

Application of response function methodology for the simultaneous determination of potential fragrance allergens and preservatives in personal care products using micellar electrokinetic chromatography

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Abstract A micellar electrokinetic chromatography method was developed for determination of 15 suspected fragrance allergens and preservatives. The target compounds are widely used as ingredients in many personal care products, and all of them are included in the European Regulation concerning cosmetic products. The method was optimized by using a central composite experimental design and response surface methodology. A modified chromatographic response function was defined to weigh the terms in the response function adequately. After optimization of experimental conditions, a background electrolyte of 100 mM sodium dodecyl sulphate and 24 mM sodium tetraborate and pH 9.0 was selected for the separation of the analytes. The developed methodology was evaluated in terms of linearity, limits of detection and quantification, precision and accuracy, showing appropriate values (i.e., $R^2 \geq 0.99$ and accuracy of 89–115 %). Finally, applicability of the micellar electrokinetic chromatography method was assessed by successfully quantifying fragrance allergens and preservatives in commercial personal care products. The most commonly found analyte was linalool (48.3 % of samples) followed by benzoic acid (37.6 %). All samples contained at least one of the target compounds, thus confirming the ubiquity of fragrance allergens and preservatives in personal care products.

Keywords Potential fragrance allergens · Preservatives · Personal care products · Response function · MEKC-DAD

Introduction

Potential risks of chemicals used in personal care products (PCPs) have received growing attention in the last decades. Considering the extensive use of PCPs, there is an increasing social concern about the safety of ingredients formulated in consumer products. The main classes of PCPs include fragrances, preservatives, antimicrobials, disinfectants, ultraviolet (UV) filters, insect repellents, phthalates, and fixatives [1]. These compounds are commonly used in many types of cosmetic products, such as gels, shampoos, soaps, creams, lotions, perfumes, and hair conditioners; and they are also used in household, food, and pharmaceutical products [2]. Among PCPs, fragrances and preservatives are the two most clinically relevant allergens in cosmetics [3].

Fragrances are ubiquitously used in scented cosmetic products for their odorous properties. Although more than 3,000 chemical substances are used for this aim, the Scientific Committee on Consumer Safety (SCCS) identified among them 26 potential fragrance allergens with a well-recognised potential to cause allergy [4]. These compounds are known in the literature as potentially allergenic substances (PAS) or suspected allergens. Preservatives are used in cosmetic products to protect them against microbial growth, both for safety and to maintain product quality. Among the many preservatives approved for use in cosmetic products, probably most attention has been focused on four esters of 4-hydroxybenzoic acid, commonly known as parabens (methyl, ethyl, propyl, and butyl paraben). Reports found in the literature indicate that parabens are endocrine disrupting chemicals and may induce hormone-related activity [5]. Parabens were also linked to breast cancer in humans [6] but the SCCS refuted this point, and concluded that there was insufficient data to establish that link [5]. Other preservatives, such as salicylic

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acid, benzoic acid, and sorbic acid are also used in commercial cosmetic and other products and, as it has been reported, they may also have negative side-effects [7–14]. In the European Union, the regulation (EC) no. 1223/2009 of the European Parliament [15] harmonized the legislation in the area of cosmetic products, emphasizing the safety aspects in human health and the environmental concerns. According to this regulation, the presence of the 26 PAS must be indicated in the list of ingredients if concentration exceeds 0.001 % in leave-on products and 0.01 % in rinse-off products. The maximum allowed concentrations of parabens (0.4 %), paraben mixtures (0.8 %), salicylic acid (0.6 to 3.0 %, depending on the product type), benzoic acid (2.5 %), and sorbic acid (0.6 %) are also specified.

As a result of this directive and the health and environmental concerns, it became necessary to develop analytical methods for identification and quantification of these ingredients. Published methods for the quantification of preservatives are mainly based on gas chromatography with mass spectrometry detection (GC-MS) or high-performance liquid chromatography (HPLC) with UV detection [16–18]. Gas and liquid chromatographies are also the dominant techniques applied to determination of potential fragrance allergens [19–21] and to simultaneous quantification of both fragrances and preservatives [22]. Moreover, over the last decade capillary electrophoresis (CE) has emerged as a powerful separation technique that can be easily automated and applied to a large number of samples. In addition, it is considered a “green” and inexpensive technique as it consumes much less solvents and chemicals when compared to other methods [23]. Among the variety of electromigration techniques, separation and quantification of preservatives (mainly parabens) has been performed by capillary zone electrophoresis [10, 24, 25] and micellar electrokinetic chromatography (MEKC) [26]. Quantification of potential fragrance allergens by microemulsion electrokinetic chromatography has also been reported in the literature [27]. However, simultaneous separation of both preservatives and potential fragrance allergens has not been accomplished.

The multivariate optimization of capillary electrophoretic methods is a field of growing interest and practicality, and it is being applied successfully as an effective tool for method development and optimization. Some of the main challenges when applying this strategy is how to evaluate quantitatively a whole chromatogram. Many objective functions, also known as chromatographic response functions (CRFs), have been developed in the literature so far. A recent review has shown the usefulness of seven of these mathematical tools [28], including Berridge’s CRF, Glajch’s chromatographic objective function, Dose’s CRF, Schlabach’s chromatographic resolution statistic, Morris’s chromatographic exponential function (CEF), Duarte’s CRF, and Stojanović’s CRF. Several restrictions have to be considered to select the most suitable

function, because results can significantly differ from one to another. Multivariate optimization of CE methods has also been recently reviewed in depth [29]. We previously reported a new modified chromatographic response function (MCRF) [30] that combines both the sigmoid normalization procedure proposed by Divjak et al. [31] and the widely used CEF introduced by Morris et al. [32]. The applicability of this modified function was demonstrated through the analysis of polycyclic musks in perfumes [30].

The aim of this study was to expand the applicability of MEKC by simultaneously separating and quantifying representative potential fragrance allergens and preservatives in PCPs. Special attention was given to the optimization procedure. A central composite experimental design (CCD) together with response surface methodology (RSM) was used to study the results of the design. To evaluate appropriately the electropherograms, the MCRF was applied. The optimized method was then assessed by quantifying the selected targets in commercial PCPs.

Materials and methods

Reagents and materials

Benzyl alcohol, anisyl alcohol, coumarin, eugenol, isoeugenol, citral, linalool, geraniol, methylparaben, ethylparaben, propylparaben, butylparaben, sorbic acid, and sodium tetraborate decahydrate (STB) were purchased from Sigma-Aldrich (Germany). Salicylic acid, benzoic acid, sodium dodecyl sulfate (SDS) and methanol (MeOH) were obtained from Panreac Química S.A. (Spain). Citral and isoeugenol were isomeric mixtures giving two peaks (*cis* and *trans* isomers), and they were considered as the sum of both isomers. All chemicals were of analytical reagent grade or better, unless otherwise stated. Doubly distilled water was taken from an Aquatron A4D system (J. Bibby Sterilin Ltd., UK).

The internal standard (IS) benzalacetone was provided by Fluka (Germany), and it was to a final concentration of 30 µg/mL. The IS was selected to be midway between the shortest and the longest migration time in the electropherogram and it was used to correct for possible variations in injection volumes or migration times.

Standard stock solutions of fragrance allergens and preservatives with concentrations 5 g/L (except for citral and linalool, 10 g/L) were separately prepared by dissolving the compounds in MeOH. Standard mixture solutions used as calibration solutions, as well as for spiking samples, were prepared by convenient dilution of the stock solutions in water with 25 % (v/v) MeOH. All solutions, standards and samples used through the study were filtered with 0.45 µm nylon filters supplied by Teknokroma (Spain), stored at 4–8 °C and warmed at room temperature before use.

Instrumentation and electrophoretic procedure

All experiments were conducted on an Agilent G7100A CE system (Agilent Technologies International, Switzerland), equipped with a diode array detector. Agilent ChemStation software (Rev. B.04.02) was used for instrumental control, data acquisition, and handling. The separations were performed using an extended light path (bubble cell) bare fused-silica capillary with an internal diameter 50 μm and a total length of 64.5 cm (effective length, 56 cm) supplied by Agilent Technologies. Prior to its first use, the capillary was conditioned by flushing with 1 M NaOH (15 min), 0.1 M NaOH (15 min), and finally water (15 min). The capillary was regenerated every day before the analysis by flushing with 0.1 M NaOH, water, and background electrolyte (BGE) for 5 min each. Between injections, the capillary was washed with 0.1 M NaOH (3 min), water (3 min), and BGE (5 min).

The optimized BGE solution consisted of 24 mM sodium tetraborate at pH 9.0 containing 100 mM SDS. This micellar electrolyte solution was prepared by dissolving the electrolyte and the surfactant in 10 % MeOH aqueous solution. MeOH was added to avoid precipitation of hydrophobic compounds. Finally, pH was adjusted to 9.0 with a few drops of 5 M hydrochloric acid.

Samples and standard solutions were injected hydrodynamically by applying a pressure of 45 mbar for 8 s. A constant voltage of 30 kV was employed in all experiments. The temperature of the capillary cartridge was also kept constant at 25 °C.

Detection wavelength was set at 210 nm, except for sorbic acid (260 nm) and citral (240 nm). Peak identification was accomplished by both comparing the spectra (scan from 190 to 400 nm) to that of the standard spectra and comparing the relative migration time of the analyte to the IS. When necessary, further identifications were performed by spiking samples with standard solutions.

Experimental design and RSM analysis was carried out using the Statistica 6.0 software (StatSoft Inc., USA).

Samples and sample preparation

PCPs, covering a wide range of product types, were obtained from commercial stores. Leave-on and rinse-off cosmetic products, such as shampoos, gels, soaps, perfumes, and creams were analyzed. Household air fresheners and oral antiseptics were also included.

Samples were treated as follows: an accurately weighed amount of each product was diluted with 25 % MeOH aqueous solution. Ultrasonication was applied for 2–4 min when necessary to dissolve the sample. Finally, IS was added, solutions were filtered through 0.45 μm nylon filters and then, they were transferred to CE vials prior to analysis. Given the

wide range of concentrations in which these compounds appear in samples, convenient dilutions were made when required.

Results and discussion

Experimental design

A three-variable CCD with six star points placed at an axial distance from the central point was employed to determine the composition of the BGE for optimal separation of analytes. The value of the axial distance was set at ± 1.682 to establish the rotatability condition. This experimental design was chosen because of its efficiency and flexibility, as it allows a reasonable amount of information while not involving an unusually large number of design points [33, 34]. The three considered variables and their low, central, and high levels were STB concentration (26, 30, and 34 mM), SDS concentration (95, 100, and 105 mM), and pH (9.0, 9.2, and 9.4). As explained previously, these ranges were extended as shown in Table 1 to establish the rotatability condition. These variables were selected on the basis of literature [35, 36] and chromatographic insight. Among the most important factors influencing MEKC separations described in the literature [37] (namely surfactant concentration, pH of the BGE, BGE type and concentration, organic modifier type and concentration, and type and concentration of any additives), MeOH concentration was kept to the minimum required to dissolve the analytes and the use of additives was unnecessary and we decided to apply maximum voltage (30 kV) based on our experience [30]. Therefore, SDS and STB concentrations and pH were the most determinant factors affecting our separation. Other variables mentioned before were set at the described values on the basis of some preliminary experiments and experience on previous works.

The experimental ranges of the studied variables were fixed according to those preliminary experiments based on established procedures for MEKC method development [38]. The experimental design consisted of 14 runs and it was completed with three replicates of the central point (C). Therefore, a total of 17 experiments were randomly performed. Analyte concentrations were selected to be intermediate between those corresponding to the studied linear range. After carrying out the experiments specified by the CCD, RSM was applied to investigate the optimum conditions of the selected variables [33, 34]. In our approach, the quality of a electropherogram was defined by resolutions between adjacent peaks ($R^{k,k+1}$) for all compounds and the total analysis time (T). The latter was determined as the time when baseline was reached after the last peak. Resolution between adjacent

Table 1 Experimental factors, levels, CCD matrix, and results

Factor	Level											
	Low	Central	High									
Sodium tetraborate (mM; STB)	26	30	34									
Sodium dodecyl sulfate (mM; SDS)	95	100	105									
pH	9.0	9.2	9.4									
Exp.	STB	SDS	pH	RP	R _{cp}	T(min)	f	g	CRF	MCRF _R	MCRF	
1	26	95	9.4	17	0	35	0.60	0.78	0.47	45.54	51.44	
2	26	95	9.0	18	0	37	0.66	0.75	0.49	40.45	47.26	
3	26	105	9.4	16	0	44	0.52	0.60	0.31	52.78	70.28	
4	26	105	9.0	19	0.5	49	0.82	0.48	0.39	27.43	40.17	
5	34	95	9.4	18	0	51	0.68	0.43	0.30	38.84	58.96	
6	34	95	9.0	17	0	55	0.55	0.34	0.19	51.40	83.28	
7	34	105	9.4	17	0	67	0.64	0.14	0.09	40.16	74.09	
8	34	105	9.0	18	0	71	0.77	0.10	0.08	28.85	54.51	
9	23.3	100	9.2	18	0	34	0.72	0.80	0.58	33.82	37.60	
10	36.7	100	9.2	19	0.5	73	0.83	0.08	0.07	26.47	50.49	
11	30	91.6	9.2	18	0	42	0.62	0.65	0.40	47.13	60.38	
12	30	108.4	9.2	17	0	49	0.52	0.48	0.25	52.78	77.31	
13	30	100	9.5	18	0	51	0.61	0.43	0.27	46.87	71.15	
14	30	100	8.9	19	1.3	62	0.95	0.21	0.20	16.46	29.10	
15(C)	30	100	9.2	19	0.7	54	0.84	0.36	0.31	26.39	42.10	
16(C)	30	100	9.2	19	0.7	55	0.85	0.34	0.29	26.10	42.28	
17(C)	30	100	9.2	19	0.7	57	0.85	0.30	0.25	25.77	42.95	

C central point, *RP* resolved peaks, *R_{cp}* resolution of the critical pair, *T* total analysis time, *f* resolution normalized term, *g* time normalized term, *CRF* chromatographic response function, *MCRF_R* resolution term in the MCRF, *MCRF* modified chromatographic response function (see text)

peaks was calculated using Eq. (1), where t_m^k , w^k , t_m^{k+1} , and w^{k+1} are the migration time and the peak width at base for each of the two consecutive peaks named k and $k+1$, respectively.

$$R^{k,k+1} = \frac{t_m^{k+1} - t_m^k}{(w^{k+1} + w^k)/2} \quad (1)$$

In some cases, peak width could not be calculated as shown due to high overlapping peaks. According to the literature [39], in such cases critical peak resolution was calculated using the ratio of the two overlapped peak heights. Experimental factors, their levels, matrix of experiments and results are presented in Table 1. Table 1 also includes some other responses used in other optimization procedures, evaluated for comparison purposes, such as the resolution of the critical pair (R_{cp}) that was calculated as the resolution of the worst resolved peak (RP) pair and the total number of RP [40]. Total analysis time and all resolutions between adjacent peaks were

also calculated for all conditions of the experimental design and these data were transformed by using the response function.

Response function and multivariate optimization

Transformation of the resolution values to desirability values ($S^{k,k+1}$) ranging between 0 and 1 was achieved using the sigmoid function described in Eq. (2) [31].

$$S^{k,k+1} = \frac{1}{1 + e^{-b_0 \cdot R^{k,k+1} + b_1}} \quad (2)$$

The $S^{k,k+1}$ value should be high (≈ 1) for maximum and low (≈ 0) for minimum acceptable values of resolution. The parameters b_0 and b_1 were determined by employing the limiting conditions $S^{k,k+1} = 0.9$ and 0.1 for $R^{k,k+1} = 2.0$ and 0.5 , respectively. These conditions were chosen according to the fact that accurate determinations of peak area are not tolerable if $R^{k,k+1} < 0.5$. Peak resolution reaches an optimum

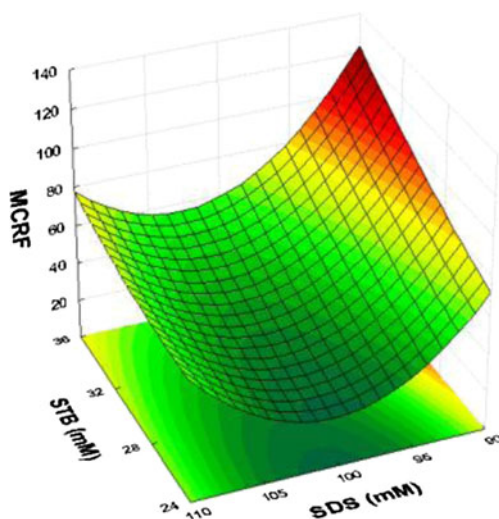


Fig. 1 Global response surface for MCRF estimated by the multivariate model as function of STB and SDS concentrations (pH fixed at 9.0)

at 2.0, so additional increases in resolution should be minimal as there are of no further benefit. The values obtained for the parameters b_0 and b_1 were 2.93 and 3.66, respectively.

The desirability function for resolution (f) was finally calculated in Eq. (3) as the geometrical average of all individual $S^{k, k+1}$ values of N analytes.

$$f = \left(\prod_{k=1}^{N-1} S^{k, k+1} \right)^{1/(N-1)} \quad (3)$$

The second performance goal was to minimize the analysis time (T) that is defined as the time when the electropherogram

reaches the baseline after migration of the last analyte. The desirability value (g) of the electropherogram in regard to analysis time was obtained using the following sigmoid transformation:

$$g = \frac{1}{1 + e^{b_2 \cdot T + b_3}} \quad (4)$$

To obtain appropriate g values, the parameters b_2 and b_3 in Eq. (4) have to be determined. This was also done by employing the limiting conditions $g=0.8$ and 0.1 for $T=34$ min and 71 min. The values obtained for the parameters b_2 and b_3 were 0.097 and -4.68 , respectively.

To compare the results of the MCRF to those of Divjak's approach, the CRF was calculated by multiplying the two desirability values f and g (Eq. 5). CRF should reach a maximum at the set optimum experimental conditions.

$$\text{CRF}(f, g) = f \cdot g \quad (5)$$

However, as in this response function the balance between resolution and time term cannot be adjusted, modified chromatographic response function (MCRF) was proposed.

$$\text{MCRF}(S, g) = \left[\sum_{i=1}^{N-1} e^{\alpha(S_{\text{opt}} - S_i)} + 1 \right] \cdot \left[2 - \frac{g}{g_{\text{opt}}} \right] \quad (6)$$

The left part of the equation combines all the normalized resolutions, so it is only referred to the resolution term, and we have called it MCRF_R . As mentioned before, experimental values of this term obtained in the experimental design are

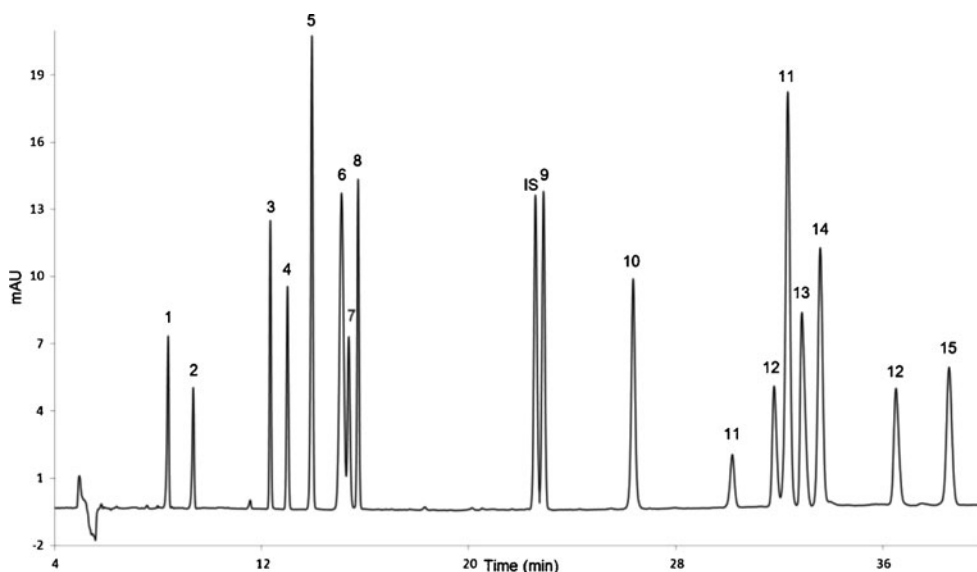


Fig. 2 Electropherogram obtained under optimal separation conditions: BGE (24 mM STB, 100 mM SDS, pH 9.0, and 10 % MeOH); applied voltage, 30 kV; sample introduction, 45 mbar for 8 s. Analyte concentrations were intermediate between those of calibration range. Peaks identification: 1, benzyl alcohol; 2, anisyl alcohol; 3, methylparaben; 4, sorbic

acid; 5, coumarin; 6, salicylic acid; 7, benzoic acid; 8, ethylparaben; 9 propylparaben; 10, eugenol; 11, isoeugenol (isomers); 12, citral (isomers); 13, linalool; 14, butylparaben; and 15, geraniol. Wavelength, 210 nm (note that citral and sorbic acid are quantified at 240 and 260 nm, respectively)

Table 2 Basic method performance parameters including linear ranges, calibration equations, R^2 , LODs, LOQs, and a summary of precision and accuracy results

	Compound	Studied linear range ($\mu\text{g/mL}$)	Slope ^a	Intercept ^a	R^2	LOD ^b ($\mu\text{g/mL}$)	LOQ ^c ($\mu\text{g/mL}$)	Concentration level ($\mu\text{g/mL}$)	Repeatability ^d	Intermediate precision ^e	Accuracy (recovery, %)
1	Benzyl alcohol	5.54–35.59	0.0395 $\pm$ 0.0022	−0.035 $\pm$ 0.048	0.9984	1.85	5.54	5.55	4.0	3.9	106.8
2	Anisyl alcohol	3.44–35.50	0.0252 $\pm$ 0.00088	−0.0082 $\pm$ 0.019	0.9994	1.15	3.44	33.78	5.2	4.3	109.3
3	Methylparaben	6.22–32.71	0.0404 $\pm$ 0.0026	−0.018 $\pm$ 0.051	0.9978	2.07	6.22	3.46	6.8	4.7	115.4
4	Sorbic acid	3.29–21.26	0.0950 $\pm$ 0.0049	−0.017 $\pm$ 0.061	0.9986	1.10	3.29	33.70	7.0	4.6	112.0
5	Coumarin	4.34–21.34	0.0712 $\pm$ 0.0049	−0.0094 $\pm$ 0.060	0.9976	1.45	4.34	6.26	6.4	5.3	104.7
6	Salicylic acid	3.51–21.38	0.0798 $\pm$ 0.0044	−0.0015 $\pm$ 0.055	0.9984	1.17	3.51	31.07	5.8	4.9	112.9
7	Benzoic acid	5.14–32.58	0.0264 $\pm$ 0.0014	0.010 $\pm$ 0.028	0.9985	1.71	5.14	3.31	3.9	4.4	113.0
8	Ethylparaben	4.25–20.90	0.0410 $\pm$ 0.0028	−0.012 $\pm$ 0.034	0.9976	1.42	4.25	20.17	6.1	4.7	95.0
9	Propylparaben	4.64–21.25	0.0386 $\pm$ 0.0028	−0.0043 $\pm$ 0.035	0.9972	1.55	4.64	4.36	2.4	2.5	107.4
10	Eugenol	8.41–28.12	0.0348 $\pm$ 0.0047	−0.051 $\pm$ 0.080	0.9947	2.80	8.41	20.25	5.9	4.6	94.4
11	Isoeugenol	8.12–44.91	0.0321 $\pm$ 0.0027	−0.0074 $\pm$ 0.086	0.9979	2.71	8.12	3.52	5.2	5.7	110.4
12	Citral	17.56–66.33	0.0290 $\pm$ 0.0031	0.099 $\pm$ 0.14	0.9941	5.85	17.56	20.29	4.2	3.7	114.6
13	Linalool	33.12–120.10	0.00553 $\pm$ 0.00067	−0.0023 $\pm$ 0.051	0.9956	11.04	33.12	5.17	10.8	8.6	100.4
14	Butylparaben	9.65–32.84	0.0305 $\pm$ 0.0031	−0.016 $\pm$ 0.060	0.9948	3.22	9.65	30.95	4.1	3.4	113.2
15	Geraniol	6.20–36.20	0.0162 $\pm$ 0.0012	0.017 $\pm$ 0.027	0.9984	2.07	6.20	4.27	2.5	2.5	113.2
								19.83	6.4	6.4	98.4
								4.68	4.2	4.4	109.4
								20.17	3.7	3.1	112.2
								8.44	7.2	12.8	99.5
								26.67	11.2	13.3	105.2
								8.13	11.1	19.2	91.9
								42.64	13.5	13.8	105.2
								17.63	13.9	14.8	99.0
								62.92	12.6	14.6	106.2
								33.19	9.8	12.7	111.6
								113.99	6.8	10.5	102.0
								9.66	7.4	11.6	110.9
								31.19	8.5	10.2	107.2
								6.23	13.5	16.7	88.9
								34.83	4.9	12.0	111.2

SD standard deviation of Y-intercepts of regression lines, *b* slope of the regression line^a Values given at 95 % confidence interval^b LOD=(3.3 × SD)/*b* (in micrograms per milliliter)^c LOQ=3 × LOD (in micrograms per milliliter)^d %RSD from six replicates of standard solutions in two consecutive days^e %RSD from duplicates of standard solutions in 4 days over a 3-week period

Table 3 Concentrations of PAS and preservatives in PCPs: mean of duplicates (in milligrams per kilograms) $\pm$ SD (%RSD)

Sample	Type ^a	Compound ^b	1	2	3	5	6	7	8	9	10	11	12	13	14	15
S1	Sh		7,200 $\pm$ 150 (2.0 %)													
S2	So		42.2 $\pm$ 5.1 (12.1 %)			123.2 $\pm$ 5.7 (4.6 %)	2,760 $\pm$ 120 (4.3 %)	5,740 $\pm$ 230 (4.0 %)			170.4 $\pm$ 13.2 (7.7 %)			161.0 $\pm$ 11.45 (7.1 %)		
S3	G					634.3 $\pm$ 7.8 (1.2 %)		3,510 $\pm$ 330 (9.5 %)						1,410 $\pm$ 160 (11.0 %)		389.9 $\pm$ 32.4 (8.3 %)
S4	G							5,130 $\pm$ 120 (2.3 %)								
S5	P					498.2 $\pm$ 0.36 (0.07 %)										
S6	P					593.8 $\pm$ 0.9 (0.15 %)					2,147 $\pm$ 67 (0.3 %)					
S7	P												69.6 $\pm$ 2.2 (3.2 %)	2,372 $\pm$ 10 (0.4 %)		222.2 $\pm$ 12.9 (5.8 %)
S8	P										1,986 $\pm$ 1 (0.1 %)			1,240 $\pm$ 10 (0.5 %)		345.5 $\pm$ 18.1 (5.2 %)
S9	P												77.7 $\pm$ 3.0 (3.9 %)	7,035 $\pm$ 83 (1.2 %)		
S10	P							273.3 $\pm$ 0.6 (0.2 %)			865.0 $\pm$ 2.1 (0.2 %)		10.8 $\pm$ 1.7 (15.4 %)	3,294 $\pm$ 6 (0.2 %)		
S11	P							87.0 $\pm$ 1.7 (1.9 %)						3,474 $\pm$ 190 (5.6 %)		
S12	P			78.7 $\pm$ 1.3 (1.7 %)		793 $\pm$ 22 (2.8 %)							60.6 $\pm$ 7.2 (11.9 %)	1,742 $\pm$ 48 (2.7 %)		150.3 $\pm$ 12.4 (8.2 %)
S13	P				770.6 $\pm$ 66.2 (8.6 %)											
S14	P												1.30 $\pm$ 0.05 (3.8 %)	464.3 $\pm$ 9.7 (2.1 %)		
S15	HTC															
S16	AC				659 $\pm$ 11 (1.7 %)								27.9 $\pm$ 1.2 (4.2 %)	3,186 $\pm$ 11 (0.35 %)		
S17	OA							4,130 $\pm$ 178 (4.3 %)								
S18	OA							4,535 $\pm$ 250 (5.6 %)								
S19	EC								602 $\pm$ 25 (4.1 %)	280 $\pm$ 13 (4.7 %)						
S20	G			2,575 $\pm$ 101 (3.9 %)			2,462 $\pm$ 56 (2.3 %)	5,437 $\pm$ 37 (0.7 %)								
S21	BM								1,465 $\pm$ 44 (3.0 %)	881 $\pm$ 28 (3.2 %)						
S22	Sh				303.5 $\pm$ 2.3 (0.8 %)			323.4 $\pm$ 4.4 (1.4 %)								
S23	G				442 $\pm$ 11 (2.4 %)						452 $\pm$ 9 (2.1 %)			1,477 $\pm$ 13 (0.9 %)		641 $\pm$ 28 (4.4 %)

Table 3 (continued)

Sample	Type ^a	Compounds ^b	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
S24	HTC				1,626±21 (1.3 %)												
S25	So	36.4±0.3 (0.9 %)			96.5±4.4 (4.6 %)	2,989±60 (2.0 %)	5,770±226 (3.9 %)					201.0±0.1 (0.1 %)				14.64±0.04 (0.3 %)	957±30 (3.0 %)
S26	HC					513±23 (4.5 %)	1,099±73 (6.6 %)										
S27	HAF			1,142±34 (3.0 %)								1,912±19 (1.0 %)	1,088±59 (5.4 %)	387.5±2.5 (0.6 %)	32,900±1,100 (3.5 %)		
S28	HAF											5,207±116 (2.2 %)			20,460±780 (3.8 %)		143±3 (2.1 %)
S29	HAF				2,636±35 (1.3 %)							1,404±105 (7.5 %)			25,300±1,600 (6.3 %)		

^a Sample types: shampoo (Sh), gel (G), soap (So), perfume (P), hand cream (HC), aftershave cream (AC), hair treatment cream (HTC), exfoliant cream (EC), body milk (BM), household air freshener (HAF), and oral antiseptic (OA)

^b Compounds are numbered as shown in Table 2

shown in Table 1. Note that as defined in previous equations, f , g , and CRF must be high at optimum conditions but MCRF optimizes at low values. The right part of Eq. (6) represents the time-related term of the MCRF. Note that the time factor was also redefined if compared with Eq. (4); as minimum MCRF value had to be reached in the optimum experimental conditions. The significance of each part of the equation can be controlled by the value of the parameter α in Eq. (6). Adjusting α will modify the slope of the resolution term of the MCRF. Increasing α means a higher slope and consequently an increase in the significance of resolution against time, and vice versa. In this work, the values of α , optimum resolution desirability S_{opt} and optimum time desirability g_{opt} in Eq. (6) were set as 3, 0.9, and 0.9 respectively. The possibility of establishing the different contribution of both resolution and time of analysis was a determinant factor in the success of the optimization procedure.

To analyze the results of the experimental design, all main effects, two-way linear interactions and quadratic effects were examined by ANOVA. From the ANOVA, it was found that quadratic effect of SDS concentration, linear effects of pH and STB concentration and interaction between SDS and pH contributed significantly on the response to MCRF for the established 95 % confidence limit. As well, the value of the adjusted determination coefficient ($R^2 > 0.87$) showed a satisfactory adjustment of the model to the experimental data.

The results of the multivariate optimization showed that the resolution term in the MCRF equation optimized at low values of STB concentration and pH, but SDS concentration had a minimum near 100 mM. However, as time term of the MCRF was oppositely affected by the last two variables, a compromise must be reached. On the basis of our criteria, the abovementioned value of $\alpha = 3$ enhanced appropriately resolution factor against the time analysis term.

Finally, the global response surface estimated by the model was visualized (Fig. 1), where minimum MCRF had to be reached in optimal conditions. RSM allowed prediction of the response at levels that were not studied experimentally, as well as more detailed analysis of the analytical variables. Derringer's desirability function was applied to optimize the three variables simultaneously. Thus, according to the RSM and the MCRF values predicted by the multivariate model, the optimum MCRF was achieved with 24 mM STB, 100 mM SDS, and pH 9.0. Therefore, these experimental conditions were selected to develop the analytical procedure. A typical electropherogram showing the separation of the standard analytes under optimum conditions is illustrated in Fig. 2.

Method performance

To evaluate the suitability of the proposed method for quantification of the target analytes the following analytical characteristics were evaluated: linearity, limits of detection and

quantification, repeatability, intermediate precision, accuracy (recovery test). Basic method performance data are listed in Table 2.

The calibration curves were constructed by plotting the normalized peak area ratios of analytes to IS (y -axis) against analyte concentration (x -axis). For this purpose, five mixed standard solutions containing all 15 compounds in the concentration ranges specified in Table 2 were analyzed in duplicate. IS was added to a final concentration of approximately 30 $\mu\text{g/mL}$ in mixed standards. Normalized peak area was calculated as the peak area divided by the corresponding migration time [41]. Results of the regression equations indicate that high linearity ($R^2 \geq 0.994$) was achieved in the analyzed concentration ranges. The concentration range was selected to be close to the limits established in the regulation, in spite of the limitations imposed by the sensitivity of UV detector and the low water solubility of mixtures of selected organic compounds. Note that, as mentioned previously, citral and isoeugenol were isomeric mixtures, so they were quantified as the sum of the two normalized peak areas.

The LOD was calculated as $(3.3 \times \text{SD}/b)$ where SD was the standard deviation of Y -intercept of regression line and b the slope of the regression line for each analyte. The LOQ was calculated as $3 \times \text{LOD}$ [42]. Under these criteria, LOD and LOQ were found to be between 1.10 and 11.04 and 3.29 and 33.12 $\mu\text{g/mL}$, respectively (Table 2). This showed that it was possible to quantify the selected compounds at concentrations equal to 0.001 % as requested by EU directive for rinse-off products (except for linalool and citral whose LOQ was 0.0018 and 0.0033 %, respectively) or 0.01 % for leave-on products [15].

Accuracy and precision of the method were verified at two concentration levels. The concentrations of all targets and results of analysis are summarized in Table 2. Low level was established at a value near the LOQ, and high level was set at a slightly lower value than the upper limit of the studied linear range. The repeatability was determined by injecting those solutions six times per day for two consecutive days, while the

intermediate precision was the average of duplicate injections of standard solutions in 4 days over a 3-week period. The repeatability and intermediate precision of the method, expressed as percentage of the relative standard deviation (RSD), varied between 2.4 and 13.5 and 2.5 and 16.7 %. The relatively high %RSD values of citral and isoeugenol (up to 13.9 and 19.2 %, respectively) can be explained by the fact that the value corresponds to the sum of two peaks. Comparison of LODs to literature reported methods based on UV detectors gave similar values [17, 25, 27].

The accuracy of the method was assessed by the recovery test. PAS and preservative recoveries were determined by spiking samples with known amounts of the compounds at two different concentrations (low and high levels). Each sample was analyzed in triplicate and the relative recovery was calculated. Several samples had to be used for this purpose, because no target-free sample was found. The obtained data demonstrated that the proposed MEKC method provided good accuracy results, as recovery values were between 88.9 and 115.4 %. Precision and accuracy results reported in the method performance section did not differ remarkably from those reported before on UV-based methodologies [17, 25, 27].

Comparison of the proposed method with other procedures reported in the literature indicated that MEKC is a good alternative to other more expensive analytical techniques such as HPLC, GC-MS or GC $\times$ GC-MS [19, 43–45]. In addition, in this procedure, special attention was given to the optimization procedure, applying for this purpose a CCD experimental design. The MCRF had the option of adjusting the weights of the resolution term relative to the time term, so it provided more advantages than other approaches used to solve similar problems that require a large number of experiments [10, 24].

Application

The developed methodology was applied to the analysis of the 15 target compounds in 29 PCPs. The studied compounds

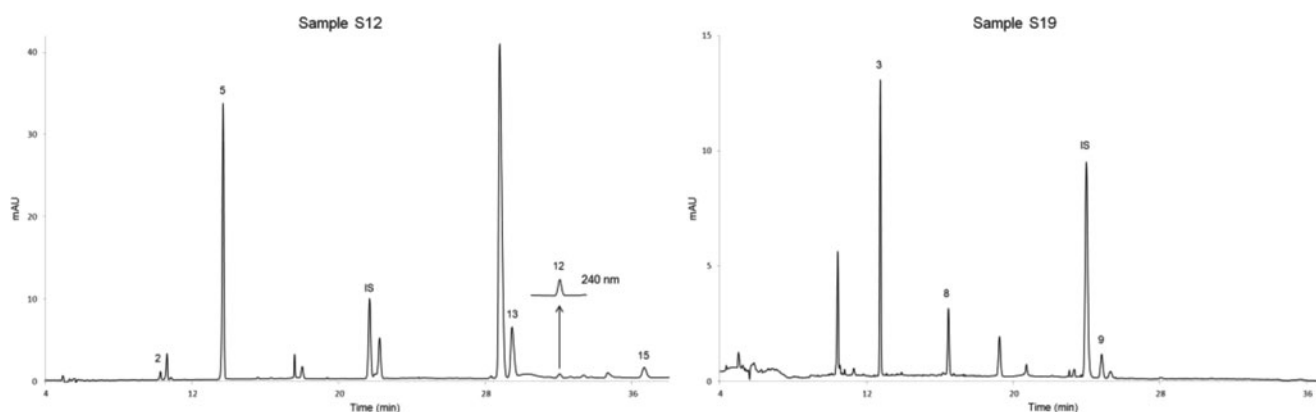


Fig. 3 Electropherograms obtained from the analysis of commercial PCPs (samples S12 and S19). Experimental conditions, see Fig. 2. Peaks identification: 2, anisyl alcohol; 3 methylparaben; 5, coumarin; 8, ethylparaben; 9, propylparaben; 12, citral isomer; 13, linalool; and 15, geraniol

were selected to be representative of the major classes of fragrance allergens and preservatives, namely: (a) alcohols (benzyl alcohol, anisyl alcohol, linalool, and geraniol), (b) lactones (coumarin), (c) carbonyl compounds (citral), (d) phenols (eugenol and isoeugenol), (e) hydrophilic preservatives (benzoic acid, salicylic acid, and sorbic acid), and (f) hydrophobic preservatives (methylparaben, ethylparaben, propylparaben, and butylparaben).

Samples were prepared as described and analyzed in duplicate. No more pretreatment was needed, except filtration. Samples were quantified by using normalized peak area ratios to IS, and the results of quantitative analysis are shown in Table 3 presented as mean of duplicates $\pm$ standard deviation. Typical electropherograms obtained from S12 and S9 are presented in Fig. 3, showing the occurrence of five fragrance allergens and three parabens, respectively.

Linalool was the most commonly found PAS (14 out of 29, 48.3 %). All targets were found in samples, except sorbic acid, with the following percentages: benzyl alcohol (31.0 %), anisyl alcohol (3.4 %), methylparaben (13.8 %), coumarin (34.5 %), salicylic acid (13.8 %), benzoic acid (37.9 %), ethylparaben (6.9 %), propylparaben (6.9 %), eugenol (31.0 %), isoeugenol (3.4 %), citral (24.1 %), butylparaben (3.4 %), and geraniol (24.1 %). All samples contained at least one of the PAS or preservative: 48.3 % of PCPs contained one or two analytes, 37.9 % three or four, and 13.4 % of the analyzed samples contained five or more compounds. It must be noted that some sample types (oral antiseptics and household air fresheners) are not affected by the aforementioned European Regulation. These values are in agreement with those typically found in similar samples by other methods [10, 25, 27, 43] and are also in accordance with the regulation, as they are appropriately labeled.

Conclusions

A rapid, simple, and low-cost MEKC method for the simultaneous separation and quantification of representative fragrance allergens and preservatives in PCPs was successfully developed. The reported method showed suitable analytical performance characteristics in terms of sensibility, linearity, LODs, LOQs, repeatability, intermediate precision and accuracy. Finally, the optimized and validated methodology was used for quantification of representative fragrance allergens and preservatives in a wide range of PCPs. Although future improvements should be aimed at enhancing some of the performance limitations, the simplicity of the developed method must be emphasized as no sample pretreatment was required.

The usefulness of the modified chromatographic response function combined with experimental design as a reliable tool for multivariate optimization of capillary electromigration

methods is showed. Finally, the results obtained from the analysis of PCPs indicate that MEKC can be successfully applied for routine analysis of simultaneous quantification of representative fragrance allergens and preservatives in commercial samples.

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