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Application of quality by design concept to develop a dual gradient elution stability-indicating method for cloxacillin forced degradation studies using combined mixture-process variable models

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ABSTRACT

Penicillins are typical of complex ionic samples which likely contain large number of degradation-related impurities (DRIs) with different polarities and charge properties. It is often a challenge to develop selective and robust high performance liquid chromatography (HPLC) methods for the efficient separation of all DRIs. In this study, an analytical quality by design (AQbD) approach was proposed for stability-indicating method development of cloxacillin. The structures, retention and UV characteristics rules of penicillins and their impurities were summarized and served as useful prior knowledge. Through quality risk assessment and screen design, 3 critical process parameters (CPPs) were defined, including 2 mixture variables (MVs) and 1 process variable (PV). A combined mixture-process variable (MPV) design was conducted to evaluate the 3 CPPs simultaneously and a response surface methodology (RSM) was used to achieve the optimal experiment parameters. A dual gradient elution was performed to change buffer pH, mobile-phase type and strength simultaneously. The design spaces (DSs) was evaluated using Monte Carlo simulation to give their possibility of meeting the specifications of CQAs. A Plackett–Burman design was performed to test the robustness around the working points and to decide the normal operating ranges (NORs). Finally, validation was performed following International Conference on Harmonisation (ICH) guidelines. To our knowledge, this is the first study of using MPV design and dual gradient elution to develop HPLC methods and improve separations for complex ionic samples.

1. Introduction

A number of impurities can be produced because of active pharmaceutical ingredient (API) degradation or other interactions on storage. To detect and profile DRIs effectively, the analytical method must take a stability-indicating approach to allow a selective determination of related substances as required by pharmaceutical guidelines [1]. Ideally, the objectives of stability-indicating methods are resolving all DRIs from the parent and from each other, and detecting and accurately quantifying all DRIs. This is often a challenge for easily degradable drugs which can produce all kinds of DRIs. Thus, the development of a stability-indicating method for impurity determination requires an in-depth understanding of the method.

HPLC is still the main method recommended by different pharmacopeias to determine degradation impurities in drugs. Currently, chromatographic method development is largely performed by the traditional quality by testing (QbT) approach or a one-factor-at-a-time (OFAT) process [2]. Such development does not provide the ability to assess robustness throughout the development process or to carry out quality risk management when the method is transferred or the experiment variables change [3]. Recently pharmaceutical regulatory guidelines have stressed the critical importance of applying quality by design (QbD) principles for in-depth process understanding to ensure that product quality is built in by design [4]. In this context, the concepts of QbD transferred to analytical method development, known as AQbD, has been well adopted by regulatory authorities. AQbD is thought of as a tool for regulatory flexibility and robust analytics as it explores scientific understanding in method implementation sequences [5]. It ensures a controlled risk-based development of the methods where quality assurance will be guaranteed and represents an advantageous alternative approach to the QbT [6,7]. AQbD principles have been successfully applied to the development of HPLC [2,8–18], ultra high-performance liquid chromatography (UHPLC) [16,19–21] and capillary electrophoresis (CE) [22–24] methods. Much more effort is

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still needed to perfect its practices and expand its application areas in developing methods with different intended purposes, such as the stability-indicating method for easily degradable drugs [3].

Penicillins are such a class of antibiotics that are commonly used in clinics. These drugs are known to yield a formidable array of degradation products of significantly different polarity and charge property, complicating the determination and separation of them [25]. It is necessary to develop the stability-indicating methods for such complex samples within the AQbD framework. Cloxacillin, a typical drug of isoxazolyl penicillin group, was selected as an example to illustrate the approach and to guide the method development for the same class of drugs.

Design of experiments (DoE) is an important tool of AQbD. It represents the interactions among the input variables that ultimately affect the method response and results [8]. Experimental variables in the chromatographic system can be generally classified into 2 types: process variables and mixture variables. PVs are statistically independent and can be freely varied within the physical and chemical constraints of the system. MVs are not independent, since their sum must always be constant and equal to 100%, such as the constitution of the mobile phase [26]. When dealing with the optimization of stability-indicating methods, the separation performance likely depends on both the values of PVs and proportions of the MVs. Thus, it is necessary to apply a combined mixture-process variable model to optimize PVs and MVs simultaneously in one experiment. The MPV approach has been proposed for pharmaceutical applications [27,28]. It was implemented widely for developing the CE methods and developed to an integrated MPV-QbD approach by S. Orlandini et al. [22,29,30].

HPLC can claim a great flexibility because the high number of involved variables make it possible to finely modulate the separation performance of solutes. The most significant variables affecting separation selectivity are usually related to the characteristics of solutes. It is often a challenge to separate all solutes in a sample that contains a large number of ionic compounds. When the eluted compound has acid/base properties, changing pH, mobile-phase type and strength are usually the most effective ways to vary separation selectivity [31,32]. Gradient elution is also useful as the varying mobile-phase composition changes the ionization degree of ionic compounds and thus their retentions. In this article, a dual gradient elution approach was applied to improve the separations of DRIs in cloxacillin by changing mobile phase pH, organic phase type and strength simultaneously, providing a good example for its application in solving poor separations of complex ionic compounds.

The aim of this article is to propose a systematic stability-indicating method development approach for cloxacillin within the AQbD framework. To our knowledge, it is the first time to use the MPV-AQbD approach and the dual gradient elution method in the development of stability-indicating HPLC methods for complex ionic samples like cloxacillin.

2. Experimental

2.1. Chemicals and reagents

The reference standards of cloxacillin (Batch No. 130423–200903) were from the National Institutes for Food and Drug Control, RP China. Cloxacillin Sodium for Injection®, labeled to contain 0.5 g cloxacillin per vial, was provided by Lukang Pharmaceutical Group Co. Ltd (Shandong, China).

HPLC-grade acetonitrile (ACN) and methanol (MeOH) were obtained from Fisher Scientific (Fairlawn, NJ). Analytical-grade hydrochloric acid (HCl), acetic acid, sodium hydroxide (NaOH) and hydrogen peroxide (H_2O_2) were purchased from Beijing Chemical Works (Beijing, China). Buffer salts and all other chemicals were of an analytical reagent grade. A milli-Q water purification system (Millipore, Billerica, MA) was used to further purify the glass-distilled water.

2.2. Instrumentation and chromatographic conditions

The HPLC system was a Waters 2695 Alliance Separation Module (Waters, Milford, MA) interfaced with a Waters 2996 Photodiode Array (PDA) Detector and a Waters Empower 2 data acquisition and manipulation system.

The chromatographic method of the initial experiments included a Capcell Pak MGII C18 column, 5 μm, 250 mm x 4.6 mm id. (SHISEIDO, Tokyo, Japan). Column temperature was maintained at 30 °C. The mobile phase was ACN–20 mmol/L solution of potassium dihydrogen phosphate, pH 5.0 (25:75, v/v) at a flow rate of 1.0 mL min^{-1}. Phosphate buffer solution was adjusted to the required pH by adding dilute sodium hydroxide solution. The UV detection wavelength was at 225 nm and the UV spectra for each peak from 200 nm to 400 nm was collected to confirm identification when running the mixtures. The injected sample volume was 20 μL.

The final chromatographic method included a C18 column, 5 μm, 250 mm × 4.6 mm id. which has a similar selectivity with the reference column Capcell Pak MGII. Based on Snyder et al.'s work, the similarity parameter (F_s) between two equivalent columns should not be greater than 3.0 [33]. Mobile phases were: Mobile phase A (MPA): 20 mmol/L solution of potassium dihydrogen phosphate, pH 5.3-ACN-MeOH (66:14:20, v/v/v) and mobile phase B (MPB): 20 mmol/L solution of potassium dihydrogen phosphate, pH 6.9-ACN (50:50, v/v) at a flow rate of 1.0 mL min^{-1}. Gradient elution was performed as follows: 0–5 min, 100% A; 5–25 min, 100% A to 60% A; 25–33 min, 60% A to 20% A; 33–50 min, 20% A; 50–51 min, 20% A to 100% A; 51–65 min, 100% A. Other conditions, such as column temperature, detection and injected sample volume, were the same as the initial experiments.

2.3. Peak tracking

For complex samples, it is common to see peak order reverse in different experimental runs in a DoE study. Therefore, a peak tracking process must be carried out. Peak tracking refers to the matching of peaks for the same compound between different experimental runs. The peak identification was carried out based on the comprehensive analysis of peak areas, peak origins from different degradation conditions, the UV spectra characteristics [34], and also the retention rules of penicillin DRIs in the RP-LC system.

2.4. Forced degradation study

Forced degradation studies were carried out under acidic, base, hydrolytic, oxidative, photolytic, and thermal conditions as ICH Q1A (R2) recommendations [1]. A mixture of ACN:H_2O (50:50, v/v) was employed as a solvent for the preparation of stock solution of the drug. Each contained 4 mg/ml of the drug, and then was diluted with an equal volume of acid/alkali and H_2O_2. The samples were withdrawn at suitable time intervals and neutralized with acid/alkali and/or diluted two times with ACN:H_2O (50:50, v/v) before HPLC injection.

Photolytic studies were carried out by exposing the drug to UV light (365 nm) in a photostability chamber in both solid and solution states. The drug in a solid state was exposed as a thin layer in a Petri plate. Dark controls were kept in parallel for comparison. Thermal forced degradation testing was carried out by sealing the drug in glass ampoules and heating them in a thermostatic block at 85 °C for 24 h. The resultant sample was dissolved in ACN:H_2O