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REVIEW

Applications of HPLC/MS in the analysis of traditional Chinese medicines

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Abstract: In China, traditional Chinese medicines (TCMs) have been used in clinical applications for thousands of years. The successful hyphenation of high-performance liquid chromatography (HPLC) and mass spectrometry (MS) has been applied widely in TCMs and biological samples analysis. Undoubtedly, HPLC/MS technique has facilitated the understanding of the treatment mechanism of TCMs. We reviewed more than 350 published papers within the last 5 years on HPLC/MS in the analysis of TCMs. The present review focused on the applications of HPLC/MS in the component analysis, metabolites analysis, and pharmacokinetics of TCMs etc. 50% of the literature is related to the component analysis of TCMs, which show that this field is the most popular type of research. In the metabolites analysis, HPLC coupled with electrospray ionization quadrupole time-of-flight tandem mass spectrometry has been demonstrated to be the powerful tool for the characterization of structural features and fragmentation behavior patterns. This paper presented a brief overview of the applications of HPLC/MS in the analysis of TCMs. HPLC/MS in the fingerprint analysis is reviewed elsewhere.

Keywords: traditional Chinese medicines (TCMs); HPLC/MS; component analysis; metabolites analysis; pharmacokinetics

Contents

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Abbrevations	Full names
2D-prep-HPLC-DAD	Two dimensional preparative high-performance liquid chromatography-diode array detector
ESI-FTICR-MS/MS	Electrospray ionization Fourier-transform ion cyclotron resonance tandem mass spectrometry
ESI-FTICR-MS	Electrospray ionization Fourier-transform ion cyclotron resonance mass spectrometry
ESI-MS^n	Multistage electrospray ionization mass spectrometry
GC-MS	Gas chromatography-mass spectrometry
HPLC/APCI-MS/MS	High-performance liquid chromatography-atmospheric pressure chemical ionization tandem mass spectrometry
HPLC/APCI-MS	High performance liquid chromatography-atmospheric pressure chemical ionization-mass spectrometry
HPLC-DAD/APCI-IT-MS	High-performance liquid chromatography-diode array detector/atmospheric pressure chemical ionization ion trap mass spectrometry
HPLC/ESI-IT-TOF/MS	High-performance liquid chromatography/electrospray ionization-ion trap-time-of-flight mass spectrometry
HPLC/ESI-MS/MS	High-performance liquid chromatography/electrospray ionization tandem mass spectrometry
HPLC/ESI-MS	High-performance liquid chromatography/electrospray ionization mass spectrometry
HPLC/ESI-Q-TOF-MS/MS	High-performance liquid chromatography/electrospray ionization quadrupole time-of-flight tandem mass spectrometry
HPLC/MS/MS	High-performance liquid chromatography/tandem mass spectrometry
HPLC/MS	High-performance liquid chromatography/mass spectrometry
HPLC-DAD/ESI-MS^n	High-performance liquid chromatography-diode array detector/tandem electrospray ionization mass spectrometry
HPLC-DAD/MS	High performance liquid chromatography-diode array detector/mass spectrometry
HPLC-PDA	High-performance liquid chromatography-photo diode array
HPLC/TOF-MS	High performance liquid chromatography/time-of-flight mass spectrometry
HPLC/IT-MS^n	High-performance liquid chromatograph/ion trap multistage mass spectrometry
MALDI-MS	Matrix-assisted laser desorption ionization mass spectrometry
Nano-LC-ESI/MS	Nano-liquid chromatography-electrospray ionization mass spectrometry
TFC-HPLC/MS	Turbulent-flow chromatography-high-performance liquid chromatography/mass spectrometry
UPLC/HDMS	Ultra-performance liquid chromatography/high definition mass spectrometry
UPLC/MS/MS	Ultra-performance liquid chromatography/tandem mass spectrometry
UPLC/MS	Ultra-performance liquid chromatography/mass spectrometry
UPLC/Q-TOF/MS	Ultra-performance liquid chromatography/quadrupole time-of-flight tandem mass spectrometry

1 Introduction

It is well known that traditional Chinese medicines (TCMs) have been used in clinical practice for thousands of years. The biologically active ingredients of these compounds play a role in their efficacy. However, TCMs comprise a complex mixture of different components and the active ingredient content is usually very low. Therefore, it is extremely difficult to study TCMs based on their components.

HPLC/MS combining the separation of components with quantitative analysis or qualitative identification provides an effective means of analyzing complex samples, and has been one of the most significant chromatographic technologies of the 21st century. Therefore, HPLC/MS was applied into TCMs research to identify the material basis of TCMs and understand the action mechanism of TCMs.

In the past 5 years, more than 350 papers have been published in international journals on HPLC/MS analysis of TCMs, and the tendency is increasing gradually (Figure 1). This trend also reflects the advantages of HPLC/MS in solving complex problems in TCMs. In the past two years, several comprehensive reviews have been published covering the majority of original publications. Yang [1] provided an overview which focused on the phytochemical analysis of

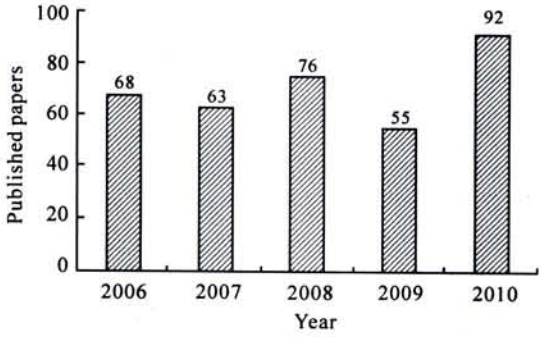

Figure 1 The annual distribution of the published papers on the analysis of TCMs by HPLC/MS

TCMs using HPLC/MS. The review indicated that HPLC/MS technique facilitated the convenient and rapid quality control of traditional medicines and their pharmaceutical preparations. Last year, Gray [2] reviewed the development of HPLC/MS and tandem MS/MS for the analysis of bioactive components and their metabolites of herbal medicines in biological fluids. In 2009, Li [3] and Zhang [4] described recent progress in the chemical analysis of Danshen and Gancao, respectively. Li described various analytical methods and their chromatographic conditions and compared their advantage/disadvantages. Zhang [5] also summarized the newly established methods. Last year, Zhang

summarized some of the applications of metabolomics in special TCMs issues with an emphasis on metabolic biomarker discovery. This will facilitate our understanding of the mechanism of action of TCMs formulae and the analysis of Chinese herbal medicines.

In this paper, we reviewed the published papers in international journals on applications of HPLC/MS in the analysis of TCMs, such as component analysis, metabolites analysis, and pharmacokinetics of TCMs (Table 1).

Table 1 The distribution of published papers on applications of HPLC/MS in the analysis of TCMs

Analytical Contents	TCMs				Total
	Active ingredients	Chinese materia medica	TCMs prescription	Others	
Component analysis	6	109	29	25	169
Metabolites analysis	20	17	18	4	59
Pharmacokinetics	29	9	14	0	52
Quality control	0	40	15	1	56
Synthetic adulterants	0	0	11	0	11
Metabolomics	1	3	2	0	6
Total					353

2 Component analysis of TCMs

50% of the literature is related to the component analysis of TCMs, which show that this field is the most popular type of research. The main focus is on: firstly, the identification of new compounds and their qualitative and quantitative method development; secondly, establishment of new technology for the rapid and simultaneous determination of multiple similar structural trace components.

2.1 Active ingredients of TCMs

In the analysis of the chemical components of TCMs, HPLC/MS technique is usually used for the separation and identification of a variety of similar structural compounds, and mass spectrometry is an important qualitative tool.

Jayaprakasam *et al*. [6] identified five flavonoids (liquiritin, liquiritigenin, isoliquiritigenin, 7, 4'-dihydroxyflavone, and isoononin) from *G. uralensis* using nuclear magnetic resonance (NMR) and HPLC/MS. They also tested the potential activity of these isolated pure compounds and glycyrrhizin to inhibit the secretion of eotaxin-1 by human fetal lung fibroblasts (HFL-1). Liquiritigenin, isoliquiritigenin, and 7, 4'-dihydroxyflavone were more effective than liquiritin, isoononin, and glycyrrhizin in suppressing eotaxin-1 secretion. Zhao *et al*. [7] developed an HPLC/ESI-MS/MS method for the separation, determination, and identification of eight pairs of diastereoisomers of podophyllotoxin and its esters. The method could be used to rapidly identify the purity and monitor the epimerization of 2-H of podophyllotoxin and its analogues from natural products, chemical reactions, and pharmaceutical metabolism.

2.2 Active parts of TCMs

Compared with the traditional plant chemical "purification-identification" research mode, the HPLC/MS method has shown high efficiency in the separation, identification and determination analysis of non-volatile components of TCMs, especially in micro and trace component analyses. Furthermore, some of the ingredients not identified by traditional methods have been found and their structures have been rapidly identified by HPLC/MS. 65% of the research topics in the international literature are related to the component analysis of TCMs, particularly Chinese herbal extracts. The classification distribution according to the structure of components is shown in Figure 2.

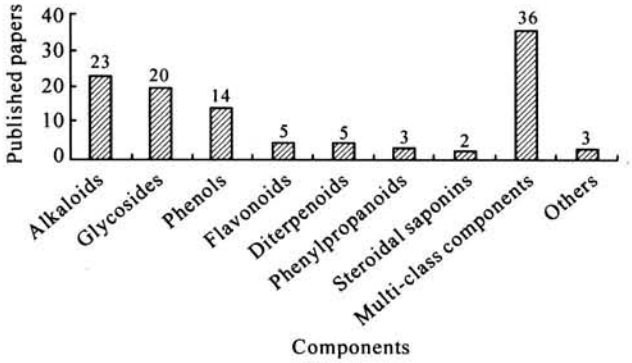

Figure 2 The distribution of the published papers on the component analysis of TCMs by HPLC/MS

2.2.1 Alkaloids

Alkaloids are one of the most important classes of compounds in natural products with biological activity. They are also used as indicators of active ingredients or toxic components in TCMs. Due to the varied structures of alkaloids, HPLC/MS is the most important technique in the qualitative and quantitative analysis of alkaloids, and its application has been very extensive.

Aconitine is an important toxic alkaloid and has been widely studied. Wang *et al*. [8] developed a MALDI-MS method and semi-qualitatively profiled the alkaloids in the Chinese herbal medicine Fuzi. Liu *et al*. [9] developed an HPLC/ESI-MS/MS method to separate and identify 32 aconitum lipo-alkaloids (LDAs) from three herbs of Aconitum genus. Yue *et al*. [10] studied aconitine-type alkaloids in the Chinese herb *Aconitum carmichaeli* by HPLC/ESI-MS/MS and ESI-FTICR-MS in positive ion mode. 111 compounds were identified including 11 monoester-diterpenoid alkaloids (MDA), 10 diesterditerpenoid alkaloids (DDA) and 81 lipo-alkaloids.

There is a rather special category in the HPLC/MS literature that combines HPLC/MS technique and receptor affinity chromatography or cell affinity screening technology to screen the active ingredients in TCMs. Wang *et al*. [11] developed an online analytical method that combined alpha

(1A)-adrenoceptor (alpha(1A)AR) cell membrane chromatography (alpha(1A)AR-CMC) with HPLC/MS for the identification of active ingredients from *Radix caulophylli* acting on the human alpha(1A)AR. Jong *et al*. [12] presented an HPLC/MS methodology for the screening of acetylcholinesterase (AChE) inhibitors in a crude extract of *Narcissus* cv "Bridal Crown" bulbs. Yuan *et al*. [13] coupled cell affinity screening (CAS) with HPLC/MS to screen the bioactive compounds related to cardiovascular diseases from the alkaloid extract derived from *Aconitum szechenyianum* Gay.

Zhou *et al*. [14] developed an HPLC/ESI-Q-TOF-MS/MS method to investigate the primary steroidal alkaloids in the extracts of eight major Fritillaria species. 41 steroidal alkaloids were selectively identified according to their MS/MS data and logical fragmentation pathways. Alali *et al*. [15] used both HPLC/MS and HPLC-PDA techniques to investigate the alkaloid rich fraction of *Colchicum brachyphyllum* Boiss. & Haussk. ex Boiss. (Colchicaceae). The spectral data of the compounds were not matched with that of the compounds isolated previously from this species or with any other colchicinoid; hence the new compounds should be pursued further.

2.2.2 Sugar and glycosides

In the study of sugar and glycosides, HPLC/MS technique showed good qualitative ability for isolating and identifying structural similar glycosides simultaneously, and provided a reliable basis for identification of different sources of Chinese herbal medicines. Zhou *et al*. [16] used HPLC/ESI-Q-TOF-MS/MS in positive mode to investigate the fragmentation behavior of four sulfur-containing iridoid glucosides isolated from *Paederia scandens* and to elucidate the main fragmentation pathways of these compounds. Lee *et al*. [17] developed an HPLC/ESI-Q-TOF-MS/MS method in negative-ionization mode to determine 12 intact glucosinolates-glucoiberin, glucocheirolin, progoitrin, sinigrin, epiprogoitrin, glucoraphenin, sinalbin, gluconapin, glucosibarin, glucotropaeolin, glucoerucin, and gluconasturtiin in 10 traditional Chinese herbs. Analysis of the glucosinolates provided scientific evidence enabling differentiation of three pairs of easily confused plants. Kite *et al*. [18] studied the major flavonoids in fruits and seeds of *Styphnolobium japonicum* (L.) Schott (syn. *Sophora japonica* L) by HPLC/MS and other spectroscopic techniques, and found two previously unreported kaempferol glycosides.

Zhang *et al*. [19] developed an HPLC/ESI-MS/MS method to simultaneously identify and quantify 6 predominant steroidal saponins in the rhizomes of *Paris polyphylla* var. *yunnanensis* and *P. polyphylla* var. *chinensis*, which are the qualified plants of "Chonglou" in Chinese. Dong *et al*. [20] established an ESI-FTICR-MS/MS method to investigate the isomers paeoniflorin and albiflorin in the extracts of the TCMs *Paeonia lactiflora* Pall. Qi *et al*. [21] developed a method of HPLC/ESI-Q-TOF-MS/MS to characterize ten major pregnane glycosides including one novel compound auriculoside Ⅳ from the roots of *Cynanchum auriculatum* Royle ex Wight when there were no reference compounds available. Xie *et al*. [22] used UPLC/Q-TOF/MS and multivariate statistical analysis to analyze 5 medicinal Panax herbs including *Panax ginseng* (Chinese ginseng), *P. notoginseng* (Sanchi), *P. japonicus* (Rhizoma Panacis Majoris), *P. quinquefolium* L. (American ginseng), and *P. ginseng* (Korean ginseng). Results indicated that the proposed method is applicable in the differentiation of complex samples that share similar chemical ingredients.

2.2.3 Phenols

Phenolic compounds are the main antioxidant ingredients in many medicinal plants. Analysis and identification of phenolic compounds are important in the research of screening antioxidant components in TCMs.

Han *et al*. [23] reported 40 phenolic compounds from *Artemisia annua* using HPLC-DAD/ESI-MS^n. C-glycosyl flavonoids were reported from *A. annua* for the first time and were found to be a new type of main ingredient, and may be responsible for its antioxidant and antiviral activity. Quinic acid derivatives were also found to be major ingredients of *A. annua*. Liu *et al*. [24] used HPLC-DAD/ESI-MS^n in negative ion mode to analyze 11 phenolic acids isolated from Danshen. Lee *et al*. [25] developed HPLC/PDA with confirmation of analyte identity by negative-ion ESI-MS/MS for determination of honokiol and magnolol in Hou Po (*Magnolia officinalis*). Hu *et al*. [26] used microwave-assisted extraction (MAE) and nano-LC-ESI/MS to determine and identify the chlorogenic acid (CA) in Honeysuckle.

2.2.4 Flavonoids

Wang *et al*. [27] established an HPLC-DAD-MS/MS method for screening and structural identification of the main ingredients in the crude extract of *Fructus aurantii Immaturus*, and 5 components were preliminarily identified as neoeriocitrin, narirutin, naringin, hesperidin and neohesperidin according to their UV and mass spectra. Han *et al*. [28] developed a bioactive lead compound screening system, composed of high-speed counter-current chromatography and HPLC/ESI-Q-TOF-MS/MS. They succeeded in discovering apoptosis inducers from gamboge, the resin of *Garcinia hanburyi*. Furthermore, gambogenic acid was identified as the lead compound. Zhao *et al*. [29] established an off-line 2-D RPLC/RPLC-Q/TOF/MS method for the separation of components in *Dalbergia odorifera* T. Chen. (Jiangxiang). In total, 637 peaks were separated in 114 fractions from the extraction of Jiangxiang. In addition, 19 flavonoids were tentatively identified from 114 fractions with Q-TOF/MS. The results showed the separation power of this two dimensional liquid chromatography system.

2.2.5 Terpenes

Yang *et al*. [30] developed an HPLC/PDA/ESI-MS/MS method for the rapid analysis of germacrane sesquiterpene lactones in the aerial part of *E. lindleyanum*. 9 germacrane sesquiterpene lactones were identified by a comparison of their characteristic data on HPLC and MS analyses with those obtained from reference compounds. Liu *et al*. [31] established a UPLC/Q-TOF-MS method for analysis of protostane triterpenoids in *Alisma orientalis* (Sam.) Juzep. A total of 20 protostane triterpenoids including 19 known compounds and a new compound were well separated within 7 min. Inbaraj *et al*. [32] developed an HPLC-DAD/APCI-IT-MS method for qualitative and quantitative analysis of carotenoids in fruits of *Lycium barbarum* Linnaeus. Huang *et al*. [33] used an HPLC/APCI-MS method for the determination of chlorophylls and their derivatives in *Gynostemma pentaphyllum* Makino, a traditional Chinese herb possessing vital biological activities.

2.2.6 Phenylpropanoids

Ahn *et al*. [34] developed an HPLC-DAD/ESI-MS^n method for the simultaneous determination of 9 coumarin compounds in the Korean medicinal herb, Cham-Dang-Gui, the dried root of *Angelica gigas* (Umbelliferae). Xie *et al*. [35] used HPLC-DAD/ESI-MS/MS to analyze the active coumarin components in *Radix angelicae dahuricae* (AE), and 10 coumarins have been identified. Five of them including xanthotoxol, osthenol, oxypeucedanin hydrate, byakangelicin and imperatorin were deemed as target ingredients for the preparative isolation through a 2D-prep-HPLC-DAD system.

2.2.7 Steroid saponins

Huang *et al*. [36] first reported P-sitosterol, stigmasterol and ergosterol coexisting in *A. roxburghii* herbs which were simultaneously identified and determined by an HPLC/APCI-MS method. Liu *et al*. [37] used UPLC/ESI-Q-TOF/MS to analyze the toad *Bufo bufo gargarizans* Cantor (toad skin). A total of 39 bufadienolides were screened out.

2.2.8 Multi-class components

In the application of HPLC/MS technique for the analysis of multi-class components from Chinese herbal medicines, many types of components can be analyzed and identified by HPLC due to its powerful separation ability. Don *et al*. [38] used HPLC/MS/MS to simultaneously separate and identify 6 main polyphenolic ingredients and four major abietane-type diterpenes from the dried rhizome of *Salvia miltiorrhiza* Bunge (Danshen) by comparing their retention time, MS and MS^2 data with those obtained from the authentic compounds. Huang *et al*. [39] also identified 15 major bioactive ingredients from the dried seeds of Oleaceae plants (*Forsythiae fructus*) by HPLC/MS. Kao *et al*. [40] developed an HPLC/ESI-Q-TOF/MS method to determine saponins and flavonoids in *Gynostemma pentaphyllum* (Thunb.) Makino.

2.3 The prescriptions of TCMs

The prescriptions of TCMs including the traditional prescription and the modern prescription is more complicated than the single herb medicine in components. The contents of the TCMs components may be changed during the preparation process or new compounds may be generated due to their interaction. Therefore, HPLC/MS has been widely used in Chinese prescription composition analysis due to its rapid and efficient isolation and identification capabilities.

2.3.1 Traditional prescriptions

As a rapid qualitative analytical technique, HPLC/MS was used by Liu for complex high-throughput screening of samples, which combined an off-line two-dimensional liquid chromatography, and HPLC-DAD/MS was used to analyze Chinese herbal formulas including Qixuebingzhi Formula, an efficient Chinese herbal formula for treating atherosclerosis. The medium- and low-polar extracts (MLPE) of the Chinese herbal formulas were separated and implemented in the production of semi-purified mixture libraries. Several bioactive compounds were quickly identified from this library through the screening and dereplication process [41]. Wen *et al*. [42] developed microdialysis coupled with HPLC-DAD/MS to study the interaction of a prescription of Danggui Buxue Decoction (CPDBD) with proteins, and 8 compounds were identified which possessed potential activities. Wang *et al*. [43] developed HPLC-DAD/ESI-MS^n to identify and characterize the flavonoids in a Chinese formulated preparation, Longdan Xiegan Decoction (LXD). In total, 51 flavonoids were characterized. Yan *et al*. [44] used UPLC/Q-TOF/MS for the global detection of aconitum alkaloids in Yin Chen Si Ni Tang.

2.3.2 Modern prescriptions

Zheng *et al*. [45] developed a diagnostic fragment-ion-based extension strategy (DFIBES) and HPLC/ESI-IT-TOF/MS method, and more than 30 ginsenosides and 20 lignans have been rapidly detected and identified from Shengmai Injection. Zhang *et al*. [46] used HPLC/TOF-MS and HPLC/IT-MS^n for screening and identification of multi-components in TCMs, and 33 ingredients from Qingkailing Injection were identified. This study is expected to provide an effective and reliable pattern for the comprehensive and systematic characterization of TCMs.

2.4 Others

Han *et al*. [47,48] developed a UPLC-MS/MS method for the simultaneous determination of 5 type B trichothecenes and 6 aflatoxins B_1, B_2, G_1, G_2, M_1 and M_2 in TCMs. Liau *et al*. [49] used an integrated method combining supercritical fluid extraction (SFE) with HPLC/APCI-MS/MS to quantify aflatoxins (AFs) in *Zizyphi Fructus* (fruits of

Zizyphus jujube), a traditional Chinese medicine.

3 Metabolites analysis of TCMs

HPLC/MS technique combining high performance liquid chromatography which has powerful separation capacity with mass spectrometry detection which has unique structural analysis capacity, has unparalleled high sensitivity and selectivity. This technique is a fast, trace, specific and accurate analytical tool and is one of the most effective methods for identification of metabolites, and has become a powerful analytical tool in the metabolic research of TCMs. One of the notable features of domestic and international research is that the active ingredients and active metabolites were characterized by studying the composition and metabolic products in the body of the prescription or extract.

30% of the literature reported the application of HPLC/MS in the analysis of metabolites of Chinese herbal medicinal ingredients. In the past 5 years, the use of HPLC/MS in the analysis of metabolites of Chinese herbal medicinal ingredients included the following aspects: (1) identification of metabolites; (2) determination of plasma concentrations of metabolites; (3) analysis of the metabolic pathways of TCMs and metabolic processes based on the metabolites; (4) analysis of the relationship between the metabolites and metabolic enzymes; (5) analysis of metabolites by the side effects of Chinese medicines and pharmacological mechanisms.

3.1 Metabolites analysis of active ingredients of TCMs

Figure 3 showed that alkaloids and flavonoids were the major components of TCMs evaluated in metabolites analysis. Psotova *et al*. [50] identified dihydrosanguinarine (DHSA) as a metabolite of sanguinarine (SA) in rats using HPLC/ESI-MS. Mitragynine is the primary active alkaloid extracted from the leaves of *Mitragyna speciosa* Korth, a plant that originates in South-East Asia and is commonly known as kratom in Thailand. Lu [51] developed HPLC/ESI-MS/MS to determine an ultra-trace amount of mitragynine in human urine. Beyer *et al*. [52] used an HPLC/ESI-MS/MS system (MRM mode) for quantification of the phenalkylamines ephedrine, pseudoephedrine, norephedrine, norpseudoephedrine, methylephedrine, methylpseudoephedrine, cathinone, mescaline, synephrine (oxedrine), and methcathinone in plasma. Wang *et al*. [53] studied the metabolism of triptolide by cytochrome P450s in human and rat liver microsomes. All the products were identified as mono-hydroxylated triptolides by HPLC/MS. Strzelecki *et al*. [54] used an HPLC/MS/MS method to identify aconitine, the main toxin of *Aconitum napellus* in the blood of a 54-year-old man. This study showed that this technique has broad application potential in the field of forensic science.

3.2 Metabolites analysis of Chinese materia medica

Kaneko *et al*. [55] developed a simple and sensitive method for measuring four types of Aconitum alkaloids (aconitine, hypaconitine, jesaconitine and mesaconitine) by HPLC/ESI-TOF/MS. This method is applicable in clinical and forensic toxicology. Kontrimaviciute [56] developed an HPLC/ESI-MS method for the determination of ibogaine and noribogaine in human plasma and whole blood. The method was successfully used in the analysis of poisoning involving *Tabernanthe iboga* root.

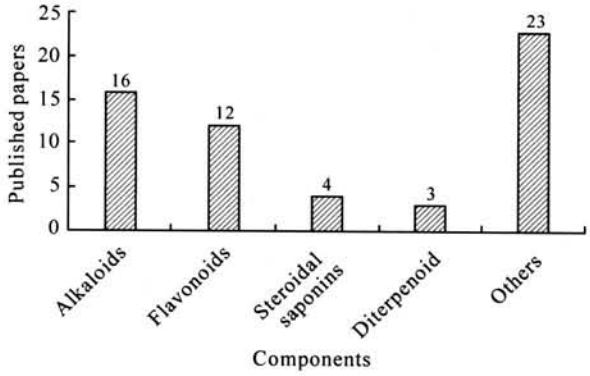

Figure 3 The distribution of the published papers on the metabolites analysis of TCMs by HPLC/MS

The domestic researchers have shown interest in: (1) the distribution of TCMs in tissue and metabolism; (2) screening the active ingredients by determination of the distribution of TCMs in tissue and the metabolic products; (3) the pharmacological mechanism of TCMs.

Wang *et al*. [57] studied the tissue distribution and excretion of resveratrol in urine and bile in rats after intragastric administration of *Polygonum cuspidatum* extract using HPLC/MS/MS. In that paper, serum chemistry and combined HPLC/DAD-MS techniques were used to study the constituents of Huangbai-Zhimu herb-pair (HBZMHP) extract absorbed into rat serum after oral administration.

Ma *et al*. [58] studied rat serum after oral administration of HBZMHP extract by HPLC/DAD-MS techniques. A total of nine characteristic HPLC peaks in the TIC chromatograms were identified as magnoflorine (1), menisperine (2), palmatine (3), berberine (4), timosaponin N or timosaponin El (5), timosaponin D (6), timosaponin 13111, anemarsaponin C or xilingsaponin B (7) timosaponin BIII (8) and timosaponin AIII (9). Ni *et al*. [59] developed UPLC/Q-TOF/MS and the MetaboLynx (TM) software combined with mass defect filtering (MDF) to provide unique high throughput capabilities for the study of drug metabolism. They have screened and identified the constituents absorbed and metabolized in studies of *G. longituba* extract after oral administration in rats. The results showed that 21 parent components of *G. longituba* extract were absorbed into the rat blood circulation and a total of 80 metabolites of 9 parent compounds were tentatively detected. This work suggests that the integrative metabolism approach make a useful template for drug metabolism research in TCMs. Li *et al*. [60] used HPLC/MS to deter-

mine the active ingredients of *Epimedium brevicornum* Maxim and its metabolites. Four active ingredients of Epimedium were found in the blood circulation of kidney-deficient rats and two of their metabolites in urine. The metabonomic approach is a potentially powerful tool to analyze the material basis and mechanism of action. In drug metabolism research, Guo *et al*. [61] developed UPLC/Q-TOF/MS with automated data analysis software (MetaboLynx (TM)) for fast analysis of the metabolic profile of flavonoids in *Abelmoschus manihot*.

3.3 Metabolites analysis of the prescriptions of TCMs

18 articles on the metabolites analysis of prescriptions of TCMs were reported within 59 articles. Li *et al*. [62] developed an HPLC/MS/MS-based method to study the multiple active licorice flavonoids (including liquiritin apioside, liquiritin, liquiritigenin, isoliquiritin apioside, isoliquiritin, and isoliquiritigenin) in rat plasma following an oral dose of Xiaochaihu Tang. Zhao *et al*. [63] developed a UPLC/Q-TOF/MS method for urinary metabonomics to study the mechanism involved after treatment of blood stasis using the TCMs prescription Xindi Soft Capsules. Lü *et al*. [64] simultaneously determined scoparone, capillarisin, rhein, and emodin in rat urine after oral administration of Yinchenhao Decoction preparation by UPLC/Q-TOF/MS.

3.4 Metabolites analysis of others

Zhang *et al*. [65] used HPLC/MS/MS to investigate the chemical components of PHY906 and its metabolites in the plasma of a patient with metastatic colorectal cancer (mCRC) treated with irinotecan and PHY906. The findings demonstrated that HPLC/MS/MS was an effective and reliable method for studying the parent chemicals of the Chinese herbal medicine PHY906 and its metabolites in this patient.

4 Pharmacokinetics of TCMs

In the pharmacokinetics research of TCMs, 76% of the studies reported in the literature used the HPLC/MS/MS method, and 24% of the studies in the literature used the HPLC/ESI-MS method. Xiong *et al*. [66] developed a UPLC/MS-MS method for the simultaneous determination of harpagoside and cinnamic acid in rat plasma and successfully applied this to the pharmacokinetic study of harpagoside and cinnamic acid in rats after oral administration of Yanyan tablets, a compound traditional Chinese medicine.

4.1 Pharmacokinetics of active ingredients of TCMs

Figure 4 showed that alkaloids, saponins and flavonoids were the major components of TCMs evaluated in pharmacokinetics analysis. Alkaloids included oxymatrine, vincristine, cepharanthine, dauricine and peimine. Guilhaumou *et al*. [67] developed an HPLC/MS/MS method for the quantification of vincristine in plasma in order to investigate the pharmacokinetics in a pediatric population. Hao *et al*. [68] determined cepharanthine in human plasma using HPLC/MS/MS. Xin *et al*. [69] developed an on-line TFC-HPLC/MS method. This method was successfully applied in the pharmacokinetic study of verticine, verticinone and isoverticine, the chemical markers of *Fritillaria thunbergii*, after oral administration of a total steroidal alkaloid extract of *F. thunbergii* in rats.

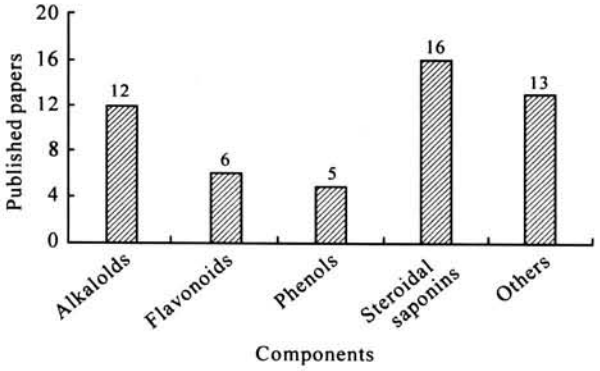

Figure 4 The distribution of the published papers on the pharmacokinetics of TCMs by HPLC/MS

The literature on saponins includes ginsenoside, baicalin, astragaloside IV, mangiferin and gastrodin. Li *et al*. [70] established an HPLC/ESI-MS method for the simultaneous determination of Panax notoginsenoside R_1, ginsenoside Rg_1, R_d, R_e and Rb_1 in rat plasma. The pharmacokinetic platform was successfully applied to the pharmacokinetic study of a multiple-constituent traditional Chinese medicine, total Panax notoginsenoside (Xuesaitong Injection). Kim *et al*. [71] used an HPLC/MS/MS method to determine the pharmacokinetics of baicalein, baicalin, wogonin and oroxylin A after intravenous administration of *Scutellariae radix* extract to male Sprague-Dawley rats. Suryawanshia *et al*. [72] developed an HPLC/MS/MS method for the simultaneous estimation of two bioactive markers, mangiferin and amarogentin along with three other components, amaroswerin, sweroside and swertiamarin in plasma after intravenous administration of a herbal preparation in male Sprague-Dawley (SD) rats.

The literature on flavonoids includes tanshinone, tanshinone IIA, silibinin, quercetin, apigenin, and genistein. Some reports include the pharmacokinetics of triptolide, bilobalide and paeonol. Xie *et al*. [73] developed an HPLC/MS/MS method for the simultaneous determination of ginkgolides (includes ginkgolide C for the first time) and bilobalide in rat plasma following intravenous administration of *Ginkgo biloba* extract. Xie *et al*. [74] used an HPLC/Q-TOF/MS technique to compare the pharmacokinetic behavior and metabolic profile in rats following oral administration of the pure paeonol alone and an herbal preparation "Qingfu Guanjieshu" (QFGJS) containing paeonol. The results indicated that other components in QFGJS could effectively influence the pharmacokinetic behavior

and metabolic profile of paeonol in rats.

4.2 Pharmacokinetics of Chinese materia medica

In the analysis of the pharmacokinetics of Chinese materia medica, *Coptis chinensis*, baikal skullcap root, ginseng berry, *Salvia miltiorrhiza* and *Schisandra chinensis* were the major materia medica. Feng *et al*. [75] developed a sensitive, rapid and selective HPLC/MS/MS method for the simultaneous determination of baicalin, baicalein, wogonin, berberine, palmatine and jatrorrhizine in Scutellaria-Coptis herb couple in rat plasma after oral administration of Yiqing Capsules and Gegen-Qinlian Tablets in rats. Wang *et al*. [76] used an HPLC/ESI-MS method for the simultaneous quantification of four active schisandra lignans (schisandrin, schisantherin A, deoxyshisandrin and gamma-schisandrin) from a traditional Chinese medicine *Schisandra chinensis* (Wuweizi) in rat plasma.

Due to the complexity of Chinese medicines, generally only one, two or three components were measured as an index of the quality of Chinese materia medica. Therefore, to comprehensively analyze both the contents and pharmacokinetics of the various components of Chinese materia medica is a great challenge.

4.3 Pharmacokinetics of the prescriptions of TCMs

In the pharmacokinetics of the prescriptions of TCMs, saponins are the major research point. The following prescriptions including saponins were studied, such as Epimedium Decoction, Shenmai Injection, Gushudan, Zishen Pills, Tangminling Pills, Shuanghuanglian Oral Liquid, Xiaochaihu Tang, Luxiancao Decoction, Huanglianjiedu Decoction and Dachengqi Decoction. Zhu *et al*. [77] compared the pharmacokinetics of baicalin and wogonoside in rats following oral administration of Xiaochaihu Tang (Minor *Radix Bupleuri* Decoction) and *Radix scutellariae* extract using an HPLC/MS method.

5 Other analysis

5.1 Quality control

Generally, one or two active ingredients in TCMs were employed for evaluating the quality of TCMs. In 2006, Ye and colleagues [78] developed a new strategy combining qualitative HPLC/MS analysis and quantitative HPLC to determine major bufadienolides for the global quality control of ChanSu crude drug. Last year, Liu *et al*. [79] established an HPLC analytical method for the quantitation of the diester-alkaloids content in the decoctions. They also investigated the components and content of alkaloids in these decoctions by semi-quantitative ESI-MS. Zhao *et al*. [80] developed an HPLC/APCI-MS method for the qualitative and quantitative analysis of steroids, as well as for the quality control of *Polyporus umbellatus*. In the same year, Han *et al*. [81] developed a reliable isotope dilution method for the simultaneous determination of fumonisins B_1, B_2 and B_3 in TCMs by UPLC/MS-MS.

Han and Ye [82] reported an HPLC/MS method for the quality control of Shuanghuanglian Oral Liquid in 2006. This will be a comprehensive quality control method of this commonly used herbal preparation. Wang and coworkers [83] developed an HPLC-MS/MS method employing both positive and negative electrospray ionization for the simultaneous determination of the nine identified compounds in the raw herbs and products of Si Wu Tang (SWT). The study proved it is a sensitive and rapid quantification approach and is a useful method in the quality control of raw herbs and products of SWT.

5.2 Analysis of synthetic adulterants

Adulteration of herbal remedies with undeclared synthetic drugs is a common problem, which may potentially cause serious adverse effects. Jung *et al*. [84] and Vidal *et al*. [85] studied the metabolites of "Lida Dai Dai Hua Capsules", a weight loss product of Chinese origin. The central nervous system drug sibutramine was identified as an additive in this recipe, and the dosage was far beyond the provisions of the German national drug dose administration.

Reepmeyer and colleagues [86] analyzed a herbal dietary supplement which can enhance sexual function. They developed an HPLC/MS and a hydrolytic technique for the detection and structure elucidation of a novel synthetic vardenafil designer drug added illegally to a "natural" herbal dietary supplement. One year later, the same group analyzed and detected a new sidenafil analogue as an adulterant in a herbal dietary supplement using HPLC with photodiode array and mass spectral detection [87].

5.3 Metabonomics study

Because metabonomics are usually used in analytical technology, with the development of analytical technology, metabonomics are now widely applied.

Last year, Ma and coworkers [88] studied the metabolic profile of plasma and kidney tissue from rats treated with Morning Glory Seed (MGS) using a UPLC/MS metabonomic approach. Their results were helpful in understanding the clinical diagnosis of TCMs-induced nephrotoxicity. Wang and colleagues [89] explored the thyroxine- and reserpine-induced changes in the metabolic profiles of rat urine and the therapeutic effect of Liu Wei Di Huang Pills employing UPLC/HDMS. Gu *et al*. [90] carried out a comprehensive metabonomic method, in combination with fingerprint analysis and target analysis to determine potential mechanisms of berberine action in the treatment of patients with type 2 diabetes and dyslipidemia.

6 Conclusion

With the development of HPLC/MS techniques, more and more TCMs and their *in vivo* analytes have been investigated. HPLC/MS techniques become the first choice for the

determination of targets in biological fluids such as blood, plasma and urine. With the high resolution, high reproducibility and high selectivity of UPLC and MS^n, UPLC/Q-TOF/MS has been demonstrated to be powerful tools for the characterization of low-abundance targets in complex samples. Some of peaks can be characterized directly online by comparing the retention time, UV spectra, and fragmentation information with the reference. During the discovery process of novel compounds, it is important to differentiate novel from known compounds in crude extracts before starting a time-consuming process of purification.

However, until now, there is no universal mass database available, because the fragment information of ESI and APCI is easily affected by ionization modes and HPLC conditions. It is necessary to establish universal database with the help of the reference substances development. Another limitation of HPLC/MS is that the peak capacity of an HPLC column is limited. Therefore, HPLC/MS in the qualitative study of TCMs is not as mature as GC-MS.

From our survey of the literature, the majority of studies only focused on determining the components of TCMs. It is insufficient in the depth of research. Therefore, more efforts should be made to explore the relationship between the effectiveness and components of TCMs by using HPLC/MS techniques. In addition, most of the authors of the published papers were from universities and research institutes, and very few from pharmaceutical companies. Therefore, it is necessary to strengthen the research cooperation between the pharmaceutical company and university or research institute.

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