Dual-stream UHPLC separations on a new, compact, sample delivery system featuring simplified software automation for increased throughput in drug discovery bioanalysis

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Introduction:

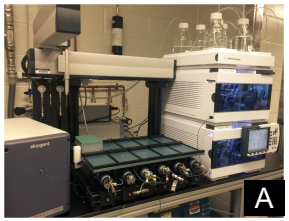
Multiplexed or multi-stream liquid chromatography systems are a well-known method for increasing bioanalytical throughput.

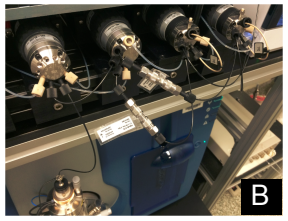
Although conceptually simple, these systems are primarily used by experts and their usage is typically confined to a few systems within the larger bioanalytical organization. The most commonly used systems still feature single stream LC.

We sought to develop a compact dual-stream LC multiplex system that could be used routinely, eliminates scheduling issues and simplifies data analysis while returning highest-quality results. We envision this system as applicable to all drug discovery bioanalysis, increasing throughput across all bioanalytical study types.

Methods:

The sample delivery system consisted of an autosampler with 10-plate capacity, 8-two position UHPLC valves (including up to 4 injection ports). The valves are arranged under the plate deck and across the front of the instrument. The system was configured with two 1290 model UHPLC pumps (Agilent). A Sciex 5500 mass spectrometer was used for targeted bioanalysis. The overall system was controlled by LeadScape software (SoundAnalytics). LeadScape handled batch creation, valve scheduling, gradient LC control, MS signal acquisition and data review).





A. LeadSampler (LS-1) configured with 2-1290 Series binary gradient UHPLC pumps (Agilent) in multiplex mode.

B. LC plumbing for dual-stream LC, injection valves/ports nos. 3&4 and 10-port scheduling valve (columns: Halo®, 2X20mm, 5µ).

C. Grounding the Turbo-V ion source to reduce system dead volume (a total of 50cm of 0.004" tubing was used, total vol. < 5µL.

LeadScape System Integration:

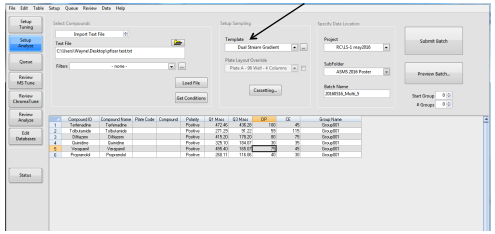


Fig. 1: LeadScape top level interface is identical to DiscoveryQuant (Version 3.0, Sciex). Autosampler and multiplex functionality is accessed via 'template' method setup

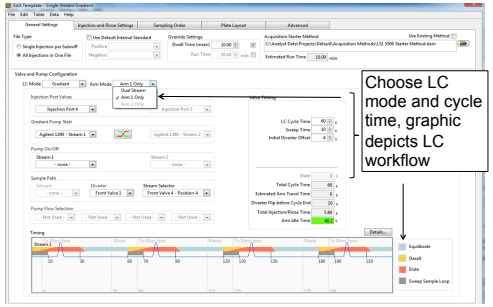


Fig. 2: Template setup panel. User chooses between single or dual stream gradient. The gradient timetable is accessed via

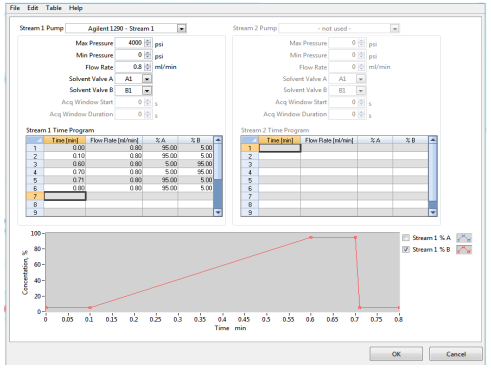
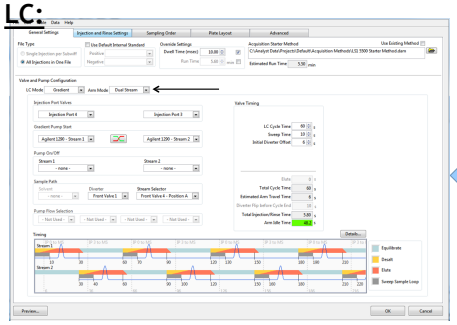


Fig. 3: single stream gradient LC timetable (Agilent 1290).

LeadScape/LS-1 Dual-Stream LC:



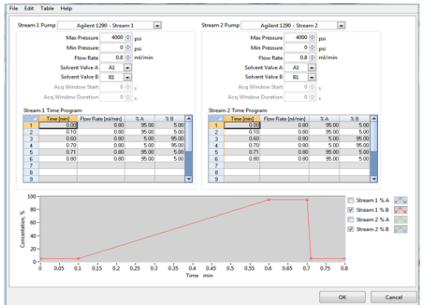
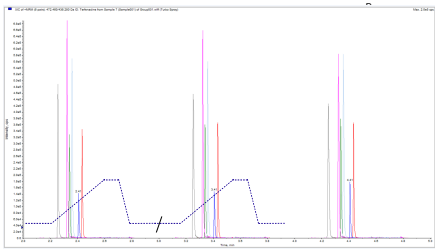


Fig. 4: Dual-stream LC. The user switches to 'Dual Stream', mode adds 2nd injection port and LC channel and sample acquisition begins. All scheduling is handled by LeadScape™.



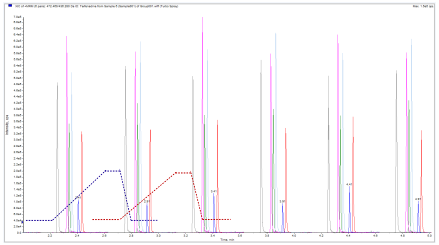
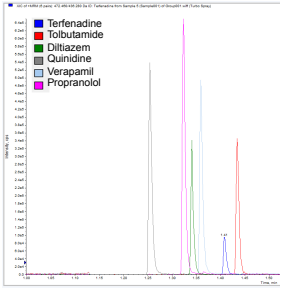


Fig. 5: Dual-stream LC. Switching from single stream (left panel) to dual stream LC (right panel) is easy. Injections are offset by one-half cycle time (30 sec in this example), Effectively doubling sample throughput.



Conclusions:

1. LeadScape™ software features the DiscoveryQuant™ workflow seamlessly integrated with a versatile sample delivery system, the LS-1.
2. LeadScape makes the switch between single and dual channel LC very easy and intuitive. These attributes should facilitate routine use of dual-channel LC methodologies.
3. The dual-stream, dual-channel setup uses two injection port valves and a single 2-position diverter valve to schedule MS sampling between streams.
4. LS-1/LeadScape doubles sample throughput by cutting single channel LC cycle time in half.
5. The LS-1 system is compact. (18X20 in., WXD) it is designed to fit on top of Sciex 5500/6500 series mass spectrometer.