

Occurrence of 1,4-Dioxane in Cosmetic Raw Materials and Finished Cosmetic Products

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Surveys of cosmetic raw materials and finished products for the presence of the carcinogen 1,4-dioxane have been conducted by the U.S. Food and Drug Administration since 1979. Analytical methods are described for the determination of 1,4-dioxane in ethoxylated cosmetic raw materials and cosmetic finished products. 1,4-Dioxane was isolated by azeotropic atmospheric distillation and determined by gas chromatography using *n*-butanol as an internal standard. A solid-phase extraction procedure based on a previously published method for the determination of 1,4-dioxane in cosmetic finished products was also used. 1,4-Dioxane was found in ethoxylated raw materials at levels up to 1410 ppm, and at levels up to 279 ppm in cosmetic finished products. Levels of 1,4-dioxane in excess of 85 ppm in children's shampoos indicate that continued monitoring of raw materials and finished products is warranted.

Polyethoxylated raw materials are widely used in cosmetic products as emulsifiers, foaming agents, and dispersants. They are made by polymerizing ethylene oxide, usually with a fatty alcohol, to form polyethoxylated alcohols which may be used to synthesize other products such as sulfated surface active agents (1). During the ethoxylation process, 1,4-dioxane can be formed as a by-product by the dimerization of ethylene oxide (1–3).

The U.S. Food and Drug Administration's (FDA) interest in 1,4-dioxane was prompted by studies which determined that 1,4-dioxane was carcinogenic in rats (4–7) and was absorbed through excised human skin and the skin of monkeys (8, 9). The contamination of polyethoxylated surfactants with 1,4-dioxane became known to the FDA when it was found at levels up to 378 ppm in food-grade polysorbates (10). Since this first report, a number of studies on the occurrence of 1,4-dioxane in cosmetic raw materials and finished products have been described (11–17).

Several gas chromatographic (GC) methods have been reported for the determination of 1,4-dioxane (1, 10, 15, 18, 19), including GC coupled to a mass spectrometer (13, 20, 21). Liquid chromatographic methods have also been reported

(11, 20). In this paper, analytical methods used by the FDA for the determination of 1,4-dioxane in cosmetic raw materials and finished products are described. The results of surveys of cosmetic raw materials and finished products for 1,4-dioxane conducted since 1979 are reported.

METHOD

Caution: 1,4-Dioxane is a known animal carcinogen. Extreme care should be exercised when handling this material.

Apparatus

(a) *Gas chromatographic system A.*—HP 5890 (Hewlett-Packard, Inc., Palo Alto, CA) equipped with flame ionization detector (FID) detector and a 6 ft × 1/8 in. od nickel column packed with 0.2% Carbowax 1500 on 80/100 mesh Carbopack C (Supelco, Inc., Bellefonte, PA) with an HP 3396 Series II integrator (Hewlett-Packard). Operating conditions: detector temperature, 230°C; injection port temperature, 210°C; oven temperature, 100°C; carrier gas, helium at 20 mL/min.

(b) *Gas chromatographic system B.*—HP 5890 Series II equipped with an FID detector (Hewlett-Packard) and a 30 m × 0.53 mm id fused silica capillary column coated with a 2 μm film of Stabilwax (Restek Corp., Bellefonte, PA). Operating conditions: detector temperature, 210°C; splitless injection mode at 150°C; oven temperature, initial, 50°C; program, 15°C/min; final, 95°C for 15 min; purge A on: 1.5 min; purge A off: 3.0 min; carrier gas, helium at 30 cm/s. The gas chromatograph was controlled by an HP 3365 Series Chem Station (Hewlett-Packard).

(c) *Distillation apparatus.*—Barrett (Fisher Scientific, Fair Lawn, NJ) trap fitted with a water cooled condenser and a 1000 mL round bottom flask.

(d) *Solid-phase extraction (SPE) cartridges.*—Bakerbond 500 mg silica gel and Bakerbond 200 mg C₁₈ columns (J.T. Baker, Inc., Phillipsburg, NJ).

(e) *Vacuum manifold.*—Visiprep (Supelco, Inc.).

(f) *Centrifuge tube.*—15 mL Pyrex (Corning, Corning, NY).

(g) *Boiling chips.*—Boileezers (Fisher Scientific Co.).

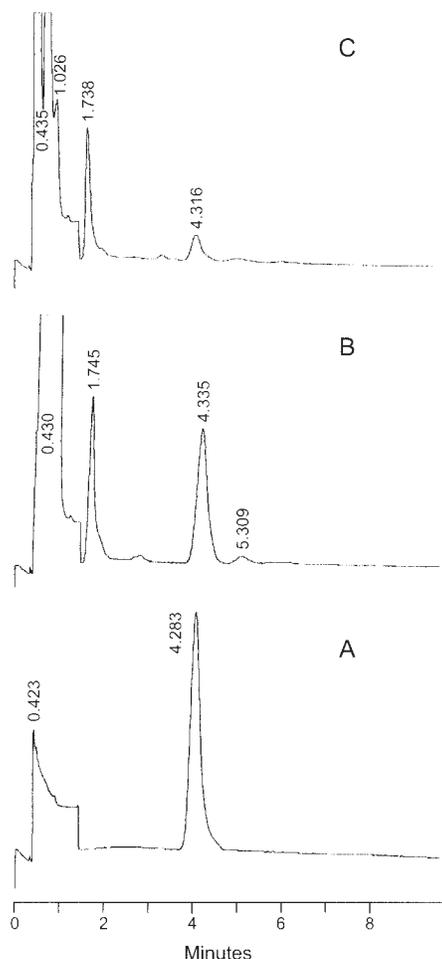


Figure 1. Chromatograms of (A), 1,4-dioxane standard (10 $\mu\text{g/mL}$); (B) and (C), extracts from 2 shampoo products.

Reagents

(a) *Solvents*.—Hexane, methylene chloride, acetonitrile, and water, UV or LC grade (Burdick & Jackson, Muskegon, MI).

(b) *Sodium carbonate*.—Reagent grade (J.T. Baker). Dissolve 10 g sodium carbonate in 100 mL LC grade water.

(c) *1,4-Dioxane*.—HPLC grade (Sigma-Aldrich, Milwaukee, WI).

(d) *1,4-Dioxane stock solution in water*.—10 mg/mL. Accurately weigh ca 1 g 1,4-dioxane in a tared 100 mL volumetric flask and dilute to volume with LC grade water.

(e) *1,4-Dioxane standard solutions in water*.—(1) 1.0 mg/mL.—Pipet 10.0 mL stock solution into a 100 mL volumetric flask and dilute to volume with LC grade water. (2) 0.01, 0.02, 0.05, 0.1, 0.25, 0.5 mg/mL.—Pipet 1, 2, 5, 10, 25, and 50 mL of 1.0 mg/mL solution into separate 100 mL volumetric flasks and dilute to volume with LC grade water.

(f) *n-Butanol*.—Reagent grade (J.T. Baker).

(g) *n-Butanol stock solution*.—8 mg/mL. Accurately weigh ca 0.8 g *n*-butanol in a tared 100 mL volumetric flask and dilute to volume with LC grade water.

(h) *n-Butanol standard solutions*.—(1) 0.8 mg/mL.—Pipet 10 mL stock solution into a 100 mL volumetric flask and dilute to volume with LC grade water. (2) 0.008, 0.016, 0.04, 0.08, 0.2, 0.4 mg/mL.—Pipet 1, 2, 5, 10, 25, and 50 mL of 0.8 mg/mL solution into separate 100 mL volumetric flasks and dilute to volume with LC grade water.

(i) *2-Octanol*.—Sigma Chemical Co., St. Louis, MO.

(j) *Antifoam A*.—Sigma Chemical Co.

(k) *1,4-Dioxane stock solution in acetonitrile*.—10 mg/mL. Accurately weigh ca 1 g 1,4-dioxane in a tared 100 mL volumetric flask and dilute to volume with acetonitrile.

(l) *1,4-Dioxane standard solutions in acetonitrile*.—(1) 1.0 mg/mL.—Pipet 10 mL stock solution into a 100 mL volumetric flask and dilute to volume with acetonitrile. (2) 0.01, 0.02, 0.05, 0.1 mg/mL.—Pipet 1, 2, 5, and 10 mL of 1.0 mg/mL solution into separate 100 mL volumetric flasks and dilute to volume with acetonitrile. (3) 4.0 $\mu\text{g/mL}$.—Transfer 20 μL of 1.0 mg/mL solution into a 5 mL volumetric flask with a syringe and dilute to volume with acetonitrile. (*Note*: Store aqueous 1,4-dioxane standard solutions at room temperature, and refrigerate [5°C] 1,4-dioxane standard solutions in acetonitrile. Prepare new standard solutions monthly.)

Raw Material/Finished Product Analysis

(a) *Azeotropic distillation*.—Accurately weigh ca 5 g cosmetic raw material or finished product into a 1000 mL round bottom flask. Add 50 mL LC grade water and 10 mL 10% sodium carbonate solution. Add 10 drops each of 2-octanol and Antifoam A, along with several boiling chips to the flask. Attach the Barrett trap and condenser and heat the solution to boiling, distilling at a moderate rate. When 10 mL distillate has been collected in the trap, drain and transfer to a 30 mL volumetric flask. Collect 2 additional 10 mL portions of distillate and transfer to the same 30 mL volumetric flask. Dilute to volume with LC grade water and reserve for GC analysis.

(b) *SPE*.—The procedure of Scalia (16) was used with minor modifications. Accurately weigh 0.18–0.22 g cosmetic product into a 15 mL centrifuge tube. Pipette 4 mL 20% methylene chloride in hexane into the centrifuge tube and mix on a Vortex mixer for several minutes. Centrifuge at 4500 rpm for 2 min. Draw off and collect the supernate. Repeat the extraction of the cosmetic product with a second 1.5 mL portion of 20% methylene chloride in hexane, and combine extracts.

Precondition a 500 mg silica gel SPE column with 2 mL acetonitrile followed by 3 mL 20% methylene chloride in hexane. Pass the combined cosmetic extract through the SPE column. Wash the column with 1 mL methylene chloride. Aspirate the SPE column to dryness by centrifugation at 3000 rpm for 1 min. Elute 1,4-dioxane from the SPE column with 3 \times 0.8 mL aliquots of acetonitrile followed by evacuation of the SPE column to dryness by vacuum to insure that all 1,4-dioxane is eluted from the column. Pass the combined acetonitrile extract through a 200 mg C_{18} SPE column that has

Table 1. Occurrence of 1,4-dioxane in ethoxylated cosmetic raw materials

Year	No. of raw materials	No. raw materials containing 1,4-dioxane	1,4-Dioxane, ppm	
			Range	Average
1979	70	58	0.6–636	49
1980	30	29	6.3–1410	207
1993	58	20	5–243	71
1996	8	8	20–653	180
1997	11	6	45–1102	348

been preconditioned with 2 mL acetonitrile. Pass an additional 2 mL acetonitrile through the C₁₈ SPE column and collect. Dilute the combined eluates to volume in a 5 mL volumetric flask with acetonitrile.

Determination

(a) *Azeotropic distillation.*—Inject 7 μ L distillate into GC system A and adjust the attenuation to give a peak height of ca 75% full scale. Determine which 1,4-dioxane and *n*-butanol standard solutions approximate the concentration of 1,4-dioxane in the test sample extract by injecting 7 μ L of the 1,4-dioxane (aqueous) and *n*-butanol standard solutions. Pipet 2 mL each of the selected 1,4-dioxane and *n*-butanol solutions into a 10 mL Erlenmeyer flask and mix thoroughly. Into a second 10 mL Erlenmeyer flask, pipet 2 mL of the same *n*-butanol solution and 2 mL test sample distillate and mix thoroughly. Alternately inject test sample/*n*-butanol and 1,4-dioxane/*n*-butanol solutions in triplicate. Calculate the ratio of 1,4-dioxane/*n*-butanol in each test sample and standard injection. Calculate the concentration of 1,4-dioxane in the sample with the following equation:

$$\text{1,4-Dioxane, ppm} = \frac{R_{\text{sam}} \times C \times 30}{R_{\text{std}} \times W}$$

where R_{sam} = average ratio of 1,4-dioxane/*n*-butanol in the test sample solution using peak heights, R_{std} = average ratio of 1,4-dioxane/*n*-butanol in the standard solution, C = concentration of 1,4-dioxane standard in $\mu\text{g/mL}$, and W = weight of sample (g).

(b) *SPE.*—Inject 2 μ L of a 4 $\mu\text{g/mL}$ standard of 1,4-dioxane (acetonitrile) into GC system B. Inject 2 μ L of the test sample extract and integrate the peak for 1,4-dioxane using peak heights. Calculate the level of 1,4-dioxane in the test sample based on the standard.

Results and Discussion

When 1,4-dioxane was identified as a contaminant in food grade polysorbates in 1979, the FDA developed analytical methods to quantitate 1,4-dioxane in polyethoxylated raw materials because of their widespread use in both foods and cosmetic products. The vacuum distillation method developed was previously described (10). An azeotropic distillation method was also developed. Azeotropic distillation was easier to perform, required less sophisticated laboratory glassware, and gave comparable results to the vacuum distillation method. The azeotropic distillation method was validated by conducting recovery studies on an ethoxylated alcohol, a shampoo, and a bubble bath. Recovery of 1,4-dioxane from an ethoxylated alcohol fortified at the 20 and 100 ppm levels averaged 99 and 97%, respectively. For shampoo fortified at the 100 ppm level, recoveries ranged from 77 to 91%, while for a bubble bath fortified at the 80 ppm level, recoveries ranged from 93 to 98%. The limit of detection and quantitation of the method was 1 and 5 ppm, respectively, for a 5–10 g sample. The GC system was linear over the range of 10 to 500 ppm. The repeatability of the method was determined by performing replicate analyses of 2 different shampoo products. In one

Table 2. Occurrence of 1,4-dioxane in ethoxylated alkyl sulfates

Year	No. of raw materials	No. raw materials containing 1,4-dioxane	1,4-Dioxane, ppm	
			Range	Average
1979	6	6	71–580	229
1980	23	22	6–1410	226
1993	17	14	16–243	80
1996	7	7	20–653	188
1997	11	6	45–1102	348

Table 3. Occurrence of 1,4-dioxane in cosmetic finished products

Year	No. of products	No. of products containing 1,4-dioxane	1,4-Dioxane, ppm	
			Range	Average
1981 ^a	11	8	2–279	50
1982 ^a	3	2	2–36	19
1983 ^a	11	6	1–8	2
1984 ^a	3	0	0	0
1992 ^b	34	31	5–141	41
1993 ^b	12	7	50–112	79
1994 ^b	27	6	20–107	45
1995 ^b	6	3	42–90	74
1996 ^a	10	7	6–34	14
1997 ^a	10	6	6–34	19

^a Products analyzed by the azeotropic distillation method.

^b Products analyzed by the solid-phase extraction method.

product, 1,4-dioxane ranged from 84–89 ppm (average, 86 ppm; standard deviation, 2.7), while for the second product, 1,4-dioxane ranged from 75–87 ppm (average, 81 ppm; standard deviation, 4.8). Typical chromatograms at 1,4-dioxane standard and sample extracts are shown in Figure 1.

Since 1979, the FDA has conducted periodic surveys of ethoxylated cosmetic raw materials for 1,4-dioxane. The raw materials were obtained directly from manufacturers. The results of these surveys are shown in Table 1. In the first survey conducted between 1979 and 1980, 70 cosmetic raw materials were analyzed for 1,4-dioxane by using the vacuum distillation method. 1,4-Dioxane was detected in 58 of the raw materials at levels up to 636 ppm, and averaging 49 ppm. The presence of 1,4-dioxane was confirmed by mass spectrometry in 17 raw materials. Six ethoxylated alkyl sulfates were analyzed during the survey; the average level of 1,4-dioxane found was 229 ppm (Table 2).

Shortly after the identification of 1,4-dioxane in ethoxylated raw materials, the FDA urged the cosmetic industry to monitor their ethoxylated raw materials for 1,4-dioxane (22). Raw material manufacturers reportedly modified their manufacturing processes to reduce the levels of 1,4-dioxane in their products (22). Subsequent surveys of cosmetic raw materials were conducted to monitor industry's progress. Another survey of 30 ethoxylated cosmetic raw materials focusing on ethoxylated alkyl sulfates was conducted from 1980 to 1981 using the azeotropic distillation method. 1,4-Dioxane was detected in 29 raw materials at levels up to 1410 ppm, and averaging 207 ppm. 1,4-Dioxane was most commonly found in ammonium laureth sulfate and sodium laureth sulfate. A summary of the levels of 1,4-dioxane found in ethoxylated alkyl sulfates is shown in Table 2.

Another survey of cosmetic raw materials was conducted from 1993 to 1994. A total of 58 raw materials was analyzed using the azeotropic distillation method. Sixteen samples of sodium laureth sulfate were included in the survey.

1,4-Dioxane was found in a total of 20 raw materials at an average level of 71 ppm. As in previous surveys, the highest levels of 1,4-dioxane were found in ethoxylated alkyl sulfates. The data showed that compared to earlier surveys, the average levels of 1,4-dioxane had declined, suggesting that modifications to the manufacturing process of ethoxylated raw materials had been made. The data also showed that ethoxylated alkyl sulfates were still the most likely raw materials to contain 1,4-dioxane. However, 3 samples of sodium laureth sulfate did not contain detectable levels of 1,4-dioxane demonstrating that manufacturing techniques were available to avoid the contaminant.

Additional surveys of cosmetic raw materials were conducted in 1996 and 1997 to monitor 1,4-dioxane levels. Ethoxylated alkyl sulfates were again the focus of the surveys. The data showed an increase in average 1,4-dioxane levels since the previous survey in 1993–94. The average level of 1,4-dioxane determined in the 1997 survey (348 ppm) was the

Table 4. Comparison of azeotropic distillation and solid-phase extraction methods for the determination of 1,4-dioxane in children's shampoo

1,4-Dioxane, ppm	
Azeotropic distillation	Solid-phase extraction
120, 115	130
82, 78	91
104, 85	91
95, 84	93
69, 74	90
86, 90	89

highest level observed since the monitoring program was initiated. The cause of the increase could not be explained.

Beginning in 1981, the FDA also conducted surveys of cosmetic finished products for 1,4-dioxane. The surveys included products that indicated the presence of ethoxylated ingredients on the label. The results of these surveys are shown in Table 3. The highest levels of 1,4-dioxane were found in a shampoo product (279 ppm) and a skin cleanser (139 ppm) analyzed in 1981. The results of these surveys suggested a downward trend in the levels of 1,4-dioxane in cosmetic finished products analyzed between 1981 and 1984. Changes in the manufacturing process of ethoxylated raw materials may be responsible for the apparent trend.

In 1992, a rapid method for the determination of 1,4-dioxane using SPE chromatography was published (16). This method was evaluated and modified to improve the recovery of 1,4-dioxane from products such as shampoos and bubble baths. The modified method was verified by conducting a series of recoveries at the 25, 50, and 100 ppm levels. Recoveries at the 50 and 100 ppm levels ranged between 81 and 117%, with an average recovery of 95%; 2 recoveries at the 25 ppm level averaged 71%. The modified SPE method was further evaluated by analyzing 6 children's shampoos by both the SPE and azeotropic distillation methods. As shown in Table 4, there was fair agreement between the 2 methods. The data were not corrected for recovery which may explain the differences in some of the values obtained.

FDA surveys of cosmetic finished products resumed in 1992 (Table 3). A total of 99 products were analyzed between 1992 and 1997. The products analyzed since 1994 focused on children's shampoos and bubble baths because children's products are typically formulated with ethoxylated raw materials, and the FDA wanted to know the degree of exposure to children. The downward trend in the levels of 1,4-dioxane previously observed in products analyzed in the 1980s was no longer evident in the products analyzed in the 1990s. Of particular concern were the levels of 1,4-dioxane observed in children's shampoos analyzed in 1994/95 manufactured by 2 companies. 1,4-dioxane was frequently present at levels in excess of 85 ppm.

The data collected by FDA since 1979 indicate that the cosmetic industry initially made progress in reducing and controlling 1,4-dioxane contamination of ethoxylated cosmetic raw materials. However, the most recent surveys indicate that not all raw material producers are effectively controlling the levels of 1,4-dioxane, especially in children's products. The low levels of 1,4-dioxane observed in some ethoxylated raw materials and finished products demonstrate that with current tech-

nology, excessive levels of 1,4-dioxane are readily avoidable. The results also indicate that continued periodic monitoring of ethoxylated cosmetic raw materials and cosmetic finished products for the presence of 1,4-dioxane is necessary.

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