Analysis of N-nitrosodiethanolamine in linoleamide DEA by high pressure liquid chromatography and UV detection

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Synopsis

A method for the analysis of N-nitrosodiethanolamine in Linoleamide DEA which uses a simple sample preparation and MICRO-SAMPLING TECHNIQUES is presented. The nitrosamine is first separated from the bulk of the matrix by normal phase high pressure liquid chromatography followed by quantitation using reverse phase HPLC. Absolute limit of detection is 1 ng.

INTRODUCTION

For the past several years, the Cosmetic, Toiletry and Fragrance Association (CTFA) has been sponsoring contract research for the development of nitrosamine analytical methodology. The main area of concentration has been the development of methodology for the analysis of N-nitrosodiethanolamine (NDE1A) in cosmetic raw materials with the use of high pressure liquid chromatography and detection using a Thermal Energy Analyzer (TEA). Methodology for the determination of NDE1A by HPLC-UV detection was also developed and validated.

Although most of the alkanolamides of diethanolamine lent themselves to HPLC-TEA methodology, Linoleamide DEA was an exception. The major problem in the development of methodology was a large TEA responding peak appearing just before the NDE1A elution area when normal phase HPLC was used. Reverse phase HPLC using TEA detection was not attempted, because good separations on reverse phase packings are usually obtained with highly aqueous solvent systems. The TEA is not compatible with highly aqueous solvent systems, thus eliminating reverse phase HPLC separation.

Rosenberg and co-workers (1), as well as Rahn and Mitchell (2), have reported on the analysis of NDE1A using reverse phase HPLC with UV detection. Likewise, Rosenberg and co-workers (3) have recently developed a two column HPLC-UV method for the micro-isolation and quantitation of NDE1A by HPLC-UV in various cosmetic raw materials and finished product. In this note, we wish to present the applicability of this method for the determination of NDE1A in Linoleamide DEA.

EXPERIMENTAL

APPARATUS

A Waters Associates (Milford, MA) Model 6000A solvent delivery system equipped with a Model U6K injector and Model 440 absorbance detector fixed at 254 nm is used throughout the method. A Spectra Physics (Piscataway, NJ) Model SP4100 computing integrator is used for quantitation. The columns are a Waters Assoc. μPorasil column (4 mm I.D. × 30 cm) and a Whatman Inc. (Clifton, NJ) Partisil PXS 10/25 ODS (C_{18}) (4.6 mm I.D. × 25 cm). Pierce Chemical Co. (Rockford, IL) "Reactivials" are used for collection vessels.

SOLVENTS

The liquid chromatographic solvents were obtained from J. T. Baker Chemical Co. (Phillipsburg, NJ) and are "HPLC Reagent" grade methanol and chloroform. The distilled water was obtained from the Electrified Water Co. (Newark, NJ). All solvents are filtered using a Millipore filtration apparatus. The N-nitrosodiethanolamine (NDE1A) was obtained from Columbia Chemical Co. (Columbia, SC).

CHROMATOGRAPHIC CONDITIONS

Table I illustrates the conditions for each of two columns utilized.

PREPARATION OF STANDARD AND SAMPLE

A NDE1A standard was prepared by weighing 50 mg of NDE1A into a 100-ml flask and bringing to volume with a solution of $CHCl_3$/MeOH (95/5) (Standard A). A working standard for HPLC was prepared by pipetting 1 ml of Standard A into a 100-ml flask and bringing to volume with $CHCl_3$/MeOH (95/5) (Standard B). This working standard has a concentration of 5 ppm or 5 ng/μl. Linoleamide DEA samples were prepared by weighing 2.5–3.0 grams into a 10-ml flask and bringing to volume with $CHCl_3$/MeOH (95/5).

PROCEDURE

Inject 5 μl of Standard B into μPorasil column to establish elution volume of NDE1A and collect NDE1A area. Inject 50 μl of sample and collect NDE1A area. Evaporate

Table I
Chromatographic Conditions for Silica and C_{18} Columns

	Columns	
	μPorasil (silica)	Partisil PXS(C_{18})
Mobile Phase	95/5 (chloroform/methanol) V/V	H_2O (100%)
Flow Rate	1 ml/min	1 ml/min
Chart Speed	10 mm/min	10 mm/min
Detector Sensitivity	0.1–2.0 aufs	0.005 aufs

both standard and sample to dryness and reconstitute in 100 μl of H_2O. Inject 25 μl of reconstituted standard and sample into Partisil PXS column (C_{18}). NDE1A is quantitated using an SP4100 computing integrator (in the external standard method mode).

RESULTS AND DISCUSSION

Numerous methods for the trace analysis of nitrosamines have been developed and published (4). The primary technique used has been High Pressure Liquid Chromatography with detection by a Thermal Energy Analyzer. The majority of nitrosamines studied have been volatile and presented little separation problem using gas chromatography, TEA detection. However, NDE1A, a polar nitrosamine, is very amenable to separation by reverse phase liquid chromatography using either water/alcohol or water as the mobile phase. The TEA's inability to handle an aqueous mobile phase is thus alleviated by quantitating the nitrosamine with a UV detector. We have found that when reverse phase liquid chromatography is extended to the analysis of commercially available alkanolamide, it is sometimes difficult to separate NDE1A from other early eluting materials, resulting in high values for the detection limit. It is also accepted that low recoveries can occur when NDE1A is isolated by multi-extractions and open column chromatography. We have developed a system by which the NDE1A is
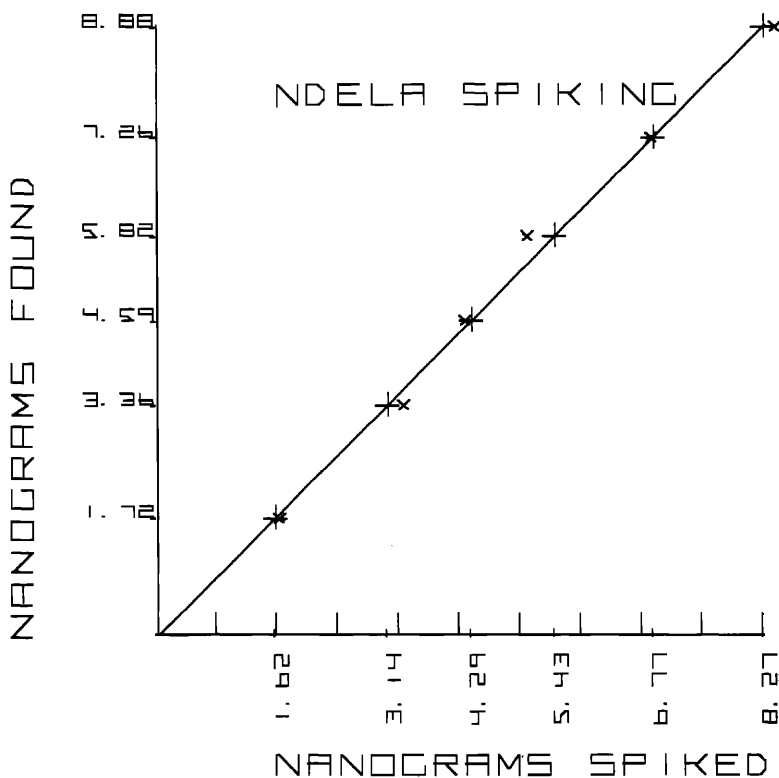

Figure 1. Linear regression line of linoleamide DEA spiked at six different levels.

isolated from the amide by normal phase HPLC, followed by quantitation on a reverse phase HPLC system (3).

Figure 1 shows a linear regression line of a sample of Linoleamide DEA spiked at six different levels, while Figure 2 shows the chromatogram of a spiked and unspiked sample. The lowest spiking level was a 1.687 ng spike, which gave 1.685 ng found in the sample. Our absolute limit of detection is 1 ng to the detector which, at a 30% dilution, gives a limit of detection in the sample of 267 ppb. By increasing sample size and final injection volume, the limit of detection for the sample can be increased without deterioration of the chromatography.

In conclusion, we have been able to determine NDE1A levels in this cosmetic raw material by a simple sample preparation, followed by micro-handling techniques. Solvent extraction and open column chromatography have been avoided and recoveries are close to 100%.
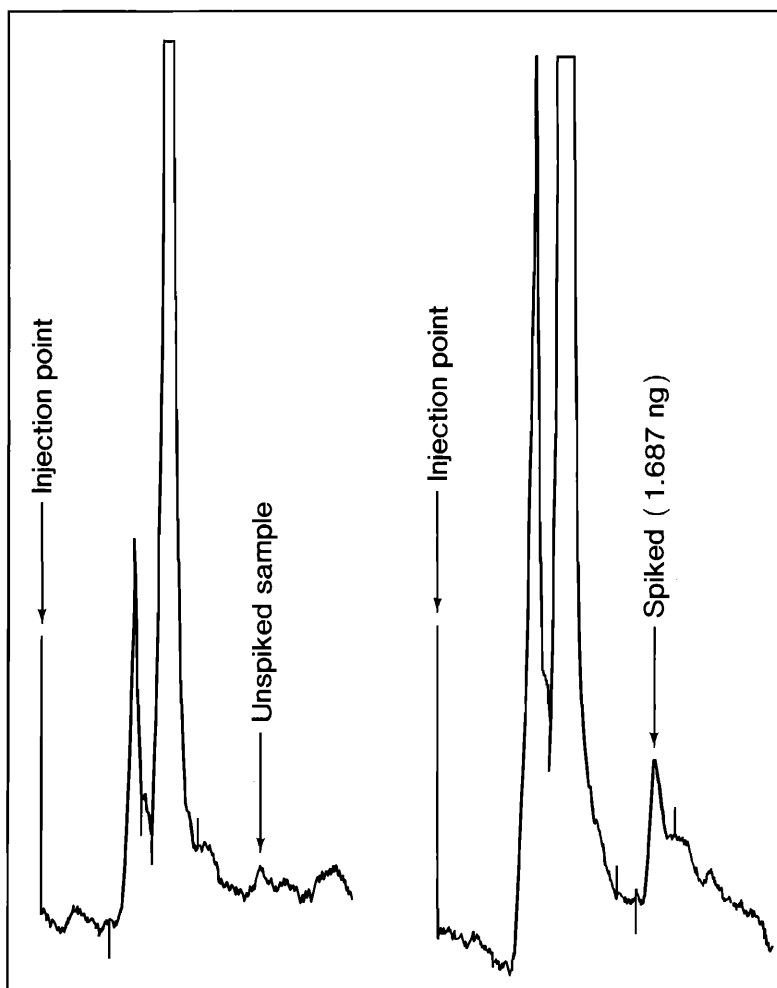

Figure 2. Reinjected collections of both spiked and unspiked samples of linoleamide DEA.

REFERENCES

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