

Standard and High-Speed Sample Enrichment and Clean-Up

Sample Preparation

Standard Sample Enrichment and Clean-Up

Typical Uses

- ▶ Online HPLC Sample Preparation
- ▶ Online SPE
- ▶ High-Throughput Sample Clean-up
- ▶ Drug Metabolism

Samples that are in complex matrices such as biological fluids, food extracts, and waste waters often must be cleaned prior to injection into an HPLC column. The matrix must be separated from the analytes of interest, so contaminants do not interfere with the separation or destroy the analytical column.

There are two types of sample preparation; offline and online. Typically, samples are cleaned offline using a solid phase extraction (SPE) cartridge; a short column packed with relatively large chromatographic particles. Gravity, vacuum, or a syringe is typically used to generate the flow. In online sample preparation, the sample carries out the matrix-analyte separation in a pre-column that connects to the analytical column by means of a switching valve.

Online processes have several advantages over offline techniques. Online analysis improves analytical precision and reduces the amount of solvent required since chromatographic pumps meter the flow rates and the volumes of mobile phase passed through both columns. The procedure can also enhance productivity, allowing automated, unattended processing of large numbers of samples using an autosampler and switching valve connected to the chromatograph's controller. Sample volume can vary from less than a microliter to many milliliters, depending on whether micro-scale columns or normal analytical columns are used.

There are two methods of online sample preparation, enrichment and stripping. In both methods, the sample is first injected onto a pre-column where the clean-up step takes place.

Enrichment Methods

In enrichment methods, the analytes are retained and concentrated onto the pre-column while the matrix components are passed to waste, bypassing the analytical column. Then the valve is switched, causing the analytes to backflush out of the pre-column and onto the analytical column. Typically, the pre-column is a reversed phase column to which analytes are injected with a mobile phase that causes them to be strongly retained. Under these conditions a large volume of sample can be injected onto the pre-column, resulting in build-up of highly concentrated sample at the pre-column inlet. Thus, in enrichment methods, samples are both cleaned up and concentrated. Depending on the volume of sample injected, enrichments can range from a factor of ten to several thousand, significantly improving the limit of detection.

- ▶ Online HPLC Sample Preparation
- ▶ Online SPE
- ▶ High-Throughput Sample Clean-up
- ▶ Drug Metabolism
- ▶ Direct Injection of Plasma and Urine
- ▶ Restricted Access Media (RAM)
- ▶ Tryptic Digest LC/MS with Trap Columns
- ▶ Sample Preparation Using Column Switching

Two popular applications illustrate the versatility of enrichment methods. One is the LC/MS analysis of drugs and metabolites by direct injection of plasma. By using a restricted access media (RAM) pre-column, the analytes are tightly bound and the plasma proteins, which 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 resulting small-volume, concentrated sample is transferred by the switching valve to a microbore 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 optimum for many MS instruments.

Figure 1.10 illustrates a typical enrichment system. The system uses a standard chromatograph, an additional LC pump, and a Rheodyne® MX Series 2-Position, 6-Port switching valve used as an automated switching valve. In Position A, the sample injects into the pre-column using mobile phase from Pump A. At the same time, the analysis of the previous sample is carried out on the analytical column using the mobile phase from Pump B. In Position B, the pre-column switches to the reverse direction so that Pump B back flushes the cleaned-up and concentrated analytes onto the analytical column. When the analytes have been transferred onto the analytical column, the valve switches back to Position A so the pre-column can be conditioned and the next sample can inject into it.

Clean-up and analysis take place concurrently. High-Speed Sample Enrichment and Clean-Up discusses techniques of increasing throughput by using parallel pre-columns.

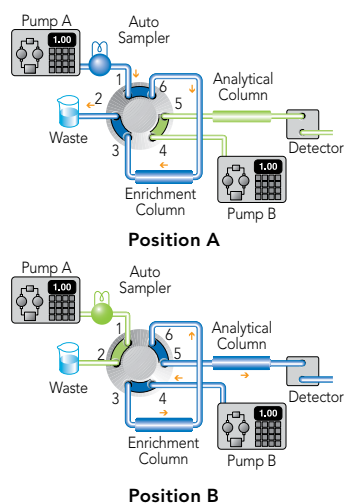


Fig. 1.10 On-line sample preparation system using the enrichment method.

Stripping Methods

In stripping methods, the analytes and matrix components are handled in the opposite way from the enrichment methods. The matrix components bind onto the pre-column while the analytes pass through the pre-column as an unretained group and into the analytical column. Then the valve switches, causing the matrix components to back flush out of the pre-column to waste, bypassing the analytical column.

Figure 1.11 illustrates a stripping method using the same equipment as used in the enrichment method previously discussed, the only difference is that the columns connect to different ports of the switching valve. In Position A, the sample injects into the pre-column using mobile phase from Pump A. The analytes pass through the pre-column and then directly onto the analytical column. The interfering matrix components are stripped onto the pre-column. In Position B,

the pre-column switches to the reverse direction so that Pump B back flushes the matrix components off of the pre-column to a waste. This back flushing prepares the pre-column for receiving the next sample. When the analysis is completed, the valve switches back to Position A, and the next sample is injected. High-Speed Sample Enrichment and Clean-Up discusses techniques of increasing throughput by using parallel pre-columns.

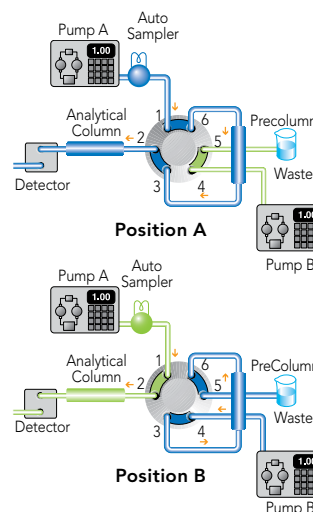


Fig. 1.11 On-line sample preparation system using the stripping method of sample clean-up.

Combination Injection and Enrichment

Rheodyne MX Series 2-Position, 10-Port switching valves can function as a combination injector and switching valve. Figure 1.12 illustrates this method. Note that this valve performs the same functions described in Figure 1.10 with the addition of a sample loop that is manipulated to accept sample and then inject it onto a pre-column.

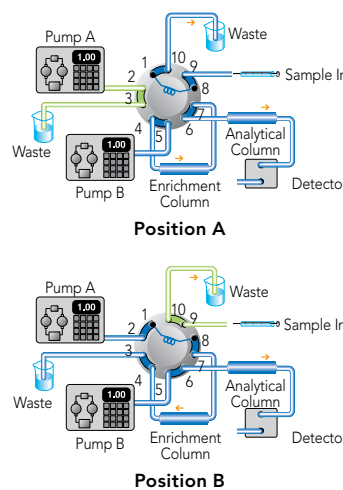


Fig. 1.12 Sample enrichment system using a ten-port valve for combination injection and switching tasks.

High-Speed Sample Enrichment and Clean-up

Typical Uses

- ▶ High-Throughput Sample Preparation
- ▶ Alternating Column Switching
- ▶ Fast Multi-Dimensional Chromatography
- ▶ Online Parallel SPE
- ▶ High-Throughput Drug Metabolite Analysis
- ▶ Alternating Enrichment Columns

The clean-up or concentration of samples before injection onto an HPLC column is often the part of the analysis that is the most time consuming. Online sample enrichment and sample clean-up using Rheodyne 2-position, 6-port switching valves can significantly reduce that time.

To further increase the speed of analysis a 2-position, 10-port switching valve can be used to operate two pre-columns in alternating parallel operation.

In parallel operation, the functions of the two pre-columns alternate: one accepts sample from the autosampler for clean-up or enrichment while the other one transfers a previously 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 when the analytical column is able to rapidly perform separations. Short, high-speed columns in an LC/MS system often run analyses quickly. Therefore, pre-columns operating in parallel significantly increase the speed of the slower sample preparation step.

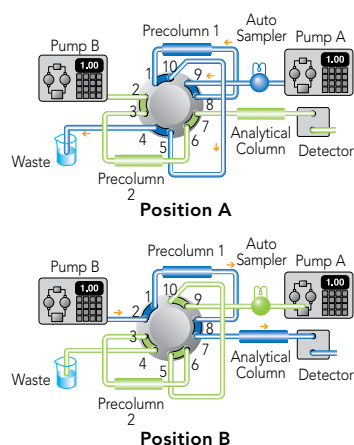


Fig. 1.13 Parallel sample enrichment system.

Figure 1.13 illustrates a typical high-speed enrichment and clean-up system. This system uses a standard chromatograph, an additional LC pump, two pre-columns,

one analytical column, and a MX Series 2-Position, 10-Port switching valve used as an automated switching valve.

In Position A, the sample injects onto Pre-column 1, using mobile phase from Pump A. Pre-column 1 enriches the sample and passes interfering matrix components to waste. In the same position, 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 both samples have been processed (either the enrichment on Pre-column 1 or the separation on the analytical column), the valve switches to Position B.

In Position B, the functions of the two pre-columns are switched. 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.

Part Number	HPLC Valves
MXP7900-000	2-position, 6-port Switching Valve, SST (6,000 psi, 414 bar)
MXP7960-000	2-Position, 10-Port Switching Valve SST (6,000 psi, 414 bar)
MXP7980-000	2-Position, 6-Port Nano Switching Valve, Ti (5,000 psi, 345 bar)
MXP7986-000	2-Position, 10-Port Nano Switching Valve, Ti (5,000 psi, 345 bar)
MXP9900-000	2-Position, 6-Port Switching Valve, PEEK™ (6,000 psi, 414 bar)
MXP9960-000	2-Position, 10-Port Switching Valve, PEEK (6,000 psi, 414 bar)
MXT715-000	2-Position, 6-Port Switching Valve, SST (10,000 psi, 1,034 bar)
MXT715-102	2-Position, 10-Port Switching Valve, SST (10,000 psi, 1,034 bar)

SST = stainless steel; Ti = titanium

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