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Cosmetics — Analytical methods — Nitrosamines: Technical guidance document for minimizing and determining N-nitrosamines in cosmetics

Cosmétiques — Méthodes analytiques — Nitrosamines: Directives techniques concernant la limitation et le dosage des N-nitrosamines dans les produits cosmétiques

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Foreword

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Introduction

N-nitrosamines are a class of compounds that have been known for over 100 years. The carcinogenicity of N-nitrosamines has been well studied and of the compounds tested, approximately 90 % have been shown to be carcinogenic across a number of animal species (Magee et al. 1976). As a result of these findings, N-nitrosamines are considered to be carcinogenic to humans (IARC 1978) and minimization of exposure to N-nitrosamines is recognized as important to the preservation of human health. N-nitrosamines are formed by the reaction of secondary amino compounds with nitrosating agents such as nitrite or oxides of nitrogen (Ikeda Challis et al. 1977, Ikeda et al. 1990). Traces of N-nitrosamine in cosmetics may result through the use of certain cosmetic ingredients and/or through the nitrosation of the precursors principally secondary amines present in finished cosmetic products (Harvey et al. 1994).

In cosmetics, secondary dialkanolamines are used in the production of dialkanolamides and secondary dialkylamines are used in the production of dialkylamides. In the presence of nitrogen oxides present as impurities or produced from other cosmetic ingredients, nitrosation of secondary amine may occur, resulting in the formation of the N-nitrosamine. Similarly, the presence of secondary amines in trialkylamines and trialkanolamines may result in the formation of N-nitrosamines following nitrosation with nitrogen oxides (SCCS/1458/11). N-nitrosamines may also be formed from nitro substituted para aminophenols in the presence of a secondary amino compound.

Concerns about N-nitrosamine contamination of cosmetics date back to at least 1979 (United States Federal Register Notice, 44 FR 21365, April 10, 1979). Although the potential for N-nitrosodiethanolamine (NDELA) contamination of cosmetic products and ingredients still exists, in principle, coordinated efforts between regulators and the regulated industry since 1979 has successfully addressed the detection, inhibition, decomposition, and prevention of NDELA formation, and resulted in a several references in the literature on analytical technical methods and formulation guidance for avoidance of the formation of NDELA and other N-nitrosamines. (US FDA Guide To Inspections Of Cosmetic Product Manufacturers). Further, vigilant testing programs by industry and inspection programs by regulators to assess their ingredients and cosmetic products for NDELA and other N-nitrosamines have demonstrated the effectiveness and have greatly reduced cosmetics as a major source of N-nitrosamine exposure to consumers.

N-nitrosamines are also covered in European cosmetics regulation. The Fifteenth European Commission Directive 92/86/EEC relating to cosmetic products does not allow the marketing of cosmetic products that contain nitrosamines. The presence of trace levels in cosmetic products is allowed, if they are technically unavoidable, as long as the product does not cause damage to human health when applied under normal or reasonably foreseeable conditions of use. This requires N-nitrosamine levels to be kept as low as reasonably practicable, although no specific level has been set for finished cosmetic products. This Directive also set a limit of 50 µg kg $^{-1}$ (ppb) for the N-nitrosodialkanolamine content of fatty-acid dialkanolamides, monoalkanolamines and trialkanolamines used as raw materials in the manufacture of cosmetic products. A similar limit 50 µg kg $^{-1}$ has been set for the N-nitrosodialkylamine content of fatty-acid dialkylamides, monoalkylamines and trialkalyamines and their salts because the properties of these compounds are similar to their respective alkanolamine analogues with respect to their potential as precursors of N-nitrosamine formation (European Commission Directive 2003/83/EC).

In order to demonstrate compliance with regulatory requirements and to allow reliable risk assessments to be performed, relevant application of appropriate analytical methods is required. A range of methods for N-nitrosamine determination are already available, two of which have become ISO Standards (ISO 15819, ISO 10130). It is important to understand the benefits and limitations of the analytical methods to provide appropriate data.

This guidance is mainly focused on the possible formation of N-nitrosamines and the analytical possibilities to detect their presence. It should be noted that the application of Good Manufacturing Practices (GMP) alone is not enough to prevent the presence of N-nitrosamines, hence this guidance also describes possible strategies for minimizing N-nitrosamine formation, methodologies available to measure N-nitrosamines and suggests a testing strategy which may be applied to both raw materials and finished products. Also included is some guidance on good analytical practice for each method, to ensure validity of the analytical data.

Cosmetics — Analytical methods — Nitrosamines: Technical guidance document for minimizing and determining N-nitrosamines in cosmetics

1 Scope

This Technical Report aims to contribute to providing general advice on strategies that can be adopted to minimize the likelihood of N-nitrosamine formation in cosmetic products and provide a description of the analytical methodologies available for the reliable determination of N-nitrosamines in cosmetic products. It also seeks to provide some insight into the relevance and limitations of each of the methods described and finally provide an analytical approach for the analysis of N-nitrosamines in cosmetic products and raw materials.

This Technical Report covers the reduction or elimination of adventitious nitrite sources, reduction or elimination of secondary amino sources, incorporation of inhibitors to N-nitrosamine formation and analytical methodologies for total N-nitroso compounds and specific methods for N-nitrosodiethanolamine (NDELA).

2 N-nitrosamine chemistry

N-nitroso compounds are characterized by a nitrosyl group (-*N* = O) bonded to a nitrogen atom, but may also contain a number of other functional groups. The N-nitrosamines are composed of the dialkyl, alkylaryl, and cyclic nitrosamine derivatives. Conditions for the formation of N-nitroso compounds can occur in a number of situations. Theoretically, N-nitroso derivatives can be formed whenever any compound containing a secondary amino group comes into contact with an active nitrosating agent (see SCCS/1458/11).

3 Minimization strategies

3.1 Reduction or elimination of adventitious nitrite sources

In line with Good Manufacturing Practices, the level of adventitious nitrite can be minimized by using purified water in manufacture and the use of nitrite-free steel or plastic containers for storage of raw materials and products. It is also important to minimize contact with air containing oxides of nitrogen during the product manufacturing process, separating production from hydrocarbon fuel equipment and open flames (e.g. using indirect heating systems). Eliminating unnecessary nitrates or nitrites from raw materials (e.g. minimizing use of raw materials manufactured in the presence of oxides of nitrogen) is essential in minimizing adventitious nitrite.

Under certain circumstances, if traces of secondary amines are present, they may be nitrosated. It should be noted that some preservatives may catalyse potential nitrosating reactions. The advice of the preservative manufacturer should be sought, if there is uncertainty about the potential for nitrosation to occur in a product.

It is important to check if specific restrictions exist in cosmetics legislation, i.e. national or regional, regarding the combination of an ingredient with a nitrosating agent. For example, in Europe, the European Cosmetics Regulation imposes a specific restriction on the use of sodium nitrite. Sodium nitrite must not be used with secondary and/or tertiary amines or other substances forming N-nitrosamines (Colipa 2009).

3.2 Avoidance of other secondary amino sources

The use of all (secondary) dialkylamines and dialkanolamines and their salts, should be minimized or not used at all. These substances may be present as impurities in intermediate ingredients. If that is a possibility, the avoidance of nitrosating systems should be considered.

Possible sources of secondary amine traces (i.e. diethanolamine, diisopropanolamine) in cosmetic products include impurities and decomposition products of raw materials such as monoalkanolamines, trialkanolamines and fatty acid mono-and di-alkanolamides. Dimethylamine and long chain methylamines may be present as impurities and decomposition products of raw materials such as amine oxides and some preservatives. Morpholine may be present as an impurity and decomposition product of certain preservatives.

For this reason, monoalkanolamines, monoalkylamines, trialkanolamines, trialkylamines, their salts and fatty acid mono- and dialkanolamides are subject to specific restrictions in the European Union. These apply to their minimum purity, maximum secondary amine content, maximum nitrosamine content, and storage in nitrite-free containers, use levels and avoidance of nitrosating systems.

In some regions there are legislations (92/86/EEC, 2003/15/EC) on levels of nitrosamine precursors that may be present in cosmetic products. Use of preservatives such as 5-bromo-5-nitro-1,3-dioxane and 2-bromo-2-nitropropane-1,3-diol in cosmetic products is restricted to a maximum authorized concentration to minimize N-nitrosamine formation.

3.3 Incorporation of inhibitors of nitrosamine formation

In addition to the selection of suitable raw materials, consideration should be given to the incorporation of an inhibition system. It must be understood that there is no “magic recipe” which will give total inhibition of N-nitrosamine formation in all possible product formulations and suitable inhibition strategies must be evaluated for each product type.

General guidelines (Colipa 2009) for the selection of a suitable inhibitory system are as follows.

— Anionic emulsifiers are far superior to nonionic or cationic emulsifiers in inhibiting nitrosation of hydrophobic amines. When nonionic or cationic emulsions are used larger amounts of inhibitors are required, regardless of the solubility characteristics of the amine.

— A hydrophilic organonitrogen ingredient in an anionic emulsion requires a nitrosation inhibitor in addition to any emulsifier used.

— Inhibitors should be selected based on their reactivity with nitrite and their oil or water-solubility characteristics.

Possible inhibitors include compounds which are traditionally classified as antioxidants and a variety of others which can preferentially react either with nitrite and nitrogen oxides (nitrite scavengers) or iminium ions produced during the formaldehyde – catalysed route to nitrosamine formation. Where low levels of formaldehyde may be present, the use of specific inhibitors of iminium ions is advised. In terms of practical application of these ideas, the following should be noted. None of these reagents will destroy N-nitrosamines already present in raw materials.

Inhibitors should be added to the formulation before any organonitrogen ingredients are added. There is a limit to how much inhibition can be achieved in real systems and there are restrictions as to which of the potential inhibitors could be incorporated into cosmetics and toiletries. In all cases, formulation, manufacture and subsequent storage should be carried out at the lowest feasible temperature.

A description of reported inhibitor systems is given in Annex A.

3.4 Potential treatment of cosmetic raw materials for N-nitrosamines decomposition

In terms of practical application of these ideas, the following should be noted. None of these reagents will destroy N-nitrosamines already present in raw materials. Another strategy used in analytical

'confirmation' steps is broad-band UV irradiation to decompose the N-NO bond in the nitrosamine, coupled with use of a NO trap to scavenge the NO that is released (Stefan et al. 2002).

4 Analytical methods

4.1 Screen cosmetic products for N-nitroso compounds by chemiluminescent detection of nitric oxide

Screen cosmetic products for total N-nitroso compounds by chemiluminescent measurement of nitric oxide liberated by the cleavage of the N-nitroso group. First, partition the cosmetic product with methylene chloride and water to separate polar and nonpolar N-nitroso compounds. Examine each extract for the presence of N-nitroso compounds by adding the cleavage reagent and sweeping the nitric oxide formed into a chemiluminescent analyser. Although the method is not intended to be quantitative, recovery studies were conducted to determine measurable levels in a cream and a lotion. The results of the study demonstrated that false-positive responses may be observed in analyses of some cosmetic products (Challis et al. 1995, Chou et al. 1987). The method therefore, is intended only for the preliminary screening of these products, and a positive response from the screening procedure should be followed by the LC-Thermal Energy Analyser or GC- Thermal Energy Analyser method for verification of specific N-nitrosamines. Using this screening method, a specific N-nitrosamine relevant to sunscreens and cosmetics was identified (Chou *et al.* 1995).

4.2 Apparent total nitrosamine content (ATNC)

The ATNC method is also a screening procedure for the analysis of cosmetic matrices. The method was evaluated by the United Kingdom Cosmetic Toiletry and Perfumery Association (CTPA) and the results of a collaborative study published (Challis et al. 1995).

Dissolve or suspend samples in water, aqueous ethanolic or aqueous tetrahydrofuran. Nitrite/nitrite ester interferences are removed by prior treatment with sulphamic acid. Denitrosate the treated test solution in a single reaction with hydrobromic acid / acetic acid in refluxing n-propyl acetate. The liberated nitric oxide is detected in a chemiluminescence reaction with ozone. Quantification is undertaken by comparison with an external nitrosamine standard.

This method is a good screening tool since it detects all sources of nitric oxide. However, it gives no indication of the identities or levels of the individual N-nitrosamines present hence the results are normally expressed in terms of N-NO.

The method does have potential for false-positive results, for example from C-nitroso, S-nitroso and some multifunctional organo-nitro compounds (present in some hair dyes). Due to the uncertainty of ensuring complete absence of such potential interferences the results are commonly referred to as "Apparent Total Nitrosamine Content" (ATNC). In addition it has been shown that the ATNC method generally gives results that are higher than the sum of the individual N-nitrosamines present.

4.3 Methods for NDELA

4.3.1 NDELA by gas chromatography: Thermal energy analyser (TEA)

The method for N-nitrosoalkanolamines can be used for specific analysis of NDELA. NDELA is extracted from cosmetic matrices by a multi-stage process, converted to a volatile derivative and analysed by Gas Chromatography (GC) with detection by Thermal Energy Analyser (Sommer et al. 1988). A sample is dissolved in water and an internal standard (e.g. N-nitroso-(2-hydroxyethyl)-(2-hydroxypropyl)-amine) is used. The sample is adsorbed onto a Kieselghur column, washed with cyclohexane/dichloromethane and eluted with n-butanol. The extract is evaporated to dryness, re-dissolved in chloroform/acetone and transferred to a silica gel column. The column is then washed and possible available NDELA eluted with acetone. The eluate is dried and the residue is treated with N-methyl N-trimethylsilyl-l heptafluorobutyramide (MSHFBA) to convert N-nitrosamines to volatile derivatives. The MSHFBA derivatives are separated by gas chromatography and detected using a Thermal Energy Analyser. In

the TEA the N-nitrosamines are cleaved by pyrolysis to release nitrosyl radicals, which are detected in a chemiluminescence reaction with ozone.

This method has good sensitivity when applied under optimum conditions and has been applied successfully for a wide variety of N-nitrosamines.

The disadvantage of this method is that it may be subject to false positives (since 1986). Particular care is required to avoid artefact nitrosamine formation. Traces of nitrous oxide present during sample clean-up may result in nitrosamine formation where samples contain free secondary amine. This can be minimized by using inhibitors such as ascorbic acid.

4.3.2 NDELA by HPLC: Post-column derivatization

Samples are prepared, depending on their solubility/dispersion in water. For samples soluble or dispersible in water a Solid Phase Extraction (SPE) method using a C_{18} phase is used. If the sample is not dispersible in water a liquid/liquid extraction method using dichloromethane is employed.

The NDELA in the sample extract is then subjected to liquid chromatography by using a reversed phase column. Post-column derivatization of the NDELA is performed via photolysis at 254 nm (to liberate nitrite) followed by a two step reaction with sulfanilamide and n-naphthylethylenediamine (Griess Reagent). Identification and quantification of the resulting coloured compound are carried out using detection at 540 nm.

The simple sample preparation methods used in this analysis makes the analysis quick and easy to use. The method has good accuracy and sensitivity and is specific for NDELA. It has been assessed by multiple laboratories in a collaborative ring trial (Flower et al. 2006) and can reliably quantify NDELA in a wide range of cosmetic matrices.

The method is specific to NDELA, however, in some cases, where certain oxidizable dyes are present in the formulation, care must be taken in following the procedure.

4.3.3 NDELA by HPLC-MS/MS

This method utilizes a similar sample preparation methodology and chromatographic separation as described in 4.3.2. Detection and quantification of NDELA is carried out using a triple quadrupole mass spectrometer / mass spectrometer (Schothorst et al. 2005).

The use of HPLC coupled to mass spectrometry to monitor fragmented ions provides a high degree of specificity for NDELA. The limits of detection and quantification for this method are typically 20 and 50 $\mu g\ kg^{-1}$ respectively, depending on the equipment and matrices.

The principal advantage of this method is that it is the only method providing an unequivocal identification.

4.3.4 High pressure liquid chromatographic–thermal energy analyser determination of N-nitrosodiethanolamine in cosmetics

The NDELA fraction was isolated from a cosmetic product by a series of solvent extractions which were designed to concentrate the NDELA and remove ingredients deleterious to the analytical system. The isolated fraction was then analysed for NDELA using high pressure liquid chromatograph (HPLC) interfaced with a thermal energy analyser (TEA). The compound was measured by comparison of detector response with those of known standards. The presence of NDELA was verified by gas chromatography-mass spectrometry of the silyl derivative (Ho et al. 1981).

4.3.5 Incorporation of an amine marker as an indicator of artefact formation

Formation of N-nitrosamine artefacts during nitrosamine analysis can be a problem because of the ease of nitrosation of secondary amines in the presence of a nitrosating agent. A secondary amine marker is

added during sample preparation. The absence of the N-nitroso derivative of the marker indicates the absence of artefact formation during sample preparation (Chou et al. 1995).

4.3.6 Tentative photolytic confirmation of N-nitrosamines

The presence of a N-nitrosamine determined by less specific methods like GC and HPLC can be further confirmed using UV photolysis. A portion of the sample extract that was previously analysed by GC or HPLC is exposed to UV light and then reanalyzed. If the peak corresponding to the N-nitrosamine nearly or completely disappears, the presence of N-nitrosamine is tentatively confirmed (Chou et al. 1995).

4.4 Determination of other specific N-nitrosamines

In cases where the presence of specific N-nitrosamines other than NDELA is suspected i.e. N-nitrosodimethylamine (NDMA), N-nitrosodiisopropanolamine (NDiPLA), it may be necessary to adapt the above methods for such analytes. In such cases it will be necessary to provide sufficient evidence to verify method performance. This would include validation parameters such as accuracy, precision, recovery, limit of detection (LoD) and limit of quantification (LoQ). Particular care should be taken to demonstrate specificity and data to support identification of the target compound should also be given.

5 Analytical approach

5.1 General

The analytical approach may be either to screen samples prior specific nitrosamine determination or undertake specific determination provided information on cosmetic ingredients is available.

5.2 Screening

The ATNC method serves as a useful screening tool for the assessment of N-nitrosamines in cosmetic products and raw materials. Due to the potential for interferences from non N-nitroso compounds this method is not applicable to all types of matrices e.g. nitro substituted amino phenol compounds that might be used in hair dye formulation could interact during the initial denitrosation phase. A result above the LoQ should be confirmed by analysis using a specific technique wherever possible. In addition, a further assessment is required to understand the likely formation of any nitrosamine. Where NDELA or other specific N-nitrosamines may be the cause of a positive result in the ATNC, the application of any of the methods described in 4.3.1, 4.3.2, 4.3.3, 4.3.4 is appropriate.

5.3 Measurement of specific N-nitrosamines

The GC-TEA methodology is very specific to N-nitroso compounds; however it may result in *in situ* N-nitrosamines formation. By including an inhibitor in the sample preparation, *in situ* N-nitrosamine formation can be minimized to undetectable levels. The absence of the nitrosated marker is an indication of no artefactual N-nitrosamine formation *in situ*. The HPLC post-column derivatization and HPLC-MS/MS methods are ISO Standards (ISO 15819, ISO 10130) and should therefore be the methods of choice, especially if discussions with regulatory authorities are likely.

A suggested analysis approach is given in the flowchart shown in Figure 1.

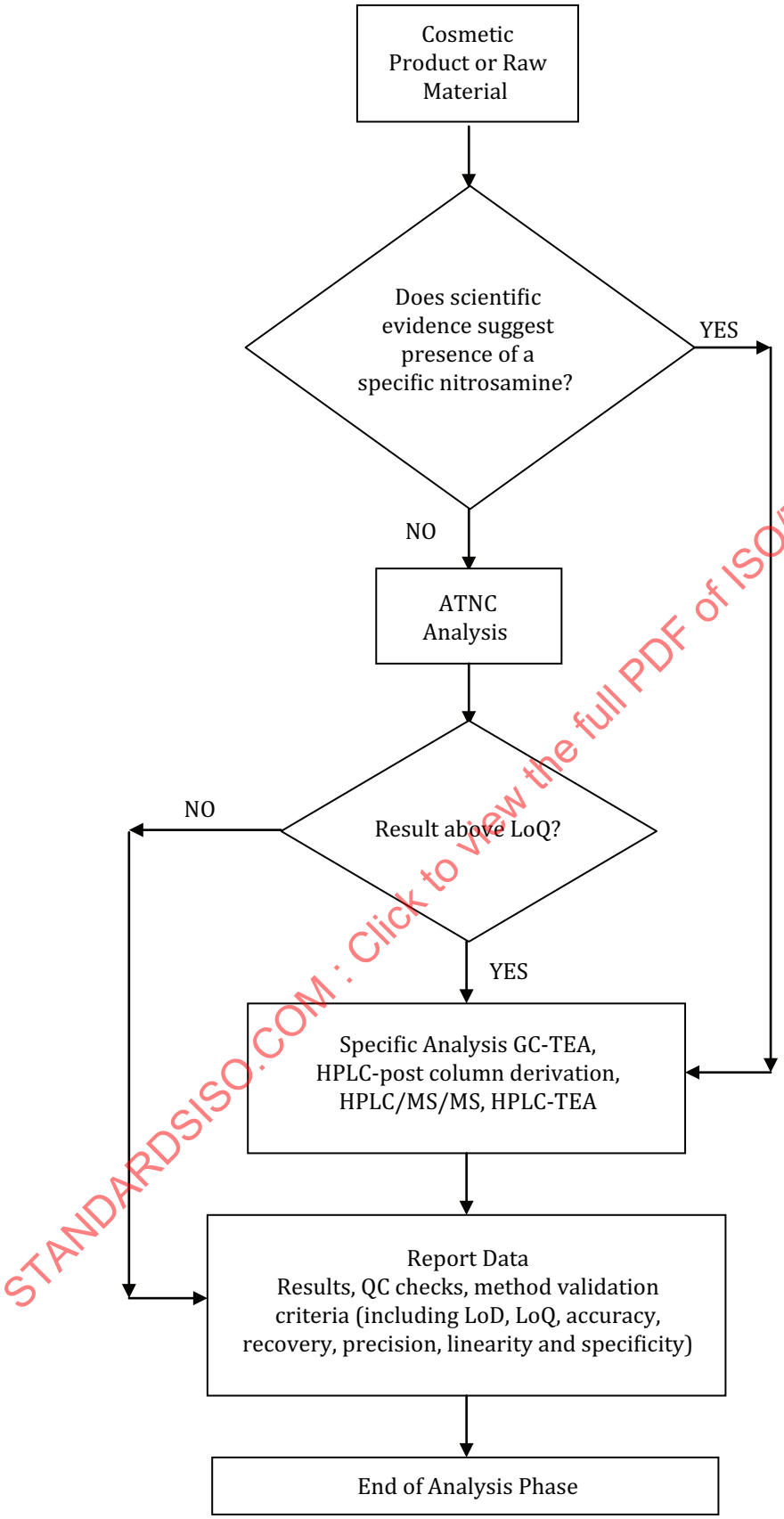

Figure 1 — Analysis of cosmetic products and raw materials (flowchart)