

# A Comparative Study on the Purification of Library Compounds in Drug Discovery Using Mass-Directed Preparative SFC and LC

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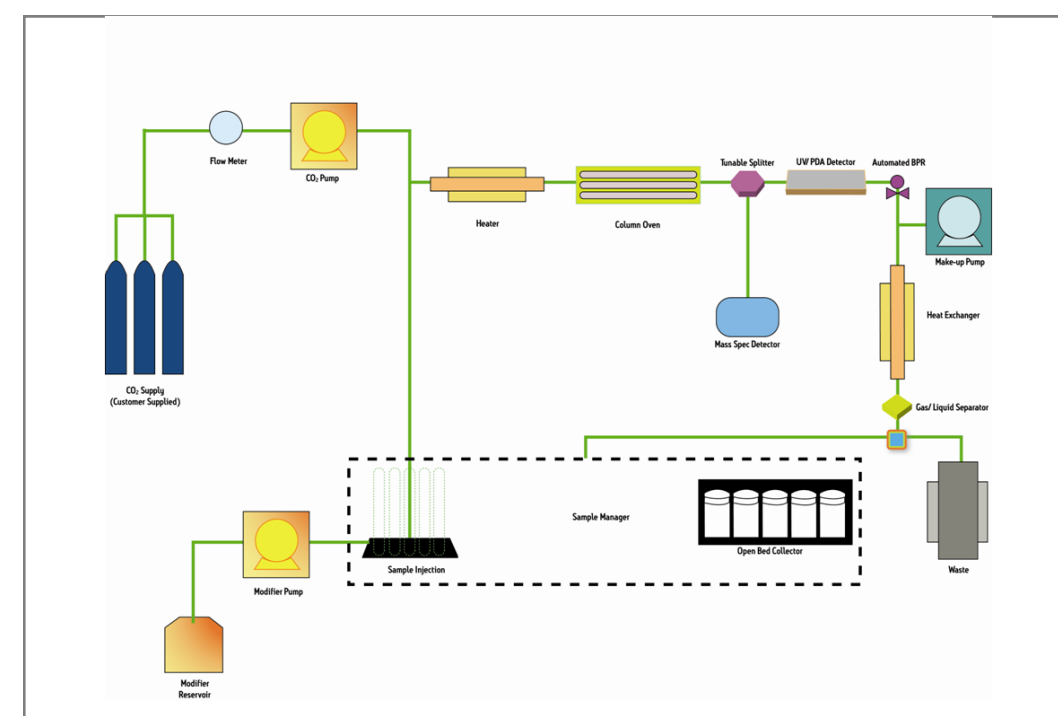
## INTRODUCTION

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, is 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 the collection of relatively small volumes of volatile organic solvents, leads to significant savings on operation costs.

A mass directed preparative SFC system, like its counterpart in LC, will expedite the work flow in drug discovery owing to the specificity that MS detection can offer. Until recently, mass-directed purification capabilities were limited to HPLC based techniques for high throughput purification of diverse libraries of drug-like compounds.

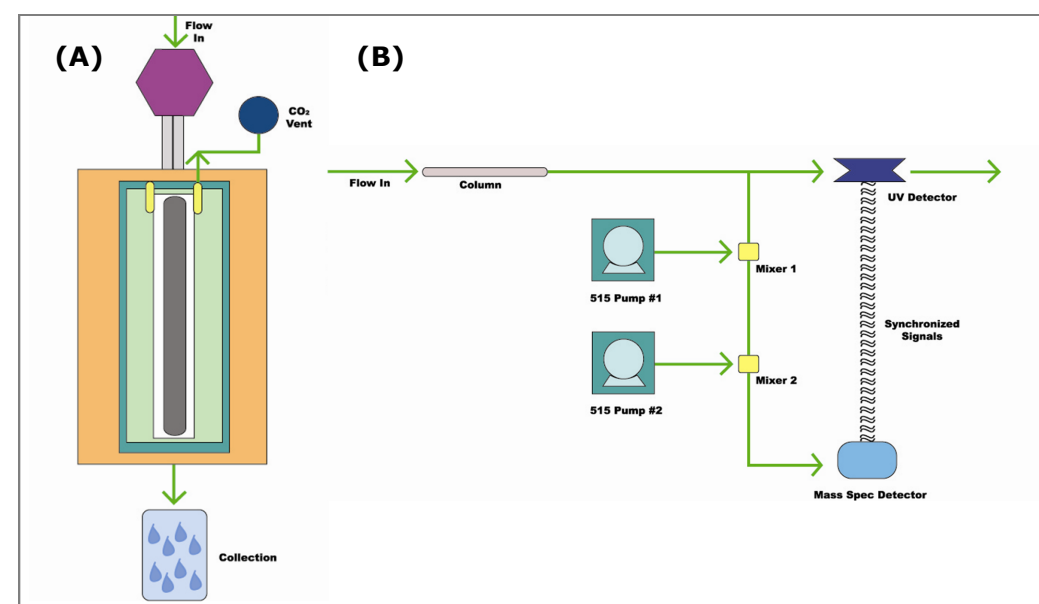
Open bed collection has been a challenge for SFC due to aerosol formation caused by the depressurization of CO<sub>2</sub>, particularly at high flow rates [1-2]. The challenge of managing those aerosols and fraction collection at high flow rates was finally overcome with the development of the Waters TharSFC™ SFC-MS Prep 100 system (Figure. 1).

In this poster, we present a comparative study of the mass-directed purification of a total of 167 library compounds by both AutoPurification® LC MS and Waters TharSFC™ SFC-MS Prep 100. The two key innovations specific to the SFC-MS Prep 100, are briefly introduced. The orthogonality of the two techniques is highlighted.



Schematic of the Waters TharSFC™ SFC-MS Prep 100 system.

## METHODS

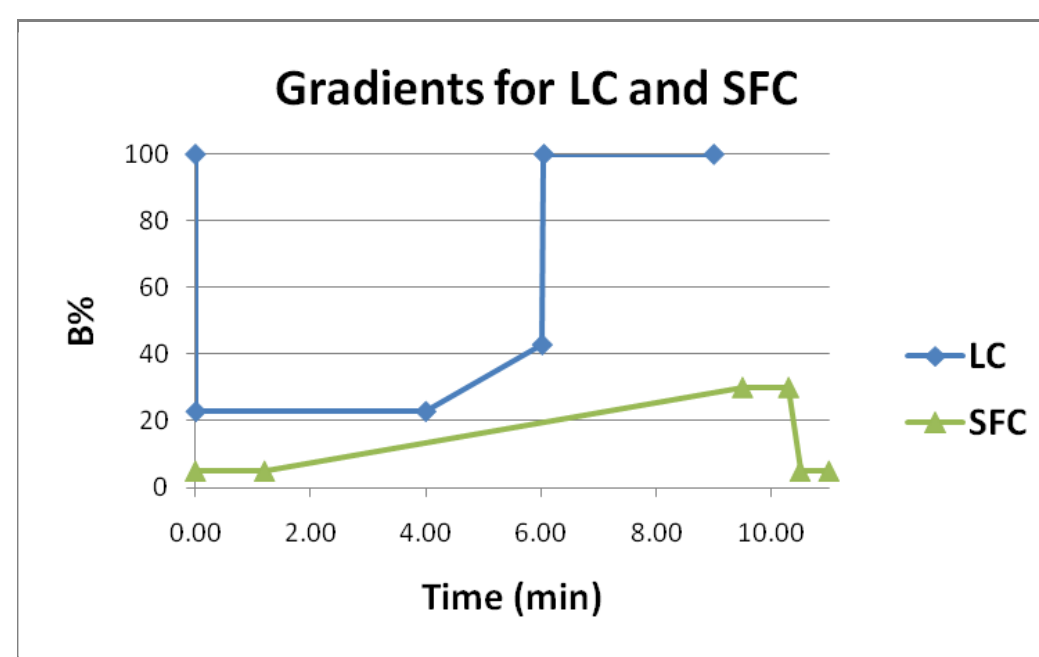


Schematics of (A) the gas/liquid separator and (B) the tunable splitter for the Waters TharSFC SFC-MS Prep 100 system.

The gas/liquid separator (A) effectively removes the CO<sub>2</sub> prior to collection in a continuous-flow mode to minimize aerosol formation. The tunable splitter (B) can achieve from 1:25,000 to 1:500,000 split ratio.

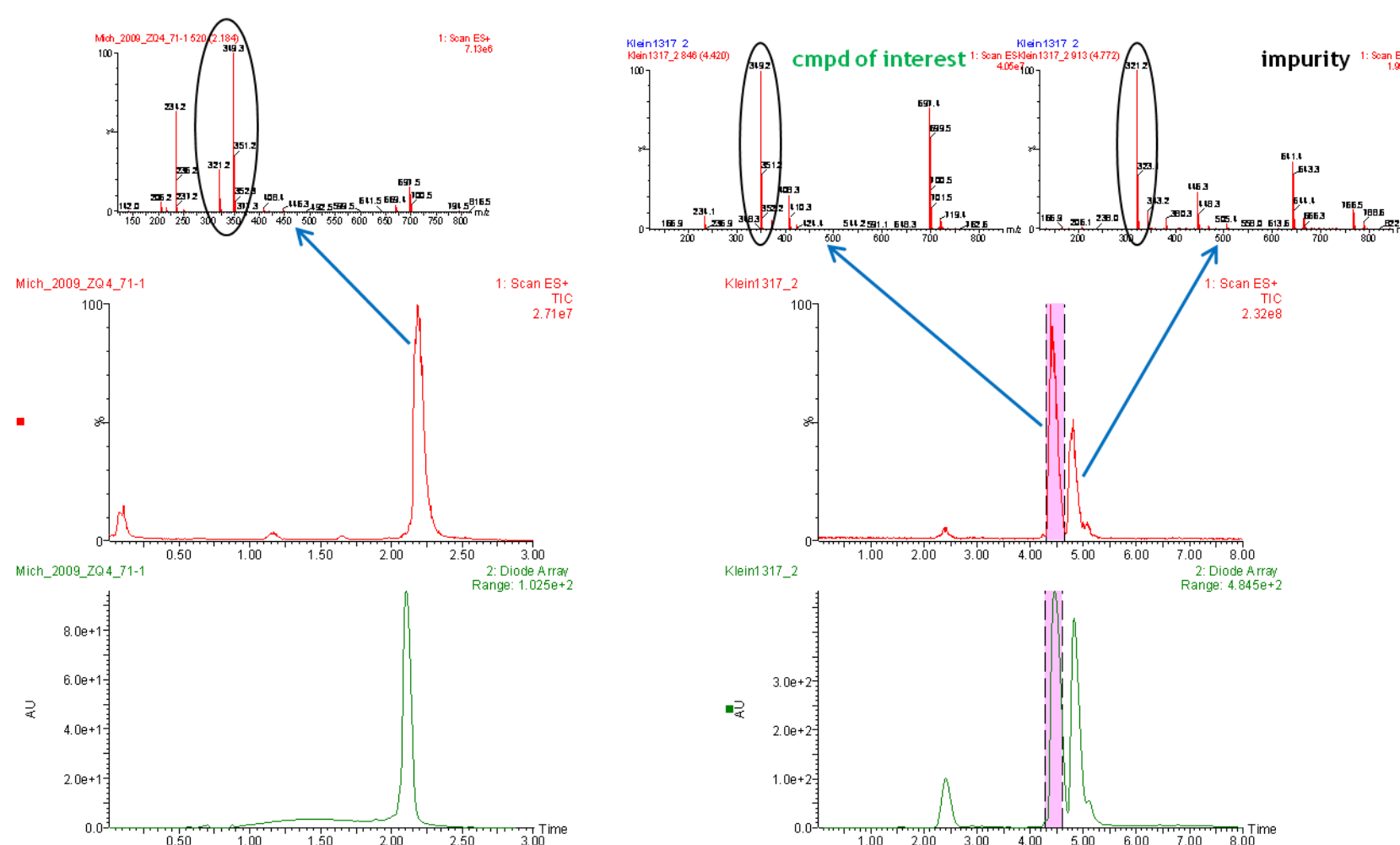
Key chromatographic parameters.

	AutoPurification® LC MS	TharSFC™ SFC MS Prep 100
sample solvents	MeOH:DMF:TFA:H <sub>2</sub> O (160:50:15:25 in volume)	TharSFC™ Ethylpyridine (30x150 mm)
column	Sunfire™ C18 (30x100 mm)	100 g/min
flow rate	50 ml/min	40°C
temperature	50°C	120 bar
back pressure	N/A	A: CO <sub>2</sub> B: methanol
mobile phase	A: 0.1% NH <sub>3</sub> in H <sub>2</sub> O B: methanol	5-30%
gradient	focused gradient	11
gradient cycle time (min)	9	2500
injection volume (µl)	2500	2500

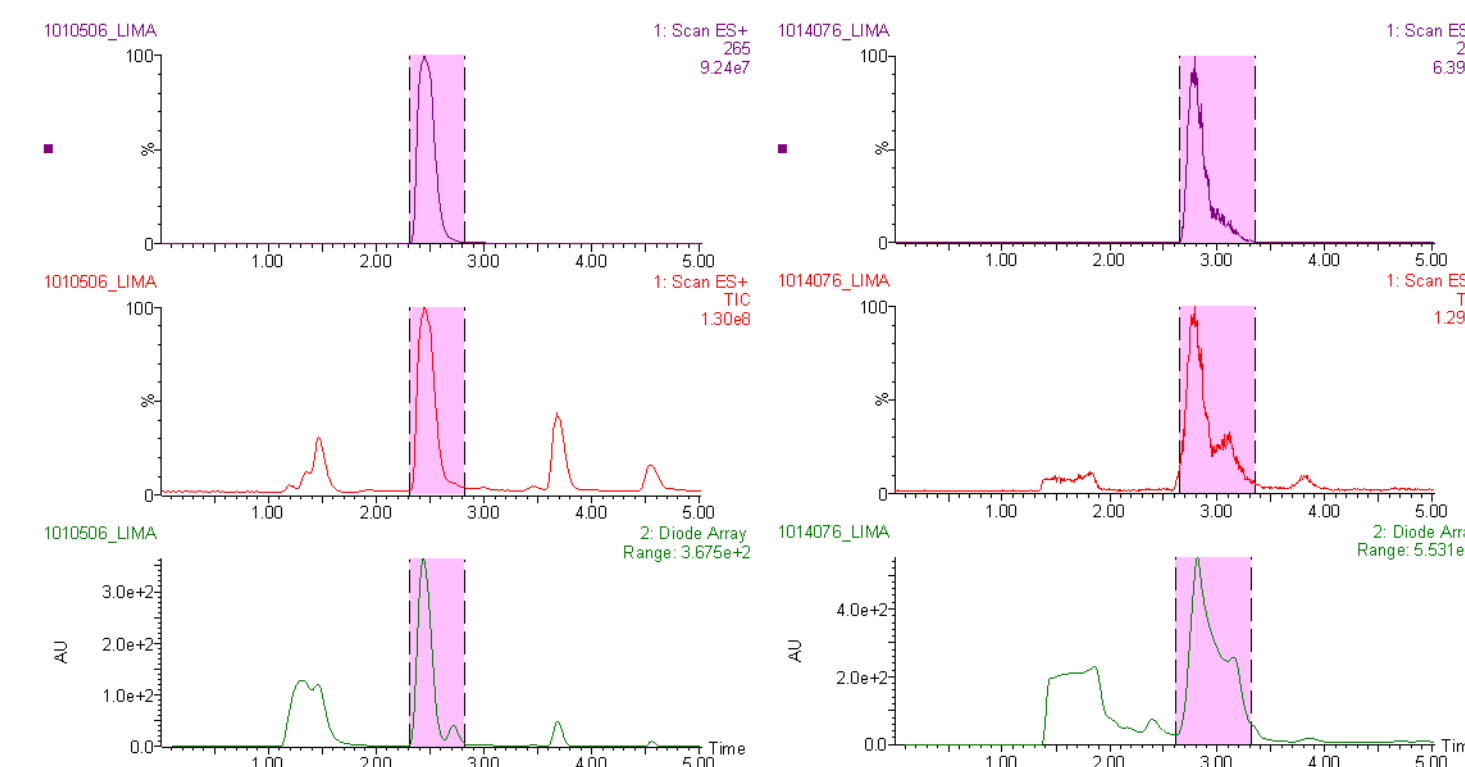


LC and SFC gradients used in this study.

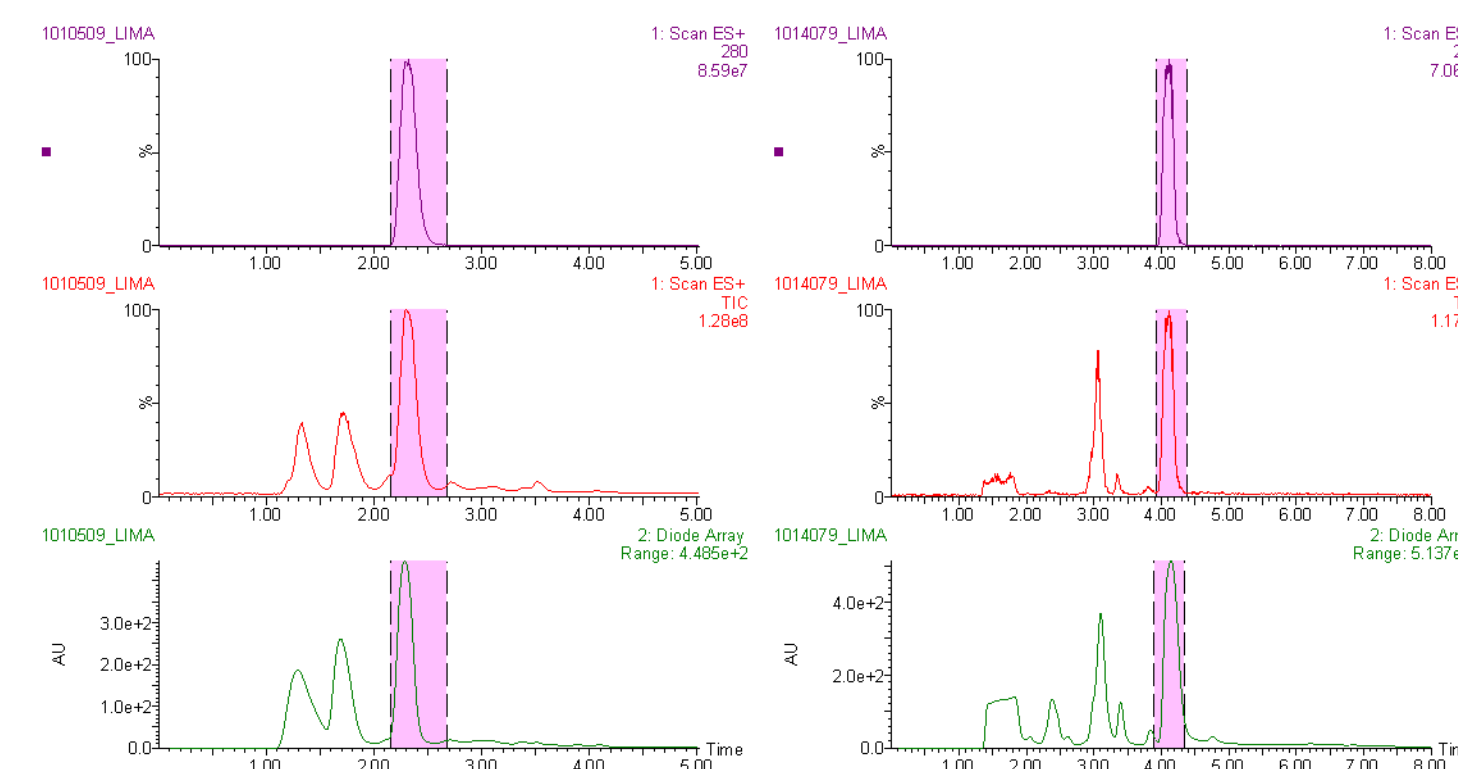
## RESULTS AND DISCUSSION



Example 1: The compound of interest (m/z=349) co-elutes with the impurity (m/z=321) in LC, but the two compounds were resolved in SFC. SFC is a normal phase separation technique, which complements RPLC.



Example 2: Both techniques were successful in collecting the desired compound. However, LC displays more symmetrical peak shapes; whereas in SFC, there is a "shoulder" in all displayed chromatograms, likely due to sample precipitation. In this study, the mobile phase for SFC is supercritical CO<sub>2</sub> and methanol, and for LC, water and methanol were used. The two mobile phases possess different solvent strengths. Depending on the nature of the compound, sample loading can be substantially different. The miscibility between samples and mobile phases should be an integral part of selecting the more suitable technique for purification.

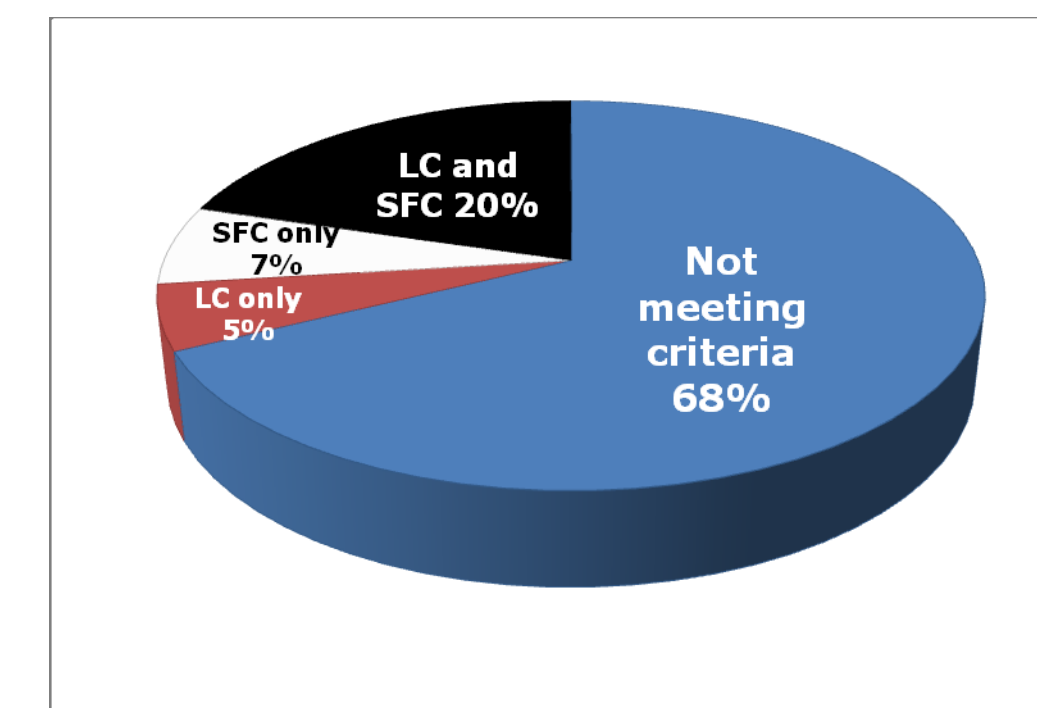


Example 3: Both techniques were successful in collecting the desired compound. However, there are more peaks present on the SFC chromatograms. This example demonstrates the different selectivity of the two techniques.

With the economic downturn and patents off protection in coming 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.

	Library A		Library B	
Number of compounds	95		72	
Registered compounds	LC	SFC	LC	SFC
Purity% by DAD (LC)	26	28	27	27
Batch cycle time (day)	95	94	95	99
	5	4	5	4

The above table summarizes the comparison between the two techniques for the purification of two libraries. Note that the registered compound is defined as the compound purified from raw mixtures that meets three pre-defined criteria: > 2mg; purity>80% by LC MS; and purity>80% by NMR. Overall, both techniques display similar performance in terms of "hit" rate and purity; and the batch time for SFC is 20% shorter than for LC. This gain in throughput is mainly due to the shorter dry-down time in SFC than in LC post-purification. It is worthy to point out that for SFC, a generic gradient from 5-30% with a cycle time of 11 min was used, whereas for LC, a focused gradient of 23-43% was used. It is envisioned that the batch cycle time for SFC can be further reduced by applying a similar focused-gradient approach. More work to optimize the work flow is currently under way.



Overall success rate from this comparative study.

It has to be pointed out that the 68% failure rate is not the true reflection of either purification technique. Based on the similar success rates of both LC and SFC, the majority of the 68% failed compounds is more likely due to the unsuccessful syntheses.

## CONCLUSION

- Both mass-directed LC and SFC were successful in purifying the two libraries.
- The success rate of purifying the raw mixtures to registered compounds was 27% for SFC and 25% for LC.
- Despite using a longer gradient in SFC, overall batch processing time for SFC was 20% less than LC. This gain in throughput was the result of shorter dry-down time post-purification.
- SFC is an orthogonal technique to LC. The combination of the two techniques resulted in an overall increase in success rate by roughly 30% compared to using each technique alone.
- No additive was used in SFC based purifications. Compared to LC based methods where TFA or formic acid are often used. The SFC approach eliminates the possibility of forming adducts that are detrimental to screening experiments downstream.
- An average of over 95% purity was achieved for the compounds that were successfully purified by mass-directed SFC in an open-bed collection format, indicating the effectiveness of the proprietary gas/liquid separator in removing CO<sub>2</sub> prior to fraction collection to eliminate cross-contamination.

## References

- Christine Aurigemma, Steve Zulli, Ziqiang Wang and Jennifer L. Leffler, "Performance evaluation of MS-directed preparative SFC with open-bed fraction collection", poster presentation, LabAutomation, 2009.
- Ray T. McClain, Anna Dudkina, James Barrow, George Hartman, and Christopher J. Welch, Journal of Liquid Chromatography & Related Technologies, 32: 483-499, 2009.