

# Using Mass-Directed Preparative SFC and RPLC for Library Compound Purification

Andreas Mich<sup>1</sup>, Burkhard Matthes<sup>1</sup>, Rui Chen<sup>2</sup> and Stefan Buehler<sup>1</sup>

<sup>1</sup> Boehringer Ingelheim Pharma GmbH & Co. KG, Biberach, Germany

<sup>2</sup> Waters Corporation, Newark Delaware, USA

## Introduction

In the past decade, supercritical fluid chromatography (SFC) has experienced a steady growth in acceptance, particularly in pharmaceutical and chemical laboratories. In SFC, "supercritical" CO<sub>2</sub> combined with one or more polar organic solvents, most commonly alcohols, are used as mobile phase. Preparative SFC is deemed by many to hold the greatest promise to attain mainstream acceptance. The reduction in solvent consumption and collection in relatively small volumes of volatile organic solvents, hence, a much less stringent post-purification endeavor, lead to significant savings on operation costs. For example, Ripka et al. calculated that 20,000 samples purified by SFC instead of reverse phase liquid chromatography (RPLC) would realize a 48 times reduction in solvent consumption.<sup>1</sup>

Mass-directed RPLC has revolutionized the workflow for chemists in drug discovery by enabling one fraction collection per injection, largely owing to the specificity that MS detection can offer. A mass-directed preparative SFC system, like its counterpart in RPLC, will certainly be welcomed by the industry for high throughput purification of diverse libraries of drug-like compounds.<sup>2</sup> However, open bed fraction collection has been a challenge for SFC as a result of aerosol formation caused by the depressurization of CO<sub>2</sub>, particularly at high flow rates. Zhang et al. demonstrated the feasibility of MS-directed SFC fraction collection, but it was limited to flow rates of 30 mL/min.<sup>3</sup> The challenge of managing aerosols in collection at high flow rates has been overcome with the development of the Waters TharSFC SFC-MS Prep 100™ system.<sup>4</sup>

In this application note, we present a comparative study of the mass-directed purification of 167 library compounds by both AutoPurification® LC-MS and Waters TharSFC SFC-MS Prep 100™. The success rates for each technique individually and the two techniques combined are compared to highlight the orthogonality of these techniques.

## Experimental

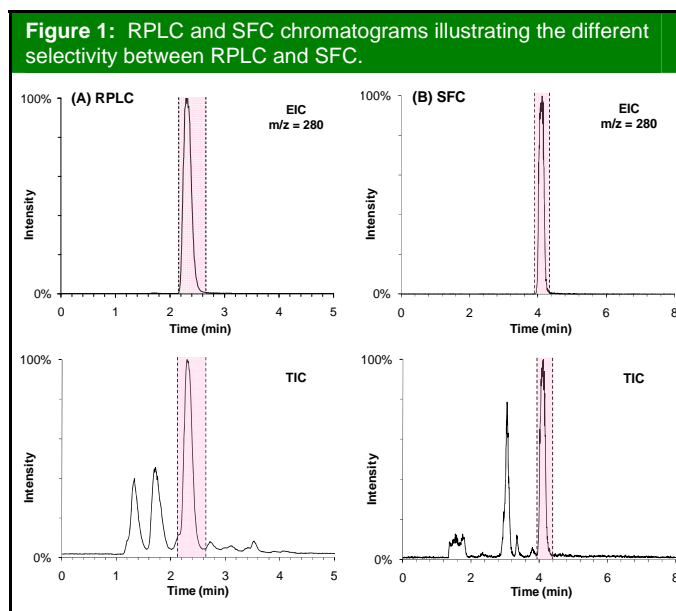
The library compounds were synthesized by the combinatorial chemistry group at Boehringer Ingelheim Pharma GmbH & Co. KG, Biberach, Germany. A detailed description of the SFC-MS Prep 100 system can be found elsewhere.<sup>4</sup> Key experimental parameters are listed in Table I.

## Results and Discussion

Figure 1 shows the representative chromatograms where both techniques were successful in collecting the desired compound. However, the chromatograms from RPLC and SFC are different, indicating the different selectivity between RPLC and SFC. Supercritical fluid chromatography is generally considered a normal phase separation technique that complements reverse phase LC in selectivity, especially for polar compounds and structurally similar compounds.<sup>5</sup> In addition, the mobile phase for SFC was supercritical CO<sub>2</sub> and methanol, and for RPLC, water and methanol were used. The two mobile phases possess different solvent strength, resulting in substantially different sample loading capacity depending on the nature of the compounds. The combination of RPLC and SFC will cover a wider sample polarity range, typical for library compounds, than each technique alone.

Table 2 summarizes the comparison between the two techniques for the purification of the two libraries. Note that the registered compound is defined as the compound purified from raw mixtures that meets three predefined criteria: (1) > 2 mg; (2) purity > 80% by LC PDA; and (3) purity > 80% by NMR. Overall, both techniques displayed similar performance in terms of success rate and purity. The success rate for SFC was 27%, 25% for RPLC, and 32% when both techniques were combined, representing a 30% increase from each technique alone. It has to be pointed out that the overall 68% "failure" rate is not the true reflection of either purification technique. Preanalysis indicated that the syntheses were not successful for more than 60% of the library compounds.

The batch time for SFC was 20% shorter than for RPLC. This gain in throughput is mainly due to the shorter dry-down time post-purification in SFC than in RPLC. It is also noted that for SFC, a generic gradient from 5-30% with a cycle time of 11 min was used, whereas for RPLC, a focused gradient of 23-43% was used based on the preceding analytical scouting experiments. It is envisioned that the batch cycle time for SFC can be further reduced by applying a similar focused-gradient approach. Efforts to optimize the work flow are in progress.



**Table I:** Key experimental parameters in this study.

AutoPurification LC-MS		SFC-MS Prep 100
Sample solvents	MeOH:DMF:TFA:H <sub>2</sub> O (160:50:15:25 in volume)	
Column	Sunfire C18 (30 x 100 mm)	TharSFC ethylpyridine (30 x 150 mm)
Flow rate	50 mL/min	100 g/min
Column temperature	50°C	40°C
Back pressure	N/A	120 bar
Mobile phase	A: 0.1% ammonia in H <sub>2</sub> O B: methanol	A: CO <sub>2</sub> B: methanol
Gradient	Focused gradient	5 – 30%
Gradient cycle time (min)	9	11
Injection volume (μL)	2500	2500

With the economic downturn and patents coming off protection in the next few years, improving productivity and more aggressive cost-savings have become top priorities in the pharmaceutical industry. In addition to the evident cost-savings as a result of higher throughput and less post-purification endeavor, the orthogonality of SFC to RPLC also provides chemists with the capability to recover more compounds from medicinal chemistry, which already has significant embedded capital investment, for ensuing research and development.

**Table II:** Comparison on the purification of the two libraries between RPLC and SFC.

	Library A		Library B	
	RPLC	SFC	RPLC	SFC
Number of compounds	95		72	
Registered compounds	26	28	27	27
Purity % by LC PDA	95	94	95	99
Batch cycle time (day)	5	4	5	4
Number of unique compounds by technique	7	9	2	2

## Conclusions

The success rate of purifying the raw mixtures to registered compounds was 27% for SFC and 25% for RPLC. The combination of the two techniques resulted in an overall increase in success rate by roughly 30% compared to using each technique alone, mainly due to the orthogonality between the two techniques. Despite using a longer gradient cycle in SFC, overall batch processing time for SFC was 20% less than RPLC. This gain in throughput was the result of shorter dry-down time post-purification.

## References

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