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REVIEW

Preparative High Performance Liquid Chromatography-based Multidimensional Chromatography and Its Application in Traditional Chinese Medicine

XIE Xiu-Man[1], SUN Wan-Yang[1], HUANG Jing-Yi[2], Navaneethakrishnan Polachi[2], TONG Ling[2,*], SUN Guo-Xiang[1,*]

[1] School of Pharmacy, Shenyang Pharmaceutical University, Shenyang 110016, China
[2] Tasly Academy, State Key laboratory of Core Technology in Innovative Chinese medicine, Tasly Pharmaceutical Analysis Institute, Tianjin 300402, China

Abstract: Separation of active components from Traditional Chinese medicine (TCM) is always difficult due to its complex matrix. The prep-HPLC-based multidimensional chromatography, which combined the characteristics of different separation techniques to improve separation capability and efficiency, is more conductive for separating and purifying complex TCMs. In this paper, we reviewed the basic principles, separation mode, key techniques of prep-HPLC-based multidimensional chromatography and its application in TCM research.

Key Words: Preparative high performance liquid chromatography; Traditional Chinese medicine; Multi-dimensional chromatography; Solvent compatibility; Interface technology; Review

1 Introduction

Traditional Chinese medicines (TCMs) are widely used in the prevention and treatment of many kinds of diseases[1]. Because the chemical composition of TCMs are extremely complex, rapid separation and purification techniques have a great importance for understanding the complex material basis and controlling the quality of TCM, indeed, discovering its potential active material as well as one of the main target in TCM research. As an amplification of analytical high performance liquid chromatography (ana-HPLC) system, the preparative high performance liquid chromatography (prep-HPLC) could not only ensure an ultra-high resolution as ana-HPLC, but also improve sample loads greatly. Consequently, highly purified target compounds could be obtained from TCMs. Currently, pre-HPLC was used for separating and purifying TCMs, biological medicines, biological products, food, etc[2–6]. However, due to the complexity of TCMs, the contents of different components are uneven in the sample, and many of the substances having hydrophobic, hydrophilic or structurally similar properties. In that case, the only use of prep-HPLC for one dimensional separation is usually difficult to meet the requirements of multi-components separation for complex samples[7,8]. Therefore, researchers have successfully constructed the multi-dimensional preparative chromatography system (prep-HPLC-based multi-dimensional chromatography) via combining different separation techniques. This kind of system could effectively solve the problems of separating and purifying of complex samples. Also it was used in the research of TCM, food, etc[9–11]. The principles, separation mode and key techniques of prep-HPLC-based multi-dimensional chromatography as well as its application on TCM research were discussed clearly in this paper.

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*Corresponding author. Email: gxswmwys@163.com
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2 Overview of pre-HPLC-based multi-dimensional chromatography

Natural products, TCM and metabolic products are always complex and multi-component. Traditional one-dimensional chromatography method cannot meet the needs of analysis and separation in a single run[12] owing to the limitation of peak capacity and resolution. According to Giddings's *et al*[13] research, total peak capacity of multi-dimensional separation system should be the arithmetic product of its various one-dimensional separation model. This kind of system makes the member of peaks that eluted from the column during a certain time achieve a certain degree of increasing. Based on this theory, multi-dimensional chromatography (MDC)[14] has got rapid development[7,15]. Prep-HPLC-based multi-dimensional chromatography was formed by combining other chromatographic techniques of different separation mechanism or different separation mode through a valve control or off-line operation. It is a new technique which can separate complex samples at a higher level of sample loads for multi-separation of different components in a single run. Moreover, it not only improve the peak capacity and orthogonality of the chromatographic system[7,15], but also reduce the overlap between chromatographic peaks[16]. Furthermore, this makes it possible for prep-HPLC-based multi-dimensional chromatography to separate and prepare multi-component samples, similar properties samples, or samples in different contents.

2.1 Separation mode

According to whether the elution fractions are continuously separated in the followed system or not, multi-dimensional chromatography could be divided into off-line and on-line mode. In off-line mode[17], the elution fractions are not for continuous separation directly. Off-line mode benefits wide selection of solvent, low instrument requirement, and high-peak capacity[18]. However, it has to suffer some drawbacks such as tedious operation, low-automatic, time-costing, and sample loss. In comparison with off-line mode, on-line mode can easily capture and enrich samples or target components by instrument system itself to realize multi-time separation[19]. On-line mode has a higher degree of automation, but the used solvents should be compatible with each other.

Both off-line mode and on-line mode are always combined in multi-dimensional preparative chromatography, which is more suitable for purifying chemical properties or structural similar samples. As the samples would loss in the process of multi-dimensional separation, adequate sample was proposed in multi-dimensional separation.

2.2 Column of preparative chromatography

The amount of sample loading in preparative chromatography is generally three to four magnitude higher than that in analytical chromatography. Prep-HPLC is not an amplification of ana-HPLC simply, but prep-HPLC and ana-HPLC could be related through a linear amplification coefficient. The basic hypothesis of linear amplification is that the chemical properties and mass transfer process of both ana-HPLC system and prep-HPLC system remain unchanged, whereas the sample quantity, flow velocity, collecting volume of prep-HPLC are multiplied for those of ana-HPLC by the linear amplification coefficient, which is the ratio of the cross-sectional area between preparation column and analysis column[20].

To reduce the solvent and sample consumption while developing a method, a same type of analytical column was firstly investigated. The condition of preparative column was then enlarged according to the following formula:

Amplification of sample load:

$$N = (D_2^2 L_2)/(D_1^2 L_1) \quad (1)$$

Amplification of flow velocity:

$$N = (D_2^2 F_1)/D_1^2 \quad (1)$$

Amplification of time:

$$T = NT_1 \quad (3)$$

where, N is the magnification factor; D_2 and D_1 are the diameter of the preparative column and the analytical column, respectively; L_2 and L_1 are the length of the preparative columns and analytical column, F_2 and F_1 are the flow velocity of preparative system and analytical system.

Selection of chromatographic column should prior consider the characteristics of a sample. As separating hydrophobic samples, reversed phase chromatographic column and hydrophilic chromatographic column are proposed. In contrast, for biological macromolecule samples, ion exchange chromatographic column is more suitable. Furthermore, hydrophobic effect columns are available for carbohydrates, and ion chromatographic columns are feasible for inorganic ions. In addition, gel chromatographic column can be used for synthetic polymer. Stereoisomerisms and racemes are proposed to be separated using cyclodextrin as stationary phase and chiral chromatographic column, respectively[21]. An experiment should be designed carefully in consideration of the characteristics of the sample, column and mobile phase to purify a sample in actual operation. Zhang *et al*[22] employed sodium bicarbonate buffer salt and 0.035% trifluoroacetic acid/water system as pH regulator. Thirty three monomeric compounds were purified from complex samples according to the different selectivities of chromatographic columns and samples under different pH values. Wei *et al*[23] developed a phenyl hexyl chromatographic column that showed a hydrophobic effect on triacylglycerols under acetonitrile-triacylglycerol system but a π-π bond interaction under methanol-triacylglycerol system. As a consequence, a variety of compounds were successfully purified from different cooking oil.