“Chiral Impurity Methods – Case Study”

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Introduction: Chiral Impurity methods

Types of Chiral Stationary Phases (CSPs) for HPLC
- Polysaccharide-CSPs
  - Coated CSPs
  - immobilised CSPs

Case study:
- Oxaliplatin Chiral HPLC method
- Lamivudine Chiral HPLC method
- Clopidogrel Bisulphate Chiral HPLC method
Chiral Impurity Methods – Introduction

- Chiral GC
- Chiral HPLC
- Chiral HPCE
- Chiral SFC
## Chiral Impurity Methods – Types of CSP

### Types of CSPs and their loading capacities

<table>
<thead>
<tr>
<th>Type</th>
<th>CSPs</th>
<th>Loading capacity (mg solute / g CSP)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Pirkle type (Brush type)</td>
<td>1-50</td>
</tr>
<tr>
<td>II</td>
<td>Polysaccharide derivatives</td>
<td>5-150</td>
</tr>
<tr>
<td>III</td>
<td>Macrocyclic type</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cyclodextrins</td>
<td>0.1-5</td>
</tr>
<tr>
<td></td>
<td>Glycopeptides</td>
<td>0.1-5</td>
</tr>
<tr>
<td></td>
<td>Chiral Crown ether</td>
<td>0.1-5</td>
</tr>
<tr>
<td>IV</td>
<td>Ligand exchange</td>
<td>0.1-1</td>
</tr>
<tr>
<td>V</td>
<td>Protein type</td>
<td>0.1-0.2</td>
</tr>
</tbody>
</table>
Chiral Impurity Methods – Types of CSP

Which type of CSP?

1999

2001

2003

2005

Polysaccharide 80%

Protein 3%

Pirkle 5%

Others 12%

Pirkle 4%

Protein 2%

Others 7%


Chiral Impurity Methods – Types of CSP

Coated polysaccharide-derived CSPs

**Cellulose derivatives**

- ‘OD-H’
- ‘OJ-H’

**Amylose derivatives**

- ‘AD-H’
- ‘AS-H’
Chiral Impurity Methods – Types of CSPs

Immobilised polysaccharide-derived CSPs

**CHIRALPAK IA**

**CHIRALPAK IB**

**CHIRALPAK IC**
Chiral Impurity Methods - Case Study

• Case study-1:
  Oxaliplatin, Chemotherapy drug

• Case study-2:
  Lamivudine, Anti retroviral drug

• Case study-3:
  Clopidogrel Bisulphate, Anti platelet drug
Chiral Impurity Methods – Case Study 1

**Oxaliplatin**

Pharmacopeial method conditions:
- **Column:** CHIRALCEL OC 4.6 x 250 mm
- **Mobile Phase:** Ethanol / MeOH (30/70)
- **Flow Rate:** 0.3 mL/min; **Injection Volume:** 20 µL
- **Detection:** 254 nm by UV; **Temp:** 40°C
- **Concentration:** 0.6 mg/mL

Rs is NLT 1.5
Limit: 0.1%
Chiral Impurity Methods – Case Study 1

**Oxaliplatin**

Cost-effective method conditions:
- Column: CHIRALPAK IC 4.6 x 250mm
- Mobile Phase: Ethanol / MeOH (30/70)
- Flow Rate: 1.0 mL/min; Injection Volume: 20 µL
- Detection: 254 nm by UV; Temp: 40°C
- Concentration: 0.6 mg/mL

Rs > 9.0
LOQ: 0.03%
Chiral Impurity Methods – Case Study 1

Oxaliplatin

Cost-effective method conditions:
Column: CHIRALPAK IC 4.6 x 250mm
Mobile Phase: Ethanol / MeOH (30/70)
Flow Rate: 1.0 mL/min; Injection Volume: 20 µL
Detection: 254 nm by UV; Temp: 40° C
Concentration: 0.6 mg/mL

Injection no: 1

Injection no: 100
Chiral Impurity Methods – Case Study 2

Lamivudine

Pharmacopeial method conditions:
Column: L45 (Cyclobond I 2000 SP, 4.6 x 250mm, 5µ)
Mobile Phase: 0.1M Ammonium acetate/MeOH (95/05)
Flow Rate: 1.0 mL/min; Conc: 0.25 mg / mL; Inj Vol: 10µL
Detection : 270 nm by UV; Temperature: 25 ºC

Rs is NLT 1.5
Impurity limit: 0.3%
Chiral Impurity Methods – Case Study 2

Lamivudine

Cost-effective method conditions:
Column: CHIRALPAK IC (4.6 x 250) mm, 5 micron
Mobile Phase: Ethanol/2-Propanol/DEA (90/10/0.1, v/v/v)
Flow Rate: 0.5 mL/min; Conc: 0.25 mg/mL; Inj Vol: 10 µL
Detection: 270 nm by UV; Temperature: 25 °C; Diluent: MP

Rs > 3.0
LOQ: 0.1%
Chiral Impurity Methods – Case Study 2

Lamivudine

Cost-effective method conditions:
Column: CHIRALPAK IC (4.6 x 250) mm, 5 micron
Mobile Phase: Ethanol/2-Propanol/DEA (90/10/0.1, v/v/v)
Flow Rate: 0.5 mL/min
Detection: 270 nm by UV; Temperature: 25 °C
Chiral Impurity Methods – Case Study 3

Clopidogrel bisulphate

Pharmacopeial method conditions:
- Column: L 57 (Ultron ES-OVM (4.6 x 150) mm)
- Mobile Phase: 10mM Phosphate buffer/ACN (75/25)
- Flow Rate: 1.0 mL/min; Conc: 0.5 mg/mL
- Detection: 220 nm by UV

Method objective:

1. Clopidogrel bisulphate related substances quantification including chiral impurity
2. Clopidogrel bisulphate assay determination
Chiral Impurity Methods – Case Study 3

Clopidogrel and its related compounds

Imp-A
Limit: 0.2%

Imp-B
Limit: 0.3%

Imp-C
Limit: 1.0%
Chiral Impurity Methods – Case Study 3
Clopidogrel bisulphate

Pharmacopeial method
SS criteria: Rs between Imp B1 and Clopidogrel NLT 2.5
Chiral Impurity Methods – Case Study 3

Clopidogrel bisulphate

Pharmacopeial method
Cost effective method conditions:
Column: CHIRALCEL OJ-H (4.6 x 250) mm, 5 micron
Mobile Phase: Methanol/DEA (100/0.1, v/v)
Flow Rate: 1.0 mL/min; Conc: 0.5 mg/mL; Diluent : Ethanol
Detection : 220 nm by UV; Temperature: 25 ºC
Chiral Impurity Methods – Case Study 3

Clopidogrel bisulphate

Cost-effective method conditions:
Column: CHIRALCEL OJ-H (4.6 x 250) mm, 5 micron
Mobile Phase: Methanol/DEA (100/0.1, v/v)
Flow Rate: 1.0 mL/min; Diluent: Ethanol
Detection: 220 nm by UV; Temperature: 25 °C
Chiral Impurity Methods – Case Study 3
Clopidogrel bisulphate

Cost-effective method conditions:
Column: CHIRALCEL OJ-H (4.6 x 250) mm, 5 micron
Mobile Phase: Methanol/DEA (100/0.1, v/v)
Flow Rate: 1.0 mL/min; Diluent: Ethanol
Detection: 220 nm by UV; Temperature: 25 °C
Chiral Impurity Methods – Case Study 3

Clopidogrel bisulphate

Chromatographic Characteristics

<table>
<thead>
<tr>
<th>Compound peak</th>
<th>Rs</th>
<th>N</th>
<th>T</th>
</tr>
</thead>
<tbody>
<tr>
<td>Imp A</td>
<td>-</td>
<td>3907</td>
<td>1.4</td>
</tr>
<tr>
<td>Imp B1</td>
<td>17.4</td>
<td>11520</td>
<td>1.3</td>
</tr>
<tr>
<td>Imp C</td>
<td>2.3</td>
<td>11554</td>
<td>1.1</td>
</tr>
<tr>
<td>Imp B2</td>
<td>2.9</td>
<td>11063</td>
<td>1.1</td>
</tr>
<tr>
<td>Clopidogrel</td>
<td>3.6</td>
<td>10235</td>
<td>1.3</td>
</tr>
</tbody>
</table>

Note: Column memory effects may influence the elution of Imp A, hence recommended to dedicate the column for this application.
Conclusions

• Chiral HPLC using polysaccharide derived CSPs is a versatile tool to estimate chiral impurities in drug substances.

• Perhaps, the chiral impurity methods described in Pharmacopeia for Oxaliplatin, Lamivudine & Clopidogrel bisulphate are utilising the columns, which are not so durable and hence results into high analytical cost.

• It would be beneficial to adapt new generation chiral column chemistry available today to develop an efficient & cost effective chiral impurity methods.
Acknowledgments

• Mr. Raghuram, General Manager, Hetero Drugs Ltd for his continuous co-operation during Lamivudine method development

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THANK YOU ALL