



Review

## Supercritical fluid chromatography in traditional Chinese medicine analysis



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### ABSTRACT

Traditional Chinese medicines (TCMs) are gaining increasing popularity throughout the world due to their long historical clinical practices. Highly efficient analytical separation tools are essential for investigating the mysterious properties of TCMs and their quality control. Supercritical fluid chromatography (SFC) showed a great potential in TCM analysis for both nonpolar and polar components. In this paper, an overview of the experimental conditions (i.e. detection mode, stationary phase, mobile phase composition, pressure and temperature) used in SFC for achiral separations of TCM components is presented and recent applications to the analysis of different classes of compounds extracted from TCMs, such as lipids, terpene and terpenoids, phenolic compounds, flavonoids, alkaloids, saponins and carbohydrates, will be briefly described.

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## 1. Introduction

Traditional Chinese medicines (TCMs) have been used in China for centuries due to their therapeutic efficacy and safety in the treatment of many diseases [1], and now they also attract worldwide attention as an important source of new bioactive compounds. It is generally believed that the active TCM components and their combined effects are responsible for the pharmacological and/or toxicological properties of TCMs. However, TCMs are very complex mixtures containing a high number of constituents with very different chemical structures, physicochemical properties and concentrations, such as phenols, flavonoids, anthraquinones, coumarins, alkaloids, organic acids, sugars and glycosides [2]. Therefore, highly efficient analytical tools are essential for isolating active compounds from TCMs and controlling their quality [3]. Various separation techniques, including reversed-phase liquid chromatography (RPLC) [4–6], capillary electrophoresis (CE) [7], normal-phase liquid chromatography (NPLC) [8], hydrophilic interaction liquid chromatography (HILIC) [9], ion-exchange chromatography (IEC) [10], gas chromatography (GC) and supercritical fluid chromatography (SFC) [11], have been applied in TCM analysis. Among these separation tools, LC is considered as the most useful technique for TCM analysis owing to its wide choice of stationary phases, the availability of different modes (RPLC, NPLC, HILIC, IEC), its high capacity, and good reproducibility, etc. [10]. However, the demand for orthogonal techniques remains high, which has largely fostered the search for other separation methods in this field.

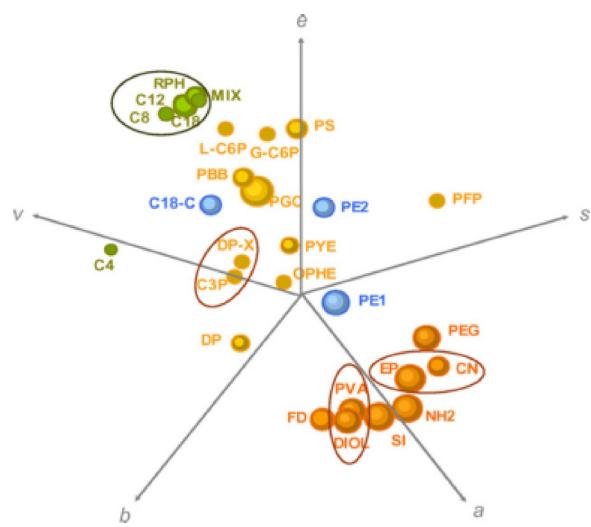
SFC, in which supercritical fluids (SF) with low viscosity and high diffusivity are used as mobile phase, such as supercritical carbon dioxide ( $s\text{CO}_2$ ), has showed great potential for the separation and isolation of active constituents in TCMs and natural products as an alternative technique to LC. This could be attributed to several interesting features of SFC, such as high separation efficiency, high mobile phase velocities and thus reduced analysis times, and eco-friendly characteristics. The application of SFC in TCM analysis could be dated back to the 1990s with the use of capillary SFC (cSFC) as an extension of GC [12]. Due to the limited application range and instrumental issues of cSFC, this research trend quickly faded away. With the development of advanced SFC instruments, the interest in SFC was revived in both the academic community and pharmaceutical industry. The revival of SFC started with chiral separations and preparative SFC (prep-SFC), and then extended to achiral separations, including TCM analysis. Nowadays, SFC coupled with different detectors has showed promising analytical applications to a wide range of TCM components, from nonpolar compounds like carotenoids, fatty acids, and terpenes, to more polar analytes like flavonoids, alkaloids, and carbohydrates.

The fundamentals of SFC including theory and instrumentation have experienced remarkable advances since it was first introduced by Klesper et al. in 1962 [13]. To date, difficulties with back-pressure regulation, consistent flow rates, modifier addition, sample injection, etc., have been largely resolved, thanks to the introduction of new state-of-the-art SFC systems by several important manufacturers [14]. Some reviews described extensively the history, theory and instrumentation of SFC [11,15–17]. This paper will outline the recent advances in achiral SFC for TCM analysis and focus on the important parameters for method development. An overview of relevant applications and potential prospects will also be given.

## 2. SFC method development for TCM analysis

### 2.1. Stationary phases

The complexity of TCM composition can make SFC separations quite challenging. Therefore, it is desirable to select an appropriate



**Fig 1.** Spider diagram for a five-dimensional representation of the solvation parameter models. Green points are non-polar alkyl phases, blue points are polar alkyl phases, yellow points are moderately polar aromatic phases and orange points are polar phases. Conditions: column temperature: 25 °C; backpressure: 150 bar; mobile phase:  $s\text{CO}_2$ -methanol = 90:10 (v/v); flow rate: 3 mL/min. Adapted from [28] with permission. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

stationary phase for obtaining satisfactory separation selectivity. A very wide range of stationary phases are currently available for SFC separation of TCM components, particularly bare silica [18] and silica-based phases modified by cyanopropyl [19], amino [20], diol and diethylamine (DEA) [21], etc. For instance, a bare silica column was used for analyzing triterpenoid saponins from *Ilex latifolia Thunb.*, *Panax quinquefolius L.* and *Panax ginseng C.A. Meyer* [18]. An amino column was applied to the analysis of magnolol and honokiol extracted from *Magnoliae cortex* [20]. Ganadera used an Acquity UPC<sup>2</sup> HSS C18 SB column for the SFC analysis of isoflavone constituents in *Glycine max*, *Trifolium pratense* and *Pueraria lobata* [22]. Additionally, alkyl-bonded stationary phases were used for the analysis of nonpolar analytes like carotenoids, fatty acids [23], and essential oils [17]. It is worth to mention the 2-ethylpyridine (2-EP) phase, which was specifically designed for improving the peak shape of strong basic compounds. This stationary phase was proved to be particularly suitable for the SFC analysis of alkaloids [24]. In order to further increase column efficiency and analysis speed, columns packed with sub-2 μm particles were also employed in SFC for TCMs analysis. Aichner et al. analyzed anthraquinones in rhubarb (*Rheum palmatum* and *Rheum officinale*) using an Acquity UPC<sup>2</sup> HSS C18 SB column (1.8 μm particles) [25]. In another report, an Acquity UPC<sup>2</sup> CSH Fluoro-Phenyl column (1.7 μm particles) was used to separate coumarins in the roots of *Angelica dahurica* [26]. Other approaches for column classification in SFC are also described in literature. Several research groups developed methodologies to characterize and classify the SFC columns according to their retention properties in order to provide guidelines for quickly and efficiently selecting the most suitable stationary phase [27]. Among these methodologies, the solvation parameter model (linear solvation energy relationship, LSER) is a widely used tool. West and Lesellier [28] compared and evaluated the specific retention properties of 28 different packed columns representative of three major types of stationary phases based on the isocratic retention data of 111 solutes including some major constituents of TCMs. By using solvation vectors and calculating of similarity factors between the different chromatographic systems, a five-dimensional classification based on LSER was performed (Fig. 1). This classification can be of great help in the selection of well suited stationary phase,

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