Recycle chromatography with the Agilent 1100 Series purification system

Application

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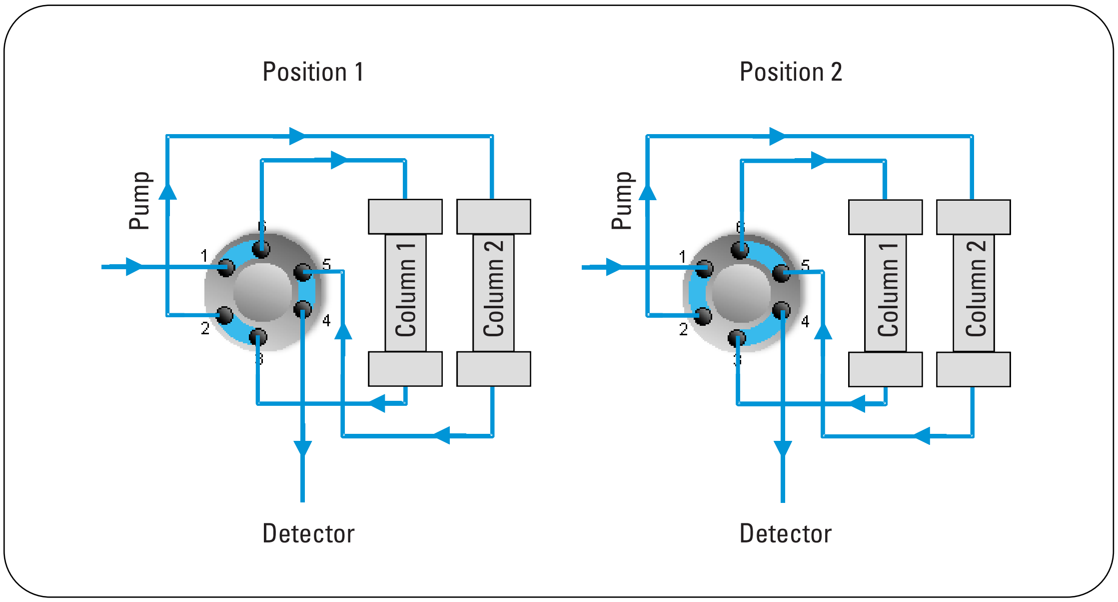

Abstract

This Application Note details how the separation of enantiomers, stereoisomers or diastereomers can be improved by recycle chromatography. The configuration of the system using two identical columns and a 2-position/6-port valve is shown, and the scope and limitations of the system are explained. An application example, which demonstrates the improvement in purity of the collected fractions when using recycle chromatography, is described.

Introduction

The separation of stereoisomers, diastereomers and especially enantiomers is an important but challenging task in compound purification in the pharmaceutical industry. Despite the development of hundreds of different chiral stationary phases over the last decades, finding the right stationary/mobile phase combination for the separation of enantiomers is still a tedious process. Very often only partial resolution of the compounds can be achieved. The usage of longer columns to get better resolution is usually restricted by the higher backpressure those columns generate. Therefore several recycling techniques[1,2] were developed, to send the compounds over the same column several times until sufficient resolution is achieved. The problem with these recycling techniques is, that the compounds are recycled through the complete system including the pump, which leads to strong off-column dispersion. In the referenced literature[3] a recycling method is described using compounds, which are only recycled within two columns and a valve leading to much lower off-column dispersion. In this Application Note we demonstrate how recycle chromatography can be performed on the Agilent 1100 Series purification system[4,5], the system configuration and method setup is described and the separation of two diastereomers is shown as an application example.

Equipment

The experiments were performed on an Agilent 1100 Series purification system containing the following modules:

- Two Agilent 1100 Series preparative pumps
- Agilent 1100 Series dual-loop autosampler PS
- Agilent 1100 Series column organizer
- Agilent 1100 Series multi-wavelength detector
- Agilent 1100 Series fraction collector PS
- Agilent 1100 Series 2-position/6-port valve

The system was controlled using the Agilent ChemStation (rev. B.01.01).

Results and discussion

Operating principle

The operating principle of this recycle chromatography technique is to move the columns in the direction of the flow of the mobile phase as shown in figure 1. After injecting the compounds, they elute from column 1 and are retained on column 2. While the compounds are further separated on column 2, column 1 is moved behind column 2 using a switching valve. After the compounds have eluted from column 2 and are retained on column 1, column 2 is moved behind column 1 and so on. The movement of the columns is achieved using a 2-position/6-port valve, the configuration and the flow connections are shown on the cover page.

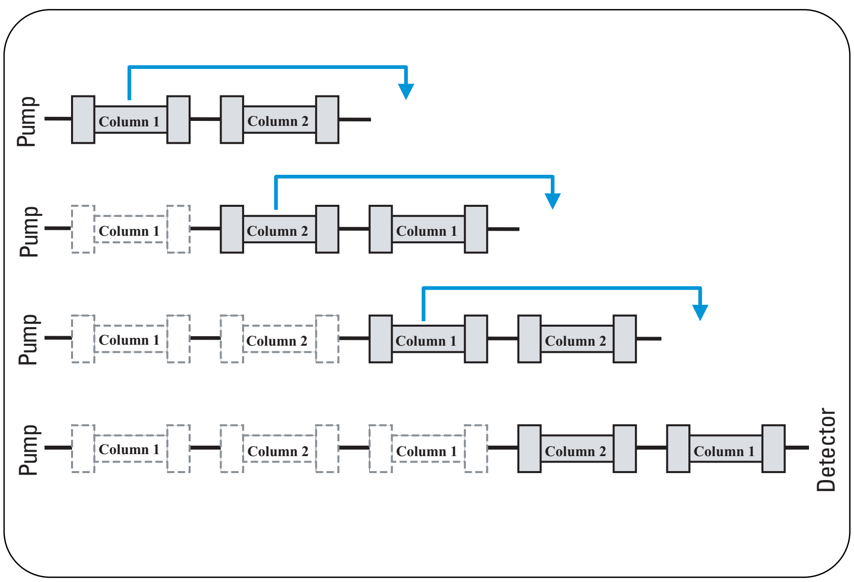

Figure 1
Operating principle of recycle chromatography.

Column:	Agilent Prep C18 21.2 x 50 mm, 5 µm
Mobile phases:	Water = A Acetonitrile = B
Isocratic:	at 42 % B
Flow:	21 ml/min
Injection:	500 µL
Column temp.:	ambient
UV detector:	DAD 245 nm/8 (ref. 360 nm/50) Prep. flow cell (3 mm pathlength)

Chromatographic results

The chromatographic results for an increasing number of virtual columns are shown in figure 2. As you can see the compounds are hardly separated using a single column, however baseline separation could be achieved using six virtual columns and four valve switches. As shown in figure 3 the valve switching times were determined from the experiments for three valve switches, which equals 5 virtual columns.

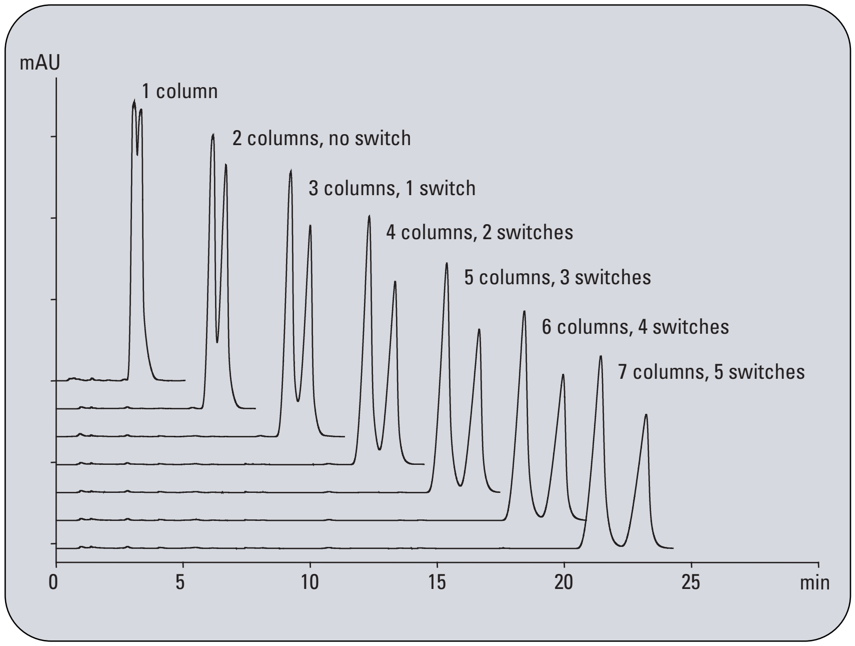

Figure 2
Resolution for various numbers of virtual columns.

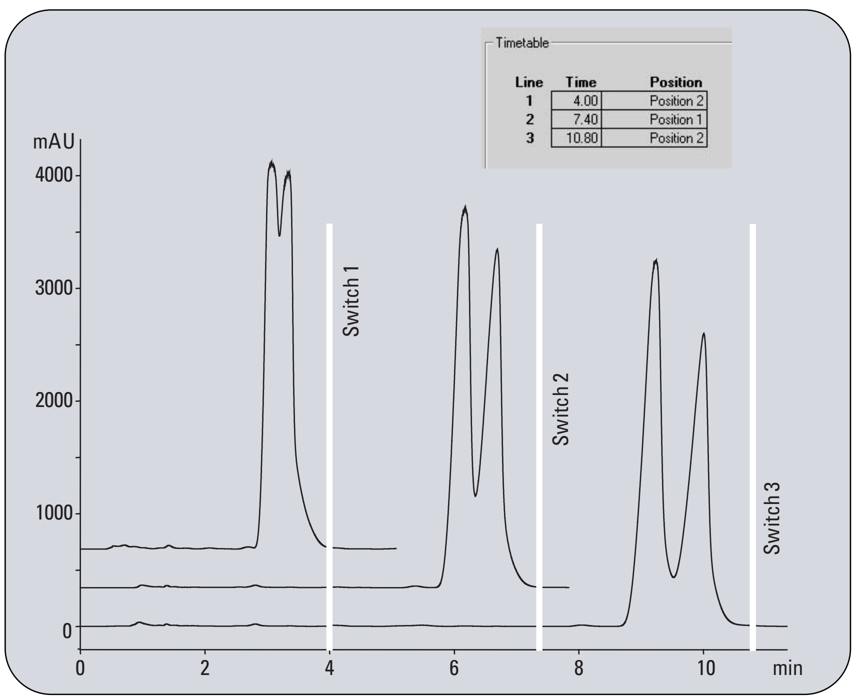

Figure 3
Determination of switching times.

Limitations

- Recycle chromatography can only be used for the separation of compounds with similar retention times, for example enantiomers, diastereomers and stereoisomers in isocratic runs. It is not suitable for gradient runs.
- The number of cycles is limited by the dwell time of the two compounds on one column. Using eight virtual columns peaks are partly cut off if the combined peak width extends the dwell time as shown in figure 4.

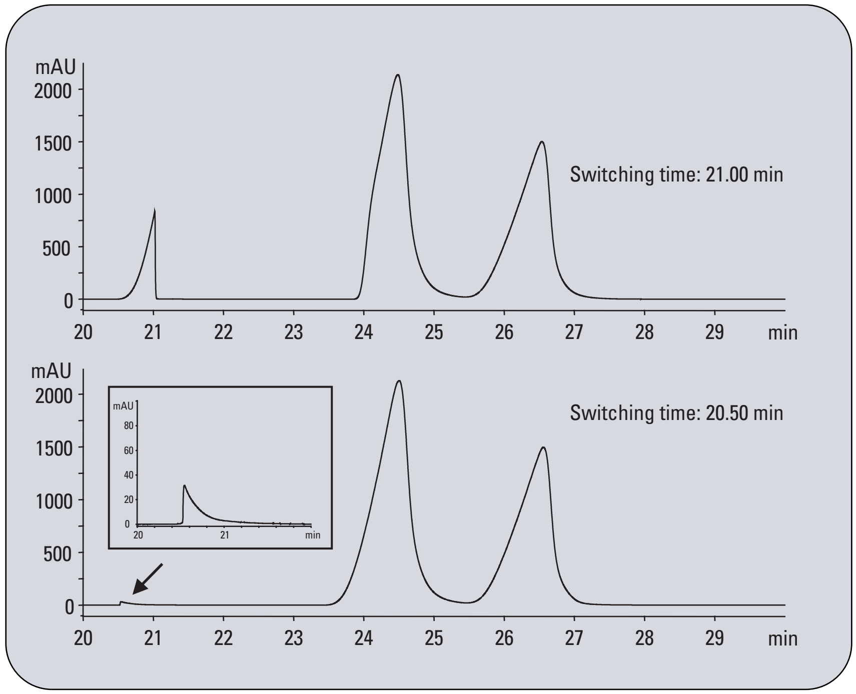

Figure 4
Recycle chromatography with eight virtual columns, six valve switching cycles.

Purification experiment

Figure 5a shows the separation of the steroid isomeres on a single column using peak-based fraction collection based on threshold and slope. The re-analysis of the fractions showed that the purity of the first fraction was 94 %, and the second fraction 84 %. Fraction collection using the recycle chromatography technique with six virtual columns resulted in two fractions of 100 % purity as shown in figure 5b.

Conclusions

In this Application Note we demonstrated how a recycle chromatography method can be performed on the Agilent 1100 Series purification system using a 2-position/6-port valve for the separation of anantiomers, stereoisomers and diastereomers. The limitation regarding switching cycles was shown as well as an application example.

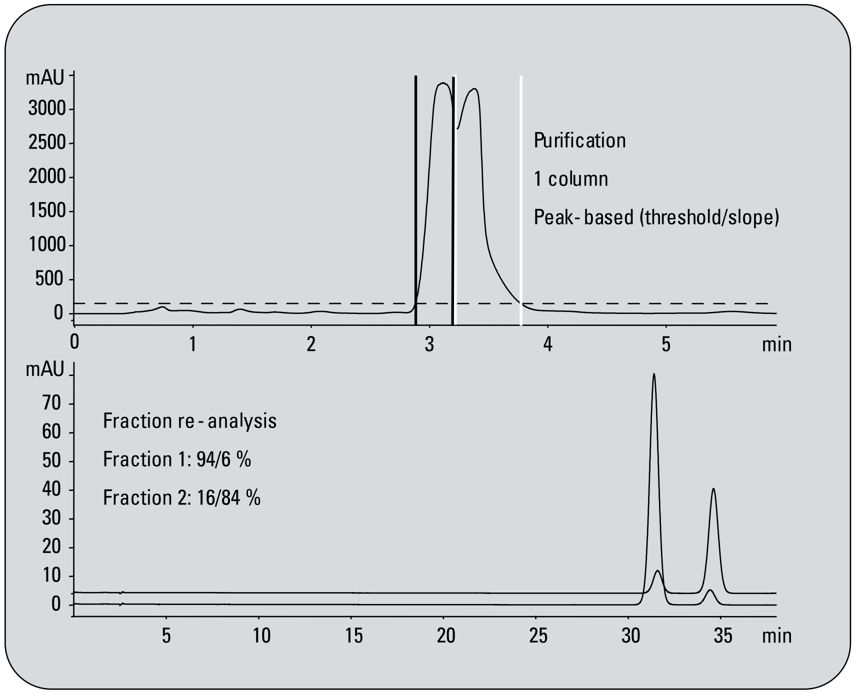

Figure 5a
Peak-based fraction collection using a single column.

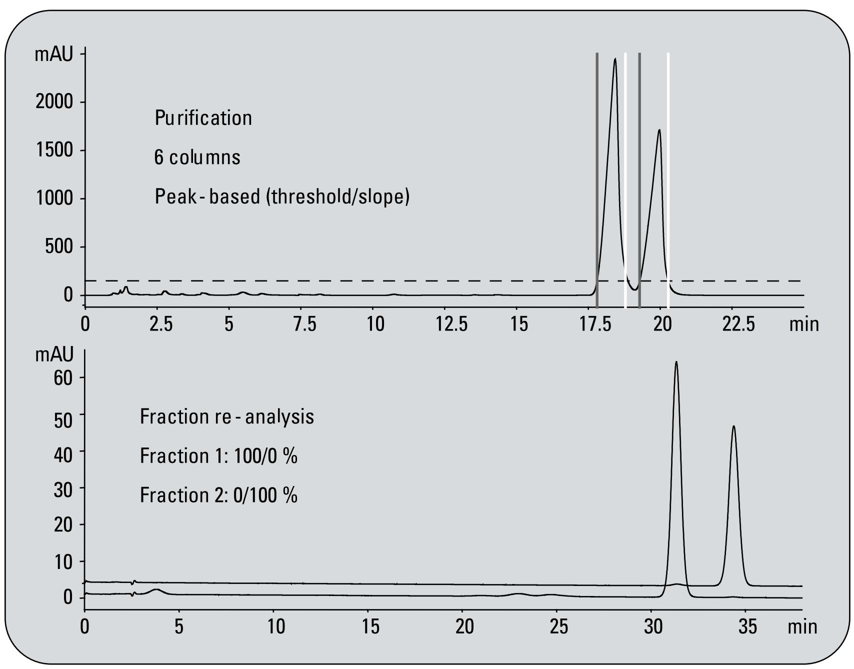

Figure 5b
Peak-based fraction collection using recycle chromatography with six virtual columns.

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www.agilent.com/chem/purification

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