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Assay of 1,4-dioxane in cosmetic products by solid-phase extraction and GC-MS

SANTO SCALIA, FABIO TESTONI, GIUSEPPE FRISINA, and MARIO GUARNERI, *Dipartimento di Scienze Farmaceutiche, Università di Ferrara, Via Fossato di Mortara 17/19, 44100 Ferrara (S.S., M.G.), and Himont Research Centre, Ferrara (F.T., G.F.), Italy.*

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Synopsis

A rapid procedure has been developed for the assay of 1,4-dioxane in cosmetic products by gas chromatography selected-ion monitoring mass spectrometry. After solid-phase extraction using Bakerbond silica- and Bakerbond C_{18}-disposable cartridges, samples were injected directly into a Poraplot Q capillary column, employing toluene as an internal standard. Recovery of 1,4-dioxane from different cosmetic matrices was between 91.1 and 93.2%, and the reproducibility of the method was better than 4.3% relative standard deviation. The minimum quantifiable amount was 3 mg/kg. Of the total commercial cosmetics investigated (n = 25), 56% were found to contain 3.4–108.4 mg/kg of 1,4-dioxane.

INTRODUCTION

Polyethoxylated surfactants are widely used in shampoo and bath preparations (1) and are generally contained in skin cleansing products and lotion formulations (2). During the polymerization of ethylene oxide to produce the polyoxyethylene moiety of the emulsifiers, 1,4-dioxane may be formed (3–5). Hence, cosmetics containing ethoxylated surfactants may be contaminated by 1,4-dioxane (6–10), which has been shown to be carcinogenic in rats and mice (11,12) and to be absorbed through the intact skin of animals (13). Furthermore, this compound has been classified as a possible carcinogen to humans (14,15). According to the European Economic Community directive on cosmetics (16), 1,4-dioxane must not be present in commercial products. Consequently, the assay of this substance in marketed cosmetics is of direct concern to consumers.

Published methods for the quantitative determination of 1,4-dioxane in finished cosmetic products are based on gas chromatography (GC) (6) or headspace GC (7,8). These techniques, however, have distinct drawbacks, such as complex and time-consuming sample pre-treatment (6), extremely long equilibrium times (7,8), the need for extensive calibrations (7,8), and unsatisfactory reproducibility and recoveries (6). More recently, an improved GC procedure that requires minimal sample preparation has been reported

(10); yet the lack of specificity of the flame ionization detector (FID) used and the fact that application of the method is restricted to shampoo products are disadvantages.

In a previous paper (9), we described the first reversed-phase high-performance liquid chromatographic (RP-HPLC) method with UV spectrophotometry for the rapid assay of 1,4-dioxane in different cosmetic preparations. However, the selection of short UV wavelengths (9) for the detection of this compound lacking a strong chromophore generally results in low selectivity, due to increased interference from matrix constituents.

Routine analyses of 1,4-dioxane in cosmetics necessitated a simple method possessing a high degree of specificity and the required sensitivity. This study describes a procedure for the assay of 1,4-dioxane in cosmetic products by GC-mass spectrometry (GC-MS) using selected-ion monitoring (SIM). Prior to GC-MS analysis, rapid and efficient purification of the complex cosmetic matrices is achieved with combined silica- and octadecylsilica-disposable cartridges. The application of the method to the determination of 1,4-dioxane in a wide range of commercial cosmetics is also reported.

EXPERIMENTAL

MATERIALS

HPLC-grade 1,4-dioxane, hexane, dichloromethane, toluene, and acetonitrile were supplied by J. T. Baker (Phillipsburg, NJ). Bakerbond C_{18} (BB-C_{18}), Bakerbond CN (BB-CN), and Bakerbond silica (BB-SiOH) cartridges were obtained from J. T. Baker. Commercial cosmetics containing ethoxylated surfactants were from retail stores or from manufacturers or importers of these products.

GAS CHROMATOGRAPHY-MASS SPECTROMETRY

GC-MS analyses were performed with an HP 5890 gas chromatograph (Hewlett Packard, Palo Alto, CA) equipped with an HP 5970 mass-selective detector (Hewlett Packard) with transfer line held at 280°C. Samples (1 μl) were introduced using on-column injection. A deactivated fused-silica pre-column (5 m × 0.32 mm i.d.) and a Poraplot Q analytical column (25 m × 0.32 mm i.d.; Chrompack Italia, Milan, Italy) were used. The operating conditions were: initial temperature, 40°C; ramp 40–220°C, rate 40°C/min; carrier gas, helium; inlet pressure, 100 kPa. The MS, connected directly to the capillary column outlet, was operated in the selected-ion monitoring mode scanning m/z 31, 58, and 88 for 1,4-dioxane and m/z 91 and 92 for toluene (internal standard) with dwell times of 350 ms. The GC-MS system was controlled by an HP 5970 MS Chemstation Rev. 3.2 data station. Quantification was on the basis of peak area for the ratio 1,4-dioxane/toluene.

STANDARD SOLUTIONS

A 1,4-dioxane stock solution was prepared at a level of 20 mg/ml in acetonitrile, and an aliquot of it was diluted to make standard solutions in the concentration range 0.3–20 μg/ml. An internal standard solution (5.9 μg/ml) was prepared by quantitative

dilution of toluene with acetonitrile based upon the expected values of 1,4-dioxane in the cosmetics.

The calibration standards were prepared by mixing 2 ml of each 1,4-dioxane standard with 2 ml of the internal standard solution, corresponding to levels of 1,4-dioxane in the cosmetic (0.2 g) in the range 3–200 mg/kg. These solutions were analyzed by GC-MS as reported above, and the response factor relative to the internal standard was determined.

SAMPLE PREPARATION

Cosmetics were processed by a modification of the method described in an earlier study (9). In brief, the cosmetic product (0.18–0.22 g) was accurately weighed into a 10-ml glass centrifuge tube; 4 ml of 20% v/v dichloromethane in hexane were added; and the sample was mixed on a vortex mixer and centrifuged at 4500 rpm for 2 min. The extraction was repeated with 1.5 ml of 20% dichloromethane in hexane, and the combined supernatants were applied to a pre-conditioned (2 ml of acetonitrile and then 3 ml of 20% dichloromethane in hexane) BB-SiOH cartridge (sorbent weight, 500 mg) at a flow rate of *ca.* 1.5 ml/min. The extraction column was then washed with 1 ml of dichloromethane, aspirated to dryness by centrifugation at 3000 rpm for 1 min, and eluted with two 0.8-ml aliquots of acetonitrile. The acetonitrile fraction was passed directly through a BB-C_{18} cartridge (sorbent weight, 200 mg), which had previously been primed with 2 ml of acetonitrile. The eluate from the BB-C_{18} cartridge was made up to volume (2 ml), diluted with the internal standard solution (2 ml), and assayed by GC-MS.

RECOVERY AND REPRODUCIBILITY

The test samples were prepared by adding 20-μl aliquots of 1,4-dioxane spiking solutions in acetonitrile, corresponding to 40 mg/kg, to the cosmetic products (0.2 g) and mixing them thoroughly. The percentage recovery was determined by comparing the amounts of 1,4-dioxane extracted from samples with those obtained by direct injections of standard solutions.

The intra-assay reproducibility was tested by analyzing, on ten different days, 1 μl of the same stock sample solution from a baby lotion. The inter-assay variability was evaluated by repeated (n = 10) extractions on Bakerbond cartridges and GC-MS analyses of the same baby lotion product.

RESULTS AND DISCUSSION

In a previous investigation (9), solid-phase extraction columns pre-packed with cyanopropylsilica were used for sample preparation prior to assay of 1,4-dioxane in cosmetics by RP-HPLC. Disposable silica cartridges were selected in this study for sample clean-up before GC-MS analysis, since they achieve effective purification of the cosmetic matrices while affording more reproducible recoveries than the cyano-packing. The eluant from the BB-SiOH cartridge was passed directly through a BB-C_{18} column (see Experimental) to obtain a clear solution suitable for GC injection.

The cool on-column injection technique was chosen because it provides highest GC sensitivity, as 1-μl volumes of liquid samples were injected directly onto the capillary column through an inlet maintained at a temperature below the boiling point of the solvent (17).

The MS of 1,4-dioxane recorded over the mass range of 30–90 daltons has four major fragments, of which m/z 31, 58, and 88 (molecular ion) were selected for SIM recording. Operation of a computer-controlled GC-MS system in the SIM mode provides both specificity and sensitivity (18). By combining the abundance of a number of characteristic ions, the MS functions as a selective detector, eliminating the interfering peaks encountered in GC-FID analysis (6,10) and the need for additional confirmatory methods (9). Moreover, the MS, when adjusted to selected masses for a defined period of time, provides detection limits that are several orders of magnitude lower than are possible in the full-scan mode.

Commercial cosmetic products, containing no detectable 1,4-dioxane, were spiked at levels corresponding to 40 mg/kg. The average recoveries (n = 6) and relative standard deviations (RSD) for a day cream, a moisturizing lotion, and a shampoo were 93.2% (RSD, 4.3%), 91.1% (RSD, 3.9%), and 92.4% (RSD, 4.7%), respectively. In contrast, a previous investigation carried out by GC-FID (6) produced poor recoveries (mean value, 63%) accompanied by a high degree of variability (RSD, 19.9%). Improved recoveries have been obtained by the GC procedures of Rastogi (8) and Italia and Nunes (10), but the lengthy sample preparation (*ca.* 16 hours) of the former and the limited applicability to shampoos of the latter are disadvantages. The working range of the present method was found to be linear over the concentration interval, 3–200 mg/kg (r^2 = 0.998). Representative GC-MS profiles (SIM mode) of a dioxane-free day cream formulation and of a liquid soap containing 4.6 mg/kg of 1,4-dioxane are shown in Figures 1 and 2, respectively. Applying the foregoing GC-MS procedure to a baby lotion, 1,4-dioxane (10.9 mg/kg) was determined with an RSD of 3.1% (n = 10) for the intra-assay reproducibility and of 4.3% (n = 10) for the inter-assay reproducibility.

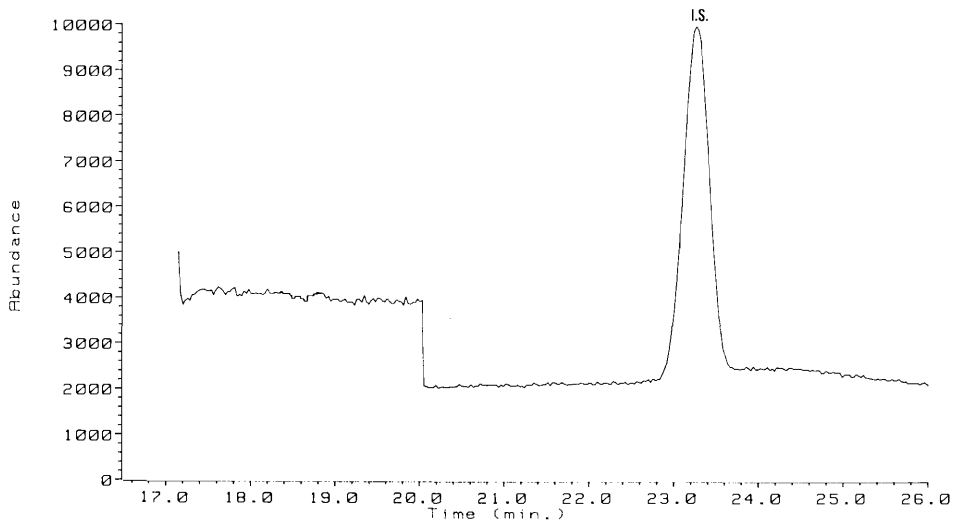

Figure 1. GC-SIM-MS chromatogram of a day cream preparation. Solvent delay, 16 min. Other operating conditions as described under Experimental; I.S. = internal standard (toluene).

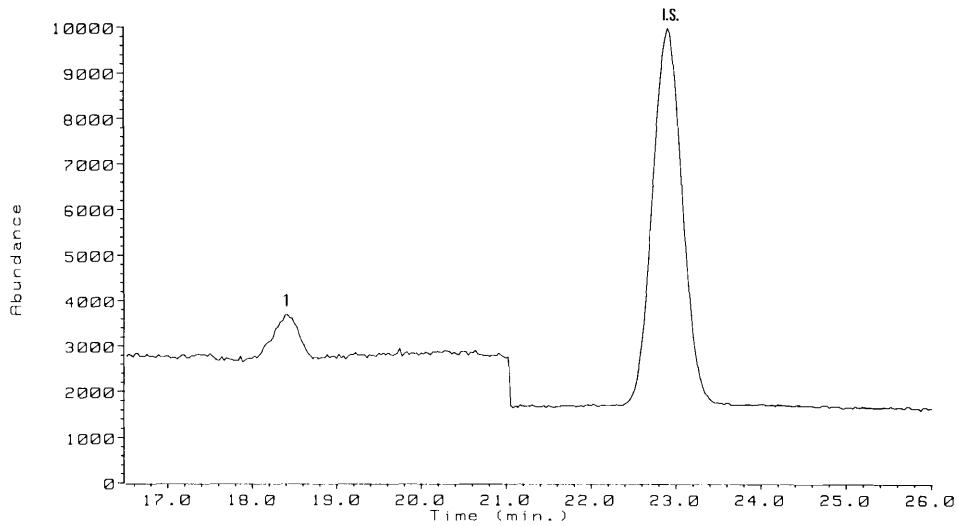

Figure 2. GC-SIM-MS trace of a liquid soap product. Conditions and peak identification as in Figure 1; 1 = 1,4-dioxane.

A variety of commercially available cosmetics were analyzed for 1,4-dioxane according to the method described here. The products (n = 25) included shampoos, liquid soaps, sun creams, bath foams, moisturizing lotions, cleansing milks, after-shave balms, baby lotions, day creams, and hair lotions. Of the total products investigated, 56% contained 1,4-dioxane with levels ranging from 3.4 to 108.4 mg/kg (Table I).

The concentrations of 1,4-dioxane in six different cosmetic preparations determined by the present GC-SIM-MS method and by the previously reported HPLC-UV procedure (9) are listed in Table II. The two methods produced consistent results, confirming the validity of the procedure developed in this study. The higher values obtained by GC-MS were traced to improved recovery during sample extraction. The MS is a more sensitive detector than the UV (9); consequently a lower amount of the cosmetic product (0.2 g) is required for the GC-MS assay, which results in a more efficient extraction into the dichloromethane-hexane (20:80) solvent.

The results presented in Table I indicate that the control of 1,4-dioxane contaminations in marketed cosmetics should be considered by national and international authorities, particularly to verify the conformance of the commercial products to the existing legislation (16). The presence of 1,4-dioxane in a baby lotion preparation (see Table II)

Table I
1,4-Dioxane Levels in Cosmetic Products Determined by GC-SIM-MS

1,4-Dioxane (mg/kg)	Percentage of total products examined (n = 25)
n.d.	44
3.4–10	16
10–50	28
50–108.4	12

n.d., not detected.

Table II
Comparison of 1,4-Dioxane Concentrations in Cosmetics Determined by GC-SIM-MS and HPLC-UV (9)

Sample	Concentration* (mg/kg)	
	GC-SIM-MS	HPLC-UV
Day cream	n.d.	n.d.
Baby lotion	10.9	9.1
Shampoo	33.4	24.6
Hair lotion	108.4	92.8
Moisturizing lotion	3.7	n.d.
Bath foam	41.4	35.7

* Mean value of three determinations.
n.d., not detected.

gives reason for particular concern. It should also be stressed that even when the 1,4-dioxane content is low, under appropriate conditions of cosmetic use, long-term application to skin is common.

CONCLUSIONS

A capillary GC method using selected-ion monitoring MS detection has been developed for the rapid (taking less than 40 min to perform) and specific determination of 1,4-dioxane in commercial cosmetics. The procedure is less laborious than others reported in the literature, as time-consuming steps including solvent partitions (6), heating (6,8), or extensive calibrations (7,8) are not required. Moreover, the use of multisample apparatus designed for solid-phase extraction cartridges enables eight isolation procedures to be performed simultaneously. In addition, the computer-controlled GC-mass selective detection system provides SIM-MS specificity at a fraction of the cost of larger MS apparatus and requires less operator training and experience. Because of the minimal sample preparation, ease of operation, accuracy, and precision, the proposed method is suitable for routine quality control analyses of finished cosmetic products.

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