A *Simplex*-Optimized Chromatographic Separation of Fourteen Cosmetic Preservatives: Analysis of Commercial Products

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Abstract

An ion-interaction high-performance liquid chromatography (HPLC)–diode-array detection method is developed and optimized for the separation of typical antimicrobial agents used in cosmetics and hygiene products. The most used preservatives contain different molecular structures, different functionalities, and are characterized by different chemical properties. Organic acids, alkyl esters of benzoic acids, alkyl *p*-hydroxy benzoic acids (parabens), phenol derivatives, and carbanilides represent the most used preservatives, and are often present in multicomponent mixtures. In order to develop a multicomponent method to be used in quality control analysis, the ion-interaction reagent reversed-phase HPLC technique seems to be particularly suitable, because it allows for the simultaneous separation of acidic, basic, and neutral species. The experimental conditions of the method are developed by OVAT (one variable at a time) treatment and further optimized by a multivariate approach based on a *Simplex* algorithm that works on a desirability function targeted to maximize the resolution in a multicomponent mixture. The new method proposed that is able to simultaneously separate fourteen preservatives is applied in the analysis of commercial products.

Introduction

The preservatives most commonly used as antimicrobial agents in cosmetics and hygiene products that are permitted by European Economic Community laws (1) belong to different classes of compounds. Good preservatives are: (*a*) organic acids (i.e., sorbic, salicylic, dehydroacetic, benzoic, and 4-hydroxybenzoic acid); (*b*) alkyl esters of benzoic acid (parabens); (*c*) alkyl esters of alkyl-*p*-hydroxybenzoic acids; (*d*) phenol derivatives (i.e., *o*-phenylphenol and 4-chloro-*m*-cresol); and (*e*) carbanilides (triclocarban). Because these preservatives are often employed in multicomponent mixtures, multiresidue methods are highly required.

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The determination of parabens is generally carried out by reversed-phase (RP) high-performance liquid chromatographic (HPLC) methods in isocratic (2–6) as well as gradient elution (7,8), and sometimes coupled with solid-phase extraction (7). High-performance thin-layer chromatography (9–11), capillary zone electrophoresis (12,13), and gas chromatography (GC)–mass spectrometry (MS) (14,15) methods are also used. For bronopol determination an HPLC method with electrochemical detection is proposed (16). Triclocarban (17,18,6), salicylic acid, and alkylbenzoates (5) are determined by HPLC with UV detection. Flow injection analysis is employed for the determination of 4-chloro-*m*-cresol in pharmaceutical preparations (19). Ion-pair HPLC is used for the determination of benzoic and sorbic acids (20), which are also separated (together with dehydroacetic acid) by GC–MS after derivatization (15). Methods in literature mainly concern the separation of preservatives characterized by a similar structure. For the separation of multifunctionality mixtures only some examples are reported that require the use of complex systems of detection (16) or gradient elution (19). Thus, for instance, only the combined and alternative use of four different sets of conditions allowed the RP-HPLC separation of 47 preservatives (5).

In order to simultaneously separate compounds characterized by different hydrophilicity and chemical properties, methods based on the ion-interaction reagent (IIR) RP-HPLC technique seem to be very suitable, because they permit the simultaneous separation of acidic, basic, and neutral species (21–24). In this study we propose a new IIR-HPLC method for the simultaneous separation of as many components out of the nineteen preservatives as possible, which would be representative of the most commonly used. The nineteen analytes considered in this study are characterized by different chemical functionalities. They are benzoic acid, salicylic acid, 4-hydroxybenzoic acid, methyl-benzoate, ethyl-benzoate, propyl-benzoate, butyl-benzoate, benzyl-benzoate, methyl-paraben, ethyl-paraben, propyl-paraben, butyl-paraben, benzyl-paraben, *o*-phenyl-phenol, 4-chloro-*m*-cresol, triclocarban, sorbic acid, bronopol, and dehydroacetic acid.

The optimization of the method's experimental conditions is performed in a first step through the OVAT (one variable at a time) treatment and then through the application of the *Simplex* algorithm. The *Simplex* explores the domain of the variables through a suitable desirability function that contains in only one parameter all of the information we wish to optimize.

The *Simplex* method

The *Simplex* technique is a widely used multivariate optimization (21–23) strategy. When p is considered as the number of experimental factors to be optimized, the method starts from a set of $p + 1$ initial experiments. The set of $p + 1$ experiments is called the *Simplex*. The optimization is based on the set of successive projections of the experiment that gives the worst response with respect to the centroid of the other ones. The experiment that gives the worst response is in turn eliminated if the projection leads to a better response and is substituted by the new one. When the projection does not lead to a better response, the second worst experiment is projected. The classical *Simplex* method stops searching when no better result can be obtained from the projection of all the experiments.

In the present research we used the modified *Simplex* version (22) in which during the search for the optimum the *Simplex* changes its shape by performing a double projection when the best result is found or by contracting on itself when the projection leads to the worst result.

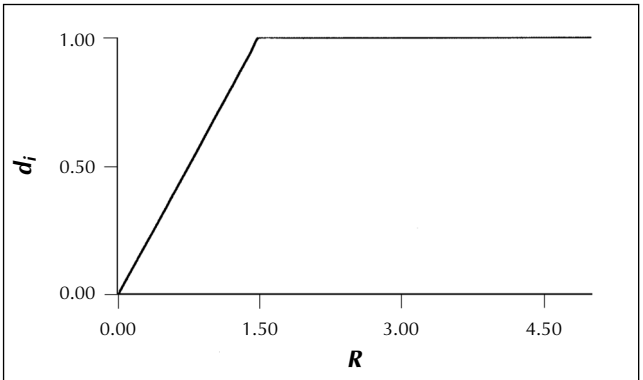

Figure 1. Plot of d_i as a function of R.

Desirability functions

The optimization for the simultaneous separation of the nineteen preservatives considered in this study can be faced by using a multicriterium selection method. The quality of the separation is evaluated on the basis of the resolution (R_{hj}) of every possible pair of peaks h and j, which is given by:

$$R_{hj} = 2\,\frac{t_{R_j} - t_{R_h}}{w_j + w_h} \qquad \text{Eq. 1}$$

where t_{R_h} is the retention time of the h-th analyte and w_h is the width of the chromatographic peak of the same analyte. The desirability of the separation of each couple of peaks is evaluated as:

$$\begin{cases} d_i = R/1.5 & \text{if } R < 1.5 \\ d_i = 1 & \text{if } R \geq 1.5 \end{cases} \qquad \text{Eq. 2}$$

The optimization of the overall resolution is searched for through the contemporary optimization of all the possible resolutions by calculating an overall desirability function (D) defined as:

$$D = (\Pi_{i\,=\,1,n} d_i)^{\frac{1}{n}} \qquad \text{Eq. 3}$$

where the product ($\Pi_{i=1,n}$) of the d_i runs on the couples of adjacent peaks or couples of peaks with a resolution less than 1.5. D, so defined, measures the overall resolution of the chromatogram. As summarized in the plot of d_i versus R (Figure 1), d_i becomes null when at least a pair of peaks coelutes (identical retention times) and it is equal to 1.0 when all the pairs of adjacent peaks show resolution equal to or greater than 1.5.

The *Simplex* optimization was performed by evaluating all the experiments on the basis of their D value, thus searching for the maximum value of the overall desirable D.

Table I. *Simplex* Optimization Procedure

Experiment	pH	F (mL/min)	%ACN	pIIR	*D*	
1	5.50	0.90	50.00	3.00	0.6786	initial *Simplex*
2	5.96	0.94	50.54	3.05	0.6178	initial *Simplex*
3	5.61	1.08	50.54	3.05	0.7793	initial *Simplex*
4	5.61	0.94	52.31	3.05	0.7936	initial *Simplex*
5	5.61	0.94	50.54	3.23	0.7521	initial *Simplex*
6	5.20	0.99	51.15	3.11	0.7638	normal reflection of 2 on 1-3-4-5
7	5.51	1.08	52.27	3.22	0.7814	normal reflection of 1 on 3-4-5-6
8	5.35	0.89	52.59	3.25	0.8492	normal reflection of 5 on 3-4-6-7
9	5.23	0.79	53.62	3.36	0.8136	double reflection of 5 on 3-4-6-7
10	5.23	1.01	53.62	3.09	0.8143	normal reflection of 6 on 3-4-7-8
11	5.24	0.88	54.84	3.26	0.6901	normal reflection of 3 on 4-7-8-10
12	5.26	0.90	52.49	3.08	0.7649	contraction of 3 on 4-7-8-10
13	5.59	1.06	52.91	3.23	0.4393	normal reflection of 12 on 4-7-8-10

Experimental

Apparatus

The analyses were carried out with a Merck-Hitachi LaChrom-HPLC equipped with a Pump Module D-7100 interfaced by Module L-7000 with two detectors (the UV detector Module L-7400 and the Diode-Array Detector Module L-7450). The data were collected and elaborated by the D-7000 Multi HPLC system manager software program.

A UV–vis Unicam Spectrophotometer Series 8700 was used for the spectrophotometric determinations, and a Crison pH2001 pH meter equipped with a combined glass–calomel electrode was employed for the pH measurements.

Reagents

Ultrapure water from Milli-Q (Millipore Corporation, Bedford, MA) was used.

Analytical-grade benzoic acid, 4-hydroxybenzoic acid, methyl benzoate, ethyl benzoate, propyl benzoate, butyl benzoate, benzyl benzoate, ethyl-paraben, propyl-paraben, butyl-paraben, 4-chloro-*m*-cresol, sorbic acid, dehydroacetic acid, butylamine, hexylamine, octylamine, and *o*-phosphoric acid were obtained from Fluka (Buchs, Switzerland). Salicylic acid, methyl-paraben, benzyl-paraben, *o*-phenylphenol, triclocarban, and bronopol were purchased from Aldrich (Milano, Italy), and HPLC-grade acetonitrile (ACN) and methanol were from Merck (Darmstadt, Germany).

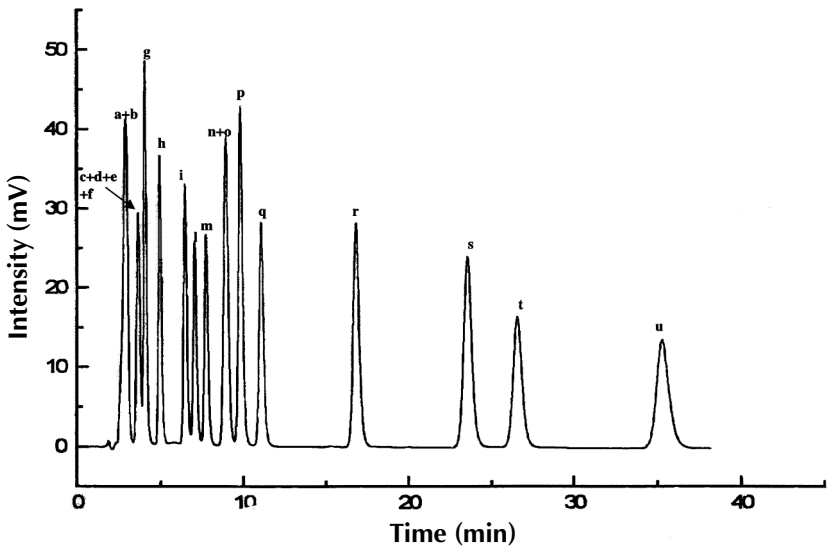

Figure 2. Chromatogram recorded for the fourteen component mixtures under the optimized conditions: benzoic acid, a; methyl benzoate, b; ethyl benzoate, c; propyl benzoate, d; butyl benzoate, e; benzyl benzoate, f; 4-hydroxy-benzoic acid, g; methyl paraben, h; ethyl paraben, i; propyl paraben, l; butyl paraben, m; benzyl paraben, n; salicylic acid, o; *o*-phenyl phenol, p; 4-chloro-*m*-cresol, q; bronopol, r; sorbic acid, s; dehydroacetic acid, t; and triclocarban, u.

Chromatographic conditions

The stationary phase used was a Merck Superspher 100 RP 18 endcapped column (250.0 × 4.6 mm, 4 µm) together with a Chrompack C_{18} (3.0 × 5.0 mm, 5 µm) guard precolumn.

The mobile phases used in the experiments of the *Simplex* design were prepared by adding to the required water–ACN mixture the required amount of alkylamine and *o*-phosphoric acid up to the required pH value.

The chromatographic system was conditioned by passing (under isocratic conditions) the eluent through the column until a stable baseline signal and reproducible retention times for two subsequent injections were obtained (approximately 1 h at a flow rate (F) of 1.0 mL/min was sufficient).

After use, the system was washed by flowing water (1.0 mL/min for 15 min), a 50:50 (v/v) water–ACN mixture (1.0 mL/min for 15 min), and 100% ACN (1.0 mL/min for 5 min).

Table II. DLs and Correlation Coefficients

	DL (µg/L)	R^2
Benzoic acid	70	0.9579
4-Hydroxy benzoic acid	50	0.9994
Salicylic acid	120	0.9876
Methyl benzoate	60	0.9696
Ethyl benzoate	70	0.9774
Propyl benzoate	70	0.9802
Butyl benzoate	40	0.9798
Benzyl benzoate	80	0.9815
Methyl paraben	9	1.0000
Ethyl paraben	60	1.0000
Propyl paraben	70	0.9999
Butyl paraben	70	0.9994
Sorbic acid	80	0.9988
Bronopol	3	0.9402
4-Chloro-*m*-cresol	820	0.9969
o-Phenyl phenol	105	0.9909
Dehydroacetic acid	730	0.9752
Triclocarban	90	0.9916
Benzyl paraben	70	0.9611

Real sample treatment

The samples were commercial cosmetic lotions from Nivea

Table III. Analysis of Commercial Cosmetic Lotions

	Retention time (min)	Concentration (mg/L)
Cosmetic lotion A		
Propyl paraben	6.82 ± 0.04	0.89 ± 0.04
Ethyl benzoate	10.64 ± 0.06	179 ± 9
Butyl benzoate	27.41 ± 0.03	11.2 ± 0.6
Cosmetic lotion B		
Propyl paraben	6.60 ± 0.03	0.76 ± 0.05
o-Phenylphenol	10.59 ± 0.04	16.7 ± 0.8
Butyl benzoate	26.45 ± 0.07	10.2 ± 0.5
Cosmetic lotion C		
4-Chloro-*m*-chresol	6.99 ± 0.02	5.7 ± 0.3
Benzyl benzoate	23.41 ± 0.04	74 ± 4
Triclorocarban	35.36 ± 0.06	3.7 ± 0.2
Cosmetic lotion D		
Benzyl benzoate	23.17 ± 0.06	3.5 ± 0.2
Triclorocarban	34.93 ± 0.07	2.2 ± 0.1

Bayersdof (Hamburg, Germany), Lancaster (New York, NY), Wendell (Zurich, Switzerland), and L'Oreal (Paris, France) that are internationally widespread diffused products that can easily be found in worldwide stores.

The commercial samples were diluted 1:10 (v/v) with ultrapure water and filtered by a microfiltration system MFS-25 (25-mm i.d., 0.20-µm pore size). The lotions in this study were generically indicated (independently on the sequence order) as lotion A, B, C, and D.

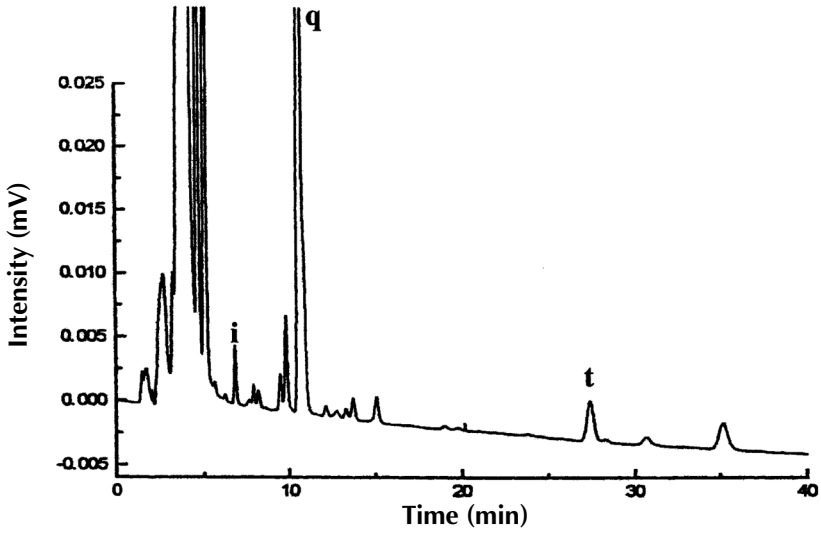

Figure 3. Chromatogram of lotion A (chromatographic conditions and peak identification are the same as Figure 2).

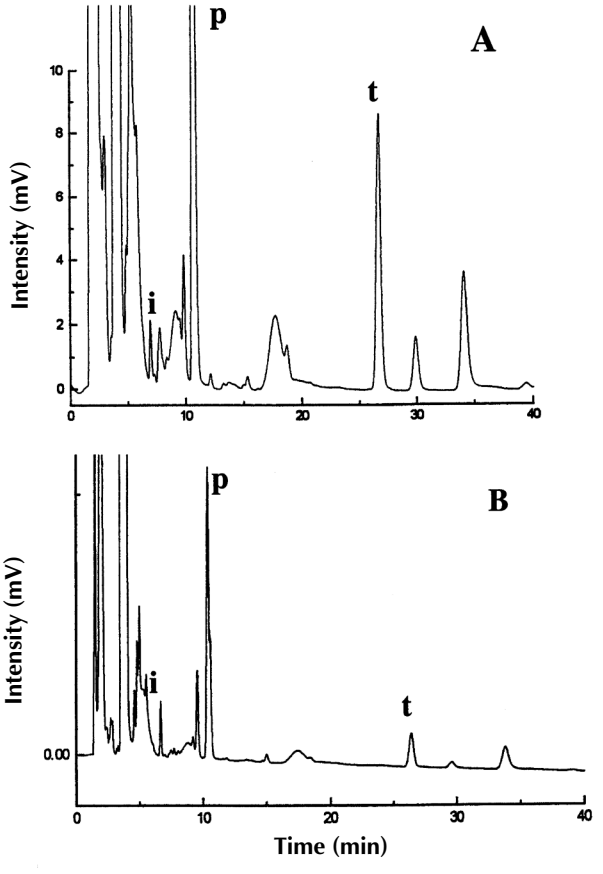

Figure 4. Chromatograms of lotion B recorded at (A) 230 nm and (B) 260 nm (chromatographic conditions and peak identification are the same as Figure 2).

Results and Discussion

The OVAT method

IIR liquid chromatography is a very versatile technique that often allows the development of methods that do not require particular sample pretreatments. The reason for the versatility and also its drawback is the dependence of the retention on many experimental factors. In particular, when using alkyl-ammonium *o*-phosphate salts as the IIRs, the principal factors that affect retention have been shown to be the alkyl chain length, the IIR concentration, the concentration of the organic modifier, and the pH of the mobile phase (24–27).

Analytes characterized by different chemical functionalities can often be separated, because the electrical double layer that forms onto the surface of the stationary phase by the IIR allows for the simultaneous separation of cationic and anionic species. Furthermore, because not all the RP sites are modified, it is also possible to achieve the simultaneous separation of neutral species, which are retained through a conventional RP mode.

In order to find out the best experimental conditions for the separation of the nineteen representative preservatives, a first-optimization process based on the OVAT method was performed. A length of 240 nm was chosen as the average best wavelength for the multicomponent analysis. On the basis of previous results obtained in our lab (24–27), ten experiments were performed by employing mobile phases containing alkylammonium *o*-phosphate with an alkyl chain between 4 and 9, a pH range between 4 and 8, and an ACN concentration ranging between 30% and 70%. The results obtained in this study confirmed the effects already observed in previous studies (24–27). Because of the more lipophilic properties assumed by the modified surface, the increasing length of the alkyl chain led to an increased retention of anions and a decreased retention of amines because of equilibria competing with alkylammonium already adsorbed onto the surface. The use of hexylamine *o*-phosphate was shown to give the best separation.

Concerning the pH of the mobile phase, it must be stressed that it exerts its effect not only on the analyte acidic dissociation equilibrium but also on the modification induced onto the stationary phase. The experiments showed that a pH of 5.5 was the most suitable for the separation studied.

The increased concentration of the organic solvent in the mobile phase lead to a decrease in the retention of both the anionic and cationic species, because the effect observed was the result of two contributions (one resulting from the

increased eluotropic strength in the mobile phase and the other from a decreased amount of the interaction reagent adsorbed onto the stationary phase surface).

These preliminary experiments gave the conditions of the mobile phase that were to be employed for the *Simplex* as 50% ACN, 1.0mM hexyl-ammonium phosphate, a pH of 5.5, and a 0.9-mL/min F value.

Simplex optimization

The experimental factors considered in the *Simplex*-based optimization were the ACN concentration (%ACN), the pH value of the mobile phase, and the hexyl-ammonium concentration (IIR), expressed as pIIR (–log [IIR]). Even if its effect is predictable, the F was also added as the fourth experimental factor in order to simultaneously optimize all of the chromatographic conditions.

The aim was to obtain a set of conditions for the best separation of all the components of the mixture. The initial *Simplex* had five vertices (one more than the number of variables), which corresponds to carrying out five experiments (experiments 1–5 in Table I) whose conditions are obtained by applying suitable changes to each factor of the starting experiment.

The results obtained in the optimization, expressed as the desirability function *D*, are reported in Table I. The first reflection of the worst experiment (experiment 2) lead to a better response. Because this was not the absolute best, a new normal reflection of the new worst experiment (experiment 1) was performed. Again, a better result (but not the absolute best) was obtained, thus a new normal reflection of the worst experiment (experiment 5) was performed. The new experiment was the very best, thus a double reflection of experiment 5 was tried, but the result did not lead to a better resolution; therefore, this normal reflection was retained. Because the following experiments did not provide any better result, the optimization procedure was interrupted and the experimental conditions were found to be 52.59% ACN, 3.25mM hexyl-ammonium phosphate, a pH of 5.35, and a 0.89-mL/min F value. A chromatographic run performed with these experimental conditions provided the chromatogram of Figure 2 in which fourteen analytes out of nineteen were separated. In these conditions that were the best we could obtained, the calibration plots were built and the detection limits (DLs) evaluated.

Calibration curves and DLs

In the optimized conditions, the calibration curve for every analyte was built. The concentration ranged between 0.125 and 2.500 µg/L for all the analytes, with the exception of bronopol and dehydroacetic acid, which showed smaller molar absorptivity values and for which a concentration range between 1.0 and 5.0 mg/L was explored. The calibration curves for all the analytes were linear with R^2 values > 0.94. In Table II are also reported the DLs evaluated for each analyte by sensitivity (peak area for concentration unit) given by the slope of the calibration plot and for a signal-to-noise ratio of 3.

Real samples analysis

Some widely diffused commercial cosmetic lotions from Nivea, Wendell, Lancaster, and L'Oreal were analyzed in order to check the capability and applicability of the method in routine analysis. All the samples were diluted 1:10 with ultrapure water and then filtered and analyzed in the optimized conditions using the diode-array detector to confirm peak identification.

The quantitative data are given as an average between the data obtained by the external calibration plots and the application of the standard addition method.

On the label of a cosmetic lotion (called A) the presence of methyl paraben was reported. This analyte was identified by the diode-array system but could not be quantitated because it

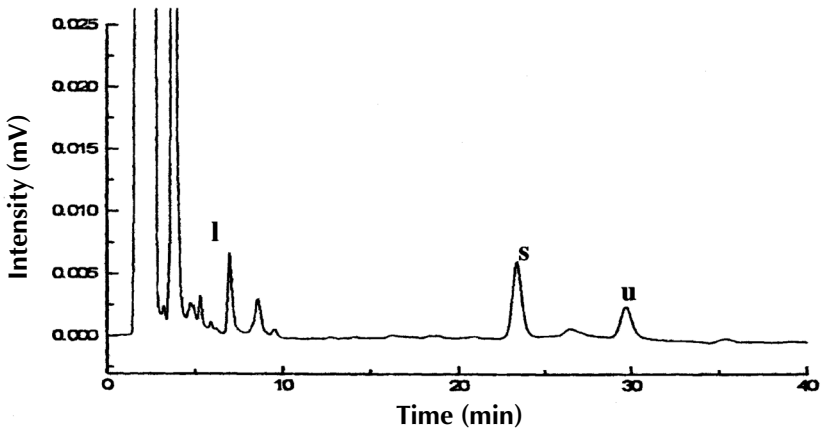

Figure 5. Chromatogram of lotion C (chromatographic conditions and peak identification are the same as Figure 2).

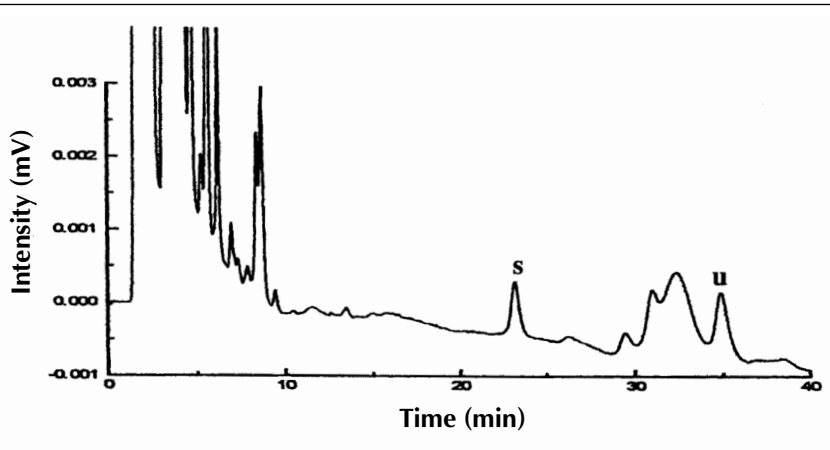

Figure 6. Chromatogram of cosmetic lotion D (chromatographic conditions and peak identification are the same as Figure 2).

coelutes with other compounds. In addition, three other preservatives not declared by the manufacturer (namely propyl paraben, ethyl benzoate, and butyl benzoate) were identified by both retention times and UV spectra and then quantitated. A fourth component was also found (possibly butyl paraben or benzyl paraben, it was indistinguishable from the UV spectra and the retention times). The chromatogram obtained is shown in Figure 3, and the concentrations that were found are reported in Table III.

Even if lotion B did not report on the label the presence of any preservative, the analysis (retention time plus UV spectra) showed the presence of three preservatives (namely propyl paraben, *o*-phenylphenol, and butyl benzoate) and a fourth compound that could have been butyl- or benzyl-paraben. Two chromatograms that were recorded for this formulation at two different wavelengths are reported in Figure 4, and the estimated concentrations are reported in Table III.

Lotion C reported on its label the presence of methyl paraben, which was confirmed but not quantitated because there was evidence of coelution. Three other preservatives not declared were identified, namely 4-chloro-*m*-cresol, benzyl benzoate, and triclocarban (the latter two were quantitated). The results are reported in Figure 5 and Table III.

The label of lotion D reported the presence of sorbic acid as a preservative. This analyte was identified by the diode-array detector but could not be quantitated because it coelutes with other compounds. The analysis also allowed for the identification and determination of benzyl benzoate and triclocarban, which were not reported on the label. The chromatogram is reported in Figure 6, and the concentrations of benzyl benzoate and triclocarban are in Table III.

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