Achiral Supercritical Fluid Chromatography (SFC) for the Purification of Pharmaceuticals

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With large quantities of small scale (<1000mg) compounds typically being needed to be purified and evaluated for discovery in-vitro and in-vivo testing regimes, a chromatographic purification technique needs to be easily integrated into discovery chemistry laboratories and highly reproducible. Within Separation Sciences Departments in the Pharmaceutical Industry there are a range of methodologies focused on the analytical separation and purification to isolate pure products [1]. High performance liquid chromatography (HPLC) has been generally accepted as the main technique of choice for analysis and purification, utilising either normal phase or reverse phase conditions, with reverse phase being the generally accepted as a high throughput approach to purify small scale drug discovery products [2, 3]. Although the use of acetonitrile within reversed phase is a widely accepted modifier, it is acutely toxic to aquatic life and the effluent from laboratories has to be controlled and incinerated which increases the cost and environmental impact. At Novartis, the integration of supercritical fluid chromatography (SFC) has several advantages such as faster run times due to lower viscosity, short equilibration times, reduced solvent consumption and, in preparative applications, fast solvent removal. SFC has rapidly become a very attractive alternative to Normal Phase and Reverse Phase purification for achiral samples [4]. An example of this efficiency is shown in Figure 1 where the same sample is purified (25mg injection). The sample was processed in a third of the time by SFC with equivalent purity and recovery.

In efforts to find new and innovative ways to improve chromatographic processes, Novartis has increasingly employed Supercritical Fluid Chromatography (SFC) to decrease the overall environmental footprint and increase productivity for small to large (mg-g) scale achiral purifications. A typical example separation comparison between SFC and HPLC is shown in Table 1 where the enormous benefits in efficiency, time, solvent consumption and evaporation time are highlighted.

The combination of SFC with high performance HPLC in parallel for achiral screening has enabled us to establish an efficient SFC platform for discovery separations. Several SFC-MS directed purification systems have been set up within Novartis globally to allow for rapid and efficient purification. This successful approach has led to an increase in the use of a SFC screening platform for achiral purification submissions within Novartis. An example of this is shown in Figure 2 where routinely more submissions are purified by SFC than traditional Reverse Phase Liquid Chromatography (RP-HPLC). The improved reliability of analytical and purification SFC instrumentation and increasing availability of a range of SFC stationary phases means this methodology is becoming method of choice for more achiral separations as well as chiral small molecule purifications [5].

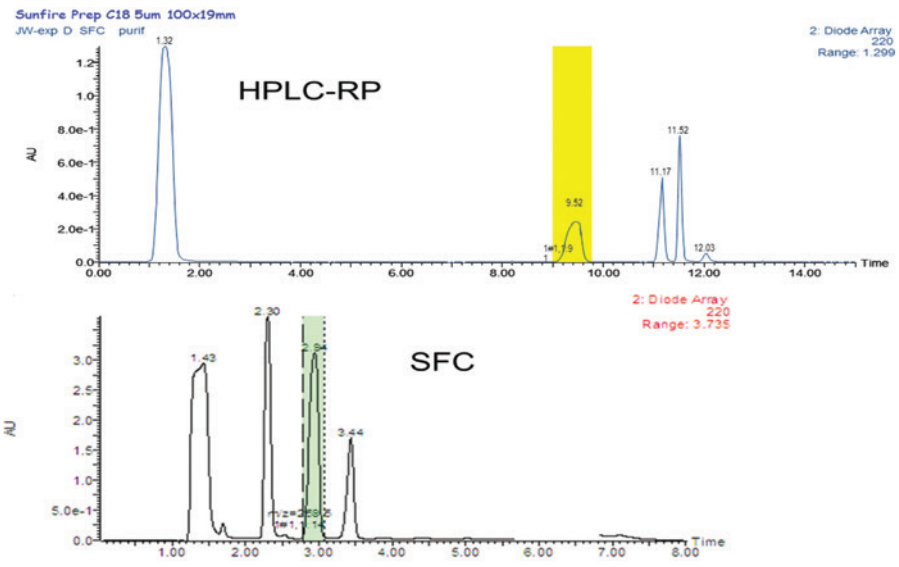

Figure 1: HPLC-RP v SFC comparison for discovery purification.

The coupling of SFC-MS collection enables focused collection of products and by-products in a small fraction of methanol which can be evaporated in a fraction of the time of aqueous-acetonitrile fractions from HPLC. There are some caveats to sample preparation for purification which need to be closely adhered too, due to a high risk of precipitation and loss of product, the samples have to be

Table 1: Comparison of Prep SFC v Prep HPLC. (Courtesy of Waters Corporation)

	Purification by SFC	Purification by HPLC
Separation Time	3 hours	46 hours
Organic Solvent Used	5L of Methanol	40L of Acetonitrile
Total Workup Time	1 hour	8 hours
Recovery	95%	80%

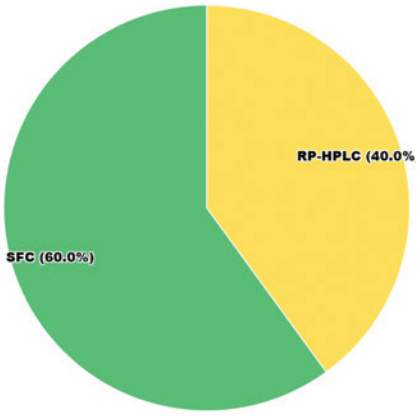

Figure 2: Typical 60:40 distribution for SFC v RP-HPLC for discovery achiral purifications.

fully dissolved in Methanol (MeOH) or a 1:1 mixture of Dichloromethane/Methanol (DCM/MeOH) to ensure solubility on-column.

Analytical Screening Strategy

Our standard screening approach is to maximise efficiency within a 2 minute analytical gradient using a range of SFC stationary phases. The gradient approach is as follows:

Mobile Phase
Line A: CO_2
Line B: Pure MeOH or MeOH (+0.1% basic modifier)

SFC Gradient

Time	% Mobile Phase A	% Mobile Phase B	Flow Rate (mL min^{-1})
0	0	5	3.0
0.1	95	5	3.0
2.00	45	55	3.0
2.40	45	55	3.0
2.50	95	5	3.0

Column: 50 mm x 3mm SFC analytical
Column Temperature: 35°C
Injection volume: µl

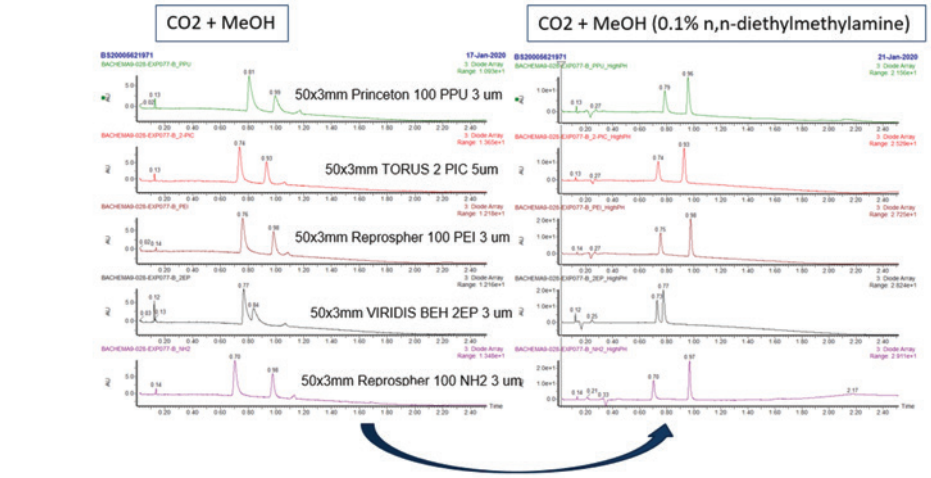

Figure 3: Addition of 0.1% n,n-diethylmethylamine to MeOH demonstrates superior peak shape and reversal of elution order.

An example where the addition of a basic modifier (0.1% n,n-diethylmethylamine) to co-solvent MeOH demonstrates the significant difference to peak shape and elution order in Figure 3.

There is no uniformly accepted 'generic' SFC column chemistry within the industry and therefore there is a need to select a range of chemistries in comparison to RP-HPLC where C18 is the more standard and unified approach. A demonstration of this is shown in Figure 4 for a test mix separation for 6 different column chemistries using the same generic SFC gradient.

Strategy for Purification

Based on the 2 minute gradient analytical result, we then can develop a 'bespoke' shallow gradient window for individual samples defining the percentage of MeOH needed to try and elute the compound of interest in the middle of the prep gradient. We have optimised the purification run times by the implementation of higher flow rates and also routine use of 3um SFC Prep columns to maximise peak efficiency. An example is shown in Figure 5 where the purification total run time is only 4 minutes using a 10-27% MeOH shallow gradient at total flow150ml mL min-1. This is now standard procedure within our laboratory and has been enabled by the recent upgrade of two waters SFC-100-MS instruments by ABsys (Oberursel, Germany).

These ABsys upgrades have enabled the laboratory to increase flow rates of up to 180 mL min-1, optimise gas-liquid separation to enhance recovery, shorten preparative methods, shorten equilibration times and implement waste collection for every run. The system is shown in Figure 6. This has enabled Achiral SFC purification processes to be optimised and has enabled an increase in the scale of separations we can employ and has been adopted by our lab as our purification system of choice.

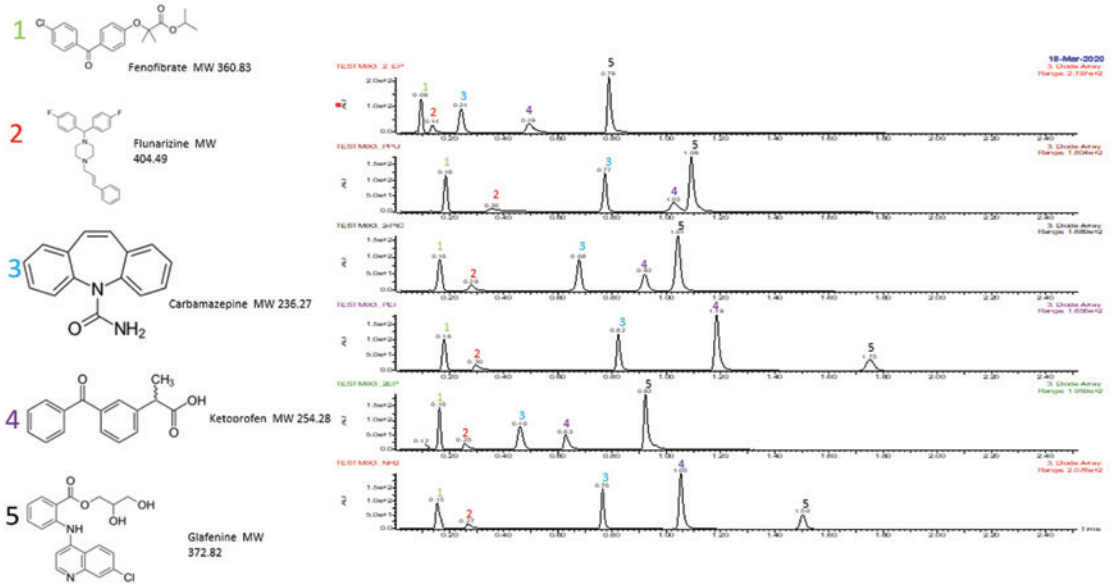

Figure 4: Testmix Selectivity on range of SFC column chemistries.

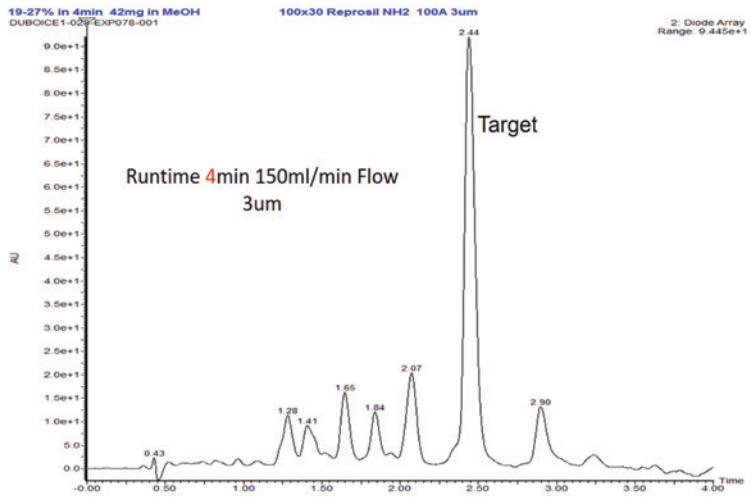

Figure 5: 42mg injection on 100 x 30mm Reprosil-NH2 100A 3um using 19-27% MeOH shallow gradient at 150ml mL min^{-1}.

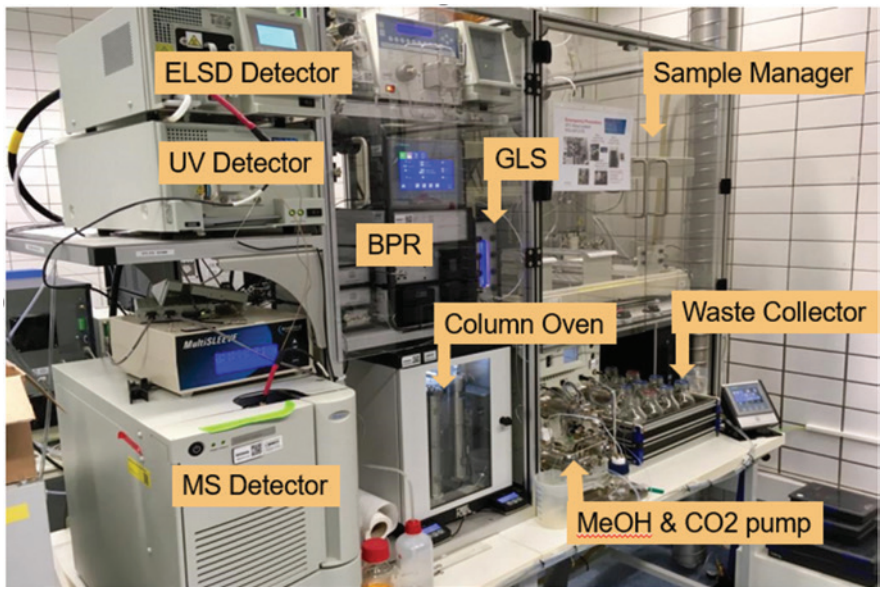

Figure 6: ABsys upgrade to Waters SFC-100 purification -
GLS = Gas Liquid Separator
BRP = Back Pressure Regulator

Conclusions

By taking advantage of the advances in SFC technology, chromatographers are also able to capitalise on the improvements improving achiral separation efficiency whilst also reducing environmental impact. Purification via SFC is ~3 to 4x faster than a traditional RP purification with purity equivalent to RP-HPLC. With the introduction of the ABsys SFC systems the reliability, speed and scale of SFC Achiral purifications has been improved. We in Novartis are now able to efficiently separate achiral novel drugs whilst significantly decreasing run times, environmental impact, maximising recovery with reduced dry down times. Analytical and Preparative Achiral SFC is standard practice and adopted by the whole Global Discovery Chemistry community. RP-HPLC will still remain a complimentary orthogonal technique for purification and first method of choice in Open Access purification.

References

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