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Note

Determination of preservatives in cosmetic products by reversed-phase high-performance liquid chromatography. IV

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In the last few years we have been interested in the analytical routine control of cosmetics with the aim of verifying their adherence to the legislation of the European Economic Community (EEC). Single preservatives, but more often combinations of preservatives, are always used in commercial samples in order to prevent degradation and contamination of the complex formulation present in the product. In this regard, the original EEC Council Directive 76/768 has been amended many times, and at present there are about 60 preservatives that are definitively or provisionally permitted for use at specified maximum concentrations.

In previous papers[1–4] we reported some high-performance liquid chromatographic (HPLC) methods suitable for the rapid identification and quantification of groups of related preservatives. In one of them[3], describing the separation of phenolic and/or halogenated preservatives, we were unable to separate triclosan from triclocarban under the chromatographic conditions used and the problem was solved by carrying out a further extraction from the cosmetic sample in order to separate the neutral (such as triclocarban) from the acidic (such as triclosan) compounds. We have now developed a reversed-phase HPLC method that allows the complete separation of 22 preservatives, including triclosan and triclocarban, with optimization of the mobile phase composition. The preservatives considered are reported in Table I together with their maximum admissible concentrations according to EEC Council Directive 76/768 (Annex VI) and subsequent adjournments. All of them are listed in the current Directive except hexachlorophene, which has recently been deleted, and dichloro-*m*-xylenol, tetrabromocresol and halocarban, which are no longer included.

EXPERIMENTAL

Chemicals

All the preservatives were kindly supplied by the Keuringsdienst van Waren

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TABLE I

PRESERVATIVE MATERIALS

Compound No.	*EEC name*	*Common name*	*Maximum authorized concentration (%)*
1	2-Phenoxyethanol		1.0
2	*p*-Hydroxybenzoic acid, methyl ester (acid)	Methylparaben	0.4
3	1-Phenoxy-2-propanol		1.0
4	Glycerol *p*-chlorophenyl ether	Chlorphenesin	0.5
5	*p*-Hydroxybenzoic acid, ethyl ester (acid)	Ethylparaben	0.4
6	2,4-Dichlorobenzyl alcohol		0.15
7	*p*-Chloro-*m*-cresol		0.2
8	4-Chloro-3,5-dimethylphenol	*p*-Chloro-*m*-xylenol	0.5
9	4-Isopropyl-3-methylphenol		0.1
10	*p*-Hydroxybenzoic acid, *n*-butyl ester (acid)	Butylparaben	0.4
11	*o*-Phenylphenol (phenol)		0.2
12	*p*-Hydroxybenzoic acid, benzyl ester (acid)	Benzylparaben	0.1
13	Sorbic acid, isopropyl ester (acid)		0.6
14	2,4-Dichloro-3,5-dimethylphenol (I.S.)	Dichloro-*m*-xylenol	–
15	5,5′-Dichloro-2,2′-dihydroxydiphenyl-methane	Dichlorophene	0.2
16	2-Benzyl-4-chlorophenol	Chlorophene	0.2
17	2,4,4′-Trichloro-2′-hydroxydiphenyl ether	Triclosan	0.3
18	3,4,4′-Trichlorocarbanilide	Triclocarban	0.2
19	Tetrabromo-*o*-cresol		–
20	4,4′-Dichloro-3-trifluoromethyl-carbanilide	Halocarban	–
21	3,3′-Dibromo-5,5′-dichloro-2,2′dihydroxy-diphenylmethane	Bromochlorophene	0.1
22	2,2′-Dihydroxy-3,3′,5,5′,6,6′-hexachloro-diphenylmethane	Hexachlorophene	–

(Enschede, The Netherlands), and were used as received. All chemicals were of analytical-reagent grade. Water was deionized and doubly distilled in glass. Methanol was of special HPLC grade (Carlo Erba, Milan, Italy). 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

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 5-μm ODS Ultrasphere (250 × 4.6 mm I.D.) (Beckman).

Chromatographic conditions

The mobile phase was methanol–water containing 1% (v/v) acetic acid with the following linear gradient of methanol concentration: 0 min, 20%; 15 min, 30%; 25 min, 40%; 35 min, 60%; 40 min, 70%; 50 min, 80%; 60 min, 85%; and 65 min, 90%. The column temperature was 25°C, injection volume 10 μl, flow-rate 2.3 ml/min, detection wavelength 280 nm and chart speed 0.25 cm/min.

Calibration graphs

Stock solutions were prepared by dissolving weighed amounts of the preservatives in 100 ml of a solution of acetic acid (1%, v/v) in tetrahydrofuran (THF) containing 1 mg/ml of dichloro-*m*-xylenol as the internal standard (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 relative to that of the I.S. were plotted against the amounts of preservative injected.

Assay of preservatives in cosmetic samples

A test portion of *ca.* 1 g of sample was accurately weighed into a glass centrifuge tube with a screw-cap. After addition of 10 ml of the solution containing the I.S., the tube was closed and immersed for 15 min in an ultrasonic bath thermostated at 60°C to melt any lipid phase and to facilitate the extraction of the preservative into the organic phase. After cooling and centrifugation, the supernatant was diluted to volume (25 ml) with THF containing 1% acetic acid. Aliquots of 10 μl of the solution were submitted to HPLC analysis.

RESULTS AND DISCUSSION

Fig. 1 shows a typical chromatogram of a standard solution of the preservatives examined and the I.S., obtained by setting the detector at 280 nm. As can be seen, good

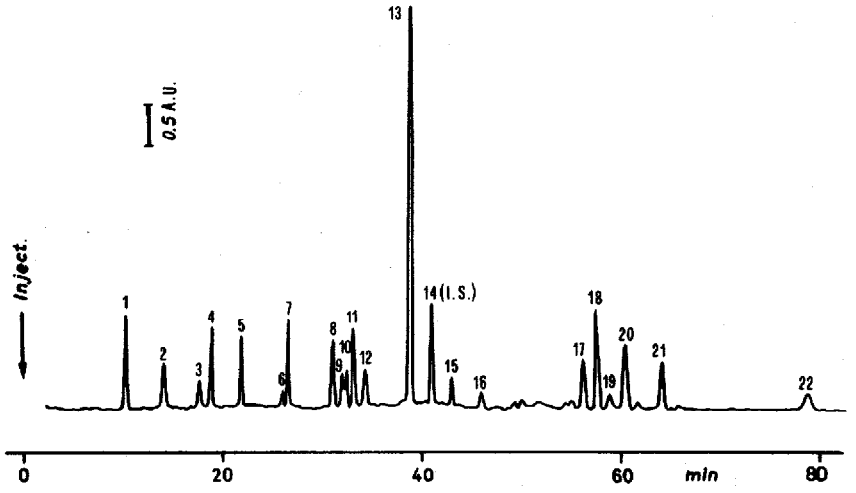

Fig. 1. Typical chromatogram of a solution containing 0.5 mg/ml of each preservative, recorded at 280 nm. Numbers on the peaks are compounds as listed in Table I.

resolution was obtained for all compounds except for slight overlapping of peaks 9 and 10. Table II reports the retention times, which were reproducible under the experimental conditions used, the detection limits (ng injected), calculated as the response three times the noise level, and the response factors relative to the I.S., calculated from the weight ratio. The photodiode-array detector allowed the purity parameter (Varian) format values[5] (λ_w), which were calculated over the wavelength range 234–311 nm, to be obtained. These values, which are shown in Table II, are useful in confirming peak identifications and in determining peak purities. When checking the compliance of a cosmetic product with the EEC legislation, a knowledge of this parameter is very helpful, particularly if the compound under investigation seems to be present at a concentration exceeding the maximum admissible level.

Calibration graphs were constructed from five consecutive injections and were linear for all the compounds considered over the range of concentrations used, *i.e.*, from 0.1 to 30 μg or from 0.02 to 6 μg injected, depending on the kind of preservative. The coefficients of linear regression ranged from 0.9979 to 0.9998 and the reproducibility was very good. In fact, if the calibration graphs were obtained on the same day, the average relative standard deviation (R.S.D.) was less than 2.0%. If the standard solutions were injected for 15 consecutive days, the variability of the assay was slightly greater, the average R.S.D. being about 3.0%.

TABLE II

RETENTION TIMES, RESPONSE FACTORS, DETECTION LIMITS AND PURITY FORMAT VALUES OF THE COMPOUNDS TESTED

Each value is the mean of five determinations.

Compound No.	*Retention time (min)*	*Relative response at 280 nm*	*Detection limit (ng injected) at 280 nm*	λ_w *(234–311)*
1	10.70	0.22	15	266.35
2	13.96	2.03	5	255.28
3	18.39	0.19	20	266.67
4	19.50	0.75	15	243.99
5	22.16	2.00	5	255.39
6	26.47	0.26	50	250.53
7	26.93	1.21	15	252.67
8	31.49	1.00	20	248.03
9	32.53	1.26	20	249.07
10	32.87	1.80	15	255.11
11	33.72	3.03	10	269.46
12	34.67	1.40	20	251.74
13	39.46	3.08	5	258.99
14 (I.S.)	41.35	1.00	20	253.79
15	42.54	1.52	20	256.71
16	43.62	1.12	30	254.08
17	57.20	1.43	15	246.45
18	58.45	3.80	5	262.66
19	59.57	0.37	60	248.43
20	61.31	3.60	5	263.64
21	65.35	0.87	20	261.39
22	79.90	0.91	40	250.28

TABLE III

RECOVERIES OF PRESERVATIVES IN COMMERCIAL PRODUCTS

Each value is the mean of five determinations.

Compound No.	*Amount added (%, w/w)*	*Day cream*			*Night cream*			*Shampoo*			*Body lotion*		
		Recovery (%)	*R.S.D. (intra-) (%)*	*R.S.D. (inter-) (%)*	*Recovery (%)*	*R.S.D. (intra-) (%)*	*R.S.D. (inter-) (%)*	*Recovery (%)*	*R.S.D. (intra-) (%)*	*R.S.D. (inter-) (%)*	*Recovery (%)*	*R.S.D. (intra-) (%)*	*R.S.D. (inter-) (%)*
1	1.00	101	1.9	2.6	100	1.7	2.9	100	1.5	1.8	96	1.9	2.1
2	0.40	96	2.1	3.2	94	1.6	2.4	98	1.8	2.9	99	1.7	2.5
3	1.00	100	1.6	1.8	96	1.5	1.9	99	1.7	2.3	96	1.1	1.4
4	0.50	98	1.4	1.9	100	1.0	1.4	98	0.9	1.0	96	1.6	2.2
5	0.40	97	2.1	2.7	94	1.5	2.0	96	2.0	2.6	96	2.1	2.8
6	0.15	97	1.5	2.2	95	1.9	2.9	93	1.2	1.7	100	1.1	1.4
7	0.20	95	1.8	2.3	96	2.0	2.8	95	2.3	3.1	94	1.5	2.1
8	0.50	94	1.8	2.7	95	1.6	2.4	96	1.2	1.9	98	1.8	2.7
9	0.10	98	2.0	2.9	93	1.9	2.5	100	1.0	1.5	97	1.5	2.0
10	0.40	100	1.6	2.2	98	2.0	3.0	98	1.6	2.7	94	1.0	1.3
11	0.20	101	1.5	2.3	99	1.1	1.7	96	1.5	2.1	98	1.2	1.8
12	0.10	92	1.9	2.7	97	2.2	3.0	94	1.8	2.9	93	1.4	2.6
13	0.60	100	1.4	2.0	99	1.9	2.6	98	0.9	1.5	96	2.1	3.0
15	0.20	93	1.7	2.6	98	1.3	2.1	97	1.1	1.9	98	2.0	3.0
16	0.20	94	1.9	2.8	96	1.8	2.9	95	2.2	3.0	99	1.0	1.7
17	0.30	96	1.1	1.8	93	2.1	3.2	93	1.8	2.7	94	1.9	2.2
18	0.20	97	1.7	2.6	94	2.0	3.0	94	1.8	2.6	93	1.9	3.0
19	0.30	98	1.5	2.2	96	2.2	3.1	93	1.6	2.9	96	2.0	2.9
20	0.30	99	1.9	2.8	95	1.5	2.1	98	1.5	2.6	100	1.7	2.2
21	0.10	96	1.0	1.9	92	2.1	3.2	97	1.8	2.5	95	1.5	2.2
22	0.10	95	1.9	2.8	94	1.6	2.2	100	1.7	2.5	98	1.8	2.8

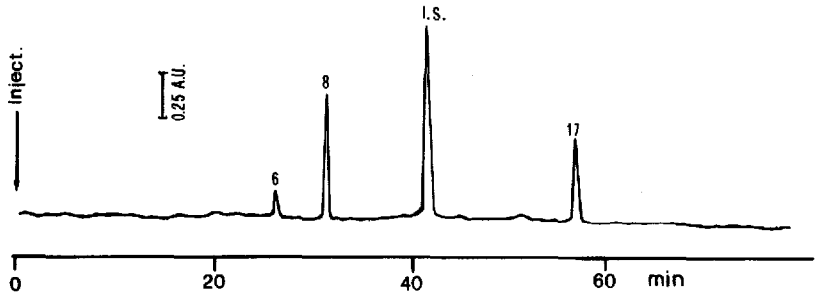

Fig. 2. Chromatogram obtained for a commercial day cream containing preservatives 6, 8 and 17.

The applicability of the proposed method for the determination of compounds 1–22 in cosmetic products was demonstrated by studying their recoveries from four different samples prepared in-house and spiked with the maximum amounts of the preservatives permitted by the EEC legislation. The recoveries obtained are shown in Table III, together with the R.S.D.s obtained either when the assays were performed on the same day (intra-assay R.S.D.) or over a period of 15 days (inter-assay R.S.D.). As the variability was almost the same, it can be inferred that the reproducibility of the method was very good even over a period of several days.

The HPLC procedure was applied to a variety of cosmetic products purchased from local outlets, and containing unknown preservatives. Fig. 2 shows a chromatogram obtained for a sample of day cream. Peak identities were confirmed by determining the purity parameters, which were in good agreement with the values calculated for the standard compounds and reported in Table II. The results of the analyses are given in Table IV. No cosmetic sample contained hexachlorophene and the levels of the preservative identified were in compliance with the EEC Directive.

To our knowledge, in the literature only the paper by Matissek[6] describes the separation and determination of a number of phenolic preservatives in cosmetic

TABLE IV

DETERMINATION OF PRESERVATIVES IN COMMERCIAL COSMETIC PRODUCTS

Each value is the mean of three determinations.

Cosmetic product	*Preservatives found and their percentages (in parentheses)*
Day cream	6(0.16) + 17(0.30) + 8(0.50)
Night cream	5(0.08) + 8(0.30) + 6(0.10) + one not identified
Shampoo	2(0.10) + 5(0.10) + 10(0.10) + 11(0.10)
Body lotion	1(0.30) + 9(0.10) + 13(0.42)
Deodorant	5(0.10) + 1(0.40) + 15(0.15)
Day cream	5(0.20) + 8(0.20) + 15(0.10) + one not identified
Hand cream	8(0.35) + 9(0.05) + 3(0.25)
Body emulsion	7(0.20) + 9(0.10) + 11(0.20)
Shampoo	2(0.20) + 1(0.40) + one not identified
Shampoo	2(0.10) + 5(0.05) + 10(0.05) + 1(0.40)
Night cream	18(0.21) + 15(0.20) + 21(0.10)

products as large as that considered here, using chromatographic techniques. Whereas gas chromatography allowed a good separation of twelve preservatives, the resolution obtained by HPLC on LiChrosorb RP-8 with isocratic elution was poor, as all the esters of *p*-hydroxybenzoic acid coelute and so do *o*-phenylphenol and *p*-chloro-*m*-xylenol.

In contrast, the chromatographic separation obtained here using an ODS column and gradient elution is very good. This fact, together with the simple extraction procedure and the good accuracy and precision, make the proposed method suitable both for rapid screening and for the quantitative determination of the 22 preservatives considered in cosmetic products.

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