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Optimization of *Panax notoginseng* extraction process using a design space approach

Xingchu Gong, Huali Chen, Jianyang Pan, Haibin Qu *

Pharmaceutical Informatics Institute, College of Pharmaceutical Sciences, Zhejiang University, Hangzhou 310058, China

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ABSTRACT

Health foods containing *Panax notoginseng* extract are developed in recent years. In this work, reflux extraction process was optimized using a design space approach to improve batch-to-batch consistency of *P. notoginseng* extract. Saponin yields and dry matter yield were identified as the process critical quality attributes (CQAs) of the extraction process. A risk assessment was applied to determine critical process parameters (CPPs), which were ethanol content (EC), amount of ethanol added (AEA), extraction time (ET) and extraction frequency (EF). Box-Behnken designed experiments were carried out to develop models between CPPs and process CQAs. Determination coefficients were higher than 0.90 for all the models. Higher ET, EF, and AEA all result in higher saponin yields and dry matter yield. Dry matter yield decrease as EC increases. Design space was calculated using a Monte-Carlo simulation method with the acceptable probability of 0.85. Normal operation ranges to attain process CQA criteria with a probability more than 94% are recommended as follows: EC of 84.0–86.0%, AEA of 5.0–7.0 mL/g, ET of 7.0–8.0 h, and EF of 2. Verification experiment results showed that operating EC, AEA, EF and ET within design space can attain CQA criteria. Most of the verification experiment results agreed well with prediction results, which means that the developed models are accurate and applicable in a larger scale extraction process.

1. Introduction

Panax notoginseng, the root of *P. notoginseng* (Burk.) F.H. Chen, is a medicinal and edible plant in China. In 1994, it was included as a dietary supplement by the US Dietary Supplement Health and Education Act in the USA [1]. *P. notoginseng* can remove blood stasis, stop bleeding, relieve swelling, alleviate pain, treat hyperlipemia and chronic infectious hepatitis [2]. Dammarane-type triterpene saponins are the main bioactive components in *P. notoginseng* [2]. The saponins contribute to many pharmacological activities, such as antithrombotic, anti-atherosclerotic, fibrinolytic, antioxidant and cardioprotective effects [3,4]. Therefore the saponins are frequently used as the main indices for *P. notoginseng* product evaluation [5,6].

Compared with directly eating *P. notoginseng*, taking its extract is more convenient. During the extraction and the following purification process, heavy metals and pesticides can be removed, which will improve the safety of products made from *P. notoginseng*. Many different methods are applied to extract saponins in *P. notoginseng* [7–13], such as heat reflux extraction, ultrasonic extraction, microwave-assisted extraction, cold percolation extraction, accelerated solvent extraction, and pressurized liquid extraction. The extraction conditions were optimized with orthogonal array design or uniform design by maximizing the extracted saponin amounts [9,10]. However, the batch-to-batch consistency of extracts is not considered in extraction condition optimizations.

For food suppliers, the batch-to-batch consistency is the key to maintaining brand equity. Because of the variability of materials and complex transformation of components in the manufacturing process, it is a challenging task to keep food batch-to-batch consistency. Recently, Quality by Design (QbD) has become a paradigm to optimize manufacturing processes [14,15]. Based on knowledge management and risk management, QbD concept is usually implemented with several steps, which are critical quality attribute (CQA) definition, risk assessment, critical process parameter

Abbreviations: AEA, amount of ethanol added (mL/g); ANOVA, analysis of variance; ARD, average relative deviation (%); C, saponin concentration; Cal, calculated values; CPP, critical process parameter; CQA, critical quality attribute; DM, the dry matter content of an extract (g/g); EC, ethanol content (v/v, %); EF, extraction frequency; ET, extraction time (h); Exp, experimental values ; EXT, extract; FMEA, Failure Mode and Effects Analysis; M, the mass; NED, the number of experimental data; PN, *Panax notoginseng*; QbD, Quality by Design; RPN, risk priority number; RSD, relative standard deviation; SC, saponin content in dry matter (mg/g); YDM, the yield of dry matter; YR, the yield of saponins.

* Corresponding author. Tel./fax: +86 571 88208428.
E-mail address: quhb@zju.edu.cn (H. Qu).

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Table 1
Coded and uncoded values for the factors.

Parameters	Symbols	Coded values		
		−1	0	1
EC (V/V%)	X_1	70	80	90
AEA (mL/g)	X_2	3	5	7
ET (h)	X_3	2	5	8
EF	X_4	2	3	4

(CPP) determination, design space development, control strategy design, and continual improvement in product lifecycle [16,17]. In these steps, the development of design space is very important because it provides guidance on the setting of critical process parameters [18].

Recently, Rozet et al. suggested that design space is "a multivariate domain of input factors ensuring that critically chosen responses are included within predefined limits with an acceptable level of probability" [18]. According to ICH Q8 (R2), parameter variations within design space are not considered to affect product quality [16]. To develop the design space of a process, the knowledge on this process is highly required to obtain quantitative mathematical models between CPPs and process CQAs. Experimental design is often applied to establish the models [19]. To calculate the probability to meet the limits of process CQAs, the Monte-Carlo simulations and Bayesian modeling are commonly applied [20–22].

In this work, the extraction process of *P. notoginseng* was optimized using a design space approach [23], which contains the steps of CQA definition, CPP identification, design space development and verification. The mixed solvent of ethanol and water was applied to extract saponins from *P. notoginseng*. In this work, the process CQAs of reflux extraction were discussed. CPPs were obtained using a risk assessment. Models between CQAs and CPPs were developed. Design space was calculated based on the Box-Behnken designed experimental results with a Monte-Carlo simulation. Finally, design space was verified.

2. Experimental

2.1. Materials and chemicals

P. notoginseng was collected from Wenshan of Yunnan Province (China). Standard substances of notoginsenoside R_1, ginsenoside Rg_1, ginsenoside Re, ginsenoside Rb_1, and ginsenoside Rd were purchased from Shanghai Winherb Pharmaceutical Technology Development. Co., Ltd. (Shanghai, China). Ethanol was purchased from Shanghai Ling Feng Chemical Reagent Co., Ltd. (Shanghai, China). Acetonitrile (HPLC grade) and methanol (HPLC grade) were obtained from Merck (Darmstadt, Germany). The formic acid (HPLC grade) was purchased from Tedia (Darmstadt, Germany). Deionized water was produced using a Milli-Q academic water purification system (Milford, MA, USA).

2.2. Procedures

A reflux extraction was used to extract *P. notoginseng* using the mixed solvent of ethanol and water as the extractant. A water bath (W501, Shanghai Shen Sheng Biotechnology Co., Ltd) operated at 95 °C was used to boil the mixed solvent. A condenser tube was applied to condense vaporized solvent. The amount of *P. notoginseng* was 50 g in each experiment. Four parameters were investigated in experiments, which were ethanol content in the mixed solvent (EC, V/V%) , amount of ethanol added (AEA, mL/g) , extraction time (ET, h) and extraction frequency (EF). In the first extraction, more solvent was added with a volume of 50 mL in all the experiments. The obtained extracts were mixed, filtered with gauze and weighed. Saponin contents and dry matter content of the extracts then were determined.

Table 2
Box-Behnken designed experiments and results.

Run	EC (X_1, V/V%)	AEA (X_2, mL/g)	ET (X_3, h)	EF (X_4)	Saponin yield (mg/g *Panax notoginseng*)				Dry matter yield (mg/g *Panax notoginseng*)
					R_1	Rg_1	Rb_1	Rd	
1	70.0	3.0	5.0	3	14.5	46.2	46.7	9.99	388.4
2	80.0	3.0	5.0	2	13.8	44.7	43.4	9.48	352.1
3	80.0	5.0	2.0	2	13.2	43.7	41.8	9.15	362.7
4	70.0	5.0	5.0	4	14.8	50.2	50.3	10.7	431.2
5	90.0	5.0	8.0	3	15.1	51.2	50.4	11.0	395.5
6	80.0	7.0	2.0	3	14.4	49.6	47.1	10.3	406.8
7	80.0	5.0	5.0	3	15.6	50.8	51.7	11.2	425.2
8	70.0	5.0	8.0	3	15.9	52.8	53.0	11.5	433.6
9	80.0	5.0	5.0	3	15.6	51.1	53.4	11.6	428.9
10	80.0	7.0	8.0	3	15.9	54.6	53.2	11.7	437.8
11	80.0	3.0	8.0	3	16.1	52.0	51.4	11.4	408.1
12	90.0	5.0	2.0	3	12.4	44.1	38.2	8.49	342.7
13	80.0	3.0	5.0	4	15.9	52.1	51.5	11.4	414.5
14	90.0	3.0	5.0	3	14.8	48.7	44.6	10.1	358.2
15	70.0	5.0	5.0	2	14.3	47.5	47.1	9.87	378.2
16	80.0	5.0	5.0	3	15.8	53.0	52.5	11.1	425.2
17	80.0	5.0	8.0	2	15.8	53.4	52.6	11.5	398.4
18	80.0	3.0	2.0	3	13.4	45.3	43.8	9.70	374.1
19	90.0	5.0	5.0	4	15.0	50.4	49.4	10.8	394.7
20	90.0	5.0	5.0	2	13.0	43.7	41.3	8.95	338.8
21	70.0	5.0	2.0	3	14.9	50.3	47.5	10.5	419.0
22	70.0	7.0	5.0	3	16.0	52.9	53.3	11.7	452.8
23	90.0	7.0	5.0	3	13.8	50.6	46.3	9.96	381.5
24	80.0	5.0	8.0	4	16.2	55.8	55.9	12.1	441.5
25	80.0	5.0	5.0	3	16.3	52.1	50.6	10.6	421.6
26	80.0	7.0	5.0	2	14.5	48.0	47.3	10.0	389.4
27	80.0	5.0	5.0	3	15.3	52.0	50.0	10.5	426.7
28	80.0	5.0	2.0	4	15.4	51.0	50.4	10.9	409.2
29	80.0	7.0	5.0	4	15.3	50.5	51.0	11.0	409.9