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REVIEW

Application of Two-dimensional Liquid Chromatography in Bioanalysis of Drugs and Toxicants

XIAO Wei-Bin, JIAN Yang, LI Hua*

Beijing Institute of Pharmacology and Toxicology, State Key Laboratory of toxicology and medical countermeasures, Beijing 100850, China

Abstract: Qualitative and quantitative analyses of drugs, toxicants and endogenous substances in biological samples play an important role in pharmacology and toxicology studies, as well as in new drug discovery and development. Analysis of biological samples is a challenging task, due to trace concentrations of analytes, complex matrix, endogenous interference, and small volume of biosamples. Analytical methods for such samples often require superior specificity, high sensitivity and good reproducibility. Two-dimensional liquid chromatography (2D-LC) technique, with advantages including high peak capacity, significant reduced matrix effect and carryover and automated sample pre-treatment, has provided a powerful solution for separation and analysis of trace drugs and toxicants in biosamples. The technique is widely used in the fields of environment, food and pharmaceutical analyses. This review summarizes the application of 2D-LC in pharmacokinetics, toxicology and analysis of endogenous substances, followed by an introduction of the principle and equipments of 2D-LC.

Key Words: Two dimensional liquid chromatography; Online solid phase extraction; Pharmacokinetics; Bioanalysis; Review

1 Introduction

Drug metabolism and pharmacokinetics (DMPK) can be used to investigate the processes and mechanisms of absorption, distribution, biotransformation and excretion of drugs and xenobiotics, and quantitatively describe their in vivo disposition and the interaction between endogenous and exogenous substances. The information and data obtained from DMPK researches provide an important basis for the development and safe clinical use of new drugs. Qualitative and quantitative analysis of drugs and their metabolites in biological samples plays a key role in DMPK research. However, the detection, quantification and structural characterization of trace amount of drugs and their metabolites always pose a great challenge to analysts[1]. Bioanalysis performed in relation to DMPK, disease discovery and diagnosis, pharmacological research, therapeutic drug monitoring, and clinical toxicology deal with various biological samples, such as plasma, serum, urine, saliva, bile, tissue samples and excreta. These samples usually contain lower levels of analytes and relatively high levels of endogenous in matrix, such as protein, fat, hormones, neurotransmitter and urea. Quantitative analysis of these samples demands that the method has high specificity, sensitivity and repeatability. Moreover, the large numbers of samples generated by DMPK research and therapeutic drug monitoring (TDM) require delivering results in a simple, rapid and high-throughput manner. Chromatography has been used as the primary separation technique for DMPK and clinical monitoring related bioanalysis. Particularly, liquid chromatography (LC) equipped with different kinds of detectors, such as UV (ultraviolet), fluorescence detector (FLD), electrochemical detector (ECD) or mass spectrometer (MS), offer the different analytical capabilities for variety of analytes. As the great stride of mass spectrometer (MS) interface technique and the enhanced analytical sensitivity in the past decade, the highly selective and sensitive LC-MS has become the first-line technique for bioanalysis of drugs and toxic substances[2]. Two-dimensional liquid chromatography (2D-LC) further strengthens the separation efficiency of LC

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* Corresponding author. Email: 78930718@qq.com

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and contributes to high sensitivity, reproducibility and accuracy[3].

Analysis of biological samples by LC-MS often requires sample pretreatment to clean-up or remove endogenous interferences. Sample treatment is not only a vital step, but also a time and labor-consuming part of bioanalysis. The outcome would directly influence the reliability of the quantitative and qualitative results. It was pointed out in previous report that tedious procedures of sample pretreatment contributed to about one third of the analytic error[4], and also took about two-thirds of total workload[5]. The 2D-LC-based on-line SPE method simplified significantly sample pretreatment procedures and made a fully automated high-throughput analysis possible. The technique was widely applied in the analysis of active ingredients from traditional Chinese medicinals, drug quality evaluation, food safety and environmental analyses[6–9]. It was also increasingly used in DMPK and toxicological studies as a promising tool for biological sample preparation.

2 Principle, configuration and features of 2D-LC

2D-LC is a separation system of tandem combination of two individual chromatographic columns with different separation mechanisms, controlling the flow between two columns by column-switching technique. During the analysis, the components are separated by first dimension (1D) column and transferred into the interface of switching valve. The fractions of the primary column are captured or cut by the interface and released onto the second dimension (2D) column and the detector. Depending on whether all parts of a sample are transferred to the second dimension, 2D-LC can be classified as comprehensive (all components are transferred) or heart-cutting (specific parts of the sample are transferred), and based on whether the 1D eluents are directly transferred into 2D column, 2D-LC can be classified as on-line or off-line modes. To date, the majority of 2D-LC approaches utilize the on-line mode which means the 1D eluate is transferred to 2D column directly.

With respect to the configuration, either one-pump or two-pump system can be employed to perform 2D-LC separation[10]. Two-pump system is accessible by temporary set-up or choosing the newly commercialized integrated platform. Figure 1A shows a two-pump system which requires an additional pump, column and switching valve to control the switch of different flow path. The injected samples are transferred into the 1D column by the 1D pump (loading pump). The eluted fractions are then trapped by interface-switch technique, such as dual sample loops, stop-flow mode or vacuum volatilization. After that, the retained compounds are flushed or back flushed from the interface or 1D column onto the 2D column by high organic solvent from the 2D pump to carry on further separation by which substantial peak capacities can be attained[11,12]. The integrated 2D-LC device incorporates two pumps connection instruction, for instance, UltiMate3000 UHPLC system offer versatility covering the maximum range of 2D-LC through software operation and the unique valve switching technique (Fig.2)[13,14]. As shown in Fig.1B, 2D-LC can also be realized by one-pump system[15,16]. The pump loads the samples into a SPE column, and non-retained components are washed to waste. After loading, the waste position is closed which forces the gradient solvent to pass through the SPE to the analytical column.

Compared with classical LC and off-line SPE method, the major benefits offered by 2D-LC include significant enhance the peak capacities, reduce the matrix effect and carryover, and improve the throughput of analysis as the result of automated sample pretreatment. In traditional 1D-LC, the peak resolution was evidently compromised if the number of components exceeded 37% of the peak capacity, whereas the separation capacities of 1D and 2D were multiplied in 2D-LC and a greatly increased resolution can be achieved[17–19], thereby good separation and multi-component analysis were achieved in complicated matrix samples. When a SPE column is configured as the first dimension of a 2D-LC, an automated on-line SPE system is then built and capable to remove salts and proteins and enrich trace amount analytes, which allows direct injections of biosamples. With automated sample pretreatment, enhanced sensitivity and excellent reproducibility were achieved for plasma and urine samples, which was advantageous for handling a large set of samples. In addition, the cross-contamination between samples and the possible degradation of analytes could be avoided as the whole analysis was performed in a sealed system[9,20,21].

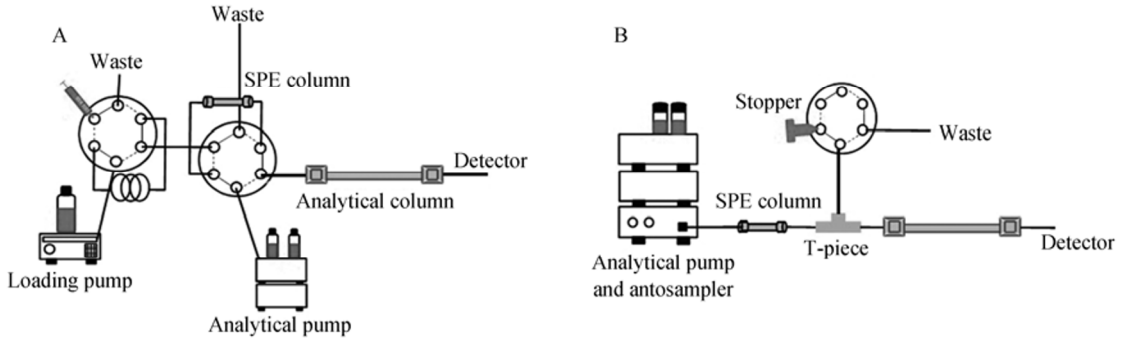

Fig.1 Common on-line SPE-LC system[10]. A. two pump system; B. one pump system