

## Use of the Eksigent NanoLC-AS1 Autosampler for Rapid Sample Injections

*Integrated autosampler control in the NanoLC software enables loading at higher flow rates for large volume samples and automated valve switching for low gradient delay*

### Introduction

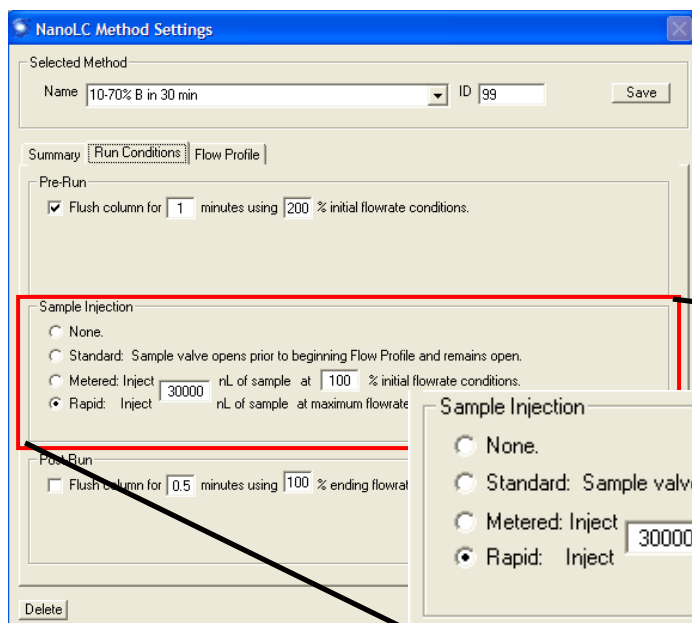
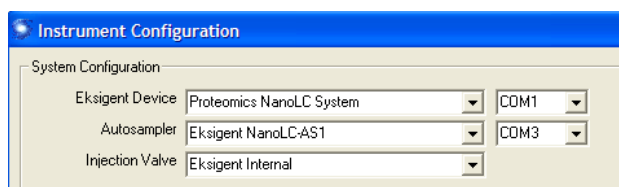
The NanoLC Proteomics system provides precise control at nanoliter per minute flow rates without the use of flow splitting, enabling reproducible, high sensitivity analyses of peptide samples by LC-MS. Although the optimal gradient flow rates for nanospray LC-MS are in the 100-200 nL/min range, the volumes of proteomics samples are often very large, in the tens of microliters, which would require long sample loading times if performed at the gradient flow rate. Use of large volume sample loops in the injection valve also results in unacceptably large gradient delay times, typically > 1 hour. The Eksigent NanoLC-AS1 autosampler includes integrated rapid and metered sample injection features, which enable large sample volumes to be loaded at higher flow rates than those used during the gradient separation. In addition, once sample injection is completed, the valve is automatically switched out of the flow path, eliminating gradient delay.

#### Benefits of the NanoLC-AS1 Autosampler

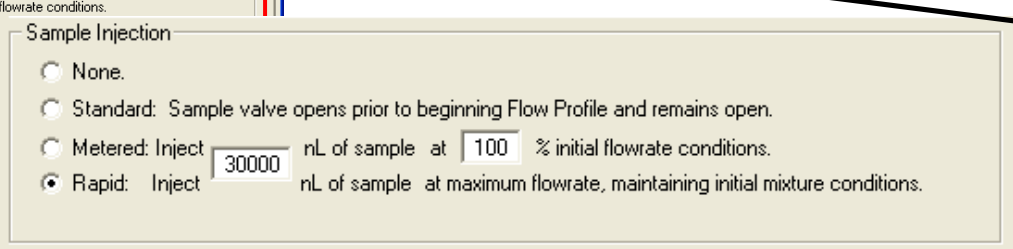
- Integrated control with the NanoLC software
- Flexible injection methods with no sample loss
- Rapid injection of large sample volumes
- Automatic switching of valve out of fluid path after injection minimizes gradient delay time

### Configuring the System for Rapid and Metered Sample Injections

In the Instrument Configuration window of the NanoLC software, the Eksigent NanoLC-AS1 autosampler and Eksigent Internal injection valve should be selected.



The type of injection (e.g.  $\mu$ L-pickup, full loop) and actual volume of sample to be injected are specified in the autosampler method (see examples below). The sample loading parameters are then specified in the Method Editor window under the Run Conditions tab. Metered injection can be used to load a specified volume at a specified percentage of the flow rate to be used during the gradient separation. Rapid injection can be used to load a specified volume at the



maximum flow rate that the system can achieve, which is typically 3-4  $\mu\text{L}/\text{min}$ , and is limited by the column backpressure. In both metered and rapid injection modes, once the specified volume has been delivered, the valve will automatically switch out of the flow path, eliminating the large gradient delay which would otherwise result from leaving the sample loop in-line. Below are outlined examples of programming metered and rapid injections using the NanoLC-AS1 autosampler.

### Example 1: Metered Injection

In this example, the gradient separation is to be performed at 200 nL/min and an injection of 3  $\mu\text{L}$  of sample at a flow rate of 1  $\mu\text{L}/\text{min}$  is desired. The sample loop on the injection valve is 10  $\mu\text{L}$ . Metered injection is selected and “10000 nL of sample” (3  $\mu\text{L}$  of sample + 7  $\mu\text{L}$  = 10  $\mu\text{L}$  loop volume) and “500% of initial flow rate conditions” (500% of 200 nL/min = 1  $\mu\text{L}/\text{min}$ ) are entered in the appropriate boxes:

Sample Injection

None.

Standard: Sample valve opens prior to beginning Flow Profile and remains open.

Metered: Inject  nL of sample at  % initial flowrate conditions.

Rapid: Inject  nL of sample at maximum flowrate, maintaining initial mixture conditions.

In the autosampler editor window, a  $\mu\text{L}$ -pickup injection method is chosen with an aspirate of 17  $\mu\text{L}$  of reagent 1 (line 4) and an aspirate of 3  $\mu\text{L}$  of sample (line 5):

Autosampler Settings

Autosampler Procedure

Name:

System Configuration: Eksigent NanoLC AS-1

1	Output	1-OFF			
2	Auxiliary		1-OFF		
3	Valve	Injector Load			
4	Aspirate	17 uL	Reagent-1	Speed: 1	Height: 5
5	Aspirate	3 uL	Sample	Speed: 1	Height: 5
6	Aspirate	5 uL	Reagent-1	Speed: 1	Height: 5
7	Output		1-ON		
8	Wait for Input		1-LOW		
9	Valve	Injector Inject			
10	Wait for Input		1-HIGH		
11	Valve	Injector Load			

### Example 2: Rapid Injection

In this example, the sample loop on the injection valve is 10  $\mu\text{L}$  and the user desires to load this amount of sample onto a trap column and then flush an additional 10  $\mu\text{L}$  of mobile phase at the initial gradient conditions. The gradient will be run at 200 nL/min. In this case, loading of the 20  $\mu\text{L}$  (10  $\mu\text{L}$  sample plus 10  $\mu\text{L}$  flush) will take 100 minutes at the 200 nL/min gradient flow rate. Furthermore, once the gradient starts there will be a 50-minute delay before the gradient reaches the column. Therefore, Rapid Inject of 20  $\mu\text{L}$  is selected in Run Conditions:

Sample Injection

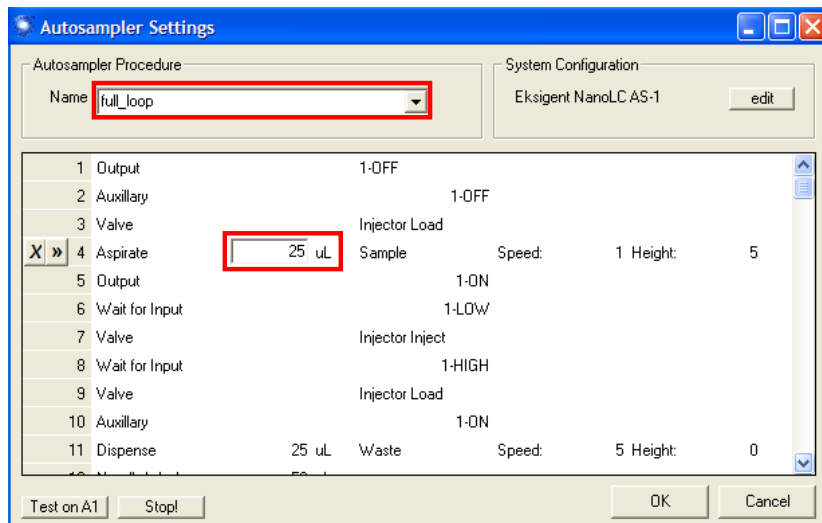
None.

Standard: Sample valve opens prior to beginning Flow Profile and remains open.

Metered: Inject  nL of sample at  % initial flowrate conditions.

Rapid: Inject  nL of sample at maximum flowrate, maintaining initial mixture conditions.

In the autosampler editor window, a full loop injection method is chosen with an aspirated volume of sample of 25  $\mu\text{L}$ .  $25 \mu\text{L} = 2 \times (10 \mu\text{L} \text{ sample loop volume} + 2.4 \mu\text{L} \text{ autosampler needle volume})$ :



This will load the trap column at the highest flow rate that the system can achieve at the starting composition; normally 3-4  $\mu\text{L}/\text{min}$ . Once the injection is complete, the valve will switch to load, removing the 10  $\mu\text{L}$  loop from the gradient flow path. The total injection will take approximately 5 minutes.

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