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Use of dichloromethane for preparative supercritical fluid chromatographic enantioseparations

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ABSTRACT

Preparative SFC is the technique of choice for small scale (mg to hundreds of grams) enantioseparations. Traditionally methanol, ethanol or isopropanol are used as a modifier for preparative SFC. When compounds being purified using preparative SFC exhibit poor solubility in carbon dioxide and these modifers, poor peak shape, low purification throughput, high solvent usage and potential sample precipitation and system blockage can occur. This paper discusses the use of dichlormethane as a modifier (alone or with other solvents) for analytical and preparative enantioseparations. It was shown that the use of dichloromethane as a modifier in preparative SFC can improve racemate solubility, leading to higher purification productivities and reduced solvent usage compared to traditional modifiers. Decreases in solvent consumption and decreased E factors were also realized. The applicability and advantages of this technique will be demonstrated during the preparative resolution of multiple enantiomers at the hundreds of gram scale.

1. Introduction

Generation of individual enantiomers is a necessary step in pharmaceutical discovery. Preparative HPLC has been used for enantioseparations for the past thirty years for separations from the mg to 100s kg scale [1–6]. Over the past fifteen years preparative SFC has appeared as the technique of choice for separations from the mg to kg scale [4,7–16]. In SFC a majority of the mobile phase has been replaced with CO_2. The critical point for CO_2 is a temperature of 31 °C and a pressure of 73 atm. Above the critical 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 a carbon dioxide containing mobile phase allows higher linear velocities relative to HPLC resulting in shorter run times. Increasing mobile phase velocities in SFC has less impact on efficiency compared to HPLC. An SFC system can flow at linear velocities two to three times those of HPLC and achieve the same chromatographic efficiency. Also the lower pressure drop in SFC allows higher flowrates than those possible with HPLC. From a purification viewpoint, the increase in flowrates results in higher productivities (racemate processed per unit time) relative to HPLC. The increased productivity of preparative SFC allows compounds to be purified in a shorter time frame, reducing the time required to generate pure enantiomers for pharmaceutical testing and accelerating the drug discovery process. Another consequence of the increased productivity of preparative SFC is potentially a reduction in the number of preparative chromatography systems in a laboratory, reducing costs and lab space requirements.

To achieve elution of most pharmaceutical compounds from an SFC column a polar modifier such as methanol or another polar solvent must be added to the carbon dioxide based mobile phase. The addition of a modifier to carbon dioxide moves the mobile phase from supercritical to subcritical under standard preparative SFC operating conditions. Even when operating under subcritical conditions many of the advantages of a supercritical mobile phase are still realized. Beyond increased linear velocities another major advantage of preparative SFC over preparative HPLC is lower solvent usage due to the replacement of the majority of the mobile phase with CO_2. After exiting the chromatographic column CO_2 is removed by a gas–liquid separator, leaving only the modifier. This results in higher product concentrations, reducing the time and energy required for post purification solvent removal and product isolation. SFC is also an environmentally conscious technology. The use of CO_2 eliminates the use of hydrocarbon based solvents (heptane/hexane) that are often used in preparative HPLC. CO_2 used in SFC is recovered as a byproduct of manufacturing processes, resulting in no net increase in CO_2. The use of CO_2 recycling units as part of the preparative SFC system can further reduce CO_2 requirements. Overall solvent volumes for preparative SFC are 2–10 times less than seen with preparative HPLC. The reduction in solvent

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requirements results in reduced time and cost to isolate purified material.

The goal of a chromatographic purification process is the introduction of maximum amount of material onto the separation column while maintaining required purity and yield of separated products. To achieve a highly productive purification process high solubility (>25 g/l) in the mobile phase is required. It is preferable to dissolve the racemate to be separated in the mobile phase. It is difficult to dissolve racemate in a carbon dioxide based mobile phase due to poor solubility of most pharmaceutical compounds in CO_2. The common approach in preparative SFC is to dissolve the sample to be purified in pure modifier. While this approach eliminates the complexity of dissolution in CO_2 and allows increased material to be applied to the column, the use of a dissolution solvent with different polarity relative to the mobile phase can lead to peak broadening and peak distortion [17]. An approach utilized by many preparative SFC scientists is to dissolve samples in a 1:1 mixture of methanol and dichloromethane (DCM). This approach will often not result in peak distortion. While the use of this mixture allows introduction of racemate onto the column, if solubility in methanol/carbon dioxide mixtures is low, poor peak shape may occur. This poor peak shape can include peak splitting and/or broadening. In a worst case situation, racemate precipitation on the column can occur once DCM flows away from the racemate. This can lead to higher system pressures and system shutdown.

A potential alternative when poor solubility in alcohol/carbon dioxide mobile phases limits or prevents chromatographic resolution using SFC is to investigate alternate modifiers that offer increased racemate solubility. These non-traditional modifiers include dichloromethane, ethyl acetate, tetrahydrofuran, methyl tert butyl ether, chloroform, acetone, toluene in addition to other organic solvents. Use of these solvents in SFC has been shown to increase preparative productivity as well as offer unique selectivity compared to traditional alcohol based modifiers [18,19]. When using dichloromethane it is often necessary to add 20–50% alcohol to allow elution from the chromatographic column in an acceptable timeframe. With non-traditional modifiers in SFC it is imperative to use a chiral stationary phase that is stable to these solvents. This includes the immobilized polysaccharide based CSP (Chiralpak IA, IB, IC, ID, IE, IF) or a Pirkle type or other type of CSP where the chiral selector is covalently bound to the silica. Use of non-traditional solvents with coated CSP can result in rapid column degradation due to dissolution of chiral selector by the mobile phase.

This paper reports on the use of methanol/dichloromethane and dichloromethane as modifiers for preparative SFC enantioseparations. Multiple examples will show how the use of these solvents can increase productivities, reduce solvent volumes and result in a greener separation process (as measured by E factor). Also included is a short discussion on the environmental impact of using dichloromethane.

2. Experimental

2.1. Equipment

The analytical SFC chromatograph was a SFC method development station sold by Waters equipped with a Waters ZQ mass spectrometer (Milford, MA, USA). The preparative SFC was a Prep 80 or Prep 350 from Waters (Milford, MA, USA).

2.2. Materials

Analytical and preparative Chiralpak and Chiralcel SFC columns were purchased as pre-packed five micron columns from Chiral Technologies (West Chester, PA, USA). Whelk-O analytical and preparative SFC columns were purchased from Regis Technologies (Morton Grove, IL, USA). All chemicals were purchased from Sigma-Aldrich, VWR or Fisher Scientific. All Amgen racemates were prepared in Amgen laboratories (Cambridge, MA, USA). The solvents were reagent grade or better and obtained from a variety of sources.

3. Results and discussion

3.1. Preparative Example #1

762 g of racemate (Compound 1, proprietary structure) enriched in the active enantiomer (ee ~20%) was submitted for preparative resolution. Standard approach for SFC method development in our laboratory utilizes a number of chiral stationary phases (CSPs) that over time have shown high success rates and methanol and isopropanol modifiers with or without basic additives. This procedure has been described previously [11]. Compound 1 did not contain basic functional groups thus no additive was utilized. The best analytical SFC separation for this molecule is shown in Fig. 1 chromatogram A. Based on the high selectivity (1.96) and short cycle time it was expected this racemate would be resolved quickly. The separation was scaled to a 5 cm i.d. Chiralpak AD-H column. The racemate was not highly soluble in methanol but concentrations of 100 mg/ml were achieved with a 1:1 mixture of methanol:dichloromethane. The use of methanol/dichloromethane mixtures for sample dissolutions is a standard approach within our laboratories and provides acceptable purification results a majority of the time. One ml of sample (~100 mg) was injected onto the column and baseline separation was observed (Fig. 1 chromatogram B). Based on the 1.5 min separation between peaks the sample volume for the next injection was increased to 6 ml (~600 mg). The results of this experiment are shown in Fig. 1, chromatogram C. Multiple tailing peaks were observed, making it difficult to determine where the individual enantiomers were eluting in the chromatogram. This chromatographic result is often an indication of poor mobile phase solubility.

To process this material a mobile phase with better solubility needed to be developed. During sample dissolution it was determined the racemate exhibited good solubility in dichloromethane/methanol mixtures. Analytical method development using immobilized chiral stationary phases (Chiralpak IA, IB, IC, ID, IE and IF) as well as a covalently bound Pirkle type phase ((S,S) Whelk-O) and a modifier of methanol/dichloromethane was explored. Previous studies have shown that dichloromethane will not elute most moderately polar pharmaceutical compounds. Stationary phase manufacturers recommend the addition of methanol to achieve elution. The enantioseparation seen under these conditions was not as large as observed in the analytical separation shown in Fig. 1. The best separation was observed using the (S,S) Whelk-O CSP and a modifier of 30% 1:1 methanol:dichloromethane. The separation of compound 1 under these conditions is shown in Fig. 2, chromatogram A. While the selectivity with the Whelk-O was significantly less than seen with Chiralpak AD (1.27 vs. 1.96) it was thought the increased racemate solubility in methanol/dichloromethane vs. methanol alone would afford better preparative peak shape than seen previously.

The preparative separation of 1100 mg of racemate using these conditions is shown in Fig. 2, chromatogram B. These conditions allowed both enantiomers to be isolated at greater than 95% yield with purities (ee) of >99%. Using overlapping injections the cycle time was reduced to 4 minutes, allowing 14.4 g of racemate to be processed per hour. Approximately 52 h of instrument time was required to process the entire batch of racemate. Using these conditions a productivity of 1.34 kkd (kilograms racemate/kg CSP/day) and a solvent usage of 0.38 l/g racemate was obtained. A high