

Determination of Reproducibility within an Analytical System via Repeat Injections

Application Note 212

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Introduction

Reproducibility within an analytical HPLC system is required in order to follow synthetic reactions, metabolic production, and mapping of tryptic digests, to name a few. Many times, on-line MS analysis is not available. In the absence of MS analysis, researchers need to be confident that their HPLC system is capable of day-to-day consistency—reproducibility that will provide them with the information they need to answer their biological and chemical questions.

The data presented in this application note shows that the Gilson HPLC system provides consistent, reproducible chromatography. A mixture of three compounds was injected repeatedly under analytical reverse-phase conditions; the results are presented below.

Materials & Methods

Chemicals and Reagents

Sample Mixture: caffeine, p-hydroxybenzoic acid ethyl ester, and biphenyl (compounds were dissolved in 60/40 acetonitrile/water at a concentration of 1 mg/mL)

Mobile Phase: solvent A: water, 0.1% TFA; solvent B: acetonitrile, 0.1% TFA

Instruments and Accessories

Gilson 215 Liquid Handler, equipped with: 175-mm Z-arm, 819 Injection Module with 7010 Rheodyne valve and 5- μ L sample loop, and beveled-tip probe (269 x 1.5 x 0.4 mm ID)

Gilson 322 HPLC Pumps, equipped with: H1 pump heads and \leq 15 mL/min. adjustable mixer set at 400 μ L

Gilson 155 UV/VIS Dual-wavelength Detector (analytical flow cell, 5.0-mm pathlength, 0.1 AUFS)

Pickering Laboratories CHX650 Column Heater

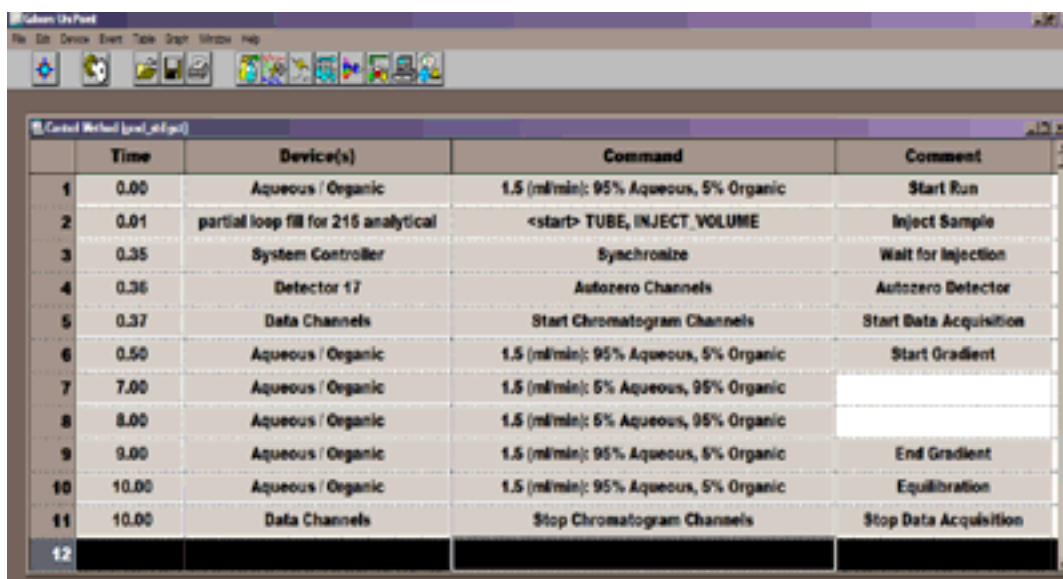
ZORBAX ODS Column (5 μ , 4.6 x 150 mm, at 40°C)

Procedure

The Gilson HPLC system was programmed to run the gradients as shown in Table 1 (total run time was 10 minutes).

0.0	1.5 mL/min.	95% A:5% B
0.5	1.5 mL/min.	95% A:5% B
7.0	1.5 mL/min.	5% A:95% B
8.0	1.5 mL/min.	5% A:95% B
9.0	1.5 mL/min.	95% A:5% B
10.0	1.5 mL/min.	95% A:5% B

Table 1: Gradient Profile



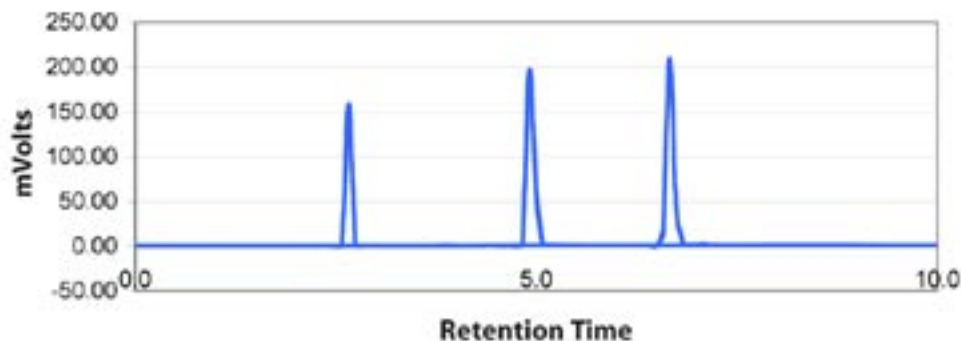
The screenshot shows the 'Control Method' window in the Gilson HPLC software. The window title is 'Control Method [method.gpc]'. The interface includes a menu bar (File, Edit, Device, Event, Table, Graph, Window, Help) and a toolbar with various icons. The main area displays a table with the following data:

	Time	Device(s)	Command	Comment
1	0.00	Aqueous / Organic	1.5 (ml/min): 95% Aqueous, 5% Organic	Start Run
2	0.01	partial loop fill for 216 analytical	<start> TUBE, INJECT_VOLUME	Inject Sample
3	0.35	System Controller	Synchronize	Wait for Injection
4	0.36	Detector 17	Autozero Channels	Autozero Detector
5	0.37	Data Channels	Start Chromatogram Channels	Start Data Acquisition
6	0.50	Aqueous / Organic	1.5 (ml/min): 95% Aqueous, 5% Organic	Start Gradient
7	7.00	Aqueous / Organic	1.5 (ml/min): 5% Aqueous, 95% Organic	
8	8.00	Aqueous / Organic	1.5 (ml/min): 5% Aqueous, 95% Organic	
9	9.00	Aqueous / Organic	1.5 (ml/min): 95% Aqueous, 5% Organic	End Gradient
10	10.00	Aqueous / Organic	1.5 (ml/min): 95% Aqueous, 5% Organic	Equilibration
11	10.00	Data Channels	Stop Chromatogram Channels	Stop Data Acquisition
12				

Figure 1: Analytical HPLC Gradient Profile

Results

The sample mixture was consecutively injected 200 times. A total-loop injection technique of introducing three times the loop volume into the injector was used (see www.rheodyne.com, tech tip #6). Over filling the injection loop (3–5 x loop size) or partial-loop injection (half or less of the loop size) minimizes variations between injections, which is directly related to fluid movement in the tubing, irrespective of the HPLC system. Dependent on the amount of sample that is available, total-loop or partial-loop injection should be incorporated in the chromatographic scheme. The raw data for each injection (retention time and mVolts) was exported to Microsoft® Excel to produce the following chromatogram:



Graph 1: Chromatogram for 200 Consecutive Injections

The chromatogram in Graph 1 represents the combined data for the series of analytical injections performed on the Gilson HPLC analytical system. The sample injected was a mixture of three components: caffeine, p-hydroxybenzoic acid ethyl ether, and biphenyl, respectively. Total-loop injection (3 x loop size) was used for injection of the sample. Chromatographic conditions are described in the *Materials & Methods* section on page 1.

The precision and reproducibility associated with the three components are presented in Table 2.

Compound	Retention Time	Area
	CV (%)	CV (%)
Caffeine	0.35	1.8
p-Hydroxybenzoic Acid Ethyl Ether	0.13	0.9
Biphenyl	0.9	0.9

Table 2: Precision and Reproducibility of Sample Mixture

Conclusion

The Gilson HPLC analytical system showed excellent precision and reproducibility for the test mixture that was repeatedly injected more than 200 times. A steep gradient and compounds that were known to elute throughout the gradient profile were chosen in order to show the capabilities of the HPLC system to equilibrate quickly between samples and consistently maintain the programmed gradient profile, and, therefore, reproducible chromatograms.

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