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# Application News

# No.L495

Supercritical Fluid Chromatography

# Automated Optimization of Chiral Separation Parameters Using Nexera UC Chiral Screening System

Chiral compounds contain asymmetric carbons in their molecules and are not superimposable on their mirror images. HPLC has been the main method used to separate such chiral compounds, but in recent years, the use of supercritical fluid chromatography (SFC) has been gaining attention. The main mobile phase used for chiral SFC is supercritical carbon dioxide, with low polarity, low viscosity, and high diffusivity, to which polar organic solvents (modifiers) are added to control solubility and polarity. Therefore, chiral compound separation by HPLC, which generally uses normal phase conditions, offers the potential for high speed, low organic solvent consumption, low cost, and low environmental impact. However, chiral SFC requires selecting a variety of separation parameters, such as columns and modifiers, which can involve large amounts of time and effort. This article describes using the Nexera UC chiral screening system to automatically optimize the large number of separation parameters by switching between up to 12 columns and various mixture ratios of four types of modifiers. This can significantly reduce the effort required.

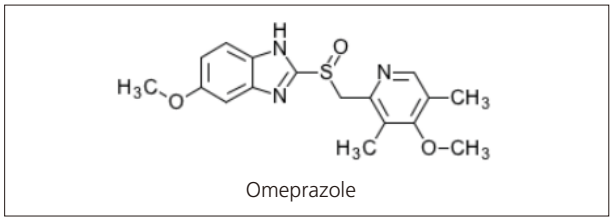



**Fig. 1 Sample Used to Evaluate the Method Scouting Function**

## Separation Parameters for the Chiral Screening System

Model sample: The structure of omeprazole is shown in Fig. 1. Daicel CHIRALPAK®/CHIRALCEL® series 12 columns for chiral analysis were used for the analysis. These columns offer a line of complementary stationary phase columns that are able to separate a wide variety of chiral compounds. When used in combination with the Nexera UC chiral screening system, which features a method scouting function, optimal chiral separation parameters can be determined easily. In addition, three types of modifiers were used, methanol, ethanol, and a mixture of acetonitrile and ethanol. Details about the separation parameters are indicated in Table 1. The optimal parameters for chiral separation were comprehensively selected from the total of 36 possible combinations of modifiers (3 types) and columns (12 types).

**Table 1 Analytical Conditions**

| | |
|---|---|
| Column | : CHIRALPAK®, CHIRALCEL® Series<br>100 mm L. × 3.0 mm I.D., 3 µm |
| Mobile Phase | : A; Super critical fluid of $CO_2$<br>B; Modifier: Methanol, Ethanol, mixture of Acetonitrile: Ethanol = 3:1 (v:v) |
| Time Program | : B Conc. 20 % (0 - 8 min) → 40 % (8 - 10 min) → 20 % (10 - 14 min) |
| Flowrate | : 3 mL/min |
| Column Temp. | : 40 °C |
| Injection Volume | : 2 µL |
| BPR Pressure | : 10 Mpa |
| Detector | : Photodiode Array Detector (Max Plot 210 - 400 nm) |

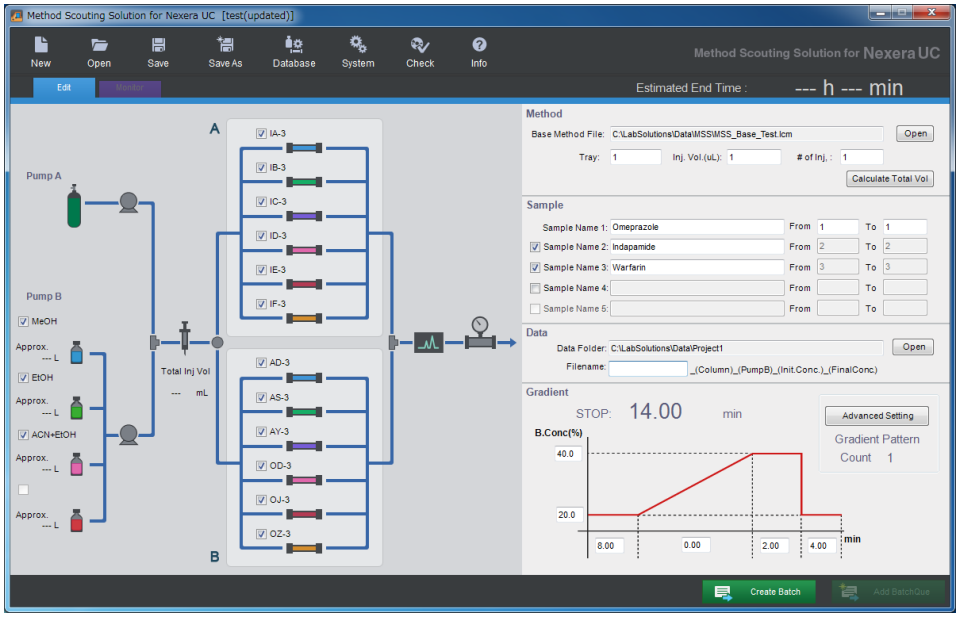
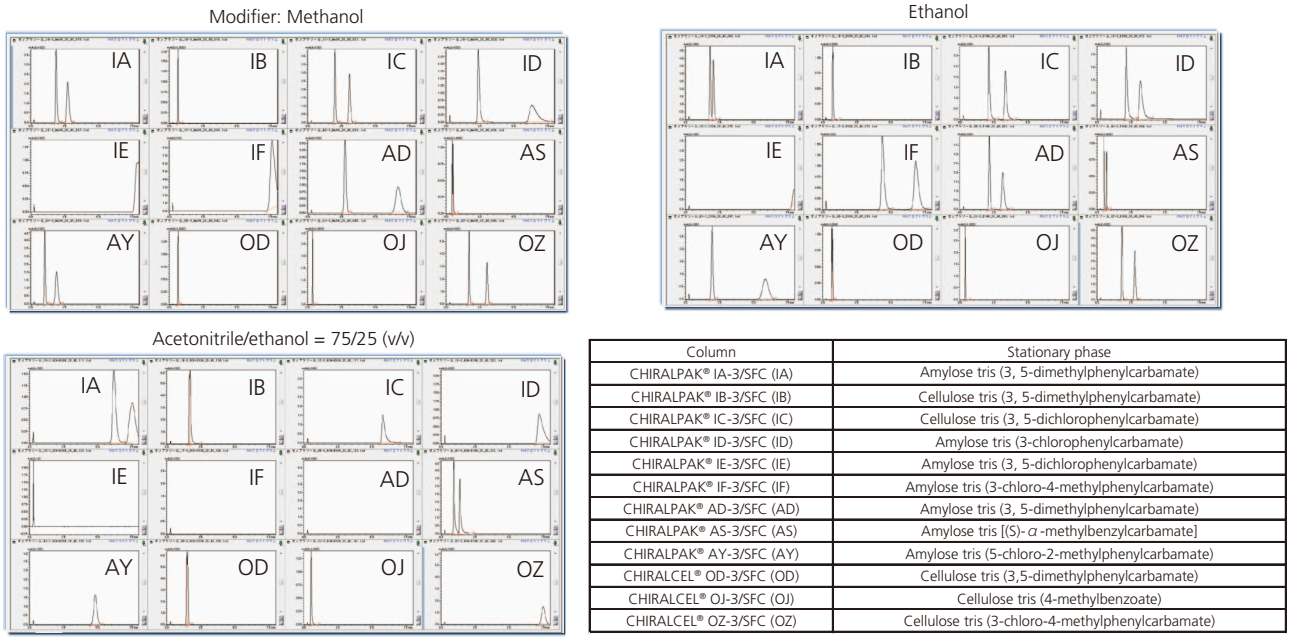



**Fig. 2 Method Scouting Solution Operating Screen for Nexera UC**

## Automated Optimization of Chiral Separation Parameters for Omeprazole

Fig. 3 shows the results from a total of 36 possible combinations of 12 chiral columns and 3 types of modifiers (methanol, ethanol, and acetonitrile/ethanol mixture).

For omeprazole, separation of peaks for two chiral forms were confirmed within 8 minutes of retention. Fig. 4 shows the separation evaluation and optimal parameter ranking results from the optional software. The software automatically ranks all the chromatograms with separation greater than a given criteria (in this case, 1.5). This confirmed the utility of using the Nexera UC chiral screening system to automatically optimize separation parameters for chiral SFC, which otherwise requires a complicated process of selecting analytical conditions.




| Column | Stationary phase |
|---|---|
| CHIRALPAK® IA-3/SFC (IA) | Amylose tris (3, 5-dimethylphenylcarbamate) |
| CHIRALPAK® IB-3/SFC (IB) | Cellulose tris (3, 5-dimethylphenylcarbamate) |
| CHIRALPAK® IC-3/SFC (IC) | Cellulose tris (3, 5-dichlorophenylcarbamate) |
| CHIRALPAK® ID-3/SFC (ID) | Amylose tris (3-chlorophenylcarbamate) |
| CHIRALPAK® IE-3/SFC (IE) | Amylose tris (3, 5-dichlorophenylcarbamate) |
| CHIRALPAK® IF-3/SFC (IF) | Amylose tris (3-chloro-4-methylphenylcarbamate) |
| CHIRALPAK® AD-3/SFC (AD) | Amylose tris (3, 5-dimethylphenylcarbamate) |
| CHIRALPAK® AS-3/SFC (AS) | Amylose tris [(S)-α-methylbenzylcarbamate] |
| CHIRALPAK® AY-3/SFC (AY) | Amylose tris (5-chloro-2-methylphenylcarbamate) |
| CHIRALCEL® OD-3/SFC (OD) | Cellulose tris (3,5-dimethylphenylcarbamate) |
| CHIRALCEL® OJ-3/SFC (OJ) | Cellulose tris (4-methylbenzoate) |
| CHIRALCEL® OZ-3/SFC (OZ) | Cellulose tris (3-chloro-4-methylphenylcarbamate) |

**Fig. 3 Comparison of Separation Using Different Combinations of 12 Chiral Columns and 3 Modifiers**

| Ranking | Run No. | Analytical Condition | Resolution | Separatoin factor | Symmetry factor | | Retention factor | | Area% | | Peak number |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Peak1 | Peak2 | Peak1 | Peak2 | Peak1 | Peak2 | |
| 1 | 32 | Omeprazole_OZ-3_MeOH_20_40 | 7.965 | 1.921 | 1.16 | 1.159 | 6.583 | 12.644 | 49.829 | 50.171 | 2 |
| 2 | 17 | Omeprazole_IC-3_MeOH_20_40 | 5.587 | 1.602 | 1.387 | 1.274 | 8.078 | 12.937 | 49.971 | 50.029 | 2 |
| 3 | 16 | Omeprazole_IC-3_EtOH_20_40 | 5.382 | 1.639 | 1.915 | 1.661 | 8.617 | 14.124 | 49.984 | 50.016 | 2 |
| 4 | 31 | Omeprazole_OZ-3_EtOH_20_40 | 5.377 | 1.599 | 1.169 | 1.162 | 7.229 | 11.561 | 49.778 | 50.222 | 2 |
| 5 | 1 | Omeprazole_AD-3_EtOH_20_40 | 3.996 | 1.509 | 1.257 | 1.404 | 8.779 | 13.25 | 50.054 | 49.946 | 2 |
| 6 | 8 | Omeprazole_AY-3_MeOH_20_40 | 3.55 | 2.08 | 1.178 | 1.145 | 3.652 | 7.597 | 49.974 | 50.026 | 2 |
| 7 | 11 | Omeprazole_IA-3_MeOH_20_40 | 3.428 | 1.523 | 1.464 | 1.312 | 7.435 | 11.327 | 49.973 | 50.027 | 2 |
| 8 | 4 | Omeprazole_AS-3_EtOH_20_40 | 2.515 | 1.673 | 1.657 | 1.518 | 1.244 | 2.081 | 49.754 | 50.246 | 2 |
| 9 | 10 | Omeprazole_IA-3_EtOH_20_40 | 1.586 | 1.157 | 1.322 | 1.279 | 7.115 | 8.234 | 49.347 | 50.653 | 2 |

Separation Parameters for Rank 1
Column: CHIRALCEL® OZ-3/SFC
Modifier: Methanol

Separation Parameters for Rank 2
Column: CHIRALPAK® IC/SFC
Modifier: Methanol

Separation Parameters for Rank 3
Column: CHIRALPAK® IC/SFC
Modifier: Ethanol

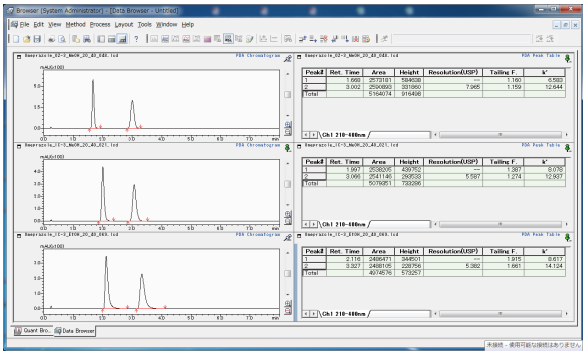


**Fig. 4 Evaluation of Separation Parameters and Chiral Separation Chromatogram Using Optimized Parameters**

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