Method Development to Separate Enantiomers by Supercritical Fluid Chromatography

The Agilent 1260 Infinity Analytical SFC Solution and Agilent Method Scouting Wizard

Application Note

Small Molecule Pharmaceuticals and Generics

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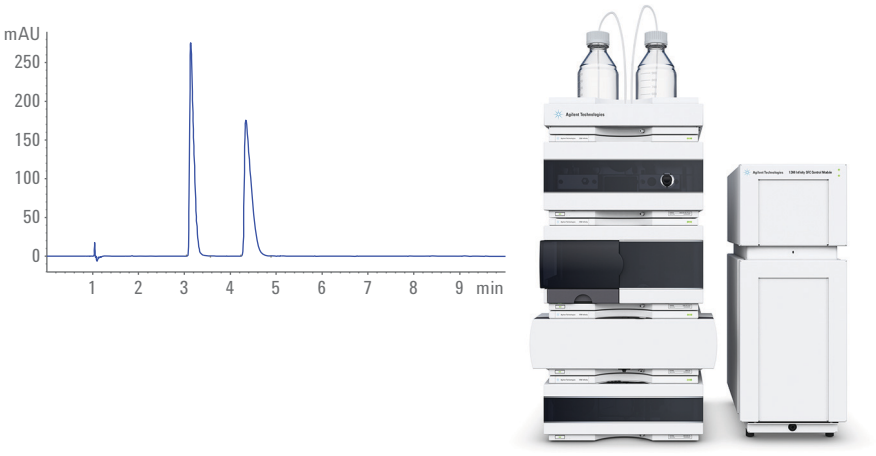
Abstract

This Application Note demonstrates the use of the Agilent 1260 Infinity Analytical SFC Solution for method screening for the separation of enantiomers. To enhance the 1260 Infinity SFC Solution for method development and screening, a second thermostatted column compartment and two column selection valves were added for column selection, together with a 12-position/13-port valve for solvent selection. The screening methods, with combinations of columns, solvents, and gradients, were set up easily with the Agilent ChemStation Method Scouting Wizard.



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Introduction

A large number of pharmaceutical compounds, for example, have one or more inherent stereogenic centers. This causes stereo isomerism, where the isomers act like mirror images. In addition, the isomers often have different physiological effects. In extreme cases, one isomer can act as a pharmaceutical substance and the other one be very harmful.

Unfortunately, stereo isomeric compounds cannot be separated by typical HPLC phases such as reversed-phase C18 to determine their relative composition. For their separation, chiral phases have to be used, which contain, for example, modified cellulose on their surface. In the past, the technique of choice for chiral analysis was normal-phase HPLC, with all its attendant problems such as long run times, unreproducible retention times, and dangerous solvents. Nowadays, SFC has proven to be an adequate alternative technique without these major drawbacks.

Since the number of new drug candidates with stereo isomers increases, fast and reliable method screening for their separation is important. This can be done by an Agilent 1260 Infinity Analytical SFC Solution with added method development capabilities. The SFC system is equipped with an additional thermostatted column compartment and two column selection valves for the use of up to four 250-mm chiral columns, a solvent selection valve for up to 12 different solvents, and a Method Scouting Wizard for fast setup of a large number of different screening methods in the ChemStation software.

Experimental

Instruments

Agilent 1260 Infinity Analytical SFC Solution (G4309A) comprising:

- Agilent 1260 Infinity SFC Control Module
- Agilent 1260 Infinity SFC Binary Pump
- Agilent 1260 Infinity High Performance Degasser
- Agilent 1260 Infinity SFC Standard Autosampler
- Agilent 1260 Infinity DAD with high pressure SFC flow cell
- Agilent 1290 Infinity Thermostatted Column Compartment (TCC) with valve drive

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

- Agilent 1290 Infinity Thermostatted Column Compartment (TCC) (G1316C) with valve drive
- Two 1200 Infinity Series Quick-Change 8-position/9-port valves (G4230A)
- Agilent 1290 Infinity Valve Drive (G1170) with 1200 Infinity Series Quick-Change 12-position/13-port valve (G4235A)
- Capillary kit for method development (p/n 5067-1595)

Instrument setup

The SFC binary pump was used with a 12-position/13-port valve for solvent selection in the Agilent OpenLAB CDS ChemStation Edition instrument configuration. The solvents were defined in the software's pump setup menu. The two TCCs were also employed within the OpenLAB CDS ChemStation Edition instrument configuration and equipped with 8-position/9-port valves each for column selection. With the method development capillary kit, up to eight columns could be used. The columns were entered into the ChemStation column database and configured in the ChemStation TCC menu.

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 & LC/MS Systems, Rev. C.01.05 with Agilent ChemStation Method Scouting Wizard, Version A02.03 (G2196AA).

Chemicals

All solvents were purchased from Merck, Germany and chemicals from Sigma-Aldrich, Corp., Germany. Fresh ultrapure water was obtained from a Milli-Q Integral system equipped with a LC-Pak Polisher and a 0.22-µm membrane point-of-use cartridge (Millipak).

SFC conditions

Parameter	Value
Solvent A	CO_2
Modifier B	Methanol, ethanol, isopropanol, acetonitrile, all with 0.1 % diethyl amine
SFC flow	3 mL/min
Isocratic elution	80 % CO_2 with 20 % modifier
Stop time	10 minutes
Backpressure regulator (BPR) temperature	60 °C
BPR pressure	120 bar
Column temperature	20 °C
Injection volume	5 µL, 3 × loop over fill
Needle wash in vial with methanol	
DAD	230 nm/bandwidth 4 nm, ref. 360 nm/bandwidth 100 nm, data rate: 10 Hz

Results and Discussion

The stereo isomeric compound metoprolol was used to demonstrate screening for chiral separation with the Agilent 1260 Infinity Analytical SFC Solution (Figure 1).

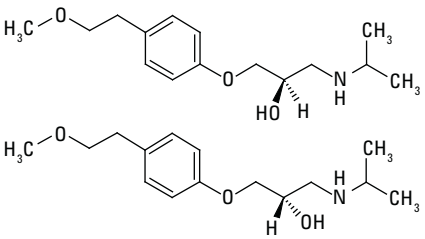
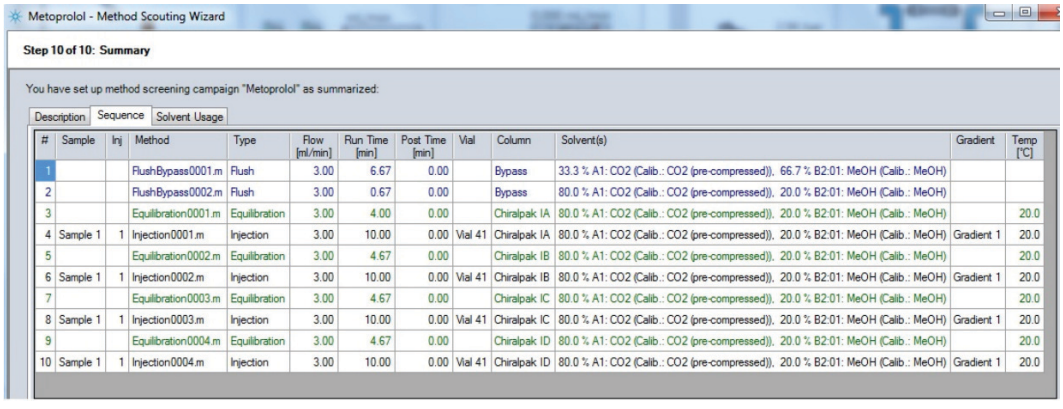


Figure 1. Stereo isomers of metoprolol.

The SFC was equipped with two clustered thermostatted column compartments for the selection of four chiral 250-mm columns and a pump/valve cluster for the selection of up to 12 solvents. To achieve better separation and peak shape for the basic compound metoprolol, diethyl amine was used as an additive (for an acidic compound, acetic acid or TFA would be an appropriate additive). A screening of the four columns with one solvent took approximately 1 hour, including all solvent exchange and equilibration steps (Figure 2).



#	Sample	Inj	Method	Type	Flow [ml/min]	Run Time [min]	Post Time [min]	Vial	Column	Solvent(s)	Gradient	Temp [°C]
1			FlushBypass0001.m	Flush	3.00	6.67	0.00		Bypass	33.3 % A1: CO2 (Calib.: CO2 (pre-compressed)), 66.7 % B2:01: MeOH (Calib.: MeOH)		
2			FlushBypass0002.m	Flush	3.00	0.67	0.00		Bypass	80.0 % A1: CO2 (Calib.: CO2 (pre-compressed)), 20.0 % B2:01: MeOH (Calib.: MeOH)		
3			Equilibration0001.m	Equilibration	3.00	4.00	0.00		Chiralpak IA	80.0 % A1: CO2 (Calib.: CO2 (pre-compressed)), 20.0 % B2:01: MeOH (Calib.: MeOH)		20.0
4	Sample 1	1	Injection0001.m	Injection	3.00	10.00	0.00	Vial 41	Chiralpak IA	80.0 % A1: CO2 (Calib.: CO2 (pre-compressed)), 20.0 % B2:01: MeOH (Calib.: MeOH)	Gradient 1	20.0
5			Equilibration0002.m	Equilibration	3.00	4.67	0.00		Chiralpak IB	80.0 % A1: CO2 (Calib.: CO2 (pre-compressed)), 20.0 % B2:01: MeOH (Calib.: MeOH)		20.0
6	Sample 1	1	Injection0002.m	Injection	3.00	10.00	0.00	Vial 41	Chiralpak IB	80.0 % A1: CO2 (Calib.: CO2 (pre-compressed)), 20.0 % B2:01: MeOH (Calib.: MeOH)	Gradient 1	20.0
7			Equilibration0003.m	Equilibration	3.00	4.67	0.00		Chiralpak IC	80.0 % A1: CO2 (Calib.: CO2 (pre-compressed)), 20.0 % B2:01: MeOH (Calib.: MeOH)		20.0
8	Sample 1	1	Injection0003.m	Injection	3.00	10.00	0.00	Vial 41	Chiralpak IC	80.0 % A1: CO2 (Calib.: CO2 (pre-compressed)), 20.0 % B2:01: MeOH (Calib.: MeOH)	Gradient 1	20.0
9			Equilibration0004.m	Equilibration	3.00	4.67	0.00		Chiralpak ID	80.0 % A1: CO2 (Calib.: CO2 (pre-compressed)), 20.0 % B2:01: MeOH (Calib.: MeOH)		20.0
10	Sample 1	1	Injection0004.m	Injection	3.00	10.00	0.00	Vial 41	Chiralpak ID	80.0 % A1: CO2 (Calib.: CO2 (pre-compressed)), 20.0 % B2:01: MeOH (Calib.: MeOH)	Gradient 1	20.0

Figure 2. Summary screen of the Agilent Method Scouting Wizard showing all individual runs for screening a chiral compound against four chiral columns and methanol as solvent. The sequence included flushing procedures, equilibration runs, and sample runs with a total run time of 1.1 hours.

The same time is needed for the screening of one solvent with four columns. In a first experiment, methanol was used as the solvent, and the sample was run on all four columns (Figure 3). It can be seen that column IA was not able to resolve the stereoisomers under the chosen screening conditions. Column IB immediately resolved both isomers. Column IC did not resolve the enantiomers and produced unusable peak shapes. Column ID again was able to resolve the metoprolol enantiomers but, compared to column IB, the isomers eluted at a later retention time with broader peaks but still with baseline resolution.

In the second screening experiment, ethanol was used as a solvent for all four columns (Figure 4). Column IA started to show initial separation of both metoprolol enantiomers with this solvent of weaker polarity. As before, the enantiomers were also clearly separated with ethanol on column IB but with higher retention times and slightly broader peaks. Column IC also showed partially separated peaks with typical peak shapes. On column ID, the retention time shifted towards the end of the screening run and peaks were partially resolved.

The screening experiment with isopropanol showed very broad and partially resolved peaks on columns IA and IC (Figure 5). On column IB, the peaks were still resolved but eluted with a very broad peak width of approximately 1 minute. On column ID, the peaks eluted from the column after the finish of the chosen run time.

The screening of the separation of the metoprolol enantiomers with acetonitrile showed no usable results (data not shown).

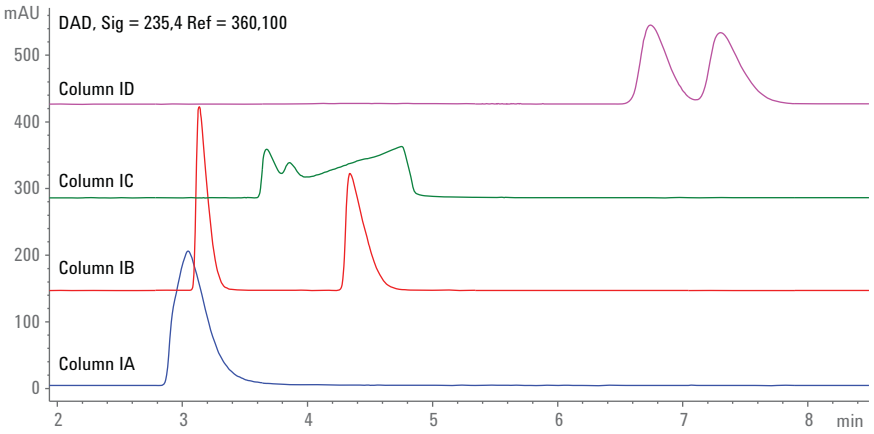


Figure 3. Separation screening of metoprolol enantiomers on four chiral columns under identical conditions using methanol (+ 0.1 % diethyl amine) as modifier.

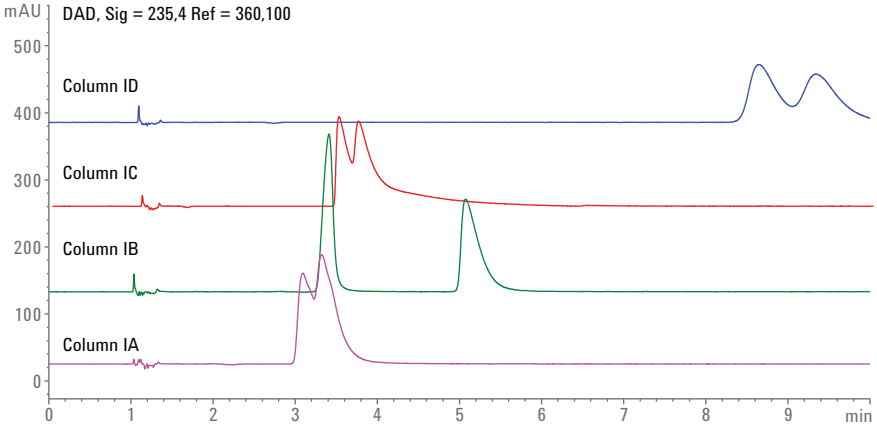


Figure 4. Separation screening of metoprolol enantiomers on four chiral columns under identical conditions using ethanol (+ 0.1 % diethyl amine) as modifier.

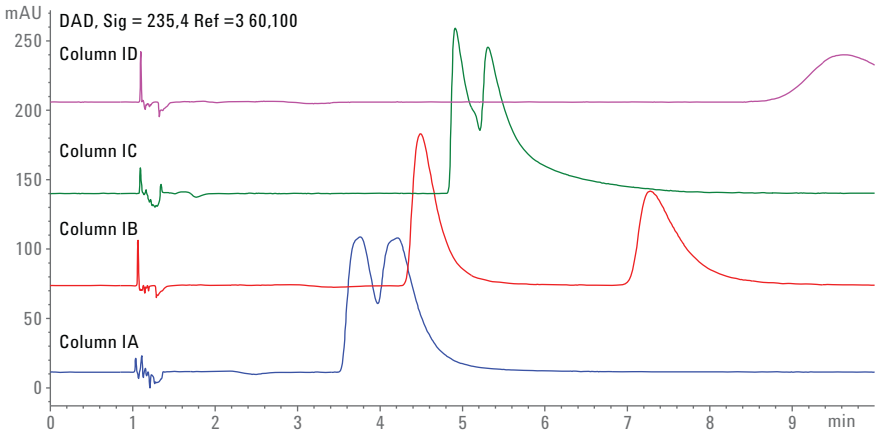


Figure 5. Separation screening of metoprolol enantiomers on four chiral columns under identical conditions using isopropanol (+ 0.1 % diethyl amine) as modifier.

The best series of separations of the metoprolol enantiomers was achieved on column IB. This is displayed for the different solvents in Figure 6.

- **A**, methanol, provided earliest retention and good peak shapes
- **B**, ethanol, resulted in higher retention and slightly broader peaks
- **C**, isopropanol, produced much broadened peaks and tailing

The separation with methanol provided earliest retention and good peak shapes. While the separation by means of ethanol resulted in higher retention times and slightly broader peaks, the separation with isopropanol resulted in much broadened peaks and tailing.

Finally, a series of 10 runs was done on column IB by means of methanol for statistical evaluation (Table 1). The retention time RSD was 0.15 %, and the area RSD < 1.3 %.

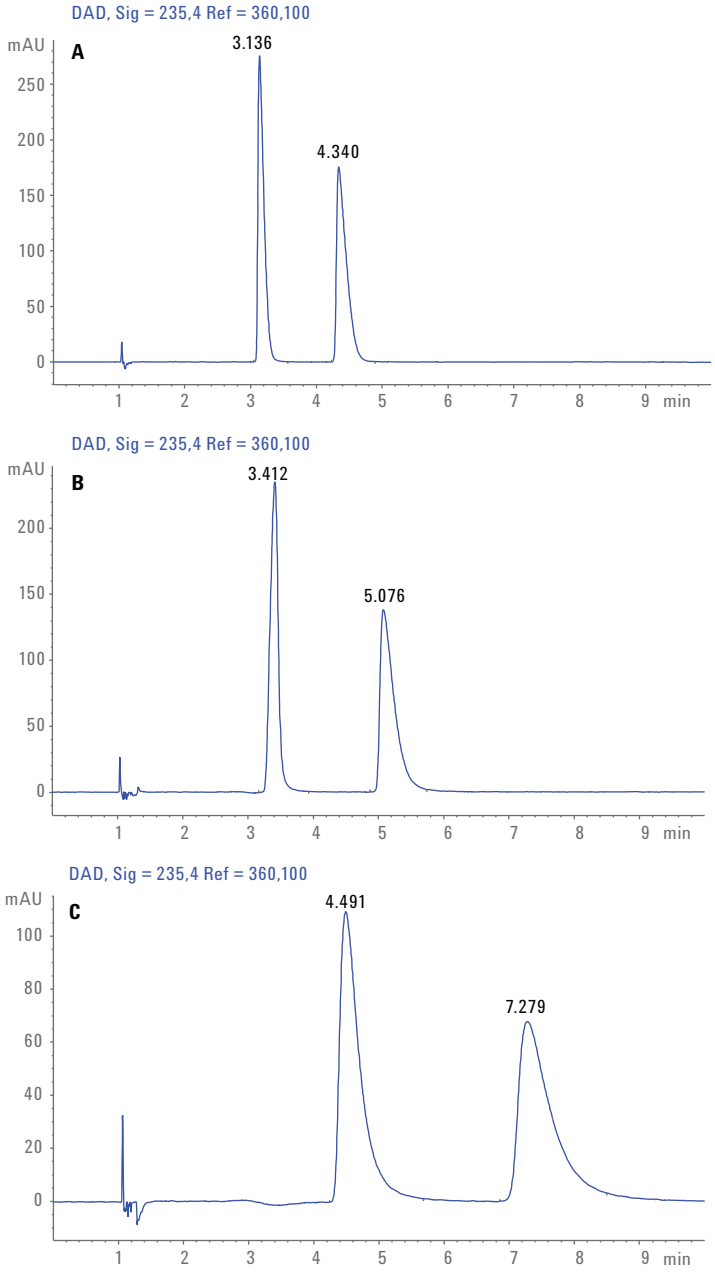


Figure 6. Separation of metoprolol enantiomers on chiral column IB with different solvents.

Conclusions

This Application Note describes the use of the Agilent 1260 Infinity Analytical SFC Solution as a method development and screening system for the separation of chiral compounds. For this application, the SFC system was enhanced with two TCCs for the use of up to eight different columns, and the SFC pump was used with a solvent selection valve for the combination of the columns with up to 12 different solvents. The results show the screening of a chiral compound on four chiral columns and four possible solvents for the separation of the enantiomers. The column showing the best separation performance was identified. After the best separation method was found, RSD values of retention time and area were determined.

Table 1. Statistical evaluation of retention time and peaks area from 10 runs.

	Enantiomer 1		Enantiomer 2	
	RT (min)	Area	RT (min)	Area
av	3.139	1910.90	4.354	1986.26
std	0.00422	24.45917	0.00637	23.31438
RSD %	0.14	1.28	0.15	1.17

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Published in the USA, September 1, 2014
5991-5145EN

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