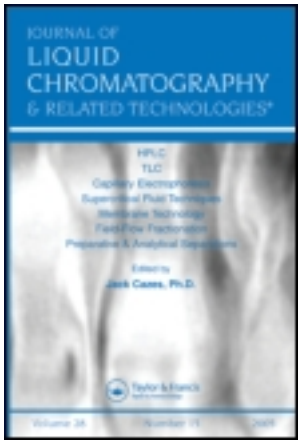

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QUANTITATIVE ANALYSIS OF FATTY ACIDS FROM SNOW LOTUS (*SAUSSUREA*) SPECIES USING HPLC WITH FLUORESCENCE DETECTION AND ATMOSPHERIC CHEMICAL IONIZATION-MASS SPECTROMETRY

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□ *Fatty acids (FA) are the basic and indispensable components in medicinal herbs. In the present work, a selective and sensitive method based on pre-column derivatization for the qualitative and quantitative analysis of FA from three snow lotus species (*S. involucrata, S. laniceps, *and* S. medusa*) was developed, and a comparative study based on the FA contents was carried out. FA were determined by HPLC with fluorescence detection, using 2-(12-oxobenzo[b]acridin-5(12H)-yl)-ethyl-4-toluenesulfonate (BAETS) as the labeling reagent and were identified with post-column APCI-MS. FA could be easily and quickly tagged by BAETS at 95°C in the presence of K_2CO_3 catalyst in N, N′-dimethylformamide (DMF). All validation procedures including linearity, limits of detection and quantification, precision, accuracy, ruggedness, and robustness were performed, and the results showed that the novel method exhibited excellent reproducibility and applicability. This proposed method was successfully applied to the analysis of total fatty acids (FA) and free fatty acids (FA) from snow lotus species. The content of FA in S. laniceps was significantly lower in comparison to the other two species that had similar FA content. Furthermore, this developed method exhibited a powerful potential for accurate detection of FA from other medicinal herbs or food stuff.*

Keywords a comparative study, BAETS, fatty acid analysis, HPLC-APCI-MS, pre-column derivatization, *Saussurea* species

INTRODUCTION

The genus *Saussurea* DC (Compositae) includes more than 400 species distributed throughout the Holarctic.[1,2] Approximately 264 species are

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found in China,[3] and some have long been used under the herbal name "snow lotus" for the treatment of rheumatic arthritis and gynecological disorders.[4–6] As a rare Tibetan medicine, snow lotus has attracted increasing attention for its significant curative effects and a series of phenolic compounds that provide consistent beneficial therapeutic effects.[7–10] However, the fatty acid (FA) composition of snow lotus remains unclear. FA, especially the essential ones, are important for human health because of their role in diverse physiological processes affecting normal health and chronic diseases, such as the regulation of cholesterol levels, insulin action, neural development, cardiovascular, and immune functions.[11] As a result, the investigation of FA composition is equally important to the pharmacological research of snow lotus and is also imperative for the quality control.[12]

Due to the absence of a specific chromophore, the majority of FA profiles are determined by GC after extraction and derivatization. The most common derivatization procedure is methylation of the combined FA to form their respective methyl esters in the presence of an acidic catalyst, such as boron trifluoride.[13] Although GC method is well-developed and fairly effective, there are some disadvantages, particularly with respect to heat-labile compounds.[14] Compared with GC, determination of FA derivatives by HPLC holds some unquestionable advantages. First, HPLC allows FA to be converted to a large number of different derivatives,[15] and derivatization can overcome some problems, such as tailing peaks and low detector sensitivity, by the formation of less polar compounds, which can be more easily analyzed by LC;[16] second, the main advantage of HPLC over GC is lower temperature during the analysis, which reduces the risk of isomerization of double bonds, and the possibility of fraction collection for further investigation;[17] and finally, availability of various solvents, column packing materials, and strong ultraviolet-absorbing or fluorescent molecules provides a high degree of selectivity and sensitivity for HPLC analysis.[17,18] Therefore, the mean of pre-column derivatization using different fluorescent labeling regents has been widely adopted to improve detection limits and to avoid matrix interferences. Until now, many fluorescent reagents for derivatization have been reported, but the results indicated that these reagents had limitations in their applications such as low detection sensitivity, short detection wavelengths, poor stability, tedious analytical procedure, and serious interferences in the biological sample analyses.[14] Thus, use of a new reagent for accurate analysis of FA is quite necessary. In the present study, a novel fluorescent reagent 2-(12-oxobenzo[*b*]acridin-5(12*H*)-yl)-ethyl-4-toluenesulfonate (BAETS) was used, which exhibited highly conjugated π-π systems and could give high fluorescent sensitivity.

The aims of the present work were: (1) to develop a selective and sensitive method for the determination of FA and FA in three species of snow lotus (*S. involucrata, S. laniceps,* and *S. medusa*) by using BAETS as the

pre-column labeling reagent with fluorescence detection coupled with online sensitive APCI-MS identification; and (2) to compare the composition of FA and FA in these three species and find the similarities and differences between species.

EXPERIMENTAL

Chemicals and Materials

S. involucrata was collected from Urumqi in Xinjiang Uighur Autonomous Region, China; *S. laniceps* was from Cuona in Tibet, China; and *S. medusa* was from Qilian in Qinghai Province, China. Taxonomic identification followed the literature[19] and representative voucher specimens were deposited at the herbarium of Northwest Institute of Plateau Biology, Chinese Academy of Sciences. Standards of seventeen FA were purchased from Sigma Reagent Co. (USA). HPLC-grade acetonitrile was 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 of analytical grade unless otherwise stated. BAETS was synthesized in our laboratory as described previously.[20]

Standards

Preparation of Standards Solution

FA standards for HPLC analysis at individual concentrations were prepared by the dilution of the corresponding stock solutions (0.01 mol L^{-1}) with DMF. The labeling solution (BAETS) was prepared by dissolving 22.15 mg of BAETS in 5 mL distilled DMF. Solutions were stored at 4°C prior to analysis.

Derivatization

Amounts of 50 mg anhydrous K_2CO_3, 30 μL mixed FA standard solution, 120 μL BAETS solution, and 150 μL DMF were consecutively added into a 2-mL vial. The vial was sealed and allowed to react in a water-bath at 95°C with shaking for 35 min. After the reaction was completed, the mixture was taken to cool at room temperature. Then, the derivatization solution was diluted with DMF and injected directly for chromatographic analysis. The procedure of derivatization is shown in Figure 1.

Samples

Preparation of Samples

Crude fat from snow lotus was extracted in a Soxhlet apparatus as follows: 5 g of sample (the dried aerial part) was refluxed with 350 mL of

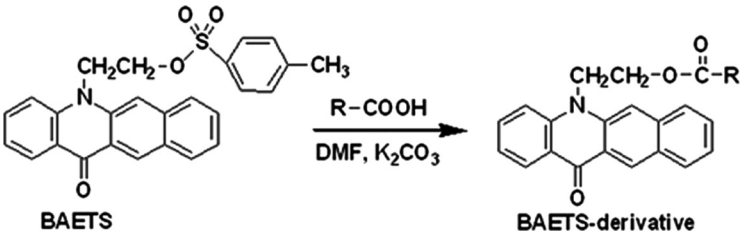

FIGURE 1 Scheme of derivatization reaction of 2-(12-oxobenzo[*b*]acridin-5(12*H*)-yl)-ethyl-4-toluenesulfonate (BAETS) with fatty acids.

petroleum ether at 75°C for 8 h. After that, the extract was concentrated to near dryness using a rotary evaporator at 60°C. The residue was further evaporated to dryness with a stream of nitrogen gas. The obtained crude fat (10 mg) was saponified,[21] re-dissolved in 2 mL DMF to estimate the contents of FA, while the unsaponified fat (50 mg) was directly dissolved in 2 mL DMF to detect the contents of FA.

Derivatization

Amounts of 50 mg anhydrous K_2CO_3, 20 μL sample solution (saponified or unsaponified), 130 μL BAETS solution, and 150 μL DMF were consecutively added into a vial. The vial was sealed and allowed to react in a water-bath at 95°C for 35 min.

HPLC-FLD-APCI-MS

The analysis was carried out with Agilent HP 1100 Series LC-MSDTrap SL liquid chromatography-mass spectrometry. The LC system was composed of a vacuum degasser (model G1322A), a quaternary pump (model G1311A), an autosampler (model G1329A), a thermostated column compartment (model G1316A), and a fluorescence detector (FLD) (model G1321A). FA-derivatives were separated on a reversed-phase Hypersil BDS C8 column (200 × 4.6 mm i.d, 5 μm) column by a gradient elution. The mobile phases consisted of acetonitrile-water (30:70) containing 0.1% ammonium formate buffer (solvent A) and 100% acetonitrile (solvent B). Gradient elution was performed as follows after injection: 50–90% B from 0 to 40 min; 90–100% B from 40 to 45 min; 100% B from 45 to 50 min. The flow rate was constant at 1.0 mL min^{-1} and the column temperature was kept at 30°C. The fluorescence excitation and emission wavelengths were set at λ_{ex} 272 nm and λ_{em} 505 nm.

The mass spectrometer was equipped with an atmospheric pressure chemical ionization (APCI) ion source and its conditions were as

follows: nebulizer pressure 413 kPa; dry gas temperature, 350°C; dry gas flow, 5.0 L min^{-1}. APCI Vap temperature 450°C; corona current (nA) 4000 (pos); capillary voltage 3.5 kV.

Validation of the Method

The analytical method was validated in terms of linearity, limits of detection and quantification (LODs and LOQs), precision, accuracy, ruggedness, and robustness.

Linearity

The calibration curves for BAETS-FA derivatives were established using triplicate sets of the FA standard mixture in the concentration range of 1.562–50 μ mol L^{-1}. The standard concentrations were 1.562, 3.125, 6.25, 12.5, 25, and 50 μ mol L^{-1}, respectively.

LODs and LOQs

LODs and LOQs were defined as the compound concentration that produced a signal-to-noise ratio of 3 (S/N = 3) and 10 (S/N = 10), respectively.

Precision

In order to estimate the instrumental precision, each derivatized standard (0.01 m mol L^{-1}) was injected three times. The precision of the chromatographic method was checked three times by applying the whole procedure, including saponification and derivatization to *S. involucrata* sample.

Accuracy

To evaluate the accuracy of the method, the recovery experiments were carried out by spiking *S. involucrata* with the FA standard mixture at levels of 1.562, 6.25, and 12.5 μ mol L^{-1}, and the overall process including saponification, derivatization, and injection was described in the Preparation of Samples procedure in the Experimental section.

Ruggedness and Robustness

Method ruggedness was estimated by comparison of the assay results that had been performed by two analysts. Robustness was investigated by varying several analytical conditions such as column temperature ±2°C, flow rate ±0.1 mL min^{-1}, pH of the eluent ±10% and capillary voltage ±0.2 kV. For all the tests, the *S. involucrata* sample was used.

RESULTS AND DISCUSSION

Stability of BAETS

The BAETS was stable in water and common organic solvents. When an anhydrous solution of BAETS in DMF was stored for two weeks in the refrigerator, the derivatization yields for FA were not obviously different. In addition, the heat stability tests for BAETS-FA derivatives were carried out at 95°C. Results showed that BAETS-FA derivatives were stable enough to be efficiently analyzed by HPLC.

Optimum Derivatization

According to our previous study,[22] DMF and K_2CO_3 were selected as the derivatization co-solvent and catalyst, respectively. With these conditions, the effects of BAETS concentrations, derivatization time, and temperature on derivatization yields were investigated.

The results indicated that constant fluorescent responses could be achieved with the addition of 8-fold molar reagent excess to total molar FA, further increasing the excess of reagent did not significantly alter detection responses. Thus, quantitative conversion of FA to their BAETS derivatives was guaranteed by using an excess of BAETS.

The optimum time for the reaction of BAETS with FA was evaluated in the time range of 10–50 min at 10 min time intervals. The fluorescence intensity of BAETS-derivatives increased with increasing reaction time. The constant fluorescent responses were achieved in the range of 30–40 min. With further increasing reaction time, there was no obvious increase in detector responses. In this experiment, the derivatization time of 35 min was selected.

When tested at different temperatures, the reaction was performed at 60, 70, 80, 90, and 100°C for 35 min, respectively. The peak heights for all FA became constant at 90–100°C. When below 90°C, the rate of reaction decreased and led to a long derivatization time. However, when the reaction temperature was set at 100°C with the reaction time of 35 min, the detector responses for the derivatized FA obviously decreased probably due to the fact that high reaction temperature resulted in the hydrolysis of derivatives. Therefore, derivatization temperature selected was set at 95°C.

LC-APCI-MS/MS for the FA-Derivatives

In order to obtain satisfactory chromatographic separation, several factors were investigated. Various columns including Hypersil C_{18} (200 mm ×

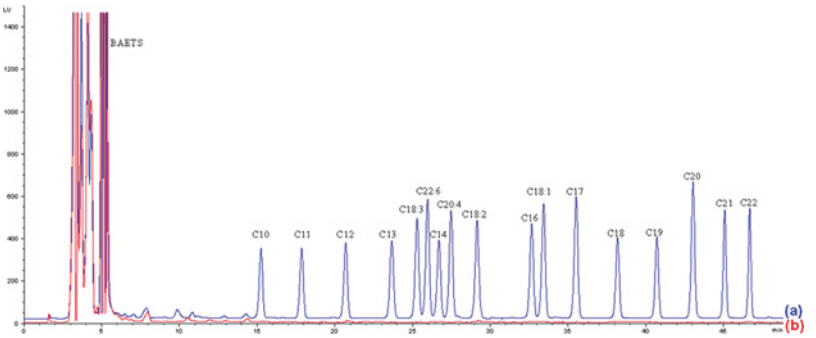

FIGURE 2 Chromatograms for fatty acid standard derivatives (a) and derivatization blank (b). (Color figure available online.)

4.6 mm, 5 μm), Hypersil BDS C_{18} (200 mm × 4.6 mm, 5 μm), Spherisorb C_{18} (200 mm × 4.6 mm, 5 μm), and Hypersil BDS C_8 (200 mm × 4.6 mm, 5 μm) were evaluated, and results indicated that Hypersil BDS C_8(200 mm × 4.6 mm, 5 μm) gave the best resolution. The solvents including methanol and acetonitrile as the mobile phases were also studied. Acetonitrile-water (30:70) containing 0.1% ammonium formate (solvent A) and 100% acetonitrile (solvent B) as the mobile phases gave the shortest separation time and the sharpest peak shapes (Figure 2).

The BAETS-FA derivatives were identified by mass spectrometry with APCI in positive-ion mode. The cleavage mode and MS, MS-MS analysis for a representative C18-derivative were shown in Figure 3. All molecular ions $[M+H]^+$ of seventeen FA derivatives were listed in Table 1. As expected, the BAETS-FA derivative exhibited an intense molecular ion peak at m/z $[M+H]^+$. The collision-induced dissociation spectra of BAETS-FA derivative produced the fragment ions at m/z 311.4, 290.5, 272.5, and 246.5. The specific fragment ion at m/z 290.5 $[M_1+H]^+$ (M_1 represented the molecular

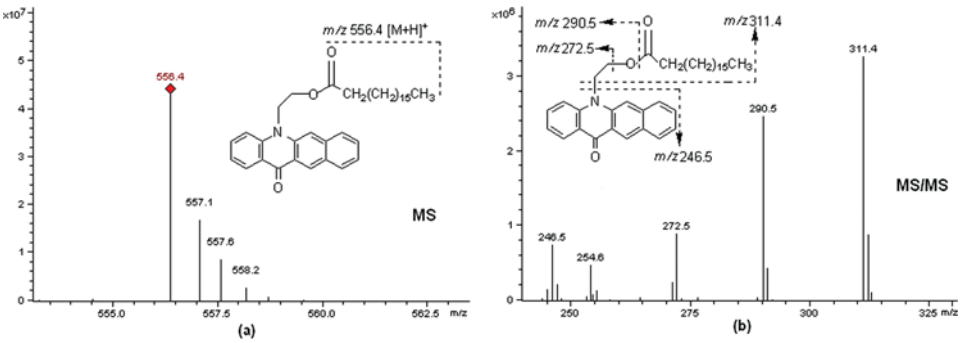

FIGURE 3 MS spectra of representative stearic acid (C18) derivative and its cleavage mode: (a) MS and (b) MS/MS. (Color figure available online.)

TABLE 1 Retention Times, Molecular Ions $[M+H]^+$, Calibration Curves, Correlation Coefficient, LODs, and LOQs for BAETS-Fatty Acid Derivatives and Precision Values, Accuracy Values for the Chromatographic Method

Fatty Acid	Retention Time (min)	$[M+H]^+$	Y = AX + B X: Injected Amount (pmol) Y: Peak Area	Correlation Coefficient r^2	LODs[a] (fmol)	LOQs[b] (fmol)	Precision		Accuracy[c]	
							Instrument Precision[d] RSD (%) ($n=3$)	Method Precision[c] RSD (%) ($n=3$)	Recovery (%)	RSD (%)
C10	15.24	443.9	Y = 10.61X +67.48	0.9996	12.32	39.87	1.12	2.14	93.57	2.13
C11	17.86	457.9	Y = 10.54X +111.46	0.9991	13.44	43.35	1.07	3.11	96.34	1.68
C12	20.69	471.9	Y = 11.57X +104.15	0.9993	12.67	40.84	0.97	2.27	92.89	2.05
C13	23.65	486.0	Y = 12.29X +103.14	0.9992	13.18	41.43	0.78	2.34	94.53	1.16
C18:3	25.28	550.1	Y = 14.99X +142.28	0.9990	9.34	29.79	0.84	2.58	102.03	1.78
C22:6	25.95	600.4	Y = 17.60X +134.06	0.9991	14.56	47.07	1.23	2.10	98.41	2.33
C14	26.69	500.0	Y = 11.81X +93.95	0.9993	16.23	51.68	1.05	3.69	95.66	1.89
C20:4	27.46	576.4	Y = 16.65X +90.71	0.9997	16.48	53.44	1.17	2.74	94.12	1.97
C18:2	29.14	552.2	Y = 15.27X +142.26	0.9991	11.89	37.51	0.96	2.63	97.37	2.56
C16	32.65	528.4	Y = 14.58X +95.93	0.9998	8.76	27.23	0.86	2.55	99.05	2.24
C18:1	33.41	554.1	Y = 17.32X +145.70	0.9993	11.25	37.14	0.91	2.19	93.94	1.59
C17	35.51	542.1	Y = 19.62X +77.42	0.9998	12.73	41.78	1.18	2.83	101.22	1.83
C18	38.17	556.4	Y = 12.06X +102.87	0.9990	12.10	38.17	0.81	3.41	100.96	2.44
C19	40.70	570.1	Y = 11.91X +98.43	0.9995	12.81	41.26	1.29	3.12	97.62	2.38
C20	43.03	584.0	Y = 19.55X +162.80	0.9994	18.21	58.01	0.99	2.21	93.91	1.87
C21	45.07	598.1	Y = 13.35X +76.01	0.9998	13.78	44.12	1.21	2.89	101.28	1.93
C22	46.67	613.5	Y = 12.16X +75.44	0.9996	11.76	36.85	1.13	2.77	103.17	2.41

[a] S/N = 3.
[b] S/N = 10.
[c] Estimated with the dried area part of *S. involucrata*.
[d] Estimated with standards.

mass of benzoacridone-5-ethanol) was from the cleavage of O-CO bond of the N-linked side chain, corresponding to the protonated benzoacridone-5--ethanol moiety. The characteristic fragment ion at m/z 272.5 $[M_1+H-H_2O]^+$ was from the cleavage of C-OCO bond (corresponding benzoacridone-5-ethanol lose one H_2O molecule). The specific fragment ion at m/z 246.5$[M_1-CH_2CH_2OH]^+$ was from the cleavage of N-C bond of the N-linked side chain. The specific fragment ion at m/z 311.4$[M+H-M_2]^+$ corresponded to the FA ethyl ester moiety (M_2 represented the molecular mass of benzoacridone).

Method Validation

Calibration curves were established using the regression of the peak area versus concentration of the each FA standard, and were listed in Table 1. All of the FA were found to give excellent linear responses at the concentration range of 1.562–50 μmol L^{-1} with coefficients >0.9990 (Table 1). LODs and LOQs provided by the proposed method ranged from 8.76–18.21 and 27.23–58.01 fmol, respectively (Table 1). The high sensitivity would enable us to accurately determine the FA from snow lotus. Furthermore, method precision, as well as instrument precision, was checked as previously described in Validation of the Method section and expressed as relative standard deviation (RSD %); the validation was in the range of 2.10–3.69% and 0.78–1.29%, respectively, indicating that the method and instrument precision was satisfactory. Moreover, the recovery experiments were performed in order to evaluate the accuracy of the method, results of which were summarized in Table 1. In addition, regarding ruggedness and robustness, slight variations of the analytical conditions and operations of different analysts did not significantly influence chromatographic retention times and peak areas. Such results further demonstrated that this method was reproducible and applicable.

Analysis of FA in *S. involucrata, S. laniceps,* and *S. medusa*

As a specific application of the proposed method, FA and FA in *S. involucrata, S. laniceps,* and *S. medusa* were analyzed. All the investigated FA were identified by their retention times and simultaneously confirmed by the post-column online APCI-MS in positive-ion mode. The typical chromatogram of these three species was showed in Figure 4, and FA contents were summarized in Table 2. Total contents of FA and FA in *S. laniceps* were 684.35 and 1416.46 μg g^{-1}, respectively, which were significantly lower than those obtained by *S. involucrata* and *S. medusa*. There were no obviously significant differences between *S. involucrata* and *S. medusa*. FA contents in *S.*

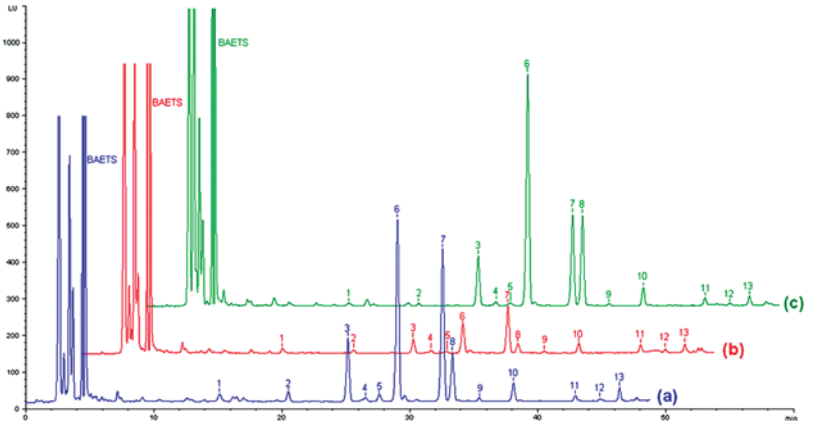

FIGURE 4 Chromatogram of free fatty acid derivatives from *Saussurea involucrata* (a), *S. laniceps* (b), and *S. medusa* (c). (1) capric acid (C10); (2) lauric acid (C12); (3) linolenic acid (C18:3); (4) myristic acid (C14); (5) arachidonic acid (C20:4); (6) linoleic acid (C18:2); (7) palmitic acid (C16); (8) oleic acid (C18:1); (9) heptadecanoic acid (C17); (10) stearic acid (C18); (11) arachidic acid (C20); (12) heneicosoic acid (C21); and (13) behenic acid (C22). (Color figure available online.)

TABLE 2 Content of FA and FA in *S. involucrata, S. laniceps,* and *S. medusa* (μg/g Dried Area Part)

		FFA			TFA		
Species		*S. involucrata*	*S. laniceps*	*S. medusa*	*S. involucrata*	*S. laniceps*	*S. medusa*
SFAs	C10	38.17	19.18	10.97	115.25	24.10	30.86
	C11	n[b]	n[b]	n[b]	n[b]	n[b]	n[b]
	C12	42.37	3.39	1.57	171.13	26.99	5.23
	C13	n[b]	n[b]	n[b]	n[b]	n[b]	n[b]
	C14	13.33	7.73	12.03	51.57	45.18	22.70
	C16	880.90	255.80	479.31	1912.97	549.64	1173.94
	C17	6.53	0.29	2.52	8.35	15.74	6.66
	C18	119.60	55.55	106.90	346.58	173.55	253.62
	C19	n[b]	n[b]	n[b]	n[b]	n[b]	n[b]
	C20	9.59	15.65	14.59	28.63	48.59	34.41
	C21	8.04	9.61	5.75	22.87	15.59	20.17
	C22	77.58	61.14	62.62	149.42	97.72	108.10
	Subtotal	1196.11	428.34	696.26	2806.77	997.10	1655.69
MUFAs	C18:1	238.28	30.22	433.08	490.61	88.54	1206.14
	Subtotal	238.28	30.22	433.08	490.61	88.54	1206.14
PUFAs	C18:2	1078.46	156.89	1304.91	2433.82	206.55	3224.23
	C18:3	358.09	67.25	266.09	922.38	96.56	575.98
	C20:4	36.35	1.65	15.46	68.89	6.16	26.92
	C22:6	n[b]	n[b]	n[b]	19.73	21.55	13.60
	Subtotal	1472.90	225.79	1586.46	3444.82	330.82	3840.73
Total content[a]		2907.29	684.35	2715.80	6742.20	1416.46	6702.56

FFA: free fatty acid; TFA: total fatty acid, that is, the sum of conjugated fatty acid and free fatty acid; SFA: saturated fatty acid; MUFA: monunsaturated fatty acid; PUFA: polyunsaturated fatty acid; nd: not detected.

[a]the sum of total fatty acids or free fatty acids.

[b]not detection.

involucrata and *S. medusa* were 2907.29 and 2715.80 $\mu g\,g^{-1}$, respectively, while FA contents of them were 6742.20 and 6702.56 $\mu g\,g^{-1}$. As can be seen from Table 2, unsaturated fatty acid (UFAs) levels were higher than saturated fatty acids (SFAs) in *S. involucrata* and *S. medusa*. On the contrary, SFAs in *S. laniceps* were predominant. Although FA levels exhibited some very significant differences, the main FA containing palmitic (C16), stearic (C18), behenic (C22), oleic (C18:1), linoleic (C18:2), and linolenic (C18:3) acids in the three snow lotus species were similar (Table 2).

In the present work, seventeen FA were investigated. Total content of SFAs calculated as the sum of twelve different FA were lowest in *S. laniceps* (997.10 $\mu g\,g^{-1}$) and highest in S. *involucrata* (2806.77 $\mu g\,g^{-1}$). Concerning monounsaturated fatty acids (MUFAs), only oleic acid (C18:1) was investigated, the content of which was lowest in *S. laniceps* (88.54 $\mu g\,g^{-1}$) and highest in *S. medusa* (1206.14 $\mu g\,g^{-1}$). Total polyunsaturated fatty acids (PUFAs) calculated as the sum of four different FA were lowest in *S. laniceps* (330.82 $\mu g\,g^{-1}$) and highest in *S. medusa* (3840.73 $\mu g\,g^{-1}$). Furthermore, the results revealed that the main SFAs were palmitic (C16), stearic (C18), and behenic (C22) acids. It was found that palmitic acid (C16) was the most abundant SFA in *S. involucrata, S. laniceps,* and *S. medusa* samples containing a high concentration of 1912.97, 549.64, and 1173.94 $\mu g\,g^{-1}$, respectively. Stearic acid (C18) was the next most abundant SFA with the concentration of 346.58, 173.55, and 253.62 $\mu g\,g^{-1}$, respectively. Behenic acid (C22) was the third most abundant SFA and the concentration was 149.42, 97.72, and 108.10 $\mu g\,g^{-1}$, respectively. As for oleic acid (C18:1), *S. medusa* revealed to be the richest species, whereas *S. laniceps* was the poorest one. Four different PUFAs including linoleic (C18:2), linolenic (C18:3), arachidonic (C20:4), and docosahexaenoic (C22:6) acids were investigated. Linoleic acid (C18:2) exhibited the highest level among the four FA and presented at the concentrations of 2433.82, 206.55, and 3224.23 $\mu g\,g^{-1}$ in *S. involucrata, S. laniceps,* and *S. medusa*, respectively. Linolenic acid (C18:3) also exhibited relatively high level, recorded at 922.38, 96.56, and 575.98 $\mu g\,g^{-1}$, respectively. However, arachidonic (C20:4) and docosahexaenoic (C22:6) acids were detected in lower amounts (see Table 2).

The FFA composition was also investigated (Table 2). Linoleic (C18:2) and linolenic (C18:3) acids were obtained in relatively high amounts in the snow lotus samples. Content of linoleic acid (C18:2) in S. *involucrata, S. laniceps,* and *S. medusa* was 1078.46, 156.89, and 1304.91 $\mu g\,g^{-1}$, respectively, while content of linolenic acid (C18:3) was 358.09, 67.25, and 266.09 $\mu g\,g^{-1}$, respectively. In addition to linoleic (C18:2) and linolenic (C18:3) acids, palmitic (C16), oleic (C18:1), stearic (C18), and behenic (C22) acids were the other abundant FA in the snow lotus. Beyond these, other FA were detected in low amounts and undecanoic (C11), tridecanoic

(C13), nonadecanoic (C19), and docosahexaenoic (C22:6) acids were even not detected using the proposed method.

CONCLUSION

A selective and sensitive method for the determination of FA using BAETS as pre-column derivatization reagent has been developed and validated in the present work. With the optimum derivatization and chromatographic separation conditions, seventeen FA were separated with good resolution. LODs and LOQs were in the femtomole range, and the chromatographic method showed better reproducibility and applicability. This proposed method was successfully applied to the qualitative and quantitative analysis of FA from snow lotus species. The results indicated that the content of FA in *S. laniceps* was significantly lower in comparison to the other two species that had similar FA content. But, the main FA containing palmitic (C16), stearic (C18), behenic (C22), oleic (C18:1), linoleic (C18:2), and linolenic (C18:3) acids in the three snow lotus species were similar. It is believed that this work would facilitate the use of snow lotus in pharmaceutical application and contribute to the quality control. Additionally, this developed method could be suitable for the routine analysis of FA from other medicinal herbs or foodstuff as it has been successfully applied to the determination of FA from snow lotus.

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