Chiral Multicolumn Method Development on the Agilent 1260 Infinity II SFC System

Application Note

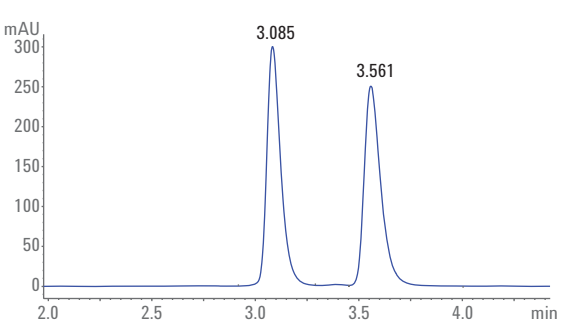
Small Molecule Pharmaceuticals

Author

Edgar Naegele
Agilent Technologies, Inc.
Waldbronn, Germany

Abstract

This Application Note demonstrates the use of the Agilent ChemStation Method Scouting Wizard for the development of a chiral separation method on the Agilent 1260 Infinity II SFC System. The SFC system was equipped with a four-column selection valve and four different chiral columns for scouting runs against different isocratic separation conditions.



Agilent Technologies

Introduction

The separation of enantiomers is one of the main application areas for modern SFC instruments. On a SFC instrument, chiral separations are typically 10 to 20 times faster than their classical separation on normal phase HPLC. In addition, the solvents used for the SFC separation are less harmful, and waste disposal is less expensive than for normal phase solvents.

This Application Note demonstrates software-assisted method development for the separation of enantiomers of a chiral pharmaceutical compound. For this purpose, four chiral columns were used and screened against different isocratic solvent compositions. The necessary methods and all flushing and equilibration steps were created automatically with Agilent ChemStation Method Scouting Wizard.

Experimental

Instrumentation

The Agilent 1260 Infinity II SFC System comprised of the following modules:

- Agilent 1260 Infinity II SFC Control Module (G4301A)
- Agilent 1260 Infinity II SFC Binary Pump (G4782A)
- Agilent 1260 Infinity II SFC Multisampler (G4767A)
- Agilent 1260 Infinity II Diode Array Detector (G7115A) with high-pressure SFC flow cell
- Agilent 1260 Infinity II Multicolumn Thermostat (G7116A)

In addition, the following parts were required to run the SFC system for automated method development:

- Agilent InfinityLab Quick Change 4-position/10-port four-column selection valve (p/n 5067-4287)
- Agilent 1290 Infinity Valve Drive (G1170A) with Agilent InfinityLab Quick Change 12-position/13-port valve (G4235A)
- Capillary kit for method development with four-column selection valve (p/n 5067-6596)

Instrumental setup

The Agilent SFC Binary Pump was clustered with an Agilent InfinityLab Quick Change 12-position/13-port valve for the selection of up to 12 different solvents in the Instrument Configuration dialog of the Agilent OpenLAB CDS ChemStation Edition software. The solvents were defined in the Pump Setup dialog. For the experiments described in Results and Discussion, only one of the solvents was used.

The Agilent 1260 Infinity II Multicolumn Thermostat was equipped with the Agilent InfinityLab Quick Change 4-position/10-port four-column selection valve, and the columns were set up in the Instrument Configuration dialog of the OpenLAB CDS ChemStation Edition software. With the method development capillary kit, up to four columns could be used. The columns were entered in the Columns Table, and assigned in the MCT dialog. All columns can be used with ID Tags (p/n 5067-5917) for automated recognition in ChemStation and assignement in the MCT dialog.

All methods necessary for column and gradient screening as well as instrument flushing and column equilibration were created with the Method Scouting Wizard (Figure 1).

Columns

- Chiral Technologies, Chiralpak IA, 4.6 × 250 mm, 5 µm
- Chiral Technologies, Chiralpak IB, 4.6 × 250 mm, 5 µm
- Chiral Technologies, Chiralpak IC, 4.6 × 250 mm, 5 µm
- Chiral Technologies, Chiralpak ID, 4.6 × 250 mm, 5 µm

Software

Agilent OpenLAB CDS ChemStation Edition for LC and LC/MS Systems, version C.01.07 SR3, including LC and CE Drivers A.02.16 with Agilent ChemStation Method Scouting Wizard, version A.02.07

Sample

Propranolol, 1 mg/mL, in methanol.

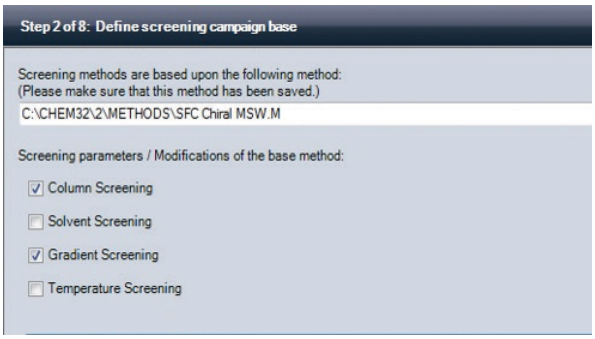
Chemicals

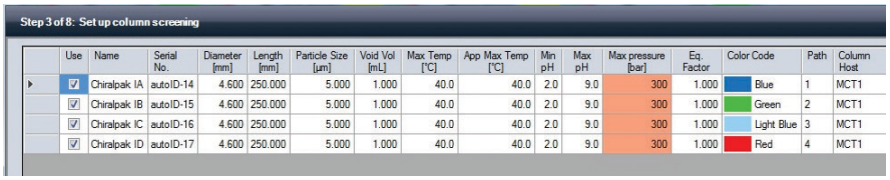
All solvents were purchased from Merck, Germany. Chemicals were purchased from Sigma-Aldrich (Germany).

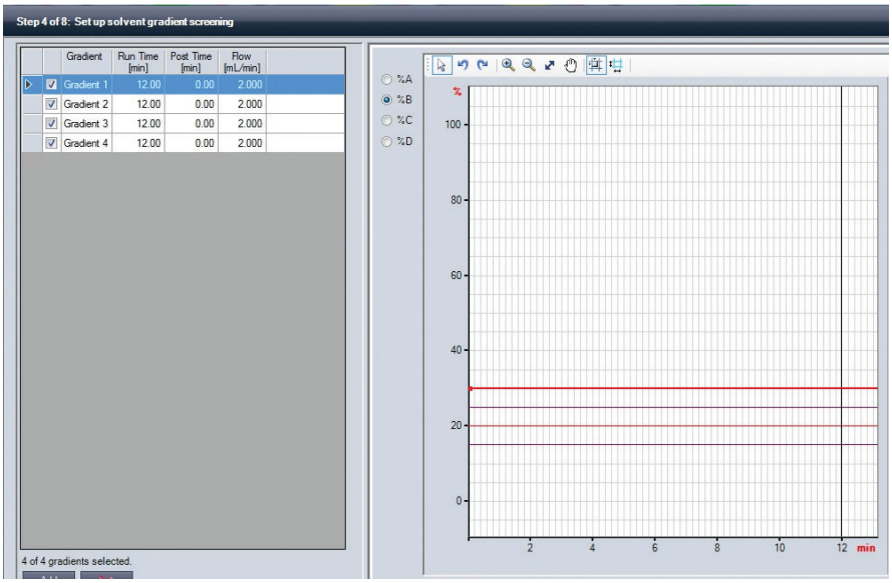
SFC method

Parameter	Value
Solvent A	CO_2
Modifier B	Methanol + 0.1 % diethyl amine
SFC flow	2.0 mL/min
Isocratic elution	15, 20, 25 and 30 % modifier
Stop time	12 minutes
Backpressure regulator (BPR) temperature	60 °C
BPR pressure	140 bar
Column temperature	30 °C
Injection volume	5 µL
Feed solvent	Methanol; feed speed 400 µL/min; over feed volume 4 µL
Needle wash	3 seconds with methanol
Diode array detection	230 nm/bandwidth 4 nm; reference 360 nm/bandwidth 100 nm; data rate 10 Hz

Method Scouting Wizard setup







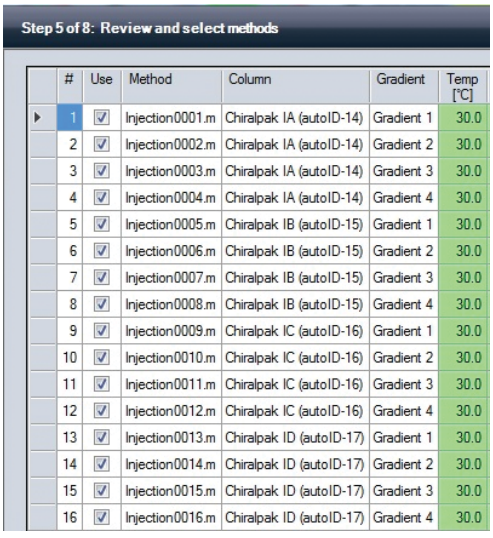


Figure 1. The Method Scouting Wizard enables the setup of the described chiral screening campaign in a 10-step procedure. This setup defines, for example, the different screening options in Step 2, here the column and gradient screening. All other parameters will be used as setup in the chosen main method. In Step 3, the columns will be selected for the column screening. Some parameters such as maximum pressure and maximum temperature will be defined here and later compared to the final method for the elimination of incompatibilities. Step 4 defines the applied gradients, here the choice of four isocratic conditions. Step 5 enables review of the methods that will be created. The other steps (not shown) will define the flushing and equilibration methods as well as sample positons.

Results and Discussion

In the described chiral screening campaign, four different chiral columns were used with the strong eluting solvent, methanol, as the modifier. The goal of the campaign was the identification of a fast method with a good baseline separation of the propranolol enantiomers within a maximum 6 minutes run time. Therefore, four isocratic compositions containing 15, 20, 25, and 30 % methanol were used. On chiral column IA, it is seen that the separation of the two enantiomers started already at 30 % methanol at a retention time of 3.25 and 3.41 minutes. However, a near baseline separation was not achieved until 15 % methanol, and at a retention time of 7.68 and 8.36 minutes. Unfortunately, this compromised the peak shape, the peak height decreased, and the absolute run time was relatively high (Figure 2).

The result of the screening showed good separation for the chiral column IB (Figure 3). The enantiomers were well separated at the baseline with 30 % methanol at 4.49 and 5.44 minutes. The retention time and the distance of both peaks increased with the decreasing content of the methanol modifier, but the peak shape was still acceptable.

By means of the chiral column IC with methanol as modifier, no separation of the propranolol enantiomers could be seen (Figure 4).

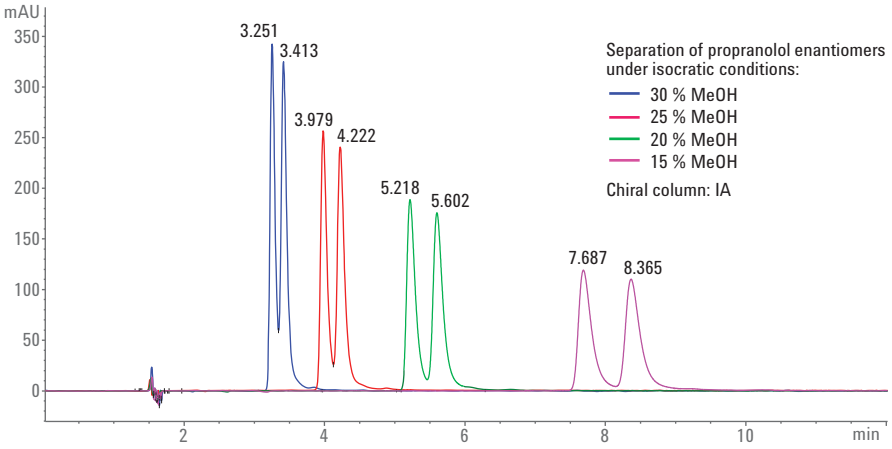


Figure 2. Separation of propranolol enantiomers on the chiral column IA with methanol as modifier.

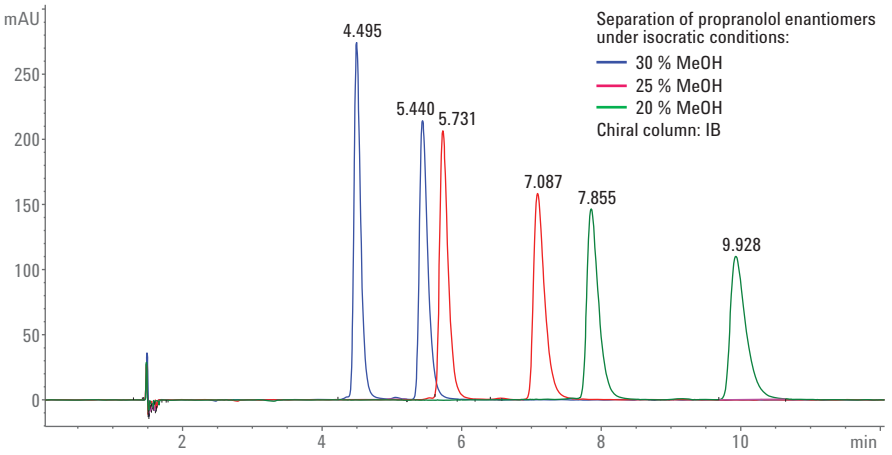


Figure 3. Separation of propranolol enantiomers on the chiral column IB with methanol as modifier.

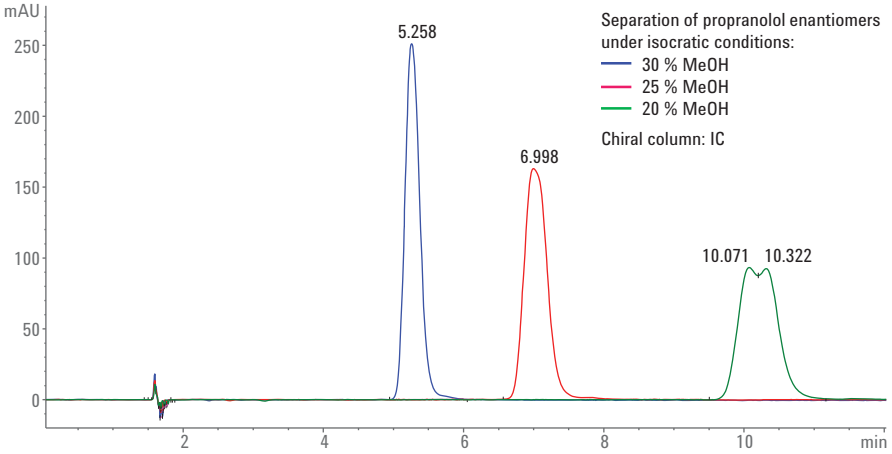


Figure 4. Separation of propranolol enantiomers on the chiral column IC with methanol as modifier.

The separation of the propranolol enantiomers started at 3.0 minutes on chiral column ID with 30 % methanol, and showed a valley at half the peak height for 25 % methanol (Figure 5). No real baseline separation could be achieved for the separation on this column.

For further optimization, chiral column IB was chosen, because there was enough distance between the peaks to increase the speed of this separation, even at high methanol content. After an increase of the flow rate from 2.0 to 2.5 mL/min, and an increase in column temperature from 30 to 40 °C, the retention times could be shifted from the range of 4.0 to 6.0 minutes down to 3.0 to 3.7 minutes (Figure 6). This yielded a short final run time of 4 minutes and the calculated retention time RSD values were 0.11 and 0.13 %, respectively.

The well separated enantiomers also offered the possibility to purify them easily on an analytical scale, and collect the enantiomers in an enantiomerically pure form in single flasks. To optimize the separation process for highest yield, a highly concentrated solution or a high injection volume was used. In this example, a high injection volume of 80 µL was used, which can be achieved by the SFC multisampler[1]. The goal of such an experiment is to find a method that overloads the column by the high concentration, but still has a sufficient separation for the collection of fractions (Figure 7). The identified method uses an isocratic composition with 25 % methanol as modifier, and separates the enantiomers on the highly overloaded column at 5.9 and 6.8 minutes with baseline separation for best fraction collection.

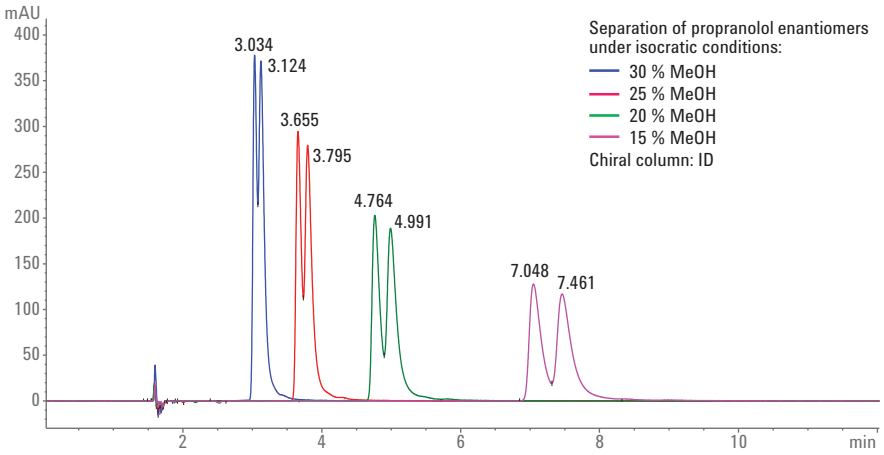


Figure 5. Separation of propranolol enantiomers on the chiral column ID with methanol as modifier.

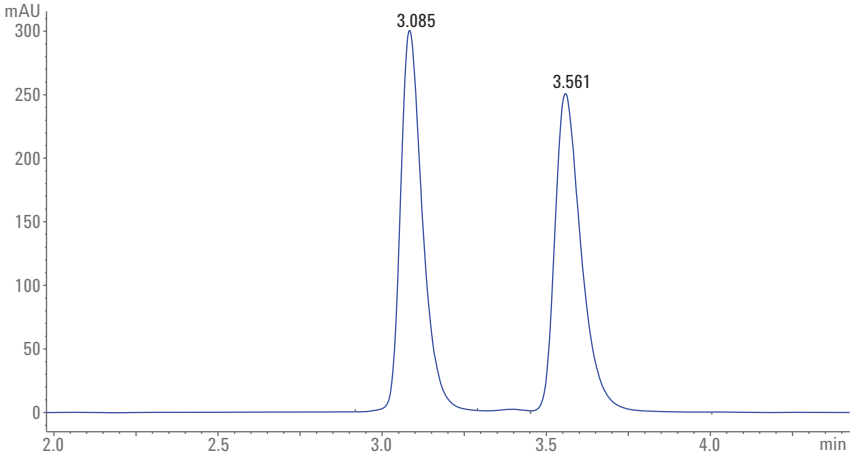


Figure 6. Final speed-optimized analytical separation method of propranolol enantiomers (flow rate: 2.5 mL/min, temperature: 40 °C, organic solvent: 30 % MeOH).

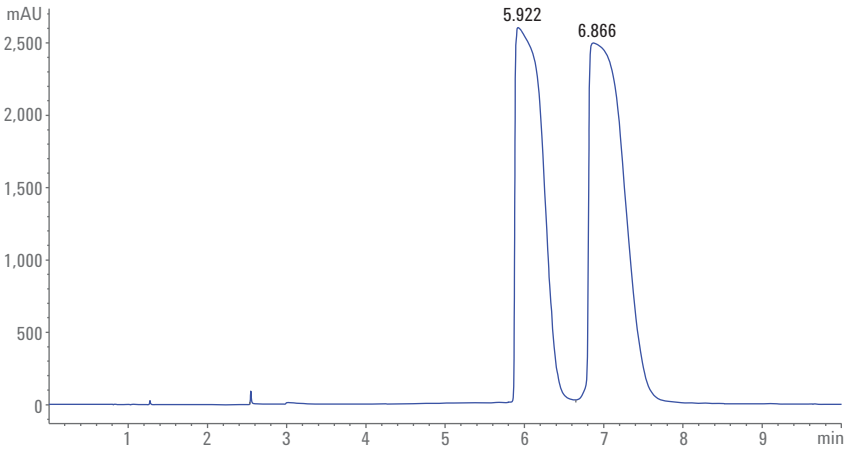


Figure 7. Column overloading experiment for the analytical preparative separation of the enantiomers of propranolol. The injected sample volume was 80 µL and the baseline separation occurred under isocratic conditions with 25 % methanol (feed speed: 100 µL/min, gradient: 5 %B at 0 minutes, 5 %B at 1.0 minutes, 25 %B at 1.1 minutes, stop time: 10 minutes).

Conclusion

This Application Note demonstrates the use of the Agilent 1260 Infinity II SFC System with the Agilent Method Scouting Wizard for software-aided method development. Four chiral columns were automatically screened under different isocratic conditions for the rapid identification of best separation conditions. After a quick optimization of the identified conditions, a separation of two enantiomers in under 4 minutes could be achieved. This is typically a factor of 10-times faster than the classical separation of enantiomers by normal phase chromatography. Finally, it is shown that the identified separation method could also be used for the analytical preparative separation of enantiomers.

Reference

1. Naegele, E. Supercritical Fluid Chromatography with Flexible Injection Volumes at Highest Precision, *Agilent Technologies Technical Overview*, publication number 5991-7623EN, **2017**.

www.agilent.com/chem

Published in the USA, October 15, 2017
5991-7624EN

Agilent Technologies