



A Single-Point, Dual-Gradient HPLC System for Increased Sample Throughput

Craig S. Young, M.S.; Curtis R. Campbell, Ph.D.; and Rich DeMuro;
Shimadzu Scientific Instruments

Modern high-throughput autosamplers for HPLC are capable of very fast injection cycles. The chromatography, including column conditioning, is usually the rate-limiting step. Here, a system is described that offers single point control of dual, independent binary gradients and increases system throughput by eliminating time lost to column conditioning. The two-column, two-sample rack approach ensures data integrity by injecting all samples from a specific sample set onto one specific column.

Sample throughput is critical to laboratories such as drug metabolism and pharmacokinetics (DMPK). Modern high-throughput autosamplers offer very fast injection cycles (15–40 s), but the rate-limiting step of sample throughput is usually the chromatography.

Shimadzu has developed an LC system that can increase sample throughput up to 100% using a two-column approach; one column equilibrates while another does a separation. Injections alternate

between columns, and the chromatographic methods need not be identical. A further benefit, especially for DMPK environments, is the preservation of data integrity by keeping all samples from a specific study tied to one specific chromatography column.

The SIL-HT Dual Gradient system uses single point control over four pumps operating as two independent pairs of binary high-pressure gradients. Injections are made into alternating column streams, controlled by two sequence tables. When a run sequence is started, an injection is made from the first sample of Study A onto Column A. During this run, Column B is conditioned for its run. The second injection is then made from the first sample of Study B onto Column B. Alternating injections continue until all samples have been injected. Each analysis stream may be directed at any time via remote controlled valve to various flow paths: UV detection, time slice for MS detection, etc.

Method

Sample racks A and B were populated with non-identical sample sets of parabens mixtures (methyl-, ethyl-, propyl-, butylparaben).

Analytical conditions:

Mobile Phase:	A: Water, B: Methanol
Gradient:	60–90% B over 1.25 min, hold for 0.3 min, condition at 60% for 1.3 min)
Columns:	Shimadzu Premier C18, 3 μm , 50 \times 4.6 mm
Injection Volume:	5 μL
Flow rate:	1 mL/min
Temperature:	Ambient
Detector:	UV 254 nm

Results and Conclusion

As shown in Figure 1, six runs from alternate sample sets were accomplished in 17 min — representing a sample throughput improvement of 100% over a single-column approach. All samples from Rack A were analyzed using Column A, while samples from Rack B were analyzed using Column B. The coefficient of variation for peak areas of constituents 1 and 3 from both sample sets was less than 0.7%. System carryover from an independent study was undetectable.

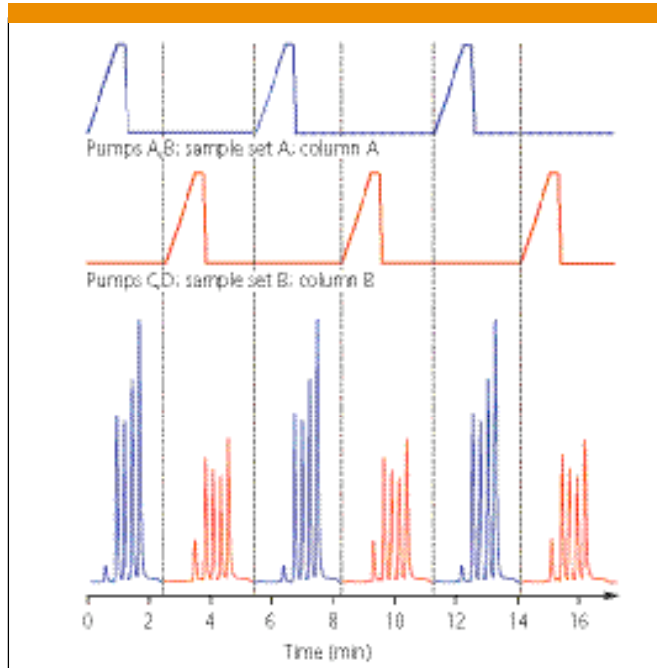


Figure 1: Continuous data taken for six alternating injections from sample sets A and B.

Shimadzu Scientific Instruments, Inc.

7102 Riverwood Drive, Columbia, MD 21046

tel. (800) 477-1227, fax (410) 381-1222

www.ssi.shimadzu.com