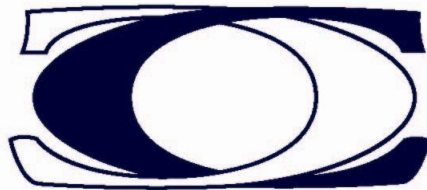


# Separation of Chiral Pesticides by SFC and HPLC

Tong Zhang<sup>(1)</sup>, Pilar Franco<sup>(1)</sup>, [Joseph Barendt<sup>\(2\)</sup>](#)

<sup>(1)</sup> Chiral Technologies Europe (Illkirch, France)

<sup>(2)</sup> Chiral Technologies, Inc (PA 19380 USA)



**CHIRAL**  
TECHNOLOGIES  
DAICEL GROUP



move easily  
move reliably  
move quickly

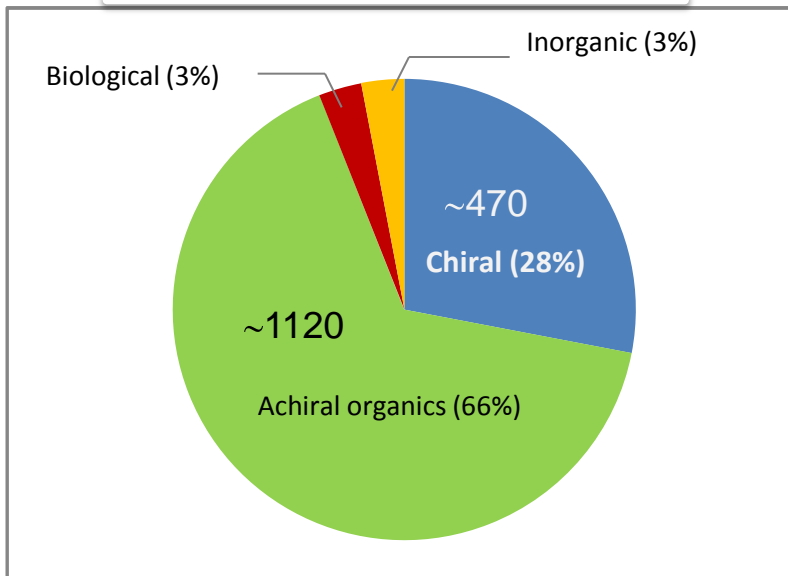
***move ahead***

# Chirality in Biologically Active Compounds

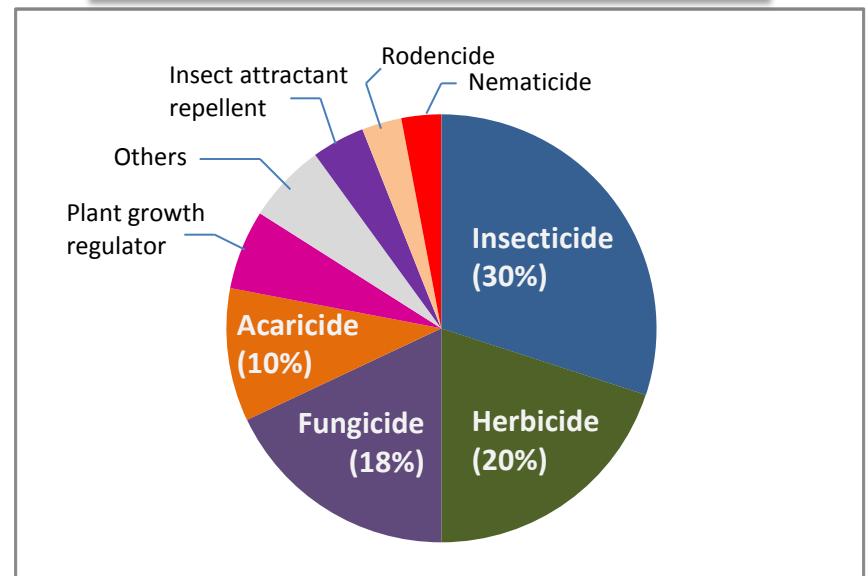
- Pharmaceuticals
- Flavor and Fragrance
- Agrochemicals
  
- Regulatory Guidance
  - Varies by application and country

# Chirality in Pesticides

Pesticides: ~1700 products



Chiral pesticides: ~ 470 products



Reference:

E.M. Ulrich et al. , Rev. Environ. Contam. Toxicol. 217 (2012) 1-74

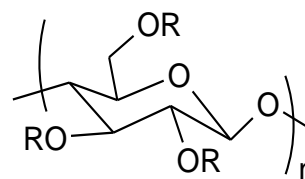
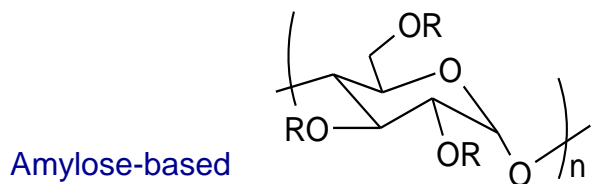
# Optical Activity

- Identical physical and chemical properties
  - In an isotropic environment
- Biological systems are distinctly *anisotropic*
  - Uptake, bio-activity, metabolism affected

# Issues Specific to Chiral Compounds

- Efficacy of each isomer
- Metabolization rates may differ
- Biodegradation in environment
  
- Drives the need for chromatographic methods
  - Analytical
  - Preparative

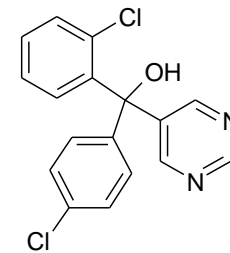
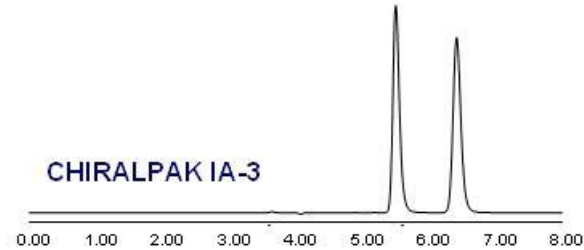
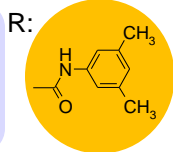
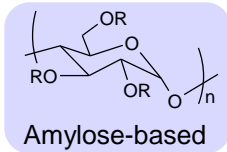
# Polysaccharide-derived Chiral Stationary Phases



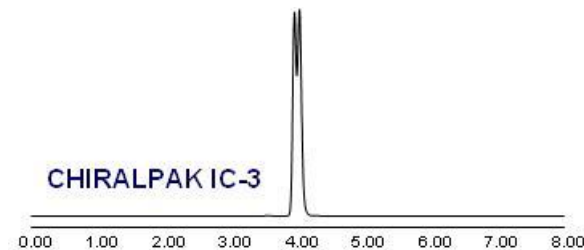
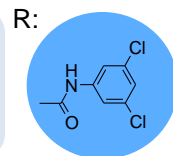
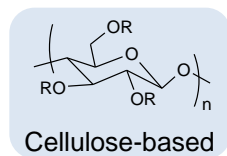
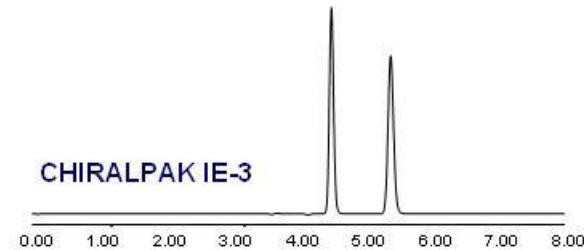
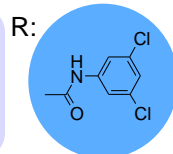
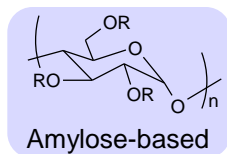
CSP	Polysaccharide	-R
CHIRALPAK IA	amylose	
CHIRALPAK IB	cellulose	
CHIRALPAK IC	cellulose	
CHIRALPAK ID	amylose	
CHIRALPAK IE	amylose	
CHIRALPAK IF	amylose	

# Experimental approaches

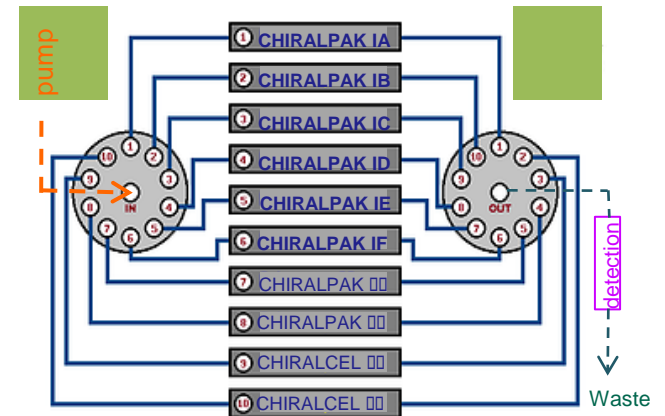
## Sample screening and effect of chiral selector



Fenarimol  
(fungicide)



Common  
Column: 150x4.6  
EtOH/DEA 100:0.1  
0.5 mL/min; 230nm

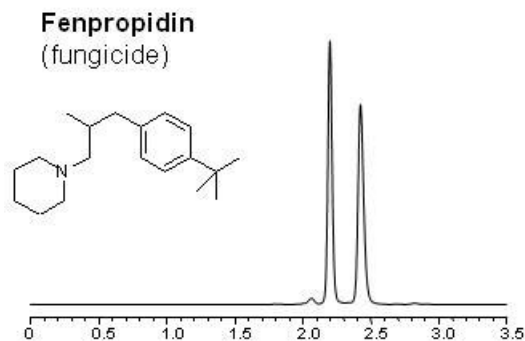


# Varying Modes

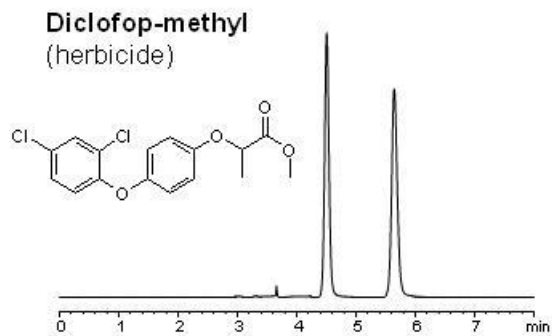
- HPLC
  - Normal Phase
    - Hexane/Alcohol, ACN, Alcohol, DCM, etc.
  - Reversed Phase
    - H<sub>2</sub>O/MeOH, H<sub>2</sub>O/ACN, H<sub>2</sub>O/THF, etc.
- SFC
  - Supercritical CO<sub>2</sub> plus modifier (~10% alcohol)



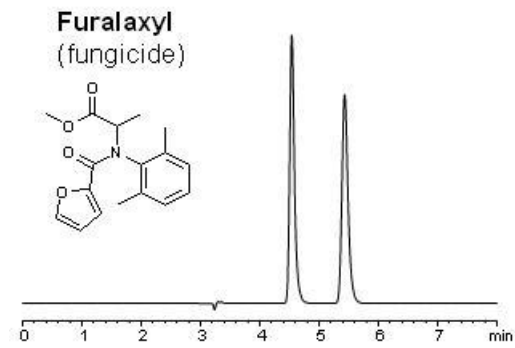
# Chiral resolution of pesticide enantiomers



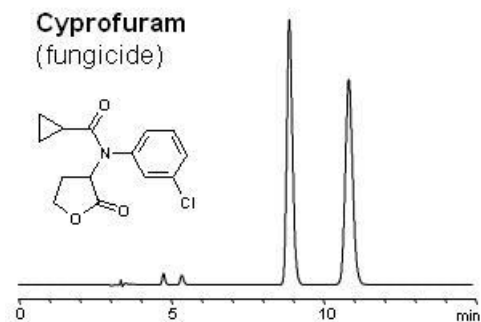
**CHIRALPAK IA**  
ACN/2-PrOH/DEA 95:5:0.1



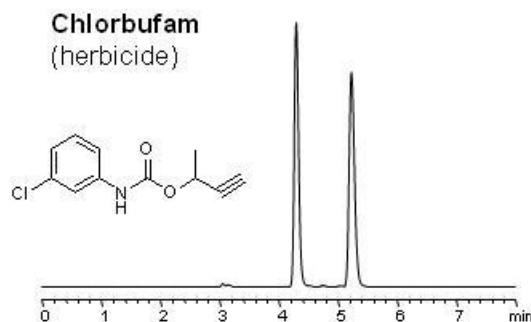
**CHIRALPAK IB**  
Hexane/2-PrOH 80:20



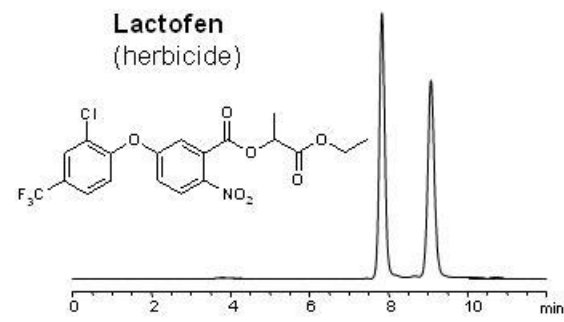
**CHIRALPAK IC**  
MeOH 100%



**CHIRALPAK ID**  
Hexane/2-PrOH 80:20

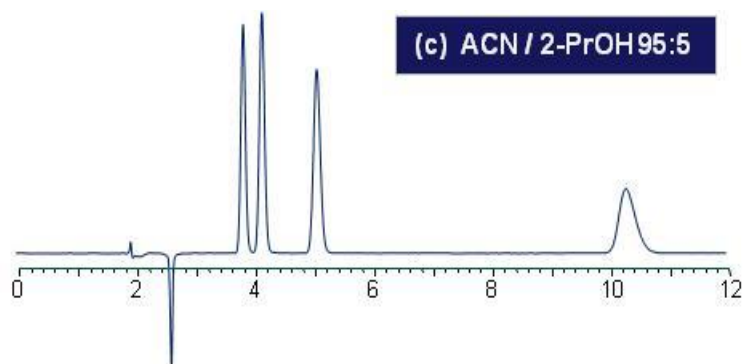
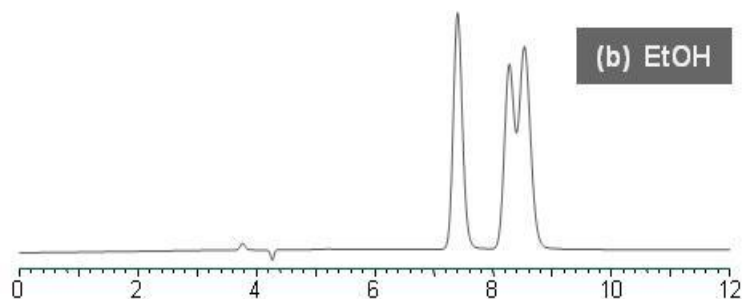
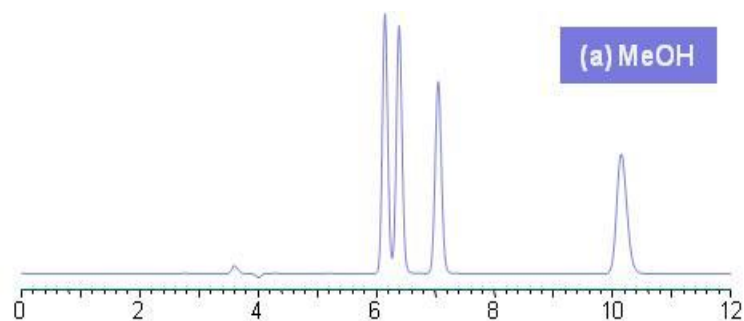
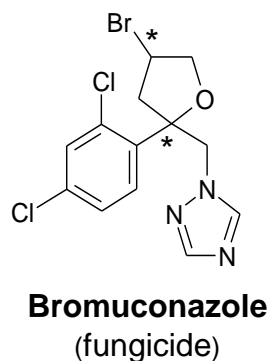


**CHIRALPAK IE**  
Hexane/EtOH 80:20



**CHIRALPAK IF**  
EtOH 100%

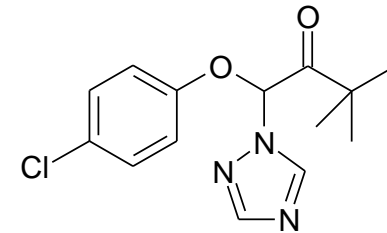
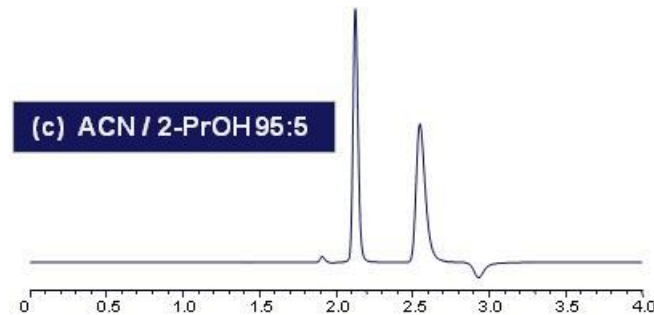
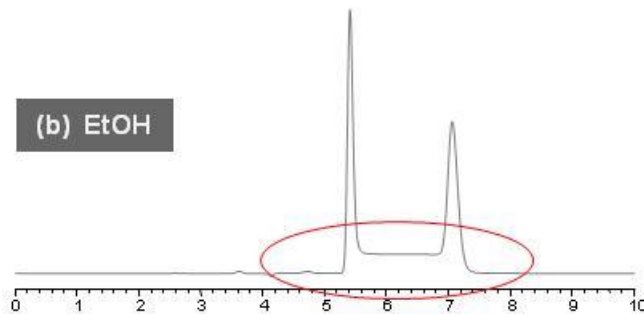
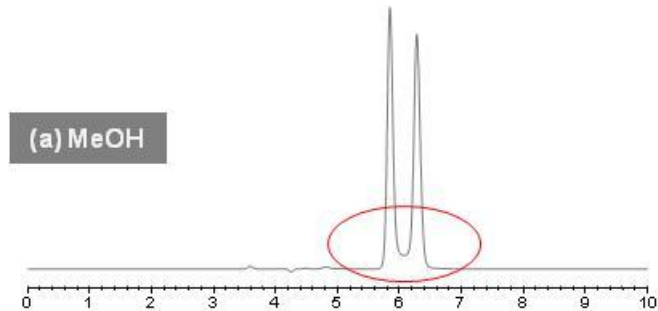
# Enantio- and stereoselective resolution of pesticides



Common  
**CHIRALPAK IC-3** (150x4.6 mm i.d.)  
Additive: 0.1% DEA

# Effect of the mobile phase

## Online racemization and its inhibition



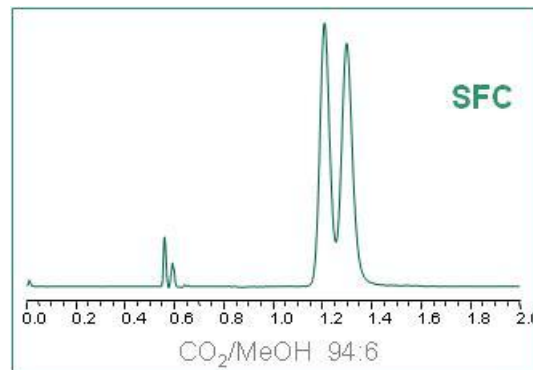
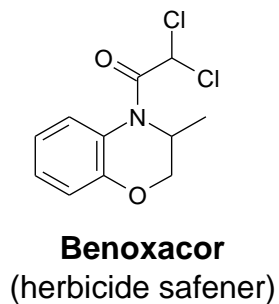
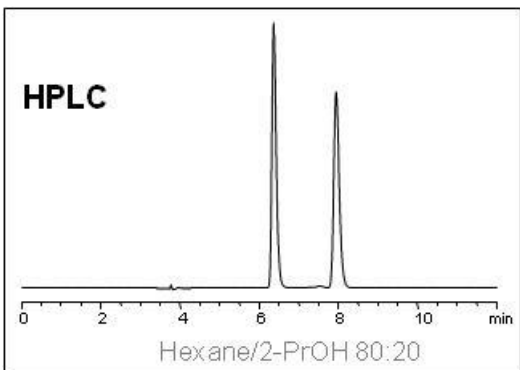
**Triadimefon**  
(fungicide)

Common  
CHIRALPAK IE-3 (150x4.6 mm i.d.)  
DEA 0.1% in MP

# HPLC and SFC

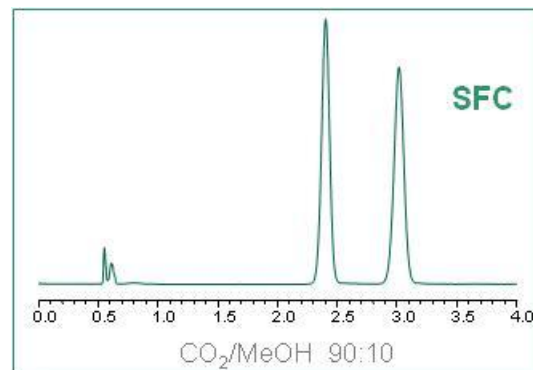
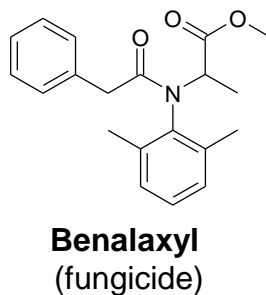
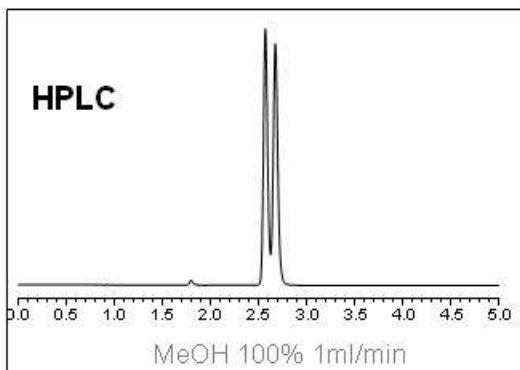
## Complementary techniques

CHIRALPAK ID



CHIRALPAK ID

CHIRALPAK IC-3

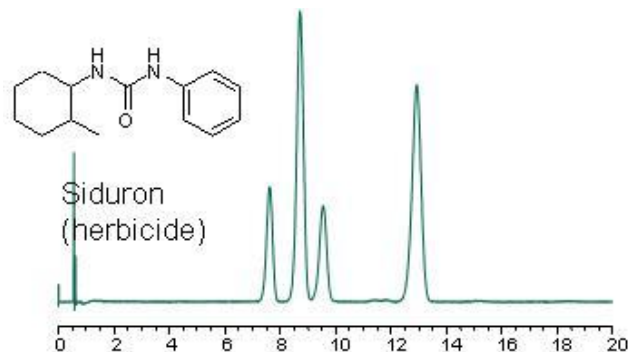


CHIRALPAK IC-3

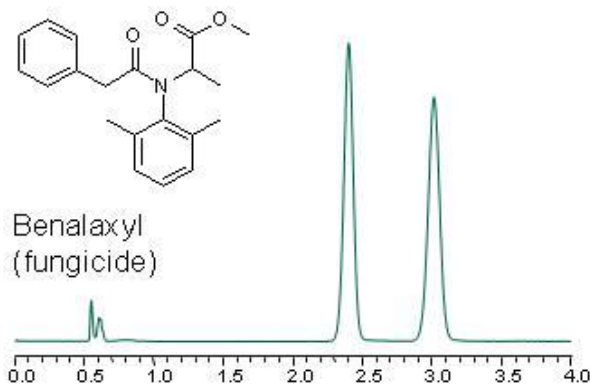
min

# Chiral resolution of pesticide enantiomers by SFC

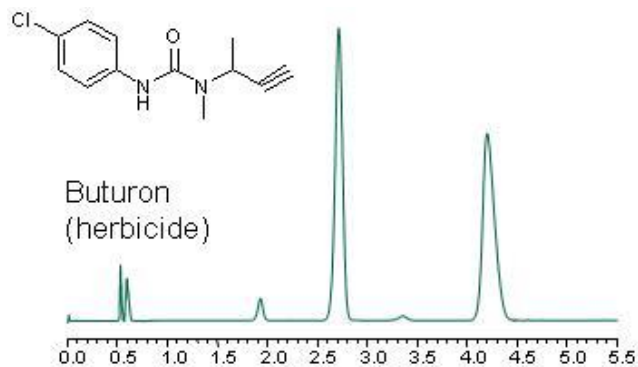
## Separation examples



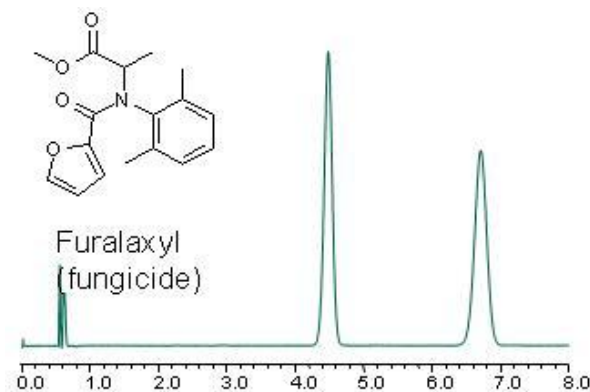
**CHIRALPAK IB-3**  
CO<sub>2</sub>/MeOH 94:6



**CHIRALPAK IC-3**  
CO<sub>2</sub>/MeOH 90:10



**CHIRALPAK IF-3**  
CO<sub>2</sub>/MeOH 90:10

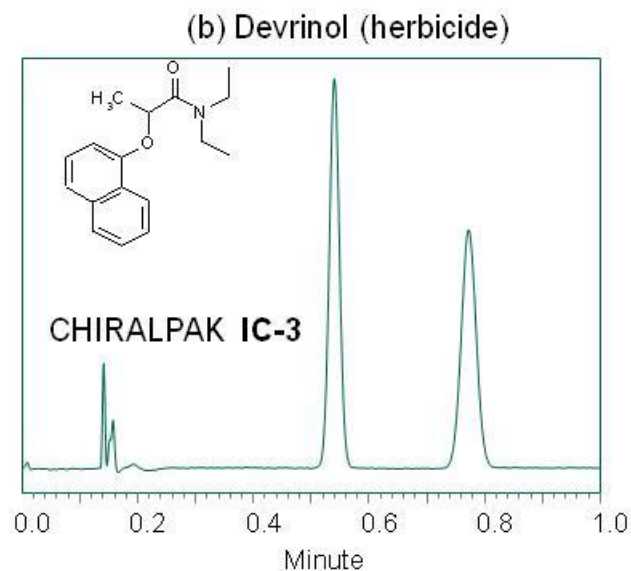
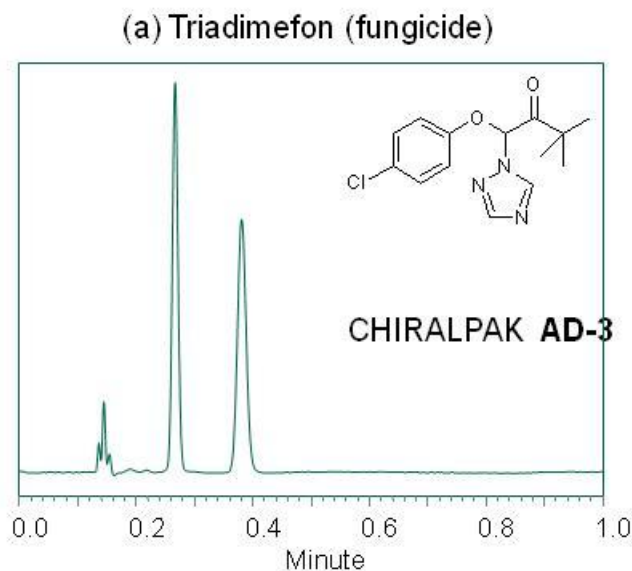


**CHIRALPAK IC-3**  
CO<sub>2</sub>/MeOH 90:10

# Enantiomer resolution of pesticides

Fast analysis by SFC by maximizing the kinetic performance of the column

Time scale: 1 minute



Column size: **100 x 3.0 mm i.d.**  
Mobile phase: CO<sub>2</sub>/MeOH 85:15 v/v  
Additive:  
(a) 1% DEA in MeOH;  
(b) None

BPr: 150 bar  
Flow rate: 3.6 mL/min  
Temperature: 40 C  
UV Detection  
System: ACQUITY UPC<sup>2</sup>™

# Preparative Separations

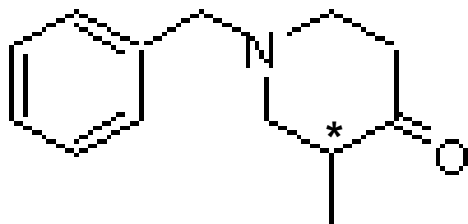
- Why Preparative?
- Need for;
  - Standard samples of each isomer
  - Differential Toxicity testing
  - Metabolism studies
  - Biodegradation studies

# Process For Prep Separation

- Similar to Analytical Method Development
  - Matrix of columns/mobile phases
  - Find best separations
- Optimize for maximum sample loading
  - Larger separation between peaks
  - Higher solubility in mobile phase
- Run “stacked injection” technique

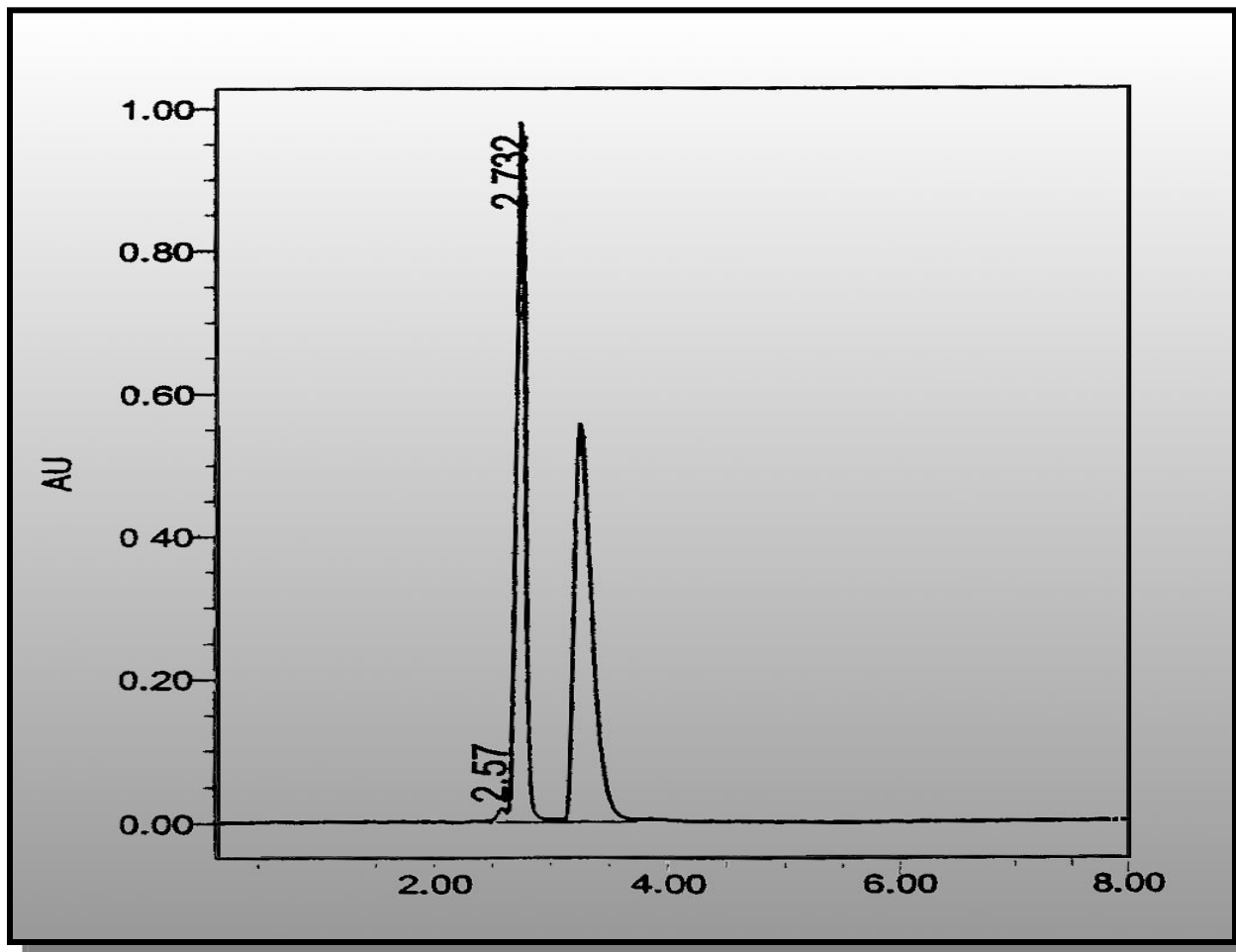


# Benzyl-3-methyl-4-piperidone



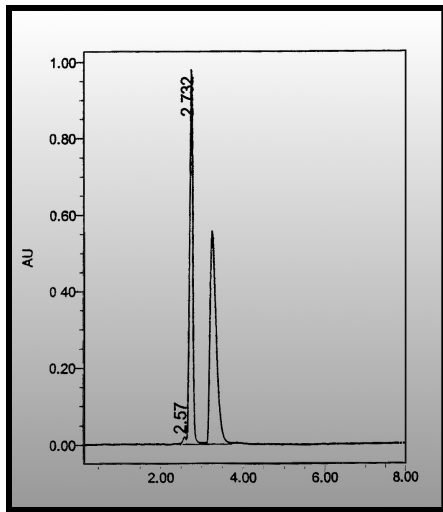
## Analytical HPLC

Column : CHIRALPAK AD-H,  
250 x 4.6 mm  
Mobile Phase : Acetonitrile, 0.1%  
DEA,  
Flow Rate : 1 ml/min  
Detection : UV, 245 nm  
Sample : 10 g/l; 0.5 µl

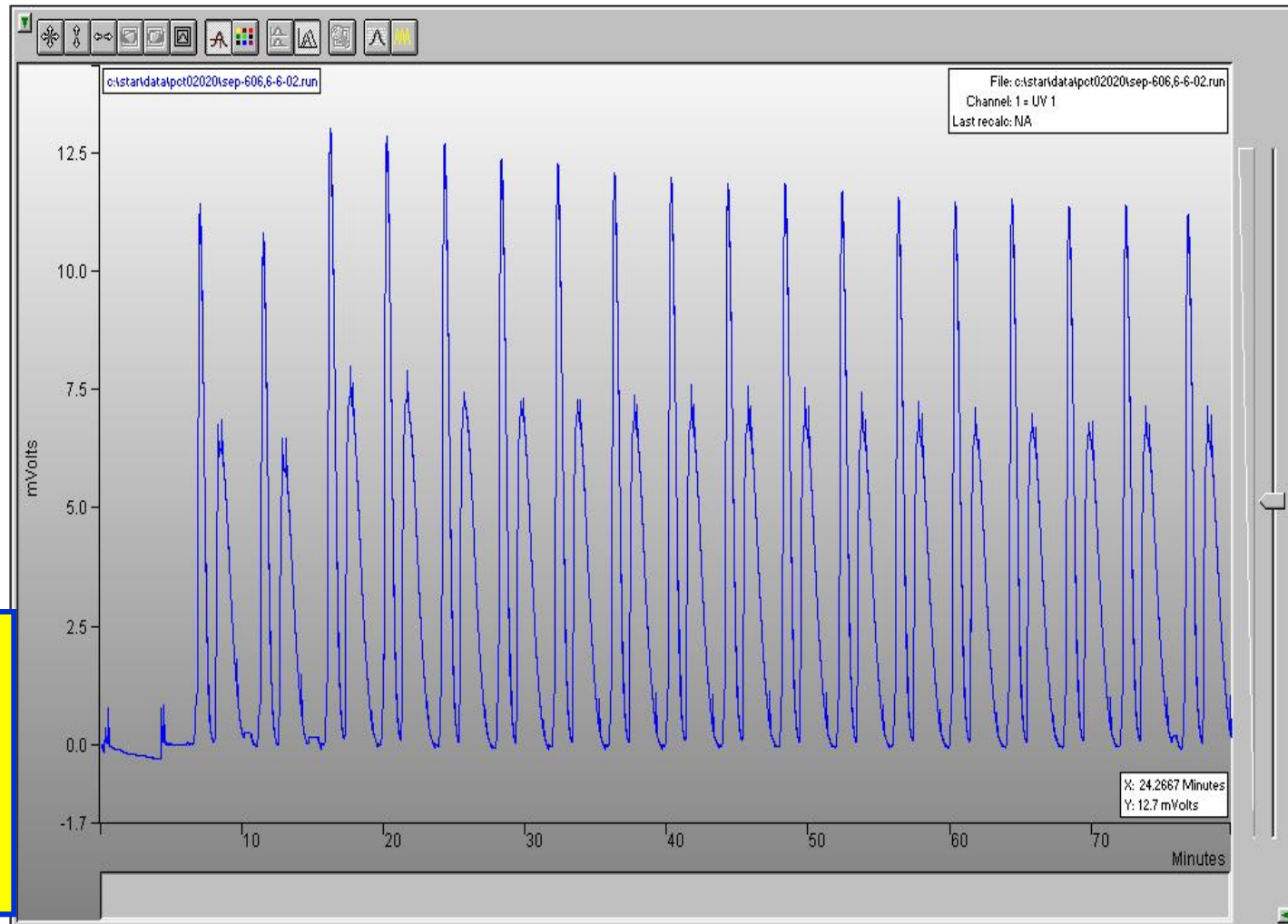


# Benzyl-3-methyl-4-piperidone

Column : CHIRALPAK AD  
20  $\mu$ m, 500 x 50 mm



150 ml/min  
675 mg / injection  
Acetonitrile  
Cycle time 4 min  
T = 25 C  
10.1 g/hr



# Suitability of Scale

- SFC/HPLC
  - Milligrams
  - Grams
  - Kilos
- Multi-Kilos and beyond - SMB

# Chromatography Equipment



SFC  
HPLC  
Batch and SMB

# Industrial Scale SMB



# Chiral Chromatography

- Effective technique for
  - Analytical determination
  - Preparative separation
  
  - Lab scale (HPLC, SFC)
    - mgs to kgs
  
  - Commercial scale (HPLC, SMB)
    - MTs



**CHIRAL**  
TECHNOLOGIES

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*THANK YOU!*