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Screening method for determining the presence of N-nitrosodiethanolamine in cosmetics by open-tubular capillary electrochromatography

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Abstract

The presence of the carcinogenic N-nitrosodiethanolamine (NDELA; CAS No. 1116-54-7) in cosmetic samples was determined using an etched, C_{18} modified capillary in the open-tubular capillary electrochromatography technique. A very simple extraction procedure leads to a sample matrix free from interferences. The calibration curve was created using UV detection at 214 nm. The detector response was linear in the range of 5–120 ppm total amount injected. Minimum detection limits (1 ppm NDELA injected on capillary) are suitable for screening a large number of cosmetic samples. Diethanolamine and triethanolamine precursors of nitrosamines are not detected at the wavelength used. Cosmetic samples were analyzed unspiked and after addition of 60 ppm of NDELA. In spiked samples recoveries varied from 94% (hand and body lotion) to 55% (lipstick sample). NDELA was found in unspiked samples of old (5–15 years old) cosmetics at concentrations of 14.0 ppm and 35.0 ppm.

Keywords: Cosmetics; Electrochromatography; Nitrosodiethanolamine

1. Introduction

N-Nitroso compounds are notorious mutagens and carcinogens that can be seriously hazardous to human health [1–3]. The possible presence of N-nitrosamines and their precursors in cosmetic products has created a constant need for the detection and positive identification of these compounds at the ppm level. Thermal energy analysis (TEA) interfaced with high-performance liquid chromatography (HPLC–TEA) or gas chromatography (GC–TEA), a chemiluminescence detector, is the most widely used system for nitrosamine analysis [4–7], but there are other detection methods as well [8–11]. Diethanolamine (DEA; CAS No. 111-42-2) and triethanolamine (TEA; CAS No. 102-71-6) based detergents are commonly used in cosmetics and toiletries including lotions, shampoos, creams and laundry soaps. They work as emulsifiers and wetting agents in many personal care products. N-Nitrosodiethanolamine (NDELA; CAS No. 1116-54-7) can be formed in these products by interaction of DEA or TEA with nitrite preservatives, such as 2-bromo-2-nitropropane-1,3-diol (Bronopol) or contaminants present in cosmetics [12,13]. NDELA is readily absorbed through the skin and accumulates in organs, such as the liver, bladder, etc. where it induces chronic toxic effects [14–18]. DEA and

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TEA themselves are not carcinogens, but they may irritate the skin and mucous membranes.

Cosmetics are difficult, often multicomplex emulsion systems, from which trace amounts of nitrosamines have to be extracted. Numerous methods describing suitable extraction procedures have been developed and published [12,19,20]. The FDA (US Food and Drug Administration) recognized the potential dangers of NDELA in cosmetics. In a study of more then 300 cosmetic samples analyzed in 1979 and early 1980 in the FDA's laboratories, 26% contained 30 ppb to 2 ppm and 7% contained between 2 and 150 ppm. In surveys of cosmetics products conducted in 1991–1992, NDELA was found in 65% of the samples at levels up to 3 ppm [21].

This manuscript deals with the application of the open-tubular capillary electrochromatography (OT-CEC) technique for the determination of NDELA in cosmetics at the ppm level. The proposed analysis can be used as a direct screening method for a large number of samples. The final confirmation of contamination of the cosmetics will require analysis by various established methods (i.e. GC–MS, HPLC–TEA or others). In our laboratory UV spectra were used to confirm contamination of the old cosmetic samples.

2. Materials and methods

All solvents used were HPLC grade. Deionized water was obtained from a Milli-Q water purification system. Triethoxysilane (TES), hexachloroplatinic acid, DEA and TEA were purchased from Aldrich (St. Louis, MO, USA). NDELA was obtained from Sigma (St. Louis, MO, USA). The buffer composition used was: pH 4.41, 0.3 *M* acetic acid+0.375 *M* γ-aminobutyric acid.

2.1. Analyzed cosmetic samples

1. Fresh sample: Klorane; wild cornflower eye make-up remover, Pierre Fabre, Paris, France.
2. Fresh sample; Charlie White Musk: scented body lotion, Revlon, New York, USA.
3. About 5-year-old sample; Charlie White Musk: scented body lotion, Revlon, New York, USA.
4. Fresh sample; Aloe Vera: moisturizing hand and body lotion, Laboratoires St. Ives, Geneva, Switzerland.
5. About 15-year-old sample; L'Air du Temps: perfumed hand cream, Nina Ricci, Paris, France.
6. Fresh sample; lipstick, double color, wine DC-30, Este Lauder, Belgium.

The experimental work was done using an Applied Biosystems 270A-HT capillary electrophoresis instrument. The temperature during the runs was kept at 30°C. Sample injections were made in the hydrodynamic mode at 5 in. (12 cmHg) vacuum. Detection was at 214 nm. The running buffer and samples were degassed with helium.

Previously described processes [22,23] were slightly modified (20 h drying with nitrogen was substituted by washing with acetone and drying for 0.5 h) and used to produce the etched C_{18} modified capillary used in this study. Before use the capillary was conditioned overnight with methanol and then by forcing at least 50 volumes of buffer through it with a syringe.

2.2. Extraction procedure

A modification of the method of Chou et al. [20] was as used as an extraction procedure for analysis of the unspiked and spiked cosmetic samples. A 1-g amount of the cosmetic product was dispersed in 10 ml of warm buffer, pH 4.41, and extracted in a separatory funnel with three 20-ml portions of methylene chloride. The aqueous (upper) layer was then separated from the methylene chloride (lower) layer. The final volume of buffer extract was brought up to 10 ml.

2.3. OT-CEC measurements

The prepared extract was directly injected onto the C_{18} modified etched capillary column (I.D. 50 μm) using hydrodynamic injection. After each 20 runs, the CEC capillary was flushed with water, methanol, water and running buffer for 5 min each using a vacuum.

Standard solutions containing 5, 10, 20, 40, 60, 80 and 100 ppm of NDELA in the pH 4.41 buffer were prepared for determining the minimum detection limit and linearity. Triplicate injections of NDELA

standards were analyzed by CEC using the C_{18} modified etched capillary at 25 kV.

3. Results and discussion

The OT-CEC separation was performed on a reversed-phase C_{18} etched modified capillary with pH 4.41 buffer. The pH of the buffer was chosen after analyzing a plot of EOF vs. pH for the capillary used (Fig. 1). Below pH 5.5 the electroosmotic flow was reversed (anodic). The exact cause of this effect is still being studied. Under these conditions all neutral species present in the cosmetic extract were carried out from the capillary never reaching the detector, which produces fewer peaks in the electrochromatogram. The NDELA peak was identified by its migration time and the presence of NDELA in extracts of cosmetics samples analyzed was confirmed by UV absorption spectra. Also the NDELA identification was confirmed using its extreme instability to UV light (after irradiation of the sample for 5 min with a mercury lamp the peak disappeared) [10].

Before the analysis of the cosmetic samples was possible the viability of quantitative determinations by the OT-CEC method was determined. Fig. 2 presents the calibration curve obtained for hydrodynamic injections of NDELA over the concentration range 5–100 ppm. The correlation coefficient is high, 0.999, indicating a good linear fit. The RSD for multiple determinations ($n=10$) of the same standard solution (40 ppm) was 1.9%. Below 5 ppm the peak area was lower than predicted by the calibration curve which may be due to adsorption of the sample on the capillary wall. This result indicates that not all surface silanols were converted to hydrides in the silation/hydrosilation process. Concentrations above 100 ppm were not studied, because C_{18} etched capillaries in the open tubular format have a low capacity and are easy to overload.

Fig. 3 shows electrochromatograms of different samples using 214 nm as the detection wavelength. The maximum absorbance of NDELA is at 234 nm, however at 214 nm fewer interfering compounds absorb and therefore this wavelength was chosen. Fig. 3A shows a 60-ppm sample of NDELA in the buffer. Next the viability of this method was checked

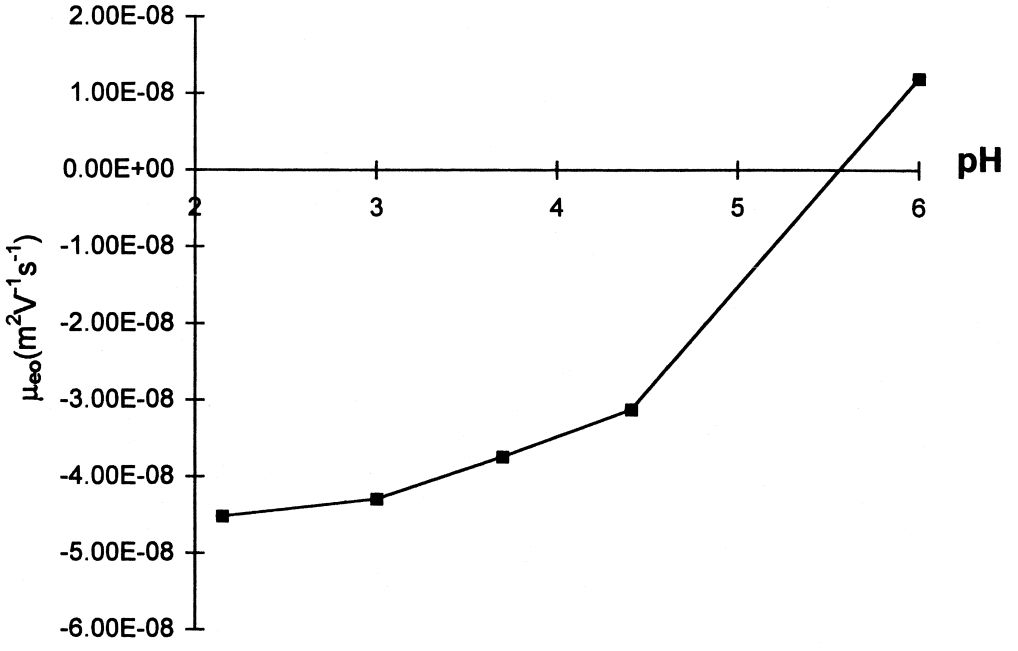

Fig. 1. Electroosmotic flow as a function of pH of the buffer in the etched C_{18} modified 50 μm I.D. capillary. Marker is dimethyl sulfoxide (DMSO) with detection at 211 nm. Each point is the average of three measurements.

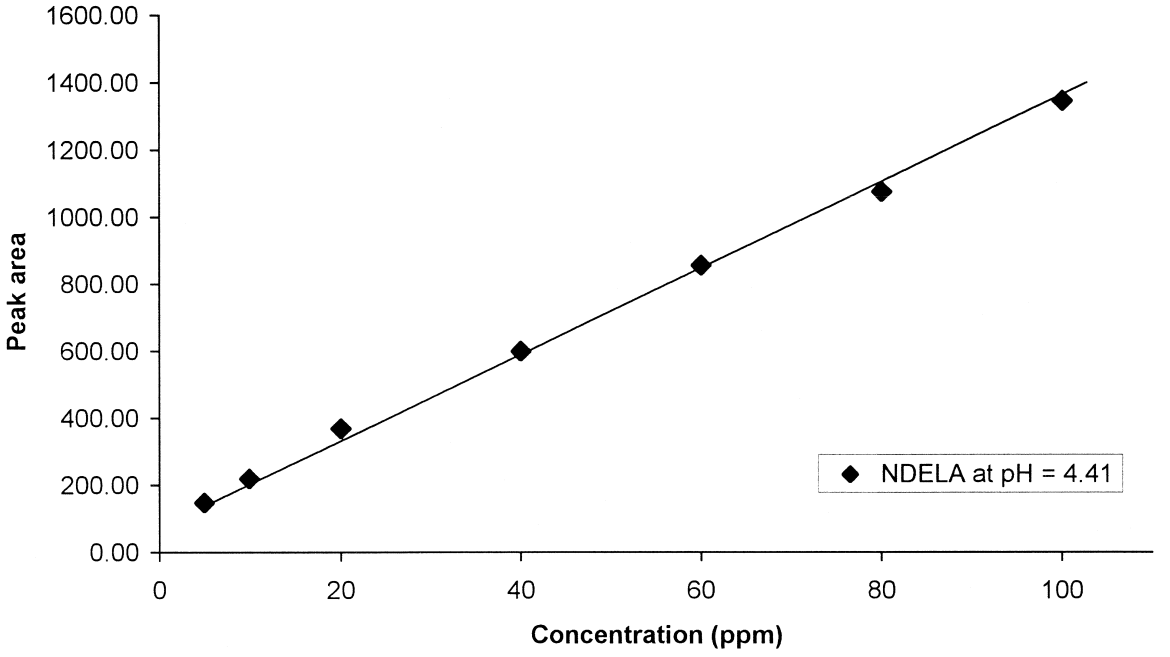

Fig. 2. Calibration curve for NDELA analysis ($y = 12.36x + 106.2$; $R^2 = 0.9991$). Conditions: column; C_{18} modified etched capillary, effective length $l = 25$ cm, total length $L = 47$ cm, buffer; pH 4.41, voltage; 25 kV, injection; 3 s, 5 in. (12.7 cmHg vacuum, 1 mmHg = 133.322 Pa). Detection at 214 nm.

on a real cosmetic — a sample of fresh Charlie White Musk body lotion was analyzed. The electrochromatogram in Fig. 3B is from the fresh body lotion after the extraction procedure described above. It shows no peaks in the eluting range of NDELA. Fig. 3C represents the same sample as in 3B spiked with 60 ppm of NDELA. The recovery was in the range 75–80% for this type of sample. Additionally a sample spiked with 120 ppm of DEA and TEA (common ingredients of cosmetic samples, which may interfere with NDELA analysis) was extracted and analyzed. There were no peaks. Several other samples of fresh cosmetics were extracted and analyzed and no peaks were detected around the elution time of NDELA. Finally two extracts of old (age of the sample was determined from personal records: there was no date on the containers) cosmetic samples were prepared. Fig. 3D shows the electrochromatogram of the same brand of body lotion as the fresh sample: Charlie White Musk — about 5-year-old sample. In this case a small peak can be seen at the migration time of NDELA. The identification of the compound was performed as described in Materials and Methods. The concentration was calculated as 14.0 ppm of NDELA in the extraction solution (140 μg per 1 g of the cosmetic sample extracted). An ~15-year-old sample of hand cream was also extracted and analyzed (Fig. 3E). In this case the concentration of NDELA was calculated as 35.0 ppm of NDELA. Table 1 shows the data for both cosmetic samples analyzed. Fig. 4A shows a 1 ppm sample of NDELA in the buffer and Fig. 4B represents an extract of the cosmetic sample Charlie White Musk spiked with 1 ppm of NDELA. An HPLC procedure of an extract containing 50 ppb of NDELA was reported [10]. In order for OT-CEC to meet this detection limit a more sensitive detector should be used (for example photodiode array detection) or the pathlength for the light should be increased (as in Hewlett-Packard bubble cells or Z cell). However, the OT-CEC procedure will facilitate the qualitative analysis of the sample and in case of analysis of large number of samples the solvent consumption would be greatly reduced.

Although the method is designed as a screening procedure for NDELA in cosmetic products it is

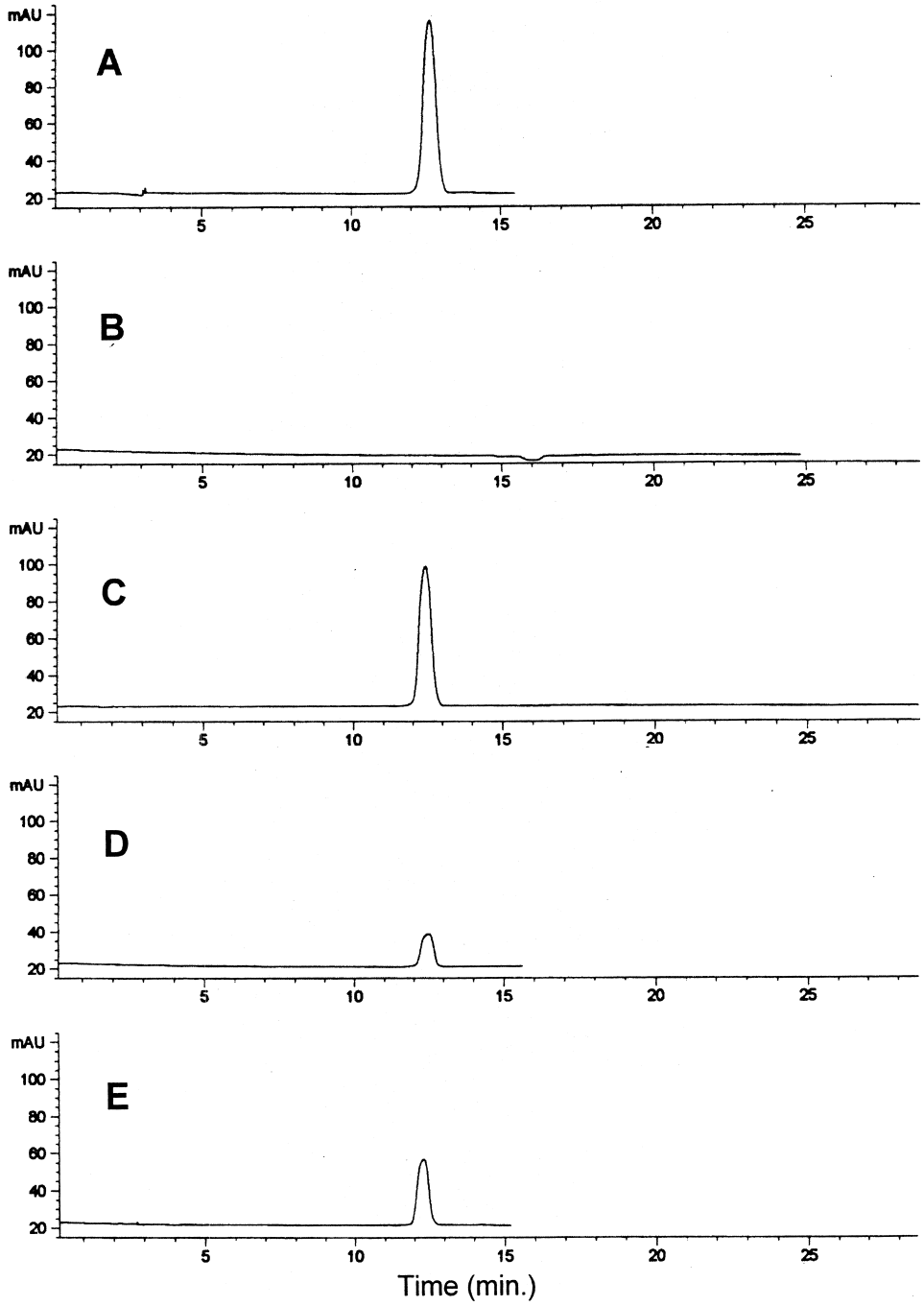

Fig. 3. Electrochromatograms of various NDELA and cosmetic samples. Conditions: same as Fig. 1. (A) 60 ppm NDELA sample in the pH 4.41 buffer; (B) extract of fresh Charlie White Musk body lotion, extraction procedure described in Materials and Methods; (C) same sample as in B spiked with 60 ppm of NDELA, (D) electrochromatogram of Charlie White Musk body lotion — about 5-year-old sample; (E) extract of about 15-year-old sample of L'Air du Temps hand cream.

Table 1
Determination of NDELA in cosmetic samples

Peak area	Area average	SD	RSD (%)	Calculated concentration (ppm)
White Musk not spiked (5-year-old sample)				
276.9	274.7	2.32	0.84	14.0
272.3				
274.9				
L'Air du Temps not spiked (15-year-old sample)				
551.8	542.0	9.00	4.92	35.3
540.3				
534.0				

useful to study the recovery of this compound for different matrices. Three types of samples were chosen and a series of recovery studies was conducted. A solution in water of 60 ppm of NDELA was added to each sample. The products were analyzed before recovery studies to determine if they contained any compounds which may give a positive response. All products after extraction did not show any peaks. The spiked products were extracted as described above and the buffer layer was analyzed for NDELA. Results are given in Table 2. From the data it is obvious that the extraction procedure cannot be used for the lipstick samples. In comparison to the other samples the lipstick has a large amount of undissolved solid that apparently retains a significant fraction of the analyte. The recoveries for the other two samples (liquid and solid) are good. Based on the data from Table 2, the OT-CEC method along with the extraction procedure can be recommended for the analysis of all types of body lotions and hand cream samples as well as all types of liquid cosmetic samples such as eye make-up removers, shampoos, etc.

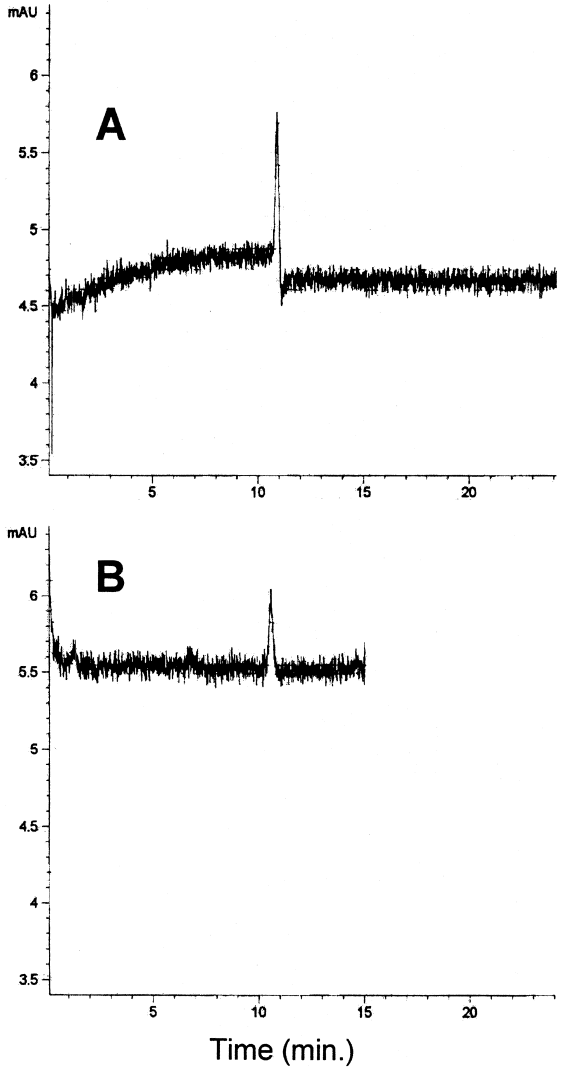

Fig. 4. Electrochromatograms of NDELA and cosmetic samples. Conditions: same as Fig. 1. (A) 1 ppm NDELA sample in the pH 4.41 buffer; (B) extract of fresh Charlie White Musk body lotion spiked with 1 ppm of NDELA, extraction procedure described in Materials and Methods.

4. Conclusions

The OT-CEC analysis presented here has been shown to be an easy method for routine screening for NDELA in cosmetic samples. A simple extraction procedure produced a sample matrix free of interferences. The detection limit can be improved by using more sensitive detection methods (for example photodiode array detection) capillaries with a bubble cell, detector with a Z cell or by reducing the sample volume of the extract by evaporation (from 10 to 1 ml). It is unfortunate that in many cases it is impossible to determine the production date of cosmetics even in the store. Results presented here indicate that most cosmetics have a finite shelf-life with respect to the formation of NDELA.

Table 2
Percent recovery in spiked cosmetic samples

Peak area	Area average	SD	RSD (%)	Calculated concentration (ppm)	Recovery (%)
Klorane spiked with 60 ppm of NDELA					
801.6 805.3 807.1	804.7	2.79	0.35	54.0	90.0
Aloe Vera spiked with 60 ppm of NDELA					
760.6 761.3 774.1	765.3	7.59	0.99	56.5	94.2
Lipstick spiked with 60 ppm of NDELA					
502.7 504.3 520.6	509.2	9.88	1.94	33.0	55.0

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