Journal of Chromatography, 469 (1989) 317–328
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 21 337

DETERMINATION OF PRESERVATIVES IN COSMETIC PRODUCTS

II. HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC IDENTIFICATION

N. DE KRUIJF*, A. SCHOUTEN, M. A. H. RIJK and L. A. PRANOTO-SOETARDHI
TNO-CIVO Food Analysis Institute, Department of Toxicological Analysis, P.O. Box 360, 3700 AJ Zeist (The Netherlands)
(First received December 6th, 1988; revised manuscript received January 18th, 1989)

SUMMARY

A high-performance liquid chromatographic (HPLC) procedure is presented for the separation and identification of preservatives that are listed in the current EEC Council Directive on cosmetic products or have been permitted in the past. The method consists of an extraction of acidified cosmetics with methanol, and separation of the extracts by HPLC. Using two isocratic and two gradient reversed-phase HPLC systems, 47 preservatives were characterized by their retention times. The preservatives in three commercial cosmetic products were tentatively identified by the procedure described. The HPLC procedure is suitable for confirmation of the presence of preservatives in cosmetic products as indicated by a previously reported thin-layer chromatographic procedure. In general this method will permit the routine detection of preservatives in cosmetics in an approximate concentration of 0.01% (w/w).

INTRODUCTION

Preservatives are antimicrobial agents that are widely used in cosmetics to protect the health of the consumer, as well as to maintain the potency and stability of the product formulations. Combinations of two or more preservatives are often used to increase the ability of the system to withstand microbial contamination and to widen the range of microorganisms against which the cosmetic is protected[1–4].

The European Economic Community (EEC) Council Directive on cosmetics includes a positive list of preservatives for cosmetics. At present some 60 preservatives or groups of related preservatives are either definitively or provisionally permitted in cosmetics at specified maximum concentrations. Methods for the identification and quantification of these preservatives in cosmetic products are necessary for checking compliance with the EEC Directive.

A screening procedure based on a combination of thin-layer chromatography (TLC) and high-performance liquid chromatography (HPLC) was developed to identify the preservatives listed. In a previous paper a TLC procedure was presented that

0021-9673/89/$03.50

enables the preliminary identification of many of the preservatives mentioned in the current EEC Council Directive on cosmetic products or which have been permitted in the past[5]. This paper describes a HPLC procedure that is suitable for confirmation of the presence of preservatives in cosmetic samples as indicated by the TLC procedure.

The use of HPLC for the analysis of preservatives permitted by the EEC Council Directive on cosmetic products has been previously described[6–14]. Most of this literature is concerned with a narrow range of related preservatives, and none of these methods enables the simultaneous determination of a wide range of unrelated preservatives in cosmetics. The HPLC procedure presented here is suitable for the separation and identification of many of the preservatives currently permitted by the EEC Council Directive on cosmetic products. The method consists of a simple sample preparation procedure and the separation of the preservatives using two isocratic and two gradient reversed-phase HPLC systems.

EXPERIMENTAL

Apparatus

The HPLC system used was a SP 8100 ternary liquid chromatograph (Spectra-Physics, San Jose, CA, U.S.A.), equipped with an SP 8110 autosampler, an SP 8440 variable-wavelength UV detector and an SP 4100 computing integrator.

Reagents and materials

Methanol, acetonitrile, tetrahydrofuran (unstabilized) and water used for the HPLC mobile phases were HPLC grade.

All preservatives were commercial grade. In a previous publication[5] EEC names and synonyms for these preservatives were tabulated along with the maximum authorized concentrations as specified in the EEC Council Directive on cosmetic products.

All other chemicals and solvents employed were of reagent-grade quality and were used without further purification.

The cosmetic samples, a hand cream, a face powder and a night cream, were obtained from local outlets.

Preparation of samples and standards

A test portion of *ca.* 1 g of sample was weighed into a 50-ml glass tube fitted with a screw cap. After addition of 0.5 ml of 4 *M* formic acid and 9.5 ml of methanol, the tube was closed and vigorously shaken for 1 min. If required, the mixture was gently heated in a water-bath maintained at 60°C, to melt any lipid phase and to facilitate the extraction of preservatives into the methanol phase. The extract was stored overnight. If necessary the mixture was filtered using a disposable filter holder. A 2-ml aliquot of the clear sample solution was pipetted into a 5-ml sample vial.

Reference solutions, each containing 0.1 mg/ml of a mixture of methanol and 4 *M* formic acid (19:1), were prepared for all preservatives.

Chromatographic system 1

A 125 mm × 4.6 mm I.D. stainless-steel column slurry-packed with 5-μm Zorbax C_8 (DuPont, Wilmington, DE, U.S.A.) was used under ambient conditions.

Mobile phase A was prepared as follows: 1.13 g of disodium hydrogen phosphate dihydrate and 2.88 g of 85% orthophosphoric acid were mixed with 1 l water. Mobile phase B was acetonitrile. The gradient profile was as follows:

Initial	80% A	20% B
Linear, 15 min to	70% A	30% B
Linear, 10 min to	40% A	60% B
Isocratic, 10 min at	40% A	60% B
Return, 2 min to	80% A	20% B
Reequilibrate, 5 min at	80% A	20% B

The flow-rate was 2.0 ml/min. The UV detector was set at 280 nm with 0.04 a.u.f.s. The injection volume was 10 μl.

Chromatographic system 2

A 125 mm × 4.6 mm I.D. stainless-steel column slurry-packed with 5-μm Nucleosil 5 C_{18} (Macherey-Nagel, Düren, F.R.G.) was used under ambient conditions. The mobile phase was acetonitrile–methanol–tetrahydrofuran–water (5:2:1:12). The flow-rate was 1.5 ml/min. The UV detector was operated at 280 nm with 0.16 a.u.f.s. The injection volume was 10 μl.

Chromatographic system 3

A 125 mm × 4.6 mm I.D. stainless-steel column slurry-packed with 5-μm ODS Hypersil (Shandon Southern, Runcorn, U.K.) was used under ambient conditions. The mobile phase was acetate buffer–acetonitrile (9:1). The acetate buffer contained 6.35 g sodium acetate trihydrate and 20 ml 96% acetic acid per litre water. The flow-rate was 2.0 ml/min. The UV detector was set at 240 nm with 0.08 a.u.f.s. The injection volume was 10 μl.

Chromatographic system 4

A 125 mm × 4.6 mm I.D. stainless-steel column slurry-packed with 10-μm μBondapak C_{18} (Waters Assoc., Milford, MA, U.S.A.) was used under ambient conditions. Mobile phase A was prepared by dissolving 5.84 g sodium chloride and 1.01 g sodium heptanesulphonate in 1 l water. To this solution 10 ml of 96% acetic acid were added. Mobile phase B was prepared as follows: 5.84 g sodium chloride and 1.01 g sodium heptanesulphonate were dissolved in 100 ml water. To this solution 900 ml of methanol and 10 ml of 96% acetic acid were added. The gradient profile was as follows:

Initial	70% A	30% B
Isocratic, 4 min at	70% A	30% B
Linear, 8 min to	35% A	65% B
Isocratic, 6 min at	35% A	65% B
Linear, 4 min to	0% A	100% B
Isocratic, 3 min at	0% A	100% B
Return, 3 min to	70% A	30% B
Reequilibrate, 5 min at	70% A	30% B

The flow-rate was 2.5 ml/min. The UV detector was set at 264 nm with 0.16 a.u.f.s. The injection volume was 10 μl.

RESULTS AND DISCUSSION

All preservatives mentioned in the current EEC Council Directive on cosmetic products, except thiomersal, phenylmercury and germall II, for which no reference material was available, were examined by the procedure described. In addition, a number of preservatives permitted previously but now deleted from the Directive were included in the investigation[5].

Of the preservatives investigated, L-usnic acid, D-usnic acid, zinc pyrithione, germall 115, potassium metabisulphite and sodium iodate were found to be insoluble in methanol–4 *M* formic acid (19:1), while others were not determined properly by means of the HPLC methods. These preservatives were either eluted too early or too late from the chromatographic columns, or were not detectable by UV detection at the wavelengths applied. The HPLC procedure consisting of the four chromatographic systems described above permitted the characterization of 47 preservatives. The chromatographic behaviour of these preservatives is summarized in Table I. Some preservatives give a large major peak as well as one or more minor peaks, indicating that impurities were present in some of the preservative reference materials. In these cases only the major peak is listed in Table I. Kathon CG, however,

TABLE I

RETENTION TIMES FOR PRESERVATIVES CHROMATOGRAPHED ON FOUR HPLC SYSTEMS

System 1: column, Zorbax C_8 (5 μm), 125 mm × 4.6 mm I.D.; mobile phase, phosphate buffer–acetonitrile gradient; flow-rate, 2.0 ml/min; detection, UV at 280 nm. System 2: column, Nucleosil 5 C_{18} (5 μm), 125 mm × 4.6 mm I.D.; mobile phase, acetonitrile–methanol–tetrahydrofuran–water (5:2:1:12); flow-rate, 1.5 ml/min; detection, UV at 280 nm. System 3: column, ODS Hypersil (5 μm), 125 mm × 4.6 mm I.D.; mobile phase, acetate buffer–acetonitrile (9:1); flow-rate, 2.0 ml/min; detection, UV at 240 nm. System 4: column, μBondapak C_{18} (10 μm), 125 mm × 4.6 mm I.D.; mobile phase, methanol–water containing sodium heptanesulphonate, sodium chloride and acetic acid ion-pair gradient; flow-rate, 2.5 ml/min; detection, UV at 264 nm.

EEC No.[a]		*Preservative*	*Retention times (min)*			
I	*II*		*System 1*	*System 2*	*System 3*	*System 4*
2.13	2.41	Pyrithione disulphide	1.3	1.3	1.5	1.7
–	2.34	8-Hydroxyquinoline	1.5	1.4	–	2.1
1.12	1.12	*p*-Hydroxybenzoic acid	2.1	1.6	1.9	1.8
1.39	2.45	Kathon CG[b]	2.4/2.1	1.6	2.5/1.5	2.0/0.9
1.34	2.51	Benzyl alcohol	3.5	3.4	4.1	2.3
1.29	2.43	Phenoxyethanol	5.4	2.4	7.4	3.2
–	2.40	Sodium pyrithione	5.5	1.8	–	1.8
1.4	1.4	Sorbic acid	5.9	2.6	7.8	4.6
1.1	1.1	Benzoic acid	6.3	2.6	6.6	4.5
1.12	1.12	Methylparaben	7.3	2.9	10.0	5.3
1.3	1.3	Salicylic acid	7.9	1.4	2.6	4.3
1.13	2.4	Dehydroacetic acid	8.1	3.0	9.6	4.8
2.2	2.3	Chlorphenesin	8.2	3.0	21.0	7.1
1.20/2.7	2.18	Bronidox	8.9	3.6	7.4	2.8

TABLE I (*continued*)

EEC No.[a]		*Preservative*	*Retention times (min)*			
I	*II*		*System 1*	*System 2*	*System 3*	*System 4*
2.14	2.56	Phenoxypropanol	9.1	3.4	18.2	6.3
1.12	1.12	Ethylparaben	12.2	4.5	29.3	9.7
1.1	1.1	Methyl benzoate	16.5	6.2	–	10.2
1.22	2.24	2,4-Dichlorobenzyl alcohol	17.4	8.0	–	12.0
1.24	2.26	*p*-Chloro-*m*-cresol	19.0	8.6	–	11.5
1.12	1.12	Propylparaben	19.1	7.8	–	12.5
1.26	2.32	*p*-Chloro-*m*-xylenol	22.5	13.6	–	13.8
1.1	1.1	Ethyl benzoate	22.6	11.0	–	12.6
1.38	2.37	4-Isopropyl-3-methylphenol	23.1	13.8	–	13.6
1.12	1.12	Butylparaben	23.2	14.5	–	14.7
1.7	1.7	*o*-Phenylphenol	23.5	15.0	–	13.3
2.19	2.6	Benzylparaben	23.7	16.4	–	14.7
1.32	2.49	Climbazol	25.4	–	–	14.2
–	2.12	Sorbic acid, isopropyl ester	25.4	18.0	–	15.4
–	2.33	Dichloro-*m*-xylenol	25.5	29.9	–	16.1
1.1	1.1	Propyl benzoate	25.8	20.8	–	15.2
–	2.29	Dichlorophene	26.2	50.4	–	17.1
2.9	2.17	Chlorophene	27.3	54.7	–	17.5
1.1	1.1	Benzyl benzoate	28.0	42.9	–	17.8
1.1	1.1	Butyl benzoate	28.2	40.5	–	18.0
1.23	2.25	Triclocarban	29.6	–	–	22.4
1.25	2.28	Triclosan	29.8	–	–	22.0
–	2.21	Tetrabromo-*o*-cresol	30.0	–	–	22.9
–	2.27	Halocarban	30.6	–	–	22.8
1.37	2.20	Bromophen	31.4	–	–	23.5
1.6	1.6	Hexachlorophene	33.3	–	–	24.4
1.21	2.19	Bronopol	–	–	1.38	–
2.3	2.9	Dibromopropamidine, diisethionate	–	–	–	12.8
2.20	2.7	Hexamidine, diisethionate	–	–	–	13.3
1.15	2.8	Dibromohexamidine, diisethionate	–	–	–	15.3
2.24	2.31	Chlorhexidine, digluconate	–	–	–	16.4
2.24	2.31	Chlorhexidine · 2HCl	–	–	–	16.6
2.15	2.53	Benzethonium chloride	–	–	–	23.5

[a] According to EEC Commission Directive 86/199/EEC of March 26th, 1986 (I); according to EEC Council Directive 82/368/EEC of May 17th, 1982 and EEC Commission Directive 83/496/EEC of September 22nd, 1983 (II).

[b] Kathon CG contains two active ingredients, and therefore two major peaks are obtained using systems 1, 3 and 4; the retention times of these peaks are presented in order of decreasing peak heights.

consists of 1.15% 5-chloro-2-methyl-4-isothiazolin-3-one, 0.35% 2-methyl-4-isothiazolin-3-one, 25% magnesium nitrate and 73.5% water[15]. As this preservative system contains two active ingredients, two major peaks were obtained, using systems 1, 3 and 4, both of which are listed in Table I in order of descending peak height.

Chromatographic system 1 is considered to be the basic screening method. Therefore, the results in Table I are given in order of ascending retention time as obtained with this system. Using this gradient procedure, many preservatives of widely different polarities are eluted. In addition, other cosmetic constituents that might interfere in the determination of the preservatives investigated are also eluted using

chromatographic system 1. Final conclusions concerning the presence of preservatives in cosmetic products can therefore be drawn only after comparison of the results from this HPLC method with the results from both the other HPLC methods and the previously reported TLC procedure[5]. The method is based on a previously described isocratic HPLC method for the determination of hexachlorophene and related bactericides in deodorant preparations[16]. The basic screening procedure is particularly useful for the simultaneous determination of *o*-phenylphenol, 2,4-dichlorobenzyl alcohol, triclocarban, *p*-chloro-*m*-cresol, triclosan, *p*-chloro-*m*-xylenol, bromophen, 4-isopropyl-3-methylphenol and hexachlorophene.

By means of chromatographic system 2 a better resolution for the neutral preservatives of medium polarity is achieved. The method is suitable for the simultaneous determination of phenoxyethanol, phenoxypropanol, methylparaben, ethylparaben, propylparaben, butylparaben and benzylparaben. Combinations of these preservatives are often used in cosmetics[17].

A number of polar preservatives that are eluted rather early when the basic screening method (system 1) is used can be resolved by means of chromatographic system 3. This method is particularly suitable for the simultaneous determination of the acid preservatives 4-hydroxybenzoic acid, salicylic acid, benzoic acid and sorbic acid.

Chromatographic system 4 has been included in the screening procedure because it enables the determination of the cationic preservatives dibromopropamidine, hexamidine, dibromohexamidine and chlorhexidine, as well as of the quaternary ammonium compound benzethonium chloride. Many preservatives of greatly varying polarities are eluted using this ion-pair gradient system, and therefore the results obtained are also very useful to corroborate the initial identifications made on the basis of retention times obtained with the other chromatographic systems. The method is derived from a previously described method for the determination of basic preservatives in cosmetics[9]. At first the suitability of this previously reported method for the determination of hexamidine, dibromohexamidine, dibromopropamidine and chlorhexidine was investigated. These initial experiments showed, however, that these preservatives gave poorly reproducible peak shapes. Sometimes fronting peak shapes were obtained, indicating an instability of the HPLC separation system. Therefore some modifications were introduced. It was found that more reproducible results and symmetrical peaks were obtained when sodium ions were added to the mobile phase constituents, and also a well defined quantity of acetic acid (1%) instead of adjusting the pH of the aqueous part and the apparent pH of the organic part of the mobile phase to 3.5. The addition of an excess of sodium ions to the mobile phase also has a stabilizing effect on the ion-pair HPLC separation system. A comprehensive study on the effect of the counter-ion concentration in the mobile phase on both pairing ion adsorption and solute retention in reversed-phase ion-pair chromatography has been reported by Bartha *et al.*[18]. In addition, the gradient profile was adjusted to enable an optimum separation of all determinable preservatives.

The column used for the determination of the cationic preservatives was μBondapak C_{18}. Several other reversed-phase materials, *viz.*, ODS Hypersil, RSIL C_{18} LL, Zorbax C_8 and Nucleosil 5 C_{18}, were also evaluated. It appeared that satisfactory results were obtained only with a μBondapak C_{18} column. A similar result was obtained by Gaffney *et al.*[19], who investigated the determination of chlorhexidine in urine.

For the identification of preservatives in unknown cosmetic formulations the following procedure proved to be most effective. The sample solutions were subjected to the complete HPLC screening procedure. The retention times of the major peaks in the chromatograms obtained were measured. Unknown preservatives were then tentatively identified by comparing these retention times with those obtained for reference substances under similar conditions (Table I). As the retention behaviour of solutes in HPLC determinations is strongly dependent on the type, the brand and the history of the stationary phase applied, and in addition rather sensitive to slight changes in several specified conditions, the retention times presented in Table I have to be regarded as indicative of the relative retention behaviour and the order of elution of the preservatives investigated using the four HPLC separation systems. Therefore, the sample solutions were subsequently analysed again by means of the appropriate chromatographic systems, together with standards of the preservatives tentatively identified in these samples. The retention times thus obtained were used to corroborate the initial identifications. The preliminary conclusions based on these results were used to confirm the presence of preservatives in the cosmetic samples as indicated by a previously reported TLC procedure[5].

The HPLC methods described have potential application not only in the identification but also in the quantification of preservatives. Although no attempt has been made in this study to optimize the sample preparation procedure, initial experiments indicated a satisfactory recovery of most investigated preservatives from cosmetics. The HPLC methods, as described, can therefore also be used to estimate the amount of a preservative that is possibly identified in a cosmetic product.

The HPLC procedure was applied to three cosmetic products previously investigated by means of the above-mentioned TLC procedure. To illustrate the practical operation of the HPLC screening procedure a detailed description of the identification of the preservatives in a commercial hand cream is given below.

The hand cream sample was subjected to the complete HPLC procedure. The chromatograms obtained using the four HPLC systems are shown in Figs. 1–4. The HPLC analyses of the cosmetic products were performed immediately after the analysis of the preservative reference materials. In this manner the retention times for standards and samples were obtained under practically identical conditions and initial identifications can be made on the basis of retention times.

As is seen in Fig. 1, using HPLC system 1 three major peaks are obtained for the hand cream sample. By comparing the retention times with those of standards (Table I), tentative peak identifications were made (Table II). Also in the chromatogram obtained by means of HPLC system 2 (Fig. 2) three major peaks are observed. The first peak (retention time 0.9 min) was found to result from an unknown compound. The retention time of this early-eluting peak does not correspond to that of any of the preservatives investigated. On the basis of the retention time (4.6 min) the second peak was tentatively identified as ethylparaben. By comparison of the sample peak height with that of the ethylparaben reference solution, the injected amount of ethylparaben represented by the peak was found to be about 1.1 μg, which corresponds to an ethylparaben level in the hand cream of *ca.* 0.11% (w/w). The third peak (retention time 8.0 min) may originate from either propylparaben or 2,4-dichlorobenzyl alcohol. The presence of 2,4-dichlorobenzyl alcohol in the hand cream can be excluded on the basis of the results obtained using HPLC system 1. In the chroma-

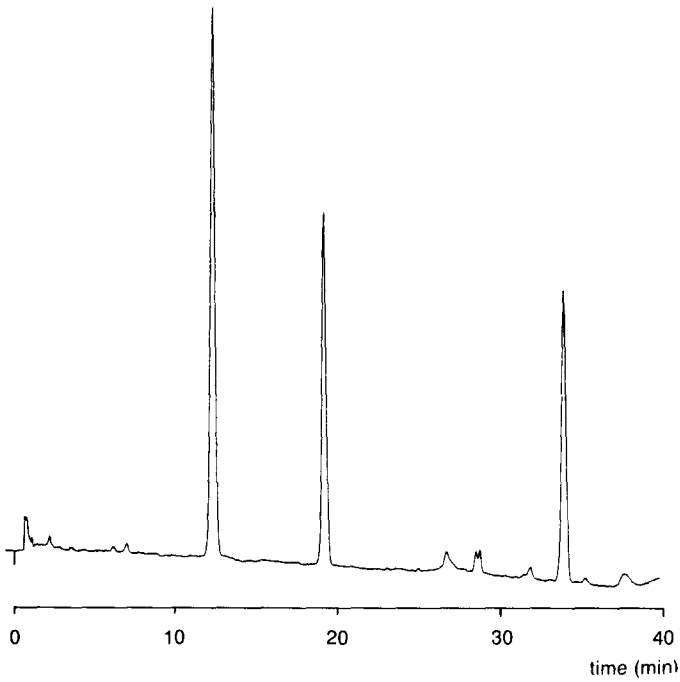

Fig. 1. Chromatogram of a hand cream extract obtained with system 1. Column: Zorbax C_8 (5 μm), 125 mm × 4.6 mm I.D. Mobile phase: phosphate buffer–acetonitrile gradient; flow-rate, 2.0 ml/min. Injection volume: 10 μl. Detection: UV at 280 nm; sensitivity, 0.04 a.u.f.s.

togram obtained with this HPLC method (Fig. 1) no peak appears to be present having the approximate retention time of 2,4-dichlorobenzyl alcohol. In this manner the third peak in the chromatogram obtained by means of HPLC system 2 was tentatively identified as propylparaben. The propylparaben content of the hand cream represented by the peak was found to be about 0.07% (w/w). The possible presence of *p*-chloro-*m*-cresol as indicated by HPLC system 1 (Table II) is not confirmed by the results obtained by means of HPLC system 2.

Using HPLC system 3 only one large peak was obtained, with a retention time corresponding to that of ethylparaben (Fig. 3). The chromatogram obtained confirms that the hand cream investigated did not contain significant amounts of polar preser-

TABLE II

INITIAL IDENTIFICATION OF CHROMATOGRAPHIC PEAKS OBTAINED FOR A HAND CREAM USING SYSTEM 1

Peak No.	*Retention time (min)*	*Possible identity*
1	12.2	Ethylparaben
2	19.0	*p*-Chloro-*m*-cresol, propylparaben
3	33.8	Hexachlorophene

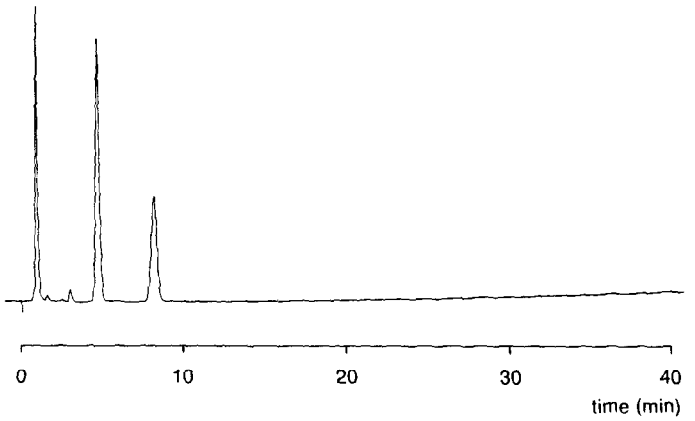

Fig. 2. Chromatogram of a hand cream extract obtained with system 2. Column: Nucleosil 5 C_{18} (5 μm), 125 mm × 4.6 mm I.D. Mobile phase: acetonitrile–methanol–tetrahydrofuran–water (5:2:1:12); flow-rate, 1.5 ml/min. Injection volume: 10 μl. Detection: UV at 280 nm; sensitivity, 0.16 a.u.f.s.

vatives, such as acid preservatives, that elute rather early when using the basic screening method (system 1), and that cannot be distinguished on the basis of the retention times obtained with HPLC system 1. The presence of ethylparaben and propylparaben in the hand cream is confirmed again by means of HPLC system 4. In the chro-

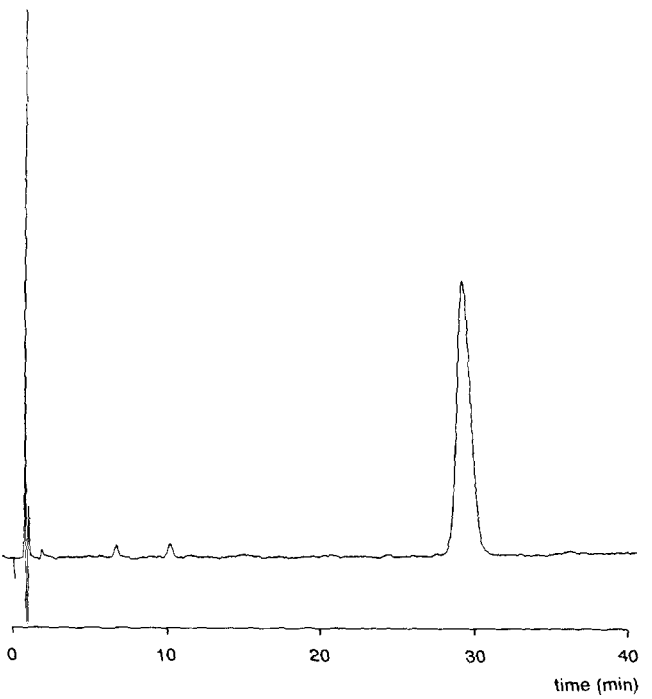

Fig. 3. Chromatogram of a hand cream extract obtained with system 3. Column: ODS Hypersil (5 μm), 125 mm × 4.6 mm I.D. Mobile phase: acetate buffer–acetonitrile (9:1); flow-rate, 2.0 ml/min. Injection volume: 10 μl. Detection: UV at 240 nm; sensitivity, 0.08 a.u.f.s.

matogram obtained with this ion-pair gradient system (Fig. 4) two major peaks are observed with retention times corresponding to those of ethylparaben and propylparaben. As no peak was found to be present in this chromatogram having the approximate retention time of hexachlorophene, the possible presence of this preservative as indicated by HPLC system 1 was not confirmed.

In summary, the results obtained by means of the complete HPLC procedure indicate the presence of ethylparaben (*ca.* 0.11%) and propylparaben (*ca.* 0.07%) in the hand cream sample. These findings confirm the results of a previously reported TLC procedure[5]. Using this TLC procedure, parabens were tentatively identified in the hand cream. Ethylparaben and propylparaben are not separated using the TLC procedure. In addition, the reaction of these related preservatives to the detection reagents applied and their behaviour under UV radiation were found to be very similar. Therefore, ethylparaben and propylparaben cannot be distinguished by means of the TLC procedure. The HPLC procedure, however, permits the identification of the individual parabens. This detailed description of the identification of preservatives in a commercial hand cream demonstrates that the initial identifications made on the basis of the TLC procedure are confirmed and supplemented by the results of the HPLC procedure, and that a final identification of the preservatives present in a cosmetic sample is achieved by an independent comparison of the results obtained for a cosmetic product by means of the TLC procedure and the HPLC procedure with results obtained for reference substances.

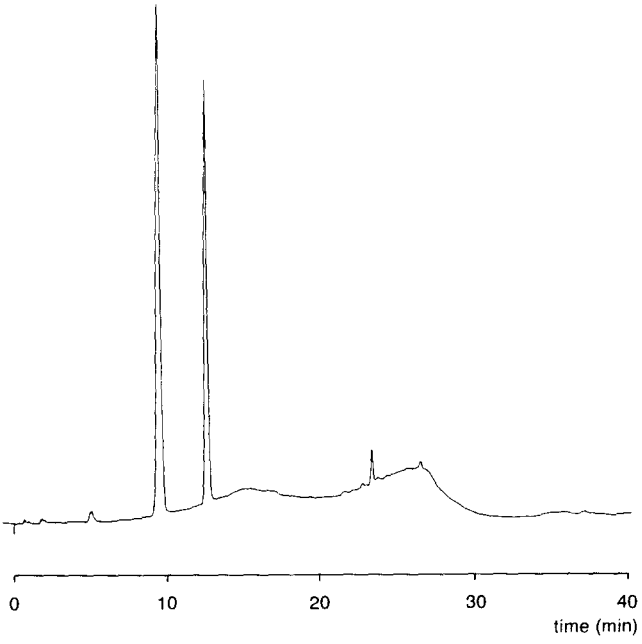

Fig. 4. Chromatogram of a hand cream extract obtained with system 4. Column: μBondapak C_{18} (10 μm), 125 mm × 4.6 mm I.D. Mobile phase: methanol–water containing sodium heptanesulphonate, sodium chloride and acetic acid ion-pair gradient; flow-rate, 2.5 ml/min. Injection volume: 10 μl. Detection: UV at 264 nm; sensitivity, 0.16 a.u.f.s.

TABLE III

QUALITATIVE DETERMINATION OF PRESERVATIVES IN COMMERCIAL COSMETIC PRODUCTS

Cosmetic product	*Preliminary identifications*		*Final identifications*
	TLC procedure[5]	*HPLC procedure*	
Hand cream	Parabens	Ethylparaben Propylparaben	Ethylparaben Propylparaben
Night cream	*p*-Hydroxybenzoic acid[a] Cationic compound[a] Parabens	*p*-Hydroxybenzoic acid Hexamidine Methylparaben	*p*-Hydroxybenzoic acid Hexamidine Methylparaben
Face powder	Parabens	Methylparaben Propylparaben	Methylparaben Propylparaben
	Benzoic acid[a] Benzyl benzoate[a]		

[a] Conclusion not clear, but the presence of this compound cannot be excluded.

In a similar manner the preservatives present in a night cream and a face powder sample were identified. The results obtained are summarized in Table III.

In conclusion, the present study has shown that the HPLC screening procedure described that consists of four separation systems, enables the characterization of 47 preservatives by their retention times. It is suitable for confirmation of the presence of preservatives in cosmetic products as indicated by a previously reported TLC procedure. In principle, the HPLC methods described can also be used for the quantitation of preservatives in cosmetics.

In general this HPLC procedure will permit the routine detection of preservatives in cosmetics in an approximate concentration of 0.01% (w/w).

ACKNOWLEDGEMENTS

Thanks are due to the Commission of the European Communities (Directorate General for the Environment, Consumer Protection and Nuclear Safety) for permission to publish this work, and to Mr. J. L. Collin and Mr. G. Gontier for continued interest and support.

REFERENCES

1 M. S. Parker, in J. J. Kabara (Editor), *Cosmetic and Drug Preservation*, Marcel Dekker, New York, 1984, p. 389.
2 R. A. Cowen and B. Steiger, *Cosmet. Toiletries*, 92 (1977) 15.
3 W. E. Rosen and P. A. Berke, *J. Soc. Cosmet. Chem.*, 24 (1973) 663.
4 B. Croshaw, *J. Soc. Cosmet. Chem.*, 28 (1977) 3.
5 N. de Kruijf, M. A. H. Rijk, L. A. Pranoto-Soetardhi and A. Schouten, *J. Chromatogr.*, 410 (1987) 395.
6 H.-J. Schmahl and R. Matissek, *Fresenius' Z. Anal. Chem.*, 307 (1981) 392.

7 R. Matissek, *Z. Lebensm.-Unters.-Forsch.*, 176 (1983) 95.
8 L. Gagliardi, A. Amato, G. Cavazzuti, V. Zagarese, E. Gattavecchia and D. Tonelli, *J. Chromatogr.*, 294 (1984) 442.
9 B. Wyhowski de Bukanski and M. O. Masse, *Int. J. Cosmet. Sci.*, 6 (1984) 283.
10 L. Gagliardi, A. Amato, A. Basili, G. Cavazzutti, E. Gattavecchia and D. Tonelli, *J. Chromatogr.*, 315 (1984) 465.
11 L. Gagliardi, A. Amato, A. Basili, G. Cavazzutti, E. Gattavecchia and D. Tonelli, *J. Chromatogr.*, 325 (1985) 353.
12 L. Gagliardi, A. Amato, A. Basili, G. Cavazzutti, E. Federici, F. Chimenti, M. G. Casanova, E. Gattavecchia and D. Tonelli, *J. Chromatogr.*, 348 (1985) 321.
13 R. Matissek, *Z. Lebensm.-Unters.-Forsch.*, 183 (1986) 273.
14 R. Matissek, R. Nagorka and M. Daase, *Lebensmittelchem. Gerichtl. Chem.*, 42 (1988) 111.
15 C. Wright, E. Gingold, S. Venitt and C. Crofton-Sleigh, *Mutat. Res.*, 119 (1983) 35.
16 J. Rooselaar and D. H. Liem, *De Ware(n)-Chemicus*, 10 (1980) 144.
17 T. E. Haag and D. F. Loncrini, in J. J. Kabara (Editor), *Cosmetic and Drug Preservation*, Marcel Dekker, New York, 1984, p. 63.
18 A. Bartha, H. A. H. Billiet, L. de Galan and G. Vigh, *J. Chromatogr.*, 291 (1984) 91.
19 M. H. Gaffney, M. Cooke and R. Simpson, *J. Chromatogr.*, 306 (1984) 303.