

Waters

Paul Lefebvre

Senior Applications Chemist

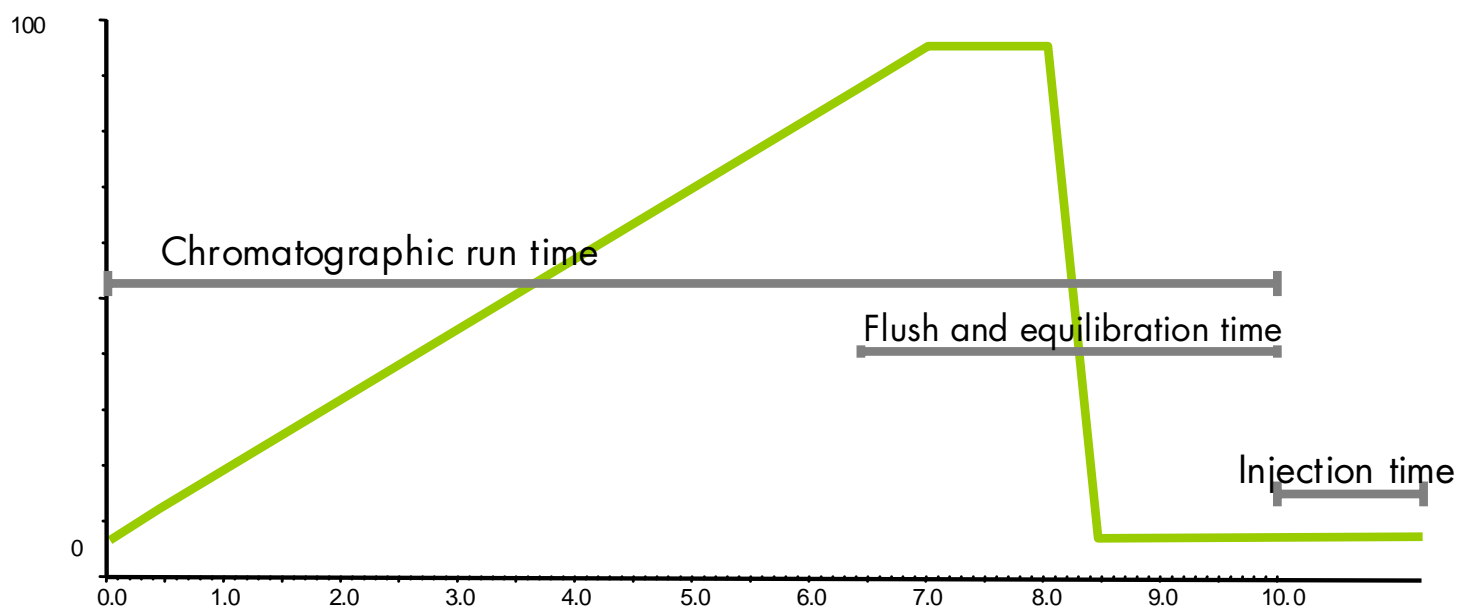
Improvements for Optimizing the Throughput of Small Molecule Purification



For Complete  Confidence

- Goal - Increase the number of samples purified / system
 - Decrease the time per sample
 - Increase the amount of time the system is in use
 - **Without impacting the purity**
- Throughput optimization can be made in several ways
 - Hardware
 - Software
 - Chemistry
 - Combinations of the above

- Places for potential time savings
 - **Chromatographic run**
 - Column equilibration
 - Injection time



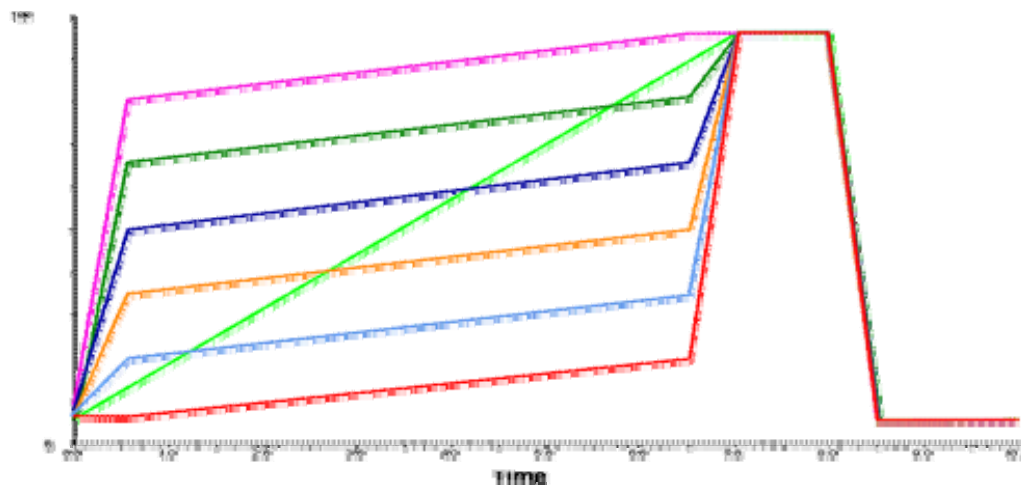
- Baseline method
 - 10 minute analysis time plus 2 minutes for the injection

	Time (min)	Flow (mL/min)	%A	%B	Curve
1	Initial	1.50	95.0	5.0	initial
2	7.00	1.50	5.0	95.0	6
3	8.00	1.50	5.0	95.0	6
4	8.25	1.50	95.0	5.0	6
5					
6					

- 10 samples
- Total run time of 120 min
- Places to save time
 - Shorten the RT
 - Decrease the equilibration time

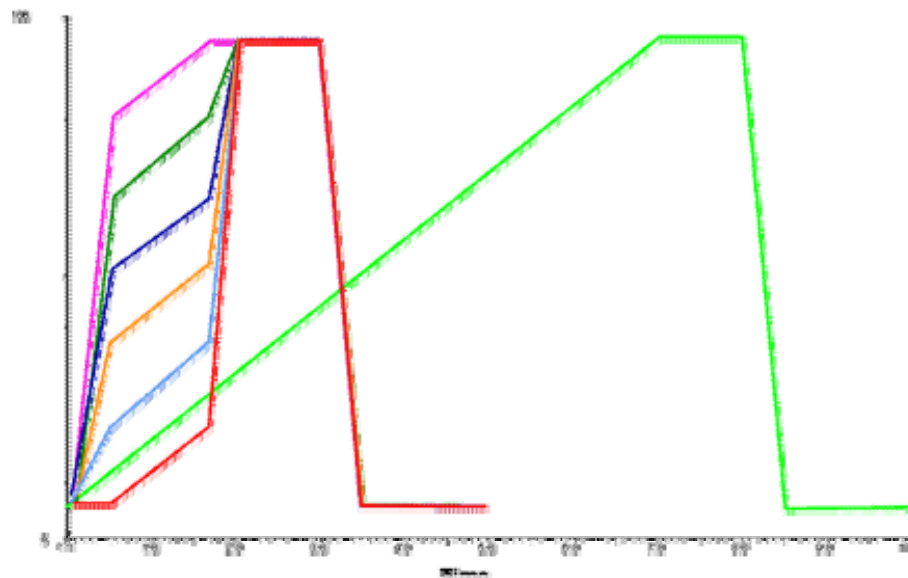
Sample	Retention Time (min)	Run Time (min)	Time Between Injections (min)
1	1.18	10	2
2	5.2	10	2
3	1.35	10	2
4	4.67	10	2
5	3.18	10	2
6	2.55	10	2
7	2.41	10	2
8	5.06	10	2
9	2.02	10	2
10	2.63	10	2
Total Run Time		120 minutes	

- By using the results from the analytical injection, it is possible to determine the % organic at which the samples elute
- Depending on requirements focused gradients can be used in different ways



- Increase resolution by reducing the gradient slope
 - Increase fraction purity but throughput remains constant

- If the resolution is adequate the slope can remain constant and the run time decreased
 - Improved throughput, purity remains constant



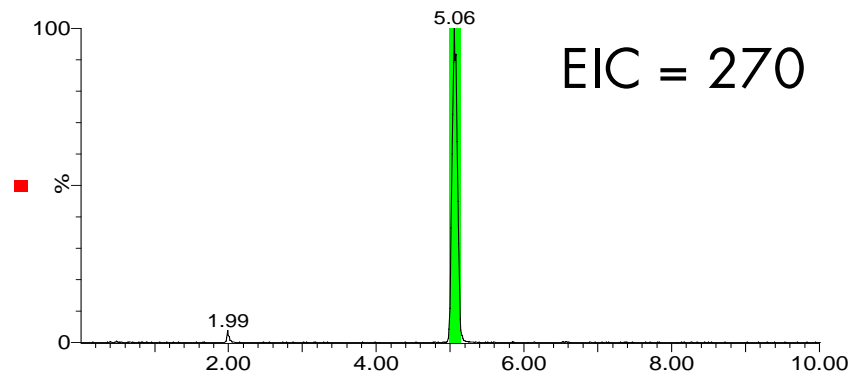
Gradient Selection

Gradient Name	Analytical Retention Time	% B Start	% B End
A	0.00 - 1.67	5	20
B	1.67 - 2.84	20	35
C	2.84 - 4.0	35	50
D	4.00 - 5.17	50	65
E	5.17 - 6.34	65	80
F	6.34 - 7.5	80	95

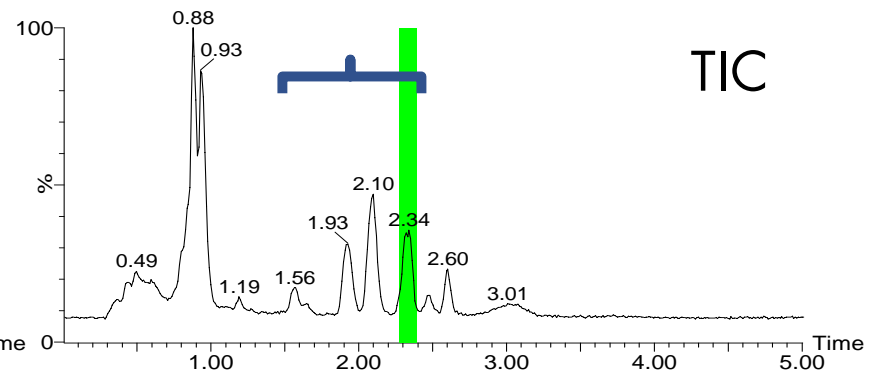
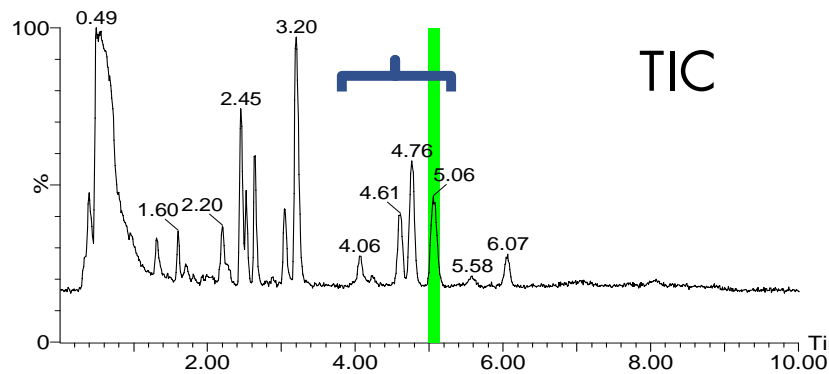
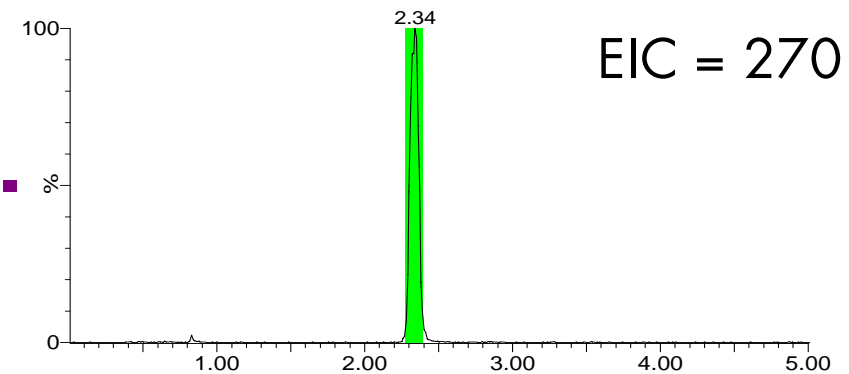
Gradient Method

Time (Minutes)	Composition (%B)
0.00–0.5	5–%B Start
0.50–1.67	%B Start–%B End
1.67–2	%B End–95
2–3	95
3 - 5	End

Generic Gradient



Narrow Gradient

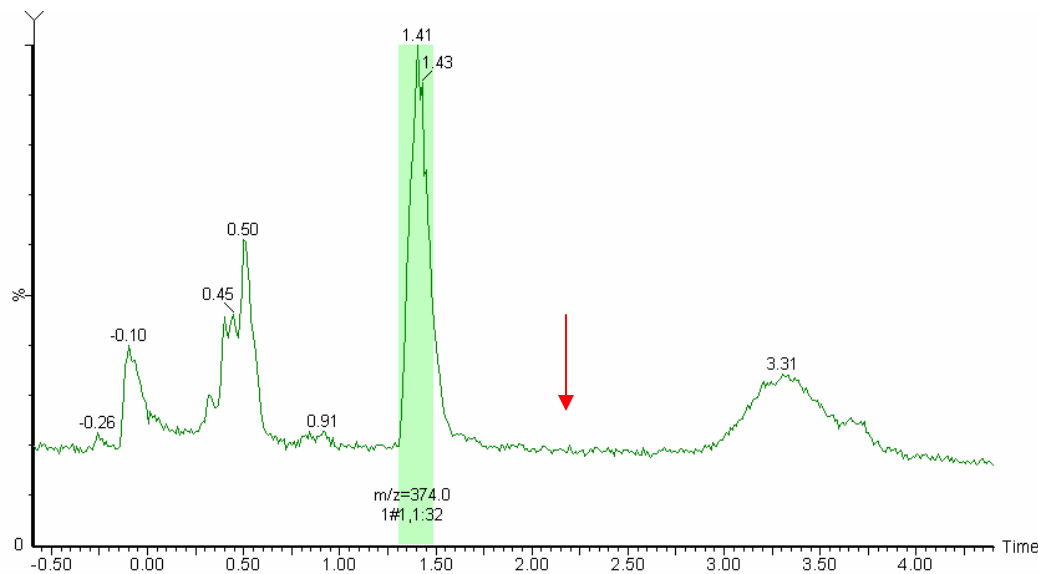


- Resolution is maintained across the gradient region

- By using focused gradients, the run time is decreased by 50%
 - However the throughput does not double, because of the time required for injecting.

Sample	Generic Retention Time (min)	Narrow Gradient	Narrow Retention Time (min)	Run Time (min)	Time Between Injections (min)
1	1.18	A	1.38	5	2
2	5.2	E	1.65	5	2
3	1.35	A	1.74	5	2
4	4.67	D	1.94	5	2
5	3.18	C	1.75	5	2
6	2.55	B	1.90	5	2
7	2.41	B	1.95	5	2
8	5.06	D	2.34	5	2
9	2.02	B	1.30	5	2
10	2.63	B	2.08	5	2
Total Run Time			70 minutes = 1.7 Fold Increase		

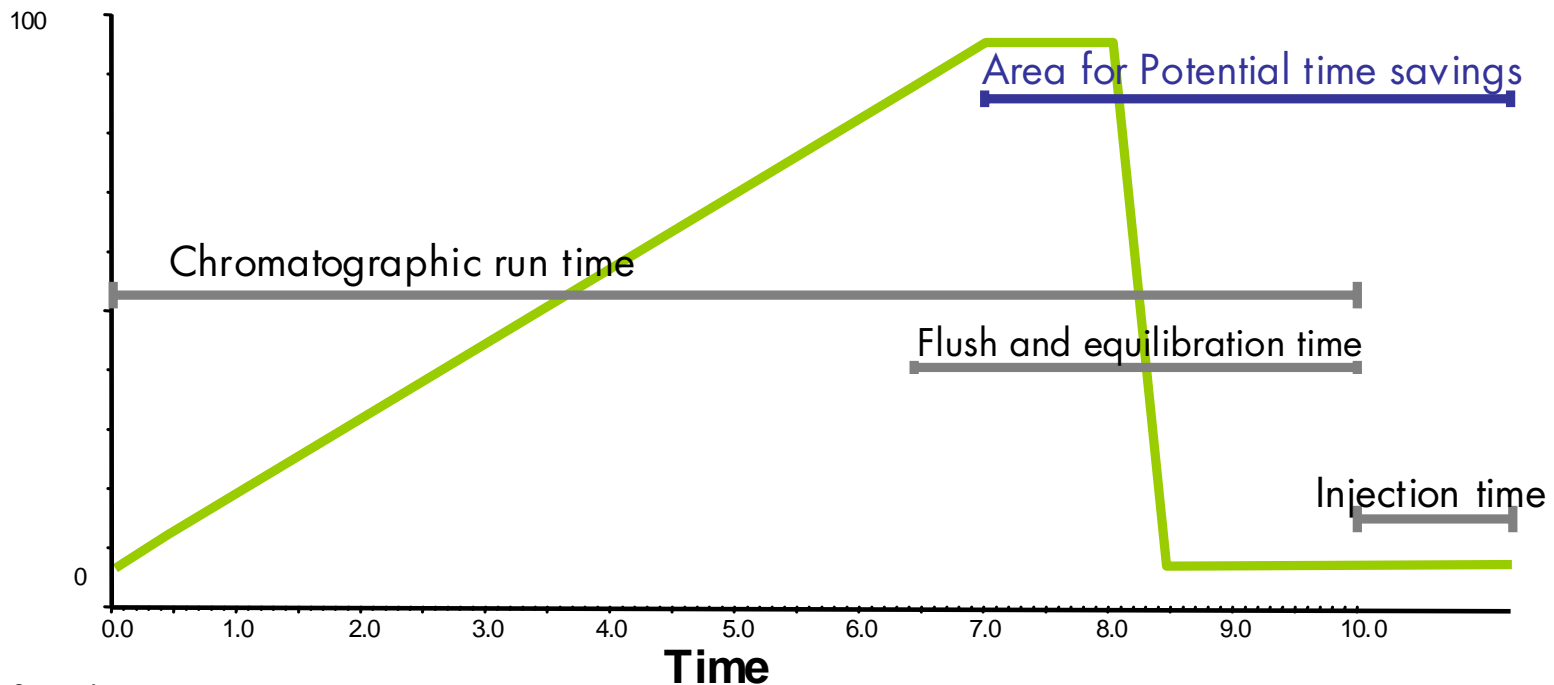
- End Run Termination - Collect the target and move on
 - Terminating acquisition after collection has occurred and moving on to the next sample has the potential for significant savings in run time, 1.75 min Vs 5 min



- Run termination alone can have a powerful effect on overall run time
- Combining the use of shallow gradients along with intelligent run termination can further increase throughput
- Run time is based on the retention time, time required to collect the fraction and inject the next sample.

Sample	Generic Run Time	Narrow Run Time
1	4.03	4.23
2	8.05	4.50
3	4.20	4.59
4	7.52	4.79
5	6.03	4.60
6	5.40	4.75
7	5.26	4.80
8	7.91	5.19
9	4.97	4.15
10	5.48	4.93
Total Run Time	58.75 min = 2.0 Fold Increase	46.53 min = 2.6 Fold Increase

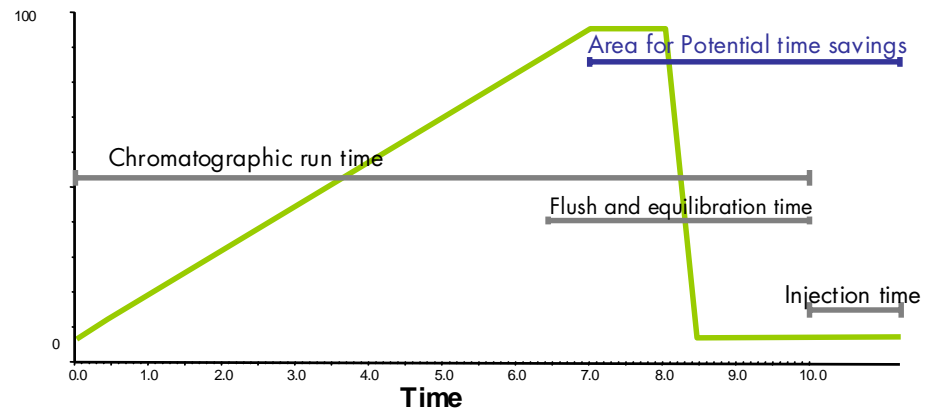
- Places for potential time savings
 - Chromatographic run
 - **Column equilibration**
 - Injection time



- A 19 X 50 mm column has a volume of approx 12ml
 - 2 column volumes are required to flush the column
 - At 25 ml/min 1min is required to flush the column
 - 5 column volumes are required to re-equilibrate the column
 - 2.5 min to equilibrate the column at 25 ml/min

- With a flow rate of 40ml/min
 - Flush time is reduced to 0.6 min
 - Equilibration time is reduced to 1.5 min

- Time savings
 - 3.5 minutes at the gradient flow
 - 2.1 minutes at the elevated flow



- Flush and re-equilibrate the first column off-line with a regeneration pump, while the next sample is running on a second column.

Method:

- Run is terminated at the end of the gradient and rinsed and equilibrated while the next sample is injected

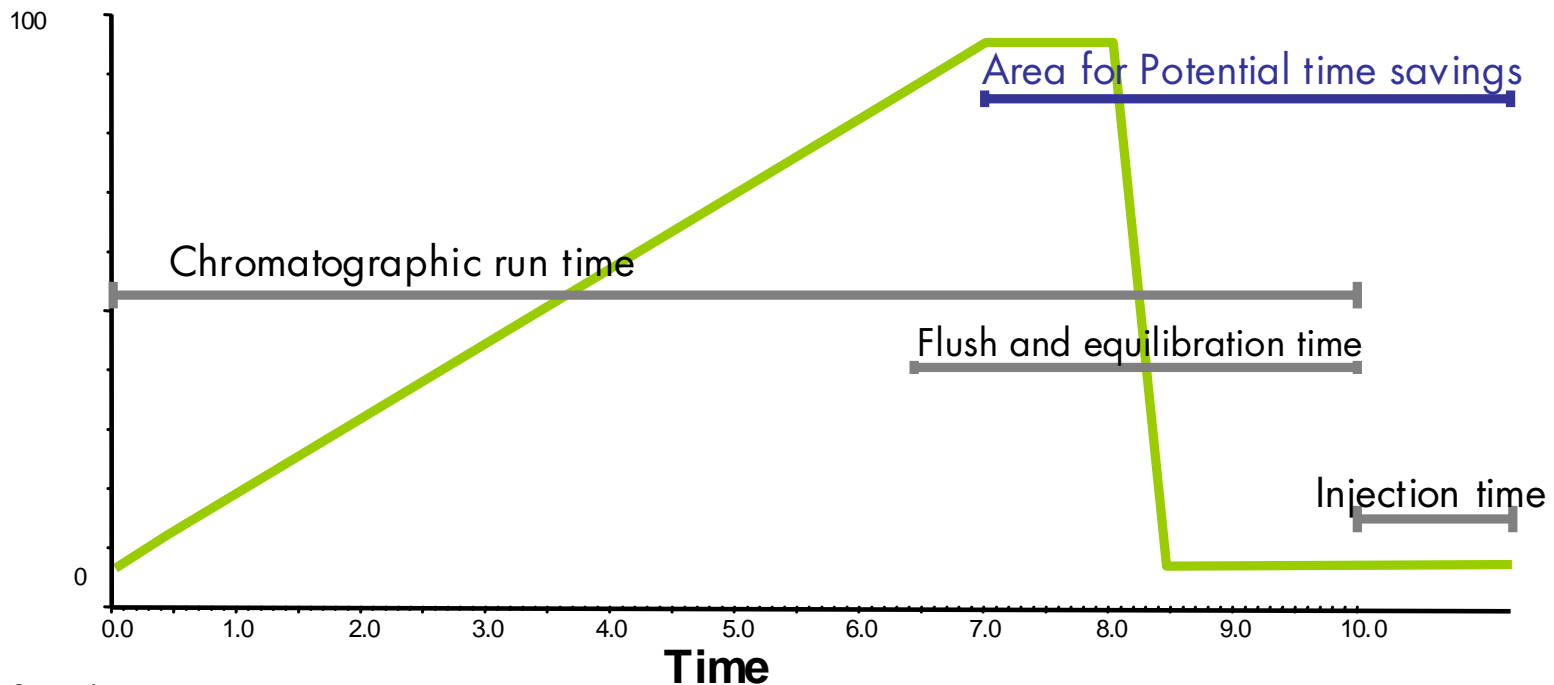
Run Time Savings:

- Generic: 3 minutes / sample = **1.2 Fold Increase**
- Narrow: 2.5 minutes / sample = **2.7 Fold Increase**

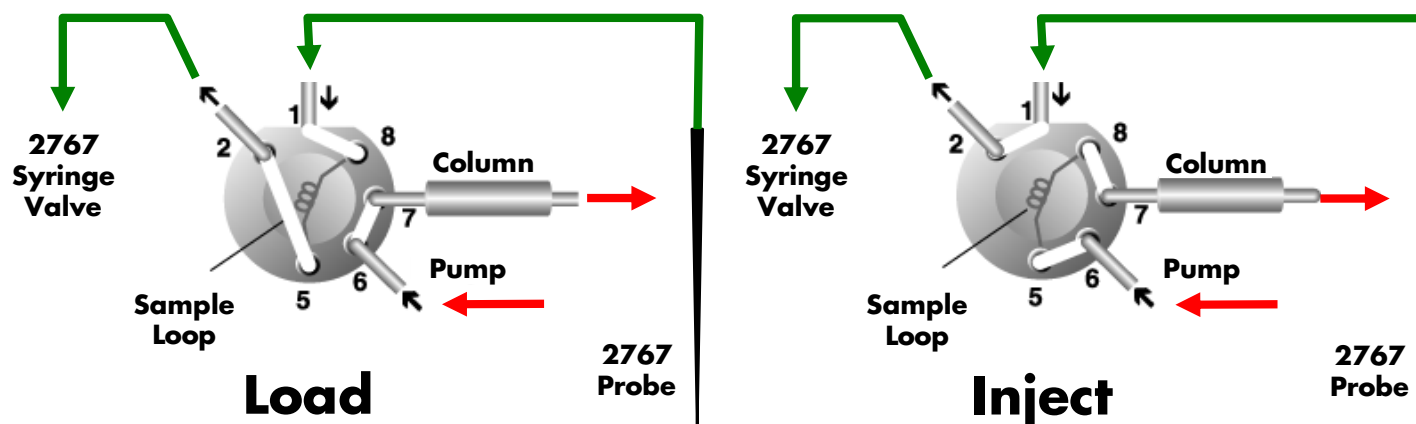
- The time savings with all of the features, shallow gradients, early run termination and column regeneration
 - Analysis time went from 120 min to 40.5 min

Sample	Generic Run Time	Generic with Regeneration	Narrow Run Time	Narrow with Regeneration
1	4.03	3.43	4.23	3.63
2	8.05	7.45	4.50	3.90
3	4.20	3.50	4.59	3.99
4	7.52	6.92	4.79	3.19
5	6.03	5.43	4.60	4.00
6	5.40	4.80	4.75	4.15
7	5.26	4.66	4.80	4.20
8	7.91	7.31	5.19	4.59
9	4.97	4.27	4.15	3.55
10	5.48	4.88	4.93	4.33
Total Run Time	58.75 min = 2.0 Fold Increase	52.75 min = 2.3 Fold Increase	46.53 min = 2.6 Fold Increase	40.53 min = 3.0 Fold Increase

- Places for potential time savings
 - Chromatographic run
 - Column equilibration
 - ***Injection time***



- By aspirating the sample directly into the sample loop, injection time is reduced
 - 1.6 minutes / sample for a 1 mL injection



- Fewer robotic steps to get sample injected
- No injection port = faster rinsing
 - Begin fraction collection sooner
 - Rinse during solvent front, rather than after collection

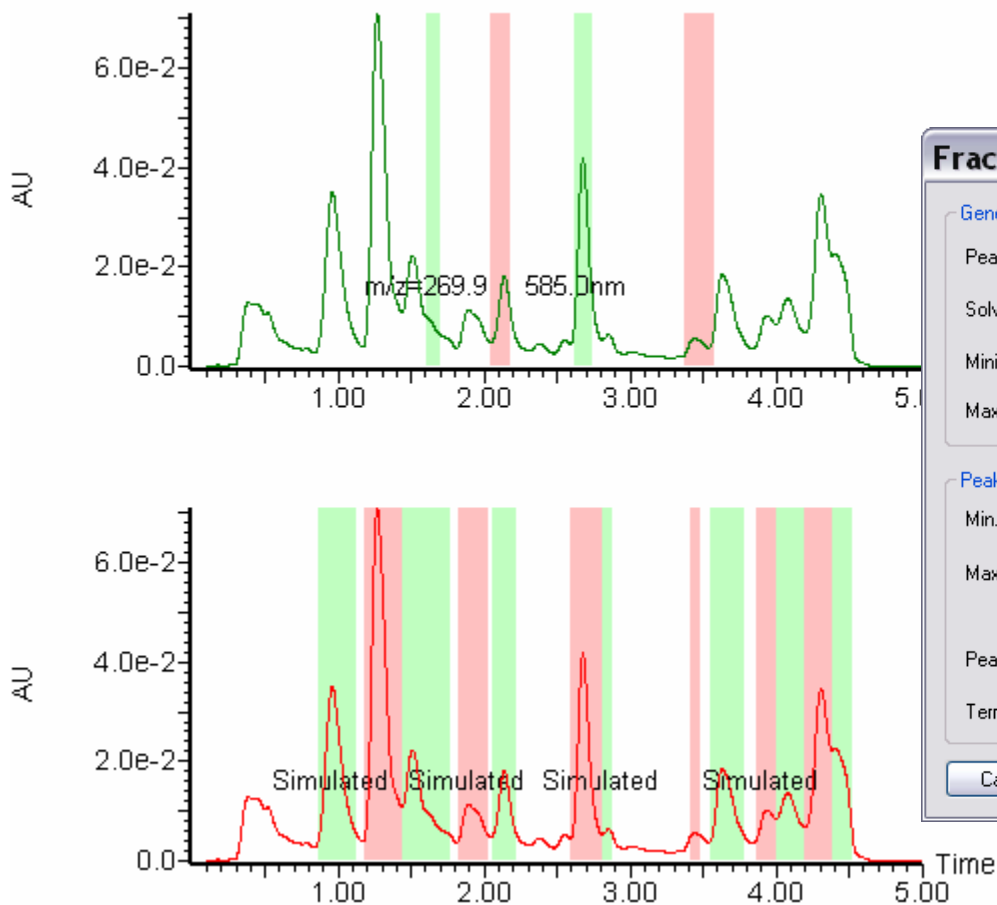
- With all of the functionality enabled, a dramatic improvement in throughput can be achieved

Tool	Original Total Run Time (min)	Direct Inject Total Run Time	Without Direct	Overall Increase using Direct Inject
Generic	120	104	—	1.2
Generic + End Run	<i>58.75</i>	<i>53.75</i>	2.0	2.2
Generic + End Run +	<i>52.75</i>	<i>36.75</i>	2.3	3.3 Fold Increase
Narrow	70	54	1.7	2.2
Narrow + End Run	<i>46.53</i>	<i>41.63</i>	2.6	2.9
Narrow + End Run + Regeneration	<i>40.53</i>	<i>24.53</i>	3.0	4.9 Fold Increase

- Collection threshold settings
 - Testing the threshold without having to do multiple injections
 - Automatic setting for each sample based on the trigger
- Automations Steps
 - Sample Login and Data processing
 - Less time with these steps, leads to improved throughput
 - Automating the steps in the process
 - No manual intervention required
 - No lag time between steps = greater throughput
- System Confidence
 - Feeling comfortable leaving the system unattended can improve throughput
 - Runs overnight and over the weekend

Testing the threshold without having to do multiple injections

- Applies the collection parameters to an acquired chromatogram
- Ideal for more focused collection
- Repeated injections



Automatic threshold setting for each sample based on the trigger

- Automatic Threshold Determination
 - Bases its determination on information obtained from the injection of a blank
 - Thresholds for any and all detectors on the system automatically determined
- No analytical injection or precious sample required
- Allows for multiple thresholds across the run
 - Compensates for changing background intensity

Automatic Sample Login

The screenshot shows the OALogin software interface. The main window is titled "OALogin" and has a "Mode Security" tab. It is divided into two sections: "Login Information" and "Sample Details".

Login Information:

- Your Name: rcleary (dropdown)
- Job ID: rcleary3 (text field)
- Method: Walk-up (dropdown)
- Description: Analytical 1 ml/min- Prep 20ml/min - Fraction Analysis. 10 min gradients 100-800Da (text field)
- Sample Holder: Waters 24 Position 4ml Vial (Single Shot - 24 wells/vials) (dropdown)
- Holder Details: 4 Rows x 6 Columns. 49.8mm depth, 6.4mm diameter. (text field)

Sample Details:

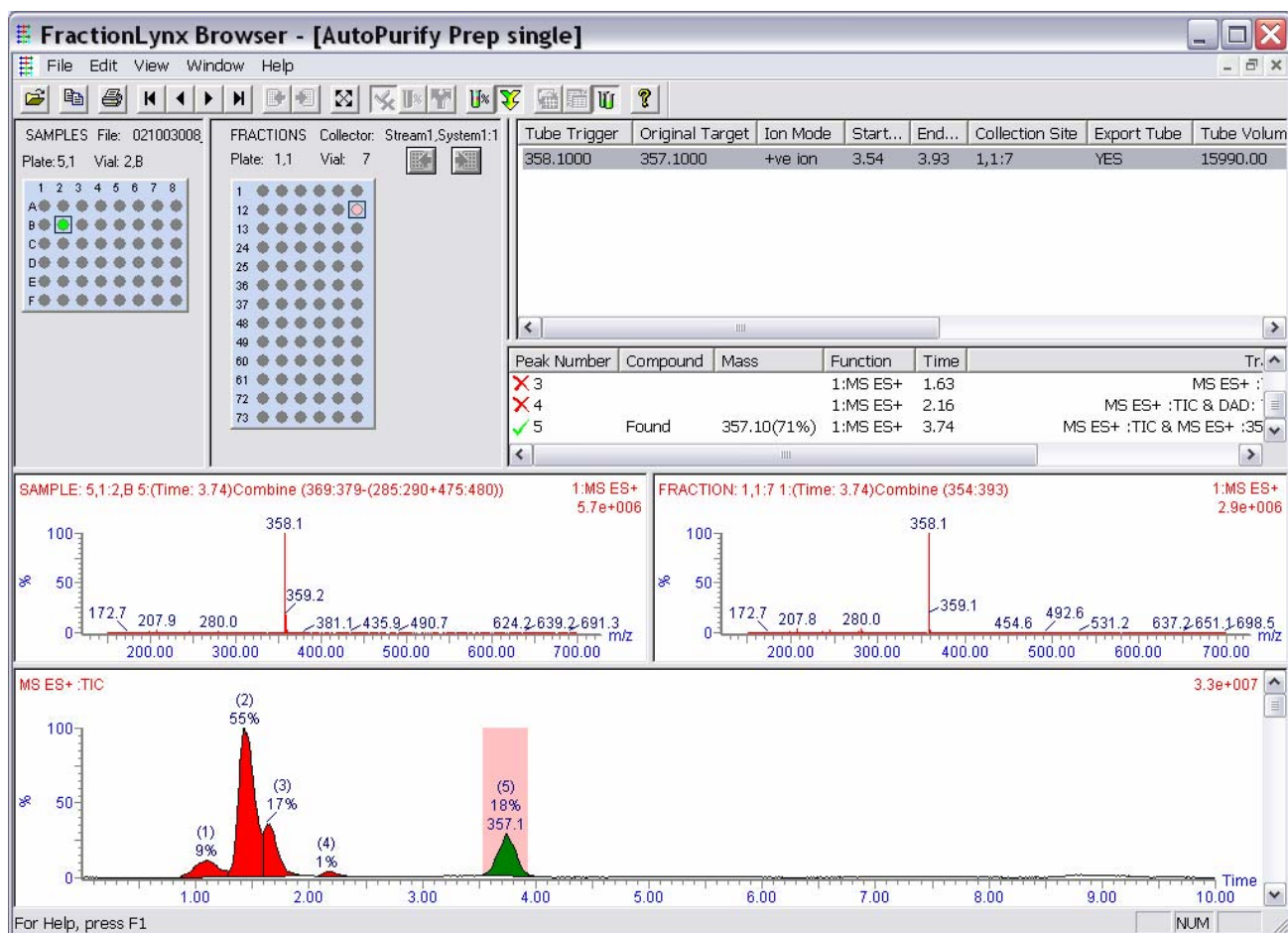
- SampleID: test sample (text field)
- Well: 3:4 (text field)
- Fraction Mass 1: 235 (text field)
- Fraction mass 2: 437 (text field)
- PDA Fraction 1: 220 (text field)
- PreInjectionVolume: 200 (text field)
- Mass to monitor: 374 (text field)

At the bottom, there is a status bar that says "Press button to login samples. There are no Jobs waiting" and a "Login" button.

Overlaid on the bottom right is a smaller dialog box titled "Place your samples(s) in the locations given below". It contains a text area with the instruction "Please put your sample(s) in the location(s) indicated." and a list box showing "'022306_01' in plate 5,3 at position 1". Below the list box is a grid representing a sample plate, with the cell at row 5, column 3 highlighted and labeled "5,3". At the bottom of the dialog box are "OK" and "Cancel" buttons.

- Step 1
 - Enter fraction information for the target compounds
- Step 2
 - Place the sample in the location indicated

Automatic Data Processing



- Step 3
 - Easy to read Browser report
 - Printed report
 - E mail
- Fraction position
- UV and/or MS spectra
- Chromatograms
- Fraction purity
- Pass/Fail result

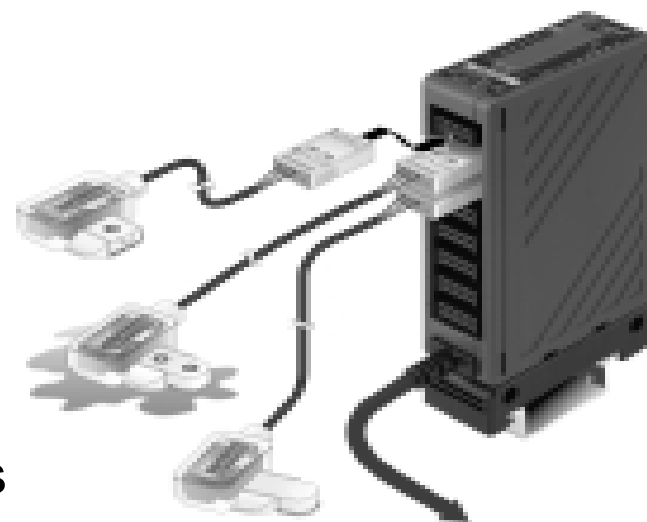
Automating the steps in the process

- AutoPurify uses the result of the analytical run to
 - determine if the sample is to be purified
 - which pre-made prep method to use
- Prep method can run automatically
- Fractions generated can be automatically analysed to assess quality

- AutoPurify is a standard element of the FractionLynx software

System Confidence

- Based on light defraction
 - Chemical resistant
 - Easy to reset
- Incorporated into main components
 - 2545, SFO, 2767
- Remainder can be placed where user wants them
- LED display in SFO for easy visualization
 - Can turn specific leads off
- Integrated into MassLynx to shutdown system



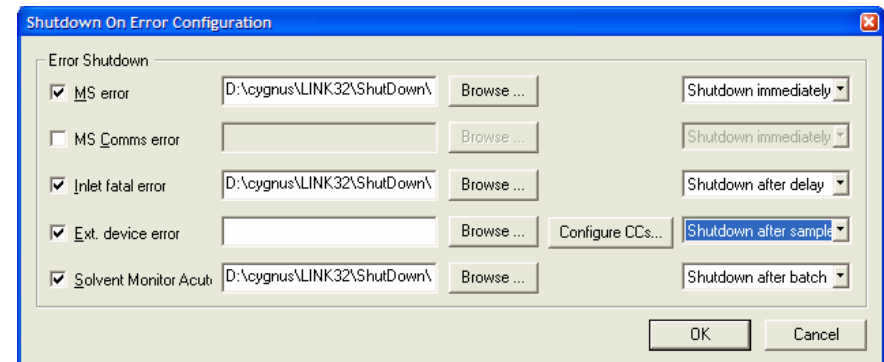
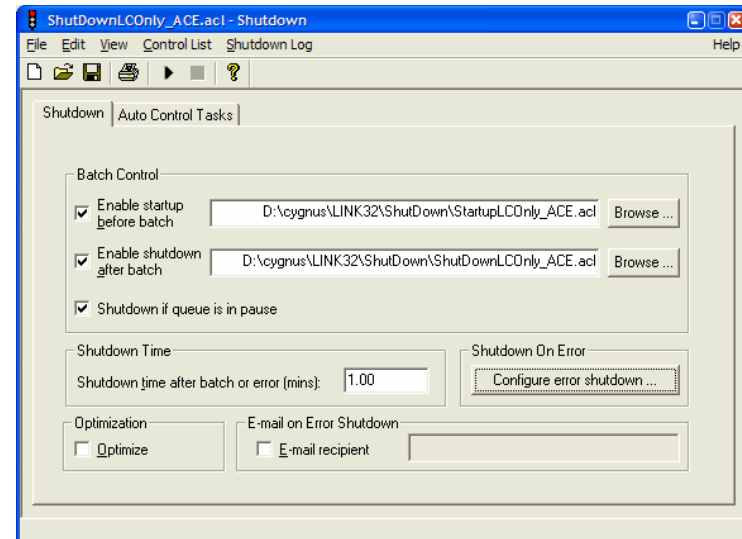
System Confidence

- MassLynx to monitor what system is doing
 - What solvents have been pumped
 - How much waste is collected
- User to define limits and actions
 - 2 set points
 - Alert for first limit
 - Action on second
- Status monitored in MassLynx



System Confidence

- User defined actions when solvent levels are met
 - Warning level
 - Shutdown after batch
 - Acute level
 - Shutdown after sample
 - Shutdown immediately



- Throughput improvements can be made in several places
 - Shallow or narrow gradients
 - Column regeneration
 - Early run termination
 - Direct inject
- Ease of use tools include
 - AutoPurify
 - OpenAccess
 - Automatic threshold determination
 - Remote status monitoring
 - Solvent and waste monitoring