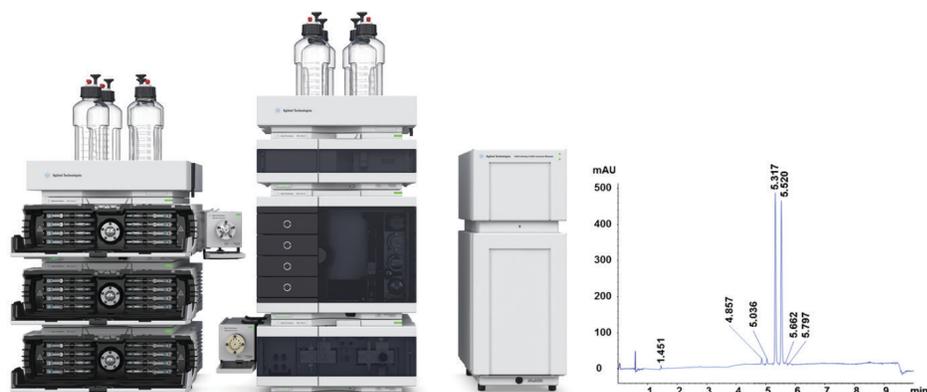


Easy Access to Rapid Chiral SFC Method Development for Non-Chromatographers



Authors

Heewon Lee
Boehringer Ingelheim
Pharmaceuticals, Inc.,
Ridgefield, Connecticut, USA

Andreas Borowiak and
Edgar Naegele
Agilent Technologies, Inc.,
Waldbronn, Germany

Abstract

This Application Note describes an easy access chiral supercritical fluid chromatography (SFC) method development system that empowers non-chromatographers, for example, synthetic organic chemists, to attain the majority of their chiral SFC method development requirements without the help of analytical specialists. The chiral SFC system is set up as a walk-up instrument with easy-to-use software and procedures. The instrument is used for two tasks:

- Method screening for new, uncharacterized samples
- Chiral analyses of known samples with existing methods

Method screening campaigns with preset chiral SFC methods are performed overnight and during the weekend, while chiral analyses are normally performed during the daytime. A method screening campaign for a racemic sample requires 20 injections (analyses), corresponding to four hours of instrument time. The system is capable of performing 600 analyses at full capacity per work week, excluding weekend time. Typically, the volume of submitted samples leads to 300 to 400 analyses performed per week in the current open access setting. The observed rate of successful separation in the initial method-screening process was roughly 75 %.

Introduction

Analytical core facilities are currently facing an increasing number of requests for the development of chromatographic methods to analyze newly developed compounds, including ligands, synthetic intermediates, and active pharmaceutical ingredients (APIs). Only a minority of the synthetic organic scientists that submit requests for method development have solid knowledge of chromatography. Therefore, they depend on method development support provided by analytical chemists with specialized knowledge and experience in chromatography.

Traditionally, chromatographic method development has been viewed as a task that requires solid knowledge of chromatography as well as significant experience. Easy access instruments have become common for routine analyses, and more tasks are assigned to non-chromatographers to expedite development processes. To save valuable and often limited resources of experienced analysts and chromatographers for the most challenging cases, concepts of easy access or walk-up systems are gaining interest in method development as well.

When performing analytical method development for chiral separations, SFC is the means of choice. SFC provides normal-phase-like separation mechanisms with short run times, better peak shape, and better resolution compared to traditional LC techniques.

This Application Note introduces an SFC method development system that is used for the initial development of chiral SFC separations. The system follows a classical method scouting approach, including automated switching of columns and solvents, to find a suitable set of chromatographic conditions that separate a chiral mixture.

Method scouting is indispensable in chiral method development since, currently, there is no software solution available that would allow prediction of chiral separation conditions from a limited set of initial experiments. To enable non-chromatographers to use method-scouting tools, the complexity of such method-scouting workflows has to be simplified to accommodate all users. The proposed workflow makes method scouting available for users of all experience levels.

Experimental

Instruments

The instrument used in this study comprises modules both from the 1200 Infinity Series and the InfinityLab Series, and provides a platform for seamless integration of the newest LC/SFC equipment into existing Agilent hardware. Modules of the 1260 Infinity Series SFC are no longer available; recommended substitutions are highlighted below. The following modules were used:

Solvent selection and delivery

- Agilent 1290 Infinity Valve Drive (G1170A) with 12-solvent selection valve head (G4235A)
- Agilent 1260 Infinity SFC Binary Pump (G4302A) (replacement available: Agilent 1260 Infinity II SFC Binary Pump (G4782A))
- 3 × Agilent 1260 Infinity High Performance Degasser (G4225A) (no longer necessary, the Agilent 1260 Infinity II SFC Binary Pump (G4782A) hosts an internal degasser)
- Agilent 1260 Infinity SFC Control Module (G4301A) with Booster Pump Upgrade Kit for increased lifetime

Sample introduction

Agilent 1260 Infinity II SFC Multisampler (G4767A)

Column selection

- Agilent 1290 Infinity II Multicolumn Thermostat (MCT) (G7116B) with 8-position/18-port InfinityLab Quick Change Valve (G4239C) for columns 1 to 8
- Agilent 1290 Infinity II MCT (G7116B) with 8-position/18-port InfinityLab Quick Change Valve (G4239C) for columns 1' to 8'
- Agilent 1290 Infinity II MCT (G7116B) with 8-position/18-port InfinityLab Quick Change Valve (G4239C) for columns 1" to 8"
- Agilent 1290 Infinity Valve Drive (G1170A) with 6-position/14-port InfinityLab Quick Change Valve (G4234A) to select the MCT selection valve

Detection

Agilent 1260 Infinity Diode Array Detector (DAD) VL+ (G1315C) with standard SFC flow cell (G4301-60100) (replacement available: Agilent 1260 Infinity II DAD WR (G7115A))

Instrumental setup

The setup used in this Application Note was tailored to the needs of the SFC walk-up method development workflow. The system is based on a standard 1260 Infinity II SFC System, and comprises both 1200 Infinity Series and InfinityLab Series modules. The system shows the possibility of upgrading existing hardware with new technology to increase functionality.

To achieve maximum flexibility in automated column selection, the Agilent valve-thermostat cluster (VTC) technology was used. VTCs enable you to use 6-position/14-port valves, normally used as a six-column selection

valve, to cluster multiple column compartments. The flow enters the valve head, can be guided to one of up to four column compartments, and is later guided back into the valve head. The valve is directly connected to a second valve that is hosted in the column compartment itself. This second valve head is connected to the actual columns.

For example, with a 6-position/14-port valve, it is possible to switch between up to four 1290 Infinity II MCTs. In addition, one position is connected to a bypass capillary for efficient flushing of the system. When fully equipped with eight columns per 1290 Infinity II MCT, a total of 32 columns can automatically be selected. Figure 1 shows a schematic of such a VTC.

Software

- Agilent OpenLab CDS ChemStation Edition for LC & LC/MS Systems, rev. C.01.07 SR4 with LC driver package A.02.18
- Agilent OpenLab Data Analysis version A.01.02, build 1.159.23
- Agilent 1260 Infinity II SFC Control Module firmware version 07.20

Columns

1. Phenomenex Lux Cellulose-1, 100 × 4.6 mm, 5 μm
2. Phenomenex Lux Cellulose-2, 100 × 4.6 mm, 5 μm
3. Phenomenex Lux Cellulose-3, 100 × 4.6 mm, 5 μm
4. Phenomenex Lux Cellulose-4, 100 × 4.6 mm, 5 μm

5. ES Industries ChromegaChiral CCA, 100 × 4.6 mm, 5 μm
6. ES Industries ChromegaChiral CCC, 100 × 4.6 mm, 5 μm
7. ES Industries ChromegaChiral CCS, 100 × 4.6 mm, 5 μm
8. ES Industries ChromegaChiral CCO-F2, 100 × 4.6 mm, 5 μm

One set of the listed eight columns was placed in each of the two column compartments. The set of eight columns in the first column compartment was used for this study. The same set of eight columns in the second column compartment was reserved for chiral method development with amine-containing modifiers. The third column compartment with different columns was not used in this study.

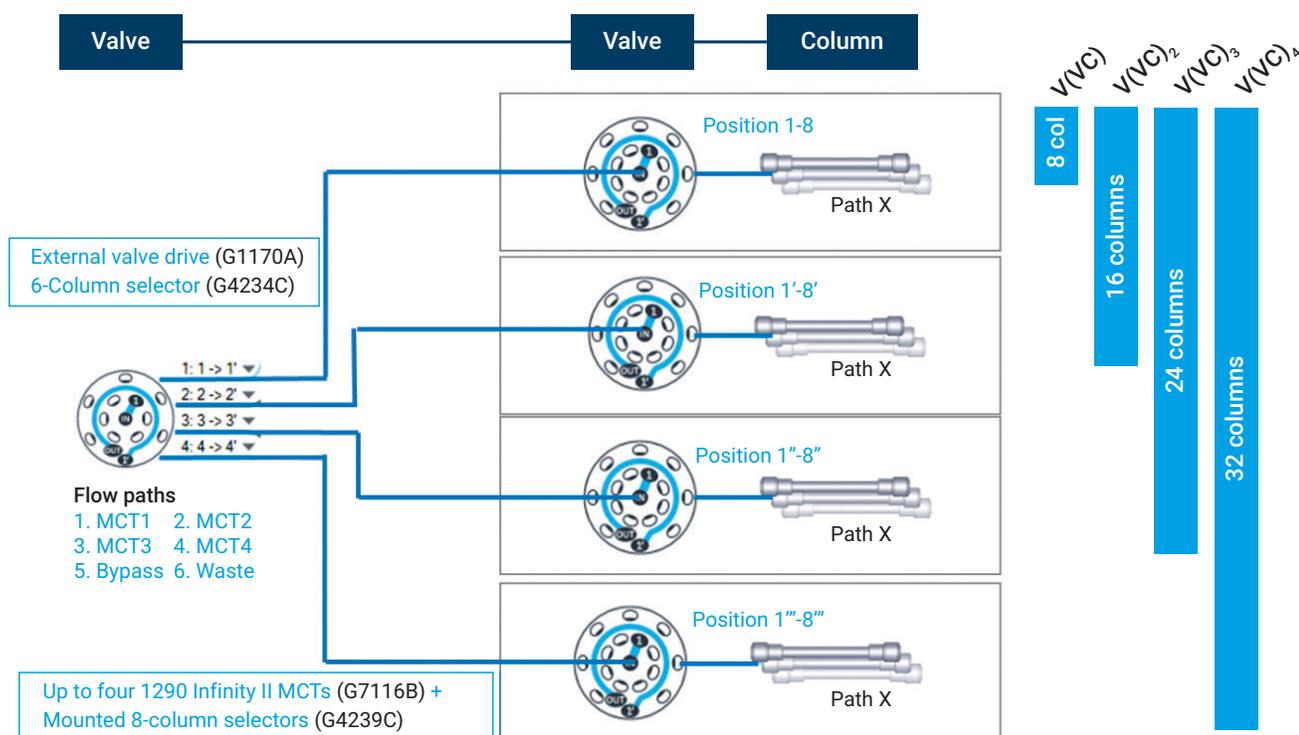


Figure 1. Schematic representation of a valve-thermostat cluster (VTC). Agilent column compartments offer the possibility to use a column compartment selection valve (left valve) that switches between up to four column selection valves (right valve), which are hosted in the column compartments. Each column selection valve can be connected to up to eight columns.

Chemicals, samples, and solvents

- Samples were compounds received from in-house sources, and are not further specified here.
- Bone dry CO₂ in a high-pressure gas cylinder was purchased from Air Gas.
- Methanol and isopropanol were purchased from EMD and Fisher.

Results and discussion

Description of the walk-up SFC method development workflow

The workflow was based on eight different enantioselective stationary phases with methanol and isopropanol modifiers, resulting in 16 different chromatographic conditions to screen. In addition, four blank injections (at the beginning of the sequence, when changing the modifier from methanol to isopropanol, and two blanks at the end of the sequence) were made, totaling 20 injections for a complete screening of predefined chiral method conditions. A template of a preset sequence of 20 analyses for blanks and samples was available to users on ChemStation. Users would change the sample vial location and the sample name in the template sequence, save the modified sequence under a new name, and run the sequence. Figure 2 gives a schematic representation of the system use.

Since one analysis takes approximately 12 minutes, and 20 injections are performed within one method screening campaign, a campaign can be run in roughly four hours. In the proposed setup, up to four method-screening campaigns can be run overnight to maximize instrument time usage. During the daytime, the walk-up system can be used for non-chromatographers to perform chiral identification and purity analyses with previously identified methods.

Predefined chiral separation method

Parameter	Value
Flow rate	3 mL/min: to avoid pressure spikes during column switching, the flow rate was reduced to 2 mL/min between analyses.
Modifiers	methanol, isopropanol
Predefined modifier gradient	Time (min) %A %B Flow (mL/min)
	0 99 1 2
	0.05 99 1 3
	3 97 3 3
	8 50 50 3
	9 50 50 3
	9.5 99 1 3
	9.95 99 1 2
	10 99 1 2
	The gradient was designed to retain and elute a wide polarity range of compounds.
Column temperature	35 °C
BPR temperature	60 °C
BPR pressure	150 bar
Total run time	10 minutes; postrun time: 1 minute (approximately 2 minutes until next injection)
Injection	5.0 µL
Feed speed	400 µL/min
Overfeed volume	4.0 µL
Feed solvent	methanol
Needle wash	3 seconds methanol
DAD detection	Signal A: 210/10 nm, ref. 500/100 nm Signal B: 220/10 nm, ref. 500/100 nm Signal C: 254/10 nm, ref. 500/100 nm Full spectra acquisition: 190 to 400 nm, 2 nm step size, 10 Hz data rate, 8 nm slit width

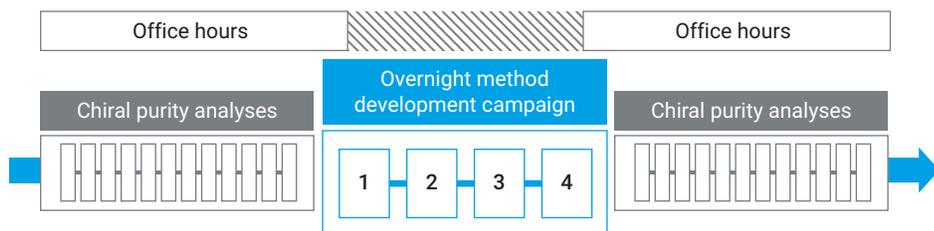


Figure 2. Workflow diagram of the walk-up SFC method development system. The alternating usage of the system as a standard and walk-up method development system increases instrument efficiency by minimizing idle instrument time.

Data analysis of chosen example compounds

To identify successful separations quickly and reliably, the OpenLab Data Analysis A.01.02 was used. The data analysis option is a standalone module delivered with OpenLab CDS ChemStation Edition. The following presents an example data analysis for compound A.

First, an integration method was created and the parameters adapted to exclude injection peaks and smaller impurities (area reject 150.00, height reject 30.00, slope sensitivity 1.0). The resulting peaks, visualized as bubbles in the Peak Explorer, highlight the retention time of the enantiomers (see Figure 3). A single bubble suggested no separation, and

two overlapping bubbles showed partial separation. Two bubbles farther apart with no overlap indicated successful enantiomeric separation. For example, the best separation conditions for compound A could be identified when using the ChromegaChiral CCA column with isopropanol as a modifier.

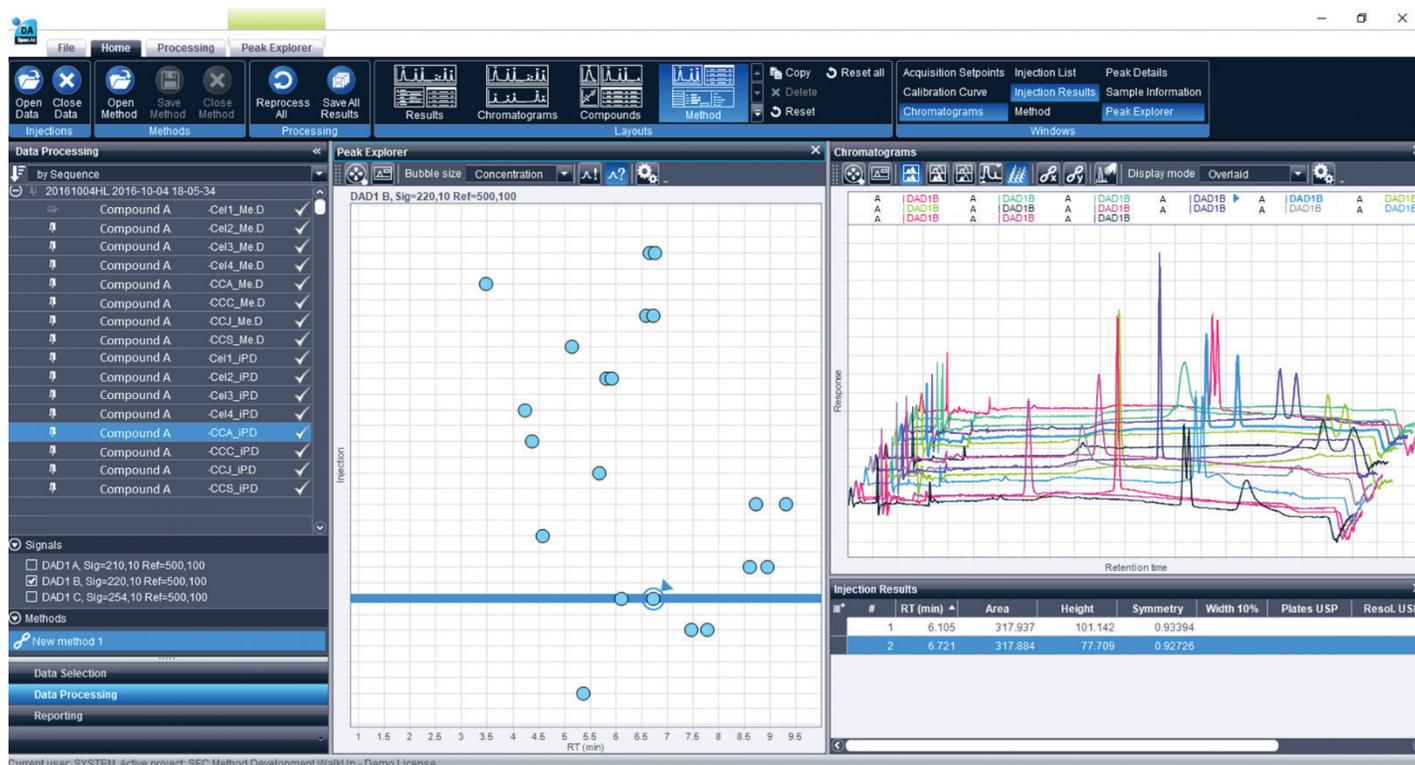


Figure 3. Data evaluation for the chiral racemic compound A using OpenLab Data Analysis A.01.02, which is part of OpenLab CDS ChemStation Edition. The 16 injections were flagged and reprocessed with an integration method that was created for this purpose. The Peak Explorer visualized peaks as bubbles. The right plot shows all 16 injections in a stacked plot. The best separation is shown in light blue in the back (highlighted by a bold blue line in the Peak Explorer).

Based on such a data evaluation workflow, one screening campaign can be processed within minutes. Figure 4 shows more examples of a chiral method screening campaign. All these campaigns have in common the fact that they led to a minimum of one set of conditions, frequently multiple conditions, that ensured baseline separation of the enantiomeric pair.

Even though methods might be further optimized in terms of run time or resolution, in most cases they were sufficient to answer basic questions about enantiomeric identification or enantiomeric purity. Successful separation methods can be stored in a database to be readily available when a chiral sample is submitted for analysis.

For example, compound A showed best results when using a Phenomenex Cellulose-1 column with isopropanol as modifier. In addition, the enantiomers of the main compound, two impurities, and their enantiomers were successfully separated. Compound B showed good separation of one enantiomeric pair as well as at least one chiral impurity when using a Phenomenex Cellulose-4 column using methanol as modifier. Compounds C and D both showed best separation on a ChromegaChiral CCA with isopropanol as an organic modifier.

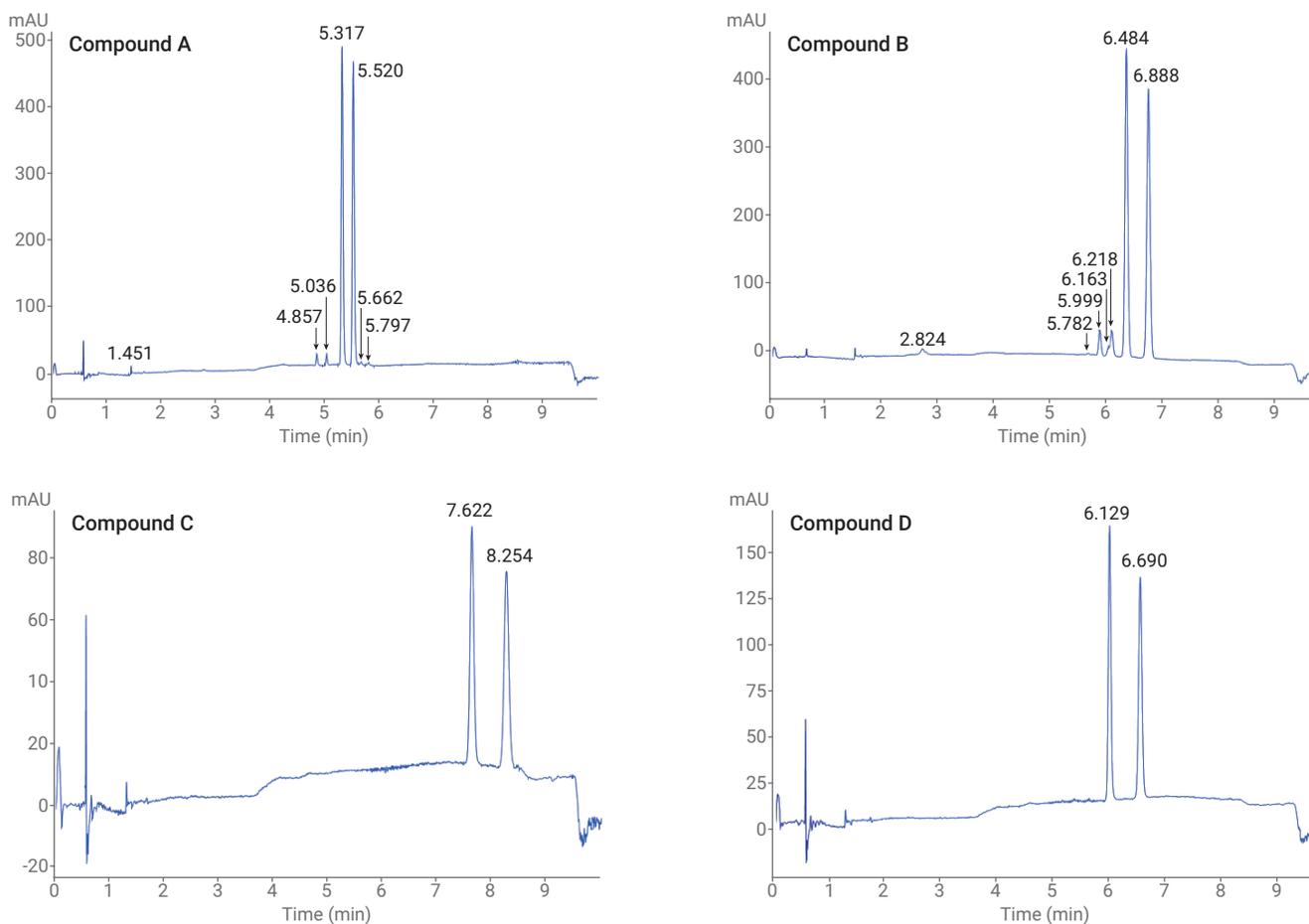


Figure 4. Examples of compounds that were successfully separated by the proposed method scouting approach. A) Separations on a Phenomenex Cellulose-1 column using isopropanol as modifier delivered the best results for compound A. B) Compound B was separated successfully on a Phenomenex Cellulose-4 column using methanol as modifier. Related compounds C and D were both separated successfully on a ChromegaChiral CCA column with isopropanol, but differ significantly in retention time. All compounds were separated with the standard gradient reported above.

Analysis of chiral identification and enantiomeric purity of compounds with established separation methods

The system was mainly used to determine chiral identification and enantiomeric purities during regular work hours. These analyses were based on previously developed and already established methods that were part of the method screening. The OpenLab Data Analysis module is designed to visualize the number of peaks identified in a simple and clear manner. Figure 5 shows how a typical analysis of enantiomeric purity may be performed for some selected compounds.

Whether a peak in your chromatogram is considered a compound and visualized as a bubble in the bubble plot (Figure 5A), or an impurity that should not be reported, can easily be defined by different thresholds in the integration method. The OpenLab Data Analysis module is able to link and reprocess integration methods to data files.

Different runs can easily be connected to individual or general integration methods that meet the purpose of the analysis.

Whether both enantiomers are present can easily be read in the bubble plot. Two bubbles indicate two peaks, and therefore, two possible enantiomers. The enantiomeric ratio of the compounds can further be investigated, for example, by comparing the area of both peaks in the peak table (Figure 5C).

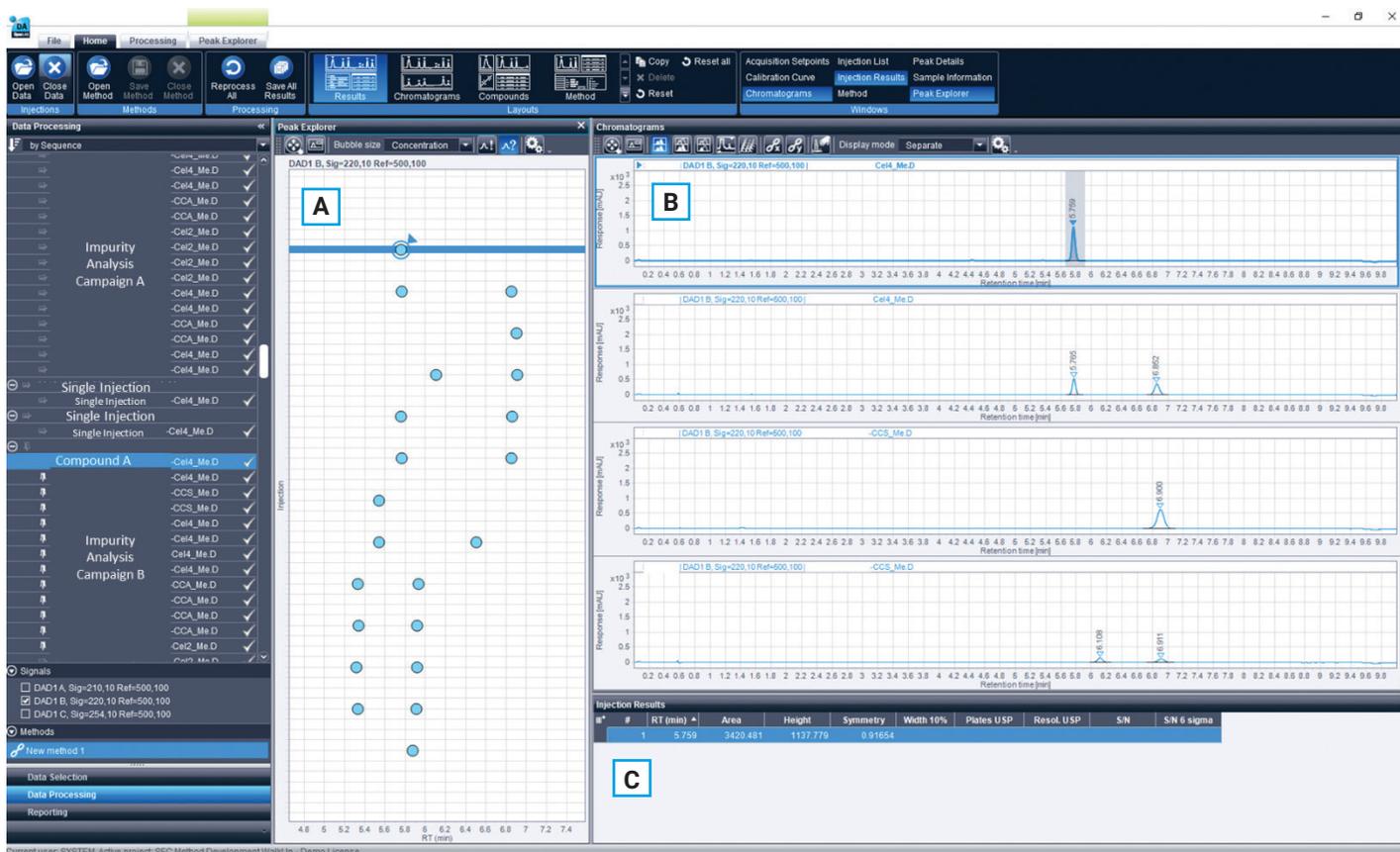


Figure 5. Visualization of analysis of enantiomeric purities for some selected compounds. The bubble plot (A) helps to visualize the number of identified peaks at a glance. Here, the bubble size is set to the same size for all compounds. The bubble size can also visualize area, area % and, if dilution and calibration data are provided, concentration. The chromatograms (B) in combination with the area reported in the peak table (C) give rough estimates of the enantiomeric ratio of the compounds.

Conclusion

Approximately 75 % of chiral compounds submitted were separated successfully with the proposed walk-up chiral SFC method development workflow. The workflow delivered a robust, reproducible, and user-friendly approach thanks to standardized methods and sequences. A single analysis was performed in roughly 12 minutes from injection to injection, including flushing, equilibration, and data acquisition. This led to a chiral screening campaign duration of roughly four hours. The workflow is fully automated, and data acquisition needs no manual interaction. Data processing to find suitable conditions after data acquisition is simple and straightforward.

We are aware that the proposed workflow will not solve all chiral separation challenges, and the success rate may vary with different structural characteristics of analytes. These methods could further be optimized in terms of peak shape, run time, or resolution based on the chiral data acquired using the standardized screening approach. Thus, the aim of the method screening workflow is not to provide optimized chiral methods for a few selected compounds, but to deliver a set of chiral data as a universal tool covering a wide range of chiral compounds. The standard methods and workflow are easy to work with, and deliver rapid solutions using a limited set of columns and solvents.

Execution of standard chiral purity analyses during regular work hours and overnight method development screen campaigns leads to a significant increase in productivity with maximum instrument usage, resulting in a high return on investment. This workflow provides the best possible trade-off by delivering quick solutions through a standardized screening approach, and allocating valuable analytical resources to focus on challenging problems that cannot be solved with the standardized methodology.

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© Agilent Technologies, Inc. 2018, 2019
Printed in the USA, January 10, 2019
5994-0011EN