Journal of Chromatography, 466 (1989) 433–437
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 21 197

Note

Determination of 4-*tert.*-butylphenol and 4-*tert.*-butylcatechol in cosmetic products by reversed-phase high-performance liquid chromatography

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(Received December 12th, 1988)

The commercial cosmetic creams producing depigmentation of the skin can contain active principles at levels of 1–2%. In conformity with the Directive of the European Communities Commission 87/137 EEC, Italy has issued departmental order 24.11.87 n. 53 (Annex II) that states which skin-lighteners can be used and their permitted levels.

We have started a study on the identification and determination of depigmenters in cosmetic products to test their compliance with EEC regulations, considering, first of all, hydroquinone and some of its ethers[1]. Among the substances excluded from use as depigmenters are 4-*tert.*-butylphenol (I) and 4-*tert.*-butylcatechol (II). To our knowledge, there are no reports in the literature concerning the determination of such phenolic compounds in cosmetic creams, whereas they have been determined by a variety of methods, especially for air- and water-pollution studies. The environment contains numerous mono- and dihydroxybenzenes derived from both natural and man-made sources. Of the developed methods, gas chromatography (GC) of the mono- and dihydroxy compounds[2,3] or of their derivatives[4] has been most widely used, especially for quantitative measurements at ppb (μg/l) levels, since the late 1950s, but these compounds have been determined also by thin-layer chromatography (TLC)[5,6] and spectrophotometry[7,8]. Advancements in the practice of high-performance liquid chromatography (HPLC) have revealed many possibilities for the determination of phenolic compounds by this technique. Several studies have been done using normal and reversed-phase HPLC with fluorescence, UV and electrochemical detection[9–12].

We report here a simple analytical method based on reversed-phase HPLC with isocratic elution for the determination of compounds I and II which is suitable for the routine analysis of vanishing creams in order to test their compliance with the EEC regulations.

0021-9673/89/$03.50

EXPERIMENTAL

Apparatus

A Model 5000 liquid chromatograph (Varian, Zug, Switzerland) equipped with a Valco AH 60 injection valve, a Varian Polychrom 9060 photodiode array detector and a Varian 4290 integrator was used. The analytical column was a 5-μm ODS Ultrasphere (150 mm × 4.6 mm I.D., Beckman).

Reagents

All reagents were of analytical reagent grade. Compounds I and II were obtained from Merck (Darmstadt, F.R.G.), 4-benzyloxyphenol, used as the internal standard (I.S.), from Fluka (Buchs, Switzerland). Acetonitrile was of solvent-for-liquid chromatography grade. All solvents and solutions for HPLC analysis were filtered through a Millipore filter, pore size 0.5 μm, and vacuum degassed by sonication before use.

Chromatographic conditions

The HPLC conditions were as follows: mobile phase, acetonitile-water containing acetic acid at 1% (40:60); flow-rate, 2 ml/min; injection volume, 10 μl; detection wavelength, 278 nm; detector sensitivity, 0.16 a.u.f.s.

Calibration graphs

Standard solutions were prepared by dissolving the appropriate amounts of compounds I and II in 100 ml of the mobile phase containing 0.1 mg/ml of I.S. These solutions and the set of solutions produced by serial dilutions were processed using the HPLC conditions described above. The ratios of the peak areas of I and II relative to the peak area of the I.S. were plotted *versus* the amounts injected.

Extraction from the cosmetic sample

About 2 g of a cosmetic cream were treated with 50 ml of methanol containing the I.S. at 0.1 mg/ml and the mixture heated at 50°C in a water-bath with shaking until sample dissolution was complete. After cooling and centrifugation, 10-μl aliquots of the solution were injected into the liquid chromatograph.

RESULTS AND DISCUSSION

Fig. 1 shows a typical chromatogram of a standard solution of compounds I, II and I.S. A good resolution was obtained. The most important parameters of the compounds investigated are summarized in Table I. Retention times were reproducible under the experimental conditions used. In an actual analysis, unknown peaks are identified using the retention times, but a more definite identification can be obtained by estimating the purity parameter (Varian) format values[13]. Table I also reports the response factors relative to the I.S., calculated from the weight ratio.

Calibration graphs were constructed from six consecutive injections. The equations obtained by linear regression analysis were $y = 1.203x - 0.011$ ($r^2 = 0.9998$) for compound I and $y = 1.470x + 0.075$ ($r^2 = 0.9994$) for II. Linearity was observed up to 10 μg injected for each compound. The reproducibility of the analysis was very

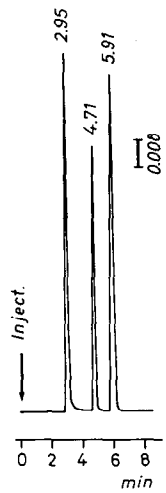

Fig. 1. Chromatogram of a standard mixture of compounds I, II and I.S.

TABLE I

RETENTION TIMES, PURITY PARAMETER FORMAT VALUES AND RELATIVE RESPONSES

Each value is the mean of six determinations.

Compound	*Retention time (min)*	*λ_m (239–311) (nm)*	*Relative response*
I	2.95	275.63	1.17
I.S.	4.71	279.70	1.00
II	5.91	273.54	1.59

TABLE II

RECOVERIES OF COMPOUNDS I AND II FROM VANISHING CREAMS

Each value is the mean of five determinations.

Cream	*Amount of I added (%, w/w)*	*Recover (%)*	*S.D.*	*Amount of II added (%, w/w)*	*Recovery (%)*	*S.D.*
A	1	98.0	1.2	1	100.0	1.8
	2	99.7	1.5	2	99.0	1.1
B	1	97.8	1.0	1	98.7	2.1
	2	99.0	1.9	2	99.4	1.8
C	1	98.4	1.9	1	101.2	1.5
D	1	98.8	1.5	1	97.9	1.7
	2	98.9	2.0	2	98.7	1.9

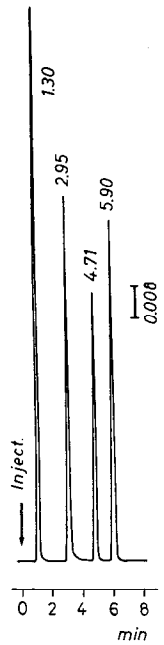

Fig. 2. Chromatogram obtained for a commercial cream containing hydroquinone and spiked with I and II at concentrations of 2% (w/w).

good, the average coefficient of variation being less than 1.7%. The detection limits, calculated as twice the noise level, were approximately 20 ng.

The applicability of the proposed method for the determination of compounds I and II in cosmetic samples was demonstrated by studying their analytical recoveries from four different creams bought on the market. The samples were tested for the presence of forbidden depigmenters. Once verified that the only skin-lightener present was hydroquinone, the creams were spiked with weighed amounts of compounds I and II and subjected to the extraction procedure described above. Five samples of the same tube of cream were analyzed. The recoveries obtained are shown in Table II. Good recoveries and precision are observed. Fig. 2 shows a chromatogram obtained for a sample of cream. The peak with a retention time of 1.3 min corresponds to hydroquinone. Peak identities were confirmed by determining the purity parameters which were in excellent agreement with the values reported in Table I.

In conclusion, the analytical method reported here for the determination of compounds I and II in cosmetic vanishing creams meets the following requirements: simplicity, rapidity of sample preparation and analysis, reproducibility and accuracy.

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