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Chiral Separation Using SFC and HPLC

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Introduction

More than half of low molecular-weight drugs have stereoisomers, and pharmacological activities of each enantiomer are different. Therefore, it is important that the efficacy and safety of compounds are evaluated as enantiomers, especially in pharmaceutical formulations and its related industries. Chiral separation using SFC and HPLC is one of the typical methods for purifying enantiomers from racemic mixtures. In this method, the suitable column and mobile phase for targeted chiral separation have to be evaluated before starting the analysis. To determine the optimized analytical conditions, a large number of candidate conditions have to be examined, a process that requires extensive method development. In these days, a more prompt and simplistic system for determining the optimized analytical conditions is needed.

We have developed a method screening system and workflow using both SFC and HPLC to evaluate chiral separation more efficiently. This system has two solvent delivery pumps and one carbon dioxide delivery pump, and can be used for SFC and HPLC with a single instrument. The system is configured by installing a column switching valve inside the oven and a solvent switching valve within solvent delivery pumps, thereby permitting comprehensive data collection while continuously switching through multiple combinations of columns and mobile phases for both SFC and HPLC automatically using dedicated control software. Here, we report the process of high efficiency method development of chiral compounds by using SFC and HPLC in a single sequence.

Experimental

System

Fig.1 shows a flow diagram of the "Nexera UC LC/SFC switching system for chiral screening" that was developed in this experiment. This system consists of a combination of supercritical fluid chromatography "Nexera UC" and ultra high-performance liquid chromatography "Nexera X2", and can be used for both SFC and UHPLC with a single instrument by switching pumps, which are used for delivering solvent or CO2, and by regulating the backpressure or not. Furthermore, solvent switching valves and column switching valves are assembled into this system, and combinations of columns and mobile phases on the screening analyses can be changed automatically.

The process of switching analytical conditions between SFC to HPLC is accomplished in about 10 min. At first, mobile phase containing CO2 in the flow line is replaced by a solvent which is miscible with both SFC and HPLC mobile phases (e.g., EtOH, IPA and MeOH). After that, a flow selecting valve is switched to the HPLC analysis line, and HPLC mobile phase is delivered to the column for the equilibration. The process of switching analytical conditions from HPLC to SFC is almost the same.

Chiral Separation Using SFC and HPLC

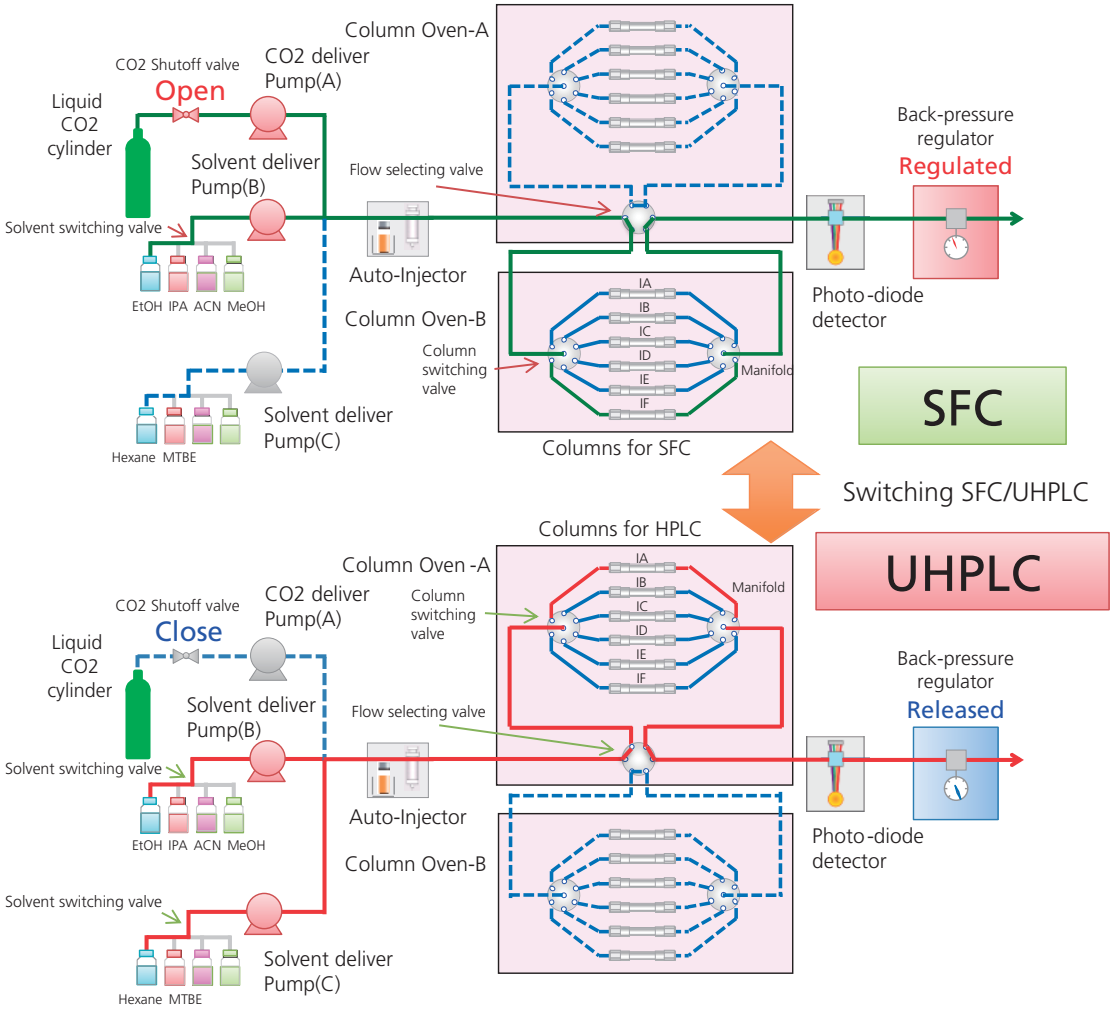
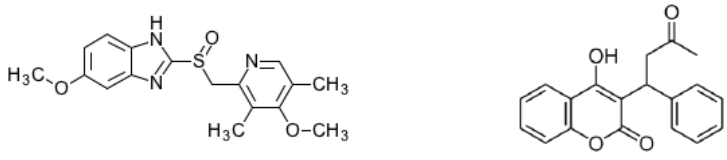


Fig. 1 Flow diagram "Nexera UC LC/SFC switching system for chiral screening"

Sample

Two standard chiral compounds (Omeprazole, Warfarin) were analyzed, as shown in Fig. 2.



①Omeprazole ②Warfarin

Fig. 2 Structures of Chiral Compounds

Chiral Column

Chiral columns "i CHIRAL-6 series (CHIRALPAK® IA/IB/IC/ID/IE/IF)" (Daicel Corp.) were selected for separation screening.

Table 1 Analytical columns for screening

Columns name	Stationary Phase	Particle size	Diameter	Length
CHIRALPAK® IA	Amylose tris (3, 5-dimethylphenylcarbamate)	3 um	SFC 3.0 mm HPLC 4.6mm	SFC 100 mm HPLC 50mm
CHIRALPAK® IB	Cellulose tris (3, 5-dimethylphenylcarbamate)			
CHIRALPAK® IC	Cellulose tris (3, 5-dichlorophenylcarbamate)			
CHIRALPAK® ID	Amylose tris (3-chlorophenylcarbamate)			
CHIRALPAK® IE	Amylose tris (3, 5-dichlorophenylcarbamate)			
CHIRALPAK® IF	Amylose tris (3-chloro-4-methylphenylcarbamate)			

Analytical condition

SFC screening conditions are shown in Table 2. Three modifier conditions were suggested, which are mixed with CO2 and solvents such as methanol, ethanol and acetonitrile at given ratios. With 3 modifiers and 6 columns, a total of 18 analytical conditions were examined with each substance. HPLC screening conditions are shown in Table 3. Three mobile phase conditions were suggested, which are mixed with solvents such as hexane , Methyl tert-butyl ether, 2-propanol and ethanol at given ratios. With 3 mobile phases and 6 columns, a total of 18 analytical conditions were examined with each substance.
A total of 36 analytical conditions were automatically screened by using SFC and HPLC.

Table 2 Analytical Conditions for SFC-Chiral analyses

Condition No.	Modifier	Modifier Conc.(%) (Isocratic)	Flow Rate	Analysis Time	Others
1	MeOH	20 %	3 mL/min	5 min	Column Temp. : 40℃ Inj. Vol. : 1 uL BPR Press : 10 MPa Detection : PDA@220 nm Step GE 0 - 5 min / 20% / Analysis 5 – 7 min / 40% / Column washing 7 – 10 min / 20% / Equilibration
2	EtOH	20 %	3 mL/min	5 min	
3	ACN / EtOH 75 / 25 (V/V)	20 %	3 mL/min	5 min	

Table 3 Analytical Conditions for HPLC-Chiral analyses

Condition No.	Mobile phase (A/B)	Solvent B.Conc(%) Isocratic	Flow Rate	Analysis Time	Others
1	Hexane / EtOH	20 %	2 mL/min	6 min	Column Temp. : 40℃ Inj. Vol. : 1 uL BPR Press : 10 MPa Detection : PDA@220 nm Step GE 0 - 5 min / 20% / Analysis 5 – 7 min / 40% / Column washing 7 – 10 min / 20% / Equilibration
2	Hexane / IPA	20 %	2 mL/min	6 min	
3	MTBE / EtOH	20 %	2 mL/min	6 min	

Results

Data processing

All chromatograms of omeprazole are shown in Fig.4. Data processing software "CLASS-Agent Report" (Shimadzu Corp.) was able to pick the best separation chromatogram quickly by comparing the resolutions, number of detected peaks, and other variables. With this software, it is possible to compare the data both visually and quantitatively and thus it helps to make data processing more efficient (Fig. 5, Table 4).

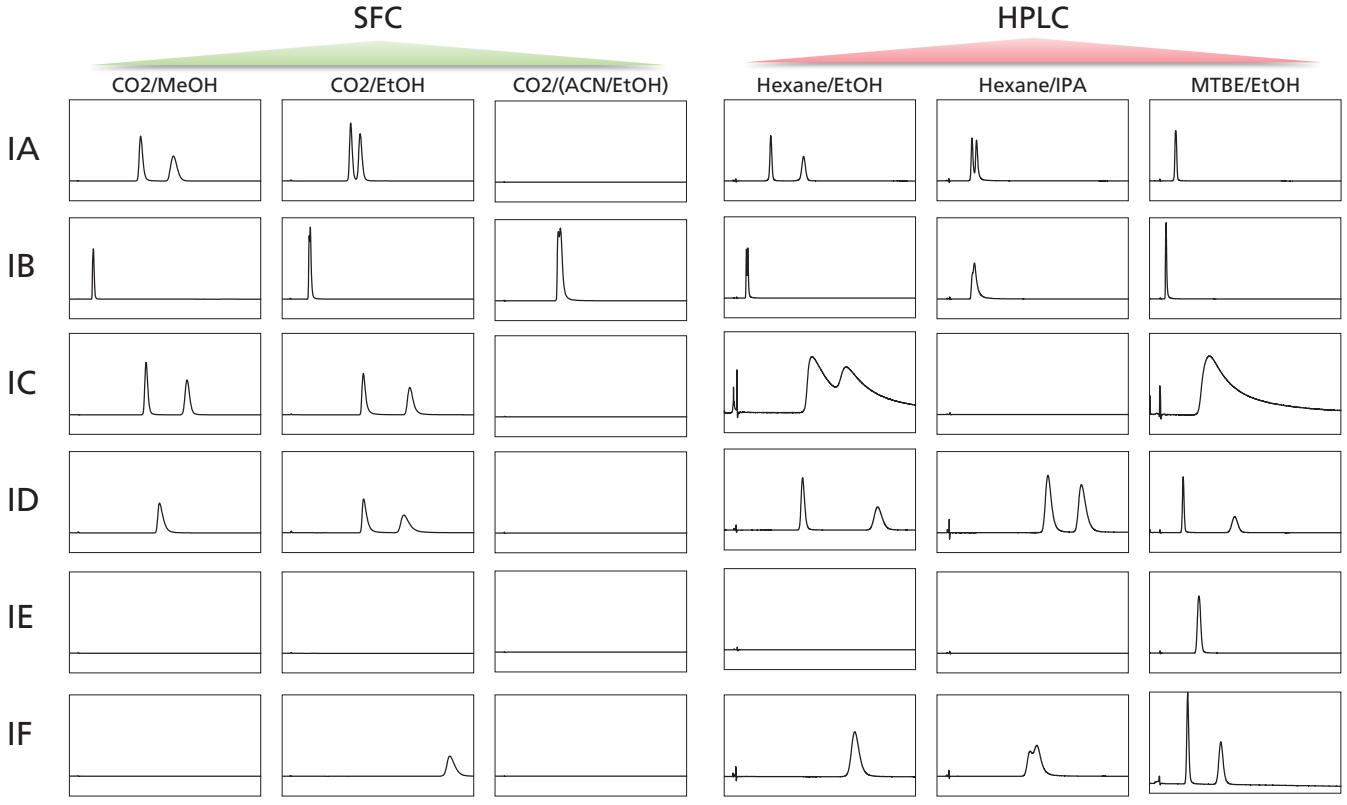


Fig. 4 Chromatograms of Omeprazole by all screening conditions

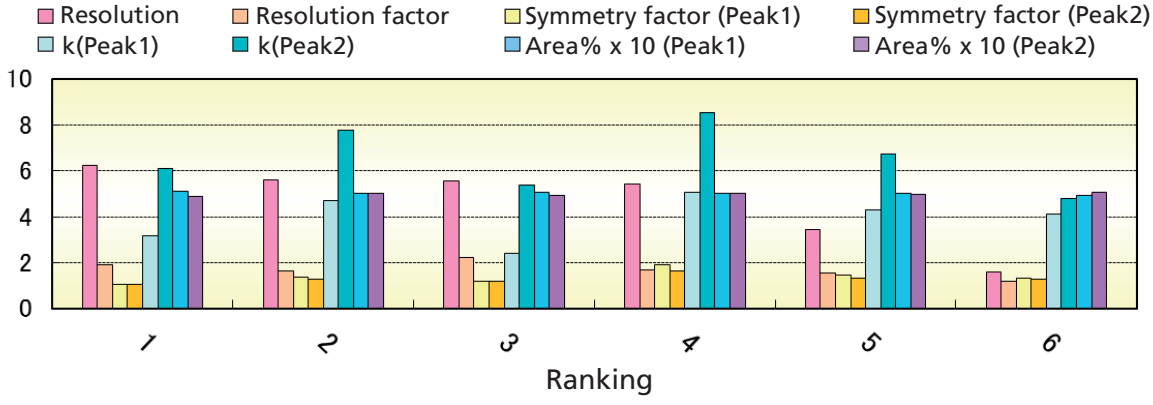


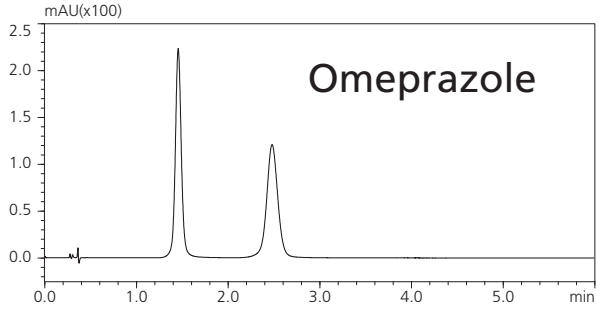
Fig. 5 Estimation result of the separation for Omeprazole by using CLASS-Agent Report

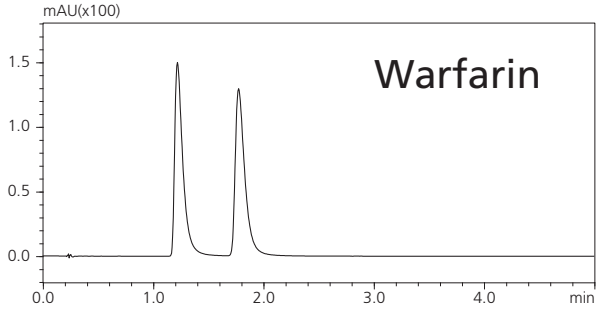
Table 4 Estimation result of the separation for Omeprazole by using CLASS-Agent Report

Ranking	Run No.	Analytical Condition	Resolution	Resolution factor	Symmetry factor		Retention factor		Area%		Detected Peaks
					Peak1	Peak2	Peak1	Peak2	Peak1	Peak2	
1	12	LC_IA_EtOH_Hexane	6.24	1.92	1.06	1.05	3.18	6.11	51.04	48.96	2
2	8	SFC_IC_MeOH	5.60	1.65	1.39	1.27	4.71	7.76	50.06	49.94	2
3	25	LC_IF_EtOH_MTBE	5.55	2.22	1.20	1.19	2.42	5.38	50.66	49.34	2
4	7	SFC_IC_EtOH	5.40	1.69	1.90	1.64	5.05	8.51	50.06	49.94	2
5	2	SFC_IA_MeOH	3.43	1.57	1.46	1.31	4.30	6.75	50.15	49.85	2
6	1	SFC_IA_EtOH	1.59	1.17	1.32	1.28	4.10	4.80	49.46	50.54	2

Screening Results

The optimized methods of each chiral compound are shown in Fig. 6. For Omeprazole, one of the HPLC conditions indicate the best separation. On the other hand, one of the SFC conditions indicate the best separation of Warfarin. This technique switching that uses SFC and HPLC in a single sequence is an effective way for efficient chiral separation method development.





HPLC CHIRALPAK IA Hexane-EtOH

Peak	Retention Time (t_n)	Retention factor (k)	Resolution (Rs)	Resolution factor (α)
1	1.463	3.180	—	—
2	2.487	6.105	6.239	1.920

SFC CHIRALPAK IA CO2 /ACN-EtOH

Peak	Retention Time (t_n)	Retention factor (k)	Resolution (Rs)	Resolution factor (α)
1	1.222	2.491	—	—
2	1.777	4.076	3.775	1.64

Fig.6 Chromatograms of Omeprazole and Warfarin with optimized conditions.

Conclusions

The combination of the "Nexera UC LC/SFC switching system for chiral screening" and chiral columns "i CHIRAL-6 series (CHIRALPAK® IA/IB/IC/ID/IE/IF)" allowed analytical conditions suitable for each chiral compound to be quickly determined. Furthermore, the data processing software "CLASS-Agent Report" (Shimadzu Corp.) was able to evaluate each chromatogram not only visually but quantitatively by comparing resolution or symmetry factors numerically, which achieved higher data processing efficiency.

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