83.5 | |
| | RSD ± 5.1 | | | RSD ± 8.4 | |

**Table 11:** Recovery precision for cosmetic sample spiked with NDELA[23]

**8. Gas chromatography with electron capture detection:**

➢ The results obtained are presented in Table 12. [25]

| PREPARATION | COSMETICS | NDELA(µg/kg) |
|---|---|---|
| 1 | Cream | 350 |
| | | 350 |
| 2 | Cream | n.d |
| | | n.d |
| 3 | Night cream | 100 |
| | | 380 |
| 4 | Slimming gel | n.d |
| | | n.d |
| 5 | Cleaning gel for eyes | |
| | Lot J810 | 300 |
| | Lot T826 | 300 |
| 6 | Cream (1.34% triethanolamine) | 170 |
| | | 250 |
| 7 | Cream (1.3% triethanolamine) | 110 |
| | | 220 |
| 8 | Cream (1.35% triethanolamine) | 100 |
| | | 100 |

**Table 12:** Levels of NDELA (µg/kg) In Cosmetic

**CONCLUSION:**

A new analytical method based on a novel modality of RP-DLLME, is proposed here for the first time to determine NDELA in cosmetic products. With this new approach, which uses water as extraction solvent unlike the conventional approach, NDELA has been determined in cosmetic samples of different nature in efficient way, besides providing good analytical features. This proposed analytical method is simple, fast, and highly sensitive, allows the determination of the analyte at lower levels.

Based upon results obtained from the LC-ESI-MS/MS demonstrated the presence of nitrites, TEA and DEA levels in sample of cosmetic products. Regarding NDELA, higher concentrations of nitrites and TEA (including nitrosating agents) resulted in higher levels of nitrosamine in cosmetic samples.

In method for the HPLC-TEA determination of NDELA has been demonstrated to give reliable and reproducible results for a large number of cosmetic products having widely different compositions.

In ion exchange chromatography the samples were examined in presence of various ionic and non-ionic polar compounds which is used in cosmetic formulations and recoveries were found to be satisfactory(80-100%).

The OT-CEC analysis presented here has been shown to be an easy method for routine screening for NDELA in cosmetics. Results presented here indicate that most cosmetics have finite shelf-life with respect to the formation of NDELA.

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