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Q-marker based strategy for CMC research of Chinese medicine: A case study of *Panax Notoginseng* saponins

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ABSTRACT

Background: To ensure pharmaceutical quality, chemistry, manufacturing and control (CMC) research is essential. However, due to the inherent complexity of Chinese medicine (CM), CMC study of CM remains a great challenge for academia, industry, and regulatory agencies. Recently, quality-marker (Q-marker) was proposed to establish quality standards or quality analysis approaches of Chinese medicine, which sheds a light on Chinese medicine's CMC study.

Purpose: Here manufacture processes of *Panax Notoginseng Saponins (PNS)* is taken as a case study and the present work is to establish a Q-marker based research strategy for CMC of Chinese medicine.

Study Design: The Q-markers of *Panax Notoginseng Saponins (PNS)* is selected and established by integrating chemical profile with pharmacological activities. Then, the key processes of *PNS* manufacturing are identified by material flow analysis. Furthermore, modeling algorithms are employed to explore the relationship between Q-markers and critical process parameters (CPPs) of the key processes. At last, CPPs of the key processes are optimized in order to improving the process efficiency.

Results: Among the 97 identified compounds, Notoginsenoside R_1, ginsenoside Rg_1, Re, Rb_1 and Rd are selected as the Q-markers of *PNS*. Our analysis on PNS manufacturing show the extraction process and column chromatography process are the key processes. With the CPPs of each process as the inputs and Q-markers' contents as the outputs, two process prediction models are built separately for the extraction process and column chromatography process of *Panax notoginseng*, which both possess good prediction ability. Based on the efficiency models of extraction process and column chromatography process we constructed, the optimal CPPs of both processes are calculated.

Conclusion: Our results show that the Q-markers derived from CMC research strategy can be applied to analyze the manufacturing processes of Chinese medicine to assure product's quality and promote key processes' efficiency simultaneously.

1. Introduction

Ensuring the quality of Chinese medicine has never been more urgent and more complicated, although CM has historically been practiced in China for thousands of years and it still significantly contributes to current healthcare system in Eastern Asian countries, as well as impacting people's daily lives in the West. In 2016, the total revenue of CM is reported to reach 865.3 B RMB, representing a significant percentage, ~30%, of pharmaceutical industry in China (Data source: Ministry of Industry and Information Technology of the People's Republic of China). Despite of being a critical scientific component of the pharmaceutical quality (Fan et al., 2006; Zhang et al., 2013; Duan et al., 2012), chemistry, manufacturing and control (CMC) of CM has long been underappreciated and underfunded. Decades of research yields few demonstrable successes on CM, which is partly due to the inherent complexity of both chemical compositions and pharmacological mechanisms. Its further modernization and globalization have been significantly hampered (Yang et al., 2017a).

Recently, to promote and develop CM globally, the concept of Q-marker was proposed (Guo, 2017; Liu et al., 2017, 2016). Q-marker can be the intrinsic chemical substances existing in the raw materials or processing/preparation-resultant ones found in the Chinese medicinal

Abbreviations: Q-marker, quality-marker; CM, Chinese medicine; *PNS*, *Panax Notoginseng Saponins*; CMC, chemistry, manufacturing and control; CQA, critical quality attribute; CPP, critical process parameter; ANN, artificial neutral network; BP-ANN, back propagating artificial neutral network; CV, cross validation; PSO, particle swarm optimization; GA, genetic algorithm; NSGA, non-dominated sorting genetic algorithm; RMSE, root mean square error; RSD, relative standard deviation; HPLC, high performance liquid chromatography

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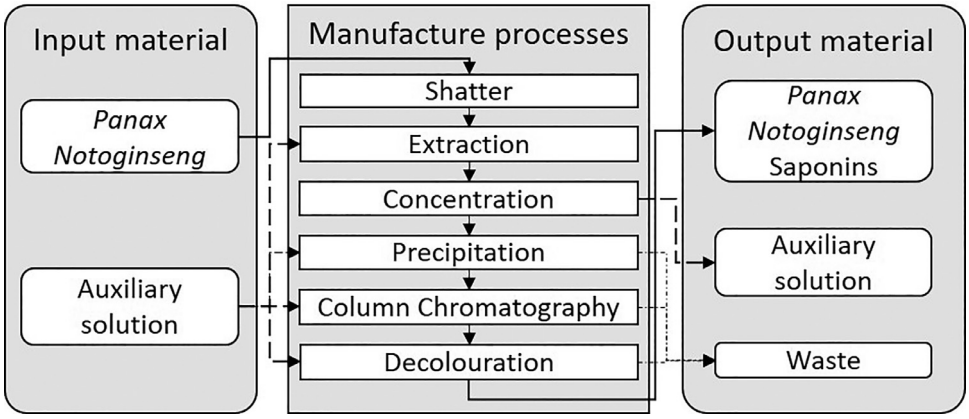

Fig. 1. The illustration of material flow in *Panax Notoginseng* total saponins manufacture processes.

products, which are closely related with the efficacy or safety of CM and can be qualitatively and quantitatively analyzed (Liu et al., 2016; Yang et al., 2017a). However, according to the methodology of "quality by test", mainstream quality control strategy of CM is established and many years of industrial practices have proven adequate assurance of products' quality cannot be achieved through corresponding approaches (Li et al., 2015). With this background, we designed and proposed a CMC technical framework for CM, which aims to control the CM quality during key manufacture processes (Cheng et al., 2017). Based on the definition, Q-markers can be deemed as the core portion of critical quality attributes (CQAs), and can therefore be used as the indicators of key processes identification, process modeling and CPPs optimization.

In present work, a Q-marker based strategy for Chinese medicine CMC study is proposed, which consists by Q-markers establishment, key processes identification, modeling between CPPs and Q-markers, as well as CPPs optimization. As a proof-of-concept, this strategy is applied to the manufacture processes of *Panax Notoginseng Saponins (PNS)* that are used to produce various formulations of Chinese medicinal products, such as Xuesaitong Injection, Xuesaitong Capsule, etc (Wang et al., 2013, 2014; Zhu et al., 2014; Dai et al., 2017). The raw material for manufacturing *PNS* is *Panax Notoginseng*, which are the dry roots and rhizomes of *Panax notoginseng* (Burk.) F.H. Chen (Araliaceae).

2. Material and methods

2.1. Reagents and instruments

Chemical standards of notoginsenoside R_1, ginsenoside Rg_1, Re, Rb_1 and Rd were purchased from Shanghai Winherb Medical Technology Co., Ltd (Shanghai, China). HPLC grade acetonitrile was purchased from Merck (Darmstadt, Germany). Distilled water was purified by Milli-Q system (Millipore). The other chemicals were of analytical grade.

The HPLC system used in this study was Agilent 1100 instrument (Agilent Technologies, USA), consisting of a quaternary solvent delivery system, an auto-sampler, an on-line degasser, a column temperature controller and a ultraviolet detector.

2.2. Q-marker establishment

According to the definition of Q-marker(Liu et al., 2016; Yang et al., 2017a), several basic properties of Q-marker can be listed: (1) Q-markers are the intrinsic or derived chemical components in Chinese medicinal materials and products; (2) Q-markers are closely related with the efficacy or safety of products; (3) Q-markers have definite chemical structure and can be qualitatively characterized and quantitatively determined (Liu et al., 2016; Yang et al., 2017a).

In our previous study (Yang et al., 2017b), we developed a method to identify a group of chemo-markers whose overall pharmacological activities are comparable to the original Chinese medicine. Using this method (Adjusted Efficacy Score), five saponins (notoginsenoside R1, ginsenoside Rg1, Re, Rb1 and Rd) was identified from 97 compounds as bioactive chemical markers of *PNS* on treating cardiovascular and cerebrovascular diseases. Apprearently, these five saponins (notoginsenoside R1, ginsenoside Rg1, Re, Rb1 and Rd) match key features required by Q-marker and can therefore be taken as Q-markers of PNS during the subsequent case study. Moreover, an HPLC method (Zhu et al., 2014; Yang et al., 2017c) by HPLC system of Agilent 1100 instrument (Agilent Technologies, USA) was adopted to measure the contents of saponins. An Agilent Zorbax C18 column (66 × 50 × 4.6 mm, 1.8 μm) (Agilent, USA) is used to perform chromatographic separation. Flow rate is 0.8 ml/min, detection wavelength is 203 nm, column temperature is 35 °C, injection volume is 3 μl. Mobile phases are water (solvent A) and acetonitrile (solvent B). The elution conditions are: 0–22 min, 17–19% B; 22–30 min, 19–27% B; 30–35 min, 27% B; 35–47 min, 27–46% B; 47–70 min, 46–90% B.

2.3. Key processes identification

In present work, key processes of *PNS* manufacturing are identified through material flow analysis. Material flow analysis is an analytical method to qualitatively or quantitatively describe the transition, transform and mixture of materials during the whole system. Specific to current study, material flow analysis (Rotter et al., 2004; Schandl and Schaffartzik, 2015) is used to quantitatively describe the transition of Q-markers and impurities in each process of *PNS* manufacturing, evaluate precision in each process and its abilities to retain Q-markers while remove impurities.

2.3.1. The material flow in PNS manufacture processes

Common *PNS* manufacture processes are consisted of shatter, extraction, concentration, water precipitation, column chromatography and decolouration. The material flow in *PNS* manufacture processes is shown in Fig. 1.

2.3.2. Medicinal materials

Information about *Panax Notoginseng* is listed in Table 1.

2.3.3. Q-marker content measurement

Notoginsenoside R_1, ginsenoside Rg_1, Re, Rb_1 and Rd contents are measured using the HPLC method described in section of Q-marker establishment.

2.3.4. Impurities content measurement

In this study, a quantitative method is used to approximate the