Development of a Twin-Column Continuous Chromatography Oligo Purification Process

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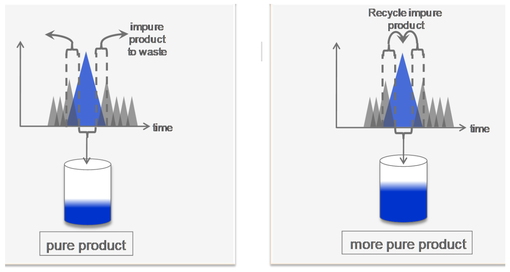
Introduction

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

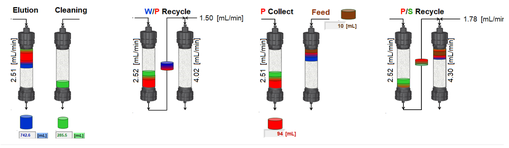
Typical Batch Process | **MCSGP Process**



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

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



The left column running a sample while the right column is being cleaned. Recycling the impure early eluting portion. Collecting the pure portion and adding more feed to the second column. 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

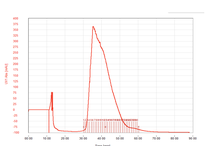
Available as 37 or 100 mL/min maximum flow rate. Capable of running MCSGP, single column batch, N-Rich, CaptureSMB

TWIN
Production Skid

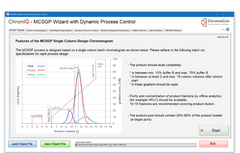
Custom built production equipment with flowrates up to 40 L/min. Capable of running MCSGP, single column batch, and separately – CaptureSMB.

3-Step MCSGP Development

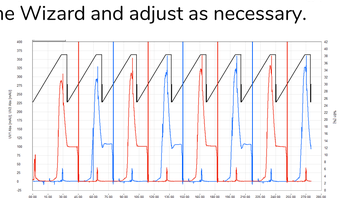
Step 1: Single column batch run with fraction collection and characterization



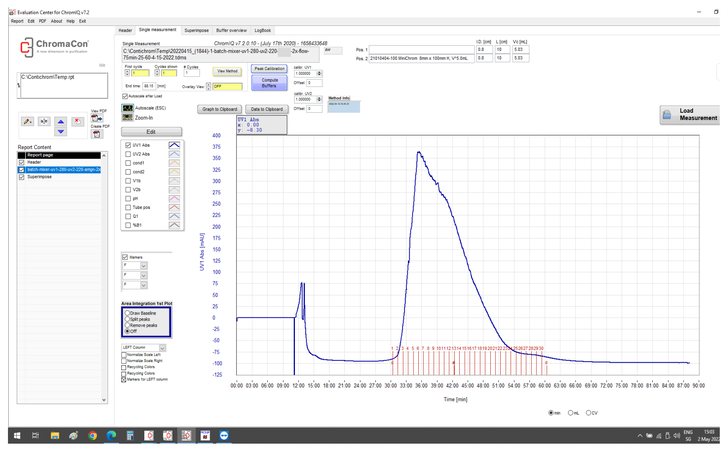
Step 2: MCSGP Wizard uses Step 1 results to develop MCSGP methodology.



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

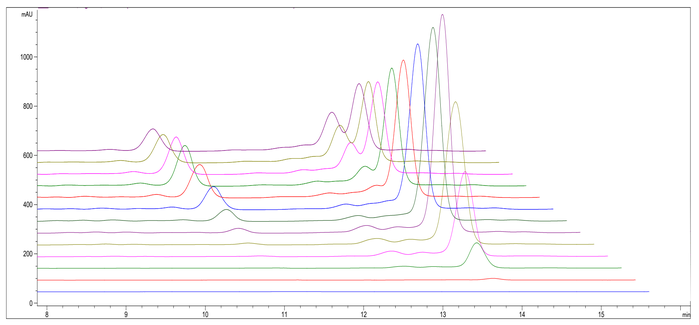


Step 1: Batch Methodology



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

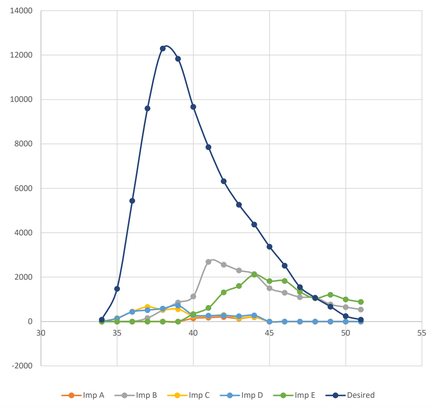
All fractions containing the desired component are analyzed with suitable analytical HPLC methodology. These chromatograms are integrated and the peak area for the desired component and every related species are recorded.



The peak area data is entered into a spread sheet. The % purity of each fraction is calculated. The % yield of each fraction is also calculated.

Fraction #	time (min)	Imp A	Imp B	Imp C	Imp D	Imp E	**Desired**	fraction % purity	fraction % yield
4	34	0.0	0.0	0.0	0.0	0.0	**85.4**	100	0.1
5	35	0.0	0.0	152.8	140.9	0.0	**1473.4**	83	1.8
6	36	0.0	0.0	447.6	439.0	0.0	**5438.0**	86	6.5
7	37	0.0	158.0	656.6	514.7	0.0	**9600.0**	88	11.5
8	38	0.0	517.6	532.9	584.2	0.0	**12298.0**	88	14.7
9	39	0.0	857.7	558.9	721.0	0.0	**11834.0**	85	14.1
10	40	141.6	1133.4	251.0	296.3	339.2	**9671.4**	82	11.6
11	41	175.5	2687.9	252.8	265.4	617.6	**7855.0**	66	9.4
12	42	200.7	2562.1	294.4	275.0	1313.9	**6319.7**	58	7.5
13	43	133.7	2300.0	162.0	240.4	1604.0	**5259.1**	54	6.3
14	44	198.8	2139.0	212.5	280.1	2113.0	**4371.9**	47	5.2
15	45	0.0	1500.0	0.0	0.0	1825.1	**3372.9**	50	4.0
16	46	0.0	1300.0	0.0	0.0	1831.4	**2517.3**	45	3.0
17	47	0.0	1100.0	0.0	0.0	1339.9	**1549.9**	39	1.9
18	48	0.0	1078.0	0.0	0.0	1049.7	**1069.7**	33	1.3
19	49	0.0	770.8	0.0	0.0	1206.3	**669.8**	25	0.8
20	50	0.0	650.5	0.0	0.0	999.7	**245.2**	13	0.3
21	51	0.0	538.9	0.0	0.0	882.4	**85.0**	6	0.1

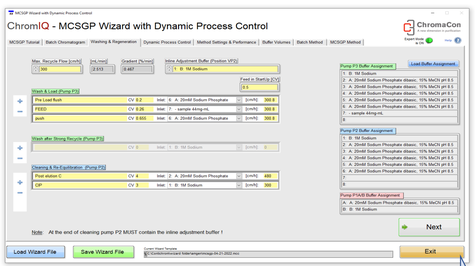
To visualize the location of impurities under the desired peak. The peak areas for each peak are plotted vs the retention time for that peak.



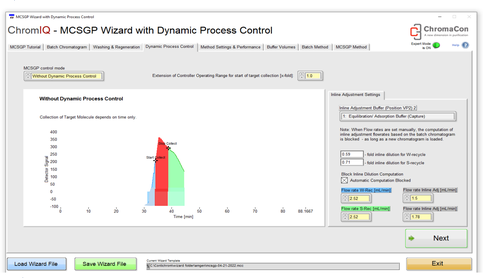
Step 2: MCSGP Wizard

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.

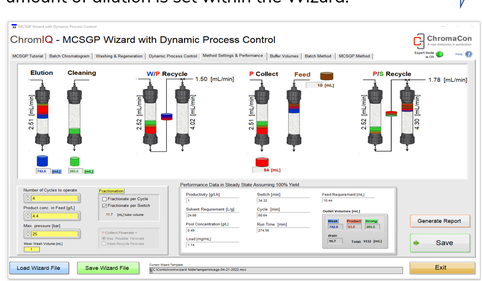
The Wizard makes an initial prediction for the chromatographic method. Adjustments can be made to sample loading, flow rates, column washes and equilibration steps parameters.



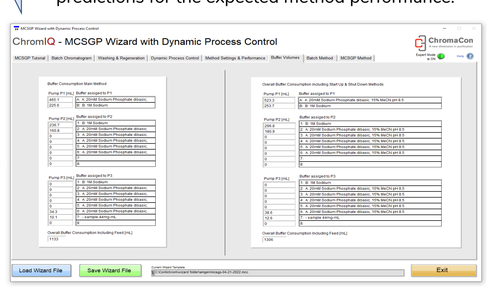
Recycling and collection can be triggered by time or by detector response. The recycled solutions are diluted before entering the next column. The amount of dilution is set within the Wizard.



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.



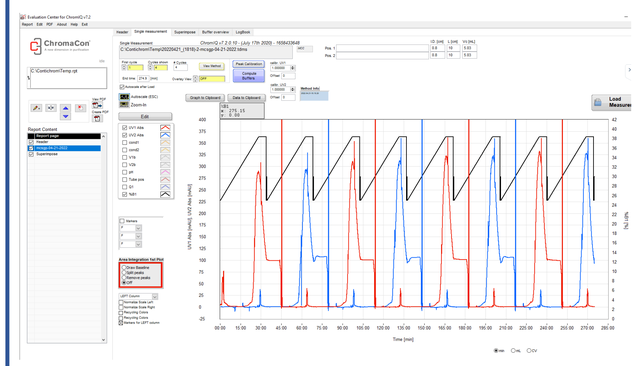
The Wizard calculates the total amount of sample feed, buffers and eluents used for the MCSGP method and for the associated startup and shutdown methods.



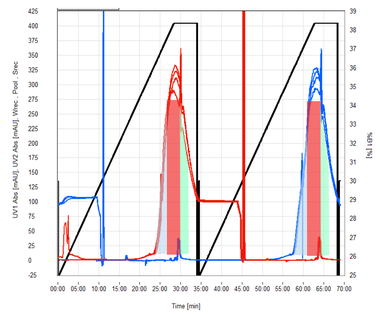
Step 3: Run MCSGP

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 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.

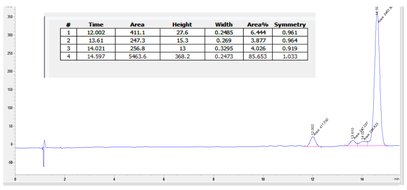


The MCSGP data can also be displayed as an overlay of the different cycles. The shaded areas represent the portions that are the early eluting recycled portion, the collected portion and the late eluting recycled portion.



Results and Discussion

Analytical chromatogram and integration results for material collected during MCSGP experiment third cycle described above in Step 3. The results were similar for the second, third and fourth cycles. The results for the first cycle were slightly different due to the steady state not being reached yet.

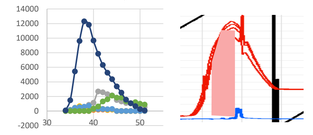


#	Time	Area	Height	Width	Area%	Symmetry
1	12.002	411.1	27.6	0.2485	6.444	0.961
2	13.61	247.3	15.3	0.269	3.877	0.964
3	14.021	256.8	13	0.3295	4.026	0.919
4	14.597	5463.6	368.2	0.2473	85.653	1.033

With data from the Batch Fraction Analysis Table, expected purity and yield estimates can be made for different batch methodology pools. Purity estimates can also be made for MCSGP methodology based on this batch method data.

Batch Pool	% purity	% yield
Fr8	88.3	14.7
Fr7-Fr8	88.1	26.2
Fr6-Fr8	87.7	32.7
Fr6-Fr9	86.7	46.8
Fr6-Fr10	85.6	58.3
Fr6-Fr11	82.4	67.7

Fraction Analysis | MCSGP results



By comparing the peak visualization data and the MCSGP Results, the collected material from the MCSGP is similar to the Fr6-Fr10 Batch Pool.

The % yield from MCSGP was 110% of the % yield obtained with the Batch Pool while the % purity for each technique was essentially identical.

	% purity	% yield
Batch Pool Fr6-Fr10	85.6	58.3
MCSGP Result	85.7	64.4

Conclusions

MCSGP can be a valuable tool for oligo and peptide purifications. The twin-column technology allows for continuous recycling of the material that has insufficient purity. This allows for increased yields without added additional processing. The development of MCSGP methodology starts the same as developing batch methodology but results are then used by the MCSGP Wizard to generate the MCSGP methodology.

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