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Common and Variation Peak Ratio Dual-Index Sequence Analysis of Vanillin-Sulfuric Acid Developing UV Fingerprint of *Panax notoginseng*

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The vanillin-sulphuric acid developing-UV fingerprint of *Panax notoginseng* extracted by chloroform, ethanol and water was analyzed by common and variation peak ratio dual index sequence analysis. The results indicated that there was same effective quality evaluation for *P. notoginseng* extractions of chloroform, ethanol and water. The common peak ratios of the different seed sources and same producing areas of *P. notoginseng* ranged from 45 to 85 %, the variation peak ratios ranged from 9.38 to 61.90 %. There were relationships between results of experiment and information of samples. This method evaluated quality of *P. notoginseng* from different seed sources and same producing areas, which provided reference for quality evaluation of Chinese herbal medicine.

Keywords: *Panax notoginseng*, Vanillin-sulphuric acid, UV fingerprint, Traditional Chinese medicine.

INTRODUCTION

Chemical fingerprint is an essential tool for the characterization of the sources of variance in plant materials[1]. Fingerprint techniques are utilized to gain a broader insight into chemical composition of medicinal plants and further to distinguish the origin of medicinal plants and classify their species[2]. Techniques like HPLC, NMR, UV and IR are commonly applied[3-5]. More specifically, UV fingerprint (UV FP) is fast and need no complex pretreatment. It has already been used to discriminate the content of biodiesel feedstock and reflected the whole component information present in the biodiesel feedstock[6]. Yuan *et al.*[7] applied the dual-index sequence analytical method to evaluate UV FP of *Gentiana rigescens* and clarified the similarity level among different areas.

Panax notoginseng (Burkill) F. H Chen (Araliaceae) as an important traditional Chinese medicine (TCM) has been cultivated in China for more than 400 years[8]. It is used to stanch blood, disperse gore, reduce pain, *etc.*[9]. The pharmacological active substances of *P. notoginseng* are sapoin components, which have been used medicinally for its haemostatic and cardiovascular properties[10].

The different seed source of *P. notoginseng* samples are very difficult to identify. Accurate evaluate quality of different seed source of *P. notoginseng* samples can facilitate seed selection for the economically field cultivation of *P. notoginseng*. The UV FP compared to HPLC and IR fingerprint had more kindly stability and reproducibility[11]. The vanillin sulphuric acid could improve the similarity of the UV FP of the *P. notoginseng*[12]. The spectral data were analyzed by the dual-index sequence analytical method, which is an efficient method for classification, identification and discriminate of components of the fingerprint spectra of traditional Chinese medicine[13].

In this paper, the vanillin sulphuric acid colorimetry-UV FP of the five different seed source of *P. notoginseng* populations were investigated by dual-index sequence analysis of common and variation peak ratio. The aim of this study was to comparative three extract ways of the chemical constituents difference and evaluate quality of different seed of *P. notoginseng* samples.

EXPERIMENTAL

UV-2500 UV/visible spectrometer (Shimadzu, Japan), with spectral range: 190-400 nm; DFT-100 type grinder (Zhejiang Wenling City Linda Machinery Co., Ltd., China); SY3200-T type ultrasonic washer (Beijing zhongxi taian technology services company, China); 100 mesh stainless steel sieve (Shanghai shenyuan ultrasonic equipment Co., Ltd., China); AR1140 electronic analytical balance (NJ, USA); UPT-I-10 Millipore Waters Milli-Q system (Ulupure, China).

All of *P. notoginseng* samples were three-year age and collected from Xiaoshao field base in Kunming, Yunnan province on October 11, 2012. All of the *P. notoginseng* samples were authenticated by Dr. Zhang Jin-yu, Institute of Medicinal

Plants, Yunnan Academy of Agricultural Sciences. Seeds of natural populations of *P. notoginseng* were from five sites of Yanshan County Yunnan Province. Each *P. notoginseng* sample group's code, number and source of seeds were listed in Table-1.

TABLE-1
SOURCE OF *Panax notoginseng* SEED

Sample group	Number of samples	Source of seeds
A	5	Ban-ge, Yanshan, Yunnan
B	5	A-meng Yanshan Yunnan
C	5	Zhe-la Yanshan Yunnan
D	5	Pan-long Yanshan Yunnan
E	5	Xiao-shuijing Yanshan Yunnan

Ethanol, chloroform, vanillin and sulphuric acid were analytical grades. Double distilled water was obtained from UPT-I-10 Millipore Waters Milli-Q system (Ulupure, China).

Sample processing: Twenty-five different roots of *P. notoginseng* were freeze-dried and grinded to fine powder by grinder. Fifteen hundred mg of each of the powder of samples was extracted with 20 mL chloroform and ultrasonication extraction for 20 min. The solid residue powder was isolated and air dried and then extracted with 20 mL ethanol and ultrasonication extraction for 20 min. The preparation of water extracts were similarly as in ethanol.

Data acquisition: Each *P. notoginseng* sample extracts 5 mL added with five drops vanillin-sulphuric acid was analyzed by UV-2500. The UV spectra were scanned by UV-2500 at 1 nm sampling interval and 0.2 nm slit width between 190 and 400 nm. For UV spectra, the data were treated by the three groups average, the first derivative and the two points smoothing.

Data processing: The data were analyzed by dual-index sequence analysis method. The UV FP of *P. notoginseng* is able to distinguish *P. notoginseng* samples from different seed source. The common and variation peak ratio of UV FP of 25 *P. notoginseng* samples were calculated, respectively. The multiple-dimensional indices were established for distinguishing varieties of *P. notoginseng* samples information. This method was effective to evaluate different *P. notoginseng* samples.

Determination of common and variation peak ratio dual-index of UV FP: Common peak ratio and variation peak ratio were calculated according to the method described by Zou *et al*.[13] as follows:

$$P = \left(\frac{N_g}{N_d}\right) \times 100\ \% \quad (1)$$

P: common peak ratio, N_g: The number of common peaks that appeared in both the UV FP compared. N_d: The total number of peaks that were different in both the UV FP compared.

$$N_d = N_g + n_a + n_b \quad (2)$$

n_a: The number of variation peaks in UV FP (a), which only belong to UV FP (a). n_b: The number of variation peaks in UV FP (b), which only belong to UV FP (b).

$$P_{va} = \left(\frac{n_A}{N_g}\right) \times 100\ \% \quad (3)$$

P_{va}: Variation peak ratio of UV FP (a).

$$P_{vb} = \left(\frac{n_b}{N_g}\right) \times 100\ \% \quad (4)$$

P_{vb}: Variation peak ratio of UV FP (b).

$$N_a = N_g + n_a \quad (5)$$

$$N_b = N_g + n_b \quad (6)$$

N_a: Total number of peaks in UV FP (a). N_b: The total number of peaks in UV FP (b).

In this method, common and variation peak ratio were used to qualitative evaluate the similarity and difference of more than two samples. The sequence values were arranged according to common peak ratio from high to low, which was called the dual-index sequence of common and variation peak ratio. Each sample stands as a reference. N samples were constructed by N sequences and N-dimensional space. Dual-index sequence of common peak ratio and variation peak ratio that could be distinguished one sample at (2 + n) dimensional spaces.

RESULTS AND DISCUSSION

Stability of method: One sample of No. C group was determined at 1, 5, 10, 15, 20, 25 and 30 h. The RSD % was calculated according to the results of wavelength of peaks in the UV FP of chloroform, ethanol and water extracts of *P. notoginseng*. The RSD % of three extracts were arranged from 0-0.62, 0-0.67 and 0.07-0.63 %, respectively. The analytical results displayed that the three extracts were stable in 30 h.

Precision of method: One sample of No. C group was tested six times. The RSD % was calculated by wavelength of peaks. The RSD % of three extracts were arranged from 0-0.63, 0-0.57 and 0-0.76 %, respectively. The results showed that this method had a good precision.

Reproducibility of method: One sample of No. C group was accurately weighted six times repeatedly. The RSD % was calculated by wavelength of peaks. The RSD % of three extracts were in the range of 0-0.58, 0-0.62 and 0-0.61 %, respectively. The results indicated that reproducibility of this method was kindly.

UV FP of the chloroform, ethanol and water extracts: The UV FP of three extracts are shown in Figs. 1-3, respectively. They are very similarity. It showed that the same more of content chemical of *P. notoginseng* extracted by the three solvent, respectively. Because the biological characteristics of Chinese herbal medicines have stability and variability inherited. It indicated the chemical compositions and content of the same producing area and different seed source of *P. notoginseng* are more influenced by environment factors than hereditary.

Dual-index sequence of chloroform extracts: The results of dual-index sequence of the common and variation peak ratio analysis of chloroform extracts are shown in Table-2. From the results, we could find that the maximum common peak ratios of A1:A2, B3:B4, C1:C5, D2:D3 and F3:F4 sequences are 65.85, 71.43, 73.68, 76.92 and 84.21 %, respectively. The minimum common peak ratios of A1:A5, B2:B5, C3:C5, D1:D4 and E4:E5 sequences are 46.67, 48.84, 58.54, 54.55 and 60.47 %, respectively. Most of the common peak ratios are more than mean of common peak ratio. For example the sequences of common peak ratio of group A of A1:A2, A2:A4,

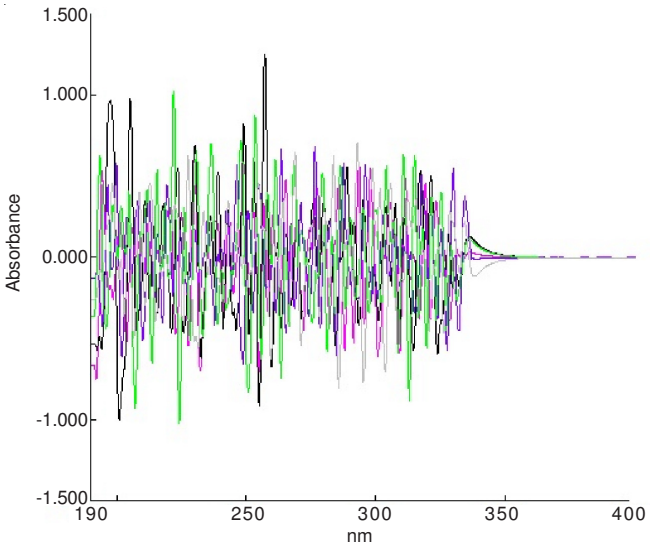

Fig. 1. UV fingerprint of the solution extracted with chloroform

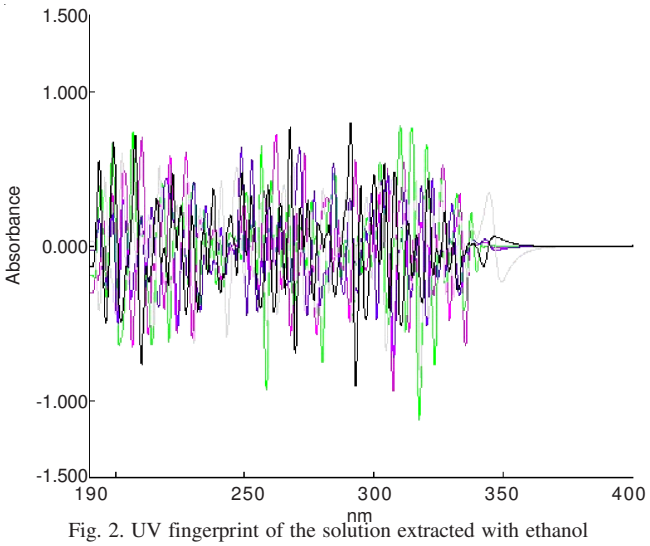

Fig. 2. UV fingerprint of the solution extracted with ethanol

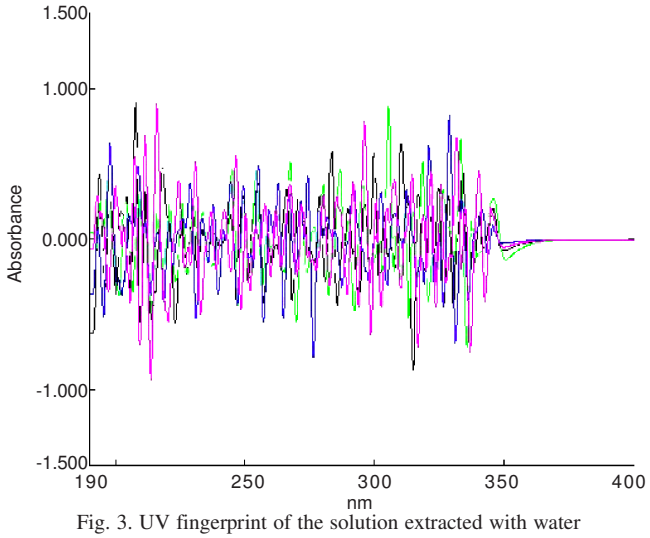

Fig. 3. UV fingerprint of the solution extracted with water

A1:A4 and A2:A3 sequences are over 57.26 %. It indicated that there are substantial similar at the different seed source of *P. notoginseng* samples, because it has stability inherited. This method could identify the different seed source of *P. notoginseng* samples.

TABLE-2
COMMON AND VARIATION PEAK RATIO IN THE UV FINGERPRINT REGIONS OF THE CHLOROFORM EXTRACTS

Sequences	P (%)	P_{va} (%)	P_{vb} (%)
Group A			
A1:A2	65.85	25.93	25.93
A2:A4	65.85	25.93	25.93
A1:A4	61.90	30.77	30.77
A3:A5	60.98	36.00	28.00
A2:A3	58.14	36.00	36.00
A2:A5	57.14	41.67	33.33
A4:A5	57.14	41.67	33.33
A1:A3	51.11	47.83	47.83
A3:A4	47.83	54.55	54.55
A1:A5	46.67	61.90	52.38
Mean	57.26	40.22	36.80
Group B			
B3:B4	71.43	23.33	16.67
B2:B3	64.29	18.52	37.04
B3:B5	64.29	37.04	18.52
B1:B5	61.90	38.46	23.08
B2:B4	59.52	28.00	40.00
B4:B5	59.52	40.00	28.00
B1:B3	58.70	33.33	37.04
B1:B4	57.78	38.46	34.62
B1:B2	54.55	50.00	33.33
B2:B5	48.84	52.38	52.38
Mean	60.08	35.95	32.07
Group C			
C1:C5	73.68	21.43	14.29
C1:C2	70.00	21.43	21.43
C2:C4	66.67	21.43	28.57
C3:C4	64.29	22.22	33.33
C2:C3	63.41	30.77	26.92
C1:C4	62.79	25.93	33.33
C4:C5	61.90	38.46	23.08
C2:C5	60.98	36.00	28.00
C1:C3	59.52	36.00	32.00
C3:C5	58.54	37.50	33.33
Mean	64.18	29.12	27.43
Group D			
D2:D3	76.92	16.67	13.33
D2:D4	72.50	20.69	17.24
D3:D4	70.00	21.43	21.43
D2:D5	60.98	40.00	24.00
D1:D2	60.47	30.77	34.62
D4:D5	58.54	41.67	29.17
D1:D5	54.76	47.83	34.78
D3:D5	54.76	47.83	34.78
D1:D3	54.55	41.67	41.67
D1:D4	54.55	41.67	41.67
Mean	61.80	35.02	29.27
Group E			
E3:E4	84.21	9.38	9.38
E2:E3	80.00	15.63	9.38
E2:E4	80.00	15.63	9.38
E1:E5	69.23	18.52	25.93
E2:E5	69.05	27.59	17.24
E1:E4	67.50	18.52	29.63
E1:E2	64.29	18.52	37.04
E3:E5	64.29	29.63	25.93
E1:E3	63.41	23.08	34.62
E4:E5	60.47	34.62	30.77
Mean	70.24	21.11	22.93

TABLE-3
COMMON AND VARIATION PEAK RATIO IN THE UV FINGERPRINT REGIONS OF GROUP A EXTRACTED BY CHLOROFORM, ETHANOL AND WATER

Sequences	P (%)	P_{va} (%)	P_{vb} (%)
Chloroform			
A1:A2	65.85	25.93	25.93
A2:A4	65.85	25.93	25.93
A1:A4	61.90	30.77	30.77
A3:A5	60.98	36.00	28.00
A2:A3	58.14	36.00	36.00
A2:A5	57.14	41.67	33.33
A4:A5	57.14	41.67	33.33
A1:A3	51.11	47.83	47.83
A3:A4	47.83	54.55	54.55
A1:A5	46.67	61.90	52.38
Mean	57.26	40.22	36.80
Ethanol			
A1:A2	68.18	40.00	6.67
A1:A5	66.67	40.00	10.00
A1:A3	65.96	35.48	16.13
A1:A4	65.22	40.00	13.33
A3:A5	60.47	38.46	26.92
A3:A4	59.09	38.46	30.77
A2:A3	54.55	33.33	50.00
A4:A5	52.27	47.83	43.48
A2:A5	51.16	45.45	50.00
A2:A4	40.43	68.42	78.95
Mean	58.40	42.74	32.62
Water			
A4:A5	71.43	16.67	23.33
A1:A4	71.43	23.33	16.67
A1:A5	70.83	20.59	20.59
A3:A5	69.77	20.00	23.33
A1:A3	69.77	23.33	20.00
A2:A3	68.29	17.86	28.57
A3:A4	65.12	28.57	25.00
A1:A2	60.47	42.31	23.08
A2:A5	59.09	26.92	42.31
A2:A4	54.55	37.50	45.83
Mean	66.07	25.71	26.87

Dual-index sequence of the group A sample: From the Table-3, the sequences of the common peak ratio of the samples are generally similar and the relationships among samples are closest. They are in range from 40-70 %. The maximum common peak ratios of chloroform, ethanol and water extracts are 65.85, 68.18 and 71.43 %, respectively. The minimum common peak ratios of the three extractions are 46.67, 40.43 and 54.55 %, respectively. It indicated that they are substantial similar among the same ages and producing areas of *P. notoginseng* samples. The mean value common peak ratios of the three extractions are 57.26, 58.40 and 66.07 %, respectively. It indicated that the three extractions could provide the effective information of resource identification. Zhang *et al*.[14] applied the common and variation peak ratio dual index sequence analysis method studied chloroform, ethanol and water extracts of the same producing areas of *Paris*. The results showed this method could clarify the similarity between the species and areas of *Paris*. The maximum variation peak ratios of the three extractions are 61.9, 45.83 and 78.95 %, respectively. The minimum variation peak ratios of them are 25.93, 16.67 and 6.67 %, respectively. It showed that there are different chemical constituents among same producing areas and age of *P. notoginseng*. The results tell us that three extractions could be used effective to identify the herbal samples *P. notoginseng*.

Conclusion

The vanillin sulduric acid colorimetry-UV FP of *P. notoginseng* could be used exactly in qualitative evaluation of *P. notoginseng* samples. This study can be used to evaluate accurately the qualities among different seed source of *P. notoginseng* samples by the common and variation peak ratio dual index sequence analysis method. The experimental results showed that there is a good correlation between UV FP and *P. notoginseng* samples of quality and there are significant of quality of five different seed source of *P. notoginseng* populations of the vanillin sulphuric acid colorimetry-UV FP, then the three extractions of the different chemical constitents of the different seed source of *P. notoginseng* could be identified accurately and quickly. This method could also be applied the basement for identification and quality evaluation of herbs.

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