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Ultrasound-assisted low-density solvent dispersive liquid–liquid extraction for the determination of alkanolamines and alkylamines in cosmetics with ion chromatography

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ABSTRACT

A new one-step sample preparation technique termed ultrasound-assisted low-density solvent dispersive liquid–liquid extraction (UA-LDS-DLLE) coupled with ion chromatography (IC) was developed for the determination of three alkanolamines and two alkylamines in complex samples. Sample matrices were rapidly dissolved and dispersed to form cloudy solutions by using two solvents, where target analytes were transferred into acid solutions, while liposoluble substances were dissolved in cyclohexane. The obtained extracts could be used directly for injection analysis without any additional purification because the potential matrix interferences had been effectively eliminated in extraction process. The extraction efficiency could be markedly enhanced and the extraction could be quickly accomplished within 13 min under the synergistic effects of ultrasound radiation, vibration and heating. Various parameters influencing extraction efficiency were evaluated using orthogonal array experimental design. The extraction performance of the approach was demonstrated for the determination of target analytes in 15 commercial cosmetics covering very different matrices. Linearity ranges of 0.3–50 mg L^{-1} and limits of detection varying from 0.072 to 0.12 mg L^{-1} were achieved. The recoveries ranged from 86.9–108.5% with the relative standard deviations (RSDs) of 1.2–6.2%. The method was proved to be a simple and effective extraction technique that provided an attractive alternative to the analysis of trace amounts of target analytes in large numbers of cosmetics.

1. Introduction

Alkanolamines are widely used as detergents, thickeners, alkalizing agents and emulsifiers in cosmetic products. But the super-scale uses of alkanolamines in cosmetics could cause potential health risks [1]. Alkylamines maybe present as contaminants in cosmetics resulting from the decarboxylation of amino acids or the use of the impure chemical raw materials. The volatile amines such as dimethylamine (DMA) and diethylamine (DIEA) emit pungent smells that are hazardous to human health [2]. DMA can also react with nitrosation agents to form carcinogenic dimethylnitrosamine compounds [3]. According to the current European and Chinese cosmetic regulations, alkanolamines including monoethanolamine (MEA) and triethanolamine (TEA) are restricted ingredients, whose maximum allowable concentrations in rinse-off products are 0.5% (w/w) and 2.5% (w/w). The total concentrations of diethanolamine (DEA) and TEA in leave-on formulations should not exceed 5% (w/w), while dimethylamine (DMA) and diethylamine (DIEA) are prohibited for use in cosmetic formulations [4,5]. The simultaneous determination of various organic compounds still remains a major challenge because of the presence of a lot of organic substances and some inorganic salts in cosmetics that may interfere with the determination.

Sample preparation is a critical step in the overall scheme of analysis, which has direct influences on the accuracy, precision of results and detection limit of method, and also it often is the most time-consuming step of the analytical process [6]. Some conventional extraction techniques such as solvent extraction [7,8], liquid–liquid extraction (LLE) [9–11] and solid phase extraction (SPE) [12–15], have been widely used for isolating the target analytes from various matrices. The former two approaches require the use of large amounts of the high-purity organic solvents and multiple clean-up steps, which are considered to be expensive and time-consuming. The latter method also requires reletively large volumes of toxic organic solvents for purification of coated fiber and elution of target analytes, which are hazardous to the operator and unfriendly to the environment [16]. As a consequence, a variety of sample preparation techniques have

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been developed to overcome the shortcomings of these classical methods by means of reducing or even avoiding the use of organic solvents [17]. One of the most efficient procedures is the development of simplified and miniaturized SPE- and LLE-based techniques such as headspace solid phase microextraction (HS-SPME) [18], solid phase microextraction (SPME) [16,19] and dispersive liquid–liquid microextraction (DLLME) [20,21], which can considerably reduce organic solvent consumption and achieve high enrichment factors for target analytes. In addition, automation of SPME requires only slight modification of a normal gas chromatographic autosampler [22]. However, the coated fibers are generally expensive and have the limited lifetimes for some applications due to the influence of the addition of salts with supersaturation or complex matrix.

DLLME was firstly introduced by Assadi and co-workers in 2006 [23], which is an improved LLE method based on the use of microliter volumes of extraction solvent. Its applications in various matrices such as cosmetics [9], fruits and vegetables [24], food and environmental samples [25–27] have been widely reviewed. However, DLLME usually suffers from two obvious drawbacks. Firstly, it generally requires high-density solvents such as chloroform, carbon tetrachloride, tetrachloroethane or chlorobenzene, which are highly toxic and environmentally unfriendly, and may limit its applicability. For these halogenated hydrocarbons, their GC peaks partially overlap with those of some analytes. Secondly, there is a lack of compatibility between the extraction solvents and detecting instruments such as reverse-phase HPLC [25] and IC [28]. Lighter-than-water organic solvents [20,26] and ionic liquids [29] are lately introduced as extraction solvents to overcome these inherent limitations. The performance of DLLME in the extraction of organic compounds from simple matrices like aqueous samples has proved to be excellent, but it is not yet perfect in complex matrices including cosmetic samples. Therefore, it needs further improvement.

The ultrasound radiation is a powerful tool to facilitate emulsification and homogenization, which provides an efficient contact between sample matrix and extractant, accelerates the mass transfer between two immiscible phases in the extraction process, leading to enhancement of extraction efficiency with a minimum equilibrium time [30]. A detailed application of the UAE technique to the environmental and food samples has been published specifically [31]. The solvent extraction-based methods including LLE, dispersive liquid–liquid extraction (DLLE), and DLLME, etc., can easily be modified or combined with other sample preparation techniques for particular purposes. In this way, ultrasound-assisted dispersive liquid–liquid extraction (UADLLE) [29], ultrasound-assisted matrix solid-phase dispersive liquid extraction (UAMSPDLE) [32] and dispersive derivatization liquid–liquid extraction (DDLLE) [33] have been developed as good alternatives to conventional LLE. An ultrasound-assisted emulsification microextraction (UAEME) enjoying the performance advantages of both UAE and DLLME has been applied to the determination of triclosan [34], phthalate ester [35], formaldehyde [36] and nitrite [37] in cosmetics. However, large amounts of organic compounds in cosmetics can also be co-extracted along with the target analytes that will cause the serious matrix interferences. Moreover, centrifugation of large-volume samples is too difficult to carry out. Therefore, these modified DLLE and DLLME approaches are still unsuitable for the direct detection of alkanolamines and alkylamines in cosmetics, and additional clean-up procedures are generally needed. Considering the characteristic ingredients of cosmetics, the dominant organic compounds can be efficiently removed by using an appropriate organic solvent while the target analytes are still remained in the acid solutions. It can significantly simplify the operation step and obtain extracts clean enough for direct injection. Thus the development of a simple, rapid and high selectivity DLLE procedure combining extraction and cleanup in one single step is of great significance.

In this study, a novel one-step sample preparation technique called ultrasound-assisted low-density solvent dispersive liquid–liquid extraction (UA-LDS-DLLE) was developed. During the extraction process, sample matrices were rapidly dissolved and dispersed to form cloudy solution by using two solvents where target analytes were transferred into acid solutions and liposoluble substances could be completely dissolved in cyclohexane. The whole procedure was performed on the synergistic effects of ultrasound radiation, heating and vibration, which could greatly improve extraction efficiency and accelerate the extraction. The approach achieved the following two improvements for conventional DLLE. One was the simplification of extraction process through integrating extraction and cleanup into one single step, which could effectively eliminate the matrix interferences from complex matrices without any further cleanup. Another was it considerably reduced the consumption of organic solvent and operating time under the synergistic effects. A cation-exchange column was adopted for the effective separation of co-existing compounds prior to IC detection. To demonstrate the feasibility of the developed approach, UA-LDS-DLLE was applied to the assays of alkanolamines and alkylamines in commercially available cosmetics.

2. Experimental

2.1. Instrumentations

UA-LDS-DLLE experiments were carried out on a Branson 2510 ultrasonic cleaner (130 W, 42 kHz, Branson Ultrasonic Corporation, Danbury, USA), a Vortex-genie 2 vibrator (Scientific Industries INC, New York, USA) and a constant temperature water bath (Chang An Scientific Instrument Co. Ltd., Beijing, China). IC analysis was performed on an ICS-2500 ion chromatography system (Dionex, Sunnyvale, CA, USA) equipped with a GP 50 high performance quaternary gradient pump with an automated vacuum degassing system, an ED 50 electrochemical detector, a LC 30 chromatography oven and an AS 50 auto-sampler with a 25 μL sample loop.

The chromatographic separation of analytes was performed using an Ion Pac SCS 1 (250 mm × 4.0 mm i.d.) analytical column fitted with an Ion Pac SCG 1 (50 mm × 4.0 mm i.d.) guard column (Dionex, Sunnyvale, CA, USA), which was eluted with 2.5 mM MSA in 5% (v/v) acetonitrile solution at a flow rate of 0.70 mL min^{-1} under isocratic conditions. The separations and non-suppressed conductivity detections were carried out at room temperature in a chromatography oven. Quantification of target analytes was performed by the integration of the peak areas using an external standardization method.

2.2. Reagents and materials

All reagents used were of high purity analytical grade or HPLC grade and the deionized water (specific resistivity 18.1 MΩ cm^{-1}) was produced by a Millipore Milli-Q water purification system (Bedford, MA, USA). Methanesulfonic acid (MSA) and acetonitrile (ACN) were obtained from Acros Organic (Geel, Belgium). MEA (≥99%), DEA (≥99%), TEA (≥99%), DMA (≥33%) and DIEA (≥99%) were purchased from Guangzhou Chemical Reagent Factory (Guangzhou, China). Cyclohexane and ether were bought from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

Individual stock standard solutions of each analyte at a concentration of 2000 mg L^{-1} were prepared by exact weighing of each compound and diluting with acetonitrile. These solutions were stored in a refrigerator at 4 °C. The accurate concentration of

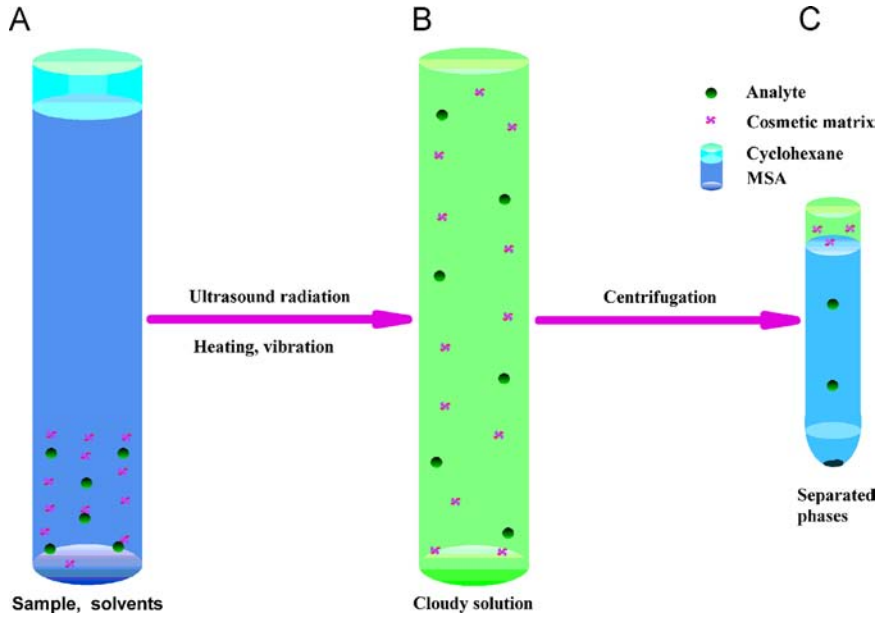

Fig. 1. Schematic description of the UA-LDS-DLLE: (A) before extraction, (B) during extraction and (C) after extraction.

DMA in solution was standardized by titration with hydrochloric acid titrant using phenolphthalein solution as indicator prior to use, while the titrant was standardized with the highest grade sodium carbonate. A series of mixed working solutions containing all analytes were prepared daily by appropriate dilution of each stock standard solution with mobile phase.

2.3. UA-LDS-DLLE system setup

The UA-LDS-DLLE system was designed and illustrated in Fig. 1. Before the extraction, the samples, 2.5 mM MSA (extraction solvent) and cyclohexane (disperser solvent), were added into the conical tube (10 mL extraction vessel) (Fig. 1A). During the extraction process, sample matrices were dispersed into extraction solutions so that cloudy solutions were formed, facilitating mass transfer of target analytes into acid solutions and lipid-soluble substances dissolution in cyclohexane (Fig. 1B). After an aliquot of the cloudy solutions (about 2 mL) was centrifuged, the water-immiscible fat and lipid were accumulated at the top of the aqueous phase while the indiscerptible solid were sedimented at the bottom of the centrifugation tube (Fig. 1C).

2.4. Sample preparation

Fifteen commercially available cosmetics including moisturizing creams (2), sunscreen creams (3), day creams (2), clean lotions (3), shampoos (2) and cosmetic powders (3) were obtained from commercial suppliers in Guangzhou, China. Several blank cosmetics samples were spiked with known concentrations of each analyte. After that, these spiked samples were homogenized thoroughly in a blender at high speed for 30 min and then kept in a refrigerator at 4 °C for 24 h to allow interactions between analytes and matrices perfectly. Subsequently each spiked sample was split into ten independent samples for method optimization and validation.

About 0.25 g of cosmetics samples and spiked samples were accurately weighed into 10 mL screw cap glass conical tubes and taken to volume with 2.5 mM MSA, followed by adding 1.0 mL of cyclohexane. These conical tubes were submitted to heating in an ultrasonic bath at 70 °C for 2 min, and the mixtures were then dispersed vigorously on a vibrator for 1 min and a further sonication step was performed under 130 W ultrasonic irradiation powers with a frequency of 42 kHz for 10 min. Subsequently, aliquots of the cloudy solutions were centrifugated at 14,000 rpm for 3 min. The upper organic phases of aqueous samples were removed with syringes and the lower aqueous phases were passed through 0.45 μm filters. Twenty five microlitre of the clear filtrates were injected into ion chromatography system.

2.5. Conventional dispersion liquid–liquid extraction (DLLE)

About 0.25 g of spiked samples were accurately weighed into 10 mL screw cap glass conical tubes and taken to volume with acetonitrile–water (v:v, 1:1). These mixtures were then dispersed vigorously for 2 min and an additional sonication step was performed for 10 min. An aliquot of a cloudy solution was centrifugated at 14,000 rpm for 3 min and 25 μL of the clear filtrates were analyzed by IC. The conditions used here were most favorable for extraction.

3. Results and discussion

3.1. Development of UA-LDS-DLLE procedure

It is well-known that ultrasound energy could cause cavitation bubbles resulting in higher temperatures and pressures in the cavitation zone, which generated physical disruption of sample matrix including mechanical erosion of solids and colloid particles rupture. The solvent would easily penetrate into the interior of substances, which facilitate mass transfer of analytes from matrix into the solvent, usually resulting to a good yield [38,39]. Heating would cause a dramatic volume expansion with subsequent structure rupture of substances, releasing the analytes into the extraction solvent. The vortex agitation was used to swirl the fluids and create a vortex, which could enable the breakup of organic phase into fine droplets and the formation of a cloudy state, resulted to the rapid extraction of analyte in aqueous sample. The extraction equilibrium could be attained within 2 min agitation time applied when the rotation speed was greater than 1500 rpm [40]. To test the performance of the synergistic effect, UA-LDS-DLLE and acetonitrile–water dispersive liquid–liquid extraction

Table 1
Experimental conditions and extraction recoveries of spiked sample extracted with an orthogonal array experimental designs L_9 (3^4) ($n=3$).

No.	Factor				Extraction recovery (mean ± SD)[a]				
	A[b] (g)	B[b] (mL)[b]	C[b] (°C)	D[b] (min)	MEA	DEA	DMA	TEA	DIEA
1	0.5	5	26	10	82.8 ± 2.1	91.2 ± 2.8	96.9 ± 3.8	81.9 ± 1.4	81.5 ± 4.5
2	0.5	10	40	30	87.1 ± 1.3	92.0 ± 6.2	86.3 ± 4.7	71.9 ± 3.8	95.8 ± 6.0
3	0.5	25	70	60	104.9 ± 2.0	101.7 ± 0.67	93.0 ± 3.7	97.0 ± 4.5	94.9 ± 2.5
4	1.0	5	40	60	88.0 ± 0.60	88.6 ± 3.1	83.1 ± 6.1	77.7 ± 5.6	74.7 ± 3.9
5	1.0	10	70	10	101.5 ± 1.3	101.2 ± 2.3	95.0 ± 4.8	91.6 ± 4.5	82.4 ± 3.0
6	1.0	25	26	30	93.6 ± 2.3	93.2 ± 1.8	95.5 ± 6.1	78.9 ± 0.8	81.9 ± 4.1
7	2.0	5	70	30	93.2 ± 1.2	95.9 ± 2.0	89.1 ± 3.5	95.4 ± 1.9	77.1 ± 1.6
8	2.0	10	26	60	88.0 ± 0.60	88.6 ± 3.1	83.1 ± 6.1	77.7 ± 5.6	74.7 ± 3.9
9	2.0	25	40	10	90.8 ± 1.6	93.9 ± 3.8	89.7 ± 4.6	89.9 ± 5.5	74.9 ± 3.3

[a] Extraction recovery (%) was the mean recovery of three independent experiments of samples spiked with 100 mg kg^{-1} for each analyte.
[b] A: Sample quantity (g); B: acid solution volume (mL); C: extraction temperature (°C); D: extraction time (min).

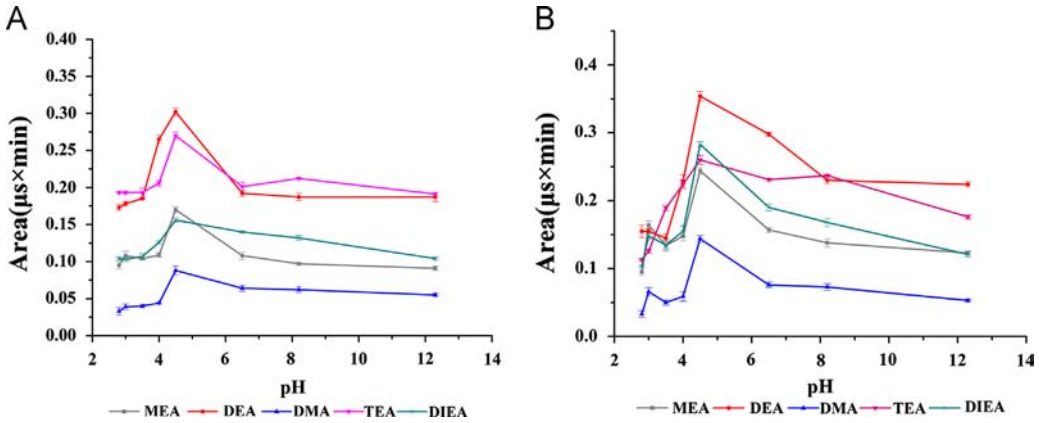

Fig. 2. Optimization of pH required for UA-LDS-DLLE keeping all other experimental conditions constant, including (A) the skin-whitening cream extract spiked with 5.00 mg L^{-1} for each analyte and (B) the clean lotion extract spiked 5.00 mg L^{-1} for each analyte. The pH values were varied from 2.8 to 12.3.

(DLLE) were comparatively studied in this study. The mean recoveries of UA-LDS-DLLE and DLLE at 100 mg kg^{-1} for each analyte were in the range of 86.1–104.5% and 65.1–80.5% with RSD_S ($n=3$) less than 5.6% and 7.1%. So a synergistic effect is more powerful than a single energy. In addition, the extraction selectivity could be adjusted by choosing a preferential solvent, controlling the concentration or pH of acid solution and the suitable ratio of sample quantity to organic solvent volume.

3.1.1. *Extraction solvent*

Alkanolamines and alkylamines can be easily dissolved in acid solution while fat and lipoid substances have a high affinity for non-polar solvents. Moreover, these amines are present as cationic forms in acid solution and cannot be extracted into non-polar solvents. In this case, two solvents (extraction and disperser solvents) were simultaneously employed for the extraction of target analytes and the elimination of interfering components, which were beneficial for the attainment of extracts clean enough and high extraction efficiency. Both clean extracts and dilute acid solutions help to subsequent chromatographic separation and accurate measurement of analytes. Various diluted acid solutions, such as MSA, sulfuric acid, hydrochloric acid and acetic acid solutions could be used as extraction solvents, but MSA was more suitable than others owing to the full compatibility with mobile phase employed. The effect of pH on the extraction was evaluated in the range from 2.8 to 12.3 by adding appropriate volumes of 0.1 M MSA or 0.1 M lithium hydroxide to the spiked skin-whitening cream and clean lotion sample solutions. Fig. 2A and B indicated that the peak areas of all the analytes increased with pH value up to 4.5, and after that, considerable decreases of the extraction happened when the pH value varied between 4.5 and 12.3, leading to the marked reductions in peak areas at a higher pH. This was probably because the analytes partially existed as their neutral molecule forms in alkalic solutions, which led to a high dissolution in organic phase but not favorable for migration into extraction solvent. The extraction efficiencies using 2.5 mM MSA (the same concentration as mobile phase) as the extraction solvent were further tested in triplicate by detecting recoveries of the sunscreen creams spiked with 100.0 mg kg^{-1} of each alkanolamine and alkylamine. The satisfactory average recoveries of the five target analytes varying from 89.1% to 102.0% with RSDs between 0.30% and 2.9% were obtained. Finally 2.5 mM MSA was selected as the most suitable extraction solvent for subsequent experiments.

3.1.2. *Disperser solvent*

In typical DLLE, the disperser solvents should possess intermediate polarity, good miscibility in extraction solvent and aqueous phase, and could enable the extraction solvent to be dispersed as fine particles in aqueous phase. In this study, non-polar solvent was used as disperser. In extraction process, due to solvent being highly dispersed in the aqueous phase, the contact surface area between solvent and sample solution became essentially infinitely large so that could shorten the extraction time. Moreover, the liposoluble substances could be completely dissolved in disperser solvent based on the principle of the dissolution in the similar material structure. Therefore, disperser solvent

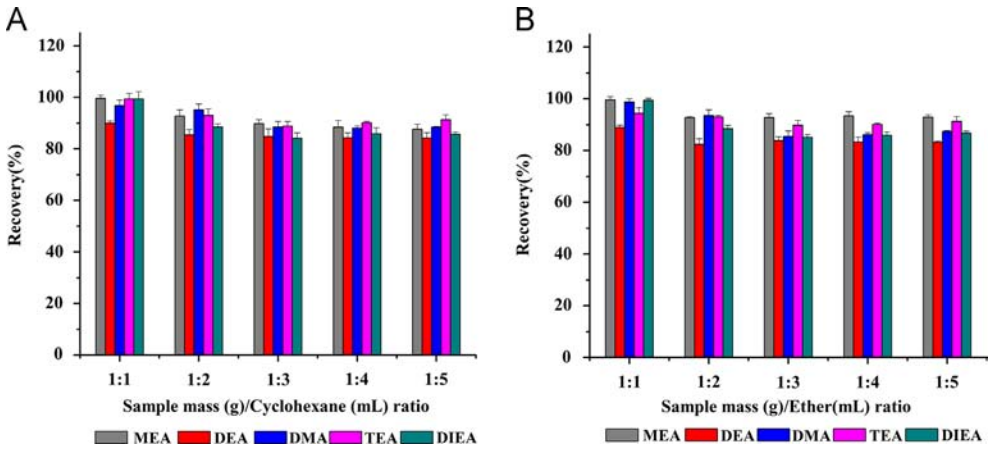

Fig. 3. Optimization of disperser solvent volume for sample preparation of sunscreen cream spiked with 100.0 mg kg^{-1} for each analyte keeping all other experimental conditions constant, including (A) cyclohexane and (B) ether. The ratio of sample quantity to disperser solvent varied from 1:1 to 1:5 (g: mL).

could directly affect the dispersion degree of sample in extraction solvents and the purification of extracts, and finally influenced the extraction efficiency of analytes. Various organic solvents have been widely used for disperser solvent, but the low-toxicity and low-density solvents including cyclohexane and ether should be preferentially selected to acquire best extraction efficiency. The optimization of the ratio of sample mass (g) to disperser solvent volume (mL) was performed by adding different amounts of cyclohexane and ether to the spiked sunscreen cream at proportions ranging from 1:1 to 1:5 at five levels, respectively. As seen in Fig. 3A and B, mean recoveries for all the analytes slightly declined with increasing the ratio and reached the relative equilibrium values in the range of 83.2–93.4%, after that any further changes did not affect the UA-LDS-DLLE. The mean recoveries using cyclohexane as disperser solvent were better than those obtained with ether. The most probable reason was that all these analytes were slightly soluble in cyclohexane and ether, but their amounts dissolved in the latter were larger than those in the former. The cleanup capability of cyclohexane was further investigated by comparing with the cosmetic sample extracted by acetonitrile–water extraction-based DLLE (Fig. S1A of the supporting information), the extracts obtained by UA-LDS-DLLE were much more clear (Fig. S1B). It was revealed that the use of cyclohexane could specifically purify the extracts. Taking into account the use of relatively large volumes of disperser solvent could obtain the more clean extracts, a sample quantity and disperser solvent volume ratio of 1:4 was selected, and 1.0 mL cyclohexane was used for all further experiments.

3.1.3. *Optimization of extraction conditions by an orthogonal array experimental design*

Apart from above two critical factors, the remaining variables that could potentially affect extraction efficiency included sample quantity, acid solution volume, extraction temperature and extraction time. These four variables were systematically investigated by means of an orthogonal array experimental design L_9 (3^4) to optimize the operation conditions. All the experiments were conducted in triplicate by detecting the above spiked cosmetic samples. The level set values of four factors (A, B, C and D) and the mean recoveries were shown in Table 1. Numerical analysis of the experimental values led to the ANOVA results listed in Table 2. The *R* factor was used for evaluating the influential variable. The variable with the largest value of *R* was the most important factor that influenced extraction efficiency. Compared with all the *R* values, extraction temperature (C) was the significant factor for four analytes including MEA, DEA, DMA and TEA, while sample quantity (A) was only significant factor for one analyte (DIEA). These two variables appeared to have a positive and a negative effect on the extraction efficiency. The other two variables (B and D) had less influence on recovery. The further analysis of variance of recoveries indicated that extraction temperature had statistically significant influence on extraction efficiency ($F=3.412$ lager than critical value $F_{0.05}$ 3.110) while the other three variables (A, B and D) had not marked influences (*F*-value less than 3.110). This is due to the fact that cosmetic sample matrices were complex colloids and had the worse diffusion ability in solution, and a high extraction temperature could decrease viscosity of cosmetic solution and enhance velocity of matrix diffusion and mass transfer of analytes, which led to the improvement of extraction efficiency in short equilibrium time. According to the largest donating rule, the largest value of *K* which affects the extraction efficiency should be the selected value. In Table 2, the K_1–K_3 values were average recoveries at three levels of the studied variables, respectively. Considering time saving, the most favorable extraction condition was established as follows: extraction solvent, 10 mL of 2.5 mM MSA; extraction time 10 min; and extraction temperature 70 °C. It indicated that the extraction efficiency could be enhanced by using

Table 2
Analysis of L_9 (3^4) test results.

Analyte	Factor[a]	K_1[b]	K_2	K_3	R[c]
MEA	A	91.6	94.4	94.3	2.8
	B	88.0	95.8	96.4	8.4
	C	91.8	88.6	99.9	11.2
	D	91.7	91.3	97.3	6.0
DEA	A	95.0	94.3	93.9	1.1
	B	91.9	95.0	96.3	4.4
	C	92.1	91.5	99.6	8.1
	D	95.4	93.7	94.1	1.7
DMA	A	92.1	91.2	90.6	1.5
	B	89.7	91.4	92.7	3.0
	C	95.1	86.4	92.4	8.7
	D	93.9	90.3	89.7	4.2
TEA	A	83.6	82.7	93.2	10.5
	B	85.0	85.9	88.6	3.6
	C	85.0	79.8	94.7	14.8
	D	87.8	82.1	89.7	7.6
DIEA	A	90.7	79.7	77.8	12.9
	B	77.8	86.6	83.9	8.8
	C	81.6	81.8	84.8	3.2
	D	79.6	84.9	83.7	5.3

[a] For key to factors, the same as Table 1.
[b] $K_i^F=(1/3)$ the extraction recovery of analyte at F_i.
[c] $R_i^F=\max\{K_i^F\}-\min\{K_i^F\}$, here *F* and *i* meant extraction factor and setting level, respectively.

the multifactorial combination at different levels in sample treatment.

3.2. Interference trial

Cosmetics commonly contain some inorganic salts such as alkali metals, alkaline earth compounds, which may interfere with the determination of analytes. Results indicated that great majority of compounds did not interfere with quantification of the target analytes owing to the good chromatographic separation. But the potassium ion peak partly overlapped with that of DEA so that the accuracy of analytical result of DEA could not be assured at the high concentrations of potassium (larger than 10 mg L^{-1}).

3.3. Optimization of chromatographic conditions

Our previous studies [41,42] have demonstrated the applications of cation-exchange columns involving a carboxylic acid polymeric stationary phase (CS12) and a weak carboxylic acid functionalized column (CS17) for the separation of alkylamines. However, because of the highly strong hydrophobic interactions of analytes with the stationary phase, chromatographic separations on a CS12 column required to apply a high concentration acid or salt gradient in the mobile phase that is normally modified with some organic solvents to elute the strongly retained cations, while CS17 column needed to utilize a relatively low concentration of MSA gradient elution to achieve good peak resolution and retention time reproducibility. To simplify the operation and improve the peak efficiency, a low capacity weak cation exchanger functionalized with carboxylic acid groups (SCS 1) was employed for the fast separation of alkanolamines and alkylamines under isocratic conditions. Acetonitrile as an effective organic modifier could reduce hydrophobic retentions and improve unsymmetrical peak shapes of the target analytes. The most appropriate percentage of acetonitrile in mobile phase was studied at seven levels varying from 2% to 10% (v/v). Results revealed that the use of an isocratic mobile phase consisting of 2.5 mM MSA and 5% (v/v) of acetonitrile resulted to high peak resolution factors for all the analytes (always larger than 1.2), Therefore, 2.5 mM MSA and 5% (v/v) of acetonitrile was used as mobile phase for the following experiments.

3.4. Analytical figures of merit of the proposed method

To evaluate the performances of the proposed method, calibration linearity range, correlation coefficients (r), limits of detection (LODs) and quantification (LOQs), and precision were investigated. The results were shown in Table 3. The calibration curves, which related the concentrations to the peak areas, were tested over a wide range of 0.3–50 mg L^{-1} (including six concentration levels). The correlation coefficients of five analytes using a least squares linear regression fit ranged from 0.9995 to 0.9998. LODs and LOQs were calculated as 3 and 10 times the standard deviation (SD) values that acquired from analyzing 11 blank skin cream extracts fortified with 0.3 mg L^{-1} for each analyte.

Potential matrix effects were studied by comparison of the slopes of the calibration curves obtained with the standard addition method and external calibration method. The calculated calibration parameters and their values were listed in Table S1. Percentage deviations between slope values were in the range of 0–4.9% in all cases. Hence, matrix components were not expected to interfere with the determination of analytes, and the external calibration with aqueous standards could be done.

The repeatability of the instrument (expressed as RSD) was measured by carrying out seven independent determinations for each multi-component standard solution at low and high concentrations. Also, the reproducibility of the method was evaluated by analyzing two group of skin cream extracts (seven independent samples for each group) spiked at two levels of all the analytes within a day (intra-day variation) and in seven non-consecutive days (inter-day variation). The precision (RSD values) varied from 0.70% to 3.6% for instrument, from 1.4% to 5.1% for the intra-day and from 1.8% to 6.1% for the inter-day variations (Table 3).

3.5. Comparison of analytical characteristics of the proposed method with traditional LLE

In order to compare the extraction efficiencies of the UA-LSD-DLLE and the LLE [10], a statistical test was carried out. The individual average values of five analytes in ten same spiked samples were assayed in order to evaluate if both sample pretreatment procedures yield similar results according to the Student's t-test. As seen in Table 4, the experimental t-values for all the analytes were always below the critical t-value 2.101(at the 95% confidence level), indicating that there were no statistically significant differences between the results obtained from the two approaches. Both methods exhibited excellent extraction efficiencies, whereas the proposed method required far less solvent, shorter extraction time and simpler operation step than the conventional LLE. In addition, it could effectively eliminate matrix interferences owing to the simultaneous use of the extraction and disperser solvents.

3.6. Application to commercial cosmetic products

To validate the feasibility of the current method, the UA-LDS-DLLE combined with IC was applied to the simultaneous determination of alkanolamines and alkylamines in 15 commercial cosmetic products. All the extracts were directly analyzed without any further concentration or purification. The results were presented in Table S2. The values ranged from 20.3 mg kg^{-1} for DEA to 1.88×10^3 mg kg^{-1} for TEA, and the contents of MEA, DEA and TEA in five cosmetics were less than the maximum authorized concentration. Among these analyzed cosmetics, three of which contained low levels of DIEA, this may be due to the usage of impure raw materials. In addition, DMA concentration in all cosmetics was always below the detection limit. Three types of

Table 3
Analytical performance of proposed method for the determination of five analytes.

Analyte	Regression equation	r	LOD	LOQ	Repeatability (RSD%, $n=7$)		Intra-day variation (RSD%, $n=7$)		Inter-day variation (RSD%, $n=7$)	
			(mg L^{-1})	(mg L^{-1})	1.00 (mg L^{-1})	50.0 (mg L^{-1})	1.00 (mg L^{-1})	50.0 (mg L^{-1})	1.00 (mg L^{-1})	50.0 (mg L^{-1})
MEA	$y=0.2250x-0.1059$	0.9997	0.084	0.28	0.72	0.70	2.1	1.4	6.1	2.5
DEA	$y=0.1347x+0.0385$	0.9996	0.081	0.27	1.0	1.2	5.1	2.0	5.6	1.8
DMA	$y=0.2431x-0.0096$	0.9997	0.090	0.30	2.1	2.2	4.5	4.1	4.4	3.2
TEA	$y=0.0838x-0.1208$	0.9995	0.12	0.42	3.6	2.3	4.3	2.4	4.2	2.6
DIEA	$y=0.1721x-0.0719$	0.9998	0.072	0.24	1.7	1.3	3.6	2.3	4.5	2.6

Table 4
Comparison of the proposed method with traditional LLE.

Sample preparation	Statistical parameter	MEA	DEA	DMA	TEA	DIEA
Proposed method	Mean ± SD[a]	89.76 ± 4.75	95.52 ± 4.29	93.03 ± 5.14	102.8 ± 8.38	99.66 ± 5.44
	Error (%)	10.2	4.5	7.0	2.8	0.34
LLE	Mean ± SD	89.10 ± 1.61	99.89 ± 6.59	95.51 ± 6.85	105.7 ± 2.73	102.9 ± 1.97
	Error (%)	10.9	0.11	4.5	5.7	2.9
	t-Value[b]	0.420	1.756	0.916	1.065	1.793

[a] Mean values of 10 independent experiments of samples spiked with 100.0 mg kg^{-1} for each analyte.
[b] The critical value $t_{0.05,18}=2.101$.

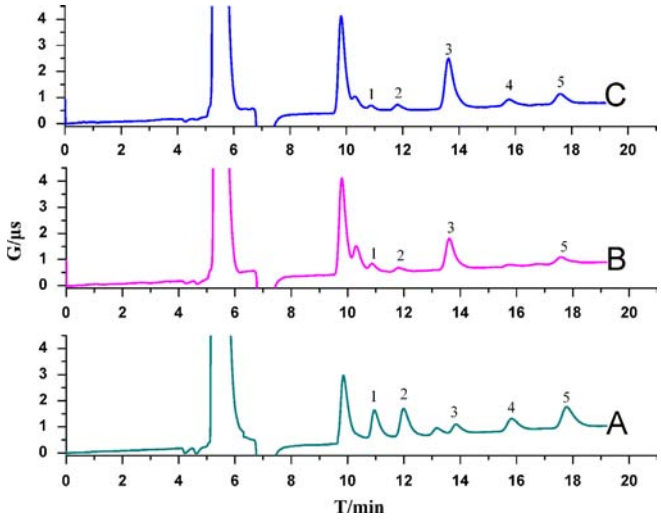

Fig. 4. Typical IC chromatograms of (A) a multi-component standard solution, (B) a representative sunscreen cream and (C) the same product spiked with five analytes after UA-LDS-DLLE. For experimental conditions see text. Peaks: 1, MEA (A, 5.00 mg L^{-1}; B, 0.81 mg L^{-1}; C, 1.22 mg L^{-1}); 2, DMA (A, 5.00 mg L^{-1}; B, 0.85 mg L^{-1}; C, 1.45 mg L^{-1}); 3, TEA (A, 1.50 mg L^{-1}; B, 3.52 mg L^{-1}; C, 7.84 mg L^{-1}); 4, DEA (A, 5.00 mg L^{-1}; C, 1.98 mg L^{-1}); 5, DIEA (A, 5.00 mg L^{-1}; B, 0.45 mg L^{-1}; C, 1.80 mg L^{-1}).

Table 5
Recovery of the five compounds from samples ($n=6$).

Analyte	Spiked	Sunscreen cream		Clean lotion		Cosmetic powder	
	(mg kg^{-1})	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
MEA	100.0	97.1	3.4	96.8	2.6	98.9	3.5
	1000.0	104.9	4.0	98.6	2.6	96.6	4.6
DEA	100.0	97.7	1.8	99.8	6.2	108.5	3.5
	1000.0	99.7	1.2	94.7	3.7	98.8	2.2
DMA	100.0	89.1	2.6	95.4	4.8	96.9	5.9
	1000.0	103.4	4.2	99.4	5.1	98.9	2.2
TEA	100.0	86.9	3.0	98.6	5.6	97.6	4.2
	1000.0	88.8	2.3	99.4	2.8	101.0	2.5
DIEA	100.0	98.4	4.1	97.5	3.3	101.6	4.0
	1000.0	104.8	3.1	95.8	2.2	94.6	3.1

cosmetics samples spiked at two concentration levels (100.0 mg kg^{-1} and 1000 mg kg^{-1} for each analyte) were analyzed independently in order to spread the range of matrices evaluated. Table 5 demonstrated that the average recoveries of five analytes for six replicate analyses ranged from 86.9% to 108.5% with the RSDs in the range of 1.2–6.2%. Typical IC chromatograms obtained from a mixed standard solution of five analytes, a representative sunscreen cream and its spiked sample obtained by UA-LDS-DLLE were displayed in Fig. 4A–C.

4. Conclusions

In the present work, an UA-LDS-DLLE integrating extraction and clean-up in one single step was originally developed for sample pretreatment. The cyclohexane serving as disperser solvent provided outstanding degreasing capability, leading to the effective elimination of matrix interferences without any further clean-up step. The synergistic effects of ultrasound radiation, vibration and heating were utilized to accelerate the formation of the cloudy solution, which could significantly enhance extraction efficiency and rapidly accomplish extraction within 13 min. The method was very favorable for the rapid quantification of quite a number of samples because it was easy to build and operate, and only required simple equipments. Compared with the conventional LLE, the proposed approach provided the satisfactory accuracy and could markedly reduce organic solvent consumption. The feasibility of the method was demonstrated by analyzing alkanolamines and alkylamines in commercially available cosmetics using ion-exchange chromatography with non-suppressed detection. UA-LDS-DLLE was a simple and effective sample pretreatment technique, which was perfectly suitable for the trace analysis of alkanolamines and alkylamines in various cosmetic products and could potentially be extended to the similar compounds in complex matrices.

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Appendix A. Supporting information

Supplementary information associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.talanta.2013.04.045.

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