Journal of Pharmaceutical and Biomedical Analysis 55 (2011) 802–809

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

Review

Strategies for quality control of Chinese medicines

S.P. Li [a,*], J. Zhao [a,b], B. Yang [b]

[a] State Key Laboratory for Quality Research in Chinese Medicine and Institute of Chinese Medical Sciences, University of Macau, Macao SAR, China
[b] Institute of Chinese Materia Medica, China Academy of Chinese Medical Sciences, Beijing, China

ARTICLE INFO

Article history:
Received 7 October 2010
Received in revised form 8 December 2010
Accepted 9 December 2010
Available online 16 December 2010

Keywords:
Chinese medicines
Quality control
Quality marker
Reference compound
Analytical approach

ABSTRACT

Chinese medicines (CM) have been attracting interest and acceptance in many countries. Quality control is vital for ensuring the safety and efficacy of CM. Usually, CM are used as whole plant and/or combination of several herbs, and multiple constituents are responsible for the therapeutic effects. Therefore, quality control of CM is very difficult. To date, the valid method for quantitatively evaluating the quality of CM is poor. In this article, the strategies for quantification, related to the markers, reference compounds and approaches, in quality control of CM were reviewed and discussed.

© 2010 Elsevier B.V. All rights reserved.

Contents

1. Introduction 802
2. Selection of markers for quality control of CM 802
   2.1. HPLC separation coupled with on-line bioassay 803
   2.2. Biospecific extraction and HPLC analysis 803
   2.3. Integrating method based on holistic effect of CM 804
3. Reference compounds for quality control of CM 805
4. Approaches for quality control of CM 806
   4.1. Sample preparation 806
   4.2. Analytical techniques 806
5. Conclusion 807
   Acknowledgements 807
   References 807

1. Introduction

Chinese medicines (CM) have been attracting interest and acceptance in many countries. An estimated 1.5 billion people now use these preparations worldwide [1]. This may be primarily because of the general belief that herbal drugs are without any side effect besides being cheap and locally available [2]. However, as more people are using CM products, there are increased reports on their adverse reactions [3]. “The biggest trouble with herbals ‘is that manufacturers don’t have to show how much of the active chemical is in a pill’. A bigger dose can mean a dangerous interaction” [4]. Therefore, quality control is crucial for ensuring the safety and efficacy of CM. However, CM are usually used as whole plant and/or combination of several herbs, which contains more than tens to hundreds or even thousands of components. Especially, effective components in most CM are unknown, which greatly increases the difficulties of quality control. Therefore, a rational and valid quality control method for CM is poor. In this article, the strategies for quantification, including the markers, reference compounds and approaches, in quality control of CM were reviewed and discussed.

2. Selection of markers for quality control of CM

The purpose of quality control is to ensure the safety and efficacy of CM. Therefore, the markers for quality control should be strongly correlated to their safety and efficacy. Actually,

* Corresponding author. Tel.: +853 8397 4692; fax: +853 2884 1358.
E-mail addresses: lishaoping@hotmail.com, spli@umac.mo (S.P. Li).

0731-7085/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2010.12.011

multiple components are frequently considered to be responsible for the therapeutic effects, which may work 'synergistically' and could hardly be separated. For well understanding the selection of markers for quality control of CM, some terminology should be carefully defined. *Effective components*, including active and relative components, are defined as the chemical compounds which can characterize the therapeutic effects of CM. *Active components* mean the chemical compounds with specific biological activity related to the therapeutic effects of CM. *Relative components* mean the compounds which may have no specific action related to the efficacy of CM but can definitely affect the therapeutic effects of active components through enhancing the solubility, improving the stability and/or increasing the bioavailability and so on of active components. Ideally, the rational markers for quality control of CM should include both active and relative components, i.e. effective components. Conventionally, systematic chemical separation followed by pharmacological activity assay or bioassay guided chemical separation are widely used for discovering active components in CM. However, chemicals in CM are complicated, which makes separation and screening extremely difficult. Over the years, the techniques for screening and identification of active components in CM have been developed considerably. High-content screening (HCS) technology has now expanded throughout all the different stages of the drug development process and is today considered a mainstream technology in drug discovery [5,6]. Nevertheless, the identification of effective components has always been the bottleneck in CM research due to the diversity of the components in CM and the complexity of their mechanism.

2.1. HPLC separation coupled with on-line bioassay

Chromatographic methods coupled with biochemical detection have been widely used for screening active components [7,8], especially antioxidants, in CM [9]. Thin layer chromatography (TLC) [10], high performance liquid chromatography (HPLC) [11,12] and high performance capillary electrophoresis (HPCE) [13–15] are the currently available techniques, and HPLC is the most commonly used. A method using HPLC coupled with DAD–MS and ABTS-based assay was employed for identification of antioxidants in essential oil of *Angelica sinensis* (*AS oil*) [16]. The sample mixture was injected into HPLC system for separation and the eluent from DAD was split into two streams. The minor stream was introduced into MS for structure elucidation, and the major one was used for ABTS assay, where the continuous flow of free radical (e.g. $ABTS^{\bullet+}$) solution was introduced into the reaction coil and interacted with the eluent. Any bleaching of the initial color was detected as a negative peak, antioxidants were easily identified based on the chromatograms and MS data. The application of this method has been well reviewed [9,11,12]. It is worth noting that the effect of CM may be the integrating action of multiple components with weak pharmacological activity. So HPLC, which could completely separate individual component in some cases, may be not available to find the active components of trace amount and/or weak activity. Indeed, the essential oil of *Curcuma wenyujin* has antioxidant activities *in vitro* [17], but no obvious antioxidant was found in the oil using HPLC separation coupled with ABTS-based assay. However, when the high-resolution method of HPLC was replaced with low-resolution technique of TLC, an obvious band with significant antioxidant activity was investigated. Finally, four peaks were found in the TLC band based on GC–MS analysis, and two of them were identified as curzerene and furanodiene, both were confirmed as antioxidants [9,18].

In fact, most drugs exert their effects through enzymes, receptors or channels rather than direct chemical reactions such as free radical scavenging. Enzymes contributed to about half of marketed small-molecule drug targets by biochemical class [19]. Therefore, it is very important and useful to develop a method for online screening of the components, which can interact with enzymes and receptors, from CM. Recently, an online method for screening α-glucosidase inhibitors, one of major oral hypoglycemia agents in China, from CM was developed. Fig. 1 illustrates the scheme of an HPLC–DAD–MS–BCD method for rapid screening and identification of α-glucosidase inhibitors from herbal extracts. As a result, two compounds namely (−)-epigallocatechingallate (EGCG) and (−)-epicatechingallate (ECG) from Pu-erh tea were easily detected as α-glucosidase inhibitors. *In vitro* assay showed that EGCG and ECG were non-competitive inhibitors, which are different from acarbose, a commercial α-glucosidase inhibitor in the market for treatment of diabetes [20].

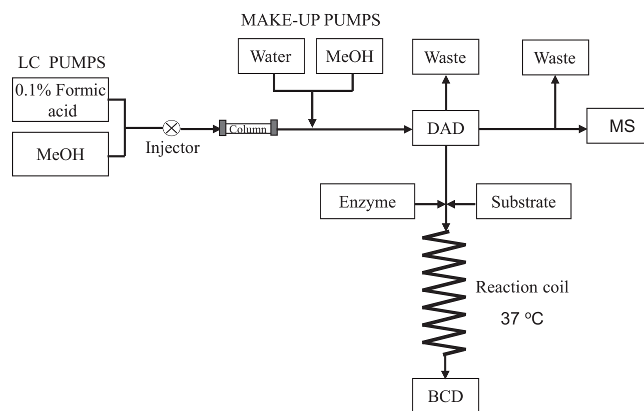

Fig. 1. Diagrammatic scheme of HPLC–DAD–MS–BCD for rapid screening and identification of α-glucosidase inhibitors from herbal extracts (modified from Ref. [19] with permission).

2.2. Biospecific extraction and HPLC analysis

Receptor and enzyme models, as the main method for high throughput screening, are applied for identifying active components aiming at specific target sites. Unfortunately, the components of CM are complicated and their targets are usually unknown. Actually, the effect of CM is a result from multi-target functions. Therefore, the disadvantages for screening active components from CM based on a specific target include low efficiency, single purpose and high cost, etc. Theoretically, modern pharmacological studies have shown that combining with some receptors or channels on cell membrane is the first step of drug action. Indeed, the ability of a drug to interact with cell membranes is very important for the behavior of drug. The same kind receptor density of cell membrane can reach $10^3 \times 10^4$ per cell, so cell material is an ideal matrix with multiple drug targets. In order to study the component binding with cell membranes, a method called retardation chromatography was introduced by Bobinski and Stein [21]. However, the cell materials are not stable and the entrapment or immobilization procedure must be adapted to the kind of material that is to be analyzed and to the kind of gel matrix used [22]. Moreover, the conditions for interaction of components in CM with biomembrane is rarely compatible to their separation on chromatography. Thus, cell materials biospecific extraction followed by HPLC analysis has been explored for screening bioactive compounds in CM [23–25]. In this method, the sample and target cells/membrane are incubated in binding buffer, the unbound components will be washed off by the same buffer. After denaturalization under suitable environment, the bound components and receptors dissociated and the former will be washed off into eluent. By using HPLC, chemical components in the eluent before and after the binding occurred are compared, and the quasi active components can be identified (Fig. 2). With

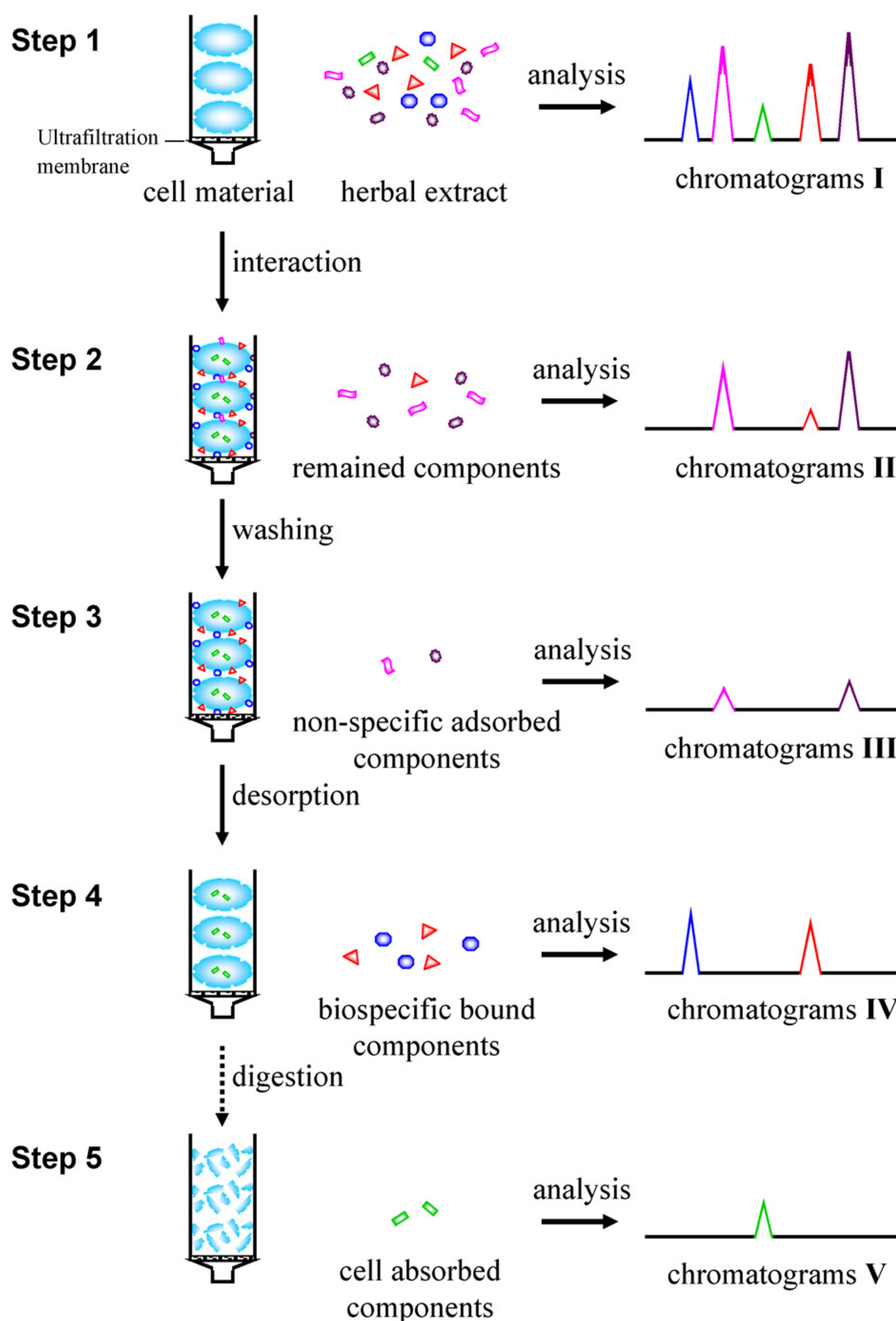

Fig. 2. Flow scheme of cell materials biospecific extraction used for screening active components in traditional Chinese medicines (from Ref. [9] with permission).

the obvious advantages, this method has been widely used for screening active components from herbal medicines, which has been reviewed recently [9,26].

2.3. Integrating method based on holistic effect of CM

The focus of the screening based on both chemical characteristics and targets properties is only the pharmacological active components. However, relative components of CM may contribute a vital role in the efficacy of active components. The main anti-depressed components of *Hypericum perforatum* are hypericin and hyperforin, but their function is closely related with the presence of rutin, a compound without anti-depressant activity, and its concentration (>3% in the extract) [27]. Similarly, the antimicrobial action of berberine could be greatly potentiated by 5′-methoxyhydnocarpin, a multidrug pump inhibitor which has no antimicrobial activity [28]. Based on the medicinal characteristics, a Chinese herb is very similar to a western medicine formulation. The difference is that the active components are well known in the western medicine formulation, and functions of the excipients are well defined. The synergist, absorption enhancer, solubilizer, stabilizer, and coloring agent correspond to individual component. Nevertheless, the components contribute to these effects, and hence in collaboration with active components in CM are unknown, which limits the characterization of CM. Therefore, modern research should reveal not only the active components, but also the relative components, which affect the efficacy of active components. Only in this way, the overall knowledge and scientificness of CM can be improved and the efficacy of medication is guaranteed. Although the holistic function of CM has been widely known, the

poor discovery approaches of the relative components are the bottleneck in modern research on quality control of CM, which eagerly await to be solved.

The component-effect correlation analysis, combination of comparative chemistry and comparative pharmacology, is a helpful method. It can possibly identify the effective compounds of CM as a whole. The strategy is by comparing the chemical components and pharmacological activities of extracts from the same CM extracted with different method or similar species of CM extracted with the same method to unravel their relationship. Based on this, the key components that influence the effects of CM could be identified [9,27,29]. However, the work poses a challenge. Up to date, the effective compounds in most CM are still unknown. Hence only one or few markers are widely employed to evaluate the quality of CM. Among 195 raw materials of CM, recorded in Chinese Pharmacopoeia (2005), with quantitative assay of specific component, the quality of 154 herbs is evaluated using a single marker, which is usually not strongly indicative of the safety and efficacy of CM. For example, *Cordyceps sinensis*, one of the valued CM, is commonly used in China because of its multiple biological activities [30]. Adenosine has been used as a marker for quality control of natural *C. sinensis* in Chinese Pharmacopoeia [31]. However, fresh natural *C. sinensis* contains very little amount of adenosine, as compared to dry and processed one [32], and more interesting is that cultured *Cordyceps* mycelium contains high level of adenosine [33]. Actually, inosine, the major biochemical metabolite of adenosine due to oxidative deamination, can stimulate axon growth *in vitro* and in the adult central nerve system [33]. Natural *Cordyceps* contain much higher amount of inosine than the cultured ones, including *C. sinensis* and *C. militaris* [21], due to their production pathway was different [34]. Therefore, having adenosine as a sole marker for good quality of *Cordyceps* may not be indicative. In order to present the holistic effect of CM, chromatographic fingerprinting is considered as a powerful alternative strategy for quality control of CM [35,36], which is already being used in China [36–39]. Compulsory fingerprint analysis was proposed, by the Chinese State Food and Drug Administration (SFDA), to control the quality of Chinese medicine injections in 2004. In last years, among 109 injections made from CM, the fingerprints of 72 injections were developed [40], but none have been compulsorily implemented. Actually, this mode only could be feasible for real quality control of CM until the blending method is permitted by SFDA in production of final products of CM.

3. Reference compounds for quality control of CM

Quality control of CM includes qualitative and quantitative analysis, while quantitative determination is usually not available without reference compound as standard. However, the shortage of reference compounds or chemical standards is the bottleneck for quality control of CM. In last decades, a great effort has been made in this area in China. However, the supply of reference compounds is far from the requirement for quality control of CM. Up to date, there are only about 400 reference compounds supplied for CM. Especially, some pure chemical compounds are difficult to obtain or store because of their instability and/or trace amount, which hinders the development of quality control of CM. Therefore, some alternative methods were developed.

Gas chromatography–mass spectrometry (GC–MS) offers a powerful tool for identification of chemical components in essential oil. Using chemical analogues as standards of analytes, an alternative method was developed to determine or estimate the contents of identified components in essential oils [41,42]. The results can be used for evaluating the quality of different samples or batches of essential oils from CM [41–44], though the quantification might be with great error. Actually, this method can be easily and accurately used for controlling the real quality of CM if the clinical dosage is calculated based on their quantification. Van Beek et al. first successfully determined ginkgolides and bilobalide in Ginkgo extracts using benzyl alcohol as the internal standard to solve the poor availability of reference substances [45]. The relative response factors of the three ginkgolides and bilobalide changed from 1.11/1.12/1.61/1.26 to 1.20/1.22/1.19/1.27 within 15 years, and this simple method was accepted in Ginkgo extracts monograph in 2006 edition of European Pharmacopoeia [46]. Recently, the strategy for resolving the shortage of reference compounds in quantitative analysis has attracted more attention [46–51]. Danshen, the rhizome of *Salvia miltiorrhiza* Bunge, is one of the most important ancient Chinese herbal drugs. More than 20 phenolic acids of water soluble constituents, which have antioxidant, anti-blood coagulation and cell protection activities, have been isolated from this plant since 1980s [52]. Salvianolic acid B, one of phenolic acids, has been used as the marker for quality control of *S. miltiorrhiza* because it not only has the actions described above, but also is the characteristic constituent of *S. miltiorrhiza* [31]. However, the preparation and purification of salvianolic acid B is difficult because of its poor stability, and the high cost for daily control work. Therefore, methylparaben was used as a reference substance of salvianolic acid B for determination of Radix Salviae Miltiorrhizae and compound Danshen tablets [47]. The assay demonstrates that methylparaben instead of salvianolic acid B is feasible for quality control of *S. miltiorrhiz* and its products. It was also reported that the contents of arctiin, arctigenin, lappaol C and lappaol H were determined using arctiin as the reference compound with corrected factor method [48].

In addition, standard extract has also been proposed as reference components for measuring each component in herbal products [53]. Silymarin, widely used for the treatment of toxic liver damage, hepatitis and cirrhosis, primarily consists of an isomeric mixture of active flavonolignans: silychristin (Sc), silydianin (Sd), and two groups of diastereoisomeric flavonolignans, silybin A (Sb A) and silybin B (Sb B), and isosilybin A (ISb A) and isosilybin B (ISb B) [54–56]. The different isomers of silymarin have been reported having different biological activities [57–59]. Currently, all six individual purified standards are not available for the quantification of silymarin although there is a wealth of literature available. The lack of standards available for the quantification of Sb A, Sb B, ISb A and ISb B leads the search of alternative reference materials. Lee et al. [53] proposed using a commercial standard silymarin extracts, with high purity of silymarin and the similar ratio profile of all six components, as the reference compounds to evaluate each active constituent in seven commercial products from different brands. Especially, a new method for quantitative analysis performed without any standard using an evaporative light-scattering detector (ELSD) is also proposed [60]. However, the peak identification is a problem without matching reference compound in these cases. Mass spectrometry plays an important role for the identification of analytes [41–44,61–63]. LC–MS was also applied for the identification of 23 flavonoids in the extract of Mexican oregano (*Lippia graveolens* H.B.K.), and ten flavonoids without matching standards among them were quantitatively estimated using eriodictyol 7-*O*-glucoside (for determination of three pentahydroxyflavanone monoglycosides), luteolin (for determination of 6-hydroxyluteolin), luteolin 7-*O*-glucoside (for determination of two 6-hydroxyluteolin glycosides and scutallarein 7-*O*-hexoside), scutellarein (for determination of 6-methoylscutallarein and 6,7-dimethoylscutallarein), galangin (for determination of methylgalangin), and phloridzin (for determination of 6-hydroxyphloretin 6″-*O*-hexoside), respectively [64]. Using substitute and/or extract as reference are currently a practical and available strategy for resolving the shortage of standards for

quality control of CM, the interest is how to improve the accuracy of quantification, and resolve the problem of peak identification.

Volatile components in CM usually contain heat labile substances, which may degrade and result in wrong results during GC analysis [65–68]. Therefore, optimization of GC conditions for analysis of volatile compounds should be based on not only the resolution but also stability of analytes [68]. However, in most cases, chemical properties of the components were unknown, so pure compounds are always, at least for first time, necessary. Alternatively, it is considered to develop a method for evaluating the heat stability of components in extracts of CM so as to determine the appropriate temperature in GC analysis.

4. Approaches for quality control of CM

4.1. Sample preparation

Sample preparation is one of the key steps which greatly influences the repeatability and accuracy of quantitative analysis. It is reported that 70–80% of analysis time is spent on sample preparation and more than 60% of analysis error derived from nonstandard sample pretreatment. Therefore, a proper sample preparation approach is very important for quality control of CM. Recent years, considerable efforts have been made to develop improved methods of extraction, and to make them commercially available. The technological development for sample preparation of CM has been reviewed [69–71]. Herein, our interest is focus on the solvent effect during sample preparation on the quantification of analytes in CM.

Currently, using organic solvent is the main trend of sample preparation for quality control of CM. Actually, decoction, which has been approved its efficacy with a long history, is the major administration form in clinical use of traditional CM. Due to the difference of organic solvent and water in soluble ability and extraction efficiency to components in CM, the quantification of analytes are certainly affected by different extraction solvent, so misleading information may hinder the unraveling of CM if no evidence for their efficacy. Therefore, sample preparation using organic solvent may be not available and rational for quality control of CM. Five methods, namely sonication (SE), pressurized liquid extraction (PLE), supercritical fluid extraction (SFE), hydrodistillation (HD) and decoction (DC), for extraction of coniferyl ferulate, as well as ferulic acid, *Z*/*E*-ligustilide and *Z*/*E*-butylidenephthalide, from *Angelica sinensis* were optimized and compared [72]. The results showed that the order of extraction efficiency was PLE ≈ SE > SFE > >HD, DC. The compositions of the SE, PLE and SFE extracts, which had a high ratio of coniferyl ferulate, were very similar, while coniferyl ferulate was not obtained by HD and DC, though they had high selectivity for the extraction of ligustilide and ferulic acid, respectively. Three extraction methods, including organic solvent (methanol) pressurized liquid extraction (OSPLE), boiling water extraction (BWE) and ambient temperature water extraction (ATWE), were also optimized and compared for sample preparation in determination of five nucleosides, i.e. uridine, inosine, guanosine, adenosine and cordycepin, from natural and cultured *C. sinensis* [73]. The results showed that the peak areas of uridine, inosine and guanosine in natural *Cordyceps* increased with the extraction time extension, and the maximum value reached at 18 h using ATWE and then decreased with the time extra increase. However, adenosine decreased with extraction time prolonged, and even disappeared when the extraction time was more than 8 h. These changes were not investigated using OSPLE and BWE. Thus ATWE, compared with OSPLE and BWE, could greatly increase the contents of uridine, guanosine and inosine but decreased and even eliminated adenosine in natural *C. sinensis*. These phenomena are attribute to that natural *Cordyceps* contains some enzymes, denatured in OSPLE and BWE, can transfer UMP, AMP and GMP into uridine, adenosine and guanosine, respectively, as well as adenosine to inosine [34,73]. In addition, analytes in CM may react with extraction solvent during sample preparation, such as alcohol solvents are not available for the extraction of cinnamaldehyde and 2-methoxycimnamaldehyde in *Cinnamomum cassia* due to aldol reaction [74]. However, alcohols are usually used as extraction solvents and/or mobile phase in most cases of chromatographic analysis [75–79], which might result in wrong results. Besides, sample solvent is one of the possible reasons for anomalous peak shapes, certainly contribute even more to peak broadening, of analytes during liquid chromatography analysis [80].

4.2. Analytical techniques

Chinese medicine is gaining popularity worldwide for health care and adjuvant therapy. However, "the quantity and quality of the safety and efficacy data on traditional medicine are far from sufficient to meet the criteria needed to support its use world-wide. The reasons for the lack of research data are due to not only to health care policies, but also to a lack of adequate or accepted research methodology for evaluating traditional medicine" [81]. According to Chinese Pharmacopoeia [31], there are more than 500 crude drugs widely used. Each of these herbs usually contains hundreds of chemical constituents but only a few components are responsible for the beneficial and/or hazardous effects. Therefore, the efficient and selective methods are required for qualitative and quantitative analysis of their effective components. In last decades, chromatography and its hyphenated techniques, such as high performance thin layer chromatography (HPTLC), gas chromatography (GC)/GC–MS, high performance liquid chromatography (HPLC)/LC–MS, as well as capillary electrophoresis (CE)/CE–MS have been extensively applied for quality control of CM, which have been well reviewed [39,82–94]. Generally, an ideal quality control method should be simple, rapid, specific and accurate. However, due to the chemical complexity of CM, an appropriate technique should be carefully investigated and optimized based on the analytical purpose and instrumental feasibility. Saponins are major components in *Panax ginseng*, *Panax notoginseng* and *Panax quinquefolius*, and usually used as markers for quality control of these three medicinal plants. Conventional HPLC is widely used for separation of these saponins, but about 60 min of analytical time is necessary so as to obtain good resolution between ginsenoside Rg1 and Re [95–106]. The advantage of mass spectrometer is that precursors and ion products of analytes can be used as a discriminating feature, and this may allow quantitative measurements of unresolved components on chromatogram. Therefore, individually quantitative determination of ginsenoside Rg1 and Re was available though they were not separated by HPLC, and finally, the analytical time could be greatly shorten [107]. With the development of UPLC, rapid quantitative analysis of saponins in *Panax* species was also available by using UV detection [108]. The appropriate analytical technique for quality control of individual CM may be varied based on the chemical properties of analytes. The genus *Epimedium*, which belongs to the family Berberidaceae, comprises about 50 species widespread in the world, and among about 40 species found in China, a few have been used as traditional Chinese medicine for a long time. It is recorded in Chinese Pharmacopoeia (2010) that the dried aerial parts of *Epimedium brevicornu* Maxim., *Epimedium sagittatum* (Sieb. et Zucc.) Maxim., *Epimedium pubescens* Maxim., *Epimedium wushanense* T.S.Ying, and *Epimedium koreanum* Nakai are used as *Yinyanghuo*. They contain a particularly high content of flavonoids which have been reported to possess multiple biological activities such as antiosteoporosis, immunomodulation, antitumor and antidepressant [109–113]. Therefore, flavonoids, considered as the

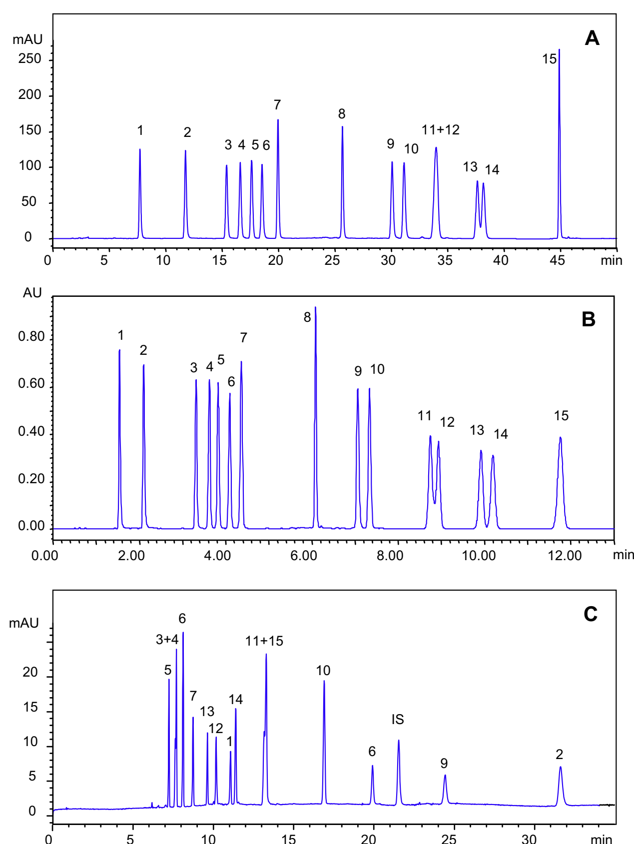

Fig. 3. HPLC (A), UPLC (B) and CZE (C) profiles of mixed 15 flavonoids from *Epimedium*. **1**, hexandraside E; **2**, kaempferol-3-*O*-rhamnoside; **3**, hexandraside F; **4**, epimedin A; **5**, epimedin B; **6**, epimedin C; **7**, icariin; **8**, epimedoside C; **9**, baohuoside II; **10**, caohuoside C; **11**, baohuoside VII; **12**, sagittatoside A; **13**, sagittatoside B; **14**, 2″-*O*-rhamnosyl icariside II; **15**, baohuoside I; **IS**, internal standard.

major active components, are usually used as the markers for quality control of *Epimedium*. HPLC [114], UPLC [115] and CZE [116] have been, respectively, developed for simultaneously determination of 15 flavonoids in *Epimedium* in our lab. The results showed that different separation methods had its characters. The investigated flavonoids in *Epimedium* were well separated by HPLC except baohuoside VII and sagittatoside A, as well as sagittatoside B and 2″-*O*-rhamnosyl icariside II. UPLC could greatly improve the resolution though complete separation between baohuoside VII and sagittatoside A was not obtained. It is interesting that poor resolution between hexandraside F and epimedin A, as well as baohuoside VII and baohuoside I were found using CZE analysis (Fig. 3). Therefore, the separation method should be carefully selected based on the analytes.

5. Conclusion

For the strategy for quality control of CM, three aspects should be considered. They are quality markers, standard substances, as well as technical approaches including sample preparation and analysis. Among them, quality markers are basis, which should be closely correlated with the safety and efficacy of CM, while the components without therapeutic activity but necessary for the efficacy of a herb also should be considered as quality marker. Standard substances are the key, and their sustainable supply is very important. Sample preparation is crucial and appropriate analysis method should be carefully considered to obtain accurate results. Some alternative methods could be employed before these problems are well resolved.

Acknowledgements

We are grateful to Miss S.K. Kuong, M. Hoi and Mr. Y.W. Chen from our institute for their assistances. The research was partially supported by grants from University of Macau (UL015/09-Y2) and Science and Technology Development Fund of Macau (028/2007/A2) as well as National Natural Science Foundation of China (No. 30928033) to S.P. Li. The Ministry of Science and Technology of the People's Republic of China (No. 2009ZX09308-005) to B. Yang, and China Post-doctoral Scientific Fund (No. 20100470) and China Academy of Chinese Medical Sciences Fund (ZZ20090111) to J. Zhao.

References

[1] I. Hosbach, G. Neeb, S. Hager, S. Kirchhoff, B. Kirschbaum, In defence of traditional Chinese herbal medicine, Anaesthesia 58 (2003) 282–283.
[2] L.M. Gupta, R. Raina, Side effects of some medicinal plants, Curr. Sci. 75 (1998) 897–900.
[3] R.J. Ko, A U.S. perspective on the adverse reactions from traditional Chinese medicines, J. Chin. Med. Assoc. 67 (2004) 109–116.
[4] J. Fischman, Herbs and prescriptions can make a risky mixture, US News World Rep. 128 (2000) 64–65.
[5] S.A. Haney, P. LaPan, J. Pan, J. Zhang, High-content screening moves to the front of the line, Drug Discov. Today 11 (2006) 889–894.
[6] F. Zanella, J.B. Lorens, W. Link, High content screening: seeing is believing, Trends Biotechnol. 28 (2010) 237–245.
[7] I.I. Koleva, H.A. Niederlander, T.A. van Been, An on-line HPLC method for detection of radical scavenging compounds in complex mixtures, Anal. Chem. 72 (2000) 2323–2328.
[8] A. Dapkevicius, T.A. van Beek, H.A. Niederlander, Evaluation and comparison of two improved techniques for the on-line detection of antioxidants in liquid chromatography eluates, J. Chromatogr. A 912 (2001) 73–82.
[9] S.P. Li, J. Zhao, Z.M. Qian, J. Li, Advanced development of chromatography in screening and identification of effective compounds in Chinese materia medica, Sci. Sin. Chim. 40 (2010) 651–667.
[10] J.B. Qu, H.X. Lou, P.H. Fan, Application of TLC-bioautography to drug screening, Chin. Tradit. Herbal Drugs 36 (2005) 132–137.
[11] S.Y. Shi, Y.P. Zhang, X.Y. Jiang, X.Q. Chen, K.L. Huang, H.H. Zhou, Coupling HPLC to on-line, post-column (bio)chemical assays for high-resolution screening of bioactive compounds from complex mixtures, Trends Anal. Chem. 28 (2009) 865–877.
[12] H.A. Niederlander, T.A. van Beek, A. Bartasiute, I.I. Koleva, Antioxidant activity assays on-line with liquid chromatography, J. Chromatogr. A 1210 (2008) 121–134.
[13] K. Tsukagoshi, T. Taniguchi, R. Nakajima, Analysis of antioxidants using a capillary electrophoresis with chemiluminescence detection system, Anal. Chim. Acta 589 (2007) 66–70.
[14] G.C. Zheng, H. Chen, Z.T. Chen, T.T. Gan, B. Feng, Z.N. Xia, Study on the on-line evaluation method for antioxidants by capillary electrophoresis with indirect chemiluminescence detection, Acta Chim. Sin. 64 (2006) 2344–2350.
[15] Z.N. Xia, Y. Pang, G.C. Zheng, Study on the antioxidantive biological fingerprints of *Astragalus* based on capillary electrophoresis, Chin. J. Anal. Chem. 36 (2008) 1646–1650.
[16] S.Y. Li, Y. Yu, S.P. Li, Identification of antioxidants in essential oil of Radix Angelicae sinensis using HPLC coupled with DAD–MS and ABTS-based assay, J. Agric. Food Chem. 55 (2007) 3358–3362.
[17] S.Y. Li, S.P. Li, Antioxidant activities of essential oil of *Curcuma longa* and *Curcuma wenyujin*, Int. J. Essential Oil Ther. 3 (2009) 31–34.
[18] J. Zhao, J.S. Zhang, B. Yang, G.P. Lv, S.P. Li, Free radical scavenging activity and characterization of sesquiterpenoids in four species of *Curcuma* using TLC bioautography assay and GC–MS analysis, Molecules 15 (2010) 7547–7557.
[19] A.L. Hopkins, C.R. Groom, The druggable genome, Nat. Rev. Drug Discov. 1 (2002) 727–730.
[20] D.Q. Li, Z.M. Qian, S.P. Li, Inhibition of three selected beverage extracts on alpha-glucosidase and rapid identification of their active compounds using HPLC–DAD–MS/MS and biochemical detection, J. Agric. Food Chem. 58 (2010) 6608–6613.
[21] H. Bobinski, W.D. Stein, Isolation of a glucose-binding component from human erythrocyte membranes, Nature 211 (1966) 1366–1368.
[22] I. Gottschalk, C. Lagerquist, S.S. Zuo, A. Lundqvist, P. Lundahl, Immobilized-biomembrane affinity chromatography for binding studies of membrane proteins, J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 768 (2002) 31–40.
[23] Z.B. Dong, S.P. Li, M. Hong, Q. Zhu, Hypothesis of potential active components in *Angelica sinensis* by using biomembrane extraction and high performance liquid chromatography, J. Pharm. Biomed. Anal. 38 (2005) 664–669.
[24] J. Wang, Z.G. Huang, H. Cao, Y.T. Wang, P. Hui, C. Hoo, S.P. Li, Screening of anti-platelet aggregation agents from *Panax notoginseng* using human platelet extraction and HPLC–DAD–ESI–MS/MS, J. Sep. Sci. 31 (2008) 1173–1180.
[25] L. Yu, J. Zhao, Q. Zhu, S.P. Li, Macrophage biospecific extraction and high performance liquid chromatography for hypothesis of immunological active

components in *Cordyceps sinensis*, J. Pharm. Biomed. Anal. 44 (2007) 439–443.
[26] J. Wang, J. Zhao, L. Yu, S.P. Li, Cell materials biospecific extraction for hypothesis of active components in Chinese medicines, in: S.P. Li, Y.T. Wang (Eds.), Pharmacological Activity-Based Quality Control of Chinese Herbs, Nova Science Publishers Inc., New York, 2008, pp. 11–19.
[27] M. Noldner, K. Schotz, Rutin is essential for the antidepressant activity of *Hypericum perforatum* extracts in the forced swimming test, Planta Med. 68 (2002) 577–580.
[28] F.R. Stermitz, P. Lorenz, J.N. Tawara, L.A. Zenewicz, K. Lewis, Synergy in a medicinal plant: antimicrobial action of berberine potentiated by 5′-methoxyhydnocarpin, a multidrug pump inhibitor, Proc. Natl. Acad. Sci. U.S.A. 97 (2000) 1433–1437.
[29] Y.W. Liu, J.L. Gao, J. Guan, Z.M. Qian, K. Feng, S.P. Li, Evaluation of antiproliferative activities and action mechanisms of extracts from two species of *Ganoderma* on tumor cell lines, J. Agric. Food Chem. 57 (2009) 3087–3093.
[30] S.P. Li, F.Q. Yang, K.W. Tsim, Quality control of *Cordyceps sinensis*, a valued traditional Chinese medicine, J. Pharm. Biomed. Anal. 41 (2006) 1571–1584.
[31] Pharmacopoeia Commission of PRC, Pharmacopoeia of the People's Republic of China, vol. I, China Medico-Pharmaceutical Science & Technology Publishing House, Beijing, 2010.
[32] S.P. Li, P. Li, T.T. Dong, K.W. Tsim, Determination of nucleosides in natural *Cordyceps sinensis* and cultured *Cordyceps* mycelia by capillary electrophoresis, Electrophoresis 22 (2001) 144–150.
[33] L.I. Benowitz, D.E. Goldberg, N. Irwin, Inosine stimulates axon growth *in vitro* and in the adult CNS, Prog. Brain Res. 137 (2002) 389–399.
[34] F.Q. Yang, D.Q. Li, K. Feng, D.J. Hu, S.P. Li, Determination of nucleotides, nucleosides and their transformation products in *Cordyceps* by ion-pairing reversed-phase liquid chromatography-mass spectrometry, J. Chromatogr. A 1217 (2010) 5501–5510.
[35] D.K.W. Mok, F.T. Chau, Chemical information of Chinese medicines: a challenge to chemist, Chemometr. Intell. Lab. 82 (2006) 210–217.
[36] P.S. Xie, A.Y. Leung, Understanding the traditional aspect of Chinese medicine in order to achieve meaningful quality control of Chinese materia medica, J. Chromatogr. A 1216 (2009) 1933–1940.
[37] Y. Liang, P. Xie, F. Chau, Chromatographic fingerprinting and related chemometric techniques for quality control of traditional Chinese medicines, J. Sep. Sci. 33 (2010) 410–421.
[38] X.K. Zhong, D.C. Li, J.G. Jiang, Identification and quality control of Chinese medicine based on the fingerprint techniques, Curr. Med. Chem. 16 (2009) 3064–3075.
[39] Y. Jiang, B. David, P. Tu, Y. Barbin, Recent analytical approaches in quality control of traditional Chinese medicines—a review, Anal. Chim. Acta 657 (2010) 9–18.
[40] http://www.39.net/focus/jkjd/174286.html (accessed 05/10/2010).
[41] S.C. Lao, S.P. Li, K.K.W. Kan, P. Li, J.B. Wan, Y.T. Wang, T.T.X. Dong, K.W.K. Tsim, Identification and quantification of 13 components in *Angelica sinensis* (Danggui) by gas chromatography-mass spectrometry coupled with pressurized liquid extraction, Anal. Chim. Acta 526 (2004) 131–137.
[42] F.Q. Yang, S.P. Li, Y. Chen, S.C. Lao, Y.T. Wang, T.T. Dong, K.W. Tsim, 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. 39 (2005) 552–558.
[43] N.Y. Qin, F.Q. Yang, Y.T. Wang, S.P. Li, Quantitative determination of eight components in rhizome (Jianghuang) and tuberous root (Yujin) of *Curcuma longa* using pressurized liquid extraction and gas chromatography-mass spectrometry, J. Pharm. Biomed. Anal. 43 (2007) 486–492.
[44] C.U. Tam, F.Q. Yang, Q.W. Zhang, J. Guan, S.P. Li, Optimization and comparison of three methods for extraction of volatile compounds from *Cyperus rotundus* evaluated by gas chromatography-mass spectrometry, J. Pharm. Biomed. Anal. 44 (2007) 444–449.
[45] T.A. Van Beek, H.A. Scheeren, T. Rantio, W.C. Melger, G.P. Lelyveld, Determination of ginkgolides and bilobalide in *Ginkgo biloba* leaves and phytopharmaceuticals, J. Chromatogr. A 543 (1991) 375–387.
[46] X.Y. Gao, Y. Jiang, J.Q. Lu, P.F. Tu, One single standard substance for the determination of multiple anthraquinone derivatives in Rhubarb using high-performance liquid chromatography-diode array detection, J. Chromatogr. A 1216 (2009) 2118–2123.
[47] Y.C. Xie, S.H. Jin, Determination of Radix Salviae miltiorrhizae and compound Danshen tablets by substitute reference substance, Chin. J. Pharm. Anal. 27 (2007) 497–502.
[48] X. Huang, Y. Zheng, Z.Y. Zeng, T. Gong, Quantitative determination of lignans in Frucyus arctii's extracted by corrected factor, J. Chendu Univ. Tradit. Chin. Med. 28 (2005) 42–44.
[49] Z.M. Wang, H.M. Gao, X.T. Fu, W.H. Wang, Multi-components quantitation by one marker new method for quality evaluation of Chinese herbal medicine, Chin. J. Chin. Mater. Med. 31 (2006) 1925–1928.
[50] Y.H. Kuang, J.J. Zhu, Z.M. Wang, Q.W. Zhang, Simultaneous quantitative analysis of five alkaloids in Rhizoma of *Coptis chinensis* by multi-components assay by single marker, Chin. Pharm. J. 44 (2009) 390–394.
[51] J.J. Zhu, Z.M. Wang, Y.H. Kuang, Q.W. Zhang, Q.P. Gao, N. Ma, A quantitative method using one marker for simultaneous assay of ginsenosides in *Panax ginseng* and *P. notoginseng*, Acta Pharm. Sin. 43 (2008) 1211–1216.
[52] X. Wang, S.L. Morris-Natschke, K.H. Lee, New developments in the chemistry and biology of the bioactive constituents of Tanshen, Med. Res. Rev. 27 (2007) 133–148.
[53] J.I. Lee, M. Narayan, J.S. Barrett, Analysis and comparison of active constituents in commercial standardized silymarin extracts by liquid chromatography-electrospray ionization mass spectrometry, J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 845 (2007) 95–103.
[54] N.C. Kim, T.N. Graf, C.M. Sparacino, M.C. Wani, M.E. Wall, Complete isolation and characterization of silybins and isosilybins from milk thistle (*Silybum marianum*), Org. Biomol. Chem. 1 (2003) 1684–1689.
[55] D.Y. Lee, Y. Liu, Molecular structure and stereochemistry of silybin A, silybin B, isosilybin A, and isosilybin B, isolated from *Silybum marianum* (milk thistle), J. Nat. Prod. 66 (2003) 1171–1174.
[56] J.I. Lee, B.H. Hsu, D. Wu, J.S. Barrett, Separation and characterization of silybin, isosilybin, silydianin and silychristin in milk thistle extract by liquid chromatography-electrospray tandem mass spectrometry, J. Chromatogr. A 1116 (2006) 57–68.
[57] V. Kren, J. Ulrichova, P. Kosina, D. Stevenson, P. Sedmera, V. Prikrylova, P. Halada, V. Simanek, Chemoenzymatic preparation of silybin beta-glucuronides and their biological evaluation, Drug Metab. Dispos. 28 (2000) 1513–1517.
[58] S. Chlopcikova, J. Psotova, P. Miketova, V. Simanek, Chemoprotective effect of plant phenolics against anthracycline-induced toxicity on rat cardiomyocytes. Part I. Silymarin and its flavonolignans, Phytother. Res. 18 (2004) 107–110.
[59] N. Skottova, V. Krecman, V. Simanek, Activities of silymarin and its flavonolignans upon low density lipoprotein oxidizability *in vitro*, Phytother. Res. 13 (1999) 535–537.
[60] S. Heron, M.G. Maloumbi, M. Dreux, E. Verette, A. Tchapla, Method development for a quantitative analysis performed without any standard using an evaporative light-scattering detector, J. Chromatogr. A 1161 (2007) 152–156.
[61] J.L. Luque-Garcia, T.A. Neubert, Sample preparation for serum/plasma profiling and biomarker identification by mass spectrometry, J. Chromatogr. A 1153 (2007) 259–276.
[62] A. Villar-Garea, M. Griese, A. Imhof, Biomarker discovery from body fluids using mass spectrometry, J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 849 (2007) 105–114.
[63] Y. Wang, K. Karu, W.J. Griffiths, Analysis of neurosterols and neurosteroids by mass spectrometry, Biochimie 89 (2007) 182–191.
[64] L.Z. Lin, S. Mukhopadhyay, R.J. Robbins, J.M. Harnly, Identification and quantification of flavonoids of Mexican oregano (*Lippia graveolens*) by LC–DAD–ESI/MS analysis, J. Food Compos. Anal. 20 (2007) 361–369.
[65] J.W. de Kraker, M.C. Franssen, A. de Groot, W.A. Konig, H.J. Bouwmeester, (+)-Germacrene A biosynthesis. The committed step in the biosynthesis of bitter sesquiterpene lactones in chicory, Plant Physiol. 117 (1998) 1381–1392.
[66] N. Baldovini, F. Tomi, J. Casanova, Identification and quantitative determination of furanodiene, a heat-sensitive compound, in essential oil by ^{13}C-NMR, Phytochem. Anal. 12 (2001) 58–63.
[67] P. Weyerstahl, H. Marschall-Weyerstahl, C. Christiansen, B.O. Oguntimein, A.O. Adeoye, Volatile constituents of *Eugenia uniflora* leaf oil, Planta Med. 54 (1988) 546–549.
[68] F.Q. Yang, S.P. Li, J. Zhao, S.C. Lao, Y.T. Wang, Optimization of GC–MS conditions based on resolution and stability of analytes for simultaneous determination of nine sesquiterpenoids in three species of *Curcuma* rhizomes, J. Pharm. Biomed. Anal. 43 (2007) 73–82.
[69] C.W. Huie, A review of modern sample-preparation techniques for the extraction and analysis of medicinal plants, Anal. Bioanal. Chem. 373 (2002) 23–30.
[70] F. Tang, Q. Zhang, Z. Nie, S. Yao, B. Chen, Sample preparation for analyzing traditional Chinese medicines, Trends Anal. Chem. 28 (2009) 1253–1262.
[71] C. Deng, N. Liu, M. Gao, X. Zhang, Recent developments in sample preparation techniques for chromatography analysis of traditional Chinese medicines, J. Chromatogr. A 1153 (2007) 90–96.
[72] J.J. Xie, J. Lu, Z.M. Qian, Y. Yu, J.A. Duan, S.P. Li, Optimization and comparison of five methods for extraction of coniferyl ferulate from *Angelica sinensis*, Molecules 14 (2009) 555–565.
[73] F.Q. Yang, S.P. Li, Effects of sample preparation methods on the quantification of nucleosides in natural and cultured *Cordyceps*, J. Pharm. Biomed. Anal. 48 (2008) 231–235.
[74] G.P. Lv, W.H. Huang, F.Q. Yang, J. Li, S.P. Li, Pressurized liquid extraction and GC–MS analysis for simultaneous determination of seven components in *Cinnamomum cassia* and the effect of sample preparation, J. Sep. Sci. 33 (2010) 2341–2348.
[75] K.N. Prasad, B. Yang, X. Dong, G. Jiang, H. Zhang, H. Xie, Y. Jiang, Flavonoid contents and antioxidant activities from *Cinnamomum* species, Innovat. Food Sci. Emerg. Technol. 10 (2009) 627–632.
[76] J. Yang, L.H. Chen, Q. Zhang, M.X. Lai, Q. Wang, Quality assessment of Cortex Cinnamomi by HPLC chemical fingerprint, principle component analysis and cluster analysis, J. Sep. Sci. 30 (2007) 1276–1283.
[77] Z.D. He, C.F. Qiao, Q.B. Han, C.L. Cheng, H.X. Xu, R.W. Jiang, P.P. But, P.C. Shaw, Authentication, quantitative analysis on the chemical profile of Cassia bark (Cortex Cinnamomi) by high-pressure liquid chromatography, J. Agric. Food Chem. 53 (2005) 2424–2428.
[78] F. Gong, Y.Z. Liang, Y.S. Fung, F.T. Chau, Correction of retention time shifts for chromatographic fingerprints of herbal medicines, J. Chromatogr. A 1029 (2004) 173–183.
[79] K. Sagara, T. Oshima, T. Yoshida, Y.Y. Tong, G. Zhang, Y.H. Chen, Determination of Cinnamomi Cortex by high-performance liquid chromatography, J. Chromatogr. 409 (1987) 365–370.

[80] S. Keunchkarian, M. Reta, L. Romero, C. Castells, Effect of sample solvent on the chromatographic peak shape of analytes eluted under reversed-phase liquid chromatogaphic conditions, J. Chromatogr. A 1119 (2006) 20–28.

[81] WHO, General Guidelines for Methodologies on Research and Evaluation of Traditional Medicines, World Health Orgnization, Geneva, 2000.

[82] C.F. Poole, Thin-layer chromatography: challenges and opportunities, J. Chromatogr. A 1000 (2003) 963–984.

[83] X. Wang, V. Kapoor, G.A. Smythe, Extraction and chromatography-mass spectrometric analysis of the active principles from selected Chinese herbs and other medicinal plants, Am. J. Chin. Med. 31 (2003) 927–944.

[84] P. Drasar, J. Moravcova, Recent advances in analysis of Chinese medical plants and traditional medicines, J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 812 (2004) 3–21.

[85] E. de Rijke, P. Out, W.M. Niessen, F. Ariese, C. Gooijer, U.A. Brinkman, Analytical separation and detection methods for flavonoids, J. Chromatogr. A 1112 (2006) 31–63.

[86] W. Oleszek, Z. Bialy, Chromatographic determination of plant saponins—an update (2002–2005), J. Chromatogr. A 1112 (2006) 78–91.

[87] G. Chen, Y. Zhu, Y. Wang, X. Xu, T. Lu, Determination of bioactive constituents in traditional Chinese medicines by CE with electrochemical detection, Curr. Med. Chem. 13 (2006) 2467–2485.

[88] M. Ganzera, Quality control of herbal medicines by capillary electrophoresis: potential requirements and applications, Electrophoresis 29 (2008) 3489–3503.

[89] M. Adahchour, J. Beens, U.A. Brinkman, Recent developments in the application of comprehensive two-dimensional gas chromatography, J. Chromatogr. A 1186 (2008) 67–108.

[90] Y.M. Lao, J.G. Jiang, L. Yan, Application of metabonomic analytical techniques in the modernization and toxicology research of traditional Chinese medicine, Br. J. Pharmacol. 157 (2009) 1128–1141.

[91] X.M. Liang, Y. Jin, Y.P. Wang, G.W. Jin, Q. Fu, Y.S. Xiao, Qualitative, quantitative analysis in quality control of traditional Chinese medicines, J. Chromatogr. A 1216 (2009) 2033–2044.

[92] M. Yang, J. Sun, Z. Lu, G. Chen, S. Guan, X. Liu, B. Jiang, M. Ye, D.A. Guo, Phytochemical analysis of traditional Chinese medicine using liquid chromatography coupled with mass spectrometry, J. Chromatogr. A 1216 (2009) 2045–2062.

[93] E. Hurtado-Fernandez, M. Gomez-Romero, A. Carrasco-Pancorbo, A. Fernandez-Gutierrez, Application and potential of capillary electroseparation methods to determine antioxidant phenolic compounds from plant food material, J. Pharm. Biomed. Anal. 53 (2010) 1130–1160.

[94] F.Q. Yang, J. Zhao, S.P. Li, CEC of phytochemical bioactive compounds, Electrophoresis 31 (2010) 260–277.

[95] N. Fuzzati, B. Gabetta, K. Jayakar, R. Pace, G. Ramaschi, F. Villa, Determination of ginsenosides in *Panax ginseng* roots by liquid chromatography with evaporative light-scattering detection, J. AOAC Int. 83 (2000) 820–829.

[96] A.J. Lau, S.O. Woo, H.L. Koh, Analysis of saponins in raw and steamed *Panax notoginseng* using high-performance liquid chromatography with diode array detection, J. Chromatogr. A 1011 (2003) 77–87.

[97] E.B. Asafu-Adjaye, S.K. Wong, Determination of ginsenosides (ginseng saponins) in dry root powder from *Panax ginseng*, *Panax quinquefolius*, and selected commercial products by liquid chromatography: interlaboratory study, J. AOAC Int. 86 (2003) 1112–1123.

[98] S. Zhu, K. Zou, S. Cai, M.R. Meselhy, K. Komatsu, Simultaneous determination of triterpene saponins in Ginseng drugs by high-performance liquid chromatography, Chem. Pharm. Bull. (Tokyo) 52 (2004) 995–998.

[99] J.B. Wan, C.M. Lai, S.P. Li, M.Y. Lee, L.Y. Kong, Y.T. Wang, Simultaneous determination of nine saponins from *Panax notoginseng* using HPLC and pressurized liquid extraction, J. Pharm. Biomed. Anal. 41 (2006) 274–279.

[100] J.B. Wan, P. Li, S. Li, Y. Wang, T.T. Dong, K.W. Tsim, Simultaneous determination of 11 saponins in *Panax notoginseng* using HPLC–ELSD and pressurized liquid extraction, J. Sep. Sci. 29 (2006) 2190–2196.

[101] J.B. Wan, F.Q. Yang, S.P. Li, Y.T. Wang, X.M. Cui, Chemical characteristics for different parts of *Panax notoginseng* using pressurized liquid extraction and HPLC-ELSD, J. Pharm. Biomed. Anal. 41 (2006) 1596–1601.

[102] J.B. Wan, S.P. Li, J.M. Chen, Y.T. Wang, Chemical characteristics of three medicinal plants of the *Panax* genus determined by HPLC-ELSD, J. Sep. Sci. 30 (2007) 825–832.

[103] S.N. Kim, Y.W. Ha, H. Shin, S.H. Son, S.J. Wu, Y.S. Kim, Simultaneous quantification of 14 ginsenosides in *Panax ginseng* C.A. Meyer (Korean red ginseng) by HPLC–ELSD and its application to quality control, J. Pharm. Biomed. Anal. 45 (2007) 164–170.

[104] Y. Wang, J. You, Y. Yu, C. Qu, H. Zhang, L. Ding, H. Zhang, X. Li, Analysis of ginsenosides in *Panax ginseng* in high pressure microwave-assisted extraction, Food Chem. 110 (2008) 161–167.

[105] C.Z. Wang, M. Ni, S. Sun, X.L. Li, H. He, S.R. Mehendale, C.S. Yuan, Detection of adulteration of notoginseng root extract with other *Panax* species by quantitative HPLC coupled with PCA, J. Agric. Food Chem. 57 (2009) 2363–2367.

[106] B.S. Sun, L.J. Gu, Z.M. Fang, C.Y. Wang, Z. Wang, M.R. Lee, Z. Li, J.J. Li, C.K. Sung, Simultaneous quantification of 19 ginsenosides in black ginseng developed from *Panax ginseng* by HPLC–ELSD, J. Pharm. Biomed. Anal. 50 (2009) 15–22.

[107] C.M. Lai, S.P. Li, H. Yu, J.B. Wan, K.W. Kan, Y.T. Wang, A rapid HPLC–ESI–MS/MS for qualitative and quantitative analysis of saponins in XUESETONG injection, J. Pharm. Biomed. Anal. 40 (2006) 669–678.

[108] J. Guan, C.M. Lai, S.P. Li, A rapid method for the simultaneous determination of 11 saponins in *Panax notoginseng* using ultra performance liquid chromatography, J. Pharm. Biomed. Anal. 44 (2007) 996–1000.

[109] H. Ji, K. Liu, S.P. Li, X.J. Gong, Effect of HEF on osteoporosis induced by retinoic acid in male rats, J. Chin. Pharm. Univ. 31 (2000) 222–225.

[110] F. Meng, Y. Li, Z. Xiong, Z. Jiang, F. Li, Osteoblastic proliferative activity of *Epimedium brevicornum* Maxim, Phytomedicine 12 (2005) 189–193.

[111] A. Ma, S. Qi, D. Xu, X. Zhang, P. Daloze, H. Chen, Baohuoside-1, a novel immunosuppressive molecule, inhibits lymphocyte activation *in vitro* and *in vivo*, Transplantation 78 (2004) 831–838.

[112] M.L. Cai, H. Ji, P. Li, M.Q. Wang, Effects of five flavonoids isolated from *Epimedium pubescens* on osteoblasts *in vitro*, Chin. J. Nat. Med. 2 (2004) 235–238.

[113] T.Z. Liu, C.Y. Chen, S.J. Yiin, C.H. Chen, J.T. Cheng, M.K. Shih, Y.S. Wang, C.L. Chern, Molecular mechanism of cell cycle blockage of hepatoma SK-Hep-1 cells by Epimedin C through suppression of mitogen-activated protein kinase activation and increased expression of CDK inhibitors $p21^{Cip1}$ and $p27^{Kip1}$, Food Chem. Toxicol. 44 (2006) 227–235.

[114] X.J. Chen, B.L. Guo, S.P. Li, Q.W. Zhang, P.F. Tu, Y.T. Wang, Simultaneous determination of 15 flavonoids in *Epimedium* using pressurized liquid extraction and high-performance liquid chromatography, J. Chromatogr. A 1163 (2007) 96–104.

[115] X.J. Chen, H. Ji, Q.W. Zhang, P.F. Tu, Y.T. Wang, B.L. Guo, S.P. Li, A rapid method for simultaneous determination of 15 flavonoids in *Epimedium* using pressurized liquid extraction and ultra-performance liquid chromatography, J. Pharm. Biomed. Anal. 46 (2008) 226–235.

[116] X.J. Chen, P.F. Tu, Y. Jiang, Y.T. Wang, S.P. Li, Effect of stability of internal standard on quantification of 15 flavonoids in *Epimedium* using CZE, J. Sep. Sci. 32 (2009) 275–281.