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FastTrack to supercritical fluid chromatographic purification: Implementation of a walk-up analytical supercritical fluid chromatography/mass spectrometry screening system in the medicinal chemistry laboratory

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ABSTRACT

Medicinal chemists often depend on analytical instrumentation for reaction monitoring and product confirmation at all stages of pharmaceutical discovery and development. To obtain pure compounds for biological assays, the removal of side products and final compounds through purification is often necessary. Prior to purification, chemists often utilize open-access analytical LC/MS instruments because mass confirmation is fast and reliable, and the chromatographic separation of most sample constituents is sufficient. Supercritical fluid chromatography (SFC) is often used as an orthogonal technique to HPLC or when isolation of the free base of a compound is desired. In laboratories where SFC is the predominant technique for analysis and purification of compounds, a reasonable approach for quickly determining suitable purification conditions is to screen the sample against different columns. This can be a bottleneck to the purification process. To commission SFC for open-access use, a walk-up analytical SFC/MS screening system was implemented in the medicinal chemistry laboratory. Each sample is automatically screened through six column/method conditions, and on-demand data processing occurs for the chromatographers after each screening method is complete. This paper highlights the "FastTrack" approach to expediting samples through purification.

1. Introduction

Walk-up or open-access LC/MS technology has been around for a number of years and has proven to be a valuable asset to the chemical synthesis process [1–7]. The utilization of standardized methods, using conventional software with an open-access interface, allows users to select the appropriate methodology without in-depth system or hardware training. LC/MS parameters can be tailored to a specific set of compounds, e.g. acidic and/or basic, to aid chemists in reaction monitoring and optimization, as well as confirmation of the intended product [8,9]. These walk-up chromatographic parameters can be scaled to preparative conditions without requiring further method development prior to purification.

SFC has been used successfully for the analysis and purification of a diverse range of compounds [10–17]. In each case, generalized mobile phase conditions were maintained and selectivity was optimized by changing the stationary phase. SFC is considered green technology, with additional advantages of fast analysis times and rapid evaporation of non-aqueous fractions. As open-access analytical instruments are primarily LC/MS and those methods are not transferable to SFC, it is reasonable to expect samples to be screened by the SFC specialists upon submission for purification. The absence of a walk-up SFC system creates a disconnect between reverse-phase LC/MS users and an SFC-based centralized lab for either analysis or purification. In addition, SFC instruments are typically more complex and their utilization is limited primarily to centralized chromatography groups.

Column screening is a rapid and efficient procedure to determine separation conditions [18–20]. In cases where compounds have no previous chromatographic analyses, e.g. chiral and new synthetic products, the empirical approach to purification method selection is routinely employed. Combined with batching or triaging samples, resources can be maximized within the centralized lab [21]. However, this could impact the timely processing of sample data necessary for purification.

In our laboratory, the standard process for submission to the purification workflow includes sample batching usually once or twice daily especially when large numbers of compounds are submitted. Chromatographer productivity was compromised as soon as the number of submissions became too large to handle; therefore, batching samples for screening made sense rather than addressing one sample at a time. This enabled the chromatographer to more efficiently perform analytical screening, method develop-

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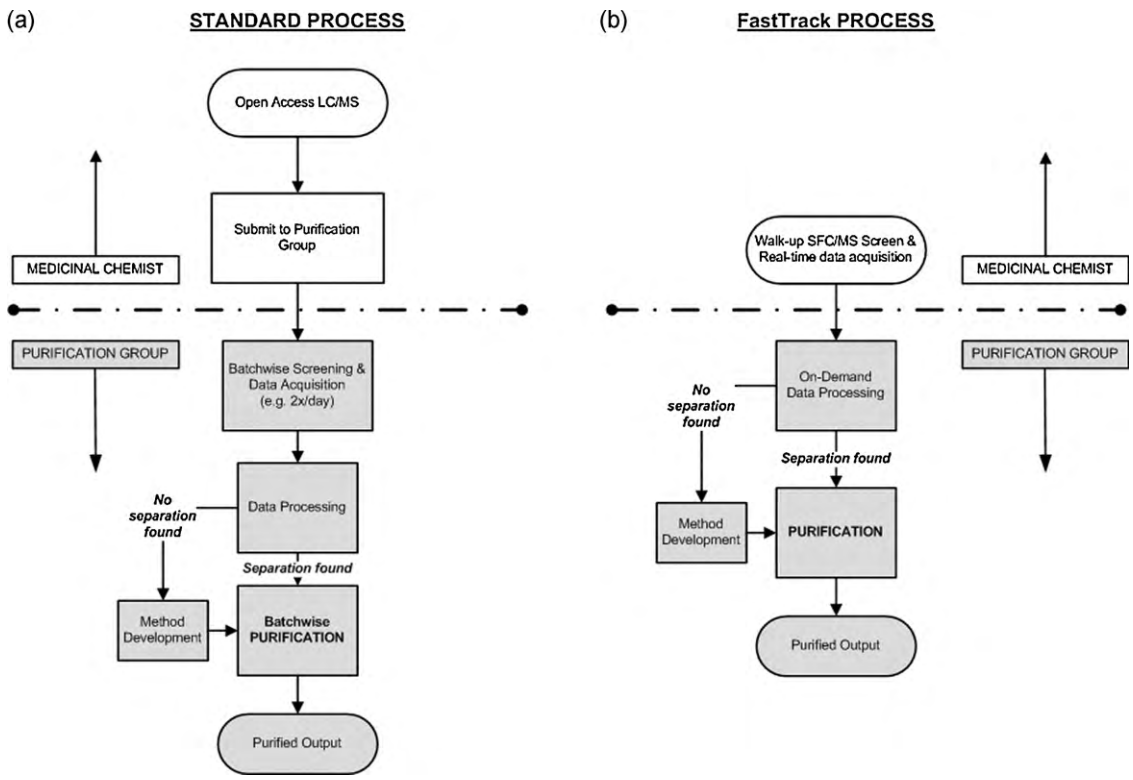

Fig. 1. Standard and FastTrack submission process workflows. (a) Standard process requires direct submission to Purification group, while (b) FastTrack process requires submission to walk-up SFC/MS screening, where purification begins pending successful analyses.

ment, and purification tasks. Analytical-scale samples are prepared for automated pre-purification screening, and each sample is injected through a screen of six different stationary phases. The sequence is ordered so that each set of column conditions is loaded and all queued samples are run through a specific 20-min condition before switching to the next column. The batch analyses time depends on the number of samples in the queue, and additional submissions are not added to the sequence once it begins because of the way the sequence is structured. As a result, the availability of screening data for manual review and subsequent method selection for scale-up SFC purification may have lengthy delays. Also, additional method development may be required to ensure sufficient separation of products and impurities. The further batching of samples for purification based on a particular column method contributes to a lack of continuity between sample submission, analysis, and purification and adversely affects sample turnaround time.

To increase efficiency and expedite the return of purified products, we implemented a walk-up analytical SFC/MS system which features the same facile software already in use on open-access LC/MS systems. Fig. 1 illustrates the reduction in workflow steps to purification using the FastTrack process. In the standard process, the chemists use open-access LC/MS to confirm the presence of their compound prior to submitting to purification. In order to purify by SFC, screening was required to be performed by the chromatographer since no open-access SFC/MS was available. The implementation of the FastTrack SFC system removes the screening step from the chromatographer's workflow and shifts that to the chemist, even though this responsibility is transparent to the chemist. Chemists need only to log their samples to the FastTrack system and initiate the runs, then submit their crude material to the purification group. While the chromatographer is purifying a group of compounds, new submissions are being screened concurrently and data is being acquired in real-time. By the time purifications are complete and the chromatographer is ready for more samples, he checks for the availability of processed new sample data (on-demand). The chromatographer then performs his own batching of the new samples by columns and resumes purification. Overall increase in work efficiency can be expected as long as samples are being screened and data is generated, so enough new compounds are available for purification without delay.

In an open-access environment, enough conditions should exist to cover most of the chemical diversity of synthesized samples without adding unnecessary time or resources to any given process. Therefore, it is necessary to initially test more than one phase to find a separation of optimal selectivity and resolution that can easily be scaled up for purification. Due to advancements in column chemistry specifically for SFC, our laboratory utilizes a number of unique stationary phases to screen highly diverse groups of small molecules. The most frequently used columns could be discerned from a large number of historical purification data, and six of these columns were selected for inclusion in the FastTrack SFC screen.

2. Experimental

2.1. Materials and supplies

Commercially available Ultra LC/MS^{TM} grade methanol was purchased from J.T. Baker (Phillipsburg, NJ, USA). Carbon dioxide (CO_2) and nitrogen (N_2) are bulk grade and purchased from AirGas West (Escondido, CA, USA). The CO_2 supplied to this system was purified and pressurized to 1500 psig using a custom booster and purifier system from Va-Tran Systems, Inc. (Chula Vista, CA, USA). Pfizer proprietary compounds were used for this study.

2.2. Analytical instrumentation

All chromatographic analyses were carried out on a supercritical fluid chromatograph configured from an Agilent 1100 Series HPLC

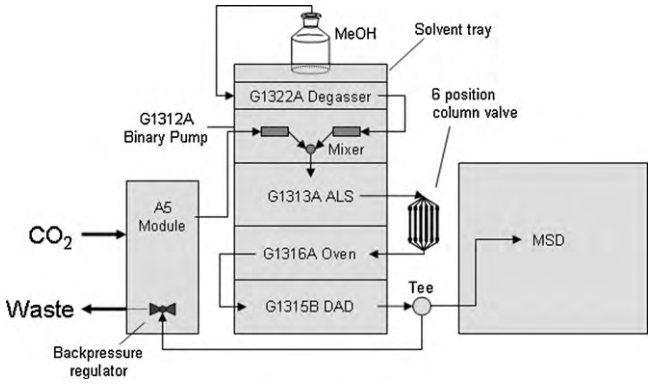

Fig. 2. Configuration of 1100 LC/MSD with an Aurora SFC A5 module.

(Palo Alto, CA, USA) including a G1322A degasser, G1312A binary pump, G1313A autosampler (ALS), G1316A column compartment and a G1315B diode array detector outfitted with a 10 mm, 13 μL high pressure flow cell (400 bar). This HPLC system was connected to an Agilent 1100 Series MSD single quadropole mass spectrometer equipped with an APCI source. The split flow was achieved using a Valco 3-way stainless steel tee (Houston, TX, USA) and a 50 cm length of 50 μm i.d. pre-cut PEEKsil tubing (Upchurch Scientific, Inc., Oak Harbor, WA, USA). To convert this HPLC/MS to a supercritical fluid chromatograph, it was integrated with an Aurora SFC Fusion™ A5 (Aurora SFC Systems, Inc., Sunnyvale, CA, USA) module and modifications were made to the ALS and binary pump as described elsewhere [22]. To accommodate multiple columns, an Agilent 1200 6-position selection valve was used. To minimize baseline noise caused by temperature differences of the mobile phase in the column and the DAD, the column outlet was plumbed to the G1316A column compartment. For this system, the columns are external to the oven and thus not temperature controlled. The oven is used to preheat the effluent to 43 °C to minimize solvent-induced noise [23]. Fig. 2 illustrates the configuration of the integrated SFC/MS system.

All data was acquired using Agilent 32-bit ChemStation™ (Version B.03.01 [317]), which controls all system components. In order to enable this SFC as a walk-up system, Agilent Easy-Access Software (Version A.5.01) was installed.

2.3. Stationary phases

A multi-column analytical screening protocol was established using a selection of commercially available stationary phases. These stationary phases were chosen based on their specific chemistries that typically result in acceptable resolution of large numbers of diverse samples without the use of additives in the mobile phase. These columns are shown in Table 1. All columns were purchased from Zymor, Inc. (Wayne, NJ, USA), and dimensions were 150 mm × 4.6 mm i.d., 5 μm particles with 100 Å pore sizes. The first six columns in Table 1 were selected for use in the FastTrack screen, while the Monol column was one of several alternative stationary phases used in cases where initial screening was unsuccessful.

Table 1
Stationary phases used for SFC screening.

Column	Type
ZymorSPHER PYR/Diol	Pyridine and diol mixed phase
ZymorSPHER HADP	HA-dipyridine
ZymorSPHER HAP	HA-pyridine
ZymorSPHER 4PYR	4-Ethylpyridine
ZymorSPHER C8/PE	C8 and pyridine (endcapped) mixed phase
ZymorSPHER Diol	Diol
ZymorSPHER Monol	Monol

Table 2
Commonly used stationary phases and the number of purifications achieved for approximately 1200 samples using these columns.

Column	Count
Diol	423
4PYR	333
PYR/Diol	223
HADP[a]	83
HAP[a]	29
C8/PE	69
Other	29

[a] Column was only recently implemented.

2.4. SFC/MS conditions

For SFC analyses, methanol was used as the modifier. No acidic or basic additives were used in the mobile phase to ensure that the free base forms of the final products are isolated. Each sample was run through each of the six columns using a 7.5–50% modifier gradient at a rate of 10%/min and held at 50% for 30 s before returning to initial conditions. Total cycle time was 5 min. A mobile phase flow rate of 3 mL/min was used, and the column outlet pressure was maintained at 140 bar. Columns were at ambient temperatures since the column switching valve was external to the column oven. UV detection was monitored at 220 and 260 nm wavelengths, the slit width was set to 8 nm and the peakwidth was set to >0.05 min (1 s response time). Product peak was identified by using positive mode APCI settings, dry gas flow and temperature were 12 L/min and 350 °C, respectively; nebulizer pressure 35 psig; vaporizer temperature 450 °C; capillary voltage 3000 V, and corona current 4.0 μA.

2.5. Purification conditions

A Berger Multigram II semi-preparative system (Waters SFC, Inc., Milford, MA, USA) was used to purify the compound in this study. Scale-up conditions were determined for the 4.6 mm i.d. analytical column and the 21.2 mm i.d. semi-preparative column, while the column lengths, particle and pore sizes remained constant. For the HADP column purification, the analytical flow rate was scaled up using a factor of 20 from 3 to 60 mL/min. This is a proportional scale-up in flow from analytical to preparative. However, the gradient was modified to start at 10% modifier. After a 0.1 min hold time, a 10–50% modifier gradient was applied at a rate of 8%/min and held at 50% for 30 s before returning to initial conditions. For the Monol column, analytical flow rate was scaled up to 50 mL/min, a factor of 16, to provide for better resolution. A 5–50% modifier gradient at a rate of 10%/min and held at 50% for 30 s before returning to initial conditions was used for this purification.

3. Results and discussion

3.1. Screening column selection

The columns used in the FastTrack SFC screen were chosen based on empirical data generated for over 400,000 compounds purified over the past several years and, specifically, on analyses of approximately 1200 recent project-based samples. Table 2 shows the number of times the most common stationary phases were used for the purifications. In each case, the sample was screened, reviewed, and purified using the selected column. Criterion for column selection included ability to scale-up while maintaining maximum resolution of impurities from the desired peak, and preserving peak shape without the use of additives in the mobile phase. The Diol, 4PYR and the PYR/Diol phases provide a suitable means for a separating a broad range of chemical matter, and 80% of the total numbers of submissions were successfully purified to accept-

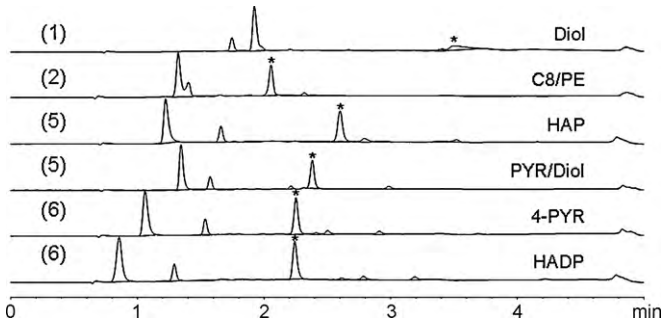

Fig. 3. UV_{220} chromatograms from a 6-column SFC screen in order of increasing separation capability for a particular sample. SFC methods are described in the text. The numbers in parenthesis indicate the number of peaks separated with a resolution >1.0. The peaks marked with an asterisk are the main products.

able purity using one of these three columns. The HADP and HAP columns have been recently introduced to supplement the other phases, and have demonstrated suitable separation with better loading. Due to their increasing acceptance for use in analytical and preparative separations, they have been added to the FastTrack SFC.

In addition to the five aforementioned columns, the C8/PE stationary phase was included in the screen since it often provides peak elution order similar to a C8 phase, but with increased resolution for Pfizer proprietary compounds. This tends to be useful if peak reversal is required for better isolating capabilities. This column has exhibited potential for minimizing time spent on separation challenges and is thus included in the FastTrack screen. If the column choice can be modified or reduced depending on the chemical matter, then a reduction in the number of columns and hence the screen time can be shortened to provide higher throughput. The difference translates directly to method development time of the analyst using other phases. Incorporating the HADP, HAP and C8/PE columns into the FastTrack screen adds 15 min to the overall screening time; however, inclusion of these phases significantly diminishes time necessary to manually fine tune methods while increasing the chances of finding a suitable separation method. Furthermore, no additional time or effort is required on the part of the submitting chemist. It is expected that the FastTrack screening process will ultimately facilitate purification efforts for faster turnaround of pure compounds by limiting method development time.

3.2. FastTrack screen results

The differences in selectivity of the FastTrack screening phases are highlighted in Fig. 3. All chromatographic peaks eluted within 4 min in each run. For this specific compound, the HADP column demonstrated the best separation of all six components in the crude mixture. While the 4PYR column also showed suitable separation of all constituents, the HADP column was chosen for purification based upon higher column loading and expected purity of the final product. The preparative SFC chromatogram (Fig. 4) has several impurities, including one coeluting on the right shoulder of the product peak. The purification method selected based on a proportional flow scale-up was successful in isolating the main peak from most impurities. Following the purification, an orthogonal LC/MS was performed. Orthogonal analysis provides the chromatographer with the ability to confirm overall purity of the collected product fraction prior to leaving the laboratory [24,25]. The inlaid HPLC trace confirms that a minor impurity indeed co-eluted with the product on scale-up in the order of 7% by UV area percent. Overall, the purification was successful in isolating the final product to approximately 93% purity by UV area percent, nearly a 30% improvement in purity from crude to final product.

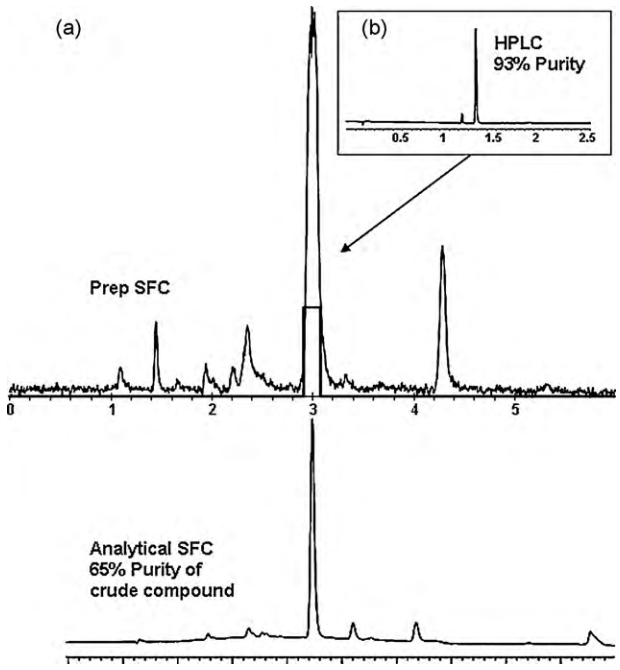

Fig. 4. (a) UV_{260} analytical and preparative SFC chromatograms of a crude compound and the collection of the main product peak on a ZymorSPHER HADP semi-prep column. See the text for purification conditions. (b) Final compound purity was determined by orthogonal HPLC analysis.

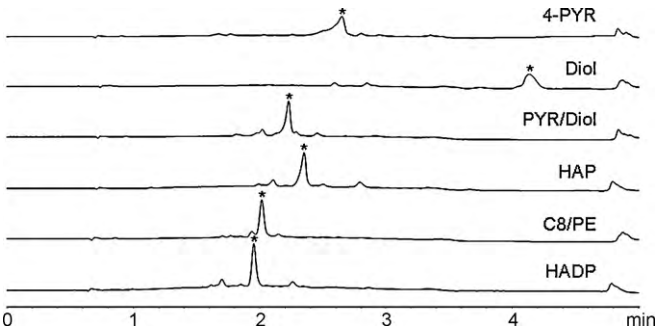

Fig. 5. Example of an unsuccessful 6-column screen due to coeluting product and impurity. Intended product is marked with an asterisk.

Not all attempts at screening result in a suitable method for purification. Fig. 5 highlights a case where no suitable separation of product and impurity peaks was found using the FastTrack column screen. While the HADP column appears to have the best separation, MS confirmed the presence of an impurity in the product. Since a partial separation was observed using the Diol column, the Monol

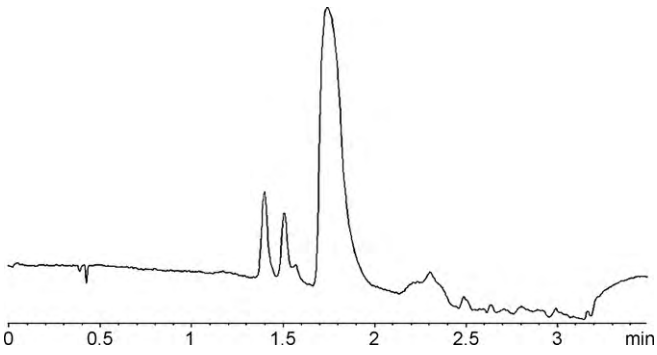

Fig. 6. Analytical SFC chromatogram using the ZymorSPHER Monol column.

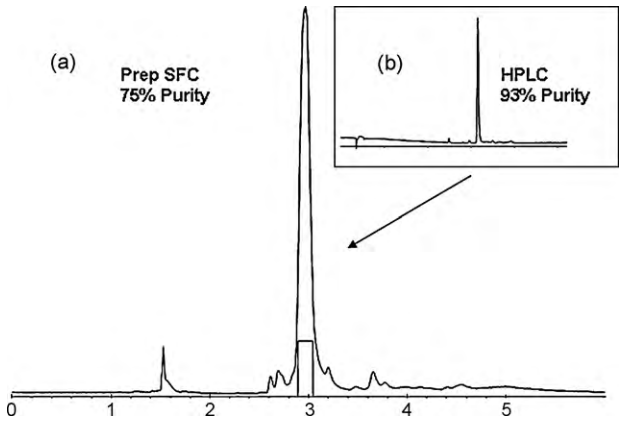

Fig. 7. (a) Preparative SFC chromatogram showing the collection of the main product peak on a Monol semi-prep column. See the text for purification conditions. (b) Final compound purity was determined by orthogonal HPLC analysis.

column was used as an alternative stationary phase to obtain the best separation by SFC despite the non-ideal peak shape (Fig. 6). Closely eluting impurities are clearly present when translating to the preparative conditions as shown in Fig. 7. However, the post-purification orthogonal LC/MS purity check confirms the success of the overall purification, with the final purity an acceptable 92.5%.

4. Conclusion

The implementation of a FastTrack SFC/MS screening system in a walk-up environment for medicinal chemists to initiate the screening across several different stationary phases has been described. The impact of the FastTrack process, coupled with the efficiencies of SFC, is an overall reduction in screening and method development time by the chromatographer. In addition to alleviating the backlog created by batching samples for screening, real-time acquisition followed by on-demand processing of data allows for review and method scale-up determination as soon as submitted compounds are received. This enables the chromatographer to redirect resources to purifications without the inconvenience of waiting for processed data. The capabilities in both SFC and reverse-phase open-access methods to facilitate and speed up the purification process allows for more choices in purification techniques and should lead to better overall quality results. Since implementing the FastTrack system, we have observed an overall increase in satisfaction from the medicinal chemists as they now have access to facile and transparent SFC data, which informs them on the purity of their samples. Since the launch of this FastTrack process, we have already observed a reduction in turnaround time from submission to purified sample return by as much as a third in most cases.

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