Method Development – Immobilized Phases

- Use: CHIRALPAK® IA™, CHIRALPAK IB™ and CHIRALPAK IC™
- Choose an appropriate additive – TFA for acidic compounds (e.g., carboxylic acids); DEA or TEA for basic compounds. ESA may be used for either acids or bases.
- Either:
  - Use starting conditions as in the diagram
  - Run scouting experiment to gauge solvent strength required (can be elution gradient)
- Find separations that show some selectivity
- Adjust solvent strength to obtain reasonable retention factor (1 < k’ < 6)
- If insufficient separation try
  - Hexane : THF
  - Hexane : ethyl acetate
  - Alternative coated phases (e.g., CHIRALCEL® OJ-H™ or CHIRALPAK AS-H™)

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**Method Development – Coated Phases**

- Or CHIRALPAK AD-3™ and CHIRALPAK OD-3™ in place of AD-H and OD-H
- Choose an appropriate additive – TFA for acidic compounds (eg carboxylic acids); DEA or TEA for basic compounds. ESA may be used for either acids or bases.
- Run screen with solvent compositions above; adjust hexane – alcohol ratio to obtain 1<k'<6.
- If no retention on polar solvents (MeOH & MeCN) there is nothing much to do
- If there is retention but inadequate separation in polar solvents try MeCN – MeOH or MeCN – IPA mixtures
- If no separation in non-polar solvents (hexane – IPA; Hexane – MeOH) try other coated phases
  - CHIRALCEL OB-H™, CHIRALCEL OC-H™, CHIRALCEL OF™, CHIRALCEL OG™ or CHIRALCEL OK™
  - Try other additives (if DEA, try ESA or cyclic amines)

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Method Development - Reversed Phase

Nature of compound

<table>
<thead>
<tr>
<th>Nature</th>
<th>Acidic</th>
<th>Neutral</th>
<th>Basic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aqueous</td>
<td>20 mM phosphate pH 2.0 40% ACN 60% MeOH</td>
<td>HCO₂H pH 2.0 40% ACN 60% MeOH</td>
<td>H₂O 40% ACN 60% MeOH</td>
</tr>
<tr>
<td>Organic</td>
<td>20 mM borate pH 9.0 40% ACN 60% MeOH</td>
<td>20 mM NH₄HCO₃ pH 9.0 40% ACN 60% MeOH</td>
<td></td>
</tr>
</tbody>
</table>

- **K'1 < 1** Partial Rs?
  - Decrease % organic
- **K'2 < 6** Baseline Rs?
  - Method OK
- **K'2 > 6**?
  - Increase % organic

- Or CHIRALPAK AD-3R™ and CHIRALCEL OD-3R™ in place of AD-RH & OD-RH
- Choose starting pH & buffer according to sample type and application (if LC-MS or not)
  - Either:
    - Start with 40% ACN and 60% MeOH
  - Run gradient to establish solvent strength required
  - Adjust % organic and / or pH to adjust retention
  - If no separation
    - If acid, try Lindner phases
    - If basic, try ion-pairing with ESA

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