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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

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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

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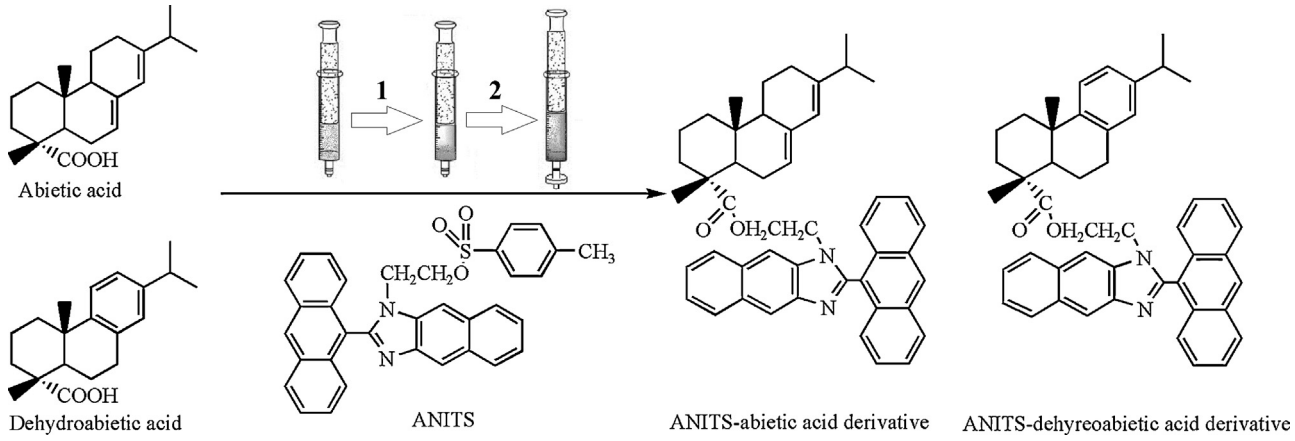

Fig. 1. Scheme of ultrasonic-assisted closed in-syringe extraction and derivatization (UCSED) technique (1: ultrasonic-assisted trace extraction and 2: in-syringe ultrasonic-assisted derivatization) and the derivatization process between the two analytes (abietic acid and dehydroabietic acid) and fluorescence 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