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Simultaneous achiral-chiral analysis of pharmaceutical compounds using two-dimensional reversed phase liquid chromatography-supercritical fluid chromatography

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ABSTRACT

A new interface was designed to enable the coupling of reversed phase liquid chromatography (RPLC) and supercritical fluid chromatography (SFC). This online two-dimensional chromatographic system utilizing RPLC in the first dimension and SFC in the second was developed to achieve simultaneous achiral and chiral analysis of pharmaceutical compounds. The interface consists of an eight-port, dual-position switching valve with small volume C-18 trapping columns. The peaks of interest eluting from the first RPLC dimension column were effectively focused as sharp concentration pulses on small volume C-18 trapping column/s and then injected onto the second dimension SFC column. The first dimension RPLC separation provides the achiral purity result, and the second dimension SFC separation provides the chiral purity result (enantiomeric excess). The results are quantitative enabling simultaneous achiral, chiral analysis of compounds. The interface design and proof of concept demonstration are presented. Additionally, comparative studies to conventional SFC and case studies of the applications of 2D LC–SFC in pharmaceutical analysis is presented.

1. Introduction

Two-dimensional (2D) chromatographic techniques have become very popular especially in the analysis of complex mixtures [1]. As compared to one-dimensional (1D) chromatography, 2D chromatographic techniques have higher selectivity and resolving power assuming the retention mechanisms are complementary. If orthogonal separation mechanisms are used in the two dimensions, the theoretical peak capacity of the system is the product of the individual peak capacities [2–4]. 2D chromatography can be performed in heart-cutting, pseudo-comprehensive or comprehensive modes. Heart-cutting provides the characterization of a selected region of the chromatogram [5], while the comprehensive mode provides the characterization of the entire chromatogram. The pseudo-comprehensive mode provides comprehensive separation of selected regions of the chromatogram [6].

The incompatibility of solvents used in the first and second dimensions can cause severe band dispersion or broadening and peak deterioration, thus posing a big challenge for the interface design [7]. To alleviate the solvent immiscibility concern, researchers have developed several 2D systems that use compatible mobile phases in both dimensions. Some examples include the following: 2D Reversed Phase Liquid Chromatography (RPLC x RPLC) [8–11], 2D Hydrophilic Interaction Liquid Chromatography (HILIC x RPLC) [12–14], 2D Normal Phase Liquid Chromatography x Supercritical Fluid Chromatography (NPLC x SFC) [15], 2D SFC x SFC [16–20] and off-line 2D RPLC x SFC [21–22]. One technique that couples the incompatible “normal phase” and “reversed phase” dimensions is 2D SFC x RPLC [23–24]. In this case, the non-polar supercritical carbon dioxide in the SFC fractions is evaporated off (when exposed to atmospheric pressure) to yield fractions with compatible mobile phases to the second RPLC dimension (usually an alcohol modifier).

Another factor to consider when developing 2D systems is the ability to operate in an on-line mode. Some advantages of this approach include the ease of automation, reproducibility of the analysis, and the accurate transfer of the fractions from the first to the second dimension without any yield loss or contamination.

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An overlooked application of 2D systems is the use in high-throughput analysis. In the pharmaceutical industry for example, Active Pharmaceutical Ingredients (APIs) have to be fully characterized per ICH guidelines [25]. For purity analysis of chiral compounds, two independent analytical methods are developed. A RPLC method usually assesses the achiral purity (impurities and related substances method), and a chiral method that would assess the enantiomeric purity (amount of undesired enantiomer). A 2D system that can generate simultaneous achiral and chiral results would have a huge impact during API process development. Sample preparation, chromatographic analysis times, and data analysis would be reduced to allow higher throughput analysis.

We have previously reported the use of 2D RPLC x RPLC analysis for simultaneous achiral-chiral analysis [6]. In the API world however, the majority of chiral methods are NPLC methods, and thus a 2D RPLC-NPLC system would have a significant bearing in achieving simultaneous achiral-chiral analysis. As mentioned earlier, the incompatibility of the reversed phase and normal phase mobile phases would make this approach very challenging. Supercritical fluid chromatography, a normal phase technique, has also been used for API chiral analysis on analytical as well as preparative scale. In addition to being a "green" technique, SFC is superior to NPLC due to its versatility, higher efficiency, higher throughput, and faster analysis times. Supercritical fluids have low viscosity and high diffusivity-to allow higher flow rates and faster re-equilibration times and have a high density-to provide a high solvating power. The first on-line 2D LC-SFC was reported by Cortes et al. in 1992 [26]. The interface that they developed is rather complicated and involves multiple stages: elimination of the first dimension solvent by the passage of nitrogen gas, using pressurized CO_2 to transfer the analytes onto an impactor interface, and then elution of the analytes from the impactor interface to the SFC capillary column by pressure programming of the CO_2 mobile phase. The adoption of this interface for conventional 2D RPLC-SFC separations would be limited due to the solvent elimination step. The authors used THF (relatively low boiling point, 66 °C) as the LC mobile phase while most conventional RPLC separations are aqueous based.

In this work, we demonstrate the development of a new automated interface to couple RPLC and SFC. The online 2D RPLC-SFC allows simultaneous achiral and chiral analysis. The API peak in a hydro-organic mixture is retained on a small volume C-18 trapping column and then back-flushed onto the second dimension SFC column. The details of 2D LC–SFC interface involving trapping column along with real world applications in pharmaceutical industry are presented.

Moreover, the effect of transfer volume in the second dimension separation, trapping column capacity is illustrated along with several case studies.

2. Materials and methods

2.1. Chemicals and reagents

Carbon dioxide (CO_2) was obtained from Praxair (Danbury, CT, USA). Acetonitrile (ACN) was purchased from Avantor's J.T. Baker (Center Valley, PA, USA). Methanol (MeOH), isopropyl alcohol (IPA), ethyl alcohol (EtOH), 98.0–100.0% formic acid, and 28.0–30.0% ammonium hydroxide (NH_4OH) were purchased from EMD chemicals (Gibbstown, NJ, USA). Ammonium formate was purchased from Sigma Aldrich (St. Louis, MO, USA). HPLC grade millipore water was obtained from Purelab ultra Millipore water dispenser. *Trans*-stilbene oxide (TSO) was purchased from TCI (Tokyo, Japan). Drug substance A used in this study was synthesized by the process chemistry department at Genentech, CA, USA.

2.2. Instrumentation

The analytical instrument is a customized two-dimensional 1260 2D-LC–SFC system with mass spectrometer from Agilent Technologies (Santa Clara, CA, USA). The RPLC unit consists of an Agilent 1260 quaternary pump (G1311B), a 1260 HiP ALS autosampler (G1367E), and an Agilent 1260 multi-wavelength UV detector (G1365C). Stainless steel fittings and tubing are used throughout the system due to high pressure considerations. The SFC unit consists of a 1260 SFC binary pump (G4302A) with a three position solvent control valve, a 1260 HiP degasser (G4225A), a 1290 thermostated column compartment (G1316C), an eight-position Agilent 1290 infinity valve drive (G1170A), an Agilent 1260 DAD (G1315C) equipped with a high-pressure flow cell, and an Agilent 1260 infinity SFC control module (G4301A). Part of the SFC flow was directed towards an Agilent 6120 quadrupole MS. An Agilent 1260 iso pump (G1310B) was used to generate a make-up flow of 0.15 mL/min was in order to compensate for the loss of $scCO_2$. An Agilent 1290 Flexcube (G4227A) was installed to enable multiple peak parking on different trapping columns using a custom built 12-port switching valve. Instrument control and data collection was done with Agilent chemstation software (Santa Clara, CA, USA).

2.3. System operating conditions

Schematics of the 2D LC–SFC interface involving an electronically controlled, 8-port, dual position valve V1 and trapping column T1 is shown in Fig. 1. The mobile phase from the HPLC pump flows through the injector to the reversed phase primary column. The eluent post detection (Detector 1) flows to the sampling valve V1. In the home/analysis position, the primary column eluent flows through the sampling loop L1 exiting to waste. The mobile phase from the SFC pump flows through trapping column T1 to the SFC column (Fig. 1A). This conditions the trapping column T1 and SFC column. There is an uninterrupted flow of mobile phase through primary and secondary columns. When components of interest elute from the primary column, the valve V1 is switched (trapping position) transferring the primary column eluent to the trapping column T1 (Fig. 1B). Switching the valve V1 back to home/analysis position back-flushes the sample components from trapping column T1 to the SFC column. The SFC column separation is monitored using UV detector 2 and/or a mass spectrometer. Interchanging positions of SFC pump and secondary column in the Valve V1 will result in co-current flow during valve switching. The chromatogram in Fig. 3 was generated using the configuration described in Fig. 1.

Schematics of the 2D LC–SFC interface using an array of trapping columns and secondary SFC columns are shown in Fig. 2. This configuration is used for analyzing multiple sections of the primary column eluent. In the home/analysis position shown in Fig. 2, the eluent from the primary HPLC column post detection flows through a fully automated, eight-port dual position valve V1 exciting to waste. The SFC mobile phase flows as follows: SFC pump through valve V1, to the parking deck valve V2, back to valve V1, and then to the SFC column/s. In valve V2, the SFC mobile phase flows either through bypass tubing or an array of trapping columns. This conditions the trapping and SFC column/s. There is an uninterrupted flow of mobile phase through primary and secondary columns. When components of interest elute from the primary HPLC column, valve V1 is switched (home to transfer) transferring the primary column eluent to parking deck valve V2. By switching the parking deck valve V2 back and forth between the bypass mode and trapping position, components are transferred to different trapping columns. Following the transfer of the primary column eluent, valve V1 is switched to home position