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

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**A B S T R A C T**

**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 ($R^2_w$) 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 $R^2_w$ was used to characterize the importance of the removed parameter. If the decrease of $R^2_w$ 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.

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**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, 2017), etc. However, these methods are not well adapted to the TCM industry.
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 ($R^2_w$) 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), Guanxinming 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 (Shaoquan, 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%), (6αR, 11αR)-9,10-dimethoxypterocarpan-3-O-β-D-glucoside (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 Anqua 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.](image-url)
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 *Astragalus 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 *Astragalus 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.

### Table 1
Coded and uncoded values of 10 potential CPPs.

| Processes                  | Potential CPPs | Symbols | Units     | Coded variables |
|----------------------------|----------------|---------|-----------|-----------------|
|                            |                | $X_1$   | mL/g *Astragalus Radix* | 5.0 | 6.0 | 7.0 |
|                            |                | $X_2$   | min       | 30 | 50 | 70 |
|                            |                | $X_3$   | mL/g *Astragalus Radix* | 0.62 | 0.66 | 0.70 |
|                            |                | $X_4$   | % (volume percent) | 91 | 93 | 95 |
|                            |                | $X_5$   | mL/mL | 2.8 | 3.0 | 3.2 |
|                            |                | $X_6$   | °C | 3.0 | 6.0 | 9.0 |
|                            |                | $X_7$   | % (volume percent) | 91 | 93 | 95 |
|                            |                | $X_8$   | mL/mL | 5.4 | 5.6 | 5.8 |
|                            |                | $X_9$   | °C | 3.0 | 6.0 | 9.0 |

### Table 2
Conditions of Plackett-Burman designed experiments.

| Run | Potential CPPs | $X_1$(/mL °C) | $X_2$/min | $X_3$(/mL °C) | $X_4$/% | $X_5$(/mL °C) | $X_6$/°C | $X_7$(/mL °C) | $X_8$/% | $X_9$(/mL °C) | $X_{10}$/°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    | 5.40          | 9.00       |
| 8   |               | 7.00          | 70.00     | 0.70          | 0.91    | 2.80          | 3.00      | 0.38          | 0.91    | 5.40          | 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     | 0.66          | 0.93    | 3.00          | 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       |

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 into 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-C18 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. 51.

A Waters XBridge BEH Amide column (4.6 mm × 250 mm, 5 μm) was used for the analysis of sugars (Gong, Wang, & Qu,
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 N2 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).

\[
\text{Yield}_i (\mu g/g) = \frac{V_{SS}(mL) \times C_i(\mu g/mL)}{M_{ar}(g)} \quad (i = 1, 2, \ldots, 10) \tag{1}
\]

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).](image)

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

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

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).

\[
Y'_i = \sum_{j=1}^{10} a_{ij}X_j \quad (j = 1, 2, \ldots, 10) \tag{3}
\]

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 (\(A_i\)), 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).

\[
A_i = \frac{1}{6} |a_{i1}| + \frac{1}{6} |a_{i2}| + \frac{1}{12} \sum_{i=3}^{4} |a_{ij}| + \frac{1}{12} \sum_{i=5}^{10} |a_{ij}| \tag{4}
\]

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

\[
R^2_w = \frac{1}{6} R^1_i + \frac{1}{6} R^2_i + \frac{1}{12} \sum_{i=3}^{4} R^2_i + \frac{1}{12} \sum_{i=5}^{10} R^2_i \tag{5}
\]

\(R^2_w\) 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 \(A_i\). To determine the number of critical parameters more objectively, a stepwise deletion method was used. Firstly, \(A_i\) was sorted numerically, and the process parameter corresponding to the minimum \(A_i\) was deleted. Second, Eq. (3) was used to establish models with the remaining process parameters. \(R^2_w\) was calculated again. The decrease in \(R^2_w\) after deleting one parameter was calculated. Deleting a CPP is assumed to result in a much larger decrease in \(R^2_w\) than deleting an unimportant parameter. According to \(A_i\) value calculated again with Eq. (4), the process parameter corresponding to the minimum \(A_i\) was deleted again. The decrease in \(R^2_w\) was observed again. If the decrease in \(R^2_w\) 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
impurities into account. The dry matter contains active ingredients and impurities. Dry matter yield (Y1) 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, Shengqi Fuzheng Injection will be unqualified. Therefore, pigment yield (Y2) is a CQA.

Water can extract the sugars in *Astragalus 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 (Y3) and sucrose (Y4) were also considered CQAs in this study. The active ingredients in *Astragalus Radix* are generally believed to include two major classes of components, which are saponins and flavonoids. In this study, the yield of astragaloside IV (Y5), the yield of astragaloside II (Y6), the yield of calycosin-7-glucoside (Y7), the yield of ononin (Y8), the yield of DG (Y9) and the yield of HDG (Y10) were considered CQAs.

### 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 *Astragalus Radix*, the ethanol precipitation of *Astragalus Radix* extract, and the concentration of *Astragalus 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 *Astragalus Radix* is fixed at three. Therefore, extraction frequency is also set equal to three in this work. Refrigeration temperature

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**Table 3**

Analysis of literature data of water extraction and ethanol precipitation of *Astragalus 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 | concentration | extraction temperature, reflux time, pH |
| Ma, 2006 | water extraction | reflux time, medicinal granularity, extraction temperature |
| Qian, 2008 | water extraction | water consumption, extraction frequency, reflux time |
| Yang & Han, 2006 | water extraction | medicinal granularity, water consumption, extraction frequency, reflux time |
| Gao, Fang, Jiang & Sun, 2000 | water extraction | water consumption, extraction frequency, reflux time |
| Wang & Zhang, 2003 | water extraction | extraction temperature, extraction frequency, reflux time |
| Hu, Zhang, Zhang, Qi & Zhao, 2007 | water extraction | extraction frequency, reflux time |
| Zhang, Chen, & Wang, 2007 | water extraction | extraction temperature, extraction frequency, reflux time |
| Zhang & Yang, 1999 | water extraction | extraction temperature, extraction frequency, reflux time, water consumption, ethanol consumption |
| Cui, Zhao, Wang, Luo & Yin, 2013 | water extraction | ethanol content, refrigeration time, ethanol precipitation frequency |
| Wen, Wang, Shao & Guo, 2017 | water extraction | ethanol consumption, extraction frequency, reflux time, extraction frequency, ethanol content, ethanol precipitation frequency, refrigeration time, concentration endpoint |
| Peng, 2015 | water extraction | water consumption, extraction frequency, reflux time, concentration method |
| Yuan, Zhang, Han & Tang, 2014 | water extraction | reflux time, extraction frequency, water consumption, pH |
| Liu & Yu, 2010 | water extraction | soaking time, soaking frequency, water consumption |
| Fu, Zhao, Li & Dai, 2016 | water extraction | reflux time, water consumption, pH |
| Tao, Wang & Gao, 2006 | water extraction | extraction temperature, reflux time, extraction frequency |
| Xu, 2012 | water extraction | medicinal granularity, water consumption, extraction temperature, ethanol content, ethanol consumption |
| He, Zhang, Zhao & Guo, 2013 | ethanol precipitation | water consumption, pH |
| Gong & Yang, 2004 | water extraction | water consumption, extraction temperature, extraction frequency, reflux time |
| Guo, Yu & Tian, 2015 | water extraction | water consumption, extraction temperature, extraction frequency, reflux time, ethanol content |
| Wang, Li & Zhang, 2008 | ethanol precipitation | water consumption, extraction frequency, reflux time, concentration endpoint |

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**Table 4**

Frequency table of reported CPPs in water extraction and ethanol precipitation process of *Astragalus 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            |
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, calcosin-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 calcosin-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 $R^2_p$ 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 $X_9$, which is the refrigeration temperature of the first ethanol precipitation. $R^2_p$ was 0.831, as shown in Fig. 4A and Table S10. Then, Eq. (3) is used to rebuild the models without $X_9$. The results were shown in Table S1 with $R^2_p$ equal to 0.809. The decrease in $R^2_p$ 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 $R^2_p$ 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 $R^2_p$ 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 $R^2_p$ 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 $R^2_p$ 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 ($X_6$) and the refrigeration temperature of the second ethanol precipitation ($X_{10}$). Compared with the conventional SPCR 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. $R^2_p$ is developed to reflect the variation proportion that can be explained.

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**Table 5**

| Run | $Y_1$ ($\times 10^{-1}$) | $Y_2$ | $Y_3$ ($\times 10^{-1}$) | $Y_4$ ($\times 10^{-1}$) | $Y_5$ | $Y_6$ | $Y_7$ | $Y_8$ | $Y_9$ | $Y_{10}$ |
|-----|-----------------|-------|-----------------|-----------------|-------|-------|-------|-------|-------|--------|
| 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   |

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**Table 6**

| Process parameters | Standard partial regression coefficients | Importance index |
|--------------------|------------------------------------------|------------------|
| $Y_1$              | 2.95 ($0.948$)                  | 0.319            |
| $X_2$              | 1.44 ($7.04$)                   | 0.308            |
| $X_3$              | 0.861 ($-0.955$)                | 0.181            |
| $X_4$              | 0.907 ($-0.955$)                | 0.293            |
| $X_5$              | 0.695 ($0.631$)                 | 0.135            |
| $X_6$              | 3.34 ($0.502$)                  | 0.161            |
| $X_7$              | -7.08 ($-5.14$)                 | 0.544            |
| $X_8$              | -2.13 ($-4.16$)                 | 0.180            |
| $X_9$              | 1.66 ($0.467$)                  | 0.341            |
| $X_{10}$           | 5.00 ($-4.34$)                  | 0.341            |
by multiple linear models. If the decrease in $R^2_m$ 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 Astragalus 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

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