A holistic strategy for quality and safety control of traditional Chinese medicines by the “iVarious” standard system

Anzhen Chen,1, Lei Sun,1, Hang Yuan,1, Aijing Wu,1, Jingguang Lu,1, Shuangcheng Ma

Abstract

An effective quality control system is the key to ensuring the quality, safety and efficacy of traditional Chinese medicines (TCMs). However, the current quality standard research lacks top-level design and systematic design, mostly based on specific technologies or evaluation methods. To resolve the challenges and questions of quality control of TCMs, a brand-new quality standard system, named “iVarious”, was proposed. The system comprises eight elements in a modular format. Meaning of every element was specifically illustrated via corresponding research instances. Furthermore, frankincense study was taken as an example for demonstrating standards and research process, based on the “iVarious” system. This system highlighted a holistic strategy for effectiveness, security, integrity and systemization of quality and safety control standards of TCMs. The establishment of “Various” integrates multi-disciplinary technologies and progressive methods, basis elements and key points of standard construction. The system provides a novel idea and technological demonstration for regulation establishment of TCMs quality standards.

1. Introduction

Traditional Chinese medicines (TCMs) refer to drugs used under the guidance of traditional Chinese medical theory. The origin, growing environment, collection time, processing method and preparation technology are all important factors influencing safety and effectiveness of TCMs. Hence, setting up an effective quality control system is the key to ensuring the quality and clinical efficacy of TCMs. Several countries issued guidelines for the establishment of quality standards. For example, the Chinese Pharmacopoeia Commission compiled the “National Drug Standards Work Manual” [1], used for research, development and application of drug standards. However, these guidelines emphasized technical requirements, writing specifications, etc. Many scholars have proposed the holistic quality control methods for TCMs in recent studies. Guo et al. [2] developed an overall quality control strategy for TCMs based on a combination of fingerprint evaluation and multi-components determination. Wang et al. [3] evaluated the safety and effectiveness of TCMs using metabolomics. Xiong et al. [4] proposed biological potency analysis for the quality control of TCMs. However, these strategies were based on specific technologies or evaluation methods, and lacked top-level design and systematic design. Therefore, a brand-new quality standard system, named “iVarious”, was proposed in the present paper. The system comprises eight elements in a modular format. The meaning of every element was specifically illustrated via corresponding research instances. Furthermore, frankincense study was taken as an example for demonstrating standards and research process, based on the “iVarious” system. The “iVarious” standard system is a novel, advanced, comprehensive and systematic quality standard research paradigm of TCMs.

2. Elements of “iVarious” standard system

The “iVarious” standard system comprises eight elements: i, V, a, r, i, o, u, and s standing for information, variety, alternative, rapidity, ion (mass spectrum), overall, uniformity, and substitute and safety, respectively (Fig. 1).

The element of information (i) involving “digitization”, “artificial intelligence”, and “Internet” is the digital form of quality standard. The other seven research elements represent sample collection (V), the set of items (a), the choice of methods (r, u and i), holistic quality evaluation (o and s), and safety (s) test, respectively. The seven
research elements are not independent but can be combined with each other.

2.1. V: principle of variety

The representativeness and accuracy of samples are the basis of standard formulation. So the variety of samples is the primary principle of standard research. Crude drug of TCMs may be collected from different original plants and different habitats (including genuine producing area and main producing area, etc). The existence of adulterants is also a problem that cannot be ignored. Therefore, all the above factors should be taken into account in sample collection of crude drug. The quality of preparation may be affected by these factors such as enterprises, raw materials and production technology. So the preparation samples should be collected from different enterprises and the batches from the same enterprise should be sufficient.

According to the National Drug Standards Work Manual (fourth edition) [1], our recommendations for sample collection are as follows.

(1) Crude drug: Samples with multiple original plants should be collected no less than 5 batches for each. Samples with single original plant should be collected no less than 20 batches. Meanwhile, samples from genuine producing area or main producing area should be collected no less than 3 batches, respectively. Confused samples and adulterants should also be collected no less than 3 batches for each.

(2) Preparation: Exclusive drug should be collected at least 10 batches for each. The samples produced by different enterprises should be collected from each enterprise. If the drugs were produced by more than five enterprises, samples should be collected from at least 5–10 enterprises. Each enterprise should be no less than 3 batches.
2.2. a: principle of alternative

The principle of alternative means that thin-layer chromatography (TLC) identification is an optional item. That is to say, when the same or similar components are tested by both TLC identification and high performance liquid chromatography/gas chromatography (HPLC/GC) assay in the same drug standard, conducting HPLC/GC assay is enough. In other cases, if HPLC/GC assay cannot be performed, TLC identification should be carried out. Fig. 2 shows the TLC identification and HPLC assay chromatogram of five different free anthraquinones in rhubarb according to "Chinese Pharmacopoeia (ChP)" [5]. As shown in Fig. 2, both TLC and HPLC provide nearly the same information involving the five types of free anthraquinones in rhubarb. Therefore, if HPLC assay was performed, TLC identification is not necessary to conduct. Applying this principle is conducive to saving resources, reducing environmental pollution and improving efficiency.

2.3. r: principle of rapidity

The principle of rapidity refers to the application of ultra-high performance liquid chromatography (UHPLC), ultra high performance liquid chromatography (UHPLC), rapid resolution liquid chromatography (RRLC) and ultra fast liquid chromatography (UFLC) with chromatographic columns packed with sub-2 µm and sub-3 µm particles. UPLC offers clear advantages over conventional HPLC in peak resolution, analytical efficiency, solvent consumption and sensitivity. It is widely used for the separation and analysis of complex systems. The advantages of UHPLC are discussed in detail in “New technology of drug detection from HPLC to UHPLC” [6]. Nováková et al. [7] and Wren et al. [8] proved that compared with the HPLC, the UPLC allows shortening analysis time up to nine times, and the separation efficiency is even higher. For the quality control of rhubarb, we found that the analytical time can be shortened from 80 min by HPLC with 5 µm particle column to 25 min by UPLC with 1.8 µm or 2.7 µm particle column, with the similar separation efficiency (Fig. 3). Using a column packed with sub-3 µm particles (Fig. 3B), the analytical speed, resolution and efficiency were basically equivalent to a sub-2 µm chromatographic column (Fig. 3C), while pressure decreased by 40%–50%. Therefore, columns packed with sub-3 µm particles may be more suitable for TCMs analysis.

At present, UPLC has been widely adopted in pharmacopoeias. In the ChP (2010 edition), the first application of UPLC was used for the determination and fingerprint analysis of danshen dropping pill [9]. The ChP (2015 edition) [5] expanded the UPLC application to 5 monographs for multi-components determination. At the end of 2016, there were 57 monographs using UPLC technology in the United States Pharmacopoeia (USP) [40].

2.4. i: principle of ion (mass spectrometry)

The principle of mass spectrometry includes structural elucidation and qualitative/quantitative analysis.

2.4.1. Structure elucidation

Mass spectrometry can provide the structural information of compounds and is widely used for chromatographic peak identification for fingerprint analysis. For example, during the investigation of an appropriate substitute of Acanthopanax senticosus Harms, Zhang et al. [10] identified and characterized 131 compounds in the leaf using ultra-performance liquid chromatography-quadrupole time-of-flight mass spectrometry (UPLC-QTOF-MS). Zhou et al. [11] identified 61 characteristic compounds in Siraitiae fructus using ultra-high performance liquid chromatography coupled with photo-diode array and quadrupole/time-of-flight mass spectrometry (UPLC-PDA-QTOF-MS). Zou et al. [12] identified 106 compounds in Wuzi Yanzong Wan by ultra-performance LC coupled with ESI-linear ion trap-Orbitrap tandem mass spectrometry (UPLC-ESI-LTQ-Orbitrap-MS).

In addition, the identification of compounds can be conducted more conveniently and quickly by establishing a database of mass spectra. For example, NIST Mass Spectral Library [13] recorded a total of 64,000 gas chromatography mass spectrometry spectra. Tu et al. [14] established a liquid chromatography-mass spectrometry-database (LC-MS-DS) containing more than 600 compounds. Qian et al. [15] established a mass spectrometry database containing more than 210 compounds, including alkaloids, flavonoids, quinones, phenylpropa-noids, and terpenoids compounds by ultra-high performance liquid chromatography electrospray ionization quadrupole time-of-flight mass spectrometry (UPLC-Q-TOF-MS).

2.4.2. Qualitative/quantitative analysis

Mass spectrometric analysis has several advantages, including ultra-high sensitivity and ultra-strong qualitative capability. Therefore, the hyphenated techniques, such as LC–MS and GC–MS, are widely used for the qualitative and quantitative analysis of TCMs. Complete chromatographic separation is not necessary for LC–MS or GC–MS analysis, especially for the detection of trace components, such as illegal addition of chemicals [16], illegal dyes [17], pesticide residues [18], endogenous toxic compounds [9], and animal gelatins [19]. Inductively coupled plasma mass spectrometry (ICP–MS) is used in elemental analysis and speciation analysis [20]. In combination with capillary electrophoresis (CE), it can be used as a supplement of LC–MS for analysis of TCMs and natural medicine [21]. The LC–MS methods used for determination of adomifoline in Senecionis Scandentis Hebra, test of toosendanin in Toosendan Fructus and Meliae Cortex, and identification of Asini CoriiColla are included in the ChP.

2.5. o: principle of overall

The principle of overall is mainly reflected in the fingerprint/characteristic chromatogram, multi-components determination, quality marker (Q-marker) and bioactivity assay.
2.5.1. Fingerprint/characteristic chromatogram

Fingerprint/characteristic chromatogram analysis has been widely used to evaluate the integrity, authenticity, consistency and stability of TCMs. Only one HPLC characteristic chromatogram was included in the ChP 2005 edition [22], and fingerprint/characteristic chromatogram tests were added in the 2010 edition, and more than 30 in the 2015 edition. It shows that the official quality standard pays more and more attention to the application of fingerprint/characteristic chromatogram in the overall quality evaluation of TCMs.

The combination of fingerprint analysis with chemometrics has been widely adopted in the overall quality control of TCMs preparations. Wang et al. [23] evaluated the inter-batch consistency of Xinkeshu tablet using four types of chromatographic fingerprints combined with chemometrics. Gong et al. [24] evaluated sample uniformity in different batches of Yindan Xinaotong soft capsules using UPLC fingerprint and similarity evaluation. Using partial least squares discriminant analysis and pattern recognition, Chen et al. [25] established two prediction models, which rapidly distinguished genuine from fake rhubarb species and three plant species.

2.5.2. Multi-components determination

The fingerprint generally pays more attention to qualitative analysis characterized by fuzziness, while the multi-components determination focuses on quantitative aspects. Therefore, fingerprint combined with multi-components determination represents an effective strategy for comprehensive quality assessment of TCMs. For example, the strategy has been used for the evaluation of Xiaoer Chaigui Tuire granules, Zhizhu pill and Saposhnikoviae Radix [26–28]. Sun et al. [29] and Zhang et al. [30] realized the identification of three species of frankincense and the determination of six boswellic acids by fingerprints and chemometrics analysis.

2.5.3. Quality marker (Q-marker)

Quality marker (Q-marker) is a new concept following the “property-effect-component” theory, first proposed by Liu et al. [31,32] and Yang et al. [33]. Q-markers are a variety of activity components from either primary or secondary metabolites generated in plants, as indicator ingredients to reflect quality safety and effectiveness of TCMs. Study and establishment of Q-markers is conducted to quality assessment and process control of TCMs products. Q-markers have both qualitative and quantitative attributes. Therefore, the new strategy for establishment of fingerprint and multi-components determination based on Q-markers is expected to promote more scientific quality research and standard system of TCMs in terms of safety and effectiveness.

2.5.4. Bioactivity assay

Biological assay is a method of determining the effectiveness of drugs using biological activities as a tool. ChP (2010 edition) [9] recorded “guidelines of bioactivity determination of TCMs”, and affirmed the role of bioactivity determination in the quality control of TCMs. However, the applications were limited to Digitalis preparations [34], Hirudo [9] and Xiaoshuan enteric-coated capsules [35] developed using the biological potency estimation. Experts used bioactivity fingerprint to evaluate the anti-inflammatory activity of aconiti radix and aconiti radix cocta [36] and the antibacterial activity of Qingkailing injection [37]. Bioactivity assays can make the quality evaluation related to the clinical safety and efficacy.

2.6. u: principle of uniformity

The principle of uniformity is to unify the sample pretreatment methods, chromatographic conditions, and quality standards of different dosage forms.

(1) The uniformity of sample pretreatment methods refers to establishing a same pretreatment method of different testing items, such as TLC/LC/GC identification, determination and fingerprint/characteristic chromatogram, as far as possible.

(2) The uniformity of chromatographic conditions refers to establish-
2.7. s: principle of substitute

The overall quality control of TCMs based on multi-components determination or fingerprint is limited because of the lack of reference substances (RS) and high cost. The “substitute reference substance” method is worth considering for resolving the problem, which includes “single indicator to determine multi-components (SSDMC)/quantitative analysis of multi-components by single marker (QAMS)”, “two reference substances for simultaneous determination of multiple components (TRSDMC)”, and “extractive reference substance (ERS)” [38].

2.7.1. SSDMC/QAMS

As a relatively mature substitute method, SSDMC/QAMS is based on relative retention time (RRT) method for peak identification and relative correction factor for quantitative analysis. It has been used in several official standards such as ChP, European Pharmacopoeia (EP) [39], and the United States Pharmacopoeia (USP) [40]. It has also been widely used in studies involving multi-components determination of *Ganoderma lucidum* [41], honeysuckle [42], rhubarb [43], *Euphorbia kansui* [44], *Semen araji* [45], *Cinnamomi ramulus* [46], *Allisma orientale* [47], *Houttuyniae herba* [48], notoginseng [49], *Mahoniae caulis* [50], and other medicinal herbs, which has a good application prospect.

2.7.2. TRSDMC

Due to the diversity of HPLC instrument and chromatographic column, the RRT was difficult to reproduce on different chromatographic systems, as well as the relative correction factor [51,52]. Hence, the practical application of RRT method is restricted. TRSDMC method is proposed [53–56] to resolve the problem of qualitative deviation. TRSDMC utilizes linear calibration using two reference substances (LCTRS) for peak identification (Fig. 4) [56]. The method is accurate and robust for more chromatographic columns. The LCTRS method has been successfully used for analysis of rhubarb [57], *Swertia Chirayita* [58], *Semen Strychni* [59] and other medicinal herbs, with better practical value and application prospect.

2.7.3. ERS

ERS is a kind of RS used for qualitative identification and multi-components determination, which is a mixture of effective or marker components obtained by specific extraction. The development and application of ERS for the overall quality control of TCMs were introduced in detail [60,61]. Based on the usage, ERS is categorized into qualitative and quantitative types.

Qualitative ERS is mainly used to identify chromatographic peaks. Qualitative ERS is widely used in EP and USP, and less in ChP. Nearly half of the crude drugs used ERS as the RS for TLC identification in EP and USP. In the USP, liquid chromatography also used ERS as the RS. In ChP (2015 edition) [5], only 15 ERS were used for TLC/LC identification and fingerprint/characteristic chromatogram analysis.

The quantitative ERS used for multi-components determination clearly indicated the content of each component. Only three quantitative ERS were contained in ChP (2015 edition) [5], including *Panax notoginseng* saponins, ginkgolides and mahonine caul. The application of quantitative ERS in EP and USP is used more extensively than in ChP.

2.8. s: principle of safety

Safety and effectiveness are the basic properties of drugs. Therefore, quality standards should not only focus on effectiveness study, but also pay attention to safety study. ChP (2000 edition), for the first time, recorded the safety index of TCMs. In the 2015 edition, four technical guidelines and seven testing methods of safety were added and revised. The detection indices of sulfur dioxide, heavy metals, pesticide residues, aflatoxin and other contaminants in more than 30 drugs were added, respectively, indicating that the safety indices represent an important part of the quality standards. Therefore, it is beneficial to develop a scientific, objective and standardized safety evaluation of TCMs by establishing the testing method, database and risk assessment system.

The principle of safety includes the analysis of endogenous and exogenous toxic compounds and the construction of risk assessment system.

2.8.1. Analysis of endogenous toxic compounds

Endogenous toxic compounds of TCMs include the chemical components containing toxicity or side effect. In order to ensure the safety, it is critical to strengthen the quality control and risk monitoring of endogenous toxic compounds, setting limits when necessary. Lots of testing methods of endogenous toxic compounds in Chinese herbs have been established in ChP (2015 edition) [5], such as HPLC detection of dieters alkaloids in *radix aconiti preparata*, *radix aconiti kusnezooffi preparata* and monkshood, strychnine in Nux vomica, cantharidin in cantharis, and other crude drugs. The testing limits of toxic compounds in *domestic senecio*, toosendan and *melliae cortex* were analyzed by HPLC-MS.

2.8.2. Analysis of exogenous toxic compounds

Exogenous toxic compounds mainly include heavy metals and harmful elements, pesticides, mycotoxins, harmful additives and organic pollutants, irradiation, veterinary drugs and antibiotics. Environmental pollution, non-standardized planting, processing and storage, and other factors contribute to excessive accumulation of exogenous toxic residues. The residues of heavy metals and harmful elements [62], pesticide residues [63], mycotoxins [64], and pigment residues [65] are considered to be the most primary exogenous toxic residues and seriously affect the safety of TCMs [66]. The testing methods and maximum residue limits (MRLs) were set in the pharmacopoeia of each country. For example, ChP includes the guidelines for the detection and MRLs of heavy metals and harmful elements, pigments, mycotoxins and other residues.

2.8.3. Risk assessment

Risk assessment is mainly used to evaluate the necessity of toxic compounds and set reasonable MRLs. ChP (2015 edition) includes “the guiding principle for the MRLs of harmful residues in TCMs” [5], guidelines setting the MRLs for exogenous toxic compounds based on the perspective of risk assessment.

Ma et al. [67] established testing methods for nearly 300 types of pesticide residues, 19 heavy metals and harmful elements, nearly 30 common pigment residues, mycotoxins and sulfite residues, and carried out screening tests for toxic residues in more than 200 commonly used TCMs. Statistical analysis of the data, for the first time, facilitated the external exposure evaluation of key exogenous toxic residues in TCMs. Based on the MRLs, the risk control of toxic residues in TCMs was explored, and the risk assessment system was preliminary established.

2.9. i: principle of information

The principle of information was integrated with concepts of “digitization, artificial intelligence, and Internet” to establish an
information platform and facilitate digital storage, update, search, comparison and statistical analysis of quality standards.

The platform of “digital standards of Chinese medicinal materials” developed by Chinese Pharmacopoeia Commission presents national standards and corresponding atlas and chromatograms in digital form, and provides statistical functions of quality standards in different national Pharmacopoeias [68]. The concept of digital reference standards (DRS) [69] has recently been proposed, substituting the qualitative and quantitative functions of physical RS in digital form. The ten characteristics corresponding to five aspects of DRS are shown in

Fig. 4. Diagrammatic sketches of LCTRS [56].
Fig. 5, which integrate digitization, data analysis and Internet of the physical RS.

The quality standard based on the information platform represents the new drug quality standard in the age of Internet. The advantages include abundant contents, rapid updates, easy-to-use and intelligent statistical functions, suggesting an important developmental trend in the future of quality standards.

3. Application and demonstration of “iVarious” standard system

Frankincense is a commonly used TCM for the treatment of inflammatory diseases such as arthritis and asthma. Therefore, frankincense study (Fig. 6) is taken as an example for demonstrating standards and research process, based on “iVarious” system. It is
generally known that quality standards include character, identification, test and assay. Based on the principles of “Various” system, research involving the quality control of frankincense includes eight steps [29,30,55]. The first step is sample collection. Following the principle of variety, a total of 55 batches of samples containing 15 batches of Somalia frankincense, 37 batches of Ethiopia frankincense and three batches of India frankincense were collected. The second step is the study of sample pretreatment methods. Following the principle of uniformity, the same pretreatment method for different items, including TLC identification, multi-components determination and fingerprint was established. Samples were ultrasonic extracted with methanol for 30 min. The third step involves the study of chromatographic conditions. Following the principle of uniformity and rapidity, the same LC analytical condition, including multi-components determination and fingerprint was established. The fourth step is the study of TLC identification. Following the principle of alternative, the TLC of frankincense showed spots of diterpene and sesquiterpenoid components, which were not all included in the HPLC chromatogram of determination [70]. So the TLC identification item cannot be alternative. The fifth step represents the study of test items. Following the principle of safety, ion and rapidity, illegally added rosin and exogenous toxic compounds were involved. The sixth step includes six boswellic acid in frankincense analyzed via UHPLC and TRSDMC, following the principle of overall and substitute. The seventh step represents the study of fingerprint analysis. Following the principle of ion and overall, the fingerprint contained 26 chromatographic peaks, of which 14 were identified by QTOF-MS method. The data were analyzed by multivariate statistical analysis including hierarchical cluster analysis (HCA) and principal component analysis (PCA). The eighth step is to build a digital platform. Based on the above studies and comprehensive data analysis, the final test items were confirmed to construct the quality standard of frankincense.

It should be noted that the “Various” standard system was proposed according to the research phase, and represented the holistic principles of quality control research. We advocated integrity, comprehensiveness, profundity and innovation in the research phase but conciseness, controllability, practicability and feasibility for the final standard. Therefore, each element of “Various” standard system should be applied according to the specific drug, adapted and simplified when necessary, to ensure the integrity, harmonization, simplicity, practicality, and controllability of the standards.

4. Conclusions

One of the key point of modernization and globalization of TCMs is to establish a scientific and rational quality standard system. In this paper, the “Various” standard system was proposed with the characteristics of informatization, comprehensiveness, systematization and simplicity. The system is based on the information platform, and integrates various evaluation methods by a modular format to ensure the safety, effectiveness and consistency of drugs. With the development of modern analytical technologies and in-depth research of quality control of TCMs, the eight elements of “Various” system may not be able to cover all the aspects of quality research. However, it provides a new strategy to conduct comprehensive quality and safety evaluation of TCMs.

Conflicts of interest

The authors declare that there are no conflicts of interest.

Acknowledgments

We are grateful for financial support from National Major Scientific and Technological Special Project for Significant New Drugs Development” (2014ZX093043407-002), Youth Development Research Foundation of NIFDC (2013WAB) and the National Natural Foundation of China (81330321).

References

[1] Chinese Pharmacopoeia Commission, National Drug Standards Manual, 4th ed., China Medicine Science and Technology Press, Beijing, 2013.
[2] D.A. Guo, W.Y. Wu, M. Ye, et al., A holistic approach to the quality control of traditional Chinese medicines, Science 347 (Suppl. 6219) (2015) S28–S31.
[3] P.C. Wang, Q.H. Wang, B.Y. Yang, et al., The progress of metabonomics study in traditional Chinese medicine research, Am. J. Chin. Med. 43 (2015) 1281–1310.
[4] Y. Xiong, D. Yan, J.B. Wang, et al., Bioprospection assay: an integrated application to quality control of Chinese medicinal materials, Chin. Herb. Med. 6 (2014) 256–264.
[5] Chinese Pharmacopoeia Commission, Chinese Pharmacopoeia, 2015 Edition Part I, China Medical Science and Technology Press, Beijing, 2015.
[6] S.C. Ma, Drug Testing New Technology: From HPLC to UHPLC, People’s Medical Publishing House, Beijing, 2012.
[7] L. Nováková, L. Matysová, P. Solich, Advantages of application of UPLC in pharmaceutical analysis, Talanta 68 (2006) 908–918.
[8] S.A.C. Wren, P. Tchelitchev, Use of ultra-performance liquid chromatography in pharmaceutical development, J. Chromatogr. A 1119 (2006) 140–146.
[9] Chinese Pharmacopoeia Commission, Chinese Pharmacopoeia, 2010 Edition Part I, China Medical Science and Technology Press, Beijing, 2010: 906–907.
[10] Y.Z. Zhang, A.H. Zhang, Y. Zhang, et al., Application of ultra-performance liquid chromatography with tandem mass spectrometry for the rapid analysis of constituents and metabolites from the extracts of Acanthopanax senticosus harms leaf, Pharmacogn. Mag. 12 (2016) 145–152.
[11] G.S. Zou, M.F. Wang, Y. Li, et al., Comprehensive analysis of 61 characteristic constituents from Sirafrutes fructus using ultrahigh-pressure liquid chromatography with time-of-flight mass spectrometry, J. Pharm. Biomed. Anal. 125 (2016) 1–14.
[12] D.X. Zou, J.F. Wang, B. Zhang, et al., Analysis of chemical constituents in Wu-zhuyang-Wan by UPLC-ESI-ITQ-orbitrap-MS, Molecules 20 (2015) 21373–21404.
[13] National Institute of Standards and Technology, NIST Mass Spectral Library, (http://www.nist.gov).
[14] P.F. Tu, S.P. Shi, Y. Jiang, Strategies and approaches on exploring material basis of Chinese materia medica, Chin. Tradit. Herb. Drugs 43 (2012) 209–215.
[15] Y.F. Qian, E.X. Shang, J.A. Duan, et al., Construction of a rapid identification method for chemical constituents in traditional Chinese medicine based on liquid chromatography-hybrid mass spectrometry-database, Chin. J. Chin. Mater. Med. 37 (2012) 3256–3263.
[16] Z.Q. Huang, S. Xiao, D. Luo, et al., Simultaneous determination of sulfathiazole and N-Deisethemylsulfathiazole in dietary supplements for weight control by HPLC-ESI-MS, J. Chromatogr. Sci. 46 (2008) 707–711.
[17] A.Z. Chen, X.R. Li, L. Sun, et al., Simultaneous determination of six illegal dyes in Die da pills by UPLC-MS/MS, Chin. J. Pharm. Anal. 35 (2015) 157–162.
[18] L.N. Chen, F.R. Song, Z.Q. Liu, et al., Multi-residue method for fast determination of pesticide residues in plants used in traditional Chinese medicine by ultra-high-performance liquid chromatography coupled to tandem mass spectrometry, J. Chromatogr. A 1225 (2012) 132–140.
[19] X.L. Cheng, F. Wei, X.Y. Xiao, et al., Identification of five gelatins by ultra performance liquid chromatography/time-of-flight mass spectrometry (UPLC-TOF-MS) using principal component analysis, J. Pharm. Biomed. Anal. 62 (2012) 191–195.
[20] E.S. Ong, T.L. Yong, S.O. Woo, Determination of arsenic in traditional Chinese medicine by microwave digestion with flow injection-inductively coupled plasma mass spectrometry (FI-ICP-MS), J. AOAC Int. 82 (1999) 963–967.
[21] J.X. Liu, Y.W. Zhang, F. Yuan, et al., Differential detection of Rhizoma coptidis by capillary electrophoresis electroosorption ionization mass spectrometry with a nanospray interface, Electrophoresis 35 (2014) 3258–3263.
[22] Chinese Pharmacopoeia Commission, Chinese Pharmacopoeia, 2005 Edition Part I, China Medical Science and Technology Press, Beijing, 2005.
[23] P. Wang, L. Nie, H.C. Zang, Useful strategy to evaluate the quality consistency of traditional Chinese medicines based on liquid chromatography and chemometrics, J. Anal. Methods Chem. 2015 (2015) 1–11.
[24] L.L. Gong, H.Y. Xu, L. Wang, et al., Identification and evaluation of the chemical similarity of Yindan xinnaotong samples by ultra high performance liquid chromatography with quadrupole time-of-flight mass spectrometry fingerprinting, J. Sep. Sci. 39 (2016) 611–622.
[25] A.Z. Chen, W.F. Jiang, H. Yuan, et al., Establishment of authenticity and species prediction models of Rhei Radix et Rhizoma based on UPLC-PLS-DA, Chin. Pharm. J. 51 (2016) 197–201.
[26] H.M. Liu, L. Nie, Quantitative analysis combined with chromatographic fingerprint for comprehensive evaluation of Xiaoxi Chaqiu Tiuer granules by HPLC-DAD, J. Chromatogr. Sci. 53 (2015) 749–756.
[27] H. Sun, X. Chen, A.H. Zhang, et al., Chromatographic fingerprinting analysis of Zhuzhu Wan preparation by high performance liquid chromatography coupled with photodiode array detector, Pharmacoag. Mag. 10 (2014) 470–476.
[28] J.L. Liao, Q. Li, R. Liu, et al., Fingerprint analysis and multi-ingredient determination using a single reference standard for Saposhnikoviae radix, Anal. Sci. 30 (2014) 1157–1163.
[29] L. Sun, C. Zhang, R.T. Tian, et al., A holistic strategy of identification and quality evaluation of Frankincense by chromatographic fingerprint combined with chemometrics, Chin. Pharm. J. 50 (2015) 140–146.
X.Y. Gao, Y. Jiang, J.Q. Lu, et al., A new concept on quality marker of Chinese materia medica: quality control for Chinese medicinal products, Chin. Tradit. Herb. Drugs 47 (2016) 1443–1457.

C.X. Liu, S.L. Chen, X.H. Xiao, et al., A new concept on quality marker for quality assessment and process control of Chinese medicines, Chin. Herb. Med. 9 (2017) 3–13.

W.Z. Yang, Y.B. Zhang, W.Y. Wu, et al., Approaches to establish Q-markers for the quality standards of traditional Chinese medicines, Acta Pharm. Sin. B 7 (2017) 439–446.

C.Q. Wang, X.H. Jia, S. Zhu, et al., A systematic study on the influencing parameters and improvement of quantitative analysis of multi-component with single marker using notoginseng as research subject, Talanta 134 (2015) 587–595.

W.G. Wang, X.L. Ma, X.Y. Guo, et al., A series of strategies for solving the shortage of reference standards for multi-components determination of traditional Chinese medicine, Mahoniae Caulis as a case, J. Chromatogr. A 1412 (2015) 100–111.

J.J. Hou, W.Y. Wu, J. Da, et al., Ruggedness and robustness of conversion factors in method of simultaneous determination of multi-components with single reference standard, J. Chromatogr. A 1218 (2011) 5618–5627.

T.W. Yang, C. Zhao, Y. Fan, et al., Design of ultraviolet wavelength and standard solution concentrations in relative response factors for simultaneous determination of multi-components with single reference standard in herbal medicines, J. Pharm. Biomed. Anal. 114 (2015) 280–287.

L. Sun, H.Y. Jin, Y. Pang, et al., Two reference substances for determination of multiple components (I): linear calibration using two reference substances for identification of chromatographic peaks, Chin. J. Pharm. Anal. 33 (2013) 1424–1430.

J.Y. Yu, W. Zhang, X.X. Hong, et al., Idea and design for digitization of Chinese medicine, Mahoniae Caulis as a case, J. Chromatogr. A 1218 (2011) 5618–5627.

B. Dai, H.Y. Jin, J.G. Tian, et al., Progress achieved in determination method of intrinsic harmful residues in traditional Chinese medicine, Chin. J. Pharm. Anal. 33 (2013) 1177–1182.

L. Sun, H.Y. Jin, R.T. Tian, et al., A simple method for HPLC retention time prediction: linear calibration using two reference substances, Chin. Med. 12 (2017) 16–27.

L. Sun, Studies on Using Substitute Reference Substance in Quality Control of Rhubarb and Rhubarb Preparations, National Institutes for Food and Drug Control, Beijing, 2014.

L.N. Liu, L. Sun, R.T. Tian, et al., Fingerprint analysis of Swertia chirita by linear calibration using two reference substances with FDA assistance, Chin. Pharm. J. 50 (2015) 287–292.

F. Qiao, L. Sun, H.Y. Jin, et al., Fingerprint analysis of Semen Strychni by chemometrics and linear calibration using two reference substances, Chin. Pharm. J. 49 (2014) 886–889.

P.S. Xie, S.C. Ma, P.F. Tu, et al., The prospect of application of extractive reference substance of Chinese herbal medicines, Chin. Med. 4 (2013) 125–136.

P. Chen, H.Y. Jin, L. Sun, et al., Application of extractive reference substance in holistic quality control of traditional Chinese medicine, Chin. Pharm. Anal. 36 (2016) 185–195.

B. Dai, H.Y. Jin, J.G. Tian, et al., Progress achieved in determination method of extrinsic harmful residues in traditional Chinese medicine, Chin. Pharm. Anal. 28 (2008) 1014–1019.

L.L. Hao, J. Xue, Multiresidue analysis of 18 organochlorine pesticides in traditional Chinese medicine, J. Chromatogr. Sci. 44 (2006) 518–522.

L.X. Fang, A.Z. Xiong, R. Wang, et al., A strategy for screening and identifying mycotoxins in herbal medicine using ultra-performance liquid chromatography with tandem quadrupole time-of-flight mass spectrometry, J. Sep. Sci. 36 (2013) 3115–3122.

A.Z. Chen, X.R. Li, L. Sun, et al., Simultaneous determination of six illegal dyed fabrics in Die Da pill by UPLC MS/MS, Chin. J. Pharm. Anal. 35 (2015) 157–162.

L.L. Wang, W.J. Kong, M.H. Yang, et al., Safety issues and new rapid detection methods in traditional Chinese medicinal materials, Acta Pharm. Sin. B 5 (2015) 38–46.

S.C. Ma, H.Y. Jin, L.N. Liu, et al., Risk control of exogenous harmful residues in traditional Chinese medicines, Chin. Pharm. J. 50 (2015) 99–103.

J.Y. Yu, W. Zhang, X.X. Hong, et al., Idea and design for digitization of Chinese Pharmacopoeia, Chin. Pharm. Aff. 29 (2015) 820–825.

Q.J. Wang, L. Sun, F. Liu, et al., Progress and challenges of reference standard and its new form: digital reference standard, Chin. Med. 7 (2016) 77–91.