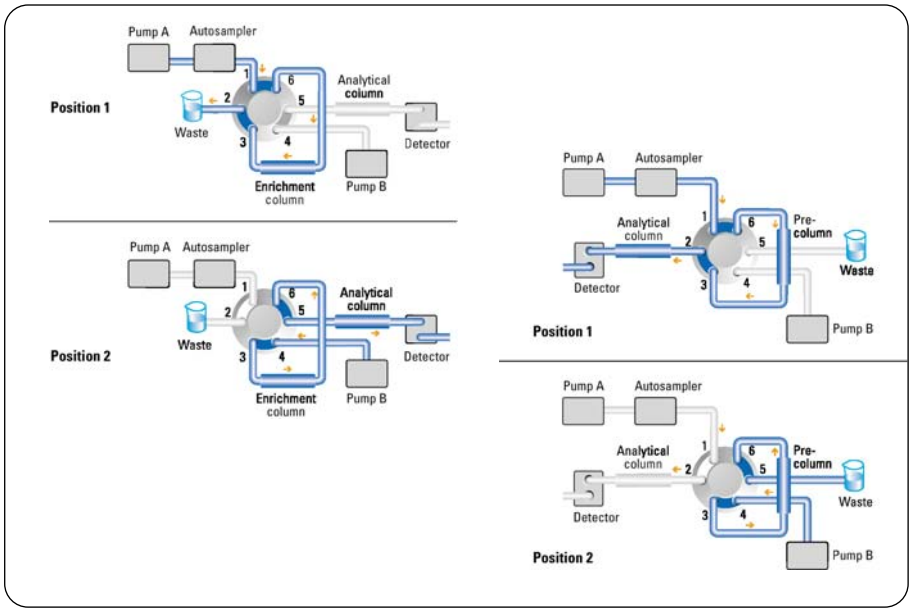
Automated sample preparation – sample enrichment and clean-up with the Agilent 1100 Series valve solutions

Application

Udo Huber



Abstract

Sample enrichment and sample stripping are two methods for automated, on-line sample preparation. In this Application Note we show the system configuration and plumbing diagrams to perform sample enrichment and sample stripping on an Agilent 1100 Series system using a 2-position/ 6-port valve. A short application example is shown and some typical applications are briefly described. Also an alternative system setup using only a single gradient pump is shown and the advantages and disadvantages of this configuration are briefly discussed.

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Introduction

Many samples applied to HPLC contain compounds that shorten the lifetime of the analytical column due to clogging or irreversible binding to the solid phase. Removing these substances can either be done off-line, for example by solid phase extraction, or on-line using sample enrichment or stripping methods with a 2-position/6-port valve[1]. The principle of both on-line methods is the same: The compounds of interest and the disturbing matrix are separated before the sample is transferred to the analytical column. The separation is usually done on a pre-column. In sample enrichment the compound of interest is retained on the pre-column while the matrix is washed to waste. In sample stripping the matrix is retained on the pre-column while the compounds of interest are transferred to the analytical column. The pre-column is then back-flushed to remove the retained matrix before the next sample is injected.

Equipment

The system used consisted of:

- Agilent 1100 Series binary pump with degasser
- Agilent 1100 Series well-plate autosampler
- Agilent 1100 Series 6-position selection valve
- Agilent 1100 Series thermostatted column compartment
- Agilent 1100 Series diode-array detector
- Agilent 1100 Series isocratic pump

The system was controlled using the Agilent ChemStation (rev. A.09.03)

Results and discussion

Figure 1 shows the configuration and plumbing diagram of the system for sample enrichment. Pump A, which can be an isocratic pump, is used to load the sample onto the enrichment column. After the matrix is washed to waste the valve switches and the compounds of interest are back-flushed to the analytical column. The gradient for the analysis is delivered by pump B, which can be a binary or quaternary pump. Another aspect of this method is, that the sample is pre-concentrated on the enrichment

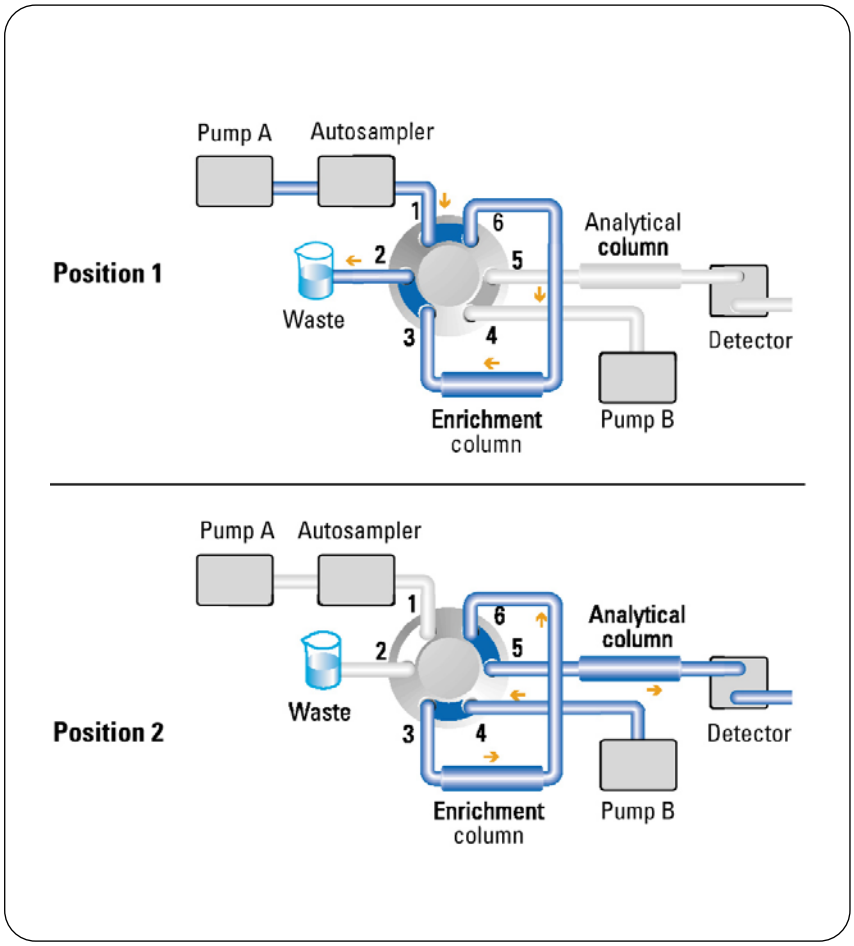

Figure 1
System configuration for sample enrichment

Column: ZORBAX SB-C18 2.1 x 100 mm, 3.5 µm
Pre-column: ZORBAX SB-C18 2.1 x 30 mm, 3.5 µm
Mobile phase: A = water + 0.1 % TFA
B = acetonitrile + 0.1 % TFA
Gradient: at 0 min 5 % B
at 5.1 min 5 % B
at 50 min 50 % B
at 55 min 95 % B
at 60 min 95 % B
Valve: at 0 min pos. 1 (Pre-column)
at 5 min pos. 2 (Pre- and anal. column)
Stop time: 60 min
Post time: 10 min
Flow rate: 0.3 mL/min
Pump A: water/acetonitrile 95:5 + 0.1 % TFA
Flow A: 0.3 mL/min
Injection: 95 µL
Column temp.: 35 °C
UV detector 1: DAD, 210/4 nm (ref. 360/60 nm)
Standard flow cell (10 mm)
UV detector 2: DAD, 210/4 nm (ref. 360/60 nm)

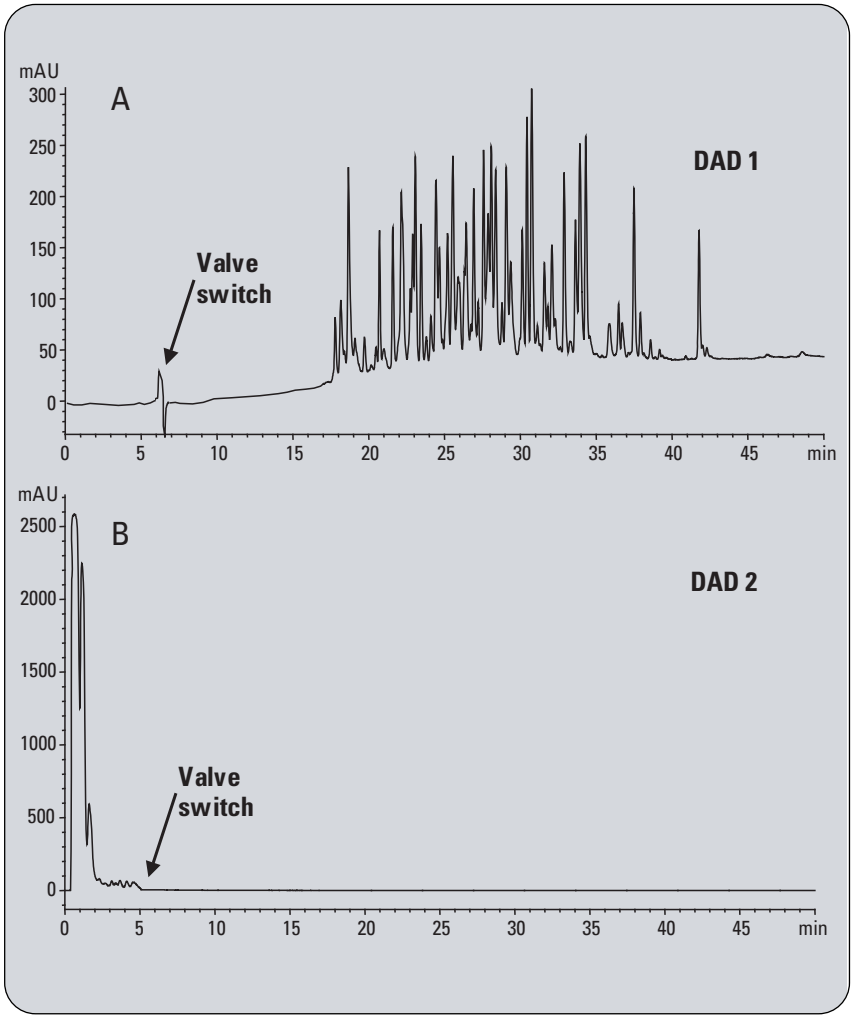

Figure 2
A) Chromatogram tryptic digest
B) Chromatogram of matrix measured with second DAD

column. If the compounds of interest are dissolved in a large solvent volume, direct injection can lead to bad chromatographic performance. Pre-concentration of the compounds on the pre-column usually results in better peak shapes and peak widths, that is, better chromatographic performance.
Some typical applications for sample enrichment include:

- Matrix removal from plasma, serum or urine samples for concentration measurements of pharmaceutical compounds. Restricted access material is often used in the pre-column.
- Analyses of tryptic digests for pre-concentration and removal of salts, reductive agents, etc.
- Removal of salts, chlorophyll etc. from natural product extracts.
- Pre-concentration of waste water or surface water samples in environmental analysis.

In the application examples in figures 2A and B a tryptic digest of bovineserum albumin was pre-concentrated and separated from the matrix on a 2.1 x 30 mm ZORBAX SB-C18 column (3.5 µm) with the mobile phase of gradient starting conditions (5 % acetonitrile) for 5 minutes at a flow rate of 0.3 mL/min. Figure 2B shows the chromatogram of the sample matrix washed through the pre-column monitored by a second diode-array detector in the waste line. Figure 2A shows the chromatogram of the peptides of the digest analyzed on a 2.1 x 100 mm ZORBAX SB-C18 column (3.5 µm).

Due to the generic software control of the valve (figure 3) the method setup for the 2-position/ 6-port valve is very easy. In the *Timetable* in the *Setup Valve* window switching time and the new position are entered. Immediately after the run is finished the valve automatically switches back to its starting position.

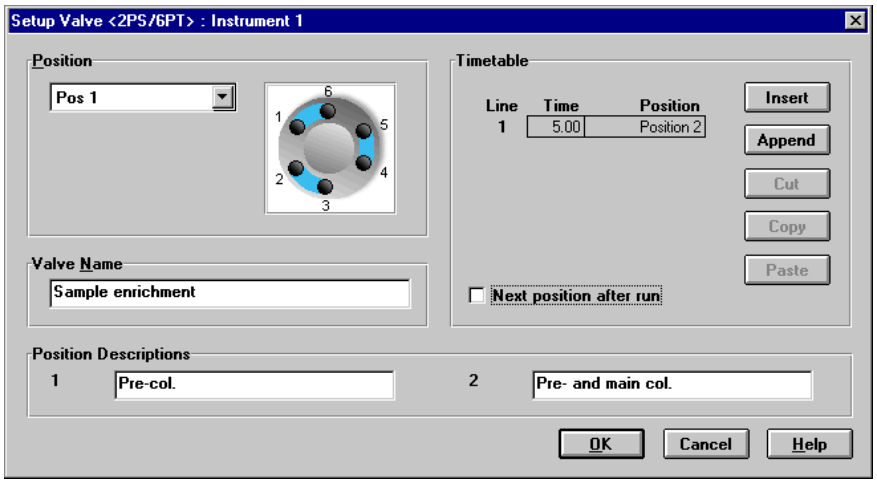

Figure 3
***Setup Valve* window for generic valve control**

Sample stripping

The configuration and plumbing (figure 4) of the system for sample stripping is very similar to the configuration for sample enrichment. The major difference is that the pump, which is connected to the autosampler (pump A) not only delivers the sample to the pre-column but also runs the gradient. Pump B, is only used for back-flushing the pre-column and can therefore be an isocratic pump. After the compounds of interest are transferred from the pre-column to the analytical column the valve removes the pre-column from the flow path of the gradient pump A. While the analysis is performed on the analytical column the pre-column can be back-flushed to waste for cleaning, which enhances the pre-column lifetime.

A typical generic application for sample stripping is to use a small, inexpensive pre-column to protect the more expensive analytical column. This approach is always useful for unknown sample matrixes, containing, for example, reagents from synthesis or residues from sample evaporation.

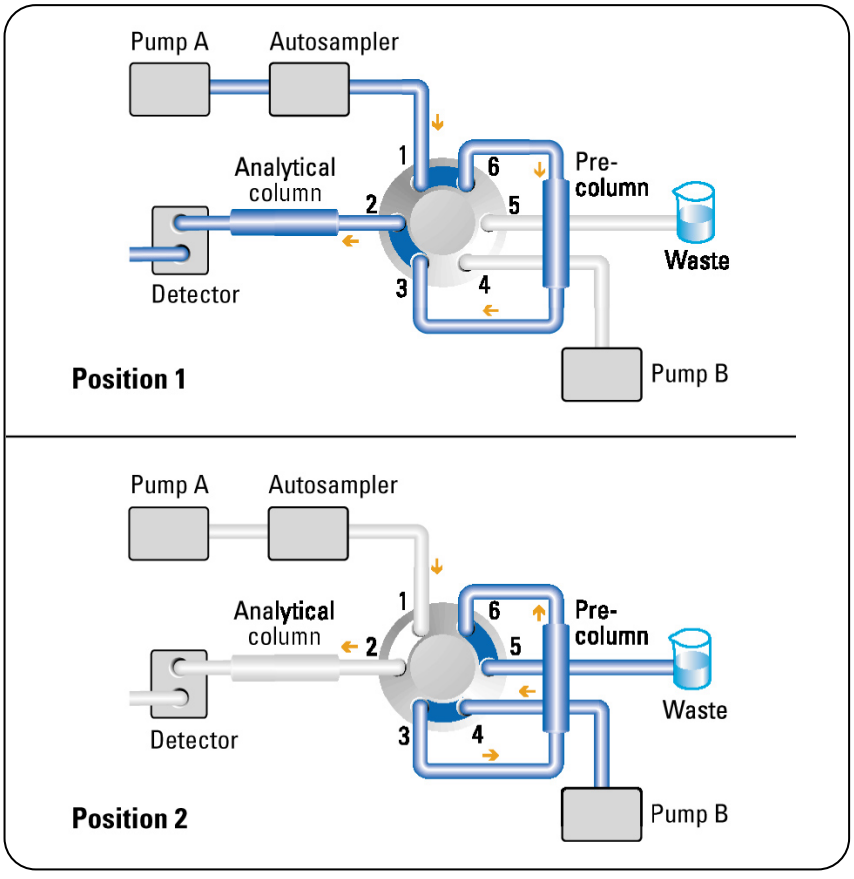

Figure 4
System configuration for sample stripping

Alternative system plumbing with a single pump

A different configuration and plumbing approach for sample enrichment and stripping, which uses a single pump is shown in figures 5A and B. For sample enrichment the valve would be switched into the position shown in figure 5A to load the compounds of interest onto the column and wash the matrix to waste. Then the valve would be switched into the position shown in figure 5B to elute the compounds of interest from the pre-column onto the analytical column. The disadvantage of this setup for sample enrichment is, that the compounds of interest are already partly separated on the pre-column. This means they are applied to the analytical column not in a single spot but over a certain time, which can lead to poorer chromatographic performance.

For sample stripping the valve would first be switched into the position shown in figure 5b to elute the compounds of interest from the pre-column and separate them on the analytical column. After the run the valve would be switched into the position shown in figure 5A to wash the pre-column. One disadvantage of this configuration is that the analytical run and the pre-column washing have to be done sequentially while they can be done in parallel with the configuration shown in the previous paragraph. Also, back-flushing of the pre-column is not possible. Therefore, matrix compounds that do not elute at all from the pre-column might lead to clogging after a few runs.

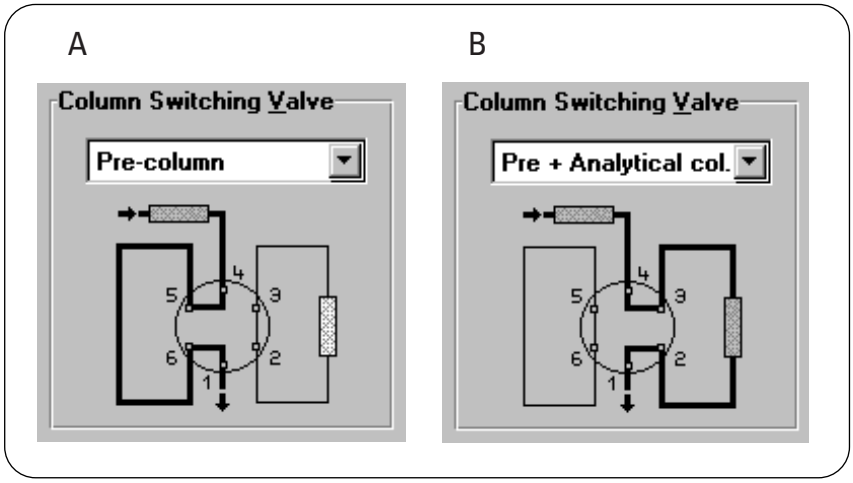

Figure 5
A) Pre-column
B) Pre- and analytical column

Conclusion

In this Application Note we showed two typical applications using a 2-position/6-port valve: sample enrichment and sample stripping. Sample enrichment can be used to pre-concentrate diluted samples on a pre-column before applying the compounds of interest to the analytical column. Furthermore, the sample matrix is washed to waste before the analytical column is switched into the flow path to minimize the contamination of the analytical column, which enhances the column lifetime. While the system configuration for sample stripping is very similar the approach to separate the compounds of interest from the matrix is different. While the matrix is retained on the pre-column the compounds of interest are transferred to the analytical column. During the run the pre-column is removed from the analytical flow path and back-flushed using a second pump. This prevents clogging of the pre-column and enhances its lifetime. Both methods, sample enrichment and sample stripping can be performed either with two pumps or a single analytical pump. Both scenarios were described in this Application Note, discussing advantages and disadvantages.

References

1.
"New dimensions for HPLC applications", *Agilent Technologies Brochure*, *Publication Number 5988-6707EN*, **2002.**

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