



Fingerprint identification of *p. notoginseng* flower, *p. ginseng* flower and *p. quinquefolium* lower based by HPLC and NIRS

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Abstract:

Aims: In view of the current study on the fingerprint of panax pseudo-ginseng, panax ginseng and panax quinquefolium, the identification features can not be clearly distinguished. HPLC and NIRS analysis technologies were used to establish the fingerprint of *p. notoginseng* flower, *p. ginseng* flower and *p. quinquefolium* flower, and to compare and analyze the differences among them.

Methods: By comparing the retention time of reference substances peaks, identified peaks, established fingerprints, and analyzed and compared the different characteristics of fingerprint of each variety. The WinISI II NIRS analysis software was used to establish the original NIRS fingerprint map of each variety, the first-order and the second-order derivative processing fingerprint map, to analyze and compare the different characteristics of the fingerprint map of each variety.

Results: Six peaks of *p. notoginseng* flower Re, R₁, Rb₁, Rc, Rb₃ and Rd were identified, five peaks of *p. ginseng* flower Re, R₁, Rb₁, Rc and Rd were identified, and five peaks of *panax quinquefolium* Re, Rb₁, Rc, Rb₃ and Rd were identified. *p. notoginseng* flower (S) and *p. ginseng* flower (R) features of HPLC fingerprint differences mainly in peak S16, S20, S22, S23, S30, S31, S32. HPLC fingerprint characteristics of *p. notoginseng* flower (S) and *p. quinquefolius* flower (X) were mainly in peak S16, X13, X14, X23, X24, X25, X35 X36, X40. HPLC fingerprint features of *p. ginseng* flowers and *p. quinquefolius* were mainly characterized by peak R27, X20, X21, X34, X36, X37, X38.

The absorption peaks of *p. notoginseng* flower NIRS original fingerprint at 2000~2200 nm were different from others. The first-derivative fingerprint of *p. notoginseng* flower and *p. quinquefolium* flower had peaks between 2300~2350 nm, while the *p. ginseng* flower were not. The peaks of *p. ginseng* flowers and American *ginseng* flowers between 2090~2150 nm, *p. notoginseng* flowers were without. The peaks between 1670~1690 nm of the fingerprint of *p. notoginseng* flower were different from others. The second derivative of *p. notoginseng* at 2250~2300 nm were different from others, and there were not difference at 2300~2350 nm between *p. notoginseng* flower and *p. quinquefolium* flower, while different from *p. ginseng* flowers.

Conclusion: HPLC and NIRS fingerprints of the flower of *p. notoginseng*, *p. ginseng* and *p. quinquefolium* could be used to distinguish and identify them. HPLC fingerprint were identified remarkable and NIRS fingerprint could quickly identify them with zero damage. The fingerprint identification method established in this paper had the advantages of obvious features and shortened analysis time. It provided a reliable reference for further identification and research and development of *p. notoginseng* flower, *p. ginseng* flower and *p. quinquefolium* flower.

Keywords: *p. notoginseng* flower; *p. ginseng* flower; *p. quinquefolium* flower; HPLC fingerprint; NIRS fingerprint

The flower buds of *p. notoginseng*, *radix Ginseng* and *p. quinquefolius*, which come from the *Ginseng* family of *Araliaceae*, respectively, and belong to rare traditional Chinese medicine^[1]. Because of *p. notoginseng* flowers, *p. quinquefolius*, *p. ginseng* flower species relationship, appearance on the small differences, indistinguishable to the naked eye, confusing, and there was mutual pseudo phenomenon and influence the quality of medicinal materials, safety usage and curative effect, *p. notoginseng* flowers, *p. quinquefolius*, *p. ginseng* flower, tastes, functions of indications, clinical application, may vary from pseudo imitation products even can cause medical accidents^[2].

There were scholars studied from the medicinal properties, microscopic characteristics, thin layer identification and fingerprint on flowers of *p. notoginseng*, *p. notoginseng* and *p. quinquefolius*, the preliminary analysis and comparison of notoginseng flower, flower of ginseng, and American ginseng flower difference characteristics, need to deal with the complex before, however, complicated operation and long analysis time, difficult to meet the practical needs, the lack of systematic study. It is need to further optimize the analysis method, set up simple operation, fast analysis speed, the results of reliability analysis method, for *p. notoginseng* flowers, American ginseng, ginseng, laying a foundation for identification and quality control, promote The flower buds of *p. notoginseng*, *radix ginseng* and *p. quinquefolius* development and utilization ^[3-6].

1. Experimental materials and equipment

Samples: 15 batches of *p. notoginseng* flowers (purchased from the Sanqi international trading center in Wenshan city, Yunnan province), 10 batches of *p. ginseng* flowers (purchased from Baishan city, Jilin province) and 10 batches of *p. notoginseng* flowers (purchased from Baishan city, Jilin province).

Reference substance: *p. notoginseng* total saponins, ginsenosides Rb3, Rc (chengdu manst biological technology co., LTD., with purity Standard, 99.65% and 99.65%, batch number: MUST-14042810, MUST-18032202, MUST-18032302), ginsenoside Rb1 (Beijing century oko biological technology co., LTD., with purity $\geq 98\%$, batch number: C54H92023).

Equipments: SHIMADZU SHIMADZU HPLC (Japan, Lc-2030), shim-pack GIST C18 (Material: 5 μ m, Dimensions:4.6 x 250 mm), Mosaic Solo (NIRS DS2500), one over ten thousand electronic analytical balance (German dolly, joint-stock company, BP121S), one over one hundred thousand electronic analytical balance (German dolly, joint-stock company, BP211D), electronic balance (SQP, dolly scientific instruments (Beijing) co., LTD.), the ultrasonic instrument (SB - 3200 type DTD ultrasound instrument, ningbo new art ultrasonic equipment co., LTD.), water bath pot (HWS - 26 electric constant temperature water bath pot, Shanghai - constant technology co., LTD.), Vacuum pump (shb-iii type circulating water multi-purpose vacuum pump, zhengzhou Great Wall science, industry and trade co., LTD.).

2. Experimental methods and results

2.1 HPLC fingerprint identification study

2.1.1 Preparation of the sample

Take 1g of the sample and place it in 150 ml conical flask, add 50 % ethanol 100ml, weigh it, and extract it by ultrasound for 40 min. After cooling, use 50 % ethanol to replenish weight.

2.1.2 Preparation of reference substance

A proper amount of total saponins, RC, Rb₁ and Rb₃ of *p. notoginseng* was accurately weighed, and put in a brown volumetric flask, which was dissolved with 50% ethanol and dissolved with fixed solution. Then, ml containing 0.95mg, 0.87mg, 0.81mg and 0.84mg of the reference substance were prepared and stored at low temperature.

2.1.3 The chromatographic conditions

SHIMADZU SHIMADZU high performance liquid chromatograph (Lc-2030, Japan); Shim-pack GIST C18 (Material: 5 μ m, Dimensions: 4.6 x 250 mm). Taking acetonitrile as mobile phase A and 0.05% phosphate water as mobile phase B, gradient elution was conducted according to the conditions in the following table. The sample injection volume was 20 L, the flow rate was 0.5 mL/min, and the detection wavelength was 203 nm.

2.1.4 Methodology study

2.1.4.1 Precision test

An appropriate amount of the control solution of RC, Rb₁ and Rb₃ was taken, and analyzed according to the chromatographic conditions under the item "2.1.3". The samples were continuously injected for 6 times, and the spectrum was recorded. RSD was 0.30%, 0.25%, 0.45%, 0.21%, 0.18% and 0.28%, respectively, to analyze and calculate the peak area and relative retention time of each chromatographic peak. It indicated that the instrument has good precision and meets the requirements.

2.1.4.2 Stability test

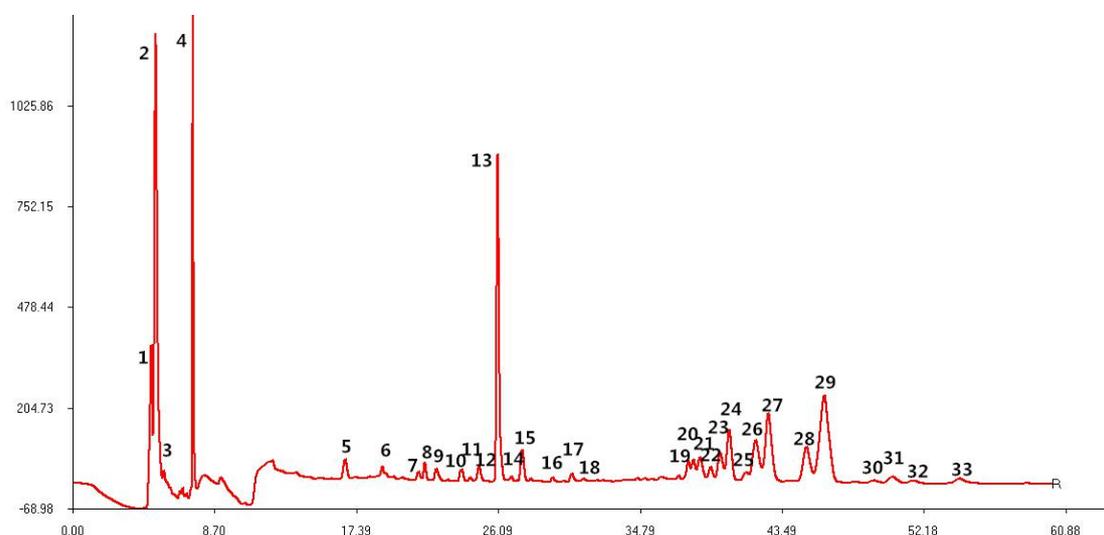
One flower sample of *p. notoginseng*, *p. ginseng* and *p. quinquefolium*, respectively, was prepared according to the preparation method of the sample under item "2.1.1", and the chromatographic conditions under item "2.1.3" were analyzed at 0 h, 8 h, 16 h and 24 h respectively. Analysis and calculation of 26 ~ 45 min of each chromatographic peak peak area and relative retention time, *notoginseng* flower RSD was 0.20% ~ 0.20%, 0.17% ~ 0.38%, *ginseng* flower RSD was 0.15% ~ 0.15%, 0.15% ~ 0.31%, American *ginseng* flower RSD was 0.21% ~ 0.21%, 0.12% ~ 0.58%, and traditional Chinese medicine chromatographic fingerprint similarity evaluation software to analyze similarity (2010 A), each sample chromatogram similarity ≥ 0.98 , The results showed that the sample solution had good stability and met the requirements within 24 h test time.

2.1.4.3 Reproducibility test

Five flower samples of *p. notoginseng*, *p. ginseng* and *p. quinquefolium*, respectively, were prepared by the preparation method of the sample under item "2.1.1", and analyzed by the chromatographic condition under item "2.1.3". Analysis and calculation of 26 ~ 45 min of each chromatographic peak peak area and relative retention time, *notoginseng* flower RSD was 0.23% ~ 0.23%, 0.14% ~ 0.32%, *ginseng* flower RSD was 0.15% ~ 0.15%, 0.15% ~ 0.31%, American *ginseng* flower RSD was 0.16% ~ 0.16%, 0.12% ~ 0.28%, and traditional Chinese medicine chromatographic fingerprint similarity evaluation software to analyze similarity (2010 A), each sample chromatogram similarity of 0.99 or higher, The results show that the pretreatment method is reproducible and meet the requirements.

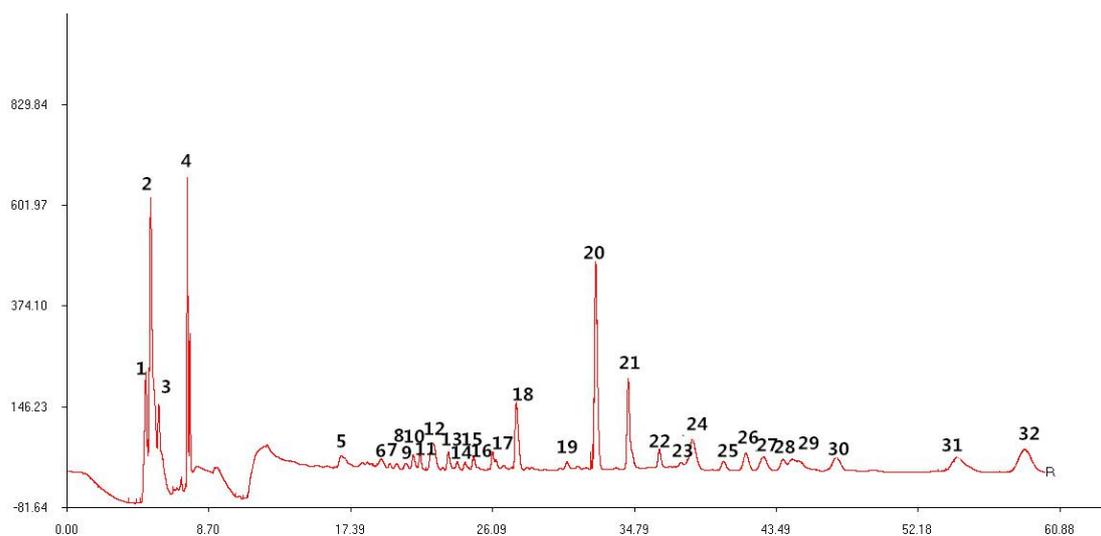
2.1.5 calibration of HPLC fingerprint characteristic peaks of *p. notoginseng*, *p. ginseng* and *p. quinquefolium*

1 g of the flower samples of *p. notoginseng*, *p. ginseng* and *p. quinquefolium*, was taken, and were prepared by the method under the item “2.1.1”. HPLC analysis was conducted according to the chromatographic conditions under the item “2.1.3” to collect the map. Application of traditional Chinese medicine chromatographic fingerprint similarity evaluation software (Version 2010.130723) for the acquisition of notoginseng flower, flower of ginseng, American ginseng flower HPLC analysis processing, generate common peak compared fingerprint (Fig. 2-1, 2-2, 2-3), confirmed the chromatographic peak of notoginseng flower fingerprint 33, ginseng flower fingerprint chromatographic peak of 31, American ginseng flower fingerprint chromatographic peak of 40. By comparing the retention time of each control sample in the mixed control sample, the characteristic peaks of each medicinal material were identified, and six chromatographic peaks of pseudo-ginseng flower Re, R₁, Rb₁, Rc, Rb₃ and Rd were identified, five chromatographic peaks of ginseng flower Re, R₁, Rb₁, Rc, Rd were identified, and five chromatographic peaks of ginseng flower Re, Rb₁, Rc, Rb₃ and Rd were identified.



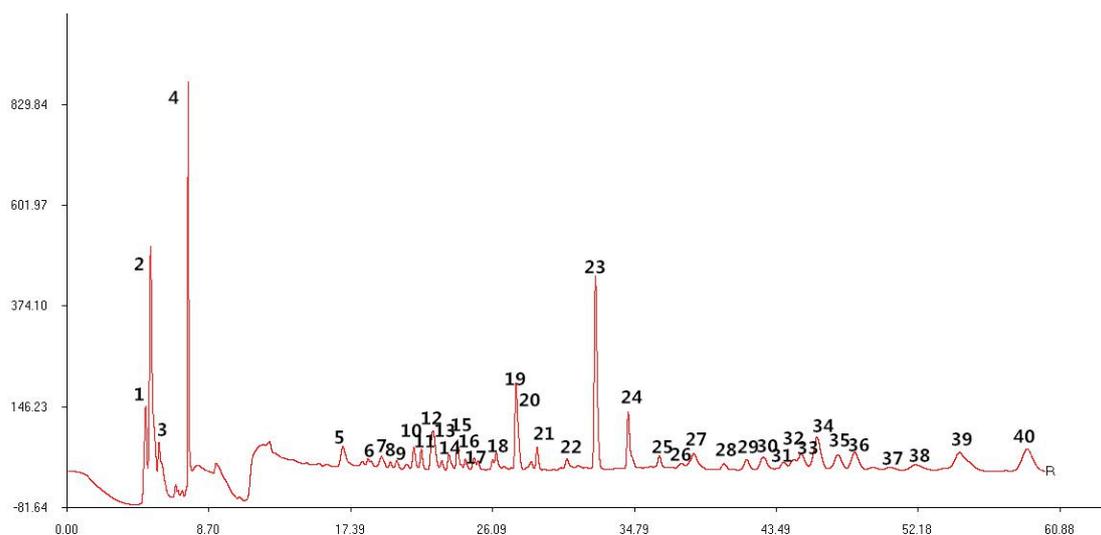
17 Re, 18 R₁, 24 Rb₁, 27 Rc, 29 Rb₃, 33 Rd

Fig. 2-1. Control fingerprint of *p. notoginseng* flowers



19 Re, 20 R₁, 25 Rb₁, 27 Rc, 31 Rd

Fig. 2-2 Control fingerprint of *p. ginseng* flowers



22 Re, 28 Rb₁, 30 Rc, 34 Rb₃, 39 Rd

Fig. 2-3 Control fingerprint of *p. quinquefolium* flowers

2.1.6 HPLC fingerprint and similarity analysis of p. notoginseng, p. ginseng and p. quinquefolium

The HPLC map AIA format of each medicinal material was exported from ShiMaZu Labsolution software, and then the whole spectrum was imported into the “Chinese Medicine Chromatogram Fingerprint Similarity Evaluation System Software” (2010.130723 version),

and the common pattern (R) of the fingerprint was generated, and the superimposed fingerprint of each batch of medicinal materials (Fig2-4, 2-5, 2-6) was established, and the similarity was calculated and analyzed.

The analysis results of similarity calculation were shown in table 2-1. The samples of each batch of sanqi flower had good similarity with the control fingerprint map, with the similarity greater than 0.913, and the average similarity was 0.951. The samples of each batch of ginseng flowers had good similarity with the reference fingerprint, with the similarity greater than 0.862, and the average similarity was 0.889. The samples of each batch of *p. quinquefolius* had good similarity with the control fingerprint, the similarity was greater than 0.856, and the average similarity was 0.913. The result of similarity analysis showed that the fingerprint of each variety was stable and the generated fingerprint met the requirements.

Table 2-1 HPLC fingerprint similarity analysis results

Notoginseng		Ginseng		American	
flower	Similarity	flower	Similarity	ginseng flower	Similarity
samples		samples		samples	
1	0.913	1	0.899	1	0.907
2	0.964	2	0.862	2	0.959
3	0.973	3	0.934	3	0.938
4	0.948	4	0.862	4	0.958
5	0.969	5	0.919	5	0.958
6	0.953	6	0.899	6	0.889
7	0.939	7	0.893	7	0.879
8	0.963	8	0.862	8	0.856
9	0.955	9	0.881	9	0.919
10	0.973	10	0.879	10	0.869

Notoginseng		Ginseng		American	
flower	Similarity	flower	Similarity	ginseng flower	Similarity
samples		samples		samples	
11	0.944				
12	0.914				
13	0.953				
14	0.963				
15	0.951				

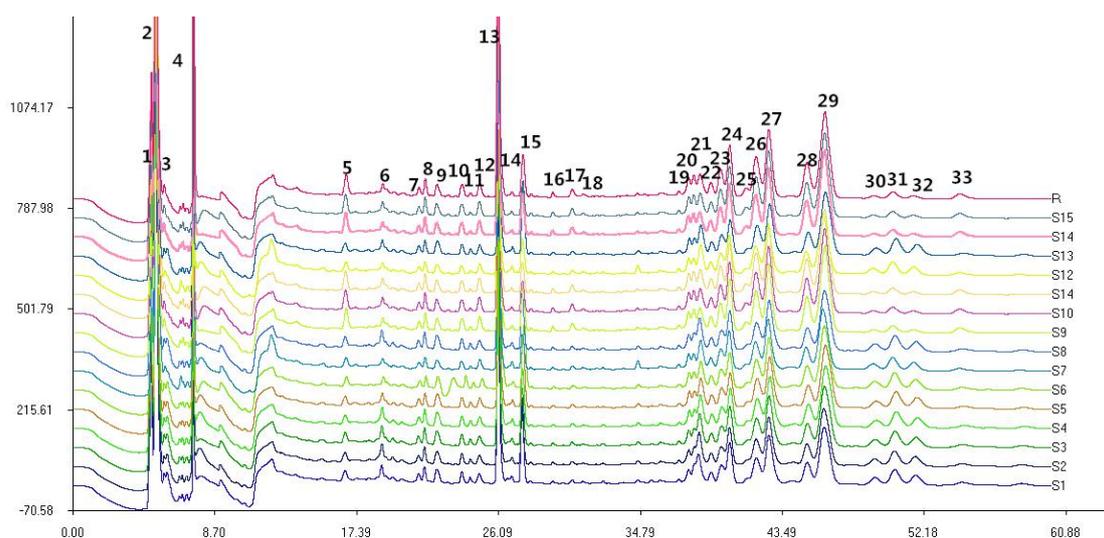


Fig. 2-4 HPLC fingerprint of p. notoginseng flower

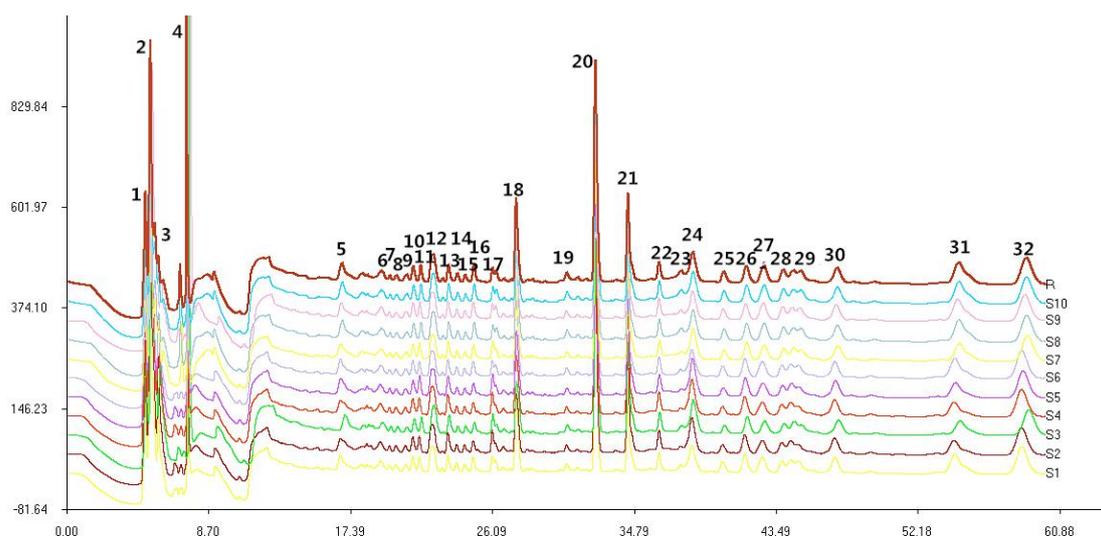


Fig. 2-5 HPLC fingerprint of *p. ginseng* flowers

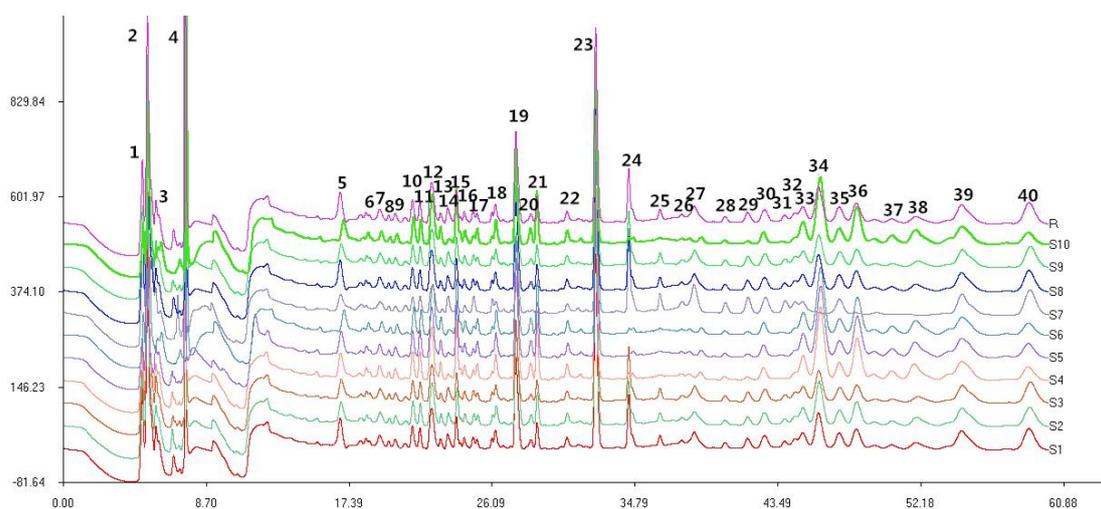


Fig. 2-5 HPLC fingerprint of *p. quinquefolium*

2.1.7 Comparison and analysis of HPLC fingerprint varieties of *p. notoginseng*, *p. ginseng* and *p. quinquefolium*

HPLC of *p. notoginseng*, *p. ginseng* and *p. quinquefolium* were combined to carry out comparative analysis and study, aiming at finding HPLC fingerprint differences of different kinds of medicinal materials, providing reliable reference for identification, laying a foundation for further identification of *p. notoginseng*, *p. ginseng* and *p. quinquefolium*.

By comparing the peak numbers of each species, 33 chromatographic peaks of *p. notoginseng* flower fingerprint, 31 chromatographic peaks of *p. ginseng* flower fingerprint, and 40 chromatographic peaks of *p. quinquefolium* flower fingerprint, the three kinds of medicinal materials had a large degree of similarity in chemical composition type, and also had significant differences. *P. otoginseng* flower (S) and ginseng flower (R) features of HPLC fingerprint differences mainly in the chromatographic peak S16, S20, S22, S23, S30, S31, S32, ginseng flower in the corresponding chromatographic peak of retention time position did not appear, *p. ginseng* flower fingerprint chromatographic peak in R13, R20, R21, R22, R32, *p. notoginseng* flower in the corresponding chromatographic peak of retention time position did not appear. HPLC fingerprint characteristics of *p. notoginseng* flowers (S) and *p. quinquefolius* flowers (X) were mainly in chromatographic peak S16, and there were no chromatographic peaks in the corresponding retention time positions of *p. quinquefolius*, and no chromatographic peaks in *p. notoginseng* flowers in the corresponding retention time positions of *p. quinquefolius* (X13, X14, X23, X24, X25, X35, X36, X40). HPLC fingerprint features of *p. ginseng* flowers (R) and *p. quinquefolium* (X) were mainly in chromatogram peak R27, and there were no chromatographic peaks in *p. ginseng* flowers in the corresponding reserved time positions of *p. quinquefolium* flowers (X20, X21, X34, X36, X37 and X38). The analysis results showed that the HPLC fingerprint characteristics significant difference, each type of *p. notoginseng*, *p. ginseng* and *p. American ginseng* flower medium basic chemical composition was present, and *p. ginseng* flowers and American ginseng had more differences in components with *p. notoginseng* flowers, at the same time, the chemical composition of *p. ginseng* flower generally exist in the American ginseng flower, American ginseng flower had differences in composition was more with *p. ginseng* flower , it was conducive to differentiate between the different varieties.

2.2 Identification of near-infrared fingerprint

2.2.1 Preparation of the sample

Take the tests, with Chinese medicine pulverizer shattered, over 200 mesh pharmacopoeia standard test screen, the fine powder vacuum drying under 60 °C for 24 h, stored in a dryer. Set aside. Proper amount of the sample powder was taken, placed in the measuring cup, and

evenly expanded about 0.5 cm thick. In integrating sphere diffuse manner, in the range of 850 ~ 2499 nm wavelength scanning, scanning each sample three times, five copies in parallel, a resolution of 2 nm, temperature 25 °C, relative humidity 70%, the average spectrum.

2.2.2 Cluster analysis of *p. quinquefolium*, *p. ginseng* and *p. quinquefolium*

Using WinISI II near-infrared analysis software, Make and Use Scores and Create Score File from Spectra File, Mean was used for principal component analysis, and GH (distance from the center) was used as the index to eliminate the map with a GH value greater than 3.0 and draw 3D graphs. The results are shown in FIG. 2-1, 2-2 and 2-3.

GH values of all samples of *p. notoginseng* were all less than 3.0, with GH mainly concentrated between 0.22 and 2.80. One map with a GH value greater than 3.0 was excluded from the *p. ginseng* flower samples, while the rest were mainly between 0.30 and 2.95. One map with a GH value greater than 3.0 was excluded from the map of *p. quinquefolium*, while the rest were mainly between 0.35 and 2.96. The results show that the near-infrared spectra of *p. notoginseng*, *p. ginseng* and *p. quinquefolium* collected by Foss were relatively stable and had good similarity.

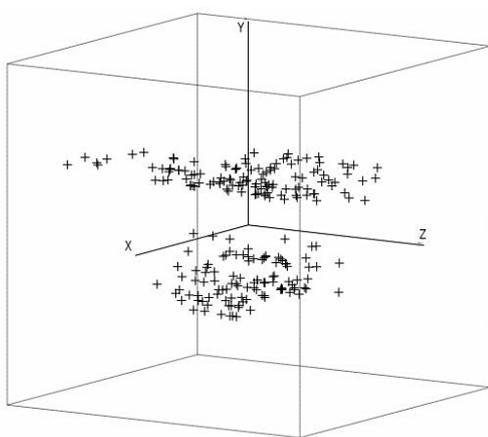


Fig. 2-1 *p. notoginseng* flower

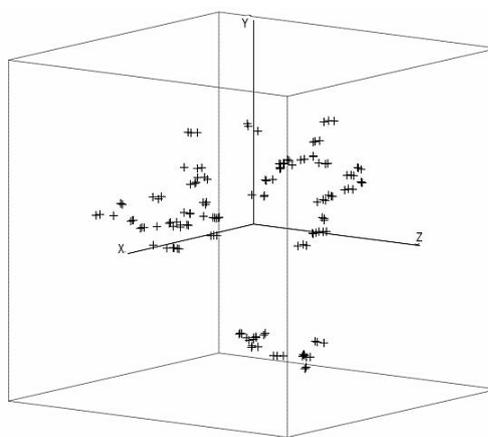


Fig. 2-2 *p. ginseng* flower

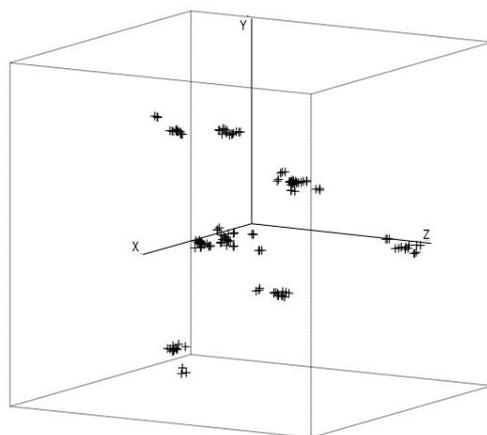


Fig. 2-3 *p. quinquefolium* flower

2.2.3 the near-infrared original map of *p. quinquefolium*, *p. ginseng* and *p. quinquefolium*

The collected nir spectra were analyzed and mapped by WinISI II near-infrared analysis software. The original nir spectra of the three varieties were obtained, as shown in FIG. 2-4, 2-5 and 2-6. The differences of the nir original spectra of *p. notoginseng*, *p. ginseng* and *p. quinquefolia* were analyzed and compared.

Comparative analysis of *p. notoginseng* flowers, *p. quinquefolium* and *p. ginseng* flower near infrared original map, three varieties of flowers of medicine between 850 ~ 850 nm had obvious absorption peak, *p. notoginseng* flowers near infrared original map between 2000 ~ 2200 nm absorption of fengfeng shape differences with *p. ginseng* and American ginseng spent flowers, *p. ginseng* flower and American ginseng flower between 850 ~ 850 nm absorption peak shape has no obvious difference.

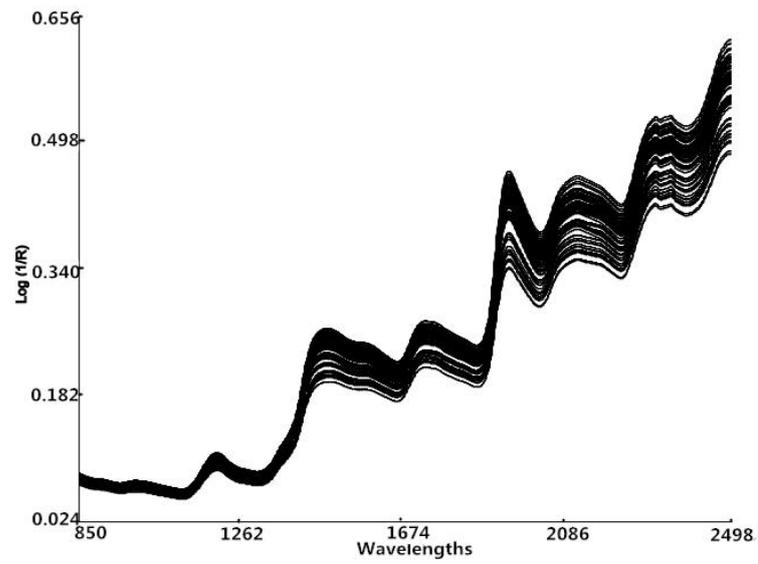


Fig. 2-4 Near-infrared original fingerprint of *p. notoginseng*

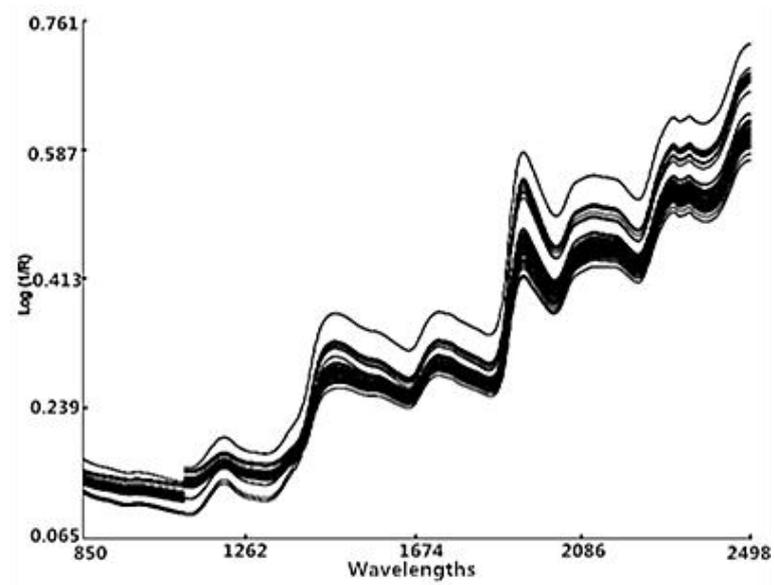


Fig. 2-5. Near-infrared original fingerprint of *p. ginseng* flowers

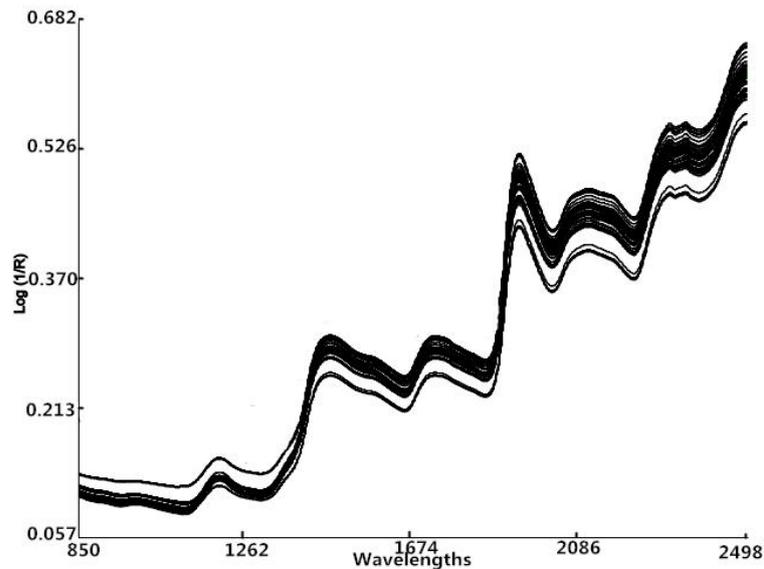


Figure 2-6. Near-infrared original fingerprint of *p. quinquefolium* flower

2.2.4 first-derivative processing and analysis of *p. quinquefolium*, *p. ginseng* and *p. quinquefolium*

The WinISI II near-infrared analysis software was used to process the original spectra of all kinds of near-infrared spectra. The near-infrared spectrum collected by scanning was selected, and the parameters Derivative, Gap, Smooth 1 and Smooth 2 were set as 1, 4, 4 and 1, respectively, for desaturated and first-order Derivative processing, and the differences between first-order Derivative processing were analyzed and compared.

The first-derivative fingerprint of *p. notoginseng* flowers, *p. quinquefolium* flowers and *p. ginseng* flowers were compared and analyzed. After the first-derivative treatment, the peak shape of each pattern was obvious, the number of peaks increased, and the differences of the three varieties were small. The first-derivative treatment fingerprint of *p. notoginseng* flowers and *p. quinquefolium* had obvious absorption peak between 2300 and 2350 nm, but no obvious absorption peak of *p. ginseng* flowers. The absorption peaks of *p. ginseng* flowers and American ginseng flowers between 2090~2150 nm and that of *p. notoginseng* flowers were not.

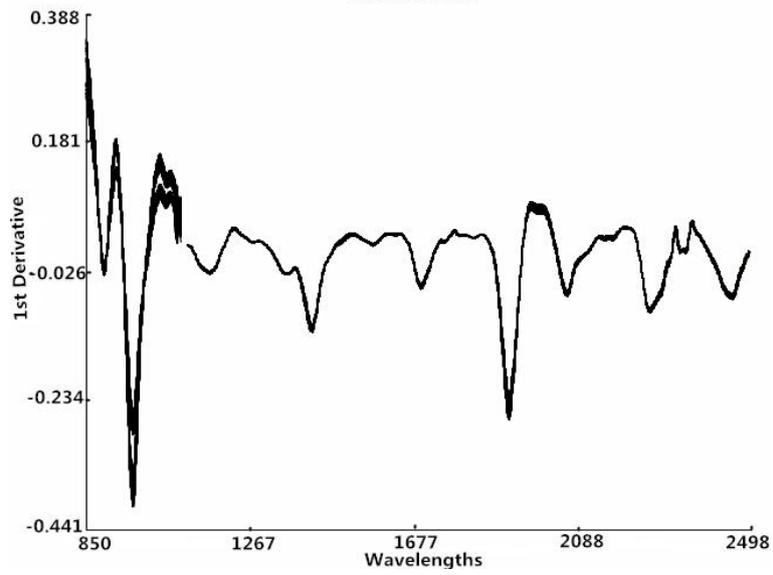


Fig. 2-4 First - derivative fingerprint of p. notoginseng

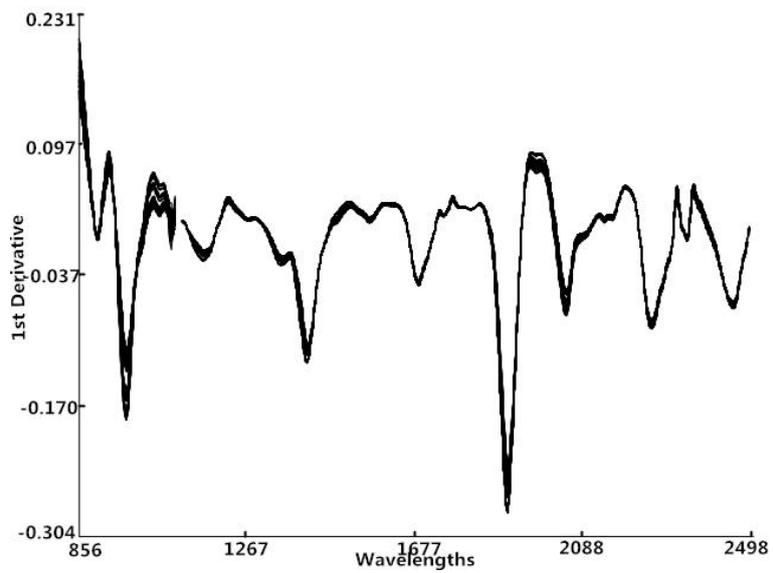


Fig. 2-5 First - derivative fingerprint of ginseng flowers

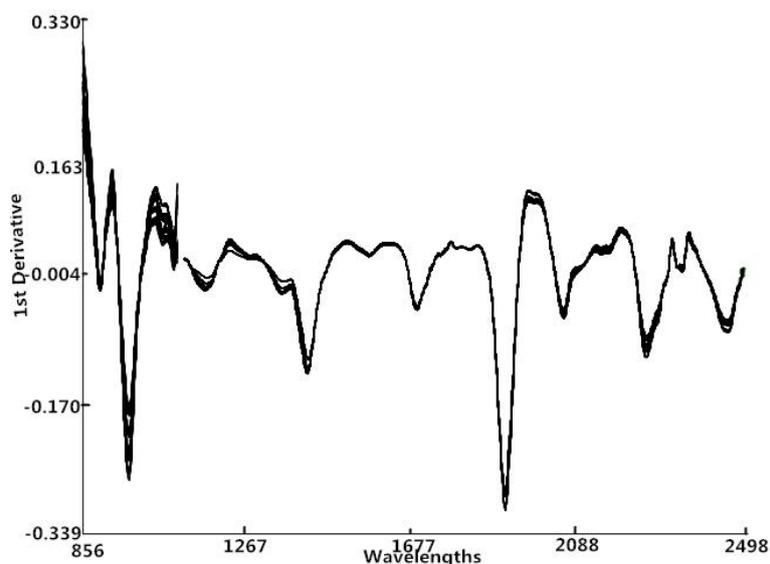


Fig. 2-6 First - derivative fingerprint of American ginseng

2.2.5 Second-derivative processing and analysis of the near-infrared spectrum of *p. quinquefolium*, *p. quinquefolium* and *p. quinquefolium*

The WinISI II near-infrared analysis software was used to process the original spectra of all kinds of near-infrared spectra. The near-infrared spectrum collected by scanning was selected, and parameters Derivative, Gap, Smooth 1 and Smooth 2 were set as 2, 4, 4 and 1 respectively. Analyze and compare the differences between the second derivative maps.

The fingerprint of the second derivative of *p. notoginseng* flowers, *p. quinquefolium* flowers and *p. ginseng* flowers was compared and analyzed. After the second derivative treatment, the peak number of the third derivative, *p. notoginseng* flowers, *p. quinquefolium* flowers and *p. ginseng* flowers was increased and the peak shape was more obvious. Between 1670 and 1690 nm, the peaks of the fingerprint of *p. notoginseng* flowers, *p. ginseng* flowers and *p. quinquefolium* flowers were different. The peak shape of the second derivative of *p. notoginseng* at 2250~2300 nm was different from that of *p. quinquefolium* flowers and *p. ginseng* flowers, while the peak shape of the second derivative of *p. notoginseng* at 2300~2350 nm was consistent with that of *p. quinquefolium* but different from that of *p. ginseng*.

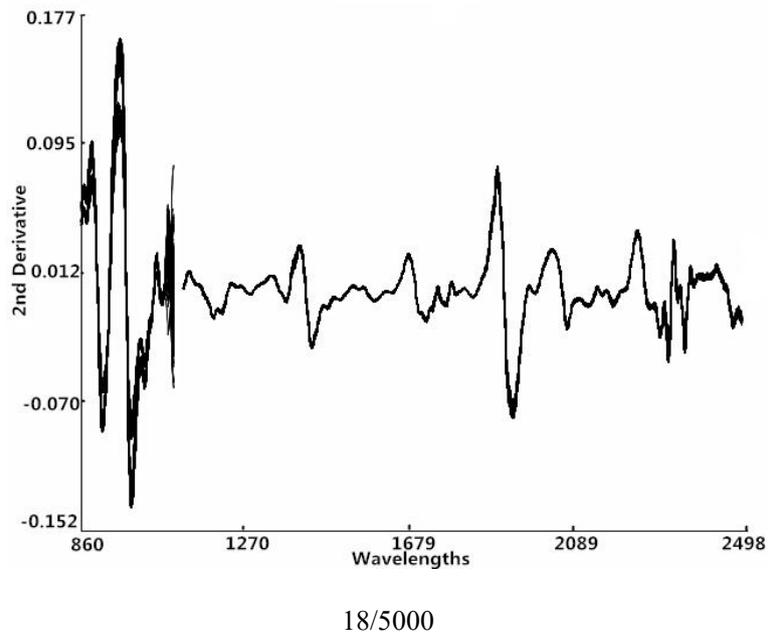


Fig. 2-7 Second - derivative processing fingerprint of p. notoginseng flowers

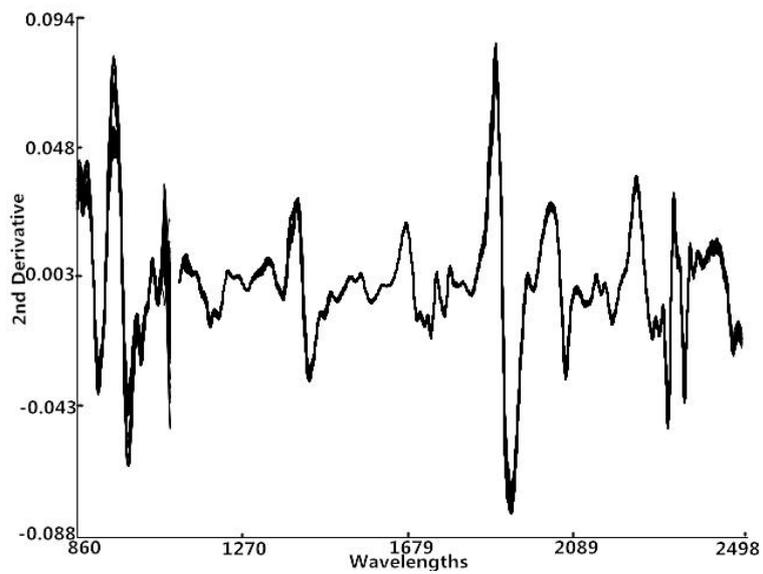


Fig. 2-8 Second - derivative fingerprint of p. ginseng flowers

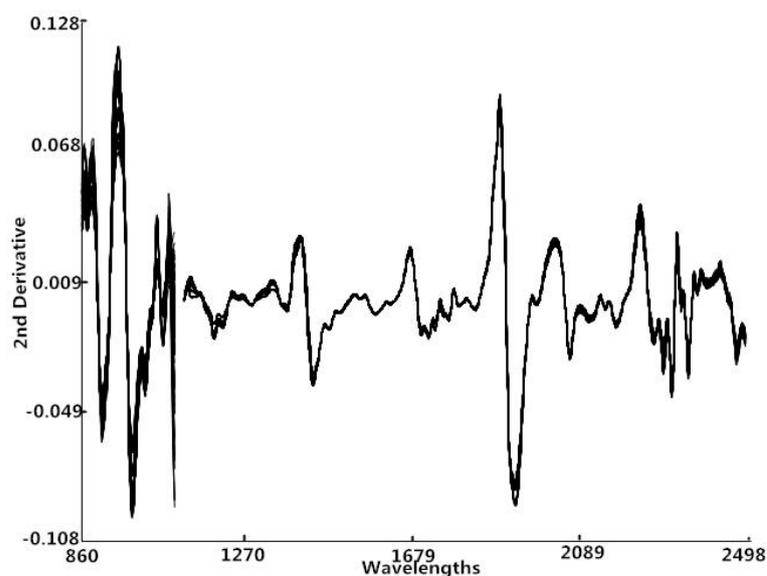


Fig. 2-9 Second - derivative fingerprint of *p. quinquefolium*

2.3 Conclusions and Discussions

There were differences in HPLC fingerprint and NIRS fingerprint of *p. notoginseng* flowers, *p. quinquefolium* flowers and *p. ginseng* flowers. HPLC fingerprint and NIRS fingerprint could be used to distinguish and identify *p. notoginseng* flowers, *p. quinquefolium* flowers and *p. ginseng* flowers. HPLC fingerprint features of them were remarkable and NIRS fingerprint could quickly and no damage identify *p. notoginseng* flowers, *p. quinquefolium* flowers and *p. ginseng* flowers. The chromatographic conditions established in this paper could shorten the analysis time, and the chromatographic peak separation effect was good, which could better reflect the chemical composition characteristics of the samples.

In this paper, the HPLC fingerprint and NIRS fingerprint of *p. notoginseng* flowers, *p. quinquefolium* flowers and *p. ginseng* flowers were studied and established for the first time in a comprehensive system, and the fingerprint features of different species of medicinal materials were analyzed comprehensively, and the characteristic peaks were found, provided reliable reference basis for the identification of *p. notoginseng* flowers, *p. quinquefolium* flowers and *p. ginseng* flowers.

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