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Analysis of 7 synthetic musks in cream by supported liquid extraction and solid phase extraction followed by GC–MS/MS

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ABSTRACT

A new method for the simultaneous determination of 7 synthetic musks (musk amberette, musk tibetene, musk moskene, musk ketone, musk xylene, phantolide, and tonalide) in cream by means of supporting liquid extraction (SLE) coupled with LC-Alumina-N SPE, then followed by GC–MS/MS has been established. In this study, 7 synthetic musks are extracted and pre-purified by a mixture solution of water and isopropanol from cream, and separated and purified by tandem columns containing SLE column and LC-Alumina-N SPE column, which were seldom reported before. Ultrasonic and mechanical shaking were applied to improve the extraction efficiency. Different experiment conditions, such as the type of extraction solution, extraction time of ultrasonic and mechanical shaking, the type of SLE and SPE column, and matrix effects were optimized and the recoveries of 7 synthetic musks for each part were above 86.61%. In addition, the use of isotope internal standards was systemically discussed. The method showed satisfactory linearity over the range assayed (5–1000 ng g^{-1}), and the limits of detections (LODs) ranged from 0.15 to 4.86 ng g^{-1}, and the limits of quantifications (LOQs) were ranging from 0.49 to 16.21 ng g^{-1}. The recoveries using this method at three spiked concentration levels (10, 100, and 1000 ng g^{-1}) range from 85.6% to 109%. The relative standard deviation was lower than 9.8% in all case. The proposed analytical method has been successfully applied for the analysis of 7 synthetic musks in commercial cream.

1. Introduction

Large quantities of synthetic musks are manufactured due to exhibiting a strong, warm, sensual and long-lasting odor, and used in a wide variety of cosmetic products, such as perfumes, skin cream, deodorants, and soaps [1,2]. There are three groups of synthetic musks according to their chemical structure: polycyclic musks (tonalide and phantolide), nitroaromatic musks (musk amberette, musk tibetene, musk moskene, musk ketone and musk xylene) and macrocyclic musk [2]. However two groups of synthetic musks, polycyclic musks and nitroaromatic musks, have been widely applied in cosmetic formulations and then aroused public attention for their potential risks to human health and environment.

In recent years, synthetic musks have been described as a new group of bioaccumulative and persistent xenobiotics [2–5]. It was found that synthetic musks could lead to different types of dermatitis, carcinogenesis and endocrine disorder [3,6–11]. In 1999, Environment Canada issued the "Environmental Protection Act", which was clearly required to reduce the use of synthetic musks [12]. In Europe, the Regulation (EC) no. 1223/2009 established the rules as follows: musk amberette, musk tibetene and musk moskene were prohibited and the concentrations of musk ketone, musk xylene, phantolide and tonalide were limited in cosmetics [13]. China and other countries have issued maximum residue limits (MRLs) for synthetic musks based on the Regulation (EC) no. 1223/2009 in cosmetics. In most of the laws and regulations, 7 synthetic musks (musk amberette, musk tibetene , musk moskene, musk ketone, musk xylene, phantolide, and tonalide) were frequently prohibited or limited in cosmetics for their high toxicity and sensitization. In order to guarantee product safety according to regulations, the development of analytical methods for the determination of synthetic musks in cosmetic is mandatory.

Most of the existing analytical methods for synthetic musks were mainly used to analyze environmental samples, such as water [14,15], sewage [16,17], sludge [16], sediment [18], and air [19]. At present, the analytical methods of synthetic musks in perfume and emulsion, since their matrixes are relatively simple, have been developed [20,21]. These methods available for the identification and quantification of musk compounds comprise a sample preparation step, including traditional liquid–liquid extraction with organic solvents (LLE) [22,23], solid phase extraction

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(SPE) [3,24], solid phase microextraction (SPME) [4,20], liquid phase microextraction (LPME) [25,26], stir bar sorptive extraction (SBSE) [27], ultrasound assisted phacoemulsification liquid phase microextraction (USAEME) [28], and molecular imprinted polymers (MIPs) reported recently [29], followed by gas chromatography–mass spectrometry (GC–MS) [3,18,19,20,27], gas chromatography–tandem mass spectrometry (GC–MS/MS) [18], liquid chromatography–mass spectrometry (LC–MS) [30], liquid chromatography–tandem mass spectrometry (LC–MS/MS) [24]. Cream is one of the most widely used cosmetics, which contains not only hydrophilic compounds like alkali, moisturizing, nutritional agent, but also a great quantity of lipophilic compounds, such as paraffin stearic acid, cetyl, and stearyl alcohol [31]. Since synthetic musks are low polarity and lipophilic compounds, it is difficult to completely separate them from the above mentioned lipophilic compounds. The interferent, which could cause the alteration of ionization efficiency in the determination of GC–MS/MS, may lead to serious matrix effects [32,33]. Due to the low selectivity of the extraction techniques, extracts from complex samples like cream have to be subjected to cleanup steps, such as by solid-phase extraction (SPE) [34,35], liquid–liquid extraction with SPME [36], and matrix solid phase dispersion method (MSPD) [37].

The aim of this study is to develop a method based on supported liquid extraction (SLE) and SPE to simultaneously determine 7 synthetics musks (musk amberette, musk tibetene, musk moskene, musk ketone, musk xylene, phantolide, and tonalide) in cream by GC–MS/MS. In this work, water was introduced in the mixed extraction solution to separate synthetic musks from hydrophilic compounds, and the isopropanol in the mixed extraction solution was used to extract synthetic musks from lipophilic compounds. In addition, SLE column and SPE column were selected to adsorb the water and separate the interferent, which could improve the purification effect and reduce the matrix effects. Tandem MS has been selected to enhance selectivity and decrease LODs of the method. The applicability of the method to real samples was tested by 28 cream products, and found that the established method is suitable for routine analysis of 7 synthetics musks in real cream.

2. Experimental

2.1. Apparatus and reagents

The instruments used in the experiment include: a Trace GC Ultra coupled to a TSQ Quantum XLS tandem mass spectrometer (Thermo Fisher Co.), UMX5 high precision electronic balance (Max=5.1 g, d=0.1 μg, METTLER TOLEDO Co.), XS205 electronic balance (Max=81 g, d=0.01 mg, METTLER TOLEDO Co.), KQ3200E ultrasonic cleaners (Kunming ultrasonic instrument Co., Ltd.), KS 260 Basic concussion instrument (IKA Co.), Vortex-Genie2T vortex mixer (Scientific Industries Co.), nitrogen evaporator (V-EVAPTM111, Organomation Associate, Jnc.) and Gradient A10 water purification System (Milli-Q Co. USA). The SPE columns used in the experiment were Supelclean™ LC-Alumina-N SPE Columns (6 mL and 2 g, Supelco, USA), Supelclean™ LC-Florisil SPE Columns (6 mL and 1 g, Supelco, USA), Supelclean™ LC-Si SPE Columns (6 mL and 1 g, Supelco, USA) and ISOLUTE SLE+ Columns (2 mL/15 mL, 5 mL/25 mL, sorbent mass/reservoir volume, Biotage, Sweden).

The studied compounds, their chemical names, CAS numbers, and purity are summarized in Table 1. Cyclohexane (99.98%), acetone (99.8%), hexanes (99.9%), dichloromethane (99.9%), isopropanol (99.9%), isooctane (99.9%) were provided by Fisher Scientific Inc. (Geel, Belgium, UK). Toluene (99.9%) and acetonitrile (99.9%) were respectively purchased from Duksan Pure Chemicals Inc. (Ansan, Kyungkido, KOREA) and Merck KGaA Inc. (Darmstadt, Germany). All above reagents were used without further purification.

The special precautions are required throughout the analytical procedure due to the widespread use of the synthetic musks in many consumer products. All the containers used in the test need to be rinsed by alcohol and acetone 3 times, respectively, before the experiment. Moreover, musk-free gloves were used and the samples were prepared in a fume hood.

Cosmetic samples from national and international brands were purchased from local markets in Beijing. Sealed samples in their original containers were stored at room temperature. The blank samples used in the experiments were found by the method of MSPD [37] from several international brand cream products. The concentrations of 7 synthetic musks (musk amberette, musk tibetene, musk moskene, musk ketone, musk xylene, phantolide, and tonalide) in this blank sample were below the instrumental detection limits (IDLs).

2.2. Extraction and cleanup

0.5 g cream was exactly weighted and placed into a 15 mL PTFE centrifuge tube, then 150 μL 2 μg g^{-1} mixed internal standard solution (D15-Musk Xylene and D3-Tonalide) in isooctane was added as a quality control for the entire procedure. 8 mL extraction solution (water: isopropanol=1:2, v/v) was added to the above sample to extract the target compounds by ultrasonic extraction 5 min, shaking extraction 5 min, and then centrifugation for 8000 rpm for 15 min. A 4 mL upper layer extraction solution was

Table 1
Target compounds: chemical names, purity, suppliers, CAS number, retention times and MS conditions.

Abbreviation	Chemical names	Purity	CAS	Retention times (min)	Parent mass (m/z)	Product mass (m/z)	Collision energy (V)	Scan time (s)	Scan width (m/z)
AHMI	Phantolide	10 ng μl^{-1} [c]	15323-35-0	14.18	229	131,173,**187** [a]	9	0.25	0.005
MA	Musk amberette	99% [b]	83-66-9	15.94	253	106,120,**223** [a]	5	0.22	0.005
MX	Musk xylene	99.5% [b]	81-15-2	16.61	282	190,248,**265** [a]	8	0.15	0.005
AHTN	Tonalide	99% [f]	21145-77-7	16.67	243	159,**187** [a],201	9	0.15	0.005
MM	Musk moskene	10 ng μl^{-1} [c]	116-66-5	17.22	263	187,216,**221** [a]	7	0.30	0.005
MT	Musk tibetene	10 ng μl^{-1} [c]	145-39-1	18.50	251	146,160,**234** [a]	8	0.35	0.005
MK	Musk ketone	98% [b]	81-14-1	19.67	279	**191** [a],247,262	11	0.35	0.005
D_{15}-MX	D_{15}-Musk xylene	100 ng μl^{-1} [d]	877119-10-3	16.26	294	248,258,**276** [a]	8	0.12	0.005
D_3-AHTN	D_3-Tonalide	100 ng μl^{-1} [e]	–	16.61	246	160,**190** [a],204	9	0.10	0.005

[a] Bold figures is quantitative mass.
[b] Dr. Ehrenstorfer GmbH (Ausburg, Germany).
[c] 10 ng μl^{-1} in cyclohexane from Dr. Ehrenstorfer GmbH (Ausburg, Germany).
[d] 100 ng μl^{-1} in acetone from Dr. Ehrenstorfer GmbH (Ausburg, Germany).
[e] 10 ng μl^{-1} in isooctane from Dr. Ehrenstorfer GmbH (Ausburg, Germany).
[f] Shanghai East's Flavors and Fragrances Co., Ltd.

purified by the tandem columns (SLE columns coupled with LC-Alumina-N SPE columns). The SPE columns were washed with 5 mL of acetone and pre-conditioned with 5 mL of isopropanol. The sorbent was kept wet during the conditioning and sample loading steps. SLE columns do not require any pre-conditions before being used. The above 4 mL upper layer extract was kept in the SLE columns to equilibrate for 5 min so that the aqueous portion and other reagent compounds were completely absorbed into the packing material. Then the tandem columns were eluted 5 times with 4 mL of dichloromethane, respectively, and all the eluting solvent was collected. The collected solution was evaporated under a gentle stream of nitrogen at 35 °C. Finally, the residue was dissolved in 0.5 mL isooctane, and then filtered through a 0.22 μm PTFE filter for GC–MS/MS analysis.

2.3. GC–MS/MS

A Trace GC coupled to a TSQ Quantum XLS triple quadruple mass spectrometer (Thermo Fisher, USA) was used. Chromatographic separation was performed on a Thermo (Thermo Fisher, USA) TG-5MS ([5%-phenyl]-methyl-polysiloxane, 0.25 μm film thickness, 0.25 mm i.d.) capillary column [25]. Helium (purity ≥ 99.999%) was applied as the carrier gas at a constant flow of 1.0 mL min^{-1}. The temperature program for the GC oven was as follows: initial temperature 60 °C (held for 2 min), increased to 150 °C at 25 °C min^{-1}, and followed by 15 °C $min^{-1}$to 260 °C (held for 10 min).The GC injection temperature was at 250 °C. Pulsed splitless mode was applied for injection. The transfer line and ion source temperatures were maintained at 280 and 230 °C. A solvent delay of 6.0 min was selected. Argon (purity ≥ 99.999%) was applied as a collision gas. The tandem MS was operated in multiple reactions monitoring (MRM) mode for mass analysis of positive ions generated using electron ionization (EI+). GC–MS/MS parameters of each compound are shown in Table 1.

3. Results and discussion

3.1. Optimization of extraction process

To ensure the precision and accuracy of measurements using the GC–MS/MS technique, extraction efficiency was cautiously evaluated. Complete extraction is a precondition of accurate and reliable for the determination of synthetic musks in cream. Synthetic musks are hardly separated from low polarity lipophilic compounds in cream matrices, due to their low polarity. In this study, a mixed solution which contains water was applied to extract and pre-purify 7 synthetic musks in terms of their different hydrophobicity from cream, and the type of mixed solution was optimized. Ultrasonication or shaking was regarded as the second factor for optimizing the separation of 7 synthetic musks from matrixes. The extraction efficiency was presented by the recoveries from spiked cream samples.

3.1.1. Optimization of mixed solution composition

The mixture of water and an organic solvent was selected as the extraction solution in terms of the different hydrophilicities of 7 synthetic musks and other compounds in cream. Firstly, the extraction efficiency was optimized by varying organic solvent (acetone, acetonitrile, isopropanol, and tetrahydrofuran) at the given ratio of water and organic solvent 1:1 (v/v). It can be seen in Fig. 1 that water–isopropanol, (1:1, v/v), shows a higher extraction efficiency. It may be due to the good solubility of isopropanol in lipid materials. Water–isopropanol was therefore applied as the extraction solvent for cream samples. After that, the extraction efficiency was examined in a series of mixed solvents with water

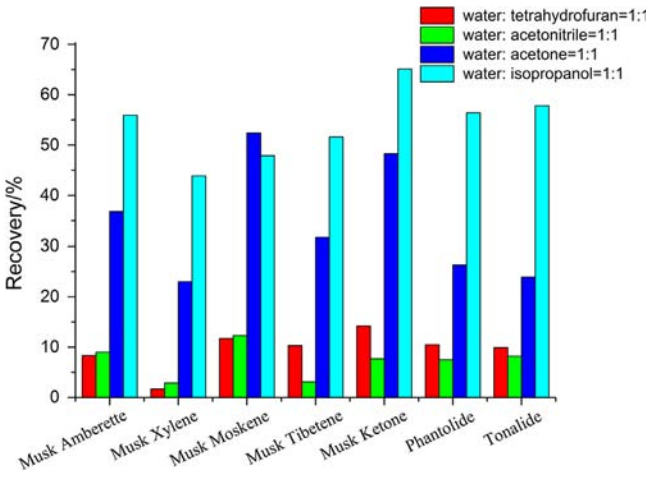

Fig. 1. Effect of different extraction solutions on the recovery of 7 synthetic musks from spiked cream samples ($n=3$).

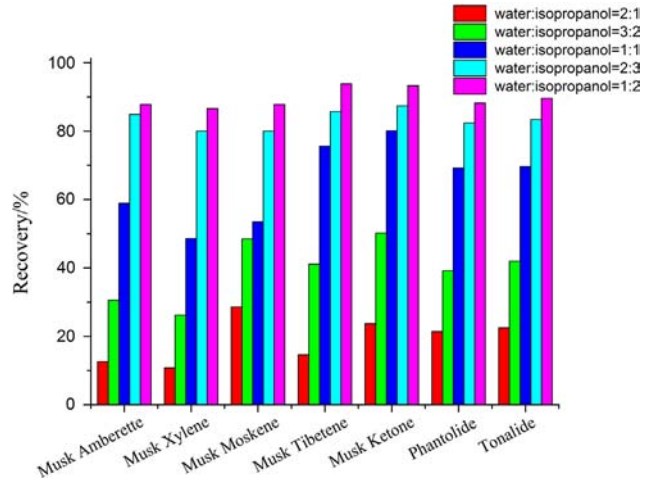

Fig. 2. Effect of different ratios of water and isopropanol on the recovery of 7 synthetic musks from spiked cream samples ($n=3$).

and isopropanol in ratios of 2:1, 3:2, 1:1, 2:3, and 1:2 (v/v). The results are listed in Fig. 2. They indicated that water–isopropanol, 1:2 (v/v), produced slightly higher extraction efficiency. Although with the amount of isopropanol in extraction solution increasing the extraction efficiency can continue to increase, the matrix effects can increase due to having too much dissolved lipid materials from cream. Hence, water–isopropanol (1:2, v/v) was chosen as the extraction solution for all subsequent experiments.

3.1.2. Optimization of ultrasonic and shaking time

The influence of the variation of the extraction times 5, 10, 15, 20 min on the recoveries of 7 synthetic musks were investigated by ultrasonic (Fig. 3) and shaking (Fig. 4) for the blank spiked cream samples, in order to obtain the good recoveries. The results in Figs. 3 and 4 show clearly that the recoveries of 7 synthetic musks reached the maximum level (over 90%) after 5 min for both ultrasonic and shaking. In order to make the extraction method to be used for the different kinds of cream, 5 min ultrasonic combined with 5 min shaking was selected as the extraction method.

3.2. Optimization of cleanup

Due to the existence of co-extracted substances during extracting the 7 synthetic musks from cream, an effective purification was needed, which could remarkably eliminate interferences of the

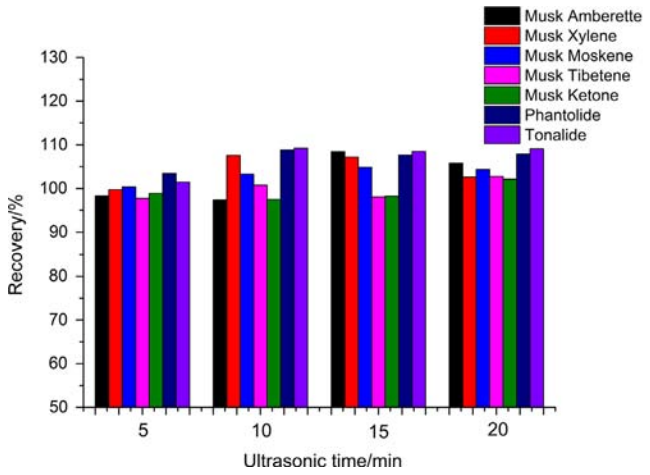

Fig. 3. Effect of ultrasonic time on the recovery of 7 synthetic musks from spiked cream samples ($n=3$).

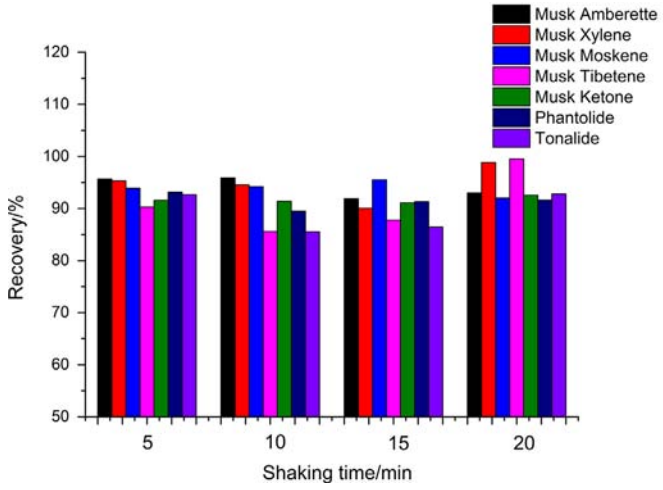

Fig. 4. Effect of shaking time on the recovery of 7 synthetic musks from spiked cream samples ($n=3$).

matrix, prolong the service of GC–MS/MS, and improve sensitivity and reproducibility. In addition, before the following SPE cleanup, the water in the supernatant solution needed to be removed. SLE columns were selected in this article to adsorb the water and a part of hydrophilic and lipophilic interferents, since the aqueous portion and other hydrophilic compounds were completely absorbed into the packing material of SLE. It is reported that SLE is a kind of newly developed sample cleanup technology, which has been used previously to analyze biological samples [38–41]. The SLE column is packed with a modified form of diatomaceous earth. When the supernatant solution is entered into the SLE columns, the aqueous portion and other hydrophilic compounds are deposited on the hydrophilic surface. Then, SPE column is needed for the further purification due to the complex composition in cream. Furthermore, a water-immiscible organic solvent is applied to the tandem columns and analytes are extracted into the organic phase and eluted. Therefore, the type of SLE and SPE columns and the volume of elution solvent were optimized in the purification process.

3.2.1. The type of SLE and SPE columns

The water adsorption capacity of SLE column depends on the volume of packing material. In this study, two types of SLE columns (2 mL/15 mL, 5 mL/25 mL, sorbent mass/reservoir volume) were evaluated and the volume of the supernatant was investigated by the recoveries of 7 synthetic musks from spiked cream samples. 0.5 g of the blank cream sample was extracted by the optimized extraction method. The supernatant, 2 mL, 3 mL, 4 mL, 5 mL and 6 mL was spiked with standards (150 ng) and internal standard (150 ng) and was added to two types of SLE columns above mentioned. It was found that the maximum volume of supernatant was 2 mL for the SLE columns (2 mL/15 mL, sorbent mass/reservoir volume) and 4 mL for the SLE columns (5 mL/25 mL, sorbent mass/reservoir volume). Considering the extraction efficiency, accuracy of the cleanup method, the SLE column (5 mL/25 mL, sorbent mass/reservoir volume) and 4 mL supernatant were chosen as the condition of SLE process.

Although the cleanup of SLE had eliminated the water and most of hydrophilic compounds, further purification was needed to remove the residual lipophilic interferents. The SPE columns can serve as chemical filters, retaining the matrix while allowing the 7 synthetic musks to be eluted. Thus, the purification effect of normal-phase SPE columns, including LC-Alumina-N SPE column, LC-Florisil SPE column, and LC-Si SPE column, were compared. It was found that when the elution solution, flowing through LC-Florisil SPE column and LC-Si SPE column was concentrated, a large amount of white impurities appeared in the concentrated eluate, which could make matrix effects affect the accuracy determination of 7 synthetic musks. However, experiment results showed that when a combination of the SLE column with the LC-Alumina-N SPE column was designed to purify the supernatant, an ideal purification effect can be achieved.

3.2.2. The volume of elution solution

In order to avoid water and hydrophilic compounds into the elution solution, dichloromethane, an immiscible water solvent, was selected as elution solution. To get the minimum elution volume, several fractions were collected at 1–2, 3–4, 5–6, 7–8, 9–10, 11–12, 13, 14–15, 16, 17, 18, and 19 mL. The relationship between the recoveries of 7 synthetic musks and the fractions of elution solution was shown in Fig. 5. The fractions of elution solution between 0 and 13 mL automatically flowed through the columns. The fractions between 14 and 15 mL through the columns were flowed by drying process, which was realized by pulling laboratory air through the cartridge using an SPE vacuum manifold. The fractions between 16 and 19 mL were used to prove that there was little synthetic musk left in the columns. The results indicated that the 7 synthetic musks were almost eluted in 0–15 mL, and their recoveries were above 98.18%. However, the

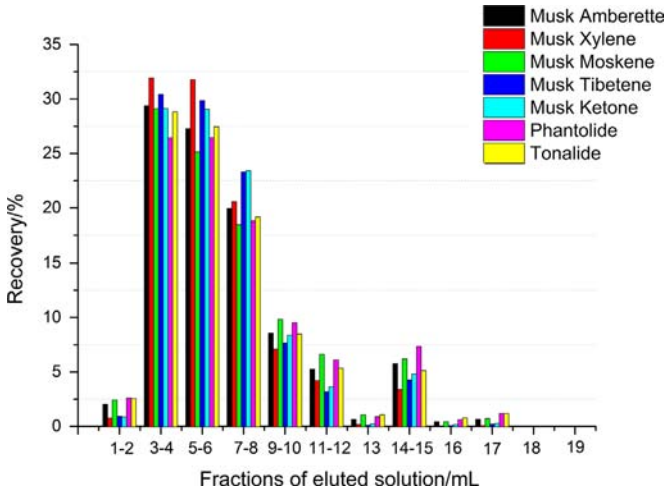

Fig. 5. The relationship between the recoveries of 7 synthetic musks and the fractions of eluted solution.

recoveries of the 7 synthetic musks increased and ranged from 3.38 to 7.35% between 14 and 15 mL (Fig. 5). The reason was that there were not any pre-conditions for the SLE column, and about 3 mL elution solution containing the analytes could be absorbed in the SLE column. Thus, the drying process was needed. Hence, 20 mL of the eluent (dichloromethane) was adopted as minimum volume to elute the compounds in this study.

3.3. Matrix effects

In order to ensure the accuracy and reliability of experimental results, it is essential to fully extract, efficiently eliminate and compensate for matrix interferences. Matrix effect (ME) can come from the sample matrix, sample preparation procedure, quality of chromatographic separation, and ionization type [42–45]. For the complicated cream matrix, the tandem columns cannot completely remove all the impurities, matrix effects still exist and become one of the main factors affecting accurate quantification and repeatability in the detection of GC–MS/MS. One of the ways to overcome matrix effects is to use internal standards, since they have similar physical and chemical properties to the corresponding analytes [46], and can not only effectively compensate for the loss of the analyte during the pre-treatment process but also compensate for the change of the analyte response value due to the matrix effects. Thus, the internal standards, D_{15}-musk xylene and D_3-tonalide, were applied to this study, and the matrix effects of external standard method (Eq. (1)) and internal standard method (Eq. (2)) were evaluated by the following two equations, respectively.

$$\mathrm{ME}(\%) = \left[\frac{A-B}{B}\right] \times 100\% \quad (1)$$

$$\mathrm{ME}(\%) = \left[\frac{C-D}{D}\right] \times 100\% \quad (2)$$

Table 2
Comparison of matrix effects of the two methods.

Synthetic musk	Matrix effects (%)	
	External standard method	Internal standard method
Musk amberette	−27.5	−0.395
Musk xylene	−29.7	11.3
Musk moskene	36.9	−10.6
Musk tibetene	−31.8	0.34
Musk ketone	−32.0	13.1
Phantolide	−24.2	3.50
Tonalide	−29.3	1.20

where A is peak area of matrix standard, B is peak area of solvent standard, C is the chromatographic peak area ratio of the quantitative ion to the internal standard in matrix standard and D is the chromatographic peak area ratio of the quantitative ion to the internal standard in solvent standard.

The generation mechanism of matrix effect (ME) is that co-eluted compounds and target compounds competition ionized droplets surface, which may suppress or enhance the ionization of target compounds and affect the detection results. The value of ME=0% represents no matrix effects, ME<0% represents an ionization suppression, ME>0% represents an ionization enhancement. The results are listed in Table 2. It shows that the matrix effects of 7 synthetic musks are from −32% to 36.9% for external standard method, −10.6% to 13.1% for internal standard method, indicating that the internal standard method can efficiently eliminate and compensate for matrix interferences.

3.4. Method performance

The chromatographic conditions were optimized to achieve an efficient separation of the 7 synthetic musks and 2 internal standards. The GC–MS/MS method parameters are listed in Table 3. Fig. 6 shows a chromatogram of a 100 ng mL^{-1} standard solution in isooctane.The instrumental linearity internal standard calibration was carried out. Calibration standards in isooctane were prepared covering a concentration range from 5 to 1000 ng mL^{-1} for 7 synthetic musks with seven calibration levels (5, 10, 50, 100, 300, 700, and 1000 ng mL^{-1}). The method (Table 3) exhibited a direct proportional relationship between the concentration of each analyte as horizontal coordinate (X) and the ratio of the quantitative ion chromatographic peak area to the chromatographic peak area of the internal standard as vertical coordinate (Y). Correlation coefficients $R \geq 0.992$ for 7 synthetic musks were obtained. Method precision on standard solutions was studied within a day ($n=3$) and among days ($n=5$) at two concentration levels (10 and 100 ng mL^{-1}). Precision for 7 synthetic musks was also satisfactory with RSD values ranging from 0.60 to 3.8% for intra-day and 2.0 to 6.0% for inter-day studies (the averages for intra-day and inter-day precision were 2.4% and 4.5%, respectively). The instrumental detection limits (IDLs) were calculated as the concentration giving a signal-to-noise ratio of 3 ($S/N=3$). These results are also presented in Table 3. The IDLs obtained range from 0.011 to 1.4 ng mL^{-1}.

To confirm that the optimized method was suitable for application, a validation process was carried out by above establishing the basic analytical parameters. Recovery and precision of the method were performed by the optimized method to real blank cream samples spiked at three concentration levels (10, 100 and 1000 ng g^{-1}) of 7 synthetic musks. Recoveries, LODs, LOQs and precision data are all summarized in Table 3. The recoveries of

Table 3
Quality parameters of the method.

Synthetic musks	Linear regression	Correlation coefficient (R^2)	IDLs (ng mL^{-1})	Intra-day RSD (%) 10 ng/g (100 ng/g)	Inter-day RSD (%) 10 ng/g (100 ng/g)	Recoveries (RSD) (%)			LOD (%w/w × 10^4)[c]	LOQ (%w/w × 10^4)[a]
						10 ng/g	100 ng/g	1000 ng/g		
Musk amberette	$y=0.0087x-0.238$	0.9921	1.0	3.8(3.6)	5.2(5.2)	90.4(6.0)	106(3.6)	106(0.68)	0.0021	0.0070
Musk xylene	$y=4E-05x-0.0002$	0.9922	1.4	3.1(2.9)	5.0(4.2)	99.2(8.9)	103(8.2)	100(1.0)	0.0048	0.016
Musk moskene	$y=0.0146x-0.4406$	0.9922	0.011	0.86(1.9)	5.7(4.2)	92.8(6.2)	106(6.2)	105(6.6)	0.0014	0.0045
Musk tibetene	$y=0.0056x-0.213$	0.9933	0.012	3.2(0.60)	5.8(3.8)	92.6(3.6)	109(5.0)	108(2.2)	0.0011	0.0036
Musk ketone	$y=0.0079x-0.1703$	0.9950	0.43	3.5(2.0)	6.0(4.5)	97.3(5.5)	98.8(4.6)	109(3.7)	0.0011	0.0037
Phantolide	$y=0.002x-0.0093$	0.9999	0.057	2.3(2.1)	2.0(2.1)	97.1(9.5)	101(4.3)	106(0.70)	0.00015	0.00049
Tonalide	$y=0.0024x-0.0072$	0.9999	0.033	1.8(2.0)	5.7(3.8)	85.6(9.8)	100(4.4)	103(1.7)	0.00069	0.0023

[a] Equivalent to μg g^{-1}.

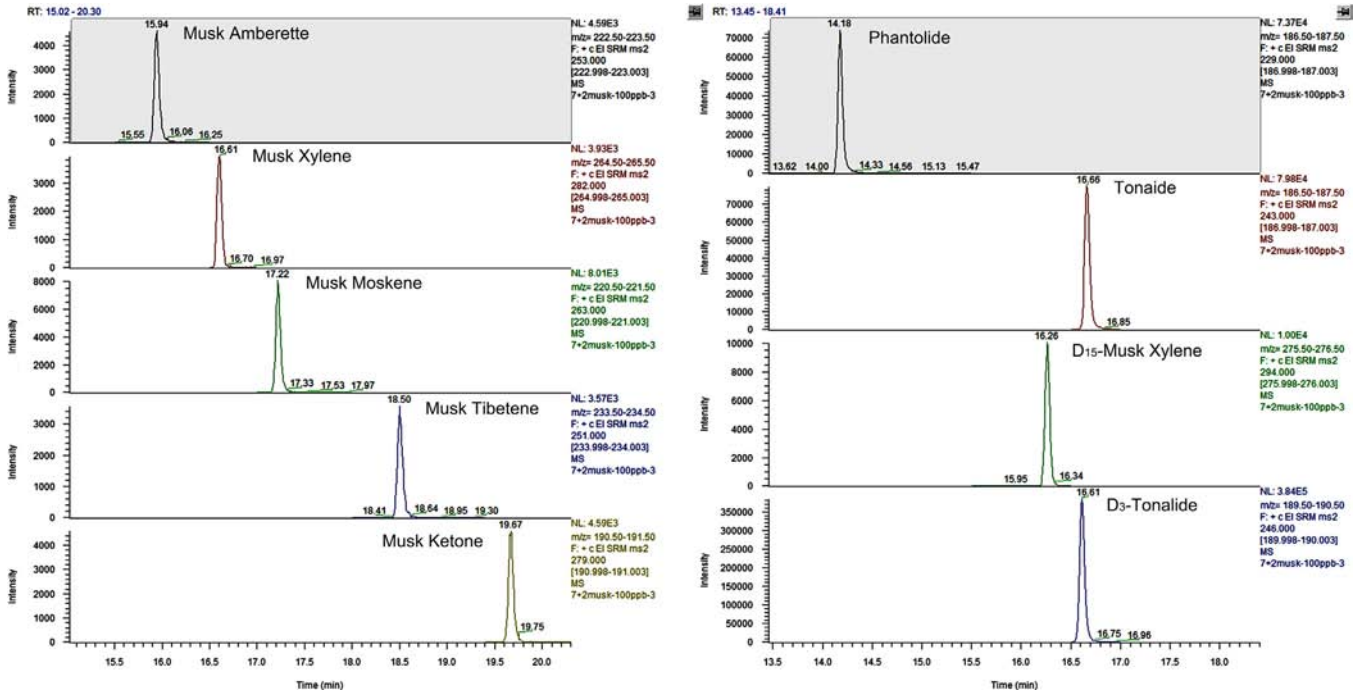

Fig. 6. GC–MS/MS chromatogram of a 100 ng mL^{-1} standard solution of 7 synthetic musks and 2 internal standards in isooctane.

Table 4
Analysis of cream samples (%w/w × 10^4)[b].

	Musk amberette	Musk xylene	Musk moskene	Musk tibetene	Musk ketone	Phantolide	Tonalide
BC[a]1[c]					0.0278	0.00231	0.00466
BC[a]2[c]					0.0117	< LOQ	0.00296
BC[a]3[c]					0.0137	< LOQ	0.00402
BC[a]4[c]					0.0157	0.00143	0.00266
NB[a]1[c]	0.00832	< LOQ			0.143		0.0514
NB[a]2[c]	< LOQ	< LOQ			0.0845	0.00695	0.00314
NB[a]3[c]	0.00881	0.564	0.00767	< LOQ	135	0.0322	0.0718
NB[a]4[c]	< LOQ	< LOQ	0.0915		0.163	0.00177	0.0272
NB[a]5[c]	0.0349	< LOQ	0.0117		0.0380	0.0108	0.0309
NB[a]6[c]	< LOQ	0.0416	0.00857		14.0	0.0115	0.0260
NB[a]7[c]	< LOQ	< LOQ			0.0905	0.0586	0.105
IB[a]1[c]					0.0131	0.00117	0.00351
IB[a]2[c]		< LOQ	< LOQ		0.0166	0.00107	0.00562
IB[a]3[c]	< LOQ	< LOQ	< LOQ		1.19	0.0143	7.14
IB[a]4[c]	< LOQ		0.0220		0.0193	0.00226	0.00432
IB[a]5[d]	< LOQ	< LOQ	0.0318		0.0696	0.0236	0.736
IB[a]6[d]		< LOQ	0.00591		0.0593		0.0198
IB[a]7[d]	< LOQ	< LOQ	< LOQ		0.173		0.0235
IB[a]8[d]	0.00839	< LOQ	< LOQ		0.0206		0.00948
IB[a]9[d]	< LOQ	< LOQ	0.0794		0.225	0.000804	0.0113
IB[a]10[d]		0.0180	0.110		0.274	0.000934	0.0156
IB[a]11[d]	< LOQ	< LOQ	0.0150		0.0228	0.0941	65.1
IB[a]12[d]		< LOQ			0.0225	0.000613	0.0214
IB[a]13[d]	< LOQ	0.0214	0.357		1.07	0.0110	0.257
IB[a]14[d]		< LOQ			0.00441		0.0110
IB[a]15[d]					0.00300	< LOQ	0.00339
IB[a]16[c]		< LOQ			0.0219		0.0277
IB[a]17[d]	0.00824	0.0879	0.287		0.799	0.00516	0.860

[a] BC: baby cream, NB: National brands and IB: International brands. Blank space: below LOD. Grey cells: forbidden compounds (EU no. 1223/2009).
[b] Equivalent to μg g^{-1}.
[c] Made in China.
[d] Made in the Country of origin.

the 7 synthetic musks ranged from 85.6 to 109%. The precision of the method was in the range 0.70–9.8% ($n=6$) described as the value of RSD. The LODs ($S/N \geq 3$) ranged from 0.15 to 4.86 ng g^{-1} and the LOQs ($S/N \geq 10$) ranged from 0.49 to 16.21 ng g^{-1}. This indicates that this method is sensitive and reliable.

3.5. Application to real samples

The proposed method was applied to the analysis of 28 cream samples including 4 kinds of baby cream and 24 kinds of adult cream, with the intention of demonstrating method adequacy for

the variety of the most common cream products. Results are shown in Table 4. Although their levels were low, musk ketone, tonalide and phantolide were found in all of the baby cream samples. All of the banned synthetic musks (musk amberette, musk moskene, and musk tibetene) and musk xylene were not found in all the baby cream samples. Regarding the adult cream, musk tibetene was found in only one sample (NB3) and the level was below LOQs (3.60 ng g^{-1} for musk tibetene). Musk amberette and musk moskene were found in 16 samples with a low levels (below 357 ng g^{-1} in Table 4). However, musk xylene was found in 21 samples and their concentrations were higher than LOQs (16.0 ng g^{-1} in Table 4) in only 5 samples (NB3, NB6, IB10, IB13, and S2). Although most of them were at low levels, tonalide and musk ketone were found in all adult cream samples. Phantolide was detected in 22 adult cream samples, but their concentrations were below 0.0941 μg g^{-1} (Table 4). The results indicated that the concentrations of all the detected 7 synthetic musks in 28 samples were below the MRL values established by the Regulation (EC) no. 1223/2009.

4. Conclusions

In this study, the method of SLE coupled with SPE has been successfully applied to extract and purify 7 synthetic musks from cream. Multivariate optimization was carried out using real cream samples and method quality parameters were also evaluated on cream samples. The results indicate that water can improve the efficiency of separation and purification of 7 synthetic musks from cream, and internal standards (IS), D_{15}-musk xylene and D_3-tonalide, are applied to eliminate the matrix effects. This pretreatment method combined with GC–MS/MS technology has been proved to be precise, accurate, and applicable to the routine analysis of 7 synthetic musks residues in cream samples. Hopefully, this paper will contribute to the simultaneous determination of synthetic musks in commercial cream, since synthetic musks are extensively used by the cosmetic industry and subjected to restriction according international regulation.

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References

[1] G.G. Rimkus, in: G.G. Rimkus (Ed.), The Handbook of Environmental Chemistry. Part X. Synthetic Musk Fragances in the Environment, 3, Springer-Verlag, Berlin, 2004, pp. 281–310.
[2] M. Gautschi, J.A. Bajgrowicz, P. Kraft, Chimia 55 (2001) 379–389.
[3] L.I. Osemwengie, S. Steinberg, J. Chromatogr. A 932 (2001) 107–118.
[4] M. Pólo, C. Garcia-Jares, M. Llompart, R. Cela, Anal. Bioanal. Chem. 388 (2007) 1789–1798.
[5] D.R. Dietrich, B.C. Hitzfeld, in: O. Hutzinger (Ed.), The Handbook of Environmental Chemistry, Springer, Berlin, Heidelberg, 2004, pp. 233–244.
[6] H. Schmeiser, R. Gminski, V. Mersch-Sundermann, Int. J. Hyg. Environ. Health 203 (2001) 293–299.
[7] M. Emig, A. Reinhardt, V. Mersch-Sundermann, Toxicol. Lett. 85 (1996) 151–156.
[8] V. Mersch-Sundermann, M. Emig, A. Reinhardt, Mutat. Res. 356 (1996) 237–245.
[9] A. Maekawa, Y. Matsushima, H. Onodera, M. Shibutani, H. Ogasawara, Y. Kodama, Y. Kurokawa, Y. Hayashi, Food Chem. Toxicol. 28 (1990) 581–586.
[10] V. Mersch-Sundermann, H. Schneider, C. Freywald, C. Jenter, W. Parzefall, S. Knasmüller, Mutat. Res. 495 (2001) 89–96.
[11] J.W. Tas, F. Balk, R.A. Ford, E.J. van de Plassche, Chemosphere 35 (1997) 2973–3002.
[12] Environment Canada, Canadian Environmental Protection Act (CEPA 1999), 1999.
[13] Regulation (EC) no. 1223/2009 of the European Parliament and of the Council of 30 November 2009 on Cosmetic Products (recast), Off. J. Eur. Union, L342/59, 2009.
[14] L. Vallecillos, E. Pocurull, F. Borrull, Talanta 99 (2012) 824–832.
[15] A.M. Peck, K.C. Hornbuckle, Environ. Sci. Technol. 38 (2004) 367–372.
[16] J.J. Yang, C.D. Metcalfe, J. Sci. Total Environ. 363 (2006) 149–165.
[17] X.L. Zhang, Y. Yao, X.Y. Zeng, G.R. Qian, M.H. Wu, G.Y. Sheng, J.M. Fu, Chemosphere 72 (2008) 1553–1558.
[18] A.M. Peck, E.K. Linebaugh, K.C. Hornbuckle, Environ. Sci. Technol. 40 (2006) 5629–5635.
[19] R. Kallenborn, R. Gatermann, S. Planting, G.G. Rimkus, M. Lund, M. Schlabanch, I.C. Burkow, J. Chromatogr. A. 846 (1999) 295–306.
[20] G.N. Wang, T. Hua, D.Z. Chen, J. Feng, L. Li, Chin. J. Chromatogr. 30 (2012) 135–140.
[21] L. Sanchez-Prado, M. Llompart, J.P. Lamas, C. García-Jares, M. Lores, Talanta 85 (2011) 370–379.
[22] P. Schmid, M. Kohler, E. Gujer, M. Zennegg, M. Lanfranchi, Chemosphere 67 (2007) S16–S21.
[23] J.L. Reiner, C.W. Wong, K.F. Arcaro, K. Kannan, Environ. Sci. Technol. 41 (2007) 3815–3820.
[24] T.A. Ternes, M. Bonerz, N. Herrmann, D. Löffler, E. Keller, B.B. Lacida, A.C. Alder, J. Chromatogr. A 1067 (2005) 213–223.
[25] F. David, P. Sandra, B. Tienpont, F. Vanwalleghem, M. Ikonomou., in: C.A. Staples (Ed.), The Handbook of Environmental Chemistry. Part Q. Phthalate Esters, 3, Springer-Verlag, Berlin, 2003, pp. 9–56.
[26] M.R. Hadjmohammadi, E. Ranjbari, Int. J. Environ. Anal. Chem. 92 (2012) 1312–1324.
[27] A.R.M. Silva, J.M.F. Nogueira, Anal. Bioanal. Chem. 396 (2010) 1853–1862.
[28] F. Kamarei, H. Ebrahimzadeh, Y. Yamini, Microchem. J. 99 (2011) 26–33.
[29] M. Lopez-Nogueroles, S. Lordel-Madeleine, A. Chisvert, A. Salvador, V. Pichon, Talanta 110 (2013) 128–134.
[30] H. Hayen, N. Jachmann, M. Vogel, U. Karst, Analyst 127 (2002) 1027–1030.
[31] H. Lautenschlager, Kosmetic Int. 11 (2004) 46–48.
[32] L. Pareja, V. Cesio, H. Heinzen, A.R. Fernández-Alba, Talanta 83 (2011) 1613–1622.
[33] P.J. Taylor, Clin. Biochem. 38 (2005) 328–334.
[34] X.Y. Zhen, J. Anal. Sci. 28 (2012) 364–368.
[35] Z. Wang, Chin. J. Chromatogr. 30 (2012) 1178–1182.
[36] C. Struppe, B. Schäfer, W. Engewald, Chromatography 45 (1997) 138–144.
[37] M. Llompart, M. Celeiro, J.P. Lamas, L. Sanchez-Prado, M. Lores, C. García-Jares, J. Chromatogr. A 1293 (2013) 10–19.
[38] K. Hara, S. Kashimura, Y. Hieda, M. Kageura, J. Anal. Toxicol. 21 (1997) 54–58.
[39] K. Matsubara, C. Maseda, Y. Fukui, Forensic Sci. Int. 26 (1984) 181–192.
[40] Z.L. Gong, K. Chandler, S. Webster, R. Kerley, S. Buist, M. McCorttipton, Talanta 91 (2012) 77–82.
[41] H. Hoja, P. Marquet, B. Verneuil, H. Lotfi, J.L. Dupuy, G. Lachatre, J. Chromatogr. B 692 (1997) 329–335.
[42] B. Kanrar, S. Mandal, A. Bhattacharyya, J. Chromatogr. A 1217 (2010) 1926–1933.
[43] R. King, R. Bonfiglio, C. Fernandez-Metzler, C. Miller-Stein, T. Olah, J. Am. Soc. Mass Spectrom. 11 (2000) 942–950.
[44] J. Xing, C.F. Xie, H.X. Lou, J. Pharm. Biomed. Anal. 44 (2007) 368–378.
[45] N.B. Cech, C.G. Enke, Anal. Chem. 72 (2000) 2717–2723.
[46] G. Häubl, F. Berthiller, R. Krska, R. Schuhmacher, Anal. Bioanal. Chem. 384 (2006) 692–696.