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應用手冊

Evaluating the Tools for Improving Purification Throughput

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Abstract

In this application note, we examine tools available for increasing the overall throughput of a purification system.

We will use information from the analytical separation to optimize the purification, and will examine the steps required between injections to then determine the most efficient way to minimize run time.

Introduction

Chemists are constantly looking for ways to improve the overall throughput of their purification system. Time is the limiting factor for throughput, and there are two areas where time savings can be achieved: the amount of time required to perform a separation, and the amount of time between injections. Making the purification system as efficient as possible requires optimizing and minimizing both of these times. The challenge, however, is minimizing these times without impacting the purity and recovery of the fractions.

In order to correctly compare time-saving techniques, we first established a baseline separation to define a standard analysis and collection time. We purified 10 drug-like compounds with a generic 10-minute preparative gradient. This baseline analysis time was then used as the comparison time for the analysis performed when the different time-saving chromatographic functionalities were applied.

The major areas for improving throughput are:

- Decreasing the time required for the analysis
- Decreasing the time between injections

One approach for decreasing the analysis time uses shallow or narrow gradients. Approaches for decreasing the time between injections include column regeneration techniques and automatically ending the purification run after the desired target has been collected.

Experimental

Components

The Waters AutoPurification System is comprised of:

- 2545 Binary Gradient Module (BGM)
- 2767 Sample Manager

- System Fluidics Organizer (SFO)
- 2996 Photodiode Array Detector
- 3100 Mass Detector
- 515 makeup pump
- Passive flow splitter, 1:1000
- All components are controlled by MassLynx and FractionLynx software

The 10-sample library consisted of various drug-like compounds at a sample concentration of about 20 mg/mL dissolved in DMSO. The chromatographic methods used water with 0.1% formic acid as mobile phase A, and acetonitrile with 0.1% formic acid as mobile phase B. Methanol was used as the makeup solvent for the preparative analysis.

Analytical Gradient

SunFire C_{18} 4.6 x 50 mm, 5 µm, 1.5 mL/min total flow gradient and a 10-minute total run time.

Generic Preparative

SunFire C_{18} 19 x 50 mm, 5 µm, 25 mL/min total flow gradient. The same gradient table, as shown in Figure 2, was used. The only difference was the flow rate.

	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					

Figure 2. Analytical gradient table.

Narrow or Shallow Preparative Gradient

SunFire C_{18} 19 x 50 mm, 5 µm, 25 mL/min total gradient. The start and end percent B composition is variable and dependant on the sample retention time during its analytical analysis.

Time (Minutes)	Composition (%B)
0.00 to 0.5	5 to %B start
0.50 to 1.67	%B start to %B end
1.67 to 2	%B end to 95
2 to 3	95
3 to 5	End

Table 1. Narrow gradient table. See Table 2 for percent B start and end.

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

Table 2. The narrow gradients used relative to the analytical retention time.

The time window in which the analytical sample eluted defines the conditions for the prep run. For example, if the compound eluted at 4.04 min, then the purification method would ramp up the organic percentage so that is was 50% at 0.5 min.

Baseline Throughput

The generic gradient was used to perform the purification of 10 samples and the overall run time was measured. This time is used to compare the improvements.

Sample	Retention Time (min)	Run Time (min)	Time Between Injections (min)
1	1.18	10	2
2	5.20	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**	

Table 3. The overall throughput with the generic gradient. The total run time was 120 minutes.

Results and Discussion

Narrow Gradients

Narrow gradients can be used to improve preparative chromatographic resolution.1 However, if the resolution is adequate in the analytical separation, a shorter narrow or focused gradient can be used to increase throughput. The short method will focus its gradient on the same organic concentration, but in a shorter time frame.

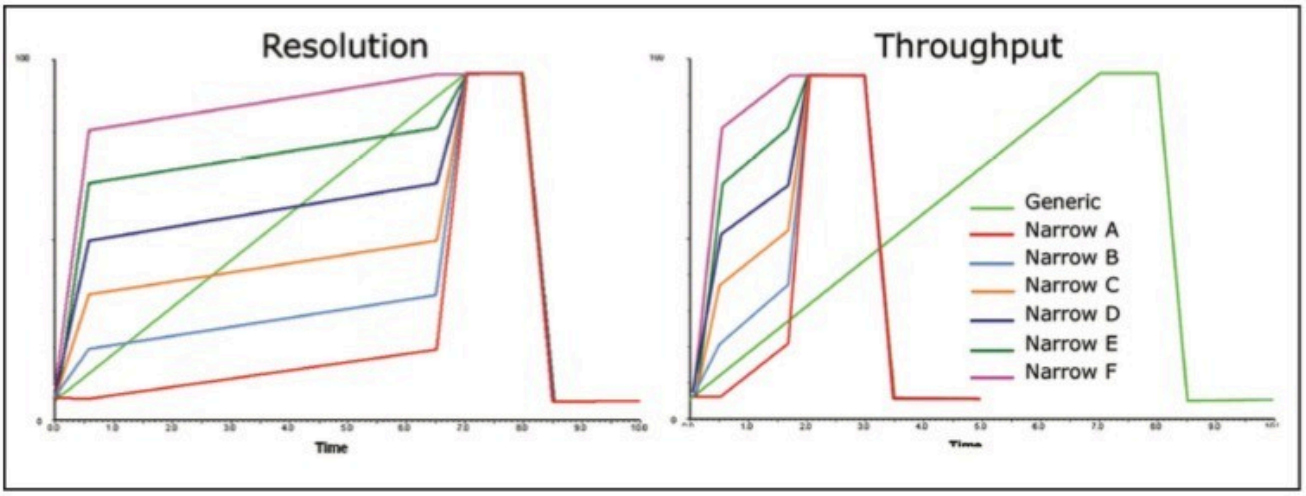

Figure 3. The different narrow gradients possible to focus on either improved resolution or throughput.

Figure 4 shows an example of one of the 10 samples being purified by both a generic and a narrow gradient. The target was successfully isolated using narrow gradient D. The results show that the resolution is maintained over the focused section of the gradient (the blue bracket). Note that there is a loss in resolution, as expected, in the non-focused areas of the gradient. This would have to be considered when the compound elutes at the very beginning or end of the focused gradient.

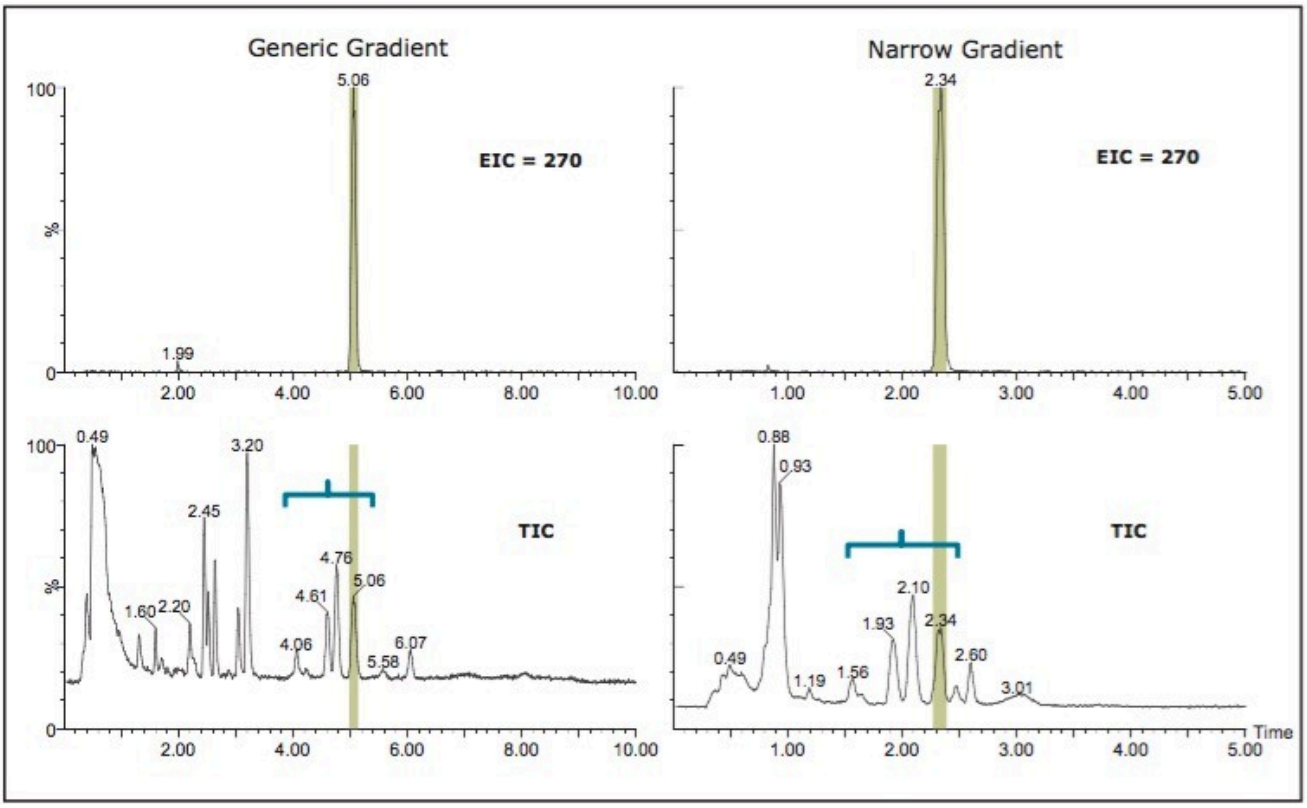

Figure 4. Comparison of the 10-minute generic and the 5-minute narrow purification. The blue bracket corresponds to the focused area of the gradient, where the resolution is maintained.

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	5.20	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 Increased Throughput**			

Table 4. The overall throughput increases by 1.7 fold when incorporating narrow gradients, compared to using a generic gradient.

Rinsing and Equilibration

It is important for high-quality chromatography that the column is rinsed and re-equilibrated with the appropriate volume of solvent, typically defined in column volumes. Insufficient rinsing can cause carryover, and equilibration time also has a significant impact on the overall throughput, with inadequate equilibration leading to retention time variability, poor chromatographic peak shape, or even sample breakthrough. The quantity of rinsing solvent is dependant upon the sample matrix, the retentiveness of the column, and the elutropic strength of the rinsing solvent. Typically, two to three column volumes is required to rinse. For equilibration, various articles report anywhere from three to 20 column volumes can be used.[2-3]

For example, a 19 x 50 mm column has a volume of about 12 mL. Two column volumes or 24 mL of 95% B were used to flush the column, and 60 mL of 5% B were used to re-equilibrate the column. With the gradient flow of 25 mL/min, the flush takes about 1 minute, and the equilibration takes about 2.5 minutes.

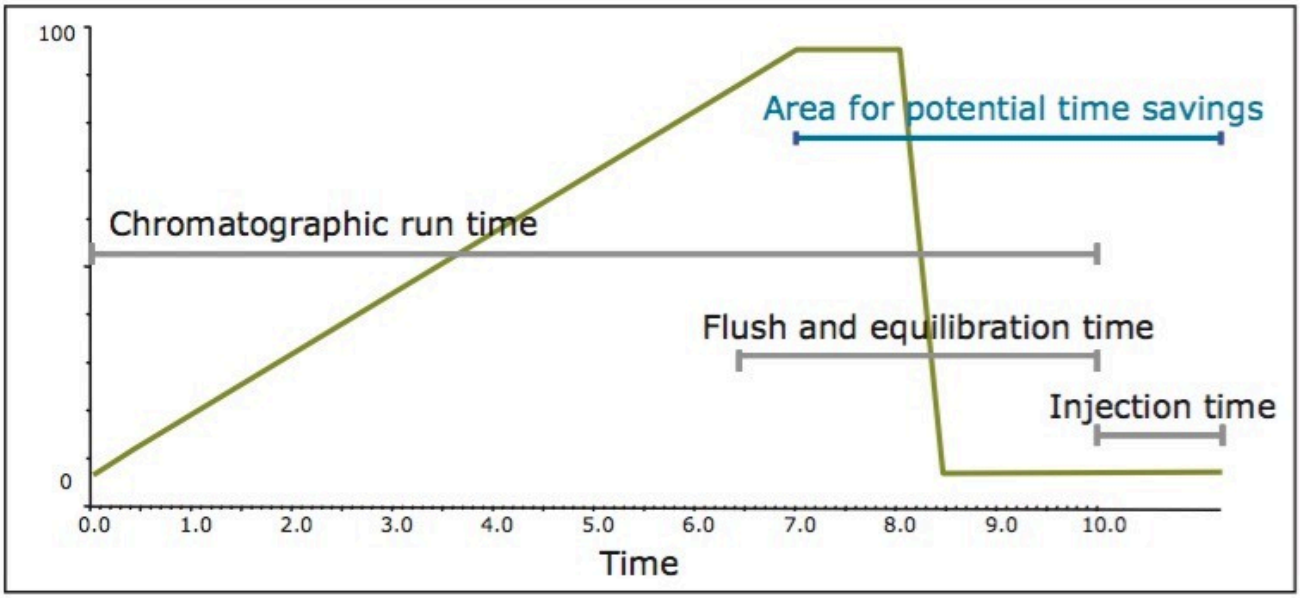

Figure 5. Illustration of an injection cycle with chromatographic analysis time, equilibration and flush time, and injection cycle for next injections time displayed. The area where time could potentially be saved is noted.

However, the flow rate can be elevated above optimal chromatographic conditions (30 mL/min for 5 µm packing), so long as the system can withstand the overall pressure increase. We found that the flow could be increased to 40 mL/min, only generating an additional 1300 psi of backpressure, reducing the flush time to 0.6 min and the re-equilibration time to 1.5 min, for a 1.5-minute savings.

Off-line Regeneration

To increase throughput, a regeneration pump can be used to flush and re-equilibrate the first column off-line, while the next sample is running on a second column.

In this method, the run is terminated at 2.5 min for the narrow gradients, or 7 min for the generic and the next injection started. The first column is switched off-line and its flush started, while the second column is put in-line to receive the next sample. As mentioned earlier, the time required for the injection to be performed is 2 min.

The run-time savings for a generic preparative saw a reduction of 3 min per sample, for a reduction in the total run time from 120 to 90 minutes, or a 1.2-fold savings.

The run-time savings for a narrow gradient was more significant. Injection-to-injection time was reduced from 12 min with the generic method to 4.5 min using narrow gradients and off-line column regeneration. This reduced the total run time from 120 to 45 minutes, a 2.7-fold savings.

Early Termination

To further reduce the time required for analysis, a software tool can be used to automatically end the run after the target has been collected. The throughput improvements of this feature will be illustrated for both generic and narrow gradients.

For either gradient approach used, once the target has finished collecting, the gradient will stop and flush with 95% B to wash the remaining material off the column. After a defined time of rinsing, the column will then be re-equilibrated with the initial gradient solvent. (Note: 2 minutes of equilibration time is performed between injections.)

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 Increased Throughput	52.75 min = 2.3 Fold Increased Throughput	46.53 min = 2.6 Fold Increased Throughput	40.53 min = 3.0 Fold Increased Throughput

Table 5. The overall throughput improvement using the run termination function can range from a two- to three-fold increase, depending on what additional tools are used. Using the regeneration pumps saves 0.6 min per injection when compared to a single column method. This corresponds to the time required to rinse the column. The re-equilibration time is incorporated into the 2 min to make an injection.

Optimized Injection Routine

Throughput can be further improved by reducing the time between injections. The injection cycle can be divided into three segments:

- Aspiration of the sample into the needle
- Dispensing the sample into the loop
- Washing the assembly

Optimizing the speed of the aspiration enables the sample to be quickly drawn into the needle and holding loop. Care must be taken to ensure the increased syringe speed does not create air bubbles in the system.

Once the sample has been drawn into the holding loop, it is dispensed at an optimized flow rate. Care must again be taken to ensure that a high-pressure condition does not occur by operating the syringe too quickly.

Two options are available for positioning the sample in the loop. The default setting is to center the sample in the loop, but the sample centering can be disabled to allow the sample to be more quickly loaded onto the front of the sample loop.

When sample centering is removed, it is possible to operate with only one wash solvent and to be able to perform this wash at the beginning of the injection sequence, decreasing the injection time.

Cumulative Time-Savings

The time required to inject and rinse was reduced from 2 min with the standard partial loop injection to 0.4 min with the new settings. Table 6 shows the throughput possible by combining optimized injection settings with the various other tools.

Tool	Original Total Run Time	Optimized Injection Total Run Time	Default Injection Routine	Overall Increase with Optimized Injection Routine
Generic	120	104	—	1.2
Generic + End Run	58.75	53.75	2.0	2.2
Generic + End Run + Regeneration	52.75	36.75	2.3	3.3-Fold Increased Throughput
Narrow	70	54	1.7	2.2
Narrow + End Run	46.53	41.63	2.6	2.9
Narrow + End Run + Regeneration	40.53	24.53	3.0	4.9-Fold Increased Throughput

Table 6. Using optimized injection routines can improve the overall throughput. The improved injection routine has a greater impact when using regeneration because the 2 min for the normal injection is used to re-equilibrate with a single column. But with regeneration, the re-equilibration is done off-line and the injection time is dead time.

Conclusion

Throughput can be increased by about five-fold using a combination of narrow gradients, early run termination, off-line column regeneration, and an optimized injection routine. This correlates to an 80 percent decrease in run time.

- Narrow gradients can be used to improve throughput, but require additional information about the target.
- Off-line column regeneration has a greater impact on throughput as the run time is reduced.
- Early run termination improves throughput and reduces the amount of consumed solvent saving both time and money.
- Optimizing the wash sequence and adjusting when it is performed will save additional time between injections.
- Various combinations of throughput-enhancing tools can be used based on the specific requirements.

References

1. P Lefebvre, A Brailsford, D Brindle, C North, R Cleary, W Potts III, B W Smith, Waters Poster Presentation, *PittCon*. 2003.
2. A P Schellinger, P W Carr, *Journal of Chromatography A*. 2006; 1109: 253-266.
3. U D Neue, *American Laboratory.* 1997; March.

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