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Quality Control of Chinese Medicines

Strategies and Methods

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Contents

Chapter 1
Key Scientific Issues in Research for Quality Control of Chinese Medicines

Shaoping Li and Jing Zhao

Abstract Quality control is the key for modernization and internationalization of traditional Chinese medicines (TCMs). Besides techniques, key scientific issues in research on quality control of TCMs are crucial for well understanding TCMs. In this chapter, science including the principles for optimization of quality markers and alternative reference compounds were reviewed and discussed.

Keywords TCMs · Quality control · Science · Quality marker · Reference compound

1 Introduction

Traditional Chinese medicines (TCMs) is the crystallization of thousands of years of history, culture, science, and wisdom in China, and has made great contributions to the prosperity of the Chinese nation and the progress of civilization. In the era of highly developed modern medicine, it stands out among the various traditional medicines in the world, and still maintains its unique charm. In the prevention and treatment of new coronary pneumonia (COVID-19), traditional Chinese medicine has played an important role [1]. With the development of society and economy, the aging of the global population, and the changes in the spectrum of human diseases, a single modern medical model can no longer adapt to the development of medical care. People all over the world have an increasing demand for traditional medicine or traditional Chinese medicine. This demand has also promoted the application and dissemination of traditional Chinese medicine on a global scale. At the same time, due to the lack of effective quality control methods, the quality and safety of TCMs

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are often questioned, seriously hindering the modernization and internationalization of TCMs.

According to the author's thinking and experience on the quality research of TCMs, we believe that three aspects should be carefully considered in the research on quality control of TCMs and the establishment of standards: (1) selection and optimization of quality markers, (2) solutions of shortage for reference substances, and (3) application and standardization of appropriate techniques. For a long time, quality control of TCMs has paid more attention to techniques, and failed to fully understand the scientific issues of quality control from the perspective of safety and effectiveness. As we all know, the strategy and idea for quality control of TCMs are different from those of chemical medicine. How to scientifically and rationally control the quality of TCMs must firstly well solve the key scientific problems of quality control, which is mainly reflected in the selection of quality markers and alternative strategy of reference substances/compounds.

2 Selection Principles of Quality Markers

2.1 Quality Markers of TCMs Should Be Closely Related to Their Safety and Efficacy

Chinese herb is composed of common components that regulate body functions and active components with specific biological activities. Common components, such as polysaccharides, proteins, and other high-molecular substances and amino acids, trace elements, vitamins, and other low-molecular substances with the functions of nutritional supplementation, immunity, and metabolic regulation, are the material basis of the overall regulatory effect of TCMs. Active components such as various alkaloids, saponins, flavonoids, and other chemical components with specific pharmacological activities, their effects are completely in line with the law of chemical drugs, and different components can play synergistic, additive, or antagonistic effects. There may be several different components working together for a certain clinical application. Therefore, quality markers of TCMs should include common components with overall regulation function and active components with specific biological activity, so that the quality markers can not only fully reflect the characteristics of TCMs, but also meet the requirements of modern medicine.

Active component refers to a chemical component that can represent a specific effect of a crude drug. Chemicals that can synergize and/or increase the specific effect of active compounds are called relative components. Combination of active and related components is known as effective components of TCMs, which can be a collection of same or different types of chemicals, and the material basis for a specific efficacy of crude drugs. Toxic components refer to those with obvious biological activity and small safety range, which are the main material basis of toxic

and side effects of TCMs. Toxic components that have nothing to do with the therapeutic effect can be removed as much as possible during the refining process of TCMs, and their amount can be controlled when necessary to ensure the safety of drug. For a long time, research of TCMs has mainly focused on active ingredients, ignoring the influence of related ingredients on their efficacy. In fact, some inactive components of TCMs may play a crucial role in the efficacy of drug [2]. Noldner et al. compared the antidepressant activity of St. John's Wort methanol extract and ethanol extract, and found that the ethanol extract was effective, but the methanol extract was not. According to the idea of active ingredient research, as long as the difference in the chemical composition of the two extracts is compared and determined, it is possible to find its antidepressant active ingredient. Although the research ideas are clear, it is not easy to truly compare the differences in chemical components of two extracts, because only when all components in the two extracts are fully separated can it be possible to truly and accurately find their chemical difference. Actually, for an extract, the number of compounds is unpredictable, so there is no clear goal for chromatographic optimization. In any case, the researchers compared the components of the two extracts based on this idea and found that the effective ethanol extract had one more component than the ineffective methanol extract—rutin, suggesting that rutin may be an antidepressant active ingredient. However, pharmacological studies have shown that a single rutin has no antidepressant effect. For this reason, the researchers added rutin to the methanol extract, which both had no antidepressant effect, and found that when the amount of rutin added in the methanol extract reached a certain level, the methanol extract which had no antidepressant effect showed antidepressant effect [3]. It is suggested that for antidepressant effect of *Hypericum perforatum*, rutin is closely related to the effect and should also be its quality marker. Similarly, the bacterial multidrug resistance efflux pump inhibitor 5'-methoxyhydnocarpin (MHC), which has no antibacterial activity itself, can significantly enhance antibacterial effect of berberine in plants of the genus *Berberis* [4]. Indeed, TCMs extracts are like a chemical drug formulation, the difference is: active ingredients in chemical medicine formulation are clear, role of individual excipient is well known, such as synergists, absorption accelerators, enhancer (assistant) solvents, stabilizers, coloring agents, etc., while active ingredients of TCMs extracts are often unclear, and the related ingredients that cooperate with active ingredients are unknown, which limits the scientific characterization of TCMs. Therefore, modern Chinese medicines research should focus on clarifying the key components that affect the action of active ingredients while revealing the core material basis (effective ingredients) of Chinese medicines. Only in this way can the scientific connotation of Chinese medicines be revealed at the level of the whole (multi-component interaction), to ensure the effectiveness of medication.

2.2 Quality Markers of TCMs Should Vary with Their Therapeutic Purpose

Clinically, the same TCMs can be used in various prescriptions, and then used for different clinical therapeutic purposes. In addition, the same TCMs often has multiple pharmacological effects. For example, "Modern Research and Application of Traditional Chinese Medicine" (Edited by Zheng Huzhan et al. Xueyuan Publishing House) introduced *Angelica sinensis* with more than 30 pharmacological effects in 15 categories. Therefore, when the same TCMs is used in different clinical applications, its active ingredients are different, and the quality markers should also be different.

Panax notoginseng, *Sanqi* in Chinese, has the effect of stopping bleeding and promoting blood circulation. The effect of promoting blood circulation is often used in the treatment of cardiovascular and cerebrovascular diseases. *P. notoginseng* also can increase the number of platelets, thereby causing a large number of platelets to aggregate and play a hemostatic effect. Therefore, if *P. notoginseng* is used for hemostasis, its quality marker should be dencichine instead of its saponins. Therefore, when we established the quality standard of *P. notoginseng* in the United States Pharmacopoeia, we proposed adding dencichine as an additional quality marker to meet quality control requirements of *P. notoginseng* in the clinical hemostasis.

2.3 Both Content and Ratio of Active Compounds Influence the Effect of TCMs

When developing quality standards for TCMs, the content of quality markers is often only focused. In fact, the ratio of active compounds will also play an important role to the effect of TCMs.

Cordyceps sinensis is a valued TCMs. At present, there are three main types of *Cordyceps* products on the market: natural *Cordyceps sinensis*, fermented Cordyceps mycelia powder, and cultured *Cordyceps militaris*, also known as *Cordyceps* flower as a common soup material on the dining table in Guangdong. Studies have shown that nucleosides are a very important type of active ingredient in *Cordyceps*. Actually, the nucleoside characteristics of the three types of *Cordyceps* products are completely different. Natural *C. sinensis*, fermented *Cordyceps* mycelia powder, and cultured *Cordyceps militaris* can be clearly distinguished through the composition and ratio of nucleosides. In order to study the influence of different nucleoside ratios on the pharmacological effects of *Cordyceps*, two nucleosides mixtures, UIG (uridine: inosine: guanosine = 8:11:5) and UAG (uridine: adenosine: guanosine = 11:7:9), were prepared according to the nucleoside characteristics of natural and cultured Cordyceps, and their effects on macrophage function were also investigated and compared.

It was found that, for normal macrophages, UIG can enhance the function of macrophages with the investigated concentration increases, but UAG can promote the function of macrophages in a certain concentration range, when the concentration reaches at a certain level, it not only does not improve the function of macrophages, but inhibits its function. For macrophages activated by LPS, UIG had no significant effect on their function, but UAG could significantly inhibit the function of macrophages. It can be seen that the effects of UIG and UAG on both normal macrophages and immune-activated macrophages show obvious differences. In fact, fermented *Cordyceps* mycelia powder is often used clinically to reduce body rejection after organ transplantation and reduce the dosage of immunosuppressants [5].

2.4 Polysaccharides Are Common Markers for Quality Control of TCMs

For a long time, the quality markers of TCMs have mainly been small molecules, such as alkaloids, flavonoids, saponins, etc., because according to Lipinski's rule, it is believed that compounds with a molecular weight below 500 Da have good druglike property. With the development of science, macromolecular biological drugs have accounted for about one-fifth of the world's innovative drugs in the past 40 years [6], such as antibodies, protein drugs, cytokines, etc. Their molecular weights range from thousands to tens of thousands, hundreds of thousands, or even millions. That is to say, modern drugs have already expanded from small molecular compounds to macromolecular polymers.

Decoction is the main traditional way of clinical administration of TCMs, which is rich in macromolecular compounds such as polysaccharides with various pharmacological effects. Although the action mechanism of orally administered polysaccharides is unclear, there is extensive literature supporting their multiple pathways of action mechanism [7, 8]. Unfortunately, in the modern development of TCMs, macromolecular compounds such as polysaccharides are often removed as impurity components. The major modern Chinese medicine industrial production process formed in the 1950s, water extraction and ethanol precipitation method, is still a classic in the production of modern Chinese medicine preparations. Actually, water is rarely used for sample preparation of quality control of TCMs, which induces that TCMs decoction's main clinical application form is out of touch with the small molecule quality control strategy of modern organic solvent extraction, which seriously affects the scientificity and rationality of quality control of Chinese medicine. Qualitative and quantitative analysis of polysaccharides is the key and bottleneck for quality control of classic formula and formula granules.

In addition, the safety of TCMs has been a hot topic in the application of traditional Chinese medicine in last decade years. In fact, in addition to the misuse of medicinal materials, inappropriate application methods are the main cause of many safety problems of TCMs. There are two unfavorable trends in the development of

modern Chinese medicine, the edible and micronization use of medicinal plants. In order to reduce the threshold for use and expand the market, the transformation of local characteristic and bulk Chinese medicines into edibles has been promoted in many places, ignoring the potential safety risks of a large number of long-term use. Besides, different from the safety of TCMs decoction has been confirmed by long-term clinical practice, direct consumption of TCMs micropowder can greatly increase the intake of water-insoluble harmful substances in the decoction, posing a safety hazard.

Polygonum multiflorum is a commonly used TCMs, and its hepatotoxicity has received widespread attention in recent years, but both clinical and research studies have shown that the results of its toxic effects varied [9, 10]. Therefore, it is necessary to prepare the water and ethanol extracts of *P. multiflorum*, and to compare the components of the extracts and investigate their hepatotoxicity. It was found that the water extract of *P. multiflorum* had no obvious hepatotoxicity, but the alcoholic extract of *P. multiflorum* gradually showed liver toxicity with the increase of concentration. Comparing the chemical components of *P. multiflorum* water and alcohol extracts, it is expected to quickly find the hepatotoxic components in *P. multiflorum.* The results showed that the content of high polarity components was similar in both extracts, but the content of low polarity compounds was significantly different. Therefore, it is believed that the hepatotoxicity of the ethanol extract has a great relationship with these low polarity compounds. Further studies have found that these low polarity compounds are mainly free anthraquinones, and they all have a certain degree of hepatotoxicity *in vitro* [9]. It can be seen that inheritance and innovation of traditional Chinese medicine, and inheritance is the first. Only on the premise of respecting tradition and ensuring safety and effectiveness, and fully understanding the characteristics of TCMs, innovation is the source of water and the tree of roots, which can truly promote the development of TCMs. TCMs decoctions may not always be the most suitable dosage forms, but long-term clinical practice has proved that they are safe and effective. If *P. multiflorum* is administered as ethanol extract or fine powder may lead to a significant increase in liver toxicity.

3 Substitution Strategy for Reference Compounds of Chinese Medicines

In addition to the scientific nature of quality control markers, the scientific nature of alternative strategies for the quality control of TCMs from the perspective of reference materials also needs to be discussed. In general, the reference substance is the reference for qualitative and quantitative analysis. Only with the reference substance can the corresponding analysis be carried out. In fact, due to the complexity of Chinese medicines and low content of most components, there is often a lack of corresponding reference substances for quality control. Therefore, how to control the quality of TCMs without reference substance, which is a scientific problem. In

the past 20 years, we have proposed three solutions to the problem of the lack of reference materials for quality control of TCMs.

3.1 Analogues Directly Referred to Quantitation

Unlike the widely used "one test multiple evaluation method" or "one standard multiple measurement method", it directly uses analogues as a reference for quantitative analysis. GC–MS is a classical method for the analysis of volatile oil from TCMs. Generally, the relative content of each chromatographic peak is calculated based on the sum of all peak areas and the qualitative analysis of chromatographic peaks retrieved from the MS database. It is not possible to evaluate the quality of volatile components in samples from different batches or origins of the same TCMs. By using the strategy of direct quantitative analysis with reference to the analogue, the problem that the quality of volatile oil of TCMs could not be evaluated due to the lack of reference materials for volatile components was successfully solved. *Angelica sinensis* is a commonly used TCMs, but in China, Japan, and South Korea, *A. sinensis*, *A. acutiloba,* and *A. gigas* were used as medicines respectively. Z-ligustilide was used as the reference compound to determine the content of butylphthalide, 3-butylene 4-hydroxylphthalide, senkyunolide A, F, H, I, E-ligustilide, 6,7-epoxyligustilide, and 6,7-dihydroxyligustilide [11]. It was found that the contents of each component in the three kinds of Angelica were significantly different, indicating that their clinical therapeutic effects should be significantly different.

Using curcumol as the reference compound, 10 sesquiterpenes in three species plants of *Curcuma* genus used as *Ezhu*, i.e. *C. wenyujin*, *C. phaeocaulis,* and *C. kwangsiensis* were determined, including germacrene D, curcumene, γ-elemene, furadienone, isocurcurenol, furadiene, gemmacone, curcumedione, curcumenol, and neocurdione. The results showed that there were significant differences in the sesquiterpene components of *Ezhu.* Cluster analysis showed that *C. wenyujin* and *C. phaeocaulis* belonged to each individual category, while *C. kwangsiensis* belonged to the two categories, respectively, suggesting that the TCMs *Ezhu* can be divided into two types of chemical characteristics according to the differences in varieties and origins. Furadienone, gemmacone, curcumadione, curcumenol, and neocurdione can be used as quality markers of *Ezhu* [12]. The direct quantitative method of analogue as reference has also been used in the quality control research of Chinese medicines *Pogostemon cablin* [13], *Cyperus cyperi* [14], *Curcuma longa* [15].

3.2 Digital or Virtual Reference Compounds

The lack of chemical reference compounds of TCMs is one of the bottlenecks in the development of quality control of TCMs. At present, there are more than 500 statutory chemical reference compounds from TCMs, which are far from meeting the

needs of their quality control. The 2020 edition of "Chinese Pharmacopoeia" contains 616 medicinal materials and decoction pieces, 47 vegetable oils and extracts, and 1607 prescriptions or single herbal preparations. Due to the complexity of chemical components, the chemical reference compounds of TCMs are basically extracted, separated, and purified from raw materials. In addition, the content of chemicals is relatively low, so it is quite difficult to extract a certain amount of pure single compound. Although scientific research project can solve the technical problems of separation and purification to a certain extent, it cannot ensure long-term and stable compensation for the consumption of reference compounds, and cannot fundamentally solve the problem of insufficient chemical reference compounds of TCMs. For this reason, we once proposed the industrialization strategy of chemical reference compounds for TCMs, aiming at fundamentally solving the problem. However, the high-purity chemical components from TCMs often have poor stability. In particular, medicinal materials containing volatile oils, which account for about 20% of the varieties recorded in Chinese Pharmacopoeia, often contain light or thermally unstable components. Even if they are sealed and stored at low temperature, their structures are easily changed. For example, aucubin will turn black when stored at room temperature for several months, and the purity of ar-tumerone will drop significantly after being stored at low temperature (−20 °C) for less than two months, making it unusable. Therefore, in view of the many technical obstacles in the preparation and storage of chemical reference compounds of TCMs, the development strategy aimed at providing physical pure compounds has the defects of high investment, long cycle, and low feasibility, and it is difficult to make a breakthrough in the short term. With the rapid progress, the research on chemical reference compounds of TCMs urgently needs to find another way, such as the application of mixed reference compounds and alternative reference compounds.

In clinic, TCMs containing volatile oil components such as Angelica, Chuanxiong, and turmeric play an important role. Of 616 medicinal materials and decoction pieces recorded in the Chinese Pharmacopoeia 2020, 55 have volatile oils and volatile components as the main active ingredients, and 10 commonly used volatile oil extracts such as patchouli oil, zedoary oil, cinnamon oil, and so on. Due to the difficulty in the separation of volatile oil components and poor stability, the lack of chemical reference compounds for volatile oil components in TCMs has greatly limited the improvement of the quality control level of TCMs containing volatile oils. In fact, gas chromatography-mass spectrometry (GC–MS) is a commonly used method for analyzing volatile oils. Its advantage is that it can use EI-MS information combining with standard mass spectral libraries (such as NIST-MS Library, etc.) for compound characterization, qualitative analysis, in the absence of reference compounds. The digital mass spectral library can effectively solve the problems of repeated preparation and difficult storage of physical chemical reference compounds. However, the MS data of the volatile components from TCMs, especially their characteristic components, collected in the commercial MS Library are very limited, which cannot solve the problem of reference compounds shortage in GC–MS analysis of TCMs. Therefore, the establishment of the EI-MS database of characteristic volatile components of TCMs, that is, the digital Chinese herbal volatile chemical

library, is an effective way to solve the shortage of reference compounds for quality control of Chinese medicines containing volatile components.

Four oils of *Curcuma* genus including *C. wenyujin*, *C. phaeocaulis*, *C. kwangsiensis,* and *C. longa* were extracted by supercritical fluid extraction and their main volatile components were fractioned by molecular distillation. The volatile components were further separated and purified by medium pressure column chromatography, countercurrent distribution chromatography, and other separation methods. Finally, 15 characteristic components were obtained. The isolated compounds were characterized by infrared spectroscopy and nuclear magnetic resonance spectroscopy, and their structures were identified by combining spectral analysis. According to the EI-MS data, AMDIS was used to establish MS data of the characteristic components in *Curcuma* genus plants.

C. wenyujin, *C. phaeocaulis*, *C. kwangsiensis,* and *C. longa* collected from three provinces in China were analyzed by HPTLC combined with DPPH bioautography. A semi-quantitative analysis method for antioxidant components in 4 *Curcuma* genus plants was established. Five sesquiterpenoids with antioxidant activity were confirmed by GC–MS analysis, and their raw materials from various origins were also evaluated [16]. GC–MS analysis method for the main volatile components in *Curcuma* and their adjacent n-alkanes was established, and the method validation was also carried out. The qualitative and quantitative parameters of the analytes were virtualized, and the accuracy of the method was verified. Qualitative and quantitative analysis of 8 main volatile components in 19 batches of *Curcuma* herbal materials was carried out by digital reference compounds method, and compared with the quantitative results by classical individual standard curve method, the results of most analytes were basically consistent.

3.3 Standardized Reference Extract Qualification and Single Analogue Quantification

The main challenge of "one test multiple evaluation method" or "one standard multiple measurement method" is the identification of chromatographic peaks. This problem can be overcome by using standard extract with the analytes. Therefore, the combination of qualitative standard extracts and direct quantification of analogue can effectively solve the qualitative and quantitative problems of TCMs quality control without individual reference compound [17].

Accuracy is very important for drug analysis, but the core goal of quality control of TCMs is to distinguish the authenticity and evaluate their quality. Due to changes in the origin and growth environment of TCMs, its component content often varies greatly. Therefore, inaccurate analysis results within a certain range will not affect the realization of the core goal of quality evaluation of Chinese medicines.

In order to solve the qualitative and quantitative problems of multi-components in TCMs, a novel strategy with standardized reference extract qualification and single

compound quantitative evaluation was used for the chromatographic peak identification and quantitative determination of five saponins, namely notoginsenoside R1 (N-R1), ginsenoside Rg1 (G-Rg1), ginsenoside Re (G-Re), ginsenoside Rb1 (G-Rb1), and ginsenoside Rd (G-Rd), in *Panax notoginseng* [17]. Quantification of 5 saponins using individual reference compound for quantification and using N-R1 as a single analogue to measure the other 4 ginsenosides (G-Rg1, G-Re, G-Rb1, and G-Rd) was performed and the results showed that their data obtained by the two methods were very similar, the maximum error is not more than 10%. Actually, although *P. notoginseng* is mainly produced in Yunnan, China, the influence of region and environment on its components content should be small, statistical analysis still shows that the proportions of samples beyond the range of 80–120% average contents of G-Rg1, G-Re, G-Rb1, and G-Rd were 26.1% (n = 153), 37.7% (n = 114), 33.3% (n = 153), and 47.1% (n = 140), respectively, much higher than the error of quantitative analysis directly use N-R1 as reference compound, and the correction coefficients of 4 saponins are 0.99–1.01. It is suggested that in the study of quality control of TCMs, due to their characteristics of large differences in the content of components, it is not necessary to pursue the accuracy of analysis too much, especially for components with good safety, direct estimation, and quantification of an analogue as reference compound can meet the requirements of quality control of TCMs.

For the analysis of saponins in *P. notoginseng*, we still have a way to obtain the components of each saponin, which makes it possible to determine the correction factor of alternative reference compound and analytes. For the quantitative analysis of polysaccharides, it is more common to have no reference substance. Due to the difficulty of separation and purification, and the polysaccharide has no definite molecular weight, it is difficult to exactly obtain the same polysaccharides in separation and purification. To solve the problem of quantitative analysis of polysaccharides without reference substances, a general method for the quantification of polysaccharides was proposed based on their increase in the refractive index (*dn/dc* value) without reference substances [18]. The *dn/dc* value is a basic characteristic parameter of a polymer. Like the specific gravity of an object, the weight of the object can be calculated when the specific gravity and volume are known. For a polymer, if its *dn/dc* value can be known and its signal response value on the refractive index detector (RID) can be measured, its content can be calculated. According to the published *dn/dc* value of polysaccharides, the average *dn/dc* value of polysaccharides is statistically calculated to be 0.151, which is used as the general *dn/dc* value of polysaccharides. The determined polysaccharides content is compared with its individual *dn/dc* value and its individual reference compounds. Their contents were very close to those measured by their standard curve method. Therefore, it is feasible to use 0.151 as the general *dn/dc* value to determine the polysaccharides content, which can fully meet the requirements for quality control of polysaccharides.

4 Conclusion

Quality control markers of TCMs should consider both active compounds and related components that affect the effect of the active ingredients, especially the related components which can better reflect the characteristics of the action of TCMs. Glycans, especially polysaccharides, are one of the main components of traditional Chinese medicine decoctions. They have multiple pharmacological effects and should be used as important marker for quality control of TCMs. For the same TCMs, the quality marker should be different with varied clinical purposes. Standardized reference extracts are easy to obtain, use, cheap, which is an important direction for the development of reference substances. For quality control of TCMs decoctions that can truly reflect the characteristics of TCMs, such as classic famous prescriptions and formula granules, existing quality control strategies and methods do not fully reflect the composition of water decoction, and the research needs to be strengthened. The quality control methods and technologies based on the water decoction, especially the analysis strategy, need to be vigorously developed.

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Chapter 2
Strategies for Quality Control of Polysaccharides in Chinese Medicines

Shaoping Li, Jing Zhao, and Chiwai Ip

Abstract Polysaccharides with multiple biological activities are usually considered as one of the major bioactive compounds in Chinese medicines (CMs). At present, the development of drugs and functional foods related to polysaccharides has attracted a great deal of attention due to their great potential effects and diverse action mechanisms. However, quality control of polysaccharides is the bottleneck and challenge due to their complexity and chemical diversity. Actually, the bioactivities of polysaccharides are closely related to their molecular structures. In order to ensure their safety and efficacy, the development of novel approaches based on the molecular structures for the improvement of quality control of polysaccharides is significantly important. Therefore, in this article, the relationship between biological activities and chemical structures, as well as the action mechanisms of polysaccharides from CMs were summarized first. Furthermore, saccharide mapping, a novel strategy for quality control of bioactive polysaccharides from CMs, was introduced and the application and perspectives were also discussed.

Keywords Strategies · Polysaccharides · Quality control · Saccharide mapping · Action mechanism

1 Introduction

Polysaccharides are chain-like structural substances formed by the condensation of 10 or more monosaccharides. Polysaccharides are widely distributed in animals, plants, and microorganisms, and play a vital role in maintaining life activities, which are known as the four basic substances of life together with protein, nucleic acid, and lipid. Studies have confirmed that polysaccharides, especially those which

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are water-soluble, often have important biological activities such as anticancer, immune potentiation, anti-oxidation, anti-coagulation, anti-inflammation, antiviral, anti-aging, blood sugar, and blood lipid reduction [1]. They are one of the most important efficacy components of Chinese medicines (CMs). In fact, the bioactivities of polysaccharides are closely correlated to their molecular structures (molecular weight distribution, constituent monosaccharides, types and configuration of glycosidic linkages, particle size, position of glycosidic linkages, solution chain conformation, etc.) [2]. Therefore, quality control of polysaccharides is essential for ensuring the efficacy and safety of CMs. However, quality control of polysaccharides has been a challenge because of their complicated structure and chemical diversity of polysaccharides.

In this chapter, we summarized the relationship between the biological activity of CMs polysaccharides and their molecular structures, proposed five mechanisms of action of polysaccharides in CMs, and clarified the necessity and importance of quality control of polysaccharides. On this basis, we introduced the quality control strategy of polysaccharides from CMs based on saccharide mapping, aiming to provide new ideas for their quality control research.

2 Biological Activity of Polysaccharides from CMs and Their Molecular Structures

The research on the biological activities and molecular structures of CMs polysaccharides in China and abroad has made great breakthroughs after the rapid development in recent decades. More and more medicinal plants and fungi polysaccharides have been studied. CMs polysaccharides, especially water-soluble polysaccharides, have very important and special biological activities and show good application prospects such as anticancer, immune potentiation, anti-oxidation, anti-coagulation, anti-inflammation, antiviral, blood sugar and blood lipid reduction. Table 1 summarizes the main biological activities and the molecular structures of polysaccharides from different CMs in recent years. In fact, the biological activities of polysaccharides are closely correlated to their physicochemical properties such as molecular weight, monosaccharide composition, types of glycosidic linkages, and higher order structure. For example, studies have shown that the antioxidant property of laminarin [3] and *Porphyridium cruentum* polysaccharides [4] increases as their molecular weight decrease. Whereas a decrease in the molecular weight of polysaccharides from *Monostroma latissimum* would reduce their anticoagulant activities [5]. Only the polysaccharides from mycelia of *Antrodia cinnamomea* (PMAC) with MW > 100 kDa are significant anti-angiogenic [6]. The type of glycosidic linkages also has an important effect on the biological activity of the polysaccharides. The majority chain types of bioactive polysaccharides from medicinal fungi are β-1, 3-glucan [7–9]. The biologically active polysaccharides of *Lycium barbarum* are mainly arabinogalactan-proteins complex [10–13]. The α-1, 4-D-galactosiduronic

and β-1, 4-mannosidic linkages could significantly affect the immunomodulatory activities of polysaccharides from *Gymnadenia conopsea* [14]. In addition, a number of specific monosaccharides play a decisive role in the biological activity of polysaccharides. For example, the immunomodulatory and anti-tumor activity of *Ganoderma lucidum* glycopeptide is closely related to fucose [15, 16]. In particular, the high-order structure of polysaccharides also affects its pharmacological activity [17]. For example, the antitumor activities of lentinan are closely related to its triple helix structure. Therefore, comparing and studying CMs polysaccharides and their molecular structures related to bioactivity, and establishing a quality control method for CMs polysaccharides based on their molecular structures are essential to ensure the safety and effectiveness of CMs polysaccharides, which is the basis for their quality control.

3 Action Mechanisms of CMs Polysaccharides

For a long time, there have been questions about the efficacy of polysaccharides taken orally based on the traditional understanding of the process in vivo. With the development of science and technology, the understanding of polysaccharide action has become more advanced. The presence of polysaccharide receptors in the body has been identified. Currently, the action mechanisms of polysaccharides in inhibiting the growth of tumor cells include at least: (1) Polysaccharides or their oligosaccharide fragments activate the immune system and release cytokines to phagocytose or kill tumor cells by binding to receptors on the surface of immune cells (Dectin-1, CR3 and TLR-2/6, etc.) [46, 47]. (2) Polysaccharides or their oligosaccharide fragments inhibit the growth of tumor cells by binding to cell growth factors (EGF, bFGF, etc.) or related enzymes (phosphatidylinositol-3-kinase, phosphorylated kinase, etc.) [48, 49]. (3) Polysaccharides or their oligosaccharide fragments bind to proangiogenic factor and proangiogenic factor receptors to regulate micro RNAs blocking angiogenesis to starving tumor cells, inhibiting tumor cell growth, or inducing apoptosis [50–53]. By synthesizing the results of modern studies, there are at least five action mechanisms for polysaccharides taken orally to exert their biological activities:

(1) A few polysaccharides or corresponding oligosaccharide active fragments are absorbed directly into the bloodstream, thus acting with target cells (e.g., macrophages and dendritic cells) [54–56].
(2) Polysaccharides or corresponding oligosaccharide active fragments pass through Peyer's patches or mesenteric lymph nodes directly [57] to activate intestinal adaptive immune response.
(3) Glycoproteins, glycolipids, and soluble oligosaccharides with similar glycan antigenic determinants with gastrointestinal epithelium recognize and bind pathogens, reducing attachment of pathogens to host cells and thereby reducing the risk of host cell infection [58, 59].

Table 1 Summary of bioactivities and chemical structures of polysaccharides from representative CMs

Sources of Polysaccharides (literature)	Molecular weight distribution (Da)	Main compositional monosaccharides	Main glycosidic bond types	Pharmacological effects[a]							
				1	2	3	4	5	6	7	8
Panax ginseng [18, 19]	3.2×10^3–4.0×10^5	Glc, Gal, Ara, Rha, GalA, GlcA	1,(3)(4)(6)-Glc, 1,(3)(5)-Ara, 1,(2)(4)-Rha, 1,(3)(4)(6)-Gal, 1,4-GalA, 1,4-GlcA	+	+	+		+	+	+	+
Panax quinquefolius [20, 21]	3.1×10^3–8.5×10^4	Glc, Gal, Man, Ara, GalA	1,4-Glc, 1,4-Gal, 1,(2)(4)-Rha, 1,4-GalA,	+	+	+	+	+	+	+	
Angelica sinensis Diels [1, 22]	5.1×10^3–5.9×10^5	Glc, Gal, Man, Ara, Rha, GalA, GlcA	1,(3)(4)(6)-Glc, 1,(4)(6)-Gal, 1,5-Ara, 1,2-Rha, 1,4-GalA	+	+	+	+	+		+	+
Lycium barbarum fruit[23]	1.0×10^4–2.3×10^6	Glc, Gal, Man, Ara, Rha, Xyl, GalA, Fuc	1,(3)(4)(6)-Glc, 1,(3)(4)(6)-Gal, 1,5-Ara, 1,4-GalA	+	+	+		+		+	+
Dendrobium sp. [24, 25]	3.0×10^3–1.0×10^6	Glc, Gal, Man, Xyl, Ara, Rha, GalA	1,(2)(3)(4)-Man, 1,(4)(6)-Glc, 1,(3)(6)-Gal, 1,2-Rha, 1,4-GalA	+	+	+	+	+	+		+
Astragalus membranaceus [26, 27]	8.7×10^3–4.8×10^6	Glc, Gal, Xyl, Ara, Rha, GalA	1,(3)(4)-Glc, 1,(3)(4)(6)-Gal, 1,5-Xyl, 1,5-Ara, 1,2-Rha, 1,4-GalA, 1,4-GlcA	+	+	+	+	+	+		+
Aloe vera [28–30]	1.0×10^4–5.7×10^6	Glc, Gal, Man, Ara, Xyl, Rha, GalA, GlcA	1,4-Glc, 1,4-Man, 1,4-Gal	+	+	+	+	+	+	+	+
Ginkgo biloba sarcotesta [1, 31–33]	3.4×10^3–9.5×10^5	Glc, Gal, Man, Rha, Xyl, GalA	1,(4)(6)-Man, 1,(4)(6)-Glc, 1,(4)(6)-Gal, 1,4-GalA	+	+	+	+	+			
Achyranthes bidentata Blume [34, 35]	1.4×10^3–5.2×10^3	Glc, Fru	2,(1)(6)-Fru, 2,1-Glc	+	+	+		+	+		

(continued)

Table 1 (continued)

Sources of Polysaccharides (literature)	Molecular weight distribution (Da)	Main compositional monosaccharides	Main glycosidic bond types	Pharmacological effects[a]							
				1	2	3	4	5	6	7	8
Cordyceps [36, 37]	6.0×10^3–1.1×10^6	Glc, Gal, Man	1,(3)(4)-Glc, 1,(2)(4)(6)-Man, 1,(4)(6)-Gal,	+	+	+		+		+	+
Ganoderma lucidum [9, 38]	5.2×10^3–2.5×10^6	Glc, Gal, Man, Fuc, Ara, Xyl	1,(3)(4)(6)-Glc, 1,(4)(6)-Gal, 1,2-Fuc, 1,(2)(4)-Man	+	+	+			+		+
Coriolus versicolor [39–41]	3.0×10^3–2.3×10^6	Glc, Gal, Man, Fuc, Rha, Xyl	1,(3)(4)(6)-Glc	+	+	+	+	+	+		+
Lentinula edodes [7, 42]	3.0×10^5–1.8×10^6	Glc. Gal, Ara, Rha, Xyl	1,(3)(6)-Glc, 1,6-Gal,	+	+	+			+		
Marine algae [43–45]	2.0×10^4–2.0×10^6	Glc, Gal, Man, Ara, Xyl, Fuc, Rha, GlcA	1,(2)(3)(4)-Rha, 1,(3)(4)-Xyl, 1,(3)(4)-Glc, 1,(2)(4)-Man, 1,(3)(4)(5)-Ara, 1,(3)(6)-Gal, 1,(3)(4)-Fuc, 1,2-GlcA	+	+	+	+	+	+		+

[a]1, anticancer; 2, immunomodulatory activities; 3, anti-oxidation; 4, anti-inflammation; 5, blood sugar and blood lipid reduction; 6, Antibacterial and antivirus; 7, Anti-radiation; 8, Liver/kidney/stomach protective effect

(4) Polysaccharides metabolized by gut microbiota to short-chain fatty acids (acetate, propionate, butyrate, etc.) regulate immune response [60, 61].
(5) Polysaccharides could induce the growth of symbiotic bacteria by acting as prebiotics for gut microbiota, which are beneficial to host health [61–63].

Besides exerting pharmacological effects through direct actions mentioned above, CMs polysaccharides also play other important roles in treating diseases. For example, enhancing or reducing the toxicity of small molecule compounds [64], acting as natural stabilizers, solubilizers, and natural drug carriers for other active ingredients [65, 66]. Therefore, investigating the effects of CMs polysaccharides helps further to elucidate the overall effects of CMs. At present, diverse biological activities and multi-pathway action mechanisms of CMs polysaccharides have made the development and research of polysaccharide-based drugs and healthcare products a hot spot in the fields of new drug development. However, quality control is a bottleneck. The improvement of quality control research for CMs polysaccharides is crucial for the future development of new polysaccharide-based products.

4 Establishment of Saccharide Mapping Method

CMs polysaccharides quality control includes qualitative analysis and quantitative detection. Due to the complexity of polysaccharides, establishing simple and rapid qualitative and quantitative methods with good accuracy and high specificity has been the key and bottleneck for quality control of CMs polysaccharides. Traditional methods of polysaccharides identification include isolation and purification, purity identification, and a range of chemical structural characterization (molecular weight, monosaccharides compositions, types and configuration of glycosidic linkages, solution chain conformation, stereochemistry, rheological properties and thermal stability analysis) [7, 67]. Despite the method being accurate and reliable for qualitative identification of polysaccharides, it is excessively cumbersome, difficult, and time-consuming to operate in daily quality control studies of CMs polysaccharides [2]. Although qualitative analysis based on chromatographic characteristics of partial acid hydrolysate [68–70] and quantitative analysis based on complete acid hydrolysis [1, 71, 72] for polysaccharides have been widely used, the specificity and accuracy are poor [73, 74]. In view of this, our group proposed a strategy of saccharide mapping for the qualitative analysis and quantitative detection of polysaccharides [75–77]. The method combines bioactive polysaccharides analysis and a series of localized enzymatic digestion coupled with chromatography (HPSEC, HPTLC, PACE, etc.) [14, 78]. Qualitative and quantitative analysis of polysaccharides based on bioactive structural features can be realized and successfully applied to the quality control of CMs polysaccharides and their products [79–87]. Figure 1 shows the schematic procedure of saccharide mapping [2]. First, establish a characteristic chromatogram of polysaccharides before localized enzymatic digestion using chromatographic techniques (Step 1); then, localized and hydrolyze polysaccharides using glycosidases

and discriminate polysaccharides based on their response to the selected enzymes (Step 2); separate and analysis polysaccharide enzymatic hydrolysates by chromatography, then, set the stable and specific polysaccharide hydrolysis fragments as the indicator, which enables qualitative and quantitative analysis of polysaccharides. Compared with other qualitative and quantitative methods for polysaccharides, saccharide mapping uses enzyme-catalyzed hydrolysis, which has the advantages of good selectivity, high specificity, mild reaction conditions, and stable products, making it an efficient and specific strategy for polysaccharide quality control.

STEP I chromatograms of polysaccharides with the same molecular weight and compositional monosaccharides but different glycosidic linkages before enzymatic digestion;

STEP II discrimination of polysaccharides based on their response to the selected enzymes;

STEP III discrimination of polysaccharides based on their enzymatic hydrolysates.

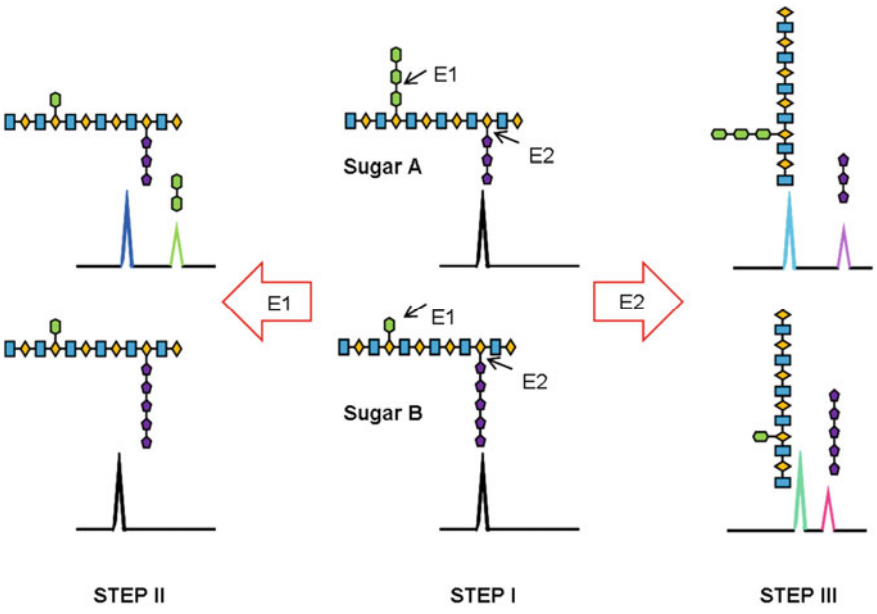

Fig. 1 The schematic procedure of saccharide mapping (Modified from Ref. [2] with permission of John Wiley and Sons)

5 Application of Saccharide Mapping

5.1 Qualitative Analysis

Based on the differences in the response of polysaccharides to hydrolysis by different enzyme localizations and the chromatographic characteristics of the hydrolysate, differential analysis of polysaccharides from different sources can be achieved. At present, there are saccharide mapping based on fluorescence-assisted gel electrophoresis (PACE), high-performance thin-layer chromatography (HPTLC), and high-performance liquid chromatography (HPLC). In particular, PACE is a convenient technique for the separation of polysaccharide enzymatic products with high resolution, reproducibility, stability, and simultaneous analysis of multiple samples [83]. Since saccharides do not carry UV or fluorescent groups, derivatization of hydrolysate is usually required before PACE analysis. 8-Aminonaphthalene-1, 3, 6-trisulfonic acid disodium salt (ANTS) and 1-aminopyrene-3, 6, 8-trisulfonic acid (APTS) are derivatization reagents for reducing terminus of glycans, both of the reagents have UV and fluorescent groups with high sensitivity. We analyzed the polysaccharide enzymatic products of different sources of *Cordyceps* [81], *Cordyceps militaris* [78], *Dictyophora indusiate* [80], *Hericium erinaceus* [79], *Gymnadenia conopsea* [14], *Lycium barbarum,* and *Ganoderma* [83] using PACE saccharide mapping. The results were useful for differentiation and identification of polysaccharides from different sources of CMs. Figure 2 is PACE fingerprints of β-1, 3-glucanase and pectinase digested polysaccharides from different Linzhi, and PACE fingerprints of α-amylase and β-glucanase digested polysaccharides from different Chongcao, respectively. The results showed that based on β-1, 3-glucanase and pectinase digested polysaccharides of *Linzhi* (*Ganoderma sinense* and *G. lucidum*) polysaccharides (Fig. 2a, b), identification of different *Linzhi* adulterants could be achieved. Based on α-amylase and β-glucanase digested polysaccharides from natural *Cordyceps* (Fig. 2c, d), their identification also could be achieved. In addition, by comparing bioactivities of polysaccharides before and after hydrolysis by different enzyme localizations, main glycosidic bond types affecting biological activities of polysaccharides can be clarified, structural characteristics analysis that related to polysaccharide activities can be realized, which is beneficial to further improve the quality control of bioactive CMs polysaccharides. Immunomodulatory activity of polysaccharides (GC6P-M and GC6P-G) of *Gymnadenia conopsea* (GC6P) enhanced by digested with β-D-mannanase (GC6P-M) and β-D-glucanase (GC6P-G) (Fig. 3a), but reduced by digested with endo-arabinanase (GC6P-E) and pectinase (GC6P-P) (Fig. 3a). The result indicates that glycosidic bonds α-1, 4-D-galactosiduronic, β-1, 4-mannosidic, α-1,5-arabinose, β-D-glucanase of polysaccharides from *Gymnadenia conopsea* are related to their immunomodulatory activity. Especially, α-1, 4-D-galactosiduronic and β-1, 4-mannosidic glycosidic bonds significantly affect the immunomodulatory activity of polysaccharides from *Gymnadenia conopsea*. Figure 3b shows the PACE fingerprints of GC6P hydrolysates by different glucosidase (β-D-mannanase, β-D-glucanase, endo-arabinanase and

pectinase). The results showed significant differences between the products [14]. This method was also successfully used for the bioactive structural characterization analysis for cultured *Cordyceps militaris* polysaccharides from different origins [78]. Immunomodulatory effect of *Cordyceps militaris* polysaccharides was found to be closely related to their 1, 4-α-D-glucosidic and 1, 4-β-D-glucosidic linkages.

Although PACE saccharide mapping has a good separation effect and sensitive fluorescence detection, the gel needs to be ready to use, which is not conducive to improving reproducibility. HPTLC is also simple, rapid, efficient, and sensitive. The sensitivity of detection with the aniline-diphenylamine colorimetric method is better than that of refractive index detection (RID) and evaporative light scattering detection (ELSD) commonly used in liquid chromatography [88]. Multiple samples can be analyzed simultaneously to facilitate the comparison of results [1]. Saccharide mapping based on HPTLC can monitor the response characteristics of polysaccharides to glycosidases and analyze hydrolysate simultaneously. Glucosidase digestion profiles and characterization of enzymes digested products of polysaccharides from 3 *Panax* species: *P. ginseng*, *P. quinquefolium*, and *P. notoginseng*, showed that

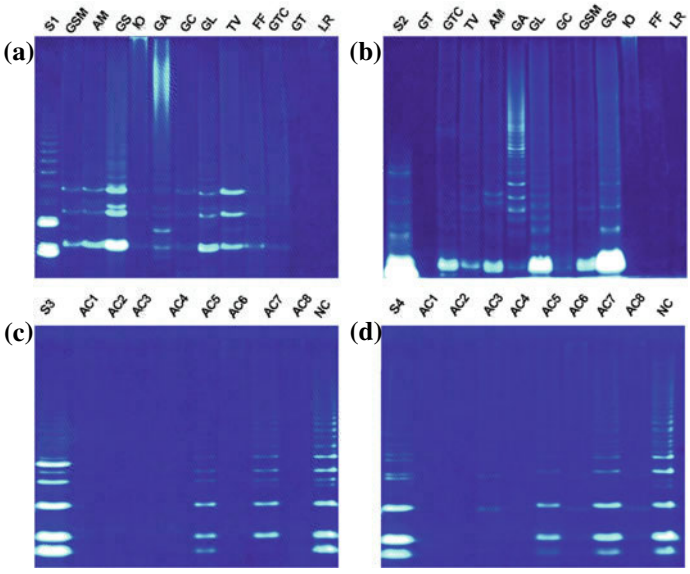

Fig. 2 PACE fingerprints of enzymatic hydrolysates of polysaccharides from *Lingzhi* (A and B) and *Chongcao* (C and D) **a**, **b** PACE fingerprints of β-1, 3-glucanase and pectinase digested polysaccharides from different Linzhi; **c**, **d** PACE fingerprints of α-amylase and β-glucanase digested polysaccharides from different Chongcao; GSM, *Ganoderma sessile*; AM, *Amauroderma* spp.; GS, *G. sinense*; IO, *Inonotus obliquuus*; GA, *G. applanatum*; GC, *G. capense*; GL, *G. lucidum*; TV, *Trametes versicolor*; FF, *Fomes fomentarius*; GT, *G. tropicum*; GTC, cultural *G. tropicum*; LR, *Lignosus rhinoceros*; AC1 and AC8, *Cordyceps gunnii*; AC2 and AC4, *C. liangshanensis*; AC3 and AC6, *C. hawkesii*; AC5, *C. gracilis*; AC7, *C. ciecadae*; **S1–S4**, enzymatic digestions of dextran, polygalacturonan, starch and oat glucan used as markers (Modified from Ref. [81] and [83] with permission of Elsevier)

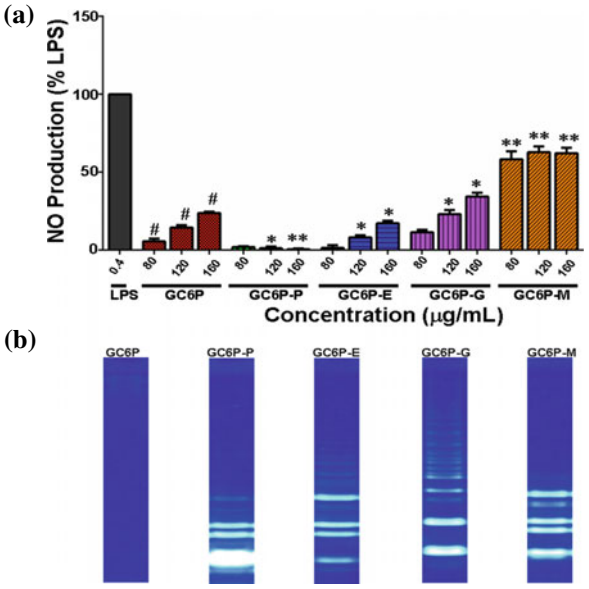

Fig. 3 Effects of glycosidic linkages on the macrophage functions and PACE fingerprints of selected enzymes digested polysaccharides from *Gymnadenia conopsea*. (Modified from Ref. [14] with permission of Elsevier). **a** Effect of glycosidic linkages on the nitric oxide (NO) released from RAW 264.7 cells; **b** PACE fingerprints of pectinase (GC6P-P), endo-arabinanase (GC6P-E), β-D-glucanase (GC6P-G) and β-D-mannanase (GC6P-M) digested polysaccharides from *Gymnadenia conopsea*, respectively; For the NO production, #$p < 0.05$ versus blank control; *$p < 0.05$, **$p < 0.001$ versus GC6P group

enzymatic reactions and enzymes digested polysaccharides by cellulase, dextranase, α-amylase, isoamylase, pectinase were basically the same, which revealing that *P. ginseng*, *P. quinquefolium*, and *P. notoginseng* have similar glucan and pectin type polysaccharides [82]. The result can provide a reference for the utilization of polysaccharides from *P. ginseng*, *P. quinquefolium*, and *P. notoginseng*. However, HPTLC is weak for the separation of saccharides with a high degree of polymerization (DP > 15) [1]. Although PACE was able to separate polysaccharides above DP > 40 [89], none of the monosaccharides were separated by PACE assisted by ANTS derivatizing reagent. The combination of PACE and HPTLC allows a more comprehensive analysis of the hydrolysate characteristics of polysaccharides. PACE and HPTLC were used to comparatively study the pectinase-digested polysaccharides from different sources *Cordyceps* [81] and from different origins of *Lycium barbarum* [90]. The results show that PACE is suitable for the analysis of polysaccharides, while HPTLC is suitable for the analysis of oligosaccharides and monosaccharides (Fig. 4). This method has the advantages of simplicity and practicality, good reproducibility, high sensitivity, and high throughput, and is expected to become a routine analytical method for quality control of polysaccharides from Chinese medicines.

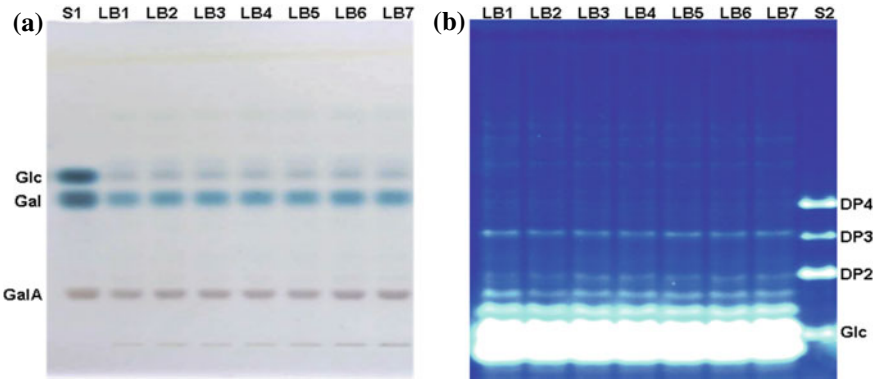

Fig. 4 HPTLC (**a**) and PACE (**b**) fingerprints of pectinase digested polysaccharides from *Lycium barbarum*. (Modified from Ref. [90] with permission of Elsevier). **LB1–LB7**, Lycium barbarum collected from different places in China; **S1**, the mixture of glucose (**Glc**), galactose (**Gal**), and galacturonic acid (**GalA**); **S2**, the mixture of laminaritetraose (**DP4**), laminaritriose (**DP3**), laminaribiose (**DP2**), and glucose (**Glc**)

HPLC is the most commonly used technique for the separation and analysis of natural compounds. According to approximately 750 papers on analysis of the chemical composition of medicinal and food plants in the past years, the use of liquid chromatography exceeds 50% [91]. The common method of LC separation for polysaccharides is size exclusion chromatography (SEC) or gel permeation chromatography (GPC), which is unable to separate polysaccharides and their hydrolysate at the same time due to its weak separation capacity. In addition, as polysaccharides have no UV absorption, sensitivity of ELSD and RID detection with them is relatively low and are usually used to identify response characteristics of polysaccharides to glycosidases. With the improvement of separation materials, HPLC, especially HPLC-MS, will play an important role in the analysis of enzymatic products by saccharide mapping. Saccharide mapping coupled with HPSEC-ELSD and HPLC-DAD-MS has been used to compare the chemical structural characteristics of natural *Cordyceps* polysaccharides with those of cultural. The results show that natural and cultured *Cordyceps* polysaccharides have similar enzymatic response characteristics. They mainly contained (1 → 4)-β-D-glucosidic linkages, (1 → 4)-α-glucosidic, (1 → 6)-α-glucosidic, and 1, 4-β-D-mannosidic. However, there are differences between natural and cultured *Cordyceps* polysaccharides and pectinase hydrolysates. Natural and cultured *Cordyceps* polysaccharides could be discriminated on the basis of HPLC profiles of pectinase hydrolysates [87]. Saccharide mapping based on HPSEC-ELSD was also successfully applied to compare and evaluate the product quality of different sources of polysaccharides from *Dendrobium* and *Ganoderma* [85, 92]. Studies show that the bioactivities of polysaccharides are closely related to their molecular weight, particle size and solution chain conformation. Although HPSEC-RID/ELSD can determine the molecular weight of polysaccharides, accurate molecular weight of polysaccharides without appropriate reference polysaccharides is difficult to obtain.

The accuracy of the determination can be significantly affected by the difference in molecular geometries between testing polysaccharides and the reference polysaccharides. In recent years, multi-angle laser light scattering (MALLS) has been rapidly developed for polymer characterization. HPSEC-MALLS-RID can directly detect the average molecular weight (Mw), particle size, and solution chain conformation of polysaccharides. Results of saccharide mapping based on HPSEC-MALLS-RID analysis shows that although lentinan injections produced by different companies in China all contained β-1, 3-d-glucanase, there are large differences in molecular weight distribution, particle size, and high-order structure between different companies and even different batches of lentinan injection from the same company [84]. It is suggested that the quality consistency of domestic lentinan injection is poor and the quality evaluation method needs to be improved urgently to ensure the safety and efficacy of lentinan polysaccharide injection.

5.2 Quantitative Analysis

Quantitative analysis methods of polysaccharides are mainly divided into colorimetry, high-performance liquid chromatography, and gas chromatography. The commonly used colorimetric methods are phenol-sulfuric acid, anthrone sulfuric acid, carbazole-sulfuric acid, and m-hydroxybiphenyl. The first two methods are mainly used for the determination of total reducing sugars, while the latter two are mainly used for the determination of acidic saccharides (alduronic acid). The accuracy of carbazole-sulfate method is easily affected by neutral saccharides, while m-hydroxybiphenyl method is not affected [93]. Currently, phenol-sulfuric acid is the most common method for quantitative determination of polysaccharides and is widely used for analysis of polysaccharides in CMs [73]. However, the accuracy of the phenol-sulfuric acid method, which uses glucose as a reference for quantification, is often significantly affected by the compositional monosaccharides of polysaccharides to be measured [77]. High-performance liquid chromatography and gas chromatography are also used for the quantitative analysis of polysaccharides. The polysaccharide content is calculated by measuring the total monosaccharide content released from complete acid hydrolysis of polysaccharides, which is more sensitive, stable, and reproducible compared with colorimetric method. However, acid hydrolysis conditions can greatly affect the result. In order to obtain satisfactory results, it is necessary to optimize acid hydrolysis conditions for different polysaccharides individually. In principle, the best condition is the complete hydrolysis of polysaccharides without degradation of compositional monosaccharides in the shortest time. No matter colorimetric, high-performance liquid chromatography, or gas chromatography are used to determine total content of saccharides. Since the biological activity of polysaccharides is closely related to their molecular weight and distribution, it is necessary to establish a rapid method for the determination of polysaccharides and their different fractions. Although HPSEC-RID and HPSEC-ELSD combined with corresponding standard curves of polysaccharides enable content determination of