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Assay of vitamin A palmitate and vitamin E acetate in cosmetic creams and lotions by supercritical fluid extraction and HPLC

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Abstract: The use of supercritical fluid extraction (SFE) as an alternative to liquid extraction was examined for the isolation of vitamin A palmitate and vitamin E acetate from cream and lotion preparations. Investigation of the factors controlling the extraction efficiency in SFE indicated that vitamin recoveries were affected mainly by the extraction pressure and by the degree of sample dispersion. The vitamins were analysed by high-performance liquid chromatography after a 30-min extraction of the cosmetic product with supercritical carbon dioxide at 40°C and at a pressure of 250 atm. Compared with conventional liquid extraction SFE produced slightly lower recoveries (>91.6%) but afforded a more effective purification of the cosmetic matrices. Moreover, SFE minimized sample handling and the use of harmful solvents and provided mild extraction conditions for the analysis of the labile vitamins. Vitamin E acetate and vitamin A palmitate were assayed in commercial cream and lotion formulations using the proposed SFE technique.

Keywords: *Supercritical fluid extraction; vitamin E acetate; vitamin A palmitate; cosmetic cream; cosmetic lotion; reversed-phase high-performance liquid chromatography.*

Introduction

Vitamins A and E are widely used in the formulation of cosmetics for the prevention or correction of skin damage [1–3]. These fat-soluble vitamins are usually present in commercial products as the more stable ester forms, namely vitamin A palmitate and vitamin E acetate [2, 4].

Owing to the photolability and sensitivity to heat and oxidation of these vitamins [4–6] their quantitative determination in cosmetic preparations is particularly important for quality control of finished products and for stability-indicating assays. To prevent degradation during analysis, rapid and simple sample pretreatment techniques which minimize the exposure to light, atmospheric oxygen and elevated temperatures are needed. The current USP methodology [7] for the assay of vitamins A and E and their esters requires saponification, liquid–liquid extraction and solvent evaporation. This procedure has inherent disadvantages including emulsion formation, analyte degradation and relatively complex sample manipulations which represent a source of possible errors. More recently, a simple method for the analysis of the foregoing vitamins in cosmetics has been reported [4] based on dissolution of the product with a tetrahydrofuran–water mixture followed by direct injection into the chromatograph. However, rapid deterioration of the column performance [8] and interference from matrix constituents are potential drawbacks of this technique as the sample is analysed without any clean-up. Moreover, large volumes of harmful solvents must be handled and eventually disposed of.

Supercritical fluid extraction (SFE) is emerging as a valuable alternative to conventional liquid extraction for the isolation of organic analytes from solid and semi-solid matrices [9]. The combined gas-like mass-transfer and liquid-like solvating characteristics of supercritical fluids lead to more rapid and efficient extractions. Furthermore, since the solvent strength of a supercritical fluid is a function of density, it can be modified by simply changing the pressure or the temperature [10]. Carbon

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dioxide, the most commonly used supercritical fluid, has the additional advantages of being non-toxic, non-inflammable, relatively inert and inexpensive and easily removed from the extract by depressurization.

The present study describes a novel method based on SFE for the rapid isolation of vitamin A palmitate and vitamin E acetate from cosmetic preparations prior to analysis by reversed-phase high-performance liquid chromatography (RP–HPLC). The application of the procedure to the assay of commercial products is also presented.

Experimental

Materials

Vitamin A palmitate and vitamin E acetate were obtained from Roche (Basle, Switzerland). HPLC-grade methanol, acetonitrile and tetrahydrofuran were supplied by Merck (Darmstadt, Germany). All other chemicals were of analytical grade (Aldrich, Steinheim, Germany). Instrument-grade liquid carbon dioxide supplied in cylinders with a dip tube was from SOL (Monza, Italy). Commercial cosmetics were from retail stores.

High-performance liquid chromatography

The HPLC apparatus (Waters Assoc., Milford, MA, USA) comprised a Model 600 pump, a Model 712 WISP auto-sampler and a Model 486 variable-wavelength UV/Vis detector. The eluent absorbance was monitored at 280 nm and 0.4 absorbance units full scale (a.u.f.s.) for vitamin E acetate, and at 325 nm and 0.6 a.u.f.s. for vitamin A palmitate using the wavelength and sensitivity range time-programming capability. Data acquisition and processing were accomplished with an APCIV computer system (NEC, Boxborough, MA, USA) using Baseline 815 software (Waters).

Separations were performed on a μBondapak C_{18} column (10-μm particle diameter, 30 cm × 3.9 mm i.d.; Waters) fitted with a Guard-Pak precolumn (10-μm particle diameter; Waters) and eluted isocratically with methanol–acetonitrile (75:25, v/v) at a flow-rate of 1.5 ml min^{-1}. The mobile phase was on-line degassed by a model ERC-3114 solvent degasser (Erma, Tokyo, Japan). Chromatography was performed at ambient temperature.

The identity of the separated compounds was assigned by co-chromatography with authentic standards. The quantification was carried out by the external standard method, using peak areas.

SFE

Supercritical fluid extractions were performed with a Dionex SFE-703 system (Dionex, Salt Lake City, UT, USA). The cosmetic product (0.10–0.15 g) was accurately weighed, mixed with celite and loaded into the stainless-steel extraction cell (3.0 ml) supplied with the extraction unit. The cell was filled with celite and inserted into the SFE-703 oven. Supercritical CO_2 was then pumped through the sample and then through the restrictor. As the CO_2 evaporated at the restrictor outlet due to depressurization, the extracted analytes were collected into 4 ml of tetrahydrofuran–methanol (4:1, v/v) in a cooled (0°C) glass vial. The content of the vial was made up to volume (5 ml) and directly analysed by HPLC. Extractions were carried out in the dynamic (continuous flow) mode for 30 min, at a pressure of 250 atm and at a temperature of 40°C. The restrictor was maintained at 100°C and the measured flow-rate for the supercritical fluid ranged from 190 to 220 ml min^{-1} (measured as gaseous CO_2). All the instrument operating parameters were controlled by the built-in microprocessor.

Accuracy and precision

The test samples were prepared by spiking a cream placebo with 50-μl aliquots of a solution of vitamin A palmitate and vitamin E acetate in tetrahydrofuran–methanol (4:1, v/v), corresponding to a level of 0.5% (w/w) of each vitamin. The percentage recovery was determined by comparing the peak areas of the two vitamins extracted from the test samples with those obtained by direct injections of equivalent amounts of the analytes.

The intra-assay precision was tested by repeated (n = 6) analyses of the same stock sample solution from a cream. The inter-assay variability was evaluated by extraction with supercritical CO_2 and HPLC analysis of independent samples (n = 6) from the same cream product.

Low-actinic glassware was used for all preparations.

Results and Discussion

In previous papers [11, 12] we investigated

the effectiveness of SFE as a sample preparation technique for the analysis of antimicrobial agents in cosmetics. Along these lines, the present work was undertaken to examine the potential use of supercritical carbon dioxide for the extraction of vitamin A palmitate and vitamin E acetate from cream and lotion matrices.

Preliminary development of the SFE conditions was performed on filter-paper spiked with 100-μl aliquots of a standard vitamin solution (10 mg ml^{-1} of each vitamin) and inserted into the extraction cell which was filled with chromatographic-grade sand to avoid significant void volumes. Initial extractions were carried out for 30 min, using supercritical CO_2 at a pressure of 200 atm and setting the extraction cell and restrictor temperatures to 40 and 60°C, respectively. Under these conditions 59.4–68.7% of the spiked vitamins were recovered. Increasing the pressure to 250 atm enhanced the recoveries to more than 92.2% due to the higher density and hence solvating power of the supercritical fluid [13].

Additional experiments were performed using a cream placebo spiked with vitamin E acetate (0.5%, w/w) and vitamin A palmitate (0.5%, w/w). The sample was smeared on filter-paper to prevent the matrix from being swept out of the cell and extracted with supercritical carbon dioxide at 40°C and 250 atm. Initial SFE experiments with the cream specimens were hampered by rapid plugging of the restrictor which caused an overpressure condition and the interruption of the operation. The problem was eliminated by raising the restrictor temperature from 60 to 100°C. However, under the SFE conditions which produced essentially quantitative recoveries of the vitamins spiked on filter-paper, only 16.4% of vitamin E acetate and 19.3% of vitamin A palmitate were extracted from the cream. The reduced extraction efficiency was traced to an ineffective penetration of the supercritical carbon dioxide into the cream matrix. Disruption of the sample continuity by mixing it with an adsorbent (i.e. celite or sand) restored the extraction efficiency for both vitamins, the highest recovery values (>93.0%) being obtained using celite as support. Consequently, this method was used for all subsequent experiments. Moreover, the extraction time was set at 30 min since there was no significant improvement in vitamin recoveries at longer operating times.

The precision of the foregoing SFE procedure was determined by assaying a day cream preparation containing 1.0% (w/w) vitamin E acetate and 0.1% (w/w) vitamin A palmitate. The results of this study gave an average relative standard deviation of 0.9% ($n = 6$) for the intra-assay reproducibility and 4.6% ($n = 6$) for the inter-assay reproducibility. Calibration curves ($n = 6$) were linear in the range 0.08–5.0% (w/w) for vitamin E acetate and 0.02–1.5% (w/w) for vitamin A palmitate with correlation coefficients greater than 0.998. The average slopes and intercepts were, respectively, 150.76 ± 2.13 and 2.70 ± 0.04 for vitamin E acetate and 337.85 ± 5.29 and 11.31 ± 0.16 for vitamin A palmitate. A representative chromatogram of a day cream product extracted according to the SFE procedure described here is shown in Fig. 1.

In order to assess the accuracy of the method developed in this study, the vitamin levels determined in four commercially available cosmetic preparations using SFE were compared to those obtained by an established technique [4] based on dissolution of the product in tetrahydrofuran-water under sonication and direct injection onto the HPLC

Table 1
Relative recoveries for vitamin A palmitate and vitamin E acetate from cosmetics using SFE compared with conventional liquid extraction [4]

	% Recovery*	
Sample	Vitamin E acetate	Vitamin A palmitate
Day cream	94.9 (3.9)	93.9 (5.4)
Anti-wrinkle cream	92.5 (4.7)	91.6 (4.8)
Hand cream	92.2 (6.1)	n.p.†
Moisturizing lotion	94.2 (4.0)	n.p.†

*Each value is the mean (relative standard deviation) of five determinations.
†Not present.

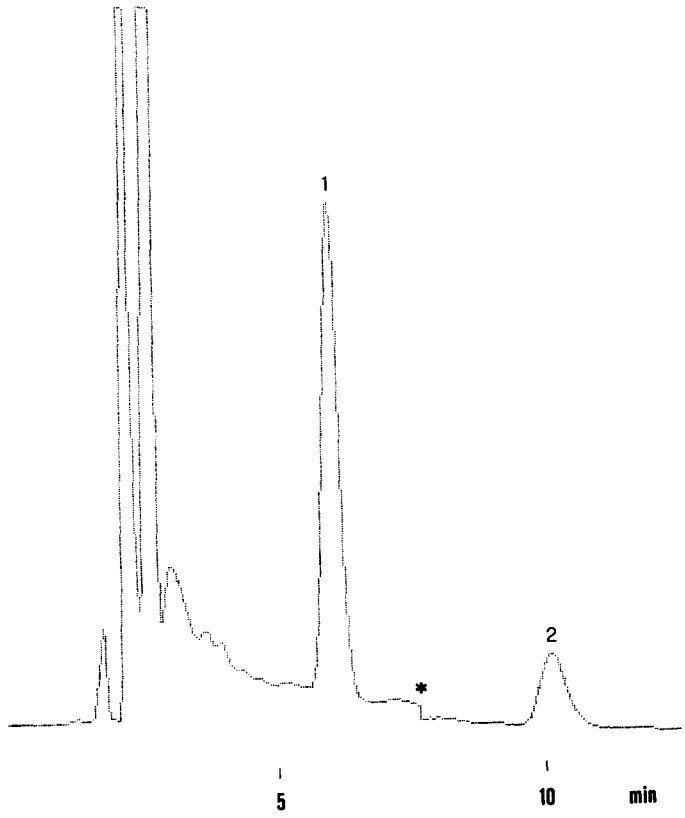

Figure 1
HPLC chromatogram of a day cream purified by SFE. Operating conditions as described under Experimental. Peaks: 1 = vitamin E acetate; 2 = vitamin A palmitate. Detection: 0 min, 280 nm and 0.4 a.u.f.s.; 7.5 min, 325 nm and 0.6 a.u.f.s. *Time-programmed change of wavelength and sensitivity range.

column after filtration (Table 1). Relative extraction efficiencies higher than 91.6% compared with liquid extraction were obtained. Less than 1% of the total vitamin content remained in the samples after SFE, indicating that the incomplete recoveries were due to the trapping system rather than the extraction process. In fact, since the analytes are collected in a solvent following the supercritical fluid decompression, losses from aerosol formation can occur [9]. The results presented in Table 1 indicate that higher vitamin recoveries were attained by liquid extraction, nevertheless SFE offers the advantage of minimizing the amount of hazardous solvents used. In addition, decreased chromatographic column lifetime is a drawback of the liquid extraction technique [8] as the cosmetic preparation after solubilization is injected directly into the HPLC without any purification. Conversely, no deterioration of the column efficiency was observed after more than 100 analyses of samples processed by SFE.

Conclusions

The first SFE method has been developed for the rapid isolation of vitamin E acetate and vitamin A palmitate from cosmetics. The proposed method is less laborious than those reported in the literature [1, 4, 7] as sample pre-treatment is reduced to loading the product adsorbed on a support into the extraction cell. Moreover, since the SFE process is fully automated, a higher sample throughput is achieved compared to conventional manual sample preparation. SFE exhibits several advantages over classical extraction techniques for the isolation of labile molecules such as vitamins A and E. In particular, it allows no sample contact with air, is conducted in the dark and can be performed at relatively low temperatures. Although satisfactory recoveries are produced by SFE, they could be further improved by increasing the efficiency of the trapping technique. The features of the SFE method outlined above make it suitable for quality control assays of vitamin A palmitate and vitamin E acetate in cosmetic products.

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