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Identification and quantification of seven volatile n-nitrosamines in cosmetics using gas chromatography/chemical ionization–mass spectrometry coupled with head space-solid phase microextraction

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ABSTRACT

An analytical method was developed for the identification and quantification of seven volatile n-nitrosamines (n-nitrosodimethylamine [NDMA], n-nitrosoethylmethylamine [NMEA], n-nitrosodiethylamine [NDEA], n-nitrosodipropylamine [NDPA], n-nitrosodibutylamine [NDBA], n-nitrosopiperidine [NPIP], and n-nitrosopyrrolidine [NPYR]) in water insoluble cream type cosmetics. It was found that the head space-solid phase microextraction (HS-SPME) was suitable for extraction, clean up, and pre-concentration of n-nitrosamines in the cream type samples so its optimal conditions were investigated. Identification and quantification of n-nitrosamines using single quadrupole gas chromatography/mass spectrometry (GC/MS) in chemical ionization (CI) mode were carried out with accurate mass measurements. Their accurate masses of protonated molecular ions were obtained within 10 mDa of the theoretical masses when sufficiently high signal was acquired from the unique calibration method using mass and isotope accuracy. For the method validation of quantification, spiking experiments were carried out to determine the linearity, recovery, and method detection limit (MDL) using three deuterated internal standards. The average recovery was 79% within 20% relative standard deviation (RSD) at the concentration of 50 ng/g. MDLs ranged from 0.46 ng/g to 36.54 ng/g, which was satisfactory for the directive limit of 50 ng/g proposed by the European Commission (EC). As a result, it was concluded that the method could be provided for the accurate mass screening, confirmation, and quantification of n-nitrosamines when applied to cosmetic inspection.

1. Introduction

The contamination of n-nitrosamines in cosmetics has been raised as a serious concern due to their carcinogenicity, which are capable of penetrating the skin and causing cancer in laboratory animals [1]. Contamination by n-nitrosamines in cosmetic products has become an issue since the 1970s and cosmetics containing n-nitrosamines have been subjected to enforcement action in the USA [2].

N-nitrosamines in cosmetics are formed by the reaction products of amines or amine derivatives with nitrosating agents such as nitrous acid (HONO), nitrites, or oxides of nitrogen as described in Eq. 1·[3].

$$R_2NH + HNO_2 \rightarrow R_2NNO + H_2O \quad (1)$$

The detailed mechanism of nitrosation of amines is as follows (Eqs. 2–4) [3].

$$2HONO \rightarrow N_2O_3 + H_2O \quad (2)$$

$$R_2NH + N_2O_3 \rightarrow R_2NH\text{–}NO + NO_2^- \quad (3)$$

$$R_2NH\text{–}NO \rightarrow R_2NNO + H^+ \quad (4)$$

Tertiary amines also act as precursors in nitrosamines' formation by undergoing nitrosative cleavage according to Eq. 5·[3].

$$R_2NR + NO_2^- \rightarrow R_2NNO \quad (5)$$

Once n-nitrosamines are formed, they are relatively stable and not easily destroyed unless exposed to ultraviolet light or nucleophiles (e.g., iodidie, thiocyanate, bromide, or chloride) [3].

Several analytical methods have been reported for the measurement of n-nitrosamines in various matrices to determine not

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only their presence but also their concentrations in samples with high precision and accuracy. For the separation and detection techniques of n-nitrosamines, gas chromatography (GC)-mass spectrometry (MS) [4], GC-thermal energy analyzer (TEA) [5,6], GC-tandem mass spectrometry (MS/MS) [7,8], and liquid chromatography (LC)/MS–MS [9,10] have been generally adopted. Among these, GC–MS is used most frequently owing to its high sensitivity and accessible mass spectral library search to assist compound identification by providing reference mass spectra for electron ionization from the National Institute of Standards and Technology (NIST) database or commercial libraries such as Wiley7n. However, parent molecular ions have generally low abundance due to the extensive fragmentation on electron ionization [11].

The aim of this study is to establish an accurate method for identification based on the new calibration approach and quantification of seven volatile n-nitrosamines in cream type cosmetics and find an optimal condition for HS-SPME as the clean-up and pre-concentration method coupled with GC–MS in CI mode. The new calibration approach, which calibrates using the mass accuracy and isotope patterns, was used for the analysis of n-nitrosamines in the cosmetic sample. From the unique calibration technique, n-nitrosamines were identified within 10 mDa of the theoretical mass when sufficiently high signal data was acquired without relying on the library search.

By adopting the HS-SPME procedure, the extraction and refinement of n-nitrosamines in emulsion type cosmetics, which are water-insoluble products including lipids, alcohols, fatty acids, and others, are simplified more than earlier approaches such as liquid–liquid extraction (LLE) or solid-phase extraction (SPE) [4]. Furthermore, the target cream type cosmetics in this study are much more complex than other cosmetic products such as lotion and skin [12]. For this reason, several steps for extraction, clean up, and pre-concentration are required for the analysis. Conventional methods such as LLE or SPE often fall short on recovery when applied to n-nitrosamines with low molecular weight [13]. The previous sample preparations were performed by the multi cleanup steps using the extruete cartridge and SPE methods to remove impurities in cosmetics which contain glycerin, alcohols, oils, parabens, and other chemicals. However, it was difficult to remove them and reliable recovery was not obtained due to the multiple steps and loss during the process of evaporation.

Therefore, the HS-SPME sample preparation is regarded as a most suitable analytical method for the volatile nitrosamines. The typical parameters consisting of salting out effect, extraction temperature, and extraction time in SPME optimization are investigated to improve the extraction efficiency. The method is validated with respect to linearity, recovery, and method detection limit (MDL). This proposed method is successfully demonstrated in the accurate mass screening, confirmation, and quantification of n-nitrosamines in cosmetic products.

2. Experiment

2.1. Chemicals and reagents

Standard solutions of seven volatile n-nitrosamines (NDMA, NMEA, NDEA, NDPA, NDBA, NPIP, and NPYR; see Table 1 for their full chemical names and information) for GC/MS analysis were purchased as a mixture at a concentration of 2000 μg/mL in dichloromethane from Supelco (Bellefonte, PA, USA). Deuterium-labeled internal standards of n-nitrosodimethylamine-d_6 (NDMA-d_6), n-nitrosodipropylamine-d_{14} (NDPA-d_{14}), and n-nitrosopiperidine-d_{10} (NPIP-d_{10}) were purchased from C/D/N Isotopes (Pointe-Claire, QC, Canada) and used for the spiking test as listed in Table 1.

Working standard solutions (1–1000 μg/mL) were prepared and then stored at −20 °C prior to use. Organic solvents (methanol, dichloromethane, and distilled water) of GC analysis grade were purchased from Burdick & Jackson (Philipsburg, NJ, USA) and a saturated solution of sodium chloride corresponding to 170 g NaCl from Wako (Osaka, Japan) for 500 mL water was used.

2.2. Preparation of samples

Real domestic cream products certificate, dated May 6, 2014, were obtained and stored at −20 °C. The most common creams in cosmetic products contain mainly glycerin, alcohols, oils, parabens, and others, even though there were minor differences related to fragrance. A 5 g cream sample, which was water insoluble emulsion type, spiked with 1 μg of each deuterium labeled internal standard, was entirely dissolved in 5 mL of dichloromethane and methanol (1:1, v/v). Sonication was carried out for 30 min and then 200 μL aliquot of extracted solvent was transferred to 4 mL of vial for the HS-SPME procedure. The total volume containing extracted solvent and saturated solution of sodium chloride was set to 2 mL to maintain the same head space volume in every sample.

In this mode, analytes should be partitioned among three phases: the aqueous, air, and fiber phases. The setting on the SPME was adjusted to 0.8 scale units to ensure that the fiber was positioned in the head space above the sample in exactly the same way from run to run. With the fiber exposed in the head space, the sample vial was placed in the heating block. 75 μm carboxen/

Table 1
Summary of retention times (min) and monitoring ions (m/z) of target nitrosamines.

Compound	Abbreviation	CAS	Retention Time (min)	Quant. Ion $[M+1]^+$	Monitoring ions	
					$[M+29]^+$	$[M+41]^+$
Group I						
n-nitrosodimethylamine-d_6	NDMA-d_6	17829-05-9	6.94	81	109	121
n-nitrosodimethylamine	NDMA	62-75-9	7.08	75	103	115
n-nitrosoethylmethylamine	NMEA	10595-95-6	7.72	89	117	129
n-nitrosodiethylamine	NDEA	55-18-5	8.20	103	131	143
Group II						
n-nitrosodipropylamine-d_{14}	NDPA-d_{14}	93951-96-3	10.54	145	173	185
n-nitrosodipropylamine	NDPA	621-64-7	10.68	131	159	171
n-nitrosodibutylamine	NDBA	924-16-3	15.49	159	187	199
Group III						
n-nitrosopiperidine-d_{10}	NPIP-d_{10}	960049-21-2	16.05	125	153	165
n-nitrosopiperidine	NPIP	100-75-4	16.12	115	143	155
n-nitrosopyrrolidine	NPYR	930-55-2	17.23	101	129	141