A method for the determination of N-nitrosoalkanolamines in cosmetics

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Ein Bestimmungsverfahren für N-Nitrosoalkanolamine in kosmetischen Mitteln


Summary. A method for the determination of N-nitrosoalkanolamines in cosmetics and toiletries is described. The ingredients used in their manufacture N-Nitroso-bis-(2-hydroxyethyl)amine (N-Nitrosodiethanolamine, NDELA) and N-Nitroso-bis-(2-hydroxypropyl)amine (NBHPA) are almost exclusively the contaminants. The method has therefore been modified for their determination. N-Nitroso-(2-hydroxy-ethyl)-(2-hydroxypropyl)amin is used as the internal standard. After dilution with water, the cosmetic is adsorbed onto a kieselguhr column and extracted with n-butanol. The extract is brought to dryness, re-dissolved in chloroform/aceton (5+1) and transferred to a silica gel column. The column is washed and eluted with acetone. The eluate is dried and the residue is treated with N-methyl-N-trimethylsilyl-heptafluorobutyramide. Trimethylsilyl (TMS)derivatives of N-nitrosoalkanolamines are determined by gas-chromatography with a thermal energy analyzer (TEA). Recovery of the internal standard is 95% and the determination limit is 5 µg/kg. Repeated analyses of a foam bath, spiked with 30 µg/kg NDELA, gave an average content of 32 µg/kg NDELA (variation coefficient 8.8%; n=10). In order to avoid artefact formation during the clean-up process, kieselguhr containing 50% sodium ascorbate has to be used when cosmetics containing free dialkanolamines are analyzed.

Introduction

N-nitrosoalkanolamines, such as NDELA and NBHPA have been shown to be potent carcinogens in several animal species [1–7]. These nitrosamines have been detected as contaminants in a variety of environmental media, especially NDELA which has been found in cutting fluids [8], pesticides [9], tobacco products [10] and in cosmetics [11, 12]. The reaction of nitrosating agents with secondary or tertiary amines yields the corresponding nitrosamines. Secondary amines are especially used as ingredients or formulation aids in cosmetics, but they are also present as contaminants. They very rapidly react with traces of nitrosating agents to form the corresponding nitrosamines. Recently, the Federal Health Office in Germany (Bundesgesundheitsamt) issued a recommendation to the manufacturers of cosmetics and toiletries to stop using free secondary amines [13]. However, bis(2-
hydroxyethyl)amine (diethanolamine) is used for the production of a wide spectrum of long-chain amides, such as coconut fatty acid amides, which are important ingredients in various cosmetics.

Methods for the analysis of NDELA have been previously reported. They are comprised of enrichment steps using silica gel [14–16], ion-exchange columns [11, 17] or other extraction methods [18, 19]. For quantification, the thermal energy analyser (TEA) is used in most cases and is coupled to a gas chromatograph (GC) or a high pressure liquid chromatograph (HPLC) [14, 15, 20]. Other methods, such as photographic assay of the nitrite liberated from a nitrosamine by photolytic or acidolytic cleavage [18], GC-electron capture detection of derivatives [16] or HPLC in combination with pulse polarography [19] have been used less frequently.

Experimental

Reagents

The reagents were of analytical grade, unless otherwise specified. They consisted of: silica gel 40 (0.063–0.200 mm) from Merck (Darmstadt, FRG), redistilled n-butanol and redistilled (twice) ethyl formate. – Solvent 1 contained cyclohexane/dichloromethane (1 + 1) and solvent 2 contained chloroform/acetone (5 + 1). – The kieselguhr column was packed with Extrelut (Merck, Darmstadt, FRG) or Chem-Elut (ICT, Frankfurt, FRG). – The chemicals used were: ammonium sulphamate, sodium sulphate, ascorbic acid and sodium chloride; N-methyl-N-trimethylsilyl-heptafluorobutyramide (NDELA) respectively.

Apparatus

Chromatography columns: glass 30 × 200 mm, teflon stop cock, frit P 3, grad. receivers [21]; splash head adapter (Fig. 1) Gas chromatography: A Hewlett-Packard (5880 A Series) Gas chromatograph connected to a thermal energy analyser (Thermo Electron Corp. Waltham, Mass., USA, Model 502) was used. – Kieselguhr column: packed tightly (using a vibrator) with 12 g Extrelut or Chem-Elut. – Second column: The bottom layer consisted of sodium sulphate (6 g) wetted with methanol/acetone (1 + 1) and the upper layer consisted of silica gel 40 (10 g) suspended in the same solvent (40 ml); the solvent was displaced by solvent 2.

Extraction and clean up

The sample (2 g) was dissolved in water (9 ml) and the internal standard, NEPHA (100 µl) and ammonium sulphamate (0.5 g) were added. The mixture was saturated with sodium chloride to break up the emulsion. In some instances, the emulsion remained stable. If this was the case, the pH was adjusted to 8.5–9.5 with NaOH and 1.5 ml chloroform was added. The mixture was applied to the kieselguhr column and the flask was rinsed with water (3 ml) which was added to the column. After equilibration (20 min) the column was washed with 100 ml solvent 1. The nitrosamines were eluted with n-butanol (150 ml) and rotary evaporated (40 °C). The vacuum was increased slowly towards the end of the evaporation so as to prevent foaming. The residue, taken up in solvent 2 (20 ml) was transferred to a second column. The flask was rinsed and the column washed with the same solvent (80 ml total). The nitrosamines were eluted with acetone (50 ml), rotary evaporated to 5 ml and transferred quantitatively into a receiver. The flask was rinsed with a small volume of acetone and the rinsing added to the final solution. The solvent was removed by a nitrogen stream.

Silylation

MSHFBA (150 µl) was added to the residue, allowed to stand for 45 min at room temperature and made up to 0.5 ml with i-octane. The external standard used was 100 ng NDELA plus NEPHA. The solvent was removed under nitrogen and derivatized in the same way.

GC Conditions

The column was 6% OV 275 on Volaspher A 2 (2 mm × 3 m) silanized glass. The carrier gas was He (20 ml/min). The injector temperature was 200 °C and the temperature programme consisted of an initial temperature of 150 °C (1 min) which was programmed at 2 °C/min to 170 °C, then at 10 °C/min to 220 °C. The pyrolyser temperature was in the range 400 °C–500 °C using an injection volume of 5 µl.

Results and discussion

Cosmetics contain a wide spectrum of surface active agents, including neutral, anionic, cationic or amphiphilic detergents in varying compositions. Therefore, to achieve reproducible and high recoveries when analysing a series of samples by GC, a thorough clean-up procedure is necessary. Satisfactory recoveries are obtained by breaking up the emulsions before proceeding further. In most instances, this can be achieved by NaCl saturation; however some samples need further treatment, as described. For on-column extraction of NDELA, n-butanol turned out to be the best choice of solvent, giving the highest yields as compared with a wide series of solvent systems examined. The first clean-up step with solvent 1 removes less polar constituents. Further clean-up is achieved by washing the second column with solvent 2 before elution of the nitrosamines with acetone, a step that removes most of the emulsifiers.

For trimethylsilylation, the conditions described were found to be appropriate; heating in a closed system at 80 °C did not improve the yields.