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Development of ultrasonic-assisted closed in-syringe extraction and derivatization for the determination of labile abietic acid and dehydroabietic acid in cosmetics

Jianjun Liu[a,b], Mengge Liu[a,b], Xiu Li[a,b], Xiaomin Lu[a,b], Guang Chen[a,b,c,*], Zhiwei Sun[a,b], Guoliang Li[a,b], Xianen Zhao[a,b], Shijuan Zhang[c], Cuihua Song[a,b], Hua Wang[a,b], Yourui Suo[c], Jinmao You[a,b,c,*]

[a] The Key Laboratory of Life-Organic Analysis, Qufu Normal University, Qufu 273165, Shandong, China
[b] Key Laboratory of Pharmaceutical Intermediates and Analysis of Natural Medicine, Qufu Normal University, Qufu 273165, Shandong, China
[c] Northwest Institute of Plateau Biology, Chinese Academy of Sciences, Xining 810001, China

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ABSTRACT

Two resin acids, abietic acid (AA) and dehydroabietic acid (DHAA), in cosmetics may cause allergy or toxicoderma, but remain inaccurately investigated due to their lability. In this work, an accurate, sensitive, efficient and convenient method, utilizing the ultrasonic-assisted closed in-syringe extraction and derivatization (UCSED) prior to high performance liquid chromatography (HPLC) coupled with fluorescence detection (FLD) and on-line tandem mass spectra (MS/MS), has been developed. Analytes are extracted by acetonitrile (10/1, v/m) in a sealed syringe under safe condition (60 °C; 15 min; nitrogen atmosphere) and then in-syringe derivatized by 2-(2-(anthracen-10-yl)-1H-naphtho[2,3-d]imidazol-1-yl) ethyl-p-toluenesulfonate (ANITS) (8-fold, 93 °C, 30 min, DMF as co-solvent, K_2CO_3 as catalyst). In UCSED, derivatization contributes to increase both analytical sensitivity and stability of analytes. Excellent linearity ($r^2 \geq 0.9991$) is achieved in wide range (75–3000 ng/mL (AA); 150–4500 ng/mL (DHAA)). Quite low detection limits (AA: 8.2–10.8 ng/mL; DHAA: 19.4–24.3 ng/mL) and limits of analyte concentration (LOAC) (AA: 30.0–44.5 ng/mL; DHAA: 70.9–86.7 ng/mL) ensure the trace analysis. This method is applied to the analysis of cosmetic samples, including *depilatory wax strip*, *liquid foundation*, *mascara*, *eyeliner*, *eyebrow pencil* and *lip balm*. No additional purification is required and no matrix effect is observed, demonstrating obvious advantages over conventional pretreatment such as solid phase extraction (SPE). Accuracy (RE: −3.2% to 2.51%), precision (RSD: 1.29–2.84%), recovery (95.20–103.63%; 95.51–104.22%) and repeatability (<0.23%; <2.87%) are significantly improved. Furthermore, this work plays a guiding role in developing a reasonable method for labile analytes.

1. Introduction

Two resin acids, abietic acid (AA) and dehydroabietic acid (DHAA) (Fig. 1), as the primary components of colophony [1], have been widely used in cosmetics as excellent adhesive or emulgator [2–4]. Cosmetics usually remain on skin for a long time to keep lasting effect of promoting attractiveness or altering appearance [5]. Unfortunately, colophony is associated closely with occupational asthma (formerly called colophony disease) [6–8] and contact allergy [9–14]. Moreover, it has been reported that allergens are produced via the oxidization of AA exposed to air [15–17] and the toxicity of resin acids mainly comes from DHAA [18–20]. According to EU legislation (Directive 67/548/EEC), a content of colophony >1% in cosmetics must be declared and marked with warning "May cause sensitization by skin contact" [21]. Therefore, it is critical to detect the two components in the increasing number of cosmetics, especially those applied to sensitive areas such as skin, face, eyes and lip [12,15,22].

AA and DHAA used to be determined by gas chromatography–mass spectrometry (GC–MS) [14,23–26]. In fact, high temperature in GC will cause isomerization of AA

* Corresponding authors at: Qufu Normal University, Key Laboratory of Pharmaceutical Intermediates and Analysis of Natural Medicine, Qufu, China.
Tel.: +86 537 4456305; fax: +86 537 4456305.
E-mail addresses: chenandguang@163.com (G. Chen), jmyou6304@163.com (J. You).

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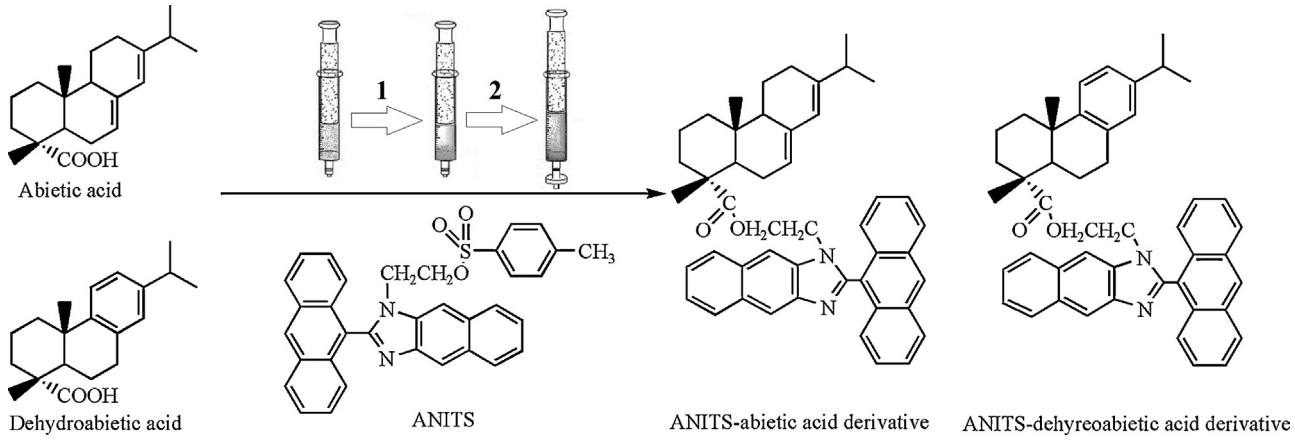

Fig. 1. Scheme of ultrasonic-assisted closed in-syringe extraction and derivatization (UCSED) technique (1: ultrasonic-assisted trace extraction and 2: in-syringe ultrasonic-assisted derivatization) and the derivatization process between the two analytes (abietic acid and dehydroabietic acid) and fluorescence reagent 2-(2-(anthracen-10-yl)-1H-naphtho[2,3-d]imidazol-1-yl) ethyl-p-toluenesulfonate (ANITS).

[27] and thus may lead to inaccurate results. High performance liquid chromatography (HPLC) coupled with ultraviolet/diode array detection (UV/DAD) [4,28–32], fluorescence detection (FLD) [29–32] or MS [25,26,31,33] has been extensively developed, acting as more accurate alternative to GC for analytes susceptible to high temperature. Due to weak chromophore in their molecules, trace resin acids are difficult to directly detect with relatively high detection limits provided by these methods. Though pre-concentration can lower the detection limits, AA and DHAA are usually present in complex matrixes and thus the interferences components will be also concentrated together. In this case, multi-step manual operations such as solid-phase extraction (SPE) [4,29,30,32] or solid-phase microextraction (SPME) [33] are usually required, otherwise it will be difficult to both avoid interferences [32] and lower detection limits [4]. However, multi-step operations are tedious, time-consuming, and more seriously, tend to cause high errors to labile analytes. In another sense, high reagent-consumption will pose a potential threat to experimenters and environment. Consequently, although a few works have been done, there are still many serious questions left to be settled in accurate and sensitive determination of the two resin acids at trace level.

Pre-column derivatization technique can be introduced to overcome these difficulties by improving sensitivity, selectivity, and accuracy as well [34]. It must be noted that abietic acid (AA) is prone to be oxidized [16]; thus, derivatization may improve the stability of analytes by modifying molecule structure [35]. Nevertheless, to the best of our knowledge, no derivatization technique is introduced to date in the determination of AA and DHAA with HPLC. Carboxylic group in analyte is derivatized commonly by probe of sulfonate ester via a complete transesterification reaction under mild conditions [36–38]. But these labeling reagents have been reported with several limitations, such as instability, short wavelengths for detection, low sensitivity, unknown by-products and serious interferences. Thus, an excellent probe 2-(2-(anthracen-10-yl)-1H-naphtho[2,3-d]imidazol-1-yl) ethyl-p-toluenesulfonate (ANITS) superior in above aspects is more competent to this work. On the other hand, for a small amount of analytes in complex matrices, trace analysis technique is playing increasingly important role by bringing about simple, efficient, inexpensive and environmentally friendly pretreatment compatible with many instruments [39–41]. Therefore, combining the ultrasonic-assisted trace extraction with the in-syringe derivatization [42,43] in closed system as a novel pretreatment technique for HPLC will make it possible to establish the desired method.

In this study, a method with ultrasonic-assisted closed in-syringe extraction and derivatization (UCSED) prior to high performance liquid chromatography (HPLC) coupled with fluorescence detection (FLD) and tandem mass spectra (MS/MS) technique has been developed and applied to the quantification of labile abietic acid (AA) and dehydroabietic acid (DHAA) in cosmetics including *depilatory wax strip*, *liquid foundation*, *mascara*, *eyeliner*, *eyebrow pencil* and *lip balm* which were usually applied in sensitive areas of body. Fluorescent reagent 2-(2-(anthracen-10-yl)-1H-naphtho[2,3-d]imidazol-1-yl) ethyl-p-toluenesulfonate (ANITS) is used to label analytes, thereby enhancing the analytical sensitivity and increasing the stability of labile analytes. UCSED technique allows for a simple, convenient operation in relatively short time, and proves to be more competent for the pretreatment of two resin acids than conventional SPE. Multi-variable optimization as well as single variable optimization is introduced to achieve the optimal conditions for labeling analytes efficiently while minimizing matrix interferences. HPLC is used to avoid the isomerization or decomposition caused by high temperature. FLD is used to quantify the analytes with quite low detection limits. Trace analysis is ensured by achieving the limit of analyte concentration (LOAC). Online MS/MS technique is introduced to monitor labile resin acids and matrix interferences, which can practically avoid additional operations for impurities. Linearity, sensitivity, accuracy, precision, recovery and repeatability are significantly improved in comparison with reported methods, making the established method a superior alternative for the determination of resin acids in micro amount of cosmetic samples.

2. Experimental

2.1. Materials and chemicals

Abietic acid (AA, ≥95%) and dehydroabietic acid (DHAA, ≥99%) were purchased from ChromaDex, Inc. (Irvine, CA) and ChemService, Inc. (West Chester, USA), respectively. Derivatization reagent 2-(2-(anthracen-10-yl)-1H-naphtho[2,3-d]imidazol-1-yl) ethyl-p-toluenesulfonate (ANITS) was synthesized as described in our previous study (supplementary text) [44]. Spectroscopically pure acetonitrile (ACN) was purchased from Sigma–Aldrich Co. (St. Louis, MO, USA). HPLC grade formic acid was purchased from Sinopharm Chemical Reagent Co. (Shanghai, China). N,N-dimethylformamide (DMF) and anhydrous potassium carbonate (K_2CO_3) were of analytical grade and bought from Tianjin Fuyu Chemical Reagent Co. (Tianjin, China). Water was purified on a Milli-Q system (Millipore, Bedford, MA, USA). All other reagents were of analytical grade unless otherwise stated. All cosmetic samples were bought from

Baiyi supermarket in Qufu Normal University (Qufu, China). Standard resin acids and cosmetic samples were stored at 4 °C in a freezer until use. All the solvents were saturated by nitrogen.

2.2. Instrumentation and conditions

2.2.1. Instrumentation

Agilent 1100 Series high-performance liquid chromatography was equipped with online vacuum degasser (G1322A), quaternary pump (G1311A), auto-sampler (G1329A), thermostated column compartment (G1316A) and fluorescence detector (FLD, G1321A, set at λ_{ex} = 250 nm and λ_{em} = 512 nm). This HPLC–FLD system was coupled with mass spectrometer 1100 Series LC-MSD Trap-SL (ion trap) with atmospheric pressure chemical ionization (APCI) source (Bruker Daltonik, Bremen, Germany). Ultrasonic-assisted closed in-syringe extraction and derivatization (UCSED) was carried out using a temperature- and time-adjustable of ultrasonic cleaner (KQ-100B, Kunshan Ultrasonic Instrument Co., Kunshan, China).

2.2.2. HPLC–FLD–MS/MS conditions

HPLC separation was performed on a reversed-phase Eclipse XDB-C_8 column (150 mm × 4.6 mm, 5 mm, Agilent Co.) by a gradient elution, where mobile phases A and B were the 20% ACN solution (ACN/H_2O: 20/80, v/v) containing 0.1% formic acid and the 100% ACN containing 0.1% formic acid, respectively. Mobile phases were filtered through a 0.20 μm nylon membrane filter (Alltech, Deerfiled, IL, USA). Gradient elution program was set as: 0–3 min, 80–100% of B; 3–10 min, 100% of B; post time: 5 min. Column temperature was set at 30 °C and flow rate was constant at 1.0 mL/min. Maximum fluorescence responses of ANITS-resin acid derivatives were achieved at excitation wavelength 250 nm (λ_{ex}) and emission wavelength 512 nm (λ_{em}). Analytes were further identified by MS in the positive ion mode with atmospheric pressure chemical ionization (APCI) source set as: nebulizer pressure constant at 35 psi, dry gas temperature at 350 °C, dry gas flow at 9.0 L/min, APCI Vap temperature at 350 °C; corona current (nA) at 4000 (pos) and capillary voltage at 3500 V.

2.3. Preparation of standard solutions

A volume of 10 mL of ANITS solution (5×10^{-2} mol/L) was prepared by dissolving 271 mg of 2-(2-(anthracen-10-yl)-1H-naphtho [2,3-d] imidazol-1-yl) ethyl-p-toluenesulfonate in DMF and then diluted with ACN to the needed low-concentration solutions. Stock solutions for AA (5×10^{-3} mol/L) and DHAA (5×10^{-3} mol/L) were prepared by dissolving 15.1 mg AA and 15 mg DHAA in 10 mL of ACN, respectively. Solution of mixed standards at the concentration of 5×10^{-4} mol/L was prepared with ACN. Working solutions for calibration curves and validation (AA: 75 ng/mL, 150 ng/mL, 300 ng/mL, 600 ng/mL, 1200 ng/mL, 1500 ng/mL and 3000 ng/mL; DHAA: 150 ng/mL, 300 ng/mL, 600 ng/mL, 1200 ng/mL, 1500 ng/mL, 3000 ng/mL and 4500 ng/mL) were prepared by diluting the stock solutions with ACN. Quality control (QC) solutions for AA and DHAA were prepared at three concentration levels (150, 750 and 1500 ng/mL). When not in use, all solutions were stored at 4 °C in darkness until HPLC analysis.

2.4. Samples pretreatment procedure

Depilatory wax strip and *lip balm* were cut into pieces (0.5 cm × 0.5 cm), *liquid foundation* and *mascara* were transferred into a vial, *eyeliner* and *eyebrow pencil* were peeled and ground to more than 100 meshes, respectively, for obtaining cosmetic samples. To improve efficiency of pretreatment, an ultrasonic-assisted closed in-syringe extraction and derivatization (UCSED) technique was developed (Fig. 1). Accurately weighed 50 mg of cosmetic sample was added into a syringe barrel (2 mL) where 500 μL of ACN was then drawn. The syringe was sealed with screw-cap and sonicated at 60 °C for 15 min. An amount of 20 mg of anhydrous K_2CO_3 was added in 200 μL of ANITS solution (5×10^{-3} mol/L), and then the obtained mixture was drawn into the syringe (more than 10 mg K_2CO_3 was drawn). The syringe was immediately re-sealed and put into an ultrasonic water bath (93 °C) for 30 min. The resulting mixture was cooled to room temperature and filtered through a syringe filter (0.2 μm) for the direct HPLC analysis. Standard sample was obtained by mixing 20 mg anhydrous K_2CO_3 and 160 μL ANITS (5×10^{-3} mol/L) with 100 μL of standard solutions (5×10^{-4} mol/L) and pretreated identically. The scheme of derivatization reaction is shown in Fig. 1, where alkoxide (R_1O^-) was produced from probe molecule ($R_1O—SO_2R_2$) via the leaving of tosyl ($R_2SO_2^+$) under alkaline condition and then attack target analyte (R_3COOH) to produce the derivative (R_3COOR_1) via a tetrahedral intermediate.

2.5. Optimization of UCSED

2.5.1. Optimization of extraction

Absolute methanol, absolute ethanol, chloroform, DMF and ACN were investigated to obtain appropriate solvent for extraction. The ratio (volume/mass) of ACN to sample (4/1–20/1), extraction temperature (20–100 °C) and time (5–25 min) for the cosmetic extraction were investigated. All the extractions were performed at three mass levels of samples (50, 100 and 500 mg).

2.5.2. Optimization of derivatization

Single-variable experiments were carried out to evaluate the factors including co-solvents, basic catalysts, molar ratio (derivatization reagent/analytes), temperature and time on the yields of derivatization. All experiments were performed at three concentration levels (150, 750 and 1500 ng/mL). Some factors such as molar ratio (derivatization reagent/analytes), temperature and time would interact with each other, and thus they were further optimized by a multivariate method. Design of multivariate experiments, data analysis and model-building were performed by the Box–Behnken design (BBD) program (Design Expert Trial Version 7.1.3, Stat-Ease Inc., Minneapolis, MN, USA) based on three levels of incomplete factorial designs [37,45,46]. Three variables, molar ratio (ANITS to resin acids) (X_1), derivatization temperature (X_2) and time (X_3), are listed in Table 1.

2.6. Method validation

2.6.1. Linearity, repeatability and sensitivity

This method was validated following the United States Food and Drug Administration (FDA) guidelines [47]. Linearity equations were established using working solutions spiked into real samples (six replicates (n = 6)): real sample was equally divided into two parts, one was analyzed directly (peak area: Y_1), the other was spiked by a working solution and then analyzed (peak area: Y_2); thus the linear equation was established by fitting the calibration curve of peak area ($Y_2 - Y_1$) versus the injected concentration (X, ng/mL). The repeatability of FLD detection was investigated by analyzing QC (quality control) solution spiked in real samples (n = 6) and was reflected by relative standard deviations (RSDs) of peak area and retention time. Analytical sensitivities were reflected by limit of detection (LOD) and limit of quantification (LOQ) tested at the signal-to-noise ratio of 3:1 and 10:1, respectively. The lowest concentration of analyte producing the quantifiable derivative whose concentration was at the LOQ level was defined as the limit of analyte concentration (LOAC) for derivatization technique, which was investigated by reducing analyte in derivatization reaction, so as to evaluate the feasibility of trace analysis.

Table 1
The Box-Behnken design method, validation by multi-criteria, nonparametric tests and optima.

Run[a]	Independent variable[b]			Response[c]		Validation[d]	
	X_1	X_2	X_3	Exp.	BBD		
1	10.00	100.00	30.00	3665.8	3638.58	Multi-criteria	
2	8.00	90.00	30.00	3965.8	3922.80	AME	47.9400
3	10.00	90.00	40.00	3870.1	3918.04	CE	0.9984
4	6.00	80.00	30.00	3287.1	3314.32	MAE	0.0000
5	8.00	90.00	30.00	3901.1	3922.80	RMSE	27.4157
6	8.00	90.00	30.00	3899.6	3922.80	MRE (%)	0.0065
7	10.00	80.00	30.00	3507.3	3479.80	R^2	0.9829
8	6.00	90.00	40.00	3830.2	3823.41		
9	8.00	80.00	20.00	3244.8	3265.51	Nonparametric tests	
10	8.00	80.00	40.00	3579.2	3558.76	p-Value	0.1204
11	10.00	90.00	20.00	3731.4	3738.19		
12	8.00	100.00	40.00	3695.7	3674.99	Optima	
13	8.00	90.00	30.00	3940.2	3922.80	X_1	8.43
14	8.00	90.00	30.00	3907.3	3922.80	X_2	92.7
15	8.00	100.00	20.00	3604.7	3625.14	X_3	30.45
16	6.00	100.00	30.00	3603.9	3631.40	BBD	3937.32
17	6.00	90.00	20.00	3708.1	3660.16	Exp.	3934.9

[a] The 17 runs from the Box-Behnken design (BBD) were given by the soft ware Design-Expert 7.1.3 Trial.
[b] Independent variables were X_1: molar ratio of ANITS to analytes, X_2: derivatization temperature (°C) and X_3: derivatization time (min).
[c] Experimental and predicted total peak areas of AA-derivative and DHAA-derivative.
[d] Validation: multi-criteria (AME: absolute maximum error, CE: coefficient of efficiency, MAE: mean absolute error, RMSE: root Mean squared error, MRE: mean relative error, R^2: correlation of determination and nonparametric tests (p-value with Wilcoxon rank sum method)).

2.6.2. Recovery, matrix effect, accuracy and precision

Real sample was equally divided into two parts: one was analyzed directly (peak area: S_1); the other was spiked by a QC solution (known peak area: S_0) and then analyzed (peak area: S_2). Thus recovery was calculated as: recovery = $(S_2 - S_1)/S_0 \times 100\%$, to investigate the influence of real sample matrix on analytes. Moreover, to investigate the matrix effect on detection, a solution used for injection was divided equally into two parts: one was analyzed directly (peak area: S_3); the other was spiked by a known QC solution (peak area: S_0) and then analyzed (peak area: S_4). The matrix effect was calculated as: matrix effect = $(S_4 - S_3)/S_0 \times 100\%$. Relative error (RE%) and relative standard deviations (RSD%) were calculated to evaluate the accuracy and precision, respectively ($n = 6$).

2.6.3. Stability

In view of the fact that AA tends to be oxidized [16], it is necessary to test the stability of two resin acids and their ANITS-labeled derivatives. According to the practical performance, following conditions were considered. Short-term and long-term stability were investigated at room temperature for 12 h and one week, respectively. Storage stability was evaluated at 4 °C for one week. Freeze–thaw stability was evaluated after two cycles of freeze (−20 °C for one week)–thaw (room temperature, spontaneously) performance. Six duplicates of both resin acids and their derivatives spiked at each of three concentration levels (150, 750 and 1500 ng/mL) were analyzed and the mean ± SD with RE% was calculated.

2.7. Application to cosmetic samples

The established UCSED-HPLC–FLD–MS/MS method was used to detect two resin acids (AA and DHAA) in six kinds of cosmetic samples including *depilatory wax strip*, *liquid foundation*, *mascara*, *eyeliner*, *eyebrow pencil* and *lip balm*, which are commonly applied to the sensitive areas of body. All of cosmetic samples were investigated at each of the three mass levels (50, 100 and 500 mg). Contents of analytes were obtained and the results were discussed in detail.

3. Result and discussion

3.1. Optimization of UCSED

3.1.1. Optimization of extraction

Solvents for extraction of analytes from cosmetics were investigated as shown in Fig. 2-1a–1e (A–F), where extraction yields (reflected by peak area) with ACN were set as 100%. From Fig. 2-1a and 1b, it could be seen that absolute ethanol provided higher extraction yield (87–95%) than methanol, probably due to the higher solubility of resin acids in ethanol than in methanol. Chloroform (Fig. 2-1c) provided a relatively high yield (89–96%), but this solvent needed additional evaporation and dryness with a stream of nitrogen gas before the HPLC analysis. In comparison with ACN, DMF (Fig. 2-1d) provided lower extraction yields for *depilatory wax strip* (Fig. 2, A-1d) and *eyebrow pencil* (Fig. 2, E-1d). Clearly, ACN provided the highest yields, probably for the reason that the good miscibility of ACN was efficient for not only the emulsification and homogenization of samples but also the extraction of the low polar resin acids. From Fig. 2-2a–2e, it could be found that the ratio (volume/mass) of ACN to sample should be kept no less than 10:1, so as to obtain the constantly high yield. Besides, investigations also showed that temperature and time should be no less than 60 °C and 15 min, respectively.

3.1.2. Optimization of derivatization

As shown in Fig. 3A, DMF provided the most intense responses. DMSO provided the responses almost similar to DMF, but several interfering peaks were observed [44]. Thus, DMF was used as co-solvent by adding the stock solution of ANITS (designedly dissolved in DMF) into the in-syringe derivatization system. Volume percentage of DMF in the resulting mixture was kept within 2–10% in order for the high yields of derivatization to be achieved. Investigations on catalyst in Fig. 3B indicated that the maximum response intensity could be achieved when more than 10 mg of K_2CO_3 was added. Investigations on limit of analyte concentration (LOAC) are illustrated in Fig. 3C. Fig. 3D indicated that the 8-fold molar ratio (ANITS to two analytes) was most favorable to derivatization. Temperature investigation (Fig. 3E) showed a response increase from 70 °C to 90 °C and a slight decrease after 95 °C, revealing the instability

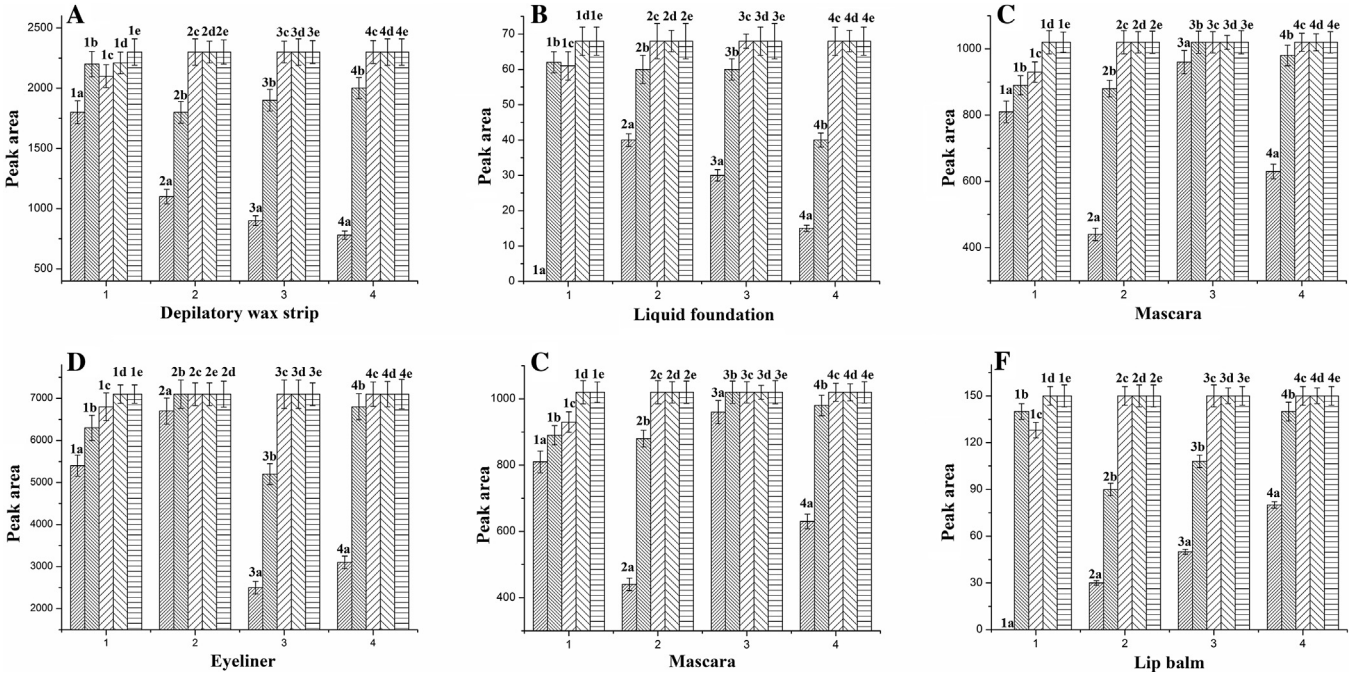

Fig. 2. Effect of extraction solvents, ratio of extraction solvent to sample (volume/mass), extraction temperature and time on extraction yield (total peak areas of derivatives) for depilatory wax strip (A), liquid foundation (B), mascara (C), eyeliner (D), eyebrow pencil (E) and lip balm (F). 1 for extraction solvents: 1a (absolute methanol), 1b (absolute ethanol), 1c (chloroform), 1d (DMF), 1e (ACN); 2 for ratio of extraction solvent to samples (volume/mass): 2a (4:1), 2b (8:1), 2c (10:1), 2d (15:1), 2e (20:1); 3 for extraction temperature: 3a (20 °C), 3b (40 °C), 3c (60 °C), 3d (80 °C), 3e (100 °C); 4 for extraction time: 4a (5 min), 4b (10 min), 4c (15 min), 4d (20 min), 4e (25 min) with standard deviations included.

of resin acids at the high temperature [27]. Fig. 3F shows that the highest derivatization yield could be obtained at 30 min.

A robust multivariable optimization method Box–Behnken design (BBD) was applied to achieve the optimal combination of the three interactive variables (X_1: molar ratio of ANITS to analytes, X_2: derivatization temperature and X_3: time) [37,45]. The 3D response surface and 2D contour plots (see supplementary figure) showed the relationship between response and variable. Experimental design, model validation [48,49] and optima prediction are listed in Table 1. Validation showed that the BBD multivariate model provided good correlation ($R^2 = 0.9829$) and coefficient of efficiency (CE = 0.9984). The p-value of nonparametric test was

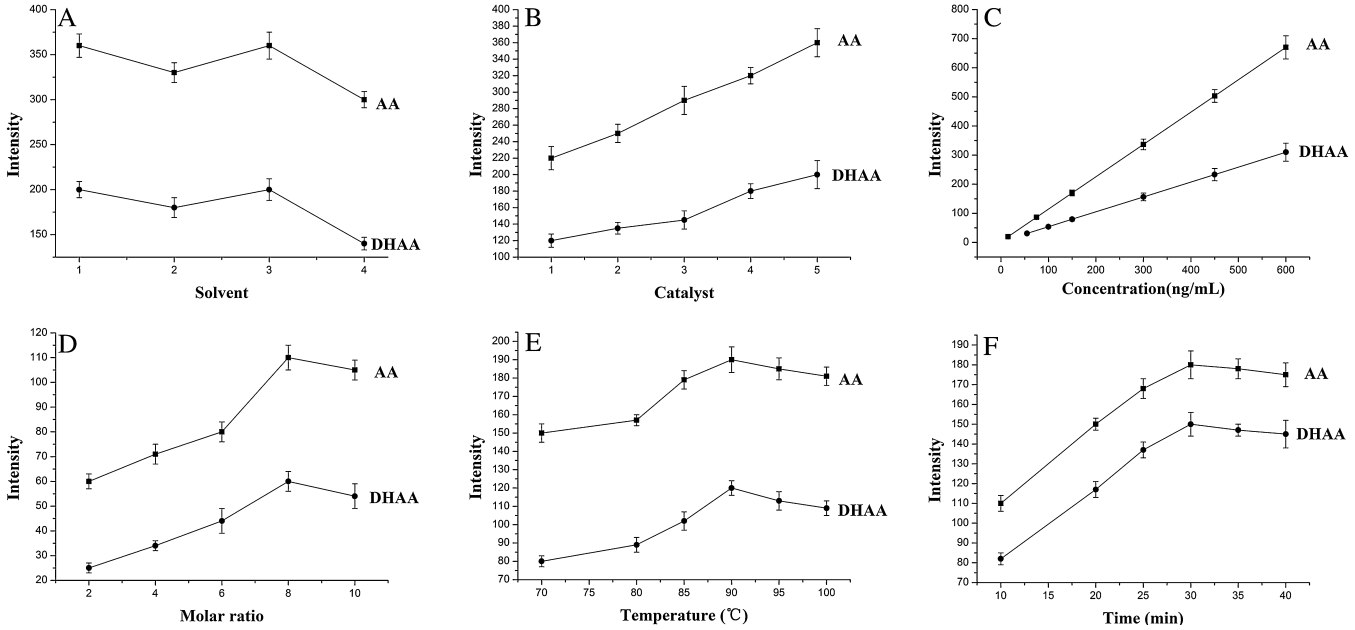

Fig. 3. The fluorescence intensity of single variable optimization for the derivatization reaction condition: (A) derivatization reaction in usual co-solvents (1: DMF, 2: ACN, 3: DMSO and 4: THF); (B) derivatization reaction in the presence of different catalysts (1: pyridine, 2: 2-methylpyridine, 3: triethylamine, 4: DMAP and 5: K_2CO_3); (C) analytes with variation of the concentration (from 15 to 600 ng/mL); (D) molar ratio of ANITS to analytes (from 2 to 10); (E) derivatization temperature (from 70 to 100 °C); (F) derivatization time (from 10 to 40 min).

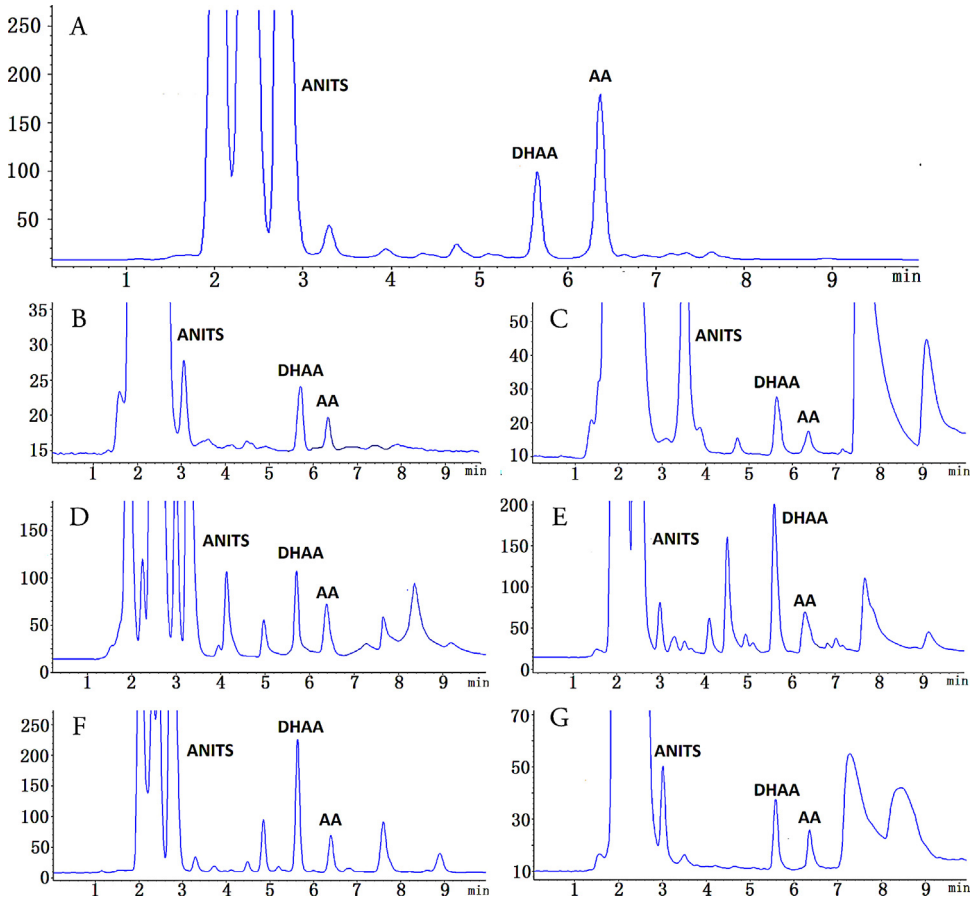

Fig. 4. The chromatograms for the standard analytes (A), depilatory wax strip (B), liquid foundation (C), mascara (D), eyeliner (E), eyebrow pencil (F) and lip balm (G).

0.1204 indicating that the differences between experimental and predicted values were not statistically significant (at the 95% confidence level) [48]. As a result, strongest response (3937.32) was predicted by regression of BBD model. The combination of variables (X_1: 8.43, X_2: 92.7 and X_3: 30.45) was applied to experiment, providing the response (3934.9) comparable to the predicted value.

3.2. The UCSED–HPLC–FLD–MS/MS method development

With the thorough optimization, a simple UCSED pretreatment for cosmetic samples has been developed. Eclipse XDB-C_8 column was selected for the low polarity of ANITS-resin acid derivatives which were detected by HPLC–FLD system at 5.63 min and 6.38 min, respectively (Fig. 4). It was noteworthy that when ANITS-labeled AA was eluted, the ratio of ACN/H_2O in mobile phase reached to 100%, which ensured the highest response of fluorescence [44]. In this sense, mobile phase composition and elution gradient were very beneficial to the sensitive detection. Analytes were separated well (Fig. 4, B–G) and no interference from matrix was observed at the retention time. Due to that the two analytes were susceptible to high temperature and oxygen and, moreover, there were peaks of impurities adjacent to targets, further strict identification by on–line MS/MS was still necessary. As shown in Fig. 5, intense molecular ion peaks ($[M+H]^+$) were observed at m/z 673.2 (AA) and m/z 671.2 (DHAA). With MS/MS, the specific fragment ions were obtained at m/z 345 and m/z $[M_A+H-345]^+$ (M_A was molecular mass of AA or DHAA) for ANITS moiety and ANITS-resin acid derivatives moiety, respectively. No fragments caused by isomerization or oxidation of analytes were monitored.

3.3. Method validation

3.3.1. Linearity, sensitivity and repeatability

Linearity, sensitivity and repeatability of this method are listed in Table 2. Linear equations were established in the range of 75–3000 ng/mL (AA) and 150–4500 ng/mL (DHAA) with excellent coefficients of determination (r^2) ≥ 0.9991, which might be ensured by the strict conditions for labile resin acids. As expected, very low LODs (8.2–10.8 ng/mL for AA; 19.4–24.3 ng/mL for DHAA) and LOQs (26.7–38.9 ng/mL for AA; 68.5–81.1 ng/mL for DHAA) were achieved, indicating the high sensitivity of this method, which might be attributable to the strong FLD responses and low interferences. The obtained LOAC values of 30.0–44.5 ng/mL (AA) and 70.9–86.7 ng/mL (DHAA) in Table 2 provided the guarantee for trace analysis of cosmetic sample. Furthermore, very good approximation between LOAC and LOQ indicated that the derivatization reaction was complete and the analysis was accurate. RSDs of retention time and peak area were lower than 0.23% and 2.87%, respectively, indicating the excellent repeatability of the determination. In addition, this and previous methods are compared in supplementary text and supplementary Table 1 in detail.

3.3.2. Accuracy and precision

Intra- and inter-day variations for AA and DHAA are listed in Table 3, where it could be seen that the intra-day and inter-day accuracy ranged from −2.73% to 2.51% and from −3.2% to 1.48%, respectively. The intra-day and inter-day precisions were found to be within 1.29–2.56% and 1.89–2.84%, respectively. In comparison with previous reports [4,29–33], this method provided more accurate and precise determination for the two resin acids AA and DHAA.

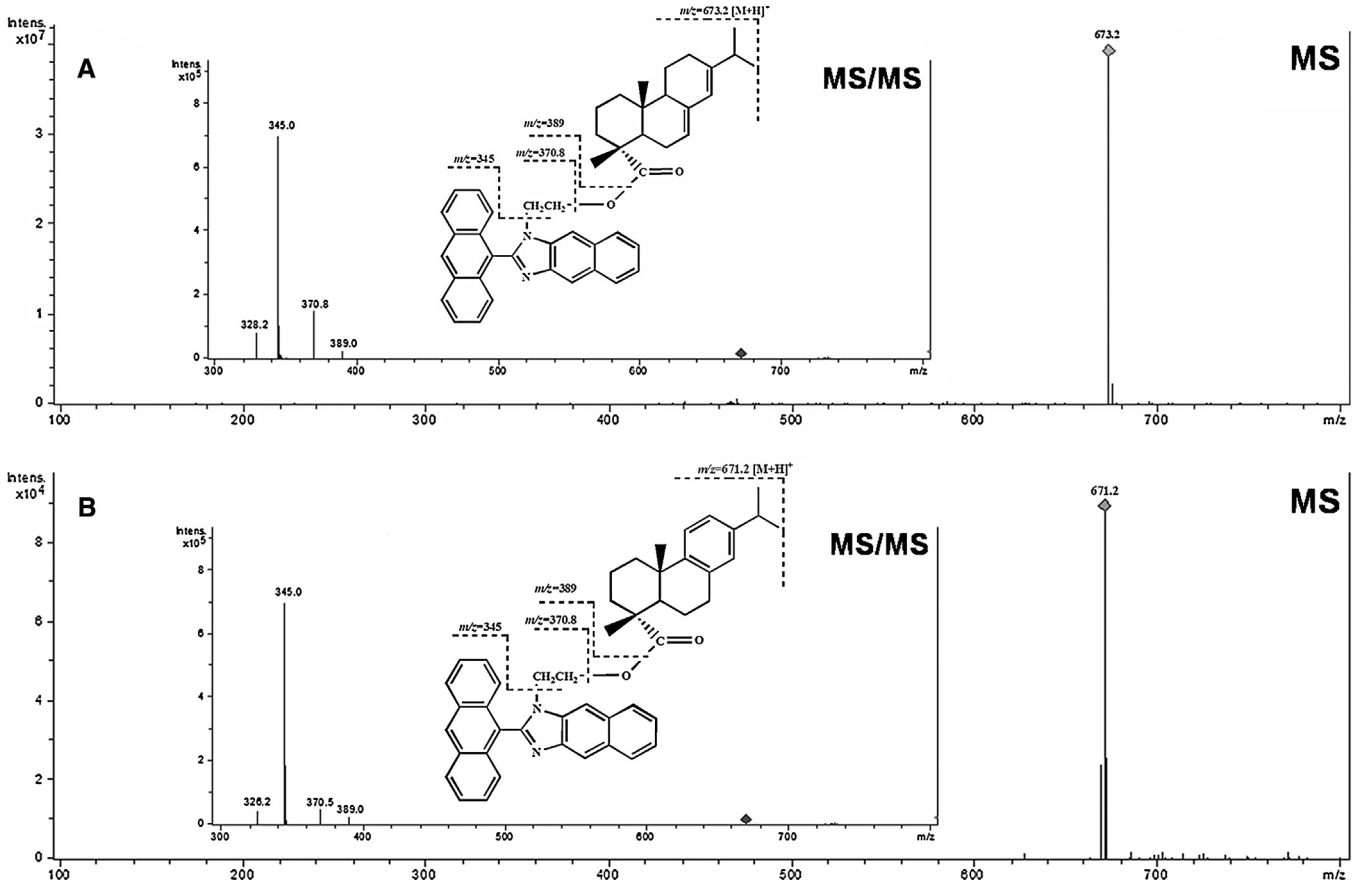

Fig. 5. The MS and MS/MS spectra for abietic acid (A) and dehydroabietic acid (B) with the cleavage mode.

Table 2
Linearity, reproducibility of retention time and peak area ($n = 6$), limit of detection (LOD), limit of quantification (LOQ) and limit of analyte concentration (LOAC) for determination of abietic acid (AA) and dehydroabietic acid (DHAA) with fluorescence detection.

Samples	Analytes	Linearity[a]		Reproducibility (RSD%)		LOD (ng/mL)[b]	LOQ (ng/mL)[b]	LOAC (ng/mL)[c]
		$Y=(a\pm SD)X+(b\pm SD)$	r^2	Retention time	Peak area			
Depilatory wax strip	AA	$Y=(1.084\pm0.018)X+(3.023\pm0.050)$	0.9996	0.06	1.33	8.7	27.5	31.8
	DHAA	$Y=(0.511\pm0.010)X+(2.717\pm0.051)$	0.9996	0.03	0.92	23.2	70.8	76.6
Liquid foundation	AA	$Y=(1.063\pm0.020)X+(2.973\pm0.056)$	0.9997	0.12	1.17	9.0	31.4	35.7
	DHAA	$Y=(0.508\pm0.004)X+(2.721\pm0.064)$	0.9998	0.08	0.98	19.4	68.5	70.9
Mascara	AA	$Y=(1.105\pm0.028)X+(3.081\pm0.078)$	0.9991	0.23	2.87	10.2	37.0	39.0
	DHAA	$Y=(0.528\pm0.005)X+(2.809\pm0.026)$	0.9999	0.11	2.30	23.6	75.4	78.3
Eyeliner	AA	$Y=(1.121\pm0.029)X+(3.128\pm0.081)$	0.9992	0.17	1.77	9.7	35.1	41.3
	DHAA	$Y=(0.522\pm0.010)X+(2.777\pm0.053)$	0.9993	0.09	1.02	24.3	81.1	86.7
Eyebrow pencil	AA	$Y=(1.126\pm0.026)X+(3.141\pm0.073)$	0.9991	0.15	2.34	10.8	38.9	44.5
	DHAA	$Y=(0.516\pm0.005)X+(2.746\pm0.025)$	0.9999	0.10	2.22	21.8	72.8	75.6
Lip balm	AA	$Y=(0.098\pm0.029)X+(3.061\pm0.081)$	0.9993	0.07	1.12	8.2	26.7	30.0
	DHAA	$Y=(0.501\pm0.013)X+(2.662\pm0.067)$	0.9994	0.07	1.06	20.3	70.0	75.7

[a] Y: peak area and X: injected concentration (ng/mL); SD: the standard deviations of six duplicates ($n = 6$); r^2: coefficients of determination.
[b] LOD and LOQ were established based on a signal-to-noise ratio of 3 and 10, respectively.
[c] LOAC was the lowest concentration of analytes required.

Table 3
Intra- and inter-day accuracy and precision of abietic acid (AA) and dehydroabietic acid (DHAA) at three concentration levels ($n = 6$).

Analytes	Spiked (ng/mL)	Intra-day			Inter-day		
		Mean ± SD	Accuracy (RE%)	Precision (RSD%)	Mean ± SD	Accuracy (RE%)	Precision (RSD%)
AA	150	148.3 ± 3.8	−1.13	2.56	147.9 ± 4.2	−1.40	2.84
	750	741.3 ± 16.9	−1.16	2.28	738.9 ± 21.0	−1.48	2.84
	1500	1459.1 ± 29.0	−2.73	1.99	1452.0 ± 31.2	−3.20	2.15
DHAA	150	151.1 ± 2.1	0.73	1.39	148.4 ± 2.8	−1.11	1.89
	750	740.2 ± 14.9	−1.31	2.01	761.1 ± 17.9	1.48	2.35
	1500	1537.7 ± 19.8	2.51	1.29	1466.9 ± 28.6	−2.21	1.95

Table 4
The stability evaluation of analytes, abietic acid (AA) and dehydroabietic acid (DHAA), and corresponding derivatives at three QC levels ($n = 6$).

Compound spiked (ng/mL)	Short-term		Long-term		Storage		Freeze–thaw	
	Mean ± SD	RE%	Mean ± SD	RE%	Mean ± SD	RE%	Mean ± SD	RE%
AA								
150	143.4 ± 4.9	−4.40	138.2 ± 8.3	−7.87	146.8 ± 2.0	−2.13	146.6 ± 1.9	−2.27
750	727.2 ± 14.8	−3.04	689.0 ± 18.9	−8.13	727.7 ± 12.3	−2.97	720.7 ± 14.5	−3.91
1500	1452.2 ± 21.0	−3.19	1336.5 ± 30.9	−10.9	1453.2 ± 18.9	−3.12	1450.5 ± 31.9	−3.30
AA-derivative								
150	147.9 ± 4.1	−1.40	146.7 ± 4.0	−2.20	148.2 ± 0.5	−1.20	146.3 ± 2.1	−2.47
750	736.4 ± 12.9	−1.81	728.4 ± 17.4	−2.88	739.0 ± 12.5	−1.47	740.3 ± 15.2	−1.29
1500	1460.4 ± 25.6	−2.64	1459.8 ± 35.0	−2.68	1486.4 ± 14.9	−0.91	1463.6 ± 25.0	−2.42
DHAA								
150	147.0 ± 3.4	−2.01	145.4 ± 5.3	−3.10	153.5 ± 1.0	2.33	151.7 ± 1.5	1.11
750	732.7 ± 13.0	−2.31	778.3 ± 16.9	3.77	739.4 ± 14.9	−1.41	732.7 ± 15.9	−2.31
1500	1546.6 ± 19.0	3.11	1546.4 ± 21.0	3.09	1469.9 ± 27.9	−2.01	1461.9 ± 20.6	−2.54
DHAA-derivative								
150	148.3 ± 2.9	−1.13	147.1 ± 4.4	−1.93	150.6 ± 0.8	0.40	147.7 ± 1.1	−1.53
750	729.2 ± 13.5	−2.78	731.3 ± 15.9	−2.49	742.8 ± 13.2	−0.97	767.3 ± 13.6	2.31
1500	1458.9 ± 18.9	−2.74	1552.0 ± 28.0	3.47	1517.0 ± 26.1	1.13	1521.0 ± 18.0	1.40

3.3.3. Stability

Investigations on the stability of analytes and their derivatives are listed in Table 4. For one thing, AA showed poorer stability than DHAA, because AA was more susceptible to oxidation [16]. For another, all the RE% values of AA were negative, indicating that the loss caused by oxidation seemed to be unavoidable. In view of these facts, it was reasonable to have pretreatment and quantification finished as soon as possible with analytes being protected. By comparing RE% value in short-term with that in long-term, it could be found that the RE% difference between the two store conditions (short-term and long term) became less after the resin acids were derivatized to corresponding derivatives, indicating that the stability might be improved after sample pretreatment. Thus the UCSED with ANITS proved to be competent enough for the analysis of labile abietic acid (AA) and dehydroabietic acid (DHAA). Besides, comparison of the RE% values between storage (4 °C) and freeze–thaw (−20 °C) indicated that 4 °C should be a beneficial temperature for the storage of stock solutions and cosmetic samples.

3.4. Application to the determination of AA and DHAA in cosmetics

With established method, micro samples of *depilatory wax strip*, *liquid foundation*, *mascara*, *eyeliner*, *eyebrow pencil* and *lip balm* were analyzed (Fig. 4). Contents of AA and DHAA in cosmetic samples are summarized in Table 5. According to the EU legislation (Directive 67/548/EEC) on dangerous substances [21], a cosmetic with colophony >1% may cause sensitization by skin contact. Overall, contents of AA and DHAA in the analyzed samples were very low (AA: 2.04–154.27 μg/g; DHAA: 10.37–1339.96 μg/g) and might probably be in the safe range. But, some following information still should be concerned. Resin acids were extensively used in

Table 5
Content of abietic acid (AA) and dehydroabietic acid (DHAA) in six kinds of cosmetic samples.

Cosmetic samples	Weighted amount[a] (mg)	Abietic acid			Dehydroabietic acid		
		Measured content (μg)	RSD (%, $n = 6$)	Average content[b] (μg/g)	Measured content (μg)	RSD (%, $n = 6$)	Average content[b] (μg/g)
Depilatory wax strip	50	3.16	1.6	64.15	19.45	1.0	395.78
	100	6.08	2.0		39.73	1.1	
	500	34.22	1.5		200.52	1.9	
Liquid foundation	50	0.11	2.6	2.04	0.51	3.2	10.37
	100	0.20	2.2		0.99	4.1	
	500	0.93	1.9		5.51	2.0	
Mascara	50	3.01	2.1	55.15	6.05	1.9	116.20
	100	5.25	2.7		11.47	1.5	
	500	26.37	1.2		56.45	1.1	
Eyeliner	50	7.55	1.9	154.27	67.65	1.2	1339.96
	100	15.54	1.4		131.26	1.5	
	500	78.21	1.1		677.14	1.2	
Eyebrow pencil	50	5.23	1.3	110.41	50.14	2.5	1005.18
	100	11.44	2.8		99.52	2.0	
	500	56.11	2.1		508.77	2.1	
Lip balm	50	0.19	4.3	4.14	1.21	3.2	24.43
	100	0.45	3.8		2.43	2.9	
	500	2.04	2.6		12.40	1.2	

[a] Weighted three amount levels of samples.
[b] The average contents of AA and DHAA in cosmetic samples with standard deviations included.

manufacture of *depilatory wax strip*. To reduce its side effects, manufacturers were urged to modify the colophony. However, as the results indicated, a certain amount of colophony remained not modified in the studied samples: 64.15 μg/g of AA and 395.78 μg/g of DHAA; thus, the allergens might be present in the wax [4]. Quantitative detection of AA (2.04 μg/g) and DHAA (10.37 μg/g) confirmed the existence of the colophony in *liquid foundation*, indicating that the allergy might be caused by this facial cosmetic [2]. As a cause of eyelid dermatitis, resin acids in *mascara* once have been verified [3], but in this work, they got the definite quantification (55.15 μg/g of AA; 116.20 μg/g of DHAA). The resin acids in *eyeliner* (154.27 μg/g of AA; 1339.96 μg/g of DHAA) and *eyebrow pencil* (110.41 μg/g of AA; 1005.18 μg/g of DHAA) were relatively high, which implied that large amounts of colophony material were used in their substrates. Similarly, the *lip balm* was found to contain 4.14 μg/g of AA and 24.43 μg/g of DHAA, which might be related to the resin acids-containing substrate materials, such as vaseline and wax matrixes. The recoveries and matrix effects of AA and DHAA in six cosmetic samples are listed in supplementary Table 2. Recoveries (95.20–104.22%) with RSD <5.59%, showed that the results were precise and reproducible; matrix effects values (93.25–101.66%) with RSD <4.05% indicated the insignificant interference. Thus, the established method provided a superior alternative for the accurate, sensitive, efficient and convenient determination of trace AA and DHAA in micro samples. Meanwhile, the application to cosmetic samples might play a guiding role for consumers to choose suitable products and hopefully for manufacturers to improve production process.

4. Conclusions

In this work, a novel method using ultrasonic-assisted closed in-syringe extraction and derivatization (UCSED) with 2-(2-(anthracen-10-yl)-1H-naphtho[2,3-d]imidazol-1-yl) ethyl-p-toluenesulfonate (ANITS) was established for labile resin acids, based on high performance liquid chromatography coupled with fluorescence detection and on-line tandem mass spectra (HPLC–FLD–MS/MS). The UCSED technique proved to be a more reliable pretreatment for labile analytes than ever reported as it simplified operation (<50 min), improved analytical sensitivity, and effectively prevented oxidation of analytes. HPLC–FLD provided selective and sensitive quantification with good resolution, excellent linearity (≥0.9991), and quite low detection limits (AA: 8.2–10.8 ng/mL; DHAA: 19.4–24.3 ng/mL). The on-line tandem mass spectra ensured a further confirmation of analytes for complex samples. Besides, accuracy, precision, recovery, and repeatability for determination of the two resin acids were significantly improved. The established UCSED–HPLC–FLD–MS/MS method was applied to six cosmetics samples of interest, demonstrating the obvious advantages for the accurate, sensitive, efficient and convenient determination of labile abietic acid and dehydroabietic acid.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.chroma.2014.10.059

References

[1] C.I. Keeling, J. Bohlmann, Diterpene resin acids in conifers, Phytochemistry 67 (2006) 2415–2423.
[2] J. Foussereau, A case of allergy to colophony in a facial cosmetic, Contact Dermatit. 1 (1975) 259.
[3] A. Karlberg, C. Liden, E. Ehrin, Colophony in mascara as a cause of eyelid dermatitis. Chemical analyses and patch testing, Acta Derm. Venereol. 71 (1990) 445–447.
[4] U. Nilsson, N. Berglund, F. Lindahl, S. Axelsson, T. Redeby, P. Lassen, A.T. Karlberg, SPE and HPLC/UV of resin acids in colophonium-containing products, J. Sep. Sci. 31 (2008) 2784–2790.
[5] Z.K. Draelos, Cosmetics: an overview, Curr. Probl. Dermatol. 7 (1995) 45–64.
[6] P.S. Burge, M.G. Harries, I.M. O'Brien, J. Pepys, Respiratory disease in workers exposed to solder flux fumes containing colophony (pine resin), Clin. Allergy 8 (1978) 1–14.
[7] K. Jones, S. Garfitt, A. Calverley, K. Channa, J. Cocker, Identification of a possible biomarker for colophony exposure, Occup. Med. 51 (2001) 507–509.
[8] P.E. Baldwin, J.R. Cain, R. Fletcher, K. Jones, N. Warren, Dehydroabietic acid as a biomarker for exposure to colophony, Occup. Med. 57 (2007) 362–366.
[9] E. Gäfvert, Allergenic components in modified and unmodified rosin. Chemical characterization and studies of allergenic activity, Acta Derm. Venereol. Suppl. (Stockh.) 184 (1994) 1–36.
[10] A.T. Karlberg, E. Gäfvert, B. Meding, B. Stenberg, Airborne contact dermatitis from unexpected exposure to rosin (colophony), Contact Dermat. 35 (1996) 272–278.
[11] S. Sadhra, C. Gray, I. Foulds, High-performance liquid chromatography of unmodified rosin and its applications in contact dermatology, J. Chromatogr. B: Biomed. Sci. Appl. 700 (1997) 101–110.
[12] A.M. Downs, J.E. Sansom, Colophony allergy: a review, Contact Dermat. 41 (1999) 305–310.
[13] L. Kanerva, Handbook of Occupational Dermatology, Springer, Berlin, Heidelberg, 2000.
[14] K. Eriksson, L. Wiklund, C. Larsson, Dermal exposure to terpenic resin acids in Swedish carpentry workshops and sawmills, Ann. Occup. Hyg. 48 (2004) 267–275.
[15] A.T. Karlberg, Contact allergy to colophony. Chemical identifications of allergens, sensitization experiments and clinical experiences, Acta Derm. Venereol. Suppl. (Stockh.) 139 (1988) 1–43.
[16] A.T. Karlberg, K. Bohlinder, A. Boman, U. Hacksell, J. Hermansson, S. Jacobsson, J.L.G. Nilsson, Identification of 15-hydroperoxyabietic acid as a contact allergen in Portuguese colophony, J. Pharm. Pharmacol. 40 (1988) 42–47.
[17] S. Sadhra, I. Foulds, C. Gray, Identification of contact allergens in unmodified rosin using a combination of patch testing and analytical chemistry techniques, Br. J. Dermatol. 134 (1996) 662–668.
[18] T.A. Söderberg, A. Johansson, R. Gref, Toxic effects of some conifer resin acids and tea tree oil on human epithelial and fibroblast cells, Toxicology 107 (1996) 99–109.
[19] G. Peng, J.C. Roberts, Solubility and toxicity of resin acids, Water Res. 34 (2000) 2779–2785.
[20] Y. Kamaya, N. Tokita, K. Suzuki, Effects of dehydroabietic acid and abietic acid on survival, reproduction, and growth of the crustacean daphnia magna, Ecotoxicol. Environ. Safe. 61 (2005) 83–88.
[21] C. Directive, 67/548/EEC of 27 June 1967 on the approximation of laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances, Off. J. Eur. Commun. 196 (1967) 1–5.
[22] K.E. Andersen, I.R. White, A. Goossens, Allergens from the standard series, in: Contact Dermatitis, Springer, Berlin, Heidelberg, 2006, pp. 453–492.
[23] H.B. Lee, T.E. Peart, J.M. Carron, Gas chromatographic and mass spectrometric determination of some resin and fatty acids in pulpmill effluents as their pentafluorobenzyl ester derivatives, J. Chromatogr. 498 (1990) 367–379.
[24] P.A. Smith, D.R. Gardner, D.B. Drown, G. Downs, W.W. Jederberg, K. Still, Detection of resin acid compounds in airborne particulate generated from rosin used as a soldering flux, Am. Ind. Hyg. Assoc. J. 58 (1997) 868–875.
[25] A. Latorre, A. Rigol, S. Lacorte, D. Barceló, Comparison of gas chromatography–mass spectrometry and liquid chromatography–mass spectrometry for the determination of fatty and resin acids in paper mill process waters, J. Chromatogr. A 991 (2003) 205–215.
[26] A. Ozaki, Y. Yamaguchi, T. Fujita, K. Kuroda, G. Endo, Safety assessment of paper and board food packaging: chemical analysis and genotoxicity of possible contaminants in packaging, Food Addit. Contam. 22 (2005) 1053–1060.
[27] H. Takeda, W.H. Schuller, R.V. Lawrence, Thermal isomerization of abietic acid, J. Org. Chem. 33 (1968) 1683–1684.

[28] E. Ehrin, A.T. Karlberg, Detection of rosin (colophony) components in technical products using an HPLC technique, Contact Dermatit. 23 (1990) 359–366.
[29] B. Lee, H. Ong, D. Koh, C. Ong, High-performance liquid chromatographie method for determination of dehydroabietic and abietic acids, the skin sensitizers in bindi adhesive, J. Chromatogr. A 685 (1994) 263–269.
[30] B. Lee, D. Koh, H. Ong, C. Ong, High-performance liquid chromatographic determination of dehydroabietic and abietic acids in traditional Chinese medications, J. Chromatogr. A 763 (1997) 221–226.
[31] K. Hroboňová, J. Lehotay, I. Skačáni, J. Čižmárik, HPLC determination and MS identification of dehydroabietic acid and abietic acid in propolis, J. Liq. Chromatogr. Relat. Technol. 28 (2005) 1725–1735.
[32] Y. Zhu, S. Zhang, Z. Geng, D. Wang, F. Liu, M. Zhang, H. Bian, W. Xu, Simultaneous determination of abietic acid and dehydroabietic acid residues in duck meat by HPLC-PAD-FLD, Food Anal. Method 7 (2014) 1627–1633.
[33] K. Mitani, M. Fujioka, A. Uchida, H. Kataoka, Analysis of abietic acid and dehydroabietic acid in food samples by in-tube solid-phase microextraction coupled with liquid chromatography-mass spectrometry, J. Chromatogr. A 1146 (2007) 61–66.
[34] R.M. Smith, Before the injection-modern methods of sample preparation for separation techniques, J. Chromatogr. A 1000 (2003) 3–27.
[35] M. Sun, L. Bai, G.J. Terfloth, D.Q. Liu, A.S. Kord, Matrix deactivation: a general approach to improve stability of unstable and reactive pharmaceutical genotoxic impurities for trace analysis, J. Pharm. Biomed. Anal. 52 (2010) 30–36.
[36] Y. Yasaka, M. Tanaka, T. Shono, T. Tetsumi, J. Katakawa, 2-(2,3-Naphthalimino) ethyl trifluoromethanesulphonate as a highly reactive ultraviolet and fluorescent labelling agent for the liquid chromatographic determination of carboxylic acids, J. Chromatogr. 508 (1990) 133–140.
[37] G. Li, X. Zhang, J. You, C. Song, Z. Sun, L. Xia, Y. Suo, Highly sensitive and selective pre-column derivatization high-performance liquid chromatography approach for rapid determination of triterpenes oleanolic and ursolic acids and application to Swertia species: optimization of triterpenic acids extraction and pre-column derivatization using response surface methodology, Anal. Chim. Acta 688 (2011) 208–218.
[38] T. Toyo'oka, Fluorescent tagging of physiologically important carboxylic acids, including fatty acids, for their detection in liquid chromatography, Anal. Chim. Acta 465 (2002) 111–130.
[39] A. Sarafraz-Yazdi, A. Amiri, Liquid-phase microextraction, Trends Anal. Chem. 29 (2010) 1–14.
[40] Y. Zhang, H.K. Lee, Liquid phase microextraction using knitting wool as the extractant phase holder before chromatographic analysis: a new approach for trace analysis, J. Chromatogr. A 1273 (2013) 12–17.
[41] S.M. Majedi, B.C. Kelly, H.K. Lee, Evaluation of a cloud point extraction approach for the preconcentration and quantification of trace CuO nanoparticles in environmental waters, Anal. Chim. Acta 814 (2014) 39–48.
[42] Y. He, H.K. Lee, Liquid-phase microextraction in a single drop of organic solvent by using a conventional microsyringe, Anal. Chem. 69 (1997) 4634–4640.
[43] J. Lee, H.K. Lee, Fully automated dynamic in-syringe liquid-phase microextraction and on-column derivatization of carbamate pesticides with gas chromatography/mass spectrometric analysis, Anal. Chem. 83 (2011) 6856–6861.
[44] J. You, F. Zhu, W. Zhao, X.E. Zhao, Y. Suo, S. Liu, Analysis of saturated free fatty acids from pollen by HPLC with fluorescence detection, Eur. J. Lipid Sci. Technol. 109 (2007) 225–236.
[45] S.L. Ferreira, R.E. Bruns, H.S. Ferreira, G.D. Matos, J.M. David, G.C. Brandao, E.G. da Silva, L.A. Portugal, P.S. dos Reis, A.S. Souza, W.N. dos Santos, Box–Behnken design: an alternative for the optimization of analytical methods, Anal. Chim. Acta 597 (2007) 179–186.
[46] M.A. Bezerra, R.E. Santelli, E.P. Oliveira, L.S. Villar, L.A. Escaleira, Response surface methodology (RSM) as a tool for optimization in analytical chemistry, Talanta 76 (2008) 965–977.
[47] S.S. Nielsen, United States Government Regulations and International Standards Related to Food Analysis, Springer, New York, 2010.
[48] R. Modarres, Multi-criteria validation of artificial neural network rainfall-runoff modeling, Hydrol. Earth Syst. Sci. 13 (2009) 411–421.
[49] W.J. Conover, W. Conover, Practical Nonparametric Statistics, Wiley, New York, 1980.