



Review paper

Development of chromatographic technologies for the quality control of Traditional Chinese Medicine in the Chinese Pharmacopoeia

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ABSTRACT

As an important branch of medicine, Traditional Chinese Medicine (TCM) has been applied for the treatment of diseases for thousands of years in China and other countries in East Asia. The Chinese Pharmacopoeia (ChP) is a drug code formulated by the Chinese government, and it includes a special volume for the monographs of TCM, which plays an important role in ensuring the quality of drugs. The use of quality control technology has always been a complex and important factor in TCM. Owing to the chemical diversity of TCM, chromatography technology has been proven to be a comprehensive strategy for the assessment of the overall quality of TCM and has become the main analytical method in the ChP. This article provides an overview of the classical and modern chromatographic technologies applied in the ChP, and summarizes the advantages and disadvantages of each technique in the TCM monographs. In 2020, the new edition of the ChP (the 2020 edition) has been implemented at the end of 2020. This paper also contains a brief introduction about the application of chromatographic technologies in the new edition of the ChP.

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1. Introduction

As a summary of the experience and wisdom of the Chinese people, Traditional Chinese Medicine (TCM) has been applied for the treatment of diseases for thousands of years. At present, TCM is attracting an increasing amount of attention from scientists owing to its unique clinical effects. The research on the quality control methods for TCM has become increasingly popular.

Each individual Chinese Materia Medica (CMM) contains several different types of chemical ingredients, such as flavones, saponins, terpenes and steroids, which poses a great challenge to analysts for the quality control of TCM. To find a suitable method for multiple components and multi-index analysis of TCM, appropriate and feasible technologies have been introduced to establish the quality standards. Chromatography-based techniques have proven a useful strategy for the assessment of the overall quality control of TCM, to reveal the working mechanisms of TCM and to comprehensively improve the intrinsic quality of TCM [1]. Chromatography allows the separation of molecules based on differences in their structure

and/or composition. Owing to the diversity of chemical components within a TCM and the complexity of its mechanisms of action, chromatographic technology has emerged as the main analytical method for the quality control of TCM [2,3].

2. The history of the Chinese Pharmacopoeia

Since the founding of the People's Republic of China in 1949, the provision of medical care and healthcare for the people has become one of the most important duties of the Chinese government. The Chinese Pharmacopoeia (ChP) is a drug code formulated by the state that plays an important role in ensuring the quality of drugs, safeguarding public health and legal rights of drug use, and promoting the healthy development of China's pharmaceutical industry.

The first edition (Ed.) of the ChP was formally published in 1953, and contained 65 medicaments from plant origins, but not the "real" TCM. Subsequently, for the first time 643 TCM monographs were included in the second Ed. of ChP (Volume I) in 1963. Since then, Volume I of the ChP has been considered as a special volume for TCM. After the publication of the fourth Ed. in 1985, the ChP is released every 5 years. The English version of ChP was first edited and released in 1985 to assist in the requirement of the internationalization of TCM.

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The current edition (2015 Ed.) is the 10th edition of the ChP, and comprises Volumes I, II, III, and IV, which contain a total of 5608 monographs. Volume I contains 2598 TCM monographs, divided into three parts: Part 1, for medicinal materials and the prepared slices of Chinese crude drugs; Part 2, for oil, fats, and extracts; Part 3, for Traditional Chinese Patent Drugs (TCPDs) and single herb preparations.

In addition to the retention of conventional testing methods, ChP has further expanded the application of new technologies and new testing methods to increase the sensitivity, specificity, and stability of tests for TCM. Several methods have been employed for the quality control of TCM, including liquid chromatography (LC), tandem mass spectrometry, high-performance liquid chromatography (HPLC), and inductively coupled plasma mass spectroscopy. Fingerprinting technology, including *fingerprint* and *characteristic chromatogram*, is referred to in more than 30 monographs in the 2015 Ed. as a means of controlling the overall quality of TCM [4].

3. Overview of chromatographic separation technology in the ChP

During the developing history of ChP, the application of chromatographic technology changed in a few phases. The first chromatographic method recorded in ChP was thin-layer chromatography (TLC) in the 1977 Ed.. To strengthen the quantitative effect of drug standards, TLC scanning (TLC-Scan) was applied to the 1985 Ed. of ChP. In this edition, gas chromatography (GC) was also used for identification and quantification of TCM, especially for those containing volatile components. But the two technologies were just applied in a few monographs. From the 1990 Ed., the development of HPLC led to great advances in chromatographic technology in the ChP. The number of HPLC applications grew rapidly. *Characteristic chromatogram* and *fingerprint chromatograms* have been used for the overall evaluation of the TCM quality since the 2010 Ed.. The number of the chromatographic methods collected in each edition of the ChP is listed in Fig. 1 [4–11].

3.1. TLC in the ChP

3.1.1. TLC and its development in the ChP

In TLC, samples are separated by exploiting the properties of solvents traveling over a solid stationary phase and the resulting chemical interactions between the solvent, solid support, and molecules of interest. TLC is used widely for the qualitative analysis of organic compounds, the isolation of individual compounds from multi-component mixtures, and quantitative analysis. Historically, the TLC was first used for the isolation of natural products [12].

TLC has many inherent advantages: the simultaneous rapid separation of a variety of samples (including chemical reference material, reference drug, and test solution) at the same time; flexibility in the selection of stationary phase and mobile phase; simple equipment; easy operation; and low cost. The above advantages laid the foundation for the widespread use of TLC in the development of the TCM standards, and it is now recognized as an essential identification method in the ChP. The number of applications of TLC in the ChP has increased edition by edition. In the 1977 Ed., TLC was applied for the identification of only one substance in each monograph, which was appropriate for the technology available in China at that time. However, in recent editions, several different TLC methods for quality control have been used in a single monograph. For example, there are two TLC identifications in the monograph of *Dalbergiae Odoriferae Lignum*, both used *Dalbergiae Odoriferae Lignum* reference drug as the reference substance but in different chromatographic systems (shown in Fig. 2 [13]). It

makes the identification more integrated and representative. For TCPDs, there is now a tendency to establish as many TLC methods as possible to identify all the ingredients in one monograph, such as Huoxue Tongmai Tablet which has 8 TLC identifications.

However, TLC is also restricted by its poor repeatability, stability, limited usage, high reliance on manual operation, and high toxicity of the developing solvents [14]. Owing to the variability and complexity of chemical components involved, the TLC identification for TCPD is different from that of individual herbs (CMM). It focuses on one or several characteristic spots in the atlas that do not interfere with other ingredients. In addition, the effect of temperature and relative humidity of the environment may greatly influence the chromatographic behavior, the image quality, and even the interpretation of the result.

3.1.2. TLC-Scan and its development in the ChP

Before HPLC was widely applied in the quality control of TCM, TLC-Scan was used for the assay of TCM. The spots indicating each component are transformed into different peaks by TLC scanner. The quality of TCM can be controlled by evaluation of the relative shift value, the peak height, and the peak area in different samples. Subsequent development included different derivatization methods enabling the detection of the compounds without ultraviolet (UV) absorption. For this reason, TLC-Scan has been used widely for the assay of saponins in TCM in the ChP since the 1985 Ed., although the number of applications reached the peak in the 2000 Ed.

Many operation factors can greatly impact the performance of TLC results, including sample preparation, development, derivatization, and scanning. A reliable TLC-Scan result is dependent on the rich experience of the operator. Thus, TLC-Scan was gradually replaced by the HPLC coupled with an evaporative light scattering detector (ELSD) in the recent editions of ChP whereas the uptake of other technologies continues to increase.

3.1.3. The future of TLC in the ChP

In recent years, high performance thin layer chromatography (HPTLC) has become a valuable quality assessment tool for the analysis of herbal drugs. The HPTLC plate is coated with finer particles than classical TLC, and numerous samples can be examined in the same plate, thus improving both the sensitivity and repeatability, and making HPTLC methods more efficient and effective [15]. Sample application can be performed automatically or semi-automatically using a TLC machine. Although each TLC instrument can perform only one TLC procedure, an automated machine reduces the influences of the operator and increases the reproducibility of TLC analysis. HPTLC and band sample application (a typical mode for the TLC machine application) were first introduced in ChP 1990 Ed., which provided specified chromatographic parameters, such as application volume and development distance. HPTLC has been regarded as one of the most important developments in modern TLC and will greatly improve the identification of TCPDs.

3.2. HPLC in the ChP

3.2.1. HPLC and its development in the ChP

Similar to other chromatographic techniques, HPLC is based on the selective partitioning of the molecules of interest between two different phases. The mobile phase of HPLC is a solvent or solvent mixture that flows over beads coated with a solid stationary phase under high pressure. During their passage through the column, molecules in the sample solution are selectively isolated between the mobile phase and the stationary phase.

For ChP, HPLC technology was first used in the 1990 Ed.; there

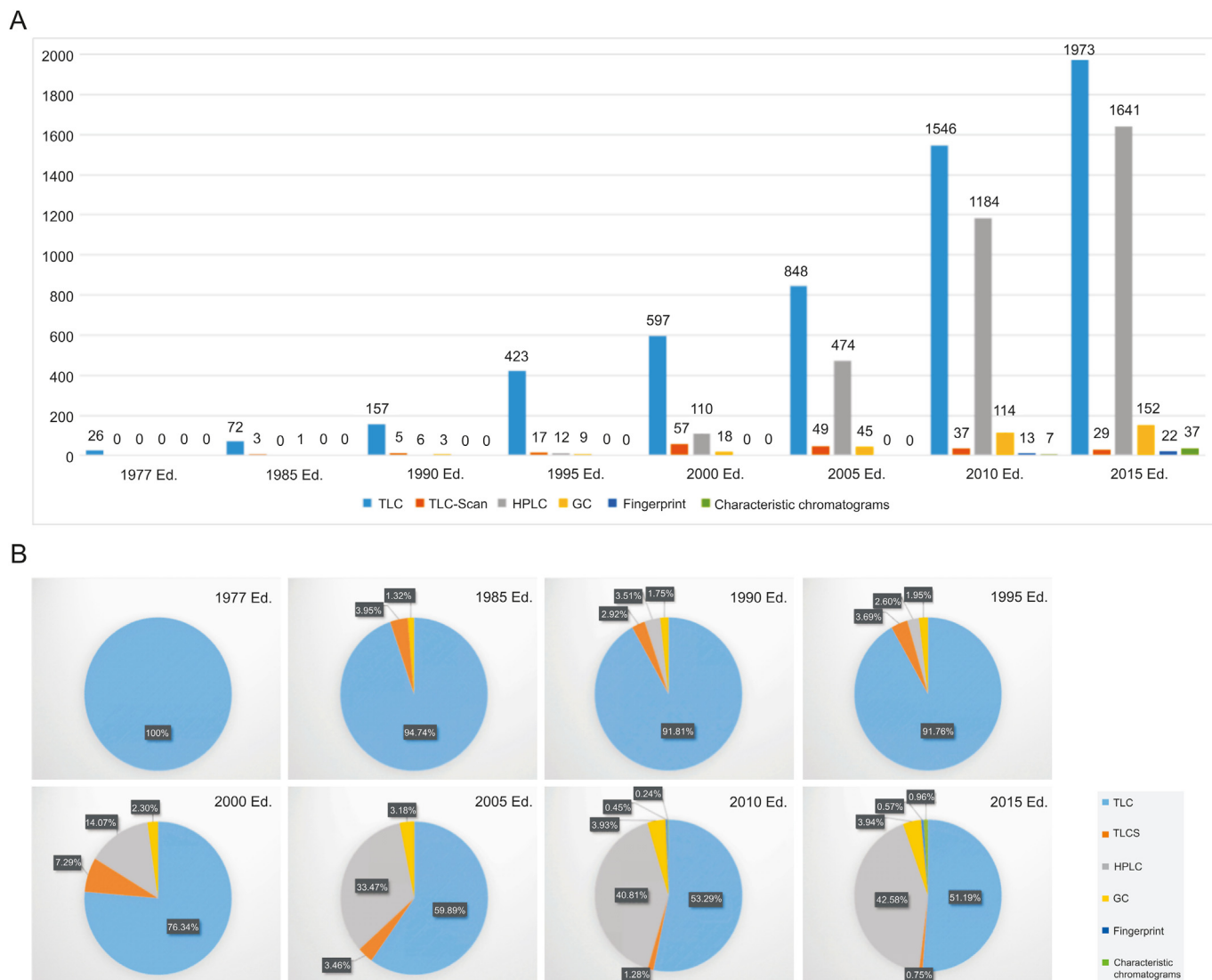


Fig. 1. (A) The number of chromatographic methods applied in each edition of the ChP. (B) The percentage of chromatographic methods applied in each edition of the ChP (Refs. [4–11]).

were six HPLC methods using UV detector in TCM monographs. Since then, the number of monographs using HPLC methods has increased rapidly. At present, HPLC is a mature technology that meets the requirement for detecting a variety of components, with the advantages of a general instrument installation, operational convenience, and low cost in detection. Thus, it has become the main technical method for the quality control of TCM in the ChP [14]. Furthermore, the development of HPLC detectors has increased its popularity for the quality control of TCM. Applications of different types of HPLC detector in each edition of the ChP are shown in Table 1. For example, ELSD makes it possible to detect chemical compounds without UV absorption, such as saponins. A representative example is the detection of astragaloside IV in *Astragali Radix* in the ChP. Mass spectrometry (MS) detector has the characteristics of high selectivity, high sensitivity, and strong separation ability, which realize the qualitative and quantitative detection of trace components. To combat the adulteration of honey, the ChP 2015 Ed. contains an analysis method for the detection of different sugars in honey such as fructose, glucose, sucrose, and maltose by using HPLC-Refractive Index Detector (RID). Although RID is not compatible with gradient elution and less

sensitive in other components, it shows great sensitivity and stability for sugar analysis. Another example is the fingerprinting of Xuezhikang Tablet and Xuezhikang Capsule by using an HPLC-Diode Array Detector (DAD). The DAD detector makes it possible to detect the ultraviolet radiation at all wavelengths at the same time, which is useful for the determination of different components with different maximum absorption wavelengths.

3.2.2. The features of HPLC in the ChP

There are many advantages that dictate the widespread use of HPLC for the analysis of herbal medicines [16]. High separation efficiency, rapid analysis, good sensitivity, and wide selection of chromatographic columns and mobile phases have made the HPLC method popular in the ChP. The application of HPLC was originally limited by the detection technology, which required the UV absorption of the test compounds in early stage. With the development of different types of HPLC detectors, the applicability has increased greatly to meet the different quality control purposes of TCM. However, each detection method has its own drawbacks. For example, high-pressure nitrogen gas or air is required for the use of ELSD, and ELSD and MS can only use nonvolatile salt solutions as

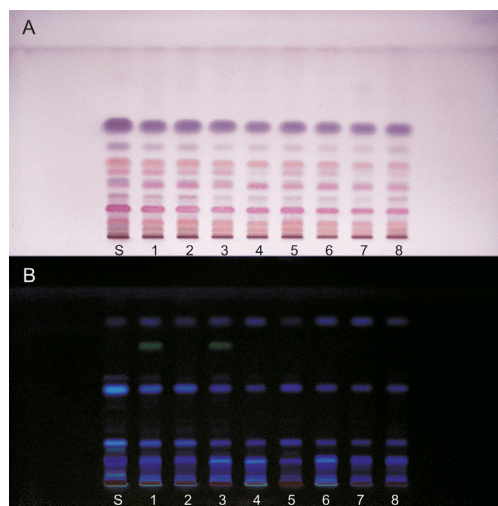


Fig. 2. TLC identifications of *Dalbergiae Odoriferae Lignum*, examined under (A) visible light and (B) ultraviolet light at 365 nm.

Track S, *Dalbergiae Odoriferae Lignum* reference drug (S); tracks 1–8, samples of *Dalbergiae Odoriferae Lignum* collected from different regions in China.

The drafter of this figure, Shanghai R&D Center for Standardization of Traditional Chinese Medicines (Ref. [13]).

the mobile phase. In addition, the complexity of the various chemical components of TCM samples may easily block the system, contaminating the chromatographic column and shortening its lifetime.

3.3. GC in the ChP

3.3.1. GC and its development in the ChP

GC is a chromatographic method that analyzes vaporized samples carried into the chromatographic column by a gaseous mobile phase. The sample is introduced into the GC column by means of a heated injector, which volatilizes all components and introduces them into the gas flowing over the stationary phase [17]. GC is of great practical significance for the quality control of TCM, especially for those containing essential oils. The first GC methods were used for the identification of camphor, mentholum, methyl salicylate, and the mixture of borneol and isoborneol in a TCPD called Shangshi Zhitong Plaster in the ChP 1985 Ed. Since then, the number of monographs using GC methods has increased slowly and reached 152 monographs in ChP 2015 Ed.

In addition, GC is widely used for the determination of residual solvents and residual pesticides [18]. The solvent remaining in the drug is a volatile organic chemical substance introduced during the preparation procedure, and it is not completely removed in the specific production process. According to the researches on the toxic and carcinogenic effects of residual solvents, their presence has gradually attracted the attention and been subject to analysis by GC. For example, some TCPD monographs choose GC for the

detection of residual solvents, such as the limit test for butanone residue in Zhike Chuanbei Pipa Dropping Pills in the 2015 Ed. of ChP. GC is a superior method for the quality control of organochlorine pesticide. The monograph of *Ginseng Radix et Rhizoma* used GC-MS to determine seven organochlorine pesticide residues with better selectivity, sensitivity, and repeatability in the 2015 Ed. of ChP. GC can be used for the separation and analysis of volatile and thermally stable substances, which makes it especially suitable for the establishing of the fingerprintings of volatile components in TCM. In the 2015 Ed., four monographs have used GC fingerprinting (including two oils, one eye drop, and one aerosol).

3.3.2. The features of GC in the ChP

Owing to its high separation efficiency and analysis speed, GC was able to eliminate interference of main components to the trace components. Electron ionization is the most popular mass interface for GC, and its accompanying spectral library brings convenience to the qualitative analysis of TCM. However, GC requires the sample to be gasified at high temperature, which may be limited by the volatility and thermal-stability of the target compound. Other components, such as alkaloids, lactones, and phenols, may also be analyzed by GC after derivatization reactions, but the derivatization process might greatly influence the repeatability of the analysis results.

4. New chromatographic technology used in the ChP

To control the comprehensive quality of TCM, some new strategies have been proposed to find out the chemical differences both in raw materials and final products by modern chromatographic techniques [19]. With the development of modern science and technology, fingerprinting, HPLC tandem mass spectrometry (HPLC-MS/MS), and other new chromatographic technologies have been applied to the analysis of CMMs and their preparations, thereby providing new research prospects and ideas.

4.1. Fingerprinting in the ChP

4.1.1. Fingerprinting and its application in the ChP

Fingerprinting, a comprehensive and quantifiable identification method, is based on the systematic study of chemical components of TCM, and used mainly to evaluate the authenticity, excellence, and stability of the quality of CMMs and TCPDs [20–22]. A fingerprinting is a chromatogram or spectrogram that precisely indicates the chemical characteristics of TCM by using proper extraction and analysis methods. For the purposes of TCM quality control, it can be divided into *Fingerprints* and *characteristic chromatograms* in the ChP. *Fingerprint* is a semi-quantitative analysis method based on the whole information of the chromatograms, and is used for the overall evaluation of the TCM quality [23]. It has been recognized as one of the most suitable technologies to control the quality of TCM by combining macroscopic qualitative and quantitative analysis from the perspective of systematization and integrity [24].

Fingerprinting technologies were initially used in 20 monographs in the ChP since 2010 Ed.; 13 of these monographs were *fingerprints* while 7 of these were *characteristic chromatograms*. The applications of *characteristic chromatogram* and *fingerprint* technologies increased the integrated quality control of these TCMs. In the ChP 2015 Ed., 59 monographs contained the methods of fingerprinting, of which most of the new admissions are TCPDs, especially in series preparations (such as Yinhuang Pills, Yinhuang Mixture, and Yinhuang Granules).

Fingerprint should reflect as much information as possible within one chromatogram [25], and should also be helpful in identifying the relationship between TCPD and its raw materials,

Table 1

The number of applications with different types of HPLC detector in each ChP edition.

HPLC detector	1990 Ed.	1995 Ed.	2000 Ed.	2005 Ed.	2010 Ed.	2015 Ed.
UV	6	12	110	463	1113	1517
ELSD	0	0	0	11	68	117
MS	0	0	0	0	3	6
DAD	0	0	0	0	0	2
RID	0	0	0	0	0	1

standardizing the production process and improving the quality of final product [26]. The quality and stability of the raw materials and its preparation can be guaranteed effectively from the *fingerprint* of the single herb preparations. The *fingerprint* of Qingkailing Injection reflects the chemical markers of *Gardeniae Fructus*, *Isatidis Radix*, *Margaritifera Concha*, *Bubali Cornu*, and *Lonicerae Japonicae Flos* in a single chromatogram, and uses Geniposide as the reference compound, as shown in Fig. 3 [4].

4.1.2. Characteristic chromatograms and their application in the ChP

A *characteristic chromatogram* is a chromatogram or spectrogram that allows the selection of one or several types of components as an identification marker for the control of the quality of herbal medicines. Here is an example of a *characteristic chromatogram* of *Notopterygii Rhizoma et Radix* which only has 4 identification markers, as shown in Fig. 4 [4]. It can be used for qualitative identification in the development of drug standards. A *characteristic chromatogram* does not require the same comprehensive evaluation of the similarity as a *fingerprint*, and its main feature is to highlight the specific different components between varieties. The components are used as characteristic peaks to locate the chromatographic peaks on the characteristic spectrum through the calculation of the relative retention times of the reference peak. These peaks may be known or unknown compounds [25].

The *characteristic chromatogram* uses different identification modes for a single chemical marker (chemical reference substance) or a single herb (reference drug), which is mainly used for the identification of TCPDs. The 2015 Ed. of ChP included 39 characteristic chromatograms, of which 24 were used for the identification of TCPDs to distinguish the authenticity. It enriched the information on the material and strengthened the specificity of identification [4].

4.1.3. Evaluation system for fingerprinting chromatograms in the ChP

As an analysis method for fingerprinting, similarity was evaluated by using Similarity Evaluation Software to show the differences between the samples and the reference materials. The ChP recommends the use of “Computer Aided Similarity Evaluation Software for Fingerprint of Traditional Chinese Medicine” to calculate the similarity of fingerprinting. This software was developed and released by the Chinese Pharmacopoeia Commission. The software contains two methods of evaluation: common peak similarity evaluation and full spectrum similarity evaluation. Common peak similarity evaluation only calculates the similarity with common peak under the provisions of the drug standard. Full

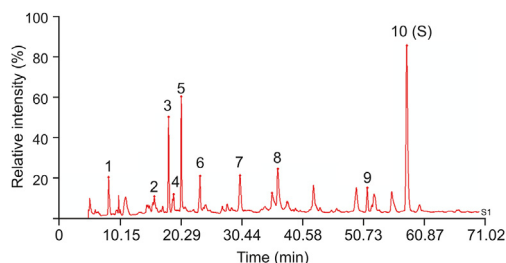


Fig. 3. The *fingerprint* of Qingkailing Injection.

1, unknown (from *Isatidis Radix*); 2, unknown (from *Margaritifera Concha*); 3, uridine (from *Isatidis Radix*); 4, unknown; 5, adenosine (from *Isatidis Radix*); 6, guanosine (from *Isatidis Radix*); 7, unknown (from *Isatidis Radix*); 8, tryptophan (from *Bubali Cornu*); 9, chlorogenic acid (from *Lonicerae Japonicae Flos*); 10 (S), geniposide (from *Gardeniae Fructus*).

The drafter of this figure, Beijing Institute for Drug Control (Ref. [4]).

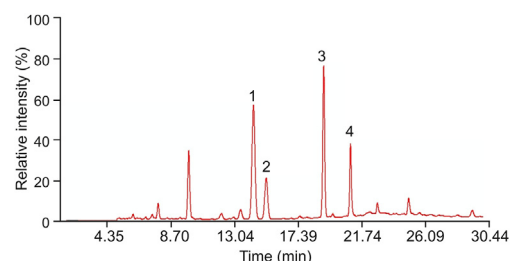


Fig. 4. The *characteristic chromatogram* of *Notopterygii Rhizoma et Radix*.

1, notopterol; 2, phenethylferulate; 3, isoimperatorin; 4, falcariindiol.

The drafter of this figure, School of Pharmaceutical Sciences, Peking University (Ref. [4]).

spectrum similarity evaluation calculates the similarity with all the peak from the chromatogram, but calculates with the common peak priority. In the 2015 Ed. of ChP, 81.8% of the monographs used full spectrum similarity evaluation, which illustrates not only the integrity of the drug, but also the specificity of each ingredient, by ensuring the stability and uniformity of the quality between batches. The rest of the fingerprinting methods used common peak similarity evaluation to ensure the specificity, stability, and consistency of preparations.

4.1.4. The future of fingerprinting chromatograms in the ChP

Fingerprint, based on the whole information of the components in sample, is often applied in the evaluation of the integrity quality of TCM. The *characteristic chromatogram* represents some important chemical markers in sample, which is often used as a qualitative identification method [23]. To further explore the synergic quality of TCM, intensive study of fingerprinting will allow the elucidation of the mechanisms of biological activities of TCM [1]. The applications of *fingerprinting* and *characteristic chromatogram* methods reached 2% of the total number of ChP monographs in 2015 Ed. In addition to these methods, the integrated quality control of TCM was increased in recent ChP editions. Many problems remain with these technologies. The isolation of peaks in the fingerprinting chromatogram is influenced by many parameters, which would affect the inter-laboratory repeatability of the chromatogram. The precision of the “Evaluation Software” is unclear because the similarity evaluation method within the software is based on a few chemometrics theories which may not be mature enough [27]. A reliable and more efficient evaluation system should be established to improve the effectiveness of the method.

4.2. TLC bioautographic method in the ChP

The TLC-bioautographic method is a rapid activity screening chromatography method which combines TLC with biological identification technology. It has been used for the screening and determination of the biological activities including antioxidant, antibacterial, and cholinesterase inhibition [28–30]. In recent years, quality evaluation guided by the clinical practices of TCM has been concerned with the establishment of the drug standards. The TLC-bioautographic method reflects the differences not only in chemical components between samples, but also in the activity of components separated by TLC, and possesses the advantages of simple operation, low cost, high sensitivity, and exclusivity [31].

Bioautography provides not only phytochemical results by chromatographic separation, but also additional information about the activity of constituents in multi-compound mixtures. Natural compounds from TCM that possess inhibitory properties on biological targets may become visible when using TLC methods [29].

The TLC-bioautographic method was first recorded in the 2010 Ed. of ChP, when a mullein glycoside in *Rehmanniae Radix* was identified.

With regard to the future prospects of TLC-bioautographic methods, research into many biological targets may be enhanced by the establishment of further effective and sensitive bioautographic assays.

4.3. New HPLC technology in the ChP

4.3.1. UPLC and its development in the ChP

Ultra-performance liquid chromatography (UPLC) is a new separation technique in liquid chromatography, which normally utilizes a column packed with 1.7 mm silica particles, and makes it possible to perform efficient separations for multiple components in a short time. It has the advantages of rapid analysis, high peak capacity, great resolution, and better sensitivity, which has proven increasingly attractive to pharmaceutical analysts [32–34].

First of all, given the continuous developments in productivity, the analysis of mass samples needs to be completed as soon as possible. Secondly, to analyze biochemical samples and natural product samples, the complexity of samples necessitates greater separation ability. UPLC is a highly efficient technique able to meet these requirements.

Fufang Danshen Dropping Pills were the first application of UPLC in the 2010 Ed. of ChP. Subsequently, three monographs applied UPLC methods in the 2015 Ed. of ChP. Despite the relative expense of the equipment and column, the finer particle size in the column means that the column operation pressure increases and the chromatographic system may be blocked much easily than normal HPLC column. These disadvantages have limited the application of UPLC in the ChP.

4.3.2. HPLC-MS and its development in the ChP

High performance liquid chromatography-mass spectrometry (HPLC-MS) techniques combine the advantage of the stronger separation ability of complex samples found with liquid chromatography with the advantages of high selectivity, and high sensitivity found with mass spectrometry [35]. Mass spectrometry provides structural information that can greatly assist research into the unknown compounds in TCM. It has become an important and irreplaceable analytical method in many research fields in TCM, such as toxic component control, trace substance determination, and gelatin drug analysis.

The application of HPLC-MS in the ChP first appeared in the 2010 Ed., in which trace amounts of toxic components, such as adoniferine and toosendanin, were determined by using HPLC-MS in a limit test to ensure the safety of CMMs.

As popular medicines in TCM, *Asini Corii Colla*, *Cervil Cornus Colla*, *Testudinis Carapacis et Plastris Colla*, and other gelatin drugs have been used to nourish *yin* and supplement blood. However, in recent years, counterfeit and adulterant products have disrupted the rights and interests of the consumers, which has attracted the attention of the pharmaceutical industry. The classical identification methods are easily influenced by subjective factors, and may lead to false negative results. Over the past decade, HPLC-MS and other methods have been used in the identification of gelatin drugs to enhance specificity [36,37]. The monographs of the three gelatin drugs named above used a HPLC-MS method to identify the characteristic peptide ions after enzymatic hydrolysis of the drugs. The method effectively evaluates the quality of all types of raw materials in animal glue, and has led to a breakthrough in research into the quality standards of glue preparations.

HPLC-MS is also used in the determination of aflatoxins, whose original standards used post-column derivatization of the HPLC.

Mass spectrometry is applied to detect 14 types of fruit seeds and animal medicinal materials that are often infected by *Aspergillus* species, such as *Platycladi Semen*, with greater sensitivity and accuracy in the 2015 Ed. of ChP.

There are still some problems with HPLC-MS technology. The matrix effect may greatly influence the qualitative and quantitative analysis of HPLC-MS, especially for the complex TCM samples. The HPLC-MS instrument is still very expensive. In contrast, HPLC-MS has become an increasingly popular instrument used in the quality control of TCM. The application of HPLC-MS in the ChP should be increased greatly in the near future. HPLC-MS will be used widely in limit tests, as the ChP seeks to effectively control exogenous pollutants in TCM [3,38,39].

4.4. Other modern chromatograph methods in the ChP

Other chromatograph technologies are used in the ChP, such as ion chromatography (IC). Sulfur fumigation is a traditional processing and storage assistance method of TCM, which can be used for the protection of raw materials from mold pollution. However, overexposure to sulfur dioxide may be a safety risk to people's health, and thus its residue in TCM should be strictly controlled. In the 2010 Ed. of ChP, iodine titration was used for the detection of sulfur dioxide residue, which is a simple and low-cost method but has poor specificity. Therefore, the IC method was established in the 2015 Ed. of ChP, with the advantages of high sensitivity and strong specificity, albeit at an increased cost. It is suggested that the method chosen for the determination of sulfur dioxide residue should be based on the real-world status of the TCM [40,41].

5. Chromatographic technology used in the 2020 Ed. of the ChP

The 2020 Ed. of ChP has already been approved by the National Medical Products Administration and National Health Commission of the People's Republic of China and will be implemented at the end of 2020. The total number of monographs of raw materials, extracts, and TCPDs reaches 2711, of which the new admissions are from TCPDs. The new ChP contains more HPLC-MS methods for TCM quality control, especially from a safety control prospective. In addition, the application of UPLC methods has increased in the new ChP and provides the allowable adjustment ranges of chromatographic parameters and the conversion formulas for method transfer between HPLC and UPLC.

HPLC-MS was used in the identification of gelatin drugs in the 2015 Ed. of ChP to enhance specificity, as mentioned above. Since the method was established and executed for 5 years, HPLC-MS has been applied in the identification of six TCPDs monographs which contain gelatin drug. The total number of the HPLC-MS method applications in the new ChP will be increased to eight.

The detection of 33 kinds of forbidden pesticide residues in TCM is another important application of HPLC-MS/MS and GC-MS/MS methods. Multiple detection indicators (including 55 compound monomers), complex technology, high sensitivity requirements, and certain applicability and controllable costs make it difficult to establish a general test method. The newly established detection method in the new edition of ChP has four steps: sample pretreatment, sample extraction, sample purification, and sample detection. To ensure applicability of the method, two to four determination methods are listed for each step.

The use of UPLC has increased in the 2020 Ed. of ChP, as shown in Table 2. A major breakthrough for the 2020 Ed. of ChP is the provision of the allowable ranges of chromatographic parameters and the formula for the conversion between HPLC and UPLC methods. Columns require a small particle size (approximately

Table 2
UPLC applications in each edition of the ChP.

Monographs	Collections in the ChP
Fufang Danshen Dropping Pills	2010/2015/2020 Ed.
Hugan Pills	2015/2020 Ed.
Hugan Capsules	2015/2020 Ed.
Hugan Granules	2015/2020 Ed.
Qishen Yiqi Dropping pills	2020 Ed.
Ginkgo Leaves Extract	2020 Ed.

2 μm) and a small inner diameter (approximately 2.1 mm) to improve the degree of resolution or reduce the analysis time, and the performance of pump, injection volume, detection cell volume, and dead volume of the system must be adjusted appropriately. The publication of this formula should provide excellent guidance for the transfer of these two methods and promote the uptake of UPLC methods in the ChP.

6. Concluding remarks

In the past few decades, the quality control procedures in TCM have been developed quickly. Adhering to scientific, advanced, normative, and practicable standards is an essential guideline and one of the basic principles of the ChP. Chromatographic technologies, such as TLC, HPLC, GC, HPLC-MS, and GC-MS, play an important role in the screening of the active components and the establishment of TCM standards. TCM is a complex multi-component system, and therefore, it is not appropriate to control the overall quality by using only a few types of analysis technologies. Accelerating the application of classical chromatographic technology to ensure the basic quality control in TCM and developing modern chromatographic technology to reveal the clinical mechanism and safety control of TCM, are the key points in the construction of the quality standards system. It is also very important to determine the chromatographic technologies that are not only appropriate for current scientific situations, but also for the development of the TCM industry.

The function of TCM is based on the comprehensive action of various complex chemical components and it is difficult to evaluate the effectiveness and specificity of TCM by using only one or a few chemical markers. Research into the effective components of TCM is not sufficiently clear to support the establishment of drug standards. Some components often appear as markers in different kinds of CMMs, which is not conducive to the identification and quality control. Enhanced research into component identification technology and the methods for active ingredient identification is the basis of TCM standards that can be used to control and strengthen the intrinsic quality of TCM in a comprehensive manner. It is necessary to figure out the clinical components of TCMs in accordance with the tenets of TCM theory to establish a TCM standard system, based on scientific and practicable studies.

However, it will take lots of time to find the clinical components and establish a proper chromatographic method in the TCM quality standards. Chromatographic technology will play an important role in the screening of active components of TCM combined with other technical fields, such as molecular biology, proteomics and metabonomics. The TLC-bioautographic method will make a greater contribution to establishment of TCM standards.

The standardization of TCM leads to internationalization. Establishment of an international standard system of TCM is the driving force and breakthrough point for the internationalization of TCM. The ChP plays an important role in the effect standardization of TCM and will make efforts to promote quality improvement and industrial development of TCM.

Declaration of competing interest

The authors declare that there are no conflicts of interest.

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