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Recent development in liquid chromatography stationary phases for separation of Traditional Chinese Medicine components

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ABSTRACT

Traditional Chinese Medicine (TCM) is an ancient medical practice which has been used to prevent and cure diseases for thousands of years. TCMs are frequently multi-component systems with mainly unidentified constituents. The study of the chemical compositions of TCMs remains a hotspot of research. Different strategies have been developed to manage the significant complexity of TCMs, in an attempt to determine their constituents. Reversed-phase liquid chromatography (RPLC) is still the method of choice for the separation of TCMs, but has many problems related to limited selectivity. Recently, enormous efforts have been concentrated on the development of efficient liquid chromatography (LC) methods for TCMs, based on selective stationary phases. This can improve the resolution and peak capacity considerably. In addition, high-efficiency stationary phases have been applied in the analysis of TCMs since the invention of ultra high-performance liquid chromatography (UHPLC). This review describes the advances in LC methods in TCM research from 2010 to date, and focuses on novel stationary phases. Their potential in the separation of TCMs using relevant applications is also demonstrated.

1. Introduction

Traditional Chinese Medicines (TCMs) have a broad range of medical applications, which were developed in ancient China, based on a tradition of more than 2000 years. TCMs are widely used to prevent and cure illness in China, and have attracted worldwide interest in recent years, due to their mild efficacy and fewer side effects. It was estimated that TCMs are used by more than 1.5 billion people worldwide [1]. TCMs are multi-component systems produced from plant materials, containing hundreds or even thousands of compounds [2–4]. However, there has been very limited knowledge known on their properties, which is a bottleneck to the modernization and globalization of TCMs. The investigation of chemical compositions of TCMs remains a research hotspot.

The separation of TCMs plays a vital role in the phytochemical identification. Various separation strategies have been used in this area [5–8]. High performance liquid chromatography (HPLC) is perceived to be one of the most powerful separation techniques, providing many advantages over other chromatographic methods, such as high capacity and good reproducibility [9]. Common chromatographic techniques include reversed-phase liquid chromatography (RPLC), normal-phase liquid phase (NPLC), hydrophilic interaction liquid phase (HILIC), and ion-exchange chromatography (IEC). These chromatographic techniques are capable of offering different separation selectivities, which enables more flexible separation. HPLC is currently used extensively in the separation of TCMs [10–14].

Stationary phases, the core of HPLC technology, are the foundation of chromatographic methods. To date, conventional RP C18 stationary phases have been the most commonly used to isolate TCM compounds [13,15–18]. Nevertheless, these phases are unable to provide satisfactory separation, such as insufficient resolution and peak capacity. This is mainly due to the limited selectivity. In recent years, there has been intense research into the development of selective stationary phases to offer an alternative but complementary separation to conventional RPLC stationary phases. And two-dimensional liquid chromatography (2D-LC) provides a great increase in separation ability and peak capacity. The selective stationary phases are useful to the construction of orthogonal multidimensional systems for TCM research [19,20]. Moreover, high-efficiency separation has become an important trend in the analysis of TCMs. high-performance liquid chromatog-

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raphy (UHPLC) with sub–2 μm particle columns are increasing concerned in this area [21–24]. In this article, we review the recent advances in LC methods in TCMs from 2010 up to date, and focus on novel LC stationary phases. The potential of these phases is demonstrated with relevant applications.

2. Selective stationary phases for TCMs

2.1. HILIC stationary phases

2.1.1. Recent development in HILIC stationary phases

HILIC, suggested by Alpert in 1990 [25], is a chromatographic technique in which analytes interact on a hydrophilic stationary phase and are eluted with a relatively hydrophobic binary eluent. In HILIC, water is the stronger eluting member (the main component is usually being 5%–40% water in acetonitrile). HILIC has been steadily gaining interest for its different separation selectivity to RPLC, and has emerged as a potent chromatographic method for polar analytes [26–28].

HILIC stationary phases are usually silica-based where the surface is modified by polar functional groups [29]. In the early days, HILIC was considered as "high aqueous NP" chromatography, and thus the stationary phases were all based on underivatized, aminopropyl-bonded, and diol-bonded silica etc. However, problems concerning hydrophilicity, selectivity, and stability were encountered. In recent years, the development of special separation materials for HILIC has attracted more and more attention [30]. The number of commercially available columns designed especially for HILIC is growing, such as amide-based, poly (succinimide)-based, saccharides-based, and zwitterion-based stationary phases. In 2014, our group reviewed the development and application of HILIC stationary phases [31].

Recently, Qiao et al. [32] prepared an imidazolium-embedded *N*,*N*-dimethylamiopropyl functionalized silica-based stationary phase (Sil-ImCl), which was used for HILIC/RPLC mixed-mode chromatography. The synthesized stationary phase was successfully applied in the separation of nucleosides, water-soluble vitamins, phenols, and positional isomers. Xiao et al. [33] reported a HILIC stationary phase based on thiourea derivative modified silica. Thiourea derivative was grafted onto the surface of silica particles via a mild addition reaction between $-NH_2$ and $-SCN$. The stationary phase performed unique selectivity in the separation of polar and hydrophilic analytes. Zhang et al. [34] developed an arginine-functionalized stationary phase for HILIC by clicking arginine onto silica gel. This stationary phase provided good separation quality of organic acids and sugars. In the same group, multiple layers of polyvinyl alcohol (PVA) coating are generated onto silica gel by thermal immobilization to form a stationary phase used for HILIC. The synthesized stationary phase showed high efficiency and high chemical stability [35].

In our group, a controlled thiol-initiated surface polymerization strategy was developed and employed to prepare hydrophilic polymer stationary phases, which performed excellent chromatographic performance and protein non-fouling properties [36]. In addition, glyco-silica materials were prepared based on thiol-ene click chemistry between alkene-saccharides and mercapto-silica, which presented good HILIC separation [37].

2.1.2. Application of HILIC stationary phases in TCMs

Considerable interest in HILIC has inspired the development of chromatographic materials. HILIC stationary phases have gradually found applications in the separation and purification of chemical components from TCMs, including glycosides, oligosaccharides, steroids, and phenolic acids.

2.1.2.1. Glycosides. The polarity of stationary phases makes HILIC columns suitable to separate glycosides (e.g., saponin, quinochalcone C-glycosides and stevioside etc.) [38–40]. These compounds commonly consist of aglycones coupled to sugar chain units, resulting in good retention on HILIC stationary phases in most cases. Besides, due to the different retention mechanisms between HILIC and RPLC, the coupling of these two chromatographic modes performs excellent orthogonality for glycosides.

Saponins, typical glycosides, are the main ingredients in many TCMs [41]. These compounds possess diverse pharmacological activities [42,43]. Jin et al. [44] developed a comprehensive off-line 2D-HILIC/RPLC method to detect saponins in extracts of *Panax notoginseng,* based on an Acchrom XAmide column and an UHPLC BEH C18 column. The orthogonality of the 2D-HILIC/RPLC was up to 81%, and the peak capacity was 10,200. In total, 224 saponins were detected, and some of them were minor amounts. In our group, a 2D-RPLC/HILIC purification method was constructed to separate saponins from *P. notoginseng* [39]. Good column orthogonality was obtained by using a C18 column and an XAmide column. As a result, eight saponins, including two pairs of isomeric saponins and one new saponin, were isolated and identified.

Natural saponins have considerable structural diversity and many are structurally similar or even isomeric. A HILIC stationary phase (named Click XIon) was used to the selective separation of isomeric saponins [45]. Compared to other commercial HILIC columns, the Click Xlon column exhibited the strongest hydrophilic retention for a set of isomeric saponins, including ginsenoside Rc (S1), ginsenoside Rb-2 (S2), and ginsenoside Rb-3 (S3) (Fig. 1). Taking the extracts of *Panax notoginseng* as an example, eleven saponins, including three sets of isomeric saponins with one new saponin were purified on the Click Xlon column, and identified by MS and NMR.

Quinochalcone C-glycosides (QCGs) are a series of pharmacologically bioactive components in *Carthamus tinctorius* L. Guo et al. [40] established an offline 2D-HILIC/RPLC system for the characterization of QCGs in *C. tinctorius* L. by the combination of an Acchrom XAmide column and a BEH Shield RP-C18 column. The evaluation results showed that the orthogonality of this 2D-LC system was 71%, and the theoretical peak capacity reached 7654. Following coupling of the 2D-LC system and linear ion-trap quadrupole/Orbitrap mass spectrometry, 163 QCG homologs were putatively characterized from *C. tinctorius* L., and 149 of these were potentially new homologs.

Anthocyanins, a class of heterosides, are widespread in natural plants, conferring attractive colors (red and blue) to them. An HILIC method was developed to efficiently purify a challenging anthocyanin from *Lycium ruthenicum* Murray with a Click Xlon zwitterionic stationary phase [46]. The experimental data showed that the use of this HILIC column could improve separation resolution, and solved the co-elution problem of anthocyanin and non-anthocyanins in one-dimensional RPLC. Consequently, the target anthocyanin and three new alkaloids were isolated from *L. ruthenicum* for the first time.

Stevioside is the major sweet component present in *Stevia rebaudiana* Bertoni. A 2D-RPLC/HILIC system was established to comprehensively identify steviol glycosides from *Stevia rebaudiana*, based on an XCharge C18 column in first dimension and an XAmide column in second dimension [38]. 30 fractions were collected in first dimension of *Stevia* aqueous extract. Then fractions 1–20 were selected for further purification and 13 compounds with high purity were obtained in second dimension.

2.1.2.2. Oligosaccharides. Raffinose family oligosaccharides (RFOs) popularity as food ingredients has intensively increased, due to their fermentation effect in the large intestine. These analytes are polar, hydrophilic and highly branched, and thus are poorly

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