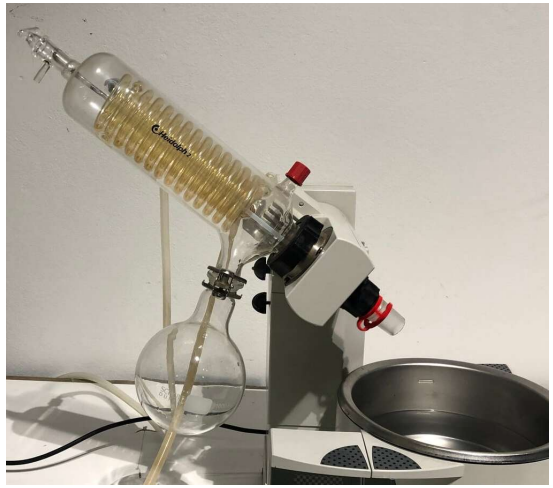
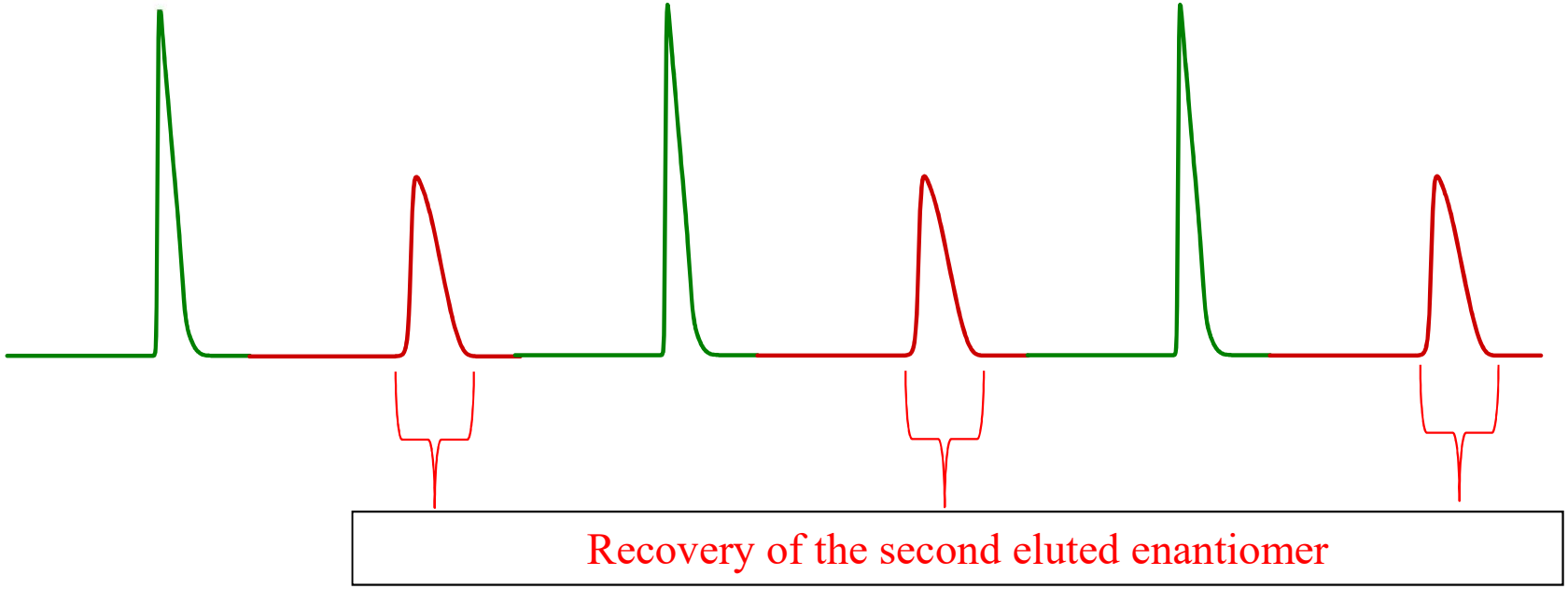
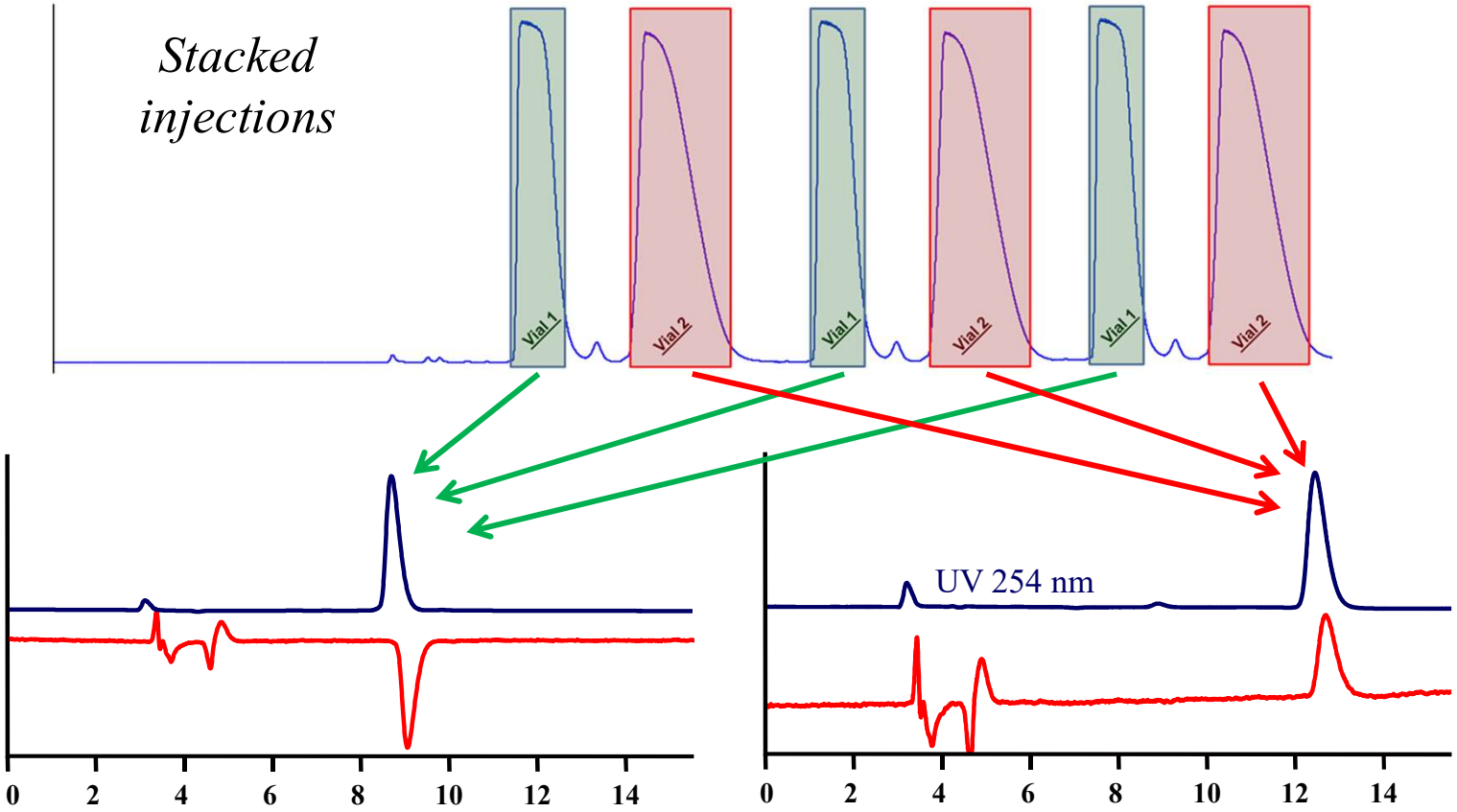
Preparative chiral resolution by enantioselective chromatography at lab scale: materials and methods





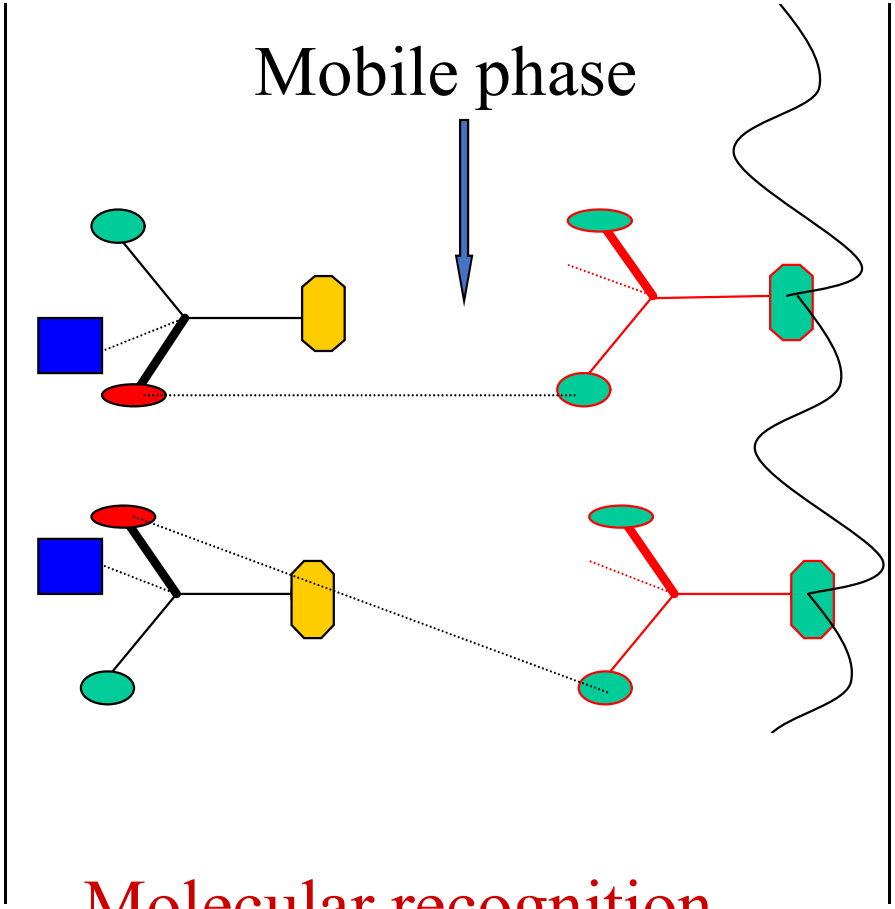
Dr. Nicolas Vanthuyne – GDR Chirafun – 19 avril 2021


Stacked injections
Vial 1
Vial 2
Vial 1
Vial 2
Vial 1
Vial 2
UV 254 nm
0
2
4
6
8
10
12
14
0
2
4
6
8
10
12
14

Enantioselective chromatography principle

Each enantiomer interacts with the chiral selector to give labile diastereomers, with different energies. The enantiomer which forms the more stable diastereomer, will be the more retained in the column.

Small energy difference

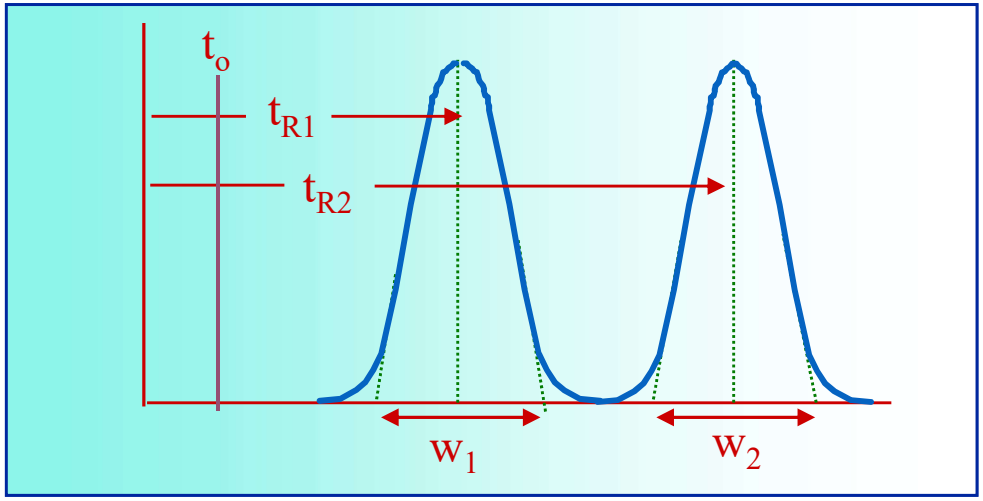


Molecular recognition

- **Column:** a chiral selector is immobilized on a support (silica)

- ***Mechanisms :*** interactions : π, hydrophobic, steric, dipolar, hydrogen bonding, inclusions, etc..

Chromatographic parameters



$$N_i = 16 \times \left[\frac{t_{R,i}}{w_i} \right]^2$$

$$k = \frac{t_R - t_o}{t_o}$$

$$\alpha = \frac{t_{R,2} - t_o}{t_{R,1} - t_o} = \frac{k_2}{k1}$$

$$Rs = 2 \times \frac{t_{R,2} - t_{R,1}}{w_1 + w_2}$$

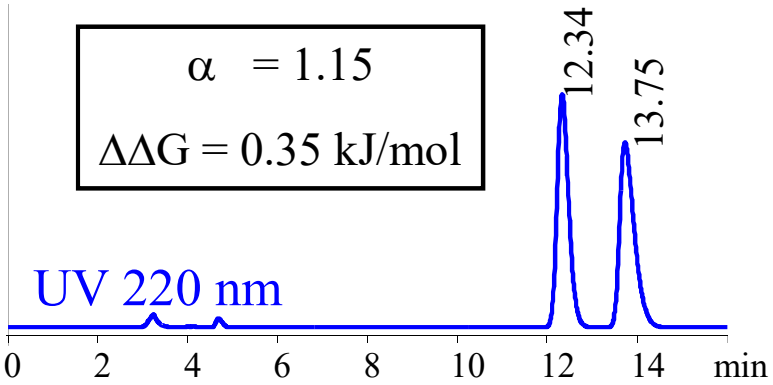
- **enantioselectivity α**

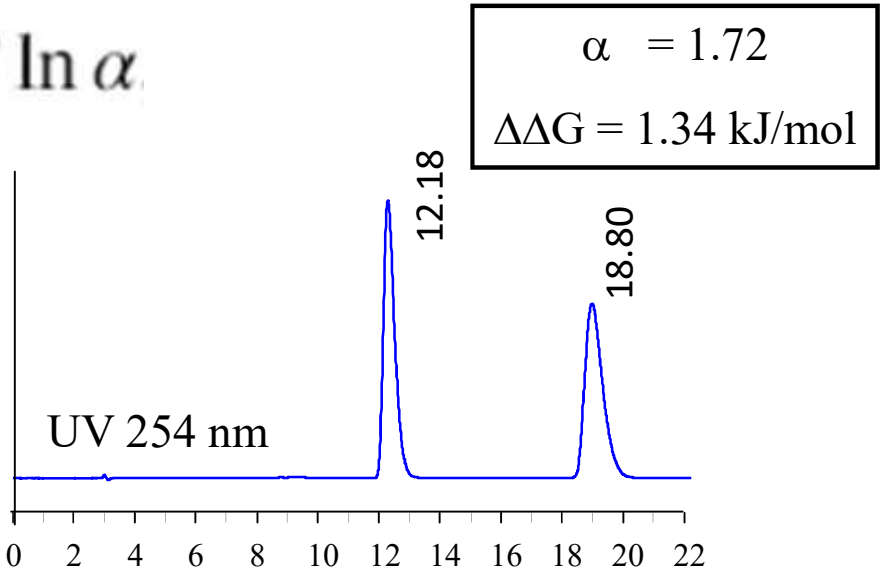
  α > 1 means that the two peaks are separated

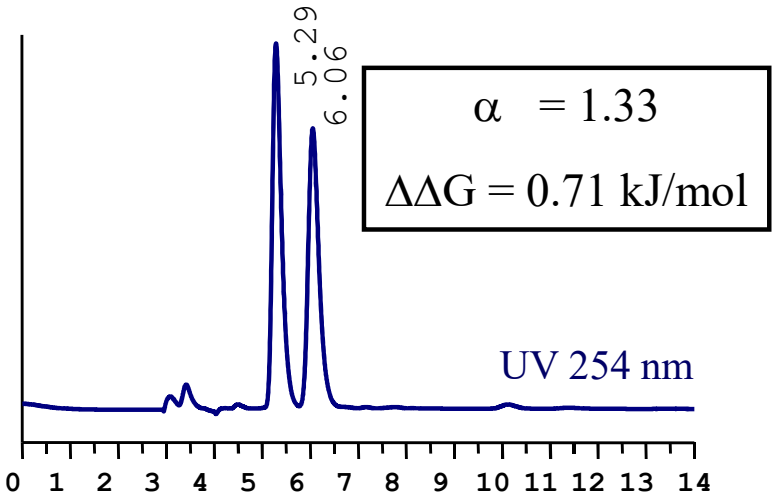
- ***Resolution***

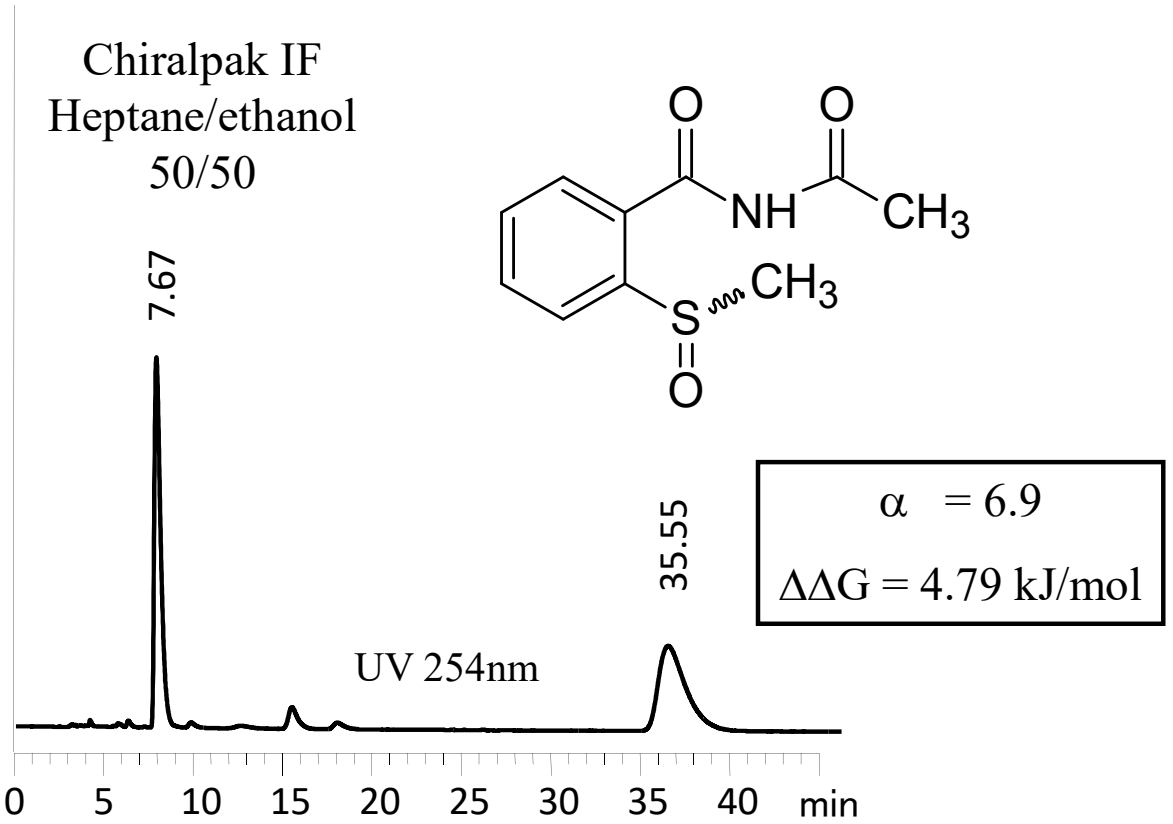
  Rs > 1,25 means a baseline separation

$$\Delta\Delta G = -RT \ln \alpha$$





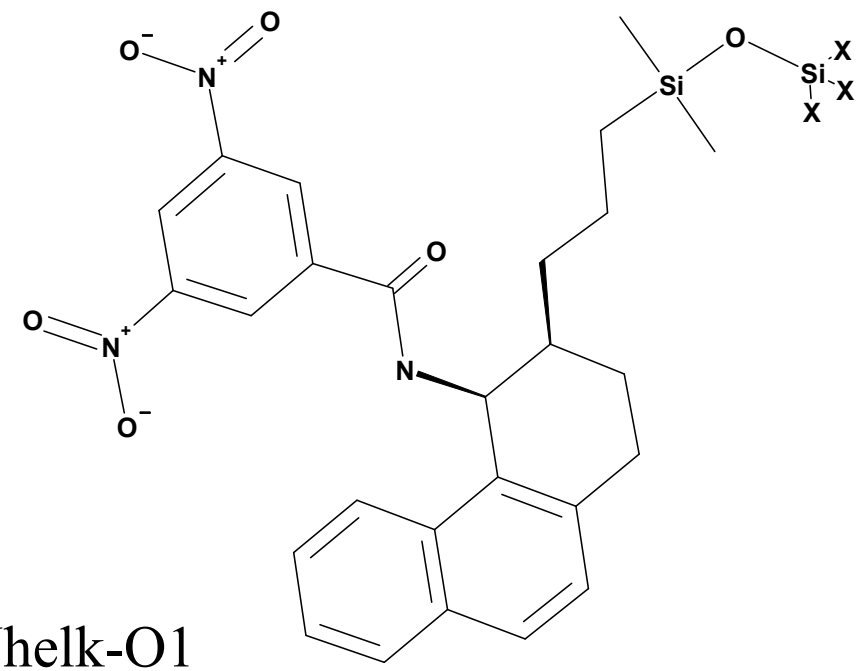




Chiral stationary phases

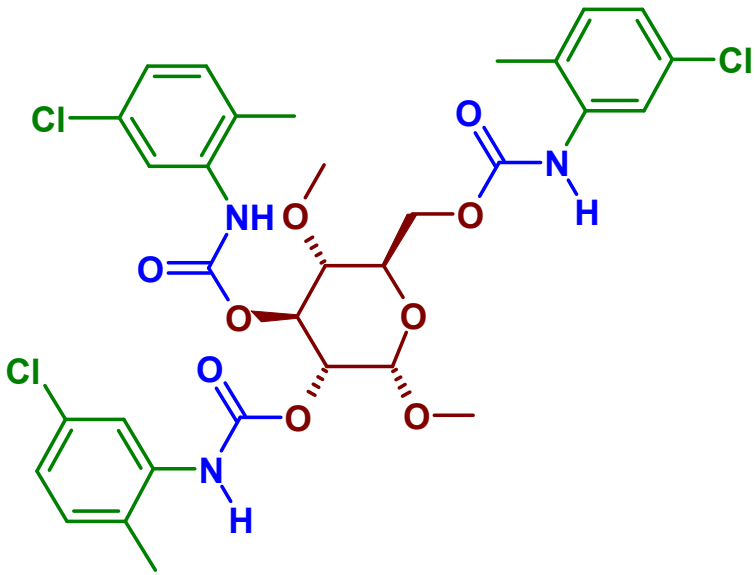
- about 2000 chiral selectors described in literature
- more than 200 commercial chiral selectors

Molecular phases



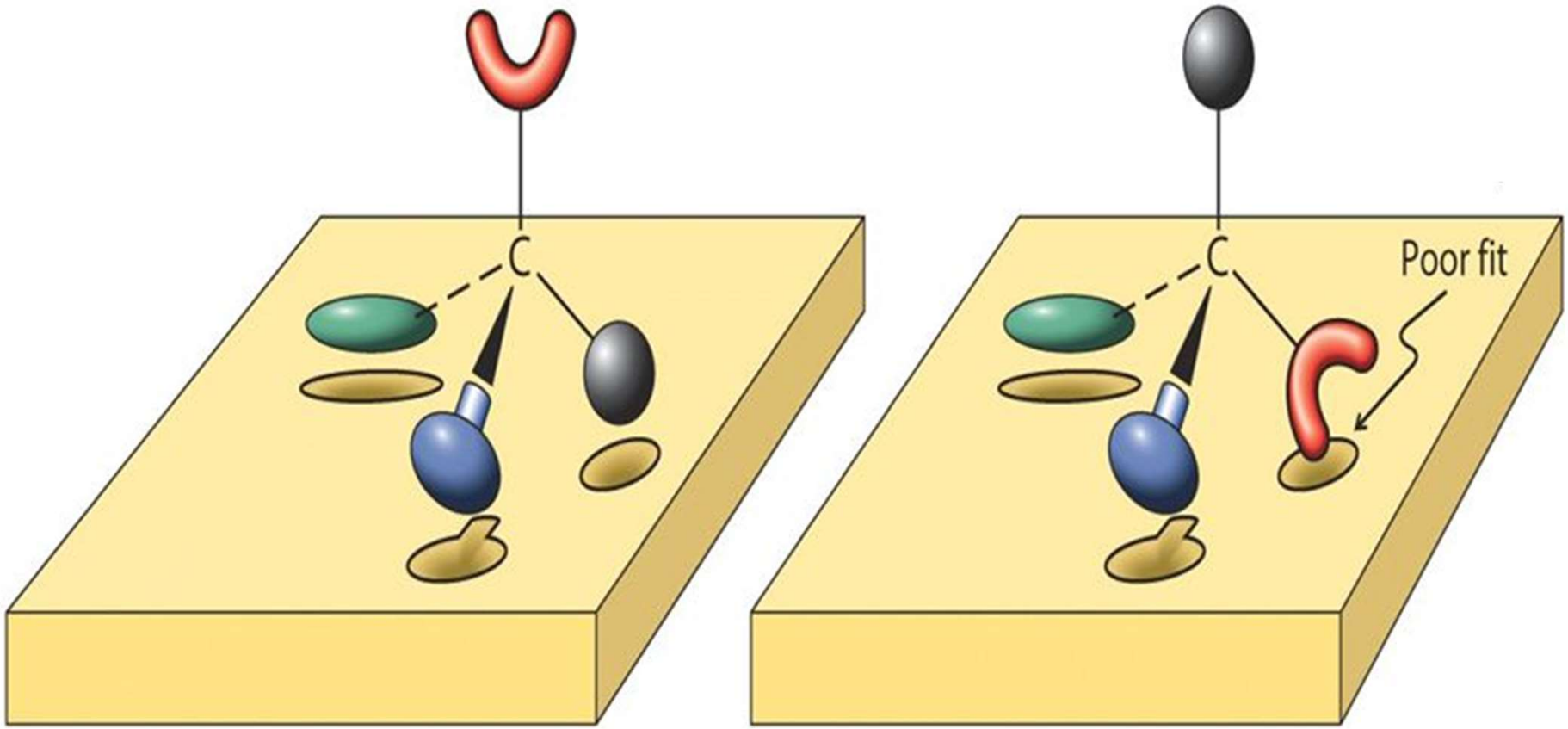
Whelk-O1

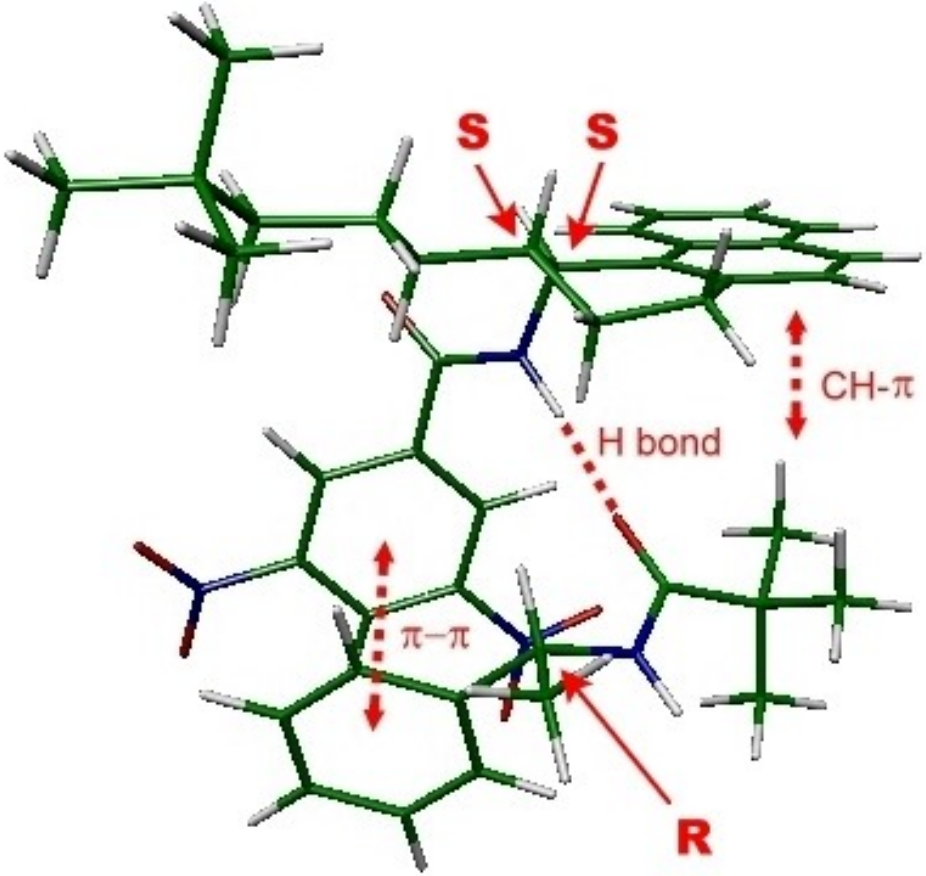
Polymeric phases

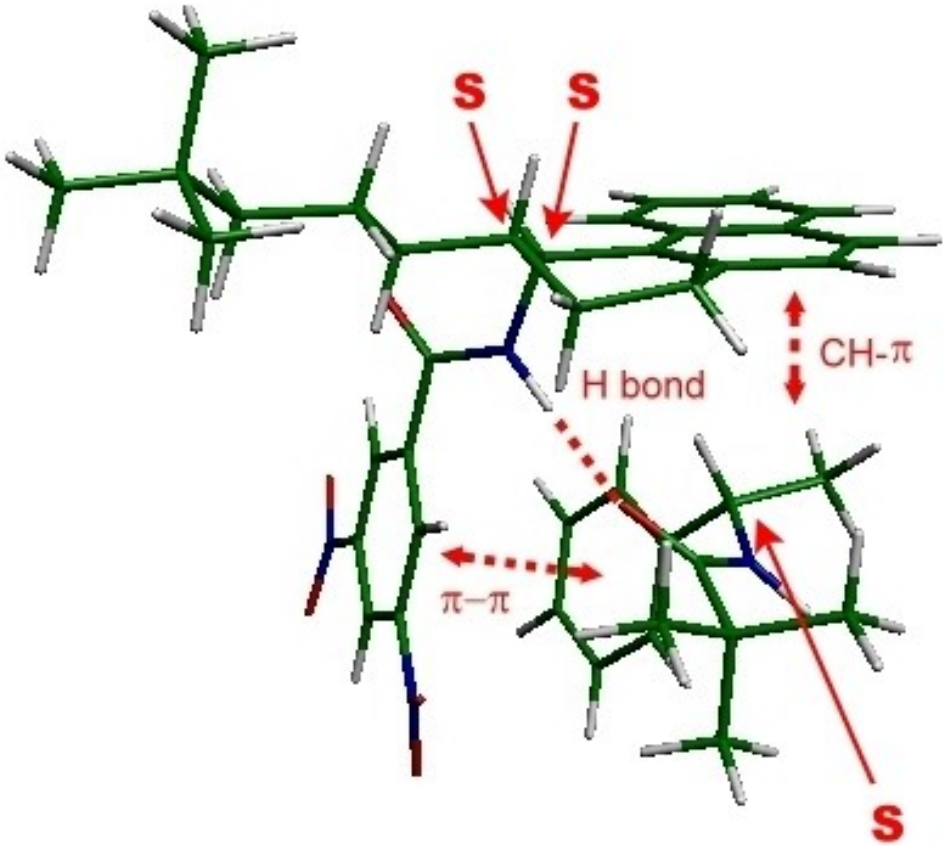


Lux-Amylose-2

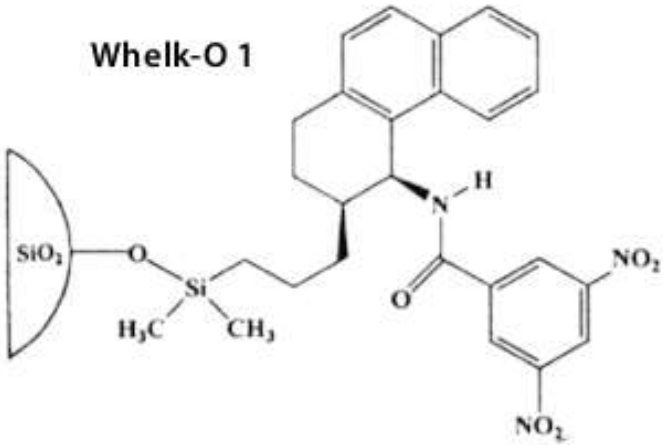
Three points interaction model

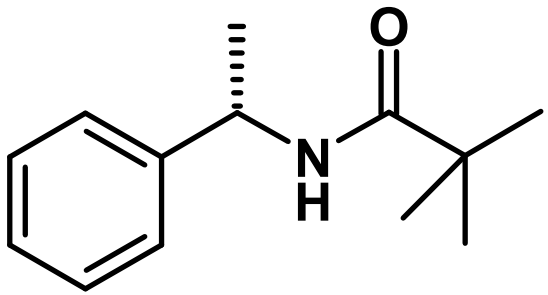


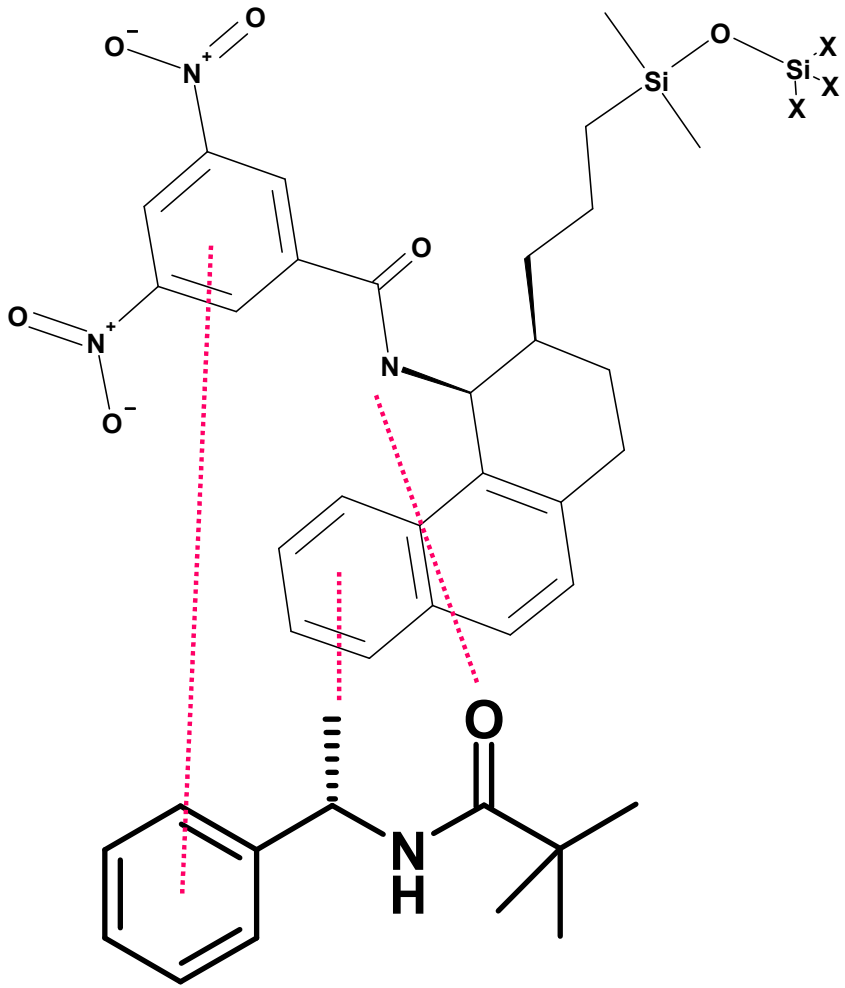

S
S
CH-π
H bond
π–π
R

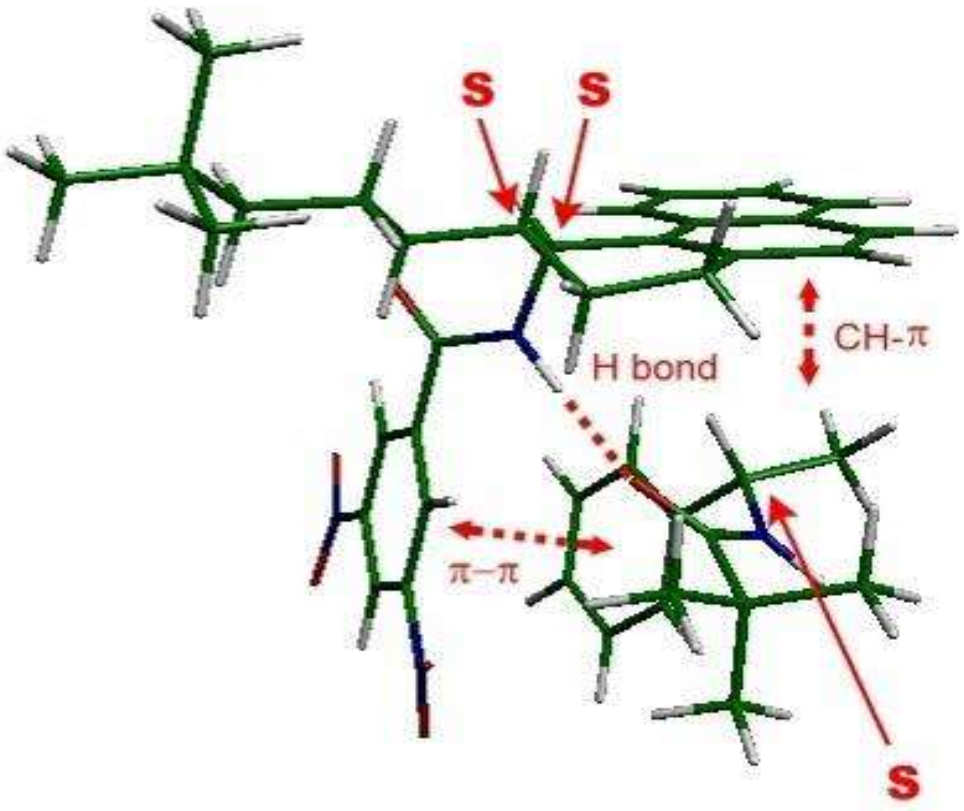

S
S
CH-π
H bond
π–π
S

Whelk-O 1

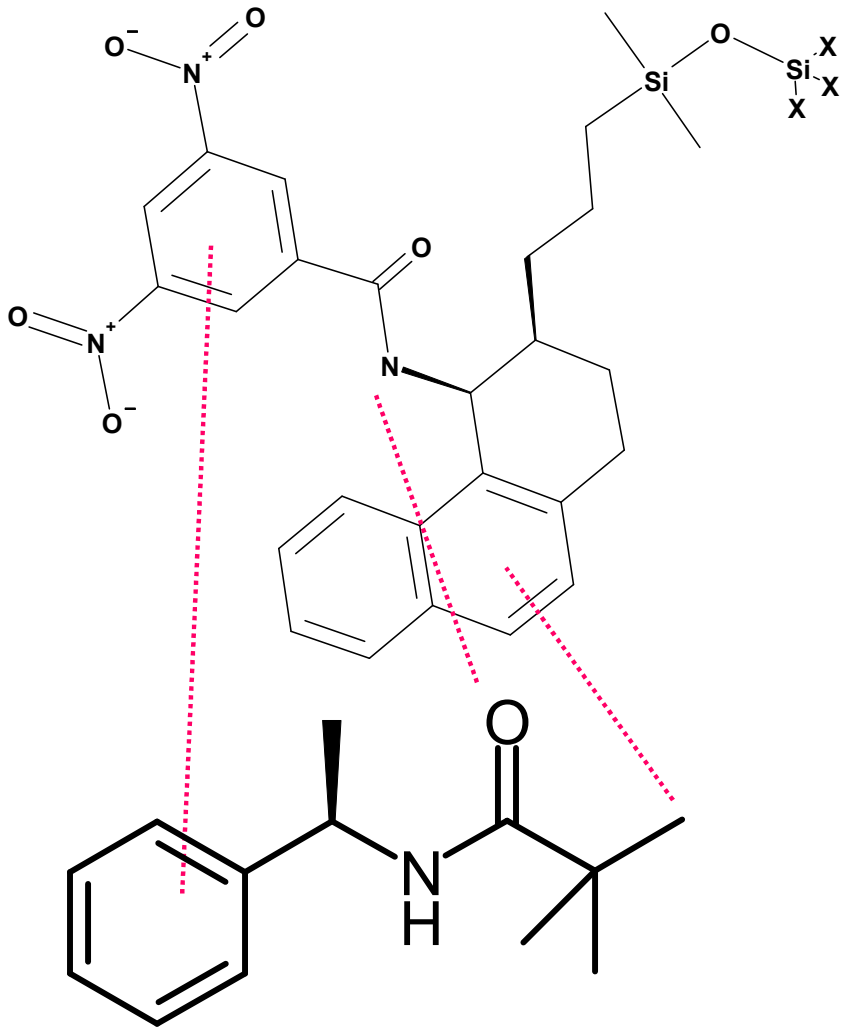

SiO₂
O
Si
H₃C
CH₃
N
H
O
NO₂
NO₂

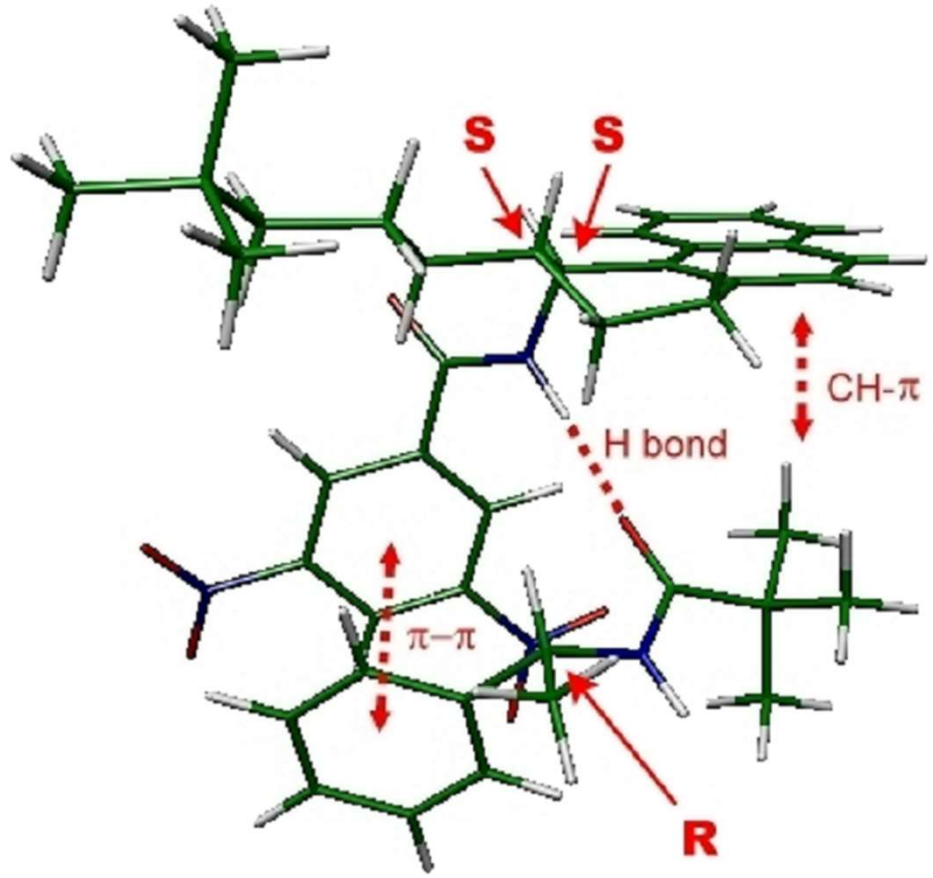

O
N
H





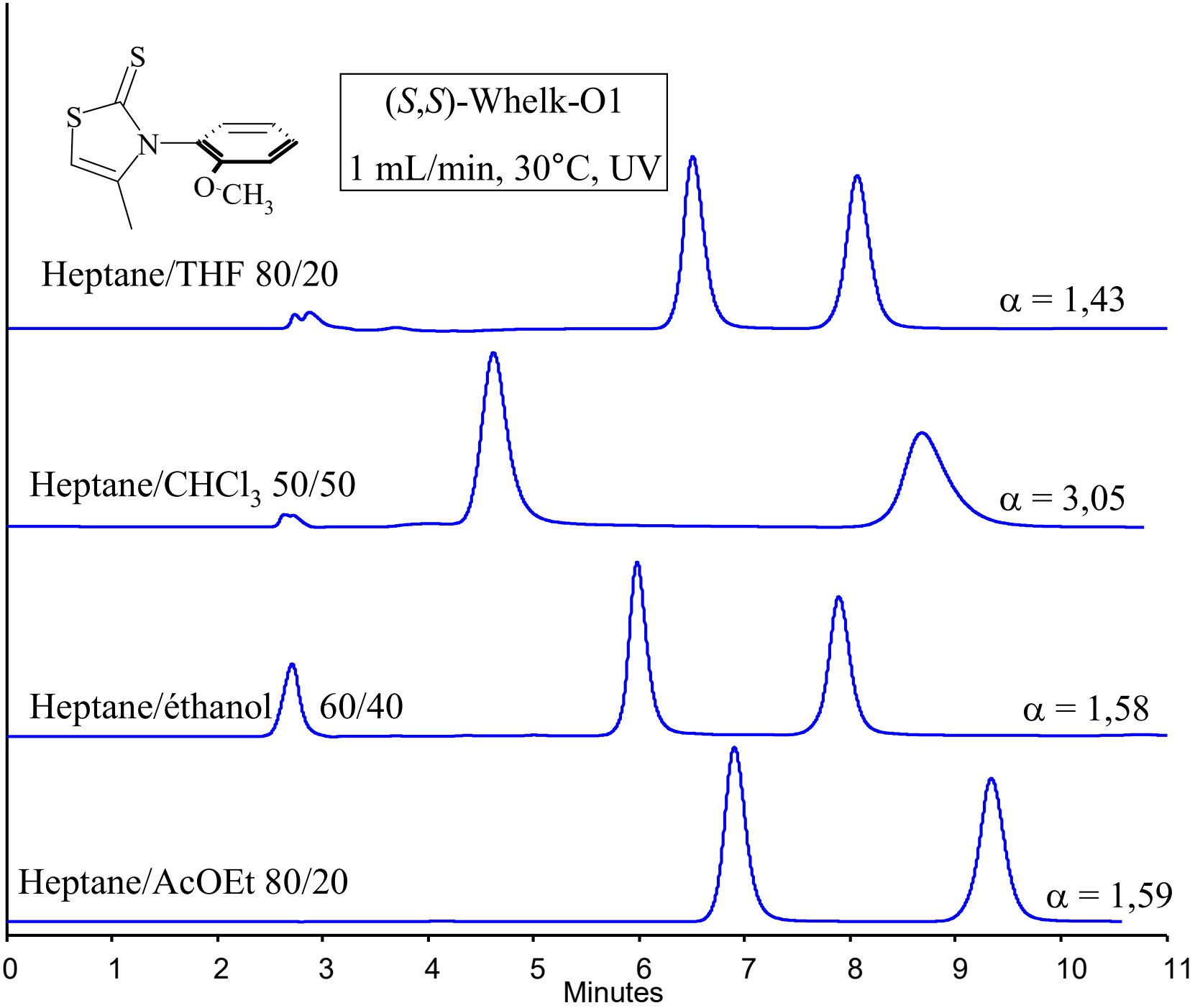
Del Rio, A.; Hayes, J.M.; Stein, M.; Piras, P.; Roussel, C.; *Chirality*, 2004, *16*, S1-S11.



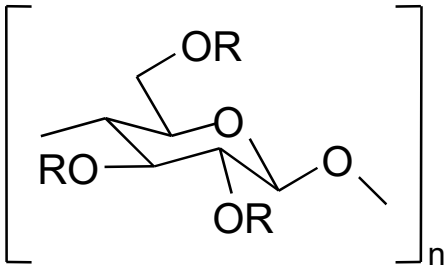


Del Rio, A.; Hayes, J.M.; Stein, M.; Piras, P.; Roussel, C.; *Chirality*, 2004, *16*, S1-S11.

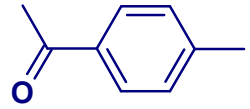
Influence of mobile phase on enantioselectivity



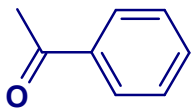
Polymeric chiral stationary phases



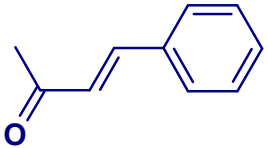
Cellulose



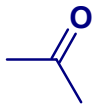
Chiralcel OJ



Chiralcel OB



Chiralcel OK



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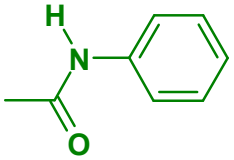

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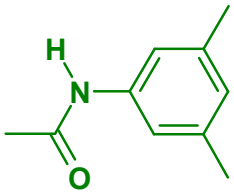
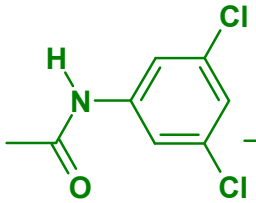
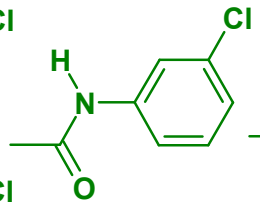


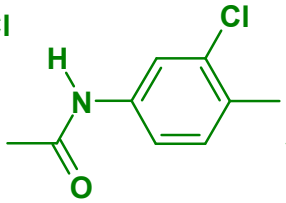
Chiralcel OD



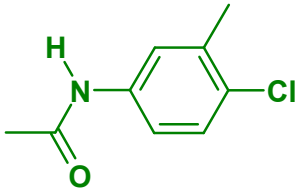
Chiralpak IC



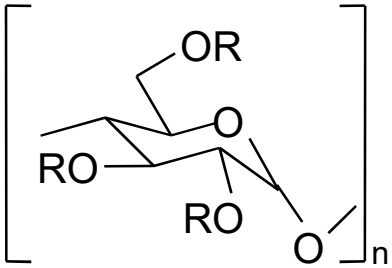
Chiralpak ID



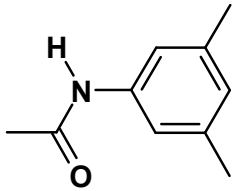
Chiralcel OZ



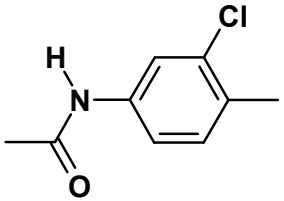
Lux-Cellulose-4



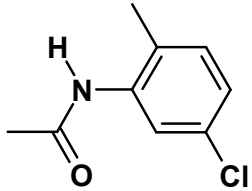
Amylose



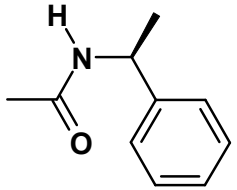
Chiralpak AD



Chiralpak AZ

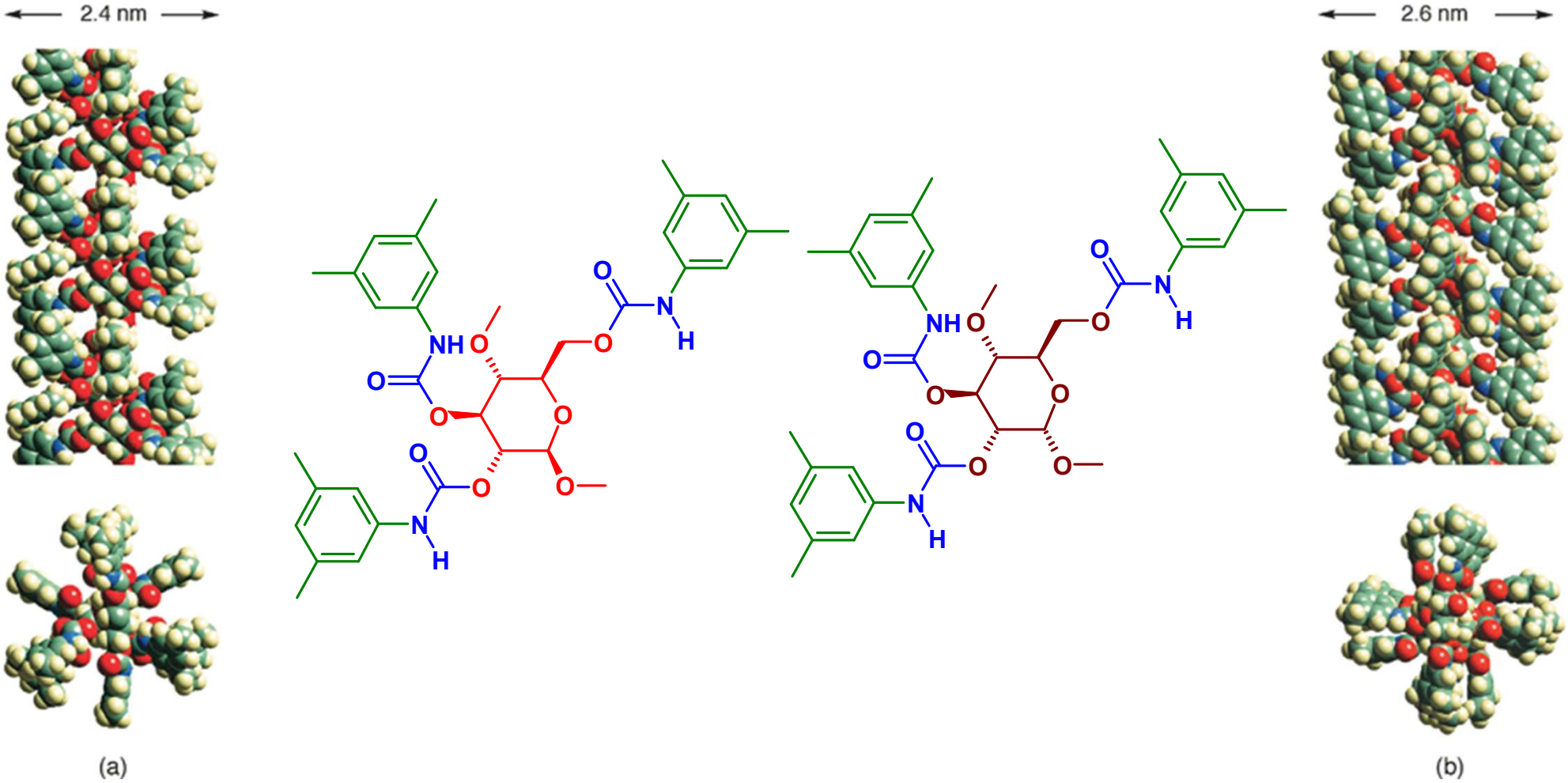


Chiralpak AY



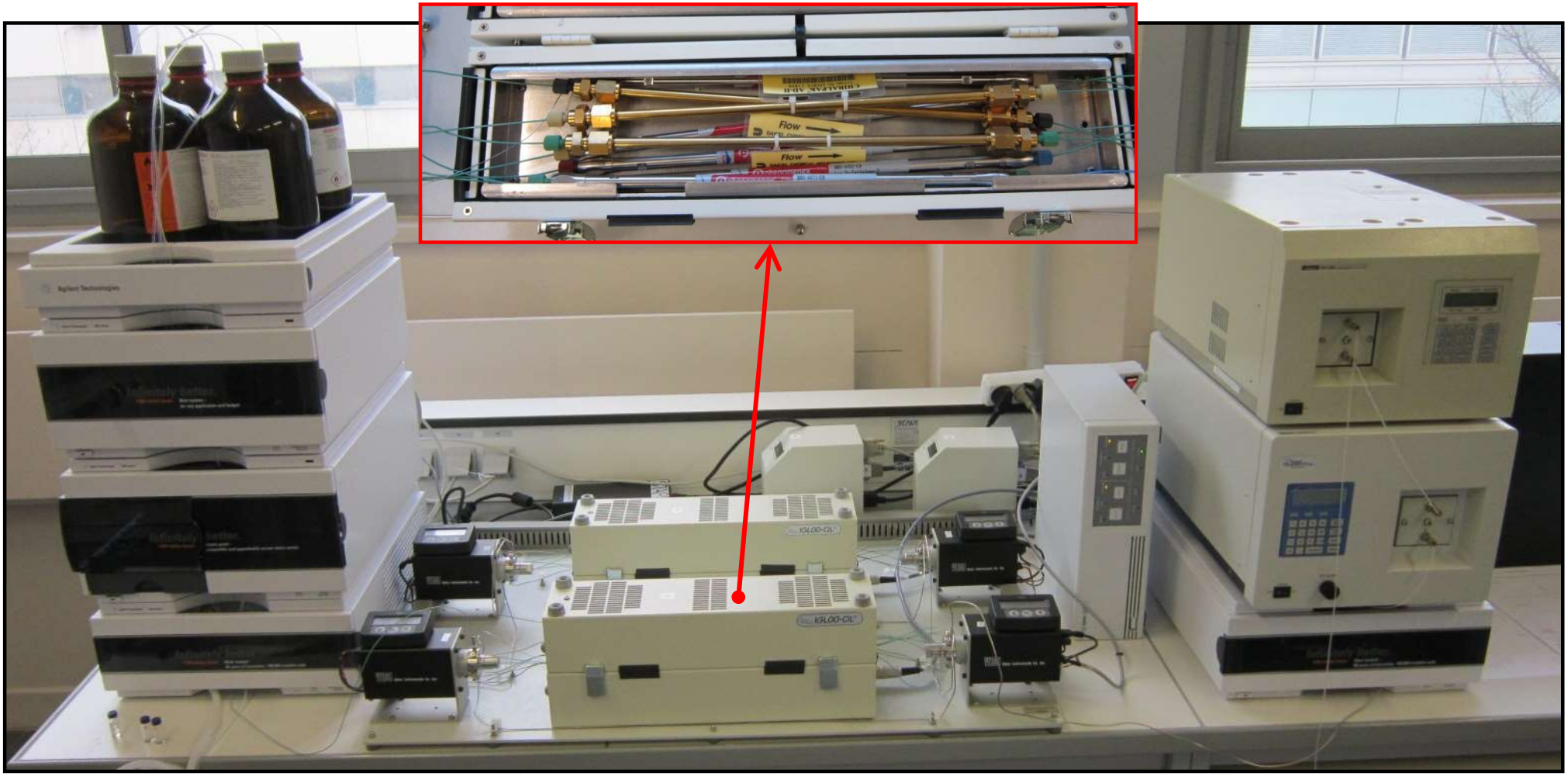
Chiralpak AS

The polysaccharide was usually coated on silica, so a lot of solvents should be avoided : chloroform, THF, ether



Optimized structures of 3,5-dimethylphenylcarbamates of cellulose **(a)** and amylose **(b).**
Along (top) and perpendicular (bottom) to the helix axis.
Yoshio Okamoto and Tomoyuki Ikai, Chem. Soc. Rev., 2008, **37, 2593-2608**

Screening unit



To develop an analytical method, we have to choose the chiral stationary phase, the mobile phase, the flow-rate, the detection (UV and chirality) and the temperature.

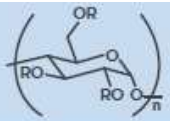
How to describe a chiral HPLC separation

Chiralpak AD-H (amylose tris (3,5-dimethylphenyl-carbamate), ChiralTechnologies Europe, 250x4.6mm), hexane / ethanol (70/30), 1 mL/min, 25°C, UV 220 nm and CD 220 nm, Rt(-, CD 220 nm) = 5.77 min, Rt (+, CD 220 nm) = 9.55 min, k(-) = 0.92, k(+) = 2.18, α = 2.37 and Rs = 8.67

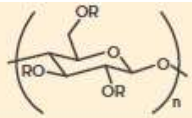
Column (name, size, seller)
Mobile phase
Flow-rate
Temperature
Detection
Chiroptical detection
Retention times
Retention factor
Enantioselectivity
Resolution
Elution order

AMYLOSE DERIVATIVES

CHIRAL TECHNOLOGIES
DAICEL GROUP

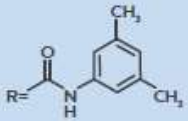


CELLULOSE DERIVATIVES



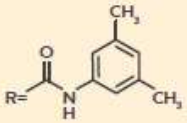
IMMOBILIZED POLYSACCHARIDE SELECTORS

CHIRALPAK® IA



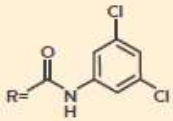
tris (3,5-dimethylphenylcarbamate)

CHIRALPAK IB (-N)



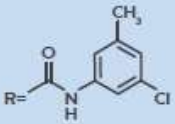
tris (3,5-dimethylphenylcarbamate)

CHIRALPAK IC



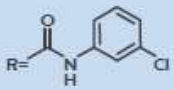
tris (3,5-dichlorophenylcarbamate)

CHIRALPAK IG



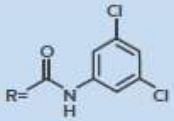
tris (3-chloro-5-methylphenylcarbamate)

CHIRALPAK ID



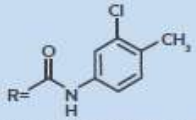
tris (3-chlorophenylcarbamate)

CHIRALPAK IE



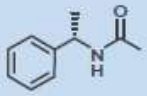
tris (3,5-dichlorophenylcarbamate)

CHIRALPAK IF



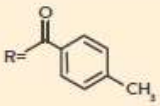
tris (3-chloro-4-methylphenylcarbamate)

CHIRALPAK IH



tris (S)-α-methylbenzylcarbamate)

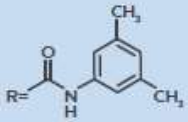
CHIRALKPAK IJ



tris (4-methylbenzoate)

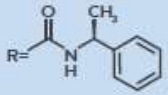
COATED POLYSACCHARIDE SELECTORS

CHIRALPAK AD



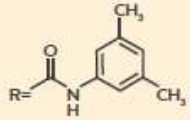
tris (3,5-dimethylphenylcarbamate)

CHIRALPAK AS



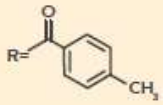
tris (S)-α-methylbenzylcarbamate

CHIRALCEL® OD



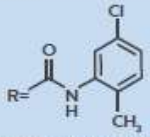
tris (3,5-dimethylphenylcarbamate)

CHIRALCEL OJ



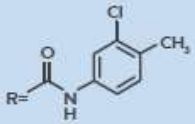
tris (4-methylbenzoate)

CHIRALPAK AY



tris (5-chloro-2-methylphenylcarbamate)

CHIRALPAK AZ

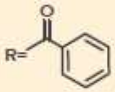


tris (3-chloro-4-methylphenylcarbamate)

CHIRALCEL® OA

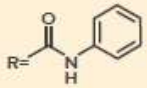
Triacetate

CHIRALCEL OB



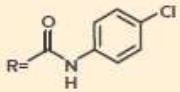
Tribenzoate

CHIRALCEL OC



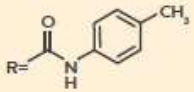
tris (phenylcarbamate)

CHIRALCEL OF



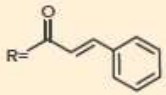
tris (4-chlorophenylcarbamate)

CHIRALCEL OG



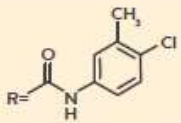
tris (4-methylphenylcarbamate)

CHIRALCEL OK



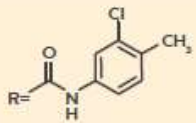
Tricinnamate

CHIRALCEL OX



tris (4-chloro-3-methylphenylcarbamate)

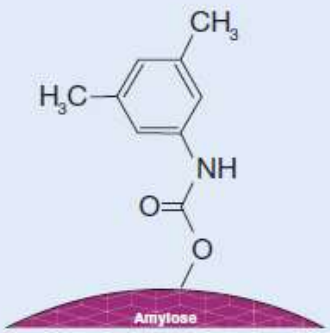
CHIRALCEL OZ



tris (3-chloro-4-methylphenylcarbamate)

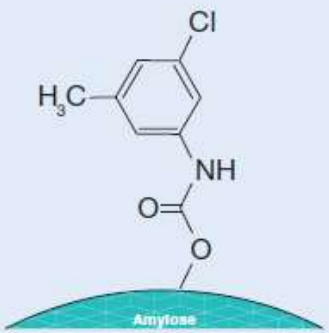
LEGEND

phenomenex®
...breaking with tradition℠



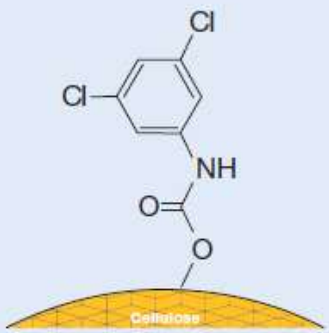
Lux i-Amylose-1

Amylose tris
(3,5-dimethylphenylcarbamate)



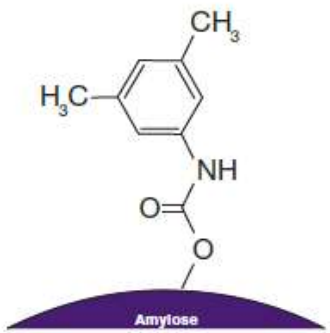
Lux i-Amylose-3

Amylose tris
(3-chloro-5-methylphenylcarbamate)



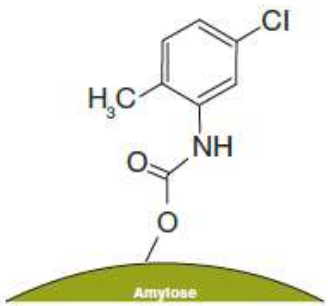
Lux i-Cellulose-5

Cellulose tris
(3,5-dichlorophenylcarbamate)



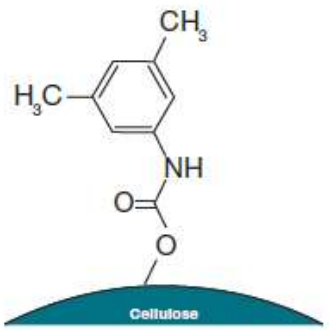
Lux Amylose-1

Amylose tris
(3,5-dimethylphenylcarbamate)



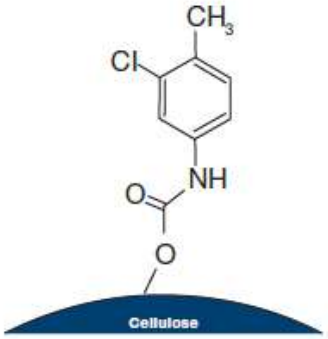
Lux Amylose-2

Amylose tris
(5-chloro-2-
methylphenylcarbamate)



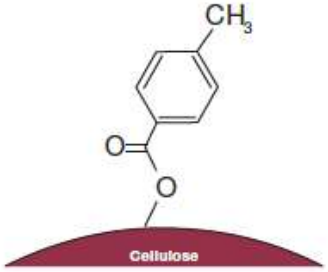
Lux Cellulose-1

Cellulose tris
(3,5-dimethylphenylcarbamate)



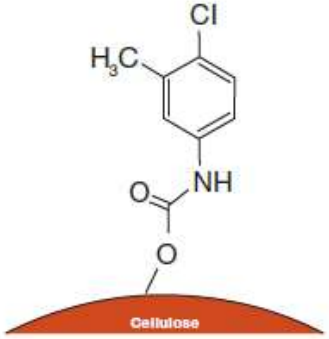
Lux Cellulose-2

Cellulose tris
(3-chloro-4-methylphenylcarbamate)



Lux Cellulose-3

Cellulose tris
(4-methylbenzoate)



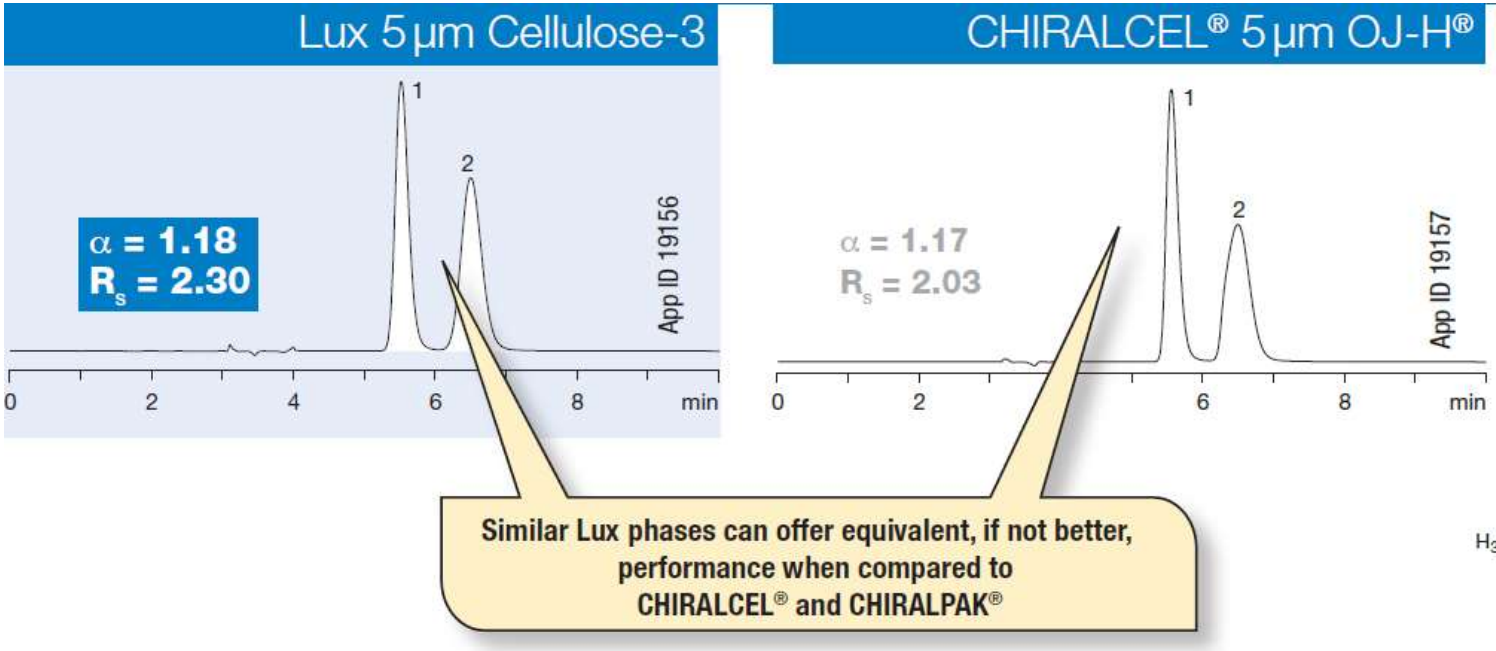
Lux Cellulose-4

Cellulose tris
(4-chloro-3-methylphenylcarbamate)

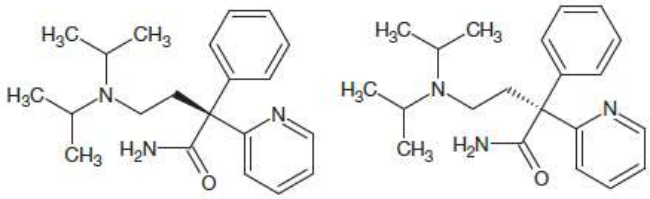
YMC EUROPE GMBH
The Selectivity Company

	CHIRAL ART Amylose-SA	CHIRAL ART Cellulose-SB	CHIRAL ART Cellulose-SC	CHIRAL ART Cellulose-SJ	CHIRAL ART Cellulose-SZ
Particle size	3, 5, 10, 20 µm				3, 5 µm
Chiral selector	Amylose tris (3,5-dimethylphenyl-carbamate)	Cellulose tris (3,5-dimethylphenyl-carbamate)	Cellulose tris (3,5-dichlorophenyl-carbamate)	Cellulose tris (4-methylbenzoat)	Cellulose tris (3-chloro-4-methyl-phenylcarbamate)

If you are using one of the DAICEL® columns below:	Guaranteed alternative:	Phase description:
CHIRALPAK® IA® and IA-3	Lux i-Amylose-1	Amylose tris(3,5-dimethylphenylcarbamate)
CHIRALPAK IG® and IG-3	Lux i-Amylose-3	Amylose tris(3-chloro-5-methylphenylcarbamate)
CHIRALPAK IC® and IC-3	Lux i-Cellulose-5	Cellulose tris(3,5-dichlorophenylcarbamate)
CHIRALPAK AD®, AD-H®, AD-3, AD-RH®, and AD-3R	Lux Amylose-1	Amylose tris(3,5-dimethylphenylcarbamate)
CHIRALPAK AY®, AY-H®, AY-3, AY-RH, and AY-3R	Lux Amylose-2	Amylose tris(5-chloro-2-methylphenylcarbamate)
CHIRALCEL® OD®, OD-H®, OD-3, OD-RH®, and OD-3R	Lux Cellulose-1	Cellulose tris(3,5-dimethylphenylcarbamate)
CHIRALCEL OZ, OZ-H®, OZ-3, OZ-RH, and OZ-3R	Lux Cellulose-2	Cellulose tris(3-chloro-4-methylphenylcarbamate)
CHIRALCEL OJ®, OJ-H®, OJ-3, OJ-RH®, and OJ-3R	Lux Cellulose-3	Cellulose tris(4-methylbenzoate)
CHIRALCEL OX-H, OX-3, OX-RH, and OX-3R	Lux Cellulose-4	Cellulose tris(4-chloro-3-methylphenylcarbamate)

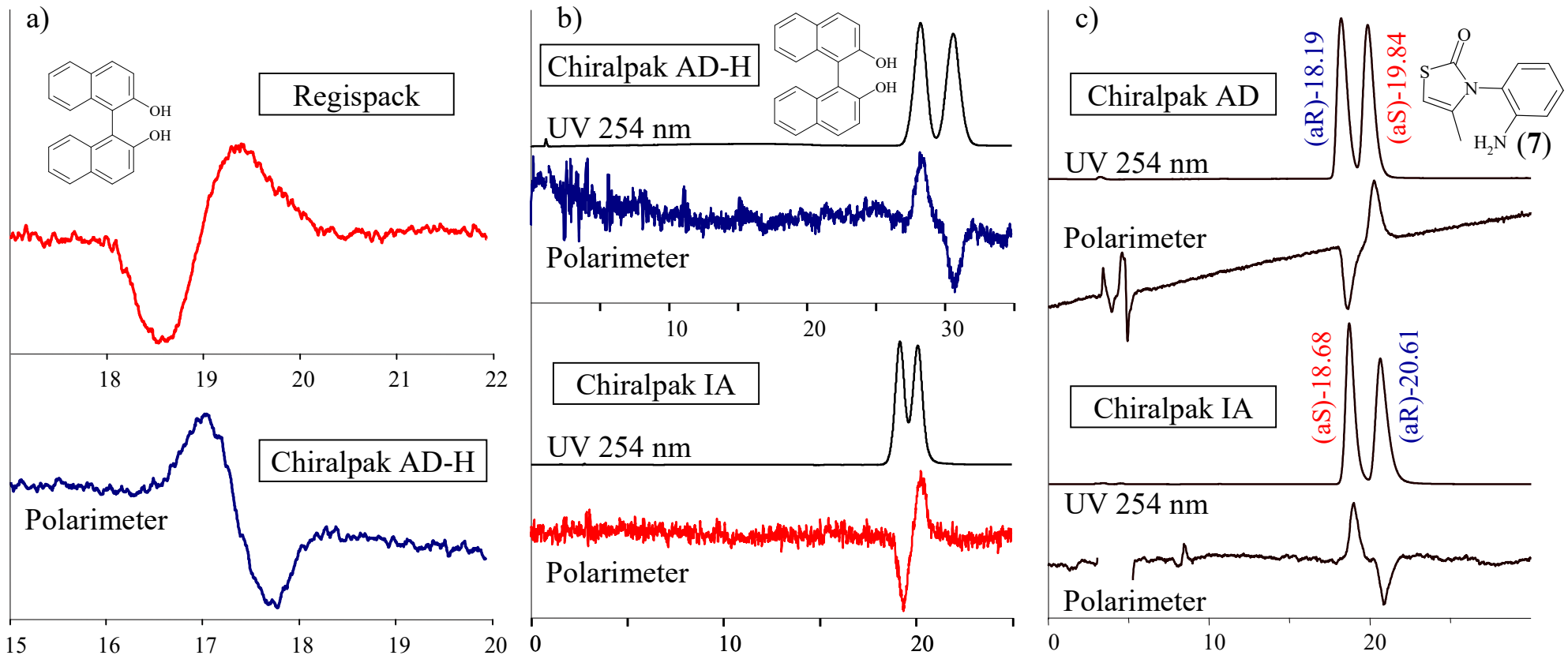


Conditions for both columns:
Dimensions: 250 x 4.6 mm
Mobile Phase: 0.1 % Diethylamine in Hexane / 0.1 % Diethylamine in Ethanol (90:10)
Flow Rate: 1 mL/min
Detection: UV @ 220 nm
Temperature: Ambient



Are separations reproducible on columns from different suppliers ?
Are separations reproducible between coated and immobilized selectors ?

3 columns with amylose tris-(3,5-dimethylphenylcarbamate) as chiral selector

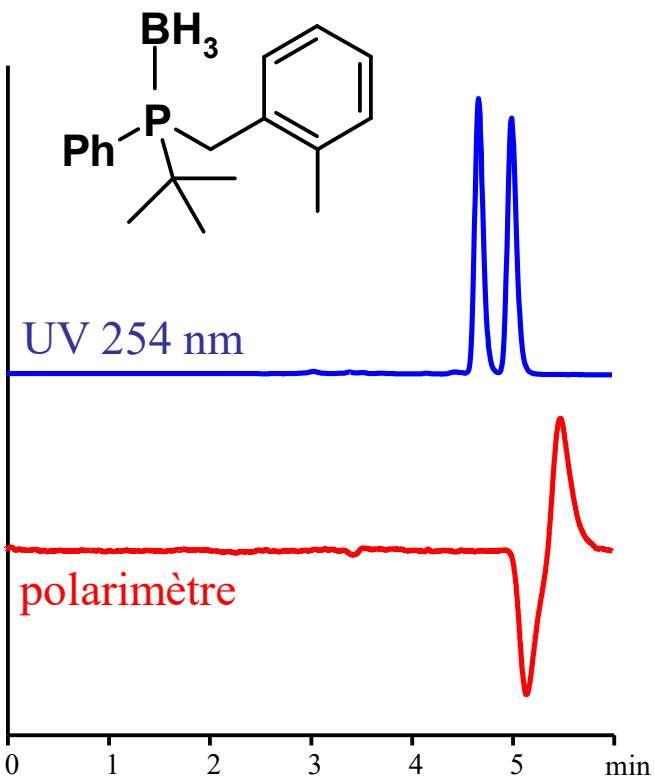


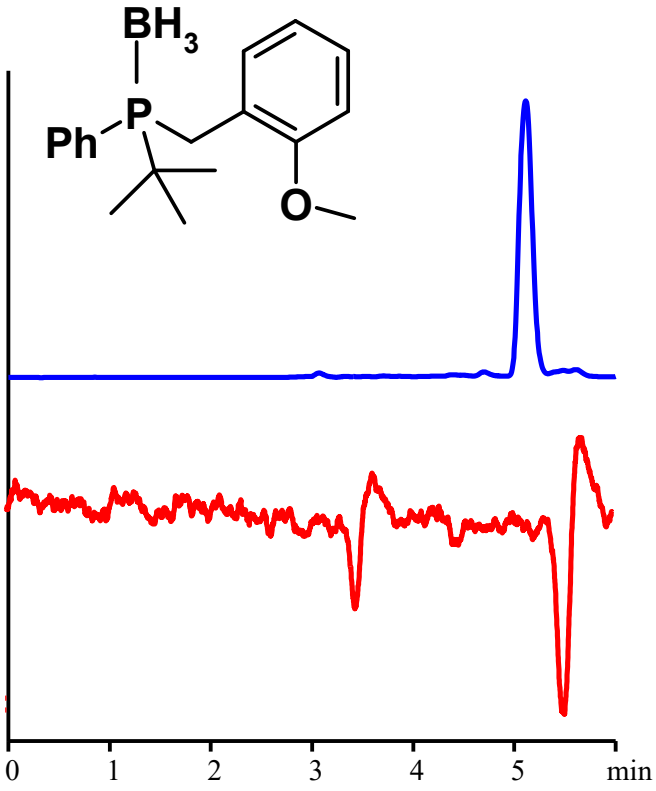
a) Polarimetric traces (OR-1590) for binaphthol, with hexane / 2-PrOH (95/5), at 40°C, on Regispack at 2 mL/min and on Chiralpak AD-H at 3 mL/min; UV chromatograms and polarimetric traces (OR-1590)

b) for binaphthol on Chiralpak AD-H and on Chiralpak IA, with hexane / 2-PrOH (95/5), at 1 mL/min and 25°C;

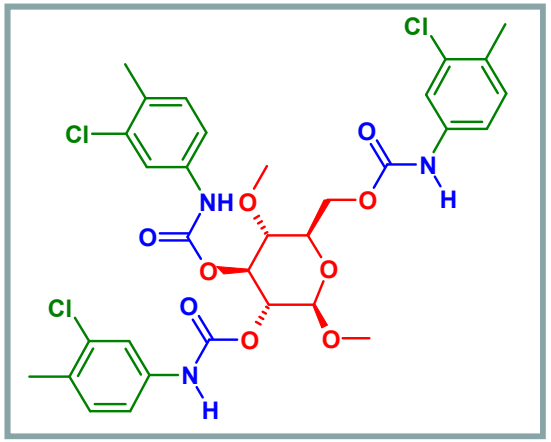
c) for (**7**) on Chiralpak AD and on Chiralpak IA, hexane / 2-PrOH (9/1), 1 mL/min, 25°C

Similar structures…





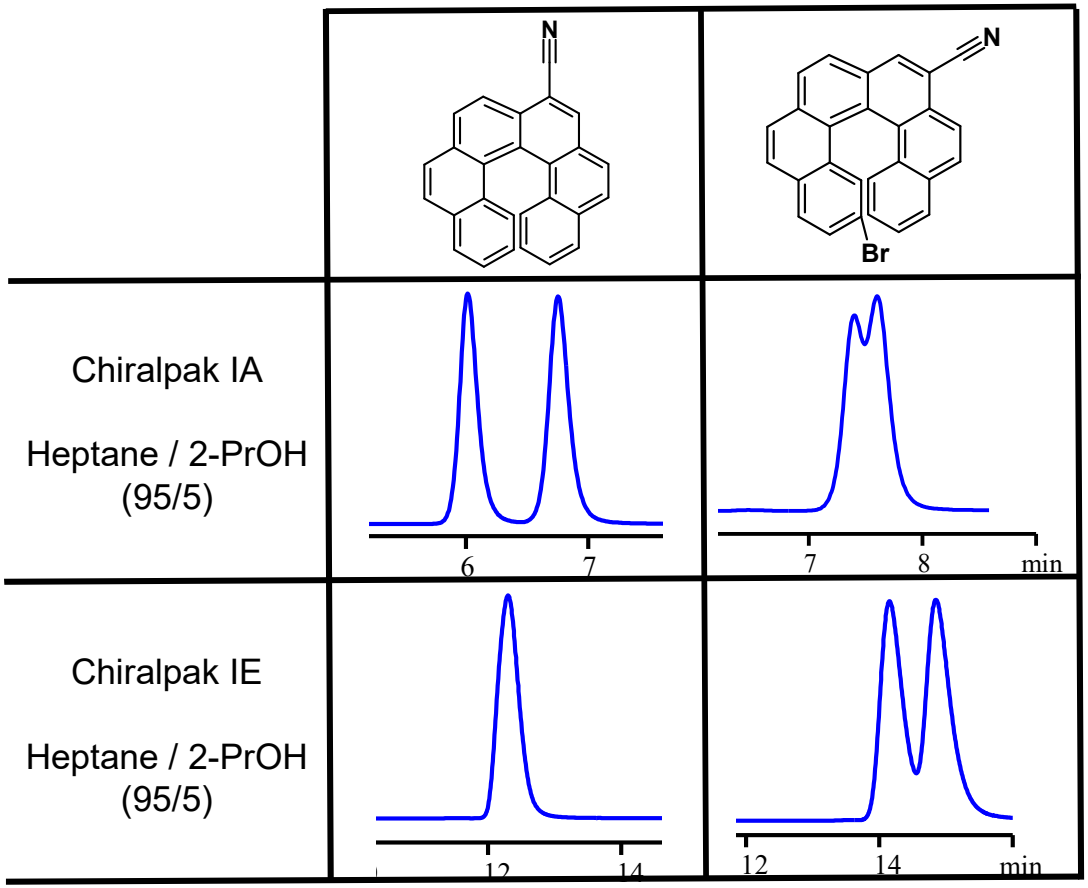
Lux-Cellulose-2
Heptane / 2-PrOH (90/10)

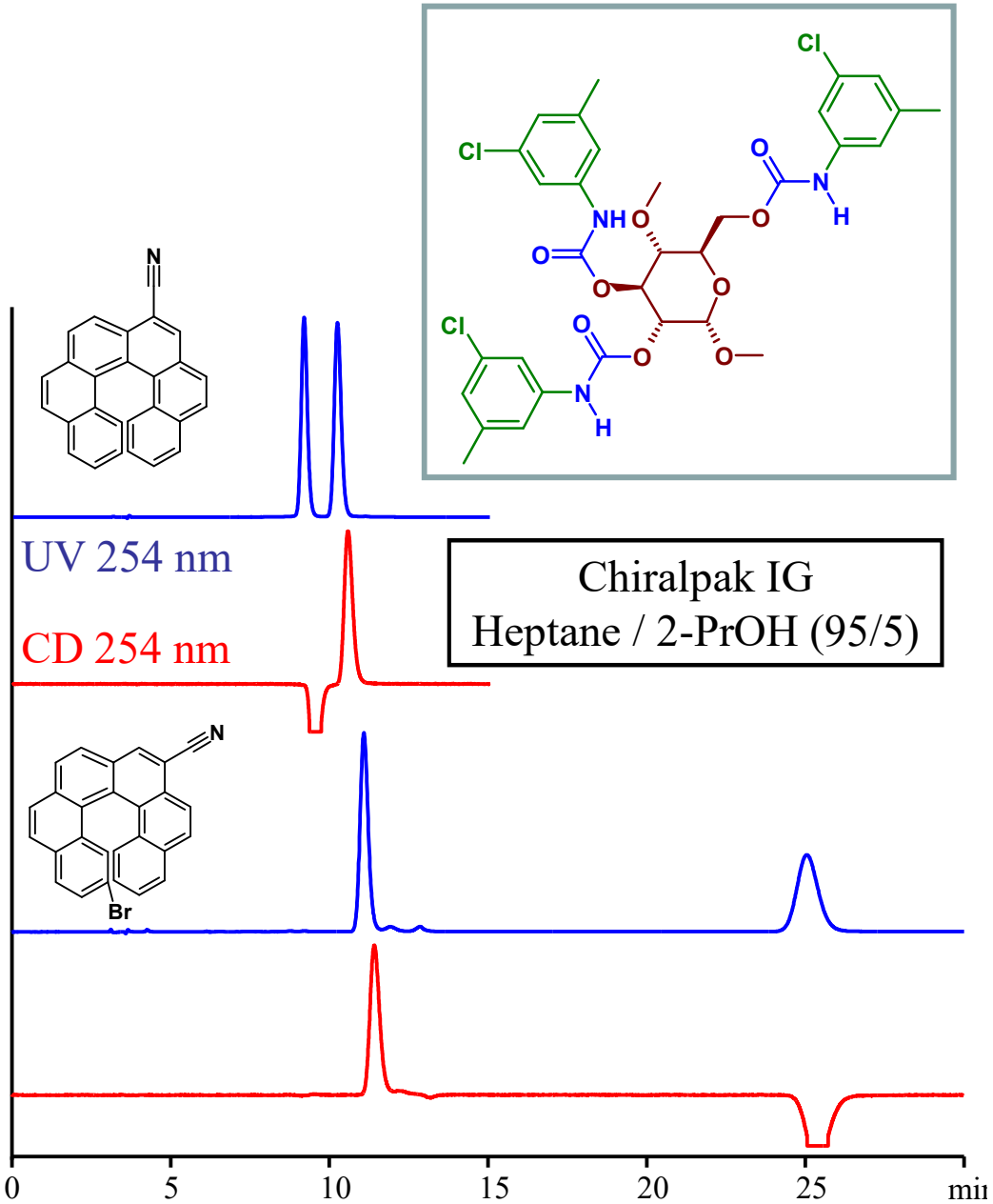


Each racemate requires a new method development

Products from Chirosciences (Marseille)

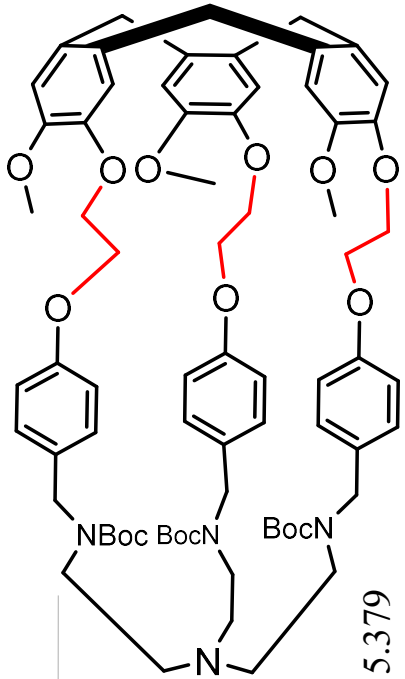
Similar structures…



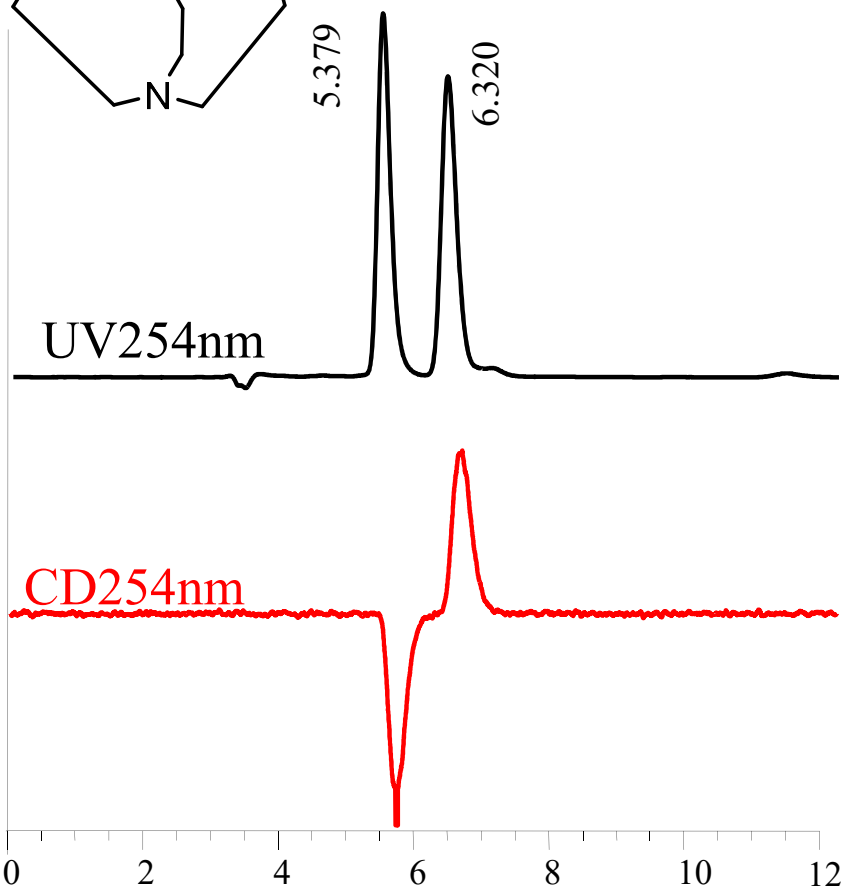


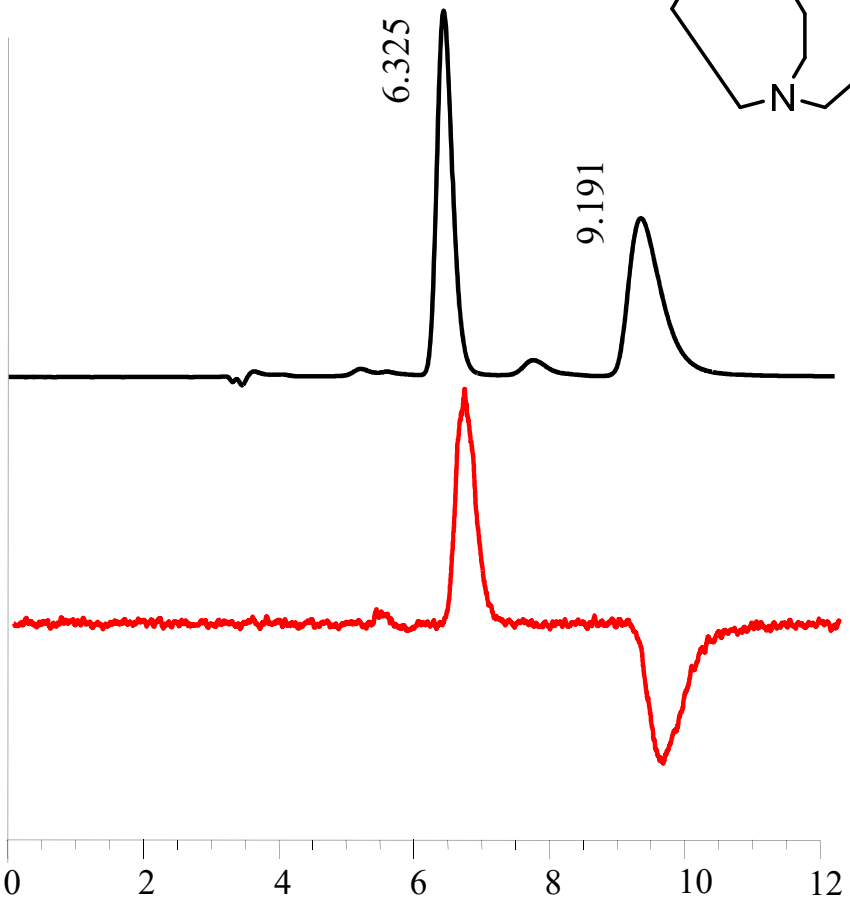
Products from Monastir University

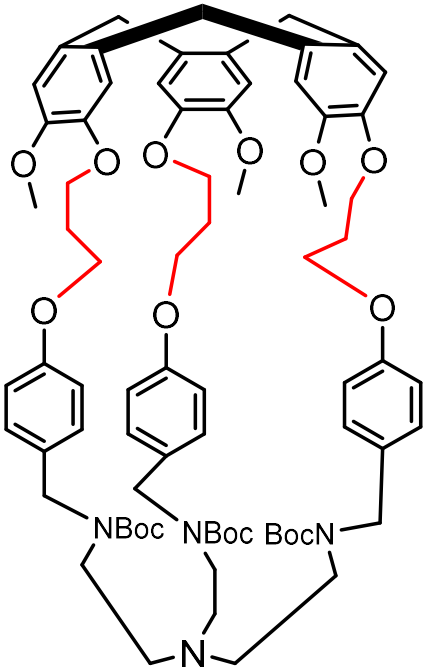
Similar structures…



Chiralpak ID
Heptane / EtOH+triethylamine / CH_2Cl_2
20/40/40



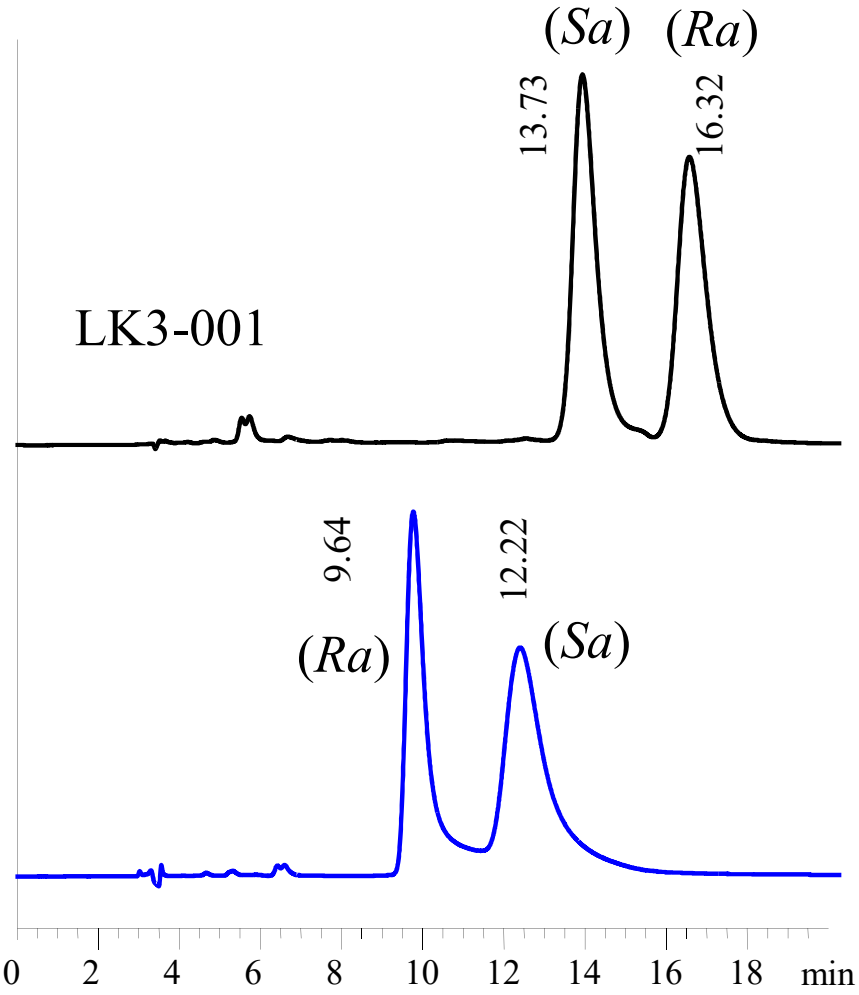


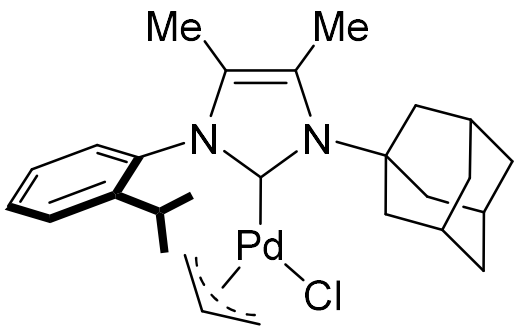


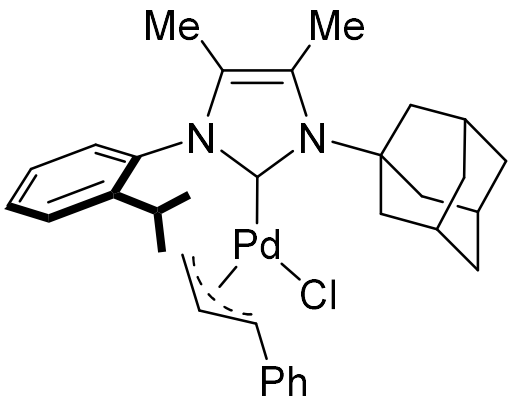
Products from Chunyang Li (Marseille)

Similar structures…

Chiralpak IG
Heptane/isopropanol/dichloromethane
80/10/10



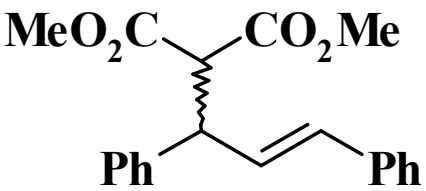




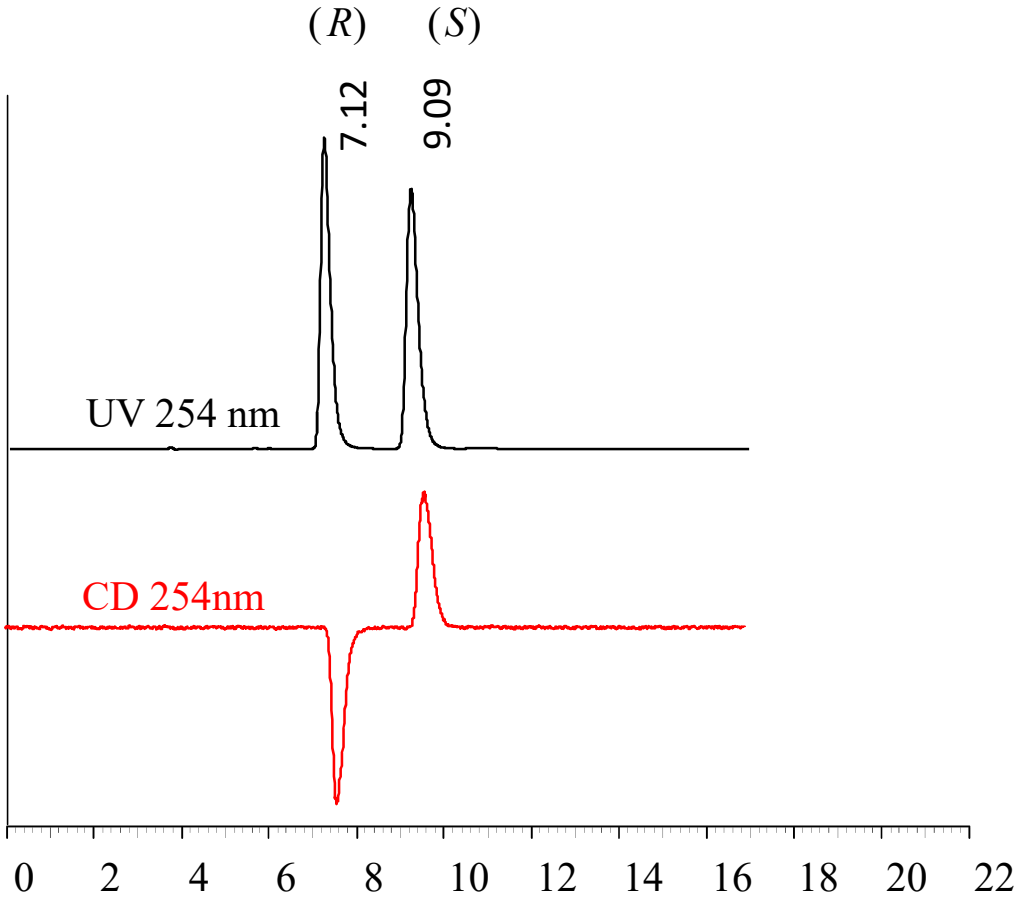
Products from Hervé Clavier (Marseille)

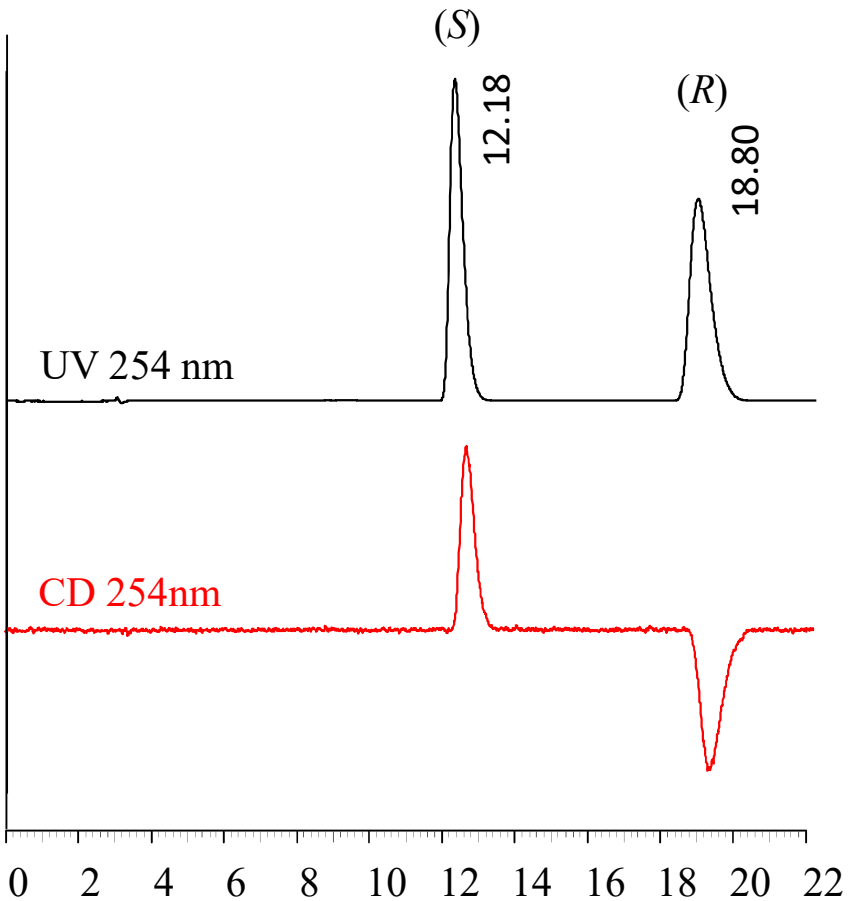
Co-solvent effect

Chiralpak AD-H
Hexane/2-PrOH (8/2)
30°C – 1 mL/min



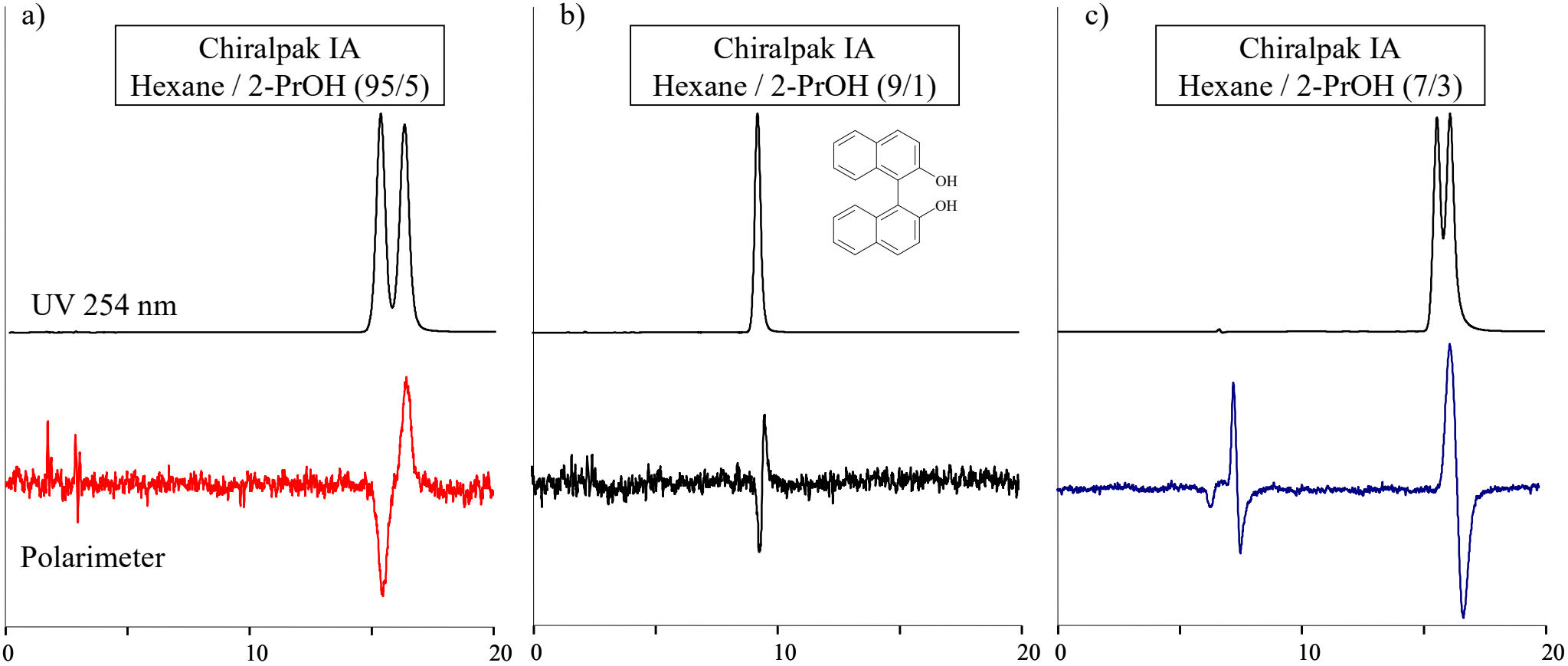
Chiralpak AD-H
Hexane/ethanol (8/2)
30°C – 1 mL/min



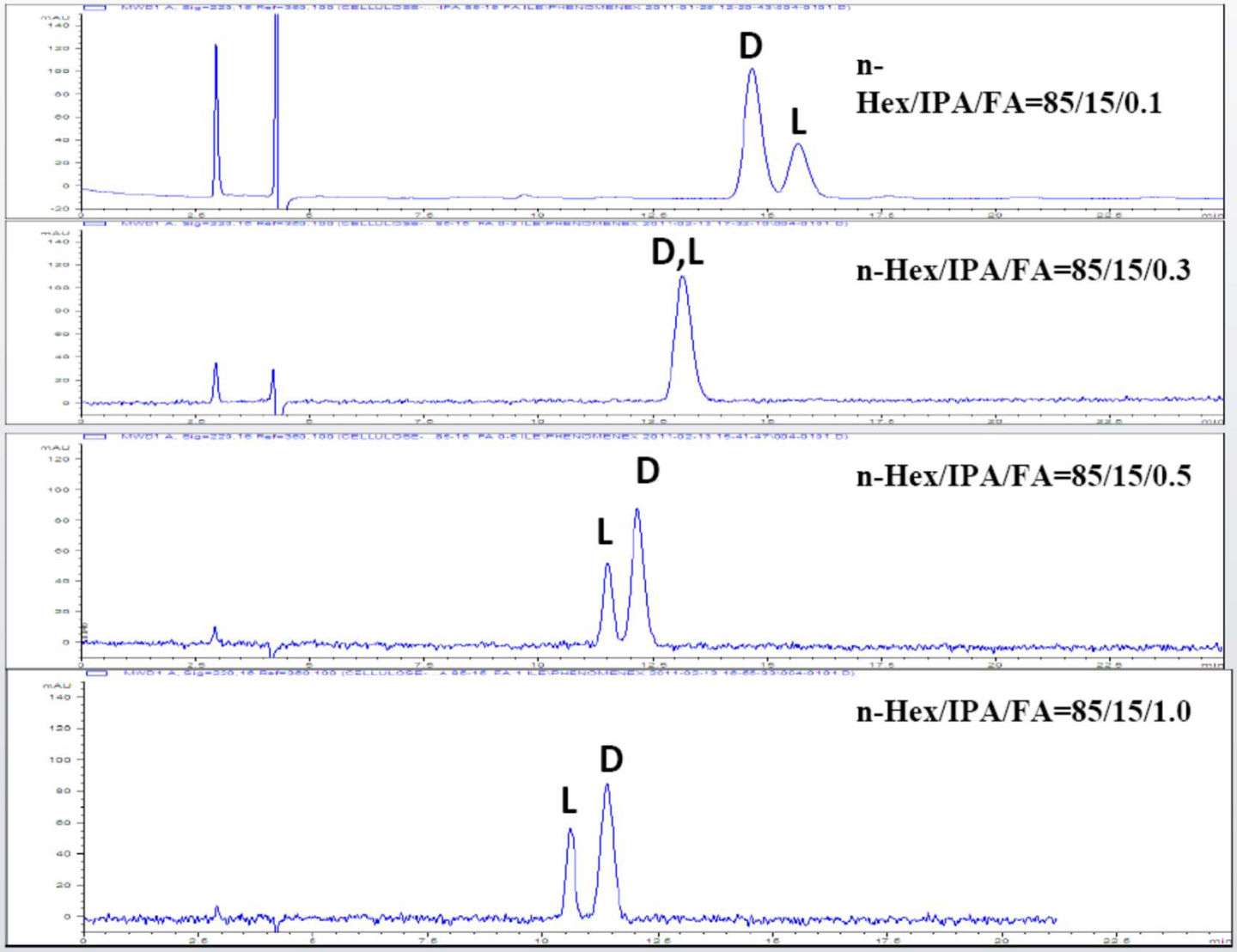


Co-solvent effect (proportion)

Temperature 35°C



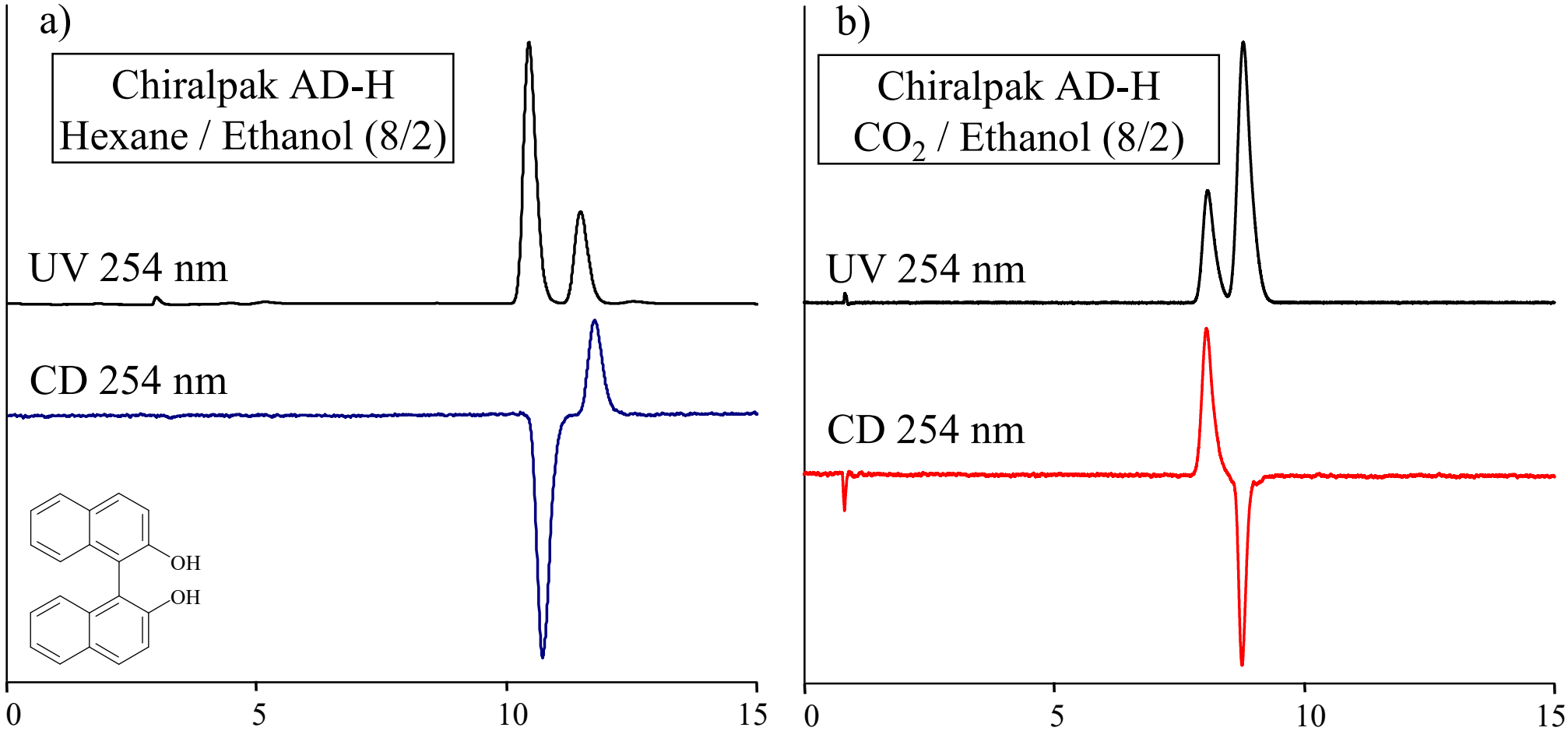
Effect of the additive



Journal of Chromatography A 1218 (2011) 6554-6560

Different proportions of formic acid for the separation of Fmoc-Isoleucine on Lux-Cellulose-1

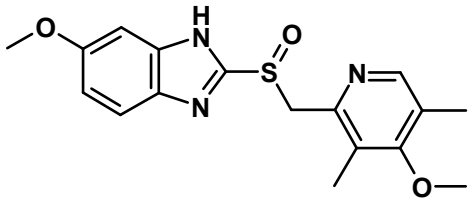
Reversal of elution order between hexane and carbon dioxide



UV and CD traces (CD-1595) at 254 nm for binaphthol, with 20% of ethanol, at 40°C, on Chiralpak AD-H a) by HPLC, hexane / ethanol (8/2), at 1 mL/min; b) by SFC, CO2 / ethanol (8/2), 4 mL/min, outlet pressure = 120 bars

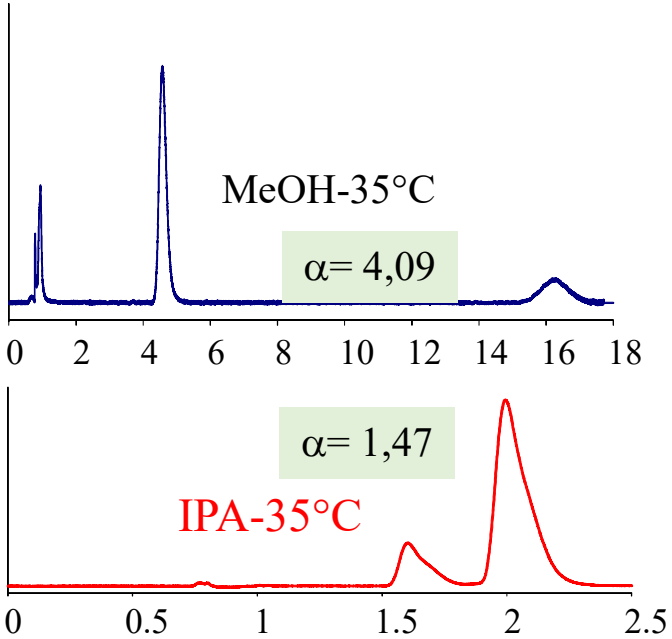
Co-solvent effect

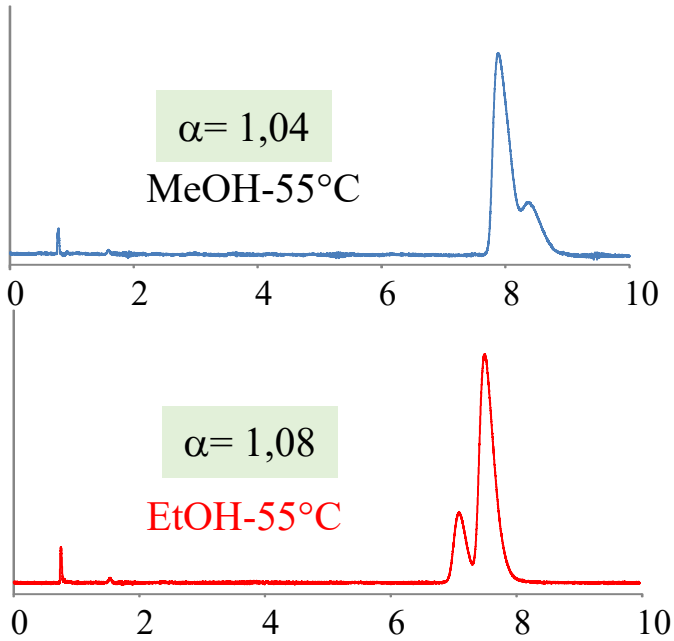
Chiralpak AD-H
CO_2 60% and 40% co-solvent
4 mL/min
120 bars



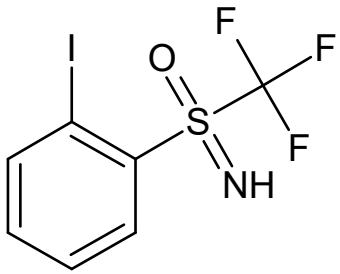
(*S*)-enriched omeprazole

Chiralpak IE
CO_2 60% and 40% co-solvent
4 mL/min
120 bars



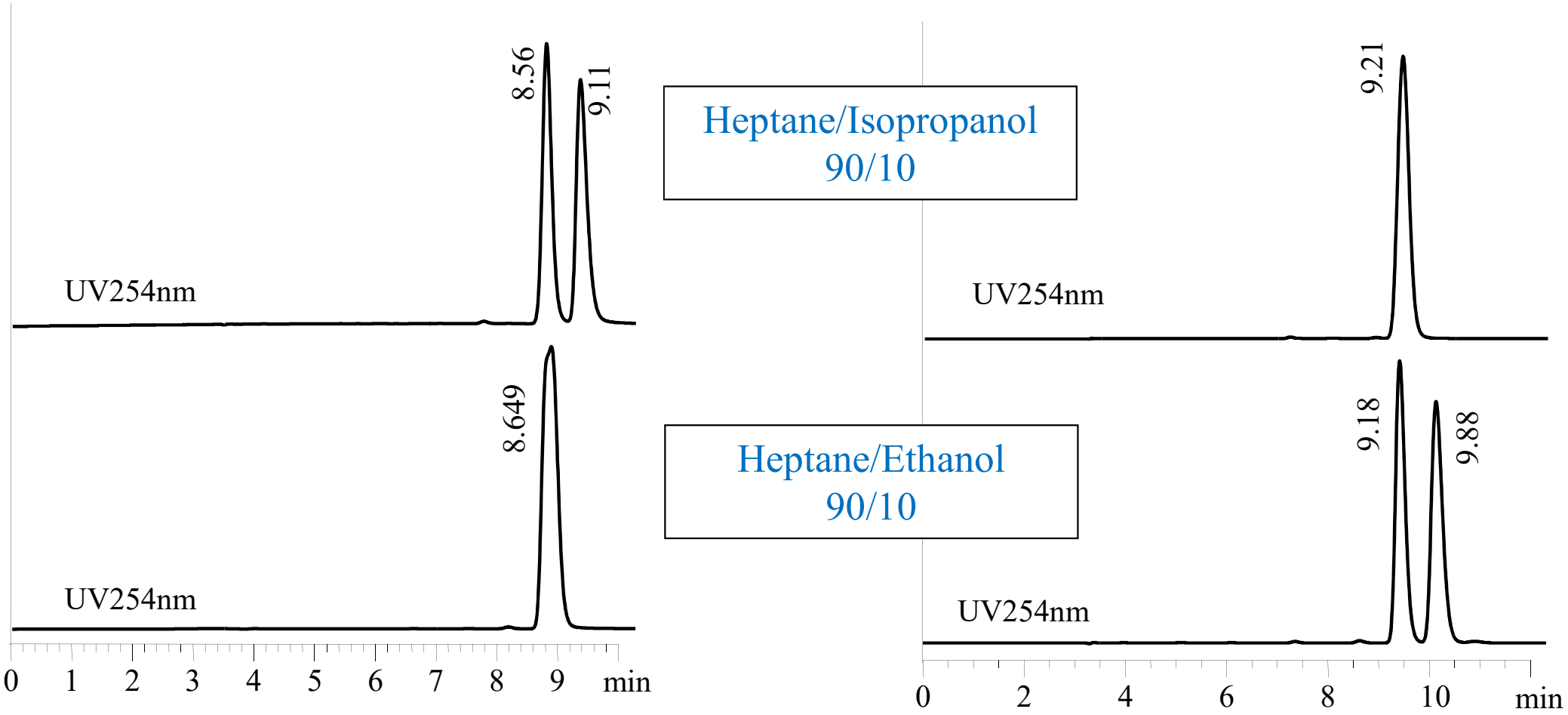


Co-solvent : ethanol or isopropanol



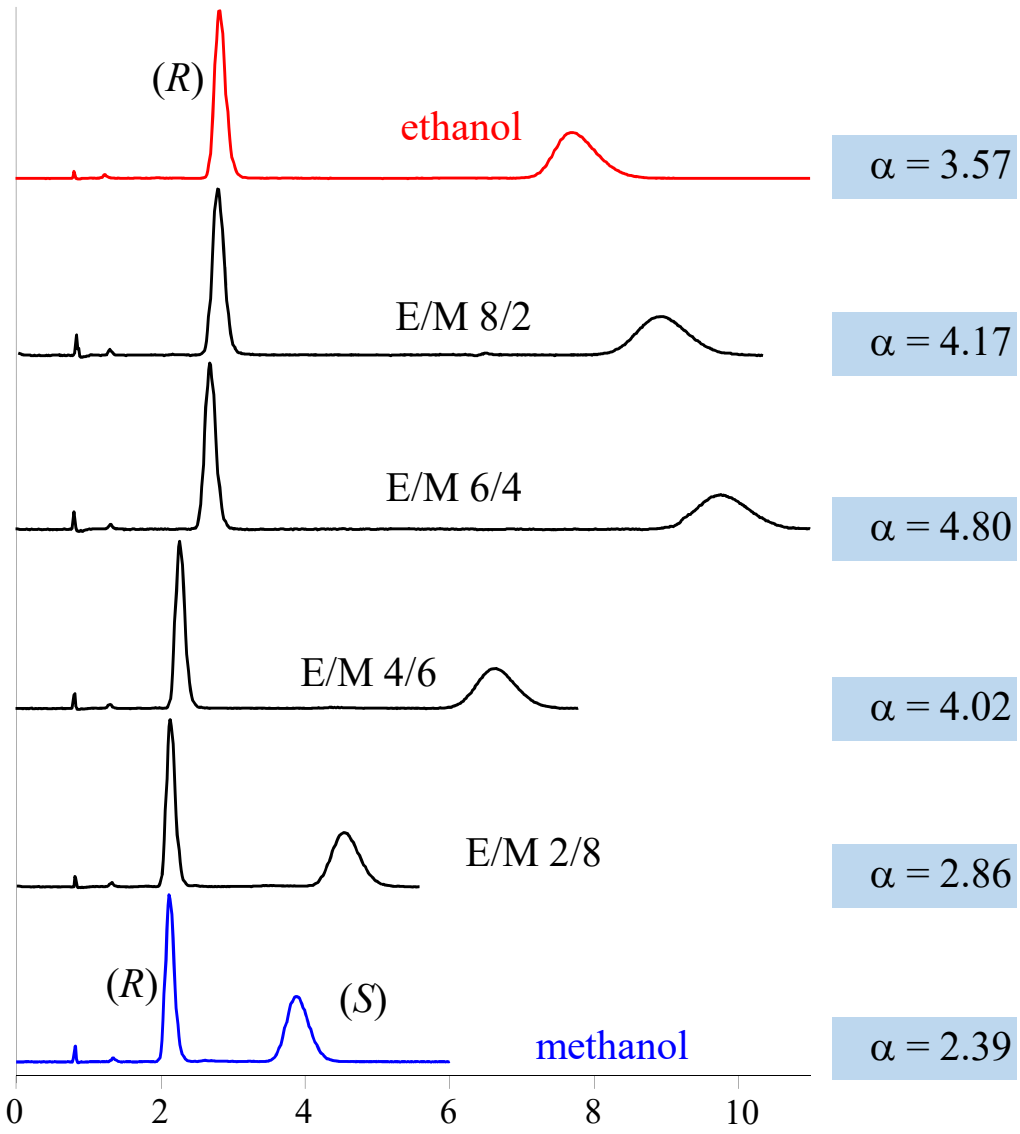
Chiralpak IE

Chiralpak AZ-H

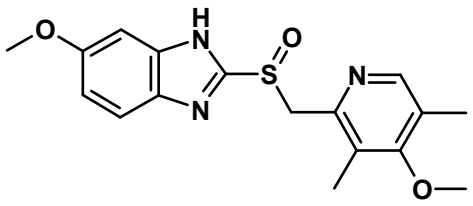


Product from E. Magnier (Versailles)

Co-solvents : synergistic effect

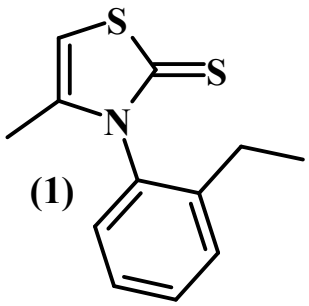


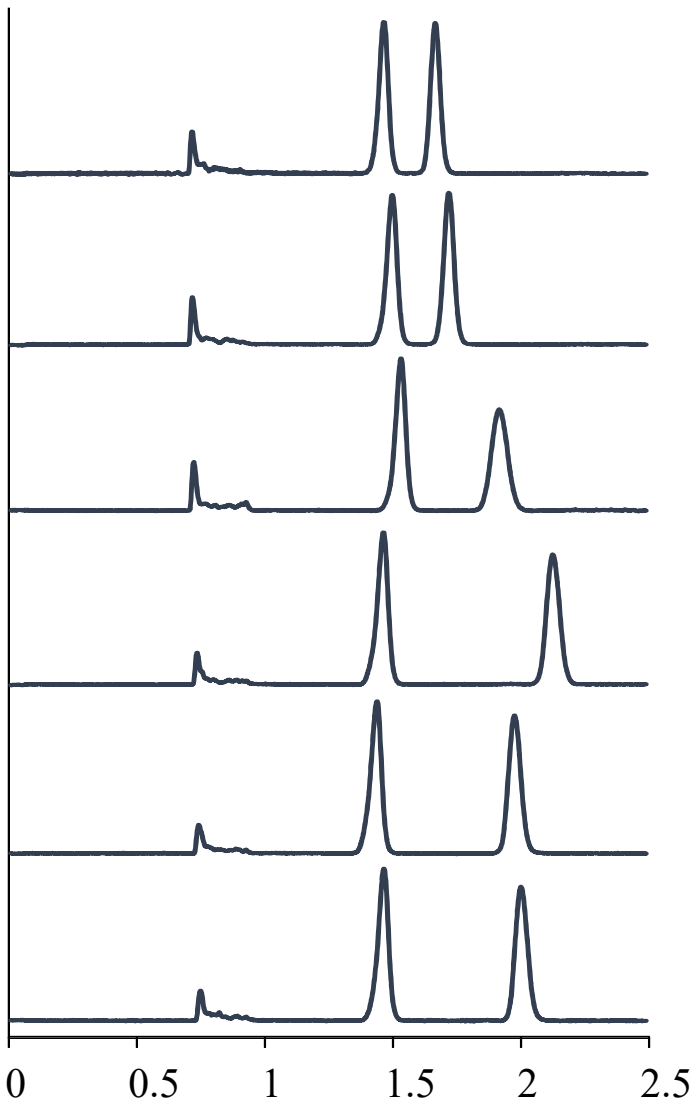
Chiralpak AY-H
CO_2/alcool 60:40
4 mL/min
35°C



CSP : memory or conditioning effect

Lux Amylose-1
CO_2/alcool 70:30
4 mL/min
30°C and 120 bars





IPA

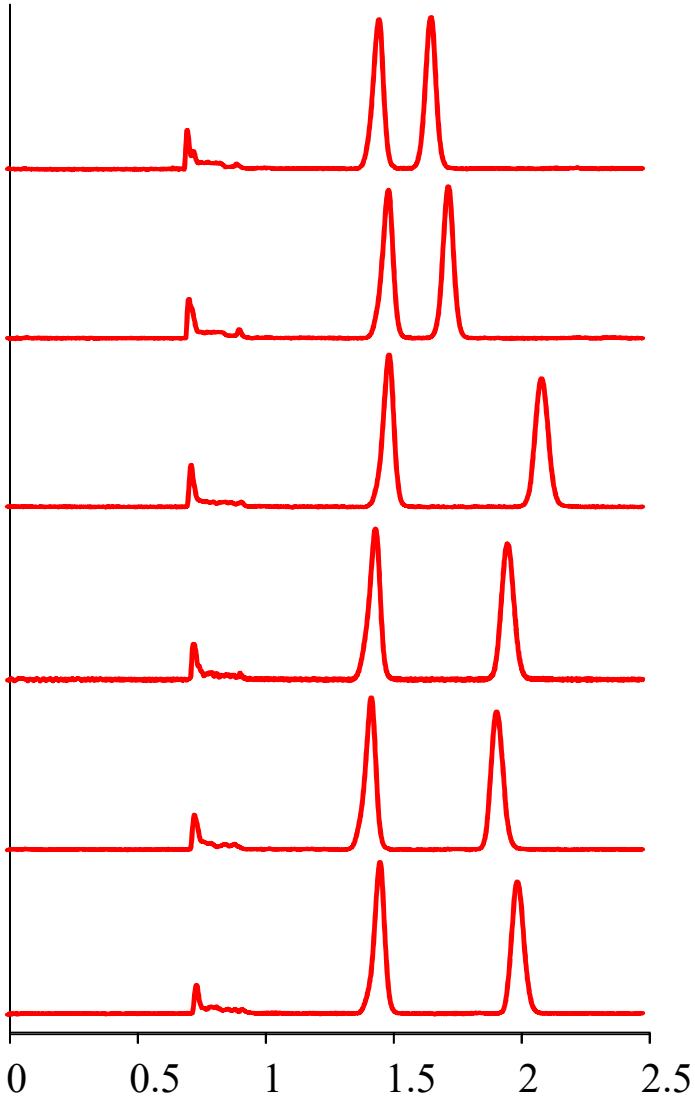
IPA/MeOH 8/2

IPA/MeOH 6/4

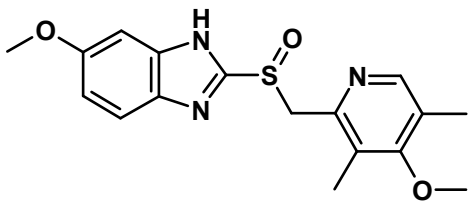
IPA/MeOH 4/6

IPA/MeOH 2/8

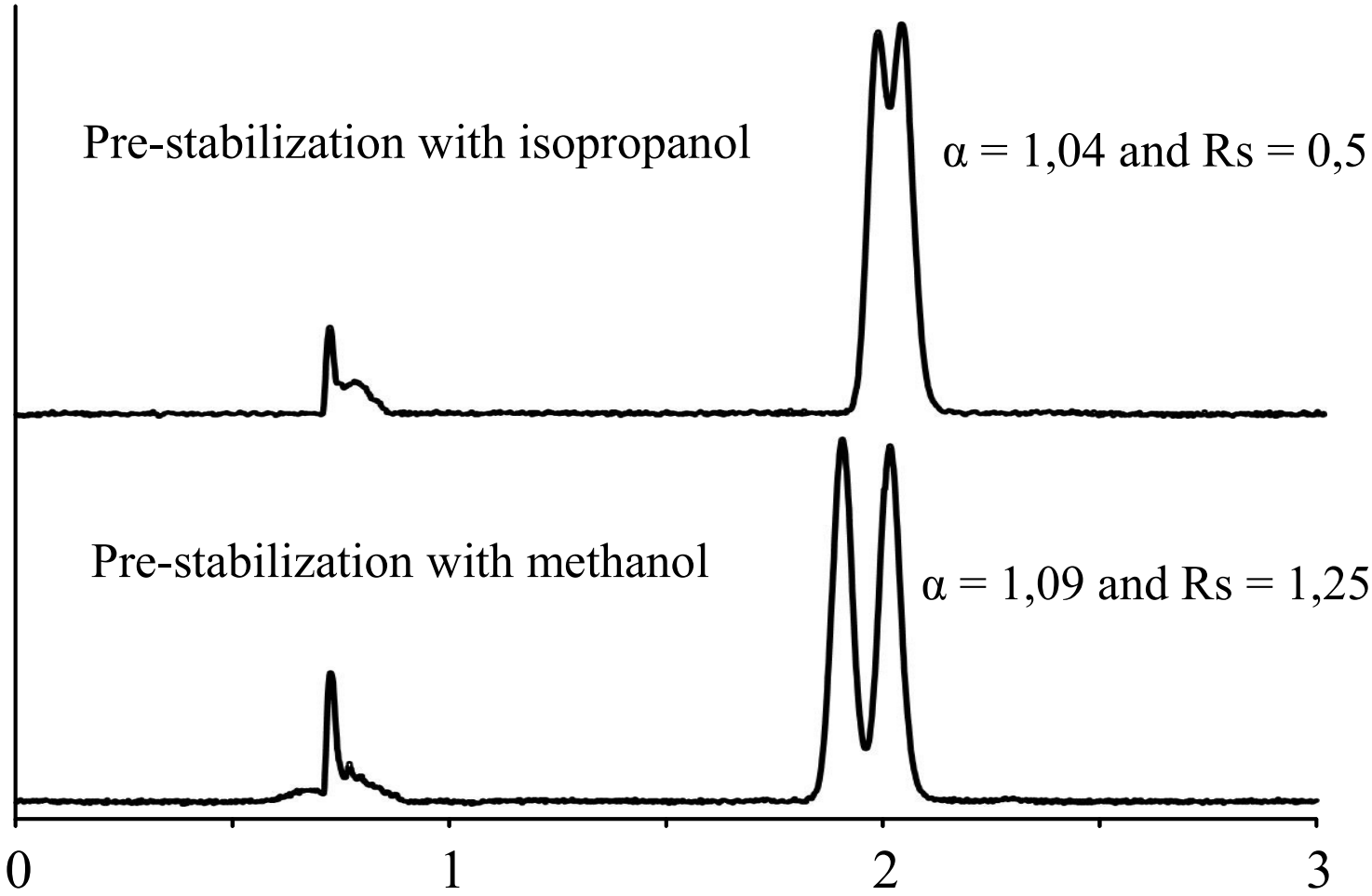
MeOH



CSP : memory or conditioning effect

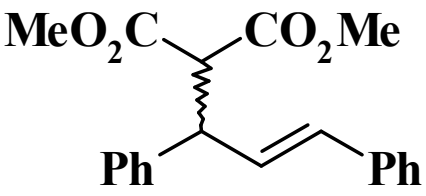


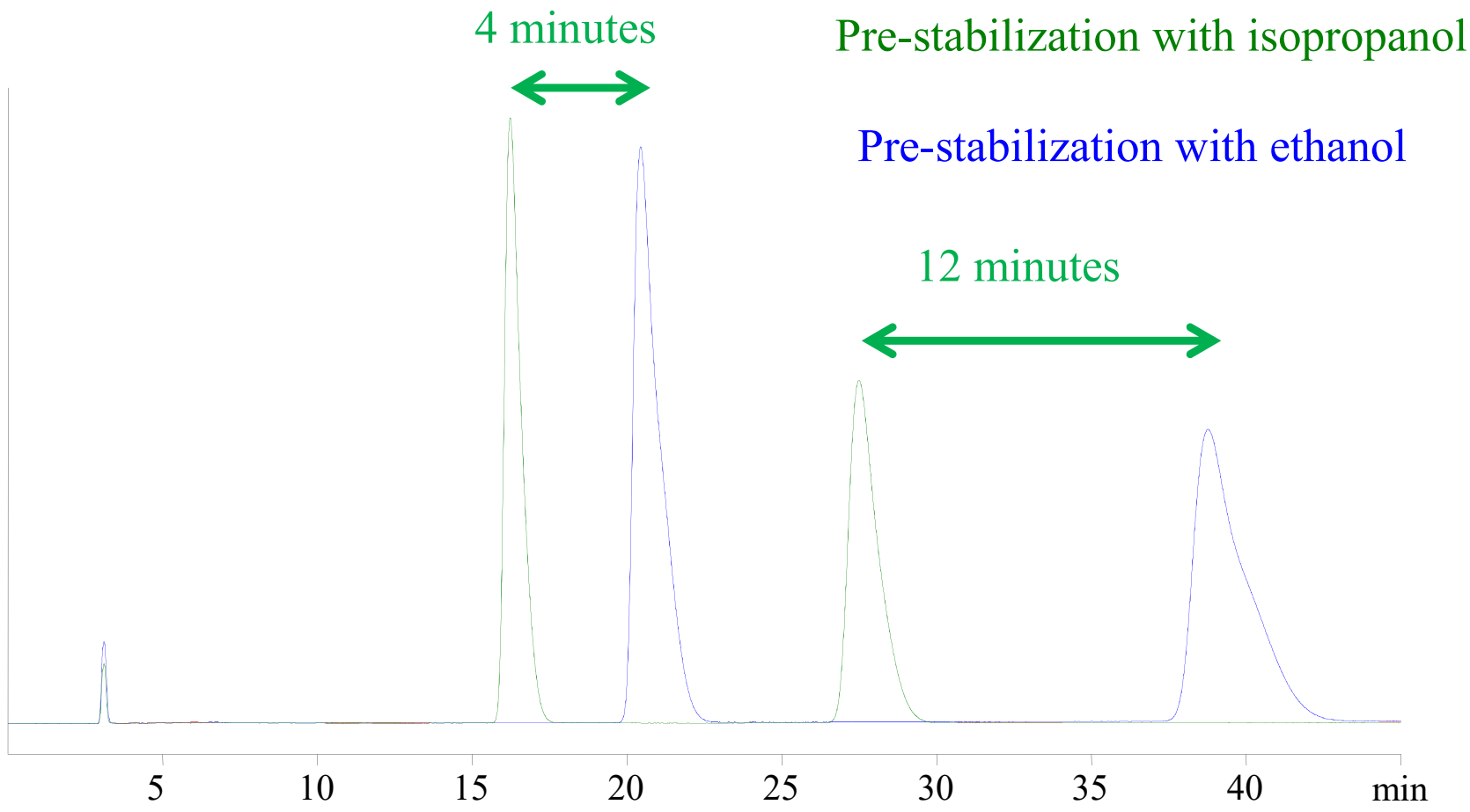
Lux Amylose-1
CO_2/ethanol 70:30
4 mL/min
30°C and 120 bars



CSP : memory or conditioning effect

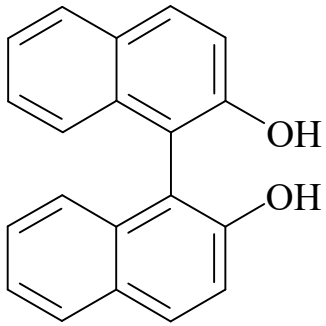
Chiralpak AD-H
Heptane / éthanol 80/20
1 mL/min et 25°C



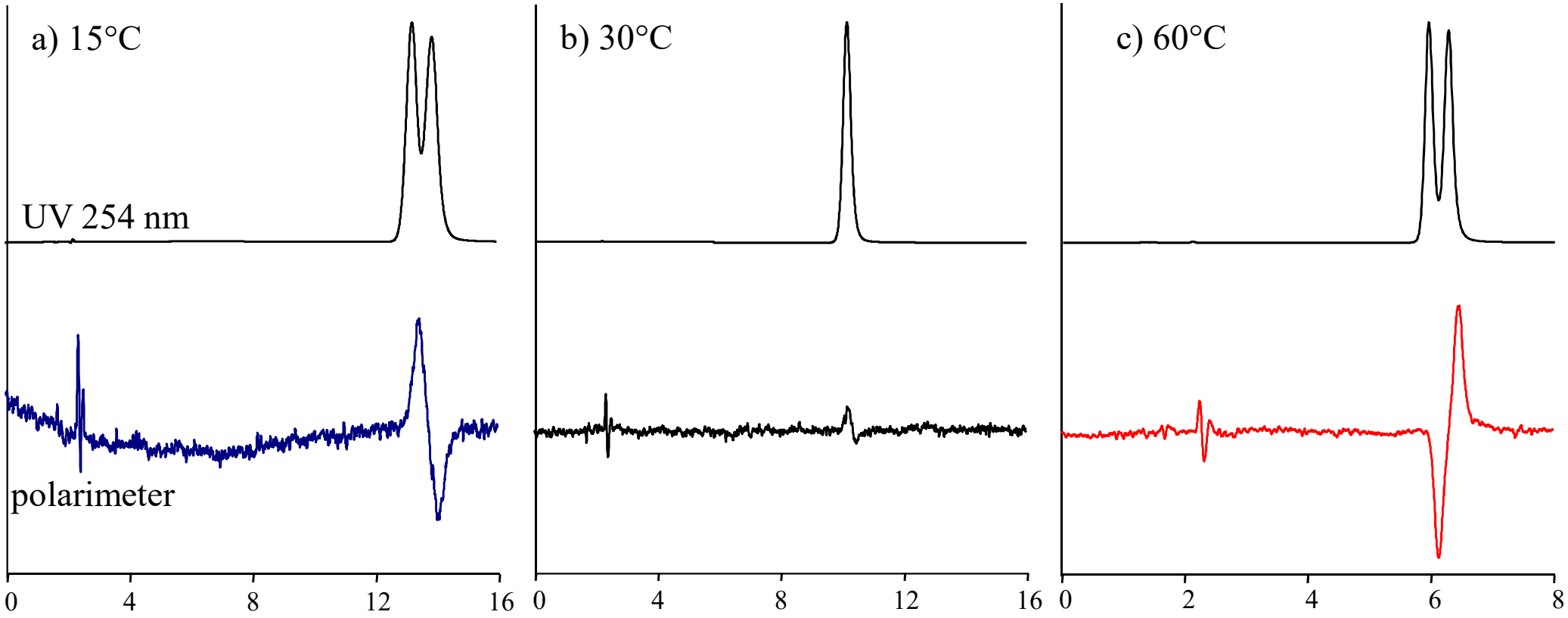


This effect is persistent, the chromatograms are reproducible for days

Isoenantioselective temperature

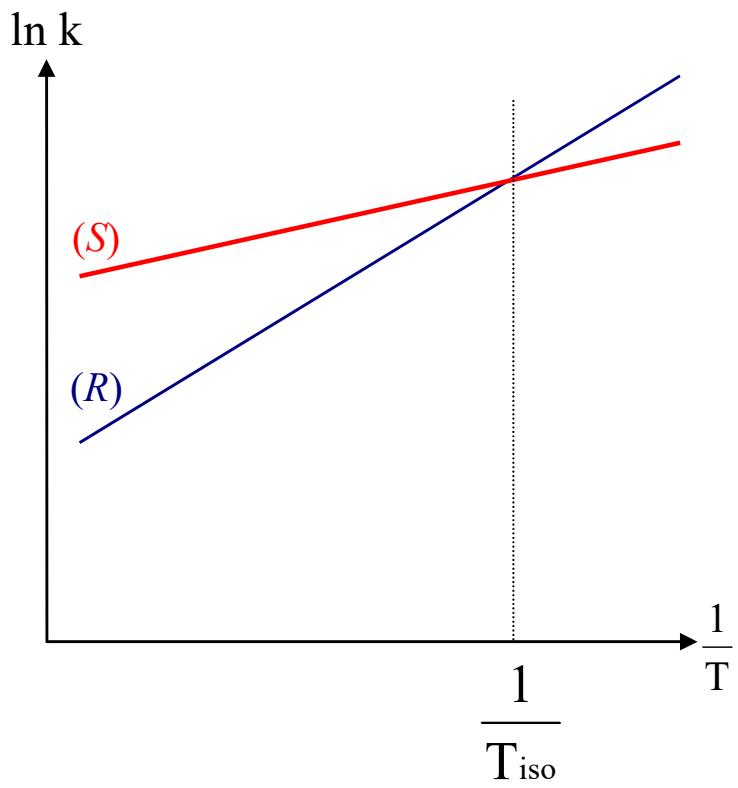


Chiralpak IA with hexane / 2-PrOH (9/1)



Temperature influence

The retention factors for each enantiomer, k_R and k_S, are dependent on enthalpy and entropy adsorptions on the chiral support, ΔH_R and ΔS_R for the (*R*)-enantiomer.

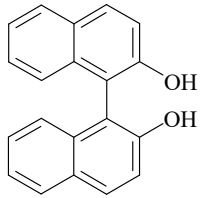


$$\ln k_R = \frac{-\Delta H_R}{RT} + \frac{\Delta S_R}{R} - \ln \beta$$

$$T_{iso} = \frac{\Delta H_R - \Delta H_S}{\Delta S_R - \Delta S_S}$$

Relationship between thermodynamic parameters for the adsorption process of enantiomers on to CSP and chromatographic parameters, β is the column phase ratio.
Accordingly, a temperature exists at which $k_R = k_S$, called isoenantioselective temperature or isoelution temperature

Temperature influence



Chiralpak IA with hexane / 2-PrOH

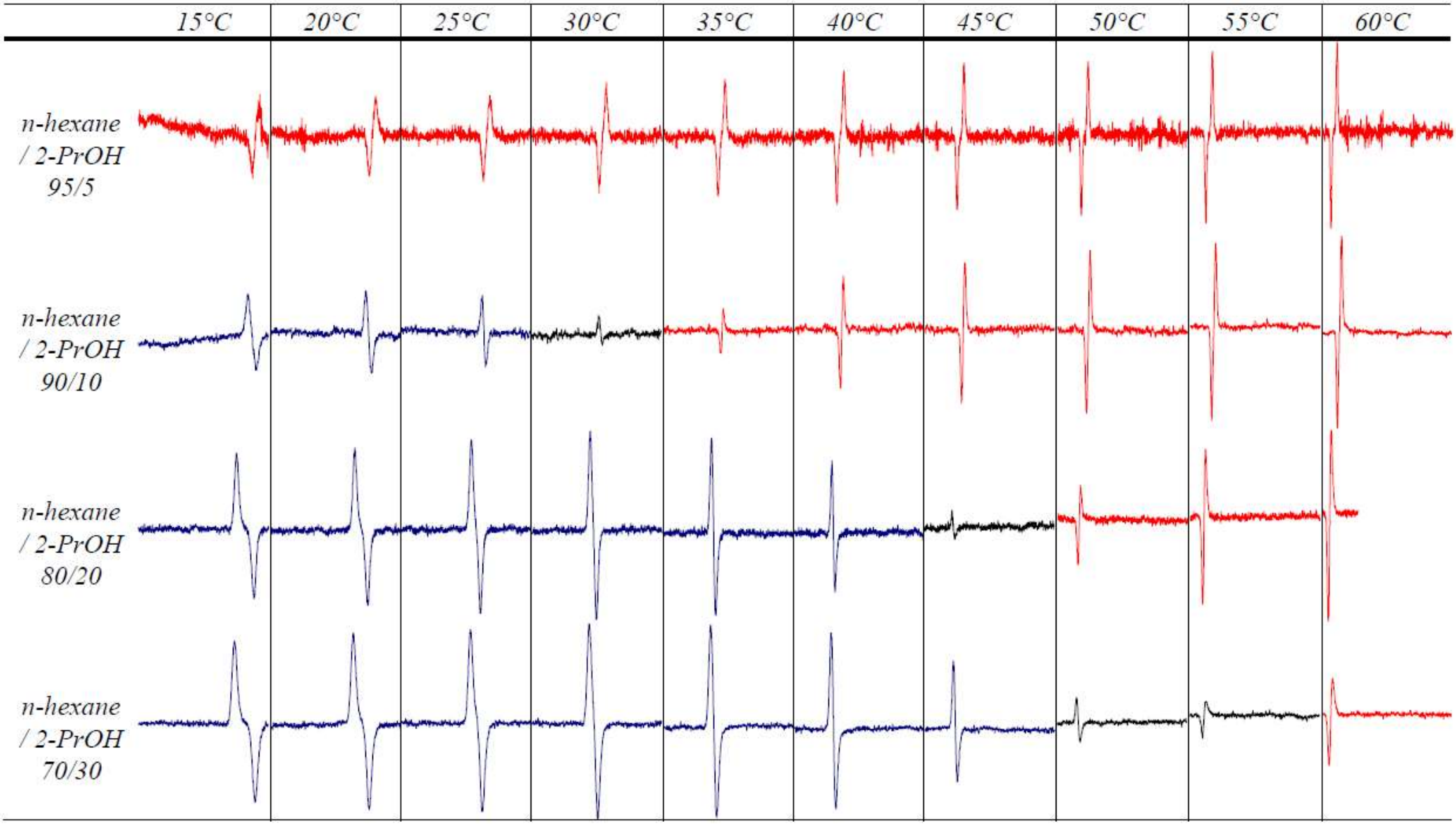
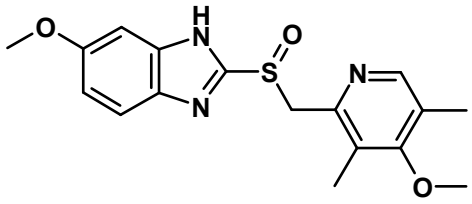


Figure 11. Polarimetric traces (OR-1590) for binaphthol on Chiralpak IA at different temperature and different percentage of 2-PrOH in the mobile phase; in blue : (*R*) enantiomer first eluted; in red (*S*) enantiomer first eluted.

Temperature influence



Chiralpak IE
polarimeter

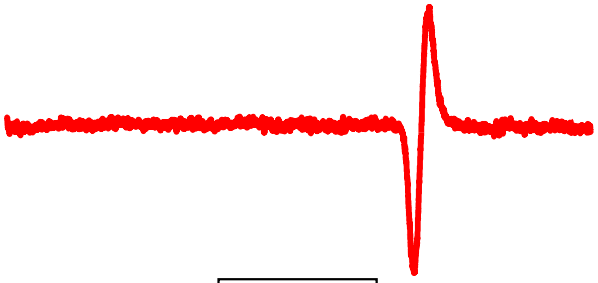
Temperature and co-solvent proportion allow the reversal of elution order

Heptane / éthanol 8/2 2 mL/min

Heptane / éthanol 5/5 1 mL/min

Heptane / éthanol 3/7 1 mL/min

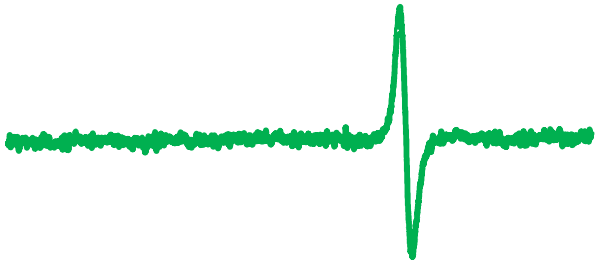
Heptane / éthanol 2/8 1 mL/min



25°C

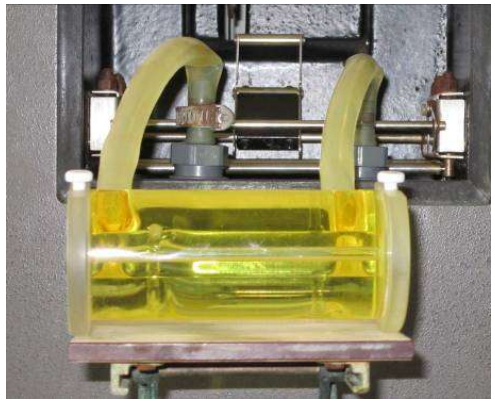
éthanol 0,5 mL/min

35°C



45°C

Differences between a bench polarimeter and a polarimetric detector



Thermostated cell

Path length = 10 cm

Volume = 1 mL

Concentration : 10 mg/mL

Lamps : Na, Hg

Specific rotation at a given wavelength : 589, 578, 546, 436, 365 nm

---

Thermostated cell

Path length = 2,5 cm

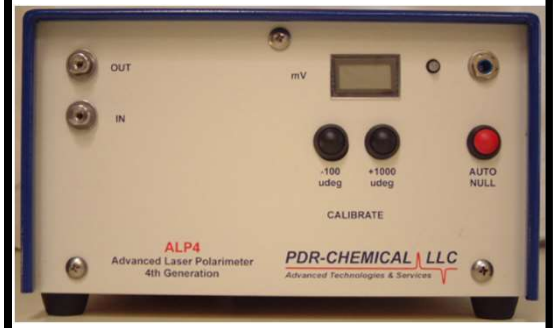
Volume = 44 µL

Concentration : 0,05 mg/mL

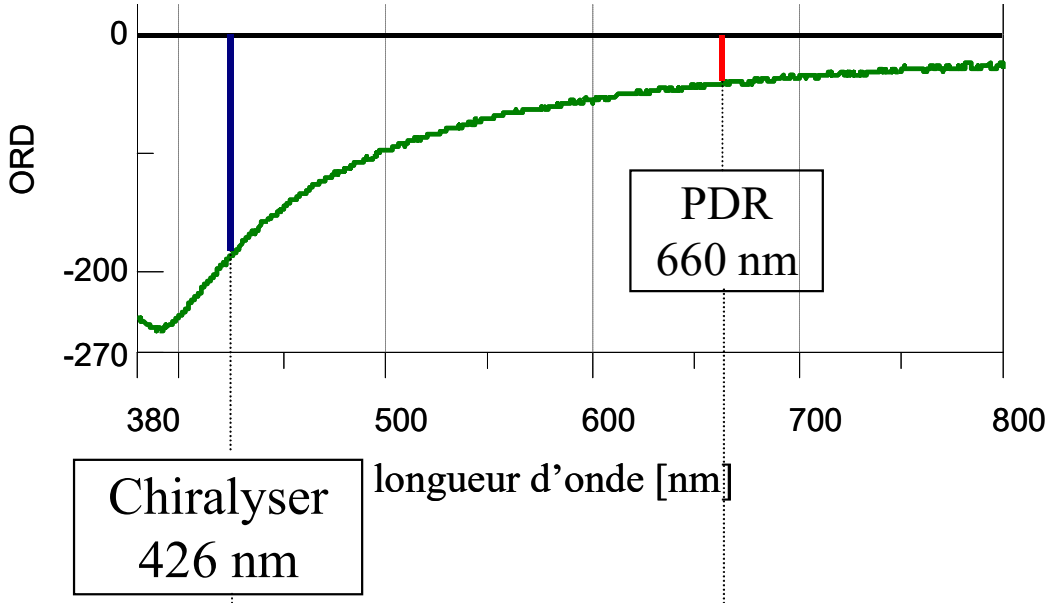
Lamps : Hg-Xe

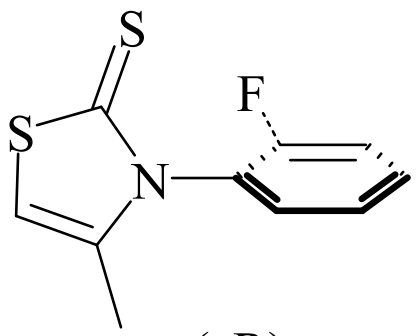
Spectral range: 350-900 nm

Polarimeters as on-line detectors

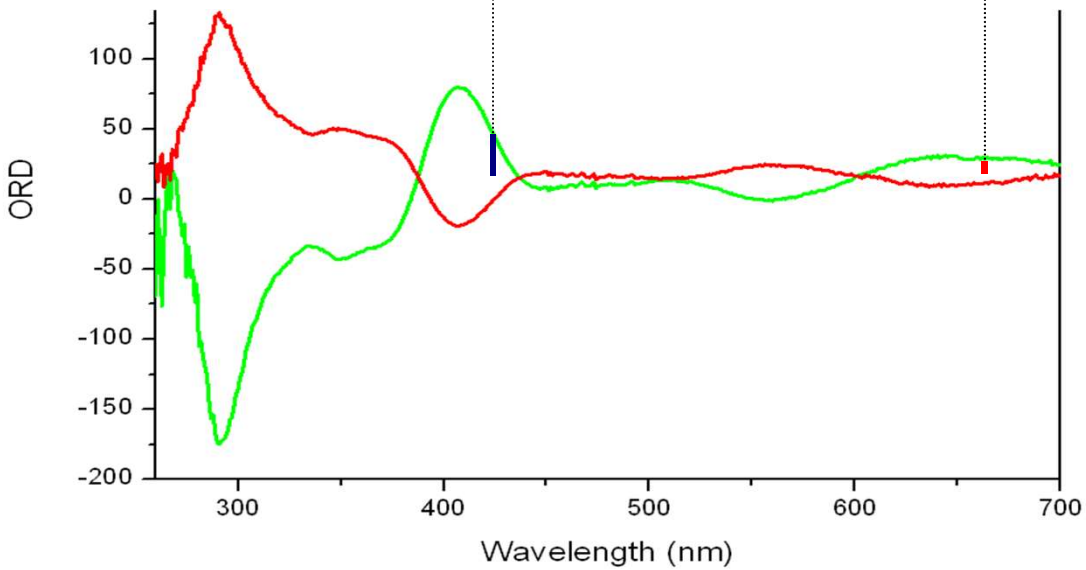
			
Model	ALP	Chiralyser	OR-1590 OR-2090
Supplier	PDR (USA)	IBZ Messtechnik (Germany)	Jasco (Japan)
Source	Laser	LED	Lamp Hg-Xe
λ (nm)	660 nm	426 nm	350-900 nm

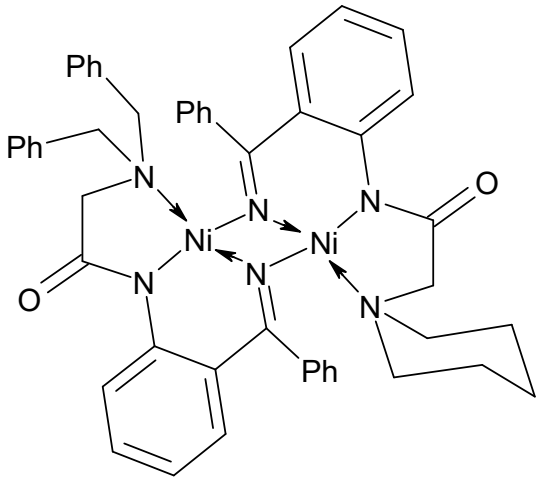
ORD: Variation of the optical rotation with the wavelength



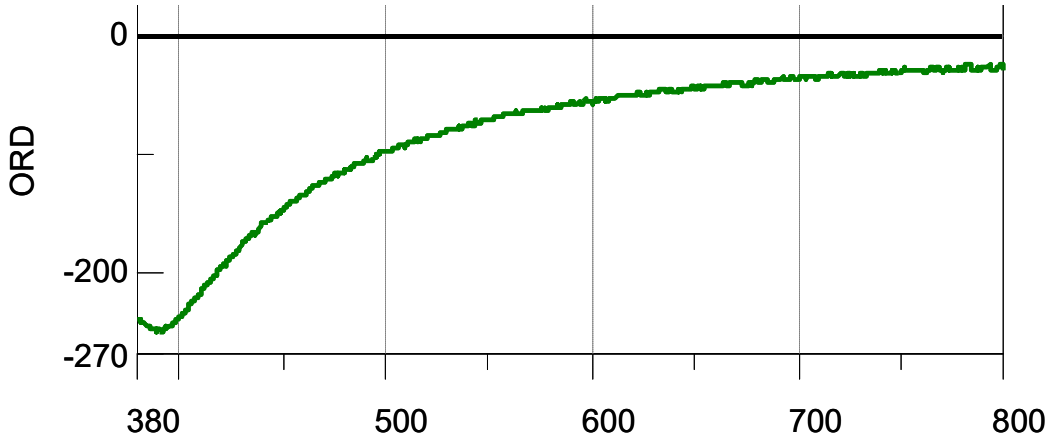


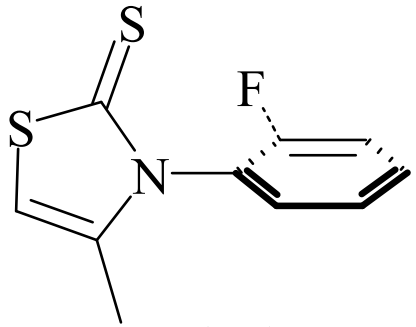
(aR)





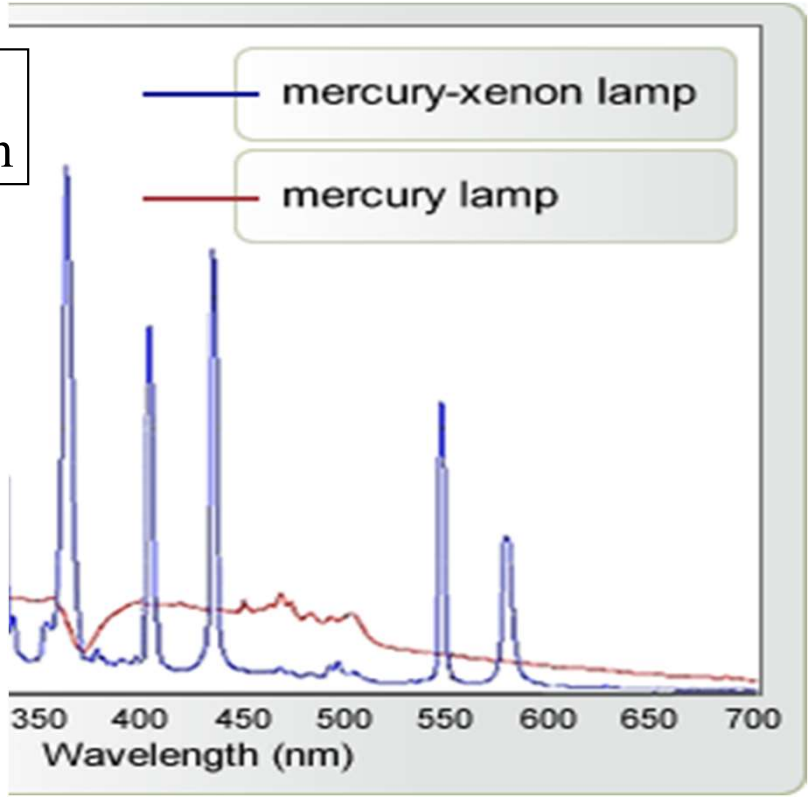
Soloshonok et al., *J. Am. Chem. Soc.* 2010, *132*, 10477

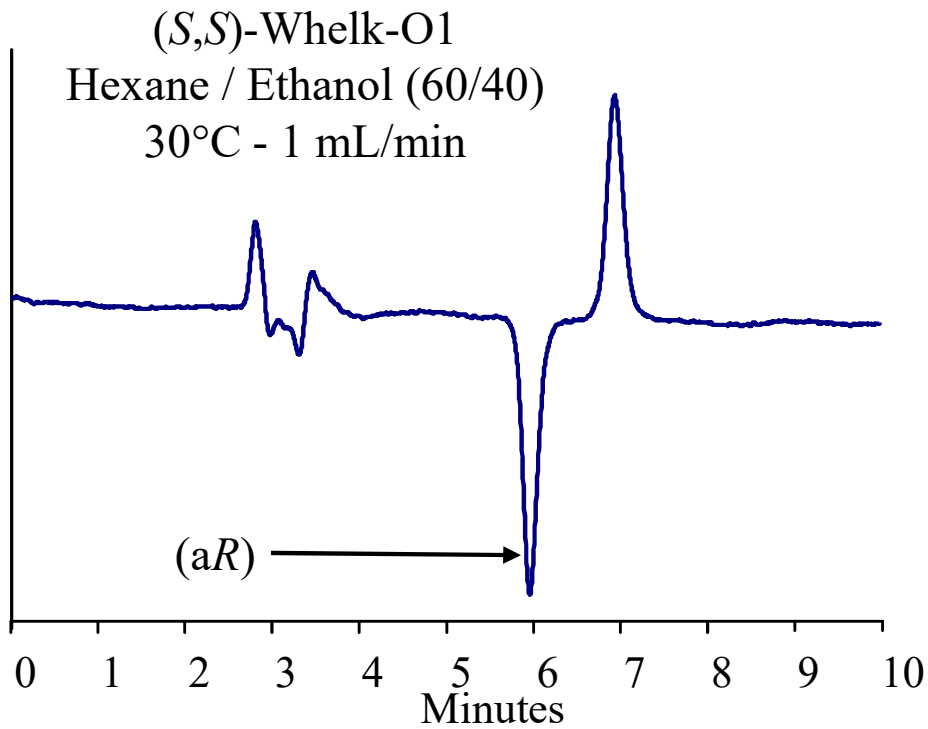


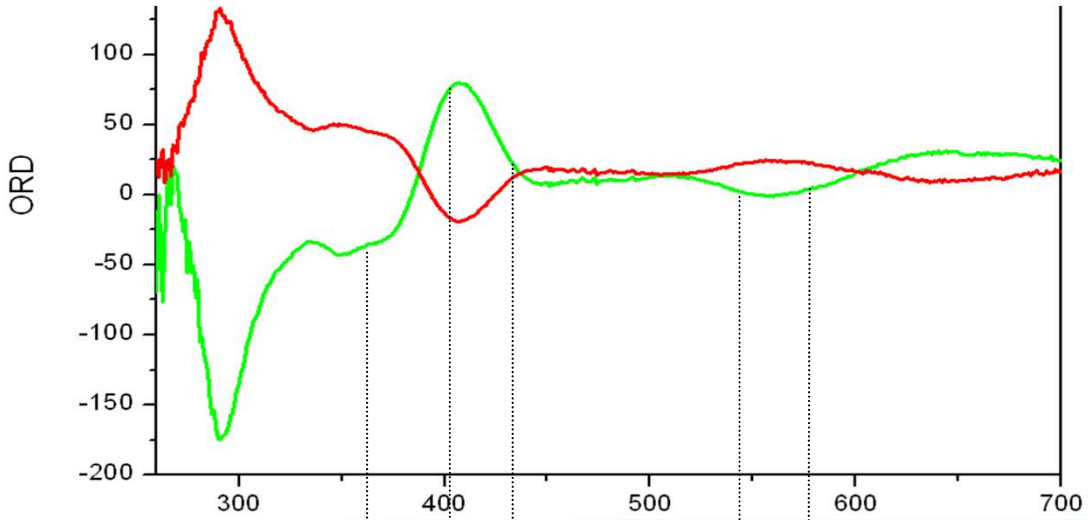


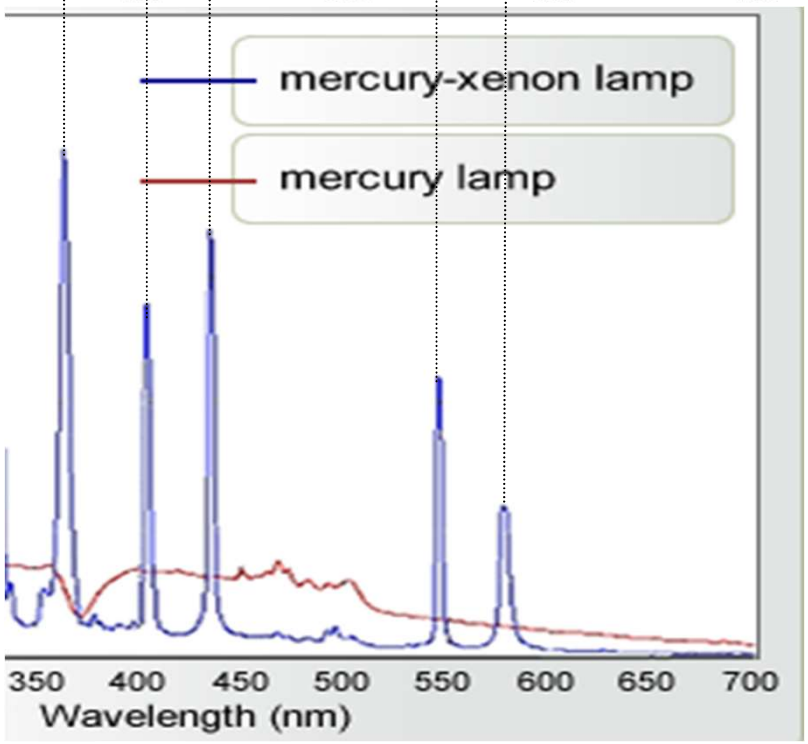
(aR)

Jasco
350-900 nm



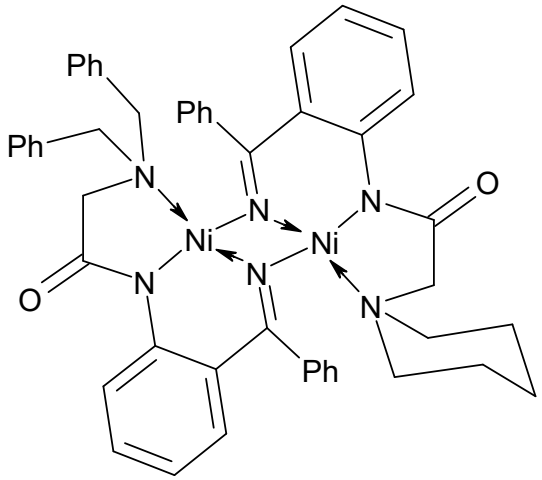


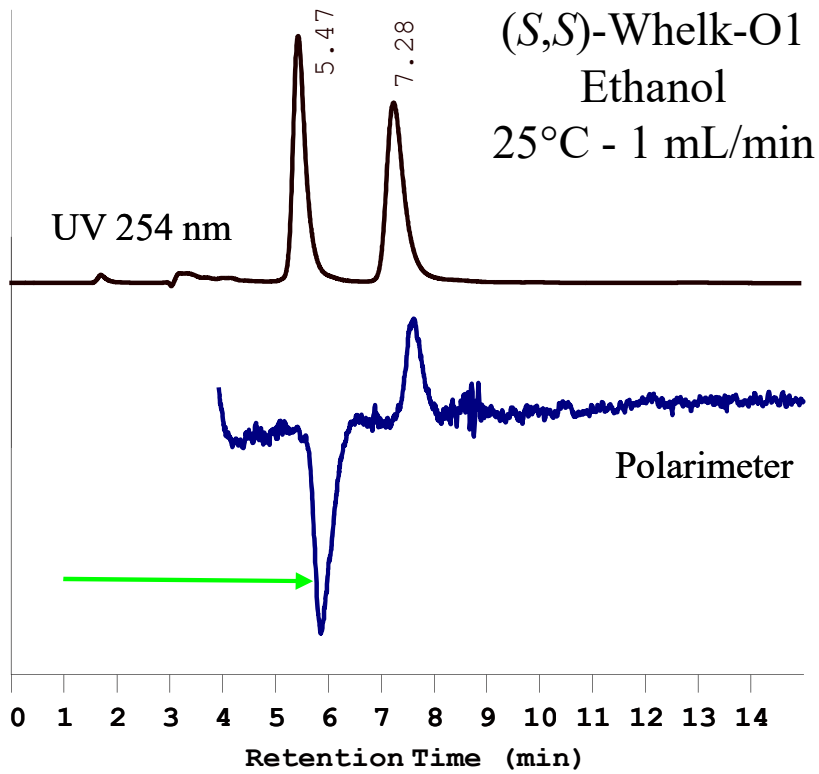




Jasco
350-900 nm

Sum of positive and negative contributions



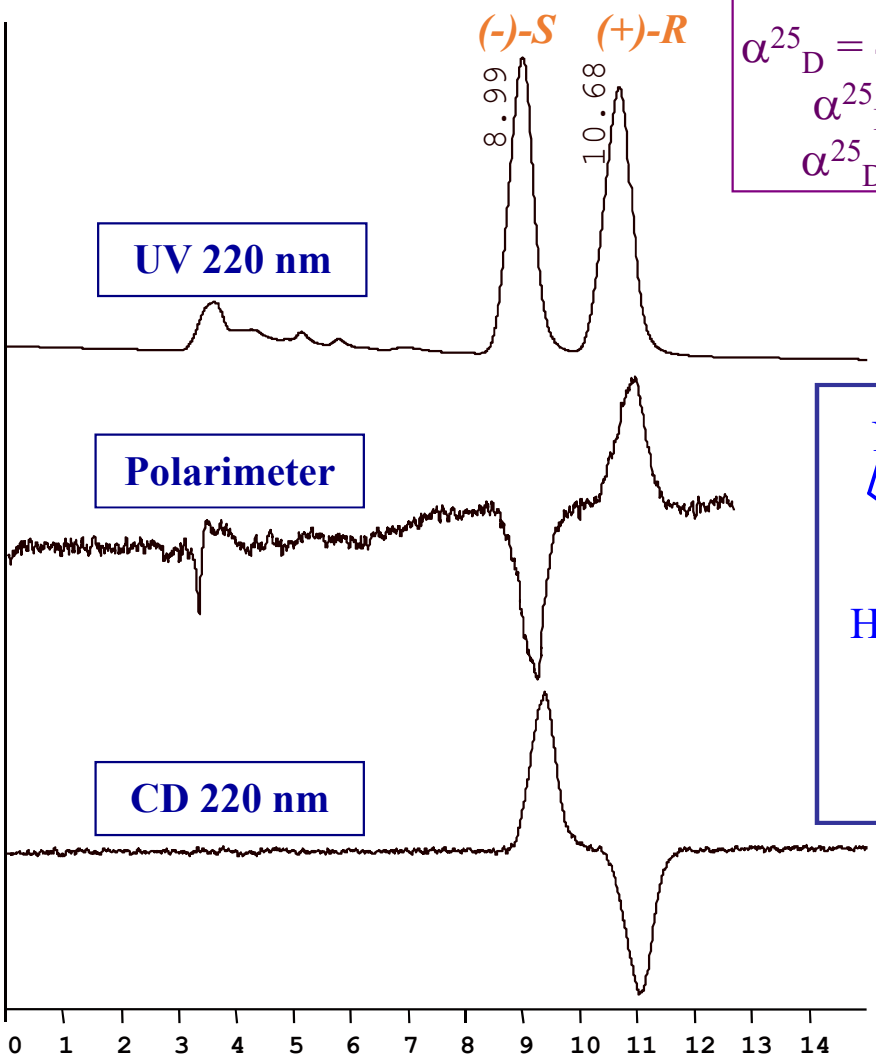


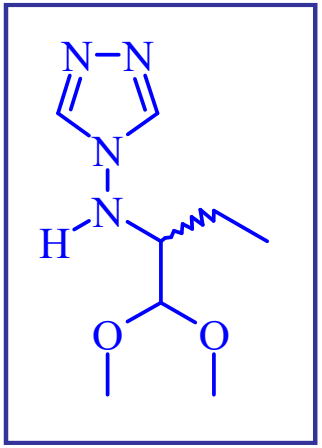
Influence of the solvent on the specific rotation

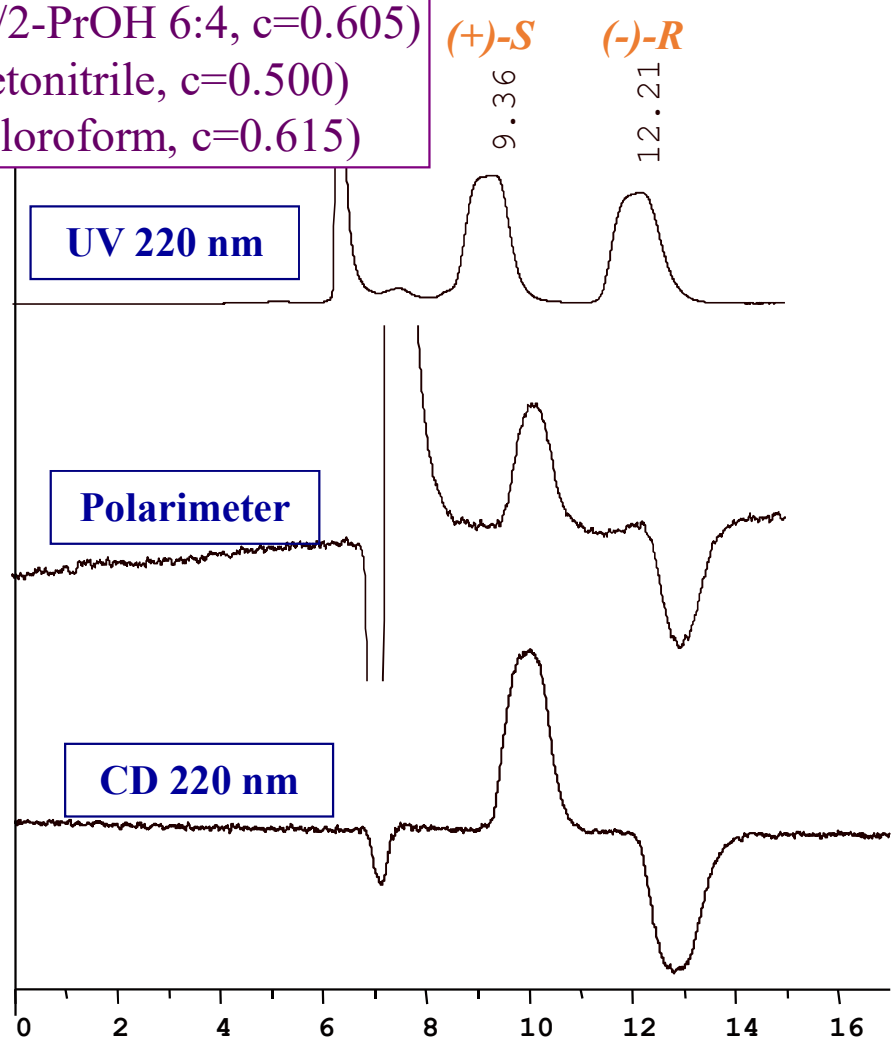
Chiralcel OD-H
hexane/2-PrOH 6:4 / 1 mL/min

For the (*S*) enantiomer :
α^{25}_{D} = **-4.55** (hexane/2-PrOH 6:4, c=0.605)
α^{25}_{D} = **+4.72** (acetonitrile, c=0.500)
α^{25}_{D} = **+18.37** (chloroform, c=0.615)

Chiralcel OD-R
Acetonitrile / 0.5 mL/min



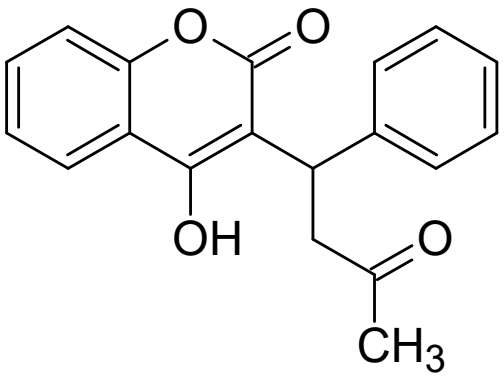


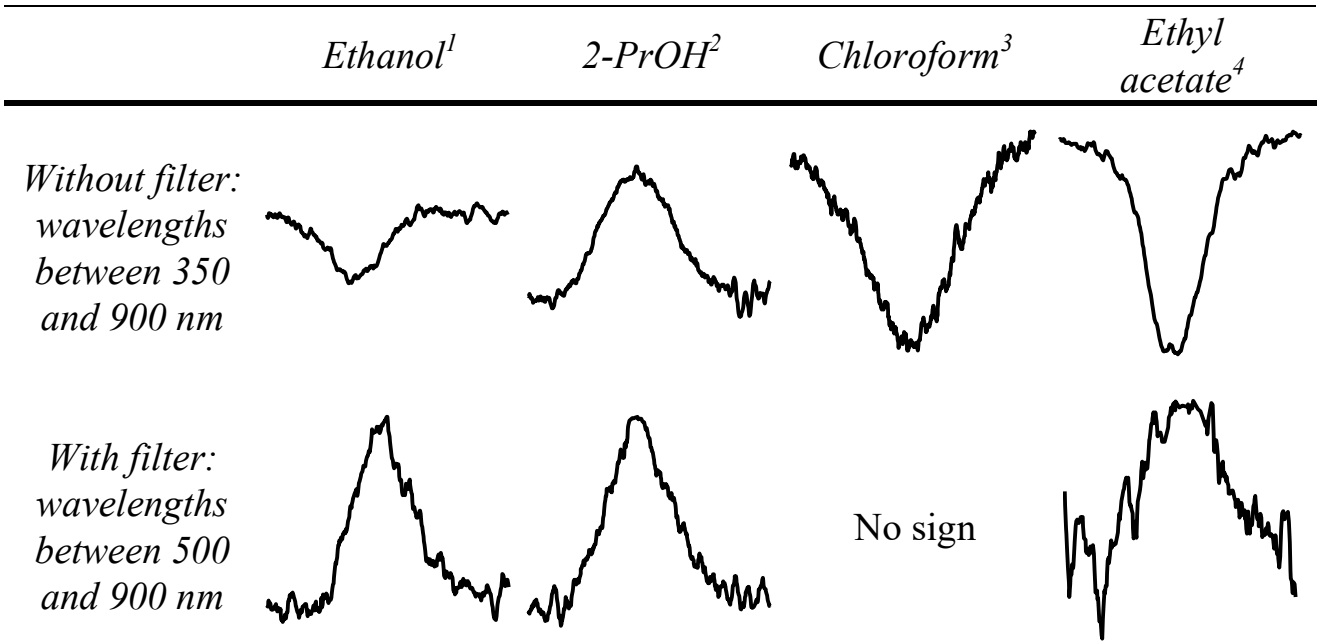


Product from Muriel Albalat (Marseille)

Influence of the solvent on the specific rotation

	NaOH (0.5N)	*Ethanol*	*2-PrOH*	*Chloroform*	*Ethyl acetate*
589 nm	- 144	+ 8.4	+ 17	- 19	+ 7.3
578 nm	- 149	+ 9.0	+ 18	- 20	+ 7.5
546 nm	- 174	+ 9.5	+ 20	- 24	+ 7.1
436 nm	- 338	+ 5.3	+ 31	- 52	- 6.6
365 nm	nd	- 51	+ 18	- 133	- 110

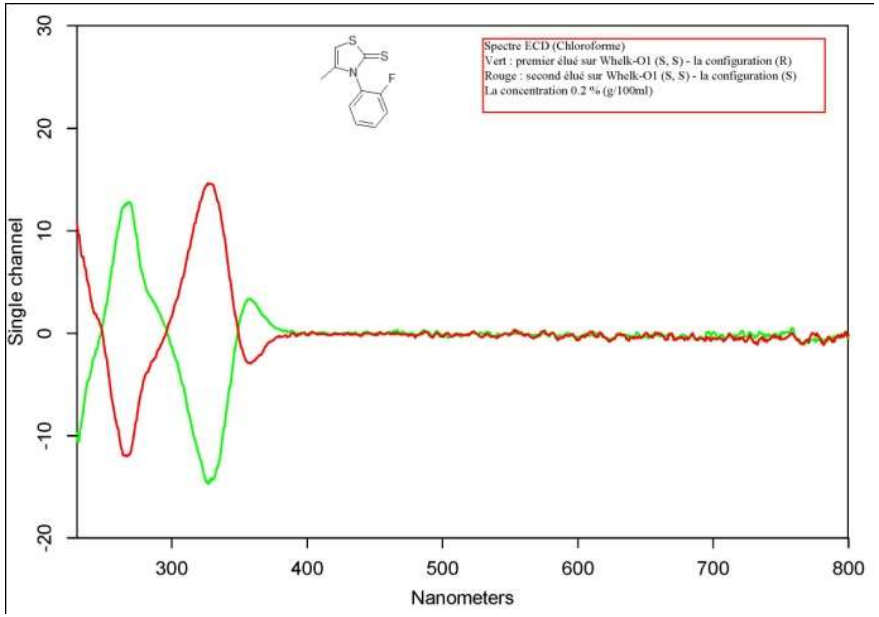




Polarimetric traces (OR-1590, Chiralpak IC) obtained for (*S*)-warfarin
1) n-hexane / ethanol (7/3); 2) n-hexane / 2-PrOH (1/1)
3) n-hexane / ethanol / chloroform (25/5/70); 4) n-hexane / ethyl acetate (1/1)

ECD

170 – 900 nm



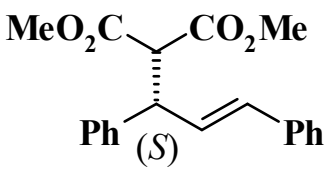
Thermostated cell

Path length = 2,5 cm

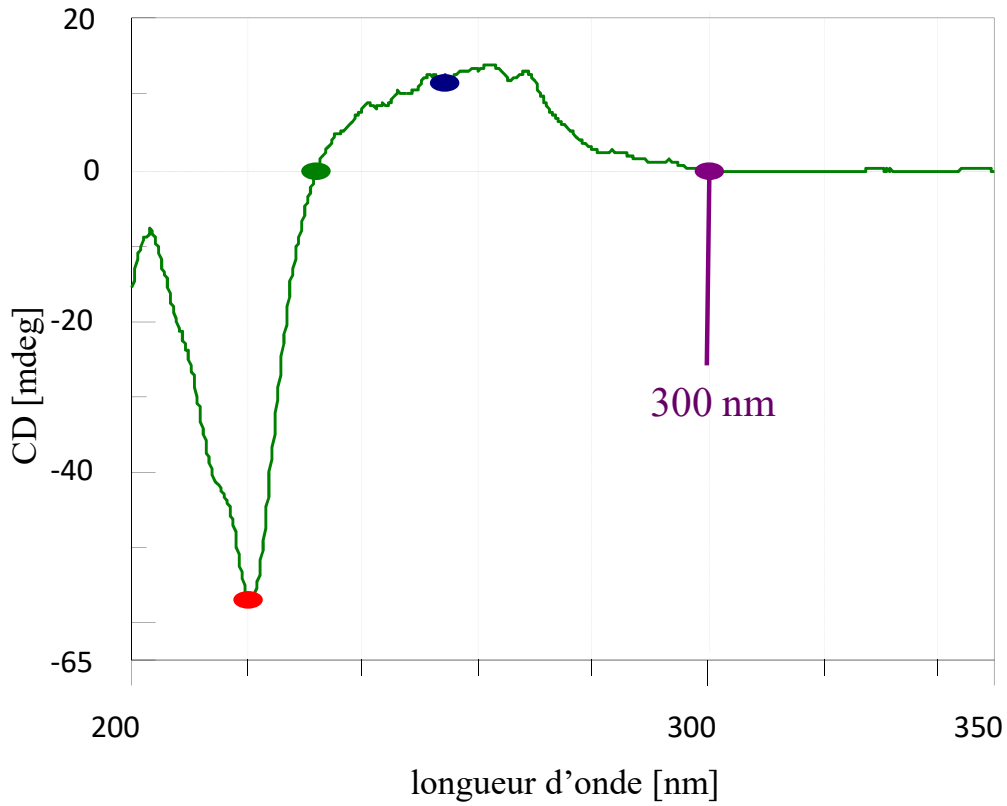
Lamps : Hg-Xe

220-420 nm

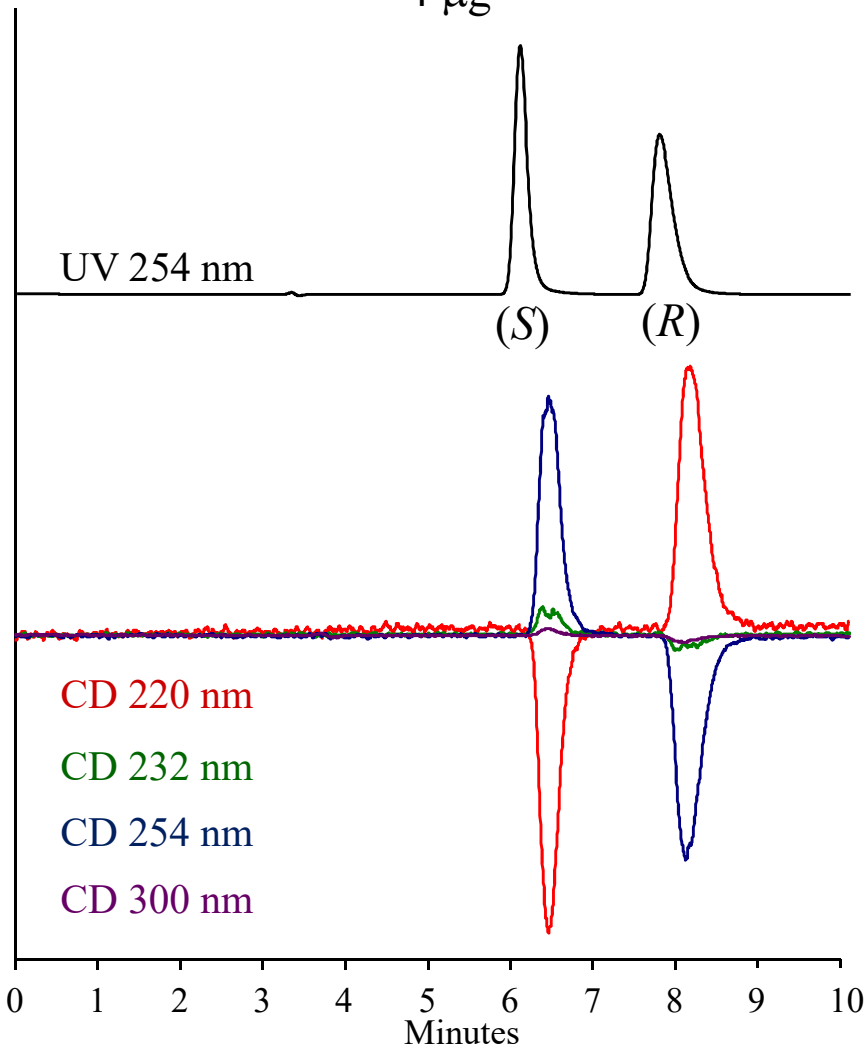
Correlation between ECD spectrum and chiroptical chromatogram



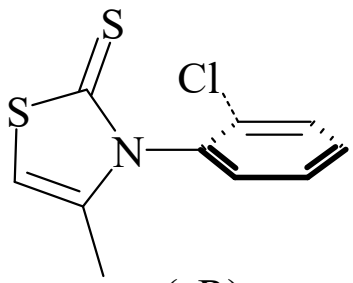
ECD spectrum in methanol for the first eluted enantiomer



Chiralpak ID
Hexane / 2-PrOH (30/70)
30°C - 1 mL/min
4 µg



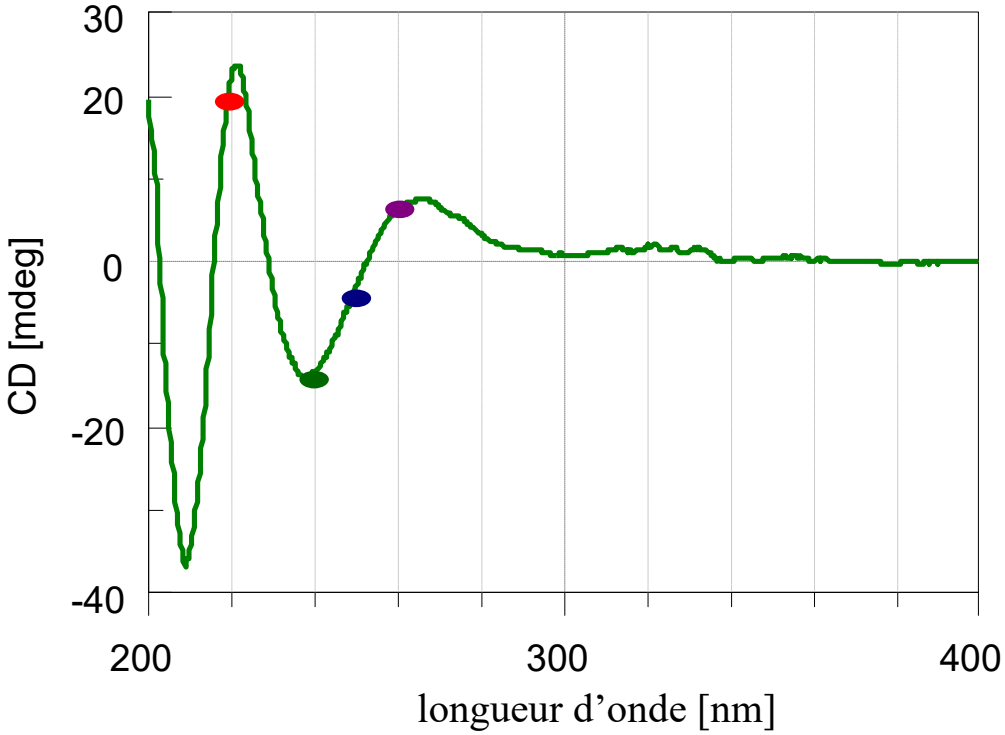
Correlation between ECD spectrum and chiroptical chromatogram

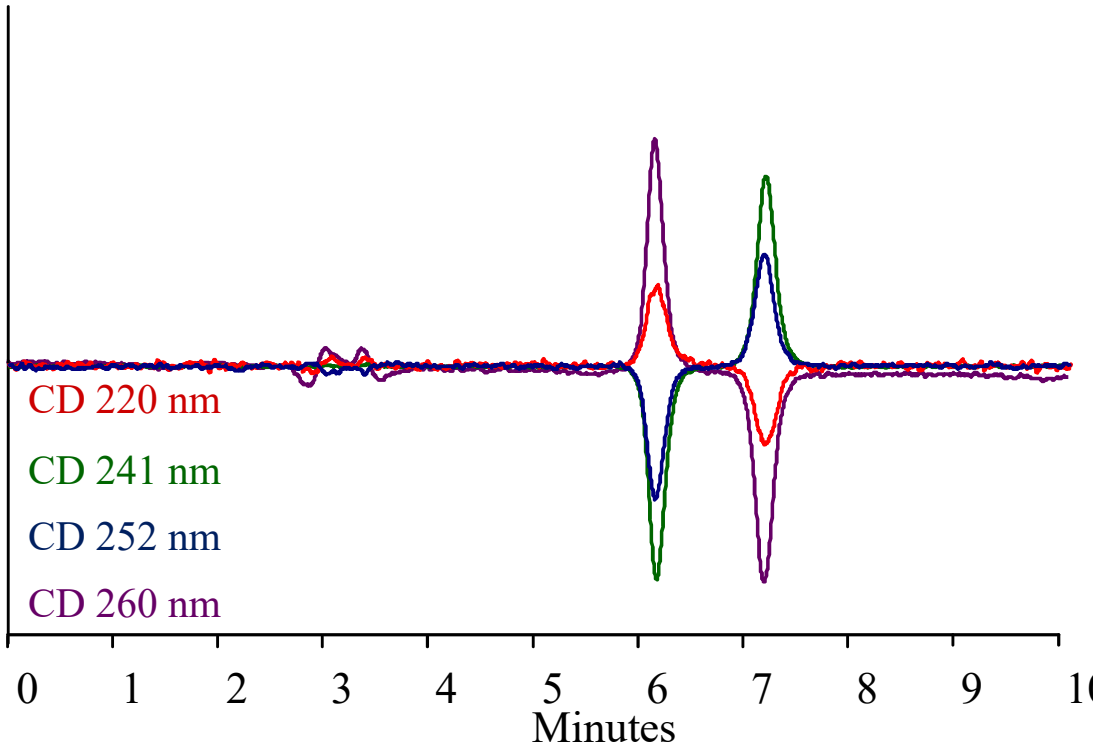


(aR)

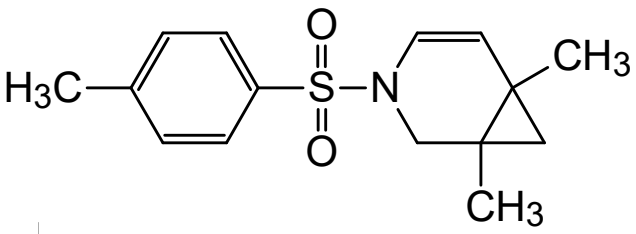
ECD spectrum in chloroform for the first eluted enantiomer

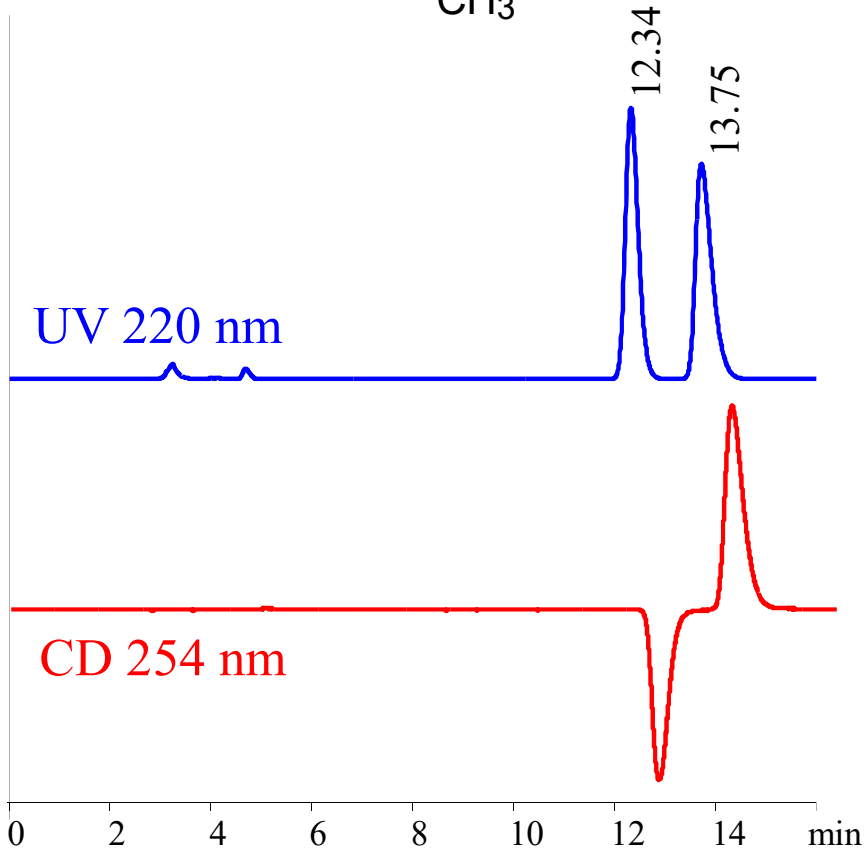
(*S*,*S*)-Whelk-O1
Hexane / Ethanol (60/40)
30°C - 1 mL/min

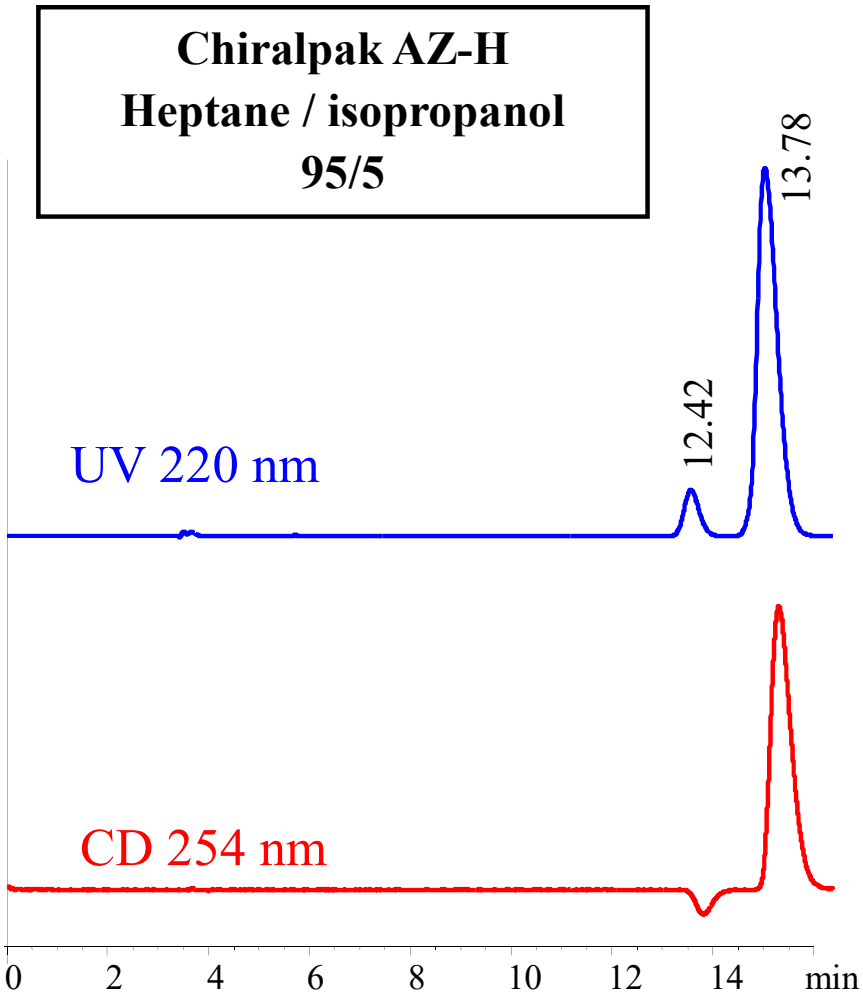




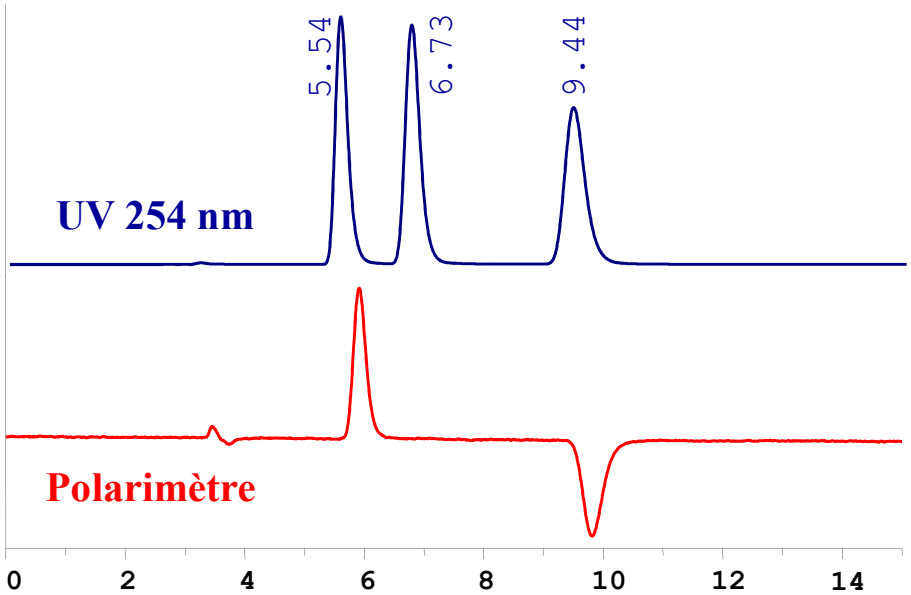
Proof of the enantiomeric ratio



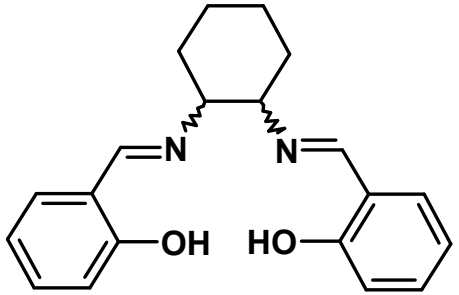


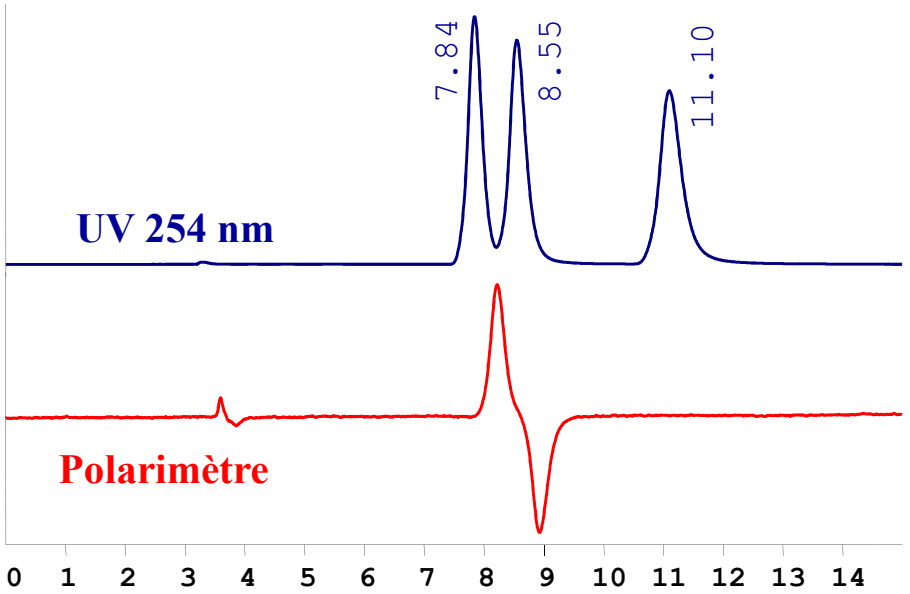


Chiroptical detection



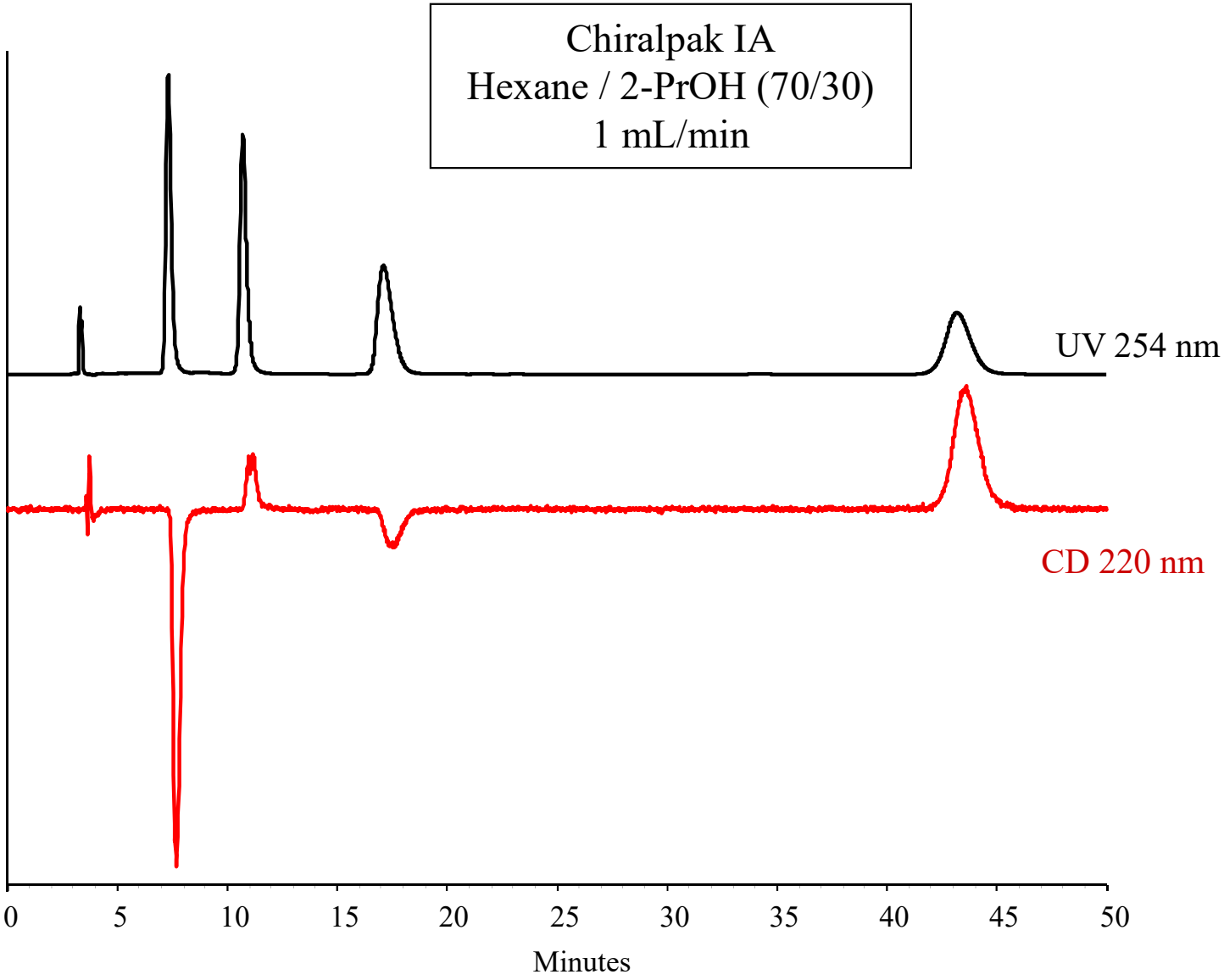
Chiralcel OD-H
Hexane/2-PrOH 90/10
1 mL/min - 25°C

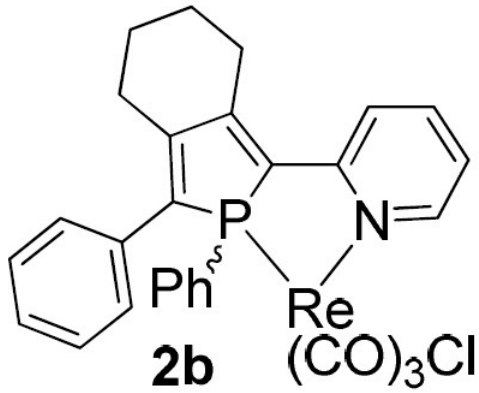




SUMICHIRAL OA-2500
Hexane/2-PrOH 90/10
1 mL/min - 25°C

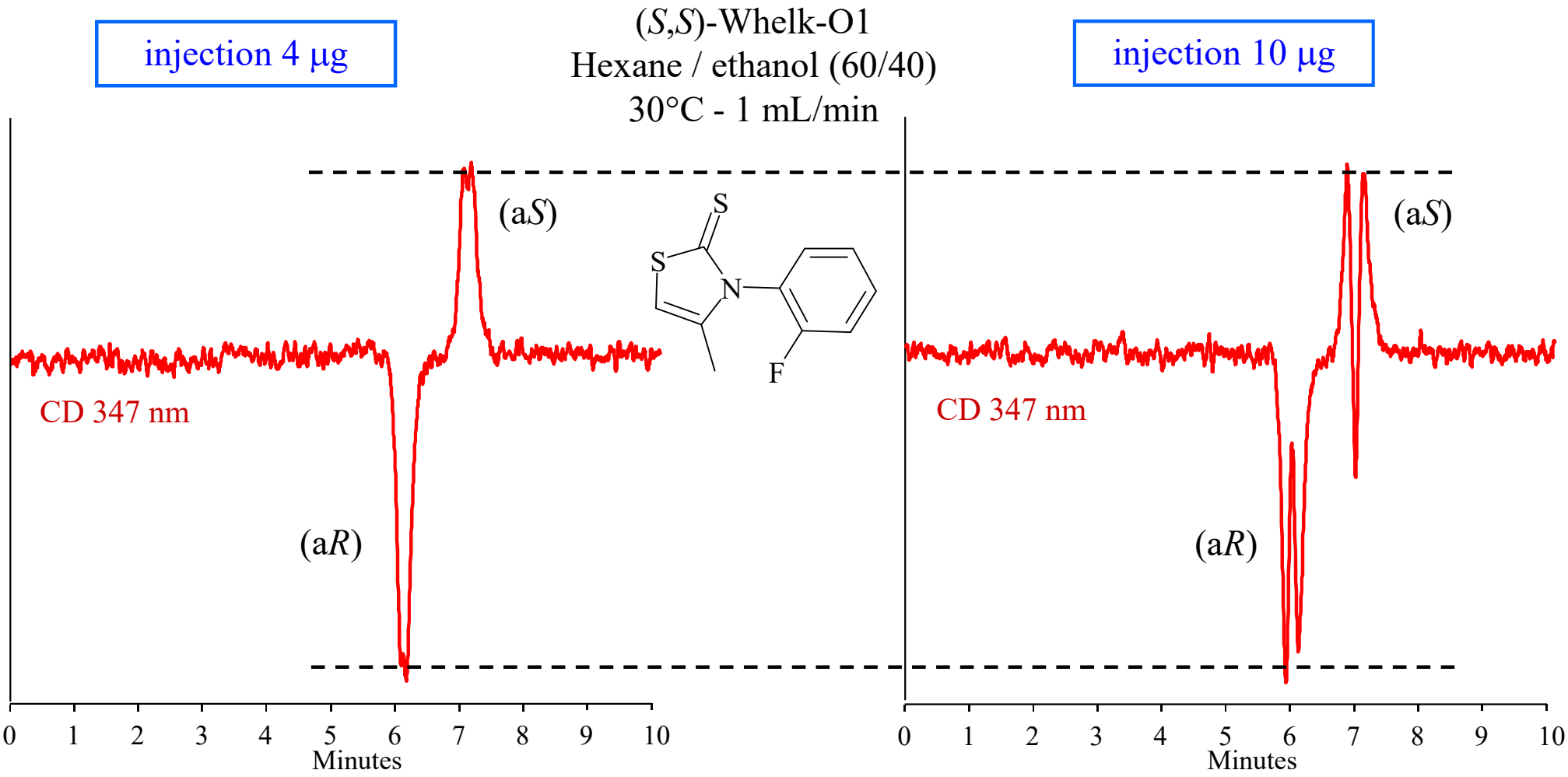
Assignment of diastereomeric pairs





Tazacs et al., *Chem. Comm.* 2012,

Detector saturation

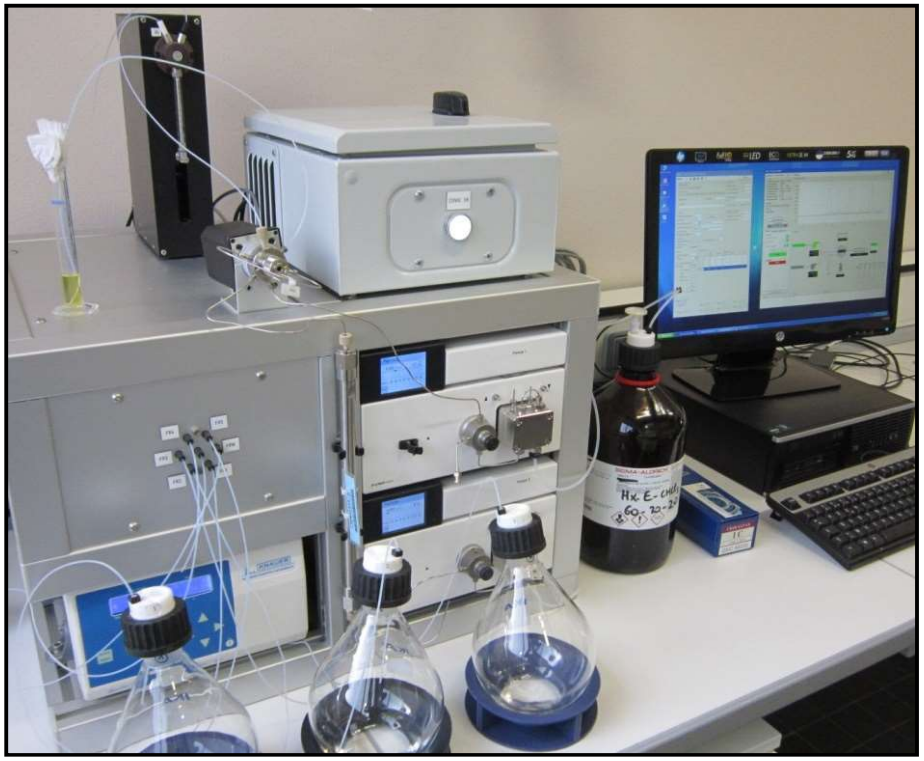


Preparative separation

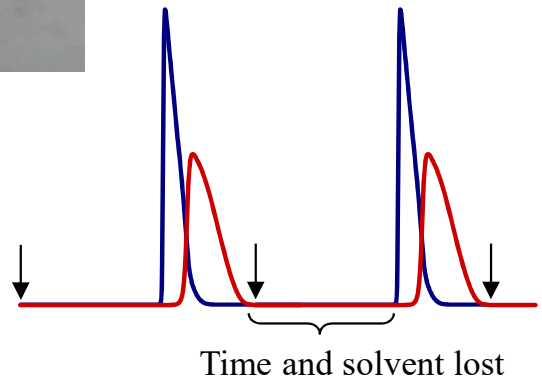
In the laboratory, we use multiple injections on columns of 1 cm in diameter. We can collect from a few milligrams to 1 gram of each enantiomer per day depending on the product.

The limiting factors are:

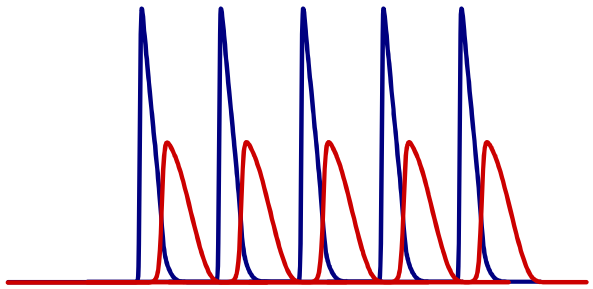
- Solubility of the product in the mobile phase
- The loading capacity of the column
- Racemization



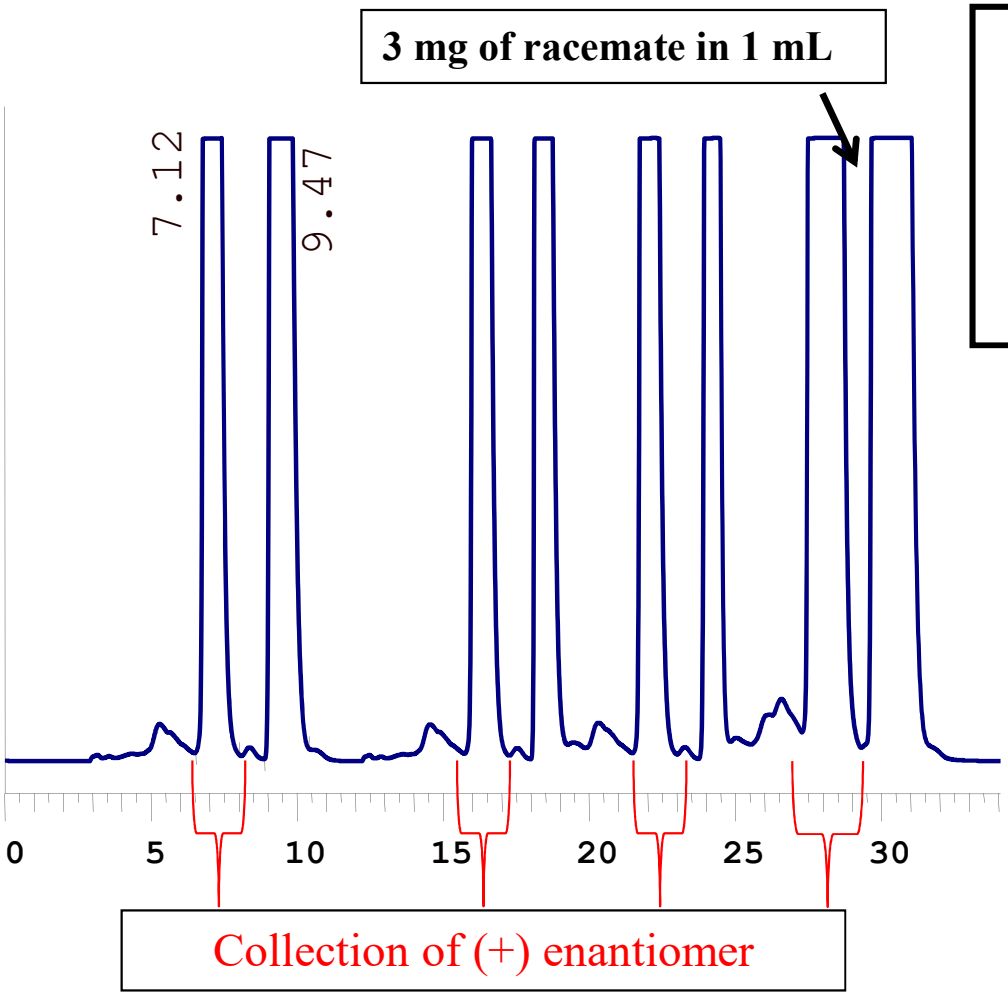
Sequential injections :



Stacked injections:

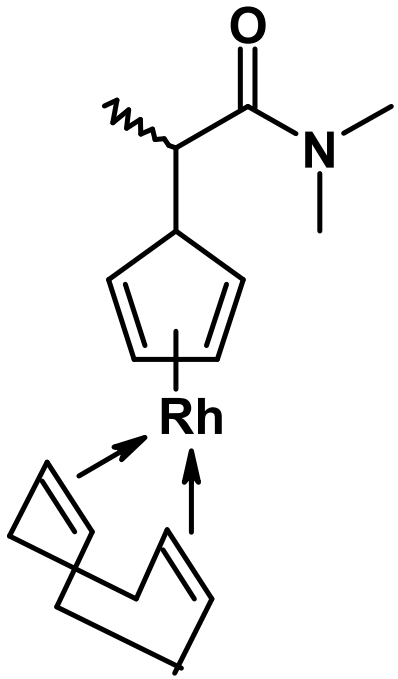


Example : Preparative separation



CHIRALPAK AD (250*10 mm)
Hexane/Isopropanol (90/10)
4,5 mL/min 20°C

Productivity : 15 mg of each enantiomer per hour

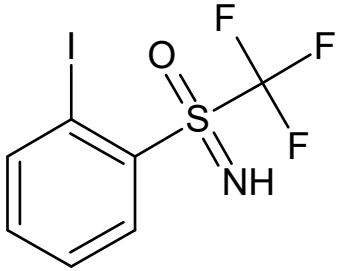


Example : Preparative separation

Lux-Amylose-2

Heptane/Isopropanol

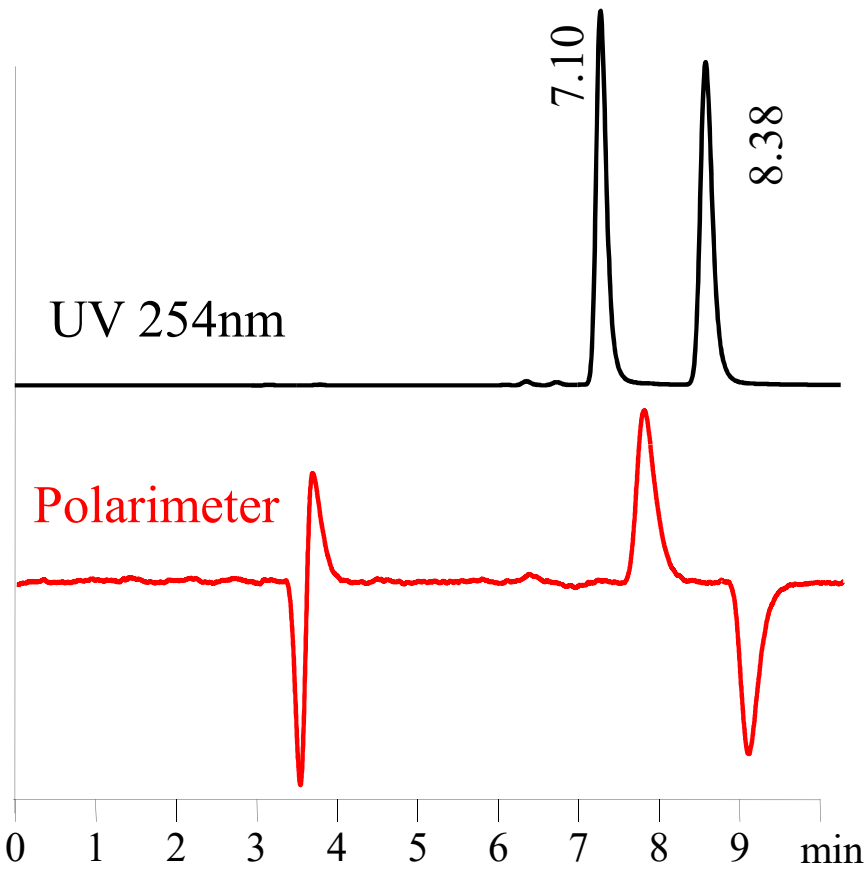
80/20

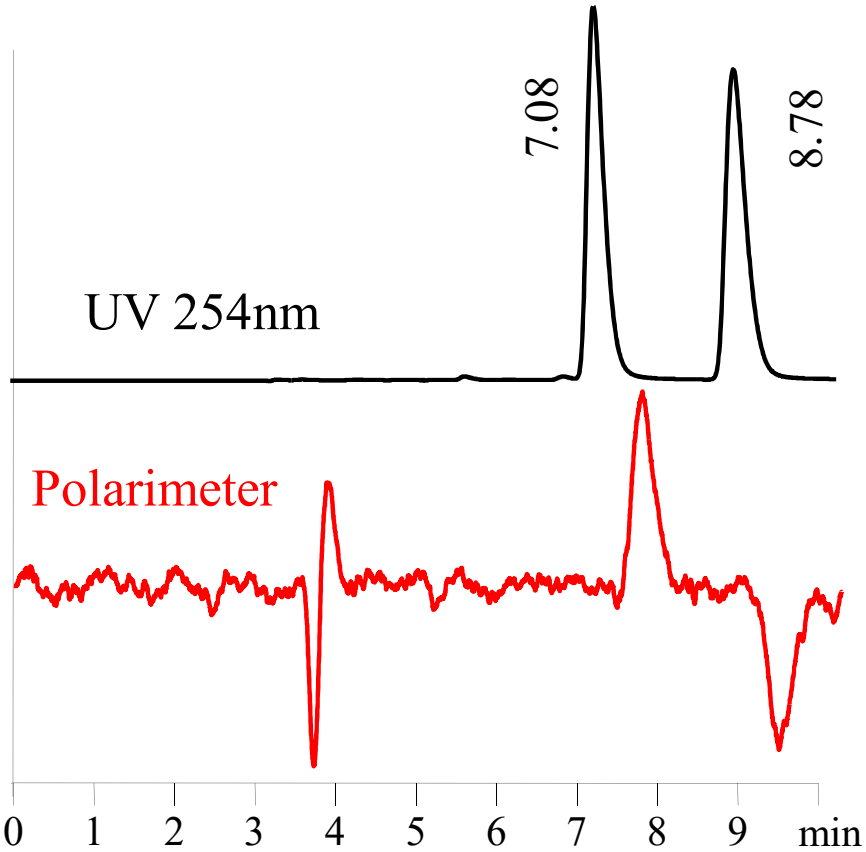


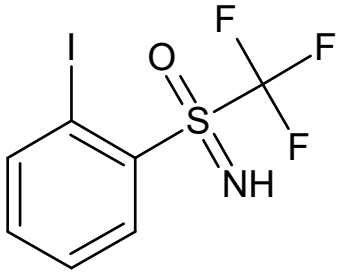
Chiralpak AS-H

Heptane/Isopropanol

90/10



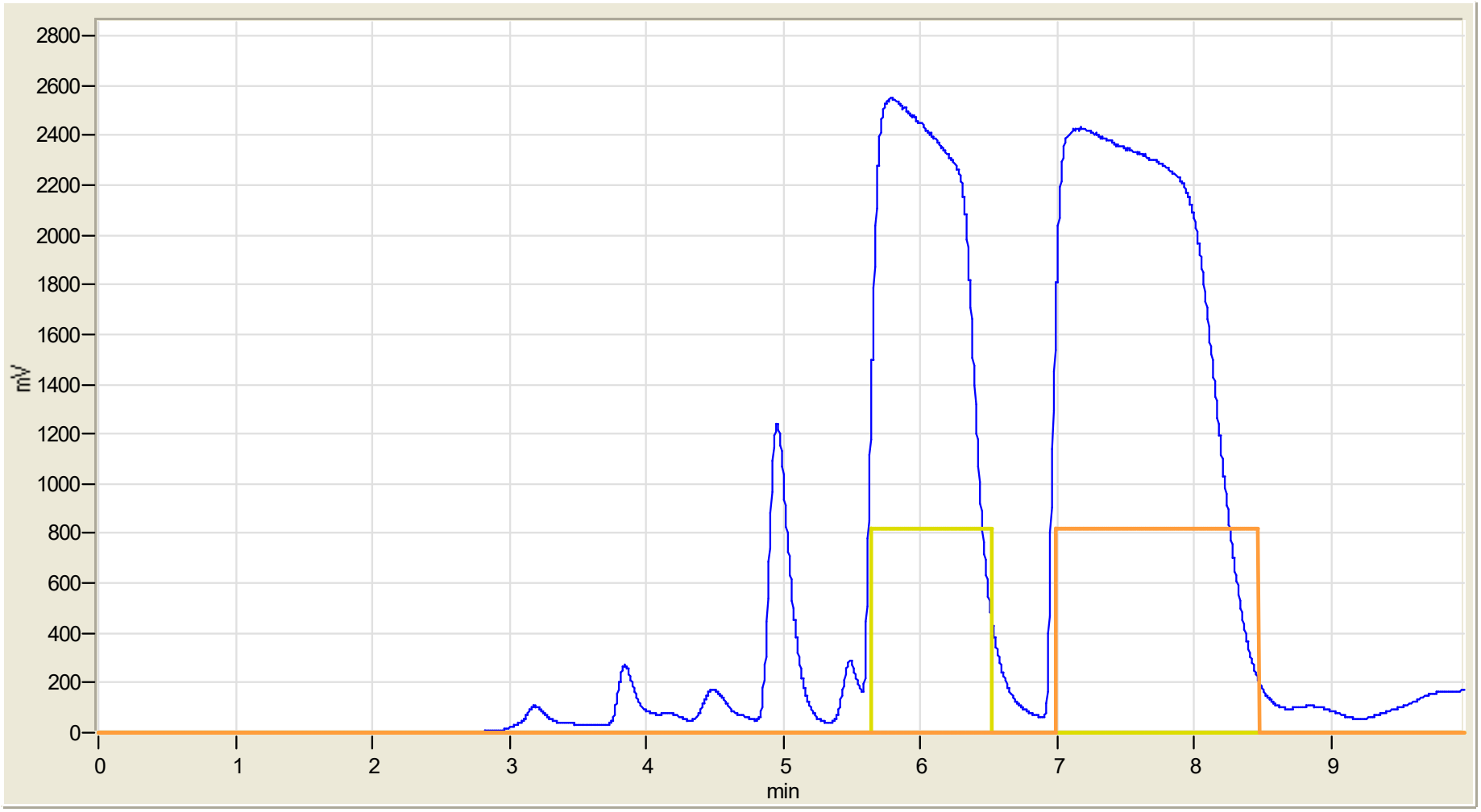




Chiralpak AS-H (250 x 10 mm)

Heptane/Isopropanol

80/20



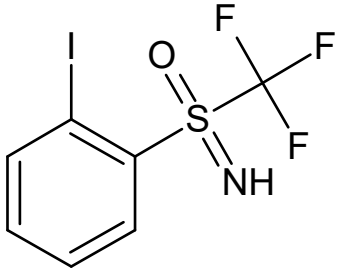
Injection of 15 mg

Different loading capacities

Lux-Amylose-2

Heptane/Isopropanol

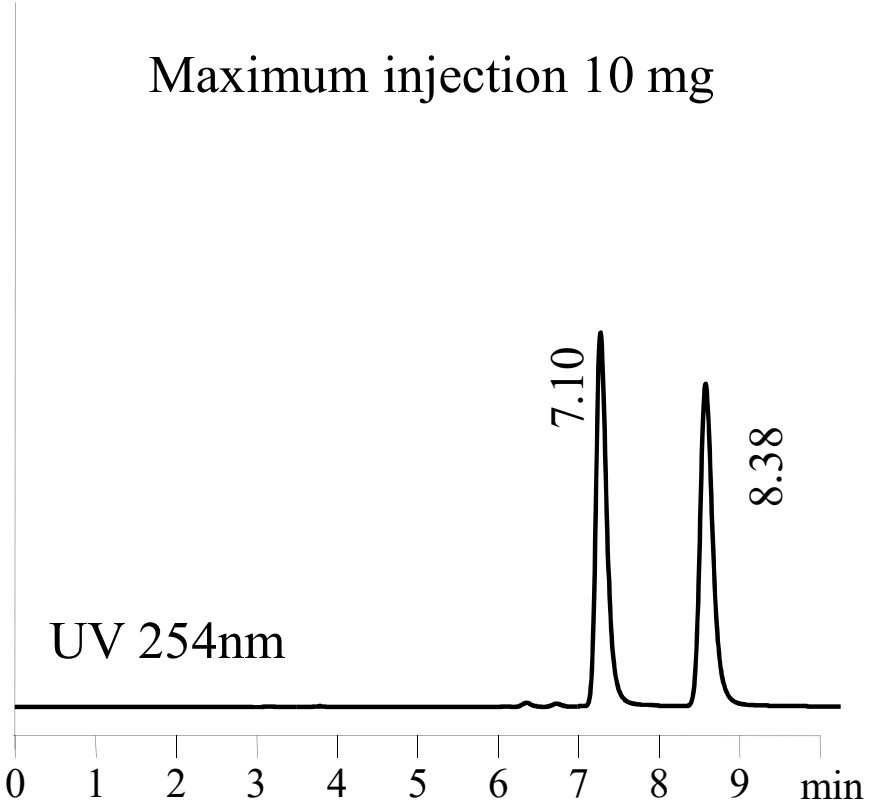
80/20

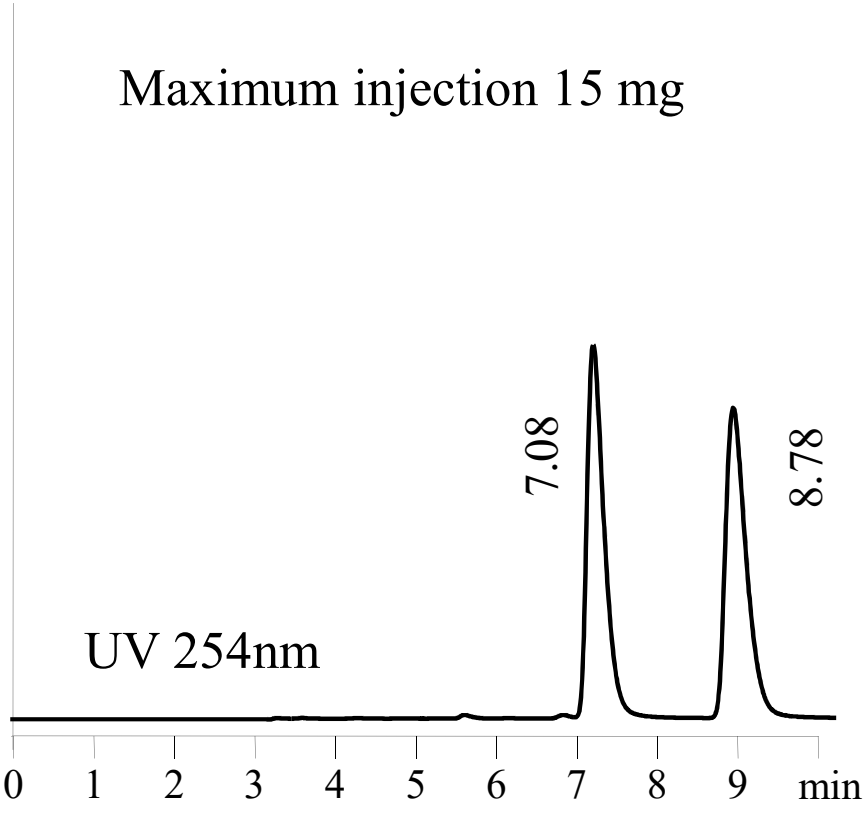


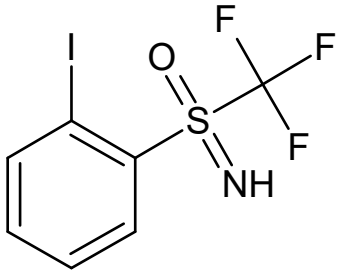
Chiralpak AS-H

Heptane/Isopropanol

90/10



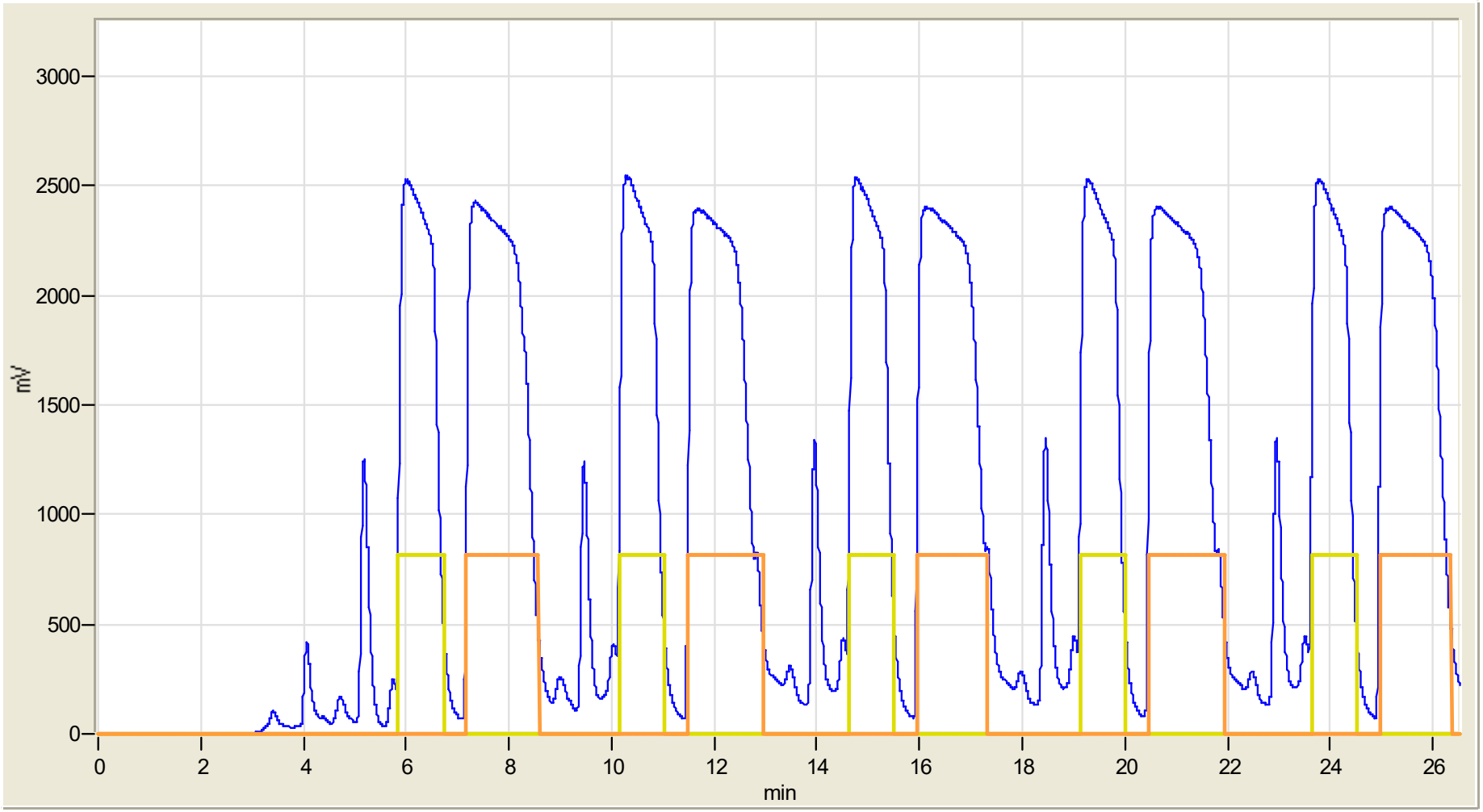




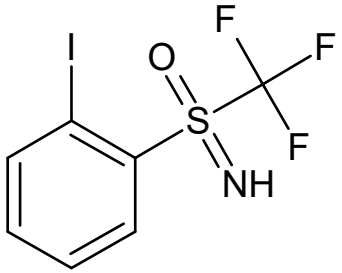
Chiralpak AS-H (250 x 10 mm)

Heptane/Isopropanol

80/20



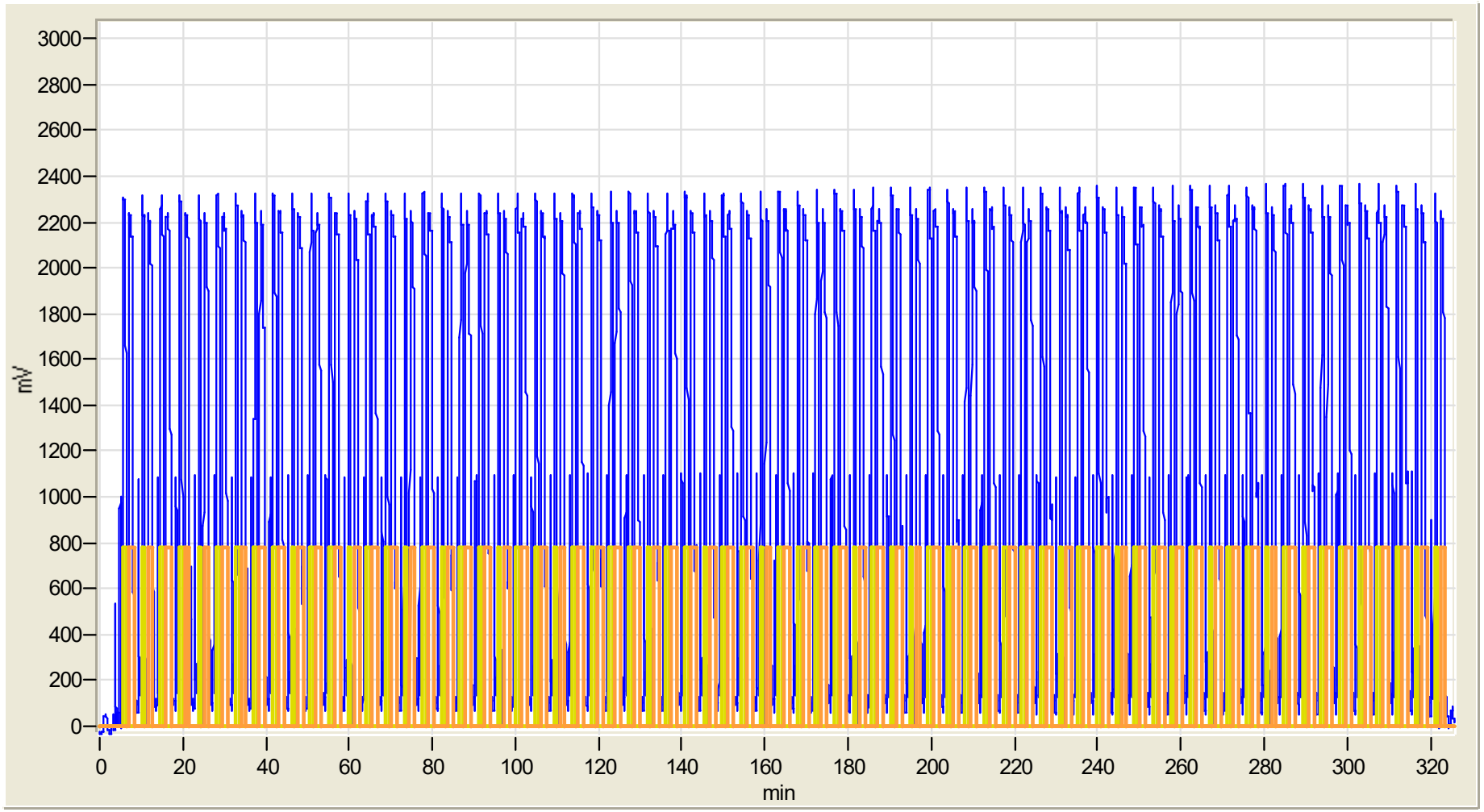
5 injections of 15 mg



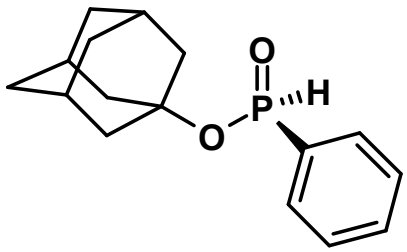
Chiralpak AS-H (250 x 10 mm)

Heptane/Isopropanol

80/20



75 injections of 15 mg

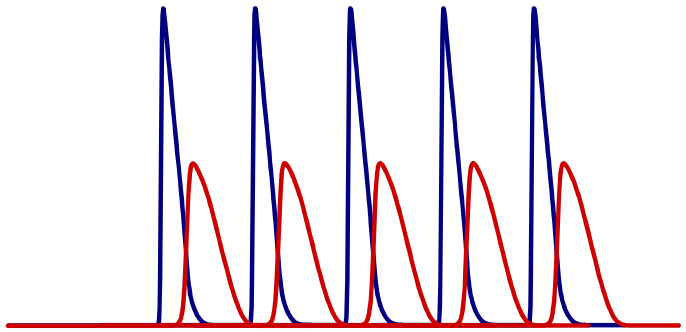


Chosen conditions

Lux-Cellulose-2

methanol / 30°C

Stacked injections 17 mg

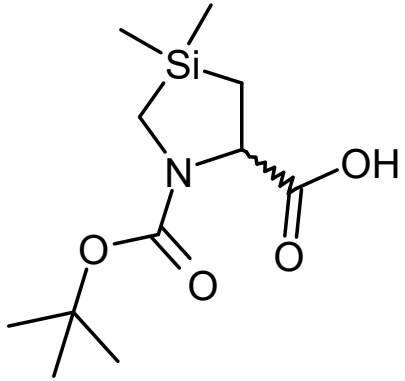


Amount of racemate by injection	17 mg
Number of injections	700
Injection every	2,4 minutes
Total time	30 hours
Volume of solvent required	9 liters
Amount collected for the first eluted	6,0 g (99% ee)
Amount collected for the second eluted	6,2 g (98% ee)

Productivity : 200 mg of each enantiomer per hour

Example : Preparative separation

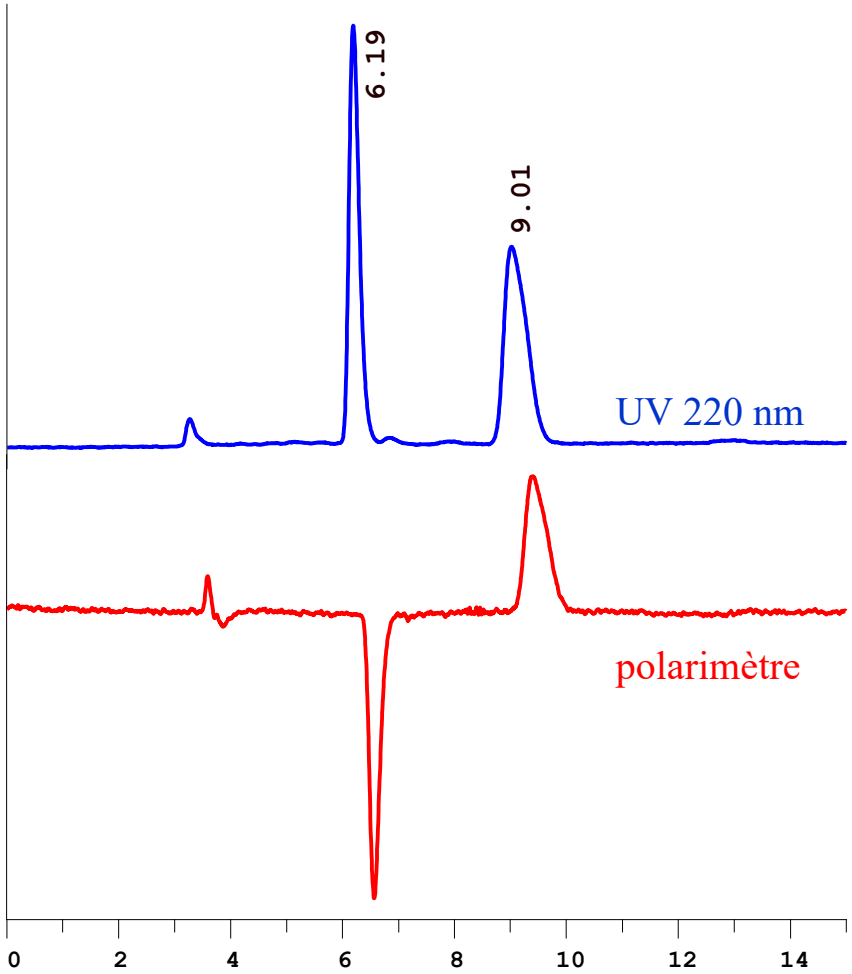
Boc-Silaproline



Chiralpak IC (250*10 mm)
Hexane/Isopropanol (90/10) / 5 mL/min / 30°C

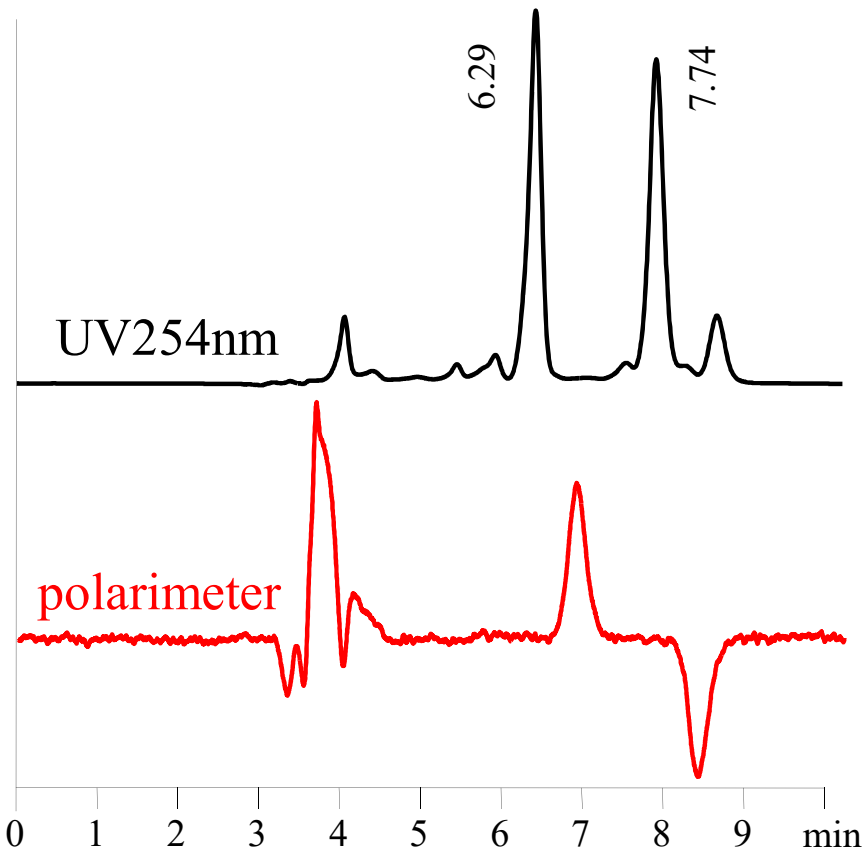
Productivity : 150 mg of each enantiomer per hour

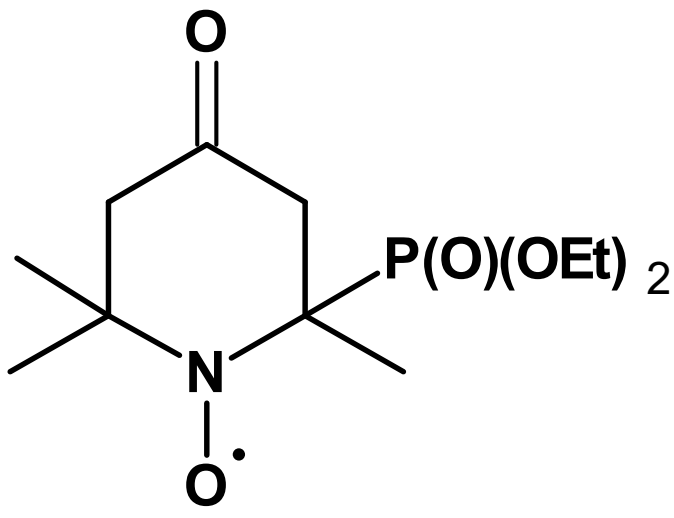
Amount of racemate by injection	67 mg
Number of injections	140
Injection every	12 minutes
Total time	30 hours
Volume of solvent required	5 liters
Amount collected for the first eluted	4,5 g (>99,5% ee)
Amount collected for the second eluted	4,5 g (>99,5% ee)



C. Martin, N. Vanthuyne, H. Miramon, J. Martinez, F. Cavelier, Amino Acids 2012, 43, 649-655.

Chiralpak IE
Heptane/Ethanol
50/50

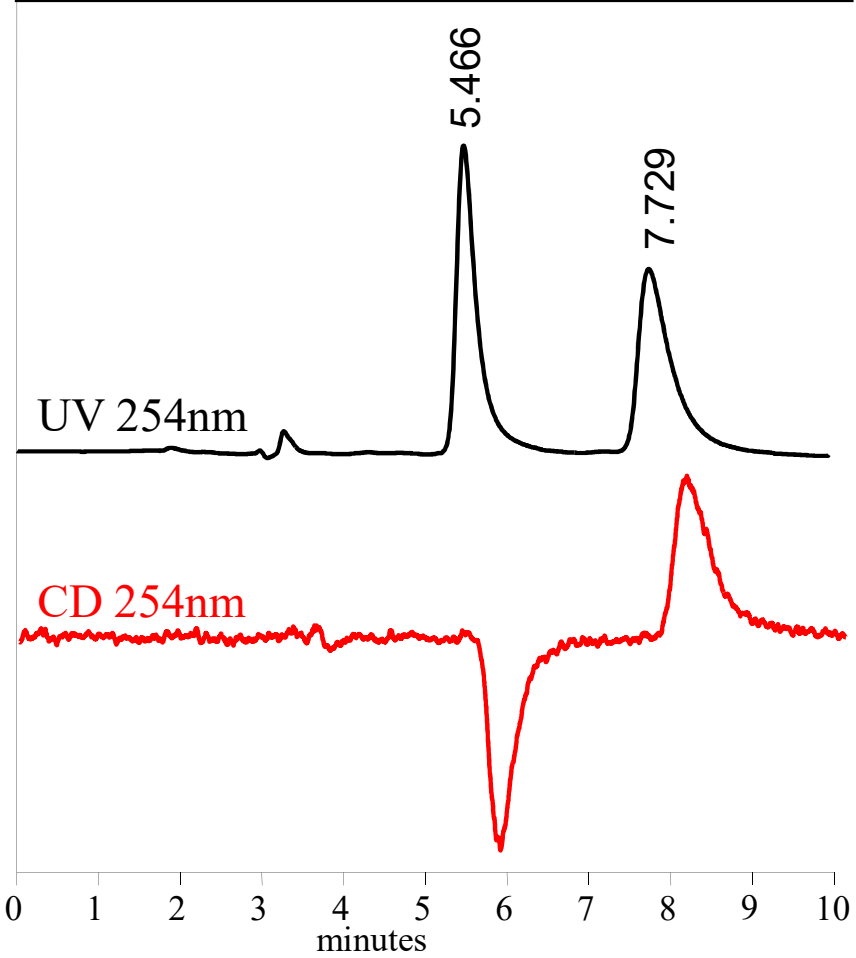


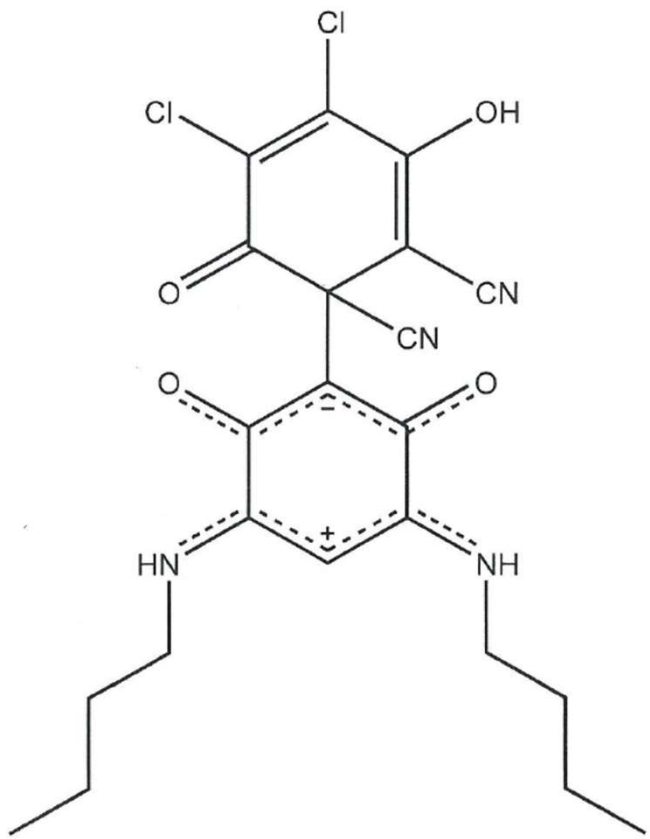


preparative : 500 mg of each enantiomer in 10 hours

N. Jugniot, …, G. Audran, P. Mellet, *Free Radical Bio Med* **2018**, *126*, 101-112.

(*S*,*S*)-Whelk-O1
Heptane / ethanol+tfa /dichloromethane
10/80/10

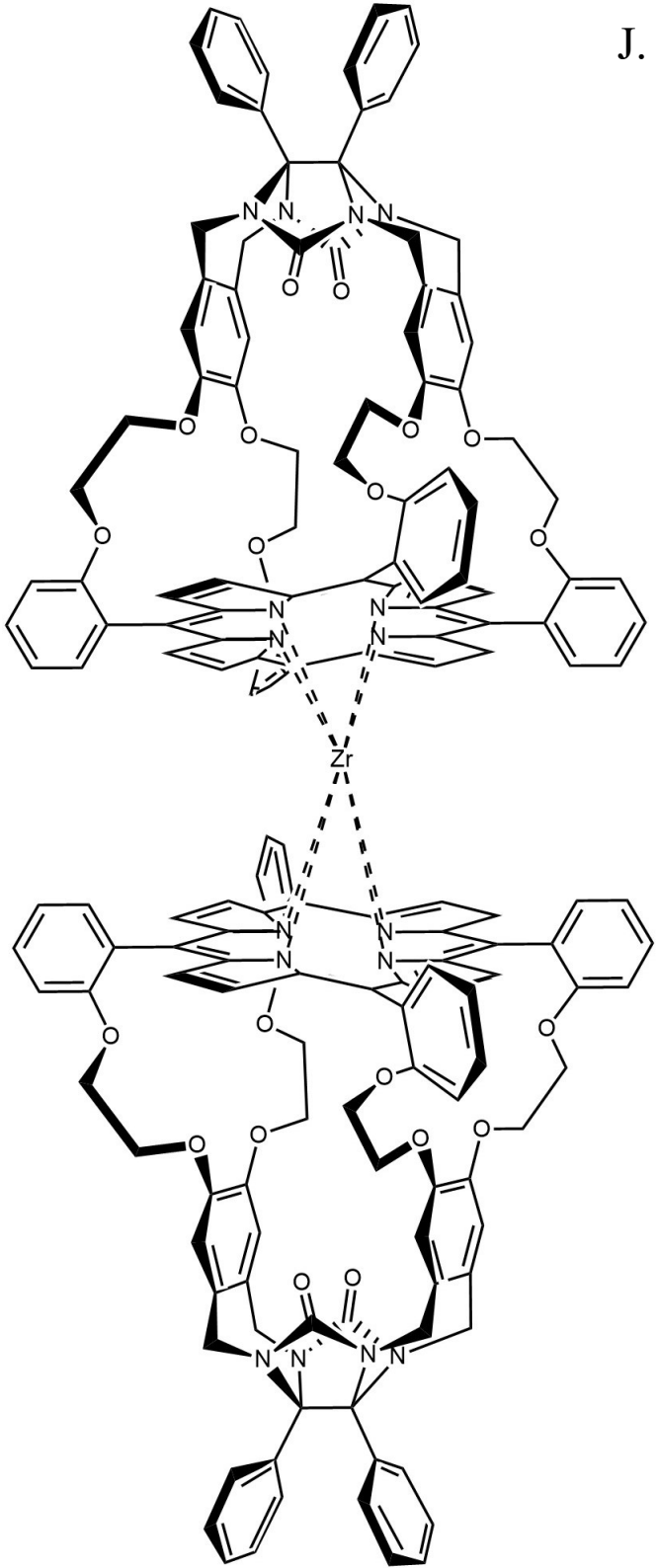


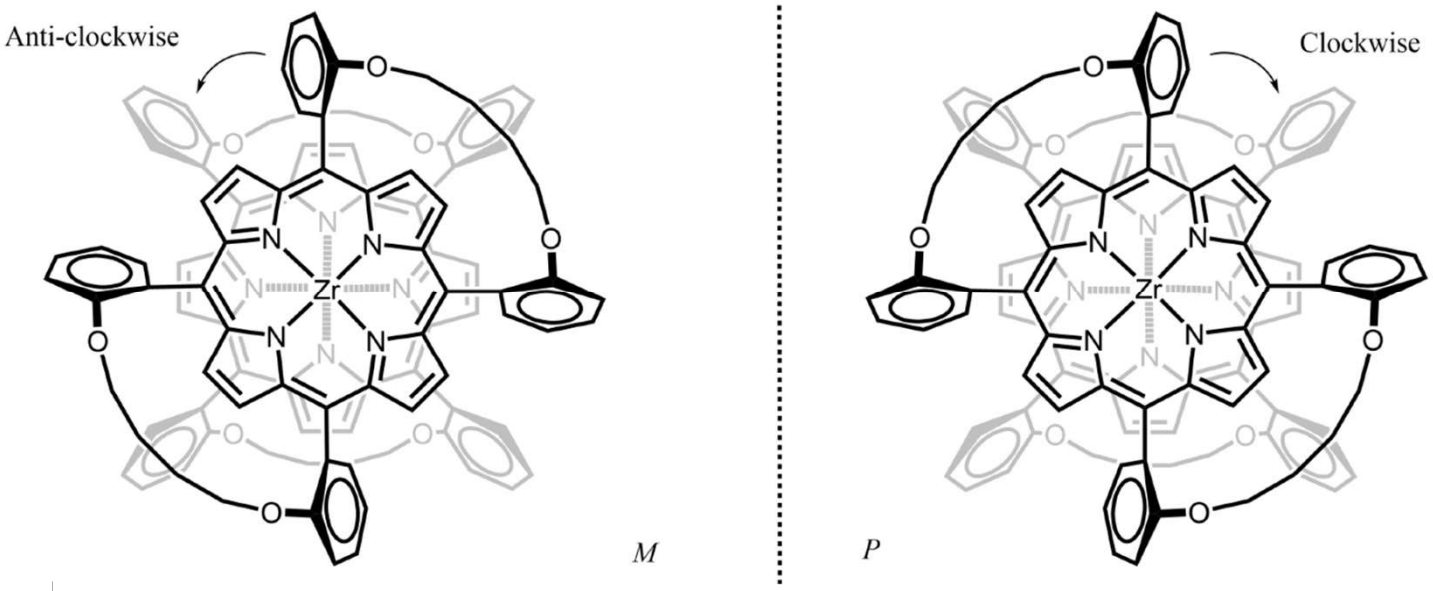


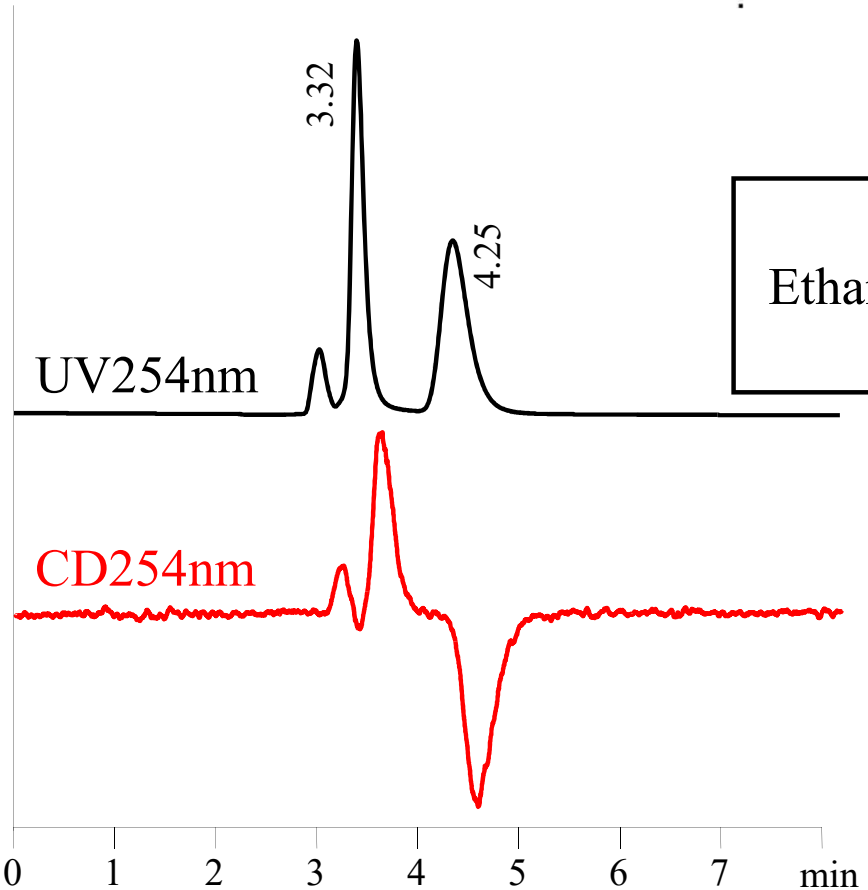
preparative : 25 mg
of each enantiomer
in 8 hours

G. Canard, …, C. Roussel, O. Siri, *New J Chem* **2018**, *42*, 8247-8252.

J. P. J. Bruekers, …, R. J. M. Nolte, *Eur J Org Chem* **2021**, *2021*, 607-617.







Chiralpak IH
Ethanol / dichloromethane
30/70

preparative : 15 mg
of each enantiomer
in 6 hours