Optimizing Performance for Fast HPLC Analysis on Short Columns

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Introduction

Obtaining HPLC results in the shortest time possible without compromising the quality of the results is an area of intense interest for chromatographers. Several new instruments have been introduced that can facilitate this desired outcome. However, one can obtain faster separations on a standard HPLC instrument by using shorter columns with narrower internal diameters. Some of the loss in efficiency that results in using shorter columns is offset by the use of narrow bore columns and by the use of smaller particle size media. However, simply switching to short, narrow bore columns packed with smaller particle size media on a standard HPLC system can result in rather disappointing results if the contributions the entire HPLC system have on performance are ignored.

"In order to fully exploit the capabilities and realize the benefits of short, narrow bore HPLC columns; it is imperative that an HPLC system be optimized to minimize system dead-volume contributions."

The effects of performing chromatographic analysis using short, narrow bore columns on an HPLC system with a standard configuration, as compared to a system that has been optimized, will be broader peaks and decreased resolution – results that compromise the quality of the HPLC analysis. This application note illustrates the importance that system optimization can have on performance.

Experimental Conditions

Instrument: Agilent 1100 with G1312A Binary pump

HPLC Instrument Configuration before Optimization:

Pump:	with on-line mixer
Detector:	G1315A DAD with standard flow cell (10 mm path, 13 µL)
Injector:	G1329A ALS with needle seat (0.17 mm ID tubing, 2.3 µL)
Tubing ID:	Pump to injector = 0.17 mm, Injector to column = 0.17 mm, Column to detector = 0.12 mm

HPLC Instrument Configuration after Optimization:

Pump:	without on-line mixer
Detector:	G1315A DAD with semi-micro flow cell (6 mm path, 5 µL)
Injector:	G1329A ALS with needle seat capillary (0.12 mm ID tubing, 1.2 µL)
Tubing ID:	Pump to injector = 0.12 mm, Injector to column = 0.12 mm, Column to detector = 0.12 mm
Column:	Luna® 3 µm C18(2), 20 x 2.0 mm (MercuryMS™ cartridge)*
Mobile Phase:	50:50 Acetonitrile/Water (isocratic)
Flow Rate:	0.2 mL/min
Injection:	2 µL Reversed Phase 2 Test Mix / Mobile Phase (1:10)
Sample:	Uracil, Acetophenone, Benzene, Toluene, Naphthalene
Detector:	UV @ 254 nm

Results

A cartridge column (MercuryMS, 20 x 2.0 mm) packed with Luna 3 µm C18(2) media was used to illustrate the importance of system optimization in order to realize the benefits offered by short, narrow bore HPLC columns.

Figure 1 shows the chromatograms obtained using this column. The top chromatogram shows the results on a standard HPLC system used for traditional analytical columns (4.6 mm ID) before optimization. The overall separation of the test mixture is good and is achieved in less than 1.5 minutes with baseline resolution of all peaks. The bottom chromatogram shows the chromatogram obtained after the HPLC system was optimized. These changes to the HPLC system included:

- Decreasing the tubing ID between the pump and injector and injector and column,
- Decreasing the tubing ID used in the injector, and
- Decreasing the volume of the UV detector flow cell from 13 µL (10 mm path) to 5 µL (6 mm path) by replacing the standard flow cell with a semi-micro flow cell.

These changes to the HPLC system result in narrower peak widths and increased resolution (see **Table 1**). The reduction in the volume between the pump and injector also results in a marked decrease in the retention for Uracil, the unretained compound in the test mixture, thus indicating that the system dead-volume has been reduced (by 0.015 mL). Furthermore, by using an injector program, the gradient delay time can be further decreased which is very useful at low flow rates (**Figure 2**).

Recommended Injector Program (from Agilent Technical Literature)

Steps	Command	Comment
1	DRAW	Draw volume of sample (injection volume) from vial
2	INJECT	Introduce sample onto column
3	WAIT 0.06 min (calculated wait time)	Flush sample loop after injection (wait time = 6 x (injection volume + 5 µL)/flow rate)
4	VALVE bypass	Direct flow from pump to column bypassing injection valve to exclude delay volume (~300 µL from auto-injector path)
5	WAIT 1.5min	The period of VALVE bypass time (Wait time = Runtime - 1min)
6	VALVE mainpass	Switch VALVE from bypass to injection position (path)

At low flow rates (≤ 0.2 mL/min), the recommended injector program allows the sample loop to be bypassed, thus reducing system delay volumes by an amount equal to the volume of the sample loop. Removing the on-line mixer from the system further reduces system delay volumes by about 400 µL.

HPLC system configuration is critical to achieving optimal performance in high-throughput HPLC analysis. As illustrated here the use of binary systems without on-line mixers and replacing connecting tubing with smaller ID allow one to more fully realize the benefits of short, narrow bore HPLC columns for fast separations.

Table 1. Peak width and resolution before and after system optimization

	Before Optimization		After Optimization	
Peak Number	Peak Width	Resolution	Peak Width	Resolution
1	0.059 min	—	0.042 min	—
2	0.066 min	1.76	0.046 min	2.60
3	0.072 min	1.96	0.057 min	2.86
4	0.085 min	2.06	0.076 min	2.73
5	0.103 min	1.89	0.099 min	2.35

Figure 1: Chromatograms obtained for a standard test mixture. Top chromatogram obtained using the standard instrument configuration before optimization. Bottom chromatogram obtained using the instrument configuration after optimization.**

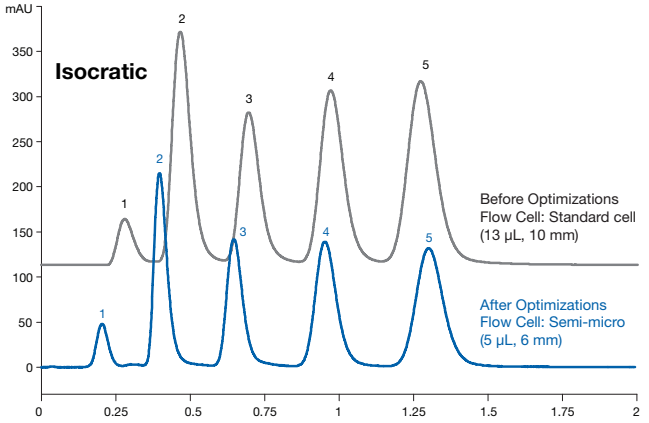
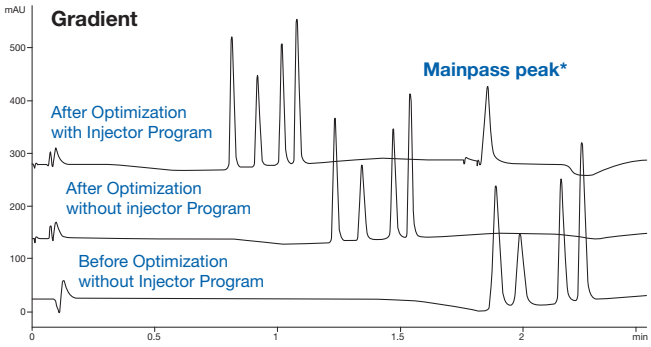


Figure 2. Effect of HPLC System Configuration**



Column: Luna 3 µm C18(2) 20 x 2.0 mm
Mobile Phase: A: 0.1 % Formic Acid in Water, B: 0.1 % Formic Acid in Acetonitrile
Gradient: 5 to 95 % B in 1.0 min; hold for 0.5 min and re-equilibrate for 1.0 min
Flow Rate: 0.6 mL/min

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* MercuryMS cartridges require a holder.
**For ordering information for HPLC Column Check Standards, please contact your Phenomenex Technical Consultant.

Ordering Information

Synergi 2.5 µm Columns (mm)

Phase	20 x 2.0	20 x 4.0	50 x 2.0	50 x 4.6
Max-RP	00M-4372-B0	00M-4372-D0	00B-4372-B0	00B-4372-E0
Hydro-RP	00M-4387-B0	00M-4387-D0	00B-4387-B0	00B-4387-E0
Polar-RP®	00M-4371-B0	00M-4371-D0	00B-4371-B0	00B-4371-E0
Fusion-RP	00M-4423-B0	00M-4423-D0	00B-4423-B0	00B-4423-E0

Synergi 2.5 µm in MercuryMS Cartridge Format (mm)

Phase	10 x 2.0	10 x 4.0	20 x 2.0	20 x 4.0
Max-RP	00N-4372-B0-CE	00N-4372-D0-CE	00M-4372-B0-CE	00M-4372-D0-CE
Hydro-RP	00N-4387-B0-CE	00N-4387-D0-CE	00M-4387-B0-CE	00M-4387-D0-CE
Polar-RP®	–	00N-4371-D0-CE	00M-4371-B0-CE	00M-4371-D0-CE
Fusion-RP	00N-4423-B0-CE	00N-4423-D0-CE	00M-4423-B0-CE	00M-4423-D0-CE

Luna® 2.5 µm C18(2)-HST Columns (mm)

Phase	50 x 2.0	50 x 3.0	100 x 2.0	100 x 3.0
Luna 2.5 µm C18(2)-HST	00B-4446-B0	00B-4446-Y0	00D-4446-B0	00D-4446-Y0

3µm Columns (mm)

Phase	20 x 2.0	20 x 4.0	30 x 2.0	50 x 4.0
Luna C18(2)	00M-4251-B0	00M-4251-D0	00A-4251-B0	00B-4251-D0
Luna Phenyl-Hexyl	00M-4256-B0	00M-4256-D0	00A-4256-B0	00B-4256-D0
Gemini C18	00M-4439-B0	00M-4439-D0	00A-4439-B0	00B-4439-D0
Gemini C6-Phenyl	00M-4443-B0	00M-4443-D0	00A-4443-B0	00B-4443-D0

3 µm in MercuryMS Cartridge Format (mm)

Phase	10 x 2.0	10 x 4.0	20 x 2.0	20 x 4.0
Luna C18(2)	00N-4251-B0-CE	00N-4251-D0-CE	00M-4251-B0-CE	00M-4251-D0-CE
Luna Phenyl-Hexyl	00N-4256-B0-CE	00N-4256-D0-CE	00M-4256-B0-CE	00M-4256-D0-CE
Gemini C18	00N-4439-B0-CE	00N-4439-D0-CE	00M-4439-B0-CE	00M-4439-D0-CE
Gemini C6-Phenyl	00N-4443-B0-CE	00N-4443-D0-CE	00M-4443-B0-CE	00M-4443-D0-CE

Standard Cartridge Holder

Part No.	Description
CH0-5846-TN	10 mm standard holder
CH0-5845-TN	20 mm standard holder

Direct-Connect Cartridge Holders

Part No.	Description
CH0-7187-TN	10 mm direct-connect holder
CH0-7188-TN	20 mm direct-connect holder

Straight PEEK Tubing

Part No.	Length	OD	ID	Color	Unit
AT0-1260-TN	5'	1/16"	0.007"/0.17 mm	yellow	ea
AT0-1259-TN	5'	1/16"	0.005"/0.12 mm	red	ea

Note: AT0-1259 recommended for system optimization

Capillary Stainless Steel Tubing

Part No.	Length	OD	ID	Unit
AT0-2996-TN	5 cm	1/16"	0.005"/0.12 mm	5/pk

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