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From analytical methods to large scale chiral SFC using chlorinated chiral stationary phases

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ABSTRACT

While traditional non-chlorinated Cellulose- and Amylose-derivatized phases have been used successfully in supercritical fluid chromatography (SFC) to resolve a broad variety of chiral compounds, some chiral pharmaceutical compounds are not well resolved on these traditional chiral stationary phases (CSP) due to the lack of chiral selectivity. Since there are no universal CSP to resolve all chiral compounds, chlorinated CSP can be complementary to the non-chlorinated CSP. Chlorinated CSP such as 4-Chloro-3-methylphenyl-carbamatecellulose (Lux-Cellulose-4), 3-Chloro-4-methylphenyl-carbamatecellulose (Lux-Cellulose-2), 5-Chloro-2-methylphenyl-carbamateamylose (Lux-Amylose-2) and immobilized 3,5-dichlorophenyl-carbamatecellulose (Chiralpak IC) have provided a range of chiral recognition mechanisms which have allowed the authors to successfully achieve chiral SFC resolution on several structurally diverse compounds, which are not well resolved in the non-chlorinated CSP. In addition, chlorinated Lux-Cellulose-4, Chiralpak IC and Lux-Amylose-2 have enabled us to utilize non-alcohol solvents as sample diluents and as co-solvents to significantly improve compound solubility and selectivity. This article will discuss the challenges associated with several SFC applications on both coated and immobilized chlorinated CSP to deliver high-quality drug candidates in large quantity. The use of dichloromethane in both sample preparation and as co-solvent in CO_2 to increase sample solubility will be presented in preparative example #2 and #3.

1. Introduction

Chiral SFC provides a direct and efficient way not only for assessing the chiral purity of compounds, but also for providing optically pure starting materials or intermediates for further synthesis, as well as final active pharmaceutical ingredients for bioassay and toxicity tests [1–7]. In supporting early discovery programs non-chlorinated chiral stationary phases (CSP) in SFC have been widely utilized for resolving a significant number of structurally diverse compounds, providing small quantities of each enantiomer in high enantiomeric excess (ee) for in-vitro potency tests [8,9]. After programs progress, larger quantities of chirally pure compounds are needed to support various biological assays. SFC using non-chlorinated CSP has been effective to provide a large quantity of chirally pure active pharmaceutical ingredients for in-vivo and toxicological studies [10–12]. There have been a number of CSP including polysaccharides, Pirkle-type and Cyclodextrins and Macrocyclic antibiotics utilized for chiral SFC resolution [13–16]. Among them, polysaccharide-based CSP appear to be the most popular because of broad chiral application scope. Non-chlorinated polysaccharide-based (Cellulose- and Amylose-derivatized) CSP have successfully resolved a vast majority of chiral compounds in a broad variety of structural classes [17–20]. However some chiral pharmaceutical compounds are not well resolved on these non-chlorinated CSP due to the lack of chiral selectivity [21–24]. In these cases chlorinated CSP can provide complementary selectivity to these non-chlorinated CSP. In addition, compound solubility and compatibility become increasingly important factors in determining throughput in SFC purifications as the scale increases. Due to their poor compatibility with non-alcohol solvents, applications of these coated non-chlorinated CSP in large-scale are limited to chiral resolution of alcohol-soluble compounds [10–12]. The solvent compatibility issue has been resolved with introduction of immobilized CSP such as Chiralpak IA and IB [1,25,26]. This article will discuss SFC applications on chlorinated CSP in delivering large quantities of high-quality drug candidates and the challenges associated with the use of dichloromethane for sample preparation and as modifier. Sample diluent influence on chiral resolution will also be discussed.

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2. Experimental

2.1. SFC instrumentation

The analytical SFC systems include a Berger analytical SFC system with six-position modifier and column switching valves, a Thar SFC method development station with a six-position modifier and a ten-position column switching valve from Waters (Milford, MA, USA), and an Aurora SFC Fusion A5 Evolution with an eight-position modifier and a six-position column switching valve from Agilent (Santa Clara, CA, USA). The preparative SFC was a SFC-350 with six fraction-collecting cyclones from Waters (Milford, MA, USA). Berger and Aurora systems are equipped with Agilent DAD/G1315B detector, while Thar is with Waters 2996 Photodiode Array Detector.

2.2. Methods

2.2.1. Analytical SFC methods

All analytical SFC experiments were performed either on Thar, Berger or Aurora analytical SFC system. All analyses were operated under isocratic conditions at a back pressure of 100 bars on Thar and Berger, and 140 bars on Aurora, with a flow rate of 3 ml/min. The injection volume is 10 μl in all the analytical experiments.

2.2.2. Preparative SFC methods

All preparative experiments were scaled up on SFC-350 at a flow rate of 120–250 ml/min, under isocratic conditions at a back pressure of 100 bars.

2.3. Materials

2.3.1. Reagents

Methanol, acetonitrile, dichloromethane (DCM), heptane, trifluoroacetic acid (TFA) and isopropanol (IPA) were HPLC grade and purchased from J.T. Baker (Phillipsburg, NJ). Ethanol, 200 proofs (99.98%) was purchased from Pharmco-AAPER (Brookfield, CT).

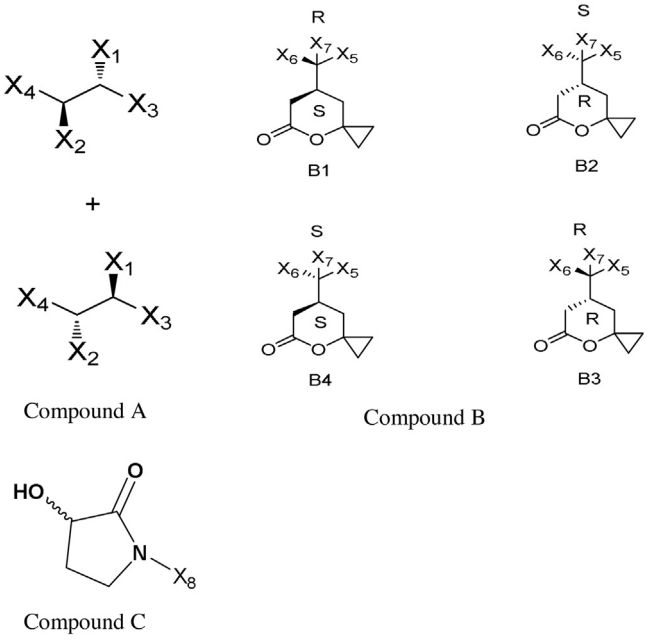


Fig. 1. Chemical structures of in-house intermediate compounds.

Carbon dioxide from Airgas (New Jersey, USA) was compressed to 1500 psi and supplied to the SFC stations.

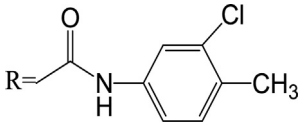
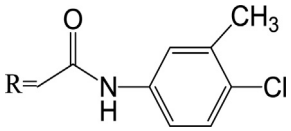
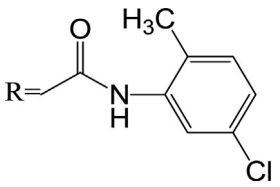
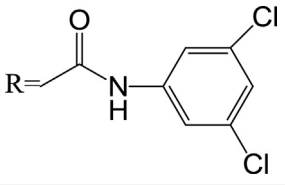
2.3.2. Compounds

Compounds synthesized in Department of Discovery Synthesis (DDS) at Discovery Chemistry (Princeton, NJ, USA) in Bristol-Myers Squibb are summarized in Fig. 1.

2.3.3. Columns

Polysaccharide-based chiral SFC columns including 3,5-dimethylphenylcarbamatecellulose (Chiralcel ODH, 0.46 × 25 cm, 5 μm), 4-methylbenzoylcellulose (Chiralcel OJH, 0.46 × 25 cm,

Table 1
Generic chemical structures of selected chlorinated CSP.

Chemical names	Structure	Brand names
3-Chloro-4-methylphenylcarbamatecellulose		Lux-Cellulose-2
4-Chloro-3-methylphenylcarbamatecellulose		Lux-Cellulose-4
5-Chloro-2-methylphenylcarbamateamylose		Lux-Amylose-2
3,5-Dichlorophenylcarbamatecellulose		Chiralpak IC