Asian Journal of Chemistry; Vol. 25, No. 4 (2013), 1866-1870

ASIAN JOURNAL OF CHEMISTRY

http://dx.doi.org/10.14233/ajchem.2013.13204

Simultaneous Analysis of Fatty Acids in *Rubus niveus* Thunb. Fruits by HPLC-MS/MS

REZENG CAIDAN[1,2], LIMAO CAIRANG[3,4], LIUBIN[1,*] and SUO YOURUI[3]

[1]Department of Chinese Medicine Chemistry, Beijing University of Chinese Medicine, Beijing, P.R. China
[2]The research Center of Chinese and Tibetan Medicine, Medicine College of Qinghai University, Xining, P.R. China
[3]Northwest Plateau Institute of Biology, Chinese Academy of Sciences, Xining, P.R. China
[4]Department of Chemistry Science, College of Nationality Normal, Qinghai Normal University, Xining, P.R. China

*Corresponding author: Fax: +86 10 84738611; Tel: +86 10 84738629; E-mail: liubinyn67@163.com

(*Received*: 8 December 2011; *Accepted*: 5 October 2012) AJC-12238

The aim of this study was to optimize the microwave extraction conditions of *Rubus niveus* Thunb. oil from Qinghai-Tibetan plateau *via* Box-Behnken design of RSM and then analyze the composition of oil by HPLC-MS/MS using 2-(12-oxobenzo[b]acridin-5(12*H*)-yl)ethyl-4-toluene-sulfonate as labeling reagent. The optimum microwave extraction conditions were as follows: irradiation power 437 W, extraction temperature 75 ºC and extraction time 12 min. Under these optimized conditions, the maximum oil extraction yield was 5.83 %. All fatty acid derivates had excellent linear responses with correlation coefficients greater than 0.9990. Detection limits (at signal-to-noise of 3:1) were 18.42 to 32.39 pmol. The results indicate that the predominant fatty acids (C10-C22) were 16:0, 18:2, 18:3, 18:0 and 22:0 in total fatty acids. And the unsaturated fatty acids were 18:3, 18:2, 18:1 and 20:4 and reached up to 40.54 % of the total mass contents of fatty acids (C10-C22) in *Rubus niveus* Thunb. from Qinghai-Tibetan plateau.

Key Words: *Rubus niveus* Thunb. fruit, Oil extraction, Response surface methodology, Fatty acids, HPLC-MS.

INTRODUCTION

Rubus niveus Thunb. is a perennial, prickly bush of the genus Rubus in family Rosaceae, generally distributed throughout the temperate Himalayan region. Beside India, *Rubus niveus* Thunb. grows in the cooler areas such as central and western China and the Philippines Islands[1]. *Rubus niveus* Thunb. fruits are sweet with a fine blend of acid, which not only have very pleasant and distinctive flavors, but also have antiphlogistic, analgesic, antidotal and antitumourous properties in Tibetan medicine. Therefore, *Rubus niveus* Thunb, fruit is very much liked by the native people and is processed into jelly, jam and syrup used in folk medicine. But *Rubus niveus* Thunb. fruit is perishable and cannot be kept under room temperature for more than 24 h. It is reported that fruits contain 58.5 % extractable juice with 3.79 mg of vitamin C per 100 mL of juice and 0.769 % mineral, 1.35 % proteins in fruit[2]. While the fatty acids composition in *Rubus niveus* Thunb. remains poorly investigated. Especially, about the unsaturated fatty acids which are easy to change by air is reported seldom. Thus, the accurate determination of fatty acids is important for better development and application of *Rubus niveus* Thunb. fruits.

Fatty acids show little UV absorption and no fluorescence response, accurate analysis of them using absorption spectrum is fairly difficult. The most used method for fatty acid analysis is based on GC or GC/MS. In contrast with GC, use of HPLC allows the fatty acids to be modified by large number of different derivatives[3] and that can overcome some problems to be more easily analyzed by LC. Therefore, derivatization of these analyses with labeling reagents, especially for the fluorescence detection, has been widely adopted. However, some reagents include 2-(2,3-anthracene-dicarboximido)ethyltrifluoromethanesulfonate (AE-OTF)[4]; 9-anthryldiazome thane (ADAM) and 4-(1-methylphenanthro [9,10]imidazole-2-yl) benzohydrazide (MPIB) reported have many limitations such as short detection wavelengths, poor stability, low detection sensitivity, tediously analytical procedure and serious interferences in the biological sample analyses in their applications[5-7]. 2-(12-Oxobenzo[b] acridin-5(12*H*)-yl) ethyl 4-toluene-sulfonate (BAETS) has advantages than many reported labeling reagents[8].

The main objectives of the presented study were: (1) to optimize the microwave- assisted extraction conditions by *n*-hexane including extraction temperature, irradiation power and extraction time for *Rubus niveus* Thunb. oil using a Box-Behnken design of RSM and obtain the optimum extraction parameters; (2) to analyze the fatty acids of extraction oil in *Rubus niveus* Thunb. using HPLC-MS/MS with sensitive and selective pre-column derivatization.

EXPERIMENTAL

Fruits of *Rubus niveus* Thunb. were obtained from Linzhi (Tibet, China). Mature fruits were harvested in September 2010.The fruit were cleaned, dried, ground and then stored in polyethylene bags until analysis.

Agilent 1100 HPLC-MS series high-performance liquid chromatography system comprises a G1322A quaternary pump, a G1329A auto-sampler, a G1316A thermo-stated column oven and a G1321A fluorescence detector (FLD). Derivatives were separated on a reversed phase Eclipse XDB-C_8 column (150 mm × 4.6 mm, 5 μm, Agilent). The mass spectrometer 1100 Series LC/MSD Trap-SL (ion trap) was equipped with an atmospheric pressure chemical ionization (APCI) source. Oil extraction was carried out by a microwave extractor (XH-1004, Beijing, China).

Fatty acids (C10-C22) used as standards were of chromatographic grade and purchased from Shanghai Chemical Reagent Co. (Shanghai, China). BAETS was synthesized in our laboratory. HPLC-grade acetonitrile was purchased from Jining Reagent Co. Formic acid ammonium was analytical grade from Shanghai Chemical Reagent Co. (Shanghai, China). Water was purified on a Milli-Q system (Millipore, Bedford, USA). N,N-Dimethyl formamide (DMF) was redistilled prior to use. All other reagents used were of analytical grade unless otherwise stated.

HPLC and MS conditions: BAETS-fatty acid derivatives were separated on a reversed phase Eclipse XDB-C_8 column by gradient elution. Eluent A was 30 % (v/v) of acetonitrile containing 30 mM ammonium formate (pH 3.7); B was 50 % (v/v) of acetonitrile containing 30mM ammonium formate; C was 95 % of acetonitrile. The percentage of the mobile phase was changed after injection as follows: A from 0-20 min; 40-80 % C from 12-35 min; B from 35-50 min; C maintained for 5 min. The flow rate was constant at 1.0 mL/min and the column temperature was set at 30 ºC. The injection volume was 10 μL. The fluorescence excitation and emission wavelengths were set at λ_{ex} 272 nm and λ_{em} 505 nm, respectively. Derivatives separated by HPLC were identified by online mass spectra equipped with an APCI source in positive-ion detection mode; nebulizer pressure 60 psi; dry gas temperature, 350 ºC; dry gas flow, 5.0 L/min. APCI Vap temperature 400 ºC; corona current 4000 nA (pos); capillary voltage 3500 V.

Extraction of oil from *Rubus niveus* Thunb. and statistic analysis: The fruiting bodies of *Rubus niveus* Thunb. (200 g) were ground in a blender to obtain powder. Each dried pretreated sample (5 g) was extracted by microwave-assisted extraction in *n*-hexane under the designed temperature, extraction time and the irradiation power. The *n*-hexane extraction solutions were separated from *Rubus niveus* Thunb. oil by using a rotary evaporator (RE-52, Shanghai, China) at 50 ºC under vacuum. The extraction yield (w/w %) was measured and calculated as follows:

$$\text{Extraction yield of oil (\%)} = \frac{m_i}{m} \times 100 \quad (1)$$

where m_i (g) is defined as the weight of dried oil; m (g) is defined as the dried raw material weight.

In this study, BBD of RSM was used to optimize the effects of processing parameters of microwave extraction from *Rubus niveus* Thunb. fruits on the yield of oil. According to previous studies, three extraction parameters *i.e.*, time (x_1), temperature (x_2) and irradiation power (x_3), were identified as key factors responsible for extraction yield. The process of microwave extraction oil from *Rubus niveus* Thunb. fruits were as follows: oil was extracted by microwave-assisted extraction (MAE) of differential irradiation power (400, 500, 600 W) from 5 g of *Rubus niveus* Thunb. fruits under different time (5, 10 and 15 min) and temperatures of (60, 70 and 80 ºC). Experiment based on a three-factor Box-Behnken design with a total of 15 experimental runs that involved 3 replicates at the center points and 3 factorial points. The experimental runs for Box-Behnken were shown in Table-1. Each experimental run was performed in duplicate except at the central point (6, 12 and 14 runs) of the design. In order to optimize the three individual variables with 15 experiment runs, the multiple regression analysis was used on the experiment data and the empirical relationship between the yield of *Rubus niveus* Thunb. oil and the variables can be approximated by the quadratic (second degree) Polynomial equation.

TABLE-1
BOX-BEHNKEN DESIGN OF THE INDEPENDENT VARIABLES (X) AND THE OBSERVED VALUES (Y)

Run	X_1: Time (min)	X_2: Temp. (ºC)	X_3: Irradiation power (W)	Y: Yield (%)
1	0 (10)	1(80)	1(600)	5.2
2	0 (10)	1(80)	-1(400)	1.8
3	-1 (5)	0(70)	1(600)	2.6
4	-1 (5)	0(70)	-1(400)	3.2
5	1 (15)	1(80)	0(500)	2.6
6	0 (10)	0(70)	0(500)	3.2
7	1 (15)	0(70)	1(600)	4.6
8	0 (10)	-1(60)	-1(400)	1.6
9	-1 (5)	-1(60)	0(500)	0.8
10	1 (15)	0(70)	-1(400)	1.2
11	-1 (5)	1(80)	0(500)	1.6
12	0 (10)	0(70)	0(500)	4.4
13	0 (10)	-1(60)	1(600)	1.6
14	0 (10)	0(70)	0(500)	3.3
15	1 (15)	-1(60)	0(500)	1.0

$$Y = \beta_0 + \beta_1X_1 + \beta_2X_2 + \beta_3X_3 + \beta_{12}X_1X_2 + \beta_{13}X_1X_3 + \beta_{23}X_2X_3 + \beta_{11}X^2_1 + \beta_{22}X_2^2 + \beta_{33}X_2^3 \quad (2)$$

where Y is the estimate response, β_0 is the constant, β_1, β_2 and β_3 are linear coefficients, β_{12}, β_{13} and β_{23} are interaction coefficients between the three variables, β_{11}, β_{22} and β_{33} are quadratic coefficients. The regression coefficients of individual linear, quadratic and interaction terms were determined. Those were used to make statistical calculation to get three-dimensional surface plots and contour plots from the fitted polynomial equation. Design-Expert (Version 7.0) software package was used to analyze the data.

Saponification of oil: 100 mg oil and 2 mL potassium hydroxide/methanol solution (2 mol/L) were added to a 10 mL test tube. The test tube was sealed and then immersed in a water bath at 93 ºC for 3 h. After cooling, 2 mL water was added and adjusted to 7 with hydrochloric acid solution (6 mol/L). This solution was extracted with chloroform (5 mL)

three times. The combined chloroform was filtered and evaporated under a stream of nitrogen. The residue was re-dissolved in 2 mL DMF, filtered through a 0.2 μm nylon membrane filter and stored at -10 ºC until HPLC /MS analysis.

Procedure of sample derivatization: 50 mg K_2CO_3, 170 μL DMF and 80 μL derivatization reagents (BAETS) solution were added into a vial which containing 100 μL of a standard fatty acid mixture. The vial was sealed and allowed to react in a water bath at 93 ºC with shaking in 5 min intervals for 50 min. After the reaction was completed, the mixture was cooled at room temperature. A 400 μL volume of the DMF was added to dilute the derivative solution. The diluted derivative solution (10 μL) was injected directly into the chromatograph.

Validation: The analytical method was validated for linearity, limit of detection (LOD), precision, accuracy and recovery. The calibration graph was established with the peak area (y axis) versus the fatty acid derivates concentration (x axis: pmol, injected amount of 10 μL), injected amount ranged from 100-10 pmol. The LODs were calculated with fluorescence detection (at a signal-to-noise ratio of 3:1). For the determination of intra-run accuracy and precision replicate analyses were performed on the same day. Inter-run accuracy and precision were determined by analysis of five batches on three consecutive days. Accuracy and precision (expressed as % CV) were evaluated. The recovery (%) was calculated by the equation: $(C_3 - C_2)/C_1 \times 100$ %, in which C_1 represents the amount of each standard spiked, C_2 represents the amount of each fatty acid in solution of oil and C_3 represents the total amount of each fatty acid in the solution. All validation analyses were performed at optimal derivatization conditions (derivazation temperature: 93 ºC; derivatization time 50 min, catalytic agent: 50 mg potassium carbonate and fivefold molar BATES excessive to total molar fatty acids; solvent: DMF).

RESULTS AND DISCUSSION

Optimization of extraction condition: Data from the total of 15 experiment results were analyzed by a least squares technique to fit the second-order polynomial model. The final mathematical equation in terms of code factors determined by Design-expert software is given below:

$$Y = 3.63 + 0.15X_1 + 0.78X_2 + 0.78X_3 - 0.20X_1X_2 + 1.0X_1X_3 + 0.85X_2X_3 - 0.89X_1^2 - 1.24X_{22} + 0.16X_3^2 \quad (3)$$

Analysis of variance (ANOVA) is a statistical technique that subdivides the total variation in a set of data into component parts associated with specific sources of variation for the purpose of testing hypotheses on the parameters of the model[8]. According to the result of ANOVA, the model has shown a good fit with the experimental data, the coefficient of determination R^2 had a value of 0.9490. This means that only 0.25 % of the total variations were not explained by the model within the range of values studied. The value of the adjusted determination coefficient (Adj. $R^2 = 0.8572$) also confirmed that the model was highly significant. At the same time, a very low value 2.02 of coefficient of the variation (CV) clearly indicated a very high degree of precision and a good deal of reliability of the experimental values. The model was found to be adequate for prediction within the range of experimental variables. The ANOVA for Y showed the linear coefficients (X_2, X_3), a quadratic term coefficient (X_1^2, X_2^2) and cross product coefficients (X_2X_3, X_1X_3) were significant, with very small p values ($p < 0.05$). The other term coefficients were not significant ($p > 0.05$). The smaller was the value of p, the more significant was the corresponding coefficient. All these results imply a satisfactory mathematical description of the extraction process by the fitted model (eqn. 3).

The response surface curves are plotted to explain the interaction of the variables and to determine the optimum level of each variable for maximum response. The graphical representations of the regression eqn. 3, called the response surfaces and the contour plots were obtained using Box-Behnken version 7.0 and the yield of *Rubus niveus* Thunb. oil affected by extraction time, temperature and irradiation power were presented in Fig. 1. In the 3-D response surface plot, each demonstrates the effect of two factors while the other factors were fixed at zero level. The values of extraction time, extraction temperature and irradiation power were 12 min, 75 ºC and 437 W, respectively. Among the three extraction parameters studied, extraction temperature and irradiation power are the most significant factor to affect the extraction of oil. The actual yield of oil was 5.83 %.

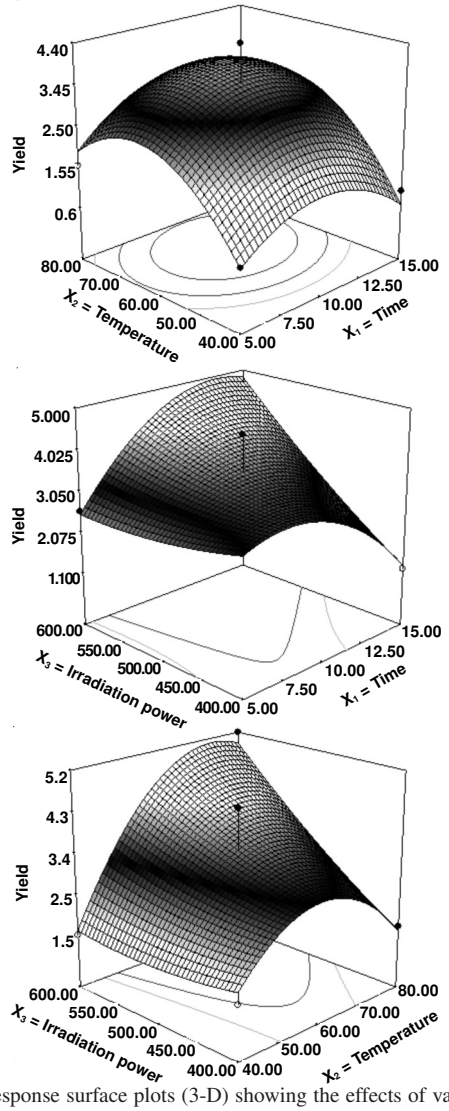

Fig. 1. Response surface plots (3-D) showing the effects of variables (X_1: extraction temperature; X_2: extraction time; X_3: extraction irradiation power) on the response Y

Optimal derivatization: The optimization process of derivatization of BAETS with 30 fatty acids (C1-C30) has been reported by our laboratory[9]. After study, we found that the derivatization conditions of BAETS with 18 kinds of fatty acids were similar to our earlier study. Therefore, we do not give unnecessary details in the present paper. The optimal derivatization conditions were as follows: reacted with fatty acids in DMF at 93 ºC for 50 min in the presence of 50 mg potassium carbonate with the addition of fivefold molar derivatization reagent excess to total molar fatty acids.

HPLC separation and MS identification: After the standard fatty acids were derivatized under the condition described above, on an Eclipse XDB-C_8 column, 18 kinds of fatty acid derivatives were separated in 50 min (Fig. 2). The ionization and fragmentation of the isolated derivative were studied by mass spectrometry with APCI in positive-ion detection mode. As expected, the BAETS-fatty acid derivatives produced an intense molecular ion peak at m/z 528.4 $[M + H]^+$. The collision-induced dissociation spectra (MS/MS) of molecular ions (MS, $[M + H]^+$ ion) produced intense and stable fragment ions at m/z 272.5, 290.6 and 283, respectively, come from the cleavage of $RNCH_2CH_2$-OCO, $RNCH_2CH_2O$-CO and RN-CH_2CH_2OCO bonds, which was specific fragment ions for DAETS-labelled fatty acid derivatives. The selected reaction monitoring, based on the m/z $[M + H]^+ \rightarrow$ m/z 283.6 and m/z 290.6 transit ion, was specific for fatty acid derivatives. The cleavage mode and MS/MS analysis for the fatty acid derivatives are shown in Fig. 3. All molecular ions $[M + H]^+$ for fatty acid derivatives are shown in Table-2.

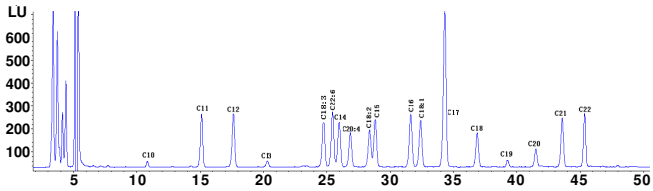

Fig. 2. Chromatogram of the standard fatty acids derivatives with BAETS (54 pmol)

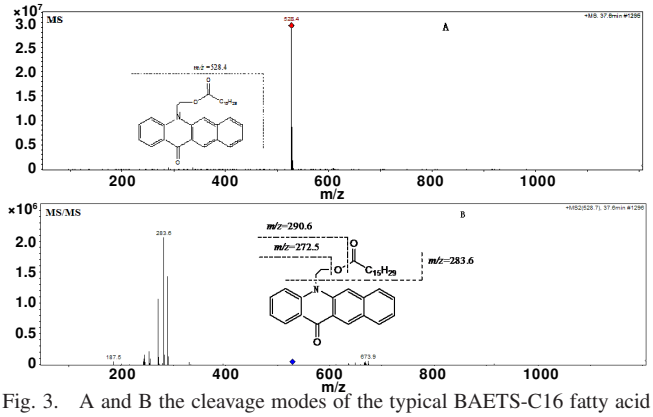

Fig. 3. A and B the cleavage modes of the typical BAETS-C16 fatty acid derivative (MS and MS/MS)

Validation of analytical method: Linear regression equations, correlation coefficients, detection limits and repeatability for all fatty acid derivatives are shown in Table-2.

Linearity and LOD: All fatty acid derivates provided excellent linear responses, with correlation coefficients greater than 0.9990. For the 1.0 pmol injections, the detection limits (at signal-to-noise of 3:1) of all of the derivatized fatty acids ranged from 18.42-32.39 pmol.

Precision: The intra-day and inter-day accuracy and precision were measured on the same day (n = 5) and on the sequential three days (n = 15), respectively. The intra-day accuracy ranged from 94.3-101.1 % with RSD % in the range of 2.5-5.8 %. The inter-day accuracy ranged from 92.9-103.7 % with RSD % in the range of 2.4-6.6 %. These results shown that quantification of fatty acid in oil can be carried out with excellent accuracy and precision using this method.

Recovery: The recovery (%) was calculated. The results were shown in Table-3. The results shown that experimental recoveries (n = 5) for free fatty acids ranged from 95.8-102.5 % with the largest mean RSD (%) < 2.0 % and for total fatty acids ranged from 93.7-103.3 % with the largest mean RSD (%) < 2.8 %.

TABLE-2
LINEAR REGRESSION EQUATIONS, CORRELATION COEFFICIENTS, DETECTION LIMITS OF THE METHOD, MS AND REPRODUCIBILITY FOR RETENTION TIME AND PEAK OF FATTY ACID DERIVATIVES

Fatty acids	Regression equation[a]	Correlation coefficients	Detection limit[b] (pmol)	MS $[M + H]^+$	Retention time[c] RSD (%)	Peak area[c] RSD (%)
C10	Y = 10.50X + 75.51	0.9997	26.58	443.9	0.26	1.29
C11	Y = 10.46X + 98.94	0.9990	32.39	457.9	0.08	1.10
C12	Y = 11.80X + 93.08	0.9993	28.53	471.9	0.64	1.52
C13	Y = 12.47X + 99.96	0.9993	21.86	486.0	0.83	1.49
C18:3	Y = 15.88X + 134.39	0.9989	28.06	552.2	0.28	1.42
C22:6	Y = 18.65X + 61.54	0.9999	24.17	600.4	1.12	2.05
C14	Y = 11.80X + 102.10	0.9990	19.05	500.0	1.03	2.62
C20:4	Y = 16.67X + 106.41	0.9996	19.57	576.4	0.19	1.32
C18:2	Y = 15.48X + 123.10	0.9995	30.48	554.3	0.73	1.88
C15	Y = 11.58X + 55.01	0.9995	22.98	514.0	0.01	2.14
C16	Y = 14.65X + 127.51	0.9990	28.43	528.4	0.06	1.68
C18:1	Y = 17.29X + 144.17	0.9992	26.49	555.5	1.08	1.28
C17	Y = 19.33X + 113.73	0.9996	22.77	542.1	1.00	1.87
C18	Y = 12.31X + 78.53	0.9994	18.56	556.4	0.56	1.48
C19	Y = 12.03X + 78.64	0.9997	27.93	570.1	1.19	2.05
C20	Y = 20.01X + 116.71	0.9998	24.07	584.0	0.37	1.44
C21	Y = 13.40X + 71.62	0.9999	18.42	598.1	0.52	1.63
C22	Y = 12.38X + 59.78	0.9999	29.66	613.5	1.06	1.18

[a]Y: peak area; X: injected amount (pmol); 10 µL injection volume, [b]Signal to noise ratio = 3, [c]Mean of five replicates.

TABLE-3
AVERAGE CONTENT OF FATTY ACIDS SAMPLES AND RECOVERY

Fatty acids	Free fatty acids[a] (mg/g)	Recovery[b] (%)	Total fatty acids[a] (mg/g)	Recovery[b] (%)
C10	0.0609	99.7	0.0139	98.0
C11	N.D	–[d]	0.0865	94.8
C12	0.0188	96.4	0.0615	99.2
C13	0.0078	96.5	0.0776	94.7
C18:3	0.0070	97.9	0.2530	102.1
C14	0.1316	97.7	0.0552	96.2
C20:4	N.D	–	0.0038	103.3
C18:2	0.0101	95.8	0.7556	100.3
C15	N.D	98.9	0.0072	98.5
C16	0.4043	98.6	0.9479	94.8
C18:1	N.D	–	0.0310	93.7
C17	0.3421	95.8	0.0010	97.6
C18	0.2359	100.1	0.1458	99.5
C20	N.D	–	0.0152	94.9
C21	0.1125	102.5	0.0083	98.3
C22	0.0150	96.3	0.1105	102.2

[a]Mean of three replicates. [b]Mean of five replicates. [c]ND: not detected. [d]None.

Analysis of *Rubus niveus* Thunb. oil by HPLC-MS/MS: Fatty acid analysis has important significance for the quality control of *Rubus niveus* Thunb. oil. The extraction oil of *Rubus niveus* Thunb. were derivatized with BAETS and analyzed by HPLC-FLD-MS/MS. The chromatogram for the analysis of total fatty acids (C10-C22) in the extracted oil is shown in Figs. 4 and 5. Fatty acids compositional data and recovery are shown in Table-3.

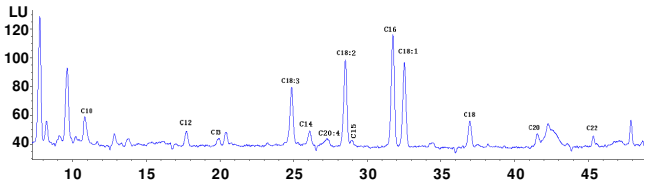

Fig. 4. Chromatogram for the analysis of free fatty acids from the extracted oil of *Rubus niveus* Thunb. fruits

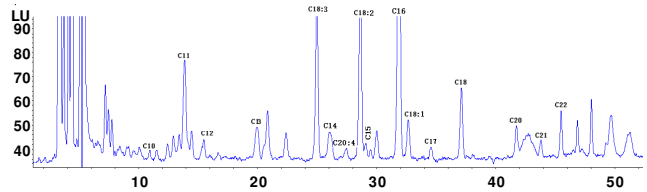

Fig. 5. Chromatogram for the analysis of total fatty acids from the extracted oil of *Rubus niveus* Thunb. fruits

The results indicated that the main unsaturated total fatty acids were 18:2, 18:3, 18:1 and 20:4, which make up 40.54 % of the total mass contents of total fatty acids (C10-C22). Results also demonstrated that *Rubus niveus* Thunb. oils were rich in unsaturated fatty acids. The predominant total fatty acids were 16:0, 18:3, 18:2, 18:0 and 22:0 in total fatty acids. The content of those were 0.9479, 0.7760, 0.7556, 0.1458 and 0.1105 mg/g, respectively. While in free fatty acids 18:2, 18:0, 16:0 were less than them in total fatty acids. These differences may be associated with differences between varieties, cultivation conditions and the level of maturation of the fruits used. These data are of important value in order to develop the products of *Rubus niveus* Thunb.

Conclusion

The MAE as a rapid and efficient extraction procedure, especially, the time used in MAE is much shorter than that of conventional extraction methods. In this study, the application of a Box-Behnken matrix became rapid, economical and efficient way of an optimization strategy of the MAE procedure. Using the proposed optimized methodology, the oil can be extracted more selectively and quickly. The MAE procedure developed for the oil extraction needs no more than 12 min irradiation at 437 W and 75 ºC and maximum yield of oil 5.83 % can be achieved. A new method using BAETS as labeling reagent for fatty acid determination was established by HPLC-MS/MS. The developed method here was capable of providing high detection sensitivity and selectivity and with minimal sample preparation. The fatty acids analysis showed that *Rubus niveus* Thunb. oils are rich in the essential fatty acids like linoleic acid, inolenic acid and arachidonic acid both in free and total fatty acid. This study should be useful for the further understanding and development of *Rubus niveus* Thunb. fruits in *Rubus niveus* Thunb. from Qinghai-Tibetan plateau.

ACKNOWLEDGEMENTS

This work was supported by the Research Fund for self-selected Topic of Beijing University of Chinese Medicine (No. 2011-MZYY-04).

REFERENCES

1. T. Flynn and D.H. Lorence, *Bishop Mus. Occas. Pap.*, **56**, 5 (1998).
2. C. Parmar and M.K. Kaushal, Wild Fruits, Kalyani Publishers, New Delhi, India, pp. 88-91 (1982).
3. W.W. Christie, High-Performance Liquid Chromatography and Lipids, Pergamon Press (English), p. 156 (1987).
4. K. Asakura, H. Ohrui and H. Meguro, *Analyst*, **118**, 765 (1993).
5. J.C. Marr, L.M. McDowell and M.A. Quilliam, *Nat. Toxins*, **5**, 302 (1988).
6. T. Iwata, T. Hirose, M. Nakamura and M. Yamaguchi, *Analyst*, **119**, 1747 (1994).
7. T. Toyo'oka, *Anal. Chim. Acta*, **465**, 111 (2002).
8. Y.P. Gao, Y.Y. Fu, Z.W. Sun, L. Kang, Z.Y. Ji, H.H. Zhao, L. Zhang and J.M. You, *J. Qufu. Normal Univ.*, **37**, 63 (2011).
9. H.P. Li, G.Q. Zhao, S.T. Niu and Y.G. Luan, *Comp. Mater. Sci.*, **38**, 561 (2007).