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Journal of Pharmaceutical and Biomedical Analysis
15 (1997) 795–801

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

Stability of vitamin C derivatives in solution and topical formulations

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Received 20 February 1996; accepted 29 May 1996

Abstract

The stability of ascorbic acid, ascorbyl palmitate and magnesium ascorbyl phosphate (VC-PMG®) in both standard solutions and topical formulations was investigated by direct RP-HPLC analysis after sample dilution with a suitable aqueous-organic solvent mixture. The results showed that, whereas the two vitamin C derivatives were more stable than ascorbic acid, the ascorbyl esters showed significant differences. Esterification with palmitic acid in 6 position did not prevent hydrolysis of the molecule, either in solution or in emulsion; only the special preparation of products with high viscoelastic properties was able to reduce the typical behaviour of this compound. Conversely, the introduction of the phosphoric group in 2 position protected the molecule from break-up of the enediol system, confirming VC-PMG as a very stable derivative of vitamin C that may be easily used in various types of cosmetic products. © 1997 Elsevier Science B.V.

Keywords: Stability; Vitamin C; HPLC; Emulsion

1. Introduction

Ascorbic acid has important physiological effects on skin, including inhibition of melanogenesis, promotion of collagen biosynthesis and prevention of free radical formation [1–5], all closely related to the well-known antioxidant properties of this compound [6–8]. Vitamin C therefore plays an important role in skin aging and may be considered an interesting ingredient of cosmetic skin care products [6].

The formulation of finished products with ascorbic acid is impractical because this substance, readily soluble in water, is extremely unstable [9–11]: it undergoes oxidation, especially in aerobic conditions (copper or heavy metals in general catalize this reaction), and with light exposure. These reactions occur quickly in basic conditions and the compound degrades itself irreversibly in a biologically inactive form (2,3-diketo-L-gulonic acid). To overcome this problem, ascorbic acid is chemically modified by esterification of the hydroxyl group with long-chain organic or inorganic acids [9,11]. Two derivatives are widely used in topical formulations: ascorbyl

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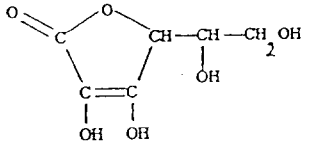
PII S0731-7085(96)01904-8

palmitate, a fatty acid ester with lipophilic properties [11], and magnesium ascorbyl phosphate (VC-PMG), an inorganic water-soluble acid ester [12]. Besides their different solubility properties, these two molecules also differ in structural features. As shown in Fig. 1, the fatty acid ester is in 6 position, while the inorganic ester group is introduced in 2 position, involving the enediol system.

The aim of this work was to compare the stability of ascorbyl palmitate and magnesium ascorbyl phosphate.



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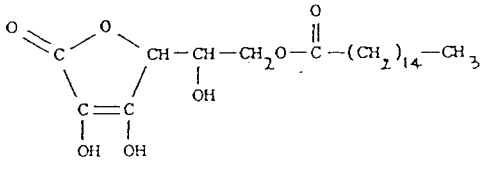

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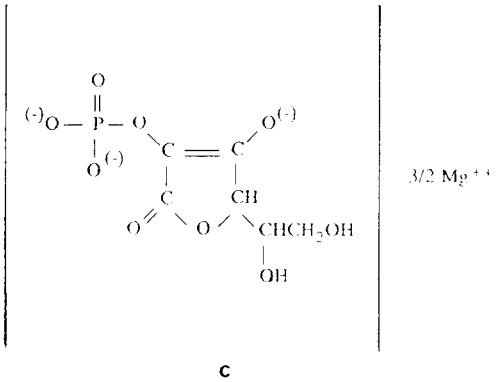


c

Fig. 1. Chemical structure of ascorbic acid (a), ascorbyl palmitate (b) and magnesium ascorbyl phosphate (c).

We first studied the behaviour of these molecules in standard solution in comparison with ascorbic acid. Then we examined their behaviour versus time in some specially prepared cosmetic emulsions, using the accelerated aging test. Samples were periodically analysed by high performance liquid chromatography and UV detection: this method allows analysis of heterophase system while avoiding any sample pretreatment, associated with a first dilution step with proper ratios of aqueous-organic solvent mixtures [13]. The procedure provides qualitative and quantitative determination of each ingredient and compatibility with other formula components with some practical advantages, such as rapidity, flexibility and reduced analytical error [14].

2. Experimental

2.1. Materials and reagents

Magnesium ascorbyl phosphate (VC-PMG®) was obtained from Nikko (Tokyo, Japan), ascorbyl palmitate and ascorbic acid from Roche (Basle, Switzerland), Lichrosorb NH_2 column (Li–NH_2 7 μm, 250 × 4 mm diameter), analytical-reagent grade reagents and solvents from Merck (Darmstadt, Germany).

2.2. Apparatus

A Gilson liquid chromatograph (Biolabo Instruments, Milan, Italy) was used, equipped with a double pump (mods. 305 and 306), Gilson 805 manometric module, Gilson 811B dynamic mixer, Rheodyne 9010 valve, Perkin–Elmer UV/VIS LC 95 detector and Shimadzu C-R5A data station.

2.3. Chromatographic conditions

The stationary phase was Li–NH_2, the eluent for VC-PMG [15] was acetonitrile (CH_3CN)-phosphate buffer (KP) (0.3 M, pH 4) (40:60, v/v). The eluent for ascorbyl palmitate and ascorbic acid [16] was methanol (MeOH)-phosphate buffer (KP) (0.02 M, pH 3.5) (70:30, v/v). UV detection was at 255 nm; injection volume 20 μl and flow-rate 1 ml min^{-1}.

2.4. Standard solutions

VC-PMG solution was prepared in KP 0.3 M, pH 4, ascorbic acid was diluted in aqueous solution and ascorbyl palmitate in MeOH. All standard solutions (1% w/v) were stored in the dark at 4°C. Calibration curves were performed in the range 5–30 μg ml^{-1}.

2.5. Cosmetic samples

2.5.1. VC-PMG analysis

About 1 g of each cosmetic sample, accurately weighed, was diluted 1:20 (w/v) with tetrahydrofuran (THF)–phosphate buffer (KP) (0.3 M, pH 4) (3:7, v/v) in a screw-capped tube and stirred in a vortex mixer until completely homogeneous. Further dilutions were performed only with phosphate buffer 0.3 M, pH 4, in the range 1:200–1:4000 (w/v), according to VC-PMG concentration in the sample in question. The obtained solutions were directly injected in the chromatographic system.

2.5.2. Ascorbyl palmitate analysis

About 1 g of each cosmetic sample, accurately weighed, was diluted 1:20 (w/v) with THF–H_20 (9:1, v/v) in a screw-capped tube and stirred in a vortex mixer until completely homogeneous. Further dilutions were performed with the same solvent mixture, to a final dilution of 1:200 (w/v). The obtained solutions were directly injected in the chromatographic system.

2.5.3. Storage conditions

All samples were stored in completely filled test-tubes of Pyrex glass equipped with a Bakelite screw-cap and a Teflon ring. During storage period the samples were kept both at room temperature and at 42°C in the dark.

3. Results and discussion

3.1. Stability of standard solutions

Chromatographic analysis of vitamin C derivatives was performed using an amino-column as stationary phase and two different mobile phases (see Section 2.3), in order to obtain very short times of analysis for each molecule.

Fig. 2 shows the chromatographic patterns of ascorbic acid solution and its derivatives. Linear calibration curves with good regression coefficients were obtained in the range 5–30 μg ml^{-1}; repeatability of analysis was verified at various concentrations, revealing good precision:

(a) $y = 4.11x + 0.07$; $r = 0.9999$;

R.S.D. = 0.8% ($n = 5$)

(b) $y = 1.75x - 1.25$; $r = 0.9999$;

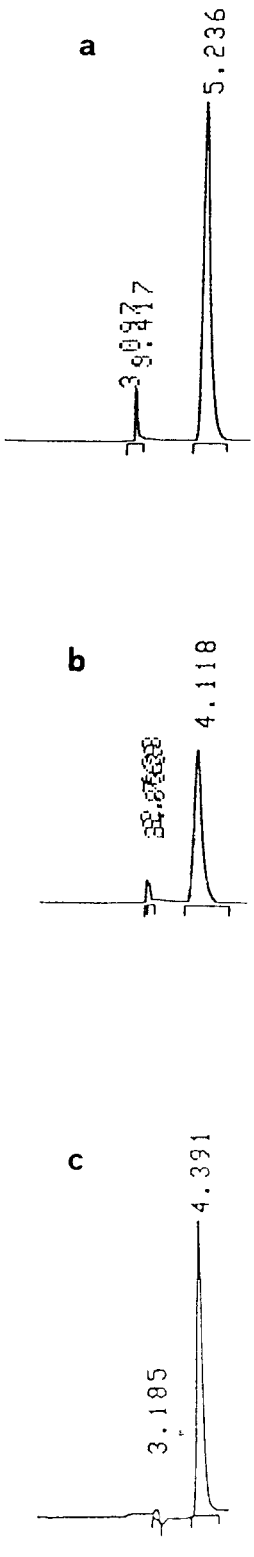
R.S.D. = 2.8% ($n = 5$)

(c) $y = 2.15x - 0.05$; $r = 1.0$;

R.S.D. = 0.5% ($n = 5$)

1% (w/v) aqueous solutions of ascorbic acid and magnesium ascorbyl phosphate, and a 1% (w/v) solution of ascorbyl palmitate in MeOH were stored both at room temperature and at 42°C in the dark for 60 days (simulating with this accelerated aging test about 12 months storage at room temperature). The solutions were periodically checked. Content of the substances is reported in Fig. 3a,b: the results showed that ascorbic acid underwent high concentration losses (recovery 37% at room temperature and none after 2 months at 42°C), confirming its great instability and inapplicability in cosmetic products [9,11]. The organic acid ester was more stable than ascorbic acid, but it also had a significant concentration loss after 60 days of storage (recovery 77% at RT, 47% at 42°C) (Fig. 3a,b. Magnesium ascorbyl phosphate showed good stability in time (95% at RT, 83% at 42°C). It is reasonable to believe that the introduction of the phosphate group in 2 position protects the enediol system of the molecule from hydrolysis better than esterification in 6 position with long lipophilic chains.

These encouraging results induced us to examine the behaviour of VC-PMG standard solutions. Specification data of the molecule provided by the producer show that neutral or basic solution guarantees the highest stability, while in acid solution this ascorbic acid derivative is extremely unstable and it may be easily hydrolyzed to ascorbic acid and inorganic phosphate [2]. Medium acid



pH, rather than basic solutions, are more suitable conditions for the formulation of topical products because this is the typical pH of the skin [17].

VC-PMG (1% w/v) solutions at various pH were prepared, and concentration losses versus time were determined after 2 months storage at room temperature.

As shown in Fig. 4, our data confirm the high instability of this molecule in strongly acid conditions (pH 3–4), while hydrolysis of the phosphoric group appeared lower, in the range of pH 5–8.5 (losses $< 10\%$).

The importance of hydrolysis of the phosphoric group from ascorbic acid, for stability studies of VC-PMG, was investigated by comparing a HCl solution with a phosphate buffer solution (KP 0.3 M), both at pH 4: VC-PMG content in buffer solution was near 99% after 5 months storage at room temperature in the dark, whereas significant loss was observed in the chloridic solution (27%).

Therefore, the presence of phosphoric ions in solutions protected the molecule from hydrolysis, due to the ion pair effect, shifting the balance of the reaction towards the phosphorylated form. These results also confirmed that the chromatographic conditions employed (KP 0.3 M, pH 4) did not negatively influence the stability of VC-PMG.

Lastly, stability studies of VC-PMG, according to pH proved that it may be employed in cosmetic samples even at neutral or weakly acid pH.

3.2. Stability of cosmetic products

We first examined the behaviour versus time of VC-PMG, emulsion (em.1a) and ascorbyl palmitate, (em.1b) in the same type of formulation, i.e. an oil-in-water emulsion prepared with 2.0% (w/w) of Eumulgin B1 (Ceteareth-12) and 1.5% (w/w) of Eumulgin B2 (Ceteareth-20) as emulsifier system.

Samples were periodically directly analysed by RP-HPLC, after dilution with the proper solvent mixture (see Section 2.5). No interference peaks were detected in the chromatographic patterns and the applicability of the method was verified using the standard addition method [13,15].

Fig. 2. Chromatographic patterns of ascorbic acid 15 μg ml^{-1} (a), ascorbyl palmitate 25 μg ml^{-1} (b) and magnesium ascorbyl phosphate 30 μg ml^{-1} (c) in standard solutionS.

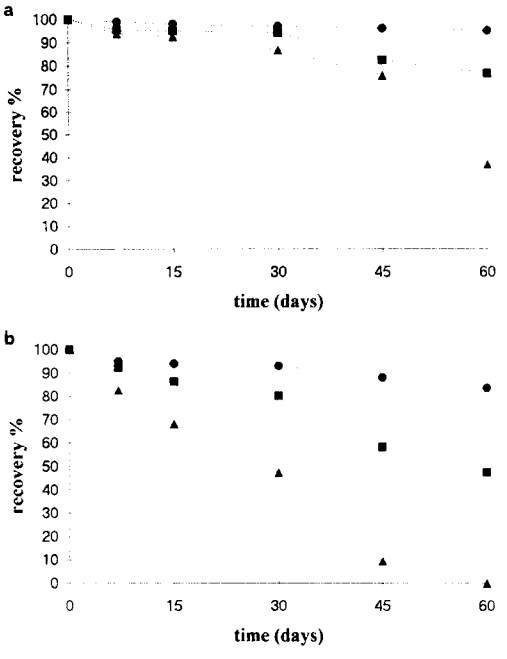


Fig. 3. Content of ascorbic acid (▲), ascorbyl palmitate (■) and magnesium ascorbyl phosphate (●) in standard solutions stored at RT (a) and 42°C (b).

The definite difference in behaviour between the two derivatives was evident: VC-PMG (em 1a) kept its stability up to 95% even after 60 days storage in the dark at 42°C (equivalent to 1 year of storage time at room temperature), while ascorbyl palmitate (em 1b) already showed great instability (27% recovery) after 2 months in the dark at RT. These results confirmed the capability of the phosphoric group to protect the enediol system from hydrolysis, even when it is included in cosmetic emulsions, while the lipophilic ester in 6 positions does not protect the vitamin from degradation.

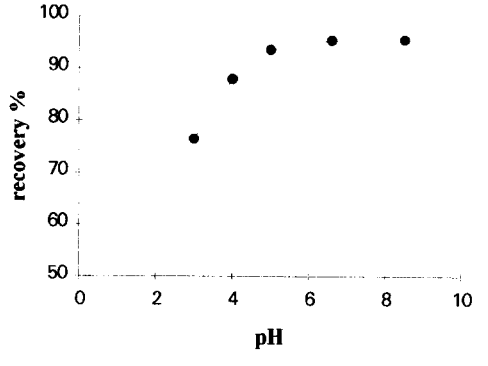


Fig. 4. Content of VC-PMG at various pH after 2 months storage at RT.

Earlier works conducted in our laboratory proved that the chemical stability of lipophilic derivatives of vitamins can also be enhanced, by improving the physical stability of the emulsion [18]. Considering the various components of an emulsion, the most important factor influencing vitamin stability is the surfactant system, which also grants stability to emulsions [19].

Starting from these results, we tried to improve the stability of ascorbyl palmitate, by changing the rheological properties of the formulations. In particular, cosmetic samples with high viscoelastic properties were considered, according to the gel network theory of emulsion stability, which relates the stability and physical properties of o/w emulsions to the viscoelastic nature of the continuous phase [20–23].

We prepared an oil-in-water emulsion (em 2) with 3.0% (w/w) of Brij 72 (Steareth-2) and 2.0% (w/w) of Brij 721 (Steareth-21) as emulsifier system, forming a typical liquid-crystalline emulsion [24]. In this way, we presumed that the stability of the molecule in relation to the ordered multilamellar structure of the emulsifiers in the dispersed phase would increase and that an additional gel phase in the external aqueous phase would form [21].

In a second test we introduced vitamin C palmitate in a two-phase o/w gel system (em 3), exploiting the solvent power of alcohol for better dispersion and using 3.0% (w/w) of Sepigel 305 (Polyacrylamide/$C_{13/14}$ Isoparaffin/Laureth-7) as thickening and stabilizing emulsifier agent.

Both emulsions 2 and 3, stored both at R.T. and at 42°C in the dark, showed a significant loss of the lipophilic derivative versus time. However, the stability of the vitamin was higher than that of em 1b: after 2 months storage sample 2 showed a striking loss in vitamin C content (about 50% at RT and 82% at 42°C), which was greater than that recorded in sample 3 (about 15% at RT and 72% at 42°C). These data show that the stability of ascorbyl palmitate depends on the structural properties of the formulations: cream-gel seems to be a more suitable vehicle for this ingredient than

Table 1
Content of VC-PMG (recovery %) in various commercial products (A–E) after 2 months at R.T. and 42°C ($n = 5$; R.S.D. < 3.00%)

Samples	R.T.	42°C
A	>99.00	94.04
B	>99.00	90.58
C	>99.00	93.17
D	>99.00	90.19
E	>99.00	90.00

oil-in-water emulsions, although these formulations are gel-like structured systems.

With regard to magnesium ascorbyl phosphate, this hydrophilic derivative of vitamin C showed very good stability in emulsion 1a. Our analysis then turned to a random examination of several commercial products, which may display incompatibility in the simultaneous presence of VC-PMG and other formula components often not certified in the label.

In particular, we considered four emulsions (A–D) and one hydrophilic gel (E), with various concentrations of active principle (1.2–6.0% w/w). Table 1 reports the results obtained after 2 months storage (accelerating aging test versus room temperature storage). All five samples stored at 42°C showed losses under 10% of the original amount found. Considering the drastic aging condition employed, VC-PMG degradation must be considered more than satisfactory, denoting its good stability even in finished cosmetic products.

In conclusion, the results reported prove that vitamin C ester solutions are more stable than ascorbic acid solutions. In particular, esterification with palmitic acid in 6 position reduces the hydrolysis of ascorbic acid but does not guarantee satisfactory stability levels in finished products, even when employed in suitable gel-like emulsions with high viscoelastic properties that may improve its chemical stability. Instead, the introduction of the phosphoric group in 2 position protects the molecule from break-up of the enediol system, confirming VC-PMG as a stable derivative of vitamin C that may be easily used in various type of cosmetic products.

Acknowledgements

Financial support from MURST (40%) and CNR Special Project on Fine Chemicals is gratefully acknowledged. The authors wish to thank Dr A. Nicolato (Zeta Farmaceutici S.p.A., Sandrigo-Vicenza-Italy) for emulsion formulations and Dr L. Crosetto and Co. for supply of raw materials.

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