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Evaluation of flash supercritical fluid chromatography and alternate sample loading techniques for pharmaceutical medicinal chemistry purifications

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ABSTRACT

Flash chromatography is the preferred approach for small molecule purification in pharmaceutical discovery. This paper will discuss the potential for flash supercritical fluid chromatography (SFC) as an alternative technology for these purifications. It was shown that the high sample loadings seen with flash LC could also be achieved using flash SFC. The dry load injection technique greatly increases the amount of sample that can be applied to a flash SFC column while still achieving separation. Flash SFC has much lower solvent usage and higher purification productivities relative to flash LC. Product concentrations post purification were higher for flash SFC vs. flash LC, reducing the time required to isolate dry product. There still exist a number of technical details to be worked out with flash SFC, mainly around the equipment and column/cartridge technology.

1. Introduction

During small molecule pharmaceutical discovery thousands of compounds are synthesized in an attempt to prepare a molecule with the desired biological and physical attributes for progression to human testing. Each of these compounds requires multiple synthetic steps and purifications. The workhorse purification technique for discovery research is normal phase flash chromatography [1–3]. Flash chromatography differs from HPLC mainly in the use of larger particle stationary phases packed in plastic cartridges. In addition most flash purifications are performed at high sample loadings (sample:silica ratios of 1:10 to 1:100) compared to preparative HPLC [4]. While flash cartridges containing reverse phase stationary phase are available; most flash purifications utilize silica gel and are operated in normal phase mode. Throughout this article flash SFC refers to the technique of preparative SFC using larger particle size silica gel stationary phases. Currently flash SFC equipment is not commercially available. All work described in this paper utilized preparative SFC equipment designed for specialty purifications.

Flash chromatography has numerous advantages. These include the availability of relatively inexpensive, automated, reliable and easy to use equipment. Flash chromatography separation conditions are easily explored via thin layer chromatography (TLC) and scale-up is easy and accurate from TLC plates to flash columns. Flash chromatography is a technique most chemists learn in graduate school. The use of disposable cartridges (for normal phase purifications) eliminates the potential for cross contamination between purifications. Finally, normal phase solvents are easy to remove post purification, reducing time to generation of pure material. The main disadvantage of flash chromatography is the lower resolution seen compared to HPLC and the large amounts of solvents used for the separations. Flash chromatography is one of the largest contributors to the use of dichloromethane in the chemistry laboratory due to its property as a non polar solvent with high dissolution power for many organic compounds. Within Amgen medicinal chemistry laboratories, flash chromatography is the main source of chemical waste.

Recently, supercritical fluid chromatography (SFC) has become a viable alternative for the analysis and purification of small molecules during drug discovery [5–11]. While the majority of SFC purifications are for chiral separations, there has been a recent increase in the use of preparative SFC for achiral separations [12–15]. With SFC a majority of the solvent in the mobile phase, usually greater than 60% is supercritical CO_2. The critical point for CO_2 is a temperature of 31 °C and a pressure of 73 atm. Above this point CO_2 exists as a supercritical fluid and has properties intermediate between a liquid and a gas. The low viscosity and high diffusivity of the SFC mobile phase allows higher flow rates relative to HPLC, resulting in shorter run times and increased efficiencies. Increasing mobile phase velocities in SFC has significantly less impact on efficiency compared to HPLC. An SFC system can flow at linear velocities at least twice those seen in HPLC and achieve approximately the same efficiencies. In addition, the lower

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pressure drop in SFC allows higher linear velocities than those possible with HPLC. This increase in flow rates often results in higher productivities (material purified per unit time) relative to HPLC. The increased productivity allows compounds to be purified in a shorter time frame, reducing the time required to generate pure compounds for pharmaceutical testing and accelerating the drug discovery process.

While supercritical CO_2 has a higher solvating power than CO_2, most pharmaceutically applicable compounds are moderately polar and CO_2 alone is insufficient for elution from a chromatographic column. In most cases a polar modifier such as methanol must be added. A major advantage of preparative SFC vs. preparative HPLC is lower solvent usage. The lower solvent usage in preparative SFC is achieved by replacing a majority of the mobile phase with CO_2. CO_2 is removed post chromatography by decreasing pressure, leaving only the modifier. This results in higher product concentrations post chromatography, reducing the time required for post purification solvent removal and product isolation. Additionally, SFC is an environmentally conscious technology. CO_2 used in SFC is generally recovered as a byproduct of manufacturing processes, resulting in no net increase in CO_2. Overall solvent volumes for preparative SFC are 2–10 times less than seen in preparative HPLC. The reduction in solvent volumes results in reduced time and cost to isolate the purified material.

Preparative separations require the introduction of larger amounts of material onto the separation column. Sample dissolution in the same solvents and solvent polarity as used for elution is ideal to minimize peak broadening and distortion and maximize preparative productivities. Poor solubility in carbon dioxide makes this approach impractical for many small molecule drug like compounds. There are currently two approaches used for sample introduction in preparative SFC. The first, mixed stream injection, introduces the sample solution just prior to the column, after carbon dioxide and the modifier solvent are mixed. This approach injects the sample just prior to the column but has the issue of decompression of the injector loop contents prior to loading with sample solution. The second approach, modifier stream injection, introduces sample solution into the modifier flow stream, prior to mixing with carbon dioxide. Modifier stream injection has been shown to improve peak shape in preparative SFC under gradient conditions [16].

Samples can be applied to flash cartridges by dissolving in an organic solvent and injecting or pumping a solution onto the head of the cartridge. For optimum results it is desirable to dissolve the sample in the initial chromatographic starting conditions and in a small volume as possible. Dissolution in different solvents (especially more polar solvents) can result in compound breakthrough or poor separations. Dissolution in large volumes of solvent can result in peak broadening and reduced purities and/or yields. Poor solubility is often a limitation in applying large amounts of sample onto a column. One technique to apply insoluble samples to a preparative column involves pre-adsorbing the sample onto the stationary phase [17–19]. This technique is known by many names including dry loading, impregnation, solid injection and dry pack. In this technique the sample is dissolved in any solvent, combined with stationary phase and the solvent evaporated to produce a dry matrix of sample coated onto the stationary phase. The coated stationary phase is packed into a column which is inserted in the chromatographic system prior to the main column. An alteration of this technique involves mixing the sample directly with stationary phase [18].

A minimal amount of work on the use of SFC for flash purifications has been reported. Burns presented a poster reporting a preparative SFC separation using 5 μm cyano TLC plates and preparative column [20]. Good correlation from TLC to SFC was reported. This approach is different from flash chromatography performed in most laboratories due to the use of small particle bonded stationary phase. Standard practice for flash purifications is single use cartridges. The expense of small particle bonded phases would make this practice impractical. Chordia et al. published a patent application describing a high pressure flash SFC system [21]. A few purifications are included as examples in the patent.

2. Experimental

2.1. Equipment

The analytical SFC chromatograph was a SFC method development station sold by Thar (Pittsburgh, PA, USA) equipped with a Waters ZQ mass spectrometer (Milford, MA, USA). The preparative SFC was a Prep 80 from Thar. (Pittsburgh, PA, USA). The preparative HPLC chromatograph was a Varian SD-1 system (Wakefield, RI, USA).

2.2. Materials

Merck KGaA silica TLC plates (20 cm × 20 cm, 60 Å, 250 μm thickness) were purchased through Sigma–Aldrich. Pre-packed flash cartridges (19 mm I.D. × 150 mm, 30 μm silica gel) were obtained from Interchim (San Pedro, CA, USA). Bulk silica gel was obtained from Interchim (San Pedro, CA, USA) and slurry packed into stainless steel columns (19 mm × 150 mm) by Waters (Milford, MA, USA). All chemicals were purchased from Sigma–Aldrich, VWR or Fisher Scientific. The solvents were reagent grade or better and obtained from a variety of sources.

3. Results and discussion

3.1. TLC to SFC correlation

The standard approach for flash chromatography users is to use TLC to evaluate various solvent combinations to identify the mobile phase offering the best separation of sample components as well as determine the solvent polarity required for elution of the compounds with an appropriate retention to produce material with required purity and yield. Implementation of flash SFC into medicinal chemistry laboratories would be easier if the process for identifying flash SFC conditions was nearly identical to the process for identifying flash LC conditions. The first component of this study was to determine if normal phase TLC could be used to estimate SFC retention times. A total of 77 drug like compounds (Table 1) were used for this study. These compounds were either neutral, acidic or basic. The TLC methods and analytical SFC conditions are summarized in Table 2. R_f values were measured for all TLC conditions and SFC R_t compared to TLC R_f. Correlation values are summarized in Table 3. Review of Table 3 shows that there is poor correlation between TLC R_f and SFC R_t for any of the solvent systems and modifiers explored. The correlation was worse for polar TLC system (10/90/1 (v/v/v) methanol/dichloromethane/ammonium hydroxide and ethyl acetate) due to a large number of compounds with R_f greater than 0.8. Based on these results it is not possible to use TLC using silica gel to predict retention or separation for SFC using silica columns.

3.2. Loading capacity for SFC using silica gel stationary phase

At the time of this study little work had been published on the use of silica for achiral SFC separations. Experiments were performed to determine whether loadings and peak shape obtained using silica were comparable to other SFC achiral phases. For this