Chiral Prep Chromatography Utilizing Twin-Column Technology

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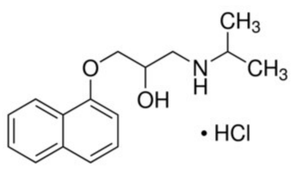
Introduction

In the early development phases of chiral pharmaceutical molecules, it is typically necessary to isolate small amounts of the different chiral isomers. Preparative chromatography is often the most straightforward way to obtain these materials. As the development process continues, the material demand increases and isolating larger amounts of material by traditional prep chromatography can become more challenging. Multi-column counter-current solvent gradient purification (MCSGP) is a twin column continuous chromatography process that has significantly more capability than a traditional single-step chromatographic process. Increases in the yield and throughput without sacrificing the purity can be achieved by utilizing the MCSGP process. The basic concept is to collect the pure early eluting component. The impure overlapping material is sent to a second column, while adding more of the initial material. The late eluting pure component is then collected. The pattern is repeated on the second column with the overlapping material being sent to the first column and repeated again and again until the initial material has been completely processed.

The work presented here will use a typical small molecule pharmaceutical compound as a case study to describe and demonstrate the MCSGP process as applied to a chiral prep chromatography project. The results and productivity estimates from this MCSGP process will be compared to those from a single column typical process.

Experimental

Propranolol (Sigma-Aldrich)
Trifluoroacetic acid (Sigma-Aldrich)
Ammonium bicarbonate (Sigma-Aldrich)
HPLC grade water (Burdick & Jackson)
Acetonitrile (Burdick & Jackson)
2-propanol (Burdick & Jackson)
All analytical columns 5µm, 4.6mm x 100mm
All prep columns 10µm, 10mm x 100mm
Agilent 1100
YMC Contichrom® Cube



Propranolol
Molar Mass: 259.18
pKA: 9.5
LogP: 3.48

Chiral HPLC Columns

YMC CHIRAL ART HPLC columns/packing materials are either coated or immobilized with chiral selectors from polysaccharide derivatives. These resins exhibit a high level of mechanical strength and chemical stability that can provide excellent peak shape without tailing.

YMC CHIRAL ART columns are suitable for the separation of a wide range of chiral compounds, cis-trans isomers, and geometric isomers. They are ideal for application areas such as measuring optical purity with analytical columns and purification of chiral materials with preparative columns and/or packing materials.

Immobilized Columns/packing materials useful for normal phase & reversed phase chromatography

Column/ Packing material	Particle size (µm)	Chiral selector	USP Classification
Amylose-SA	3 5 10 20	Amylose tris(3,5-dimethylphenylcarbamate)	L99
Cellulose-SB		Cellulose tris(3,5-dimethylphenylcarbamate)	—
Cellulose-SC		Cellulose tris(3,5-dichlorophenylcarbamate)	—
Cellulose-SJ		Cellulose tris(4-methylbenzoate)	—

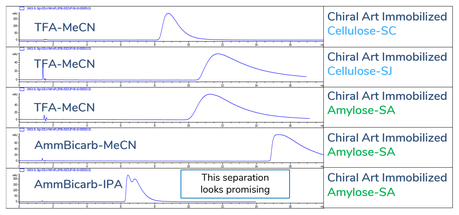
Coated Columns/packing materials with high resolution for various compounds

Column/ Packing material	Particle size (µm)	Chiral selector	USP Classification
Amylose-C Amylose-C Neo	3 5 10 20	Amylose tris(3,5-dimethylphenylcarbamate)	L51
Cellulose-C		Cellulose tris(3,5-dimethylphenylcarbamate)	L40

Method Development

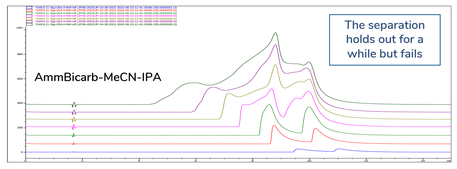
Analytical Scale Chromatography

Screening
All chiral chromatography method development starts with column screening. pH 2 and pH 8 reversed phase conditions with acetonitrile and 2-propanol were evaluated. The conditions with pH 8 and alcohol were the only promising result from the initial screen.



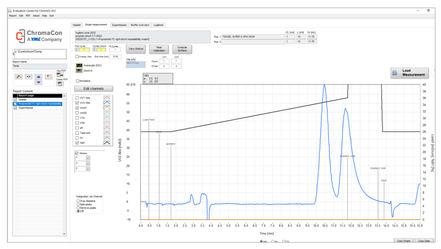
Optimization
The promising conditions were quickly optimized. The peak shape was not ideal, but the peaks were completely separated.

Loading
A simple loading study was conducted with the optimized conditions. The chiral separation did not scale well. Batch chromatography would be difficult with these conditions.

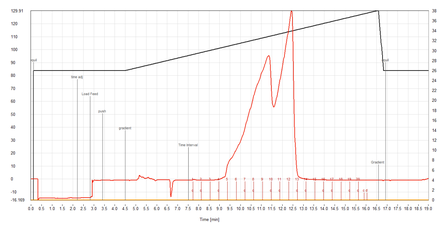


CUBE Scale Chromatography

Scale-up
The optimized analytical conditions were scaled from 5µm 4.6mm x 100mm columns to 10µm 10mm x 100 mm columns. The chromatographic profile scaled well.

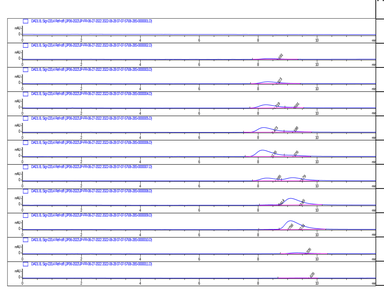


Fractionation
The loading was increased on the 10µm 10mm x 100 mm column until the two peaks were significantly overlapping. Fractions were collected every 30 seconds across the eluting material.



Fraction Analysis

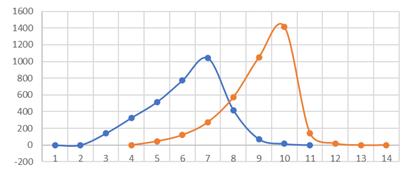
A slightly modified version of the optimized analytical method was utilized to assay the collected fractions. Both peaks were integrated and the peak areas were tabulated. It is important that fractions were collected before and after the desired material was eluted.



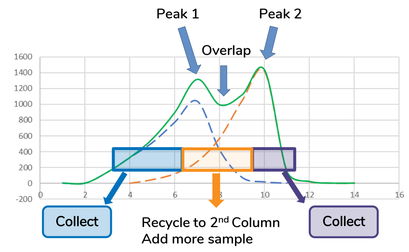
Fraction	Peak Area Peak 1	Peak Area Peak 2
1	0	0
2	140	0
3	326	0
4	515	46
5	775	121
6	1040	275
7	417	574
8	71	1049
9	15	1416
10	0	145
11	0	21

Recycling Plan Development

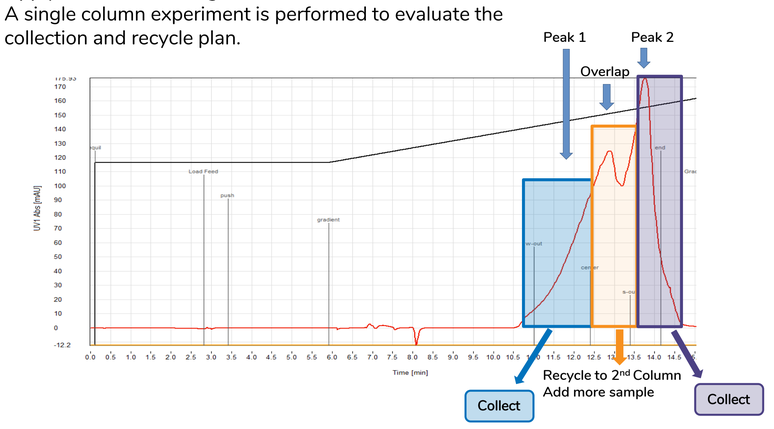
Plot of Fractionation Data
From the fractionation data, the peak areas are plotted versus the fraction number. This provides a view of the components as individual peaks relative to each other.



Recreate the Chromatogram
The peak area data from the fractionation experiment can be combined to recreate the chromatogram. This provides a view of where the pure peaks can be collected.

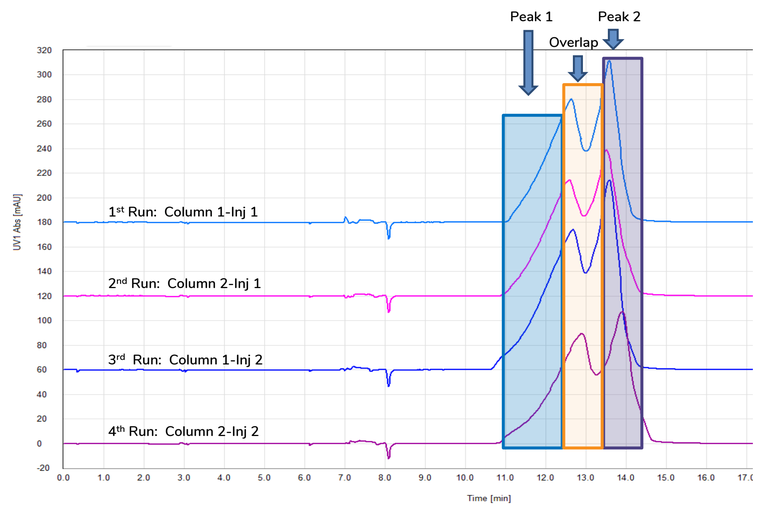


Apply the Plan to a Single Column Batch Run
A single column experiment is performed to evaluate the collection and recycle plan.

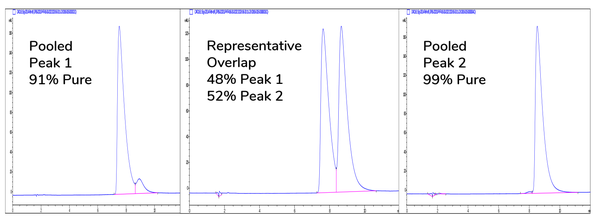


Results

Chromatograms with Recycling
To demonstrate the recycling plan, four runs were made. There were two injections on each of the columns. The Peak 1 and Peak 2 materials were collected, and the overlap material was recycled onto the next column.



Evaluation
Three samples were evaluated by HPLC. The collected Peak 1 material from all four runs were combined to make a single pooled sample. The same was done for the Peak 2 material. Overlap material from a single column run was collected as a representative overlap sample.



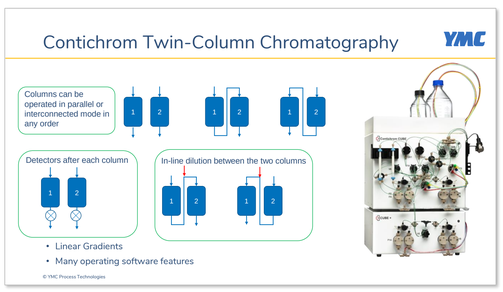
Summary

Chiral isolations are necessary for pharmaceutical development. Chiral chromatography is a straightforward and direct isolation technique for chiral compounds. Most small molecule compounds can have a chromatographic method developed and material isolated within a few days. Twin column technology is very useful for a wide variety of isolation situations.

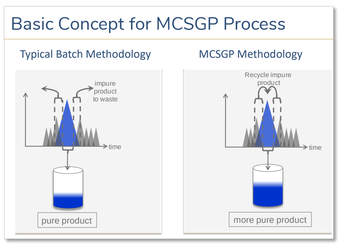
The RP example presented here demonstrates a way to isolate chiral material without a significant amount of development.

The CUBE, MCSGP, and Chiral Purifications

The CUBE
The Contichrom® CUBE is a continuous chromatography development system with integrated dynamic process control functions. It offers unique capabilities for mastering complex separation challenges using the proprietary Twin-column processes. The user-friendly software enables rapid process development.



MCSGP
MCSGP stands for multicolumn countercurrent solvent gradient purification. This process is typically used for the purification of a single species from a multi-component mixture with both high purity and high yield. It is a continuous countercurrent multicolumn chromatography process that utilizes a linear gradient. High purity is achieved by collecting the portion of the desired component with suitable purity. High yield is achieved by combining the material that contains the desired component, but doesn't meet the target purity, with more of the initial crude material that needs to be purified.

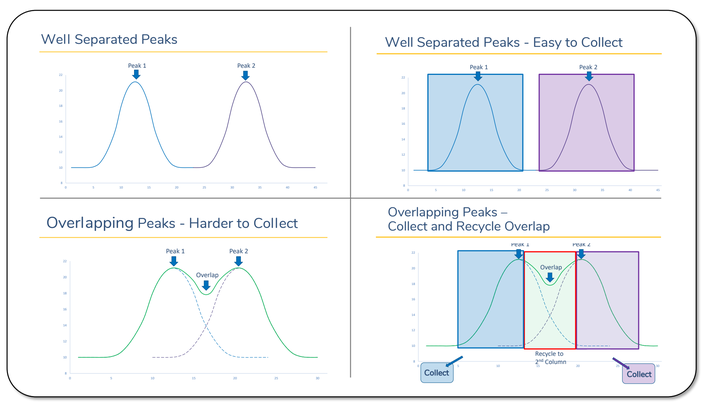


Chiral Purifications
Chiral separations are among the most difficult chromatographic processes. With enantiomeric separations, the two components are nonsuperimposable mirror images that are virtually identical in all physical and chemical properties. They have the same atoms, bonded together in the same way, but their 3D arrangement is different.

Polysaccharide-based chromatography columns are the work horse for chiral separations.

Well Separated Peaks
When the two components are well separated, chiral purifications are straightforward. Typical single column batch chromatography is very effective for isolating purified material.

Overlapping Peaks
When the two components are not well separated, chiral purifications can be very difficult. Typical single column batch chromatography can be utilized for purifications but with a lot of extra work. The front part of the early eluting peak is pure and collected. The overlapping portion is a mixture of both components, and it can be collected and later reprocessed. The back part of the late eluting peak is pure and collected. Twin-Column chromatography can eliminate the extra work by automatically recycling the overlapping material.



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