

Method Development for Gypenosides Fingerprint by High Performance Liquid Chromatography with Diode-Array Detection and the Addition of Internal Standard

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In this paper, a new method for liquid chromatographic fingerprint of saponins in *Gynostemma pentaphyllum* (THUNB.) MAKINO was developed. The *G. pentaphyllum* powder was defatted by Soxhlet extraction with petroleum ether and then gypenosides were extracted from the residue with methanol by sonicating. Column chromatography with macro pore resin was then used to separate and enrich gypenosides. HPLC fingerprint analysis of gypenosides fraction was performed on a C18 column, with an isocratic elution of 34% acetonitrile for 60 min at 0.8 ml/min, sample injection volume was 20 μ l and the wavelength was 203 nm. To cover the lack of standard compounds, the addition of an internal standard of ginsenoside Rb₂ was employed in the gypenosides fingerprint profile. The relative retention time (RRT) and relative peak area (RPA) of the gypenosides peaks in the fingerprint were calculated by setting the ginsenoside Rb₂ as the marker compound. The relative standard deviation (RSDs) of RRT of five common peaks vs. ginsenoside Rb₂ in precision, repeatability and stability test were less than 1%, and the RSDs of RPA were less than 5%. The method validation data proved that the proposed method for the fingerprint with internal standard of *G. pentaphyllum* saponins is adequate, valid and applicable. Finally, three batches of crude drug samples collected from Shanxi province were tested by following the established method.

Key words *Gynostemma pentaphyllum*; gypenoside; HPLC; fingerprint; internal standard

Gynostemma pentaphyllum (THUNB.) MAKINO (Jiaogulan) is a perennial creeping herb, which belongs to the Cucurbitaceae family. The earliest record of the plant is in the book "Herbs for Famine," which was published in the Ming Dynasty (1368–1644 A.D). As one of important traditional Chinese medicines, *Gynostemma pentaphyllum* exhibits a variety of biological activities, including increasing the resistance to infection, anti-inflammation, reducing cholesterol, anti-hyperlipidemic and hypoglycemic.^{1,2)} Some of the saponins of *Gynostemma* were reported to be effective in the treatment of tumours.³⁾ The water extracts of *G. pentaphyllum* were proven not to produce any significant chronic toxic effect.⁴⁾

The contents in *G. pentaphyllum* include saponin, flavonoid, sterol, polysaccharides, vitamins, minerals and amino acids. The saponins in *G. pentaphyllum*, which were also named gypenosides, are the most effective contents because of the biological activities. Over 100 saponins were isolated and identified from *G. pentaphyllum*.⁵⁾ Gypenosides exist mainly as dammarane type-triterpene glycosides (Fig. 1). Mostly, the branches of R₁ and R₂ are sugars. The main sugar types are β -D-glucose, β -D-xylose, α -L-arabinose and α -L-rhamnose. The content of the saponins varies among dif-

ferent species, geographical sources and time of collection. Eight gypenosides were identified to be the same as the protopanaxadiol-type ginsenosides Rd, Rb₁, Rb₃, F₂, Rc, Rg₃, malonyl-Rb₁ and malonyl-Rd.^{6–12)}

Since the early 1990s, many brands of herb products, functional foods and beverage based on *G. pentaphyllum* such as "Fuzhenghuayu" tablets and total Jiaogulan saponin tablets have been available in the Chinese markets. The majority of the reported methods for the quality control of *G. pentaphyllum* were the determination of the total quantity of gypenosides by thin layer chromatography (TLC)^{13–15)} or colorimetry.^{16–18)} However, these methods are not sufficient enough for the quality assessment of the distribution of gypenosides in the raw plant or products.

As an important quality control method, chromatographic fingerprint technique has attracted great attention in recent years.¹⁹⁾ Different from the quality control method of the synthetic medicines, the fingerprint technique emphasizes on the systematic characterization of the components of samples and the evaluation of the stability of the plants. Fingerprint analysis has been introduced and accepted by WHO as a strategy for the assessment of herbal medicines. Since *G. pentaphyllum* is such a complex herbal medicine, fingerprint will help the understanding of the chemical composition of this species. However, it was seldom exploited previously, and few works have been reported in its chemical fingerprinting. The reason is that the study of *G. pentaphyllum* encounters many difficulties; the internal standard of gypenosides is not commercially available. What's more, it's very difficult to analyze the saponins of *G. pentaphyllum* by HPLC-UV without any pretreatment because the UV absorbance of gypenosides is very weak and the amount of gypenosides is rela-

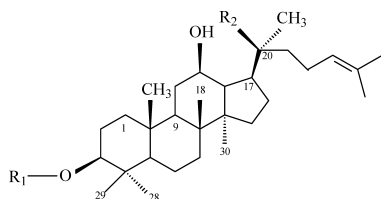


Fig. 1. Structure of a Typical *G. pentaphyllum* Saponin

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tively low comparing to other interfering components co-existing in the plant. Also it's well known that saponins in *G. pentaphyllum* vary among species and growing locations.

Considering the difficulties in obtaining gypenosides, an internal standard which is not contained in *G. pentaphyllum* could be added in the fingerprinting as the marker compound for the calculation of relative retention (RRT) times and relative peak areas (RRA), which is very important for the setting of criterion of fingerprint. Hence, ginsenoside Rb₂, a kind of saponin with similar structure and properties to gypenosides, was considered to be the internal standard.

In this study, the HPLC fingerprint profile of saponins of *G. pentaphyllum* from Shanxi province was developed for evaluating the quality of this herbal medicine. *G. pentaphyllum*, after defatted by Soxhlet extraction, was extracted by sonicating in methanol. Then the sample was applied to the column chromatography with macro pore resin to enrich and purify the saponins in the extract. Before injecting the saponins fraction onto HPLC for fingerprint analysis, ginsenoside Rb₂ was added as a marker compound, which was used to calculate several important parameters for the quality assessment of *G. pentaphyllum*. To our best of knowledge, this method of obtaining a chemical fingerprint of a sample with an internal standard, which does not exist in the sample is new and novel. Method validation experiment proves that this fingerprinting with internal standard is feasible. Finally, three *G. pentaphyllum* samples were analyzed by the new method.

Experimental

Materials and Reagents Three batches of *G. pentaphyllum* samples were obtained from Shanxi province, China.

HPLC grade acetonitrile (TEDIA Company, U.S.A.) and purified water (Coca Cola Company, U.S.A.) were the mobile phases. Petroleum ether (Zhenxing Chemical Factory, Shanghai, China) and methanol (Zhenxing Chemical Factory, Shanghai, China) used for extraction were analytical reagent (AR) grade. Macro pore resin HZ-818 (stylenedivinylbenzene, non-polarity, 60 mesh, Huazhen Company, Shanghai) and AB-8 (copolymer of stylenedivinylbenzene and acrylate, weak polarity, 60 mesh, Nankai University, China) were used in column chromatography.

Sample Preparation The dried aerial part of *G. pentaphyllum* was powdered to about 40 mesh. Then 2.00 g of the powder were extracted with petroleum ether for 12 h in a Soxhlet apparatus. After volatilization of petroleum ether, the residue was extracted successively with 30 ml of MeOH in the supersonic water bath for 30 min. Then the extract was condensed into about 10 ml and subjected to HZ-818 resin column. In this procedure, saponins and flavones were eluted while chlorophyll retained on the resin column. The eluents were collected and dried into yellow residue, which was then mixed thoroughly with water. The suspension was passed through AB-8 resin column, eluted in turn with water-ethanol (100:0, 50:50, 5:95) to separate flavonoids and saponins. The 95% alcohol eluent was collected. After evaporation of the solvent under reduced pressure, the residue was redissolved in 10 ml 60% MeOH.

About 1 mg ginsenoside Rb₂ was dissolved in 1 ml 60% methanol aqueous solution, then mixed with gypenosides solution described above (sample: ginsenoside Rb₂ solvent = 6:1, v/v). The solution was filtered through a 0.45 μm syringe prior to HPLC analysis.

HPLC Analysis An Agilent 1100 series equipped with G1312A Binary pump, G1329A automatic sample injector and G1315A Diode Array Detector (DAD) was used in this study. HPLC analysis for the optimization of extraction method was performed on an Alltima C18 column (4.6 mm × 250 mm, 5 μm, Alltech, U.S.A.). Mobile phases were water and acetonitrile (B) with gradient elution at 30 °C as 5—100% B from 0 to 60 min. The flow rate was 0.8 ml/min and the wavelength 203 nm. The sample injection volume was 20 μl.

The fingerprinting analysis of saponins in *G. pentaphyllum* was carried out on an Eclipse XDB C18 column (4.6 mm × 250 mm, 5 μm, Agilent, U.S.A.) at 30 °C, eluted by 34% acetonitrile for 60 min at 0.8 ml/min, with

sample injection volume 20 μl and the wavelength 203 nm.

Method Validation and Sample Analysis This procedure assesses the precision, repeatability and stability of the retention time and peak area.

Test solution of one batch of sample prepared according to 2.2 was analyzed continuously for 6 times for the injection precision test and the relative standard deviation (RSDs) of relative retention time and relative peak area of the five characteristic peaks were calculated. The evaluation of intermediate precision of the fingerprinting method was carried out over 6 d, and three different batches of packed columns of Eclipse XDB C18 were also compared by triplicate analysis of the same test solution on each column.

Samples from the same batch was separated into 8 aliquots by quarter separation method. Six aliquots of them were selected and analyzed for repeatability validation according to the analysis procedure.

For stability validation, one aliquot was analyzed at 0, 2, 4, 8, 12, 24 h after the preparation.

Three batches of *G. pentaphyllum* samples from Shanxi province were analyzed according to the method described above.

Results and Discussion

Method Development. Optimization of Extraction

Methods The extraction methods of gypenoside from *G. pentaphyllum* were optimized in our study. Different extraction methods such as reflux and supersonic extraction were explored, and each extract was analyzed by HPLC. The chromatograms of *G. pentaphyllum* samples in different extracting methods are illustrated in Fig. 2. In HPLC, qualitative identification of a single component can be achieved via comparison of retention data with that of standard compound. However, the results can be considered unequivocal only if additional independent physical/chemical methods are applied. For this reason the coupling of HPLC to diode array detector (DAD), yielding online UV spectra is of paramount interest.^{20,21)} The UV spectra shows that the peak 1, 2, 3 and 4 in Fig. 2 might be flavone compounds which were eluted before 25 min, and peak 5 and peak group 6 were saponins whose retention time were longer than 25 min.

The chromatogram of the extract of *G. pentaphyllum* sample refluxed by methanol for 2.5 h after defatted by Soxhlet extraction with petroleum ether for 12 h is shown in Fig. 2b. Compared with the Fig. 2a, which is the HPLC chromatogram of the herb treated with the same process without defatting, it can be noticed that in Fig. 2b more saponins with retention times longer than 25 min were extracted. One explanation is that gypenosides are contained in the cytoplasm of *G. pentaphyllum*, when the sample is extracted by Soxhlet extraction with petroleum ether, the cell wall is broken partially, so that gypenosides can be extracted readily with methanol.

To compare the methods of refluxing and supersonic extraction, samples were extracted by the two methods respectively after defatted by petroleum ether. Comparing with the sample extracted by refluxing, as shown in Fig. 2c, gypenosides were successively extracted in the similar quantity in the *G. pentaphyllum* sample extracted by methanol in supersonic bath, but the extracting time was greatly shortened. From time and economic point of view, extracting the sample using supersonic bath takes only 30 min and therefore was chosen for all further analysis.

Purification of Gypenosides The UV absorbance of saponin is very weak and the detection wavelength is usually set at 203 nm. At this wavelength, the impurity such as flavone, pigment etc. in the extract would cause many disturbing peaks; hence column chromatography with AB-8

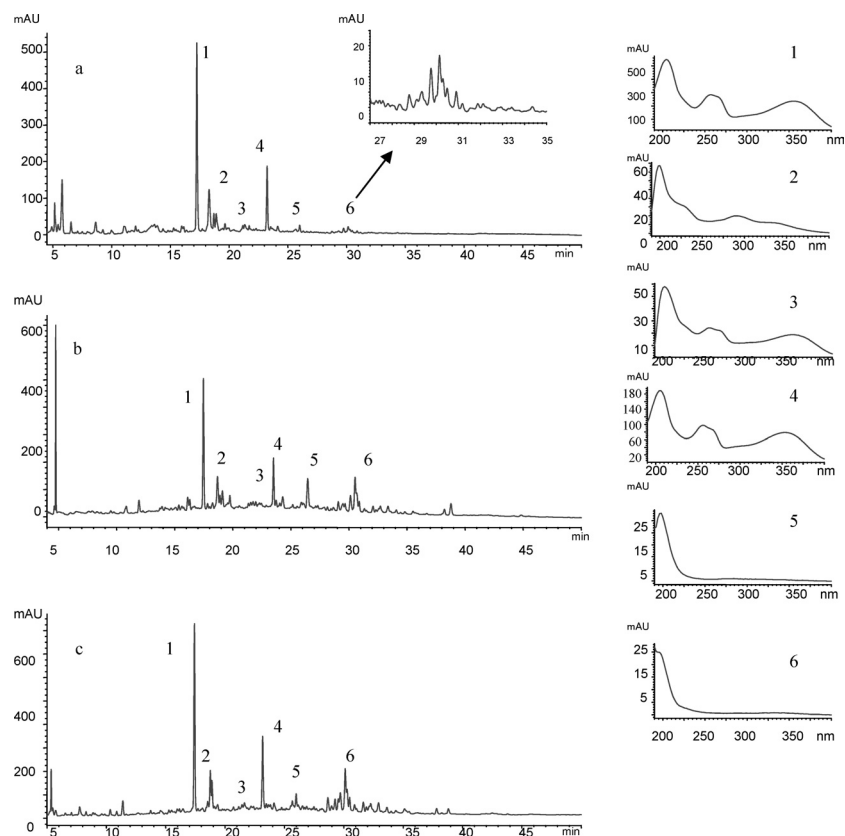


Fig. 2. HPLC Chromatograms of *G. pentaphyllum* Sample Using Different Extracting Methods

(a) Extracted by refluxing with methanol. The UV spectra of typical compounds in peak group 1—6 are shown at right. (b) Extracted by refluxing with methanol after defatted by Soxhlet extraction. (c) Extracted by sonication with methanol after defatted by Soxhlet extraction. HPLC conditions: Alltima C18 column (4.6 mm×250 mm, 5 μ m). Mobile phases were water and acetonitrile (B) with gradient elution at 30 °C as 5—100% B from 0 to 60 min. The flow rate was 0.8 ml/min and the wavelength 203 nm. The sample injection volume was 20 μ l.

resin was explored to purify the extract of *G. pentaphyllum*. The extract was subjected to AB-8 resin column, and eluted sequentially with water, 20%, 50%, 75% and 95% ethyl alcohol. Each fraction was collected and injected onto HPLC for analysis. The chromatograms are shown in Fig. 3. As can be deduced from the figure, the 50% eluent are flavones, and the 95% eluent saponins.

However, the eluents also contain a certain amount of chlorophyll, which may remain on the HPLC column and reduce its lifetime. To solve this problem, the extract was injected onto the HZ818 resin column before the AB-8 resin column, then the column was eluted with 95% ethyl alcohol, and the fraction was collected as enriched gypenosides extraction.

Optimization of the HPLC Condition Phosphoric acid aqueous solution and acetonitrile as candidate mobile phases were tested initially in this study. It was found that the separation effect for saponin was almost the same as by using the mobile phases of water and acetonitrile. Probably because most of saponins in this extract are neutral, and the acidic solvent would not improve the separating effect, therefore water and acetonitrile were used as mobile phases. Considering the similar polarity and capacity of gypenosides, isocratic elution was adopted. On Eclipse XDB C18 column (4.6 mm×250 mm, 5 μ m), using the mobile phase of 34% acetonitrile, gypenosides can well be separated. The other benefit of using isocratic elution was that a better repeatability of retention times of gypenosides could be achieved.

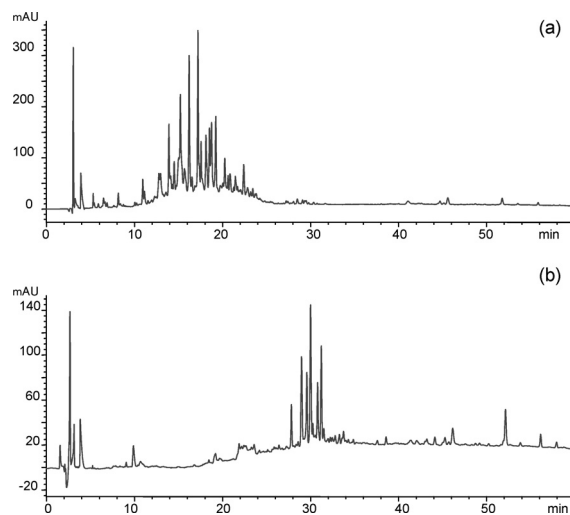


Fig. 3. HPLC Chromatograms of *G. pentaphyllum* Extract Eluents on AB-8 Resin Column

(a) Eluted by 50% alcohol in AB-8 column; (b) eluted by 95% alcohol in AB-8 column. HPLC conditions: Alltima C18 column (4.6 mm×250 mm, 5 μ m). Mobile phase was water and acetonitrile (B) with gradient elution at 30 °C as 5—100% B from 0 to 60 min. The flow rate was 0.8 ml/min and the wavelength 203 nm. The sample injection volume was 20 μ l.

Three columns, Eclipse XDB C18 column (4.6 mm×250 mm, 5 μ m, Agilent U.S.A.), Alltima C18 column (4.6 mm×250 mm, 5 μ m, Alltech) and Eclipse XDB C18 column (4.6 mm×150 mm, 5 μ m, Agilent U.S.A.), were eval-

uated in this study. The HPLC conditions were optimized for each column, and Eclipse XDB C18 column (4.6 mm × 250 mm, 5 μm) was considered as a most suitable column for the separation of gypenosides due to its good separation ability for saponins in *G. pentaphyllum*.

Selection of Internal Standard In the fingerprint analysis of herbal medicine, choosing an appropriate marker compound is very important. With the marker peak, relative retention time and relative peak area can be calculated more reliably than purely assessing the retention time and area of a single peak. However, the standard of gypenosides was not commercially available, also it was very difficult to separate a single saponin standard from *G. pentaphyllum* in a common analytical laboratory because the structures of saponins in this species are quite similar. In consideration of the difficulties in obtaining gypenosides, another compound which was not contained in *G. pentaphyllum* was added into the test solution as marker.

An internal standard should be similar in structure and chemical character with target constituents, and also an ideal internal standard should have similar signal response with those of target compounds. Ginsenoside Rb₁, Rb₂, R_c and R_d, with chemical properties similar to gypenosides, were added to the extract of *G. pentaphyllum*, respectively. HPLC chromatograms of the mixtures demonstrated that ginsenoside Rb₁ had almost no retention under the optimized separation conditions, while the peaks of R_c and R_d partly overlapped with those of some other gypenosides peaks in the fingerprint. Fortunately, ginsenoside Rb₂ was separated well from the other peaks of saponins in a reasonably elution time. As a result, ginsenoside Rb₂ was selected to be the internal standard in this fingerprint method. The typical fingerprint profile of *G. pentaphyllum* from Shanxi province with internal standard is shown in Fig. 4. By carefully analyzing the fingerprint profiles of these samples, five peaks with reasonable heights and good resolutions were selected as characteristic peaks for the identification of the crude drug because these peaks represented saponins identified by DAD detector and further conformed by LC-MS-MS analysis.

Method Validation. Precision The precision of the fingerprint method is demonstrated by performing the developed procedure optimized previously. The validation results

are summarized in Table 1. The RSD values of five characteristic peaks were less than 0.3% for the relative retention time and 3% for the relative peak area.

One test solution was injected into HPLC in 1 d, 2 d, 3 d, 4 d, 5 d, 6 d, respectively to test the intermediate precision of the HPLC method in different time. The RSDs of the five characteristic peaks were less than 0.3% for the relative retention time and 3% for the relative peak area.

The intermediate precision on three different batches of packed columns were studied. The RSDs of the five characteristic peaks were less than 2% for the relative retention time and 5% for the relative peak area.

Stability One test solution was injected into HPLC at 0 h, 2 h, 6 h, 8 h, 12 h, 24 h, respectively to investigate the stability of the sample. The results are shown in Table 1. The RSDs of five characteristic peaks were less than 0.3% for the relative retention time and 4% for the relative peak area, which means the samples were stable for at least 24 h.

Repeatability The repeatability of the present method was evaluated by performing 6 reduplicate experiments (Table 1). The RSDs of five characteristic peaks were less than 0.3% for the relative retention time and 5% for relative peak area.

From the results of the above method validation experiments, it can be concluded that this fingerprinting method of *G. pentaphyllum* saponins is adequate, valid and applicable.

Sample Analysis The fingerprint profiles of three batches of *G. pentaphyllum* samples from Shanxi are shown in Fig. 5. The fingerprint profiles of the three batches of sample were similar. Mean relative retention times of the five characteristic peaks were 1.56, 2.05, 2.39, 2.54, 2.82 with

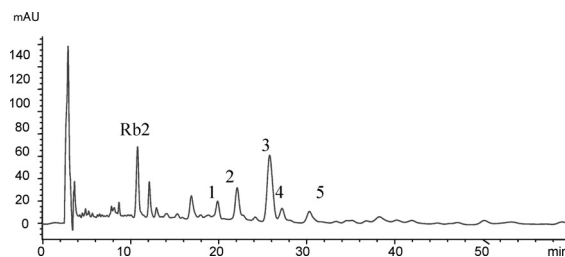


Fig. 4. The Fingerprint Profile of *G. pentaphyllum* Saponins from AnKang Shanxi with Internal Standard

Table 1. Validation Data of Fingerprint Method of *G. pentaphyllum*

Item		Marker peak Ginsenoside Rb ₂		Peak No.									
				Peak 1		Peak 2		Peak 3		Peak 4		Peak 5	
		RRT ^{a)}	RPA ^{b)}	RRT	RPA	RRT	RPA	RRT	RPA	RRT	RPA	RRT	RPA
Injection precision	Mean	1.00	1.00	1.84	0.24	2.05	0.41	2.40	1.50	2.83	0.21	3.60	0.35
	RSD %	0.00	0.00	0.15	2.01	0.13	2.89	0.13	1.83	0.10	2.83	0.10	2.62
Intermediate precision Intra-day precision	Mean	1.00	1.00	1.84	0.24	2.05	0.41	2.40	1.50	2.83	0.21	3.60	0.35
	RSD %	0.00	0.00	0.29	2.52	0.11	2.81	0.18	1.92	0.13	2.86	0.10	2.64
Precision of different batch of columns	Mean	1.00	1.00	1.84	0.23	2.05	0.41	2.39	1.50	2.83	0.21	3.60	0.35
	RSD %	0.00	0.00	0.67	2.13	1.41	2.82	1.92	2.61	1.70	3.08	1.45	4.16
Stability	Mean	1.00	1.00	1.84	0.22	2.05	0.41	2.39	1.49	2.83	0.21	3.59	0.35
	RSD %	0.00	0.00	0.25	2.52	0.20	3.04	0.17	1.88	0.12	2.01	0.12	2.51
Repeatability	Mean	1.00	1.00	1.84	0.21	2.05	0.40	2.39	1.47	2.83	0.21	3.59	0.35
	RSD %	0.00	0.00	0.12	2.42	0.12	4.92	0.15	0.78	0.17	1.65	0.23	2.36

a) RRT: retention time of characteristic peak/retention time of marker peak of ginsenoside Rb₂. b) RPA: peak area of characteristic peak/peak area of marker peak of ginsenoside Rb₂.

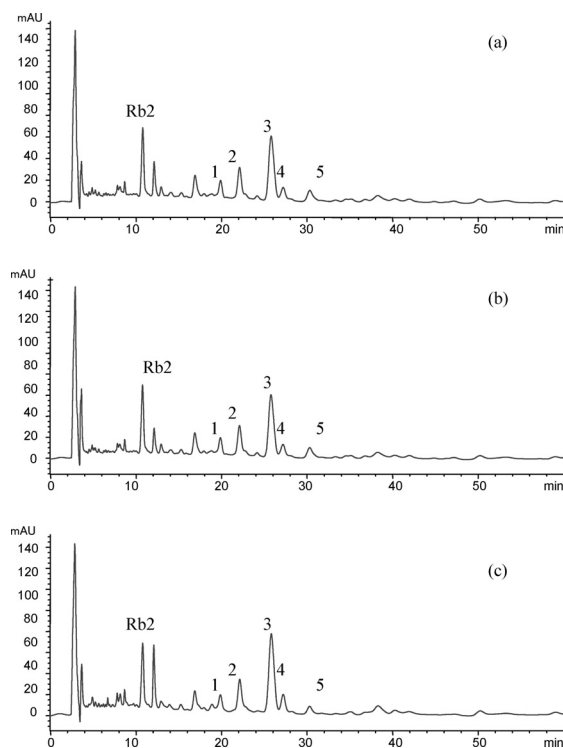


Fig. 5. Chromatograms of Three *G. pentaphyllum* Samples from Shanxi Province

RSDs of 0.14%, 0.09%, 0.06%, 0.07%, 0.08% and 0.05%, respectively, and the RSDs of relative peak area were 7.79%, 11.19%, 14.63%, 3.41%, 15.16% and 10.92%, respectively. These results indicate that the RRTs of characteristic peaks are stable enough to be set as the parameter for authentication of *G. pentaphyllum* samples. The RSDs of RPA indicate that the concentration of saponins in *G. pentaphyllum* differs even cultured in the same place. Based on this finding, it is important to widen the limits of RPA when specify the quality control data of *G. pentaphyllum*.

Conclusion

Although *G. pentaphyllum* is a famous Chinese herbal medicine, little work has been done to the development of a chromatography fingerprinting of saponins for quality evaluation of *G. pentaphyllum* due to the lack of gypenoside standards. In this paper, a new approach to develop the HPLC fingerprint of *G. pentaphyllum* with an internal standard is proposed.

Ginsenoside Rb₂, as a marker compound, was added into the sample solution before the fingerprint analysis. With the marker compound, the components represented in the fingerprint were analyzed with their relative retention time and relative peak area. The validation studies of precision, stability

and repeatability have confirmed that this method for the fingerprinting analysis of *G. pentaphyllum* saponins is adequate, valid and applicable. Three samples were analyzed by using this method, the RSDs of the relative retention time of the five characteristic peaks were 0.14%, 0.09%, 0.06%, 0.07%, 0.08%, 0.05%, respectively. Thus, the sample which has the similar HPLC chromatographic pattern and RRT value with the typical fingerprint chromatogram could be authenticated as genuine *G. pentaphyllum* from Shanxi province.

Clearly, this study put forward a promising method for the quality control of *G. pentaphyllum*. Addition of internal standard as marker compound would be helpful to improve the quality control ability of fingerprinting based strategy for TCM, not only for *G. pentaphyllum* but also for other complex herbal medicines.

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References

- 1) Megalli S., Davis N. M., Roufogalis B. D., *J. Pharm. Pharm. Sci.*, **9**, 281—291 (2006).
- 2) Circosta C., Pasquale D. R., Occhinto F., *Phytomedicine*, **12**, 634—638 (2005).
- 3) Wang Y. Q., *Zhong Xi Yi Jie He Za Zhi*, **8**, 262—286 (1988).
- 4) Attawish A., Chivapat S., Bansiddhi J., Techadamrongsin Y., Mitrijit O., Chaorai B., Chavalittumrong P., *Fitoterapia*, **75**, 539—551 (2004).
- 5) Razmovski N. V., Hsun W. H. T., Tran V. H., Qian L. G., Duke C. C., Roufogalis B. D., *Phytochem. Rev.*, **4**, 197—219 (2005).
- 6) Kuwahawa M., Kawanish F., Komiya T., Oshio H., *Chem. Pharm. Bull.*, **37**, 135—139 (1989).
- 7) Cui J. F., Eneroth P., Bruhn J. G., *Eur. J. Pharm. Sci.*, **8**, 187—191 (1999).
- 8) Yin F., Hu L. H., *Helv. Chim. Acta*, **88**, 1126—1134 (2005).
- 9) Liu X., Ye W. C., Hsiao H. W. W., Che C. H., Zhao S. X., *J. Chin. Pharm. Univ.*, **34**, 21—23 (2003).
- 10) Yin F., Hu L. H., Pan R. X., *Chem. Pharm. Bull.*, **52**, 1440—1444 (2004).
- 11) Yin F., Hu L. H., Lou F. C., Pan R. X., *J. Nat. Prod.*, **67**, 942—952 (2004).
- 12) Hu L. H., Chen Z. L., Xie Y. Y., *J. Nat. Prod.*, **59**, 1143—1145 (1996).
- 13) Xie P. S., Yan Y. Z., *J. High Resolu. Chromatogr. Chromatogr. Commun.*, **10**, 607—613 (1987).
- 14) Zheng J., He W., *Guangdong Yaoxueyuan Xuebao*, **16**, 203—204 (2000).
- 15) Wang B. Q., "Research on the Quality of Chinese Preparative Medicines and Reference Substances," China Medical and Pharmaceutical Science and Technology Press, Beijing, China, 1994.
- 16) Ding S. L., Zhu Z. Y., *Chinese Traditional and Herbal Drugs*, **23**, 527—629 (1992).
- 17) Jing D. Y., Bian J., Gong L., Ye K., *China Pharmaceuticals*, **15**, 19—20 (2006).
- 18) Chi M. Y., Mei X. W., Han C. L., Guan S. B., *Lishizhen Medicine and Materia Medica Reseach*, **17**, 2004—2005 (2006).
- 19) Pavel D., Jitka M., *J. Chromatogr. B*, **812**, 3—21 (2004).
- 20) Engelhardt H., König T., *Chromatographia*, **2**, 341—353 (1989).
- 21) Springfield E. P., Eagles P. K. F., Scott G., *J. Ethnopharmacol.*, **101**, 75—83 (2005).