Walk-up SFC-MS for Fast Purification of reaction Mixtures within Discovery Chemistry

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Purification is often regarded as the bottleneck to delivering pure compounds for biological assays within Discovery Research. Medicinal chemists depend on simple processes and robust instrumentation for rapid purification of intermediates and final products, whilst expending as little time and effort as possible. Reverse phase LC-MS platforms are typically used, often with an open-access interface which for the chemist, simplifies sample submission, method selection and batching of samples. The widely accepted limitation of reverse phase LC purification, however, is the time required to remove the post purification aqueous solution and provide a dry isolated product. Supercritical Fluid Chromatography (SFC) is a technique that delivers fractions in a small volume of organic solvent, significantly reducing the post purification processing time. SFC is an established technique within Lilly for queue-based chiral and achiral purification submissions. Until recently, SFC instrumentation has not had a robust hardware and software interface, and as such, has been generally inaccessible to the medicinal chemist in a walk-up environment. In this short application note, we describe our experience with the deployment of SFC-MS for walk-up purification, considering both the challenge to the analytical chemist, and the benefit it affords to the medicinal chemist.

Within our Discovery Chemistry synthesis laboratories, normal phase low pressure flash purification tends to be used as the first pass purification method by the medicinal chemist, for front line achiral purification of intermediates and final products. In an attempt to reduce purification touch time for the chemist, and to free up time for synthetic design, we have explored and tested different purification service concepts. One example is a fee for service flash purification laboratory where a third party provides instrumentation and staff to deliver a two-hour turnaround for flash purifications, using a method provided by the chemist. Although this workflow delivers speed, we occasionally find the quality of collected material to be compromised due to the selection of inappropriate solvent conditions, resulting in no elution of target compound or co-elution of target compound with impurities. Medicinal chemists have limited time for method development; the selection of generic solvent conditions increases the probability of unsuccessful purification, which then impacts productivity through purification re-work. Instead, we have implemented a screening strategy using open access analytical instrumentation, to quickly identify the right technique and the right method for each reaction mixture.

The benefits and application of SFC are well documented and recently published [1-2]. Farrell's group was one of the first adopters of SFC for fast track purification [3]. The speed, separation orthogonality and solvent handling benefits of SFC offer a win-win scenario for both the analyst and medicinal chemist. To complement reverse phase and normal phase HPLC, we have implemented a four-column SFC-MS screen to quickly scout for mass directed SFC purification conditions. Both analytical SFC-MS and preparative SFC-MS instruments are located in a shared equipment zone for easy access, and configured with walk-up software for simple sample submission. The walk-up (open access) interface enables a chemist to submit samples into a queue, without interacting with the instrument operating system, using methods set up by an administrator. Generating analytical data in open access, across multiple techniques and methods, offers a diverse and fast screening approach, which significantly improves the probability of a successful first pass purification within an acceptable timeframe. Generating the analytical screening data 'up-front' also provides the flexibility to the chemist of running their own open-access purification, or to hand the sample over to experts where method selection, purification, evaporation and characterisation is delivered as a service.

Experimental

CO_2

Carbon dioxide was supplied by BOC Gases (Worsley, Manchester, UK). CO_2 gas (99.9% purity) was delivered from a bulk tank and pressurised to 1500 psi using a booster system supplied from Va-Tran Systems, Inc. (Chula Vista, CA, USA).

Instrumentation

The analytical (UPC²) and preparative (Prep100) SFC systems were both configured with a QDa MS, supplied by Waters. The analytical configuration included a column oven and switching valve to accommodate eight columns in total, supporting four chiral columns for enantiomeric excess (EE) determination, in addition to the four achiral columns used for purification method screening. The preparative configuration includes column switching between four columns (achiral only).

SFC Stationary Phase

A four-column analytical screen was established using a selection of commercially available stationary phases including (i) GreenSep Naphthyl, (ii) Torus 1-Aminoanthracene (1-AA), (iii) Propyl-

Pyridyl-Urea (PPU) and (iv) GreenSep Nitro (NO_2). The analytical column dimensions were 100 x 3.0 mm i.d., 5 µm and the preparative column dimensions were 150 x 30.0 mm i.d., 5 µm. The Naphthyl and Nitro columns were supplied by ES Industries, the 1-AA by Waters and the PPU by Princeton Chromatography. The four columns were selected based on their different selectivity when tested on novel compound mixtures across different Lilly compound libraries. Figure 1 illustrates the distribution of SFC columns selected for the isolation of each target compound, from 1,000 sequential purifications across multiple chemistry projects. This exemplifies the need for a column screen, where one single column is the preferred choice for only 30% of the entire sample set.

SFC-MS methodology

The analytical and preparative SFC-MS experimental conditions are described in Figure 2. One generic analytical SFC-MS gradient method is used across all columns, with a single modifier of methanol with ammonia (20 mM). Analytical retention time windows determine the appropriate tailored purification method. See Table 1 for the five tailored gradient programs set up within the open access interface. Analytical SFC-MS screens, submitted towards the end of the day, are automatically batched by method to reduce the frequency of switch methods. Eliminating venting and pressure stabilisation steps, by minimising column switching, increases the efficiency of queued batches and helps to reduce unplanned maintenance intervention. Analytical SFC-MS screens, submitted during core hours, are batched by sample because the set of results are required more urgently to enable purification within the same day. In our experience, it is not necessary to perform scale-up calculations to account for the difference in CO_2 density and mass flow between the analytical and preparative systems. Chromatography from the preparative separation closely resembles the chromatography from the analytical separation and is generally 'good enough' without flow rate or backpressure adjustment.

Results and Discussion

SFC is the preferred technique for small scale, front line, achiral purifications. This assumes reasonable separation, adequate solubility in MeOH and a sample amount of less than 1.5 g. Submitted weight amount per injection, in a walk-up environment, can vary considerably. Loading up to 800 mg of material is possible, but a general guideline of 50 mg per injection is advertised as an appropriate starting point. Sample quantities greater than 1.5 g require too many injections for daytime processing. Larger scale samples are directed towards flash chromatography or submitted to run by SFC overnight, using a night-time submission feature within the open access software.

Table 1: Modifier compositions used for the tailored preparative SFC-MS methods.

Analytical Retention Time Window (min)	Gradient Program	% Modifier starting conditions	% Modifier at time 4.5 minutes	% Modifier at time 5.0 minutes	% Modifier at time 6.0 minutes
0.10 – 0.45	1	5	10	55	55
0.45 – 0.75	2	10	20	55	55
0.75 – 1.05	3	15	25	55	55
1.05 – 1.20	4	20	30	55	55
1.20 – 1.60	5	25	40	55	55

One of the main challenges to the application of MS directed SFC purification in open access, is the unknown solubility of the mixture in methanol as it hits the stream of CO_2 post injection valve. Complex mixtures contain many impurities with unknown solubility characteristics, as a result filters are often blocked and require intervention from an expert. Implementation of a simple solid phase extraction (SPE) sample pre-treatment step, performed by the chemist, significantly reduces (i) the risk of blockages and (ii) the overall quantity of material to process. We have also found it beneficial to make a small change in the configuration of the preparative SFC system. The standard instrument configuration introduces the CO_2 stream, to the sample flow path, directly after the injection valve, where the sample is then carried some distance to the column. If solubility is an issue, precipitation occurs between the CO_2 mixing point and the separation column. The frequency of manual intervention (to replace filters) can be reduced by moving the CO_2 mixing point to inside the column oven, just before the column switching valve. Reducing the length of tubing between the mixing point and the column, combined with heating to 55°C, helps to maintain sample dissolution and reduces the risk of sample plugging.

To mitigate risk of instrument down time, we have found it highly beneficial to support an assisted open access service where chromatographers are available to collaborate with chemists, by monitoring and assisting the purification workload. Facilitating open access purification, using a super-user, also improves efficiency by maximising instrument utilisation throughout the day and by stacking sequences to run into the evening.

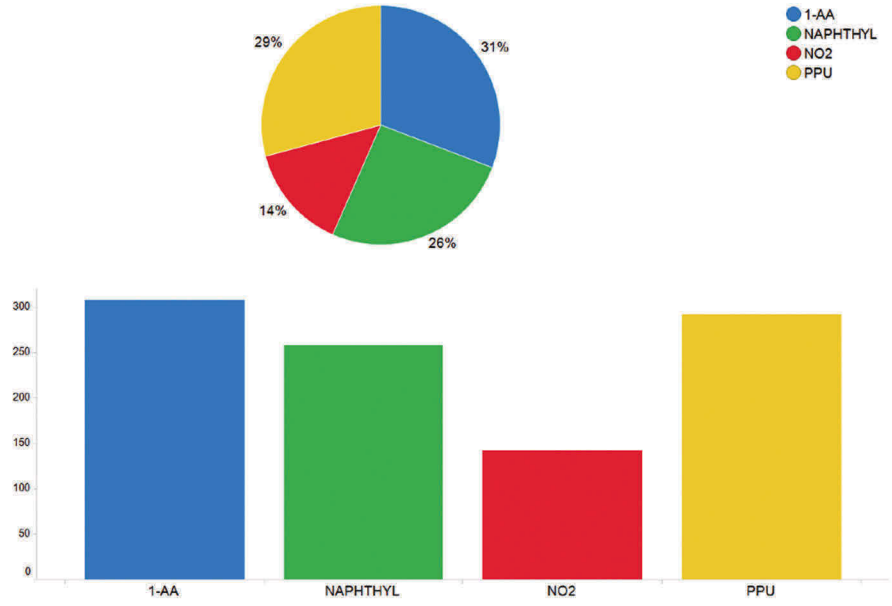

Figure 1: Column type selected for SFC purification, for a set of 1,000 sequential purifications across multiple chemistry projects.

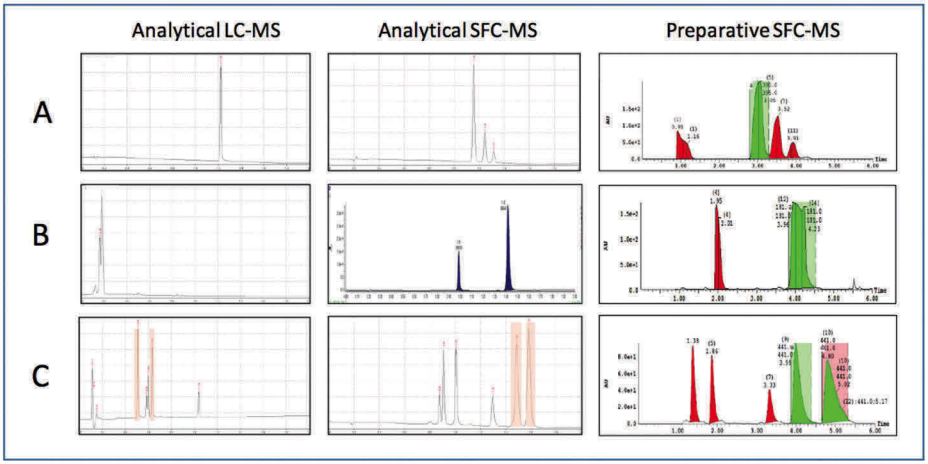

Figure 2: LC-MS method : 5-95% MeCN in 1.5 min and holding at 95% for 0.5 min, where the organic solvent is MeCN and the aqueous solvent is 10 mM ammonium carbonate pH 9; flow rate is 1.2 ml/min; temperature is 50 °C; stationary phase is the Waters XBridge C18 column with dimensions 50 mm x 2.1 mm i.d.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

Acknowledgement

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References

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