Mass-Directed Preparative SFC with Open-Bed Fraction Collection

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INTRODUCTION

In recent years, improving productivity and cost-savings have become major initiatives in pharmaceutical discovery and development. The introduction of new technologies for high-throughput synthesis has enabled preparation of up to 48 compounds in the same time frame previously required for a single compound. Consequently, there has been increasing pressure on development and implementation of efficient purification platforms to support the overall high throughput process.

Mass-directed reverse phase liquid chromatography (RPLC) purification has revolutionized the workflow for chemists in drug discovery by enabling one fraction collection per injection, largely due to the specificity that MS detection offers. As a result, mass-directed RPLC has quickly become the most commonly used technique in high throughput purification environments. Considerable effort has been put into streamlining the process so that minimal user intervention is required. However, a major limitation in the process is the long dry-down time of aqueous fractions downstream.

Adopted primarily for chiral analysis and purification by the pharmaceutical industry in the early 1990s, supercritical fluid chromatography (SFC) has experienced a remarkable resurgence in the past decade. The reduction in solvent consumption and collection in relatively small volumes of volatile organic solvents in preparative SFC has led to significant savings on operational costs. For example, Ripka et al. calculated that 20,000 samples purified by SFC instead of RPLC would realize a 48 times reduction in solvent consumption.

A mass-directed preparative SFC system, like its counterpart in RPLC, is an ideal platform for high-throughput purification of diverse libraries of drug-like compounds. However, open-bed fraction collection has been a challenge for SFC due to aerosol formation caused by the depressurization of CO₂, particularly at high flow rates. Kassel et al. demonstrated the feasibility of mass-triggered SFC fraction collection at 15 mL/min and Zhang et al. reported their results with flow rates up to 30 mL/min. The challenge of managing aerosols in collection at high flow rates has been overcome with the development of Waters® Prep 100 SFC MS Directed System.

In this application note, we present our research results to illustrate one of the key innovations of the Prep100 SFC MS Directed System: the Gas-Liquid Separator (GLS). Some comparative results on library compound purification using both AutoPurification® LC/MS and Prep 100 SFC MS Directed systems are also presented to highlight the orthogonality of the two techniques.
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SYSTEM CONFIGURATION

All preparative experiments were performed on a Prep 100 SFC MS Directed System, as shown in Figure 1, which consists of: Waters 2767 Sample Manager, 3100 MS Detector, 2998 PDA Detector, 515 Pumps and Waters CO₂ Pump and Co-solvent Pump, Analytical-2-Prep Column Oven, Automated Back Pressure Regulator (ABPR), GLS, and Tunable Splitter. The system was controlled by Waters MassLynx™ Software.

RESULTS AND DISCUSSION

One of the major challenges in implementing open-bed fraction collection at high flow rates in SFC is CO₂ expansion. After exiting the ABPR, high pressure liquid CO₂ decompresses to its gaseous form at a volume ratio of approximately 1:500. This rapid expansion causes aerosol formation, which can lead to sample loss and cross contamination. This challenge is addressed by a proprietary GLS, as shown in Figure 2. The geometry of the GLS, the dimension of the initiator, and its angle with respect to the inner wall effectively eliminate the majority of gaseous CO₂. Another key aspect of the GLS implementation is to preserve the peak integrity during a
gradient before and after the GLS, such that time delay between detectors and collector tip across a gradient can be accounted for correctly. To accomplish that, a make-up flow of methanol with a reverse gradient is introduced to ensure a constant flow passing through the GLS, especially at the point in the gradient when the co-solvent percentage is low. To prove the efficiency of the GLS, two detectors, one placed in the main flow path before the GLS and the other after the GLS in place of the collector tip, were used to compare the pre- and post-GLS peak profiles. Figure 3 shows the two traces at different modifier percentages from the two UV detectors. It is evident that peak profiles were well maintained passing through the GLS. The maximum band broadening due to GLS is 1.2 s (results not shown) at the peak tail, representing a less than 2% sample loss.

The effectiveness of the GLS is demonstrated by the high recovery and high purity of collected fractions. For example, Aurigemma et al. reported their evaluation of the Prep 100 SFC MS Directed System. In their experiment, a mixture containing 8 mg/mL each of (A) flavone, (B) carbamazepine, (C) acetaminophen, and (D) sulfamethazine was injected and collected over three replicate injections, as shown in Table 1. Overall, sample recovery and purity was greater than 95% (with the exception of peak C in injection 1) during gradient elution. In particular, the high purity of two closely eluting components (C and D) indicated a negligible cross contamination between peaks.

In a separate experiment, similar evaluations were performed using a mixture of caffeine, sulconazole, and bendroflumethazide. On average, greater than 90% recovery and greater than 99% purity were achieved for all compounds using UV triggering, MS triggering, and UV+MS triggering, respectively.

An additional built-in advantage of the Prep 100 SFC MS Directed System is the software continuity from the analytical platform, Resolution™ SFC MS System, capable of both pre-purification scouting and post-purification analysis. Both platforms are controlled by the MassLynx Software package. After the analytical scouting run, data is processed and the retention time for the ion of interest is used to match one of the pre-defined focused gradients on the preparative system. The purification strategy is automatically determined and exported to the preparative platform. The whole process is automated by AutoLynx™ and FractionLynx™ application managers, part of MassLynx Software control. Furthermore, since both Prep 100 SFC MS Directed and AutoPurification LC/MS systems are controlled by MassLynx Software, it should be effortless for existing AutoPurification LC/MS users to adapt Prep 100 SFC MS Directed into their workflow.
Implementing the Prep 100 SFC MS Directed System into high-throughput purification environments provides a cost-savings that results from reduction in solvent consumption and dry-down time. In addition, the orthogonality between SFC and RPLC offers increased cost-savings by pushing more compounds from medicinal chemistry through the pipeline. SFC is loosely considered a normal phase chromatographic technique and complements RPLC in selectivity. For example, Figure 4 shows the comparison between mass-directed RPLC and SFC in purifying a real-world pharmaceutical compound directly from combinatorial synthesis; in which, the impurity was resolved from the target compound in SFC but not in RPLC. In our previous study, we reported a 30% increase in success rate when combining the two techniques compared to using each technique alone. In the same study, we also reported a 20% reduction in overall batch processing time for SFC compared to RPLC as a result of shorter dry-down time post-purification.

<table>
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<tr>
<th>Inj.</th>
<th>Peak</th>
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Table 1: Purities and recoveries of SFC collections.
CONCLUSIONS

- The GLS, the key innovation in the Prep 100 SFC MS Directed System, has been able to overcome one of the main historical challenges in SFC open-bed fraction collection.
- Greater than 90% recovery and greater than 95% purity were achieved in two independent evaluations, which indicates the effectiveness of the GLS.
- The level of automation available through the software between the analytical and preparative platforms enables a streamlined analytical to purification process with minimal user intervention.
- The orthogonality between mass-directed RPLC and SFC can potentially offer users additional cost-savings by recovering more compounds from medicinal chemistry for ensuing research and development.

References: