Cosmetics Europe
the personal care association

COSMETICS EUROPE:

TECHNICAL GUIDANCE DOCUMENT ON MINIMISING AND DETERMINING NITROSAMINES IN COSMETICS

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1. INTRODUCTION

Nitrosamines[1] are a class of compounds that have been known for over 100 years. The carcinogenicity of nitrosamines has been well studied and of the compounds tested, approximately 90% have been shown to be carcinogenic across a number of animal species. As a result of these findings, nitrosamines are considered to be carcinogenic to man.

Formation of relevant nitrosamines can occur by the reaction of secondary amino compounds with nitrosating agents.

Traces of nitrosamine in cosmetics may result through the use of certain cosmetic ingredients and/or through the nitrosation of the precursors present in finished cosmetic products.

The most likely sources of nitrosatable agents are the dialkanolamines used in the production of dialkanolamides, of which diethanolamide is the most widely used in cosmetic products. The most likely sources of nitrosating agents are nitrates as potential impurities in raw materials, or gaseous nitrogen oxide.

Under certain conditions, nitrosation of diethanolamine yields the nitrosamine, N-nitrosodiethanolamine (NDELA), a polar, non-volatile, N-nitrosamine compound. Traces of NDELA have been reported in cosmetics. NDELA is generally regarded as one of the more potent carcinogens amongst the N-nitrosamine family of chemicals.

In the European cosmetics regulation, nitrosamines are covered in Annex II and III of the European Cosmetics Directive (76/768/EEC).

Annex II states that nitrosamines must not form part of the composition of cosmetic products. However, according to Article 4.2, they are accepted as traces that are technically unavoidable in Good Manufacturing Practice, as long as the finished product conforms with Article 2 of the Cosmetics Directive (it does not cause damage to human health when applied under normal or reasonably foreseeable conditions of use).
Annex III sets maximum limits (50 $\mu g.kg^{-1}$ or 50 ppb) for the level of nitrosamines in mono and tri- alkylamines and alkanolamines and in fatty acid dialkylamines and dialkanolamides. In addition, it should be noted that certain

[1] Throughout this document we talk about nitrosamines. Alternative nomenclature for these compounds includes: N-nitroso-compounds and N-nitrosamines.

levels of NDELA in products have previously resulted in a RAPEX[2] notification within the EU.

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 nitrosamine determination are already available, some of which are currently the subject of ISO standardisation, and it is important to understand the benefits and limitations of the methods to provide appropriate data.

This guideline describes possible strategies for minimising nitrosamine formation, some of the methodologies available to measure nitrosamines and suggests a testing strategy which may be applied to both raw materials and finished products. Also included are some guidelines on good analytical practice for each method, to ensure validity of the analytical data.

2. OBJECTIVES

The main objectives of this guidance document are to:

- provide general advice on strategies that can be adopted to minimise the likelihood of nitrosamine formation in cosmetic products;
- describe the analytical methodologies available and the relevance and limitations of each of them;
- propose an analytical approach for the analysis of nitrosamines in cosmetic products and raw materials.

3. SCOPE

The guidance provided in this document covers the following:

- reduction or elimination of adventitious nitrite sources;
- reduction or elimination of secondary amino sources;
- incorporation of inhibitors to nitrosamine formation;
- analytical Methodologies for Total Nitrosamines and specific methods for N-nitrosodiethanolamine (NDELA);
- analytical approach

This document does not provide formulation-specific strategies for prevention of nitrosamine formation; it is the responsibility of the formulator to demonstrate the efficacy of the strategies employed.

[2] Rapid Alert System for non-food consumer products

4. MINIMISATION STRATEGIES

4.1 Reduction or Elimination of Adventitious Nitrite Sources

In line with Good Manufacturing Practices, the level of adventitious nitrite can be minimised by:

a) Using purified water in manufacture.

b) Using nitrite-free steel or plastic containers for storage of raw materials and products.

c) Minimising contact with air containing oxides of nitrogen during the product manufacturing process.

d) Separating production from hydrocarbon fuel equipment and open flames (e.g. using indirect heating systems).

e) Eliminating unnecessary nitrates/nitrites from raw materials.
e.g. minimising use of raw materials manufactured in the presence of oxides of nitrogen.

Under certain circumstances, if secondary amine contaminants are present, 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 regarding the combination of an ingredient with a nitrosating agent.

The Cosmetics Directive imposes a specific restriction on the use sodium nitrite. Sodium nitrite must not be used with secondary and/or tertiary amines or other substances forming nitrosamines.

4.2 Reduction or Elimination of Secondary Amino Sources

The Cosmetics Directive, Annex II, specifically prohibits the use of all (secondary) dialkyl- and dialkanolamines and their salts, diethanolamine, diisopropanolamine and bis (hydroxyalkyl) amines not further N-substituted. These substances may be present as impurities in other ingredients. If that is a possibility, the avoidance of nitrosating systems should be considered.

Possible sources of secondary amine traces in cosmetic products include the following:

- Diethanolamine and diisopropanolamine (which may be present as impurities and decomposition products of raw materials such as monoalkanolamines, trialkanolamines, and fatty acid mono-and di-alkanolamides)

- Dimethylamine and long chain methylamines (which may be present as impurities and decomposition products of raw materials such as amine oxides and some preservatives)

- Morpholine (which may be present as an impurity and decomposition product of certain preservatives)

Hence, monoalkanolamines, monoalkylamines, trialkanolamines, trialkylamines, their salts and fatty acid dialkylamides and dialkanolamides are subject to specific restrictions in Annex III of the Cosmetics Directive. These apply to their minimum purity, maximum secondary amine content, maximum nitrosamine content, storage in nitrite-free containers, use levels and avoidance of nitrosating systems.

4.3 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 nitrosamine formation in all possible product formulations and suitable inhibition strategies must be must be evaluated for each product type.

General guidelines 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.

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

- Nonionic or cationic emulsions require larger amounts of inhibitors than do anionic emulsions, regardless of the solubility characteristics of the amine.

- 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 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 1.

5. ANALYTICAL METHODS

5.1 Apparent Total Nitrosamine Content (ATNC)

Description of the method

The ATNC method is a screening procedure for the analysis of cosmetic matrices. The method has been evaluated by the UK Cosmetic Toiletry and Perfumery Association (CTPA) and the results of a collaborative study have been published[3].

Samples are dissolved or suspended in water or aqueous tetrahydrofuran (THF), and nitrite/nitrite ester interferences are removed by prior treatment with sulphamic acid. The treated test solution is denitrosated 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 carried out by comparison with an external standard (e.g. N-nitrosodiethanolamine, N-nitrosodiisopropylamine). The authors claim a determination limit for N-NO of 10 µg kg^{-1}.

Advantages and limitations

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 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 nitrosamines present.

[3] A Screening procedure for total N-nitroso contaminants in personal care products: results of collaborative studies undertaken by a CTPA Working Group. Challis, B.C., Cromie, D.D.O., Guthrie, W.G., Pollock, J.R.A., Taylor, P., Telling, G.M., Wallace, L. International Journal of Cosmetic Science **17**(6) 219-231 (1995).

5. 2 Methods for NDELA

5.2.1 NDELA by Gas Chromatography – Thermal Energy Analysis

Description of the method

The method for N-nitrosoalkanolamines[4] 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 (TEA).

Sample is dissolved in water and an internal standard (e.g. N- nitroso-(2-hydroxyethyl)-(2-hydroxypropyl)-amine) added. 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 mixture and transferred to a silica gel column, the column is then washed and eluted with acetone.

The eluate is dried and the residue is treated with N-methyl N-trimethylsilyl-l heptafluorobutyramide. (MSHFBA) to convert nitrosamines to volatile derivatives. The MSHFBA derivates are separated by gas chromatography and detected using a Thermal Energy Analyser. In the TEA the nitrosamines are cleaved by pyrolysis to release nitrosyl radicals, which are detected in a chemiluminescence reaction with ozone. Recovery of the internal standard is typically 95% and the authors claim a determination limit for NDELA of 5 µg kg^{-1}.

Advantages and limitations

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

The disadvantage of this method is that it can be prone to give false positives. Particular care is required to avoid artefactual nitrosamine formation. Traces of NOx absorbed onto the Kieselguhr column can result in nitrosamine formation during sample clean-up where samples contain free secondary amines. This can be minimised by using inhibitors such as ascorbic acid.
This method is also the most time consuming of the specific methods for NDELA.

[4] A method for the determination of N-nitrosoalkanolamines in cosmetics. Sommer, H., Eisenbrand, G.Z Lebensm Unters Forsch (1988), **186**, 235-238

5.2.2 NDELA by HPLC – post-column derivatisation

This method has been collaboratively evaluated by the CTPA nitrosamine working group[5].

Description of the method

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 can be employed.

The NDELA in the sample extract is then separated from the matrix on a reversed phase column. Post-column derivatisation of the NDELA is performed via photolysis at 254nm (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 540nm.

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. The CTPA collaborative trial demonstrated that the method can accurately and reliably quantify NDELA in a wide range of cosmetic matrices for which, under optimum conditions, this method gives limits of detection and quantification of 5 and 10 µg kg^{-1} respectively.

Advantages and limitations

This method has the following advantages: it is simple to set up, has a relatively rapid sample preparation time, has good accuracy and sensitivity.

The method is specific to NDELA vs other nitrosamines. However, in some cases, where certain oxidisable dyes are present in the formulation, care must be taken to follow the procedure proposed by the authors to ensure specificity.

Calibration using Internal Standard is not recommended for this method since there is potential for slightly different recovery of internal standard from the sample matrix.

5.2.3 NDELA by HPLC/MS/MS.

Description of the method

This method[6] utilises a similar sample preparation methodology and chromatographic separation as described in section 4.2.2. Detection and

[5] A method for the determination of N-nitrosodiethanolamine in personal care products - collaboratively evaluated by the CTPA Nitrosamines Working Group. Flower, C., Carter, S,. Earls, A., Fowler, R., Hewlins, S., Lalljie, S., Lefebvre, M., Mavro, J., Small, D., Volpe, N. International Journal of Cosmetic Science,(2006), **28** , 21-33

[6] Determination of N-nitrosodiethanolamine in cosmetic products by LC-MS-MS. Schothorst, R. C., Somers, H. J. Anal Bioanal Chem (2005), **381**, 681-685

quantification of NDELA is carried out using a triple quadrupole mass spectrometer (MS/MS).

The use of HPLC coupled to tandem 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 µg kg^{-1} respectively, depending on the equipment and matrices.

Advantages and limitations

The principal advantage of this method is that it is the only method providing an unequivocal identification. However, this method is less sensitive than the HPLC post-column derivatisation method and the GC-TEA method.

5.3 Determination of other specific nitrosamines

In cases where the presence of specific nitrosamines other than NDELA is suspected, it may be possible to adapt the above methods for such analytes. However, in such cases it is necessary to provide sufficient evidence to verify the method performance. This could include accuracy, precision, recovery, detection limit and limit of quantification.

Particular care should be given to demonstrating specificity and data to support identification of the target compound should also be given.

6. ANALYTICAL APPROACH

6.1 Screening

The ATNC method serves as a useful screening tool for the assessment of nitrosamines in cosmetic products and raw materials. It is relatively simple and inexpensive to set up and application to majority of cosmetic samples is possible. Since the method is not susceptible to false negative results, if samples are shown to be below acceptable limits further testing should not be required.

Due to the potential for interferences from non N-nitroso compounds, a positive result above acceptable limits should be confirmed by analysis using a specific technique wherever possible. Where the nature of the cosmetic matrix suggests that NDELA or other specific nitrosamines may be the cause of a positive in the ATNC, the application of any of the methods described in sections 5.2 and 5.3 is appropriate.

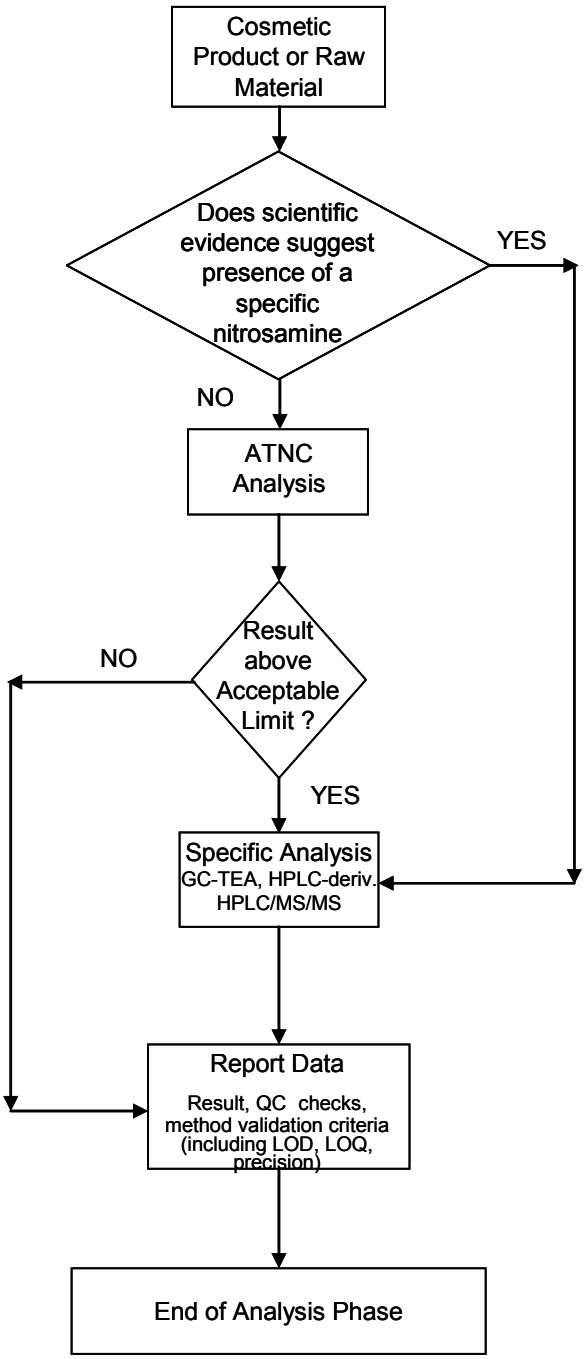
6.2 Measurement of specific nitrosamines

The GC-TEA methodology is very specific, however it is time consuming and may result in *in situ* nitrosamines formation as discussed. The HPLC post-column derivatisation and HPLC/MS/MS methods are both subject to standardisation by ISO and should therefore be the methods of choice especially if discussions with regulatory authorities are likely.

When using these methods it is necessary to include appropriate quality control checks such as fortified recovery samples to demonstrate satisfactory method performance.

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

Figure 1. Analysis of Cosmetic Products and Raw Materials (flowchart)



Note: 16 ug kg^{-1} (ppb) in ATNC method is equivalent to 50 ug kg^{-1} (ppb) NDELA

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This list has been compiled to the best of the authors' knowledge; it may be not exhaustive and, therefore, it is subject to regular update.

ANNEX 1 Compounds reported to act as inhibitors

A wide range of materials have been reported in the literature as inhibiting the formation of N-nitroso compounds under certain conditions. These materials are listed below, but it must be emphasised that the effectiveness of any potential inhibitor must be established for each individual application. Under the European Cosmetics Directive, use of any of these inhibitors is considered as introduction of a cosmetic ingredient and the requirements regarding product safety and product information as well as specific ingredient regulations needs to be considered.

Water Soluble Inhibitors

Ascorbic acid
Gentisic acid
Cysteine
Erythorbic acid
Glutathione
Hydroquinone*
Magnesium ascorbyl phosphate
Ethanolamine
Potassium sorbate
Sulphamic acid
Sodium adipate
Sodium ascorbate
Sodium ascorbyl phosphate
Sodium bisulfite
Sodium citrate
Sodium erythorbate
Sodium tartrate

* the use of hydroquinone is subject to specific restrictions under the Cosmetics Directive.

Oil Soluble Inhibitors

Ascorbyl palmitate
Tocopherol
Di-*t*-butyl hydroquinone
Ethoxyquin
Octyl gallate
Propyl gallate

Water/Oil Soluble Inhibitors

Gallic acid

Inhibitors of Formaldehyde-Catalysed Reactions

Effective inhibition requires the use of combinations of inhibitors that prevent iminium ion formation as well as scavenging nitrite. Suitable iminium ion traps are citrate, adipate and tartrate anions. Suitable nitrite scavengers to be used in conjunction with the iminium ion traps are ascorbic acid and its salts, erythorbic acid and its salts, sodium ascorbyl phosphate or magnesium ascorbyl phosphate. These latter two are the preferred nitrite scavengers from the aspect of stability in formulations.

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