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Simultaneous HPLC determination of multiple components in a commercial cosmetic cream[1]

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Abstract

A high-performance liquid chromatographic method for the simultaneous determination of magnesium ascorbyl phosphate (**I**), imidazolidinylurea (**II**), a mixture of methyl-(**III**), ethyl-(**IV**), propyl-(**V**), butyl-(**VI**) parabens dissolved in phenoxyethanol, and ascorbyl palmitate (**VII**), was studied by using a cyano-propyl column and a methanol gradient at 220 and 240 nm. Calibration curves were found to be linear in the 0.05–5 mg ml^{-1} range (compounds **I**, **II**, **VII**) and 0.9–160 mg ml^{-1} (compounds **III–VI**). Linear regression analysis of the data demonstrates the efficacy of the method in terms of precision and accuracy. An extraction method is developed and validated in order to apply this chromatographic method to a commercial cosmetic cream. The precision of this method, calculated as the relative standard deviation (RSD) of the recoveries (1.57–2.21%) was excellent for all compounds **I–VII**. © 1998 Elsevier Science B.V. All rights reserved.

Keywords: Cosmetic cream; Paraben mixture; Imidazolidinylurea; Mg ascorbyl phosphate; Ascorbyl palmitate; HPLC

1. Introduction

In the near future, cosmetic industries are going to have to comply with the sixth amendment to the 76/768/EEC Council Directive, which introduced, amongst other innovations, the availability of both a full dossier and labelling of cosmetic ingredients. On the other hand, the cosmetic formulations are complex mixtures of different chemical compounds with different chemical properties. Therefore, new analytical methods, which can detect and quantify such compounds in commercial products, have to be found.

In particular, we studied a commercial cosmetic cream with leukogen properties, which contains parabens and imidazolidinylurea as preservatives, magnesium ascorbyl phosphate and ascorbyl palmitate as skin whiteners [1,2].

The simultaneous HPLC determination and quantitation of such ingredients has not yet been reported in literature. A paraben mixture is usually quantified by an ODS column, whereas imidazolidinylurea and ascorbyl phosphate, owing to

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their polar character, tend to elute early with the above column and are respectively analyzed by octyl silica and aminopropyl columns [3,4]. On the contrary, ascorbyl palmitate, which shows greater affinity to the ODS stationary phase, is not eluted in an experimentally acceptable time, also with increasing proportions of organic modifier; for this reason it was analyzed with a more polar column [5].

In the present work, we would like to demonstrate that the cyano-propyl stationary phase can simultaneously detect the above components when used together with a phosphate buffer-methanol mobile phase in a gradient elution system. The use of an UV multi-wavelength detector can overcome some problems arising from the extreme differences in the composition of compounds to be determined at the same time.

This chromatographic method has been successfully utilized for routine analysis and quality control analysis in commercial batches. We developed a previous dilution method [4,6] which is based on a sample dilution with an aqueous–organic mixture.

2. Experimental

2.1. Reagents and standards

The methyl-, ethyl-, propyl- and butylparaben, imidazolidinylurea, magnesium ascorbyl phosphate, ascorbyl palmitate, and the commercial creams were purchased from Mastelli, S.r.l. (Sanremo, Italy). All chemicals used were of analytical grade. Methanol and water were of HPLC grade. Solvents were filtered through a 0.45 μm membrane and degassed.

2.2. Instrumentation

HPLC was performed using a HP-1100 liquid chromatograph (Hewlett-Packard, PA) equipped with quaternary pumps, a Rheodyne 7125 (Berkeley, CA) injector valve with a 20-μl loop and a diode-array UV multi-wavelength detector. The chromatograms were recorded and the peak areas were calculated using HP Chemstation software. A 200×4.6 mm i.d. stainless-steel Hypersil BDS cyano-propyl column was used.

2.3. Chromatographic conditions

The mobile phase consisted of methanol: monobasic sodium phosphate (pH 3.5; 0.025 M) (40:60, v/v). Subsequently, the composition was altered gradually to 80% of methanol over 8 min. This composition was maintained for an additional 4 min. The initial eluent composition was restored in 5 min. Flow rate was 1.0 ml min^{-1}. The determinations were performed at room temperature at 220 and 240 nm, respectively.

2.4. Calibration curves

Five standard solutions were prepared for each product by dissolving weighed amounts of compounds **I–VII** in a water–methanol mixture in order to obtain the ranges of concentration as reported in Table 2. Calibration curves were constructed by plotting the peak areas of compounds **I–VII** (y) against their concentration (x); regression analysis of the data ($n = 5$) for each component gave the values for slope, along with the intercept and correlation coefficient for each calibration curve (Table 2). The calibration curves were used for the quantification of compounds **I–VII** in six samples of commercial cream.

2.5. Sample preparation

Approximately 1 g of our commercial cream, accurately weighed, was diluted 1:10 with tetrahydrofurane-phosphate buffer (pH 3.5; 0.025 M) (3:7, v/v) in a screw-capped tube. It was stirred in a vortex mixer and slightly warmed until a homogeneous suspension was obtained. This suspension was injected into the chromatograph.

3. Results and discussion

3.1. Validation of the method

The aim of the present chromatographic method was to separate a complex mixture con

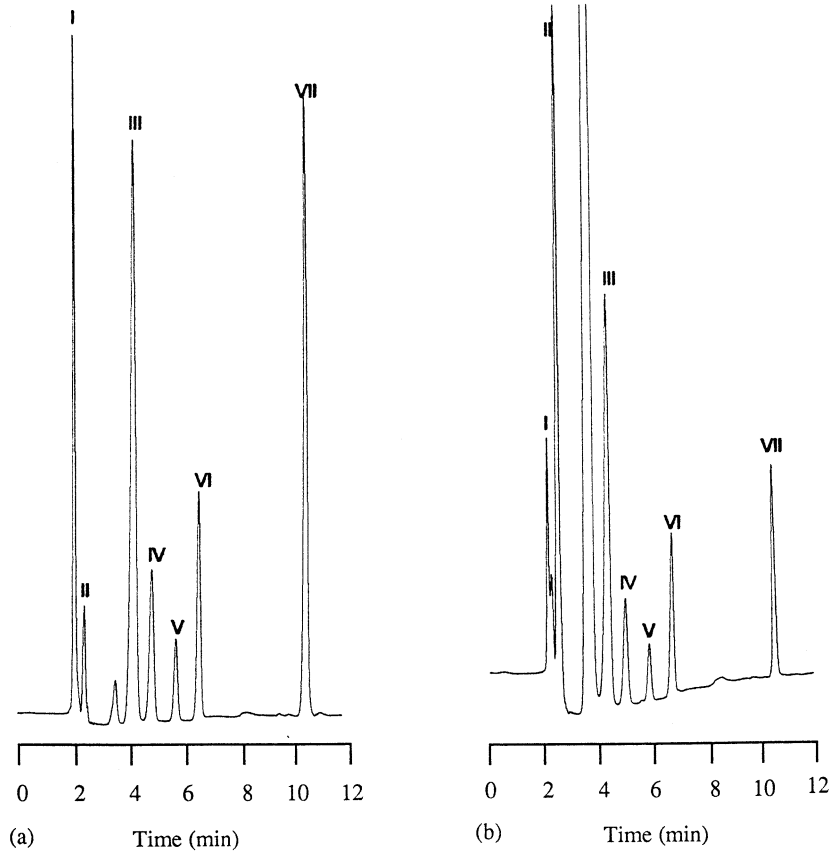

Fig. 1. Chromatograms of standard mixture at: (a) 240 nm; and (b) 220 nm.

taining seven components of a very different nature. Since imidazolidinylurea and magnesium ascorbyl phosphate are very polar, the parabens medium apolar, and ascorbyl palmitate very apolar, they are usually quantified by different columns. Here, on the contrary, we used only a cyano-propyl column with a methanol gradient for all of them. Fig. 1 shows the separation of a standard mixture of the **I–VII**. In Table 1, the retention times are reported.

One crucial problem was to maintain both a good separation of the paraben mixture, as in the ODS column, and to elute the ascorbyl palmitate in a short period of time. Actually, this methanol gradient reached the above, and the final 80% of methanol showed that it did not disturb the separation of parabens and allowed for the elution of ascorbyl palmitate in about ten min.

Furthermore, in comparison with the tested C8 and C18 columns, this method needs a short time of column restoring (5 min) without any negative effects on the subsequent paraben separation.

Another crucial separation was the elution of ascorbyl magnesium phosphate and imidazolidinylurea which in reverse phase columns were

Table 1
Retention times (min) of compounds **I–VII**

Compound	RT	
I	Mg ascorbyl phosphate	2.08
II	Imidazolidinylurea	2.49
III	Methyl-paraben	4.11
IV	Ethyl-paraben	4.73
V	Propyl-paraben	5.55
VI	Butyl-paraben	6.36
VII	Ascorbyl palmitate	10.40

Table 2
Validation data ($n = 5$)

Comp.	Conc. range	Calibration curves	Corr. Coeff.	Recovery average%	Recovery RSD%
I	0.05–5 mg ml^{-1}	$Y = 2.36X - 0.8$	0.9999	100.1	1.69
II	0.01–1 mg ml^{-1}	$Y = 1.33X + 1.11$	0.9988	96.89	2.04
III	4–160 μg ml^{-1}	$Y = 2.34X - 3.1$	0.9997	100.97	1.55
IV	1.1–35 μg ml^{-1}	$Y = 1.13X + 1.13$	0.9986	98.9	1.61
V	0.9–30 μg ml^{-1}	$Y = 0.75X - 0.4$	0.9999	99.2	1.92
VI	1.9–65 μg ml^{-1}	$Y = 1.98X + 0.9$	0.9995	99.9	1.34
VII	0.05–5 mg ml^{-1}	$Y = 1.13X + 1.2$	0.9998	97.8	1.76

Y, peak area; X, concentration.

not at all retained and eluted together. Although our method separated compounds **I** and **II** in a good manner (Fig. 1(a)), in order to improve their quantification, we took advantage of the different UV absorption of these components, and using a multiwavelength UV-DAD, collected the peak areas of compound **II** at 220 nm, while all the others at 240 nm. Fig. 1(b) shows the high sensitivity at 220 of **II** in respect to the other components. In this range of UV detection, no interferences on baseline were noted from the mobile phase.

The method was validated for linearity, accuracy and precision. Linearity was observed in the range of concentration indicated in Table 2. This range includes the concentrations of **I–VII** in the commercial cosmetic cream (Table 3). The correlation coefficient ranged from 0.9986 to 0.9999 ($n = 5$). The precision of the method was calculated as the relative standard deviation (RSD) of assays containing compounds **I–VII** in the same range of concentration. The RSD ranged from 1.34 to 2.04%. The accuracy was assessed by calculating relative recoveries of **I–VII**. The analytical recoveries were 97.8–100.1%.

3.2. Application to quality control

The validated method was applied to a series of industrial batches so as to estimate both the concentrations of compounds **I–VII** in the commercial cream and the reproducibility of the extraction method.

Fig. 2 shows a cromatogram of **I–VII** at the 240 nm after extraction from a commercial sample. Other ingredients in the cream, i.e. water, fat, surface active agents, are not quantified in this method, which determines only the preservatives and the active compounds; however they do not disturb the quantification of **I–VII** in the sample.

In Table 3, the recovery of compounds **I–VII** in

Table 3
Recoveries % of compounds **I–VII** in commercial batches

	I	**II**	**III**	**IV**	**V**	**VI**	**VII**
	5 mg ml^{-1}	0.3 mg ml^{-1}	100 μg ml^{-1}	22 μg ml^{-1}	18.6 μg ml^{-1}	40 μg ml^{-1}	5 mg ml^{-1}
1	89.12	97.95	101.68	101.83	97.50	87.22	90.47
2	85.59	102.31	101.03	100.91	101.61	88.99	89.25
3	86.07	96.51	99.12	98.68	97.90	88.20	87.15
4	86.01	99.37	98.39	97.63	98.19	88.75	86.48
5	88.54	98.74	97.41	100.01	101.31	89.34	87.51
6	88.66	96.38	99.14	99.01	97.38	92.51	85.32
Mean	87.33	99.23	99.77	98.52	98.02	89.17	85.11
SD	1.60	2.13	1.61	1.54	1.94	1.80	1.87
RSD	1.83	2.21	1.61	1.57	1.98	2.01	2.20

six samples of the commercial cream are shown. The precision of this method, calculated as the RSD of the recoveries (1.57–2.21) was excellent for all compounds **I–VII**. Nevertheless, the accuracy of the recoveries was satisfied only with compounds **II–V**, in fact with **I**, **VI**, **VII**, it ranged between 85 and 90%.

Although this accuracy allows for a precise determination in the quality control, commercial reasons and the theoretical possibility of having a different range of concentration of compounds **I–VII** in the industrial batches led us to make many attempts at improving the extraction system.

We carried out many changes in this extraction mixture, such as changes in buffer composition and ionic strength; changes of organic modifier, such as tetrahydrofurane, methanol, ethilic ether, and their percentage composition, however, we did not find a good solvent mixture for all components **I–VII**.

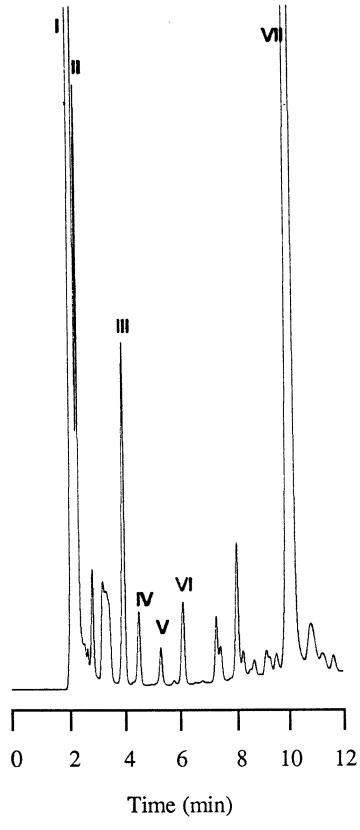

Fig. 2. Chromatogram of mixture extracted from commercial cream at 240 nm.

Another reason for improving the extraction system is the short lifetime of the column. In fact, in this condition only about a hundred analyses of commercial batches are possible. On the other hand, when we used a guard-column, the accuracy and precision of the determination of compounds **I** and **II** worsens.

4. Conclusions

The cyano-propyl column demonstrated that it could quantify a complex mixture that contained compounds which were quite different among themselves. In particular, this allowed for the contemporary analysis of even other very polar and very apolar components without damaging the separation of the parabens, which are usually well separated in the reverse-phase column.

In order to overcome the problems due to the short lifetime and a not-complete recovery from the commercial cream, we are studying a possible extraction in two steps, which would be able to thoroughly extract compounds **I–VII** without losing the simplicity and the directness of this extraction method.

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