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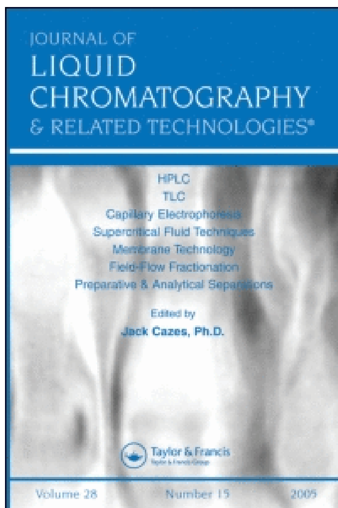


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# Simultaneous Determination of Antioxidants and Preservatives in Cosmetics and Pharmaceutical Preparations by Reversed-Phase HPLC

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# SIMULTANEOUS DETERMINATION OF ANTIOXIDANTS AND PRESERVATIVES IN COSMETICS AND PHARMACEUTICAL PREPARATIONS BY REVERSED-PHASE HPLC

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## ABSTRACT

A high performance liquid chromatographic method for the simultaneous determination of multiple additives in o/w cosmetic and pharmaceutical formulations was developed by using a RP-8 Select B column, a linear gradient elution and UV detection. A very simple extraction procedure was required. The separation obtained for nine antioxidants and seven preservatives was very good under the chromatographic conditions used. Their analysis was carried out in commercial samples and satisfactory results were obtained both for the recovery and the coefficient of variation.



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## INTRODUCTION

Many chemicals are permitted as additives to protect food, pharmaceuticals, cosmetics, drugs and other materials from the effects of oxidation and/or bacterial contamination. Antioxidants are added to products containing fats or oils to prevent rancidification. They can be naturally occurring compounds, especially present in the vegetable products, or synthetic molecules based on phenolic structures with varying degrees of hydroxylation and side-chain substitutions.

Antioxidants are molecules that react readily with oxygen and, as a result, act as inhibitors being oxidized themselves. They can be present single or as combinations, and are permitted at a maximum concentration range from 0.01 to 0.3%. It has been demonstrated that some compounds, like ascorbyl palmitate may act as synergists: either it enhances the effect of a phenolic antioxidant or it forms complexes with traces of copper and iron, and hence deactivates their prooxidant effect.

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Preservatives, particularly esters of 4-hydroxybenzoic acid, commonly known as parabens, are widely added to food., drugs and cosmetics[1]. Because of the critical role of antioxidants and preservatives in the improvement of shelf-life of a product, various methods of analysis have been developed for their quantitative determination including TLC,[2,3] GC,[4,5] HPLC,[6-12] and, recently, capillary electrophoresis.[12]

We describe here a simple analytical procedure for the simultaneous determination of 16 additives, 9 antioxidants and 7 preservatives, in o/w cosmetic or pharmaceutical formulations, which involves a one-step extraction or a dilution of the sample and analysis by reversed phase HPLC.

## EXPERIMENTAL

### Standards and Reagents

All reagents used were of analytical-reagent grade and used without further purification. Acetonitrile and methanol was 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. Commercially available antioxidant and preservative agents used in this study are listed in Table 1.

**Table 1**

**List of the Antioxidants and Preservatives Studied**

| Chemical Name | Abbreviation | Supplier |
|---|---|---|
| n-propyl gallate | PG | Fluka |
| octyl gallate | OG | Fluka |
| dodecyl gallate | DG | Fluka |
| 3-tert-butyl-4-hydroxy anisole | BHA | Fluka |
| 2,5-di-tert-butyl-4-hydroxy toluene | BHT | Fluka |
| 2-tert-butyl hydroquinone | BHQ | Acros |
| 2,5-di-tert-butyl hydroquinone | DBHQ | Acros |
| ascorbyl palmitate | AP | Fluka |
| tocopheryl acetate | TA | Fluka |
| methyl-4-hydroxy benzoate | MP | Formenti |
| ethyl-4-hydroxy benzoate | EP | Formenti |
| propyl-4-hydroxy benzoate | PP | Formenti |
| benzyl-4-hydroxy benzoate | BeP | Formenti |
| butyl-4-hydroxy benzoate | BP | Formenti |
| sorbic acid | SA | Fluka |
| 2,4,4'-trichloro-2'-hydroxydiphenyl ether (triclosan) | TC | Ciba |

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### Apparatus

Chromatography was performed on a Varian 9012 liquid chromatograph equipped with two pumps, a 10 μL sample loop, a Hewlett Packard 1050 photodiode-array detector, and a personal computer Vectra HP 486.

The analytical column was of stainless-steel (250 mm x 4.0 mm I.D.) packed with 5 μm SelectB RP-8 (Merck,Darmstadt, Germany).

### HPLC Conditions

The composition of mobile phase was acetonitrile (A), methanol (B), water containing $10^{-3}$ M perchloric acid (C). The following conditions of linear gradient elution were used: t=0 , 35% A, 10% B, 55% C; t=30, 20% A, 65% B, 15% C; t=35, 70% A, 30% B.

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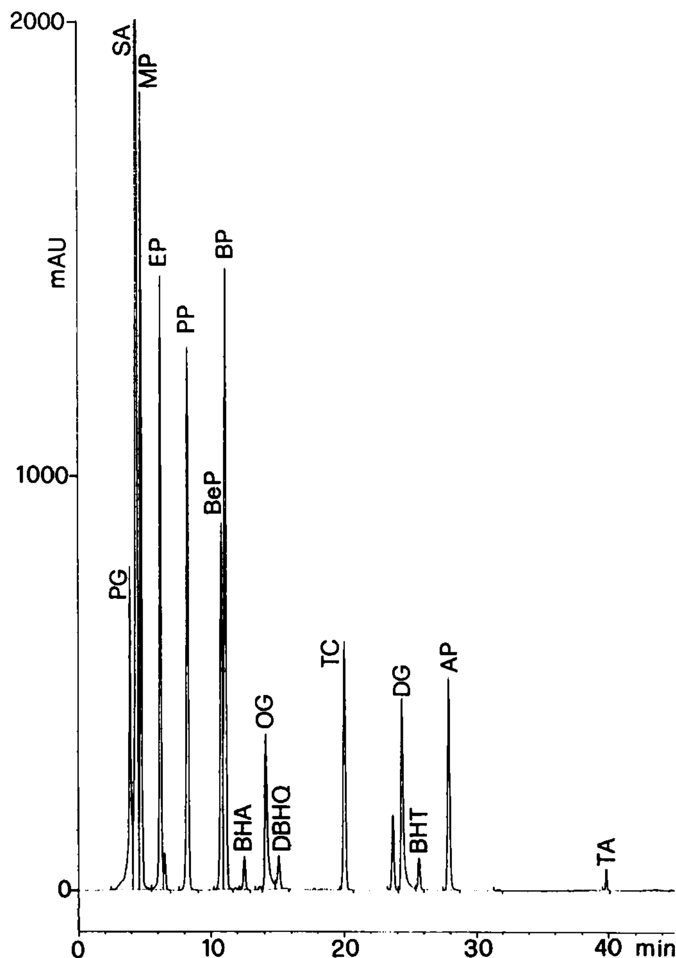


**Figure 1.** Typical chromatogram obtained at 254 nm for a standard solution containing 1 mg mL$^{-1}$ of all the antioxidants and preservatives studied with the exception of SA, (0.4 mg mL$^{-1}$).

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 1.0 mL·min$^{-1}$ and detection was performed at 254, 263, and 280 nm. The temperature of the column was maintained at 25 °C.

**Table 2**

**Analytical Parameters**

| Compound | Retention Time (min) | Detection Wavelength (nm) | Detection Limit (ng Injected) |
|---|---|---|---|
| PG | 4.02 | 280 | 10 |
| SA | 4.43 | 263 | 5 |
| MP | 4.91 | 254 | 10 |
| EP | 6.37 | 254 | 10 |
| BHQ | 6.71 | 280 | 20 |
| PP | 8.46 | 254 | 10 |
| BeP | 10.81 | 254 | 10 |
| BP | 11.03 | 254 | 10 |
| BHA | 12.74 | 280 | 15 |
| OG | 14.39 | 280 | 10 |
| DBHQ | 15.43 | 280 | 15 |
| TC | 19.98 | 280 | 10 |
| DG | 24.62 | 280 | 10 |
| BHT | 25.90 | 280 | 20 |
| AP | 28.25 | 254 | 30 |
| TA | 39.79 | 280 | 20 |

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### Calibration Standard Solutions

Stock solutions were prepared by dissolving the appropriate amounts of the standard additives in a solvent consisting of A and B in the ratio 7:3. A set of working standard solutions was prepared by diluting aliquots of the stock solutions to give concentrations ranging from 5.0 to 1000 $\mu g \cdot mL^{-1}$ for each compound studied. The calibration graphs were constructed by plotting the peak areas obtained at the optimum wavelength of detection versus the amounts (μg) injected.

### Sample Preparation

Eleven oil-rich products (five pharmaceutical specialties and six cosmetic products) were analyzed by the following procedure. About 2 g of the o/w emulsions was dissolved by sonication with 8 mL of a mixture methanol-acetonitrile (1:1, v/v), transferred to a 10 mL volumetric flask and brought to

**Table 3**

**Calibration Curves: Linear Regression of the Amount Injected (x) Versus the Peak Area (y)**

| Compound | Intercept[a] | Slope[a] | $R^2$ |
|---|---|---|---|
| PG | (-0.11 ± 0.02)E2 | (2.04 ± 0.01)E3 | 0.9996 |
| SA | (-2.22 ± 0.23)E2 | (10.33 ± 0.15)E3 | 0.9997 |
| MP | (0.10 ± 0.03)E2 | (1.64 ± 0.02)E3 | 0.9995 |
| EP | (0.71 ± 0.04)E2 | (1.52 ± 0.03)E3 | 0.9997 |
| BHQ | (-0.04 ± 0.01)E2 | (0.70 ± 0.02)E3 | 0.9998 |
| PP | (0.15 ± 0.02)E2 | (1.43 ± 0.02)E3 | 0.9987 |
| BeP | (-0.06 ± 0.02)E2 | (1.08 ± 0.04)E3 | 0.9996 |
| BP | (0.58 ± 0.09)E2 | (1.57 ± 0.11)E3 | 0.9998 |
| BHA | (0.02 ± 0.02)E2 | (0.69 ± 0.11)E3 | 0.9999 |
| OG | (-0.14 ± 0.05)E2 | (1.20 ± 0.01)E3 | 0.9984 |
| DBHQ | (0.07 ± 0.04)E2 | (0.74 ± 0.07)E3 | 0.9989 |
| TC | (0.11 ± 0.04)E2 | (1.33 ± 0.03)E3 | 0.9992 |
| DG | (-0.01 ± 0.02)E2 | (1.39 ± 0.01)E3 | 0.9993 |
| BHT | (0.09 ± 0.04)E2 | (0.55 ± 0.01)E3 | 0.9981 |
| AP | (0.01 ± 0.02)E2 | (0.095 ± 0.001)E3 | 0.9980 |
| TA | (-0.03 ± 0.01)E2 | (0.14 ± 0.01)E3 | 0.9999 |

[a] mean value ± standard deviation at 95% confidence interval (t=3.18; n=5).

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volume. After centrifugation at 3000 rpm for 5 min, the supernatant was filtered through a 0.45 μm filter. In the case of lipid fusions (lipsticks) about 2g of product was treated with 10 mL of N,N dimethylformamide, left under reflux for 10 min, and then filtered through a 0.45 μm filter.

## RESULTS AND DISCUSSION

Figure 1 shows the chromatogram of a standard solution of the 16 additives studied at the concentration of 1mg · $mL^{-1}$ with the exception of SA (0.4 mg · $mL^{-1}$). The chromatogram was recorded at 254 nm. In order to obtain maximun sensitivity the detection wavelength could be selected according to the values reported in Table 2, in dependence on the molar absorptivity of the compound concerned.

**Table 4**

**Analysis of Pharmaceutical Formulations***

| Formulation | Additive | Amount Declared (%) | Found (%) | C. V. |
|---|---|---|---|---|
| A | AP | 4.0 E-1 | 3.7 E-1 | 1.3 E-2 |
| | TA | 2.5 E-1 | 2.4 E-1 | 0.83 E-2 |
| | BHA | 1.5 E-1 | 1.3 E-1 | 0.46 E-2 |
| | MP | 2.0 E-1 | 1.9 E-1 | 0.51 E-2 |
| | PP | 2.0 E-1 | 1.9 E-1 | 0.46 E-2 |
| B | BHT | 0.15 E-1 | 0.16 E-1 | 0.43 E-3 |
| | BHA | 0.15 E-1 | 0.14 E-1 | 0.42 E-3 |
| | MP | 1.8 E-1 | 1.7 E-1 | 0.59 E-2 |
| | PP | 0.20 E-1 | 0.19 E-1 | 0.79 E-3 |
| C | BHT | 1.0 E-1 | 0.95 E-1 | 0.28 E-2 |
| | MP | 1.5 E-1 | 1.4 E-1 | 0.53 E-2 |
| | PP | 1.0 E-1 | 0.94 E-1 | 0.35 E-2 |
| D | BHA | 0.30 E-1 | 0.27 E-1 | 0.95 E-3 |
| | MP | 1.0 E-1 | 1.0 E-1 | 0.34 E-2 |
| | EP | 1.0 E-1 | 0.96 E-1 | 0.40 E-2 |
| E | BHT | 0.70 E-1 | 0.66 E-1 | 1.9 E-3 |
| | MP | 1.0 E-1 | 1.0 E-1 | 0.38 E-2 |
| | PP | 0.5 E-1 | 0.49 E-1 | 1.7 E-3 |

*mean of five determinations.

The chosen elution conditions allowed a very good separation of all the agents taken into account. Retention characteristics are summarized in Table 2. The retention times were reproducible under the experimental conditions used, the coefficient of variation (C.V.) ranging from 1.7 to 2.4 for within-day and from 2.6 to 4.1% for between-day studies.

The photodiode-array detector allowed the estimation of the peak purity factors. These values were calculated over the range 190-600nm and resulted > 99.5% for all the agents studied, thus confirming the good resolution achieved.

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**Table 5**

**Analysis of Cosmetic Formulations***

| Formulations | Additive | Found (%) | C.V. |
|---|---|---|---|
| F | BHA | 1.1 E-1 | 0.54 E-2 |
| | BHT | 1.0 E-1 | 0.49 E-2 |
| G | BHA | 0.45 E-1 | 0.19 E-2 |
| | BHT | 0.10 E-1 | 0.45 E-3 |
| | MP | 1.5 E-1 | 0.70 E-2 |
| | PP | 1.5 E-1 | 0.79 E-2 |
| H | BHA | 0.25 E-1 | 1.2 E-3 |
| | TA | 5.0 E-1 | 2.3 E-2 |
| | MP | 0.7 E-1 | 0.29 E-2 |
| | PP | 0.5 E-1 | 0.25 E-2 |
| I | BHA | 0.15 E1 | 0.72 E-3 |
| | TA | 0.5 E-1 | 2.5 E-3 |
| | MP | 1.0 E-1 | 0.39 E-2 |
| | PP | 0.5 E-1 | 2.0 E-3 |
| L | TA | 1.2 E-1 | 0.54 E-2 |
| | EP | 0.50 E-1 | 2.0 E-3 |
| | MP | 1.0 E-1 | 0.43 E-2 |
| | PP | 0.5 E-1 | 1.8 E-3 |
| M | TA | 1.0 E-1 | 0.49 E-2 |
| | TC | 3.0 E-1 | 1.5 E-2 |

* mean of five determinations.

The calibration graphs were constructed from five consecutive injections over the covered range of concentration, as indicated in the experimental section. The least square regression fit showed good linearity, passing through the origin. The data obtained for the calibration lines are shown in Table 3. The detection limits, calculated as a signal-to-noise ratio of 3:1, are reported in Table 2.

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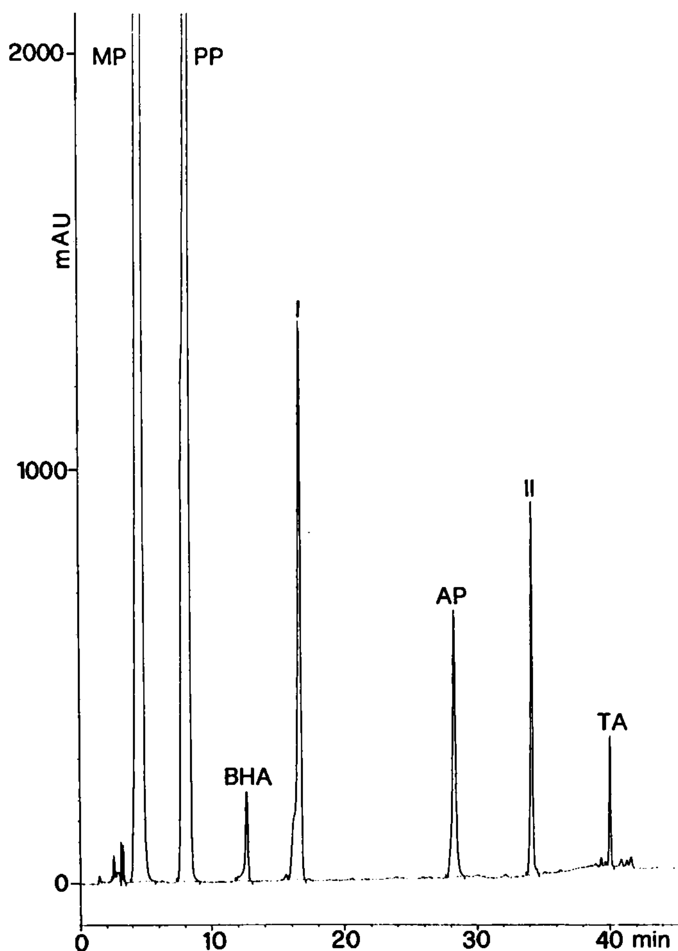


**Figure 2.** Chromatogram obtained at 254 nm for the pharmaceutical formulation A (see Table 4). Peak I and II correspond to dexamethasone valerate and pyridoxine tripalmitate, respectively.

Recovery tests were carried out to evaluate the reproducibility and accuracy of the proposed method. An oil-water emulsion and a lipstick, prepared in our laboratory so that their composition was similar to that of the formulations found on the market, were spiked with 0.05% (w/w) of all the

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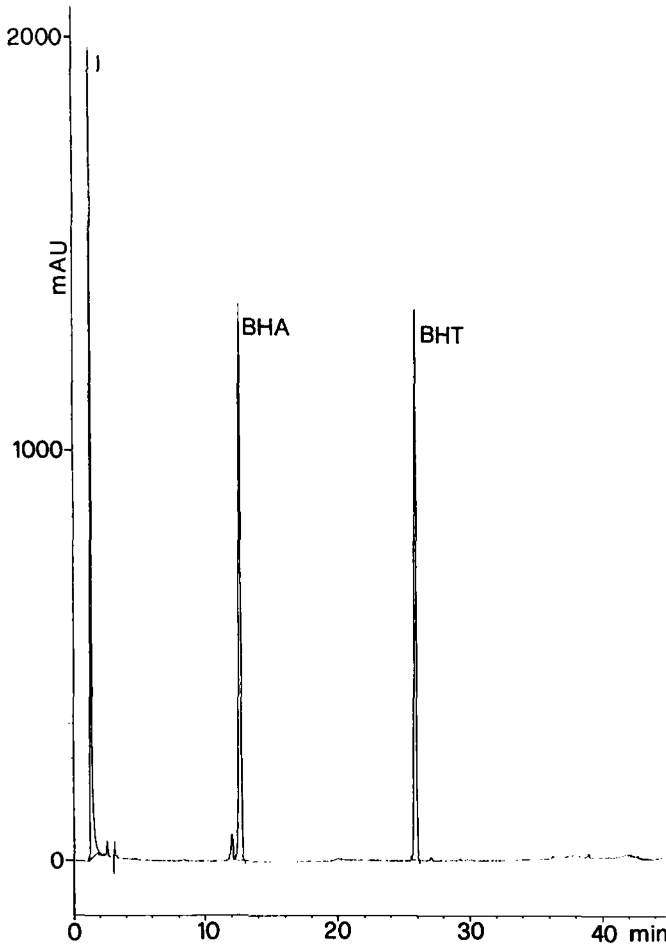


**Figure 3**. Chromatogram obtained at 280 nm for the lipstick E (see Table 5). Peak I corresponds to the dye C.I.15850.

antioxidants and preservatives studied and subjected to the described analytical procedure. The mean recoveries (n = 4) for all the compounds ranged from 94.5 to 99.6% with a C.V. < 3.7%. Therefore good recovery and precision were observed.

The content of the agents under study in 11 commercially available pharmaceutical and cosmetic formulations was determined in triplicate by using the proposed method. The samples contained combinations of the antioxidants and preservatives which were identified by comparing the retention times and the purity values of the peaks observed, with those obtained from the standard solutions. The assay results are shown in Tables 4 and 5. The quantities found for the pharmaceutical formulations were in conformity with the values claimed by the manufacturer. Typical chromatoghrams (for the cream A and the lipstick F) are shown in Figures 2 and 3. In Figure 2 the peaks named I and II correspond to the active principles dexamethasone valerate and the additive pyridoxine tripalmitate, respectively. In Figure 3 the peak named I correspond to a water-soluble dye (C.I. 15850) present in the composition of the lipstick, which is practically unretained by the column. From both chromatograms we can also affirm that interferences from the excipients were not observed.

The analytical results obtained lead to the conclusion that the developed method can be successfully adopted for an accurate determination of the sixteen additives considered in oil-rich cosmetics and pharmaceuticals.

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