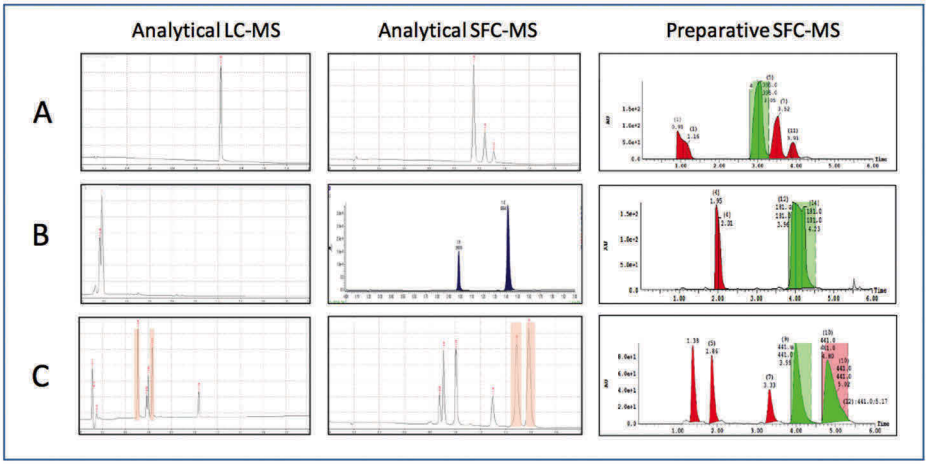

Figure 2: LC-MS method : 5-95% MeCN in 1.5 min and holding at 95% for 0.5 min, where the organic solvent is MeCN and the aqueous solvent is 10 mM ammonium carbonate pH 9; flow rate is 1.2 ml/min; temperature is 50 °C; stationary phase is the Waters XBridge C18 column with dimensions 50 mm x 2.1 mm i.d. (3.5 µm).

Analytical SFC-MS method : 5-55% methanol with ammonia (20 mM) in 1.5 min and holding at 55% for 0.5 min; flow rate is 3 ml/min; temperature is 55 °C; back pressure is 105 bar; stationary phase is the Naphthyl column (sample A) and the 1-AA column (samples B and C), with dimensions 100 mm x 3.0 mm i.d. (5.0 µm).

Preparative SFC-MS method: tailored gradient program # 5 (see table 1) for samples A, B and C; methanol with ammonia (40 mM) is used as the SFC modifier; flow rate is 100 ml/min; temperature is 55 °C; back pressure is 120 bar; column type is as stated analytically, with dimensions 150 mm x 30.0 mm i.d. (5.0 µm).

Figure 2 illustrates the benefit of screening purification samples by SFC, to compliment traditional reverse phase LC-MS. Sample 'A' delivers one peak by HPLC, but separates into three components by SFC. Sample 'B' is an example of a more polar reaction mixture. Poor retention and separation is achieved by HPLC, whereas adequate retention and very good separation is achieved by SFC. In this example, favourable solubility and separation enabled the injection of 830 mg of material. Sample 'C' illustrates an example where both HPLC and SFC separate two regio-isomers. SFC is the obvious choice for purification due to the resolution of impurities from the target compound.

Conclusion

SFC-MS is now embedded as a primary tool for front line purification of reaction mixtures. For samples in the region of 200 mg to 1.5 g the median cycle time, from dissolution of the crude material to the isolation of pure solid, is 3 hours. Smaller quantities (less than 200 mg) are normally processed and fractions dried within 90 minutes. Although SFC methods are much shorter compared to HPLC, the average loading per injection is generally lower. For the medicinal chemist, the overall speed benefit of SFC stems from the collection of target compound in small volumes of methanol, and the much-reduced time for solvent evaporation compared to reverse phase HPLC. It is important to note however, the different level of oversight required across the two techniques to achieve the robustness required in a walk-up environment. In our experience, the success of open access preparative SFC-MS depends on the availability of an analytical chemist or super-user, to partner with submitters and to help monitor the performance of instruments across the working day.

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