SFC-UV AND SFC-MS METHOD DEVELOPMENT: THE NEW STATE OF THE ART

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INTRODUCTION

Improving productivity and aggressive cost-savings have been one of the most persistent initiatives in the pharmaceutical and related industries. To that end, supercritical fluid chromatography (SFC) has been of great interest as a viable complement to liquid chromatography (LC). The advantages of SFC include the reduction in solvent consumption, shorter dry-down time post-purification, and its orthogonality to RPLC that enables chemists to recover more compounds from medicinal chemistry for ensuing research and development.

One "bottleneck" in SFC applications is the extensive method development required due to the lack of a "universal" column. Currently, most commercial instruments employ automated column and solvent switching to test each condition individually. To expedite this process, multi-column (parallel) screening approaches in SFC have been introduced [1]. Compared to sequential screening, the parallel approach enables multiple columns being screened simultaneously, significantly improving the throughput of method development and optimization.

In this poster, we introduce the Waters Method Station X5 SFC system, a multi-channel SFC system with UV and/or MS detector. We will present some application examples to demonstrate its design objectives and operational principles.

METHODS

A detailed description of the Method Station X5 SFC system can be found elsewhere [1]. A schematic of the SFC X5 system is shown Figure 1. In the first two experiments, SuperChrom™ was used for data acquisition. A Resolution X5 SFC MS system controlled by MassLynx™ software was used for the last experiment.

Table 1. Chromatographic conditions for screening experiments.

	Chiral	Achiral	Achiral-MS
Flow rate	12.5 mL/min	15 mL/min	20 mL/min
No. of channels	4	5	5
System pressure	120 bar	120 bar	120 bar
Injection volume	10 µL	50 µL	50 µL
Modifier	methanol	methanol	methanol
Gradient	5% for 1 min 5 to 40% in 5 min 40% for 1 min 40 to 5% in 1 min 5% for 1 min	5 to 40% in 5 min 40% for 1 min 40 to 5% in 2 min 5% for 2 min	5 to 30% in 5 min 30% for 1 min 30 to 5% in 2 min 5% for 2 min

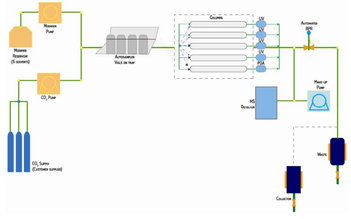


Figure 1. Schematic of the Method Station X5 SFC system.

RESULTS

Despite the lack of one universal column for chiral separation by SFC, it is many users' experience that more than 75% of the pharmaceutical relevant chiral molecules can be separated by one or multiple of the following chiral stationary phases: OD-H, AD-H, OJ-H and AS-H [2].

Figure 2 shows the SFC chromatograms of 4-benzoyloxy-2-azetidinone obtained in parallel screening mode. Both AD-H and AS-H columns yielded baseline resolution of the enantiomers. OD-H generated partial separation and OJ-H offered no resolution. Based on the screening results, the system should then be switched to the single channel mode with either AD-H or AS-H for an optimal isocratic method. However, for illustration purposes, the OD-H column was selected for further method optimization to mimic a common scenario where only partial separation is achieved with a gradient in the parallel mode. The system was then switched to the single channel mode with the OD-H column for further method optimization. The optimized method using 5% methanol is shown in Figure 3.

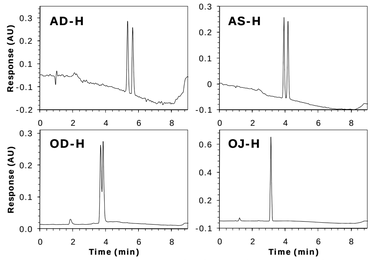


Figure 2. Parallel screening of 4-benzoyloxy-2-azetidinone (2.5 mg/ml racemic) on four chiral columns simultaneously.

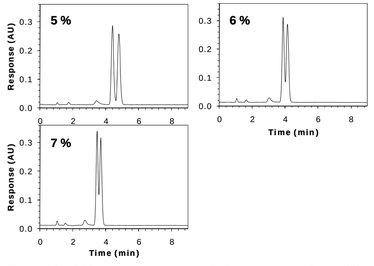


Figure 3. SFC chromatograms of 4-benzoyloxy-2-azetidinone on an OD-H column under different isocratic conditions. Injection volume: 2 µL; flow rate: 2.5 mL/min.

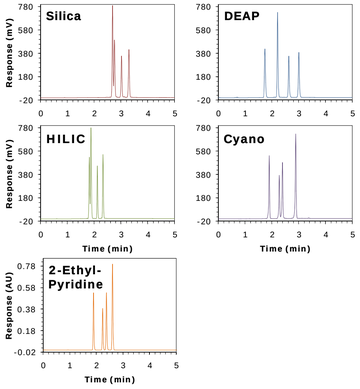
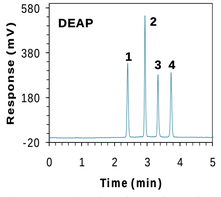


Figure 4. Parallel screening of theophylline, amcinonide, thymine and uracil (1 mg/mL each) on 5 different achiral columns.



Components

1.Theophylline
2.Amcinonide
3.Thymine
4.Uracil

Figure 5. Single channel analysis of achiral mix on DEAP column. Injection volume: 10 µL; flow rate: 3 mL/min.

The same principle can be applied for achiral applications as well, as long as the selected stationary phases (up to 5) encompass a sufficiently wide selectivity range.

Figures 4 and 5 demonstrate achiral method development and optimization for a 4-compound mixture. The software controlled switching between parallel and single channel modes enables a streamlined process with minimal user intervention. Depending on the downstream purification platform, bulk or batch, the method optimization can be done in either isocratic or gradient fashion.

For compounds without chromophores or structural analogs including degradents and metabolites (Figure 6) , it is beneficial to have a MS detector for structural confirmation. The Method Station X5 SFC system can be seamlessly integrated with Waters 3100 MS controlled by MassLynx™.

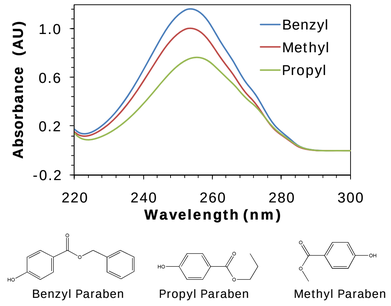


Figure 6. UV Spectra and chemical structures of benzyl paraben, methyl paraben and propyl paraben.

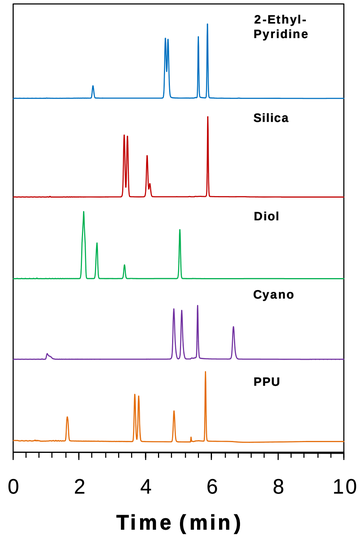


Figure 7. Parallel screening of paraben mixture: acetaminophen, caffeine, benzyl paraben, methyl paraben and propyl paraben (1 mg/mL each).

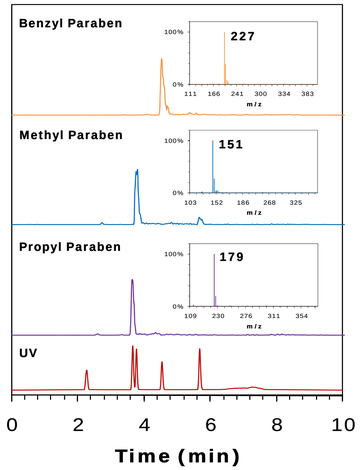


Figure 8. SFC UV chromatogram of the mixture and the extracted ion chromatograms (EICs) of 3 parabens obtained under APCI negative mode. Injection volume: 10 µL; flow rate: 4 mL/min.

Figures 7 and 8 demonstrate the parallel screening and single channel optimization with MS detection for a 5-compound mixture. With MS, the identities of the 3 structurally similar parabens were readily confirmed.

CONCLUSION

- **The Method Station X5 SFC system offers a streamlined process for chiral and achiral SFC method development and optimization in an automated fashion.**
- **The Method Station X5 SFC system can be seamlessly integrated with Waters 3100 MS detector, which is often required for structural confirmation in achiral**

References

1. *L. Subbarao, J. Cole, and R. Chen, LC GC Europe Application Notebook, Dec.* ***2009***
2. *E. R. Francotte, J. Chromat. A 906 (****2001****) 379-397.*

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