

# Solutions Guide

MX Series II  
Modular Automation for Nano and  
Analytical Scale HPLC  
And Low Pressure Fluid  
Switching Applications

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03/09/2017 2321498B

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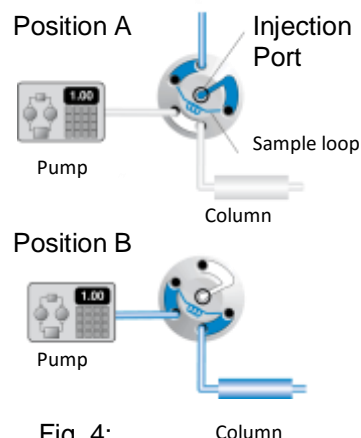
# Sample Injection

The sample and the sample's injection into the system are the most critical factors in any analytical process. Therefore, the quality, reproducibility, and flexibility of the sample injection valve are important.

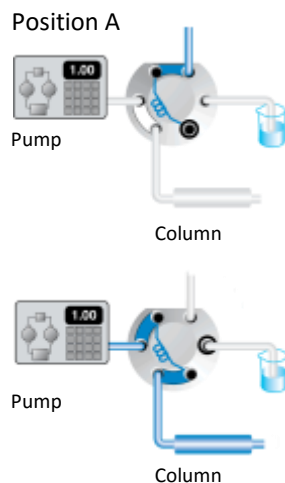
Sample injectors commonly have two positions, (1) LOAD and (2) INJECT. The LOAD position allows sample loading into the sample loop. The INJECT position flushes the sample from the sample loop onto a column.

Sample injectors are situated after the pump and before the column. It is common practice to have a filter before the injector to minimize any pump seal debris from entering the injector. A filter between the injector and the column will trap particles that otherwise may block the column frit.

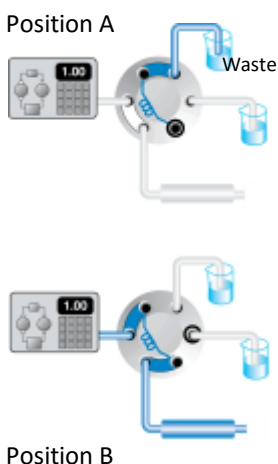
**Fig. 1:**  
Syringe Loading Injector



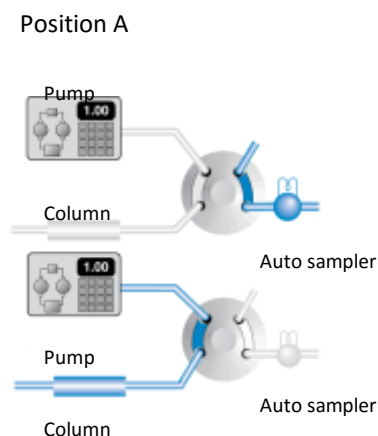
**Fig. 2:**  
2-Position 6-Port  
Vertical Port Injector



**Fig. 3:**  
2-Position 6-Port  
Analytical Scale Injector



**Fig. 4:**  
2-Position 4-Port  
Nano Injector



**Table 1. MX Series II Automated Valves**

Part Number	Description
Analytical-Scale	
MXP7900-000	Two-Position Six-Port (DuraLife™)
MXP9900-000	Two-Position Six-Port (PEEK)
MXP7920-000	Two-Position Six-Port Vertical Port (DuraLife™ II) with needle port
Fast Chromatography	
MXT715-000	Two-Position Six-Port (UltraLife™)

## Two-Column Selection

Column selection can help separation scientists to develop reproducible, rugged methods in the shortest time possible. Automated column selection can dramatically cut time, save costs, and increase productivity while improving analytical results. With a variety of columns from which to choose, any laboratory can optimize selectivity (different phases), resolution (different particle sizes), time (different lengths), and load/sensitivity (different diameters, injection volumes, and concentration strategies) for shared users.

Column selection allows multiple users to select methods using the correct column for their application and conveniently flush and store columns. Columns are stored in the solvent of choice and require no manual changes. With multiple columns, different types of samples can run at night and over weekends using different methods resulting in better utilization of existing equipment and increased productivity.

In a validated environment, use column selection for column to column reproducibility, method ruggedness, cross method conformational purity analysis using columns with different selectivity, sample long-term stability, solute specificity, column equilibration, and column quality control. The MX Series II Two-Position, Six-Port Switching Valve, illustrated below, is a self-contained, electrically actuated valve for automated two-column selection.

Fig. 5: 2 Column Selection Using a 2-Position 6-Port Switching Valve

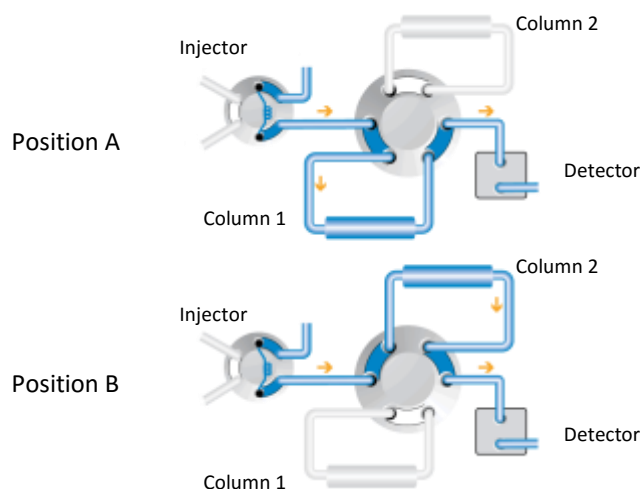


Table 2. MX Series II Automated Valves

Part Number	Description
Nano-Scale	
MXP7980-000	Nano Two-Position Six-Port (DuraLife™ II)
Analytical-Scale	
MXP7900-000	Two-Position Six-Port (DuraLife™)
MXP9900-000	Two-Position Six-Port (PEEK)
MXP7960-000	Two-Position Ten-Port (DuraLife™)
MXP9960-000	Two-Position Ten-Port (PEEK)
Fast Chromatography	
MXT715-000	Two-Position Six-Port (UltraLife™)

## Alternating Column Regeneration

Alternating column regeneration using two matched columns can increase sample throughput. This timesaving process involves equilibrating one column while using the other for analysis. A switching valve enhances the convenience of column regenerating by switching between the analyzing column and the equilibrating column.

For example, consider an analysis with a total time of 30 minutes: 20 minutes for the analysis and 10 minutes for equilibration in preparation for the next sample. Using alternating column regeneration, the 10 minute regeneration time occurs during the analysis thus reducing the total analysis time to 20 minutes. In this example, alternating column regeneration reduces the analysis time by one-third. Time saved will depend on the column equilibration time.

Column regeneration is beneficial for laboratories running large quantities of samples such as for drug discovery and quality assurance. This application increases efficiency and saves money for repetitive chromatographic analyses. The Nano-Scale MX Series II Ten-Port Valve (MXP7986-000) and Analytical-Scale Ten-Port Valves (MXP7960-000 or Biocompatible MXP9960-000) make unattended operation possible without the wait of long equilibration time.

See High Speed Sample Enrichment and Cleanup (Page 6) and Sample Clean Up/Sample Enrichment (Page 7) for alternating pre-columns.

Fig. 6: Alternating Column Regeneration Using a 2-Position 10-Port Switching Valve

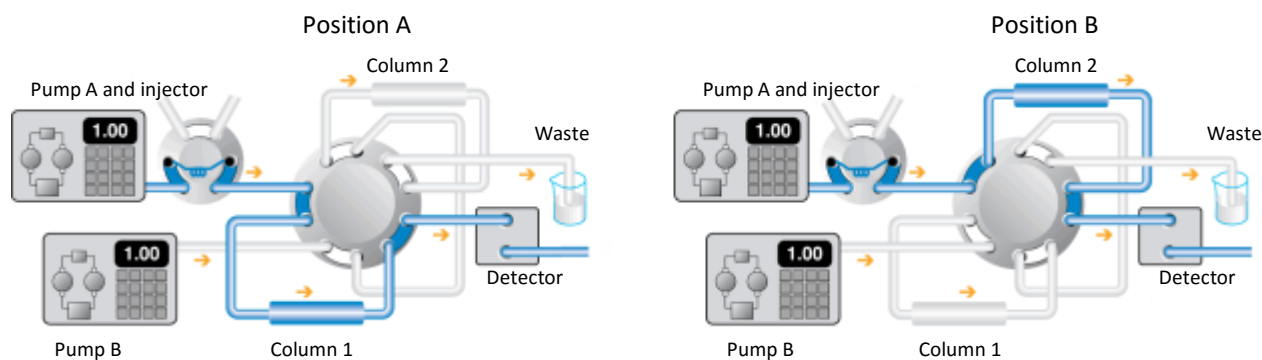


Table 3. MX Series II Automated Valves

Part Number	Description
Nano-Scale	
MXP7986-000	Nano Two-Position Ten-Port (DuraLife™ II)
Analytical-Scale	
MXP7960-000	Two-Position Ten-Port (DuraLife™)
MXP9960-000	Two-Position Ten-Port (PEEK)

## High Speed Sample Enrichment and Cleanup

The clean up or concentration of sample before injection onto an HPLC column is often the part of the analysis that requires the most time. The on-line sample preparation methods described here and on page 7 can significantly reduce that time. Furthermore, using two pre-columns in alternating parallel operation can increase the speed of these on-line methods even more.

In parallel operation, the functions of the two pre-columns alternate: one column accepts sample from the autosampler for clean up or enrichment while the other column transfers an already purified sample to the analytical column. A switching valve in the system then interchanges the functions of the pre-columns. This technique is especially effective in increasing overall analysis speed if the analytical column rapidly performs separations. For example, short, high-speed columns in a LC-MS system often run analyses quickly. Therefore, pre-columns operating in parallel significantly increase the speed of the relatively slow sample preparation step.

A typical high-speed enrichment and clean-up system is illustrated below. This system uses a standard chromatograph, an additional LC pump, two pre-columns, one analytical column, and a Nano-Scale Ten-Port Switching Valve. In Position 1, the sample injects onto Pre-Column 1, using mobile phase from Pump A. Pre-Column 1 enriches the sample and passes interfering sample components to waste. Meanwhile, the previously injected sample, now enriched and free of interfering compounds, backflushes out of Pre-Column 2 and onto the analytical column using the mobile phase from Pump B. After completion of the slowest step (either the enrichment on Pre-Column 1 or the separation on the analytical column), the valve switches to Position 2.

Position 2 interchanges the functions of the two pre-columns. The sample backflushes out of Pre-column 1 and onto the analytical column. Pre-column 2 now receives the next sample from the autosampler to carry out the next enrichment step.

Note some of the similarities that this alternating parallel pre-column method has to the alternating column regeneration method described on page 5. The purpose of alternating columns is to reduce the analysis time by allowing one column to separate sample components while another column re-equilibrates with the initial mobile phase. Various on-line sample preparation schemes and column switching schemes use a single system to eliminate more than one rate-limiting step, producing a robust, automated high-speed analysis system.

Fig. 7: High Speed Sample Enrichment Using a 2-Position 10-Port Switching Valve

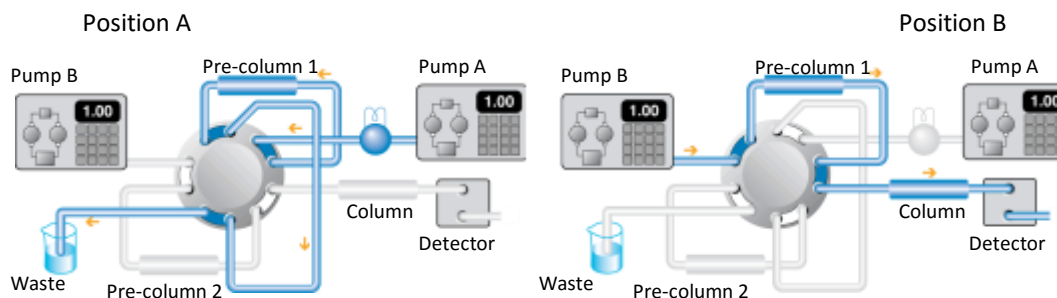


Table 4. MX Series II Automated Valves

Part Number	Description
Nano-Scale	
MXP7986-000	Nano Two-Position Ten-Port (DuraLife™ II)
Analytical-Scale	
MXP7960-000	Two-Position Ten-Port (DuraLife™)
MXP9960-000	Two-Position Ten-Port (PEEK)

## Sample Clean Up / Sample Enrichment

Complex samples such as biological fluids, food extracts, and waste waters often must be cleaned up and enriched. On-line sample clean up and enrichment uses a pre-column connected to an analytical column using a switching valve. This procedure can enhance productivity by allowing automated, unattended processing of large numbers of samples using an autosampler and a switching valve connected to the controller of the chromatograph.

### Methodologies

In enrichment, analytes are retained and concentrated on an enrichment column while unwanted components bypass the analytical column to waste. When the switching valve is actuated it backflushes analytes out of the enrichment column onto the analytical column. See illustration below. The injection of a large volume of sample onto the enrichment column results in a highly concentrated sample at the enrichment column inlet.

Two popular applications illustrate the versatility of sample enrichment. One is the LC/MS analysis of drugs and metabolites by direct injection of plasma with minimal previous work-up. By using a restricted access media (RAM) pre-column, the tightly bound analytes and plasma proteins that would otherwise destroy the analytical column pass through the pre-column to waste.

Another popular application is the LC/MS analysis of protein tryptic digests. A very small pre-column, often called a trap column, allows the injection of several microliters of digest under conditions where the peptides are highly retained. The switching valve transfers the small volume of concentrated sample onto a micro-bore or packed capillary column. Sample volumes are large enough for convenient handling, yet enrichment allows the actual gradient elution separation of the peptides to take place on micro-columns using low flow rates that are optimal for MS instruments. The system uses a standard chromatograph, an additional LC pump, and an Analytical- or Nano-Scale Two-Position, Six-Port Automated Valve.

Fig. 8: 2-Position 6-Port Switching Valve

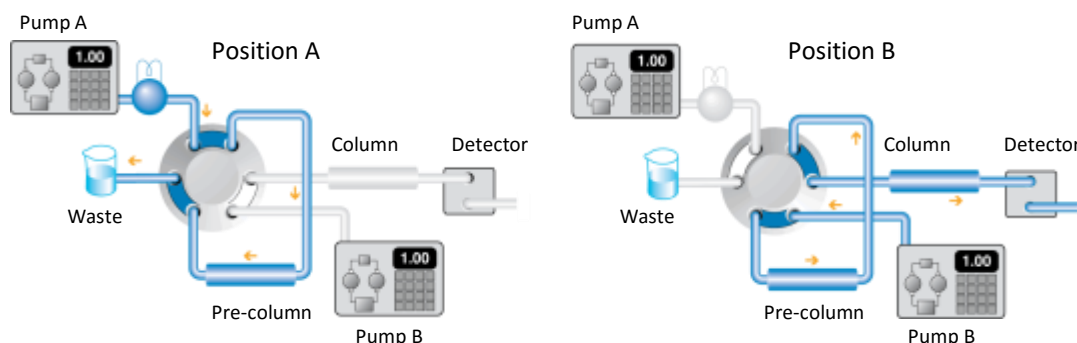


Table 5. MX Series II Automated Valves

Part Number	Description
Nano-Scale	
MXP7980-000	Nano Two-Position Six-Port (DuraLife™ II)
Analytical-Scale	
MXP7900-000	Two-Position Six-Port (DuraLife™)
MXP9900-000	Two-Position Six-Port (PEEK)
Fast Chromatography	
MXT715-000	Two-Position Six-Port (UltraLife™)

## Column Backflushing

Column backflushing is an analytical method in which a switching valve reverses the flow through a column. This method allows automatic clean up of a column without disconnection in situations where sample mixture is trapped on the head of a column. Rather than force highly retained compounds through the full length of the column, the reversed flow permits the elution of compounds in a shorter time period.

For automated column backflushing, connect a Nano-Scale MX Series II Six-Port Switching Valve (MXP7980-000) or an Analytical-Scale MX Series II Six-Port Switching Valve (MXP7900-000, MXP9900-000, or MXT715-000) to the system in place of the column between the autosampler (or injector) and the detector. The illustration to the right shows the column connected to Ports 6 and 3 and a jumper tube connected to Ports 1 and 5 of a MX Series II Two-Position, Six-Port Switching Valve to enable flow reversal.

Switching the valve from one position to another only changes the direction of flow through the column. Choosing the appropriate tubing size is an important factor in using this application to minimize volume added to the system that could increase dispersion.

Fig. 9: Column Backflushing Using a 2-Position 6-Port Switching Valve

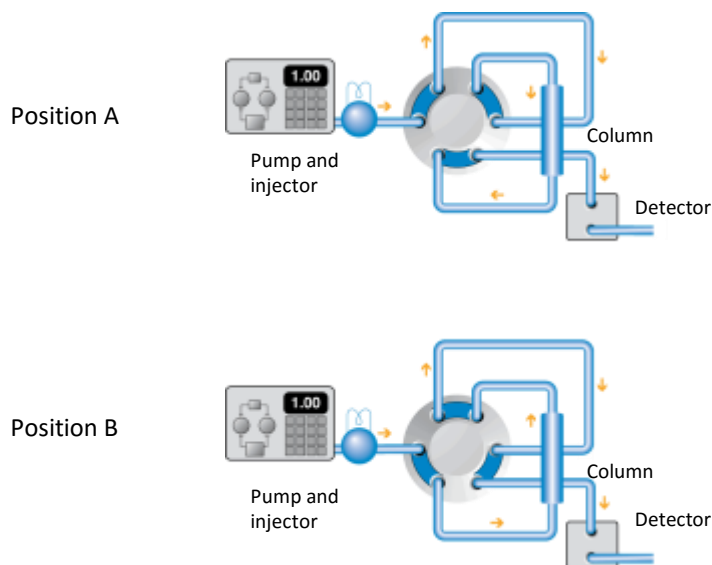


Table 6. MX Series II Automated Valves

Part Number	Description
Nano-Scale	
MXP7980-000	Nano Two-Position Six-Port (DuraLife™ II)
Analytical-Scale	
MXP7900-000	Two-Position Six-Port (DuraLife™)
MXP9900-000	Two-Position Six-Port (PEEK)
Fast Chromatography	
MXT715-000	Two-Position Six-Port (UltraLife™)



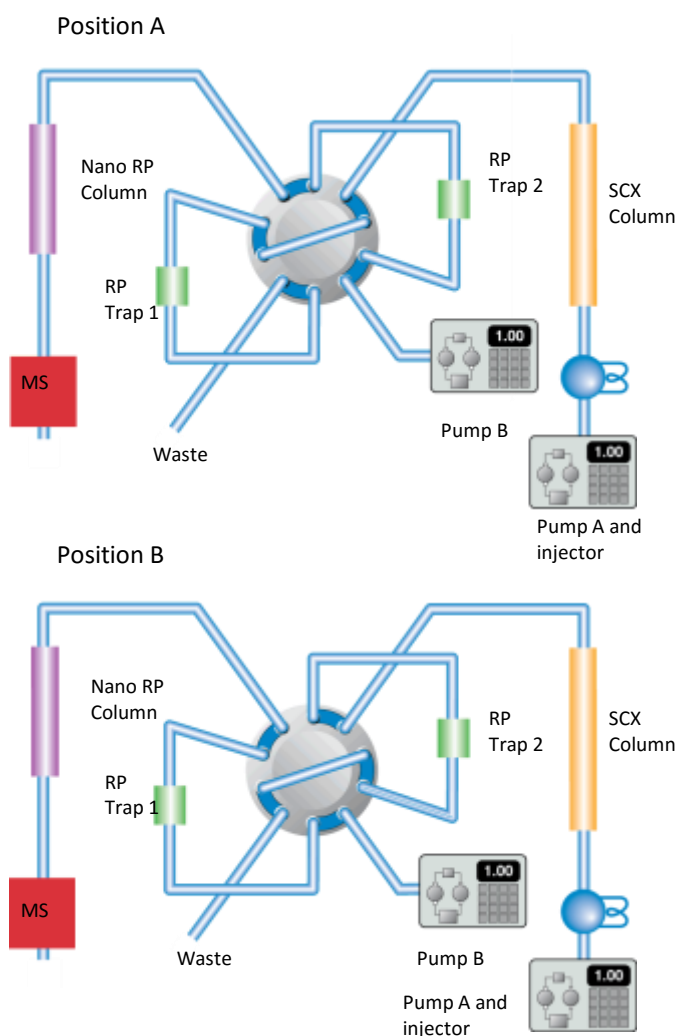
## Multi-dimensional Proteomic Peptide Separation

Reverse phase (RP) chromatography, a one-dimensional separation, is a common technique used in proteomic peptide separation. A multi-dimensional separation system can enhance peptide separations. An autosampler in-line with an MX Series II Ten-Port Switching Valve (MXP7986-000, or MXP7960-000 or MXP9960-000) creates a two-dimensional chromatography system.

The first dimension of the analysis is separation of a protein on an ion exchange (SCX) column. Salt fractions eluted from this column trap on an RP trap column. Switching the valve from Position 1 to Position 2 elutes the peptides from the trap column for further resolution using a gradient nano pump. The peptides are then detected and analyzed by ESI-MS or MS-MS. The peptide eluent can be spotted onto a MALDI target for further analysis.

In Position 1, effluent from the SCX column traps on the RP Trap Column 1. While Nano RP Column 1 analyzes the sample, RP Trap Column 2 traps the next salt fraction. In Position 2, the SCX column is in-line with RP Column 2 while RP Trap Column 1 traps the next salt fraction.

**Fig. 10:**  
Multi-dimensional Proteomic  
Peptide Separation Using a  
2-Position 10-Port Switching Valve



**Table 7. MX Series II Automated Valves**

Part Number	Description
Nano-Scale	
MXP7986-000	Nano Two-Position Ten-Port (DuraLife™ II)
Analytical-Scale	
MXP7960-000	Two-Position Ten-Port (DuraLife™)
MXP9960-000	Two-Position Ten-Port (PEEK)

## Solvent Selection

Solvent selection helps achieve chromatographic selectivity to optimize separation. Automated solvent selection improves analysis time by switching to a stronger eluting solvent or buffer. Stronger solvents or buffers are also useful for column clean-up and to eliminate undesired sample components that cause retention time reproducibility problems. Simple switching between two (or more) solvents, such as the mobile phase and rinse solution, saves time by eliminating disconnecting and reconnecting fittings. Solvent selection allows step gradients using an isocratic pump to elute strongly retained samples.

The selectors can be used for column clean-up eliminating undesired sample components that cause retention time reproducibility problems. Using a variety of solvents simplifies maintenance by rinsing the entire system from the solvent inlet filter through the detector flow cell to eliminate potential salt precipitation. This time-saving technique automates flushing, column equilibrating, QC testing procedure routines, and can easily add molecular weight calibrants into an MS inlet.

Manual solvent selection can be very tedious and difficult to reproduce. Selection using hands-off automation saves time, money, and increases laboratory productivity. Time-based collection using solvent, buffer, and eluent selection is achieved by using a MX Series II module with a six-position seven-port or a ten-position eleven-port selector valve. An example of automated selection is shown. A Solvent Selector platform switches among six different solvents to save disconnection time.

Fig. 11: Solvent Selection using a 6-Position 7-Port Selector Valve

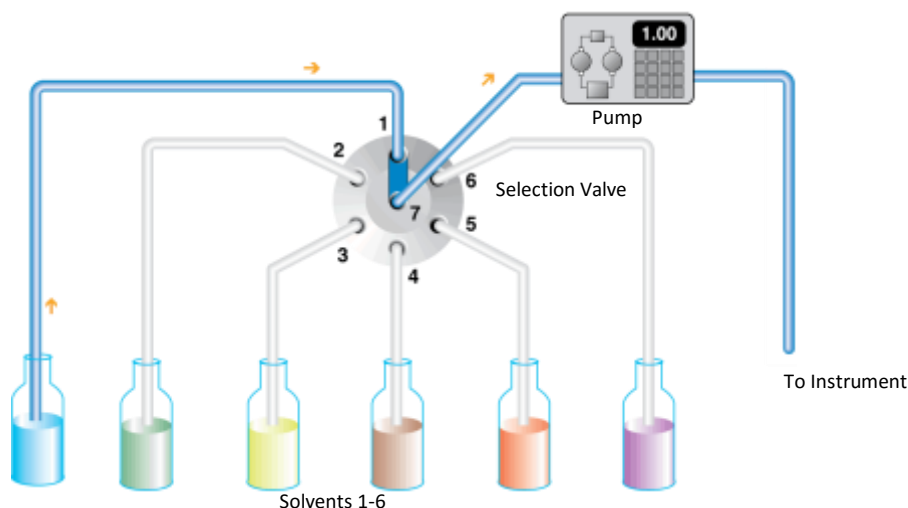


Table 8. MX Series II Automated Valves

Part Number	Description
Analytical-Scale	
MXP7970-000	Six-Position Seven-Port (UltraLife™)
MXX777-605	Six-Position Seven-Port (RPC-7)
Preparative Scale	
MXX777-616	Six-Position Seven-Port (RPC-7)
MXX778-605	Ten-Position Eleven-Port (RPC-7)
Fast Chromatography	
MXT715-105	Six-Position Seven-Port (UltraLife™ II)

## Fraction Collection

Fraction collection is desirable to isolate separated components of the sample as they elute from a chromatographic column. Fraction collection can be used over a broad range, from micro-, semi-preparative-, to preparative-scale depending on the pump flow rate. Selection of an MX Series II module for fraction collection should be based on the anticipated solvents, pH, buffers, salt concentration, and material biocompatibility. Normally the fraction collection platform will be located after the detector where pressure considerations are less important. However, in some situations, the valve may be located in a high-pressure region (e.g., cutting fractions from one column onto individual columns for desalting) where pressure limitations should be considered.

Hands-off, automated collection can be accomplished using a ten-position, eleven-port selector or a six-position, seven-port selector. Time-based collection eliminates manual operation of repetitive analysis.

The illustration below shows an example of automated fraction collection using a ten-position selector. In this case one position is dedicated to waste and nine ports are available for fraction collection.

Fig. 12: Fraction Collection using a 10-Position 11-Port Selection Valve

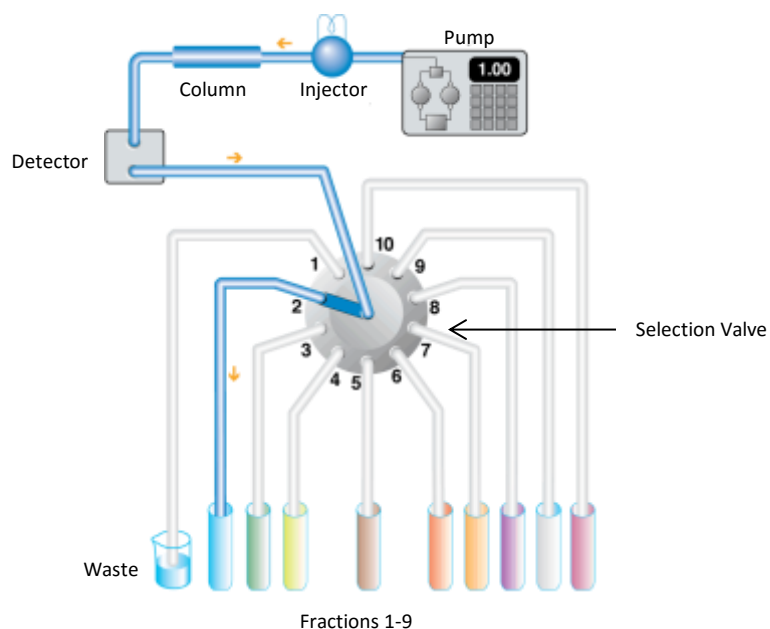


Table 9. MX Series II Automated Valves

Part Number	Description
Analytical-Scale	
MXP7970-000	Six-Position Seven-Port (UltraLife™)
MXX777-605	Six-Position Seven-Port (RPC-7)
Preparative Scale	
MXX777-616	Six-Position Seven-Port (RPC-7)
MXX778-605	Ten-Position Eleven-Port (RPC-7)
Fast Chromatography	
MXT715-105	Six-Position Seven-Port (UltraLife™ II)

## Six Column Selection

In today's high throughput laboratory environment multiple column selection capability can increase overall productivity and help maintain method reproducibility by eliminating time consuming column changes. With six-column selection, six different column chemistries or column dimensions can be accommodated on one system. Rapid method development is enabled by easily switching to another column when one column is not providing adequate separation. For routine HPLC pressures (up to 6,000 psi /400 bar), two MXP7970-000 units operated in tandem are recommended. For Fast Chromatography pressures (up to 15,000 psi /1000 bar), two MXT715-105 units in tandem are recommended. Both units are programmed to switch position simultaneously if a different column is desired. The MX units are stackable which allows for use of minimal bench space. Using two units in tandem also allows for the column inlet and outlet to be plumbed as close as possible to the valve head, thereby eliminating added system volume from extra transfer tubing.

Fig. 13: Column Selection Using Two 6-Position 7-Port Selector Valves

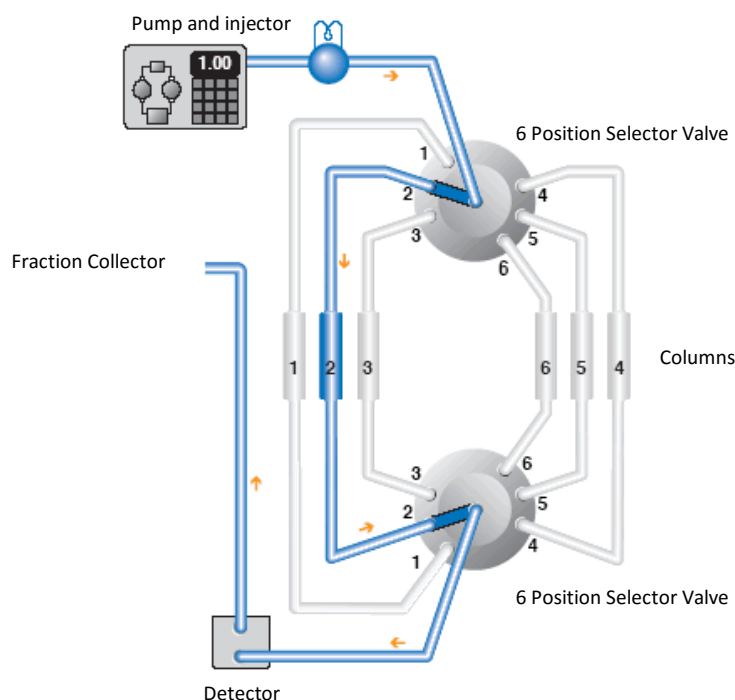


Table 10. MX Series II Automated Valves

Part Number	Description
Analytical-Scale	
MXP7970-000	Six-Position Seven-Port (UltraLife™)
Fast Chromatography	
MXT715-105	Six-Position Seven-Port (UltraLife™ II)