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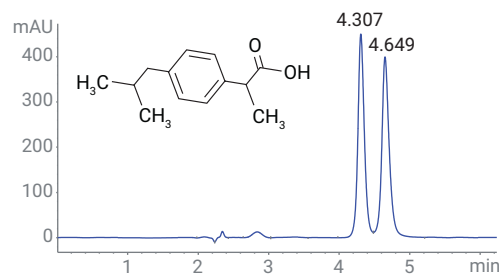
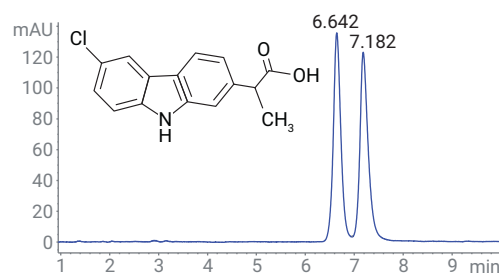
# Chiral Screening for SFC and UHPLC with the Agilent 1260 Infinity II SFC/UHPLC Hybrid System

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## Abstract

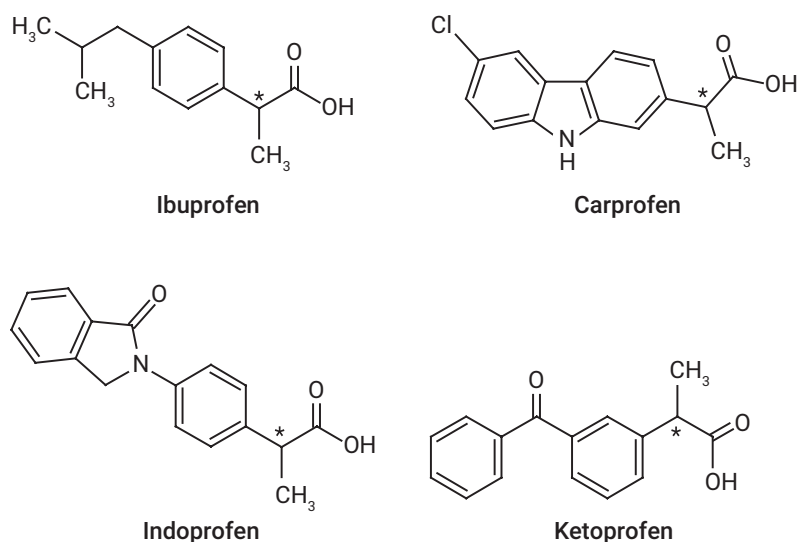
This Application Note demonstrates the use of an Agilent 1260 Infinity II SFC/UHPLC Hybrid System for fast method development in SFC and UHPLC mode by means of the Agilent Method Scouting Wizard software. As an example, a series of racemic pharmaceutical mixtures was screened using columns with identical chiral stationary phases in SFC and UHPLC mode, and the results of separations compared.



## Introduction

The modern 1260 Infinity II SFC/UHPLC Hybrid System can be operated robustly and reliably in SFC or UHPLC mode with the same level of performance as standalone SFC and UHPLC systems. This enables switching between SFC and UHPLC modes within one sequence of samples<sup>1</sup>. Commonly, standalone SFC and UHPLC instruments are used for software-aided method scouting during development of analytical separation methods<sup>2</sup>. Modern SFC/UHPLC hybrid systems could be used for this task in both modes. This provides an economical approach to collect initial scouting data for the analytical method development process.

This Application Note demonstrates the use of the 1260 Infinity II SFC/UHPLC Hybrid System in analytical method development with software-aided method scouting in SFC and UHPLC mode. As an example, the fast scouting of different chiral compounds (Figure 1) using several combinations of chiral columns and solvents is presented, and subsequent method optimization is discussed.



**Figure 1.** Chiral compounds used in this study (the asterisk indicates the chiral center).

## Experimental

### Instrumentation

Agilent 1260 Infinity II SFC/UHPLC Hybrid System comprises:

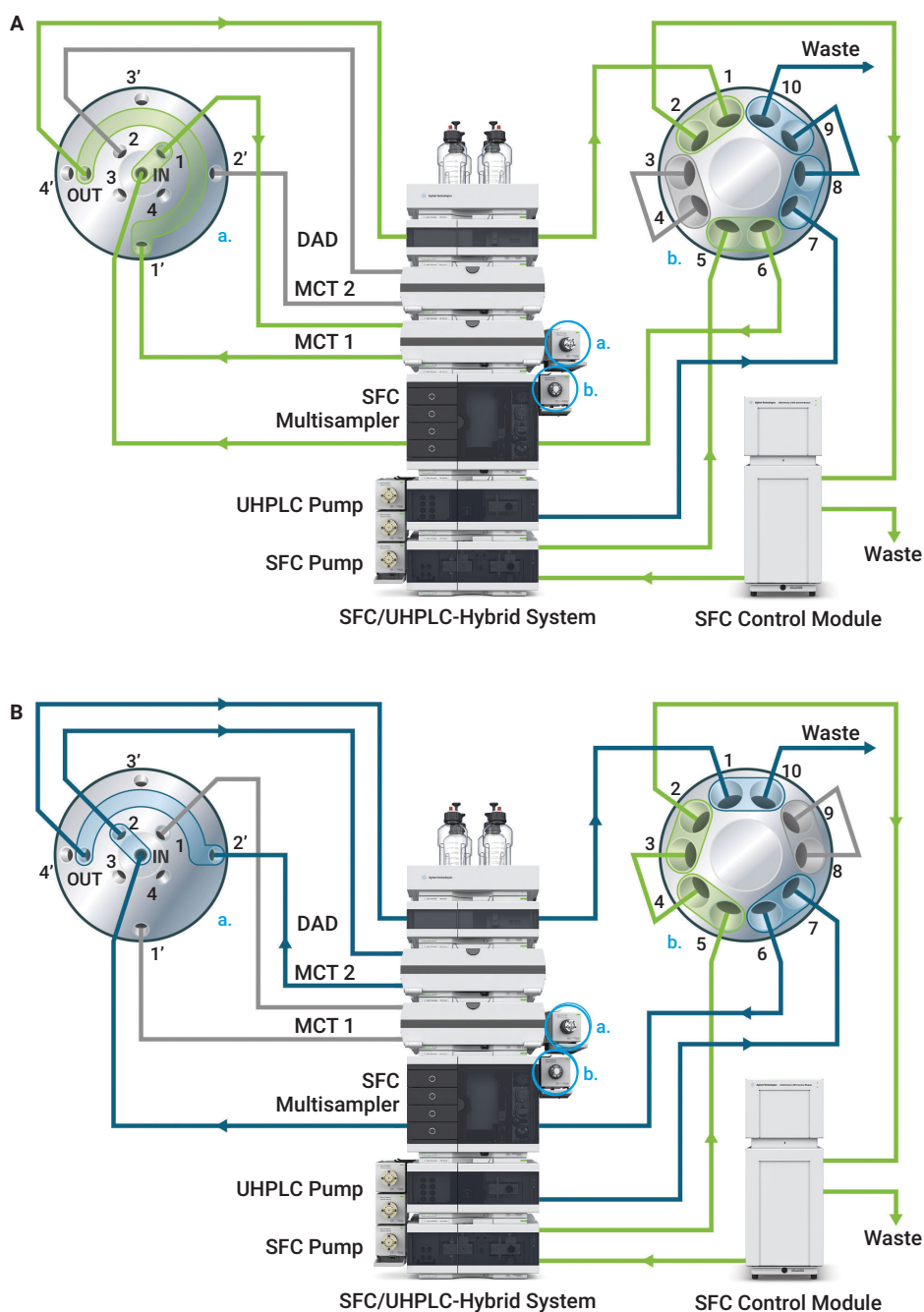
- Agilent 1260 Infinity II SFC Control Module (G4301A)
- Agilent 1260 Infinity II SFC Binary Pump (G4782A)
- Agilent 1260 Infinity II SFC Multisampler (G4767A)
- Agilent 1260 Infinity II Diode Array Detector (G7115A) with High-Pressure SFC Flow Cell (G4301-60100).
- Two Agilent 1290 Infinity II Multicolumn Thermostats (MCT) (G7116B) with Agilent InfinityLab Quick Change 8-Position/18-Port Valve (G4239C)
- Agilent 1260 Infinity II Quaternary Pump (G7111B) or Agilent 1260 Infinity II Binary Pump (G7112B; these will be subsequently referred to as the "UHPLC pump")
- Agilent 1290 Infinity Valve Drive (G1170A) with Agilent InfinityLab Quick Change 2-Position/10-Port Valve (G4232C)
- Agilent 1290 Infinity Valve Drive (G1170A) with Agilent InfinityLab Quick Change 4-Position/10-Port 4-Column Selector Valve Head (G4237A)
- Three Agilent 1290 Infinity Valve Drives (G1170A) with Agilent InfinityLab Quick Change 12-Position/13-Port Solvent Selector Valve Head (G4235A)

## Instrumental setup

For the conversion of the 1260 Infinity II SFC System into the 1260 Infinity II SFC/UHPLC Hybrid System, a quaternary or binary UHPLC pump is added. This is connected by an InfinityLab Quick Change 2-Position/10-Port Valve, which allows direct switching between SFC and UHPLC modes (Figure 2). Additionally, the described system is equipped with two MCTs. They are connected to the system with an InfinityLab Quick Change 4-Position/10-Port 4-Column Selector Valve for switching between the two MCTs (with one position for bypass). One MCT is used for SFC columns and the other for UHPLC columns. Each MCT contains an InfinityLab Quick Change 8-Position/18-Port 8-Column Selector Valve (not shown in Figure 2).

For solvent selection, the SFC pump is clustered with an InfinityLab Quick Change 12-Position/13-Port Solvent Selector Valve at channel B. The UHPLC pump is also connected with two InfinityLab Quick Change 12-Position/13-Port Solvent Selector Valve heads on channels A and B.

The central features, as outlined in Figure 2, are the plumbing at the InfinityLab Quick Change 2-Position/10-Port Valve and the plumbing at the InfinityLab Quick Change 4-Position/10-Port Valve. The position of the valve shown in Figure 2A connects the SFC Binary Pump and the SFC Control Module to the shared modules of the instrument. The position at the InfinityLab Quick Change 4-Position/10-Port Valve connects to MCT 1, which hosts the SFC columns. After flowing through the autosampler, the MCT with the SFC columns, and the detector, the CO<sub>2</sub> stream is connected back to the SFC Control Module for backpressure regulation. In this position, the UHPLC pump is connected to the waste.

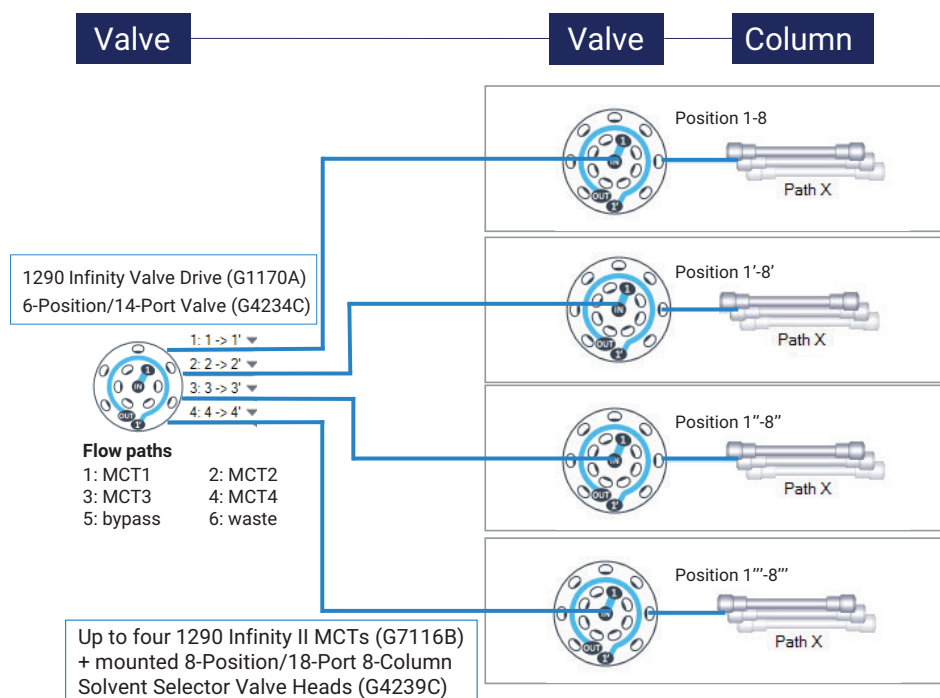


**Figure 2.** Instrumental setup of the Agilent 1260 Infinity II SFC/UHPLC Hybrid System. A) SFC mode: the InfinityLab Quick Change 2-Position/10-Port Valve (b.) connects the SFC Control Module and the SFC Binary Pump to the shared modules. The InfinityLab Quick Change 4-Position/10-Port Valve (a.) connects MCT 1 with the SFC columns (contains an InfinityLab Quick Change 8-Position/18-Port 8-Column Selector Valve). B) UHPLC mode: the InfinityLab Quick Change 2-Position/10-Port Valve (b.) connects the UHPLC pump to the shared modules. The InfinityLab Quick Change 4-Position/10-Port Valve (a.) connects MCT 2 with the UHPLC column (contains an InfinityLab Quick Change 8-Position/18-Port 8-Column Selector Valve).

After switching the InfinityLab Quick Change 2-Position/10-Port Valve to the UHPLC position and the InfinityLab Quick Change 4-Position/10-Port Valve to MCT 2, which hosts the UHPLC columns, the UHPLC pump is connected to the shared modules (Figure 2B). To maintain backpressure, the SFC Binary Pump is directly connected to the SFC Control Module.

For solvent selection, the SFC Binary Pump is clustered with an InfinityLab Quick Change 12-Position/13-Port Solvent Selection Valve at channel B. The UHPLC pump is also connected with two InfinityLab Quick Change 12-Position/13-Port Solvent Selector Valves on channels A and B.

The described SFC/UHPLC hybrid system could be enhanced by up to four MCTs for 32 columns. Of course, this cannot be built up in one stack of modules. In this case, two stacks of modules are necessary. Such a system would require an Agilent InfinityLab Quick Change 6-Position/14-Port Valve for the selection of the MCTs (Figure 3).



**Figure 3.** General setup of an MCT cluster: up to four MCTs hosting 32 columns. The MCTs are accessed by an InfinityLab Quick Change 6-Position/14-Port Valve, and inside each MCT there is an InfinityLab Quick Change 8-Position/18-Port 8-Column Selector Valve for final column selection. All valves are switched automatically to the correct position by choosing the desired column in the analytical method.

### Columns (one set for SFC mode in MCT 1 and one set for UHPLC mode in MCT 2)

- InfinityLab Poroshell 120 Chiral-CF, 4.6 × 100 mm, 2.7 µm (p/n 685975-609)
- InfinityLab Poroshell 120 Chiral-CD, 4.6 × 100 mm, 2.7 µm (p/n 685975-607)
- InfinityLab Poroshell 120 Chiral-V, 4.6 × 100 mm, 2.7 µm (p/n 685975-604)
- InfinityLab Poroshell 120 Chiral-T, 4.6 × 100 mm, 2.7 µm (p/n 685975-603)

### Software

- Agilent OpenLab CDS ChemStation Edition for LC & LC/MS Systems, Rev. C.01.08
- Method Scouting Wizard Rev. A02.08.011

### Software setup

For the generation of different methods of separation screening in SFC and UHPLC mode, the Method Scouting Wizard was used. On the first page of the Method Scouting Wizard, the pump was chosen according to the mode used for the screening. Solvents, columns, and gradient separation conditions were then set up. Finally, all necessary separation methods as well as flushing and equilibration methods were generated and executed automatically by the Method Scouting Wizard. For data evaluation, Method Scouting Reports, which are integrated in ChemStation Intelligent Reporting, were used to filter the successful separations by resolution.

### Samples

Carprofen, ketoprofen, ibuprofen, indoprofen, each 1 mg/mL in methanol.

### Chemicals

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

### SFC starting conditions

Parameter	Value
Solvent A	CO <sub>2</sub>
Modifier B	Methanol + 0.1 % TEA/0.1 % TFA Ethanol + 0.1 % TEA/0.1 % TFA Isopropanol + 0.1 % TEA/0.1 % TFA
SFC flow	2 mL/min
Gradient	0 minutes to 10 % B, 10 minutes to 50 % B
Backpressure regulator (BPR) temperature	60 °C
BPR pressure	140 bar
Column temperature	30 °C
Injection volume	2.0 µL
Feed solvent	methanol
Over feed volume	4 µL
Feed speed	400 µL/min
Needle wash	3 seconds in methanol

### UHPLC starting conditions

Parameter	Value
Solvent A	Water + 15 mM ammonium formate, pH 4.0
Solvent B	Acetonitrile
Flow rate	0.5 mL/min
Isocratic	30, 40, 50 %B Stop time: 10 minutes
Column temperature	30 °C
Injection volume	1.0 µL
Needle wash	3 seconds in methanol

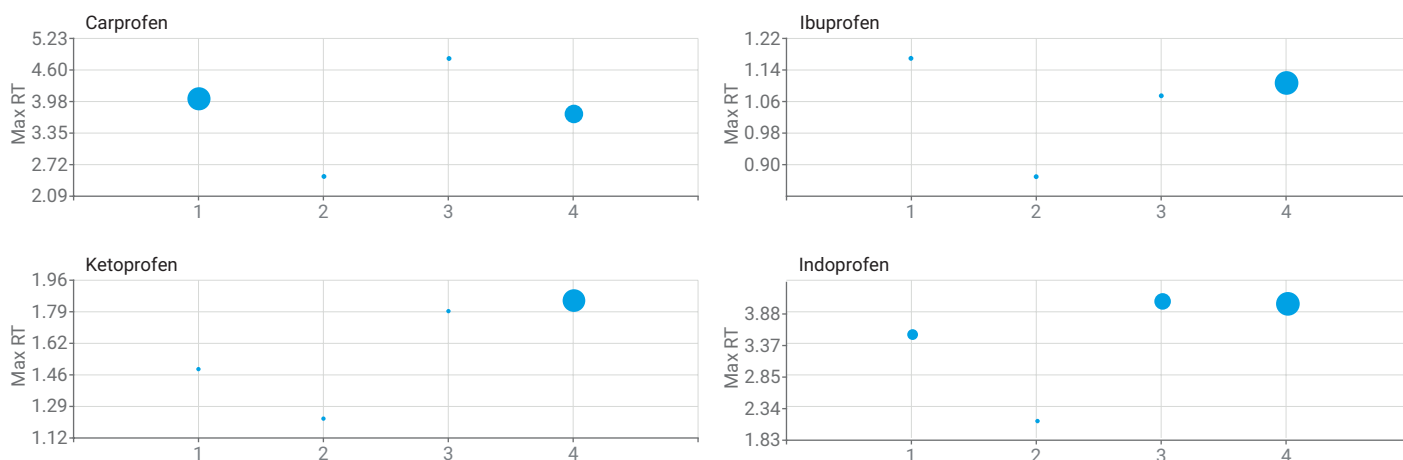
## Results and Discussion

### Method scouting and method optimization in SFC mode

For the initial scouting runs, a short 10 minute gradient run with modifier concentration of 10 to 50 % was applied at a column temperature of 30 °C in SFC mode. Four different chiral columns were screened against three solvents (methanol, ethanol, and isopropanol, each with 0.1 % TEA/TFA). Only methanol as modifier could resolve the enantiomers of the chiral test mixtures using different chiral columns. For a brief overview, the Method Scouting Report, which shows the achieved resolution in bubble charts, was used (Figure 4). The bubble chart report shows that all compounds start to separate under the initial scouting conditions on the InfinityLab Poroshell 120 Chiral-T column (more detailed information is outlined in Table 1).

**Table 1.** Results of the initial chiral screening separation by means of SFC on four different chiral columns with methanol (0.1 % TEA/TFA) as modifier.

Peaks	RT	Resolution	Symmetry	Column
<b>Carprofen</b>				
2	3.867 4.033	2.10	0.86 0.69	Poroshell 120 Chiral-CD
1	2.490		0.80	Poroshell 120 Chiral-CF
1	4.840		0.64	Poroshell 120 Chiral-V
2	3.585 3.731	1.63	0.92 0.80	Poroshell 120 Chiral-T
<b>Ketoprofen</b>				
1	1.489		0.68	Poroshell 120 Chiral-CD
1	1.225		0.87	Poroshell 120 Chiral-CF
1	1.800		0.91	Poroshell 120 Chiral-V
2	1.797 1.855	0.65	1.08 0.79	Poroshell 120 Chiral-T
<b>Ibuprofen</b>				
1	1.170		0.63	Poroshell 120 Chiral-CD
1	0.870		1.18	Poroshell 120 Chiral-CF
1	1.080		1.25	Poroshell 120 Chiral-V
2	1.067 1.107	0.52	1.82 0.67	Poroshell 120 Chiral-T
<b>Indoprofen</b>				
2	3.467 3.535	0.66	0.94 0.64	Poroshell 120 Chiral-CD
1	2.149		0.95	Poroshell 120 Chiral-CF
2	3.929 4.075	1.23	0.81 0.65	Poroshell 120 Chiral-V
2	3.827 4.028	1.90	0.71 0.63	Poroshell 120 Chiral-T



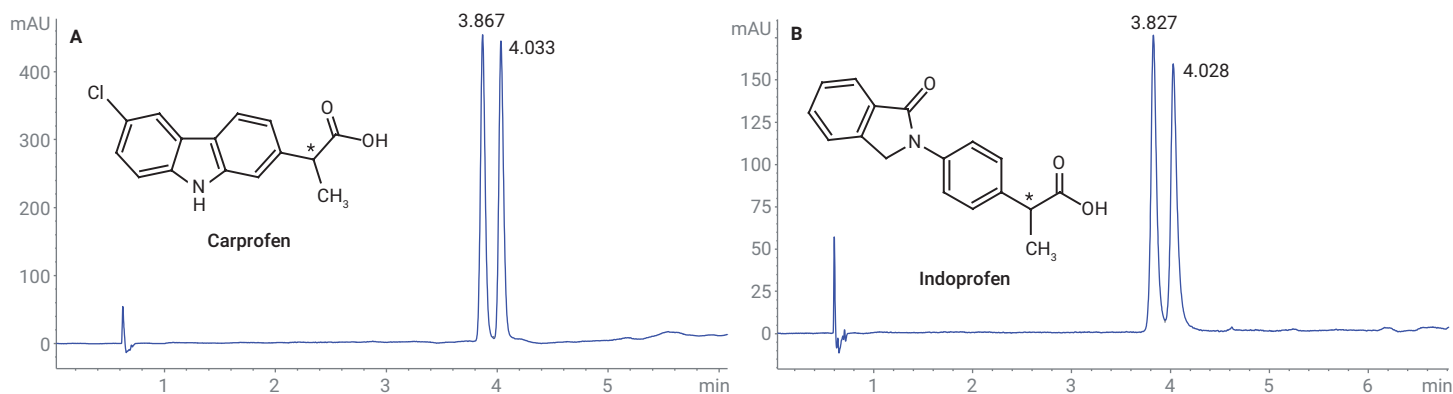
**Figure 4.** Brief overview of resolution achieved in the SFC scouting runs. The bubble size shows the maximum resolution sum achieved for each compound and column combination. The retention time of the compounds is given on the Y-axis. Columns: 1) InfinityLab Poroshell 120 Chiral-CD; 2) InfinityLab Poroshell 120 Chiral-CF; 3) InfinityLab Poroshell 120 Chiral-V; 4) InfinityLab Poroshell 120 Chiral-T.

The enantiomers of carprofen and indoprofen separate on other phases as well. The carprofen enantiomers in particular separate well on the InfinityLab Poroshell 120 Chiral-CD column (Figure 5A). Figure 5B shows the separation of the indoprofen enantiomers on the InfinityLab Poroshell 120 Chiral-T

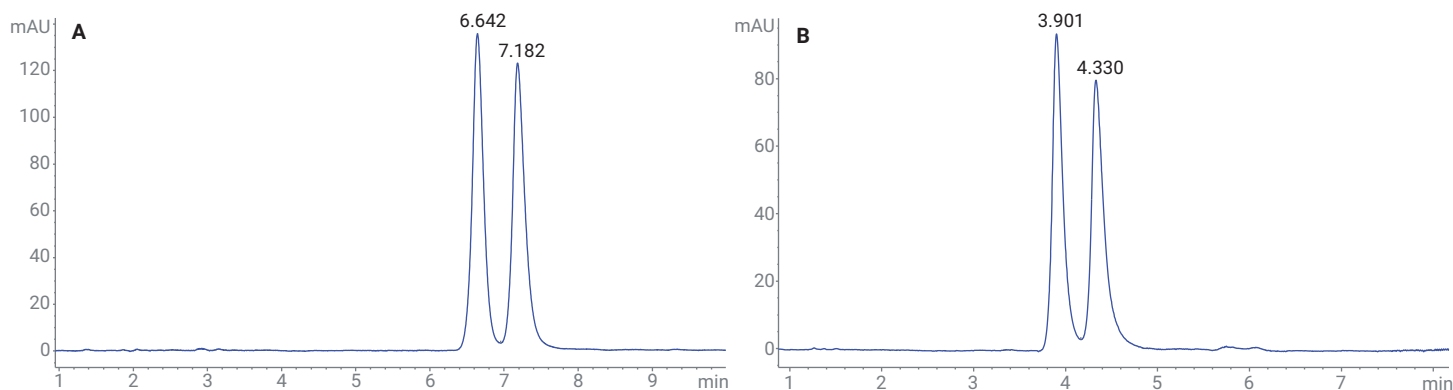
120 Chiral-T column. The initial screening took approximately three and a half hours for all compounds and columns combined with one solvent.

For further optimization of the separation, isocratic separation conditions were tested with the InfinityLab Poroshell 120 Chiral-T

column. Figures 6A and 6B respectively show isocratic separations of carprofen and indoprofen enantiomers on this column. The ketoprofen racemic mixture could only be separated with lower resolution under the tested conditions.



**Figure 5.** Separation of enantiomers of A) carprofen (InfinityLab Poroshell 120 Chiral-CD) and B) indoprofen (InfinityLab Poroshell 120 Chiral-T) under initial SFC chiral screening conditions.

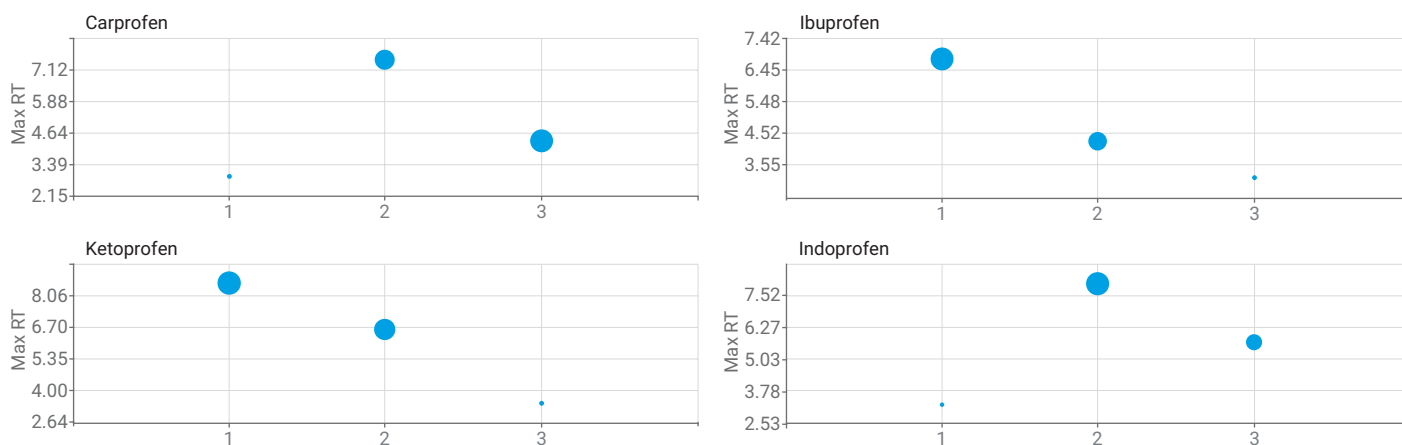


**Figure 6.** Separation of enantiomers of A) carprofen (InfinityLab Poroshell 120 Chiral-T, isocratic, 10 %B) and B) indoprofen (InfinityLab Poroshell 120 Chiral-T, isocratic, 15 %B).

## Method scouting and method optimization in UHPLC mode

Under UHPLC conditions, three different mobile phases (methanol, 30, 40, and 50 %, with 15 mM ammonium formate at pH 4.0) were screened against the four racemic test mixtures and the InfinityLab Poroshell 120 Chiral-T column. Bubble chart reports created from the results show the maximum resolution sum,

and identify the best solvent for the separation of each compound together with the maximum retention time on the given column (Figure 7). Table 2 summarizes the detailed results. To optimize the separation, which was achieved with a level of 40 % methanol, the temperature was decreased from 30 to 20 °C (Table 3).



**Figure 7.** Brief overview of resolution achieved in UHPLC scouting runs. The bubble size shows the maximum resolution sum achieved for each compound and solvent on the InfinityLab Poroshell 120 Chiral-T column. Solvents: 1) 30 % methanol; 2) 40 % methanol; and 3) 50 % methanol, each with 15 mM ammonium formate at pH 4.0.

**Table 2.** Results of the initial chiral screening separation by means of UHPLC mode of the four racemic mixtures with three solvents on one chiral column at 30 °C.

	30 % methanol			40 % methanol			50 % methanol		
	RT	Resolution	Symmetry	RT	Resolution	Symmetry	RT	Resolution	Symmetry
Carprofen				7.184 7.606	1.37	0.60 0.53	4.149 4.318	1.06	0.85 0.71
Ibuprofen	6.303 6.811	1.83	0.49 0.44	3.823 4.057	1.65	0.84 0.78	3.067 3.187	1.06	0.95 0.91
Ketoprofen	8.227 8.566	0.99	0.72 0.56	5.851 5.989	0.64	1.03 0.66	3.465		
Indoprofen				7.358 7.920	1.71	0.57 0.49	5.584 5.850	1.49	0.79 0.70

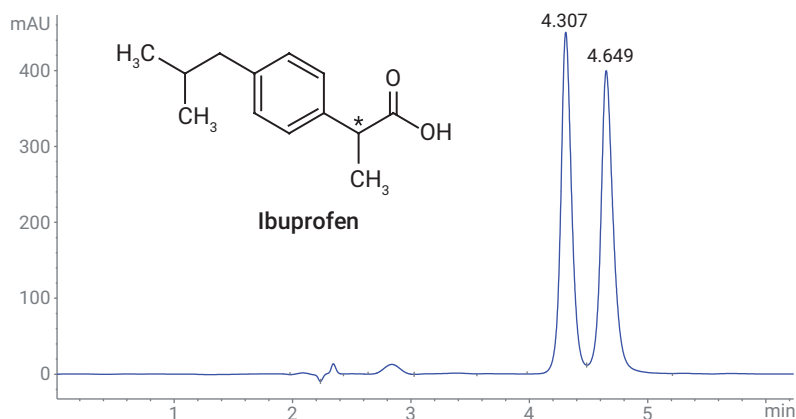
**Table 3.** Temperature optimization of the separation achieved with 40 % methanol on the InfinityLab Poroshell 120 Chiral-T column.

	RT	Resolution	Symmetry
Carprofen	8.228 8.857	1.48	0.56 0.51
Ibuprofen	4.307 4.649	2.03	0.83 0.72
Ketoprofen	6.379 6.566	0.72	0.91 0.66
Indoprofen	8.783 9.645	1.98	0.63 0.64

Figure 8 shows, as an example, the separation finally achieved for ibuprofen enantiomers. The ketoprofen racemic mixture could only be separated with insufficient resolution under the tested conditions.

## Conclusion

This Application Note demonstrates the use of an enhanced 1260 Infinity II SFC/UHPLC Hybrid System for combined method development in SFC and UHPLC modes for chiral screening. This system can host up to 16 columns, up to 12 solvents for SFC, and up to 24 solvents for UHPLC separations. By means of four MCTs, it is possible to use up to 32 columns. The methods necessary for the screening were created with the aid of the Method Scouting Wizard software. It was shown that a set of racemic mixtures could be separated into their component enantiomers by means of the same InfinityLab Poroshell 120 chiral columns in SFC mode as well as UHPLC mode. After a short optimization of the screening results, a good method for separation of the enantiomers under isocratic conditions could be achieved. The applied concept of chiral screening was recently realized by a high throughput walk-up system running in a pharmaceutical environment<sup>3</sup>.



**Figure 8.** Separation of the enantiomers of ibuprofen under UHPLC conditions.

## References

1. Orthogonal Separations Using the Agilent 1260 Infinity II SFC/UHPLC Hybrid System, *Agilent Technologies Technical Overview*, publication number 5991-8276EN.
2. Chiral Multicolumn Method Development on the Agilent 1260 Infinity II Analytical SFC System, *Agilent Technologies Application Note*, publication number 5991-7624EN.
3. Easy Access to Rapid Chiral SFC Method Development for NonChromatographers, *Agilent Technologies Application Note*, publication number 5994-0011EN.

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