Application of Mid-Infrared Spectroscopy in the Quality Control of Traditional Chinese Medicines

Authors
Suqin Sun, Jianbo Chen, Qun Zhou, Guanghua Lu, Kelvin Chan

Affiliations
The affiliations are listed at the end of the article

Abstract
Chinese herbal medicines are often referred to as Chinese materia medica (CMM). Composite formulae containing mixtures of CMM are prescribed for treatment and prevention of diseases in the practice of traditional Chinese medicine (TCM). Some of the well-known CMM formulae (Fufang in Chinese) are manufactured and marketed as proprietary Chinese medicines (PCM). Quality assessment and assurance of these products are difficult; they are a challenging task. Mid-infrared spectroscopy, a classic molecular structure analysis method, has been innovatively applied in the quality control of TCM, and has gained significant impact and advancement in analytical fields. Infrared fingerprinting features appear particularly suitable for the identification of multicomponent matrices in samples whose chemical integrity has not been altered or destroyed because no extraction procedure is needed. This review summarizes and gives an overall view on the application of mid-infrared and two-dimensional correlation infrared (2D-IR) spectroscopy as well as chemometric techniques in the identification of CMM, investigation of TCM processing procedures, and analysis of herb extracts and preparations.

Abbreviations
2D-IR: two-dimensional correlation infrared
ANN: artificial neural network
ATR: attenuated total reflectance
CMM: Chinese materia medica
FSD: Fourier self-deconvolution
FT-IR: Fourier transform infrared spectroscopy
HCA: hierarchical clustering analysis
IRMFA: infrared spectroscopic macro-fingerprints analysis
M-IR: mid-infrared spectroscopy
NNM: nearest neighbor method
PCA: principal component analysis
PCM: proprietary Chinese medicines
PLS: partial least square
RBF: radial basis function
SD-IR: second derivative infrared
SIMCA: soft independent modeling of class analogies
SVM: support vector machine
TCM: traditional Chinese medicine
TIRIA: tri-level infrared spectroscopic identification analysis

Introduction
Traditional Chinese medicines (TCM) include Chinese medicinal materials (CMM), CMM extracts, and proprietary Chinese medicines (PCM)/composite formulae, which contain complex chemical compositions. In general, pharmacological screenings and clinical tests show that multi-chemical compounds in TCM preparations are bioactive contributing to the overall therapeutic effect for that specific preparation. Similarly, for a holistic approach towards disease treatment in individual patients, the TCM doctor prescribes selected CMM in the form of herbal decoctions. This is essentially how CMM are used for prevention and treatment of diseases. It is difficult to identify a single chemical marker or the most representative marker contributing to the medicine function of a CMM. Thus, the quality of TCM products still remains a problem to be effectively evaluated, controlled, and assured. Over the past decade two directions on the quality control of TCM have been reported in the literature and adopted by the Pharmacopoeia using different chemometric methods.
The mid-infrared spectrum is considered as the overlapped spectrum of all chemical compositions. The infrared spectral peaks for a particular function group in the molecular structure are located at the same spectral region. The information for a class of chemical compounds with similar molecular structures can be deduced. For example, the peak at 1745 cm$^{-1}$ is assigned to the stretch vibration of C=O bonds in pure glycerin tripalmitate. If a peak is located at this position of the infrared spectrum, it may indicate that this herbal sample contains ester compounds and related groups. Therefore, chemical information about TCM samples can be obtained by comparing the positions of overlapped peaks to those of authenticated TCM reference samples or chemical standards. With the help of multivariate calibration models, some chemical compounds can be quantified in TCM samples.

The derived pattern of IR spectrum of a TCM sample can be considered as a spectroscopic fingerprint for this specific sample. This fingerprint is defined as “macro-fingerprint” to be differentiated from the features of the pure compounds. The changes in peak position and intensity of the spectra can be related to the changing variety of chemical compositions in the sample. Hence, various TCM samples might be differentiated by their infrared spectra although their chemical compositions have not been exactly revealed or identified. Based on this principle, the true or fake herbs, good (defined upon the fact that their qualities have been proved in practice for thousands of years) or bad quality samples might be identified or referred to by their infrared spectra.

In the literature, tri-level infrared spectroscopic identification analysis (TIRIA) is used to identify CMM [6]. CMM samples can initially be identified and differentiated by comparing their FT-IR spectra, known as the primary identification. The secondary identification analysis is based on second derivative infrared (SD-IR) spectroscopy with a greater resolution than the primary infrared spectrum. The resolution can differentiate and separate some overlapped peaks on the primary infrared spectrum resulting in better SD-IR spectra to distinguish the CMM samples. In the case that differentiation using infrared and SD-IR spectra is not possible for some samples, two-dimensional correlation infrared (2D-IR) spectroscopy can be used; which is known as tertiary identification analysis. 2D-IR or two-dimensional correlation spectroscopy was originally introduced and expanded by Noda [7–9] and provides a 3D-plot of the spectrum. The 2D-IR plots of the CMM samples can then be used to tell the differences apart.
ricae Cornu (the horn of Saiga tatarica L.), Scorpio (the whole body of Buthus martensi Karsch), and Hirudo (the whole body of Hirudo nipponica Whitman) can be visualized as bands of amide I and II on their infrared spectra. The fact that Cervi Pantotrichum Cornu contains inorganic salt Ca₃(PO₄)₂ and Scorpio sulfates could be verified from their infrared spectra [10].

Coptidis Rhizoma (the rhizome of Coptis chinensis Franch.) contains a high level of berberine as visualized in the fingerprinting peaks found both on its infrared and SD-IR spectra, with the pattern being more obviously shown in the latter than in the former spectrum (Fig. 1). We also observed that the intensities of characteristic peaks changed with the varying amount of berberine in tested samples, which was in agreement with the result of the HPLC analysis [11].

Pei and coworkers analyzed Epimedi Herba (the branch and leaf of Epimedium brevicornu Maxim.) by infrared and HPLC methods. They figured out that the peak at ~1259 cm⁻¹ on its infrared spectrum was related to the 4′-methoxyl-prenylflavonols, which were considered as the main bioactive compounds in this herb [12]. Cheung et al. also identified this characteristic peak by wavelet analysis and radial basis function (RBF) using neural network [13]. Therefore, the absorption peak at ~1259 cm⁻¹ on the infrared spectrum may be used as a characteristic peak to rapidly and effectively assess the quality of Epimedi Herba.

Differentiation of Genuine and Fake TCM Herbs

Genuine and fake TCM herbs can be identified by infrared macro-fingerprinting because the fake herbs must contain different chemical compositions compared to the true ones. Cao et al. distinguished the authentic Gastrodiae Rhizoma (the tuber of Gastrodia elata BL.) from its counterfeit (the rhizome of Canna edulis Ker) by 2D-IR spectroscopy [14]. Although their infrared spectra were found to be similar, their 2D-IR spectra were significantly different. There were two strong auto-peaks located at 1237 and 1415 cm⁻¹ in the range of 1500–800 cm⁻¹ on synchronous 2D-IR spectrum of the genuine Gastrodiae Rhizoma, whilst the auto-peaks in the counterfeit samples appeared at 1024, 1055, 1194, and 1225 cm⁻¹. Zhou et al. [15] identified the authentic Rhe Radix et Rhizoma (the root and rhizome of Rheum tanguticum L.) and its fake one (the rhizome of Rheum franezbachii Munt.) by infrared and 2D-IR spectra with thermal perturbation. The peak position and intensity on infrared spectra of these pair of herbs were very similar, but their 2D-IR spectra were drastically different. In the region of 1700–1000 cm⁻¹, only two auto-peaks located at 1460 and 1080 cm⁻¹ occurred in the fake herb, whilst two additional auto-peaks occurred at 1560 and 1060 cm⁻¹ in the genuine herb.

TIRIA is often utilized to identify genuine or fake TCM herbs. Sun et al. differentiated the genuine Pinellia Rhizoma (the rhizome of Pinellia ternata [Thunb.] Breit.) from its counterfeit with this technique [16]. In addition, the authentic and fake herbs, namely Pinellia Rhizoma [16], Asini Corii Colla (donkey hide stewed and concentrated as gelatinous mass, Equus asinus L.) [17] (Fig. 2), Glycyrrhizae Radix et Rhizoma (the root and rhizome of Glycyrrhiza uralensis Fisch.) [18], Anisi Stellati Fructus (the fruit of Illicium verum Hook. f.) [19], Codonopsis Radix (the root of Codonopsis pilosula [Franch.] Nannf.) [20], Rosae Rugosae Flos (the flower bud of Rosa rugosa Thunb.) [21], Cistanches Herba (the fleshy stem of Cistanche desertica Y.C. Ma) [22] and Cordyceps (the stroma formed Cordyceps sinensis [Berk.] Sacc., a parasite of the larva of Hepialus armoricanus Oberthru.) [23] were successfully identified by this method.

The derivative infrared spectra and the Fourier self-deconvolution (FSD) method can separate overlapped peaks and enhance the resolution of the spectra during analysis. Cheng et al. differen-
entiated genuine Dioscorea Rhizoma samples from their counterfeits by the FSD-IR spectra [24]. The genuine and fake Dioscorea Rhizoma (the tuber of *Dioscorea tutschanovii* Bess. f. yunnanensis Y. H. Chou et C. C. Hsu) [25] and Ophiopogonis Radix (the root tuber of *Ophiopogon japonicus* [Thunb.] Ker-Gawl.) [26] were differentiated by combining the derivative infrared spectra and statistic test methods. RBF neural network was also used to identify the genuine and fake Atractylodis Macrocephalae Rhizoma (the rhizome of *Atractylodes macrocephala* Koidz.) [27] and Rhei Radix et Rhizoma [28] on the basis of infrared spectra.

**Differentiation of Chinese Herbs Collected from Different Geographical Regions**

The proper and successful practice of Chinese medicine depends on the availability of good quality CMM samples, which should originate from their original cultivation areas. It is generally accepted that CMM originating from these areas are of the best quality. These CMM are referred to as “trueborn” (“Daodong” in Chinese transliteration) from the original cultivation area. Those not grown in their geographical origins are considered as “non-trueborn” CMM. Samples from these different sources may result in various therapeutic effects. Prices between trueborn and non-trueborn samples are usually different in herbal markets. Infrared techniques were used to differentiate these kinds of samples based on the variation in their chemical compositions.

Han and coworkers [29] analyzed Puerariae Lobatae Radix (the root of *Pueraria lobata* [Willd. Ohwi]) samples collected from three different regions (Tianjin, Hunan, and Chongqing) in China by infrared and 2D-IR spectroscopy. All samples showed similar infrared spectra identified as starch but different intensities of the characteristic peaks characterized as puerarin. The samples collected from Tianjin showed stronger intensity than those of other regions, and their infrared spectra differed most from the starch. Similar observations were obtained from the SD-IR spectra. These results indicated that the quality of the samples collected from Tianjin might be better than the others. Other investigations using infrared and 2D-IR on CMM collected from different geographical areas were reported, e.g., Fritillariae Bulbus [30], Panacis Quinquefolii Radix (the root of *Panax quinquifolium* L.) [31], and Citri Reticulatae Pericarpium (the pericarp of *Citrus reticulata* Blanco) [32].

Some statistic classification methods are feasible to enhance the resolution of the infrared spectra for large numbers of samples. For the identification of trueborn and non-trueborn samples of Dioscoreae Rhizoma (the rhizome of *Dioscorea opposita* Thunb.), three different classification methods were applied. Sun et al. differentiated, with the aid of standard samples, trueborn from non-trueborn samples of Dioscoreae Rhizoma using the correlation coefficients of infrared spectra [33]. The correlation coefficients among the spectra of trueborn samples to those of standard samples were greater than 0.98, whereas those of the non-trueborn samples were smaller than 0.98. Xu and coworkers differentiated the samples of Dioscoreae Rhizoma collected from different cultivation areas by PCA analysis of FT-IR spectra. The scores of the samples on the second and third principal components were effective to differentiate the trueborn samples from non-trueborn ones [34]. The SIMCA classification method was also applied to differentiate the trueborn samples of Dioscoreae Rhizoma from the others [35]. Zhou and coworkers also used the SIMCA method to identify the samples of Lycii Fructus (the fruit of *Lycium bar-
sample of Scutellariae Radix by three kinds of BP-ANN methods. The recognition rate for the best model was more than 97% [46].

Identification of Different Species of Chinese Herbs

Huang and coworkers [47] analyzed some typical herbal samples belonging to different families, such as Araliaceae, Campanulaceae, Magnoliaceae, Lauraceae, Leguminosae, Berberidaceae, and Cruciferae. The similarities and differences among the herbal samples in a specific family were also analyzed. The results indicated that the FT-IR technique was an effective method for the chemotaxonomy, which would be a supplement of the morphologic taxonomy.

The infrared spectra of Ginseng Radix et Rhizoma, Panacis Quinquefolii Radix and Notoginseng Radix et Rhizoma (the root of Panax notoginseng [Burk.] F.H. Chen) were much similar for the same matrix compositions. But the three groups of herbal samples were differentiated by either the SIMCA method or the SD-IR and 2D-IR spectra [48]. Wang et al. identified samples of Cimicifugae Rhizoma (the rhizome of Cimicifuga spp.) from 15 species of plants by infrared spectra. The differences between samples of different families were quite obvious [49]. The samples of Lycii Fructus (Gouqizi in Chinese transliteration) from 10 species of plants were identified by infrared spectra [50]. Pei et al. identified samples of Epimedi Herba from 5 species of plants by infrared and SD-IR spectra [51].

For the identification works using the TIRIA method, the abovementioned examples are normally compared using number, position, and approximate intensity of auto- and cross peak of the 2D-IR spectra. However, Chen et al. [52] introduced the quantitative analysis method by 2D-IR spectra and discriminated samples of Atraglali Radix (Huangqi in Chinese transliteration) coming from different genera by the symmetry analysis of hetero 2D-IR spectra (Fig. 4) and statistical test methods [53].

Differentiation of CMM in Various Parts, Storage Duration, and Morphological Features

Lu and coworkers differentiated the main root from the rootlets of Angelicae Sinensis Radix (the root of Angelica sinensis (Oliv.) Diels) by infrared and 2D-IR spectra [54]. Different spectra between the main root and the rootlets of the same plant indicated the inhomogeneous distribution of amino acids, essential oil, and sugar. Jin et al. [55] identified the root, stem, and leaf of Acanthopanax senticosus (Rupr. et Maxim.) Harms by infrared and 2D-IR spectra. It was found that starch and calcium oxalate were abundant in the root and stem, whilst the leaves contained much more flavones than the other two plant parts. Xu et al. analyzed different parts of the stem of Cistanche deserticola Y.C. Ma by infrared and 2D-IR spectra and revealed that the chemical compositions were different in the cortex and core of this stem [56]. Hong et al. found that peoniflorin in the xylem of Paeoniae Alba Radix (the root of Paeonia lactiflora Pall.) was more abundant than that in the cortex by infrared spectra [57].

Zhan and coworkers applied wavelet transform to improve the resolution of 2D-IR spectra and successfully differentiated the various age samples of Ginseng Radix et Rhizoma [58]. During storage of Citri Reticulatae Pericarpium samples, the peak intensities at 2851, 1716, and 1516 cm⁻¹ on the FT-IR spectra of its extract were increased, and peak positions were changed to 1734, 1517, and 1276 cm⁻¹, which resulted from the increased amount of hesperidin, organic acids, and esters. The results reflected the fact that “the longer the storage duration of the Citri Reticulatae Pericarpium, the better quality of the herb” [59]. Moreover, Sun et al. successfully differentiated samples of Lycii Fructus in a variety of colors, shapes, tastes, and water content by FT-IR spectra [60]. Liu et al. analyzed the samples of Paeoniae Alba Radix collected from the Good Agricultural Practice base, herb markets, and purchased standard herbs [61].

Quality Assessment of CMM during Processing

Some CMM must be processed by physical and/or chemical procedures before clinical use in order to decrease the side effects or improve therapeutic effects. It is valuable to reveal the fundamental physical and chemical processing to effectively control the quality of the processed sample and differentiate it from raw materials.

Yu and coworkers [62] investigated the processing of Rehmanniae Radix (the root of Rehmannia glutinosa Libosch, raw material) by yellow wine to produce Rehmanniae Radix Praeparata (processed sample) by infrared and 2D-IR spectra. Based on the changes of the infrared (Fig. 5A), SD-IR (Fig. 5B), and 2D-IR spectra of the samples stewed in yellow wine for various durations, it was revealed that stachyose was hydrolyzed into galactose, glucose, and fructose during herb processing. The mixture of glucose and fructose in the processed sample gave an overlap peak at ca. 777 cm⁻¹, which was different from the peak at ~771 cm⁻¹ in raw materials.
Meanwhile, melanoidin was produced by the chemical reaction between amino acids and monosaccharides. Hence, the processed sample appeared blacker in color. These results explained the reason why regular processed samples (Rehmanniae Radix Praeparata) should be sweet and appear black in color. It is possible that the processing procedure can be monitored and controlled by infrared techniques.

The processing procedure of Sinapis Semen was also studied using infrared and 2D-IR spectra [63]. The decreasing of amide I and II bands at about 1657 and 1546 cm\(^{-1}\), respectively, during herb processing indicated the loss of the proteins, which was consistent with the conventional processing principles. The absorption peak of cellulose at ~ 1055 cm\(^{-1}\) was significantly decreased after processing for 10 min resulting in herbal samples turning yellow in color.

The raw and processed Aconiti Radix (the root of *Aconitum carmichaeli* Debx.) [64] and Aconiti Kusnezoffii Radix (the root of *Aconitum kusnezoffii* Reichb.) [65] were differentiated by infrared and 2D-IR spectra, as well as the Aconiti Lateralis Radix Praeparata processed in three different ways [66]. Bao et al. studied the effects of Chrysanthemi Flos (the capitulum of *Chrysanthemum morifolium* Ramat.) processing on the infrared spectra and found that the existence of the peak at 1714 cm\(^{-1}\) could be chosen as a marker to control the processing procedure [67]. Xu et al. investigated the changes of chemical compositions in *Vitis* Fructus (the fruit of *Vitis trifoliata* L. var. *simplicifolia* Cham.) by infrared and 2D-IR spectra during the processing procedures and further successfully differentiated the patterns of various processed samples [68].

### Quality Control of Herbal Extracts and Formula Granules

Extracting CMM in water or other solvents can eliminate unwanted constituents such as cellulose and starch, resulting in a herbal extract with high content of bioactive components. The herbal extract is further processed to herbal preparations such as granules of individual CMM or, if CMM composite formula (mixture of several CMM) is involved, CMM formula granules, CMM injection preparations, and other dosage forms. Liu and coworkers studied the extracts of Angelicae Sinensis Radix extracted by different procedures and observed that a high content of Z-ligustilide was found in the extracts of petroleum ether and water distillation, but the divergence of infrared, 2D-IR, and 2D-IR spectra among these extracts was significantly different [69–70]. Extracts of *Chrysanthemi Flos* collected from seven cultivation regions using different solvents were analyzed by infrared and 2D-IR spectra [71]. The compositions in these extracts were found to vary with the geographical origins and extracting solvents. Wu et al. analyzed the water and alcohol extracts of Coptidis Rhizoma by infrared spectra and found that the amount of berberine in these extracts was greater than that in raw materials [72].

Formula granules are a new type of TCM preparation. They are usually manufactured from CMM using mixtures of solvent-free extracts of CMM with inert excipients such as starch and dextrin during the evaporation procedure of the herbal extract. These formula granules are produced by various manufacturers, and their qualities may be different in the amount of bioactive compounds. Their quality should be assessed and controlled to assure their therapeutic effects. Huang et al. [73] analyzed hundreds of formula granules made by different manufacturers. Their infrared spectra were compared with those generated from extracts of raw materials. Generally, the contents of bioactive components in formula granules were greater than those in raw materials. Formula granules made from different CMM sources or by various manufacturers could be discriminated by infrared spectra. The similarities and differences among different batches of formula granules manufactured by the same herbal industry could be assessed. Zhou and Tang [74, 75] investigated the types and contents of excipients added to formula granules by infrared spectra. They observed that dextrin and lactose were common ingredients and that mixtures of different types of excipients were also used. It was observed that the contents of excipients in formula granules generally varied among manufacturers.

Wu and coworkers [76] analyzed formula granules of *Salviae Milltorrhizae Radix et Rhizoma* made by nine different manufacturers by comparing the infrared spectra of the herbal extract, dextrin, and starch. It was found that two formula granules contained a very small amount of excipients, four contained some dextrin, two contained a high content of dextrin, and one contained a high amount of starch (Fig. 6). The correlation coefficients of infrared spectra among each formula granule to the reference could give the quantitative evaluation for their similarity.

### Quality Control of TCM Injections and Preparations

The normal and expired ‘*Qing Kai Ling*’ injections were identified by infrared and 2D-IR spectra, as well as the mechanism of the deteriorative processes [77, 78]. The differences between the 2D-IR spectra of the normal and expired ‘*Qing Kai Ling*’ injections suggested that its degradation mainly resulted from the oxidation of flavonoids and the decomposition of glycosides [78]. Zhou et al. also discriminated ‘*Qing Kai Ling*’ injections collected from different manufacturers by infrared and 2D-IR spectra [79]. Chen et al. differentiated three types of TCM injections, and all of them were found to contain the extracts of *Ginseng Radix et Rhizoma* [80]. Zhang et al. analyzed the similarities and differences between two injections made from *Chrysanthemi Indici Flos* (the capitulum of *Chrysanthemum indicum* L.) and *Carthami Flos* (the flower of *Carthamus tinctorius* L.) by infrared and 2D-IR spectra [81].

Yan and coworkers [82] established calibration models by applying ATR techniques and a PLS algorithm to quantify the contents of baicalin and chlorogenic acid in ‘*Shuang Huang Lian*’ injections. The determination coefficients (R\(^2\)) of calibration models were
over 0.99. The average relative deviations between the predicted contents of the two compounds by infrared spectroscopy models and the amount measured by HPLC were less than 4%. This result indicates that infrared spectroscopy could be a rapid method for the quality control of TCM injections.

‘Red Flower Oil’, a widely used TCM preparation, is a mixture of several essential oils consisting of wintergreen oil, turpentine oil, clove oil, and cinnamon leaves oil. Wu et al. [83] observed that infrared spectroscopy could be used to identify methyl salicylate as the main compound in wintergreen oil (Fig. 7 A), α-pinene in turpentine oil (Fig. 7 B), and eugenol in clove oil and cinnamon leaves oil (Fig. 7C). These ‘Red Flower Oil’ samples collected from different manufacturers could be discriminated by infrared and 2D-IR spectra. The same author [84] also established calibration models by ATR spectrum and a PLS algorithm to quantitatively analyze methyl salicylate, α-pinene, and eugenol in different samples. All determination coefficients (R²) of calibration models were more than 0.99 for the three compounds. Their values predicted by the infrared spectroscopy models were consistent with those measured by GC.

Conclusions

The practice and use of TCM is not only popular in China and some Asian regions, it is also finding appreciation worldwide [85]. As TCM is a multi-composition remedy, its quality is difficult to effectively assess, control, and assure so as to provide the therapeutic actions that the TCM practitioner expects the patient will receive. Fingerprinting is accepted as one of the approaches for quality control of TCM products using analytical techniques such as chromatography, electrophoresis, or spectroscopy pattern-recognition in research publications. Most of these techniques involve “invasive” extraction procedures and do not reflect the...
true” chemical characteristics of the CMM. Mid-infrared and 2D-IR spectroscopy, which do not require an invasive or extensive sample preparation procedure, combined with appropriate chemometric techniques has been shown to be a useful, rapid, additional, or alternative approach for quality control of CMM and PCM used in TCM treatment.

The main advantages of mid-infrared spectroscopy for the quality control of TCM products are as follows. Firstly, an infrared spectrum provides a “holistic” spectroscopic fingerprinting of all compositions in a tested TCM sample. The variation of both bioactive compounds and unwanted ingredients in tested samples can be shown in the holistic spectroscopic fingerprint thus helping to differentiate and identify good quality from poor quality CMM. Secondly, the operation procedure for sample testing by infrared spectroscopy is simple and rapid. Most CMM samples and PCM products can be directly tested without any extraction, separation, or other preparation. Therefore, chemical composition in tested samples is considered as non-changed, non-damaged. With the availability of software integrating databases, pattern recognition, and calibration models, the quality control of TCM products can be rapidly completed. Currently mid-infrared procedure has been applied to monitor the production of pharmaceutical dosage forms as good manufacturing practice (GMP) in the pharmaceutical industry. With the advancement of modern and database handling technology such an application may be possible in GMP of CMM processing and PCM products in herbal industry. Furthermore, the identity and contents of some chemical compounds in CMM samples can be obtained from infrared spectra by applying some calibration models. Therefore, it is promising and encouraging that mid-infrared spectroscopy offers a rapid alternative or an additional analytical approach for the quality control of TCM products, particularly useful for herbal manufacturers to upgrade the GMP procedure.

Acknowledgements

This work was sponsored by the State Administration of Traditional Chinese Medicine of China (2001DDZX01), the grant of Ministry of Science and Technology of China (2002BA906A29-4), and a Sichuan Provincial grant in China (04SG011-035-1, 2009FR20053).

Affiliations

1 Key Laboratory of the Ministry of Education in China on Bioorganic Phosphorus Chemistry & Chemical Biology, Department of Chemistry, Tsinghua University, Beijing, China
2 Key Laboratory of the Ministry of Education in China on the Standardization of Chinese Materia Medica, School of Pharmacy, Chengdu University of Traditional Chinese Medicine, Chengdu, China
3 Herbal Medicine Research & Education Centre, Faculty of Pharmacy, University of Sydney and Centre for Complementary Medicine Research, College of Health & Science, University of Western Sydney, Sydney, Australia

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