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Optimization by experimental design and artificial neural networks of the ion-interaction reversed-phase liquid chromatographic separation of twenty cosmetic preservatives

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Abstract

Particular attention are recently receiving antimicrobial agents added as preservatives in hygiene and cosmetics commercial products, since some of them are suspected to be harmful to the human health. The preservatives used belong to different classes of chemical species and are generally used in their mixtures. Multi-component methods able to simultaneously determinate species with different chemical structure are therefore highly required in quality control analysis. This paper presents an ion interaction RP-HPLC method for the simultaneous separation of the 20 typical antimicrobial agents most used in cosmetics and hygiene products, that are: benzoic acid, salicylic acid, 4-hydroxybenzoic acid, methyl-, ethyl-, propyl-, butyl-, benzyl-benzoate, methyl-, ethyl-, propyl-, butyl-, benzyl-paraben, *o*-phenyl-phenol, 4-chloro-*m*-cresol, triclocarban, dehydroacetic acid, bronopol, sodium pyrithione and chlorhexidine. For the development of the method and the optimization of the chromatographic conditions, an experimental design was planned and models were built by the use of artificial neural network to correlate the retention time of each analyte to the variables and their interactions. The neuronal models developed showed good predictive ability and were used, by a grid search algorithm, to optimize the chromatographic conditions for the separation of the mixture.

Keywords: Optimization; Artificial neural network; Experimental design; Cosmetics

1. Introduction

Many are the preservatives commonly used as antimicrobial agents in cosmetics and hygiene products. The compounds admitted and their concentration are ruled by the European Economic Community laws by the directive 76/768: the maximum admitted concentrations are, respectively: 0.4% (w/w) if only one preservative is present and 0.8% (w/w) expressed as *p*-hydroxybenzoic acid content if more than one preservatives are present [1]. Preservatives belong to different classes of compounds as organic acids (sorbic, salicylic, dehydroacetic, benzoic, 4-hydroxybenzoic, etc.), alkyl esters of benzoic acid, alkyl esters of alkyl-*p*-hydroxybenzoic acids (parabens), phenol derivatives (*o*-phenyl-phenol, 4-chloro-*m*-cresol, etc.), carbanilides (triclocarban), etc. Since they are often employed in multi-component mixtures, multi-residue methods are highly requested.

Benzoic acid is generally determined by UV-ion-pair HPLC [2] and by GC-MS after derivatization [3]. Parabens are determined by RP-HPLC with UV-Vis detection in isocratic [4–8] and in gradient elution [9–11], by HP-TLC [12–14], by CZE with UV and DAD detection [11,15–17] and by GC-MS [3,18] methods. HPLC with electrochemical detection is employed for the determination of bronopol [19,20] and HPLC with UV detection for the determination of triclocarban [8,21,22], salicylic acid and alkyl benzoates [7]. 4-Chloro-*m*-cresol in pharmaceutical preparations is quantified by flow injection analysis [23]. Few examples of separation of mixtures are reported that require complex systems of detection [19] or gradient elution [23]. The RP-HPLC separation of 47 preservatives was achieved through the combined and alternative use of four different sets of conditions [7]. The extraction of preservatives from cosmetic products normally is performed by diluting the sample [4], or by an extraction by solvent or solid phase

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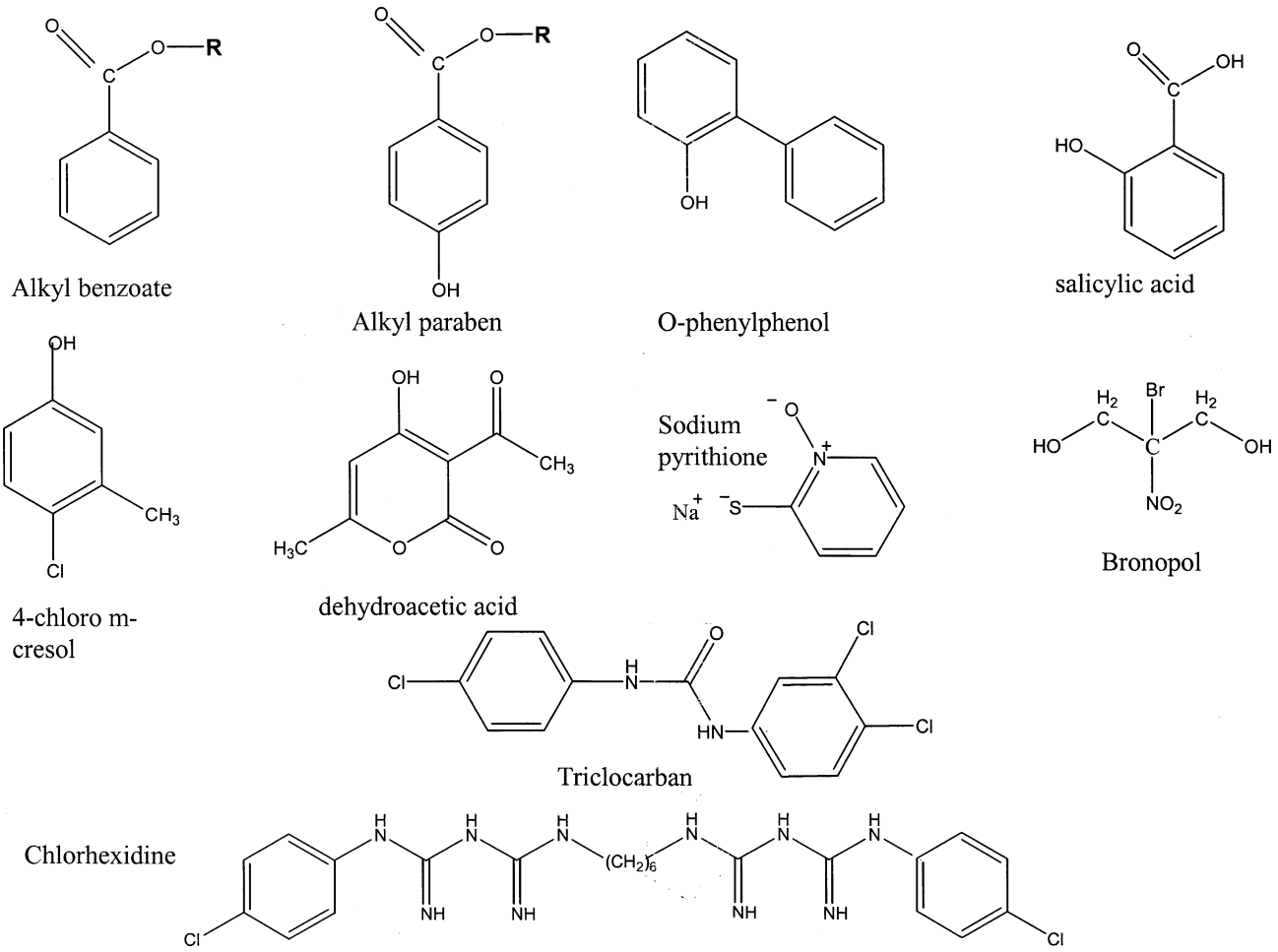

Fig. 1. Analytes structures (R represents the different alkylic functional groups: methyl, ethyl, propyl, butyl, benzyl).

[6]; In alternative method a supercritical fluid extraction (SFE) with supercritical carbon dioxidewas performed [9].

In this lab a method for the separation of 14 preservatives has been previously developed, based on ion interaction RP-HPLC technique, that is very suitable for mixtures of species with different hydrophilicity and chemical properties [24–27]: the optimization of the chromatographic conditions was obtained by a simplex algorithm [28].

In this paper we propose another application of the IIR-HPLC technique for the simultaneous separation of the 20 most commonly used preservatives and namely: benzoic acid, salicylic acid, 4-hydroxybenzoic acid, methyl-, ethyl-, propyl-, butyl-, benzyl-benzoate, methyl-, ethyl-, propyl-, butyl-, benzyl-paraben, *o*-phenyl-phenol, 4-chloro-*m*-cresol, triclocarban, dehydroacetic acid, bronopol, sodium pyrithione and chlorhexidine. Their chemical structures are reported in Fig. 1.

The experimental conditions to be optimized are: the concentration of the organic modifier (acetonitrile), the pH value of the mobile phase, the ion interaction reagent concentration and the elution flow rate. Flow rate effect is easily predictable: it was introduced among the experimental factors in order to be able shorten the total analytical time while maintaining a satisfactory resolution of the analytes. The experimental domain was explored by experimental design and the optimization of the chromatographic conditions was performed by modeling the retention times using artificial neural networks (ANN), that are mathematical algorithms which permit to model complex behaviors of the investigated systems by simulating the brain functioning.

2. Theory

In order to model analyte retention times as a function of the four experimental factors, back-propagation ANN was used.

In back-propagation ANN, the neural network is constituted by: (i) an input layer, where each neuron is associated to an experimental factor; (ii) layers of processing neurons, the so-called hidden layers; and (iii) an output layer, where each neuron is associated to the response.

The signal moves from the input layer towards the output layer (Fig. 2a), and in this process each neuron uploads all

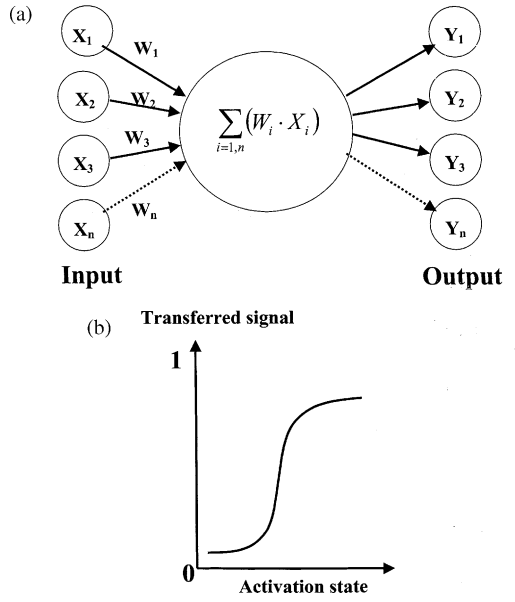

Fig. 2. (a–b) Functional schemes of the neural network.

the neurons of the successive layers, transferring a portion of the signal that it has been accumulated. The portion of signal transferred is regulated by a transfer function, similar to the one represented in Fig. 2b. For central values of the signal, the portion transferred is approximately proportional to the signal itself, while at the extreme values of the signal, the portion transferred is either null or 1. In every neuron of the hidden layers and of the output layer the signals coming from every neuron of the previous layer are accumulated applying a multiplying weight.

$$\sum_{i=1,n} (W_i X_i)$$

The weights are optimized during the training of the neural network in the process called network training. The back-propagation algorithm, largely described elsewhere [29,30], allows the optimization of the weights associated to each couple of connected neurons, so that the network is able to provide the correct output when certain input vector is entered. In this process every experiment of the training set is in turn presented to the network and the weights are corrected in such a way to decrease the error committed by the network in estimating the corresponding responses. In each cycle which constitutes a learning epoch, all the experiments are presented once to the network; the iterations of the learning epochs are repeated until the network produces satisfactory results.

The number of hidden layers and of neurons in each hidden layer must be selected to achieve a satisfactory fitting ability of the network, associated to a satisfactory predictive ability. By increasing the number of hidden layers and/or neurons in the hidden layers, it is possible to obtain very flexible networks, with incredible modeling ability, but this may cause the network to learn the data by heart, with no generalization of the rules which determine the system behavior and functioning. So it is very important to check the predictive ability of the artificial neural networks by cross-validation techniques. For example, by partitioning the available data into a training set and a test set.

3. Experimental

The analyses are carried out with a Merck-Hitachi (Darmstadt, Germany) 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 are collected and elaborated by the D-7000 Multi HPLC System Manager Software.

A UV-Vis Unicam Spectrophotometer series 8700 is used for the spectrophotometric determinations.

The stationary phase is a Merck Superspher 100 RP 18 endcapped column (250.0 mm × 4.6 mm, 4 μm). together with a Chrompack C_{18} (3.0 mm × 4.6 mm, 5 μm) guard pre-column. The mobile phases used in the experiments of the experimental design are prepared by adding to the water/acetonitrile mixture the required amount of alkylamine and of *o*-phosphoric acid up to the required pH value.

The chromatographic system is conditioned by passing, under isocratic conditions, the eluent through the column until a stable baseline signal and reproducible retention times for two subsequent injections are obtained (about an hour is enough). The injection volume is 100 μl, controlled by a calibrated loop. After use, the system is washed by flowing water (1.0 ml/min for 15 min), a 50/50 (v/v) water/acetonitrile mixture (1.0 ml/min for 10 min) and 100% acetonitrile (1.0 ml/min for 5 min).

A Crison pH200l pH-meter equipped with a combined glass-calomel electrode is employed for the pH measurements. Ultrapure water from Milli-Q (Millipore Corporation, Bedford, MA, USA) is used.

Benzoic acid, 4-hydroxybenzoic acid, methyl-, ethyl-, propyl-, butyl-, benzyl-benzoate, ethyl-, propyl-, butyl-paraben, 4-chloro-*m*-cresol, dehydroacetic acid, butylamine, hexylamine, octylamine and *o*-phosphoric acid are Fluka (Buchs, Switzerland) analytical grade chemicals. Salicylic acid, methyl-, benzyl-paraben, *o*-phenyl-phenol, triclocarban, bronopol and chlorhexidine were purchased from Aldrich (Milano, Italy); acetonitrile and methanol were Merck (Darmstadt, Germany) HPLC grade chemicals.

3.1. Samples preparation

One gram of shaving foam was dissolved in 20 ml of ultrapure water and stirred in a vortex mixer, then diluted 1:100 with the mobile phase and filtered through a 0.2 μm syringe filter. One milliliter of deodorize sample was delivered, sonicated for 15 min and then diluted 1:100 with the mobile phase and filtered.

Table 1
Extreme values of the variables in the full factorial design and of the star design

	C_{ACN}	pH	C_{IIR}	F
Min FFD	35.0	3.5	1.0	0.8
Max FFD	55.0	7.5	10.0	1.2
Min STAR	40.0	4.5	2.2	0.9
Max STAR	50.0	6.5	8.8	1.1
Central	45.0	5.5	5.5	1.0

C_{ACN} is the percentage of the organic modifier in the mobile phase; C_{IIR} is the concentration of ion-interaction reagent in the mobile phases (mg/l); F is the flow rate of the mobile phase (ml/min).

4. Result and discussion

The exploration of the experimental domain is started with a full factorial design (FFD), consisting of 16 experiments, whose extreme values are reported in Table 1. Three replicates of the central experiment were performed along the FFD (at the beginning, in the middle and at the end of the FFD) in order to check the analysis repeatability and to estimate the experimental error.

The treatment permitted to calculate the effect of the principal factors and of their interactions. Since the test of curvature [31] showed that several analytes did not exhibit a linear behavior, a star design was added, leading to a total of 25 experiments; in order to introduce a higher number of levels in the experimentation and since the extreme values employed in the FFD were already the largest possible absolute values, an internal star design was employed (Table 1).

The 25 experimental retention times were used to build a dendrogram, representing the analyte similarities as a function of their dependence on the experimental factors. The dendogram, reported in Fig. 3, was obtained by the similarity between the analytes, described by their retention times in the different experimental conditions explored in the experimental design. The distance to define the objects similarity was the r, Pearson correlation, so that similar behaviors and not the absolute retention times were responsible of high similarities. The Ward method was used as the aggregation method. In the dendrogram, it is quite evident that there are two groups of analytes: trichlocarban, *o*-phenyl-phenol, benzoate derivative and paraben derivatives on one side and the acids plus bronopol and two single outliers (chlorhexidine and sodium pyrithione) on the other. This result confirms the different physico-chemical behaviors, which was expected on the basis of the different molecular structures and functionalities. Chlorhexidine and sodium pyrithione, which have rather complex chemical structures, exhibit a peculiar behavior.

The complete set of 25 experiments was first employed to calculate for each analyte a second-order regression model, but many of these models were not satisfactory, the worst being those of sodium pyrithione ($R^2 = 0.30$), of chlorhexidine ($R^2 = 0.59$), of 4-Cl-*m*-chresol ($R^2 = 0.77$) and of *o*-phenyl-phenol ($R^2 = 0.79$). The two worst ordinary least square (OLS) regression models were thus obtained for the two outliers emerged from the cluster analysis. The behavior of the systems was probably too complex to be modeled with OLS models, and suggested to face the problem by means of the artificial neural networks. In particular a non-linear effect of pH versus C_{ACN} can be envisage. pH could possibly introduce plateau dependencies which cannot be properly modeled by second-order regression models.

Several architectures of the ANN network with one hidden layer and different numbers of neurons in this layer were explored. The networks were built modeling all together the retention times of the analytes; this means that the output layer was constituted by 20 neurons: one for each analyte. A maximum of 20,000 training epochs was investigated and few experiments have been performed according to the experimental design techniques. A leave-one-out scheme then

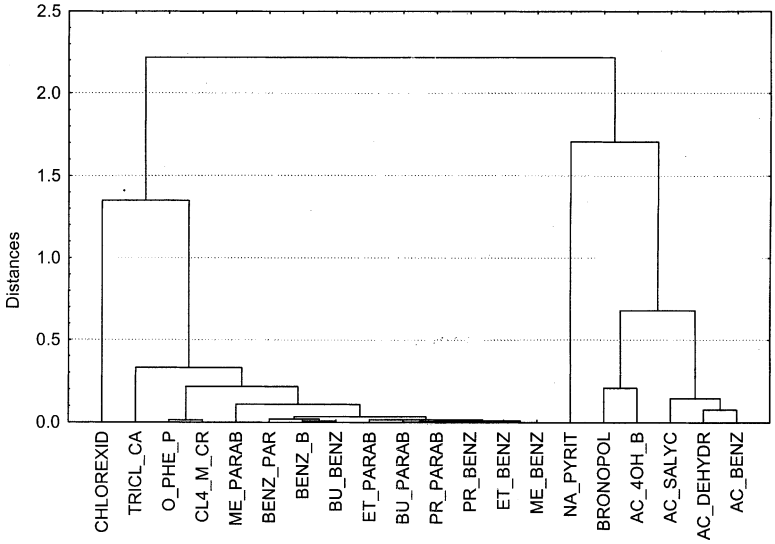

Fig. 3. Dendrogram representing the analytes similarities in function of the retention times.

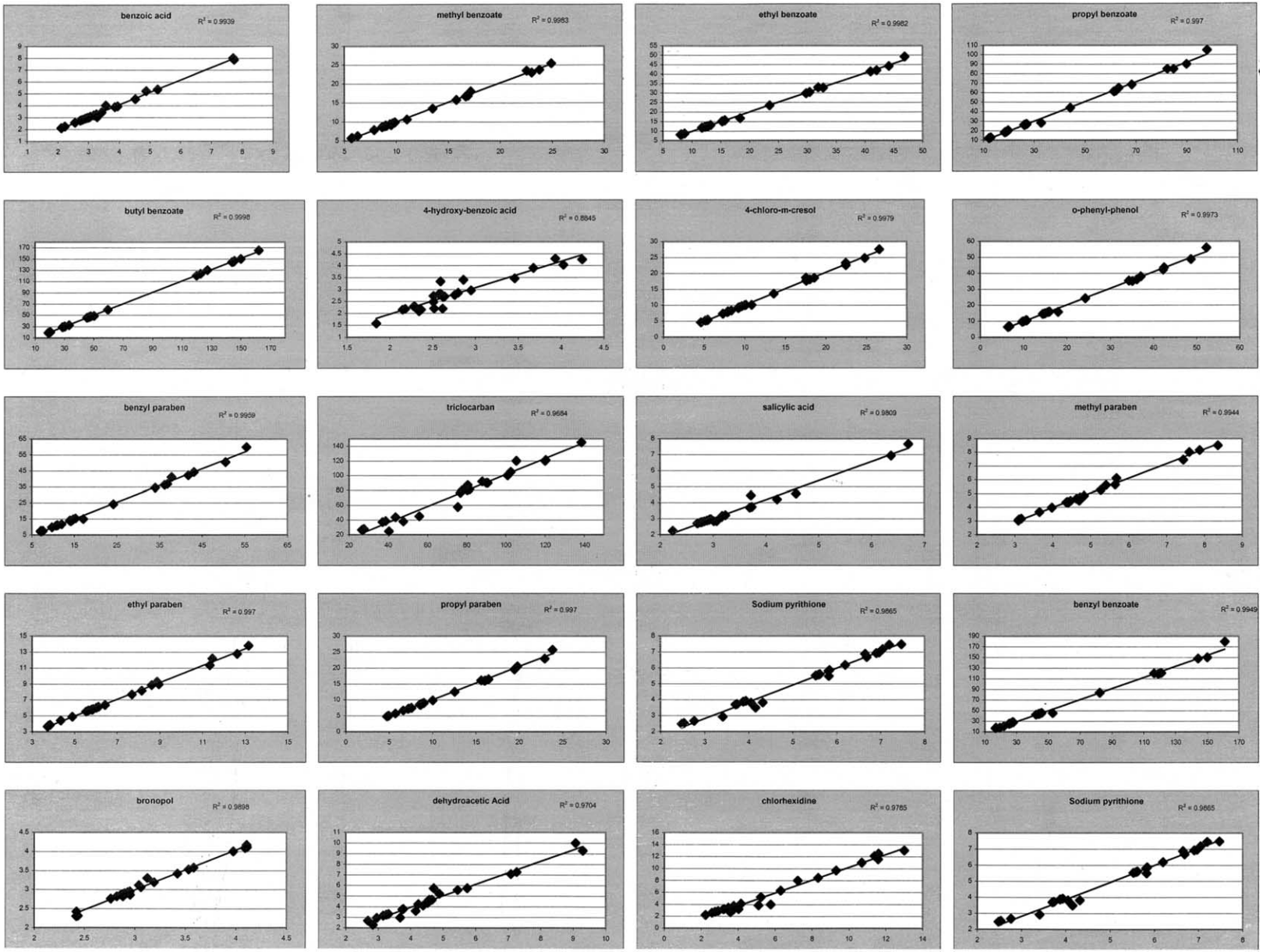

Fig. 4. Leave-one-out cross-validation (LOD-CV) results for each analyte.

has been applied, in which the iteration of the network training which provided the best prediction of the experiment left out. Correlation coefficient of the fitted experiments above the threshold of 0.80, was selected as that providing the optimal network. This procedure guarantees for the capacity of the network to generalize the information present in the data and to work as a predictive tool. Afterwards the network to be employed for the optimization is calculated for all the experiments, in order to exploit all the information available.

To check the predictive ability of each architecture, a leave-one-out cross-validation (LOD-CV) scheme was followed, that is the only approach possible because the number of available experiments is restricted. There is anyway a high risk to obtain, for some experiments, a good predictive neural model by chance. This was demonstrated by some good predictive abilities for the left out experiment, corresponding to rather poor fitting abilities on the training set. Usually these situations correspond to a very low number of learning epochs. In fact, the network learn very soon to model the training set. In order to avoid the risk of good but

Table 2
Predicted and experimental retention time in the optimized conditions

	Predicted	Observed
Chlorexidine	2.74	2.74
4OH benzoic acid	3.15	2.66
Salicylic acid	3.98	2.82
Benzoic acid	4.52	3.00
Bronopol	3.56	3.66
Methyl-paraben	5.04	4.72
Dehydroacetic acid	6.50	5.05
Ethyl-paraben	7.00	6.51
Sodium pyrithione	7.75	6.76
Propyl-paraben	10.17	9.95
4-Chloro-*m*-cresol	11.20	10.84
Methyl-benzoate	11.63	11.31
Butyl-paraben	15.40	16.28
Benzyl-paraben	15.80	16.98
o-Phenyl-phenol	16.89	17.97
Ethyl-benzoato	18.43	18.62
Propyl-benzoate	30.75	32.66
Butyl-benzoate	45.80	55.52
Benzyl-benzoate	47.65	59.02
Triclocarban	94.93	106.10

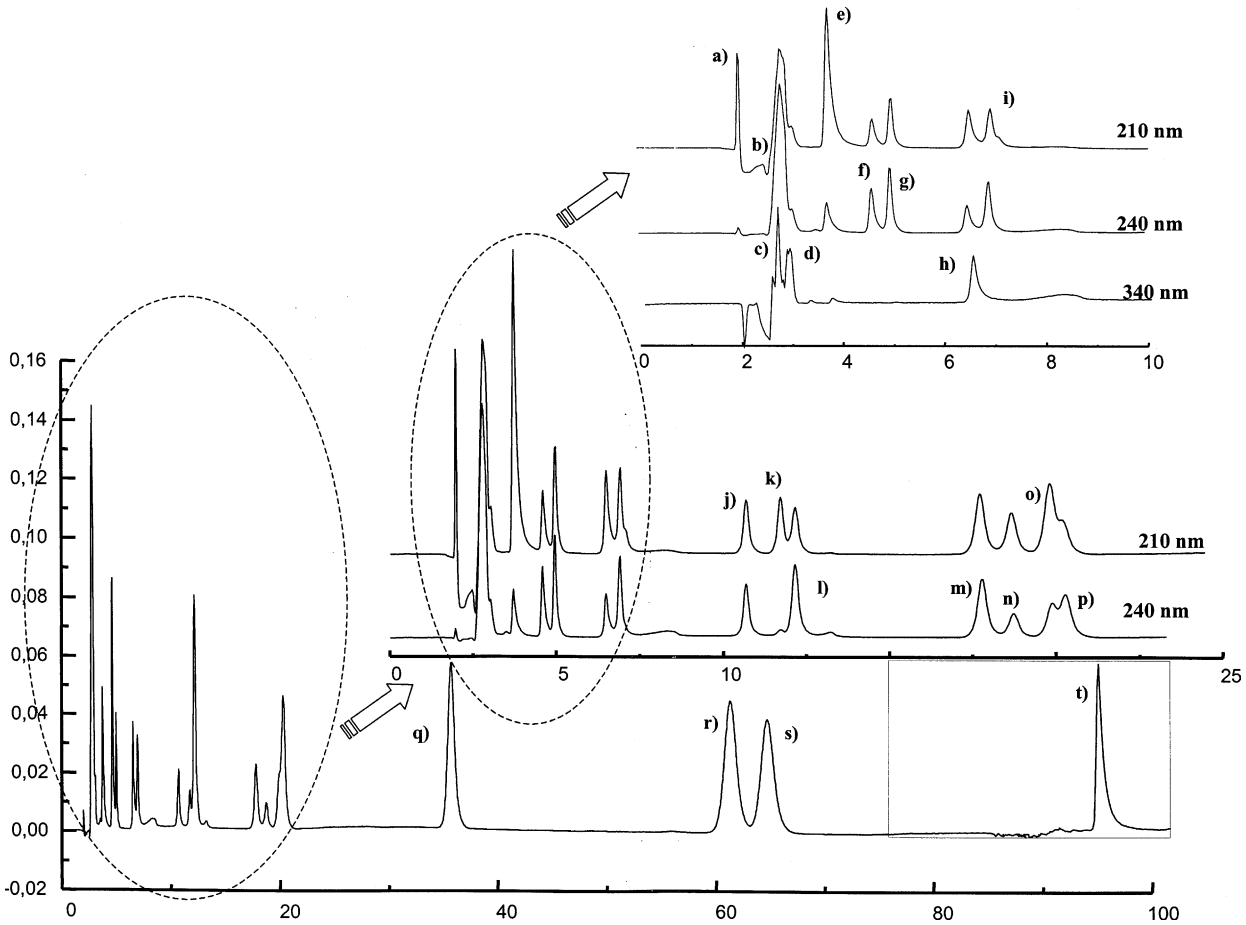

Fig. 5. Chromatograms obtained in the optimized conditions at different λ stationary phase: Merck Superspher 100 RP18 endcapped column (250.0 mm × 4.6 mm, 4 μm), together with a Chrompack C_{18} (3.0 mm × 5.0 mm, 5 μm) guard pre-column. Mobile phase: C_{ACN} = 22.5%, pH = 4.1, F = 0.9 ml/min, C_{IIR} = 1.4 mM; peaks identification: (a) 4-hydroxybenzoic acid; (b) chlorhexidine; (c) salicylic acid; (d) benzoic acid; (e) bronopol; (f) methyl-paraben; (g) dehydroacetic acid; (h) ethyl-paraben; (i) sodium pyrithione; (j) propyl-paraben; (k) 4-chloro-*m*-cresol; (l) methyl benzoate; (m) butyl-paraben; (n) benzyl-paraben; (o) *o*-phenyl-phenol; (p) ethyl benzoate; (q) propyl benzoate; (r) butyl benzoate; (s) benzyl-benzoate; (t) triclocarban.

unreliable predictive results, a threshold on the fitting ability of the best predictive neural networks was introduced. This means that the good predictive results are accepted only if the fitting ability of the network is satisfactory: a multiple correlation coefficient higher than 0.80 was selected as the threshold.

Even if for several analytes two neurons in the hidden layer were sufficient to obtain good results, for others five neurons were necessary, so this was the final architecture selected. The results obtained from the LOD-CV are reported in Fig. 4. The predictions are all very satisfactory. The worst predicted ($R^2_{CV} = 0.8845$) is 4OH benzoic acid; but the result can be: considered acceptable, given the narrow range of the retention times observed for this analyte; The other analytes have R^2_{CV} greater than 0.9700.

Since it had been demonstrated that the neural network was able to effectively predict the retention times of the left-out experiments, a final network with the same architecture was used for the optimization purpose. The chromatographic optimization was performed by a grid search algorithm, exploring the region defined by the experimental design extremes, dividing each factor in 21 intervals. A total of 21^4 situations was evaluated, simulating the corresponding retention times by mean of the neural network. The target function was the difference between the nearest peaks, which had to be maximized in order to obtain the best chromatographic resolution. The best conditions are found as: $C_{ACN} = 22.5\%$, pH = 4.1, $F = 0.9$ ml/min, $C_{IIR} = 1.4$ mM; Table 2 reports the experimental and predicted retention times in these conditions. It can be observed that the neural network predictive ability is very satisfactory. Fig. 5 shows the chromatogram obtained in the optimized conditions at 210 nm. Since not all analytes absorb at the same UV wavelength, a further analysis was performed by mean of the diode array (Fig. 5).

Under the optimized conditions repeatability and reproducibility of the method were evaluated. Repeatability is obtained by independent trials of the same method, in the same laboratory, by the same operator in a short time period; in these conditions three different injections of a mixture containing all the analytes at concentration of 2.00 mg/l each are performed and the averages value of the retention times, the standard deviation and the percent variation coefficient are calculated. The method repeatability results very good as is never higher than 0.86% (Table 3). To evaluate the reproducibility is necessary to prepare a new mobile phase and a new mixture of all the analytes. The new mixture is analyzed three times by a different operator in a different day. The average retention times obtained with the two mixture are compared and the standard deviation and the CV% are estimated. CV% is always lower than 3.5 with the only exceptions of 4OH benzoic acid and salicylic acid (CV of about 6%).

For each analyte a calibration plot reporting the peak area versus standard concentration is constructed for three different standard concentrations, injected with increasing concentration to overcome possible memory effects. Due to the different sensitivity of the analytes the concentrations range was between the quantitation limits (LOQs) and 7.00 mg/l. The linearity of the response (peak area versus concentration) is verified in the whole range investigated. The correlation coefficients R^2 are always >0.9574.

In order to express the sensitivity (given as the peak area for 1.0 mg/l concentration as obtained by the slopes of

Table 3
Validation parameters for repeatability and reproducibility evaluation

	Day 1			Day 2			Reproducibility				
	$t_{R,Av.1}$ (min)	S.D.	CV (%)	$t_{R,Av.2}$ (min)	S.D.	CV (%)	$t_{R,Av.1}$ (min)	$t_{R,Av.2}$ (min)	Average (min)	S.D.	CV (%)
Chlorexidine	2.67	0.03	0.40	2.81	0.07	1.00	2.67	2.81	2.74	0.03	0.48
OH benzoic acid	2.78	0.02	0.62	2.54	0.03	1.36	2.78	2.54	2.66	0.17	6.38
Salicylic acid	2.91	0.03	0.87	2.74	0.02	0.63	2.91	2.74	2.82	0.12	4.17
Benzoic acid	3.06	0.02	0.57	2.94	0.02	0.59	3.06	2.94	3.00	0.08	2.83
Bronopol	3.67	0.02	0.47	3.64	0.01	0.32	3.67	3.64	3.66	0.02	0.52
Methyl-paraben	4.73	0.02	0.37	4.71	0.03	0.74	4.73	4.71	4.72	0.01	0.30
Dehydroacetic acid	5.16	0.04	0.78	4.94	0.03	0.54	5.16	4.94	5.05	0.15	3.03
Ethyl-paraben	6.53	0.03	0.44	6.49	0.04	0.62	6.53	6.49	6.51	0.03	0.43
Odium pyrithione	6.71	0.03	0.41	6.82	0.03	0.58	6.71	6.82	6.76	0.01	0.32
Propyl-paraben	9.99	0.03	0.35	9.91	0.05	0.53	9.99	9.91	9.95	0.06	0.57
4-Chloro-*m*-cresol	10.90	0.06	0.52	10.79	0.08	0.76	10.90	10.79	10.84	0.08	0.70
Methyl-benzoate	11.35	0.03	0.31	11.27	0.05	0.47	11.35	11.27	11.31	0.06	0.50
Butyl-paraben	16.36	0.06	0.39	16.21	0.11	0.65	16.36	16.21	16.28	0.11	0.65
Benzyl-paraben	17.08	0.08	0.44	16.88	0.15	0.87	17.08	16.88	16.98	0.14	0.85
o-Phenyl-phenol	18.08	0.09	0.51	17.85	0.14	0.78	18.08	17.85	17.97	0.17	0.93
Ethyl-benzoato	18.69	0.06	0.29	18.54	0.11	0.61	18.69	18.54	18.62	0.11	0.57
Propyl-benzoate	32.83	0.12	0.35	32.49	0.20	0.62	32.83	32.49	32.66	0.24	0.73
Butyl-benzoate	55.89	0.24	0.44	55.15	0.39	0.71	55.89	55.15	55.52	0.53	0.95
Benzyl-benzoate	59.37	0.22	0.37	58.67	0.33	0.56	59.37	58.67	59.02	0.49	0.84
Triclocarban	107.71	0.58	0.54	104.5C	1.11	1.07	107.71	104.50	106.10	2.27	2.14

Table 4
Correlation coefficients, LODs and LOQs for the analytes (injection volume = 100 μl)

	R^2 calibration plot	LOD (mg/l)	LOQ (mg/l)
Chlorexidine	0.9959	0.045	0.150
4OH benzoic acid	0.9998	0.015	0.051
Salicylic acid	0.9898	0.127	0.422
Benzoic acid	0.9998	0.329	1.097
Bronopol	0.9898	0.021	0.071
Methyl-paraben	0.9574	0.053	0.176
Dehydroacetic acid	0.999	0.024	0.079
Ethyl-paraben	0.9884	0.034	0.112
Sodium pyrithione	0.9999	0.020	0.066
Propyl-paraben	0.9998	0.019	0.064
4-Chloro-*m*-cresol	0.9999	0.045	0.149
Methyl-benzoate	0.9977	0.022	0.075
Butyl-paraben	0.9996	0.018	0.061
Benzyl-paraben	0.9976	0.014	0.045
o-Phenyl-phenol	0.9993	0.071	0.236
Ethyl-benzoato	0.9598	0.013	0.043
Propyl-benzoate	0.9988	0.050	0.167
Butyl-benzoate	0.9999	0.012	0.042
Benzyl-benzoate	0.9986	0.018	0.062
Triclocarban	0.9965	0.095	0.318

the calibration plots) into concentration units, in the chromatogram an area which corresponds to a signal to noise ratio around 3 is identified and used to proportionally transform sensitivity into concentration units (mg/l). LOD values, reported in Table 4, are always lower than 0.35 mg/l. Quantitation limits also reported in Table 4 are evaluated by the calibration plots as the lowest concentration that can be quantified and are estimated as a signal greater than a signal to noise ratio equal to 10.

4.1. Real samples analysis

The optimized method has been applied to the analysis of two shaving foams and one deodorizer purchased in a hard discount and in a perfume shop.

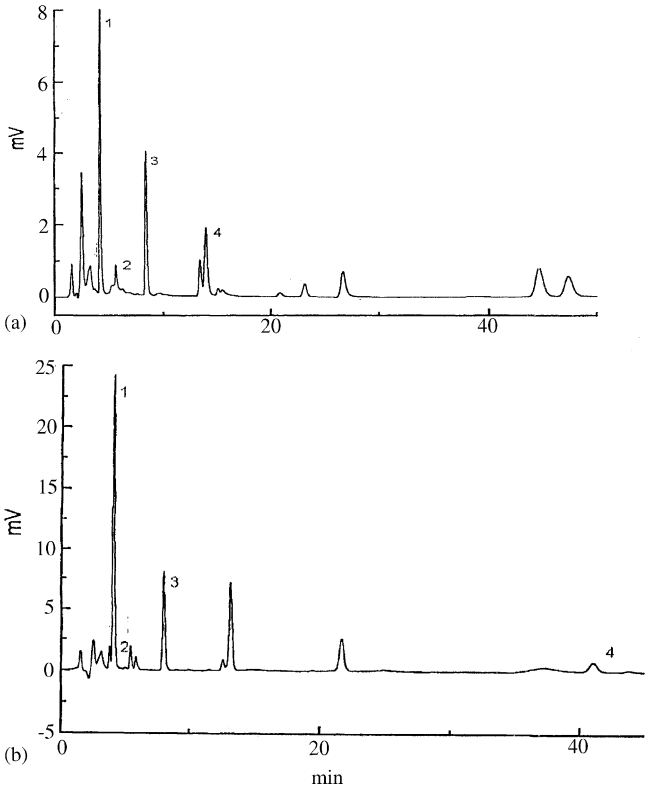

Fig. 6. Chromatograms of the two shaving foams (mobile phase and stationary phase as in Fig. 5). (a) Sample more expensive; peaks identification: (l) methyl-paraben; (2) ethyl-paraben; (3) propyl-paraben; (4) butyl-paraben. (b) Sample purchased in a hard discount; peaks identification: (1) methyl-paraben; (2) ethyl-paraben; (3) propyl-paraben; (4) benzyl-benzoate.

Fig. 6 reports the chromatograms of the two foams shows the presence of a number of different preservatives that have been identified with diode array and with standard addition methods. The number of preservatives identified is greater than that of those declared on the label, while the label indicate the presence of ethyl-, propyl-, butyl- and

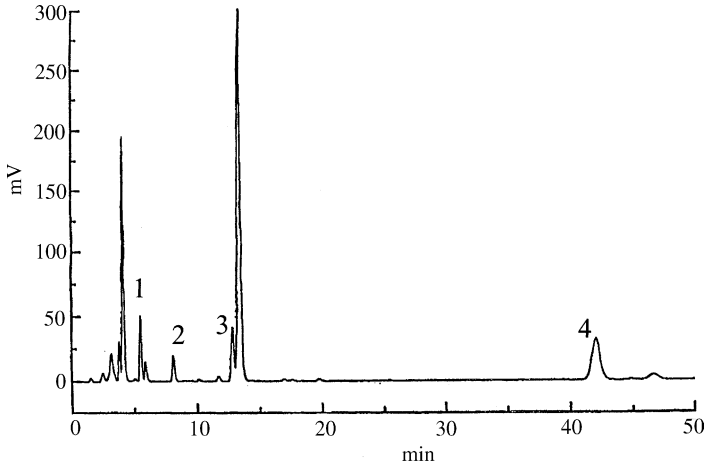

Fig. 7. Chromatograms of the deodorizer (mobile phase and stationary phase as in Fig. 5); peaks identification: (1) ethyl-paraben; (2) propyl-paraben; (3) butyl-paraben; (4) benzyl-benzoate.

Table 5
Concentration (mg/l) obtained from the real samples analysis

	RS1	RS2	RS3
Ethyl-paraben	6.0 + 0.3	12.7 + 0.6	30.4 + 1.5
Propyl-paraben	36.5 + 1.8	60.0 + 3.0	13.7 + 0.7
Butyl-paraben	27.4 + 0.3		43.7 + 2.1
Benzyl-benzoate		**62.1 + 3.1**	**110 + 5**
Methyl-paraben	45.4 + 2.2	**116 + 5.8**	

In bold are reported the analytes not declared in the product labels.

methyl-paraben and the preservatives identified are: ethyl-, propyl-, butyl- and methyl-paraben and benzyl-benzoate. In particular it can be noticed that the sample more expensive (Fig. 6a) is characterized by an higher content of parabens respect the more cheap one (Fig. 6b).

The third sample analyzed is an herbal deodorizer (Fig. 7); product label reports the presence of parabens, but the analysis have revealed also the presence of benzyl-benzoate in concentration of about 100 mg/l.

All the results of real samples analysis are reported in Table 5.

5. Conclusions

The multiresidue method here developed can be advantageously used in quality control of hygiene products, since it permits in only one chromatographic run the identification and the determination of a great number of preservatives, each with different chemical structure. In the application of the method to real samples it was possible to identify the presence of a greater number of preservatives with respect to those indicated on the label.

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