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Preparative two-dimensional liquid chromatography/mass spectrometry for the purification of complex pharmaceutical samples

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ABSTRACT

A new preparative two-dimensional liquid chromatography/mass spectrometry system (2D LC–LC/MS) has been designed and implemented to enhance capability and resolving power for the separation and purification of pharmaceutical samples. The system was constructed by modifications of a conventional preparative LC/MS instrument with the addition of a set of switching valves and a sample loop, as well as interfacing a custom software program with MassLynx. The system integrates two chromatographic separations from the first and second dimensions into a single automated run to perform the purification of a target compound from a complex mixture without intermediate steps of sample preparation. The chromatography in the first dimension, operated in the heart-cutting mode, separates the target compound from the impurities by mass-triggered fractionation based on its molecular weight. This purified fraction from the first dimension is stored in the sample loop, and then gets transferred to the second column by using at-column dilution. A control software program, coined Prep 2D LCMS, was designed to integrate with MassLynx to retrieve data acquisition status. All of the chromatographic hardware components used in this preparative 2D LC–LC/MS system are from the original open access preparative LC/MS system, which has high level of robustness and affords easy and user-friendly operation. The new system is very versatile and capable of collecting multiple fractions with different masses under various purification modes as configured in the methods, such as conventional one-dimensional (1D) purification and/or 2D purification. This new preparative 2D LC–LC/MS system is therefore the ideal tool for medicinal chemistry lab in drug discovery environment.

1. Introduction

Rising costs to introduce new medicine into the market drive pharmaceutical industry to improve the efficiency and reduce the attrition rate of the discovery and development pipelines [1,2]. In drug discovery laboratories, at stages of lead generation and lead optimization, it is quite common to require new compounds to be synthesized with weekly turn-around time for quantities ranging from low milligrams to hundreds of milligrams. The compounds made in chemistry laboratory, either in a compound library or in a single batch, will need to pass a purity criterion before registering in the laboratory information management system (LIMS) for enzymatic and physical chemical assays. To obtain the compounds that meet the purity requirement in a given timeframe, the purification process sometimes becomes critical. If target molecules are produced in relatively high yield (purity more than 80%) from the synthetic reactions, the compounds can be obtained in purity of more than 95% using conventional one-dimensional methods for preparative separations, such as high-performance liquid chromatography coupled with ultraviolet (HPLC/UV) [3–12] or with mass spectrometry (HPLC/MS) [8,13–29], and supercritical fluid chromatography coupled with UV (SFC/UV) [8,24,30–34] or with MS (SFC/MS) [35–39]. If the compounds were made in reaction mixture with low yield (purity less than 50%), the purification process would be quite complex and the goal to make the weekly registration would become very challenging, if not impossible. To avoid such situation, it is more desirable to optimize the synthetic reaction to improve the yield and reduce by-products generated from the reactions before purifying the compound. Otherwise, it will require extensive chromatographic method development over combinations of columns and mobile phase conditions, either by using reversed phased liquid chromatography (RP-LC), or normal phase liquid chromatography (NP-LC), and/or supercritical fluid chromatography (SFC). Automated method screening is often conducted in an overnight process and the data are evaluated the next day to select optimal conditions for separating a desired product from impurities, which are then applied to preparative purifications. When the first purification fails to provide the desired product at required purity (≥95%), additional rounds of method development and purification may be performed using orthogonal

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chromatography. Such process is tedious and challenging and may still have difficulties in delivering pure compounds, which may potentially delay the progress of the drug discovery process.

Two-dimensional liquid chromatography (2D LC) is considered to have higher selectivity and resolving power compared to one-dimensional HPLC for the separations of complex samples [40–42]. Preparative 2D LC methodology has been reported in the literature [43–47]. The development of preparative 2D LC instrumentation and technology has great potential for the applications of the preparation and purification of organic compounds from complex mixtures as the streamlined process of higher efficiency and productivity. For preparative scale purification, the heart-cutting mode of 2D LC is generally recognized to be the most suitable choice [43], because only the fraction containing the target compound from the effluent of the first dimension is transferred to the second dimension for subsequent purification. Currently, only semi-preparative scale 2D LC instruments have been reported in the literature, the example of which was applied for the separation and purification of natural products, such as coumarins [44], lignans [45], isoflavonoids [46], and iridoid glycosides [47]. The reported methods utilizes UV detectors in both first and second dimensions, either by using a sample loop to trap the fraction eluted from the first dimension and then load the trapped fraction stored in the sample loop onto the second dimension, or by using a set of valve interface to load the effluent from the first dimension directly onto the second dimension. The method without using the sample loop is able to achieve the maximum sample recovery from the first dimension to the second dimension [45], or is able to have higher capacity to load large volume of sample solution onto the second dimension [47]. However, using the method without the sample loop, the two columns elute sequentially at the same time during fraction transfer process, similar to a configuration of two columns coupling together. The flow rate in such case becomes a limiting factor for the preparative HPLC purifications due to high pressure build up. Another challenging factor for such no-loop preparative 2D LC configuration is the mobile phase compatibility of two different solvents from the first and second dimensions. The mobile phase compatibility issue can be critical because the peak shape may become broader due to different mobile phases used in the first and second dimensions, therefore only the same mobile phase in isocratic mode can be utilized [44,45]. In some cases, when the gradient condition used in the first dimension, the result of broadening peak was observed in the second dimension [47]. To further improve the efficiency of sample loading and cycle time, a configuration of preparative 2D LC system with one column in the first dimension and two columns in the second dimension for parallel elution of two heart-cutting fractions from the first dimension have been experimented [47].

Here we report the design and implementation of a preparative 2D LC–LC/MS system for purification of the complex samples using mass-triggered approach [13,14,48] to perform the heart-cutting and fractionation from the first and second dimensions. The system is modified from a conventional open access preparative LC/MS system, which has high level of robustness and affords easy and user-friendly operation. The new system is very versatile and capable of collecting multiple fractions with different masses under various purification modes as configured in the methods, such as conventional one-dimensional (1D) purification and 2D purification.

2. Experimental

2.1. Chemicals

HPLC grade methanol (MeOH) and acetonitrile (ACN) were acquired from Fisher Scientific (Pittsburgh, PA, USA). Trifluoroacetic acid (TFA) and ammonium bicarbonate were purchased from Aldrich (Milwaukee, WI, USA). HPLC grade water was generated through a Milli-Q system (Millipore, Bedford, MA, USA). The standard test compounds, Fmoc-alanine and Fmoc-tryptophan were purchased from Calbiochem (San Diego, CA, USA). All other test compounds, including samples, were obtained from our synthetic laboratory.

2.2. Analytical HPLC–mass spectrometry

All analytical LC/MS were carried out on a Waters Acquity UPLC-SQD single quadrupole mass spectrometer (Waters Corp., Milford, MA, USA) equipped with an electrospray ion source (ESI). Data were acquired in the full scan positive mode (200–800 Da). Dwell time was 0.1 s with an inter-spray scan delay time of 0.1 s. The ion source parameters were used as following: sprayer voltage, 3.2 kV; cone voltage, 25 eV; desolvation temperature, 350 °C; and source temperature, 150 °C. The instrument resolution was 1000 (10% valley definition). The analytical LC/MS for method development was performed on a Waters Acquity UPLC system equipped with an Acquity BEH 2.1 mm × 50 mm, 1.7 μm fully porous particle column and flow rate at 0.6 mL/min with mobile phase of an acidic condition (0.035% TFA in acetonitrile and 0.05% TFA in water at pH 1) or a basic condition (10 mM ammonium bicarbonate, pH adjusted to 9.5) in a 1.5 min gradient profile (5–95% gradient in 1.2 min). MassLynx 4.1 software was used for data acquisition.

2.3. Preparative two-dimensional (2D) LC–LC/MS system

Preparative 2D LC–LC/MS purifications were carried out on a new designed system which is a modified Waters preparative HPLC instrument (Waters Corp., Milford, MA) equipped with the following components: two Waters 2525 pumps with a set of valves for both 1D and/or 2D preparative purifications; a sample loop (in Fig. 1, Loop 2, size in 0.08 in. × 50 ft) to hold heart-cutting fraction from the first dimension (also for loading the trapped fraction to the second dimension); a Waters system fluid organizer (SFO), two Waters 515 pumps for delivering the make-up flow (515 Pump 1) and providing at-column dilution while loading the trapped fraction (515 Pump 2) onto the second column; a Waters 2767 automated sample manager to control sample injection of the first dimension as well as fraction collection for both first and second dimensions; a Waters 2487 dual wavelength UV detector; and a Waters ZQ 2000 single quadrupole mass spectrometer equipped with an electrospray ion source. Data were acquired in the full scan mode (200–800 Da). Dwell time was 0.1 s with an inter-spray scan delay time of 0.1 s. The ion source parameters were as the following: sprayer voltage, 3.0 kV; cone voltage, 25 eV; desolvation temperature, 300 °C; source temperature, 120 °C; cone gas flow at 50 L/h; and desolvation gas flow at 400 L/h. The instrument resolution was 1000 (10% valley definition). MassLynx 4.1 software was used for data acquisition and the FractionLynx 4.1 for mass triggered fractionation (Loop 1 in Fig. 1 as the delay loop, size in 0.04 in. × 50 ft, for mass triggered fractionation). The chromatographic separations in the first and second dimensions of preparative 2D LC–LC/MS were performed on an XBridge C18 OBD, 30 mm × 75 mm, 5 μm, fully porous particle column (Waters Corp., Milford, MA, USA). Both acidic and basic mobile phases can be applied onto the first and second dimensions. The acidic mobile phase was consisted of A1, water – 0.05% TFA (v/v, pH 1), and B1, acetonitrile – 0.035% TFA (v/v), whereas the basic mobile phase was consisted of A2, water with10 mM ammonium bicarbonate (pH adjusted to 9.5) and B2, 20% ACN in water (v/v) with 10 mM ammonium bicarbonate. The flow rate for both first and second dimensions was at 50 mL/min. Sample concentrations varied from 20 to 200 mg/mL (sample quantities from 50 mg to 1.5 g) and a 500–1000 μL aliquot was injected onto the first dimension. To