



Extreme cosmetics and borderline products: an analytical-based survey of European regulation compliance

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Abstract

The cosmetic industry currently focuses on products with magnified or exaggerated effects or extremely long-lasting characteristics. There are also a number of related commercial products for which the regulatory framework is far from clear; they are called ‘borderline’, and the European authorities only recommend which regulations they need to comply with. In any case, all these products must be safe under reasonable conditions of use in accordance with the applicable laws in force in the European Union (EU) framework. In this context, adequate analytical methodology is needed to evaluate the degree of compliance. Ultrasound Assisted Extraction (UAE) procedures for the analysis of 70 cosmetic ingredients have therefore been developed in this work. Moreover, for cosmetics with plastic applicators, a *Supported-UAE* (*Sup-UAE*) method was also opportunely optimized to check if a partial transfer of plasticizers to the cosmetics—and thereby to the consumers—could happen. In a survey of 50 commercial products (30 ‘extreme’ and 20 ‘borderline’), the methods afforded mean recoveries of about 100% and RSD values lower than 5% for UAE and 10% for *Sup-UAE*, and with detection limits far below the legal requirements, for all the target compounds, thereby demonstrating their analytical suitability. Results are discussed in detail for phthalates, fragrances (musks and allergens) and some frequent preservatives. Additionally, a labelling study was performed to check if the consumer is correctly and fully informed.

Keywords Phthalates · Fragrances · Preservatives · Ultrasound assisted extraction · Gas chromatography–mass spectrometry

Introduction

There is a growing marketing trend in the cosmetic sector for products with very particular characteristics that can be considered as ‘extreme cosmetics’; these include long-lasting make-up products, remarkably waterproof eye mascaras and lipsticks, eyelash lengtheners and volumizers, eye pencils

with tattoo effect and lip plumpers. These cosmetics must, like all other cosmetics, comply with the European Cosmetic Products Regulation (EC) No 1223/2009 in order to ensure, as far as possible, product safety [1].

On the other hand, a number of products or categories of products are the so-called borderline products, for which the interpretation of the EU Cosmetics Regulation [1] is proposed through the development of a Manual on the Scope of Application of the Cosmetics Regulation, which evaluates them on a case-by-case basis, taking account of all the characteristics of the product in question [2]. The manual does not ‘prescribe’ what regulatory framework applies; rather, it suggests if a particular product may or may not fall within the scope of application of the Cosmetics Regulation [1]. Moreover, the manual also proposes if any other concerned Directive (Medicinal Products [3, 4], Biocidal Products [5, 6], Toys [7], Textiles [8]) may apply alongside with the Cosmetics one; however, the views expressed are not legally binding. Of course, as in all consumer goods, the chemical substances present in both ‘extreme cosmetics’ and ‘borderline’ products must also comply with the restrictions set out in

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the REACH (Registration, Evaluation, Authorisation and restriction of Chemicals) Regulation (EC 1907/2006) [8].

Another regulation intended to ensure, as far as possible, the safety of cosmetics' users is the Directive on Food-Imitating Products [9] that prohibits the marketing, import and manufacture of products that look like food-stuffs but are not edible. Children are particularly vulnerable as they could be tempted to try to eat such products. As it will be shown, it is easy to find products on the market that are clearly in breach of this directive.

In any case, many of these products have been already launched on the market, and as far as the consumer's safety can be compromised, a global overview about their regulatory compliance is advised. Thus, the main goal of this work is to carry out a preliminary survey of 'extreme cosmetics' and 'borderline' products in order to check their analytical composition, mainly focused on some particular groups of ingredients such as phthalates, fragrances (musks and allergens) and some frequent preservatives.

From the point of view of their chemical composition, cosmetic products must comply with the Regulation [1], and this compliance must be analytically verifiable. In this way, the analytical methodology needed to check the legal compliance has been considerably improved in recent years, as it is shown in what can be considered a reference manual for general cosmetic analysis [10] and in a recent review by the authors based on the analysis of ingredients included in the positive lists of the Regulation EC No 1223/2009 [1, 11]. Instrumental methods are generally based on gas (GC) and liquid (LC) chromatographic techniques hyphenated with single (MS) or tandem mass spectrometry (MS/MS) together with some particular applications of conventional detectors. These multianalyte approaches have proven to be the most useful ones for cosmetics control, as it will be shown in this paper. From the perspective of cosmetics products surveillance, official bodies can carry out in-market control inspections and, in these cases, simplified workflows are of interest, such as those methods based on miniature ion trap mass spectrometry with ambient ionization for on-site screening; but they are in the early stages of development [12]. Regarding the extraction step, most cosmetics are complex samples requiring a sample pretreatment before analysis; however, in some cases, simple strategies as well as ready-to-shot approaches can perform really well. There is also a general trend towards microextraction techniques and green processes [10, 11].

Many cosmetics have applicators. The function of an applicator is to facilitate the transfer of product from the primary cosmetic container to the targeted area of application in a relatively controlled way. Following the criteria of the Manual on Borderline Products [2], an applicator is neither a substance nor a mixture, but it is an article. In this sense, the applicator is not part of the product, and it could be considered part of the primary packaging (material in direct contact with

the cosmetic product); as such, it should follow the corresponding regulation depending of the type of applicator (e.g. textile, if a wipe). Nevertheless, it should be demonstrated that there is no migration of potentially harmful substances (and/or forbidden ones) from the applicator to the cosmetic product once applied to the target part of the body.

It is also true that, according to the Cosmetic Regulation (Article 17) [1]: 'The non-intended presence of a small quantity of a prohibited substance, stemming from impurities of natural or synthetic ingredients, the manufacturing process, storage, migration from packaging, which is technically unavoidable in good manufacturing practice, shall be permitted provided that such presence is in conformity with Article 3' on safety [1].

In this context, extraction strategies based on ultrasound assisted extraction (UAE), a fast and simple technique, are proposed. The aim is to evaluate their viability for the analytical control of 70 cosmetic ingredients (phthalates, fragrances and preservatives) in a broad range of cosmetic products. First, an UAE method for wipes, one of the broadest borderline categories with very particular characteristics, has been optimized. Besides, a broad-spectrum method useful for 'extreme cosmetics' and some 'borderline' products was also proposed. Moreover, in order to check if a partial transfer of plasticizers from the plastic applicators to the cosmetics—and thereby to the consumers—can happen, *Supported-UAE* (*Sup-UAE*) was also employed upon optimization. In the development of all these procedures, the smallest solvent amount and the minimum energy consumption (short extraction times at room temperature) have been sought.

Finally, using the reliable multicomponent analytical methods developed, a survey of 50 commercial cosmetics—designed to impart very pronounced and lasting effects to the user—along with some selected 'borderline' products will be performed, in order to verify whether these products complied with the European legislation [1]. Additionally, a labelling study will be also carried out with the aim of checking if the information that reaches the consumer is correct and complete, as stated in Article 19 of the EC Regulation 1223/2009 [1]. Cosmetics labels must also comply with EC Regulation 1272/2008 [13], which aims to contribute to the global harmonization of classification, labelling and packaging criteria; it states that substances and mixtures, especially those for the general public, should be supplied with the necessary labelling information.

Experimental

Chemicals and reagents

The studied compounds, their CAS numbers, and legal restrictions and EU limits are summarised in Table 1. All the target

Table 1 Studied compounds, CAS number, European legislation restrictions [1], retention time, linear range, determination coefficients (R^2) and instrumental detection limits (IDL)

Code	Compound	CAS number	MCA ^a [1]	Retention time (min)	Linear range (ng mL ⁻¹)	R^2	IDL (ng mL ⁻¹)
Fragrance allergens							
1	Pinene	80-56-8		4.50	2–1000	0.9998	0.22
2	Limonene	5989-27-5		6.23	2–1000	0.9996	0.41
3	Benzyl alcohol	100-51-6		6.31	5–1000	0.9980	1.2
4	Linalool	78-70-6		7.28	5–1000	0.9974	1.5
5	Methyl-2-octynoate	111-12-6		8.45	5–1000	0.9970	1.5
6	Citronellol	106-22-9		8.71	10–1000	0.9967	2.1
7	Citral	5392-40-5		8.71; 9.1 ^b	10–1000	0.9969	2.8
8	Geraniol	106-24-1		8.94	10–1000	0.9961	2.8
9	Cinnamaldehyde	104-55-2		9.23	10–1000	0.9988	2.2
10	Hydroxycitronellal	107-75-5	1%	9.26	5–1000	0.9952	1.3
11	Anise alcohol	105-13-5		9.32	20–1000	0.9978	6.1
12	Cinnamyl alcohol	104-54-1		9.46	20–1000	0.9982	4.4
13	Eugenol	97-53-0		9.81	5–1000	0.9973	1.2
14	Methyleugenol	93-15-2	0.0002–0.01% ^c	10.17	5–1000	0.9995	1.0
15	Isoeugenol	97-54-1	0.02%	10.11; 10.4 ^b	10–1000	0.9993	2.6
16	Coumarin	91-64-5		10.45	10–1000	0.9991	2.2
17	α -Isomethyl ionone	127-51-5		10.63	2–1000	0.9983	0.61
18	Lilial®	80-54-6		10.97	2–1000	0.9978	0.44
19	Amylcinnamal	122-40-7		11.79	10–1000	0.9960	3.2
20	Lyrall®	31,906-04-4	Prohibited ^d	11.96	10–1000	0.9960	3.0
21	Amylcinnamyl alcohol	101-85-9		12.08	20–1000	0.9990	6.0
22	Farnesol	4602-84-0		12.09; 12.12 ^b	250–1000	0.9988	70
23	Hexylcinnamal	101-86-0		12.65	10–1000	0.9979	3.0
24	Benzyl benzoate	120-51-4		12.94	10–1000	0.9990	1.9
25	Benzyl salicylate	118-58-1		14.20	10–1000	0.9951	2.3
26	Benzyl cinnamate	103-41-3		17.94	10–1000	0.9961	2.3
Synthetic musks							
27	Musk xylene	81-15-2	0.03–1.0% ^c	13.83	2–1000	0.9978	0.47
28	Musk ketone	81-14-1	Prohibited–1.4% ^f	15.64	10–1000	0.9930	3.0
29	Musk ambrette	83-66-9	Prohibited	13.49	5–1000	0.9903	1.5
30	Musk moskene	116-66-5	Prohibited	14.18	2–1000	0.9925	0.61
31	Musk tibetene	145-39-1	Prohibited	14.93	2–1000	0.9927	1.5
32	Galaxolide	1222-05-5		13.81	2–1000	0.9954	0.22
33	Celestolide	13,171-00-1		12.32	2–1000	0.9946	0.16
34	Phantolide	15,323-35-0	2% ^g	12.71	2–1000	0.9957	0.16
35	Cashmeran	33,704-61-9		10.84	2–1000	0.9969	0.53
36	Traseolide	68,140-48-7		13.70	2–1000	0.9901	0.16
37	Tonalide	1506-02-1	Prohibited–2.5% ^h	13.88	2–1000	0.9968	0.21
38	Ambrettolide	7779-50-2		15.13	2–1000	0.9959	2.0
Plasticizers							
39	Bisphenol A (BPA)	80-05-7	Prohibited	18.78	2–1000	0.9989	0.20
40	Dimethylphthalate (DMP)	131-11-3		10.45	2–1000	0.9995	0.26
41	Diethylphthalate (DEP)	84-66-2		11.33	2–1000	0.9995	0.13
42	Diisobutylphthalate (DIBP)	84-69-5	Prohibited ⁱ	13.87	2–1000	0.9966	0.18
43	Dibutylphthalate (DBP)	84-74-2	Prohibited	15.34	2–1000	0.9975	0.44
44	Dimethoxyethylphthalate (DMEP)	117-82-8	Prohibited	15.97	2–1000	0.9946	0.59

Table 1 (continued)

Code	Compound	CAS number	MCA ^a [1]	Retention time (min)	Linear range (ng mL ⁻¹)	R ²	IDL (ng mL ⁻¹)
45	Diisopentylphthalate (DIPP)	605-50-5	Prohibited	17.25	2–1000	0.9941	0.66
46	Dipentylphthalate (DPP)	131-18-0	Prohibited	18.48	2–1000	0.9977	0.37
47	Benzylbutylphthalate (BBP)	85-68-7	Prohibited	20.09	5–1000	0.9999	0.88
48	Diisooheptylphthalate (DIHP)	41,451-28-9		20.65	1000–10,000	0.9970	200
49	Di(ethylhexyl)phthalate (DEHP)	117-81-7	Prohibited	21.06	2–1000	0.9957	0.36
50	Dicyclohexylphthalate (DCHP)	84-61-7		21.01	2–1000	0.9931	0.22
51	Diphenylphthalate (DPhP)	84-62-8		21.16	2–1000	0.9952	0.51
52	Di- <i>n</i> -octylphthalate (DNOP)	117-84-0		22.16	2–1000	0.9965	0.48
53	Diisononylphthalate (DINP)	28,553-12-0		22.48	1500–10,000	0.9992	400
54	Diisodecylphthalate (DIDP)	89-16-7		23.52	1000–10,000	0.9998	200
55	Dimethyladipate (DMA)	627-93-0		8.83	2–1000	0.9991	0.30
56	Diethyladipate (DEA)	141-28-6		9.96	2–1000	0.9982	0.58
57	Di(ethylhexyl)adipate (DEHA)	103-23-1		20.31	2–1000	0.9901	0.39
Preservatives							
58	Bronidox	30,007-47-7	0.1%	8.61	10–10,000	0.9971	2.6
59	Phenoxyethanol (PhEtOH)	122-99-6	1%	8.66	5–1000	0.9954	1.1
60	Butylhydroxyanisole (BHA)	121-00-6		10.60	2–1000	0.9910	0.50
61	Butylhydroxytoluene (BHT)	128-37-0		10.81	2–1000	0.9954	0.15
62	Iodopropynilbutylcarbamate (IPBC)	55,406-53-6	Prohibited–0.02% ^j	11.92	50–1000	0.9918	10
63	Triclosan	3380-34-5	0.2–0.3% ^k	17.99	20–1000	0.9934	3.2
64	Methylparaben (MeP)	99-76-3	0.4–0.8% ^l	10.44	10–1000	0.9936	3.0
65	Ethylparaben (EtP)	120-47-8	0.4–0.8% ^l	10.87	10–1000	0.9954	3.2
66	Isopropylparaben (iPrP)	4191-73-5	Prohibited	11.06	20–1000	0.9971	3.2
67	Propylparaben (PrP)	94-13-3	0.14–0.8% ^m	11.54	20–1000	0.9942	3.1
68	Isobutylparaben (iBuP)	4247-02-3	Prohibited	11.99	20–1000	0.9936	3.9
69	Butylparaben (BuP)	94-26-8	0.14–0.8% ^m	12.38	10–1000	0.9914	2.8
70	Benzylparaben (BzP)	94-18-8	Prohibited	18.40	20–1000	0.9965	6.2

^a Maximum concentration authorized in ready-to-use preparation^b These compounds are mixtures of isomers^c Maximum permitted concentration in the final product: 0.01% (fine fragrance), 0.004% (eau de toilette), 0.002% (fragrance cream), 0.0002% (other leave-on products) and 0.001% (rinse-off products)^d Forbidden in all type of cosmetics from 23 August 2019^e 1.0% (fine fragrance), 0.4% (eau de toilette) and 0.03% in other products^f Forbidden in oral products. Maximum permitted concentration in the final product: 1.4% in fine fragrance, 0.56% in eau de toilette and 0.042% in other products^g 2% in leave-on products, no limit in rinse-off products^h Forbidden in oral products. 0.2% (rinse-off products), 0.1% (leave-on products excluding 1% (hydroalcoholic products), 2.5% (fine fragrance) and 0.5% (fragrance cream))ⁱ Prohibited: the European Cosmetics legislation also prohibits the use of substances classified for carcinogenic, mutagenic and reprotoxic (CMR) hazards (Article 15)^j Forbidden in products for children under 3 years, except in bath products. Rinse-off products, 0.02%; leave-on products, 0.01% (not to be used in body lotion and body cream); deodorants, 0.0075%^k 0.3% (toothpaste, hand soap, body soap, shower gel, non-spray deodorants, face powders and blemish concealers, nail products for cleaning the fingernails and toenails before the application of artificial nail systems; and 0.2% (mouthwashes)^l 0.4% (as acid) for single ester 0.8% (as acid) for mixtures of esters^m 0.14% (as acid) for individual esters and 0.8% (as acid) for mixtures of esters, where the sum of their individual concentrations and their salts does not exceed 0.14%. Not to be used in leave-on products designed for application on the nappy area of children under 3 years of age

compounds have purity levels $\geq 95\%$ (excluding α -isomethyl ionone and Lilial[®], $\geq 85\%$). Individual stock solutions of each compound were prepared in methanol or isooctane (Fluka Analytical, Steinheim, Germany), and further dilutions and mixtures were prepared in ethyl acetate or acetone (Sigma Aldrich, Steinheim, Germany) for calibration studies or spiked solutions, respectively. Stock solutions were stored in amber glass vials protected from light at $-20\text{ }^{\circ}\text{C}$, and working solutions were prepared weekly. All solvents and reagents were of analytical grade.

Since one of the studied compound families is plasticizers (mainly phthalates and adipates), the plastic material was substituted with metallic and glass material to avoid possible phthalate contamination during the experimental procedure and overestimation in the obtained results. All material was also maintained at $230\text{ }^{\circ}\text{C}$ for 12 h and allowed to cool down wrapped with aluminium foil before their use.

Sampling and sample treatment

Thirty cosmetic products (Table 2) considered as ‘extreme cosmetics’, owing to their particular characteristics to magnify their functions and/or their duration of action, were purchased from different local markets and including a broad range of brands. Therefore, long-lasting lipsticks and eye masks, eyelash lengtheners, waterproof make-up, eye pencils with tattoo effect, face masks, and lip plumpers, among others, were analysed. In addition, 20 ‘borderline’ products [2] were tested. These products, as mentioned in the “Introduction”, exist at the boundaries that are not always clear between cosmetic products and other products and articles such as toys, biocidal products, pharmaceuticals, medical devices etc.; or the product may be multifunctional, e.g. a sunscreen (cosmetic function) with an insect repellent added (biocidal function). Therefore, 50 samples were received in their original containers and perfectly closed to avoid contamination. Once they have been registered according to the standardised protocol in the laboratory, they must be kept in their own container at ambient temperature, and protected from direct exposure to light until they are analysed. The classification into ‘extreme cosmetics’ (Table 2) and ‘borderline products’ (Table 3) has been done to facilitate the discussion of results.

Ultrasound assisted extraction (UAE) and Supported-UAE (Sup-UAE)

UAE was employed in all cases to extract the studied analytes from the selected samples. In this way, three different approaches were applied: two classical UAE procedures, and a modified one named *Supported-UAE*. This last one was applied for samples containing applicators (eyeliner, eyelash lengtheners, lip plumper etc.) and temporary tattoos.

Ultrasound assisted extraction (UAE)

The classical UAE was first intended for one type of ‘borderline product’, namely wipes and face masks (Fig. 1a). An individual wipe, previously weighed, was introduced into a 40-mL glass vial with the aid of metallic tweezers (for face masks, a quarter of them was placed into the vial, see Fig. 1a). Then, 20 mL of ethyl acetate was added. The vial was sealed with an aluminium cap furnished with a PTFE-faced septum, and it was placed into an ultrasound bath (Hoyer Handel GmbH, Hamburg, Germany) at 50 W for 10 min at $25\text{ }^{\circ}\text{C}$. Afterwards, the obtained extracts were diluted (1:5, v/v) in ethyl acetate, filtered through $0.20\text{-}\mu\text{m}$ PTFE microporous filters and directly injected into the GC-MS instrument.

For the recovery studies (fortification level, $100\text{ }\mu\text{g/wipe}$), once the samples were introduced into the 40-mL glass vial, they were spiked with $20\text{ }\mu\text{L}$ of the surrogate acetonetic solution ($50\text{ }\mu\text{g mL}^{-1}$ of benzyl alcohol- d_7 , MeP- d_4 and DEHP- d_4) and with $100\text{ }\mu\text{L}$ of the $1000\text{ }\mu\text{g mL}^{-1}$ standard solution of all the target compounds prepared in acetone and the UAE procedure was then carried out as described above.

For cosmetics others than wipes and masks (Fig. 1b), 100 mg of cosmetic sample and 2 mL of ethyl acetate were placed in a 10-mL glass vial and the UAE procedure was carried out as described above.

UAE blanks were performed together with each batch of sample analysis placing 20 mL or 2 mL , depending on the analysed samples, into a 40-mL or 10-mL glass vial, respectively, and the UAE procedure was performed following the procedure described above.

Supported-ultrasound assisted extraction (Sup-UAE) procedure

A sheet of filter paper was cut into small pieces ($3\text{ cm} \times 2.4\text{ cm}$, weight about 50 mg) and about 50 mg of cosmetic sample was applied with an applicator over the filter paper (Fig. 1c). Then, the filter paper with the sample was carefully placed into a 10-mL glass vial, and 5 mL of ethyl acetate was added. Afterwards, the vial was sealed with an aluminium cap furnished with PTFE, and it was placed into an ultrasound bath (Hoyer Handel GmbH, Hamburg, Germany) at 50 W for 10 min at $25\text{ }^{\circ}\text{C}$. Afterwards, the obtained extracts were filtered through $0.20\text{-}\mu\text{m}$ PTFE microporous filters and directly injected into the GC-MS instrument.

For the recovery studies, fortified samples were prepared at $100\text{ }\mu\text{g g}^{-1}$. For that, the 50 mg of sample ‘painted’ over the filter paper was spiked with $20\text{ }\mu\text{L}$ of the surrogate solution in acetone ($50\text{ }\mu\text{g mL}^{-1}$ of benzyl alcohol- d_7 , MeP- d_4 and DEHP- d_4) and with $50\text{ }\mu\text{L}$ of $100\text{ }\mu\text{g mL}^{-1}$ acetonetic standard solution of all the target compounds. Then the filter paper containing the sample

Table 2 Summary of 'extreme cosmetics' samples analysed

Samples	Product	Application site	Claim (function and/or 'extreme' distinctive feature)	Analytical compliance monitoring (ingredients/labelling)
ExC1	Liquid eyeliner	Eyes	24 h. Heavy metal glitter. Paint razor-thin or bold sweeps of glitter for jewel-encrusted sparkle that won't budge	Pass/pass
ExC2	Eyelash mascara	Eyes	Effect tattoo. Ultracreamy volumizing formula coats each lash with triple-black pigments and a deeply nourishing ingredient blend for bigger, blacker, badder, lashes... it will not quit until you say when	Pass/pass
ExC3	Liquid eyeliner	Eyes	Waterproof, tattoo effect	Fail ^a /pass
ExC4	Waterproof cream eye shadow	Eyes	Smoky eye effect/waterproof	Fail/pass
ExC5	Eye sparkling liquid gel	Eyes	Reflective effect/long-lasting	Fail ^a /pass
ExC6	Cream-gel brow	Eyebrows	Waterproof/long-lasting	Fail/pass
ExC7	Brow pencil	Eyebrows	Waterproof/long-lasting	Fail ^a /pass
ExC8	Lash extension kit	Lashes	Nylon fibres are placed on the eyelashes prior to the prime and seal mascara application to obtain a false lash look. Instant effect. Long-lasting	Fail ^a /pass
ExC9	Clay face mask	Face	Express face detox: nourishing/purifying (avocado)	Pass/pass
ExC10	Overnight face mask	Face	Express face detox: relaxing (pumpkin)	Fail/pass
ExC11	High definition radiance face cream	Face	Skin perfecter (the cream changes its colour on application)	Fail/pass
ExC12	Face cream with bee venom	Face	Peptide-based anti-wrinkle effect	Pass/fail
ExC13	Anti-wrinkle snake venom cream	Face	Peptide-based anti-wrinkle effect	Pass/fail
ExC14	Serum snake venom	Face	Anti-aging/lifting/Botox effect	Fail/pass
ExC15	Facial base infused with coconut extract	Face	Revitalizing effect (infused with coconut water)	Fail/fail
ExC16	Face cleansing with microfoam	Face	Microfoam face cleansing	Fail/fail
ExC17	Gel-type mask with cocoa	Face	Pudding jelly mask sheet	Fail/fail
ExC18	Illumination mask	Face	Pore-purifying steaming pack	Pass/pass
ExC19	Face in-shot illumination blister	Face		Fail/pass
ExC20	Face in-shot illumination mask	Face		Fail/pass
ExC21	Hot pore cleansing	Face	Hot effect	Fail/pass
ExC22	Cold pore cleansing	Face	Cold effect	Fail/pass
ExC23	Make-up setting spray	Face	Long-lasting	Fail ^a /pass
ExC24	Banana hand milk	Hands	'Magic', moisturizing (milk protein extracts); external appearance of food	Fail/fail
ExC25	Lipstick	Lips	Permanent	Fail/fail
ExC26	Liquid lipstick	Lips	Everlasting	Fail ^a /pass
ExC27	Banana lip balm	Lips	Nourishing (banana and coconut oil extracts); external appearance of food	Fail ^b /pass
ExC28	Liquid lip colour	Lips	Full metal impact/transfer-proof	Fail ^a /pass
ExC29	Liquid lip colour	Lips	Long-lasting/transfer-proof	Fail ^a /pass
ExC30	Liquid lip colour	Lips	Full metal impact/transfer-proof	Fail ^a /pass

All samples must comply with Cosmetics Regulation (EC) No 1223/2009 [1]

^aThis fail is due in part to the migration of substances from the applicator^bThis fail is entirely due to the migration of substance from the applicator

Table 3 Summary of commercial 'borderline' products analysed

Samples	Product	Application site	Claim (function and/or 'extreme' distinctive feature)	Suggested category in the Borderline Products Manual	Must comply with Cosmetics Regulation (EC) No 1223/2009	Additional regulatory framework applies ^a	Analytical compliance monitoring (ingredients/labelling)
BP1	Wet wipe	Armpit	Cleansing and antiperspirant	Wipe	Yes	None	Fail/pass
BP2	Bust plumper	Bust	Plumping up care for the bust	Topical breast augmentation product	Yes ^b	Medicinal Products	Fail/fail
BP3	Waterproof mascara	Eyelash	- ultra concentrated length Waterproof, smudge-proof, all-day wear	Products that make eyelashes grow	Yes	Unclear	Fail ^f /fail
BP4	Make-up removal wipe	Face	Make-up removal	Wipe	Yes (cosmetic content)	None	Fail/pass
BP5	Face mask	Face	Moisturizing/soothing	Wipe	Yes (cosmetic content)	Mask tissue ^d	Pass/pass
BP6	Face mask	Face	Moisturizing/energizing	Wipe	Yes (cosmetic content)	Mask tissue ^d	Pass/fail
BP7	Face mask	Face	Moisturizing	Wipe	Yes (cosmetic content)	Mask tissue ^d	Fail/pass
BP8	Face mask	Face	Flash effect	Wipe	Yes (cosmetic content)	Mask tissue ^d	Fail/fail
BP9	Face mask	Face	Rebalance excess oil on oily skin	Wipe	Yes (cosmetic content)	Mask tissue ^d	Fail/pass
BP10	Lip injection	Lips	Lip plumper	Products that make the lips swell	Yes	Medicinal Products	Fail ^f /fail
BP11	Lip injection	Lips	Lip plumper	Products that make the lips swell	Yes	Medicinal Products	Fail ^f /fail
BP12	Decorative nail polish stickers	Nails	Changing nails appearance	General product	Yes	None	Fail/pass
BP13	Nappy area baby wipe	Nappy area, baby	Cleaning	Wipe	Yes	None	Pass/pass
BP14	Temporary tattoo, dragonfly	Skin	Changing skin appearance	Washable, temporary 'tattoos'	Yes	Toys Directive	Fail ^f /lack
BP15	Temporary tattoo, butterfly	Skin	Changing skin appearance	Washable, temporary 'tattoos'	Yes	Toys Directive	Fail ^f /lack
BP16	Temporary tattoo, dragon	Skin	Changing skin appearance	Washable, temporary 'tattoos'	Yes	Toys Directive	Fail ^f /lack
BP17	Temporary tattoo, wolf	Skin	Changing skin appearance	Washable, temporary 'tattoos'	Yes	Toys Directive	Fail ^f /fail
BP18	Temporary tattoo, dragonfly	Skin	Changing skin appearance	Washable, temporary 'tattoos'	Yes	Toys Directive	Fail ^f /lack
BP19	Sexual gel	External genital organs	Pleasure gel	Products intended to stimulate sexual activity	No	Borderline with other legislation undisclosed	Lack
BP20	Sexual gel	External genital organs	Pleasure gel	Products intended to stimulate sexual activity	No	Borderline with other legislation undisclosed	Lack

^a As suggested by the Working Group on Cosmetic Products (Sub-Group on Borderline Products) [2]

^b If just to improve the breast's firmness

^c If is a topical breast augmentation product

^d Must comply with restrictions included in Annex XVII of REACH (Regulation (EC) No 1907/2006) [8]

^e If acts through inflammation and/or irritation; as it is the case: the effect is based, at least partly, on '*Capsicum frutescens* resin'

^f This fail is due in part to the migration of substances from the applicator

^g This fail is entirely due to the migration of substance from the applicator

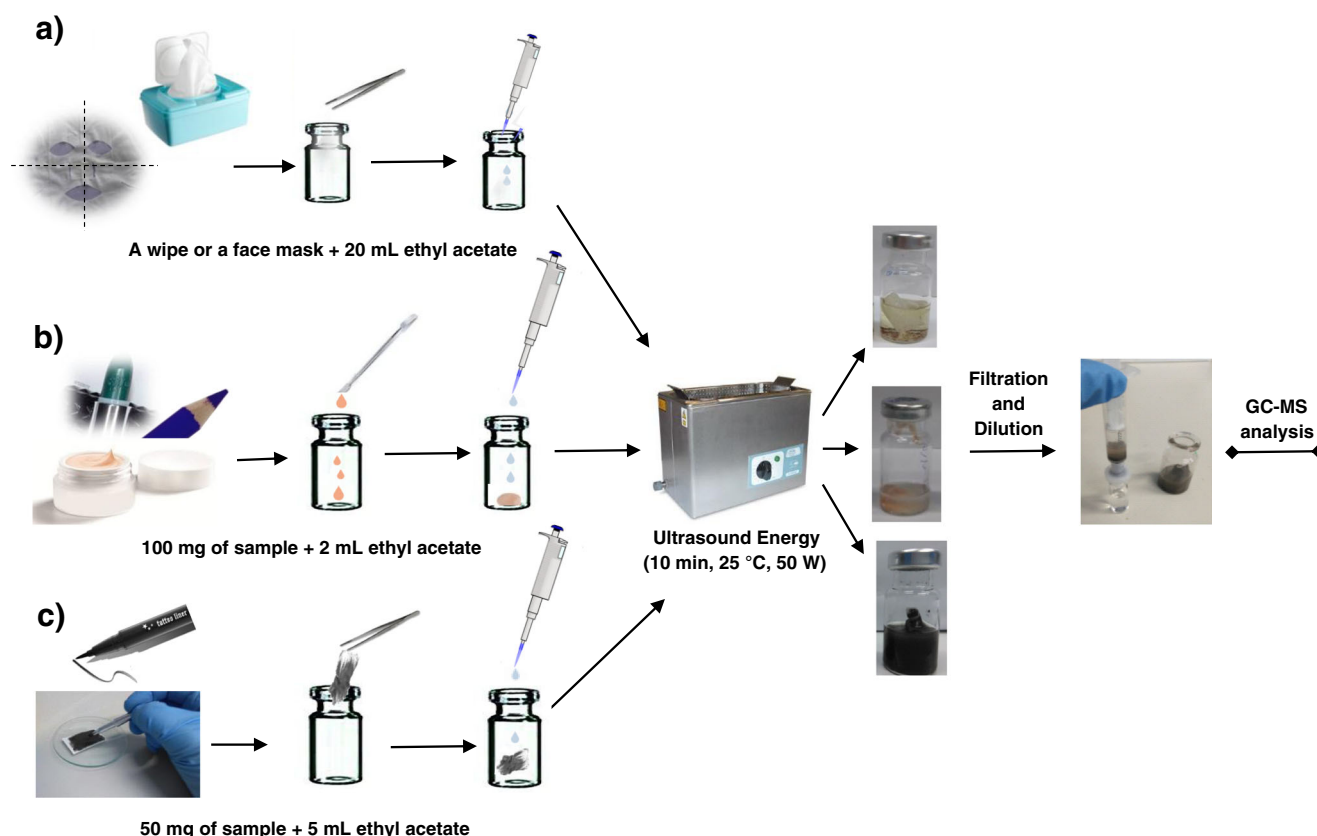


Fig. 1 Schematic representation of the three ultrasound energy-based extraction procedures: a) UAE for borderline products (wipes and masks); b) broad-spectrum UAE for extreme cosmetics; c) *Supported*-ultrasound assisted extraction (*Sup-UAE*)

and the spiked compounds was introduced into the vial and the procedure was carried out as described above.

For the analysis of a specific ‘borderline’ product such as temporary tattoos, the experimental procedure was similar. In this case, the corresponding tattoo sample was ‘tattooed’ on a filter paper with the same size as the sample, weighed and introduced into a 10-mL glass vial. Then, the corresponding volume of ethyl acetate was added to obtain a sample dilution of about 1:100 (w/v) and the UAE procedure was carried out as described above prior to the GC-MS analysis.

GC-MS analysis

The 70 cosmetic ingredients belonging to different chemical families could be determined in a single run. The GC-MS analysis was performed using an Agilent 7890A coupled to an Agilent 5975C inert mass spectra detector (MSD) with triple-axis detector and an Agilent 7693 autosampler from Agilent Technologies (Palo Alto, CA, USA). The temperatures of the transfer line, the quadrupole and the ion source were set at 290, 150 and 230 °C, respectively. The system was operated by Agilent MSD ChemStation E.02.00.493 software.

Separation was carried out on a ZB-Semivolatiles (30 m × 0.25 mm i.d., 0.25 µm film thickness) obtained from Phenomenex (Torrance, CA, USA). Helium (purity

99.999%) was employed as carrier gas at a constant column flow of 1.0 mL min⁻¹. The instrumental conditions were optimized in a previous work [14] to achieve an efficient separation of the 70 target compounds: 26 fragrance allergens, 12 musks, 19 plasticizers (including 3 adipates, 15 phthalates and bisphenol A) and 13 preservatives (including two very common synthetic antioxidants BHA and BHT). The GC oven temperature program is summarised in the inset of Fig. 2. Splitless mode (1 min, 75 mL min⁻¹) was used for 1-µL volume injection (injector temperature was set at 260 °C). The mass spectral detector (MSD) was operated in the selected ion monitoring (SIM) mode checking three ions per compound as in previous works [14, 15], to achieve an unequivocal identification of the analytes, and the electron multiplier was set at a nominal value of 1600 V. Full scan mode was also employed to carry out a screening of non-target compounds. In this case, the mass range was 40–700 *m/z*, from 4 to 35 min.

Calibration standards were prepared in ethyl acetate covering a range from 2 to 1000 µg L⁻¹ (nine levels and three replicates per level) with the exception of DIHP, DIDP and DINP (1000–10,000 µg L⁻¹; four levels and three replicates per level) (see specific range in Table 1). Results showed a direct proportional relationship between the amount of each analyte and the chromatographic response with determination coefficients (*R*²) higher than 0.9900 in all cases (Table 1). Instrumental detection limits

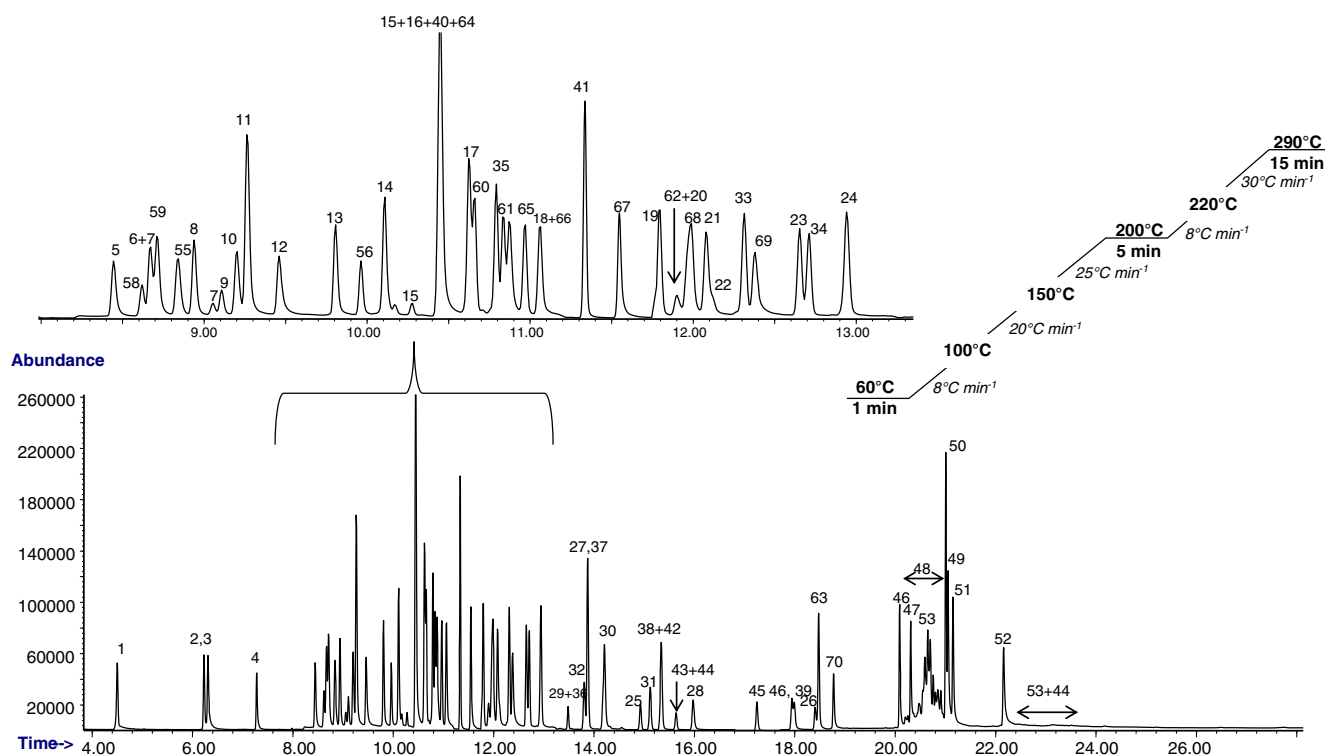


Fig. 2 SIM chromatogram of a $1 \mu\text{g mL}^{-1}$ (or $4 \mu\text{g mL}^{-1}$ for DIHP, DIDP and DINP) standard solution of the 70 target compounds in ethyl acetate. See codes in Table 1

(IDLs) were calculated as the concentration giving a signal-to-noise ratio (S/N) of 3 in all cases, since any of the target compounds were detected in the solvent blanks. The obtained values (Table 1) were at the low nanogram per milliliter level for most compounds, excluding farnesol, DIHP, DIDP and DINP, although these were also in the nanogram per milliliter range. Figure 2 shows a SIM chromatogram of a 1000 ng mL^{-1} (4000 ng mL^{-1} for DIHP, DIDP and DINP) standard solution of the 70 target compounds in ethyl acetate.

Results and discussion

Two methods were optimized for the extraction of the target analytes from the cosmetic products: a procedure based on UAE and *Sup*-UAE one. Additionally, the extraction efficiency of the proposed broad-spectrum UAE method was compared with a μ -matrix solid-phase dispersion (μ -MSPD) method, which was previously developed and validated by the authors [15–17], in a subset of cosmetic samples.

Ultrasound assisted extraction (UAE) procedure

Solvent is the main variable potentially affecting the UAE procedure and its influence was evaluated in preliminary experiments in order to obtain the highest extraction efficiency. Hence, a solvent screening was carried out, testing

acetonitrile, methanol (MeOH), ethyl acetate and hexane/acetone (1:1, v/v). The results for a subset of target analytes (sample BP13) are shown in Fig. S1 (see Electronic Supplementary Material (ESM)). Use of methanol as the extraction solvent afforded the lowest chromatographic responses for all compounds, whereas with acetonitrile the extraction efficiency varied greatly depending on the type of compound tested; therefore, these two solvents were discarded. The highest extraction efficiency was obtained using ethyl acetate and hexane/acetone. However, with the use of the binary solvent mixture a biphasic extract was observed, probably because of different relative solubilities of the diverse compounds in each particular solvent, and its use was also discarded. Thus, ethyl acetate was finally chosen to perform the UAE; with this solvent, clean extracts were obtained and, in addition, it is directly compatible with the chromatographic system. The detailed procedure is described in Sect. ‘[Ultrasound assisted extraction \(UAE\)](#)’.

The experimental procedure has been optimised and validated in three types of wipes currently on the market: a wipe to clean the nappy area in children under 3 years old (sample BP13), a face and eye cleansing wipe (sample BP4) and a moisturising face mask (sample BP5). This procedure has also been successfully applied to other types of cosmetics marketed in wipe format, such as deodorants and other types of face masks (anti-aging and exfoliating).

To optimize and validate the UAE method the three aforementioned representative types of wipes were chosen,

selecting articles that did not contain, as far as possible, the compounds under study. The experimental procedure to perform the recovery studies was described in Sect. ‘[Ultrasound assisted extraction \(UAE\)](#)’. Results are summarised in Table 4, and as can be seen, mean recoveries of about 98% and relative standard deviation (RSD) values lower than 15% were obtained in most cases.

Supported-ultrasound assisted extraction procedure (*Sup-UAE*)

The proposed methodology based on the *Supported-UAE* procedure was also validated in order to demonstrate its suitability to analyse samples with applicators used to apply the cosmetic formulations in eyelashes, nails or lips. In this case, to carry out the recovery studies, an eyebrow cream sample containing an applicator (sample ExC6, see Table 2) was selected. The experimental procedure was detailed in Sect. ‘[Supported-ultrasound assisted extraction \(*Sup-UAE*\) procedure](#)’. As can be seen in Table 4, recovery values were in all cases satisfactory with mean values of about 100% for all the studied compounds and with RSD values lower than 5%.

In addition, to demonstrate that the filter paper employed as support does not influence the extraction process by retaining the compounds during the extraction procedure, a sheet of filter paper (3 cm × 2.4 cm, weight about 50 mg) was spiked with the surrogates and target compounds, and the *Sup-UAE* procedure was carried out as was described in Sect. ‘[Supported-ultrasound assisted extraction \(*Sup-UAE*\) procedure](#)’. In this case, as can be seen also in Table 4, recovery percentages between 80% and 108% and RSD values lower than 10% were obtained for most studied compounds, demonstrating that the support does not absorb the target compounds during the extraction procedure.

Therefore, the two proposed approaches, UAE and *Sup-UAE*, can be considered suitable for the simultaneous determination of fragrance allergens, synthetic musks, plasticizers and preservatives in different types of cosmetic products.

Application to real samples

Fifty ‘extreme cosmetics’ and ‘borderline’ products including face masks, deodorant and make-up remover wipes, lipsticks, eyelash lengtheners, waterproof make-up, tattoo effect eyeliners, anti-aging creams, brilliant eyeliners, artificial nails, lips plumper, sexual lubricant gels and temporary tattoos among others were analysed. The quantification results for the studied compounds (in micrograms per gram) are summarised in Tables S1 and S2 (see ESM) for ‘extreme cosmetics’ and ‘borderline’ products, respectively.

UAE and *Sup-UAE* must be seen as complementary techniques when the cosmetics to be treated have applicators. In the case of fragrance allergens, UAE is preferred as some of

the substances may be lost during on the paper surface the application procedure, particularly the more volatile ones; however, for some products no significant differences were detected. This dissimilar behaviour can be related to the different ability to retain the volatiles depending on the matrix of the analysed cosmetic.

However, the use of *Sup-UAE* is recommended to analyse the possible migration of certain substances from the applicators. Figure 3 shows the comparison of the total sum of phthalates obtained by UAE and *Sup-UAE* in four selected samples: two ‘borderline’ (BP3 and BP10) and two ‘extreme cosmetics’ (ExC3 and ExC26). As can be seen, the total amount of phthalates extracted by *Sup-UAE* is higher in all cases, demonstrating that applicators are an additional source of these compounds. DEHP and DBP are prohibited according to Annex II of Regulation EC No 1223/2009 [1] and DIBP is prohibited as a carcinogenic, mutagenic or toxic for reproduction (CMR) substance [18]. On the other hand, the other found phthalate, DEP, is allowed as an ingredient in cosmetic formulations. Specifically, the amount of phthalates provided by the applicator varies between 70% and 90% (see Tables 2 and 3). In addition, in some cases, a different qualitative profile appears, demonstrating that some phthalates come exclusively from the applicator, as is the case with DEP in sample BP3 and DEP and DEHP in sample BP10. On the other hand, *Sup-UAE* is also the most recommended option for analysing temporary tattoos since, although they do not contain applicators, they are analysed once they are ‘placed’, simulating what happens in real life.

Three cosmetic products: a colour correcting cream (ExC11), a topical bust plumper (BP2) and a lip injection plumper (BP10) were analysed by the proposed broad-spectrum UAE procedure and a previously validated extraction procedure based on μ -MSPD [15–17]. Qualitatively, the compositional profile of the samples is similar, the same compounds being detected by both techniques. A statistical comparison of the concentration for each analyte obtained by both extraction techniques was also carried out. Results are summarised in Table S3 (see ESM) showing no significant statistical differences for the detected compounds. It should be noted that the analytes considered are in concentration ranges covering several orders of magnitude: from less than 1 $\mu\text{g g}^{-1}$ for most phthalates to tens and hundreds of micrograms per gram for fragrances and thousands of micrograms per gram for preservatives (see concentrations in ESM Tables S1 and S2); regardless, both techniques work well for either concentration range.

The chromatographic profiles of the 50 commercial products were highly variable and dependent on their composition as can be seen in Fig. 4, which shows the profiles of several samples taken as representative of the products analysed: an anti-wrinkle cream (ExC13, Fig. 4a), a liquid

Table 4 Percentage of recovery and precision (coefficient of variation, %) of the 70 target compounds for both the UAE and the *Supported*-UAE methods

Target compounds	UAE (100 µg/wipe)			<i>Sup</i> -UAE (100 µg g ⁻¹)	
	Baby nappy wipe (BP13)	Make-up remover wipe (BP4)	Moisturizing face mask (BP5)	Paper support	Cream-gel brow (ExC6)
Fragrance allergens					
Pinene	100 (8.2)	97.2 (6.9)	41 (14) ^a	77.3 (12)	107 (6.1)
Limonene	–	100 (6.7)	43 (6.1) ^a	89.4 (6.5)	90.7 (0.42)
Benzyl alcohol	–	110 (6.3)	98.3 (8.1)	107 (10)	104 (6.9)
Linalool	–	110 (7.8)	95.2 (9.2)	104 (10)	96.0 (11)
Methyl-2-octynoate	120 (3.8)	99.1 (6.7)	80.7 (2.7)	86.8 (5.4)	115 (1.4)
Citronellol	107 (8.8)	84.1 (6.4)	99.3 (6.0)	103 (2.0)	97.0 (4.8)
Citral	–	104 (6.2)	91.6 (8.5)	96.6 (2.5)	110 (4.1)
Geraniol	119 (8.9)	89.0 (4.2)	87.8 (9.2)	97.7 (5.6)	103 (4.1)
Cinnamaldehyde	101 (8.4)	96.4 (7.5)	88.7 (10)	96.8 (3.1)	104 (4.0)
Hydroxycitronellal	103 (7.6)	97.4 (6.8)	89.6 (10)	89.7 (6.1)	103 (5.9)
Anise alcohol	105 (8.5)	97.6 (6.5)	98.9 (8.4)	94.8 (1.0)	102 (5.3)
Cinnamyl alcohol	113 (9.1)	102 (7.8)	104 (10)	89.4 (19)	106 (6.4)
Eugenol	111 (8.8)	106 (7.7)	91.6 (10)	89.1 (11)	105 (5.0)
Methyleugenol	105 (9.2)	99.2 (7.1)	85.8 (8.9)	98.8 (5.6)	99.2 (1.7)
Isoeugenol	108 (9.0)	106 (7.7)	91.4 (9.3)	90.4 (6.3)	100 (7.5)
Coumarin	105 (14)	103 (2.0)	93.4 (13)	97.9 (3.8)	99.7 (5.2)
α-Isomethyl ionone	103 (8.6)	105 (7.6)	74.6 (7.7)	88.2 (11)	101 (4.2)
Lilial®	–	104 (7.5)	74.1 (4.8)	90.0 (7.4)	104 (4.4)
Amyl cinnamal	95.6 (12)	107 (3.7)	80.8 (8.3)	87.0 (5.8)	97.0 (1.3)
Lylal®	108 (8.9)	114 (8.6)	85.3 (11)	89.7 (11)	111 (6.0)
Amylcinnamyl alcohol	113 (1.1)	107 (11)	96.4 (5.1)	94.1 (8.7)	102 (14)
Farnesol	103 (7.9)	108 (7.7)	93.5 (9.4)	97.9 (14.0)	102 (6.3)
Hexylcinnamaldehyde	106 (10)	109 (8.8)	96.4 (11)	97.1 (2.2)	106 (3.3)
Benzyl benzoate	105 (9.1)	101 (8.4)	–	87.9 (12)	110 (3.4)
Benzyl salicylate	114 (9.4)	116 (11)	89.6 (3.6)	86.0 (8.5)	109 (3.8)
Benzyl cinnamate	98.7 (12)	103 (14)	94.0 (10)	95.9 (3.3)	111 (3.0)
Synthetic musks					
Musk xylene	84.1 (2.1)	106 (4.9)	100 (5.3)	98.7 (8.5)	104 (4.4)
Musk ketone	90.3 (4.4)	115 (2.1)	94.4 (4.2)	103 (8.6)	107 (4.9)
Musk ambrette	113 (2.1)	116 (1.4)	72.5 (5.2)	86.4 (16)	103 (4.9)
Musk moskene	97.1 (8.3)	119 (14)	79.2 (11)	93.4 (6.6)	105 (4.7)
Musk tibetene	82.4 (7.3)	105 (1.1)	81.3 (14)	89.1 (14)	106 (4.2)
Galaxolide	104 (4.3)	107 (2.3)	95.0 (3.6)	101 (1.8)	112 (4.6)
Celestolide	113 (13)	108 (2.0)	82.0 (8.6)	92.8 (7.3)	108 (4.5)
Phantolide	88.6 (1.3)	89.2 (1.9)	74.7 (2.1)	94.4 (8.2)	108 (4.1)
Cashmeran	98.8 (12)	97.5 (1.7)	107 (3.7)	89.4 (15)	91.0 (6.6)
Traseolide	102 (2.3)	112 (2.9)	90.1 (3.4)	90.1 (2.1)	102 (8.3)
Tonalide	87.7 (14)	88.5 (3.5)	83.8 (2.8)	88.4 (15)	104 (3.3)
Ambrettolide	117 (10)	119 (3.3)	72.1 (6.6)	102 (3.0)	107 (3.6)
Plasticizers ^b					
BPA	86.2 (13)	103 (6.9)	84.1 (9.4)	94.2 (3.0)	110 (3.1)
DMP	105 (11)	101 (2.5)	90.6 (7.4)	116 (14)	90.2 (2.4)
DEP	120 (11)	116 (2.0)	94.7 (7.0)	92.4 (11)	106 (2.6)
DIBP	115 (12)	116 (0.52)	71.1 (4.9)	90.1 (16)	105 (2.9)

Table 4 (continued)

Target compounds	UAE (100 µg/wipe)			<i>Sup</i> -UAE (100 µg g ⁻¹)	
	Baby nappy wipe (BP13)	Make-up remover wipe (BP4)	Moisturizing face mask (BP5)	Paper support	Cream-gel brow (ExC6)
DBP	108 (1.4)	113 (1.1)	73.0 (4.8)	89.5 (16)	107 (3.2)
DMEP	114 (13)	116 (0.62)	108 (10)	93.5 (2.6)	108 (5.6)
DIPP	111 (3.4)	104 (1.6)	70.3 (3.9)	100 (1.1)	112 (3.8)
DPP	116 (14)	115 (11)	75.0 (3.7)	89.2 (14)	107 (3.4)
BBP	91.8 (15)	99.2 (4.1)	90.6 (8.3)	90.6 (16)	103 (4.1)
DIHP	105 (14)	84.5 (13)	75.3 (2.0)	90.7 (8.1)	79.8 (3.2)
DEHP	89.2 (14)	104 (5.9)	72.9 (6.5)	94.6 (11)	106 (4.0)
DCHP	82.4 (15)	82.6 (3.2)	81.1 (4.4)	96.8 (2.5)	103 (3.7)
DPhP	112 (1.2)	110 (5.9)	77.3 (6.9)	87.1 (13)	106 (4.3)
DNOP	102 (10)	97.1 (5.3)	72.6 (8.6)	91.8 (9.0)	108 (5.5)
DIDP	108 (1.7)	91.1 (8.3)	84.2 (13)	103 (16)	106 (8.4)
DMA	114 (12)	102 (3.5)	96.5 (6.2)	94.7 (1.0)	101 (4.7)
DEA	119 (11)	113 (2.1)	101 (7.9)	97.7 (2.4)	91.9 (4.6)
DEHA	81.0 (2.1)	97.5 (3.1)	79.9 (4.5)	93.6 (9.2)	103 (4.1)
Preservatives					
Bronidox	91.9 (3.1)	113 (7.9)	99.3 (7.0)	99.9 (6.0)	100 (3.5)
PhEtOH	101 (2.8)	–	–	91.4 (4.8)	–
BHA	109 (1.8)	117 (4.2)	76.2 (8.6)	88.9 (11)	105 (5.1)
BHT	103 (2.3)	115 (3.6)	–	94.7 (15)	104 (4.7)
IPBC	94.6 (4.2)	118 (13)	70.6 (11)	89.2 (0.81)	109 (1.8)
Triclosan	82.0 (5.4)	95.9 (12)	96.1 (4.3)	79.0 (1.3)	97.1 (5.8)
MeP	88.2 (4.0)	93.3. (7.0)	–	100 (13)	102 (2.6)
EtP	110 (3.5)	113 (5.2)	102 (7.0)	84.0 (10)	105 (3.9)
iPrP	86.9 (2.1)	93.6 (5.5)	75.4 (8.0)	98.2 (8.3)	96.2 (4.2)
PrP	111 (2.5)	117 (13)	102 (9.3)	97.7 (10)	110 (4.7)
iBuP	96.8 (2.4)	117 (6.4)	71.8 (10)	90.6 (0.2)	99.6 (4.6)
BuP	92.7 (2.8)	90.6 (7.2)	89.3 (11)	84.2 (8.9)	110 (4.7)
BzP	82.8 (5.6)	106 (1.9)	92.4 (11)	89.5 (11)	77.8 (3.8)

– Recovery not calculated since the concentration of the compound present in the sample is greater than the fortification level (see concentration in Tables S1 and S2)

^a As a result of the high volatility of these fragrance allergens, in the case of face masks it is recommended to process the sample (cut and weigh) just before analysis

^b DINP is undetectable at this level

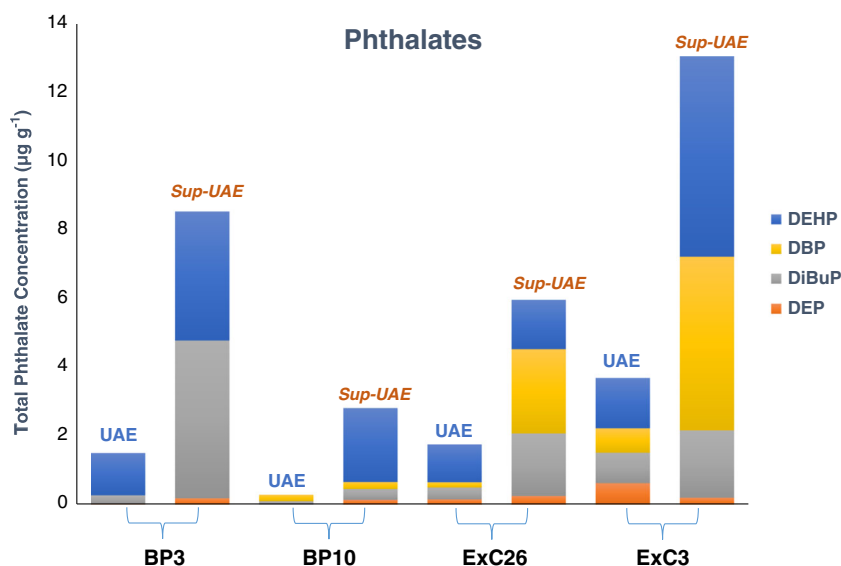
eyeliner (ExC3, Fig. 4b) and a sex gel (BP20, Fig. 4c). In the following sections, the results obtained by UAE and *Sup*-UAE methods are discussed for the different groups of target compounds. A summary of the results for the ingredients checkout was added to the descriptive tables (Tables 2 and 3, last column) for the sake of simplicity.

Phthalates

Eighteen plasticizers (15 phthalates and 3 adipates) were successfully determined using the proposed analytical methodology, in addition to bisphenol A (banned as an ingredient for

cosmetic formulations, but used in packaging materials from where trace migration can occur). Figure 5a shows the distribution of permitted and prohibited phthalates in the tested products and the occurrence of five detected phthalates out of a possible 15. It is extremely important to remark here that, as stated above, the amount of phthalates provided by the applicator varies between 70% and 90% and, in some cases, these compounds come exclusively from the applicator (see Tables 2 and 3). In this section, phthalate data are discussed globally, but this perspective should not be lost. It should also be clarified that Regulation EC No 1223/2009 [1] allows the presence of traces of prohibited substances in cosmetic

Fig. 3 Contribution of phthalates from the product applicators to the cosmetic phthalate content: differences in the phthalates composition (qualitative and quantitative) obtained by UAE and *Sup*-UAE techniques



products as long as they are technically unavoidable. It is also true that a safety assessment of a cosmetic product must be carried out before it is placed on the market, including the verdict from a qualified safety assessor about whether the cosmetic is safe, safe with restrictions or unsafe (in the last case, the launch is cancelled). DMP and DEP are the most widely used phthalates in cosmetics in the EU, because they have not been restricted or associated with any risks for health or the environment; however, in this study DEP was found in 82% of the samples, whereas DMP was found in just 6%. Regarding the use of low molecular weight orthophthalates (those with 3–6 carbon atoms in their backbone), only DBP and DIBP were ever used in cosmetics or body care products [19] but they should no longer be found in products manufactured and commercialised in the EU, because the European Cosmetics legislation prohibits the use of substances classified as CMR in cosmetic products [18, 20]. However, these two phthalates along with the banned DEHP appear in a high percentage of the analysed samples (80%), having almost all, two or three of the banned phthalates. This highlights the presence of banned phthalates (at tens of micrograms per gram, see ESM Table S1) in the temporary tattoo samples, since these products do not contain applicators, although they are packaged in contact with plastic material. No phthalates with chain substituents longer than hexyl have been found in the tested products, except for DINP in the artificial nails (sample BP12). On the other hand, only one out of the three analysed adipates, DMA, was found in four samples, three temporary tattoos (BP14, BP15 and BP17) and the artificial nails (BP12); and all the cosmetic products tested were bisphenol A free.

The restriction levels of phthalates in different goods are a matter of current concern. EC communications from 2014 [20] and 2018 [18] discussed the opportunity of adopting a Danish proposal to tighten the restriction on four phthalates at

EU level (DEHP, DBP, BBP and DIBP) because of their potential reproductive toxicity and endocrine-disrupting properties. After carefully considering the available information, the Commission initially decided not to adopt the proposed restriction [20]; however, the four phthalates were finally classified as substances of very high concern due to their endocrine-disrupting properties [18].

However, these communications also contain very positive data: the socio-economic report states that the phasing out of the four phthalates and/or phthalates in general has already taken place, or is underway. The reasons behind this fact are twofold: the implementation of the EU legislation on phthalates in food contact materials, cosmetics, childcare articles and toys; and, the substitution of the phthalates with other plasticizers, which is expected to continue, because there are affordable and technically available alternatives for the majority of applicators already on the market. Besides, other market factors, such as a trend of using non-phthalates plasticizers and uncertainties of long-term price and availability of phthalate feedstocks, support this trend of substitution. So, the current studies and the future projections indicate continued considerable decrease of the presence of the phthalates in articles in the EU. On this basis, the decrease in volume taking place will further reduce the exposure to a level which is of no concern to human health [18, 20].

Fragrance ingredients: allergens and musks

Thirty-eight per cent of the tested products were fragrance-free (12 ‘extreme cosmetics’ and 7 ‘borderline’ products), confirming this current trend; additionally, one liquid eyeliner (ExC3) was free of allergenic fragrances. The remaining 30 products presented one or more allergenic fragrances among their scented ingredients, and eight of them were not correctly labelled in this aspect. According to the European Cosmetics

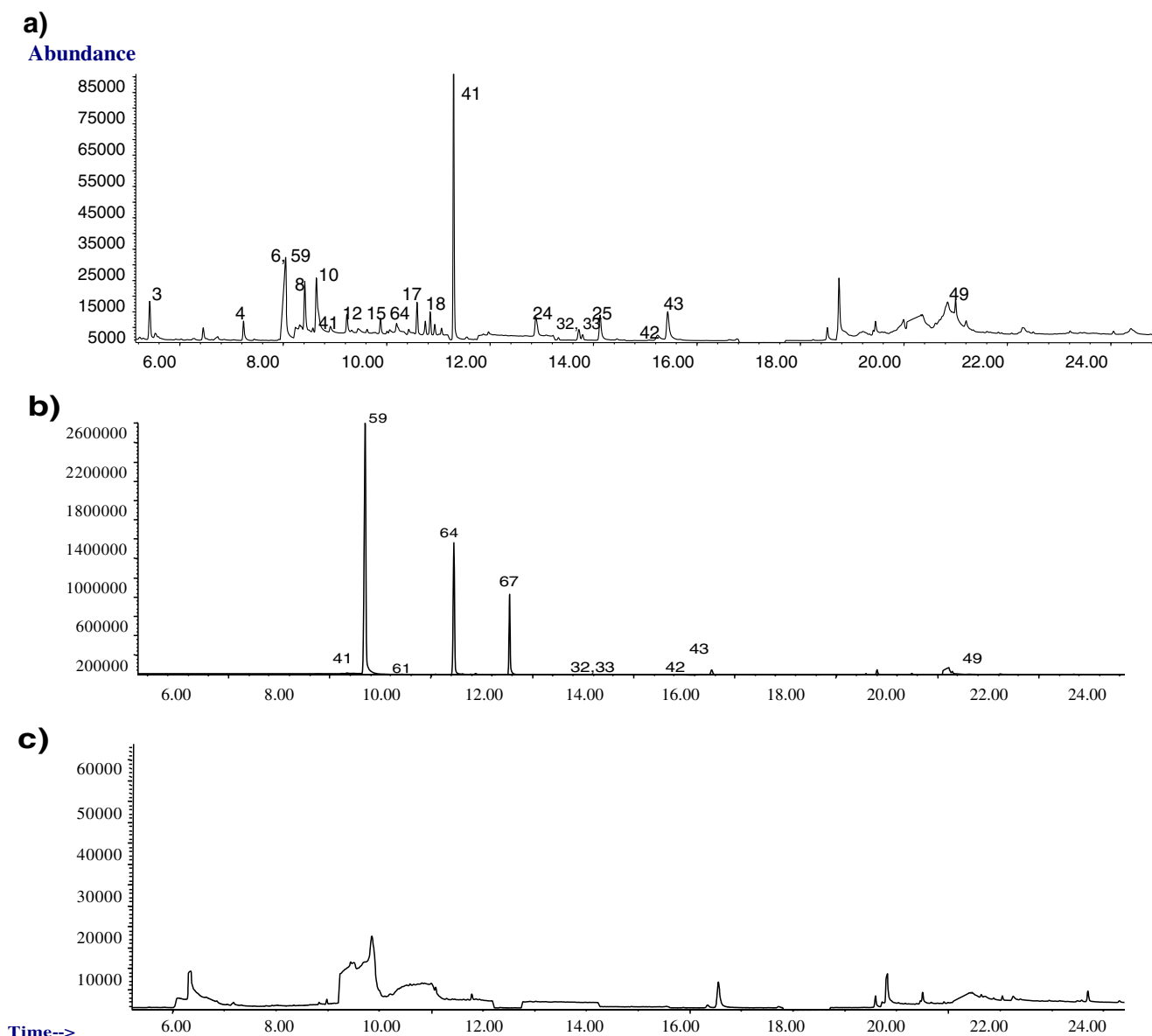


Fig. 4 GC-MS total ion chromatograms (TIC) of three commercial samples: a) snake venom serum (ExC14); b) waterproof liquid eyeliner tattoo effect (ExC3); and sexual pleasure gel (BP20)

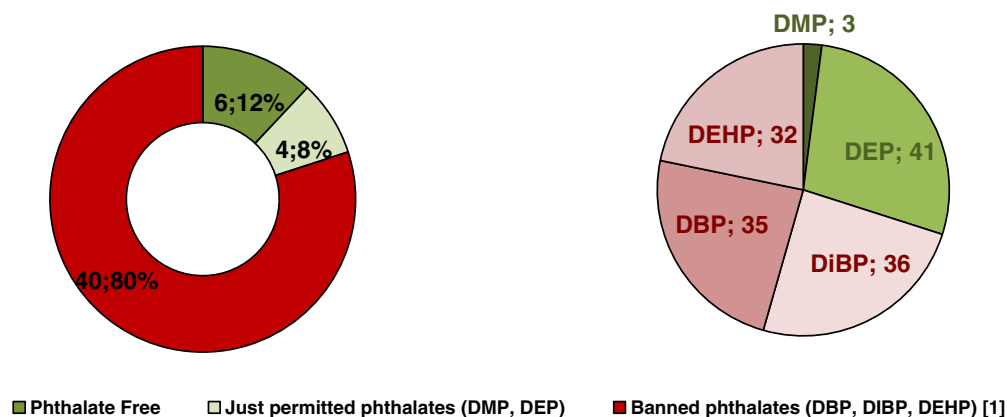
Regulation [1], fragrance allergens must be indicated in the list of ingredients when their concentration in the final product exceeds 0.001% (w/w) for leave-on and 0.01% (w/w) for rinse-off products. In this way, two analysed products were over-labelled (ExC16 and BP8), since they declare more allergens than they contain; whereas the remaining six samples were under-labelled as they contain allergens in a concentration above the labelling limit which, consequently, should appear on the label so that the user is correctly informed.

Twenty of the 26 allergenic fragrances and four of the 12 analysed synthetic musks were detected, and their frequency of occurrence is summarised in Fig. 5b. The most common allergens were limonene, linalool and benzyl alcohol, which are also the most volatile (other than pinene),

followed by α -isomethyl ionone and benzyl benzoate. Regarding musks analysis, the trend that began in the 1990s towards a decline in the use of nitromusks and their replacement with polycyclic musks is noted, galaxolide and tonalide being the most detected ones. The macrocyclic musk ambrettolide appears in only one sample.

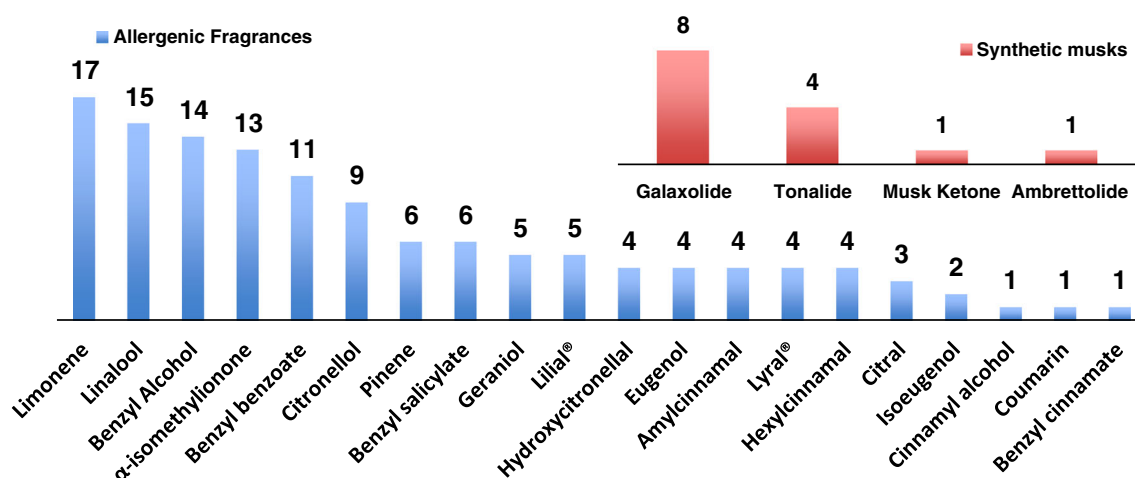
The snake venom-based cosmetics anti-wrinkle cream (ExC13) and serum (ExC14) are by far the samples with more fragrance ingredients in their composition: 10 and 11 allergens, respectively, and galaxolide and tonalide; the cream (ExC13) also contains the only nitromusk detected, musk ketone, although with a concentration within the established limits. Satisfactorily, no prohibited nitromusks were detected in any of the analysed products. The sexual

a)



b)

Fragrance ingredients



c)

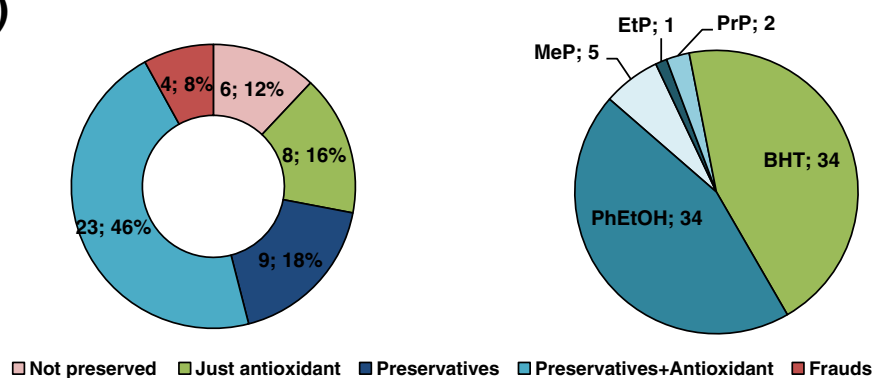


Fig. 5 Occurrence, distribution and frequency of the analysed compounds in the survey of marketed products: a) phthalates; b) fragrances, number on top of bars indicates how many products contain each one; c) preservatives. [For phthalates and preservatives, on the left

gels (BP19 and BP20) and three out of the four analysed temporary tattoos (BP15, BP17 and BP18) were also free

the data represent the number of products and detection rate; on the right, the data represent the number of products containing each particular compound] (see legends in the graphics for additional information)

of fragrances, excluding one of them (BP16), which is a ‘scented’ dragon.

Preservatives

Only four of the 11 target preservatives were detected in any of the 50 tested products and in most of them in combination with the antioxidant BHT, which was detected in the 68% of the analysed samples (Fig. 5c). The obtained data demonstrate two recent trends in cosmetic formulation: phenoxyethanol is undoubtedly the most widely used preservative currently applied in a broad range of cosmetics, while the use of parabens is minimal because some of them are considered to be endocrine disrupters or potential allergens, which has led to an intensive marketing campaign ('parabens free') that resulted in a decrease in their use as preservatives by manufacturers. In fact, only 5 out of 50 tested products have parabens, namely methyl paraben, only coupled with propyl in one sample, which also contains ethyl paraben (ExC3). In contrast, phenoxyethanol was detected in 34 of the 50 products, although some of them present regulatory nonconformities associated with this compound. Thus, three of the analysed samples (Exc3, Exc5, Exc8) clearly exceed the legal limit of 1% w/w ($10,000 \mu\text{g g}^{-1}$ in sample) and another one (Exc1) is at the limit. All of them are 'extreme cosmetics' for eyelashes or eyes, which are very sensitive areas. More surprising is the detection of four fraudulent samples linked to this compound, in this case because its presence is declared on the corresponding labels; however, there is no trace of phenoxyethanol in the samples. Two of them are lip plumpers (BP10, BP11) which must also comply with the Medical Products Directive [3, 4] and the others are 'borderline' products: a gel mask (Exc17) and a lip balm (Exc27). In addition, while two of these samples have at least the antioxidant BHT, the other two become unpreserved, at least according to the Regulation. Moreover, five additional samples are not preserved in any way (sexual gels, lipstick, waterproof cream eye shadow and less worrying, the false nails); that is, they do not contain any of the target preservatives and none are mentioned on the product label. It is important to recall that, in the EU framework, any preservative used in a cosmetic formulation must be in the corresponding positive list (Annex V of the Regulation 1223/2009 [1]) and must be labelled. A balance must be maintained between the correct use of preservatives and the need to preserve samples from bacterial contamination.

Labelling check-up and other compliance concerns

Tables 2 and 3 also summarise whether the labels of each of the products analysed and checked are correct or not, and the main non-compliances are explained and discussed below.

The labelling of allergenic fragrances in cosmetic products is regulated by law [1] and is of particular importance for the safety of consumers. In this way, these compounds must be indicated in the list of ingredients when their concentrations exceed $10 \mu\text{g g}^{-1}$ (for leave-on products)

or $100 \mu\text{g g}^{-1}$ (for rinse-off products). It is important to note that in this respect, recalls of products from the market due to incorrect labelling of allergens are not uncommon (11 recalls so far this year in EU countries [21]).

The label of sample BP13 (a nappy area baby wipe) deserves special mention: benzyl alcohol was detected in this product at a concentration of $10,795 \pm 349 \mu\text{g g}^{-1}$ (i.e. $1.08 \pm 0.035\%$ w/w). This compound is an allergenic fragrance but can also act as a preservative. The specific function of this ingredient in the baby wipe is not declared. If it acts as a preservative it is out of the required legal limit (1%, w/w in ready for use preparations); if it acts as a fragrance ingredient, it is incorrectly labelled. The fragrances should be ordered by content and benzyl alcohol appears fifth, while by concentration it should be the first, and the content is surprisingly high for a fragrance (two order of magnitude higher than the remaining fragrances).

Interestingly, the labelling on temporary tattoos samples (BP14, BP15, BP16 and BP18) is non-existent, although these products show a sentence on the package saying: 'phthalates test: passed' even though all of them have the three forbidden phthalates detected. Another temporary tattoo sample, BP17, has an extensive but incorrect labelling, because it also contains prohibited phthalates as well as more than $50 \mu\text{g g}^{-1}$ of the adipate DMA, although this substance is not regulated (BP17 label reads as follows: 'The products comply with following standards. China standard GB6675-2014. EU newest regulation EC1223/2009. The USA FDA regulation. Related testing reports are available. FDA, TRA, CPSR, MSDS, Microbial contamination test report, Heavy metals test report, EN71. Full sets cosmetic PIF documents to ensure our products are safe and non-toxic to human'). It is useful to remember here that tattoos must comply with both the regulations of cosmetics [1] (because they change the appearance of the skin) and toys [7] (see Table 3).

The 'extreme cosmetics' Exc24 and Exc27 should be directly withdrawn from the market, as they do not comply with the Directive on Food-Imitating Products [9] since they have external appearance of food (bananas) and even the word 'food' appears on the label in small print. They also say 'keep away from children' but this warning does not discourage compliance with the law. Similarly, sample Exc17 looks like a cocoa pudding and has such shape and these words on the wrapper. Moreover, these three products (for hands, lips and eyes, respectively) have forbidden phthalates in their compositions. Currently, some products are being recalled from the market for this reason [22]: soaps in the shape of doughnuts, sweets and fruits or soaps that imitate ice creams (even including plastic spoons).

Three of the selected samples are venom-based cosmetics: two of them claim to contain snake venom (Exc13 and Exc14) and the other one, bee venom (Exc12). Snake venom products do not actually contain natural snake venom but a

synthetic compound known as dipeptide diaminobutyroyl benzylamide diacetate that mimics the action of the full-length natural peptide Waglerin-1 [23]. Similar to Botox, it blocks signal transmission and relaxes the muscles at the area of application in order to reduce the formation and appearance of wrinkles [24]. One of the tested products (ExC13) is correctly labelled and clarifies that the declared peptide is the active principle, its concentration in the cosmetic and its synthetic origin. On the contrary, the second one (ExC14) just declares the peptide as an ingredient without additional explanations. On the web site of the supplier, it can be found that the main ingredient of the serum is a polypeptide found in the venom of the temple snake (*Tropidolaemus wagleri*) so that one could think of it as being of natural origin. The bee venom that appears as an ingredient in the facial cream (ExC12) contains mostly mellitin, an oligopeptide that is the main active ingredient of apitoxin; however, the label does not specify exactly its content, as is often the case [25].

Conclusions

Two ultrasound-based methods (UAE and *Sup*-UAE) were optimized for the extraction of 70 compounds that can appear in cosmetics as ingredients (fragrances, preservatives) or as unexpected forbidden components (phthalates, nitromusks). The efficiency of an additional UAE procedure was compared with a μ -MSPD method previously validated. From an analytical perspective, the proposed UAE method meets the performance requirements, and is fast, simple and green. Moreover, it has been demonstrated that *Sup*-UAE complements the aforementioned techniques, giving additional information mainly related to compounds than can migrate from applicators, and it becomes the recommended option for temporary tattoos and similar products.

Fifty commercial products were tested according to the methodology proposed, and the ingredients and labelling compliance were checked on the basis of the analytical results. In short, from the regulatory viewpoint, 80% of the cosmetics classified as 'extreme' are non-compliant with respect to ingredients and 23% fail in labelling. In fact, just four out of 30 products comply with the legislation in relation to the 70 ingredients evaluated and are also correctly labelled. For the 20 analysed 'borderline' products, 75% of them are non-compliant in terms of ingredients; seven products are fail, whereas another seven pass regarding the labelling. However, it is worth noting that six items lack a label: the temporary tattoos (BP14, BP15, BP16, BP18) and the sexual gel samples (BP19, BP20); in these articles not one of the 70 ingredients screened was found.

In summary, the analytical methodology developed is appropriate for the analysis of both 'extreme cosmetics' and 'borderline' products, but the regulatory situation of all of

them is clearly open to improvement. Moreover, some of the products analysed should be withdrawn from the market because of banned phthalate content, over-preservation or because they resemble food.

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Compliance with ethical standards

Competing interests The authors declare that they have no competing interests.

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