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IPC-USP 8th ASM 2009

"Chiral Impurity Methods – Case Study"

Ch. LAKSHMI NARAYANA
DAICEL CHIRAL TECHNOLOGIES (INDIA) PVT LTD

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Overview

- 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

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Chiral Impurity Methods – Introduction

- Chiral GC
- Chiral HPLC
- Chiral HPCE
- Chiral SFC

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Chiral Impurity Methods – Types of CSP

Types of CSPs and their loading capacities

Type	CSPs	Loading capacity (mg solute / g CSP)
I	Pirkle type (Brush type)	1-50
II	Polysaccharide derivatives	**5-150**
III	Macrocyclic type	
	Cyclodextrins	0.1-5
	Glycopeptides	0.1-5
	Chiral Crown ether	0.1-5
IV	Ligand exchange	0.1-1
V	Protein type	0.1-0.2

Drug discovery today, volume 10, Number 8, April 2005

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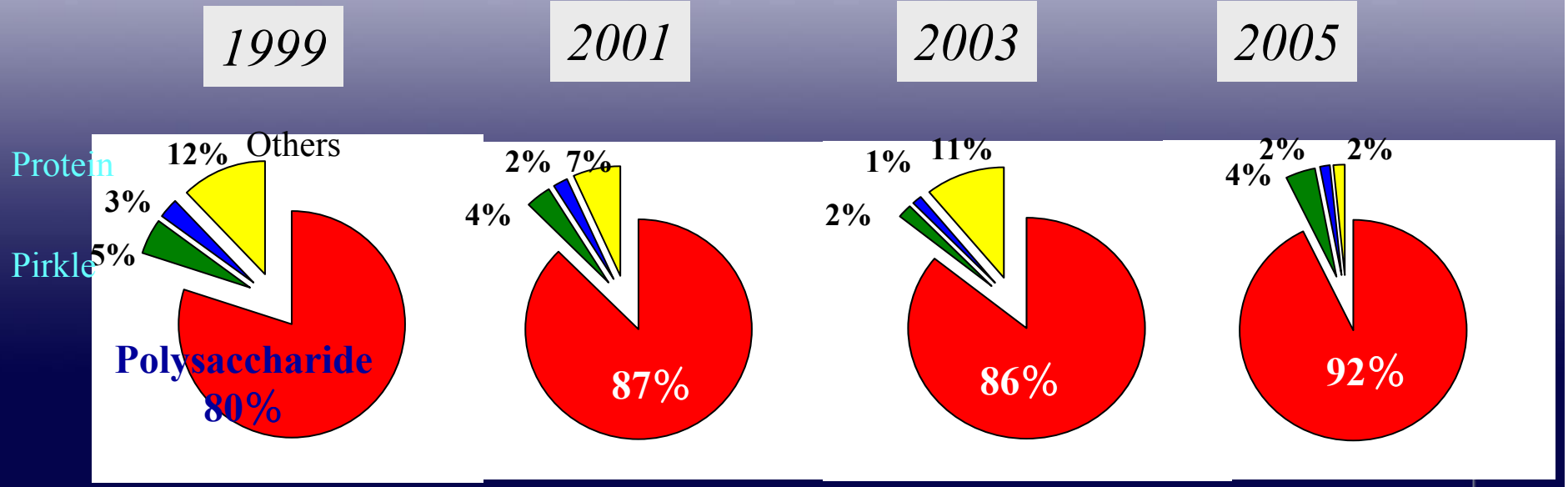
Chiral Impurity Methods – Types of CSP

Which type of CSP ?



1995-2003 : Tetrahedron Asymmetry

2005 : J. Am. Chem. Soc.

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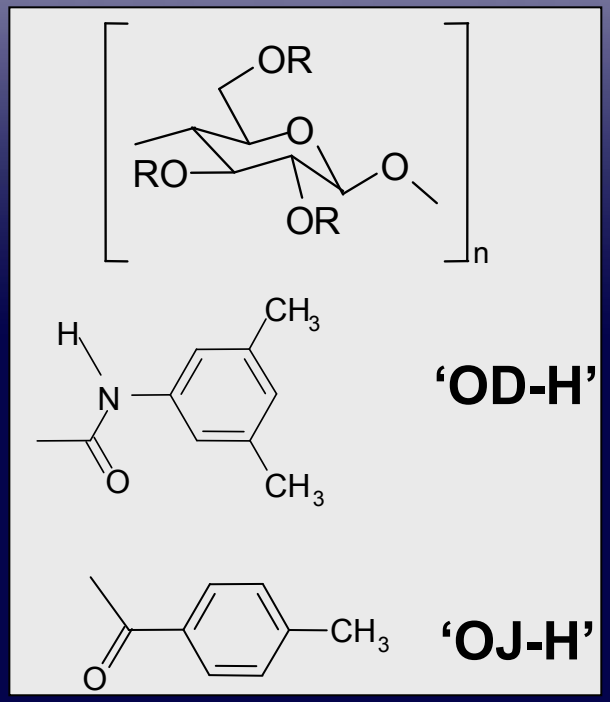
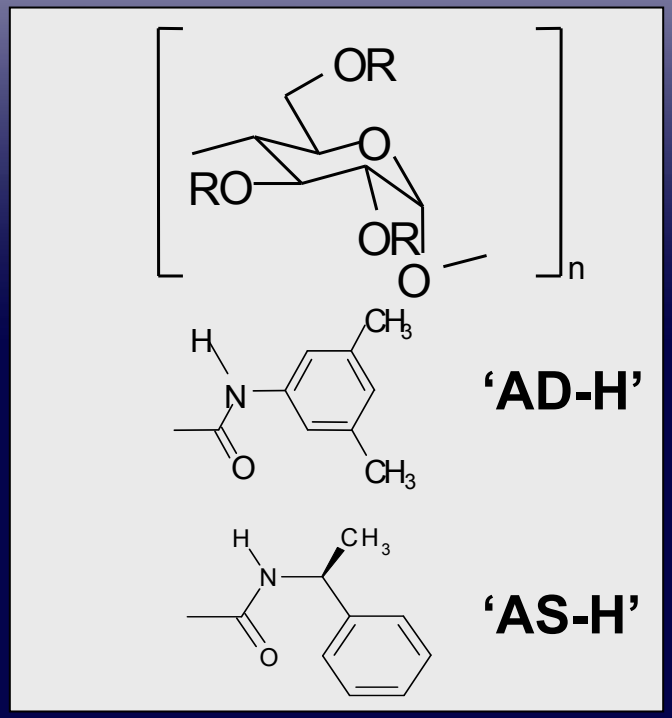
Chiral Impurity Methods – Types of CSP

Coated polysaccharide-derived CSPs

Cellulose derivatives



Amylose derivatives



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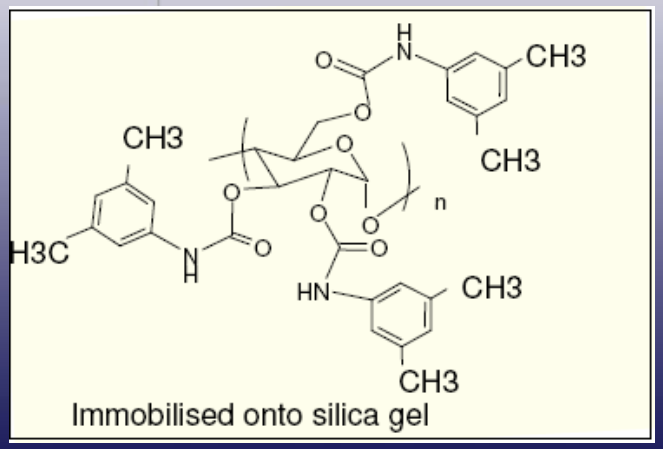
Chiral Impurity Methods – Types of CSPs

Immobilised polysaccharide-derived CSPs



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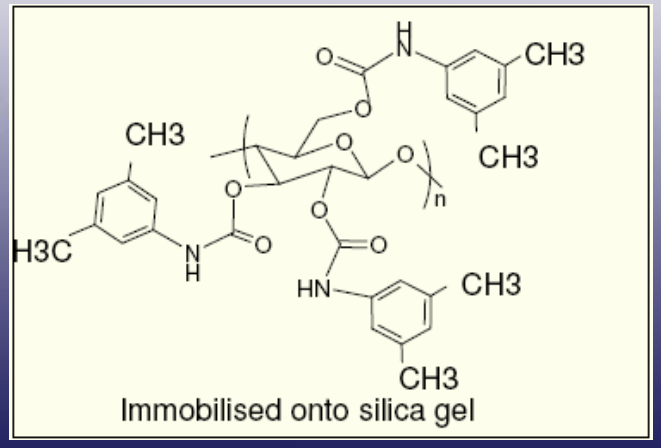

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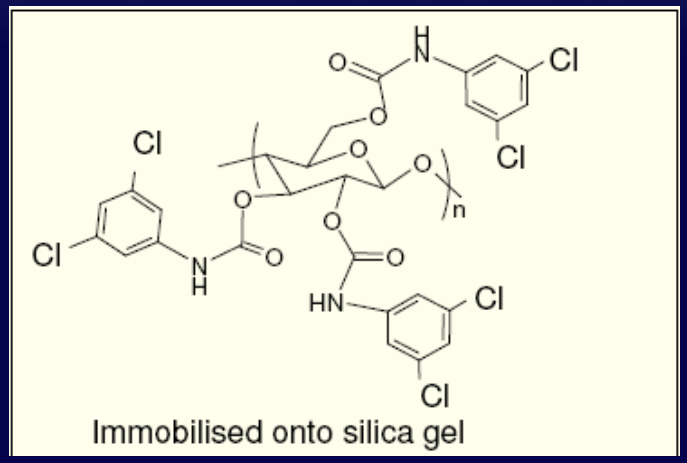

CHIRALPAK IC

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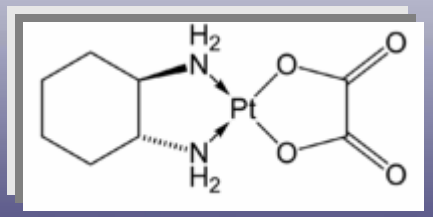
Chiral Impurity Methods-Case Study

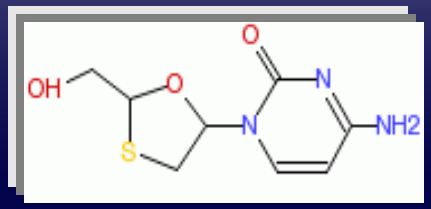
- Case study-1:

Oxaliplatin, Chemotherapy drug



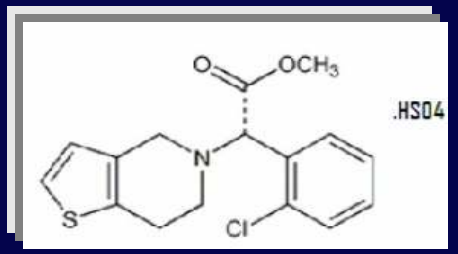
- Case study-2:

Lamivudine, Anti retroviral drug



- Case study-3:

Clopidogrel Bisulphate, Anti platelet drug



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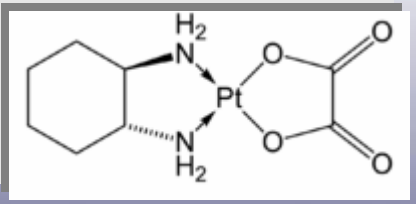
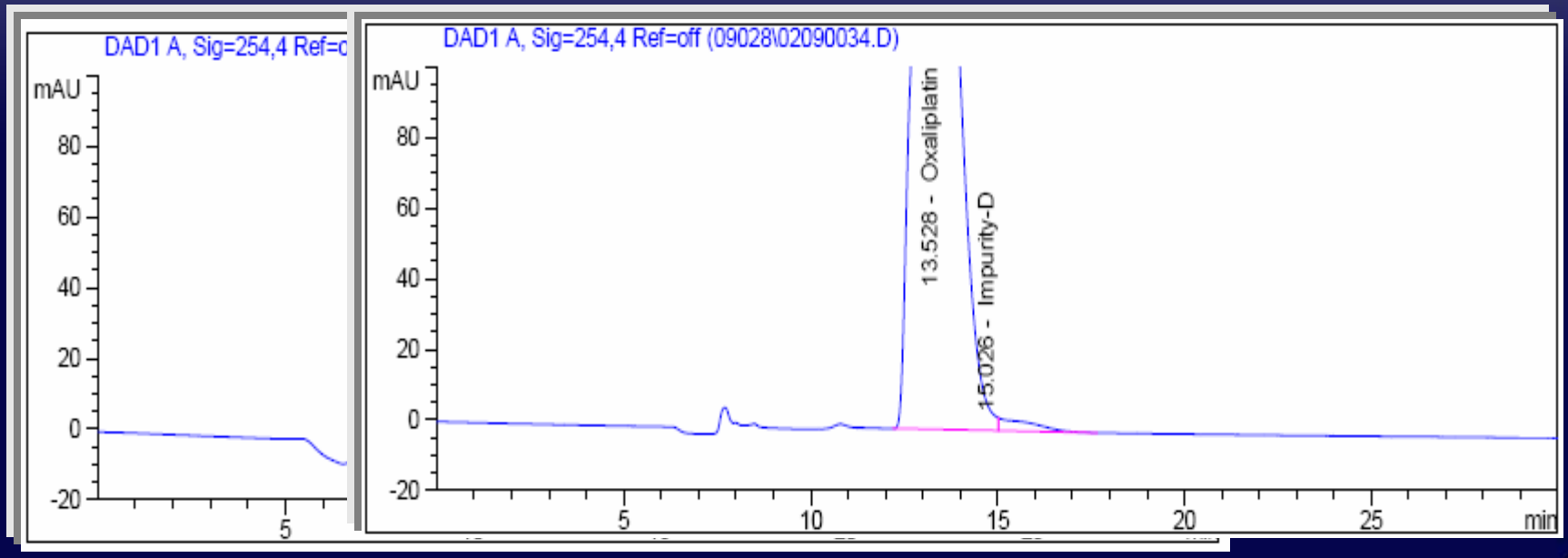
Chiral Impurity Methods – Case Study 1

Oxaliplatin



Pharmacopeial method conditions:
Column: CHIRALCEL OC 4.6 x 250mm
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%



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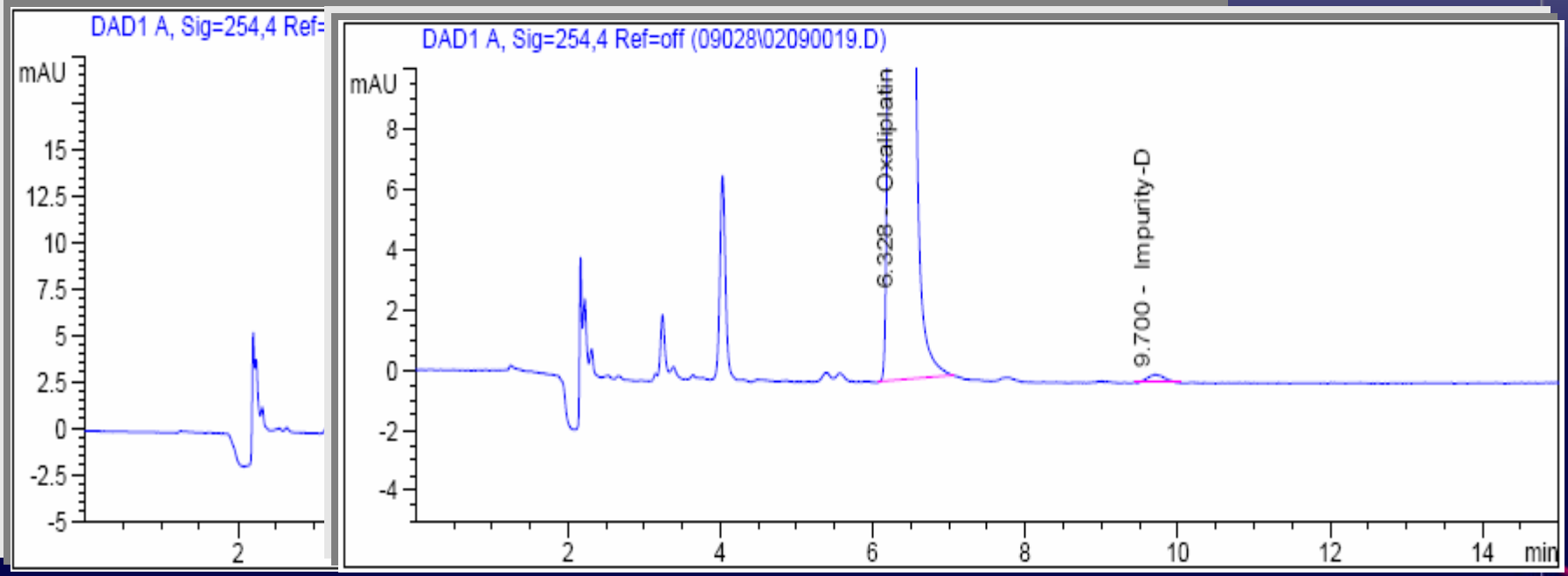
Chiral Impurity Methods – Case Study 1

Oxaliplatin

Cost-effecive 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%



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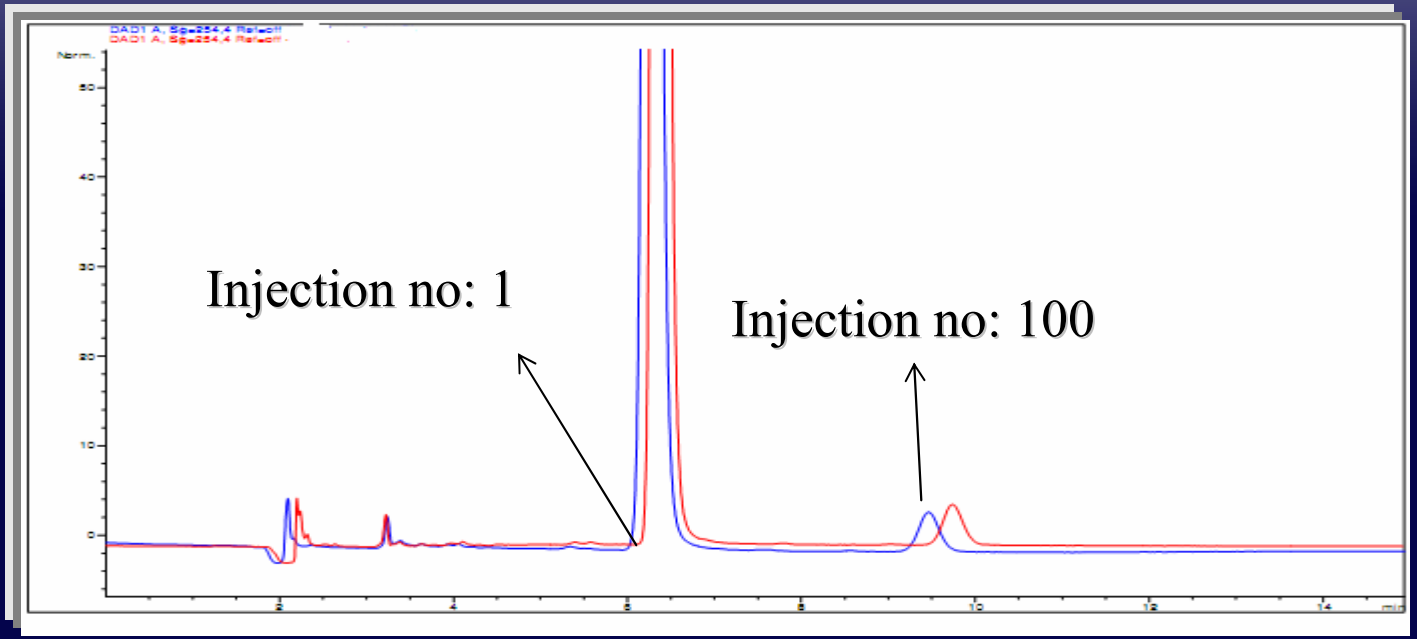

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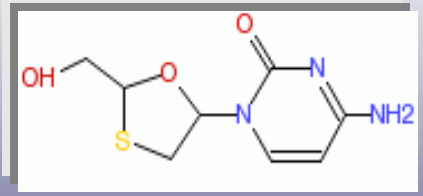
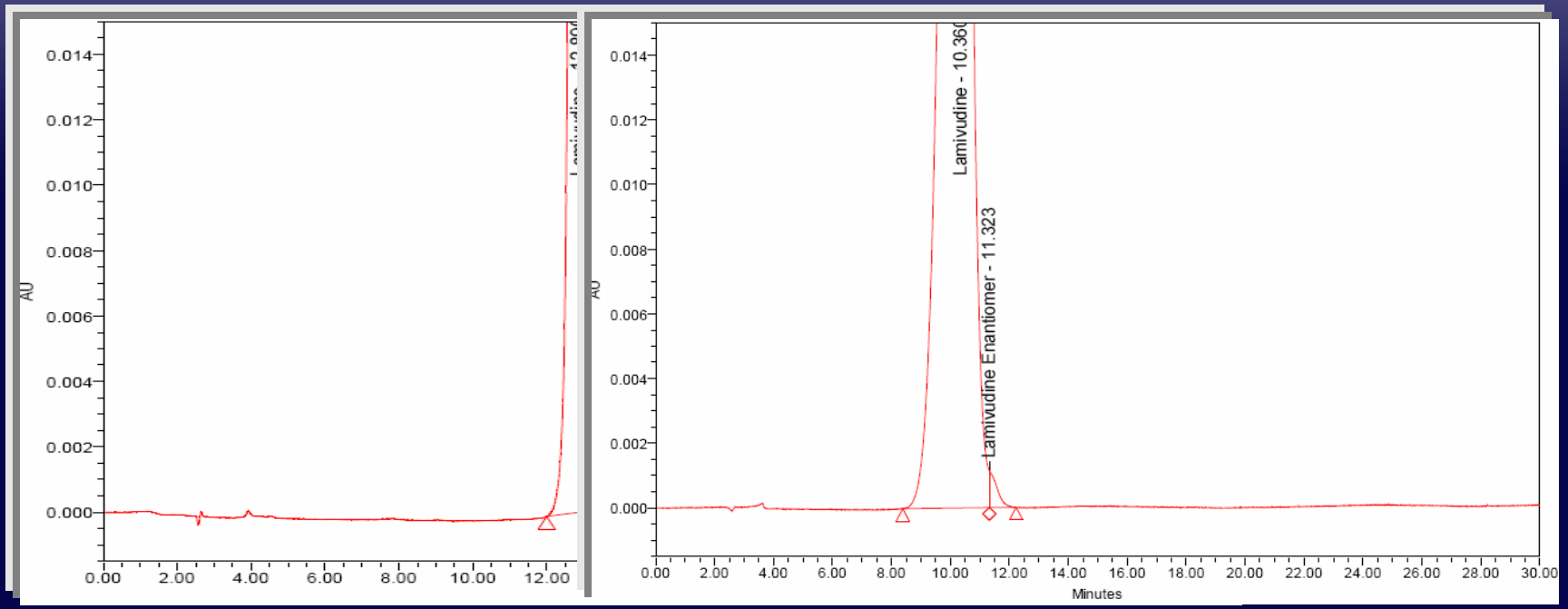
Chiral Impurity Methods – Case Study 2

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 ° C

Rs is NLT 1.5
Impurity limit: 0.3%



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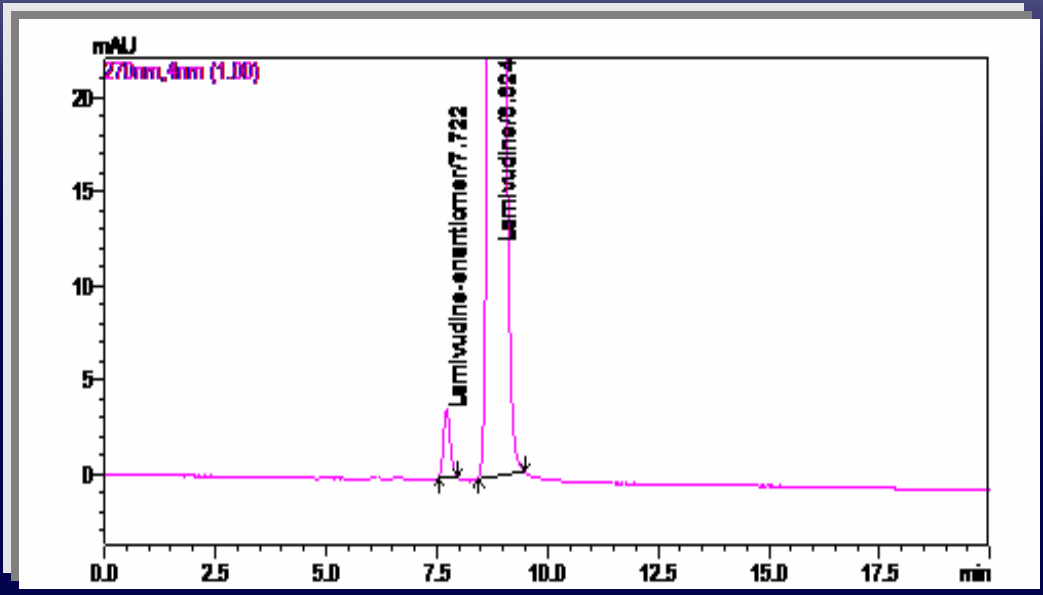
Chiral Impurity Methods – Case Study 2

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: 10µL
Detection : 270 nm by UV; Temperature: 25 ° C; Diluent : MP



Rs > 3.0
LOQ: 0.1%

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Chiral Impurity Methods – Case Study 2

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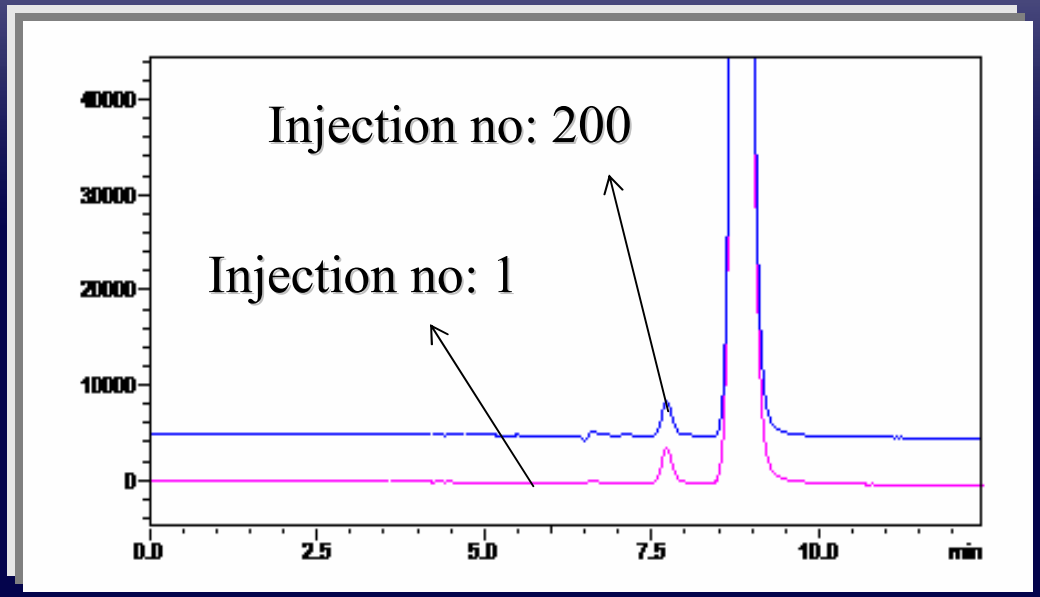
Detection : 270 nm by UV; Temperature: 25 ° C



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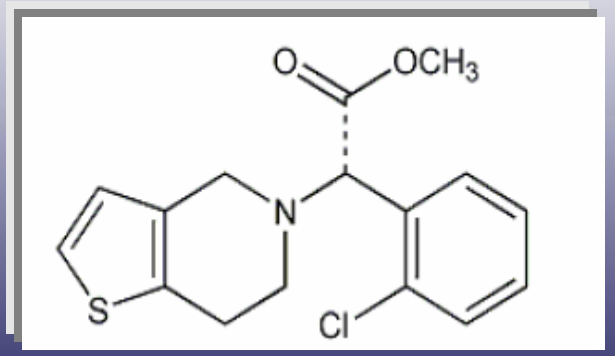
Chiral Impurity Methods – Case Study 3

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 bisuphate assay determination**

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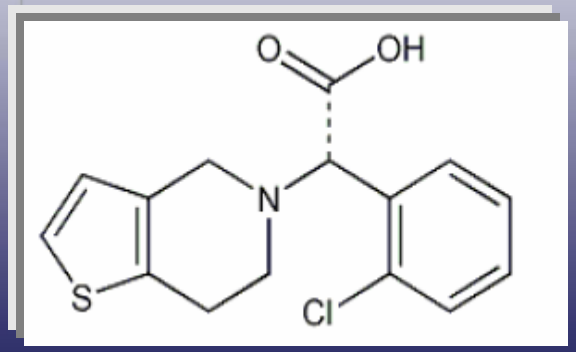
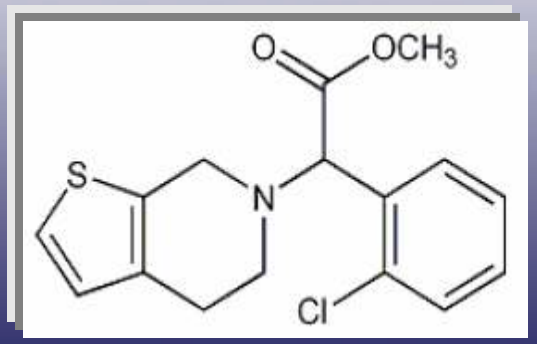
Chiral Impurity Methods – Case Study 3

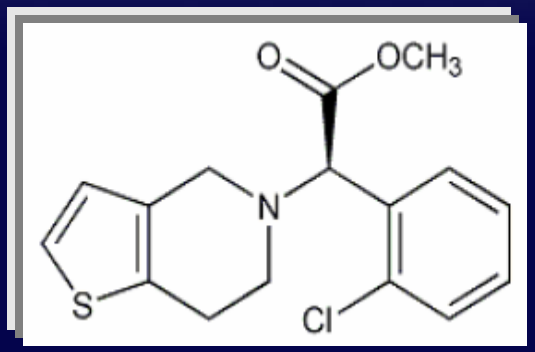
Clopidogrel and its related compounds



Imp-A
Limit: 0.2%



Imp-B
Limit: 0.3%



Imp-C
Limit: 1.0%

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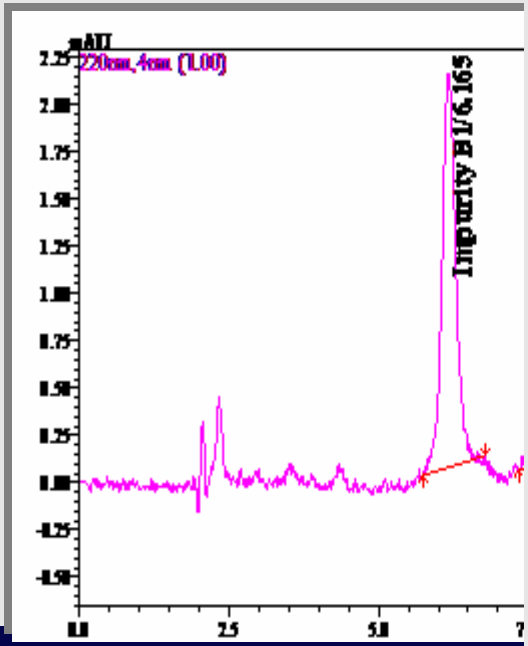
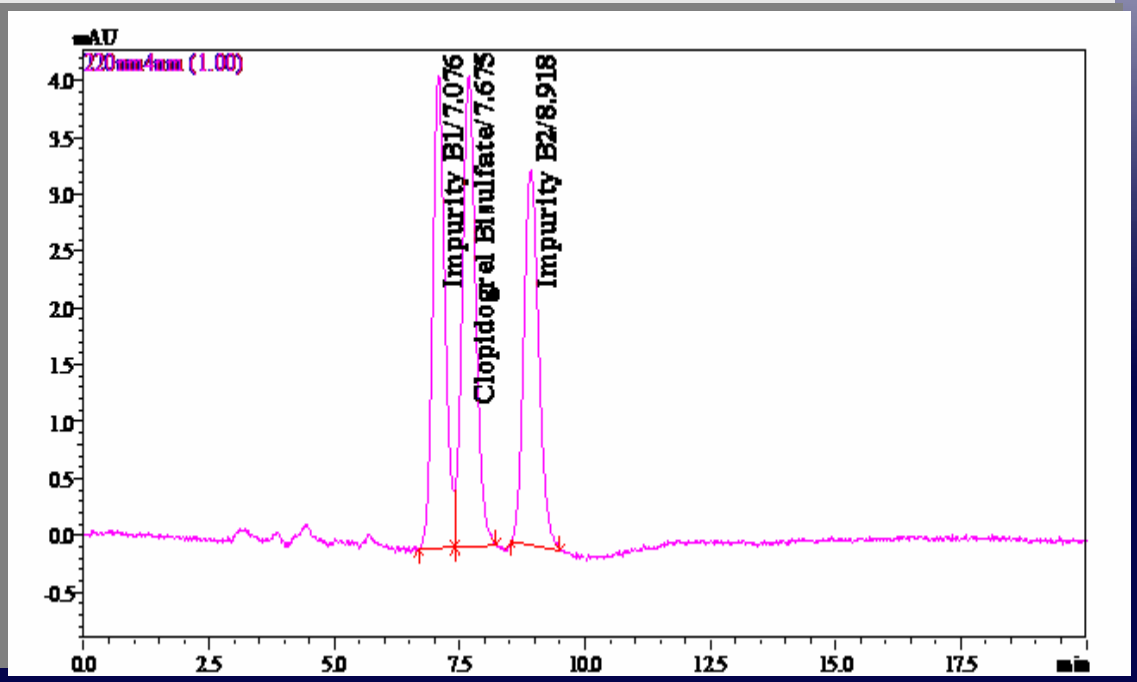
Chiral Impurity Methods – Case Study 3

Clopidogrel bisulphate

Pharmacopeial method

SS criteria: Rs between Imp B1 and Clopidogrel NLT 2.5





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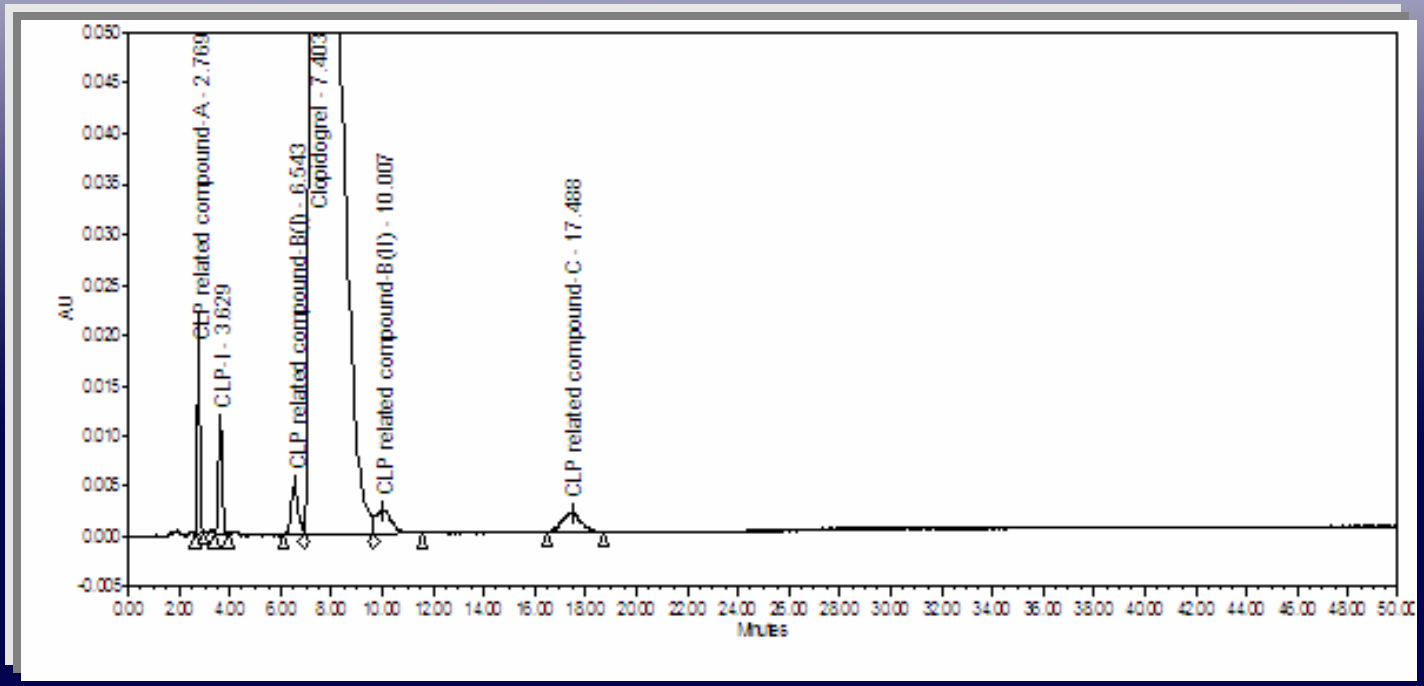
Chiral Impurity Methods – Case Study 3

Clopidogrel bisulphate

Pharmacopeial method



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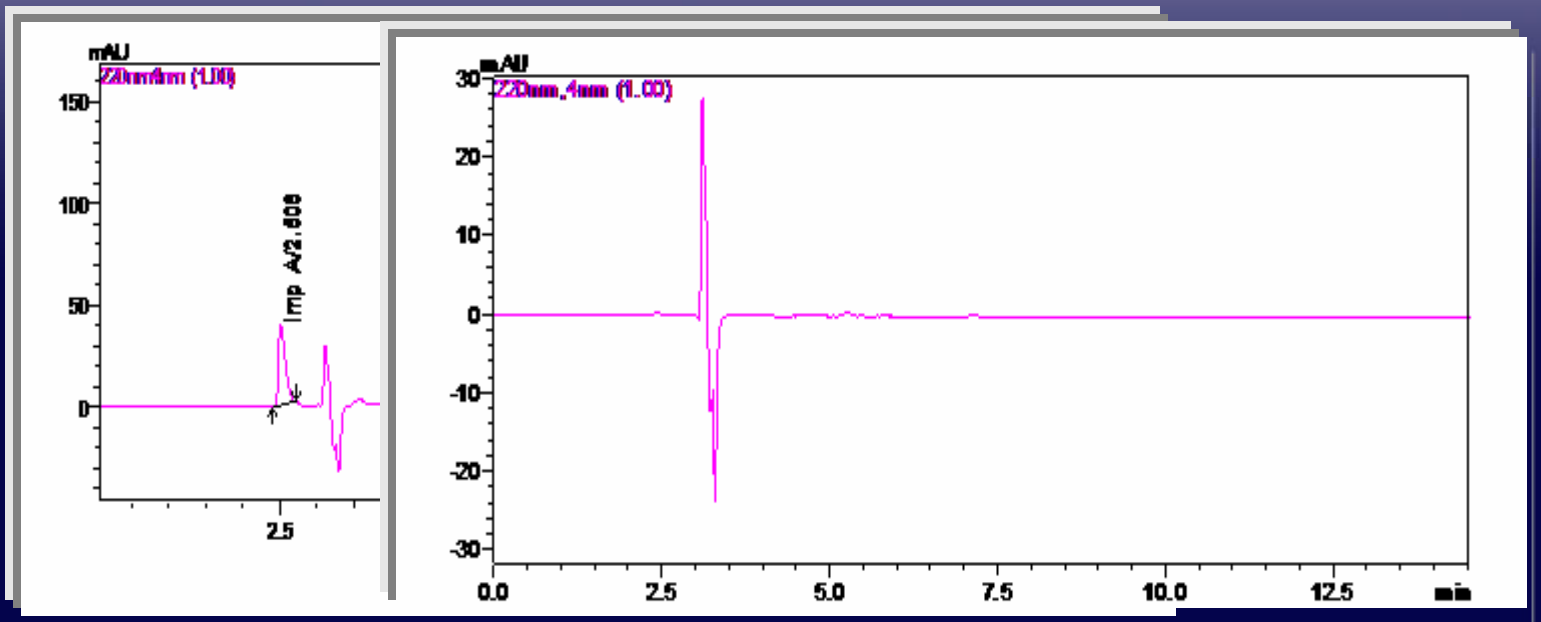
Chiral Impurity Methods – Case Study 3

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; Conc: 0.5 mg/mL; Diluent : Ethanol
Detection : 220 nm by UV; Temperature: 25 ° C



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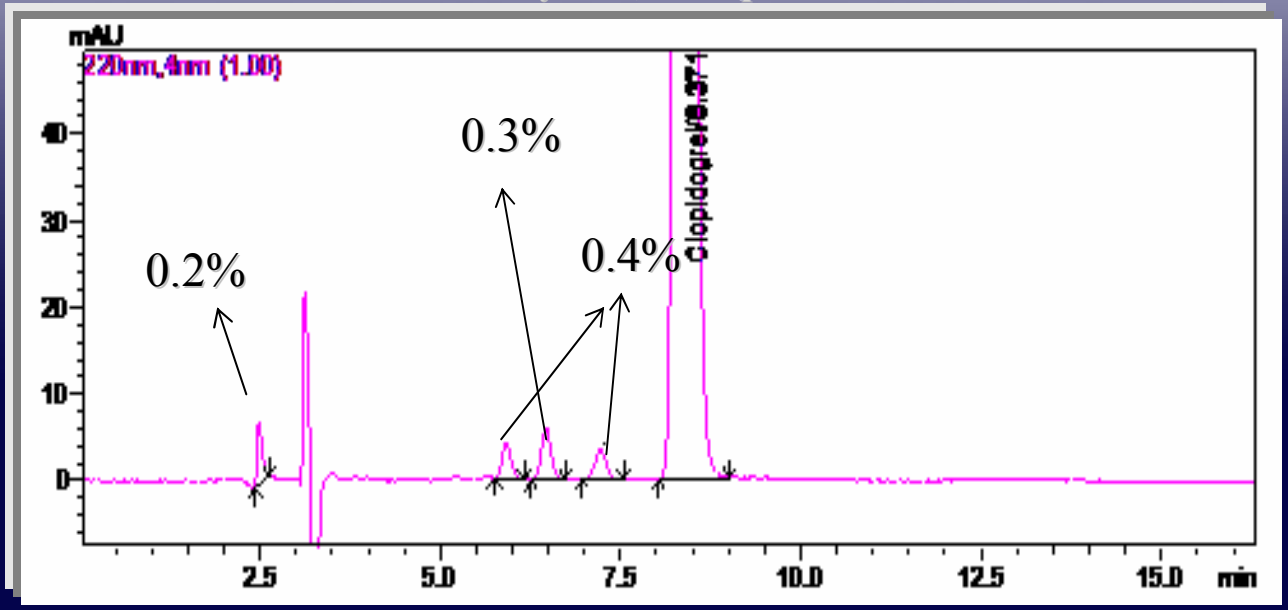

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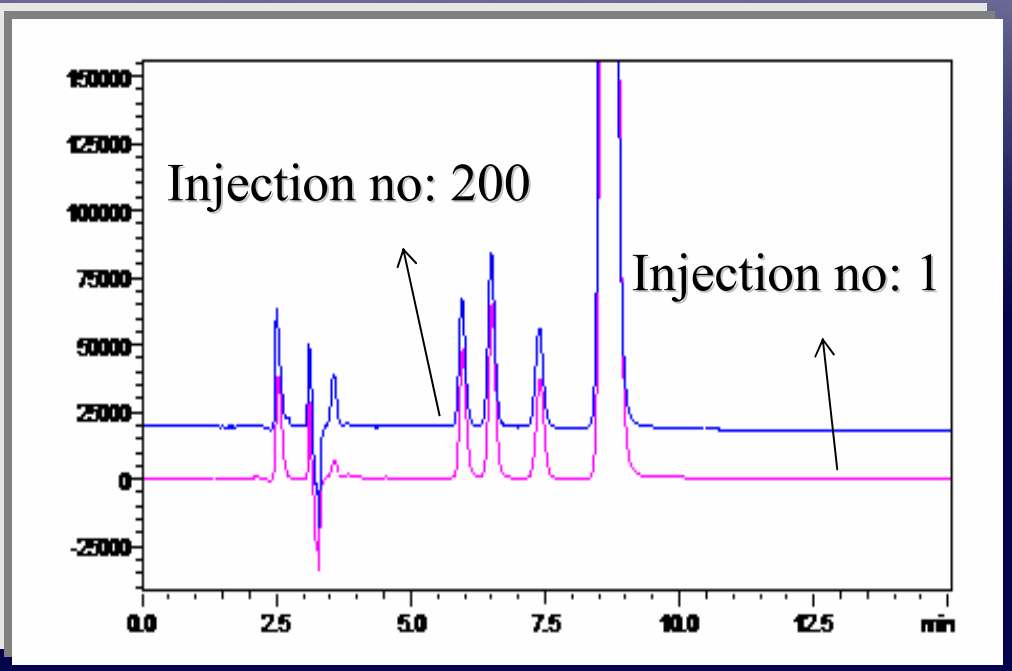

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Chiral Impurity Methods – Case Study 3

Clopidogrel bisulphate

Chromatographic Characteristics

Compound peak	Rs	N	T
Imp A	-	3907	1.4
Imp B1	17.4	11520	1.3
Imp C	2.3	11554	1.1
Imp B2	2.9	11063	1.1
Clopidogrel	3.6	10235	1.3

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

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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.

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Acknowledgments

- Mr. Raghuram, General Manager, Hetero Drugs Ltd

  for his continuous co-operation during Lamivudine method development
- My Colleagues Mr. Thirupathi & Mr. Srinivasu for their significant contribution during method development
- USP India for the lecture opportunity

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THANK YOU ALL