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Influence of sodium metabisulfite and glutathione on the stability of vitamin C in O/W emulsion and extemporaneous aqueous gel

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Abstract

Vitamin C exerts several functions on skin as collagen synthesis, depigmentant and antioxidant activity. Vitamin C is unstable in the presence of oxygen, luminosity, humidity, high temperatures and heavy metals, which presents a significant challenge to the development of cosmetic formulations. Therefore, the utilization of an effective antioxidant system is required to maintain the vitamin C stability. The purpose of this research work was to develop prototypes of cosmetic formulations, as O/W emulsion and extemporaneous aqueous gel, containing vitamin C and to evaluate the influence of sodium metabisulfite (SMB) and glutathione (GLT), as antioxidants, on the stability of the active substance. A HPLC stability-indicating method was developed and validated for this study and stability assays were performed in 90 and 26 days and storage conditions were 5.0 ± 0.5, 24 ± 2 and 40.0 ± 0.5 °C. The HPLC stability-indicating method showed linearity ($r^2 > 0.99$), specificity, R.S.D. < 1.22% and accuracy/recovery ranging from 95.46 to 101.54%. Preparations with SMB or GLT and the antioxidant-free presented results statistically distinct, demonstrating the necessity of the antioxidant system addition. O/W emulsions with SMB or GLT retained the vitamin C content >90.38% stored at 5.0 ± 0.5 and 24 ± 2 °C. For the aqueous gel with SMB or GLT, the active substance concentration was maintained >94.03%. Considering the vitamin C stability, the SMB and the GLT showed to be statistically adequate, as antioxidants, for the cosmetic formulations.

Keywords: Vitamin C; Chemical stability; O/W emulsion; Aqueous gel; Sodium metabisulfite; Glutathione

1. Introduction

Cosmetic industry has been employing the vitamin C (ascorbic acid) especially as a function of its effects as: skin depigmentant, collagen synthesis stimulation, antioxidant against the formation of free-radicals, consequently, exerting an anti-aging activity. This active substance is able to penetrate the stratum corneum and, hence, the concentration, pH value and cosmetic dosage form must be considered to attain the optimal activity of the ascorbic acid (Colven and Pinnel, 1996; Tokgoz, 1996).

The vitamin C possesses limited stability, which represents a significant challenge to cosmetic industry. To assess the vitamin C chemical stability in semisolid cosmetic formulations, several aspects must be regarded like: exclusion of the oxygen, protection against light and temperature, maintenance of the acid pH and the utilization of an efficient antioxidant system. The development of cosmetic dosage forms requires several stages, initiating with the rigorous selection of raw and packaging materials to the appropriate manufacture technique and stability assays (Asker et al., 1985; De-Ritter, 1982; Rubin et al., 1976).

Sodium metabisulfite (SMB) and glutathione (GLT) have been indicated and used as antioxidants for vitamin C. The employment of the SMB may be designated for acidic preparations and the GLT has been used in aqueous solutions and blood samples to reduce the vitamin C degradation (Wade and Weller, 1994; Touitou et al., 1996; Karg et al., 1987). Besides the rigorous selection of the antioxidant system, the excipients or the formulation may collaborate with the active substance stability, such the use of non-ionic surfactants/self-emulsifying waxes,

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multiple emulsions, the presence of chelating agents and cosmetic forms possessing acid pH (Blaug and Hajratwala, 1974; Gallarate et al., 1999; Touitou et al., 1996).

The assessment of the vitamin C chemical stability requires a sensitive and selective analytical methodology and titrimetric, spectrophotometric, electrochemical, fluorimetric, enzymatic and chromatographic methods were previously described (Jaffe, 1984; Pelletier, 1985; Moser and Bendich, 1991; Ball, 1994; Arya et al., 2000). The high-performance liquid chromatography (HPLC) is able to target the goals of this active substance determination and can be used as a stability-indicating assay, which is a analytical method employed to the analysis of stability samples in pharmaceutical and cosmetic industries (Bakshi and Singh, 2002; Marshall et al., 1995). Although, the method must be previously validated to achieve those characteristics.

The purpose of this research work was to develop prototypes of cosmetic formulations, as O/W emulsion and extemporaneous aqueous gel, containing vitamin C and to evaluate the influence of sodium metabisulfite (SMB) and glutathione (GLT), as antioxidants, on the stability of the active substance.

An O/W emulsion was obtained with non-ionic self-emulsifying wax contemplating the low potential of skin irritancy, especially for the pH value of the formulation ≈3.0, which is required to achieve optimal activity of the vitamin C on the skin, and the maintenance of chemical stability of the product (Blaug and Hajratwala, 1974). Extemporaneous aqueous gel was based on non-ionic gelling agent hydroxyethylcellulose. Sorbitol, humectant agent, was introduced on the formulation due to its capability to diminish the presence of oxygen, reducing the vitamin C oxidation (Blaug and Hajratwala, 1974; Macek, 1960).

To reduce the oxidation level of the active substance, a preventive antioxidant/chelating agent, HEDTA, was added to O/W emulsion and extemporaneous aqueous gel. This category of ammine polycarboxylic acid possesses efficacy in preparations containing ascorbic acid when compared with similar antioxidant/chelating agents, for example, EDTA (Kassem et al., 1971). Besides the antioxidant preventive mechanism, due to the vitamin C instability, an important stage during the development of cosmetic formulations was the selection of a corrective antioxidante system. SMB is usually indicated to be added in formulations with acid pH, thus, being appropriate for those prototypes developed in this research. GLT has been reported as an adequate ascorbic redox agent in solutions and blood samples (Lowik et al., 1990; Wade and Weller, 1994; Touitou et al., 1996).

2. Experimental

2.1. Equipments and standards

Samples were chromatographed at 24.0 ± 2.0 °C, using metaphosphoric acid 0.2%/methanol/acetonitrile (90:8:2, v/v/v; pH 2.8) as the mobile phase with flow rate of 1.0 ml min^{-1} and detection at 254 nm employing a Shimadzu LC-10AD HPLC integrated with a Class-LC10 system with an UV–vis detector. A Shimadzu SPD-10A software was used for data acquisition. Separations were achieved using a 250 mm × 4.6 mm Phenomenex® LiChrospher 5 μm C_{18} column.

2.2. Standards and reagents

Ascorbic acid was provided from Roche (São Paulo, Brazil) and nicotinic acid, employed as internal standard, was purchased from LabSynth (São Paulo, Brazil), both with purity of 99.0%. HPLC grade methanol and acetonitrile from Mallinckrodt were used to prepare the mobile phase, together with metaphosphoric acid 0.2% of reagent grade from Carlo Erba. Pure water was produced by a Millipore Milli-Q Plus System (Molsheim, France). All chemicals and reagents were used without any further purification.

2.3. Prototype cosmetic formulations

O/W emulsions were prepared with the following substances of pharmaceutical grade: emulsifying wax NF (10.0%, w/w), ethylhexyl stearate (3.0%, w/w), mineral oil (4.0%, w/w), sodium carboxymethyl betaglucan (3.0%, w/w), HEDTA (0.2%, w/w), water (and) glycerin (and) sodium lactate (and) TEA-lactate (and) serine (and) lactic acid (and) urea (and) sorbitol (and) sodium chloride (and) lauryl diethylenediaminoglycine (and) lauryl aminopropylglycine (and) allantoin (2.5%, w/w), phenoxyethanol (and) methylparaben (and) ethylparaben (and) propylparaben (and) butylparaben (0.3%, w/w), vitamin C (ascorbic acid, 10%, w/w), glutathione (1.0%, w/w) or sodium metabisulfite (0.25%, w/w), methyldibromo glutaronitrile (0.15%, w/w), fragrance and distilled water. The extemporaneous aqueous gels were developed with hydroxyethylcellulose (0.7%, w/w), methylparaben (0.2%, w/w), HEDTA (0.2%, w/w), sorbitol (5.0%, w/w), water (and) glycerin (and) sodium lactate (and) TEA-lactate (and) serine (and) lactic acid (and) urea (and) sorbitol (and) sodium chloride (and) lauryl diethylenediaminoglycine (and) lauryl aminopropylglycine (and) allantoin (2.5%), vitamin C (ascorbic acid, 10%, w/w), glutathione (1.0%, w/w) or sodium metabisulfite (0.25%, w/w), methyldibromo glutaronitrile (0.8%, w/w), fragrance and distilled water. All components were obtained from commercial sources and used as received.

2.4. HLPC stability-indicating method validation

2.4.1. Linearity

Aliquots of standard ascorbic acid were diluted to concentrations ranging from 1.0 to 12 μg ml^{-1}. Appropriate amount of nicotinic acid was transferred to each solution of ascorbic acid achieving constant concentration of 20 μg ml^{-1}. Two samples of each concentration were prepared. The least-squares fit method was employed to evaluate statistically the results for linearity by regression line and coefficient of linear correlation (r^2) (Jenke, 1996; Rolim et al., 2006).

2.4.2. Specificity

2.4.2.1. Analyte-free formulations without sodium metabisulfite and glutathione. About 250 mg of O/W emulsion and extemporaneous aqueous gel were weighed and dispersed, separately,

in 50 ml of the mobile phase. Samples were centrifuged at 3000 rpm for 5 min at 24.0 ± 2.0 °C. Analyte-free samples were diluted to final concentration of 10.0 μg ml^{-1}, which would correspond in vitamin C.

2.4.2.2. Analysis of standards (ascorbic and nicotinic acids), antioxidants (glutathione and sodium metabisulfite) and degradation product of vitamin C (oxalic acid). About 25 mg of ascorbic acid, nicotinic acid, glutathione, sodium metabisulfite and oxalic acid were weighed and transferred individually to volumetric flasks and dissolved in 50 ml of the mobile phase. Aliquots of each sample were diluted to required concentration of 10.0 μg ml^{-1}.

2.4.3. Accuracy/recovery and precision

To achieve accuracy/recovery, aliquots of samples were diluted to final concentrations of 2.0, 6.0 and 10.0 μg ml^{-1} of standard ascorbic acid and 20.0 μg ml^{-1} of nicotinic acid on the mobile phase. To obtain intra- and inter-run precision, samples were diluted to final concentration of ascorbic acid of 6.0, and 20.0 μg ml^{-1} of nicotinic acid (US Pharmacopeia XXVII, 2004).

2.4.4. Limits of detection (LoD) and quantification (LoQ)

LoD and LoQ were determined by dilutions of ascorbic acid in order to obtain signal/noise ratios of ≈3:1 for LoD and ≈10:1 for LoQ (US Pharmacopeia XXVII, 2004; Q2B Validation of Analytical Procedures: Methodology, 1996).

Appropriate amounts of standard ascorbic acid were diluted to concentrations of 0.03, 0.05, 0.1 and 1.0 μg ml^{-1}. Three samples of each concentration were prepared.

2.5. Stability assay

Formulations (antioxidant-free, with SMB or GLT) were weighted (30 g) and packaged in opaque white polyethylene flasks with 50 g of content capacity. Samples were analyzed after the resting period of 24 h of preparation (t_0). All assays were conducted at 24 ± 2 °C.

Storage conditions were 5.0 ± 0.5, 24 ± 2 and 40.0 ± 0.5 °C. Samples had their chemical stability obtained by vitamin C determination achieved with the previously validated HPLC method. For each day of study there were two samples of each preparation for all the storage conditions. At the pre-determined times, samples were removed from the storage conditions and allowed to warm to room temperature (24 ± 2 °C) prior to the evaluation of the chemical stability.

2.6. Statistical treatment

Statistical data applied on the evaluation of the chemical stability of the vitamin C were obtained with Greek-Latin square design (Box et al., 1978), which time, temperature and antioxidant parameters were combined. Statistical treatments were carried out using the Statigraphics™ software Version 6.0.

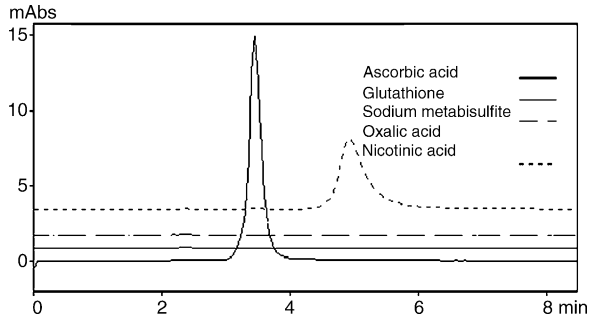

Fig. 1. Chromatogram of standard ascorbic acid, glutathione, sodium metabisulfite, oxalic acid and internal standard nicotinic acid.

3. Results and discussion

The mobile phase metaphosphoric acid 0.2%/methanol/acetonitrile (90:8:2, v/v/v) provided an active substance retention time of 3.5 min, differentiating the resolution of the ascorbic acid from nicotinic acid, shown in Fig. 1, with sharp and symmetrical peaks.

Linearity was studied over a concentration range of 1.0–12.0 μg ml^{-1}. The results demonstrated satisfactory and consistent behavior of the HPLC stability-indicating method. Least-squares regression analysis was used to evaluate the concentration range data that showed excellent linearity, with $r^2 \geq 0.99$ (Jenke, 1996). Linear regression was $y = 0.0732x - 0.0141$, where y is the ratio of ascorbic acid/nicotinic acid peak areas and x is the concentration of ascorbic acid (μg ml^{-1}).

Specificity for ascorbic acid quantification on the prototype formulations was investigated in order to obtain an indication of possible interferents from excipients, nicotinic acid and active substance degradation product, the oxalic acid. As shown in Figs. 1 and 2, the presence of the excipients of O/W emulsion, extemporaneous aqueous gel and oxalic acid did not cause any interference with the ascorbic acid peak. Under these conditions, ascorbic acid was observed to be well resolved from components of formulations and the potential degradation product of the vitamin C.

Accuracy/recovery ranged from 98.97 to 100.84% and 95.46 to 101.54% for O/W emulsion and extemporaneous aqueous gel, respectively. Precision, intra- and inter-days tests, was expressed

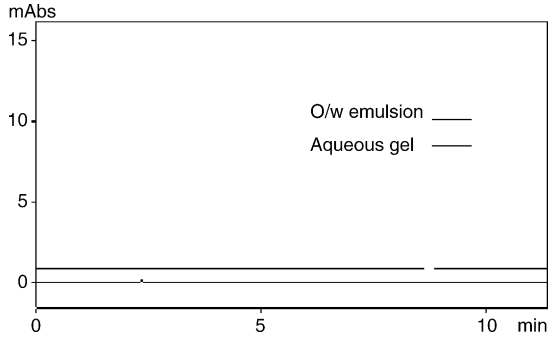

Fig. 2. Chromatogram of analyte-free o/w emulsion and extemporaneous aqueous gel.

Table 1
Ascorbic acid concentration (%) on the o/w emulsion and extemporaneous aqueous gel antioxidant-free (Af) and with glutathione (GLT) or sodium metabisulfite (SMB) during the stability test

	t_0	5.0 ± 0.5 °C							24 ± 2 °C							40.0 ± 0.5 °C						
		3 days	7 days	14 days	15 days	26 days	30 days	60 days	90 days	3 days	7 days	14 days	15 days	26 days	30 days	60 days	90 days	3 days	7 days	14 days	15 days	26 days	30 days	60 days	90 days
O/W emulsion																									
Af	99.33	98.84	97.40	–	98.33	–	97.92	96.28	93.13	98.04	96.60	–	96.58	–	94.23	91.29	87.27	96.75	94.11	–	86.67	–	77.32	60.10	42.34
GLT	100.00	98.86	98.73	–	98.78	–	97.09	96.05	95.00	98.07	97.03	–	97.01	–	96.12	94.51	91.89	97.88	95.54	–	93.20	–	90.17	77.31	57.31
SMB	99.07	98.89	99.07	–	98.47	–	97.81	96.45	95.56	98.02	96.71	–	96.30	–	96.16	93.86	90.38	97.45	95.15	–	95.51	–	85.67	67.02	47.49
Extemporaneous aqueous gel																									
Af	98.98	97.78	96.23	96.21	–	95.53	–	–	–	96.31	94.07	93.62	–	92.29	–	–	–	92.02	88.13	82.17	–	69.50	–	–	–
GLT	98.93	96.88	96.28	96.33	–	97.10	–	–	–	97.79	95.92	94.60	–	94.03	–	–	–	95.42	93.61	90.26	–	81.11	–	–	–
SMB	98.02	98.15	97.42	97.51	–	97.57	–	–	–	96.64	96.26	95.46	–	94.54	–	–	–	96.06	93.71	87.48	–	78.69	–	–	–

t_0, resting period (24 h after preparation).

Table 2
Ascorbic acid concentration (%, w/w) on the O/W emulsion as a function of time (1–3), temperature (I–III) and antioxidants (A–C)

	1	2	3
I	A: 100.31, 98.36	B: 96.76, 97.42	C: 96.11, 95.02
II	C: 99.42, 98.72	A: 93.67, 94.79	B: 91.51, 92.28
III	B: 99.53, 100.48	C: 85.34, 86.01	A: 47.63, 37.05

Time: 1, resting period (24 h after preparation); 2, 30 days; 3, 90 days. Temperature: I, 5 °C; II, 24 °C; III, 40 °C. Antioxidant: A, antioxidant-free; B, GLT; C, SMB.

as relative standard deviation (R.S.D., %) in terms of area ratio of ascorbic acid/nicotinic acid. Low percentage values of R.S.D. (0.38 and 1.22%) were an evidence of the appropriate precision of this stability-indicating method which provided an irrelevant variability of the data. LoD and LoQ values were found to be ≈0.05 and ≈0.17 μg ml^{-1}, respectively.

Data in Table 1 showed that vitamin C was stable in all prototype formulations for 90 days for O/W emulsion, and 26 days for the extemporaneous aqueous gel when stored at 5.0 ± 0.5 °C (>93.13%). Storage in low temperatures, regularly, sustains the active substance rate of degradation. The retained content of vitamin C presented a similar profile in all O/W emulsion prototypes (antioxidant-free and containing SMT or GLT), although, the emulsion with GLT presented slightly superior stability results after 90 days of the study at 24 ± 2 and 40 ± 0.5 °C.

O/W emulsion samples stored at 5.0 ± 0.5 and 24 ± 2 °C (room temperature) suffered analogous reduction profile of the active substance. These data demonstrated that storage conditions did not exert influence on the stability of the vitamin C and, consequently, on the rate of degradation. These results were confirmed with the statistical treatment, reported in Tables 2–4, for the O/W emulsion, and in Tables 5–7, for the aqueous gel, which there were no significant differences ($p < 0.05$) until the 30th day of the study; after this period, it was verified an opposite behavior.

The O/W emulsions stored at 40.0 ± 0.5 °C had an accentuated degradation of ascorbic acid. High temperature condition demonstrated that the degradation of the active substance occurred as a function of temperature and, statistically, presented significant difference ($p < 0.05$) from the storages at 5.0 ± 0.5 and 24 ± 2 °C. This result was expected due to the vitamin C sensitivity to high temperature (Gallarate et al., 1999). The comparison of the emulsion samples, the antioxidant-free one presented the higher reduction of the vitamin C. Samples con-

Table 3
Analysis of variance (ANOVA) of the ascorbic acid concentration on the O/W emulsion as a function of time, temperature and antioxidants

Variation	Sum of squares	Degrees of freedom	Mean squares	F-value	Significance level
Time	1642.96	2	821.48	8.95	0.0049
Temperature	1646.07	2	823.03	8.96	0.0049
Antioxidant	1081.15	2	540.57	5.89	0.0183
Residual	1010.06	11	91.82		
Total	5380.23	17			

Table 4
Analysis of the ascorbic acid concentration (%, w/w) on the O/W emulsion as a function of time, temperature and antioxidants

Parameter	O/W emulsion			
	Level (comparison)	Number of analysis (difference)	Mean (±confidence interval)	Homogeneous groups[a]
Time (days)	1 (0 day) (1–2)	6 (7.14)	99.47 (12.18)	a
	2 (30 days) (1–3)	6 (22.87)	92.33 (12.18[b])	a
	3 (90 days) (2–3)	6 (15.73)	76.60 (12.18[b])	b
Temperature	I (5 °C) (I–II)	6 (2.26)	97.33 (12.18)	c
	II (24 °C) (I–III)	6 (21.32)	95.06 (12.18[b])	c
	III (40 °C) (II–III)	6 (19.06)	76.00 (12.18[b])	d
Antioxidant	A (antioxidant-free) (A–B)	6 (−17.69)	78.63 (12.18[b])	e
	B (GLT) (A–C)	6 (−14.80)	96.33 (12.18[b])	f
	C (SMB) (B–C)	6 (2.89)	93.43 (12.18)	f

[a] Homogeneous groups for means indicated with same letters are not significantly different.
[b] Statistically different.

Table 5
Ascorbic acid concentration (%, w/w) on the extemporaneous aqueous gel as a function of time (1–3), temperature (I–III) and antioxidants (A–C)

	1	2	3
I	A: 99.06, 98.91	B: 97.15, 95.42	C: 97.28, 97.86
II	C: 97.53, 98.51	A: 94.63, 93.51	B: 94.39, 93.67
III	B: 100.06, 97.80	C: 93.17, 94.26	A: 71.71, 67.30

Time: 1, resting period (24 h after preparation); 2, 7 days; 3, 26 days. Temperature: I, 5 °C; II, 24 °C; III, 40 °C. Antioxidant: A, antioxidant-free; B, GLT; C, SMB.

Table 6
Analysis of variance (ANOVA) of the ascorbic acid concentration on the extemporaneous aqueous gel as a function of time, temperature and antioxidants

Variation	Sum of squares	Degrees of freedom	Mean squares	*F*-value	Significance level
Time	418.07	2	209.03	7.8	0.0078
Temperature	347.02	2	173.51	6.5	0.0139
Antioxidant	317.20	2	158.60	5.9	0.0180
Residual	295.06	11			
Total	1377.35	17			

taining GLT presented a better stability profile for the active substance at 40.0 ± 0.5 °C than for those samples without GLT (antioxidant-free and with SMB).

Statistical analysis of the vitamin C content on the O/W emulsions antioxidant-free and with SMB or GLT, stored at 24 ± 2, 5.0 ± 0.5 and 40.0 ± 0.5 °C, showed that there were significant differences among the results, demonstrating the necessity of the antioxidant system addition on prototype formulations, since a decrease of the active substance occurred more slowly on the presence of the SMB and GLT. Between those antioxidants, there were not significant statistical differences ($p < 0.05$).

The vitamin C concentration obtained from the emulsion samples containing sodium metabisulfite and glutathione, as antioxidants, were >90.38% when stored at 5.0 ± 0.5 and 24 ± 2 °C for 90 days, being considered acceptable (US Pharmacopeia XXIV, 2000).

For the extemporaneous aqueous gel samples were verified that the decrease of the active substance stored at 5.0 ± 0.5 and 24 ± 2 °C presented a similar profile to emulsion samples with acceptable chemical stability, possessing minimum vitamin C content ≈92% for the antioxidant-free aqueous gel, after 26 days of analysis. Extemporaneous gels containing SMB were found to be more stable, at the end of the assay, with active concentrations of 97.57 and 94.54% for the storage conditions of 5.0 ± 0.5 and

Table 7
Analysis of the ascorbic acid concentration (%, w/w) on the extemporaneous aqueous gel as a function of time, temperature and antioxidants

Parameter	Extemporaneous aqueous gel			
	Level (comparison)	Number of analysis (difference)	Mean (±confidence interval)	Homogeneous groups[a]
Time (days)	1 (0 day) (1–2)	6 (3.95)	98.64 (6.58)	a
	2 (7 days) (1–3)	6 (11.61)	94.69 (6.58[b])	a
	3 (26 days) (2–3)	6 (7.65)	87.03 (6.58[b])	b
Temperature	I (5 °C) (I–II)	6 (2.24)	97.61 (6.58)	c
	II (24 °C) (I–III)	6 (10.23)	95.37 (6.58[b])	c
	III (40 °C) (II–III)	6 (7.99)	87.38 (6.58[b])	d
Antioxidant	A (antioxidant-free) (A–B)	6 (−8.89)	87.52 (6.58[b])	e
	B (GLT) (A–C)	6 (−8.91)	96.41 (6.58[b])	f
	C (SMB) (B–C)	6 (−0.02)	96.43 (6.58)	f

[a] Homogeneous groups for means indicated with same letters are not significantly different.
[b] Statistically different.

$24 \pm 2\,^{\circ}C$, respectively. Statistical treatment demonstrated no significant differences ($p < 0.05$) between those temperatures of storage and for the presence of SMB and GLT. Antioxidant-free gels also presented acceptable chemical stability for vitamin C, although, samples with SMB or GLT resulted to a lesser decrease of active, for the conditions mentioned above.

At $40.0 \pm 0.5\,^{\circ}C$, the augment of the temperature was a determinant factor on the acceleration of the vitamin C degradation for all prototypes extemporaneous aqueous gels, confirmed by the statistical analysis. The presence of SMB and GLT provided better stabilization of the ascorbic acid with higher retained concentrations (±10%) when compared with the antioxidant-free aqueous gel. It was observed that the gel prototypes had a decrease of the vitamin C as a function of the temperature and the storage period of 26 days.

4. Conclusions

A HPLC stability-indicating method was validated to quantify vitamin C incorporated in cosmetic formulations developed as an O/W emulsion and extemporaneous aqueous gel containing sodium metabisulfite or glutathione, as antioxidants. Method provided linearity, specificity for the vitamin C, adequate precision, sufficient accuracy/recovery and low limits of detection and quantification.

The presence of antioxidants statistically demonstrated the necessity of their addition on the prototype formulations, although, between them, there were no significant differences ($p < 0.05$). At 5.0 ± 0.5 and $24 \pm 2\,^{\circ}C$, all samples presented similar results for the vitamin C chemical stability for 90 and 26 days for O/W emulsion and extemporaneous aqueous gel, respectively, being considered acceptable. Storage condition of $40.0 \pm 0.5\,^{\circ}C$ has accelerated the rate of degradation of the vitamin C, even on the presence of the antioxidants.

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