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Multicomponent analytical methodology to control phthalates, synthetic musks, fragrance allergens and preservatives in perfumes

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ABSTRACT

A simple, fast, robust and reliable multicomponent analytical method applicable in control laboratories with a high throughput level has been developed to analyze commercial brands of perfumes. Contents of 52 cosmetic ingredients belonging to different chemical families can be determined in a single run. Instrumental linearity, precision of the method and recovery studies in real samples showed excellent results, so that quantification by external calibration can be effectively applied. Relevant limits of detection and quantification were obtained for all the targets considered, far below the legal requirements and amply adequate for its accurate analytical control.

A survey of 70 commercial perfumes and colognes has been performed, in order to verify whether these products complied with the recent changes in European legislation: regarding the maxima allowed concentrations of the ingredients and/or ingredient labelling. All samples contained some of the target ingredients. Several samples do not comply with the regulations concerning the presence of phthalates. Musks data confirmed the trend about the replacement of nitromusks by polycyclic musks; as well as the noticeable introduction of macrocyclic musks in the perfumes composition. The prohibited musk moskene has been detected in one sample in an appreciable concentration. The average number of fragrance allergens is twelve per sample; their presence must be indicated in the list of ingredients when its concentration exceeds the 0.001%, but values higher than 1% have been found in some samples. Preservatives data show that parabens, although ubiquitous in other cosmetic products, are not widely used in perfumery. In contrast, the presence of BHT is indeed widespread. The degree of compliance with the European Regulation on the labelling has been evaluated in a subset of samples, and only about the 38% of the perfumes were properly labelled for the allergens tested.

1. Introduction

The safety of fragrance ingredients is a top priority for the cosmetics industry in general and, particularly, for perfumes manufacturers. New scientific data are constantly evaluated to ensure that the highest standards are applied to the creation of a fragrance. But a perfume may contain hundreds of substances, and any analytical method designed to analyze as many ingredients as possible with minimal effort would be welcome for both the industry and the control bodies worldwide.

The free circulation of cosmetic products in the market and the safety of cosmetics placed on it, which obviously include perfumes, have to be ensured and guaranteed by the respective governments. Recently, on 30 November 2009, in the European Union context, a new Cosmetic Products Regulation [1] (which is, to a great extent, a recast of the previous Cosmetics Directive [2] and its successive amendments and adaptations) has been adopted in order to strengthen certain elements of the regulatory framework for cosmetics, such as in-market control, with a view to ensuring a high level of protection of human health. This Regulation is already into force and it will apply with some exceptions from 11th July 2013. Among the new features of the Regulation, Article 19 from Ch. VI ("consumer information") is dedicated specifically to "labelling" and directly affects the groups of ingredients selected in this study. Previous (but currently applied) regulatory aspects for fragrance ingredients in cosmetics have been well reviewed and discussed [3,4].

Because of the nature of the use of perfumes (leave-on cosmetics), there is a high potential of human exposure. So, it is important that the ingredient labelling is correct, because this can be used by consumers to avoid the use of the products containing specific chemical(s) that they cannot tolerate; and by dermatologists, as a guide to the compounds that may be the cause of skin reactions [5] or other adverse effects. Among the different groups of chemicals used as additives in perfumes, the following four have been selected to be controlled in

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this study: phthalates, synthetic musks, fragrance allergens and preservatives.

Phthalates are commonly found in perfumes mainly as carriers or solvents for synthetic musks. Among the principal phthalates used in cosmetics, dimethyl phthalate (DMP) and diethyl phthalate (DEP) are not banned in Europe, but they are defined as contaminants to be controlled by the U.S. Environmental Protection Agency (EPA), together with the di-n-octyl phthalate (DNOP); so their analytical control is also considered here. Backing up this decision, recent findings suggest that long-term exposure to DEP, one of the widely used phthalate esters, can lead to serious health problems, and most perfumes contain non-negligible amounts of DEP [6]. The rest of phthalates measured in this study are forbidden by the European Union [1,2]: di-n-butyl phthalate (DBP), bis(2-ethylhexyl) phthalate (DEHP), bis(2-methoxyethyl) phthalate (DMEP) and di-n-pentyl phthalate (DPP).

Synthetic musks are used as an alternative for the natural musk, an intensely smelling secretion of the male musk deer. Nitromusks dominated the market for many years but declined significantly in the 90 s [7] due to their bioaccumulative properties and health adverse reactions, which eventually led to the prohibition of musk tibetene, musk moskene and musk ambrette; while musk ketone and musk xylene can still be used in cosmetics but with restrictions. There was a parallel increase in the use of polycyclic musks, a second group of synthetic musks which comprises several high-volume use products, such as tonalide® (AHTN) and galaxolide® (HHCB). However, reports on the presence of polycyclic musks in water, fish and human samples damped enthusiasm and caused production levels to decrease [8]. Nevertheless, these compounds are still largely used in personal care products, sanitation products and fragrances [9]. Although polycyclic musks have been tested in the past and showed no toxicological and dermatological effects, their high levels of use, chemical stability and low biodegradability have the consequence that have been gradually replaced by a third group of fragrances, consisting in partially artificial and partially nature-identical members, the macrocyclic musks [10].

European legislation requires monitoring 27 volatile compounds used in perfumery as they might elicit skin sensitization, the so-called potentially allergen substances (PAS) or fragrance allergens. Of these 27 substances, 25 are chemically defined volatile compounds whereas the other two are natural moss extracts and do not correspond to defined chemicals. Without presuming whether their possible sensitizing properties will be confirmed or invalidated, their occurrence in fragrance concentrates needs to be determined [11]. Recent changes in EU regulations include the transfer of methyleugenol from the Annex II (List of substances prohibited in cosmetic products) to the Annex III (List of substances which cosmetic products must not contain except subject to the restrictions laid down) and thus this compound should also be considered for controlling. Besides, pinene is a monoterpene that may be allergenic (although it is not regulated as such) and its presence is very common in perfumes -as it will be shown below-, so its control is also included in this study; making a total of 26 compounds monitored (25 regulated and 1 proposed).

Preservatives are used in cosmetics to protect them against microbial growth, both to care for consumers and to maintain product integrity. The esters of p-hydroxybenzoic acid or parabens are the most widely used preservatives in cosmetic products, especially the mixtures of methyl-, propyl-, ethyl-, butyl- and/or benzylparaben. This ubiquity led us to select them for control in perfumes. Butylated hydroxytoluene (BHT) is a synthetic antioxidant widespread used as preservative. The Final Report on the safety assessment of BHT indicates that the compound itself is not generally considered genotoxic, although it can modify the genotoxicity of other agents [12]; however the report concludes that BHT is safe at the low concentration currently used in cosmetic formulations (from 0.0002% to 0.5%).

Analytical aspects related to perfumes involve, overall, the characterization and the quality control of the extracts obtained by perfume manufacturers, of new extracts obtained from different sources or with different methods, or of perfumery raw materials [3]. Nevertheless, there is an increasingly pressing demand to know perfumes composition in depth, in particular due to the possible biological activities of some ingredients [13], like many of the compounds whose control is proposed in this study. Due to the numerous interfering compounds, the analysis of fragrances composition remains a very challenging task for perfume and cosmetic manufacturers. In this sense, most of the advances in multicomponent analysis of cosmetics have been made in the determination of different groups of preservatives [14–17] or combinations of preservatives (parabens) and phthalates [18] or preservatives including antioxidants [19–22], but in no case focused on the analysis of perfumes. Otherwise, in the case of fragrances allergens, the developed methodology has been based mainly on GC-MS [11,23–25] or comprehensive two-dimensional gas chromatography with MS detection [26,27] or, more recently, with FID detection combined with chemometrical tools [28]. In all the cited cases, the methodologies have been applied to very few perfume samples.

In addition, in none of the previous work, the analytical effort solves more than a group of ingredients focusing on perfumes and neither focused on the analysis of a large number of perfume samples to survey the levels of such ingredients in commercial products, as it is the case in the present study, with the exception of a recent survey by an international NGO, in which phthalates and synthetic musks have been determined in a random selection of 36 perfume brands [10].

The objective of the present work is to put forward a reliable multicomponent analytical method applicable in control laboratories, from companies and institutions, with a high throughput level. A total of 52 target compounds belonging to four different types of ingredients have been selected to be determined in a single GC-MS run: six preservatives (parabens and BHT), twelve synthetic musks (five nitromusks, six polycyclic and one macrocyclic), twenty-six fragrance allergens, and eight phthalates. Instrument and analytical conditions have been optimized; no pre-treatment of samples other than dilution is needed. The ruggedness of the proposed methodology is demonstrated by its application using two different instrumental set-ups, involving external and internal ion trap configurations as well as two different GC stationary phase polarities. To assess the performance of the proposed method, accuracy, precision, linearity and detection limits (LODs) have been evaluated obtaining quantitative recoveries and low RSD values. The obtained LODs are far below the established restrictions as regards labelling in the European Cosmetics Regulation [1]. A survey of a variety of perfumes and colognes for the target ingredients was conducted. Seventy samples, fragrances for men, women, children and babies, have been analyzed with the proposed methodology to check whether these products complied with the new European Regulation with respect to both the maximum allowed concentration (MAC) of the ingredients present and the correct labelling.

2. Material and Methods

2.1. Reagents and materials

The studied compounds, their chemical names and the purity of the standards are summarized in Table 1. Ethyl acetate and acetone (analytical grade) were provided by Merck (Darmstadt, Germany). Individual stock solutions of each compound were prepared in

Table 1
Retention times, quantification and identification ions of the targets.

N	Compound	Chemical name	CAS number	Purity (%)	GC–MS detection	
					Retention time (min)	Quantifier and qualifiers
1	Pinene	2,6,6-Trimethylbicyclo[3.1.1]hept-2-ene	80-56-8	≥99^{a}	6.75	77,91,*93*
2	Limonene	(4R)-1-Methyl-4-(1-methylethenyl)cyclohexene	5989-27-5	97^{b}	8.68	67,93,121
3	Benzyl alcohol	Benzene methanol	100-51-6	99^{c}	8.94	77,79,108
4	Linalool	3,7-Dimethyl-1,6-octadien-3-ol	78-70-6	97^{b}	9.84	43,93,121
5	Methyl-2-octynoate	Methyl heptin carbonate	111-12-6	≥99^{d}	11.09	67,79,95
6	Citronellol	(±)-3,7-Dimethyloct-6-en-1-ol	106-22-9/26489-01-0	95^{b}	11.36	69,81,95
7	Citral	3,7-Dimethyl-2,6-octadienal	5392-40-5	95^{b}	11.51 11.78	39,69,109
8	Geraniol	3,7-Dimethyl-(2E)-2,6-octadien-1-ol	106-24-1	≥96^{a}	11.60	41,69,111
9	Cinnamal	3-Phenyl-2-propenal	104-55-2	≥93^{d}	11.90	77,103,131
10	Hydroxycitronellal	7-Hydroxy-3,7-dimethyloctanal	107-75-5	≥95^{d}	11.94	43,59,81
11	Anise alcohol	4-Methoxybenzyl alcohol	105-13-5	98^{b}	11.99	109,121,138
12	Cinnamyl alcohol	3-Phenyl-2-propen-1-ol	104-54-1	98^{c}	12.18	77,92,134
13	Eugenol	2-Methoxy-4-(2-propenyl) phenol	97-53-0	99^{b}	12.49	103,131,164
14	Methyleugenol	1,2-Dimethoxy-4-(2-propenyl)-benzene	93-15-2	99^{b}	12.78	147,163,178
15	Isoeugenol	2-Methoxy-4-(1-propenyl) phenol	97-54-1	98^{b}	12.86 13.15	103,131,164
16	DMP	Dimethyl phthalate	131-11-3	98^{a}	13.14	77,163,194
17	Coumarin	2H-1-benzopyran-2-one	91-64-5	99^{b}	13.18	89,118,146
18	MeP	Methyl 4-hydroxybenzoate	99-76-3	99^{a}	13.21	65,121,152
19	α-Isomethyl ionone	3-Methyl-4-(2,6,6-trimethyl-2-cyclohexen-1-yl)-3-buten-2-one	127-51-5	≥85^{a}	13.29	107,135,150
20	BHT	Butylated hydroxytoluene	128-37-0	99^{b}	13.45	57,205,220
21	Cashmeran	1,1,2,3,3-Pentamethyl-2,5,6,7-tetrahydroinden-4-one	33704-61-9	>95^{e}	13.50	135,191,163
22	EtP	Ethyl 4-hydroxybenzoate	120-47-8	99^{a}	13.61	65,121,138
23	Lilial®	2-(4-*tert*-Butylbenzyl) propionaldehyde	80-54-6	≥95^{a}	13.63	131,147,189
24	DEP	Diethyl phthalate	84-66-2	98^{a}	14.01	149,176,177
25	PrP	Propyl 4-hydroxybenzoate	94-13-3	99^{a}	14.28	65,121,138
26	Amyl cinnamal	2-Benzylideneheptanal	122-40-7	97^{b}	14.47	115,129,202
27	Lyral®	Hydroxyhexyl-3-cyclohexene carboxaldehyde	31906-04-4	≥97^{a}	14.65	79,91,136
28	Amylcinnamyl alcohol	2-Pentyl-3-phenylprop-2-en-1-ol	101-85-9	≥85^{a}	14.77	91,115,133
29	Celestolide	4-Acetyl-6-tert-butyl-1,1-dimethylindane	13171-00-1	>98^{e}	14.97	173,229,244
30	Farnesol	3,7,11-Trimethyldodeca-2,6,10-trien-1-ol	4602-84-0	95^{b}	14.99	41,69,81
31	BuP	Butyl 4-hydroxybenzoate	94-26-8	99^{a}	15.14	65,121,138
32	Hexylcinnamal	2-Benzylideneoctanal	101-86-0	≥95^{d}	15.34 15.60	115,129,216
33	Phantolide	6-Acetyl-1,1,2,3,3,5-hexamethylindan	15323-35-0	>98^{f}	15.38	145,187,229
34	Benzyl benzoate	Phenylmethyl benzoate	120-51-4	98.5^{c}	15.66	105,194,212
35	Musk ambrette (MA)	6-*tert*-Butyl-3-methyl-2,4-dinitroanisole	83-66-9	99^{g}	16.21	91,251,253
36	Traseolide	5-Acetyl-3-isopropyl-1,1,2,6-tetramethylindane	68140-48-7	Tech. ^{f}	16.39	131,173,215
37	Galaxolide	1,3,4,6,7,8-Hexahydro-4,6,6,7,8,8-hexamethylcyclopenta(g)-2-benzopyran	1222-05-5	75^{f}	16.50	171,213,243
38	Musk xylene (MX)^{h}	1-*tert*-Butyl-3,5-dimethyl-2,4,6-trinitrobenzene	81-15-2		16.56	43,282,297
39	Tonalide	6-Acetyl-1,1,2,4,4,7-hexamethyltetralin	1506-02-1	98^{f}	16.58	159,187,243
40	Musk moskene (MM)^{h}	1,1,3,3,5-Pentamethyl-4,6-dinitro-2H-indene	116-66-5		16.92	263,264,278
41	Benzyl salicylate	Benzyl-2-hydroxybenzoate	118-58-1	≥ 99^{a}	16.98	65,91,228
42	Musk tibetene (MT)^{g}	1-*tert*-Butyl-3,4,5-trimethyl-2,6-dinitrobenzene	145-39-1		17.70	43,251,266
43	Ambrettolide	17-Oxacycloheptadec-6-en-1-one	7779-50-2	>97^{e}	17.87	67,81,95
44	DBP	Dibutyl phthalate	84-74-2	99^{b}	18.10	149,150,223
45	Musk ketone (MK)a	4-*tert*-Butyl-3,5-dinitro-2,6-dimethyl acetophenone	81-14-1		18.45	279,280,294
46	DMEP	Bis(2-methoxyethyl) phthalate	117-82-8	94^{g}	18.79	58,59,149
47	Benzyl cinnamate	3-Phenyl-2-propenoic acid phenylmethyl ester	103-41-3	99^{b}	20.80	91,131,192
48	DPP	Dipentyl phthalate	131-18-0	99.2^{g}	21.24	149,150,237
49	BzP	Benzyl hydroxybenzoate	94-18-8	99^{a}	21.34	65,91,121
50	BBP	Benzyl butyl phthalate	85-68-7	98^{b}	22.83	91,149,206
51	DEHP	Bis(2-ethylhexyl) phthalate	117-81-7	99.5^{a}	23.89	149,150,167
52	DNOP	Di-n-octyl phthalate	117-84-0	≥98^{a}	25.31	41,149,279

Obtained from: [a]Fluka Chemie GmbH, Germany (MK: 100 ng μL^{-1} in acetonitrile); [b]Sigma–Aldrich Chemie GmbH, Germany; [c]ChemService, West Chester, USA; [d]SAFC Supply Solutions, St. Louis, USA; [e]Ventos, Barcelona, Spain; [f]LGC Standards GmbH, Germany; [g]Dr. Ehrenstorfer, Germany (MT: 10 ng μL^{-1} in cyclohexane); [h]100 ng μL^{-1} in acetonitrile, Riedel de Haën, Germany.

acetone. Further dilutions and mixtures were prepared in ethyl acetate and then stored in amber glass vials at -20 ◦C.

2.2. Gas chromatography-mass spectrometry

The samples were analyzed in two GC-MS equipments. The first one was a Varian 450-GC gas chromatograph (Varian Chromatography Systems, Walnut Creek, CA, USA) coupled to an ion trap mass spectrometer Varian 240-MS (Varian Chromatography Systems) with a waveboard for multiple MS (MS^n) analysis; and a sampler model CP-8400. The system was managed by Varian MS workstation v6.9.1 software. Separation was carried out on a HP5 capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness) from Agilent Technologies (Palo Alto, CA, USA). The ion trap mass spectrometer was operated in the electron impact (EI) ionization mode (+70 eV) using an external ionization configuration. Manifold, ion trap, ion source and transfer line temperatures, were maintained at 40, 150, 200 and 280 °C, respectively. The filament emission current was 25 μA. The acquisition mass range was from 39 to 400 m/z at 3 μscans, starting at 5 minutes and ending at 30 min.

The second equipment used was a Varian 3800-GC gas chromatograph (Varian Chromatography Systems, Walnut Creek, CA, USA) coupled to a Varian Saturn 2000 ion trap mass spectrometer (Varian Chromatography Systems) equipped with a 1079 split/splitless injector. The system was operated by Saturn GC-MS workstation version 5.4 software, and separation was carried out on a VF-1701ms capillary column (30 m × 0.25 mm i.d. × 0.39 mm o.d., 0.25 μm film thickness) from Varian, Inc. (Lake Forest, CA, USA). The ion trap mass spectrometer was operated in the electron impact (EI) ionization mode (+70 eV). Manifold, ion trap, and transfer line temperatures, were maintained at 110, 200, and 280 °C, respectively. The filament emission current was 10 μA. The acquisition mass range was the same described for the other equipment.

Common GC conditions to both systems were as follows. Helium (purity 99.999%) was employed as carrier gas at a constant column flow of 1.0 mL min^{-1}. The GC oven temperature was programmed from 45 °C (held 2 min) to 100 °C at 8 °C min^{-1}, to 130 °C at 20 °C min^{-1} (held 3 min) and, to 200 °C at 25 °C min^{-1}; (total analysis time = 25 min). Splitless mode (held 2 min) was used for injection, the split flow was set at 20 mL min^{-1} and the injector temperature was kept at 220 °C. The injection volume was 2 μL.

The analytes were positively identified by comparison of their mass spectra and retention times to those of standards. The identification and quantification ions and retention times for each target compound are listed in Table 1.

2.3. Sampling and sample pre-treatment

Seventy commercial perfumes and eau de toilettes (for men, women, children and babies) were purchased in perfumeries, supermarkets, and convenience stores from Galicia (Northwest Spain). The samples included international and national brands, covering a wide range of prices. Samples were stored at room temperature until analysis. All samples were clear liquids and no special pre-treatment was applied apart from homogenization and dilution 1:10 with ethyl acetate. Due to the huge range of concentrations in which these compounds can be included in the samples (from ng per mL to mg per mL), dilutions 1:100 and 1:1000 with ethyl acetate were also injected in some cases.

The general use of the targets in all classes of personal-care products and cleaning products, as well as the well-known ubiquitous presence of phthalates, demand special precautions during all analytical procedures to minimize contamination risk.

3. Results and Discussion

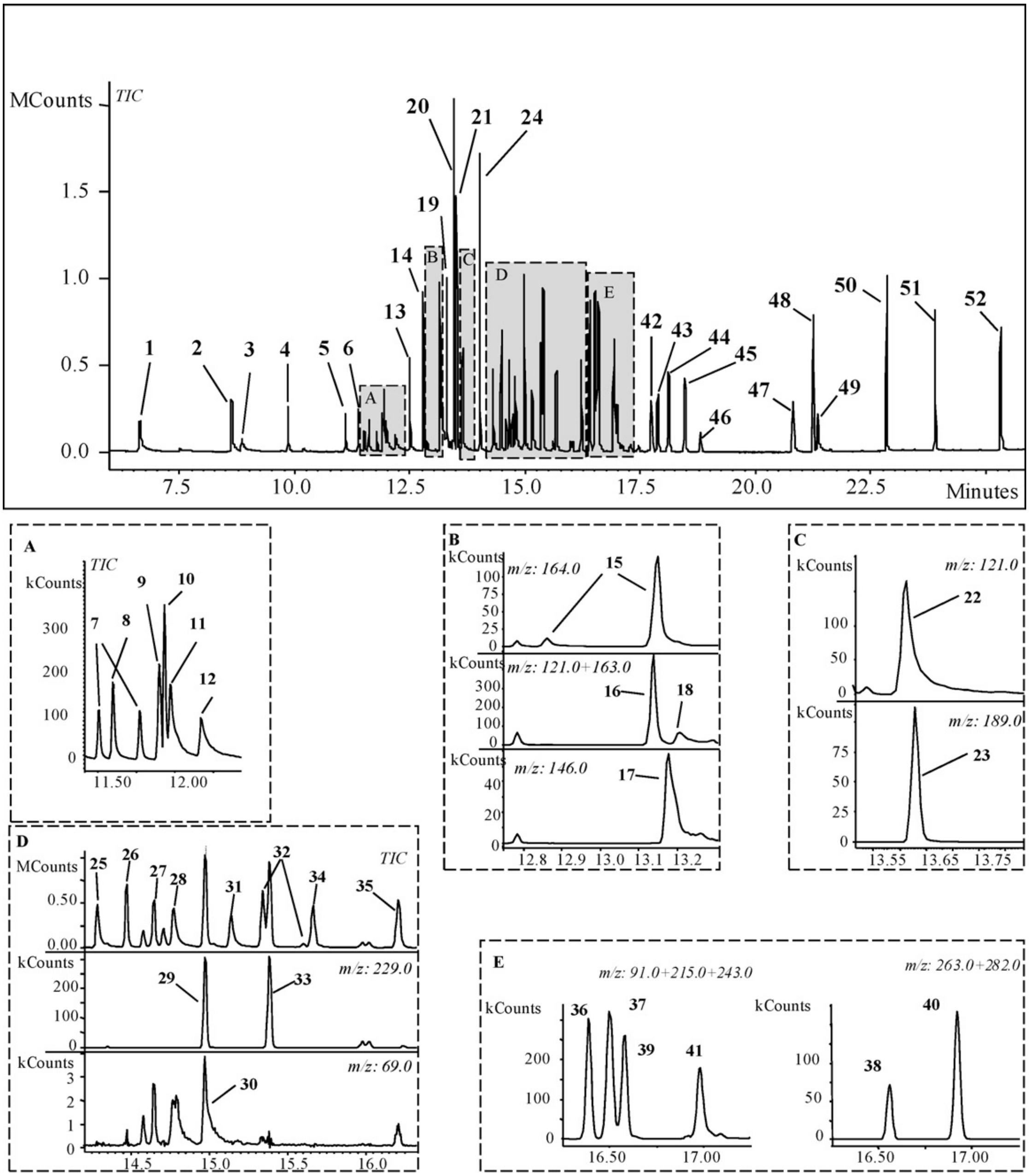
In this study we develop a chromatographic method useful to separate and identify 52 analytes with very different chemical and physical properties, and belonging to different cosmetic additive families including fragrance allergens (26), phthalates (8), synthetic musks (12), and preservatives (6). The objective is to make possible the application of the chromatographic method to the analysis of these important groups of regulated cosmetic ingredients in cosmetic analysis in a single run. As previously commented, most of the studies found in the literature deals with only one group of these ingredients, and, in addition, the number of compounds considered is low. Some studies about the determination of regulated suspected allergens [29], one of the families included in our study, show the difficulties to achieve an effective separation of these compounds. Previously, we had developed a GC-MS method that makes possible the quantification of 24 of the fragrance allergens [30,31]. This was the starting point to develop a chromatographic method including the other four analyte families. Different chromatographic columns and different temperature programs were tested to achieve the best possible separation conditions. The final selected chromatographic conditions are summarized in the material and methods section. Taking into account the high number of analytes considered, the global method resolution can be considered quite good; although some analytes were not (completely) resolved, the extracted ion chromatograms, permitted the proper quantification of all compounds. The selection of the quantification ions was based on the attainment of the most favourable resolution and the maximum signal-to-noise ratio. Fig. 1 shows a chromatogram composition obtained for a 5 μg mL^{-1} standard mixture including all target compounds. The 52 cosmetic ingredients could be analyzed in only 25 min.

3.1. Method Validation

It is well known that the most important problem concerning phthalate analysis is the risk of contamination, resulting in false positive results and over-estimated concentrations [32]. The sources of contamination can be present in any step of the analytical procedure. Special care was taken to avoid the contact of reagents and solutions with plastic materials. Laboratory glassware was washed prior to use with ultrapure water and dried at 300 °C. This material was stored in aluminium foil to avoid adsorption of phthalates from the air. Besides, due to the occurrence of musk fragrances as ingredients of all kind of cleansing products and cosmetics, the risk of sample contamination when they are manipulated in the laboratory is not negligible, so it is advisable to extreme precautions to avoid sources of interference in the laboratory environment.

Blank runs of the chromatographic system and direct injections of the solvent selected, ethyl acetate, must be daily done. In spite of all the precautions adopted, one of the phthalates, DEP, was found in all solvent blank runs and, in consequence, detection limit was calculated considering the average blank signal.

To verify that the developed GC-MS method was suitable for the quantitative determination of the selected groups of ingredients in perfumery commercial products, method quality parameters were estimated and summarized for each family of compounds in Table 2. Regarding to instrumental linearity, the method exhibited a direct proportional relationship between the amount of each analyte and the chromatographic response with correlation coefficients $R \geq 0.999$ for phthalates and musks fragrances, $R \geq 0.996$ for fragrance allergens and $R \geq 0.998$ for preservatives (Table 2). The sensitivity of the method, expressed as the slope of the calibration curve, is also included in Table 2.



1: Pinene; 2: Limonene; 3: Benzyl alcohol; 4: Linalool; 5: Methyl-2-octynoate; 6: Citronellol; 7: Citral; 8: Geraniol; 9 Cinnamal; 10: Hydroxycitronellal; 11: Anise alcohol; 12: Cinnamyl alcohol; 13: Eugenol; 14: Methyleugenol; 15: Isoeugenol; 16: DMP; 17: Coumarin; 18: MeP; 19: α-Isomethyl ionone; 20: BHT; 21: Cashmeran; 22: EtP; 23: Lilial; 24: DEP; 25: PrP; 26: Amyl cinnamal; 27: Lyral®; 28: Amylcinnamyl alcohol; 29: Celestolide; 30: Farnesol; 31: BuP; 32: Hexylcinnamal; 33: Phantolide; 34: Benzyl benzoate; 35: MA; 36: Traseolide; 37: Galaxolide; 38: MX; 39: Tonalide; 40: MM; 41: Benzyl salicylate; 42: MT; 43: Ambrettolide; 44: DBP; 45: MK; 46: DMEP; 47: Benzyl cinnamate; 48: DPP; 49: BzP; 50: BBP; 51: DEHP; 52: DNOP

Fig. 1. Total and extracted ion chromatograms for a standard mixture (5 μg mL^{-1}).

Method precision was studied within a day and among days at several concentration levels between 0.01 and 10 μg mL^{-1}. The results for the levels 0.1 and 1.0 μg mL^{-1} are also included in Table 2. For phthalates, RSD values ranged from 0.81 to 5.9% (intra-day precision, average 3.00%), and between 2.1 and 5.8% (inter-day precision, average 3.99%). Precision for musk fragrances was also satisfactory with RSD values ranging from 0.52 to 6.7% for intra-day and 1.5 and 5.7% for inter-day studies (the averages for intra-day and inter-day precision were 2.81 and 3.90%, respectively). For the suspected fragrance allergens, RSDs for the intra-day precision ranged from 0.38 to 7.7% with an average value of 4.04%, while the RSDs for the inter-day precision ranged from 1.1 to 7.5% with an average value of 4.61%. Finally, preservatives RSD values ranged from 1.6 and 5.7% (intra-day precision, average 3.34%), and between 2.3 and 6.5% (inter-day precision, average 5.00%).

Instrumental detection limits (IDLs) were calculated as the concentration giving a signal-to-noise ratio of three (S/N = 3) in all cases with the exception of DEP, which appeared in all the blanks. For

Table 2
Performance of the method for the target compounds.

	Sensitivity	Linearity *R*	Precision (% RSD)				IDLs (ng mL^{-1})	Recovery[f] (RSD)(%)	LOD[g] (%, w/v)
			Intra-day (*n* = 3)		Inter-day (*n* = 6)				
Phthalates[a]			0.1[e]	1.0[e]	0.1[e]	1.0[e]			
DMP	178	1.000	0.96	4.7	2.1	5.8	1.0	105 (2.7)	0.0000010
DEP	189	1.000	5.9	4.3	4.7	5.4	15	n.c.	0.000015
DBP	271	1.000	4.3	3.8	3.6	4.8	1.0	97.3 (4.4)	0.0000010
DMEP	10	1.000	0.90	1.5	2.9	4.0	6.4	105 (1.0)	0.0000064
DPP	187	1.000	1.7	1.1	3.7	3.9	0.65	106 (5.3)	0.00000065
BBP	113	1.000	2.9	3.7	2.1	3.8	5.0	97.9 (8.1)	0.0000050
DEHP	192	0.999	0.81	3.7	3.5	3.9	0.81	88.6 (5.4)	0.00000081
DNOP	297	0.999	4.8	3.0	4.7	5.0	0.65	95.1 (5.7)	0.00000065
Musk fragrances[a]									
Cashmeran	78	1.000	1.9	6.7	1.5	4.9	0.48	107 (2.9)	0.00000048
Celestolide	157	1.000	4.2	2.0	3.6	5.5	0.20	92.8 (5.1)	0.00000020
Phantolide	179	1.000	4.4	3.7	3.6	4.4	0.19	101 (2.8)	0.00000019
Musk ambrette	23	0.999	0.52	1.7	1.9	3.9	1.0	99.2 (3.5)	0.0000010
Traseolide	197	0.999	0.83	3.2	3.3	3.5	0.25	96.8 (4.8)	0.00000025
Galaxolide	319	1.000	2.6	4.4	2.5	5.5	0.21	n.c	0.00000021
Musk xylene	56	0.999	2.3	3.7	2.7	5.1	1.1	100 (2.1)	0.0000011
Tonalide	183	1.000	3.6	3.5	2.8	5.6	0.27	94.1 (3.6)	0.00000027
Musk moskene	151	1.000	1.7	4.5	2.9	5.7	0.23	102 (2.3)	0.00000023
Musk tibetene	50	0.999	1.5	2.7	2.9	4.9	1.1	107 (6.1)	0.0000011
Ambrettolide	66	1.000	2.0	0.54	3.0	4.0	0.83	110 (8.0)	0.00000083
Musk ketone	83	1.000	1.4	3.9	4.7	5.1	0.88	99.6 (7.0)	0.00000088
Fragrance allergens									
Pinene[b]	56	1.000	6.2	1.0	5.0	6.0	0.98	94.9 (8.9)	0.0000010
Limonene[b]	58	0.999	6.6	3.2	6.6	3.5	0.26	n.c.	0.00000026
Benzyl alcohol[c]	50	0.999	n.c.	7.2	n.c.	7.5	25	106 (2.0)	0.0000033
Linalool[b]	25	0.999	7.7	2.4	5.8	2.4	1.7	n.c.	0.0000017
Methyl-2-octynoate[c]	18	0.996	n.c.	2.1	n.c.	6.5	15	105 (6.3)	0.000015
Citronellol[d]	38	0.998	7.4	7.3	6.7	2.9	15	104 (5.8)	0.000012
Citral[c]	4	0.998	n.c.	6.4	n.c.	5.0	29	105 (5.0)	0.000029
Geraniol[c]	5	0.997	n.c.	0.81	n.c.	5.9	27	97.7 (2.7)	0.000027
Cinnamal[c]	65	0.998	n.c.	5.8	n.c.	5.4	6.0	105 (8.0)	0.0000060
Hydroxycitronellal[b]	31	0.999	4.0	1.2	6.2	1.4	1.5	96.0 (5.2)	0.0000033
Anise alcohol[c]	23	0.999	n.c.	5.3	n.c.	4.1	21	106 (3.0)	0.000021
Cinnamyl alcohol[c]	17	1.000	n.c.	2.1	n.c.	6.5	26	103 (3.8)	0.000026
Eugenol[b]	56	0.999	3.6	3.6	7.1	2.9	4.5	93.9 (8.7)	0.0000015
Methyleugenol[b]	77	0.999	3.6	1.4	5.7	1.1	0.42	101 (2.5)	0.00000042
Isoeugenol[c]	65	0.999	n.c.	2.8	n.c.	3.5	6.3	106 (3.7)	0.0000063
Coumarin[d]	50	0.998	6.6	4.1	5.9	5.5	22	95.5 (6.6)	0.00000072
α-Isomethyl ionone[b]	77	0.999	4.9	3.5	6.7	3.8	0.75	101 (7.1)	0.00000075
Lilial®[b]	37	0.998	4.8	2.3	4.4	1.7	0.30	n.c.	0.00000030
Amyl cinnamal[b]	32	0.998	3.8	1.3	5.4	2.6	2.4	100 (5.7)	0.0000024
Lyral®[b]	33	0.998	2.5	4.1	2.5	3.8	3.0	n.c.	0.0000030
Amylcinnamyl alcohol[d]	24	0.999	3.3	4.4	6.9	4.2	18	89.4 (2.5)	0.000018
Farnesol[c]	3	0.999	n.c.	5.7	n.c.	4.6	29	n.c.	0.000069
Hexylcinnamal[b]	53	0.999	5.5	2.2	5.8	2.9	0.73	103 (1.3)	0.00000073
Benzyl benzoate[b]	76	0.999	6.0	4.9	4.9	3.8	2.4	90.7 (0.5)	0.0000024
Benzyl salicylate[b]	160	0.999	0.60	4.4	3.2	3.8	2.3	98.6 (5.1)	0.0000023
Benzyl cinnamate[d]	35	0.999	6.8	0.38	4.4	3.7	7.5	102 (2.2)	0.0000075
Preservatives									
MeP[d]	31	0.999	2.2	3.3	6.0	3.8	15	108 (4.5)	0.000015
BHT[a]	174	0.998	3.6	1.6	3.2	4.8	0.16	100 (4.8)	0.0000016
EtP[d]	106	1.000	4.6	4.8	6.5	6.2	15	98.3 (6.3)	0.000015
PrP[d]	118	1.000	1.8	2.4	5.7	3.9	9.0	105 (5.6)	0.0000090
BuP[d]	94	1.000	5.7	4.5	5.4	5.8	15	105 (2.6)	0.000015
BzP[d]	87	0.998	2.7	2.9	2.3	6.4	50	97.5 (5.9)	0.000050

Linearity ranged from:

[a] 0.005 to 10 μg mL^{-1}.

[b] 0.010 to 20 μg mL^{-1}.

[c] 0.200 to 20 μg mL^{-1}.

[d] 0.050 to 20 μg mL^{-1}.

[e] Concentration levels (μg mL^{-1}).

[f] Spiked amounts on real samples ($n = 3$) ranged from 0.00070 to 0.0028% (w/v) for phthalates; from 0.00010 to 0.0027% (w/v) for musks; from 0.0011 to 0.0083% (w/v) for allergens; and from 0.0024 to 0.013% (w/v) for preservatives.

[g] Calculated on real samples for a sample dilution factor of 1:10. n.c., not calculated.

this reason its IDL was calculated as the blank signal plus three times the standard deviation. IDLs are also shown in Table 2. The calculated values were, in general, in the low ng per mL. Derivatization, and, particularly, acetylation of parabens could improve the peak shape of these quite polar compounds and, therefore, their IDLs [17]. The inconvenient of this process, which is carried out in a basic medium, is that can alter other targets containing for example alcohol groups. Since the aim of this work is to establish a reliable multicomponent analytical method with a high throughput level and the obtained IDLs without derivatization are far below the regulation limits, derivatization of parabens was discarded.

One of the most important difficulties regarding cosmetic analytical control is the wide range of concentrations at which the regulated ingredients can be found (between the parts per million and the high percentage). This problem becomes a challenge when the objective is the control of different compound families, enlarging significantly the number of analytes to determine. Due to the high concentration of some of the target ingredients, all real perfume samples analyzed were diluted by a factor of 10 to 1000. Frequently, and due to the broad range of concentrations found, several dilutions must be analyzed to achieve proper quantification of all compounds.

Recovery studies were carried out with real samples and they were quite challenging for several reasons. For these studies, three different real samples were used (S41, S42 and S57). The selection of the samples was difficult, since all the samples contained several of the targets. When the original target concentration of the sample is quite high, it was not possible to calculate the recovery; in the rest of the cases, the initial concentration was taken into account to calculate the recoveries. Another difficulty was the selection of the spiked amount, since the addition of the lowest possible percentage of solvent is preferable to avoid changes in the matrix and, in some cases, enough concentrated standards are not available. The spiked concentrations ranged from 0.00070 to 0.0028% (w/v) for the phthalates, from 0.00010 to 0.0027% (w/v) for the musk fragrances, from 0.0011 to 0.0083% (w/v) for the fragrance allergens, and 0.0024 to 0.013% (w/v) for the preservatives. The recovery of DEP, galaxolide, limonene, linalool, lilial and lyral could not be evaluated since they were already present in the samples in a high concentration. On the other hand, the recovery of farnesol was not evaluated, since a high concentrated standard solution was not available.

Recoveries are shown in Table 2, and were satisfactory in all cases, with a minimum of 88.6% and a maximum of 110%. Therefore, quantification by external calibration can be effectively employed.

Method detection limits were also evaluated in real samples and they are referred to a sample dilution factor of 1:10. As can be seen in the Table 2, these limits for most analytes correspond to ten times the IDLs, excluding few exceptions for which the chromatographic background was higher than in the corresponding standard solution chromatogram, increasing in this way the LODs of the method.

3.2. *Application to Real Samples*

The validated method was applied to the analytical control of the 52 target ingredients in 70 different commercial products, designed to be used by men, women, children or babies. Individual data on the presence and found concentrations (%, w/v) of each of the ingredients in the real samples (named S1 to S70) can be found in Tables I to IV of the supplementary material. As a graphical example, Fig. 2 shows the analysis of the sample S11, in which a total of 22 ingredients have been detected in a single run: DEP and DEHP as phthalates; galaxolide, tonalide and MK as musks; 15 of the 26 allergenic fragrances and the preservatives MeP and BHT.

A statistical summary of the data has been performed to facilitate the discussion of results, considering just the analytes detected in more than 10% of the consumer products.

3.2.1. *Phthalates*

The degree of prohibition of phthalates in cosmetics is not homogeneous. Some of them can not be part of the composition of cosmetic products as DBP, DMEP, DPP, BBP and DEHP. Besides, some of the principal phthalates used in cosmetics, DMP and DEP are permitted in Europe, but are considered pollutants in USA -together with DNOP-, and thus their analytical control is also preventively considered in this study. A practical approach to control those banned phthalates can be that the LOQs of the selected method should be less than or equal to 0.0001%. Thus, the average LOD obtained for phthalates with the proposed methodology was 0.0000038%; with minima and maxima in the 10^{-6}% and 10^{-5}% order for LODs. In a previous USA Food and Drug Administration (FDA) study of consumer cosmetic products for phthalate esters [33], levels lower than 10 μg mL^{-1} (0.001%) were reported as not detected.

Table 3 shows a statistical distribution of the phthalates in the evaluated samples. The most common phthalates were DEP, DBP and DEHP present in 81.4%, 72.8% and 55.7%, of the perfumes tested, respectively; as it has been just commented, DBP and DEHP are banned by the EU. The presence of DMP, DPP and BBP was very limited, but again three of the samples did not comply with the regulations in this regard; and finally, DNOP and DMEP were not detected in any sample. Six samples are completely free of phthalates (S23, S43, S45, S48, S55 and S59). Table I (see supplementary material) shows the complete distribution of the phthalates in the samples. Fig. 3 is a partial extract showing the distribution of the banned phthalates in the irregular samples; mainly emphasizing the sample whose DBP concentration reaches 4% (S54). The number of irregular samples may seem high, but it should be noted that these compounds can come from many different sources (e.g. packaging of raw materials or perfumes themselves) and not necessarily from the manufacturing process.

3.2.2. *Musk Fragrances.*

There are three nitromusks that are not allowed in cosmetic formulations: ambrette, moskene and tibetene, and a LOQ lower or equal to 0.0001% can also be applied to their control. Other two nitromusks may be part of the composition of cosmetic products with some constraints, being the maximum permitted concentrations for musk xylene and musk ketone 1.0% and 1.4% in fine fragrance, 0.4% and 0.56% in eau de toilette, and 0.03% and 0.042% in other products, respectively. Although right now the polycyclic musks are considered safer than the nitromusks, it is likely that, given its vast global production and the amount and variety of potential sources, they will be in the spotlight sooner or later; thereby implementing their analytical control in cosmetics is to go one step ahead. An appropriate LOD average value was also obtained for all the twelve musks considered in this study: 0.00000056%; with minima and maxima values between 10^{-7} and 10^{-6}%. Musks real data (Tables 3 and II) confirm the commented trend about the replacement of nitromusks (present in the 21.4% of the perfume samples) by polycyclic musks (present in the 97.1%) with a clear prevalence of galaxolide and tonalide; as well as the noticeable introduction of macrocyclic musks such as ambrettolide, determined in a 55.7% of the perfumery products (Fig. 4). Two samples were completely free of musk fragrances (S10 and S59) and one else virtually free of them (S12). It is pertinent to project out here that the prohibited musk moskene has been detected in one sample (S16) in an appreciable concentration (0.12%).

3.2.3. *Fragrance allergens.*

The presence of any potentially allergenic fragrance must be declared in the product label when present in concentrations greater than 0.001% for leave-on cosmetics and 0.01% in rinse-off products. The former limit has to be considered here, since perfumes are designed to remain on the skin. Thus, suitable LOD data were obtained for this group of substances, with an average value of 0.000010%, far below the legal requirements; being the minima and maxima in the 10^{-7}% and 10^{-5}% order, respectively.

The information concerning the allergenic fragrances is summarized in Tables 3 and III. The average amount of fragrance allergens is 12 per sample. Four fragrance allergens appeared in

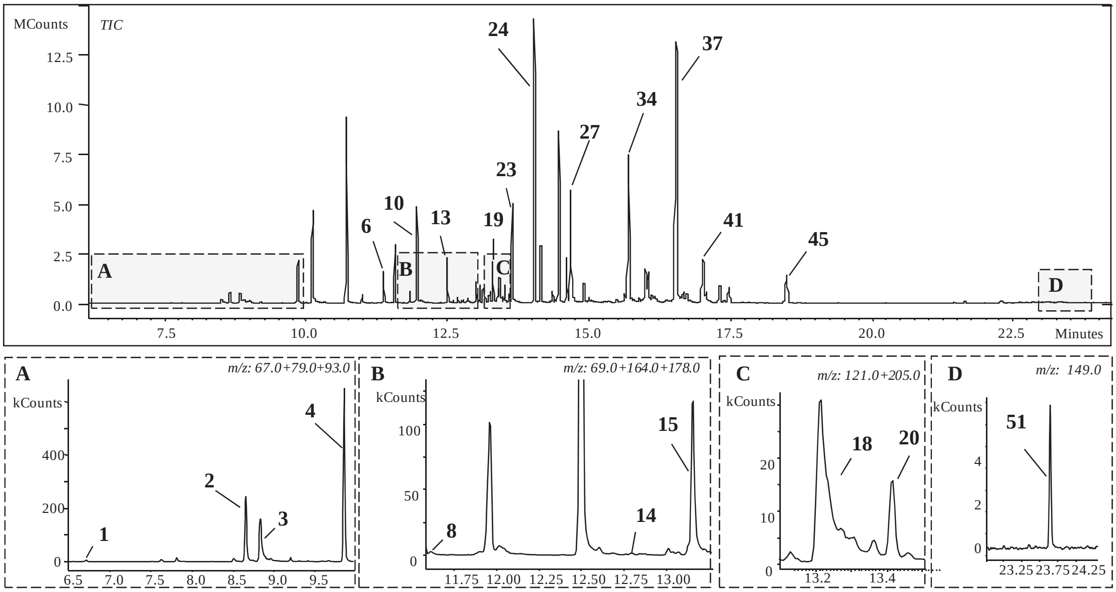


Fig. 2. Total and extracted ion chromatograms obtained for sample S11 (key numbers as in Fig. 1).

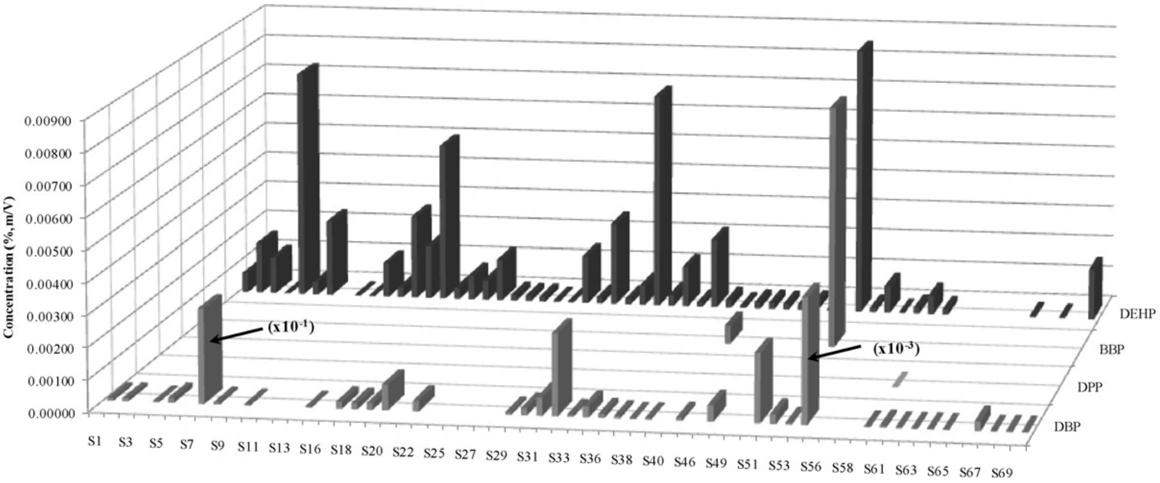


Fig. 3. Partial distribution of the phthalates in the samples. The plot shows the irregular samples containing phthalates banned in the EU.

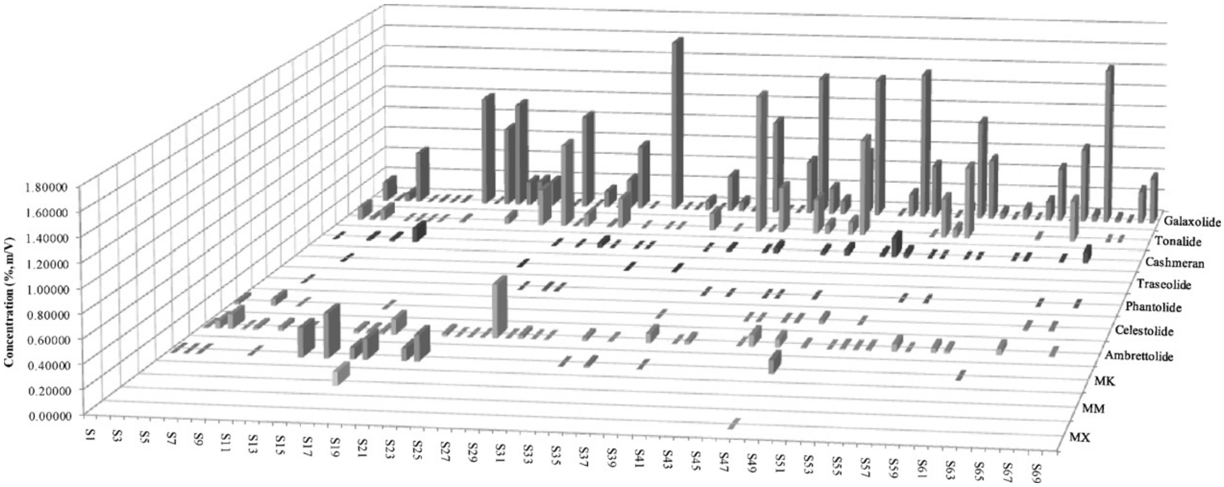


Fig. 4. Distribution of the musk compounds in the samples. The prohibited musk moskene has been detected in sample S16.

Table 3
Concentration of the target compounds in real samples. Maximum concentration and statistic parameters (%, w/v).

Compound	Maximum concentration	25th percentile	50th percentile Median	75th percentile	90th percentile	N out of 70
Phthalates						
DMP	0.00259	0.00007	0.00017	0.00039	0.00089	9
DEP	3.158	0.2987	0.4686	0.7569	1.327	57
DBP	3.928	0.00004	0.00009	0.00030	0.00108	39
DMEP	<LOD	–	–	–	–	0
DPP	0.00002	–	–	–	–	1
BBP	0.00772	–	–	–	–	2
DEHP	0.00861	0.00016	0.00032	0.00133	0.00265	51
DNOP	<LOD	–	–	–	–	0
Musk fragrances						
Cashmeran	0.1712	0.00093	0.00740	0.0328	0.0680	27
Celestolide	0.0582	0.00080	0.00376	0.0105	0.0308	13
Phantolide	0.00482	0.00023	0.00055	0.00091	0.00205	14
Musk ambrette	<LOQ	–	–	–	–	0
Traseolide	0.00683	0.00289	0.00366	0.00452	0.00591	4
Galaxolide	1.600	0.00096	0.1225	0.4846	0.9491	63
Musk xylene	0.00065	–	–	–	–	1
Tonalide	1.268	0.00138	0.0703	0.3051	0.5799	34
Musk moskene	0.1164	–	–	–	–	1
Musk tibetene	<LOQ	–	–	–	–	0
Ambrettolide	0.4570	0.00325	0.0157	0.0348	0.0862	39
Musk ketone	0.3761	0.00099	0.0172	0.1436	0.2385	15
Fragrance allergens						
Pinene	0.1182	0.00137	0.00660	0.0162	0.0347	68
Limonene	1.123	0.0229	0.1353	0.3779	0.5735	69
Benzyl alcohol	0.3159	0.00936	0.0253	0.0413	0.1156	32
Linalool	1.085	0.1078	0.2137	0.3953	0.7327	70
Methyl-2-octynoate	<LOQ					0
Citronellol	2.325	0.0101	0.0485	0.1185	0.2720	63
Citral	0.1590	0.00739	0.0197	0.0800	0.2459	34
Geraniol	1.648	0.00453	0.0126	0.0318	0.0436	51
Cinnamal	0.00735	0.00046	0.00095	0.00139	0.00293	10
Hydroxycitronellal	1.024	0.00417	0.0232	0.1138	0.2241	45
Anise alcohol	0.00871	–	–	–	–	2
Cinnamyl alcohol	0.0484	0.0284	0.0343	0.0405	0.0446	6
Eugenol	0.5676	0.00086	0.00298	0.0366	0.1185	43
Methyleugenol	0.0301	0.00030	0.00044	0.00120	0.00541	22
Isoeugenol	0.0568	0.00154	0.00227	0.0119	0.0474	9
Coumarin	0.8971	0.00705	0.0269	0.0808	0.1942	39
α-Isomethyl ionone	1.115	0.0181	0.0625	0.201	0.3176	56
Lilial®	5.812	0.0261	0.1641	0.3383	1.069	59
Amyl cinnamal	0.0312	–	–	–	–	4
Lyral®	2.252	0.0373	0.0961	0.1791	0.4995	28
Amylcinnamyl alcohol	0.00432	–	–	–	–	3
Farnesol	0.0733	–	–	–	–	1
Hexylcinnamal	1.462	0.0104	0.1366	0.2819	0.7379	35
Benzyl benzoate	0.7233	0.00500	0.0373	0.1143	0.2992	44
Benzyl salicylate	2.318	0.00271	0.0326	0.1925	0.4676	59
Benzyl cinnamate	0.1452	0.00089	0.00107	0.0659	0.1275	9
Preservatives						
MeP	0.21481	–	–	–	–	2
BHT	0.24812	0.00098	0.00494	0.0191	0.0546	48
EtP	<LOQ	–	–	–	–	0
PrP	<LOQ	–	–	–	–	0
BuP	<LOQ	–	–	–	–	0
BzP	<LOQ	–	–	–	–	0

more than 90% of the samples, i.e. pinene in 68 samples, limonene in 69, linalool in all the samples, and citronellol in 63. The highest found concentrations corresponded to lilial (5.81239%), citronellol (2.32510%), benzyl salicylate (2.31787%), and lyral (2.25157%). Values higher than 1% were also obtained for six compounds more.

The degree of compliance with the Regulation on the labelling has been evaluated in a subset of 29 samples (41.4% of all perfumes tested). The in depth analysis of the results (see Table III of supplementary material) showed that only eleven of the twenty-nine samples were properly labelled for all the allergens tested (about the 38% of the subset). Seven samples were mislabelled with respect to a single allergen, since the compound should be included as an ingredient but it is not: methyleugenol in S1, amyl-cinnamyl alcohol in S4 and S48, citronellol in S57; lilial in S65, cinnamal in S66, and α-isomethyl ionone in S67. The remaining 11 samples showed labelling errors of varying degrees, highlighting S6 (with 14 missing names in the label of the 17 allergens determined), S42 (13 of 17), S49 (9 of 11) and S61 (12 of 14). Finally, in a different subgroup of another 11 samples one or more compounds were labelled as ingredients but were not found in the perfume: 1 in S10, S51, S60, S64 and S68; 2 in S24 and S67; 3 in S59, 4 in S66, 5 in S63, 9 in S70, and 12 in S69. The latter situation may be due to a strategy of some manufacturers of perfumes, which use a label with all allergens controlled for all consumer products that might contain them, regardless of the identity and actual amount of each allergen compound in each of the products. This is not a good practice; it does not help the consumer to choice informed, not to dermatologists who are looking for the possible source of a specific allergy.

3.2.4. Preservatives.

Regarding to parabens, the MAC is 0.4% (expressed as acid) for one ester and 0.8% for mixtures of esters, so an average LOD of 0.000018% properly allows the application of the method for the analytical control of parabens in perfumery products. The CIR (Cosmetic Ingredient Review)[1] found BHT safe as used [12]; nevertheless, BHT is also in the crosshairs of some opinion groups that opt for the so-called "safe cosmetics". Given that the CIR indicates that its presence is safe at concentrations up to 0.5%, LOD values in the 10^{-5}% order are amply adequate for its accurate analytical control.

Preservatives data are shown in Tables 3 and IV. The only ester of p-hydroxybenzoic acid detected was methylparaben (in just 2 of the 70 samples). In contrast, BHT appeared in the 68.5% of the perfumes tested, confirming that its presence is indeed widespread.

4. Conclusions

The proposed multicomponent analytical methodology has proven to be useful for the control of 52 target ingredients (8 phthalates, 12 synthetic musks, 26 fragrance allergens and 6 preservatives) in 70 commercial perfumes from different brands, designed to be used by men, women, children or babies. The robustness of the methodology has been demonstrated using two different instrumental set-ups (external and internal ion traps with different GC stationary phase polarities). The good performance of the GC-MS method has been demonstrated in terms of linearity, accuracy, precision, IDLs, quantitative recoveries (> 88%) and LODs far below the European Cosmetics Regulation established limits of either control or restriction.

Only 10 samples accomplished with the Regulation on phthalates. Two of the three phthalates found as the most common in the analyzed samples are banned by the EU: 39 perfumes contained DBP and 51 perfumes, DEHP. Besides, three samples did not comply with the regulations regarding the presence of DMP, DPP and BBP, although their levels were very limited.

Musks real data confirm the trend about the replacement of nitromusks by polycyclic musks and the perceptible introduction of macrocyclic musks. The prohibited musk moskene has been detected in one sample in an appreciable concentration.

The average number of fragrance allergens was 12 per sample. Four fragrance allergens appeared in more than 90% of the samples (pinene, limonene, linalool and citronellol). The degree of compliance with the Regulation on the labelling has been evaluated in a subset of samples (about 41% of the total), resulting that only 11 perfumes were properly labelled for all the allergens tested.

The generic use of BHT in perfumes has been confirmed, as well as the practical absence of parabens.

From a positive point of view, all perfumes met the Regulation either relating to the labelling or to the established control or restriction limits regarding to preservatives, and all but one fulfill the Regulation regarding to musks.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.talanta.2011.03.079.

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[1] Independent organization that thoroughly reviews and assesses the safety of ingredients used in cosmetics in an open, unbiased, and expert manner; and publishes the results in the peer-reviewed scientific literature.