

Short communication

# High-performance liquid chromatographic determination of ionic compounds in cosmetic emulsions: application to magnesium ascorbyl phosphate

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First received 18 October 1994; revised manuscript received 31 January 1995; accepted 31 January 1995

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## Abstract

A rapid, reliable method based on reversed-phase high performance liquid chromatography for the qualitative and quantitative determination of magnesium ascorbyl phosphate (VC-PMG) in both standard solutions and cosmetic products is reported. The procedure, based on an aminic stationary phase and acetonitrile–0.3 M phosphate buffer (pH 4) (40:60) as the mobile phase, allows the determination of VC-PMG in cosmetic samples after dilution with a tetrahydrofuran–0.3 M phosphate buffer (pH 4) (3:7) solvent mixture.

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## 1. Introduction

The analysis of untreated heterophase systems may easily be performed after dilution with aqueous–organic solvent mixtures [e.g., water–tetrahydrofuran (1:9)]. This dilution step avoids any pretreatment of the sample with some practical advantages, such as rapidity, flexibility and decrease of the analytical error [1]. This procedure, combined with RP-HPLC, allows the qualitative and quantitative determination of each ingredient, so that its stability in the finished product and compatibility with other formula components may be studied [2]. Although this method has been successfully applied to the determination of non-polar and polar molecules in multi-component systems [3,4], it

cannot be considered as optimum for the determination of ionic compounds.

In this paper, we report a new approach to the direct determination of ionic molecules in cosmetic products; we investigate the chromatographic behaviour of the magnesium salt of ascorbyl phosphoric acid (VC-PMG), a new derivative recently introduced commercially and used in cosmetic emulsions as a skin whitener and radical scavenger [5–7].

It is well known that ascorbic acid has important physiological effects on the skin, including the inhibition of melanogenesis, promotion of collagen formation and prevention of free radical formation [8–10]; these effects are closely related to the well known antioxidant properties of this molecule [11–13].

The use of vitamin C in finished products is limited by its chemical properties: this molecule, readily soluble in water, is extremely unstable in

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aqueous solution [14,15] and is poorly absorbed through the skin [14,15]. The reactivity of ascorbic acid and its solubility properties may be modified by esterification with organic or inorganic acids [14,15]. Of the various derivatives available on the market, magnesium ascorbyl phosphate (VC-PMG) (Fig. 1) seems to be the most stable molecule in aqueous solution [8,14].

Previous publications have only described spectroscopic procedures for the determination of magnesium ascorbyl phosphate [8,14], and no chromatographic study for a reproducible, objective evaluation of its behaviour in finished products has yet been performed. The aim of this study was to develop a rapid, reliable method based on RP-HPLC for the qualitative and quantitative determination of magnesium ascorbyl phosphate in cosmetic products [16,17]. As VC-PMG is a polar molecule, its chromatographic separation may be carried out using an anion-exchange column. However, direct analysis of heterophase systems on such a column is not easy to perform, because they need a high percentage of organic modifiers, often incompatible with high-molarity buffer solutions. For these reasons, we investigated the chromatographic behaviour of VC-PMG on reversed and bonded phases with increasing polarity.

The determination of VC-PMG in untreated cosmetic samples may be performed using an aminic stationary phase and acetonitrile-phosphate buffer as the mobile phase after sample

dilution with a tetrahydrofuran–0.3 M phosphate buffer (pH 4) (3:7) solvent mixture.

## 2. Experimental

### 2.1. Materials and reagents

Magnesium ascorbyl phosphate (VC-PMG) was obtained from Nikkol (Tokyo, Japan). LiChrosorb RP-18 (Li-RP18), LiChrosorb CN (Li-CN) and LiChrosorb NH<sub>2</sub> (Li-NH<sub>2</sub>) columns (7 μm, 250 mm × 4 mm I.D.) and analytical-reagent grade reagents and solvents were obtained from Merck (Darmstadt, Germany).

### 2.2. Apparatus

A Gilson liquid chromatograph (Biolabo Instruments, Milan, Italy) equipped with two pumps (Models 305 and 306), a Gilson Model 805 manometric module, a Gilson Model 811B dynamic mixer, a Rheodyne Model 9010 valve, a Perkin-Elmer LC 95 UV-Vis detector and a Shimadzu C-R5A data station was used.

### 2.3. Chromatographic conditions

The stationary phase was Li-NH<sub>2</sub> (7 μm), the eluent was acetonitrile–0.3 M phosphate buffer (pH 4) (40:60) at a flow-rate of 1 ml/min, UV detection at 255 nm was applied and the injection volume was 20 μl.

### 2.4. Standard solutions

VC-PMG standard solution (1%, w/v) was prepared in 0.3 M phosphate buffer (KP) (pH 4) and stored in the dark at 4°C. Further dilutions were performed, using the same solvent, in the range 5–30 μg/ml for obtaining calibration graphs.

### 2.5. Samples

About 1 g of each cosmetic sample, accurately weighted, was diluted 1:20 with tetrahydrofuran–0.3 M phosphate buffer (pH 4) (3:7) in a screw-

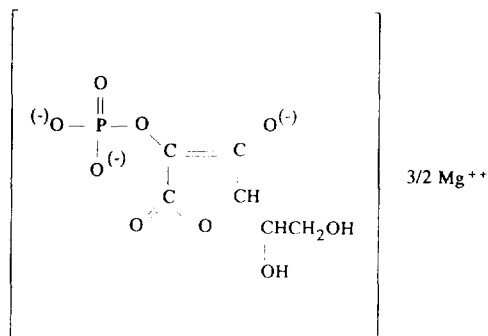


Fig. 1. Structure of magnesium ascorbyl phosphate (VC-PMG).

capped tube and stirred in a vortex mixer until completely homogeneous. Further dilutions were performed only with 0.3 M phosphate buffer (pH 4) until a final dilution of 1:200–4000, depending on the VC-PMG concentration in the cosmetic sample considered. The solutions obtained were injected directly into the chromatographic system.

### 3. Results and discussion

#### 3.1. VC-PMG analysis

The chromatographic behaviour of magnesium ascorbyl phosphate in aqueous solution was investigated using three stationary phases of increasing polarity and acetonitrile to acid phosphate buffer mobile phase ratios. As expected, no interaction between VC-PMG and cyano-propylic or octadecylcyclic column was detectable, as the molecule eluted with the solvent.

In contrast, a chromatographic separation occurred on the aminopropyl column (Fig. 2), which can be considered a weak anionic ex-

changer under the conditions employed. Moreover, when the amount of organic modifier in the mobile phase was increased, no significant change in the retention process could be noted, confirming the ionic nature of the interaction.

The capacity factor ( $k'$ ) of magnesium ascorbyl phosphate on the aminic column closely depended on the ionic strength of the buffer. As reported in Fig. 2, it gradually decreased with increasing molarity, and was not affected by acetonitrile at concentrations up to 40%.

The chromatographic process also was significantly influenced by the pH of the mobile phase: acidity of the eluent is essential for the anion-exchange mechanism, as it provides salt dissociation and protonation of aminic groups. This chromatographic process did not occur if a neutral mobile phase was used (Fig. 3).

In conclusion, the chromatographic elution of VC-PMG on an aminic stationary phase closely depends on the molarity and acidity of the buffer, although in order to determine this molecule directly in a cosmetic sample, avoiding pretreatment steps (i.e., extraction of the oily phase), a significant percentage of organic modifier had to be used.

Considering the time of analysis, peak res-

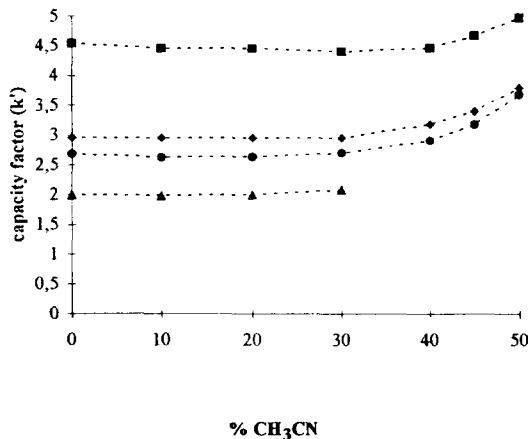


Fig. 2. Influence of ionic strength on capacity factor ( $k'$ ) of VC-PMG. Column, Li-NH<sub>2</sub>; eluent, acetonitrile–phosphate buffer (pH 4); flow-rate, 1 ml/min; detection at 255 nm. ■ = 0.15; ◆ = 0.25; ● = 0.3; ▲ = 0.5 M. An acetonitrile concentration over 30% is not compatible with 0.5 M phosphate buffer.

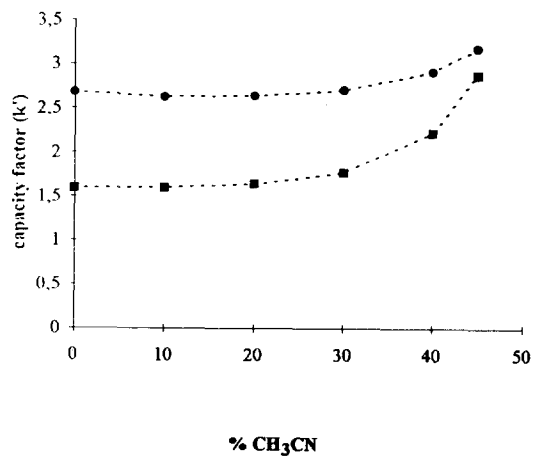


Fig. 3. Influence of pH on capacity factor ( $k'$ ) of VC-PMG. Column, Li-NH<sub>2</sub>; eluent, acetonitrile–0.3 M phosphate buffer; flow-rate, 1 ml/min; detection at 255 nm. ■ = pH 6.5; ● = pH 4.

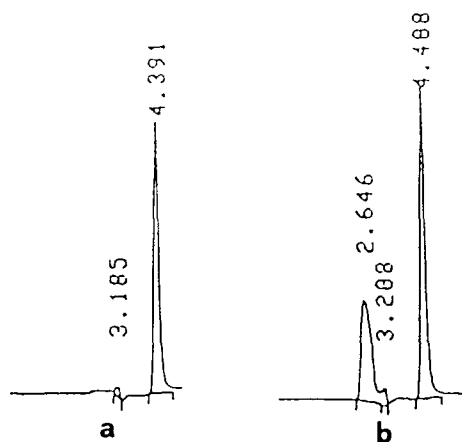


Fig. 4. Chromatographic patterns of VC-PMG (30  $\mu\text{g/ml}$ ) in (a) standard solution and (b) a cosmetic emulsion.

olution and compatibility between acetonitrile and phosphate buffer, the optimum chromatographic parameters were as given in Section 2.3.

Fig. 4a shows the chromatographic pattern of a standard solution of VC-PMG in 0.3 M phosphate buffer (pH 4) (30  $\mu\text{g/ml}$ ); linear calibration graphs [ $y$  = peak area;  $x$  = concentration ( $\mu\text{g/ml}$ )] were obtained in the range 5–30  $\mu\text{g/ml}$  with good regression coefficients ( $y = 2.15x - 0.05$ ;  $r = 1.00$ ). Repeatability of analysis was verified at various concentrations, and revealed good precision and accuracy (recovery 99.10–101.00%; R.S.D. 0.17–0.48%).

The stability of VC-PMG standard solutions in 0.3 M phosphate buffer (pH 4) was studied in order to verify its compatibility with the mobile phase employed; 1% standard solutions, stored at room temperature in the dark and periodically analysed for 5 months, did not show any significant concentration loss (Table 1).

Table 1  
Recovery of VC-PMG standard solutions after storage for 5 months at room temperature

Standard solution (1%)	Recovery (%) ( $n = 5$ )
H <sub>2</sub> O	91.27 $\pm$ 0.22
KP (pH 4)	99.12 $\pm$ 0.28
Dil. HCl (pH 4)	73.58 $\pm$ 0.24

### 3.2. Application to cosmetic samples

Analysis of cosmetic formulations can easily be performed, avoiding any extraction step, by dilution of samples with suitable aqueous–organic mixtures [12,13]. The procedure normally employed for heterophase systems is based on sample dilution with a tetrahydrofuran–water (9:1) solvent mixture, followed by direct RP-HPLC. This mixture is not suitable for the determination of magnesium ascorbyl phosphate, as the complete solubilization of this molecule can be achieved only using a highly polar medium. For this reason, we investigated the solubilization power of various mixtures prepared with tetrahydrofuran and phosphate buffer; good results were obtained using tetrahydrofuran–0.3 M phosphate buffer (pH 4) (3:7).

The determination of VC-PMG in cosmetic samples can be performed by first applying a dilution step (1:20) of about 1 g of the sample, accurately weighed, with tetrahydrofuran–0.3 M phosphate buffer (pH 4) (3:7) solvent mixture. Under these conditions, we obtained a homogeneous suspension of the ingredients, which could be directly analysed in the chromatographic system. Depending on the VC-PMG concentration in the sample, further dilutions can be performed using only phosphate buffer.

Fig. 4 compares the chromatographic patterns of VC-PMG (a) in standard solution and (b) in a cosmetic emulsion, and shows a good peak resolution. The matrix effect was evaluated using the standard addition method (standard solution,  $y = 2.15x - 0.05$  ( $r = 1.00$ ); standard solution + sample,  $y = 2.20x + 13.90$  ( $r = 1.00$ )).

In order to evaluate the applicability of this method to cosmetic emulsions, several oil–water systems containing known concentrations of VC-PMG were prepared. Recovery trials and relative standard deviations for each sample confirmed the reproducibility and flexibility of the method, which appears to be suitable for the rapid, sensitive determination of VC-PMG (Table 2).

VC-PMG was then determined in six “skin whitener” emulsions marketed in Italy; no inter-

Table 2  
Assay results for VC-PMG in various oil–water emulsions

Concentration found (%, w/w) (n = 5)	Recovery (%)	R.S.D. (%)
1.04 ± 0.01	104.00	0.96
1.07 ± 0.01	97.27	0.93
1.07 ± 0.03	97.27	2.80
1.06 ± 0.02	96.36	1.89
1.53 ± 0.01	102.00	0.65

ferences with other formula components were detected, confirming the applicability of the methodology.

In conclusion, chromatographic determination of this highly polar molecule can easily be directly performed in cosmetic emulsions using a tetrahydrofuran–buffer solvent mixture and a weak anion exchanger as the stationary phase, and can be used to perform stability studies of this molecule in cosmetic products.

In order to overcome the problems due to the short lifetime of the silica aminic column (hydrolysis of aminic groups), the use of a polymeric aminic stationary phase can be considered. This kind of column, more expensive than the former, provides the same chromatographic results with stable performance.

#### Acknowledgements

Financial support from MURST (40%), CNR Special Project on Fine Chemicals, and L. Crosetto is gratefully acknowledged.

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