Analysis of free fatty acids in *Notopterygium forbesii* Boiss by a novel HPLC method with fluorescence detection

Shijuan Zhang, Jinmao You, Guoying Zhou, Chunli Li, Yourui Suo

**Abstract**

A new labeling reagent for fatty acids, 1-(9H-carbazol-9-yl) propan-2-yl-methanesulfonate (CPMS), has been synthesized and successfully applied to the HPLC determination of fatty acids in traditional Chinese herb *Notopterygium forbesii* Boiss. The reaction of CPMS with fatty acids could proceed easily and quickly in the presence of K₂CO₃ catalyst within 30 min. The derivatives exhibit excellent fluorescence property with excitation and emission wavelengths of 293 nm and 360 nm respectively. The 34 derivatives of fatty acids were separated on a BDS C8 reversed-phase column with gradient elution. Good linear correlations were observed for all fatty acids with correlation coefficients of >0.996. The detection limits at a signal-to-noise ratio of 3 were in the range 0.032–0.312 μg g⁻¹. Free fatty acids in the roots, stem, leaves and petioles of *N. forbesii* Boiss from different places were analyzed by the developed method. This is the first time that the fatty acids composition of *N. forbesii* Boiss has been reported. This method also shows powerful potential for the trace analysis of fatty acids or other carboxylic acids from complex samples.

**1. Introduction**

*Notopterygium forbesii* Boiss (NF), a plant belonging to the umbelliferae family, is known as Qianghuo in Chinese. It has been used for centuries as a common traditional Chinese medicine because of its anti-inflammatory, diaphoretic, and analgesic properties [1–3]. The rhizomes and roots of *Qianghuo* have been widely used for the treatment of common cold, rheumatism, and headache in folk, and at least 13 prescriptions used *Qianghuo* as ingredients [3]. In Hong Kong, NF is one of the five Chinese herbs in a formula used clinically to treat heroin addiction [4]. However, there has been limited research on the chemical constituents of *Qianghuo*. Till now, we have not found any report of the fatty acids in NF.

Fatty acids are present in all organisms and constitute essential structural elements of biological membranes. They play an important role in the prevention and treatment of cancer [5,6], retinitis pigmentosa [7], schizophrenia [8], inflammatory diseases [9], cardiovascular disease [10], and so on [11–14]. The pharmacological significance of fatty acids has attracted many people's attention such as nutritionists and pharmacists [15]. Many researches indicate that free fatty acids (FFAs) are indispensable components for the efficacy of medicinal plants [16,17]. Therefore, sensitive and accurate determination of fatty acids in traditional Chinese medicines (TCMs) is of great importance.

Since fatty acids showed neither natural UV absorption nor fluorescence property, direct LC methods were rarely applied to the analysis of them. Most of the methods applied to the analysis of fatty acids are gas chromatography(GC) [18–20] or gas chromatography–mass spectrometry (GC–MS) [21–24]. GC have been widely used in the micro-scale analysis of fatty acids in different research areas since 1950s [25]. However, there are some obvious drawbacks of GC methods. For example, long chain polyunsaturated fatty acids are unstable during the GC analysis, therefore, the accuracy was greatly affected [18]. It has been proposed that the injection technique, especially in vaporizing injectors, is the main source of error in quantitative GC [26]. Besides, the often used derivatizing reagent, such as boron trifluoride and diazomethane, are very dangerous or harmful. Compared with GC methods, the analysis of fatty acids by HPLC can be carried out at much lower temperature, therefore, the risk of damaging heat-labile compounds is greatly reduced. HPLC analysis also offers an alternative to GC for accurate quantitative analysis of fatty acids where GC is not available.

Sensitive HPLC analysis of fatty acids can be achieved by derivatization of the carboxyl moiety with a suitable chromophore or fluorophore [27–32]. In this paper, a new fluorescence reagent 1-(9H-carbazol-9-yl) propan-2-yl-methanesulfonate...
grade and purchased from Sigma Reagent Co. (USA). HPLC grade
2.2. Reagents and chemicals
be less than 1% during sample analysis process. The relative standard deviations (RSDs) in retention times should
dimethylsulfoxide (DMSO), potassium carbonate (K₂CO₃), tetrahy-
from Yuwang Company, China. Dimethylformamide (DMF),
m₅ min before the next injection. The injection volume was 10
0 to 20 min and then held for 10 min; 83–92% B from 30 to
gradient condition of mobile phase was as follows: 45–83% B from
sil BDS C8 column (200
m/i.d., Dalian Elite Analytical Instruments Co., Ltd., China). Solvent A was 5% acetonitrile in water and B was acetonitrile. The flow rate was constant at
1.0 mL min⁻¹ and the column temperature was kept at 30°C. The gradient condition of mobile phase was as follows: 45–83% B from
2.1. Instruments
The HPLC analysis was performed using an Agilent 1100 Series
HPLC system, equipped with an on-line-degasser, a quaternary
pump, an autosampler and a thermostated column compartment. A fluorescence detector (model G1321A, Agilent, USA) was
adjusted at wavelengths of 293 and 360 nm for excitation and emission. Chromatographic separation was achieved on a Hyper-
sil BDS C8 column (200 x 4.6 mm, 5 µm i.d., Dalian Elite Analytical Instruments Co., Ltd., China). Solvent A was 5% acetonitrile in water and B was acetonitrile. The flow rate was constant at
1.0 mL min⁻¹ and the column temperature was kept at 30°C. The gradient condition of mobile phase was as follows: 45–83% B from
0 to 20 min and then held for 10 min; 83–92% B from 30 to
50 min; 92–100% B from 50 to 55 min and then held for 15 min. The column was equilibrated with the initial mobile phase for 5
min before the next injection. The injection volume was 10 µL. The relative standard deviations (RSDs) in retention times should be less than 1% during sample analysis process.

2.2. Reagents and chemicals
All fatty acids used as standards were of chromatographic grade and purchased from Sigma Reagent Co. (USA). HPLC grade acetonitrile, methanol, ethanol and n-hexane were purchased from Yuwang Company, China. Dimethyldiformamide (DMF), dimethylsulfoxide (DMSO), potassium carbonate (K₂CO₃), tertaryl-
hydroxide (THF), pyridine, diethyl ether and chloroform were of analytical grade obtained from Shanghai Chemical Reagent Co. (Shanghai, China). Water was purified on a Milli-Q system (Millipore,
Bedford, MA, USA). All other reagents used were also of analytical grade unless otherwise stated. CPMS was synthesized in authors’ laboratory as described in the synthesis section and Fig. 1.

2.3. Samples
Wild NF 01 was collected from Tongde, Qinghai province. Wild
NF 02 was collected from Weiyuan, Gansu province. Cultivated NF
01 and 02 were from Qinghai province and Sichuan province,
respectively. According to the procedure described in Chinese Pharmacopoeia (2005 edition) [33], the leaves, root, petioles and stem of NF samples were dehydrated, milled and passed through 0.25 mm sieve prior to analysis.

2.4. Synthesis of CPMS
2.4.1. Synthesis of 1-(9H-carbazol-9-yl) propan-2-ol
Carbazole (16.7 g), KOH (7.0 g) and 2-butane (200 mL) were
mixed in a 500 mL round-bottom flask and rapidly cooled to 0°C
with ice-water by vigorous stirring. A freezing mixture of 1,
2-epoxyethane (5.8 g) in 50 mL of 2-butane solution was added
dropwise within 1 h. After stirring at 0°C for 2 h, the contents
were heated to 50°C for another 2 h and concentrated by a rotary evaporator. After cooling, the residue was transferred into 200 mL
of ice-water with vigorous stirring for 0.5 h. The precipitated solid
was recovered by filtration, washed with water and 30% aqueous ethanol. Solid was then dried at room temperature for 48 h. The crude product was recrystallized three times from methanol
(200 mL x 3) to afford a white crystal, yield 81.4%.

2.4.2. Synthesis of 1-(9H-carbazol-9-yl)
propan-2-yl-methanesulfonate (CPMS)
To a solution of 2.3 g methylsulfonyl chloride in 30 mL pyridine
(0°C) in a 100 mL round-bottomed flask, a mixture of 1-(9H-
carbazol-9-yl) propan-2-ol (4.5 g) in 50 mL of pyridine was added
dropwise within 30 min under vigorous stirring. After stirring
at 0°C for 4 h, the contents were left to stand at ambient temperature for another 4 h with vigorous stirring. The mixture was then transferred into 100 mL of ice water with vigorous stirring for 30 min; the precipitated solid was recovered by filtration, washed
with distilled water and then dried at ambient temperature for
48 h. The crude products were recrystallized twice from methanol
to give white crystals of 4.4 g, yield (71.7%), m.p. 128.5–128.8°C,
found (%): C 63.32, H 5.67, N 4.64, O 15.83, S 10.54; calculated (%): C 63.34, H 5.65, N 4.62, O 15.82, S 10.57. IR (KBr): 1598.74, 1559.74, 1465.15 (Ph), 1452.20, 1402.28 (C–H), 1125.75, 1348.51 (C–SO₂–); MS: m/z [M + H]⁺: 304.3.

2.5. Synthesis time and stability
The synthesis of CPMS could be finished within 6 days. A shorter synthesis time could be achieved by drying the product in
an oven at 50°C, but not at room temperature for 48 h. Under this
condition, CPMS could be synthesized within 3 days. CPMS is a
stable compound and can be placed at 4°C for long time storage. It could also be placed at room temperature for 5 months without any decomposition. Strong acidic or basic conditions should be avoided because CPMS may hydrolyze under such conditions.

2.6. Preparation of solutions
A mixed standard stock solution containing 34 fatty acids was
prepared by dilution of the corresponding stock solution (1.0 x
10⁻² mol L⁻¹) with acetonitrile. CPMS solution (1.0 x 10⁻² mol L⁻¹)
was prepared by dissolving 30.3 mg CPMS in 10 mL acetonitrile.
The corresponding low concentration of derivatization reagent solution (1.0 x 10⁻³ mol L⁻¹) was obtained by diluting the stock
solution with acetonitrile. When not in use, all solutions were stored at 4°C in a refrigerator until HPLC analysis.
2.7. Sample preparation

The prepared sample (30 mg) was weighed in a 5 mL glass centrifuge tube and then mixed with 2 mL of ethanol. Extraction was performed with ultrasonication for 30 min. Sample was centrifuged at 4000 r/min for 10 min, then the supernatant was collected, and 1 mL of ethanol was added into the residue for further extraction. The twice supernatant was united and evaporated to dryness under a gentle stream of nitrogen. Then it was redissolved in acetonitrile for the next analysis.

2.8. Derivatization procedure

To a solution containing a standard fatty acid mixture or sample in a 2 mL vial, 100 µL derivatization reagent solution, 10 mg K$_2$CO$_3$ and 100 µL DMF were added. The vial was sealed and allowed to react in a water bath at 90 °C for 30 min. The derivatization procedure is shown in Fig. 1. After the reaction was completed, the mixture was cooled to room temperature, then an appropriate volume of acetonitrile solution was added to dilute the derivatization solution to 0.6 mL. The diluted solution was syringe filtered using a 0.22 µm nylon filter and injected directly for HPLC analysis (10 µL).

3. Results and discussion

3.1. Optimization of derivatization conditions

3.1.1. Effect of co-solvents and basic catalysts on derivatization

CPMS was stable in common organic solvents. After keeping the stock solution of CPMA in acetonitrile at room temperature for 1 week, the corresponding derivatization yields for fatty acids did not change obviously with peak area deviations of less than 5%. Therefore, acetonitrile and some other solvents such as THF, DMF and DMSO were tested as co-solvents for the derivatization procedure. The results indicated that DMF gave the most intense fluorescence responses. The signal intensity obtained in DMF solution was about 140–200% of that in other solvents. In addition, DMF used as the derivatization co-solvent can avoid the problem of precipitation of hydrophobic long-chain fatty acid derivatives. It should be noted that the vial should be sealed during the derivatization process to avoid the evaporation of DMF at 90°C. Several basic catalysts, including pyridine, triethylamine, 4-dimethylaminopyridine (DMAP), K$_2$CO$_3$ and Na$_2$CO$_3$, were evaluated for their derivatization efficiency. The signal intensity obtained by using K$_2$CO$_3$ as catalyst was about 2–5-fold of that of the other catalysts. The effect of the K$_2$CO$_3$ amount on derivatization yield was also tested and maximum response was obtained when the usage of K$_2$CO$_3$ was 10 mg. Increasing the added amounts of K$_2$CO$_3$ to 15 mg, no increase in response was observed. However, when the amounts of K$_2$CO$_3$ exceeded 20 mg, a decrease in response was observed. Therefore, 10 mg K$_2$CO$_3$ was applied in all subsequent experiments.

3.1.2. Effect of CPMS concentration and temperature on derivatization

The effects of CPMS 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 a six-fold molar reagent excess to total molar fatty acids. Further increasing the excess of reagent beyond this level had no significant effect on the yields. The effect of the reaction temperature on derivatization was also evaluated. The result indicated that a lower reaction temperature needed a longer reaction time. However, when the temperature was increased to 100 °C, the response was slightly decreased. That is probably due to the partial hydrolysis of the products at 100 °C. Maximum and constant peak heights were obtained by the reaction of CPMS with fatty acids at 90 °C for 30 min. Thus, six-fold molar reagent excess and 90 °C were employed for derivatization.

3.2. Optimization of extraction

A lot of methods have been applied for the extraction of fatty acids, such as soxhlet extraction [34,35], supercritical fluid extraction [36,37] and ultrasonic extraction [28,35]. Though most of the conventional methods are in common with soxhlet extraction, they are time-consuming and require large amount of solvent. Supercritical fluid extraction is very popular in recent years because it is non-toxic and environmental friendly. However, the system is complicated and expensive. Recent study indicated that extraction yields of supercritical CO$_2$ extraction was the lowest compared with other methods [36]. Ultrasonic extraction is simple and available in many laboratories. Therefore, it was applied in our experiment. Four types of extraction solvents, including ethanol, chloroform–methanol (1:1, v/v), chloroform–methanol–water (2:1:0.8, v/v/v) and n-hexane–diethyl ether (1:1, v/v), were compared for the extraction efficiency. The results indicated that chloroform–methanol (1:1, v/v) was slightly better than the others for the extraction of linoleic acid, oleic acid and hexadecanoic acid. However, considering the toxicity of chloroform, nontoxic ethanol was selected for the extraction. Its extraction efficiency, though a bit lower than chloroform–methanol (1:1, v/v), was still satisfying (recovery > 85%).

3.3. Chromatographic separation

To get a good command of the fatty acids that may exist in NF, 34 fatty acids were chosen in this paper. 26 of them are saturated fatty acids and 8 are unsaturated fatty acids (UFAs). It is challenging to separate the 34 fatty acids in a plain HPLC column since unsaturated fatty acids often coelute with saturated fatty acids containing one or two fewer carbon atoms. C18 columns showed much better separation property than C8 columns for fatty acids. However, the separation time was quite long (more than 120 min). Therefore, C18 column was not considered for latter experiment. Several kinds of C8 columns have been compared for their separation efficiency. The results indicated that BDS C8 column in conjunction with a gradient elution could separate the 34 fatty acid derivatives within 68 min (see Fig. 2A). Thus, BDS C8 column was applied in this paper.

3.4. Method valuation

The method was validated for linearity, limits of detection (LOD), precision and accuracy. Linearity data was generated by plotting the peak areas versus concentrations in the range 2.0–100 ng mL$^{-1}$. 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 1.6–15.6 ng mL$^{-1}$, while method LODs were in the range of 0.032–0.312 µg g$^{-1}$ for the 34 fatty acids. Precision of the method was estimated by applying the whole procedure to real sample analysis in triplicate, and the obtained RSDs were ≤ 6.7%. Accuracy of the method was measured by analyzing samples spiked with 1.0 µg g$^{-1}$ fatty acids. The recoveries were calculated based on the formula of (measured value – endogenous value)/added value × 100. All analyses were carried out in triplicate. The results indicated that the recoveries of all fatty acids were in the range 85.3–96.2% (Table 1).
3.5. Analysis of fatty acids in NF

The proposed method was applied to the analysis of FFAs in the roots, stem, leaves and petioles of NF. Standard solution was injected after every 5 samples to compensate for the possible deviation in retention times. To get an accurate information of the identities of FFAs, the RSDs in retention times should be less than 1% as described above. Fig. 2 shows a representative chromatogram of FA standard solution and a chromatogram of FFAs in the root of wild NF. The results indicated that NF was rich in UFAs. The highest content of UFAs was found in the root of wild NF collected from Qinghai (82% of the total FFAs). The second highest content of UFAs was found in the root of wild NF collected from Gansu. The content of UFAs in the root of cultivated NF was much lower than that in wild plants, but their content were still higher than 60% of the total FFAs. Though the stems, leaves and petioles of NF were rarely applied for medical purpose, their UFAs contents still accounted for more than 53% of the total FFAs. The highest content of linolenic acid was found in the leaves of NF (266.6 mg g⁻¹), while the highest content of linoleic acid was found in the roots of NF (1155 mg g⁻¹). The UFAs existed in NF are mainly linoleic acid (C18:2), oleic acid (C18:1) and linolenic acid (C18:3). Saturated FFAs are mainly hexadecanoic acid (C16), octanoic acid (C8) and behenic acid (C22). Besides, there are small amounts of sheptanoic acid (C7), octanoic acid (C8), nonanoic acid (C9), pentadecanoic acid (C15) octadecanoic acid (C18), arachidic acid (C20), tricosanoic acid (C23) and so on. The compositional data for FFAs is shown in Table 2.

3.6. Comparisons of the proposed method with previously reported methods

To demonstrate the merit of the proposed method, the sensitivity of this method was compared with some other methods reported before. As shown in Table 3, the sensitivity of this method was much higher than those of GC [19], HPLC [38], GC–MS [22,23] and LC–MS [39] methods. CPMS was also compared with some other derivatizing reagents for their signal enhancement effects. The HPLC sensitivity of using CPMS as derivatizing reagent was higher than those applying traditional labeling reagents such as phenacyl bromide [29], 9-anthryldiazomethane [30] and 2-nitrophenylhydrazine [31]. In addition, the proposed method also shows the merit of less sample consumption. The sample amount used in this method is only 30 mg, therefore, it can be well applied in the analysis of FFAs in precious samples.

4. Conclusions

A novel fatty acid-reactive fluorescent labeling reagent (CPMS) has been well synthesized and successfully applied to the analysis of FFAs in NF. The synthesis procedure of CPMS contains only two
Table 1
Linearity, LODs, precision and accuracy for the method.

<table>
<thead>
<tr>
<th>Fatty acids</th>
<th>Correlation coefficient</th>
<th>Instrument LOD (ng mL⁻¹)</th>
<th>Method LOD (µg g⁻¹)</th>
<th>Recovery (%)</th>
<th>RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C5</td>
<td>0.99753</td>
<td>1.6</td>
<td>0.032</td>
<td>92.5</td>
<td>2.5</td>
</tr>
<tr>
<td>C6</td>
<td>0.99608</td>
<td>1.8</td>
<td>0.036</td>
<td>91.4</td>
<td>2.3</td>
</tr>
<tr>
<td>C7</td>
<td>0.99720</td>
<td>2.1</td>
<td>0.042</td>
<td>90.2</td>
<td>3.2</td>
</tr>
<tr>
<td>C8</td>
<td>0.99883</td>
<td>2.4</td>
<td>0.048</td>
<td>93.6</td>
<td>1.9</td>
</tr>
<tr>
<td>C9</td>
<td>0.99756</td>
<td>2.9</td>
<td>0.058</td>
<td>94.8</td>
<td>4.2</td>
</tr>
<tr>
<td>C10</td>
<td>0.99694</td>
<td>3.0</td>
<td>0.060</td>
<td>95.7</td>
<td>2.6</td>
</tr>
<tr>
<td>C11</td>
<td>0.99823</td>
<td>3.7</td>
<td>0.074</td>
<td>94.6</td>
<td>3.7</td>
</tr>
<tr>
<td>C12</td>
<td>0.99759</td>
<td>4.0</td>
<td>0.080</td>
<td>93.0</td>
<td>3.1</td>
</tr>
<tr>
<td>C13</td>
<td>0.99683</td>
<td>4.3</td>
<td>0.086</td>
<td>90.8</td>
<td>4.8</td>
</tr>
<tr>
<td>C18:3</td>
<td>0.99692</td>
<td>4.6</td>
<td>0.092</td>
<td>96.2</td>
<td>5.2</td>
</tr>
<tr>
<td>C22:6</td>
<td>0.99733</td>
<td>7.3</td>
<td>0.146</td>
<td>95.3</td>
<td>4.6</td>
</tr>
<tr>
<td>C20:4</td>
<td>0.99647</td>
<td>5.3</td>
<td>0.106</td>
<td>91.8</td>
<td>3.5</td>
</tr>
<tr>
<td>C16:1</td>
<td>0.99753</td>
<td>4.5</td>
<td>0.090</td>
<td>92.8</td>
<td>5.4</td>
</tr>
<tr>
<td>C18:2</td>
<td>0.99823</td>
<td>5.6</td>
<td>0.112</td>
<td>90.7</td>
<td>4.7</td>
</tr>
<tr>
<td>C15</td>
<td>0.99756</td>
<td>5.9</td>
<td>0.118</td>
<td>93.5</td>
<td>3.6</td>
</tr>
<tr>
<td>C20:1</td>
<td>0.99803</td>
<td>5.8</td>
<td>0.116</td>
<td>94.7</td>
<td>2.6</td>
</tr>
<tr>
<td>C19</td>
<td>0.99721</td>
<td>6.4</td>
<td>0.128</td>
<td>90.2</td>
<td>3.7</td>
</tr>
<tr>
<td>C20</td>
<td>0.99807</td>
<td>6.7</td>
<td>0.134</td>
<td>92.1</td>
<td>4.5</td>
</tr>
<tr>
<td>C22:1</td>
<td>0.99713</td>
<td>7.0</td>
<td>0.140</td>
<td>91.6</td>
<td>5.0</td>
</tr>
<tr>
<td>C21</td>
<td>0.99832</td>
<td>7.5</td>
<td>0.150</td>
<td>90.5</td>
<td>5.6</td>
</tr>
<tr>
<td>C22</td>
<td>0.99728</td>
<td>8.0</td>
<td>0.160</td>
<td>89.6</td>
<td>3.8</td>
</tr>
<tr>
<td>C23</td>
<td>0.99697</td>
<td>8.3</td>
<td>0.166</td>
<td>91.3</td>
<td>4.5</td>
</tr>
<tr>
<td>C24</td>
<td>0.99715</td>
<td>7.9</td>
<td>0.158</td>
<td>92.4</td>
<td>4.9</td>
</tr>
<tr>
<td>C25</td>
<td>0.99759</td>
<td>8.9</td>
<td>0.178</td>
<td>90.0</td>
<td>5.8</td>
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<tr>
<td>C26</td>
<td>0.99658</td>
<td>9.2</td>
<td>0.184</td>
<td>87.6</td>
<td>5.2</td>
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<tr>
<td>C27</td>
<td>0.99742</td>
<td>9.9</td>
<td>0.198</td>
<td>86.8</td>
<td>6.3</td>
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<tr>
<td>C28</td>
<td>0.99683</td>
<td>9.5</td>
<td>0.200</td>
<td>88.4</td>
<td>4.9</td>
</tr>
<tr>
<td>C29</td>
<td>0.99663</td>
<td>11.5</td>
<td>0.230</td>
<td>86.2</td>
<td>6.5</td>
</tr>
<tr>
<td>C30</td>
<td>0.99665</td>
<td>15.6</td>
<td>0.312</td>
<td>85.3</td>
<td>6.7</td>
</tr>
</tbody>
</table>

Table 2
Main content of free fatty acids in *Notopterygium forbesii* Boiss.

<table>
<thead>
<tr>
<th>Samples</th>
<th>Free fatty acids (µg g⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C8</td>
</tr>
<tr>
<td>NF (Qinghai, wild) Leaves</td>
<td>5.79</td>
</tr>
<tr>
<td>Petioles</td>
<td>5.11</td>
</tr>
<tr>
<td>Stem</td>
<td>8.25</td>
</tr>
<tr>
<td>Root</td>
<td>12.99</td>
</tr>
<tr>
<td>NF (Qinghai, cultivated) Root</td>
<td>2.37</td>
</tr>
<tr>
<td>NF (Gansu, wild) Root</td>
<td>17.82</td>
</tr>
<tr>
<td>NF (Sichuan, cultivated) Root</td>
<td>15.00</td>
</tr>
</tbody>
</table>
| *a* Total fatty acids listed in the table.

Table 3
Comparison of the proposed with other methods.

<table>
<thead>
<tr>
<th>Analytes</th>
<th>Sample amount</th>
<th>Method</th>
<th>Derivatizing reagent</th>
<th>LOD</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alcoholic beverages</td>
<td>500 µL</td>
<td>GC</td>
<td>–</td>
<td>0.61–1.27 µg g⁻¹</td>
<td>[19]</td>
</tr>
<tr>
<td>Bakery products</td>
<td>25 g</td>
<td>GC-MS</td>
<td>Sodium methyle in methanol</td>
<td>0.98–3.93 µg g⁻¹</td>
<td>[22]</td>
</tr>
<tr>
<td>Cordyceps</td>
<td>0.1 g</td>
<td>GC-MS</td>
<td>Trimethylsilyl</td>
<td>1.2–3.3 µg g⁻¹</td>
<td>[23]</td>
</tr>
<tr>
<td>Plasma</td>
<td>0.5 mL</td>
<td>HPLC</td>
<td>Phenacyl bromide</td>
<td>10 µmol L⁻¹</td>
<td>[29]</td>
</tr>
<tr>
<td>Serum</td>
<td>75 µL</td>
<td>HPLC</td>
<td>ADAM</td>
<td>10 µmol L⁻¹</td>
<td>[30]</td>
</tr>
<tr>
<td>Oil</td>
<td>1 mg</td>
<td>HPLC</td>
<td>2-NPH</td>
<td>15 µmol L⁻¹</td>
<td>[31]</td>
</tr>
<tr>
<td>Vegetable oils</td>
<td>100 mg</td>
<td>HPLC</td>
<td>–</td>
<td>0.1–0.6 µg g⁻¹</td>
<td>[38]</td>
</tr>
<tr>
<td>Shellfish</td>
<td>0.2 g</td>
<td>LC-MS</td>
<td>CPMS</td>
<td>30 µg g⁻¹</td>
<td>[39]</td>
</tr>
<tr>
<td><em>Notopterygium forbesii</em> Boiss</td>
<td>30 mg</td>
<td>HPLC</td>
<td>–</td>
<td>0.032–0.312 µg g⁻¹ or 0.31–1.1 µmol L⁻¹</td>
<td>This work</td>
</tr>
</tbody>
</table>

*a* No derivatizing reagent was used.

b ADAM, 9-anthryldiazomethane.

c 2-NPH, 2-nitrophenylhydrazine.
simple steps and can be synthesized in laboratories with slight experience. The proposed method showed much higher sensitivity than traditional methods in the analysis of FFAs and only trace amount of sample was needed. The result indicated that NF was rich in unsaturated FFAs. The content of UFAs in the roots of wild NF collected from Qinghai was much higher than those obtained from other places or cultivated ones. To the best of knowledge, this is the first time that FFAs in NF was reported. A possible drawback of the proposed method is the necessity to synthesize CPMS before analysis. However, the synthesis procedure is simple and the synthesized CPMS can be used for a long time.

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References