Oligo Purification Process Improvements with Twin-Column Chromatography

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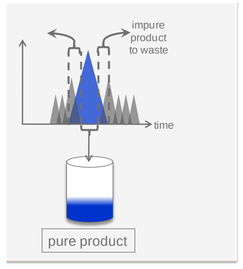
Introduction

Peptide and Oligo synthesis technologies are well developed for large scale manufacturing of pharmaceutical materials. Isolation and purification is a critical step in these manufacturing processes, and almost always utilizes chromatography. 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 batch process. The product yield is greatly improved with MCSGP while avoiding many complications encountered with a batch process.

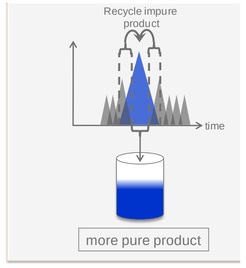
MCSGP Technique Overview

Typical Batch Process



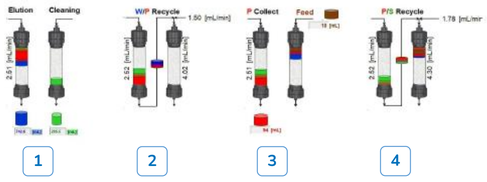
A typical batch process collects the pure portion of the desired peak. The portions with early and late impurities are discarded or retained for later reprocessing

MCSGP Process



An MCSGP process collects the pure portion of the desired peak. The portions with early and late impurities are sent to a second column while adding more feed material and the purification continues

2-Column MCSGP Process



1. The left column runs a sample while the right column is being cleaned.
2. Recycling the impure early eluting portion
3. Collecting the pure portion and adding more feed to the second column
4. Recycling the impure late eluting portion

The next step would be the right column running a sample while the left column is being cleaned.

CUBE
Bench-Top System

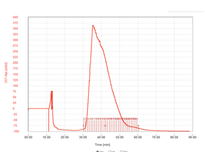
Available as 37 or 100 mL/min maximum flow rate. Capable of running MCSGP, single column batch, N-Rich and CaptureSMB

TWIN
Production Skid

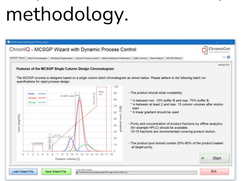
Custom built production equipment with flowrates up to 40 L/min. Capable of running MCSGP, single column batch, and separately – CaptureSMB.

3-Step MCSGP Development

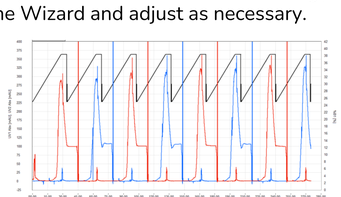
Step 1: Single column batch run with fraction collection and characterization



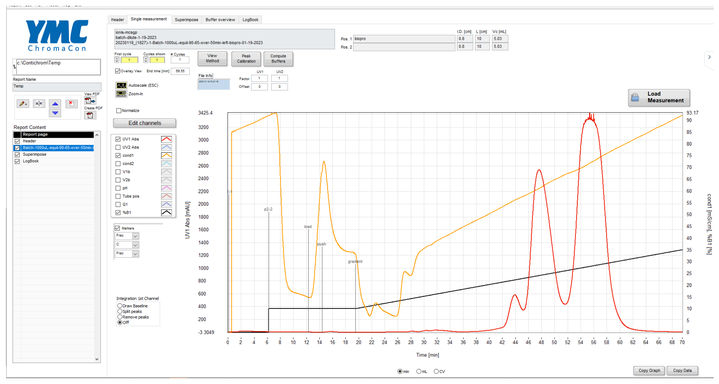
Step 2: MCSGP Wizard uses Step 1 results to develop MCSGP methodology.



Step 3: Run the MCSGP methodology from the Wizard and adjust as necessary.



Step 1: Batch Methodology

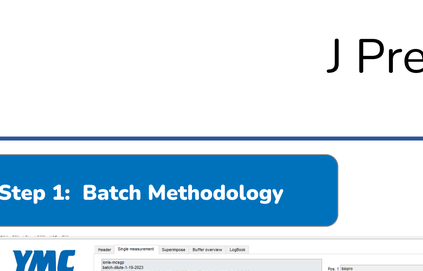


A single column, linear gradient batch run is made on a CUBE. There must be a segment of the desired peak with suitable purity that can be collected. Fractions are collected that capture all of the desired material.

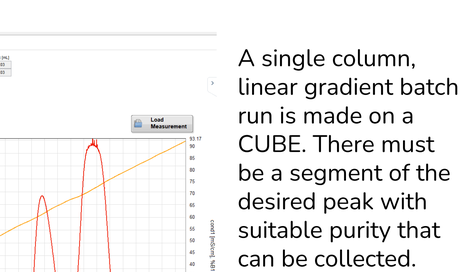
Step 2: MCSGP Wizard

The batch chromatogram from Step 1 is loaded into the MCSGP Wizard. The boundaries are set for recycling the early eluting material, the material to be collected and recycling the late eluting material.

The Wizard makes an initial prediction for the chromatographic method. Adjustments can be made to sample loading, flow rates, column washes and equilibration steps parameters.

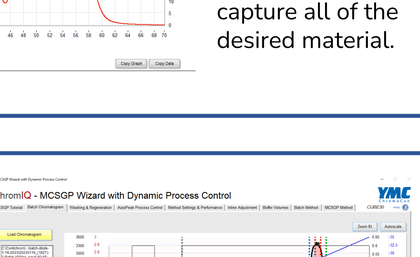


Recycling and collection can be triggered by time or by detector response. The recycled solutions are diluted before entering the next column. The amount of dilution is set within the Wizard.



The number of cycles, maximum system pressure and how the collected product is pooled are set within the Wizard. The Wizard also makes predictions for the expected method performance.

The Wizard calculates the total amount of sample feed, buffers and eluents used for the MCSGP method and for the associated startup and shutdown methods.

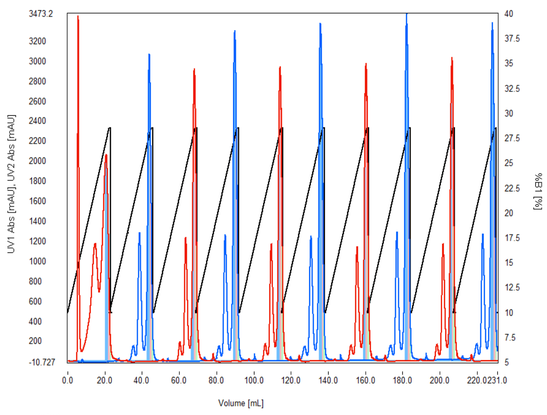


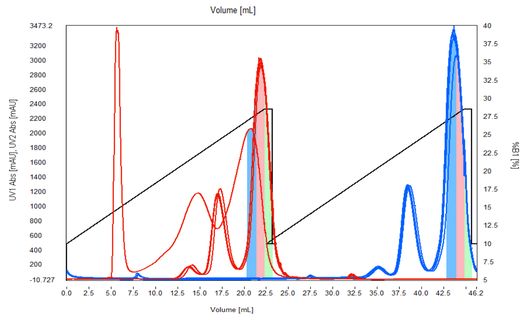
Step 3: Run MCSGP

With MCSGP, a "cycle" is defined as both columns completing a gradient run. A "switch" is when a single column completes a gradient run.

It is best to run at least 4 cycles (8 switches) to evaluate when the MCSGP methodology has reached steady state. The data can be viewed linearly as displayed below. The black trace below is the elution gradient. The red trace is the UV signal from column 1. The blue trace is the UV signal from column 2.

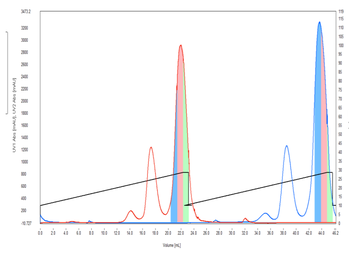
The MCSGP data can also be displayed as an overlay of the different cycles. The shaded areas represent the portions that are the early eluting recycled portion, the collected portion and the late eluting recycled portion.



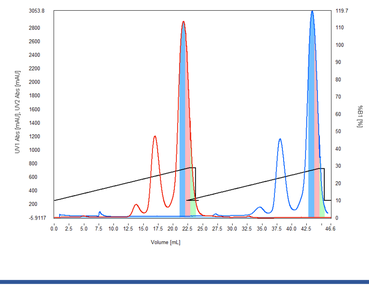


Triggering MCSGP Recycling and Collection

Time-based triggered recycling and collection is easy to set up but can be inaccurate because of the differences in the two columns or changes in the system over time.

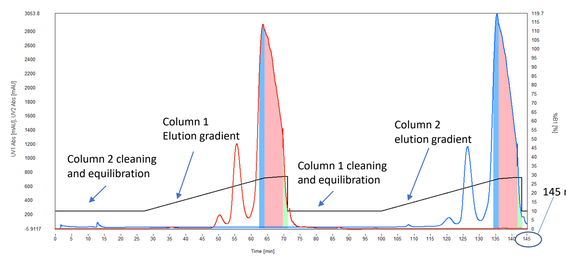


UV detector-based triggered recycling and collection can account for differences in the two columns or changes in the chromatographic profile.

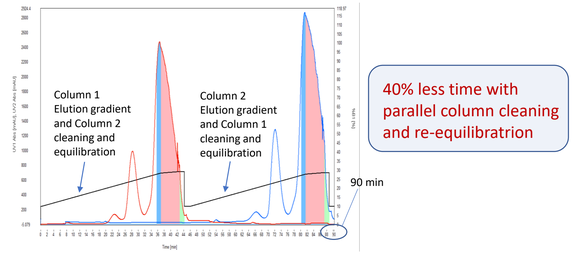


Optimized Column Cleaning and Re-equilibration

Single column batch processes need to clean and equilibrate the column between runs. This can be accomplished as part of the method before each injection or after the separation is completed. MCSGP can operate sequentially like this, but the total run time becomes long.



MCSGP allows for column cleaning and equilibration to be performed in parallel. One column is cleaned and washed while the other is running a separation. This parallel processing allows for significant time saving.



Conclusions

Product yield is a critical attribute for oligo and peptide purifications. The Twin-Column MCSGP process can be a valuable tool for increasing the product yield during the chromatography step. The desired material with insufficient purity is continuously recycled during the MCSGP process. This allows for significant increases in yields without added additional processing. Throughput during the Twin-Column MCSGP process is improved by cleaning and re-equilibrating one column while the other is running a separation.

Developing MCSGP methodology starts with the development of batch methodology. The single column method is used by the MCSGP Wizard to generate the MCSGP methodology.

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