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Application News

Supercritical Fluid Chromatography System Nexera™ UC
Software for Efficient Method Development LabSolutions™ MD

Efficient Scouting of Chiral Separation Conditions Using LabSolutions MD

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User Benefits

- Supercritical fluid chromatography offers quicker separation of chiral compounds.
- LabSolutions MD offers more efficient scouting of analytical conditions for new compounds.
- Peak shapes of acidic and basic analytes can be improved by adding suitable additives to the modifier.

■ Introduction

Chiral compounds contain at least one asymmetric carbon, so they cannot be superimposed on their mirror image. Although HPLC is the dominant chromatographic technique used to separate chiral compounds, there is growing interest in chiral separation that uses supercritical fluid chromatography (SFC). SFC is normally performed using a mobile phase of supercritical carbon dioxide, which offers low polarity, low viscosity and high diffusivity. Polar organic solvents (modifiers) are then added to this mobile phase to control interactions between the analytes and the stationary phase. Chiral compounds are typically separated by HPLC under normal phase conditions, but the unique properties of SFC mentioned above offer advantages, such as higher separation speeds and lower organic solvent consumption, which reduce costs and lower the environmental impact.

Therefore, the pharmaceutical sector uses SFC because it can provide rapid optical resolution in the synthesis of novel drugs. Finding the best column and mobile phase (modifier) combination for a given analyte species from the wide variety of chiral columns available is a very time-consuming and labor-intensive process, so there is demand for faster and more streamlined methods for scouting chiral separation conditions.

This article describes a workflow for developing separation conditions for chiral compounds that uses the Nexera UC chiral screening system and LabSolutions MD analytical method development software.

■ Nexera UC Chiral Screening System

The Nexera UC chiral screening system consists of an SFC system, a solvent switching valve, and a column switching valve. It can automatically and continuously switch the modifiers between up to 12 columns, enabling the comprehensive collection of analytical data. Because it can switch between up to seven different modifiers and investigate a variety of separation conditions, the Nexera US chiral screening system can identify high-resolution separation conditions for chiral compounds quicker and with less work than other systems.

Fig. 1 Nexera™ UC Chiral Screening System

■ Analyte Compounds

This article describes a process for optimizing the analytical conditions for three analyte compounds: chlormezanone (neutral compound), flurbiprofen (acidic compound), and disopyramide (basic compound) (Fig. 2). Each of these compounds has one asymmetric carbon and two isomers.

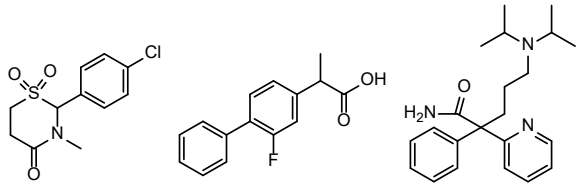

Fig. 2 Structural Formula of Analyte Compounds
(From Left: Chlormezanone, Flurbiprofen, and Disopyramide)

■ Creating Column Screening Schedules

LabSolutions MD offers excellent ease of use and can create multiple analytical conditions automatically in a single operation and then screen these conditions efficiently. The modifier and column conditions can also be set by simply selecting from the modifiers and columns that are pre-registered in the software database (Fig. 3). Scouting for analytical conditions manually is a labor-intensive process that involves manually switching between modifiers and columns and manually preparing analytical methods and batch schedules. However, LabSolutions MD can configure and automate these tasks with ease.

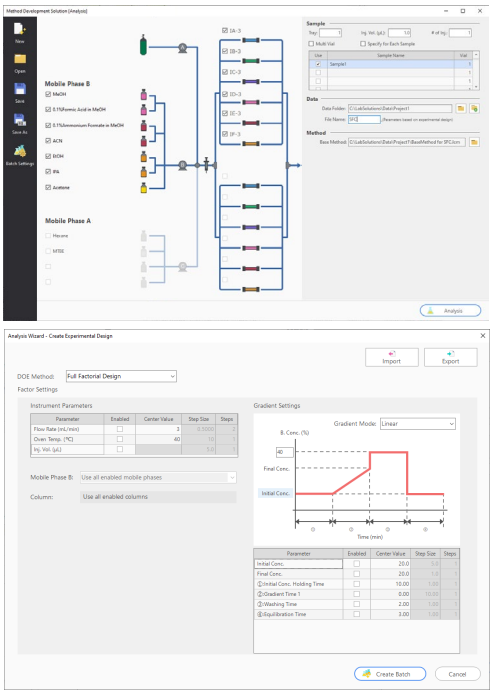

Fig. 3 Analysis Schedule Preparation Window

LabSolutions MD was used to scout 42 analytical conditions for each analyte compound from a combination of six columns and seven modifiers. The screening conditions are shown in Table 1. The screening results for chlormezanone are shown in Fig. 4.

Table 1 Modifier and Column Scouting Conditions

System:	Nexera UC Chiral Screening System
Column:	CHIRALPAK® IA-3 (100 mm x 3.0 mmI.D., 3 µm) CHIRALPAK® IB-3 (100 mm x 3.0 mmI.D., 3 µm) CHIRALPAK® IC-3 (100 mm x 3.0 mmI.D., 3 µm) CHIRALPAK® ID-3 (100 mm x 3.0 mmI.D., 3 µm) CHIRALPAK® IE-3 (100 mm x 3.0 mmI.D., 3 µm) CHIRALPAK® IF-3 (100 mm x 3.0 mmI.D., 3 µm)
Mobile Phase A:	CO_2
Mobile Phase B:	Methanol Acetonitrile Ethanol 2-Propanol Acetone 0.1 % Formic acid in methanol 0.1 % Ammonium formate in methanol
Flowrate:	3.0 mL/min
Time Program:	B. Conc. 20 % (0-8 min) → 40 % (8.01-10.0 min) → 20 % (10.01-12.0 min)
Column Temp.:	40 °C
Injection Volume:	2 µL in ethanol
BPR Pressure:	10 MPa
Detection:	220 nm (for chlormezanone) 245 nm (for flurbiprofen) 260 nm (for disopyramide) (PDA with a high-pressure flow cell)

■ Rapid Identification of Optimized Conditions from Screening Results

The column screening stage yields as many chromatograms as the conditions that are screened (Fig. 4). All these chromatograms must then be assessed to determine which conditions offer the best separation. This assessment step is typically very labor intensive and requires experience in chromatography. However, LabSolutions MD can evaluate separation conditions that are based on the resolution between the target peaks. This removes the need for intuition and experience to identify the optimal conditions, so this task can now be performed by anyone quickly and easily (Fig. 5).

When analyte compounds have multiple asymmetrical carbons and are expected to separate into many peaks, LabSolutions MD can rank separation conditions that are based not just on the resolution between the target peaks but also on the number of detected peaks or a score (Equation 1) that is calculated from the number of detected peaks (P) and the resolution (Rs).

$$(\text{Score}) = P \times (Rs1 + Rs2 + \ldots + RsP - 1) \qquad \text{(Equation 1)}$$

The chromatogram with the highest score in this analysis is outlined in red in Fig. 4. In this analysis, LabSolutions MD showed that using methanol as a modifier with the CHIRALPAK® IC-3 column offered the best chiral separation of chlormezanone isomers.

			Response
Sample Name	MPB Nick Name	Column Nick	Resolution
Chlormezanone	MeOH	IA-3	11.049
Chlormezanone	0.1%Ammo···	IF-3	10.872
Chlormezanone	0.1%Ammo···	IA-3	10.727
Chlormezanone	EtOH	IF-3	10.629
Chlormezanone	0.1%Formi···	IA-3	10.628
Chlormezanone	EtOH	IA-3	10.445
Chlormezanone	MeOH	ID-3	10.193
Chlormezanone	0.1%Ammo···	ID-3	10.005
Chlormezanone	0.1%Formi···	ID-3	9.928
Chlormezanone	MeOH	IF-3	8.102

Fig. 5 Separation Conditions Ranked by Score
(Top 10 Scoring Separation Conditions are Shown.)

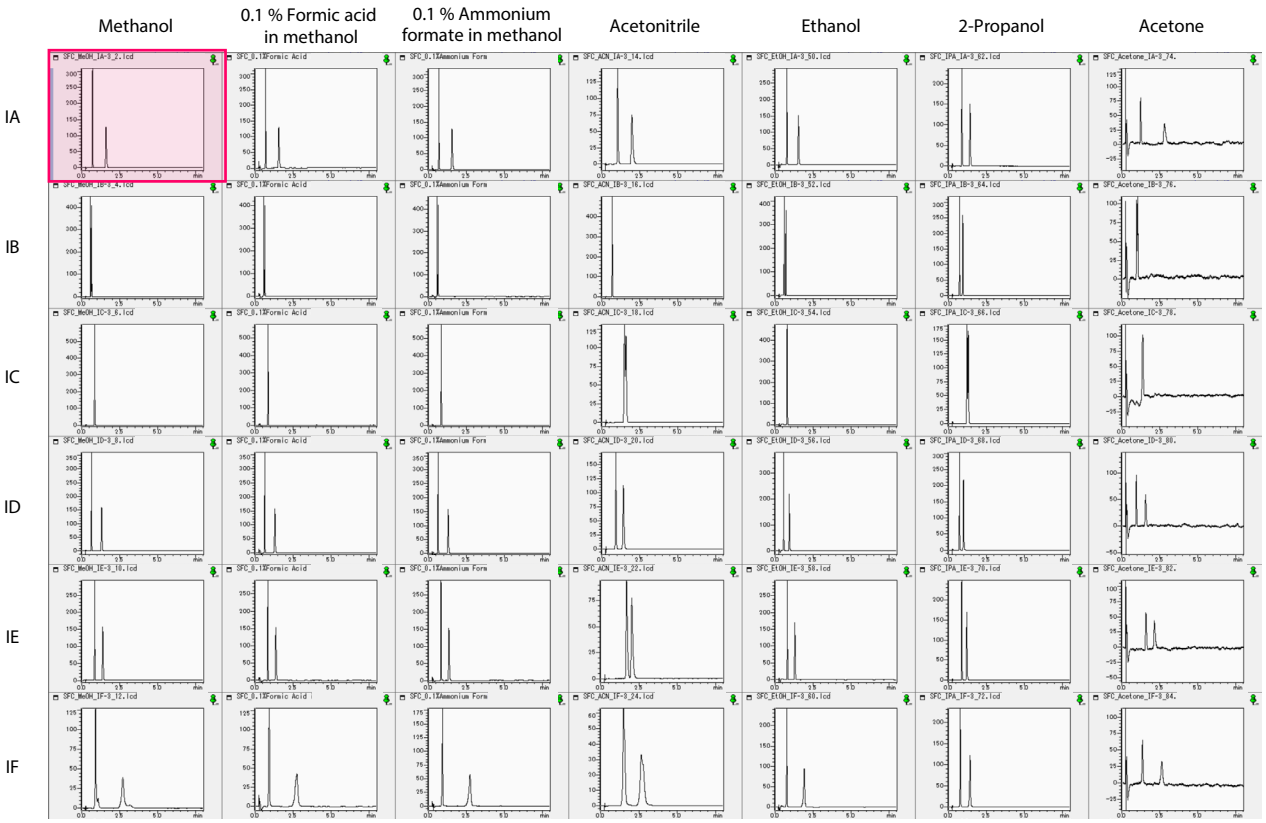

Fig. 4 Chlormezanone Chromatograms Obtained during Screening
(Highest Scoring Chromatogram Outlined in Red)

■ Tuning Separation and Improving Peak Shapes with Additives

With HPLC, buffers and ion-pair reagents are sometimes added to the mobile phase to tune the selectivity of a separation or to improve peak shapes. Additives are also added for similar purposes with SFC, but they are added to the modifier and not the supercritical carbon dioxide mobile phase. Adding acids, such as formic acid, and bases, such as amines, in SFC can improve peak shapes by preventing the ionization of target components and masking secondary functional groups in the stationary phase.

■ Optimum Separation Conditions for Flurbiprofen and Disopyramide

The optimum conditions for chiral separation of the acidic compound flurbiprofen and the basic compound disopyramide were scouted by a method that is also used for the neutral compound chlormezanone.

Chromatograms that were obtained from flurbiprofen and disopyramide during this scouting process are shown in Fig. 6.

Good peak shapes were obtained from the acidic compound flurbiprofen even without adding an acidic additive to the modifier. This is because unlike LC, SFC uses a slightly acidic mobile phase (carbon dioxide), so good peak shapes can be obtained from many acidic compounds without additives. Peak shapes from some acidic compounds can also be improved by adding additives, such as formic acid and trifluoroacetic acid.

For the basic compound disopyramide, adding a basic additive to the modifier reduced nonspecific absorption by preventing ionization of the target components and masking secondary functional groups in the stationary phase, which improved separation and peak shapes.

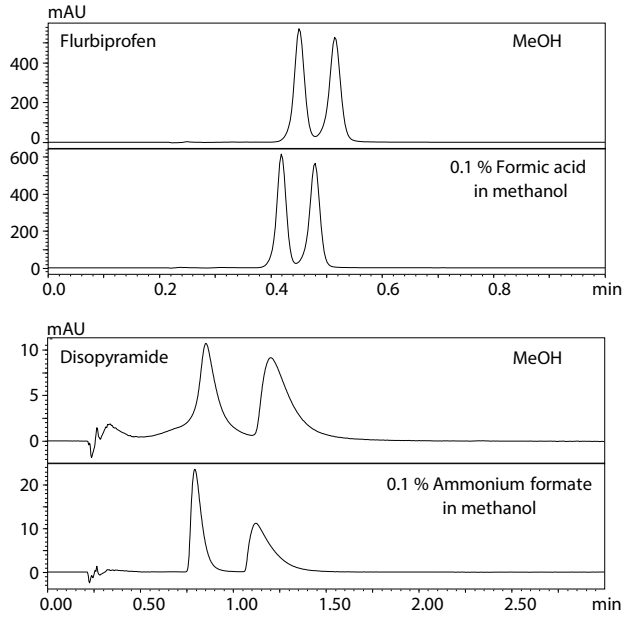

Fig. 6 Chromatograms from Flurbiprofen (Top) and Disopyramide (Bottom)

■ Conclusion

This article describes a workflow for finding separation conditions for chiral compounds using the Nexera UC chiral screening system and LabSolutions MD analytical method development software. LabSolutions MD allowed analytical conditions to be investigated with less work and greater efficiency.

Because SFC uses a slightly acidic mobile phase (carbon dioxide), it can produce good peak shapes from many acidic compounds without using additives. This article also shows that SFC can produce good separation of even basic compounds when suitable additives are added to the modifier. The Nexera UC chiral screening system can switch between up to seven different modifiers in an analysis sequence, enabling the efficient investigation of additives that are not just organic solvents.

Furthermore, the carbon dioxide used by SFC is cheaper than the organic solvents commonly used by HPLC and reduces disposal costs, which promises lower running costs for SFC. SFC can also be used for preparative purification and it offers significant time and labor savings in chiral purification.

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