Journal of Chromatography, 206 (1981) 158–163
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 13,382

Note

Determination of N-nitrosodiethanolamine in cosmetics by gas chromatography with electron capture detection

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(First received August 6th, 1980; revised manuscript received October 2nd, 1980)

N-Nitrosodiethanolamine (NDELA) has been shown to be weakly carcinogenic in rats after oral administration[1] and in hamsters after subcutaneous injections[2]. Hepatocellular carcinomas and renal adenomas are induced in rats, and adenocarcinomas of the nasal cavity, papillary tumours of the trachea, hepatocellular adenomas and local fibrosarcomas are produced in hamsters. NDELA has also been reported to be mutagenic on *Actinomyces* either *olivaceus* or *griseoflavus*[3], as well as on *Salmonella typhimurium* strains TA 100 and TA 1535 in the absence of a metabolic activation system[4].

NDELA has been detected in the urine of a human wearing a NDELA-contaminated facial cosmetic, thereby demonstrating its ready absorption in the skin[5]. Very high levels (up to 3%) of NDELA have been found in nitrite–triethanolamine based cutting and grinding fluids used in metal working[6,7]. Levels up to 4.9×10^4 ppb have also been shown to be present in facial cosmetics, hand and body lotions and hair shampoos[8]. Several methods have been described for the analysis of NDELA, including high-performance liquid chromatography combined with a thermal energy analyser (TEA), and gas chromatography (GC) coupled either with a TEA or with a high resolution mass spetrometer (MS).

As those sophisticated instruments are not available in most laboratories, we have developed a method for the determination of NDELA using conventional GC with electron capture detection. This sensitive and selective method has been applied to the detection of NDELA present at the ppb level in several cosmetics and dermopharmaceuticals.

EXPERIMENTAL

Reagents

All reagents were of analytical grade. Diethanolamine, 2-chloroethanol, 3-hydroxy-*n*-propylamine, thionyl chloride and trifluoroacetic anhydride were obtained from Aldrich Europe (Beerse, Belgium) and hydrogen peroxide from Solvay (Brussels, Belgium). The reagents were used without further purification, except for thionyl chloride and trifluoroacetic anhydride which were purified by distillation.

NDELA and N-nitrosopropanolethanolamine (NPELA), used as the internal

0021-9673/81/0000–0000/$02.50

standard, were synthesized by reaction of the corresponding amines with sodium nitrite according to Jones and Wilson[9] with minor modifications: the pH of the medium was maintained at the optimal value of 3 and the N-nitrosamines were purified by extraction with *p*-dioxan after elimination of water and solvents under vacuum. Their purity was checked by thin-layer chromatography (TLC) and GC–MS. *n*-Propanolethanolamine was obtained by direct reaction between 2-chloroethanol and 3-hydroxy-*n*-propanolamine in water at 120°C during 24 h, and subsequently purified.

Peroxotrifluoroacetic acid (PTFA) reagent was prepared as follows: 0.5 ml 98% hydrogen peroxide was added to 7 ml ethyl acetate kept at 0°C. Five 0.5-ml aliquots of trifluoroacetic anhydride were successively added under constant shaking to the solution cooled at 0°C. The reagent was kept in the dark and in ice; a new amount was prepared every week.

Apparatus and conditions

A Perkin-Elmer Model 3920 gas chromatograph, equipped with a ^{63}Ni electron capture detector (ECD) was used. A spiral borosilicate glass column (2 m × 2 mm I.D.) packed with 3% OV-225 on Supelcoport (100–120 mesh) was employed. The operating conditions were as follows: column temperature, 210°C; injector and detector temperature, 250°C; carrier gas (argon–methane, 95:5) flow-rate, 100 ml/min; standing current for the ECD, 2 nA.

A LKB 9000 S gas chromatograph–mass spectrometer was used for confirmation of the identity of the N-nitrosamine derivatives. The operating conditions were as follows: spiral glass column (2 m × 3 mm I.D.) packed with 1% OV-1 on chromosorb W (60–80 mesh); carrier gas (helium) flow-rate, 30 ml/min; oven temperature, 200°C; injector temperature, 230°C; separator temperature, 240°C; ion-source temperature, 270°C; trap current ,60 nA; energy, 70 eV; accelerating voltage, 3.5 kV.

Clean-up procedure

In order to prevent nitrosation artifacts during the analytical procedure, ammonium sulphamate (1 g) was added to 1 g of the cosmetic to be analysed; NPELA (100 μl of an aqueous solution of 1 μg NPELA/ml) was added as the internal standard. The sample was stirred at 30°C for 20 min in a mixture of 10 ml of 2,2-dimethoxypropane, 25 ml of methyl *tert.*-butyl ether and 100 μl of 10% aqueous oxalic acid solution. The mixture was then neutralized with an excess of calcium carbonate and the solvent removed under vacuum in a rotary evaporator operated at 30°C.

The residue was extracted four times with 50 ml light petroleum (b.p. 45–60°C) and four times with 50 ml ethyl acetate. The extracts were successively loaded on a silica gel column (20 × 2 cm, Merck silica gel 60A, 70–230 mesh). Ethyl acetate (150 ml) was used to wash out the impurities and 250 ml acetone subsequently eluted the N-nitrosamines. The acetone fraction was evaporated to dryness under vacuum at 50°C and the residue dissolved in 5 ml acetone.

Derivatization

A 2-ml aliquot of the final acetone solution was evaporated under a stream of nitrogen in a reaction vial; the residue was dissolved in 2 ml ethyl acetate, 10 μl of thionyl chloride were added and the vial was then heated at 55°C for 1 h. The organic phase was washed by shaking with 1 ml of 1 *M* sodium hydroxide and the two phases

were separated after freezing the aqueous phase at −50°C. A 200-μl volume of the PTFA reagent was added to the ethyl acetate solution, followed by standing at 55°C for 1 h. The organic solution was washed with 1 ml of 1 *M* NaOH and an aliquot (1 μl) of the reaction mixture used for GC analysis.

Photolysis

Two aliquots (1 ml) of the acetone solution were evaporated to dryness under nitrogen. The residues were dissolved in 500 μl ethyl acetate and the solution placed in photolysis tubes of soft glass (16 × 0.25 I.D. × 0.35 cm O.D.) which were then flame sealed. One tube was irradiated with UV light for 3 h; the other was kept in the dark for the same period and served as a control. The UV light was emitted from a 8-W germicide lamp situated 4 cm from the samples and submitted to a oscillatory movement so as to make the irradiation uniform. The solutions were then diluted in 1.5 ml of ethyl acetate and 10 μl of thionyl chloride, heated at 55°C for 1 h and finally derivatized as above.

RESULTS AND DISCUSSION

Denitrosation of N-nitrosamines with thionyl chloride in methylene chloride, leading to the formation of nitrosyl chloride, has been used by others[10] as a method for the analysis of non-volatile nitrosamines. It was therefore essential to carefully select the reaction conditions (nature of the solvent, temperature and duration of the treatment, thionyl chloride concentration) to enable the specific transformation of NDELA into volatile bis(2-chloroethyl)-N-nitrosamine with minimal denitrosation.

The reaction has been carried out in different solvents and the products examined by TLC. Bis(2-chloroethyl)-N-nitrosamine was found to be the only derivative formed when ethyl acetate was used as the solvent; in other solvents, such as dichloromethane or diethyl ether, two or more other products were present. Under our conditions, 5 μg thionyl chloride/ml has been found to be the optimal concentration. The results presented in Fig. 1 indicate that the reaction is complete after standing for 1 h at 55°C. At higher temperatures the derivative formed starts to break down.

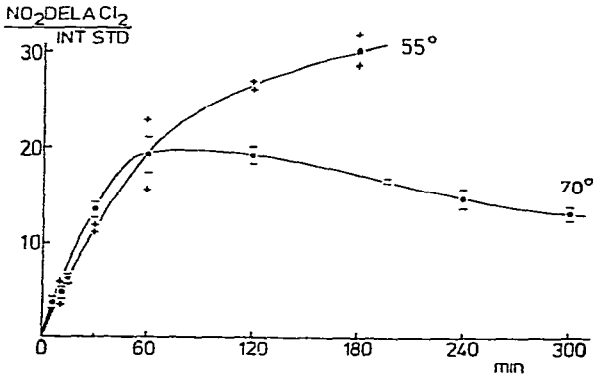

Fig. 1. Effect of time on the reaction of $SOCl_2$ (8 mg/ml) with NDELA (5 μg/ml) at 55 and 70°C.

Fig. 2 indicates that similar reaction conditions were also the most appropriate for the subsequent transformation of bis(2-chloroethyl)-N-nitrosamine into the corresponding nitramine with the PTFA reagent. Moreover, the utilization of ethyl acetate as solvent permits aliquot samples to be taken of the organic phase, previously washed with a sodium hydroxide solution, for direct injection into the gas chromatograph–ECD; the hydroxide solution is used to destroy the excess of PTFA reagent.

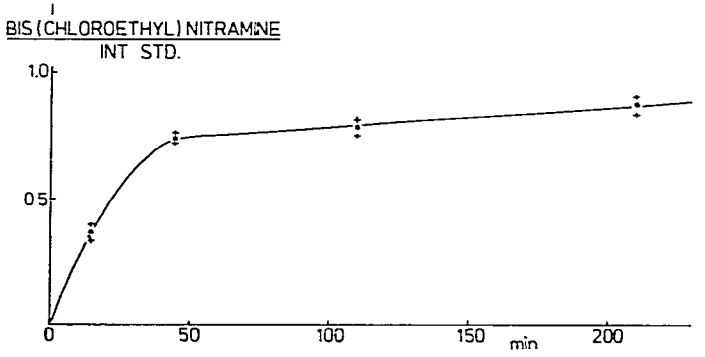

Fig. 2. Effect of time on the oxidation of bis(2-chloroethyl)-N-nitrosamine to nitramine with peroxotrifluoroacetic acid (100 μl PTFA reagent per ml mixture) in ethyl acetate at 55°C.

When pure NDELA was treated, a linear relationship was found between ECD response and NDELA concentration, from 0 to 200 ng NDELA/ml. The sensitivity of the method allows the determination of less than 5 ng NDELA/ml.

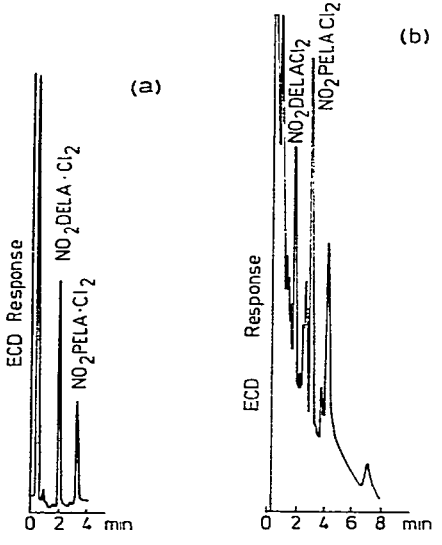

Fig. 3. Gas chromatograms: a, pure NDELA and NPELA (500 ng/ml of each) after derivatization; b, NDELA (1 ppm) after extraction from a cosmetic preparation and derivatization (sample 1, Table I).

With cosmetic samples, the chromatograms are more complex and it is not possible to determine less than 50 ng NDELA/ml (Fig. 3). The concentrations of NDELA present in the analysed samples were computed from a standard graph (Fig. 4) constructed from chromatograms of cosmetic preparations containing known added amounts (0–400 μg/kg) of NDELA and a fixed amount (600 μg/kg) of NPELA used as internal standard. The results obtained are presented in Table I, and were

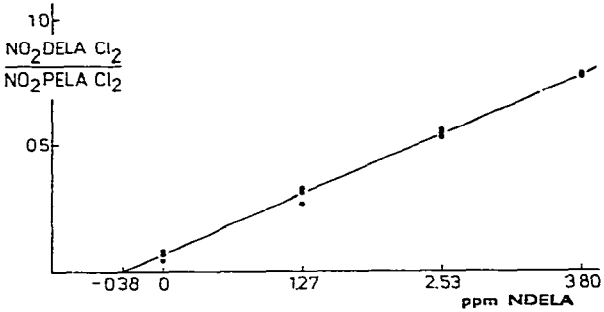

Fig. 4. Calibration curve for NDELA in cosmetic preparation, obtained by addition of increasing amounts of NDELA to sample (1 in Table I).

TABLE I

LEVELS OF NDELA (μg/kg) IN COSMETIC AND DERMOPHARMACEUTICAL PREPARATIONS COMMERCIALLY AVAILABLE IN BELGIUM

For each preparation, two different samples purchased in separate shops were submitted to analysis. n.d. = Not detectable.

Preparation	*Utilization*	*NDELA (μg/kg)*
	Cosmetics	
1	Cream	350
		350
2	Cream	n.d.
		n.d.
3	"For night" cream	100
		380*
4	Cream	100
		100
	Dermopharmaceutical preparations	
5	Slimming gel	n.d.
		n.d.
6	Cleaning gel for eyes	
	Lot J810	300
	Lot T826	300
7	Cream	n.d.
		200
8	Cream (1.34% triethanolamine)	170
		250
9	Cream (1.3% triethanolamine)	110
		220
10	Cream (1.35% triethanolamine)	100
		100

* Confirmed by photolysis.

confirmed for samples with the highest levels of NDELA by photolysis according to Doerr and Fiddler[11].

ACKNOWLEDGEMENT

This work was supported by a grant from the Centre de Recherche et d'Information des Consommateurs (C.R.I.O.C.).

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