Natural Medicine

Quality Control of Chinese Medicine Based on Technology Innovation

Jianbo Wan, PhD, Shaoping Li, PhD, Yitao Wang, PhD, Jing Liu, MD, PhD, Jing X. Kang, MD, PhD

Abstract

With the aim to promote the establishment of international standardization of Chinese medicine (CM), the key scientific techniques, including the selection of the markers, preparation of reference chemical compounds, and the optimization of evaluation methods for standardization of quality control of CM, were discussed and explored in the present paper. A systematic strategy was used to develop the quality control of several CMs attributed to "Huo xue hua yu" and "Fu zheng gu ben". The key techniques and approaches being used included computational clustering analysis of chromatographic data, biochromatographic technique for identification of biological active compound, the combination of different separation techniques for preparation of reference compound, the application of Pressurized Liquid Extraction (PLE) technique for raw herbal sample preparation, and the optimization of quality control analytical techniques. The results showed that the strategy is feasible for the quality control of CM.

[N A J Med Sci. 2009;2(4):152-155.]

Key Words: *Quality control, Markers for quality control, Reference compound; Techniques for quality control, Standardization*

1. Introduction

Chinese medicine (CM), with mild nature and few side effects, is widely considered to be effective in disease prevention and health maintenance, as well as in curing chronic diseases. In recent years, with the rise of the back-to-nature trend and growing concern for treating ageing problem and chronic diseases, the demand of CM has been on the increase in recent years, and the growth potential of the global market is promising. However, it is well-known that quality control is the bottle-neck for CM enters into the world market. Therefore, how to control the quality such as safety, efficacy and stability of Chinese medicine or herbal medicine is very important for globalization of CM.

The obstacles in front of quality control of CM are not only lagging in relative techniques, but also the idea or model of research. To establishment of platform for standardization of quality control of CM, the three main aspects, including the rational marker of quality control, high quality and abundant reference compounds, and feasible analytical means, should be highlighted and tackled well. In the present paper, the systematic strategies and the key scientific techniques were used to develop international standards for quality control of several CMs attributed to "*Huo xue hua yu*" and "*Fu zheng gu ben*".

2. Rational Markers of Quality Control

Scarcity of scientific and rational markers largely limits the development of CM quality control. Actually, the genuine discrimination and grade evaluation are two major functions of quality control of CM. Therefore, according to different aims, the markers of quality control are divided naturally into two levels as follows.

2.1. The marker for discrimination

The substitute and adulterant of CMs, especially the valuable CM, has appeared on occasion in the current market, which was caused perhaps by the faults made during the cultivation, harvest and marketing procedures, usage of the multiple origins of CMs, and substituting and adulterating on purpose as well. In the past decade, the approach for genuine discrimination has been developed from the traditional adjustment of appearance and description of CM, to evaluation using the modern analytical means, the former was based on the rough markers, such as color, smell, micro appearance, and simple physical and chemical property, and the experience should be required, the later was developed by the detection of the major chemical ingredients. Actually, the method to aim directly at its simple component wasn't enough accurate. For example, the ursolic acid was the major ingredient of cherry fruit of *Cornus officinalis*, which was the main component of the famous prescription named "*Liu Wei Di Huang Wan*". If only ursolic acid was selected as the

Jianbo Wan, PhD, Shaoping Li, PhD
Institute of Chinese Medical Sciences, University of Macau, Macau SAR, China
Jianbo Wan, PhD, Jing Liu, MD, PhD, Jing X. Kang, MD, PhD
Department of Medicine, Massachusetts General Hospital and Harvard Medical School, Boston, MA, 02114, USA

Prof. Yi-Tao Wang, PhD (Corresponding Author)
Institute of Chinese Medical Sciences, University of Macau Taipa, Macau, China
Tel: 853-397-4691 Fax: 853-2884-1358
Email: ytwang@umac.mo

marker, the prescription couldn't be distinguished from the substitute with *Crataegus pinnatifida*, which contained the higher ursolic acid than *Cornus officinalis*.[1,2]

Hierarchical cluster analysis, one of the multivariate statistics approaches, is used to distinguish relatively homogeneous clusters of cases based on measured characteristics,[3] which can be applied for selecting the marker of quality control. Hierarchical cluster analysis was performed based on 32 peaks characteristics from electrophoretic profiles of natural and cultured *Cordyceps*. The tested 12 samples of *Cordyceps* are divided into two main clusters, which attributed to the cultured or natural *Cordyceps* respectively. Furthermore, the statistic method was also performed based the characteristic peaks of adenosine and inosine, which optimized by principle component analysis, the result was very similar to the one derived from 32 peaks characteristics. Therefore, the adenosine and inosine could be used as markers for discrimination of natural and cultured *Cordyceps*.[4,5] Using the same methods, the ratios of Rg1/Rb1 and Rb3/Rb1 characteristics were elucidated as the marker to clarify the underground parts and aerial parts of *Panax notoginseng*.[6]

Usage of the multiple origin plants is one of the CM feature. It is estimated that 27 percent of CMs recorded in China Pharmacopoeia (version 2005) are the multiple origin, including 92 di-origin CMs, 42 tri-origin CMs, 7 quadr-origin CMs, 3 penta-origin CMs, and 1 hexa-origin CMs. Actually, the content and category of main ingredients from the different species of multiple origin CMs are highly variable, therefore, the exact species identity of assurance of safety and efficacy of medication. The rhizomes of three species *Curcuma* plants, *Curcuma phaeocaulis*, *Curcuma kwangsiensis* and *Curcuma wenyujin*, are used as *Ezhu*.[7] Five components, including furanodienone, germacrone, curdione, curcumenol and neocurdione, were optimized as markers for discrimination of the three species *Curcuma* using the hierarchical cluster analysis.[8-10] In addition, the two characteristic ingredients of *Pogostemon cablin*, patchouli alcohol and pogostone, were used to distinguish *"Shipai Huoxiang"* from *"Hainan Huoxiang"*.[11] Therefore, because the chemical ingredients of CM should be contributed to its efficacy and safety, it is necessary to develop the further research focused on the components and efficacy of multiply origin CM, which can help to elucidate the scientific evidence on application of multiply origin CM, and also provide the useful information for standardized agriculture process of CM.

2.2. The marker for grade evaluation

In most cases, bioactive compounds and action mechanisms of CM are not or only partially known, which limit the development of CM grade evaluation. A conventional procedure of finding marker for grade evaluation is the separation of individual compounds from CM followed by pharmacological screening or chemical isolation in the direction of bioactivity. However, the whole procedure is time-consuming, arduous, and hardly reflecting the CM feature of entirety and synergistic action.

Modern pharmacological studies have shown that the combination with the receptors or ion channels on cell membrane is the first step of drug action in most cases. Therefore, the capability of a drug to interact with cell membranes is very important for the behavior of the drug in the organism. A new method of biomembrane chromatography has been developed for screening potential active components from CM. This method in the characteristics of combination of the bioactive screening with the analytical and separation techniques would provide the efficient tool for finding the marker of quality control of CMs, and an integrative result of interaction of multiple target and multiple bioactive compounds, can be also partly elucidated. Using red cell biomembrane extraction and HPLC, the potential active components in *Angelica sinensis* was screened and determined. Four compounds, identified as ferulic acid, ligustilide, senkyunolide H and senkyunolide I based on their UV, MS and NMR spectrums, were found in the desorption eluate of red cell membrane extraction for aqueous extract of *Angelica sinensis*. The results suggested that the ligustilide, senkyunolide H and senkyunolide I, beside ferulic acid, could be considered as potential marker for quality control of *Angelica sinensis*.[12,13]

3. Preparation of Reference Chemical Compounds

The preparation of reference chemical compounds is the key step of international standardization and modernization of CM. In the past decade research focused on the separation of chemical ingredients from CM has been developed rapidly in China. However, it is far way from the standardization requirement of CM.

According to the physical and chemical property of the CM ingredients, the combination of different extraction and isolation techniques should be selected to the efficient preparation of the reference substances. As for the CM contained non-polarity components, samples can be extracted using the CO_2 supercritical fluid extraction (SFE), then chemical components can be pre-fractioned and enriched using molecular distillation (MD), followed by further purification with preparative middle pressure chromatography (MPLC) and high performance liquid chromatography (Pre-HPLC). The substances obtained should be identified chemical structure using the contemporary spectrum techniques, such as Ultraviolet (UV), high resolution mass spectroscopy (HRMS), nuclear magnetic resonance (1H-NMR, 13C-NMR) etc. Finally, the purity or the content should be clarified using the analytical method, such as HPLC and GC. **Figure 1** showed the summary for the preparation of reference compounds, including the polarity and non-polaruty components in CMs. The method embodies flexibility and standardization of isolation with the high repeatability. Under the model, the most of major components in the GC-MS profile of Ezhu Oil, 11 compounds, were abundantly isolated and identified in our lab.

4. Feasible and Optimum Analytical Approach

4.1. Efficient sample preparation procedure

Sample preparation procedure often take up 60-80 percent of the total analysis time, contributing highly to the total cost of analysis, and affect error generation through incomplete dissolution of analytes and contamination from reagents or vessels.[14] The conventional techniques for sample preparation, such as reflux extraction, soxhlet extraction, and ultrasonic extraction, are time consuming and require large amount of reagents. Therefore, the standardization of CM requests more rapid and automated procedures to take into account the constant increase in the number of samples to be tested. During the past few years, one of the most promising sample preparation techniques is pressurized liquid extraction (PLE; Dionex trade name ASE for accelerated solvent extraction) which has been widely applied in environmental, [15,16] food[17] and pharmaceutical fields.[18] A systemic research was firstly launched to application of the PLE technique to sample preparation of quality control of CMs.[13,19-25] PLE was applied to extract the triterpenoid saponins from *Panax notoginseng* using the 20 ml methanol as solution and 20 min time-consuming, the extract efficiency was equivalent to that using the sohlex extraction with 120 ml solution and 5h cost.[19] Several methods including PLE, ultrasonication, soxhlet extraction and immersion were also used for sample preparation of *Panax notoginseng* and their extraction efficiency was compared,[20] the results showed that the different data would be obtained using different sample preparation methods, which is the reason why the standardization of sample preparation should be emphasized, and PLE has the highest extraction efficiency and repeatability. Therefore, PLE technique is the most promising sample preparation technique for quality control of CM.

4.2. Feasible analytical technique

With the development of analytical chemistry technology, more and more detective techniques and approaches are applied into the quality control of CM. The HPLC was conventional analytical technique, and its application area has been gotten wider with the development of its detector, such as the diode array detector (DAD), refractive index detector (RID), evaporative light scattering detector (ELSD), and mass spectrometric detector (MSD). However, how to choose the best one from the numerous analytical approaches is problem for development of CM quality control. Optimum analytical technique is selected based on the analytes property and comparison of different quality control techniques. The curcumol was considered as the marker of Ezhu injection, and determined using chromatometry method described in China Pharmacopeia,[26] however, this method was interrupted seriously by the other component of Ezhu injection, which could be avoided using the GC and HPLC. Due to the mutual inversion of the ingredients in Ezhu under the high temperature of GC, the HPLC-UV was optimum analytical method for quality control of Ezhu.[8-10] The ginsenoside was the major ingredient and considered as the marker of *Panax notoginseng*. The thin layer chromatography (TLCs) was firstly applied for determination of saponin, however, TLCs method was limited by its low repeatability and sensitivity, even used HPTLC system equipped with applicator, auto-development, densitometer[27] to reduce the factitious error, the result was far from the requirement of quality control. Then, HPLC coupled with UV detector has been extensively used for analysis of ginsenosides in last two decades. However, the saponins in *P. notoginseng* show poor UV absorptivity and low-wavelength UV (198–205 nm) is required for the detection, which greatly increases the baseline noise and lowers the sensitivity of the detection.[20] ELSD is a universal, non-specific detector, and can provide a stable baseline even with steep gradients.[6,28] Therefore, the HPLC-ELSD is optimum analytical method for the quality control of *P. notoginseng*.

5. Conclusion

In the present article, the systematic strategies of international standardization of Chinese medicine, including the selection of the markers for quality control of CM, preparation of reference chemical compounds, and the establishment of platform for standardization of quality control of CM, were discussed and explored. The results showed that the strategies are feasible for the quality control of CM.

References

1. Yan LX, Tang WM, Ji YQ. Determination of ursolic acid in the pulp of Fructus Corni by HPLC-ELSD. Chin J Pharm Anal. 2003;23(5):358-359.
2. Hao RX, Yan CP, Sun XF, Wang H. Analysis of usolic acid in Cratacgus Pinnatifida Bge var major N.E. Br. By HPLC. Chin J Pharm Anal. 1994;14(2):31-32.
3. Shannon W, Culverhouse R, Duncan J. Analyzing microarray data using cluster analysis. Pharmacogenomics. 2003;4(2):41-52.
4. Gong YX, Li SP, Li P, Liu JJ, Wang YT. Simultaneous determination of six main nucleosides and bases in natural and cultured Cordyceps by capillary electrophoresis. J Chromatogr A. 2004;1055(1-2):215-221.
5. Li SP, Li P, Lai CM, et al. Simultaneous determination of ergosterol, nucleosides and their bases from natural and cultured Cordyceps by pressurised liquid extraction and high-performance liquid chromatography. J Chromatogr A. 2004;1036(2):239-243.
6. Wan JB, Yang FQ, Li SP, Wang YT, Cui XM. Chemical characteristics for different parts of Panax notoginseng using pressurized liquid extraction and HPLC- ELSD. J Pharm Biomed Anal. 2006;41(5):1596-1601.
7. Pharmacopeia Commission of PRC (Ed.), Pharmacopoeia of the People's Republic of China, Chinese ed., Chemical Industry Press, Beijing, PR China, 2005.
8. Yang FQ, Li SP, Chen Y, et al. Identification and quantitation of eleven sesquiterpenes in three species of Curcuma rhizomes by pressurized liquid extraction and gas chromatography-mass spectrometry. J Pharm Biomed Anal. 2005;39(3-4):552-558.
9. Yang FQ, Li SP, Chen Y, et al. Fingerprint of Ezhu by GC-MS. Acta Pharm Sin. 2005;40(11):1013-1018.
10. Yang FQ, Li SP, Chen Y, et al. Quantitative Determination of Curcumol, Germacrone and Curdione From Zedoray Rhizome by GC-MS. Chin J Pharm Anal. 2005;25(7):80-83.
11. Hu LF, Li SP, Cao H, et al. GC–MS fingerprint of Pogostemon cablin in China. J Pharm Biomed Anal. 2006;42(2):200-206.
12. Dong ZB, Li SP, Hong M, Zhu Q. Hypothesis of potential active components in Angelica sinensis by using biomembrane extraction and high performance liquid chromatography. J Pharm Biomed Anal. 2005; 38(4):664-669.
13. Lao SC, Li SP, Kan KW, et al. Identification and quantification of 13

components in Angelica sinensi (Danggui) by gas chromatography-mass spectrometry coupled with pressurized liquid extraction, Anal Chim Acta. 2004;526(2):131-137.
14. Buldini PL, Ricci L, Sharma JL. Recent applications of sample preparation techniques in food analysis. J Chromatogr A. 2002;975(1): 47-70.
15. Dabrowski L, Giergielewicz-Mozajska H, Biziuk M, Gaca J, Namiesnik J. Some aspects of the analysis of environmental pollutants in sediments using pressurized liquid extraction and gas chromatography-mass spectrometry. J Chromatogr A. 2002;957(1):59-67.
16. Pörschmann J, Plugge J. Extraction of polar and hydrophobic pollutants using accelerated solvent extraction (ASE). Fresenius J Anal Chem. 1999;364(7):643-644.
17. Palma M, Pineiro Z, Barroso CG, Palma M, Pineiro Z, Barroso CG. In-line pressurized-fluid extraction-solid-phase extraction for determining phenolic compounds in grapes. J Chromatogr A. 2002;968(1-2):1-6.
18. Alonso-Salces RM, Korta E, Barranco A, Berrueta LA, Gallo B, Vicente F. Pressurized liquid extraction for the determination of polyphenols in apple. J Chromatogr A. 2001;933(1-2):37-43.
19. Li P, Wan JB, Li SP, Wang YT. Studies on the Accelerated Solvent Extraction of Panax notoginseng saponins. Chin J Nat Med. 2004; 2(3):157-161.
20. Wan JB, Lai CM, Li SP, Lee MY, Kong LY, Wang YT. Simultaneous determination of nine saponins from Panax notoginseng using HPLC and pressurized liquid extraction. J Pharm Biomed Anal. 2006;41(1):274-279.
21. Li P, Li SP, Fu CM, Kan KW, Wang YT. Pressurized solvent extraction in quality control of Chinese herb. Chin J Chin Mater Med. 2004;29(2):723-726.
22. Wan JB, Xu C, Li SP, Kong LY, Wang YT. Studies on the Pressurized Solvent Extraction of Isoflavonoids From Pueraria Lobata. Chin J Anal Chem. 2005;33(10):1435-1438.
23. Li P, Li SP, Gong YX, Wang YT. Simultaneous determination of ergosterol, nucleosides and their bases from natural and cultured Cordyceps by pressurized solvent extraction and high performance liquid chromatography. Acta Pharm Sin. 2004;39(2):917-920.
24. Li P, Li SP, Lao SC, Fu CM, Kan KK, Wang YT. Optimization of pressurized liquid extraction for Z-ligustilide, Z-butylidenephthalide and ferulic acid in Angelica sinensis. J Pharm Biomed Anal. 2006;40(5):1073-1079.
25. Gong YX, Li SP, Wang YT, Li P, Yang FQ. Simultaneous determination of anthraquinones in Rhubarb by pressurized liquid extraction and capillary zone electrophoresis. Electrophoresis. 2005;26:1778-1782.
26. Pharmacopeia Commission of PRC (Ed.), Pharmacopoeia of the People's Republic of China, Chinese ed, Chemical Industry Press, Beijing, PR China, 2000.
27. Wan JB, Li SP, Jian JR, Kong LY, Wang YT. Analysis of Saponins from Panax notoginseng Using Pressurized Solvent Extraction Coupled with High Performance Thin Layer Chromatography. Chin J Nat Med. 2004;2(4):215-218.
28. Wan JB, Li P, Li SP, Wang YT, Dong TX, Tsim WK. Simultaneous determination of 11 saponins in Panax notoginseng using HPLC-ELSD and pressurized liquid extraction. J Sep Sci. 2006;29(14):2190-2196.

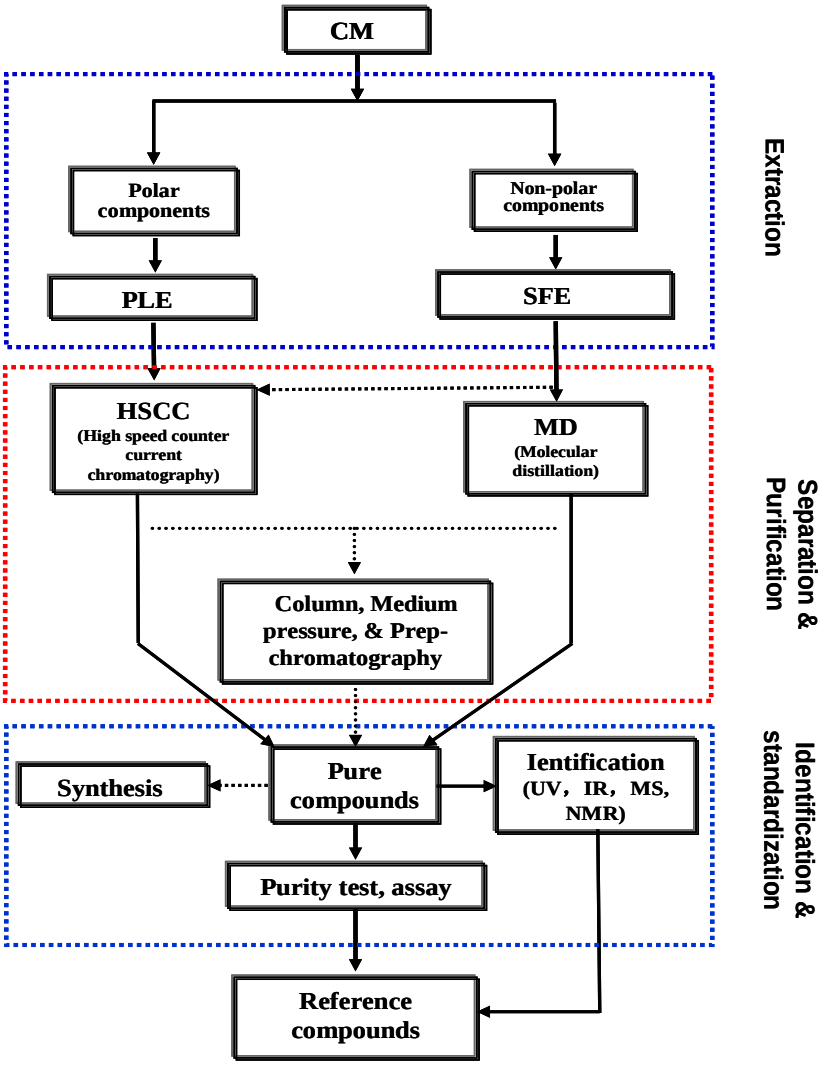

Figure 1. Flow chart for preparation of reference compounds.