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Microemulsion electrokinetic chromatography: An application for the simultaneous determination of suspected fragrance allergens in rinse-off products

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ABSTRACT

A mixture of 18 neutral UV-active compounds with different characteristics of polarity was determined by capillary electrophoresis using a pseudostationary phase constituted by a microemulsion. The test analytes were volatile fragrance compounds, included in a list of 24 chemicals classified as suspected allergens according to Directive 2003/15/CE.

The considered compounds were detected at 195 nm and *p*-anisaldehyde was chosen as internal standard. The background electrolyte consisted of a standard microemulsion made of 90.95% 10 mM borax buffer, pH 9.2, 1.05% *n*-heptane, 8.00% SDS/*n*-butanol in 1:2 ratio, to which 40 mM methyl-β-cyclodextrin was added. Temperature and voltage were set at 20 °C and 25 kV, respectively. These experimental conditions allowed separation of the compounds to be obtained in about 20 min. The method was applied to real samples made up of rinse-off scented products.

The results obtained using the standard microemulsion as pseudostationary phase showed its high resolution power, capable of effectively separating a complex mixture of analytes. Microemulsion electrokinetic chromatography was confirmed to have a great potential for different analytical challenges, holding up the possibility of using this technique as a good and complementary alternative to HPLC methods for routine analysis.

1. Introduction

Capillary electrophoresis (CE) has gained increasing attention due to its ability for rapid separation, high efficiency, low sample consumption, on-line detection and easy automation [1]. Furthermore, it is an analytical technique which falls within green chemistry as small amounts of organic solvents are needed, and it presents high selectivity thanks to the wide range of separative approaches that allow complex analytical problems to be solved.

An apparent drawback of CE is the lack of standardized systems suitable to routinely solve different analytical challenges. In fact, when dealing with a new sample consisting of a complex mixture of analytes, it is often necessary to test in parallel different operative modes and/or pseudostationary phases to determine the initial starting conditions for method development.

In this work, a microemulsion system previously optimised and used by the authors as starting point for further optimisation studies [2–4] was directly applied to the determination of 18 fragrance allergens in scented products, with the aim of demonstrating the potential and suitability of this pseudostationary phase for the separation of complex mixtures and thus its possible routine use for the analysis of different samples.

According to Directive 2003/15/EC of the European Parliament and Council, amending Council Directive 76/768/EEC [5], the identity of 26 raw materials, classified as suspected allergens (SAs), should be labelled in cosmetics in descending order of weight if their concentration exceeds certain limits. These limits correspond to 0.001% (10 ppm) in products intended to remain on the skin, or 0.01% (100 ppm) in products intended to be rinsed off the skin. Among the 26 ingredients, 24 are volatile chemicals, while two are natural extracts. This regulatory requirement necessitates reliable procedures able to detect and quantify low levels of these ingredients in highly complex mixtures, and this represents an analytical challenge in terms of sensitivity and selectivity. For these reasons, the practical impossibility of determining all target compounds in all different matrices using one single method [6] and of quantitatively analysing most fragrance mixtures by simply using just one analytical dimension [7] has been pointed out.

Due to the volatility of the 24 SAs, many analytical strategies for their determination are based on gas chromatography, and a classification of the GC procedures has been recently proposed [6]. The main approach followed by researchers has been the devel-

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opment of a GC–MS procedure for analysing a partial [8–13] or a complete [14–19] test mixture of the 24 SAs. Comprehensive two-dimensional GC [7,20], targeted multidimensional gas chromatography [7] and GC × GC–MS [21–24] have also been proposed. Only one HPLC procedure has been developed [25] where the ingredients were separated in a running time of 40 min.

To our knowledge, up to now no CE method for the analysis of SAs has been presented. Thus, the novelty of this study consists in the use of Microemulsion electrokinetic chromatography (MEEKC) [26–28] for the quantitative analysis of 18 compounds included in the original group of 24 SAs. These 18 ingredients, listed in Table 1, have been selected as target of this work due to their property of being UV-active compounds and thus detectable by CE on-column diode-array detector (DAD). The use of a pseudostationary phase was necessary due to the neutral characteristics of the 18 analytes, and this opened the way to direct application of the standard microemulsion system previously cited and thus to evaluate its potential for the separation of another complex sample. A suitable cyclodextrin (CD) was also added to the background electrolyte (BGE), improving the separation and giving rise to CD-MEEKC [3,4,29].

The separation of the compounds was obtained in about 20 min and the analytical performance of the method was tested in terms of selectivity, robustness, linearity, accuracy and precision. The developed procedure was then applied to the analysis of allergens in real rinse-off scented products (i.e. a shampoo and a bath gel).

2. Materials and methods

2.1. Chemicals

The reference standards of the 18 SAs (Table 1) and of the internal standard *p*-anisaldehyde (AN) were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Sodium tetraborate decahydrate (borax) was from BDH Laboratory Supplies (Poole, UK) and *n*-butanol was obtained from Merck (Darmstadt, Germany). Acetonitrile (ACN) (HPLC grade), *n*-heptane, sodium dodecyl sulfate (SDS), methyl-β-cyclodextrin (MβCD), heptakis(2,6-di-O-methyl)-β-cyclodextrin (DMβCD), heptakis(2,3,6-tri-O-methyl)-β-cyclodextrin (TMβCD), (2-hydroxypropyl)-β-cyclodextrin (HPβCD), (2-hydroxypropyl)-γ-cyclodextrin (HPγCD), (2-hydroxyethyl)-β-cyclodextrin (HEβCD) were from Sigma-Aldrich (St. Louis, MO, USA).

A Simplicity 185 system (Millipore, Billerica, MA, USA) was employed to purify water previously treated by a deionisation treatment using an Elix system (Millipore).

2.2. Solutions and microemulsions

The standard stock solutions of the tested SAs (10 mg mL^{-1}) and AN (1 mg mL^{-1}) were prepared in ACN and were stored in a freezer at −20 °C. A 0.5 mg mL^{-1} mixture (mixture A) of all the analytes was made weekly by proper dilution of the stock solutions with ACN and stored at 4 °C. Working standard solutions were prepared daily by diluting mixture A with water directly in a vial to 500 μL, together with the appropriate volume of AN standard stock solution. In this way the final concentration for the fragrance allergens was included in the range 0.010–0.200 mg mL^{-1}, while the concentration of AN was kept constant at 0.050 mg mL^{-1}.

Microemulsion buffer was composed of 90.95% aqueous phase (10 mM borax, pH 9.2), 1.05% oil phase (*n*-heptane), 8.00% surfactant/cosurfactant (SDS/*n*-butanol) in 1:2 ratio. This microemulsion was obtained by sequentially mixing in a beaker suitable amounts of aqueous phase, cosurfactant, surfactant and finally oil, taking care to add each component only after reaching a complete dissolution of the previously mixed compounds. The optimum BGE was prepared by adding to the microemulsion 40 mM MβCD.

2.3. Sample preparation

The considered real samples were commercially available rinse-off products, namely a shampoo and a bath gel. For sample preparation, an accurately weighed portion of the cosmetic product, corresponding to about 1 g, was transferred into a beaker to which 2 mL of water were added. This mixture was gently stirred for 2 min. A 300 μL aliquot of this mixture together with 25 μL of internal standard stock solution and 175 μL of water were added to a 500 μL vial. In this way, if the concentration of the fragrance allergens present in the original sample was 0.01%, the final test concentration for the CE analysis would be 0.020 mg mL^{-1}.

2.4. Instrumentation and capillary electrophoresis analysis

A multiple magnetic stirrer Multipoint HP15 (Ney Company, Bloomfield, USA) was used to stir microemulsions. All electropherograms were obtained on an Agilent Technologies ^{3D}CE system (Agilent Technologies, Waldbronn, Germany) equipped with a UV–vis DAD. The capillary was thermostated by air. Instrument control and data acquisition and analysis were performed by ^{3D}CE ChemStation software (Rev. A.09.01, Agilent Technologies).

Fused-silica capillaries (50 μm inner diameter) were from Composite Metal Services (Ilkley, UK). The length of the capillary to the detector was 56.0 cm (total length, 64.5 cm). The capillaries were cut using a Capillary Cleaving™ tool (Supelco, Bellefonte, PA, USA). The detection window was built-in by burning off the polyimide coating on the capillary using The Windowmaker™ (MicroSolv, Postnova Analytics, Landsberg/Lech, Germany).

CE separation conditions were: voltage 25 kV; temperature 20 °C; detection wavelength 195 nm; hydrodynamic sample injection for 5 s at 50 mbar. A generated current of 45 μA was observed. Each new capillary was initially conditioned with 1 M NaOH and water for 5 min each. Between the electrophoretic runs, the capillary was rinsed for 2 min with methanol, 2 min with 0.1 M NaOH, 1 min with water and 3 min with the BGE.

2.5. Calibration curves, calculations, software

The calibration method used was the internal standard procedure. The calibration curves ranged from 0.010 mg mL^{-1} to 0.200 mg mL^{-1}, apart from cetone alpha and hexyl cinnamal, for which the range was from 0.020 mg mL^{-1} due to their lower UV absorbance. For each curve five concentration values were analysed, performing two replicates for each sample.

The curves were constructed by plotting the corrected peak area ratios of analyte/internal standard *versus* analyte concentrations. For cetone alpha, which corresponded to two separated peaks in the electropherogram related to the two isomers of this compound (15A and 15B), the sum of 15A and 15B corrected areas/internal standard corrected area ratio was plotted *versus* cetone alpha concentration.

Resolution values R were calculated on the basis of the formula $R = 2(t_{RB} - t_{RA}/w_B + w_A)$, where t_{RA} and t_{RB} are the migration times and w_A and w_B the widths at the bases of adjacent peak pairs, respectively [30].

The experimental design software used to set-up the experimental plan for testing robustness and for statistically treating the obtained data was NEMROD-W [31].

Table 1
Target fragrance allergens.

	Compound	CAS	IUPAC name
1	Benzyl alcohol	100-51-6	α-Hydroxytoluene
2	Anisyl alcohol	105-13-5	(4-Methoxyphenyl)-methanol
3	Coumarin	91-64-5	2H-1-benzopyran-2-one
4	Cinnamyl alcohol	104-54-1	3-Phenyl-2-propen-1-ol
5	Cinnamal	104-55-2	3-Phenyl-2-propenal
6	Eugenol	97-53-0	2-Methoxy-4-(2-propenyl) phenol
7	Linalool	78-70-6	3,7-Dimethyl-1,6-octadien-3-ol
8	Isoeugenol	97-54-1	2-Methoxy-4-(1-propenyl) phenol
9	Citral	5392-40-5	(E)-3,7-dimethylocta-2,6-dienal
10	Geraniol	106-24-1	3,7-Dimethyl-2,6-octadien-1-ol
11	Citronellol	106-22-9	3,7-Dimethyl-6-octen-1-ol
12	Methylheptin carbonate	111-12-6	Methyl oct-2-ynoate
13	Benzyl benzoate	120-51-4	Benzoic acid phenylmethyl ester
14	Benzyl salicylate	118-58-1	2-Hydroxybenzoic acid phenylmethyl ester
15	Cetone alpha	127-51-5	4-(2,6,6-Trimethylcyclohex-2-en-1-yl)-3-methylbut-3-en-2-one
16	Benzyl cinnamate	103-41-3	Benzyl 3-phenyl propenoate
17	Amyl cinnamal	122-40-7	2-Phenylmethylene heptanal
18	Hexyl cinnamal	101-86-0	2-Phenylmethylene octanal

3. Results and discussion

3.1. Standard microemulsion as pseudostationary phase

The 18 considered fragrance allergens may be divided into four main classes according to their functional groups, namely alcohols, esters and lactones, carbonyl compounds and phenols (Fig. 1). They present different hydrophilic/lipophilic characteristics but they all have neutral properties, and in order to obtain their electrophoretic separation a suitable charged pseudostationary phase is necessary. Moreover, due to the analytical challenge represented by the separation of such a high number of chemicals, it would be preferable to take advantage of a large migration window.

A standard microemulsion system made up of 90.95% aqueous phase (*W*, 10 mM borax, pH 9.2), 1.05% oil phase (*O*, *n*-heptane), 8.00% surfactant/cosurfactant (*S*/*CoS*, SDS/*n*-butanol) in 1:2 ratio

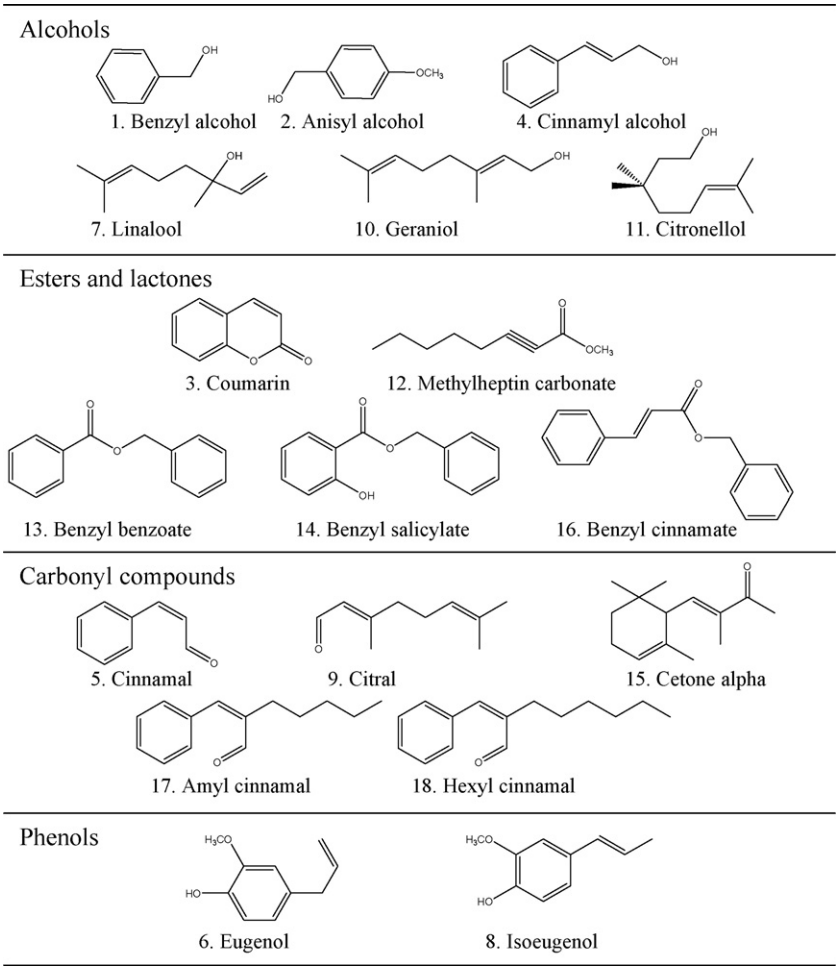

Fig. 1. Molecular structures of the target fragrance allergens.

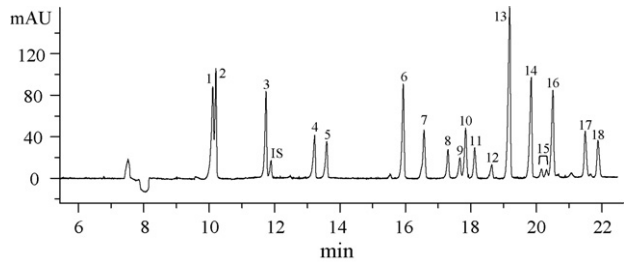

Fig. 2. Electropherogram of the fragrance allergens referring to the optimal CD-MEEKC conditions: BGE, standard microemulsion composed by 90.95% aqueous phase (10 mM borax, pH 9.2), 1.05% oil phase (*n*-heptane), 8.00% surfactant/cosurfactant (SDS/*n*-butanol) in 1:2 ratio; MβCD concentration, 40 mM. Voltage, 25 kV; temperature, 20 °C; hydrodynamic injection 50 mbar, 5 s; detection wavelength: 195 nm. Numbered peaks correspond to those listed in Table 1.

was previously optimised and employed by the Authors as a powerful starting point to obtain the MEEKC or the cyclodextrin-MEEKC separation of different mixtures of compounds, namely neutral and basic and/or acidic substances [2–4]. In these studies, the standard microemulsion was selected as the centre point of a mixture design [32] planned to find the optimum composition of the microemulsion for the analysis of the different considered test samples. The experimental range for the components was 88.0–93.9% for the aqueous phase, 0.1–2.0% for the oil phase and 6.0–10.0% for the mixture S/CoS in 1:2 ratio. For all the different groups of analytes investigated the optimal composition of the microemulsion was found inside this experimental space, pointing out the wide versatility of this mixture and its high resolving power.

Thus, it was decided to evaluate the possibility of directly using the standard microemulsion as pseudostationary phase for the analysis of the mixture of fragrance allergens, with the aim of confirming its suitability to rapidly obtain the separation of complex mixtures in a few steps. The resolving ability of the system was further increased by adding a suitable cyclodextrin chosen from the cyclodextrins mentioned in Section 2.1. In this way a secondary equilibrium which tuned the separation process was introduced. Adding 40 mM MβCD to the standard microemulsion used as background electrolyte, the separation of the analytes was obtained in about 20 min.

The composition of the microemulsion can be further modulated in order to improve its performance in terms of selectivity and analysis time, and with this purpose the same mixture design successfully employed in the previously cited papers was applied [2–4]. Unfortunately, in this case mixture design failed to reach the above target. In fact, the responses, identified by the critical resolution values between the peaks, could not be statistically treated due to practical problems encountered with the high number of compounds involved and the lack of control of the peak pattern. This is

Table 2
Mean resolution values (R) and confidence limits ($n = 4$, $\alpha/2 = 0.025$).

	Compound	$R \pm$ limit
1	Benzyl alcohol	0.85 ± 0.04
2	Anisyl alcohol	15.79 ± 0.49
3	Coumarin	1.61 ± 0.15
IS	*p*-Anisaldehyde	12.84 ± 0.48
4	Cinnamyl alcohol	3.23 ± 0.17
5	Cinnamal	21.60 ± 0.34
6	Eugenol	5.55 ± 0.38
7	Linalool	6.48 ± 0.55
8	Isoeugenol	3.59 ± 0.54
9	Citral	1.46 ± 0.25
10	Geraniol	2.23 ± 0.36
11	Citronellol	4.80 ± 0.53
12	Methylheptin carbonate	3.51 ± 0.48
13	Benzyl benzoate	5.19 ± 0.51
14	Benzyl salicylate	2.43 ± 0.44
15	Cetone alpha	2.49 ± 0.62
16	Benzyl cinnamate	8.16 ± 0.65
17	Amyl cinnamal	2.99 ± 0.44
18	Hexyl cinnamal	–

Resolution values refer to the separation between the indicated peak and the following peak in the electropherogram. For cetone alpha resolution value was taken from its second migrating peak.

Table 3
Robustness testing: four-run Plackett–Burman experimental plan and measured responses.

Exp. no.	T (°C)	V (kV)	*CD conc.* (mM)	R_1	R_2	R_3
1	21	26	39	0.99	1.63	1.61
2	19	26	41	1.08	1.67	1.43
3	21	24	41	1.06	1.70	1.48
4	19	24	39	1.24	1.63	1.65

Table 4
Linearity data.

	Compound	a	s_a	b	s_b	R^2
1	Benzyl alcohol	53.8839	1.0132	−0.0481	0.1242	0.9972
2	Anisyl alcohol	53.5227	1.2463	0.4900	0.1527	0.9957
3	Coumarin	40.1602	0.5223	0.0866	0.0640	0.9986
4	Cinnamyl alcohol	23.6055	0.3435	0.0687	0.0421	0.9983
5	Cinnamal	13.7316	0.1625	0.1383	0.0199	0.9989
6	Eugenol	42.6404	0.4659	0.0058	0.0571	0.9990
7	Linalool	22.9891	0.6937	−0.0726	0.0850	0.9928
8	Isoeugenol	10.4148	0.2032	0.0611	0.0249	0.9970
9	Citral	7.3854	0.1741	0.0030	0.0213	0.9961
10	Geraniol	20.8766	0.4287	0.0428	0.0525	0.9966
11	Citronellol	11.6168	0.2150	0.0257	0.0264	0.9973
12	Methylheptin carbonate	6.7896	0.1927	−0.0144	0.0236	0.9936
13	Benzyl benzoate	62.6596	2.0335	−0.2302	0.2492	0.9916
14	Benzyl salicylate	33.3364	1.1671	−0.2004	0.1430	0.9903
15	Cetone alpha	2.9323	0.0551	−0.0033	0.0068	0.9972
16	Benzyl cinnamate	20.9707	0.6579	0.0405	0.0806	0.9922
17	Amyl cinnamal	13.2257	0.4037	0.1327	0.0495	0.9926
18	Hexyl cinnamal	11.6268	0.2415	−0.0562	0.0297	0.9966

Regression equation, $y = ax + b$; s_a, standard deviation for the slope; s_b, standard deviation for the intercept.

a well-known issue which often arises during the optimisation step of a CE procedure dealing with a complex sample. In other words, by modifying experimental conditions, inversions and changes in the migration order of the peaks may occur, leading to a loss of information about the identity of the peaks and making it difficult to be aware of the effects of different experimental conditions [33]. Nevertheless, even if it was not possible to identify an optimum by means of response surfaces [34], all the electropherograms obtained were visually inspected and from them it was possible to define the optimum experimental conditions as those just in the centre of the experimental range, corresponding to the standard microemulsion. The related electropherogram represented the best compromise with respect to selectivity and analysis time.

The effect of voltage was then investigated by decreasing this factor to 15 kV, but no particular improvement in the separation pattern was noticed. On the contrary, an increase of analysis time was evidenced together with a general decrease of the efficiency of the peaks. Thus, the final optimal experimental conditions were identified using as background electrolyte the standard microemulsion with the addition of 40 mM MβCD and setting voltage at 25 kV. The corresponding electropherogram is shown in Fig. 2, where the concentration of the compounds was set at 0.125 mg mL^{-1}.

3.2. Analytical performances of the method

In order to verify the suitability of the established method for quantitative analysis, selectivity, robustness, linearity, accuracy and precision of the procedure were investigated.

Selectivity of the method was assessed by measuring the values of resolution between peaks at the higher level of the calibration curve (0.200 mg mL^{-1}). The mean calculated values and the confidence limits ($n = 4$, $\alpha = 0.05$) are reported in Table 2. Identification of the peaks was based on migration time for each compound separately and confirmed by spiking each compound in sequence in a mixture.

Robustness of the method was assessed at 0.125 mg mL^{-1} (near the middle of the linearity range) by means of a multivariate strategy [35,36], using a 4-run Plackett–Burman matrix [34]. The selected factors were the independent variables temperature, voltage and concentration of the cyclodextrin and the effect of small changes in the value of these factors was investigated on the critical resolution values. These latter were selected as those corresponding to values around 1.5 at the higher concentration values of the linearity range, namely R_1 between peaks 1 and 2 (benzyl alcohol and anisyl alcohol), R_2 between peaks 3 and internal standard (coumarin and *p*-anisaldehyde), and R_3 between peaks 9 and 10 (citral and geraniol). The experimental domain of the factors was centred on the optimised conditions and corresponded to the following: X_1, temperature (T), 19–21 °C; voltage (V), 24–26 kV; MβCD concentration, (*CD conc.*) 39–41 mM. The planned experiments are reported in Table 3 together with the obtained responses. Statistical treatment of the data [34] showed that none of the considered factors exerted a significant effect on the responses in the considered experimental range, thus confirming the robustness of the method.

Linearity was evaluated in the range 0.010–0.200 mg mL^{-1} apart from cetone alpha and hexyl cinnamal for which it was evaluated in the range 0.020–0.200 mg mL^{-1} due to the low absorbance of these two compounds. The related data are reported in Table 4. The R^2 values were all above 0.99. From the linearity range data it appears that high slopes were obtained for benzyl benzoate, benzyl alcohol, and anisyl alcohol, thus showing a high method sensitivity for these compounds. On the contrary the lowest slope, and thus the lowest method sensitivity, was for cetone alpha. Anyway also for this analyte it was possible to determine concentrations equal to 100 ppm as requested by EU Directive [5].

Table 5
Accuracy and precision data ($n = 3$, $\alpha/2 = 0.025$).

	Compound	Concentration level (mg mL^{-1})	Accuracy (recovery, %)	Repeatability (RSD, %)
1	Benzyl alcohol	0.020	102.3 ± 9.5	3.7
		0.125	97.6 ± 2.9	1.2
		0.175	100.6 ± 5.3	2.1
2	Anisyl alcohol	0.020	94.0 ± 8.9	3.8
		0.125	104.5 ± 1.2	0.5
		0.175	101.1 ± 1.6	0.6
3	Coumarin	0.020	95.8 ± 4.6	1.9
		0.125	100.3 ± 4.9	2.0
		0.175	102.2 ± 3.0	1.2
4	Cinnamyl alcohol	0.020	94.9 ± 6.0	2.6
		0.125	102.4 ± 5.0	2.0
		0.175	101.6 ± 3.7	1.5
5	Cinnamal	0.020	101.2 ± 14.6	5.8
		0.125	96.9 ± 6.6	2.7
		0.175	102.6 ± 3.3	1.3
6	Eugenol	0.020	99.0 ± 10.2	4.1
		0.125	100.1 ± 2.8	1.1
		0.175	102.6 ± 2.2	0.9
7	Linalool	0.020	96.3 ± 9.9	4.1
		0.125	97.7 ± 14.6	6.0
		0.175	105.4 ± 1.6	0.6
8	Isoeugenol	0.020	93.2 ± 10.6	4.6
		0.125	106.0 ± 9.5	3.6
		0.175	102.9 ± 7.2	2.8
9	Citral	0.020	105.6 ± 8.7	3.3
		0.125	98.5 ± 10.3	4.2
		0.175	101.8 ± 1.8	0.7
10	Geraniol	0.020	94.6 ± 8.3	3.5
		0.125	99.2 ± 4.9	2.0
		0.175	101.9 ± 4.4	1.7
11	Citronellol	0.020	106.7 ± 6.9	2.6
		0.125	101.7 ± 5.7	2.2
		0.175	104.5 ± 3.2	1.2
12	Methylheptin carbonate	0.020	103.9 ± 14.5	5.6
		0.125	93.1 ± 1.6	0.7
		0.175	102.7 ± 10.6	4.2
13	Benzyl benzoate	0.020	105.8 ± 12.9	4.9
		0.125	94.5 ± 8.0	3.4
		0.175	106.7 ± 9.1	3.4
14	Benzyl salicylate	0.020	96.9 ± 3.9	1.6
		0.125	95.2 ± 1.8	0.8
		0.175	107.2 ± 1.5	0.6
15	Cetone alpha	0.030	101.8 ± 13.8	5.4
		0.125	95.0 ± 7.7	3.2
		0.175	104.2 ± 5.5	2.1
16	Benzyl cinnamate	0.020	94.0 ± 6.7	2.9
		0.125	106.2 ± 12.8	4.9
		0.175	101.5 ± 13.3	5.3
17	Amyl cinnamal	0.020	93.5 ± 8.5	3.7
		0.125	95.6 ± 11.9	5.0
		0.175	97.7 ± 11.0	4.5
18	Hexyl cinnamal	0.030	106.6 ± 8.9	3.4
		0.125	96.5 ± 5.0	2.1
		0.175	105.2 ± 7.4	2.8

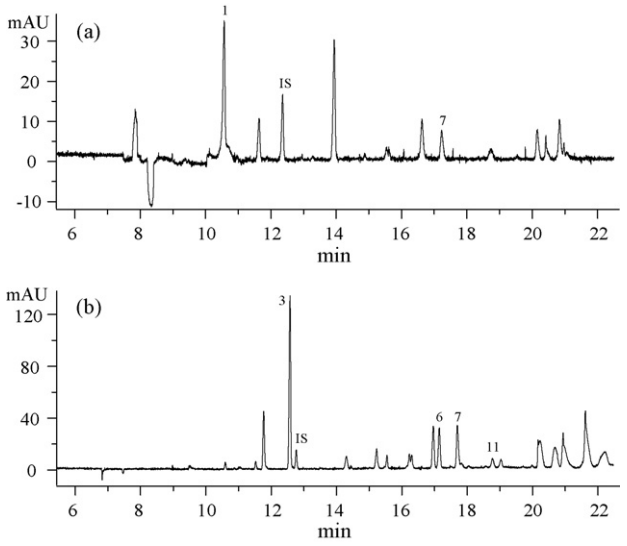

Fig. 3. Electropherogram of the real samples: (a) shampoo; (b) bath gel. Experimental conditions as in Fig. 2. Numbered peaks correspond to those listed in Table 1.

Accuracy and precision of the method were verified at three concentration levels (0.020–0.125–0.175 mg mL^{-1}), each with three replicates, evaluating the recovery values together with their confidence interval and the RSD. For cetone alpha and hexyl cinnamal the lower concentration level was set at 0.030 mg mL^{-1}. The data obtained are presented in Table 5.

3.3. Analytical applications

The developed method was applied to the analysis of two real samples of rinse-off scented products. In the shampoo, whose electropherogram is depicted in Fig. 3a, two labelled allergens were detected, corresponding to benzyl alcohol (peak 1) and linalool (peak 7). For these compounds the determined percentages ($n = 3$, $\alpha/2 = 0.025$) were: benzyl alcohol, 0.031 ± 0.003%, RSD 3.4%; linalool, 0.013 ± 0.001%, RSD 4.2%. In the bath gel, four labelled allergens were detected, namely coumarin, eugenol, linalool, and citronellol (peaks 3, 6, 7 and 11, respectively), and the related electropherogram is shown in Fig. 3b. The percentages of these compounds were ($n = 3$, $\alpha/2 = 0.025$): coumarin, 0.126 ± 0.007%, RSD 2.1%; eugenol, 0.025 ± 0.002%, RSD 3.2%; linalool, 0.055 ± 0.007%, RSD 5.3%; citronellol, 0.027 ± 0.003%, RSD 4.9%.

4. Conclusions

A rapid and selective CD-MEEKC method for the simultaneous determination of 18 fragrance allergens in rinse-off scented products has been developed. The method can be considered a useful approach to detect most allergenic compounds in cosmetics. The main advantages of this method are the use of inexpensive instrumentation and small amounts of solvents, for which CE is recognised as a "green" technique. The main drawback is that not all the 24 compounds mentioned in the European directive can be determined, however this fact is not due to the efficacy of the separative system but rather to the DAD detector. On the contrary, the standard microemulsion separative system demonstrated its great versatility and resolving power. The possibility of rapidly screening 18 fragrance compounds in real samples can be considered a convenient advantage to reaching a cost-effective compromise with respect to other more expensive analytical techniques.

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