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Short communication

Simultaneous determination of 10 active components in traditional Chinese medicine "YIGONG" capsule by RP-HPLC–DAD

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Abstract

A simple, reliable and accurate method for the simultaneous separation and determination of 10 active components (psoralen, isopsoralen, emodin, oleanolic acid, stachydrine hydrochloride, ammonium glycyrrhizinate, glycyrrhizinate, schizandrol, imperatorin and isoimperatorin) in traditional Chinese medicine "YIGONG" capsule was developed using reverse phase high-performance liquid chromatography (RP-HPLC) coupled with diode array detection. The chromatographic separation was performed on a Eurospher C_{18} column (250 mm × 4.6 mm i.d. with 5.0 μm particle size) with a acetonitrile–water gradient containing 0.5% (v/v) aqueous phosphoric acid as mobile phase. Two detection wavelengths (210 and 250 nm) were utilized for the quantitative analysis due to the different UV spectra of these components. Good linear behaviors over the investigated concentration ranges were observed with the values of R^2 higher than 0.999 for all the analytes. The recoveries, measured at three concentration levels, varied from 95.0 to 105.3%. The validated method was successfully applied to the simultaneously determination of these active components in "YIGONG" capsule from different production batches.

Keywords: High-performance liquid chromatography; Active components; Chinese medicine; "YIGONG" capsule; Quality control

1. Introduction

In ancient China and Asian countries, natural materials are traditionally used as legal medicines or folk medicines. Also they have ever been applied as sole drug to the treatment of human and animal diseases. By scientific medical system of some developed countries in Europe and America, they have increasingly become recognized now. "YIGONG" capsule (YGC) is derived from a traditional Chinese formula named "Yi-Gong-Ning-Xue" oral solution (origined from Hubei Shientang Pharmaceutical Co., Ltd. of China, National Drugs surveillance administrative bureau standard WS-5092 (B-0092)-2002, country medicine accurate character B20020100) was consists of 12 commonly used Chinese drugs including Radix Fructus Psoraleae, Coastal Glehnia Root, Radix Polygoni Multiflori, Fructus Ligustri Lucidi, Fructus Schisandrae, Radix Rubiae, Herba Leonuri and Radix Glycyrrhizae, etc. [1]. Accordingly, the derived botanical drug YGC is used for the treatment of invigorating vital energy, nourishing YIN, fastness kidney and hemostasis. Moreover, it is also an effective adjunctive therapy for the cure of menometrorrhagia, prolonged and/or irregular menstruation [2].

The chemical constituents of YGC were investigated and over 20 compounds were isolated with column chromatography and semipreparative HPLC. On the basis of spectroscopic analysis and comparison with the data of known compounds, they are elucidated as glycyrrhizin (GLY), ammonium glycyrrhizinate (AG), psoralen (PS), isopsoralen (IS), schizandrol (SC), imperatorin (IM), isoimperatorin (ISO), emodin (EM), oleanolic acid (OA), stachydrine hydrochloride (SH), respectively. These components have bioactivity, and their chemical structures are shown in Fig. 1. According to the literature, the major constituents in YGC, such as GLY [3,4], AG [5,6], PS, IS [7,8], SC [9], IM, ISO [10,11], EM [12], OA [13], and SH [14], were reported to possess arrays of biological activities. Although HPLC methods have been applied to determine some of the constituents in crude drugs and Chinese patented medicines [15–22], no analytical method has been reported for simultaneous determination of 10 major constituents in YGC. Hence, it is very important to establish such a method for quality control of these bioactive

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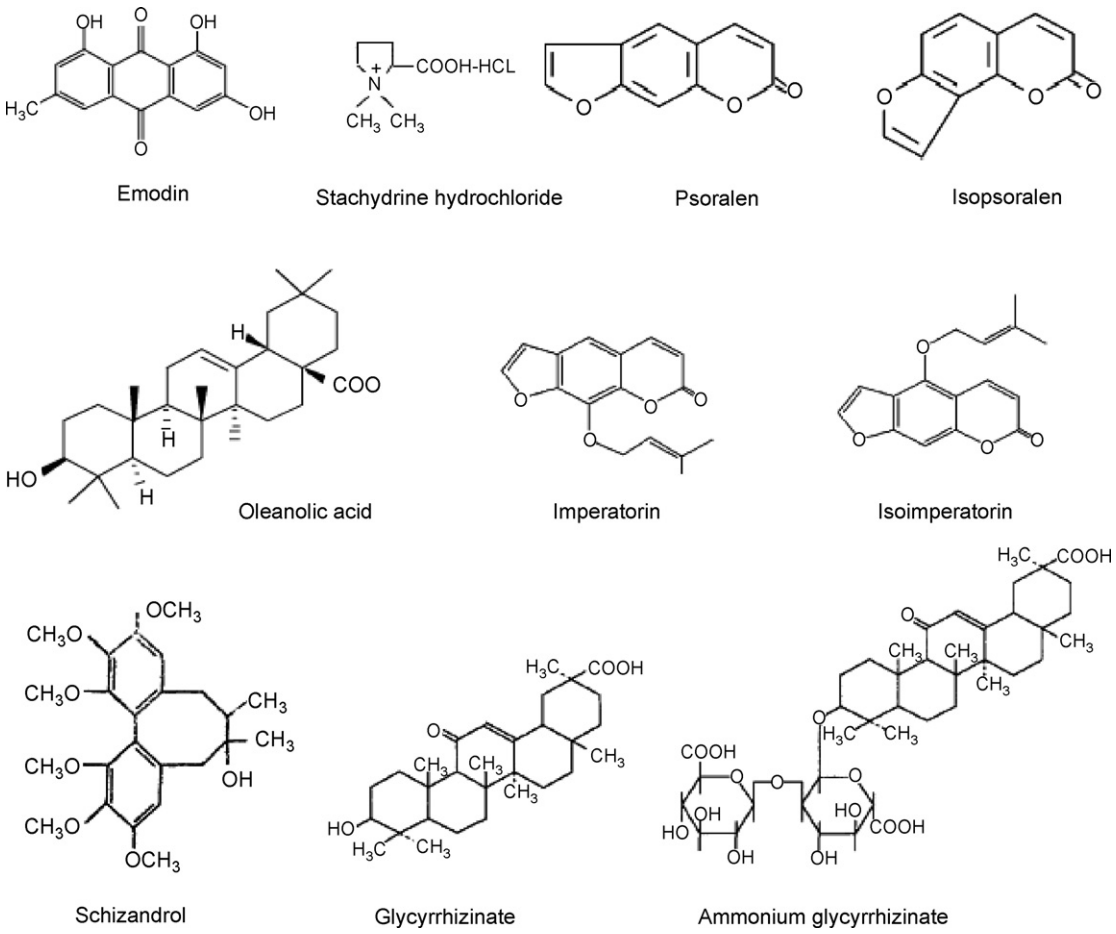

Fig. 1. Molecular structures of the investigated components. EM: emodin; SH: stachydrine hydrochloride; PS: psoralen; IS: isopsoralen; OA: oleanolic acid; IM: imperatorin; ISO: isoimperatorin; SC: schizandrol; GLY: glycyrrhizin; AG: ammonium glycyrrhizinate.

compounds, which could help to evaluate the quality of the herbal formula. In the work discussed in this communication, a simple, rapid and accurate RP-HPLC–DAD method was developed for simultaneous quantification of these 10 compounds of YGC.

2. Experimental

2.1. Chemicals and reagents

Sample YGC was supplied by Hubei Shientang Pharmaceutical Co., Ltd. (Hubei, China). Standard substances (GLY, AG, PS, IS, SC, IM, ISO, EM, OA and SH) were provided from National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). HPLC grade methanol and acetonitrile were purchased from Merck (Darmstadt, Germany). Phosphoric acid (analytical reagents) was purchased from Gaojing chemical industry company (Hangzhou, China). Other reagents were all of analytical grade. HPLC-grade water was purified by use of Milli-Q system (Milford, MA, USA).

2.2. Instrumentation and analytical conditions

The HPLC system 1100 series (Agilent Technologies, Palo Alto, CA, USA) equipped with the ChemStation software (Agilent Technologies) and comprised of a quaternary solvent delivery pump, an online vacuum degasser, an autosampler, a thermostated compartment and a diode array detector, were used for the chromatographic analysis. All separations were carried out on a Eurospher C_{18} column (250 mm × 4.6 mm i.d. with 5.0 μm particle size) from Hanbang Science and Technology (Jiangsu, China). Mobile phase A was 0.5% (v/v) phosphoric acid aqueous solution (pH 3.0) and phase B was acetonitrile. The elution was performed using a linear gradient of 5–40% B at the beginning of 20 min, then 40–85% B within 20–45 min and 85–100% B at the last 5 min. The flow-rate was 1.0 mL min^{-1}, column temperature was maintained at 30 °C, effluent was monitored at 210 and 250 nm, and injection volume was 10 μL. The peak identification was based on the retention time and the DAD spectrum against the standard presented in the chromatogram.

2.3. Standard solution preparation

A stock solution containing the 10 standards (AG 0.2 mg mL^{-1}, GLY 1.0 mg mL^{-1}, PS 0.12 mg mL^{-1}, IS 0.12 mg mL^{-1}, IM 0.14 mg mL^{-1}, ISO 0.10 mg mL^{-1}, EM 0.06 mg mL^{-1}, SC 0.25 mg mL^{-1}, OA 0.10 mg mL^{-1} and SH 0.50 mg mL^{-1}) was prepared in methanol and stored away from light at 4 °C when not in use. Working solutions of the lower

concentration were prepared by appropriate dilution of the stock solution.

2.4. Sample solution preparation

The powder of YGC (about 0.5 g) was extracted with 20.00 mL solvent composed of 80% methanol in water (v/v) for 30 min in an ultrasonic bath. The operation was repeated for three times. After cooling, the total solution was filtered, concentrated, and then dried with a rotatory evaporator under reduced pressure to give a residue. The residue was then dissolved in methanol by ultrasonication and transferred to a 10-mL volumetric flask. This solution was diluted to a proper consistency and then filtered through a syringe filter (0.45 μm) before injected into the HPLC system for analysis.

3. Results and discussion

3.1. Chromatographic separation

In order to optimize the extraction conditions for achievement of quantitative extraction, variables involved in the procedure such as solvent, extraction method and extraction time were optimized. Pure and aqueous methanol or ethanol solutions also were tried as the extraction solvent, the best solvent was found to be 80% methanol in water (v/v), which gave rise to optimum extraction of all the 10 components with broad range of polarity in high yield. Compared with refluxing extraction, the ultrasonic treatment procedure was found to be the better extraction method for all the 10 components. In order to investigate extraction time, powdered YGC samples were extracted with 20 mL 80% methanol in water (v/v) for 10, 20, 30, 45, and 60 min, respectively. The results suggested that all the 10 components were almost completely extracted by extracting with 80% methanol in water (v/v) three times for 30 min each.

Due to the complex composition of the sample solution, different mobile phases (CH_3OH–H_2O, CH_3OH–H_2O–H_3PO_4, CH_3CN–H_2O, CH_3CN–CH_3OH H_2O, CH_3CN–H_2O–H_3PO_4) were attempted to elute the investigated 10 components. In order to enhance resolution and eliminate tailing of the peaks of the target compounds, formic acid, acetic acid, or trifluoroacetic acid (compared with phosphoric acid) were added in the mobile phase. Considering the total resolution of the chromatographic separation, the running time and solvent/reagent consumption, the mobile phase CH_3CN–H_2O–H_3PO_4 was chosen for the separation. The typical chromatographic profiles of the blank, standard solution and the real sample solution were shown in Fig. 2. The blank sample used here was the mixture of 0.1 M H_3PO_4 aqueous solution and acetonitrile (1:200, v/v), which was applied in the extraction procedure. Almost no interference was presented in the chromatographic separation, and each target peak had a good resolution. On the basis of the absorption maxima of the 10 compounds in UV spectra acquired by use of the diode-array detector, and the monitoring wavelength was set at 250 nm for PS, IS, EM, AG, GLY, IM, ISO and 210 nm for OA, SH, SC, respectively. The detections at two wavelengths were carried out to improve the sensitivity and selectivity for the quantitative analysis and provided good response with minimum interference.

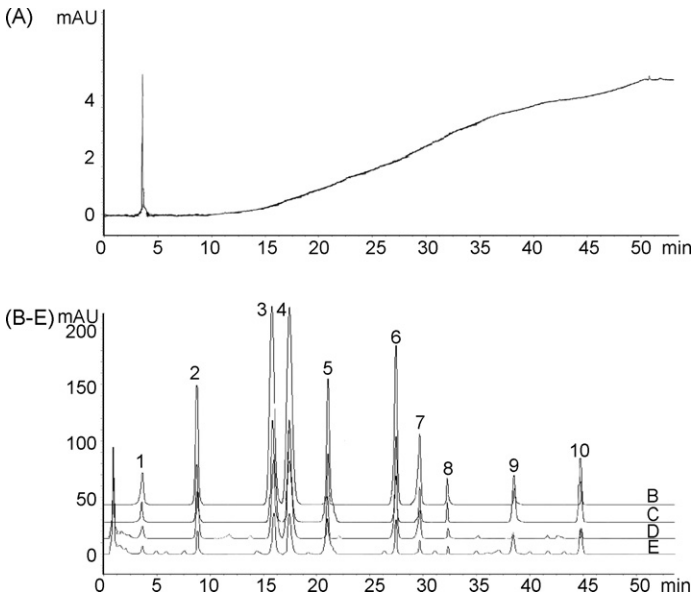

Fig. 2. The superimposed typical HPLC chromatograms of the blank (A). Standard solution (B) and the real sample solution (D) at 250 nm; standard solution (C) and the real sample solution (E) at 210 nm. The peaks marked were 1 = GLY, 2 = AG, 3 = PS, 4 = IS, 5 = SC, 6 = IM, 7 = ISO, 8 = EM, 9 = OA, 10 = SH, respectively.

Table 1
Linear relationships between peak area and concentration

Components	Regression equation ($y = ax + b$)[a]	R^2	Linear range (mg/L)	LOD (mg/L)	LOQ (mg/L)
PS	$y = 16{,}814x - 3837$	0.9995	4.79–286.5	0.71	1.42
IS	$y = 14{,}438x - 10{,}205$	0.9991	4.21–274.3	0.68	1.39
OA	$y = 290{,}000x + 15{,}700$	0.9999	0.75–75	0.15	0.31
SH	$y = 1966x + 26$	0.9999	0.8–39.5	0.19	0.38
EM	$y = 65.44x - 3.34$	0.9999	0.50–26.0	0.30	0.60
AG	$y = 1034x + 11$	0.9994	0.90–80.0	0.40	0.79
GLY	$y = 5695x - 3089$	0.9998	0.60–30.0	0.05	0.15
SC	$y = 52{,}803x + 10{,}452$	0.9999	1.55–83.7	0.19	0.38
IM	$y = 51{,}000x + 20{,}609$	0.9999	2.82–84.6	0.04	0.10
ISO	$y = 59{,}000x + 66$	0.9996	2.02–40.4	0.60	0.20

[a] y is peak area, x the concentration of the compounds (mg/ml), a the slope and b is the intercept of the regression line, respectively. Both values of a and b are given as the form of mean ± S.D and R^2 is the correlation coefficient of the equation. *Note*: All the abbreviations see in Fig. 1.

3.2. Linearity, range and limits of detection

In order to obtain the calibration curves, integrated chromatographic peak areas were plotted against the corresponding concentration of the injected standard solutions. Limits of detection (LOD) and quantification (LOQ), detected as the injection concentration providing the peak height, 3- and 10-fold of the ratio of signal-to-noise (s/n) were acquired. The regression equations were established using seven concentration levels on the consecutive 6 days. In Table 1, the detailed descriptions of the regression curves were presented. The good linearity (coefficient of determination $R^2 > 0.999$) was achieved in the investigated ranges for all the analytes. What is more, the developed calibration curves were considerable stable because the relative standard derivation (RSD) values of the slope were all less than 2.0%.

3.3. Precision, repeatability and stability

The injection of continuous six times using the same sample was employed for the injection repeatability, and the injection of six different samples which were obtained through the same sample preparation procedure was used for the analysis repeatability. Furthermore, the test of injection repeatability was determined by the mixture standard solutions at three concentration levels, and the test of analysis repeatability was investigated by the real sample solution. The results of injection repeatability of the solution at a modest concentration were shown in Table 2, and all the RSD values were lower than 0.2%. Meanwhile, the RSD values of the analysis repeatability were lower than 3.5% both for the retention time and peak area.

The instrument precision was examined by the performance of the intra-day and inter-day assays by six replicated injections of the mixture standard solutions used above. The intra-assay precision was performed with the interval of 4 h in 1 day, and the inter-assay precision was performed over 3 days. The precision result of the solution at medium concentration was presented in Table 3, and it was shown that the RSD values of retention time were lower than 0.25%, while the RSD values of peak area were lower than 2.5% both for the intra-assay and inter-assay precision. The same real sample was analyzed within 24 h at

Table 2
Repeatability of the method ($n = 6$)

Components	RSD of retention time (%)		RSD of peak area (%)	
	Injection	Analysis	Injection	Analysis
PS	0.07	0.09	0.77	1.42
IS	0.04	0.05	0.74	1.84
OA	0.09	0.10	0.85	2.07
SH	0.03	0.04	0.95	3.40
EM	0.03	0.04	1.54	3.39
AG	0.03	0.04	1.55	3.08
GLY	0.04	0.05	1.54	3.01
SC	0.10	0.11	0.65	1.66
IM	0.08	0.10	0.71	1.50
ISO	0.07	0.08	0.93	3.28

Note: All the abbreviations see in Fig. 1.

Table 3
Intra-assay and inter-assay precision of the method ($n = 6$)

Components	Intra-assay RSD (%)		Inter-assay RSD (%)	
	Retention time	Peak area	Retention time	Peak area
PS	0.11	1.80	0.23	2.40
IS	0.09	1.40	0.22	2.35
OA	0.04	0.98	0.08	1.76
SH	0.05	1.18	0.09	1.89
EM	0.03	0.86	0.11	1.59
AG	0.06	1.29	0.18	2.04
GLY	0.02	0.75	0.04	0.98
SC	0.07	1.27	0.19	2.15
IM	0.08	1.35	0.21	2.26
ISO	0.05	1.08	0.10	1.67

Note: All the abbreviations see in Fig. 1.

Table 4
Recoveries of compounds 1–10 from YGC ($n = 3$)

Compound	Quantity added (mg/L)	Quantity found (mg/L)[a]	Recovery (%)[b]	RSD (%)
PS	25.83	26.08	101.0	3.62
	51.66	51.42	99.5	2.99
	77.49	76.99	99.4	1.18
IS	25.20	26.40	104.8	3.45
	50.40	50.92	101.0	2.37
	75.60	73.81	97.6	2.20
OA	3.32	3.21	96.8	2.30
	6.64	6.54	98.5	1.83
	9.96	10.13	101.7	2.17
SH	4.42	4.43	100.2	2.26
	10.49	10.72	102.2	1.84
	29.46	28.98	98.4	1.62
EM	2.53	2.66	105.3	2.98
	5.06	5.12	101.2	3.43
	7.59	7.04	97.5	3.07
AG	10.01	9.95	99.4	1.08
	20.02	19.88	99.3	2.67
	30.03	29.81	99.3	1.12
GLY	2.21	2.18	98.6	2.42
	6.63	6.58	99.3	1.82
	9.44	9.47	98.0	2.07
SC	10.12	10.03	99.1	1.66
	20.24	20.36	100.6	2.86
	30.36	30.44	100.3	1.77
IM	23.76	24.85	104.6	0.92
	47.52	48.57	102.2	3.26
	71.28	71.35	100.1	1.10
ISO	4.83	4.59	95.0	1.26
	9.82	9.63	98.1	1.38
	19.64	19.72	100.4	2.68

Note: All the abbreviations see in Fig. 1.

[a] Calculated by subtracting the total amount after spiking from the amount in the herb before spiking. Data are means from three experiments ($n = 3$).

[b] Calculated as [(amount detected)/(amount added)] × 100. Data are means from three experiments ($n = 3$).

Table 5
Determination of the active components in YGC by the developed HPLC method

No. of batches	Content ($n=5$, mean ± S.D., mg/g)									
	PS	IS	OA	SH	EM	AG	GLY	SC	IM	ISO
20061204	18.4 ± 0.2	17.1 ± 0.3	3.7 ± 0.1	6.2 ± 0.1	2.7 ± 0.1	9.3 ± 0.2	3.7 ± 0.1	13.9 ± 0.1	15.1 ± 0.1	6.4 ± 0.1
20070115	19.9 ± 0.1	19.6 ± 0.2	3.5 ± 0.1	5.1 ± 0.2	2.5 ± 0.2	9.8 ± 0.2	3.9 ± 0.1	12.9 ± 0.2	15.2 ± 0.1	6.3 ± 0.1
20070317	20.6 ± 0.5	17.6 ± 0.3	4.0 ± 0.0	5.3 ± 0.2	2.3 ± 0.2	10.6 ± 0.1	4.1 ± 0.0	13.6 ± 0.1	14.6 ± 0.2	5.9 ± 0.1
20070407	19.1 ± 0.4	19.5 ± 0.1	3.8 ± 0.1	6.1 ± 0.1	2.9 ± 0.1	10.8 ± 0.0	4.0 ± 0.0	12.8 ± 0.2	13.9 ± 0.3	6.1 ± 0.1
20070503	19.8 ± 0.2	18.1 ± 0.4	3.4 ± 0.3	5.5 ± 0.2	2.4 ± 0.1	9.1 ± 0.3	3.5 ± 0.2	13.5 ± 0.2	14.5 ± 0.2	6.5 ± 0.0
20070513	21.5 ± 0.3	19.9 ± 0.2	3.8 ± 0.1	5.4 ± 0.2	2.8 ± 0.1	10.7 ± 0.1	3.8 ± 0.1	13.3 ± 0.2	14.7 ± 0.2	5.8 ± 0.2
20070525	18.6 ± 0.2	17.7 ± 0.5	3.6 ± 0.2	5.6 ± 0.1	2.6 ± 0.1	10.4 ± 0.1	3.6 ± 0.1	12.4 ± 0.3	14.3 ± 0.2	5.7 ± 0.2
Average	19.7	18.5	3.7	5.6	2.6	10.1	3.8	13.2	14.6	6.1
RSD (%)	5.6	6.1	5.5	7.3	8.3	6.9	5.7	4.0	3.1	5.1

Note: All the abbreviations see in Fig. 1.

the room temperature for the stability test, and the solution was found to be stable (RSD values of the retention time and peak area were both lower than 2.0%).

3.4. Recovery test

There has neither a standard method for the determination of these active components nor a standard reference because of the complexity of Chinese medicines. So recovery of the standard from samples is generally used to evaluate the accuracy of the newly developed analytical method. Three different quantities (low, medium and high) of the authentic standards were added into the known real sample. The mixtures were extracted as described in Section 2.4, and was analyzed using the developed HPLC method mentioned above. Then, the quantity of each component was subsequently achieved from the corresponding calibration curves. The recovery of the investigated components ranged from 95.0 to 105.3%, and their RSD values were all less than 4.0% (show in Table 4). It was known from the recovery tests that the developed method manifested the reliability and accuracy for the measurement of these components.

3.5. Application

For the simultaneous determination of 10 major components in Chinese medicine YGC from different production batches, the developed HPLC method was used by comparing the retention times and on-line UV spectra with those of standards. The amounts of the 10 compounds in the samples were then calculated. The results, shown in Table 5, indicate that the content variability was lower for imperatorin, schizandrol between batches than for the other components. However, the content of some components such as oleanolic acid, psoralen, isopsoralen, isoimperatorin, glycyrrhizinate, emodin, stachydrine hydrochloride, ammonium glycyrrhizinate had a fluctuation with the RSD values between 5 and 8.5%, which might influence the quality stability of this botanical drug. Therefore, the simultaneous determination of all these active components contained in YGC is necessary to improve the quality control level of this botanical drug.

4. Conclusion

This is the first report of an accurate and reliable analytical method for the simultaneous determination of 10 major bioactive constituents (glycyrrhizin, ammonium glycyrrhizinate, psoralen, isopsoralen, schizandrin, imperatorin, isoimperatorin, emodin, oleanolic acid and stachydrine hydrochloride) in Chinese medicine YGC by using reverse phase high-performance liquid chromatography coupled with diode array detection. High linearity, repeatability, intra-day and inter-day assay precision, accuracy and reliability were presented in the method validation procedure. The proposed method is promising to improve the quality control of "YIGONG" capsule and other related botanical drugs.

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