Determination of 1,4-Dioxane in Cosmetic Products by High-performance Liquid Chromatography

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A rapid high-performance liquid chromatographic procedure has been developed for the assay of 1,4-dioxane in cosmetic products. After solid-phase extraction, using Bond Elut CN and Bond Elut C_{18} cartridges, samples were analysed directly on a LiChrospher CH-8 reversed-phase column with spectrophotometric detection at 200 nm and acetonitrile - water as eluent. Recovery of 1,4-dioxane from different cosmetic matrices was between 81.5 and 90.1% in the 30–90 μg g^{-1} range. The minimum quantifiable amount was 6.5 μg g^{-1}. The method is simple, reproducible and specific and is suitable for routine analyses of commercial cosmetics.

Keywords: *Reversed-phase high-performance liquid chromatography; solid-phase extraction; 1,4-dioxane; cosmetic product*

Emulsion-based cosmetics, containing ethoxylated surfactants, may be contaminated by 1,4-dioxane,[1–3] a carcinogen in rats and mice,[4] which is absorbed through the intact skin of animals.[5,6] 1,4-Dioxane may be formed during the polymerisation of ethylene oxide to produce the polyoxyethylene portion of the emulsifiers.[7] Hence, the determination of levels of 1,4-dioxane in commercial cosmetic products is of direct concern to the consumer. At present, the assay of this substance in cosmetics is carried out by gas chromatography (GC).[3,7,8] This technique, however, suffers from several drawbacks such as the need for extensive sample pre-treatment, unsatisfactory accuracy and reproducibility and a high degree of variability in the recovery of 1,4-dioxane from different cosmetic formulations.[7]

For routine determinations of this substance in cosmetics, an accurate, precise and simple method was required. This paper describes a reversed-phase high-performance liquid-chromatographic (RP-HPLC) procedure for the assay of 1,4-dioxane in cosmetic products. Prior to RP-HPLC analysis, rapid and efficient purification of the complex cosmetic matrices is achieved with disposable cyanopropyl- and octadecyl-silica cartridges. The method is applicable to the determination of 1,4-dioxane in a wide range of commercially available cosmetic preparations.

Experimental

Materials

High-performance liquid chromatography grade 1,4-dioxane, hexane, dichloromethane, acetonitrile, methanol and water were supplied by Farmitalia Carlo Erba (Milan, Italy). Bond Elut C_{18} (BE-C_{18}), Bond Elut Si (BE-Si) and Bond Elut CN (BE-CN) cartridges were obtained from Analytichem International (Harbor City, CA, USA). Commercial cosmetics, containing polyoxyethylene derivatives, were from various manufacturers.

Chromatography

The HPLC apparatus consisted of a Jasco chromatographic system (Model BIP-I pump, Model GP-A40 solvent programmer and Model UVIDEC-100-V variable-wavelength UV detector; Jasco, Tokyo, Japan) linked to an injection valve with a 20-μl sample loop (Rheodyne, Cotati, CA, USA) and a chromatographic data processor (Chromatopac C-R3A, Shimadzu, Kyoto, Japan). The detector was set at 200 nm and 0.01 a.u.f.s. Sample injections were effected with a Hamilton Model 802 RN 10-μl syringe (Hamilton, Bonaduz, Switzerland).

Separations were performed either on a LiChrospher CH-8 column (particle diameter 5 μm, 250 × 4.0 mm i.d.; Merck, Darmstadt, FRG) or on a Supelcosil LC-18-DB column (particle diameter 5 μm, 150 × 4.6 mm i.d.; Supelco, Bellefonte, PA, USA) under gradient conditions at a flow-rate of 1.0 ml min^{-1}. Solvent A and solvent B acetonitrile in water were 5 and 50% v/v, respectively. The elution programme was as follows: isocratic elution with 5% solvent B - 95% solvent A for 5 min, then a 2-min linear gradient to 95% solvent B; the mobile phase composition was finally maintained at 95% solvent B for 1 min. Samples were injected 0.5 min after the start of the elution programme. The mobile phase was filtered through HVLP-type 0.45-μm filters (Millipore, Molsheim, France) and de-gassed on-line by a Model ERC-3311 automatic solvent de-gasser (Erma, Tokyo, Japan). Chromatography was carried out at ambient temperature.

Gas chromatographic analyses were performed, according to Black *et al.*,[7] using a Fractovap 4200 gas chromatograph (Carlo Erba) fitted with a flame-ionisation detector. The glass column (2.0 m × 4 mm i.d.) was packed with Chromosorb 106 (Alltech, Eke, Belgium). The operating conditions were: column temperature, 210 °C; injector port temperature, 230 °C; detector temperature, 230 °C; carrier gas (nitrogen) flow-rate, 40 ml min^{-1}.

The identity of the 1,4-dioxane peak was assigned by co-chromatography with the authentic compound on two different reversed-phase columns (LiChrospher CH-8 and Supelcosil LC-18-DB) and confirmed by comparison of the GC retention time with that of the authentic compound.

Peak areas were measured with an integrator, which was calibrated with standard solutions of pure 1,4-dioxane.

Sample Processing

The cosmetic product (0.5–0.6 g) was weighed accurately into a 10-ml glass centrifuge tube; 4 ml of 10% v/v dichloromethane in hexane were added, and the sample was mixed vigorously on a vortex-mixer and centrifuged at 2000 *g* for 2 min. The extraction was repeated with 2 ml of 10% dichloromethane in hexane and the combined supernatant solutions were applied to a pre-conditioned (3 ml of acetonitrile followed by 5 ml of 10% dichloromethane in hexane) BE-CN cartridge (sorbent mass, 500 mg) at a flow-rate of *ca.* 1.5 ml min^{-1}. The column was then aspirated to dryness by centrifugation at 1000 *g* for 1 min, and elution was carried out with two 0.8-ml aliquots of 15% v/v acetonitrile in water. In order to obtain a clear solution, the eluate was passed directly through a BE-C_{18} cartridge (sorbent mass, 200 mg), which had previously been primed with 3 ml of acetonitrile and 5 ml of

15% acetonitrile in water. A further 0.4-ml portion of 15% acetonitrile in water was passed through the column to complete the elution of 1,4-dioxane. The eluate from the BE-C_{18} cartridge was made up to volume (2 ml) and analysed directly by RP-HPLC.

Recovery and Reproducibility

"Spiked" solutions were obtained by quantitative dilution of 1,4-dioxane with methanol. The test samples were prepared by adding 50-μl aliquots of the spiked solutions, corresponding to 30 and 90 μg g^{-1}, to the cosmetic products (0.5 g) and mixing them thoroughly. The percentage recovery was determined by comparing the peak areas of 1,4-dioxane extracted from samples with those obtained by direct injections of standard solutions.

The intra-assay reproducibility was tested by analysing, on ten different days, 10 μl of the same stock sample solution from a cleansing lotion. The inter-assay variability was evaluated by replicate (n = 10) extractions on Bond Elut cartridges and HPLC analyses of the same cleansing lotion product.

Results and Discussion

Liquid Chromatography

Because of the weak UV absorptivity of 1,4-dioxane, the determination of low levels of this compound by HPLC with UV spectrophotometry required detection at 200 nm and a low background absorbance of the eluent. Accordingly, a predominantly aqueous mobile phase and acetonitrile, as the organic modifier, were employed. Under these conditions, the determination of as little as 20 ng of 1,4-dioxane per injection was achieved.

The influence of the packing on the chromatographic behaviour of 1,4-dioxane was also examined by using the eluent described under Chromatography and different RP supports (*i.e.*, octadecyl-, octyl-, phenyl- and cyano-silica columns). Optimum retention and peak symmetry were attained by use of the C_{18} and C_8 stationary phases, which were selected for the assay of 1,4-dioxane in cosmetic samples. Although the actual separation was effected isocratically in *ca.* 6 min [Fig. 1(*b*)], a rapid gradient elution was carried out before the next injection (see under Experimental) to ensure the elution of strongly retained substances.

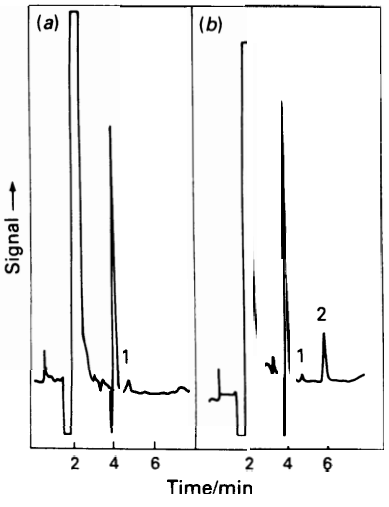

Fig. 1. RP-HPLC traces of (*a*) a day cream extract; and (*b*) the same product spiked with 30 μg g^{-1} of 1,4-dioxane. Column, LiChrospher CH-8. Other operating conditions as described under Experimental. 1, Unknown compound; and 2, 1,4-dioxane

Sample Preparation

In order to minimise the sample handling steps prior to RP-HPLC analysis, purification procedures based on solid-phase extraction techniques were examined. Mixtures of hexane and dichloromethane were tested as extraction solvents in combination with cartridges pre-packed with polar sorbents such as silica and cyanopropyl-bonded silica.

The dichloromethane content of the hexane - dichloromethane mixture strongly influenced the recovery of 1,4-dioxane. Lower recoveries (<60%) were observed at dichloromethane concentrations higher than 10% v/v owing to incomplete adsorption of 1,4-dioxane on the extraction column during sample application. Dichloromethane - hexane (10 + 90 v/v) was therefore used as the solvent for sample extraction.

Both BE-Si and BE-CN cartridges were found to retain 1,4-dioxane efficiently. The cyanopropylsilica column, however, afforded the most reproducible recovery and a more effective sample clean-up. The cyano-packing is of intermediate polarity and can be used in the reversed-phase mode and also in the normal mode.[9] By this means, improved selectivity is obtained, which combines the two separation principles.

Different cosmetic products, containing no detectable 1,4-dioxane, were spiked with the amounts reported in Table 1 and subjected to the assay procedure. Representative RP-HPLC traces are illustrated in Fig. 1. As shown in Table 1, the recovery of 1,4-dioxane was at least 81.5%; losses were traced to incomplete extraction into the dichloromethane - hexane (10 + 90 v/v) solvent. The recovery was not affected significantly by the type of cosmetic matrix or by the presence of ethanol in the original sample (Table 1). In contrast, a previous investigation,[7] carried out by GC, produced a mean recovery of 63.0% (relative standard deviation, 19.9%), with values lower than 20% for cosmetic formulations containing ethanol.

The recovery of the method developed in this study was found to be reproducible between different batches of BE-CN; moreover, the extraction and solid-phase isolation were achieved with small volumes of the appropriate solvent mixtures, in contrast to the purification procedure used in an earlier study.[7]

A linear correlation was obtained between peak area and concentration of 1,4-dioxane in the range 6.5–300 μg g^{-1} [r = 0.999, a (slope) = 0.07, b (intercept) = 0.33]. A representative chromatogram of a cleansing lotion product containing 7.3 μg g^{-1} of 1,4-dioxane is shown in Fig. 2. A lower limit of determination (*ca.* 2 μg g^{-1}) was attained[7] using a GC assay,

Table 1. Recovery of 1,4-dioxane added to cosmetic products

Sample	Amount added/ μg g^{-1}	Recovery,% (mean ± SD,* n = 6)
Day cream	30	83.9 ± 2.2
	90	90.1 ± 1.1
After-shave emulsion	30	82.6 ± 2.8
	90	84.4 ± 2.7
After-shave emulsion containing 10% m/m ethanol	30	81.5 ± 1.2
Moisturising lotion	30	86.4 ± 3.1
	90	89.5 ± 2.6
Suncream	30	82.4 ± 2.5
	90	83.6 ± 2.8
Shampoo	30	84.8 ± 4.3
	90	88.3 ± 3.0

* SD = standard deviation.

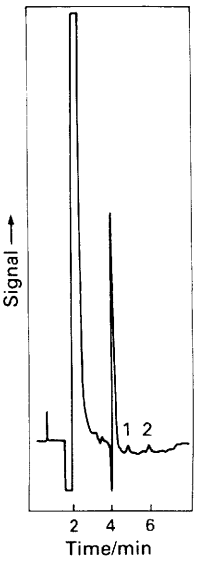

Fig. 2. Chromatogram of a cleansing lotion extract. Conditions and peak indentification as in Fig. 1

but the poor accuracy and reproducibility of this method were disadvantages.

Applying the RP-HPLC procedure to a cleansing lotion, 1,4-dioxane (7.3 μg g^{-1}) was determined with a relative standard deviation of 4.4% (n = 10) for the intra-assay reproducibility and 9.6% (n = 10) for the inter-assay reproducibility.

Virtually identical values were obtained for 1,4-dioxane when the same cosmetic preparation, purified according to the procedure described here, was analysed by RP-HPLC or GC (Table 2). This indicates that the RP-HPLC assay is not subject to any interference from the formulation matrix.

Conclusions

Until now, the assay of 1,4-dioxane in cosmetic products has been carried out by GC. This technique requires several sample manipulations, which represent a source of possible errors.

An RP-HPLC method for the rapid (taking less than 30 min to perform) and selective determination of 1,4-dioxane in cosmetics has been developed. To the best of our knowledge this is the first report on a liquid-chromatographic analysis of dioxane. The proposed procedure is less laborious than others reported in the literature, as time-consuming steps including solvent partitions, heating and several column purifications are not required. Moreover, multiple samples can be processed simultaneously with specially designed vacuum manifolds. Because of the ease of operation, minimal sample preparation, good accuracy and precision, the method is well suited to routine quality control analyses of cosmetics.

Table 2. Comparison of amounts of 1,4-dioxane in cosmetic products determined by RP-HPLC and GC

	Concentration*/μg g^{-1}	
Sample	RP-HPLC	GC
Cleansing lotion	9.1	9.5
Suncream (spiked with 30 μg g^{-1})	25.4	24.6

* Mean value of three determinations.

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