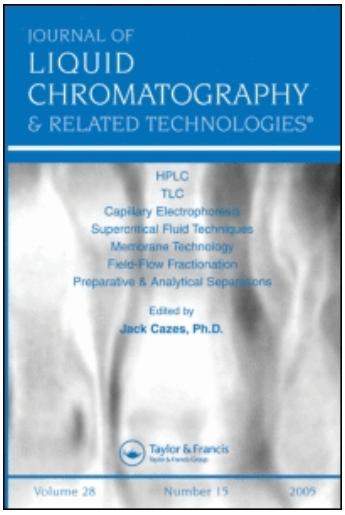

Journal of Liquid Chromatography & Related Technologies

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

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To cite this Article McClain, Ray T. , Dudkina, Anna , Barrow, James , Hartman, George and Welch, Christopher J.(2009) 'Evaluation and Implementation of a Commercially Available Mass-Guided SFC Purification Platform in a High Throughput Purification Laboratory in Drug Discovery', Journal of Liquid Chromatography & Related Technologies, 32: 4, 483 — 499

To link to this Article: DOI: 10.1080/10826070802671325

URL: http://dx.doi.org/10.1080/10826070802671325

Journal of Liquid Chromatography & Related Technologies®, 32: 483–499, 2009

ISSN: 1082-6076 print/1520-572X online
DOI: 10.1080/10826070802671325

Evaluation and Implementation of a Commercially Available Mass-Guided SFC Purification Platform in a High Throughput Purification Laboratory in Drug Discovery

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Abstract: The evaluation and implementation of a commercially available, off the shelf, analytical SFC/MS and a mass directed, semi-preparative SFC-MS system for use in a high throughput purification laboratory is described. The advantages of the use of SFC to support high throughput purification includes rapid evaporation of the chromatographic mobile phase in isolated fractions, resulting in faster sample turnaround time. In addition, SFC provides a complimentary separation technique based on normal phase, adsorption chromatography mechanisms. The screening scheme employed on the associated analytical SFC-MS and the methodology developed for the mass directed, semi-prep SFC-MS are presented in detail. The utilization of standard FractionLynx/AutoPurify functionalities to enable rapid incorporation into high throughput environments is also emphasized.

Keywords: Drug discovery, High throughput, Mass guided SFC, Preparative SFC, Purification, SFC-MS

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INTRODUCTION

In recent years, there has been increasing pressure to improve the productivity and efficiency of pharmaceutical discovery and development. In response to the need to identify clinical candidates more rapidly, a variety of technologies have been introduced for high throughput synthesis of new lead compounds.[1–4] Consequently, as many as 24–48 compounds can often be prepared in the time previously required to synthesize a single compound. In order for purification to avoid becoming rate limiting, there has been a corresponding development of purification technologies to support the overall high throughput synthesis enterprise. Mass directed reversed phase preparative HPLC systems are now routinely employed to support high throughput synthesis,[5–8,28] with the vast majority of compounds encountered in medicinal chemistry being amenable to this approach. The principal advantage of mass guided preparative HPLC is that the collection of fractions can be limited to only those compounds eluting within the target mass range. Mass directed systems accomplish this through monitoring the extracted ion signal from the mass spectrometer to trigger fraction collection. This triggering mechanism is compound specific, in contrast to the previous unselective UV triggering mode.

A major problem with this approach is the fact that evaporation of aqueous fractions coming from reversed phase chromatography can be exceedingly slow, with fraction evaporation adding 8 hours or more to the purification cycle time. This critical step of the process adds a minimum of one day to the turnaround time for purification. The use of Supercritical Fluid Chromatography (SFC) has been proposed as a possible solution to the excessive evaporation times experienced with reversed phase HPLC.[9,28] SFC is a normal phase separation technique utilizing polar stationary phases and CO_2 compressed and heated to its super/subcritical state as the mobile phase.[10–15] A polar co-solvent, commonly methanol, is added to the CO_2 to increase solvent strength and to control elution of compounds from the stationary phase. Several of the major advantages of SFC are provided by the physical nature of the CO_2. The CO_2 has diffusivity similar to that of a gas with a solvating power similar to that of a liquid. The CO_2 portion of the mobile phase converts to a gas by expanding 500 times its volume upon being exposed to atmospheric pressure during fraction collection. This leaves the analyte in a small volume of the methanol co-solvent in the fraction vessel. Evaporating several milliliters of methanol can be accomplished in 1–2 hours compared to an entire day for the aqueous reversed phase fractions. The reduced viscosity and enhanced diffusivity of the CO_2 minimizes resistance to mass transfer, allowing faster flow rates than HPLC at comparable backpressures. A four fold increase in linear

velocity compared to reverse phase HPLC can often be achieved without compromised efficiency. The combined advantages of shorter evaporation time and increased linear velocity dramatically reduce the overall purification cycle time per compound. In addition, SFC offers a complimentary technique to reversed phase HPLC, with the combination of the chemical properties of super/subcritical CO_2 with the normal phase columns often affording separation enhancements. Several groups have carried out comparative evaluations of the performance of SFC and reversed phase HPLC for resolution of diverse sets of proprietary compounds in the pharmaceutical discovery environment.[16–18] The results conclusively support SFC as being comparable to reverse phase HPLC in its ability to retain and controllably elute achiral molecules common to medicinal chemistry. Two studies also demonstrate comparable recovery when samples were purified by both techniques.[17,19]

The use of preparative SFC for high throughput purifications initially focused on UV based fraction triggering systems.[10,20–23] The early work of Berger, Farrell, and White in this area clearly showed that the proposed advantages of the SFC approach could indeed be realized. Productivity enhancements demonstrated with these UV based preparative SFC systems quickly led to the desire to have mass directed preparative SFC capabilities, and several customized mass directed systems were described soon thereafter.[24,25] These modified systems overcame several of the problems that prevented the commercialization of mass directed SFC systems to be distributed in an off the shelf format. The super/subcritical CO_2 exiting the fraction collection device is converted to a gas upon being exposed to atmospheric pressure, with the resulting 500:1 expansion often leading to problems with uncontrollable spray, poor recovery, and contamination of other vessels in close proximity in the fraction collector. This collection problem was solved by Zhang through customization of a Water's 2757 fraction collector delivering mass directed SFC. The absence of a standard software package to control all aspects of the chromatographic process through a single computer was also an issue preventing widespread acceptance. We now describe the evaluation of a commercial mass guided preparative SFC platform that addresses both of these issues and that has been implemented in a high throughput purification work flow to support pharmaceutical discovery.

EXPERIMENTAL

Reagents/Chemicals

Industrial grade CO_2 in cylinders containing siphon tubes was obtained from Airgas Inc. (Radnor, Pa.). Chromasolv gradient grade methanol

serving as the co-solvent was purchased from Sigma Aldrich (St Louis, MO). Commercially available standards were screened to identify components that would chromatograph across the entire gradient range of the analytical method being examined. The potential compounds were then examined to ensure a diverse range of function groups would be present in the final mix. The final compounds selected for the five part mix include 2,2-diphenylethanol and bumetanide from Sigma (St Louis, MO), ibuprofen from Acros Organics (NJ), and acetazolamide and ketoprofen from MP Biomedicals LLC (Solon, Ohio).

Instrumentation/Columns

The analytical SFC/MS instrument used in the screening of crude products and in the purity assessment of isolated fractions was the Thar SuperPure Discovery Series SFC/MS. The modules consist of the fluid delivery module, 6 column oven, automated back pressure regulator, THAR injection module, Water's 2996 photodiode array detector, Water's 3100 mass detector, and a Thermo Fisher DigitalOne water circulator. MassLynx version 4.1 was used to control the analytical SFC/MS and the mass directed semi-preparative system. The analytical columns used were 2-ethylpyridine 4.6 mm (i.d.) $\times$5 cm, 5 um from Princeton Chromatography (Princeton, NJ), Chromegabond Aminophenyl 4.6 mm (i.d.) $\times$5 cm, 5 um from ES Industries (West Berlin, NJ), Chromegabond NO_2 4.6 mm (i.d.) $\times$5 cm, 5 um from ES Industries (West Berlin, NJ) and Chromegasphere Si100 4.6 mm (i.d.) $\times$ 5 cm, 5 um from ES Industries (West Berlin, NJ).

The preparative SFC/MS instrument used in the purification studies was the SFC-MS Prep 30, a commercially available 30 g/minute minute system offered by THAR Technologies. The modules consist of a THAR semi-preparative fluid delivery module, 10 column oven, automated back pressure regulator, Leap Technologies/CTC Analytics HTC Pal autosampler, Water's 2996 photodiode array detector, Water's 3100 mass detector, Water's 2757 fraction collector, Water's Series III makeup pump, and a Thermo Fisher DigitalOne water circulator. MassLynx version 4.1 and FractionLynx version 4.1 were used to control the preparative SFC/MS instrument. The preparative columns used were 2-ethylpyridine 10 mm (i.d.) $\times$10 cm, 5 um from Princeton Chromatography (Princeton, NJ), Chromegabond Aminophenyl 10 mm (i.d.) $\times$10 cm, 5 um from ES Industries (West Berlin, NJ), Chromegabond NO_2 10 mm (i.d.) $\times$10 cm, 5 um from ES Industries (West Berlin, NJ), and Chromega sphere Si100 10 mm (i.d.) $\times$10 cm, 5 um from ES Industries (West Berlin, NJ).

Chromatographic Methods

The specific method parameters for both the analytical and preparative systems as well as the rationale for arriving at these methods will be described in the results and discussion section.

RESULTS AND DISCUSSION

We have previously relied on a rapid reversed phase gradient analytical HPLC method coupled with a set of correlated focused gradient preparative HPLC methods to handle the large number of compounds processed in our high throughput purification laboratory.[5] In this approach, preparative elution times are quickly estimated based on analytical results, affording a rapid and dependable basis for preparative scaleup. The focused gradient approach eliminates the need to examine the entire solvent gradient profile, thereby saving time and maximizing resolution in the area of interest. Furthermore, the use of mass detection insures that the proper mass of interest is isolated. In this approach, only one analytical chromatogram is performed prior to the actual preparative separation. This approach generally allows for unknown compounds to be purified in a single preparative injection, greatly facilitating high throughput purification of compounds in support of drug discovery. For preparative SFC purifications, White and coworkers have reported a similar approach using a fast gradient analytical SFC method, combined with a longer associated UV based preparative SFC method for rapid purification.[21] White's preparative retention time and corresponding

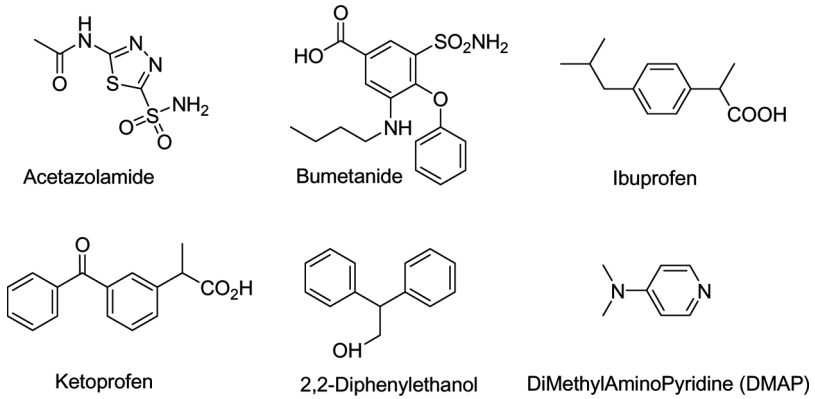

Figure 1. Collection of commercially available standards used as a standard test mixture in evaluation of SFC-MS purification.

collection windows are set through calibration of the analytical SFC and preparative SFC retention times for a standard mixture. We now report on a combined strategy in which analytical SFC-MS using a standard gradient is followed by mass guided preparative SFC using a set of focused gradients. The resulting methodology has been optimized for utilization on this commercial SFC platform interfaced through Water's standard version of MassLynx with all associated features being active.

A number of commercially available compounds of varying polarity, acidity, basicity, and heterogenicity were investigated *via* analytical SFC/MS in order to develop an in house standard mixture of analytes that would be representative of the diversity of compounds encountered in drug discovery. The collection of molecules selected to be used in our evaluation is shown in Figure 1.

The columns used for achiral SFC purifications have traditionally been normal phase columns such as amino, cyano, diol, and silica. Specialty phases such as 2-ethylpyridine, aminophenyl, nitro, benzamide, and

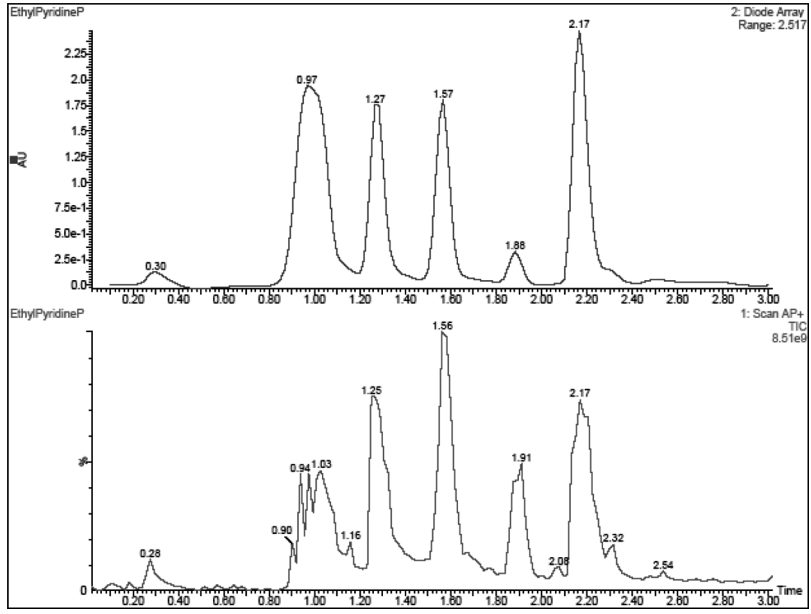

Figure 2. Chromatographic analysis demonstrating techniques wide applicability to varying functionalities. Top trace: Diode array signal at 214 nm of 5 standard mix evaluated per analytical method detailed in Table 1. The 2-ethylpyridine column was used with neat methanol as the co-solvent. Bottom trace: Total ion current trace from the mass spectrometer obtained during the same chromatographic analysis.

propylacetamide were subsequently introduced as SFC became more widely used for achiral analysis and purification, with the 2-ethylpyridine column becoming something of a column of choice for many high throughput laboratories carrying out SFC separations. Our investigations also found the 2-ethylpyridine column to be a generally useful stationary phase for carrying out SFC analysis of a variety of different compounds, including both acidic and basic compounds. The standard mixture was initially analyzed on a 4.6 mm (i.d.) × 15 cm 2-ethylpyridine column at a flow rate of 4 mL/min with a 6 minute gradient ranging from 2–60% methanol in CO_2. The efficiency and resulting resolution for the test mixture exceeded our needs, presumably arising from the diffusivity of the super/subcritical CO_2 . This facilitated stepping down to a shorter 4.6 mm (i.d.) × 10 cm 2-ethylpryidine columns with a 4 minute gradient and then, ultimately to an even shorter 4.6 mm (i.d.) × 5 cm 2-ethylpyridine column with a 2 minute gradient.

The system back pressure was evaluated at 100, 120, 150, and 200 bar using the standard mixture, and 120 bar was found to deliver acceptable chromatographic selectivity and peak shape while minimizing pressure,

Table 1. Summary of analytical and semi-preparative methodology

	Analytical	Preparative
Flow Rate	4 mL/min	20 mL/min
Injection Volume	5 uL	333 uL
Co-Solvent	Methanol	Methanol
Gradient	10 Sec 2% MeOH Hold	360 Sec Initial–Final % MeOH gradient
	120 Sec 2–60% MeOH gradient	60 Sec 60% MeOH Hold
	50 Sec 60–2% MeOH gradient	120 Sec 60–2% MeOH gradient
Column Dimension	4.6 mm (i.d.) × 5 cm, 5 um	10 mm (i.d.) × 10 cm, 5 um
Column Temperature	40°C	40°C
Back Pressure Regulator	120 Bar	120 Bar
UV Wavelength Scan	214–234 nm	214–234 nm
Scan Range	150–650 m/z	150–650 m/z
Capillary Voltage	3000 V	3000 V
Cone Voltage Ramp	17–30 V	17–30 V
Extractor Voltage	2 V	2 V
RF Lens	0.1	0.1
Source Temperature	150°C	150°C
Desolvation Gas Temp	590°C	590°C
Desolvation Gas Flow	650 L/Hr	650 L/Hr

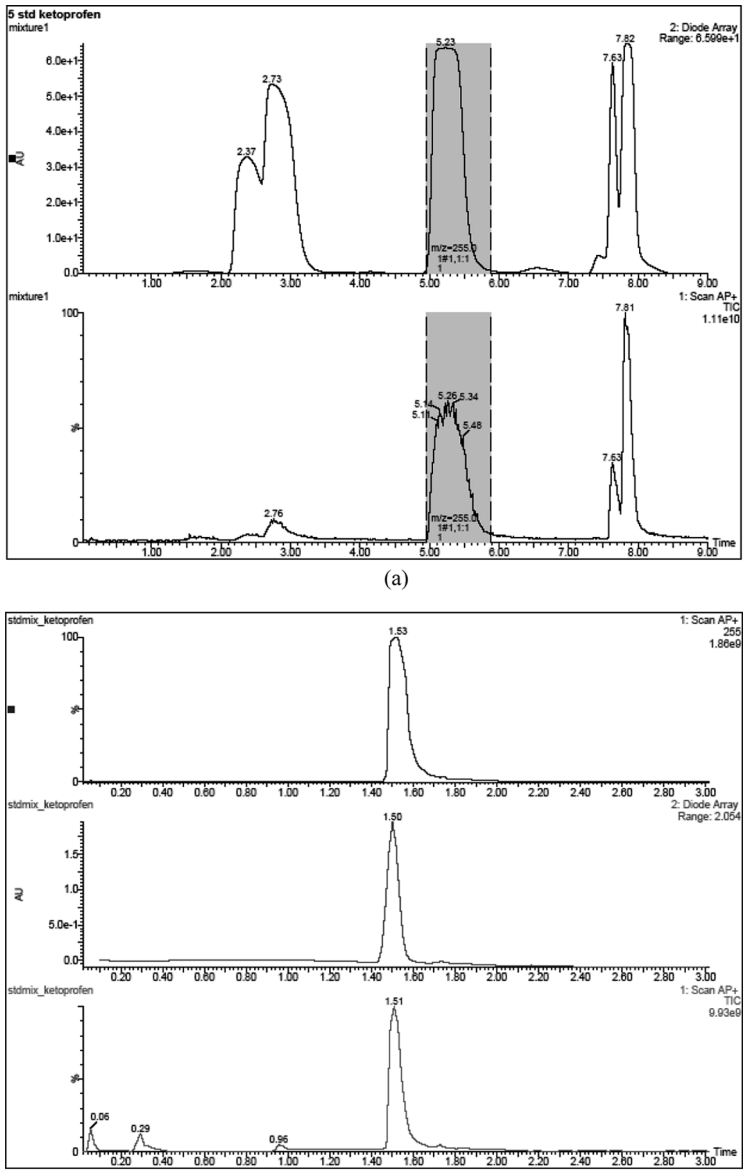

Figure 3. (a) Preparative example demonstrating utilization of focused gradient approach and the systems ability to isolate fraction with high percent recovery. Top trace: Diode array signal at 214 nm of 5 standard test mix evaluated per preparative method detailed in Table 1. The 2-ethylpyridine column was used with neat methanol as the co-solvent. The focused gradient range of 5–10% was assigned through cross referencing the narrow gradient table in FractionLynx.

which may be beneficial on the preparative scale. The mass spectrometer settings were next evaluated to optimize sensitivity and to minimize fragmentation of the molecular ion, as an intact molecular ion is key to the success of the high throughput purification process. The corona and extractor voltages were found to have a wide range of comparable performance and were set at traditional values of 3000 and 2 volts, respectively. The cone voltage was originally set at 30 volts and excessive fragmentation was observed. A cone voltage ramp of 17–30 volts over the m/z range of 150–650 was implemented, thereby reducing most of the fragmentation. The critical mass spectrometer parameters influenced by the CO_2 expansion in the APCI probe were the desolvation gas and probe temperatures.[26] Before optimizing these temperature to 590°C and 150°C, respectively, very little TIC signal was observed.

A chromatogram showing the separation of the test mixture components with the optimized standard analytical SFC/MS method is shown in Figure 2. The optimized method parameters are summarized in Table 1. This standard method has proven useful for rapid screens of crude reaction products and purity evaluation of isolated fractions.

This analytical screening method provides adequate peak shape for the majority of compounds, but some SFC separation peak shapes are enhanced by the addition of additives such as trifluoroacetic acid (TFA), isopropylamine ($iPrNH_2$), diethylamine (DEA).[19,21,27] The use of additives is not favored in our high throughput laboratory, so samples requiring better peak shape than what is delivered by the 2-ethylpyridine column are analyzed with comparable gradients on the aminophenyl, nitro, and silica columns. The best alternative stationary phase is then utilized during purification of the difficult samples.

The creation of a set of semi-preparative SFC methods that would correspond to various elution time windows within the standard analytical gradient was next investigated. After some experimentation, a standard preparative column size of 10 mm (i.d) × 10 cm was selected based upon considerations of optimal loading capacity and limitations of the 30 mL/min flow rate achievable by the system. Historically, 21 mm i.d. semi-prep columns are used in our HPLC purification platform but such

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Bottom trace: Total ion current signal with highlighted fraction representing isolated ketoprofen possessing the mass of 254 specified in the sample list. (b) Analytical analysis of isolated fraction confirming purity of ketoprofen. Top trace: Extracted ion current for 255, the M + 1 adduct of ketoprofen, analyzed per the analytical method detailed in Table 1. The 2-ethylpyridine column was used with neat methanol as the co-solvent. Middle trace: Diode array signal at 214 nm of isolated ketoprofen. Bottom trace: Total ion current trace obtained during the same chromatographic purity analysis.

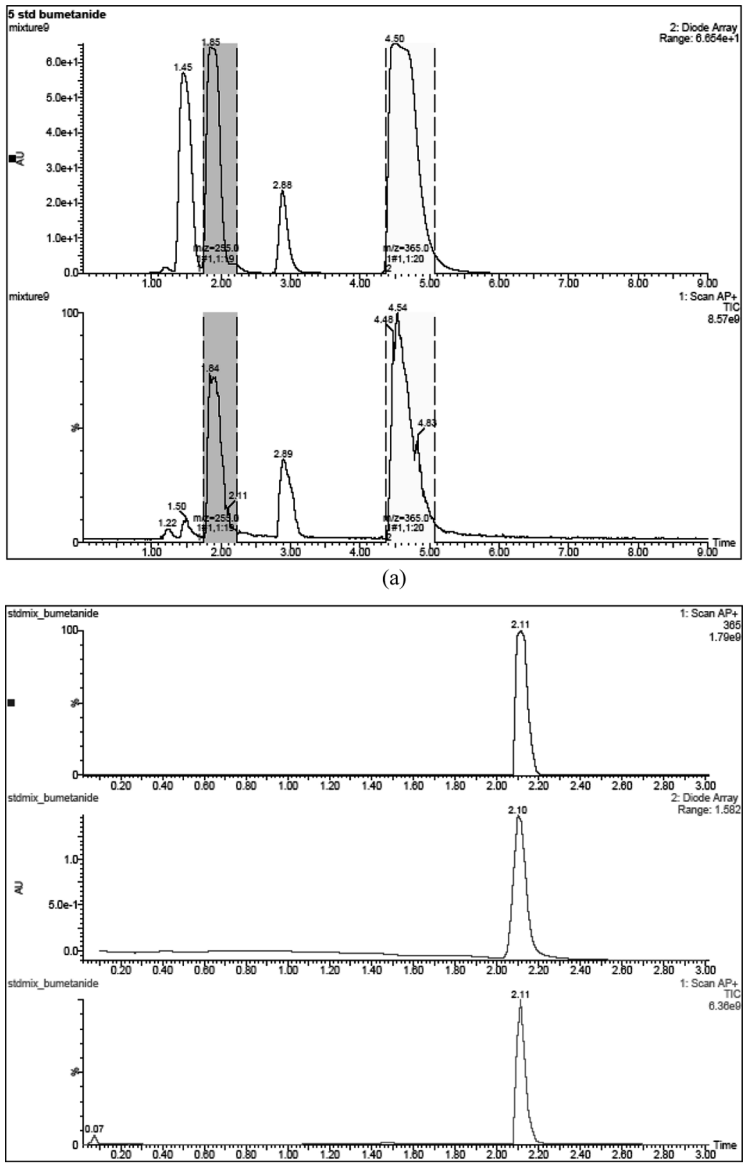


Figure 4. (a) Preparative example demonstrating utilization of focused gradient approach and the systems ability to isolate fraction with high percent recovery. Top trace: Diode array signal at 214 nm of 5 standard test mix evaluated per preparative method detailed in Table 1. The 2-ethylpyridine column was used with neat methanol as the co-solvent. The focused gradient range of 20–25% was assigned through cross referencing the narrow gradient table in FractionLynx.

columns would clearly be oversized for a 30 mL/min SFC system, where flow rates in the range of 100 mL/min or even higher would afford maximum productivity. As a concession to the doubling of the column bed length of the preparative column, the gradient was extended to 6 minutes on the semi-preparative system to compensate for the column extension. The mass spectrometer parameters were transferred directly from the analytical system as the goal of maintaining an intact molecule is the common to both systems.

With the standard analytical and preparative SFC methods in hand, we next set out to establish a high throughput automated purification workflow incorporating this methodology. We have previously been successful in the use of Agilent and On-the-Mark Solution's A2PREP (Analytical to Preparative) software for automated assignment and execution of focused gradient preparative HPLC methods based on initial results obtained using a standard analytical method. A similar approach has been used for the SFC platform employing the Water's AutoLynx/FractionLynx software to automate the narrow gradient SFC method assignment. Six semi-preparative SFC methods with focused gradient ranges of approximately 5% have been developed and correlated to six time ranges in the analytical SFC screening method. The Water's data acquisition software on the analytical SFC/MS, processes the analytical data file, extracts the ion of interest from the total ion chromatogram (TIC), and then matches the observed retention time to one of the six available narrow gradients. The sample list is then automatically saved to a networked drive, and then imported into the semi-preparative SFC/MS with the sample names appended to include –PREP after the initial name. The one minute pre-run to ensure adequate column equilibration and the automatically assigned fraction triggering parameters contained in the autoMIT file are assigned in the automated transfer and saved to the sample list. As different autosamplers are used on the analytical and semi-preparative systems, the sample position must be converted to 96-well plate format on the semi-preparative system. This entails entering 2:1 for the first sample and filling series for the remainder of the samples. The use of shallow gradients maximizes resolution of the

Bottom trace: Total ion current signal with highlighted fraction representing isolated bumetanide possessing the mass of 364 specified in the sample list. (b) Analytical analysis of isolated fraction confirming purity of bumetanide. Top trace: Extracted ion current for 365, the M + 1 adduct of bumetanide, analyzed per the analytical method detailed in Table 1. The 2-ethylpyridine column was used with neat methanol as the cosolvent. Middle trace: Diode array signal at 214 nm of isolated bumetanide. Bottom trace: Total ion current trace obtained during the same chromatographic purity analysis.

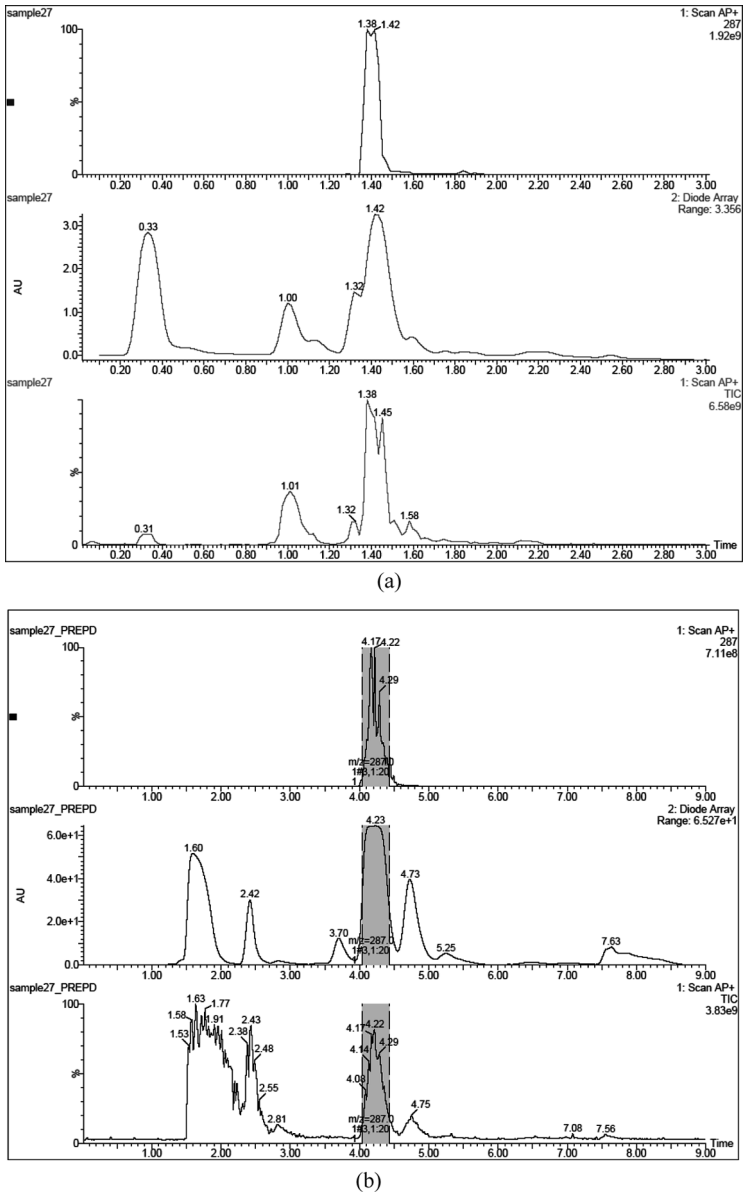
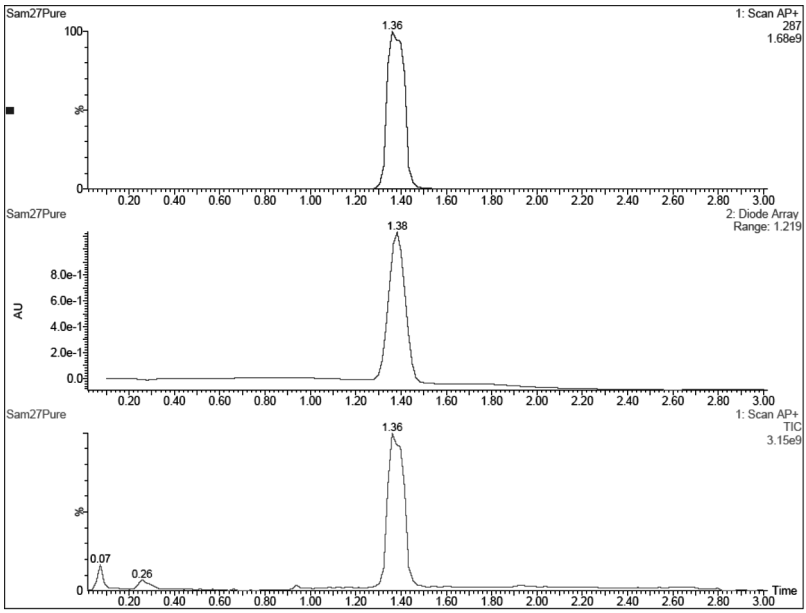
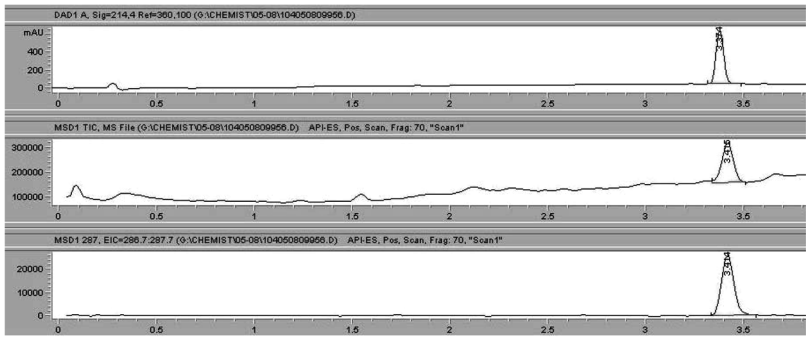


Figure 5. (a) Analytical screen of an internally synthesized crude product. Top trace: Extracted ion current for 287, the M + 1 adduct of the desired compound, analyzed per the analytical method detailed in Table 1. The 2-ethylpyridine column was used with neat methanol as the cosolvent. Middle trace: Diode array signal at 214 nm of the crude product. Bottom trace: Total ion current trace obtained during the same crude screening analysis. (b) Preparative example of



(c)



(d)

Figure 5. Continued.

an actual internally synthesized crude product. Top trace: Extracted ion current for 287.0, the M + 1 adduct of the desired component evaluated per preparative method detailed in Table 1. The 2-ethylpyridine column was used with neat methanol as the cosolvent. The focused gradient range of 7–12% was assigned automatically through AutoLynx/FractionLynx. Middle trace: Diode array signal at 214 nm of the crude sample product. The 2-ethylpyridine column was used with neat methanol as the cosolvent. Bottom trace: Total ion current signal with highlighted fraction representing isolated desired compound possessing the

desired compound, despite the short residence time of the mixtures on the column, and is also helpful in reducing purification time. The well known fast equilibration time provided by the high diffusivity of super/subcritical SFC, enables minimal column cleaning and re-equilibration time.

The five component standard mixture examined in Figure 2 was used to demonstrate the effectiveness of the narrow gradient SFC methods. The semi-preparative method detailed in Table 1, with an initial mobile phase containing of 5% methanol, and a final mobile phase containing 10% methanol, was used to purify the ketoprofen component of the mix. The 5–10% methanol gradient method was assigned through cross referencing the narrow gradient table in FractionLynx and the resulting semi-preparative chromatogram can be observed in Figure 3a. The peaks with retention times of 2.37, 2.73, 5.23, 7.63, and 7.82 correspond to the standard components 2,2-diph enylethanol, ibuprofen, ketoprofen, acetazolamide, and bumetanide, respectively. The mass loaded on the column for this injection totaled 37.9 mg with 10.0 mgs being ketoprofen. The recovery was found to be 82% (average of 3 injections), while the purity was found to be 100% when the isolated fraction was re-examined on the same analytical SFC/MS method used for screening. The data file demonstrating purity of the isolated ketoprofen can be observed in Figure 3b. The high percent recovery of the isolated ketoprofen supports the vendor claim of the ability of the commercialized system to handle fraction collection accurately without the sample being carried away with the expanding CO_2. The high recovery of ketoprofen also suggests that the timing of the ion's detection in the mass spectrometer is simultaneous with the main flow of the system arriving at the fraction collector's diverter valve. As discussed in the introduction, these were the two main issues that had to be overcome for development of a robust and useful MS guided preparative SFC system.

The semi-prep method detailed in Table 1, with an initial mobile phase consisting of 20% methanol and a final mobile phase consisting of 25% methanol, was used to purify the bumetanide component of the

mass of 286 specified in the sample list. (c) Analytical SFC/MS analysis of isolated fraction confirming purity of isolated compound Top trace: Extracted ion current for 287.0, the M + 1 adduct, analyzed per the analytical method detailed in Table 1. The 2-ethylpyridine column was used with neat methanol as the cosolvent. Middle trace: Diode array signal at 214 nm of isolated compound. Bottom trace: Total ion current trace obtained during the same chromatographic purity analysis. (d) Analytical LC/MS analysis of isolated fraction confirming purity of isolated compound. Top trace: Diode array signal at 214 nm of isolated compound. Middle trace: Total ion current trace obtained during the same chromatographic purity analysis. Bottom trace: Extracted ion current for 287.0, the M + 1 adduct of the purified compound.

mix. The 20–25% methanol gradient method was assigned through cross referencing the narrow gradient table in FractionLynx and the resulting semi-preparative chromatogram can be observed in Figure 4a. The peaks with retention times of 1.45, 1.85, 2.68, and 4.50 correspond to the standard components 2,2-diphenylethanol/ibuprofen, which co-eluted, ketoprofen, acetazolamide, and bumetanide, respectively. The mass loaded on the column for this injection totaled 37.9 mgs with 11.5 mg being bumetanide. The recovery was found to be 84% while the purity was found to be 100% when the isolated fraction was reexamined on the same analytical SFC/MS method used for screening. The data file demonstrating purity of the isolated bumetanide can be observed in Figure 4b. The fraction collected at 1.85 minutes in Figure 4a is the ketoprofen component of the mix. It's collection was executed to evaluate carryover from one fraction to the next in a single chromatographic purification. The high percent recovery of bumetanide coupled with the absence of ketoprofen in Figure 4b convincingly demonstrates the lack of carryover of the system.

Internally synthesized crude products were next evaluated through the work flow already optimized by the analysis and purification of standards. The chromatographic data in Figure 5 documents the successful purification of a randomly selected library compound subjected to this work flow. Figure 5a represents the original screening of the crude reaction product by the analytical SFC/MS method described in Table 1, confirming the presence of the desired mass of interest, 286.0, at the analytical retention time of 1.38 minutes. This analytical data file was processed collectively with the other samples in the analytical sample list by FractionLynx for automated foc used gradient assignment in the preparative sample list. Figure 5b represents the resulting purification of the crude product by the focused gradient of 7–12% automatically selected by FractionLynx. Figure 5c represents the purity confirmation of the isolated fraction via the original screening method. The 2.5 mgs of compound isolated was found to be 100% pure. The purified material was also analyzed by our traditional LC/MS purity determining method on a C_{18} column with a generic 5–100% acetonitrile gradient and was confirmed to be 100% pure. This LC/MS data is captured in Figure 5d. The results representing the real word sample supports that the rapid screening of crude product, automated assignment of a focus gradient through FractionLynx, and subsequent mass directed SFC purification provides successful isolation of desired compounds.

CONCLUSION

An achiral SFC platform consisting of an analytical SFC/MS and a mass directed, semi-prep SFC has been shown to be a valuable addition to a

high throughput purification laboratory supporting medicinal chemistry. The analytical SFC allows for rapid screening of crude reaction products and purity confirmation analysis of isolated fractions on a 2-ethylpyridine column using a 2 minute gradient. The mass directed system provides rapid separation of libraries of compounds on 10 mm (i.d.) × 10 cm semi-preparative columns with no modifiers necessary for the vast majority of the time. The automated narrow gradient assignment achieved through AutoLynx/FractionLynx enables rapid purification of compounds. The expansion and controlled release of CO_2 upon fractionation leaves the desired isolated compound in several milliliters of methanol, decreasing evaporation time from greater than 8 hours previously encountered with mass directed reversed phase HPLC to one hour. The complimentary separation mechanism provided by this normal phase technique provides an additional separation tool for compounds previously unable to be chromatographed successfully in the high throughput environment. The commercially available platform has successfully overcome the issues that previously delayed delivery of such a system to market; mainly uncontrollable fract ionation of super/subcritical CO_2 and absence of a single software package utilized as the user interface to the system.

While the current system represents a much needed advance over previously available commercial preparative SFC systems for high throughput purification, a number of improvements can be imagined that could deliver enhanced performance or capabilities. For example, use of small particle stationary phases may allow for increased speed of analysis or increased loading capacity. In addition, fraction collection directly into tared, bar coded vials would greatly streamline the overall process. Finally, a mass directed semi-preparative system with a higher capacity for increased flow would allow the use of 21.2 mm (i.d.) × 10 cm semi-preparative columns for superior loading. These and other improvements to the system will be the focus of our future efforts in this area.

ACKNOWLEDGMENTS

The authors would like to thank THAR, Waters, Bill Wranitz, Matt Przybyciel, Denise Heyburn, Chuck Ross, and Vince Van Nostrand for their assistance in achieving SFC capabilities.

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Received March 16, 2008
Accepted September 17, 2008
Manuscript 6389