Journal of Pharmaceutical and Biomedical Analysis 56 (2011) 1089–1093

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

Short communication

Discrimination of the seeds of *Notopterygium incisum* and *Notopterygium franchetii* by validated HPLC-DAD–ESI-MS method and principal component analysis

Kaijie Xu[a], Shunyuan Jiang[b], Yan Zhou[a,*], Yanxia Zhang[a], Bing Xia[a], Xuemin Xu[b], Yi Zhou[b], Yufei Li[a], Mingkui Wang[a], Lisheng Ding[a,*]

[a] Key Laboratory of Mountain Ecological Restoration and Bioresource Utilization, Chengdu Institute of Biology, Chinese Academy of Sciences, Chengdu 610041, PR China
[b] Sichuan Key Laboratory of Quality and Innovation Research of Chinese Materia Medica, Sichuan Academy of Chinese Medicine Sciences, Chengdu 610041, PR China

ARTICLE INFO

Article history:
Received 12 April 2011
Received in revised form 18 July 2011
Accepted 22 July 2011
Available online 30 July 2011

Keywords:
Notopterygium incisum
N. franchetii
Seeds
HPLC-DAD–ESI-MS method
Principal component analysis

ABSTRACT

A validated HPLC-DAD–ESI-MS method has been developed to simultaneously quantify 12 bioactive compounds in the seeds of *Notopterygium incisum* Ting ex H.T. Chang and *Notopterygium franchetii* H. de Boiss whose rhizomes and roots are widely used as traditional Chinese medicine. This method was validated to be sensitive, precise and accurate and was applied to evaluate the difference in the chemical profiles and contents of these analytes in 37 batches of *N. incisum* and 31 batches of *N. franchetii* samples collected from different locations. Principal component analysis showed that the two species were separated into two groups obviously. This study established a validated method for identification of the authenticity of the seeds of *N. incisum* and *N. franchetii* and supplied effective guidance for artificial cultivation.

1. Introduction

Notopterygium incisum Ting ex H.T. Chang and *Notopterygium franchetii* H. de Boiss (family Umbelliferae) are two medicinal herbs of Qianghuo, distributing in high altitude region (2500–4000 m) in Tibet, Sichuan, Qinghai and Gansu of China. Their rhizomes and roots are widely used as antirheumatic and analgesic medicine for the treatment of rheumatism, headache, subcalorism, etc. [1,2]. The chemical constituents from the rhizomes and roots of the two plants were coumarins, phenoloids and essential oils [3–9]. Pharmacological studies have shown that coumarins such as isoimperatorin, notopterol and bergapten possess anti-inflammatory, analgesic and anti-cancer activities [10–14].

There were some reports about the qualitative and quantitative analysis of coumarins from Qianghuo by chromatographic fingerprinting techniques such as thin layer chromatography (TLC) [15] and high performance liquid chromatography (HPLC) [16,17]. HPLC coupled with mass spectrometry (HPLC–MS) has been used for the quality control of Qianghuo in recent years [18–22].

Because of the predatory excavation and environment destruction, wild resources of *N. incisum* and *N. franchetii* were almost exhausted. In order to protect wildlife resources and solve the supply and demand contradiction, artificial cultivation was conducted. However, the seeds of *N. incisum* and *N. franchetii* are always blent due to the similarities in their physical traits in the medicinal material market. However, no effective method was established to distinguish the seeds of the two species, and it is of difficulty for artificial cultivation. The rhizomes and roots of *N. incisum* are more commonly used herbal medicine than those of *N. franchetii*, so the price of *N. incisum* semina is much higher than that of *N. franchetii* in the market. In order to identify the authenticity of two species semina and supply effective guidance for artificial cultivation, a validated HPLC-DAD–ESI-MS method has been developed to simultaneously quantify 12 main compounds for the first time. Dataset obtained from HPLC–MS were processed by principal component analysis (PCA) to compare the difference of the two species.

2. Experimental

2.1. Chemicals and materials

HPLC grade methanol (Fisher, USA), acetic acid (China) and deionized water obtained from a Milli-Q water system (Millipore Corp., Bedford, MA, USA) were used for sample preparation procedures and HPLC analysis. Twelve standard compounds

* Corresponding authors.
E-mail addresses: zhouyan@cib.ac.cn (Y. Zhou), lsding@cib.ac.cn (L. Ding).

0731-7085/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2011.07.034

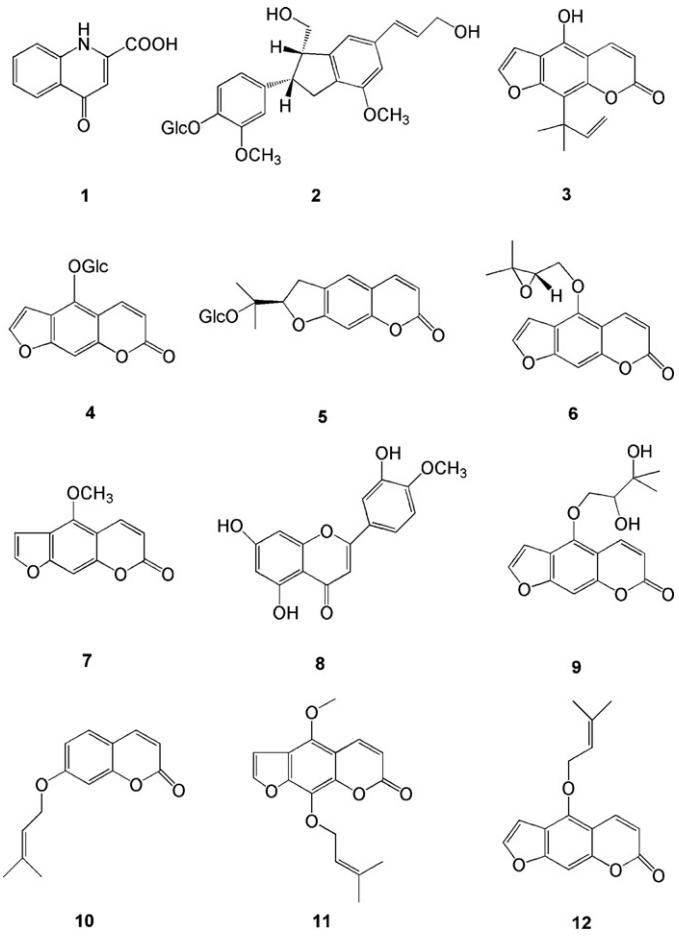

Fig. 1. The chemical structures of compounds **1**–**12**.

namely kynurenic acid (**1**), alaschanioside C (**2**), umbelliferone (**3**), bergaptol-*O*-β-D-glucopyranoside (**4**), nodakenin (**5**), oxypeucedanin (**6**), bergapten (**7**), diosmetin (**8**), oxypeucedanin hydrate (**9**), *O*-prenyl-umbelliferone (**10**), phellopterin (**11**) and isoimperatorin (**12**) were isolated in our laboratory from the seeds of *N. incisum* and *N. franchetii* and their structures (Fig. 1) were elucidated by comparison of UV, MS, ^{1}H NMR and ^{13}C NMR spectral data with the literature values [4,5,23–29]. The purity of each compound was determined to be higher than 98% by HPLC–UV analysis.

A total of 68 batches of *N. incisum* and *N. franchetii* semina samples (Nos. **1**–**68**) were collected from different sources in China. All samples were authenticated as the seeds of *N. incisum* and *N. franchetii* by Dr. Shunyuan Jiang (Sichuan Academy of Chinese Medicine Sciences) and the corresponding voucher specimens (SACMS No. in Supplementary Table) were deposited in the Sichuan Academy of Chinese Medicine Sciences.

2.2. Preparation of sample solutions

An accurately weighed sample of 0.5 g grinded powder and 20 mL 70% methanol were added to a conical flask and extracted by ultrasonication for 30 min, and then filtered off and repeat this extraction process twice. All extracts were combined and evaporated under vacuum, and then diluted to volume with 70% methanol in a 25 mL volumetric flask. A volume of 2 mL of the solution was filtered through 0.45 μm membranes, and a 20 μL aliquot was injected into the HPLC for analysis.

2.3. Instrumentation and chromatographic condition

A TSP HPLC system consisting of a vacuum degasser, quaternary pump, autosampler and DAD detector (Thermo Separation Products Inc., USA) was used for acquiring chromatograms and UV spectra. For chromatographic analysis, TIANHE kromasil C18 column (5 μm, 250 mm × 4.6 mm) with a suitable guard column (C18, 5 μm, 4.0 mm × 3.0 mm) was used. The mobile phase consisted of methanol (A) and 0.1% acetic acid solution (B) using a gradient of 35–40% A for 0–10 min, 40–55% A for 10–20 min, 55–75% A for 20–45 min, 75% A for 45–50 min and 75–87.5% A for 50–55 min. The flow rate was 0.7 mL/min, and the column temperature was maintained at 35 °C The injection volume was 20 μL. DAD detector was set to scan from 190 to 400 nm. UV detector was set at 240 nm for quantitative analysis of compounds **2**, **4**, **6**, **7**, **9**, **11**, **12** and 330 nm for compounds **1**, **3**, **5**, **8**, **10**.

A ThermoQuest Finnigan LCQ^{DECA} system equipped with an electrospray ionization (ESI) source (ThermoQuest LC/MS Division, San Jose, CA, USA) was used for mass spectrometric measurements. The ESI-MS spectra were acquired in both positive and negative ion modes. The mass spectrometry parameters were as follows: nebulizer sheath gas and auxiliary gas, N_2 (80 unit) and (20 unit); capillary temperature, 300 °C; spray voltage, 4.5 and 5.0 kV in negative and positive ion mode, respectively; capillary voltage, −13 V in (−) ESI, 25 V in (+) ESI. All data were processed by Finnigan Xcalibur^{TM} core data system Rev. 1.2 (ThermoQuest Corporation, San Jose, CA, USA).

2.4. Validation procedure

2.4.1. Calibration curves, limits of detection and quantification

Stock solutions containing 12 reference compounds were prepared and diluted to appropriate concentrations for construction of calibration curves. All calibration graphs were plotted based on linear regression analysis of the integrated peak areas (y) versus concentrations (x, μg/ml) of the 12 standards at eight different concentrations. The dilute solution was further diluted to a series of concentrations with methanol for the gain of the limits of detection (LOD) and limits of quantitation (LOQ). The LODs and LOQs under the present chromatographic conditions were determined by diluting the standard solution when the signal-to-noise ratios (S/N) of analytes were almost 3 and 10, respectively. The results were shown in Table 1.

2.4.2. Precision, accuracy and repeatability

Intra- and inter-day variations were chosen to determine the precision of the developed method. For intra-day variability test, the mixed standard solutions were analyzed for six replicates within 1 day, while for inter-day variability test; the solutions were examined in duplicates for three consecutive days. Variations were expressed by relative standard deviation (RSD).

The recovery was used to evaluate the accuracy of the method. Recovery was carried out by spiking accurate amounts of 12 standards into the sample Nf 239A for five parallel measurements. The mixture was extracted and analyzed using the method mentioned above.

The sample stability test was evaluated by testing the sample Nf 239A at room temperature at 0 h, 2 h, 4 h, 8 h, 12 h and 24 h within 1 day. The RSD% was taken as a measure of repeatability and stability. All the results were shown in Table 2.

2.5. Chemometric data analysis

The HPLC–MS data of 68 seed samples of *N. incisum* and *N. franchetii* were analyzed by the statistical software SIMCA-P 12.0.1 (Umetrics) to reveal the potential difference between the seed of

Table 1
Regression equation, correlation coefficients, linearity ranges and limit of detection (LOD) and quantitation (LOQ) for the 12 markers of Qianghuo semina.

No.	Compounds	Regression equation	Range of linearity (μg/ml)	R^2	LOD (μg/ml)	LOQ (μg/ml)
1	Kynurenic acid	$y = 62{,}058x + 109{,}859$	1–150	0.9986	0.20	0.70
2	Alaschanioside C	$y = 30{,}183x - 36{,}304$	5–100	0.9988	1.50	5.00
3	Umbelliferone	$y = 80{,}633x + 298{,}674$	1–100	0.9983	0.20	0.70
4	Bergaptol-*O*-β-D-glucopyranoside	$y = 60{,}482x + 49{,}622$	5–100	0.9989	1.50	5.00
5	Nodakenin	$y = 82{,}241x + 29{,}162$	0.5–50	0.9986	0.10	0.30
6	Oxypeucedanin	$y = 83{,}480x + 103{,}908$	1–75	0.9985	0.30	1.00
7	Bergapten	$y = 106{,}393x + 322{,}677$	5–100	0.9980	1.00	3.50
8	Diosmetin	$y = 74{,}074x + 50{,}136$	1–100	0.9982	0.30	1.00
9	Oxypeucedanin hydrate	$y = 76{,}005x + 130{,}639$	1–100	0.9986	0.30	1.00
10	*O*-Prenyl-umbelliferone	$y = 8910.2x + 5 \times 106$	10–850	0.9993	0.15	0.50
11	Phellopterin	$y = 60{,}368x + 140{,}711$	5–100	0.9987	0.25	0.75
12	Isoimperatorin	$y = 94{,}410x + 209{,}456$	1–100	0.9992	0.25	1.00

Table 2
Precision, repeatability, stability, recovery for the 12 markers of Qianghuo semina.

No.	Precision RSD (%)		Repeatability ($n = 6$)	Stability ($n = 6$)	Mean recovery (%) ($n = 5$)
	Intra-day ($n = 6$)	Inter-day ($n = 6$)			
1	2.85	2.54	2.16	2.43	94.97
2	2.42	1.95	2.11	2.98	101.73
3	2.68	2.87	2.64	2.56	96.31
4	1.78	2.84	2.17	2.77	94.16
5	1.82	2.22	1.64	1.75	96.74
6	2.30	2.44	2.29	2.67	94.70
7	2.15	2.40	1.89	2.76	103.78
8	2.20	2.48	2.02	1.77	95.48
9	1.83	2.65	2.22	1.61	93.27
10	2.07	2.36	2.41	1.54	103.09
11	2.61	2.11	1.71	2.11	93.14
12	1.01	1.58	1.15	2.46	93.73

N. incisum and *N. franchetii*. Firstly, the HPLC–MS dataset was input into Excel and the 12 standard compounds were set as variables. Secondly, the Excel was imported to SIMCA-P 12.0.1. Finally, the data was analyzed by invoking the order "*t*[1]/*t*[2] Scatter Plot" in the "Favorite".

3. Results and discussion

3.1. Optimization of chromatographic conditions

DAD detector was set to scan from 190 to 400 nm. According to the maximum absorption of the standards, UV detector was set at 240 nm for quantitative analysis of compounds **2**, **4**, **6**, **7**, **9**, **11**, **12** and 330 nm for compounds **1**, **3**, **5**, **8**, **10**. Comparing with acetonitrile, methanol showed better separation. An acidified mobile phase could minimize peak tailing and improve resolution, and facilitating ionization. Therefore, methanol (**A**) and 0.1% acetic acid solution (**B**) were chosen as mobile phases with a flow of 0.7 mL/min in the above gradient.

3.2. Validation of the method

As shown in Table 1, acceptable results of the regression analysis, the correlation coefficients (r^2), LODs and LOQs were obtained for all analytes: all calibration curves showed good linear regression ($r^2 \geq 0.9980$) within the test ranges; the LODs and LOQs of the 12 standard components were in the range of 0.15–1.50 μg/mL and 0.30–5.00 μg/mL, respectively. The intra- and inter-day variations were less than 3% and the percentage recoveries were in the range of 93.14–103.78% with RSD less than 4% (Table 2). The results of the repeatability and stability tests shown in Table 2 demonstrated that the developed assay was reproducible and stable (RSD <3%).

3.3. Identification of constituents from N. incisum and N. franchetii

HPLC-DAD–MS method was employed to analyze the main components of the seeds samples of *N. incisum* and *N. franchetii*. Under

Table 3
The retention time (t_R), UV and MS characteristics of the main detected peaks detected in *N. incisum* semina and *N. franchetii* semina samples.

No.	t_R (min)	λ_{max} (nm)	Positive ions (m/z)	Negative ions (m/z)	Identification	Mixed standards solution	Ni 152	Nf 239A
1	7.60	241, 332	190 $[M+H]^+$		Kynurenic acid	+	+	+
2	12.08	229, 273	541 $[M+Na]^+$		Alaschanioside C	+		+
3	13.47	229, 320	163 $[M+H]^+$		Umbelliferone	+		+
4	14.25	247, 308	387 $[M+Na]^+$	363 $[M-H]^-$	Bergaptol-*O*-β-D-glucopyranoside	+	+	+
5	18.28	229, 330	431 $[M+Na]^+$	407 $[M-H]^-$	Nodakenin	+	+	+
6	26.58	248, 308	287 $[M+H]^+$		Oxypeucedanin	+		+
7	30.82	246, 306	217 $[M+H]^+$		Bergapten	+	+	+
8	34.48	251, 341	301 $[M+H]^+$	299 $[M-H]^-$	Diosmetin	+	+	+
9	42.55	248, 308	305 $[M+H]^+$	303 $[M-H]^-$	Oxypeucedanin hydrate	+	+	+
10	44.38	231, 315	231 $[M+Na]^+$		*O*-Prenyl-umbelliferone	+	+	+
11	45.55	241, 265	323 $[M+Na]^+$		Phellopterin	+		+
12	50.12	248, 308	271 $[M+H]^+$	269 $[M-H]^-$	Isoimperatorin	+	+	+

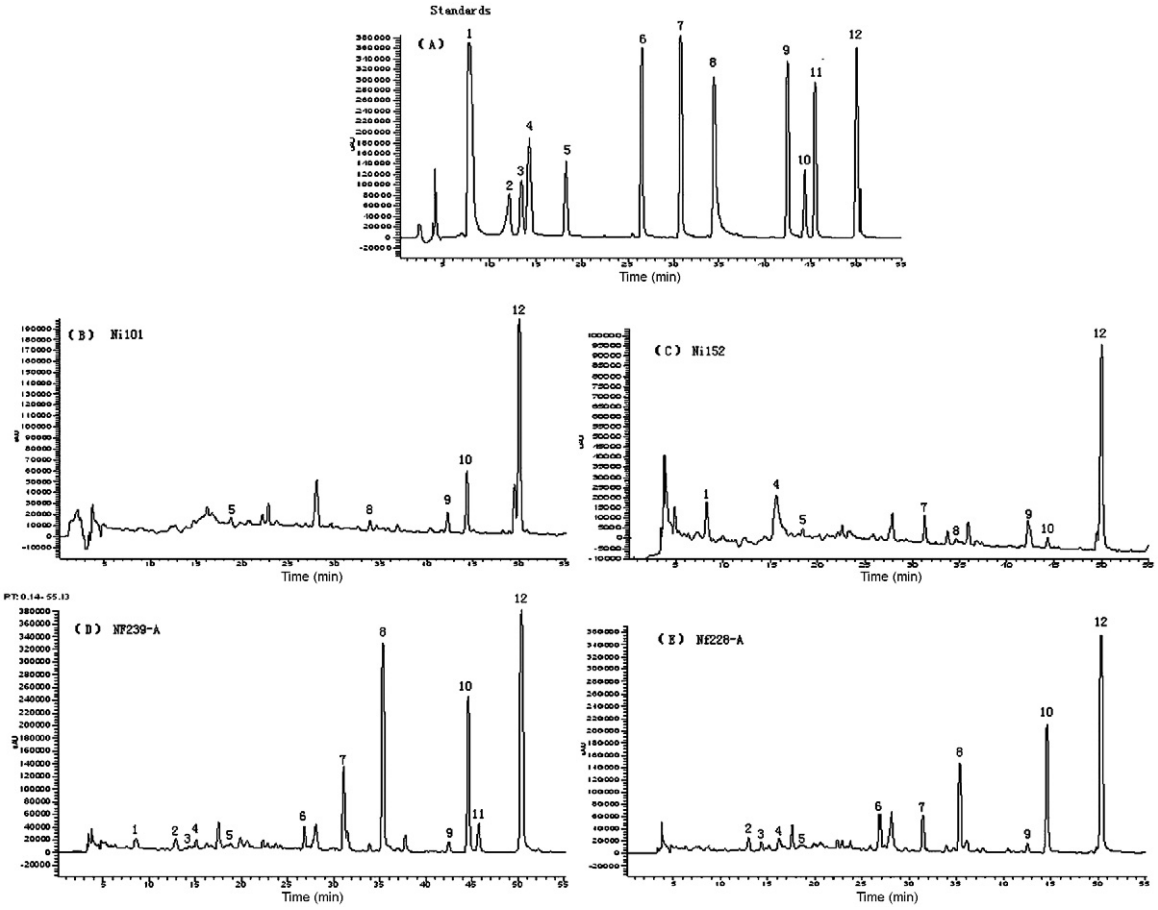

Fig. 2. The HPLC chromatograms of 12 standards and four representative samples of Qianghuo semina. (A) Standards, (B) Ni 101, (C) Ni 152, (D) Nf 239-A and (E) Nf 228-A.

the optimized MS conditions, both positive and negative modes were used to identify the peaks. By comparing the retention time, UV and MS spectra data of the peaks with that of the standard compounds and literatures [3–9], eight compounds were unambiguously identified in *N. incisum* samples and thirteen compounds were unambiguously or tentatively identified in *N. franchetii* samples. The on-line detected chromatographic and spectrometric data of the determinated compounds were given in Table 3. The representative HPLC chromatograms of standard solution and the extracts of the seeds samples of *N. incisum* and *N. franchetii* are presented in Fig. 2.

3.4. Sample analysis

In order to reveal the chemical constituents diversity of *N. incisum* and *N. franchetii* semina, the developed HPLC-DAD–ESI-MS method was successfully applied to simultaneous quantification of 12 standards in 37 batches of *N. incisum* semina and 31 batches of *N. franchetii* semina samples from different collections in China. The 12 compounds included nine coumarins (**3**–**7**, **9**–**12**); two flavonoids (**2**, **8**) and one alkaloid (**1**) were quantified by external standard methods and the analytical results were summarized (as shown in Supplementary Table). The contents of alaschanioside C (**2**), bergaptol-*O*-β-D-glucopyranoside (**4**), nodakenin (**5**), diosmetin (**8**) and isoimperatorin (**12**) are generally high in the seeds of *N. incisum* and *N. franchetii*. Nevertheless, the seeds of *N. franchetii* contained more compounds than those of *N. incisum*. Umbelliferone (**3**), oxypeucedanin (**6**), bergapten (**7**), *O*-prenyl-umbelliferone (**10**) and phellopterin (**11**) were detected in the seeds of *N. franchetii*; in contrast these compounds were detected at trace level in the seeds of *N. incisum*. Moreover, the contents of kynurenic acid (**1**) and oxypeucedanin hydrate (**9**) were higher in seeds of *N. incisum* than those of *N. franchetii.*

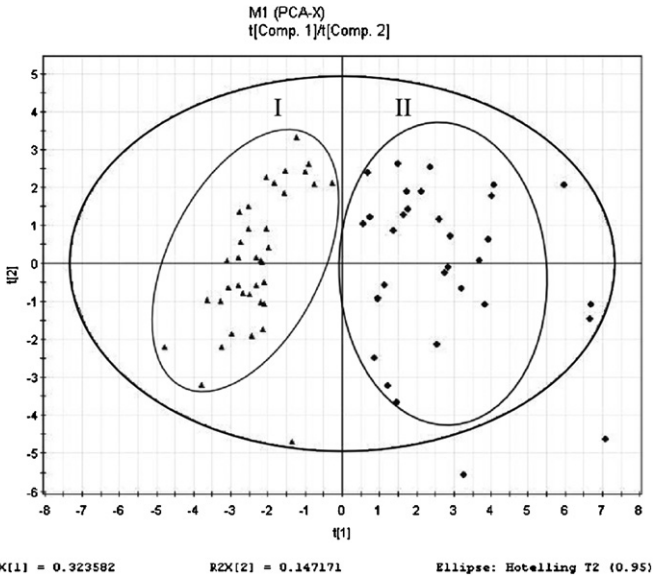

Fig. 3. Two-component PCA scatter plot of 68 Qianghuo semina samples. "▲" represents *N. incisum* semina samples (**1**–**37**); "♦" represents *N. franchetii* semina samples (**38**–**68**).

3.5. Principal component analysis (PCA)

To identify the differences between the seeds of *N. incisum* and *N. franchetii*, unsupervised principal component analysis (PCA) was performed. As can be seen from the scores plot (Fig. 3), the determined samples could be divided into two major clusters, one for samples of *N. incisum* semina (samples 1–37) and the second for samples of *N. franchetii* semina (samples 38–68), respectively. According to the results of PCA, it was revealed that the species was the dominant factor among the many factors which cause the obvious differentiation. The contribution of source was smaller than the species. Furthermore, little variation were observed within the different size of samples (Nf 228, Nf 239 and Ni 157) and the samples collected from different harvest years in the same region (Rangtang base, Ni 156-I, Ni 156-II, Ni 156-III, Ni 156-V and Ni 156-VI).

4. Conclusions

In order to identify the authenticity of two *Notopterygium* species semina and supply effective guidance for artificial cultivation, a validated HPLC-DAD–ESI-MS method has been developed to simultaneously quantify 12 main compounds in the seeds of *N. incisum* and *N. franchetii* for the first time. The newly developed method was validated to be sensitive, precise and accurate. The seeds of *N. franchetii* were found to contain more compounds than those of *N. incisum*. PCA statistical analysis was used to for the differential analysis within the two *Notopterygium* species. Overall, the *N. incisum* semina were clearly separated from *N. franchetii* semina, and the difference between these samples is a result of them coming from different *Notopterygium* species.

Acknowledgements

The work was supported by the Grant from the National Natural Sciences Foundation of China (30973634; 21072185), technological talent program of the Chinese Academy of Sciences granted to Dr. Y. Zhou.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jpba.2011.07.034.

References

[1] Chinese Pharmacopoeia Commission, Pharmacopoeia of the People's Republic of China, vol. I, People's Medical Publishing House, Beijing, 2010, p. 170.
[2] J. Shi, S.Y. Jiang, X.J. Ma, H. Sun, Y. Zhou, Studies on seeds germination and seedlings growth of *Notopterygium incisum*, Chin. J. Chin. Mater. Med. 32 (2007) 1841–1844.
[3] L.M. Li, B.D. Liang, S.W. Yu, H.D. Sun, Chemical constituents of *Notopterygium incisum*, Chin. J. Nat. Med. 5 (2007) 351–354.
[4] Y.Q. Xiao, K. Baba, M. Taniguchi, X.H. Liu, Y.F. Sun, M. Kozawa, Coumarins from *Notopterygium incisum* Ting, Acta Pharm. Sin. 30 (1995) 274–279.
[5] S. Wang, T.Z. Wang, Study on chemical constituents of *Notopterygium franchetii*, Chin. J. Chin. Mater. Med. 21 (1996) 295–296.
[6] P. Zhang, X.W. Yang, Studies on chemical constituents in roots and rhizomes of *Notopterygium incisum*, Chin. J. Chin. Mater. Med. 33 (2008) 2918–2921.
[7] S.B. Wu, Y.H. Yu, Y.H. Hu, J.F. Hu, A new dimeric furanocoumarin from *Notopterygium incisum*, Chin. Chem. Lett. 19 (2008) 940–942.
[8] F.Q. Guo, Y.Z. Liang, C.J. Xu, L.F. Huang, Determination of the volatile chemical constituents of *Notoptergium incium* by gas chromatography–mass spectrometry and iterative or non-iterative chemometrics resolution methods, J. Chromatogr. A 1016 (2003) 99–110.
[9] X.W. Yang, P. Zhang, H.Y. Tao, S.Y. Jiang, Y. Zhou, GC–MS analysis of essential oil constituents from rhizome and root of *Notopterygium franchetii*, J. Chin. Pharm. Sci. 15 (2006) 200–205.
[10] Y.P. Sun, Q. Xu, Effects of aqueous extract from rhizoma *Notopterygii* on the delayed-type hypersensitivity and inflammatory reactions and its mechanisms, J. Chin. Pharm. Univ. 34 (2003) 51–54.
[11] L. Santana, E. Uriarte, F. Roleira, N. Milhazes, F. Borges, Furocoumarins in medicinal chemistry. Synthesis, natural occurrence and biological activity, Curr. Med. Chem. 11 (2004) 3239–3261.
[12] Y.H. Guo, M. Sha, X.S. Meng, A.M. Cao, The anti-viral studies of *Notopterygium incisum*, Lishizhen Med. Mater. Med. Res. 16 (2005) 198–199.
[13] S.Y. Tang, H.S. Wang, W.X. Zhang, B. Halliwell, *Notopterygium franchetii* Boiss extract and its active constituents increase reactive species and heme oxygenase-1 in human fetal hepatocytes: mechanisms of action, Chem. Res. Toxicol. 21 (2008) 2414–2423.
[14] S.B. Wu, F. Pang, Y. Wen, H.F. Zhang, Z. Zhao, J.F. Hu, Antiproliferative and apoptotic activities of linear furocoumarins from *Notopterygium incisum* on cancer cell lines, Planta Med. 76 (2010) 82–85.
[15] Y. Tan, H.P. Zhang, Y.F. Sun, Determination the content of nodakenin from *Notopterygium incisum* by TLC densitometry, Chin. J. Chin. Mater. Med. 21 (1996) 486–487.
[16] F. Zhang, D.B. Li, Extracting isoimperatorin from *Notopterygium incisum* by HPLC, J. Nanchang Coll. 3 (2007) 107–108.
[17] Z. Chen, Y.H. Wang, Determination of ferulic acid in rhixoma et radix *Notopterygii* by HPLC, Chin. J. Inform. TCM 12 (2005) 51–52.
[18] Y.H. Li, S.Y. Jiang, Y.L. Guan, X. Liu, Y. Zhou, L.M. Li, S.X. Huang, H.D. Sun, S.L. Peng, Y. Zhou, Quantitative determination of the chemical profile of the plant material "Qiang-huo" by LC–ESI-MS–MS, Chromatographia 64 (2006) 405–411.
[19] X. Liu, S.Y. Jiang, K.J. Xu, H. Sun, Y. Zhou, X.M. Xu, J.H. Yi, Y.C. Gu, L.S. Ding, Quantitative analysis of chemical constituents in different commercial parts of *Notopterygium incisum* by HPLC-DAD–MS, J. Ethnopharmacol. 126 (2009) 474–479.
[20] G.S. Qian, Q. Wang, K.S.Y. Leung, Y. Qin, Z.Z. Zhao, Z.H. Jiang, Quality assessment of rhizoma et radix Notopterygii by HPTLC and HPLC fingerprinting and HPLC quantitative analysis, J. Pharm. Biomed. Anal. 44 (2007) 812–817.
[21] Y. Jiang, B. David, P.F. Tu, Y. Barbin, Recent analytical approaches in quality control of traditional Chinese medicines – a review, Anal. Chim. Acta 657 (2010) 9–18.
[22] C. Tistaert, B. Dejaegher, Y.V. Heyden, Chromatographic separation techniques and data handling methods for herbal fingerprints: a review, Anal. Chim. Acta 690 (2011) 148–161.
[23] R. Torres, F. Faini, B. Modak, F. Urbina, C. Labbe, J. Guerrero, Antioxidant activity of coumarins and flavonols from the resinous exudate of *Haplopappus multifolius*, Phytochemistry 67 (2006) 984–987.
[24] Z.J. Cai, F.J. Dan, F. Cheng, J.Z. Wang, K. Zou, Chemical constituents of antibacterial activity fraction of *Angelica polymorpha*, J. Chin. Med. Mater. 31 (2008) 1160–1162.
[25] F. Guo, Q.L. Liang, Z.D. Min, Study on the flavonoids from *Elephantopus scaber* Linn, Chin. Trad. Herb. Drugs 33 (2002) 303–304.
[26] Z.M. Gu, D.X. Zhang, X.W. Yang, M. Hattori, T. Namba, Isolation of two new coumarin glycosides from *Notopterygium franchetii* and evaluation of a Chinese crude drug, qiang-huo, the underground parts of *N. incisum* and *N. franchetii*, by high-performance liquid chromatography, Chem. Pharm. Bull. 38 (1990) 2498–2502.
[27] G.F. Liu, J. Wang, Y.Q. Fu, S.S. Yang, Chemical constituents of *Stellera chamaejasme*, J. Chin. Pharm. Sci. 6 (1997) 125–128.
[28] I. Saracoglu, M. Varel, I. Calis, Neolignan, flavonoid, phenylethanoid and iridoid glycosides from *Phlomis integrifolia*, Turk. J. Chem. 27 (2003) 739–747.
[29] A.K. Suleiman, Transtorine, a new quinoline alkaloid from *Ephedra transitoria*, J. Nat. Prod. 61 (1998) 262–263.