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Optimization of a chromatographic process for the purification of saponins in *Panax notoginseng* extract using a design space approach

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ABSTRACT

Quality by Design principle was applied to optimize the load and wash steps in the chromatographic process of *Panax notoginseng* extracts. Saponin recovery, saponin purity and saponin yield per unit volume of resin were identified as the process critical quality attributes (CQAs) of the chromatographic process. The critical process parameters (CPPs) were determined by an Ishikawa diagram combined with a Failure Mode and Effect Analysis. Column height/diameter ratio, load rate, load volume, total saponin concentration in loading solution and wash volume were identified as CPPs for the load and wash steps. The models between CPPs and process CQAs were developed according to the data obtained from central composite designed experiments. Determination coefficients (R^2) were higher than 0.83 for all the models. Loss mechanism of saponins was discussed, overloading may lead to the loss of all the saponins and wash with water may lead to the loss of notoginsenoside R_1, ginsenoside Rg_1 and ginsenoside Re. Design spaces with different acceptable probabilities were calculated using a Monte-Carlo simulation method. Recommended normal operation region is located in height/diameter ratio of 13.5–15.0, load rate of 1.5–1.6 BV/h, load volume of 5.5–6.0 BV, total saponin concentration of 10.4–12.2 mg/g, wash volume of 2.0–2.6 BV when acceptable probability was set to 0.80. The verification experimental values were in a good agreement with the predicted values. The design space is reliable and working within it can attain process CQAs criteria with a high probability.

1. Introduction

The root of *Panax notoginseng*, *Sanqi* in Chinese, is a highly valued and commonly used Chinese medicinal herb. It has been a main material in many traditional Chinese medicines and health foods, such as Xuesaitong injection, Xueshuantong injection, *Sanqi* tablet and Yun-Nan-Bai-Yao. The major bioactive compounds of *Sanqi* are widely recognized as the dammarane triterpene saponins among which notoginsenoside R_1, ginsenosides Rg_1, Re, Rb_1 and Rd are five main components [1–4]. The saponins possess extensive bioactivities, such as anticarcinogenic, antioxidant, anti-aging and cardioprotective effects [5–8]. The above-mentioned five saponins were used as quality control indices in the manufacturing of *Sanqi* related preparations [9,10].

Column chromatography, typically used to remove impurities, is one of the key unit operations in the manufacturing of *Sanqi* related botanical injections, such as Xueshuantong injection [10,11]. The performance of a chromatographic process is influenced by many factors, such as the composition of load solution, flow rate and the composition of elution solvent [12–15]. In addition, in the chromatographic process for traditional Chinese medicine purification, the compositions of load solution are complicated [10,11,16,17]. Therefore maintaining batch-to-batch consistency is a challenging task in the chromatographic process for traditional Chinese medicine purification.

Recently, Quality by Design (QbD) and design space concept are gained increasing popularity in pharmaceutical manufacturing process, such as ethanol precipitation [17,18], powder blending [19], tablet manufacturing [20], pharmaceutical co-precipitation [21] and chromatographic process [22–25]. The application of

Abbreviations: ARD, average relative deviation; BV, bed volume; CPPs, critical process parameters; CQAs, critical quality attributes; C_0^i, concentration of saponin *i* in the loading solution (mg/g); C_e^i, concentration of saponin *i* in the elution solution (mg/g); C_e^{TS}, total saponin concentration in elution solution (mg/g); DM, dry matter content in the elution solution (mg/g); EV, experimental values; *i*, component identifier; m_0, mass of loading solution (g); m_e, mass of elution solution (g); PV, predicted values; QbD, Quality by Design; TOR, total saponin obtained using per milliliter resin (g/mL); SRR, saponin recovery ratio; PU, total saponin purity in the elution solution; RSD, relative standard deviations; V_R, volume of HPD-100 resin (mL).

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QbD and design space concept can promote science-based regulatory policy development, and help to ensure or improve pharmaceutical product quality and product batch-to-batch consistency [19]. In order to develop the design space, the modeling of chromatographic process is required. Guiochon reviewed the mechanism models for chromatographic processes, such as general rate model and relative simplified models [26]. Multicomponent adsorption behavior, column properties, and mass transfer inside or outside resins are considered. However, many compounds and their quantity are unknown for a herbal mixture. Therefore it is very difficult to measure adsorption isotherm accurately. Accordingly, very few published work was found to model chromatography process using a mechanism model for the purification of a herbal extract. Using statistical model is an alternative way. Recently, Jiang et al. [23] and Ji et al. [24] have applied QbD principles to develop and optimize chromatographic process for protein purification. Process critical quality attributes (CQAs) and critical process parameters (CPPs) were identified and design spaces were mapped. However, the economic efficiency has not been considered in their studies, and design spaces were developed without considering the uncertainty of model parameters. Rozet et al. suggested that the design space should take into account the uncertainty of model parameters and provide information of the probability of meeting the specifications [27]. The Monte-Carlo simulation is one of the commonly used methods to include uncertainty of the parameters of the models during the design space development [25,28]. In this work, the chromatographic load and wash steps for the purification of saponins in *Sanqi* extract were developed using design space approach as a case study. Process CQAs of the chromatographic process were first obtained. Ishikawa diagram and Failure Mode and Effect Analysis were conducted to determine the CPPs. A design space for load and wash steps was calculated based on the central composite design experimental results with a Monte-Carlo simulation method. Finally, the obtained design space was verified.

Table 1
Coded and uncoded values for the parameters.

Critical process parameter	Symbols	Coded values		
		−1	0	1
Height/diameter ratio	X_1	9	12	15
Load rate (BV/h)	X_2	0.6	1.0	1.6
Load volume (BV)	X_3	3.0	4.5	6.0
Total saponin concentration (mg/g)	X_4	10.4	20.9	31.6
Wash volume (BV)	X_5	2.0	5.0	8.0

2. Materials and methods

2.1. Materials and reagents

Sanqi was collected from Wenshan of Yunnan Province, China. Notoginsenoside R_1, ginsenosides Rg_1, Re, Rb_1 and Rd (Shanghai Winherb Medical Science Co., Ltd., Shanghai, China) were used as standard compounds without further purification. HPLC grade formic acid and acetonitrile were supplied by Roe Scientific Inc. (Newark, USA) and Merck (Darmstadt, Germany), respectively. 95% (*v*/*v*) ethanol was obtained from Zhejiang Changqing Chemical industry Co., Ltd. (Zhejiang, China). Ultrahigh-purity water was prepared in a Milli-Q purification system from Millipore (Milford, PA, USA).

2.2. Preparation of crude Sanqi extracts

According to Chen et al.'s work [10], *Sanqi* powder was extracted twice by 86% (*v*/*v*) ethanol using a water bath (W501, Shanghai Shensheng Biotechnology Co., Ltd) for 8 h. The extracts were filtered and the filtrates were mixed. The mixed filtrates then

Table 2
Central composite design and results.

Run	X_1	X_2 (BV/h)	Corresponding superficial velocity (mL/min)	X_3 (BV)	X_4 (mg/g)	X_5 (BV)	Saponin recovery ratio					Saponin purity	TOR (g/mL)
							R_1	Rg_1	Re	Rb_1	Rd		
1	12	1.1	0.7	4.5	20.9	5.0	0.59	0.74	0.68	0.89	0.99	0.84	0.075
2	12	1.1	0.7	4.5	20.9	2.0	0.67	0.82	0.85	0.88	1.01	0.85	0.080
3	9	1.1	0.5	4.5	20.9	5.0	0.60	0.73	0.76	0.87	0.96	0.88	0.075
4	12	1.6	1.0	4.5	20.9	5.0	0.65	0.76	0.70	0.90	0.95	0.85	0.077
5	12	1.1	0.7	4.5	20.9	5.0	0.61	0.76	0.77	0.91	0.98	0.87	0.078
6	12	1.1	0.7	4.5	31.6	5.0	0.36	0.45	0.40	0.65	0.97	0.85	0.080
7	9	1.6	0.7	6.0	31.6	2.0	0.26	0.37	0.33	0.44	0.73	0.80	0.079
8	15	1.6	1.2	3.0	31.6	2.0	0.78	0.85	0.79	0.83	0.98	0.90	0.082
9	15	1.6	1.2	6.0	31.6	8.0	0.21	0.28	0.25	0.46	0.77	0.79	0.073
10	9	0.6	0.3	3.0	31.6	2.0	0.78	0.75	0.75	0.78	0.81	0.77	0.085
11	12	1.1	0.7	6.0	20.9	5.0	0.32	0.49	0.44	0.76	0.96	0.81	0.077
12	12	1.1	0.7	3.0	20.9	5.0	1.00	1.01	0.99	0.99	0.98	0.87	0.063
13	15	1.1	0.8	4.5	20.9	5.0	0.62	0.79	0.79	0.97	0.97	0.88	0.081
14	9	1.6	0.7	3.0	31.6	8.0	0.59	0.73	0.78	0.78	0.91	0.87	0.073
15	12	1.1	0.7	4.5	10.4	5.0	1.05	1.05	1.06	1.04	0.99	0.88	0.049
16	15	0.6	0.5	3.0	10.4	2.0	1.02	1.05	1.11	1.03	0.97	0.87	0.033
17	15	1.6	1.2	3.0	10.4	8.0	1.02	1.04	1.04	1.03	0.94	0.87	0.032
18	15	1.6	1.2	6.0	10.4	2.0	0.97	1.05	1.11	1.05	0.99	0.89	0.066
19	9	1.6	0.7	3.0	10.4	2.0	0.98	1.03	0.89	1.03	1.12	0.87	0.032
20	9	1.6	0.7	6.0	10.4	8.0	1.01	1.00	0.85	1.01	1.12	0.88	0.063
21	9	0.6	0.3	6.0	10.4	2.0	1.08	1.02	1.01	1.05	1.06	0.89	0.065
22	9	0.6	0.3	3.0	10.4	8.0	1.03	1.00	0.94	1.04	1.11	0.92	0.032
23	15	0.6	0.5	6.0	31.6	2.0	0.26	0.34	0.30	0.47	0.89	0.78	0.081
24	15	0.6	0.5	3.0	31.6	8.0	0.54	0.65	0.67	0.84	1.01	0.88	0.071
25	12	1.1	0.7	4.5	20.9	8.0	0.59	0.70	0.65	0.90	1.04	0.85	0.075
26	15	0.6	0.5	6.0	10.4	8.0	1.03	0.99	0.98	1.00	1.05	0.87	0.063
27	9	0.6	0.3	6.0	31.6	8.0	0.19	0.27	0.21	0.47	0.92	0.78	0.074
28	12	0.6	0.4	4.5	20.9	5.0	0.70	0.77	0.83	0.94	1.03	0.87	0.081
29	12	1.1	0.7	4.5	20.9	5.0	0.64	0.72	0.67	0.87	0.98	0.86	0.075