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Selection of reference standard during method development using the analytical hierarchy process

Wan-yang Sun [a], Ling Tong [b], Dong-xiang Li [b], Jing-yi Huang [c], Shui-ping Zhou [b], Henry Sun [b], Kai-shun Bi [a,*]

[a] School of Pharmacy, Shenyang Pharmaceutical University, Shenyang 110016, China
[b] Tasly R&D Institute, Tianjin Tasly Group Co., Ltd., Tianjin 300402, China
[c] China Pharmaceutical University, Nanjing 210009, China

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ABSTRACT

Reference standard is critical for ensuring reliable and accurate method performance. One important issue is how to select the ideal one from the alternatives. Unlike the optimization of parameters, the criteria of the reference standard are always immeasurable. The aim of this paper is to recommend a quantitative approach for the selection of reference standard during method development based on the analytical hierarchy process (AHP) as a decision-making tool. Six alternative single reference standards were assessed in quantitative analysis of six phenolic acids from *Salvia Miltiorrhiza* and its preparations by using ultra-performance liquid chromatography. The AHP model simultaneously considered six criteria related to reference standard characteristics and method performance, containing feasibility to obtain, abundance in samples, chemical stability, accuracy, precision and robustness. The priority of each alternative was calculated using standard AHP analysis method. The results showed that protocatechuic aldehyde is the ideal reference standard, and rosmarinic acid is about 79.8% ability as the second choice. The determination results successfully verified the evaluation ability of this model. The AHP allowed us comprehensive considering the benefits and risks of the alternatives. It was an effective and practical tool for optimization of reference standards during method development.

1. Introduction

Reference standard is widely used in absolute quantification analytical procedure. Unlike conventional external standard method, an increasing number of methods, such as the substitute standard method [1–3] and internal standard method [4,5], use them to calculate the contents of other analytes or to calibrate the variations of analytical procedures. In these situations, reference standard is critical for ensuring reliable and accurate method performance. However, no systematical evaluation method of reference standards has been developed. The selection of reference standard is still largely experience-based with more consideration of reference standard characteristics, and less of method performance [6,7]. We can only know whether the selected one is good enough until the end of the method validation. This increases the risk of method development, decreases the reliability of the analytical method, and restricts the discovery of better reference standards.

To the best of our knowledge, considering reference standard characteristics and method performance of alternative reference standards simultaneously during method development is necessary. Reference standard characteristics, such as similar structure, similar physical and chemical properties, purity and stability, are essential to a reference standard. What's more, method performances like precision, accuracy and robustness are pivotal criteria of a reference standard. However, these two kinds of criteria are difficult to compare simultaneously because the criteria are measured in different units, while some of them are even immeasurable. To solve this issue, we proposed a commonly applied multi-criteria decision analysis method, i.e., the analytical hierarchy process (AHP) [8]. The AHP is a theory of measurement through pairwise comparisons and relies on the judgements of experts to derive priority scales for each alternative. It can facilitate decision-making in complex situations involving tradeoffs by explicitly considering the benefits and risks of alternatives [9], and is widely applied in problems involving priority setting and selection among alternatives [10,11].

* Corresponding author.Tel.: +86 24 23986012; fax: +86 24 23986012.
E-mail address: kaishunbi.syphu@gmail.com (K.-s. Bi).

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In this study, an AHP model for the selection of ideal reference standard during method development was established. As a typically reference standard scarce and chemically unstable herbal medicine [12,13], *Salvia Miltiorrhiza* Radix et Rhizoma (Danshen) and its preparations were selected as an example. We aimed at developing a single standard quantification method that simultaneously determining six phenolic acids using only one reference standard and their relative response factors. Each analyte was treated as alternative single reference standard. The AHP model simultaneously contained the criteria of feasibility to obtain, abundance in samples, chemical stability, accuracy, precision and robustness according to the requirement of this method. Ideal reference standard was selected using the priorities obtained by the AHP model. Determination results obtained using the selected single reference standard were verified on three analytical systems and compared with those obtained using external standard method.

2. Experimental

2.1. Chemicals, reagents and materials

Danshensu (DSS), protocatechuic aldehyde (PAL), rosmarinic acid (RA), salvianolic acid B (Sal B) were obtained from National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China); lithospermic acid (LA) and salvianolic acid A (Sal A) were purchased from Yifang Technology Co. Ltd. (Tianjin, China). The structures of the standard compounds together with their UV spectra are presented in Fig. 1.

HPLC grade acetonitrile was from Merck (Darmstadt, Germany). HPLC grade formic acid (>95% pure) was from Sigma (Saint Louis, MO, USA). High purity deionized water was purified by Milli-Q system (Bedford, MA, USA).

Salvia Miltiorrhiza Radix et Rhizoma (SM) samples assigned as S01-S19 were collected from several provinces in China. Voucher specimens were deposited at Tasly Institute. Cardiotonic pill (CP) assigned as S20-S36 were provided by Tasly Pharmaceutical Co. Ltd (Tianjin, China). Fufang Danshen tablet (FDT) samples assigned as S37-S49 were purchased from pharmacies in several cities in China.

2.2. Chromatography analysis

Analysis was primarily performed by a Waters ultra performance liquid chromatography (UPLC) System, comprised a binary solvent delivery pump, an on-line degassers, an auto-samplers, a column temperature controller and a photodiode array detector coupled with an analytical workstation (Empower 3 software) (Waters Corp, Milford, MA, USA). Two additional UPLC instruments were used. One was Waters H-Class System equipped a tunable UV detector, another was a Shimadzu LC-30 System equipped a photodiode array detector (Shimadzu Corp, Tokyo, JP). Samples were primarily separated at 30 ± 0.1 °C on a Waters Acquity UPLC® BEH C18 column (1.7 μm particles, 2.1 mm i.d. × 100 mm) coupled with a Waters Acquity UPLC® BEH C18 guard column (1.7 μm particles, 2.1 mm i.d. × 5 mm). The mobile phase was composed of (A) aqueous formic acid (0.2%, v/v) and (B) acetonitrile using a gradient elution of 8% B at 0 min, 12% B at 1 min, 17% B at 3 min, 20% B at 6 min, 23% B at 8 min and 27% B at 10 min. The flow rate was 0.4 mL/min, and the column temperature was maintained at 30 °C. The photodiode array detector was operated at 281 nm and 329 nm with 1.2 nm bandwidth and no reference wavelength. The sample injection volume was 3 μL.

2.3. Preparation of solution

2.3.1. Preparation of standard solutions and system test samples

Stock solutions of DSS, PAL, RA, LA, Sal B and Sal A were prepared and diluted to about 50 μg/mL in 70% methanol. The mixed stock solution of six analyte was prepared by dissolving the reference standards (517.3 μg/mL for DSS, 222.5 μg/mL for PAL, 542.0 μg/mL for RA, 470.7 μg/mL for LA, 499.0 μg/mL for Sal B, 495.2 μg/mL for Sal A) in 70% methanol. Reference testing samples at three concentrations were prepared by diluting mixed stock solution with 70% methanol (dilution factor = 5, 20, 100).

2.3.2. Preparation of sample solutions

Powdered SM samples of 0.25 g was accurately weighted and sonicated in 50 mL of 70% methanol for 30 min. For FDTs, ten tablets of FDT were crushed into fine powder in a mortar after the sugar coating was scraped off. The FDT powder (0.25 g) and CPs (25 pills) were accurately weighted and sonicated in 25 mL of 70% methanol for 30 min, which was performed on KQ-500DE ultrasonic bath (300 W, 50 kHz) (Shanghai, China). After cooling down to room temperature, supernatant was collected and filtered through a membrane filter (0.22 μm) for further analysis. All the solutions were stored in refrigerator prior to analysis.

2.4. Evaluation of reference standard

All the six phenolic acids were treated as the alternative reference standards because of the similar structure, similar physical and chemical properties. Critical reference standard characteristics and method performance of each reference standard were investigated.

2.4.1. Evaluation of reference standard characteristics

Feasible to obtain, abundance in samples and chemical stability of each reference standard were investigated. The characteristic of feasible to obtain was reflected by chemical purity and price. These informations were gathered from the specifications and vendors of the reference standards. Information of abundance in sample was summarized from SM related literatures. Chemical stability of each reference standards was measured by the peak areas of single stock solutions at 0 h, 2 h, 4 h, 6 h, 8 h, 10 h, 12 h, 24 h and 48 h, and described as relative standard deviation (R.S.D.).

2.4.2. Evaluation of method performance

To reflect the method performance during method development, preliminary relative response factor (F_x) between the single reference standard and analyte was obtained using a reference testing sample (dilution factor = 20) and calculated as followed:

$$F_x = \frac{C_s \cdot A_x}{A_s \cdot C_x} \quad (1)$$

where A_s and A_x are the peak areas, C_s and C_x are the concentrations of the single reference standard and analyte. And the concentration of each analyte was calculated as followed:

$$C_x = \frac{F_x \cdot C_s \cdot A_x}{A_s} \quad (2)$$

Accuracy of each reference standard was evaluated by assaying five replicates of reference testing samples at low, medium and high concentrations. Results were described as relative errors (RE) of the total content between determined and original. Precision and robustness was evaluated by assaying a reference testing samples at middle concentration. In the precision test, six columns were investigated using three instruments in turns. Detailed informations of columns were shown in Table S1. In the robustness test,