Enhancing Chiral Bioanalysis with Supercritical Fluid Chromatography for NCE molecules

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Abstract

A supercritical fluid, existing above its critical temperature and pressure, combines gas-like and liquid-like properties, offering unique advantages for the separation of various compounds. In this study, we used the power of SFC for the analysis of chiral new chemical entity molecules, achieving remarkable results. Our investigation focused on chiral NCEs, encompassing one enantiomer and two diastereomers, totalling four distinct analytes. The separation of diastereomers using traditional LC was a challenging task, as it required extended runtimes of 40-45 minutes. In contrast, SFC enabled the separation of stereo-isomers in a significantly shorter timeframe, typically within 20 minutes.

With SFC, we achieved exceptional chiral separation and notably reduced run times compared to conventional RPLC. Specifically, within a 20-minute runtime, we successfully separated the parent molecule, one enantiomer, and two diastereomers.

In another application, we achieved rapid separation of analytes, metabolites, and their enantiomers in as little as 10 minutes, while traditional LC systems required 25-30 minutes. Additionally, SFC exhibited superior baseline stability. (Data not presented in this poster)

Introduction

The traditional LC-MSMS method has set the gold standard for bioanalysis of drugs in human plasma samples due to its ease of instrumentation, accommodation, reverse-phase capabilities, and technology transfer capabilities. However, these techniques lack the resolution needed to separate enantiomers for higher throughput while retaining their resolution.

SFC played a significant role when we applied this fundamental principle in the separation of stereo-isomers for NCE molecules. We applied this principle to the simultaneous determination of NCE2, NCE3, NCE4, NCE1 in K2EDTA Human plasma Samples. All of these are stereo-isomers and require chiral separation to achieve PK profiling of individual components for qualitative and quantitative characterization of the safety and efficacy of the drug molecule, including in-vivo interconversion.

Methods and Materials

The bioanalytical sample extraction employed solid-phase extraction, taking into consideration NCE physicochemical properties for higher selectivity and sensitivity. Chiral separation of enantiomers and diastereomers required a normal-phase chiral column. We used the Phenomenex cellulose lux-4 stationary phase column after evaluating various chiral columns, which provided the desired selectivity. The previous method could achieve separation in a 25-minute runtime, but the trial did not yield acceptable chromatographic peak elution, despite trying different alternatives one by one. Improved chromatography required a longer elution time to remove the compound, which was not suitable for the high throughput requirement of Phase I trial samples. The SFC setup employed a much more versatile approach, using carbon dioxide as the SFC mobile phase, which provided a high number of theoretical plates, resulting in higher resolution and peak symmetry.

Table 1. SFC MSMS conditions

Analytical Technique	Supercritical fluid chromatography coupled with mass spectroscopy
MS/MS	Shimadzu 8050
Ion source	Turbo ion spray
Column type	Phenomenex lux cellulose 4 150*4.6mm
Mobile Phase	Pump A: CO2 Pump B (05:92:03 % v/v) Line A: Acetonitrile Line B: 0.1% formic acid in 10mM ammonium formate in methanol Line C: 10mM Ammonium Trifluoro acetate in water

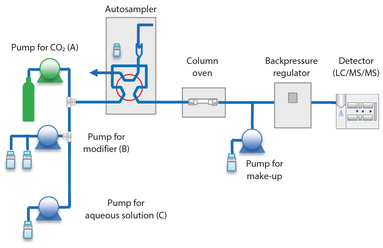

Figure 1: Scheme of a supercritical fluid chromatography instrument. Adapted from Supercritical Fluid Chromatograph, SFC Basic Guide, C190-E270A, Shimadzu

Table 2. Chromatographic Peak element comparison

	Peak Elements - LC			Peak Elements - SFC		
	Ret. time (min)	Resolution*	Tailing Factor	Ret. time (min)	Resolution*	Tailing Factor
NCE2	12.06	2.05	1.267	11.36	5.15	1.098
NCE3	13.63	2.59	1.319	13.65	NA	1.082
NCE4	16.03	2.90	1.480	8.92	3.69	1.098
NCE1	19.16	NA	1.433	10.18	2.89	1.104

* Resolution calculated with subsequent peak.

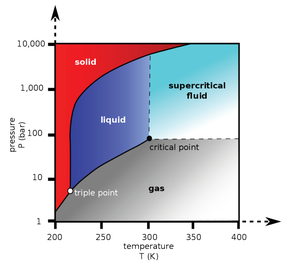

Figure 2 - Phase diagram for carbon dioxide Adapted from HYPERLINK "https://commons.wikimedia.org/wiki/File:Carbon_dioxide_pressure-temperature_phase_diagram.svg" File:Carbon dioxide pressure-temperature phase diagram.svg - Wikimedia Commons by Ben Finney and Mark

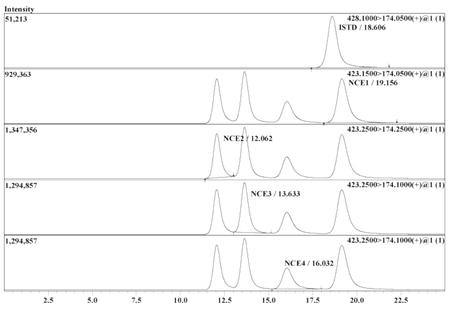

Figure 3. Traditional LCMSMS chromatography (FR: 1mL/min);

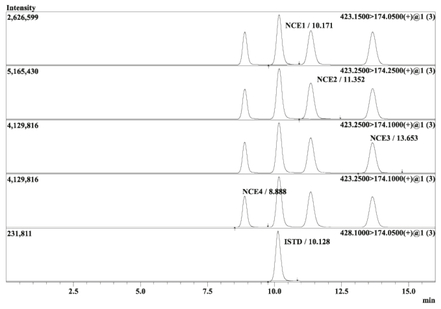

Figure 4. SFCMSMS chromatography (FR: 3ml/min)

Table 3. Precision and Accuracy with the SFC method in chiral bioanalysis

	% Accuracy					% CV				
	HQC	MQC1	MQC2	LQC	LLOQ QC	HQC	MQC1	MQC2	LQC	LLOQ QC
NCE2	99.6	93.4	99.6	99.2	99.2	6.9	2.4	2.1	3.2	3.0
NCE3	99.9	93.2	98.3	99.0	97.9	4.3	3.4	1.5	3.6	2.5
NCE4	94.0	90.8	98.3	99.5	98.6	4.2	3.6	1.5	3.0	2.8
NCE1	98.0	91.7	98.3	99.3	97.9	2.1	2.3	2.2	3.7	2.9

Results & Discussion

SFC technique allowed us to reduce the run time effectively by more than 40% for chiral sample analysis while increasing the resolution and peak symmetry. SFC allows us to use a flow rate as high as 3 mL/min, providing quick separation thanks to its characteristics, such as higher diffusion coefficients, lower viscosity, and higher temperature. With advancements in technology, SFC machines can be combined with MSMS with minimal changes in the configuration while still providing better intensity with normal phase chromatography, thanks to the use of mobile phase modifiers, which expands the range of substances that can be separated in shorter run times.

SFC is more environmentally friendly, as the CO2 generally used in SFC is sourced from industrial processes and recycled, making it a sustainable choice. It does not require post-usage disposal measures, is more energy-efficient, and provides improved resolution and faster run times.

Conclusions

In summary, the utilization of supercritical fluid chromatography presents a transformative approach, particularly beneficial for chiral applications. It does not only enhance analytical efficiency but also aligns with green technology principles, as it employs environmentally friendly carbon dioxide as the supercritical fluid, in contrast to traditional chromatography's use of organic solvents. Further usage of this technique should be employed to non-chiral analysis as well which may bring the sustainable procurement, high resolution and reduced run time for all bioanalysis.

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