

# [ ACQUITY UPLC SYSTEM CONSIDERATIONS FOR SIZE-EXCLUSION CHROMATOGRAPHY (SEC) ]

SYSTEM TYPE	ACQUITY UPLC System	ACQUITY UPLC H-Class System	ACQUITY UPLC H-Class Bio System	ACQUITY UPLC I-Class System
<b>System Overview</b>	Uses a Binary Solvent Manager (BSM) coupled to an autosampling sample manager.	Flexibility of Quaternary Solvent Blending and Auto-Blend Plus™ Technology with direct-inject autosampling designed for routine sample analysis using non-salt containing eluents.	Flexibility of Quaternary Solvent Blending and Auto-Blend Plus Technology with direct-inject autosampling designed for the separation of biomolecules. The system's flowpath is engineered with non-stainless steel, iron-free materials to maximize system robustness.	Comprised of a Binary Solvent Manager (BSM) and a sample manager with fixed-loop or flow-through-needle options. This system has the lowest system dispersion specifications and the lowest sample carryover for demanding LC-MS applications.
<b>Solvent Delivery Considerations for SEC</b>	The BSM includes solvent degassing, switching, blending, and delivery elements. For SEC applications, Waters recommends a 10x increase in seal wash.	The Quaternary Solvent Manager (QSM) is a high pressure pump that can simulatenously pump four degassed solvents (A, B, C, and D). For SEC applications, Waters recommends a 10x increase in seal wash.	Constructed of bio-inert materials, the bio-quaternary solvent manager is a high pressure pump designed to accommodate the aqueous, high-ionic-strength solvents used in biopharmaceutical applications. No changes are needed for SEC with NaCl.  <a href="#">(Reference technical brief: "Size Exclusion Using ACQUITY UPLC H-Class Bio System")</a>	The BSM is a high pressure pump that can simultaneously pump and proportion two degassed solvents (A1 or A2 with B1 or B2). The BSM utilizes two serial flow solvent delivery mechanisms operating in parallel to enable automatic linear and non-linear gradient profile implementation. For SEC applications, Waters recommends a 10x increase in seal wash.
<b>Sample Management Considerations for SEC</b>	The Sample Manager includes sample transport mechanics, sample temperature control, and sample injection fluidics. Sample injection volumes of 0.1-250 µL, in either a fixed-loop or partial-loop mode. For more viscous samples, reduce syringe draw rates.  Waters® <u>does not</u> recommend use of non-volatile buffers over 500 mM in needle wash solvents.  <b>For SEC applications, Waters recommends 90% aqueous/10% organic mixtures for seal washes.</b>	The Sample Manager-FTN uses a flow-through-needle design to aspirate samples. Follow Waters service guidelines for removing salt deposits and for routine maintenance of the sample syringe for SEC applications. For more viscous samples, reduce syringe draw rates.  Waters <u>does not</u> recommend use of non-volatile buffers over 500 mM in needle wash solvents.  <b>For SEC applications, Waters recommends 90% aqueous/10% organic mixtures for seal washes.</b>	The bioSM-FTN uses a flow-through needle design to aspirate samples. The biocompatible needle, made of MP35N material, serves as part of the injection flow path when the sample is pushed onto the column.  Waters <u>does not</u> recommend use of non-volatile buffers over 500 mM in needle wash solvents.  <b>For SEC applications, Waters recommends 90% aqueous/10% organic mixtures for seal washes.</b>	The Sample Manager-FTN uses a flow-through needle design to aspirate samples and hold it in the sample needle in preparation for injecting the sample onto the column. Sample injection volumes of 0.1-10 µL are supported by the standard configuration. Sample temperature is regulated over a range of 4 to 40 °C using peltier technology. For more viscous samples, reduce syringe draw rates.  Waters <u>does not</u> recommend use of non-volatile buffers over 500 mM in needle wash solvents.  <b>For SEC applications, Waters recommends 90% aqueous/10% organic mixtures for seal washes.</b>
<b>Column Heaters for SEC</b>	Accommodates one column, 20-150 mm length x 1.0-4.6 mm internal diameter (I.D.).  <b>30-CHC:</b> Accommodates multiple columns, serially connected columns supported (up to six); 20-300 mm length x 2.1-7.8 mm I.D., plus either a guard column or an in-line filter (20 mm maximum).	<b>CH-A:</b> Single column, up to 4.6 mm internal diameter (I.D.), up to 150 mm in length with filter or guard column.  <b>CH-30A:</b> Single column, up to 4.6 mm I.D., up to 300 mm length with filter or guard column.  <b>30-CHC:</b> Accommodates multiple columns, serially connected columns supported (up to six); 20-300 mm length x 2.1-7.8 mm I.D., plus either a guard column or an in-line filter (20 mm maximum).	<b>CH-A:</b> Single column, up to 4.6 mm internal diameter (I.D.), up to 150 mm in length with filter or guard column.  <b>CH-30A:</b> Single column, up to 4.6 mm I.D., up to 300 mm length with filter or guard column.  <b>30-CHC:</b> Accommodates multiple columns, serially connected columns supported (up to six); 20-300 mm length x 2.1 7.8 mm I.D., plus either a a guard column or an in-line filter (20 mm maximum).	<b>CH-A:</b> Single column, up to 4.6 mm internal diameter (I.D.), up to 150 mm length with filter or guard column.
<b>UV/Vis Detector and Flow Cell Considerations for SEC</b>	<b>ACQUITY UPLC TUV Detector:</b> 500 nL (analytical flow cell volume); Waters recommends use of titanium (1.5 µL, 5 mm pathlength) or stainless steel optical flow cells (1.5 µL, 5 mm pathlength) when performing SEC applications under aqueous conditions. <u>Other flow cell material (i.e., Teflon®) may cause peak tailing.</u>  <b>ACQUITY Refractive Index Detector:</b> 1.3 µL (flow cell volume).  <b>ACQUITY PDA Detector:</b> Waters recommends TaperSlit™ for SEC applications.	<b>ACQUITY UPLC TUV Detector:</b> 500 nL (analytical flow cell volume); Waters recommends use of titanium (1.5 µL, 5 mm pathlength) or stainless steel optical flow cells (1.5 µL, 5 mm pathlength) when performing SEC applications under aqueous conditions. <u>Other flow cell material (i.e., Teflon) may cause peak tailing.</u>  <b>ACQUITY Refractive Index Detector:</b> 1.3 µL (flow cell volume).  <b>ACQUITY PDA Detector:</b> Waters recommends TaperSlit for SEC applications.	<b>ACQUITY UPLC TUV Detector:</b> 500 nL (analytical flow cell volume); Waters recommends use of titanium (1.5 µL, 5 mm pathlength) or stainless steel optical flow cells (1.5 µL, 5 mm pathlength) when performing SEC applications under aqueous conditions. <u>Other flow cell material (i.e., Teflon) may cause peak tailing.</u>  <b>ACQUITY Refractive Index Detector:</b> 1.3 µL (flow cell volume).  <b>ACQUITY PDA Detector:</b> Waters recommends TaperSlit for SEC applications.	<b>ACQUITY UPLC TUV Detector:</b> 500 nL (analytical flow cell volume); Waters recommends use of titanium (1.5 µL, 5 mm pathlength) or stainless steel optical flow cells (1.5 µL, 5 mm pathlength) when performing SEC applications under aqueous conditions. <u>Other flow cell material (i.e., Teflon) may cause peak tailing.</u>  <b>ACQUITY Refractive Index Detector:</b> 1.3 µL (flow cell volume).  <b>ACQUITY PDA Detector:</b> Waters recommends TaperSlit for SEC applications.
<b>Typical UPLC System Dispersion Volume: No Column Heater</b>	10-15 µL	10-15 µL	10-15 µL	10-15 µL