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SIMULTANEOUS DETERMINATION OF TWO ANTIOXIDANTS IN COSMETC PRODUCTS BY GAS CHROMATOGRAPHY

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ABSTRACT. A gas chromatographic method for the simultaneous determination of two antioxidants in o/w („oil in water") cosmetic formulations was developed by using GC with FID detection. A simple extraction procedure of the sample was required. The sample treatment proposed consists of dissolving the oil-rich products sample (Anti-Wrinkle Eye Contour Cream, Intensive Moisturizing Day Lift Cream and Replenishing Night Lift Cream) by sonication with organic solvent, centrifugation and filtration of the supernatant. The analytes were separated on a fused silica capillary column coated with DB-5 (5% diphenyl-95% dimethylpolysiloxane) and good separation was obtained for analysed antioxidants. The analysis was carried out in commercial samples, and satisfactory results were obtained for the recovery of this compounds.

Keywords: *cosmetic product, antioxidants, GC-FID.*

INTRODUCTION

Antioxidants have great popularity as primary ingredients in cosmetics [1]. Latest generation of cosmetic treatments developed against wrinkles, rely on antioxidant properties of some ingredients [2]. Products containing substances with antiradical activity were created mainly to satisfy expectations of treatment and prevention of photo-aging [3]. There are considerable data to suggest the benefits of such ingredients in cosmetics [4].

Antioxidant preservatives are able to inhibit reactions promoted by oxygen, thus avoiding the oxidation and rancidity of commonly used fats, oils, waxes, surfactants, perfumes, etc.. In cosmetics they are usually reducing agents and free radical scavangers [5]. Antioxidants can be naturally occuring compounds, or synthetic molecules based on phenolic structures with varying degrees of hydroxylation and side-chain substitutions [6]. Natural antioxidants

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are mainly represented in cosmetic products by tocopherols [7], which is the primary active form of vitamin E. The vitamin E isomers are usually eserified to acetates for use in commercial vitamins and topical formulations because the esters are far more stable [8].

Some of the synthetic antioxidants used in cosmetic formulas, such as butylated hydroxyanisole (BHA) and butylated carcinogenic [9, 10] so that extensive use of such raw materials in cosmetics may represent a potential health risk [11]. Long term and hydroxytoluene (BHT) are widespread studies indicate that the use of synthetic antioxidants in cosmetic products cand result in potential health risks associated with their intake [12].

3-tert-butyl-4-hydroxy anisole (BHA) is one of the widely used synthetic antioxidant in cosmetic preparations. This substance is generally recognized as safe for use in products, when the total content of antioxidants is not over 0,02% of fat or oil content, including essential (volatile) content of the product.

Determination of antioxidant compound from cosmetics is often difficult due to the matrix complexity, therefore great attention must be devoted to developing suitable extraction procedures and reliable evaluation of the mean recovery values. The procedure used to extract antioxidants from cosmetics depends on the nature of the formulaton (emulsion, cream, shampoos, etc.) and also on the characteristic of the analytical techniques employed for determination of active substances [5].

Various analytical tehniques have been developed to determine antioxidants in cosmetics, pharmaceutical preparations and drugs. The methods include high - performance chromatography (HPLC) [11, 13, 14, 15, 16, 17, 18, 19, 20], gas chromatography (GC) [12, 21, 22], capillary elecrophoresis (CE) [23] and micellar electrokinetic chromatography (MEKC) [24, 25].

Cosmetic formulations contain very often mixtures of antioxidant preservatives belonging to different chemical classes and characterized by different functional groups. Therefore, multicomponent analysis methods are required. In this sense, chromatographic techniques are those most commonly used to determine antioxidants and preservatives in cosmetic products. Gas chromatography (GC) with flame ionization detector (FID), electron capture detector (ECD) or mass spectrometry (MS) are widely used for the detection and determination of this compounds in cosmetics. Liquid chromatography (LC) is also one of the most commonly used technique to separate and determine antioxidant preservatives, in particular both ion-pair and reversed-phase LC with UV/VIS detection [5].

The official methods approved by the different legislations are not enough to carry out the necessary analytical control of cosmetics [5]. That is why, it is necessary to establish new efficient analysis methods, for monitoring the use of this controversal antioxidants in a adequate mode, and for the determination, and for the admissibility limit control of antioxidants permitted, and used in the cosmetic industry [26].

In view of functional group similarities between tocopherols and the synthetic antioxidants, it was decided to investigate the same approach to the latter group of antioxidants. The objective of this study was the simultaneous identification and determination of 3-tert-butyl-4-hydroxy anisole (BHA) and tocopherol acetate (α-TA) in cosmetic formulas by gas chromatography with FID detection.

In the present study, there is applied a method based on simple extraction procedure with organic solvent of the studied antioxidants in anti-aging cosmetic preparations prior to analysis by reversed-phase gas chromatography with FID detection. The application of this procedures to the assay of commercial products is presented.

RESULTS AND DISCUSSION

Good separation of standards was obtained for GC analysis with retention characteristics of $t_{R,BHA}$=8.073 and $t_{R,\alpha\text{-}TA}$=23.301. The choosen chromatographic conditions allowed a good separation of the two compounds taken into account. On the basis of conducted experiments methods for the simultaneous determination of BHA and α-TA in cosmetic formulas by GC-FID was established.

Confirmation of peak identity for the natural antioxidant α-TA was obtained by the GC determination in cosmetic samples. The retention parameters of compound from cosmetic formulation are listed in Table 1. The synthetic antioxidant BHA and the natural antioxidant α-TA, respectively, could be eluted with acceptable peak shape by method developed.

Table 1. t_R values of BHA and α-TA in the analized samples

Sample	t_R Value (min.)	
	BHA	α-TA
Anti-Wrinkle Eye Contour Cream	8.053	23.281
Intensive Moisturizing Day Lift Cream	8.057	23.284
Replenishing Night Lift Cream	8.055	23.280

Stock solutions were prepared by dissolving the appropiate amounts of the standard antioxidants in ethanol. A set of working solutions was prepared by dilluting aliquots of the stock solution to give concentrations ranging from 10 to 1000 mg/L for the two studied compounds.

The standard solutions of the two antioxidants were injected, to determine the individual retention times of the synthetic and natural antioxidants. Figure 2 shows the chromatogram of a standard solution of the two antioxidants studied for the gas chromatographic method at the concentration of 50 mg/L BHA and 500 mg/L α-TA respectively:

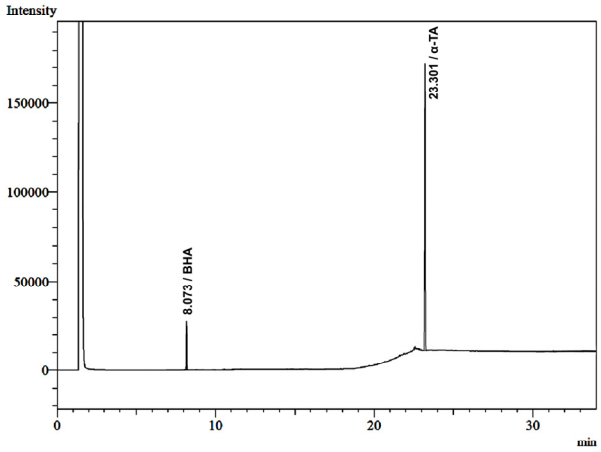
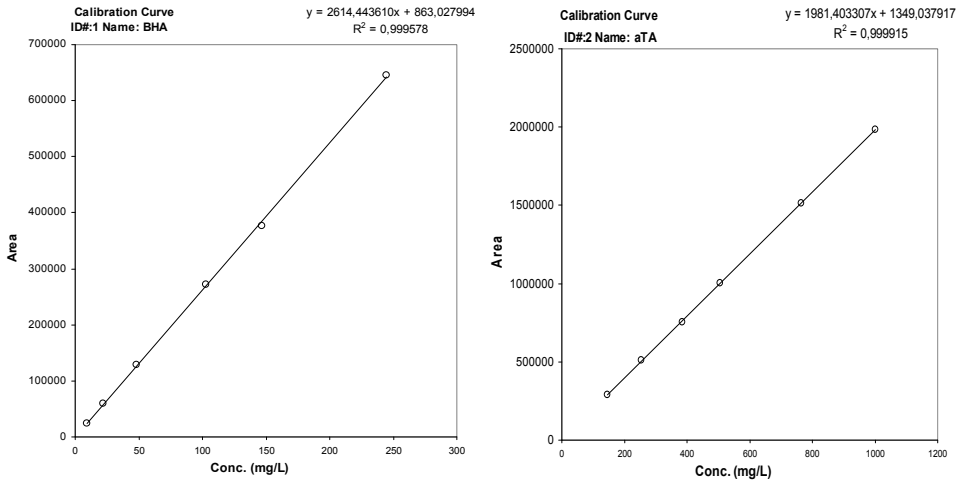


Figure 1. Chromatogram of a BHA and α-TA Standard

The calibration graphs for BHA and α-TA were constructed over the covered range of concentration, as indicated in the experimental part and are presented in Figure 2a for BHA and Figure 2b for α-TA respectively:



a b

Figure 2. Calibration curves for standards.

Calibration curves were constructed in order to study method liniarity. The linearity obtained was satisfactory and present a correlation coefficient of 0.999578 for BHA and 0.999915 for α-TA for the proposed GC method.

The detection limit (LOD) and quantitation (LOQ) expressed as signal/noise = 3 and signal/noise =10, were determined based on standard deviation through responses and the slope of regression equation of a curve constructed concentration levels of 10,25, 50, 100, 150 and 250 mg/L for BHA, and 150, 250, 375, 500, 750 and 1000 mg/mL for α-TA, respectively. The LOD value obtained for BHA was 0,131 and 0,383 for α-TA, respectively. There was calculated a LOQ of 0,396 for BHA and 1,160 for α-TA.

Three anti-aging cream samples- Anti-Wrinkle Eye Contour Cream, Intensive Moisturizing Day Lift Cream and Replenishing Night Lift Cream were prepared in the laboratory by adding 0,05% BHA and 0,5% α-TA (w/w). The recovery, defined as (found concentration/spiked concentration) × 100%, was calculated for each cream sample. The concentrations of the synthetic antioxidant BHA and the natural antioxidant α-TA, respectively, determined from the cosmetic samples are listed in Table 2. Complete triplicate analysis was performed on all cream samples to allow the calculation of average deviations as a measurement of chromatographic reproductibility.

Table 2. Concentration values of synthetic and natural antioxidants in the three anti-aging cream samples

Cream	Concentration (g/100g)±SD (n=3)		Recovery (%)	
	BHA	**α-TA**	**BHA**	**α-TA**
Anti-Wrinkle Eye Contour	0,0447±0,003	0,4241±0,002	**89,4**	**84,8**
Intensive Moisturizing Day Lift	0,0468±0,002	0,4414±0,003	**93,6**	**88,3**
Replenishing Night Lift	0,0470±0,001	0,4159±0,004	**94,0**	**83,2**

Commercial samples of three anti-aging creams (Anti-Wrinkle Eye Contour Cream, Intensive Moisturizing Day Lift Cream and Replenishing Night Lift Cream) that contained combinations of these two antioxidants were analysed and the target compounds were identified by comparing the retention times of the observed peaks with those obtained from the standard solutions.

The GC chromatogram of Anti-Wrinkle Eye Contour Cream, Intensive Moisturizing Day Lift Cream and Replenishing Night Lift Cream are presented in Figure 3a, Figure 3b and Figure 3c, respectively.

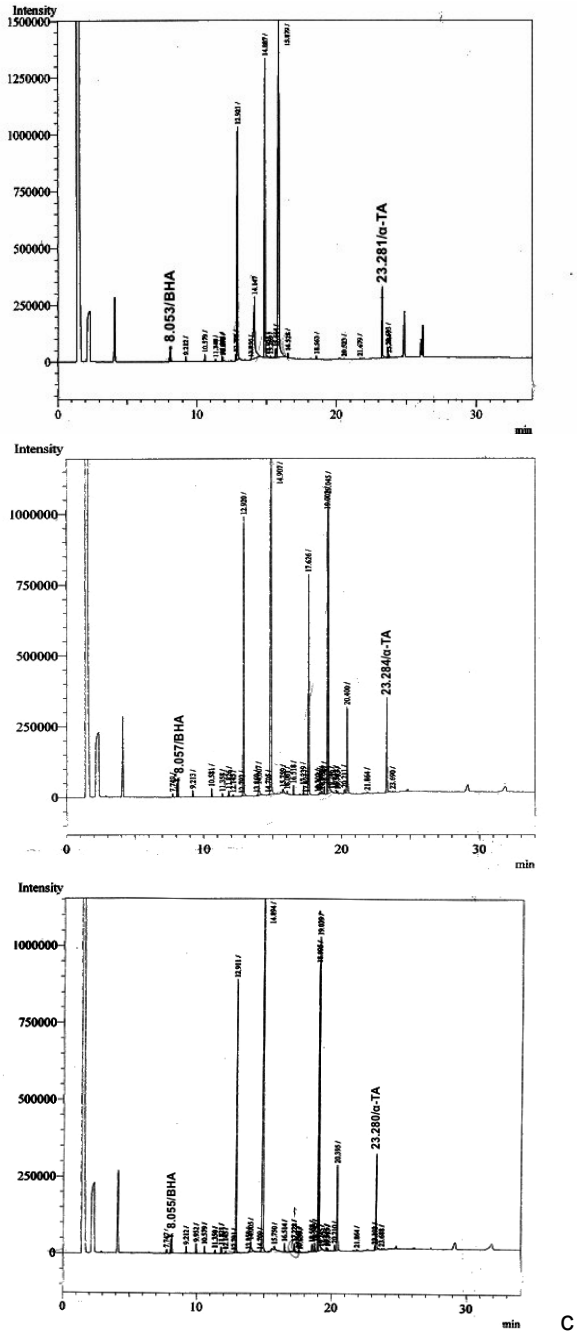


Figure 3. Chromatograms of the samples: a-Anti-Wrinkle Eye Contour Cream, b-Intensive Moisturizing Day Lift Cream, c-Replenishing Night Lift Cream

The concentrations of the synthetic antioxidant BHA and the natural antioxidant α-TA respectively determined from the cosmetic samples are listed in Table 3:

Table 3. Concentration values of synthetic and natural antioxidants in the commercial anti-aging cream samples

Cream	Concentartion (g/100g) (n=3)		Claimed Concentartion (g/100g)	
	BHA	**α-TA**	**BHA**	**α-TA**
Anti-Wrinkle Eye Contour	0,0413±0,002	0,4391±0,002	0,05	0,5
Intensive Moisturizing Day Lift	0,0438±0,002	0,4663±0,003	0,05	0,5
Replenishing Night Lift	0,0406±0,002	0,4284±0,003	0,05	0,5

The developed method can be succesfully applied for the identification and determination of these antioxidants in cosmetic preparations. Good recovery of the natural antioxidant α-TA of 84,8% for a Anti-Wrinkle Eye Contour Cream 88,3% for a Intensive Moisturizing Day Lift Cream and 83,2% for a Replenishing Night Lift Cream by GC analysis were obtained. BHA could be eluted with acceptable peak shapes, and there were obtained satisfactory recoveries by the method: 89,4% for a Anti-Wrinkle Eye Contour Cream, 93,6% for a Intensive Moisturizing Day Lift Cream and 94% for a Replenishing Night Lift Cream. The quantities found for the cosmetic formulations were in conformity with the values claimed by the manufacturer.

CONCLUSIONS

Anti-aging products present a real interest, but there are few studies that can be found, regarding the analysis and monitoring methods of antioxidants in this category of products.

A GC-FID method following a simple and rapid extraction with ethanol was used for the determination of BHA and α-TA in three oil-rich cosmetic products (Anti-Wrinkle Eye Contour Cream, Intensive Moisturizing Day Lift Cream and a Replenishing Night Lift Cream).

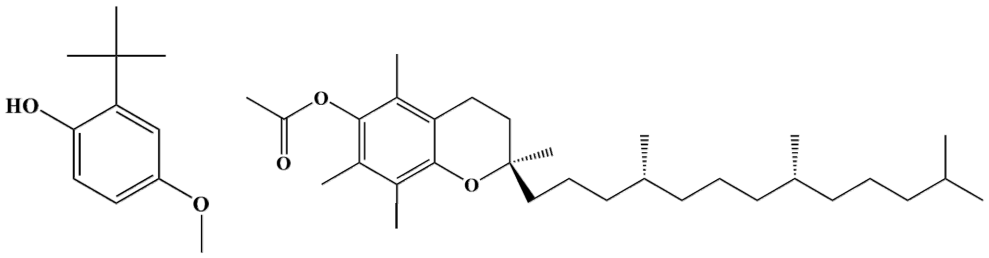
Calibration curves of standard solution were linear in the range from 10 to 1000 mg/mL for the studied compounds. Good recoveries were obtained for the natural and synthetic antioxidants by the used gas chromatographic method.

This method allowed a simultaneous, simple, rapid and accurate determination and confirmation of BHA and α-TA in cosmetic products containing various ingredients. The described method presents good selectivity, adequate detection and quantitation limits and can be applied in the routine analysis for quality control of cosmetic preparations containg α-TA and BHA.

EXPERIMENTAL SECTION

Standards and reagents

All reagents used were of analytical grade and were used without further purification. Commercially available antioxidants used in the study are 3-tert-butyl-4-hydroxy anisole (JAN DEKKER, Nederland B.V.) and tocopheyl acetate (BASF SRL, Bucureşti, Romania). The molecules structures of the two investigated compounds are shown in Figure 4:



BHA **α-TA**

Figure 4. Structure of BHA and α-TA

Sample Preparation

Three oil-rich products (Anti-Wrinkle Eye Contour Cream, Intensive Moisturizing Day Lift Cream and Replenishing Night Lift Cream) were analyzed by dissolving 1.0 g of each o/w emulsions by sonication (HF-Freq. 35 kH, Transsonic T460, Elma GmbH&CO) with 10 mL of ethanol. After 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.).

Calibration Standard Solutions

Stock solutions were prepared by dissolving the appropiate amounts of the standard antioxidants (0,0125 g BHA and 0,0500 g α-TA) in 10 mL ethanol, transfered to a 50 mL volumetric flask and brought to volume. A set of working solutions was prepared by dilluting aliquots of the stock solution to give concentrations ranging from 10 to 1000 mgL for the studied compounds.

Chromatographic Conditions

Chromatography was performed on a Shimadzu 2010 Model gas cromatograph equipped with a FID detector. Fused silica capillary column DB-5, 30m x 0,25 mm ID, coated with a 0,25 µm film of 5% diphenyl-95% dimethylpolysiloxane, was used. The owen temperature was held at 120ºC for five minutes, then increased at a rate of 10ºC/min to 310ºC and held for 10 minutes. Detector conditions were 320ºC, N_2-30 ml/min and H_2-40 ml/min flow-rate. The injection temperature was 350ºC. 1 µl aliquots of the standard and sample solutions were injected and analyzed under the operation conditions described above.

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