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PROGRAMA DE PÓS-GRADUAÇÃO EM FÁRMACOS E MEDICAMENTOS

PHOTOPROTECTION / PHOTOSTABILIZER POTENTIAL IN A QUERCETIN SUNSCREENS: DETERMINATION OF PHYSICAL-CHEMICAL, SAFETY AND EFFICACY PROPERTIES

Mirela Cardoso Garcia

São Paulo
2019

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MIRELA CARDOSO GARCIA

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Dedication

I dedicate all this work and my life to my parents and to God.
Without them nothing would be possible.

To my advisor Dr. André Rolim Baby for enabling this work to be carried out.

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First, I thank to God for the opportunity to grow as a life.

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Just Thank You!

"Some people are satisfied with what they already know, it's as if their knowledge fits into the pool. They take a few strokes to one side, others arm-strokes to the other, they cling to the edges and touch the bottom with their feet: they feel safe in this restricted range. But nothing like diving into a sea of endless knowledge where there are no limits, the depth is oceanic and the idea is to swim without reaching the mainland, simply keep moving. It tires, but it also revitalizes. "

Medeiros, Martha

ABSTRACT

GARCIA, M.C. Potential photoprotector / photostabilizer of sunscreens associated with quercetin: physical-chemical and pre-clinical tests. 2019. (Doctoral Thesis). Faculty of Pharmaceutical Sciences, University of São Paulo, São Paulo, 2019.

Ultraviolet radiation (UV) is related to the development of skin cancer and photoaging, also produces free radicals or reactive oxygen species (ROS) that cause premature aging. Therefore, sunscreens with extended UV protection capacity have been studied associated with antioxidant compounds. Different components can be incorporated with the purpose of improving, the photoprotection efficiency, as well as other cosmetic attributes, creating a multifunctional product. Among the compounds, there are some to be used from natural origin that are excellent, because it reduces the side effects and toxicity. Flavonoids are good examples of natural agents, because have demonstrated photoprotective antioxidant action in food and it is also promising for topical use. The aim of this work is to develop photoprotective formulations with quercetin, evaluating potential photoprotective and antioxidant of each formulation through tests already established and described in the literature, with emphasis on *in vitro* testing of potential photoprotective, antioxidant and security (*het-cam*); *ex vivo* antioxidant potential (*tape-stripping*). The results show us the photoprotective and antioxidant capacity of quercetin and the ideal concentration for this potential, thereby contributing to the synthesis of new molecules and development of new products, concerning stable, safe and effective exposure to solar UV radiation, preventing new incidences of skin cancer and reduction of photoaging.

Keywords: flavonoid, sunscreen, antioxidant, photoaging.

RESUMO

GARCIA, M.C. Potencial fotoprotetor / fotoestabilizador de filtros solares associados à quercetina: ensaios físico-químicos e pré-clínicos. 2019. (Tese de doutorado). Faculdade de Ciências Farmacêuticas, Universidade de São Paulo, São Paulo, 2019.

A radiação ultravioleta (UV) está relacionada ao desenvolvimento de câncer de pele e fotoenvelhecimento, também produz radicais livres ou espécies reativas de oxigênio (EROs) que causam o envelhecimento precoce. Portanto, protetores solares com capacidade de proteção UV amplo espectro foram estudados associados a compostos antioxidantes. Diferentes componentes podem ser incorporados com o objetivo de melhorar a eficiência da fotoproteção, assim como outros atributos cosméticos criando um produto multifuncional. Entre os compostos a serem utilizados, de origem natural são excelentes, pois reduzem os efeitos colaterais e a toxicidade. Os flavonóides são bons exemplos de agentes naturais, porque demonstraram ação antioxidante fotoprotetora nos alimentos e também são promissores para uso tópico. O objetivo deste trabalho é desenvolver formulações fotoprotetoras com quercetina, avaliando potenciais fotoprotetores e antioxidantes de cada formulação através de testes já estabelecidos e descritos na literatura com ênfase em ensaios *in vitro* de potenciais fotoprotetores, antioxidantes e de segurança (*het-cam*); potencial antioxidante *ex vivo* (*tape-stripping*). Os resultados nos mostram a capacidade fotoprotetora e antioxidante da quercetina e a concentração ideal para este potencial, contribuindo para a síntese de novas moléculas e desenvolvimento de novos produtos estáveis, seguros e eficazes em relação à exposição à radiação solar UV, prevenindo novas incidências de câncer de pele e redução do fotoenvelhecimento.

Palavras-chave: flavonoide, fotoprotetor, antioxidante, fotoenvelhecimento.

List of abbreviations

ANVISA	**-**	National Health Surveillance Agency
AVO	**-**	Avobenzone
BMBM	**-**	Avobenzone
CEP	**-**	Research Ethics Committee
CIE	**-**	*Comission Internationale de l'Eclairage*
COLIPA	**-**	Cosmetics Europe: The Personal Care Association
cP	**-**	centiPoise
DEM	**-**	Minimal Erythematogenesis Dose
DNA	**-**	Deoxyribonucleic acid
DPPH	**-**	2,2-diphenyl-1-picrylhydrazyl
FDA	**-**	Food and Drug Administration
HPLC	-	High performance liquid chromatography
INCI	**-**	International Nomenclature of Cosmetics Ingredients
O/A	**-**	Oil in water
OMC	**-**	Octyl methoxycinnamate
pH	**-**	Hydrogen potential
PMMA	**-**	Polymethylmethacrylate
QRC	**-**	Quercetin
RDC	-	Resolution of the Collegiate of Directors
ROS	-	Reactive Oxygen Species
SPF	**-**	Solar protection factor
UV	**-**	Ultraviolet
UVA-PF	**-**	UVA protection fator

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Summary

1. Introduction

1. Introduction

The ultraviolet (UV) rays have positive and negative aspects, as they can treat diseases like psoriasis and vitiligo, but they can also cause erythema and lead to a more advanced stage of skin cancer. Exposure to sun rays and their attribution to erythema have been described since the 1800s, but studies of sunscreens appeared in the early 1900s and had grown in the market since 1978, when the Food and Drug Administration (FDA) took a special care to protect against sunburn and skin cancer (MATSUMURA; ANANTHASWAMY, 2004; NATARAJAN et al., 2014; URBACH, 2001).

It is a growing concern with health and, body aesthetics has increased the demand for cosmetic products capable of preventing skin aging and the appearance of pathologies as a skin cancer (COLOMBO, 2018). Skin cancer is already considered a public health problem in the United States and in many countries, including Brazil (VAN NAGELL et al., 2013). According to INCA (National Cancer Institute), it was expected about 171,840 new cases of skin cancer in Brazil in 2018, being: 165,580 cases of non-melanoma skin cancer and 6,260 melanomas. This is due, among the predisposition and other factors, to the prolonged exposure to UV radiation (INCA - INSTITUTO NACIONAL DE CÂNCER, 2018).

Although studies on sunscreens have started more than 100 years ago, their use in combination with natural products is still controversial, especially regarding information on the stability of the components against UVA and UVB radiation (CORRÊA, 2012).

As it is known, all individuals are subject to sun exposure and there is a real need for protection. Chemical (or organic) UV filters are effective substances against UV rays, but with potential for adverse events. For this reason, safe strategies, preferably of natural origin, are for formulations of sunscreens available in the market that meet the prerequisites of quality control, preclinical and clinical tests (DEORE; KOMBADE, 2012).

A sunscreen, in addition to absorbing UV radiation, must be stable when in contact with human skin and during the period of protection and heat, photostable, in which it provides protection for several hours without degradation of its components. In addition, it can not be irritating, toxic and should not penetrate the skin, avoiding systemic exposure of the formulation components (NOHYNEK; SCHAEFER, 2001).

It is of extreme interest the development of a formulation containing sunscreen that remains on the skin over a long period of time, together with antioxidant, which

neutralizes ROS (DEORE; KOMBADE, 2012). Natural compounds, such as high molecular weight polyphenols, flavonoids and phenolic acids, are indicated to prevent the harmful effects of UV radiation on the skin, since they have protective and antioxidant action, the ability to stimulate blood circulation on the skin and remove dead cells, depending on how it is used, among others (ICHIHASHI et al., 2003; RATES, 2001).

Quercetin is a flavonoid, which has antioxidant capacity five times more than vitamins E and C, which also acts synergistically with vitamin E, being quite promising in the cosmetic area (HARBORNE; WILLIAMS, 2000). This work is consisted on the preparation of sunscreens containing quercetin for photoprotective, photostabilizing and antioxidant potential, in addition to clinical trials, in order to observe safety, efficacy and photostability.

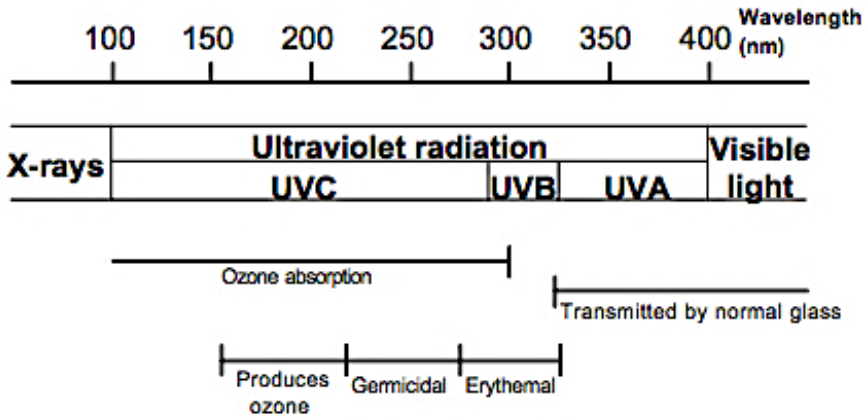
2. Literature review

2. Literature Review

2.1. UV Radiation

Humans were exposed daily to solar radiation due to the occupational fact, which caused several damages. Currently the radiation is used for recreational purposes as tanning (during the time that the exposure to rays are lighter), and regardless of the intensity all UV radiation will promote some damage (D'ORAZIO et al., 2013). UV radiation has been one of the main environmental complications for skin, causing effects in several layers and leading, sometimes, to skin cancer. In the last decade there has been an increase in the incidence of skin cancer due to changes in people's lifestyle patterns in which there is a significant increase in the amount of UV radiation received. Approximately 5% of the solar radiation is emitted in forms of UV rays and it is divided according to its wavelength **(Figure 1)** (AFAQ, 2011; MATSUMURA; ANANTHASWAMY, 2004).

Figure 1: Spectrum of UV radiation and the chemical, physical and biological effects (MATSUMURA; ANANTHASWAMY, 2004).

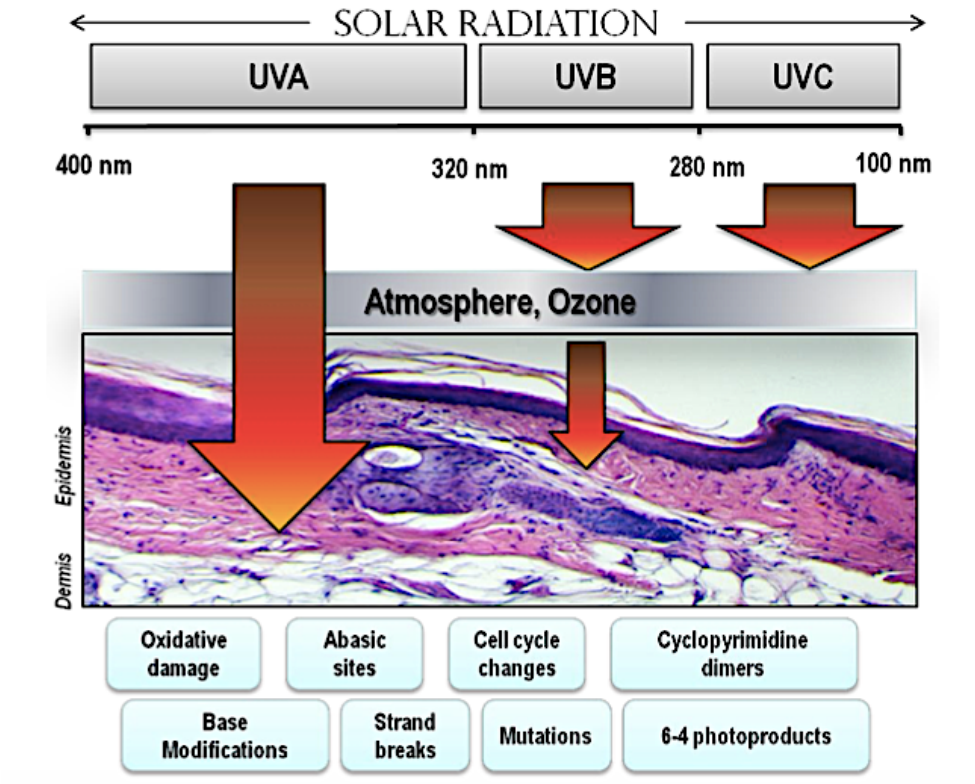


- UVA: 320-400nm, reaches deeper layers of the skin, promoting aging of skin tissues, increasing the formation of ROS, causing oxidative stress. This radiation is widely absorbed by our DNA and can cause lesions and mutagenic photoproducts by their reactions (FISHER; KRIPKE, 1977; WIDEL et al., 2014)

- UVB: 290-320nm, it makes up only 4-5% of the radiation, can cause severe damages to the DNA, but penetrates less when compared to the UVA rays, promoting erythema, burns, blistering and mutation of the p53 gene. It also contributes to the formation of free radicals (AFAQ, 2011; WIDEL et al., 2014)
- UVC: 180-290nm, does not reach the Earth's surface because it is absorbed by the ozone layer (WIDEL et al., 2014).

UVA radiation absorbed by the skin surface at the dermal level is also capable of producing ROS **(Figure 2),** which is harmful, activate inflammatory mediators and the entire intracellular cascade of transcription of the genes regulating inflammation, proliferation, cell cycle and apoptosis, in addition to decrease synthesis, promote collagen degradation, accelerating the photo-aging process (MONTAGNER; COSTA, 2009).

Figure 2: Damages caused by ultraviolet rays UVA, UVB and UVC (D'ORAZIO et al., 2013).



As previously stated, UVA radiation penetrates deeper into our skin, being more dangerous than UVB radiation, favoring the formation of ROS and free radicals like hydroxyl, damaging lipids, protein and cellular DNA. UVB radiation penetrates the skin

only up to the epidermis, so it is responsible for damage such as erythema, tanning, induction of oxidative stress, DNA damage and premature aging of the skin. UVB is eventually penetrated by the epidermis, but can also lead to DNA damage, mainly due to cumulative effect (D'ORAZIO et al., 2013; ICHIHASHI et al., 2003).

Oxidative stress refers to elevated intracellular levels of ROS, which causes damage to lipids, proteins and DNA. They are usually associated with several pathologies, including carcinogenesis, in which they are present at high levels (SCHIEBER; CHANDEL, 2014).

2.1.1. Benefits and harms of UV radiation

UV radiation gives the human being benefits, such as vitamin D synthesis, mediation of certain endorphins in the skin and influence on the circadian rhythm. However, it can be classified as a tumor promoter because it is mutagenic depending on the tumor exposure (D'ORAZIO et al., 2013). In addition to the mutagenic propensity, UV radiation interrupts keratinocytes in the epithelium, causing inflammatory disorders, can modify levels of photosensitizers of the skin leading to phototoxic stresses (NATARAJAN et al., 2014).

2.1.2. Impacts of UV radiation on the skin

The skin is the first barrier between our organism and the environment, therefore it is a target of UV irradiation, chemicals among others, causing oxidative stress that is a beginner of photoaging and skin cancer (SAIJA et al., 1998). Among the impacts of the radiation under the skin are those with initial response, which includes erythema and immediate pigmentary darkening, and the later response such as persistent pigmentation, epidermal hyperplasia, free radical formation and vitamin D synthesis (RIGEL, 2008).

Erythema: sunburn, mainly caused by exposure to UVB radiation, with a peak between 6 and 24 hours after exposure.

Immediate pigment darkening: predominantly caused by UVA radiation, often grayish in color, developed within minutes by photooxidation and redistribution of existing melanin in epidermal melanocytes and disappears within 24 hours.

Late pigmentation dimming: induced by UVA and UVB radiation, begins about 3 days after exposure, with increased tyrosinase leading to melanin synthesis and remains for weeks.

Epidermal hyperplasia: an adaptive process that occurs during days of exposure to UVA and UVB radiation, especially in light-skinned individuals (HÖNIGSMANN, 2002).

Free radical formation: induced by UV rays. The free radicals formed include oxygen, hydrogen peroxide and superoxide radicals, causing damage to DNA, proteins and cell membranes, being directly related to mutagenesis.

Vitamin D synthesis: exposure to UVB radiation converts 7-dehydrocholesterol into Vitamin D3 (cholecalciferol) (VANCHINATHAN; LIM, 2012).

Photoaging: UVA radiation penetrates deeper into the skin due to its longer wavelengths, having a more important role in photoaging, which includes wrinkles, lentigines, loss of elasticity, blackheads, among others (MAEDA, 2018).

Photocarcinogenesis: also known as skin cancer, in which UV radiation is directly linked to the mutations caused in the DNA of skin cells, being documented the increase of melanomas with increased sun exposure (RIGEL, 2008).

2.2. Skin Cancer

Skin cancer has an incidence and increase of about 8% per year in the world. The same is subdivided into categories besides malignant and benign, there are melanomas, carcinomas (basocellular, squamous cell) in which it is interconnected to the layer of the skin in which the cancerous cells appear (GORDON, 2013). Repeated exposure to sunlight throughout life is epidemiologically shown to be the leading cause of skin cancer and that is why sun protection since childhood should be instituted (ARMSTRONG; KRICKER, 2011). Skin cancer is more common in people over 40 years and uncommon in children and black people, except for those who have skin diseases, but nowadays this average age has been decreasing since the constant exposure to solar radiation in young people (INCA - INSTITUTO NACIONAL DE CÂNCER, 2018).

2.3. Photoprotection

Photoprotection is directly linked to how many hours of sun exposure, wearing hats and sunglasses, sunscreens among other cautions (ARMSTRONG; KRICKER,

2011; ICHIHASHI et al., 2003). Photoprotection is one of the main preventive and therapeutic strategies for image control and skin cancer, as well as recommendations for exposure to the sun during peak sessions (GILABERTE; GONZÁLEZ, 2010).

Photoprotection is critical for prophylaxis and remedy in the fight against radiation and its effects. Nowadays several photoprotection media have been developed and perfecting old media, such as clothing, in which today there are photo-protective materials, as well as physical protection (GONZÁLEZ; FERNÁNDEZ-LORENTE; GILABERTE-CALZADA, 2008).

From a few years to the present, society has been extremely concerned with aesthetics and consequently aging, particularly photo-aging, and thus requires multifunctional products, with anti-aging, antioxidant, photo-protective action among other benefits (KULLAVANIJAYA; LIM, 2005). Sunscreens are one of the well-accepted forms of photoprotection, and it has been widely prescribed by dermatologists because of the number of cancer cases and complaints of photoaging, as well as the marketing developed by companies about them (WANG; BALAGULA; OSTERWALDER, 2010).

2.4. Sunscreen and main filters

In Brazil, photoprotective substances are regulated by ANVISA (RDC N ° 69, OF MARCH 23, 2016) and 34 substances are regulated as UV filters permitted in Brazil and Mercosur **(Table 1)**, such as methoxycinnamate, avobenzone, zinc oxide, titanium dioxide, PABA (para-aminobenzoic acid) and their maximum authorized concentrations. The combination of these is well accepted (BRASIL, 2016; WANG; BALAGULA; OSTERWALDER, 2010).

Table 1: List of permitted ultraviolet filters for toiletries, cosmetics and perfumes by ANVISA (BRASIL, 2016).

	Substance (INCI name)	**Maximum Concentration Allowed**
1	N, N, N-trimethyl-4- (2, oxoborn-3-ylidenemethyl) anilium methyl methacrylate / CAMPHOR BENZALKONIUM METHOSULFATE	6%
2	3, 3 '- (1,4-phenylenedimethylene) bis (7,7-dimethyl-2-oxo-bicyclo- (2.2.1) 1-heptylmethanesulfonic acid and its salts TEREPHTHALYLIDENE DICAMPHOR SULFONIC ACID (& SALTS)]	10% (expressed as acid)
3	*1 -(4 – terc – butilfenil) – 3 – (4 –metoxifenil) propano – 1, 3 – diona BUTYL METHOXYDIBENZOYLMETHANE* [1- (4-tert-butylphenyl) -3- (4-methoxyphenyl) propane-1,3-dione BUTYL METHOXYDIBENZOYLMETHANE]	5%
4	*Ácido alfa – (2 – oxoborn – 3 –ilideno) tolueno – 4 – sulfônico e seus sais de potássio, sódio e trietanolamina BENZYLIDENE CAMPHOR SULFONIC ACID & SALTS* [Alpha - (2-oxoborn-3-ylidene) toluene-4-sulfonic acid and its potassium, sodium and triethanolamine salts BENZYLIDENE CAMPHOR SULFONIC ACID & SALTS]	6% (expressed as acid)
5	*2 – Ciano – 3, 3´– difenilacrilato de 2 –etilexila OCTOCRYLENE* [2-Cyano-3,3-diphenylacrylate OCTOCRYLENE]	10% (expressed as acid)
6	*4 – Metoxicinamato de 2 – etoxietila CINOXATE* [4-methoxycinnamate 2-ethoxyethyl CINOXATE]	3%
7	*2, 2' – dihidroxi – 4 – metoxibenzofenona BENZOPHENONE–8* [2,2'-dihydroxy-4-methoxybenzophenone BENZOPHENONE-8]	3%
8	*Antranilato de mentila MENTHYL ANTHRANILATE* [MENTHYL ANTHRANILATE Mentane anthranilate]	5%
9	*Salicilato de trietanolamina TEA-SALICYLATE* [Triethanolamine Salicylate TEA-SALICYLATE]	12%
10	*Ácido 2 – fenilbenzimidazol – 5 – sulfônico e seus sais de potássio, sódio e trietanolamina PHENYLBENZIMIDAZOLE SULFONIC ACID (& SODIUM , POTASSIUM ,TEA SALTS)* [2-phenylbenzimidazole-5-sulfonic acid and its salts of potassium, sodium and triethanolamine PHENYLBENZIMIDAZOLE SULFONIC ACID (& SODIUM, POTASSIUM, TEA SALTS)]	8% (expressed as acid)
11	*4 – Metoxicinamato de 2 – etilhexila 4 – Metoxicinamato de 2 – etilhexila* [4-methoxycinnamate 2-ethylhexyl 4-methoxycinnamate 2-ethylhexyl methoxycinnamate]	10%

12	*2 – Hidroxi – 4 – metoxibenzofenona BENZOPHENONE–3 (1)* [2-Hydroxy-4-methoxybenzophenone BENZOPHENONE-3 (1)]	10%
13	*Ácido 2 – hidroxi – 4 – metoxibenzofenona – 5 – sulfônico BENZOPHENONE–4 (ACID)* [BENZOPHENONE-4 (ACID) 2-Hydroxy-4-methoxybenzophenone-5-sulfonic acid]	10% (expressed as acid)
14	*Sal sódico do ácido 2 – hidroxi – 4 – metoxibenzofenona – 5 – sulfônico BENZOPHENONE–5* [2-Hydroxy-4-methoxybenzophenone-5-sulfonic acid sodium salt BENZOPHENONE-5]	5% (expressed as acid)
15	*Ácido 4 – aminobenzóico PABA* [4-Aminobenzoic acid PABA]	15%
16	*Salicilato de homomentila HOMOSALATE* [HOMOSALATE homomethyl salicylate]	15%
17	*Polímero de N – {(2 e 4)[(2 – oxoborn – 3 –ilideno) metil] benzil} acrilamida POLYACRYLAMIDOMETHYL BENZYLIDENE CAMPHOR* [N - {(2 and 4) [(2-oxoborn-3-ylidene) methyl] benzyl} acrylamide POLYACRYLAMIDOMETHYL BENZYLIDENE CAMPHOR polymer]	6%
18	*Dióxido de titânio TITANIUM DIOXIDE*	25%
19	*N – Etoxi – 4 – aminobenzoato de etila PEG-25 PABA* [PEG-25 PABA ethyl N-ethoxy-4-aminobenzoate]	10%
20	*4 – Dimetil-aminobenzoato de 2 –etilhexila ETHYLHEXYL DIMETHYL PABA* [4-Dimethyl-aminobenzoate 2-ethylhexyl ETHYLHEXYL DIMETHYL PABA]	8%
21	*Salicilato de 2- etilhexila ETHYLHEXYL SALICYLATE* [2-ethylhexyl salicylate ETHYLHEXYL SALICYLATE]	5%
22	*4 – Metoxicinamato de isopentila ISOAMYL p–METHOXYCINNAMATE* [4 - Isopentyl methoxycinnamate ISOAMYL p-METHOXYCINNAMATE]	10%
23	*3 – (4' – metilbenzilideno) – d – l –cânfora 4–METHYL BENZYLIDENE CAMPHOR* [3- (4'-methylbenzylidene) -d-1-camphor 4-METHYL BENZYLIDENE CAMPHOR]	4%
24	*3 – Benzilideno cânfora 3-BENZYLIDENE CAMPHOR* [3-Benzylidene camphor 3-BENZYLIDENE CAMPHOR]	2%
25	*2, 4, 6 – Trianilin – (p – carbo – 2'- etil –hexil – 1' – oxi) – 1, 3, 5 – triazina ETHYLHEXYL TRIAZONE* [Trialkyl- (p-carbo-2'-ethylhexyl-1'-oxy) -1,3,5-triazine ETHYLHEXYL TRIAZONE]	5%
26	*Óxido de zinco ZINC OXIDE*	25%
27	*2-(2H-benzotriazol-2-il)-4-metil-6-{2 -metil-3-(1,3,3,3,-tetrametil-1-((trimet ilsilil)oxi)-disiloxanil)propil}fenol DROMETRIZOLE TRISILOXANE* [2- (2H-benzotriazol-2-yl) -4-methyl-6- {2-methyl-3- (1,3,3,3-tetramethyl-1 -((trimethylsilyl) oxy) disiloxanyl) propyl DROMETRIZOLE TRISILOXANE]	15%

28	*Ácido benzóico,4,4'-[[6-[[4-[[(1,1-dimetil-etil)amino]carbonil]fenil]amino]-1,3,5-triazina-2,4-diil]diimino]bis-,bis(2-etil hexil)éster DIETHYLHEXYL BUTAMIDO TRIAZONE* [4,4 '- [[6 - [[4 - [[(1,1-dimethyl-ethyl) amino] carbonyl] phenyl] amino] -1,3,5-triazine-2,4-diyl] -diimino] bis-, bis (2-ethyl hexyl) ester DIETHYLHEXYL BUTAMIDE TRIAZONE]	10%
29	*2,2'-metileno-bis-6-(2H-benzotriazol- 2-il)-4-(tetrametil-butil)-1,1,3,3-fenol METHYLENE BIS-BENZOTRIAZOLYL TETRAMETHYLBUTYLPHENOL* [2,2'-methylene-bis-6- (2H-benzotriazol-2-yl) -4- (tetramethyl-butyl) -1,1,3,3-phenol METHYLENE BIS-BENZOTRIAZOLYL TETRAMETHYLBUTYLPHENOL]	10%
30	*Sal monosódico do ácido 2,2'-bis-(1,4-fenileno)- 1H-benzimidazol-4,6-dissulfônico DISODIUM PHENYL DIBENZIMIDAZOLE TETRASULFONATE* [2,2'-Bis- (1,4-phenylene) -1H-benzimidazole-4,6-disulfonic acid monosodium salt DISODIUM PHENYL DIBENZIMIDAZOLE TETRASULFONATE]	10% (expressed as acid)
31	*(1,3,5)-triazina-2,4-bis{[4-(2-etil-hexi loxi)-2-hidróxi]-fenil}-6-(4-metoxifen il) BIS-ETHYLHEXYLOXYPHENOL METHOXYPHENYL TRIAZINE* [(1,3,5) -triazine-2,4-bis {[4-(2-ethylhexyloxy) -2-hydroxy] phenyl} -6- (4-methoxyphenyl) BIS-ETHYLHEXYLOXYPHENOL METHOXYPHENYL TRIAZINE]	10%
32	*Dimeticodietilbenzalmalonato POLYSILICONE-15* [Dimethyldiethylbenzalmalonate POLYSILICONE-15]	10%
33	*Éster hexílico do ácido 2-[4-(dietilamino)-2-hidroxibenzoil]-, benzóico DIETHYLAMINO HYDROXYBENZOYL HEXYL BENZOATE* [2- [4- (Diethylamino) -2-hydroxybenzoyl] benzoic acid, hexane DIETHYLAMINO HYDROXYBENZOYL HEXYL BENZOATE]	10%
34	*1,3,5-Triazina, 2,4,6-Tris([1,1'-Bifenil]-4-il)- TRIS-BIPHENYL TRIAZINE (2)* [1,3,5-Triazine, 2,4,6-Tris ([1,1'-Biphenyl] -4-yl) TRIS-BENHENYL TRIAZINE (2)]	10%

In the 1920s the most used sunscreens used to contain PABA, over the years studies have shown that PABA was carcinogenic and have started to use other photoprotective compounds, such as benzophenone and inorganic ones, as titanium dioxide and zinc oxide (BURNETT; WANG, 2011)**.**

The effectiveness of sunscreens is expressed by the Sun Protection Factor (SPF) which is defined as the UV energy required to produce a minimal dose of erythema on the protected skin divided by the UV energy required to produce a minimal dose of erythema on the unprotected skin the first approach to knowing the product SPF, determined "*in vivo*" (MARTO et al., 2016).

2.4.1. Chemical and physical filters

The agents with photo-protective capacity can be divided into 2 classes:

- inorganic or physical: reflect or spread UV radiation and has a large spectrum, for example titanium dioxide, widely used in photo-protective formulations, makeup with photoprotection and other cosmetics.

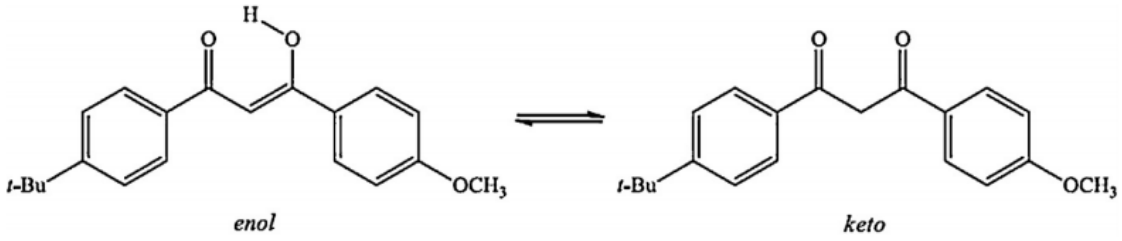
- organic or chemical: they are able to absorb UV radiation and convert it into thermal energy, but they have low / medium spectrum and therefore are used in combination, such as octyl methoxycinnamate (UVB filter) and avobenzone (UVA filter) (KIM et al., 2015).

Modern formulations incorporate organic filters specific to particular wavelengths, UVA and UVB filters together (AFONSO et al., 2014). Inorganic filters can also be incorporated, inhibiting photo-aging by UV light and have a wide wavelength range from which they absorb, scatter and reflect the photon (SERPONE; DONDI; ALBINI, 2007). Currently, the sunscreens provide large spectrum UV protection, made by combining filters. They should be photostable, water resistant, easy formulation and with new molecules that give these characteristics to them (NOHYNEK; SCHAEFER, 2001).

2.4.2. Avobenzone

Avobenzone is one of the most common UVA filters used in sunscreens, 360nm maximum absorption, molecular weight 310.39, melting point of 83.5°C, boiling point 464°C and widely known to be photo-labile, so methods that decrease this instability are used, such as the addition of antioxidants (AFONSO et al., 2014). Avobenzone exists in two tautomeric forms: enol (with a hydroxyl) and keto (with a carboline), but in solar filters the predominance is of the enol form by its better absorbance of the UVA rays **(Figure 3)** (TREBŠE et al., 2016; TROSSINI et al., 2015).

Figure 3: Representation of the enol and keto forms of the avobenzone molecule respectively (TREBŠE et al., 2016).

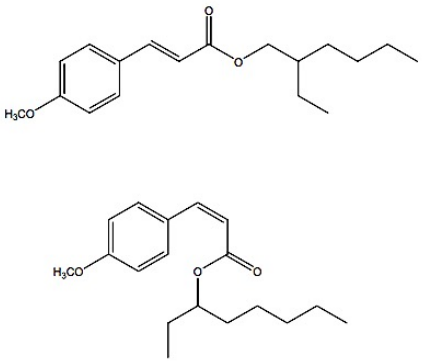


> *"Although avobenzone is stable in organic solvents under UV-irradiation the presence of water brings to photochemical degradation of the molecule with formation of the two pairs of corresponding substituted benzoic aldehydes and acids. The longer is the irradiation, the higher are the levels of these products"* (TREBŠE et al., 2016).

2.4.3. Octyl methoxycinnamate

Octyl methoxycinnamate (molecular weight 290.40; melting point -25°C, boiling point 198°C) is a chemical compound widely used in the cosmetic area as a UVB filter and when exposed to solar radiation the molecule modifies from octyl trans-methoxycinnamate to octyl cis-methoxycinnamate, according to **Figure 4** (PATTANAARGSON et al., 2004).

Figure 4: Structure of octyl meth methoxycinnamate and cis octyl methoxycinnamate respectively (PATTANAARGSON et al., 2004)**.**



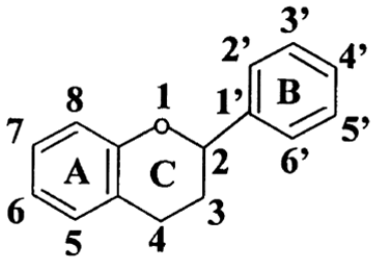
Chemical compounds are combined in order to improve their absorbance of UV rays and consequently increase their sun protection, such as the combination of octicyl methoxycinnamate with avobenzone. Octyl methoxycinnamate is a UVB filter with maximum absorption of about 310nm, however this combination is fully photo-labble (KIM et al., 2015).

2.4. Flavonoids

As previously mentioned, exposure to UV radiation promotes the formation of ROS, which can cause skin cancer in addition to other pathologies. A product with antioxidant activity has the ability to neutralize these ROS, in addition to its photoprotection, such as flavonoids (DEORE; KOMBADE, 2012). The use of natural products such as plants (leaves, fruits, stems) with therapeutic effects is already widely used since the existence of human civilization (RATES, 2001).

The flavonoids are phenolic compounds, which have in their structure aromatic rings and three acetate units, according to **Figure 5**, but there is structural diversity among their classes due to the reactions that may suffer (methylation, hydroxylation, glycosylation among others) (PIETTA, 2000).

Figure 5: Base structure of flavonoids (HEIM; TAGLIAFERRO; BOBILYA, 2002).



Flavonoids can be divided into 14 classes, however six are major:

• flavanoids: have hydroxyl at position 3, for example catechins.

• flavonois: have carbonyl group at position 4, hydroxyl at 3 and double bond between positions 2-3, for example quercetin.

• flavones: have carbonyl group at position 4 and double bond between 2-3, for example apigenins.

• isoflavonoids: have a carbonyl group at the 4-position and ring B is attached to the molecule by means of carbon 3, and may or may not have a double bond between carbons 2-3.

• flavanones: have a carbonyl group at the 4-position (for example myricetin)

• anthocyanidins: have hydroxyl group in position 3 and double bonds, one between 1-2 and the other between 3-4 (HEIM; TAGLIAFERRO; BOBILYA, 2002).

Noroozi et al. (1998) showed that rutin, quercetin and myricetin were more effective than vitamin C in inhibiting hydrogen peroxide-induced oxidative damage in human lymphocyte DNA. In addition to mention that quercetin is one of the most potent antioxidant flavonoids (NOROOZI; ANGERSON; LEAN, 1998).

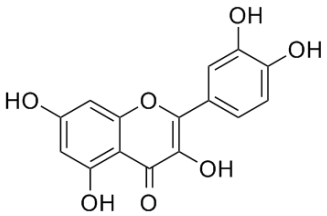
The flavonoids present in certain plants have UVB action, thus being able to adapt in environments that contain large amount of UV rays, thus having the same effects as photoprotector (HARBORNE; WILLIAMS, 2000).

2.4.1. Quercetin

Quercetin, molecular weight 302.24 **(Figure 6)** belongs to the class of flavonois. It has yellow coloration and is practically insoluble in water, but highly soluble in other apolar solvents. Like rutin, it has numerous pharmacological activities, such as antitoxic, antioxidant, anti-aging, antimicrobial, anticarcinogenic, among others (BORSKA et al., 2010; KHALID et al., 2016).

There are several therapeutic effects from quercetin but in topical formulations quercetin does not work properly permeation into the skin, therefore several systems in order to increase their permeation and facilitate their manipulation (since it is insoluble) are studied, such as microparticles, liposomes, nanoparticles and colloidal silica- based emulsion (KHALID et al., 2016; MERKEN; BEECHER, 2000; PRIPREM et al., 2008; SCALIA et al., 2013; SCALIA; MEZZENA, 2009).

Figure 6: Schematic representation of the quercetin structure (MEDCHEM, 2019).



Quercetin has the property of retarding the oxidative injury, removing the oxygen radicals and thus protecting against lipid peroxidation. Also studies showed that formulations containing quercetin prevent oxidative stress of the skin induced by UVB radiation (CASAGRANDE et al., 2007; SCALIA et al., 2013).

3. Aims

3. Aims

3.1. General aim

This research contemplated the comparative analysis of photoprotective and antioxidant potential of sunscreens samples associated with quercetin.

3.2. Specific aims

- Production of formulations containing UV filters associated with quercetin.
- Determination of the organoleptic and physicochemical characteristics of the samples.
- Functional evaluation of isolated active compounds and formulations:
  - antioxidant activity *in vitro*
  - sun protection factor *in vitro*
  - critical wavelength (nm)
  - sample photostability
- Safety assessment:
  - *in vitro* (HET-CAM)
  - *in vivo* (cutaneous biocompatibility)
- Evaluation of the antioxidant potential of the epidermis *ex vivo* (*tape-stripping*).

4. Material

4. Material

4.1. Reagents and solvents

Table 2: Reagents and solvents used in the research.

Material Chemical or commercial name	INCI name	Provider
DPPH	*2,2-Diphenyl-1-picrylhydrazyl*	**Sigma-Aldrich®**
Ethanol	*Alcohol*	**Synth®**
Methanol HPLC grade		**Merck®**
Buffer solutions		**Synth®**

4.2. Raw materials

Table 3: Raw materials used in the research.

Material Chemical or commercial name	INCI name	Provider
Avobenzone	*Butyl Methoxydibenzoylmethane*	**VOLP**
Octyl methoxycinnamate	*Ethylhexyl methoxycinnamate*	**VOLP**
Aristoflex® AVC	*Ammonium Acryloyldimethyltaurate/ VP Copolymer*	**Pharma Special**
Glyceryl cocoate	*Glyceril cocoate*	**Cognis Brasil Ltda.**
Glycerin	*Glycerin*	**Mapric**
Phenonip®	*Phenoxyethanol (and) methylparaben (and) ethylparaben (and) propylparaben (and) butylparaben*	**Pharma Special**
Disodium EDTA	*Dissodium EDTA*	**Deg Impor. De Produtos Químicos**
Quercetin	*Quercetin*	**Viafarma**

4.3. Equipment and accessories

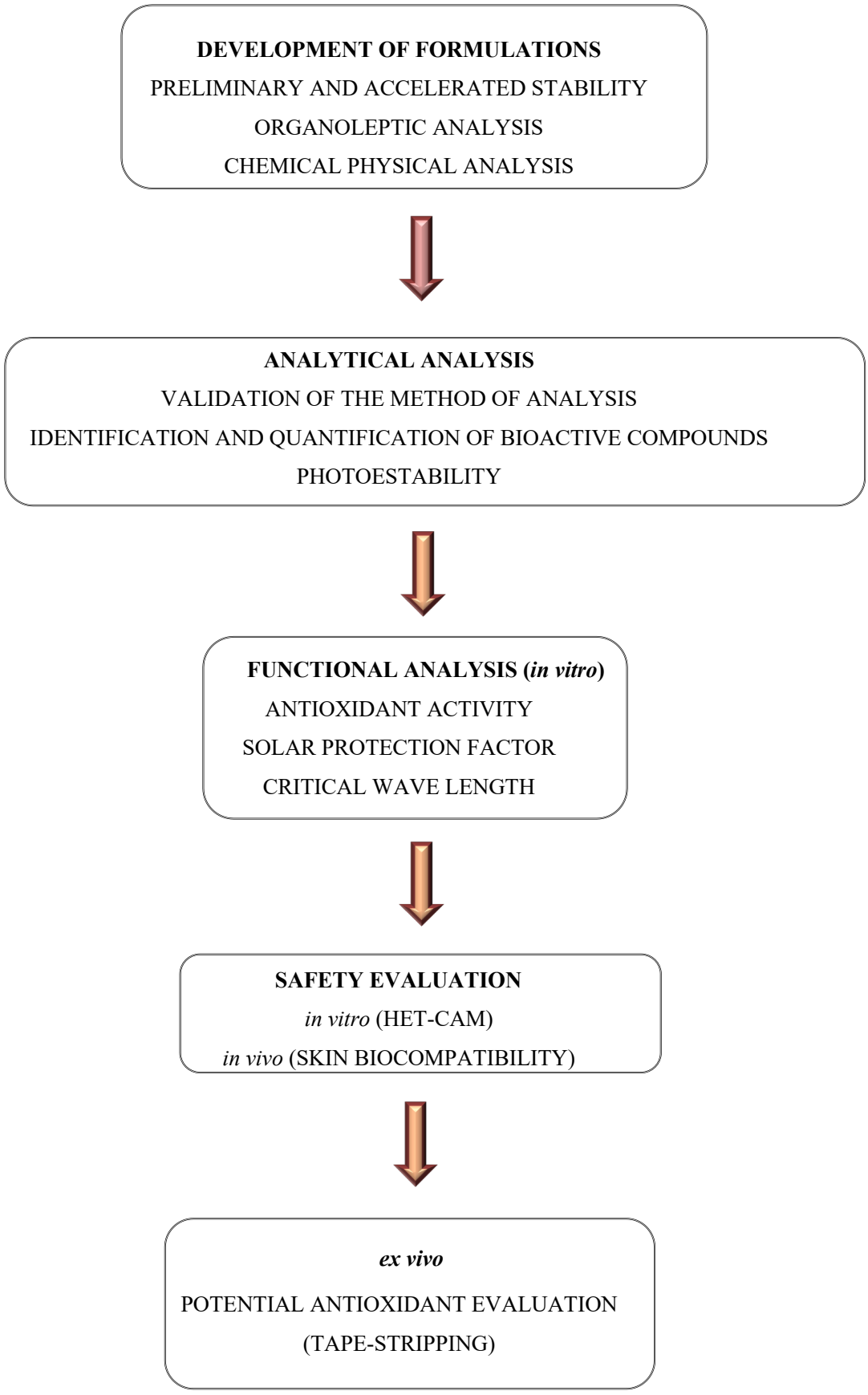
Table 4: Equipment or accessories used in the research.

Equipment or accessories	Brand and model
Adhesive tape for *tape-stripping*	Scoth 750 25mmx50m 3M
Analytical balance	Shimadzu® AUY 220
Camera and software for HET-CAM	Model AM-211, DinoLite®
Centrifuge	Hitachi® RX2
Corneometer CM825	CK Electronics GmbH, Germany
Diffuse reflectance spectrophotometer with integration sphere	Labsphere® UV 2000S
Epicutaneous patches	Finn®Chambers, Epitest®, Finland
Filter membrane with 0.45 μm porosity	Milipore ®
Glass beaker	Laborglas®
Heating plate	HS10, IKA®
HPLC Prominence Auto Sampler	Shimadzu®
Incubator	Chocmaster®
Mechanical stirrer	IKA® RW 20n
Micropipettes 10.0-100.0 μL and 100.0-1.000.0 μL	Eppendorf®
Minolta Chroma Meter CR-300	Minolta Camera Co., Japan
pH meter	Quimis® Q400 AS
Photo-stability camera	Atlas Suntest® CPS+
Polymethylmethacrylate plates (PMMA)	HelioScreen®Helioplate
Polypropylene beaker 150.0mL	Nalgon®
Refrigerator	Consul® *Forst Free* 60
Reverse phase column (250L X 4.6/ Serial No. 2112328	Model Shim-pack VP-ODS- Shimadzu®
Semi-analytic balance	Gehaka® BG 4000
Test tubes	
Tewameter TM 300	CK Electronics GmbH, Germany
Thermostatic bath	Nova Ética® N480
Ultrasonic bath	Unique® Ultracleaner 1600A

Uv-Visible Spectrophotometer	Thermo Scientific® Evolution 600
Volumetric flasks 25.0mL / 50.0mL / 100.0mL	Pyrex®
Vortex Genius 3	IKA®
Water purifier system	Gehaka® OS20 LX

5. Work Flow Chart

5. Work Flow Chart



6. Methods

6. Methods

6.1. Formulations and organoleptic analysis

Formulations, as O/W emulsions, were obtained with compatible ingredients and suitable organoleptic characteristics according to **Table 5**. The choice of the emulsifier was very important to the production of sunscreens. Aristoflex® was selected in considering it is compatible with UV filters (organic and inorganic), good stabilizing properties, viscosity in a wide pH range and sensory properties (CLARIANT, 2019).

Table 5: Composition of the O / W emulsion formulations and the function of the components.

Composition (chemical / commercial name)	**Function**	**F1**	**F2**	**F3**	**F4**
Avobenzone	UVA filter	4.00	4.00	4.00	4.00
Octyl methoxycinnamate	UVB filter	8.00	8.00	8.00	8.00
Aristoflex®	Emulsifying base	4.00	4.00	4.00	4.00
Glyceryl cocoate	Emollient / Stabilizer	4.00	4.00	4.00	4.00
Glycerine	Humectant	5.00	5.00	5.00	5.00
Phenonip®	Preservative system	0.75	0.75	0.75	0.75
Disodium EDTA	Sequestrant	0.30	0.30	0.30	0.30
Quercetin	Flavonoid	0.10	0.20	0.30	----
Ethanol	Solubilizer / co-solvent	20.00	20.00	20.00	20.00
Purified water	Vehicle	q.e.*	q.e.*	q.e.*	q.e.*

*q.e.: quantity enough to

The preparation method of the formulations was the following:

⇒ **Oil phase (A):** avobenzone + octyl methoxycinnamate + glyceryl cocoate were placed in a beaker (A) and heated in a heating plate so that the components solubilized. After solubilizing (approximately 85 °C) they were removed from the plate and cooled until room temperature (25° ± 2°C).

⇒ **Aquous phase (B):** in another beaker (B), quercetin was dissolved in ethanol under stirring and after complete solubilization, the aqueous phase (glycerin + Phenonip®, Disodium EDTA and water) were added and homogenized.

The preparation method was cold emulsification. Therefore, the Aqueous Phase (B) was dropped onto the Oil Phase (A) and Aristoflex® was added under constant mechanical stirring (1500 rpm) until complete emulsification.

The organoleptic analysis was performed macroscopically, evaluating the color, appearance and odor of each formulation (BRASIL, 2004).

Samples classified as:

M: modified; formulations (for instance, visual alteration, color change, precipitation and phase separation) were eliminated from this study (BRASIL, 2004).

N: normal (no change), samples were considered approved and submitted to further tests.

6.2. Preliminary stability

Preliminary stability test was performed after a 24h rest period of the formulations was finished. Centrifugation and thermal stress tests were used in which the samples were analyzed for appearance (physical integrity), color and odor (BABY et al., 2008; BRASIL, 2004).

6.2.1. Centrifugal Testing

Aliquots of 5.0g of each sample were weighed, in triplicate, in semi-analytic balance and transferred to centrifuge tubes and, then, centrifuged at 3000 rpm for 30 minutes (25° ± 2°C) (BRASIL, 2004). Samples were classified as described at **6.1.**

6.2.2. Thermal stress test

Aliquots of 5.0g of each sample were weighed, in triplicate, in semi-analytic balance and transferred to closed glasses and set down to the thermostated bath with temperatures controlled from 40 ± 2 to 80 ± 2 °C, raising the temperature by 10 °C every 30 minutes. Formulations were evaluated at the end of the test, after returning to room temperature (25 ± 2 °C). Samples were analyzed for color change, phase separation or precipitation (BABY et al., 2008). The samples were analyzed for their appearance and classified as described at **6.1**.

6.3. Physicochemical characterization by pH value

The pH values were determined in a pH meter by the direct immersion of the electrode in each sample at room temperature (25 ± 2 °C) (BRASIL, 2004). The pH meter was previously calibrated with buffer solutions pH 4.0 and 7.0 (Synth®). Triplicates were performed.

6.4. Analytical quantification

6.4.1. Method Validation for quantification of UV filters and quercetin

The validation of a method of analysis must be performed in order to guarantee suitability and reliability of the method used according to ICH, 2005.

The HPLC system was used to quantify quercetin, avobenzone and octyl methoxycinnamate in the samples. The data obtained were analyzed by the LC-Solution® Multi-PDA program. Reverse phase column was used (250L X 4.6/ Serial No. 2112328-Model Shim-pack VP-ODS- Shimadzu®). The mobile phase was 88:12 methanol: water,

with a flow rate of 0.8 mL / min. Detection was obtained at 325.0 nm at a temperature of 25.0 ± 1.0 ° C.

6.4.1.1. Linearity

The linearity was determined from the average of three analytical curves obtained by HPLC analysis of solutions containing chemical standards.

Component Purity:
- Quercetin: ≥ 95.0%
- Avobenzone: 99.5%
- Octyl Methoxycinnamate: 99.2%

In order to determine linearity, at least 5 (five) different concentrations of the standard substance should be used for the solutions prepared in at least triplicate (BRASIL, 2017). Dilutions were made in order to obtain concentrations within the working range of each component:

- Avobenzone: 0.40-122ug/mL
- Octyl methoxycinnamate: 0.20-124ug/mL
- Quercetin: 0.20-122ug / mL

The samples were filtered on a 0.45µm membrane (Millipore®) and placed in vials. The analytical curves were constructed from the concentration values as a function of the peak area. The calculation of the equation of the straight line of the analytical curve was performed by means of the linear regression by least squares method (BRASIL, 2017).

6.4.1.2. Specificity and interferent evaluation

The specificity was obtained by comparing the chromatograms of each component used to obtain the samples, in order to verify the time of the retention peak of each substance and by the chromatogram of the sample in order to verify the presence of interferents in the same peaks of the substances analyzed (quercetin, avobenzone and octyl methoxycinnamate).

Dilutions of the sample and the standard solutions were made with methanol in volumetric flasks to obtain total solubilization of the same and after they were filtered on a 0.45µm membrane (Millipore®) and placed in vials.

6.4.1.3. Precision

The determination of the intra-run and inter-run precision was performed by the preparation of quercetin, avobenzone and octyl methoxycinnamate solutions, the primary reference standard, a minimum of 9 determinations covering the specified range for the procedure (e.g., 3 concentrations/3 replicates each) (ICH, 2005).

The precision was calculated by describing the standard deviation (SD) and percentage of variation coefficient (CV%) of the absorbance values obtained in the HPLC analysis of three samples with known concentrations of each substance (avobenzone, octyl methoxycinnamate and quercetin) that were within the working range. The tests were done in triplicate. **Equation 1** describes the calculation of CV%.

Equation 1: $$CV\ \% = \frac{SD}{DMC}$$

Where:

CV % = coefficient of variation

SD = standard derivation

DMC = mean concentration determined

6.4.1.4. Accuracy

Evaluated by the recovery method, using known concentrations (low, medium and high) in order to verify the proximity of the obtained results to the true value (ICH, 2005).

Accuracy (%) was performed in replicates of three and **Equation 2** was applied to determine the recovery percentage.

Equation 2: $$Recovery = \frac{Experimental\ Average\ Concentration}{Real\ Concentration} \times 100$$

6.4.1.5. Detection Limit

The detection limit was calculated according to **Equation 3** (ICH, 2005), described below:

Equation 3: $$DL = 3{,}3 \cdot \sigma / S$$

Where: σ: the standard deviation of the response S

S: the slope of the calibration curve

6.4.1.6. Quantification limit

The quantification limit is smaller sample quantity (precise and accurate) achieved by the instrument used. The quantification limit was calculated according to **Equation 4** (ICH, 2005), described below:

$$QL = 10 \cdot \sigma / S$$

Where: σ: the standard deviation of the response S

S: the slope of the calibration curve

6.5. Quantification of UV filters and quercetin in formulations and in PMMA plates.

Solutions: 40mg of each formulation was (in triplicate) dissolved in a 50mL volumetric flask completed with methanol. Theses flasks were vigorously stirred until complete solubilization of the formulation. Samples were filtered on a 0.45μm membrane (Millipore®) and placed in vials for HPLC assay.

PMMA: 2mg / cm^2 were spread out circularly on the PMMA plates and dried for 24 hours at room temperature (25 ± 2 °C). After, plates were immersed in methanol for 30 minutes and, then, another 30 minutes of ultrasound. Methanol was removed, filtered on 0.45μm membrane (Millipore®) and placed in vials for HPLC analysis.

The data obtained were analyzed by the LC-Solution Multi-PDA program. Reverse phase column was used (250L X 4.6/ Serial No. 2112328- Model Shim-pack VP-ODS- Shimadzu®). The mobile phase was 88:12 methanol: water, with a flow of

0.8mL/min. Detection was obtained at 325.0 nm at a temperature of 25.0 ± 1.0 ° C and compared by the percentage of recovery.

6.5. Functional analysis (*in vitro*)

6.5.1. Antioxidant activity

The reaction with DPPH is a colorimetric reaction, since the DPPH before the reaction has violet color and, after, it turns yellow. It can thus be easy measured in a spectrophotometer at 515nm (MISHRA; OJHA; CHAUDHURY, 2012).

6.5.1.1. Preparation of DPPH solutions

A DPPH amount of 2.40 mg of DPPH was weighed in an analytic balance and transferred to a 100 mL volumetric flask. Volume was completed with methanol, homogenized at room temperature (25 ± 2 °C) and, after, stored in an appropriate labeled amber glass bottle. DPPH solutions were prepared for each day of test and concentration was 60μM.

6.5.1.2. Determination of the antioxidant activity of the formulations containing quercetin

Aliquots of 5.0 mg of each formulation (in triplicate), were weighed in an analytic balance and transferred to test tubes. Methanol (2mL) was added and vigorously stirring used until complete solubilization. After 2.0 mL of the DPPH solution were added and rest in dark for 30 minutes at room temperature (25 ± 2 °C). Afterwards, they were analyzed in a Uv-Visible Spectrophotometer at a wavelength of 515 nm in a 1.0 cm path quartz glass. Methanol was used as the negative control, and, the positive control, was the methanolic solution of DPPH • at 60 μM.

The antioxidant activity was expressed by the DPPH • percent inhibition of the samples, according to the **Equation 5.**

Equation 5: $$\%inhibition = \frac{Abs\ control - Abs\ sample}{Abs\ control}\ x\ 100$$

Where:
Abs control = absorbance of the DPPH solution;
Abs sample = absorbance of the DPPH solution with the sample to be analyzed.

6.5.2. Photoprotective efficacy

The *in vitro* protective efficacy of the samples was determined using the diffuse reflectance spectrophotometer with integration sphere. Samples of 2.0 mg / cm^2 were spread out circularly on PMMA plates, and dried for 30 minutes at room temperature (25 ± 2 °C). The blank was a PMMA plate with glycerin. The spectrophotometric values of the samples applied to the substrate, comprising the wavelength range (λ) between 290.0 and 400.0 nm and records at each 1.0 nm, determined the values of SPF, UVA / UVB ratio and critical wavelengths (λc) through the UV-2000S® program. For each sample, replicates of three were analyzed and seven readings per plate evaluated (COSMETICS EUROPE, 2011).

The SPF was calculated according to the **Equation 6** (DIFFEY; ROBSON, 1989).

Equation 6 : $$SPF = \frac{\sum_{250nm}^{450nm} S(\lambda)EA(\lambda)}{\sum_{250nm}^{450nm} S(\lambda)EA(\lambda)T(\lambda)}$$

Where:
S(λ)= spectral solar irradiance;
EA(λ)= spectral erythematogenicity of CIE (*Comission Internationale de l'Eclairage*);
T(λ)= spectral transmittance of the sample.

6.5.3. Critical wavelength (λc)

The critical wavelength (λc) was calculated according to the **Equation 7**.

$$\textbf{Equation 7:} \qquad \lambda c = \frac{\sum_{\lambda=250}^{\lambda=\lambda c} A\,(\lambda)}{\sum^{\lambda=450} A\,(\lambda)}$$

Where:

A(λ) = spectral absorbance of the sample.

The results will be analyzed statistically according to the most adequate test and its variables.

6.5.4. *In vitro* and chemical evaluation of photostability

After performing the *in vitro* photoprotection test, the PMMA plates containing the samples were irradiated in a photo-stability camera prior to reading in diffuse reflectance spectrophotometer with integration sphere. Samples were applied in PMMA plates, as a thin film, in the ratio of 2.0 mg/cm^2 and were irradiated with a fixed dose of 800 J/m^2 (four times the eritematogenic dose). Temperature was controlled to avoid overheating. (SOOD; PH, 2011).

The SPF and critical wavelength parameters, pre and post-irradiation, were analyzed and compared. For each sample, replicates of three were analyzed (SCALIA; MEZZENA, 2010).

After measuring the SPF and wavelength in Labsphere® the plates were immersed in methanol for 30 minutes and, then, another 30 minutes of ultrasound. Methanol was removed, filtered on 0.45μm membrane (Millipore®) and placed in vials for HPLC analysis.

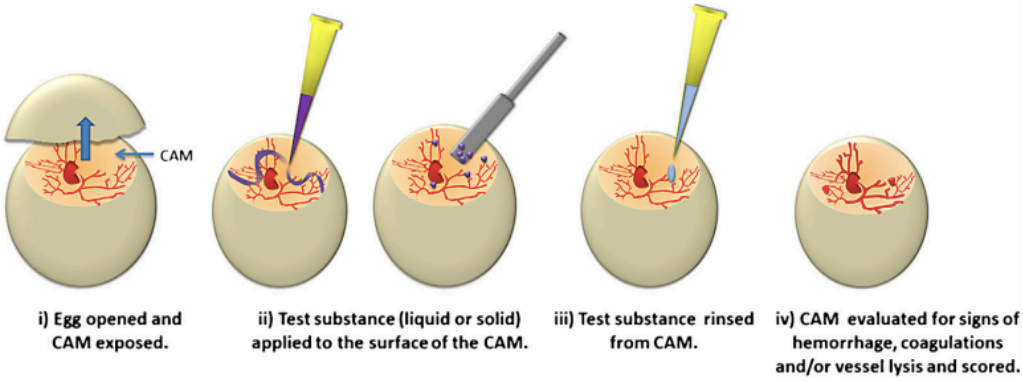
6.6. *In vitro* safety evaluation by HET-CAM – Test of the choroid-allantoic membrane of the chicken egg

The methodology used was based on the official method of assessing the irritant potential described in *Journal Officiel de La Republique Française – Arreté du 29 Novembre 1996*. The formulation was applied to the chorioallantoic membrane (MCA) of the chicken egg on the tenth day of incubation, and the presence or absence of irritant

effects such as hyperemia, hemorrhage and coagulation or opacity were observed. For each formulation, three fertilized eggs of *Leghorn* breed were used.

The eggs were incubated (incubator Chocmaster®), for 10 days at 37°C ± 0.5°C with relative humidity of 70%. After this procedure, the eggs were placed in a vertical position, on a support, with the air chamber facing upwards. The eggshell was removed with the aid of metallic clamp which exposed the chorioallantoic membrane, that was humidified with physiological solution (NaCl 0.9%). An aliquot of 300 Ml of the formulation was applied to the chorioallantoic membrane and after 20 seconds of contact, it was washed with 5 Ml of physiological solution for removal of samples **(Figure 7).**

Figure 7: Schematic representation of Hen´s Egg Test – Chorioallantoic Membrane (HET-CAM) (WILSON; AHEARNE; HOPKINSON, 2015).



For 5 minutes, vascular effects were recorded by the DinoLite® model AM-211 software. Subsequently the images were evaluated according to period of 5 minutes, according to the scale described in **Table 6**. The irritant phenomena observed were graded in numerical values (1, 3, 5, 7 and 9), time dependent.

Table 6: Graduation of the irritant phenomena determined by time.

Phenomenon	Less than 30 seconds	Between 30-60 seconds	Between 60-300 seconds
Hyperemia	5	3	1
Hemorrhage	7	5	3
Coagulation/Opacity	9	7	5

Each formulation was analyzed with 3 eggs and a positive control was made with sodium dodecylsulfate (SDS) at 1% and sodium hydroxide (NaOH) at 1% and negative control with physiological solution (NaCl 0.9%). The classification of each formulation was obtained with the average of the graduation values of the 3 eggs, the degree of irritation was divided into four categories, described in **Table 7**.

Table 7: Mean of the graduation of the irritant phenomena and the final classification of the degree of irritation of the evaluated formulations.

Mean of the graduation values of irritant phenomena	Final classification of the degree of irritation of the formulations evaluated
0.00 to 0.99	Non-irritating (NI)
1.00 to 4.99	Mild irritant (LI)
5.00 to 8.99	Moderate irritant (MI)
9.00 to 21.00	Severe irritant (SI)

6.7. *In vivo* analysis: Skin biocompatibility

The cutaneous biocompatibility test should be done in conjunction with the Lusófona University, at the CBIOS (Center for Research in Biosciences & Health Technologies), located in Lisbon, Portugal for a period of two months. The analysis should be done with volunteers based on pre-established criteria, such as age, sex, conditions for the experiment and consent of the responsible Ethics Committee. Skin patches containing each of the formulations to be tested and water (negative control) will be applied to the forearms of the volunteers for 24 hours. Values were measured prior to the application of the patches and 2 hours after the patches were removed. To minimize the effect of variability the results were analyzed from the ratio between the values obtained 2 hours after the application of the patches and the values before the application.

The cutaneous compatibility was tested in a panel of 11 male and female volunteers, mean age 23.8±4.0 years, after informed consent. The procedures were performed in accordance with the ethical standards of the responsible committee on human experimentation and with the Helsinki Declaration. The volunteers were interviewed and evaluated by a professional, considering the inclusion and exclusion criteria:

Criteria for inclusion:
(i) healthy participants;
(ii) both sexes;
(iii) age between 18 and 60 years;
(iv) whole skin in the test region;
(v) agreement to join the study procedures.

Exclusion criteria:
(i) gestation or lactation;
(ii) history of phototoxic reactions, photoallergic or photoinduced pathologies;
(iii) personal or family of skin cancer;
(iv) use of anti-inflammatory medications, immunosuppressive or photosensitizing agents (topical or systemic) one month before the start of the study;
(v) the presence of sunburn or tan at the test site;
(vi) uneven skin tone in the region of the study;
(vii) presence of inflammatory dermatoses, nevus lesions, spots, seborrheic keratosis, active pathologies or excess hair at the test site;
(viii) known allergies to any component of formulations.

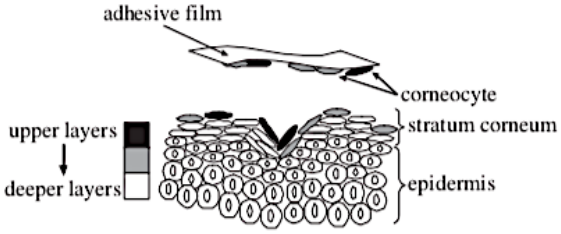
Epicutaneous patches (Finn® Chambers, Epitest®, Finland) were applied to the volar forearms of the volunteers for 24 h. Each patch had five chambers, each containing either one of the formulations tested or water. Non-invasive measurements of cutaneous biophysical properties were made at each site tested. Skin barrier function was probed with a Tewameter® TM 300, according to published guideline (PINNAGODA et al., 1989) and stratum corneum (SC) hydration was assessed with a Corneometer® CM825. In colorimetry, the parameter a* reflects the red chromaticity and was therefore used to quantify an increase in erythema, using a Minolta Chroma Meter® CR-300. All measurements were performed in triplicate using the CIE Lab system. The basal values were determined before patch application, and further measurements were made at 24 h, 1 h after patch removal. To minimize the effect of inter-individual variability, the results were analyzed as the ratio between the values obtained after patch application and the basal values (PIÉRARD, 1998).

6.8. "*Ex vivo*" functional characterization

6.8.1. Antioxidant activity – Tape-stripping

Aliquots of 2.0 mg / cm² of the formulations were applied uniformly over previously cleaned pig ears (the fat was removed and the hairs cut). Three applications in two-hour intervals between each formulation were performed, according to the current recommendations for reapplications of sunscreens (MILESI; GUTERRES, 2002). After two hours of the last application the tape-stripping technique was used to removal the stratum corneum **(Figure 8).**

Figure 8: Schematic of the tape-stripping test (ZAHOUANI, 2011).



Same pressure was placed on each tape for 3 seconds and removed quickly. Up to 20 consecutive tapes were used, but the first two were discarded (KLANG et al., 2012).

Then, all the tapes were exposed to UV radiation in a photo-stability chamber, with a dose of 4177 KJ / m^2. Subsequently, the stratum corneum was extracted from the tapes with methanol followed by ultrasound for 15 minutes. Dispersions with the stratum were added 2.0 mL of DPPH solution (waiting about 60 minutes) and evaluated for antioxidant activity through the DPPH assay (both the irradiated tapes and the non-irradiated tapes), previously described in **6.6.1.**

The ear skins used were cut into small pieces and vortexed for 5 minutes with methanol and, after, they were added 2mL of DPPH solution (60 minutes of reaction). At the end of this time, the samples were evaluated at Uv-Visible spectrophotometer at a wavelength of 515 nm in a 1.0 cm optical path quartz glass. As a negative control, methanol was used.

6.9. Statistical analysis of results

Data were evaluated in the MiniTab® program version 18. The analyzes were performed in triplicate, and the significance level was 5.0% ($p \leq 0.05$). In comparisons between two samples, the results were evaluated according to the t-Student statistical test. The comparisons among three or more samples were ANOVA One-Way statistical test followed by the Tukey test was used.

7. Results and Discussion

7. Results and discussion

7.1. Formulations and organoleptic analysis

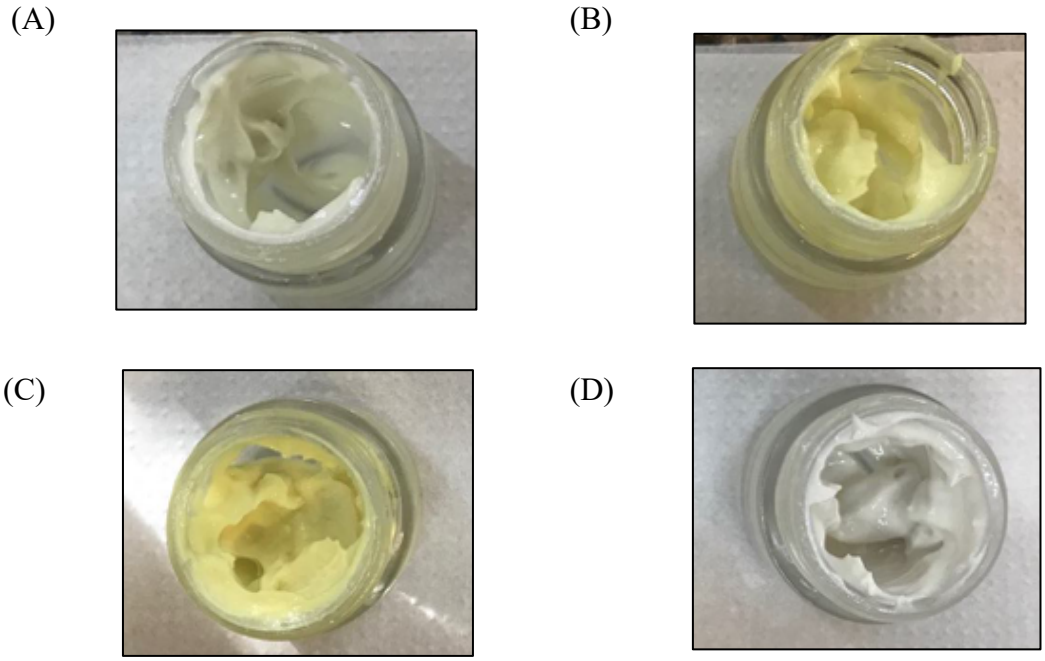
Emulsions are widely used in cosmetic products since they have excellent visual appearance (homogeneity) and they are soft to the touch (COLOMBO, 2018). In order to choose the components of the formulation, the characteristics of the product must be taken into account, since the interaction among the components, pH value, polarity can displace the UV filter absorption, modifying the effectiveness of the sunscreens (VELASCO et al., 2012).

Sunscreens are made in different cosmetic forms (emulsion, gel, aerosol), the O/W emulsion selected for this study being one of the most frequent (BALOGH; PEDRIALI; KANEKO, 2011). The effectiveness of a sunscreen is directly related to its components, so that the choice of the ingredients is as important as the selection of the other into the formula. In a emulsion containing UV filters, the solvent, the emollient and the emulsifier are determining factors of its substantivity and stability (MILESI; GUTERRES, 2002).

The production of the formulations was by a cold emulsification process as previously stated. During the preparation, the raw materials and actives were used in order to obtain safe, effective and cosmetically acceptable samples to the consumer.

The organoleptic analysis was evaluated by visualization and verification of color, phase separation and odor. The three formulations obtained yellowish coloration, but the higher the concentration of quercetin, the more yellowish the formulation (**Figure 9**). Appearance was uniform and odor characteristic was of the raw materials and unchanged throughout the test.

Figure 9: Final appearance of solar filters associated with quercetin formulations. (A) 0.1% quercetin; (B) 0.2% quercetin; (C) 0.3% quercetin and (D) control.

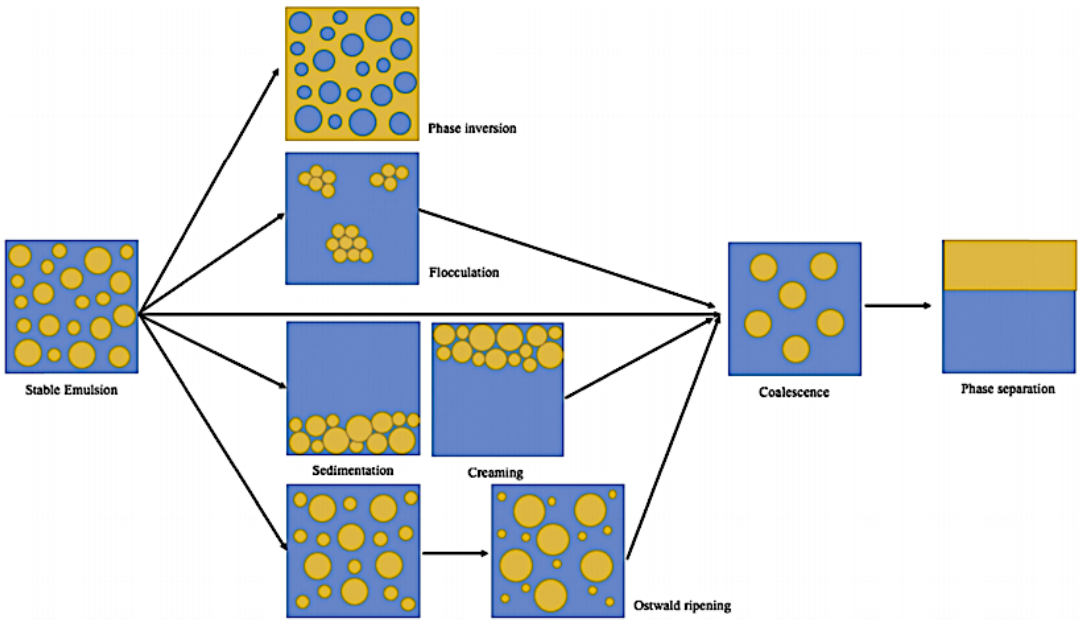


The vehicle in which quercetin is employed influences its effectiveness, as well as, its release when topically applied. However there is the adversity of quercetin solubilization of in vehicles (CASAGRANDE et al., 2007). Topical administration of antioxidants may be a good alternative to reduce the oxidative damage caused by UV radiation in the skin (SAIJA et al., 1998).

7.2. Preliminary stability

Studies of the stability of cosmetic products help to guide the development of the formulation (choice of raw materials) and the choice of packaging; estimate the expiration date, as well as assist in monitoring of physic-chemical, organoleptic and stability, producing information about the reliability and safety of products (BRASIL, 2014). The stability of the emulsion is due to the behavior of the phases of the dispersions, for example the an hydrophilic and the lipophilic parts (COLOMBO, 2018). Some of these instabilities are described in **Figure 10.**

Figure 10: Representation of the phenomena of physical instability of emulsions (HU et al., 2016)**.**



The stability of the four sunscreens samples was verified by the centrifugation and thermal stress tests in order to accelerate possible instabilities of the system, as seen in **Table 8**.

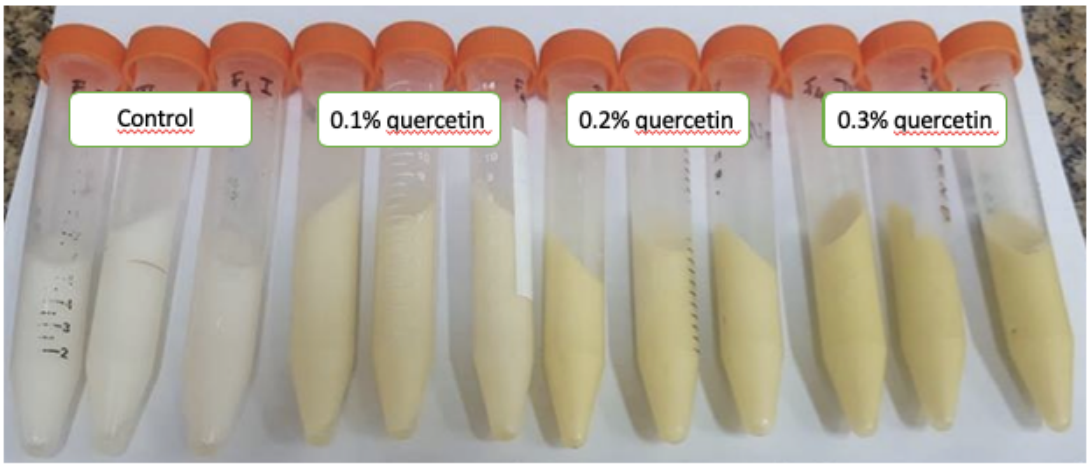
Table 8: Characteristics of sunscreens samples subjected to thermal stress and centrifugation tests, with N (normal) and M (modified).

	Preliminary stability	
Formulations	**Centrifugation tests**	**Thermal Stress Tests**
F1	N	N
F2	N	N
F3	N	N
F4	N	N

The emulsions presented a stable and homogeneous appearance (**Figure 11**). In some studies, quercetin was microencapsulated or carried by silica nanoparticles in order to increase its stability in topical formulations, obtaining positive results compared to its free form (SCALIA et al., 2013; SCALIA; MEZZENA, 2009).

The centrifugation and thermal stress tests were efficient to select the emulsions that were submitted to the next tests.

Figure 11: Photo of the formulations after the centrifugation test, being, respectively, control, 0.1% quercetin, 0.2% quercetin and 0.3% quercetin and in triplicate each formulation.



According to Scalia & Mezzena (2009) the concentrations that obtained better stability reached 1.0% and in the present study with up to 0.3% we obtained excellent results of stability in the emulsions. None of the emulsions showed flocculation, phase inversion, coalescence, sedimentation or phase separation during all the tests.

7.3. Physicochemical characterization by pH value

The pH of the formulations should have the pH closer to the skin (4.6 - 5.8). The acid pH assists in the bactericidal and fungicidal functions of the skin surface. Some skin secretions have a buffering capacity, since the skin has its pH altered by several inappropriate topical products (LEONARDI; GASPAR; CAMPOS, 2002). The results for pH are in **Table 9**, in which they were all according to the literature and, consequently assisting the function of the activities for topical use.

Table 9: pH of the photoprotective samples measured in pH meter.

Samples	pH
F1	5.5 ± 0,1
F2	5.3 ± 0,1
F3	5.3 ± 0,1
F4	5.1 ± 0,2

The pH value of a formulation shall ensure the stability of the components, their effectiveness and safety, as well as being compatible with the biological fluids according to the intended route of administration, in the case of topical application. The value of the pH of the preparations was considered adequate and no correction was necessary (BRASIL, 2004).

7.4. Analytical quantification

7.4.1. Method Validation

The validation of an analytical method is a key part of its reliability in the purpose to be analyzed, in this case the quantification of quercetin and UV filters in the samples by HPLC (VAN ZOONEN et al., 1999). The validation of an analytical method is used so that we can confirm or not the suitability of the method chosen for the analysis to be done. Therefore, all results of analytical parameters must comply with current standards (GIUDICE, 2008).

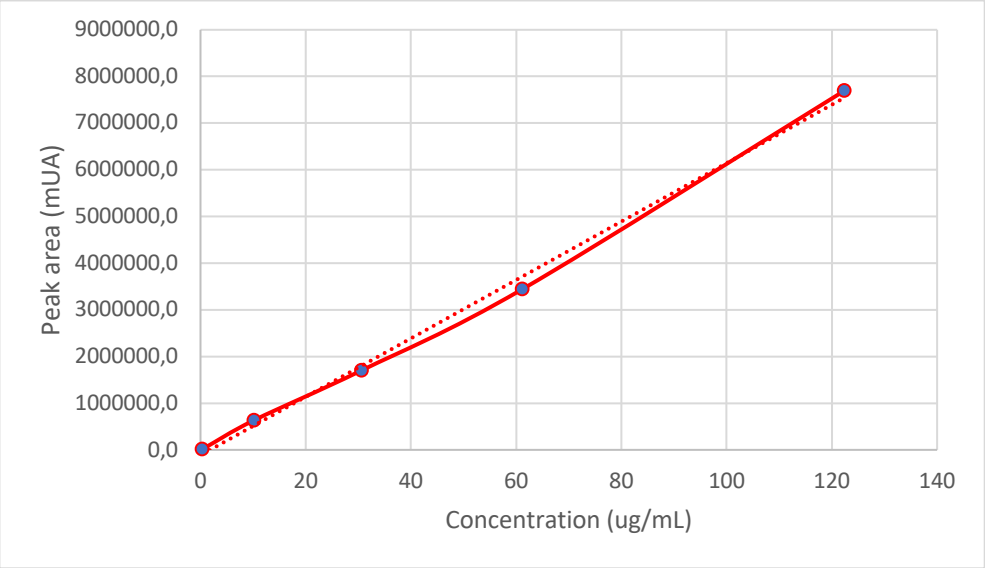
The first use of HPLC in the characterization of flavonoids occurred in 1976 and since then, several studies have used this methodology for its quantification. HPLC is a highly reliable instrument for analyzing complex samples as creams as it provides the separation and quantification of components and removes most interferents (CASAGRANDE et al., 2009; MERKEN; BEECHER, 2000).

7.4.1.1. Linearity

Linearity determines the range of analyte concentrations to be analyzed. It ensures that the results are proportional to the analyte concentration (ICH, 2005).

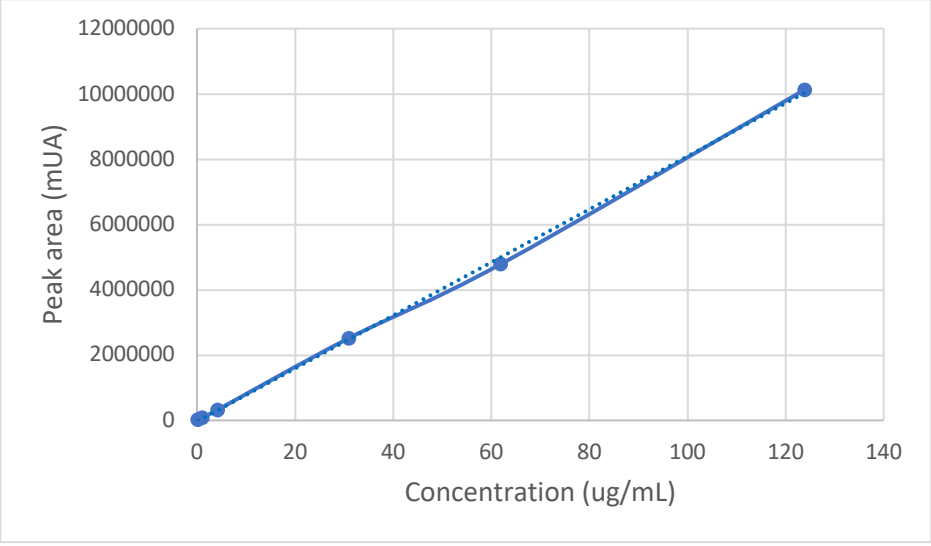
Linearity was done with substances of interest: avobenzone **(Figure 12)**, octyl methoxycinnamate **(Figure 13)** and quercetin **(Figure 14).** And in the chosen concentrations (within the working range) the three substances were linear, that is, results proportional to the concentrations.

Figure 12: Avobenzone standard curve on HPLC. The concentrations chosen were between 0.4 – 122.0ug/mL.



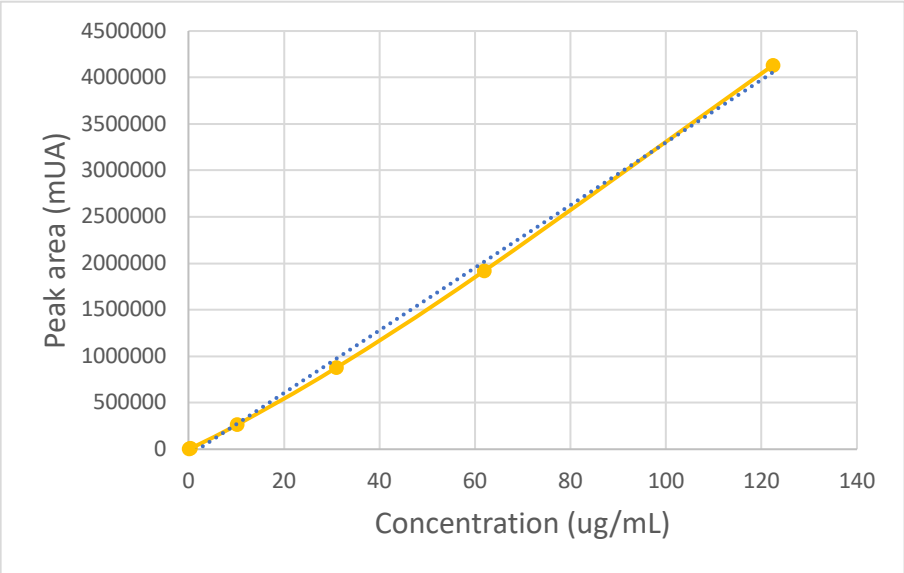
Legend: Chromatographic conditions: Reverse phase column was used (250L X 4.6/ Serial No. 2112328- Model Shim-pack VP-ODS- Shimadzu®). The mobile phase was 88:12 methanol:water, with a flow of 0.8 mL / min. Detection was obtained at 325.0 nm at a temperature of 25.0 ± 1.0 ° C.

Figure 13: Standard curve of octyl methoxycinnamate on HPLC. The concentrations chosen were between 0.2-124.0 ug/mL.



Legend: Chromatographic conditions: Reverse phase column was used (250L X 4.6/ Serial No. 2112328- Model Shim-pack VP-ODS- Shimadzu®). The mobile phase was 88:12 methanol:water, with a flow of 0.8 mL / min. Detection was obtained at 325.0 nm at a temperature of 25.0 ± 1.0 ° C.

Figure 14: Standard curve of quercetin in HPLC. The concentrations chosen were between 0.2-122.0ug/mL.



Legend: Chromatographic conditions: Reverse phase column was used (250L X 4.6/ Serial No. 2112328- Model Shim-pack VP-ODS- Shimadzu®). The mobile phase was 88:12 methanol:water, with a flow of 0.8 mL / min. Detection was obtained at 325.0 nm at a temperature of 25.0 ± 1.0 ° C.

The analytical curve of the standard solutions (avobenzone, octyl methoxycinnamate and quercetin) was constructed by plotting the area of the main plot versus active concentration. They were found to be linear over a wide concentration range with correlation coefficient of at least 0.99 for all (**Table 10).** According to the literature (BRASIL., 2003), the correlation coefficient (r^2) must be above 0.990 The analytical curves presented characteristics of linearity, in agreement with the normative determinations (BRASIL., 2003; ICH, 2005).

Table 10: Linear Regression Analysis.

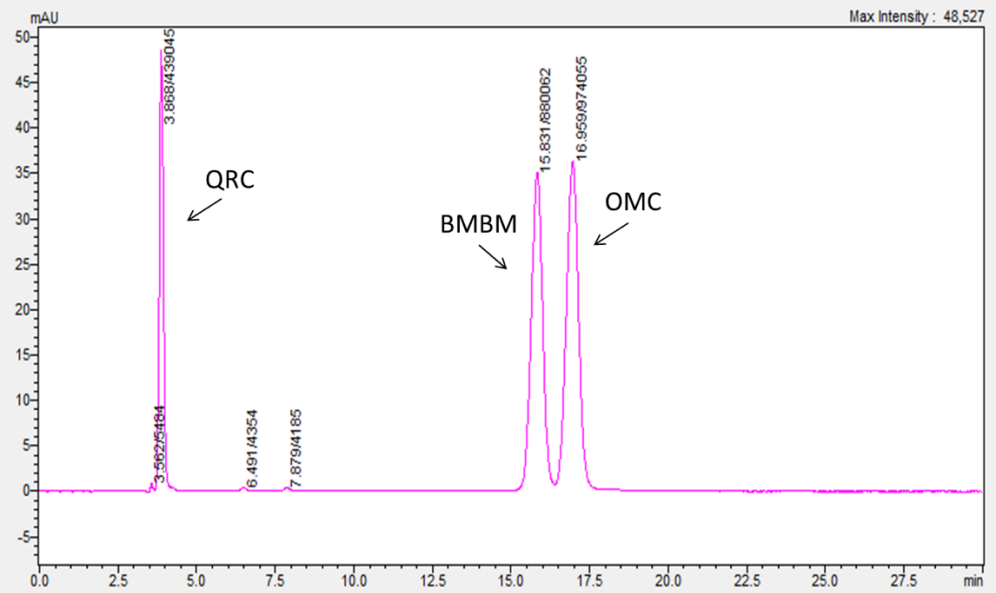
	Equation of the line	**Correlation Coefficient**
Avobenzone	y = 62586x - 114758	R^2 = 0,9966
Octyl methoxycinnamate	y = 81262x - 35167	R^2 = 0,9993
Quercetin	y = 33699x - 69163	R^2 = 0,9974

Legend: y = peak area, x = concentration of analyte and r = correlation coefficient.

7.4.1.2. Specificity and interferent evaluation

For the evaluation of the specificity it was performed the comparison of the chromatograms of the standards solutions. The first test was made with the three substances, according to **Figure15.**

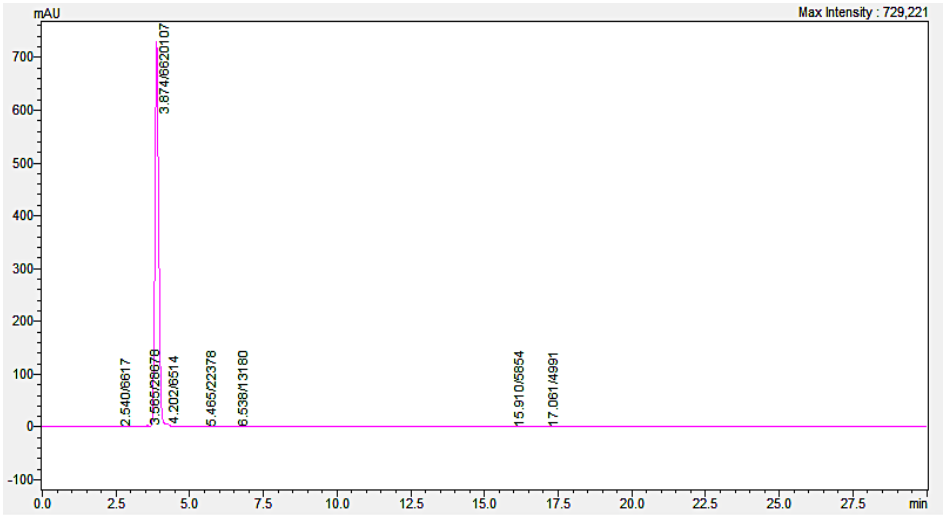
Figure 15: Chromatogram of avobenzone, octyl methoxycinnamate and quercetin by HPLC.



Legend: QRC: quercetin; BMBM: avobenzone; OMC: octyl methoxycinnamate. Chromatographic conditions: Reverse phase column was used (250L X 4.6/ Serial No. 2112328- Model Shim-pack VP-ODS- Shimadzu®). The mobile phase was 88:12 methanol:water, with a flow of 0.8 mL / min. Detection was obtained at 325.0 nm at a temperature of 25.0 ± 1.0 ° C.

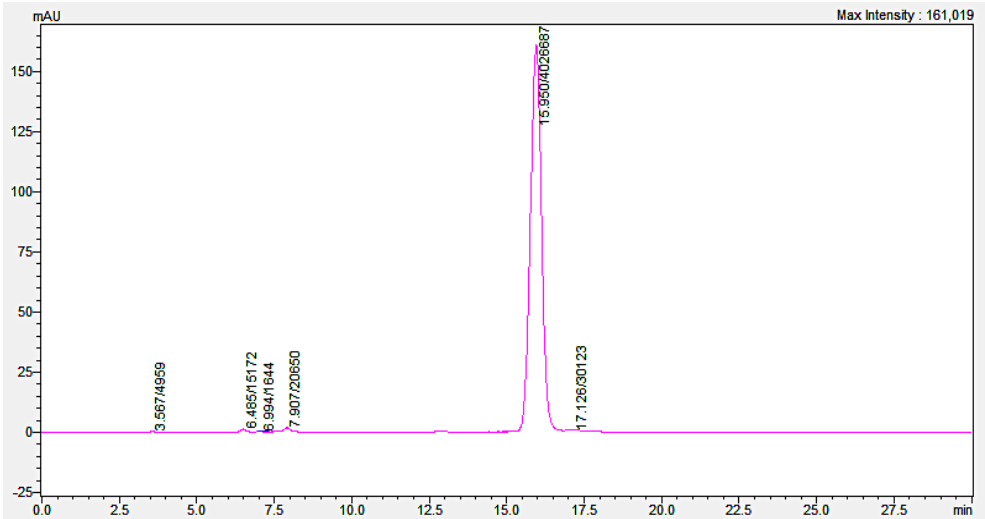
The second step was to analyze the peaks of each substance separately. The analysis of the chromatograms indicated that the flavonoid quercetin had the retention time of 3.8 minutes **(Figure 16)** avobenzone had the retention time of 15.95 minutes **(Figure 17)** and octyl methoxycinnamate had the retention time of 17.11 minutes **(Figure 18)**.

Figure 16: Quercetin chromatogram by HPLC and retention time 3.8 minutes.



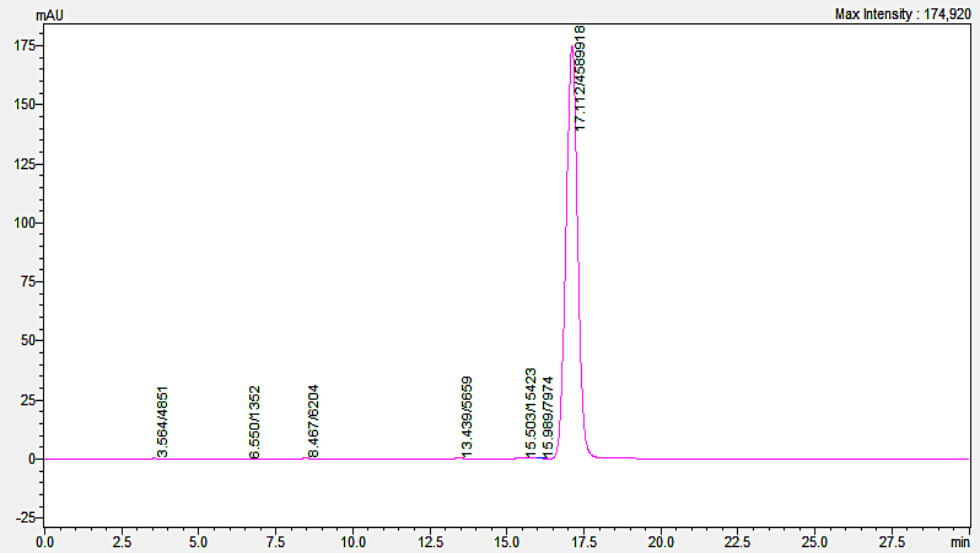
Legend: Chromatographic conditions: Reverse phase column was used (250L X 4.6/ Serial No. 2112328- Model Shim-pack VP-ODS- Shimadzu®). The mobile phase was 88:12 methanol:water, with a flow of 0.8 mL / min. Detection was obtained at 325.0 nm at a temperature of 25.0 ± 1.0 ° C.

Figure 17: Avobenzone chromatogram by HPLC and retention time 15.95 minutes.



Legend: Chromatographic conditions: Reverse phase column was used (250L X 4.6/ Serial No. 2112328- Model Shim-pack VP-ODS- Shimadzu®). The mobile phase was 88:12 methanol:water, with a flow of 0.8 mL / min. Detection was obtained at 325.0 nm at a temperature of 25.0 ± 1.0 ° C.

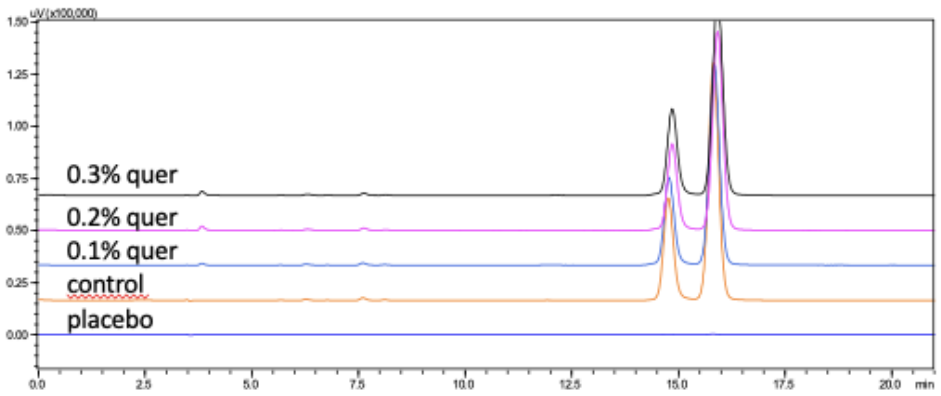
Figure 18: Chromatogram of octyl methoxycinnamate by HPLC and retention time 17.11 minutes.



Legend: Chromatographic conditions: Reverse phase column was used (250L X 4.6/ Serial No. 2112328- Model Shim-pack VP-ODS- Shimadzu®). The mobile phase was 88:12 methanol:water, with a flow of 0.8 mL / min. Detection was obtained at 325.0 nm at a temperature of 25.0 ± 1.0 ° C.

First, the substances were analyzed in isolation in order to know the retention time of each of them and to know if they overlapped the peaks. Since the retention times were different, the formulations were analyzed in order to investigate the presence of interferents **(Figure 19).**

Figure 19: Chromatogram of the samples (0.1%, 0.2%, 0,3% of quercetin and control) and placebo.



Legend: Chromatographic conditions: Reverse phase column was used (250L X 4.6/ Serial No. 2112328- Model Shim-pack VP-ODS- Shimadzu®). The mobile phase was 88:12 methanol:water, with a flow of 0.8 mL / min. Detection was obtained at 325.0 nm at a temperature of 25.0 ± 1.0 ° C.

Chromatograms showed that the method was specific since the peaks in the chromatograms were independent in the established concentration range and there were no interferences at the peaks of interest.

7.4.1.3. Precision and Accuracy

Precision refers to the variability of the results in repeated analyzes of the sample under similar experimental conditions. The method was validated by intra- and inter-day precision evaluation (ICH, 2005). Evaluates the proximity of the results to be obtained in a series of measurements (three samples with known concentrations: low, medium and high) of different solutions. Calculate the standard deviation and coefficient of variation.

The precision and accuracy are presented in **Table 11**. The method is considered precise, since all the samples obtained Relative Standard Deviation below 5%, according to the current norms**.** Accuracy expresses closeness between the actual value and the analytical result obtained. Values close to 100% are desirable, but accepted between 80-120% (BRASIL, 2017). The results presented were within the established limits.

Table 11: Method of precision and accuracy for quercetin, avobenzone and octyl methoxycinnamate (OMC) quantified by HPLC.

	Concentration ug /mL	Average	Standard deviation	CV %	Accuracy
QUERCETIN	0.40	8269.99	161.66	2.0	98.04%
	61.20	2094421.56	36500.02	1.7	98.03%
	122.40	4655225.56	54954.37	1.2	99.67%
AVOBENZONE	1.02	54472.00	782.06	1.4	98.04%
	2.04	110122.00	1269.89	1.2	98.04%
	4.08	224811.30	2863.24	1.3	98.03%
OMC	0.41	25306.33	150.60	0.6	97.56%
	2.04	154333.00	1296.67	0.8	98.04%
	10.32	1101071.33	993.31	0.1	96.90%

Legend: Chromatographic conditions: Reverse phase column was used (250L X 4.6/ Serial No. 2112328- Model Shim-pack VP-ODS- Shimadzu®). The mobile phase was 88:12 methanol:water, with a flow of 0.8 mL / min. Detection was obtained at 325.0 nm at a temperature of 25.0 ± 1.0 ° C.

7.4.1.4. Detection Limit

The detection limit is the lowest detectable and acceptable concentration that can be detected by the instrument used, but not necessarily quantified with acceptable precision and accuracy (BRASIL, 2017).

By using Equation 3 and the information presented in Table 7, the estimated detection limits of the reference standard solutions are:

- Quercetin: 0.0933 ug /mL
- Avobenzone: 1.978 ug /mL
- Octyl Methoxycinnamate: 2.468 ug /mL

7.4.1.5. Quantification Limit

By using Equation 4 and the information presented in Table 7, the estimated quantification limits of the reference standard solutions are:

- Quercetin: 0.3112 ug /mL
- Avobenzone: 6.594 ug /mL
- Octyl Methoxycinnamate: 8.229 ug /mL

7.5. Quantification of UV filters and quercetin in PMMA plate.

The quantification of the actives (avobenzone, octyl methoxycinnamate and quercetin) was compared from the ones in solution and applied on PMMA plates. Recovery near 100% was desired as we can observed in **Tables 11**. Amounts of UV filters and quercetin quantified from the PMMA plates were similar to those in solution.

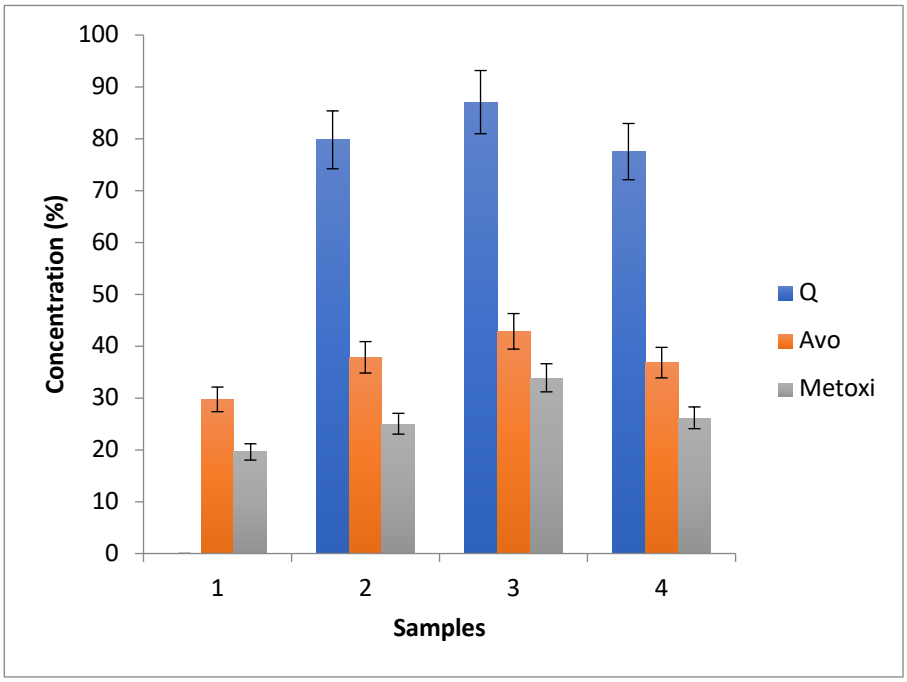
Table 12: Quantification and comparison of solution x PMMA plates of 0,1% quercetin by HPLC.

	STANDARD DEVIATION	RELATIVE STANDARD DEVIATION	% RECOVERY
0.1% quercetin	0.05	1.56	99.38
Avobenzone	1.95	3.27	102.24
Octyl Methoxycinnamate	1.64	1.65	99.13

Relative standard deviations below 5% were also required, as it was seen for all actives. The percentage of recovery of the substances on the plaque was high, indicating a viable method.

After irradiation of the PMMA plates the formulations showed good photostability of the components, and the formulation containing 0.2% quercetin presented better results **(Figure 20).**

Figure 20: Graphic corresponding to the components of the formulation present in the PMMA plates after irradiation.



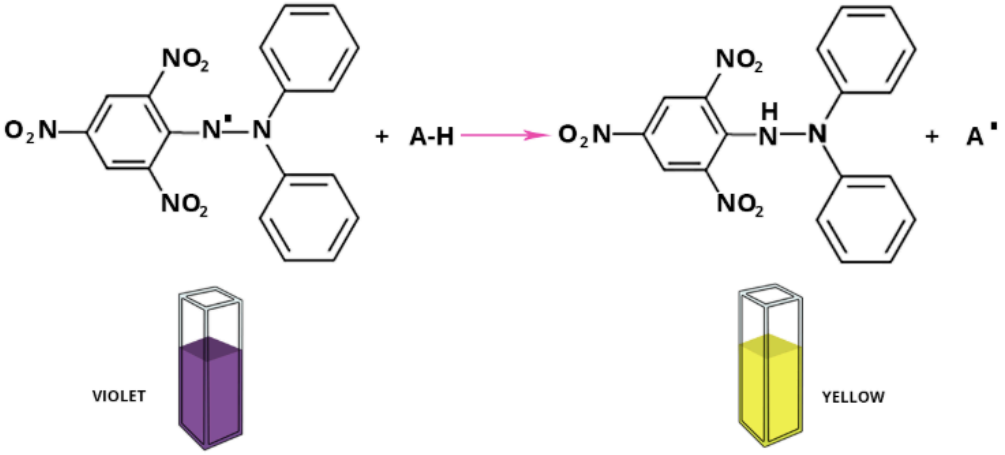
Legend: 1 (control sample); 2 (0.1% of quercetin sample); 3 (0.2% of quercetin sample) and 3 (0.3% of quercetin sample).

7.5. Functional analysis (*in vitro*)

7.5.1. Antioxidant activity

This method employed to verify the antioxidant potential of in *vitro* compounds will be based on the study of Brand-Williams et al. (1995). They generated an assay to identify the antioxidant activity of the compounds by the evaluation of the 2,2-diphenyl-1-picrylhydrazyl (DPPH •) radical sequestering activity. In this assay the compounds react with DPPH in methanol solution. The DPPH absorbs 515nm, but if its reduction occurs by oxidizing agents or free radicals this absorption disappears, indicating its positive activity as seen in **Figure 21** (BRAND-WILLIAMS; CUVELIER; BERSET, 1995).

Figure 21: Reduction reaction of the DPPH molecule. Where A = antioxidant radical and H = antioxidant radical reduced; (CHIMIACTIV, 2019).



Quercetin may delay the formation of ROS and consequently cell death, protecting primarily against lipid peroxidation (CASAGRANDE et al., 2006). Quercetin is the flavonoid with the highest antioxidant activity (ALEXANDROVA et al., 2006; RATNAM et al., 2006; SCALIA; MEZZENA, 2010).

7.5.1.1. Determination of the antioxidant activity of the formulations containing quercetin

It is known that UVA radiation produces ROS, so adding antioxidants to sunscreens is an efficient method of photoprotection, in which antioxidant actives neutralize ROS induced by UVA radiation (WANG; OSTERWALDER; JUNG, 2011). The literature already shows the ability of flavonoids to reduce the formation of ROS, as well as quercetin being the flavonoids with the highest antioxidant activity found in foods (AGATI et al., 2009; OWENS et al., 2008; RICE-EVANS; MILLER; PAGANGA, 1997; STEVANATO; BERTELLE; FABRIS, 2014). For this reason, the study of the antioxidant activity of quercetin in formulations is important, and particularly here in, we obtained satisfactory results, with the increase of the quercetin concentration in the formulations, there was an increase of the antioxidant activity, according to **Figure 22** and **Table 13.**

Figure 22: Test tubes containing diluted solutions of photoprotective formulations containing 0.1%; 0.2%; 0.3% quercetin; control (without quercetin) and DPPH (positive control) after 30 minutes in the dark for reduction reaction of the DPPH molecule.

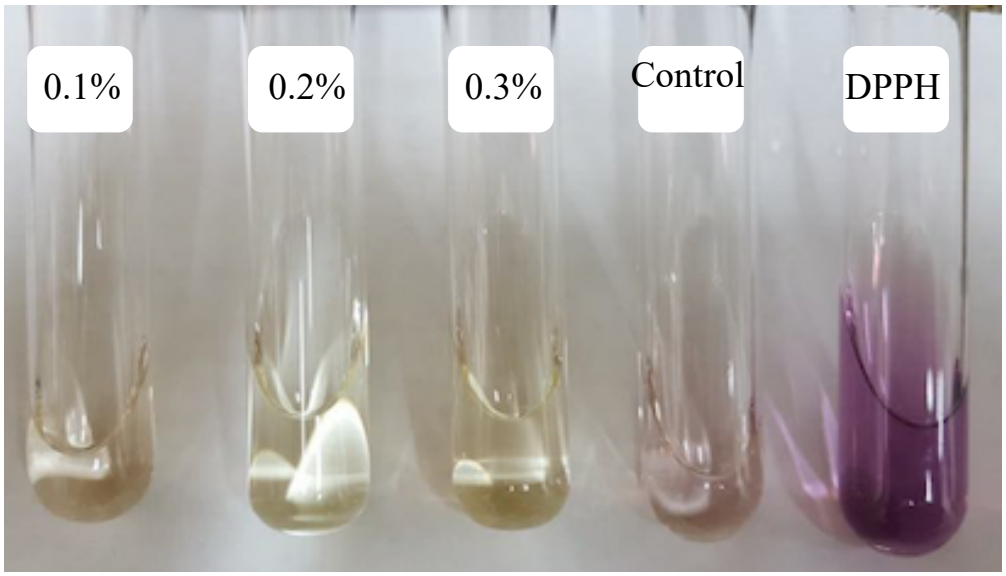


Table 13: Antioxidant activity of sunscreen formulations containing 0.1% quercetin; 0.2%; 0.3% and control (without quercetin).

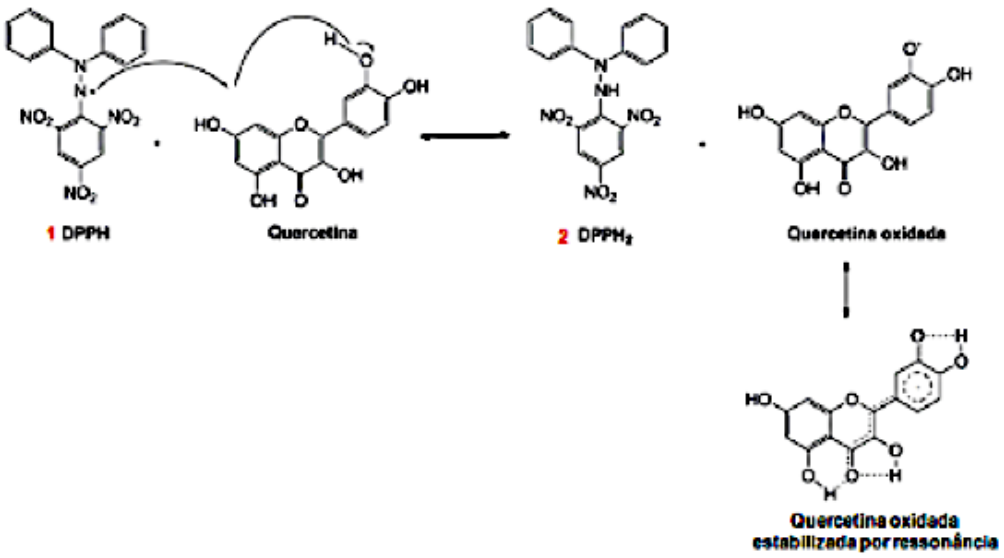
Formulations	Antioxidant Activity %
Control	3.62 ± 0.3 ^{A}
0.1%	27.68 ± 0.7 ^{B}
0.2%	53.49 ± 1.2 ^{C}
0.3%	74.36 ± 1.0 ^{D}

Legend: Antioxidant activity expressed as mean ± standard deviation n = 3. Results were evaluated according to ANOVA One-Way statistical test, followed by Tukey test for comparison between groups (significance level = 0.05) (Annex I); Different letters represent significant statistical differences between the groups

Figure 23 shows the reaction between DPPH and quercetin, in which DPPH undergoes reduction and quercetin oxidation when together in reaction. Quercetin reacts with DPPH by donating H^{+} of one of its free hydroxyls, forming $DPPH_2$ and oxidized

quercetin, in which it undergoes resonance between the oxygen and H^+ of another nearby free hydroxyl.

Figure 23: Reaction of DPPH reduction and quercetin oxidation.



7.5.2. Photoprotective efficacy *in vitro*

Diffuse reflectance spectrophotometer with integration sphere depends on the preparation of the film formation and its thickness on the substrate. The sunscreens have distinct absorbances and few of them cover a wide range of the UV reaction, so the need to add UV filters in a single formulation in required (SPRINGSTEEN et al., 1999). Flavonoids have antioxidant properties and also photoprotective potential due to their ability to absorb UV radiation (SAEWAN; JIMTAISONG, 2013).

Table 14: SPF and UVA-PF in *vitro* of the developed formulations.

Formulations	SPF	UVA-PF
0.1% quercetin	10± 1.2[a]	2.0 ± 0.1[e]
0.2% quercetin	11,33± 1.3[b]	2.0 ± 0.1[e]
0.3% quercetin	8,67± 0.7[c]	2.0 ± 0.1[e]
control	12± 1.2[d]	2.0 ± 0.1[e]

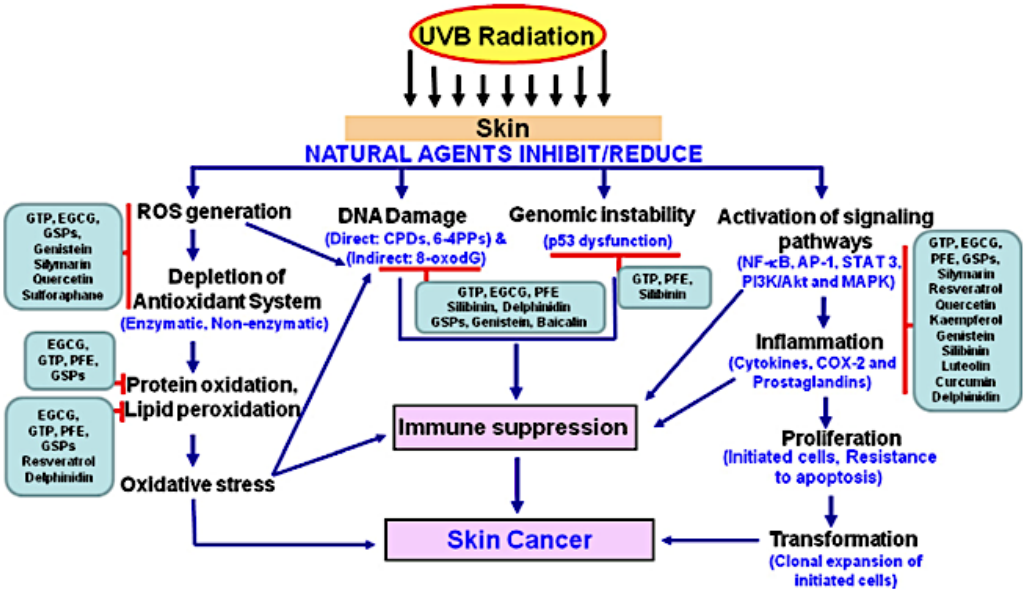
Legend: Results were evaluated according to ANOVA One-Way statistical test, followed by Tukey test for comparison between groups (significance level = 0.05) (Annex II); Different letters represent significant statistical differences between the groups.

Formulations were not considered of wide spectrum, since they did not reach SPF values at least 15 and after irradiation, about 83% of their SPF decreased (GASPAR; MAIA CAMPOS, 2006; KIM et al., 2015). The SPF is still the main circumstance about the photoprotective efficacy of a sunscreen, but it should not be based only on the numerical value itself, and the appropriate way of using the product should also be considered, in terms of quantity applied and regularity in reapplication (SCHALKA; MANOEL, 2011).

Natural substances have gained considerable dedication in the use of cosmetics, since they prevent the damages caused by the UV radiation and consequently avoid ROS formation, oxidative stress, immunosuppression, gene mutation among other cellular and molecular inhibitory effects induced by the skin, have positive effects when employed. In the case of quercetin, it acts with suppression of UVB-induced transactivation of AP-1, NF-jB and phosphorylation of MAPK **(Figure 24)** (AFAQ, 2011; NICHOLS; KATIYAR, 2010).

Quercetin in topical application has been shown to be effective in inhibiting skin damage induced by UVB radiation, as well as in peroxidation, however, there was no significant increase in UVA protection when associated with the physical filter titanium dioxide (CASAGRANDE et al., 2007; KIM et al., 2015; SAEWAN; JIMTAISONG, 2013).

Figure 24: Reactions caused by UV radiation from which they are inhibited by natural agents, such as quercetin (AFAQ, 2011).



7.5.3. Photo-stability assay and critical wavelength (λc)

The sunscreen components should reflect or absorb and scatter radiation all over the period of time they are intended to provide protection for and consequently should remain photostable. Many filters, in particular avobenzone and octyl methoxycinnamate, are photo unstable (ANTONIOU et al., 2008). Avobenzone and octyl methoxycinnamate were the sunscreens selected with the aim of developing a photoprotector with a broad spectrum and with a photo unstable profile in order to verify a possible photostabilization associated with quercetin.

The results of SPF before and after irradiation are presented in **Table 15**, while **Graph 1** illustrates the photostability of quercetin containing photoprotective formulations during the 4 hours of irradiation.

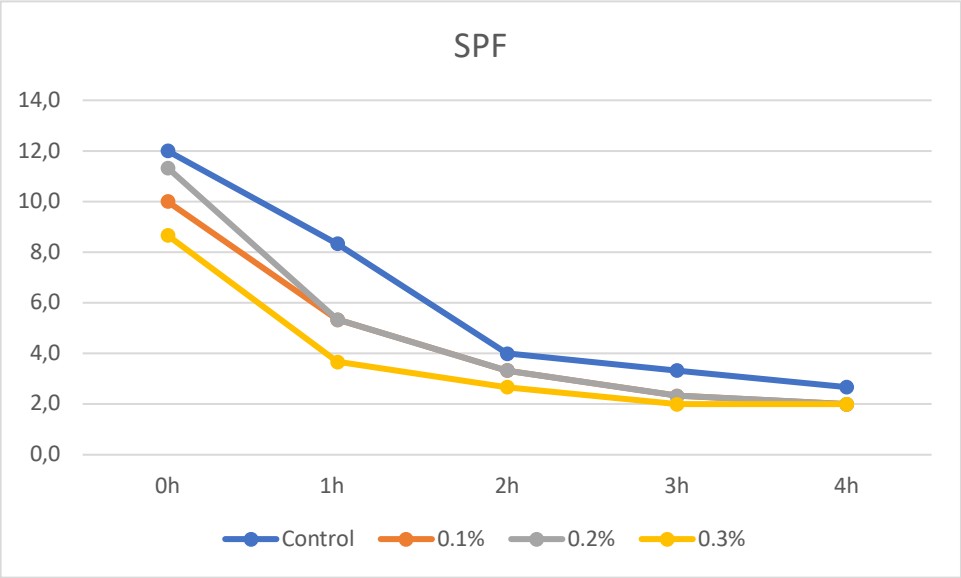
Table 15: SPF and critical wavelength (λc) of quercetin-containing photoprotection formulations before and after irradiation

	SPF		critical wavelength(λc)	
	pre-irradiation	**pos-irradiation**	**pre-irradiation**	**pos-irradiation**
Control	12.0^{A}	2.7^{E}	380.67^{G}	363.33^{J}
0.1% quercetin	10.0^{B}	2.0^{F}	381.0^{H}	363.67^{K}
0.2% quercetin	11.3^{C}	2.0^{F}	378.0^{I}	365.33^{L}
0.3% quercetin	10.0^{D}	2.0^{F}	381.0^{H}	363.67^{K}

Legend: Results were evaluated according to ANOVA One-Way statistical test, followed by Tukey test for comparison between groups (significance level = 0.05) (Annex III); Different letters represent significant statistical differences between the groups.

Again, the formulation containing 0.2% quercetin showed better results when compared to the others.

Graph 1: Profile of the photostability of quercetin containing photoprotective formulations during the 4 hours of irradiation measured by FPS.



It was desired that quercetin had synergistic effect associated with sunscreens or even stability of the filters used (avobenzone and octyl methoxycinnamate), as well as rutin (another flavonoid of the same quercetin class), but it was not found (VELASCO et al., 2008).

Among the differences in results is the photoinstability characteristic of some combinations of UV filters, such as avobenzone and octyl p-methoxycinnamate used in the formulations.

After irradiation the formulations showed a reduction in the critical wavelength value (Table 15), as well as in the FPS values shown in Table 14. Avobenzone and Octyl-methoxycinnamate are organic filters that absorb UV radiation when applied to the skin, leaving - those susceptible to photodegradation and generation of free radicals. Among several strategies to circumvent this profile is the association of natural photostabilizers, but studies did not find photostability of avobenzone with octyl methoxycinnamate along with quercetin in its free form. When associated with liposomes, nanoparticles or silica may have a better result (KOCKLER et al., 2012; SCALIA; MEZZENA, 2010).

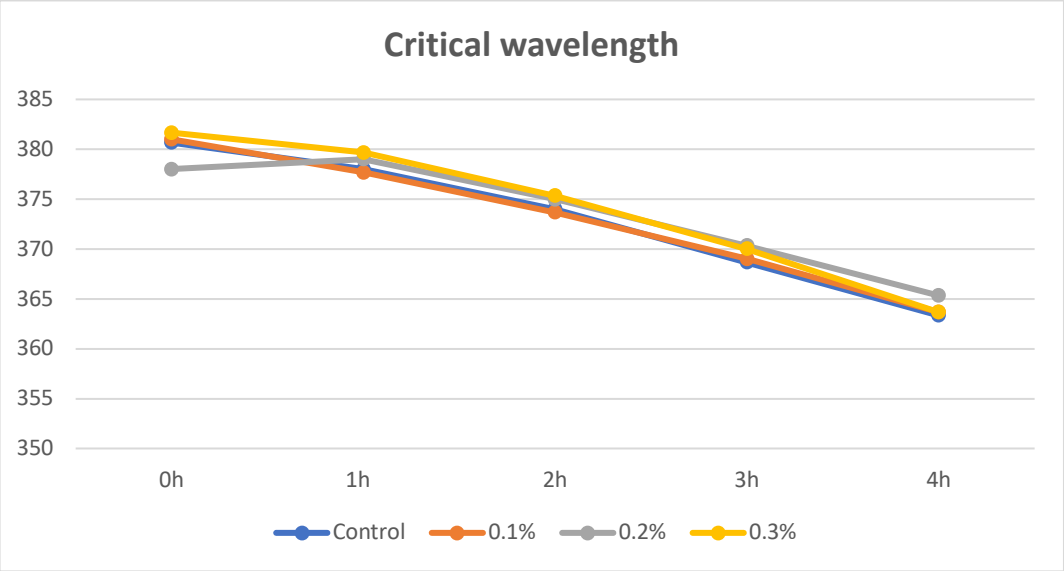
The critical wavelength is the wavelength at which the area under the integrated optical density curve starting at 290.0 nm equals 90% of the integrated area between 290.0 and 400.0 nm. Sunscreens must have a minimum critical wavelength of 370.0 nm.

The sunscreens shall have a minimum critical wavelength of 370.0 nm. On this parameter, the values obtained showed that there was no statistical difference between the results when the pre-irradiation values were within the range and showed desirable critical wavelength values for broad spectrum substances as shown in **Table 15** (BRASIL, 2012).

The **Graph 2** represents the decay of critical wavelength of photoprotective formulations containing quercetin during the 4 hours of irradiation.

In relation to the critical wavelength, all formulations had the same value (λc = 380.0), probably referring to the spectral profile of avobenzone (KIM et al., 2015).

Graph 2: Profile of critical wavelength of photoprotective formulations containing quercetin during the 4 hours of irradiation.



According to the current legislation of ANVISA and FDA, the samples must be previously irradiated to determine the UVA protection factor (UVA-PF) and the photostability of photoprotective formulations. The photo stability assay evaluates changes in the photoprotective potential of the samples by the radiation emitted by the camera (BRASIL, 2012; SOOD; PH, 2011).

The photoinstability of the filters used was verified with both the FPS values and the critical pre-irradiation and post-irradiation wavelength values.

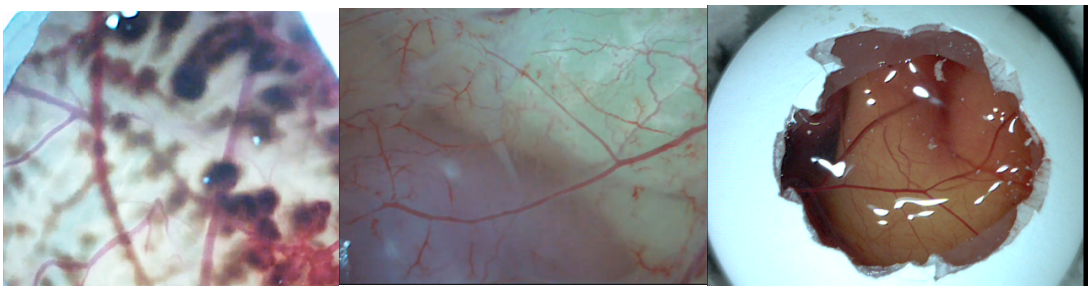
7.6. *In vitro* safety evaluation by means of HET-CAM - Test of the choroid-allantoic membrane of the chicken egg

The CAM is a highly vascular tissue, it responds to injury in a similar manner as our ocular tissue. One of the benefits of the HET-CAM is the possibility for using different kinds of formulations, such as solid or insoluble substances (BATISTA-DUHARTE et al., 2016).

Figure 25 shows the lysis, clots and hemorrhage of the CAM vessels when in contact with NaOH, Lysis and hemorrhage when in contact with SDS and without any change when in contact with physiological solution.

Figure 25: Photographs of the positive and negative controls used during the test, where NaOH: sodium hydroxide, SDS: sodium dodecylsulfate and NaCl 0.9%: physiological solution.

NaOH 1 % SDS 1 % NaCl 0,9 %



The *HET-CAM (chorioallantoic membrane)* test, proposed as a non-animal model for pre-viewing ocular irritation of chemicals. One of the advantages of HET-CAM is that method can be a valuable tool for assessing the potential for irritation to a wide variety of adjuvant forms including emulsion, gels and particles (BATISTA-DUHARTE et al., 2016).

Table 16 shows the results of the HET-CAM assay of each sample (in triplicate). The formulations for containing a reasonable amount of ethanol to solubilize quercetin, end up having potential ocular irritative, and the use of the products around the eyes is not recommended.

Table 16: Results of the HET-CAM assay

Item Number (Chicken Egg)	Reagent (20 secs.)	Post-wash reaction time *	Results	Degree of irritation
1	Positive Control (NaOH)	30 Secs.	Lise, clots and hemorrhage	**Strongly irritating**
2	Positive Control (SDS)	30 Secs.	Lise and hemorrhage	**Strongly irritating**
3	Formulation with flavonoid quercetin 0,1%	60 Secs.	hemorrhage	**Slightly irritating**
4				
5				
6	Formulation with flavonoid quercetin 0,2%	40 Secs.	Increased blood vessels / hemorrhage	
7		60 Secs.	Increased caliber in blood vessels	
8		60 Secs.		
9	Formulation with flavonoid quercetin 0,3%	120 Secs.	hemorrhage	
10		120 Secs.	hemorrhage and Increased caliber	
11		160 Secs.	hemorrhage	
12	Control Base	45 Secs.	hemorrhage	**Moderately Irritant**
13		50 Secs.		
14		40 Secs.		
15	Dissolved NaOH	30 Secs.	Lise, clots and hemorrhage	**Strongly irritating**
16				
17				
** Wash physiological saline solution*				

According to the HET-CAM test samples containing quercetin were shown to be slightly less irritant than the control sample, as well as when compared to the positive controls. These data corroborate the fact that quercetin has a potential vascular protective and vasoprotective effect (LUANGARAM; KUKONGVIRIYAPAN; PAKDEECHOTE, 2007; MAHMOUD et al., 2013).

7.7. *In vivo* analysis: Skin biocompatibility

All the formulations had a good performance in the biocompatibility tests. After 24h contact, no erythema was detected, in accordance with the negative control. The results obtained with Corneometer® showed values slightly above the hydration level when compared to water according to **Table 20.**

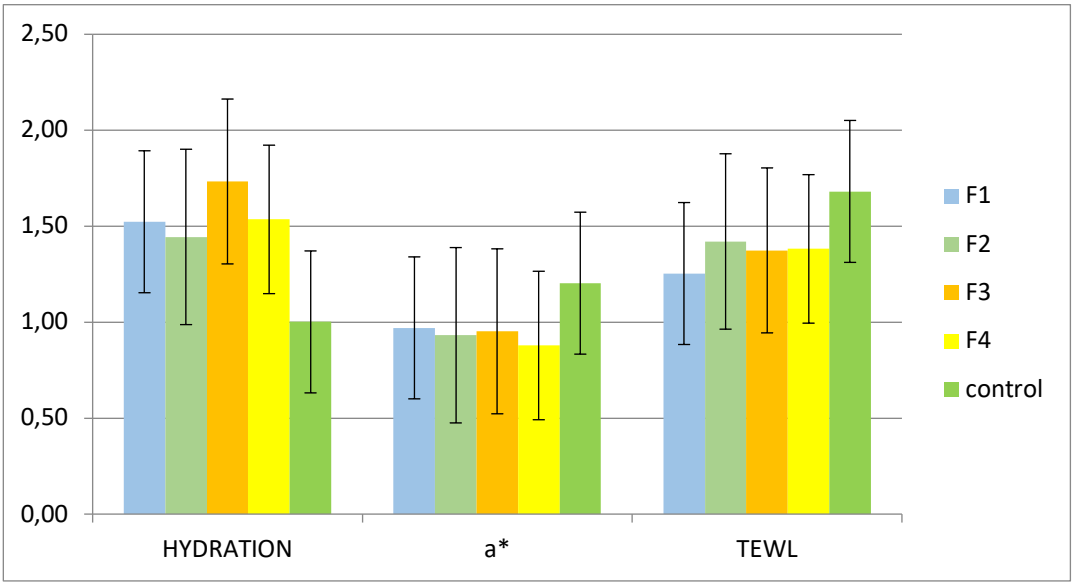
Table 17: Comparison between the results of the hydration profile, transepidermal loss of water and colorimetry of the skin of the volunteers.

	HYDRATION	a*	TEWL
control	1,00	1,20	1,68
F1	1,52	0,97	1,25
F2	1,44	0,93	1,42
F3	1,73	0,95	1,37
F4	1,54	0,88	1,38

Legend: F1 is a control sample; F2 is 0.1% of quercetin; F3 is 0.2% of quercetin; F4 is 0.3% of quercetin and the control is water.

The **Graph 6** show us the comparison between the results of the hydration profile, transepidermal loss of water (TEWL) and colorimetry (a*) of the skin of the volunteers, showing a great hydration profile, mainly in the formulation containing 0.2% quercetin.

Graph 6: Comparison between the results of the hydration profile, transepidermal loss of water and colorimetry of the skin of the volunteers.



Legend: F1 is a control sample; F2 is 0.1% of quercetin; F3 is 0.2% of quercetin; F4 is 0.3% of quercetin and the control is water.

The skin biocompatibility tests indicated the samples did not interfere in the stratum corneum barrier and caused no inflammatory reactions. Biocompatibility with the skin is a safety prerequisite for the use of cosmetics, and in this case the photoprotective formulations containing quercetin have been shown to be biocompatible with human skin and are therefore promising for use in bioactive cosmetics (BLITTERSWIJKR; PONEC, 1994; LABSKY, 2005).

7.8. "*Ex vivo*" functional characterization

7.8.1. Antioxidant activity – *Tape-stripping*

Pinkus (1966) described the technique of tape-stripping in the dermatological scope and not only in the pharmacist. This technique has been modified over the years and will be used for the purpose of evaluating the antioxidant activity of the formulations (PINKUS, 1966).

The tape stripping method is an extremely important and efficient device for dermopharmacokinetic experiments *in vitro* and the calculation of a cosmetic's or drug's

skin penetration behavior, besides being minimally invasive if carried out *in vivo* (NAGELREITER et al., 2015; ZAHOUANI, 2011).

Several studies have demonstrated the efficiency of using pig ear skin compared to human skin, but there are still differences between them such as the permeation is a little higher in the human skin, although in many studies this difference was not significant (HOJEROVÁ; MEDOVCÍKOVÁ; MIKULA, 2011; JACOBI et al., 2007; LADEMANN et al., 2009). Tape-stripping analyzes the substances applied topically, mainly in the stratum corneum of the epidermis, since the cell layers of the stratum corneum are successively removed from the skin area using adhesive (KLANG et al., 2012).

The present experiment was to performed observe how much antioxidant activity exists in the stratum corneum after topical application of formulations containing quercetin (antioxidant flavonoid). The tapes contain the amount of formulation that corresponding amount of the penetrated formulation, which can be determined by classical analytical chemical methods. The area of application, adhesive tape model, application pressure, application time as well as removal rate are essential factors to be standardized in the tape-stripping test (LADEMANN et al., 2009).

We obtained a higher percentage of antioxidant activity in the formulation containing 0.3% quercetin, followed by the ones containing 0.2%, and 0.1%, as show **Table 18**.

Table 18: Determination of the antioxidant activity of the formulation containing 0.1% ; 0,2% and 0,3% of quercetin.

	Antioxidant activity (%)			
	0.1%	0.2%	0.3%	Control
Average	22.70	40.58	62.12	3.86

The antioxidant potential of a substance corresponds to the amount of DPPH used in a certain of time and according to researches quercetin has a high antioxidant power, which was confirmed in the calculations of antioxidant activity in item 7.5.1.1. and in tape-stripping, in which the antioxidant activity was also increased along with the quercetin concentration in the formulations, reaching 62.12% with the formulation

containing 0.3% quercetin (ANDREA, 2015; CASAGRANDE et al., 2007; VICENTINI et al., 2007).

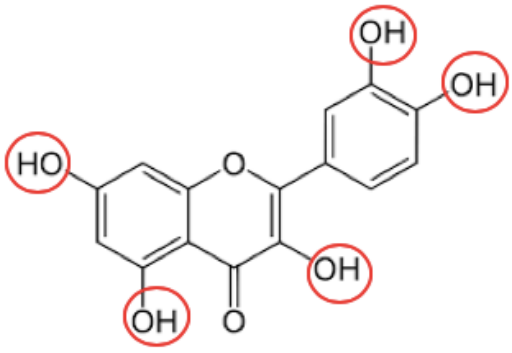
After irradiation the tapes containing the samples maintained a high antioxidative power, a little below when compared to the tapes before irradiating, but with high percentages and with greater antioxidant capacity the formulation containing 0.3% of quercetin (**Table 19**).

Table 19: Determination of the antioxidant activity of the formulation containing 0.1% ; 0,2% and 0,3% of quercetin pos-irradiation.

	Antioxidant activity (%) pos-irradiation			
	0.1%	0.2%	0.3%	Control
Average	20.82	38.33	60.23	1.66

Antioxidants in sunscreens for a topical application can be potentially neutralize the UVA-induced free radicals (WANG; OSTERWALDER; JUNG, 2011)**.** The antioxidant capacity of quercetin is directly correlated to the number of free hydroxyl groups shown in **Figure 21** (LESJAK et al., 2018; RICE-EVANS; MILLER; PAGANGA, 1996). In each molecule of quercetin, five groups of free hydroxyl are observed, providing a great antioxidant power.

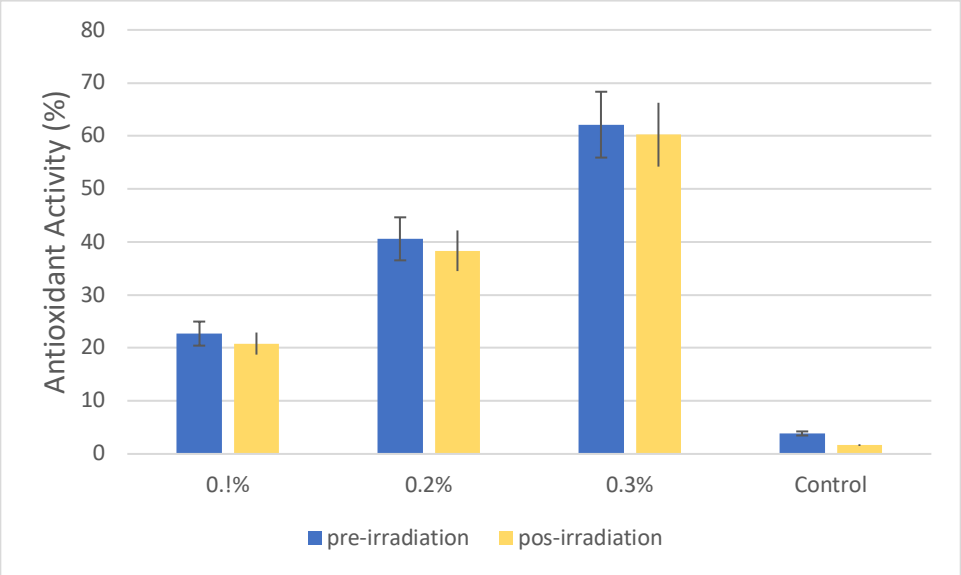
Figure 21: Schematic representation of the quercetin structure modified, contrasting their free hydroxyls, which are responsible for their antioxidant capacity (MEDCHEM, 2019).



It is expected that antioxidant activity will increase with increasing quercetin concentration, since quercetin is considered the flavonoid with the highest antioxidant

potential (SAEWAN; JIMTAISONG, 2013), an assertion was confirmed in the analysis (Graph 7) . Even after irradiation the quercetin continued to maintain its high antioxidative power and with increase proportional to its concentration in the samples.

Graph 7: Antioxidant activity of photoprotective formulations containing quercetin and control.



Several scientific studies report that quercetin in topical formulations has up to 1% permeation in the skin, even in nanoemulsions or the use of surfactants that contribute to permeation. Therefore, the formulation containing quercetin tends to be in the stratum corneum, which proves the tape-stripping test. Pig skin is slightly less permeable than human skin which explains the percentage of antioxidant activity found, as well as the use of alcohol may also cause damage to the barrier function of the utilized skin (BARTEK; LABUDDE; MAIBACH, 1972; JACOBI et al., 2007; LADEMANN et al., 2009).

The permeation or absorption of substances by the skin is influenced by several factors, such as lipophilicity, pH, concentration of the active in the formulation, skin hydration, molecular size of the active and temperature. The pig ears used did not obtain significant antioxidant activity, understanding that quercetin is not permeated by the skin (CASAGRANDE et al., 2007; NAIR et al., 2013; SAIJA et al., 1998).

8. Conclusions

8. Conclusions

⇒ **Production of formulations containing UV filters associated with quercetin and determination of the organoleptic and physicochemical characteristics of the samples.**

Formulations were considered stable, not having their characteristics modified during the tests, as well as their compatibility with skin pH. The color was yellowish characteristic of quercetin, homogeneous aspect, indicating the compatibility between the chosen ingredients.

⇒ **Functional evaluation of isolated active compounds and formulations:**

- antioxidant activity *in vitro*

The results of the antioxidant activity proved the antioxidant action of quercetin in the formulation, since it presented an increase directly proportional to the increase of the concentration of the flavonoid in the manipulated samples.

- sun protection factor *in vitro*

The SPF value showed that the formulation containing 0.2% quercetin had a higher one when compared to the other formulations, but they were not considered broad spectrum, however, the values of critical wavelength.

- sample photostability and critical wavelength (nm)

According to the results presented by the SPF tests after irradiation, quercetin did not contribute to the photostability of the filters. The critical wavelength also decayed according to the irradiation time, asserting the photoinstability of the filters.

⇒ **Safety assessment:**

- *in vitro* (HET-CAM)

Formulations containing quercetin were less irritant than the positive controls and the formulation without quercetin, an indicative of quercetin vasoprotective function.

9. References

9. References

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Annex A: Statistical Analysis of Antioxidant Activity

ANOVA com um fator: Control; 0.1%; 0.2%; 0.3%

Método

Hipótese nula	Todas as médias são iguais
Hipótese alternativa	Nem todas as médias são iguais
Nível de significância	α = 0,05

Assumiu-se igualdade de variâncias para a análise

Informações dos Fatores

Fator	Níveis	Valores
Fator	4	Control; 0.1%; 0.2%; 0.3%

Análise de Variância

Fonte	GL	SQ (Aj.)	QM (Aj.)	Valor F	Valor-P
Fator	3	8533,73	2844,58	428830,46	0,000
Erro	8	0,05	0,01		
Total	11	8533,78			

Sumário do Modelo

S	R2	R2(aj)	R2(pred)
0,0814453	100,00%	100,00%	100,00%

Médias

Fator	N	Média	DesvPad	IC de 95%
Control	3	3,62000	0,01000	(3,51157; 3,72843)
0.1%	3	27,6800	0,0100	(27,5716; 27,7884)
0.2%	3	53,4800	0,0100	(53,3716; 53,5884)
0.3%	3	74,4633	0,1620	(74,3549; 74,5718)

DesvPad Combinado = 0,0814453

Comparações Emparelhadas de Tukey

Informações de Agrupamento Usando Método de Tukey e Confiança de 95%

Fator	N	Média	Agrupamento
0.3%	3	74,4633	A
0.2%	3	53,4800	B
0.1%	3	27,6800	C
Control	3	3,62000	D

Médias que não compartilham uma letra são significativamente diferentes.

Teste T para Duas Amostras e IC: Control; 0.1%

Método

μ_1: média de Control
μ_2: média de 0.1%
Diferença: $\mu_1 - \mu_2$

Não assumiu-se igualdade de variâncias para esta análise.

Estatísticas Descritivas

Amostra	N	Média	DesvPad	EP Média
Control	3	3,6200	0,0100	0,0058
0.1%	3	27,6800	0,0100	0,0058

Estimativa da diferença

Diferença	IC de 95% para a Diferença
-24,0600	(-24,0827; -24,0373)

Teste

Hipótese nula $H_0: \mu_1 - \mu_2 = 0$
Hipótese alternativa $H_1: \mu_1 - \mu_2 \neq 0$

Valor-T	GL	Valor-p
-2946,74	4	0,000

Teste T para Duas Amostras e IC: Control; 0.2%

Método

μ_1: média de Control
μ_2: média de 0.2%
Diferença: $\mu_1 - \mu_2$

Não assumiu-se igualdade de variâncias para esta análise.

Estatísticas Descritivas

Amostra	N	Média	DesvPad	EP Média
Control	3	3,6200	0,0100	0,0058
0.2%	3	53,4800	0,0100	0,0058

Estimativa da diferença

Diferença	IC de 95% para a Diferença
-49,8600	(-49,8827; -49,8373)

Teste

Hipótese nula $H_0: \mu_1 - \mu_2 = 0$
Hipótese alternativa $H_1: \mu_1 - \mu_2 \neq 0$

Valor-T	GL	Valor-p
-6106,58	4	0,000

Teste T para Duas Amostras e IC: Control; 0.3%

Método

μ_1: média de Control
μ_2: média de 0.3%
Diferença: $\mu_1 - \mu_2$

Não assumiu-se igualdade de variâncias para esta análise.

Estatísticas Descritivas

Amostra	N	Média	DesvPad	EP Média
Control	3	3,6200	0,0100	0,0058
0.3%	3	74,463	0,162	0,094

Estimativa da diferença

Diferença	IC de 95% para a Diferença
-70,8433	(-71,2464; -70,4402)

Teste

Hipótese nula $H_0: \mu_1 - \mu_2 = 0$
Hipótese alternativa $H_1: \mu_1 - \mu_2 \neq 0$

Valor-T	GL	Valor-p
-756,15	2	0,000

Annex B: Statistical Analysis of SPF

ANOVA com um fator: control; 0.1%; 0.2%; 0.3%

Método

Hipótese nula	Todas as médias são iguais
Hipótese alternativa	Nem todas as médias são iguais
Nível de significância	α = 0,05

Assumiu-se igualdade de variâncias para a análise

Informações dos Fatores

Fator	Níveis	Valores
Fator	4	control; 0.1%; 0.2%; 0.3%

Análise de Variância

Fonte	GL	SQ (Aj.)	QM (Aj.)	Valor F	Valor-P
Fator	3	19,6244	6,54148	65414,75	0,000
Erro	8	0,0008	0,00010		
Total	11	19,6252			

Sumário do Modelo

S	R2	R2(aj)	R2(pred)
0,01	100,00%	99,99%	99,99%

Médias

Fator	N	Média	DesvPad	IC de 95%
control	3	12,0100	0,0100	(11,9967; 12,0233)
0.1%	3	10,0100	0,0100	(9,9967; 10,0233)
0.2%	3	11,3200	0,0100	(11,3067; 11,3333)
0.3%	3	8,67000	0,01000	(8,65669; 8,68331)

DesvPad Combinado = 0,01

Teste T para Duas Amostras e IC: control; 0.1%

Método

μ_1: média de control
μ_2: média de 0.1%
Diferença: $\mu_1 - \mu_2$

Não assumiu-se igualdade de variâncias para esta análise.

Estatísticas Descritivas

Amostra	N	Média	DesvPad	EP Média
control	3	12,0100	0,0100	0,0058
0.1%	3	10,0100	0,0100	0,0058

Estimativa da diferença

Diferença	IC de 95% para a Diferença
2,00000	(1,97733; 2,02267)

Teste

Hipótese nula H_0: $\mu_1 - \mu_2 = 0$
Hipótese alternativa H_1: $\mu_1 - \mu_2 \neq 0$

Valor-T	GL	Valor-p
244,95	4	0,000

Teste T para Duas Amostras e IC: control; 0.2%

Método

μ_1: média de control
μ_2: média de 0.2%
Diferença: $\mu_1 - \mu_2$

Não assumiu-se igualdade de variâncias para esta análise.

Estatísticas Descritivas

Amostra	N	Média	DesvPad	EP Média
control	3	12,0100	0,0100	0,0058
0.2%	3	11,3200	0,0100	0,0058

Estimativa da diferença

Diferença	IC de 95% para a Diferença
0,69000	(0,66733; 0,71267)

Teste

Hipótese nula H_0: $\mu_1 - \mu_2 = 0$
Hipótese alternativa H_1: $\mu_1 - \mu_2 \neq 0$

Valor-T	GL	Valor-p
84,51	4	0,000

Teste T para Duas Amostras e IC: control; 0.3%

Método

μ_1: média de control
μ_2: média de 0.3%
Diferença: $\mu_1 - \mu_2$

Não assumiu-se igualdade de variâncias para esta análise.

Estatísticas Descritivas

Amostra	N	Média	DesvPad	EP Média
control	3	12,0100	0,0100	0,0058
0.3%	3	8,6700	0,0100	0,0058

Estimativa da diferença

	IC de 95% para a
Diferença	Diferença
3,34000	(3,31733; 3,36267)

Teste

Hipótese nula $H_0: \mu_1 - \mu_2 = 0$
Hipótese alternativa $H_1: \mu_1 - \mu_2 \neq 0$

Valor-T	GL	Valor-p
409,06	4	0,000

Annex C: Statistical Analysis of SPF pre-irradiation

ANOVA com um fator: control; 0.1%; 0.2%; 0.3%

Método

Hipótese nula	Todas as médias são iguais
Hipótese alternativa	Nem todas as médias são iguais
Nível de significância	α = 0,05

Assumiu-se igualdade de variâncias para a análise

Informações dos Fatores

Fator	Níveis	Valores
Fator	4	control; 0.1%; 0.2%; 0.3%

Análise de Variância

Fonte	GL	SQ (Aj.)	QM (Aj.)	Valor F	Valor-P
Fator	3	8,84143	2,94714	22103,56	0,000
Erro	8	0,00107	0,00013		
Total	11	8,84249			

Sumário do Modelo

S	R2	R2(aj)	R2(pred)
0,0115470	99,99%	99,98%	99,97%

Médias

Fator	N	Média	DesvPad	IC de 95%
control	3	12,0100	0,0100	(11,9946; 12,0254)
0.1%	3	10,0100	0,0100	(9,9946; 10,0254)
0.2%	3	11,3000	0,0100	(11,2846; 11,3154)
0.3%	3	10,0167	0,0153	(10,0013; 10,0320)

DesvPad Combinado = 0,0115470

Teste T para Duas Amostras e IC: control; 0.1%

Método

μ_1: média de control
μ_2: média de 0.1%
Diferença: $\mu_1 - \mu_2$

Não assumiu-se igualdade de variâncias para esta análise.

Estatísticas Descritivas

Amostra	N	Média	DesvPad	EP Média
control	3	12,0100	0,0100	0,0058
0.1%	3	10,0100	0,0100	0,0058

Estimativa da diferença

Diferença	IC de 95% para a Diferença
2,00000	(1,97733; 2,02267)

Teste

Hipótese nula H_0: $\mu_1 - \mu_2 = 0$
Hipótese alternativa H_1: $\mu_1 - \mu_2 \neq 0$

Valor-T	GL	Valor-p
244,95	4	0,000

Teste T para Duas Amostras e IC: control; 0.2%

Método

μ_1: média de control
μ_2: média de 0.2%
Diferença: $\mu_1 - \mu_2$

Não assumiu-se igualdade de variâncias para esta análise.

Estatísticas Descritivas

Amostra	N	Média	DesvPad	EP Média
control	3	12,0100	0,0100	0,0058
0.2%	3	11,3000	0,0100	0,0058

Estimativa da diferença

Diferença	IC de 95% para a Diferença
0,71000	(0,68733; 0,73267)

Teste

Hipótese nula H_0: $\mu_1 - \mu_2 = 0$
Hipótese alternativa H_1: $\mu_1 - \mu_2 \neq 0$

Valor-T	GL	Valor-p
86,96	4	0,000

Teste T para Duas Amostras e IC: control; 0.3%

Método

μ_1: média de control
μ_2: média de 0.3%
Diferença: $\mu_1 - \mu_2$

Não assumiu-se igualdade de variâncias para esta análise.

Estatísticas Descritivas

Amostra	N	Média	DesvPad	EP Média
control	3	12,0100	0,0100	0,0058
0.3%	3	10,0167	0,0153	0,0088

Estimativa da diferença

Diferença	IC de 95% para a Diferença
1,9933	(1,9598; 2,0269)

Teste

Hipótese nula $H_0: \mu_1 - \mu_2 = 0$
Hipótese alternativa $H_1: \mu_1 - \mu_2 \neq 0$

Valor-T	GL	Valor-p
189,10	3	0,000

Annex D: Statistical Analysis of SPF pos-irradiation

ANOVA com um fator: control; 0.1%; 0.2%; 0.3%

Método

Hipótese nula	Todas as médias são iguais
Hipótese alternativa	Nem todas as médias são iguais
Nível de significância	α = 0,05

Assumiu-se igualdade de variâncias para a análise

Informações dos Fatores

Fator	Níveis	Valores
Fator	4	control; 0.1%; 0.2%; 0.3%

Análise de Variância

Fonte	GL	SQ (Aj.)	QM (Aj.)	Valor F	Valor-P
Fator	3	1,10250	0,367500	980,00	0,000
Erro	8	0,00300	0,000375		
Total	11	1,10550			

Sumário do Modelo

S	R2	R2(aj)	R2(pred)
0,0193649	99,73%	99,63%	99,39%

Médias

Fator	N	Média	DesvPad	IC de 95%
control	3	2,70000	0,01000	(2,67422; 2,72578)
0.1%	3	2,00000	0,01000	(1,97422; 2,02578)
0.2%	3	2,0000	0,0200	(1,9742; 2,0258)
0.3%	3	2,0000	0,0300	(1,9742; 2,0258)

DesvPad Combinado = 0,0193649

Comparações Emparelhadas de Tukey

Informações de Agrupamento Usando Método de Tukey e Confiança de 95%

Fator	N	Média	Agrupamento	
control	3	2,70000	A	
0.3%	3	2,0000		B
0.2%	3	2,0000		B
0.1%	3	2,00000		B

Médias que não compartilham uma letra são significativamente diferentes.

Teste T para Duas Amostras e IC: control; 0.1%

Método

μ_1: média de control
μ_2: média de 0.1%
Diferença: $\mu_1 - \mu_2$

Não assumiu-se igualdade de variâncias para esta análise.

Estatísticas Descritivas

Amostra	N	Média	DesvPad	EP Média
control	3	2,7000	0,0100	0,0058
0.1%	3	2,0000	0,0100	0,0058

Estimativa da diferença

Diferença	IC de 95% para a Diferença
0,70000	(0,67733; 0,72267)

Teste

Hipótese nula $H_0: \mu_1 - \mu_2 = 0$
Hipótese alternativa $H_1: \mu_1 - \mu_2 \neq 0$

Valor-T	GL	Valor-p
85,73	4	0,000

Annex E: Statistical Analysis of critical wavelength(λc) pos-irradiation

ANOVA com um fator: control.; 0,1%; 0,2%; 0,3%

Método

Hipótese nula	Todas as médias são iguais
Hipótese alternativa	Nem todas as médias são iguais
Nível de significância	α = 0,05

Assumiu-se igualdade de variâncias para a análise

Informações dos Fatores

Fator	Níveis	Valores
Fator	4	control.; 0,1%; 0,2%; 0,3%

Análise de Variância

Fonte	GL	SQ (Aj.)	QM (Aj.)	Valor F	Valor-P
Fator	3	7,30680	2,43560	24356,00	0,000
Erro	8	0,00080	0,00010		
Total	11	7,30760			

Sumário do Modelo

S	R2	R2(aj)	R2(pred)
0,01	99,99%	99,98%	99,98%

Médias

Fator	N	Média	DesvPad	IC de 95%
control.	3	363,330	0,010	(363,317; 363,343)
0,1%	3	363,670	0,010	(363,657; 363,683)
0,2%	3	365,330	0,010	(365,317; 365,343)
0,3%	3	363,670	0,010	(363,657; 363,683)

DesvPad Combinado = 0,01

Comparações Emparelhadas de Tukey

Informações de Agrupamento Usando Método de Tukey e Confiança de 95%

Fator	N	Média	Agrupamento		
0,2%	3	365,330	A		
0,3%	3	363,670		B	
0,1%	3	363,670		B	
control.	3	363,330			C

Médias que não compartilham uma letra são significativamente diferentes.

Annex F: Statistical Analysis of Antioxidant Activity by Tappe-stripping pre-irradiation

ANOVA com um fator: control; 0.1%; 0.2%; 0.3%

Método

Hipótese nula	Todas as médias são iguais
Hipótese alternativa	Nem todas as médias são iguais
Nível de significância	α = 0,05

Assumiu-se igualdade de variâncias para a análise

Informações dos Fatores

Fator	Níveis	Valores
Fator	4	control; 0.1%; 0.2%; 0.3%

Análise de Variância

Fonte	GL	SQ (Aj.)	QM (Aj.)	Valor F	Valor-P
Fator	3	5575,85	1858,62	18586182,75	0,000
Erro	8	0,00	0,00		
Total	11	5575,86			

Sumário do Modelo

S	R2	R2(aj)	R2(pred)
0,01	100,00%	100,00%	100,00%

Médias

Fator	N	Média	DesvPad	IC de 95%
control	3	3,86000	0,01000	(3,84669; 3,87331)
0.1%	3	22,7000	0,0100	(22,6867; 22,7133)
0.2%	3	40,5700	0,0100	(40,5567; 40,5833)
0.3%	3	62,1200	0,0100	(62,1067; 62,1333)

DesvPad Combinado = 0,01

Comparações Emparelhadas de Tukey

Informações de Agrupamento Usando Método de Tukey e Confiança de 95%

Fator	N	Média	Agrupamento		
0.3%	3	62,1200	A		
0.2%	3	40,5700		B	
0.1%	3	22,7000			C
control	3	3,86000			D

Médias que não compartilham uma letra são significativamente diferentes.

Teste T para Duas Amostras e IC: control; 0.1%

Método

μ_1: média de control
μ_2: média de 0.1%
Diferença: $\mu_1 - \mu_2$

Não assumiu-se igualdade de variâncias para esta análise.

Estatísticas Descritivas

Amostra	N	Média	DesvPad	EP Média
control	3	3,8600	0,0100	0,0058
0.1%	3	22,7000	0,0100	0,0058

Estimativa da diferença

Diferença	IC de 95% para a Diferença
-18,8400	(-18,8627; -18,8173)

Teste

Hipótese nula H_0: $\mu_1 - \mu_2 = 0$
Hipótese alternativa H_1: $\mu_1 - \mu_2 \neq 0$

Valor-T	GL	Valor-p
-2307,42	4	0,000

Teste T para Duas Amostras e IC: control; 0.2%

Método

μ_1: média de control
μ_2: média de 0.2%
Diferença: $\mu_1 - \mu_2$

Não assumiu-se igualdade de variâncias para esta análise.

Estatísticas Descritivas

Amostra	N	Média	DesvPad	EP Média
control	3	3,8600	0,0100	0,0058
0.2%	3	40,5700	0,0100	0,0058

Estimativa da diferença

Diferença	IC de 95% para a Diferença
-36,7100	(-36,7327; -36,6873)

Teste

Hipótese nula H_0: $\mu_1 - \mu_2 = 0$
Hipótese alternativa H_1: $\mu_1 - \mu_2 \neq 0$

Valor-T	GL	Valor-p
-4496,04	4	0,000

Teste T para Duas Amostras e IC: control; 0.3%

Método

μ_1: média de control
μ_2: média de 0.3%
Diferença: $\mu_1 - \mu_2$

Não assumiu-se igualdade de variâncias para esta análise.

Estatísticas Descritivas

Amostra	N	Média	DesvPad	EP Média
control	3	3,8600	0,0100	0,0058
0.3%	3	62,1200	0,0100	0,0058

Estimativa da diferença

Diferença	IC de 95% para a Diferença
-58,2600	(-58,2827; -58,2373)

Teste

Hipótese nula $H_0: \mu_1 - \mu_2 = 0$
Hipótese alternativa $H_1: \mu_1 - \mu_2 \neq 0$

Valor-T	GL	Valor-p
-7135,36	4	0,000

Annex G: Statistical Analysis of Antioxidant Activity by Tappe-stripping pos-irradiation

ANOVA com um fator: control.; 0,1%; 0,2%; 0,3%

Método

Hipótese nula	Todas as médias são iguais
Hipótese alternativa	Nem todas as médias são iguais
Nível de significância	α = 0,05

Assumiu-se igualdade de variâncias para a análise

Informações dos Fatores

Fator	Níveis	Valores
Fator	4	control.; 0,1%; 0,2%; 0,3%

Análise de Variância

Fonte	GL	SQ (Aj.)	QM (Aj.)	Valor F	Valor-P
Fator	3	5608,92	1869,64	18696387,00	0,000
Erro	8	0,00	0,00		
Total	11	5608,92			

Sumário do Modelo

S	R2	R2(aj)	R2(pred)
0,01	100,00%	100,00%	100,00%

Médias

Fator	N	Média	DesvPad	IC de 95%
control.	3	1,66000	0,01000	(1,64669; 1,67331)
0,1%	3	20,8200	0,0100	(20,8067; 20,8333)
0,2%	3	38,3200	0,0100	(38,3067; 38,3333)
0,3%	3	60,2200	0,0100	(60,2067; 60,2333)

DesvPad Combinado = 0,01

Comparações Emparelhadas de Tukey

Informações de Agrupamento Usando Método de Tukey e Confiança de 95%

Fator	N	Média	Agrupamento
0,3%	3	60,2200	A
0,2%	3	38,3200	B
0,1%	3	20,8200	C
control.	3	1,66000	D

Médias que não compartilham uma letra são significativamente diferentes.

Teste T para Duas Amostras e IC: control; control.

Método

μ_1: média de control
μ_2: média de control.
Diferença: $\mu_1 - \mu_2$

Não assumiu-se igualdade de variâncias para esta análise.

Estatísticas Descritivas

Amostra	N	Média	DesvPad	EP Média
control	3	3,8600	0,0100	0,0058
control.	3	1,6600	0,0100	0,0058

Estimativa da diferença

Diferença	IC de 95% para a Diferença
2,20000	(2,17733; 2,22267)

Teste

Hipótese nula H_0: $\mu_1 - \mu_2 = 0$
Hipótese alternativa H_1: $\mu_1 - \mu_2 \neq 0$

Valor-T	GL	Valor-p
269,44	4	0,000

Teste T para Duas Amostras e IC: 0.1%; 0,1%

Método

μ_1: média de 0.1%
μ_2: média de 0,1%
Diferença: $\mu_1 - \mu_2$

Não assumiu-se igualdade de variâncias para esta análise.

Estatísticas Descritivas

Amostra	N	Média	DesvPad	EP Média
0.1%	3	22,7000	0,0100	0,0058
0,1%	3	20,8200	0,0100	0,0058

Estimativa da diferença

Diferença	IC de 95% para a Diferença
1,88000	(1,85733; 1,90267)

Teste

Hipótese nula H_0: $\mu_1 - \mu_2 = 0$
Hipótese alternativa H_1: $\mu_1 - \mu_2 \neq 0$

Valor-T	GL	Valor-p
230,25	4	0,000

Teste T para Duas Amostras e IC: 0.2%; 0,2%

Método

μ_1: média de 0.2%
μ_2: média de 0,2%
Diferença: $\mu_1 - \mu_2$

Não assumiu-se igualdade de variâncias para esta análise.

Estatísticas Descritivas

Amostra	N	Média	DesvPad	EP Média
0.2%	3	40,5700	0,0100	0,0058
0,2%	3	38,3200	0,0100	0,0058

Estimativa da diferença

Diferença	IC de 95% para a Diferença
2,25000	(2,22733; 2,27267)

Teste

Hipótese nula H_0: $\mu_1 - \mu_2 = 0$
Hipótese alternativa H_1: $\mu_1 - \mu_2 \neq 0$

Valor-T	GL	Valor-p
275,57	4	0,000

Teste T para Duas Amostras e IC: 0.3%; 0,3%

Método

μ_1: média de 0.3%
μ_2: média de 0,3%
Diferença: $\mu_1 - \mu_2$

Não assumiu-se igualdade de variâncias para esta análise.

Estatísticas Descritivas

Amostra	N	Média	DesvPad	EP Média
0.3%	3	62,1200	0,0100	0,0058
0,3%	3	60,2200	0,0100	0,0058

Estimativa da diferença

Diferença	IC de 95% para a Diferença
1,90000	(1,87733; 1,92267)

Teste

Hipótese nula H_0: $\mu_1 - \mu_2 = 0$
Hipótese alternativa H_1: $\mu_1 - \mu_2 \neq 0$

Valor-T	GL	Valor-p
232,70	4	0,000

Annex H: Statistical Analysis of Hydration

ANOVA com um fator: F1; F2; F3; F4; control

Método

Hipótese nula	Todas as médias são iguais
Hipótese alternativa	Nem todas as médias são iguais
Nível de significância	α = 0,05

Assumiu-se igualdade de variâncias para a análise

Informações dos Fatores

Fator	Níveis	Valores
Fator	5	F1; F2; F3; F4; control

Análise de Variância

Fonte	GL	SQ (Aj.)	QM (Aj.)	Valor F	Valor-P
Fator	4	0,880307	0,220077	1737,45	0,000
Erro	10	0,001267	0,000127		
Total	14	0,881573			

Sumário do Modelo

S	R2	R2(aj)	R2(pred)
0,0112546	99,86%	99,80%	99,68%

Médias

Fator	N	Média	DesvPad	IC de 95%
F1	3	1,51667	0,01528	(1,50219; 1,53114)
F2	3	1,44000	0,01000	(1,42552; 1,45448)
F3	3	1,73000	0,01000	(1,71552; 1,74448)
F4	3	1,54000	0,01000	(1,52552; 1,55448)
control	3	1,00000	0,01000	(0,98552; 1,01448)

DesvPad Combinado = 0,0112546

Teste T para Duas Amostras e IC: control; F1

Método

μ_1: média de control
μ_2: média de F1
Diferença: $\mu_1 - \mu_2$

Não assumiu-se igualdade de variâncias para esta análise.

Estatísticas Descritivas

Amostra	N	Média	DesvPad	EP Média
control	3	1,0000	0,0100	0,0058
F1	3	1,5167	0,0153	0,0088

Estimativa da diferença

Diferença	IC de 95% para a Diferença
-0,5167	(-0,5502; -0,4831)

Teste

Hipótese nula H_0: $\mu_1 - \mu_2 = 0$
Hipótese alternativa H_1: $\mu_1 - \mu_2 \neq 0$

Valor-T	GL	Valor-p
-49,02	3	0,000

Teste T para Duas Amostras e IC: control; F2

Método

μ_1: média de control
μ_2: média de F2
Diferença: $\mu_1 - \mu_2$

Não assumiu-se igualdade de variâncias para esta análise.

Estatísticas Descritivas

Amostra	N	Média	DesvPad	EP Média
control	3	1,0000	0,0100	0,0058
F2	3	1,4400	0,0100	0,0058

Estimativa da diferença

Diferença	IC de 95% para a Diferença
-0,44000	(-0,46267; -0,41733)

Teste

Hipótese nula H_0: $\mu_1 - \mu_2 = 0$
Hipótese alternativa H_1: $\mu_1 - \mu_2 \neq 0$

Valor-T	GL	Valor-p
-53,89	4	0,000

Teste T para Duas Amostras e IC: control; F3

Método

μ_1: média de control
μ_2: média de F3
Diferença: $\mu_1 - \mu_2$

Não assumiu-se igualdade de variâncias para esta análise.

Estatísticas Descritivas

Amostra	N	Média	DesvPad	EP Média
control	3	1,0000	0,0100	0,0058
F3	3	1,7300	0,0100	0,0058

Estimativa da diferença

Diferença	IC de 95% para a Diferença
-0,73000	(-0,75267; -0,70733)

Teste

Hipótese nula H_0: $\mu_1 - \mu_2 = 0$
Hipótese alternativa H_1: $\mu_1 - \mu_2 \neq 0$

Valor-T	GL	Valor-p
-89,41	4	0,000

Teste T para Duas Amostras e IC: control; F4

Método

μ_1: média de control
μ_2: média de F4
Diferença: $\mu_1 - \mu_2$

Não assumiu-se igualdade de variâncias para esta análise.

Estatísticas Descritivas

Amostra	N	Média	DesvPad	EP Média
control	3	1,0000	0,0100	0,0058
F4	3	1,5400	0,0100	0,0058

Estimativa da diferença

Diferença	IC de 95% para a Diferença
-0,54000	(-0,56267; -0,51733)

Teste

Hipótese nula H_0: $\mu_1 - \mu_2 = 0$
Hipótese alternativa H_1: $\mu_1 - \mu_2 \neq 0$

Valor-T	GL	Valor-p
-66,14	4	0,000

Annex I: Statistical Analysis of a*

ANOVA com um fator: F1; F2; F3; F4; control

Método

Hipótese nula	Todas as médias são iguais
Hipótese alternativa	Nem todas as médias são iguais
Nível de significância	α = 0,05

Assumiu-se igualdade de variâncias para a análise

Informações dos Fatores

Fator	Níveis	Valores
Fator	5	F1; F2; F3; F4; control

Análise de Variância

Fonte	GL	SQ (Aj.)	QM (Aj.)	Valor F	Valor-P
Fator	4	0,185160	0,046290	462,90	0,000
Erro	10	0,001000	0,000100		
Total	14	0,186160			

Sumário do Modelo

S	R2	R2(aj)	R2(pred)
0,01	99,46%	99,25%	98,79%

Médias

Fator	N	Média	DesvPad	IC de 95%
F1	3	0,97000	0,01000	(0,95714; 0,98286)
F2	3	0,93000	0,01000	(0,91714; 0,94286)
F3	3	0,95000	0,01000	(0,93714; 0,96286)
F4	3	0,88000	0,01000	(0,86714; 0,89286)
control	3	1,20000	0,01000	(1,18714; 1,21286)

DesvPad Combinado = 0,01

Comparações Emparelhadas de Tukey

Informações de Agrupamento Usando Método de Tukey e Confiança de 95%

Fator	N	Média	Agrupamento		
control	3	1,20000	A		
F1	3	0,97000		B	
F3	3	0,95000		B	C
F2	3	0,93000			C
F4	3	0,88000			D

Médias que não compartilham uma letra são significativamente diferentes.

Teste T para Duas Amostras e IC: control; F1

Método

μ_1: média de control
μ_2: média de F1
Diferença: $\mu_1 - \mu_2$

Não assumiu-se igualdade de variâncias para esta análise.

Estatísticas Descritivas

Amostra	N	Média	DesvPad	EP Média
control	3	1,2000	0,0100	0,0058
F1	3	0,9700	0,0100	0,0058

Estimativa da diferença

Diferença	IC de 95% para a Diferença
0,23000	(0,20733; 0,25267)

Teste

Hipótese nula H_0: $\mu_1 - \mu_2 = 0$
Hipótese alternativa H_1: $\mu_1 - \mu_2 \neq 0$

Valor-T	GL	Valor-p
28,17	4	0,000

Teste T para Duas Amostras e IC: control; F2

Método

μ_1: média de control
μ_2: média de F2
Diferença: $\mu_1 - \mu_2$

Não assumiu-se igualdade de variâncias para esta análise.

Estatísticas Descritivas

Amostra	N	Média	DesvPad	EP Média
control	3	1,2000	0,0100	0,0058
F2	3	0,9300	0,0100	0,0058

Estimativa da diferença

Diferença	IC de 95% para a Diferença
0,27000	(0,24733; 0,29267)

Teste

Hipótese nula H_0: $\mu_1 - \mu_2 = 0$
Hipótese alternativa H_1: $\mu_1 - \mu_2 \neq 0$

Valor-T	GL	Valor-p
33,07	4	0,000

Teste T para Duas Amostras e IC: control; F3

Método

μ_1: média de control
μ_2: média de F3
Diferença: $\mu_1 - \mu_2$

Não assumiu-se igualdade de variâncias para esta análise.

Estatísticas Descritivas

Amostra	N	Média	DesvPad	EP Média
control	3	1,2000	0,0100	0,0058
F3	3	0,9500	0,0100	0,0058

Estimativa da diferença

Diferença	IC de 95% para a Diferença
0,25000	(0,22733; 0,27267)

Teste

Hipótese nula $H_0: \mu_1 - \mu_2 = 0$
Hipótese alternativa $H_1: \mu_1 - \mu_2 \neq 0$

Valor-T	GL	Valor-p
30,62	4	0,000

Teste T para Duas Amostras e IC: control; F4

Método

μ_1: média de control
μ_2: média de F4
Diferença: $\mu_1 - \mu_2$

Não assumiu-se igualdade de variâncias para esta análise.

Estatísticas Descritivas

Amostra	N	Média	DesvPad	EP Média
control	3	1,2000	0,0100	0,0058
F4	3	0,8800	0,0100	0,0058

Estimativa da diferença

Diferença	IC de 95% para a Diferença
0,32000	(0,29733; 0,34267)

Teste

Hipótese nula $H_0: \mu_1 - \mu_2 = 0$
Hipótese alternativa $H_1: \mu_1 - \mu_2 \neq 0$

Valor-T	GL	Valor-p
39,19	4	0,000

Annex J: Statistical Analysis of TEWL

ANOVA com um fator: F1; F2; F3; F4; control

Método

Hipótese nula	Todas as médias são iguais
Hipótese alternativa	Nem todas as médias são iguais
Nível de significância	α = 0,05

Assumiu-se igualdade de variâncias para a análise

Informações dos Fatores

Fator	Níveis	Valores
Fator	5	F1; F2; F3; F4; control

Análise de Variância

Fonte	GL	SQ (Aj.)	QM (Aj.)	Valor F	Valor-P
Fator	4	0,289560	0,072390	723,90	0,000
Erro	10	0,001000	0,000100		
Total	14	0,290560			

Sumário do Modelo

S	R2	R2(aj)	R2(pred)
0,01	99,66%	99,52%	99,23%

Médias

Fator	N	Média	DesvPad	IC de 95%
F1	3	1,25000	0,01000	(1,23714; 1,26286)
F2	3	1,42000	0,01000	(1,40714; 1,43286)
F3	3	1,36000	0,01000	(1,34714; 1,37286)
F4	3	1,38000	0,01000	(1,36714; 1,39286)
control	3	1,67000	0,01000	(1,65714; 1,68286)

DesvPad Combinado = 0,01

Comparações Emparelhadas de Tukey

Informações de Agrupamento Usando Método de Tukey e Confiança de 95%

Fator	N	Média	Agrupamento			
control	3	1,67000	A			
F2	3	1,42000		B		
F4	3	1,38000			C	
F3	3	1,36000			C	
F1	3	1,25000				D

Médias que não compartilham uma letra são significativamente diferentes.

Teste T para Duas Amostras e IC: control; F1

Método

μ_1: média de control
μ_2: média de F1
Diferença: $\mu_1 - \mu_2$

Não assumiu-se igualdade de variâncias para esta análise.

Estatísticas Descritivas

Amostra	N	Média	DesvPad	EP Média
control	3	1,6700	0,0100	0,0058
F1	3	1,2500	0,0100	0,0058

Estimativa da diferença

Diferença	IC de 95% para a Diferença
0,42000	(0,39733; 0,44267)

Teste

Hipótese nula H_0: $\mu_1 - \mu_2 = 0$
Hipótese alternativa H_1: $\mu_1 - \mu_2 \neq 0$

Valor-T	GL	Valor-p
51,44	4	0,000

Teste T para Duas Amostras e IC: control; F2

Método

μ_1: média de control
μ_2: média de F2
Diferença: $\mu_1 - \mu_2$

Não assumiu-se igualdade de variâncias para esta análise.

Estatísticas Descritivas

Amostra	N	Média	DesvPad	EP Média
control	3	1,6700	0,0100	0,0058
F2	3	1,4200	0,0100	0,0058

Estimativa da diferença

Diferença	IC de 95% para a Diferença
0,25000	(0,22733; 0,27267)

Teste

Hipótese nula H_0: $\mu_1 - \mu_2 = 0$
Hipótese alternativa H_1: $\mu_1 - \mu_2 \neq 0$

Valor-T	GL	Valor-p
30,62	4	0,000

Teste T para Duas Amostras e IC: control; F3

Método

μ_1: média de control
μ_2: média de F3
Diferença: $\mu_1 - \mu_2$

Não assumiu-se igualdade de variâncias para esta análise.

Estatísticas Descritivas

Amostra	N	Média	DesvPad	EP Média
control	3	1,6700	0,0100	0,0058
F3	3	1,3600	0,0100	0,0058

Estimativa da diferença

Diferença	IC de 95% para a Diferença
0,31000	(0,28733; 0,33267)

Teste

Hipótese nula H_0: $\mu_1 - \mu_2 = 0$
Hipótese alternativa H_1: $\mu_1 - \mu_2 \neq 0$

Valor-T	GL	Valor-p
37,97	4	0,000

Teste T para Duas Amostras e IC: control; F4

Método

μ_1: média de control
μ_2: média de F4
Diferença: $\mu_1 - \mu_2$

Não assumiu-se igualdade de variâncias para esta análise.

Estatísticas Descritivas

Amostra	N	Média	DesvPad	EP Média
control	3	1,6700	0,0100	0,0058
F4	3	1,3800	0,0100	0,0058

Estimativa da diferença

Diferença	IC de 95% para a Diferença
0,29000	(0,26733; 0,31267)

Teste

Hipótese nula H_0: $\mu_1 - \mu_2 = 0$
Hipótese alternativa H_1: $\mu_1 - \mu_2 \neq 0$

Valor-T	GL	Valor-p
35,52	4	0,000

Annex K: Student Sheet

25/04/2019

Janus - Sistema Administrativo da Pós-Graduação

Universidade de São Paulo
Faculdade de Ciências Farmacêuticas
Documento sem validade oficial
FICHA DO ALUNO

9139 - 9215614/1 - Mirela Cardoso Garcia

Email:	mirelacga@usp.br
Data de Nascimento:	13/02/1985
Cédula de Identidade:	RG - 34.114.873-8 - SP
Local de Nascimento:	Estado de São Paulo
Nacionalidade:	Brasileira
Graduação:	Farmacêutico - Universidade São Judas Tadeu - São Paulo - Brasil - 2010
Mestrado:	Mestre em Ciências Farmacêuticas (1) - Faculdade de Ciências Farmacêuticas - Universidade Estadual Paulista "Júlio de Mesquita Filho" - São Paulo - Brasil - 2014

Curso:	Doutorado
Programa:	Fármaco e Medicamentos
Área:	Produção e Controle Farmacêuticos
Data de Matrícula:	27/02/2015
Início da Contagem de Prazo:	27/02/2015
Data Limite para o Depósito:	29/04/2019
Orientador:	Prof(a). Dr(a). André Rolim Baby - 27/02/2015 até o presente. Email: andrerb@usp.br
Proficiência em Línguas:	Inglês, Aprovado em 27/02/2015
Trancamento(s):	60 dias Período de 28/11/2018 até 26/01/2019
Data de Aprovação no Exame de Qualificação:	Aprovado em 11/04/2017
Data do Depósito do Trabalho:	
Título do Trabalho:	
Data Máxima para Aprovação da Banca:	
Data de Aprovação da Banca:	
Data Máxima para Defesa:	
Data da Defesa:	
Resultado da Defesa:	
Histórico de Ocorrências:	Primeira Matrícula em 27/02/2015 Trancado em 28/11/2018

Aluno matriculado no Regimento da Pós-Graduação USP (Resolução nº 6542 em vigor de 20/04/2013 até 28/03/2018).
Última ocorrência: Matrícula de Acompanhamento em 27/01/2019
Impresso em: 25/04/2019 08:34:52

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Universidade de São Paulo
Faculdade de Ciências Farmacêuticas
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FICHA DO ALUNO

9139 - 9215614/1 - Mirela Cardoso Garcia

Sigla	Nome da Disciplina	Início	Término	Carga Horária	Cred.	Freq.	Conc.	Exc.	Situação
FBF5704-6/3	Análise Espectrométrica de Fármacos	09/03/2015	22/06/2015	150	10	95	A	N	Concluída
EDM5103-2/1	Aprendizagem Colaborativa e Mapas Conceituais: Fundamentos, Desafios e Perspectivas (Faculdade de Educação - Universidade de São Paulo)	12/03/2015	03/06/2015	120	8	92	B	N	Concluída
EDM5804-7/2	Tópicos de Epistemologia e Didática (Faculdade de Educação - Universidade de São Paulo)	13/03/2015	04/06/2015	120	8	95	A	N	Concluída
FBF5779-3/1	Preparo de Artigos Científicos na Área de Farmácia	10/03/2017	19/05/2017	90	6	89	B	N	Concluída
FBF5830-1/1	Formulações Cosméticas e Dermatológicas: Aspectos Clínicos	09/10/2017	12/11/2017	75	5	80	A	N	Concluída

	Créditos mínimos exigidos		Créditos obtidos
	Para exame de qualificação	Para depósito de tese	
Disciplinas:	0	20	37
Estágios:			
Total:	0	20	37

Créditos Atribuídos à Tese: 167

Observações:
1) Curso com validade nacional, de acordo com o disposto na Portaria MEC nº 1.077, de 31.08.2012..

Conceito a partir de 02/01/1997:
A - Excelente, com direito a crédito; B - Bom, com direito a crédito; C - Regular, com direito a crédito; R - Reprovado; T - Transferência.

Um(1) crédito equivale a 15 horas de atividade programada.

Última ocorrência: Matrícula de Acompanhamento em 27/01/2019
Impresso em: 25/04/2019 08:34:52

Janus - Sistema Administrativo da Pós-Graduação

Universidade de São Paulo
Faculdade de Ciências Farmacêuticas
Documento sem validade oficial

FICHA DO ALUNO

9139 - 9215614/1 - Mirela Cardoso Garcia

Comissão julgadora da tese de doutorado:			
NUSP	Nome	Vínculo	Função
3755071	André Rolim Baby	FCF - USP	Presidente

Última ocorrência: Matrícula de Acompanhamento em 27/01/2019
Impresso em: 25/04/2019 08:34:52

Mirela Cardoso Garcia

Endereço para acessar este CV: http://lattes.cnpq.br/9412300458221250
Última atualização do currículo em 23/04/2019

Graduada em Ciências Biológicas (2006) pela Universidade Presbiteriana Mackenzie e graduada em Farmácia (2009) pela Universidade São Judas Tadeu. Possui Mestrado, bolsa FAPESP, em Ciências Farmacêuticas pelo programa de pós-graduação em Ciências Farmacêuticas pela Universidade Estadual Paulista Júlio Mesquita Filho (FCFAR- UNESP), área de Farmacotecnia. Atualmente doutoranda na Universidade de São Paulo (USP) na área de Cosmetologia, precisamente fotorpotetores contendo flavonoides e docente no curso de Farmácia na Universidade de Guarulhos (UnG), Experiência na área de indústria farmacêutica com marketing farmacêutico, cosmetologia, desenvolvimento de fotoprotetores, farmacotecnia e controle de qualidade físico-químico de medicamentos e cosméticos. **(Texto informado pelo autor)**

Identificação

Nome Mirela Cardoso Garcia
Nome em citações bibliográficas GARCIA, M. C.

Endereço

Endereço Profissional
Universidade de São Paulo, Faculdade de Ciências Farmacêuticas.
Avenida Professor Lineu Prestes 580, bloco 15
Butantã
05508000 - São Paulo, SP - Brasil
Telefone: (11) 30912358
URL da Homepage: http://www.fcf.usp.br/

Formação acadêmica/titulação

2015
Doutorado em andamento em Fármaco e Medicamentos.
Universidade de São Paulo, USP, Brasil.
Orientador: André Rolim Baby.
Palavras-chave: cosmetologia; famacotecnia; fotoprotetor; flavonoides; antioxidante.
Grande área: Ciências da Saúde
Grande Área: Ciências da Saúde / Área: Farmácia / Subárea: Farmacotecnia.

2012 - 2014
Mestrado em Ciências Farmacêuticas (Conceito CAPES 6).
Universidade Estadual Paulista Júlio de Mesquita Filho, UNESP, Brasil.
Título: MICROPARTÍCULAS DE METOTREXATO E ÁCIDO POLI LÁTICO-CO-GLICÓLICO OBTIDAS POR ?SPRAY-DRYING?,Ano de Obtenção: 2014.
Orientador: Maria Virgínia Costa Scarpa.
Bolsista do(a): Fundação de Amparo à Pesquisa do Estado de São Paulo, FAPESP, Brasil.
Palavras-chave: metotrexato; micropartículas biodegradáveis; spray drying; liberação prolongada; poli (ácido lático-co-ácido glicólico) PLGA.
Grande área: Ciências da Saúde
Grande Área: Ciências da Saúde / Área: Farmácia / Subárea: Controle de Medicamentos.

2006 - 2009
Graduação em Farmácia.
Universidade São Judas Tadeu, USJT, Brasil.
Título: "Utilização de derivados de células-tronco vegetais para a redução dos sinais do envelhecimento".
Orientador: Ricardo D'Agostino Garcia.

2002 - 2006
Graduação em Ciências Biológicas.
Universidade Presbiteriana Mackenzie, MACKENZIE, Brasil.
Título: "Incidência de anticorpos anti-citomegalovírus (CMV) numa população de adultos jovens".
Orientador: Dra. Sofia Rocha Lieber.

Formação Complementar

2014 - 2014

Rodada Tecnológica de Química Aplicada a Estética. (Carga horária: 8h).
Associação Brasileira de Cosmetologia, ABC, Brasil.

2012 - 2012 Tópicos Avançados em Biofarmácia e Farmacocinétic. (Carga horária: 40h).
Universidade Estadual Paulista Júlio de Mesquita Filho, UNESP, Brasil.

2011 - 2011 Aperfeiçoamento Profissional. (Carga horária: 200h).
Universidade Estadual Paulista Júlio de Mesquita Filho, UNESP, Brasil.

2009 - 2009 Farmacologia. (Carga horária: 8h).
Universidade São Judas Tadeu, USJT, Brasil.

2006 - 2006 Coleta de Sangue a Vácuo. (Carga horária: 4h).
Greiner Bio-one Brasil, GBB, Brasil.

2003 - 2003 Saúde Pública. (Carga horária: 4h).
Universidade Presbiteriana Mackenzie, MACKENZIE, Brasil.

2002 - 2002 Fundamentos de Biologia Marinha. (Carga horária: 60h).
ARGOS - Centro de Estudos de Biologia Marinha, ARGOS, Brasil.

Atuação Profissional

Universidade de Guarulhos, UNG, Brasil.

Vínculo institucional
2016 - Atual Vínculo: Professor Visitante, Enquadramento Funcional: Professora, Carga horária: 4

Centro Estadual de Educação Tecnológica Paula Souza, CEETEPS, Brasil.

Vínculo institucional
2015 - 2016 Vínculo: Professor Visitante, Enquadramento Funcional: Professor Associado I, Carga horária: 20
Outras informações Professora por tempo determinado de Química Geral, Contexto Profissional e Projeto de Desenvolvimento de Produto Cosmético AA

Universidade de São Paulo, USP, Brasil.

Vínculo institucional
2014 - Atual Vínculo: estudante, Enquadramento Funcional: Doutoranda em Cosmetologia, Regime: Dedicação exclusiva.

Universidade Estadual Paulista Júlio de Mesquita Filho, UNESP, Brasil.

Vínculo institucional
2012 - 2014 Vínculo: Colaborador, Enquadramento Funcional: Mestranda, Regime: Dedicação exclusiva.
Vínculo institucional
2011 - 2011 Vínculo: estágio de aperfeiçoamento, Enquadramento Funcional: estágio de aperfeiçoamento, Regime: Dedicação exclusiva.
Outras informações Estágio de Aperfeiçoamento no laboratório de Controle de Fármacos e Medicamentos.

Editora Manole Ltda., MANOLE, Brasil.

Vínculo institucional
2010 - 2011 Vínculo: funcionária, Enquadramento Funcional: Gerente de Produto (Lexi-Comp)/ vendedora, Carga horária: 44, Regime: Dedicação exclusiva.
Outras informações Gerente de Produto (Lexi-Comp)/ vendedora ? Visitas em farmácias hospitalares de determinados hospitais. ? Desenvolvimento de planos estratégicos de marketing e vendas. ? Desenvolvimento, revisão e gestão de materiais promocionais do Lexi-Comp e outras campanhas desenvolvidas pela editora como Tratado de Psiquiatria 2011. ? Gestão de senhas ?teste? do produto e propostas.

Mogami Importação e Exportação Ltda., MOGAMI, Brasil.

Vínculo institucional
2010 - 2010 Vínculo: funcionária, Enquadramento Funcional: farmacêutica, Carga horária: 44, Regime: Dedicação exclusiva.
Outras informações Representate Técnica na área ginecológica (Gynecare J&J) ? Visitas médicas em centros cirúrgicos ou ambulatórios em determinados hospitais; ? Acompanhamento do uso de produtos vendidos por meio de licitações; ? Negociações com os hospitais sobre as licitações e compra dos produtos Gynecare

CSL Behring, CSL, Brasil.

Vínculo institucional

2006 - 2007 Vínculo: funcionária, Enquadramento Funcional: funcionária, Carga horária: 44, Regime: Dedicação exclusiva.

Outras informações área de Marketing Farmacêutico: suporte ao gerente de produto ? negociações com fornecedores gráficos - escolha do material adequado para mídias diversas, negociação de prazos de entrega e preços ? gestão de eventos: negociação com agências de turismo, montadoras de estandes, organizadoras de congressos médicos, realização de workshops, reunião de treinamentos, coffe-break, negociação de brindes em geral para congressos e outros eventos da empresa. ? gestão de materiais de propaganda medica: envio de materiais promocionais durante ciclos de visitação, entrada e saída de materiais e brindes via SAP. ? marketing farmacêutico: busca de materiais científicos em bibliotecas como BIREME, Lilacs entre outras, planilha de artigos já comprados de acordo com o produto, correção de materiais técnicos científicos para uso em propaganda médica, revisão dos materiais científicos produzidos pela empresa e contato direto com os representantes de vendas e terceirizados. ? atualização do web site da empresa através Fat Wire ? software proprietário em linguagem html ? planilha de custos de marketing ? lançamento de pagamentos e patrocínios para analise de budget do gerente de produto.

Universidade Presbiteriana Mackenzie, MACKENZIE, Brasil.

Vínculo institucional

2006 - 2006 Vínculo: estagiária, Enquadramento Funcional: estagiária, Carga horária: 20

Outras informações Estágio em Virologia: pesquisa de anticorpos anti-CMV

Colégio Lumiere, LUMIERE, Brasil.

Vínculo institucional

2003 - 2004 Vínculo: estagiária, Enquadramento Funcional: estagiária, Carga horária: 20

Outras informações Estágio em Licenciatura: ministrar aulas de Ciências/Biologia para alunos de 5ª à 8ª séries 1º ao 3º colegial

Associação Nacional de Assistência ao Diabético, ANAD, Brasil.

Vínculo institucional

2003 - 2003 Vínculo: Colaborador, Enquadramento Funcional: estagiário, Carga horária: 20

Outras informações Estágio em aconselhamento genético e assistência para diabéticos.

Faculdade Método de São Paulo, FAMESP, Brasil.

Vínculo institucional

2019 - Atual Vínculo: Professor Visitante, Enquadramento Funcional: Professora, Carga horária: 8

Outras informações Professora no Curso Superior de Estética nas disciplinas de Introdução à Cosmetologia e Cosmetologia Aplicada

Áreas de atuação

1. Grande área: Ciências da Saúde / Área: Farmácia / Subárea: Garantia e controle de qualidade farmacêuticos.

2. Grande área: Ciências da Saúde / Área: Farmácia / Subárea: Farmacotécnica e tecnologia farmacêutica/Especialidade: Cosmetologia.

3. Grande área: Ciências Humanas / Área: Educação / Subárea: Ensino-Aprendizagem.

4. Grande área: Ciências da Saúde / Área: Farmácia / Subárea: Farmacotecnia.

Idiomas

Inglês Compreende Bem, Fala Bem, Lê Bem, Escreve Bem.

Francês Compreende Bem, Fala Bem, Lê Bem, Escreve Bem.

Espanhol Compreende Bem, Fala Bem, Lê Bem, Escreve Bem.

Prêmios e títulos

2018 3º lugar no 31º Congresso Brasileiro de Cosmetologia, Associação Brasileira de Cosmetologia.

Produções

Produção bibliográfica

Apresentações de Trabalho

1. TOKUNAGA, V. K. ; **GARCIA, M. C.** ; OLIVEIRA, C. A. ; ESCUDEIRO, C. C. ; BLOCH, L. D. ; SARRUF, F. D. ; SAUCE, R. ; PARISE-FILHO, R. ; ROSADO, C. ; VELASCO, M. V. R. ; BABY, A. R. . Propriedades fotoprotetora e fotoestabilizadora da cafeína. 2018. (Apresentação de Trabalho/Congresso).
2. **GARCIA, M. C.**. Gel para barbear e pós-barba. 2018. (Apresentação de Trabalho/Conferência ou palestra).
3. **GARCIA, M. C.**; CANDIDO, T. M. ; FERNANDES, L. S. ; VELASCO, M. V. R. ; BACCHI, E. M. ; BABY, A. R. . EXTRATOS DE JACARANDA: POTENCIAL EFEITO FPS BOOSTER EM PROTETORES SOLARES BIOATIVOS. 2015. (Apresentação de Trabalho/Congresso).
4. **GARCIA, M. C.**; CANDIDO, T. M. ; FERNANDES, L. S. ; VELASCO, M. V. R. ; BACCHI, E. M. ; BABY, A. R. . Extratos de Jacaranda: Potencial efeito FPS booster em protetores solares bioativos. 2015. (Apresentação de Trabalho/Congresso).
5. **GARCIA, M. C.**; SCARPA, M. V. . PLGA MICROPARTICLES CONTAINING METHOTREXATE OBTAINED BY ?SPRAY-DRYING?: PROFILE RELEASE COMPARED WITH SCANNING ELECTRON AND ATOMIC FORCE MICROSCOPIES. 2014. (Apresentação de Trabalho/Congresso).
6. **GARCIA, M. C.**; MUSSARELI, K. R. ; DEPAOLI, A. C. C. ; SCARPA, M. V. . COMPARISON OF PROFILE RELEASE MICROPARTICLES ACID POLY-LÁTICOCO GLYCOLIC (PLGA) OBTAINED WITH METHOTREXATE 'SPRAY-DRYING' WITH DIFFERENT CONCENTRATIONS. 2013. (Apresentação de Trabalho/Congresso).
7. **GARCIA, M. C.**; MUSSARELI, K. R. ; DEPAOLI, A. C. C. ; SCARPA, M. V. . EVALUATION OF THE RELEASE PROFILE, IN VITRO PERMEATION AND CUTANEOUS RETENTION OF CARBOPOL GEL WITH EXTRACT OF Physalis angulate. 2013. (Apresentação de Trabalho/Congresso).
8. SCARPA, M. V. ; MUSSARELI, K. R. ; DEPAOLI, A. C. C. ; **GARCIA, M. C.** . 'Complexation drug-dendrimer: evaluation of the mucoadhesive properties'. 2013. (Apresentação de Trabalho/Congresso).
9. SCARPA, M. V. ; ANGELIERI, N. F. ; DEPAOLI, A. C. C. ; MUSSARELI, K. R. ; **GARCIA, M. C.** . EVALUATION OF THE RELEASE PROFILE, IN VITRO PERMEATION AND CUTANEOUS RETENTION OF CARBOPOL GEL WITH EXTRACT OF Physalis angulate. 2013. (Apresentação de Trabalho/Congresso).
10. MUSSARELI, K. R. ; DEPAOLI, A. C. C. ; **GARCIA, M. C.** ; SCARPA, M. V. . Complexation dendrimer-drug: strategy for increasing the solubility of ibuprofen. 2013. (Apresentação de Trabalho/Congresso).
11. MUSSARELI, K. R. ; DEPAOLI, A. C. C. ; **GARCIA, M. C.** ; SCARPA, M. V. . Qualitative analysis of the dendrimer-drug complex by atenuated total reflectance fourier tranform infrared spectroscopy. 2013. (Apresentação de Trabalho/Congresso).
12. DEPAOLI, A. C. C. ; MUSSARELI, K. R. ; **GARCIA, M. C.** ; SCARPA, M. V. . Characterization of Solid Lipid Nanoparticles (SLN) containing different concentrations of ibuprofen by comparing the technique of high pressure homogenization versus sonicator.. 2013. (Apresentação de Trabalho/Congresso).
13. DEPAOLI, A. C. C. ; MUSSARELI, K. R. ; **GARCIA, M. C.** ; SCARPA, M. V. . CHARACTERIZATION OF SOLID LIPID NANOPARTICLE BY FOURIER TRANSFORM INFRARED SPECTROSCOPY. 2013. (Apresentação de Trabalho/Congresso).
14. DEPAOLI, A. C. C. ; MUSSARELI, K. R. ; **GARCIA, M. C.** ; ANDREANI, T. ; SCARPA, M. V. . Estudo do comportamento do ibuprofeno em lipídios sólidos para o desenvolvimento de nanopartículas lipídicas sólidas. 2013. (Apresentação de Trabalho/Congresso).
15. DEPAOLI, A. C. C. ; MUSSARELI, K. R. ; **GARCIA, M. C.** ; ANGELIERI, N. F. ; SCARPA, M. V. . Desenvolvimento de nanopartículas lipídicas sólidas: homogeneizador de alta pressão x sonicador. 2013. (Apresentação de Trabalho/Congresso).
16. MUSSARELI, K. R. ; DEPAOLI, A. C. C. ; **GARCIA, M. C.** ; SCARPA, M. V. . Complexetion dendrimer-drug: Qualitative analysis by infrared spectroscopy. 2013. (Apresentação de Trabalho/Congresso).
17. DEPAOLI, A. C. C. ; MUSSARELI, K. R. ; **GARCIA, M. C.** ; SCARPA, M. V. . Qualitative analysis of solid lipid nanoparticle containing ibuprofen by infrared spectrophotometry. 2013. (Apresentação de Trabalho/Congresso).
18. **GARCIA, M. C.**; DEPAOLI, A. C. C. ; MUSSARELI, K. R. ; SCARPA, M. V. . Microparticles of poly(D,L-lactic-co-glycolic acid) (PLGA) with methotrexate obtained by 'spray-drying' qualitatively analyzed by infrared spectroscopy. 2013. (Apresentação de Trabalho/Congresso).

Produção técnica

Trabalhos técnicos

1. **GARCIA, M. C.**; RUAS, G.W. . Manual de Monografia. 2015.

Demais tipos de produção técnica

1. **GARCIA, M. C.**; RUAS, G. W. . Manual de Monografia FATEC Luigi Papaiz. 2015. (Desenvolvimento de material didático ou instrucional - Manual de Monografia).

Bancas

Participação em bancas de trabalhos de conclusão

Trabalhos de conclusão de curso de graduação

1. ITO, R. K.; **GARCIA, M. C.**. Participação em banca de Juliana Penasso Silva.Desenvolvimento de sabonete liquido para acne utilizando semente e extrato da folha de Psidium guajava L... 2015. Trabalho de Conclusão de Curso (Graduação em Tecnologia em Cosméticos) - Faculdade de Tecnologia Diadema.
2. ALMASY, A.G.; **GARCIA, M. C.**; LIZARRAGA, A.. Participação em banca de Rodrigo Juliano Pellin.Sabonete repelente em aerossol. 2015. Trabalho de Conclusão de Curso (Graduação em Tecnologia em Cosméticos) - Faculdade de Tecnologia Diadema.
3. ITO, R. K.; **GARCIA, M. C.**; NICOLETTI, M.A.. Participação em banca de Beatriz Gomes Domingos.A biodiversidade nas indústrias cosméticas no Brasil. Estudo baseado em levantamento bibliográfico de dados relacionados a produtos contendo ativos vegetais. 2015. Trabalho de Conclusão de Curso (Graduação em Tecnologia em Cosméticos) - Faculdade de Tecnologia Diadema.
4. PAIS, M.C.N.; **GARCIA, M. C.**; BANZATO, T.P.. Participação em banca de Leticia Gabrielle Garcia.Levantamento bibliográfico e técnico do risco de retinoide, hidroquinona e ureia em formulações cosméticas para gestantes. 2015. Trabalho de Conclusão de Curso (Graduação em Tecnologia em Cosméticos) - Faculdade de Tecnologia Diadema.

Eventos

Participação em eventos, congressos, exposições e feiras

1. 31º Congresso Brasileiro de Cosmetologia. Propriedades fotoprotetora e fotoestabilizadora da cafeína. 2018. (Congresso).
2. 28º Congresso Brasileiro de Cosmetologia. EXTRATOS DE JACARANDA: POTENCIAL EFEITO FPS BOOSTER EM PROTETORES SOLARES BIOATIVOS. 2015. (Congresso).
3. Maison de la Beautè. 2015. (Outra).
4. 9th World Meeting on Pharmaceutics, Biopharmaceutics and Pharmaceutical Technology. PLGA MICROPARTICLES CONTAINING METHOTREXATE OBTAINED BY ?SPRAY-DRYING?: PROFILE RELEASE COMPARED WITH SCANNING ELECTRON AND ATOMIC FORCE MICROSCOPIES. 2014. (Congresso).
5. 3rd Conference on Innovation in Drug Delivery - Advances in Local Drug Delivery APGI. "Evaluation of the release profile of supercritical extract of Physalis angulata incorporated in solid lipid nanoparticles". 2013. (Congresso).
6. 3rd Conference on Innovation in Drug Delivery - Advances in Local Drug Delivery APGI. "Complexation drug-dendrimer: evaluation of the mucoadhesive properties". 2013. (Congresso).
7. CIFARP- 9º International Congress of Pharmaceutical Sciences. COMPARISON OF PROFILE RELEASE MICROPARTICLES ACID POLY-LÁTICOCO GLYCOLIC (PLGA) OBTAINED WITH METHOTREXATE "SPRAY-DRYING" WITH DIFFERENT CONCENTRATIONS. 2013. (Congresso).
8. III Congresso Farmacêutico da UNESP. Estudo do comportamento do ibuprofeno em lipídios síolidos para o desenvolvimento de nanopartícula lipídica sólida. 2013. (Congresso).
9. III International Symposium on Drug Discovery.EVALUATION OF THE RELEASE PROFILE, IN VITRO PERMEATION AND CUTANEOUS RETENTION OF CARBOPOL GEL WITH EXTRACT OF Physalis angulate. 2013. (Simpósio).
10. I International Symposium on Cosmetology.I International Symposium on Cosmetology. 2013. (Simpósio).
11. XII Encontro SBPMAt. Microparticles of poly(D,L-lactic-co-glycolic acid) (PLGA) with methotrexate obtained by "spray-drying" qualitatively analyzed by infrared spectroscopy. 2013. (Congresso).
12. 26º Congresso Brasileiro de Cosmetologia. 2012. (Congresso).
13. International Workshop on Imune System Dietary Factors and Nanotechonology.International Workshop on Imune System Dietary Factors and Nanotechonology. 2012. (Outra).
14. 23 Congresso Brasileiro de Cosmetologia. Inovação e sustentabilidade. 2009. (Congresso).
15. I Simpósio de Atualização em Farmacologia Clínica do Curso de Farmácia. 2007. (Simpósio).
16. IX JOFARMA -Jornada Farmacêutica do Curso de Farmácia. 2006. (Encontro).
17. XXII Semana da Ciência. 2005. (Encontro).
18. XII Simpósio de Educação e V Encontro de Professores."Inclusão e Exclusão: Contrapontos da Educação Brasileira". 2003. (Simpósio).

Orientações

Orientações e supervisões em andamento

Trabalho de conclusão de curso de graduação

1. CARLOS FACHIN JUNIOR. ESTRATÉGIAS DE MARKETING DESENVOLVIDAS NAS INDÚSTRIAS FARMACÊUTICAS. Início: 2016. Trabalho de Conclusão de Curso (Graduação em Farmácia) - Universidade de Guarulhos. (Orientador).
2. LETÍCIA AZEVEDO VENÂNCIO. COMPARAÇÃO DE CREMES DENTAIS CONTENDO PERÓXIDO DE HIDROGÊNIO E METABISSULFITO DE SÓDIO COMO AGENTES BRANQUEADORES. Início: 2015. Trabalho de Conclusão de Curso (Graduação

em Tecnologia em Cosméticos) - Faculdade de Tecnologia Diadema. (Orientador).

Orientações e supervisões concluídas

Trabalho de conclusão de curso de graduação

1. Amanda Galastri dos Santos. USO DE PELE HUMANA RECONSTRUÍDA COMO ALTERNATIVA AOS TESTES EM ANIMAIS PARA PRODUTOS COSMÉTICOS. 2018. Trabalho de Conclusão de Curso. (Graduação em Farmácia) - Universidade de Guarulhos. Orientador: Mirela Cardoso Garcia.
2. Paula Leal da Costa. ESTUDO DE ATIVIDADE ANTIOXIDANTE ? TAPE STRIPPING DE FORMULAÇÕES CONTENDO FILTROS SOLARES E QUERCETINA. 2018. Trabalho de Conclusão de Curso. (Graduação em Farmácia) - Universidade de Guarulhos. Orientador: Mirela Cardoso Garcia.
3. ISABEL RODRIGUES CALIXTO LINHARES. COSMÉTICOS ORGÂNICOS. 2018. Trabalho de Conclusão de Curso. (Graduação em Farmácia) - Universidade de Guarulhos. Orientador: Mirela Cardoso Garcia.
4. PATRÍCIA GOMES CORRÊA. USO DA ISOTRETINOÍNA ORAL E TÓPICA NO TRATAMENTO DA ACNE. 2018. Trabalho de Conclusão de Curso. (Graduação em Farmácia) - Universidade de Guarulhos. Orientador: Mirela Cardoso Garcia.
5. CRISTIANA SANTOS DE SOUZA SILVA. MERCADO DE LUXO DOS COSMÉTICOS. 2018. Trabalho de Conclusão de Curso. (Graduação em Farmácia) - Universidade de Guarulhos. Orientador: Mirela Cardoso Garcia.
6. AMANDA DE SOUSA FRANÇA. POTENCIAL DE IRRITAÇÃO OCULAR DE FORMULAÇÕES CONTENDO QUERCETINA POR HET-CAM. 2018. Trabalho de Conclusão de Curso. (Graduação em Farmácia) - Universidade de Guarulhos. Orientador: Mirela Cardoso Garcia.
7. Célio Ricardo Moura de Sousa. O USO DA FOSFOETANOLAMINA SINTÉTICA. 2017. Trabalho de Conclusão de Curso. (Graduação em Farmácia) - Universidade de Guarulhos. Orientador: Mirela Cardoso Garcia.
8. Bruna Larissa. Utilização de cosméticos em pacientes oncológicos. 2017. Trabalho de Conclusão de Curso. (Graduação em Farmácia) - Universidade de Guarulhos. Orientador: Mirela Cardoso Garcia.
9. Matheus Gabriel Pereira. VANTAGENS E DESVANTAGENS DA NANOTECNOLOGIA EM COSMÉTICOS. 2017. Trabalho de Conclusão de Curso. (Graduação em Farmácia) - Universidade de Guarulhos. Orientador: Mirela Cardoso Garcia.
10. Ana Kezia da Silva Lemos. ÁCIDO HIALURÔNICO NA ESTÉTICA. 2017. Trabalho de Conclusão de Curso. (Graduação em Farmácia) - Universidade de Guarulhos. Orientador: Mirela Cardoso Garcia.
11. Angélica Maria de Souza. USO DE FORMOL EM ALISANTES CAPILARES. 2017. Trabalho de Conclusão de Curso. (Graduação em Farmácia) - Universidade de Guarulhos. Orientador: Mirela Cardoso Garcia.
12. Ludmila da Silva Morais. METAIS PESADOS EM TINTURA CAPILAR. 2017. Trabalho de Conclusão de Curso. (Graduação em Farmácia) - Universidade de Guarulhos. Orientador: Mirela Cardoso Garcia.
13. Mayra Ataide Souza. FOTOPROTEÇÃO EM PRODUTOS INFANTIS. 2017. Trabalho de Conclusão de Curso. (Graduação em Farmácia) - Universidade de Guarulhos. Orientador: Mirela Cardoso Garcia.
14. Gabriela Alves dos Santos. Evolução dos produtos fotoprotetores: de simples emulsão aos produtos multifuncionais. 2016. Trabalho de Conclusão de Curso. (Graduação em Farmácia) - Universidade de Guarulhos. Orientador: Mirela Cardoso Garcia.
15. Alexander Rodrigues dos Anjos. Diabetes Mellitus - Insulinas. 2016. Trabalho de Conclusão de Curso. (Graduação em Farmácia) - Universidade de Guarulhos. Orientador: Mirela Cardoso Garcia.

Educação e Popularização de C & T

Apresentações de Trabalho

1. **GARCIA, M. C.**; MUSSARELI, K. R. ; DEPAOLI, A. C. C. ; SCARPA, M. V. . COMPARISON OF PROFILE RELEASE MICROPARTICLES ACID POLY-LÁTICOCO GLYCOLIC (PLGA) OBTAINED WITH METHOTREXATE 'SPRAY-DRYING' WITH DIFFERENT CONCENTRATIONS. 2013. (Apresentação de Trabalho/Congresso).
2. **GARCIA, M. C.**; MUSSARELI, K. R. ; DEPAOLI, A. C. C. ; SCARPA, M. V. . EVALUATION OF THE RELEASE PROFILE, IN VITRO PERMEATION AND CUTANEOUS RETENTION OF CARBOPOL GEL WITH EXTRACT OF Physalis angulate. 2013. (Apresentação de Trabalho/Congresso).
3. SCARPA, M. V. ; MUSSARELI, K. R. ; DEPAOLI, A. C. C. ; **GARCIA, M. C.** . 'Complexation drug-dendrimer: evaluation of the mucoadhesive properties'. 2013. (Apresentação de Trabalho/Congresso).
4. SCARPA, M. V. ; ANGELIERI, N. F. ; DEPAOLI, A. C. C. ; MUSSARELI, K. R. ; **GARCIA, M. C.** . EVALUATION OF THE RELEASE PROFILE, IN VITRO PERMEATION AND CUTANEOUS RETENTION OF CARBOPOL GEL WITH EXTRACT OF Physalis angulate. 2013. (Apresentação de Trabalho/Congresso).
5. MUSSARELI, K. R. ; DEPAOLI, A. C. C. ; **GARCIA, M. C.** ; SCARPA, M. V. . Complexation dendrimer-drug: strategy for increasing the solubility of ibuprofen. 2013. (Apresentação de Trabalho/Congresso).
6. MUSSARELI, K. R. ; DEPAOLI, A. C. C. ; **GARCIA, M. C.** ; SCARPA, M. V. . Qualitative analysis of the dendrimer-drug complex by atenuated total reflectance fourier tranform infrared spectroscopy. 2013. (Apresentação de Trabalho/Congresso).
7. DEPAOLI, A. C. C. ; MUSSARELI, K. R. ; **GARCIA, M. C.** ; SCARPA, M. V. . Characterization of Solid Lipid Nanoparticles (SLN) containing different concentrations of ibuprofen by comparing the technique of high pressure homogenization versus sonicator.. 2013. (Apresentação de Trabalho/Congresso).
8. DEPAOLI, A. C. C. ; MUSSARELI, K. R. ; **GARCIA, M. C.** ; SCARPA, M. V. . CHARACTERIZATION OF SOLID LIPID NANOPARTICLE BY FOURIER TRANSFORM INFRARED SPECTROSCOPY. 2013. (Apresentação de Trabalho/Congresso).
9. DEPAOLI, A. C. C. ; MUSSARELI, K. R. ; **GARCIA, M. C.** ; ANDREANI, T. ; SCARPA, M. V. . Estudo do comportamento do ibuprofeno em lipídios sólidos para o desenvolvimento de nanopartículas lipídicas sólidas. 2013. (Apresentação de Trabalho/Congresso).

10. DEPAOLI, A. C. C. ; MUSSARELI, K. R. ; **GARCIA, M. C.** ; ANGELIERI, N. F. ; SCARPA, M. V. . Desenvolvimento de nanopartículas lipídicas sólidas: homogeneizador de alta pressão x sonicador. 2013. (Apresentação de Trabalho/Congresso).
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12. DEPAOLI, A. C. C. ; MUSSARELI, K. R. ; **GARCIA, M. C.** ; SCARPA, M. V. . Qualitative analysis of solid lipid nanoparticle containing ibuprofen by infrared spectrophotometry. 2013. (Apresentação de Trabalho/Congresso).
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15. **GARCIA, M. C.**; CANDIDO, T. M. ; FERNANDES, L. S. ; VELASCO, M. V. R. ; BACCHI, E. M. ; BABY, A. R. . Extratos de Jacaranda: Potencial efeito FPS booster em protetores solares bioativos. 2015. (Apresentação de Trabalho/Congresso).
16. TOKUNAGA, V. K. ; **GARCIA, M. C.** ; OLIVEIRA, C. A. ; ESCUDEIRO, C. C. ; BLOCH, L. D. ; SARRUF, F. D. ; SAUCE, R. ; PARISE-FILHO, R. ; ROSADO, C. ; VELASCO, M. V. R. ; BABY, A. R. . Propriedades fotoprotetora e fotoestabilizadora da cafeína. 2018. (Apresentação de Trabalho/Congresso).
17. **GARCIA, M. C.**. Gel para barbear e pós-barba. 2018. (Apresentação de Trabalho/Conferência ou palestra).

Desenvolvimento de material didático ou instrucional

1. **GARCIA, M. C.**; RUAS, G. W. . Manual de Monografia FATEC Luigi Papaiz. 2015. (Desenvolvimento de material didático ou instrucional - Manual de Monografia).

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