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Enantiomeric separation of chiral polycyclic musks by capillary electrophoresis: Application to the analysis of cosmetic samples

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ABSTRACT

The enantiomeric separation of four chiral polycyclic musks (Galaxolide, Tonalide, Traseolide and Phantolide) using CE was achieved for the first time in this work. Two chiral methodologies were developed by CD-MEKC using SDS as surfactant in a CHES buffer (pH 9.0). One methodology enabled the fast enantiomeric separation of individual polycyclic musks with analysis times lower than 10 min for Tonalide, 13 min for Traseolide and Phantolide, and 17 min for Galaxolide. Enantiomeric resolutions obtained were higher than 1.5 using different separation media for each compound. A second methodology was also developed enabling the simultaneous enantioseparation of the four musks. In this case, the use of a dual CD system containing two neutral CDs was necessary to achieve the separation of all enantiomers from three out of four musks in 45 min. Although a coelution between Galaxolide and Phantolide was observed, the use of different UV absorption wavelengths allowed the simultaneous analysis of both musks. In addition, a sweeping strategy was performed in order to increase the sensitivity of the method. Appropriate analytical characteristics (linearity, LOD and LOQ, precision and absence of matrix interferences) were obtained for conventional and sweeping methodologies. Finally, the usefulness of the method was demonstrated in the determination of the enantiomers of the polycyclic musks in personal care products as perfumes.

1. Introduction

Musks are widely used as fragrance fixative in a huge spectrum of personal care products and house commodities. The first synthetic compounds replacing natural musks were aromatic nitro musks. However, their amino metabolites showed a photo allergic reaction and a high bioaccumulation potential in human and animals. For this reason, other synthetic compounds called polycyclic musks (PCMs) have replaced them during this decade [1–4]. This type of musks include Galaxolide (1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethylcyclopenta[g]-2-benzopyran), Tonalide (7-acetyl-1,1,3,4,4,6-hexamethyl-1,2,3,4-tetrahydronaphthalene), Traseolide (1-(2,3-dihydro-1,1,2,6-tetramethyl-3-(1-methylethyl)-1H-inden-5-yl)-*trans*-ethanone), Phantolide (1-(2,3-dihydro-1,1,2,3,3,6-hexamethyl-1H-inden-5-yl) ethanone), Celestolide (4-acetyl-6-tert-butyl-1,1-dimethyl indan) and Cashmeran (1,2,3,5,6,7-hexahydro-1,2,3,3-pentamethyl-4h-inden-4-one). Nowadays, the most commonly employed in the fragrance industry is Galaxolide followed by Tonalide [4–7].

Nevertheless, their trend to bioaccumulation in different matrices due to their lipophilic and persistent nature has been reported not only in surface water and aquatic organisms [5,7–9], but also in humans (mother milk, human adipose tissue and blood plasma) [1,10–14]. Thus, these chemicals are considered as emergent contaminants [4], and the Official Journal of the European Union [15] has established a maximum authorized concentration in finished cosmetic products: (i) in the case of Tonalide, values of 0.1% in leave-on products except for hydro alcoholic products (1%), fine fragrance (2.5%) and fragrance cream (0.5%), as well as, 0.2% in rinse-off products; and (ii) for Phantolide, a 2% in leave-on products and no restriction in rinse-off products.

All PCMs except Celestolide are chiral compounds (see Fig. 1). Tonalide and Phantolide exhibit one stereogenic center, while Galaxolide and Traseolide have two stereogenic centers leading to one pair or two pairs of enantiomers, respectively. However, Traseolide is commercialized as *trans* isomer (contains more than 95% of this isomer) and it can be considered as one pair of enantiomers. Several works have reported that isomer (3S) for Tonalide and the isomers (4S7R and 4S7S) for Galaxolide are responsible for their strong musk odor while the other enantiomers are slightly less intense [4,16–18]. In addition, some authors postulated that due to the typical musk smell of the two active Galaxolide

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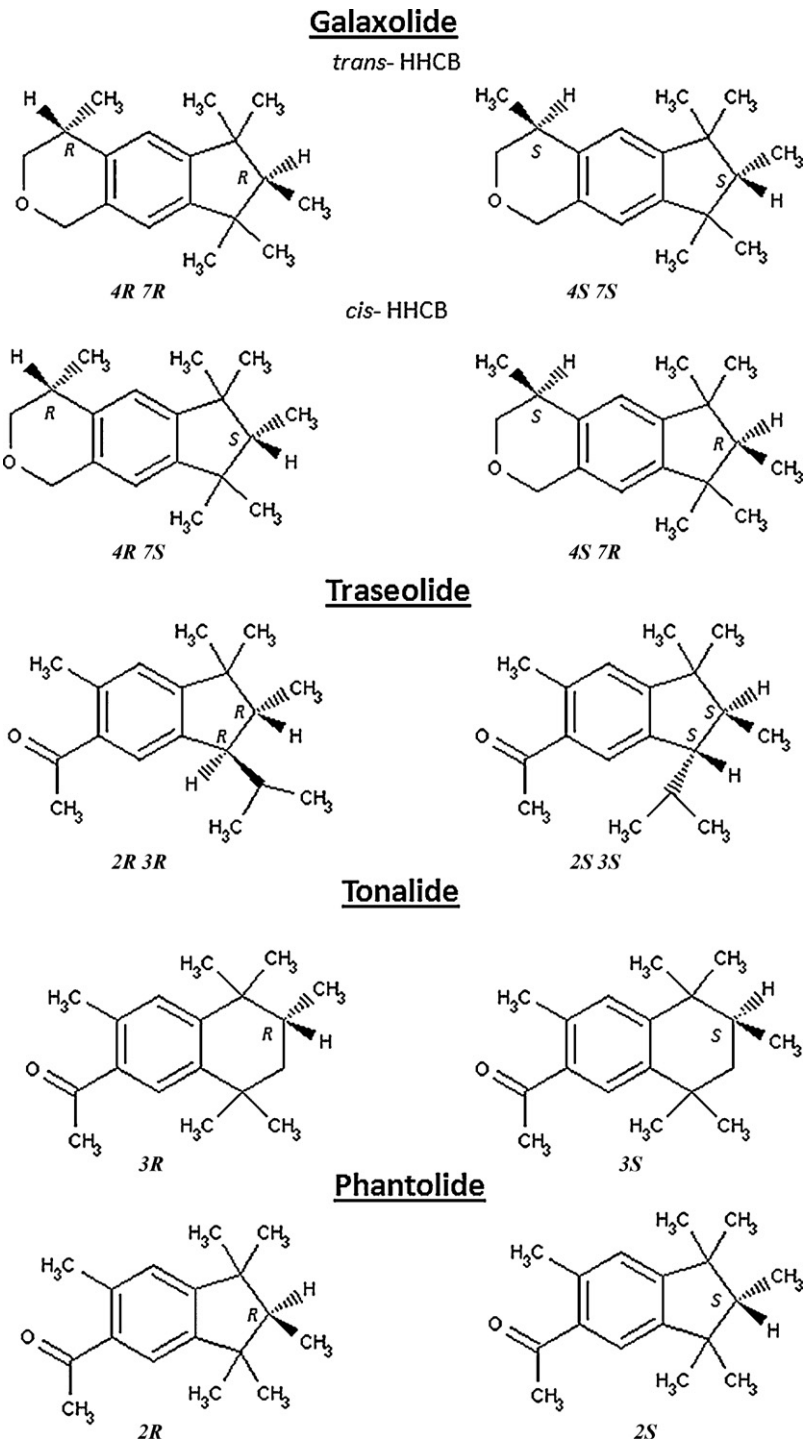

Fig. 1. Stereochemical structures for Galaxolide, Traseolide, Tonalide and Phantolide.

enantiomers and the three-dimensional structural similarity to the pheromone 5-α-androst-16-en-3-one, they can be considered as artificial pheromones [16,17].

Although there is a lack of toxicological information available about the biodegradation and toxicity of their enantiomers, a chiral pollutant liberated to the environment as racemate can suffer a species-dependent enantioselective metabolism and can display different biological activities. Thus, for example, in the case of Galaxolide and Tonalide evidences of their enantioselective metabolism have been found in different aquatic organisms [4,17–21].

Up to date, a few methods using GC [19–22] or HPLC [23] have been reported for the chiral analysis of PCMs. Thus, enantioselective GC was used to separate the different enantiomeric/diastereomeric PCMs in wastewater, sewage sludge and biota species using chiral stationary phases modified with cyclodextrin (CD). The enantioselective GC phase heptakis(6-*O*-*tert*-butyldimethylsilyl-2,3-di-*O*-methyl)-β-CD was the most commonly employed to perform these enantioseparations [19,20,22]. The fastest analyses for a mixture of the most frequently used compounds Galaxolide and Tonalide, were obtained by Franke et al. [19] with nearly baseline ($R_S < 1$) enantioseparation of Tonalide in 33 min and baseline

enantioseparation of the two pairs of enantiomers of Galaxolide in 39 min. However, the best simultaneous enantioseparation of four PCMs (Galaxolide, Tonalide, Traseolide and Phantolide) was achieved by Gatermann et al. [20] who obtained the baseline separation of all enantiomers in about 60 min. On the other hand, a preparative enantioselective HPLC separation methodology for Galaxolide and Traseolide using a chiral stationary phase (monofunctionalized permethyl-β-CD on 3-aminopropyl silica gel) was developed. A nearly baseline separation of the enantiomers of *trans*-Traseolide and baseline enantioseparation of only the third and fourth eluting isomers of Galaxolide were achieved in about 30 and 50 min, respectively [23].

The literature shows that GC is the most common technique for enantioselective determination of PCMs. In most cases, analysis times reported for the GC methods developed were about 60 min although analysis times larger than 200 min have been described for Galaxolide [20,21], one of the most PCM employed. The few methods described so far for the enantiomeric determination of PCMs as well as their high analysis times, justify the need to develop new analytical enantioselective methodologies. Since CE has shown in the last years a great potential in the chiral field, this technique can be presented as an interesting alternative to GC or HPLC. The advantages of CE over HPLC and GC are its high efficiency combined with short analysis times, versatility and wide range of application, reduced sample pre-treatment, and cheapness (a low consumption of reagents and the absence of chiral columns) [24]. However, to our knowledge, there is no reference about the enantioseparation of these musks by CE. Therefore, the aim of this work was to develop a fast CE method for the enantiomeric separation of PCMs and to apply it to the analysis of personal care products.

2. Experimental

2.1. Reagents and samples

All reagents employed for the preparation of the separation buffers were of analytical grade. NaOH, dodecyl sulfate sodium salt (SDS) and dichloromethane (DCM) were obtained from Merck (Darmstadt, Germany). Methanol (MeOH) was from Scharlau (Barcelona, Spain); dimethyl sulfoxide (DMSO), urea and boric acid were obtained from Fluka (Buchs, Switzerland) and 2-[N-cyclohexylamino]ethane sulfonic acid (CHES), sodium deoxycholate (SDC), sodium taurodeoxycholate (STDC), sodium cholate (SC), and hexane were supplied from Sigma–Aldrich (Steinheim, Germany). Anhydrous sodium sulphate was obtained from J.T. Baker (Deventer, The Netherlands) and NaCl was from Panreac Quimica S.A. (Barcelona, Spain). Distilled water was purified through a Milli-Q System from Millipore (Bedford, MA, USA).

Chiral selectors, γ-CD, acetyl-γ-CD (Ac-γ-CD, degree of substitution (DS) = 7), 2-hidroxypropyl-γ-CD (HP-γ-CD, DS = 4.5), permethyl-γ-CD (DS = 10), octakis (2,3,6-tri-O-acetyl)-γ-CD (2,3,6-Ac-γ-CD) and acetyl-β-CD (Ac-β-CD, DS = 7) were purchased from Cyclolab (Budapest, Hungary); β-CD, 2-hidroxypropyl-β-CD (HP-β-CD, DS = 0.6), methyl-β-CD (M-β-CD, DS = 2), and sulfated-β-CD were obtained from Fluka (Buchs, Switzerland) and permethyl-β-CD (DS = 10.5–14.7) was from Sigma–Aldrich (Steinheim, Germany).

Stock solutions of each PCMs Galaxolide, Tonalide, Traseolide and Phantolide from LGC Standards (Teddington, United Kingdom) were firstly prepared by dissolving them in DMSO or hexane. For their injection in CE, the stock solutions in DMSO were diluted with either BGE without chiral selector or a specified matrix (e.g. aqueous MeOH with NaCl) up to the desired concentration. These solutions were stored at 4 °C and warmed at room temperature before use.

2.2. Sample treatment

Perfumes were supplied from stores of Madrid, Spain. Sample preparation was performed as in a previous work [25]. The extraction method consisted of taking 100 mg of the sample and adding 0.5 ml water and 3 ml hexane. The samples were shaked during 3 min and centrifuged for 10 min. The upper organic layer was isolated and the extraction procedure was repeated twice. Hexane layers were added together and concentrated to approximately 0.5 ml. Silica SPE cartridges (Bond Elut-SI, 500 mg, 3 ml), supplied from Varian (Palo Alto, USA), were covered with approximately 300 mg of Na_2SO_4 before the extraction. Since the concentrated extract was added, the elution was performed by adding 2 ml of hexane, which was discarded, and subsequently 6 ml of DCM, which was collected. The eluate was evaporated to dryness at 30 °C under a stream of nitrogen. The residue was dissolved in 1 ml of MeOH with NaCl, and they were diluted 1:6 with MeOH prior to analysis by CE.

2.3. CE conditions

CE experiments were carried out with an HP^{3D} CE instrument (Agilent Technologies, Waldbron, Germany) equipped with an on-column DAD. The instrument was controlled by a PC running the 3D-CE ChemStation from Agilent Technologies. Separations were performed on uncoated fused-silica capillaries with a total length of 48.5 cm (40 cm effective length) or 72.5 cm (64 cm effective length) and 50 μm I.D. × 375 μm O.D. purchased from Composite Metal Services (Worcester, England). Injections were made at the cathodic end using a pressure of 50 mbar for different times. The electrophoretic separation was achieved with a voltage of 20 or 30 kV (normal polarity mode). The temperature of the capillary was 15 °C.

Buffer solutions were prepared diluting the appropriate amount of CHES or boric acid with Milli-Q water, adjusting the pH to the desired value (pH 9.0) with NaOH 1 M before completing the volume with water to get the desired buffer concentration (100 mM). A pH meter (Metrohn 744, Herisau, Switzerland) was used for the pH adjustment of the separation buffers. The background electrolyte (BGE) was elaborated dissolving the suitable amount of chiral selector, urea and/or surfactant in the separation buffer. An ultrasonic bath (Ultrasons-H) was used for the preparation and degassing of the BGEs and all prepared solutions. All solutions were stored at 4 °C, warmed at room temperature before use, and filtered prior use through 0.45 μm pore size disposable nylon filters from Sugelabor (Madrid, Spain).

Before its first use, the new capillary was rinsed with MeOH for 5 min, 1 M NaOH for 25 min, followed by 5 min with water and conditioned with BGE for 30 min. Between injections of samples, the capillary was conditioned with BGE (2 min), NaOH (3 min), water (1 min) and BGE (5 min). A pressure of 1 bar was applied in all steps.

2.4. Data treatment

Resolution was obtained from the Chemstation software using the equation:

$$R_s = 1.18\frac{t_2 - t_1}{w_{1/2,1} + w_{1/2,2}}$$

where t_1 and t_2 are the migration times of the first and second migrating enantiomers and $w_{1/2,1}$, $w_{1/2,2}$ are their respective peak widths at the half height.

Corrected peak areas (Ac) were used to compensate fluctuations in electrophoretic conditions and to obtain a good reproducibility of data. They were calculated dividing the peak area (A_i) by the corresponding migration time (t_i), that is, $Ac = A_i/t_i$.

Experimental data analysis and parameters were calculated using Excel Microsoft XP® and Origin 7.0. Graphs with different electropherograms were composed in Origin 7.0.

3. Results and discussion

3.1. Development of analytical methodologies for the enantiomeric separation of PCMs

The most commonly PCMs used in cosmetic industry, Galaxolide and Tonalide, as well as Traseolide and Phantolide, were selected as representative chiral PCMs in the present work [4–7]. Since they are neutral compounds, three different systems based on CD-MEKC with SDS and neutral CDs, MEKC with bile salts (chiral anionic surfactants) and EKC with anionic CDs were tested.

First, a CD-MEKC system using SDS as achiral anionic surfactant and neutral CDs was tested because according to our experience in the separation of neutral hydrophobic compounds by CE (log *P* for PCMs range from 5.10 to 6.20 [26]), the excellent solubilization power of SDS confers this option the highest probability of success. In addition, borate buffer at high pH value (pH 9.0) was selected on the basis of the results previously obtained by our research team. On the other hand, urea was also used as additive at a constant concentration in order to increase the solubility of the neutral highly hydrophobic musks and CDs in the aqueous buffer solution [27]. Therefore, a 50 mM borate buffer at pH 9.0 with 50 mM SDS, 2 M urea, and a neutral CD was chosen as initial conditions. Moreover, since it is well known that the enantiomeric R_s of chiral compounds can increase when decreasing the working temperature, a temperature of 15 °C was selected [28–30].

A screening of ten neutral CDs (five β-CDs and five γ-CDs) in the CD-MEKC system described was carried out to explore their chiral discrimination against the musks studied. All CDs were tested at a concentration of 25 mM except 2,3,6-Ac-γ-CD which was employed at 2 mM concentration due to its low solubility. Sudan Red was used as marker of the migration time of the micelle due to its high hydrophobic character (log *P* = 6.66) [26]. As shown in Table 1, only the γ-CDs offered enantioselectivity for the compounds studied, except in the case of 2,3,6-Ac-γ-CD, possibly because of the low concentration employed. In addition, all γ-CDs except 2,3,6-Ac-γ-CD, gave larger elution windows and lower retention factors. These facts can be explained taking into account that the addition of γ-CD to the buffer displaces the distribution of the PCMs from the micellar phase to the aqueous phase as a function of the possible interaction between the water soluble CD and the PCMs enantiomers. Although the cavity size of β-CD and its derivatives is enough to include the PCMs inside, the monomeric surfactants in aqueous phase can be included by the CD because of the presence of the lipophilic hydrocarbon chain. This may prevent the solute from inclusion, and only CDs with wider cavity such as γ-CD have the capability of including the solute together with the surfactant monomer. Thus, co-inclusion would be expected to require a larger cavity size, such as that of γ-CDs [31,32].

The best chiral discrimination for the four PCMs was obtained using γ-CD, Ac-γ-CD and HP-γ-CD, so they were chosen as chiral selectors in further experiments. Also, since broad and tailed peaks were observed in some cases, a CHES buffer was tested because its organic nature increases the solubility of hydrophobic compounds in aqueous media. The results showed that the symmetry and shape of the peaks improved when this organic buffer was employed. The new conditions consisting of a 50 mM CHES buffer at pH 9.0 with 50 mM SDS and 2 M urea, enabled to obtain the baseline enantioresolution ($R_s \geq 1.5$) for Tonalide and Traseolide, with analysis times of only 10 min, when Ac-γ-CD and HP-γ-CD were used, respectively. However, only a partial enantioseparation ($R_s \approx 1.0$) for Phantolide was achieved with γ-CD, while for Galaxolide only three peaks of the four possible isomers were obtained in the best cases (with γ-CD), which could not be assigned to enantiomers or diastereoisomers due to the absence of such standards.

A second strategy consisting of the use of bile salts as anionic chiral surfactants in MEKC was considered. The effect of different bile salts (SC, STDC, and SDC) at a concentration of 50 mM was tested. However, none of them gave rise to the chiral separation of the compounds studied. Moreover, the combination of one bile salt (SDC) at a higher concentration (100 mM) to enhance the elution window, together with the previously selected γ-CDs (γ-CD, Ac-γ-CD and HP-γ-CD) and the corresponding β-CDs was also evaluated. SDC was selected to combine with CDs since it gave previously better shape of peaks. Only γ-CD gave a slight discrimination for Galaxolide under these conditions ($R_s < 1$ for two isomers out of four). Nevertheless, any enantioseparation for the other compounds was obtained. Finally, a third strategy consisting of using an anionic CD was employed. Sulfated-β-CD was selected as chiral selector at different concentrations in both normal polarity (at pH 9.0 with 0.6%, w/v) and reverse polarity (at pH 2.0 with 2%, w/v) according to Lin et al. [33]. However, no peaks were observed. It can be indicated

Table 1
Retention time (t_r), enantiomeric resolution (R_s) and retention factor (k) for the four polycyclic musks, Galaxolide, Tonalide, Traseolide and Phantolide obtained with different types of CDs using a CD-MEKC system. Experimental conditions: BGE, CD in 50 mM borate buffer (pH 9.0) with 2 M urea and 50 mM SDS; uncoated fused-silica capillary, 48.5 cm (40 cm to the detector window) ×50 μm ID; UV detection at 205 nm (for Galaxolide) and 215 nm (for Tonalide, Traseolide, and Phantolide); applied voltage, 20 kV; temperature, 15 °C; injection by pressure, 50 mbar for 3 s.

CDs[a]	t_{eof} (min)	t_{mc} (min)	Elution window	Galaxolide			Tonalide			Traseolide			Phantolide		
				t_r (min)	R_s[b]	k	t_r (min)	R_s	k	t_r (min)	R_s	k	t_r (min)	R_s	k
Without CD	3.45	14.22	10.77	13.91	–	138.5	14.05	–	248.4	13.74	–	88.2	13.33	–	46.3
γ-CD	3.91	16.11	12.20	10.75	1.2	5.2	12.12	0.4	8.4	12.19	–	8.7	10.20	-	4.5
				10.99		5.7	12.27		8.9						
Ac-γ-CD	4.29	17.69	13.40	7.12	1.0	1.1	9.29	1.0	2.4	11.06	–	4.2	7.46	0.8	1.3
				7.23		1.2	9.57		2.7				7.59		
HP-γ-CD	3.97	14.58	10.61	8.04	1.0	1.9	9.49	–	4.0	9.70	1.1	4.3	7.88	–	2.2
				8.37		2.2				9.86		4.6			
2,3,6-Ac-γ-CD	3.27	12.02	8.74	10.61	–	19.0	10.71	–	20.7	10.73	–	21.2	10.69	–	20.4
Permethyl-γ-CD	4.06	14.90	10.84	6.68	0.6	1.2	7.96	0.4	2.0	8.39	0.8	2.4	6.9	–	1.3
				6.74		1.2	8.01		2.1	8.70		2.7			
β-CD	3.75	12.80	9.04	12.58	–	140.5	12.72	–	420.4	12.69	–	277.9	12.38	–	70.1
Ac-β-CD	4.00	13.65	9.65	10.52	–	7.3	10.73	–	8.0	10.73	–	8.1	10.68	–	7.8
HP-β-CD	3.84	13.08	9.25	10.60	–	9.0	10.70	–	9.6	10.81	–	10.2	10.59	–	9.1
M-β-CD	3.99	13.61	9.62	8.94	–	3.6	9.41	–	4.4	9.66	–	4.9	9.38	–	4.5
Permethyl-β-CD	4.05	14.85	10.81	9.46	–	3.8	9.51	–	3.8	9.64	–	4.0	9.40	–	3.7

[a] All CDs were tested at a 25 mM concentration except 2,3,6-Ac-γ-CD which was employed at a 2 mM concentration.
[b] Non-possible to assign unequivocally to enantioseparation.

that PCMs cannot be soluble in a aqueous solution only with this anionic CD.

3.2. Optimization of the CD-MEKC methodology for the enantiomeric separation of PCMs

On the basis of the best enantioseparation obtained for all musk compounds using SDS as anionic surfactant and γ-CD, Ac-γ-CD or HP-γ-CD as neutral chiral selectors, an optimization of the CD-MEKC methodology was carried out. Thus, experimental parameters such as buffer, surfactant and urea concentration were varied. An increment in their concentration reduces the EOF making possible to open the separation window and to improve the enantioseparation [34,35]. Although the retention time was also increased, a better enantioselectivity was generally observed for all PCMs when CHES and SDS concentration was increased up to 100 mM. Moreover, higher concentration of urea (up to 5 M) in the BGE not only allowed a better solubility of PCMs and CDs in the buffer but also improved the chiral separation of musks. As a consequence, these concentrations (100 mM CHES, 5 M urea and 100 mM SDS) were employed in further experiments.

The influence of the concentration (25 and 50 mM) of the selected CDs on the enantiomeric resolution of PCMs was studied. Table 2 shows that in all cases, increasing the CD concentration lead to a decrease in the migration time due to the higher interaction between the neutral CD and the musks and therefore the lower interaction with the anionic micelle. In most cases (8 out of 12), the enantiomeric resolution increased when the CD concentration was increased. As shown in Table 2, the use of 50 mM HP-γ-CD enabled the chiral separation of the four stereoisomers of Galaxolide and the two enantiomers of Traseolide, with high resolution and the shortest analysis times. In addition, the use of 50 mM Ac-γ-CD and γ-CD gave the best enantioselectivity and shorter migration time for Tonalide and Phantolide, respectively.

Taking into account that only HP-γ-CD offered chiral discrimination for all compounds studied, also providing the shortest analysis times, a study of the effect of its concentration between 25 and 75 mM was carried out in order to assess whether this CD could be the best choice for all musks studied. However, neither increasing nor decreasing the CD concentration a better enantioseparation for musks was obtained except for Traseolide, for which an increase in the CD concentration improved its enantioresolution (see Table 2). As a compromise among the resolution, peaks shape and analysis time for each PCM, different electrophoretic conditions were chosen as optimal for each compound. Moreover, in the case of Traseolide an increase in the temperature from 15 to 30 °C improved the resolution decreasing the analysis time. The electropherograms obtained under the optimized conditions for each PCM are shown in Fig. 2.

3.3. Optimization of the chiral separation of a multicomponent mixture of the four PCMs

Since none of the above conditions allowed the baseline separation of the enantiomers of all studied PCMs in the same analysis, and with the purpose of achieving the simultaneous chiral separation of a greater number of PCMs, the use of a combination of two chiral selectors was considered as a promising alternative. Thus, different mixtures of HP-γ-CD with γ-CD or Ac-γ-CD were tested (see Table 3). Since the use of Ac-γ-CD alone gave the worst enantioselectivity for Galaxolide, while HP-γ-CD or γ-CD gave the best chiral separations for Galaxolide, Traseolide and Phantolide, a higher number of dual CDs systems were carried out with HP-γ-CD and γ-CD.

As shown in Table 3, the combination of HP-γ-CD and γ-CD at a 1:1 ratio (25 mM) gave partial resolution for Tonalide and Traseolide, and $R_S > 1.5$ for Phantolide. However, only two out of four isomers of Galaxolide could be separated showing the importance

Table 2
Effect of the CD concentration on the enantioseparation of Galaxolide, Tonalide, Traseolide and Phantolide. Experimental conditions: BGE, CD in 100 mM CHES buffer (pH 9.0) with 5 M urea and 100 mM SDS. Other experimental conditions as in Table 1.

CD	Concentration (mM)	Galaxolide		Tonalide		Traseolide		Phantolide	
		t_r (min)	R_s	t_r (min)	R_s	t_r (min)	R_s	t_r (min)	R_s
HP-γ-CD	25	23.09 23.30 23.56 24.10	1.0 1.2 2.6	26.96	–	27.59 28.06	2.0	22.79 22.95	0.6
HP-γ-CD	35	18.85 19.03 19.29 19.78	0.9 1.2 2.5	23.44	–	24.20 24.75	2.1	19.25 19.40	0.6
HP-γ-CD	50	15.77 15.95 16.18 16.66	1.7 2.1 4.5	20.72 20.79	0.4	22.05 22.76	3.9	16.53 16.68	1.0
HP-γ-CD	75	13.10 13.23 13.41 13.83	0.8 1.0 2.3	18.22	–	19.54 20.49	4.8	13.50 13.61	0.5
γ-CD	25	25.69 26.23 26.50 27.63	1.6 0.8 3.1	35.49 36.02	0.8	36.36	–	25.01 25.77	2.2
γ-CD	50	16.87 17.75 19.23	2.8[a] 5.1[a]	29.28 30.60	1.7	30.83 31.68	1.1	16.45 17.42	2.8
Ac-γ-CD	25	19.60 19.85 20.40	0.8[a] 2.0[a]	30.01 31.10	2.0	32.97 33.10	0.4	19.91 20.42	2.3
Ac-γ-CD	50	15.02 15.19	1.0[a]	26.01 27.99	4.8	27.12	–	16.43 16.84	2.1

[a] Non-possible to assign unequivocally to enantioseparation.

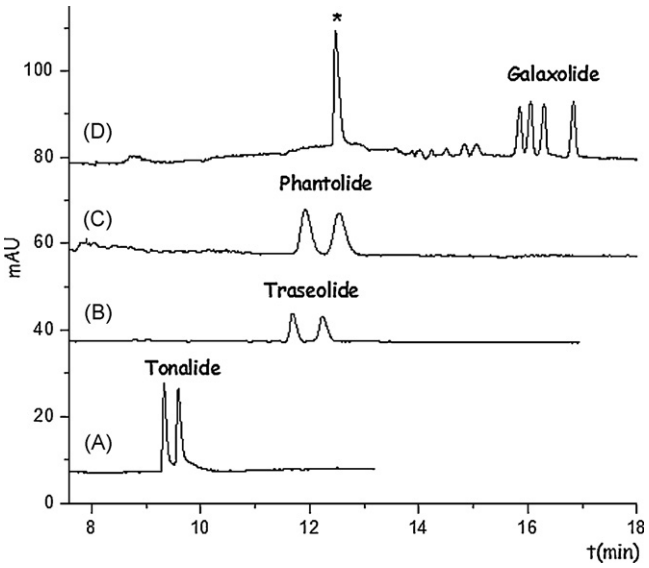

Fig. 2. Electropherograms corresponding to the faster individual enantioseparation of the four individual musks: (A) Tonalide; BGE, 25 mM Ac-γ-CD in 50 mM CHES buffer (pH 9.0) with 50 mM SDS and 2 M urea; applied voltage, 20 kV; temperature, 15 °C; (B) Traseolide; BGE, 50 mM HP-γ-CD in 50 mM CHES buffer (pH 9.0) with 100 mM SDS and 5 M urea; applied voltage, 30 kV; temperature, 30° C; (C) Phantolide; BGE, 50 mM γ-CD in 50 mM CHES buffer (pH 9.0) with 100 mM SDS and 5 M urea; applied voltage, 30 kV; temperature 15 °C (D) Galaxolide; BGE, 50 mM HP-γ-CD in 100 mM CHES buffer (pH 9.0) with 100 mM SDS and 5 M urea; applied voltage, 20 kV; temperature, 15 °C. Other experimental conditions: uncoated fused-silica, 50 μm × 48.5 cm; injection by pressure, 50 mbar for 3 s of sample (250 mg/L racemic mixture); UV detection at 215 nm for Tonalide, Traseolide and Phantolide, and at 205 nm for Galaxolide. (*) Byproduct of Galaxolide.

of HP-γ-CD on its enantioselectivity. The use of a higher concentration of γ-CD (50 mM) and a concentration of HP-γ-CD 25 mM gave no enantioresolution for Traseolide and Phantolide, and a decrease in the enantioseparation for Galaxolide. An increase in the HP-γ-CD concentration up to 50 mM mixed with γ-CD or Ac-γ-CD was then tested. A mixture 50 mM in HP-γ-CD and 25 mM in Ac-γ-CD originated a significant improvement in the resolution for Traseolide (R_s = 4.4) in comparison with the use of Ac-γ-CD alone (R_s = 0.4). Likewise, a mixture 50 mM in HP-γ-CD and 25 mM in γ-CD gave rise to an increase in the resolution for Traseolide (R_s = 2.7) with respect to the value obtained when using γ-CD alone (R_s = 1.1). However, poorer resolutions were obtained under both of the above-mentioned conditions for Tonalide and Phantolide, and only a partial enantioseparation of the four isomers of Galaxolide was achieved. Finally, a dual system 35 mM HP-γ-CD with γ-CD (15 or 25 mM) or Ac-γ-CD (15 mM) was also tested. The use of a mixture 35 mM HP-γ-CD and 15 mM γ-CD was selected for further experiments since this concentration originated a resolution higher than 1.5 for all musk enantiomers except for Tonalide for which a partial resolution was obtained.

The effect of an organic solvent was also investigated under the selected conditions. Organic solvents in the separation media can reduce the retention factors of strongly bound solutes to micelles (those with higher migration times) and therefore improve the interaction with the chiral selector (i.e., CD). A slight improvement in enantioselectivity for Tonalide but an important increase in the migration time (∼44 min) was obtained with a 10% MeOH as buffer modifier. Thus, the use of organic solvent was rejected.

A further effort was made to improve the enantioseparation of Tonalide as well the other compounds. Hence, the effect of instrumental parameters such as the capillary length and operating temperature was studied. The effect of the temperature on the enantiomeric separation was tested in the range from 15 to 40 °C. An increase in the capillary temperature resulted in a decrease in the migration times for all musks due to a smaller electrolyte viscosity, but it also produced a decrease in the resolution values except for Traseolide for which an improvement in the enantioselectivity was observed. Finally, the use of a longer capillary (total length 72.5 cm) keeping constant the electric field, lead to a better enantioselectivity for all musks and excellent baseline separation for all musks was obtained in about 45 min as shown in Fig. 3. As a result, a temperature of 15 °C, a capillary length of 72.5 cm and an applied voltage of 30 kV were chosen as optimal electrophoretic conditions. Although an excellent separation of the enantiomers of each individual PCM was obtained, a comigration of two of the four isomers of Galaxolide with the two Phantolide enantiomers was observed in a mixture of them (see Fig. 3B and C). Nevertheless, Galaxolide does not absorb at 260 nm while Phantolide can be detected at this

Table 3
Effect of the use of a mixture of CDs on the enantioseparation of Galaxolide, Tonalide, Traseolide and Phantolide. Experimental conditions as in Table 2. (*) Broad and tailed peaks.

CD (1)	Concentration (mM)	CD (2)	Concentration (mM)	Galaxolide		Tonalide		Traseolide		Phantolide	
				t_r (min)	R_s	t_r (min)	R_s	t_r (min)	R_s	t_r (min)	R_s
HP-γ-CD	25	γ-CD	25	15.72 16.27	2.7[a]	22.68 23.04	0.9	23.93 24.27	1.0	15.70 16.06	1.9
HP-γ-CD	25	γ-CD	50	11.71 11.83 12.05 12.34	0.6 1.2 1.5	19.42 20.06	2.2	21.36	–	20.93	–
HP-γ-CD	35	γ-CD	15	15.97 16.16 16.38 16.68	1.4 1.6 2.2	22.50 22.71	0.6	24.33 24.91	2.2	16.43 16.71	1.6
HP-γ-CD	35	Ac-γ-CD	15	15.53 15.78 15.89 16.11	1.7 0.7 1.7	21.36 21.57	0.8	23.83 24.48	2.4	15.99 16.25	1.6
HP-γ-CD	35	γ-CD	25	14.72 15.36	1.8[a]	21.98 22.37	1.2	23.62 24.13	1.7	14.66 15.15	1.3
HP-γ-CD	50	γ-CD	25	13.02 13.31 13.46	1.0[a] 0.6[a]	19.74 20.08	1.1	21.47 22.20	2.7	12.98 13.21	1.0
HP-γ-CD	50	Ac-γ-CD	25	(*)	–	17.59 17.89	1.5	22.43 23.52	4.4	14.31 14.61	1.5

[a] Non-possible to assign unequivocally to enantioseparation.

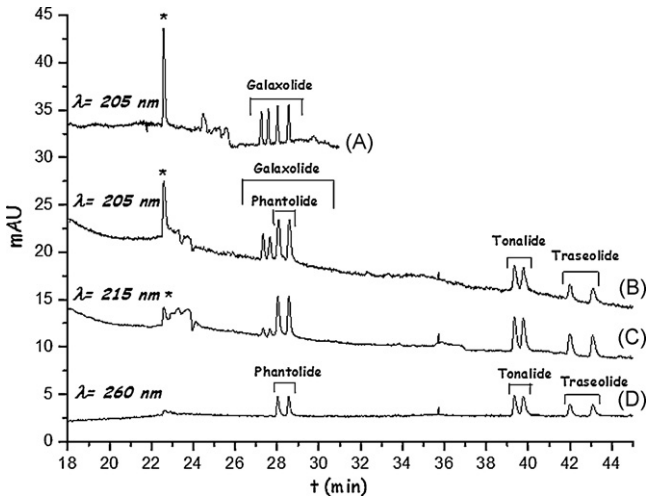

Fig. 3. Electropherograms corresponding to the chiral separation of the PCMs at different detection wavelengths: (A) Galaxolide at 205 nm; (B–D) mixture of the four musks (Galaxolide, Tonalide, Traseolide and Phantolide) at 205 nm, 215 nm and 260 nm, respectively. Experimental conditions: BGE, 35 mM HP-γ-CD and 15 mM γ-CD in 100 mM CHES buffer (pH 9.0) with 100 mM SDS and 5 M urea; uncoated fused-silica, 50 μm × 72.5 cm; hydrodynamic injection, 50 mbar for 3 s of sample; applied voltage, 30 kV; temperature, 15 °C. (*) Byproduct of Galaxolide.

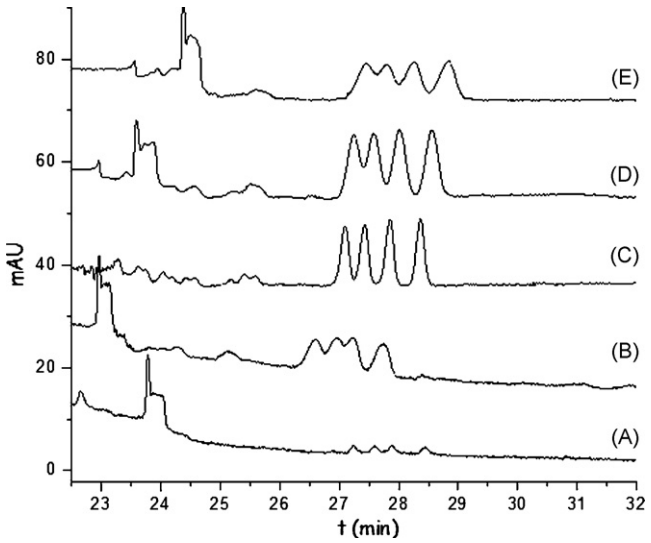

Fig. 4. Electropherograms corresponding to the effect of sample matrix and the injection time using sweeping injection strategy for Galaxolide (100 mg/L) at 50 mbar: (A) 3 s of sample dissolved in 100 mM CHES, 5 M urea and 100 mM SDS; (B) 30 s of sample dissolved in 100 mM CHES, 5 M urea and 100 mM SDS; (C) 30 s of sample dissolved in 100 mM CHES, 5 M urea and 30% MeOH (v/v) and prepared adjusting the conductivity; (D) 45 s of sample dissolved in 100 mM CHES, 5 M urea and 30% MeOH (v/v) and prepared adjusting the conductivity; (E) 30 s of sample dissolved in 100 mM CHES, 5 M urea and 30% MeOH (v/v) but prepared without adjusting the conductivity. UV detection at 205 nm. Other conditions as in Fig. 3.

wavelength. Thus, the detection of Phantolide could be made at a wavelength at which Galaxolide cannot be seen in UV (see Fig. 3D). The identification of each isomer for the four PCMs was not possible due to the lack of pure enantiomer standards.

3.4. On-line sample preconcentration by sweeping conditions

An in-capillary preconcentration strategy was employed in this work to preconcentrate the analytes inside the capillary before their chiral separation and detection. The sweeping injection method was chosen since it enables to achieve improved responses for neutral and hydrophobic compounds. Sweeping is based on the interaction between the analyte and an additive in the BGE which acts as pseudophase (such as micelles). Picking and accumulation of analytes occurs due to partitioning or interaction with the pseudophase. It should be emphasized that sweeping is possible if the sample matrix is free of the pseudophase, although the sample matrix could have a lower, similar, or higher conductivity than the separation solution [36,37]. In this way, when a voltage is applied, micelles sweep the analyte in a narrow band producing a sample preconcentration. This injection strategy compresses analyte band within the capillary to increase the volume of sample that can be injected in CE without loss of efficiency.

First, sweeping injection was performed by preparing the sample in a matrix without micelles but otherwise similar to the running buffer, i.e., its conductivity was adjusted with NaCl to be the same as that of the separation medium, so a homogeneous electric field is maintained along the separation. In view of the fact that the Galaxolide solution (100 mg/L) was not soluble in a matrix without SDS, an addition of 30% MeOH (v/v) was necessary. Next, sweeping was performed by prolonging the sample zone by applying longer injection times (from 3 to 45 s) and it was found that injection times higher than 30 s lead to broad peaks and to a decrease in the enantioselectivity (see Fig. 4A–D). As a comparative between a conventional injection of 3 s and the sweeping injection of 30 s, a concentration factor up to 12-fold can be obtained with sweeping injection over the traditional method of injection.

Second, a comparative of injection time in homogeneous and heterogeneous electric field was also performed. Thus, dissolving the sample in BGE free of SDS but without adjusting the conductivity (see Fig. 4E), in a heterogeneous electric field, the sweeping strategy did not work and an increasing in the injection time over 30 s was not possible.

Nevertheless, only a maximum concentration of musks up to ~175 mg/L was soluble with 30% MeOH (v/v) in the matrix sample. An increase in the percentage of MeOH to 50% (v/v) allowed to increase the concentration of the musks up to ~400 mg/L. However, an injection time of only 20 s could be used as maximum time in the sweeping method to avoid a decrease in the enantioselectivity of the four musks studied. Thus, the sweeping strategy could be applied only for samples with a low concentration of PCMs.

3.5. Calibration, precision and detection limits

Table 4 summarizes data for calibration curves, precision and LODs and LOQs obtained for the three musks which can be simultaneously analyzed using conventional injection (50 mbar for 3 s) and sweeping injection (50 mbar for 20 s). Since enantiomeric standards were not available, calibration curves were constructed for each racemate by plotting the sum of the corrected peak areas (peak area to migration time ratio) of all isomers of a given musk *versus* the corresponding concentrations of the racemate.

All calibration curves were linear over the concentration range of 100–1000 mg/L of Galaxolide racemate and 50–500 mg/L for Tonalide and Traseolide racemates for conventional injection and a concentration range of 20–200 mg/L of Galaxolide racemate and 10–100 mg/L for Tonalide and Traseolide racemates analyzed for sweeping injection. The linearity of the plots was assessed in terms of correlation coefficients (r), with r as high as 0.991 and 0.997 for conventional and sweeping injections, respectively. In addition, all the confidence intervals at 95% for intercept included the 0 value.

Precision was evaluated in terms of: (i) *instrumental repeatability* obtained from three consecutive injections on the same day of a standard solution analyzed using conventional and sweeping injections; and (ii) *intermediate precision* assessed from three standard solutions freshly prepared on three different days analyzed using conventional injection. Good repeatability in terms of

Table 4
Linearity, precision, LOD and LOQ obtained using conventional (50 mbar × 3 s) and sweeping (50 mbar × 20 s) injections.

Chiral musk	Galaxolide		Tonalide		Traseolide	
Type of injection	Conventional	Sweeping	Conventional	Sweeping	Conventional	Sweeping
Linearity[a]						
Correlation coefficient	0.998	0.997	0.9991	0.995	0.997	0.991
Confidence intervals at 95% for slope	$(7.6 \pm 0.42) \times 10^{-3}$	$(67.9 \pm 4.8) \times 10^{-3}$	$(19.7 \pm 1.4) \times 10^{-3}$	$(149.0 \pm 4.9) \times 10^{-3}$	$(7.08 \pm 0.43) \times 10^{-3}$	$(59.5 \pm 8.8) \times 10^{-3}$
Confidence intervals at 95% for *y*-intercept	0.005 ± 0.253	0.457 ± 0.590	0.0273 ± 0.411	0.123 ± 0.611	0.071 ± 0.124	-0.020 ± 0.458
Precision in migration times						
Repeatability[b]	2.4	0.5	1.9	0.9	2.2	1.3
Intermediate[c]	2.6	–	2.8	–	2.7	–
Precision in peak areas						
Repeatability[b]	2.3	3.4	3.0	2.5	5.4	3.4
Intermediate[c]	6.5	–	5.2	–	6.5	–
LOD[d]	49	12	25	8	26	10
LOQ[e]	147	37	75	24	78	31

[a] y = sum of the corrected peak area of the isomers, x = concentration of the racemate; 5 concentration levels: 100–1000 mg/L for Galaxolide and 50–500 mg/L for Tonalide and Traseolide for conventional injection, and 20–200 mg/L for Galaxolide and 10–100 mg/L for Tonalide and Traseolide for sweeping injection; average values of duplicate measurements.
[b] RSD in % from three replicate injections of a standard solution of 400 mg/L for Galaxolide and 200 mg/L for Tonalide and Traseolide for conventional injection, and 100 mg/L for Galaxolide and 50 mg/L for Tonalide and Traseolide for sweeping injection.
[c] RSD in % from three freshly prepared standard solutions of 400 mg/L for Galaxolide and 200 mg/L for Tonalide and Traseolide injected in three different days.
[d] LOD = $3.29\sigma/m$ (in mg/L); σ = standard error of the y-intercept of the regression line, m = slope of the regression line.
[e] LOQ = $10\sigma/m$ (in mg/L); σ = standard error of the y-intercept of the regression line, m = slope of the regression line.

migration times and corrected peak areas was evident based on the low RSD values (*migration time* $RSD_{n=3} < 2.4\%$; *corrected peak area* $RSD_{n=3} < 5.4\%$) for both types of injection.

LODs obtained were ≤49 and ≤12 mg/L of racemate for conventional and sweeping injections, respectively (values that correspond approximately to half for the enantiomers of Tonalide and Traseolide, and a quarter for the enantiomers of Galaxolide). LOQs determined using conventional injection were ≤147 mg/L while LOQ values were ≤37 mg/L when samples were loaded using sweeping injections. Therefore, LOD enhancement factors obtained with the sweeping method developed were from 3 to 4 times depending on each PCM. In addition, the existence of possible matrix interferences was investigated by comparing the calibration slopes obtained by the external standard and the standard addition calibration methods. The results obtained by comparing the two calibration lines (confidence limits of the slopes, $P < 0.05$) showed that there were not statistically significant differences between the slopes obtained by the two calibration methods for all PCMs.

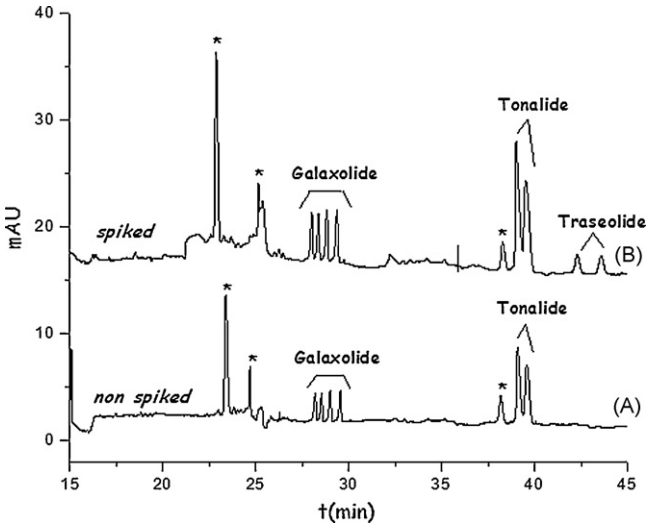

Fig. 5. Representative electropherograms of (A) perfume, (B) perfume spiked with 400 mg/L of racemic Galaxolide and 200 mg/L of racemic Tonalide and Traseolide. UV detection at 205 nm. Other conditions as in Fig. 3.

3.6. Application of the developed methodology to the analysis of perfume samples

The developed method was applied to the determination of racemic PCMs in perfume samples. Very sharp peaks were obtained only when conventional injection (50 mbar for 3 s) was used. This was attributed to sample treatment after SPE procedure since samples were reconstituted in 100% organic solvent (MeOH) due to their low solubility, unfavorable conditions for sweeping.

Fig. 5 shows the electropherograms corresponding to a perfume sample, unspiked or spiked with 400 mg/L of racemic Galaxolide and 200 mg/L of racemic Tonalide and Traseolide. Two racemic musks were detected in the unspiked perfume sample. The recovery values obtained for the different musk racemates ranged from 90% to 116%. Quantitation of Galaxolide and Tonalide in the perfume sample gave concentrations of 14.5 ± 1.3 ($n = 3$) and 9.6 ± 0.7 mg/g ($n = 3$), respectively. These values are in good agreement with those reported in literature by GC [3,25].

4. Conclusions

The enantiomeric separation of four chiral PCMs (Galaxolide, Tonalide, Traseolide, and Phantolide) was successfully achieved in this work using CD-MEKC. It is the first time that CE has been applied to the enantiomeric separation of PCMs. Two chiral methodologies were developed. First, the simple and fast chiral analysis of each individual PCM was carried out obtaining enantiomeric resolutions higher than 1.5 under different conditions (analysis time about 17 min for Galaxolide and about 10 min for Traseolide, Phantolide and Tonalide). Furthermore, a chiral methodology enabling the simultaneous enantiomeric separation of the four PCMs was also developed. The best enantiomeric separations were achieved with a dual CD system composed by 35 mM HP-γ-CD, 15 mM-γ-CD in 100 mM CHES buffer (pH 9.0) with 5 M urea and 100 mM SDS at 30 kV and 15 °C. The four chiral PCMs were enantiomerically separated within 45 min although a comigration between Galaxolide and Phantolide was observed. However, the

detection of Phantolide at 260 nm, wavelength at which Galaxolide does not present absorption could be achieved.

An optimization of a sweeping CD-MEKC methodology was also performed in order to enhance the sensitivity in the analysis for samples with a low concentration in these PCMs.

A sweeping injection of up to 30 s could be selected as maximum time to avoid a decrease in the enantioselectivity in the separation of the musks studied. A concentration factor up to 12-fold can be obtained with sweeping injection over the conventional method of injection. However, this sweeping strategy could only be applied for sample solutions with a low concentration of PCMs (<200 mg/L). Both methods with conventional and sweeping injection were validated in terms of linearity, accuracy, precision, and LODs and LOQs. Finally, the applicability of the developed method with conventional injection was successfully demonstrated by achieving the analysis of personal care products. The most commonly PCMs used in cosmetic industry, Galaxolide and Tonalide, were determined in a perfume sample showing the usefulness of the developed methodologies for the chiral determination of PCMs in these cosmetic products.

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