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Technical Report

Rapid Method Scouting of Chiral Compounds

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Abstract:

Enantiomer resolution by chiral column chromatography is a variation of column chromatography that is actively being researched in the pharmaceutical field of drug discovery. One drawback of this method, however, is the extensive time and effort required to determine the optimum mobile phase conditions and the most suitable column for separation of the analyte among a wide variety of available chiral columns. This has spurred the demand for faster scouting of chiral separation conditions.
Here, using the Nexera Method Scouting system with the *i*Chiral-6 polysaccharide-based columns (Daicel Corporation), we report an example in which high-resolution column conditions are constructed for analysis of chiral compounds.

Keywords: UHPLC, Nexera Method Scouting, chiral column, *i*Chiral-6, chiral compounds

1. Introduction

The safe and secure use of chiral compounds in their pure form (enantiomers) is essential for direct biological applications, specifically in the field of pharmaceuticals. It is often the case where one enantiomer has detrimental or toxic effects while the other enantiomer has curative properties, so it is critical that enantiomers be fully separated. Optical separation (chiral separation) by HPLC is one method typically used to obtain chiral compounds. This HPLC method normally involves a labor-intensive, time-consuming search for an appropriate mobile phase and column suitable for a particular chiral compound, a process referred to as method scouting. Much effort has been directed recently at accelerating such method scouting to speed up drug synthesis and the production of pharmaceutical intermediates.

As an associated technology, ultra high performance liquid chromatography (UHPLC) has been attracting much attention as an HPLC technique capable of increasing throughput in commercial analytical enterprises by improving productivity and efficiency. Introduction of UHPLC is also being promoted for method scouting as a means of increasing overall throughput while minimizing solvent consumption.

Here we introduce the results obtained in the analysis of three chiral compounds: bromacil, α-methyl-α-acethyl-γ-butylrolactone, and methylclothiazide using the Nexera Method Scouting system with the high-resolution conditions that are possible with the *i*Chiral-6 polysaccharide-based columns.

2. Experiment

2-1. System

Fig. 1 shows the flow diagram depicting the Nexera Method Scouting system that was constructed for this application. The system is configured by installing a column switching valve inside the oven and a solvent switching valve within each of the Nexera ultra high performance pump, thereby permitting comprehensive data collection while continuously switching through a maximum of 96 unique combinations of columns and mobile phases.

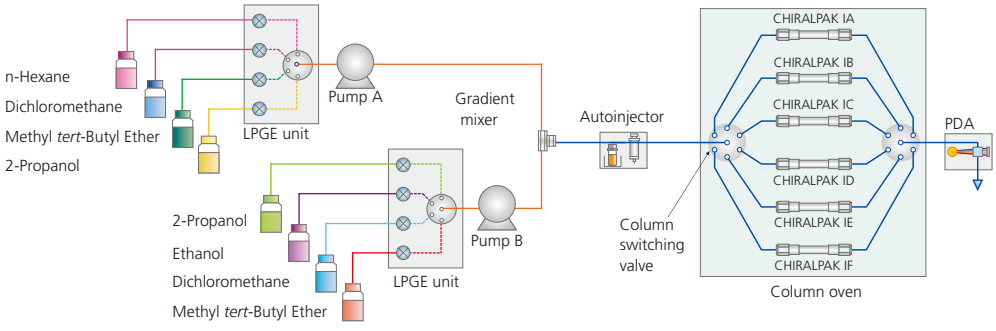


Fig. 1 Flow Diagram of the Nexera Method Scouting System

1 Analytical & Measuring Instruments Division

2-2. Sample Information

Three standards of chiral compounds (bromacil, α-methyl-α-acethyl-γ-butylrolactone, and methylclothiazide) were analyzed, as shown in Fig. 2. These standards were prepared by dissolving in hexane and MTBE as needed at 1.0 mg/mL.

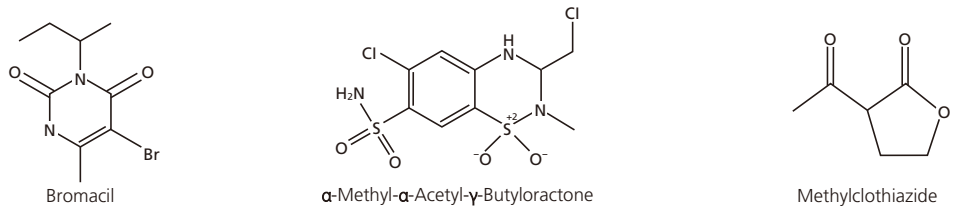


Fig. 2 Structural Formulas of Analyte Chiral Compounds

2-3. Chiral Columns

The *i*Chiral-6 high-resolution polysaccharide-based columns (CHIRALPAK® IA/IB/IC/ID/IE/IF) available from Daicel Corporation were utilized to provide the high-resolution conditions for the displayed chiral compounds. Fig. 3 shows the respective column functional groups. Since the *i*Chiral-6 columns are compatible with the range of organic solvents specified here, these columns are applicable to method scouting analysis of chiral compounds.

(A) CHIRALPAK® IA (150 mmL. × 4.6 mmI.D., 5 µm)
Amylose tris(3,5-dimethylphenylcarbamate)

(B) CHIRALPAK® IB (150 mmL. × 4.6 mmI.D., 5 µm)
Cellulose tris(3,5-dimethylphenylcarbamate)

(C) CHIRALPAK® IC (150 mmL. × 4.6 mmI.D., 5 µm)
Cellulose tris(3,5-dichlorophenylcarbamate)

(D) CHIRALPAK® ID (150 mmL. × 4.6 mmI.D., 5 µm)
Amylose tris(3-chlorophenylcarbamate)

(E) CHIRALPAK® IE (150 mmL. × 4.6 mmI.D., 5 µm)
Amylose tris(3,5-dichlorophenylcarbamate)

(F) CHIRALPAK® IF (150 mmL. × 4.6 mmI.D., 5 µm)
Amylose tris(3-chloro-4-methylphenylcarbamate)

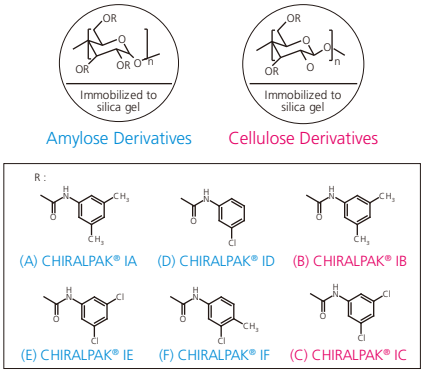


Fig. 3 Chiral Selector

2-4. Separation Conditions

For the mobile phases, eight different solvent mixtures were used, consisting of various combinations of hexane, 2-propanol, ethanol, dichloromethane, and methyl-t-butyl ether. Details of the separation conditions are shown in Table 1. Using a total of 8 mobile phase combinations and 6 different columns, the 48 unique separation conditions yielded an exhaustive search for the conditions suitable for the separation of each of the chiral compounds.

Table 1 Analytical Conditions

Separation Conditions No.	Mobile Phase	Flow Rate	Analysis Time	Other
1	Hexane / 2-Propanol = 9 / 1(v/v)	3 mL/min	9 min	Column temperature: 40°C Injection volume: 10 µL Detection: 230 nm
2	Hexane / 2-Propanol = 6 / 4(v/v)	3 mL/min	9 min	
3	Hexane / Ethanol = 8 / 2 (v/v)	3 mL/min	14 min	
4	Ethanol	1 mL/min	18 min	
5	Hexane / Dichloromethane = 9 / 1(v/v)	3 mL/min	4 min	
6	Dichloromethane / Ethanol = 100 / 2(v/v)	3 mL/min	4 min	
7	Hexane / Methyl *tert*-Butyl Ether = 9 / 1(v/v)	3 mL/min	4 min	
8	Methyl *tert*-Butyl Ether / Ethanol = 9 / 1(v/v)	3 mL/min	4 min	

Note: For analysis of methylclothiozide, 0.1% diethylamine was added to each mobile phase.

3. Results

The chromatograms obtained for bromacil using all 48 sets of conditions are shown in Fig. 4, and the optimum conditions for each of the chiral compounds are shown in Fig. 5.

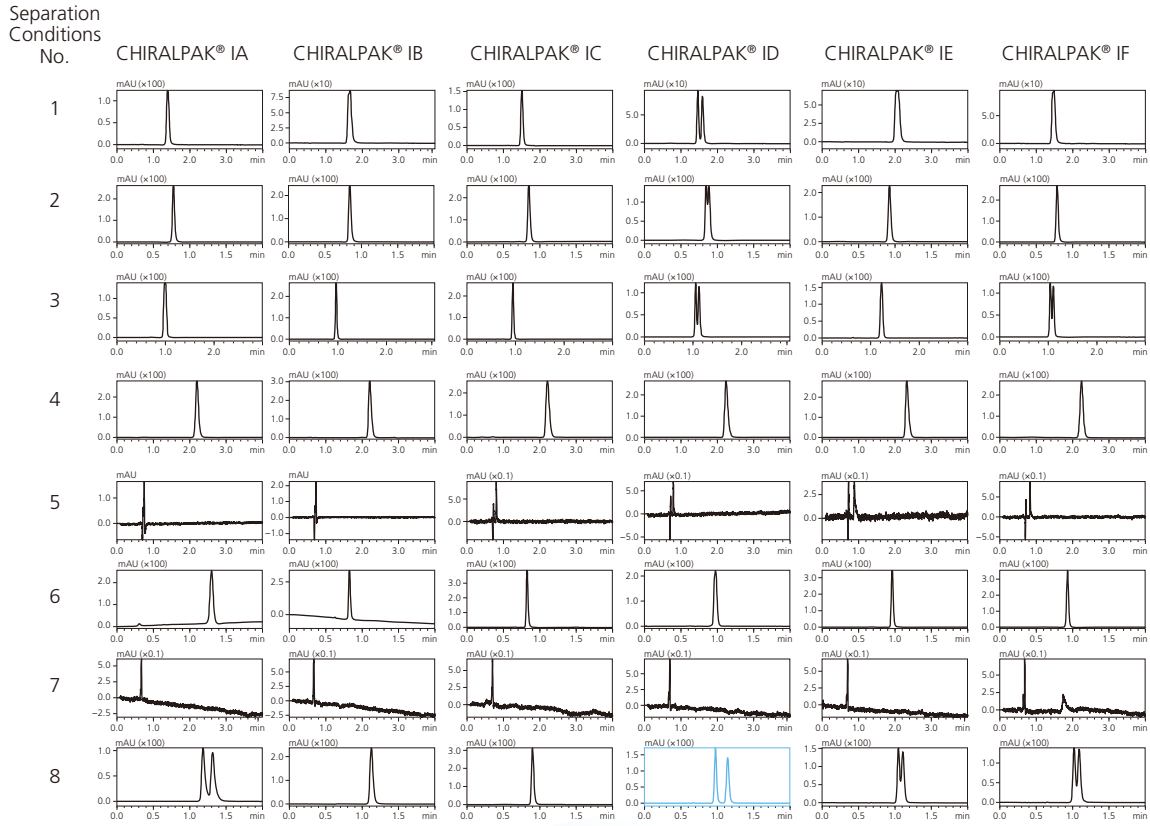
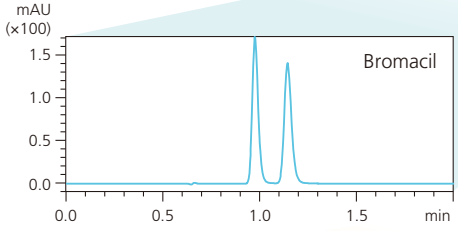
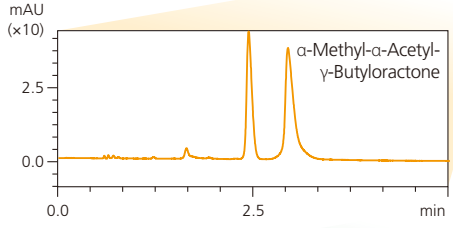


Fig. 4 Method Scouting Results for Bromacil



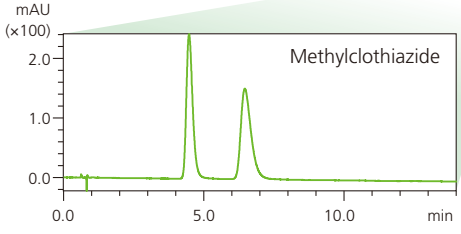
Separation Conditions 8 CHIRALPAK® ID

Peak	Retention Time t_n	Retention Factor k	Resolution Rs	Separation Factor α
1	0.974	0.522	—	—
2	1.142	0.784	2.643	1.504



Separation Conditions 3 CHIRALPAK® IF

Peak	Retention Time t_n	Retention Factor k	Resolution Rs	Separation Factor α
1	2.434	3.125	—	—
2	2.936	3.977	3.167	1.273



Separation Conditions 3 CHIRALPAK® ID

Peak	Retention Time t_n	Retention Factor k	Resolution Rs	Separation Factor α
1	4.465	6.202	—	—
2	6.449	9.401	3.724	1.516

Fig. 5 Chromatograms of Bromacil, α-Methyl-α-Acetyl-γ-Butyloractone and Methylclothiazide

4. Analysis

CLASS-Agent Report data processing software (Shimadzu Corp.) can quickly select the best separation conditions by comparing the data trace along with chromatographic parameters such as resolution, number of detected peaks, etc. With this software, it is possible to compare data both visually and quantitatively, thus making data processing more efficient.

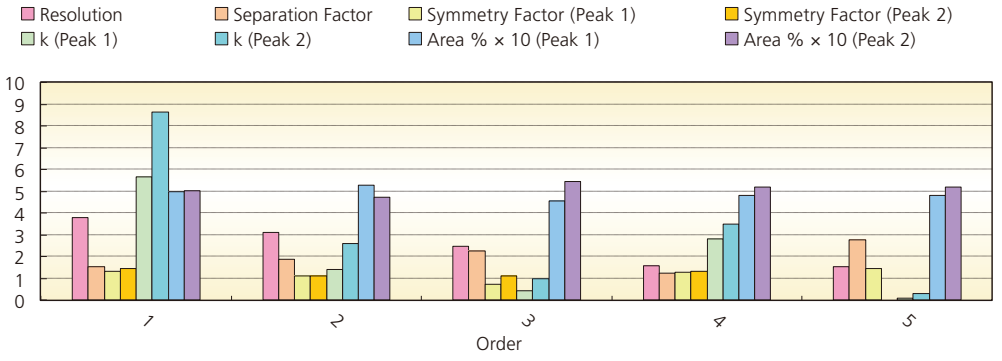


Fig. 6 Quantitative Comparison of Chromatograms Using CLASS-Agent Report

Table 2 Analysis Results for Methylclothiazide

Order	Run No.	Analytical Conditions	Resolution	Separation Factor	Symmetry Factor		k		Area %		No. of Peaks Detected
					Peak 1	Peak 2	Peak 1	Peak 2	Peak 1	Peak 2	
1	18	Methylclothiazide_ID_n-Hex_EtOH_3_analysis_B20%_14 min	3.785	1.523	1.31	1.463	5.665	8.626	49.777	50.223	2
2	27	Methylclothiazide_IF_MC_EtOH_6_analysis_B2%_4 min	3.086	1.858	1.127	1.094	1.39	2.583	52.748	47.252	2
3	6	Methylclothiazide_IB_MC_EtOH_6_analysis_B2%_4 min	2.456	2.248	0.715	1.094	0.443	0.995	45.633	54.367	2
4	13	Methylclothiazide_IC_n-Hex_EtOH_3_analysis_B20%_14 min	1.577	1.238	1.264	1.3	2.821	3.493	47.96	52.04	2
5	30	Methylclothiazide_IF_n-Hex_EtOH_4_analysis_B100%_18 min	1.515	2.759	1.465	0	0.102	0.282	48.153	51.847	2

5. Conclusion

Using the Nexera Method Scouting system and the *i*Chiral-6 high-resolution polysaccharide-based columns, it was possible to quickly determine through exhaustive analysis the best column and mobile phase for each of three types of chiral compounds. Further, by comparing such numerical values representing the resolution and symmetry index of each chromatogram using CLASS-Agent Report, it was possible to conduct a comparative evaluation of the chromatograms as well as a visual comparison, permitting greatly improved efficiency of data analysis.

We believe that the system and columns used in this research will be useful in a variety of markets including the synthetic drug discovery sector and pharmaceutical intermediates sector responsible for chiral analysis, new method development in pharmaceutical CMC departments, and chemical and food R&D.

References

1) Technical Report "Ultra Fast Method Scouting" (C190-E158)
2) Technical Report "Improved R&D Efficiency Through Speedier Method Development (3)" (C190-E159)

Acknowledgment

We wish to express our deep appreciation to Daicel Corporation for their guidance and cooperation for us to proceed with this study.

Note: CHIRALPAK is a registered trademark of Daicel Corporation.

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