Research Article

# A sensitive high-performance liquid chromatography method with fluorescence detection for the determination of fatty acids as exemplified for *Dendrobium* species

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A novel high-performance liquid chromatography (HPLC) method using 2-(9-oxoacridin-10(9H)-yl) acetohydrazide (OAAH) as pre-column labeling reagent was applied to the analysis of fatty acids in four kinds of *Dendrobium* species for the first time. The reaction of OAAH with fatty acids could proceed easily and quickly in the presence of 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC) as condensing reagent within 20 min. The derivatives exhibit excellent fluorescence property with excitation and emission wavelengths of 255 and 420 nm, respectively. The 20 fatty acid derivatives were separated on a SB C18 reversed-phase column with gradient elution. Good linear correlations were observed for all fatty acids with correlation coefficients of $>0.996$. When 50 mg of sample was used for analysis, the detection limits at a signal-to-noise ratio of 3 were in the range of 0.10–0.42 μg/g. Free fatty acids and total fatty acids in four kinds of *Dendrobium* species were analyzed by the developed method. The results indicated that the main fatty acids in *Dendrobium* species were unsaturated linoleic acid (C18:2n-6) and saturated hexadecanoic acid. This work provides a useful tool for the safety assessment and quality control of *Dendrobium* species.

**Practical applications:** *Dendrobium* species, the stems of which can be used for medical purpose or as health foods, are precious plants in China. Their significant medical effects have recently attracted increasing attention. However, there is little research on the fatty acid composition of *Dendrobium* species. The proposed method provided a sensitive method for the HPLC analysis of FFAs and TFAs in four kinds of *Dendrobium* species. This is the first report of the fatty acid compositions of *Dendrobium* species. It would be helpful for the comprehensive safety or quality assessment of *Dendrobium* species.

**Keywords:** *Dendrobium* species / Fatty acids / Fluorescence / HPLC

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## 1 Introduction

*Dendrobium* species, known as *Shihu* in Chinese, are precious herbal plants in Chinese traditional medicine. The most precious specie of *Dendrobium* was called *Dendrobium candidum*, the stems of which were regarded as the gold in herb and as famous as the ginseng cultivated in the northeast of China. In ancient China, it belonged to only imperial family. Nowadays, it becomes easily available and is often used as a health food rather than a medicine. *D. devoninum* is a *Dendrobium* specie second to *D. candidum* in quality. It possesses similar functions with *D. candidum* but is a bit cheaper. The other species such as *D. aphyllum* are widespread and much cheaper. *Dendrobium* species possess the functions of curing cataract, fever, throat inflammation, and chronic superficial gastritis. They can also be used as a tonic for the promotion of body fluid production and immunomodulatory effects [1–3].

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**Abbreviations: OAAH,** 2-(9-oxoacridin-10(9H)-yl) acetohydrazide; **EDC,** 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride; **FA,** fatty acids; **TFA,** total fatty acids; **FFA,** free fatty acids; **LC,** liquid chromatography; **LOD,** limit of detection; **UFA,** unsaturated fatty acid

 

The stems of *Dendrobium* species have been traditionally used for medical purpose, and the stems of *D. candidum* and *D. devoninum* are often used in cooking to improve people's health status. To make better use of *Dendrobium* species, it is necessary to get a comprehensive understanding of them. There are some precious studies on the chemical constituents of *Dendrobium* [2, 4–7], among which polysaccharides have been studied more frequently. Fatty acids (FAs) play an important role in the prevention and treatment of lots of diseases such as cancer [8, 9], inflammatory diseases [10], retinitis pigmentosa [11], schizophrenia [12], cardiovascular disease [13], and so on [14–16]. Some researches indicate that FAs are effective components for the efficacy of medicinal plants [17]. Till now, we have not found any report on the FA composition of *Dendrobium* species.

FAs are nonvolatile and exhibit neither natural UV absorption nor fluorescence property. Therefore, GC or liquid chromatography (LC) method in combination with derivatization were the most often used methods for the analysis of them [18–23]. GC and GC–MS methods have been traditionally applied to the analysis of fatty acids since 1950s [24]. However, there are also some drawbacks of GC methods. For example, the long chain polyunsaturated fatty acids are unstable during the GC analysis. Besides, the often used derivatizing reagent such as boron trifluoride and diazomethane are dangerous to operate. In contrast, the HPLC analysis of FAs can be carried out under mild conditions. Thus, the risks of damaging heat-liable compounds are greatly reduced. Therefore, HPLC method can be used as an alternative to GC method for the accurate analysis of FAs.

In this study, a sensitive pre-column derivatization HPLC method with fluorescence detection was developed for the analysis of FAs. 2-(9-Oxoacridin-10(9H)-yl) acetohydrazide (OAAH) with excellent fluorescence properties was chosen as derivatization reagent because it is easy to synthesize. Besides, the derivatization of OAAH with FAs could be carried out under mild conditions within 20 min, much less than the often used 30 min of other derivatization methods [22, 23]. The proposed method was successfully applied to the analysis of total fatty acids (TFAs) and free fatty acids (FFAs) in four kinds of *Dendrobium* species. To the best of knowledge, this is the first report of the FAs of *Dendrobium* species.

## 2 Materials and methods

### 2.1 Reagents and chemicals

All FAs used as standards were of chromatographic grade and purchased from Sigma–Aldrich (USA). 1-Ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC), acetonitrile, methanol, ethanol, chloroform, and *n*-hexane were of HPLC grade and purchased from Sigma–Aldrich (USA). Pure distilled water was purchased from Watsons (Guangzhou, China). All other reagents used were of analytical grade unless otherwise stated. OAAH was synthesized in authors' laboratory according to the method described by Wang [25].

### 2.2 Instruments

The HPLC analysis was performed using an Agilent 1290 Series HPLC system, equipped with an on-line-degasser, a binary pump, an autosampler, and a thermostated column compartment. A fluorescence detector (model G1321B, Agilent, USA) was adjusted at wavelengths of 255 and 420 nm for excitation and emission. Chromatographic separation was achieved on a SB C18 column ($2.1 \times 50$ mm$^2$, 1.8 μm inner diameter, Agilent, USA). Solvent A was 5% acetonitrile in water and B was acetonitrile. The flow rate was constant at 0.25 mL/min and the column temperature was kept at 30°C. The gradient condition of mobile phase was as follows: 40–70% B from 0 to 15 min; 70–100% B from 15 to 28 min and then held for 2 min. The column was equilibrated with the initial mobile phase for 4 min before the next injection. The injection volume was 5 μL. A F7000 fluorescence spectrophotometer (Hitachi, Tokyo, Japan) was used to obtain the fluorescence excitation and emission spectra of the derivative.

### 2.3 Samples

Four kinds of dried stems of *Dendrobium* species were purchased from Zibu Tang drugstore in Hangzhou, China. Samples were milled and passed through a 0.25 mm sieve prior to analysis.

### 2.4 Synthesis of OAAH

The OAAH was synthesized according to the method described by Wang [25] in our laboratory with some modifications. The synthesis of commercial available acridone and 2-(9-oxoacridin-10(9H)-yl) acetic acid was abandoned, and the synthesis procedures of OAAH were reduced from 4 to 2 simple steps. The synthesis route is depicted in Fig. 1.

#### 2.4.1 Synthesis of ethyl-2-(9-oxoacridin-10(9H)-yl) acetate

2-(9-Oxoacridin-10(9H)-yl) acetic acid (5.0 g) and ethanol (150 mL) were mixed in a 250 mL round-bottom flask and stirred for 10 min at RT. A solution of 20 mL concentrated sulfuric acid was added drop wise within 10 min. The mixture was heated at 80°C for 10 h with vigorous stirring. After cooling, ethanol was removed by rotary vacuum evaporator. The residue was transferred into a glass beaker with 200 mL water. The result solution was neutralized to pH 7.0 with saturated $Na_2CO_3$ solution. The precipitated solid was

 

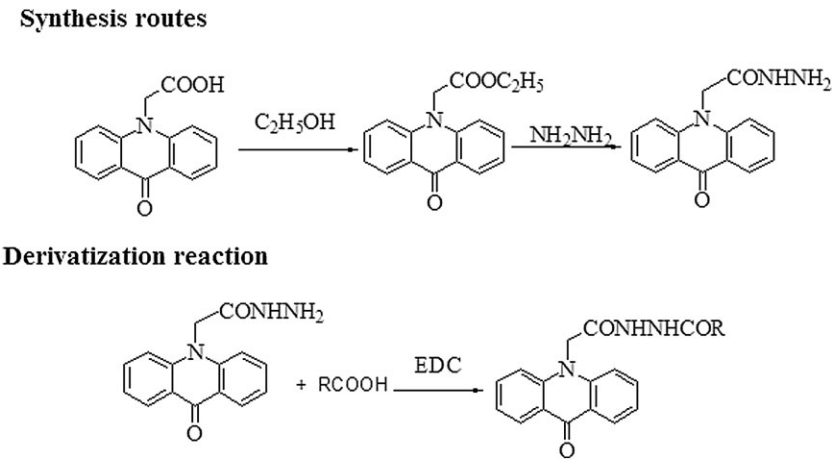


**Figure 1.** Synthesis routes of OAAH and derivatization scheme of OAAH with fatty acids.

recovered by filtration, washed three times with ethanol. The product was dried at RT for 48 h to obtain a yellow crystal, yield 85.2%.

### 2.4.2 OAAH

Ethyl-2-(9-oxoacridin-10(9H)-yl) acetate (5.6 g), 80% hydrazine hydrate (10 mL), ethanol (50 mL) were mixed in a 250 mL round-bottom flask, and reacted at 90°C for 6 h with vigorous stirring. After cooling, the precipitated solid was recovered by filtration and washed with ethanol. The crude product was dried at RT for 48 h and recrystallized from acetonitrile to afford a faint yellow crystal. Yield 4.3 g (82.0%). m.p. > 280°C. Found: C, 68.02; H, 5.65; N, 14.90. Calculated: C, 68.07; H, 5.71; N, 14.88. IR (KBr): 3327.12(N–H), 1662.71, 1631.01(C=O), 1597.65, 1607.44 (Ph–C=N–), 1489.83, 1460.46 (C–H), 750.51, 672.92 (Ar–H); MS: *m*/*z* $[M+H]^+$: 350.7.

## 2.5 Preparation of solutions

Individual stock solution of 100 mg/L for each fatty acid was prepared in dimethylformamide (DMF) and stored at 4°C in the dark. Standard solutions containing all compounds were mixed and diluted with acetonitrile. Working solutions of all compounds and calibration concentrations were prepared by appropriate dilution of the stock solutions on the day of analysis. OAAH solution ($1.0 \times 10^{-3}$ mol/L) was prepared by dissolving 3.5 mg OAAH in 10 mL acetonitrile. When not in use, all solutions were stored at 4°C in a refrigerator until HPLC analysis.

## 2.6 Sample preparation

The prepared sample (50.0 mg) was weighed in a 5 mL glass centrifuge tube and then mixed with 3 mL of petroleum ether. Extraction was performed with ultrasonication for 30 min. Sample was centrifuged at 4000 r/min for 10 min, then the supernatant was collected, and 2 mL of petroleum ether was added into the residue for further extraction. The twice supernatants were united, and 200 μL of the extract was evaporated to dryness under a gentle stream of nitrogen. The dried sample was ready for the analysis of FFAs, which were naturally present in the samples. For the analysis of TFAs, samples were saponified according to the method described by Zhao et al. [26] to convert FAs existed in ester form to molecular form. The saponified samples were then extracted by petroleum ether and evaporated to dryness for later derivatization.

## 2.7 Derivatization procedure

To a vial containing 100 μL of mixed standard of FAs or dried sample, 100 μL acetonitrile, 100 μL derivatization reagent solution and 10 μL (0.02 mol/L) EDC were added. The vial was sealed and allowed to react in a water bath at 85°C for 20 min. The derivatization procedure is shown in Fig. 1. After the reaction was completed, the mixture was cooled to RT. Acetonitrile solutions of 690 and 790 μL were respectively added to dilute the derivatized standard and sample solution to 1.0 mL. The diluted solution was syringe filtered using a 0.22 μm nylon filter and injected directly for HPLC analysis. Each sample was analyzed in three replicates.

## 2.8 Optimization of derivatization parameters

Derivatization parameters were optimized by changing one parameter at a time. The effect of OAAH concentration on derivatization was studied from 1- to 10-fold molar reagent excess to total molar fatty acids, whereas the influence of temperature was studied from 60 to 95°C. The molar ratio of EDC to carboxylic acids was studied in the range of 0.5–10. Parameters, which provided the highest responses, were chosen in later experiments.

 

### 2.9 Method validation

The analytical method was validated by linearity, limit of detection (LOD), accuracy, and precision. Calibration curve was constructed for each compound by plotting peak area versus concentration (2–200 ng/mL). All target compounds from extracted samples were injected in this concentration range. Higher concentrations were diluted to meet this standard. LODs were calculated at a signal-to-noise (S/N) ratio of 3. Quantitative analysis was carried out by using external standard method. Recoveries were carried out by spiking blank samples with three different concentrations of standard solutions. The recoveries were calculated based on the formula of (measured value − endogenous value)/added value × 100. Intra-day precision was determined by running a sample with spiked standard of 5.0 μg/g with six replicates, and inter-day precision was determined by running a sample with spiked standards at the same level with three replicates on three different days over a period of one week.

## 3 Results and discussion

### 3.1 Extraction of FAs from *Dendrobium* species

Various methods have been applied to the extraction of FAs [27–30]. Soxhlet extraction is widely used but it is time- and solvent-consuming. Supercritical fluid extraction is non-toxic and environmentally friendly, but the system is complicated and expensive, and a large sample amount was needed. Ultrasonication extraction is quite popular in many laboratories because of its simplicity. It was therefore applied in our experiment. Ethanol and petroleum ether were compared for their extraction efficiency. Recoveries of higher than 89% for FFAs were observed for both of the two extraction solvents. However, for the analysis of TFAs, petroleum ether showed better performance. Therefore, petroleum ether was chosen for the extraction.

### 3.2 Optimization of derivatization conditions

#### 3.2.1 Fluorescence properties of OAAH-FA derivative

For a HPLC method with fluorescence detection, it was important to study the fluorescence performance of the derivatives in different mobile phases. The HPLC analysis of FAs was performed in mobile phase composed of water and acetonitrile. Therefore, the fluorescence property of OAAH-FA derivative was studied by changing the volume ratios of acetonitrile to water. As shown in Fig. 2, no obvious red- or blue-shift was observed when solvents were changed from pure water to pure acetonitrile. The fluorescence intensity of the derivatives in mixed solvent of water and acetonitrile was higher than those in single water or acetonitrile. Therefore, solvents composed of water and acetonitrile were suitable for the analysis of OAAH-FA derivatives.

#### 3.2.2 Effects of EDC concentrations on derivatization

Owing to the excellent condensation and dehydration property, EDC became one of the most often used condensing agents for the condensation of hydrazide reagent with carboxylic acids in many studies [31–33]. EDC was reported to form a highly unstable activated acid intermediate, which can react easily with amine groups [33]. To achieve the complete reaction of FAs, the amount of EDC should be sufficient enough, but excess EDC is not a good choice due to the possible contaminations it may bring in. The molar ratios of EDC to carboxylic acids were then studied in the range of 0.5–10. As shown in Fig. 3, the fluorescence response increased when the EDC concentration was increased. The

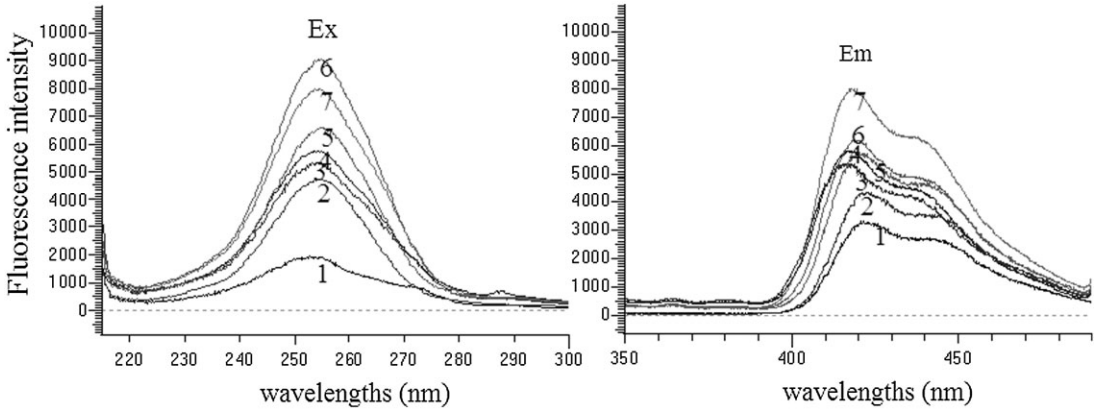


**Figure 2.** Fluorescence spectra of OAAH–C11 derivative in solvents composed of water and acetonitrile (1–7 were volume ratios of acetonitrile to water: 1:0; 0:1; 4:1; 2:1; 1:4; 1:2; 1:1).

 

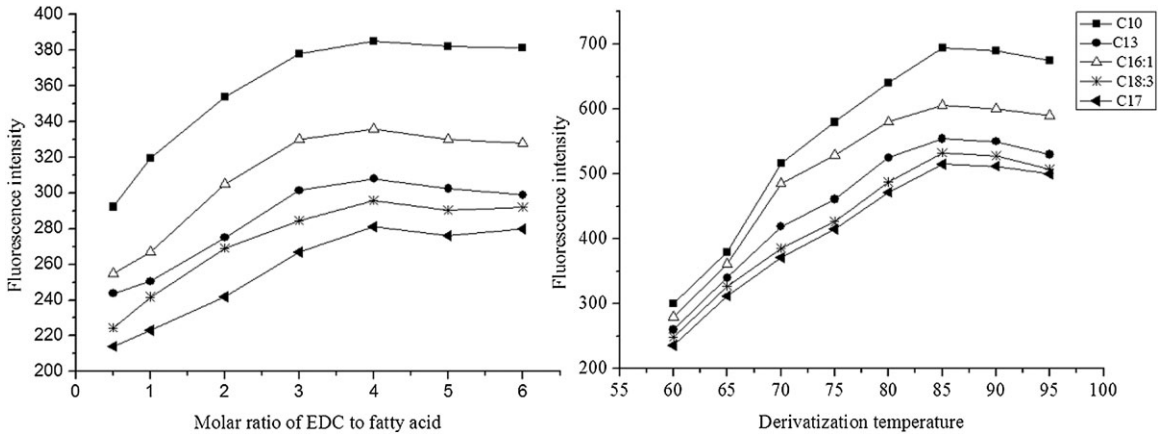


**Figure 3.** Effects of EDC concentrations and derivatization temperature on fluorescence intensity.

fluorescence intensity reached a maximum and was constant at the molar ratio of 4. It should be pointed out that EDC is sensitive to moisture and should be prepared everyday just before analysis.

### 3.2.3 Effects of OAAH concentration and reaction temperature on derivatization

The effects of OAAH concentrations on derivatization were studied in detail to ensure sufficient reaction of the analytes. The results indicated that constant fluorescence intensity was achieved with the addition of an eightfold molar reagent excess to total molar FAs. Further increasing the excess of reagent beyond this level had no significant effects on the yields. For the convenience of operation, $1.0 \times 10^{-3}$ mol/L OAAH was applied for derivatization. This concentration was sufficient enough for most samples because only 50 mg of sample was applied for analysis. The effect of the reaction temperature on derivatization was also evaluated. As can be seen from Fig. 3, maximum fluorescence intensity was obtained at 85°C. Lower reaction temperature needed longer reaction time to obtain steady response. When the temperature was higher than 90°C, the response decreased slightly. Maximum and constant fluorescence intensity was obtained by the reaction of OAAH with fatty acids at 85°C for 20 min. Thus, $1.0 \times 10^{-3}$ mol/L OAAH and 85°C were employed for derivatization.

### 3.2.4 Stability of OAAH and FA derivatives

Anhydrous acetonitrile solution of OAAH could be stored at 4°C for 2 wk without obvious decrease in derivatization yields for FAs compared to those newly prepared OAAH solution. The stabilities of the corresponding derivatives were also investigated. A standard solution containing 5 μg/L of FAs and a real sample solution were analyzed according to the procedures described above. These solutions were repeatedly analyzed by HPLC–FLD after being placed at RT for 0, 4, 8, 12, 24, 48, 72, and 96 h, respectively. The corresponding derivatives were stable for normalized peak areas with relative SDs (RSDs) of $<5.6\%$, and therefore it can be concluded that the stability of OAAH-FA derivatives is sufficient for chromatographic analysis.

## 3.3 Chromatographic separation

To get a good understanding of the FAs that may exist in *Dendrobium* species, 20 FAs were chosen in this paper. Fourteen of them are saturated fatty acids and 6 are unsaturated fatty acids (UFAs). Complete HPLC separation of the 20 FA derivatives could be achieved on a SB C18 column with good resolution and sharpest peaks. Mobile phase was simply consisted of acetonitrile and water and was sufficient enough to separate the 20 derivatives with good resolution and peak shapes. Thus, SB C18 column in conjunction with gradient elution with acetonitrile and water as mobile phase was applied in this paper.

## 3.4 Method valuation

The method was validated according to the procedures described in Section 2 and the results were listed in Table 1. The correlation coefficients were found to be $>0.996$, indicating excellent linearity of the analytes. Instrumental LODs calculated at a signal-to-noise ratio (S/N) of 3 were in the range of 0.20–0.85 ng/mL, while method LODs were in the range of 0.10–0.42 μg/g for the 20 FAs due to the low sample amount used and 500 times dilution. The intra-day precision for the tested samples was in the range of 2.7–5.8%, while the inter-day precision was between 3.9 and 7.6%. Accuracy of the method was measured by analyzing samples spiked with 2.0, 5.0, and 20 μg/g FAs, respectively. The results indicated that the recoveries of all FAs were in the range of 89.0–96.5%.

 

**Table 1.** The linearity, LOD, precision, and accuracy of the method

| Fatty acids | Correlation coefficient | Instrument LOD (ng/mL) | Method LOD (μg/g) | Recovery (%) | | | RSD (%) | |
|---|---|---|---|---|---|---|---|---|
| | | | | 2 μg/g | 5 μg/g | 20 μg/g | Intraday ($n = 6$) | Interday ($n = 9$) |
| C10 | 0.997 | 0.20 | 0.10 | 94.6 | 95.1 | 96.2 | 2.7 | 3.9 |
| C11 | 0.999 | 0.24 | 0.12 | 94.5 | 93.1 | 95.6 | 3.6 | 4.6 |
| C12 | 0.998 | 0.26 | 0.13 | 92.5 | 94.3 | 93.4 | 3.1 | 4.5 |
| C13 | 0.997 | 0.27 | 0.24 | 90.6 | 91.2 | 91.5 | 4.8 | 6.2 |
| C18:3n-3 | 0.998 | 0.30 | 0.15 | 95.3 | 96.1 | 96.5 | 5.0 | 7.3 |
| C14 | 0.998 | 0.24 | 0.12 | 94.1 | 95.3 | 96.4 | 4.5 | 5.3 |
| C16:1 | 0.998 | 0.25 | 0.12 | 92.6 | 93.3 | 92.7 | 2.9 | 4.7 |
| C15 | 0.997 | 0.29 | 0.14 | 91.5 | 92.6 | 93.8 | 3.4 | 4.8 |
| C18:2n-6 | 0.998 | 0.31 | 0.16 | 94.1 | 94.8 | 95.7 | 5.6 | 7.3 |
| C16 | 0.999 | 0.29 | 0.14 | 92.5 | 93.2 | 94.1 | 4.7 | 6.2 |
| C18:1n-9 | 0.998 | 0.40 | 0.20 | 91.8 | 92.4 | 93.5 | 3.5 | 4.8 |
| C17 | 0.998 | 0.40 | 0.20 | 90.5 | 91.7 | 92.4 | 4.6 | 6.1 |
| C18 | 0.998 | 0.51 | 0.26 | 92.4 | 93.2 | 93.6 | 3.4 | 4.7 |
| C20:1n-9 | 0.998 | 0.46 | 0.23 | 92.8 | 92.1 | 94.3 | 2.9 | 4.4 |
| C19 | 0.997 | 0.61 | 0.30 | 90.1 | 91.5 | 92.4 | 3.6 | 5.2 |
| C20 | 0.998 | 0.70 | 0.35 | 91.5 | 92.6 | 90.7 | 4.5 | 6.8 |
| C22:1n-9 | 0.998 | 0.70 | 0.35 | 90.2 | 90.5 | 91.6 | 5.1 | 7.4 |
| C21 | 0.998 | 0.75 | 0.38 | 90.6 | 91.4 | 92.2 | 5.8 | 7.6 |
| C22 | 0.997 | 0.80 | 0.40 | 89.5 | 89.0 | 90.7 | 4.8 | 6.8 |
| C23 | 0.997 | 0.85 | 0.42 | 89.7 | 89.2 | 89.4 | 5.3 | 7.1 |

RSD, relative standard deviation

### 3.5 Comparison of the proposed method with previously reported methods

GC and GC–MS methods played an important role in FA analysis, and the sensitivity obtained by some authors could be as low as 0.61 to 3.3 μg/g [19, 34]. The sensitivity of this method (0.10–0.42 μg/g) was on the same level or a bit higher than those of traditional GC and GC–MS methods. A series of fluorescence reagents have been synthesized in our laboratory and successfully applied to the analysis FAs. The merit of simplicity distinguished OAAH from them. For example, 2-(2-(anthracen-10-yl)-1H-phenanthro[9,10-d]imidazol-1-yl)ethyl 4-methylbenzenesulfonate (APIETS) [22] and 2-(12-oxobenzo[b]-acridin-5(12H)-yl)-ethyl-4-toluenesulfonate (BAETS) [23] had been successfully applied to the analysis of FAs, but the application of them was hampered by the complicated synthesis procedures. The synthesis of CPMS also needed only two steps [21], but the reaction time was 30 min, longer than the 20 min of this method. Therefore, the proposed method is simple and sensitive enough to be used as a good alternative to GC methods. It can be well applied in laboratories where GC is not available.

### 3.6 Application of the proposed method

The proposed method can be well applied to the analysis of FAs in various samples due to the high sensitivity and selectivity. It is especially suitable for the analysis of samples, which are rich in heat-liable FAs since the temperature applied in this method is much lower than those of GC methods. Besides FAs, compounds with carboxyl groups could also be analyzed by the proposed method. The reaction could be carried out under exactly the same conditions.

As an example, the proposed method was applied to the analysis of TFAs and FFAs in four kinds of *Dendrobium* species. Figure 4 shows a representative chromatogram of FA standard solution and a chromatogram of TFAs in *D. candidum*. As shown in Table 2, the unsaturated free fatty acids (UFFAs) and unsaturated total fatty acids (UTFAs) existed in *Dendrobium* species are mainly linoleic acid (C18:2n-6), oleic acid (C18:1n-9) and linolenic acid (C18:3n-3). Saturated free fatty acids and saturated total fatty acids are mainly hexadecanoic acid (C16), ctadecanoic acid (C18), myristic acid (C14), pentadecoic acid (C15), and arachidic acid (C20). C18:2n-6 was the most abundant UFA existed in the four kinds of *Dendrobium* species, while the concentration of C16 was higher than those of the other saturated FAs.

The most expensive *D. candidum* had the lowest amounts of FFAs and TFAs. Its UFAs constituted 45.9 and 57.9% of the FFAs and TFAs, respectively. The omega-6/omega-3(linoleic acid to linolenic acid) FFA ratio was the optimal ratio of 4 suggested by many researchers. For *D. devoninum*, whose quality and price were a bit lower than those of *D. candidum*, the UFA content was about 46.4% of the FFAs and 65.0% of the TFAs. Its omega-6/omega-3 FFA ratio was 6. *D. aphyllum* was the cheapest and most easily available, but its concentration

 

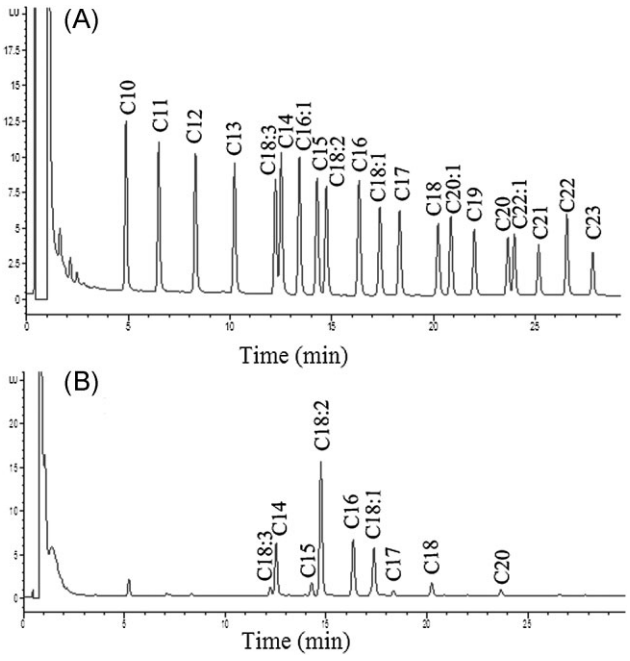


**Figure 4.** Chromatograms of fatty acid derivatives from (A) 20 fatty acid standards and (B) Chromatogram of FFAs in *D. candidum*. Peak labels: C10 (decoic acid); C11 (undecanoic acid); C12 (dodecanoic acid); C13 (tridecanoic acid); C18:3n-3 (linolenic acid); C14 (myristic acid); C16:1(2-hexadecenoic acid); C15 (pentadecanoic acid); C18:2n-6 (linoleic acid); C16 (hexadecanoic acid); C18:1n-9 (oleic acid); C17 (heptadecanoic acid); C18 (octadecanoic acid); C20:1n-9 (11-eicosenoic acid); C19 (nonadecanoic acid); C20 (arachidic acid); C22:1n-9 (13-docosenoic acid); C21 (heneicosoic acid); C22 (docosanoic acid); C23 (tricosanic acid).

of C18:2 (1003 μg/g), FFAs and TFAs were the highest among the four *Dendrobium* species. Its omega-6/omega-3 ratio was the highest value of 17. The results that different *Dendrobium* species vary both in their FA concentrations and in the omega-6/omega-3 FFA ratios is helpful for people to make informed choice according to their individual needs.

In conclusion, a sensitive HPLC method with OAAH as labeling reagent was developed for the analysis of FAs. The reaction of OAAH with FAs was fast and robust and could be finished under mild conditions. By introducing OAAH with excellent fluorescence property into the FA molecules, the HPLC sensitivity of FAs were greatly enhanced. Meanwhile, the sample amount was greatly reduced. These features are especially useful for the analysis of precious samples which are either very expensive or in great shortage. The usefulness of the proposed method was well exemplified in the analysis of FAs in *Dendrobium* species. The results indicated that *Dendrobium* species were mainly composed of linoleic acid (C18:2n-6) and hexadecanoic acid. The fact that different *Dendrobium* species show great differences in their FA contents will be helpful for prescription and diet. The omega-6/omega-3 FFA ratios of *Dendrobium* species were inversely proportional to their price. *Dendrobium* species with highest quality not only had the lowest content of FAs, but also had the lowest omega-6/omega-3 FFA ratio. This information will be helpful for the quality control of *Dendrobium* species. This method also shows powerful potential for the analysis of trace carboxylic acids from complex samples.

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*The authors have declared no conflict of interest.*

**Table 2.** Main contents of fatty acids in *Dendrobium* species ($n = 3$)

| | FFA (μg/g)[a)] | | | | TFA (μg/g)[a)] | | | |
|---|---|---|---|---|---|---|---|---|
| Fatty acid | *D. candidum* | *D. devoninum* | *Guanjie shihu*[a)] | *D. aphyllum* | *D. candidum* | *D. devoninum* | *Guanjie shihu*[b)] | *D. aphyllum* |
| C18:1n-9 | 39.8 | 51.9 | 26.0 | 102 | 241 | 618 | 334 | 295 |
| C18:2n-6 | 107 | 167 | 97.3 | 542 | 507 | 788 | 493 | 1003 |
| C18:3n-3 | 26.9 | 28.3 | 7.3 | 31.9 | 30.9 | 78.4 | 20.2 | 55.3 |
| C14 | 24.4 | 34.4 | 12.8 | 41.3 | 145 | 205 | 142 | 85.0 |
| C15 | 19.4 | 18.0 | 14.2 | 62.5 | 25.7 | 67.5 | 38.5 | 65.5 |
| C16 | 94.5 | 131 | 81.1 | 407 | 227 | 285 | 170 | 545 |
| C17 | 12.2 | 16.3 | 11.6 | 45.3 | 34.1 | 29.3 | 29.6 | 50.8 |
| C18 | 40.7 | 60.3 | 45.9 | 92.4 | 95.1 | 140 | 89.7 | 247 |
| C20 | 13.6 | 24.9 | 22.6 | 43.6 | 38.9 | 71.3 | 51.7 | 87.2 |
| UFAs | 174 | 247 | 131 | 676 | 779 | 1484 | 847 | 1353 |
| Sum of FAs | 378 | 532 | 319 | 1368 | 1345 | 2282 | 1369 | 2434 |

[a)]Concentration means μg/g sample.
[b)]A widespread new cultivated specie.

 

## References


[1] Zha, X. Q., Luo, J. P., Wei, P., Identification and classification of *Dendrobium candidum* species by fingerprint technology with capillary electrophoresis. *S. Afr. J. Bot.* 2009, *75*, 276–282.

[2] Xu, J., Guan, J., Chen, X. J., Zhao, J., Li, S. P., Comparison of polysaccharides from different Dendrobium using saccharide mapping. *J. Pharm. Biomed. Anal.* 2011, *55*, 977–983.

[3] Xiao, L., Ng, T. B., Feng, Y.-B., Yao, T. et al., Dendrobium candidum extract increases the expression of aquaporin-5 in labial glands from patients with Sjögren's syndrome. *Phytomedicine* 2011, *18*, 194–198.

[4] Yang, L., Wang, Z., Xu, L., Simultaneous determination of phenols (bibenzyl, phenanthrene, and fluorenone) in Dendrobium species by high-performance liquid chromatography with diode array detection. *J. Chromatogr. A* 2006, *1104*, 230–237.

[5] Zha, X.-Q., Luo, J.-P., Luo, S.-Z., Jiang, S.-T., Structure identification of a new immunostimulating polysaccharide from the stems of *Dendrobium huoshanense*. *Carbohydr. Polym.* 2007, *69*, 86–93.

[6] Zhang, G.-N., Zhong, L.-Y., Annie Bligh, S. W., Guo, Y.-L. et al., Bi-bicyclic and bi-tricyclic compounds from *Dendrobium thyrsiflorum*. *Phytochemistry* 2005, *66*, 1113–1120.

[7] Hsieh, Y. S. Y., Chien, C., Liao, S. K. S., Liao, S.-F. et al., Structure and bioactivity of the polysaccharides in medicinal plant *Dendrobium huoshanense*. *Biorg. Med. Chem.* 2008, *16*, 6054–6068.

[8] Bougnoux, P., Hajjaji, N., Maheo, K., Couet, C., Chevalier, S., Fatty acids and breast cancer: Sensitization to treatments and prevention of metastatic re-growth. *Prog. Lipid Res.* 2010, *49*, 76–86.

[9] Larsson, S. C., Kumlin, M., Ingelman-Sundberg, M., Wolk, A., Dietary long-chain n-3 fatty acids for the prevention of cancer: A review of potential mechanisms. *Am. J. Clin. Nutr.* 2004, *79*, 935.

[10] Calder, P., C., n-3 polyunsaturated fatty acids, inflammation, and inflammatory diseases. *Am. J. Clin. Nutr.* 2006, *83*, S1505.

[11] Freeman, M. P., Hibbeln, J. R., Wisner, K. L., Davis, J. M. et al., Omega-3 fatty acids: Evidence basis for treatment and future research in psychiatry. *J. Clin. Psychiatr.* 2006, *67*, 1954–1967.

[12] Arvindakshan, M., Supplementation with a combination of ω-3 fatty acids and antioxidants (vitamins E and C) improves the outcome of schizophrenia. *Schizophr. Res.* 2003, *62*, 195–204.

[13] Mozaffarian, D., Wu, J. H. Y., Omega-3 fatty acids and cardiovascular disease. *J. Am. Coll. Cardiol.* 2011, *58*, 2047–2067.

[14] Paradiso, V. M., Gomes, T., Nasti, R., Caponio, F., Summo, C., Effects of free fatty acids on the oxidative processes in purified olive oil. *Food Res. Int.* 2010, *43*, 1389–1394.

[15] Kuhnt, K., Degen, C., Jaudszus, A., Jahreis, G., Searching for health beneficial n-3 and n-6 fatty acids in plant seeds. *Eur. J. Lipid Sci. Technol.* 2012, *114*, 153–160.

[16] Hon, G. M., Hassan, M. S., van Rensburg, S. J., Abel, S. et al., Non-esterified fatty acids in blood cell membranes from patients with multiple sclerosis. *Eur. J. Lipid Sci. Technol.* 2012, *114*, 703–709.

[17] Bent, S., Ko, R., Commonly used herbal medicines in the United States: A review. *Am. J. Clin. Nutr.* 2004, *116*, 478–485.

[18] Schreiner, M., Quantification of long chain polyunsaturated fatty acids by gas chromatography. *J. Chromatogr. A* 2005, *1095*, 126–130.

[19] Takahashi, K., Goto-Yamamoto, N., Simple method for the simultaneous quantification of medium-chain fatty acids and ethyl hexanoate in alcoholic beverages by gas chromatography-flame ionization detector: Development of a direct injection method. *J. Chromatogr. A* 2011, *1218*, 7850–7856.

[20] Silva, L., Cachada, A., Pereira, R., Freitas, A. C. et al., Gas chromatography – Optical fiber detector for assessment of fatty acids in urban soils. *Talanta* 2011, *85*, 222–229.

[21] Zhang, S., You, J., Zhou, G., Li, C., Suo, Y., Analysis of free fatty acids in *Notopterygium forbesii* Boiss by a novel HPLC method with fluorescence detection. *Talanta* 2012, *98*, 95–100.

[22] Sun, Z., You, J., Song, C., Xia, L., Identification and determination of carboxylic acids in food samples using 2-(2-(anthracen-10-yl)-1H-phenanthro[9,10-d]imidazol-1-yl) ethyl 4-methylbenzenesulfonate (APIETS) as labeling reagent by HPLC with FLD and APCI/MS. *Talanta* 2011, *85*, 1088–1099.

[23] Jing, N., Shi, J., Li, G., Sun, Z., You, J., Determination of fatty acids from mushrooms using high performance liquid chromatography with fluorescence detection and online mass spectrometry. *Food Res. Int.* 2012, *48*, 155–163.

[24] Ruiz-Rodriguez, A., Reglero, G., Ibañez, E., Recent trends in the advanced analysis of bioactive fatty acids. *J. Pharm. Biomed. Anal.* 2010, *51*, 305–326.

[25] Wang, C. M. S., Thesis, Qufu Normal University, Qufu (China) 2011.

[26] Zhao, X., Liu, Y., Wang, H., Suo, Y., HPLC and GC–MS analysis of fatty acids in Tibetan medicine herpetospermum seed oil (in chinese). *Nat. Prod. Res. Dev.* 2009, *21*, 76–83.

[27] Rui, H., Zhang, L., Li, Z., Pan, Y., Extraction and characteristics of seed kernel oil from white pitaya. *J. Food Eng.* 2009, *93*, 482–486.

[28] Péres, V. F., Saffi, J., Melecchi, M. I. S., Abad, F. C. et al., Comparison of soxhlet, ultrasound-assisted and pressurized liquid extraction of terpenes, fatty acids and Vitamin E from Piper gaudichaudianum Kunth. *J. Chromatogr. A* 2006, *1105*, 115–118.

[29] Bernardo-Gil, M. G., Casquilho, M., Esquível, M. M., Ribeiro, M. A., Supercritical fluid extraction of fig leaf gourd seeds oil: Fatty acids composition and extraction kinetics. *J. Supercrit. Fluids* 2009, *49*, 32–36.

[30] Abbasi, H., Rezaei, K., Rashidi, L., Extraction of essential oils from the seeds of pomegranate using organic solvents and supercritical $CO_2$. *J. Am. Oil Chem. Soc.* 2007, *85*, 83–89.

[31] Saito, M., Ushijima, T., Sasamoto, K., Ohkura, Y., Ueno, K., 2-(5-Hydrazinocarbonyl-2-oxazolyl)-5, 6-dimethoxybenzothiazole as a precolumn fluorescence derivatization reagent for carboxylic acids in high-performance liquid chromatography and its application to the assay of fatty acids in human serum. *J. Chromatogr. B* 1995, *674*, 167–175.

[32] McKnight, T. E., Peeraphatdit, C., Jones, S. W., Fowlkes, J. D. et al., Site-specific biochemical functionalization along the height of vertically aligned carbon nanofiber arrays. *Chem. Mater.* 2006, *18*, 3203–3211.


 

[33] Wissink, M., Beernink, R., Pieper, J., Poot, A. et al., Immobilization of heparin to EDC/NHS-crosslinked collagen. Characterization and in vitro evaluation. *Biomaterials* 2001, *22*, 151–163.

[34] Yang, F. Q., Feng, K., Zhao, J., Li, S. P., Analysis of sterols and fatty acids in natural and cultured Cordyceps by one-step derivatization followed with gas chromatography–mass spectrometry. *J. Pharm. Biomed. Anal.* 2009, *49*, 1172–1178.

 