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STUDY ON FINGERPRINT OF *RADIX FALLOPIAE MULTIFLORAE* BY HPLC-DAD-MS

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ABSTRACT

To establish a fingerprint of *Radix Fallopiae multiflorae* by HPLC-DAD-MS. The chromatographic column was Agilent Eclipse XDB-C18 (4.6 mm x 250 mm, 5 µm). The mobile phase was acetonitrile – acid formic 0.5%, a gradient elution was conducted and the detection wavelength was at 254 nm. According to the comparative study of fingerprint, we found 15 communal peaks and identify 12 peaks by MS and reference compounds. The HPLC fingerprint of *Radix Fallopiae multiflorae* has good repeatability and strong characteristic, and it can provide scientific basis for the quanlity evaluation of *Radix Fallopiae multiflorae*.

KEYWORDS: Fingerprint, *Radix Fallopiae multiflorae*, HPLC-DAD-MS.

1. INTRODUCTION

Radix Fallopiae multiflorae (RFM) is a common medicinal plant in China. It has been also widely used in Vietnamese traditional medicine for treatment of depression, anemia, hair-loss and constipation. RFM contains anthraquinones (emodin, physcion, physcion-8-*O*-*β*-D-glucopyranoside, emodin-8-*O*-*β*-D-glucopyranoside, emodin-1,6-dimethylether, emodin-8-*O*-(6′-*O*-malonyl)-glucoside); stilbenes (2,3,5,4′-tetrahydroxystilbene-2-*O*-*β*-D-glucopyranoside, 2,3,5,4′-tetrahydroxystilbene-2,3-*O*-*β*-D-glucopyranoside) and flavonoids (tricin, quercetin-3-*O*-galactoside), as well as gallic acid, catechin, torachrysone-8-*O*-*β*-D-glucopyranoside, *N*-transferuloyl tyramine, *N*-transferuloyl-3-methyldopamine.[1,2]

The technology of fingerprint has the characteristics of macroscopic and integrity. It can reflect the overall characteristics of the samples analysed. It is very suitable for the study of complex systems such as herbal medicine.[3] China has begun to use fingerprint technology to supervise and manage Chinese herbal medicines since 2004.[4] In this paper, HPLC-DAD-MS technique was used to analyse RFM and establish fingerprints of RFM to provide scientific and effective methods for the quality control and evaluation of RFM.[5]

2. MATERIALS AND METHODS

2.1 Materials and chemicals

Forty-eight RFM samples (from **No-1** to **No-48**) were collected from different parts of Vietnam and were authenticated by Department of Medicinal Material Resources, NIMM.

All standards were purchased from Sigma-Aldrich. All solvents for HPLC were purchased from Merck. Distilled water was produced by a Milli-Q purification system (Millipore, USA).

2.2 Preparation of analytical samples

Weigh accurately 1 g of the powder to a stoppered conical flask, add 50 mL of methanol. Heat under reflux for 1 hour, cool and filter, use the successive filtrate as the test solution.[6]

2.3 HPLC analysis

The method was performed on a Shimadzu (Kyoto, Japan) HPLC system equipped with a LC-20AD pump, DGU-20As degasser, SIL-20A HT autosampler, CTO-10AS VP column oven, SPD-20A UV/VIS detector and MS/MS detetctor. Data acquisition and integration were performed using LC Solution software. Chromatographic separation was carried out on an C_{18} column (250 mm × 4.6 mm; 5 µm). A reverse phase HPLC assay was carried out using an isocratic elution with a flow rate of 0.5 mL/min, a column temperature of 25°C, a mobile phase of acetonitrile (A) and 0.5% (v/v) formic acid (pH = 3) (B). The gradient was as follow: 0-22 min: 16%A; 22-45 min: 16-34%A; 45-50 min: 34-95%A: 50-80 min. The injection volume was 10 µl of each solution. The UV detector was set at 254 nm. The MS detector with Q1 SCAN (m/z: 100 – 900).

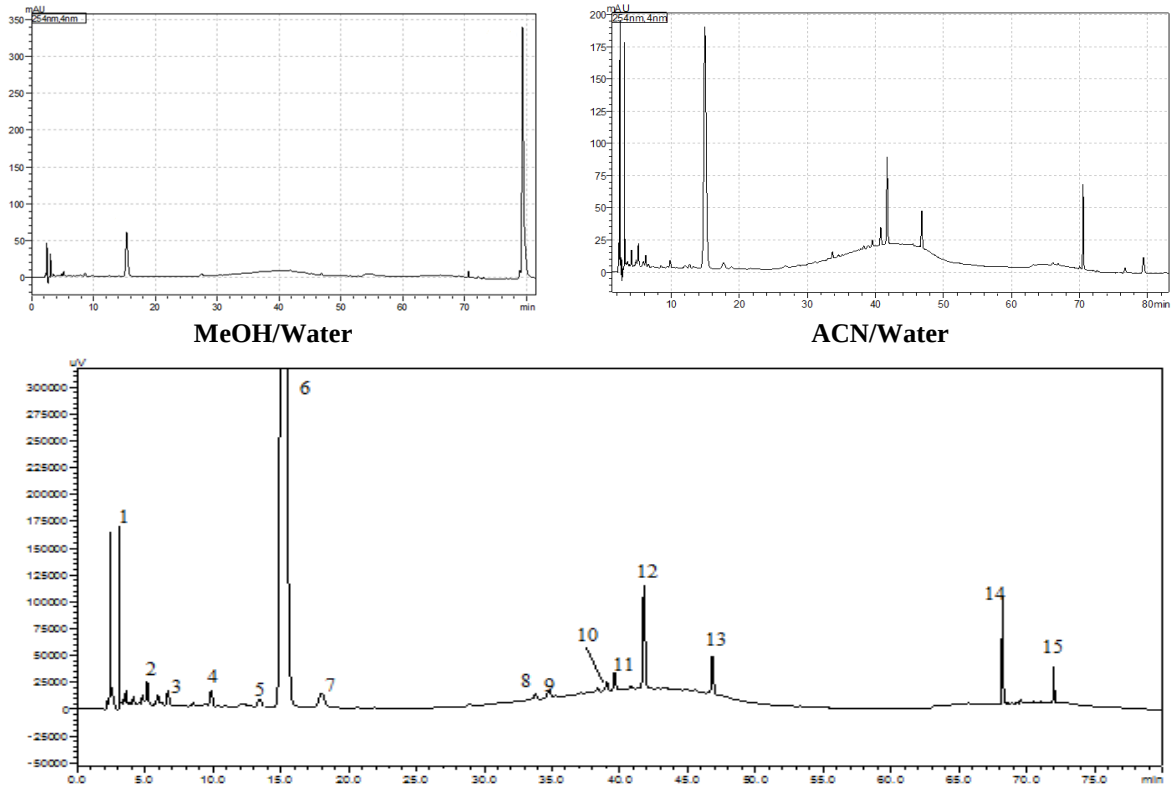
3. RESULTS AND DISCUSSION

3.1 Blank test

Precision absorbing mobile phase 10 µl, analyzing the samples according to the method of chromatographic condition in chromatographic conditions. The results showed that the blank solvent had no interference to the experiment.

3.2 Optimization of chromatographic conditions

To develop a reliable chromatographic fingerprinting method, an optimized strategy for HPLC conditions was performed. To obtain sharp and symmetrical peaks, different mobile phase systems, including methanol-water, acetonitrile-water, acetonitrile-water (containing 0.5% acid formic) were tested. As a result, high resolution, good baseline, sharp and symmetrical peaks were obtained by using acetonitrile-water (containing 0.5% acid formic) system (Figure 1).



MeOH/Water **ACN/Water**

ACN/acid formic 0.5%

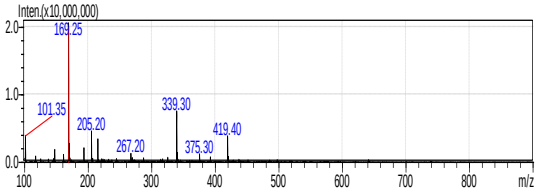
Figure 1: HPLC chromatograms of RFM sample in different HPLC conditions.

3.3 Repetitive study

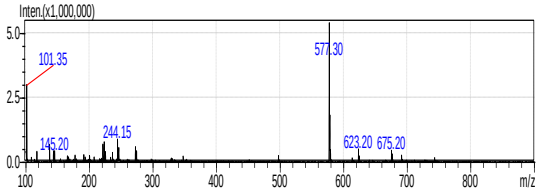
Take the same batch of medicinal powder, 5 sample solutions were prepared according to the method in preparation of solution. Analyzing the samples according to the method of chromatographic condition in chromatographic conditions. The retention time and peak area of each characteristic peak are measured. The %RSDs were lower than 2% and show good repeatability of the test.

3.4 Assignments of the characteristic peaks

Figure 1 shows the 15 characteristic peaks detected at 254 nm in RFM sample. The structural identification of each peak was carried out by careful studies on MS and MS^2 –pectra and by comparison with the reference compounds and literature (Table 1).



Peak-1.



Peak-2.

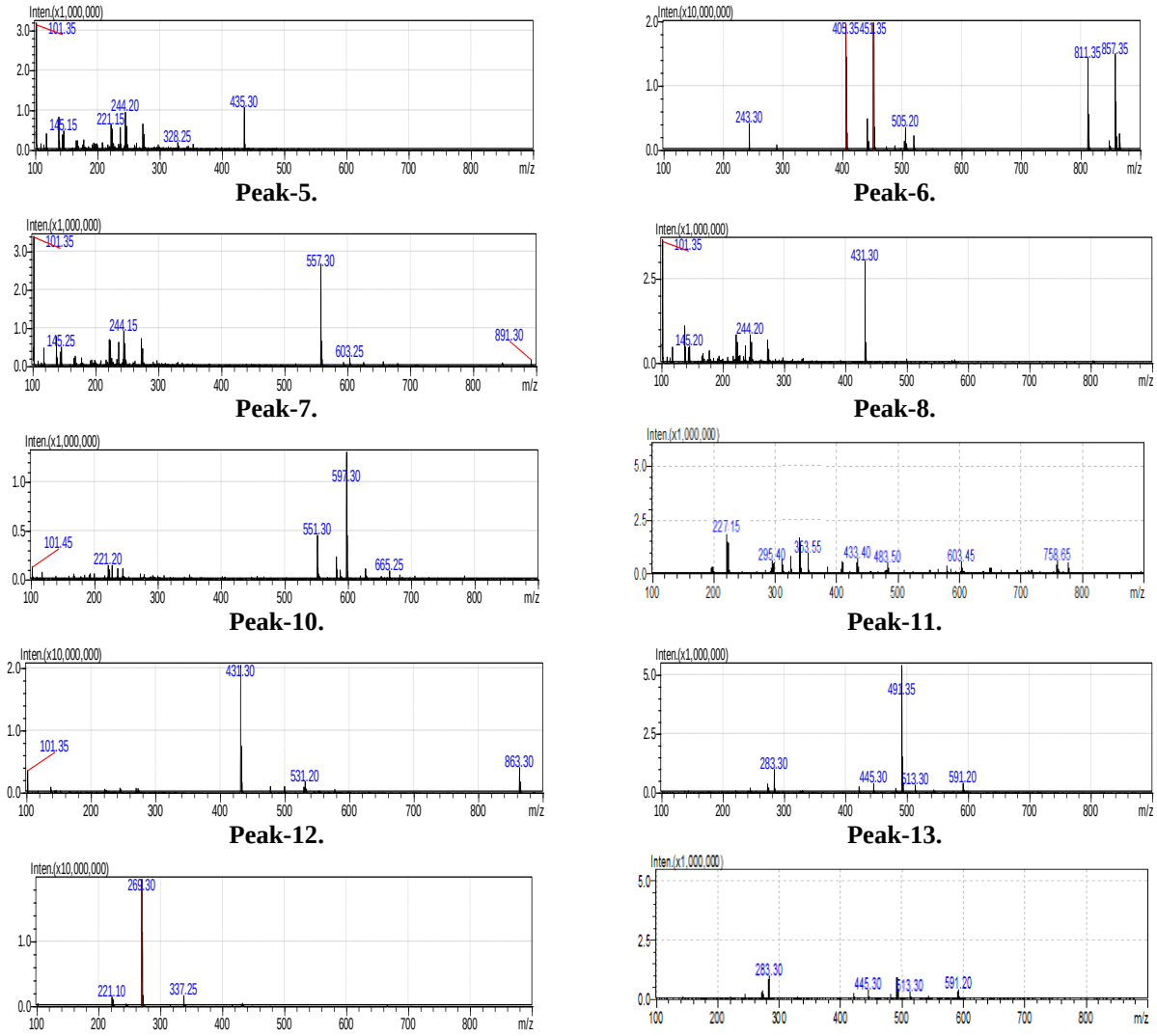


Figure 2: Mass spectra of 12 common peaks.

Table 1: Assignments of the characteristic peaks by HPLC-DAD-MS.

No	RT (min)	MS	MS^2	Assignment	
				Molecular	Name
1	3.098	169.25 [M-H]	MS^2: 125	$C_7H_5O_5$	Acid gallic
2	5.165	577.30 [M-H]	MS^2: 125; 288; 406	$C_{30}H_{25}O_{12}$	Procyanidin B
3	6.705	*Not detected*	*Not detected*	*Not detected*	*Not detected*
4	9.888	*Not detected*	*Not detected*	*Not detected*	*Not detected*
5	13.462	435.30 [M+FA-H]		$C_{20}H_{22}O_8$	Piceid
6	15.165	405.35 [M-H] 811.35 [2M-H]	MS^2: 243.10	$C_{20}H_{21}O_9$	2,3,5,4′-tetrahydroxystilbene-2-*O*-*β*-D-glucopyranoside (THSG)
7	17.152	577.30 [M-H]	MS^2: 313; 243	$C_{27}H_{25}O_{13}$	Tetrahydroxystilben-O-galloyl-glucoside
8	34.779	431.30 [M-H]	MS^2: 269; 239	$C_{21}H_{19}O_4$	Emodin-1-O-glucoside
9	35.244	*Not detected*	*Not detected*	*Not detected*	*Not detected*
10	39.039	597.30 [M+FA-H]	MS^2: 243; 145	$C_{29}H_{27}O_{11}$	Tetrahydroxystilbene-2-O-coumaroyl-glucoside
11	39.611	227.15 [M-H]	MS^2: 112	$C_{14}H_{12}O_3$	resveratrol
12	41.778	431.30 [M-H]	MS^2: 269	$C_{21}H_{19}O_{10}$	Emodin-*8*-*O*-*β*-D-glucoside
13	46.819	491.35[M-H]	MS^2: 283; 445	$C_{22}H_{21}O_{10}$	Physcion-*8*-*O*-*β*-D-glucoside
14	68.198	269.30[M-H]	MS^2: 225; 241	$C_{15}H_9O_5$	Emodin
15	71.973	283.30[M-H]	MS^2: 240	$C_{16}H_{11}O_5$	Physcion

Take RFM samples (from No-1 to No-48), the test sample solution was prepared according to the method in preparation of solution. Analyzing the samples according to the method of chromatographic condition in chromatographic conditions. By comparing the fingerprints of 48 RFM samples, 12 common peaks were identified as acid gallic (peak **1**), procyanidin B (peak **2**), piceid (peak **5**), THSG (peak **6**), tetrahydroxystilbene-O-galloyl-glucoside (peak **7**), emodin-1-O-glucoside (peak **8**), tetrahydroxystilbene-2-O-coumaroyl-glucoside (peak **10**), resveratrol (peak **11**), emodin-8-*O*-*β*-*D*-glucoside (peak **12**), physcion-8-*O*-*β*-*D*-glucoside (peak **13**), emodin (peak **14**), physcione (peak **15**). Stilbenes are the main components of RFM, through the study on the fingerprints of 48 samples of RFM, we choose peak **6** (THSG) as a reference HPLC fingerprint of RFM cause its better retention time, peak area and the degree of separation.[6]

3.5 Fingerprinting and principle component analyses

Fingerprinting and chemometrics analyses can show the chemical similarities between one and another one holistically and visually. PCA, one of the chemometrics, is an unsupervised mathematical produre that transforms a number of possibly correlated variables into a smaller number of uncorrelated variables called principle components. Its operation can be thought of as revealing the internal structure of the data in a way which best explains the variance.

In this test, the variables of each sample consisted of percentage of peak area (each peak area divided by the total of area of 12 peaks). The data were exported to Excel to form a two-dimensional matrix (48 samples versus 12 variables) which was then exported to minitab 14 for PCA.

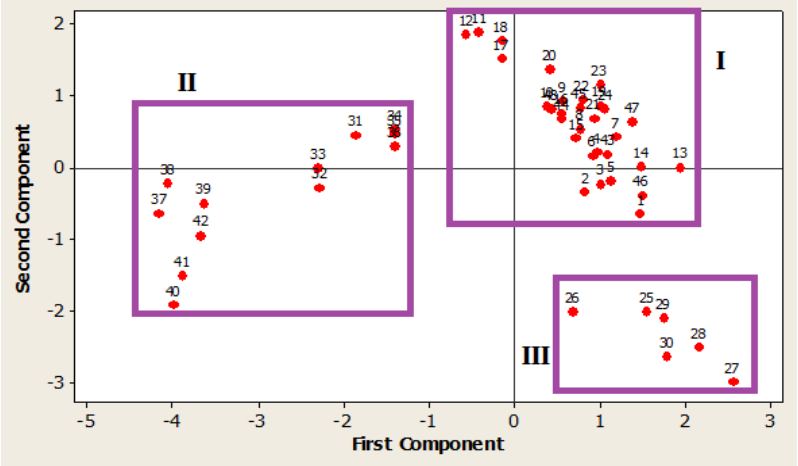


Figure 3: PCA scores plot of RFM samples.

All samples of Group I (No-1→No-24, No-43→No-48) were closer due to the similarities of the percentage of peak area of the anthraquinone (peaks **8,12,13,14,15**). These samples were in the Red River Delta.

The same way, the similarity between the RFM samples from Lai Chau and Son La can be observed (Groups II) due to the similarities in relation to the percentage of peak area of stilbenes (peaks **6,7,10,11**). And resveratrol appeared in the samples from Sin Ho (Lai Chau) and Thuan Chau (Son La) with the height of more than 1000m, but not appeared in the samples from Moc Chau (Son La) with the height of less than 1000m.

In relation to the components of the samples in Group III, there was a similarity in relation to the percentage of peak area of THSG (peak **6**), especially, the content of THSG with above 3% level – these were much higher than other samples. These informations contributed to the research on traceability of RFM from Ha Giang.

4. CONCLUSION

HPLC fingerprints of *Radix Fallopiae multiflorae* are established by HPLC combined DAD and MS detectors. 15 common peaks are identified as characteristic peaks, and 12 components of them are figured. The HPLC fingerprints of 48 *Radix Fallopiae multiflorae* samples were obtained. PCA analysis were used to analyse the fingerprint of these samples. The result can distinguish *Radix Fallopiae multiflorae* in different locations, and evaluate the quality and authenticity of *Radix Fallopiae multiflorae*. It further proves the specificity of the fingerprint and provides a new idea for the study of the fingerprint of herbal medicine in Vietnam.

ACKNOWLEDGEMENTS

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