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Monitoring batch-to-batch reproducibility using direct analysis in real time mass spectrometry and multivariate analysis: A case study on precipitation

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ABSTRACT

Batch-to-batch variability of traditional Chinese medicine (TCM) has already heavily limited its industrial development. By utilizing direct analysis in real time mass spectrometry (DART-MS) and multivariate statistical analysis, the present study introduced a novel and rapid methodology for TCM manufacturing process monitoring. *Danshen* alkaline precipitation was employed as an example operating unit to demonstrate the effectiveness of this new technique. A total of 15 batches run under normal operating conditions were used to develop a reference principal component analysis model and then enabled the establishment of multivariate control charts. Hotteling T^2 and DModX charts were applied to examine batch-to-batch reproducibility of 12 test batches. Artificial variations including starting material change and process fault were identified, which was in a good agreement with conventional analysis result. Further understanding of the deviating process behavior was achieved by investigating the contribution plot of abnormal batches. The results showed that DART-MS, in conjunction with multivariate analysis, provided valuable information on model process and gave us a new perspective for TCM manufacturing process monitoring.

1. Introduction

Traditional Chinese medicine (TCM) has the unique characteristics of deriving from botanical material and preparing as complex mixture. The manufacture of TCM is usually performed in batch mode, but batch-to-batch variability in drug constituents has already heavily limited its industrial development [1]. On the other hand, although TCM has been clinically demonstrated effective for thousand years, neither its active constituents nor its biological activities are completely characterized [2]. Therefore, contrast to that of synthetic or highly purified drug, batch-to-batch consistency of TCM is especially difficult to be maintained by measuring the product with a single analytical mean. In June of 2004, U.S. Food and Drug Administration addressed this issue through the "Guidance for Industry: Botanical Drug Products". In order to ensure the consistently good quality of botanical drug, combination use of multiple tests was recommended for drug substance and finished product. These multiple analytical methods included chemical identification by spectroscopic and/or chromatographic fingerprint, chemical assay of characteristic markers, biological assay and so on [3].

Ambient desorption/ionization mass spectrometry (MS), as a new technique for fingerprint profiling, has a reputation for its remarkable feature of direct analysis of complex samples. Ambient MS techniques enable: (1) surface ionization in the open environment, (2) determination of sample with any size and shape, (3) high-throughput analysis with minimal sample pre-treatment [4–6]. Since the breakthrough invention of desorption electrospray ionization (DESI) [7], a variety of ambient ionization methods have been reported [8,9]. According to the dominant desorption and ionization approach, these ambient techniques are generally classified into three categories: (1) spray-based, (2) electric discharge-based and (3) gas-, heat- or laser-assisted [10]. In brief, the ionization processes in the spray-based techniques are as follows: reagent is firstly ionized through spraying, and the charge and energy of ionized reagent are then transferred to the analytes. The primary ions in the electric discharge-based techniques, on the other hand, are usually generated through corona discharge, plasma discharge or glow discharge. In the third subcategory, the samples are directly ionized or matrix-assisted ionized by gas, heat or laser. Direct analysis in real time (DART), the one most prevalently studied in the electric discharge-based subset, allows instantaneous ionization of small molecular species with a wide range of polarities [11]. DART is a glow discharge plasma ion source. The gas exiting its discharge chamber directly flows into a second chamber containing a perforated disk electrode, a gas heater and a grid electrode, and finally into mass spectrometer. Ionization happens when the metastable gas meets sample in the gap between DART outlet and

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mass spectrometer orifice [12,13]. The main advantage of DART-MS, compared with other ambient ionization techniques, lies in that it is not necessary to expose the analyte to high voltage, laser or radiation.

Coupled with various conventional mass spectrometers, DART was employed by some researchers for characterization of tomato and pepper fruit [14], oak wood [15], arabidopsis seed [16], betel leaf [17], hairy root [18] and umbelliferae root [19]. These studies demonstrated successful applications of DART-MS for fingerprint analysis of herbal products. However, there were still few reports on herbal medicine, especially on TCM. Recently, we published our study on a famous Chinese herbal preparation (*Danshen* injection) [20]. Fingerprint analysis was carried out on its final product and then analytical markers of *Danshen injection* were identified with chemometric method. In the current work, the capacity of DART-MS will be further evaluated for monitoring batch-to-batch reproducibility of herbal drug substance. For the purpose of batch monitoring during TCM manufacture, some attempts have been made using near-infrared (NIR) spectroscopy [21,22]. Comprehensive sample information is provided by NIR, but it is worth noting that the technique lacks specificity. Alternatively, DART-MS fingerprint can offer a high specificity through characteristic mass-to-charge ratio (m/z).

Routine approach for batch process monitoring relies on a statistical model, which is established on normal operating batches. New batch is then compared with the model to investigate batch-to-batch consistency or variation [23]. However, batch process typically displays nonlinear behavior and does not operate at a steady state [24]. These characteristics make the resulting spectral data problematic in collinearity and noise. Classical univariate analysis methods are insufficient to deal with such a dataset. Instead, the multivariate statistical method would be effective: one or two latent variables are firstly extracted and then enable the specification of control limit [25]. By means of plotting, batch monitoring result is able to be conveniently visualized through multivariate control charts. In industry, three kinds of multivariate control charts (score, Hotelling T^2 and DModX chart) are popular in batch performance monitoring [26–28].

Chinese herbal injection is a relatively new sort of TCM, whose manufacture usually consists of several operation units. Among these operations, pH adjustment is frequently used to separate impurity from drug substance. Alkaline precipitation is such a purification unit: by treating herbal extract with alkaline reagent, base-insoluble impurity tannin polymers are removed. The removal of tannin is of utmost importance, since the interaction between tannin and plasma protein will cause significant adverse reaction [29,30]. However, the processing of alkaline precipitation in practice is generally based on the operator's experience, which varies from one individual to another. Changes of input materials and operating conditions finally lead to undesired batch-to-batch variation. In order to improve batch-to-batch consistency of alkaline precipitation, it is necessary to develop a feasible approach for process monitoring.

Danshen injection is a common cardiovascular-protecting Chinese herbal injection. It is produced from the root of *Salvia miltiorrhiza* (Chinese *Danshen*) and administered to patients via vessels or muscles. On the basis of previous studies, phenolic acids including salvianic acid A, protocatechuic aldehyde, caffeic acid and salvianolic acid B were proposed as the main bioactive constituents in *Danshen injection* [31]. However, little is known about its manufacture. Especially, there were few reports on alkaline precipitation of *Danshen injection* yet. Therefore, the alkaline precipitation of *Danshen injection* was selected as the model batch process in this study to examine the potential of DART-MS for batch monitoring.

Table 1
Experimental runs.

Batch No.	Batch information	pH[a]	Temperature[b] (°C)	Stir[c]
A1–A15	Reference, normal	8.5	10	Yes
B1–B3	Test, normal	8.5	10	Yes
C1–C3	Test, abnormal	7.0	10	Yes
D1–D3	Test, abnormal	8.5	10	No
E1–E3	Test, abnormal	8.5	30	Yes

[a] pH value of alcohol solution immediately after adding NaOH.
[b] Temperature during the 48 h standing procedure.
[c] Stir or not while adding NaOH.

2. Materials and methods

2.1. Chemicals and samples

The alcohol solution used for alkaline precipitation was obtained after reconstituting the concentrated alcohol solution of *Danshen* with 95% EtOH at a 1:4 (w/w) ratio, with the assistance of stirring as needed. The concentrated *Danshen* alcohol solution was supplied by Chiatai Qingchunbao Pharmaceutical Co., Ltd. (Zhejiang, China). Sodium hydroxide and diluted hydrochloric acid was purchased from Er-kang Pharmaceutical Co., Ltd. (Hunan, China). Glucose and fructose were supplied by Haotian Biotechnology Co., Ltd. (Heilongjiang, China), all with the purity >99.0%. Standard substances including salvianic acid A, caffeic acid and rosmarinic acid were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China), all with the purity not less than 98.0%. Protocatechuic aldehyde (purity > 98.0%), salvianolic acid A (purity > 97.0%) and salvianolic acid B (purity > 95.0%) from Tauto Biotech Co., Ltd. (Shanghai, China) were used. HPLC-grade formic acid and acetonitrile were purchased from Tedia (OH, USA) and Merck (Darmstadt, Germany). Water for HPLC analysis was prepared by a Milli-Q purification system (MA, USA).

A total of 27 batches of alkaline precipitation samples were prepared. These samples were divided into two groups. The first group was composed by 15 samples (batches A1–A15) prepared under normal operating conditions and taken as reference group. These samples were prepared as following: the alcohol solution of *Danshen* (55.0 mL) was placed into a glass bottle with a volume of 70 mL. The magnetic stirrer worked consistently while manually adding 35% NaOH into the alcohol solution, and the pH value of alcohol solution after NaOH addition was controlled around 8.5. The solution was allowed to stand for 20 min with stirring and then for 48 h at 10 °C without stirring. After that, the supernatant was withdrawn for pH adjustment to 3.0 with diluted hydrochloric acid. The second group including 12 batches was taken as the test group: batches B1–B3 were prepared in the same way as batches in the reference group; others had changes, and the alterations were present in Table 1. Samples for DART-MS and HPLC–UV method were centrifuged at 10,000 rpm for 10 min prior to analysis. Samples for HPLC–ELSD analysis were further treated by acetonitrile (1:3, v/v) to remove large molecules such as polysaccharide and protein.

2.2. DART-MS analysis

IonSense DART-SVP ionization source (MA, USA) under negative ion mode was employed for sample ionization. The grid electrode was set as +50 V, and the distance between ion source and MS orifice as 15 mm. The pressure and temperature of high-purity argon (Ar) were 0.17 MPa and 400 °C, respectively. Samples for DART analysis were introduced automatically with an IonSense Dip-it sampler (MA, USA), at a constant speed of 0.2 mm s^{-1}. The ion source was equipped with an Applied Biosystems/MDS SCIEX API4000 triple–quadrupole MS (CA, USA). The declustering and