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Determination of N-nitrosodiethanolamine in cosmetic products by reversed-phase dispersive liquid-liquid microextraction followed by liquid chromatography

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ABSTRACT

A new analytical method for the determination of N-nitrosodiethanolamine (NDELA), a very harmful compound not allowed in cosmetic products, is presented. The method is based on a new approach of dispersive liquid–liquid microextraction (DLLME) useful for extraction of highly polar compounds, called reversed-phase DLLME (RP-DLLME), followed by liquid chromatography–ultraviolet/visible (LC-UV/Vis) determination. The variables involved in the RP-DLLME process were studied to provide the best enrichment factors. Under the optimized conditions, a mixture of 750 μL of acetone (disperser solvent) and 125 μL of water (extraction solvent) was rapidly injected into 5 mL of toluene sample solution. The extracts were injected into the LC-UV/Vis system using ammonium acetate 0.02 M as mobile phase. After chromatographic separation, the eluate passed throughout a photolysis unit in order to convert NDELA to nitrite, and then it was merged with a flow stream of Griess Reagent and passed throughout a post-column reactor at 50 °C to derivatize nitrite into an azo-dye, which was finally measured spectrophotometrically at 540 nm. The method was successfully validated showing good linearity, an enrichment factor of 31.5 ± 0.9, limits of detection and quantification of 1.1 and 3.6 ng mL^{-1}, respectively, and a good repeatability (RSD < 8%). Finally, the proposed analytical method was applied to the determination of NDELA in commercial cosmetic samples of different nature, specifically three lipophilic creams and a hydrophilic shower gel, with good relative recovery values (87 – 117%) thus showing that matrix effects are negligible. These results were compared with those obtained by applying the ISO 10130 official method, which uses the same detection approach. It was concluded that a great improvement in the sensitivity was achieved, whereas the use of organochlorine solvents is avoided and therefore it can be considered as a greener approach.

1. Introduction

The growing social concern about health and beauty has encouraged in recent years a remarkable increase in the use of cosmetic products. Because of this widespread use, it is necessary to carry out adequate quality controls to not only ensuring the effectiveness, but also the safety in users [1]. In this respect, European legislation on cosmetic products [2] includes a list of compounds whose use in cosmetic products is banned.

It should be emphasized that, given the high responsibility of the European cosmetic enterprises, the presence of the banned substances in the cosmetic products as consequence of their intentional use is not expected. Therefore, their presence could be due to unintentional causes, as for example, deficiencies in the purification of raw materials, degradation of some cosmetic ingredients, migration of compounds from the containers, or even reaction between cosmetic ingredients. An example of this is the so-called N-nitrosamines [3]. Their presence in cosmetic products was banned in 1992 [4], since mutagenic, carcinogenic and teratogenic effects are associated to these compounds [5,6]. Nevertheless, different studies have evidenced their presence in cosmetic products [7–9], although fortunately at trace levels. This could be attributed to the fact that these compounds are formed with relative ease by reaction of an amine (usually a secondary one, although it can be also formed from a tertiary one) [10] and a nitrosating agent such as nitrite or oxides of nitrogen [5,11]. Moreover, some preservatives such as bronopol or bronidox, which contain nitro moieties in their structures, can also act as nitrosating agents and thus form nitrosamines when react with amines [11,12]. Among the N-nitrosamines found in cosmetics, N-nitrosodiethanolamine (NDELA) is undoubtedly the most typical one [10]. It is formed from the diethano-

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lamine that could be present as impurity of triethanolamine, which is a common amine employed in the cosmetic industry as buffering agent. Hence there is a growing need to develop new sensitive and selective analytical methods for its determination at trace levels.

Different analytical methods for NDELA determination can be found in the literature. Liquid chromatography (LC) is usually employed for its determination, preferably coupled to thermal energy analyzer (TEA) [13–16], owing to its selectivity and sensitivity for N-nitrosamines determination. Nevertheless, mass spectrometry (MS) [17–19], ultraviolet/visible spectrometry (UV/Vis) [20–22] and differential pulse polarography [23] have been also used as LC detectors. Nevertheless, despite NDELA is a highly polar compound with low volatility, gas chromatography (GC) with TEA [24,25], MS [20,26], electron capture detector [27] or electrolytic conductivity detector [28] has also been employed for its determination, but a derivatization step was usually conducted to obtain a more volatile derivative, *e.g.*, as trimethylsilyl [24,25,28] or acetate [27] esters.

It should be mentioned that two official analytical methods, i.e., ISO 10130 [29] and ISO 15819 [30] based on the papers published by Flower et al. [21] and Schothorst et al. [17], respectively, have been approved for the determination of NDELA in cosmetic products. In both of them, sample pre-treatment is similar, and it depends significantly on the dispersibility of the cosmetic samples in water. If the nature of the cosmetic product is hydrophilic (*e.g.*, shampoos or shower gels) and it is easily dispersible in water, solid-phase extraction (SPE) is used to retain, and thus to remove, matrix compounds. One the other hand, if the cosmetic product is hydrophobic (*e.g.*, milks or creams) and it is not easily dispersible in water, sample is dissolved in dichloromethane and NDELA is extracted by liquid-liquid extraction (LLE) using water, taking into account the high polarity and water-solubility of NDELA. The extracts were analyzed by LC-UV/Vis in the ISO 10130 method or by LC-MS/MS in the ISO 15819 method. In any case, sample pre-treatment is focused for cleaning-up purposes, but enrichment, which could improve the limits of detection and quantification, is not pursued. In this sense, microextraction techniques could play a crucial role. However, despite the great impact that microextraction techniques are having currently [31–33], either in the solid- or in the liquid-phase, there is just one case in which NDELA has been determined in cosmetic products [26] by using solid-phase microextraction (SPME).

Nevertheless, among microextraction techniques currently developed, it should be cited dispersive liquid-liquid microextraction (DLLME) [34], which has become a very popular sample preconcentration and clean-up technique [35,36]. DLLME is based on a ternary component solvent system, where a mixture of the extraction organic solvent and the disperser solvent is injected into the sample rapidly forming the so-called cloudy solution. Afterwards, phase separation is accomplished by centrifugation, and the analytes remain in the organic phase. However, the high polarity and water-solubility of NDELA makes no possible the use of DLLME as such. Nevertheless, recently Hashemi et al. [37] proposed for the first time a modification of the original DLLME called reversed-phase DLLME (RP-DLLME), where a small volume of water, used as extraction solvent, is dispersed into a bulk organic solution containing the polar target analyte. They determined, firstly, oleuropein in olive's processing wastewaters and in olive leave extracts [37], and later, tyrosol and hydroxytyrosol in olive oils [38]. More recently, RP-DLLME was also employed for the determination of phenolic compounds [39], bisphenol A [40] and amygdalin [41] in different kind of oils. However, despite the high potential of this new approach, which opens a way to extract and concentrate highly polar compounds from lipophilic samples, no more applications have been found in the literature.

The aim of the present work is to improve the analytical determination of NDELA in cosmetic products described in the two above-mentioned ISO methods, by exploiting the high potential of the RP-DLLME as clean-up and enrichment step before its determination by LC. For comparison purposes, it is taken the approach followed in the ISO 10130 method based on that N-nitroso compounds exhibit photohydrolysis when they are subjected to UV radiation, since the N-nitroso bond is cleaved giving nitrite ions. Then, nitrite can react with a primary amine (R_1–NH_2) to give a diazonium salt (R_1–$N{\equiv}N^+$) by means of a diazotization reaction and further it can be coupled to an aromatic amine (R_2) to give an azo-dye (R_1–$N{=}NH^+$–R_2), which exhibits intense reddish colours susceptible to be determined by UV/Vis. This same detection approach was previously used by Shuker and Tannenbaun [42] for the determination of different N-nitroso compounds in biological fluids, and later by Bellec and co-workers [43] to determine N-nitrosamines in gastric juice and alcoholic beverages. More recently, Flower and co-workers [21], in which ISO 10130 is based, also relied in the same approach to determine NDELA in cosmetic samples.

2. Experimental

2.1. Apparatus and materials

The LC system comprised of a degasser, a quaternary pump, an autosampler, a thermostated column oven and a UV/vis detector from Agilent Technologies. The column was a LiChrospher® RP-18 (12.5 cm×4 mm id, 5 μm particle size) from Merck (Darmstadt, Germany).

The photolysis unit for cleaving the N-nitroso bond of NDELA and thus give nitrite consisted of a PTFE tube (5 m×0.3 mm i.d.) coiled around a T-8C UV lamp tube (1.5 cm diameter, 26 cm length, 8 W) emitting at 254 nm from Vilber Lourmat (Torey, France).

The post-column reactor for derivatizing nitrite into azo-dye consisted of a coiled PTFE tube (5 m×0.3 mm i.d.) immersed in a thermostatic water bath at 50 °C. The derivatization reagent was pumped by using a Hitachi® (Tokyo, Japan) L-7100 high-pressure pump.

Sep-Pak® Vac C18 (500 mg, 6 mL) cartridges from Waters (Massachusetts, USA) were used in the sample treatment of gel samples by the reference method.

A Crison® (Alella, Spain) micropH 2000 pHmeter was used for the pH measurements.

A Hettich® (Tuttlingem, Germany) EBA 21 centrifuge was also employed.

2.2. Reagents and samples

N-Nitrosodietanolamine (NDELA) used as standard was purchased from Sigma-Aldrich (Steinheim, Germany).

LC-grade ethanol and acetonitrile, ultrapure acetone, toluene > 99.5%, analytical reagent-grade chloroform, ethyl acetate and dichloromethane, were all obtained from Scharlau Chemie (Barcelona, Spain). N-decane > 99% was purchased from Sigma-Aldrich (Steinheim, Germany) and n-hexane > 95% from J.T.Baker (Deventer, Holland).

Sulfanilamide > 99% from Sigma-Aldrich (Steinheim, Germany), phosphoric acid 85% (1.71 g mL^{-1} density) and N-(1-naphthyl)ethylenediamine (NED) dihydrochloride > 98% all from Merck (Darmstadt, Germany) were used for the Griess Reagent.

Reagent-grade ammonium acetate from Scharlau Chemie (Barcelona, Spain) was used in the mobile phase.

Extra pure anhydrous sodium sulfate powder from Scharlau Chemie (Barcelona, Spain) was used as drying agent for the samples.

Deionized water (resistivity ≥18 MΩ cm) was obtained by means of a NANO pure II water purification system from Barnstead (Boston, MA, USA).

Three commercial lipophilic creams samples and a hydrophilic shower gel were analyzed. They were from different brands and the names are not shown for reasons of confidentiality.