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Control the effects caused by noise parameter fluctuations to improve pharmaceutical process robustness: A case study of design space development for an ethanol precipitation process

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ABSTRACT

To provide a new method to develop a design space to improve the robustness of drug manufacturing processes based on the control of noise parameter influences, the ethanol precipitation process of Danhong injection was investigated as a sample. Water content in the concentrated extract (WCCE), the concentration of ethanol (CEA), and the amount of ethanol added (AEA) are three adjustable parameters. The effects of refrigeration temperature (RT) was investigated on three levels for simulating its fluctuations. The models between parameters and process critical quality attributes (CQAs) were obtained using a simplified central composite design with determination coefficients more than 0.84. The decrease of RT led to lower active ingredient recoveries and higher DMR. The increase of CEA and the decrease of WCCE caused more precipitation. The decrease of CEA or AEA resulted in higher active ingredient recoveries. The design space was calculated using an exhaustive search-Monte Carlo method and normal operation ranges were also obtained. The results of verification experiments agreed well with prediction results. The proposed method can be used to develop a design space applicable in a larger scale manufacturing process with the negative effects caused by the fluctuations of noise parameters controlled.

1. Introduction

Quality by design (QbD) is a concept based on knowledge management and risk management [1,2]. It helps to develop new drugs and improve drug quality control [3,4]. There are several steps to implement QbD concept in drug manufacturing, including critical quality attribute (CQA) definition, critical process parameter (CPP) identification, risk assessment, design space development, control strategy design, and continual improvement in drug lifecycle [5,6]. Design space development is a key component in the implementation of QbD [7]. According to the ICH Q8(R2) guideline, working within the design space will not result in drug quality changes [5]. To establish a design space, quantitative relationships between process parameters and CQAs must be obtained [8–13]. Response surface methodology is the most widely applied method to develop a design space.

Recently, Rozet et al. define a design space as "a multivariate domain of input factors ensuring that critically chosen responses are included within predefined limits with an acceptable level of probability" [7]. Because response surface methodology cannot give any guarantee that CQAs will attain the defined criteria with high probability [7], several other methods, such as the Monte-Carlo simulations and Bayesian modeling, are applied to calculate the probability [7,14–16].

In the production of drugs, some CPPs are difficult to be controlled in a narrow range. For example, because of high control expenses, refrigeration temperature (RT) of a precipitation process is a parameter often fluctuates with seasons. While pH value is a parameter often fluctuates because of the control difficulties caused by big time lag. These CPPs can be considered as the noise

Abbreviations: AC, active ingredient content (mg g^{-1}); ACR, active ingredient recovery (%); AEA, the amount of ethanol added (mL g^{-1}); ANOVA, analysis of variance; ARD, average relative deviation (%); CE, concentrated extract; CEA, the concentration of ethanol (g g^{-1}); CPP, critical process parameter; CQA, critical quality attribute; DM, dry matter content (mg g^{-1}); DMR, dry matter removal (%); DSS, Danshensu; EV, experimental value; FEP, first ethanol precipitation; HSYA, hydroxysafflor yellow A; LA, lithospermic acid; MAS, mass of supernatant (g); MCE, mass of concentrated extract (g); NOR, normal operating ranges; PV, predicted value; QbD, quality by design; RA, rosmarinic acid; RSD, relative standard deviation; RT, refrigeration temperature (°C); SaB, Salvianolic acid B; SP, supernatant; WCCE, water content in concentrated extract (%).

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parameters in drug manufacturing [17]. Contrarily, parameters that can be easily controlled with acceptable costs are considered as the adjustable parameters [17]. For a specific process, a design space should only define the ranges for adjustable parameters. To increase process robustness, the negative effects caused by the fluctuations of the noise parameters must be controlled using the adjustable parameters. However, to the author's knowledge, there is no published works focusing on the control of noise parameter effects in the design space development.

In this work, a design space is developed aiming to increase process robustness by the control of noise parameter effects. The first ethanol precipitation (FEP) process of Danhong injection is investigated as a sample. Danhong injection is a botanical injection clinically for the treatment of coronary heart disease, angina, myocardial infarction, and cerebral diseases [18]. The sales of Danhong injection have reached more than 4 billion RMB per year. Phenolic acids and flavones are considered as the active ingredients of Danhong injection, such as Danshensu (DSS), rosmarinic acid (RA), lithospermic acid (LA), Salvianolic acid B (SaB) and hydroxysafflor yellow A (HSYA) [19]. Danhong injection is made from *Salviae miltiorrhizae Radix et Rhizoma* (Danshen) and *Carthami Flos* (Honghua) with a series of processes, including water extraction, concentration, ethanol precipitation, and adsorption. The FEP process deals with the concentrated extract of mixed Danshen and Honghua. Highly polar impurities in the concentrated extract are usually removed in the ethanol precipitation process, such as saccharides, salts, and proteins [20–25]. However, the losses of active ingredients, including phenolic acids and flavonoids, are also observed in published works [23,26,27]. Because of the important impacts on drug safety and efficacy, the development of a design space for the FEP process will help to improve the quality control of Danhong injection [3].

The ethanol precipitation process is an easily operating process without using toxic solvent [28,29]. However, the mechanism of the ethanol precipitation process is usually very complicated when dealing with the water extract of medicinal plants. Active ingredients may lose because of several different reasons, such as precipitation, degradation, or encapsulation [30]. Therefore response surface methodology, such as central composite design or Box-Behnken design, was often applied to investigate the ethanol precipitation process [31]. Simple mathematical models can also be obtained with response surface methodology [32,33].

In this work, a risk assessment was carried out to obtain the CQAs of the FEP process. Experimental design was applied to establish the quantitative relationships among the adjustable parameters, the noise parameter, and the CQAs of the FEP process. The probability to attain the defined criteria was calculated using the Monte-Carlo method. A design space was obtained and verified using the adjustable parameters to control the effects caused by noise parameter fluctuations.

2. Materials and methods

2.1. Materials and chemicals

Danshen was purchased from Nepstar Drugstore (Zhejiang, China), and Honghua was purchased from Daily Healthy Drugstore (Zhejiang, China). Ethanol (>99%) was obtained from Tianjin Damao Chemical Reagent Factory (China). Standard substances including DSS, RA, and LA were purchased from Winherb Medical S&T Development Co., Ltd. (Shanghai, China). SaB was obtained from Chengdu Biopurify Phytochemicals Ltd. (Sichuan, China). HSYA was purchased from Aladdin Industrial Inc. (Shanghai, China). Deionized water was produced using a Mill-Q academic water purification system (Milford, MA, USA). The HPLC-grade acetonitrile was purchased from Merck (Darmstadt, Germany). The HPLC-grade formic acid was obtained from ROE SCIENTIFIC INC. The HPLC-grade ammonium formate was purchased from Alfa Aesar China (Tianjin) Co., Ltd. All materials were used as received without any further purification.

2.2. Design of experiments

In this work, four parameters of refrigeration temperature, concentration of ethanol added (CEA), water content of concentrated extract (WCCE), and amount of ethanol added (AEA), were investigated with a simplified central composite design. Table 1 shows the coded and uncoded values of parameters. The effects of RT was investigated on three levels to simulate its fluctuations. The central point was repeated for 3 times. Other points were repeated for twice. Therefore a total of 47 experiments were carried out. The run orders are listed in Table 2. The ranges of the four parameters were set based on production experiences. The experimental conditions for the verification of the design space are listed in Table 3.

2.3. Experimental procedure

2.3.1. Preparation of concentrated extract

Reflux extraction was carried out to extract mixed 6 kg of Danshen and 2 kg of Honghua with 80 L of distilled water for 1 h. The extract then was filtered and collected. The extraction was repeated twice. Two water extracts then were combined and concentrated under reduced pressure. After concentration, the water content of the obtained concentrated extract was 26.8% ± 0.32%. The concentrated extracts with higher water content were obtained by dilution with deionized water.

2.3.2. Ethanol precipitation

The ethanol precipitation experiments were carried out in run orders. Ethanol solution of designed concentration was added into 20 g of a concentrated extract in a conical flask under magnetic stirring with a flow rate of 5 mL/min. After the addition of ethanol, the flasks then were kept in a low-temperature thermostat bath (THD-1008 W, Ningbo Tianheng Instrument Factory) for 20 h. After that, the supernatants were collected and weighed. The contents of DSS, LA, RA, SaB, and HSYA and dry matter in the supernatants were determined.

2.4. Analytical methods

The concentrations of DSS, HSYA, RA, LA, and SaB were determined by high performance liquid chromatography analysis according to the method published in previous work [34]. The method is briefly described as follows. The HPLC system HP 1100 series (Agilent Technologies, Waldbronn, Germany) was equipped with a Chemstation software (Agilent Technologies). The separations were carried out at 30 °C on a ZORBAX Eclipse Plus C18

Table 1
Coded and uncoded values of parameters for central composite design.

Coded values	Uncoded values			
	X_1 (WCCE, %)	X_2 (CEA, g g^{-1})	X_3 (AEA, mL g^{-1})	X_4 (RT, °C)
−1.5	40.0	0.89	1.1	
−1	43.0	0.90	1.3	5.0
0	49.0	0.92	1.7	15.0
1	55.0	0.94	2.1	25.0
1.5	58.0	0.95	2.3	