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Note

Determination of quinine in hair preparations by reversed-phase high-performance liquid chromatography

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Quinine salts (sulphates, bisulphates, hydrochlorides, etc.) are widely used in cosmetics for their rubefacient and bacteriostatic action. They can be common constituents of hypoallergic cosmetic formulations, in particular antidandruff lotions and sunscreen preparations. As the individual human sensitivies to a cosmetic preparation are quite different, the choice of the ingredients during formulation studies, especially of hypoallergic cosmetics, must be done in such a way as to avoid substances which can produce contact dermatitis in susceptible subjects.

On the market there are many hair preparations designed for the treatment of baldness, dandruff, greasy or non-greasy hair, which include in the formulation quinine salts as germicidal bacteriostatic agents.

Various analtyical techniques have been proposed for the determination of quinine, but most possess some drawbacks when applied to cosmetic preparations. Colorimetric methods[1] lack the necessary specificity while spectrophotometric methods[2], although satisfactory from this point of view, are limited by the time required for sample preparation. Thin-layer chromatography[3] has been used for the determination of quinine, either by scanning the plates *in situ* after spraying with suitable reagents or by cutting off the spots followed by detection by fluorimetry or spectrophotometry. Gas–liquid chromatography[4] shows high selectivity and sensitivity but the extraction steps involved are time-consuming and it is affected by thermal decomposition. Consequently, most recently published work on the analysis of quinine has concentrated upon the use of high-performance liquid chromatography (HPLC) employing mainly columns packed with silica[5,6]. However, this packing material is rapidly inactivated by the excipients which are commonly present in cosmetic preparations unless the sample is subject to laborious extraction procedures. HPLC analyses of quinine in biological samples and pharmaceuticals have in general been carried out using reversed-phase systems[7,8].

The aim of the present study was to develop a simple assay for quinine in hair preparations using reversed-phase HPLC which meets the following requirements: reproducibility, rapidity of sample preparation, prolonged column life. The need for such a method arises from the application of European Economic Community Instruction No. 76/768 (enclosure III, first part) which states that the quinine base con-

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tent in cosmetic products formulated for hair hygiene cannot exceed the following limits: 0.5% in shampoos and 0.2% in hair lotions.

EXPERIMENTAL

Reagents

Anhydrous quinine base was supplied by Carlo Erba (Milan, Italy). Its purity was confirmed by HPLC on an ODS column after dissolving the drug in methanol. The quinine solution should be kept refrigerated at 0°C in the dark. Methanol and acetonitrile were of special HPLC grade. The water used was deionized and doubly distilled in glass. Phosphoric acid, potassium dihydrogen phosphate and tetramethylammonium bromide were all of laboratory reagent grade and were used without further purification. All solutions and solvents were filtered through a Millipore filter, pore size 0.45 μm (Millipore, Bedford, MA, U.S.A.).

Apparatus

The instruments used were a Varian Model 5000 liquid chromatograph equipped with a variable-wavelength UV detector (Varichrom UV 50), a Valco AH60 injection valve and a Varian Model 9176 recorder (Varian, Zug, Switzerland). The analytical column was 250 × 4.6 mm I.D. ODS-coated silica (Whatman, Partisil 10 ODS). Peak areas were determined by electronic integration (Varian Model CDS-111).

HPLC conditions

The chromatographic conditions were as follows: mobile phase, 0.1 *M* phosphoric acid–0.1 *M* potassium dihydrogen phosphate–0.1 *M* tetramethylammonium bromide–water–acetonitrile, pH 2.4 (10:50:100:340:88 v/v) (this pH was higher than the limit value (2.2) which is recommended for the packing performance); flow-rate, 1 ml/min; pressure, 900 p.s.i.; column temperature, 25°C; injection volume, 10 μl; detector wavelength, 332 nm; detector sensitivity, 0.16 a.u.f.s.

Preparation of standard quinine solution

A standard solution was made by dissolving 10 mg of pure substance in 100 ml of methanol. This solution was then chromatographed using the HPLC conditions described above.

Preparation of sample

A quantity of the sample equivalent to approximately 10 mg of anhydrous quinine base was weighed into a 100-ml volumetric flask and 20 ml of methanol were added. The flask was sonicated for 30 min to facilitate solubilization of the quinine, and then the solution was made up to volume. An aliquot of this solution was centrifuged, filtered through a Millipore filter and kept for HPLC analysis.

RESULTS AND DISCUSSION

Under the chromatographic conditions used the retention time of quinine was

TABLE I

PRECISION OF THE HPLC METHOD

Amount injected (μg)	*n*	*Coefficient of variation (%)*
0.2	10	1.38
0.4	10	1.36
0.6	10	1.40
0.8	10	1.38

TABLE II

RECOVERY STUDY

Each value is the mean from five determinations.

Cosmetic	*Theoretical content (%, w/w)*	*Recovery (%)*
Shampoo A	0.1	98
Shampoo B	0.05	99
Lotion C	0.1	97
Lotion D	0.05	98

TABLE III

HPLC ASSAY OF QUININE IN HAIR COSMETIC PRODUCTS

Each value is the mean from five determinations.

Cosmetic	*Actual content (%, w/w)*	*Recovery (%, w/w), mean ± S.D.*
Shampoo I	0.060	0.0582 ± 0.0005
Shampoo II	0.090	0.0934 ± 0.0007
Shampoo III	0.030	0.0317 ± 0.0002
Lotion IV	0.015	0.0151 ± 0.0001
Lotion V	0.012	0.0135 ± 0.0003

quite reproducible, even over several days, 7.9 min (coefficient of variation 0.98%). The detector wavelength was set at 332 nm to avoid possible interferences from other substances present in the extract, so obtaining a higher specificity. A fluorescence detector was not utilized because the relatively high amounts of quinine to determine did not require a greater sensitivity. The calibration graph obtained was linear up to 1 μg quinine injected. The detection limit was approximately 5 ng, calculated on a response of twice the noise level. The precision of the HPLC method was very satisfactory as shown by the data of Table I.

Recovery experiments were carried out by adding known amounts of quinine to four commercial products not containing quinine. The results are given in Table II

and indicate that quinine is quantitatively extracted by the procedure described. The applicability of the proposed technique for the assay of quinine in cosmetic hair preparations is demonstrated by the data in Table III.

In conclusion, the proposed HPLC method is suitable for the analysis of hair preparations containing quinine and compares favourably with the previous methods with regard to speed and precision.

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