Extending the Range of Laboratory-Scale Purification by Utilising Supercritical Fluid Chromatography for Unattended Overnight Operation

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Supercritical Fluid Chromatography (SFC) remains the preferred choice for preparative enantioseparation within Drug Discovery Research, where semi-preparative SFC is routinely employed to purify intermediates and final products in quantities up to 50 g. As compounds progress through the Medicinal Chemistry pathway, greater quantities of material are required; this can be in the region of 200 g of pure enantiomer for those compounds requiring toxicological evaluations. To meet the increasing demand for larger scale purification within Discovery, and to deliver in a timely manner, SFC can be applied in an uninterrupted batch process where purification campaigns are run unattended overnight across multiple days. In this article the challenges in extending the upper limit of laboratory-scale purification from 50 g to 500 g (racemate) are highlighted and the controls required to satisfy Health and Safety when handling large volumes of SFC co-solvents. Examples of chiral SFC purifications are presented to illustrate the benefit of utilising night-time capacity to process up to 1 kg of material.

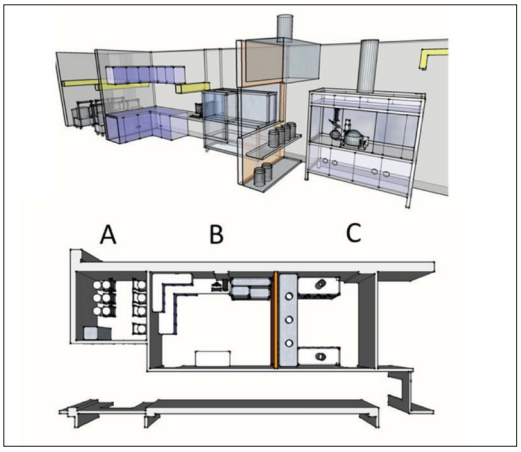

Figure 1: Concept design image of the SFC Purification laboratory with the solvent delivery area (A), the purification area (B) and fraction manipulation and evaporation area (C).

Introduction

The application and benefits of SFC are well documented and recently published [1-8]. Within Lilly SFC remains the technique of choice for the chiral purification of Discovery samples less than 50 g [9]. The low viscosity of supercritical fluids enables 3-5 times higher flow rates compared to HPLC, with increased efficiencies due to the higher diffusivity of analytes in a CO_2 based mobile phase. Compared to the organic solvents employed in HPLC, CO_2 is inexpensive, non-toxic and non-flammable. Most importantly, SFC significantly reduces organic solvent consumption, is easier to handle and permits rapid fraction drying. Handling large volumes of organic solvent, within a Discovery laboratory environment, is highly restrictive and in our opinion the solvent handling benefits of SFC over HPLC are understated.

Miller [10] and Zhang et.al [11] discuss the application of single-column batch preparative SFC for material up to 1 kg in quantity. At the Lilly Research site in the UK, we recently reviewed our purification capabilities to meet the increasing demand for larger scale samples. The result of the review was the development of a SFC workflow to accommodate the following requirements:

i. unattended overnight operation at a flow rate up to 400 g/min.

ii. the capacity to deliver and collect up to 100 litres of co-solvent across a 12 hour purification period.

iii. semi-automated solvent top up and fraction solvent manipulation without interrupting the purification campaign.

iv. parallel fraction solvent evaporation.

v. simple and quick sample to sample changeover.

vi. compliance to local solvent handling and waste stream protocols.

Experimental

Carbon dioxide was supplied by BOC Gases (Worsley, Manchester, UK). CO_2 gas was purified and pressurised to 1500 psi using a booster system supplied from Va-Tran Systems, Inc. (Chula Vista, CA, USA). Methanol, ethanol and isopropanol were HPLC grade and pre-blended with diethylmethylamine (DEMA) additive (0.2%), supplied by Romil (Cambridge, UK). Coated Polysaccharide chiral SFC Columns packed with Chiralpak AD and Chiralpak AS (amylose derivatives) and Chiralcel OD and OJ (cellulose derivatives) were used. Immobilised Polysaccharide chiral SFC columns packed with Chiralpak IA (amylose derivative) and Chiralpak IC (cellulose derivative) were also used. All columns were supplied by Chiral Technologies (Chiral Technologies Europe, Illkirch, France). Preparative column dimensions were 25 cm x 3 cm i.d. or 25 cm x 5 cm i.d. 5 µm particle size.

Figure 2: SFC co-solvent delivery room and flammable solvent cabinets for each solvent type methanol (A), ethanol (B), isopropanol (C) and special solvent blend as required (D).

Figure 3: SFC co-solvent delivery solution with two 50 litre containers daisy-chained together. The container on the lower platform is replaced when empty, allowing solvent top-up without interrupting the purification campaign.

Figure 4: Fraction collection containers located inside a dedicated fume hood. The solvent level sensor (highlighted) is fitted to each container to prevent overfilling.

Instrumentation

SFC purification was performed using a Novasep Supersep 400 system equipped with a CO_2 recycling option (Novasep, Pompey, France). Flow rates specific to the Novasep system are reported as g/min, the unit used by Novasep to measure the flow rate of the dual head diaphragm pump.

Control Measures and Lab Infrastructure

Preparative SFC methods typically use 5-20% of methanol, ethanol or isopropanol as the running co-solvent but occasionally a higher percentage of modifier is required. Using the extreme scenario of 40% modifier, a maximum volume of 100 litres was estimated to maintain an overnight run. The purification workflow therefore needed to accommodate a bank of 3 x 100 litres of solvent with a semi-automated switch over system. This is a very high solvent inventory for a laboratory within Discovery.

Any process involving the handling of large volumes of organic solvent requires careful planning with consideration of the Dangerous Substance and Explosive Atmospheres Regulations 2002 [12], these regulations are the UK's implementation of the European Union-wide ATEX 137 Directive [13,14]. Risks were assessed, laboratory areas were classified into hazardous zones and control measures were implemented.

The SFC purification laboratory was divided into three areas for solvent delivery, purification and fraction manipulation / evaporation (Figure 1). For each type of modifier solvent, two 50 litre containers were daisy-chained together and housed in a ventilated flammable solvent cabinet. A small positive pressure of nitrogen was applied to enable the transfer of solvent to the leading (static) container allowing a simple solvent top up process without stopping the purification process (Figures 2 and 3). Fractions are collected directly from the preparative SFC instrument into a dedicated fume hood containing 50 litre and 25 litre collection containers which were each fitted with solvent level sensors to prevent overfilling (Figure 4). Collected fractions are transferred, using closed lines, to 20 litre evaporators (Buchi, Switzerland) and waste solvent is transferred to a 200 litre waste container using air operated diaphragm pumps operating at a flow rate of 3 litres/min. Fractions are dried down without interrupting the purification run, speeding up the return of dry product to the medicinal chemist. External alarms from solvent and CO_2 detectors trigger an automated shutdown procedure to isolate the modifier and CO_2 solvent supply from the purification instrument, in addition to venting nitrogen from the solvent delivery containers.

Results and Discussion

Instrument reliability and chromatographic reproducibility are critical to a successful multi-day purification campaign, especially when there are no manual intervention opportunities overnight. The first purification we attempted exemplifies the excellent hardware reliability and injection-to-injection

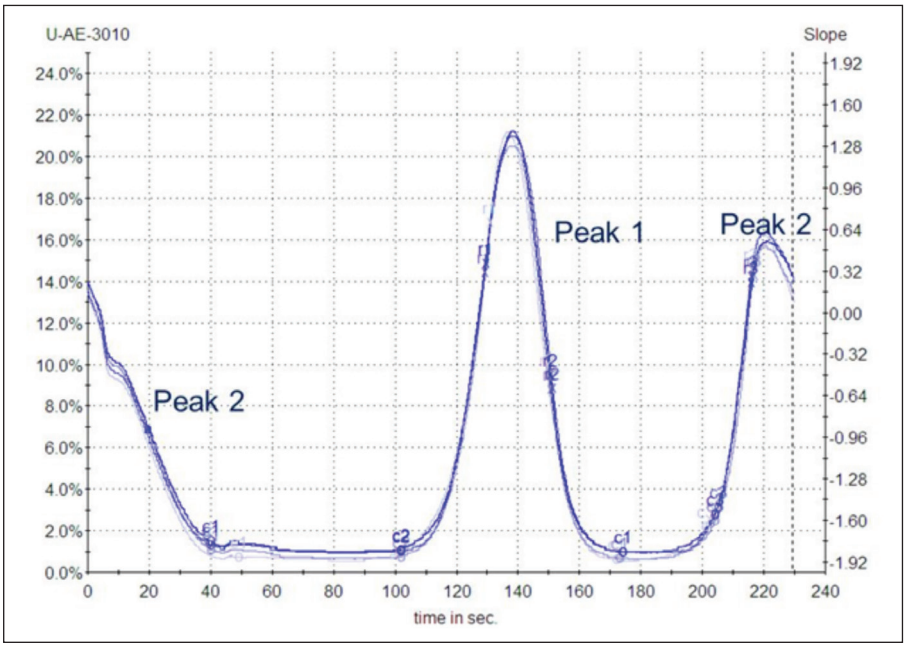

Figure 5: Preparative chiral SFC example (145 g) showing four overlaid chromatograms from a run of 1,309 injections across four days (injection number 40 on the 12th February 2013 at 17:51; injection number 450 on the 13th February 2013 at 20:03; injection number 900 on the 15th February 2013 at 00:48 and injection number 1034 on the 15th February 2013 at 09:22). The flow rate was 160 g/min using 15% isopropanol with diethylmethylamine (0.2%) using a AS-H column of dimensions 3 cm i.d. x 25 cm (5µm). Injection volume was 3.5 ml (127 mg). The first eluting isomer was collected into position 2 and the second eluting isomer collected into position 3.

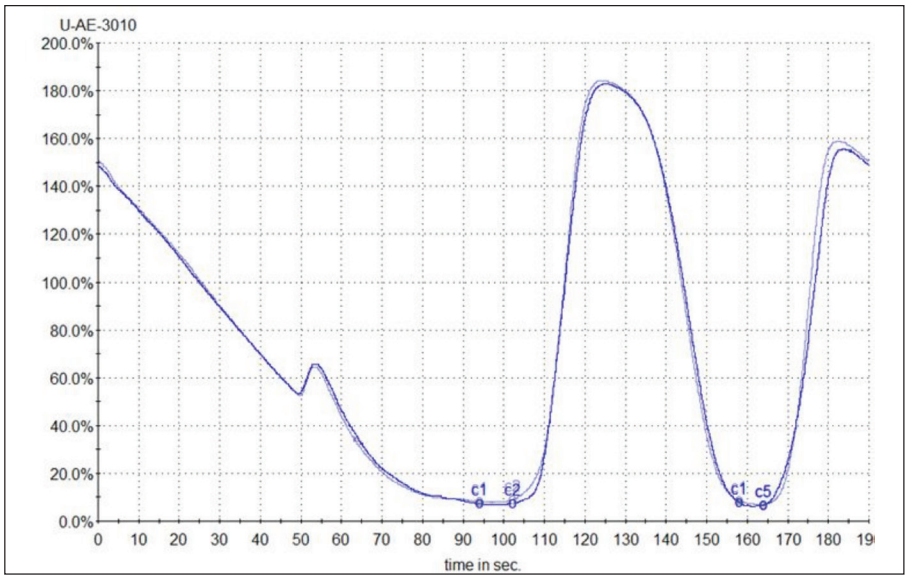

Figure 6: Preparative chiral SFC example (902 g) showing two overlaid chromatograms from a run of 820 injections across two days. The flow rate was 350 g/min using 22% ethanol with diethylmethylamine (0.2%) using a AD-H column of dimensions 5 cm i.d. x 25 cm (5µm). Injection amount was 1.1 g (20.8 g/hr). The first eluting isomer was collected into position 2 and the second eluting isomer collected into position 5. The peak at 50 seconds is a system peak following injection.

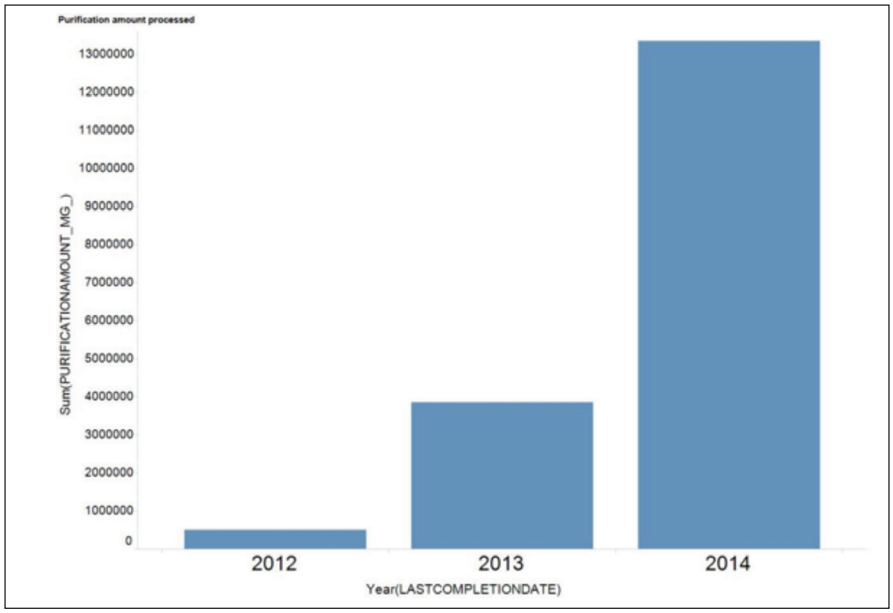

Figure 7: Metrics showing the total amount of material processed (mg) using preparative chiral SFC between 2012 and 2014. In 2012 approximately 500 g of material was processed. In 2013 approximately 4 kg of material was processed and in 2014 approximately 13 kg of material was processed.

reproducibility observed during the last three years of operation. Figure 5 shows four example chromatograms selected across a four-day run. Fractions were collected by time and 1,309 injections were completed successfully without any intervention. Greater efficiency is expected with a 5 cm I.D column (unavailable at the time of purification); accepting a sub-optimal column size it was still possible to process 145 g within a reasonable and acceptable timeframe.

High productivity is required to process up to 500 g of racemate within a few days. Figure 6 illustrates the potential of SFC to purify larger amounts of material when used in automation. For this example 902 g of material was processed, and pure enantiomers returned, within 3 working days of submission. Approximately 1.1 g of racemate was injected every 190 seconds using only 260 litres of organic solvent - a significantly lower volume compared to an equivalent preparative HPLC run.

Figure 7 shows the impact of the new preparative SFC workflow. Following the commissioning of the new laboratory in 2013 there has been a dramatic increase in the amount of material purified (intermediates and final products) by chiral SFC in support of Discovery Chemistry, 13 kg in total for 2014 compared to 500 g in 2012.

Conclusion

SFC offers significant benefits over HPLC for Discovery Research purification laboratories. The reduced organic solvent consumption and fast chromatography changes the purification landscape within the Discovery environment enabling purification work which would otherwise not be considered, due to solvent handling complications. By utilising night-time capacity for single column batch preparative SFC, purifications up to 1 kg are possible. A one-week cycle time is a reality for samples in the region of 500 g. This capability is highly valued for those compounds synthesised at scale for toxicological evaluations, where speed is the key driver.

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