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Original Article

Critical process parameter identification of manufacturing processes of *Astragali Radix* extract with a weighted determination coefficient method

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ABSTRACT

Objective: Critical process parameters (CPPs) identification is an important step of the implementation of quality by design (QbD) concept. There are many CPP identification methods, such as risk analysis method, sensitivity analysis method, multiple linear regression method, standard partial regression coefficient (SPRC) method, and so on. The SPRC method can consider multiple process critical quality attributes (CQAs) simultaneously, but the determination of CPP number is subjective. Therefore, new CPP identification method is still required.

Methods: The manufacturing process of *Astragali Radix* extract, which contained water reflux extraction, concentration, and ethanol precipitation, was used as an example. First, the multiple process CQAs were determined to be the yield of pigment, dry matter, sugars, and active ingredients. Second, the potential CPPs were determined by a knowledge organization method. Plackett-Burman designed experiments were then performed. A weighted determination coefficient (R_w^2) method was presented to identify CPPs. In this method, the importance of different CQAs was considered. Process parameters were removed one-by-one according to their importance index. The decrease in R_w^2 was used to characterize the importance of the removed parameter. If the decrease of R_w^2 was less than a preset threshold, the removed parameter was not a CPP.

Results: During the manufacturing process of *Astragali Radix* extract, the potential CPPs determined by the knowledge organization method were water consumption, reflux extraction time, extraction frequency, ethanol content, ethanol consumption, and concentration endpoint. Reflux extraction time, the first ethanol consumption, the second ethanol consumption, and the second ethanol precipitation refrigeration temperature were found to be CPPs using the weighted determination coefficient method with the threshold of 10%.

Conclusion: Using the weighted determination coefficient method, CPPs can be determined with all the CQAs considered based on their importance. The determination of CPP number is more objective compared with the SPRC method.

1. Introduction

The quality by design (QbD) concept, which is based on knowledge management and risk management (Yu et al., 2014), has been gradually accepted and implemented in the traditional Chinese medicine (TCM) industry. Most of the advances were on the optimization of manufacturing processes (Chen, Gong, Zhang, Chen & Qu, 2015; Dai et al., 2016; Gong, Chen, Pan & Qu, 2015). In the implementation of the QbD concept, the identification of critical process parameters (CPPs) is important because process control strategy will be established by the control of CPPs.

There are several methods that can be applied to identify CPPs, such as the risk analysis method (Zhao, Gong & Qu, 2017), sensitivity analysis method (Degerman, Westerburg & Nilsson,

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2009), stepwise regression method (Liu, Shen, Li, Qu & Gong, 2016), multiple linear regression method (Liu et al., 2016), and standard partial regression coefficient (SPRC) method. Compared with the stepwise regression method and multiple linear regression method, the SPRC method possesses the following two important advantages (Liu et al., 2016). First, the importance index is calculated by considering all the process critical quality attributes (CQAs) of the target process. Second, when multiple process CQAs have different importance indices, different weights can be used in the calculation of the importance index. Therefore, the SPRC method was used in CPP identification of ethanol precipitation (Yan, Guo, Qu & AL., 2013), lime milk precipitation process (Shen, Gong, Pan & Qu, 2017) and HPLC analysis process (Shao, Cao, Qu, Pan, & Gong, 2018) recently. However, in the implementation of the SPRC method, determining the CPP number was subjective in reported works (Shao, Cao, Qu, Pan, & Gong, 2018; Shen et al., 2017). Therefore, the SPRC method must be improved to determine the number of CPPs more objectively.

In this work, a weighted determination coefficient (R_w^2) method was proposed based on the SPRC method. The manufacturing process of *Astragali Radix* extract was studied as an example. *Astragali Radix* extract is an intermediate of Shenqi Fuzheng Injection, which is a TCM injection made from *Astragali Radix* and *Codonopsis Radix* for the treatment of leukocyte dysfunction, weakness and adjuvant therapy for cancer (Dai et al., 2008; Wang, Tong, Li, Cao & Su, 2012). *Astragali Radix* extract was prepared using a water extraction-ethanol precipitation combination process, as seen in Fig. 1. There is a second ethanol precipitation followed by the first ethanol precipitation to remove additional impurities. It is quite common for the manufacturing of TCM injections to assure drug safety, such as the Danshen Injection (Gong, Wang & Qu, 2011a), Guanxinning Injection (Gong, Yan & Qu, 2014), and Compound Kushen Injection (Liu et al., 2011). The water extraction-ethanol precipitation combination process is commonly used in the production of TCM. After extracting the active ingredients, impurities can be removed by changing the polarity of solvent. If water extraction and ethanol precipitation can be investigated simultaneously, it helps to find CPPs from a more global perspective. However, there are only literatures on water extraction or ethanol precipitation alone at present. To the best of the author's knowledge, there is no work on CPP identification of the combination process of water extraction-ethanol precipitation.

In this work, process CQAs for *Astragali Radix* extract was determined. Potential CPPs were selected using a knowledge organization method. Then, Plackett–Burman designed experiments were performed and the results were analyzed. CPPs were identified with a weighted determination coefficient method.

2. Methods

2.1. Materials and chemicals

Astragali Radix was kindly provided by Limin Pharmaceutical Factory (Shaoguan, China). *D*-fructose (99.5%) was purchased from Aladdin Chemistry Co., Ltd. (Shanghai, China). Sucrose (99%) was purchased from Sigma-Aldrich Co., Ltd. (Shanghai, China). The standard substances of calycosin-7-glucoside (> 98%), ononin (> 98%), (6aR, 11aR)−9,10-dimethoxypterocarpan-3-*O*-β-*D*-glycoside (DG, > 98%), 2′–hydroxy-3′,4′- dimethoxyisoflavan-7-*O*-β-*D*-glucopyranoside (HDG, > 98%), astragaloside IV (> 98%), and astragaloside II (> 98%) were purchased from Winherb Medical Technology Co., Ltd. (Shanghai, China). HPLC-grade acetonitrile and methanol were obtained from Merck (Darmstadt, Germany). HPLC-grade formic acid was obtained from Anaqua Chemicals Supply (Huston, TX, USA). Triethylamine was of guaranteed reagent grade and purchased from Aladdin Chemistry Co., Ltd. (Shanghai, China). Dimethyl sulfoxide was of guaranteed reagent grade and purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Ethanol was of guaranteed reagent grade and purchased from Shanghai Lingfeng Chemical Reagent Co., Ltd. (Shanghai, China). Ultra-high-purity water was produced using a Milli-Q water purification system from Millipore (Milford, MA, USA).

2.2. Procedures

First, 150 g of *Astragali Radix* was placed in a glass round bottom flask. Then, water was added for reflux extraction three times. For the first extraction, the amount of water added was 2 mL/g more than the set value. For the other two extractions, the amount of water was equal to the set value. After extraction, the extract was filtered through a 200-mesh gauze. The filtrate from the three extractions was merged as the water extract. The water extract was concentrated under reduced pressure to obtain a certain volume of concentrated extract of *Astragali Radix*. Then, a certain volume of ethanol solution was pumped into the concentrated extract under magnetic stirring (85–1, Hangzhou Instrument Motor Co., Ltd.). The magnetic stirring was stopped after the ethanol solution was completely added. The ethanol precipitation system was placed in a low-temperature thermostat bath (THD-1008 W, Ningbo Tianheng Instrument Co., Ltd) for more than 12 h. Then, the supernatant of

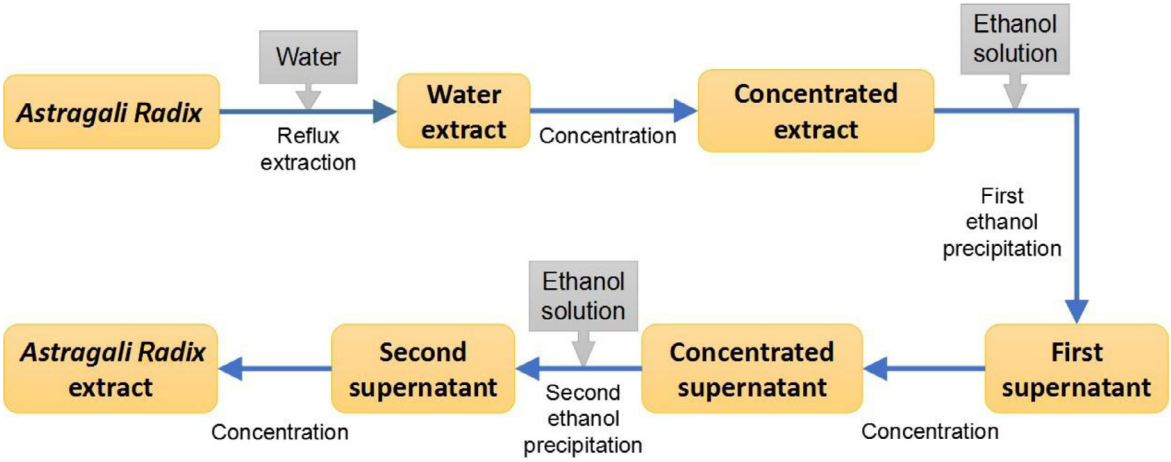

Fig. 1. Manufacturing processes of *Astragali Radix* extract.

the first ethanol precipitation was collected by filtration. The supernatant was concentrated under reduced pressure to obtain a concentrated supernatant, which was the material of the second ethanol precipitation. A certain volume of ethanol solution was pumped into the concentrated supernatant under magnetic stirring. Next, the second ethanol precipitation was placed in a low-temperature thermostat bath for more than 12 h. After refrigerating, the second ethanol precipitation supernatant was collected by filtration. Then, the pigment content, dry matter content, sugar contents, and active ingredient contents were determined.

2.3. Experimental design

The Plackett–Burman design was used to investigate the effects of potential CPPs on the manufacturing process of *Astragali Radix* extract. A total of 10 parameters were investigated: reflux extraction water consumption (X_1), reflux extraction time (X_2), water extract concentration endpoint (X_3), ethanol content of the first ethanol precipitation (X_4), ethanol consumption of the first ethanol precipitation (X_5), refrigeration temperature of the first ethanol precipitation (X_6), supernatant concentration endpoint (X_7), ethanol content of the second ethanol precipitation (X_8), ethanol consumption of the second ethanol precipitation (X_9), and refrigeration temperature of the second ethanol precipitation (X_{10}). The water extract concentration endpoint was represented as the volume of water extract concentrate per gram of *Astragali Radix*. In this work, the concentration endpoints were 0.62, 0.66, and 0.70 mL/g, respectively. The coded and uncoded values of potential CPPs were shown in Table 1. The conditions of the Plackett–Burman designed experiments were shown in Table 2.

2.4. Analytical method

The flavonoids and saponins in the second ethanol precipitation supernatant were analyzed by an HPLC-UV-ELSD method (Luo et al., 2016). First, a mixed stock solution was prepared by dissolving appropriate amounts of flavonoid and saponin standards in 50% methanol solution. In this process, a small amount of dimethyl sulfoxide was added to help dissolve the standards. Then, the stock solution was diluted to obtain a series of standard solutions with different concentrations. The supernatant samples were prepared using the same method as standard solutions. For the sugar determination in the supernatant samples, an HPLC-ELSD method was applied (Shao, Cao, Qu, Pan, & Gong, 2018). Calibration curves were carried out with mixed sugar standard solutions quantitatively diluted with 85% acetonitrile solution. The supernatant samples were required to be diluted to an appropriate concentration. All standards and samples were filtered through 0.22 μm Millipore membranes before analysis.

The separation of flavonoids and saponins was performed on an Agilent Zorbax SB-C_{18} column (4.6 mm × 250 mm, 5 μm) (Luo et al., 2016). The chromatographic conditions were as follows: mobile phase: 0.2% formic acid-water (phase A) and acetonitrile (phase B); linear gradient elution (0 – 16 min, 15%–23% B; 16–20 min, 23%–28% B; 20–25 min, 28%–30% B; 25–30 min, 30%–30% B; 30–40 min, 30%–55% B; 40–50 min, 55%–95% B); flow rate: 0.8 mL/min; injection volume: 10 μL; column temperature: 30 °C; UV detection wavelength: 270 nm; ELSD atomization temperature: 30 °C; drift tube temperature: 80 °C; and N2 flow rate: 1.6 L/ min. A typical chromatogram was shown in Fig. S1.

A Waters XBridge BEH Amide column (4.6 mm × 250 mm, 5 μm) was used for the analysis of sugars (Gong, Wang, & Qu,

Table 1
Coded and uncoded values of 10 potential CPPs.

Processes	Potential CPPs	Symbols	Units	Coded variables		
				−1	0	1
Reflux extraction	Water consumption	X_1	mL/g *Astragali Radix*	5.0	6.0	7.0
	Reflux extraction time	X_2	min	30	50	70
Concentration of water extract	Concentration endpoint	X_3	mL/g *Astragali Radix*	0.62	0.66	0.70
First ethanol precipitation	Ethanol content	X_4	% (volume percent)	91	93	95
	Ethanol consumption	X_5	mL/mL	2.8	3.0	3.2
	Refrigeration temperature	X_6	°C	3.0	6.0	9.0
Concentration of supernatant	Concentration endpoint	X_7	mL/g *Astragali Radix*	0.30	0.34	0.38
Second ethanol precipitation	Ethanol content	X_8	% (volume percent)	91	93	95
	Ethanol consumption	X_9	mL/mL	5.4	5.6	5.8
	Refrigeration temperature	X_{10}	°C	3.0	6.0	9.0

Table 2
Conditions of Plackett-Burman designed experiments.

Run	Potential CPPs									
	X_1/(mL · g^{-1})	X_2/min	X_3/(mL · g^{-1})	X_4/%	X_5/(mL · mL^{-1})	X_6/°C	X_7/(mL · g^{-1})	X_8/%	X_9/(mL · mL^{-1})	X_{10}/°C
1	6.00	50.00	0.66	0.93	3.00	6.00	0.34	0.93	5.60	6.00
2	5.00	30.00	0.62	0.91	2.80	3.00	0.30	0.91	5.40	3.00
3	5.00	70.00	0.62	0.95	3.20	3.00	0.38	0.95	5.80	3.00
4	7.00	70.00	0.62	0.91	2.80	9.00	0.30	0.95	5.80	3.00
5	7.00	30.00	0.70	0.95	3.20	3.00	0.30	0.91	5.80	3.00
6	7.00	70.00	0.62	0.95	3.20	9.00	0.30	0.91	5.40	9.00
7	5.00	30.00	0.62	0.95	2.80	9.00	0.38	0.91	5.80	9.00
8	7.00	70.00	0.70	0.91	2.80	3.00	0.38	0.91	5.80	9.00
9	5.00	30.00	0.70	0.91	3.20	9.00	0.30	0.95	5.80	9.00
10	7.00	30.00	0.62	0.91	3.20	3.00	0.38	0.95	5.40	9.00
11	5.00	70.00	0.70	0.95	2.80	3.00	0.30	0.95	5.40	9.00
12	6.00	50.00	0.66	0.93	3.00	6.00	0.34	0.93	5.60	6.00
13	7.00	30.00	0.70	0.95	2.80	9.00	0.38	0.95	5.40	3.00
14	5.00	70.00	0.70	0.91	3.20	9.00	0.38	0.91	5.40	3.00
15	6.00	50.00	0.66	0.93	3.00	6.00	0.34	0.93	5.60	6.00

2011). The chromatographic conditions were as follows: mobile phase: 0.3% triethylamine-water (phase A) and 0.3% triethylamine-acetonitrile (phase B); linear gradient elution (0–37 min, 85%–76% B); flow rate: 0.9 mL/min; injection volume: 5 μL; column temperature: 34 °C; ELSD atomization temperature: 60 °C; drift tube temperature: 65 °C; and N_2 flow rate: 1.8 L / min. A typical chromatogram was shown in Fig. S2.

The dry matter content was determined by a gravimetric method. An appropriate amount of accurately weighed sample was placed in a dried weighing bottle and was dried in a 105 °C drying oven (DHG-9146A, Shanghai Jing Hong Laboratory Instrument Co., Ltd.) for 3 h (Xu et al., 2015). The dried sample was cooled to room temperature in a desiccator before being weighed. The dry matter content was calculated based on the change in sample mass before and after drying. The pigment content in the sample was calculated in terms of tartrazine equivalent. The absorbance at 420 nm was measured by a spectrophotometer (T6, Beijing Purkinje General Instrument Co., Ltd). First, the standard curve was obtained using tartrazine solutions. Then, the sample was diluted five times with ethanol solution and measured. Finally, the pigment content was calculated.

2.5. Data processing

In this work, all the yields are considered the mass collected in the second supernatant per gram of *Astragali Radix*, as seen in Eq. (1).

$$Yield_i(\mu g/g) = \frac{V_{SS}(mL) \times C_i\ (\mu g/mL)}{M_{AR}(g)}\ (i = 1,\ 2, \ldots, 10) \quad (1)$$

Where M is mass, V is volume, subscript SS refers to the second supernatant, subscript AR refers to the *Astragali Radix*, C is the concentration in the second supernatant, and subscript i refers to dry matter, pigment, fructose, sucrose, astragaloside IV, astragaloside II, calycosin-7-glucoside, ononin, DG, and HDG, respectively.

The weighted determination coefficient method was developed based on the SPRC method. A schematic diagram of the novel method was shown in Fig. 2. In this method, the comprehensive influences of the process parameters on all the process CQAs can be reflected.

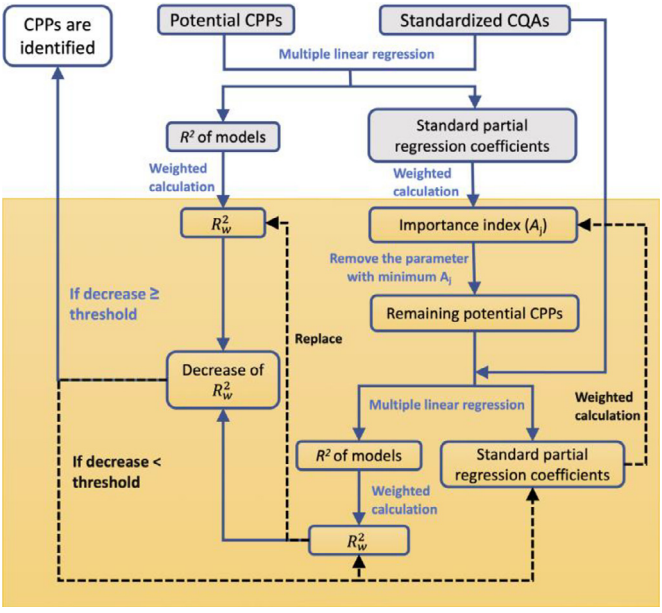

Fig. 2. Schematic diagram of weighted determination coefficient method (Yellow section will be repeated until CPPs are obtained).

The process CQAs of Y_1 to Y_{10} are standardized according to Eq. (2).

$$Y_i' = \frac{Y_i - \bar{Y}_i}{SD_i}\ (i = 1, 2, \ldots, 10) \quad (2)$$

Where SD is the standard deviation; Y, Y' and $\bar{Y}$ are the measured value, standardized value, and average value of a process CQA, respectively. A quantitative model of the process parameters and a process CQA was established using multiple linear regression according to Eq. (3).

$$Y_i' = \sum_{j=1}^{10} a_{j,i} X_j\ (j = 1, 2, \ldots, 10) \quad (3)$$

Where X is the coded value of a process parameter, subscript j refers to a process parameter, and a is a SPRC. Then, the absolute values of each SPRC were weighted and summed, and the result was called the importance index (A_j), which indicates the importance of the process parameters. In this study, the yield of dry matter, the yield of pigment, the yields of fructose and sucrose and the yields of active ingredients account for 1/6, 1/6, 1/6 and 1/2, respectively. Therefore, the fructose yield and sucrose yield account for 1/12 and 1/12, respectively. Because astragaloside IV, astragaloside II, calycosin-7-glucoside, ononin, DG, and HDG are all considered as active ingredients, the yield of each active ingredient accounts for 1/12. The importance index was calculated according to Eq. (4).

$$A_j = \frac{1}{6} \times \left|a_{j,1}\right| + \frac{1}{6} \times \left|a_{j,2}\right| + \frac{1}{12} \times \sum_{i=3}^{4} \left|a_{j,i}\right| + \frac{1}{12} \times \sum_{i=5}^{10} \left|a_{j,i}\right| \quad (4)$$

After fitting the models for process CQAs, the weighted determination coefficient was calculated using Eq. (5).

$$R_w^2 = \frac{1}{6} \times R_1^2 + \frac{1}{6} \times R_2^2 + \frac{1}{12} \times \sum_{i=3}^{4} R_i^2 + \frac{1}{12} \times \sum_{i=5}^{10} R_i^2 \quad (5)$$

R_w^2 was used to evaluate the weighted proportion of data variation that was explained by the models. The weights of the various process CQAs were the same as those used in the calculation of A_j. To determine the number of critical parameters more objectively, a stepwise deletion method was used. Firstly, A_j was sorted numerically, and the process parameter corresponding to the minimum A_j was deleted. Second, Eq. (3) was used to establish models with the remaining process parameters. R_w^2 was calculated again. The decrease in R_w^2 after deleting one parameter was calculated. Deleting a CPP is assumed to result in a much larger decrease in R_w^2 than deleting an unimportant parameter. According to A_j value calculated again with Eq. (4), the process parameter corresponding to the minimum A_j was deleted again. The decrease in R_w^2 was observed again. If the decrease in R_w^2 exceeds the preset threshold after deleting a parameter, then this parameter and all the remaining parameters are considered the CPPs. In this work, multiple linear regression was performed using Design-Expert 8.0.6 (Stat-Ease, USA).

3. Results and discussions

3.1. Process CQA determination

The purpose of the water extraction process is to fully extract the active ingredients, while the ethanol precipitation process removes impurities based on retaining the active ingredients. Therefore, the process CQAs of the manufacturing processes of *Astragali Radix* extract should take the active ingredients and

Table 3
Analysis of literature data of water extraction and ethanol precipitation of *Astragali Radix*.

Literatures	Process researched	CPPs in researches
Zhou et al., 2015	water extraction	soaking time, water consumption, reflux time, extraction frequency
Xu, 2016	water extraction	reflux time, extraction frequency, water consumption
Wu, 2017	water extraction concentration	water consumption, soaking time, reflux time, extraction frequency; concentration endpoint, concentration temperature
Ma, 2006	water extraction	extraction temperature, reflux time, pH
Qian, 2008	water extraction	reflux time, extraction frequency, water consumption
Yang & Han, 2006	water extraction	reflux time, medicinal granularity, extraction temperature
Gao, Fang, Jiang & Sun, 2000	water extraction	water consumption, extraction frequency, reflux time
Wang & Zhang, 2003	water extraction	water consumption, extraction frequency, reflux time
Hu, Zhang, Zhang, Qi & Zhao, 2007	water extraction	medicinal granularity, water consumption, extraction frequency, reflux time
Zhang, Chen, & Wang, 2007	water extraction	water consumption, extraction frequency, reflux time
Zhang & Yang, 1999	water extraction	water consumption, extraction frequency, reflux time
Cui, Zhao, Wang, Luo & Yin, 2013	water extraction ethanol precipitation	extraction temperature, extraction frequency, reflux time, water consumption, ethanol consumption
Wen, Wang, Shao & Guo, 2017	ethanol precipitation	ethanol content, refrigeration time, ethanol precipitation frequency
Feng, 2015	water extraction ethanol precipitation concentration	water consumption, extraction temperature, reflux time, extraction frequency, ethanol content, ethanol precipitation frequency, refrigeration time, concentration endpoint
Yuan, Zhang, Han & Tang, 2014	water extraction concentration	water consumption, extraction frequency, reflux time, concentration method
Liu & Yu, 2010	water extraction	reflux time, extraction frequency, water consumption, pH
Fu, Zhao, Li & Dai, 2016	water extraction	soaking time, soaking frequency, water consumption
Tao, Wang & Gao, 2006	water extraction	reflux time, water consumption, pH
Xu, 2012	water extraction	extraction temperature, reflux time, extraction frequency
He, Zhang, Zhao & Guo, 2013	water extraction ethanol precipitation	medicinal granularity, water consumption, extraction temperature, ethanol content, ethanol consumption
Gong & Yang, 2004	water extraction	water consumption, pH
Guo, Yu & Tian, 2015	water extraction	water consumption, extraction temperature, extraction frequency, reflux time
Wang, Li & Zhang, 2008	water extraction ethanol precipitation	water consumption, extraction temperature, reflux time, extraction frequency, ethanol content, ethanol consumption

impurities into account. The dry matter contains active ingredients and impurities. Dry matter yield (Y_1) was considered a CQA. The Maillard reaction that occurs during the extraction process will gradually deepen the color of the extract (Coca, Garcia, Gonzalez, Pena & Garcia, 2004). If the color is too dark, Shenqi Fuzheng Injection will be unqualified. Therefore, pigment yield (Y_2) is a CQA. Water can extract the sugars in *Astragali Radix*, while ethanol can remove a large amount of the sugars. According to the solubility measurement results (Gong, Wang, & Qu, 2011; Bouchard, Gerard, & Witkamp, 2007), some monosaccharides and oligosaccharides are dissolved in the ethanol precipitation supernatants. The yields of fructose (Y_3) and sucrose (Y_4) were also considered CQAs in this study. The active ingredients in *Astragali Radix* are generally believed to include two major classes of components, which are saponins and flavonoids. In this study, the yield of astragaloside IV (Y_5), the yield of astragaloside II (Y_6), the yield of calycosin-7-glucoside (Y_7), the yield of ononin (Y_8), the yield of DG (Y_9) and the yield of HDG (Y_{10}) were considered CQAs.

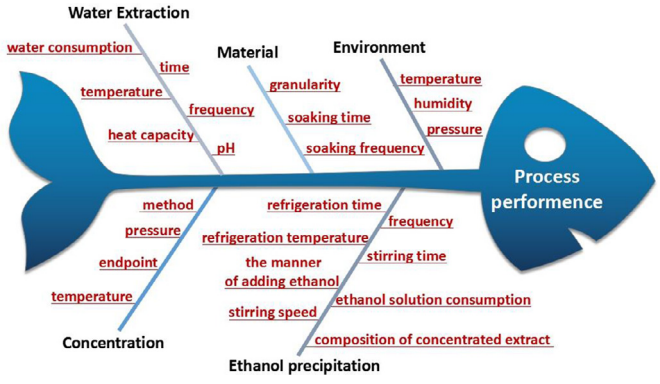

Fig. 3. Ishikawa diagram of manufacturing process of *Astragali Radix* extract.

3.2. Potential CPPs

There are many process parameters for water extraction and ethanol precipitation. All the influencing factors were shown in Fig. 3 in the form of an Ishikawa diagram. Five main causes, including environment, material, water extraction, concentration, and ethanol precipitation, and their related sub-causes were considered. To identify potential CPPs, a knowledge organization method was used (Cui et al., 2016). References on the water extraction of *Astragali Radix*, the ethanol precipitation of *Astragali Radix* extract, and the concentration of *Astragali Radix* extract were searched. Twenty-three works were obtained, and their information was shown in Table 3. The frequency of each process parameter considered important in the 23 works was counted, and the results were shown in Table 4. According to the frequency, extraction time, extraction water consumption, extraction frequency, ethanol consumption, ethanol content, and concentration endpoint were considered potential CPPs. In industry, the extraction frequency of *Astragali Radix* is fixed at three. Therefore, extraction frequency is also set equal to three in this work. Refrigeration temperature

Table 4
Frequency table of reported CPPs in water extraction and ethanol precipitation process of *Astragali Radix*.

Process parameters	Research processes	Frequency of CPPs
Reflux time	water extraction	19
Water consumption	water extraction	19
Extraction frequency	water extraction	16
Extraction temperature	water extraction	7
pH value	water extraction	4
Medicinal granularity	water extraction	3
Soaking time	water extraction	3
Soaking frequency	water extraction	1
Ethanol content	ethanol precipitation	4
Ethanol consumption	ethanol precipitation	3
Refrigeration time	ethanol precipitation	2

Table 5
Results of Plackett–Burman experimental design (μg/g *Astragali Radix*).

Run	Y_1 ($\times 10^{-3}$)	Y_2	Y_3 ($\times 10^{-3}$)	Y_4 ($\times 10^{-3}$)	Y_5	Y_6	Y_7	Y_8	Y_9	Y_{10}
1	88.5	19.2	2.90	55.9	90.8	87.6	219	55.4	90.7	51.9
2	97.6	26.6	2.70	66.3	88.7	90.4	207	57.5	89.0	59.0
3	83.5	22.3	3.70	43.0	87.6	84.3	241	53.3	111	79.8
4	76.0	14.8	2.94	55.6	89.9	87.8	212	54.3	83.5	47.7
5	95.6	23.4	2.69	84.9	92.5	93.5	220	61.1	95.8	38.7
6	120	50.3	3.75	84.9	122	127	330	90.1	135	86.4
7	104	24.4	3.31	63.9	152	154	372	107	149	84.9
8	124	38.1	4.40	79.2	101	103	249	70.0	99.8	70.5
9	75.5	18.0	2.58	53.8	92.0	92.0	170	47.7	72.5	31.3
10	89.9	30.6	2.50	51.9	60.1	70.6	173	42.8	71.0	44.4
11	82.3	27.9	3.23	75.5	112	107	316	83.4	116	79.7
12	82.2	26.0	2.75	55.6	77.7	81.8	288	68.2	117	73.1
13	103	22.1	2.82	52.7	76.7	79.3	182	46.5	69.9	32.9
14	108	29.0	4.34	69.7	84.5	92.9	240	66.9	91.6	72.9
15	67.9	15.0	2.00	42.2	116	113	240	63.7	97.0	55.5

was found to be a CPP in ethanol precipitation processes of many other drugs, such as Danhong injection (Gong, Li, Guo & Qu, 2014) and Guanxinning injection (Gong, Wang, Li & Qu, 2013). Therefore, refrigeration temperature is also considered a potential CPP.

3.3. Results of Plackett–Burman designed experiments

The results of the Plackett-Burman designed experiments were shown in Table 5. After water extraction and ethanol precipitation, the yield of dry matter was between 10.4 and 108 mg/g; The yield of pigment varied from 14.8 to 50.3 μg/g; The yield of fructose was between 2.00 and 4.40 mg/g; And the yield of sucrose was between 42.2 and 84.9 mg/g. Most of the dry matter consisted of sugars. In addition, the active ingredient yields of astragaloside IV, astragaloside II, calycosin-7-glucoside, ononin, DG, and HDG were 60.1 – 116, 70.6 – 154, 170–372, 42.8 – 107, 69.9 – 149, and 32.9 – 86.4 μg/g, respectively. The yield of calycosin-7-glucoside was greater than that of any other active ingredient.

3.4. CPP identification

The weighted determination coefficient method was applied to identify CPPs with the R_w^2 decrease threshold equal to 0.1. The standard partial regression coefficient and importance index values were shown in Table 6 when all the potential CPPs were considered. The minimum importance index is 0.135 for X_6, which is the refrigeration temperature of the first ethanol precipitation. R_w^2 was 0.831, as shown in Fig. 4A and Table S10. Then, Eq. (3) is used to rebuild the models without X_6. The results were shown in Table S1 with R_w^2 equal to 0.809. The decrease in R_w^2 was 2.98%, which was less than 0.1, as seen in Fig. 4B. This means the refrigeration temperature of the first ethanol precipitation is not a CPP. In Table S1, the minimum importance index is 0.152 for X_5, which is the ethanol consumption of the first ethanol precipitation. Then, Eq. (3) is used to rebuild the models without X_5. The results were shown in Table S2. The decrease in R_w^2 is 3.49%, which was also less than 0.1. This means the ethanol consumption of the first ethanol precipitation is also not a CPP. The above process is repeated until X_4, which is the ethanol content of the first ethanol precipitation, is removed. The decrease in R_w^2 is 15.41%, which is greater than 0.1. This means the ethanol content of the first ethanol precipitation is a CPP. The results can be seen in Tables S2-S7.

In order to verify the remaining parameters are CPPs, the above process was continued. The results can be seen in Tables S8-S10. The decrease in R_w^2 was greater than 0.1, as shown in Fig. 4B. This shows the weighted determination coefficient method worked satisfactorily. In Fig. 4A, as the process parameters decrease, the value of R_w^2 decreases gradually, which indicates that less variation in the data can be explained by the models.

The CPPs were found to be extraction time (X_2), ethanol content of the first ethanol precipitation (X_4), ethanol content of the second ethanol precipitation (X_8) and the refrigeration temperature of the second ethanol precipitation (X_{10}). Compared with the conventional SPRC method, determining the CPP number is more objective. However, the amount of calculation greatly increases with the weighted determination coefficient method.

4. Conclusion

A weighted determination coefficient method was presented in this study. In this method, the importance of process parameters is tested with one-by-one exclusion in model building. R_w^2 is developed to reflect the variation proportion that can be explained

Table 6
Standard partial regression coefficient and importance index values when all potential CPPs are considered.

Process parameters	Standard partial regression coefficients										Importance index
	Y_1	Y_2	Y_3	Y_4	Y_5	Y_6	Y_7	Y_8	Y_9	Y_{10}	
X_1 ($\times 10$)	2.95	2.83	−0.948	9.05	−2.83	−2.37	−2.55	−2.43	−2.72	−3.82	0.319
X_2 ($\times 10$)	1.44	3.40	7.04	2.058	1.320	0.891	3.70	2.61	3.23	6.41	0.308
X_3 ($\times 10$)	0.861	−0.955	1.41	2.99	−1.55	−1.81	−2.21	−1.41	−3.37	−3.35	0.181
X_4 ($\times 10$)	0.907	1.21	0.0348	1.69	4.78	4.30	5.79	4.84	6.15	3.37	0.293
X_5 ($\times 10$)	−0.758	1.80	0.207	−0.296	−3.10	−2.46	−2.32	−2.71	−1.14	−0.935	0.152
X_6 ($\times 10$)	0.695	−0.953	0.631	−1.20	2.81	3.30	1.42	2.11	0.705	−0.709	0.135
X_7 ($\times 10$)	3.34	0.502	3.89	−3.61	−1.32	−0.532	0.0263	−0.342	0.0301	1.87	0.161
X_8 ($\times 10$)	−7.08	−5.14	−4.17	−6.94	−4.59	−5.51	−4.58	−5.91	−4.94	−4.25	0.544
X_9 ($\times 10$)	−2.13	−4.16	0.341	−1.23	2.67	1.87	0.255	0.310	1.42	−0.979	0.180
X_{10} ($\times 10$)	1.66	4.67	0.708	2.21	4.51	5.00	4.34	4.81	3.74	2.91	0.341

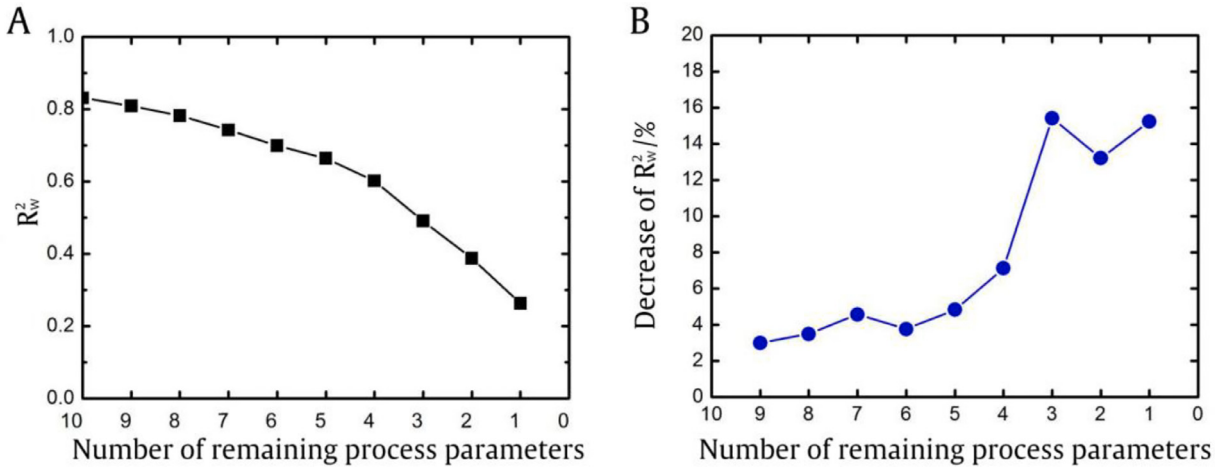

Fig. 4. R_w^2 values (A) and decrease of R_w^2 (B).

by multiple linear models. If the decrease in R_w^2 after a process parameter is removed in model building is greater than a preset threshold, CPPs can be identified. The CPPs of the preparation process of *Astragali Radix* extract, which uses the combination process of water extraction and ethanol precipitation, are identified as an example. First, the CQAs are determined to be the yield of pigment, dry matter, sugars, and active ingredients. Second, the potential CPPs are determined by a knowledge organization method. There was a small possibility of missing more important factors with the knowledge organization method. Then, Plackett–Burman designed experiments are performed. The weighted determination coefficient method is used to identify CPPs. With 10% as the threshold, the CPPs are extraction time, ethanol content of the first ethanol precipitation, ethanol content of the second ethanol precipitation, and the refrigeration temperature of the second ethanol precipitation.

Compared with the stepwise regression method and multiple linear regression method, the present method possesses the following two advantages. First, instead of considering a single CQA, the importance index is calculated by considering all the CQAs of the target process. Second, different weights for multiple process CQAs can be used in the calculation of the importance index. When compared with SPRC method, the determination of CPP number is more objective. Generally speaking, the present method has a large amount of calculation, and is suitable for finding the CPPs of the processes with many evaluation indices.

Declaration of Competing Interest

The authors declare no conflict of interest.

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Supplementary material

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.chmed.2019.11.001.

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