Investigation of Chiral Separation Conditions of Bupivacaine by Unified Fluid Chromatography Methods Scouting System

Introduction

In the synthesis of medicinal drugs and agrichemical fields, synthetic compounds with optical activity are continuing to gain more attentions. These compounds can show the same physical chemical properties, but show different biological activity.

It has been reported that using only one enantiomer of the optical isomer can enhance the medicinal effects and reduce the side effects when the biological activity varies between the enantiomers.

Our Methods Scouting System makes it simple for users to search and select the appropriate measurement conditions using various solvents and columns for both chiral and achiral separations.

Supercritical fluid chromatography (SFC) is well known for quick separations, easy solvent replacement, easy sample treatment after preparation, decreasing solvent cost over HPLC and simple scale-up from analytical to preparative.

In this application, method scouting of bupivacaine, used as local analgesic, with three modifiers which diethylamine (DEA) was added to for addition agent and six columns is carried out by using UFC (Unified Fluid Chromatography) and Method Scouting Program, which is optional program of ChromNAV Ver.2.

**Keyword:** Bupivacaine, SFC, UFC, ChromNAV Ver.2, Method Scouting Program, i-CHIRAL 6, Chiral separation

Experimental Condition

**Column:** CHIRALPAK IA, IB, IC, ID, IE and IF/SFC

(i-CHIRAL 6), (4.6 mm I.D. x 150 mmL, 5 mm)

**Eluent:** CO2/modifier (75/25)

**Modifier:**
1) methanol + 0.4% diethylamine
2) acetonitrile/ethanol (80/20) + 0.4% diethylamine
3) methyl tert-butyl ether (MTBE)/ethanol (80/20) + 0.4% diethylamine

**Flow rate:** 3.0 mL/min

**Column temp.:** 40°C

**Wave length:** UV: 220 nm, CD: 230 nm

**Back pressure.:** 15 MPa

**Injection volume:** 5 µL

**Standard:** 1.0 mg/mL bupivacaine in ethanol/acetic acid (90/10)

**Structure**

![Bupivacaine Structure](image)

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Schematic diagram

1: CO₂ Cylinder, 2: CO₂ pump, 3: Modifier solvents, 4: Solvent switch valve, 5: Modifier pump, 6: Stop valve, 7: Mixer, 8: Pre-heat coil, 9: Autosampler, 10: Column oven, 11: Switching valve, 12: Columns (i-CHIRAL 6), 13: PDA detector, 14: Circular dichroism detector, 15: Back pressure regulator, 16: Chromatography data system (ChromNAV Ver.2)

Result

Figure 1 and 2 show the ChromNAV software results Previewer of Method Scouting by UV (PDA) detector and CD detector. This view provides easy viewing of all screening conditions and quick determination of the best for further optimization if needed.

Fig. 1 Result of Method Scouting (UV detector)
Fig. 2 Result of Method Scouting (CD detector)

Table 1 shows the degree in separation of bupivacaine. As shown in the table, MTBE/ethanol with 0.4 % DEA and CHIRALPAK IA is suited to this measurement.

<table>
<thead>
<tr>
<th>Modifier</th>
<th>Column</th>
<th>IA</th>
<th>IB</th>
<th>IC</th>
<th>ID</th>
<th>IE</th>
<th>IF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanol + 0.4% DEA</td>
<td>N.S.</td>
<td>N.S.</td>
<td>N.S.</td>
<td>N.S.</td>
<td>N.S.</td>
<td>N.S.</td>
<td>N.S.</td>
</tr>
<tr>
<td>Acetonitrile/Ethanol (80/20) + 0.4% DEA</td>
<td>3.70</td>
<td>N.S.</td>
<td>N.S.</td>
<td>N.S.</td>
<td>N.S.</td>
<td>2.40</td>
<td></td>
</tr>
<tr>
<td>MTBE/Ethanol (80/20) + 0.4% DEA</td>
<td>5.49</td>
<td>N.S.</td>
<td>0.683</td>
<td>N.S.</td>
<td>N.S.</td>
<td>2.34</td>
<td></td>
</tr>
</tbody>
</table>

N.S.: Not Separated

Table 1 Degree in separation