Determination of UV Filters In Cosmetics by HPLC

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ZADÁNÍ BAKALÁŘSKÉ PRÁCE

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Zásady pro vypracování

I. Teoretická část:
V teoretické části zpracujete literární rešerši na zadané téma.
Stručně představte nejdůležitější UV filtry používané v kosmetických přípravcích.
Věnujte se rovněž metodě vysokoúčinné kapalinové chromatografie.
Proveďte shrnutí současného stavu řešené problematiky.
II. Praktická část:
V praktické části navrhněte HPLC metodu vhodnou pro stanovení vybraných UV filtrů používaných v kosmetických přípravcích.
Proveďte optimalizaci této metody a ověřte její vhodnost pro analýzu reálného kosmetického přípravku.
Výsledky zpracujte, přehledně uspořádejte a diskutujte.

Forma zpracování bakalářské práce: **tištěná/elektronická**

Seznam doporučené literatury:

[1] NAŘÍZENÍ EVROPSKÉHO PARLAMENTU A RADY (ES) č. 1223/2009 ze dne 30. listopadu 2009 o kosmetických přípravcích.
[2] ČSN EN 16344 Kosmetika – Analýza kosmetických přípravků – Screening UV filtrů v kosmetických přípravcích a kvantitativní stanovení 10 UV filtrů metodou HPLC.
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ABSTRAKT

Táto bakalárska práca sa zameriava na vývoj RP-HPLC metódy na identifikáciu a kvantifikáciu UV filtrov používaných v kozmetických produktoch. Konkrétnejšie sa práca zaoberá vývojom optimálnej metódy na separáciu vybraných filtrov prítomných v kozmetických formuláciách. UV filtre chránia pokožku pred UV žiarením, ktoré je známe svojím škodlivým účinkom, hlavne v oslabení bariérovej funkcie kože, urýchlení procesu starnutia a značnom prispievaní k vzniku rakoviny kože. K separácii filtrov bolo vyvinutých niekoľko metód, z ktorých tri sa ukázali vhodné na analýzu komerčných kozmetických krémov na opaľovanie.

Kľúčové slová: UV žiarenie, koža, UV filtre, krémy na opaľovanie, HPLC

ABSTRACT

This Bachelor's thesis is aimed at development of RP-HPLC method for identification and quantification of UV filters used in cosmetic products. It specifically deals with finding optimal conditions for separation of particular filters present in formulations. UV filters protect skin from UV radiation which is known for its harmful effects, especially in compromising the barrier function of the skin, speeding up the ageing process and significantly contributing to the formation of skin cancer. For the separation of filters several methods had been developed, three of which were useful in analysing the commercial sunscreen products.

Keywords: UV radiation, skin, UV filters, sunscreen products, HPLC

I would like to express my sincere gratitude to my advisor doc. Ing. Věra Kašpárková, CSc. for her invaluable guidance, support, patience and advice through the whole process of making this thesis. Without her expertise and encouragement, this endeavor would not have been possible.

I hereby declare that the print version of my Bachelor's/Master’s thesis and the electronic version of my thesis deposited in the IS/STAG system are identical.

CONTENTS

INTRODUCTION

UV radiation has been proved to cause significant changes in the skin structure, having impact not only on its barrier function but also on the skin cancer formation. This has been a big concern, especially over the past few years, as the number of cases of cancer has increased, raising awareness of this topic. Therefore, there has been a significant emphasis put on the usage of sunscreen cosmetic products as a useful protection from UV radiation and its harmful effects. These products contain UV filters, compounds responsible for the photoprotection, which are strictly regulated by Legislation. Filters minimize and prevent the effects of UV radiation by scattering and reflecting UV rays in case of inorganic filters or by absorbing UV energy and converting it to heat in case of organic filters. Several UV filters are typically added to a formulation in order to ensure a broad spectrum protection and increase the efficacy of the cosmetic product [1]. Sunscreen products come to the direct contact with epidermis, therefore their safety has to be closely examined in order to protect the end users. The highest concentrations of filters allowed in cosmetic products are given by The Regulation No. 1223/2009 of the European Parliament and of the Council [2] This emphasizes the importance of developing reliable analytical methods for evaluating the properties of such products and determining the concentrations of used UV filters [1]. One of the most frequently used analytical methods to determine UV filters in cosmetic products is RP-HPLC, chromatographic technique allowing simultaneous qualitative and qualitative analysis of several UV filters in a product [3].

This thesis is aimed at developing optimal chromatographic conditions for determination of organic UV filters in commercial sunscreen products using RP-HPLC with PDA detector.

I. THEORY

1 SKIN AS A BARRIER AGAINST UV RADIATION

Skin, as the largest organ of the human body and the first barrier of defense against various kinds of external factors, possesses many indispensable functions. The barrier function of the skin is ensured by the outermost layer *stratum corneum* (SC). Its structure is composed of lipids arranged as lamellar bilayers, primarily consisting of three main groups of lipids – ceramides, free fatty acids and cholesterol. Their main function is to contribute (together with keratinocytes) to the reduction of water loss through the epidermal layer because levels off water lost through the skin play an important role in its ability to protect human´s body [4]. UV radiation is well known to be essential for the vitamin D production, but it also exhibits a number of harmful properties. These include mainly skin photoaging, damage and malignancies resulting in skin cancer. Exposure to UV radiation dramatically decreases the protective function of the skin barrier. The specific consequences of the UV radiation exposure depend on different factors and the effect on SC is still not well understood. Nevertheless, studies show that after UV radiation exposure there is an apparent increase in damage and decrease in the natural capacity of skin to resist external factors, influencing its substantial barrier function [5].

1.1 Melanocytes and Langerhans Cells

Melanocytes are dendritic cells present in human skin, specifically in *epidermis* and occasionally in small amounts in *dermis*. Their main function is to produce and transfer a pigment called melanin to keratinocytes, hence the production of skin pigmentation [6]. When exposed to UV radiation they increase production of this pigment in order to protect the skin. However, it should be taken into consideration that not only does UV radiation initiates melanin production, it also plays a vital role in senescence of melanocytes, influencing their proliferation and morphology [7]. Repetitive irradiation with UV effectively induces senescent-like characteristics in primary melanocytes, for instance expression and secretion of pro-inflammatory cytokines, growth factors, proteinases [8] and cell shape alternations together with changes in their metabolism [7]. After irradiation melanocytes show a host of gene ontology terms, including those connected to stress-induced premature senescence, such as “cell cycle arrest” and “not signaling” [8]. Senescent melanocytes furthermore contribute to the ageing process of the skin, which prohibits neighboring keratinocytes from proliferation. They also show a higher rate of autophagy,

process closely bound to synthesis and elimination of melanosomes. The malfunction of melanocytes might potentially cause pigmentary disorders [7].

One of the melanocytic disorders is an epidermal melanocyte derived tumor called melanoma. Its formation is directly linked to sunlight exposure and with its occurrence increasing rapidly, it currently has one of the highest mortality rates among different types of cancers [6]. UV radiation causes various changes in DNA in melanocytes and even though these cells are naturally capable of error repair, some of the affected cells are prone to accumulation of UV-induced mutations in their genetic information and progress further to evolve into melanoma [8].

Langerhans cells (LC) are dendritic cells present in epidermis. They are so called immunocompetent cells, and are one of the key components of the skin immune system [9] involved in immune response to allergens bound to epidermal proteins. It has been shown that UV exposure has a significant effect, both quantitative and qualitative, on the Langerhans cells, causing decrease in the number of these cells as well as alternation of their morphology which results in immunosuppression [10]. A number of studies have been done to examine the impact of UV radiation on the LCs, including exposure to sunlight as well as solar simulated UV radiation. There are two phenomena thought to be responsible for decrease in number of Langerhans cells, first of them being migration to the lymph nodes and second one being apoptosis. It is important to mention that these changes are in the majority of cases dependent on the radiation dose. A study on human skin, in which people were irradiated with UVB radiation with a dose of 1.5 MED (Minimal Erythema Dose) over a period of 10 consecutive days showed that the number of LCs lowered by 30 to 50 %. Another study carried out *in vitro* on human LCs showed that a radiation dose of 200 J/m^2 had a cytotoxic effect leading to rapid decrease in number of viable cells caused by apoptosis. Decrease in the number of LCs, as well as changes in their morphology can have a major impact on human health. If it is taken into consideration that LCs play an important role in immunological response, UV irradiation may impair immune surveillance mechanisms, such as their ability to induce elimination of cancer cells or ability to activate T-cells [9].

1.2 UV Radiation and its Impact on the Skin

Skin barrier consists primarily of corneocytes set in lipid matrix containing cholesterol, free fatty acids, ceramides and sterol/wax esters. Long-term UV exposure is proven to be harmful to the barrier function of the skin, increasing *stratum corneum* thickness as well as TEWL

(trans-epidermal water loss). It also significantly affects coherence of membrane junctions and alternates membrane protein expression. As a result, prolonged UV irradiation has a significant impact on the structure of the skin and its integrity, causing premature photoaging, pigmentation, inflammation and carcinoma. The longer the wavelength of UV, the deeper it can penetrate into the skin. Most of the effects on the skin are also cumulative and dose-dependent [11].

Cellular cohesion is vital for the proper function of protective mechanisms of the skin. The energy needed for the detachment of intracellular links between cells is called delamination energy and it decreases in direct proportion to increasing intensity of UVB radiation, regardless of location of studied skin on the body or age group. The changes in the intracellular lipid and desmosome cohesion have also been observed in the deeper parts of the skin, suggesting that UV has a consequential impact not only on the upper parts of SC, leading to increased permeability and weakened integrity, but also leads to inflammatory processes in the skin [5] [12].

Influence on the adherent junctions in *stratum granulosum* layer is observably dose-dependent, disruption being connected primarily to increasing and cumulative exposure to UV radiation. With higher doses, however, the skin enters a so-called reparative state, trying to solve the changes in the barrier. Hematoxylin and eosin staining of skin explants later treated with UV radiation of different intensities have shown that cells in *stratum granulosum* exhibit localized cell death. This study had been carried out on an *ex vivo* human skin irradiated by a solar simulator equipped with a filter that allowed a balanced ratio of 4 % UVB and 96 % UVA. Such model has been chosen because the UVA/UVB ratio provided physiologically relevant UV energies. Daily exposure to UV radiation was represented by energy of 20 J/cm^2 applied in five consecutive doses. Extremely high UV dose (100 J/cm^2) was used to demonstrate epidermal cell death. Exposure conditions play an essential role here, repetitive exposure to smaller doses of UV shows great number of apoptotic cells, confirming the previously mentioned fact that the effects of irradiation are of a cumulative character [11].

2 UV FILTERS

2.1 Types of UV Filters and the Mechanism of their Function

UV filters or sunscreens should minimize the harmful effects of ultraviolet radiation on the skin. Based on the mechanism of protection, UV filters can be classified into two main categories – organic and inorganic [1].

Organic or chemical sunscreens absorb UV radiation, resulting in excitation to a higher energy state. They are further subdivided into either UVA or UVB filters. Only a few organic filters offer protection against both types of UV radiation. Moreover, in majority of sunscreen products chemical filters need to be combined because none of them provide high enough broad-spectrum protection at the currently allowed concentration levels [13].

Inorganic (particle-based) UV filters function on the principle of reflection and scattering. Therefore, they are also called physical UV filters. These include for example titanium dioxide and zinc oxide. Both are also allowed to be used in cosmetic products in form of nanoparticles. The maximum concentration of both ZnO and TiO_2 currently allowed in the nano form is of 25 % as given by European legislation [2]. In addition, two more UV filters in nano form are currently allowed by the Regulation, namely Methylene bis-benzotriazolyl tetramethyl butylphenol (nano) and Tris-biphenyl triazine (nano).

2.2 Regulation 1223/2009 on Cosmetic Products

The Regulation No. 1223/2009 of the European Parliament and of the Council provides collective rules about cosmetic products available on the European market. It is superior to individual national laws, which prevents individual member states of European Union from adapting regulations according to their needs [2].

The Regulation No. 1223/2009 of the European Parliament and of the Council defines UV filters as "*substances which are exclusively or mainly intended to protect the skin against certain UV radiation by absorbing, reflecting or scattering UV radiation. This clearly defines the main function of UV filters.*" The Annex VI of this regulation provides the list of all UV filters allowed in cosmetic products as well as the maximum concentration for use in formulations [2].

2.3 Frequently Used UV Filters

As mentioned above, the allowed UV filters are listed in the Annex VI of The Regulation No. 1223/2009 of the European Parliament and of the Council. Recently, Jesus et al. published review paper identifying UV filters most frequently used in commercial cosmetic products intended for used in cosmetics for both adults and children. They analyzed 444 sunscreen formulations and found out that Avobenzone (BDM), Octocrylene (OCR) and Bis-ethylhexyloxylphenol methoxyphenyl triazine (EMT) are currently the most frequently used filters (> 40 %). Among other frequently used filters the authors included Ethylhexyl triazone (ET), Ethylhexyl salicylate (ES) or Ethylhexyl methoxycinnamate (EHMC). They also observed increase in application of TiO_2 usage. Here, some of the most common ones are described [14].

2.3.1 Avobenzone (BDM)

Avobenzone, also known under its chemical name [4-(1,1-dimethylethyl)phenyl]-3-(4-methoxyphenyl)propane-1,3-dione (INCI Butyl methoxydibenzoylmethane), is one of the most common chemical UV filters used in sunscreens. It shows higher rates of photodegradation and its photostability distinctly depends on the solvent used in the specific formulation, with polarity having the greatest impact. Its photodegradation products, such as arylglyoxals and benzyls may potentially be the cause of photoallergic and cytotoxic reactions [15]. The highest allowed concentration of avobenzone in cosmetic products is 5 % [2].

2.3.2 Oxybenzone (BP3)

Oxybenzone, also known as 2-hydroxy-4-methoxybenzophenone, is used as a short-wave absorber in sunscreen products. In smaller concentrations it is also used as a photo-stabilizer which minimizes colour and odour. It is also known for its allergenic properties, specifically, it’s been proven to induce contact allergy, photo-contact allergy and contact urticaria reactions in humans. Its efficacy is dependent on how the specific formulation has been formulated where in lower concentrations it demonstrates little absorption through the skin. One of the highly discussed and of course not very welcome effects are connected to its major impact on coral reefs due to its ability to cause coral bleaching, inhibit reproduction of corals and cause their demise [16]. The Regulation No. 1223/2009 of the European Parliament and of the Council allows its use in cosmetic products in three concentrations

based on the type of the cosmetic product containing Oxybenzone. In face products, hand and lip products its concentration levels cannot exceed 5,5 % when used as a UV filter. In body products, if used as an UV filter, its concentration cannot exceed 1,7 % and in other products or when used to protect product formulation its concentration cannot exceed 0,5 % [2].

2.3.3 Octinoxate (EHMC)

Octinoxate, also known by its chemical name 2-ethylhexyl-4-trimethoxycinnamate, is currently approved for use in cosmetic products at the highest concentration 10 % [2]. It belongs among commonly used UV filters in sunscreen products and belongs to the group of organic UVB filters. Concerning cosmetic formulations, it can often be combined with nanoparticles or water-resistant liposomes to decrease the risk of percutaneous absorption [17].

2.3.4 Enzacamene (4MBC)

Enzacamene, also known under its chemical name 4-methylbenzilidene camphor, is one of the most commonly used chemical UV filters used in cosmetic products. This camphor derivate is mostly effective in the absorption of UV-B radiation. Currently it has been identified as an endocrine disruptor affecting growth and reproduction of aquatic organisms making it a concerning threat for the safety of the environment [18]. Currently allowed concentration in cosmetic products cannot exceed 4 % [2].

2.3.5 Octisalate (ES)

Octisalate, also known as 2-ethylhexyl salicylate, is another commonly used UV filter, providing protection against UVB radiation. Due to its lower effectiveness, higher doses are required in formulations [19]. The Regulation No. 1223/2009 of the European Parliament and of the Council states that its concentration level of 10 % cannot be exceeded [2].

2.3.6 Ethylhexyl triazone (ET)

Ethylhexyl triazone is a strong UVB absorber which not only provides effective protection but also shows very good photostability. It is frequently used in sunscreen products for children [14]. Maximum concentration allowed to be used in ready to use formulation is 5 % [2].

2.3.7 Octocrylene (OCR)

Octocrylene, also known as 2-Cyano-3,3-diphenylacrylic acid, 2-ethylhexyl ester, is a filter frequently used in cosmetic products absorbing UVA and UVB radiation respectively. According to the article written by Ana Jesus et al. concerning recent trends on UV filters, octocrylene is one of the three most used chemical UV filters used in sunscreen products. In terms of photostability, this compound is able to react and transform into a phototoxic benzophenone derivative. Its properties, however, include a so-called dual activity, which means that it not only provides UV protection but also acts as a photo-stabilizer for other filters, such as avobenzone, in combination with which it improves photoprotective abilities of the final product [14]. Octocrylene is allowed to be used in cosmetics up to 9 % concentration in propellant sprays and up to 10 % in other products [2].

2.3.8 Titanium oxide

Titanium oxide is a physical UV-filter working on a principle of reflecting and scattering UV radiation. Its use as an inorganic physical sunscreen has recently gained popularity, due to the fact that it is allowed to be incorporated into cosmetic formulations in the form of nanoparticles. The nanoparticles with sizes between 1 nm and 100 nm are of transparent appearance, which is a favourable attribute when it comes to cosmetic formulations. It is also said to prevent skin irritation, which could be beneficial especially for people with sensitive skin [20]. The Regulation No. 1223/2009 of the European Parliament and of the Council allows its use in cosmetic products both in micro and nano particles, specifically stating that it can be used *"in powder form containing 1 % or more of particles with aerodynamic diameter ≤ 10 μm"*, which applies specifically to the particles not falling into the nano category. Nanoparticles can be used in the maximum concentration of 25 % in ready for use preparation and nanomaterials have to be of ≥ 99 % purity and are required to be photostable in the final formulation [2].

2.3.9 Zinc Oxide

Zinc oxide is another inorganic physical UV filters possessing similar properties as TiO_2. The Regulation No. 1223/2009 states that its maximum concentration in ready for use preparation cannot exceed 25 % and it is not allowed *"to be used in applications that may lead to exposure of the end user's lungs by inhalation"*. Usage of nanoparticles has to follow

special requirements, regarding for example purity, which cannot be lower than 96 % and water solubility, which must be lower than 50 mg/l [2].

It should also be noted that the impact of nanoparticles on human health is not fully understood, and as a result, there are currently no clear regulations in place among international authorities concerning their usage [20].

3 METHODS FOR DETERMINATION OF UV FILTERS

The content of UV filters in cosmetic products determines the efficacy of the final formulations. This means that analytical control of sunscreens present in formulations is necessary. The concentration levels of UV filters cannot exceed the limits allowed by legislation in order to ensure safety of the end users. All of the above-mentioned reasons highlight the necessity to thoroughly analyse products containing UV filters. There are many methods applicable for their determination, some of them more preferred than other. Below the most frequently used methods are briefly characterized.

3.1 UV-Vis Spectroscopy

UV-Vis spectroscopy is an analytical technique used to observe the absorbance of energy or electromagnetic radiation, which is able to excite electrons to the first singlet excited state of the compound or material, providing both qualitative and quantitative information about a given compound or molecule The principle can be easily explained using The Beer-Lambert Law (Equation 1) [21].

$$A = \varepsilon c l \tag{1}$$

where

A – absorbance [-]

l – optical path length (thickness of the cuvette) [cm]

c – molar concentration of the compound [$mol \cdot dm^{-3}$]

ε – molar absorptivity of the compound [$mol^{-3} \cdot dm^{-3} \cdot cm^{-1}$]

In the determination of UV filters, spectroscopic methods are used either as stand-alone methods or for detection of UV filters after they have been separated by other analytical methods, such as HPLC [22]. A calibration curve showing relation between absorbance and concentration of a solution, obtained using UV-Vis Spectroscopy is crucial in the determination of concentration of UV filters, used for example in final formulations of cosmetic products [22].

3.2 High Performance Liquid Chromatography

High Performance Liquid Chromatography is an analytical method used to analyse individual components of a studied sample. It is one of the most used chromatographic techniques used for both qualitative and quantitative determination of UV filters in cosmetic products. The principle of the method is separation of the sample components, which move with the mobile phase within the chromatographic column. The separation is based on their interaction with the stationary phase followed by the determination of quantity or concentration based on the area of retention peak [23]. Method preferred for separation of UV filters is reversed-phase chromatography, which means that there is a octadecyl-modified silica gel column used as a stationary non-polar phase, and a polar mobile phase, such as methanol or acetonitrile. Regarding the polarity of mobile and stationary phase, a much less used procedure is on normal-phase column like polar silica gel. In such case solvents like hexane, ethyl acetate or acetone are used. The temperature in the column oven has a direct impact on the retention time of some UV filters and therefore their separation, thus it can be helpful in the improvement of the resolution between coeluting compounds [22]. The results of chromatographic separation are highly dependent on the optimal experimental conditions, such as the composition of mobile phase, the type of stationary phase, temperature in the oven, flow rate of the mobile phase and other factors.

3.3 Thin Layer Chromatography (TLC)

Thin layer chromatography is a method used to separate individual compounds based on their affinity to polar stationary phase. It enables detection of substances with very high affinity to the stationary phase. Mobile phase is a non-polar solvent mixture [23]. TLC was previously mostly used for isolation and identification purposes, after scraping the spots from the plate followed by measuring IR and UV spectra and quantification using gravimetric and photometric methods [22].

3.4 Gas chromatography

Gas chromatography is an analytical method not often used to determine UV filters in cosmetic products due to the fact that most of these compounds have high boiling points, hence they do not meet the standard criteria such as volatilisation and thermostability required when choosing this type of method. However, there are studies, which describe application of gas chromatography for identification and quantification of ionizable UV

filters. In such cases, gas chromatography can be used together with mass spectroscopy detector or flame ionization detector [22].

3.5 Electrochemical techniques

Electrochemical techniques are not typical for UV filter determination, nevertheless, they can be applied for instance to determine and quantify BDM, BP3, EMC or ES in cosmetics applying voltammetry by using glassy carbon and mercury film electrodes or epoxy-carbon composite electrodes [22].

4 CURRENT STATE OF THE TOPIC BEING SOLVED

Analysis of cosmetic products containing UV filters is required to determine whether the concentrations comply with currently allowed concentration limits given by legislation (Regulation 1233/2009), which is closely related to safety of the end user and effectiveness of the formulations. There are currently two international standards with analytical methods established for determination of UV filters. The first *EN 16344 Cosmetics – Analysis of cosmetic products – Screening for UV-filters in cosmetic products and quantitative determination of 10 UV-filters by HPLC* and the second *EN 17156 Cosmetics. Analytical methods. LC/UV method for the identification and quantitative determination in cosmetic products of the 22 organic UV filters in use in the EU*.

One of the most frequently used methods is liquid chromatography, which allows direct analysis of matrices as well as target analytes. The type of cosmetic product aimed for analysis as well as the target analytes determine sample preparation process; however, the preparation doesn't always require a complex sample pre-treatment since the concentration of UV filter is usually sufficiently high enough [24].

The most frequently used techniques for analysis of UV filters in cosmetic products include liquid chromatography-UV/Vis (LC-UV/Vis), high-performance liquid chromatography-UV/Vis (HPLC/UV-Vis) and micellar electrokinetic capillary chromatography-UV/Vis (MEKC-UV/Vis). Parameters of the aforementioned techniques are summarised below.

- HPLC-UV/Vis: The matrices used for analysis by this method can include emulsions, creams, lotions, foundations, lipsticks etc, detecting a wide variety of organic UV filters such as EMC, MBC, ET, BDM, OCR, BP3 and other. The column temperature ranges between 30 °C and 40 °C and the mobile phase is in all of the cases listed in the study set to gradient elution [24].
- LC-UV/Vis: This method can be used to analyse sunscreens, facial creams, lip balms, emulsions, etc. containing a variety of chemical filters such as OCR, ES, BP3, EMC, etc. Using a C18 column at the temperature as high as 60 °C and mobile phase consisting of ethanol/formic acid (aq.) the recovery rate can be as high as 104 % [24].
- MEKC-UV/Vis: This method can be used to analyse products like shampoos, gels, perfumes, or creams using uncoated silica-fused capillary and a surfactant (sodium dodecyl sulphate) with recovery rate up to 107,4 % [24].

Experimental conditions for determination of UV filters by HPLC depend on the types of compounds to be determined. Commonly available sunscreen products usually contain only a couple of UV filters, however, when developing specific conditions of analysis, researchers aim to find such methods which can be used to determine a broader spectrum of compounds in one mixture, so that the applicability is not strictly subjected to a very small sample of filters.

A detailed overview of the analytical methods used to determine organic UV filters in cosmetic products is provided in scientific literature, for example a review article by Narloch and Wejnerowska [24]. This paper lists different analytical techniques performed to determine concentration of UV filters as well as it shows the treatment of the samples with different matrices. Based on the given data, the analysis of completely dissolved samples reached high recoveries between 80 and 113 % suggesting that the techniques used were effective and therefore provide reliable data concerning successful separation and analysis of studied compounds.

Dencausse, J.L. et al [3] published scientific paper aimed to determine the optimal experimental conditions when using the HPLC method for quantitative determination of sunscreens in high-protection cosmetic products. In this study, the selectivity, response function, precision, linearity and accuracy of the method have been observed through the comparison of results obtained in three different types of samples; 1) standard solutions without matrix, 2) simulated matrix standard solutions and 3) blank matrix. The data were collected in 3D between 210 and 400 nm, injection volume was 10 µl, flow rate was set to 1 $ml \cdot min^{-1}$, the samples were eluted with a specific gradient profile of mobile phase consisting of acetonitrile, tetrahydrofuran and acetic acid. Because Avobenzone (BDM) and Ethylhexyl methoxycinnamate (EHMC) were partially co-eluted, tetrahydrofuran has been added as an organic modifier together with acetic acid, which lead to better separation results. The peaks obtained during the separation were symmetrical and well resolved at the wavelength 330 nm. All studied sunscreens have been eluted within 30 minutes [3].

De Orsi D. et al [25] developed determination procedure for eleven UV filters by HPLC, however, in order to reach baseline separation of all the filters, three different methods had to be used in this study, based on the specific properties of the filters included in the experiment. The first chromatographic method was carried out under following conditions:

mobile phase acetonitrile/water in the ratio 10:90 followed by linear gradient elution up to 90 % acetonitrile in 30 minutes and after that returning to the initial mobile phase. Flow rate was 1 $ml \cdot min^{-1}$, injection volume was 10 µl and column temperature 35 °C. UV filters analysed using this procedure were for example Phenylbenzylimidazole sulphonic acid, Benzophenone-4, Benzophenone-3, 4-Methylbenzyliden camphor, Diethylamino-hydroxybenzoyl-hexylbenzoate, Ethylhexyl methoxycinnamate and Octocrylene. Second chromatographic method was carried out as follows: mobile phase was set to isocratic elution with methanol/acetonitrile 1:1, flow rate was 1 $ml \cdot min^{-1}$, injection volume 10 µl and column temperature 35 °C. UV filters determined using this procedure were Methylene bis-benzotryzolyl tetramethylbutylphenol, Bis-ethylhexyloxyphenol methoxyphenyl triazine, Ethyhexyl triazine. The third method was carried out as follows: mobile phase was set to isocratic elution methanol/water 65:35, flow rate 2 $ml \cdot min^{-1}$, injection volume 10 µl and column temperature 35 °C. Under these conditions, UV filter Butyl methoxydibenzoyl-methane was quantified. The detector used in this study was DAD. The efficiency and accuracy of the method were tested using three different types of cosmetic formulations, and the results obtained were consistent with the real contents of filters in the formulations, suggesting that the proposed methods are effective [25].

Kicheol Kim et al. [26] developed and evaluated a HPLC method for analysis of nine common UV filters together with parabens. The main aim of this study was to provide method sufficiently robust and simple to allow analysis of sun-care products in accordance with the existing legislation. The conditions of the analysis were set as follows: mobile phase acetonitrile/water at gradient elution, flow rate 0,9 $ml \cdot min^{-1}$ and temperature 30 °C. In addition, 0,5 % acetic acid was added to acetonitrile/water mobile phase to prevent ionization of parabens, which were analysed simultaneously with UV filters. The results of the analysis showed resolution factors above 1,8 proving that these conditions are favourable for a quantitative analysis. The recovery tests of the proposed method were performed on eight UV filters and the results raged between 98 and 102 %. Finally, 101 sun-care products containing UV filters were analysed using this method and in each one of them at least one filter was quantified. Therefore, it can be concluded that method proposed by Kicheol Kim et al. is indeed efficient and suitable for quality control of commercial skincare products [26].

5 AIM OF THE THESIS

The aim of this thesis is to develop method for determining UV filters in commercial cosmetic products. Theoretical part discusses the importance of using sunscreen products based on the proven influence of UV radiation on the skin and the health risks connected to its exposure, as well as the current trends in UV filter analysis. The objective of the experimental part was to develop an HPLC method for identification and quantification of UV filters and to verify whether it is suitable for cosmetic product analysis.

II. EXPERIMENTAL PART

6 MATERIALS AND METHODS

6.1 Instruments and devices

The HPLC system (Shimadzu) consisting of:

- Pump LC-20AD
- Mobile phase degasser DGU-20A5R
- Autosampler SIL-20A HT
- Oven CTO-20AC
- PDA diode array detector
- Program LabSolutions
- Columns
    - XSelect CSH C18 5 µm – hybrid particles with end-capped C18 and Phenyl-Hexyl (Waters)
    - Kinetex C18 5 µm – CoreShell technology (Phenomenex)
- Sonication bath (Kraintek)
- Analytical balances (Adam Equipment)
- Syringes and syringe needles
- Syringe filters (0,45 µm, 0,22 µm, Millipore)
- Laboratory glassware, pipettes, vials and other common laboratory equipment were also used

6.2 Chemicals and samples

6.2.1 Chemicals

- Acetonitrile for HPLC (Fisher Scientific UK)
- Ethanol for HPLC (Penta)
- Acetic acid for HPLC (≥99,98%) 1 % (Lukeš)
- Demineralized water

6.2.2 UV filters

Following standards of UV filters were used in the thesis (Table 1).

Table 1: UV filters used in the thesis

CAS	Name	INCI	Supplier
70356-09-1	Avobenzone (BMD)	Butyl methoxydibenzoylmethane	Syncare , s.r.o
131-57-7	Oxybenzone (BP3)	Benzophenone-3	Sigma-Aldrich
131-56-6	Benzophenone-1 (BP1)	Benzophenone-1	Sigma-Aldrich
5466-77-3	Octinoxate (EHMC)	Ethylhexyl methoxycinnamate	SynCare, s.r.o
187393-00-6	Bemotrizinol (EMT)	Bis-ethylhexyloxyphenol methoxyphenyl triazine	SynCare, s.r.o
118-60-5	Octisalate (ES)	Ethylhexyl salicylate	Sigma-Aldrich
88122-99-0	Ethylhexyl Triazone (ET)	Ethylhexyl triazone	SynCare, s.r.o
38102-62-4/ 36861-47-9	Enzacamene (4MBC)	4-methylbenzylidene camphor	Sigma-Aldrich
103597-45-1	Bisoctrizol (MBT)	Methylene bis-benzotriazolyl tetramethylbutylphenol	Sigma-Aldrich
21245-02-3	Padimate-O	Ethylhexyl dimethyl PABA	Sigma-Aldrich
6197-30-4	Octocrylene (OCR)	Octocrylene	Sigma-Aldrich
1843-05-6	Benzophenone-12 (BP-12)	Benzophenone-12	Sigma-Aldrich

6.2.3 Samples

Commercially available creams listed in the Table 2 were used in the thesis.

Table 2: Cosmetic products

Cosmetic Product (Manufacturer)	UV filters (INCI)	Photo
Product 1 SunDance SPORT Sonnencreme - Gel SPF 30 (dm-drogerie markt)	Octocrylene, Ethylhexyl salicylate, Titanium dioxide (nano), Butyl methoxydibenzoylmethane	
Product 2 Garnier Ambre Solaire Hydra 24H Protect SPF 20 (Garnier)	Ethylhexyl salicylate, Ethylhexyl triazone, Butyl methoxydibenzoylmethane, Bis-ethylhexyloxyphenol methoxyphenyl triazine	
Product 3 Nubian Milk for Sunbathing SPF 6 (Herba Drug, s.r.o.)	Octocrylene, Ethylhexyl salicylate, Butyl methoxydibenzoylmethane	
Product 4 Aloha Milk for Sunbathing SPF 20 (VIVACO s.r.o.)	Octocrylene, Butyl methoxydibenzoylmethane	
Product 5 Garnier Ambre Solaire Sensitive Advanced SPF 50+ (Garnier)	Ethylhexyl salicylate, Bis-ethylhexyloxyphenol methoxyphenyl triazine, Titanium dioxide (nano), Butyl methoxydibenzoylmethane, Drometrizole trisiloxane, Diethylhexyl butamido triazone	

6.3 Preparation of standard solutions

6.3.1 Stock solutions of UV filters

For the purposes of measurement, stock solutions of standards were prepared as follows: 0,05 g of each of the UV filters was accurately weighed and dissolved in 10 ml ethanol in volumetric flask to concentration of 5 mg/ml.

6.3.2 Calibration Solutions

Standard solutions of UV filters for calibration were prepared by diluting stock solution to concentrations ranging from 2 to 0,1 mg/ml. Five calibration standards were prepared. This concentration range was used for each of the UV filters analyzed (contained in analyzed sunscreen products).

6.3.3 Sample Solutions of Sunscreen Products

0,1 g of each of commercial sunscreen product was accurately weighted and dissolved in 10 ml of ethanol for HPLC in volumetric flask. After dissolving, solutions were sonicated for approximately 10 minutes and filtered through syringe filters, firstly through filter with 0,45 μm pore size and afterwards through syringe filter with 0,22 μm pore size (both Millipore). Sunscreen formulations usually contain high concentrations of individual UV filters, therefore some of the samples were diluted. Dilutions used together with mass of the samples used for analysis are listed in Table 3.

Table 3: Mass of the sunscreen products (m) used and dilution of sunscreen solutions

Sample	m [g]	Dilution factor
Product 1	0,1044	6
Product 2	0,1013	Not diluted
Product 3	0,1026	Not diluted
Product 4	0,1025	3

6.4 Methods

During the development of chromatographic method, several variables were tested. Methods used for chromatographic analysis of sunscreens in this thesis can be divided into two main categories based on the column used. Conditions applied in each of the respective method are listed in the Tables below. In all methods PDA detector and injection volume of 10 µl were used.

6.4.1 Methods using XSelect column: I – IV.

The methods are listed in Tables 4 to 7.

Table 4: Chromatographic conditions – method I

Type of elution	Mobile Phase	Type of Column	Flow Rate [ml/min]	Temperature [° C]
isocratic	acetontrile, acetic acid (90:10)	XSelect CSH C18	0,8	35

Table 5: Chromatographic conditions – method II

Type of elution	Mobile Phase	Type of Column	Flow Rate [ml/min]	Temperature [° C]
isocratic	acetontrile, acetic acid (90:10)	XSelect CSH C18	0,8	45

Table 6: Chromatographic conditions – method III

Type of elution	Mobile Phase	Type of Column	Flow Rate [ml/min]	Temperature [° C]
isocratic	ethanol, acetic acid (85:15)	XSelect CSH C18	0,8	45

Table 7: Chromatographic conditions – method IV

Type of elution	Mobile Phase	Type of Column	Flow Rate [ml/min]	Temperature [° C]
isocratic	ethanol, acetic acid (75:25)	XSelect CSH C18	0,8	45

6.4.2 Methods using Kinetex column: V - IX.

The methods are listed in Tables 8 to 14.

Table 8: Chromatographic conditions – method V

Type of elution	Mobile Phase	Type of Column	Flow Rate [ml/min]	Temperature [° C]
isocratic	ethanol, acetic acid (80:20)	Kinetex C18	0,7	35

Table 9: Chromatographic conditions – method VI

Type of elution	Mobile Phase	Type of Column	Flow Rate [ml/min]	Temperature [° C]
isocratic	ethanol, acetic acid (75:25)	Kinetex C18	0,5	30

Table 10: Chromatographic conditions – method VII (gradient Table 11)

Type of elution	Mobile Phase	Type of Column	Flow Rate [ml/min]	Temperature [° C]
gradient	ethanol, acetic acid	Kinetex C18	0,5	30

Table 11: Method VII gradient elution conditions

Time [min]	Acetic acid concentration [%]
7	30
12	50
17	0

Table 12: Chromatographic conditions – method VIII

Type of elution	Mobile Phase	Type of Column	Flow Rate [ml/min]	Temperature [° C]
isocratic	ethanol, acetic acid (95:5)	Kinetex 18	0,8	35

Table 13: Chromatographic conditions – method IX (gradient Table 14)

Type of elution	Mobile Phase	Type of Column	Flow Rate [ml/min]	Temperature [° C]
gradient	ethanol, acetic acid	Kinetex C18	0,8	35

Table 14: Method IX gradient elution conditions

Time [min]	Acetic acid concentration [%]
3	15
5	0

7 RESULTS AND DISCUSSION

7.1 Determination of maximum wavelength

Each one of the UV filters shows a specific wavelength value λ_{max} at which the absorbance in UV spectrum is the highest. The wavelength of maximum absorbance is crucial in spectrophotometry because it tells the wavelength at which the sample absorbs the most radiation. This will enable determination of the maximum concentration of the UV filter in the real sample. The wavelength range of interest in this analysis was 250 - 400 nm recorded by a PDA detector. This detector allows measuring absorbance in the entire wavelength range in real time. The λ_{max} values for studied UV filter extracted from spectrum are listed in the Table 15 and examples of spectra are shown in the Figures 1 to 4.

Table 15: Values of λ_{max} for analysed UV filters determined from UV-Vis spectra recorded by PDA detector

UV filter	**λ_{max} [nm]**
BP1	288
BP3	287
MBC	298
OCR	303
EHMC	308
BDM	**357**
PDO	309
ES	305
BP12	289
ET	**313**
EMT	**341**
BMT	310

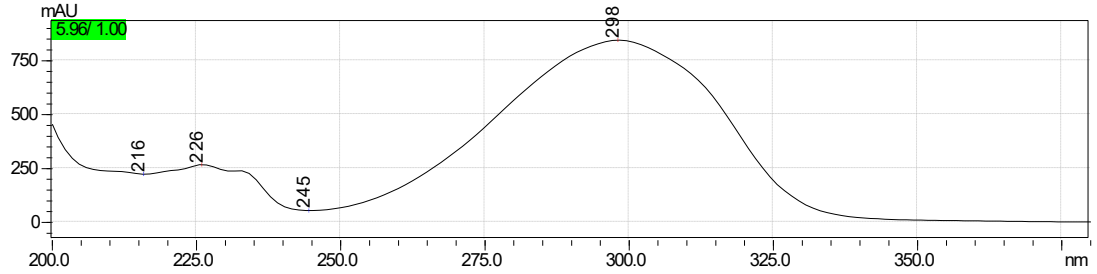

Figure 1: Absorption spectrum of MBC

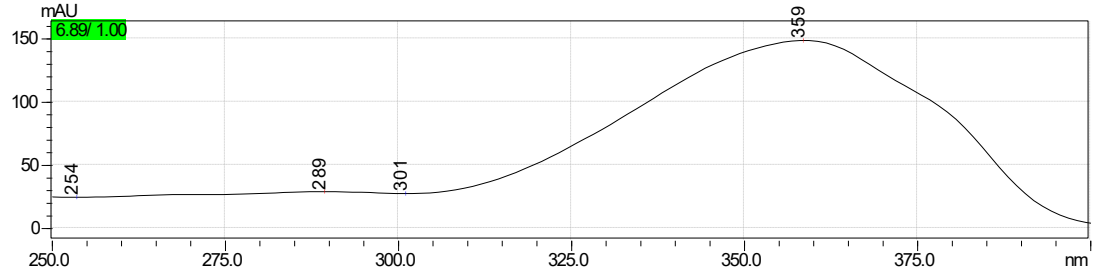

Figure 2: Absorption spectrum of BDM

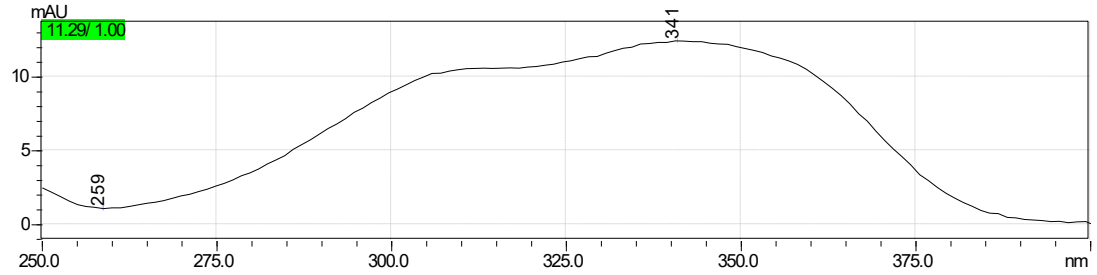

Figure 3: Absorption spectrum of EMT

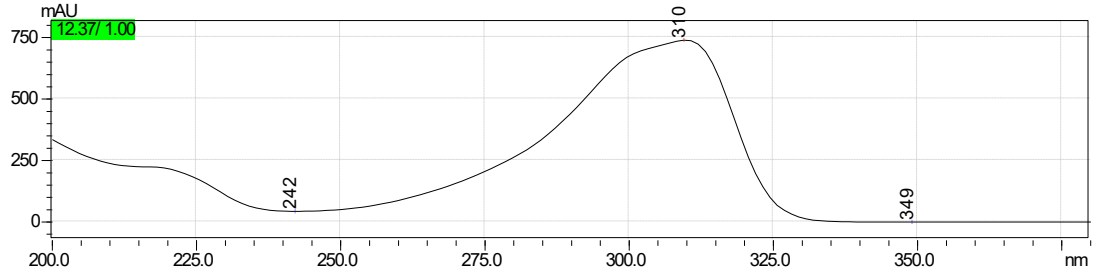

Figure 4: Absorption spectrum of MBT

The data summarized in the Table 15 illustrate that most of the UV filters studied in the thesis exhibit λ_{max} at UVB range (280-315 nm). Here, MBC (Figure 1) can be given as an example. At UVA range (315-400 nm), only BDM (λ_{max} = 357 nm, Figure 2) and EMT (λ_{max} = 341 nm) are included. Spectrum of EMT, which is shown in Figure 3 also proves that this sunscreen exhibits absorbance at a broad spectrum of wavelengths. The Standard EN 16344 [27] recommends using the range of λ_{ma} 220 – 420 nm for HPLC analysis of

sunscreens. Filters studied in this thesis absorb approximately in the range 287 – 313 nm. When comparing the data here presented with data from the literature, the values of λ_{max} can slightly differ. For instance, Dondi et al. state the absorption maximum of Avobenzone (BDM) at 360 nm whereas in this thesis the maximum was stated at 356 nm [28]. Rai, et al. [29] report absorption of Octocrylene at 303 nm and Octisalate at 307 nm, which almost corresponds with findings in this thesis. The reason is that the maximum absorbance can be influenced by the composition of solvent (mobile phase) used in analysis. Based on the results summarized in this chapter, λ_{max} values of 300 nm were chosen in HPLC analysis.

7.2 HPLC screening of UV Filters

In this part, the main objective of the work was to develop a suitable method for identification of UV filters. Filters available at the Department of Fat, Surfactant and Cosmetic Technology were studied. According to A. Jesus et al. [14], these include also the filters most used in cosmetic products

The following chromatographic variables were tested during the method development:

- Column type
    - XSelect CSH C18 5 μm
    - Kinetex C18 5 μm
- Composition of mobile phase
- Column temperature
- Flow rate

The samples of UV filters were analysed in three different mixtures (Mix A, B, C), compositions of which are specified in Table 16.

Table 16: Composition of individual mixtures of UV filter used for HPLC method development

Sample	UV filters
Mix A	BP1, BP3, MBC, OCR, EHMC
Mix B	BDM, PDO, ES, BP12
Mix C	BP1, BP3, MBC, OCR, EHMC, BDM, PDO, ES, BP12

Firstly, mix A and mix B were analysed using isocratic methods (Method I and Method II), which are described in detail in chapter 6.4 (Tables 4 and 5). Both methods used XSelect column with mobile phase consisting of acetonitrile (ACN) and 1% aqueous acetic acid (AA) in the ratio ACN:AA 90:10. The methods only differed in oven temperature. In Method I oven temperature was set to 35 °C and in Method II to 45 °C. Based on the retention times of the analysed UV filters (Table 17), it can be concluded that the increase in temperature by 10 °C shortened the time of elution and therefore made the process more effective.

Table 17: Retention times of UV filters in Mix A and Mix B recorded with X-Select column, mobile phase ACN:AA 90:10 at two different temperatures

		Retention time [min]	
Sample	**UV filter**	**Method I** **Temperature 35 °C**	**Method II** **Temperature 45 °C**
Mix A	BP1	3,97	3,82
	BP3	4,62	4,38
	MBC	6,50	5,94
	OCR	7,61	6,71
	EHMC	8,32	7,26
Mix B	BDM	7,79	6,95
	PDO	8,50	7,51
	ES	9,37	8,23
	BP12	12,61	10,57

Figure 5 shows the chromatogram obtained from the analysis of mix A using Method II. As the baseline separation indicates, this method was suitable for separation of five UV filters present in Mix A. Figure 6 shows the chromatogram obtained from the analysis of Mix B using method II. In this case, the separation was also successful.

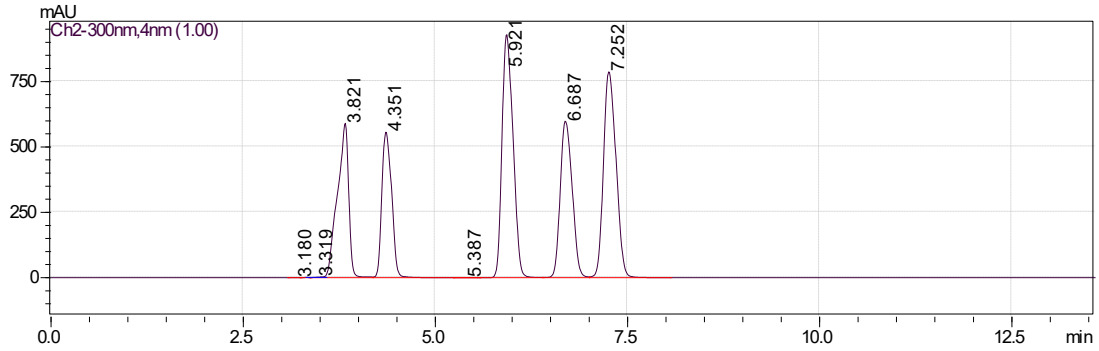

Figure 5: HPLC chromatogram of Mix A (BP1, BP3, MBC, OCR and EHMC) recorded with X-Select column, mobile phase ACN:AA 90:10 at 45 °C.

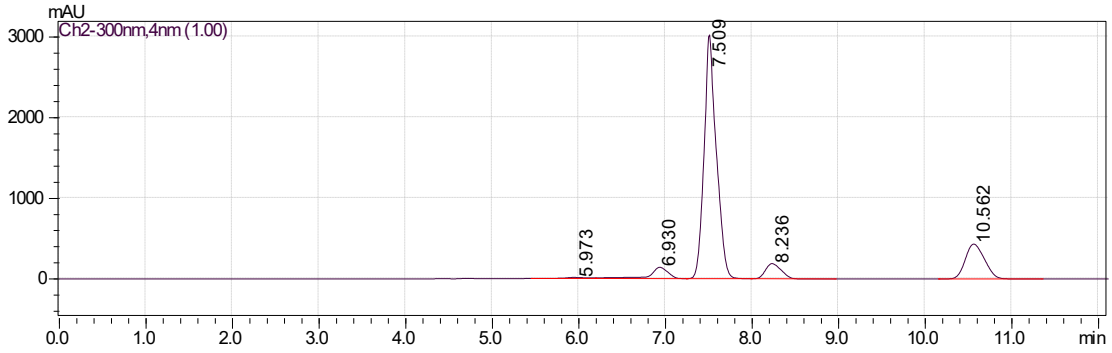

Figure 6: HPLC chromatogram of Mix B (BDM, PDO, ES, BP12) recorded with X-Select column, mobile phase ACN:AA 90:10 at 45 °C

After the successful separation of filters in Mix A and Mix B, Mix C was prepared combining filters from Mixes A and B. Therefore, Mix C contained a total number of nine filters. Firstly, the analysis was carried out using Method II, however, the separation was poor and some peaks were hardly seen. Therefore, Method III was developed. In this method, ethanol (EtOH) was used instead of acetonitrile in ratio EtOH:AA 85:15. Conditions of this method are described in detail in part 6.4 Table 6. Chromatogram from the analysis of Mix C using Method III is shown in Figure 7.

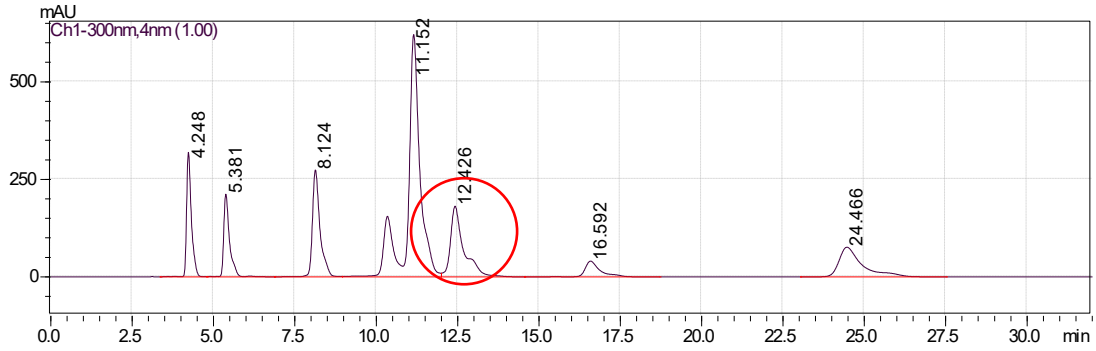

Figure 7: HPLC chromatogram of Mix C (BP1, BP3, MBC, OCR, EHMC, BDM, PDO, ES, BP12) recorded with X-Select column, mobile phase EtOH:AA 85:15 at 45 °C

Separation was still relatively poor, around retention time of 12 minutes, where 6th and 7th peak (circle in Figure 7) were not separated and BDM is visible only as a shoulder on the peak with Rt = 12.425 min. Therefore, Method IV (see Table 7) was introduced. Ratio of ethanol to acetic acid was changed to 75:25, which ensured a better separation as it is shown in Figure 8. However, peaks MBC, BDM and OCR were not base-line separated.

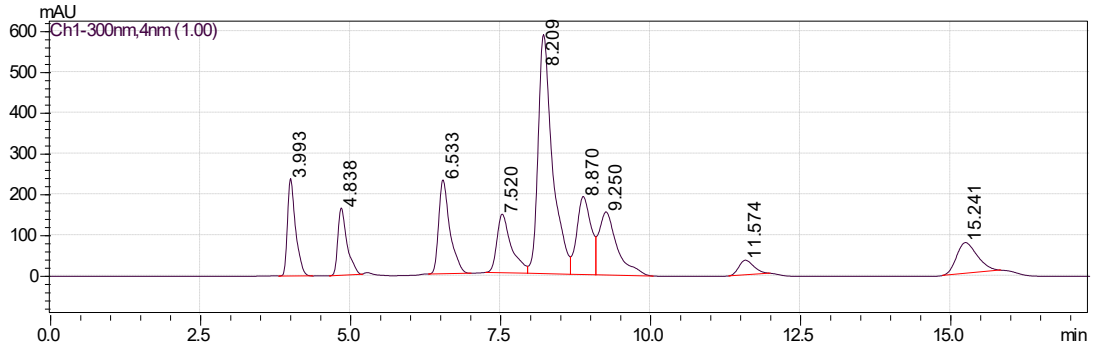

Figure 8: HPLC chromatogram of Mix C (BP1, BP3, MBC, OCR, EHMC, BDM, PDO, ES, BP12) recorded with X-Select column, mobile phase ACN:AA 75:25 at 45 °C

Afterwards, a new type of column was introduced into the analysis. Instead of XSelect column, Kinetex column was used. First method developed using the new column was Method V (EtOH:AA 80:20) which, however, did not provide base-line separation, but later was useful in analysis of cosmetic products, which did not contain as many as filters an in Mix C. The analysis of real samples will be discussed in detail later in this thesis.

Second method using Kinetex column was Method VI described in detail in Table 9. Mobile phase consisted of ethanol and acetic acid in ratio 75:25 and flow rate was lowered to 0,5 ml·min^{-1}. Chromatogram obtained from analysis of Mix C using this method is shown in Figure 9.

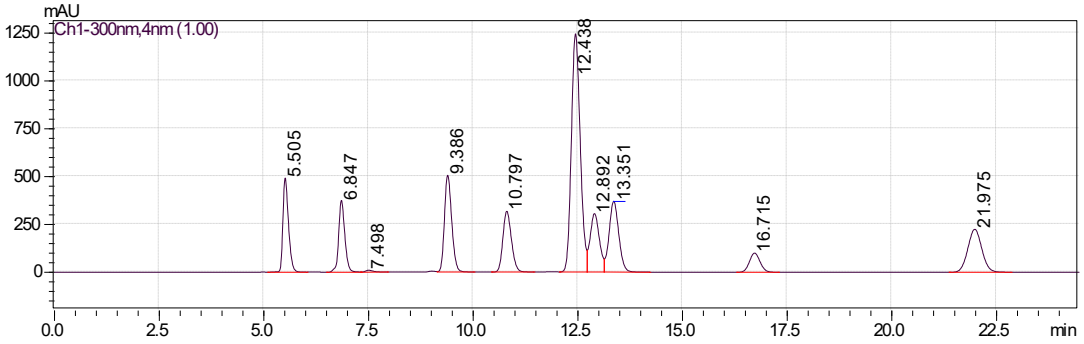

Figure 9: HPLC chromatogram of Mix C (BP1, BP3, MBC, OCR, EHMC, BDM, PDO, ES, BP12) recorded with Kinetex column, mobile phase EtOH:AA 75:25 at 30 °C

Since the goal was to reach baseline separation, Method VI was modified and from isocratic to gradient elution into Method VII. Gradient conditions are described in detail in Table 11. As it is visible in Figure 10, change from isocratic to gradient type of elution enabled baseline separation of filters from Mix C.

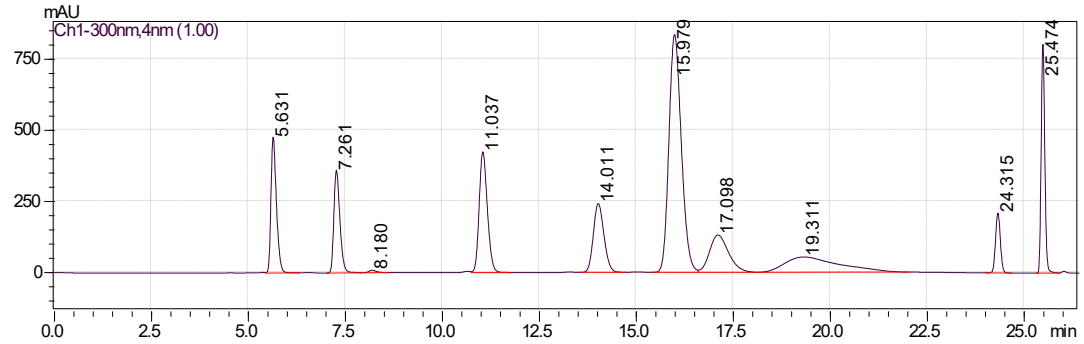

Figure 10: HPLC chromatogram of Mix C (BP1, BP3, MBC, OCR, EHMC, BDM, PDO, ES, BP12) recorded with Kinetex column, gradient elution at 45 °C

Method VII proved to be the most suitable for screening of UV filters since it enabled separation all nine filters from Mix C. Nevertheless, commercial products normally contain smaller number of UV filters and the analytical method has to be carefully developed due to different nature of their properties.

All the methods discussed in the part 7.2 of the thesis have one thing in common. None of them were able to separate filters ET, EMT and BMT. Because of their higher molar masses, these filters showed extremely long retention times. However, elution can be easily accomplished, in this case, by using an organic phase (ethanol or acetonitrile) in the absence of acetic acid.

7.3 Cosmetic Product Analysis

In this part, analysis of real samples containing studied UV filters will be discussed in detail. There were five commercial sunscreen products analysed in this thesis. All of them contained the most frequently used UV filters [14] and were chosen based on the list of ingredients (given on the product label) so that the filters present in these products would align with the filters screened during the method development (Part 6.4 of the thesis). Detailed information about composition of these products can be found in part 6.2.2 Table 2.

7.3.1 Product 1 - SunDance SPORT Sonnencreme - Gel SPF 30

The product was analysed using **Method V** which is described in detail in Table 8. This method was not suitable for screening of many UV filters in one sample but proved to be efficient in cosmetic product analysis. It used Kinetex column and isocratic type of elution of mobile phase consisting of ethanol and acetic acid in ratio 80:20, flow rate 0,7 ml·min^{-1}, temperature 35 °C and chromatograms were recorded at λ = 300 nm. Product 1 contained following UV filters: Octocrylene (OCR), Ethylhexyl salicylate (ES) and Butyl methoxydibenzoylmethane (BDM). Chromatogram obtained from this analysis is shown in Figure 11.

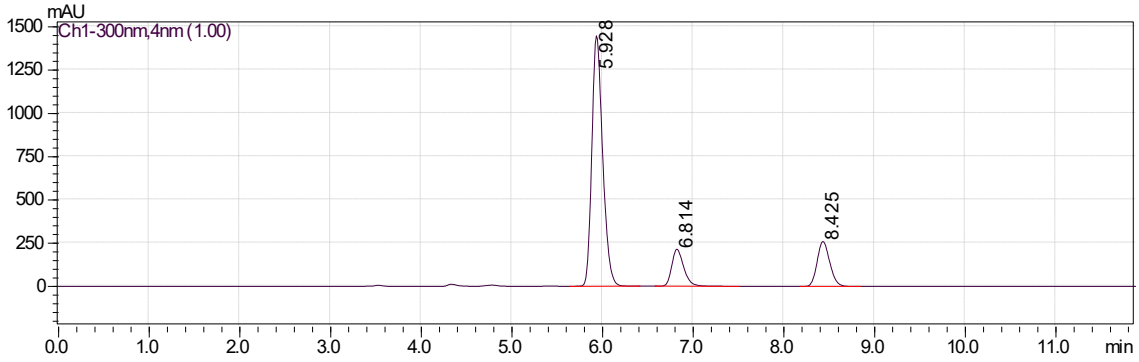

Figure 11: HPLC chromatogram of Product 1: OCR (Rt = 5.9 min), BDM (Rt = 6.8 min), ES (Rt = 8.4 min), recorded with Kinetex column, mobile phase EtOH:AA 80:20 at 35 °C

7.3.2 Product 2 - Garnier Ambre Solaire Hydra 24H Protect SPF 20

This sample was analysed using **Method VIII** (Kinetex C18 column, mobile phase EtOH:AA 95:5 at flow rate 0,8 ml/min, temperature 35 °C, λ = 300 nm, see Table 12). This product contained following UV filters: Ethylhexyl salicylate (ES), Ethylhexyl triazone (ET), Butyl methoxydibenzoylmethane (BDM), Bis-ethylhexyloxyphenol methoxyphenyl triazine (EMT) and the chromatogram is displayed in Figure 12.

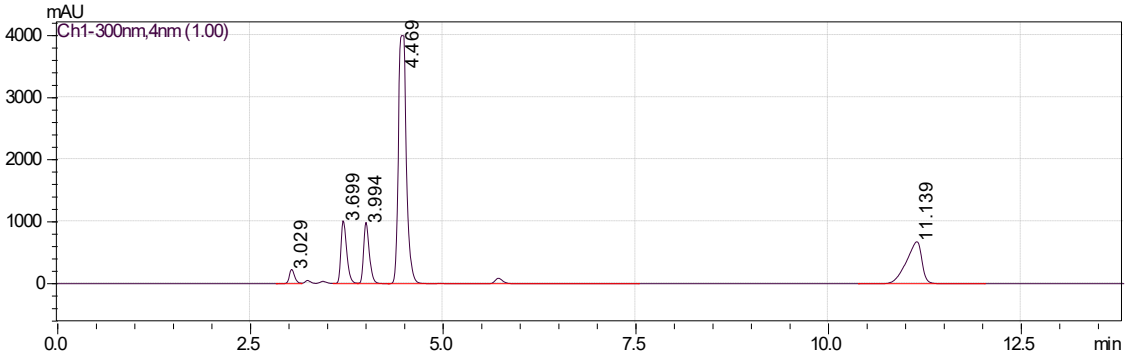

Figure 12: HPLC chromatogram of Product 2 : BDM (Rt = 3.7 min) , ES (Rt = 3.9 min), ET (Rt = 4.5 min) , EMT (Rt = 11.1 min) recorded with Kinetex column, mobile phase EtOH:AA 95:5 at 35 °C

7.3.3 Product 3 - Nubian Milk for Sunbathing SPF 6

Nubian milk was analysed using **Method V**, the same as Product 1. It contained following UV filters: Octocrylene (OCR), Ethylhexyl salicylate (ES), Butyl methoxydibenzoylmethane (BDM). Chromatogram of their analysis is shown in Figure 13.

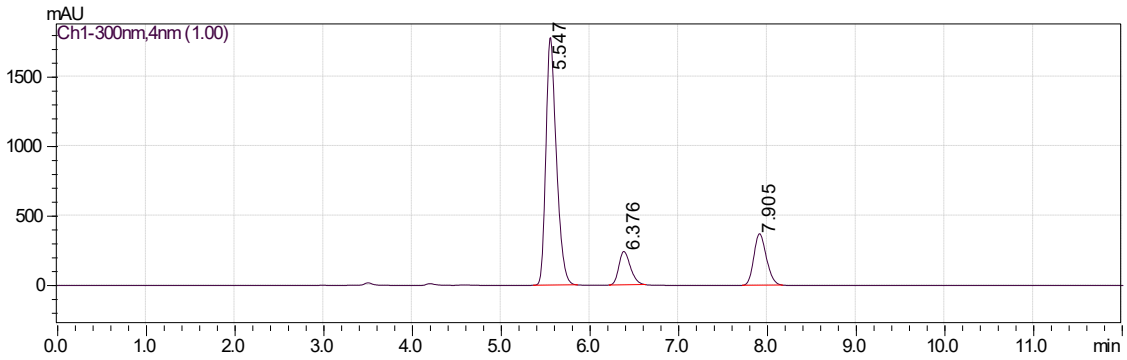

Figure 13: HPLC chromatogram of Product 3: OCR (Rt = 5.5 min), BDM (Rt = 6.4 min), ES (Rt = 7.9 min), recorded with Kinetex column, mobile phase EtOH:AA 80:20 at 35 °C

7.3.4 Product 4 - Aloha Milk for Sunbathing SPF 20

The product was also analysed using **Method V**. It contained following UV filters: Octocrylene (OCR), Butyl methoxydibenzoylmethane (BDM). Chromatogram obtained during this analysis is shown in Figure 14.

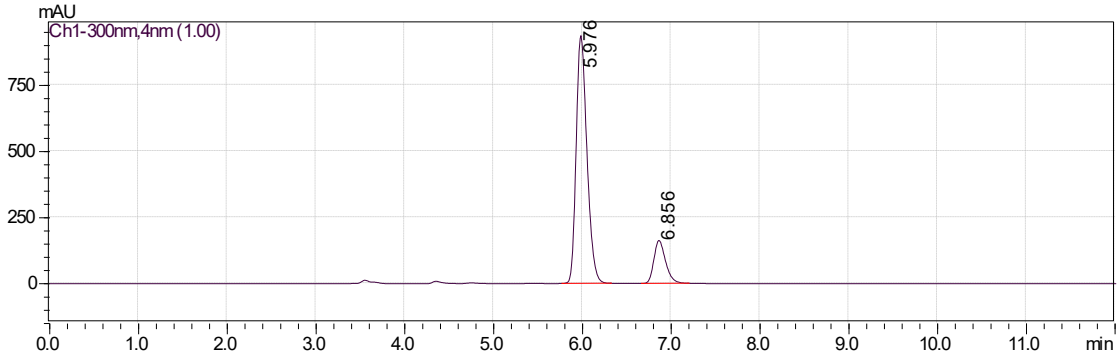

Figure 14: HPLC chromatogram of Product 4: OCR (Rt = 5.98 min), BDM (Rt = 6.9 min), recorded with Kinetex column, mobile phase EtOH:AA 80:20 at 35 °C

7.3.5 Product 5 - Garnier Ambre Solaire Sensitive Advanced SPF 50+

The sample contained following UV filters: Ethylhexyl salicylate (ES), Bis-ethylhexyloxyphenol methoxyphenyl triazine (EMT), Butyl methoxydibenzoylmethane (BDM), Drometrizole trisiloxane, Diethylhexyl butamido triazone and was analysed using **method IX**. This method used gradient elution conditions of which are described in detail in Table 13 and Table 14. Gradient elution shortened the time of analysis. Corresponding chromatogram is shown in Figure 15.

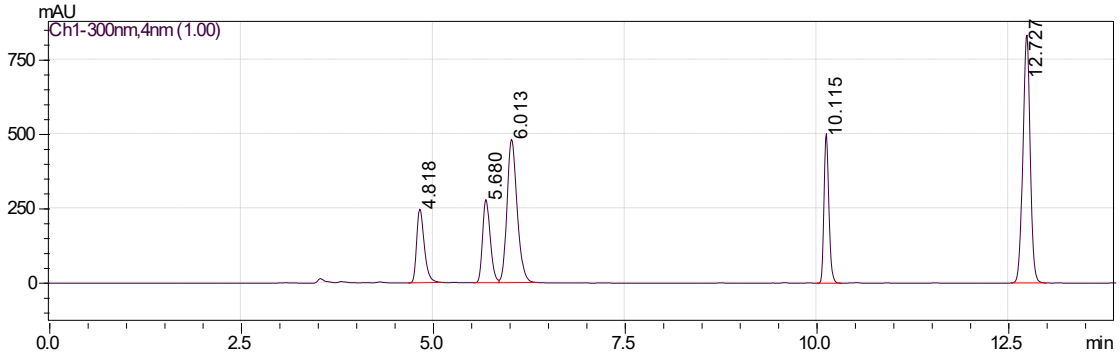

Figure 15: HPLC chromatogram of Product 5 (BDM, ES, EMT) recorded with Kinetex column, gradient elution

Chromatograms of sun-care products (Figures 11 to 15) show base line separation of UV filters in all studied products.

7.4 UV Filters – Quantification

Concentrations of UV filters in four commercial sunscreen products were determined in this thesis. Products 1, 3 and 4 were all analysed using Method V and this method was also used to quantify the filters present in them. Calibration curves for individual filters present in each

of the samples are listed in part 7.4.1 and 7.4.2. All of the filters were analysed at the wavelength of λ = 300 nm except for ET which was analysed at λ = 320 nm. Product 2 was analysed with Method VIII (see 7.4.2).

The concentrations of filters in the Product 5 (Garnier Ambre Solaire Sensitive Advanced SPF 50+) were not quantified, as not all calibration standards were available. However, the chromatogram in Figure 15 shows that knowledge gained during the bachelor's thesis enabled to develop HPLC method, which separated well all filters in this sample.

7.4.1 Calibration Curves – Method V

Calibration was conducted using external standard calibration method. The method is commonly used when the sample preparation is simple and small instrumental variations are observed. To create a curve, standard solutions with known concentrations of the analyte are prepared and a fixed volume injected into the column. The resulting areas of the peaks in the chromatogram are calculated and plotted versus the concentration injected. Calibration curves are given in Figure 16 (Octocrylene), Figure 17 (Avobenzone) and Figure 18 (Octisalate). Calibration equations for studied UV filters obtained from a linear regression analysis are given in Table 18.

Table 18: Linear regression equation obtained for UV filters analysed with Method V (y is the peak area corresponding to the concentration, x) together with correlation coefficient R2 recorded at λ = 300 nm

Sample	Rt [min]	Equation	R^2
Octocrylene (OCR)	5,99	y = 9595349x - 128383	0,9998
Avobenzone (BDM)	6,88	y = 3259837x - 95435	0,9998
Octisalate (ES)	8,49	y = 3745388x - 167620	0,9995

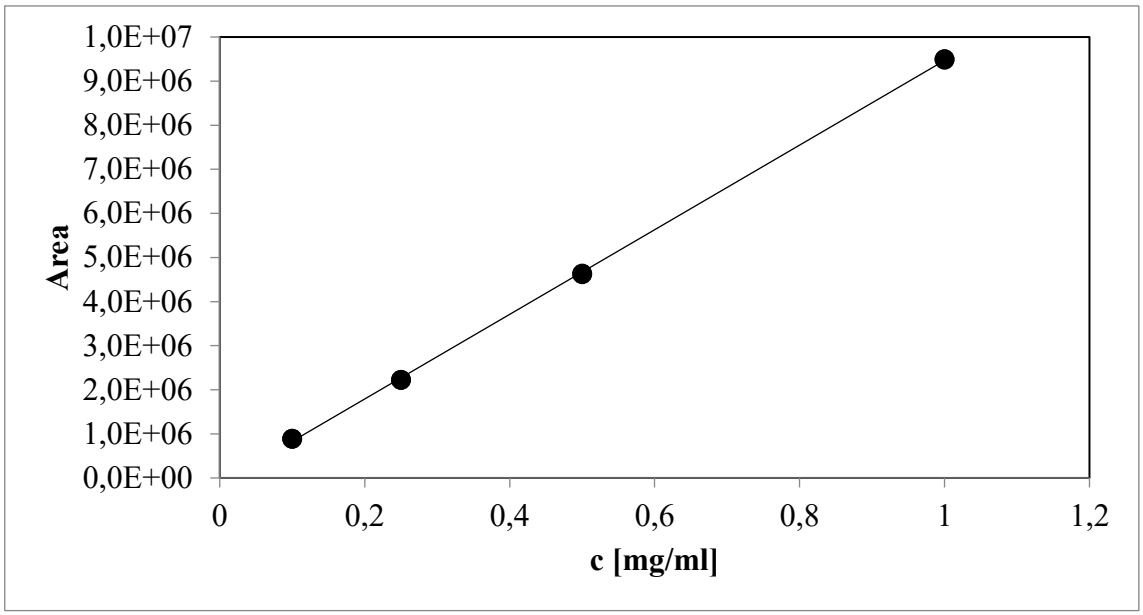

Figure 16: Octocrylene calibration curve, Method V

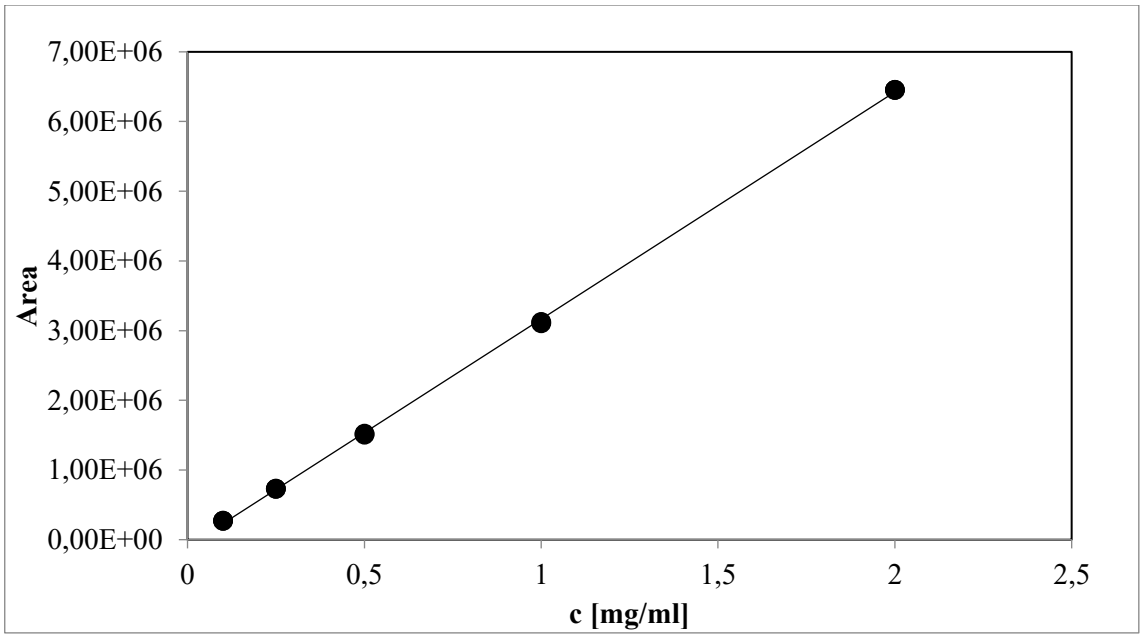

Figure 17: Calibration curve – Avobenzone, Method V

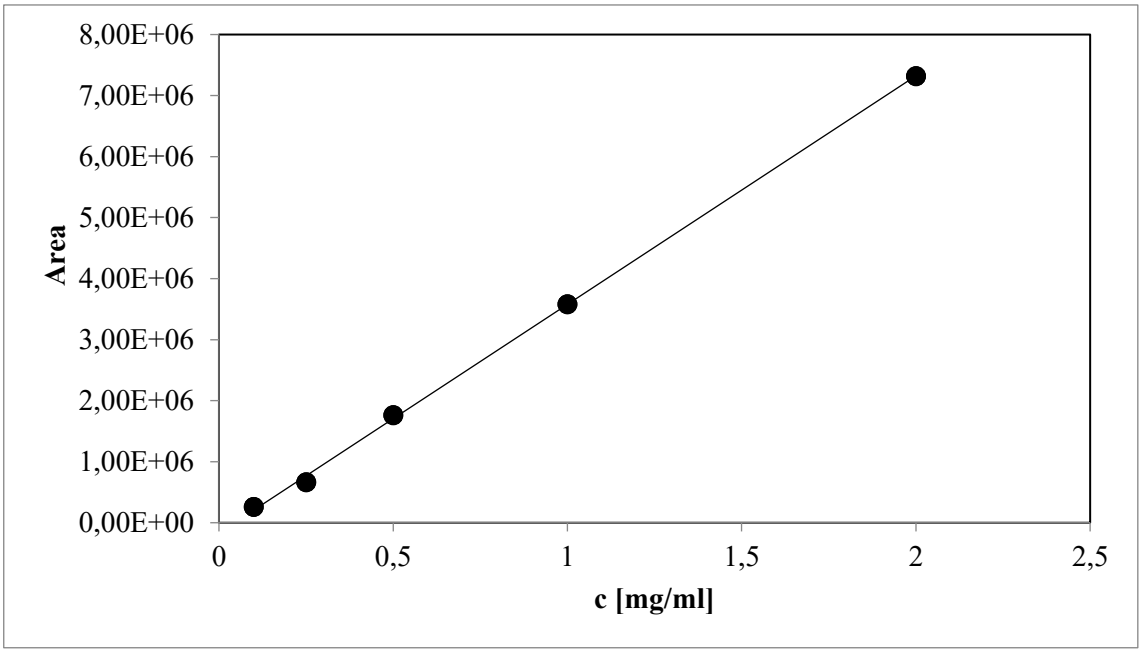

Figure 18: Calibration curve – Octisalate, Method V

7.4.2 Calibration Curves – Method VIII

Calibration curves for UV filters analysed by method VIII are presented in Figure 19 (Avobenzone), Figure 20 (Octisalate), Figure 21 (Ethylhexyl Triazone) and Figure 22 (Bemotrizinol). Calibration equations obtained from a linear regression analysis are given in Table 19.

Table 19: Linear regression equation obtained for UV filters analysed with Method VIII (y is the peak area corresponding to the concentration, x) together with correlation coefficient R2 recorded at λ = 300 nm (ET was recorded at λ = 320 nm)

Sample	Rt [min]	Equation	R^2
Avobenzone (BDM)	3,47	y = 3015546x - 68548	0,9998
Octisalate (ES)	4,03	y = 3368483x - 150031	0,9996
Ethylhexyl Triazone (ET)	4,55	y = 13133842x + 199354	0,9987
Bemotrizinol (EMT)	11,36	y = 1310925x - 19416	1,000

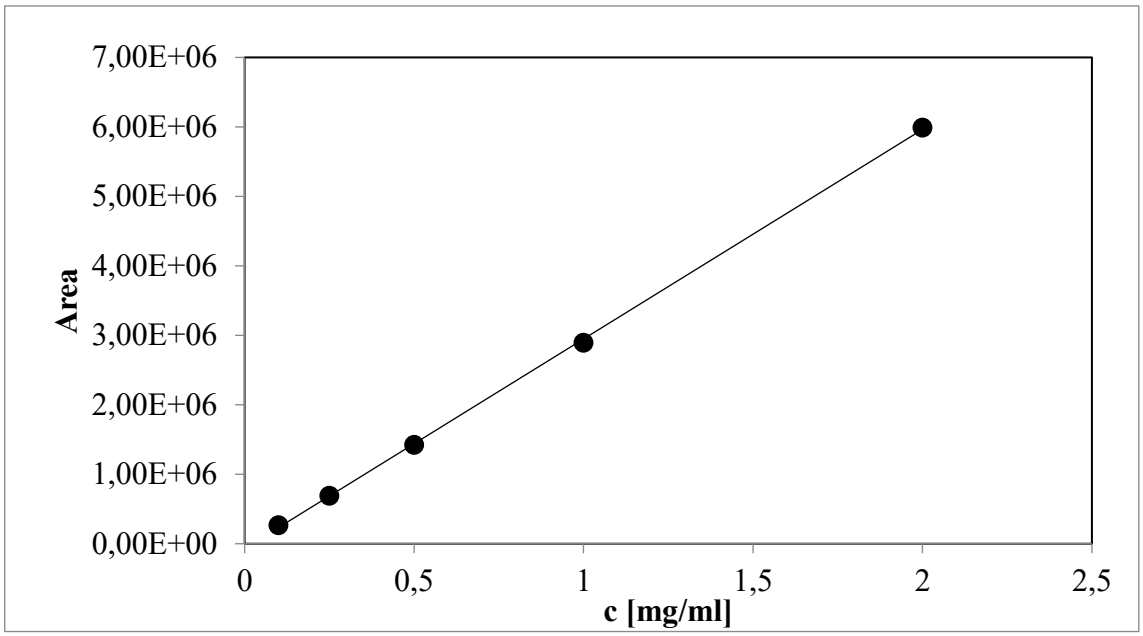

Figure 19: Calibration Curve – Avobenzone, Method VIII

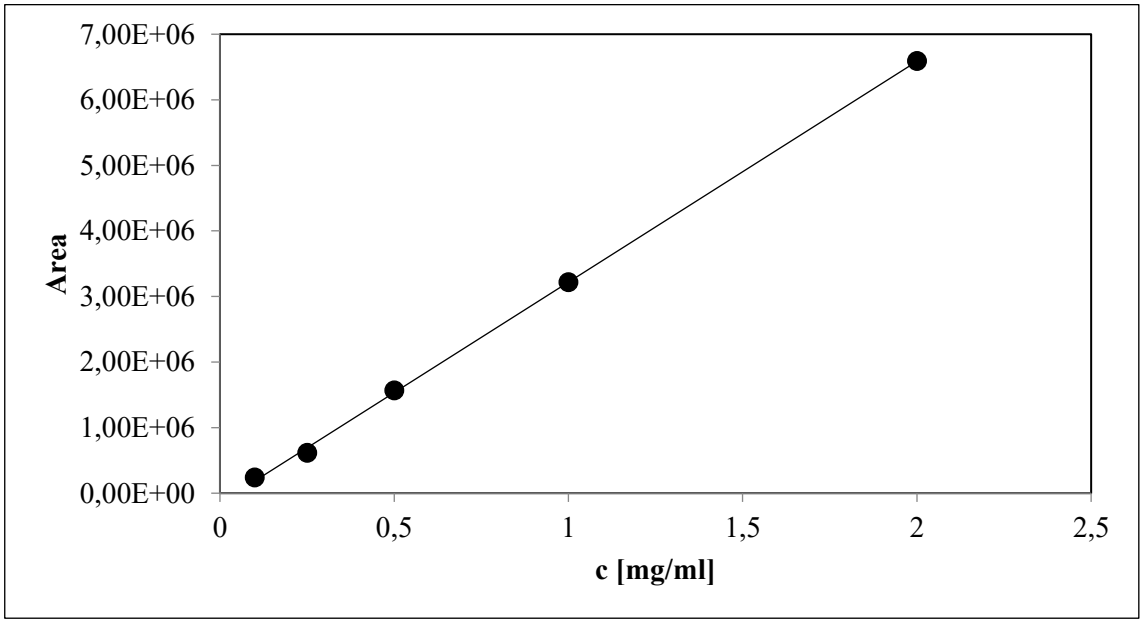

Figure 20: Calibration curve – Octisalate, Method VIII

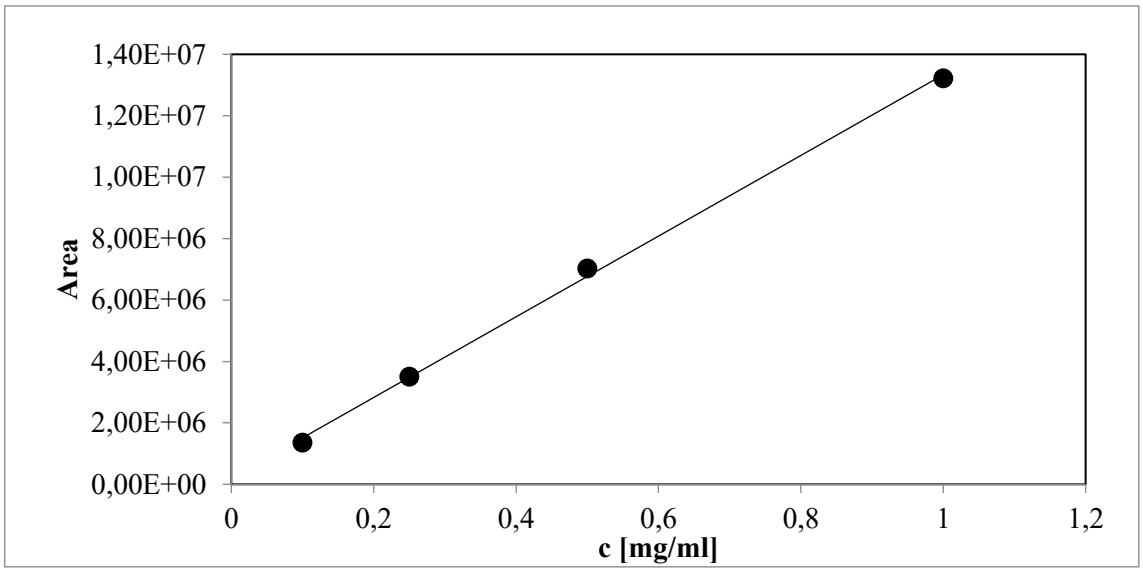

Figure 21: Calibration curve – Ethylhexyl Triazone, Method VIII

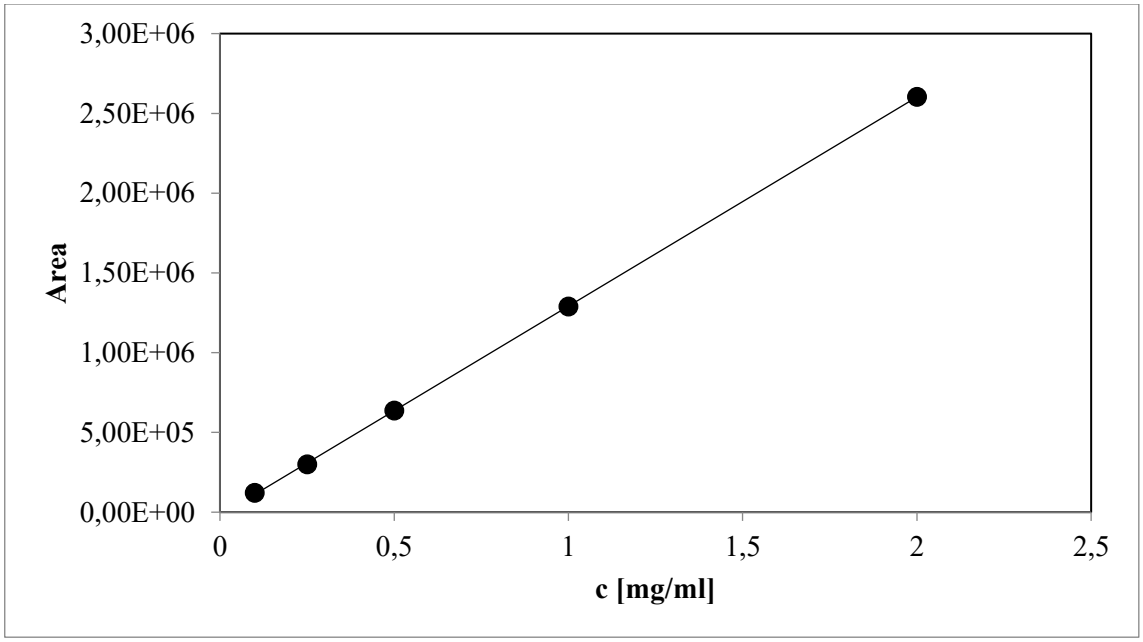

Figure 22: Calibration curve – Bemotrizinol, Method VIII

7.4.3 Concentrations of UV Filters in Analysed Cosmetic Products

Above presented calibration equations were used to calculate concentrations of UV filters in individual samples, which are summarized in Table 20. The results are presented as averages of three replicate analyses (AVG) ± standard deviations (SD) and are given in percentage contained in the sample.

Table 20: Concentrations of UV-filters in analysed cosmetic products

Product	**c [%]**				
	OCR	BDM	ES	ET	EMT
(1) SunDance SPF 30	3,62±0,003	1,95±0,002	2,18±0,003	n.p.	n.p.
(2) Garnier SPF 20	n.p.	0,57±0,010	0,53±0,000	0,62±0,000	2,39±0,010
(3) Nubian SPF 6	1,60±0,001	0,76±0,000	1,06±0,000	n.p.	n.p.
(4) Aloha SPF 20	2,56 ±,010	1,51±0,020	n.p.	n.p.	n.p.
Maximum concentration *)	10	5	5	5	10

n.p. not present

*) *according to REGULATION (EC) No 1223/2009, Annex VI updated 31.1.2023*

The data presented in the Table 20 show that all analysed products contained Avobenzone (BDM), which provides protection against radiation in UVA region of the spectrum (Figure 2) and belongs among the most frequently used filters in cosmetics [14]. Sunscreen Bemotrizinol (EMT) known for its ability to provide protection in broad-spectrum of wavelengths (see Figure 3) was present in only one analysed product (Garnier). Octocrylene (OCR) and Octisalate (ES) are also frequently used filters, which is reflected by their presence in the analysed samples.

Octocrylene, present in Products 1, 3 and 4 is currently allowed to be used in ready to use formulations in a maximum concentration of 10 %. Its concentration in products analysed in this thesis was significantly lower. Its highest concentration was recorded in Product 1 (SunDance) which also claimed to have the highest protection factor (SPF 30) from the analysed creams. Its second highest concentration was recorded in Product 4 (Aloha) with SPF 20 and the lowest concentration was recorded in Product 3 with the lowest SPF 6. Avobenzone (BDM) is allowed to be used in ready to use formulations in a maximum concentration of 5 %. This filter is responsible for protection in UVA region of the spectrum and since all of the products claimed to provide broad-spectrum protection this filter was present in all of them. Its highest concentration was recorded in Product 1 (SunDance) and lowest in Product 2 (Garnier). Both Ethylhexyl salicylate (ES) and Ethylhexyl triazon (ET) are allowed in a maximum concentration 5 %. ES was present in Products 1, 2 and 3 again with highest concentration in Product 1 (SunDance) and ET was present only in Product 2.

Product 2 also contained Bemotrizinol (EMT), which provides broad-spectrum protection. Its maximum concentration allowed by the Regulation (EC) No 1223/2009 is 10 %.
When comparing the results of quantification, final concentrations of analysed UV filters don't seem to have direct correlation with the SPF of the products. This corresponds with the paper written by B. Herzog and U. Osterwalder [30] which says that *"The sun protection factor is not unequivocally related to the overall UV absorbance spectrum of a sunscreen (absorbance, extinction, and optical density are synonymous terms). That means, it is possible to have quite different levels of UVA protection with the same SPF value."*

The HPLC methods and results obtained during the analysis of commercial products exhibit introductory effort to establish analysis of sunscreens at the Department of Fat, Surfactant and Cosmetic Technology. Due to the lack of time, none of the methods developed in this thesis have been validated or studied in more details with respect to influence of chromatographic parameters on method performance. The same applies to the procedure used for extraction of filters from the products. Validation is required here as well.

CONCLUSION

For the analysis of commercial sun-care products, nine different methods have been developed and tested. Three of these methods were chosen as the most suitable ones. Method V, which is isocratic, with composition of mobile phase ethanol/acetic acid 80:20, using Kinetex C18 column, flow rate 0,7 ml/min and oven temperature 35 °C, was used to analyse three different products: SunDance SPORT Sonnencreme (Product 1), Nubian Milk (Product 3) and Aloha Milk (Product 4). Method VIII was developed and used to analyse sun-care Product 2 (Garnier Ambre Solaire, SPF 20). Conditions in this method were as follows: Kinetex C18 column, mobile phase EtOH:AA 95:5 at flow rate 0,8 ml/min, temperature 35 °C, λ = 300 nm. Both mentioned methods allowed identification and quantification of all aforementioned UV filters present in these formulations.

Product 5, Garnier Ambre Solaire Sensitive, was analysed using method IX with mobile phase ethanol/acetic acid with gradient elution using Kinetex C18 column, oven temperature 35 °C and flow rate 0,8 ml/min. Here, the filters were not quantified as this sample contained Drometrizole trisiloxane and Diethylhexyl butamido triazone, which weren't among the filters screened during method development, however, this method allowed baseline separation of all present filters.

Therefore, this goes to show that methods developed in this thesis could be used to analyse products containing variety of different UV filters.

External standard calibration method was used to find the concentrations of analysed filters in products. Butyl methoxydibenzoylmethane (BDM) was present in all of the formulations in concentrations ranging from 1,95±0,002 to 0,57±0,010 %. Octocrylene (OCR) was present in Product 1 (3,62±0,003 %), Product 3 (1,60±0,001 %) and in Product 4 (2,56 ±,010 %). Ethylhexyl salicylate (ES) was present in Product 1 (2,18±0,003 %), Product 2 (0,53±0,000 %) and in Product 3 (1,06±0,000 %). Moreover, product 2 contained Ethylhexyl triazone (ET) in concentration 0,62±0,000 % and Bemotrizinol (EMT) in concentration 2,39±0,010 %. When compared to the percentages allowed by the Regulation No. 1223/2009, the concentrations are well below the maximum concentration levels.

In conclusion, it can be stated that the development of HPLC methods for separation and quantification of organic UV filters present in commercial sun-care products was successful and these methods can be used to analyse wide spectrum of filters currently allowed in cosmetics.

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LIST OF ABBREVIATIONS

SC	stratum corneum
UV	ultra violet
UVA	ultra violet radiation A
UVB	ultra violet radiation B
LC	Langerhans cells
MED	minimal erythema dose
TEWL	trans epidermal waster loss
TiO_2	titanium dioxide
ZnO	zinc oxide
BDM	Avobenzone
OCR	Octocrylene
PDO	Padimate-O
EMT	Bemotrizinol
ET	Ethylhexyl triazone
ES	Ethylhexyl salicylate, Octisalate
EHMC/EMC	Ethylhexyl methoxycinnamate
BP3	Benzophenone-3
BP1	Benzophenone-1
4MBC	4-methylbenzylidene camphor
MBT	Bisoctrizole
BP12	Benzophenone 12
HPLC	High Performance Liquid Chromatography
cm	centimetre
dm	decimetre
TLC	Thin Layer Chromatography
IR	infrared
A	absorbance
ε	molar absorptivity of the compound
c	molar concentration of the compound
l	optical path length
LC-UV/Vis	Liquid Chromatography-UV/Vis
HPLC/UV-Vis	High Performance Liquid Chromatography-UV/Vis

MEKC-UV/Vis	Micellar Electrokinetic Capillary Chromatography-UV/Vis
aq	aqueous
nm	nanometres
DAD	diode array detector
SPF	sun protection factor
EtOH	ethanol
AA	acetic acid
ml	millilitres
V	volume
mg	milligram
PDA	photo diode array
µl	microliters
λ_{max}	maximum wavelength
ACN	acetonitrile
R_t	retention time

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