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Determination of retinol, retinyl palmitate, and retinoic acid in consumer cosmetic products

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Synopsis

Retinol and retinyl palmitate are frequently used in cosmetic products. A simple, rapid, and sensitive reversed-phase high-performance liquid chromatography (HPLC) method with ultraviolet (UV) detection was developed for the quantitation of retinol, retinyl palmitate, and retinoic acid in cosmetic preparations. The analytes were extracted from a cosmetic/Celite mixture using a solvent system composed of equal amounts of hexane, isopropanol, and ethyl acetate, and the extract was injected directly into an HPLC chromatograph with a C18 column and UV detector set at 330 nm. Chromatographic separation was achieved by gradient elution with a mobile phase, starting with aqueous ammonium acetate buffer/methanol that was gradually changed to methanol/dichloromethane. The average recoveries of retinol, retinyl palmitate, and retinoic acid from spiked cosmetic products were 95% or higher. In a survey of twenty-nine consumer cosmetic skin care products labeled to contain retinoids, most products were found to contain either retinol or retinyl palmitate at concentrations up to 2.2% (w/w), while a few products contained both ingredients. A number of products also contained *cis* isomers of retinol that could be quantitatively distinguished from the all-*trans* compound. The method can be used to quantitate several retinoids and their isomers in cosmetic products. The method will be useful for obtaining information needed to estimate levels of exposure to retinoids from cosmetic products.

INTRODUCTION

Retinol (vitamin A) is the parent compound of a large number of natural and synthetic compounds collectively referred to as retinoids. In addition to retinol, the primary biologically important and naturally occurring retinoids are retinyl palmitate, retinaldehyde, and retinoic acid (Figure 1). These retinoids are essential for the development, growth, and health of vertebrates. One of the earliest biological functions discovered for retinoids was their critical role in the development and maintenance of healthy epithelial tissue, including the skin (1–3). Studies have demonstrated that the normal structure and function of the skin is dependent on the orchestration of cellular division, differentiation, and keratinization by retinoids (4).

Discovery of the powerful effects elicited by retinoids in the skin has led to their widespread use in dermatologic drug products and cosmetics. Both topically and orally administered retinoids are currently used to treat dermatologic conditions such as acne and disorders of keratinization (e.g., psoriasis and ichthyosis) and to reduce the clinical signs

of aging in the skin (5,6). Retinoids used in these dermatologic drug products are primarily isomers and synthetic analogues of retinoic acid. Commonly used active pharmaceutical ingredients include all-*trans*-, 13-*cis*- and 9-*cis*-retinoic acid, commercially known as tretinoin, isotretinoin, and alitretinoin, respectively (Figure 1). Retinoids are also widely used in cosmetics. An indication of the frequency of use for retinoids in cosmetics may be obtained from the U.S. Food and Drug Administration's Voluntary Cosmetic Registration Program (VCRP). In 2008, approximately 30,000 cosmetic products were registered in the VCRP. Registered formulations included products containing retinol (160 products), retinyl acetate (28 products), retinyl palmitate (1778 products), and retinoic acid (three products). Since participation in the VCRP is voluntary, these data may underestimate the frequency of use of retinoids in cosmetics. Retinoid-containing products registered through the VCRP include moisturizers, skin cleaners, skin conditioners, lipstick, makeup foundations and bases, shampoos, and, increasingly, products marketed to reduce the appearance of aging and photoaging. The use of retinoids in this array of product categories increases the likelihood that a consumer may receive multiple daily exposures to retinoids due to the use of cosmetic products.

Information on the levels of retinoids in cosmetic products is very limited. In 1987, the Cosmetic Ingredient Review (CIR) Expert Panel, an independent safety advisory group established in 1976 by the Cosmetic, Toiletry & Fragrance Association, reported that retinol and retinyl palmitate are generally used in cosmetics at concentrations ≤1% (7). A small number of products were reported to contain between 1% and 5% retinol (w/w) and between 5% and 10% retinyl palmitate (w/w) (7). The reported concentrations were

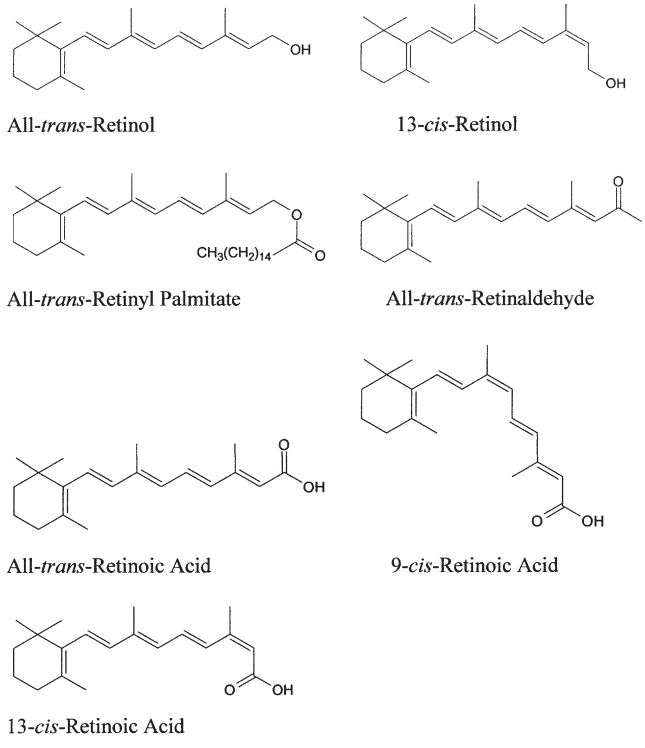

Figure 1. Selected retinoid structures.

not obtained from direct chemical analysis but from formulators of cosmetics who reported the number of their products falling within several ranges of retinoid concentration. In 2005, the CIR Expert Panel re-examined the use of retinoids in cosmetics and found that the concentrations of retinol and retinyl palmitate used to formulate cosmetics had not substantially changed (8).

Although there are currently uncertainties in the information on the frequency and level of use, it is clear that the use of retinoids in cosmetics is widespread. After reviewing animal and clinical data available in 1987 and 2005, the CIR Expert Panel concluded that retinyl palmitate and retinol were safe as cosmetic ingredients in the then-current practices of use and concentration. However, questions have persisted concerning the safety of exposure to retinoids in cosmetic products. Because topically applied retinoids such as retinol and retinyl palmitate readily penetrate the skin, systemic increases in retinoid levels could result from exposure to retinoid-containing cosmetics (9). Clinical studies have shown that no significant increase in serum levels of retinol is observed following multiple topical applications of retinol or retinyl palmitate (10,11). However, since serum levels of retinol are in tight homeostatic control under most physiological conditions, serum concentrations of retinol may be insensitive indicators of vitamin A status under exposure conditions leading to vitamin A toxicity (12). Therefore, additional information is needed about the effects of topically applied retinol and retinyl palmitate on retinoid levels in tissues, such as tissues in the developing fetus, which are sensitive targets for retinoid toxicity (9). In addition, animal studies have shown that topically applied retinoic acid can be photocarcinogenic (13). While these results are controversial and are found to vary with experimental conditions (13), concerns about the effects of topically applied retinoids such as retinyl palmitate on photocarcinogenesis have been raised and are being addressed by a study funded by the National Toxicology Program (14).

Chemical identification and quantitation of retinoids in cosmetics are needed to address the uncertainties in currently available information on the levels of retinoids in cosmetics and to provide data on exposure levels for use in risk assessments. Several reports of analysis of cosmetic products for retinoids have appeared. Scalia *et al.* have reported the use of supercritical fluid extraction and isocratic, reversed-phase high-performance liquid chromatography (HPLC) for analysis of tocopheryl acetate and retinyl palmitate in cosmetic products (15). Using this method, they successfully analyzed one day cream containing 0.1% retinyl palmitate. Ceugniet *et al.* have described the use of an isocratic, reversed-phase HPLC method suitable for analysis of cosmetic skin creams (16). These investigators were able to chromatographically resolve and identify, through use of chromatographic standards, retinaldehyde and its isomers, all-*trans*-retinol, 5,8-peroxyretinal, and 5,6-epoxyretinal. The method was used to analyze a commercial cosmetic formulation containing 0.05% retinalaldehyde. Similarly, an isocratic, reversed-phase HPLC method, optimized for separation of retinol, retinyl acetate, and retinyl palmitate, has been used for analysis of a body lotion containing 0.075% retinyl palmitate (17). The fat-soluble vitamins A, D, E, and K were also chromatographically separated using this method. Electrodeposition of retinal, retinol, and isomers of retinoic acid has also been investigated as an approach for determining the levels of retinoids in cosmetics (18). Failloux *et al.* have reported the use of Raman spectroscopy for the analysis of vitamin A degradation products in cosmetic product type matrices (19). In this work, specific conditions under which retinol degrades were examined. Retinol decomposition products were then identified and quantified by Raman spectroscopy. Flores-Perez *et al*. report the use of

cantilever-based sensors for the detection of the three retinoids, 9-*cis* retinol, 13-*cis* retinol, and all-*trans* retinol, in solution (20). This method utilized changes in the resonance frequency of the micro-cantilever sensor, due to the attachment of the analyte molecule to the micro-cantilever's surface, to detect and quantify retinoid molecules.

While these studies demonstrate utility for the analysis of retinoids in cosmetics, none of the methods involved the simultaneous analysis of a wide variety of cosmetic matrices, using real cosmetic samples, for all three retinoids—from the highly polar retinoic acid, to the less polar retinol, to the non-polar retinyl palmitate. Nor did these methods involve solid-phase extraction of the retinoid from the cosmetic matrix prior to analysis. Given the diversity of cosmetic products, the complexity of their formulations, and the many potential interfering compounds contained in them, purification prior to HPLC analysis is critical. Therefore, we sought to develop and validate a new procedure suitable for use in a survey of consumer cosmetics for retinoic acid, retinol, and retinyl palmitate. In addition, a limited survey of cosmetic products labeled to contain retinoids was performed to demonstrate the applicability of this procedure.

MATERIALS AND METHODS

REAGENTS AND MATERIALS

The following HPLC grade reagents were used: hexane and ethyl acetate (Burdick and Jackson, Muskegon, MI), isopropanol and ammonium acetate (Fisher Scientific Corporation, Fair Lawn, NJ), acetic acid (J. T. Baker, Phillipsburg, PA), and methanol and dichloromethane (Mallikrodt, Phillipsburg, N.J.). Butylated hydroxytoluene (BHT) was obtained from Sigma-Aldrich (St. Louis, MO). Retinoids for preparation of standards were obtained from Sigma-Aldrich and were stored at −20°C. Purities reported by Sigma-Aldrich were as follows: 13-*cis*-retinoic acid (98%), 9-*cis*-retinoic acid (98%), all-*trans*-retinoic acid (98%), 13-*cis*-retinol (90%), all-*trans*-retinol (95%), all-*trans*-retinaldehyde (98%), and all-*trans*-retinyl palmitate (99%). De-ionized water was prepared with a Milli-Q purification system from Millipore (Billerica, MA). The extraction tube and adaptors were obtained from Supelco (Bellefonte, PA). The Symmetry C18 analytical column (250 mm × 4.6 mm with a 5-μm particle size) was obtained from Waters Corporation (Milford, MA). Celite 545 was obtained from Fisher Scientific (Fairlawn, NJ).

SAMPLES OF COSMETIC PRODUCTS

Twenty-nine samples were purchased via the Internet or from local stores and included a wide range of different types of skin care products with retinol or retinyl palmitate listed as ingredients. Samples were stored in their original containers and packaging at room temperature until opened for analysis.

CALIBRATION STANDARDS

Actinic glassware was used for preparation and storage of all solutions of standards and samples. Seven stock solutions were prepared in a solvent comprised of 1/3 (v/v) hexane,

1/3 (v/v) isopropanol, 1/3 (v/v) ethyl acetate, and 0.1% BHT. Each stock solution contained 1 mg/ml of one of the following retinoids: 9-*cis*-retinoic acid, 13-*cis*-retinoic acid, all-*trans*-retinoic acid, 13-*cis*-retinol, all-*trans*-retinol, all-*trans*-retinaldehyde, or all-*trans*-retinyl palmitate. Working standard solutions were prepared from the stock solutions and were used for HPLC peak identification and calibration of detector response. Each working standard contained equal concentrations of all seven retinoids appropriately diluted in the hexane/isopropanol/ethyl acetate/BHT solvent (above). Ten working standards containing the following concentrations of all seven retinoids were prepared: 0.0003, 0.001, 0.002, 0.003, 0.01, 0.02, 0.03, 0.1, 0.2, and 0.3 mg/ml. Three sets of calibration curves were used to cover the range of expected retinoid concentrations in the samples. One set of calibration curves was derived from data obtained by duplicate injections of the standards having retinoid concentrations of 0.0003, 0.001, 0.002, and 0.003 mg/ml. Data from duplicate injections of the 0.003, 0.01, 0.02, and 0.03 mg/ml standards were used to obtain a second calibration set of curves. The third set of calibration curves was obtained from data collected following duplicate injections of the 0.03, 0.1, 0.2, and 0.3 mg/ml standards. Each set of calibration curves was obtained using a linear regression analysis of peak areas versus standard concentrations for each of the seven retinoids. Retinoids isolated from samples were identified by comparing their retention times and UV absorbance maxima with those of standards, and then were quantified by using the appropriate standard calibration curve for each retinoid. When not in use, standards and sample extracts were tightly sealed, stored at 2°C, and protected from light; they were stable for up to three months.

SAMPLE EXTRACTION

Approximately 300 mg of each sample was weighed into a 40-ml beaker, mixed thoroughly with about 1.7 g of Celite, and then transferred to a 10-ml extraction tube. The sample/Celite mixture was covered with a filter disk and compacted. The extraction tube was eluted into a 10-ml volumetric flask with enough extraction solvent (1/3 (v/v) hexane, 1/3 (v/v) isopropanol, 1/3 (v/v) ethyl acetate, and 0.1% BHT) to fill the volumetric flask to the mark. At the end of the extraction period, the eluate was thoroughly mixed and a 20-µl aliquot was immediately injected into the HPLC. Duplicate injections were performed for all samples. During preparation and analysis, exposure of standards and extracted samples to air, light, and heat was minimized to prevent oxidation or decomposition. When not in use, standards and sample extracts were tightly sealed, stored at 2°C, and protected from light.

HPLC METHOD

Chromatographic analyses were carried out with an Agilent 1100 series HPLC chromatograph, equipped with quaternary pumps, a vacuum degasser, an auto-injector, a variable-wavelength diode array UV-visible absorbance detector, and a personal computer with chromatographic and spectrographic software. The mobile phase was degassed with an in-line degasser. Chromatographic separation was achieved using a Symmetry C18 analytical column (250 mm × 4.6 mm with a 5-µm particle size).

The column was eluted at 1 ml/min using a gradient starting at 25% solvent A (0.4 M ammonium acetate/1.0% acetic acid pH 5.3 buffer) and 75% solvent B (methanol),

changing linearly to 80% solvent B and 20% solvent C (dichloromethane) in 30 minutes, and ending at 70% solvent B and 30% solvent C (35 minutes), with a final ten-minute holding period. The HPLC system was then gradually returned to the initial conditions at a flow rate of 1 ml/min in preparation for the next sample (approximately ten minutes). Peak areas of analytes detected at 330 nm were used for quantitation. Additionally, absorption maxima for retinoic acid and retinaldehyde at 350 and 380 nm, respectively, were monitored to further confirm identification of these two analytes. HP Chemstation™ software was used for the treatment of data and the generation of reports.

RECOVERY STUDIES

Mass recovery was determined by spiking four different retinoid-free sample matrixes (three different lotions and a cream, Table I) at 50, 500, 5000, and 50000 µg/g, with each *trans*-retinoid isomer followed by extraction and HPLC analysis as described above. Levels of retinoids in the unspiked lotions and creams used for recovery studies were well below the limits of detection (LOD). Further details on LODs for each retinoid are described in the Method Performance section (p. 495). Levels of retinoids found in analysis of consumer cosmetic products were in the range of retinoids used in the recovery studies (Table III). The average recovery at each spiking concentration was determined using the following formula (21): mass recovery (%) = (retinoid found/retinoid spiked) × 100%.

QUANTIFICATION

External standard calibrations were used for quantification. To determine analyte concentrations, peak areas were calculated for each retinoid, and the amount of analyte was

Table I
Mass Recovery of Retinoic Acid, Retinol, and Retinyl Palmitate from Consumer Cosmetic Skin Care Product Sample Matrixes

Spike/sample	Matrix	Retinoic acid	Retinol	Retinyl palmitate
50 µg/g/Sample A	Lotion	95.7%	100.3%	88.9%
50 µg/g/Sample B	Lotion	94.7%	102.7%	94.8%
50 µg/g/Sample C	Cream	98.0%	97.6%	93.7%
50 µg/g/Sample D	Lotion	102.0%	100.4%	95.3%
500 µg/g/Sample A	Lotion	99.6%	99.8%	94.4%
500 µg/g/Sample B	Lotion	102.2%	105.9%	98.8%
500 µg/g/Sample C	Cream	102.0%	104.3%	102.5%
500 µg/g/Sample D	Lotion	98.9%	100.4%	95.5%
5000 µg/g/Sample A	Lotion	98.2%	99.0%	96.4%
5000 µg/g/Sample B	Lotion	97.8%	98.5%	96.9%
5000 µg/g/Sample C	Cream	100.7%	98.7%	96.9%
5000 µg/g/Sample D	Lotion	95.3%	91.6%	91.3%
50000 µg/g/Sample A	Lotion	101.4%	98.8%	96.8%
50000 µg/g/Sample B	Lotion	99.4%	99.4%	97.0%
50000 µg/g/Sample C	Cream	103.4%	100.9%	98.2%
50000 µg/g/Sample D	Lotion	103.5%	95.4%	96.2%

determined by using a four-point calibration curve, with the concentration range used depending on the expected concentration of analyte. All peak areas were evaluated for accuracy of integration and manually reintegrated if necessary.

RESULTS AND DISCUSSION

OPTIMIZATION OF THE HPLC METHOD

In most samples, each analyte's peak was baseline separated and could be quantified unambiguously. This separation was achieved using gradient elution with a mobile phase that was initially more polar (25% ammonium acetate buffer and 75% methanol) to resolve the highly polar analyte, retinoic acid. The mobile phase was gradually changed to a less polar solvent (80% methanol and 20% dichloromethane) to elute retinol, which has an intermediate polarity. Finally, the mobile phase was gradually changed to an elutant with even lower polarity (70% methanol and 30% dichloromethane) to elute the extremely lipophilic analyte, retinyl palmitate. During optimization of the mobile phase composition, the aqueous buffer concentration and pH were found to be important for ensuring good resolution of retinoic acid and to minimize peak broadening due to ionization of the retinoic acid. However, retinol is unstable if the pH is too low. An acetic acid/ammonium acetate buffer at pH 5.5 was found to provide optimum separations.

IDENTIFICATION OF RETINOIDS

Retinoids were identified by comparison of peak retention times and UV spectra with known standards. Figure 2A shows the chromatographic separation and elution order of standard retinoids, 13-*cis*-retinoic acid, 9-*cis*-retinoic acid, all-*trans*-retinoic acid, 13-*cis*-retinol, all-*trans*-retinol, all-*trans*-retinaldehyde, and all-*trans*-retinyl palmitate, whose retention times were 17.6, 18.5, 19.1, 20.5, 20.7, 22.0, and 38.9 minutes, respectively. As expected, the retention time was found to be inversely correlated with the polarity and correlated with the lipophilicity of the retinoid. Although available information suggests that all-*trans*-retinyladehyde is not used in cosmetics, it is included in the chromatography standard mixture to demonstrate the ability of the chromatographic system to resolve this retinoid from retinoic acid, retinol, and retinyl palmitate. All-*trans*-retinylaldehyde appears as a negative chromatographic peak (Figure 2A) due to selection of 360 nm as a reference wavelength. Figures 2B and 2C show typical chromatographic separations of retinoids in extracts of cosmetic products containing either all-*trans*-retinol or all-*trans*-retinyl palmitate. Figures 2D and 2D′ show the chromatographic separation of retinoids in an extract from a lotion containing all-*trans*-retinol. The additional peaks seen in Figure 2D may be attributable to other isomers or oxidation products of retinoids in the lotion.

Tests with standard 9-*cis*- and 13-*cis*-retinoic acid and 13-*cis*-retinol showed that the HPLC method could distinguish between these *cis* isomers and the respective *trans* isomers. Most products containing all-*trans*-retinol were also found to contain 11-*cis*-, 9-*cis*-, or 13-*cis*-retinol from less than 10 µg/g to 347 µg/g. Levels of all-*trans*-retinaldehyde from less than 10 µg/g to 138 µg/g were also observed in some products (data not shown). The levels of these components may reflect differing amounts of 11-*cis*-, 9-*cis*-, 13-*cis*-retinol, and all-*trans*-retinaldehyde impurities in the all-*trans*-retinol raw material and/or

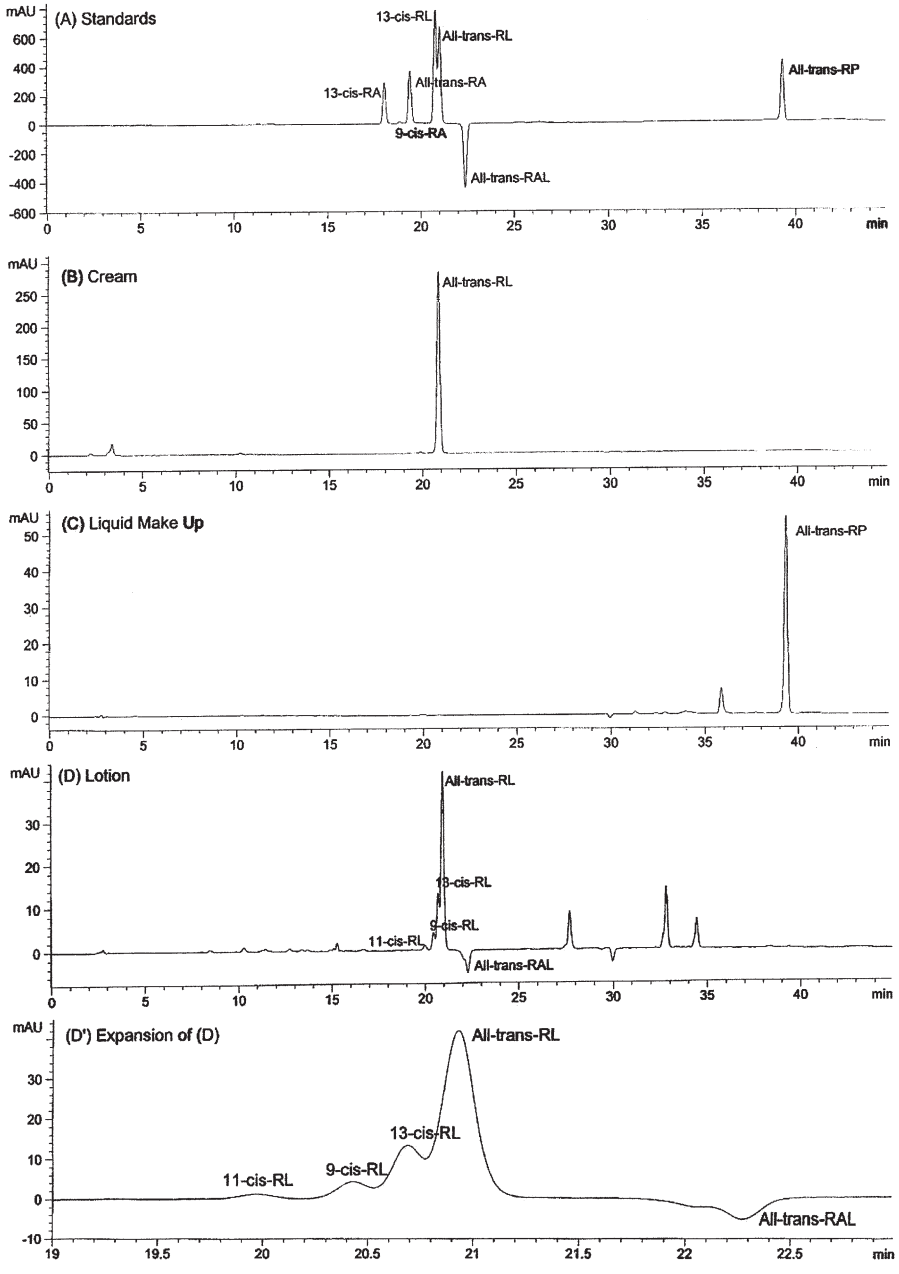

Figure 2. (A) Chromatographic separation and elution order for standard retinoids 13-*cis*-retinoic acid (13-*cis*-RA), 9-*cis*-retinoic acid (9-*cis*-RA), all-*trans*-retinoic acid (all-*trans*-RA), 13-*cis*-retinol (13-*cis*-RL), all-*trans*-retinol (all-*trans*-RL), all-*trans*-retinaldehyde (all-*trans*-RAL), and all-*trans*-retinyl palmitate (all-*trans*-RP) (note: the negative peak for all-*trans*-retinaldehyde is due to the use of 360 nm as reference wavelength). (B) Chromatographic separation of extracted cosmetic cream containing all-*trans*-retinol with no evidence of other isomers or oxidation products. (C) Chromatographic separation of extracted cosmetic liquid makeup containing all-*trans*-retinyl palmitate with no evidence of other isomers. (D) Chromatographic separation of extracted cosmetic lotion containing all-*trans*-retinol with evidence of other isomers and/or oxidation products (note additional, unidentified peaks). (D′) Expansion of D.

varying rates of isomerization and oxidation of all-*trans*-retinol due to different product formulations, packaging, storage conditions, and the length of time the product was stored prior to sale. Other mono-*cis* isomers (e.g., 7-*cis*-) and di-*cis* isomers (e.g., 7,9-*cis*-, 9,11-*cis*-, 9,13-*cis*- and 11,13-*cis*-) were not studied due to their reported instability and probable low concentrations.

Although chromatographic separation (peak-to-peak) of most retinoids was between 0.5 and 1.0 minutes or more, the peak-to-peak separation of 13-*cis*- and all-*trans*-retinol was only 0.2 minutes. It was necessary to confirm the identity of these analytes using the HP Chemstation™ software purity factor based on their UV spectra. In addition, two other retinol isomers, 11-*cis*-retinol and 9-*cis*-retinol, for which standards were not commercially available, were identified based on their UV absorbance spectra and λ-max in comparison to published reference data (18). Concentrations for these two isomers were determined, based on their peak area and the calibration curve for the all-*trans* isomer. Retention times for 11-*cis*-retinol and 9-*cis*-retinol were 19.7 and 20.2 minutes, respectively. Since *cis* retinoid isomers are known to generally have lower extinction coefficients than the corresponding *trans* isomers (22), levels reported for 11-*cis*-retinol and 9-*cis*-retinol that are calculated using the extinction coefficients for the *trans* isomers must be considered overestimates or upper bounds for their concentrations. Retinyl palmitate isomers, other than the all-*trans* isomer, remain unidentified since neither standards nor UV absorbance and λ-max data are available for these isomers.

Figure 3A shows the UV spectra for standard retinoids 13-*cis*-retinoic acid, 9-*cis*-retinoic acid, all-*trans*-retinoic acid, 13-*cis*-retinol, all-*trans*-retinol, all-*trans*-retinaldehyde, and all-*trans*-retinyl palmitate. With the exception of all-*trans*-retinaldehyde, all of the retinoids have UV absorption maxima at or near 330 nm. The UV spectra of the chromatographic peaks identified as all-*trans*-retinol (Figure 3B), extracted from a cosmetic cream sample, and all-*trans*-retinyl palmitate (Figure 3C), extracted from a liquid makeup sample, were consistent with UV spectra for the corresponding chromatographic standards. Figure 3D shows the UV spectra for the chromatographic peaks identified in Figures 2D and 2D′. UV spectra confirmed the identification of peaks in chromatographic analysis of the products' extracts.

RETINOID STABILITY

Due to the instability of retinoids from oxidation and isomerization, antioxidants or other stabilizers must be included in cosmetic formulations containing retinol or other retinoids to prevent decomposition; thus BHT, EDTA, vitamin C, vitamin E, and/or other stabilizing agents were listed as ingredients in most of the samples analyzed (23,24). Many samples were also labeled with recommendations to seal tightly after opening.

All products were opened and analyzed within one day. During sample preparation, extraction, and analysis, an effort was made to minimize exposure of samples to air, light, and heat. BHT was added to the extraction solvent to prevent oxidation during the extraction. Samples were found to be stable during sample preparation and extraction. This stability was confirmed by the mass recovery results for all-*trans*-retinoic acid, all-*trans*-retinol, and all-*trans*-retinyl palmitate, and by the chromatographic results, which indicated no significant isomerization, oxidation, or decomposition of all-*trans*-retinoic acid, all-*trans*-retinol, and all-*trans*-retinyl palmitate during extraction and HPLC analysis.

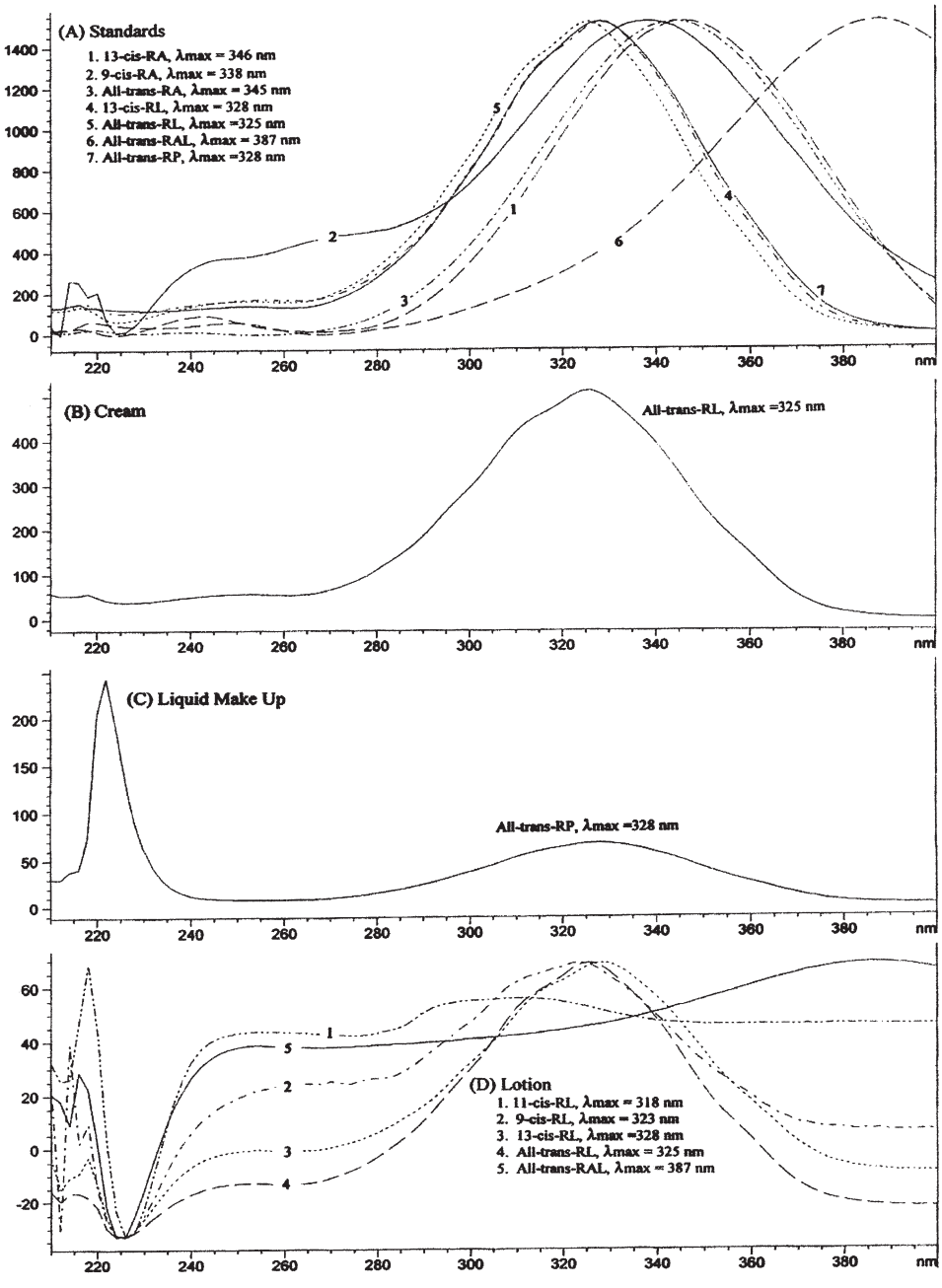

Figure 3. (A) UV spectra for peaks in Figure 2A (standard retinoids 13-*cis*-retionic acid (13-*cis*-RA), 9-*cis*-retinoic acid (9-*cis*-RA), all-*trans*-retinoic acid (all-*trans*-RA), 13-*cis*-retinol (13-*cis*-RL), all-*trans*-retinol (all-*trans*-RL), all-*trans*-retinaldehyde (all-*trans*-RAL), and all-*trans*-retinyl palmitate (all-*trans*-RP) in the order of their elution). (B) UV spectrum for Figure 2B (extracted cosmetic cream containing all-*trans*-retinol with no evidence of other isomers or oxidation products). (C) UV spectrum for Figure 2C (extracted cosmetic liquid makeup containing all-*trans*-retinyl palmitate with no evidence of others isomers). (D) UV spectrum for peaks eluting in chromatographic analysis of an extract of a cosmetic lotion containing all-*trans*-retinol (see Figure 2D).

Also, the standard solutions for all-*trans*-retinoic acid, all-*trans*-retinol, and all-*trans*-retinyl palmitate exposed to air and light at room temperature remained stable during the extraction time.

However, to determine long-term cosmetic product stability, the 29 cosmetic products were stored under normal conditions (at room temperature in their original packaging) and re-analyzed after three to six months for all-*trans* retinol and all-*trans* retinyl palmitate. By comparing the results of previous analysis for each product with the subsequent result, it was possible to determine the extent to which the samples had become isomerized and/or oxidized or self-decayed over time. Most products showed some decomposition to a varying degree.

In addition, to determine the stability of standard retinoid solutions at 0.1 mg/ml, the stability of each standard was evaluated in terms of the effect of heat, light, and air with time. In the dark protected from air at 2°C, the standards generally lasted for about three months without significant decomposition; in the dark protected from air at room temperature, the standards lasted for approximately one month; in the dark not protected from air at room temperature, the standards lasted for approximately one week; exposed to light but protected from air, the standards lasted for several days; and exposed to light and air, the standards decomposed within one day.

The specific decomposition path of oxidation or isomerization and final products differed depending on the retinoid and the specific conditions. However, in general, retinoic acid was most sensitive to light. Retinol was sensitive to air and light. Retinyl palmitate was most sensitive to heat.

METHOD PERFORMANCE

Twenty microliters of each standard solution was injected directly into the HPLC chromatograph to determine the linearity of response. Calibration curves were constructed from a plot of peak area vs concentration for each retinoid. The calibration curves obtained were found to be linear, from 0.0003 mg/ml to 0.3 mg/ml. Regression correlation coefficients were greater than 0.995. The limit of detection (LOD), defined as three times the baseline noise (21), was different for each retinoid and ranged from 0.34 µg/g to 1.08 µg/g (average, 0.6 µg/g). Specific LODs for each retinoid analyzed were as follows: 13-*cis*-retinoic acid (1.08 µg/g); all-*trans*-retinoic acid (0.63 µg/g); 13-*cis*-retinol (0.34 µg/g); all-*trans*-retinol (0.54 µg/g); all-*trans*-retinaldehyde (0.45 µg/g); and all-*trans*-retinyl palmitate (0.62 µg/g). The limit of quantification (LOQ), defined as ten times the baseline noise (21), averaged 2.0 µg/g. Samples with retinoid concentrations exceeding the highest standards were appropriately diluted to allow use of calibration curves for quantitation.

INTERFERENCE

Sample extracts were not further purified after the Celite extraction, to minimize degradation of the samples. At the detection wavelength of 330 nm, unidentified peaks eluting at retention times near the retinoid analytes were rare. Extracts treated using a C18 cartridge or filtration through a 0.45-µm filter disk gave the same analytical results as

extracts without further treatment. To confirm the identity of analyte peaks, chromatographic peak identifications were confirmed by retention time, peak width, and the UV spectrum of the chromatographic peak.

RECOVERY STUDIES

A tertiary solvent mixture was chosen that contained mutually miscible solvents: 1/3 (v/v) hexane, 1/3 (v/v) isopropanol, and 1/3 (v/v) ethyl acetate. This solvent mixture gave good recoveries of retinoids from a wide range of sample matrices. The solvent mixture was also compatible with direct injection onto the column without further sample manipulation, which helped minimize the decomposition of the extracted retinoids.

Table I shows results obtained for four different sample matrices spiked with four different levels of retinoic acid, retinol, and retinyl palmitate. Recoveries were generally 95% or greater. Since additional studies showed that increasing the volume of extractant did not lead to better recoveries, a single liquid/solid extraction using 10 ml of extractant was chosen for simplicity and rapidity.

COSMETIC SKIN CARE PRODUCTS SURVEYED

The 29 consumer cosmetics surveyed in this study included anti-wrinkle, anti-aging, skin renewal, line removal, skin-whitening, skin-moisturizing, and skin-cleansing products, and were in the form of creams, lotions, complexes, and serums (see Table II for more information on each cosmetic product analyzed). Many of the products highlighted the presence of vitamin A and/or other vitamins in the formulation (e.g., "vitamin enriched," "a natural vitamin A wrinkle cream," "retinol formula," "a retinol facial treatment with multi-vitamins," "refining night cream with 0.5% pure retinol," "vitamin A cream extra strength," "max retinol vitamin A," and "double retinol wrinkle treatment"). Seventeen (59%) of the products included retinol, either in the product name or in the list of ingredients, four included retinyl palmitate, and six included both. One product found to contain retinoid did not indicate the presence of either retinol or retinyl palmitate in the list of ingredients.

Twenty-five (86%) of the products were labeled to contain anti-oxidants or stabilizers such as vitamin C, vitamin E, BHT, and di- or tetra-sodium EDTA to prevent oxidation or decomposition of the retinol or retinyl palmitate. In addition, 22 products (76%) were labeled to contain a sunscreen, recommended use with a sunscreen, or recommended night-time use only due to the possibility of increased photosensitivity. Finally, 18 products (62%) provided product use warnings such as "for external use only," "avoid contact with the eyes or mucous membranes," "discontinue use if persistent irritation occurs," or "keep out the reach of children."

SURVEY RESULTS

The results of the analysis are given in Table III. Eighteen products (62%) were found to contain retinol. Six (21%) were found to contain retinyl palmitate, and five (17%) were found to contain both retinol and retinyl palmitate. No products were found to contain

Table II
Sample Descriptions

Product type	Labeled ingredient	Anti-oxidant or stabilizer	Sunscreen included or recommended	Product use warning
Anti-wrinkle facial serum	RP	Isostearic acid Vitamin E EDTA	Not listed	Not listed
Wrinkle-corrector cream	RL	BHT	Apply morning or evening	Not listed
Line defense complex	RL	Not listed	Use SPF 15 daily	Yes
Liquid makeup	Not listed	Not listed	SPF 20	Yes
Anti-wrinkle cream	RP	Vitamin E	Not listed	Not listed
Anti-cellulite treatment cream	RL	BHT EDTA	Not listed	Yes
Anti-wrinkle cream	RL	EDTA Vitamin E BHT	SPF 15	Yes
Nightly renewal cream	RL	Vitamin C BHT EDTA	Night-time use	Yes
Line eliminator	RL	Vitamins C and E BHT EDTA	Night-time use only	Yes
Renew body serum	RP	EDTA	Not listed	Not listed
Refining night cream	RL	BHT EDTA	Use sunscreen	Yes
Wrinkle corrector	RL	Not listed	Not listed	Not listed
Day cream	RP	BHT Vitamin E EDTA	SPF 20	Yes
Anti-wrinkle treatment cream	RL	Vitamin E EDTA BHT	Use sunscreen	Not listed
Anti-wrinkle night complex	RL	Vitamin E EDTA	Use sunscreen	Yes
Skin-refining lotion	RL	EDTA	Not listed	Yes
Skin-whitening serum	RL	EDTA Vitamins E and C BHT	Use nightly	Yes
Anti-wrinkle cream	RL	Vitamin C BHT EDTA	Use nightly	Not listed
Anti-wrinkle cream	RL/RP	Vitamin E EDTA	SPF 20	Yes
Wrinkle treatment	RL	EDTA BHT Vitamins C and E	Use nightly	Not listed
Anti-age complex	RL	Vitamin E BHT EDTA	Nightly use	Yes
Smoothing serum	RL	Not listed	Nightly use	Yes
Moisturizer	RL/RP	Vitamins E and C EDTA	Nightly use	Yes
Clean complex	RL/RP	EDTA	Use sunscreen	Yes

(Continued)

Table II (Continued)
Sample Descriptions

Product type	Labeled ingredient	Anti-oxidant or stabilizer	Sunscreen included or recommended	Product use warning
Skin care complex	RP/RL	Vitamin E EDTA	Not listed	Yes
Anti-aging cream	RL	Vitamins E and C BHT EDTA	Nightly use	Not listed
Skin transformation serum	RL/RP	Vitamin E	Not listed	Not listed
Anti-acne rejuvenating cream	RL	EDTA	Nightly use	Yes
Skin brightener	RP/RL	Vitamin E	Use morning or evening	Not listed

Table III
Survey Results for Retinol and Retinyl Palmitate Isomers (in µg/g)[a]

		Retinol		Retinyl palmitate	
Sample no.	Retinol all-*trans*-	9-*cis*-[b]	11-*cis*-[b]	13-*cis*-	all-*trans*-
1	139	ND	20	<10	14984
2	1726	ND	ND	347	ND
3	3370	15	28	31	ND
4[c]	ND	ND	ND	ND	563
5	ND	ND	ND	ND	502
6	377	<10	ND	58	ND
7[c]	1503	ND	ND	ND	ND
8	3103	<10	ND	ND	ND
9	3242	22	ND	ND	ND
10	<10	ND	ND	ND	912
11	13949	95	ND	175	ND
12	1819	70	ND	103	ND
13[c]	ND	ND	ND	ND	7982
14	206	<10	11	13	ND
15	6021	19	ND	ND	ND
16	410	<10	<10	<10	ND
17	1681	<10	ND	<10	ND
18	3565	53	<10	58	ND
19[c]	ND	ND	ND	ND	6565
20	500	<10	ND	<10	ND
21	5634	30	<10	ND	ND
22	2364	39	ND	180	ND
23	1503	46	<10	41	<10
24	<10	<10	<10	<10	260
25	62	ND	ND	<10	4160
26	21817	133	ND	177	15
27	718	17	<10	155	ND
28[d]	2559	25	<10	41	178
29	59	26	ND	ND	34

ND = Not detected, i.e., below level of detection (0.6 µg/g).

[a]9-*cis*-, 13-*cis*-, and all-*trans*-retinoic acid were not detected in any of the samples.

[b]Estimated concentrations based on calibration curve for all-*trans*-retinol.

[c]Product classified as both a cosmetic and a drug due to cosmetic and sunscreen claims.

[d]Product may be classified as both a cosmetic and a drug due to cosmetic and apparent anti-acne claims.

retinoic acid. Concentrations of all-*trans*-retinol ranged from 59 μg/g to 21817 μg/g. Concentrations for all-*trans*-retinyl palmitate ranged from 15 μg/g to 14984 μg/g.

Retinoid concentrations and identity were found to be consistent with the label information in 23 products (80%). One product, without any listed ingredients, was found to contain retinyl palmitate at 563 μg/g. Another product that listed only retinyl palmitate on the label was found to contain all-*trans*-retinol (139 μg/g) in addition to all-*trans*-retinyl palmitate (14984 μg/g). This is possibly due to the hydrolysis of the retinyl palmitate during manufacturing or during storage. Two products were found to contain retinyl palmitate in addition to retinol, the listed ingredient. The most likely explanation for this is the presence of retinyl palmitate in the retinol ingredient as an impurity. Alternatively, retinyl palmitate may have been used as a partial substitute for retinol. Two other products, although labeled to contain both retinol and retinyl palmitate, were found to contain only retinol in one product, and only retinyl palmitate in the other product.

CONCLUSIONS

In this report, a rapid method for the determination of retinoic acid, retinol, and retinyl palmitate in consumer cosmetic products is described. The method allows determination of all retinoids commonly used as cosmetic ingredients. Twenty-nine consumer cosmetic products, including anti-wrinkle, anti-aging, skin renewal, line removal, skin-whitening, moisturizing, and skin-cleansing preparations, were analyzed. Seventeen of the surveyed products included retinol on the ingredient label, and four listed retinyl palmitate. Six included both retinol and retinyl palmitate. One product did not indicate the presence of either retinol or retinyl palmitate. Overall, the retinoids found in the products agreed with retinoids identified as ingredients on the labeling of the product. Retinoic acid was not found in any of the analyzed products. The range of concentrations determined for retinol and retinyl palmitate was found be generally ≤1%, consistent with earlier reports (7,8). The analytical method described here is appropriate for use in larger surveys of retinoids in cosmetic products and can provide data needed for estimating exposure levels to these commercially important cosmetic ingredients.

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