A gas chromatographic (GC) method is described for the determination of coumarin in fragrance products. Coumarin was tentatively identified by retention time and confirmed by GC/mass spectrometry. The amount of coumarin was determined by external standard. The method was validated by conducting recovery studies from fortified fragrance products at several concentrations. Recoveries of coumarin ranged from 99 to 110%, with a relative standard deviation of 3.24. The method was used to survey a variety of fragrance products purchased in the metropolitan Washington, DC area, for coumarin. Seventy percent of the products were found to contain coumarin at concentrations ranging from 0.002 to 0.61%.

Coumarin (2H-1-benzopyran-2-one) is a naturally occurring compound found mostly at trace levels in a wide variety of plants and essential oils, including Tonka beans and sweet clover (1). Synthetic coumarin, the principle source of coumarin used in commercial products, was formerly used in food (1) but was banned by the U.S. Food and Drug Administration (FDA) in 1954 (2). Now it is used primarily as a fragrance ingredient and fixative in fragrance products and other types of cosmetics including creams and lotions, toothpastes, hair preparations, bath oils, and toilet soaps (1, 3). Coumarin is also used as a fragrance ingredient in products such as detergents and tobacco products manufactured outside the United States (4), as additive in plastic and rubber materials, and as a masking agent in paints and sprays with unpleasant odors (5). Seventeen hundred metric tons of coumarin were used as a fragrance ingredient worldwide in 1995 (6). Approximately 250,000 pounds of coumarin were reportedly used annually in the United States as a fragrance ingredient at concentrations ranging from 0.3 to 0.8% in perfumes, 0.015 to 0.1% in creams and lotions, and 0.03 to 0.2% in soaps (1).

Serious concerns were raised about the safety of coumarin in the mid-1950s when Hazeltin et al. found that rats and dogs developed liver lesions from coumarin supplied in the diet (7). These observations led to a ban on coumarin as a food additive in the United States (1, 2) and later in several other countries (6). The effects of ingested coumarin on the livers of other animal species have also been studied. Fentem et al. (8) observed serious damage to the livers of Wistar rats fed a diet containing coumarin, but no lesions were found in the livers of Mongolian gerbils. Ueno and Hironomo (9) found that coumarin did not damage the livers of Syrian hamsters, while Evans (10) found that coumarin only caused negligible harm to the livers of baboons. Carlton observed liver tumors in rats on a diet containing coumarin (4). A National Toxicology Program study in 1993 found kidney and lung cancers in certain strains of mice fed a diet containing coumarin (11). Other studies on the metabolism of coumarin have been reported (3, 12–14). Results from these studies indicated that coumarin was metabolized by several different species-dependent mechanisms. The major metabolite found in humans exposed to coumarin (13) was found to be identical to that observed in animal species which did not exhibit liver damage. The apparent species dependant metabolism of coumarin raised questions about the potential toxicological effects in humans. Deyoung (15) found that coumarin had teratogenic activity and was especially harmful to human embryos. Yourick (16) found that >95% of the coumarin applied to human skin in a cosmetic emulsion was absorbed through the skin.

There are conflicting results on the sensitivity of human skin to coumarin. In studies sponsored by the Research Institute of Fragrance Materials (RIFM), coumarin was not found to be a sensitizer (1). Both Wilkenson (17) and Malten (18) however, observed that coumarin was a sensitizer in clinical studies. The International Fragrance Association (IFRA) has not recommended any restrictions on the use of coumarin in fragrance products.

Several different analytical methods have been developed for the determination of coumarin in foods and beverages (19–24). TLC was used to determine trace quantities of coumarin in cinnamon (25). Bogan and O’Kennedy (26) determined coumarin in human serum and plasma by HPLC and Jirovetz (27) developed a method for the determination of coumarin in blood.

A few methods have been published on the determination of coumarin in fragrance products. A high-performance liquid chromatographic (HPLC) method developed by Bettero and Benassi (28) was used to determine coumarin in various cosmetic products including colognes and perfumes. A gas chromatographic (GC) method was developed by Rastogi for the determination of coumarin and other fragrance ingredients in a variety of cosmetic products including toilet waters (29).
The GC method described in this paper uses a capillary chromatography column interfaced to an electron capture detector (ECD) and external standards to determine coumarin in a variety of fragrance products. The method provides improved sensitivity and selectivity over previously published methods. The method was validated by performing recoveries from a fragrance fortified with coumarin at different concentrations. The method was also used to survey commercial fragrance products purchased in 1995 and 1997 from retail stores in the metropolitan Washington, DC area. The results of these surveys are also presented.

METHOD

Apparatus

(a) Gas chromatograph.—Model 5890 Series II (Hewlett-Packard, Palo Alto, CA) equipped with electronic pressure control (EPC), split/splitless capillary inlet system containing 4 mm id liner with a fixed silanized glass wool plug (Focusliner, SGE, Inc., Austin, TX), NiECD, Model 7673A autosampler system (Hewlett-Packard) with 10 μL syringe set for 1 μL delivery volume at fast injection speed, 30 m × 0.32 mm id fused silica capillary column with 0.25 μm film of 50% cyanopropylphenyl 50% dimethylpolysiloxane (Hewlett-Packard), and a Vectra PC 486 computer with HP 3365 ChemStation. Operating conditions: carrier gas, helium; make up gas, argon–methane (95 + 5, v/v), combined argon–methane plus helium flow, 71 mL/min; EPC carrier gas program, initial, 60 psi, hold for 0.5 min, reduced from 60 psi to 10 psi at 99 psi/min, hold for 0.5 min, reduced from 10 psi to 8 psi at 0.2 psi/min, hold for 26 min, and raised from 8 psi to 60 psi at 30 psi/min, hold for 40 min; inlet purge time for splitless injection, initial, off from 0 to 1.8 min, on from 1.8 to 8.0 min and off after 8 min; oven temperature, initial, 120°C for 4 min, programmed from 120°C to 150°C at 2°C/min, hold 18 min, then programmed from 150°C to 210°C at 10°C/min, hold 20 min; injector temperature, 300°C; detector temperature, 150°C; carrier and make up gas purifiers, OMI-1 indicating purifier tubes (Supelco, Inc., Bellefonte, PA).

(b) Gas chromatograph with mass selective detector (MSD).—GC Model 5880A (Hewlett Packard) with dedicated split/splitless capillary inlet system, and 30 × 0.25 mm id capillary column with 0.25 μm film of bonded polyethylene glycol (Supelco, Inc.), directly interfaced to Model 5970B MSD (Hewlett Packard). GC operating conditions: carrier gas, helium, at 0.8 mL/min with 5 psi head pressure; splitless time, inlet purge off from 0 to 1.5 min; oven temperature, initial, 80°C for 2 min, program from 80°C to 220°C at 30°C/min, hold for 35 min; injector temperature, 200°C; GC–MSD interface...
temperature, 280°C; injection volume, 1 μL, manual; carrier gas purifier, Oxisorb (Supelco, Inc.). MS parameters: electron impact ionization, 70 ev; positive ion mode; full scan mass range, 50–450 m/z.

Reagents

(a) Acetone.—High purity (Burdick & Jackson, Muskegon, MI).
(b) Coumarin.—Aldrich Chemical Co., Milwaukee, WI.
(c) Coumarin standard solutions (CM).—(1) Stock solution.—12.5 mg/mL. Accurately weigh 625 mg coumarin in 10 mL tared beaker to nearest 0.1 mg, dissolve in acetone, quantitatively transfer solution to 50 mL volumetric flask, dilute to volume with acetone, and mix. (2) CM working solution.—0.25 mg/mL. Accurately pipet 1.0 mL CM stock solution into a 50 mL volumetric flask, dilute to volume with acetone, and mix. (3) CM standard solutions (CMS).—(I) 25 µg/mL.—Accurately pipet 5 mL of 0.25 mg/mL CM working solution into 50 mL volumetric flask, dilute to volume with acetone, and mix. (II) 12.5 µg/mL.—Accurately pipet 5 mL of 25 µg/mL CMS into 10 mL volumetric flask, dilute to volume with acetone, and mix. (III) 5.0 µg/mL.—Accurately pipet 2 mL of 25 µg/mL CMS into 10 mL volumetric flask, dilute to volume with acetone, and mix.

Coumarin Determination

Inject 1 μL portions of CMS standard solution (III) into the gas chromatograph at an attenuation which gives a peak height of ca 1/3 full scale. The difference in coumarin peak heights from 3 consecutive injections should be less than 2%. Inject CMS standard solutions (I) and (II) and calculate the linear regression equation constants and the corresponding correlation coefficient. If the correlation coefficient is less than 0.996, repeat standard injections.

For fragrance product dilution pipet 1.0 mL fragrance product into a 5 mL volumetric flask, dilute to volume with acetone, and mix. Inject 1.0 μL into the GC system at the same attenuation used for CMS standard solution (III). If a peak is observed at the retention time of coumarin that is outside the range of peak heights obtained for the CMS standard solutions, accurately prepare a more dilute solution of the fragrance product in acetone and reanalyze by GC.

Determine coumarin concentration in the diluted fragrance product using the linear regression equation. Calculate percent coumarin in the fragrance product using the following equation:

\[
\text{Coumarin, } \% = \frac{(Y-A)}{B} \times (D)(10^4)
\]

where Y = peak height of coumarin in diluted fragrance product; A = slope of regression line; B = y intercept of regression line; D = dilution factor.

Results and Discussion

Fragrance products, prepared from a variety of synthetic ingredients, essential oils, and botanical extracts, frequently contain hundreds of chemical compounds. A limited number of these compounds have been found to have adverse toxicological properties by RIFM, an independent organization established by the fragrance industry to study the safety of fragrance ingredients. Based on the data obtained from RIFM and other sources, IFRA makes recommendations to the industry on prohibitions or use levels of fragrance ingredients which are published in the IFRA Code of Practice.

RIFM and IFRA are the primary organizations which make up the fragrance industry’s self-regulatory program. Because U.S. government regulations do not require manufacturers to disclose the identity of fragrance ingredients in their products, the FDA established a program to develop analytical methods to survey fragrance products for potentially harmful ingredients. For example, analytical methods were developed to determine the levels of synthetic nitromusks in fragrance products (30, 31).

The complexity of typical fragrance products required that the procedures/techniques selected for the determination of coumarin be optimized for selectivity. A capillary column was used to separate coumarin from as many chromatographic interferences as possible. An ECD was used for its high sensitivity and specificity to oxygenated organic chemical compounds. An electronic pressure control program for the helium carrier gas helped to rapidly and quantitatively transfer the volatilized coumarin from the splitless injection inlet to the head of the capillary column, which improved the repeatability of the retention time of coumarin and shortened the analysis time. A 4 mm id borosilicate glass injection liner containing a fixed plug of silanized glass wool was found to give the most repeatable chromatographic results. The glass wool was situated to quantitatively wipe residual analyte from the outside of the syringe needle during each injection.

The ECD response to coumarin was found to be temperature-dependent. The ECD responded strongly to coumarin at a detector temperature of 150°C, but sensitivity decreased rapidly as the temperature increased, and almost disappeared at 280°C. At 150°C, the detector gave a linear response to coumarin standards ranging in concentration from 5 to 25 µg/mL.

The GC liner was evaluated by repetitively injecting one of the coumarin standard solutions. The coumarin peak height repeatability, RSDx, was 0.44. The detection limit was 5 ng/mL corresponding to a calculated coumarin concentration of 0.0000025% in a fragrance product. An example of a GC–ECD chromatogram of a fragrance product containing coumarin is shown in Figure 1.

<table>
<thead>
<tr>
<th>Table 2. Levels of coumarin in fragrance products</th>
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<tr>
<td>Coumarin, %</td>
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<tr>
<td>0.002–0.009</td>
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<tr>
<td>0.01–0.03</td>
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<td>0.04–0.09</td>
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<td>0.1–0.7</td>
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The accuracy of the method was determined by conducting a recovery study of coumarin from a fragrance product previously found not to contain coumarin. The product was fortified with coumarin at 4 different concentrations averaging 33, 413, 2067, and 8773 μg/mL, equivalent to approximately 0.003, 0.04, 0.20, and 1.0% coumarin in the fragrance product. The results of the recovery study are shown in Table 1. Recoveries ranged from 100 to 110%, with an RSDX of 3.24. Recoveries above 100% were attributed to a small positive interference from the fragrance product matrix.

The analytical method described in this paper was used to survey fragrance products purchased in the metropolitan Washington, DC area, for coumarin. Sixty-six products were purchased from 1995 to 1997, and included colognes, eau de parfums, perfumes, toilet waters, and after shaves. Forty-seven (71%) of these products were found to contain coumarin at concentrations ranging from 0.002 to 0.61% (Table 2). The average concentration of coumarin found was 0.14%. The presence of coumarin was confirmed in most of the products by GC–MSD.

Surveys of fragrance products for coumarin have been previously published. Rastogi (32) found coumarin in 11 of 22 surveyed at concentrations ranging from 0.046 to 6.043%. Bettero and Benassi (33) found a range of 0.0011 to 0.0038% coumarin in 3 eau de colognes and 0.00278% in an after shave. De Groot and Frosch listed coumarin as the 6th most commonly used fragrance ingredient in the United States (34). It was present in 272 out of 400 (68%) cosmetics and toiletry products. In the Netherlands (35), coumarin was the 15th most common fragrance ingredient in commercial fragrance products. In the Washington, DC area, for coumarin. Sixty-six products were surveyed at concentrations ranging from 0.046 to 6.043%.

There are currently no U.S. government regulations on the use of coumarin in fragrance products. IFRA has also not issued restrictions on its use. The results of this study together with data on skin absorption and metabolism (16) will be helpful in evaluating human exposure. The FDA will continue to monitor fragrance products for coumarin and develop analytical methods for other ingredients or chemical components as toxicological issues are identified.

References

(34) De Groot, A.C., & Frosch, P.J. (1997) *Contact Derm.* 36, 57–86