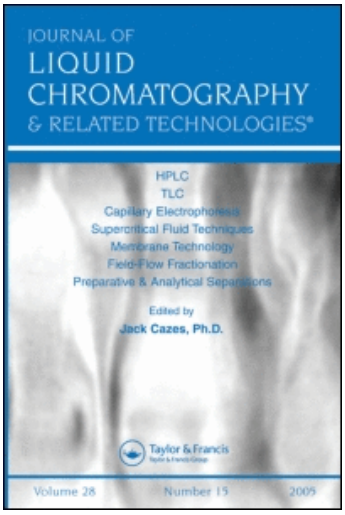

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Determination of Coptis Chinensis Franch and Phellodendron Amurense Rupr in Qingwei-Huanglian Wan by Pls-HPLC Method

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Original Article

DETERMINATION OF COPTIS CHINENSIS FRANCH AND PHELLODENDRON AMURENSE RUPR IN QINGWEI-HUANGLIAN WAN BY PLS-HPLC METHOD

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ABSTRACT

Coptis chinensis Franch contains berberine(1), palmatine(2), jatrorrhizine(3) and etc. And phellodendron amurense Rupr also contains above components. Qingwei-Huanglian Wan is made from Coptis chinensis Franch, phellodendron amurense Rupr and etc. Berberine, palmatine and jatrorrhizine in Qingwei-Huanglian Wan were determined by HPLC. The optimal composition of mobile phase CH_3COOEt-HCOOH-EtOH (15:3:2) for HPLC separation of berberine, palmatine and jatrorrhizine was successfully determine by using window diagram technique. Detection wavelength was 345nm. Flow rate: 1.5ml/min. Calibration graphs for (1), (2) and (3) were rectilinear for 0.06-0.39μg, 0.06-0.61μg and 0.01-0.12μg respectively. The basic principle and method of partial least squares method (PLS) is presented in this paper. The data from HPLC were treated with PLS program to obtain the contents of Coptis chinensis

Franch and amurense phellodendron Rpur. The results were contained. No information has ever been available in the literature for the application of PLS in HPLC of pharmaceutical analysis. Compared with other traditional computing methods, PLS is a more perfect multicomponent determination method. It has faster speed, more accurate and reliable results. PLS-HPLC provides a new method for HPLC to obtain the contents of medicinal materiales from chinese patent medicines.

Qingwei-Huanglian Wan is the Chinese Patent Medicine which is used to treat headache, fever, aphtha, glossitics, constipation and diarrhoea. It contains Phellodendron Amurense Rupr, Coptis chinensis Franch, Rehannia glutinosa libosch and Scrophularia ningpoensis Hemsl etc.. Phellodendron Amurense Rupr and Coptis chinensis Franch are the two main components in the above medicine[1]. The effective components of Phellodendron Amurense Rupr and Copits Chinensis Franch are berberine, palmatine and jatrorrhizine etc.. The applications of HPLC in Chinese Patent Medicine were reported in many literatures. However, in every previous literature, only some chemical components in a Chinese Traditional Patent Medicine were determined by HPLC, and the crude drugs in it can not be determined. The Partial Least Squares method is a superior multivariate statistical method. The application of PLS-HPLC was studied in the determination of effective components in Qingwei-Huanglian Wan. The results are satisfactory.

Experimental

Chemicals

Berberine hydrochloride(I) (supplied by Pharmaceutical Factory of The Second Millitary Medicine University), Palmatine hydrochloride(II) and jatrorrhizine(III) (supplied by Chinese Medicine College of China Pharmaceutical University), Coptis Chinensis Franch(IV) and Phellodendron Amurense Rupr(V) (obtained from Medicinal Crops Corperation of Jiangsu Province), and Qingwei-Huanglian Wan(VI) (supplied by Anqing Pharmaceutical Factory in China) were used in the preparation; acetic ester, formic acid and anhydrous alcohol were of AR grade.

Apparatus

The equipment consisted of a Waters' HPLC equipped with a silica column (2.5cm * 3.9mm), an injector (U6K) and a variable wavelength UV detector (490). The detector was

se: at 346nm at a range of 0.01 AUFS. The flow rate was 1.5ml/min.

Standard Solution and Mobile Phase

Six mg of I, II and III, accurately weighed, was transfered to a 5ml volumetric flask respectively. Methanol was added to volume and mixed. The optimum of mobile phase CH_3COOEt-HCOOH-EtOH proportion was 15:3:2 by using window diagram technique[2].

Extraction Procedure

The proper amount of IV, V and VI, accurately weighed, were transfered into sandy extractor respectively. After soaked for one night in 50ml methanol, the solution must be refluxed for 40min., than filtered, refluxed again plus 50ml methanol for 40min., filtered and the filter liquor was added together. The total filter liquor was concentrated to about 5ml. It can be diluted to the desired concentration by adding methanol. The filter residue was refluxed again by using proper amount of methanol. Upon the examination, there were not I, II and III in the filter residue. It indicated that the extraction above was complete.

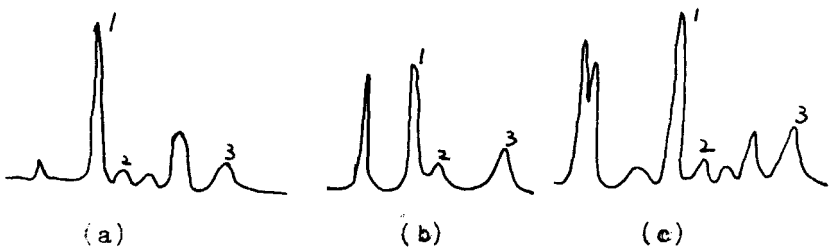
Calibration Curve and Linearity Range

Five μl of each standard solution was injected. The individual peaks was separated under optimal chromatographic condition. The linearity regression between the peak aera(A) and concentration(C) (μg) was processed. The linearity equation for I in the range of 0.06-0.39.μg was $A=2.328*10^7 C-4.656*10^4$ (r=.9997, n=6); for II in the range of 0.06-0.61 μg was $A=2.396*10^7 C-3.080*10^4$ (r=0.9994, n=5); for III in the range of 0.01-0.12μg was $A=4.279*10^6 C-6.342*10^3$ (r=0.9995, n=5).

Experimental Method

Some amount of powder of IV and V, accurately weighed, were extracted according to above extraction procedure. The chromatographic spectra of IV and V were obtained by HPLC method under the selected condition (optimum) (see Fig. 1(a.b)). The percentage of I, II and III in IV and V can be calculated from their peak-areas respectively.

Seven sets of calibration samples, prepared by different proportions of IV and V according to the



(a) Coptis Chinensis Franch

(b) Phellodendron Amurense Rupr

(c) Qingwei-Huanglian Wan

1. Berberine; 2. Jatrorrhizine; 3. Palmatine

FIG 1

Chromatograms of Constituents in Coptis Chinensis Franch, Phellodendron Amurense Rupr and Qingwei-Huanglian Wan

prescription of VI, were extracted with above extraction procedure. The content of each component was determined by HPLC. The data were used by the PLS model in the calibration procedure.

The appropraite amount of powder of simulated and real samples of VI, accurately weighed, were processed under above procedure. The chromatographic spectra, Fig. 1(c), was obtained by HPLC method. The content of each component was calculated easily. The concentrations of IV and V in simulated and real samples were obtained with the PLS program.

The Principle of PLS Method

The Chinese Patent Medicine which consists of m kinds of crude drug containing p chemical components each was considered in the text. Take the Qingwei-Huanglian Wan(VI) as an example, it includes IV(i) and V(j) which containing same components, I(B), II(Pa) and III(C), so p equals 3, m equals 2. After the content of each

chemical component in the sample of VI was determined by HPLC method, the following equations was obtained,

$$x_{i,\beta}=y_{i,i} \cdot k_{i,\beta}+ y_{i,j} \cdot k_{j,\beta} \quad (1)$$

$$x_{i,\alpha}=y_{i,i} \cdot k_{i,\alpha}+ y_{i,j} \cdot k_{j,\alpha} \quad (2)$$

$$x_{i,c}=y_{i,i} \cdot k_{i,c} +y_{i,j} \cdot k_{j,c} \quad (3)$$

where $x_{i,\beta}$ in eq.(1) is the content of I in one sample of VI, which equals the sum of the concentration of IV(i) in the sample ($y_{i,i}$) multiplied by the percentage of I in IV ($k_{i,\beta}$) and the concentration of V(j) in the sample($y_{i,j}$) multiplied by the percentage of I in V($k_{j,\beta}$). The meaning of eq.(2) or eq.(3) is the same as above.

The equations of (1), (2) and (3) can be changed into following matrix format:

$$X_{i\times p}=Y_{i\times m} \cdot K_{m\times p} \quad (4)$$

when the sample number is n, then the eq. (4) can be defined as follows:

$$X_{n\times p}=Y_{n\times m} \cdot K_{m\times p} \quad (5)$$

where $Y_{n\times m}$ represents the concentration matrix of n samples for m kinds of crude drug, $X_{n\times p}$ is the content matrix measured at p components' position for n samples, $K_{m\times p}$ is the percentage coefficient matrix of p components for m kinds of crude drug.

The mathematical model between the content of the chemical components in the Chinese Patent Medicine and the concentration of the crude drugs in the Medicine, having deduced above, can be solved by Partial least squares method, a kind of multivariate statistical method[5-6]. In PLS approch, both matrix X and matrix Y are processed by principal components analysis. X and Y may be represented by a product of two small matrices plus "random" errors respectively:

$$X_{(n\times p)}=T_{(n\times a)} \cdot Lx_{(a\times p)}+ E_{(n\times p)} \quad (6)$$

$$Y_{(n\times m)}=U_{(n\times a)} \cdot Ly_{(a\times m)}+ F_{(n\times m)} \quad (7)$$

where T is the latent matrix of X with n rows (mixtures) and a columns (number of dimenssions), Lx represents the loading matrix with a rows and p columns, U is the latent matrix of Y, Ly is the loading matrix of Y, and E and F are error matrices that have the same dimensions as the original matrix X and matrix Y respectively. The matrices Lx and Ly are so calculated that both $\|X-TLx\|$ and $\|Y-ULy\|$ are "small" and that T and U are correlated with each other column-wise by using a diagonal matrix D:

$$U_{(n\times a)}=T_{(n\times a)} \cdot D_{(a\times a)}+ G_{(n\times a)} \quad (8)$$

where a (in eq.(6), eq.(7) and eq.(8)) is the number of abstract components, and it can be calculated by cross-validation .

The contents of each component in calibration samples which were prepared with different proporations of IV and V according to the prescription of VI, were determined by HPLC method. Then some parameters such as

TABLE 1

Recovery Test

No.	Coptis chinensis Franch			Phellodendron amurense Rupr		
	Added (μg)	Found (μg)	Recovery (%)	Added (μg)	Found (μg)	Recovery (%)
1	2.002	1.984	99.10	5.010	4.946	98.72
2	3.402	3.409	100.2	8.624	8.573	99.41
3	1.802	1.779	98.72	4.376	4.372	99.91
4	3.812	3.527	97.65	9.101	8.931	98.13
5	2.402	2.379	99.04	6.002	5.875	97.88
6	2.402	2.362	98.33	5.963	5.975	100.2
7	3.003	3.021	100.6	7.611	7.441	97.77
8	4.203	4.125	98.14	10.25	10.20	99.51
9	3.003	2.986	99.43	7.423	7.453	100.4
10	4.003	3.947	98.60	9.833	9.811	99.78
Mean Recovery (%)			98.98±0.91			99.17±0.98

Lx, Ly, T, D and a, can be obtained by PLS program after the data above inputted.

Once the model have been determined, concentrations Y′ in n′ unknown samples may be computed from the contents X′ of corresponding components in the following steps. At first, determine T′ of unknown samples by means of the following equation:

$$X'(n\times p) = T'(n\times a) \cdot Lx(a\times p) \qquad (9)$$

where Lx may be gotten from calibration section. Then compute the latent variables U′ of Y′:

$$U'(n\times a) = T'(n\times a) \cdot D(a\times a) \qquad (10)$$

The final result Y′ is obtained from the following equation:

$$Y'(n\times m) = U'(n\times a) + Lx(a\times m) \qquad (11)$$

Results

Recovery Test

As the other kinds of crude drugs in VI didn′t contain components of I, II and III, there were no disturbance in the determination for HPLC method. Based on above mentioned "experimental method " and orthogonal design rule, 7 sets of calibration samples and 10 sets of simulation samples were prepared and analyzed. The

TABLE 2
Assay of Qingwei-Huanglian Wan

No.	Coptis chinesis Franch (%)		Phellodendron amurense Rupr (%)	
	PM	PLS-HPLC	PM	PLS-HPLC
I		5.78		14.01
II	5.56	5.56	13.89	13.78
III		5.83		13.94
Mean(%)		5.73±0.13		13.91±0.12

PM: Percentage in the prescription

results of HPLC analysis were inputted to the PLS program. After the parameters of PLS model were determined by 7 sets of calibration samples, the recovery ratioes of 10 sets of simulation samples, listed in table 1, were obtained.

Analysis of The Actual Samples

Three sets of powder samples of Qingwei-Huanglian Wan weighed, were extracted and analyzed according to above mentioned "experimental method". The contents of IV and V in 3 sets of VI samples were determined by PLS program. The avarages of predicted percentage of IV and V in 3 sets of samples are 5.73% and 13.91% respectively, and are close to the percentages 5.56%(IV) and 13.89% (V) in the prescription. The results listed in Table 2, especially showed that the predicted proporation of IV and V, 1:2.498, are good agree with the proporation in prescriptiom, 1:2.5.

Discussion

1. Ussually, in order to control the quanlity of the products, we must known the contents of crude drugs in Chinese Patent Medicine during the production. At present, the popular method used to predict the contents of crude drugs is to determine the contents of chemical components in crude drugs. However the crude drugs IV and V in VI have same chemical components, in this situation, the contents of crude drugs can not be predicted by above mentioned method. This problem can be solved by HPLC-PLS method, and its results are

corresponding with the requirement. All these indicate that PLS-HPLC method is new and feasible for the determination of crude drugs in Chinese Patent Medicine.

2. PLS is a new multivariate statistical method, and its performance is better than other traditional methods such as ordinary multivariate regression and principal components regression. This is because the calibration technique, which makes good use of the information in concentration matrix Y and content matrix X, is used in PLS method.

3. The method is applicable to the quanlity control of the products. The contents of IV and V in VI can be predicted accurately fast, if the method described here is used by factories making VI.

REFERENCES

1. Chinese Medicine Institute. Handbook of Chinese Medicine Preparation, People's publishing house of health, Beijing, 1975, p332.

2. Wang, Y., Zhao, L., Lin, S., Dong, S. and An, D., Determination of Berberine and Palmatine in Cortex Phellodendron and Chinese Patent Medicines by HPLC, Acta Pharm. Sinica 24(2): 275-279, U1989

3. Luo, G. A., Wang, Y. M., Wang, Z. P. and Zhou, G. H., Determination of Two Components in Sulfatene Compound Tablets by Partial Least Square Method, J. of China Pharm. Univ., 19(2), U104, U1988.

4. Wold, s., Martens, H. and Wold, H., in Ruhe, A. and Kagstrom, B. Eds., "Proceedings on the Symposium Matrix Pencils, Pitea, 1982". Springer Verlag, Berlin and Heidelberg, 1982, Lecture Notes in Mathematics.

5. Luo, G. A., Lan, Q. T., Wang, Z. P. and Zhou, G. H., Determination of Three Components in Aspirin Compound Tablets by Use of UV-PLS Method, Acta Pharm. Sinica, 24(9), 684, 1989.

6. Wang, Z. P., Zhou, G. H., and Luo, G. A., Partial Least Squares Method (PLS) and Its Application in Analytical Chemistry, Anal. Chem.(Chinese), 17(7), 662, 1989.

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