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Short Research Article

Supercritical fluid chromatography and radiolabeled compound synthesis†

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Introduction

Packed column supercritical fluid chromatography (SFC) has proven to be a very useful tool in our laboratory for the production of radiolabeled tracers for use in drug metabolism studies. The initial driver for its adoption was chiral chromatography where SFC had clear advantages over high-performance liquid chromatography (HPLC). A primary requirement in the production of tracers for drug metabolism today is speed. It is usually more time efficient to synthesize racemic mixtures and then separate the isomers than to develop novel enantioselective syntheses. SFC is faster than HPLC for enantiomeric resolution due to inherently lower viscosities, higher diffusivity rates and lower pressure drops which allow higher flow rates, faster equilibration rates, higher efficiencies and improved resolution. Higher speed, increased loadability, improved recoveries, longer column life and ease of use all lead to higher throughput, i.e. shorter time to delivery. With the advent of newer column technologies and eluent modifiers, these same advantages lead to increased use of SFC in our laboratory for achiral separations as well, particularly for in-process cleanup of intermediates to improve yields in subsequent steps. In order to facilitate the maximum use of SFC, we have successfully mated radiochemical flow monitors, evaporative light scattering detectors (ELSD) and mass spectrometers to our instruments.

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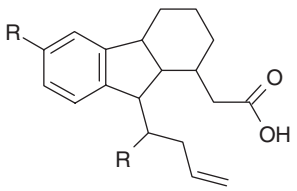
Results and discussion

Example 1: Chiral resolution of four stereoisomers (all four required for the study).



Initially separated using two separate HPLC methods in sequence on a Chiralcel OJ-H column followed by a Chiralpak AD-H column with hexane and ethanol as eluents. Three fractions were collected in the hexane/ethanol eluent from the first column and all were then evaporated to dryness and redissolved in ethanol. One of the fractions was rechromatographed on the second column with the two remaining isomers collected in hexane and ethanol, evaporated to dryness and redissolved in ethanol.

Conversely, the isomers could be separated by SFC in one pass using an AX QN column with CO_2 and methanol as eluent. The isomers were collected in methanol, evaporated to dryness and redissolved in the requested ethanol diluent (Figure 1).

Example 2: Chiral resolution of two isomers using stacked injections. Eluent is CO_2 and methanol. Typically recovery is > 90% at > 99% purity (Figure 2).

Example 3: Achiral SFC chromatography. Removal of the halobenzylamine starting material from the halobenzylnitrile product mixture was desired in order to improve the radioincorporation in the next step. The compounds were poorly separated by reverse-phase

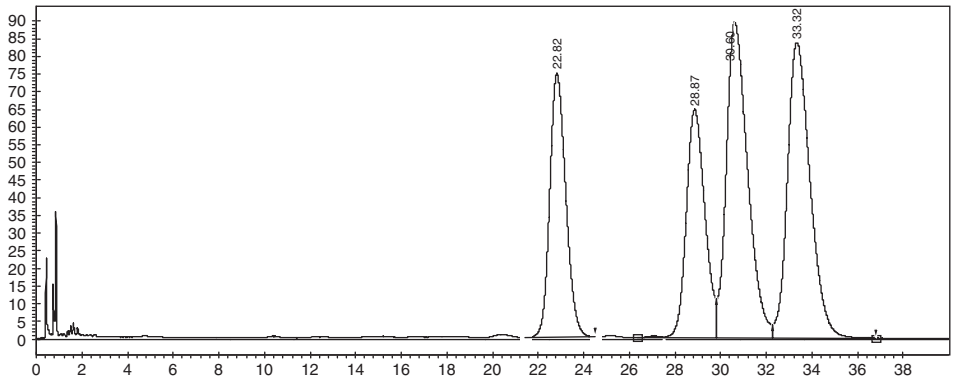


Figure 1

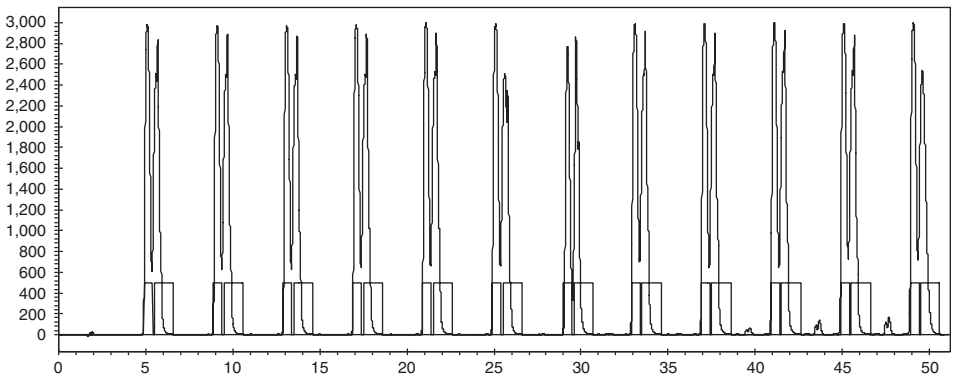


Figure 2

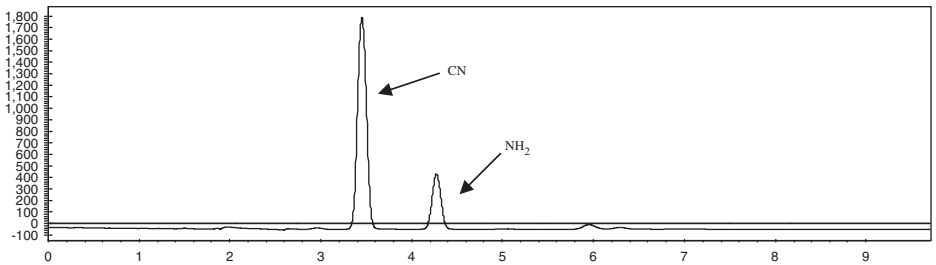


Figure 3

chromatography. The eluent was CO_2 and methanol and the product was recovered in pure methanol (Figure 3).

Example 4: Achiral SFC chromatography. Compounds are poorly soluble in water. Eluent is CO_2 and methanol:tetrahydrofuran (Figure 4).

Example 5: SFC and radiochemical detection. Decompression of CO_2 during mixing with scintillation cocktail caused cooling of the flow cell and led to the formation of condensation and ice on the cell, which led to loss of signal. Adding 0.5 ml/min isopropanol to the eluent stream just before the backpressure regulator and moving the addition of scintillation cocktail to immediately after the backpressure regulator rather than at the flow cell solved the problem.

Chiral analysis: AD-H column, CO_2:0.5% v/v cyclohexylamine in isopropanol, 2.0 ml/min, 100 bar, 35°C, UV detection at 305 nm (Figure 5).

Radiochemical detection: Tritium, Radiomatic 625TR flow monitor, 500 µl flow cell, Ultima Gold M scintillant at 3.0 ml/min (Figure 6).

Example 6: Radiochemical detection. Achiral analysis: Berger CN column, 6µ, 4.6 × 250 mm. Eluents: A: CO_2; B: ethanol, 2.0 ml/min, 35°C, 100 bar, 500 µl flow

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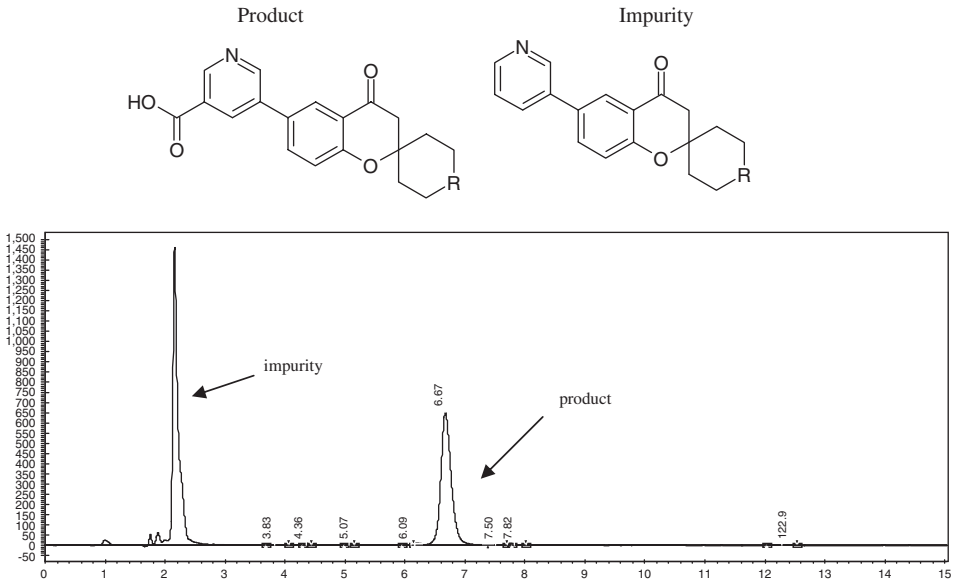

Figure 4

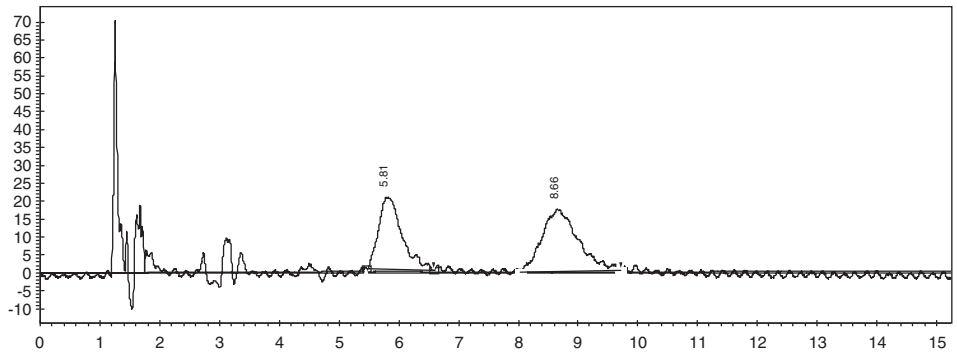

Figure 5

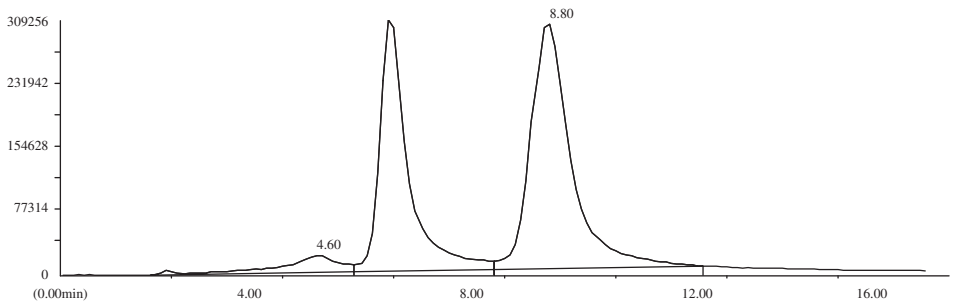

Figure 6

cell, Ultima Gold M scintillant at 3.0 ml/min, Radiomatic 625TR flow monitor 95A:5B to 65A:35B in 30 min (Figure 7).

Example 7: SFC with ELSD. As with the radiochemical detectors, decompression of the CO_2 at the heated nebulizer caused cooling with the formation of solid CO_2 which led to noise in the background due to the breakoff of solid particles of CO_2. Addition of isopropanol to the eluent at 0.5 ml/min just before the backpressure regulator eliminated the problem. Using

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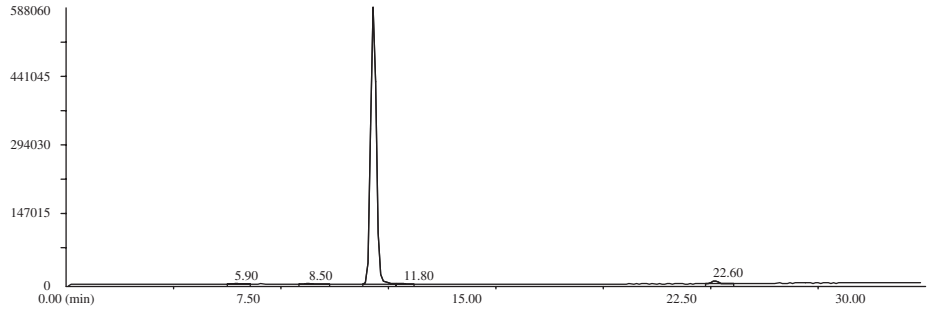

Figure 7

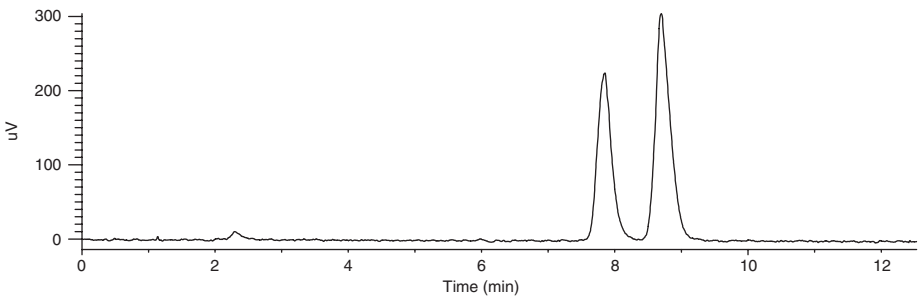

Figure 8

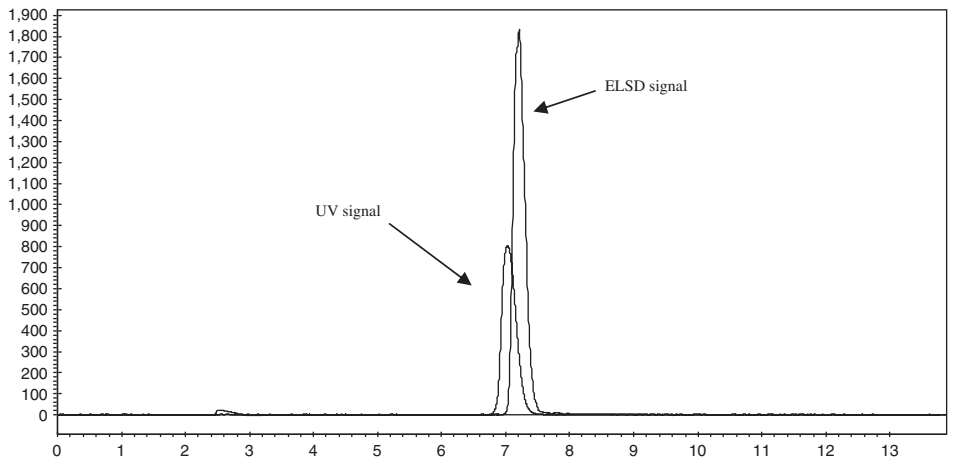

Figure 9

methanol instead of isopropanol made the problem worse.

Chiral SFC analysis: Chiralcel OJ-H, 5μ, 4.6 × 250 mm. Eluents: A: CO_2; B: isopropanol, 70A:30B, 2.0 ml/min, 35°C, 100 bar, Polymer Labs PL-ELS 2100 detector, nebulizer = 30°C, evaporator tube = 30°C, 0.8 l/min N_2 +0.5 ml/min IPA pre-BPR (Figure 8).

Example 8: Achiral SFC with ELSD detection. Caffeine at 1 mg/ml in methanol, Berger Ethyl Pyridine column, 6μ, 4.6 × 250 mm. Eluents: A: CO_2; B: methanol, 90A:10B, 2.0 ml/min, 100 bar, 35°C, UV detection at 210 nm. ELSD: PL-ELS 2100, 30°C/30°C/0.8 l/m (nebulizer T/evaporator T/N_2 flow), 3 μl injected +0.5 ml/min IPA (Figure 9).

SFC and mass spectrometry: Caffeine as per above with 0.5 ml/min isopropanol added before the back-pressure regulator, peeksil™ tubing from backpressure regulator to mass spectrometer (PE Sciex 2000 with Turbospray™). Total organic flow: 0.5 ml/min IPA

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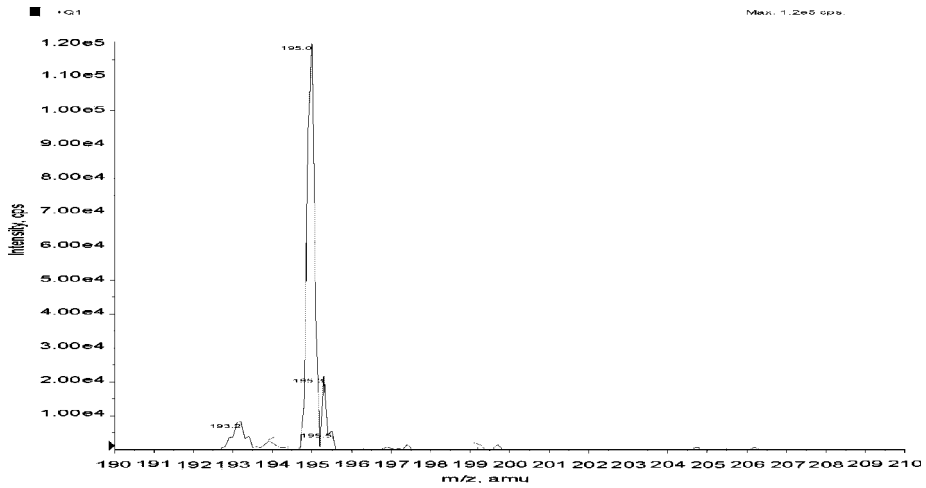

Figure 10

+0.2 ml/min methanol (10% methanol at 2.0 ml/min total SFC flow) = 0.7 ml/min. Isopropanol flow adjusted so that total organic flow is kept less than 1.0 ml/min (otherwise splitter is added just after backpressure regulator). At analytical flow rates, 1.0–3.0 ml/min, increasing the nebulizer temperature of the mass spectrometer is unnecessary (Figure 10).

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