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Article

Characterization and identification of ascorbyl methylsilanol pectinate for cosmetic formulations application

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The *ascorbyl methylsilanol pectinate* (AMP) presents the same functional properties of ascorbic acid (AA). Besides antioxidant and depigmentant activity, the AMP presents silanol in its chemical structure. The aim of this work was to characterize and indentify the AMP alone and in cosmetic formulations. The following techniques were employed: Fourier Transform Infrared Spectrophotometry, particle size distributions, *in vitro* antioxidant activity with 2.2-diphenyl-1-picrylhydrazyl (DPPH) and Oxigen Radical Absorbance Capacity Assay and High Performace Liquid Chromatography (HPLC) (developed and validated method) for the active ingredient; Microscopy, HPLC and Normal Stability Assay (NSA) for the emulsions. Particle size distributions results showed that the average size of AMP was 1.0 µm and polydispersity index was 0.1. In DPPH assay AA and AMP were statistically the same. The value of ORAC obtained for AMP was 0.74 and for AA in the literature was 0.95. In the NSA the formulations were stable in conditions of 5.0 and 45.0 ± 2.0 ºC for 90 days. Adequate stability at ambient temperature out of reach of light was also observed. Thus, this works presented an acceptable method for quantification of AMP alone and in cosmetic formulations. AMP was an adequate choice for the incorporation in emulsions with antioxidant efficacy.

Keywords: Derivative of ascorbic acid. HPLC analysis. Antioxidant activity.

INTRODUCTION

Topical application of ascorbic acid (AA) or vitamin C and its derivatives has stimulated the interest by the scientific community, since its effects on skin hydration, stain attenuation and antioxidant action have been increasingly observed by cosmetic, dermatological and by the users themselves (Spiclin, Gasperlin, Kmetec *et al*., 2001; Kim, Lee, 2018). The scientific literature has reduced the erythema induced by UVB radiation with topical treatment of vitamin C (Gonçalves, 2001; Aguilera *et al*., 2012).

The AA acts as a cofactor in the hydroxylation of hydroxyproline, an important amino acid in connective tissue and collagen fibers, significantly contributing to the formation of new collagen fibers, thus improving skin elasticity and firmness considered as the major target of aging (Gaspar, Campos, 2007; Kim, Lee, 2018). In addition to directly eliminating the reactive oxygen and nitrogen species (ROS/RNS), ascorbic acid regenerates the α-tocopherol and, therefore,it participates in the protective mechanism against lipoperoxidation (Halliwell, Gutteridge, 1999; Stamford, 2012). However, the use of ascorbic acid in cosmetic formulations has low stability in aqueous solution due to the change to the oxidized form of dehydroascorbic acid in formulations, such as gels and Oil and Water (O/W) emulsions.

A lot of research has sought to develop derivatives of ascorbic acid with similar action, but which exhibit superior chemical stability and equivalent percutaneous permeation (Sheraz *et al*., 2015). Among these substances the *ascorbyl methylsilanol pectinate* (AMP) has the cosmetic properties of ascorbic acid with the addition of the benefits of silanotriol which is present in its structure.

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Thus, its presence in topical formulations aims to restore the endogenous silicium, providing the regeneration of the tissues (Exsymol, 1998). This derivative is widely used in the cosmetic industry, included in patents (Clauss, Baeza, 1993; Xu, Richards, 2004; Burke-colvin, Hines, Gan, 2011). However, there are not papers in our literature that characterize the AMP (to the best of our knowledge).

Cosmetic emulsions O/W have been developed with several ingredients in order to obtain the most stable and to provide the formation of liquid crystals with biomimetic effect to the skin. The AMP (10.0% w/w) incorporation and the physical stability of these formulations and particle size analysis were verified. The *in vitro* determination of antioxidant activity by the methods of DPPH and ORAC (Oliveira *et al*., 2016; Ou, Woodill-hampsch, Prior, 2001) were used. For the identification and quantification, chromatographic techniques of ultraviolet spectrophotometry, infrared spectrophotometry and High Performace Liquid Chromatography (HPLC) were applied.

The objective of the study was to characterize and identify the AMP through its quantification using HPLC and antioxidant activity, aiming at the antiaging action of the preparation. The evaluation of the stability of the formulations will be verified under various temperature conditions (high, refrigerator and ambient) in order to observe the influence of light and heat that impact the shelf life and cosmetic effectiveness. The use of AMP instead of AA in formulations may be an interesting alternative because the scientific literature records signs of its degradation, compromising the antirradical efficacy and, in addition, the benefits of organic silicium coupled to the active ingredient.

MATERIAL AND METHODS

Material

Ascorbyl methylsilanol pectinate dispersion 1% (0.6% of ascorbic acid and 0.3% of methylsilanol) w/v (Exsymol), ascorbic acid (Sigma Chemical Co) analytical reference standard of purity of 99%.

Methods

Characterization of AMP

AMP is widely used as a derivative of ascorbic acid by adding the properties of organic silicium. In the literature no information was found about the characterization of the same, techniques were employed to heal this purpose.

Infrared Spectrophotometry with Fourier transform (FTIR)

Infrared (IR) spectroscopy analyses were performed on a Bomem Spectrophotometer® with Fourier transform, model MB-100, operating in the region of 4000 to 400 cm^{-1} with a resolution of 4 cm^{-1}. Samples of AMP were analyzed using cells for liquid, KBr pellet technique and films deposited on KBr crystals, prepared by dispersion.

Droplet diameter and Polydispersity Index (PDI)

In order to evaluate the particle size and polydispersity index of the ascorbic acid derivative, the AMP sample (1.0% w/v aqueous solution) was subjected without dilution to dynamic light scattering using the Zetasizer Nano system ZS, Malvern® equipment. The sample was introduced in a 1 cm quartz cuvette and the measurements were performed at 25.0 ºC. The equipment has a 4 mW He-Ne laser operating at a wavelength of 633 nm and performs non-invasive measurements by backscatter optics (NIBS). The measurements were made at a detection angle of 173º and the internal diameter of the particles was obtained based on the measurements, as well as the distribution, represented by the polydispersity index (PDI).

In vitro antioxidant potential - DPPH method

In order to compare the antioxidant capacity of ascorbic acid and its derivative, the DPPH method was used. The capacity of the AMP antioxidant activity was evaluated, based on its reaction with the radical DPPH (2.2'-diphenyl-1-picrylhydrazyl). Five different dilutions of AMP (7.5, 15.0, 50.0, 90.0, 120.0 and 150.0 µg/mL) were prepared in 10 mL of methanol. Exactly 1.0 mL of each dilution was transfered to a test tube and 3.0 mL of DPPH solution was added. The reaction occurred at 25.0 ºC for 2 hours in the absence of light. After, the reaction product was carried out at 517 nm in a UV/VIS spectrophotometer. The value that would designate the antioxidant capacity can be given only by the proportion of the absorbances (ABS) measured between the sample and the control (Oliveira *et al*., 2016).

$$\% \text{ FRS} \equiv \frac{Abs_{control} - Abs_{sample}}{Abs_{control}} \times 100 \quad (1)$$

Legend: **%** *FRS:* percentage of free radical scavenging; *Abs control:* Absorbance of negative control sample; *Abs sample:* Absorbance of samples.

Antioxidant Potential by Oxigen Radical Absorbance Capacity Assay (ORAC)

AMP samples (1% w/v aqueous solution) were diluted in 75 mM phosphate buffer (pH 7.0). 25 µL of (6-hidroxi-2,5,7,8-tetrametilcromo-2-ácido carboxílico/Trolox®) and 150 µL of fluorescein 40 nM (diluted in 75 mM phosphate buffer pH 7.0) at each concentration (6.25; 12.5; 25.0; 100.0 Mm) of the sample in 96 well microplate were added. To the control, 200 µl of buffer were added in the 5 wells. After incubation at 37 ºC for 30 minutes, 25 µL of AAPH (2.2'-azobis (2-amidinopropane) dihydrochloride solution 153 mM were added (in 75 mM phosphate buffer pH 7.0) and shaked in high intensity for 10 seconds. The measurement was made every minute during one hour with a wavelength of excitation and 485/20 nm emission 528/20 nm. The external wells were filled with 300 µL water to ensure the thermal homogeneity of the plate. The final value was calculated on the basis of the linear regression equation between the Trolox® concentration and the area under the decay curve of fluorescence (AUC) The results are expressed in µM equivalent of Trolox®/g of the sample, the calculation being based on the following equation (López-alarcón, Lissi, 2006):

$$\frac{(AUC\ sample - AUC\ blank)}{(AUC\ Trolox^{®} - \text{AUC blank})} \times \frac{molarity\ Trolox^{®}}{concentration\ sample} \quad (2)$$

Legend: AUC_{sample}=area under the curve of sample, AUC_{blank}=area under the curve of blank.

Formulations

Preparation

F1 and **F1A** (Table I) Formulations were obtained by manually stirring with glass stickcontinuously at room temperature, by the method cold emulsification. **F2, F2A, F3** and **F3A** (Table I) Formulations were obtained by heating at 70.0-75.0 ºC, of aqueous and oily phases, separately. The aqueous phase was poured into the oil phase by manually stirring with glass stick until cooling. After the emulsions reached 25.0 ºC, thermolabile ingredients were added. The method is known as phase inversion emulsification method (Baby, 2007; Prista, Alves, Morgado, 1995). These formulations are close to commercially available cosmetic or cosmeceutic formulations that incorporated many ingredients as emollients, humectants and antioxidants, which improve the sensorial performance of the product.

The formulations tested were designed to be manipulated in the master pharmacy. **F1** corresponds to a simpler base consisting of: 1 thickener and 1 emulsifier, 2 humectants, 1 emollient and 1 preservative system. In the formulation **F2,** hydrolyzed wheat protein and glycerin were added for superficial skin wetting. *Propyleneglycol* as humectant was removed and *Methyl Gluceth - 20* was retained. The moisturizing active ingredient NMF (widely used in the cosmetic area) and emollients of vegetable origin were added, seeking greater natural appeal with butters rich in essential fatty acids and it is biomimetic for the skin. The skin microbiome rebalancing is more likely to occur due to the use of plant components, which justifies its inclusion. *Cyclopentaloxane* (silicon) used at 5% w/w provides a sticky feel to the formulation and its proportion in **F2** and **F3** is reduced. In the **F3** formulation vegetable oils were added as emollients, following the trend of cosmetic products with vegetable ingredients for greater rebalancing of the cutaneous flora. Emollients are used for greater skin softness and improving sensory characteristics of the formulation. The proportion of silicone has decreased because many oils have been added. The sensory perception was adequate, which indicates that the formulations have potential for consumer acceptance.

TABLE I – Qualitative and quantitative composition (% w/w) of the preparations

Ingredients*/Function	F1	F1A	F2	F2A	F3	F3A
Polyacrylamide and C_{13-14} Isoparaffin and Laureth-7/thickener	2.5	2.5	-	-	-	-
Propyleneglicol/humectant	2.0	2.0	-	-	-	-
Methyl Gluceth – 20/ humectant	2.0	2.0	2.0	2.0	3.0	3.0
Dissodium EDTA/chelator	0.1					
Hydrolyzed Wheat Protein/ active moisturizing	2.0					

(continuing)

TABLE I – Qualitative and quantitative composition (% w/w) of the preparations

Ingredients*/Function	F1	F1A	F2	F2A	F3	F3A
Hidraction NMF/ active moisturizing	-	-	1.0	1.0	1.0	1.0
Glycerin/humectant	-	-	2.0	2.0	2.0	2.0
Hydroxypropyl Methylcellulose/thickener	-	-	1.0	1.0	1.0	1.0
Polysorbate 80/ nonionic surfactant	-	-	-	-	3.5	3.5
Acqua*/vehicle q.s.p.	100.0					
Cetearyl Alcoho/thickener and co-emulsifier	-	-	2.5	2.5	4.0	4.0
PEG-7 Sheabutterate/ emollient	-	-	1.0	1.0	2.0	2.0
PEG-23 Shea butterate/ nonionic surfactant	-	-	4.1	4.1	-	-
Shea Butter Cetyl Esters/emollient	-	-	2.0	2.0	3.0	3.0
Shea Butter Oleyl Esters/emollient	-	-	2.0	2.0	-	-
Butyl Hydroxytoluene/ antioxidant	0.1					
Macadamia ternifolia Seed Oil/emollient	-	-	-	-	2.0	2.0
Olea europaea (olive) oil/emollient	-	-	-	-	3.0	3.0
Cyclopentasiloxane/ emollient	5.0	5.0	2.0	2.0	2.0	2.0
*Phenoexyethanol, Methylparaben, Ethylparaben, Propylparaben and Butylparaben/*preservative	0.5	0.5	0.5	0.5	-	-
*Ginkgo biloba Leaf Extract/*antioxidant active	-	-	2.0	2.0	-	-
Ascorbyl Methylsilanol Pectinate/antioxidant active	-	10.0	-	10.0	-	10.0

*Legend:*q.s.p.:* sufficient quantity to, *INCI Name: International Nomenclature of Cosmetic Ingredient; - not added. **F1, F2** and **F3** – Base e **F1A, F2A** and **F3A** – Base + AMP (*Ascorbyl Methylsilanol Pectinate)*

Microscopic analysis

The procedure for microscopic analysis of the formulations was the same for all formulations. A small amount of formulations was placed on a glass slide, covered with a cover slip, and then subjected to microscopic analysis (Axioplan-2 Optical Microscope, Carl Zeiss®). The homogeneity of dispersion was evaluated and, with the aid of polarization, the presence of anisotropy areas, indicative of liquid crystals, was observed. The analyses were performed at 25 ºC.

Normal Stability Assay (NSA)

The stability of the emulsions was tested by simultaneously maintaining separate samples in the oven (45.0 ± 2.0 ºC), in the refrigerator (5.0 ± 2.0 ºC) and at room temperature (25.0 ± 2.0 ºC) and in photostability chamber under 200J/s with 320-400 nm (25.0 ± 2.0 ºC) for 90 days (Dario *et al.,* 2016; Brazil, 2004). Possible changes in organoleptic properties (appearance, color, and odor) were evaluated. The pH was determined with a 10% dilution of the sample with Digimed® pHmeter.

Determination of AMP (HPLC) in the formulations obtained

Chromatographic method was developed and validated for the determination of AMP content in cosmetic formulations. The HPLC system Shimadzu® LC-10 AD VC consisted of detector UV–VIS diode array detector, coupled to software Class VP. The system was

run isocratically using a C18 column (Coluna Synergi-Hydro® RP Phenomenex, 5 µm, 150.0 x 4.6 mm). Mobile phase with metanol: tetrabutyilammonium (TBA) 30 mM (pH 5.94) ion pair (30:70; v/v) was used at a flow rate of 1.0 mL/min at 25 ºC and UV detection at 254 nm (Snyder, Kirkland, Glajch, 1996).

The liquid chromatographic separation of AMP alone and in cosmetic formulations, followed by UV detection was validated. Linearity, precision, accuracy, quantification and detection limits and search for interferons (ICH, 1996).

Stock solutions (2.5 mg/mL) of AMP were prepared by addding 0.5 mL of AMP (1.0% w/v aqueous solution) in 9.5 mL of mobile phase and frozen. Linearity was assessed with five levels of standard concentration (25, 50, 100, 200, 400 µg), prepared by serial dilution of AMP stock solution. Each point concentration was analyzed in triplicate. Three calibration curves were used to establish the linear calibration equation *(y=mxv+c)* and the correlation coefficient (R^2). Repeatability (precision) was determined by determination of AMP in cosmetic formulations (10.0% w/v) expressed as relative standard deviation (RSD). Accuracy was investigated by recoveries at three different levels of the standard AMP (300.0, 200.0 and 1000.0 µg). The limits of detection (LD) and quantification (LQ) were calculated according to ICH guidelines.

Sample preparation

Exactly 1.5g of each developed formulation containing 10% of AMP were weighed analytically and the volume of the 10 mL volumetric flask was completed with the mobile phase. 3 mL was transferred to a volumetric flask and the volume (10 mL) was completed with mobile phase to final concentration of 4.5 mg/mL AMP. The samples were centrifuged (Centrifuge Eppendorf® model: 5804 R) by 15 min cycles at 15000 rpm at 25.0 ºC. Then 7 mL of the solvent was added to 3mL of the soluble fraction with shaking for 10 minutes (magnetic stirrer) and centrifugation at 15000xG for 15 minutes. Soluble fraction collected and injected into the chromatograph. Six replicates were obtained.

Statistical analysis

Statistical analysis was performed using Statistica® software, by 2-sample Student's T-Test. Differences were considered statistically significant at a value of $p<0.05$.

RESULTS AND DISCUSSION

Characterization of AMP

Similarity between AMP *and ascorbic acid by Infrared Spectrophotometry with Fourier transform (FTIR)*

The spectra in the infrared region of the AMP presented absorption bands in the region of 625 to 4000 cm-1 (Table II), characteristics of ascorbic acid. AMP presents, in its structural formula, an ascorbic acid molecule linked to silanol; this way bands characteristic of ascorbic acid are present.

The AMP showed an OH band stretch in the 3243.14 region. In the region of 1644.54 it presented a characteristic band of C=O. There was a band stretch characteristic of the Si-OH (1160.58) function and band deformation in the 760.70 region as a function of OH.

TABLE II – Absorption bands obtained in the determination of the infrared absorption spectrum of AMP on film under KBr plate

AMP	Attribution
3243.14	ν (OH)
1644.54	C=O
1160.58	ν (Si-OH)
760.70	δ (OH)

Legend: (ν)=stretch, (δ)=angular deformation, **AMP**=Ascorbyl methylsilanol pectinate, **KBr**= potassium bromide

Particle size and distribution by Dynamic Light Scattering (DLS)

The AMP (1.0% w/v dispersion) presented a mean droplet diameter equal to ~ 1 µm, corroborating with the aspect of slightly opalescent due to light refraction (Srilatha *et al*., 2013). According Kundu *et al.,* (2013), the droplets of macroemulsion are usually in the size range of 1–50 µm. The Polydispersity Index (PDI) of 0.1 was considered adequate according to the literature (<0.3) (Kaszuba, 2015;

Dario *et al*., 2016; Anton, Vandamme, 2009). According to Murdock *et al.* (2008) dynamic light scattering (DLS) is a simple method for analyzing suspension stability and measurement of particle size in solution.

In vitro antioxidant potential of AMP

The antioxidant activity by the reaction with DPPH (2.2′-diphenyll-1-picrylhydrazyl), the AA presented IC_{50} (50% of the initial amount)value 6.65 µg/mL (37.76 µM) and AMP (1.0% w/v aqueous solution) presented IC_{50} value 5.92 µg/mL (33.61 µM). The IC_{50} value 2.07 µg/mL (11.8 µM) when the reaction was done in methanol was low as compared to this work (Sharma, Bath, 2009). There was no statistical difference as the p value was 0.1408. This means that these molecules have similar antioxidant potential by this method. In the determination of the antioxidant activity by the reaction with ORAC, the value obtained for AMP was 0.74. According to Ou *et al.,* (2001), the value of ORAC to AA was 0.95, close to the result obtained for the AMP, which indicates that in the concentrations studied of AMP they have practically the same antioxidant point as the AA.

Formulations

Normal Stability Assessment

During the NSA, all formulations were considered normal in relation to organoleptic properties. The pH analysis (Table III) did not show significant changes for the formulations under the storage conditions when the pH was compared at t0 and at the end of the study (t90). Considering the pH results, it was possible to affirm that the developed formulations were stable for the period of 90 days under the established stress conditions. The addition of AMP in the formulations under 25.0 ± 2.0 ºC has modified color and aspect, specially color with 60-90 days. According to Maia (2002) O/W emulsions and aqueous gel with AA (10% w/w) maintained pH value and content of AA with addition of sodium metabissulfite or glutathione. In emulsions with AMP the addition of another antioxidant was not necessary, remembering that AMP is a dispersion of 1% w/v, that is, with 10% of this dispersion there was 6% of AA w/w (active ingredient) and in the cosmetic products it is usual the concentration of 10% of AA plus addition of antioxidant to fulfill its action.

TABLE III – Organoleptic characteristics (aspect, color and odor) and pH value submitted to the Normal Stability Assessment (**NSA**) during the 90 days

	Storage Temperature															
Test	**25.0 ± 2.0 ºC**						**5.0 ± 2.0 ºC**					**45.0 ± 2.0 ºC**				
	Time (days)															
F1	0	7	15	30	60	90	7	15	30	60	90	7	15	30	60	90
Color	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Aspect	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Odor	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
pH	5.3	5.3	5.3	5.3	5.3	5.4	5.4	5.3	5.3	5.4	5.4	5.4	5.4	5.3	5.5	5.4

(continuing)

TABLE III – Organoleptic characteristics (aspect, color and odor) and pH value submitted to the Normal Stability Assessment (**NSA**) during the 90 days

	Storage Temperature															
Test	**25.0 ± 2.0 °C**						**5.0 ± 2.0 °C**					**45.0 ± 2.0 °C**				
F1A	0	7	15	30	60	90	7	15	30	60	90	7	15	30	60	90
Color	N	SM	SM	SM	M	M	N	N	N	N	N	N	N	N	N	N
Aspect	N	SM	SM	SM	SM	SM	N	N	N	N	N	N	N	N	N	N
Odor	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
pH	5.5	5.4	5.4	5.3	5.3	5.3	5.5	5.5	5.5	5.5	5.5	5.5	5.5	5.5	5.5	5.4
F2	0	7	15	30	60	90	7	15	30	60	90	7	15	30	60	90
Color	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Aspect	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Odor	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
pH	5.3	5.3	5.3	5.3	5.3	5.3	5.2	5.3	5.3	5.2	5.2	5.3	5.3	5.3	5.4	5.4
F2A	0	7	15	30	60	90	7	15	30	60	90	7	15	30	60	90
Color	M	SM	SM	SM	M	M	N	N	N	N	N	N	N	N	N	N
Aspect	N	SM	SM	SM	SM	SM	N	N	N	N	N	N	N	N	N	N
Odor	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
pH	5.4	5.4	5.4	5.4	5.4	5.4	5.4	5.4	5.5	5.4	5.3	5.4	5.4	5.5	5.4	5.4
F3	0	7	15	30	60	90	7	15	30	60	90	7	15	30	60	90
Color	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Aspect	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N

(continuing)

TABLE III – Organoleptic characteristics (aspect, color and odor) and pH value submitted to the Normal Stability Assessment (**NSA**) during the 90 days

	Storage Temperature															
Test	**25.0 ± 2.0 °C**						**5.0 ± 2.0 °C**					**45.0 ± 2.0 °C**				
Odor	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
pH	5.4	5.6	5.6	5.6	5.4	5.4	5.5	5.6	5.3	5.4	5.4	5.4	5.4	5.3	5.4	5.4
F3A	0	7	15	30	60	90	7	15	30	60	90	7	15	30	60	90
Color	N	SM	SM	SM	M	M	N	N	N	N	N	N	N	N	N	N
Aspect	N	SM	SM	SM	SM	SM	N	N	N	N	N	N	N	N	N	N
Odor	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
pH	5.6	5.6	5.6	5.6	5.6	5.5	5.6	5.6	5.5	5.5	5.5	5.5	5.5	5.5	5.5	5.5

Legend: **N:** Normal; **SM:** Slightly Modified; **M:** Modified.

Microscopic analysis of the formulations

Formulations **F2** and **F2A** obtained unimodal particle size distribution. Formulations **F1, F1A, F3** and **F3A** obtained a bimodal distribution. The non-active formulation (**F2**) obtained a larger number of internal phase droplets when compared to active formulation (**F2A**). The incorporation of 10.0% w/v of the active (water soluble liquid) increased the external (aqueous) phase volume, leading to a reduction in the ratio of internal phase/external phase volume (Figure 1). Also shown macroscopically by the reduction of viscosity.

In cosmetic emulsions the most common type of liquid crystal found is the lyotropic, with a structural arrangement designated as lamellar phases that are responsible for the stabilization of the emulsions. Liquid crystals stabilize the emulsion against coalescence, but not against flocculation (Santos, 2006). According to Yamada (2011) liquid crystals are similar to the lipids of stratum corneum, so the presence of the same is desirable in cosmetic emulsions, the formulation being easily assimilated by the skin with a biomimetic action. The formation of liquid crystals depends on the balance of surfactants (lipo and hydrophilicity), type of oil and preparation temperature of emulsion (Suzuki *et al*., 1988). Liquid crystals of the lamellar type were identified in the **F3** and **F3A** formulations, which was an objective of the result of mixing the ingredients in the formulations. The incorporation of AMP in the formulation did not interfere in the formation of liquid crystals.

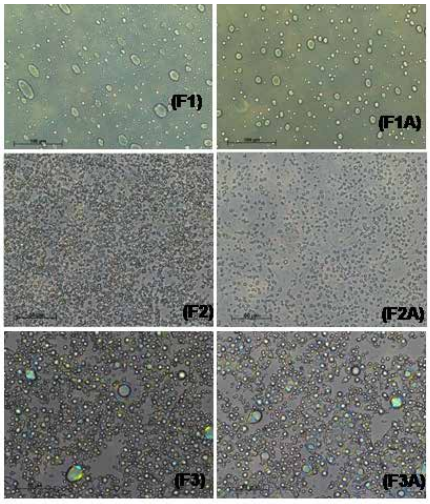

FIGURE 1 – Photomicrographs under polarized light of the formulations (**F1, F1A, F2, F2A, F3 and F3A**).

Validation of the method and determination of AMP in formulation

Table IV shows the values obtained for precision and accuracy.

The method was linear, with R^2=1. No peak was detected that interfered in the determination, demonstrating adequate selectivity for the method (Figure 2). Experimental concentration (µg/mL)±SD of AMP in the formulations **F1A, F2A** and **F3A** presented content of 99.49±0.84, 100.41±0.82 and 90.11±0.86%, respectively. The method was suitable for repeatability and recovery with adequate standard deviation values. The limits of detection and quantification were respectively 0.084 and 0.281 µg/mL.

TABLE IV – Precision (repeatability), average of six determinations and accuracy of AMP at three different levels of concentrations

Precision	AMP theoretical concentration (%)	AMP experimental concentration (%)	Content/ RSD%
F1A	10	9.94	99.49/0.84
F2A	10	10.04	100.41/0.82
F3A	10	9.01	90.11/0.86

(continuing)

TABLE IV – Precision (repeatability), average of six determinations and accuracy of AMP at three different levels of concentrations

Accuracy	Recovery of AMP SD (%)		
	100 µg/mL	200 µg/mL	300 µg/mL
F1A	102.35%	99.60%	95.36%
F2A	99.69%	104.84%	96.69
F3A	102.35%	101.49%	100.6

*Legend:***RSD**=relative standard deviation,**SD**=standard, **AMP**=Ascorbyl methylsilanol pectinate.

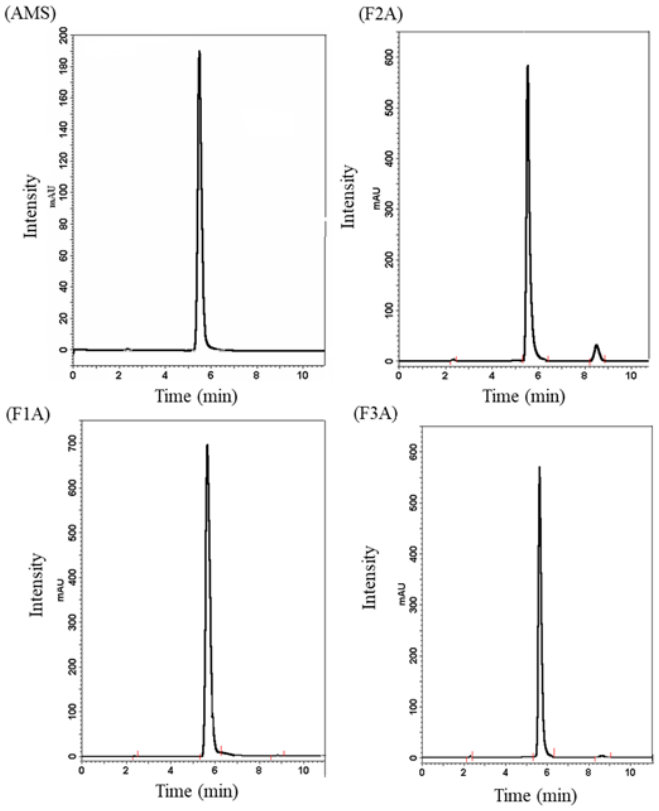

FIGURE 2 – HPLC chromatograms of AMP, F1A, F2A and F3A by UV detection.

CONCLUSIONS

The chromatographic method developed for quantification and identification of the AMP was

satisfactory according to the ICH Guidelines. The AMP showed to be an excellent alternative as a derivative of AA, because it presents similar *in vitro* antioxidant activity with addition of the benefits of organic silicium present in its molecule. It had satisfactory stability because it practically maintained the characteristics of the emulsions in relation of the beginning of the study: pH value, color, odor and aspect under the conditions of 5.0 ± 2.0 ºC and 45.0 ±2.0 ºC. In the temperature of 25.0 ± 2.0 ºC (ambient condition) the formulation was exposed to indirect light and presented slight modification in appearance and color up to 30 days and with 60-90 days the color was modified influenced by the UV radiation. The results suggest the AMP can be used in antiaging formulations because of the antioxidant action and it is indicated to protect from the action of light, with adequate packaging material.

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