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The Use of Parallel HPLC to Speed Up Chiral Column Screening

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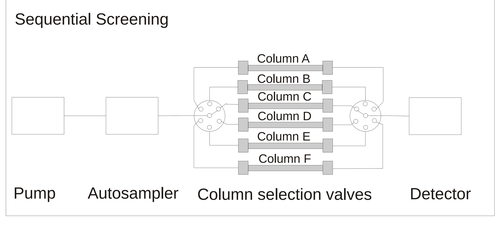
Overview

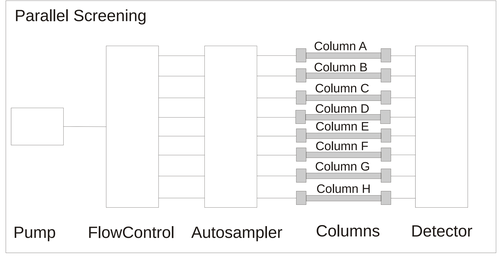
A new approach for improving the throughput in chiral method development is introduced.
The truly parallel HPLC system allows the screening of eight different columns with chiral stationary phases simultaneously. Thus the screening for the optimal separation of chiral samples is eight times faster.
With a specially developed software tool the decision which separation conditions are the best for a given problem is achieved easily.

Introduction

In the absence of any good theory or empirical correlations of chiral columns and the types of molecules separated on any of the chiral stationary phases (CSPs) currently available, the only solution is to screen each separation problem over a number of CSPs with different solvents in order to find separation conditions.
The traditional way to screen the CSPs is testing each column sequentially. The sequential screening can be automed in a standard single HPLC system by the use of column selection valves. With this setup and a runtime of 30 min the screening of 6 different columns with four different solvents each results in a total runtime of 12 hours.



The approach discussed here uses parallel HPLC to screen up to eight different columns simultaneously. In this setup the solvent from one pump is splitted into 8 channels. The FlowControl module guarantees equal flowrates in each channel. The time for the above mentioned experiment with 6 columns and 4 solvents could be reduced from 12 hours to only 2 hours.



Methods

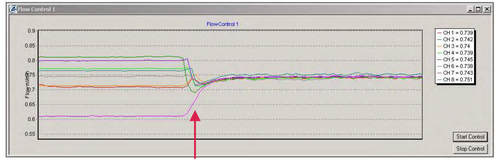
The screening system is assembled from the following modules:
- HPLC Pump 1100, Agilent
- Solvent Selection Valves, Sepiatec
- Sepmatix 8x FlowControl, Sepiatec
- Sepmatix 8x Autosampler (Screening setup), Sepiatec
- Sepmatix 8x DADetector

HPLC Conditions:
Flowrate: 1 ml/min per channel
Eluents: Hexane with different additives Ethanol, Methanol and Isopropanol
Detection: DAD 200-600 nm, online 254 nm
Injection: 20µl in each channel
Columns: 8x 250x4.6 mm with eight different CSPs from different supliers
Sample: 0.1% in Ethanol

Sepmatix System

The optional Screening Setup allows injection of sample from one vial to eight loops.

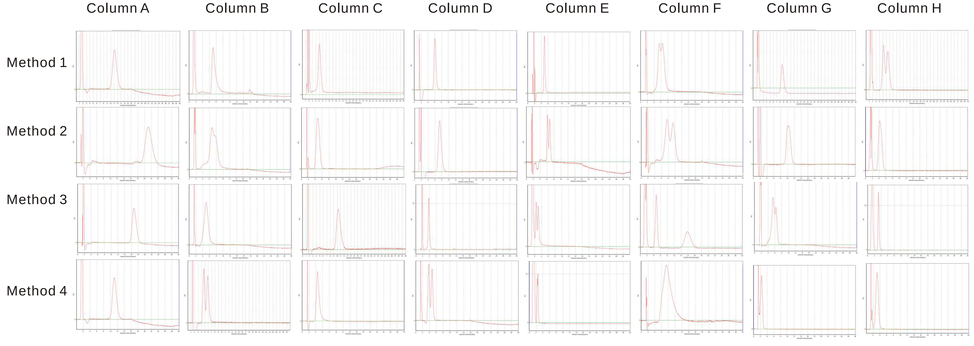
The FlowControl module of the Sepmatix system measures the flow in the columns and regulates it by adjusting the internal backpressure regulators. Due to this automated system, columns and solvents in the setup can be easily exchanged without compromising the equal flowrates in each line.



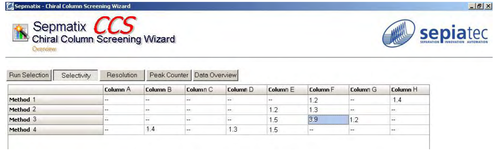
Start of the FlowControl

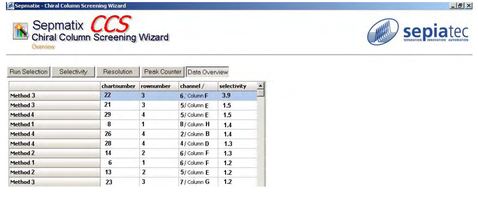
Results

The 32 chromatograms were achieved in 2 hours chromatographic run time. Each channel has a different column, each method uses a different solvent. Since the sample throughput is enlarged, the main bottleneck of the process is the manual evaluation of the data to find the optimal combination of stationary phase and solvent. With the new Chiral Column Screening Wizard it is possible to reprocess up to 80 chromatograms (10 sepmatix runs) in a few minutes. Visual inspection of the chromatograms shows the best separation on Column F with the solvents used in Method 3. Also the resulting parameters from the integration are used to build up a hitlist.



The selectivity is calculated for each run which shows a separation of the enantiomers. Comparison of the calculated values shows the best selectivity on Column F with Method 3.





Conclusions

With the upcoming of more stationary phases for the separation of chiral samples, enhancing the throughput of column screening is a key issue for a thorough test of possible combinations. With parallel HPLC systems the simultaneous screening of columns is possible. Due to the FlowControl module of the suggested system, the exchange of columns between runs is possible without the necessity of manual adjusting of backpressure regulators, which makes the process easier for the operator.

Saving the time of sequential scanning of different columns can either speed up the process or allows to scan the influence of other parameters in the same time. For screening the influence of the temperature, the Sepmatix System can be equipped with the optional column oven.

If the finding of optimal parameters for a separation is reduced to screening, reliable automation is an important tool for systematic work.