Rapid and Sensitive Determination of N-Nitrosodiethanolamine in Baby Shampoo

Application Note

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Abstract

This application note describes a fast and sensitive quantification for N-nitrosodiethanolamine (NDELA) in baby shampoo by means of UHPLC liquid chromatography-electrospray ionization-tandem mass spectrometry in positive ion mode. An Agilent 6400 Series Triple Quadrupole LC/MS System was used in multiple reaction monitoring (MRM) mode. The transition was *m/z* 135.1 to 74.1 and confirmed by *m/z* 104.0. A linearity coefficient of $R^2 > 0.995$ was observed over a wide range of 0.2 ppb to 40 ppb of NDELA in standard solution. The accuracy of method was studied by spiking at three concentrations in a sample. The results show that recoveries were better than 90.0 %.

Introduction

N-nitrosamines have been determined to be human carcinogens because evidence of their carcinogenicity has been demonstrated in experimental animals.[1] Animal carcinogens induce cancer by a genotoxic mechanism,[1] and therefore should be regarded as potential human carcinogens.

N-nitrosodiethanolamine is not produced commercially; however, it is widespread in the environment. Dermal contact, ingestion, and inhalation (HSDB 2009) are potential methods of human exposure to N-nitrosodiethanolamine. It is a contaminant of products that humans come into contact with daily, such as cosmetics, lotions, shampoos, cutting fluids, certain pesticides, antifreeze, and tobacco at concentrations up to 130 ppm.

The quantity of n-nitrosamines measured in typical consumer products can vary widely. They have been detected in facial cosmetics at concentrations of 42 to 49,000 µg/kg, in lotions at up to 140 µg/kg, and in shampoos at up to 260 mg/kg (IARC 1978). Cosmetics at least five years old have higher values of N-nitrosodiethanolamine than new samples of the same products, indicating that the formation of N-nitrosodiethanolamine may limit the shelf-life of cosmetic products.[2]

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Nitrosamines are the reaction products of amines or amine derivatives with nitrosating agents such as nitrous acid (HNO_2), nitrites, or oxides of nitrogen and can be formed as shown in the equation below:

$R_2NH + HNO_2 \rightarrow R_2NNO + H_2O$

Experimental

LC/MS conditions

This experiment was performed using an Agilent 1290 Infinity LC System and an Agilent 6460 Triple Quadrupole LC/MS System with an Agilent Jet Stream source. The Agilent MassHunter Workstation was used to capture and analyze the data. LC/MS parameters used are shown in Tables 1 and 2.

SPE sample preparation

Weigh approximately 1.0 g of baby shampoo, add 100 µL of 1 ppm ISD working solution, and adjust to 10.0 mL with water. Shake for 10 minutes and centrifuge at 5,000 rpm for 10 minutes. Condition the solid phase extraction C18 column with methanol and water, respectively. Load 2 mL of supernatant and discard the first mL. Collect the following 0.5 mL for injection to the LC/MS.

Results and Discussion

The N-nitrosodiethanolamine response was measured by MRM in positive ESI mode with a fragmentor voltage of 80 V. The collision energy of the compound was 3 and 9 V for product ions of 74.1 and 104.0, respectively. The fragmentation pattern for N-nitrosodiethanolamines is shown in Figure 1.

Table 1. LC/MS conditions.

LC conditions		
Column	Agilent ZORBAX Eclipse Plus C-18, 3.0 mm × 100 mm (1.8 µm)	
Column temperature	45 °C	
Mobile phase	A: 2 mmol NH_4Ac in water B: 2 mmol NH_4Ac in 90 % MeOH/water	
Flow-rate	0.3 mL/min	
Gradient	Time (min)	%B
	0	10
	1.8	10
	2	98
	5	98
Post time	3 min	
Injection volumes	5 µL	
Agilent 6460 Triple Quadrupole source conditions		
Gas heater	300 °C	
Gas flow	5 L/min	
Nebulizer pressure	45 psi	
Sheath gas heater	320 °C	
Sheath gas flow	10 L/min	
Vcap	3,000 V	
Nozzle voltage	500	
Delta EMV	300 V	

Table 2. MRM settings.

Compound	Precursor	Product	Dwell (ms)	Fragmentor (V)	CE (V)
NDELA	135.1	74.1	50	80	3
NDELA	135.1	104.0	50	80	9
NDELA-d8 (ISTD)	143.0	111.0	50	80	3

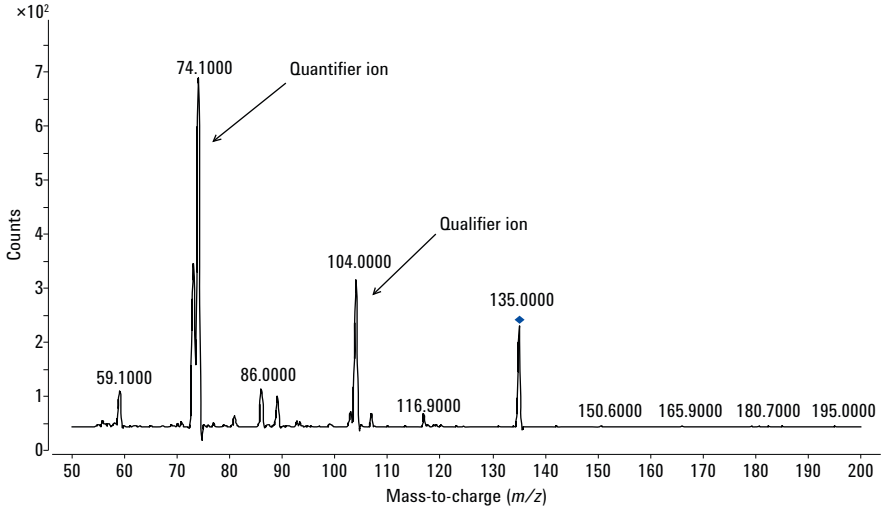

Figure 1. Product ion spectrum of N-nitrosodiethanolamine.

Figure 2 shows a chromatogram of NDELA obtained using a ZORBAX Eclipse Plus C-18 column, 3.0 × 100 mm. The retention time of the compound was 1.9 minutes and chromatographic cycle time was 8 minutes (Figure 2). Confirmation was obtained by comparing both the ion ratios and the retention time with NDELA standards. The ion ratio between qualifier ion and quantifier ion was approximately 55 %.

A serial dilution was used to check the linearity of the application. Figure 3 shows the relative response over the calibration range using the deuterated analog d8-N-nitrosodiethanolamine. Results for the calibration curve showed a very good linearity ($R^2 > 0.995$) over the concentration range of 0.2 to 40 ppb NDELA.

For method accuracy, the recoveries of NDELA in shampoo sample were performed by spiking three concentration of standard into a baby shampoo sample. The recoveries of the compound were very good and are shown in Table 3.

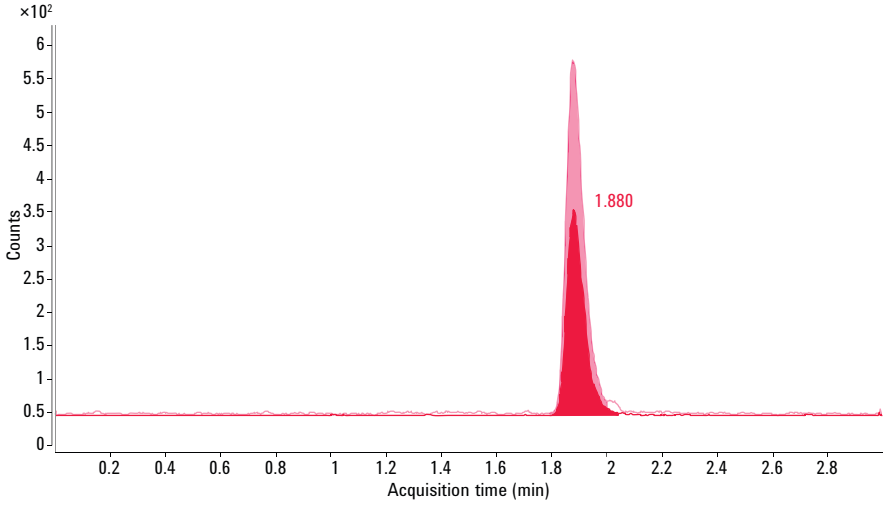

Figure 2. Overlaid chromatogram of the two measured transitions at a level of 5 ppb.

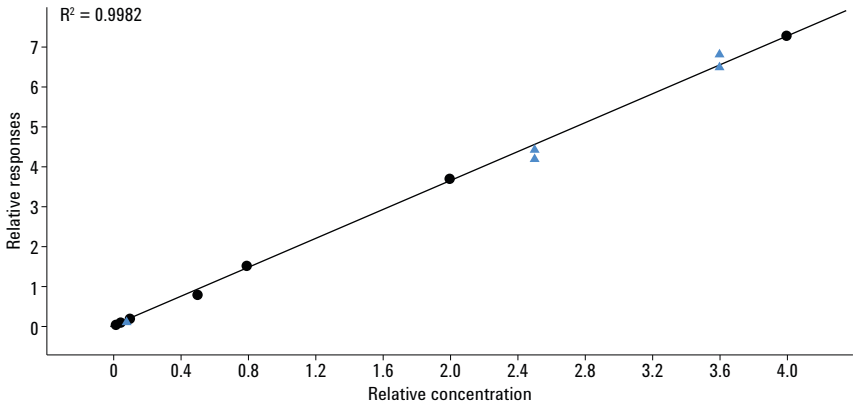

Figure 3. Calibration curve for N-nitrosodiethanolamine.

Table 3. Accuracy results from spiked shampoo samples.

Spiked concentration	Reported concentration (ppb)	% Accuracy	Ion ratio
LQC (0.8 ppb)	1st spiked = 0.85 2nd spiked = 0.77	1st spiked = 106.5 2nd spiked = 95.0	52.3 55.7
MQC (25 ppb)	1st spiked = 24.52 2nd spiked = 23.21	1st spiked = 98.2 2nd spiked = 92.8	55.3 55.0
HQC (26 ppb)	1st spiked = 36.04 2nd spiked = 37.86	1st spiked = 100.1 2nd spiked = 105.2	54.2 54.7

Figure 4 shows a spiked shampoo sample at a level of 0.8 ppb N-nitrosodiethanolamine. Based on this performance, the limit of quantitation(LOQ) (S/N >10 peak to peak) is 0.4 ppb and limit of detection (LOD) (S/N > 3 peak-to-peak) is 0.1 ppb.

Conclusions

By coupling the 1290 Infinity LC System with a 6460 Triple Quadrupole LC/MS System, a rapid and sensitive method was developed for the analysis of N-nitrosodiethanolamines (NDELA) in baby shampoo. The Infinity Series LC System is capable of performing fast analysis with narrow peaks. Together with multiple MRM transitions in the 6460 Triple Quadrupole LC/MS System, this method provides excellent sensitivity, linearity, and accuracy of trace analysis showing both quantitation and confirmation of NDELA.

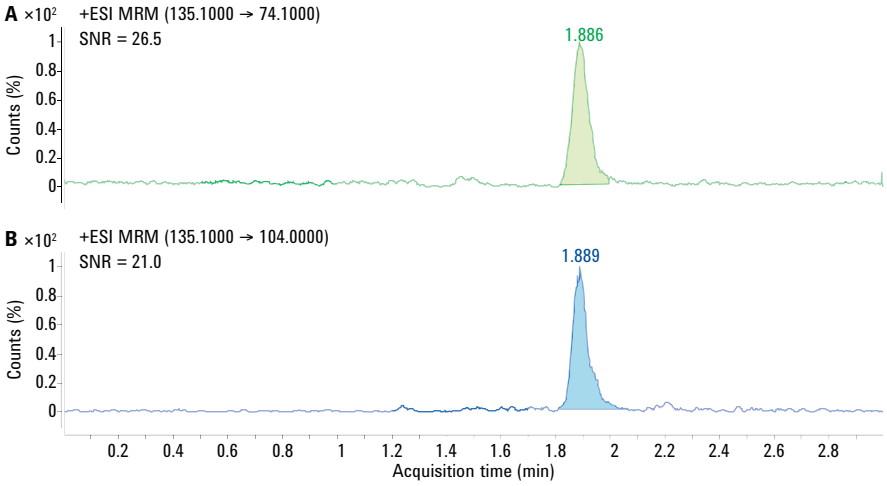

Figure 4. Chromatogram of N-nitrosodiethanolamine spiked at a level of 0.8 ppb, main signal (A) and qualifier (B).

References

1. Schothorst, R. C. and Somers, H. H. J. Determination of N-nitrosodiethanolamine in cosmetic products by LC–MS–MS. *Anal Bioanal Chem.*, **2005**, 381:681–685.

2. Matyska, M., Pesek, J.J. and Yang, L. Screening Method for Determining the Presence of N-nitrosodiethanolamine in Cosmetics by Open Tubular Capillary Electrochromatography. *Journal of Chromatography A*, **2000**, 887:497-503.

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