Journal of Chromatography A, 1371 (2014) 1–14

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Review

Application of two-dimensional chromatography in the analysis of Chinese herbal medicines

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ARTICLE INFO

Article history:
Received 1 June 2014
Received in revised form 7 October 2014
Accepted 25 October 2014
Available online 31 October 2014

Keywords:
Two-dimensional chromatography
GC × GC
LC–LC
LC × LC
Chinese herbal medicines

ABSTRACT

For the purpose of better understanding the complex Chinese herbal medicines (CHMs) and controlling their quality, powerful analytical techniques are essential. Although conventional one-dimensional (1D) chromatographic approaches have been widely used for the analysis of multiple components in CHMs, the complexity of CHM samples often exceeds the maximal capacity of any single separation mode. Therefore, in past decades, many researchers have attempted to explore the coupling of independent separation techniques to improve the resolving power for complex CHM samples. Two-dimensional (2D) separation systems, based on two independent columns with different separation mechanisms, have proven to be more powerful than 1D techniques and have been used successfully to separate and analyze CHM samples with excellent performance. This article aims to review the most recent advances in the strategies for analyzing CHMs using 2D chromatography. For this purpose, some remarkable applications of the commonly used couplings, mainly including 2D-GC and 2D-LC for analysis of CHMs, are described. Moreover, their major advantages and shortcomings are discussed, which might be helpful to the researchers who focus on quality control of CHMs.

Contents

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http://dx.doi.org/10.1016/j.chroma.2014.10.078
0021-9673/

1. Introduction

Chinese herbal medicines (CHMs), under the guidance of traditional Chinese medicine theory, have been widely used in China and some Asian countries to treat and prevent diseases for thousands of years. Unlike Western medicines, which usually contain a single chemical entity with clear efficacy, CHMs are rather complex samples containing a large number of compounds ranging from polar to nonpolar, from inorganic to organic, and from small molecules to macrobiomolecules. Therefore, for the purpose of better understanding the complex CHMs and controlling their qualities, powerful analytical techniques for adequately separating chemical constituents and tracking potentially bioactive components are essential.

Conventional one-dimensional (1D) chromatographic approaches such as thin-layer chromatography (TLC), gas chromatography (GC), liquid chromatography (LC), GC–mass spectrometry (GC–MS), LC–mass spectrometry (LC–MS), capillary electrophoresis (CE), capillary electrochromatography (CEC) and counter-current chromatography (CCC) have been widely used for analysis of multiple components in CHMs and have obtained satisfactory analytical results. Unfortunately, the complexity of CHM samples often exceeds the maximal capacity of any single separation mode, which suffers from problems of co-elution, low abundance or high background. As a consequence, in past decades, many researchers have attempted to explore the coupling of independent separation techniques to improve the resolving power for complex samples, like CHMs. Thus, two-dimensional (2D) separation systems, based on two independent columns with different separation mechanisms, have appeared. Because the maximal theoretical peak capacity of a 2D system is equal to the product of the individual dimensions of that system, 2D chromatography has proven to be more powerful than 1D techniques with respect to total peak capacity and resolution, as well as sensitivity.

To date, 2D chromatographic systems have been used successfully in the analysis of petroleum samples [1], environmental samples [2], food samples [3] and some other complex mixtures encountered in the biotechnology and life sciences. In recent years, with the requirements of completely profiling complex samples and tracking potentially bioactive components in CHMs, many researchers have paid special attention to the analysis of CHMs by combining two effective separation modes, such as 2D-GC, 2D-LC and LC-CEC.

Therefore, the main objective of this review is to summarize the most recent advances in the strategies for analyzing CHMs using 2D chromatography. For this purpose, the different and commonly used couplings, mainly including 2D-GC and 2D-LC, are described, and their major advantages and shortcomings are further discussed. Considering that several beneficial reviews about 2D techniques have already introduced the general aspects (such as fundamentals, set-up, data processing and applications) of these different couplings in detail [4–7], only a brief description of the approach of each coupling is given in this review.

2. Comprehensive two-dimensional gas chromatography (GC × GC)

For a long time, 1D-GC has provided a great deal of valuable information to unravel the samples with simple matrices. However, when confronting some highly complex samples such as CHMs, peak overlap would occur, resulting in ambiguous peak identification and inaccurate quantification. Although this problem can be partly resolved through the coupling of the MS to the GC (GC–MS) either for a single peak assignment and quantitation [6] or for the correct identification of co-eluting peaks with the help of deconvolution methods [8,9], the low capacity and poor resolution of 1D-GC for complex samples are still the main obstacles to complete component separation and identification.

An effective technique for improving the separation capability is multidimensional gas chromatography (MDGC), which generally combines two or more independent columns via an interface. Since MDGC was first introduced by Simmons and Snyder [10] in 1958, MDGC has been significantly developed and widely applied to the analysis of complex samples in different areas with satisfactory results [11–14]. Conventional MDGC is defined as a heart-cutting technique involving the transfer of selected regions or zones of unresolved components from the first dimension (^{1}D) column into the secondary dimension (^{2}D) column for further separation, where column selectivity can give enhanced resolution of the heart-cutting parts. However, when many peaks are of interest, instead of isolating just a few targeted analytes, such heart-cutting MDGC is unsuitable because the sampled fractions must be of small duration to prevent recombination of adjacent cuts in the ^{2}D column. Moreover, if the sample composition is extremely complex, a long running time is required because only one heart-cut can be performed per injection for the common MDGC analysis. For example, in the analysis of flue-cured tobacco essential oil, 23 heart-cuts with a duration of a few minutes across the entire ^{1}D separation were employed, resulting in a 48-h analysis time [15].

As an advancement to MDGC, the comprehensive two-dimensional GC (GC × GC) technique was described by Liu and Phillips in 1991 [16]. Using fast, continuous heart-cutting with a sampling period less than the width of a ^{1}D peak, GC × GC can achieve complete 2D separation and comprehensive analysis of an entire sample in a single analytical run. As a better solution for complex samples, GC × GC can offer not only increased peak capacity but also higher sensitivity than 1D-GC. Especially once coupled with MS, this technology can be sufficiently powerful to provide unparalleled separation and identification of analytes and is particularly suitable for trace analyte analysis. When proper orthogonal conditions are employed, chemically related compounds display as ordered structures, and this property could greatly benefit both group-type analysis and provisional classification of unknowns. In addition, thanks to the high separation capability of GC × GC, the always tedious and time-consuming sample pretreatment procedures can be minimized or even abandoned in the analysis of some complex samples. In addition to the benefits offered by GC × GC, this technique also suffers some limitations, such as the need for complicated instruments and costly maintenance. Moreover, because GC × GC could generate a large amount of raw data, the lack of software for data processing is a significant impediment to the adoption of GC × GC for complex samples.

To date, a certain number of applications based on GC × GC have been successfully developed for the CHM analysis. Some of the most remarkable applications are summarized and shown in Table 1. The types of modulators and the column arrangement, as well as the detector selection, are the primary factors that should be considered when developing these methods.