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Direct analysis in real time mass spectrometry, a process analytical technology tool for real-time process monitoring in botanical drug manufacturing

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ABSTRACT

A promising process analytical technology (PAT) tool has been introduced for batch processes monitoring. Direct analysis in real time mass spectrometry (DART-MS), a means of rapid fingerprint analysis, was applied to a percolation process with multi-constituent substances for an anti-cancer botanical preparation. Fifteen batches were carried out, including ten normal operations and five abnormal batches with artificial variations. The obtained multivariate data were analyzed by a multi-way partial least squares (MPLS) model. Control trajectories were derived from eight normal batches, and the qualification was tested by R^2 and Q^2. Accuracy and diagnosis capability of the batch model were then validated by the remaining batches. Assisted with high performance liquid chromatography (HPLC) determination, process faults were explained by corresponding variable contributions. Furthermore, a batch level model was developed to compare and assess the model performance. The present study has demonstrated that DART-MS is very promising in process monitoring in botanical manufacturing. Compared with general PAT tools, DART-MS offers a particular account on effective compositions and can be potentially used to improve batch quality and process consistency of samples in complex matrices.

1. Introduction

Process analytical technology (PAT), presented by the United States Food and Drug Administration (USFDA), is the use of analytical techniques for real-time process characterization in the pharmaceutical industries [1]. Meanwhile, botanical drugs are well-known for their complicated multi-compound, multi-ingredient formulation and preparations, and the large quality variability associated with the products made by different manufacturers or different batches by the same manufacture, thus the process monitoring in botanical manufacturing has been a challenging topic for many years. Near-infrared (NIR) spectrometry, a general PAT tool, has been commonly used for multi-composition non-destructive analysis and real-time monitoring of botanical manufacturing processes [2]. However, little is provided of chemical composition by spectroscopic assessments with absorption spectrogram of characteristic functional groups, which remains one of the barriers in spectrometry application to qualitative and quantitative determination of botanical drugs [3].

Recent advances in ambient ionization technology have opened up the opportunity of mass spectrometry (MS) to be an innovative PAT tool in botanical manufacturing [4,5]. Direct analysis in real time (DART), developed by Cody, is a versatile, emerging ambient ion source undergoing rapid development. The ionization mechanism of DART is based on the reactions of electronic or excited-state species with carrier gas and polar or non-polar analytes [6]. Recent studies have presented direct analysis in real time mass spectrometry (DART-MS) as a sample preparation-free MS analysis tool for the monitoring of ongoing organic chemical reactions, or control of reaction kinetics [7]. Because its response is instantaneous, DART-MS exhibits great potential for real-time information [8]. Furthermore, since the molecular weight of species is directly measured and most chemical compounds can be ionized [9], DART-MS is especially sensitive in acquiring multi-constituent spatial information from a given sample in complex matrix [10,11]. These features offer great flexibilities in the coupling or integration of dynamic systems to DART-MS, in order to determine the physical and chemical composition and properties of the desired industrial pharmaceuticals during a manufacturing process [12,13].

Batch control on key manufacturing processes, aimed at delivering consistent products with intended performance, occupies a decisive position in guaranteeing the quality and stability of natural medicines [14]. Percolation extraction, well suited for the purification of heat-sensitive substances, has gained wide acceptance

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Table 1
Overview of the design of experiments and batch information introduced to the batch process model of percolation.

Batch	Particle size (mm)	Flow rate (mL/min)	Acetic acid (%)	Remarks
1–10	0.71–0.20	0.4	1	Normal operated batches
11	1.25–0.71	0.4	1	Raw material variation (powder size)
12	0.71–0.20	1.0	1	Operation condition variation (flow rate)
13	0.71–0.20	0.4	5	Operation condition variation (HAc percentage in solvent)
14	0.71–0.20	0.4	1	Process upset (solvent was HAc of 5% during 0–5 h)
15	0.71–0.20	0.4	1	Process upset (flow rate was 1.0 mL/min during 11–12 h)

for various preparations of herbs [15]. According to the Chinese National Drug Standards [16] and practical production data, the percolation process yields the majority of bioactive constituents from raw materials, and it is undoubtedly the first pivotal step during many herbal productions. However, the whole percolation process often takes about 60 h, so the traditional analytical approaches on the final products require a too long turnaround time to accommodate any needed process adjustments, and thus are uneconomical and inefficient. Thereby, batch process control and real-time quality verification during the percolation process are still far from perfection in pharmaceutical industries.

Alkaloid drugs of *Sophora Flavescens* Ait., a type of alkaloid with similar chemical structures and proven anti-tumor/anti-cancer activity, are the main antineoplastic herbal agents used in clinical practice [17,18]. Combined with radiation and chemotherapy, these medicines are effective in relieving the pain and improving life qualities of terminal cancer patients [19]. In this study, the potentials of DART-MS in improving the batch quality and process consistency of products that contain a myriad of compounds in complex matrices is demonstrated. A case study has been carried out by investigating a percolation process during the production of an anti-cancer alkaloid. MS spectra are collected during the batch processes with fixed time intervals. Control trajectories, generated from multivariate batch analysis, are used to identify and predict the batch abnormalities during the process evolution.

2. Materials and methods

2.1. Materials

Dried medical materials of *Radix Sophorae Flavescentis* (RSF) and *Heterosmilax Japonica Kunth* (HJK) were provided by Shanxi Zhendong Pharmaceutical Co. Ltd. (Shanxi, China). Standard reagents, including sophocarpine, matrine, oxysophocarpine, and oxymatrine, were received from the National Institutes for Food and Drug Control (Beijing, China). D-Glucose and D-fructose were purchased from Sigma–Aldrich (Missouri, USA). Sodium 1-pentanesulfonate monohydrate ($C_5H_{11}SO_3Na{\cdot}H_2O$) of guaranteed grade was purchased from J & K Chemical Ltd. (Shanghai, China). HPLC-grade acetic acid (HAc) and acetonitrile were obtained from Merck (Darmstadt, Germany), and analytical grade phosphoric acid (H_3PO_4) was from Shanghai Lingfeng Chemical Reagent (Shanghai, China). Ultrapure water was produced in the laboratory with a Milli-Q water purification system (Molsheim, France).

2.2. Percolation process and design of experiments

Adapted from the regulations published in Chinese Pharmacopeia (2010 edition) [20] and Drug Standards of China, 1% aqueous solution of HAc was selected as the extraction solvent throughout the whole procedure, and the particle size of dried medical materials was screened in the range of 0.20–0.71 mm. Before each percolation process, a dried powder (100 g, RSF: HJK = 7: 3 w/w) was soaked in the HAc solution in a volume 4 times of the powder for 48 h. Normal operation conditions of the percolation processes were as follows: sufficiently soaked materials were dynamically extracted by the HAc aqueous solution with a flow rate of 0.40 mL per min, until the effective constituents were completely extracted out. During the proceeding of the percolation processes, sampling analysis was performed hourly. To compare the trajectories of tested batches, batch lengths were aligned to duration of 28 h.

Previous studies have reported that, for a satisfactory percolation performance, the flow rate of solvent and particle size of medical materials should be strictly controlled during the process [21,22]. Since alkaloids were the primary compounds investigated in this work, acid concentration of the solvent was studied as well [23]. All the designed experiments are summarized in Table 1. The batch process study included ten normal batches, along with five abnormal batches with variations on raw materials (batch 11), operation conditions (batches 12 and 13), and intentional process upsets (batches 14 and 15).

2.3. DART-MS analysis

The adopted DART-SVP ionization source (IonSense, MA, USA) was interfaced to a triple-quadrupole mass spectrometer (MDS SCIEX API 4000, Applied Biosystems, CA, USA). High-purity argon (Ar) was used as the run gas, and high-purity nitrogen (N_2) was the standby gas. During the sample analysis, Ar was heated to 450 °C and transferred under the pressure of 0.17 MPa, to form a hot gas stream for thermo-desorption of analytes. Sample coated glass tubes were placed on the dip-it sampler (IonSense, MA, USA), and passed through the gas beam at a speed of 0.20 mm s^{-1}. The samples were positioned away from the DART outlet with a distance of 15 mm. Grid electrode at the DART exit gun was +100 V. MS was adjusted to the positive ion mode with selected ion monitoring (SIM). Operations of DART and MS were controlled by DART v.3.0.3b and Analyst 1.5.0 software, respectively. Taking the uncertainty of a single measurement on DART-MS into account, three replicates on each sample were performed, and their mean value was recorded.

2.4. HPLC analysis

High performance liquid chromatography (HPLC), a classical assay method, was utilized to evaluate the DART-MS analysis. The chromatographic system used in this study was an Agilent 1100 HPLC system (Agilent Technologies, California, USA), which consisted of a vacuum degasser, a quaternary pump, an autosampler, a column heater–cooler, an ultraviolet detector and the ChemStation software. The analytical column used was a Diamond C_{18} column (5.0 μm, 4.6 mm × 250 mm, Diamond, Beijing, China) coupled with an Agilent C_{18} pre-column (5.0 μm, 4.0 mm × 10 mm). Solvent A was aqueous solution of 0.040% phosphoric acid and 10 mmol L^{-1} sodium 1-pentanesulfonate, and solvent B was pure acetonitrile. The flow rate was 1.2 mL/min and the column temperature was set at 30 °C. The solvent gradient was as follows: 0–35 min, 3–7% B; 35–50 min, 7% B; 50–60 min, 7–10% B; and 60–70 min, 10% B. The UV detection wavelength was 210 nm. Four alkaloids in the sample, including sophocarpine, matrine,