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Letter

Quantification of phenolphthalein in cosmetic products

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Abstract. A simple method for the quantification of phenolphthalein in cosmetic formulations is described. The sample, acidified with acetic acid, is extracted with methanol and analysed by reversed-phase HPLC on a column packed with 5 µm SelectB RP-8. The initial mobile phase is acetonitrile-aqueous 0.1 M acetic acid (10:90), then a linear gradient up to 90% acetonitrile is applied in 30 min. The limit of determination is 1 ng injected when detection is performed at 230 nm.

Key words. Phenolphthalein – high-performance liquid chromatography – cosmetics.

Introduction

Phenolphthalein, 3,3-bis(p-hydroxyphenylphtalide) (I) is one of the most common indicators for titrations and it is a drug of the group of stimulant laxatives. With this action, it has been commonly used in many commercial non-prescription formulations, alone or in combination with other agents like anthraquinones, during the most of the twentieth century [1].

Recent studies have demonstrated that phenolphthalein may be a significant source of oxidative stress in physiological systems [2] and may cause multiple carcinogenic effects in rats and mice. One of the sites for neoplasm that is of particular concern is the ovary, and epidemiology studies are under way to identify any potential effects of (I) exposure at this site in humans [3].

Vary few methods have been reported in the literature for the determination of phenolphthalein. They are based on titrimetry [4], conductimetry [5], spectrophotometry [6], and HPLC [7-8]. The official method described in the British Pharmacopoeia involves the measurement of the colour developed as a result of adding glycine buffer to the drug substance, but no formulations are taken into account in this compendium. The USP XXII [9] and the USP XXIII [10] describe a high-performance liquid chromatographic method for the determination of phenolphthalein as the raw material and in tablets.

In the cosmetic field it has been employed in colour-changeable toothpastes or antiplaque mouthrinses, especially marketed in the USA, with the aim of indicating the duration of adequate tooth-brushing. Recently, after the approval of the XVIII EEC Directive, phenolphthalein has been inserted in the Enclosure II, with the serial number 417, i.e., in the list of substances forbidden in the cosmetic field and, therefore, all the countries of the European Union must comply with the above cited Directive.

This event has stimulated us to develop an analytical method for identification and quantitation of (I) in cosmetic formulations intended for oral hygiene, which could be used for the routine control of commercial samples to verify their conformity to legislation.

This paper describes a procedure for phenolphthalein determination based on a very simple extraction of the sample and analysis by reversed-phase HPLC.

Experimental

Standards and reagents

Phenolphthalein was supplied by Carlo Erba (Milan,Italy). Triclosan, 2,4,4'-trichloro-2'-hydroxydiphenylether (TC) was purchased from Ciba-Geigy (Basel, Switzerland), methyl-4-hydroxy benzoate (MP), ethyl-4-hydroxy benzoate (EP), *n*-propyl-4-hydroxy benzoate (PP), *n*-butyl-4-hydroxy benzoate (BP) were purchased from Formenti (Italy). All reagents used were of analytical-reagent grade and used without further purification. Methanol and acetonitrile were of HPLC grade. Water was deionized and doubly distilled from glass apparatus. All solvents and solutions for HPLC analysis were filtered through a Millipore filter (pore size 0.45 µm) and vacuum degassed by sonication before use.

Apparatus

The HPLC system consisted of a Shimadzu LC-10AD liquid chromatograph equipped with an external Rheodyne injector valve, fitted with a 10 µL sample loop, and a Hewlett Packard 1050 photodiode-array detector. The chromatographic data were processed with a personal computer Vectra HP 486, utilising an HP 3DChemstation software. The analytical column was of stainless-steel (250 mm ×

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4.0 mm I.D.) packed with 5 μm SelectB RP-8 (Merck, Darmstadt, Germany).

HPLC conditions

The initial composition of mobile phase was: acetonitrile-water containing 0.1 M acetic acid in the ratio 10:90 (v/v), then a linear gradient up to 90% acetonitrile in 30 min. At the end of the elution, the initial mobile phase was passed through the column for 10 min to allow a good re-equilibration of the chromatographic system. Flow-rate was 1.5 mL min^{-1}; injection volume, 10 μL; column temperature, 25 °C. The preferential wavelength of detection was 230 nm. The range of wavelengths examined by the photodiode-array detector was 200 – 400 nm.

Calibration standard solutions

Stock solutions of phenolphthalein and preservatives were prepared by dissolving the appropriate amount of the standards in acetonitrile-0.1 M acetic acid (4:1, v/v). A set of working standard solutions was prepared by diluting aliquots of the stock solutions to give concentrations ranging from 2 to 1000 μg mL^{-1}. The calibration graphs were constructed by plotting the peak areas obtained at the optimum wavelength of detection versus the amounts (μg) injected.

Sample preparation

About 1 g of the cosmetic sample (toothpaste or mouthrinse) was accurately weighed and treated with 0.5 – 1 mL of an aqueous solution of 2 M acetic acid until a possible pink-violet colour disappeared; 5 mL of methanol were added and the dispersion was submitted to ultrasonic treatment for 5 min. After centrifugation, the supernatant was collected and the residue re-extracted with 4 mL of methanol. The combined extracts were transferred to a 10 mL volumetric flask and taken to volume with methanol. Before injecting into the liquid chromatograph the solution was filtered through a nylon filter (0.45 μm).

Results and discussion

Due to the critical role of preservatives in the improvement of the shelf-life of a product, they are widely added to cosmetics, food and drugs. The esters of 4-hydroxybenzoic acid, known as parabens, and triclosan are the preservatives most frequently used. Since these agents could interfere in the chromatographic determination of (I), we have also submitted to HPLC analysis the preservatives listed in table I. Figures 1A and 1B show the chromatograms of a standard solution, containing all the compounds studied, at the concentrations indicated in the legend, recorded at 230 and 280 nm, respectively. Depending on the molar absorptivity of the compound, the optimum detection wavelength for (I) was at 230 nm, whereas 280 nm could be chosen for the quantitation of the phenolphthalein itself and of the preservatives when analysing a real cosmetic sample, because of the "cleaner" chromatogram obtained at the wavelengths higher than 230 nm.

The designed gradient elution allowed a good separation of all the agents considered and a good symmetry of the peaks. In particular the resolution factor between (I) and PP, resulted 2.05. Capacity factors were reproducible under the experimental conditions used, the coefficient of variation (C.V.) being less than 2.5% for between-day studies. The photodiode-array detector allowed the estimation of the peak purity, which was calculated over the range 200 – 400 nm and resulted greater than 0.995 for all the compounds tested, so confirming the identity of the active principle.

The calibration graphs for phenolphthalein and the preservatives taken into account were constructed from five consecutive injections. The linearity was evaluated over the range of concentrations reported in the experimental section. The equations obtained by the least square regression fit are reported in table II. The limit of determination (LOD) for phenolphthalein, defined as the concentration giving a signal-to-noise ratio of 3, resulted 1 ng at 230 nm and 5 ng at 280 nm, respectively.

Table I. Chromatographic parameters*.

Compound	*Retention volume (mL)*	*Capacity factor factor*	*Peak asymmetry*
MP	15.54(0.10)	5.06(0.09)	0.95(0.04)
EP	18.99(0.09)	6.45(0.08)	0.89(0.05)
(I)	21.73(0.08)	7.52(0.08)	0.88(0.04)
PP	22.32(0.08)	7.75(0.11)	0.94(0.04)
BP	25.53(0.10)	8.93(0.12)	0.85(0.06)
TC	32.99(0.13)	11.94(0.14)	0.83(0.07)

* Each value is the mean of five determinations. SD in parentheses.

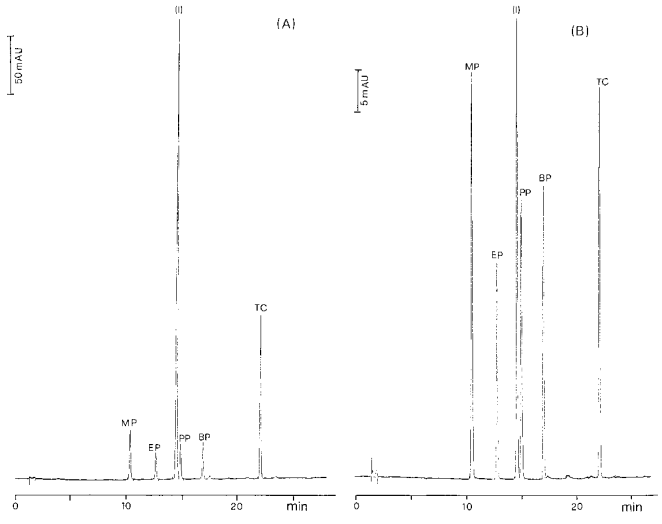

Fig. 1. Typical chromatograms obtained at 230 nm (A) and 280 nm (B) for a standard solution containing 66 μg mL^{-1} of phenolphthalein and TC, and 25 μg mL^{-1} of MP, EP, PP, and BP.

Table II. Calibration curves for the compounds investigated: linear regression of the amount injected (*x*) *versus* the peak area (*y*); mean value ± standard deviation at 95% confidence interval (t = 3.18; n = 5).

Compound	*Detection wavelength*	*Intercept*	*Slope*	R^2
MP	230	–10 ± 3	1316 ± 35	0.9997
EP	230	–34 ± 9	736 ± 19	0.9998
(I)	280	6 ± 1	513 ± 14	0.9996
(I)	230	16 ± 3	4774 ± 52	0.9997
PP	230	–23 ± 9	1016 ± 29	0.9998
BP	230	18 ± 4	1043 ± 27	0.9995
TC	230	6 ± 1	1723 ± 45	0.9996

Table III. Analysis of phenolphthalein in cosmetic samples*.

Sample	*Amount found (%, w/w)*	*Amount added (%, w/w)*	*Recovery (%)*
Toothpaste 1	ND	0.02	95.6 (2.5)
Toothpaste 2	ND	0.02	100.8 (2.1)
Toothpaste 3	ND	0.02	96.2 (1.8)
Toothpaste 4	0.031(6E-4)	=	=
Toothpaste 5	0.039(7E-4)	=	=

*Mean of five determinations. SD in parentheses. ND = not detectable.

The proposed procedure was applied to the analysis of five samples of toothpastes, commercially available on the European market. Two of them was found to contain phenolphthalein at the concentrations shown in table III. The other cosmetic products, for which the absence of any peaks at the retention time corresponding to (I) had been verified, were used to carry out recovery tests. They were spiked with 0.02% (w/w) of phenolphthalein and analysed. The results obtained are reported in table III. As can be seen good recovery and precision were observed. A typical chromatogram obtained for an analysed commercial sample, the toothpaste named 5 in table III, is reported in figure 2. This product, in addition to phenolphthalein, estimated at a concentration of 0.039%, contained triclosan, as a preservative, at a concentration of 0.19%. At the detection wavelength of 230 nm, no interference was observed from the other components of the matrix, as confirmed by the values of the peak purity.

The analytical results obtained lead to the conclusion that the method proposed for the identification and quantitation of phenolphthalein in cosmetic formulations for oral hygiene is simple, rapid, and displays good accuracy and precision. Therefore, it could be successfully adopted for the analysis of cosmetics to verify the absence of phenolphthalein, or, in the opposite case, to establish its concentration.

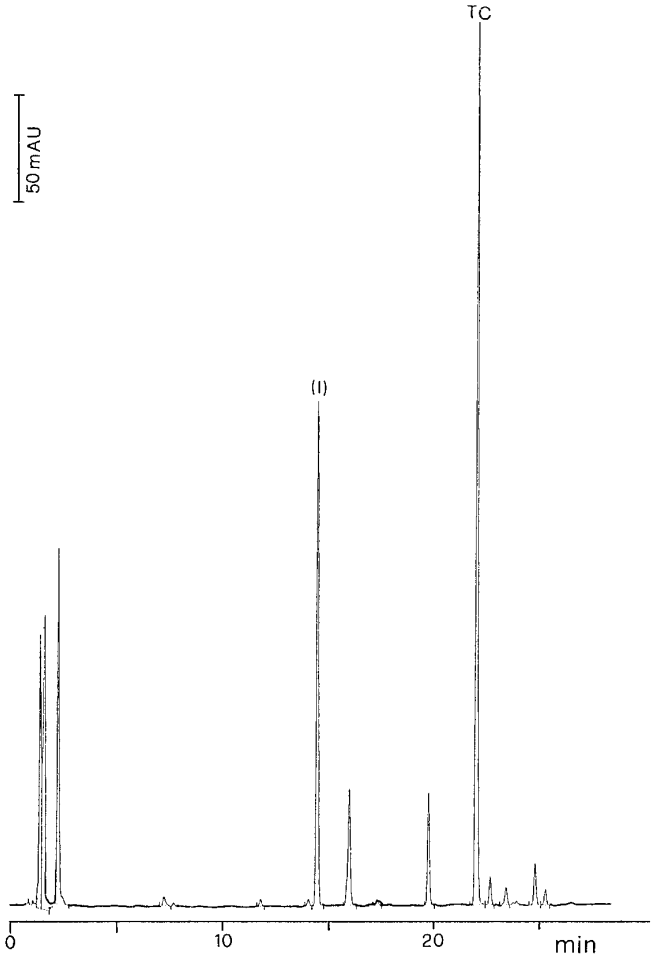

Fig. 2. Chromatogram obtained at 230 nm for the sample of toothpaste 5 (see Tab. III).

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