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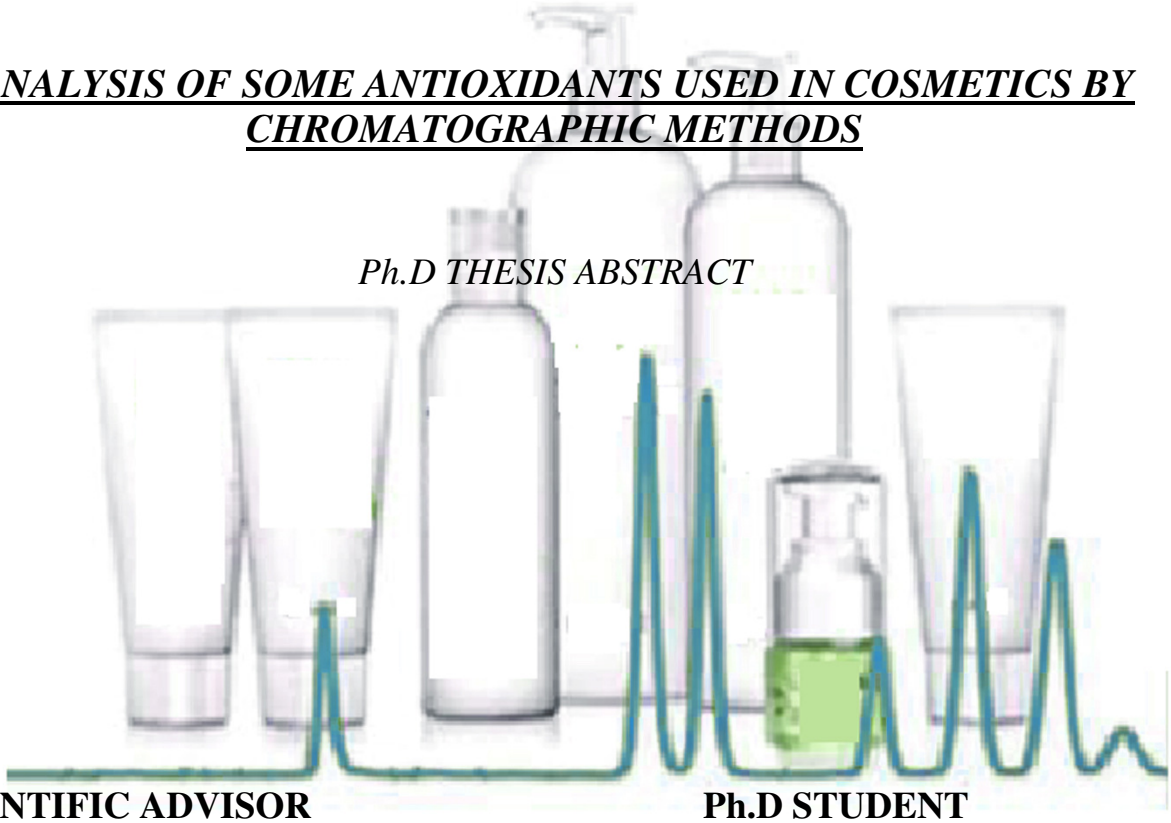
FACULTY OF CHEMISTRY AND CHEMICAL ENGINEERING

ANALYSIS OF SOME ANTIOXIDANTS USED IN COSMETICS BY CHROMATOGRAPHIC METHODS

Ph.D THESIS ABSTRACT



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KEY WORDS:

Cosmetic products

Antioxidant compounds

FTIR (ATR) analysis

Gas chromatography with flame ionization detection (GC-FID)

High performance liquid chromatography with UV-VIS detection (HPLC-UV/VIS)

I. Introduction

The broad definition of *cosmetics* includes all substances and devices used in any way to improve the appearance of the body[1].

Currently on the market there are products, that renew, restore, and rejuvenate, not just cleanse, protect, and moisturize. There is probably no greater focus of interest currently than the incorporation of vitamins and antioxidants in skin care products. There are considerable data to suggest the benefits of such ingredients in cosmetics [6].

In recent years, the cosmetics market has been enriched by numerous skin care products accompanied by advertising claimes centred on their antioxidant activity. These products, containing substances with antiradical activity, were created mainly to satisfy expectations of treatment and prevention of photo-agin [6]. Their topical use is believed to be particularly effective; when applied to the skin surface, the antioxidant compounds concentrate first in the horny layer, which is the structure most exposed to oxidative stress. In most of these products, the antioxidant capacity has been entrusted not to a single substance, but to an association of different principles.

Vitamins and antioxidants have great popularity as primary ingredients in cosmetics. There have been considerable scientific data with these bioactive cosmetics to suggest definite benefits to consumers. For any ingredient to be beneficial it must be stable in production, storage, and use, be nontoxic to the consumer, and have activity at the target site once applied. Market-driven economics clearly reflect great popularity and apparent satisfaction with antioxidant and vitamin formulations. The cosmetics market offers consumers many hydrosoluble or liposoluble active principles for protection of the skin from antioxidant stimuli. By this mechanism, the products should prevent photo-ageing and maintain the skin in a cosmetically pleasing condition.

Many substances, with more or less complex chemical structures, have been found to posess antiradical activity and have been introduced onto the market as anti-ageing products [9].

Antioxidants, such as the synthetic antioxidants Butylated Hydroxy Toluene (BHT) and Butylated Hydroxy Anisole (BHA) or the natural antioxidants tocopherols (Vitamin E), are defined as any substance, which inhibits or delays an oxidative change when it's concentration is lower than the cosmetic substance, which is being oxidized [10].

These synthetic antioxidants are approved cosmetic additives, international regulations tend to establish more and more restrictions to their use and the consumer increasingly prefers to avoid synthetic additives in favour of those perceived as natural.

Cosmetological research has increasly focused on process leading to the formation of anatomical-functional damage to the skin, identified with ageing. At the same time, every possible means to counteract the injurious effects have been evaluated. Great interest in this topic has been

aroused by the study of substances able to prevent cutaneous damage by free radicals: these substances are currently named as antioxidants [11].

The interaction between antioxidants and free radicals in the skin is of special intersest, as it plays an important role in the prevention of skin ageing [12].

Antioxidants, which contain a phenolic group, play an important role in cosmetics, pharmaceutical products and food. Antioxidants can be classifeid in two categories: natural antioxidants, which are mainly represented by tocopherols, and synthetic antioxidants, like 2,6-di-tert-butyl-p-hydroxy toluene (BHT), tert-butyl-4-hydroxy anisole (BHA), propyl, octyl and dodecyl gallate, tert-butylhydroquinone (TBHQ) and nordihydroguaiaretic acid (NDGA) [15].

Some of the synthetic antioxidants used in cosmetic formulas, such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are carcinogenic [174, 175] so that extensive use of such raw materials in cosmetics may represent a potential health risk [125]. 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.

Long term and widespread studies indicate that the use of synthetic antioxidants in cosmetic products cand result in potential health risks associated with their intake [139]. A particular case in these sense can be represented by the category of make-up cosmetics, like lipsticks.

Matrix of anti-ageing cosmetics is very complex, usually containing a high number of ingredients. 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 and also on the features of the analytical techniques employed for the determination of active substances. That why it is necessary to develop performant methods of analysis, that can exactly, and with precission determine the type and amount of antioxidants present in cosmetic products.

This Ph.D thesis delineates the role of antioxidants in cosmetology and also aims to improve some analysis methods for this compounds in anti-ageing products. The purpose of this study was the implementation of rapid determination methods of synthetic and natural antioxidants in cosmetic products. At the same time, there was developed a original cosmetic formula and the analysis of this cosmetic cream by the proposed chromatographic methods.

PART I
THEORETICAL CONSIDERATIONS

II. Antioxidants in cosmetics

II.1 Definition

Antioxidant is "an organic compound added to natural fats and oils and food products, to retard oxidation, deterioration, and rancidity". Many antioxidants are substituted phenolic compounds (butylated hyroxyanisole, di-tert-butyl-para-cresol and propyl gallate).

Maximum concentration of antioxidants approved by FDA (Food and Drug Administration) is 0.02%-0.05% [16].

II.2 Classification of antioxidanţilor

Antioxidants can be classified after their nature in natural and synthetic antioxidants. Antioxidants fall into two classes after their principal function and namely in: true (proper) antioxidants which function by breaking the free radical chain, and which are themselves destroyed during the induction period and synergists which generally have low effect themselves but enhance the action of proper antioxidants and also represent other functions [4].

II.3 Considerations for use of antioxidants in cosmetic formulas

The best approach to the preservation of any cosmetic formulation is empirical. This is not meant to imploy that many publications on the suject of antioxidants do not have their place in the selection of the antioxidant of choice [24]. However, the published data should serve only as a guide in setting up actual tests with the formulas to be preserved.

II.3.1 Choice of antioxidants in cosmetic formulas

Boehm and Williams [25] pointed out that in addition to antioxidant activity, an antioxidant should possess certain physical and physiological properties if it is to be of practical value in cosmetic, pharmaceutical and food preparation:

- *the antioxidant should not impart odour or taste to the preparation to which it is added;*
- *it should be nearly neutral in reaction;*
- *it should be easily and definitely soluble in the substrate;*
- *it must be pharmacologically safe and must not be strongly toxic to animal tissues.*

Whilst the above criteria apply to pharmaceutical and food products they may equally well be applied to cosmetic preparations, with the addition that the antioxidant must be dermatologically

innocuous and free from primary irritating effects and from sensitizing properties. Since the skin is often more sensitive to affront than is, for example, the stomach and in the case of lipsticks in particular, great care should be taken in choosing a suitable antioxidant material.

III.3.2 Cosmetic application: Concepts and formulas

Vitamins can be used in all cosmetic products. Vitamins A (Retinyl Palmitate), C (Ascorbic Acid) and E (Tocopherol or Tocopheryl Acetate) are the most popular antioxidants in skincare. There are also some natural ingredients which are rich in antioxidants. Some of these include green tea, rose hip oil, sweet almond oil, avocado oil and grapeseed extract, also the minerals selenium and zinc. The most used antioxidants in cosmetic formulas are listed in Figure 3.

In creams and lotion and a dosage range of Vitamin E up to 25% in sun tan and body oils [26].

II.4 Mechanism action of antioxidants

Cosmetic preparations containing fats and oils, particularly those characterized by a high incidence of unsaturated linkages, are susceptible to oxidative deterioration. The composition of the fat or oil affects the degree and ease of such oxidation [28]. If two or more double bonds or a conjugated system of unsaturated linkages exist, oxidation will take place at more rapid place. The presence of highly unsaturated compounds, even in very small quantities, is usually sufficient to start a typical chain reaction of oxidation in a system of a lower degree of unsaturation [31]. Almost all fixed oils, as well as their fatty acids are subject to oxidative deterioration since they contain components with two or more unsaturated linkages.

Antioxidants are added to fats and oils in very low quantities, because of cost factors and regulatory requirements. Another reason is inversion, when an excess of antioxidants acts in the opposite way, it actually promotes oxidation. Antioxidants provide an alternate path for oxidation which does not involve the substrate, e.g. fats and oils. The antioxidant does not function indefinitely; it is destroyed in the process.

II.4.2 Mechanism action of antioxidants

When considering the inhibition of lipid oxidation, antioxidants are classicaly devided into two types: chain-breaking antioxidants and preventive antioxidants [31]. Chain-breaking antioxidants are substances inhibiting the propagation step, they interrupt the autoxidation chains. This period of strong inhibition of lipid oxidation is called the induction period or lag time. Preventive inhibitors decrease the rate of autoxidation by suppressing the rate of initiation reactions.

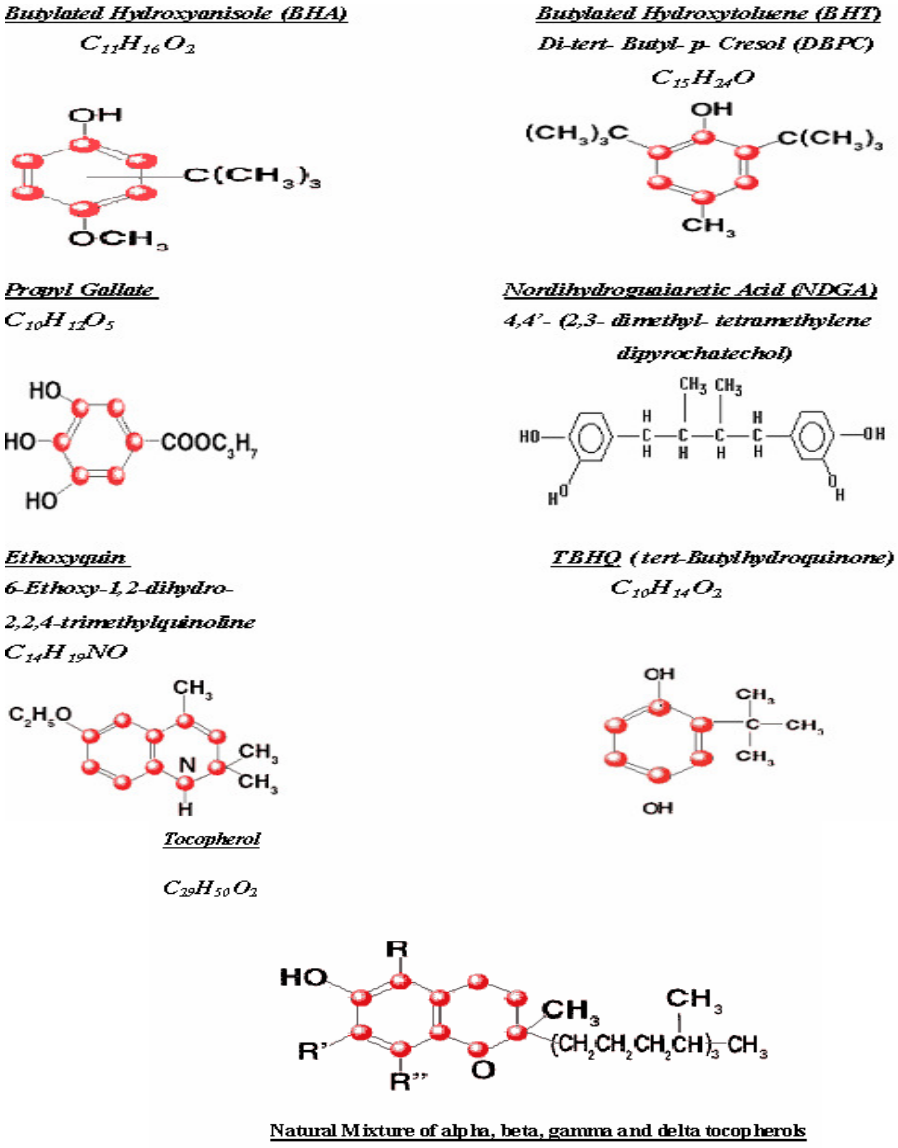

Figure 3: **Antioxidants used in cosmetic products**

Other mechanisms of preventive antioxidants include singlet oxygen queching, oxygen scavenging and hydroperoxide reduction [33]. Part of these compounds may act as synergists with chain-breaking antioxidants. Synergists are defined as compounds that have little or no antioxidant activity of their own but which can enhance the activity of chain-breaking antioxidants. Antioxidants may also have multiple effects on their mechanism of action and may therefore be difficult to interpret. Moreover, the antioxidative effect of a compound may change to a pro-oxidative effect in certain reaction conditions or level of addition.

As a free radical chain reaction, autoxidation can be divided into three separate processes: *initiation, propagation* and *termination* [34], [35] (Table 4):

Table 4: *Mechism action of antioxidants*

Initiation:	**RH → R• + H•**		(1)
Propagation:	**R• + O_2 → ROO•**		(2)
	ROO• + RH → ROOH + R•		(3)
Termination:	**ROO• + ROO• → ROOR + O_2**		(4)
	ROO• + R• → ROOR	*non- radical products*	(5)
	R• + R• → R-R		(6)

During the initiation step an alkyl radical (R•) is formed from an unsaturated fatty acid (RH) (Reaction 1). Once formed, an alkyl radical (R•) reacts very rapidly with oxygen from a peroxyl radical (ROO•) (Reaction 2). The second step of propagation, the abstraction of a peroxyl radical (ROO•) to generate hyperoxide (ROOH), and another radical (R•), is the rate determing step (Reaction 3). Propagation steps may be more complicated than the simple transfer, and addition steps. The reactions terminate when radicals react with each other, forming more stable products that are not capable of propagating the chain reactions (Reactions 4- 6).

III. Extraction methods of antioxidants used in cosmetics

Because of the fact, that antioxidants used in cosmetic products are contained in the cosmetic matrix, their extraction is difficult, and it depends on the nature of the sample, which can be solid or liquid. The extraction procedure depends also by the techniques trough the compounds will be analysed: spectrophotometrical, electrochemical or chromatographic.

The extraction step of the sample in order of the analysis of antioxidants in cosmetic products by chromatographic methods is necessary because of the preconcentration of the concerned alalytes. More approaches, like organic solvent extraction, solid phase extraction and supercritical fluid extraction [38] were developed in this sense, and than applied for the determination of antioxidants and vitamins in different samples.

There are two majore approaches to extract the antioxidants from the matrix:

- *direct extraction of the antioxidants without fat saponification*
- *extraction of the antioxidants after fat saponification*

IV. Analysis methods of antioxidants used in cosmetics

Generally, the use of antioxidants in cosmetics is done as a mixture of these compounds, and the combination of antioxidants brings a increase of their antioxidant power. In this way the determination processes are difficult, because of the interferences that can appear, and finally there can appear errors, concerning the expression of the final results.

The analysis methods of antioxidants are multiple, and depend most of time of the laboratory equipment, and also of the sensibility that is intended to obtain trough analysis. The most important analysis methods of antioxidants used in cosmetics are:

- *Spectophotometric methods*
- *Electrochemical methods*
- *Chromatographic methods*
-

Chromatgraphy methods in all forms, such as thin-layer [91], liquid [93-95] and gas [44] chromatography, have been previously applied.

Metodele cromatografice de toate tipurile, ca de exemplu cromatografia pe strat subţire [91], cromatografie de lichide [93-95] şi de gaze [44] au fost aplicate în acest sens.

The most used chromatographic analysis methods are:

- *Gas Chromatography*
- *Thin Layer Chromatography*
- *High Performance Liquid Chromatography*

PART II
ORIGINAL CONTRIBUTIONS

V. Application of the FTIR (ATR) spectrometric method for the characterisation of some anti-aging cosmetic products abd active ingredients with antioxidant potential

Determination and quantitation of active ingredients in cosmetic and hair care products frequently proves to be challenging to analytical chemists. Often the active ingredients are present in low concentrations and the formulations have complex compositions. A typical "oil in water" (o/w) formulation might contain ingredients such as water, glycerin, stearic acid, mineral oil, triethanolamine, cetyl alcohol, carbomers and be preserved with parabens and antioxidants, which mostly occur in mixtures in cosmetic fromulations [140].

Firstly for a developed cosmetic formula to fulfill all the requirements, it is necessary to have a suitable vehicle (that encompasses emultions, emollients, moisturizers, preservatives, perfume, colorants) and to include in its formulation all the active ingredients (UV filters, botanical or biotechnological extracts), necessary to attain what is claimed by its advertising [143]. Ingredients, that make the object of a cosmetic formulation can be generically classified in three categories: substances with lipophilic character- oil, fats, tocopherols (Vitamin E), synthetic preservatives with antioxidant effect, emulsifiers-siliconic oils, C16-C18 fatty alcohols and hydrophilic compounds, like glycerin, emollient that is often used in cosmetic formulations.

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.

Considering the importance of Vitamin E for skin protection against oxidative damage, and the necessity of a proper evaluation of antioxidant potential in cosmetic formulation, it is of great interest to determine this property for tocopherols or for topical formulations containing this ingredients, by measuring the hydrogen donor capability [145].

The IR spectrometric analysis, along its improved version (FTIR) allows the highlighting of the specific fingerprint based on functional groups, and also of some chemical changes taking place during the oxidation processes. The FTIR method allows qualitative and quantitative analysis of the chemical composition of emulsions, lotions, hair care products and other categories of cosmetic products [146]. FTIR is one of the most widely used methods to identify the chemical constituents and elucidate the compounds structures, and has been used as a method to identify medicines in Pharmacopoeia of many countries. Owing to the fingerprint characters and extensive applicability to the samples, FTIR has played an important role in cosmetic and pharmaceutical analysis [148].

The aim of this study is represented by the survey of FTIR (ATR) specific fingerprints for the characterisation of three anti-aging cosmetic formulations and the identification of specific recognition markers of the active ingredients. The antioxidant potential of alpha tocopherol acetate and of the preservative BHA, usually used in cosmetic formulations or added in a controlled way at known levels of concentration, in a standard cream were evaluated by the DPPH method [157].

V.1 MATERIALS AND METHODS

V.1.1 Materials

Three types of cosmetic formulations, obtained from a local supplier, were used; these were denominated according to their intended pupropse: *Anti-Wrinkle Eye Contour Cream*, *Intensive Moisturizing Day Lift Cream* and *Replenishing Night Lift Cream*.

V.1.2 Methods

V.1.2.2 Determination of the antioxidant activity using the DPPH method

The antioxidant activity of the cream ingredients was evaluated using two types of measurements: by the controlled dosing of known concentrations of alpha-tocopherol acetate (α-TA) and of the preservative butylated hydroxyanisole (BHA) (Experiment A) and by direct measurement of the respective antioxidant activity, at the concentrations levels usualy mixed into the three studied creams (Experiment B).

Experiment A. The antioxidant activity of the alpha-tocopherol acetate and of the preservative butylated hydroxyanisole (BHA) was tested on a Intensive Moisturizing Day Lift Cream cream, by the controlled addition of various concentrations of alpha-tocopherol acetate (α-TA) and/or BHA. Table 9 shows the C0-C6 concentrations of alpha-tocopherol acetate (α-TA) and/or BHA that were added to the Intensive Moisturizing Day Lift Cream, in order to evaluate the antioxidant activity via the DPPH method.

Experiment B. The antioxidant activity was determined for creams 1-3, which previously had a stated concentration of 0,5% α-TA and 0,05% BHA.

Table 9: *Concentrations of α-TA and BHA added in a controlled manner to the Intensive Moisturizing Day Lift Cream in order to determine the antioxidant activity*

Intensive Moisturizing Day Lift Cream		
Concentration (%)	**α-Tocopherol acetate**	**BHA**
C0	-	-
C1	0,1%	-
C2	0,2%	-
C3	0,5%	0,05%
C4	0,5%	-
C5	-	0,05%
C6	0,1%	0,05%

V.1.2.3 Application of the FTIR method

The unsaturation, peroxidation and carbonyl indices [6, 17, 18], were calculated starting from data obtained by FTIR spectrometry, using absorbance values for certain frequencies and considering following formulas:

Unsaturation index (respective the absorption band $\nu_{C=CH}$ at 3006 cm^{-1}):

$$\frac{A_{3006}}{A_{3006} + A_{2921} + A_{2851}} \qquad (V.1)$$

Carbonyl index (respective the absorption band $\nu_{C=O}$ at 1746 cm^{-1}):

$$\frac{A_{1746}}{\sum A_i} \qquad (V.2)$$

where $\sum A_i$ stands for the sum of surfaces between 1800 and 650 cm^{-1}.

The *peroxidation index* (the widening of the 3460-3480 cm^{-1} band).

V.2 RESULTS AND DISCUSSION

V.2.1 Reference data regarding the composition of the creams and FTIR spectra specific of some oily products.

Figure 9 shows by comparison, the composition of the three studied creams. The different ratios between lipophilic and hydrophilic components are noticed.

Whilst the Anti-Wrinkle Eye Contour Cream contains Stearic Acid, Cetyl alcohol, Octyldodecanol and glycerol, cucumber extract and Potassium Cetyl Phosphate, all organic components, the Intensive Moisturizing Day Lift Cream has a higher ratio of non-polar components (glyceryl stearate, avocado oil, soja oil, Ceteareth 20) balanced with glycerol and cucumber extract. On the other hand, the Replenishing Night Lift Cream contains less Soja oil and Cetyl alcohol but more Ceteareth 20 and Cocoa butter.

To properly interpret the FTIR fingerprint specific to this creams, the FTIR spectra specific to oily products was chosen as guide, and the specific frequencies of the functional groups and fingerprint areas were evidenced (Figure 2).

Based on this spectrum, the specific frequency regions (I-IV) were identified, which can be assigned to some functional groups from ingredients and creams (Table 10).

Table 10: *Identification of specific frequency regions characteristic for some oils and suspensions (creams) and their designations*

Frequency area (cm^{-1})	**Characteristic frequencies (cm^{-1})**		**Assignment**
Zone IV **< 1000**	721 921 954 995	- C = C trans - C = C cis	Compounds with conjugated double bonds: polyunsaturated fatty acids, carotenoids, tocopherols
Zone III **1100 - 1800**	1743 1718 1643 1465 1379 1111 1109 1165 1205 1043	COOH C = O OR -CH2-CH3 - υ O-CH_2 - υ C - O C- OR	Esters Free fatty acids Aldehydes and ketones (flavours) Saturated fats Glycerin
Zone II ***2270 - 2400***	2358 2331	C = O	CO_2
Zone I ***> 2800***	2850 2916 2954	C – H (C = C) cis	Natural fatty acids Cholesterol or fitosterols

Based on this remarks, the specific spectra of the components of the studied creams and namely the natural emollients, synthetic emollients, of emullsifier and of antioxidants were characterized.

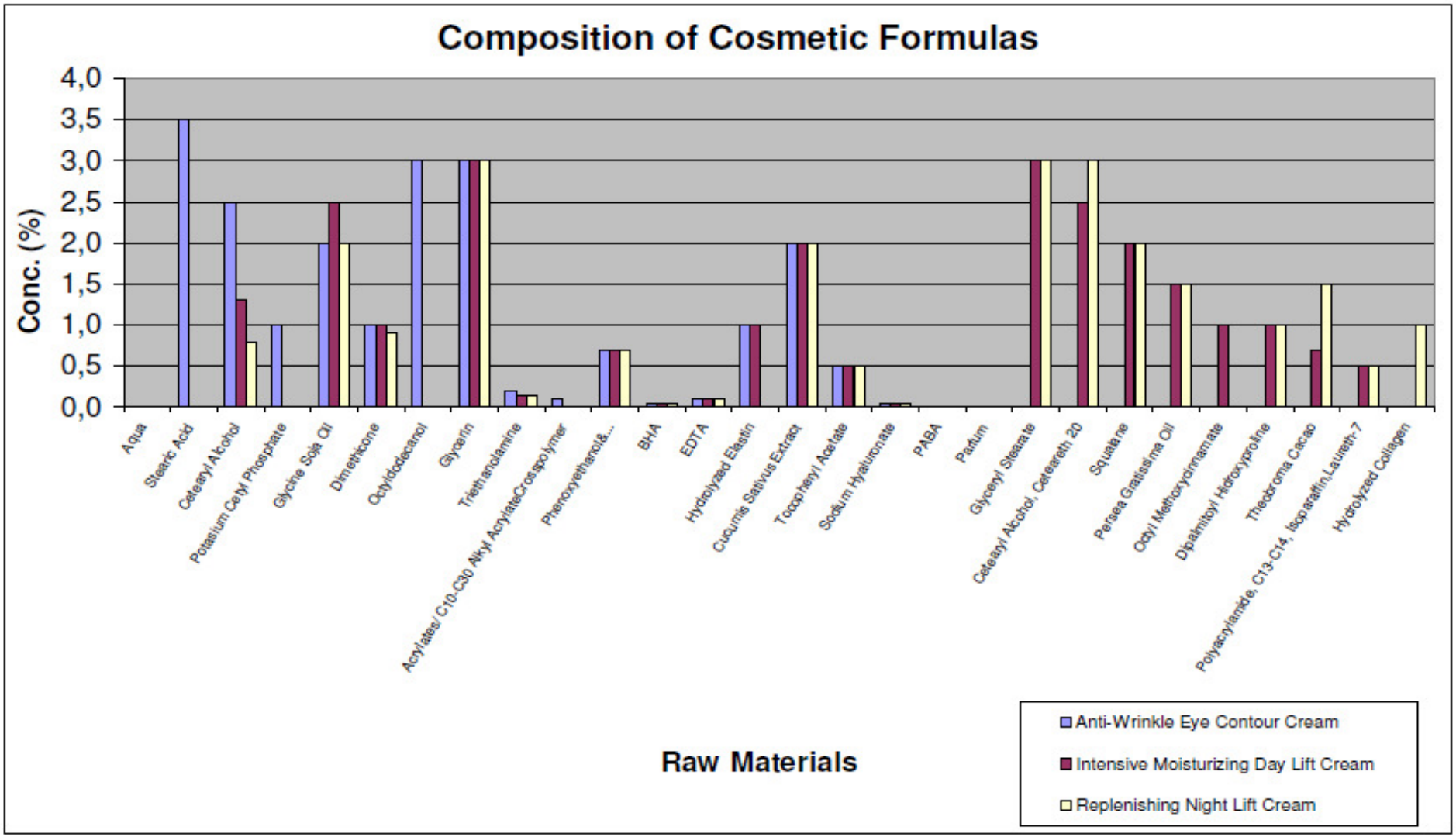

Figure 9: **The composition of the used creams in a graphical representation**

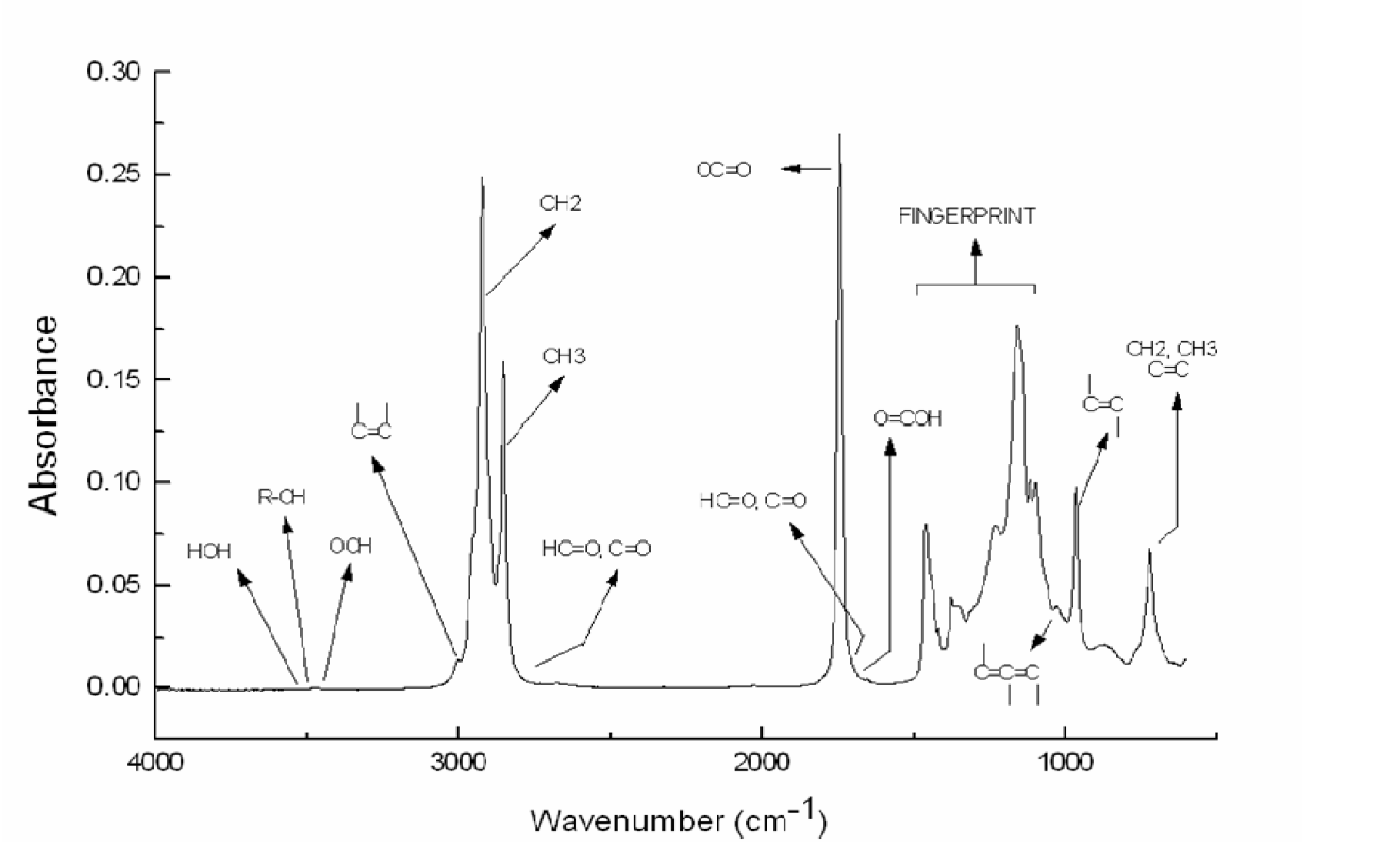

Figure 10: **FTIR spectrum characteristic to oily products, with the evidence of the characteristic frequencies of functional groups and fingerpirnt areas**

V.2.4 Cosmetic cream characterisation by generic FTIR spectra and fingerprint areas.

Figures 16 represents the characteristic FTIR (ATR) fingerprint areas of the studied creams. It shows the comparatively the FTIR spectra of the creams with α-TA (a), the general spectra with the four characteristic areas (I-IV) marked for the studied creams (b) and details about fingerprint zone IV (c).

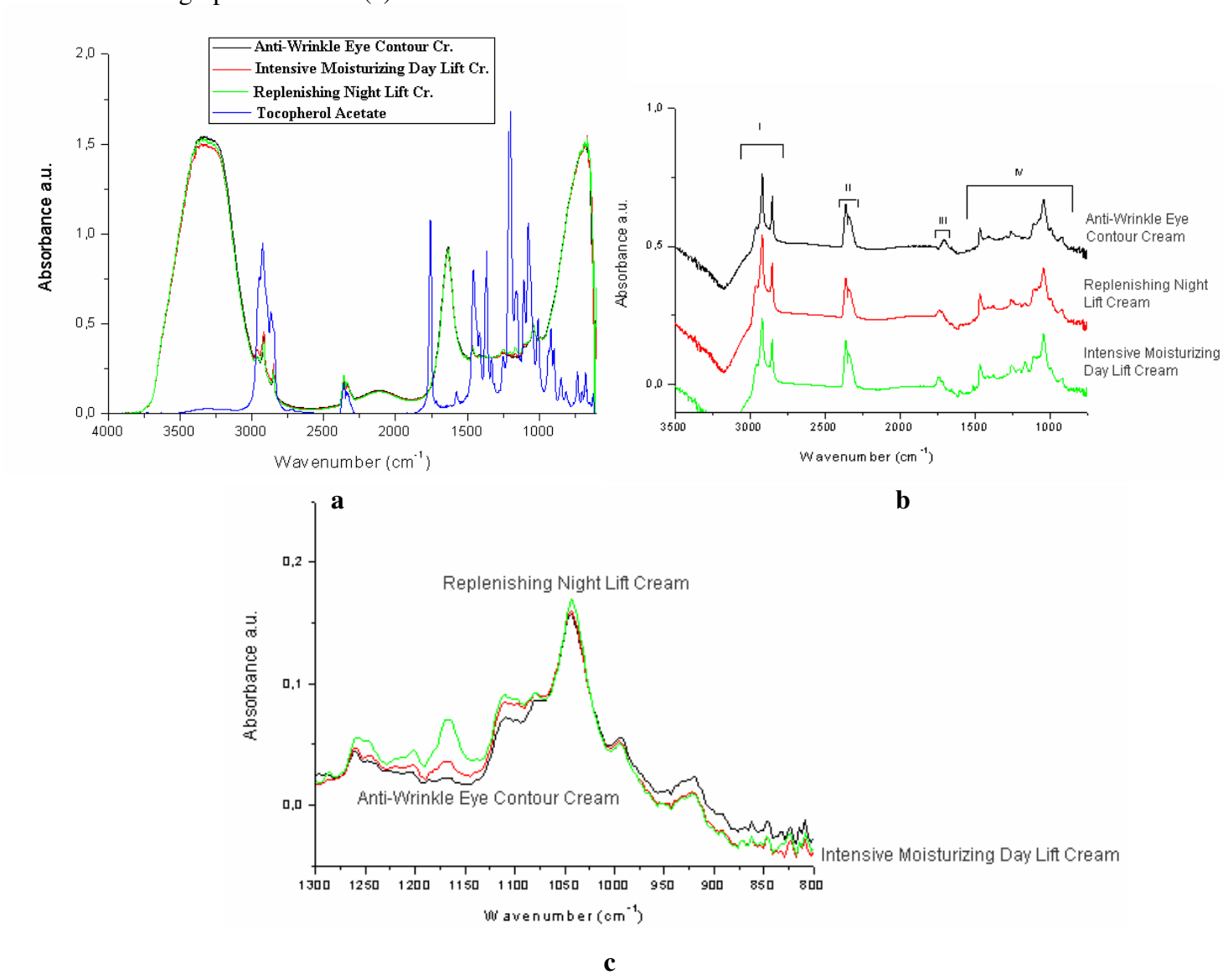

Figure 16: Characteristic FTIR (ATR) fingerprint areas of the studied creams: *a*- FTIR spectra of the creams comparatively with α-TA *; b*- General *FTIR (ATR)* spectra with the four characteristic areas (I-IV) for the studied creams*; c*- Detailed fingerprint zone (IV), comparatively for the three cream types

All three sreams have a similar FTIR spectrum and are hard to tell apart; the differences in composition shown in Fig. 9 are too reduced to affect the IR absorptions. The fingerprint area IV (Figure 16 c) however does show higher intensities for the region 1150-

1200 cm^{-1} (esters and fats) for the Intensive Moisturizing Day Lift Cream and 800-1000 cm^{-1} for the Anti-Wrinkle Eye Contour Cream (contains more polar compounds).

Table 16 includes the values of characteristic frequencies and signal strength for the three creams:

Table 16: *Characteristic frequencies and signal strength for the three creams*

Frequency zone (cm^{-1})	Anti-Wrinkle Eye Contour Cream		Intensive Moisturizing Day Lift Cream		Replenishing Night Lift Cream	
	Wave Number	*Intensity*	*Wave Number*	*Intensity*	*Wave Number*	*Intensity*
	920	0,331	921	0,0218	921	0,021
IV	993	0,0662	995	0,0623	995	0,063
	1043	0,1674	**1043**	0,1815	**1043**	0,1699
	1078	0,0968	1080	0,1043	1080	0,1025
	1109	0,0824	1111	0,1027	1109	0,0955
	1165	0,0327	1168	0,0826	1170	0,0464
	1205	0,038	1201	0,0571	1203	0,0433
	1247	0,0468	1257	0,068	1246	0,0508
	1261	0,0544	1286	0,0389	1259	0,0569
	1379	0,0299	**1379**	0,0353	**1379**	0,0351
			**1419	0,03	***1417	0,0297
	1465	0,0648	**1465**	0,0725	**1465**	0,0771
	1701	0,0234	1718	0,0147	1718	0,0137
III						
			**1743	0,0286	***1743	0,0171
	2331	0,0993	**2331**	0,1011	**2331**	0,0864
II	**2358**	0,1521	**2358**	0,1586	**2358**	0,1349
	2848	0,182	2850	0,1606	2850	0,1891
	2916	0,2633	**2916**	0,2372	**2916**	0,2891
I	**2954**	0,0712	**2954**	0,0732	**2956**	0,1086

Only two types of characteristic frequencies, marked with ** and *** respectively, for the Intensive Moisturizing Day Lift Cream and Replenishing Night Lift Cream creams.

V.2.5 Antioxidant activity of the investigated creams

The antioxidant activity couldn't be evidenced by the DPPH method. The obtained result is also confirmed by other studies showing that tocopherol acetate, although more stable than tocopherol, does not show any antioxidant activity [3, 6, 20, 21] by individual use, since it is a synergic antioxidant. Table 17 shows the values of the unsaturation degree (UI) and of the carbonyl index (CI), considering tha values of measured absorptions at diferent IR frequencies (described in Materials and methods).It has been observed that all three creams have simiular values for the degree of unsaturation and carbonyl index, however the *Anti-Wrinkle Eye Contour Cream* has a higer degree of unsaturation while the *Intensive Moisturizing Day Lift Cream* has a higher CI. The lack of the peroxidation band shows the

oxidative stability of these creams.

Table 17: *Unsaturation degree and carbonyl indices for the studied creams*

	UI	**CI**
Anti-Wrinkle Eye Contour Cream	$\frac{0,3631}{0,3631+0,3707+0,2315}=0,3761$	$\frac{0,1621}{0,1008+1,3627}=0,1107$
Intensive Moisturizing Day Lift Cream	$\frac{0,3401}{0,3401+0,3801+0,2295}=0,3581$	$\frac{0,1956}{0,0942+1,3875}=0,1320$
Replenishing Night Lift Cream	$\frac{0,3447}{0,3447+0,4296+0,2553}=0,3347$	$\frac{0,1854}{0,0972+1,3594}=0,1272$

V.3 CONCLUSIONS

The use of FTIR spectrometry has allowed the evidence of the characteristic fingerprint of the three types of creams studied, and also of the ingredients from these products, depending of their role in the specific cream.

Comparing the diferent compositions of the creams, depending on the ratio of lipophylic to hydrophylic ingredients, of emollient-emulsifier type, antioxidant and active ingredients (cucumber extract, tocopherol acetate) there have been noticed:

1. similarities regarding the shape of FTIR spectra between different natural emollients with the evidence of some characteristic frequencies and recognition markers, especially for Soja oil.
2. similarities of the FTIR fingerprints between the stearate type and cetearyl type emulsifiers.
3. clear differences between acrylates and respectively stearates and Ceteareth.
4. significant differences between synthetic emollients, dimethicone and Oktyldodecanol.
5. identification of differences between natural antioxidants (α-tocopherol) and synthetic ones (BHA) and characteristic marker highlighting (frequencies and signals).
6. evidence of the active principles (flavours) from cucumber extract and it's fingerprint in propylene glycol compared to glycerine – both ingredients being hydrophil.

For the studied creams, the FTIR the spectrum is similar, but some differences in band intensities can be noted and identified in the areas 1150-1200 and 800-1000 cm^{-1}.

Concluding, the data that was obtained from the analysis of the ingredients is very useful and does replace a database for their individual or class of compounds identification. For creams with a complex composition, especially when they differ by reduced percentages between the polar and nonpolar ingredients, it is hard to evidentiate the recognition markers by the FTIR (ATR) method. The data obtained by FTIR analysis need to be correlated with

chromatographic methods (HPLC and/or GC) in order of the validation for the individual components determination from cosmetic formulations.

The antioxidant effect of creams which include molecules with antioxidant potential seems to be a complex issue needing further studies. Even if molecules like BHA or α-tocoferol, considered individually, with antioxidant effect (by the DPPH method), in complex mixtures with lipides, especially when their concentration is below 1%, the antioxidant action cannot be registred by usual measurements. Higher sensibility methods, like electronic spin resonance (ESR) could probably deliver additional information about the antioxidant potential of some complex mixtures, like anti-aging creams.

VI. Analysis of some synthetic and natural antioxidants in cosmetics by chromatographic methods

Cosmetic formulations contain very often mixtures of antioxidant preservatives belonging to different chemical classes and characterized by different functional groups[143]. Therefore, multicomponent analysis methods are required; in this sense, chromatographic techniques are those most commonly used to determine antioxidants and preservatives in cosmetic products.

For chromatographic analysis, based on liquid or gas chromatographic tehniques, sample preparation, depending on the nature and matrix compexity, it is generally possible to use simple dilution procedures with organic solvents or mixtures of adecvate solvents, or more complex operations for extracting the analite from the solid matrix. Mostly, the extraction process also needs a clean-up step using a suitable solid phase extraction. Sample preparation is necessary in case of antioxidant and preservatives determination from complex matix or heterogeneos samples, in which interference problems are likely. The easiest method for sample pretreatment in o/w cosmetic formulas is represented by the dilution and homogenisation of the sample, centrifugation and filtration.

The procedure used to extract preservatives and antioxidants from cosmetics depends on the nature of the products- emulsions, creams, shampoos, etc. and also the characteristics of the analytical techniques to be employed to determine the active substances. Reported procedures in the reviewed papers have been grouped according to the analysed cosmetic product and classified acording of the categories proposed in Annex I of the EU Cosmetics Directive (Council Directive 76/768/EEC) [163] (Figure 17):

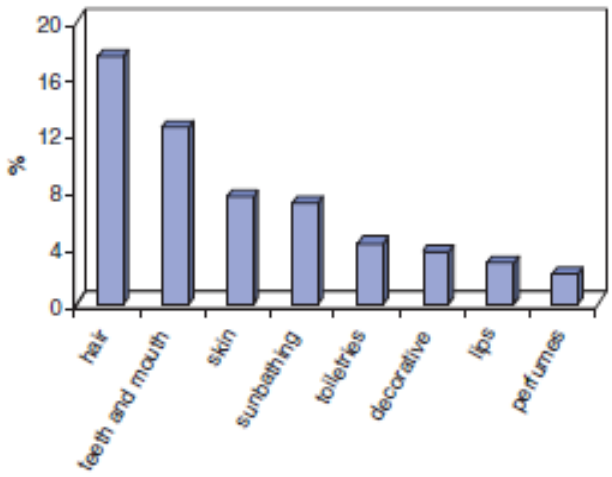

Figure 17: **Percentages of analysed categories of cosmetic products**

Matrix of cosmetic samples is complex, cosmetic formulas contain a high number of ingredients, and often the analysis of formulation requires extensive treatments (such as solubilization, purification and/or preconcentration). Dissolution of the samples can be carried out using suitable chemicals and/or solvents assisted by physical complementary treatments like heating, or exposure to ultrasound or microwave radiation. Sometimes, analytical procedure requires a purification/preconcentration process step of the analytes of interest. To this either solid phase extraction or liquid-liquid extraction are employed (Figure 18):

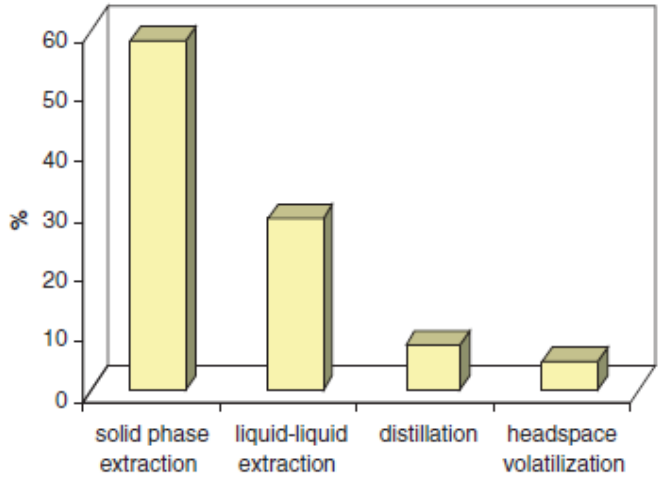

Figure 18: **Relative frequency of treatments for purification/preconcentration of analytes**

In some cases, analytes should be transformed by means of derivatisation to compounds with better analytical features for the analytical tehnique to be used, for instance, gas chromatography requires that low volatile analytes be transformed into volatile derivates. Other reactions, like saponification can be applied fpr sample preparation (Figure 19):

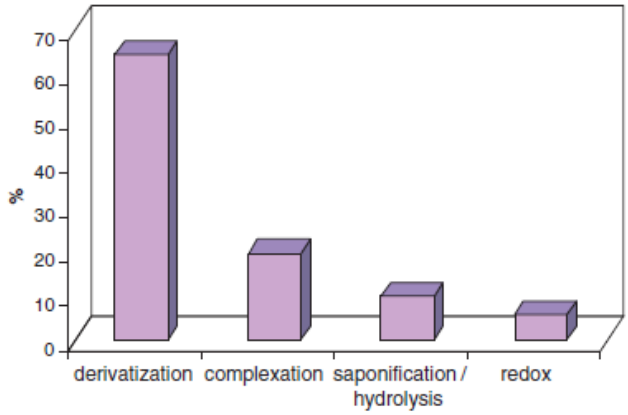

Figure 19: **Relative frequency of chemical reactions of analytes to be determined**

Revised references on cosmetic analysis have been classified into five groups according to the analytical tehnique used (Figure 20), namely, chromatographic and related tehniques, such as electrophoresis (69%), molecular spectroscopy (15%), electrochemical measurements (8%), atomic spectroscopy (5%) and others (3%) [143].

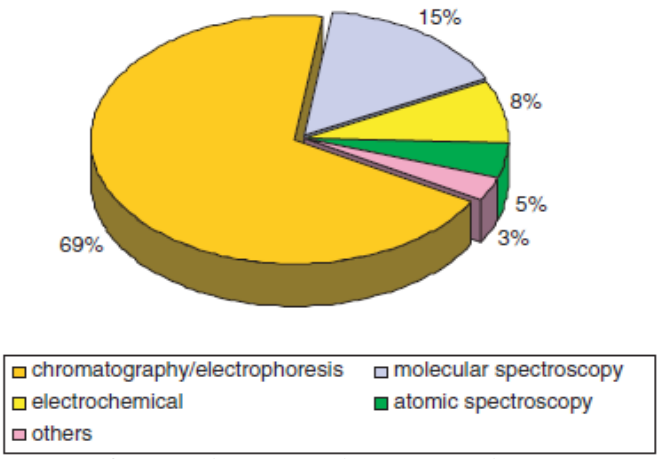

Figure 20: **Percentages of analytical techniques used in reported methods for cosmetic analysis**

The official methods aproved by the different legislations are not enough to carry out the necessary analytical control of cosmetics. The development and validation of new methods with suitable analytical features is still a field under development, reasearchers hoping to improve cosmetic quality control feasibility of cosmetic [173].

The use of antioxidants in cosmetic products can be individual or in mixture, when the antioxidant capacity increases. One of the widly used synthetic antioxidants is BHA (3-tert-butyl-hydrxy anisole), and the most used natural antioxidant is α-TA in cosmetic formulas. Figure 21 presents the chemical structure of both antioxidants:

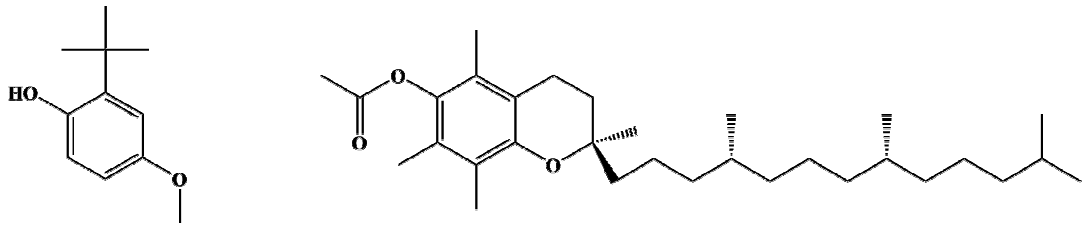

Figure 21: Structure of BHA and α-TA

The present study delineates the role of antioxidants in cosmetology and also aims to improve some analysis methods for this compounds in anti-ageing products. There were concerned two aspects of the analysis: the sample preparation, which was performed by extraction with different solvent systems, solid phase extraction and saponification and the analysis in fact, by GC-FID and/or HPLC-UV, aiming the determination of the optimal

analysis conditions. Analysis were performed on synthetic samples (anti-aging type cosmetic formulas spiked with BHA at concentration levels of 0,05% and α-TA at concentration levels of 0,5%), on commercial available products and on a original, self developed cosmetic formula.

VI.1 Analysis of some synthetic and natural antioxidants used in cosmetic producst by chromatographic methods

The antioxidants used in the study are lisetd in Table 18 and are commercially available.

Table 18: *List of studied antioxidants*

List of studied antioxidants		
Chemical Name	*Formua*	*Supplier*
3-tert-butyl-4-hydroxi anisole	BHA	JAN DEKKER Nederland B.V.
Tocopherol Acetate	α-TA	BASF

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.

VI.1.1 Simulatneous determination of synthetic and natural antioxidants in anti-aging cosmetic formulations by ethnol extraction and subsequent GC-FID analysis

A gas chromatographic method was developed for the simultaneous determination of two antioxidants in o/w („oil in water") cosmetic formulations by using GC with FID detection. A simple extraction procedure of the sample was required and the separation of both compounds was obtained under the used chromatographic conditions [178].

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.

Figure 22 presents the cromatogram of a standard solution of the two studied antioxidants:

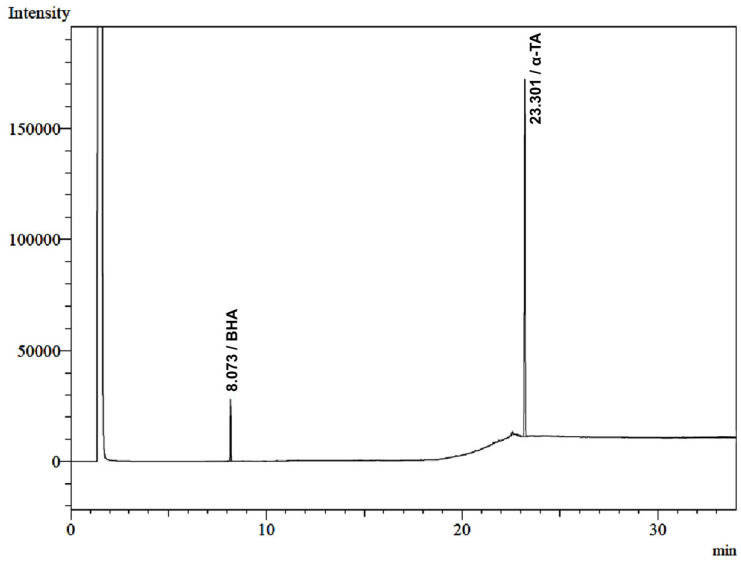

Figure 22: GC Chromatogram of a BHA and α-TA standard

The calibration graphs for BHA and α-TA were constructed over the covered range of concentrations, and are presented in Figure 23A for BHA and Figure 23B for α-TA respectively:

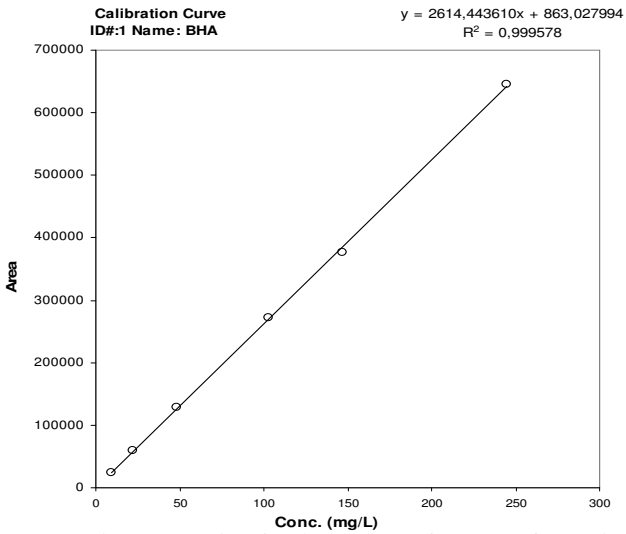

Figure 23A: Calibration Curve of BHA

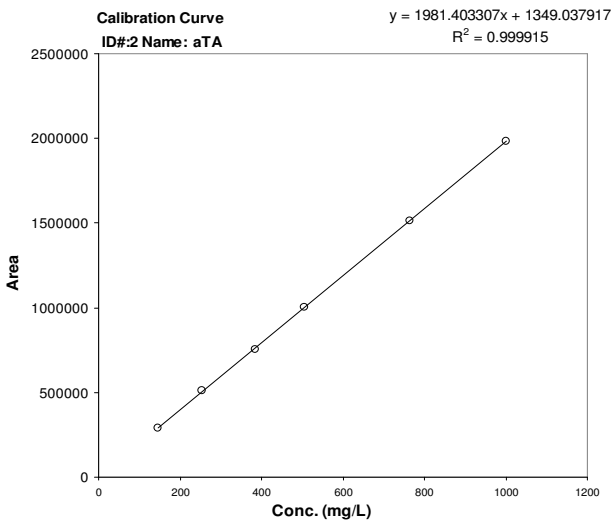

Figure 23B: Calibration Curve of α-TA

The LOD value obtained for BHA was 0,131 mg/L and 0,383 for α-TA mg/L, respectively. There was calculated a LOQ of 0,396 mg/L for BHA and 1,160 mg/L 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). Complete triplicate analysis was performed on all cream samples to allow the calculation of average deviations as a measurement of chromatographic reproductibility.

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* is presented in Figure 24. The chromatogram of a *Intensive Moisturizing Day Lift Cream* and that one for a *Replenishing Night Lift Cream* are presented in Figure 25, and respectively in Figure 26:

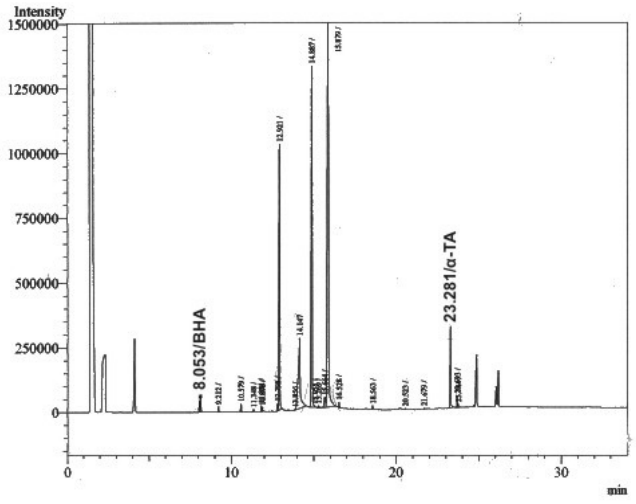

Figure 24: GC Chromatogram of a Anti-Wrinkle Eye Contour Cream

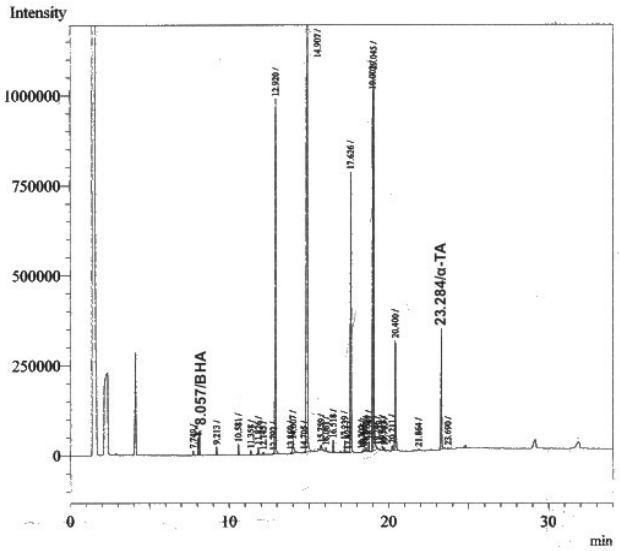

Figure 25: GC Chromatogram of a Intensive Moisturizing Cream

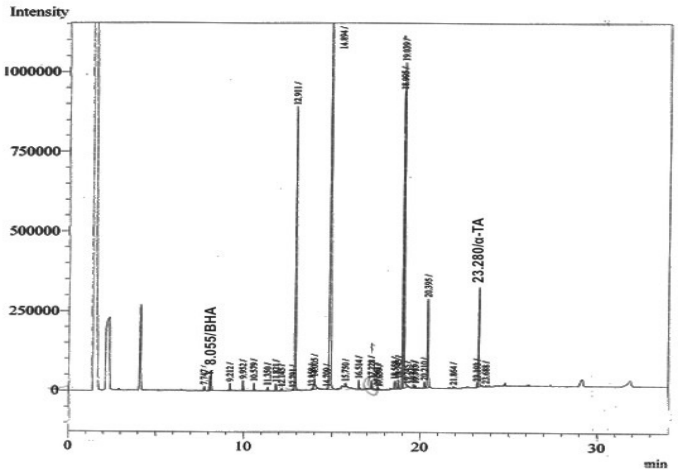

Figure 26: GC Chromatogram of a Replenishing Night Lift Cream

The concentrations of the synthetic antioxidant BHA and the natural antioxidant α-TA respectively determined from three anti-aging creams in the cosmetic samples, the recovery rates and antioxidant concentrations in commercial cream samples are listed in Table 25:

Table 25: *Concentration values of antioxidants in the analysed cream samples*

Sample	Concentration ± DS Sample (g/100g)		Recovery rate (%)		Concentration ± DS Commercial Sample (g/100g)		Claimed Concentration (g/100g)	
	BHA	***α-TA***	***BHA***	***α-TA***	***BHA***	***α-TA***	***BHA***	***α-TA***
Anti Wrinkle Eye Contour Cream	0,0447 ± 0,003	0,4241 ± 0,002	89,4	84,8	0,0413 ± 0,002	0,4391 ± 0,002	0,05	0,5
Intensive Moisturizing Day Lift Cream	0,0468 ± 0,002	0,4414 ± 0,003	93,6	88,3	0,0438 ± 0,002	0,4663 ± 0,003	0,05	0,5
Replenishing Night Lift Cream	0,0470 ± 0,001	0,4159 ± 0,004	94,0	83,2	0,0406 ± 0,002	0,4284 ± 0,003	0,05	0,5

n = 3

This method allowed a simultaneous, simple, rapid and accurate determination and confirmation of BHA and α-TA in cosmetic products containing various ingredients. The obtained results demonstrate that the proposed method for the analysis of antioxidants with a high incidence of interference from cosmetic products is appropriate.

In wiev 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 quantitative determination of alpha tocopherol acetate and 3-tert-butyl-4-hydroxy anisole in cosmetic formulas by GC-FID analysis.

VI.1.2 Simultaneous determination of synthetic and natural antioxidants in cosmetic formulations by ethanol extraction and subsequent GC-FID analysis

A gas chromatographic method was developed for the simultaneous determination of synthetic and natural antioxidants-α-tocopherol acetate and BHA respectively, in o/w cosmetic formulations by using GC with FID detection. A simple extraction procedure of the

sample with methanol:acetonitrile organic solvent mixture was required and the separation of both compounds was obtained under the used chromatographic conditions [182].

Good separation of standards was obtained for GC analysis with retention characteristics of $t_{R,BHA}$=10.118 and $t_{R,\alpha\text{-}TA}$=26.074. The choosen chromatographic conditions allowed a good separation of the two compounds taken into account.

Figure 29 presents the cromatogram of a standard solution of the two studied antioxidants:

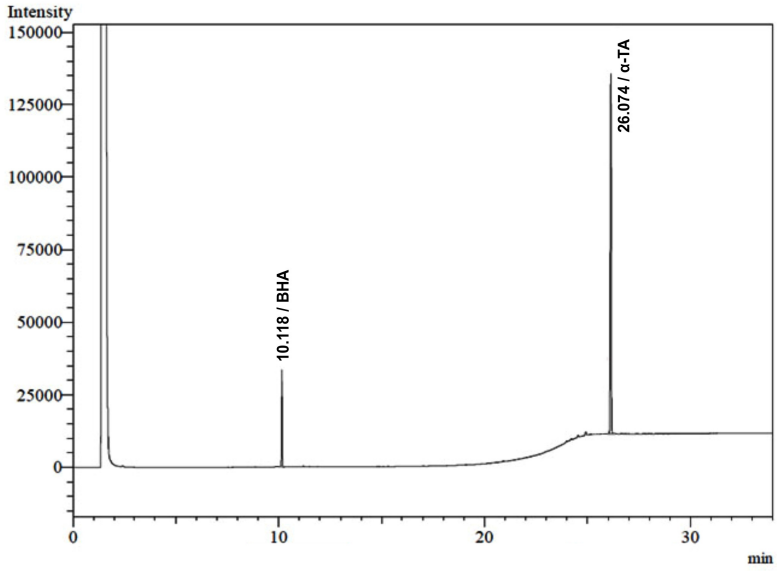

Figure 29: GC Chromatogram of a BHA and α-TA standard

The calibration graphs for BHA and α-TA were constructed over the covered range of concentrations, and are presented in Figure 30A for BHA and Figure 30B for α-TA respectively:

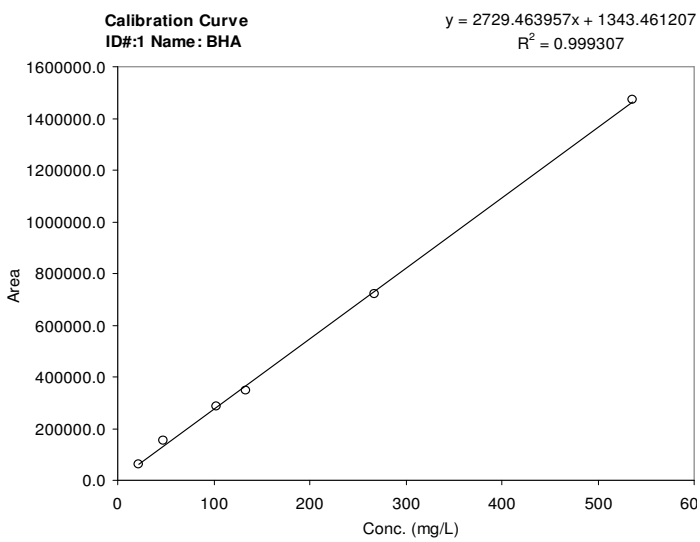

Figure 30A: Calibration Curve of BHA

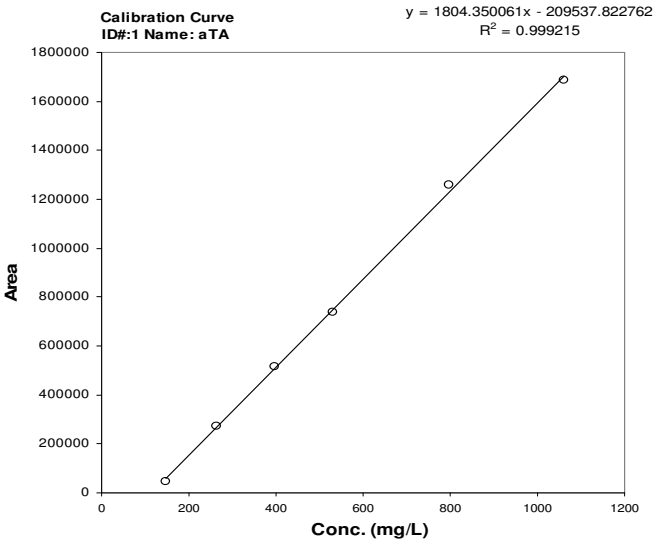

Figure 30B: Calibration Curve of α-TA

The LOD value obtained for BHA was 0,136 mg/L and 1,237 for α-TA mg/L,

respectively. There was calculated a LOQ of 0,413 mg/L for BHA and 3,749 mg/L 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).

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* is presented in Figure 31:

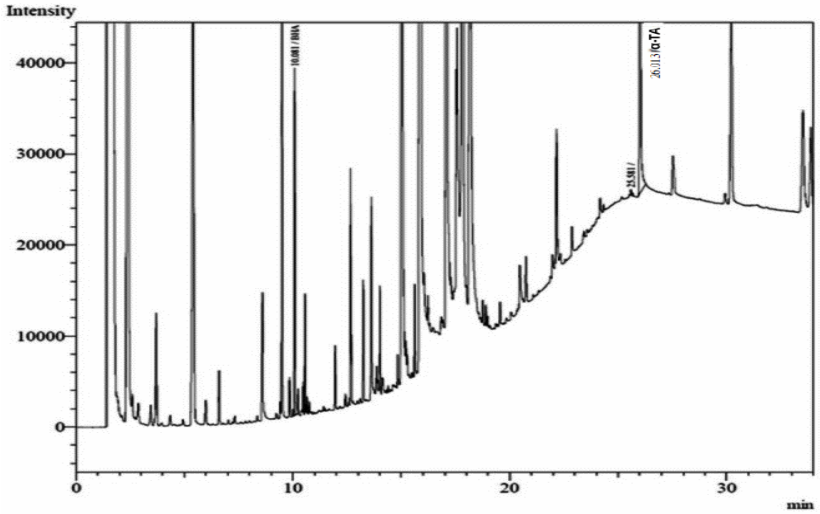

Figure 31: GC Chromatogram of a Anti-Wrinkle Eye Contour Cream

The chromatogram of a *Intensive Moisturizing Day Lift Cream* and that one for a *Replenishing Night Lift Cream* are presented in Figure 32 and Figure 33 respectively:

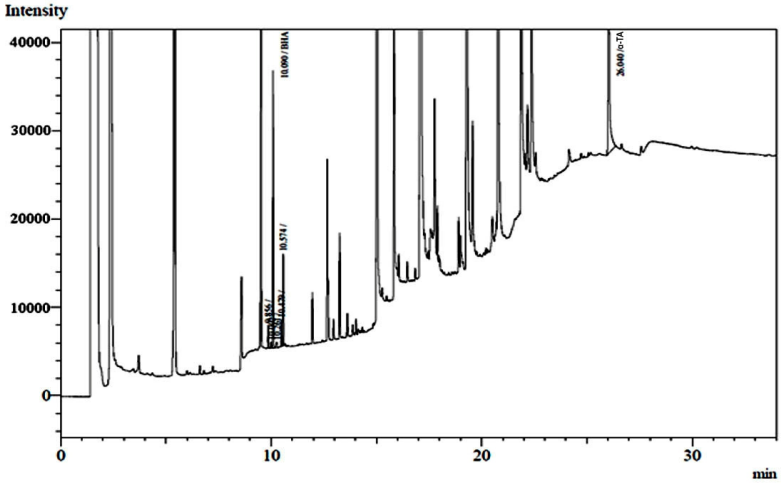

Figure 32: GC Chromatogram of a Intensive Moisturizing Day Lift Cream

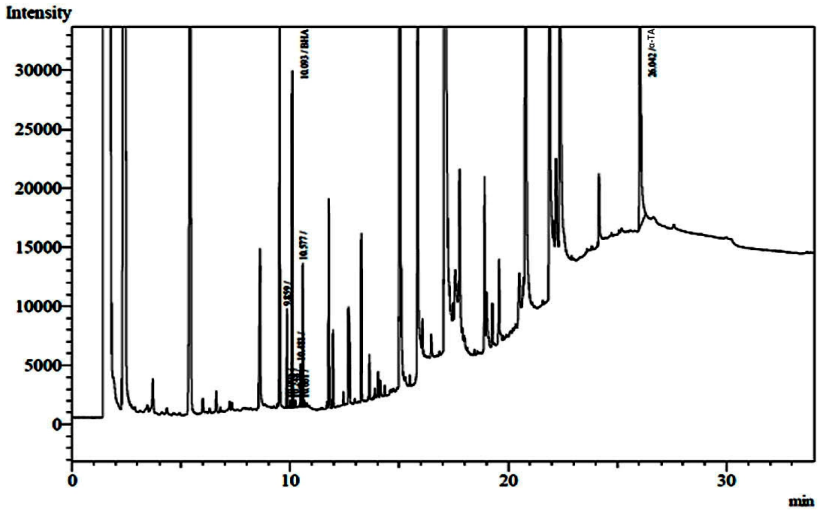

Figure 33: GC Chromatogram of a Replenishing Night Lift Cream

The concentrations of the synthetic antioxidant BHA and the natural antioxidant α-TA respectively determined from three anti-aging creams in the cosmetic samples-*Anti-Wrinkle Eye Contour Cream*, *Intensive Moisturizing Day Lift Cream* and *Replenishing Night Lift Cream*, the recovery rates and antioxidant concentrations in commercial cream samples are listed in Table 32:

Table 32: *Concentration values of antioxidants in the analysed cream samples*

Sample	**Concentration ± DS Sample (g/100g)**		**Recovery Rate (%)**		**Concentration ± DS Commercial Sample (g/100g)**		**Claimed Concentration (g/100g)**	
	BHA	***α-TA***	***BHA***	***α-TA***	***BHA***	***α-TA***	***BHA***	***α-TA***
Anti-Wrinkle Eye Contour Cream	0,0455± 0,002	0,4846± 0,012	90,9	96,9	0,0595± 0,008	0,4819± 0,002	0,05	0,5
Intensive Moisturizing Day Lift Cream	0,0467± 0.001	0,4408± 0,007	93,3	88,2	0,0491± 0,003	0,5348± 0,001	0,05	0,5
Replenishing Night Lift Cream	0,0456± 0.001	0,4663± 0.006	91,3	93,3	0,0478± 0,001	0,5368± 0,002	0,05	0,5

n = 3

The method allowed a simultaneous, simple, rapid and accurate determination and confirmation of BHA and α-TA in cosmetic products containing various ingredients. The obtained results demonstrate that the proposed method for the analysis of antioxidants with a high incidence of interference from cosmetic products is appropriate. The determined antioxidant concentrations in the analysed anti-aging products were in conformity with those claimed by the manufacturer.

VI.1.3 Simultaneous determination of synthetic and natural antioxidants in a anti-aging cosmetic formulation by methanol sample preparation and diclormethan extraction and subsequent GC-FID analysis

A gas chromatographic method was developed for the simultaneous determination of synthetic and natural antioxidants-α-tocopherol acetate and BHA respectively, in a o/w cosmetic formulation by using GC with FID detection. A simple extraction procedure of the sample with dichlormethan was required and the separation of both compounds was obtained under the used chromatographic conditions.

Good separation of standards was obtained for GC analysis with retention characteristics of $t_{R,BHA}$=10.118 and $t_{R,\alpha\text{-}TA}$=26.074. The choosen chromatographic conditions allowed a good separation of the two compounds taken into account. Figure 29 presents the cromatogram of a standard solution of the two studied antioxidants:

The calibration graphs for BHA and α-TA were constructed over the covered range of concentrations, and are presented in Figure 30A for BHA and Figure 30B for α-TA respectively. The obtained liniarity was satisfactory and presented a correlation coefficient of 0.999307 for BHA and 0.999215 for α-TA by the developed analysis method. The LOD value obtained for BHA was 0,136 mg/L and 1,237 for α-TA mg/L, respectively. There was calculated a LOQ of 0,413 mg/L for BHA and 3,749 mg/L for α-TA.

A anti-aging cream sample-*Anti-Wrinkle Eye Contour Cream* was prepared in the laboratory by adding 0,05% BHA and 0,5% α-TA and the recovery rate was calculated from the analysed cream sample.

A commercial sample of a anti-aging cream (*Anti-Wrinkle Eye Contour Cream*) that contained combinations of these two antioxidants was analysed and the target compounds were identified by comparing the retention times of the observed peaks with those obtained from the standard solutions. The enlarged GC chromatogram of *Anti-Wrinkle Eye Contour Cream* is presented in Figure 36B for the elution evidence of both antioxidant compounds.

The concentrations of the synthetic antioxidant BHA and the natural antioxidant α-TA

respectively, determined from the anti-aging creams-*Anti-Wrinkle Eye Contour Cream*, the recovery rates and antioxidant concentrations in commercial cream samples are listed in Table 36.

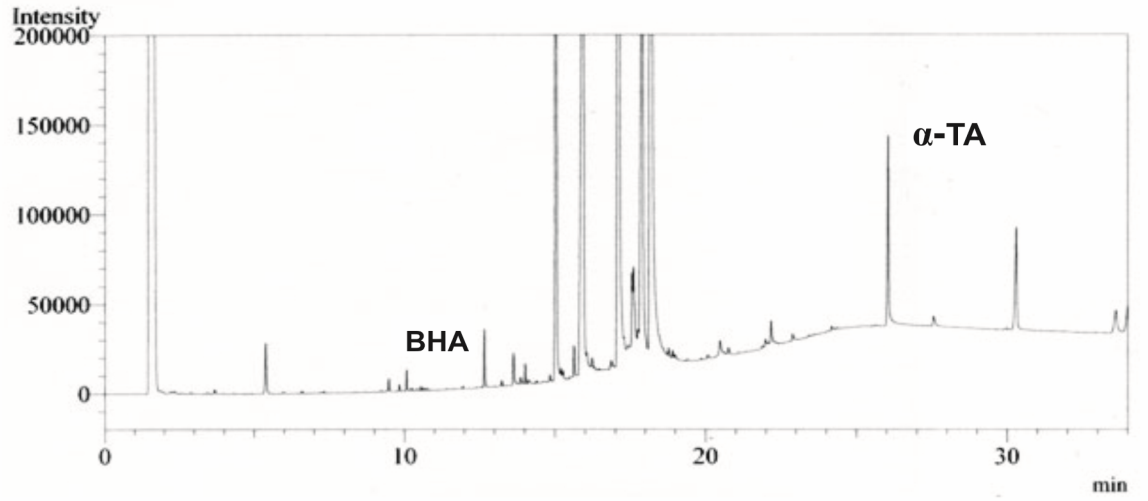

Figure 36B: Enlarged GC Chromatogram of a Anti-Wrinkle Eye Contour Cream

Table 36: *Concentration values of antioxidants in the analysed cream sample*

Sample	Concentration ± DS Sample (g/100g)		Recovery Rate (%)		Concentration ± DS Commercial Sample (g/100g)		Claimed Concentration (g/100g)	
	BHA	***α-TA***	***BHA***	***α-TA***	***BHA***	***α-TA***	***BHA***	***α-TA***
Anti-Wrinkle Eye Contour Cream	0,0149±0,002	0,4977±0,0004	29,8	99,5	0,0146±0,001	0,5967±0,001	0,05	0,5

n = 3

The method allowed a simultaneous, simple, rapid and accurate determination and confirmation of BHA and α-TA in a cosmetic product containing various ingredients. The obtained results for the natural antioxidant α-TA demonstrate that the proposed method for the analysis of natural antioxidants with a high incidence of interference from cosmetic products is appropriate. By the applied method there was obtaied a very low recovery rate for the BHA determination in the analysed cream sample.

VI.1.4 Simultaneous determination of synthetic and natural antioxidants in a anti-aging cosmetic formulation by organic solvent mixture sample preparation and diclormethan extraction and subsequent GC-FID analysis

A gas chromatographic method was developed for the simultaneous determination of

synthetic and natural antioxidants-α-tocopherol acetate and BHA respectively, in a o/w cosmetic formulation by using GC with FID detection. A diclormethan extraction procedure of the sample was required and the separation of both compounds was obtained under the used chromatographic conditions [183].

Good separation of standards was obtained for GC analysis with retention characteristics of $t_{R,BHA}$=10.118 and $t_{R,\alpha\text{-}TA}$=26.074. The choosen chromatographic conditions allowed a good separation of the two compounds taken into account. Figure 29 presents the cromatogram of a standard solution of the two studied antioxidants.

The calibration graphs for BHA and α-TA were constructed over the covered range of concentrations, and are presented in Figure 30A for BHA and Figure 30B for α-TA respectively. The obtained liniarity was satisfactory and presented a correlation coefficient of 0.999307 for BHA and 0.999215 for α-TA by the developed analysis method. The LOD value obtained for BHA was 0,136 mg/L and 1,237 for α-TA mg/L, respectively. There was calculated a LOQ of 0,413 mg/L for BHA and 3,749 mg/L for α-TA.

A anti-aging cream sample-*Anti-Wrinkle Eye Contour Cream* was prepared in the laboratory by adding 0,05% BHA and 0,5% α-TA and the recovery rate was calculated from the analysed cream sample. The synthetic antioxidant eluted with a unsatisfactory peak shape and high, that does not enable the cuantification of BHA from the analysed cream sample by the developed analysis method.

A commercial sample of a anti-aging cream (*Anti-Wrinkle Eye Contour Cream*) that contained combinations of these two antioxidants was analysed and the target compounds were identified by comparing the retention times of the observed peaks with those obtained from the standard solutions. The enlarged GC chromatogram of *Anti-Wrinkle Eye Contour Cream* is presented in Figure 39B for the elution evidence of the natural antioxidant α-TA:

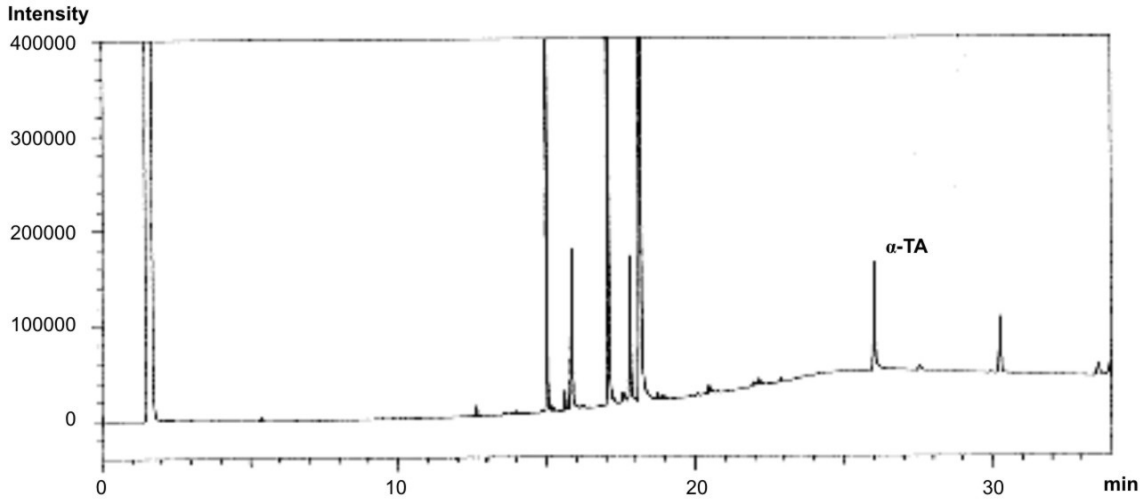

Figure 39B: Enlarged GC Chromatogram of a Anti-Wrinkle Eye Contour Cream

The concentrations of the natural antioxidant α-TA, determined from the anti-aging cream sample-*Anti-Wrinkle Eye Contour Cream*, the recovery rates and antioxidant concentrations in commercial cream samples are listed in Table 40:

Table 40: *Concentration values of antioxidants in the analysed cream sample*

Sample	Concentration ± DS Sample (g/100g)	Recovery Rate (%)	Concentration ± DS Commercial Sample (g/100g)	Claimed Concentration (g/100g)
Anti-Wrinkle Eye Contour Cream	0,4360±0,02	87,2	0,6871±0,001	0,5

n = 3

The method allowed a simultaneous, simple, rapid and accurate determination and confirmation of BHA and α-TA in a cosmetic product containing various ingredients.

The obtained results for the natural antioxidant α-TA demonstrate that the proposed method for the analysis of natural antioxidants with a high incidence of interference from cosmetic products is appropriate.

By the applied method there was obtaied a very low recovery rate for the BHA determination in the analysed cream sample. BHA eluted with a unsatisfactory peak shape and high, that does not enable the cuantification of BHA from the analysed cream sample by the developed analysis method. The very low concentration obtained for the synthehtic antioxidant does not allow a quantitative determination of this compound by the applied analysis method.

The developed analysis method can be successfully applied for the qualitative and quantitaive determination of natural antioxidants from cosmetic products. The determined concentrations of the natural antioxidant in the analysed anti-aging product were in conformity with those claimed by the manufacturer.

VI.1.5 Simultaneos determination of synthetic and natural antioxidants in anti-aging cosmetic formulations after fat saponification by hexane:ethyl acetate organic solvent mixture extraction and subsequent GC-FID analysis

A gas chromatographic method was developed for the simultaneous determination of synthetic and natural antioxidants-α-tocopherol acetate and BHA respectively, in three o/w cosmetic formulations by using GC with FID detection. Fat saponification was performed for

cream sample preparation, succeed by hexane:ethyl acetate organic solvent mixture extraction, and the separation of both compounds was obtained under the used chromatographic conditions.

Good separation of standards was obtained for GC analysis with retention characteristics of $t_{R,BHA}$=10.118 and $t_{R,\alpha\text{-}TA}$=26.074. The choosen chromatographic conditions allowed a good separation of the two compounds taken into account. Figure 29 presents the cromatogram of a standard solution of the two studied antioxidants.

The calibration graphs for BHA and α-TA were constructed over the covered range of concentrations, and are presented in Figure 30A for BHA and Figure 30B for α-TA respectively. The obtained liniarity was satisfactory and presented a correlation coefficient of 0.999307 for BHA and 0.999215 for α-TA by the developed analysis method. The LOD value obtained for BHA was 0,136 mg/L and 1,237 for α-TA mg/L, respectively. There was calculated a LOQ of 0,413 mg/L for BHA and 3,749 mg/L for α-TA.

Three anti-aging cream sample-*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. The synthetic antioxidant eluted with a unsatisfactory peak shape and high, that does not enable the cuantification of BHA from the analysed cream sample by the developed analysis method. The recovery rate was calculated for α-TA in the analysed cream sample.

The three commercial samples of the 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 enlarged GC chromatograms of *Anti-Wrinkle Eye Contour Cream* is presented in Figure 41A, in Figure 41B for the *Intensive Moisturizing Day Lift Cream* and in Figure 41C for the *Replenishing Night Lift Cream* for the elution evidence of the natural antioxidant α-TA:

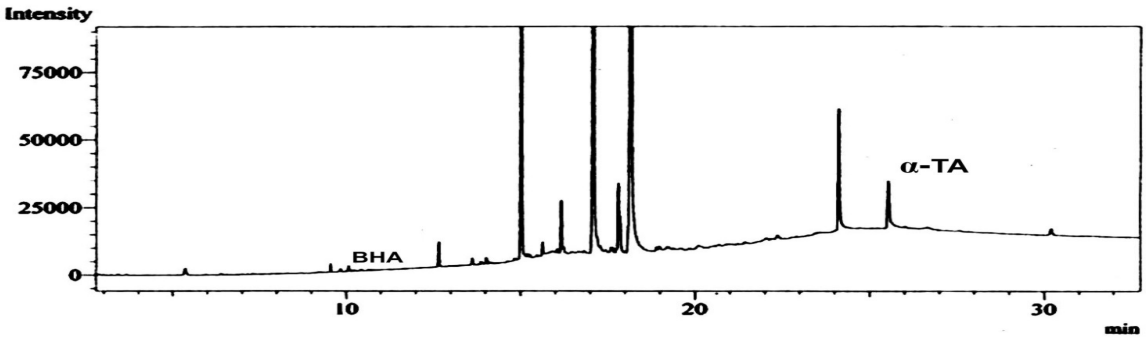

Figure 41A: Enlarged GC Chromatogram of a Anti-Wrinkle Eye Contour Cream

The concentrations of the natural antioxidant α-TA, determined from the anti-aging cream samples-*Anti-Wrinkle Eye Contour Cream*, *Intensive Moisturizing Day Lift Cream* and *Replenishing Night Lift Cream*, the recovery rates and antioxidant concentrations in commercial cream samples are listed in Table 44.

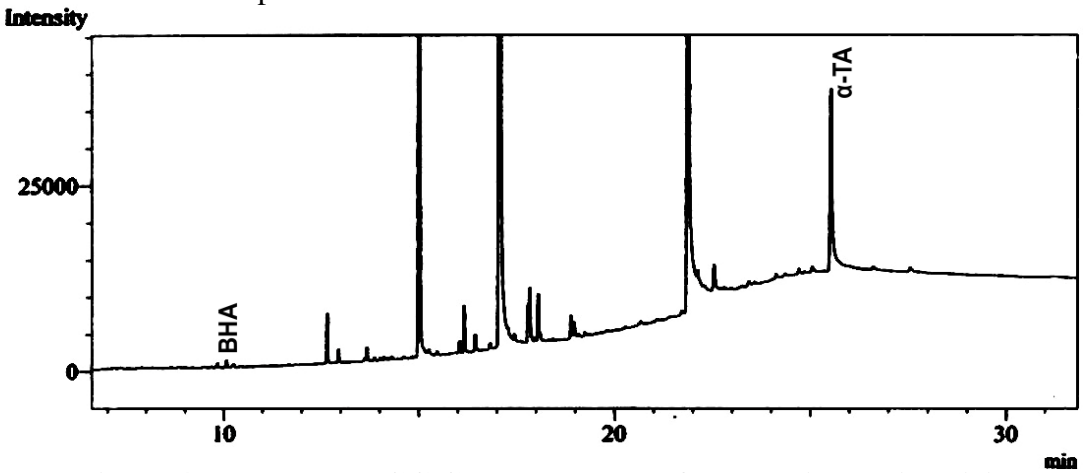

Figure 41B: Enlarged GC Chromatogram of a Intensive Moisturizing Day Lift Cream

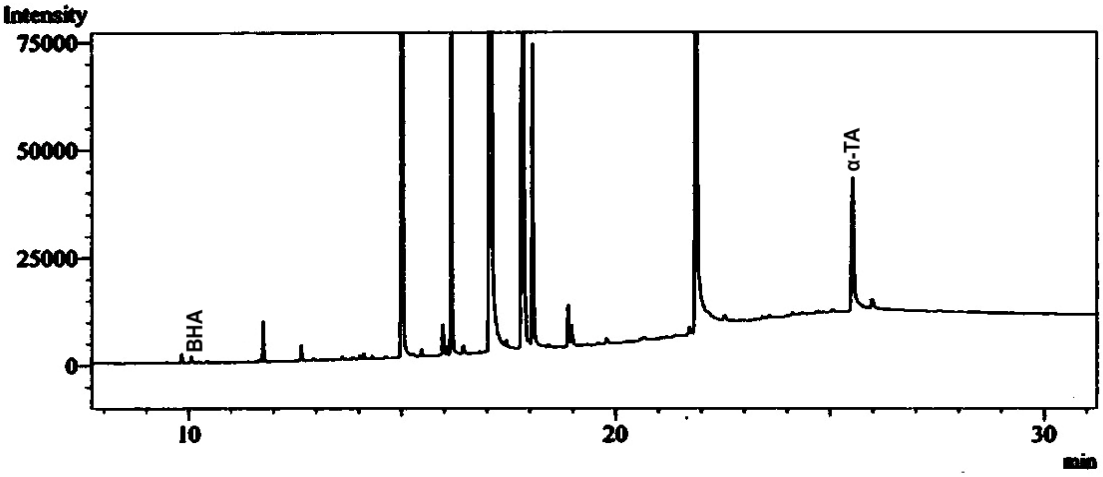

Figure 41C: Enlarged GC Chromatogram of a Replenishing Night Lift Cream

Table 44: *Concentration values of antioxidants in the analysed cream samples*

Sample	Concentration ± DS Sample (g/100g)	Recovery Rate (%)	Concentration ± DS Commercial sample (g/100g)	Claimed Concentration (g/100g)
Anti-Wrinkle Eye Contour Cream	0,3345±0,001	66,9	0,3100±0,002	0,5
Intensive Moisturizing Day Lift Cream	0,3367±0,001	67,3	0,3428±0,002	0,5
Replenishing Night Lift Cream	0,3268±0,003	65,4	0,3640±0,001	0,5

n = 3

The very low concentration obtained for the synthetic antioxidant, that does not enable the qunatitative determination of this compound from the analysed cream sample by the developed analysis method. The α-TA concentrations determined in cosmetic formulation prepared in the laboratory, and also in commercial cream samples were relatively low by the developed abalysis method. The obtained results confirm that this compounds have to be isolated from the lipidic matrix by a prior preparation procedure, but saponification or sample extraction often brings a analyte loss in the analysis of antioxidants from cosmetic cream samples.

VI.1.6 Simultaneous determination of synthetic and natural antioxidants in anti-aging cosmetic formulations by methanol extraction and subsequent GC-FID analysis

A gas chromatographic method was developed for the simultaneous determination of natural and synthetic antioxidants-α-tocopherol acetate, and BHA respectively, in o/w cosmetic formulations by using GC with FID detection. A simple methanol extraction procedure of the sample was required and the separation of both compounds was obtained under the used chromatographic conditions.

Good separation of standards was obtained for GC analysis with retention characteristics of $t_{R,BHA}$=10.108 and $t_{R,\alpha\text{-}TA}$=26.101. The choosen chromatographic conditions allowed a good separation of the two compounds taken into account. Figure 43 presents the cromatogram of a standard solution of the two studied antioxidants.

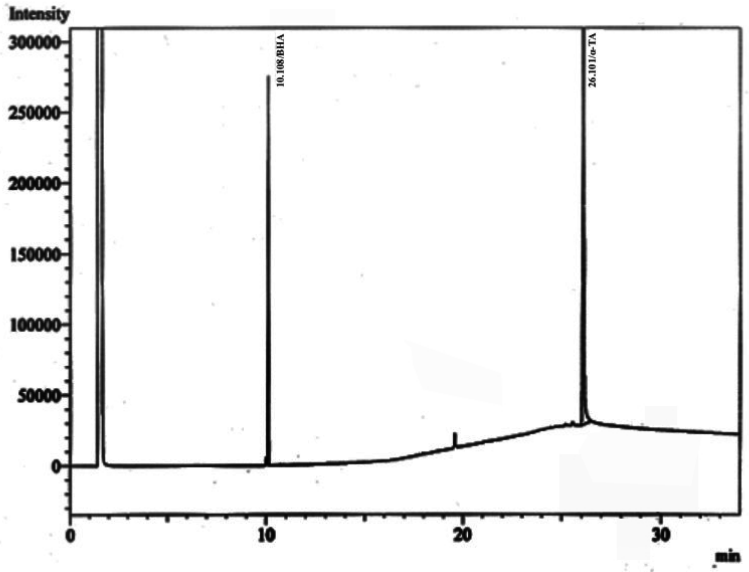

Figure 43: GC Chromatogram of a BHA and α-TA standard

The calibration graphs for BHA and α-TA were constructed over the covered range of concentrations, and are presented in Figure 44A for BHA and Figure 44B for α-TA

respectively. The LOD value obtained for BHA was 0,238 mg/L and 0,726 for α-TA mg/L, respectively. There was calculated a LOQ of 0,721 mg/L for BHA and 2,1999 mg/L for α-TA.

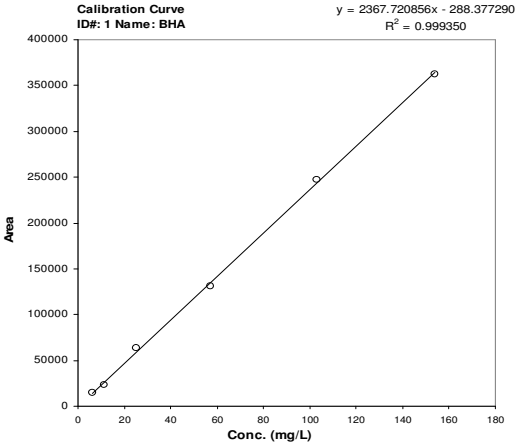

Figure 44A: Calibration Curve of BHA

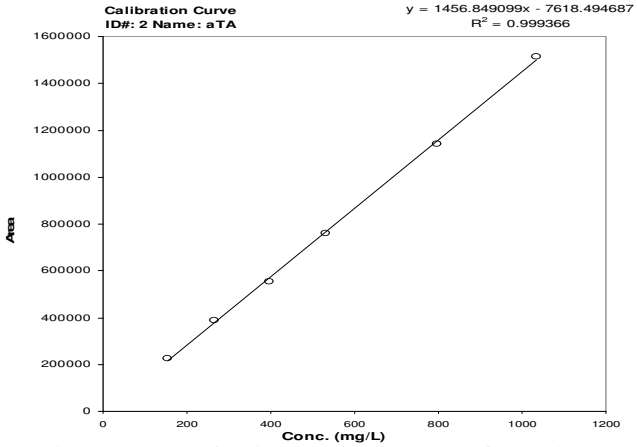

Figure 44B: Calibration Curve of α-TA

Two anti-aging cream sample-*Anti-Wrinkle Eye Contour Cream* and *Intensive Moisturizing Day Lift Cream* were prepared in the laboratory by adding 0,05% BHA and 0,5% α-TA. The chromatographic peak corresponding to the synthetic antioxidant is distorted, it does not presnet a satisfactory peak shape and high, which leads to the impossibility of the quantitaive determination of this compound in cream samples by the developed analysis method. The recovery rates were calculated for α-TA in the analysed cream samples.

Two commercial samples of the anti-aging creams (*Anti-Wrinkle Eye Contour Cream* and *Intensive Moisturizing Day 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 chromatograms of *Anti-Wrinkle Eye Contour Cream* is presented in Figure 45, and Figure 46 presents the chromatogram of the *Intensive Moisturizing Day Lift Cream*.

The concentrations of the natural antioxidant α-TA, determined from the anti-aging cream type samples-*Anti-Wrinkle Eye Contour Cream* and *Intensive Moisturizing Day Lift Cream*, the recovery rates and antioxidant concentrations in commercial cream samples are listed in Table 51.

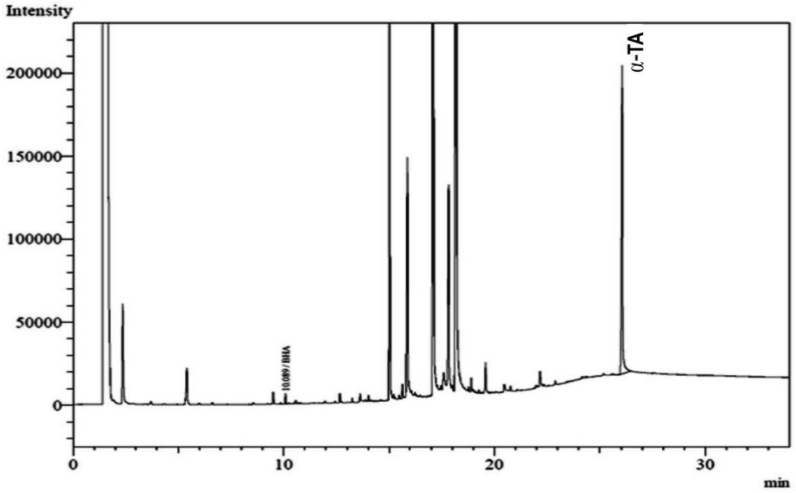

Figure 45: GC Chromatogram of a Anti-Wrinkle Eye Contour Cream

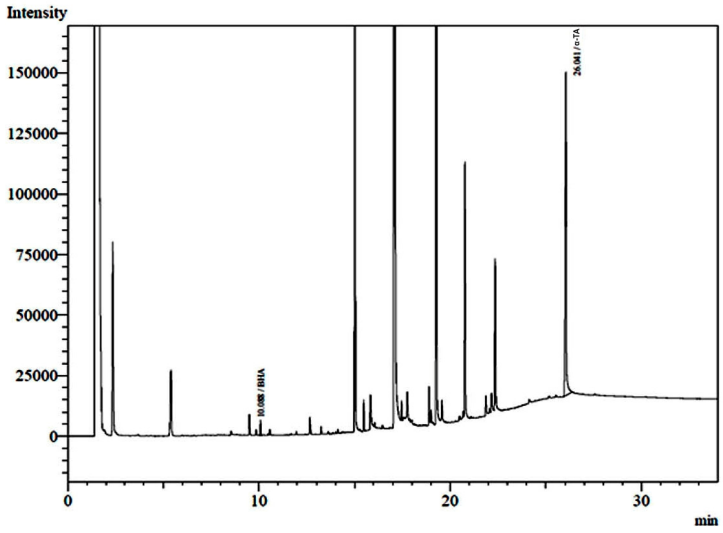

Figure 46: GC Chromatogram of a Intensive Moisturizing Day Lift Cream

Table 51: *Concentration values of antioxidants in the analysed cream samples*

Sample	Concentration ± DS Sample (g/100g)	Recovery Rate (%)	Concentration ± DS Commercial Sample (g/100g)	Claimed Concentration (g/100g)
Anti-Wrinkle Eye Contour Cream	0,4625±0,002	92,5	0,4619±0,002	0,5
Intensive Moisturizing Day Lift Cream	0,4131±0,002	82,6	0,3268±0,001	0,5

n = 3

The synthetic antioxidant eluted with a unsatisfactory peak shape and high, that does not enable the cuantification of BHA from the analysed cream samples by the developed analysis method. The very low concentration obtained for the synthetic antioxidants does not

permit a quantitative determination of this compound in cosmetic formultaions by the applied analysis method.

The developed analysis method cand be successfully applied for qualitative and quantitative determination of α-TA in cosmetic formulations. The determined concentrations of the natural antioxidant in the analysed anti-aging product were in conformity with those claimed by the manufacturer.

CONCLUSIONS

Cosmetic products formulations comprise natural and synthetic antioxidants, thereby simultaneous identification and determination of this compound would be ideally.

Generally, the most used antioxidants in cosmetic formulations are the synthetic antioxidant BHA, and the natural antioxidant α-T, in the acetate form α-TA, at concentration levels of 0,05%, and 0,5% respectively.

Different preparation procedures of the cream samples, for synthetic samples, obtained by impregnation at a concentration level of 0,05% BHA and 0,5% α-TA, and also for commercial available cosmetic product samples were developed, and analysis of this samples was performed by GC coupled with FID. The most appropiate method proved to be cream sample dilution with acetonitrile:methanol organic solvent mixture by sonication, filtration and simultaneous antioxidant determination by GC. Figure 48A and Figure 48B respectively, presents the natural and synthetic antioxidant concentrations determined in the analysed cream samples, and in commercial available cosmetic products by GC-FID:

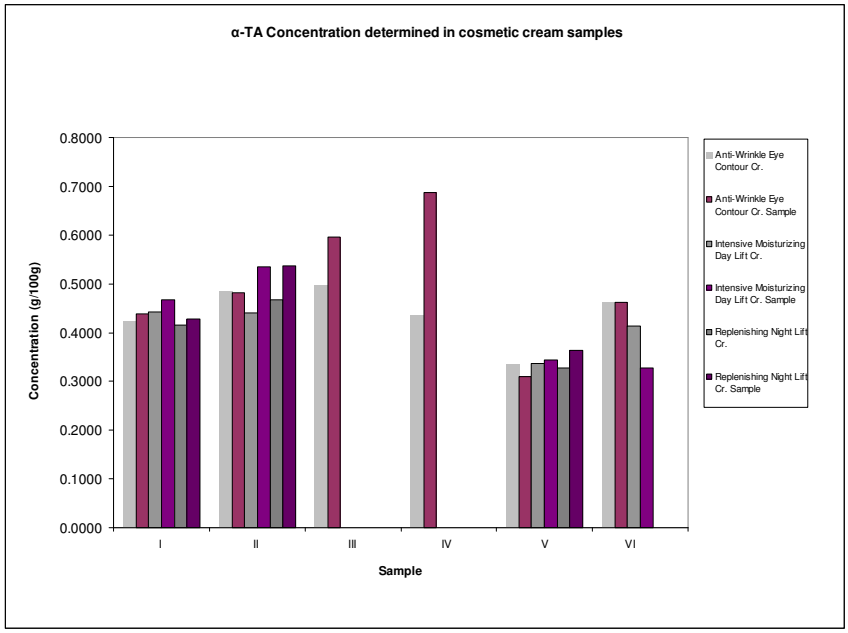

Figure 48A: α-TA Concentration determined in cosmetic cream samples by GC-FID

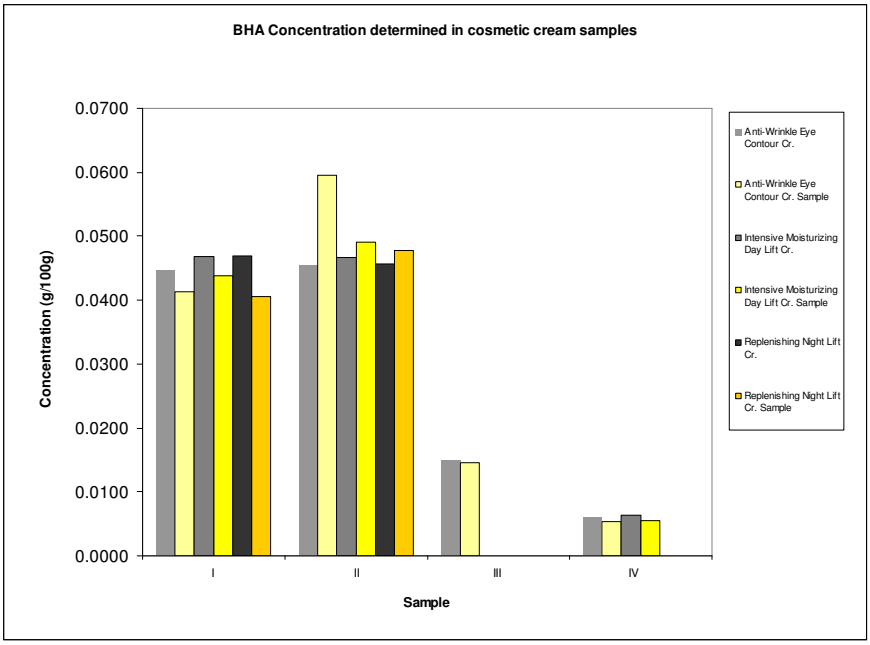

Figure 48B: BHA Concentration determined in cosmetic cream samples by GC-FID

I.2 Analysis of alpha tocopherol acetate in a anti-aging cosmetic formulation preserved with multifunctional additives by gas chromatography coupled with FID

A gas chromatographic method was applied by using the GC-FID tehnique for the determination of the natural antioxidant α-tocopherol acetate, in a o/w proper developed cosmetic formulations, and preserved with multifunctional additives. A simple methanol:acetonitrile organic solvent mixture (50:50 v/v) extraction procedure of the sample was required and the separation of both compounds was obtained under the used chromatographic conditions.

The antioxidant used in the study is lisetd in Table 53 and is commercially available.

Table 53: *INCI nomenclature of the studied antioxidant*

Commercial name	*INCI*	*Supplier*
Dermofeel® E 74 A	***Tocopheryl Acetate***; *Helianthus annuus (sunflower) seed oil*	Dr. Straetmans GmbH

Cosmetic formulation

In the developed and analysed cosmetic formulation, α-TA is comprised in sunflower seed oil, raw material that is commercialy denominated as Dermofeel® E74A. The α-TA concentration claimed by the supplier is > 73,5 % α-TA in sunflower seed oil. The developed cosmetic formulation and the manufacuring procedure is presented in Figure 50.

Figure 51 presents the cromatogram of a standard solution of the studied natural antioxidant.

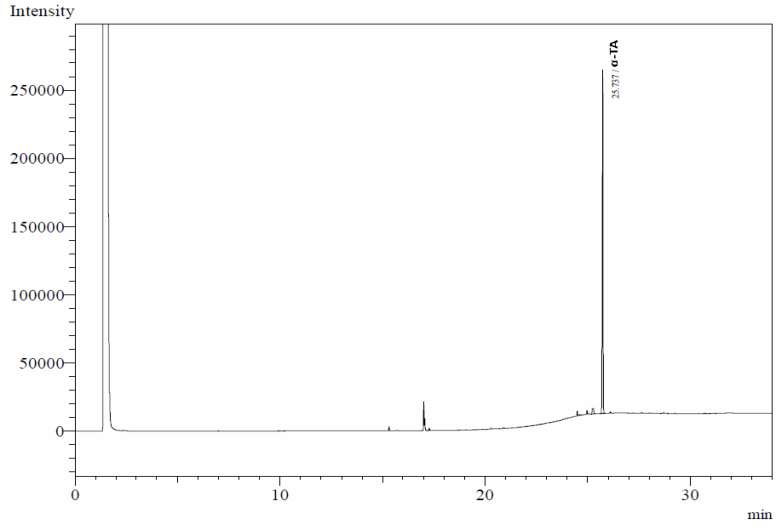

Figure 51: GC Chromatogram of a α-TA standard

Anti Aging Light Day Cream

Claims: **Preservative free, PEG-free**
UV Protection, estimated SPF 10
Elegant skin feeling

Phase	Ingredient	INCI	Supplier	%
A	Deionised Water	Aqua		56.50
	Glycerol	Glycerin	ROBOT Import Export SRL	3.00
	dermofeel® PA-3	Sodium Phytate, Aqua	Dr. Straetmans	0.10
	dermosoft® LP	*Caprylyl Glycol, Glycerin,Glyceryl Caprylate, Phenylpropanol*	*Dr. Straetmans*	*0.80*
A1	Rapithix A 100	Sodium Polyacrylate	ISP	0.20
	Keltrol RD	Xanthan Gum	CP Kelco	0.30
B	symbio®muls CG	Glyceryl Stearate Citrate, Cetearyl Alcohol, Glyceryl Caprylate	Dr. Straetmans	5.00
	Plantec Natural Shea Butter	Butyrospermum Parkii	CRM International	2.00
	Fitoderm	Squalane	Cognis	5.00
	dermofeel® sensolv	Isoamyl Laurate	Dr. Straetmans	8.00
	DC 345 Fluid	Cyclopentasiloxane, Cyclohexasiloxane	Dow Corning	3.00
	DC 200 Fluid	Dimethicone	Dow Corning	1.00
	Salisol AB	Butyl Methoxydibenzoylmethane	SALICYLATES& CHEMICALS PVT. LTD.	2.80
	Salisol OMC	Octyl Methoxycinnamate	SALICYLATES& CHEMICALS PVT. LTD.	5.20
	dermofeel® E 74 A	**Tocopheryl Acetate, Helianthus Annuus (Sunflower) Seed Oil**	**Dr. Straetmans**	**0.50**
C	Extrapone Green Tea GW	Camelia Sinensis Extract, Glycerin, Aqua	Symrise	4.00
	Nutrilan I-50	Hydrolyzed Collagen	Cognis	2.00
D	Parf. Lucky Light	Parfum	CPL	0.30
	Sodium Hydroxid (10%)	Sodium Hydroxid	Prod Alma	q.s
				100.00

Manufacturing Procedure:

1. Heat Phase A up to 78°C and disperse Keltrol and Cosmedia SP. Heat phase B up to 78°C.
2. Emulsify phase B into phase A under stirring. Homogenize for 1-2 min. using an Ultra Turrax.
3. Start to cool down under medium stirring.
4. Add C and D below 40°C and cool down under stirring. Adjust pH value if necessary.

Specification Values:

Appearance: Soft light yellow emulsion.
pH: 5.5 – 6.0.
Viscosity (Brookfield: Helipath TF; Speed 10): 30.000 - 40.000 mPas.
Centrifugation (15 min., 4.000 rpm): No separation.

Stability: More than 3 months stable at 20°C, 40°C and 4°C.

Microbiological Stability: Proven.

Figure 50: Developed cosmetic formulation and manufacturing procedure

The calibration graph for α-TA was constructed over the covered range of concentrations, and is presented in Figure 52:

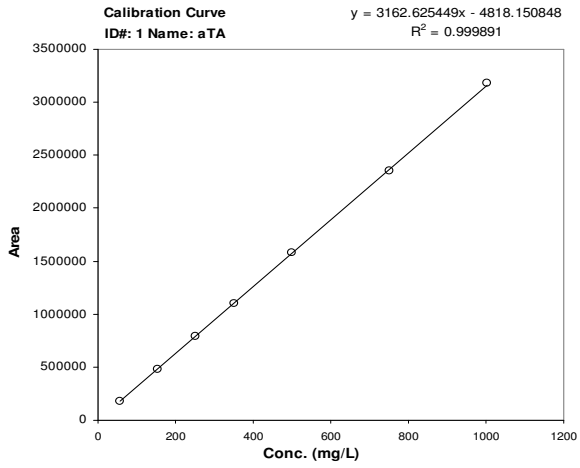

Figure 52: : Calibration Curve of α-TA

The LOD value obtained for α-TA by the proposed analysis method was 0,044 mg/L

and there was calculated a LOQ of 0,134 mg/L for α-TA.

The analysed cream sample that contained the natural antioxidant was analysed and the target compound was identified by comparing the retention time of the observed peak with that obtained from the standard solution. The GC chromatograms of a *Anti Aging Light Day Cream* is presented in Figure 53:

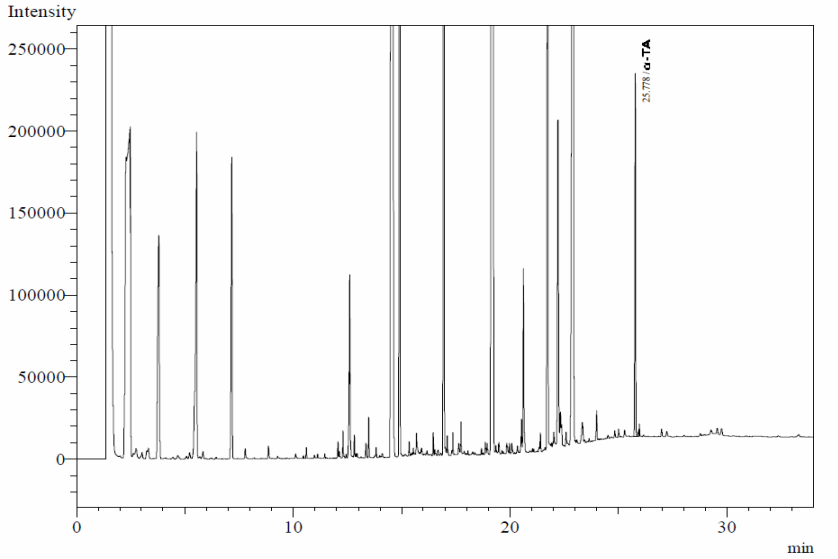

Figure 53: GC Chromatogram of a Anti Aging Light Day Cream

Table 58: *Concentration values of the natural antioxidant in the analysed Anti Aging Light Day Cream samples*

Sample	Concentration ± DS Sample (g/100g)	Recovery Rate (%)	Claimed Concentration (g/100g)
Anti Aging Light Day Cream	0,3537 ±0,002	94,3	0,375

n = 3

CONCLUSIONS

The formula of a anti-aging cream, preserved with multifunctional additives was developed.

This method allowed a simple, rapid and accurate determination and confirmation of α-TA in the analysed cosmetic product containing various ingredients. The obtained results demonstrate that the proposed method for the analysis of antioxidants with a high incidence of interference from cosmetic products is appropriate.

VI.3 Analysis of synthetic and natural antioxidants in cosmetic products by high performance liquid chromatography

The antioxidants used in the study are lisetd in Table 18 and are commercially available.

Chromatographic conditions

Chromatography was performed on a Agillent 1100 Series liquid cromatograph equipped with quaternary pump, degasifier, column thermostat, auto-sampler and a UV/VIS detector. Separations were performed on a Eclipse XDB-C18 (4,6mm Id x 250mm (5μm)) . The composition of 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.

VI.3.1 Simultaneous determination of synthetic and natural antioxidants in anti-aging-cosmetic formulations by dilution with acetonitrile:methanol organic solvent mixture and subsequent HPLC/UV analysis

A high performance liquid chromatographic method was developed for the simultaneous determination of two antioxidants- α-tocopherol acetate, and BHA respectively, in o/w cosmetic formulations by using the RP-HPLC-UV tehnique. A simple dilution procedure of the sample with methanol:acetonitrile organic solvent mixture was required and the separation of both compounds was obtained under the used chromatographic conditions.

Good separation of standards was obtained for HPLC analysis with retention characteristics of $t_{R,BHA}$=1.849 and $t_{R,\alpha\text{-}TA}$=13.767.

Figure 56 presents the cromatogram of a standard solution of the two studied antioxidants:

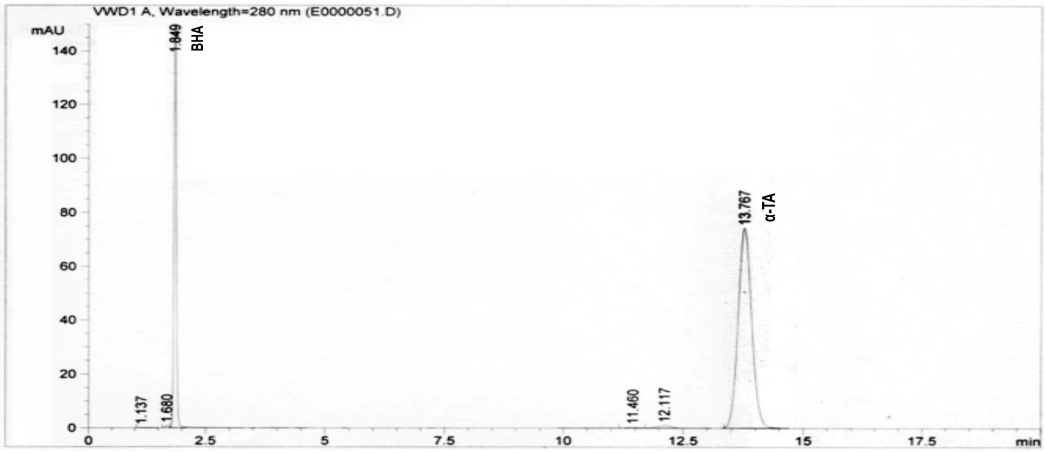

Figure 56: HPLC Chromatogram of a BHA and α-TA standard

The calibration graphs for BHA and α-TA were constructed over the covered range of concentrations, and are presented in Figure 57A for BHA and Figure 57B for α-TA respectively:

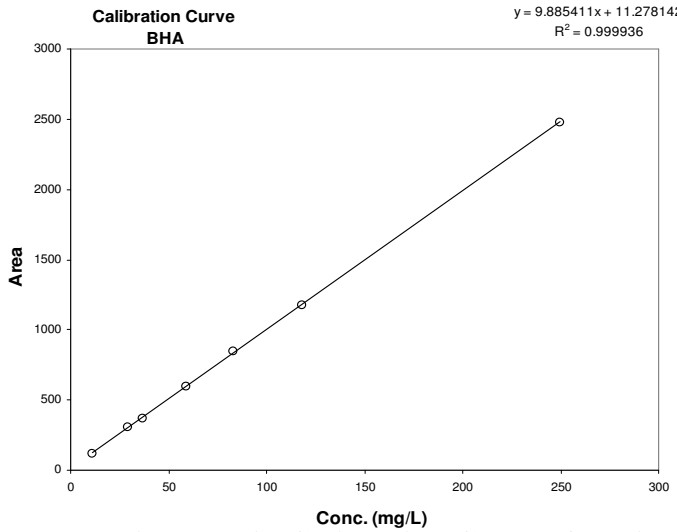

Figure 57A: Calibration Curve of BHA

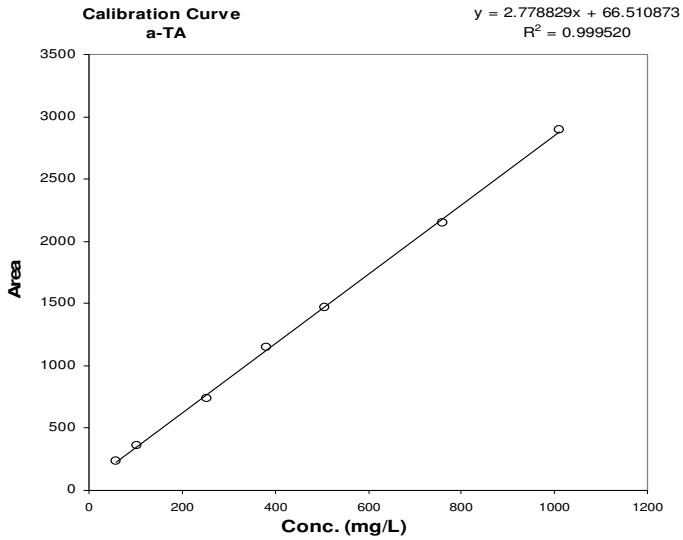

Figure 57B: Calibration Curve of α-TA

The LOD value obtained for BHA was 0,666 mg/L and 0,532 mg/L for α-TA, respectively. There was calculated a LOQ of 2,019 mg/L for BHA and 1,613 mg/L 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). Complete triplicate analysis was performed on all cream samples to allow the calculation of average deviations as a measurement of chromatographic reproductibility.

Commercial samples of three anti-aging creams 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 HPLC chromatogram of *Anti-Wrinkle Eye Contour Cream* is presented in Figure 58. The chromatogram of a *Intensive Moisturizing Day Lift Cream* and that one for a *Replenishing Night Lift Cream* are presented in Figure 59, and respectively in Figure 60.

The concentrations of the synthetic antioxidant BHA and the natural antioxidant α-TA respectively determined from three anti-aging creams in the cosmetic samples, the recovery rates and antioxidant concentrations in commercial cream samples are listed in Table 66.

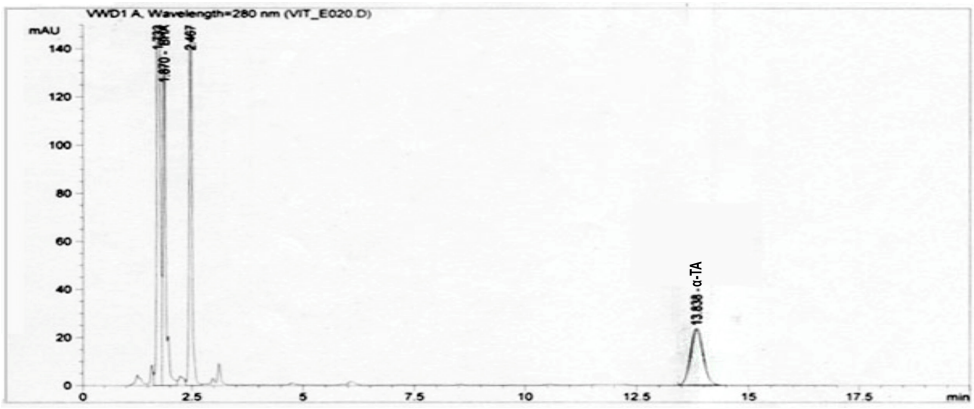

Figure 58: HPLC Chromatogram of a Anti-Wrinkle Eye Contour Cream

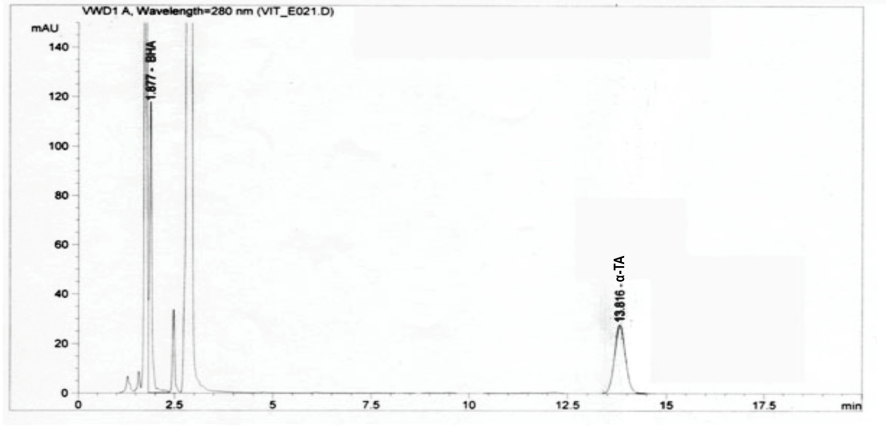

Figure 59: HPLC Chromatogram of a Intensive Moisturizing Day Lift Cream

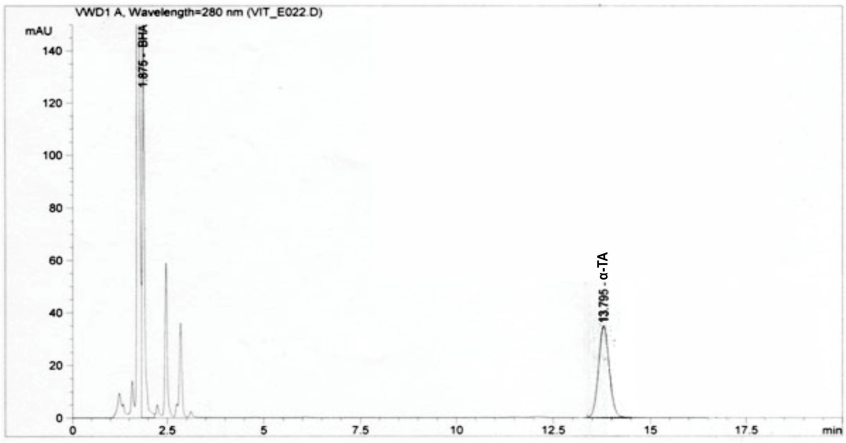

Figure 60: HPLC Chromatogram of a Replenishing Night Lift Cream

The method allowed a simultaneous, simple, rapid and accurate determination and confirmation of BHA and α-TA in cosmetic products containing various ingredients. The obtained results demonstrate that the proposed method for the analysis of antioxidants with a high incidence of interference from cosmetic products is appropriate.

Table 66: *Concentration values of antioxidants in the analysed cream samples*

Sample	Concentration ± DS Sample (g/100g)		Recovery Rate (%)		Concentration ± DS Commercial sample (g/100g)		Claimed Concentration (g/100g)	
	BHA	***α-TA***	***BHA***	***α-TA***	***BHA***	***α-TA***	***BHA***	***α-TA***
Anti-Wrinkle Eye Contour Cream	0,0499±0,0008	0,2605±0,0012	99,7	52,1	0,0606±0,0011	0,1419±0,0008	0,05	0,5
Intensive Moisturizing Day Lift Cream	0,0454±0,0012	0,2503±0,0007	90,8	50,1	0,0502±0,0014	0,1675±0,0003	0,05	0,5
Replenishing Night Lift Cream	0,0522±0,0005	0,2530±0,0005	104,4	50,6	0,0658±0,0006	0,2173±0,0008	0,05	0,5

n = 3

VI.3.2 Simultaneius determination of synthetic and natural antioxidants in anti-aging cosmetic formulations by methanol extraction and subsequent HPLC/UV analysis

A high performance liquid chromatographic method was developed for the simultaneous determination of natural and synthetic antioxidants- α-tocopherol acetate, and BHA respectively, in two o/w cosmetic formulations (*Intensive Moisturizing Day Lift Cream* and *Replenishing Night Lift Cream*), by using the RP-HPLC-UV tehnique. A simple extraction procedure of the sample with organic solvent was required and the separation of both compounds was obtained under the used chromatographic conditions.

Good separation of standards was obtained for HPLC analysis with retention characteristics of $t_{R,BHA}$=1.849 and $t_{R,\alpha\text{-}TA}$=13.767. Figure 56 presents the cromatogram of a standard solution of the two studied antioxidants:

Two anti-aging cream samples-*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.

Commercial samples of the two anti-aging creams 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

chromatogram of a *Intensive Moisturizing Day Lift Cream* and the chromatogram of a *Replenishing Night Lift Cream* are presented in Figure 63, and respectively in Figure 64:

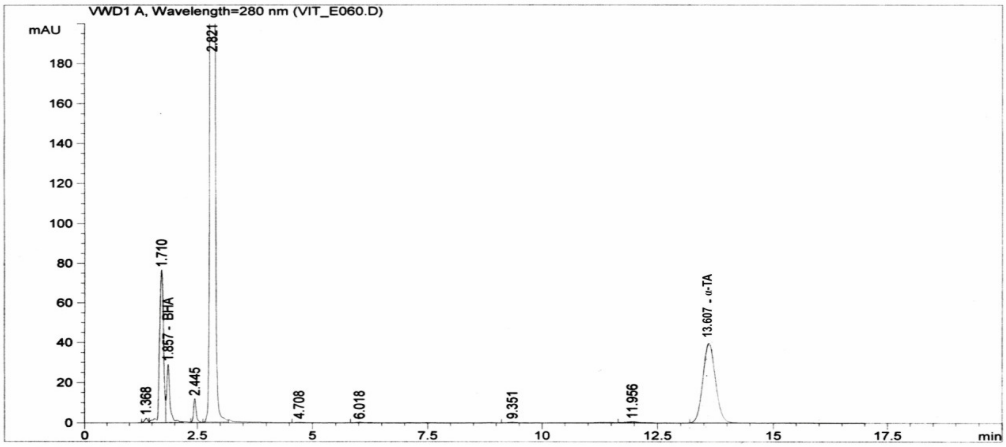

Figure 63: HPLC Chromatogram of a Intensive Moisturizing Day Lift Cream

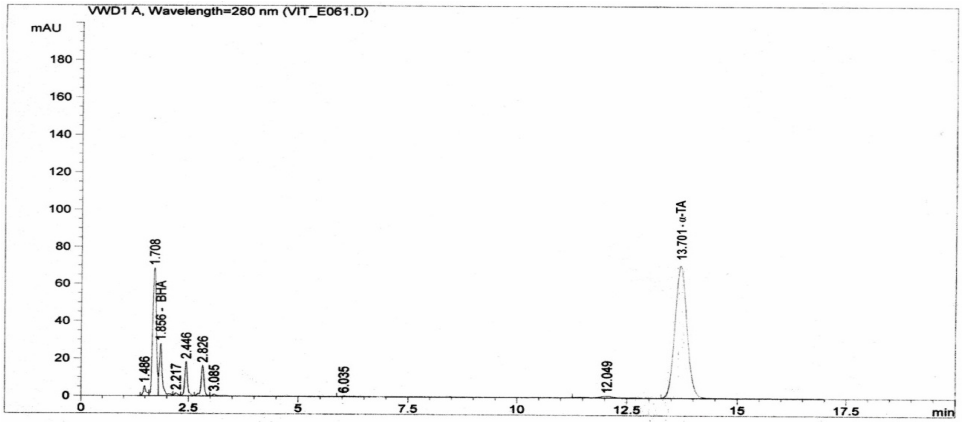

Figure 64: HPLC Chromatogram of a Replenishing Night Lift Cream

The concentrations of the two antioxidants determined from three anti-aging creams in the cosmetic samples, the recovery rates and antioxidant concentrations in commercial cream samples are listed in Table 70.

The method allowed a simultaneous, simple, rapid and accurate determination and confirmation of BHA and α-TA from anti-aging cosmetic products. There were obtained good recovery rates for the synthetic antioxidant BHA with values >80,9%, but the concentration obtained for this compound in the studied commercial cream samples were lower than that ones claimed by the manufacturer. Good recovery rates were obtained for the natural antioxidant, with values of 102% α-TA for the *Intensive Moisturizing Day Lift Cream* and 108,1% α-TA for the *Replenishing Night Lift Cream* by the applie method and HPLC/UV determination.

Table 70: *Concentration values of antioxidants in the analysed cream samples*

Sample	Concentration ± DS Sample (g/100g)		Recovery Rate (%)		Concentration ± DS Commercial sample (g/100g)		Claimed Concentration (g/100g)	
	BHA	***α-TA***	***BHA***	***α-TA***	***BHA***	***α-TA***	***BHA***	***α-TA***
Intensive Moisturizing Day Lift Cream	0,0421± 0,001	0,5102± 0,001	84,3	102,0	0,0262± 0,001	0,4880± 0,002	0,05	0,5
Replenishing Night Lift Cream	0,0405± 0,001	0,5404± 0,001	80,9	108,1	0,0249± 0,001	0,9112± 0,0005	0,05	0,5

n = 3

VI.3.3 Simultaneous determination of synthetic and natural antioxidants in a anti-aging cosmetic formulation by tetrahydrofuran:methanol dilution and subsequent HPLC/UV analysis

A high performance liquid chromatographic method was developed for the simultaneous determination of natural and synthetic antioxidants- α-tocopherol acetate, and BHA respectively, in a o/w cosmetic formulations (*Anti-Wrinkle Eye Contour Cream*), by using the RP-HPLC-UV tehnique. A simple dilution procedure of the sample with organic solvent mixture was required. Just the separation of the natural antioxidant α-TA was achieved under the used chromatographic conditions.

Good separation of standards was obtained for HPLC analysis with retention characteristics of $t_{R,BHA}$=1.849 and $t_{R,\alpha\text{-}TA}$=13.767. Figure 56 presents the cromatogram of a standard solution of the two studied antioxidants:

A anti-aging cream sample- *Anti-Wrinkle Eye Contour Cream* was prepared in the laboratory by adding 0,05% BHA and 0,5% α-TA.

Commercial sample of the anti-aging cream that contained combinations of these two antioxidants was analysed and the target compounds were identified by comparing the retention times of the observed peaks with those obtained from the standard solutions. The chromatogram of a *Anti-Wrinkle Eye Contour Cream* is presented in Figure 67.

The concentrations of the natural antioxidant determined from a anti-aging cream in the cosmetic samples, the recovery rates and antioxidant concentrations in commercial cream samples are listed in Table 74.

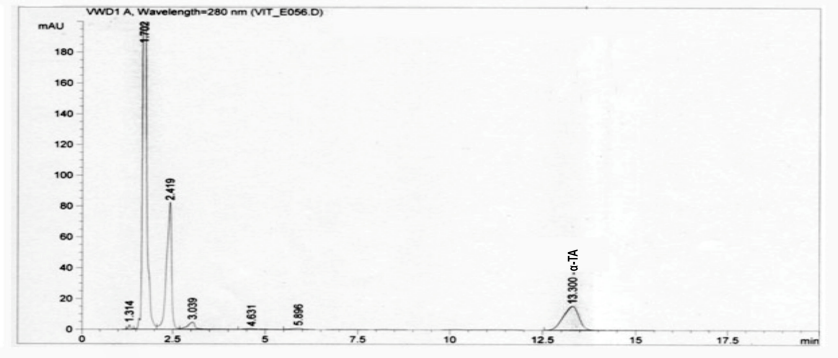

Figure 67: HPLC Chromatogram of a Anti-Wrinkle Eye Contour Cream

Table 74: *Concentration values of antioxidants in the analysed cream samples*

Sample	Concentration ± DS Sample (g/100g)	Recovery Rate (%)	Concentration ± DS Commercial sample (g/100g)	Claimed Concentration (g/100g)
Anti-Wrinkle Eye Contour Cream	0,4221±0,001	84,4	0,3454±0,001	0,5

n = 3

The method allowed a simple, rapid and accurate determination and confirmation of α-TA from anti-aging cosmetic products. There was obtained a good recovery of 84,4% for α-TA from a *Anti-Wrinkle Eye Contour Cream* by the applied method and HPLC/UV determination. α-TA concentration in the analysed anti-aging product was in conformity with those claimed by the manufacturer.

CONCLUSIONS

Different preparation procedures of the cream samples, for synthetic samples, obtained by impregnation at a concentration level of 0,05% BHA and 0,5% α-TA, and also for commercial available cosmetic products were developed, and analysis of this samples was performed by HPLC coupled with UV/VIS detector. All determination methods by this techinque proved to be applied for the identification and quantification of natural and synthetic antioxidants from cosmetic products. The synthetic antioxidant could not be elutetd by the determination method which used THF:methanol sample dilution and HPLC analysis. This method allowed the quantiative determination of the natural antioxidant from the analysed cosmetic product. Figure 69A and Figure 69B respectively, presents the natural and synthetic antioxidant concentrations determined in the analysed cream samples, and in

commercial available cosmetic products by HPLC/UV:

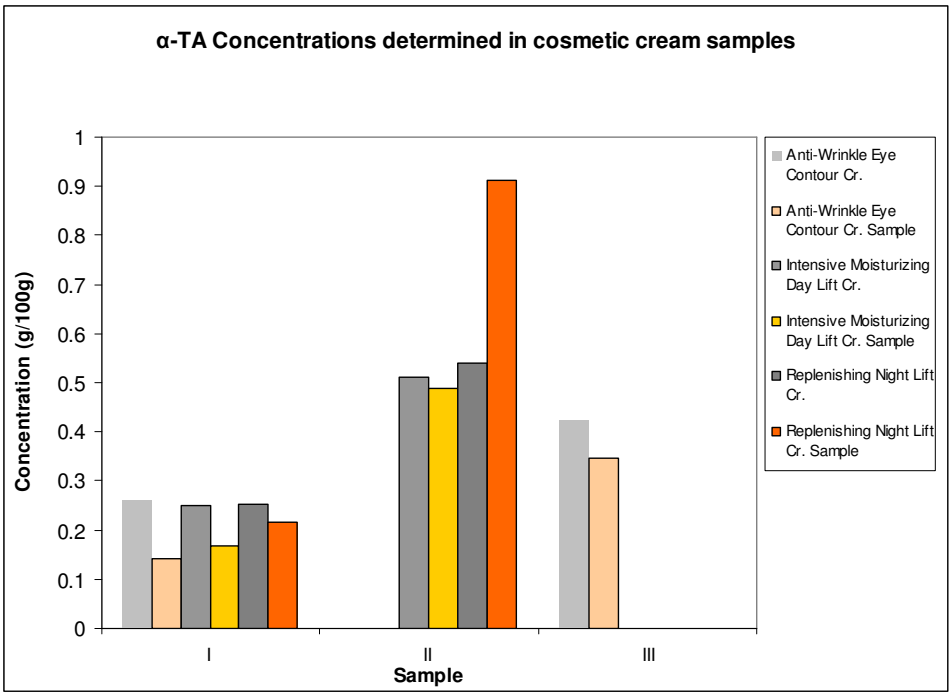

Figure 69A: α-TA Concentration determined in cosmetic cream samples by HPLC-UV

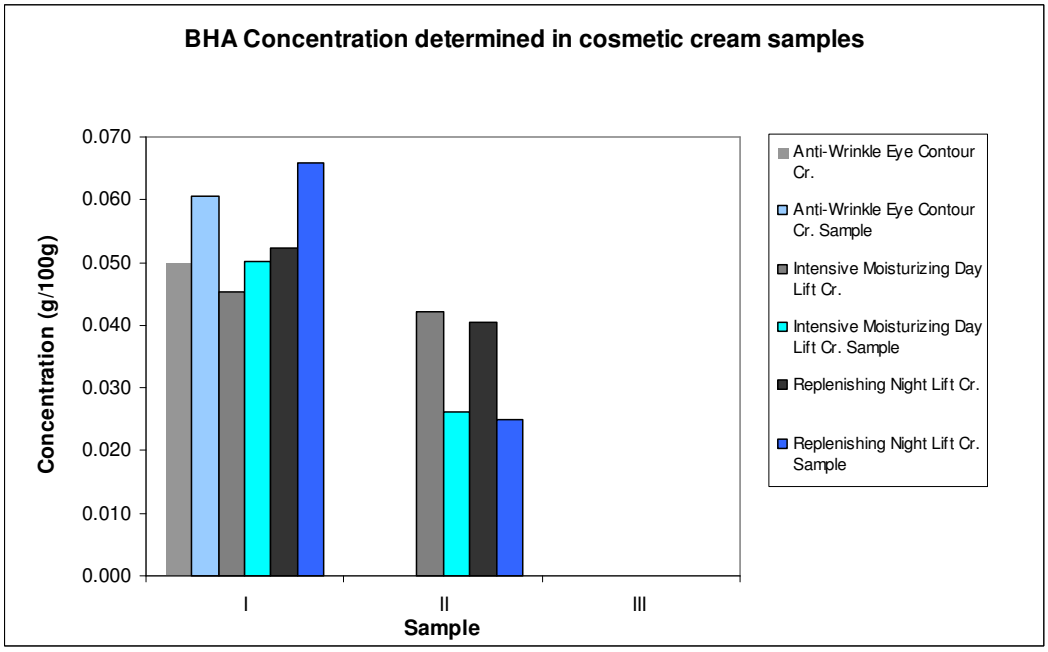

Figure 69B: BHA Concentration determined in cosmetic cream samples by HPLC-UV

VII. Final Conclusions

The use of antioxidants in mixture in cosmetic formulations makes a difficult determination of those compounds in cosmetic products, because of the multitude of compounds used as cosmetic antioxidants, and the variety of cosmetic products that are comercialized. That why it is necessary to develop performant analysis methods that can exactly, and with precission determine the type and amount of antioxidants present in cosmetic products.

The present thesis delineates the role and the importance of antioxidants in cosmetic formulations and also aims to improve some analysis methods for this compounds in anti-ageing products, products that contain a complex composition of raw materials.

The antioxidant potential of alpha tocopherol acetate and of the preservative BHA, usually used in cosmetic formulations or added in a controlled way at known levels of concentration, in a standard cream were evaluated by the DPPH method. The antioxidant effect of creams which include molecules with antioxidant potential seems to be a complex issue needing further studies. Even if molecules like BHA or α-tocoferol, considered individually, with antioxidant effect (by the DPPH method), in complex mixtures with lipides, especially when their concentration is below 1%, the antioxidant action can not be registred by usual determinations.

The use of FTIR (ATR) spectrometry has allowed the evidence of the characteristic fingerprint of the three studied cream types, and also of the ingredients from these products, depending of their role in the specific cream.

For creams with a complex composition, especially when they differ by reduced percentages between the polar and nonpolar ingredients, it is hard to evidentiate the recognition markers by the FTIR (ATR) method. The data obtained by FTIR analysis need to be correlated with chromatographic methods (HPLC and/or GC) in order of the validation for the individual components determination from cosmetic formulations.

For the simultaneous determination of synthetic and natural antioxidants from anti-aging cosmetic products, there was used gas chromatography with FID and high performance chromatography with UV, after optimizing of the analysis conditions, so that good separation of the compounds of interest can be achieved. There were developed sample preparation procedures of cosmetic products, as simple and which can present a high recovery rate of the compounds.

Matrix of anti-ageing cosmetics is very complex, usually containing a high number of ingredients. 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 and also on the features of the analytical techniques employed for the determination of active substances.

Different preparation procedures of the cream samples were developed, and analysis of this samples was performed by GC coupled with FID. The most appropiate method proved to be cream sample dilution with acetonitrile:methanol organic solvent mixture by sonication, filtration and simultaneous antioxidant determination by GC.

For the determination of the natural antioxidant α-tocopherol acetate, in a proper developed cosmetic formulations (*Anti Aging Light Day Cream*), and preserved with multifunctional additives it was used a organic solvent mixture methanol:acetonitrile (50:50 v/v) succeeded by gas chromatographic separation coupled with FID. In the developed and analysed cosmetic formulation, α-TA is comprised in sunflower seed oil. The α-TA concentration claimed by the supplier is > 73,5 % α-TA in sunflower seed oil, and confirmed by the GC analysis. The method allowed a simple, rapid and accurate determination and confirmation of α-TA in the developed and analysed cosmetic product.

Simultaneous determination of BHA and α-TA in cosmetic products by the RP-HPLC-UV analysis method was performed by organic solvent mixture acetonitrile:methanol sample dilution and sonication in the first developed methodand THF:methanol dilution in the second one. Another determination method by HPLC-UV, used solid phase extraction prior to the chromatographic analysis. All determination methods by this techinque proved to be applied for the identification and quantification of natural and synthetic antioxidants from cosmetic products.

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