Mass-Directed Isolation and Profiling of Small Molecule Analytes with SFC

Lu Dai, May Kong, Anne-Marie Beausoleil, Tim Fass, Andersen Yun, Qifeng Xue, Kanaka Hettiarachchi

Theravance Biopharma US, Inc., 901 Gateway Boulevard, South San Francisco, CA 94080

ESI-MS (electrospray ionization mass spectrometry) coupled with SFC (supercritical fluid chromatography) is routinely being used in our laboratory for identification and isolation of small molecule analytes in supporting Theravance Biopharma's drug discovery efforts in PK analyses as well as other bioanalytical and clinical applications. SFC is a preferred technique in separating isomers and enantiomers, as well as in rapid purification of achiral compounds. [1] This study demonstrates the utility of the preparative SFC-MS system (Waters MD SFC100) to rapidly purify small molecule analytes for toxicology measurements and metabolite identification. We show that SFC-MS is an effective way of isolating these highly sensitive compounds and other complex mixtures.

The purification of one deuterated chiral isomer from its closely eluting 5% impurity (Figure 1) at the gram scale was accomplished within a day using 10 min mass-directed SFC methods. This isolation is uniquely applicable to SFC-MS due to the chiral separation and sensitivity of sample. Ultrapure molecular biology grade ethanol (200 proof) was used for the purification of sample due to issues with trans-esterification.

Integrating ESI-MS with SFC is ideal, as CO_2 is highly volatile, enhancing the ionization process. The conditioning solvent and tuning parameters for the mass spectrometer were adjusted to achieve an ideal signal trace with good linearity ($r^2 > 0.980$) over a range of concentrations and minimal noise for accurate chiral peak detection and isomer isolation (Figure 2). The split ratio to the mass spectrometer was 50,000 : 1 for SFC. The limit of quantitation (LOQ) was 1 ng.

The unique separation abilities and high measurement sensitivity prove the SFC-MS technique to be well suited for supporting drug discovery and clinical applications.

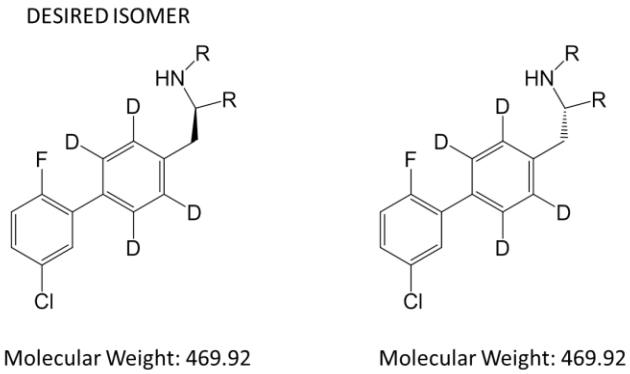

Figure 1. Deuterated compound structures

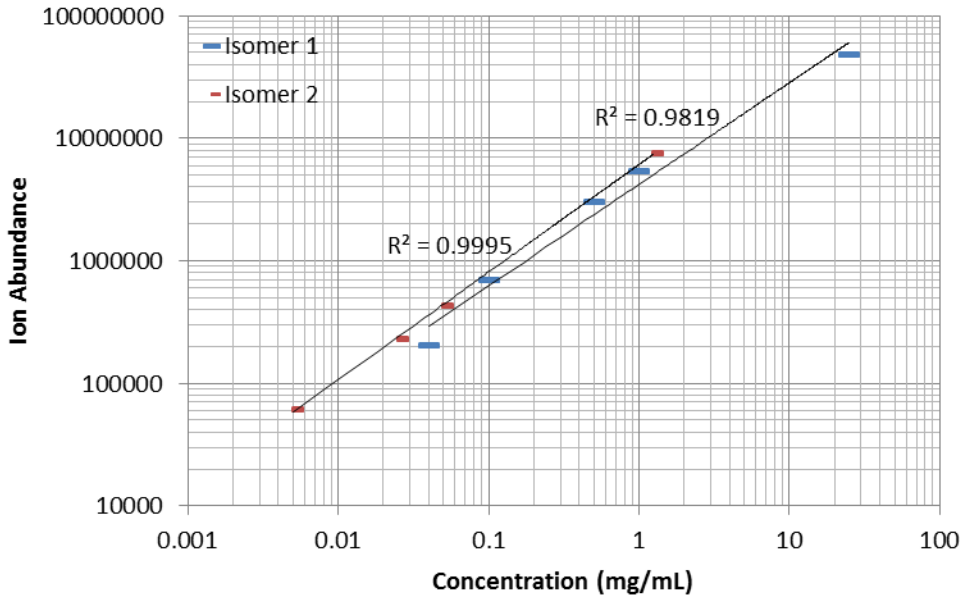

Figure 2. MS signal intensity vs. concentration for a chiral mixture by SFC-MS in positive-ion mode. The makeup flow rate is maintained at approximately 750 µL/min from a 50:000 : 1 split.

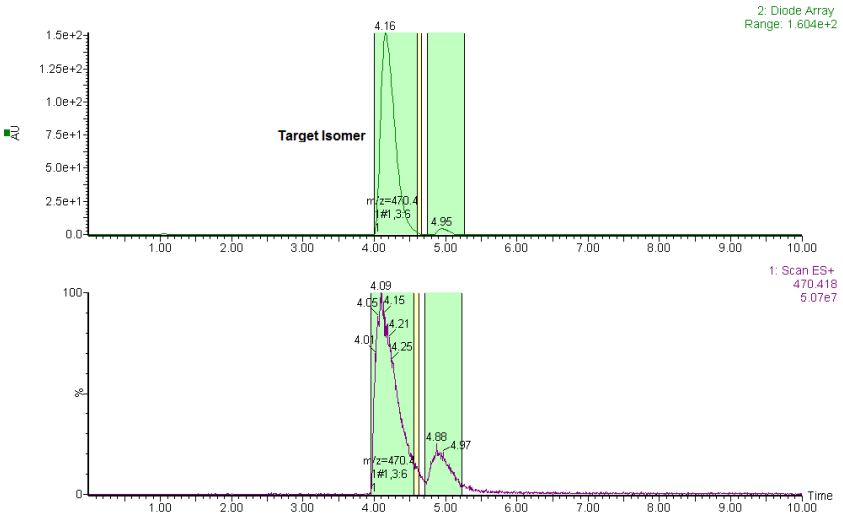

Figure 3. Chiral separation with the SFC-MS system. The SFC chromatogram of the sample shows the separation of two isomers. SFC condition: 40 °C, 70.0 mL/min, ethanol/CO2 10-30%B in 10 min, Chiralpak AS-H column.

References:

[1] Hettiarachchi, K., Kong, M., Yun, A., Jacobsen, J. R. and Xue, Q. (2014), Development of an automated dual-mode supercritical fluid chromatography and reversed-phase liquid chromatography mass-directed purification system for small-molecule drug discovery. J. Sep. Science, 37: 775–781.