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TECHNOLOGIES EUROPE

“Determination of enantiomeric excess with Evaporative Light Scattering Detectors (ELSD):

Why racemic mixtures do not show a 50:50 ratio”

T. Zhang, D. Nguyen, P. Franco

Overview

- Introduction: need for Evaporative Light Scattering Detection (ELSD) in the separation of enantiomers

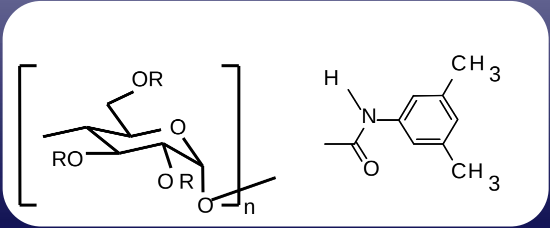
- Principles of ELSD for quantification of enantiomers

- Practical examples of quantification on
  - ⇨ CHIRALPAK® IA
  - ⇨ CHIRALPAK® IB
  - ⇨ CHIRALPAK® QD-AX

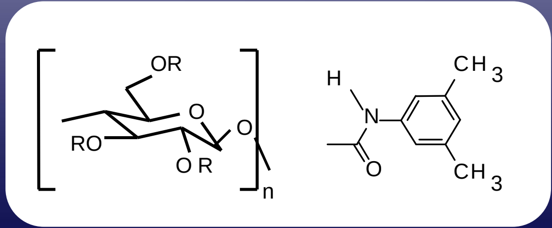
Need for ELSD

The immobilized polysaccharide-derived chiral stationary phases

CHIRALPAK® IA

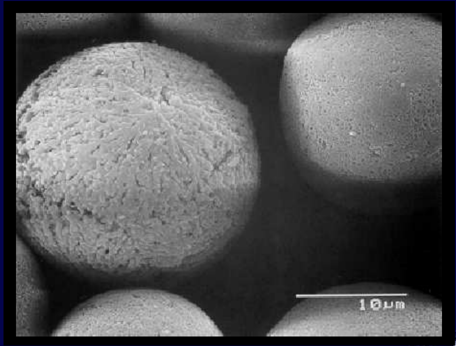


CHIRALPAK® IB

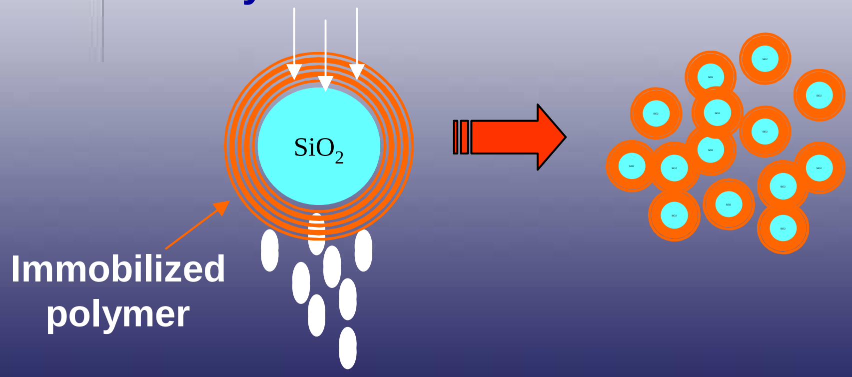


- Based on amylose and cellulose *tris*-(3,5-dimethylphenylcarbamate)
- Immobilization onto 5 µm silica gel by a proprietary process

REVOLUTIONARY generation of CSPs



Need for ELSD



- Compatible with all solvents
- New selectivity profile
- Robustness
- Extented durability

Normal phase conditions:

- Alkane/alcohol

Polar mode:

- Acetonitrile
- Ethanol
- Methanol
- Other alcohols

Extended solvent range:

- MtBE
- Toluene
- Choroform
- Dichloromethane
- Ethyl acetate
- THF
- 1,4-dioxane
- Acetone
- DMSO or DMF (as injection solvents)

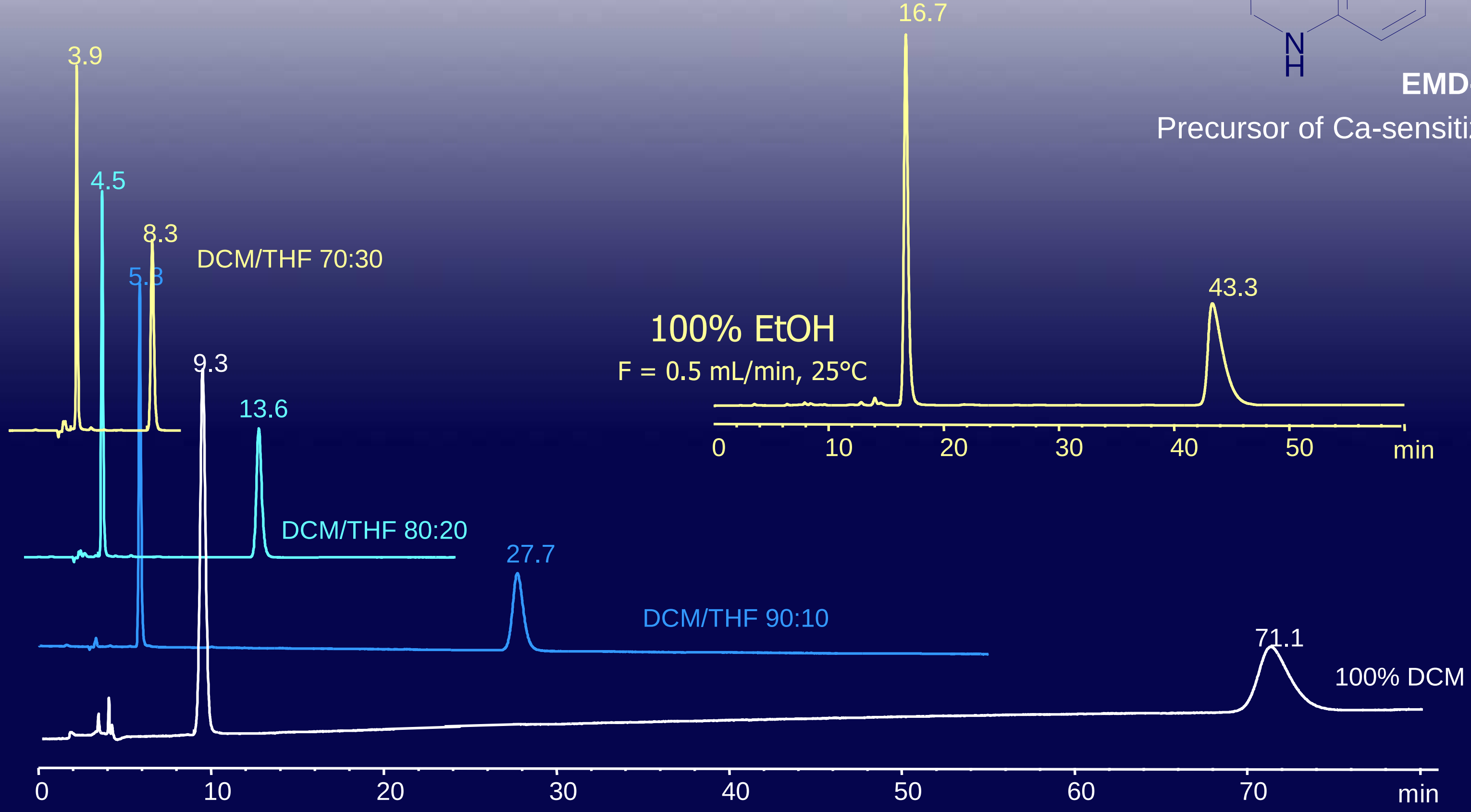
Eluting strength

Need for ELSD

➢ T. Zhang *et al., J. Chromatogr. A* 1083 (2005) 96-101

EMD-53986

Precursor of Ca-sensitizing drug



F = 1 mL/min, 25°C
(Column 25 x 0.46 cm)

CHIRALPAK® IA

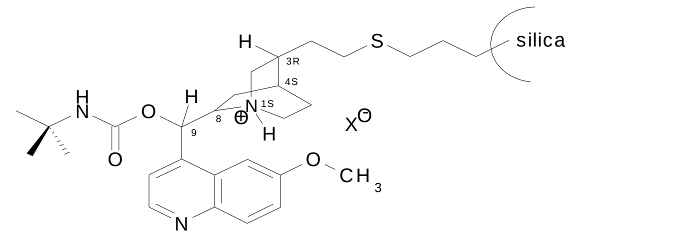
UV cut-off of common HPLC solvents

Feasibility of UV detection

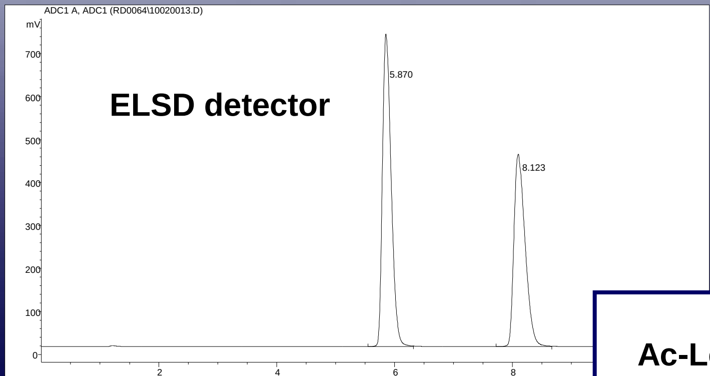
Solvent	UV cut-off (nm)	UV detection feasibility
Hexane	195	Good
2-Propanol	205	Good
Ethanol	205	Good
Methanol	205	Good
Acetonitrile	190	Good
Dichloromethane	233	Troublesome *
Chloroform	245	Troublesome *
Ethyl acetate	256	Troublesome *
Acetone	330	Failure
Toluene	284	Failure

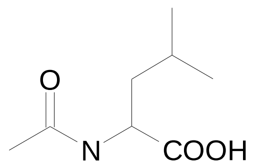
* but still possible with compounds which are chromophoric at λmax > 250 nm

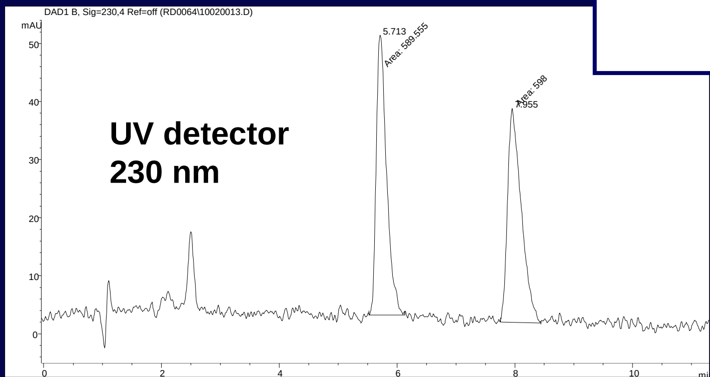
Detection of molecules with low absorbing chromophores
Example acetyl-D,L-Leu



CHIRALPAK® QN-AX: (8S,9R)
CHIRALPAK® QD-AX: (8R,9S)







CHIRALPAK® QD-AX

methanol / formic acid

F = 1 mL/min, 25°C
(Column 15 x 0.46 cm)

Advantages of ELSD detection

- Compatible with all organic solvents
- Able to detect analytes which do not bear strong UV absorbing groups (i.e. Boc- or Ac-derived amino acids)
- Produces stable baselines during gradient chromatographic elution regardless of the spectral properties of the different mobile phases
- Adapted for the analysis of fatty acids, glycerides, lipids, surfactants and pharmaceuticals …
- Rare applications in enantiomeric resolution until present!!

Basic principle of ELSD

- ELSD is based on the differences in volatility between the mobile phase and the analyte molecules in the outlet stream
- It operates by:
  - nebulizing the effluent coming out of the column
  - vaporizing the solvent in the formed droplets through a heated drift tube
  - leaving behind the non-volatile solute particles, which are carried through a beam of light
- The incident light is scattered by the particles and collected by a photomutitplier

- The response does not follow Beer's Law.
- Instead, *the measured peak area (A) is related to the sample mass (m):*

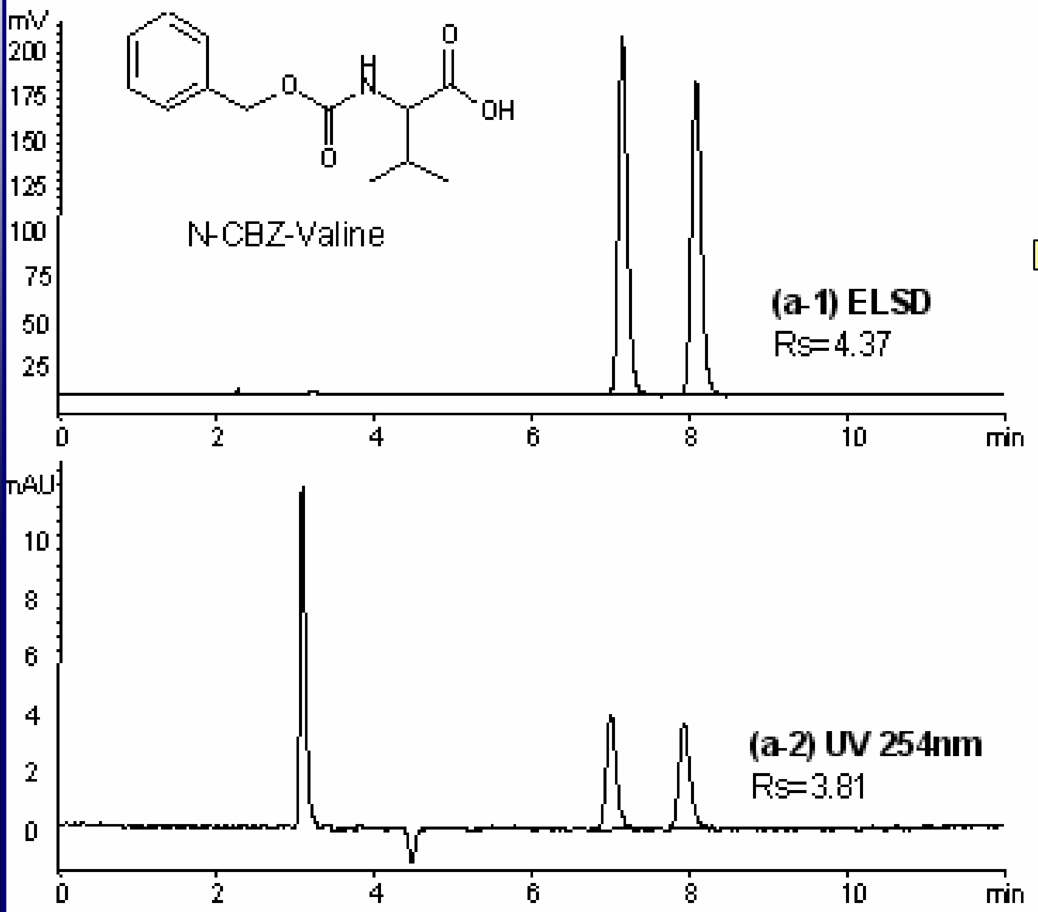
$$A = a\, m^b$$

$$\text{Log } A = \log a + b \log m$$

(exponential relationship between the peak area and the sample mass)

Coefficients a and b are related to the nature of solute, droplet size, concentration, mobile phase compositions, …

ELSD *versus* UV detection
Solvents with high UV cut-off



Better signal-to-noise ratio

CHIRALPAK® IB

n-hexane / ethyl acetate / TFA

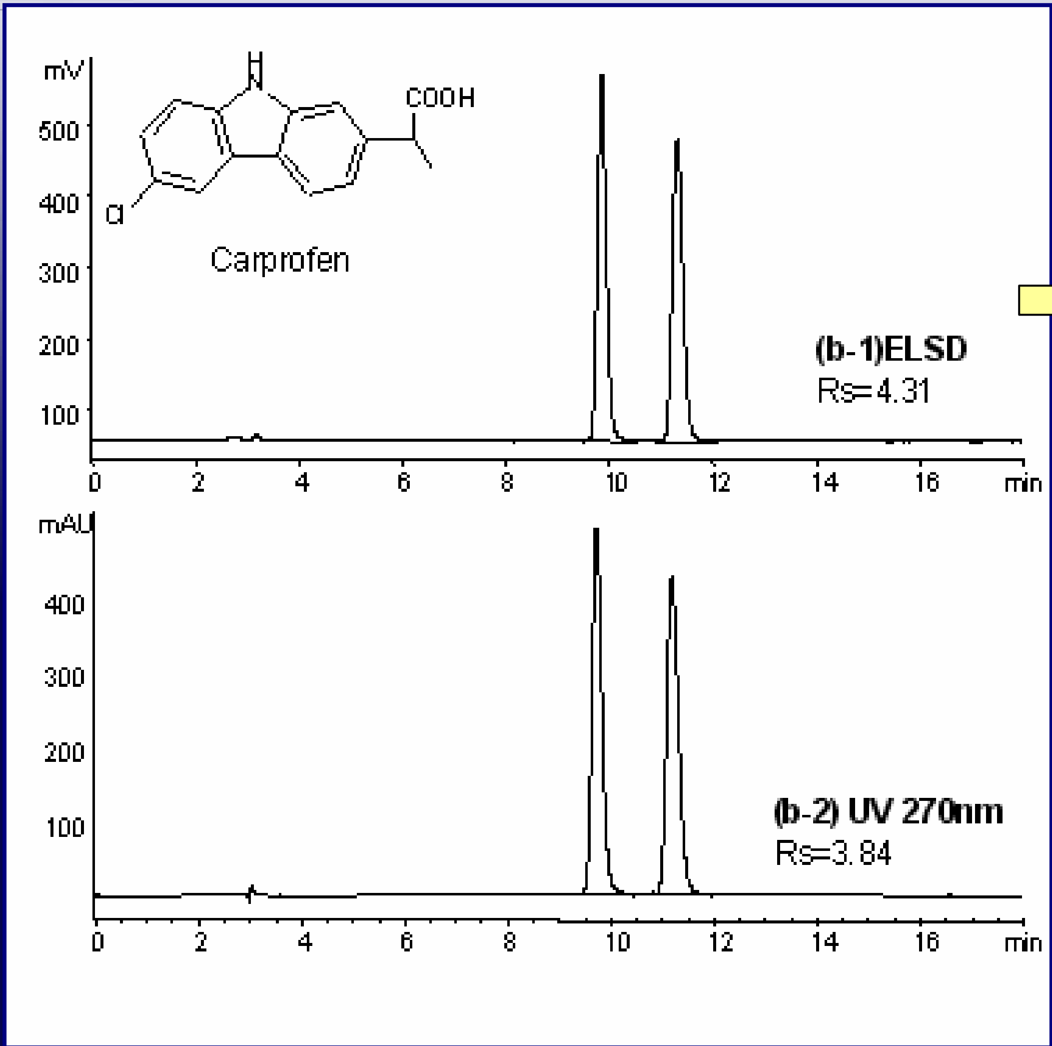
70/30/0.1

F = 1 mL/min, 25°C

(Column 25 x 0.46 cm)

ELSD *versus* UV detection

Solvents with high UV cut-off



Higher resolution values

CHIRALPAK® IB

n-hexane / ethanol / TFA

90/10/0.1

F = 1 mL/min, 25°C

(Column 25 x 0.46 cm)

Differences in peak shape

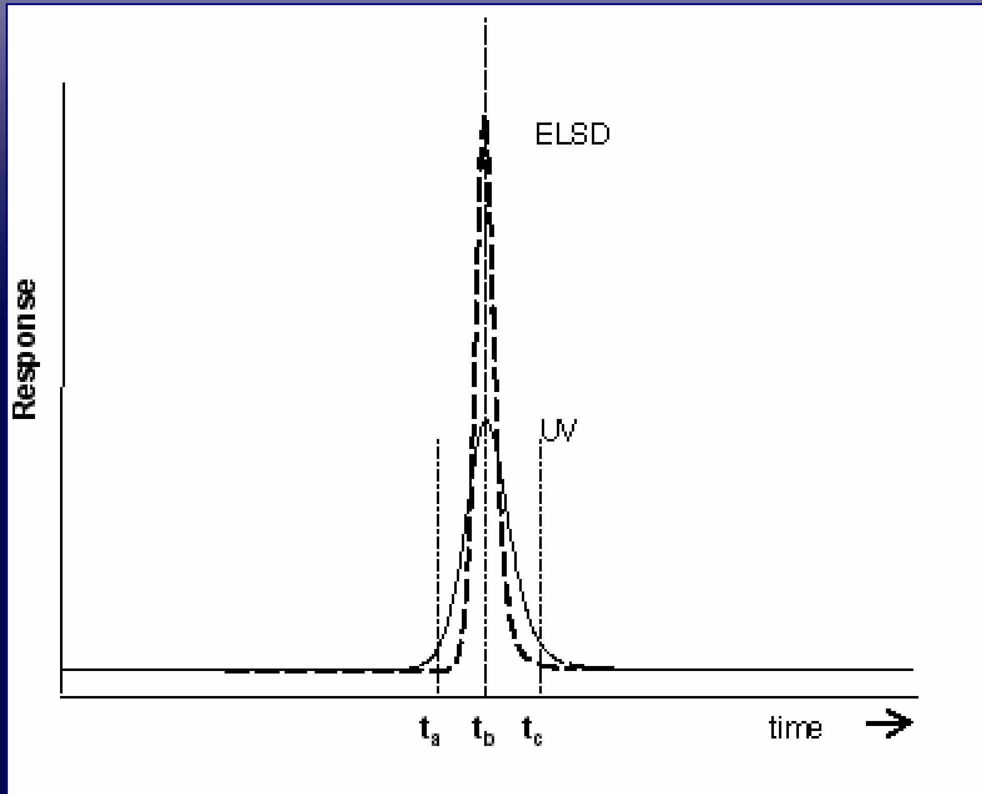
between ELSD and UV detection

- Due to the exponential correlation between the ELSD response and the sample mass

⇨ the instant response intensity at the maximum is significantly « amplified »

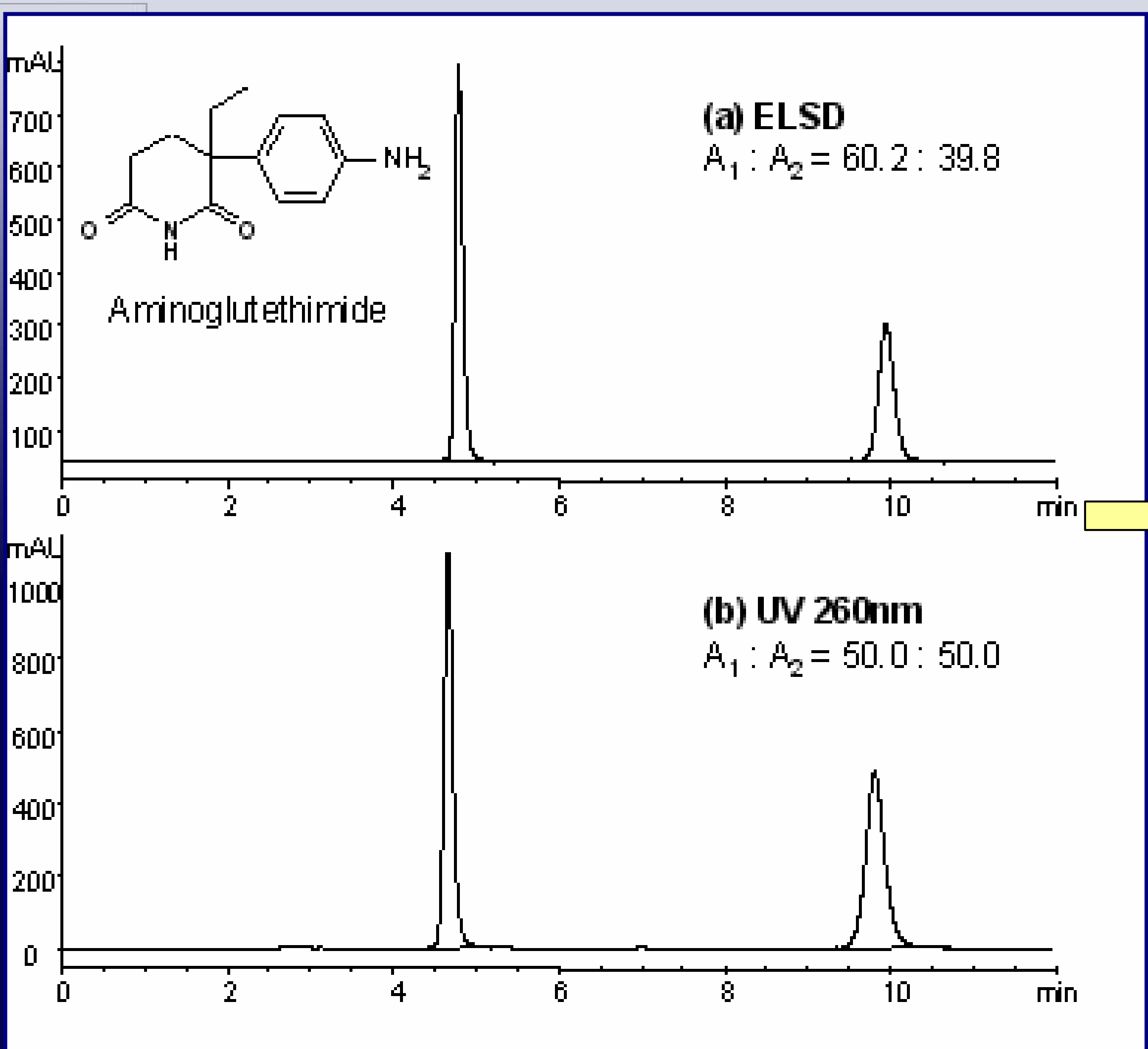
⇨ while the response for the very low sample mass points at the « foot » of the peak are « shaved »

⇨ ⇨ HIGHER EFFICIENCY



Deviation of peak area by ELSD

Example ELSD *versus* UV detection



Racemic mixture!!

CHIRALPAK® IA

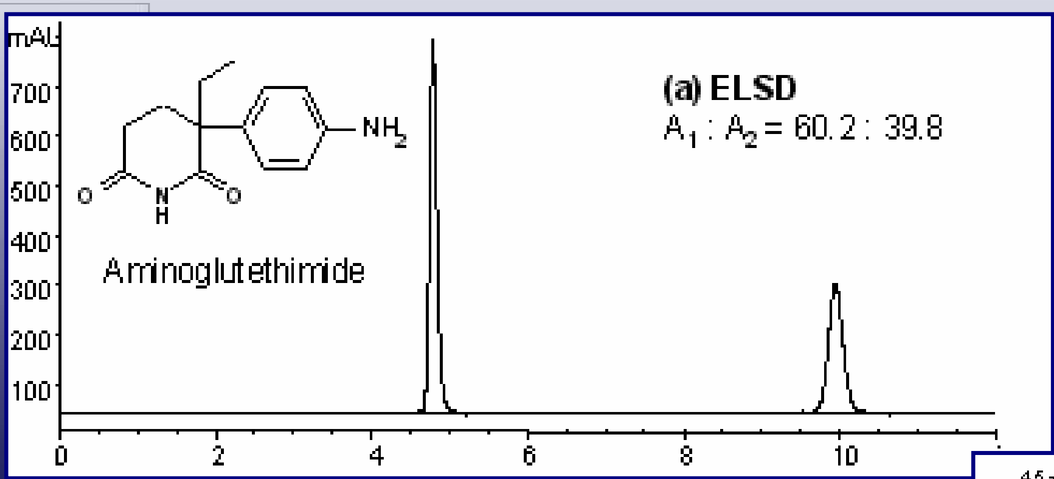
ethyl acetate 100%

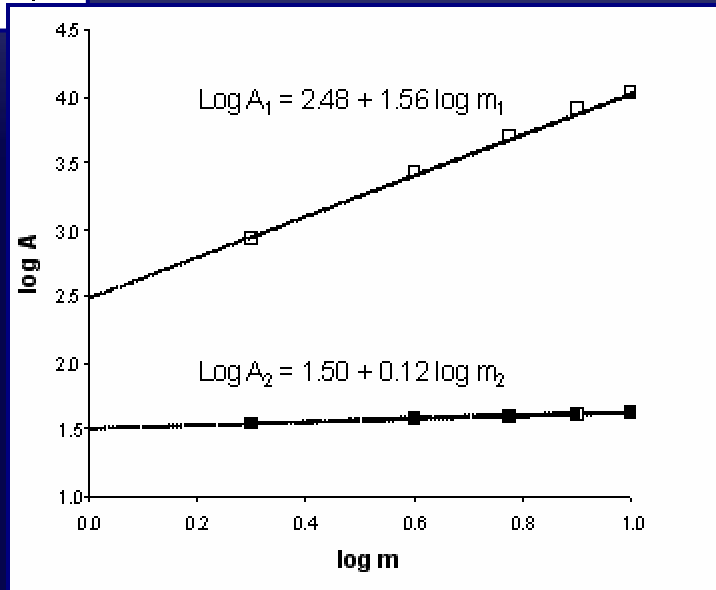
F = 1 mL/min, 25°C

(Column 25 x 0.46 cm)

Deviation of peak area by ELSD

Correlation areas both enantiomers





CHIRALPAK® IA

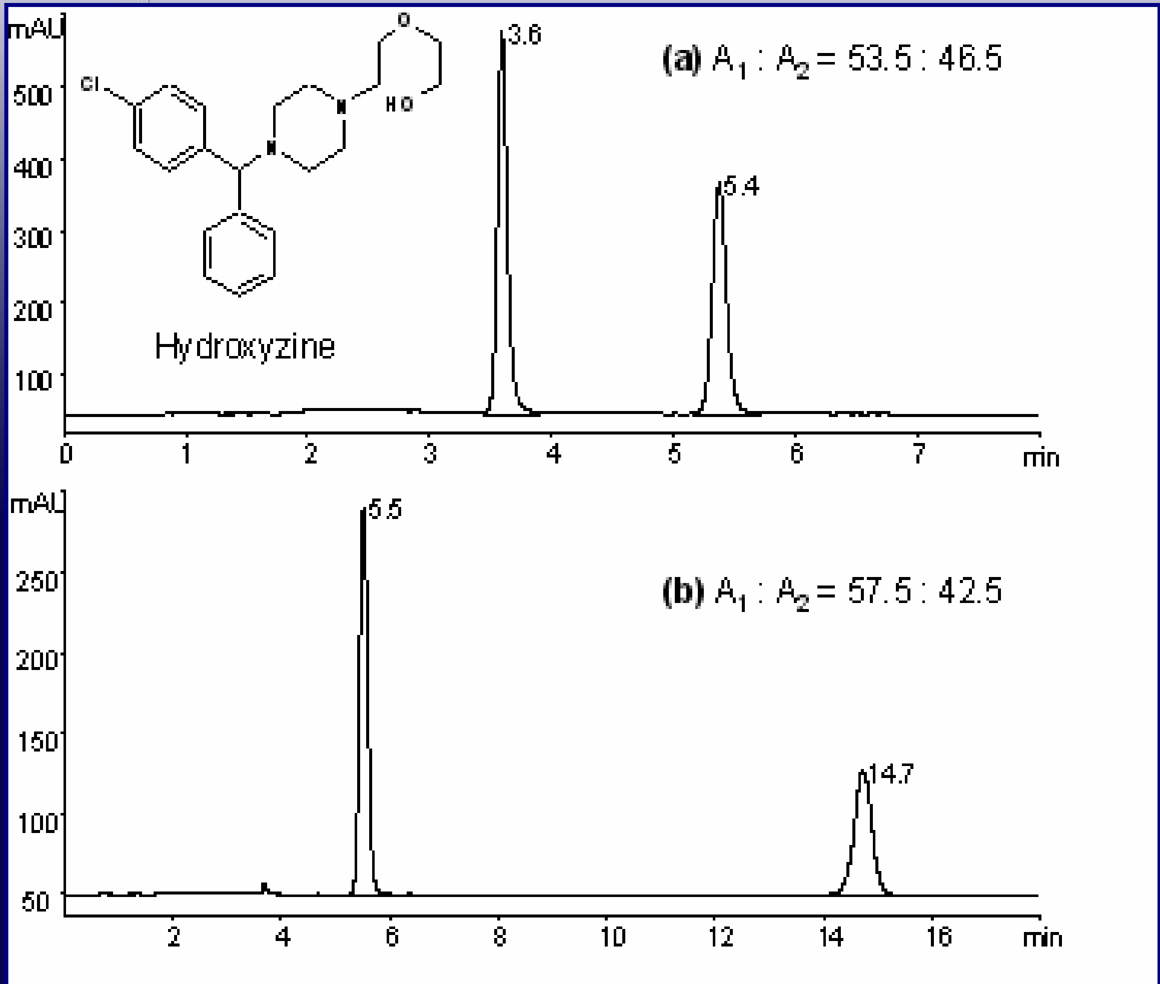
ethyl acetate 100%

F = 1 mL/min, 25°C

(Column 25 x 0.46 cm)

Variation of peak area percentage by ELSD

Influence of peak interval



CHIRALPAK® IA

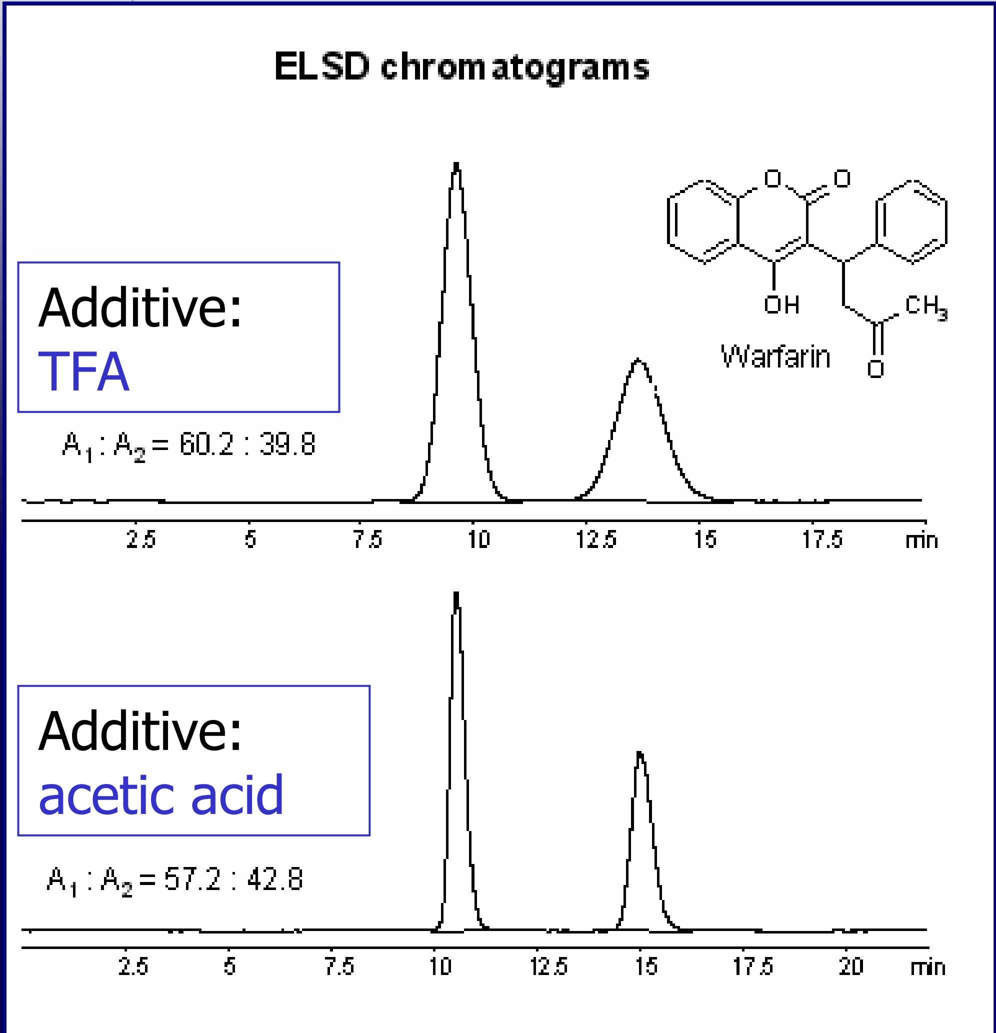
toluene / methanol / DEA
90/10/0.1

toluene / methanol / DEA
98/2/0.1

F = 1 mL/min, 25°C
(Column 25 x 0.46 cm)

Variation of peak area percentage by ELSD

Influence of peak width



CHIRALPAK® IB

n-hexane / chloroform / acidic addtive

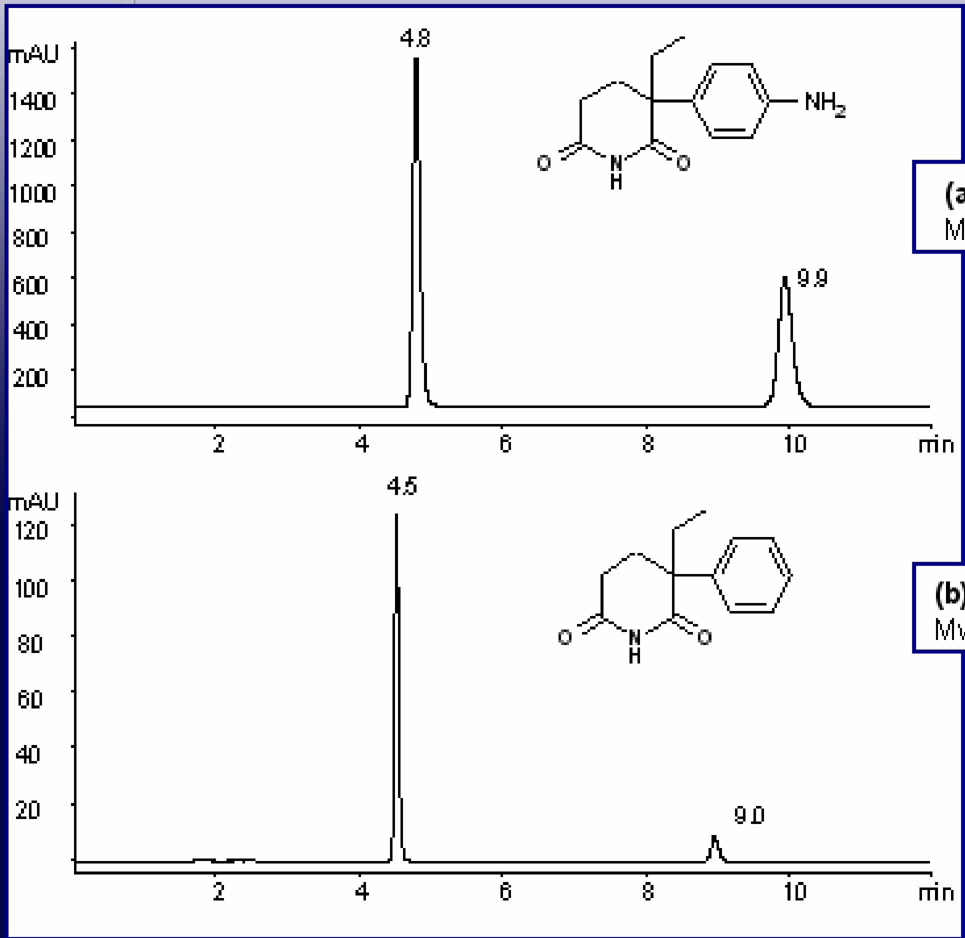
50/50/0.1

F = 1 mL/min, 25°C

(Column 25 x 0.46 cm)

Variation of peak area percentage by ELSD

Influence of MW of the compound



(a) Aminoglutethimide
Mw: 232.3

$A_1:A_2$ 58:42

$A = a\,m^b$

$\text{Log } A = \log a + b \log m$

(b) Glutethimide
Mw: 217.3

$A_1:A_2$ 89:11

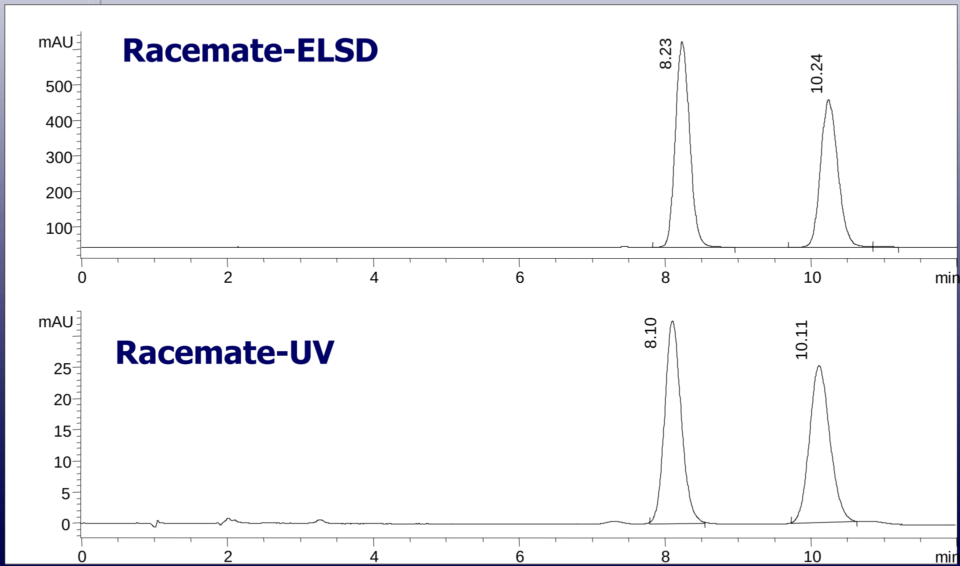
CHIRALPAK® IA
ethyl acetate 100%

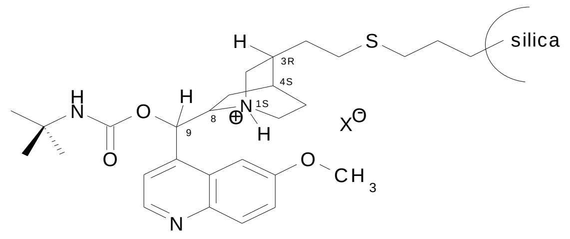
F = 1 mL/min, 25°C
(Column 25 x 0.46 cm)

Calibration curve for quantification by ELSD

Separation of amino acid derivative - 1

N-CBZ-D,L-Phe





CHIRALPAK® QN-AX: (8S,9R)
CHIRALPAK® QD-AX: (8R,9S)

CHIRALPAK® QD-AX
methanol / formic acid
100/0.3

F = 1 mL/min, 25°C
(Column 15 x 0.46 cm)

Enriched samples

Rac:L (mg/ml)	V_{rac}:V_L (µl)	UV 254 nm A_1	A_2	A_1%	ELSD A_1	A_2	A_1%
2:1	10:0	513.9	501.9	50.6	7897.8	6851.9	53.5
2:1	3:12	814.7	148.7	84.6	14685.3	973.4	93.8
2:1	5:10	793.2	254.9	75.7	14038.7	2180.9	86.6

racemate

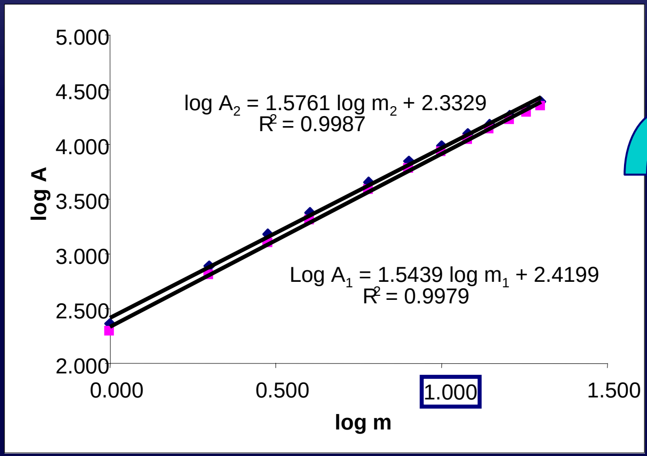
Calibration curve for quantification by ELSD

Separation of amino acid derivative - 2

Enriched samples Rac:L (mg/ml)	$V_{rac}:V_L$ (µl)	UV 254 nm A_1	A_2	$A_1\%$	ELSD A_1	A_2	$A_1\%$
2:1	10:0	513.9	501.9	50.6	7897.8	6851.9	53.5
2:1	3:12	814.7	148.7	84.6	14685.3	973.4	93.8
2:1	5:10	793.2	254.9	75.7	14038.7	2180.9	86.6

racemate

« Apparent » values directly calculated with the ELSD detection



Percentages calculated with the ELSD areas in the calibration curves

50.2%
83.9%
75.2%

N-CBZ-D,L-Phe

CHIRALPAK® QD-AX

methanol / formic acid

Conclusions

➢ T. Zhang *et al., J. Separation Sci.* 29 (2006) 1517

- Evaporative Light Scattering Detectors (ELSD) are useful tools for the qualitative and quantitative analysis of enantiomeric mixtures
- High versatility for their use with CHIRALPAK® IA and CHIRALPAK® IB with UV-absorbing mobile phases
- Well adapted for the detection of non-UV absorbing molecules on use with CHIRALPAK® IA, CHIRALPAK® IB and CHIRALPAK® QD-AX

Acknowledgements

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CHIRAL

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