Solutions Guide

MX Series
Modular Automation for
Nano, Micro, and Analytical Scale
HPLC Applications

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Solutions Guide
MX Automated Valves

Sample Injection

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.

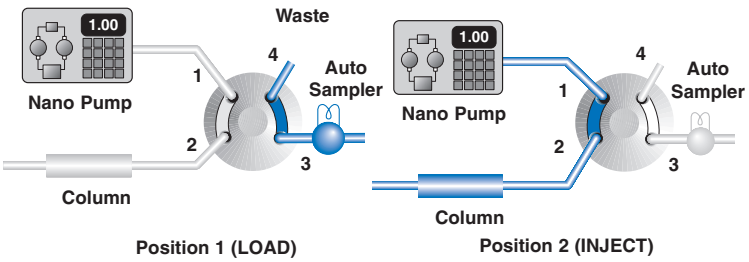
Sample injectors are situated after the pump and before the column. It is common practice to have a filter before the injector to minimize any pump seal debris from entering the injector. A filter between the injector and the column will trap particles that otherwise may block the column frit.

Nano-Scale and Analytical-Scale Injectors

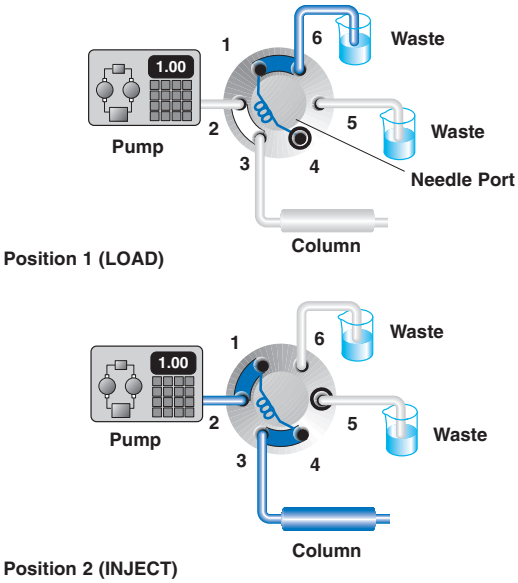
Various MX Valves for sample injection are available:

Nano-Scale MX Four-Port Injection Valve,
Analytical-Scale MX Six-Port Injection Valve, and
Analytical-Scale MX Syringe Loading Injection Valve.

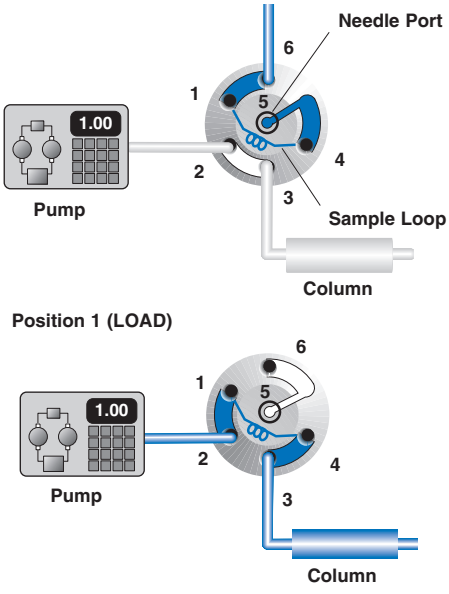
Nano-Scale



Analytical-Scale 2-Position, 6-Port



Analytical-Scale Syringe Loading Injector



MX Automated Valves

Part Number	Description
Nano-Scale	
MX7984-000	MX Nano Injector,10 nL internal loop (Biocompatible DuraLife™ II)
Analytical-Scale	
MX7900-000	MX Two-Position, Six-Port (Stainless Steel)
MX9900-000	MX Two-Position, Six-Port (Biocompatible)
MX7925-000	MX Syringe Loading Injector (Stainless Steel)
MX9925-000	MX Syringe Loading Injector (Biocompatible)

To order, contact your authorized Rheodyne MX dealer. See www.rheodyne.com for a complete list of dealers.

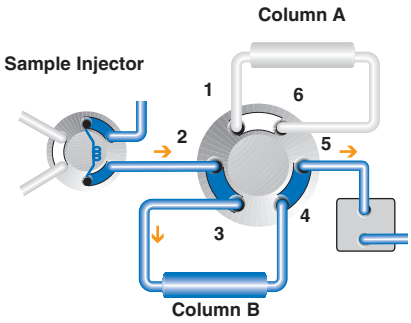
Two-Column Selection

Two-Column Selection

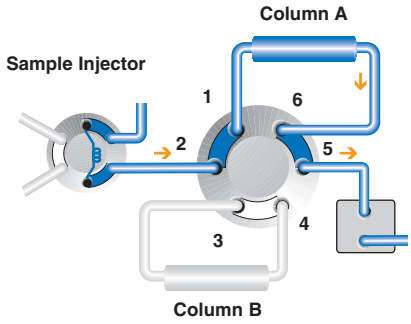
Column selection can help separation scientists to develop reproducible, rugged methods (frequently validated) in the shortest time possible. Automated column selection can dramatically cut time, save costs, and increase productivity while improving analytical results. With a variety of columns from 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 assay and conveniently washout and store columns. Columns are stored in the solvent of choice and require no manual changes. 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 on columns with different selectivity, sample long-term stability, solute specificity, column equilibration, and column quality control. The MX Two-Position, Six-Port Switching Valve, illustrated to the right, is a self-contained, electrically actuated valve for automated two-column selection.



Position 1 (LOAD)



Position 2 (INJECT)

MX Automated Valves

Part Number	Description
Nano-Scale	
MX7980-000	MX Nano-Scale Two-Position, Six-Port Switching Valve (Biocompatible DuraLife™ II)
Analytical-Scale	
MX7900-000	MX Two-Position, Six-Port Switching Valve (Stainless Steel)
MX9900-000	MX Two-Position, Six-Port Switching Valve (Biocompatible)

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Alternating Column Regeneration

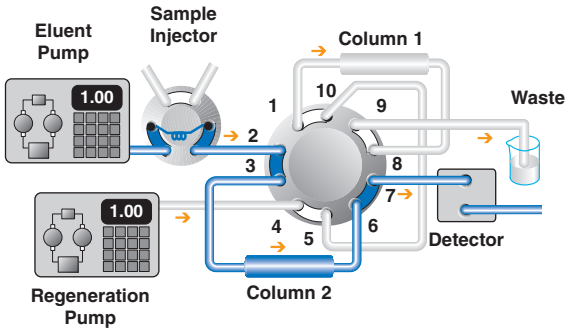
Alternating Column Regeneration

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.

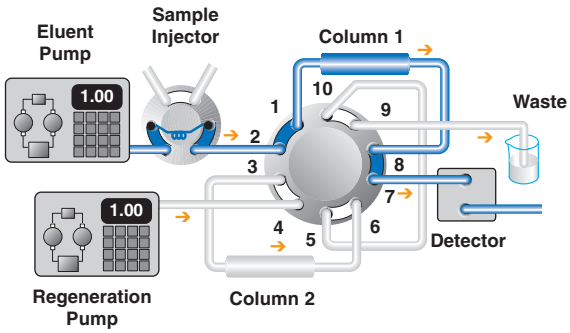
For example, consider an analysis with a total time of 30 minutes: 20 minutes for the analysis and 10 minutes for equilibration in preparation for the next sample. Using alternating column regeneration, the 10 minute regeneration time occurs during the analysis thus reducing the total analysis time to 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 Ten-Port Valves (Stainless Steel MX7986-000 and Analytical-Scale Ten-Port Valves (Stainless Steel MX7960-000) make unattended operation of this technique possible without the wait of a long equilibration time.

See High Speed Sample Enrichment (Page 5) and High-Speed Sample Clean Up and Enrichment (Page 6) for alternating pre-columns.



Position 1



Position 2

MX Automated Valves

Part Number	Description
Nano-Scale	
MX7986-000	MX Nano-Scale Two-Position, Ten-Port Switching Valve (Biocompatible DuraLife™ II)
Analytical-Scale	
MX7960-000	MX Two-Position, Ten-Port Switching Valve (Stainless Steel)

To order, contact your authorized Rheodyne dealer. See www.rheodyne.com for a complete list of dealers.

High Speed Sample Enrichment

High Speed Sample Enrichment

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 6 can, when properly optimized, significantly reduce that time. Furthermore, using two pre-columns in alternating parallel operation can increase the speed of these on-line methods even more.

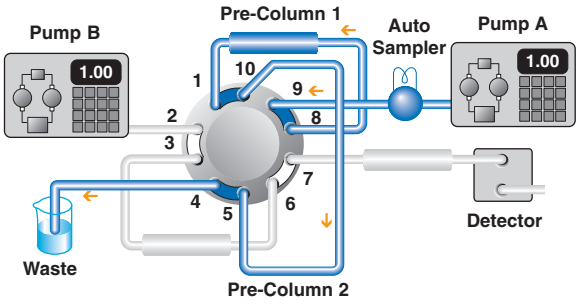
In parallel operation, the functions of the two pre-columns alternate: one column accepts sample from the autosampler for clean up or enrichment while the other column transfers an already purified sample to the analytical column. A switching valve in the system then interchanges the functions of the 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 to the right. This system uses a standard chromatograph, an additional LC pump, two pre-columns, one analytical column, and a Nano-Scale MX Ten-Port Switching Valve.

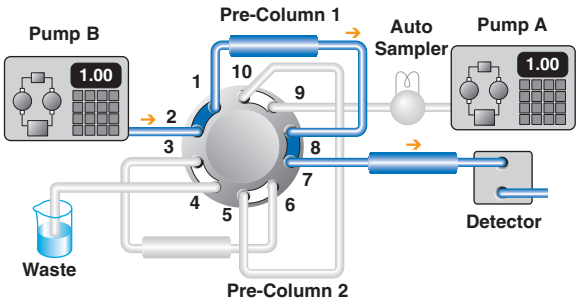
In Position 1, the sample injects onto Pre-Column 1, using mobile phase from Pump A. Pre-Column 1 enriches the sample and passes interfering sample components to waste. Meanwhile, the previously injected sample, now enriched and free of interfering compounds, backflushes out of Pre-Column 2 and onto 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. Sample back-flushes 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 4. 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.



Position 1



Position 2

MX Automated Valves

Part Number	Description
Nano-Scale	
MX7986-000	MX Nano-Scale Two-Position, Ten-Port Switching Valve (Biocompatible DuraLife™ II)
Analytical-Scale	
MX7960-000	MX Two-Position, Ten-Port Switching Valve (Stainless Steel)

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High Speed Sample Clean Up and Enrichment

High Speed Sample Clean Up and Enrichment

Complex samples such as biological fluids, food extracts, and waste waters often must be cleaned up and enriched. Before injection onto an HPLC column, the analytes of interest and contaminants must be separated so they will not interfere with the separation or destroy the analytical column. Sample enrichment will also enhance detection.

On-line sample clean up and enrichment uses a pre-column connected to an analytical column using a switching valve. Automation has advantages over off-line techniques. On-line analysis improves analytical precision and reduces solvent volumes since LC pumps meter the flow rates and the volumes of mobile phase passed through both columns. This on-line 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

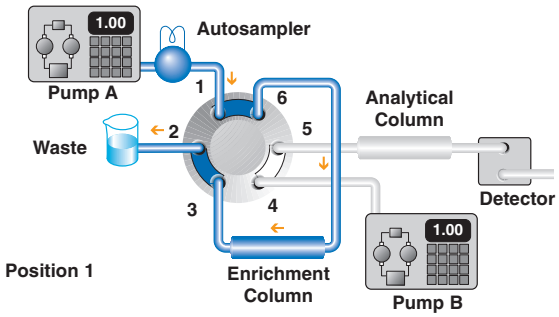
In enrichment, analytes are retained and concentrated on an enrichment column while unwanted components bypass the analytical column to waste. When the switching valve is actuated it backflushes analytes out of the enrichment column onto the analytical column. See illustration below. The enrichment column is typically a reverse phase column where analytes are injected using a mobile phase composition that causes them to be strongly retained.

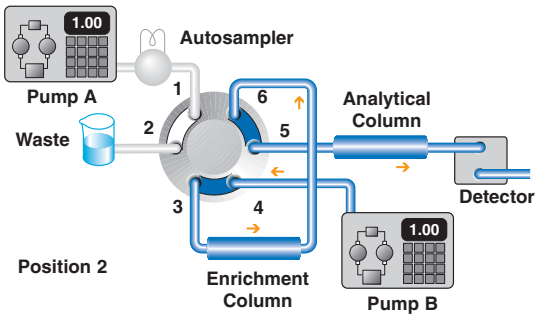
Under these conditions, the injection of a large volume of sample onto the enrichment column results in a highly concentrated sample at the enrichment column inlet. In the process illustrated, samples are both concentrated and cleaned up. Enrichment can range from a factor of ten to several thousand (depending on the volume of sample injected) to significantly improve detection.

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 MX Two-Position, Six-Port Automated Valve (Model MX7900-000 or MX 7980-000). In Position 1, the sample injects onto the enrichment column using mobile phase from Pump A. Meanwhile the analysis of the previous sample is carried out on the analytical column using the mobile phase from Pump B. In Position 2, the enrichment column switches to the reverse direction so Pump B backflushes the cleaned-up and concentrated analytes onto the analytical column. After the transfer of the analytes onto the analytical column, the valve switches back to Position 1, so the enrichment column is conditioned and the next sample can inject onto it. Enrichment and analysis take place concurrently.





MX Automated Valves

Part Number	Description
Nano-Scale	
MX7980-000	MX Nano-Scale Two-Position, Six-Port Switching Valve (Biocompatible DuraLife™ II)
Analytical-Scale	
MX7900-000	MX Two-Position, Six-Port Switching Valve (Stainless Steel)
MX9900-000	MX Two-Position, Six-Port Switching Valve (Biocompatible)

To order, contact your authorized Rheodyne MX dealer. See www.rheodyne.com for a complete list of dealers.

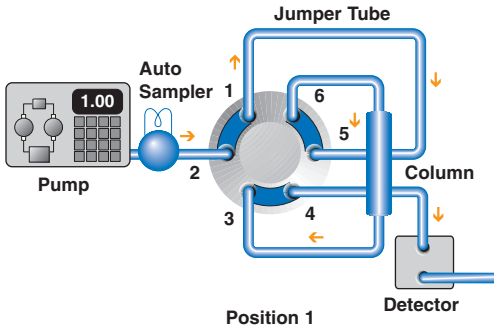
Column Backflushing

Column Backflushing

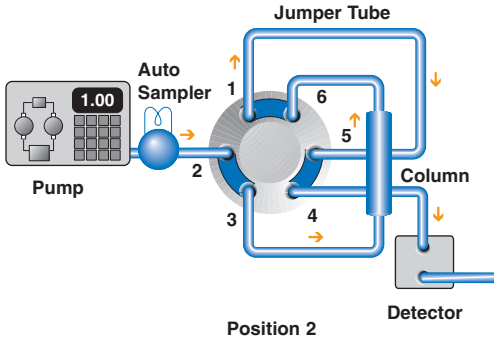
Column backflushing is an analytical method in which a switching valve reverses the flow through a column. This method allows automatic clean up of a column without disconnection in situations where sample mixture is trapped on the head of a column. Rather than force highly retained compounds through 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 Six-Port Switching Valve (Stainless Steel MX7980-000) or an Analytical-Scale MX Six-Port Switching Valve (Stainless Steel MX7900-000 or Biocompatible MX9900-000) 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 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.



Position 1



Position 2

MX Automated Valves

Part Number	Description
Nano-Scale	
MX7980-000	MX Nano-Scale Two-Position, Six-Port Switching Valve (Biocompatible DuraLife™ II)
Analytical-Scale	
MX7900-000	MX Two-Position, Six-Port Switching Valve (Stainless Steel)
MX9900-000	MX Two-Position, Six-Port Switching Valve (Biocompatible)

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MS Solvent Diversion

MS Solvent Diversion

In order to enhance the capability of an analytical method, analysts may combine two or more traditionally separate analytical techniques. Hyphenated Techniques is the common term for combining two techniques into a single system such as liquid chromatography-mass spectrometry (LC-MS).

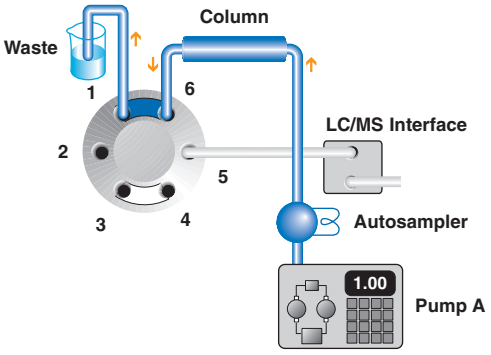
Combining LC with MS presents an enormous range of possibilities for sample analysis. LC offers the ability to separate almost any mixture, and MS offers extremely sensitive detection along with qualitative power for target analyte identification. An LC-MS creates an efficient analysis technique to eliminate a two-step method for obtaining comprehensive data on an unknown sample. For example, further identification of the separated sample from the column is possible with data from the mass spectrometer.

A MX Switching Valve can help make a working combination of the two techniques. An example of an automated LC-MS system consists of a Nano-Scale MX Six-Port Switching Valve, or Analytical-Scale MX Six-Port Switching Valve, in-line with an autosampler, a column (LC System), and the mass spectrometer (MS System).

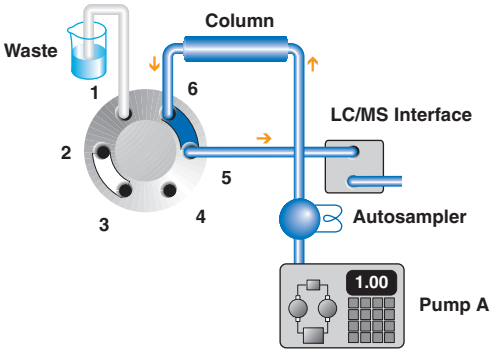
The MX Switching Valve operates as an automatic switching valve to divert undesired portions of the sample from the LC system to waste before the sample enters the MS. This flow path diversion removes the unwanted portion of the sample from the entire system thus keeping any non-volatile salts away from the mass spectrometer.

Non-volatile salts in the mobile phase can quickly degrade the performance of a mass spectrometer should they enter the high vacuum region. Even if the mobile phase is free of non-volatile salts, the sample may contain salts. Since any salts present in the sample should remain in the unretained portion, switching the column effluent away from the mass spectrometer as the unretained portion emerges from the column is a good way to keep mass spectrometer maintenance to a minimum.

The illustration below shows that the undesired portion of the sample is diverted to waste in Position 1, and the sample flow travels first through the LC system and then the MS system in Position 2.



Position 1



Position 2

MX Automated Valves

Part Number	Description
Nano-Scale	
MX7980-000	MX Nano-Scale Two-Position, Six-Port Switching Valve (Biocompatible DuraLife™ II)
Analytical-Scale	
MX7900-000	MX Two-Position, Six-Port Switching Valve (Stainless Steel)
MX9900-000	MX Two-Position, Six-Port Switching Valve (Biocompatible)

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Multi-Dimensional Proteomic Peptide Separation

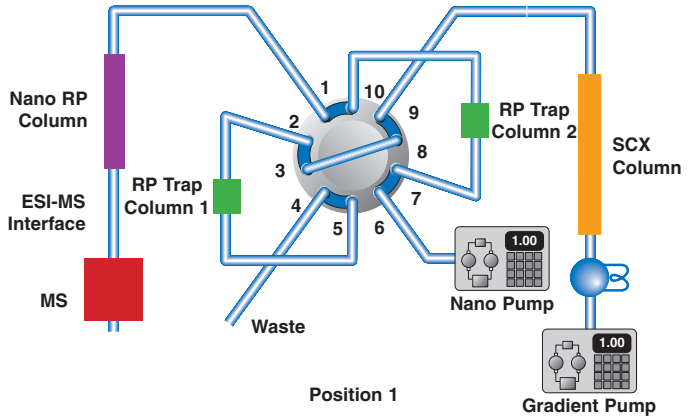
Multi-Dimensional Proteomic Peptide Separation

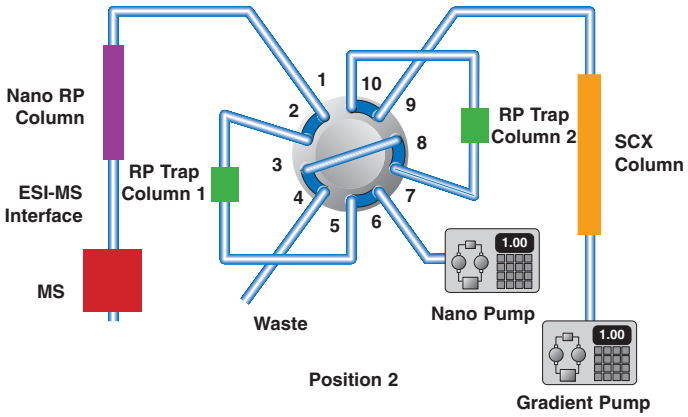
Reverse phase RP chromatography, a one-dimensional separation, is a common technique used in proteomic peptide separation. A multi-dimensional separation system can enhance peptide separations.

An autosampler in-line with a Nano-Scale MX Ten-Port Switching Valve (Stainless Steel MX7986-000) or an Analytical-Scale MX Ten-Port Switching Valve (Stainless Steel MX7960-000) 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 eluate 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.





MX Automated Valves

Part Number	Description
Nano-Scale	
MX7986-000	MX Nano-Scale Two-Position, Ten-Port Switching Valve (Biocompatible DuraLife™ II)
Analytical-Scale	
MX7960-000	MX Two-Position, Ten-Port Switching Valve (Stainless Steel)

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