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Review

Separation methods for antibacterial and antirheumatism agents in plant medicines

Dawei Wen, Yuping Liu, Wei Li, Huwei Liu*

Key Laboratory of Bioorganic Chemistry and Molecular Engineering of Ministry of Education, College of Chemistry and Molecular Engineering, Peking University, Beijing 100871, PR China

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Abstract

Traditional oriental medicines (TOM), with a very long history and many remarkable features, are very popular in Asian countries, especially in China, Japan and Korea. With the development of advanced analytical techniques, the modernization of traditional medicine has become a hot area in recent years and some herbal medicines have been increasingly accepted in western countries. Separation and determination of active components in various herbal medicines are considered to be critical for the modernization process. Antibacterial and antirheumatism agents are widely distributed in many medical plants and commonly used in clinical treatment. Therefore, the development of effective separation methods for the quality control of herbal medicines is absolutely important. In this article, the separation methods for the analysis of antibacterial and antirheumatism compounds in TOM were reviewed, including thin layer chromatography (TLC), gas chromatography (GC), supercritical fluid chromatography (SFC), high-performance liquid chromatography (HPLC), capillary electrophoresis (CE) and related hyphenation techniques. Sample preparation procedures and further development of these methods were also discussed.
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* Corresponding author. Tel.: +86 10 62754976; fax: +86 10 62751708.
E-mail address: hwliu@chem.pku.edu.cn (H. Liu).

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1. Introduction

Some herbs and plants have been used by our ancestors to treat their diseases for several centuries in the world. For instance, traditional Chinese medicines (TCMs), the most organized traditional medicine, have been used for clinical treatment for about two thousand years: there are 252 species of crude drugs from plants recorded in Shennong Materia Medica [1], the earliest Pharmacopoeia of China in Eastern Han (24–220 A.D.), where the properties and usage of herbal medicines and their clinical efficacy and toxicity were also demonstrated. Nowadays, there are more than 500 species of medicinal plants recorded in the Chinese Pharmacopoeia [2]. In those plant medicines, antibacterial and antirheumatism agents are important active components, and their therapeutic effects have been verified in numerous modern medical investigations, which are often complementary to those of Western drugs.

Although many plant medicines have distinct effect in treating diseases, their pharmacological, pharmacokinetical and toxicological mechanisms have not been understood well because these drugs are usually complex mixtures containing up to more than hundreds of different constituents. The separation and determination of the active components in medicinal plant extracts represent an advisable method to these problems. The major compound types of antibacterial and antirheumatism agents in herbs usually involve phenolic, stilbene and polyphenol compounds, flavonoids, terpenoids, alkaloids, anthraquinones, lignans, polysaccharides and peptides [3,4]. Since the extracts often contain large amount of proteins, sugars, mucilage and tannin, making the isolation and measurement of the active constituents in crude drugs and their medical preparations extremely difficult, powerful separation techniques with high efficiency and sensitivity are necessary. Therefore, chromatographic methods have been mainly applied in the analysis of herbal medicines, including gas chromatography (GC), supercritical fluid chromatograph (SFC), high-performance liquid chromatography (HPLC) and thin layer chromatography (TLC). Hyphenated techniques such as GC–MS, LC-MS, LC-MS–MS have been increasingly used to enhance the sensitivity and provide a wealth of information for on-line compositional and structural analysis. On the other hand, as a micro-column technique developed since 1980s, capillary electrophoresis (CE) has been exploited to separate major compounds in plant medicines, demonstrating high efficiency and fast separation speed. Applications of different separation modes of CE, including capillary zone electrophoresis (CZE), micellar electrokinetic chromatography (MEKC), microemulsion electrokinetic capillary chromatography (MEEKC) and capillary electrochromatography (CEC), in this area have been comprehensively studied. The present review summarizes the applications of chromatographic and electromigration methods to the isolation and determination of antibacterial and antirheumatism agents in medicinal plants, especially in traditional Chinese medicines during recent years, and compared the characteristics of those methods by using several examples.

2. Chromatographic methods

For the analysis of plant medicines, chromatographic techniques (TLC, GC, SFC and HPLC) are frequently used methods and act as standards for identification and quality control of most regulated herbal medicines as illustrated in the Pharmacopoeia Commission of People's Republic of China.

2.1. Thin layer chromatography (TLC)

TLC is an important method for the isolation, purification and confirmation of natural products. Comparing with other chromatographic methods, TLC is often considered to be deficient in reproducibility and accurateness, but some distinctive attributes of this tool should be considered: low-cost analysis, high-throughput screening of samples, minimal sample preparation, full sample integrity, disposable stationary phase [5]. Some typical analysis of antibacterial compounds in plant medicines based on TLC in the recent years is demonstrated in Table 1. For the tentative isolation and preseparation of unfamiliar plant medicine extracts and complicated samples, TLC has its specific advantages [9,10], i.e. it is free of time constraints for identification or confirmation. To identify the structures of active constituents requires post-chromatography treatment for detection, such as ^{1}H and ^{13}C NMR and MS [6–9,11–18]. In addition, for the confirmation of antibacterial ability of unknown constituents, TLC naturally suits for bioautography technique [7–15]. Some new detection methods, such as Surface Enhanced Raman Scattering (SERS), were put on trail to be connected with TLC in situ for the analysis of berberine in *Coptis Chinensis* France [16]. In summary, thin layer chromatography (TLC) still acts as an important screening and preparative tool for antibacterial and antirheumatism agents in plant medicines and has its feasibility in hyphenation procedures in the future.

2.2. Gas chromatography (GC), gas chromatography–mass spectrometry (GC–MS) and supercritical fluid chromatography (SFC)

Gas chromatography (GC) is a powerful method for the analysis of active components in herbal medicines, although its application is limited to volatile analytes, comparing with HPLC method. Because of its fast speed, low analytical cost, high reproducibility, tunable polarity of stationary phases and various sample introduction modes, GC has been applied to the separation and determination of volatile and semi-volatile components in crude drugs. In Chinese pharmacopoeia [2], GC acts as an official identification and quality control method for some traditional Chinese medicines.

Table 1
Applications of TLC in analysis of plant medicines

Medicinal plant	Functional part	Active constituents separated	Stationary phase	Mobile phase	Remarks	Reference
45 Indian medicinal plants		Tentative preseparation only	Silica gel G.		Screening by bioautography evaluated by antimicrobial assay	[10]
Thirteen Indian herbs	Essential oil from Radix seed flower grass needle, stem and berry	Carotol, Cedrol, Carvone, Zingiberene, Linalool, D-limonene, tagetone, linalool, α,β-thujone, vetiverone, thymol, bornyl acetate, geraniol	Silica gel Merck 60 F 254	Hexane/ethyl acetate	Screening by bioautography Identified by GC and GC–MS	[9]
Citrus paradisi	Peel	Naringin	silica gel	Chloroform–methanol	Identified by ^{1}H and ^{13}C NMR	[6]
Euphorbia fisheriana steud	Radix	2,4-Dihydroxy-6-methoxy-3-methyl-1-acetophenone	Silicon GF(254)		Identified by ^{1}H and ^{13}C NMR	[18]
Morus macroura	Stem bark	Terpenoids	Silicon GF(254)		Identified by IR, ^{1}H and ^{13}C NMR and Mass spectra;	[17]
Mentha piperita	Essential Oil	Menthol, menthone and other terpenes displaying synergistic effects	Silica gel 60 F 254	*n*-Hexane/ethyl acetate	Screening by bioautography identified by GC–MS	[7]
Carpobrotus edulis L.	Leaf	Flavanoids	SILICA gel 60 F 254; Silica + Indicator, 1 mm, G 1510/LS 254	Ethyl acetate-methanol-water	Screening by bioautography	[11]
Bocconia arborea	Aerial parts	Dihydrochelerythrine Dihydrosanguinarine	Silica gel 60 F 254	*n*-Hexane–chloroform–methanol	Screening by bioautography	[15]
Westringia fruticosa, Westringia viminails	Aerial parts	Cinnamate esters of catalpol	Silica gel	Chloroform-methanol	Identified by UV, IR, ^{1}H and ^{13}C NMR and Mass spectra; screening by bioautography	[12]
Eriosema Tuberosum	Radix	Eriosemaones A–D and other polyphenols	Silica gel 60 F 254	Chloroform–methanol	Identified by ^{1}H and ^{13}C NMR and MS spectra screening by bioautography	[13]
Bridelia retusa	Stem bark	Terpenoids	Silica gel 60 F 254	Chloroform–methanol	Identified by ^{1}H and ^{13}C NMR and MS spectra screening by bioautography	[14]
Evodiarutaecarpa Juss Benth (Wuzhuyu)	Fruit	Alkyl methyl quinolone alkaloids	Silica gel	MeOH–phosphate buffer	Identified by IR spectra, NMR and Mass spectra; against Helicobacter pylori in vitro	[8]
Coptis Chinensis France	Radix	Berberine	Silicon GF(254)	*n*-Butanol–acitic acid-H_2O	Combined with Surface Enhanced Raman Scattering (SERS)	[16]

In antibacterial and antirheumatism plant medicines, terpenoids [19–21] are the most typical kind of volatile active components. As listed in Table 2, a lot of literature is focused on the separation and determination of these compounds [22–35], and a comprehensive review on the separation of cardioactive and antibacterial terpenoids from some Salvia species was presented by Ulubelen reasently. [44]. Some other compounds, such as phenolic compounds [24,36,42], isoquinoline alkaloids [37,38], tetracyclic alkaloids [39], triterpenic acids [40,41] and lignans [43], have been determined by GC. Besides the identification by retention time, coupling with the mass spectrometry (MS) is a powerful technique for the precise identification of active components in complicated plant medicine extracts. In the past ten years, GC–MS was already popularized as a kind of routine equipment, and the characterization of the active components in antibacterial and antirheumatism herbals is based on this technique [22–24,26–28,30–33,35,37–39,42].

Chiral recognition of bioactive compounds in natural products can also be achieved by GC. For example, the chiral separation of terpenoids was reported by Ochocka, et al., who separated some enantiomers of monoterpenic hydrocarbons in essential oils from *Juniperus communis* on a glass column, packed with α-cyclodextrin coated Chromosorb W NAW [45]. Sjodin et al. utilized two types of capillary chiral columns (a permethyl-β-cyclodextrin column and a dipentylbutyryl-γ-cyclodextrin column) coupled in series with an ordinary DB-WAX capillary column to separate enantiomers of monoterpenic hydrocarbons in different tissues of *Pinus sylvestris* [46]. Sybilska and Asztemborska compared the performance of three cyclodextrin columns (β-DEX120, γ-DEX120 and γ-DEX255) for the chiral recognition of terpenoids in pharmaceuticals derived from natural sources [47]. Recently, some organic alcohols and acids in Rhubarb (*Rheum rhabarbrum* L.) were separated on a capillary column coated with heptakis(2,3,6-tri-*O*-ethyl)-cyclodextrin and OV-1701 [48].

For sample pretreatment in GC analysis of herbal medicines, headspace solid phase microextraction (HS-SPME) and headspace injection are convenient techniques. Czerwinski et al. reported a HS-SPME procedure for the GC–MS analysis of terpenoids in herb-based formulations. Analytical results possessed satisfying accuracy and precision and demonstrated that, as a fast and inexpensive technique for the isolation of organic analytes, HS-SPME coupled to GC–MS can be successfully employed for the quality control of herbal medicines and other formulations containing herb extracts [49]. Hamm et al. optimized the conditions of HS-SPME for GC–MS analysis of different terpenoids in olibanum. Comparing with dichloromethane extraction, HS-SPME demonstrated its preferable capability for sesqui- and diterpenes (see Fig. 1) [50]. For phenolic compounds, Chen et al. established a direct headspace GC method to identify and determinate paeonol in radix-barks of *Cortex moutan radicis, Radix cynanchi paniculati* and *Paeonia lactiflora* [51]. Cinnamic aldehyde, an important constituent of antirheumatism plant medicines Rougui (bark of *Cinnamomum cassia* Presl), was determined by a similar method and the results were compared with that of other two *Cinnamomum* species [52].

The application of development of supercritical fluid chromatography (SFC) in the separation of antibacterial and antirheumatism agents in plant medicines has been limited so far, though there have been some publications on the analysis in this respect. Some tentative studies demonstrated the superiority of SFC, i.e. the derivatization procedure can be eliminated for the non-volatile components. Kohler et al. established a SFC-FID procedure for the determination of artemisinin and artemisinic acid extracted from aerial parts of *Artemisia annua* with supercritical fluid extraction (SFE) [53]. They also used an evaporative light scattering detector (ELSD) coupled to SFC for the quantitative determination of both compounds [54]. A separation procedure of underivatized triterpene acid standrads by SFC-FID was reported by another group [55].

2.3. High-performance liquid chromatography (HPLC) and hyphenation techniques

2.3.1. High-performance liquid chromatography (HPLC)

In general, antibacterial and antirheumatism components in herbal medicines can be divided into several categories [3,4]: isoquinoline alkaloids [56,77], anthraquinone and naphthoquinone [66], phenylpropionic acid [57,78], lignans [79], flavones [72,74], terpenoids [61,67] and some of their glycosides. These compounds have been separated routinely by reversed-phase HPLC on C18 columns with UV and other common detectors through the optimization of separation conditions, such as solvent strength, mobile phase composition, pH, gradient and temperature. Some typical examples are demonstrated in Table 3. Furthermore, some studies focused on TOM, and quality control for these plant medicine preparations (see Table 4 and Fig. 2) can be achieved by analyzing selected primary active components.

While RP-HPLC has demonstrated powerful ability for the analysis of antibacterial and antirheumatism bioactive components in crude drugs, other HPLC modes were barely exploited. Diozan and Assadi reported a normal-phase HPLC worked out for the determination of anthraquinones in rhubarb roots, dock flowers and senna leaves by using a Spherisorb-CN column and mobile phase composed of $CHCl_3$ and HAc (95:5) [81]. A procedure for the combination of ion-pair extraction and ion-pair HPLC was developed for isolation of alkaloids (in particular quaternary alkaloids), from plant materials. Sodium perchlorate was utilized as the ion-pairing reagent. The arising ion-pairs of quaternary alkaloids were extracted by 1,2-dichloroethane under acidic conditions and dissolved in a 1:1 mixture of DMSO and 0.5 mol/L $NaClO_4$. The HPLC separation was carried out on a Cosmosil 5 C18-AR column with multi-step linear gradient elution over 120 min. Seven quaternary alkaloids were

Table 2
Applications of GC in analysis of plant medicines and their preparations

Medicinal plant	Functional part	Active constituents separated	Column	Detector	Remarks	Reference
Pelargonium species	Essential oils from leaves	Terpenoids	OV-101, 50 m × 0.25 mm i.d and HP-5, 30 m × 0.25 mm i.d	FID; MS		[22]
Thymus kotschyanus, Thymus persicus	Essential oils from leaves	Terpenoids	DB-1, 60 m × 0.25 mm i.d, 0.25 μm	MS		[23]
Ishpingo (*Ocotea quixos*, Lauraceae)	Essential oils from fruit calices	Terpenoids, *trans*-cinnamaldehyde and methyl cinnamate	MEGA SE52 poly-5% diphenyl/95%-dimethyl-siloxane bonded phase, 30 m × 0.32 mm i.d, 0.15 μm	FID; MS		[24]
Satureja hortensis L.	Essential oil and methanol extracts from aerial parts	Terpenoids	HP-5, 30 m × 0.25 mm i.d, 0.25 μm	FID		[25]
Lamiaceae (*Mentha piperita* L.)	Essential oils	Terpenoids	Thermon 600 T, 50 m × 0.25 mm i.d, 0.20 μm; HP-Innowax FSC, 60 m × 0.25 mm i.d, 0.25 μm	FID; MS		[26]
Mentha haplocalyx Briq., spikes of *Schizonepeta tenuifolia* Briq., *Folium perilla frutescens* (L.) Britt.	Crude drugs	Terpenoids	HP-5, 30 m × 0.25 mm i.d, 0.25 μm	FID; MS		[27]
Origanum species	Essential oils	Terpenoids	Supelcowax 10 capillary column 60 m × 0.25 mm i.d	FID; MS		[28]
marjoram *(Majorana hortensis* M.*)*	Essential oils	Terpenoids	HP-1, 25 m × 0.32 mm i.d, 0.25 μm	FID;	Changes of marjoram during storage	[29]
Piper nigrum, Piper guineense	Essential oils	Terpenoids	FSOT RSL-200, 30 m × 0.32 mm i.d, 0.25 μm; HP-5 MS or Stabilwax,	FID;MS		[30]
Salvia officinalis	Essential oils from leaves	Terpenoids	DB5-MS, 30 m × 0.32 mm i.d, 0.25 μm;	MS		[31]
Lonicera japonica Thunb. (jinyinua)	Flower	Terpenoids and other organic acids	HP-5MS, 30 m × 0.25 mm i.d, 0.25 μm	MS		[32]
Cnidium monnieri (L.) (Shechuangzi)	Seed	Terpenoids	SE-54, 25 m × 0.25 mm i.d, 0.25 μm	MS		[33]
Niuhuang Jiedu tablets	Traditional preparation	Borneol compounds	SE-30 Chromosorb WAW DMCS 80/100, 2 m × 3 mm i.d	FID		[34]
Dendranthema indicum (L.) Des Monl. Var. aromaticum (Shennong Xiangju)	Flower	Terpenoids	RTS-5MS, 15 m × 0.25 mm	MS		[35]
Niuhuang Jiedu tablets	Traditional preparation	Paeonol	SE-30, 28 m × 0.28 mm i.d, 0.26 μm	FID		[36]
Standards		Phenolic acids	HP-5MS, 30 m × 0.25 mm i.d, 0.25 μm	MS	Research of microwave-accelerated derivatization processes	[42]
Fumaria species	Aerial parts	Isoquinoline alkaloids	HP-1, 12 m × 0.2 mm i.d, 0.33 μm	MS		[37]
Croton (Euphorbiaceae) species	Crude ethanol extracts from leaves	Isoquinoline alkaloids	Duran 50, coated with OV-1701-OH, 20 m × 0.3 mm i.d, 0.1 μm	MS	On-column injection high temperature GC, without derivatization.	[38]
Erythrina (Leguminosae) species	Seeds	Tetracyclic alkaloids	PAS-1701, 25 m × 0.32 mm i.d, 0.25 μm	MS	Derivatized by *N*,*O*-bis (trimethylsilyl)acetamide	[39]
*Officinalis*Species (Houpo)		Triterpenic acids: oleanolic acid, ursolic acid	BP1, 12 m × 0.15 mm, 0.25 μm	FID	Derivatized by *N*,*O*-bis-(trimethylsilyl) trifluoro- acetamide	[40]
Spica Prunellae (*Prunella vulgaris* L.) (Xiakucao)	Spica	Triterpenic acids: oleanolic acid, ursolic acid	10% SE-30, 2 m × 3 mm i.d	FID	Derivatized by CH_2N_2	[41]
Magnolia officinalis Rehd et Wils (Houpo)	Stem and radix barks	Magnolol, honokiol	SE-30, 40 m × 0.53 mm	FID		[43]

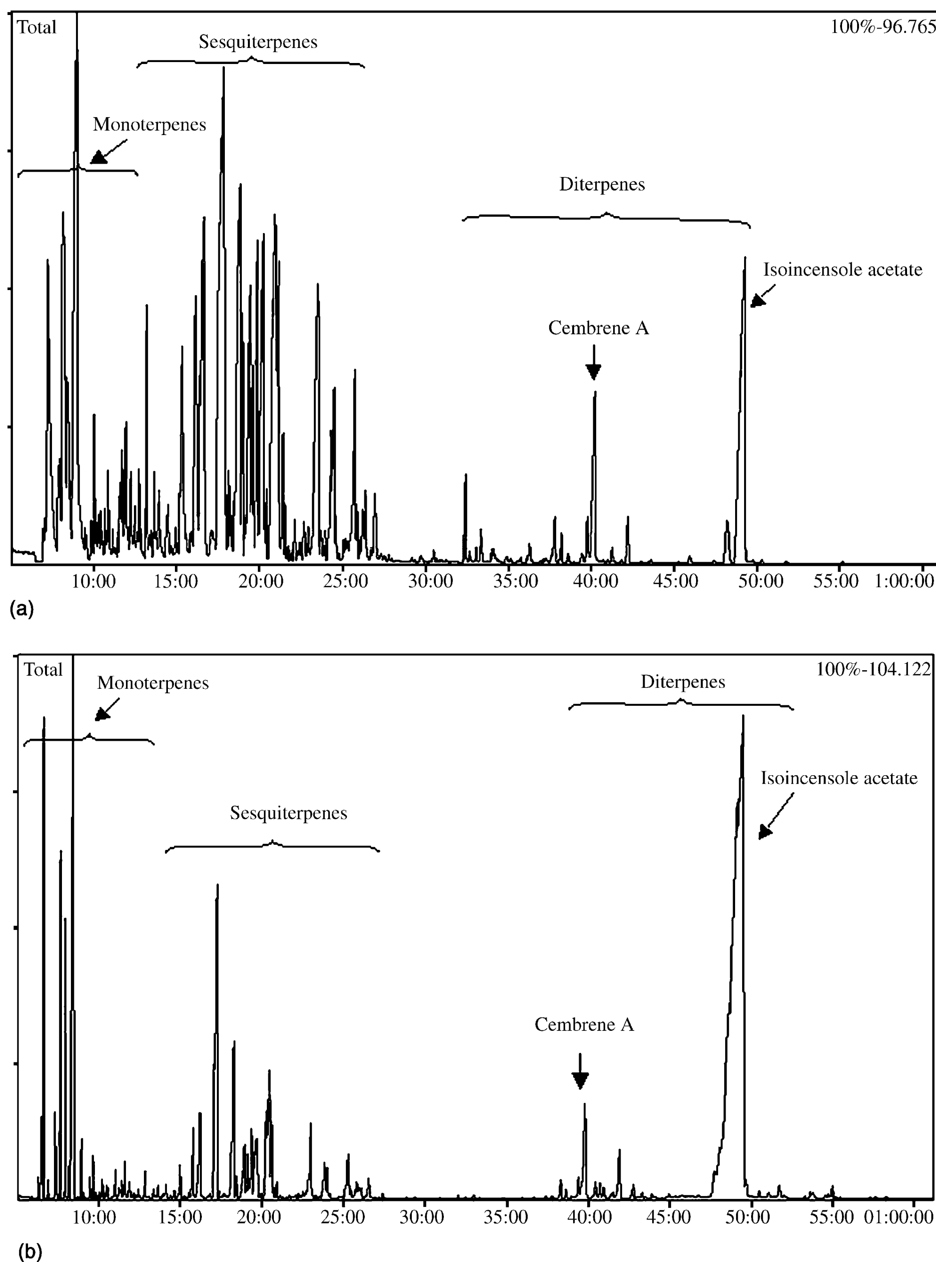

Fig. 1. Comparison between SPME and dichloromethane extraction for terpenoids in frankincense. Reprinted from [50] with permission.

isolated from *Magnolia obovata* [80]. In addition, the aristolochic acid contained in the root of *Aaristolochia* Species (Qingmuxiang) was separated on a Hypersil C18 column using *tert*-butyl ammonium bromide as the ion-paring agent [82]. Furthermore, an ion-pair HPLC–ESI-MS–MS method has been developed for direct and rapid characterization of isoquinoline alkaloids in a crudely purified extract of the aerial parts of *Eschscholtzia californica* (Papaveraceae) by Fabre et al. [83].

Application of a wide number of packing materials, such as silica-based ODS, C8 and C18-OH, specially designed packing materials including alumina or polymeric packings as well as some new kinds of bonded stationary phases, and their behavior in the chromatographic analysis of catharanthus alkaloids (an important category of antirheumatism agents) was reported by Theodoridis et al. in 1997 [86]. The result demonstrated that a complete separation was easily achieved on ODS columns, while polymeric materials also gave acceptable results. The performance and selectivity of two kinds of chiral columns, Chiral-AGP and Chiral-HAS, were reported by Fitos et al. for catharanthus alkaloids stereoisomers [87]. Chiral-AGP showed better stereoselectivity than Chiral-HAS for this type of alkaloids. Another kind of commercial AGP-based chiral selective columns, Chrialcel OD series, were used to the enantioselective separation of some lignans [84] and naphthoquinone derviatives [85].

Table 3
Applications of HPLC in analysis of plant medicines

Medicinal plant	Functional part	Active constituents separated	Column	Eluent	Detector	Reference
Paeonia lactiflora Pall. (Shaoyao)	Radix	Phenolic compounds and Phenolic glycosides	Cosmosil C18-MS, 250 mm × 4.6 mm i.d, 5 μm	MeCN/50 mmol L^{-1} KH_2PO_4–0.1% H_3PO_4 in water	UV 254 nm	[98]
Lonicera japonica Thumb. (Rendong), *Lonicera macranthoides* Hand et Mazz.	Flower	Chlorogenic acid	ODS, 250 mm × 4.6 mm i.d	MeCN/0.1% H_3PO_4 in water 20:80	UV 326 nm	[62]
Artemisia scpoaria Waldst. et Kit. (Yinchen)	Shoot	Chlorogenic acid	Zorbax SB-ODS, 250 mm × 4.6 mm i.d, 5 μm	MeOH/3% HAc in water 15:85	DAD 327 nm	[70]
Taraxacum species (Pugongying)	Aerial part	Caffeic acid, Chlorogenic acid	Hypersil C18, 200 mm × 2.1 mm i.d, 5 μm	MeOH/KH_2PO_4-H_3PO_4 in water (pH = 4.2)	DAD 328 nm	[57]
Bupleurum Chinense DC. (Chaihu)	Radix	Saikosaponins	Nucleosil C18, 250 mm × 4.6 mm i.d, 5 μm	Water/MeOH 34:66	UV 208 nm	[99]
Gentiana Manshurica Kitag. (Longdan)	Radix	Glycosides	Zorbax ODS, 250 mm × 4.6 mm i.d, 5 μm	Water/MeOH gradient	UV 254 nm	[100]
Gentiana species	Radix	Gentiopicrin	Diamonsil C18	MeOH/water 3:7	UV 254 nm	[60]
Hypericum perforicum L. (Guanye Jinsitao)	Leaf	Quercetin, Hyperin, Avicularin, Rutin	Shim-pack CLC-ODS, 150 mm × 6 mm i.d, 5 μm	Water (pH3.1–3.5 adjusted by H_3PO_4)/MeCN gradient	UV 254 nm	[72]
Morus alba L. (Sangye)	Leaf	Rutin, Quercetin	YWG-C18, 250 mm × 4.6 mm i.d,	MeOH/water/$H_3PO_4$60:40:0.4	UV 257 nm	[101]
Euphorbia humifusa Willd. (Dijincao)	Aerial part	Quercetin	Kromasil C18, 250 mm × 4.6 mm i.d, 5 μm	0.4% H_3PO_4 in water/ MeCN 60:40	DAD 370 nm	[71]
Pueraria lobata (Willd.) Ohwi (Gegen)	Radix	Isoflaones: Puerarin, Daidzin, Daidzein	Symmetry C18, 150 mm × 3.9 mm i.d, 5 μm	MeOH/1% HAc in water gradient	DAD 250 nm	[74]
Vaccinium species (Yueju)	Fruit	*trans*-Resveratrol	XTerra MS C18, 100 mm × 2.1 mm i.d, 3.5 μm	0.1% HCOOH in Water/ 0.1% HCOOH in MeCN gradient	ESI-MS	[58]
Polygonum cuspidatum varieties (Huzhang and Mexican Bamboo)	Radix	Stilbenes	Discovery C18, 250 mm × 4.6 mm i.d, 5 μm	0.15% TFA and 0.18% HCOOH in water/MeCN gradient	DAD 220–320 nm	[59]
Cinnamomum cassia Presl (Rougui) ⟨1⟩ *Paeonia lactiflora* Pall. (Shaoyao) ⟨2⟩	Stem bark ⟨1⟩ Radix ⟨2⟩	Cinnamic acid ⟨1⟩ Paeoniflorin ⟨2⟩	LiChropher R-18, 125 mm × 4 mm i.d, 5 μm	Water/MeCN/MeOH/HAc 61:34:5:0.1 ⟨1⟩ 80:15:5:0.1 ⟨2⟩	UV 280 nm ⟨1⟩ 250 nm ⟨2⟩	[78]
Notoperygium incisum Ting (Qianghuo)	Rhizoma and Radix	Isoimperaterin, Nodakenin, Notopterol	Inertsil ODS, = 2,150 mm × 4.6 mm i.d, 5 μm	MeOH/water 1:2	UV 330 nm	[102]
*Aaristolochia*Species (Guanmutong and Qingmuxiang)	Radix and seed	Aristolochic acid I and II	LiChrospher 100 RP-18, 250 mm × 3 mm i.d, 5 μm	0.3% $(NH_4)_2CO_3$ in water/MeCN 75:25	UV 254 nm	[64]

Table 3 (*Continued*)

Medicinal plant	Functional part	Active constituents separated	Column	Eluent	Detector	Reference
Curculigo orchioides Gaerth. (Xianmao)	Radix	Curculigoside	Inertsil ODS-3, 150 mm × 4 mm i.d, 5 μm	MeOH/water/HAc 45:80:1	UV 283 nm	[63]
Inula japonica Thumb. (Xuanfuhua)	Flower	Inunolide	Spherisorb ODS, 200 mm × 4.6 mm i.d, 5 μm	MeOH/water 40:55	UV 210 nm	[65]
Sideritis (Labiatae) Species		Diterpenoids	Hypersil ODS, 150 mm × 4.6 mm i.d, 5 μm	MeOH/water 70:30	DAD 220 nm (190–390 nm were monitored)	[61]
Hedyotis diffusa Willd. (Baihuashecao) *Hedyotis corymbosa* (L.) Lam. (Shuixiancao)	Aerial part	triterpenic acids: Oleanolic acid, Ursolic acid	Kromasi ODS, 250 mm × 4.6 mm i.d, 5 μm	MeOH/water 82/18	UV 210 nm	[67]
Perilla frutescens (L.) Britt. (Zisu)	Leaf	Tormentic acid Oleanolic acid, Ursolic acid	Spherisob ODS, 250 mm × 4.6 mm i.d, 5 μm	MeCN/1.25% H_3PO_4 in water 86:14	DAD 206 nm	[75]
Staphylea holocarpa Hemsl.	Radix and leaf	Ursolic acid	Shim-pack CLC-CN C18, 150 mm × 6 mm i.d, 5 μm	MeOH/water/HAc	APCI-MS	[76]
Verbena officinalis L. (Mapiancao)	Aerial part	Ursolic acid	Kromasi ODS, 250 mm × 4.6 mm i.d, 5 μm	MeOH/KH_2PO_4-H_3PO_4 in water 89:11	DAD 210 nm	[73]
Coptis Species (Huanglian)	Rodix	Coptisine, Epicoptisine, Berberine, Palmatine, Jatrorrhizine	Shimpack CLC-ODS, 150 mm × 6 mm i.d	25 mmol L^{-1} KH_2PO_4 25 mmol L^{-1} SDS in 3:7 MeCN/water. Add H_3PO_4 to pH 5.0	UV 345 nm	[68]
Scutellaria baicalensis Georgi (Huangqin) Pueraria lobata (Willd.) Ohwi (Gegen)	Radix	Baicalin and Puerarin	Cosmosil C18-MS, 150 mm × 4 mm i.d, 5 μm	0.03% H_3PO_4 in water/ MeCN 87:13 or 2% HAc in water/ MeOH 79:21	DAD 250 and 270 nm	[103]
Cassiae species (Juemingzi)	Seed	Anthraquinone compounds	Symmetry C18, 250 mm × 3.9 mm i.d, 5 μm	MeOH/0.1% H_3PO_4 in water 90:10	DAD 440 nm	[66]
Cnidium monnieri (L.) Cuss. (Shechuangzi)	Fruit	Xanthone compounds	YMC-pack ODS-AQ312, 150 mm × 6 mm i.d, 5 μm	MeOH/MeCN/water 220:70:210	UV 320 nm	[69]
Corydalis bungeana (Diding)	Aerial part	Isoquinoline Alkaloids	TSK-120A, 150 mm × 4 mm i.d	50 mmol L^{-1} NaH_2PO_4 in MeOH/0.1% SDS in water 35:65	UV 289 nm	[77]
Hydrastis Canadensis (Goldenseal)	Radix	Isoquinoline Alkaloids	Zorbax Eclipse-XDB, 250 mm × 4.6 mm i.d, 5 μm	10 mmol L^{-1} NH_4Ac in water /MeCN 70:30	UV 235 nm	[56]
Coptis and *Evodia* species (Huanglian, Wuzhuyu)	Radix	Alkaloids	Cosmosil C18-MS, 250 mm × 4.6 mm i.d, 5 μm	MeOH/50 mmol L^{-1} NaAc and 0.25% HAc in water gradient	UV 250 nm	[104]
Magnolia officinalis Rehd. et Wils. (Houpo)	Bark	Magnolol, Honokiol	NucleosilC18, 250 mm × 4 mm i.d, 5 μm	MeCN/0.1% H_3PO_4 in water 65:35	DAD 209 and 218 nm	[79]

Table 4
Applications of HPLC in analysis of plant medicine preparations

Medicinal plant	Active constituents separated	Column	Eluent	Detector	Reference
Zhuifeng touguo wan (pill)	Paeoniflorin	Diamonsil C18, 150 mm × 4.6 mm i.d, 5 μm	MeOH/water 30:70	UV 230 nm	[105]
Ganmao qingre chongji (powder)	Forsythoside A	ODS, 150 mm × 4.6 mm i.d, 5 μm	MeCN/H_3PO_4–10 mmol L^{-1} HK_2PO_4 (pH3.2) gradient	UV 332 nm	[106]
Lianqiao powder	Forsythoside A	Zorbax Extend C18, 150 mm × 4.6 mm i.d, 5 μm	MeOH/water 40:60	UV 280 nm	[107]
Houttuynia cordata Thunb (Yuxingcao) and Ganmaoling-diji (decoction)	Quercitrin	Shim-pack CLC-ODS, 150 mm × 6 mm i.d, 5 μm	MeOH/water 45:55 adjusted to pH 2.5 by H_3PO_4	UV 254 nm	[108]
Bike Chongji (powder)	Sinomenine	Kromasil C18, 250 mm × 4.6 mm i.d, 5 μm	MeOH/Water/ethylidene diamine	UV 264 nm	[109]
Liuwei dihuang capsule	Ursolic acid	ODS C18, 250 mm × 4.6 mm i.d, 5 μm	MeOH/water	DAD 206 nm	[111]
Leigongteng liniment	Triptolide	Zorbax ODS C18, 250 mm × 4.6 mm i.d, 5 μm	MeOH/water 40:60	UV 218 nm	[110]
Liandan xiaoyan tablet	Dehydro-andrographolide	Diamonsil C18, 250 mm × 4.6 mm i.d, 5 μm	MeOH/water 70:30	UV 255 nm	[113]
Huang-Lian-Jiee-Dwu Tang (decoction) and some medicinal herbs	Berberine	LUNA phenyl-hexyl, 250 mm × 4.6 mm i.d, 5 μm Zorbax SB-phenyl, 150 mm × 4.6 mm i.d, 5 μm	MeAC/MeOH/20 mmol L^{-1} HK_2PO_4 in water 35:20:45 adjusted to pH 3.0 by H_3PO_4 MeAC/20 mmol L^{-1} HK_2PO_4 in water 30:70 adjusted to pH 3.0 by H_3PO_4	UV 346 nm	[112]
Sann-Joong-Huey-Jian tang (decoction)	Gentiopicroside, Mangiferin, Berberine, Palmatine, Baicalin, Glycyrrhizin, Wogonin	Cosmosil C18-MS, 150 mm × 4.6 mm i.d, 5 μm	MeCN/0.03% H_3PO_4 gradient	UV 254 nm	[114]
Niuhuang qingxin wan (pill)	Berberine, Baicalin	Spherisorb C18, 250 mm × 4.6 mm i.d, 5 μm	0.7% TFA in 75:25 water/MeCN	UV 274 nm	[115]
Niuhuang Jiedu tablet	Baicaloin, Sennoside, Glycyrrhizin	Intersil ODS, 250 mm × 4.6 mm i.d, 5 μm	MeCN/0.1% HAc in water	DAD 240–450 nm	[116]
Wuu-Ji-San (powder)	Liquiritin, Glycyrrhizin, Hesperidin, Cinnamic acid, Cinnamldehyde, Magnolol, Honokiol	Cosmosil C18-MS, 150 mm × 4.6 mm i.d, 5 μm	MeCN/0.03 H_3PO_4 gradient	UV 254 nm	[117]
Hsiao-Cheng-Chi-Tang (decoction)	Hesperidin, Magnolol, Honokiol, Naringin, Sennoside A&B, Gallic acid, Emodin	Cosmosil C18-MS, 250 mm × 4.6 mm i.d, 5 μm	A: NaAc-HAc in water/MeCN 9:1; B: MeOH/MeCN/1% HAc in water gradient	UV 280 nm	[118]

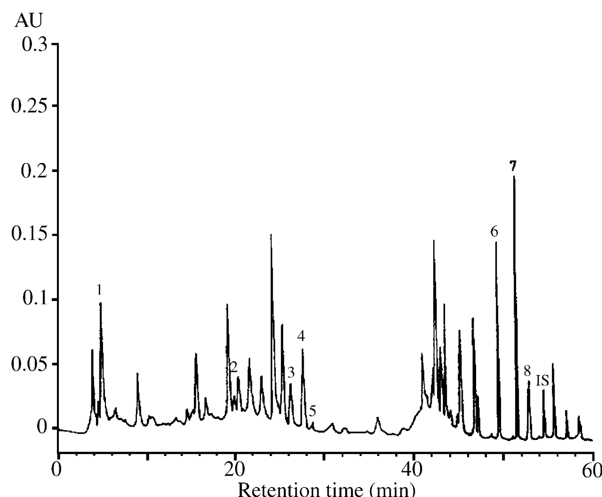

Fig. 2. Chromatogram of Hsiao-Cheng-Chi-Tang. Peaks – (1) gallic acid; (2) sennoside b; (3) sennoside a; (4) naringin; (5) hesperidin; (6) honokiol; (7) magnolol; (8) emodin. Reprinted from [118] with permission.

Besides the UV detector, also fluorescence and electrochemical detectors were used to detect the pharmacologically active compounds. A compound with antibacterial and antioxidant activity, *trans*-resveratrol (a stilbene derivative), which has both the fluorescence and electrochemical activities [88], can serve as a typical example. Several publications compared the determination ability of DAD with other detection modes in the case, for instance, fluorescence detection [89], multi-channel electrochemical detection [90], ESI-MS detection (negative mode) [91] and ESI-MS–MS detection (positive mode) [92].

In recent years, evaporative light scattering detector (ELSD) has proven its potential for the analysis of nonvolatile natural products, particularly in the quantitative analysis of the compounds possessing low or no UV absorbance at all. ELSD can be also the detector of choice if a steep gradient elution is applied. Three terpene compounds, actein, 27-deoxyactein and cimicifugoside, which cannot be detected by UV detection, in *Cimicifuga foetida* L. (Shengma) were analyzed by RP-HPLC on a Hypersil ODS column with MeOH/water gradient elution [93]. The detection limits of ELSD for actein, 27-deoxyactein and cimicifugoside were 40, 33 and 33 ng, respectively, while the limit of DAD (200 nm) were 606, 880 and 427 ng. A further research conducted by Li et al. [94] led to a validated HPLC method, obtaining a baseline separation of eighteen major constituents, triterpene glycosides, phenolic acids and flavonoids in Black cohosh, on a C-18 column with water-TFA/MeCN gradient elution and in-line DAD and ELSD detections (see Fig. 3). The determination of another antibacterial agent, andrographolide in commercial andrographis (*Andrographis paniculata*) products, was achieved by this detection mode effectively with the detection limit of 50 ng for andrographolide [95]. An HPLC-ELSD coupling with ESI-MS for the analysis of furostanol saponins from *Tribulus terrestris* was reported by De Combarieu in 2003 [96]. In addition, a high-throughput analysis method of natural products was founded by Cremin and Zeng [97], using an eight-way fully automated parallel LC-MS–ELSD system to analyze a library of 96 structurally diverse natural products. This rapid characterization of plant constituents in terms of compound libraries is quite helpful for screening of new biologically active compounds.

2.3.2. Hyphenated techniques of HPLC

For the identification of unknown active constitutes in crude plant extracts separated by HPLC, some spectrometric methods including mass spectrometry (MS), nuclear magnetic resonance (NMR), circular dichroism (CD), and laser polarimetric detection were used in various cases. MS and MS^n are the most important detection techniques for the identification of active compounds in herbal medicines [58,76,91,92]. In the literature, the quantification ability of MS was also demonstrated as a competitive method to DAD.

For various LC-MS instruments, the ionization techniques used were mainly atmospheric-pressure chemical ionization (APCI), electrospray ionization (ESI), thermospray ionization (TSI) and continuous-flow fast atom bombardment (CF-FAB). CFFAB source was less frequently utilized as it is limited to lower flow rates of the mobile phase. A comparative research of two ion sources, ESI and CFFAB, was reported for the analysis of saponins from Black Bean (*Vigna mungo*) [119]. A comprehensive review [120] on the applications of HPLC–DAD–MS indicated that the on-line identification of phytochemical constituents in botanical extracts can be easily achieved. This review [120] primarily focusd on ESI- and TSI-MS and their applications for the qualitative analyses of phenolic compounds, saponins, alkaloids and other classes of natural products in botanical extracts. Because most of antibacterial and antirheumatism components in herbal plants are polar compounds, both ESI and APCI ion sources are suitable for their analysis. Recently, a comparison of ESI and APCI techniques for the analysis of the main constituents from *Rhodiola rosea* extracts was reported by Tolonen et al. [121], and the fragmentation patterns of ten components both in the positive and negative mode were reported.

Unlike LC-MS technique, NMR, CD and laser polarimetric detection coupling to LC are barely used in applications. Extortionate request of technique levels and expensive instrument and running costs limited the development of these hyphenation technologies. In 1999, Bringmann et al. [122] constructed an on-line HPLC–NMR instrumentation for the rapid identification of isoquinoline alkaloids in *Dioncophyllum thollonii* in phytochemical screening studies. All components were identified and investigated by stop-flow ^{1}H NMR, 2D total correlation spectroscopy (TOCSY), and 2D nuclear Overhauser effect spectroscopy (NOESY). The same group constructed an instrument, which includes HPLC-CD on-line coupling to HPLC-NMR and HPLC-MS–MS, for the determination of the full absolute stereostructure of new metabolites in plant extracts [123]. In 2001, they utilized this instrument to establish a photometric screening method for dimeric naphthylisoquinoline alkaloids and complete on-line struc-

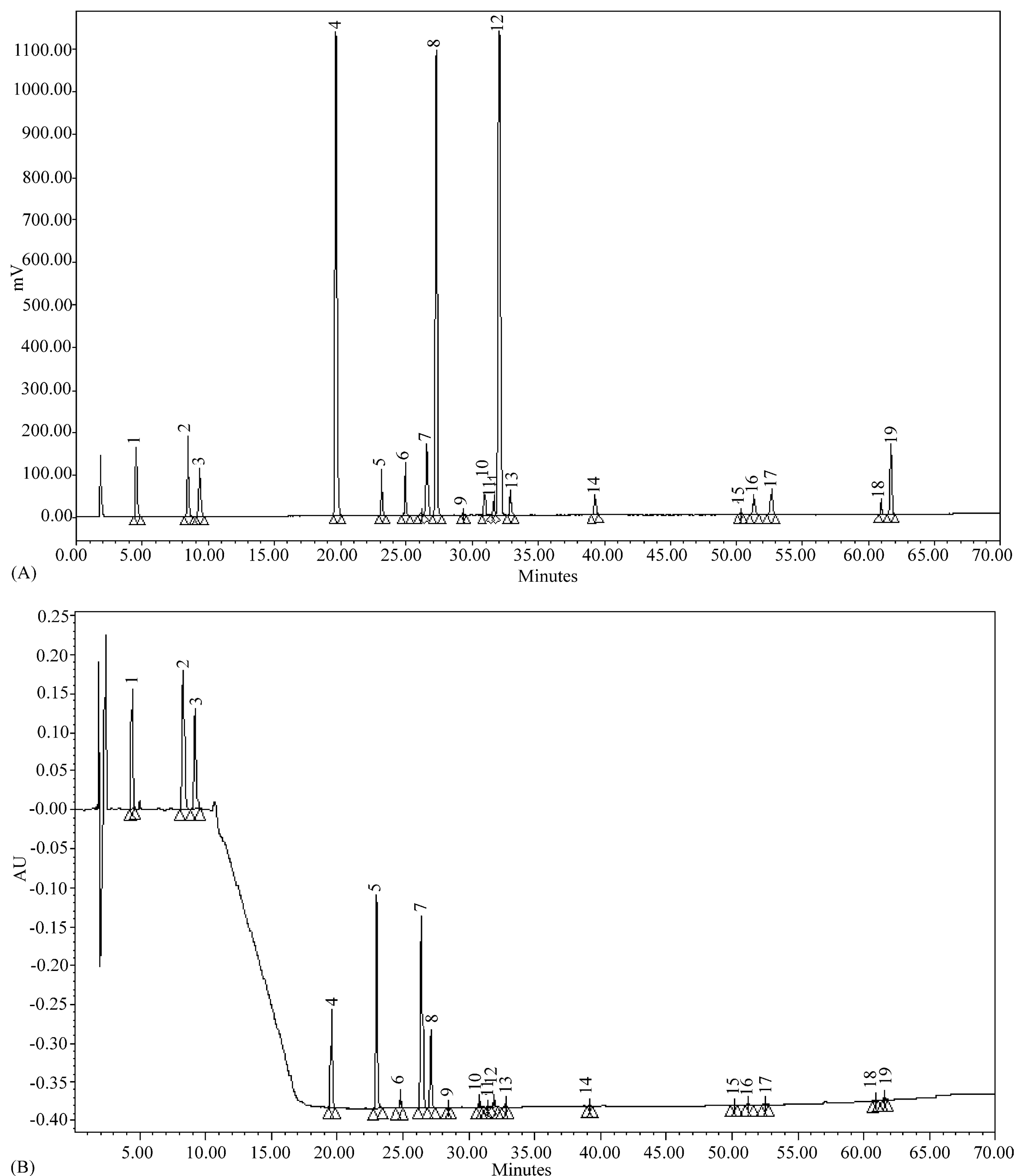

Fig. 3. Typical Chromatogram of 18 reference standards ((A): HPLC-ELSD, (B): HPLC-UV at 203 nm). (1) Caffeic acid; (2) ferulic acid; (3) isoferulic acid; (4) cimicifugoside H-2; (5) kaempferol; (6) cimiracemoside A; (7) formononetin; (8) cimicifugoside H-1; (9) (26*R*)-actein; (10) 26-deoxycimicifugoside; (11) (26*S*)-actein; (12) 23-epi-26-deoxyactein; (13) 23-*O*-Ac-SHENGMANOL-3-*O*-β-D-xyloside; (14) 26-dexoyactein; (15) 25-*O*-Ac-cimigenol-3-*O*-α-l-arabinoside (24*S*); (16) 25-*O*-Ac-cimigenol-3-*O*-β-D-xyloside (24*S*); (17) cimigenol-3-*O*-α-l-arabinoside (24*S*); (18) cimigenol-3-*O*-β-D-xyloside (24*S*). Reprinted from [94] with permission.

tural elucidation of a dimer in *Dioncophyllum thollonii* crude extracts [124]. Another hyphenation technique, HPLC–laser polarimetric detection, was reported by Halls and Lewis [84], achieving a chiral separation of lignans in *Forsythia intermedia*, and a good linear response over the concentration range examined was obtained with a detection limit of 0.4 nmol for (+)-pinoresinol.

3. Electromigration methods

As a micro-column separation technique developed in past two decades, electromigration methods, in particular different capillary electrophoresis (CE) modes, have their immense potential for drug analysis. CE mode can provide fast-speed and lower-cost analysis, compared to HPLC which was

Table 5
Applications of CE in analysis of plant medicines and their preparations

Medicinal plant	Functional part	Active constituents separated	Mode	Effective length and i.d.	Buffer	Voltage	Detector	Reference
Rhizoma Picrorhizae (Huhuanglian)	Radix	Phenolic acids	CZE	50 cm × 50 μm i.d	100 mmol L^{-1} borate buffer pH = 9.5	30 kV	DAD 210 nm	[165]
Securidaca inappendiculata (Chanyiteng)	Stem	Xanthones	CZE	50 cm × 50 μm i.d	200 mmol L^{-1} borate buffer pH = 9.510 mmol L^{-1} β-CD sulphonate	30 kV	DAD 265 nm	[166]
Cassia siamea (Rougui)	Bark	Anthraquinones	CZE	50 cm × 50 μm i.d	100 mmol L^{-1} borate buffer pH = 9.050 mmol L^{-1} hydroxypropyl-γ-CD, 10% MeCN	20 kV	DAD	[127]
Apocynum Venetum (Luobuma)	Leaf	Rutin Quercetin D-catechin	CZE	75 cm × 25 μm i.d	25–50 mmol L^{-1} borate buffer pH = 8.4	16 kV	EC +950mV	[128]
Polygonum cuspidatum Sied. et Zucc. And Zijin capsule	Leaf, capsule	*trans*-Resveratrol	CZE	65 cm × 25 μm i.d	100 mmol L^{-1} borate buffer pH = 9.24	30 kV	EC +850mV	[129]
Coptidis Rhizoma (Huanglian), Phellodendri Cortex (Huangbai)	Radix	Berberine, palmtine	CZE	50 cm × 75 μm i.d	50 mmol L^{-1} borate buffer-15% MeOH pH = 7	14 kV	DAD 223 nm	[130]
Standards		Matrine, oxymatrine	CZE	50 cm × 50 μm i.d	20 mmol L^{-1} phosphate buffer (pH = 8.0)	30 kV	DAD 214 nm	[131]
Cassia species (Fanxieye)	Leaf	Sennoid A	CZE	50 cm × 75 μm i.d	37.5 mmol L^{-1} Tris-H_3PO_4 buffer-25% MeOH	15 kV	DAD 214 nm	[132]
Magnolia officinalis Rehd. et Wils.	Bark	Magnolol, honokiol	CZE	64.5 cm × 75 μm i.d	80 mmol L^{-1} borate buffer pH = 10	18 kV	DAD 294 nm	[133]
Cinnamomum cassia Presl (Rougui) and *Coptis* Species (Huanglian)	preparation	Isoquinoline alkaloids	CZE	75 cm × 75 μm i.d	200 mmol L^{-1} borate buffer-15% MeOH pH = 7	14 kV	UV 254 nm	[134]
Gegenqinlian decoction		Berberine, palmtine, jatrorrhizine	CZE	40 cm × 50 μm i.d	60 mmol L^{-1} phosphate buffer-40% MeOH (pH = 8.0)	22 kV	UV 254 nm	[135]
Decoction		Precipitation recction between berberine and rheinic acid	CZE	31.6 cm × 50 μm i.d	12.5 mmol L^{-1} phosphate buffer pH = 7.0	12 kV	UV 254 nm	[136]
Pericarpium Citri Reticulatae	Fruit	Hesperidin, synephrine	CZE	40 cm × 25 μm i.d	50 mmol L^{-1} borate buffer pH = 9.0	12 kV	EC +900 mV	[137]
Chinese prepared medicine		Aristolochic acids I&II	CZE	35 cm × 50 μm i.d	120 mmol L^{-1} borate buffer pH = 9.5	18 kV	DAD 254 nm	[138]
Lonicera japonica Thumb. (Rendong)	Flower	Chlorogenic acid	CZE	52 cm × 75 μm i.d	10 mmol L^{-1} borate-19 mmol L^{-1} phosphate buffer-10% MeOH	16 kV	UV 214 nm	[139]
Lianqiao Huaimihua	Fruit, flower	Baicailin, quercetin, frosythin	CZE	40 cm × 50 μm i.d	80 mmol L^{-1} borate-30 mmol L^{-1} SDS buffer-MeCN	12 kV	UV 254 nm	[140]

African Ancistrocladus Species	Root bark	Naphthylisoquinoline alkaloids	NACE	21 cm × 50 μm i.d for UV 57 cm × 50 μm for MS	100 mmol L^{-1} ammonium acetate buffer pH = 3.1/MeCN 50:50	18 kV	DAD 232 nm and ESI-MS–MS	[141]
Rhizoma corydalis (Yuanhu)		tetrahydropalmatine	NACE	52.5 cm × 75 μm i.d	50 mmol L^{-1} of NaAc in MeOH containing 2 mol L^{-1} HAc	25 kV	DAD 214 nm	[142]
Rhizoma coptidis (Huanglian)	Radix	Berberine	NACE	52.5 cm × 75 μm i.d	25 mmol L^{-1} of NaAc in MeOH containing 1 mol L^{-1} HAc	25 kV	DAD 254 nm	[143]
Thalictrum species	Aerial part	Isoquinoline alkaloids	NACE	50 cm × 50 μm i.d	75 mmol L^{-1} of NaAc in MeOH containing 1 mol L^{-1} HAc	30 kV	DAD 200 nm	[144]
Ligustrum lucidum Ait		Oleanolic acid, ursolic acid	MEKC	52 cm × 75 μm i.d	15 mmol L^{-1} phosphate, 15 mmol L^{-1} tetreborate, 10 mmol L^{-1} SDS/5% MeOH	16 kV	UV 214 nm	[146]
Andrographis paniculata Nees (Chuanxinlian)	Aerial part	Andrographlide, dehydroandrographlide,	MEKC	42.2 cm × 75 μm i.d	15 mmol L^{-1} phosphate, 10 mmol L^{-1} SDS, 5% MeOH pH = 6.8	20 kV	UV 240 nm	[147]
Citrus species	Fruit	Naringin, hesperidin	MEKC	56 cm × 50 μm i.d	80 mmol L^{-1} borate, 50 mmol L^{-1} SDS, 10% MeCN	20 kV	DAD 213 nm	[148]
Tripterygium wilfordii	Aerial part	Diterpenoid triepoxides	MEKC	52.4 cm × 75 μm i.d	60 mmol L^{-1} borate, 10 mmol L^{-1} SDS, pH = 8.0	20 kV	DAD 214 nm	[149]
Rhei Rhizoma (Rhubarb)	Radix	Anthraquinoids	MEKC	86.5 cm × 75 μm i.d	60 mmol L^{-1} borate, 12.5 mmol L^{-1} prosphate, 10 mmol L^{-1} SDS, pH 9.34	25 kV	UV 254 nm	[150]
Rhei Rhizoma (Rhubarb)	Radix	Anthraquinoids	MEKC	60 cm × 75 μm i.d	50 mmol L^{-1} borate-NaOH, 25 mmol L^{-1} SDS, pH 11	18 kV	UV 254 nm	[151]
Rhei Rhizoma (Rhubarb)	Radix	Anthraquinoids	MEKC	40 cm × 50 μm i.d	15 mmol L^{-1} phosphate, 80 mmol L^{-1} borate, 15% MeOH pH = 9.7	15 kV	UV 254 nm	[152]
Rhei Rhizoma (Rhubarb)	Radix	Anthraquinoids	MEKC	45 cm × 50 μm i.d	15 mmol L^{-1} phosphate, 20 mmol L^{-1} SDS, 20 mmol L^{-1} SC 20 mmol L^{-1} β-CD pH = 10.4	14 kV	UV 254 nm	[153]
Rhei Rhizoma (Rhubarb)	Radix	Anthraquinoids	MEKC	50 cm × 50 μm i.d	15 mmol L^{-1} phosphate, 20 mmol L^{-1} SDS, 20 mmol L^{-1} β-CD 10–15% MeOH pH = 10.38	14 kV	UV 254 nm	[154]
Rhei Rhizoma (Rhubarb)	Radix	Anthraquinoids	MEKC	60 cm × 50 μm i.d	15 mmol L^{-1} phosphate, 60 mmol L^{-1} borate, 30 mmol L^{-1} SDS, 17% MeCN pH = 8.6	28 kV	UV 270 nm	[155]
Rhei Rhizoma (Rhubarb)	Radix	Anthraquinoids	MEKC	40 cm × 50 μm i.d	20 mmol L^{-1} SC, 20 mmol L^{-1} STC, 80 mmol L^{-1} borate, 15 mmol L^{-1} β-CD, pH = 11	16 kV	UV 254 nm	[156]
Thalictrum species	Aerial part	Isoquinoline alkaloids and saponins	MEKC		Anionic and cationic surfactants			[157]
Thalictrum species	Aerial part	Isoquinoline alkaloids	MEEKC	50 cm × 50 μm i.d	10 mmol L^{-1} phosphate, 140 mmol L^{-1} SDS, 100 mmol L^{-1} *n*-heptane, 12% *n*-butanol, 1% methanol and 5 mmol L^{-1} SC	25 kV	DAD 200 nm	[158]

considered as standard method for the determination of antibacterial and antirheumatism components in natural products. However, CE procedures cannot act as universal approaches for the determination or quality control of various plant drugs and their preparations at the moment, owing to their lower reproducibility and detection limits than as compared to HPLC. Nevertheless, the numbers of publications on CE application in the analysis of TOM has increased very rapidly.

For the analysis of antibacterial and antirheumatism components in plant medicines, some recent developments in CE analysis are demonstrated in Table 5. It can be seen that capillary zone electrophoresis (CZE) invented in 1981 [125] is a fundamental mode among the others and its applicability to the analysis of a large number of natural products has been moved [126]. As the separation mechanism in CZE is based on charge differences of the analyzed solutes. It appears quite suitable for the analysis of antibacterial and antirheumatism active components (flavonoids, coumarins, organic acids and alkaloids) [127–140,165,166]. For hydrophobic analytes, nonaqueous CE (NACE) demonstrated its advantages regarding selectivity and separation efficiency [141–144]; however, Micellar Electrokinetic Chromatography is the most common used approach [145–151]. A series of investigations concentrated on anthraquinoids in Rhubarb were reported by Yuan's group [152–156] using different buffer systems, including borate, phosphate, with the addition of SDS, β-cyclodextrin, sodium cholate and sodium taurocholate. On the other hand, Bo et al. compared different CE modes including NACE [144], MEKC [157] and MEEKC [158] for the separation of isoquinoline alkaloids in *Thalictrum* species, and the optimization of separation conditions for different CE modes was comprehensively discussed. The same group compared cyclodextrin-modified CZE [166,167], MEKC and MEEKC [168,169] for the separation of nine xanthones in *Securidaca inappendiculata* species, and a more integrated work (including CZE, MEKC, MEEKC, CEC and HPLC) [163,170] has been accomplished by Bo et al. resently (see Fig. 4). Other similar investigations have compared the separation ability between different electrophoresis modes [159,160], or between chromatographic and electromigration methods [161,162], indicating that CE methods could represent either alternative, or complementary procedures, to HPLC for the analysis of active components in some TOMs.

In addition, a comprehensive two-dimensional instrument coupling capillary LC and MEKC was investigated by Zhang et al. [164]. The overall system performance was verified in the separation of complex neutral components and resolution of hundreds of compounds in traditional Chinese medicines was demonstrated.

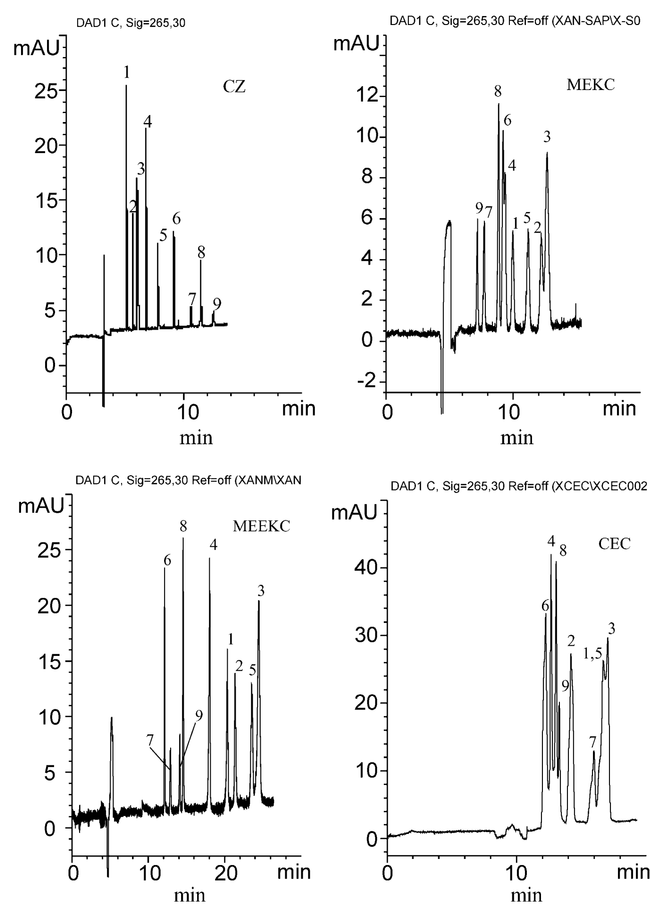

Fig. 4. Separations by different CE modes for nine xanthones. The structures of nine xanthones and separation conditions please refer to [163]. Reprinted from [170] with permission.

4. Quantitation and validation

For the quantitation of antibacterial and antirheumatism agents in plant medicines, HPLC represent a universal method with high accuracy and reproducibility. DAD and UV detection have been widely used. For some specific compounds, a lower detection limit can be obtained by using electrochemical (EC) or fluorescence (FL) detectors [89,90]. It was also demonstrated that ELSD can be a more suitable detection tool than UV for UV poorly detectable organic components or in gradient elution procedures [93–97]. In addition, MS has a lower detection limit, yet a strait linear range in usual [91,92]. Thus, to quantitate trace compounds in plant medicines, MS appears more favorable, while for the quantitation of volatiles, GC-FID is a standard method that can barely be replaced by other techniques (see Table 2).

For CE method, the validation procedure is similar to HPLC. Limited by absolute injection amount, the sensitivity and accuracy is lower than HPLC in most cases.

5. Conclusion

For the analysis of antibacterial and antirheumatism agents in plant medicines, the methods mentioned above have been used incoordinately, from academic researches to universal methods in applications. Chromatographic approaches, par-

ticularly GC and RP-HPLC demonstrated their applicabilities for most samples. The design of the apparatus, pretreatment procedure, the very separation, as well as optimization of analytical conditions seem to be mature. On the other hand, electromigration methods, which have already demonstrated their advantages in terms of the analysis time and costs, seem to be competitive and have their potential to take the place of chromatographic methods in some realms.

As standard quality control methods for targeted active components in crude plant drugs and their preparations, a tradeoff between analytical time and detection limit should be considered for GC, HPLC and CE. Sample preparation procedures are often the key step for fast analysis. Solid phase extraction (SPE) and solid phase microextraction (SPME), as well as other automated procedures will be more and more frequently used. If the disadvantages in reproducibility and sensitivity of electromigration methods can be resolved, these methods are likely to replace the chromatographic methods to some extent.

For the comparison of a particular component in various plant medicines, or for the scan of all bioactive compounds in a particular plant medicine, high-throughput analysis methods, e.g. multi-dimensional chromatographic and electromigration methods, parallel multi-way fully automated systems are likely to show their potential in the future.

Acknowledgements

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