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Improvement of total analytical workflow by using online SFE-SFC

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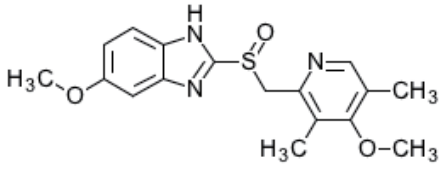
Introduction

Total analysis time is composed of sample preparation, a chromatographic separation of the target compounds and column equilibration. Online SFE-SFC can decrease the total analysis time, because after simply placing a sample in a special extraction vessel, it automatically performs the entire process from extraction of target components to acquisition of data. Furthermore, SFC can be expected to separate isomers in a shorter time than HPLC.
Online SFE-SFC is particularly time-saving in the pharmaceutical industry. 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 accurately 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. A 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 and this process requires extensive method development. A more prompt and simplistic system for determining the optimized analytical conditions has been needed. A HPLC/SFC switching system meets and exceeds those needs.
We demonstrated high-resolution separations using a HPLC/SFC switching system, which automatically switches analytical modes between HPLC and SFC in a single sequence. Here, we report the process of a high-efficiency method development workflow for chiral compounds by using SFC and HPLC in a single sequence. In addition, an online SFE-SFC method was developed and a plasma sample was analyzed under the optimized conditions.

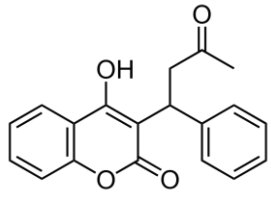
Materials and Method

Sample and column

Two standard chiral compounds (Omeprazole, Warfarin) were analyzed, as shown in Fig. 1. For HPLC / SFC chiral analytical columns, the i CHIRAL-6 series (CHIRALPAK® IA/IB/IC/ID/IE/IF, Daicel Corporation) were used. Column specifications, such as stationary phase and particle size, are shown in Table 1 and Fig. 2.



(1) Omeprazole



(2) Warfarin

Fig. 1: Structures of Chiral Compounds

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Table 1: Chiral Columns used in this study

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)			

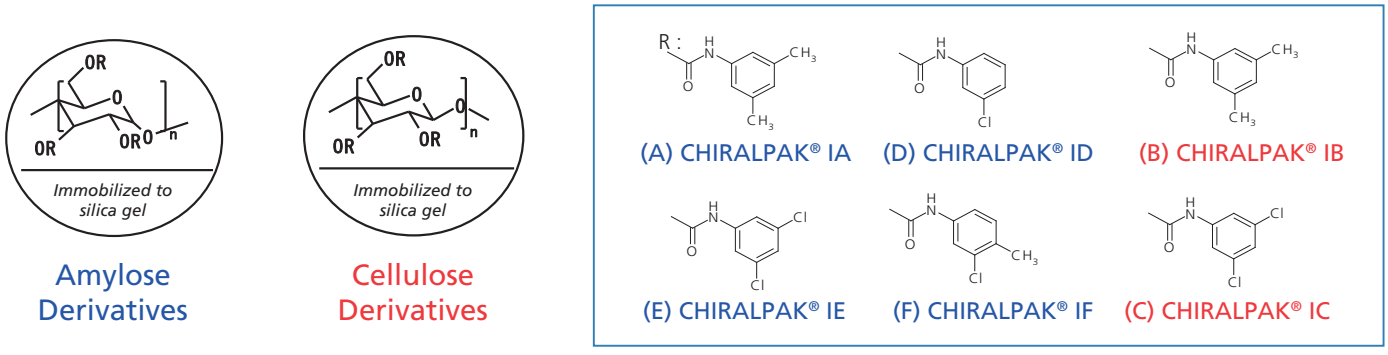

Fig. 2: Chiral Columns

System

Fig. 3 shows a flow diagram of the "Nexera UC online-SFE-HPLC/SFC switching system" that was developed in this experiment. This system consists of the online SFE-SFC system "Nexera UC", which combines supercritical fluid extraction and supercritical fluid chromatography, and ultra high-performance liquid chromatography "Nexera X2". The single instrument can be used for both SFC and HPLC by switching pumps for delivering solvent or CO_2, and by regulating the backpressure or not. Furthermore, solvent switching valves and column switching valves are assembled into this system, allowing combinations of columns and mobile phases for the scouting analyses to be changed automatically.
It allows for online SFE-SFC analysis without reconnecting flow lines.

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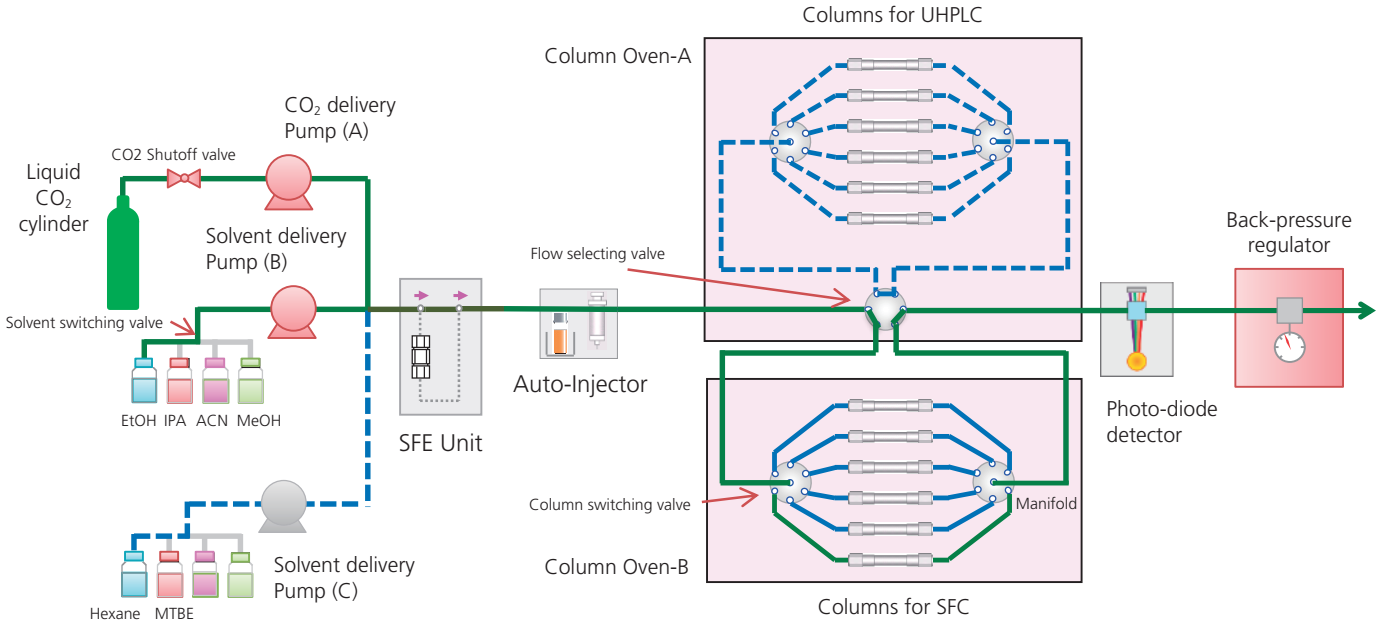
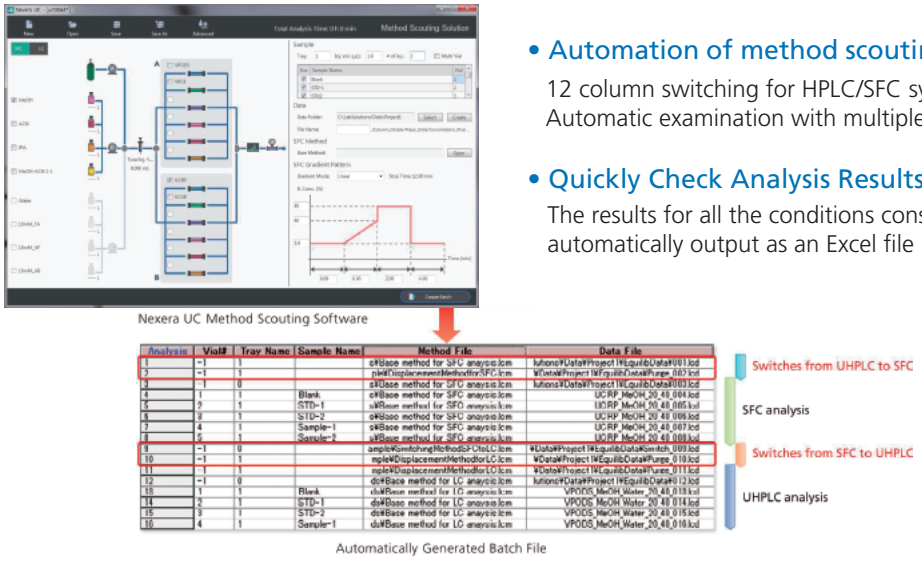


Fig. 3: Flow diagram "Nexera UC online-SFE-HPLC/SFC switching system" (for online SFE-SFC analysis)

Software

Software performs the work of switching between HPLC and SFC. Purging of mobile phases, which is required when switching between SFC and HPLC, is accomplished by simply executing a batch table automatically generated by the dedicated software: "Nexera UC Method Scouting Solution Ver.2". It also enables multiple mobile phases and columns to be used for method scouting.



- **Automation of method scouting process**
  12 column switching for HPLC/SFC system
  Automatic examination with multiple gradient patterns
- **Quickly Check Analysis Results**
  The results for all the conditions considered can be automatically output as an Excel file

Fig. 4: Method scouting software

Analytical conditions

HPLC / SFC analytical conditions are shown in Table 2 / Table 3. By using 3 mobile phases and 12 columns (HPLC 6 columns and SFC 6 columns), a total of 36 analytical conditions were automatically examined. Online-SFE-SFC analytical conditions for a rat plasma sample are shown in Table 4.

Table 2: HPLC chiral analytical conditions

No.	Mobile phase (A/B)	Others
1	Hexane/ Ethanol	B Conc.(%) : 20% (Isocratic) Time program : 20 % (0 -6min) - 40 % (6-8 min : wash) - 20 % (8 -12min) Flow Rate : 2mL/min Column Temp : 40 °C Inj. Vol. : 1 uL Detection : PDA@220 nm
2	Hexane / Isopropyl alcohol	
3	Methyl tertiary butyl ether / Ethanol	

Table 3: SFC chiral analytical conditions

No.	Modifier	Others
1	Methanol	Modifier Conc. (%) : 20% (Isocratic) Time program : 20 % (0 -5min) - 40 % (5-7 min : wash) - 20 % (7 -10min) Flow Rate : 3mL/min Column Temp. : 40 °C Inj. Vol. : 1 uL BPR Press : 10 MPa Detection : PDA@220 nm
2	Ethanol	
3	Acetonitrile Ethanol = 75 / 25 (v/v)	

Table 4: Online-SFE-SFC analytical conditions

SFE	
Extraction Time	: 15 min
Mobile Phase	: A: CO_2 B: Ethanol
B Conc.	: 0 % (v/v)
Flowrate	: 5.0 mL/min
Back Pressure	: 10 MPa

SFC	
Column	: CHIRALPAK® IA (100 mm L. × 3.0 mm I.D., 3 µm)
Mobile Phase	: A: CO_2 B: Ethanol
Time Program	: 0 (0min) - 20 % (1 to 4 min) → 40 % (4 to 9min)
Flowrate	: 3.0 mL/min
Column Temp.	: 40 °C
Back Pressure	: A) 10 MPa, B) 40 MPa
Detector	: PDA@220 nm

Results

Column and modifier scouting

All chromatograms of warfarin are shown in Fig. 5. In some conditions, isomers were successfully separated. A comparison of SFC/HPLC chromatograms using 36 analytical conditions indicated that SFC parameters provided better separation for warfarin.

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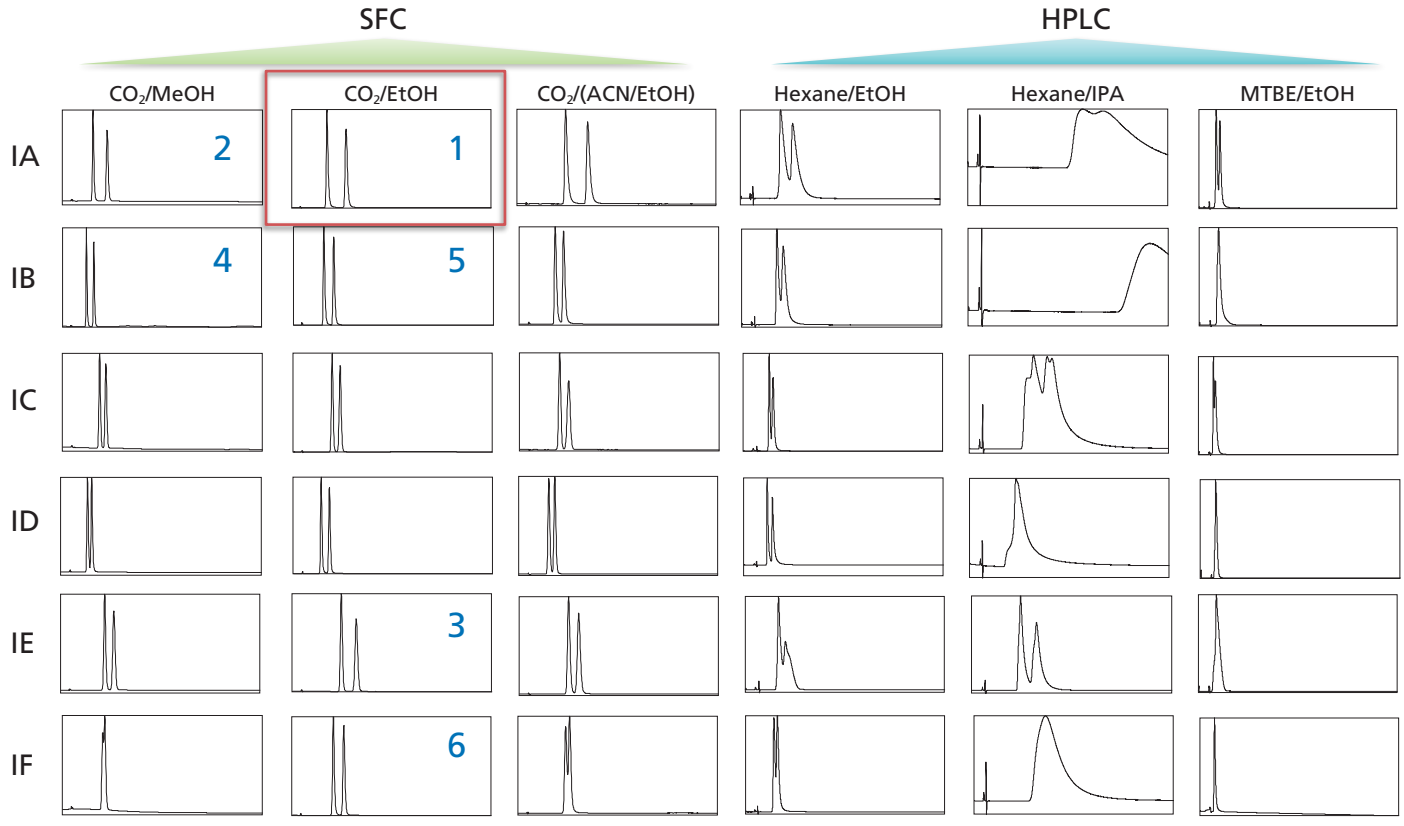

Fig. 5: Chromatograms of Warfarin with all scouting conditions.
(The number in chromatograms shows rank of Fig. 6)

Data processing software "Multi-data Report (MDR)" 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 quantitatively and thus it makes data processing more efficient (Fig. 6).

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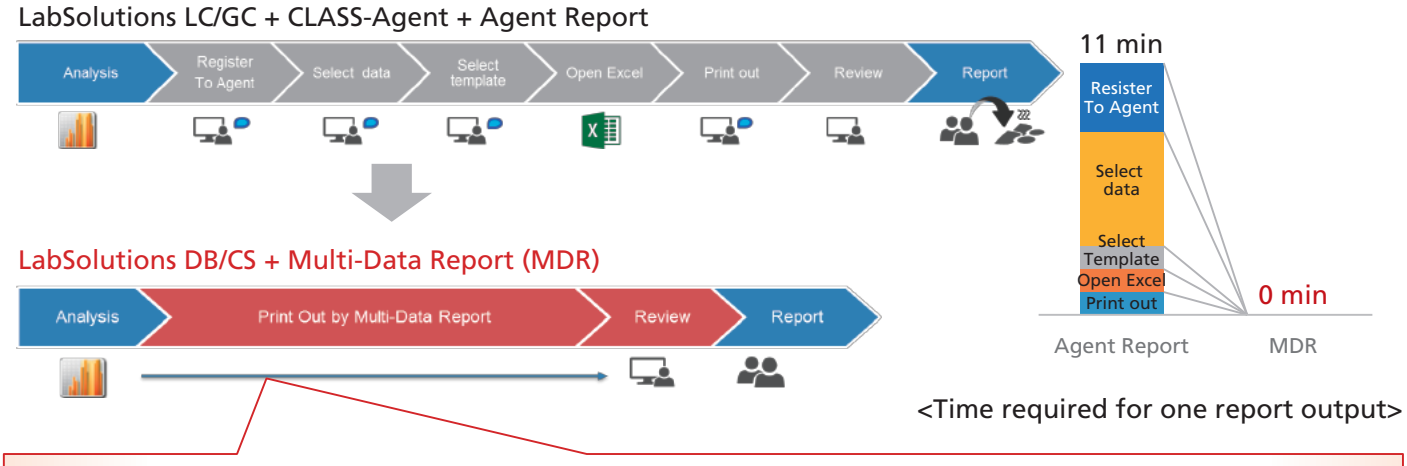

<Time required for one report output>

Ranking	Run No.	Analytical Condition	Resolution	Selectivity	Symmetry factor		Retention factor		Area %		Detected Peaks
					Peak 1	Peak 2	Peak 1	Peak 2	Peak 1	Peak 2	
1	2	SFC_IA_EtOH	4.61	1.89	1.65	1.49	1.53	2.89	49.93	50.07	2
2	3	SFC_IA_MeOH	4.08	1.86	1.35	1.28	1.17	2.17	49.90	50.10	2
3	14	SFC_IE_EtOH	3.16	1.43	1.19	1.13	2.51	3.57	50.01	49.99	2
4	6	SFC_IB_MeOH	2.96	1.73	1.38	1.28	0.72	1.25	49.83	50.17	2
5	5	SFC_IB_EtOH	2.85	1.60	1.59	1.43	1.18	1.88	49.79	50.21	2
6	17	SFC_IF_EtOH	2.72	1.38	1.29	1.20	1.96	2.71	49.84	50.16	2

Fig 6: Workflow of outputting report which shows estimation result of the separation for Warfarin

Online SFE-SFC analysis

Glass filter was prepared by dropping 5 µL of rat blood plasma spiked with a warfarin standard. Fig. 7 shows the results from analyzing the warfarin isomer by online SFE-SFC (analytical conditions in Table 4). Warfarin chiral compounds were detected and separated enough after only fifteen minutes of SFE extraction.

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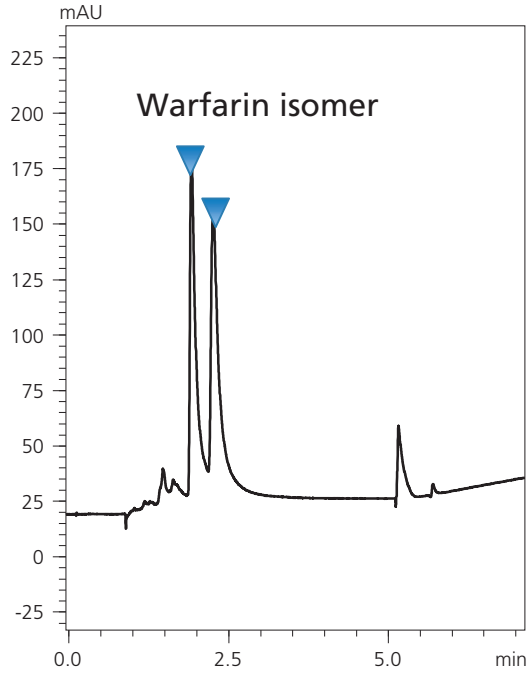

Fig. 7: Chromatogram of rat blood plasma spiked with a warfarin standard

Conclusion

- With the "Nexera UC HPLC/SFC switching system for chiral screening ", analytical conditions suitable for chiral compounds could be quickly determined. Furthermore, the data processing software "LabSolutions DB/CS + Multi-Data Report " achieved higher efficiency.
- Online SFE-SFC system can be used to reduce the work involved in pretreatment processes that were previously performed manually for research in pharmaceutical fields. The evaluation of quantitative analysis will be examined in future work.

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