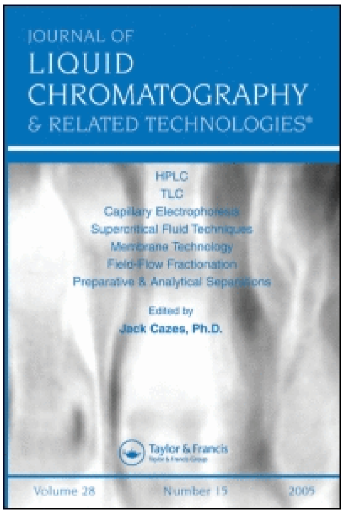

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HPLC DETERMINATION OF CICLOPIROX, OCTOPIROX, AND PYRITHIONES IN PHARMACEUTICALS AND ANTIDANDRUFF PREPARATIONS

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ABSTRACT

A simple and rapid HPLC method for the determination of ciclopirox, octopirox, and pyrithiones in pharmaceutical or antidandruff formulations has been developed. HPLC was carried out on a Purospher® RP-18 endcapped column using acetonitrile-water containing 0.02 M ortophosphoric acid and 0.5 mM EDTA disodium salt, (68:32, v/v) as the mobile phase and UV detection at 305 or 340 nm. The extraction procedure has been validated analysing samples spiked with known amounts of the active principles. The recoveries were greater than 94% and the reproducibility was within 3%.

INTRODUCTION

Some N-hydroxypyridone compounds are employed as antiseborrheic agents in cosmetic products and as antimycotics in pharmaceuticals. Ciclopirox olamine (I), the 2-aminoethanol salt of 6-ciclohexyl-1-hydroxy-4-methyl-2-pyridone, is a broad-spectrum antifungal drug which is present in preparations for topical use at a concentration of 1%. Octopirox (II), the monoethanolamine salt of 1-hydroxy-4-methyl-6-(2,4,4-trimethylpentyl)-2-pyridone, is a preserva-tive mentioned in the Italian law n. 713 (10/11/1986) and subsequent adjournments, issued in compliance with the EEC Directive concerning cosmetic products, which is permitted at a maximum concentration of 1% for those products which are rinsed off after use and 0.5% for the other products. However, octopirox is normally present at concentrations between 0.5 and 1.0% in the rinse-off hair products, between 0.05 and 0.10% in the leave-on products, and can be used at concentrations greater than 1.0% in the antidandruff formulations.

Zinc pyrithione (IV) the zinc complex of 2-mercaptopyridine-1-oxide, is one of the most effective antidandruff agents, which finds extensive use in shampoos, conditioners, rinses, and hair dressings. It shows bactericidal and fungicidal activities and it is reported in the above cited law for use in the products which must be rinsed off, with a maximum allowed concentration of 0.5%, but it is forbidden in products for oral hygiene. Since the maximum limit can be overcome in the antidandruff formulations, shampoos are normally formulated with one or two percent of IV, while the other products typically contain lower concentrations. IV is substantially insoluble in water and is present in aqueous based products as a dispersion of fine solid particles; related soluble compounds must be absent. One of them is the sodium salt of 2-mercaptopyridine-1-oxide (III) which has been used in the past as antidandruff agent, but at present it is forbidden in the cosmetic field as stated by the EEC legislation.

The analytical methods reported in the literature for the determination of I-IV are essentially based on TLC,[1-3] spectrophotometry,[4,5] and HPLC.[6-11]

After separation on silica gel plates, ferrous sulfate is used as a selective reagent to visualise the spots, which in turn are quantitated by means of spectrodensitometry. The easy formation of Fe(II) complexes with N-hydroxy-pyridone group is at the basis of the spectrophometric determination of I and II. The United States Pharmacopoeia reports this kind of determination for the purity assay of ciclopirox olamine.[12]

Zinc pyrithione too can be quantitated by transchelation to the Fe(II) complex, since the latter is much stronger than the former. Spectrophotometric methos obviously suffer from a lack of selectivity and can be applied only when the active principle is alone. Quantitative TLC is often not very precise or accurate.

Methods of determination employing HPLC have been somewhat hampered by the complexing properties of pyritiones and N-hydroxypyridone compounds which strongly interact with silica gel-based stationary phases or stainless-steel components of the liquid cromatograph. In the case of ciclopirox or octopirox the problems have been overcome by blocking the weak acidic N-hydroxyl group ($pK_a \simeq 7$) by means of an alkylation reaction.[11] The resulting 1-alkyloxypyridones show a regular chromatographic behaviour, i.e. without irreversible adsorption on the column or peak tailing.

In the case of pyrithiones the HPLC analysis has been successfully performed by a transchelation of the active principle to the Cu(II) complex and subsequent extraction in methylene chloride.[8-10] Since the cupric complex is extremely strong, a quantitative displayment is easily achieved.

In this paper we propose the use of a novel stationary phase for reversed-phase chromatography (Purospher® RP-18), which has been submitted to a multi-step chemical modification and deactivation of the surface in order to minimise sylanol groups and heavy metals cations content and, therefore, the possibility of chelate complexes formation.

EXPERIMENTAL

Standards and Reagents

Standards II, III, and IV were purchased from Keuringsdienst van Waren (Enschede, The Netherlands), standard I was kindly supplied by Laboratorio Bruschettini (Genova, Italy). All reagents used were of analytical-reagent grade and used without further purification. Acetonitrile and methanol were of HPLC grade. Water was deionized and doubly distilled from glass apparatus.

All solvents and solutions for HPLC analyses 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 Varian 9012 liquid chromatograph equipped with an external Rheodyne injector valve, fitted with a 10 µL sample loop, and a Hewlett Packard 1050 photodiode-array detector. HP processed the chromatographic data with a personal computer Vectra HP 486, utilizing software developed. The analytical column was of stainless-steel (250 mm x 4.0 mm I.D.) packed with 5 µm Purospher® RP-18 endcapped (Merck, Darmstadt, Germany).

HPLC conditions

The conditions used for the separation were: mobile phase, acetonitrile-water containing 0.02 M ortophosphoric acid and 0.5 mM EDTA disodium salt, (68:32, v/v); flow-rate, 1.0 $mL \cdot min^{-1}$; injection volume, 10 µL; ·column temperature, 25°C; detection wavelengths, 305 and 340 nm.

Calibration standard solutions

Stock solutions were prepared by dissolving the appropriate amounts of standards I-III in methanol-water, 1:1 (v/v). A set of working standard solutions was prepared by diluting aliquots of the stock solutions to give concentrations ranging from 0.001 to 0.1 $mg \cdot mL^{-1}$·. Due to the insolubility of zinc pyrithione in the water-methanol solution, the stock and working solutions was prepared in methanol-water containing 0.02 M acetic acid and 5mM EDTA disodium salt (80:20, v/v). The range of concentration analyzed was 0.002 to 0.2 $mg \cdot mL^{-1}$. The calibration graphs were constructed by plotting the peak areas obtained at the optimum wavelength of detection versus the amounts (µg) injected.

Extraction Procedure

Since IV is present in shampoos as a dispersion of solid particles in a viscous liquid matrix, the samples were shaken on a mechanical shaker for 20 min prior to analysis to ensure homogeneity. One gram of the sample was then accurately weighed into a 100 mL volumetric flask and added with the same solvent (S1) used for the preparation of the standard solutions. The dispersion was submitted to ultrasonic treatment for 10 min, centrigufed, and filtered through a nylon filter (0.45 µm) before injecting into the liquid chromatograph. A similar extraction was performed for the samples containing I-III, except for the solvent, which was a mixture methanol-water, 70:30 (v/v) (S2).

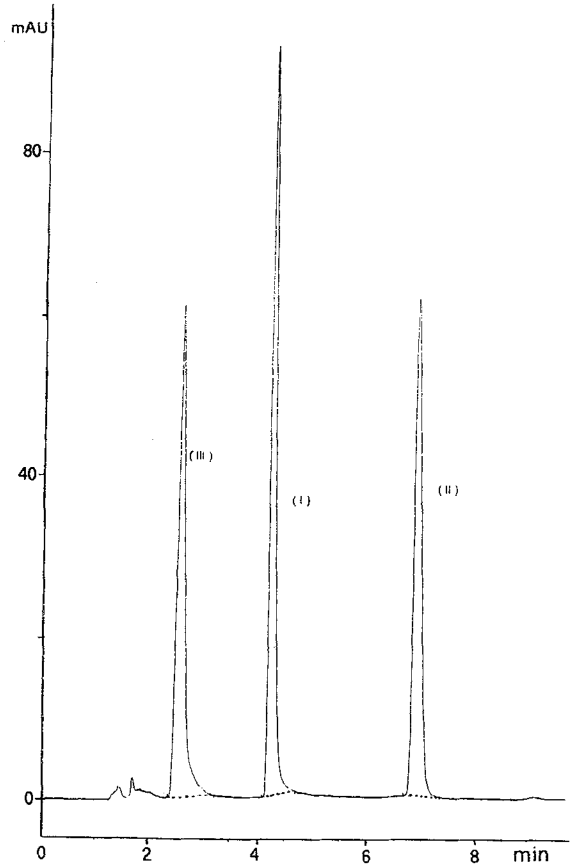

Figure 1. Typical chromatogram obtained at 305 nm for a standard solution containing 50 μg mL^{-1} of compounds I, II, and III.

RESULTS AND DISCUSSION

Figure 1 shows the chromatogram, recorded at 305 nm, of a standard solution containing compounds I-III at the concentrations indicated in the legend. The proposed stationary phase and the chosen elution conditions allowed a satisfactory simmetry of the peaks (the asimmetry factors are reported in Table 1) without appreciable tailing. Obviously sodium and zinc pirythiones eluted with the same retention time.

Table 1

Retention Parameters for Compounds I-IV*

Compound	Retention Volume (mL)	Capacity Factor	Peak Asymmetry Factor
I	4.25 (0.04)	3.25 (0.04)	0.81 (0.03)
II	6.90 (0.06)	5.90 (0.07)	0.88 (0.04)
III, IV	2.60 (0.03)	5.23 (0.06)	1.13 (0.05)

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

Table 2

Recovery Studies*

Sample	Active Principle	Amount Added (%, w/w)	Recovery
Shampoo 1	II	1.0	98.6 (2.5)
Shampoo 2	II	0.4	97.5 (2.8)
Hair cream	II	0.1	99.2 (3.0)
Cream 1	I	1.0	98.4 (2.2)
Cream 2	I	1.0	97.4 (2.9)
Lotion	I	1.0	98.8 (2.5)
Shampoo 1	III	0.5	96.9 (2.5)
Shampoo 2	III	0.2	93.9 (3.1)
Shampoo 3	III	0.08	94.1 (2.9)
Shampoo 1	IV	0.5	98.2 (3.0)
Shampoo2	IV	1.1	97.2 (2.6)
Shampoo 3	IV	0.9	98.7 (2.8)

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* Mean of five determinations, SD in parentheses.

The detection wavelength of 305 nm was chosen since this value permitted the simultaneous analysis of the compounds investigated with a good sensitivity. In case the active principles are to be determined alone, the wavelength can be fixed at 305 nm to obtain the maximum sensitivity for I and II, and at 340 nm for III and IV.

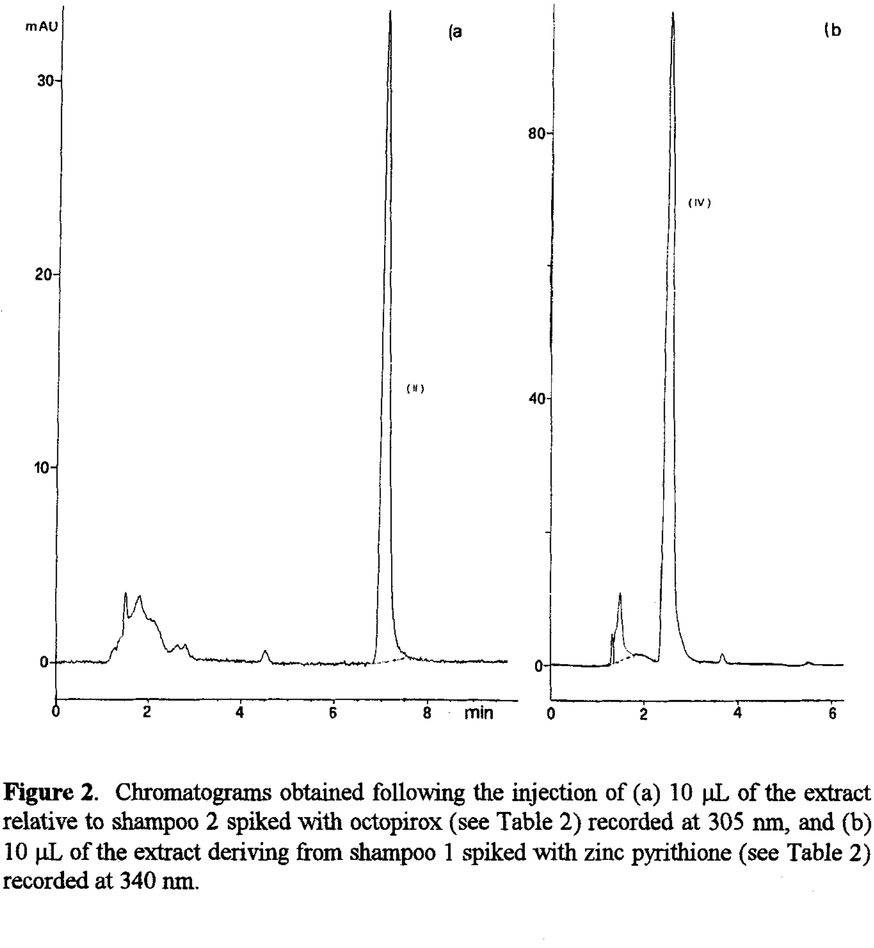

Figure 2. Chromatograms obtained following the injection of (a) 10 μL of the extract relative to shampoo 2 spiked with octopirox (see Table 2) recorded at 305 nm, and (b) 10 μL of the extract deriving from shampoo 1 spiked with zinc pyrithione (see Table 2) recorded at 340 nm.

Retention characteristics are summarized in Table 1. Capacity factors were reproducible under the experimental conditions used, the coefficient of variation (C.V.) ranging from 0.7 to 1.1% for within-day and from 1.0 to 1.6 % for between-day studies, respectively.

The calibration graphs were constructed from five consecutive injections. The least square regression fit showed good linearity ($R^2 > 0.9998$) over the range of concentrations reported in the experimental section. The limit of detection, defined as the concentration giving a signal-to-noise ratio of 3 at the optimum detection wavelength, resulted 3 ng injected for each compound.

The photodiode-array detector allowed the estimation of the peak purity, which was calculated over the range 220-400 nm for compounds I and II, and 300-400 nm for III and IV because of the low capacity factor shown by pyrithiones. The use of wavelengths in the near UV reduces to a minimum the possible interferences by the other components of the cosmetic formulations, and therefore a more definitive confirmation of the identity of the active principle can be achieved.

To validate the proposed extraction procedure of I-IV from pharmaceuticals or cosmetics, some commercial formulations, known not to contain the active principles under investigation and previously examined for confirming the absence of any peaks at the retention volumes reported in Table 1, were spiked with weighed amounts of I-IV and analysed according to the present method. The recoveries obtained, as well as the standard deviations, were good, as shown in Table 2. Figures 2a and 2b show the chromatograms obtained for two of these samples, namely shampoo 2 and shampoo 1 spiked with octopirox and zinc pyrithione, respectively.

The method was further evaluated by analysing pharmaceutical and cosmetic products, commercially available on the Italian market. Replicate analyses (n=5) were carried out and the results obtained were in agreement with the nominal content of the analytes, when this information was claimed by the manufacturer. In all the shampoos tested, sodium pyrithione was not found, while the average percentages of zinc pyrithione and octopirox were 1.1 and 0.8, respectively.

Due to the rapidity and good recovery of the sample processing, the HPLC procedure here described for determining compounds I-IV is a valuable tool for quality control studies, especially to verify the compliance of antidandruff formulations with the current legislation. Obviously, when pyrithiones are to be analysed, the search for the sodium salt must be performed firstly, and this can be easily accomplished by using the extracting solvent S2 for the sample processing.

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