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Review

Recent advances in analysis of Chinese medical plants and traditional medicines

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Abstract

Chinese herbal medicine is gaining increasing popularity worldwide for health promotion and adjuvant therapy. Thus, selective and efficient analytical methods are required not only for quality assurance but also for authentication of the plant material. Applications of both chromatographic and electrophoretic techniques to the analysis of medicinal plants and Chinese traditional medicine preparations over the last 3 years are outlined in this review. The role of chemical fingerprinting is highlighted and a brief survey of determination of toxic components, natural and synthetic adulterants is also included. Moreover, different sample pretreatment and extraction methods are discussed.

Keywords: Analysis; Chromatography; Separation; Fingerprint; TCM; Chinese medicine; Drugs; Preparations

Contents

Abbreviations: APCI, atmospheric pressure chemical ionization; CCC, counter-current chromatography; CODA, component detection algorithm; CZE/CE, capillary zone electrophoresis; DAD, diode-array detector; ELSD, evaporative light scattering detector; ESI, electrospray ionization; ILC, immobilized liposome chromatography; LC–MS, liquid chromatography with mass spectrometry detection; LC–MS/MS, liquid chromatography with double mass spectrometry detection; LOD, limit of detection (S/N = 3); MEEKC, microemulsion electrokinetic chromatography; MEKC, micellar electrokinetic chromatography; MRM, multiple reaction monitoring; N, noise; R, correlation coefficient; R.S.D., relative standard deviation; S, signal; SDS, sodium dodecylsulfate; SIM, selective ion monitoring; SPE, solid phase extraction; TCM, traditional Chinese medicine; TOF, time-of-flight

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1. Introduction

There is a long history of herbal medicine in far Eastern countries; in particular Chinese people have utilized herbs and plants to treat various diseases for more than 8000 years. Traditional Chinese medicine (TCM) has been modified to some extend in other countries, such as Korea and Japan and has attracted considerable attention even in European and North American countries [1]. In the Chinese Pharmacopoeia, there are recorded more than 500 examples of crude drugs from plants and 400 TCMs that are widely used all over the world [2,3]. These drugs are complex mixtures, containing usually hundreds of chemically different constituents but only a few, if not one, compounds are responsible for the beneficial and/or hazardous effects [4]. Furthermore, biologically active compounds form just a minute part of herbs being diluted with a large amount of proteins, sugars or tannins, which, in some cases, does not contribute to the pharmaceutical effect but they make the quality control of crude drugs and their medical preparations extremely difficult. Therefore, very efficient and selective methods, including the extraction techniques are required for identification and quantitative analysis of the active compounds or drug standardisation. Chromatography and electromigration methods represent main techniques applied in this field due to their powerful separation efficiency combined with sensitive detection as indicated by several recent reviews covering the TCM preparations [5–11] or selective active compounds in a variety of sample matrices (*Cinchona* alkaloids [12], indole alkaloids [13] or tropane alkaloids [14]).

Traditionally, chromatography and relative techniques are also used to evaluate the quality of plant raw materials, as the diversity of components and their content vary with not only the species but also with the growing conditions, the season when plants are harvested, the process methods and storage duration. In the medicine plant production, it is not always necessary to produce very pure preparations. Herbal drugs are then standardized to selected component to assure comparability of different preparations of plant origin (herbal teas, extracts, extract evaporates, preparations, formulations, decoctions i.a.). In this respect, fingerprint analysis has been accepted by WHO, as a methodology for the assessment of herbal medicines [15]. Fingerprint is also used to control quality of TCM and their raw materials as stated by the Chinese Pharmacopoeia [16]. Conventional research focuses mainly on the determination of the most active or hazardous components, while fingerprinting can offer integral characterization of a complex system with a quantitative degree of reliability. Chromatography and electromigration methods again are recommended by responsible authorities. It should be noted that it is very important for assessment of the quality of various samples to maintain the reproducibility of retention characteristics of particular peaks as high as possible. It means to minimize unavoidable shortcomings, such as imperfect resolution, peak tailing and separation columns from different producers despite the same type of stationary phase characteristic despite the fact, that chemometric and/or computer data analysis (e.g. CODA) can overcome many separation difficulties.

Whatever analytical method requires an appropriate isolation of analyte of interest, in particular from a complex matrix. Selection of an extraction strategy is always challenging and depends upon the type of herb, the accessibility of plant material as well as the content of analyte [17].

Another problem in the evaluation of TCM is associated with inconsistent terminology arising from the feet that a significant number of studies have been published in Chinese by Korean and Japan authors. This situation has led to confusion in some extend as researchers of each of the three countries seem to use different English name for identical or similar Chinese medical formulae. As the number of publications written in English has permanently increased and literature search has been computerized (see e.g. Chinese Medicine and Acupuncture information site created by West TCM, Vancouver Canada [18]), standard names for these formulae should be established by a consensus between these countries. This matter is clearly documented in a comparative review summarized all investigations of TCMs published in the American Journal of Chinese Medicine from 1997 to March 2001 [19].

Advances in chromatographic and spectroscopic techniques now permit the isolation and structural analysis of potent biologically active plant constituents that are present in quantities too minute to have been previously characterized. In addition, advances in plant cell and tissue culture and genetic manipulation could provide new means for economic production of rare plants and the chemicals that they produce. These new chemical and biological technologies will serve to enhance the continued usefulness of higher plants as renewable resources of chemicals. The importance of secondary metabolites analyses is obvious as it can serve as a qualified tool, i.e. in chemotaxonomy, chemical ecology, agriculture, food industry, plant pathology and stress analysis. Here, we would rather expect some difficulty in drawing a dividing line around the field defines as, e.g. the subject of secondary metabolites isolation and structure elucidation will definitely interfere with it. On the other side, the development in the field, emerging and vanishing techniques sometimes can yield into a feeling that some older ways or results do not have the “proper” significance.

Hence, we will try to cover relevant sources (with cross-check based on the Chemical Abstracts) from the period of last 2 years to depict as true overview as possible, knowing, however, the difficulty of such a task. Also, as the information is extremely vast and in most cases the separation schemes of secondary metabolites deal with different compounds polarities we decided not to give exact compound names or chemical structures. In this type of information survey, we value most the type of metabolite and proper literature quotation. The present review summarized the application of separation methods to analysis of medical plants and TCM.

There are also comprehensive electronic sources of separation information, which in larger part includes secondary

metabolites and their derivatives, e.g. Cumulative CAMAG Bibliography Service (CCBS), which brought since 1997, the most comprehensive compilation of literature in the field of planar chromatography (TLC/HPTLC) available as database. It contains all abstracts of CBS issues beginning with CBS 51 (May 1983). The database is updated after every other CBS edition. Currently, it includes about 7700 abstracts of publications from 1982 to 2003 [20].

Similarly, ACD/Chromatography Applications Database is a summary of submissions of Agilent Technologies, Alltech, Astec, Beckman Coulter, Chiral Technologies, Eprogen (former Eichrom Technologies Inc.), GL Sciences, GROM Analytik + HPLC, Hamilton Company, Argonaut Technologies (former Jones Chromatography), Phenomenex, Polymer Laboratories, Regis Technologies, Restek Corporation, Thermo Hypersil-Keystone, Varian, and ZirChrom Separations. Version 8.0 of the Database contains 5444 applications, including over 500 chiral, 1200 gas chromatography (GC) and 100 capillary electrophoresis (CE) applications, covering a broad range of organic and natural product chemistry. The ACD/Chromatography Applications Database brings "live" chromatograms with all necessary raw and elucidation data with peak to structure assignment in fully searchable format (Fig. 1) [21].

2. Quantitative analysis of components from TCMs

In most cases, pharmaceutical preparations are made from a few or many plants. According to the practice of TCM, toxic herbal materials might be used for specific therapeutic application; however, the toxic ingredient compounds also act as active principles of the herbal material concerned, e.g. in *Digitalis* preparations. Thus, quantitative and selective determination of principal constituents is crucial to the quality control of TCM preparations; therefore, much attention has been paid to the development of suitable separation methods in this field.

Five toxic alkaloids were determined in aconitine root (*Radix aconitini praeparata*), seeds of *Strychnos pierrian* and TCM preparation Shen Jin Huo Luo Wan by non-aqueous CE for the first time in 2002 [22]. Aconitine, hypoconitine, mesaconitine, strychnine and brucine were separated on a fused-silica capillary, the separation voltage was set at 15 kV and electrolyte consisted of 40 mM ammonium acetate, 80% methanol and 0.1% acetic acid. Atropine was selected as internal standard and analysis was completed in less than 15 min. Detection was at 200 nm. The calibration curves showed a linear range from 2 to 200 mg/L for these alkaloids with correlation coefficient (R) between 0.9988 and

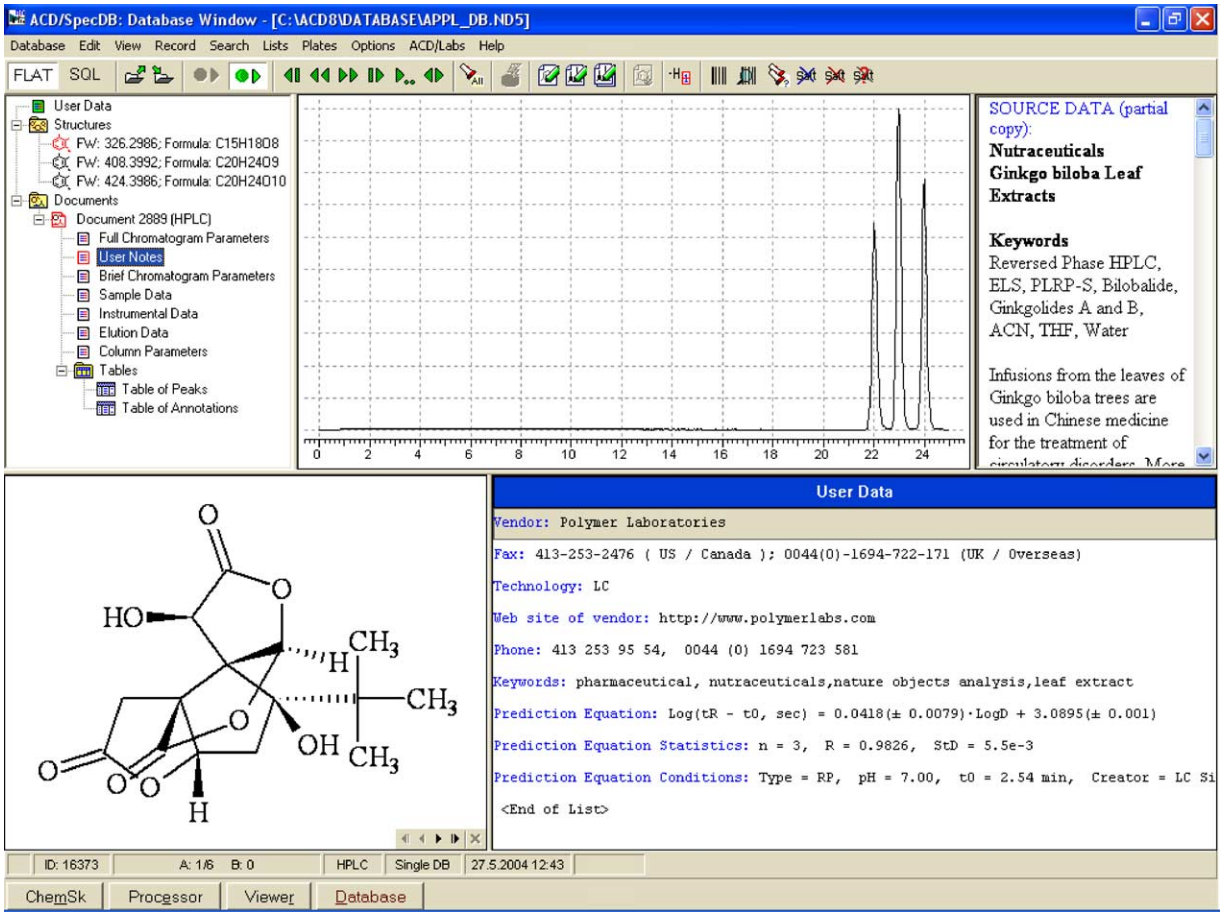

Fig. 1. ACD/Chromatography Applications Database window.

0.9999. The limit of detections (LOD) varied from 0.85 to 1.90 mg/mL and recoveries ranged from 95 to 108.8% if a sample was spiked with 20 mg/mL each of the alkaloids. Analytes were extracted from pulverized drugs with methanol at room temperature for 10 h and extracts were sonicated for 30 min. In the following paper [23], the separation conditions were further optimized to establish a precise CE–MS method enable to assess the influence of preparation procedure on concentration of the alkaloids. Alkaloids in TCM Maqianzi were separated in ammonium acetate (80 mM) and 0.1% acetic acid dissolved in water–methanol (40:60, v/v). The calibration curves exhibited linear dynamic ranges with $R = 0.9920$ for strychnine and 0.8806 for brucine if examined with concentration level from 0.2 to 50 mg/L. The respective LOD at S/N = 3, were 0.12 and 0.13 mg/L indicating that sensitivity of MS detection using the selective ion monitoring (SIM) mode was better than that of UV detection [22]. Four main alkaloids were found in the unprepared herb: strychnine, brucine, novacine and icajine. Separation of Maqianzi alkaloids and products of their decomposition was carried out in ammonium acetate (40 mM) and 0.1% acetic acid dissolved in pure methanol. As the resolution of nine alkaloids (aconitine, hypaconitine, mesaconitine, benzoylaconine, benzoylhypaconine, benzoylmesaconine, aconine, hypaconine and mesaconine) was still unsatisfactory; SIM mode had to be applied to identify the compounds.

Three aconitine alkaloids (hypoconitine, aconitine and mesaconitine) and other unknown compounds coexisting in the five TCM, Chuanwu, Caowu, Fuzi, *Aconitum tanguticum* Maxim. and *Aconitum gymnandrum* were completely separated by non-aqueous CE within 13 min [24]. The electrophoretic medium was 20 mM sodium tetraborate–70% (v/v) methanol (pH 8.5) and an uncoated capillary was used. Detection was carried out with a UV detector at 214 nm. Calibration curves were linear giving $R > 0.99$ for the concentration range of 0.02–0.8 g/L and each analyte. The recoveries of the three compounds were 104.0, 93.5 and 96.0%, respectively. Sample powder was alkalised with ammonia and then refluxed with diethyl ether, the supernatant was discarded and the residue was extracted with methanol in an ultrasonic apparatus. A rapid and sensitive HPLC method was developed for the simultaneous determination of aconitine, mesaconitine and hypoconitine in blood and urine samples [25]. Analytes were concentrated by solid phase extraction using Oasis MCX cartridges and then separated on an XTerra RP 18 column with a gradient, ammonium hydrogen carbonate buffer—acetonitrile. Calibration curves were linear in the range of 2.75–550 ng for aconitine and hypoconitine and 3–600 ng for mesaconitine. The LOD for each analyte was 0.1 ng. Metabolites of aconitine were analysed on a Hypersil column with acetonitrile–water–formic acid (105:95:1, v/v/v) [26].

A new approach based on a LC–MS/MS technique was introduced for simultaneous qualitative and quantitative analysis of TCM products for the presence of toxic compounds [27]. Indole alkaloids (brucine and strychnine), quinolizidine alkaloids (matrine and oxymatrine), diterpenoid alkaloids (hypaconitine, mesaconitine and aconitine) and bufadienolides (cinobufotalin, cinobufagin, resibufogenin and bufalin) were determined in extracts of *Semen Strychni*, *Radix Sophorae Tonkinensis*, *Radix Aconiti Lateralis* and *Venenum Bufonis*, respectively. The mobile phase system was optimised separately for each group of target compounds (Table 1) and was also employed as a solvent for extraction. A triple quadrupole LC–MS system allowing the simultaneous monitoring of more than one pair of parent/daughter fragment ions was used and quantitative analysis was conducted by multiple reaction monitoring (MRM). Parameters, such as reproducibility, spiking recovery, linearity and detection limit were assessed to validate performance of the method (Table 2). Matrine and oxymatrine are the major quinolizidine alkaloids in the roots of *Sophora subprostata* that form a commonly used TCM, Shan-dou-gen [28]. To evaluate the quality of *S. subprostata*, a rapid CE method on uncoated capillary was developed. The electrolyte was a buffer solution, containing 75% 130 mM sodium diphosphate-phosphoric acid (pH 3.5) and 25% acetonitrile. Applied voltage was 10 kV, temperature was 30 °C and UV detector was set at 200 nm. Samples were extracted with water by ultrasonic at 40 °C and then a solution of 2-(4-hydroxyphenyl)ethylammonium chloride as internal standard was added. Calibration graphs were constructed in the range 22.0–440.0 μg/mL for matrine and 10.8–216.0 μg/mL for oxymatrine and showed a good linear relationship between the peak areas and the concentrations giving $R > 0.9975$. The recoveries of matrine and oxymatrine ranged from 86.1 to 92.5% and LODs were 1.1 and 1.0 μg/mL, respectively. The coefficient variations of intra- and inter-days studies as well as the results of the day-to-day experiment revealed the precision and accuracy of the method. Authors suggested that these two analytes could be used as markers to assess the quality of *S. subprostata* drugs.

Dichlormethane extract of Chinese thin-plate Ch'an Su, preparation close to *Venenum bufonis* quantitatively analysed on silica gel column, with a hexane–acetone gra-

Table 1
Optimized HPLC conditions for the determination of toxic compounds [27]

Analytes	HPLC column	The composition of mobile phase[a] (v/v)
Indole alkaloids	Amino column Restek	Acetonitrile with 0.4% acetic acid–10 mM ammonium acetate (85:15)
Quinolizidine alkaloids	Alltima C_{18} column	Acetonitrile–20 mM ammonium formate with 0.5% formic acid (10:90)
Diterpenoid alkaloids	Hypersil C_{18} column	Acetonitrile–0.4% acetic acid with 0.01% TMAH (50:50)
Bufadienolides	Hypersil C_{18} column	Methanol with 0.1% acetic acid–0.1% acetic acid (55:45)

[a] Flow rate was 200 μL/min.

Table 2
The validation of LC–MS method for the determination of target toxic compounds [27]

Analyte	Linearity range (mg/L)	*R*	LOD (ng)	Recovery (%)	Repeatability (% R.S.D.)
Mesaconitine	0.5–20	1.000	0.6	90.3	5.4
Aconitine	0.5–20	1.000	0.4	87.3	5.4
Hypaconitine	0.5–20	1.000	0.5	87.0	4.3
Brucine	0.1–5	0.999	1.5	88.9	3.6
Strychnine	0.1–5	1.000	1.8	96.6	4.1
Matrine	0.5–20	1.000	1.4	97.3	2.7
Oxymatrine	0.5–20	1.000	1.0	89.6	1.3
Cinobufotalin	0.5–20	0.999	0.6	101.1	4.5
Cinobufagin	0.5–20	0.998	1.5	99.7	3.5
Resibufogenin	0.5–20	0.997	1.6	100.4	2.4
Bufelin	0.5–20	0.999	0.7	100.8	3.9

dient (acetone 0–100%), and subsequently, by hexane-ethyl acetate gradient (ethyl acetate 10–100%) and finally on Sephadex LH-20 revealed 20*S*,21-epoxyresibufogenin, 20*R*,21-epoxyresibufogenin, and resibufogenin, 3-*O*-formyl-20*S*,21-epoxyresibufogenin, 3-*O*-formyl-20*R*,21-epoxyresibufogenin and 3-oxo-20*S*,21-epoxyresibufogenin [29].

A simple and rapid micellar electrokinetic chromatography (MEKC) was described for the simultaneous determination of quercetin, gentiopicrin, forsythin, chlorogenic acid and caffeic acid in three anti-viral TCM preparations, which were composed of several Chinese herbs [30]. Among them, *Forsythia suspensa* (Thunb.) Vahl, *Lonicera japonica* Thump, *Scutellaria baicalensis* Georgi and *Gentiana rigescens* Franch are the major components, and therefore, bioactive ingredients in these herbs can be considered as the index compounds for quality assessment. The analytes were sufficiently separated within 11 min under conditions of 40 mM sodium tetraborate (pH 9.65), containing 20 mM SDS, 20 kV at 25 °C. In the tested concentration range (8.5–1000.0 μg/mL), regression equations revealed good linear relationship (R = 0.9920–0.9991) between peak areas and corresponding concentration. Furthermore, a multiple linear regression model of quantitative structure–property relationship (QSPR) was constructed to predict the migration times of analytes. The method was validated by analysis of three representative TCM samples with recoveries ranging from 89.2 to 106.6%. Sample 1 (injection) was injected directly; Samples 2 (oral liquid) and 3 (syrup for babies) were injected after dilution. Samples of *Scutellaria baicalensis* were alternatively analysed by the high-speed counter-current chromatography (CCC) in two-phase system (1-butanol–water, 1:1, v/v) with 86% recovery [31].

Other *Gentiana* samples from Tibetian medicines (Shiweilongdankeli, i.a.), *G. rhodantha*, *G. kitag*, *G. scabra*, *G. rigescens*, and *G. macrophylla* were analysed by micellar electrokinetic electrophoresis and MEKC to reveal gentiopicroside and sweriamarin in 70 mM borate, 10 mM SDS and 6% isopropanol (pH 9.0) at 15 kV with detection at 254 nm with excellent recoveries [32].

Gastrodin and tetramethylpyrazine were simultaneously determined in three TCM preparations, Zhennaoning jiaonang, Yangxue shengfa and Xiaoshuan zaizao wan, by MEKC [33]. Analytes were isolated by a simple extraction with water under ultrasonication. Running buffer comprised 50 mM sodium tetraborate and 15 mM SDS, pH 9.50 and experiments were performed at 20 kV with UV detection at 200 nm. The linear calibration ranges were 2.5–200 μg/mL (R = 0.999) for gastrodin and 5.0–200 (R = 0.997) for tetramethylpyrazine; the detection limits were 0.5 and 0.8 μg/mL, respectively. Recoveries of the two analytes from the samples were between 94.2 and 104.5%.

Synephrine level was estimated in *Evodia* fruit and eight samples of TCM, containing the crude drug using a CE method [34]. Separation was carried out on an AccuSep capillary column with a solvent of 40 mM monosodium phosphate-acetonitrile (9:1, v/v, adjusted to pH 4.5 with phosphoric acid) as the running buffer and 12.5 kV as the electrophoretic voltage. Migration time of synephrine was 20 min and 4-dimethylaminopyridine was employed as an internal standard.

A MEKC method was established for the identification and determination of diterpenoid triepoxides in the Chinese herb *Tripterygium wilfordii* Hook F. and its preparations [35]. A fused-silica capillary was used and direct UV detection was performed at 214 nm. Optimised separation conditions consisted of 20 mM boric acid and 10 mM sodium tetraborate buffer (pH 8.0), containing 10 mM SDS as electrolyte and voltage set at 20 kV. Roots of *T. wilfordii* were extracted in a Soxhlet apparatus with one of three solvents: ethanol, chloroform or ethyl acetate; the last method yielded the higher amounts of triptonide, triptolide and tripdiolide in extract. The MEKC exhibited good accuracy and precision giving the correlation coefficients of calibration greater than 0.9994 and recoveries of the triepoxides within the range 98.72–101.94%.

A capillary zone electrophoresis (CZE) method with amperometric detection was elaborated for the determination of five active components in the TCM preparation, Huangdan Yinchen Keli, in which *Herba artemisiae* and *Rhubarb root* are the main components [36]. The optimal conditions were 30 mM borate buffer (pH 9.5) as running electrolyte, 18 kV as separation voltage and 1.00 V as detection potential. The LODs were 0.11, 0.3, 0.15, 0.29 and 1.4 μmol/L for aloe-emodin, emodin, rhein, chlorogenic acid and caffeic acid, respectively.

A novel microemulsion electrokinetic chromatography (MEEKC) method for separation and determination of two sesquiterpene lactones, alantolactone and isoalantolactone, in *Radix inulae* and Liuwei Anxian San was developed [37]. The optimised microemulsion system was composed of hexane (0.32%, w/w), SDS (1.24% w/w), 1-butanol (2.64%, w/w), acetonitrile (10%, w/w) and 10 mM sodium tetraborate (85.80%, w/w, pH 9.2). The applied voltage was 20 kV and analytes were detected at 214 nm. Regression equations revealed linear relationship (R > 0.9946) between the

peak area of each analyte and the concentration in the range 5–500 μg/mL. The LODs were, approximately 0.45 μg/mL (alantolactone) and 0.56 μg/mL (isoalantolactone) and recoveries ranged from 98.2 to 104.3%. Three different methods were used to prepare extracts; the highest yield was achieved with a procedure in which the material was shaken with methanol, then extracted by ultrasonic agitation and finally, centrifuged.

Three bioactive components, rhein, baicalin and berberine, were determined in TCM preparations, Sanhuangpian, Niuhuangjiedu-pian and Huanglianshangqing-pian, by a CZE method [38]. Separation was performed on a fused-silica capillary with 10 mM sodium borate (pH 9.2, adjusted with 0.5 M NaOH). The wavelength of UV detector was set at 254 nm and the applied voltage was 25 kV. Powdered samples were extracted with ethanol in an ultrasonic bath. Repeatability of migration indices determined according to the two-marker technique with glycyrrhizin acid and cefalexin as marker compounds was superior to that calculated by using absolute migration times. The spiking of extracts revealed the recoveries from 96.7 to 104.6% for the three analytes. The relationships between the concentration and the corresponding peak area were linear with $R > 0.998$.

MEKC method using an uncoated capillary eluted with 15 mM SDS in 30 mM borate buffer (pH 9.5) was compared with an HPLC method consisted of a C_{18} column eluted with mobile phase of methanol–water (55:45, v/v) at a flow rate of 0.9 mL/min [39]. Three bioactive compounds (andrographolide, deoxyandrographolide and neoandrographolide) in ethanol–water (40:60, v/v) extracts of *Andrographis paniculata* were determined by the two methods. Both method exhibited acceptable calibration data and the recoveries but MEKC yielded a speedier separation, while HPLC gave a slightly better reproducibility in retention time, however, HPLC method was substantially more sensitive than MEKC (Table 3). Two TCM preparations, Chuanxinlian and Xiaoyan Lidan tablets, were analysed by MEKC because the method was considered to be more suitable owing to its fast analysis time and the absence of column contamination [40]. Chuanxinlian, prepared i.a. from *Andrographis paniculata* and *Clerodendron cyrtophyllum* was also analysed with HPLC on YWG C_{18} column with methanol–water (1:1, v/v) as mobile phase and UV detection at 225 nm. Calibration curve was linear ($R = 0.9997$) and recovery over 98% was achieved.

The MEKC separation of morroniside, loganin and gallic acid was improved if β-cyclodextrin (1 mM) was added into a running buffer consisted of 10 mM phosphate, 5 mM sodium tetraborate, 140 mM SDS and 5% (v/v) methanol [41]. Based on experiments, 12.5 kV was selected as the optimum voltage and direct UV detection was employed at 214 nm. The three effective components of *Fructus corni* (Shanzhuyu), the fruit of *Cornus officinalis* Sieb. et Zucc., were separated in 22 min. Calibration graphs were constructed for the concentration up to 1000 μg/mL of each analyte and strongly linear relationship with $R > 0.99$ was found. The LODs were 3.5, 3.2 and 0.079 μg/mL for morroniside, loganin and gallic acid, respectively. The recovery experiments were performed by adding accurate amounts of three analytes to a real sample and the results ranged from 95.7 to 102.6%.

Using a LC–MS/MS method, three species of herbs (*Stellera chamaejasme* L., *Euphorbia ebracteolata* Hayata and *E. fischeriana* Steud.) were identified in Langdu, a well-recognized TCM for the treatment of hydrothorax, carbuncles and scrofula [42]. The chromatographic conditions involved an Alltech Hypersil C_{18} column eluted with a gradient of methanol and water in which the amount of methanol increased from 40 to 100% in 55 min. In order to enhance the ionisation efficiency at the electrospray interface, acetic acid was added to the mobile phase at the concentration of 0.1%. After the separation, the compounds were analysed by tandem MS which could alternately record a product ion MS and MRM signal for the target analyte eluted from column. The structural information provided by the product ion MS was used to characterize individual marker compound, whereas, the MRM signal constituted a profile of the separated compounds. The LC–MS/MS analysis was divided into time segments such that different optimum MS/MS experimental conditions could be applied for the respective marker compounds. As a result, the chromatogram obtained could be regarded as a segmental MRM profile, with each segment selectively optimised for a particular representative chemotaxonomic marker in the herb extract. The profiles indicated that *S. chamaejasme* contained daphnetin, skimmetine, stellerin, chamaechromone and neochamaejasmin, *E. fischeriana* contained ebracteolata compound B, ingenol, jolkinolide B and fischeriana A, whilst *E. ebracteolata* contained ebracteo-

Table 3
Characteristics of MEKC and HPLC method developed for the analysis of *Andrographis paniculata* (**1**, andrographolide; **2**, deoxyandrographolide; **3**, neoandrographolide) [39]

Method analyte	MEKC			HPLC		
	1	**2**	**3**	**1**	**2**	**3**
Concentration range of calibration (mg/L)	68–580	40–480	50–600	8–164	27–863	19–122
Correlation coefficient	0.9984	0.9980	0.9978	0.9999	0.9993	0.9986
Recovery (%)	101.0	97.9	98.2	101.0	99.1	98.5
Migration time (min)	8.02	12.7	11.7	4.28	16.2	10.4
Reproducibility, R.S.D. (%)	0.70	1.19	1.18	0.17	0.27	0.22
Detection limit (mg/L)	8.64	30.0	60.1	0.17	2.00	3.11

lata compounds B and C along with ingenol. The method was successfully applied to differentiate these herbs from related species *Alocasia macrorrhiza* (L.) Schott and *E. kansui* Liou.

A deep insight into composition of extracts made from traditional Chinese herb *Ligusticum chuanxiong* Hort. and its preparations was achieved by LC–DAD–MS [43]. Altogether, 17 main constituents were simultaneously separated and nine of them unequivocally identified (vanillin, ferulic acid, senkyunolide I, senkyunolide H, senkyunolide A, coniferyl ferulate, Z-ligustilide, neocnidilide and 3-butylidenphtalide) by comparison with the authentic standards. Additional eight compounds were tentatively identified based on their MS data. Another LC–diode array detector (DAD)–MS method with atmospheric pressure chemical ionisation (APCI) was applied to extracts of rhizome of *L. chuanxiong* (Chuanxiong) [44]. Six phtalides were unambiguously identified and structure often other phtalides was confirmed according to MS data.

For the analysis of the TCM formula, Si-Wu-Tang, a LC method was elaborated using both DAD and electrospray ionisation (ESI) detection system [45]. Based on the baseline separation on Hypersil C_{18} column with water–acetonitrile–acetic acid as a mobile phase, 12 compounds, including phenolic acids, phthalides and terpene glycosides were identified and seven of them were quantified. Most of these compounds derived from *Paeonia lactiflora* and *Ligusticum chuanxiong*. The linear calibration curves were acquired with $R > 0.99$ and LODs were between 0.75 and 5 ng. The recovery rates were in the range of 96.64–105.21%. Paeoniflorin from *P. lactiflora* Pall, was purified with high-speed CCC using two-phase system 1-butanol–ethyl acetate–water (1:4:5, v/v/v) with 94.3% recovery in a single run [46].

HPLC on a C_{18} column was investigated for the analysis of eight lignans (syringaresinol, lirioresinol B dimethyl ether, xanthoxylol, phillygenin, fargesin, sesamin and asarinin) [47]. A baseline separation was achieved under gradient elution in 25 min. The method was proven to be facile and reliable in routine quantification of asarin and sesamin in TCM preparations, containing *Asarum sieboldii* where corresponding recoveries were found to be 95.7 and 96.1%, respectively.

Protocatechuic acid, protocatechualdehyde, paeniflorin and ferulic acid were successfully separated on an Agilent Zorbax extend C_{18} column [48]. The mobile phase was a mixture of methanol and 0.5% acetic acid employing gradient elution at a flow rate of 0.15 mL/min. Detection was accomplished with DAD. Regression equations revealed good linear relationship ($R = 0.99938$–0.99996) between peak areas of the analytes and their concentration and the average recoveries were between 97.57 and 100.68%. The method was applied to the simultaneous determination of the above bioactive constituents in the TCM formula, Guanxin II.

Puerarin is the major isoflavone in the TCM preparation, Ganmao Qingre granules, consisting of fresh or dried roots of plants, such as *Pueraria lobata* (Willd.) Ohwi or *P. thomsonii* Benth. Using an HPLC method, puerarin was monitored in several samples obtained from four different manufactures [49]. Analyses were performed on a Supelco Discovery RP-Amide C_{16} column with methanol and 5 mM potassium dihydrogen phosphate (pH 4.0) as mobile phase (27:73, v/v) and UV detection at 248 nm. Linear range over 0.2–200 μg/mL of puerarin was estimated and the LOD was 0.1 μg/mL and recovery within 99.7–103%. Puerarin and magnolol were determined in TCM preparative, Po Chai pills by HPLC on a Hypersil ODS column with methanol–water (30:70 or 77:33, v/v) as mobile phase and UV detection at 252 and 294 nm [50]. The calibration curve was linear ($R = 0.99826$) over the concentration range 4–21 μg/mL. Pills were only diluted with methanol and a clear filtrate was analysed. Also a MEKC with electrochemical detection was described for the determination of puerarin and daidzein in *Puerariae radix* and its TCM preparations, Zhennaolin capsules and Jingfukang granules [51]. Samples were extracted with methanol in an ultrasonic bath. Under the optimum conditions, the analytes were base line separated within 11 min in a SDS-borax (pH 7.8) running buffer, and excellent linearity ($R > 0.9998$) was obtained in the concentration range from 5×10^{-4} to 5×10^{-6} mol/L. LODs were 0.6×10^{-6} and 1.1×10^{-6} and recoveries 93.3 and 114% for puerarin and daidzein, respectively. Furthermore, a LC–MS/ESI method allowing simultaneous quantification of nine phytoestrogens (biochanin, baicalin, daidzein, daidzin, flavone, genistein, genistin, glycitin and puerarin) and rutin (internal standard) in *Pueraria radix* (Kudzu root) was also reported [52]. The HPLC column was an Agilent, Zorbax C_{18} eluted with solvent A (water–glacial acetic acid, 1000:1, v/v) and B (acetonitrile–water–acetic acid, 800:200:1, v/v/v) in a gradient mode: 5 min, 5% B; increasing B to 80% in 50 min; decreasing B back to 5% in 2 min. Recoveries of all analytes varied from 90 to 110%. HPLC–UV yielded sensitivity ranging from 30 to 100 pg, while HPLC–MS yielded sensitivity ranging from 50 to 200 pg. Authors concluded that the former method could be used for routine assay and the later method for further confirmation.

HPLC was employed to the determination of six marker compounds (mangiferin, jateoirhizine, palmatine, berberine, cinnamic acid and cinnamaldehyde) in the TCM, Zi-Shen pill [53]. The separation was performed on a C_{18} column by stepwise gradient elution with water (0.2% triethylamine, v/v, adjusted to pH 4 with phosphoric acid)–methanol–acetonitrile system (98:0:2, 20 min; 80:5:15, 10 min; 65:13:22, 25 min) at a flow rate of 0.9 mL/min and with UV detection at 280 nm. Regression equations showed good linear relationship between the peak area of each marker and its concentration. The recoveries were 95.5, 98.3, 96.8, 99.5, 101.7 and 102.1%, respectively. The repeatability (2.5%) and reproducibility (3.3%) indicated that the method is suitable for routine analyses.

A gradient HPLC was also elaborated for the analysis of amygdalin in the TCM prescriptions, containing semen

Armeniacae amarum or semen *Persicae* [54]. Separation was carried out on a Hypersil C_8 column with mobile system consisted of methanol–acetonitrile–water–glacial acetic acid in the volume ratio of 1:1:16:0.02 at a flow rate of 0.8 mL/min and the eluate was monitored at 210 nm. The detector response was linear in the range 0.1104–1.840 mg/mL ($R = 0.9998$). The recoveries varied from 96.6 to 103.2%. In similar samples of semen *Armeniacae amarum* (Kyounin) or semen *Persicae* (Tounin) quantitative HPLC analysis was able to establish gentiobioside and glucopyranoside of mandelic acid, benzyl gentiobioside and glucopyranoside along with amygdalin and prunasin. Kyounin was found not to contain benzyl glucopyranoside [55].

An isocratic HPLC method was applied to the determination of geniposide in *Gardenia jasminoides* Ellis (Zhi-Zi), *G. jasminoides* Ellis var *grandiflora* Nakai (Shui-Zhi-Zi) fruits and two TCM preparations using a mobile phase of acetonitrile–methanol–5 mM monosodium phosphate (pH 4.6) (5:15:80, v/v/v) [56]. Separation was carried out on a Nova-Pak RP-C_{18} column and detection signal was set at 240 nm. Intra-assay and inter-assay accuracy and precision of the analyses were <10% in the range of 0.1–50 μg/mL. The concentration of geniposide in the TCM preparations, Huang-Lian-Jiee-Dwu-Tang (66.27 mg/g) and In-Chern-Tang (68.54 mg/g) was lower than this in the herb *Gardenia jasminoides* Ellis (73.44 mg/g).

The first report on the analysis of main constituents in the Shenbao tablet described a sensitive and selective LC–MS/MS method allowing simultaneous separation and identification of 20 compounds [57]. The samples were separated on an Alltima C_8 column by a linear gradient elution using water–acetic acid (A; 100:0.5, v/v) and acetonitrile (B, 0 min; 76:24, 15 min; 70:30, 40 min; 53:47, 50 min; 30:70 and kept 10 min) as the mobile phase at a flow rate of 1.0 mL/min. ESI coupled with ion trap mass spectrometer was used as a detection system.

A combination of flow injection analysis with CE was designed for the analysis of TCM preparations on the content of ephedrine and pseudo-ephedrine [58]. A high resolution was obtained using 100 mM borate buffer (pH 9.8) within 8 min in 25 μm separation channel (UV detection at 215 nm). The linear calibration range was 50–1500 μg/mL for both analytes ($R > 0.998$); LODs were 2.65 for ephedrine and 2.92 μg/mL for pseudo-ephedrine. The content of both analytes in five TCM preparations was determined with R.S.D. in range 1.16–4.51% and recoveries in range 90.4–114.6%.

Wangla, Tibetian medicine, dried rhizomes from *Coeloglossum viride* (L.) Hartm. var. *bracteatum* (Willd.) Richter was analysed for composition of coelovirin A–D. The ethanolic extract of the dried rhizomes was suspended in water and then extracted sequentially with petroleum ether and ethyl acetate. Polar fraction was chromatographed successively on macroporous resin, silica gel and analysed by RP-HPLC in 40% methanol in water [59].

To identify and quantify naringin, hesperidin and neohesperidin in rat serum after orally administrating the decoction of *Bulpleurum falcatum* L. and *Fractus aurantii* a LC–MS/MS method was developed and validated [60]. The separation was performed on a Nova Pak C_{18} column with acetonitrile–water (25:75, v/v) as mobile phase at the flow rate 0.8 mL/min. According to the recoveries, calibration data, precision and accuracy the method seems to be highly suitable for carrying out preclinical or pharmacokinetics studies of TCM.

A HPLC method for separation and detection of pterostilbene in Dragon's blood, a resin of *Dracanea cochinchinensis*, was established on Phenomemex C_{18} RP column with acetonitrile-1% acetic acid (41:59, v/v) mobile phase and detection at 310 nm [61].

Two alkaloids, sterculinine I and II, together with 13 known compounds were isolated from the 95% ethanol extract of Pangdahai (Boat-fruited Sterculia Seed) a TCM preparation produced from *Sterculia lychnophora* Hance [62]. Extract was separated between water and cyclohexane and ethyl acetate, then analysed by HPLC on a Waters RP-18 column with acetonitrile–water (1:9, v/v) and DAD (334 nm).

Cordycepin (3′-deoxyadenosine) was identified and determined in *Cordyceps kyushuensis* Kob., Chinese insect pathogenic fungus that infects the *Clanis bilineata* Walker [63]. A CZE method on uncoated fused-silica capillary coupled with a PDA detector (258 nm) was investigated; borate-running buffer (pH 9.4) was used and applied voltage was 20 kV.

The 95% ethanolic extract of TCM fruit body of fungus *Phellinus igniarius*, was pretreated by partitioning between ethyl acetate and 1-butanol and chromatographed over normal phase silica gel column, followed by Sephadex LH-20 and RP-HPLC to reveal ratio of phelligrins A and B [64].

TCM preparation Jin Gu Cao made from *Schnabelia tetradonta* (Sun) Wu and Chen was analysed on content of immunosuppressive cyclic peptides, schnabepeptide and schnabepeptide B [65]. Ethanolic extract was partitioned between water and petroleum ether and then ethyl acetate. The latter was analysed by silica gel column chromatography.

Chinese phytomedicinal formulation Sanqi Zongdai Pian, traditionally prepared from the crude extracts from roots of *Panax notoginseng* was analysed by high-speed CCC for level of highly polar dammarane saponins with two-phase system from hexane–1-butanol–water (3:4:7, v/v/v) [66]. Comprehensive analysis of ginsenosides in *P. notoginseng* suspension cultures was developed with HPLC using Merck Superspher RP-18 end-capped column with UV detection [67,68].

TCM gegenqinlian decoction was analysed for quantities of quaternary alkaloids of *Coptis chinensis* by high performance CE in 60% sodium phosphate (60 mmol/L)–40% methanol with 2-(4-hydroxyphenyl)ethylammonium chloride as internal standard with detection at 254 nm to reveal concentrations of berberine, jatrorrhizine, and palmatine with high recovery of 97–99% [69].

3. Quantitative analysis of herbs

Comprehensive two-dimensional (2D) GC–MS with time-of-flight (TOF) analyser was successively introduced into analysis of *Pogostemon cablin* Benth (Cablin Patchouli) volatile oil [70]. The first, a polyethylene coated capillary column SOLGELWAX was connected with the second chiral column, Cyclodex-B, and both columns were placed into a same oven. For comparison, GC–MS using the same SOLGELWAX column was also investigated. Under the same operation conditions, GC chromatograms displayed only 79 peaks, while about 800 peaks were resolved with GC × GC (Fig. 2). Moreover, 394 compounds with the similarity over 800 were identified. Based on the zone volume normalization it was found that the contents of pogostone, patchoulol, α-patchoulene and β-patchoulene were 24,28,6 and 2%, respectively.

Eight phenylpropanoids were quantified in *Rhodiola rosea* L. extracts by a LC–DAD method [71]. The method utilized Xterra RP 18 column eluted with 10 mM ammonium acetate/acetonitrile gradient and detection was performed at 276 and 254 nm. Good linearity was obtained over the range 0.2–50 μg/mL for salidroside and 0.2–100 μg/mL for rosavin. In the next paper, a MS detection was introduced [72]. Ionisation process was optimised with ESI or APCI; best results were obtained with ESI when using pure water without any additives as the mobile phase. If SIM mode was performed ten analytes were determined in one run. Calibration curves were linear over the concentration range 0.5–500 ng/mL for salidroside and 2–2000 ng/mL for rosavin.

Chinese medical herbs, including *Raphanus sattvus* L., *Brassica alba* L. Boiss., *Lepidium apetalum willd.*, *Rorippa* sp., *Polygonum tinctorium* Fort., *Isatis indigotica* Fort. and *I. tinctoria* L. and seeds of commonly consumed vegetables were screened for total isothiocyanates content by a novel GC–MS method [73]. Drugs were extracted with hot water; extract was mixed with myrosinase solution and incubated for 2 h. A solution of 1,2-benzenedithiol was added and corresponding 1,3-benzodithiole-2-thione were extracted with chloroform. The GC–MS analysis was performed on a capillary HP-5MS phenyl methyl silicone column with helium as a carrier gas and column temperature was 50 °C for 3 min increasing to 270 °C in rate of 10 °C/min. Diphenylamine was used as an internal standard. The linear dynamic range covered from 0.12 to 200 μM and LOD was found to be 35 nM. The total content of isothiocyanates varied considerably for different herbs; *Raphanus sativus* L. was their richest source.

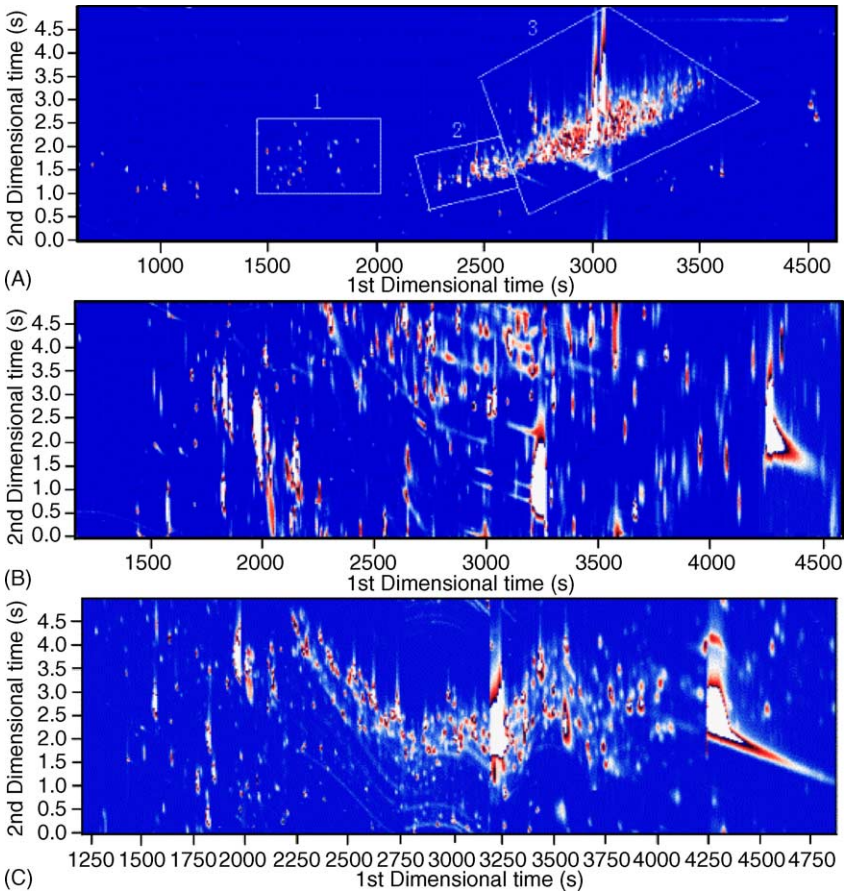

Fig. 2. The GC × GC/MS–TOF contour plots of *Pogostemon cabltn Berth* volatile oil on different column systems [70]. (A) Column set 1; (B) column set 2; (C) column set 3. Zones 1–3 in (A) are mainly oxygenated monoterpenes, sesquiterpenes and oxygenated sesquiterpenes and pogostone, respectively.

The 2D data obtained from GC–MS were used qualitatively and quantitatively to determine the components of the volatile fractions of *Schisandra chinensis* [74]. Analyses were carried out on an OV-1 capillary column held initially at 40 °C and then heated to 230 °C at a rate of 5 °C/min. Helium was employed as carrier gas at a constant flow rate of 0.7 mL/min. Mass spectra were recorded at 70 eV ionisation energy. Plant material was extracted by six extraction techniques, which were compared from the point of yields, a number of resolved peaks and determined compounds. It was demonstrated that different extraction methods gave rise to volatile fractions, which differ substantially. Data were processed by sub-window factor analysis and other chemometric techniques that seemed to be particularly valuable with respect to the analysis of complex samples, such as TMC preparations. Altogether, more than 80 compounds were identified.

Extracts of *Radix Angelica Sinensis* were analysed with immobilized liposome chromatography (ILC) to show more than 10 components [75]. Two of them, ligustilide and ferulic acid, were identified from their MS spectrum and with standard samples. A buffer, containing 10 mM sodium phosphate (pH 7.0) and 20 mM sodium chloride was used as mobile phase (Fig. 3). Individual fractions from ILC were further analysed with HPLC on an ODS column coupled with UV and MS detector employing methanol–water (50:50, v/v) as a mobile phase. The method might be applied to predict the penetration ability of multiple compounds in TCM simultaneously.

Liquiritigenin and isoliquiritigenin from *Glycyrrhiza uralensis*, liquorice tablets and liquorice complex were determinated by CE with electrochemical detection. A fused-silica capillary was used for the separation and a wall jet configured 300 μm carbon disk electrode at +1000 mV was employed for analysis [76]. Samples were extracted with methanol in an ultrasonic bath. The appropriate running buffer consisted of 50 mM sodium borate (pH 8.7) and baseline separation was achieved within 8 min. Recoveries and the linear calibration curves indicated that this method is sufficiently accurate, sensitive and reproducible for determination of both analytes.

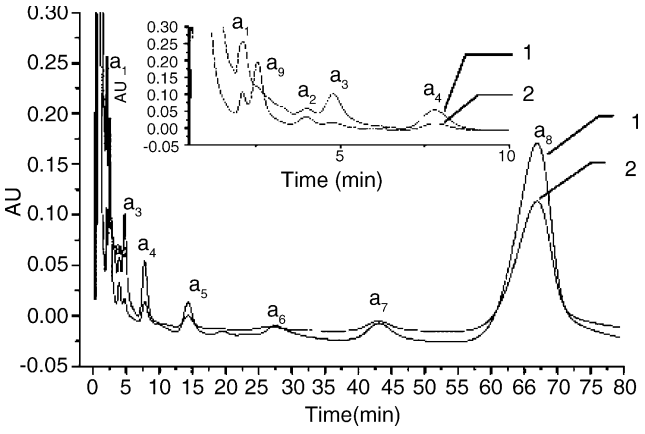

Fig. 3. ILC chromatogram of *Radix A. Sinensis* extract [75]. Detection wavelength 210 nm (1) and 280 nm (2), the inset shows an expansion of the first 10 min of separation.

Curcumin was determined in *Curcuma longa*, *C. wenyujin* and *C. kwangsiensis* by a simple HPLC method on Supelcosil LC_{18} column with methanol–2-propanol–0.5% acetic acid (19:25:56, v/v/v) as mobile phase at a flow rate of 0.6 mL/min [77]. The calibration curve was linear ($R = 0.9995$) over the concentration range 14–56 μg and the average recovery was 99.82%. More efficient CZE method was elaborated for the determination of curcumin, demethoxycurcumin and bisdemethoxycurcumin in *Curcuma domestica* Val. and *C. xanthorrhiza* [78]. The three major curcuminoids were fully separated in less than 5 min using standard fused-silica capillary and DAD. An electrolyte solution of 20 mM phosphate, 50 mM sodium hydroxide and 14 mM β-cyclodextrin was found to be appropriate. LOD was 0.01 mg/mL and 3,4-dimethoxy-*trans*-cinnaniic acid was employed as internal standard. The procedure was able to distinguish between individual *Curcuma* species. Alternatively, curcumin in turmeric was established by CE with amperometric detection (CE–AD) after solid phase extraction (SPE) based on the tributyl phosphate resin. CE–AD conditions were optimised to 15 mM phosphate buffer at pH 9.7 and 16 kV separation voltage [79].

Radicis Cortex Moutan, the dried root bark of *Paeonia moutan* Sims. was analysed for a series of water-soluble tannins [80]. Samples were extracted with 70% methanol at room temperature followed by centrifugation. An HPLC–UV system utilised a Cosmosil $5C_{18}$-MS column eluted at a flow rate of 1 mL/min with a linear solvent gradient of A (20 mM potassium phosphate adjusted to pH 4 with 10% phosphoric acid) and B (methanol–water, 7:3, v/v). In the same paper, a CE–UV analysis was investigated using fused-silica capillary and the electrolyte consisting of 20 mM SDS and 25 mM sodium borate (adjusted to pH 5.5 with phosphoric acid)–2-propanol (93:7, v/v). The LOD of each of eight marker substances varied from 0.04 to 0.93 μg/mL (HPLC) and 0.02 to 0.36 μg/mL (CE). Good linearity was found ($R > 0.999$) for both methods along the six points of concentration within the range 15–336 μg/mL. The recoveries of all constituents determined by either method were around 96–103%.

Analysis of *Rhodiola sachalinensis* by high-speed CCC in two-phase system consisted of 1-butanol–ethyl acetate–water (2:3:5, v/v/v) yielded quantitative evaluation of content of pure salidroside [81].

Flower buds of *Lonicera japonica*, *L. dasystyla*, *L. hypoglauca* and *L. confusa* (*Flos Lonicerae*) were analysed on the content of iridoid glucosides by HPLC with evaporative light scattering detector (ELSD) on a C_{18} column in methanol–water (3:7, v/v), containing 0.5% of acetic acid. Five major iridoid glucosides, 7-*epi*-loganin, sweroside, loganin, 7-*epi*-vogeloside, and secoxyloganin were analysed simultaneously with very good quantification [82]. Samples of *Flos Lonicerae* were also analysed with high-speed CCC developed for the separation of chlorogenic acid in butanol–acetic acid–water (4:1:5, v/v/v) two-phase solvent

system (upper phase used as a mobile phase). The 342 ml column was able to separate 300 mg sample of the crude extract with 90% recovery [83].

Chloroform extracts of the TCM *Semen Cuscutae* were analysed by GC and GC–MS techniques [84]. A fused-silica capillary column HP-5 was used for the separation; nitrogen was a carrier gas and oven temperature was initially held at 200 °C for 2 min, then programmed to 300 °C at 10 °C/min, and finally, maintained at 300 °C for 10 min. For GC–MS, a capillary column RTX-5M5 and helium were employed. Compounds were identified by mass spectra and also by programmed-temperature retention indices. *Semen Cuscutae* showed a high content of odd-carbon number *n*-alkanes and, moreover, bioactive (+)-sesamin was identified there for the first time. For its quantification, an external standard calibration was established in the concentration ranging from 0.05 to 1.1 mg/mL and a linear regression equation with $R = 0.99924$ was obtained. Recovery of (+)-sesamin was about 96% and LOD was evaluated to be 10 μg/mL. Characterization of several species of *Cuscuta* exemplified the applicability of this method for fingerprinting. *Cuscuta chinensis* Lam., *C. australis* R. Br. and *C. japonica* Choisy are source of *Semen Cuscutae*, therefore, 40 samples of their ripe seeds or stem were collected from different areas and analysed by HPLC [85]. Five principal flavanoids, quercetin 3-*O*-β-D-galactoside-7-*O*-β-D-glucoside, quercetin 3-*O*-β-D-apiofuranosyl-(1 → 2)-β-D-galactoside, hyperoside, quercetin and kaempferol were separated simultaneously on a Hypersil-ODS column eluted by solvent A (0.025 M phosphoric acid) and B (100% methanol). The initial mixture was 82% A and 18% B (v/v) for 3 min; linearly gradient to 55% A in 11 min and then held for 3 min. The analysis was monitored at 360 nm. The recovery of the method was 97.0–102.9% and all flavanoids showed good linearity ($R > 0.999$) in a wide concentration range (0.6–96 μg/mL). LODs ranged from 72 to 103 ng/mL. The results indicated that contents of flavanoids varied significantly from species to species, therefore, authors concluded that the HPLC method developed represented an excellent technique for quality control of this vegetable drug.

Marker substances of *Atractylodis rhizoma* were analysed by a MEKC method. Atractylenolide III was clearly separated from other components and the analysis was performed according to Japanese Pharmacopeia [86].

Bioactive triterpenes were analysed in *Perilla frutescens* (L.) Britt. HPLC on a Sperisorb ODS column with acetonitrile and aqueous phosphoric acid as the mobile phase and detection at 206 nm. The method was precise with R.S.D.s that ranged between 0.6–1.5% (intra-day) and 0.7–2.6% (inter-day) and revealed the presence of tormentie, oleanoic and ursolic acids [87].

Quantitative analysis of volatile constituents of *Notoptergium incium* was performed with GC–MS using 2D data resolved by correlative chemometric methods; 65 of the 98 constituents in essential oil, accounting for 92.13% of the total content, were identified [88]. The samples were prepared by hydro-distillation and then analysed on OV-101 capillary column with helium as carrier gas. The column temperature was maintained at 50 °C for 6 min and then programmed at 8 °C/min to 250 °C. The method could be an example how the modern way enables analysis of complex mixtures if the overlapping peaks are resolved with evolving window orthogonal projection. The analogous methodology was applied to the analysis of the essential oil isolated from *Artemisia capillaris* herba allowing the identification of 42 compounds that represented 89.03% of the total content (Fig. 4) [89].

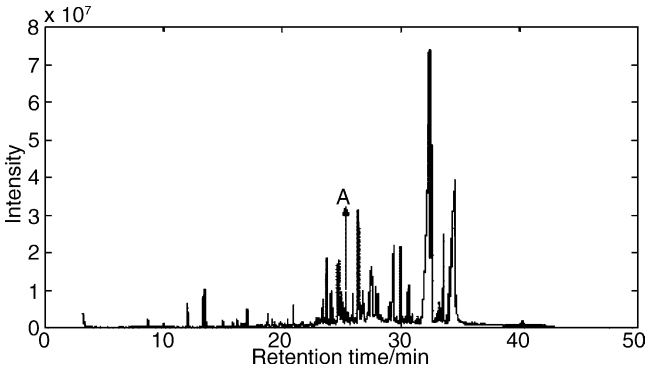

Fig. 4. The GC–MS chromatogram of volatiles isolated from *A. capillaris* [89].

Non-volatile terpenes from *Cimicifuga foetida* L. were analysed with RP-HPLC with ELSD in comparison with UV. As the best matrix for extraction of terpenes from the raw material was selected methanol–water (8:2, v/v). Actein, 27-deoxyactein and cimicifugoside glycosides were found and their analyses made quantitative with good calibration curves [90]. Rhizome of this drug (Shengma) was also analysed by chromatographic separation on silica gel and Sephadex LH-20 revealing cimicifugic acid, esculetin, caffeic acid methyl ester, 4-*O*-acetylcaffeic acid, sinapic, caffeic and ferulic acids [91].

Active ingredients of *Rhododendron dauricum* L. were analysed by CE with electrochemical detection. Analysis on a wall jet configured 300 μm carbon disk electrode with good response at +950 mV during 16 min in borax buffer (pH 8.7) with excellent linearity showed farrerol, quercetin, syringic, vanilic, 4-hydroxybenzoic, and protocatechic acids as major active ingredients [92].

Honokinol and magnolol were isolated from cortex of *Magnolia officinalis* Rehd. et Wils. (Houpu) by high-speed CCC in a two-phase system hexane–ethyl acetate–methanol–water (10:4:10:4, v/v/v/v) and the fractions were analysed with HPLC with high efficiency and reasonable time [93].

Compounds from *Rhizoma chuanxiong* (roots of *Ligusticum chuanxiong* Hort.) were separated and identified by 2D LC coupled with DAD that was directly connected to APCI–MS [94]. As the separation system Kromasil-CN and Kromasil-ODS columns with methanol–water and acetonitrile–acetic acid buffer, respectively, were used. Totally, more than 52 components in the methanol extract were

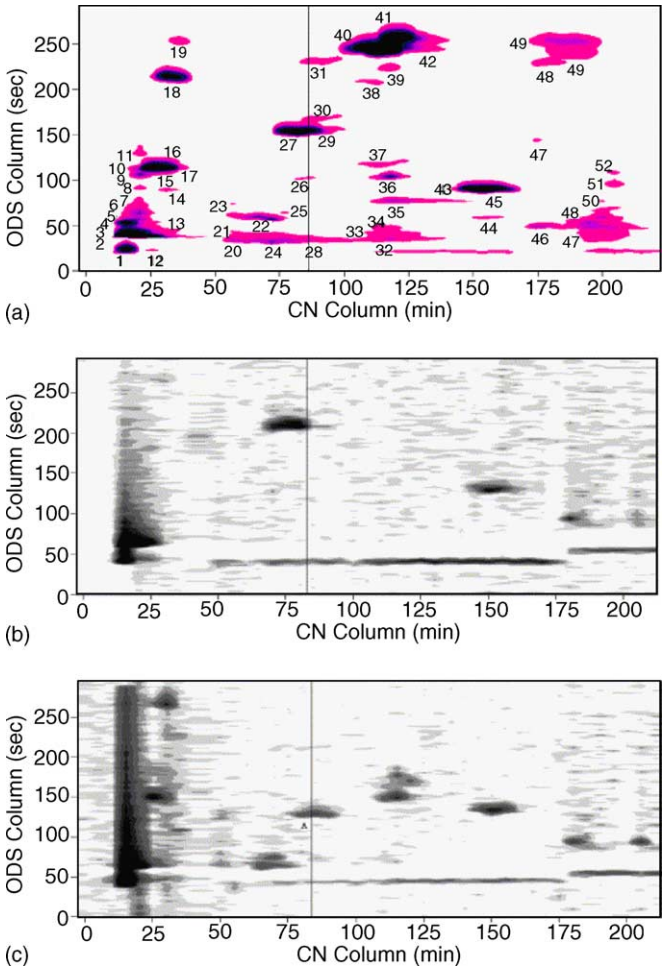

Fig. 5. 2D chromatograms of 52 components of *Rhizoma ehuanxiong* [94]. The heights of the 2D pot determined by: (a) the relative UV absorbance; (b) the counts per second using APCI positive ion mode; (c) the counts per second using APCI negative ion mode.

separated in less than 215 min (Fig. 5) and 11 of them were identified.

CZE method was developed for simultaneous determination of eukovoskle, cinnamic acid and ferulic acid in the flower and stem of *Euphrasia regelii* [95]. Extraction was carried out with methanol in an ultrasonic bath. Fused-silica capillary was eluted with 20 mM sodium borate, containing 10% (v/v) methanol (pH 8.5) and UV detection was employed at a wavelength of 254 nm. The recoveries were in the range 95.5–104.6% and LODs were 1.78, 0.80 and 0.33 mg/L, respectively. All analytes were separated within 10 min.

The simultaneous determination of 20-hydroxy ecdysone, 3,7-dimethoxy-quercetin, acteoside and rutin in the mixture of leaf and stem, and flower of *Lamium maculatum* was investigated by CZE [96]. With an electrolyte, containing 30 mM sodium borate at pH 9.47 and 20 kV applied voltage, the four active compounds were completely separated within 5 min. Regression equation revealed linear relationship ($R > 0.9998$) between the peak area and the concentration; recoveries ranged from 98.3 to 105.0%.

Diterpenoid dimer annonebinide A was determined and isolated from Chinese Pond apple, *Annona glabra* L. air dried stems. Ethyl acetate extract of the drug was chromatographed on a silica gel column with good efficiency [97].

Oleanolic acid 28-*O*-β-D-glucopyranosyl-3-*O*-α-L-arabinopyranosyl-(1 → 3)-(6′-butyl ester)-β-D-glucuropyranoside and 28-*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranoside were determined and isolated from the roots of *Hemsleya penxianensis* var. *gulinensis*. The butanol extract was chromatographed on a D-101 silica gel column with ethanol–water (50%) and chloroform–methanol–water (8:3:0.3, v/v/v) [98].

Morphinane mecoquitupline was determined in an ethanolic extract of the air-dried and ground whole plants of traditional Tibetian medicine *Meconopsis quintuplinervia* Regel Meconopsis by column chromatography on macroporous adsorbent resin, normal phase and reverse phase silica gels and Sephadex LH-20, followed by RP-HPLC [99].

Ethanol extract of the aerial parts of Tibetan herb *Clematis tangutica* (Maxim.) Korsh. was analysed by the antifungal assay-guided fractionation to reveal triterpene saponins with oleanolic acid as aglycone [100].

Bromophenols composition of ethanolic extract of air-dried Qingdao red alga *Rhodomela confervoides* was studied [101]. At first, extract was partitioned between ethyl acetate and water and organic phase was then subjected to column chromatography over silica gel eluting with a gradient increasing methanol (0–100%) in chloroform. The fraction eluted by 30% methanol was separated again by size-exclusion chromatography over Bio-Beads SX-3 with chloroform-ethyl acetate (1:1, v/v) [102], or alternatively, the fraction eluted by 10% methanol was purified by size-exclusion chromatography over Bio-Beads SX-3 with chloroform-ethyl acetate (1:1, v/v) and analysed by RP-HPLC.

High-speed CCC was used for determination of rhein in samples *of Rheum officinale* Baill (Dahuang) with a two-phase solvent system composed of hexane–ethyl acetate–methanol–water (3:7:5:5, v/v/v/v) [103]. The same technique was used in shikonin quantitative analysis of crude extract of *Lithospermum erythrorhizori* Sieb. et Zucc. with hexane–ethyl acetate–ethanol–water (16:14:14:5, v/v/v/v) giving 96.9% recovery [104].

Quantitative analysis of six non-glycosidic iridoids from *Cymbaria mongolica* was developed using the crude extract combined column chromatography Sephadex LH-20 and silica gel [105]. Similar combined stepwise column chromatography gave quantitative content of six taraxastanes from *Saussurea petrovii* [106].

High-speed CCC was used to determine quantitative amounts of osthol and xanthotoxol in Common Cnidium Fruit, *Cnidium monnieri* (L.) Cusson in hexane–ethyl acetate–methanol–water (1:1:1:1 and 5:5:6:4, v/v/v/v, respectively) [107].

An HPLC–DAD method was developed for the simultaneous determination of noradrenaline and dopamine in dif-

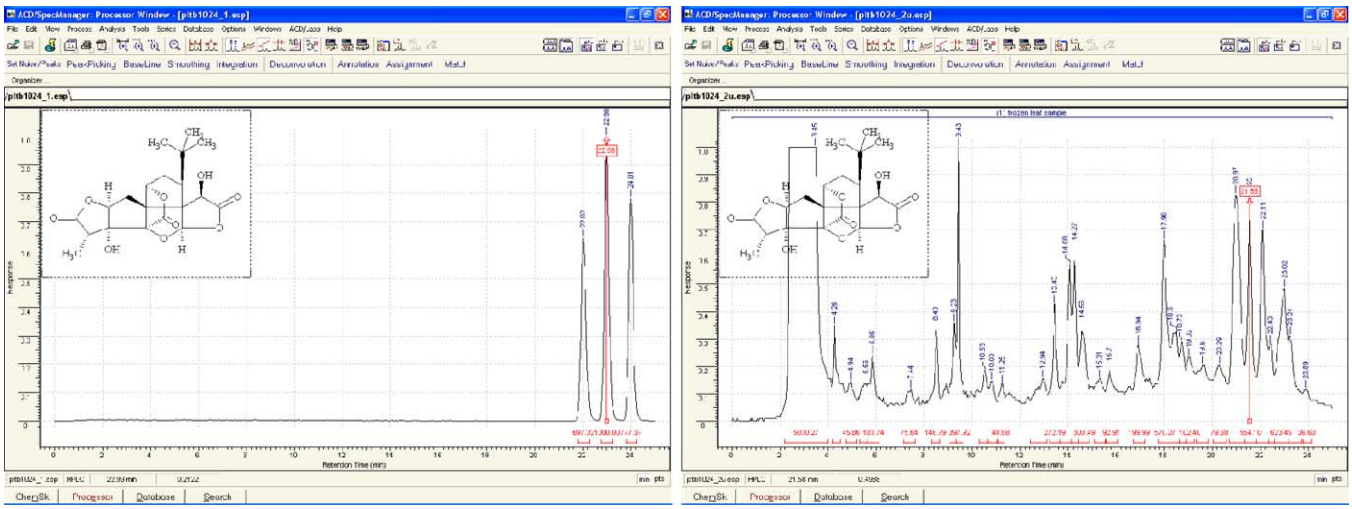

Fig. 6. ACD/Labs Chromatography Database in processor window shows active structural assignment of peaks in real run as well as in mixed standards analysis.

ferent parts of *Portulaca oleracea* L. [108]. The separation was achieved within 10 min on a Kromasil C_{18} column by a mobile phase, consisting of 70% (v/v) methanol and 0.02 M potassium dihydrogen phosphate solution, which contained 30 mM SDS and was adjusted to pH 3.0 with phosphoric acid. Good linear relationship ($R > 0.999$) was shown in the range of 0.004–6.00 μg and 0.011–8.25 μg and the LODs were 0.40 ng and 0.55 ng, respectively.

Chloroform soluble material from ethanol extract of *Poria cocos* (Fuling) was analysed on content of dehydrotumulosic acid by HPLC on a C_{18} column with methanol–acetonitrile–2% glacial acetic acid with detection at 242 nm and testosterone propionate as internal standard with high reproducibility [109].

Ginger, the rhizome of *Zingiber officinale* Roscoe gave 7 diarylheptanoids on quantitative analysis of alcohol extract by column chromatography on silica gel with a gradient of petroleum ether–acetone (from 20:1 to 0:100) [110].

Well developed separation of nutraceuticals (Fig. 6) from *Ginkgo biloba* leaf extracts and a commercial phytopharmaceutical preparation (Natures Aid) was achieved by RP-HPLC with ELSD on a PLRP-S 100 Å column and showed bilobalide, ginkgolides A and B, using acetonitrile tetrahydrofurane and water ternary gradient [111].

4. Fingerprint in quality control

The identification of crude herbs based on geographical origins is crucial in order to ensure authenticity, quality, safety and efficacy of the raw material before it is converted to the final product. Among all the quality control systems, fingerprint has gained more and more attention due to its ability to identify a particular herb and, moreover, to distinguish it from closely related species. Chromatographic fingerprints sometimes exhibit variations in peak height and retention time of a given sample running through identical columns under the same separation conditions. Because of this, appropriate consideration must be given to proper normalization of chromatographic fingerprints.

An HPLC fingerprint method was described for the fast and simple identification of *Isatis indigotica* Fort, in the TCM preparations [112]. Method employed C_{18} reversed phase column with water–methanol (96:4, v/v) as the mobile phase at a flow rate of 1.0 mL/min and UV detection at 260 nm. A combination of HPLC and ELSD was established for the fingerprinting of the TMC, QingKaiLing injection [113].

In order to create an electrophoretic profile of genuine *Flos Carthami*, a standardized procedure was used to develop the CE fingerprint [114]. Three active compounds of *Flos Carthami* with available reference standards, adenosine, rutin and quercetin were used as marker compounds and rifampicin was selected as internal standard; 50 mM sodium tetraborate (pH 9.7) with 18% methanol was chosen as the running buffer, a voltage of 24 kV was applied and the UV detection was at 210 nm. The intra- and inter-day precisions were below 0.8 and 1.8%, respectively. Under these conditions, almost baseline separation of 29 peaks was observed (Fig. 7). Furthermore, raw herb samples from nine sources in China were investigated giving fingerprints exhibited a fair consistency in the constituents and their contents. In contrast, *Flos Carthami* was well distinguished from *Stigma Croci*, a possible substitute in TCM, and *Flos Hemerocallis*, a commercial adulterant, by comparing the fingerprints of each herb. Sample preparation is a key step in fingerprint analysis, so three methods were evaluated: extraction with ethanol–water solution (3:1, v/v) in an ultrasonic bath or in a Soxlet apparatus, and extraction with boiled water. Recoveries of over 95% for marker compounds were achieved in alcohol–water system and ultrasonic extraction was selected due to its simplicity.

High-speed CCC was applied to develop fingerprint for the first time only this year [115]. Preparative separation was performed on a three coils connected in

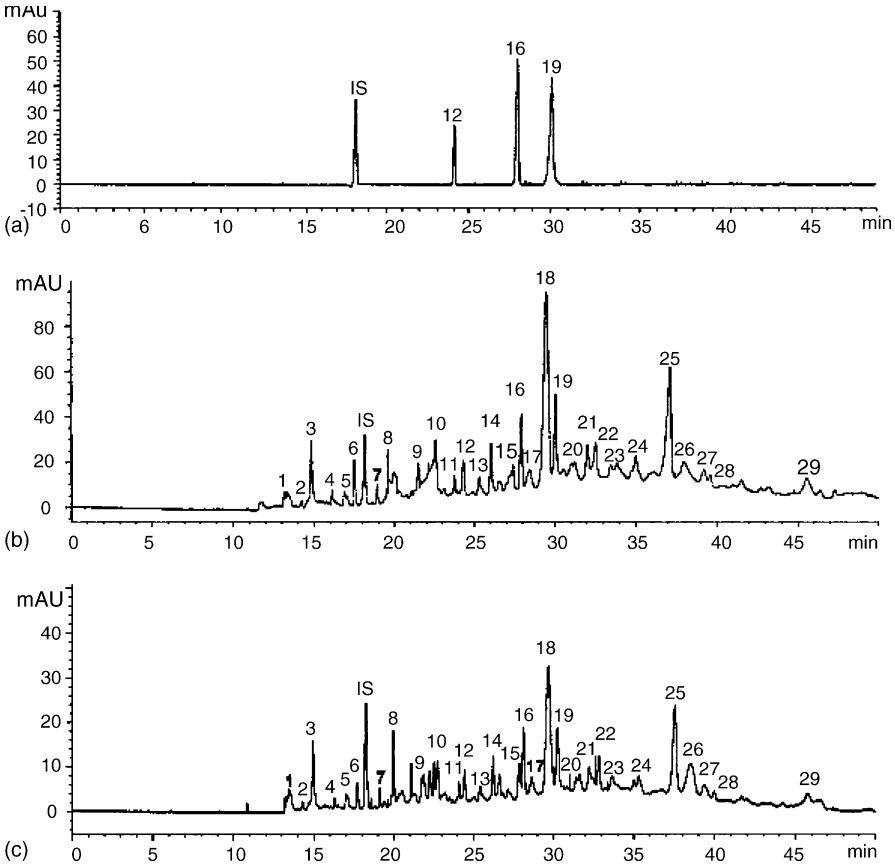

Fig. 7. Electropherogram of (a) standard substances, (b) fingerprinting of *Flos Carthami* from Fengqiu, (c) and from Sichuan [114]. Peaks represent 12 adenosine, 16 rutin, 19 quercetin, I.S. rifampicin.

series (total volume 300 mL) using a stepwise elution with both upper and lower phase of solvent system A (hexane–ethanol–water, 10:5.5:4.5, v/v/v) and the lower phase of system B (hexane–ethanol–water, 10:7:3, v/v/v). Altogether, 12 distinct peak fractions were eluted from the crude extract (100 mg) of *Salvia miltiorrhiza* Bunge within 13 h, whereas, analytical HPLC identified 11 peaks in 45 min. Each fraction was analysed by gradient HPLC on a Ultrasphere C_{18} column with 0.1% trifluoroacetic acid diluted with either water or acetonitrile as the mobile phase. The content of corresponding fractions isolated from three samples of *S. miltiorrhiza* of different origin varied greatly according to the HPLC chromatograms and absorption spectra, therefore it was concluded that location and climate had great impact on the quality of this TCM herb. Retention times of all 12 peaks were reproducible giving relative standard deviation less than 3% which was a little bit higher than that of HPLC (0.66%). Nevertheless, authors summarized that CCC was proven to be feasible as a new method for fingerprinting especially if semi-preparative CCC would be replaced by analytical one with 50 mL capacity. In this case, precision could be increased and run time decreased. The identity of individual components in fractions obtained by the CCC separation of a crude extract from *S. miltiorrhiza* was further studied by LC–MS [116]. Cryptotanshinone, tanshinone I and tanshinone HA were the principal compounds in fractions 7, 8 and 11, respectively. High-speed CCC with hexane–ethanol–water (10:5.5:4.5 and 10:7:3) was used for analytical determination of six diterpenoids from *S. miltiorrhiza*, namely dihydrotanshinone I, cryptotanshinone, methylenetanshiquinone, tansinone I, tanshinone HA and danshenxikun B [117]. With hexane–ethyl acetate–ethanol–water (3:7:1:9, v/v/v/v) the method showed quantification of salvianoic acid B content [118]. High-speed CCC coupled with ESI–MS/MS was also proved suitable to purify and detect tanshinone IIA separated from its ethyl ester in crude extract of *S. miltiorrhiza* [119].

The method of spectral correlative chromatography (SCC) was proposed as a mean of solving the problems arising from chromatographic shortcomings [120]. In conjunction with a local least squares procedure, it was applied to the HPLC analysis of *Ginkgo biloba* methanol extract and *Astragali seu* Hedysari ether extract. 2D data sets for *G. biloba* were obtained by LC–UV on two C_{18} columns (Spherisorb ODS2 C_{18} of two suppliers) at wavelength 260 nm, while *A. seu* Hedysari was analysed using another HPLC instrument and UV spectra were recorded. These results indicated that it is feasible for correlative spectrum-oriented local lest squares analysis to correct the shift of retention time of pure peaks from diversities and to help reveal chemical natures of various chromatographic fingerprints.

A chromatographic response function (CRF) was also introduced to compare quantitatively HPLC fingerprint chromatograms obtained under different experimental conditions [121]. The chromatographic separation of several drugs, components of TCM preparation Langdu, was carried out with a Hypersil BDS column. Ebracteolata compound b, jolkinolide B and neochamaejasmin were recognized as the characteristic marker compounds for *Euphorbia ebracteolata* Hayata, *E. fischeriana* Steud and *Stellera chamaejasme* L., respectively. A new CRF named as the fingerprint index was proposed to identify the optimum experimental conditions for the establishment of characteristic fingerprint chromatogram. The mobile phase was methanol–water with composition changing from 40:60 to 100:0 in 50 min and was kept at the final composition for 20 min. The detection wavelength was set at 230 nm.

The acetone extracts of 24 species of *Ephedra*, including *E. sinica*, known as Ma Huang, were analysed on a Waters XTerra RP_{18} column with the mobile phase consisted of water (A) and acetonitrile (B) [122]. A gradient elution (10 min, 75% A, 25% B; 100% B over 45 min and then hold 10 min) produced a fingerprint chromatogram, containing the series of characteristic peaks between 52 and 64 min. Separation was scanned at 320 nm. This fingerprinting allows distinguishing between *Ephedra* species from Euroasia, North America and South America. More importantly, the series of peaks were not present in the ephedrine alkaloid spiked with *Ginkgo biloba* or *Ginseng* extracts, suggesting that the fingerprint is capable of determining if ground plant material is from an *Ephedra* species.

The analytical power of GC × GC was demonstrated for the differentiation of ginseng by obtaining fingerprints of the volatile oils present in three ginseng species [123]. Essential oils were isolated using conventional solvent extraction procedures. In the next paper, the coupling of headspace SPE with comprehensive 2D GC equipped with flame ionisation detector was shown to be more powerful technique for the rapid sampling and analysis of volatile oils in complex herbal materials [124]. Volatiles were extracted by exposing polydimethylsiloxane fiber to the headspace of a sample vial that was maintained at 70 °C and agitated for 30 min. GC × GC analyses were performed on a HP-5 capillary column coupled to a BP20 capillary column with nitrogen as a carrier gas. The columns were heated from 80 to 120 °C at a rate 10 °C/min and then increased to 170 °C at 2 °C/min, followed by a ramp to 200 °C at 10 °C/min and then held for 2 min. More than 20 marker compounds belonging to *Panax quinquefolius* could be observed within the 2D contour plots of ginseng itself as well as mixtures that contained ginseng and three another important herbs.

Tianmendong, dried roots of *Asparagus cochinchinensis* (Lour.) Merr., which is sometimes commercially substituted by *A. filicinus*, *A. meioclados*, or *A. spinasissimus*, was studied on indicative compounds and fingerprinting, which revealed two new furostanol glycosides aspacochiosides A, B and C. The ethanolic extract of the air-dried and ground roots of *A. cochinchinensis* was subjected to column chromatography on macroporous adsorbent resin, normal phase and reverse phase silica gels and Sephadex LH-20 successively to afford characteristic aspacochioside C [125].

Chinese traditional remedy Shiwei from *Pyrrosia* species *P. lingua*, *P. sheareri* and *P. petiolosa*, was subjected a study to control its quality by HPLC fingerprint techniques. From the 95% ethanolic extract, a characteristic flavone diglycoside pyrropetioside A was isolated by chromatography on macro porous resin, normal phase silica gel, and Sephadex LH-20 successively [126].

Shuanghuang Shaofutie plaster made from Chuanxiong and Danshen was analysed by TLC using silica gel plates G with benzene–hexane (4:1, v/v) as a fingerprinting tool for quality of the preparation [127].

It is noteworthy, e.g. Chinese thin-plate Ch'an Su, preparation close to *Venenum Bufonis* could be analysed for fingerprinting by the specific TLC colour reactions with sulfuric acid during heating of the solvent developed TLC plate [29].

5. Safety assurance tools

Some herbs are known to be toxic and, when appropriate, need to be used with care under supervision of highly qualified practitioners [128]. Labelling of herbal products may not accurately reflect their content and adverse events or interactions attributed to specific herbs may actually be due to misidentified plants, pharmaceutical drugs or heavy metals. It is therefore, important to determine the presence of toxic components and adulterants in herbal medicine to ensure safety of the patients. More than 48 cases of renal poisoning attributed to Fang-Ji (*Stephania tetrandra*) in a weight-loss preparation were actually caused by Guang-Fang-Ji (*Aristolocholia fangchi*); aristolochic acid is known to be nephrotoxic and carcinogenic [129]. The confusion in this case seems to have arisen from the similarity of the names in Chinese. Aristolochic acids are present in plants of *Aristolochia*, *Bragantia* or *Asarum* species and are the most monitored toxic adulterants so far.

Two HPLC methods employing a Cosmosil $5C_{18}$-MA column, one combined with DAD and another with MS, were developed for the analysis of aristolochic acids I and II in herbal medicines [130]. In the LC–UV mode, the separation was performed with linear gradient elution (eluent A: 30 mM ammonium dihydrogenphosphate, eluent B: acetonitrile) according to the following profile: 0 min, 80% A, 20% B; 25 min, 40% A, 60% B; 30 min, 80% A, 20% B, then maintained for 5 min. The linear gradient (eluent A: 20 mM ammonium acetate, eluent B: acetonitrile) was also applied in the LC–MS mode (0 min, 90% A, 10% B; 25 min, 40% A, 60% B; 30 min, 90% A, 10% B). The flow rate was always set at 1.0 mL/min. MS detection was performed in an electrospray positive ion mode. Calibration graphs (LC–UV) were constructed within the concentration range: 0.99–79.91 μg/mL for aristolochic acid I, and 0.37–7.42 μg/mL for aristolochic

acid II. The correlation coefficients of regression equations gave a strong evidence of linearity. The LODs for aristolochic acids I and II were 0.20 and 0.19 μg/mL, respectively. Both analytes were extracted from herbal materials with hexane and subsequently with methanol, while sonicated. Because, the peaks of aristolochic acid I or II usually overlap with nearby peaks of similar molecular weight of fragment ions, additional SPE should be included before LC–MS analysis. The presence of aristolochic acids I or II was proved in all five TCM preparations investigated (*Aristolochia fanghi*, *A. manshuriensis*, fruit of *A. debilis*, stem of *A. debilis*, root of *A. debilis*) where their total content was 0.352, 0.368, 0.972, 0.772 and 0.596, respectively. It is noteworthy that even eight herbal slimming formulations of 18 tested contained aristolochic acids. Another HPLC study showed that *A. fanghi* and *A. fructus* contained 3.9 and 2.3 mg/g aristolochic acid [131].

Isocratic separation on ODS Hypersil C_{18} column with methanol–water (60:40, v/v) allowed a rapid determination of both aristolochic acids within 5 min [132]. The LODs were 7.3 and 4.2 ng and recoveries 107.6 and 114.2% for aristolochic acids I and II, respectively. Furthermore, correlation coefficients were >0.99. In this work, 10 kinds of *A. fanghi* and 21 kinds of *Caulis Aristolochiae Manshuriensis* from different cultivation regions in China were analysed but only *A. fanghi* collected in the Guangdong province of China had the concentration of aristolochic acids below the LOD.

A C-18 symmetry column was also efficient for the separation of aristolochic acid I in methanol extracts of slimming preparations based on TCM herbs [133,134]. Two analytical methods, LC–UV and LC–MS/APCI, with linear gradient conditions (60:40–100:0 in 21 min) were used (eluent A: methanol, 0.5% acetic acid; eluent B: 0.5% acetic acid). To performed quantitative analysis, crude extracts were further purified by SPE. Out of 42 analysed preparations, four were found to contain aristolochic acid I. For the preliminary TLC screening, the samples were applied to a silica gel 60 F_{254} plate and eluted with chloroform–methanol–acetic acid (65:20:2, v/v/v). Visualization was made by spraying with a solution of diphenylamine in sulfuric acid and subsequent heating.

A facile HPLC method for simultaneous resolution and quantification of aristolochic acids, aristolactams and their analogues, altogether 17 compounds, under gradient conditions was developed [135]. A C_{18} column was eluted with a solvent mixture of sodium acetate (eluent A, pH 5) and acetonitrile (eluent B) and retention times were found to be strongly dependent on pH. At higher pH, the elution reversal between aristolochic acid and aristolactam was detected. In the next paper, the method was adopted to fulfil criteria for fingerprinting [136]. The same solvents as mentioned above were employed for a gradient elution (B: 5 min, 20%; 33 min, 44%; 43 min, 50%; 53 min, 68%; 59 min, 80%). Under the optimized conditions, chromatographic profiles of methanol extract from crude drug, such as *A. fangi*, *A. manshuriensis*,

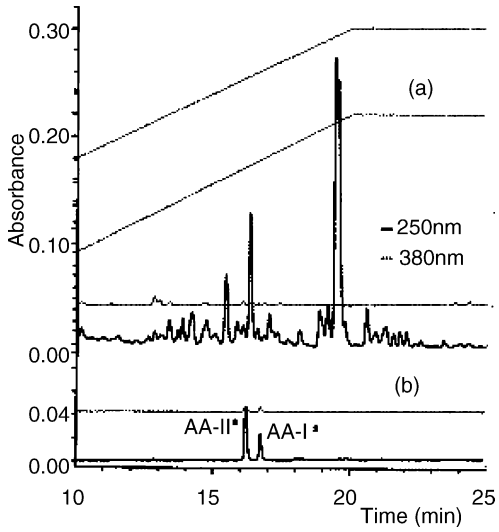

Fig. 8. LC–UV chromatograms of (a) an extract of *Asarum sieboldii* (Xixin sample) and (b) a standard solution of aristolochic acid I (500 ppb) and II (778 ppb).

Stephania tetrandra and *Clematis armandii* were measured allowing easy identification of each of these homonymic Chinese drugs. Furthermore, the aristolochic acids and aristolactams composition of seven species of *Aristolochia* was investigated.

Methanol extracts of nine *Asarum* species (a TCM preparation Xixin) were analysed by both LC–UV (Fig. 8) and LC–MS with APCI [137]. For separation, a Merck Lichrocart Purospher RP-18e column was used with a flow rate of 1.0 mL/min. A linear gradient elution (0 min, 100% A; 20 min 100% B, then kept for 10 min) was established with eluent A (20% acetonitrile, v/v, containing 0.005% trifluoroacetic acid, pH 3.42) and B (acetonitrile) for LC–UV, whereas, the LC–MS solvent system consisted of a gradient starting at 20% methanol (v/v, containing 0.005% trifluoroacetic acid, pH 3.42), increasing to 100% methanol over 15 min, holding for another 6 min and then returning to 20% methanol over 2 min. The linearity of the peak area (LC–MS) with respect to concentration was studied at concentrations from 10 to 1000 ng/mL of each aristolochic acid and calculated R was higher than 0.9971. The reproducibility was expressed as R.S.D., which was 6.7% at a concentration of 1000 ng/mL and 13.6% at 25 ng/mL. The recoveries were 99.5, 110.2 and 94.1% from the extracts, containing aristolochic acid I in concentrations of 42.2, 3376.9 and 11.7 ng/mg, respectively. The LC–MS method was more sensitive and accurate than a traditional LC–UV system. The content of aristolochic acid I was found to range from 3.3 ng/mg in *A. sieboldii* to 3376.9 ng/mg in *A. crispulatum*.

Aristolochic acids were also quantified in six TCM tinctures consisting of a water–alcoholic extract of *A. argentina* by means of a CE method [138]. Sufficient resolution was achieved in working electrolyte composed of sodium dihydrogenphosphate, sodium borate and SDS at pH 6.5–7 in a few minutes.

Co-administration of herbs and synthetic drugs can enhance the pharmacological effects but synergistic therapeutic effects may also complicate long-term medication. Over the past several years, the adulteration of TCM by synthetic substances has been reported for several times [139,140]. These incidences highlight the importance of detecting the presence of any synthetic drugs in herbal medicine to ensure its safety. Thus, four synthetic anti-diabetic drugs, acetohexamide, chlorpropamide, glibenclamide and tolbutamide were assayed simultaneously using a CE method with UV detection at 200 nm in 4 min [141]. The electrolyte was a solution, containing 100 mM sodium phosphate and borate buffer (pH 7.5); applied voltage was 15 kV and temperature 30 °C. 2-(4-Hydroxyphenyl) ethyl ammonium chloride was used as an internal standard. Correlation coefficients of the regression equations measured over the concentration range of 4.0–80.0 μg/mL were above 0.999 and LODs were at least 0.4 μg/mL. The R.S.D. of these anti-diabetic drugs for intra- and inter-day analyses were 0.23–4.27 and 1.23–6.33%, respectively. The recoveries of the adulterants ranged from 81.3 to 105.5% when herbal preparation Liow Wey Dih Hwang Wan was spiked with respective analytes. Using the CE method developed, chlorpropamide (0.5 mg/pill), was actually determined in a real sample of TCM.

Synthetic anorexics (fenfluramine, phentermine, benzphentermine, chlorphentermine, clobenzorex, phendimetrazine, phenmetrazine, amfepramone) were separated simultaneously by a GC technique coupled with nitrogen–phosphorus or mass selective detector [142]. LODs were lower than 100 μg/L for all the eight analytes in different matrixes of two real samples Chinese adulterated weight-reducing tonic purchased from Chinese market and fenfluramine was found on both of the samples.

A sensitive and selective LC–MS/MS method was developed for the determination of phenylbutazone, caffeine and oxyphenbutazone in a traditional Indonesian herbal product, Serbuk Jarem (a mixture of *Myristicae semen*, *Blumaeae folium*, *Kaemferiae rhizoma*, *Curcumae rhizoma* and *Retrofracti fructus*) [143]. Ethanol extracts of the samples were separated on a Phenomenex Luna C_{18}(2) column and eluted at a flow rate of 200 μL/min under individually set isocratic conditions comprising mobile phases A (water with 2 mM ammonium formate and 50 mM formic acid) and B (95% acetonitrile, v/v, with 2 mM ammonium formate and 50 mM formic acid). Phenylbutazone was eluted with 40% A for 6 min, caffeine with 85% A for 5 min, while oxyphenbutazone was eluted with 50% A for 5 min. The LODs were 3.69, 0.84 and 2.0 ng/mL, respectively. Linear calibration curves of phenylbutazone and caffeine were obtained for the range 1–50 μg/mL ($R > 0.9975$) and 0.1–10 μg/mL ($R > 0.9999$) and the recoveries were 108 and 106%, respectively. Each sachet of the herbal product contained 43.2 mg phenylbutazone and 3.2 mg caffeine, while content of oxyphenbutazone was below the quantification level.

6. Conclusion

The utilization of all kinds of beneficial information from the sources of TCM and adjacent fields brings the necessity of good, repeatable and precise analytical methods for the quality and safety reasons. The fast growing area could be characterized i.a. by the strengthening of the domestic Chinese research papers, both in count and quality. Their harder availability could be sometimes a small drawback. The selection for this review was based on the significance of the information for separation techniques and not on the level of sophistication of the analytical science used. The "elemental" information could be very helpful especially in the field of natural product chemistry, or for simplier possibility of development of analytical or chemotaxonomical methods.

The chemistry behind TCM is exciting and extremely rewarding as the wisdom of several 1000 years of experiments brings, however, endless amount of samples to be analysed.

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