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Quality Control Method for Herbal Medicine - Chemical Fingerprint Analysis

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1. Introduction

The herbal medicines (HMs) and their preparations have been widely used for thousands of years in many oriental countries, such as China, Korea, Japan, etc, (Liang Y.-Z. et al.,2004) and it is attracting more and more attention from all over the world. But the uncontrollable quality of HMs is the obstacle for internationalization and modernization. For HMs, both single and combinations contain a myriad of compounds, and multiple constituents of HMs represent their therapeutic effects. Moreover, the chemical constituents in herbs in the HM products may be diverse due to various harvest seasons, plant origins, origins, processing and other factors (Liang Y.-Z. et al.,2004).As to one kind of herbal medicine, any difference from above aspects can lead to variant efficacy. This point was illustrated in many literatures. (Pan Ruijing, et al., 2011;Qiu YQ, et al.,2007;Zeng Y.-X., et al., 2007;Zhang YY, et al., 2008) The content of bioactive components in the roots of *Salvia miltiorrhizae* (danshen) was closely related to germplasm and harvest time (He Chun'e, et al., 2010).As for HMs, the specific quality control of the tested samples usually was achieved through the identification and determination. Because of uncertainty and complexity, there is great difficulty in establishing a specific method of quality control of HMs. The techniques of authentication are not powerful enough to identify all the ingredients in a HM, target setting is too general to determine the active ingredient and the components that are regarded as markers are often not active. Hereby there are some problems in developing specific and objective quality standards of herb medicines. And just on based of this point, adulteration or inferior quality drugs appeared more and more in markets.

Subsequent evaluation revealed some products to be adulterated with other related plants from the same genus(Ma C.-H.,2011), for instance, in the U.S. market black cohosh products are adulterated widely with other species of *Actaea*, which are lower-priced and exert unknown effect on menopausal symptoms (Jiang B.,et al.,2006). At the same time also a large number of substitutes enjoy the same name but have different ingredients and efficiency, which causes confusion medicinal species and affects the safety and efficacy of HMs. In order to eliminate these quality problems, on one hand we must actively foster the concept of pharmaceutical production of good quality and the concept of legal system, and on the other hand, the scientific and technologically advanced quality control standards should be established as soon as possible.

The quality control standardization of HM contains standardization of herb medicinal substances, cut crude drug and preparations, and nowadays the emphasis is laid on that of HM substance. Without Quality control standardization of herb medicinal substances, there is not standardization of cut crude drug and preparations at all. The morphological identification and microscopic identification are utilized to determine the authenticity of HMs, and the physical and chemical characters are used to evaluate the quality of herbs in the existing quality standards. But by all above methods, the complexity of herbal medicines can not be elaborated, yet the chromatographic fingerprinting analysis was featured by the fundamental attributions of "integrity" and "fuzziness" or "sameness" and "difference", which can chemically represent the characteristics of the HM investigated(Xie P. S., 2001 as cited in Liang Y. Z. et al., 2004). Two key issues are involved in the development of a fingerprint method: (i) how to gain more effective and stable information and (ii) how to evaluate the similarity and difference with chemometric method(Liang X.-M. et al., 2009).

Mork and Chau grouped authentication and quality control of herbal medicines into the 'component-based' approach and 'pattern-based' approach (D.K.W. Mok & F.T. Chau, 2006). In 2008, Zeng et.al (Zeng Z.D. et al., 2008) refined these two approaches into compound-oriented approach and pattern-oriented approach respectively. The compound-oriented approach includes the marker approach and the multi-compound approach. Generally, marker approach is utilized when the active ingredient was identified, such as paclitaxel and ephedrine. And multi-compound approach will be used when a single compound can not represent the efficacy of the HMs. Pattern-oriented approach namely fingerprint analysis is more popular now, because most HMs' chemical ingredients have been studied, but it is difficult to determine which compound is effective. In recent years, the combination of qualitative fingerprint profiles and quantitative multi-compounds detection was proposed and was applied into the quality control of HMs (Kong W. J. et al., 2009; Zhou F. Z. et al., 2008; Su J et al., 2008).

So far, during the production and circulation of HMs, there is no comprehensive and integrated quality control measure to reflect the variations of HM products, and to effectively control the quality in the whole process. The research and establishment of fingerprints contributed much to solving the problem. It can evaluate the integrative and holistic properties of herbal medicines by comparing the similarity and correlation of the analytes among the whole producing process, such as manufacture, processing and storage of raw materials for preparation, intermediate products, finished products and distribution products. The fingerprint analysis have been internationally accepted as one of the efficient methods to control the quality of herbal medicines (Liang Y.Z. et al., 2004). In this chapter we reviewed the quality control methods of HMs, especially fingerprint analysis methods. Moreover, a section named case study was given at the end of fingerprinting techniques. In this part, three carefully selected cases were described in details, aiming to demonstrate the whole process of fingerprint analysis.

2. Compound approach

On basis of the Chinese Pharmacopoeia, identification and quantification of chemical markers are crucial to the quality control of herbal medicines. Totally, 525 quantitative monographs by chemical markers were documented in the Chinese Pharmacopoeia (2005 edition) for control and evaluation of herbal medicinal materials, prepared slices, herbal extracts and preparations. For example, a HPLC method was used to identify and quantify

the syringoside (content $\geq$ 0.050%) in *radix acanthopanacis* and chrysophano (content $\geq$ 0.20%) and aurantio-obtusin (content $\geq$ 0.080%) in *semen cassiae* (CPh.2005,pp.288). Of course, additionally, the combination with morphological, phytochemical and physicochemical identification is essential. M.H. Jeon et al. developed a reversed-phase high-performance liquid chromatography-pulsed amperometric detection (RP-HPLC-PAD) method for the detection of albiflorin and paeoniflorin in *Paeoniae Radix* (Min-Hwan Jeon et al.,2009).As many herb medical substances contain the same compounds, thus just one or several markers approach fails to confirm the identity of a specific plant, let alone make any evaluation regarding its quality. This problem will appear when a specific class of compounds simultaneously exist in two substances (Xie P.S. et al., 2006). So the multi-compound approach and fingerprint approach are proposed, and the fingerprint approach will be discussed in the next section. Compared with marker approach, multi-compound approach study on more compounds, and does not necessarily require specific chemical markers. Chemometric deconvolution and resolution are the main methods in this approach (Zeng Z.D.,2008). Such as iterative OPA or non iterative EWOP (Guo F.Q., 2004), Artificial Neural Networks (ANNs), k-nearest neighbor (k-NN) (Tian R.T. et al., 2008.) et.al. Chemometric methods for evaluation of chromatographic separation quality from two-way data were reviewed in the literature (Xu L et al., 2008). For instance, total terpene lactones and total flavonol glycosides were detected for the quality control of ginkgo substances, ginkgo extraction and ginkgo tablet (CPh.2005, pp.292/322/663).A method, HPLC-DAD-ESI-MS, was established to qualitatively identify and quantitatively determine the 10 major active coumarins of Zushima (Su J. et al., 2009).And a HPLC-DAD method is firstly established for the simultaneous determination of 10 major components in different origins of *Carthamus tinctorius*, by L. Fan et al. (Fan L et al., 2009).

3. Fingerprint approach

By definition, a chromatographic fingerprint of a HM is, in practice, a chromatographic pattern of the extract of some common chemical components of pharmacologically active or chemically characteristics (Liang Y.Z.et al., 2004). Specifically, fingerprints of herbal medicine refer to the profiles which can illustrate the specific properties of the analyte including raw materials, slices, semi-finished products and finished products after appropriate processing, and be obtained by certain analysis techniques. The research of fingerprinting of herbal medicines is really an interdisciplinary and comprehensive research, which is based on the chemical composition of traditional Chinese medicine system. It needs crossover of herbal medicine, separation science, analytical science, and bioinformatics to provide a platform for the quality control of traditional herbal medicines. Those features make fingerprint analysis especially suitable for research on HMs which bearing characteristics of a complex mixture of chemical compounds.

Fingerprinting is now globally accepted as a quality evaluation model of herbal medicine. Due to different national conditions and traditions, the research thoughts and methods of fingerprinting are diverse in different countries. Japanese scientists accept the decoction of a prescription consisting of trueborn crude drug slices as standard extraction, and the fingerprint obtained from the standard extraction is taken for standard fingerprint. Food and Drug Administration has also started to accept fingerprint, because fingerprint method can be utilized to the quality control of the Botanical Drug Substances and Botanical Drug Products in application material named chemistry, manufacture and control (CMC) of

Investigation New Drug (IND). Furthermore, France, Germany, Britain, India and the WHO, adopted fingerprinting to evaluate the quality of medicinal plants. And Chinese manufacturers are required by the Chinese State Food and Drug Administration (SFDA) to standardize the injections and their corresponding raw materials made from Traditional Chinese Medicine, by using chromatographic fingerprinting method.

Just as in any single HM and its combinations, there are lots of unknown components and many of them are in low amount, researchers applied more and more techniques into this field. Fingerprinting, generally, was divided into chemical and biological fingerprint patterns. Chemical fingerprint is used to analyze the chemical constituents in HMs, consisting chromatographic fingerprint, such as thin layer chromatography (TLC), high-performance liquid chromatography (HPLC), gas chromatography (GC), capillary electrophoresis (CE) and spectral fingerprint, for instance, UV, IR, MS, X-ray and so on, and their hyphenated techniques. The biological fingerprints mainly refer to genomics fingerprints. Since genetic composition is unique for each individual, DNA methods for HMs' identification are less affected by age, physiological conditions, environmental factors, harvest, storage and processing methods. Genomic fingerprint has been used widely for the differentiation of plant individual, genus, homogeneity analysis, and detection of adulterants (Cheng KT et al., 1997; Huang Y et al., 2002; Wang C.Z. et al., 2007; Zhang X. et al., 2006). However, as for herbal instances processed or extractions of plants, DNA fingerprinting techniques usually can not do anything. Moreover, the efficiency of Chinese herbal medicines is based on the chemical components they contain, chemical analysis therefore better reflect the intrinsic quality of medicine. Consequently, DNA fingerprinting should be used as a complement tool of other quality control techniques.

In this part, the emphasis was put on chemical fingerprinting techniques, of which the classification and application were discussed in details, and at the end of this section, several carefully selected and systematical cases were presented to illustrate the methodology of fingerprints.

3.1 Chromatographic fingerprint analysis techniques and classification

3.1.1 Thin layer chromatography

TLC is the common fingerprint method for herbal analysis because of its simplicity, rapidity and economy. A major advantage of TLC is that it can provide the light images and fluorescence images, which is one more visual parameter than Chromatograms, and you can get different levels of profiles and corresponding integral data with chromatography scanning and digital processing, especially for routine analysis and on-site inspection test. But TLC analysis also has shortcomings: low resolution, low sensitivity and the difficulty of detection of trace components, etc.

Four species of herb medicines *bailahuén*-species *Haplopappus remyanus*, *Haplopappus multifolius*, and *Haplopappus taedaare* are all used in popular medicine for the same purpose, we supposed that chemical compounds would be very similar. But H. Vogel et al. identified them easily by TLC of the resins (Hermine Vogel et al., 2005). With this technique, authentication of various species of *Ginseng* and *Radix Puerariae* is possible, as well as the evaluation of stability and consistency of their preparations from different manufactures (Xie P.S et al., 2006). HPTLC fingerprint is mainly used to study the compounds with low or moderate polarities, but Di et al. established a fingerprint of fungal polysaccharide acid hydrolyzates by using automated multiple development (AMD) (Di X et al., 2003). Micro

emulsion thin layer chromatography (ME-TLC) which differs significantly from conventional TLC, provides higher detection sensitivity and separation resolution and reproducibility (Cui S.F. et al., 2005). Researchers from Hong Kong Polytechnic University applied high performance thin layer chromatography (HPTLC) combining with digital imaging technology to compare the fingerprints of *Radix Puerariae Lobatae* and *Radix Puerariae Thomsonii*, and directly revealed the chemical outline of two different species (Chen SB et al., 2006).

3.1.2 GC and its hyphenated techniques

Gas chromatography (GC) and GC-MS with high specificity, high sensitivity, stability and small amount of sample characteristics, are unanimously accepted as the method for the analysis of volatile constituents of HMs (Jiang Y, et al.). The combination of GC-MS fingerprinting analysis along with relative retention index (RRI) and related Chemometrics methods was developed to distinguish *Scutellaria Barbata D. Don* (SB) and its adulterants, simultaneously witness the consistent of SB from nine different origins (Pan R.J. et al., 2011). GC can detect almost all the volatile chemical compounds with high sensitivity, which is especially true for the usual FID detection and GC-MS. Moreover, the high selectivity of capillary columns enables separation of many volatile compounds simultaneously within very short time. However, it is not convenient for the analysis of samples of polar, non-volatile and heat-labile ingredients (Liang Y.Z. et al., 2004). The samples must be gasified by the tedious sample pretreatment such as derivatization, but the ingredients in most herbal instances are high polar compounds, which limits Gas chromatography's application in the chemical identification and authentication of HMs. To solve this problem, and expand the gas chromatography in the identification of herbal medicine, Chinese University of Hong Kong and Shanghai Innovative Research Center of Traditional Chinese Medicine combined to firstly set up off-line pyrolysis - gas chromatography - mass spectrometry fingerprint method to obtain the fingerprints of HMs (Chen W.D. et al., 2007).

3.1.3 HPLC and its hyphenated techniques

HPLC has been a popular and commonly used method in the research field of fingerprint analysis for Chinese medicines because it is easy to learn and use and is not limited by the volatility or stability of the sample compound (Liang Y.-Z. et al., 2004). Thus HPLC is utilized more widely than GC as the complement. One of the main advantages of HPLC is that many detectors can be connected to it, such as UV, DAD, ELSD, FLD, RID, MS, and NMR, etc, and even connect with two or more of them (Wang H.L. et al., 2010), which supplies much more possibilities for detecting different constituent types (Qi L.W. et al., 2008). HPLC fingerprinting technique is advisable for good quality control of Wuweizi (Lu Y. & Chen D.F., 2008). A method of HPLC-UV/MS was developed to determine the flavonoid profile of *S. baicalensis* in (Liu G.Z. et al., 2009). Another method using liquid chromatography coupled to electrospray ionization mass spectrometry (LC-ESI-MS) has been optimized and established for the qualitative and quantitative analysis of ten active phenolic compounds originating from the pigeon pea leaves and a medicinal product (Tongluo Shenggu capsules) (Liu W et al., 2010). To investigate the possibility of using two non-official species of *Herba Cistanche* as alternatives to the official species, a high-performance liquid chromatography-diode array detection-mass spectrometry (HPLC-DAD-MS) fingerprint method was developed to comparatively analyze the crude herbs of

these four species (Jiang Y. et al, 2008). C. Ma et al. used HPLC-ESI-TOF-MS/MS and chemometric analysis to identify marker compounds in the assessment of 4 species of *Actaea*. This method successfully distinguished different species of *Actaea* and made the black *cohosh* products high quality and unadulterated (Ma C.H. et al., 2011). And in recent years, coulometric electrode array detection (HPLC-CEAD) and charged aerosol detection (CAD) (Bai C.C., et al., 2009) have also been used for the analysis of HMs. CAD is a quality detector (R.W. Dixon & D.S. Peterson, 2002), and has similar properties with ELSD, suitable for detecting the compounds without or with weak UV absorption, but with higher sensitivity, wider test range and better reproducibility (Jiang Y. et al., 2010; Bai C.C., et al., 2009). A binary chromatographic fingerprint analysis was developed using hydrophilic interaction chromatography (HILIC) and reversed-phase liquid chromatography (RPLC) to gain more chemical information about two parts of water-extracted fraction: the polar compounds and weakly polar compounds. The fingerprint of the polar compounds was analyzed with HILIC, whereas the fingerprint of the weakly polar compounds was analyzed with RPLC (Jin Y et al., 2008).

Except availability of more sensible detectors for HPLC, the appearance of capillary HPLC and ultra-performance (UPLC) have increased the analysis efficiency, so that shorter analytical time and better separation have been achieved (Zheng X.T. et al., 2008; Chen J.H. et al., 2008). UPLC also can be connected with different detectors, for instance, UV, TOF/MS, Q/TOF/MS, PAD and even two or more of them combined together. Those hyphenated techniques with the virtue of the high resolution, high speed of UPLC and the accurate mass measurement of TOF-MS, can get more information about the complex characteristics of herb medicine and can identify and quantify multi-components in HMs. Thus in recent years, UPLC technique, especially the hyphenated techniques are usually utilized in this field (Li S.-L. et al., 2009; Cheng J et al., 2010; H. Liu et al., 2009; Kong W.J. et al, 2009). Moreover, the UPLC-PDA-Q-TOF-MS was used to evaluate decocting-induced chemical transformations (S.-L. Li et al., 2010a) and chemical consistency between traditional and dispensing granule decoctions (S.-L. Li et al., 2010b).

3.1.4 Capillary electrophoresis and its Hyphenation

Capillary electrophoresis is the combination of a classic electrophoresis and modern micro-column separation technology, and in recent years it presents a rapid development and has been widely used in the medicine field. Capillary electrophoresis (CE), with high resolution, minimal sample and solvents consumption, short analysis time and high separation efficiency, is an effective tool for drug quality control. The methodology of CE was established to evaluate one herb drug in terms of specificity, sensitivity and precision, and the results were in agreement with those obtained by the HPLC method. Furthermore, the analysis time of the CE method was two times shorter than that in HPLC and solvent consumption was more than 100-fold less (Sombra L.L., et al., 2004). A characteristic fingerprint of *Flos Carthami* established using CE, simultaneously contributed to several objects in a study: identifying the raw herb, helping distinguish the substitute or adulterant and further assessing the differences of *Flos Carthami* grown in various areas of China (Sun Y et al., 2003). In addition, CE has a wide range of applications such as for use of organic, inorganic, neutral molecules, biological macromolecules, and so on. The hyphenated CE instruments, such as CE-DAD, CE-MS and CE-NMR, have been utilized in the past decades. However, Overlapping peaks in complex samples and irreproducible migration times are

unintelligible defects of CE and hyphenations (Christophe Tistaert et al., 2011). Comparison of the CE and HPLC fingerprints of *radix Scutellariae* showed a decrease in analysis time from 40 to 12min for CE, but also a decrease in detected peaks from 14 to 11 (Wang L.C., et al., 2005).

3.2 Evaluation of chemical fingerprint of herbal medicine

The whole process of evaluation of chemical fingerprint of herbal medicine was showed in fig.1. Evaluation of fingerprinting is important and critical for the quality assessment and quality control of herbal medicine. Due to experimental variations and column aging, shifts in retention time between fingerprints occur. However, data handling techniques require the same variable to contain synchronous information in every fingerprint. So when the fingerprints were obtained, peak-alignment or warping techniques are commonly applied to compensate for minor shifts in retention times. Many techniques in peak alignment have been proposed including target peak alignment (TPA), dynamic time warping (DTW), fuzzy warping (FW), parametric time warping (PTW), semi-parametric time warping (STW) and correlation optimized warping (COW) (Christophe Tistaert et al., 2011). And then, data information of fingerprint profiles should be normalized by being organized in a data matrix.

Fig. 1. The whole process of evaluation of chemical fingerprint of herbal medicine

The objective of this step is to achieve the relative proportions of the data (either peak height or peak area can be selected to express peak intensity) obtained from fingerprints. A chromatographic peak inherent in each profile is addressed as reference, and the ratio of peak height/area between other peaks and reference peak should be calculated in every sample. On the basis of the normalization processing, peaks whose peak height/area ratios change slightly in the same species but vary seriously between different species, could be picked out as candidate feature peaks using Stepwise Discriminant Analysis. The selected candidate feature peaks would be developed into characteristic spectrums that are used to evaluate the similarity and difference of fingerprints of analytes by some chemometric techniques such as Similarity Analysis, Principal Component Analysis, Clustering Analysis, etc.

Because of complexity and multiplicity of information from the fingerprints, pulsing minor differences in concentrations of some detected samples, more and more chemometric methods were applied to quality control and evaluation of HMs. Similarity comparison as one of the most common and earliest tools representing the equivalence of fingerprints of herbs, consists of both the point-point approach and the peak-peak approach. And the relationship within a set of fingerprints could be analyzed by (dis)similarity comparison of the substances with a certain standard fingerprint. So a reasonable standard (reference) should firstly be achieved. Popularly, the reference may be derived from not only standard extract or proportioned mixture of HMs (e.g. EGb761) but also computation by some mathematical methods (e.g. Principle Component Analysis) (Liang, Y.-Z., et al., 2004). Similarity evaluation could be achieved by many methods including congruence coefficient, correlation coefficient, distance coefficient, Nei coefficient, improved Nei coefficient, etc. These methods have their own characteristics and scope of application, which has been discussed in the reference (Nie L et al., 2005). Generally, congruence coefficient and correlation coefficient (Jin Y. et al., 2008) was the workhorse of chemometrics in the similarity analysis of data sets. However, it has been aware that the (dis)similarities of the herbal objects have shortcomings (Liang, Y.-Z., et al., 2004; Christophe Tistaert et al., 2011): a reference fingerprint is necessary, but in many instances, the herbal medicines, selected for producing reference fingerprints, often do not have the best efficacy, which might influence the outcome. And a second concern is the subjective threshold consideration for quality control and discrimination. Different thresholds may be used in different studies (Li Y. et al., 2010; Fan X.H. et al., 2005; Wei H. et al., 2010). And sometimes masking and swamping effects might occur, explicitly and implicitly.

In order to overcome the drawbacks of similarity evaluation, more methods appeared. Principal component analysis (PCA) and hierarchical cluster analysis (HCA) were used to discern HPLC fingerprints of 30 *Cortex cinnamomi* samples from different species and regions (Yang, J. et al., 2007). Ni et al. using pattern recognition models, PCA and HCA, successfully distinguished the fingerprints of 46 *Eucommia bark* samples originating from different locations, produced by the combination of LC-DAD and LC-MS techniques (Ni, Y. et al., 2008). Both PCA and HCA are exploratory data analysis tools which might be able to represent a multivariate data table as a low-dimensional plane: original variables are transformed into latent variables (LV) summarizing the systemic patterns of variation between the samples. Robust PCA (rPCA) (M.Hubert et al., 2001), Projection Pursuit (PP) (Daszykowski M. et al., 2002) also were devoted to gain insight in the structure of a multivariate data table as exploratory data analysis besides PCA and CA. Clustering techniques are divided into two subtypes: hierarchical (HCA) and non-hierarchical (FC, Fuzzy Clustering). And Hierarchical Clustering Analysis (HCA) is one of the most popular clustering techniques applied on herbal fingerprints, because of its flexibility to alter the similarity measurement criterion and the applied linkage method to suit different applications (Massart D.L. et al., 1997, as cited in Christophe Tistaert et al., 2011).

Another approach commonly used is Pattern Recognition that diverges from all above methods, as it makes use of discrete information on the samples in the calibration set. This information is described in a response vector Y . Recently, Pattern Recognition has been intensively used for the classification of data sets, including numerous linear and non-linear classification techniques. The most popular methods for the classification of herbal products include Linear Discriminant Analysis (LDA) (Lerma-García M.J et al., 2009; Ni Y. et al., 2008), K-nearest neighbors (k-NN), Artificial Neural Networks (ANN) (Tian R.T. et al., 2008),

Soft Independent Modelling of Class Analogy (SIMCA) (Chen Y. et al., 2008), Partial Least Squares-Discriminant Analysis (PLS-DA) (Toh D.F. et al., 2009) and Orthogonal Projections to Latent Structures-Discriminant Analysis (OPLS-DA) (Li S.L. et al., 2010). Advantage and disadvantage of each technique were reviewed in the literature (Christophe Tistaert et al., 2011).

3.3 Case study

The details of 3 carefully selected cases were discussed in this section, presenting establishment and evaluation of fingerprints.

Case I: Development, optimization and validation of a fingerprint of *Ginkgo biloba* extracts by high-performance liquid chromatography (Ji Y.B. et al., 2005)

This case presented four-step strategy for method development, optimization and validation of an HPLC fingerprint of *Ginkgo biloba* extracts (GBE 20030106): 1) Selection of a suitable chromatographic system including separation mode, stationary phase and mobile phase; 2) Screening design of some controllable parameters and the range of the factors; 3) Gradient optimization using a uniform design; 4) Methodology validation identical with that discussed in other cases. The theory of Screening design and uniform design could be discovered in the reference (Ji Y.B. et al.,2005,2006).

1) Selection of a suitable chromatographic system

Most compounds of interest are high polar components consisting of glycoside derivatives of quercetin, kaempferol and isorhamnetin, so reversed-phase liquid chromatography methods with ODS columns and acetonitrile–water mobile phases are recommended. Three columns were screened with a 30min-linear gradient elution, where acetonitrile/0.1% phosphoric acid (B/A; B%: 15%-30%) as mobile phase. And the Alltima C18 column (C) was selected according to selectivity and resolution. Then several mobile phases were investigated: methanol/ 0.1% phosphoric acid, acetonitrile/0.1% phosphoric acid and isopropanol and tetrahydrofuran added in mobile phase as organic modifier. Finally it was decided to use the chromatographic system consisting of the Alltima C18 column and an acetonitrile–0.1%phosphoric acid mobile phase combination.

Factor	Units	Level (-1)	Level (+1)
X1: concentration of phosphoric acid	%	0.1	0.5
X2: detector wavelength	nm	350	370
X3: column temperature	°C	30	50
X4: initial concentration of acetonitrile (B%)	%	12	18
X5: gradient time	min	30	50
X6: injection volume	μl	5	10
X7: concentration of isopropanol in mobile phase	%	0	10

Table 1. Factors and levels investigated in the screening design

2) Screening design

A fractional factorial design was utilized as screening design to identify significant main effects. The peak number was used as response and seven factors were examined (table 1). And the main effect plot determining the important factors and the best factor setting was shown in fig. 2. The main effect plots (Fig. 3) illustrated that detection wave-length and column temperature have main effects on the peak capacity (the total number of peaks observed). And it is reasonable that more information can be obtained using a higher injection volume.

Fig. 2. Main effect plots from the screening design

From the results of the screening experiments, the values of some important controllable parameters can be selected and determined: detection wavelength (350nm), column temperature (30°C), acid concentration (0.1%) and injection volume (10 μ l), while no isopropanol was needed in the mobile phase. Another two factors, the initial concentration of organic solvent and the gradient time did not seem very important as presented in fig.2. However, the gradient optimization always be considered as a point of a general strategy to improve the HPLC separation. Hereby, it was selected for the next step.

3) Gradient optimization

Two parameters were chosen as optimization factors, the gradient time (t_G) and the concentration of organic solvent at the beginning of the gradient (B%). The optimization region selected was 28–52min for t_G and 11 to 17% for B%, according to the result of the screening experiment of step two. Uniform table U_7 (7^2) was given (table 2). And both the specific level investigated and the results including the values of hierarchical chromatographic response function (HCRF) were presented in table 3. HCRF can simultaneously assess the number of peaks that may be detected, the resolution and time of analysis.

No.	B ₀ (%)	t _G (min)	HCRF
1	14	40	43,068,057
2	13	52	41,068,045
3	15	28	39,056,069
4	12	32	39,034,066
5	11	44	40,067,053
6	17	36	41,080,057
7	16	48	46,068,049

Table 2. Uniform design table of U₇ (7²)

No.	Factor	
	X1	X2
1	4	4
2	3	7
3	5	1
4	2	2
5	1	5
6	7	3
7	6	6

Table 3. The result of the uniform design

Fig. 3. HPLC fingerprints of *Ginkgo extract* in two different experiment conditions. (A) No. 1 (B) No. 7 experimental runs in a U₇ (7²) design, HPLC conditions as in text

From the results of Table 3, the conditions of Experiment Nos.1 and 7 were selected (Fig. 3a and b). These two chromatograms are very similar, but shorter analysis time can be obtained in Fig. 3a, and the resolution of the peaks around 10min is better. So the conditions of Experiment No. 1 was chosen as optimized conditions: a linear gradient elution with acetonitrile/0.1% phosphoric acid (from 14/86 to 30/70 in 40min) as mobile phase, a column temperature of 30 °C, a detection wavelength of 350 nm, and an injection volume of 10 μ l.

4) Methodology validation

The methodology of HPLC fingerprints from *Ginkgo biloba* extract was validated by identification and purity determination of chromatographic peaks, injection precision, repeatability and sample stability.

Conclusion and discussion

The process of development, optimization and validation of HPLC fingerprints of *Ginkgo biloba* extract was introduced in this case. The methods of development, optimization and validation of fingerprints are not limited to those discussed above, but all fingerprinting methods can be established imitating the process detailed in this part. A sequential procedure based on a uniform design approach was also applied in the development of CE fingerprints of *Ginkgo biloba* extract (Y.B. et al., 2006). Yet the authentic sample should be obtained or extracted from crude herbs before establishing the fingerprints, which is also very important for the final result. Recent developments in sample preparation techniques for the analysis, such as the extraction, clean-up, and concentration of analytes from HMs can be found in the references (Deng C.H et al., 2007). (Huie C.W., 2002).

Case II: Quantitative and chemical fingerprint analysis for quality control of *Rhizoma Coptidis chinensis* based on UPLC-PAD combined with chemometrics methods (Kong W.J. et al., 2009)

Rhizoma coptidis (Huanglian in Chinese), on one hand, as a traditional Chinese medicine, has been commonly used for the efficacy of suppressing fever, dispelling dampness, removing toxicosis and anti-microbes for many centuries, and on the other hand, phytochemical and modern pharmacological studies revealed that alkaloids therein it have the significant efficacy of anti-virus, anti-inflammatory, anti-cancer and anti-microbes (Huang et al. 2006; Enk et al. 2007; Hsu et al. 2007; Yan et al. 2008).

Since application of *Rhizoma Coptidis* is growing steadily, a quality control method using UPLC-PAD was developed to differentiate the *Rhizoma coptidis* from various sources by chemical fingerprints which were investigated by SA, HCA and PCA. Simultaneously, five markers were selected to carry out quantitative diagnosis. All the ten samples are dried and share the same origin: *Coptis chinensis* Franch. Sources and origins of raw herbs studied is presented in table 4.

Sample number	Sources	Harvesting time
S1	Shizhu, Sichuan	August, 2007
S2	Longchi, Sichuan	August, 2007
S3	Shuanghe, Hubei	September, 2007
S4	Huangshui,Chongqing	August, 2007
S5	Shizhu, Sichuan	December, 2007
S6	Huangshui,Chongqing	August, 2007
S7	Shizhu, Sichuan	February, 2007
S8	Wangsi, Sichuan	August, 2007
S9	Moudao, Hubei	September, 2007
S10	Xinchang, Sichuan	August, 2007

Table 4. Sources and origins of raw herbs

Development of fingerprint

In this study, the UPLC-PAD method was validated by linearity, detection limit and quantification limit, precision, reproducibility and stability after optimization. And then, ten fingerprints of *Rhizoma Coptidis* with reasonable heights and good resolutions were obtained.

Evaluation of fingerprints

There were 11 characteristic peaks (from peak 1 to peak 11) in the chromatogram, as shown in fig. 4B. Peaks purity was identified by comparison of retention time and PAD spectra. Peak 11 (berberine) with the biggest area peak, was chosen to calculate the relative retention time (RRT) and relative peak area (RPA) of all the other peaks.

Fig. 4. Chromatogram of mixed standard compounds (A) and standardized chromatographic fingerprint of 10 batches *Rhizoma Coptidis* samples obtained by UPLC-PAD method at 350 nm(B)

Similarity analysis (SA)

The simulative mean chromatogram as reference fingerprint was derived from computation by Similarity Evaluation System for Chromatographic Fingerprint of Traditional Chinese Medicine. And the correlation coefficients between each fingerprint of *Rhizoma Coptidis* samples and the simulative mean chromatogram were 0.997, 0.981, 0.980, 0.983, 0.995, 0.971, 0.994, 0.981, 0.965 and 0.977, respectively. As we can see from the above results, different samples have different correlation coefficients and diverse internal quality. The correlation coefficients of the samples from the same source were similar, such as S1, S5 and S7, illustrating that productive origin is more influential than harvest time.

Hierarchical clustering analysis (HCA)

A hierarchical agglomerative clustering analysis was performed based on the relative peak areas of all the 11 common chromatographic peaks of *Rhizoma Coptidis* samples, using SPSS. The results of HCA as shown in Fig. 5, revealed the quality characteristics more clearly. Supposing an appropriate distance level (Level I) chosen, the samples could be classified into three quality clusters. Cluster-I including the samples collected from Shizhu city,

Sichuan province. The cluster II comprised the samples collected from other cities of Sichuan province and Chongqing city and cluster III was formed by samples collected from Hubei province. If a higher distance level (Level II) was adopted, the quality of samples was changed to two clusters, the first cluster comprised all samples collected from Sichuan province and Chongqing city, and the samples from Hubei province consisted in the second cluster.

Fig. 5. Dendrogram of clustering of *Rhizoma coptidis* samples. This dendrogram was performed using SPSS 13.0 software (Chicago, USA). The Ward's method as the amalgamation rule and the squared Euclidean distance as metric were used to establish clusters

Principle component analysis (PCA)

PCA was employed to evaluate the discrimination ability of these common components used in SA and HCA, using the relative peak areas of common peaks as input data instead of the full fingerprints. The first two principal components PC1 and PC2 are used to present the inhomogeneity in the data sets. Fig. 6 showed the clear differentiation of samples in terms of cultivating locations, which is correspondence with the SA and same to HCA.

PCA analysis was also used to find the possible main chemical markers which have the most influence on the discrimination among different samples. For the log-centered data set with 81.26% of explained variance by the first two principal components, the loadings plot of the scores (Fig. 7) indicated that circled peaks 5, 8 (epiberberine), 9 (coptisine), 10 (palmatine), and 11 (berberine) may have more influence on the discrimination of the samples from different sources than other components. These peaks could be found in Fig. 4. The sequence of the total contents of the five alkaloids in different samples was $S1 > S7 > S2 > S5 > S4 > S3 > S8 > S10 > S6 > S9$. The higher content of five alkaloids, the better quality raw herbs have. This finding is also corresponding with the above results. So the five compounds may be used to qualitatively identify *Rhizoma coptidis* from various regions and harvest times.

Conclusion and discussion

A method with the combination of UPLC-PAD and chemometrics techniques in this study successfully classified ten samples of *Rhizoma Coptidis* from different regions, which is more effective than that in the official Chinese pharmacopoeia (China Pharmacopoeia Committee 2005). In Chinese pharmacopoeia berberine alone was detected for the quality evaluation of *Rhizoma Coptidis*.

Fig. 6. The loadings plot from PCA for the common components. The loadings plot were performed with the original relative peak areas of the common compounds as input data using software of Unscrambler 9.7 from Camo AS (Trondheim, Norway); possible markers were marked with a circle

Fig. 7. Scores plot of PCA of *Rhizoma Coptidis* samples from various sources on the first two PCs. This plot of PCA on the first two PCs with the original peak areas of the 11 common compounds as input data was obtained using software of Unscrambler 9.7 from Camo AS (Trondheim, Norway). The labels of the sample refer to Table 4

The combination of fingerprint with multi-component quantification would be more powerful, because the qualitative and quantitative information of the analytes can be obtained at the same time. It has been proved to be a comprehensive method to assess the quality of herbs and herbal products. In this method, Zushima, *Carthamus tinctorius* and some preparations, such as ShuangHuang-lian oral liquid, xuesetong injection, etc. were successfully analyzed (Su J. et al., 2009; Fan L. et al., 2009; Cao Y.H. et al., 2006; Lai C.M. et al., 2005)

Case III: Combinative method using HPLC fingerprint and quantitative analyses for quality consistency evaluation of an herbal medicinal preparation produced by different manufacturers (Li Y, et al., 2010)

Yiqing is a medicinal preparation derived from the well-known traditional Chinese medicine (TCM) formula named San Huang Xie Xin (SHXX) decoction, composed of three herbs including *Rhizoma Coptidis* (*Coptis chinensis*), *Radix et Rhizoma Rhei* (*Rheum officinale*) and *Radix Scutellariae* (*Scutellaria baicalensis*). Fifteen samples (S1-15) of Yiqing preparations from twelve manufacturers were assessed using a combinative method of HPLC fingerprint and quantitative determination through analyzing nine bioactive compounds from Yiqing preparations. Meanwhile, four samples (S1-4) are different batches from one manufacture.

Development of fingerprint

Sample pretreatment conditions and HPLC chromatographic conditions were first optimized by investigating the effect of extraction solvents, extraction time and methods on the extraction efficiencies, and the effect of mobile phase and detection wavelength on the chromatographic separation efficiencies for marker compounds. And the optimal extraction and chromatographic conditions for Yiqing used in this study can be found in the literature (Li Y, et al., 2010). Consequently, a typical HPLC chromatogram fingerprint with satisfactory resolution of the nine chemical markers was shown in Fig. 8. Chromatographic fingerprints were achieved for 15 Yiqing samples from 12 manufacturers, and about 40 peaks were found in each individual sample (Fig9. A). A simulative median fingerprint was generated by the professional software by analyzing all the 15 samples (Fig. 9B).

Fig. 8. The typical HPLC chromatographic profile of nine standards. The peaks marked with 3, 6, 10, 15, 16, 17, 19, 21 and 22 are berberine, baicalin, wogonoside, baicalein, wogonin, aloe-emodin, rhein, emodin and chrysophanol, respectively

Evaluation of fingerprints

15 samples' similarity values compared with simulative median fingerprint were 0.929, 0.982, 0.978, 0.978, 0.995, 0.997, 0.985, 0.987, 0.969, 0.781, 0.844, 0.980, 0.988, 0.780 and 0.978 respectively. Most of the similarity values were in the range of 0.992–0.997 for samples S1–S9, S12, S13, and S15, suggesting that similar chemical components were present in these samples regardless of manufacturer. But another three samples (S10, S11, and S14) demonstrated low similarity values of 0.871, 0.884 and 0.780, indicating that these three products were different from the other samples. Overall, the results from the chromatographic fingerprints illustrate that the principal components of Yiqing preparations were relatively stable.

Quantitative determination of the nine marker compounds

The Quantitative determination method was validated by the investigation of correlation coefficients, linear ranges, limit of detection (LOD) and limit of quantification (LOQ), intra-day and inter-day precision, repeatability and Recovery. The validation results indicated that the conditions used in the quantitative determination were acceptable. The contents of the nine marker compounds in 15 samples from 12 manufacturers were determined with above method. And the contents of each individual marker between manufacturers were different, and the RSD values are 118.20, 36.53, 115.20, 125.09, 116.31, 124.39, 128.05, 118.24, 100.92 respectively, almost of which beyond 100% except for baicalein (36.53%), indicating the large variations in their quality.

Fig. 9. HPLC chromatographic fingerprints of 15 Yiqing samples (A) and simulative median chromatogram obtained by Similarity Evaluation System for Chromatographic Fingerprint of Traditional Chinese Medicine software (B). The chromatograms marked with S1–S15, and R represents 15 Yiqing samples and the simulative median chromatogram, respectively. The peaks marked with 1–22 in the simulative median chromatogram represent 22 characteristic peaks

The HPLC fingerprint method combined with multi-component quantification utilized in this study is an efficient and comprehensive tool for quality control or consistency evaluations of herbal drugs and preparations. In terms of utilization of complex and large amount of information of fingerprints, chemometric techniques should be introduced into the study. It is believed that with chemometric techniques, much more principles could be found.

4. Conclusion

Due to long historical clinical use and reliable therapeutic efficiency, herbal medicines are attracting and increasing global attention. However, the herbal medicine's efficiency is based on their complex chemical constituents, so quality control of herbal medicine also raised the researchers' attention. Fingerprinting of herbal medicines is utilized on the authenticity and quality control of HMs and the total producing process of herbal preparation. The combination of qualitative fingerprinting and quantitative multi-component analysis is a novel and rational method to address the key issues of quality control of herbal medicine. This method is thought of more effective and rational than simple fingerprinting.

Generally speaking, fingerprinting technique concludes three phases: information acquisition, information processing and information excavation. Only if the specificity, reproducibility, stability of fingerprinting were guaranteed, a set of developed quality control standards could be established. However, to achieve good reproducibility and stability is not an easy task, because of the diversity of HMs' collection sources and time, processing methods, etc and the difference of experimental conditions (e.g. columns, solvents, equipment type, etc.) when analysis was undergoing. This is the main reason that fingerprinting technique is still hard to be generalized.

Fingerprinting analysis should be interdisciplinary collaborative researches involving the multi-dimensional fingerprint, efficacy testing and information processing. In fact, the fingerprint discussed in this chapter is the first step of quality control, because the discussed methods can only solve the problem of comparing the integrated sameness and difference of HMs, and controlling the stability of the available herbal products, the next step should combine the fingerprinting profiles with the therapeutic efficiency. In recent years, the fingerprint-efficacy study caused the scientists' interests, and many studies have been down or ongoing. For instance, for quality control of artificial *Calculus bovis*, an attempt on fingerprint-efficacy study of artificial *C. bovis* was developed in the literature (Kong W.J, et al., 2011). In this study, the chemical fingerprints of 10 batches of artificial *C. bovis* sample from various sources were established by UPLC-ELSD. Then, the antibacterial effects of these samples on *E. coli* growth were determined using microcalorimetry. The relationship between the fingerprints and efficacy of artificial *C. bovis* was elucidated. The fingerprint of most therapeutical HM should be determined as a reference for the quality control, and the result will be more convincing. Generally the fingerprint-efficacy study will provide a powerful way and feasible ideas for the quality control of HMs.

5. References

- Bai C. C., Han S. Y., Chai X. Y., Jiang Y., Li P. & Tu P.F. (2009). Sensitive Determination of Saponins in *Radix et Rhizoma Notoginseng* by Charged Aerosol Detector Coupled with HPLC. *J. Liq. Chromatogr. Relat. Technol.* 32, (2009), pp.242-260, 1082-6076.

- Cao Y. H., Wang L. C., Yu X. J. & Ye J. N. (2006). Development of the chromatographic fingerprint of herbal preparations Shuang-Huang-Lian oral liquid. *J. Pharm. Biomed. Anal.* 41, (June 2006), pp. 845-856, 0731-7085
- Chen J.H., Wang F.M., Liu J., Lee F.S.C., Wang X.R. & Yang H.H. (2008). Analysis of alkaloids in *Coptis chinensis* Franch by accelerated solvent extraction combined with ultraperformance liquid chromatographic analysis with photodiode array and tandem mass spectrometry detections. *Anal. Chim. Acta.* 613, (2008), pp.184-195, 0003-2670
- Chen S.B., Liu H.P., Tian R.T., Yang D.J., Chen S.L., Xu H.X, Albert S.C. Chana & Xie P.S.. (2006). High-performance thin-layer chromatographic fingerprints of isoflavonoids for distinguishing between *Radix Puerariae Lobate* and *Radix Puerariae Thomsonii*. *Chromatogr. A.* 1121, (July 2006), pp.114-119, 0021-9673
- Chen Y., Zhu S.B., Xie M.Y., Nie S.P., Liu W., Li C., Gong X.F. & Wang Y.X. (2008). Quality control and original discrimination of *Ganoderma lucidum* based on high-performance liquid chromatographic fingerprints and combined chemometrics methods. *Anal. Chim. Acta.* 623, (August 2008), pp.146-156, 0003-2670
- Chen W.D. , Zhan Q.F., Wu P, Ge X.W., Shu J.Z., Hui Y.Z., Wang J & Xu H.X. (2007). Study on Fingerprint of Herbal Material by Pyrolysis-GC/MS. *Journal of Chinese Mass Spectrometry Society.* 28, (2007), pp.267-274
- Cheng K T, Chang H C, Su C H, & FL Hsu (1997). Identification of dried rhizomes of *coptis* species using random amplified polymorphic DNA. *Bot Bull Acad Sinica*, 38, (1997), p.241-244, 0006-8063
- Christophe Tistaert, Bieke Dejaegher & Yvan Vander Heyden. (2011). Chromatographic separation techniques and data handling methods for herbal fingerprints: A review. *Analytica Chimica Acta.* 690, (2011), pp.148-161, 0003-2670
- Cui S.F., Fu B.Q., F.S.C. Lee & Wang X.R. (2005). Application of microemulsion thin layer chromatography for the fingerprinting of *licorice* (*Glycyrrhiza* spp.). *J. Chromatogr. B* 828 (December 2005), pp.33-40, 0378-4347
- Di X, Kelvin K. C. Chan, Hei Wun Leung & Carmen W. Hui. (2003). Fingerprint profiling of acid hydrolyzates of polysaccharides extracted from the fruiting bodies and spores of *Lingzhi* by high-performance thin-layer chromatography. *J. Chromatogr. A.* 1018, (2003), pp.85-95, 0021-9673
- Daszykowski M., Walczak B. & Massart D.L. (2002). Projection methods in chemistry. *Chemometr. Intell. Lab. Syst.* 65, (January 2003), pp. 97-112, 0169-7439
- Deng C.H, Liu N., Gao M.X & Zhang X.M. (2007). Recent developments in sample preparation techniques for chromatography analysis of traditional Chinese medicines. *J. Chromatogr. A.* 1153, (June 2007), pp. 90-96, 0021-9673
- D.K.W. Mok & F.T. Chau (2006). Chemical information of Chinese medicines: A challenge to chemist. *J. Chemometrics and Intelligent Laboratory Systems* 82, (Sep. 2005), pp.210-217, 0269-3879
- Fan L, Zhao H.Y., Xu M., Zhou L., Guo H., Han J., Wang B.R. & Guo D.A. (2009). Qualitative evaluation and quantitative determination of 10 major active components in *Carthamus tinctorius* L. by high-performance liquid chromatography coupled with diode array detector. *J. Chromatogr. A.* 1216, (2009), pp.2063-2070, 0021-9673

- Fan X.H., Y.Y. Cheng, Z.L. Ye, R.C. Lin & Z.Z. Qian (2005). Multiple chromatographic fingerprinting and its application to the quality control of herbal medicines. *Anal. Chim. Acta.* 555, (January 2006), pp.217–224, 0003-2670
- Guo F.Q., Liang Y.Z., Xu C.J., Li X.N. & Huang L.F. (2004). Analyzing of the volatile chemical constituents in *Artemisia capillaris herba* by GC-MS and correlative chemometric resolution methods. *J. Journal of Pharmaceutical and Biomedical Analysis.* 35, (2004), pp.469–478, 0731-7085
- He C.E, Wei J.-H., Jin Y & Chen S.-L. (2010). Bioactive components of the roots of *Salvia miltiorrhizae*: Changes related to harvest time and germplasm line Industrial .*Crops and Products* .32,(May 2010), pp.313–317, 0926-6690.
- Huie C.W. (2002). A review of modern sample-preparation techniques for the extraction and analysis of medicinal plants. *J.Anal Bioanal Chem.* 373, (2002), pp.23–30, 1618-2642
- Huang Y, Qin M. J., Yang G., Xu L.S. & Zhou K.Y., *Chinese Traditional and Herbal Drugs* Vol. 33, No.10,(2002)pp.935-937
- Hermine Vogel, Mauricio González, Francesca Faini, Iván Razmilic, Jaime Rodríguez, José San Martín & Francisco Urbina. *Journal of Ethnopharmacology* 97 (2005), pp.97–100, 0378-8741
- Ji Y.B., Xu Q.S., Hu Y.Z. & Yvan Vander Heyden (2005). Development, optimization and validation of a fingerprint of *Ginkgo biloba* extracts by high-performance liquid chromatography. *J. Chromatogr. A* 1066, (2005), pp. 97–104,0021-9673
- Ji Y.B., Alaerts G., Xu C.J., Hu Y.Z. & Heyden Y.V. (2006). Sequential uniform designs for fingerprints development of *Ginkgo biloba* extracts by capillary electrophoresis. *J. Chromatogr. A.*1128, (2006), pp.273–281,0021-9673
- Jiang B., F. Kronenberg, P. Nuntanakorn, Qiu M.H. & E.J. Kennelly (2006). Evaluation of the botanical authenticity and phytochemical profile of *black cohosh* products by high-performance liquid chromatography with selected ion monitoring liquid chromatography-mass spectrometry. *J. Agric. Food Chem.* 54, (May 2006), pp.3242–53, 0021-8561
- Jiang Y, Li S.P., Wang Y.T., Chen X.J. & Tu P.F. (2008). Differentiation of *Herba Cistanches* by fingerprint with high-performance liquid chromatography–diode array detection–mass spectrometry. *J. Chromatogr. A* 1216 (March 2009) 2156–2162, 0021-9673
- Jiang Y, Bruno David, Tu P.F. & Yves Barbin (2010). Recent analytical approaches in quality control of traditional Chinese medicines—A review. *J. Analytica Chimica Acta.* 657, (2010), pp.9–18, 0003-2670
- Jin Y, Liang T, Fu Qi, Xiao Y.S., Feng X.T, Ke Y.X. & Liang X.M. (2008). Fingerprint analysis of *Ligusticum chuanxiong* using hydrophilic interaction chromatography and reversed-phase liquid chromatography. *J. Chromatogr. A.*1216, (March 2009), pp.2136–2141,0021-9673
- Jin C, Kong W, Luo Y, Wang Jo, Wang H, Li Q & Xiao X. (2010). Development and validation of UPLC method for quality control of *Curcuma longa*Linn.: Fast simultaneous quantitation of three curcuminoids. *Journal of Pharmaceutical and Biomedical Analysis.* 53, (2010), pp.43–49 0731-7085
- Kong W.J., Zhao Y.L., Xiao X.H., Jin C & Z L.L. (2009). Quantitative and chemical fingerprint analysis for quality control of *Rhizoma Coptidis chinensis* based on UPLC-PAD combined with chemometrics methods. *Phytomedicine* 16, (2009), pp.950–959, 0944-7113

- Kong W.J, Wang J.B, Zang Q.C, Xing X.Y., Zhao Y.L., Liu W., Jin C., Li Z.L. & Xiao X.H. (2011). Fingerprint-efficacy study of artificial *Calculus bovis* in quality control of Chinese materia medica. *J.Food Chemistry*. 3, (2011), PP.1342-1347, 0308-8146
- Lai CM, Li SP, Yu H Wan J.B., Kan K.W. & Wang Y.T. (2005). A rapid HPLC-ESI-MS/MS for qualitative and quantitative analysis of saponins in "XUESETONG" injection. *J Pharm and Biomed Anal*.40, (2006), pp.669-678,0731-7085.
- Lerma-García M.J., Lantano C., Chiavaro E., Cerretani L., Herrero-Martínez J.M. & Simó-Alfonso E.F. (2009). Classification of extra virgin olive oils according to their geographical origin using phenolic compound profiles obtained by capillary electrochromatography. *Food Res. Int.* 42, (2009), pp.1446-1452, 0963-9969
- Li S.L., Song J.Z., Qiao C.F., Zhou Y., Qian K.D., Kuo-Hsiung Leeb & Xu H.X. (2009). A novel strategy to rapidly explore potential chemical markers for the discrimination between raw and processed *Radix Rehmanniae* by UHPLC-TOFMS with multivariate statistical analysis. *Journal of Pharmaceutical and Biomedical Analysis*. 51, (2010), pp.812-823, 0731-7085
- Li S.L., Shuk-Fan Lai, Song J.Z., Qiao C.F., Liu X, Zhou Y, Cai H, Cai B.C. & Xu H.X. (2010). Decocting-induced chemical transformations and global quality of Du-Shen-Tang, the decoction of ginseng evaluated by UPLC-Q-TOF-MS/MS based chemical profiling approach. *Journal of Pharmaceutical and Biomedical Analysis* 53 (2010) 946-957,0731-7085
- Li S.L., Song J.Z., Qiao C.F., Zhou Y., Qian K.D. & Xu H.X. (2010). UPLC-PDA-TOFMS based chemical profiling approach to rapidly evaluate chemical consistency between traditional and dispensing granule decoctions of traditional medicine combinatorial formulae. *Journal of Pharmaceutical and Biomedical Analysis*. 52, (2010), pp.468-478, 0731-7085
- Li Y., Wu T., Zhu J., Wan L., Yu Q., Li X., Cheng Z. & Guo C. (2010). Combinative method using HPLC fingerprint and quantitative analyses for quality consistency evaluation of an herbal medicinal preparation produced by different manufacturers. *J. Pharm. Biomed. Anal.* 52, (August 2010), pp.597-602,0731-7085
- Liang, Y.-Z. Xie P.S. & Chan Kelvin. (2004). Quality control of herbal medicines. *Chromatogr. B* 812, (August 2004) pp.53-70,1570-0232.
- Liang X.M., JinY, WangY.P, Jin G.W., Fu Q & Xiao Y.S. (2009). Qualitative and quantitative analysis in quality control of traditional Chinese medicines. *J. Chromatogr. A* .1216, (July 2008), pp.2033-2044, 0021-9673
- Liu G.Z., Ma J.Y., Chen Y.Z., Tian Q.Q., Shen Y, Wang X.S., Bo C & Yao S.Z. (2009). Investigation of flavonoid profile of *Scutellaria bacalensis* Georgi by high performance liquid chromatography with diode array detection and electrospray ion trap mass spectrometry *J. Chromatogr. A* 1216 (June 2009) 4809-4814, 0021-9673
- Liu W, Kong Y, Zu Y.G., Fu Y.J, Luo M, Zhang L & Li J. (2010). Determination and quantification of active phenolic compounds in *pigeon pea leaves* and its medicinal product using liquid chromatography-tandem mass spectrometry. *J. Chromatogr. A*. 1217, (July 2010), 4723-4731, 0021-9673
- Liu H, Du Z.X. & Yuan Q.P. (2009). A novel rapid method for simultaneous determination of eight active compounds in silymarin using a reversed-phase UPLC-UV detector. *J. Chromatogr. B*. 877, (2009), pp.4159-4163, 1570-0232

- Lu Y. & Chen D.F. (2008). Analysis of *Schisandra chinensis* and *Schisandra sphenanthera*. *J. Chromatogr. A* 1216, (2009), pp.1980–1990, 0021-9673
- Ma C.-H., Adam R. Kavalier, Jiang B. & Edward J. Kennelly. Metabolic profiling of *Actaea* species extracts using high performance liquid chromatography coupled with electrospray ionization time-of-flight mass spectrometry. *Chromatogr. A* 1218, (2011), pp.1461–1476, 0021-9673
- Min-Hwan Jeon, Ha-Jeong Kwon, Ji-Seon Jeong, Yong-Moon Lee & Seon-Pyo Hong (2009). Detection of albiflorin and paeoniflorin in *Paeoniae Radix* by reversed-phase high-performance liquid chromatography with pulsed amperometric detection. *J. Chromatogr. A* 1216, (March 2009), pp.4568–4573, 0021-9673
- M.Hubert P. J. Rousseeuw & S.Verboven (2001). A fast method for robust principal components with applications to chemometrics. *Chemometr. Intell. Lab. Syst.* 60, (January 2002),pp.101–111, 0169-7439
- Ni Y., Peng Y. & Kokot S. (2008). Fingerprinting of complex mixtures with the use of high performance liquid chromatography, inductively coupled plasma atomic emission spectroscopy and chemometrics. *Anal. Chim. Acta.* 616, (May 2008), pp.19–27, 0003-2670
- Ni, Y., Peng, Y. & Koko, t S. (2008). Fingerprint analysis of *Eucommia bark* by LC-DAD and LC-MS with the aid of chemometrics. *Chromatographia* 67, (2008), pp.211–217, 0009-5893
- Nie L, Cao J, Luo G.A. & Wang Y.M. (2005). Comparison of different methods for evaluating the similarity of the fingerprints of traditional Chinese medicines. *J. Chinese Traditional Patent Medicine.* 27,(2005),PP.249-252
- Pan R.-J., Guo F.-Q., Lu H.-M., Feng W.-W. & Liang Y.-Z. (2011). Development of the chromatographic fingerprint of *Scutellaria barbata* D. Don by GC-MS combined with Chemometrics methods. *Journal of Pharmaceutical and Biomedical Analysis*, 55,(2011),pp.391–396, 0731-7085.
- Qi L.W., Li P., Ren M.T., Yu Q.T., Wen X.D. & Wang Y.X. (2008). Application of high-performance liquid chromatography-electrospray ionization time-of-flight mass spectrometry for analysis and quality control of *Radix Astragali* and its preparations. *J. Chromatogr. A* 1216, (2009), 2087–2097,0021-9673
- Qiu YQ, Lu X, Pang T, Zhu S.-H., Kong H.-W. & Xu G.W. (2007). Study of traditional Chinese medicine volatile oils from different geographical origins by comprehensive two-dimensional gas chromatography - time-of-flight mass spectrometry (GC×GC-TOFMS) in combination with multivariate analysis. *Journal of Pharmaceutical and Biomedical Analysis.*43, (January 2007) pp.1721–1727, 0731-7085.
- R.W. Dixon & D.S. Peterson (2002). Development and Testing of a Detection Method for Liquid Chromatography Based on Aerosol Charging. *Anal. Chem.* 74, (2002), pp.2930-37, 0003-2700
- Sombra L. L., Gómez M.R., Olsina R., Martínez L. D. & Silva M.F. (2004). Comparative study between capillary electrophoresis and high performance liquid chromatography in 'guarana' based phytopharmaceuticals. *Journal of Pharmaceutical and Biomedical Analysis.* 36, (2005), pp.989–994, 0731-7085
- Su J, Zhang C, Zhang W, Shen Y.H., Li H.L., Liu R.H., Zhang X, Hu X.J. & Zhang W.D. (2008). Qualitative and quantitative determination of the major coumarins in

- Zushima by high performance liquid chromatography with diode array detector and mass spectrometry. *J. Chromatogr. A* 1216 (2009) 2111–2117
- Sun Y, Guo T, Sui Y & Li F. (2003). Fingerprint analysis of *Flos Carthami* by capillary electrophoresis. *J. Chromatogr. B* 792, (2003), pp.147–152, 1570-0232
- Tian R.T., Xie P.S. & Liu H.P. (2008). Evaluation of traditional Chinese herbal medicine: Chaihu (*Bupleuri Radix*) by both high-performance liquid chromatographic and high-performance thin-layer chromatographic fingerprint and chemometric analysis. *J. Chromatogr. A* 1216, (2009), pp.2150–2155, 0021-9673
- The State Pharmacopoeia Commission of People's Republic of China (2005). Pharmacopoeia of the People's Republic of China Beijing: Chemical Industry Press (2005), 7-117-06983-X/R 6984
- Toh D.F., New L.S., Koh H.L. & Chan E.C.Y. (2009). Ultra-high performance liquid chromatography/time-of-flight mass spectrometry (UHPLC/TOFMS) for time-dependent profiling of raw and steamed *Panax notoginseng*. *J. Pharm. Biomed. Anal.* 52, (May 2010), pp.43–50, 0731-7085
- Wang H.L., Yao W. F., Zhu D.N. & Hu Y.Z. (2010). Chemical Fingerprinting by HPLC-DAD-ELSD and Principal Component Analysis of *Polygala japonica* from Different Locations in China. *J. Chinese Journal of Natural Medicines* 8, sep.2010), pp.343–348
- Wei H., Sun L., Tai Z., Gao S., Xu W. & Chen W. (2010). A simple and sensitive HPLC method for the simultaneous determination of eight bioactive components and fingerprint analysis of *Schisandra sphenanthera*. *Anal. Chim. Acta* 662, (2010), pp.97–104, 0003-2670
- Xie P.S., Chen S.B., Liang Y.Z., Wang X.H., Tian R.T. & Roy Upton (2006). Chromatographic fingerprint analysis—a rational approach for quality assessment of traditional Chinese herbal medicine *J. Chromatogr. A* 1112 (2006), pp.171–180, 0021-9673
- Xu L, Li-Juan Tang, Chen-Bo Cai, Hai-Long Wu, Guo-Li Shen, Ru-Qin Yu & Jian-Hui Jiang. (2008) Chemometric methods for evaluation of chromatographic separation quality from two-way data—A review. *analytica chimica acta* 613, (2008), pp.121–134, 0003-2670
- Yang, J., Chen, L.H., Zhang, Q., Lai M.X., & Wang Q. (2007). Quality assessment of Cortex cinnamomi by HPLC chemical fingerprint, principle component analysis and cluster analysis. *Journal of Separation Science* 30, (2007), pp.1276–1283, 1615-9306
- Zeng Y.-X., et al. (2007). Comparative analysis of volatile components from *Clematis* species growing in China. *Analytica Chimica Acta*, 595, (2007) pp.328–339, 0003-2670
- Zhang YY, Dan M, Wu J.-B, Yang H.-Z, Huang H, Qi Y, Wei S.-D., Toru Okuyama, & Kaoru Nakajima. (2008). Study on the Chromatographic Fingerprinting of *Schisandra chinensis* (Turcz.) Baill. by LC Coupled with Principal Component Analysis. *Chromatographia* 68 (July 2008), pp.101-104, 0009-5893
- Zeng Z.D., Foo-tim Chau, Hoi-yan Chan, Chui-yee Cheung, Tsui-yan Lau, Wei S.Y., Daniel Kam-wah Mok, Chi-on Chan & Liang Y.Z. (2008). Recent advances in the compound-oriented and pattern-oriented approaches to the quality control of herbal medicines. *Chinese medicine*. (August 2008), pp.3:9
- Zhou F.R., Zhao M.B., Tu P.F. (2008). Qualitative evaluation and quantitative determination of 10 major active components in *Carthamus tinctorius* L. by high-performance

- liquid chromatography coupled with diode array detector. *J. Chromatogr. A* 1216, (2009), pp.2063–2070, 0021-9673
- Wang C.Z., Li P., Ding J.Y., Peng X. & Yuan C.S. (2007). Simultaneous identification of *Bulbus Fritillariae cirrhosae* using PCR-RFLP analysis. *Phytomedicine*. 14, (2007), pp. 628-32, 0944-7113
- Wang L.C., Cao Y.H., Xing X.P. & Ye J.N. (2005). Fingerprint Studies of *Radix Scutellariae* by Capillary Electrophoresis and High Performance Liquid Chromatography. *Chromatographia*. 62, (July 2005), pp.283–288, 0009-5893.
- Zhang X., Xu Q., Xiao H., Liang X., Huang L. & Liu (2006). *J. World Sci. Tech./Modern Trad. Chin. Med. Mat. Med.* 8, (2006), pp.33.
- Zheng X.T., Shi P.Y., Cheng Y.Y. & Qu H.B. (2008). Rapid analysis of a Chinese herbal prescription by liquid chromatography–time-of-flight tandem mass spectrometry. *J. Chromatogr. A*. 1206, (2008), pp.140-146, 0026-9673

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The authors of this thematic issue provide a comprehensive summary of most recent knowledge and references on quality control in wide fields. Quality control is essential for natural products like natural medicine and related food products. In this issue fifteen chapters have been included, discussing in detail various aspects of quality control. It will certainly prove useful not only for phytochemical researchers, but also many scientists working in numerous fields. Much effort has been invested by the contributors to share current information. Without their efforts and input 'Quality Control of Herbal Medicine and Related Areas' could not exist.

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