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Determination of sunscreen agents in cosmetic products by supercritical fluid extraction and high-performance liquid chromatography

Santo Scalia*

Department of Pharmaceutical Sciences, University of Ferrara, Via Fossato di Mortara 17, 44100 Ferrara, Italy

Abstract

A rapid supercritical fluid extraction (SFE) procedure for the isolation of five of the most common sunscreen agents (2-ethylhexyl-*p*-dimethylaminobenzoate, 2-hydroxy-4-methoxybenzophenone, 2-ethylhexyl-*p*-methoxycinnamate, 4-methylbenzylidene camphor and 4-*tert*.-butyl-4′-methoxydibenzoylmethane) from cosmetic products is described. Investigation of the factors affecting the extraction efficiency in SFE indicated that sunscreen recoveries were affected mainly by the supercritical CO_2 pressure and by the trapping method. The sunscreens were analyzed by reversed-phase high-performance liquid chromatography after a 10-min extraction of the cosmetic product with CO_2 at 250 bar and 40°C, using sequential glass surface and C_{18} sorbent as collection system. A quantitative comparison of SFE with a liquid extraction procedure was performed on commercial cosmetics. The SFE method yielded recoveries higher than 94.8% compared with conventional liquid extraction and exhibited a precision better than 5.3% relative standard deviation. Moreover, SFE minimized sample handling, reduced the consumption of harmful solvents and afforded a more effective purification of the cosmetic matrices.

Keywords: Cosmetics; Sunscreen agents

1. Introduction

Because of the expanding knowledge about the harmful effects of the solar UV rays on human skin [1,2], the use of topical sunscreen agents has become increasingly widespread [1,3,4]. Chemical sunscreens are compounds which absorb deleterious UV light, thereby decreasing the amount of the solar radiation energy reaching the skin. In recent years, the trend toward products with high protection factors and screening efficiency against both UV-B (290–320 nm) and UV-A (320–400 nm) wavelengths has resulted in the development of cosmetic preparations containing different sunscreen compound combinations [5,6]. Lists of approved UV absorbers with their maximum allowed concentrations have been set by various regulatory authorities in Europe, USA, Australia and Japan [5,7]. Hence, the assay of sunscreen agents in commercial products is important for quality control purposes and for checking their conformance to the existing legislation. In addition, in order to ensure an adequate photoprotective action during usage, the stability of the sunscreen in the finished product needs to be determined.

Published procedures for the isolation of sunscreen agents from cosmetic matrices, prior to chromatographic analysis, require several sample manipulations including solvent extraction, liquid–liquid ex-

*Tel.: +39-532-291-277; fax: +39-532-291-296.
E-mail address: sls@dns.unife.it (S. Scalia)

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traction, sonication, centrifugation and filtration [6,8–10]. These processes are laborious and time-consuming and therefore are not suitable for routine analyses of cosmetic products. Moreover, large volumes of hazardous solvents must be handled and disposed of.

Supercritical fluid extraction (SFE) is being recognized as a valuable alternative to traditional sample preparation methods for the isolation of organic analytes from solid and semisolid matrices [11–14]. The combined liquid-like solvating capabilities and gas-like transport properties of supercritical fluids lead to improved mass-transfer and reduced extraction time [11,13]. Moreover, the dissolving power of a supercritical fluid can be modified by simply changing the applied pressure and/or temperature [11,15]. Finally, the most commonly used supercritical fluid, carbon dioxide, has the additional advantages of being non-flammable, fairly non-toxic, cost-effective and easily removed from the extract following decompression.

The present study reports on the development of an SFE method for the rapid and efficient isolation of five of the most common [10,16] sunscreen compounds (2-ethylhexyl-*p*-dimethylaminobenzoate, 2-ethylhexyl-*p*-methoxycinnamate, 4-methylbenzylidene camphor, 2-hydroxy-4-methoxybenzophenone and 4-*tert*.-butyl-4′-methoxydibenzoylmethane; see Fig. 1) from cosmetic preparations, prior to analysis by high-performance liquid chromatography (HPLC). The application of this technique to the assay of commercial products is also presented.

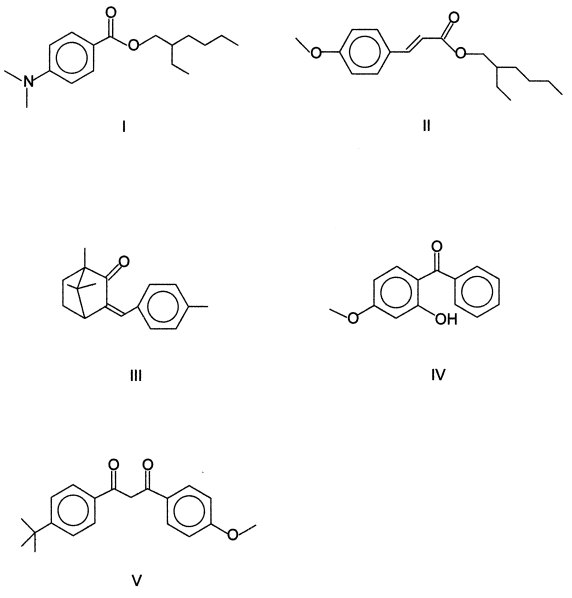

Fig. 1. Chemical structures of the sunscreen agents. (I) 2-Ethylhexyl-*p*-dimethylaminobenzoate; (II) 2-ethylhexyl-*p*-methoxycinnamate; (III) 4-methylbenzylidene camphor; (IV) 2-hydroxy-4-methoxybenzophenone; (V) 4-*tert*.-butyl-4′-methoxydibenzoylmethane.

2. Experimental

2.1. Reagents

Instrument-grade liquid carbon dioxide supplied in cylinders with a dip tube was from Sapio (Monza, Italy). Methanol, acetonitrile and tetrahydrofuran of HPLC-grade were obtained from Merck (Darmstadt, Germany). 2-Ethylhexyl-*p*-dimethylaminobenzoate (EH-DMAB; CAS No. 21245-02-3), 2-ethylhexyl-*p*-methoxycinnamate (EH-MC; CAS No. 5466-77-3) and 2-hydroxy-4-methoxybenzophenone (HM-B; CAS No. 131-57-7) were provided by Van Dyk (Belleville, NJ, USA) with purities of at least 98%. 4-Methylbenzylidene camphor (M-BC; CAS No. 38102-62-4) and 4-*tert*.-butyl-4′-methoxydibenzoylmethane (BM-DBM; CAS No. 70356-09-1) were supplied, respectively, by Haarmann&Reimer (Holzminden, Germany) and Givaudan (Geneva, Switzerland) with purities of at least 98%. Hydromatrix (diatomaceous earths) was from Applied Separations (Allentown, PA, USA). All other chemicals were of analytical grade (Sigma, St. Louis, MO, USA). Commercial cosmetics were from retail stores.

2.2. High-performance liquid chromatography

The HPLC apparatus comprised a Model LabFlow 3000 pump (LabService Analytica, Bologna, Italy), a Model 7125 injection valve with a 5-μl sample loop (Rheodyne, Cotati, CA, USA) and a Model 975-UV variable-wavelength UV–Vis detector (Jasco, Tokyo, Japan) set at 320 nm, which is a compromise absorption wavelength to obtain satisfactory UV responses for all analytes. Data acquisition and

processing were accomplished with a personal computer using Borwin software (JBMS Developpements, Le Fontanil, France). Sample injections were effected with a Model 80365 syringe (Hamilton, Bonaduz, Switzerland). Separations were performed on a 5-μm Hypersil BDS Phenyl column (150×3.0 mm I.D.; Hypersil, Runcorn, UK) eluted with methanol–acetonitrile–tetrahydrofuran–water (45:10:10:35, v/v) containing 0.5% (v/v) acetic acid. The column temperature was maintained at 40°C using a Model 7990 Space Column Heater (Jones Chromatography, Hangoed, UK). Chromatography was performed under isocratic conditions, at a flow-rate of 0.4 ml/min. The identity of the separated peaks was assigned by co-chromatography with the authentic standards. Quantification was carried out by integration of the peak areas using an external standard method.

2.3. SFE

Supercritical fluid extractions were performed with a Spe-ed SFE system (Model 7010/680 atm; Applied Separations) which comprises an air-driven pump to deliver the CO_2 to the extraction cell (10-ml stainless steel vessel with 2-μm frits at either ends) housed within a temperature-controlled oven. The CO_2 pump head was cooled by means of circulating water at 4°C (Dese Lab., Padua, Italy). The outlet of the extraction cell was connected to a thermally-controlled variable restrictor which maintains supercritical pressure conditions in the system. The cosmetic product (0.1–0.15 g) was accurately weighed and, in the case of lipsticks, was cut into small pieces. The sample was mixed with hydromatrix and loaded into the extraction cell which was filled with hydromatrix. A plug of polypropylene wool was inserted into the cell at both ends. Extractions were carried out in the dynamic (continual flow) mode for 10 min at 40°C at a pressure of 250 bar. The restrictor was maintained at 80°C and the measured flow-rate for the supercritical fluid was 1.5 l/min of expanded gas. As the CO_2 evaporated at the restrictor outlet due to decompression, the extracted material was collected into an empty glass vial fitted with a septum and a needle vent. A conventional solid-phase extraction C_{18} cartridge (Applied Separations), inserted at the needle vent outlet, was used as a secondary trapping device. The analytes were eluted from the cartridge and rinsed off the surface of the collection vial with ethanol. The combined ethanol fractions were adjusted to a known volume (10 ml) and analyzed by HPLC.

2.4. Accuracy and precision

The accuracy of the SFE method was determined by comparing the sunscreen assay results obtained for the same cosmetic preparation by the proposed SFE technique with a liquid extraction procedure [9].

The method precision was calculated by extraction with supercritical CO_2 and HPLC assay of independent samples ($n=5$) from the same lipstick product. The chromatographic precision was evaluated by repeated analyses ($n=5$) of the same sample solution from a lipstick.

3. Results and discussion

Initial development of the SFE conditions was performed on hydromatrix (a relatively inert matrix) spiked with a 100-μl aliquot of a standard solution containing ca. 5 mg/ml of each sunscreen agent in methanol. These experiments were carried out to evaluate analyte solubility in supercritical CO_2 and the trapping efficiency of the collection system. After the solvent was allowed to evaporate, the spiked hydromatrix was loaded into the extraction cell which was filled with hydromatrix to avoid significant void volumes. Preliminary extractions were carried out for 5 min, with supercritical CO_2 at 200 bar, using ethanol as collection solvent and setting the extraction cell and restrictor temperature to 40 and 80°C, respectively. Under these conditions, 62–66% of the spiked UV filters were recovered. Increasing the pressure to 250 bar enhanced the recoveries to 75–86%. No significant improvement in the extraction efficiency was observed at higher pressure (300 bar), suggesting that the obtained yields are not limited by analyte solubility, as this parameter is a function of the applied pressure and hence density of the supercritical fluid. Since less than 2.1% of the sunscreen spikes remained in the hydromatrix after SFE (as determined by hydromatrix extraction with ethanol under sonication and

HPLC analysis), the observed incomplete recoveries could be traced to the trapping system, based on collection of the extracted sunscreens in a liquid solvent (20 ml of ethanol) during depressurization of the supercritical CO_2. In fact, this technique is prone to analyte loss due to its purging from the collecting solvent [17,18]. In order to overcome this problem, solid trapping with decompression of the supercritical CO_2 into an empty vial was examined. This method, even with cooling (ca. 4°C) of the glass surface, did not achieve better results. The use of tandem trapping systems has been reported to enhance the collection efficiency in SFE [17]. Accordingly, this approach was employed in the present study by inserting an adsorbent trap in-line after the empty receiving vial. Cartridges pre-packed with silica or octadecylsilica (C_{18}) were examined as sorbents for sunscreen collection after SFE. The C_{18} stationary phase was more effective than silica in recovering the analytes vented from the collector vial by the depressurized supercritical fluid and therefore it was selected as the secondary trapping device. The use of the combined glass surface–C_{18} sorbent trapping system improved the sunscreen recoveries to more than 92%, thus demonstrating that for this application tandem trapping is more efficient than conventional single trap methods. Moreover, no improvement in recoveries resulted from longer operating time (15 min) and consequently the extraction duration was set at 5 min.

Additional experiments were then performed on cosmetic samples to evaluate the influence of matrix effects on sunscreen extraction rates and yields by supercritical CO_2. As spiked samples are not truly representative of real samples, in order to optimize the operating parameters for the isolation of sunscreens from cosmetics by SFE, the assay results obtained with this technique were compared to those determined on the same commercial product by an independent method. Among the procedures reported in the literature for conventional liquid extraction of sunscreen agents from cosmetic preparations, those developed by Gagliardi et al. [8], Ikeda et al. [9], DiNunzio and Gadde [6] and Jiang et al. [10] were tested. The highest sunscreen levels were obtained using the sample preparation scheme of Ikeda et al. [9], based on dissolution of the product in tetrahydrofuran under sonication followed by various sample transfers and filtration. Consequently, this method was selected for the quantitative comparison between the traditional and the SFE techniques.

Using the optimized SFE conditions (CO_2 at 250 bar and 40°C for 5 min, two-step collection procedure) the sunscreen extraction from a lipstick product containing EH-MC and HM-B and from a cream formulation containing M-BC and BM-DBM was investigated. Compared to the yields from triplicate liquid extractions, SFE recovered after 5 min, 94% of HM-B, 91% of EH-MC, 93% of M-BC and 91% of BM-DBM. Increasing the extraction time to 10 min, improved the relative extraction efficiencies of the foregoing compounds to more than 94.8% (lipstick 1 and cream from Table 1). Further extending the operating time to 15 min, did not produce any significant improvement in the extraction of sunscreens. Therefore, in order to achieve satisfactory analyte recoveries from cosmetic matrices, an extraction duration of 10 min is required. This difference can be ascribed to analyte diffusion and interaction within the matrix. Increasing the pressure of the supercritical fluid from 250 to 300 bar did not yield higher recoveries. Consequently, all further SFE experiments were carried out at 250 bar, 40°C with 10-min extractions.

Applying the optimized SFE parameters to the extraction of a commercial lipstick product, HM-B (4.77%, w/w) and EH-MC (8.12%, w/w) were determined with a relative standard deviation (RSD) of 0.8 and 1.7% ($n=5$), respectively, for the chromatographic precision and 1.1% and 3.8% ($n=5$), respectively, for the method precision. Calibration curves for each sunscreen agent were linear over the ranges 45–80 μg/ml and 450–800 μg/ml, with correlation coefficients greater than 0.996.

Five different preparations, all commercially available, were analyzed and the relative extraction efficiencies (compared to the yields from triplicate liquid extractions) measured by reversed-phase HPLC are listed in Table 1. The contents of sunscreens in the investigated products are in compliance with the European Union (EU) legislation [7] and indicate that SFE produced in 10 min sunscreen recoveries comparable to those attained by conventional liquid extraction performed for 20 min. Moreover, statistical analysis of the results demonstrated that there were no significant differences between the

Table 1
Levels of sunscreen agents extracted from cosmetic products, using SFE compared with conventional liquid extraction [9]

Sample	Concentration[a] (%, w/w)				
	EH-MC	HM-B	M-BC	BM-DBM	EH-DMAB
Lipstick 1					
Liquid extraction	8.15 (10.1)	4.81 (8.1)			
SFE	8.12 (3.8)	4.77 (1.1)			
Relative recovery[b]	99.6%	99.2%			
Cream					
Liquid extraction			1.93 (3.6)	1.70 (5.3)	
SFE			1.88 (2.1)	1.61 (4.9)	
Relative recovery[b]			97.4%	94.8%	
Lotion					
Liquid extraction	4.63 (0.9)			1.75 (5.1)	3.53 (1.1)
SFE	4.67 (4.5)			1.70 (5.3)	3.49 (2.3)
Relative recovery[b]	100.9%			97.1%	98.9%
Lipstick 2					
Liquid extraction	4.54 (2.9)			0.79 (10.1)	
SFE	4.45 (2.5)			0.77 (3.9)	
Relative recovery[b]	98.0%			97.5%	
Lipstick 3					
Liquid extraction	7.54 (2.2)				
SFE	7.30 (1.9)				
Relative recovery[b]	96.8%				

[a] Each value is the mean (RSD) of at least three determinations.
[b] Percentage recovery based on the amount extracted by the liquid extraction method.

two techniques ($P>0.05$). Furthermore, sample processing by SFE is less laborious than the classical methods currently used [8–10], as pretreatment of the cosmetic product is reduced to mixing the sample with hydromatrix and loading it into the extraction cell.

A chromatogram of a typical separation of the sunscreens object of the study is presented in Fig. 2. Representative HPLC traces of SFE extracts from a lipstick product containing EH-MC and HM-B and a lotion containing EH-MC, BM-DBM and EH-DMAB are shown in Figs. 3 and 4, respectively. No interference was observed in the sunscreen retention windows from the placebo extracts of the examined samples (possible interfering cosmetic ingredients include vitamin A esters, vitamin E esters, β-carotene, butylated hydroxyanisole and butylated hydroxytoluene). A rapid decrease of the column efficiency was produced by the liquid extraction technique [9]. This was not found to be the case with sample preparation by SFE, thus indicating that this method reduces the amount of co-extracted formulation excipients.

4. Conclusions

An SFE procedure for the rapid and efficient isolation of sunscreen agents from cosmetics has been developed. SFE exhibits several advantages over the currently adopted conventional methods, including minimum sample manipulation and ease of automation of various processes. In addition, it allows a drastic reduction of the volume of hazardous liquid solvents used. Because of the features outlined above, the SFE method is suitable for quality control assays of sunscreen agents in cosmetic products.

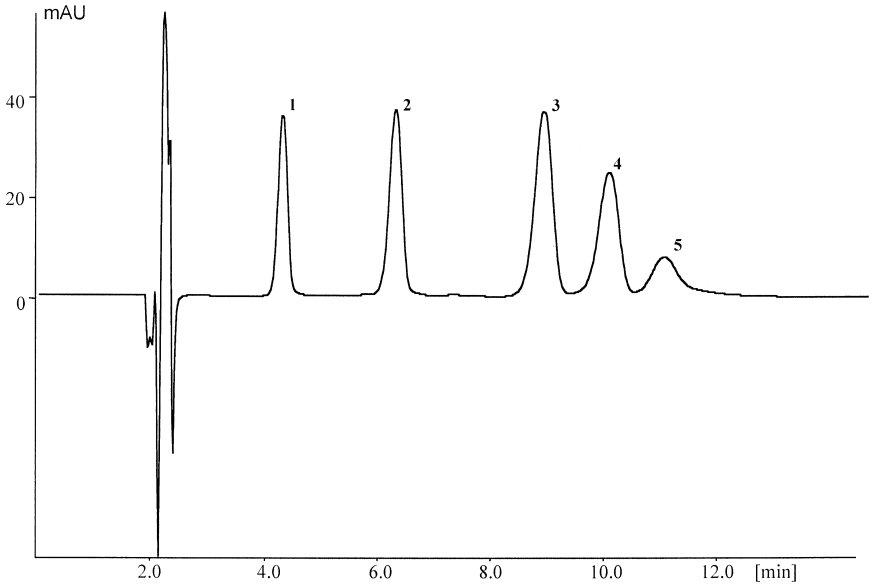

Fig. 2. HPLC separation of a standard mixture of sunscreen agents. Operating conditions as described in Experimental. Peaks: 1=HM-B; 2=EH-DMAB; 3=M-BC; 4=EH-MC; 5=BM-DBM.

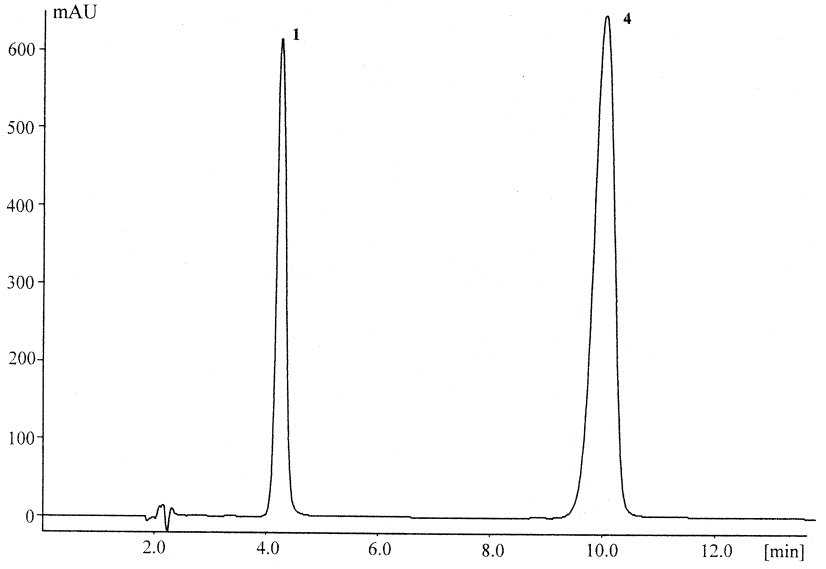

Fig. 3. HPLC chromatogram of a lipstick product purified by SFE. Conditions and peak identification as in Fig. 2.

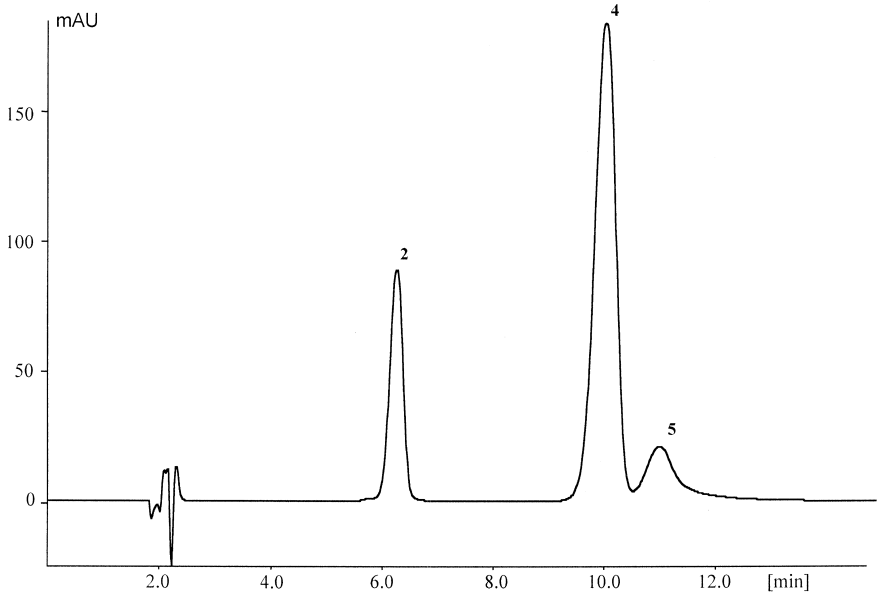

Fig. 4. HPLC trace of a lotion preparation purified by SFE. Conditions and peak identification as in Fig. 2.

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