SIMULTANEOUS ANALYSIS OF NATURAL AND SYNTHETIC ANTIOXIDANTS IN COSMETIC CREAMS BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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Abstract

A high performance liquid chromatographic method for the simultaneous determination of two additives in o/w cosmetic formulations was developed by using HPLC and UV detection. A simple dilution procedure of the sample was required. The separation obtained for the two antioxidants was good under the chromatographic conditions used. Analysis was carried out on commercial samples, and satisfactory results were obtained for their recovery.

Keywords: cosmetic product, antioxidants, antiradical activity, chromatographic analysis methods, HPLC.

INTRODUCTION

Cosmetological research has increasingly focused on processes leading to the formation of anatomical-functional damages of the skin, identified as aging. Great interest in this topic has been generated by the study of substances able to prevent cutaneous damage by free radicals: these substances are termed as antioxidants [1].

Cosmetics are commercially available products used to improve the aspect of skin. Consumer’s demand for more effective products, that substantively beautify the aspect of the skin, has resulted in increased scientific research and product development in cosmetics industry [2]. Numerous data suggest the benefits of such ingredients in cosmetics [3]. In recent years, the cosmetics market has been enriched by numerous skin care products accompanied by advertising claims centred on their antioxidant activity. These products, containing substances with antiradical activity, were created mainly to satisfy the expectations of treatments and to prevent photo-aging [4].

Studies in this area indicate a main association between a potential health hazard and ingestion, and the use of antioxidants in cosmetic products. That why, it is necessary to establish new efficient analysis methods, for monitoring the use of these controversial antioxidants in an adequate mode, as well as for determining the admissibility limit control of antioxidants permitted in cosmetic industry.

Cosmetic formulations frequently contain mixtures of preservatives and antioxidants belonging to different chemical classes, characterized by different functional groups. Therefore, a multicomponent analysis is required. In this respect, the chromatographic techniques are most commonly used to determine the presence of preservatives and antioxidants in cosmetics [5].

EXPERIMENTAL

Standards and reagents

All reagents used were of analytical grade. Acetonitrile and methanol were of HPLC grade. The commercially available antioxidants used in the study are listed in Table 1 [6, 7].

Commercial cosmetics were purchased from a local cosmetic manufacturer.

The chemical structure of the two antioxidants is given in Figure 1.
Multiple studies have been performed on both tocopherol and its acetyl ester derivate, tocopheryl acetate. While tocopherol is the primary active form of vitamin E, the esters of vitamin E have also been shown to penetrate the epidermis when applied topically \[2\]. The isomers of vitamin E are usually eserified to acetates for use in commercial vitamins and topical formulations, because the esters are far more stable \[8\].

**HPLC conditions**

Chromatography was performed on an Agilent 1100 Series liquid chromatograph equipped with quaternary pump, degasifier, column thermostat, auto-sampler and a UV/VIS detector.

Separations were performed on a Eclipse XDB-C\(_18\) (5 µm particle diameter, 250 mm x 4.6 mm). The composition of the mobile phase was acetonitrile:methanol (25:75 v/v). The flow-rate was 1.5 mL/min, and detection was performed at 280 nm. Chromatography was performed at ambient temperature.

**Calibration standard solutions**

Stock solutions were prepared by dissolving appropriate amounts of the standard antioxidants in a solvent consisting of acetonitrile and MeOH, in a 7:3 ratio \[9\]. A set of working solutions was prepared by diluting aliquots of the stock solution to give concentrations ranging from 25.0 to 1000 µg/mL for the studied compounds.

**Sample preparation**

Three oil-rich products (Anti-Wrinkle Eye Contour Cream, Intensive Moisturizing Day Lift Cream and a Replenishing Night Lift Cream) were analyzed by the following procedure: 0.5 g of each o/w emulsions were dissolved by sonication with 10 mL of a methanol:acetonitrile (1:1 v/v) mixture, transferred to a 25 mL volumetric flask and brought to volume. After an approx. 5 min. centrifugation, the supernatant was filtered through a 0.45 µm filter (Chromafil PET-45/25, 0.45 µm, Macherey-Nagel GmbH&CO. KG.).

**RESULTS AND DISCUSSION**

Figure 2 shows the chromatogram of a standard solution of the two antioxidants studied at concentrations of 25 µg/mL BHA and, respectively, 250 µg/mL α-TA.

Figure 3 shows the chromatogram of a standard solution of the two antioxidants studied at concentrations of 50 µg/mL BHA and 500 µg/mL α-TA, respectively.

Figure 4 shows the chromatogram of a standard solution of the two antioxidants studied at concentrations of 100 µg/mL BHA and 1000 µg/mL α-TA, respectively:
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Figure 2. Chromatogram of standard BHA (25 µg/mL) and α-TA (250 µg/mL)

Figure 3. Chromatogram of standard BHA (50 µg/mL) and α-TA (500 µg/mL)

Figure 4. Chromatogram of standard BHA (100 µg/mL) and α-TA (1000 µg/mL)
Figure 5. Calibration graphs for BHA and α-TA

Figure 6. Chromatogram of anti-wrinkle eye contour cream
The chromatograms were recorded at 280 nm. The calibration graphs for BHA and α-TA, constructed over the covered concentration range, as indicated in the experimental part, are presented in Figure 5.

The chromatograms of the Anti-Wrinkle Eye Contour Cream, Intensive Moisturizing Day Lift Cream, and Replenishing Night Lift Cream are presented in Figures 6, 7 and respectively, 8.

It can be easily shown that the determined $R_F$ values are similar in all samples, which demonstrates the determination of the synthetic and natural antioxidants, and also that the above-described method can be successfully applied for the qualitative determination of this compound in cosmetic preparations.
CONCLUSIONS

Cosmetological research has increasingly focused on the processes leading to the formation of the anatomical-functional damage of the skin, identified as aging. Great interest has been generated by the study of substances able to prevent cutaneous damage by free radicals, termed as antioxidants. Antioxidants are substances which, once added in cosmetic, pharmaceutical, and food products, retard their oxidative degradation processes – rancidity.

Natural antioxidants, specially tocopherols (α-, β-, γ-, δ-tocopherols) are recognized for their large applications (as due to their antioxidative character and also because tocopherols are forms of vitamin E). Usually, the most frequently used antioxidants are the synthetic ones, because of their stability during preparation processes.

Out of the new products launched for skin and body care at global level, (11,724 products), 11% (almost 1,290 cosmetic products) contain the most frequently used antioxidant in cosmetic products: alpha tocopherol acetate.

Anti-aging products present a real interest, however only few studies have been devoted to the analysis and monitoring methods of antioxidants in such products.

A high performance liquid chromatographic method following a SPE extraction was applied for the determination of BHA and alpha tocopherol acetate, in three oil-rich commercially available cosmetic products (Anti-Wrinkle Eye Contour Cream, Intensive Moisturizing Day Lift Cream and Replenishing Night Lift Cream). The presence of the two – synthetic and natural – antioxidants in the study of three commercially available cosmetic products was determined by a HPLC method with UV detection, allowing a simultaneous, simple and rapid determination and the confirmation of BHA and α-TA presence in o/w emulsions, cosmetic products containing many ingredients. The obtained results demonstrate the suitability of the proposed method for analyzing antioxidants in cosmetics with high levels of interference.

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References