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The Fingerprinting of Huangjinju Powder for Injection on Chinese Patent Medicine by XRD Fourier

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ABSTRACT : The purpose is to study the identification method of Huangjinju powder for injection and the medicinal materials by the fingerprint of X-ray Diffraction Fourier (XRDF). We used the same method on both the studying of Huangjinju and the medicinal materials. Then we selected a few components alignment to compare. We analyzed the data by setting up the deviation *d*(Å) as ±0.05 to calculate the rate of special mark on the sample (*Px*) and on the patent (*P*). The special XRDF of Huangjinju[*d*(Å)/(I/I$_0$)] have 5 peaks that have not expressed in medicinal materials. Therefore *Px* is 22.73%. Flos Trollii Chinensis has 3 special marks and *Px* is 17.65%. Flos Chrysanthemi Indici has 1 special mark and *Px* is 3.57%. Its coincided interplanar spacing with the patent is 2.907Å. Flos Lonicerae Japonicae has 6 special marks and *Px* is 23.08%. Its special mark in the patent are 4.95/14 and 4.50/15, respectively. The *P* is 9.09%. Its coincided interplanar spacing with the patent is 3.93Å and 3.05Å, respectively. The number of special XRDF mark peaks of baicalin is 9 and *Px* is 18.37%. Its coincided interplanar spacing with the patent is 2.910Å. It has visible mark and specificity adopting XRDF fingerprint to identify Huangjinju and medicinal materials. Establishing the quality standard is a synthetic index that depends both on special marks in the medicinal materials of the patent and on the coincidence peak data.

Key words : Huangjinju powder, XRDF Fingerprinting, Special mark, Evaluating quality

INTRODUCTION

Adopting scientific identification methods on traditional Chinese medicine (TCM) has been a matter of concern lately. Consequently, the quality standard that can reflect the intrinsic characters of TCM should be set up. The study of identification standard on Chinese patent medicine and medicinal materials is always an important subject in evaluating the quality of TCM. This paper implies the need of studying new drugs of TCM. Huangjinju powder for injection and other medicinal materials such as Flos Chrysanthemi Indici were used. Then we selected a few intermediate productions and some constituents as a contrast group. We analyzed the geometric topology and marked the peak value of XRDF fingerprint on Huangjinju. Then we marked crystalline components for assurance. The identification method of Huangjinju and medicinal materials by fingerprint of X-ray Diffraction Fourier (XRDF) has been established to supply with the scientific data and applied technique (Zhang, 2005). 5 batches of Huangjinju, 31 medicinal materials collected in different time, some extractions and baicalin were chosen to assay systematically.

MATERIALS AND METHODS

Materials

The numbers of Huangjinju are Q1010-1~5 (these samples are supplied by Beijing Hongtaikangda Medicinal technology Co. LTD.).

These samples were collected and identified by Prof. Zhang Gui-Jun (Table 1).

Extraction of Flos Trollii Chinensis (Zou, 2004 and Zou, 2005) are intermediate production of Huangjinju and it mainly contains of orientin and flavonoids.

The method of preparation

Flos Trollii Chinensis was extracted with water 3 times. The liquid of extract was merged, filtered and lyophilized (The number of sample is Q1010-6).

Extraction of Flos Chrysanthemi Indici is also intermediate production of Huangjinju. They mainly contain of terpenes and flavonoids.

Flos Chrysanthemi Indici was extracted with water 3 times. The liquid of extract was merged and concentrated to 1 : 1.

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Table 1. Medicinal materials of Huangjinju

Name	Number	Area	Collective time	Habitat
Flos Trollii Chinensis (*Trollius chinensis* Bunge.)	148-1	Aershan city	2004.07	wild
	148-2			
	148-3			
	148-4		2001.07	
	148-5			
	148-6			
	148-7			
	148-9			
	148-12		2002.07	
	148-13			
	148-14			
	148-10	Jiagedaqi city	2001.07	
	148-16			
	148-15			
	148-17		2002.07	
	148-18			
	148-8	Chengde city Beijing Merchandise	2001.07	
	148-11			
	148-19	Ningxia borough	2002.07	cultivation
	148-20			
Flos Chrysanthemi Indici (*Chrysanthemum indicum* L.)	77-1	Jinzhai county	2000.10	wild
	77-2			
	77-3			
	77-4		2002.10	
	77-5			
	77-6			
	77-7			
	77-8		2001.10	
Flos Lonicerae Japonicae (*Lonicera japonica* Thunb.) (149-1~149-4. merchandise; 149-5~149-7. xiao mao hua; 149-8~149-10. da mao hua; 149-11~149-12. zhen hua)	149-1	Zhengcheng town, Pingyi county	2001.05	cultivation
	149-2			
	149-3			
	149-4		2002.05	
	149-5			
	149-6			
	149-7			
	149-8			
	149-9			
	149-10			
	149-11			
	149-12			

The concentrated liquid was deposited by 95% ethanol, filtered and lyophilized (The number of sample is Q1010-7).

Baicalin is the standard and was bought from the National Institute for the Control of Pharmaceutical and Biological Products.

The instrument of Rigaku D/max-2550 powder X-ray diffractometer was used in the research.

Methods(Zhang, 2005 and Lu, 2002)

The XRD condition are CuK_{α} radiation; divergent slit (DS) : 1°; receipt slit (RS) : 0.15 mm; graphite monochromator; pipe pressure/pipe flow : 40 kV/150 mA; 2θ scanned area : 3°~60°; scanning rate : 8°/min; step size : 0.02°.

Preparation of samples and measurement of XRDF fingerprint

The samples, Huangjinju, extraction of Flos Trollii Chinensis, Flos Chrysanthemi Indici and baicalin, were sifted by sieve with diameter of 150 μm ± 6.6 μm. Then they were measured on machine; the medicinal materials are dedusted, dispersed and filtrated by the same sieve.

Data analysis

XRDF character data are expressed by interplanar spacing d (Å) and relative intensity (I/I_0), labeled as $d/(I/I_0)$. The data were analyzed by setting up the deviation of d (Å) to ± 0.05.

The rate of special mark in the patent calculation: the rate of special mark (P) = The number of special XRDF mark peaks in the patent of samples/the number of XRDF mark peaks of the patent×100%.

The rate of special mark on the sample calculation: the rate of special mark on the sample (Px) = The number of special XRDF mark peaks of samples / the number of XRDF mark peaks of samples×100%.

The mark peaks of medicinal materials, intermediate productions and baicalin in fingerprinting of Huangjinju are labeled with*.

The samples which contain α-SiO_2 and α-SiO_2 is labeled with (ICDDPDF-05-0490.

RESULTS AND DISCUSSION

Fingerprint character of XRDF on Huangjinju

The number of special XRDF mark peaks of Huangjinju is 22 (n=4). They are 11.51/25△, 9.27/33, 6.64/25, 5.55/46, 5.13/38, 4.97/82△, 4.65/59, 4.47/73, 4.32/71^, 4.20/78, 4.06/43△, 3.93/42, 3.77/64, 3.61/39△, 3.46/44△, 3.05/39^, 2.909/38, 2.616/44△, 2.369/46, 2.226/40△, 2.105/36, 2.012/46 (Fig. 1, Fig. 2).

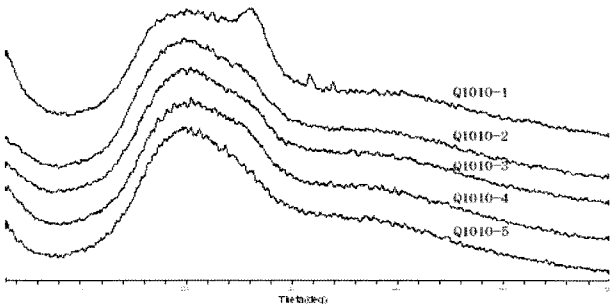

Fig. 1. XRDF fingerprint of Huangjinju.

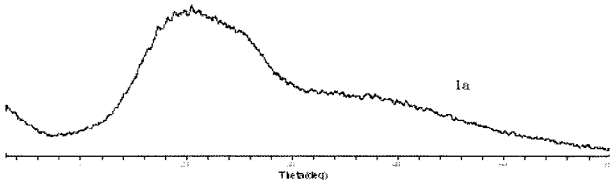

Fig. 2. XRDF control fingerprint of Huangjinju.

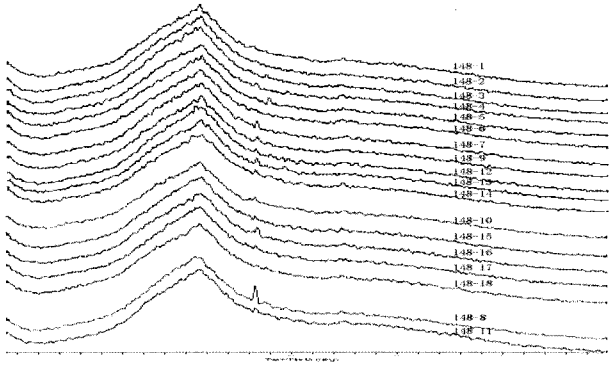

Fig. 3. XRDF fingerprint of wild Flos Trollii Chinensis.

Finger character of XRDF on medicinal materials

The diffraction figures and special XRDF mark peaks of wild Flos Trollii Chinensis (148-1~148-18) showed that: the geometric topology of diffraction figures on 18 samples are coincident. The results showed that the components of wild Flos Trollii Chinensis of different collective times, areas, habitats and storage periods are coincident (Fig. 3, Fig. 5-1a).

The number of special XRDF mark peaks on Flos Trollii Chinensis collected in Aershan city is 17 (n = 11). They are 9.94/17, 8.17/14, 7.19/15, 5.42/21, 4.25/70*, ^, 4.14/98, 3.57/20*, 3.34/41^, 3.22/27, 3.03/25*, 2.873/29*, 2.781/21, 2.572/33*, 2.423/20*, △, 2.231/19*, ^, 2.137/18*, ^, 1.986/17*, △.

The number of special XRDF mark peaks on Flos Trollii Chinensis collected in Jiagedaqi city is 18 (n = 5). They are

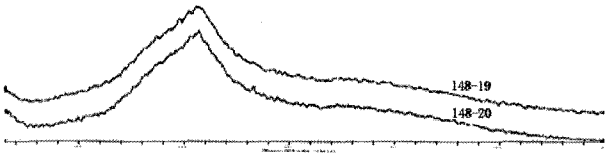

Fig. 4. XRDF fingerprint of cultivated Flos Trollii Chinensis.

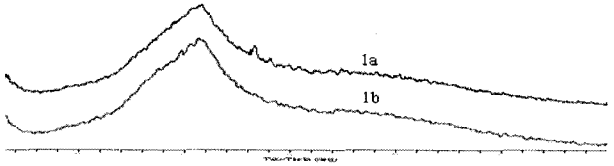

Fig. 5. XRDF control fingerprint and reference fingerprint of Flos Trollii Chinensis. 1a. Diffraction topology of the wild Flos Trollii Chinensis. 1b. Diffraction topology of the cultivated Flos Trollii Chinensis.

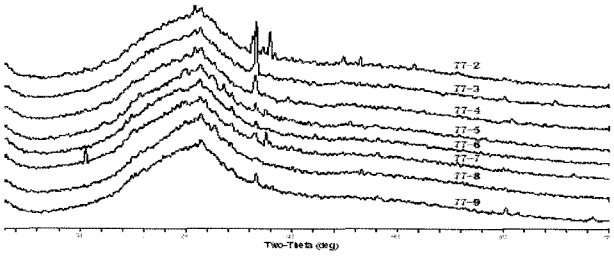

Fig. 6. XRDF fingerprint of Flos Chrysanthemi Indici.

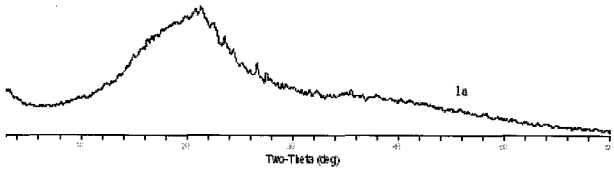

Fig. 7. XRDF control fingerprint of Flos Chrysanthemi Indici.

10.05/15, 5.13/38, 4.94/19, 4.33/21, 4.18/91$^{\triangle}$, 4.12/100, 3.43/24, 3.33/48$^{\triangle}$, 3.21/21, 3.11/18, 2.98/21, 2.846/23, 2.644/27, 2.565/34, 2.479/20, 2.387/24$^{\triangle}$, 2.24/23, 1.987/17$^{\triangle}$.

The geometric topology of diffraction figures on wild and cultivated Flos Trollii Chinensis (148-19~148-20 is coincident (Fig. 4, Fig. 5-1b)). It showed that the components of wild and cultivated Flos Trollii Chinensis are coincident.

The number of special XRDF mark peaks on Flos Chrysanthemi Indici is 28 (n = 8). They are 8.38/35, 7.23/21, 5.88/16, 4.63/19*, 4.44/27*, 4.26/32$^{\triangle}$, 4.15/82*, 4.04/29*, 3.92/30*, 3.76/21*, 3.66/27*, 3.53/13, 3.34/77$^{\triangle}$, 3.24/39, 3.02/18*, 2.972/17, 2.907/14*, 2.777/21, 2.58/20*, 2.513/16, 2.463/17$^{\triangle}$, 2.356/23*, 2.289/14*, $^{\triangle}$, 2.247/13*, $^{\triangle}$, 2.137/24*, $^{\triangle}$, 1.971/15*, 1.819/20$^{\triangle}$, 1.691/10$^{\triangle}$ (Fig. 6, Fig. 7).

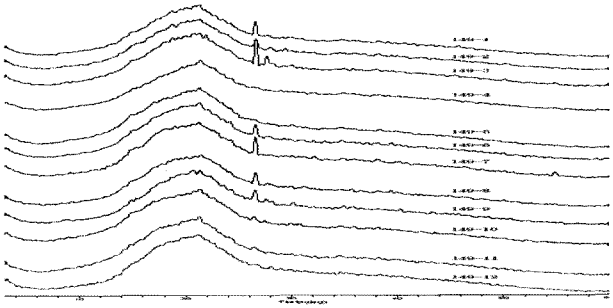

Fig. 8. XRDF fingerprint of Flos Lonicera Japonicae.

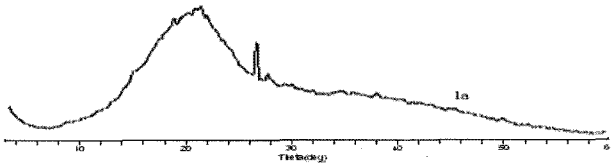

Fig. 9. XRDF control fingerprint of Flos Lonicerae Japonicae.

The number of special XRDF mark peaks on Flos Lonicerae Japonicae is 26 (n = 12). They are 8.84/7, 7.57/11, 5.91/16, 5.05/21, 4.95/14*, 4.83/15, 4.67/19*, 4.50/15*, 4.28/27*, $^{\triangle}$, 4.16/67*, 3.93/12*, 3.78/15*, 3.65/13*, 3.49/12*, 3.35/74$^{\triangle}$, 3.19/24, 3.05/19*, 2.974/22, 2.879/18*, 2.79/16, 2.59/24*, 2.455/15$^{\triangle}$, 2.356/22*, 2.29/15$^{\triangle}$, 2.128/15*, $^{\triangle}$, 2.055/16* (Fig. 8, Fig. 9).

Finger character of XRDF on some intermediate productions

The number of special XRDF mark peaks of extract from Flos Trollii Chinensis is 33 (n = 3). They are 11.684/36, 10.723/3, 8.615/23, 7.894/6, 7.132/6, 6.339/26, 6.095/14, 5.801/32, 5.381/49, 5.007/50, 4.721/44, 4.354/45*, 4.10/7*, 3.86/20, 3.651/29*, 3.548/100, 3.461/12*, 3.317/27, 3.064/9*, 2.855/2, 2.723/6, 2.665/2*, 2.608/3*, 2.543/2, 2.464/6, 2.303/7, 2.23/5*, 2.137/7*, 2.078/5*, 1.976/3*, 1.867/2, 1.77/3, 1.729/2 (Fig. 10).

Special XRDF mark peaks of extractions

The number of special XRDF mark peaks of extraction from Flos Chrysanthemi Indici is 30 (n = 3). They are 13.545/22, 7.357/1, 6.702/100, 5.824/2, 5.514/1*, 5.175/6*, 4.865/2, 4.697/18*, 4.586/24, 4.145/6, 4.048/4*, 3.897/2*, 3.684/8, 3.61/9*, 3.453/2*, 3.329/4, 3.225/2, 3.072/1*, 2.976/1, 2.774/2, 2.725/1, 2.65/1*, 2.518/1, 2.428/2*, 2.336/1*, 2.194/1*, 2.133/1*, 2.097/1*, 2.068/1*, 2.022/1* (Fig. 11).

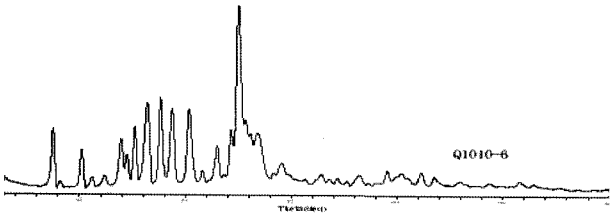

Fig. 10. XRDF fingerprint on the extraction of Flos Trollii Chinensis.

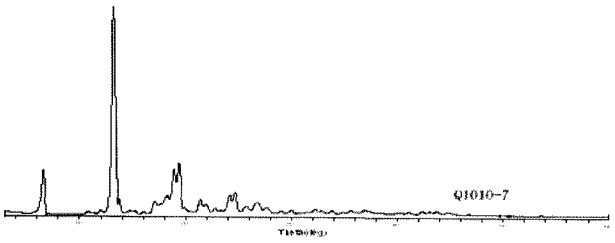

Fig. 11. XRDF fingerprint on the extraction of Flos Chrysanthemi Indici.

The number of special XRDF mark peaks of baicalin (Shang, 2002 and Chen, 2004) is 49 (n=3). They are 10.34/62, 8.59/23, 7.15/54, 6.49/10, 6.05/34, 5.23/44, 4.44/20*, 4.30/83*, 4.19/43*, 4.08/38*, 3.97/36*, 3.86/28, 3.74/61*, 3.57/23*, 3.51/71*, 3.44/33*, 3.30/16, 3.18/100, 3.04/31*, 2.96/15*, 2.91/15*, 2.859/15*, 2.789/14, 2.713/20, 2.622/22*, 2.586/21*, 2.557/16, 2.478/14, 2.449/13, 2.396/13*, 2.312/15, 2.239/12*, 2.215/15*, 2.159/17*, 2.139/21*, 2.130/17*, 2.080/12*, 2.057/13*, 2.047/12*, 2.016/12*, 1.928/13, 1.921/16, 1.881/10, 1.869/9, 1.822/9, 1.776/10, 1.752/10, 1.713/10, 1.689/9.

Data analysis and results

The number of special XRDF mark peaks [d(Å)/(I/I$_0$)] of Huangjinju is 5. They are 11.51/25, 9.27/33, 6.64/25, 5.55/46 and 5.13/38. They are not expressed in medicinal materials, thus the Px is 22.73%. The special interplanar spacing is from 5.13 to 11.51.

When compared with the fingerprinting of other medicinal materials, the number of special XRDF mark peaks of Flos Trollii Chinensis is 3. They are 9.94/178.17/145.42/21, Px = 17.65%. The special interplanar spacing is from 5.42 to 9.94. Flos Trollii Chinensis gave 9 mark peaks in fingerprint. Among the 9 mark peaks, the 6 peaks are the same with those of Flos Chrysanthemi Indici. Also, the 4 peaks are the same with those of Flos Lonicerae Japonicae and 5 peaks are the same with those of baicalin. The identified special interplanar spacing in the patent is from 1.986Å to 4.25Å.

Wild Flos Trollii Chinensis in Aershan city has 5 mark peaks (n = 11). They are 8.17/14, 7.19/15, 5.42/21, 3.57/20, 2.781/21. Flos Trollii Chinensisin in Jiagedaqi city has 7 mark peaks (n = 5). They are 10.05/15, 5.13/38, 4.94/19, 3.43/24, 3.11/18, 2.644/27, 2.479/20. Cultivated Flos Trollii Chinensis has no mark peak of d = 3.22 Å.

Compared with fingerprinting of other medicinal materials, the number of special XRDF mark peaks of Flos Chrysanthemi Indici is 1. It is 8.38/35Px = 3.57%. There are 15 mark peaks of Flos Chrysanthemi Indici in fingerprinting. In the 15 mark peaks 9 peaks are the same with those of Flos Lonicerae Japonicae and 6 peaks are the same with those of Flos Trollii Chinensis and 12 peaks are the same with those of baicalin. The interplanar spacing d(Å) of the one mark peak is 2.907, which is the same with mark peak of Huangjinju. The identified special interplanar spacing in the patent is from 1.971 to 4.63.

Compared with fingerprinting of other medicinal materials, the number of special XRDF mark peaks of Flos Lonicerae Japonicae is 6. They are 8.84/7, 7.57/11, 5.05/21, 4.95/14, 4.83/15, 4.50/15; Px = 23.08%. The identified special interplanar spacing is 4.50 to 8.84. Flos Lonicerae Japonicae gives 15 mark peaks in fingerprinting. Among the 15 mark peaks, 5 peaks are the same with those of Flos Trollii Chinensis, 10 peaks are the same with those of Flos Chrysanthemi Indici, and 11 peaks are the same with those of baicalin. It has 2 special mark peaks (4.95/15, 4.50/16) P = 9.09%; the interplanar spacing d(Å) of the two mark peaks are 3.93 and 3.05, respectivey, which is the same with mark peak of Huangjinju. The identified special interplanar spacing in the patent is from 2.055 to 4.95.

Special analysis of XRDF fingerprint on some intermediate productions

Compared with other medicinal materials, extraction of Flos Trollii Chinensis has 7 special mark peaks. They are 11.684/36, 10.723/3, 7.894/6, 6.339/26, 5.801/32, 5.381/49, 4.721/44, Px = 21.21%. Compared to medicinal material of Flos Trollii Chinensis, 12 mark peaks and the interplanar spacing d(Å) (2.23, 2.137) of 2 mark peaks of Flos Trollii Chinensis extraction are the same. 11 mark peaks of Flos Trollii Chinensis extraction are the same with those of Huangjinju. Also 7 mark peaks of Flos Trollii Chinensis extraction are the same with those of Flos Chrysanthemi Indici; Its 5 mark peaks are the same with Flos Lonicerae Japonicae and its 9 mark peaks are the same with baicalin. The interplanar spacing d(Å) (3.461) of 1 mark peak coincided with that of Huangjinju. The identified special interplanar spacing in the patent is from 1.976Å to 4.354Å.

Compared with other medicinal materials, extraction of Flos Chrysanthemi Indici has 8 special mark peaks. They are

13.545/22, 7.357/1, 6.702/100, 5.824/2, 5.514/1, 5.175/6, 4.586/24, 2.518/1; $Px = 26.67\%$.

Compared with medicinal material of Flos Chrysanthemi Indici, 18 mark peaks and the interplanar spacing d(Å) (2.976, 2.774, 2.133) of 32 mark peaks of Flos Chrysanthemi Indici extraction are the same.

16 mark peaks of extraction of Flos Chrysanthemi Indici are the same when it was compared with the fingerprinting of Huangjinju.

Also the 5 mark peaks of extraction of Flos Chrysanthemi Indici are the same with those of Flos Trollii Chinensis. Its 9 mark peaks are the same with Flos Lonicerae Japonicae and its 9 mark peaks are the same with baicalin. It has 3 special mark peaks (5.514/1, 5.175/6, 4.697/18) $P = 13.64\%$. The interplanar spacing d(Å) (3.61) of 1 special mark peak coincided with that of Huangjinju. The identified special interplanar spacing in the patent is from 2.022 Å to 5.514 Å.

Compared with other medicinal materials, baicalin has 9 special mark peaks. They are 10.34/62, 8.59/23, 6.49/10, 6.05/34, 5.23/44, 3.86/28, 2.713/20, 1.881/10, 1.752/10; $Px = 18.37\%$.

25 mark peaks of baicalin are the same with Huangjinju. 14 mark peaks of baicalin are the same with those of Flos Trollii Chinensis. Its 19 mark peaks are the same with Flos Lonicerae Japonicae and 17 mark peaks are the same with Flos Chrysanthemi Indici. The interplanar spacing d(Å) (2.910) of 1 mark peak coincided with that of Huangjinju. The identified special interplanar spacing in the patent is from 2.016Å to 4.44Å.

Conclusions and discussions

Adopting XRDF fingerprint to identify Huangjinju and medicinal materials gave us visible and specific standard. XRDF fingerprint can be used to identify Chinese patent medicine and its medicinal materials, as well as half manufactured drug or chemical components. Therefore XRDF fingerprint is one of the important indices to evaluate drug quality. Establishing the quality standard is a synthetic index that depends both on special marks in the medicinal materials of the patent and the coincidence peak data (Table 2). The method is simple, exact, specific, thorough, scientific and suitable.

The XRDF fingerprint of every kind of medicinal materials, half manufactured drug and chemical components shares the same characteristic mark peaks of Huangjinju. These peaks can be used as special characteristic index for identification. The diffraction peaks of Q1010-1 Huangjinju are somewhat different from other samples. Perhaps this is due to the different technological process to lead to unanalyzed Q1010-1 sample.

Identified characters of XRDF on medicinal materials are

Table 2. Data analysis of XRDF special mark peaks

Sample		Number of special mark peaks	Special mark peaks of samples*			Shared mark peaks with powder for injection *[d(Å)=±0.05]					d(Å) of Anastomotic peaks with powder for injection
			Number of peaks	Varied range of peaks d(Å)	Px (%)	Number of peaks	Number of special mark peaks	P (%)	Varied range of peaks d(Å)	The total rate of special mark%	
Huangjinju powder for injection		22	5	5.13~11.51	22.73	17		77.27	2.012~4.97	77.27	–
Medicinal materials	Flos Trollii Chinensis	17	3	5.42~9.94	17.65	9	–	–	1.986~4.25		–
	Flos Chrysanthemi Indici	28	1	8.38	3.57	15	–	–	1.971~4.63	9.09	2.907
	Flos Lonicera Japonica	26	6	4.50~8.84	23.08	15	2	9.09	2.055~4.95		3.933.05
Baicalin		49	9	1.752~10.34	18.37	25	–	–	2.016~4.44		2.910
Intermediate productions	Extract of Flos Trollii Chinensis	33	7	4.721~11.684	21.21	11	–	–	1.976~4.354		3.461
	Extract of Flos Chrysanthemi Indici	30	8	2.518~13.545	26.67	16	3	13.64	2.022~5.514	13.64	3.61

*the data can be used when quality standard is established.

not all coincident with those of medicinal materials in patent. The fact shows that they are not the same components.

Changes in the identified special interplanar spacing on different samples are small. This fact shows that the classification of these crystal components is the same. A few of mark peaks of medicinal materials and patent have visible relativity and they also have their own specificity. Characteristics of effective components are labeled obviously. All these results show that crystal of chemical components in medicinal materials are accumulated after being handled in preparing process. Its characters have changed but it still has special characteristics for identification. Thus when quality standard is established, the effect of components caused by preparing processes should be considered. The method of identification and index of evaluation should be decided according to the integrative characteristics of medicinal materials, intermediate productions and patent medicine.

The geometric topology of diffraction figures on Flos Trollii Chinensis of different collective times is similar. Thus their components are presumed to be almost the same. Flos Trollii Chinensis of different areas, different habitats and different storage periods each has obviously special mark peaks. Flos Trollii Chinensis in Jiagedaqi city has 7 mark peaks and Aershan samples has 5 mark peaks. Therefore this method can be used to identify TCM of different areas and different habitats. Also the method is especially suited to evaluate the quality of genuine medicinal materials. The geometric topologies of diffraction figures on wild and cultivated Flos Trollii Chinensis are almost the same, however there is no 3.22 Å special mark peak in geometric topology of diffraction figures on cultivated Flos Trollii Chinensis. It shows that there are some differences between wild and cultivated Flos Trollii Chinensis.

The range of special mark peaks of Flos Trollii Chinensis extraction and that of Flos Trollii Chinensis as raw material has no significant difference.

The geometric topology of diffraction figures of Flos Chrysanthemi Indici of different collective times is similar. Therefore their components are presumed to be the same. The contents of α-SiO_2 are different in 8 samples. The sequence is as followes: 77-2 > 77-11 > 77-3 > 77-6 > 77-8 > 77-4 > 77-5 > 77-7.

The analysis of diffraction peaks are as follows: The peak of 4.44 is more obvious in the samples 77-4 ~ 77-7 than in the samples of 77-1 ~ 77-3. In the sample of 77-8 the peak is weak. The peaks of 4.15 ~ 3.34 changed depending on storage times. The crystalline components increased with storage time. The intensity of 4.15 peak is the same in 8 samples. The peak of 8.40 in 77-6 sample may be caused by the components of different area.

The geometric topology of diffraction figures of Flos Lonicerae Japonicae of different processing method and collective times is similar. Thus their components are the same. there is no difference in the XRDF Fingerprint of Flos Lonicerae Japonicae of different processing. The contents of α-SiO_2 are different in 12 samples. The sequence is as follows: 149-3 > 149-7 > 149-1 ≈ 149-6 ≈ 149-8 > 149-9 > 149-2 > 149-10 ≈ 149-11 ≈ 149-12 > 149-5 > 149-4.

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