Review

Recent advances in the compound-oriented and pattern-oriented approaches to the quality control of herbal medicines

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Abstract

The current approaches to the quality control of herbal medicines are either compound-oriented or pattern-oriented, the former targeting specific components with some known chemical properties and the latter targeting all detectable components. The marker approach uses specific chemical compounds with known molecular structures, while the multi-compound approach uses both chemical compounds with known structures and those with partial chemical information e.g. retention times, mass spectra and ultraviolet spectra. Apart from chromatographic techniques, new techniques such as oscillating and electrochemistry fingerprints have been developed for quality control. Chemometric resolution methods are widely used for component deconvolution and data comparison. Pattern recognition techniques are used for authentication of herbal medicines.

Background

High chemical complexity of herbal medicines makes quality control through chemical analysis difficult. For example, a common Chinese medicinal herb *Cortex Cinnamomi* (*Rougui*) revealed over 90 volatile components in a gas chromatography-mass spectroscopy (GC-MS) experiment, only 60 of which were chemically identified [1]. Little chemical information is known about herbal medicines [2]. Mok and Chau categorized authentication and quality control of herbal medicines into the 'component-based' and 'pattern-based' approaches [3]. For a refined classification, we refer to these two approaches in this article as the compound-oriented approach and pattern-oriented approach respectively. The compound-oriented approach includes the marker approach and the multi-compound approach. The marker approach takes into account herbal medicines with known components, while the multi-compound approach may include some unknown components whose chemical properties are known. The pattern-oriented approach, such as fingerprint analysis, evaluates all data acquired from analytical instrument. For example, fingerprints and bioactivities have recently been correlated to the quality control of herbal medicines [4-6]. Instead of measuring all elements

within a biological system, systems biology aims to reveal intrinsic trends of the system [7,8]. Metabonomics focuses on the investigation of high-throughput data from metabolite profiling. The key advantage of metabonomics is to provide an integrative and systematic view of metabolism, which may also reveal the quality of herbal medicines [9,10].

Different ingredients within a herbal medicine may have synergistic effects. These active ingredients must be identified and quantified for better understanding of the action mechanisms of herbal medicines. For the moment, the conventional marker approach is not successful as only a few chemical markers are available for herbal medicines in the American Herbal Pharmacopeia and Chinese Pharmacopoeia. For example, in the Chinese Pharmacopoeia (2005 edition) only one chemical marker chlorogenic acid ($C_{16}H_{18}O_9$) is recommended for the identification of *Flos Lonicerae* (*Jinyinhua*) (content ≥ 1.5%) and *Flos Chrysanthemi* (*Juhua*) (content ≥ 0.2%) [11]. These two herbs cannot be differentiated with the only chemical marker. Likewise, *Fufang Danshen Diwan* is a formulated herbal medicine that consists of three herbs but there is only one chemical marker salvianic acid A ($C_9H_{10}O_5$) recommended for this particular medicine in the Chinese Pharmacopoeia (2005 edition) [11].

The pattern-oriented approach such as fingerprinting is more useful than compound-oriented approach in most cases. Chemical fingerprints are characteristic for herbal medicines, and are therefore useful in the quality control of herbal medicines [12-15]. The information-rich chemical fingerprints can be obtained from advanced instruments [16-21]. All detectable chemical components of a herbal medicine may be shown in fingerprints. Chemometrics helps analyze and interpret useful information from raw data, e.g. alignment of shifts of retention time in chromatography, data assessment and comparison, smoothing and filtering, deconvolution and resolution of overlapping peak clusters, in determination of the chromatographic and spectral profiles of pure components [22-27]. This article summarizes recent advances in both compound-oriented and pattern-oriented approaches in terms of experimentation, instrumentation and data processing.

Compound-oriented approach
Marker approach
According to the Chinese Pharmacopoeia (2005 edition), identification and quantification of chemical markers are crucial to the quality control of herbal medicines. A total of 525 quantitative assays of chemical markers were documented in the Chinese Pharmacopoeia (2005 edition) for assessment of herbal medicinal materials, plant lipids, herbal extracts and formulations [11]. Chemistry of these

markers is known and their analytical procedures and reference standards are available for quality control. Chau *et al.* used near infrared spectroscopy (NIR) to quantitatively determine the content of berberine and total alkaloid in *Cortex Phellodendri* (*Guanhuangbo*) [28]. The content of berberine determined by high-performance liquid chromatography-diode array detection (HPLC-DAD) was used as a critical parameter to confirm the accuracy of the data obtained from NIR according to a linear model of partial least squares (PLS) regression. In another study, high-performance liquid chromatography (HPLC) and HPLC-DAD were used to assess the quality consistency of a formulated Chinese medicine *Qingfu Guanjie Shu* (capsule) using four marker compounds, namely sinomenine, paeoniflorin, paeonol and curcumin. [29]. Lin *et al.* used liquid chromatography-tandem mass spectrometers (LC-MS/MS), solid phase extraction, and the marker glycyrrhetic acid to simultaneously validate *Radix Glycyrrhizae* (*Gancao*) and quantify the target compound in the samples [30]. Quantitative studies of markers and identification of active ingredients were carried out for the quality control of herbal medicines [31-34]. Gas chromatography (GC), gas chromatography-mass spectroscopy (GC-MS), thin layer chromatography (TLC), thin layer chromatography-ultraviolet spectrophotometry (TLC-UV), capillary electrophoresis (CE) and capillary zone electrophoresis (CZE) were also proposed for the quality control of herbal medicines [35-39].

Multi-compound approach
Compared with the marker approach, the multi-compound approach uses multiple compounds with known chemical properties and does not necessarily require chemical markers. Chemometric deconvolution and resolution are major methods in this approach. In the Chinese Pharmacopoeia (2005 edition) [11], multiple compounds, instead of a single compound, are recommended for the quality control of herbal medicines. For example, total flavonol glycosides (i.e. quercetin, isorhamnetin and kaempferol) as well as total terpene lactones (i.e. bilobalide, and ginkgolides A, B and C) were used for the quality control of a ginkgo leaf product [11]. However, analyzing multiple compounds in a single chromatogram may not be easy. These chromatograms often contain overlapping peaks, which may not be resolved by changing chromatographic conditions. One possible solution is the use of chemical and/or instrumental methods that take advantage of spectra with very close retention times, e.g. mass spectra, ultraviolet spectra or other chemical properties containing variations large enough to resolve overlapping chromatographic profiles [40-43].

Chemometric resolution methods (CRM) were used extensively in the past decades to 'purify' chromatographic peak profiles of complex mixture systems such as

herbal medicines [13]. The qualitative and quantitative chemical information obtained by CRM did help discover the active ingredients of herbal medicines and study the synergistic effects of the ingredients [1].

Previously, both iterative and non-iterative resolution methods were used to study the volatile and non-volatile components in herbal medicines [44-46]. Many non-iterative resolution methods such as heuristic evolving latent projection (HELP), alternative moving window factor analysis (AMWFA), (subwindow factor analysis) SFA, evolving window orthogonal projection (EWOP) were useful in discovering more than ten components of herbal medicines [47-51]. Using GC-MS coupled with HELP, Li *et al.* identified 38 volatile chemical components of *Radix Paeoniae Rubra* (*Chishao*), which accounted for 95.21% of all detectable components [52]. In another study, 69 components of *Radix Rehmanniae Preparata* (*Shudihuang*) were separated, of which 59 were identified using standard spectra in the database of the National Institute of Standards and Technology (NIST). The 26 identified methyl esters accounted for about 94.29% of the total number of components [53]. Most of the iterative methods including (orthogonal projection approach) OPA and (iterative orthogonal projection resolution) IOP were applied to determine the chemical composition of herbal medicines [45,46]. With these chemometric methods, 65 volatile chemical constituents of *Rhizoma et Radix Notopterygii* (*Qianghuo*) were identified out of the 98 separated chemicals. Qi *et al.* resolved the overlapping chromatographic peaks in *Resina Draconis* (*Xuejie*) using HPLC-DAD. Therefore, using chemometric methods with hyphenated instruments was powerful in the analysis of herbal medicines [54]. Zeng *et al.* used PCA and generalized rank annihilation factor analysis (GRAFA) to process HPLC-DAD data sets obtained from *Radix Salviae Miltiorrhizae* (*Danshen*) and *Radix Notoginseng* (*Sanqi*) [55]. Ye *et al.* simultaneously analyzed seven major saponins of *Danshen Diwan* with HPLC-DAD and electron spray ionization-mass spectrometry (ESI-MS) [56].

Chemometric methods including spectral correlative chromatography (SCC), multi-component spectral correlative chromatography (MSCC), AMWFA were proposed for comparing three-dimensional (3D) or two-dimensional (2D) chromatographic profiles and integrating presence or absence information [49,57-59]. SCC was used to compare pure or selective herbal medicine components [59]. Both MSCC and AMWFA were used to analyze complex herbal medicines. The main feature of MSCC is the construction of an orthogonal projection matrix using abstract spectra acquired from decomposition of original fingerprint data sets. For comparison, the pure and mixed spectra are projected to the matrix for presence or absence information of target components.

AMWFA was used to extract pure chromatographic and spectral profiles of common components of related herbal medicines [49,58,59]. All these new chemometric algorithms were applied in the identification, quantification, comparison of chemical components and quality control of herbal medicines [60-66].

Pattern-oriented approach
Single pattern approach
Fingerprint analysis
The pattern-oriented approach analyzes fingerprints obtained from one-, two- or higher dimensional chromatographic and/or spectral instruments. Single pattern approach focuses on one type of pattern (e.g. chemical fingerprints of chromatograms and spectra) for the quality control of herbal medicines. Significant progress was made in 2D chromatographic profiles obtained from HPLC, CE, GC as well as 3D ones from hyphenated instruments [18,19,67-70]. Yan *et al.* combined 3D fingerprints from HPLC-DAD instruments with principal component analysis (PCA) to monitor *Qingkailing* (a proprietary Chinese medicine injection formulation) produced by various manufacturers [71]. High-speed counter-current chromatography (HSCCC) and high-performance liquid chromatography-coulometric electrode array detector (HPLC-CEAD) were used to obtain the fingerprint of *Radix Salviae Miltiorrhizae* (*Danshen*) collected from various localities [72-74]. Binary chromatographic fingerprints from HPLC-DAD and GC-MS were used to analyze the aporphinoid and quinolizidine alkaloids of *Caulophyllum robustum* (*Leiyemudan*). Similarity index and the cluster analysis method were used to analyze the quality of ten batches of samples of *Caulophyllum robustum* [75]. Fan *et al.* developed multiple chromatographic fingerprinting including two HPLC fingerprints for the quality assessment of *Danshen Diwan* [76]. Thin layer chromatography scan (TLCS) provided unique fingerprints which differentiated passiflora and other herbs grown under various conditions [21,77]. Fourier transform infrared spectroscopy (FT-IR), NIR and two-dimensional correlation infrared spectroscopy (2D-IR) spectroscopy were used to construct spectral fingerprints of complex herbal medicines [20,28,78,79]. New fingerprinting techniques have recently emerged, such as oscillating fingerprints [80], electrochemistry fingerprints [81,82], X-ray diffraction (XRD) second derivative fingerprints [83] and high performance liquid chromatography electrospray ionization tandem mass spectrometry (HPLC-ESI-MSn) [84].

Chemometric methods for fingerprint analysis
It is important to extract useful information from chromatographic/spectral fingerprints which contain chemical information of all detectable components of herbal medicines. Chau *et al.* developed a software package Computer Aided Similarity Evaluation (CASE) for data processing

under Matlab [85]. Gong *et al.* developed a series of chemometric methods to extract information from fingerprints [24,86,87]. Xu *et al.* used target peak alignment (TPA) to correct the retention time shifts and multiplicative scattering correction (MSC) for response correction [88]. In addition, wavelet technique was used for data preprocessing of fingerprints [89]. Comparison, assessment and discrimination analysis are key steps to process the 'standardized' fingerprints of herbal medicines. Chemometric methods are used to compare data among herbs with hundreds or even thousands of chemical components. This information can be further used to identify the origins of herbs, or to authenticate herbs. For example, research has made significant progress since the introduction of the similarity index and pattern recognition analysis [90-94]. Information fusion and determination of relative entropy critical value were proposed for the similarity analysis of fingerprints of herbal medicines [90-93]. PCA, orthogonal projection technique (OP), 2D-IR worked well on data discrimination analysis of fingerprints [94-97]. Data deconvolution methods helped obtain chromatographic and spectral profiles of individual pure components [47-53]. Furthermore, chemical fingerprint databases of herbal medicines were developed according to chemometric methods [98,99].

Multi-pattern approach
The multi-pattern approach assesses the quality of herbal medicines according to multiple patterns. For example, both chromatographic fingerprints and biological activity profiles are used for the quality control of herbal medicines. This approach targets the discovery of bioactive ingredients, assessment of medical effects, correlation between chemical fingerprints and pharmacological indices, and quality control [100-105]. Some studies used chromatographic fingerprints from liquid chromatography-diode array detection-atmospheric pressure chemical ionization-mass spectroscopy (LC-DAD-APCI-MS) followed by data processing. Wang *et al.* found 32 potential bioactive components of *Radix Angelicae Sinensis* (*Danggui*) in rabbit plasma and over ten new compounds [100]. Using a *Herba Houttuyniae* (*Yuxingcao*) injection, Lu *et al.* evaluated its anti-inflammatory effects and established the correlation between the chemical components of the injection and the bioactivities of the active ingredients [101,102]. Wang *et al.* devised a method which combined metabolic profiling and liquid chromatography-diode array detection-mass spectroscopy (LC/DAD-MS) with chemometrics to study *Danggui Buxue Tang*, a Chinese medicine formulation [103]. Aided by PCA, Yu *et al.* discovered major bioactive components of *Aquilegia oxysepala* (*Jianeloudoucai*) [104]. Evidently, the multi-pattern approach provides a reliable means for the quality control of herbal medicines.

Conclusion
The compound-oriented and pattern-oriented approaches to the quality control of herbal medicines have been significantly improved in terms of analytical instruments, biological screening methods and chemometrics. Among all the advanced techniques, multi-pattern approach will have a great potential for further development.

Abbreviations
2D-IR: two-dimensional correlation infrared spectroscopy; AMWFA: alternative moving window factor analysis; CE: capillary electrophoresis; CRM: chemometric resolution methods; CZE: capillary zone electrophoresis; DAD: diode array detection; ESI-MS: electron spray ionization-mass spectrometry; EWOP: evolving window orthogonal projection; FSMWEFA: fixed-size moving window evolving factor analysis; FT-IR: Fourier transform infrared spectroscopy; GC: gas chromatography; GC-MS: gas chromatography-mass spectroscopy; GRAFA: generalized rank annihilation factor analysis; HELP: heuristic evolving latent projection; HPLC: High-performance liquid chromatography; HPLC-CEAD: high-performance liquid chromatography-coulometric electrode array detector; HPLC-DAD: high-performance liquid chromatography-diode array detection; HPLC-ESI-MSn: high-performance liquid chromatography electrospray ionization tandem mass spectrometry; HSCCC: high-speed counter-current chromatography; IOP: iterative orthogonal projection resolution; LC-DAD-APCI-MS: liquid chromatography-diode array detection-atmospheric pressure chemical ionization-mass spectroscopy; LC/DAD-MS: liquid chromatography-diode array detection-mass spectroscopy; LC-MS: liquid chromatography-mass spectroscopy; LC-MS/MS: liquid chromatography-tandem mass spectrometers; MS: mass spectroscopy, MSCC: multi-component spectral correlative chromatography; NIR: near infrared spectroscopy, OP: orthogonal projection technique; OPA: orthogonal projection approach, PCA: principle component analysis; PLS: partial least squares; SCC: spectral correlative chromatography; SFA: subwindow factor analysis, TLC: thin layer chromatography; TLC-UV: thin layer chromatography-ultraviolet spectrophotometry; XRD: X-ray diffraction.

Competing interests
The authors declare that they have no competing interests.

Authors' contributions
ZZ and FC conceived the classification of approaches to quality control of herbal medicines. ZZ drafted the manuscript. FC supervised the project and revised the manuscript. HC and CYC provided references and helped revise the manuscript. TL and SW advised on the manuscript. DM, COC and YL were responsible for some studies in

this work. All authors read and approved the final manuscript.

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