Pre-column Models and Configurations

Part Number	Inlet flow rate (µL/min.)	Split flow range (µL/min.)
650-250-010	250	0.10 to 0.50
650-250-050	250	0.50 to 2.50
650-250-100	250	1.0 to 5.0
650-250-500	250	5.0 to 25.0
650-250-0CS	250	Custom
650-0CS-0CS	Custom	Custom

Note: Please refer to the chart below for optimum flow rates for various capillary HPLC columns.

Column ID	Column Vol.	Peak Volume, k = 1	Typical Injection Volume*	Typical Injection Volume Range	Flow Rate for equivalent v**
4.6 mm	1,500 µL	148 µL	20 µL	5 - 50 µL	1.0 µL/min.
3.0 mm	640 µL	44 µL	10 µL	3 - 30 µL	0.42 µL/min.
2.1 mm	320 µL	22 µL	2 µL	0.5 - 15 µL	0.21 µL/min.
1.0 mm	70 µL	4 µL	0.5 µL	0.1 - 3 µL	47 µL/min.
0.5 mm	15 µL	1 µL	150 nL	40 - 500 nL	12 µL/min.
0.3 mm	6 µL	0.3 µL	50 nL	15 - 250 nL	4.2 µL/min.
0.1 mm	700 nL	32 nL	10 nL	1 - 10 nL	472 nL/min.
0.075 mm	400 nL	18 nL	2 nL	0.5 - 5 nL	266 nL/min.

Chart 2 Column diameter vs. flow rate and injection volumne
Column length = 150 mm, N = 13,000
* Typical injection volume = 10 - 30% of peak volume of first elution peak
** Maintain equivalent mobile phase linear velocity when scaling down in column diameter

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Automated Flow Splitter AS650

AS650 Flow Splitter Features & Benefits

- **Cost effective**
  Converts any analytical HPLC system to nano or capillary flow rates without the investment in a dedicated system
- **Modular design**
  Easy to install, operate & remove
- **Dynamic feedback flow control**
  Delivers constant split flow rate regardless of mobile phase viscosity and HPLC column back pressure
- **Reproducible results**
  Robust and simple design of key components insures reproducible run to run results
- **Fast equilibration times**
  Since equilibration time is determined by the HPLC system flow, equilibration is faster and less prone to thermal errors run to run results
- **Keypad entry of split flow parameters**
  Push button control eliminates manual hardware adjustments and flow rate measurements
- **Windows PC program monitors pressure and flow rate and provides real time diagnostics of system performance**
- **Configurable for a wide range of flow rates**
  Available in a wide variety of models for pre-column application

Product Specifications

Split flow range:	100 nL/min. to 50 µL/min.
Input flow range:	200 µL/min. to 500 µL/min.
Internal volume low flow:	Est. 0.5 µL, depends on configuration
Maximum pressure:	5,000 psi
Split flow accuracy:	Max flow deviation +/- 3% or 10 nL/min. whichever is greater
Outputs:	RS232
Power:	85 - 264 VAC; 47 - 63 Hz.; 60 Watts
Display:	4 line, 20 character LED
Dimensions:	6.5” W x 7.8” H x 13.6” D
Weight:	15Lbs.

Description

The *QuickSplit AS*650 Automated Flow Splitter finally takes the guess work out of flow splitting. Push button control eliminates the need for tedious adjustments and flow measurements for flow rates as low as 100 nL/min. The *AS*650 is compatible with all HPLC systems and connects with standard chromatography fittings. The modular design of the *AS*650 allows the unit to be used in a plug and play fashion. The autosplitter connects to any HPLC system between the pump and autoinjector. Once connected, the *AS*650 will deliver programmed split flows ranging from 100 nL/min. to 250 µL/min. If your applications calls for a return to analytical flow rates, simply disconnect the unit and return the HPLC system to normal operation.

The *AS*650 is the first fully automated modular flow splitter for HPLC and LC/MS. The *AS*650 is a convenient and cost effective way to convert any HPLC System to deliver nanoliter and microliter flow rates. Inlet and outlet flow rates are digitally set on the user interface, an alpha numeric keypad, and a 4 line LED displays inputs and status. Windows software provides real time pressure and flow rate outputs displayed on your PC. Because the *AS*650 dynamically compensates for any pressure changes which occur down stream from the device, it is possible to use the instrument in a pre-column split mode and maintain a constant split ratio and flow rate during gradient HPLC. Split ratios are stable and not effected by pressure fluctuations generated by mobile phase viscosity changes during gradient HPLC. This feature makes the *AS*650 ideal for separating proteins, peptides, and small molecules by capillary LC/MS. Since equilibration time is determined by the HPLC system flow, automated split control also minimizes the long and tedious equilibration times associated with dedicated nano/capillary HPLC systems. This feature also makes the *AS*650 much less prone to flow rate errors associated with temperature fluctuations. The design of the *AS*650 is optimized to minimize delay volume and gradient distortion while maintaining a flexible layout of key components to accommodate a wide range of flow rate configurations. Although the instrument is designed primarily for pre-column applications to eliminate split ratio changes caused by the HPLC column.

Theory of Operation

Incoming flow from the HPLC system is split by a static splitter S into low flow (LF) and high flow (HF) channels (Figure 1). Pressure drop across both channels is measured by low volume transducers P0, P1, and P2. The ratio of the differential pressures P0-P1 and P0-P2 are measured and maintained by automatic feedback control of variable resistor R3. The low volume of the splitter and resistors allows an accurate means to calculate and deliver a split flow rate which is independent of solvent viscosity.

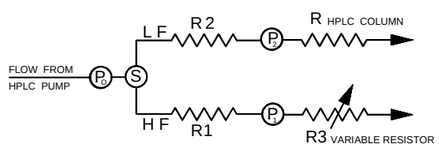
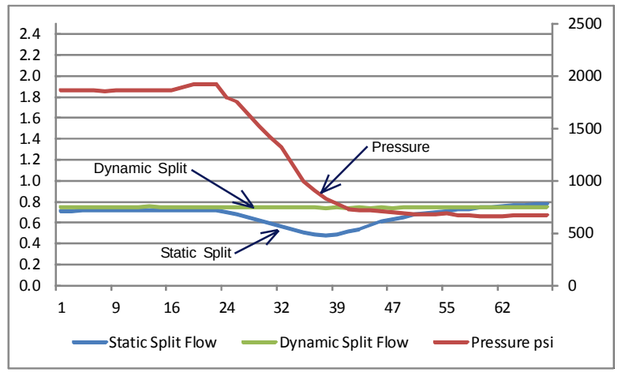


Figure 1 Schematic diagram of Model AS650

ASI Performance Data

The data below demonstrates the unique dynamic flow rate compensation underlying the performance of the *AS*650 Autosplitter. The chart includes three traces. The pressure trace depicts a pressure gradient which is typical of capillary chromatography. The shape of the pressure gradient is determined by the mobile phase, flow rate, and gradient profile. The static split trace represents the split flow profile associated with a static flow splitter. The final trace represents the split flow profile generated by the *AS*650. By comparing the split flow profiles of static and dynamic flow splitters you can see that the dynamic split flow remains constant throughout the gradient run while the static split flow changes during the run. Like the gradient profile itself, the magnitude of the static split flow deviation will be determined by several factors including, but not limited to, HPLC column geometry, mobile phase, gradient profile, and gradient time.

Chart 1 Comparison of dynamic and static splitter flow profiles



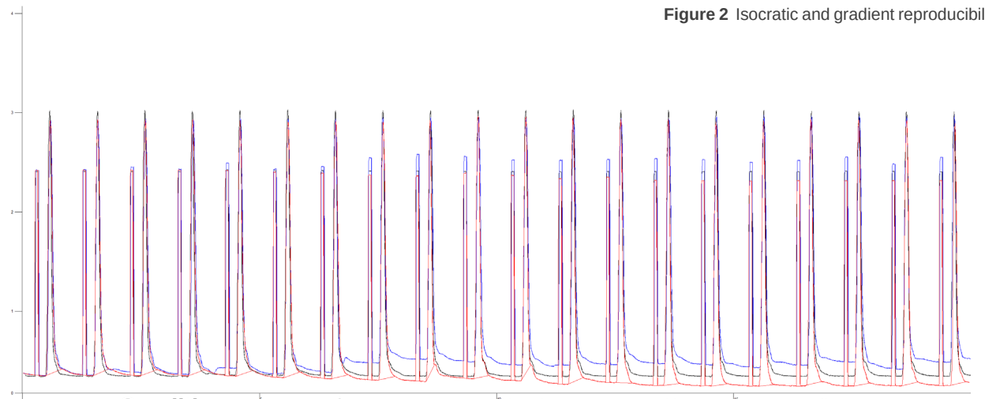
Conditions:

Gradient:	ACN 5% to 95% in 25 min., 10 min. hold
Column:	0.150 mm x 100 mm, C-18, 3 µm
Static splitter:	Tee type splitter with fused silica resistors
Dynamic splitter:	ASI AS650 Automated Flow Splitter

Dynamic Flow Control and Measurement

The key question for any automated splitting device is, of course, how accurately does it control the split flow under gradient conditions, and secondarily, how reproducible is it. To measure these parameters, *ASI* has developed a chromatographic model to dynamically measure flow rate. The measurement is based on injecting a nonretained UV absorbing peak at a specified dwell or cycle time marker. Any change in time between the marker and peak is directly proportional to changes in flow rate. To demonstrate accuracy and reproducibility we have overlayed 3 chromatographic runs described below in Figure 2. Under constant flow conditions, retention times for isocratic and gradient runs should be nearly identical and that is exactly what is seen. To emphasize robustness and challenge the *AS*650 we have purposely chosen challenging isocratic (5% and 95% 2-prop) and gradient (5 to 95% B in 10min.) conditions.

Figure 2 Isocratic and gradient reproducibility



Conditions:

Instrument:	Shimadzu LC 10ADvp with ASI *AS*650 Autosplitter
Solvents:	A = H^2O, B = 50/50 H^2O/2-propanol
Column:	0.30 mm x 50 mm, C-18, 3 µm, HAI
Inlet flow:	0.250 mL/min.
Split flow:	8.0 µL/min.
Injection:	Approx. 1 µL neat acetone, 1 min. dwell time
Detection:	UV, oncolumn
Runs (3):	5% isocratic, 95% isocratic, gradient 5 - 95% B in 10 min., 5 min. hold