

2nd Joint CE user Meeting  
KNCV - KVCV

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SAC

Friday, November 17th, 2006  
Aldhem Hotel, Grobbendonk  
More information and registration at:  
[analytische.kvcv.be/#ce](http://analytische.kvcv.be/#ce)

Sectie Analytische  
KVCV

# Evaluation of Capillary Electrochromatography as Chiral Separation Technique

## Definition of a Chiral Separation Strategy

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**sanofi aventis**

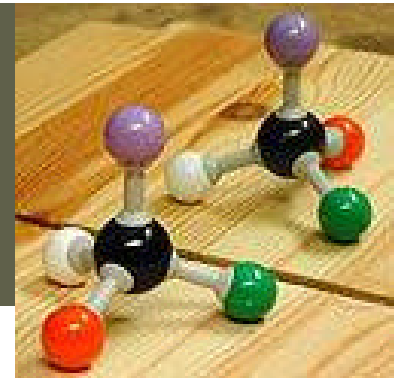


Vrije Universiteit Brussel

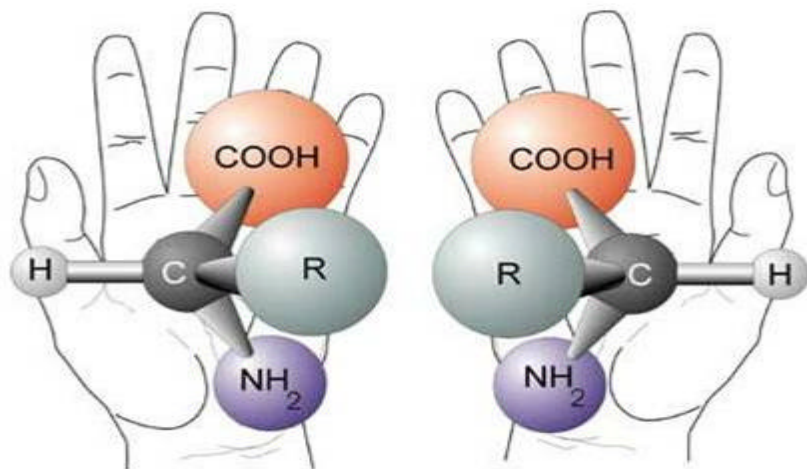
# Outline

1. Introduction
  - Chirality
  - Capillary Electrochromatography
2. Aim
3. Methodology
4. Strategy
  - Acidic
  - Non-acidic
5. Conclusions & Future Perspectives

# Chirality



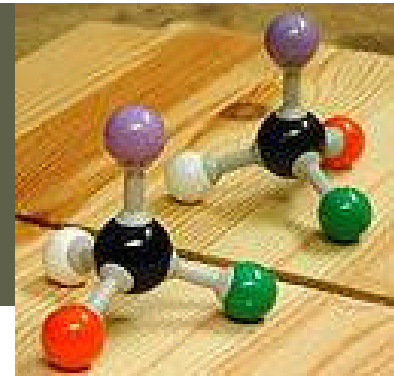
“Existence of non-superimposable forms of an asymmetric molecule that are mirror images of each other”



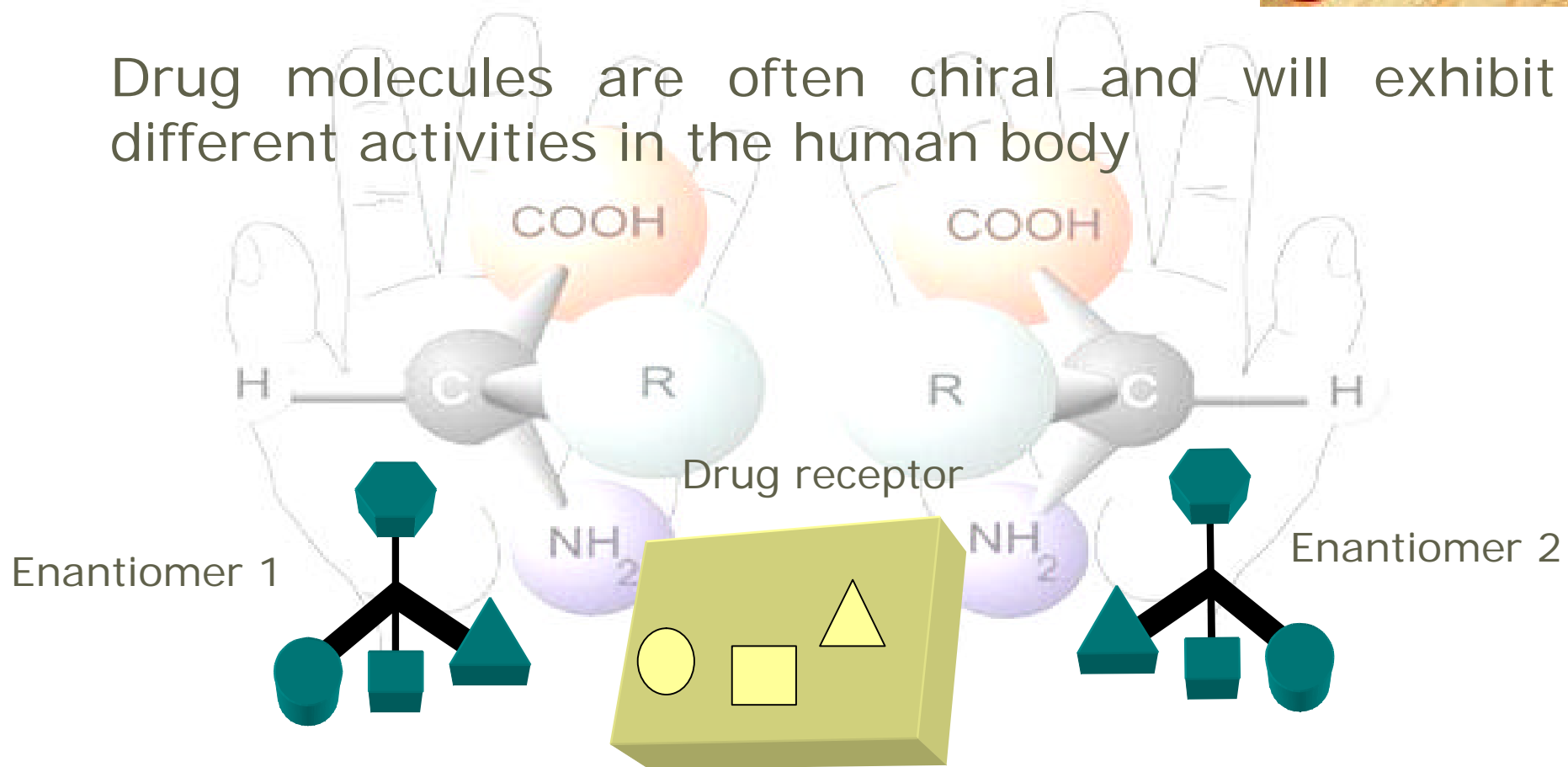
Enantiomers

*Chirality ptilavids*

# Chirality

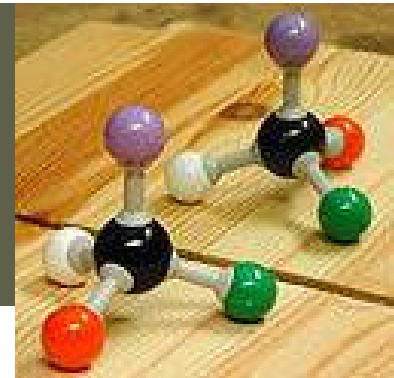


Drug molecules are often chiral and will exhibit different activities in the human body



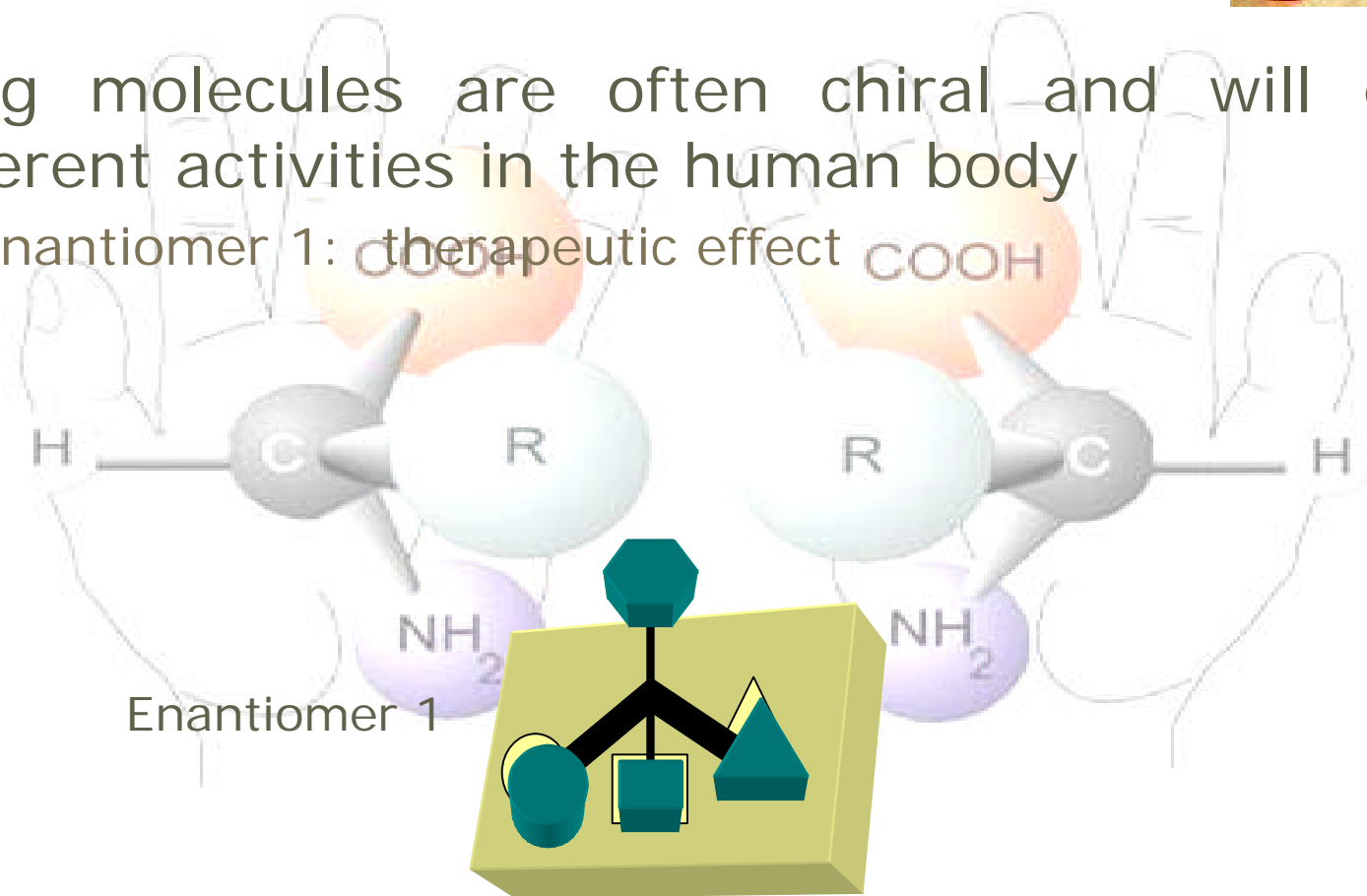
*Chirality of drugs*

# Chirality



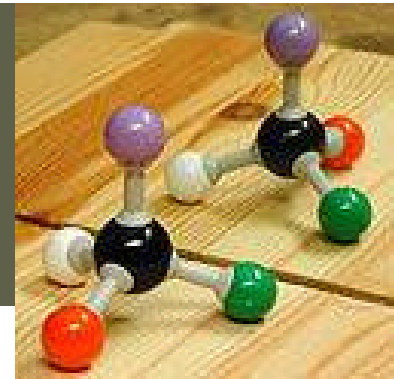
Drug molecules are often chiral and will exhibit different activities in the human body

- Enantiomer 1: therapeutic effect



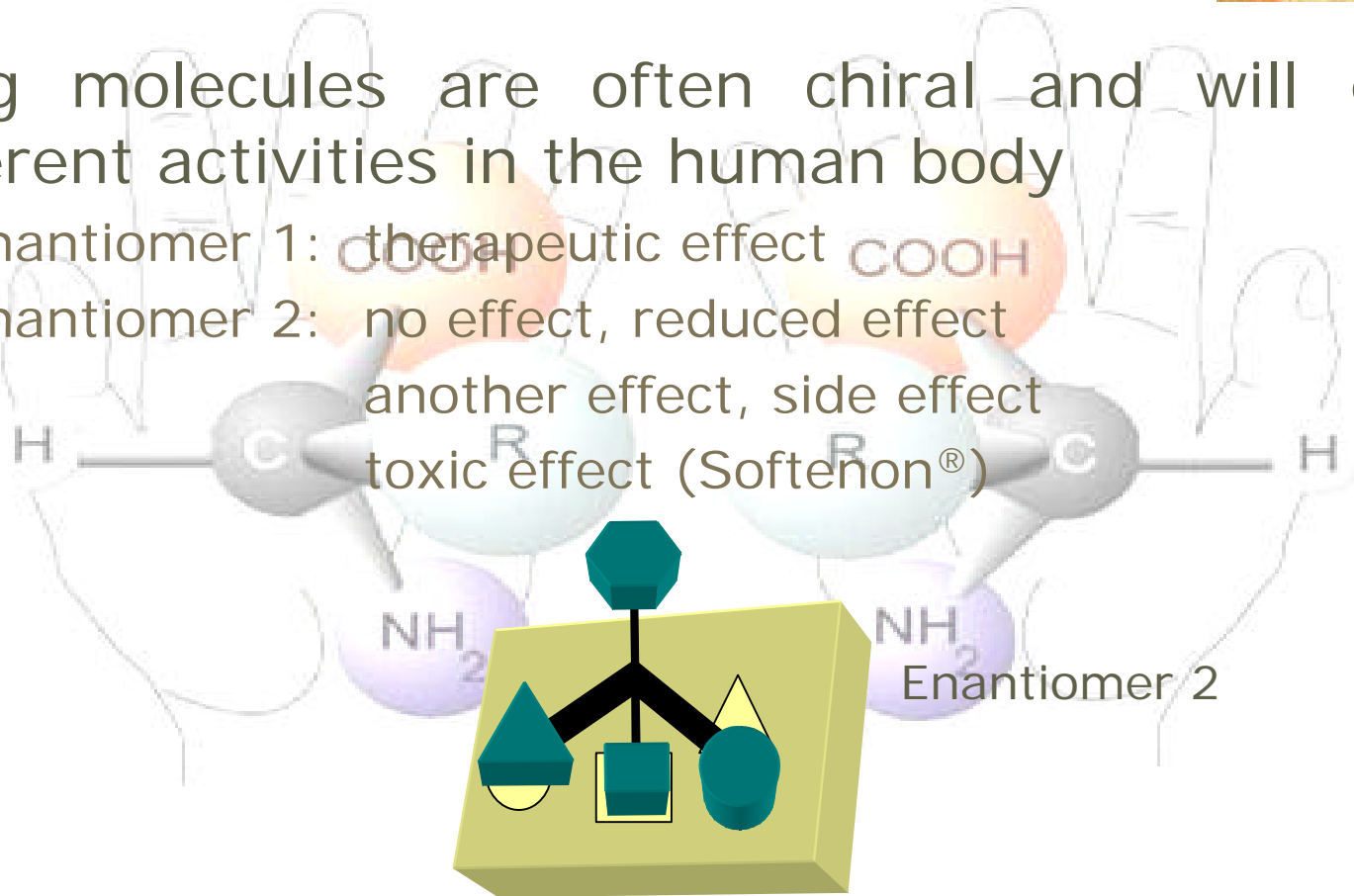
*Chirality of drugs*

# Chirality



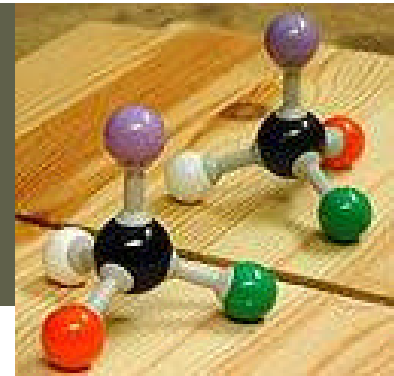
Drug molecules are often chiral and will exhibit different activities in the human body

- Enantiomer 1: therapeutic effect
- Enantiomer 2: no effect, reduced effect  
another effect, side effect  
toxic effect (Softenor<sup>®</sup>)



*Chirality of drugs*

# Chirality



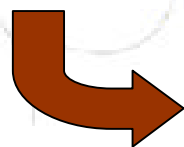
Single-enantiomer drugs preferred

Methods to separate and quantify enantiomers are needed for registration of a drug molecule

In early drug development:

- Racemates (mixture of enantiomers) synthesized
- Fast screening of potential chiral drug molecules is performed → reduces method development time

Generic screening and optimization strategies can be useful



*Chirality ntislarids*

# Chirality

Separation techniques for chiral compounds

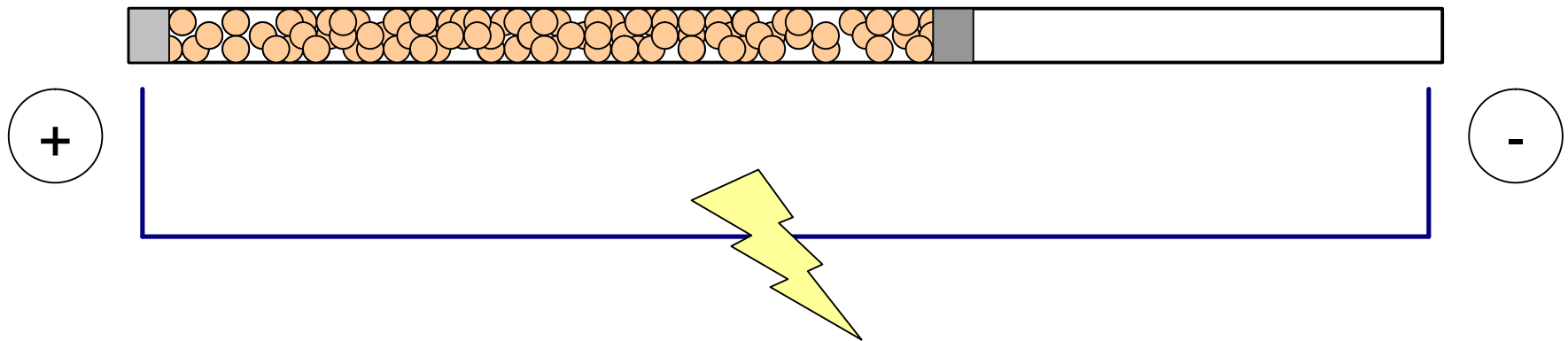
- **NPLC** : **N**ormal-**P**hase **L**iquid **C**hromatography
- **RPLC** : **R**eversed-**P**hase **L**iquid **C**hromatography
- **POSC** : **P**olar **O**rganic **S**olvent **C**hromatography
- **SFC** : **S**upercritical **F**luid **C**hromatography
- **GC** : **G**as **C**hromatography
- **CE** : **C**apillary **E**lectrophoresis
- **CEC** : **C**apillary **E**lectro **C**hromatography???

Strategies + Chiral knowledge-based system



# Capillary Electrochromatography

Hybrid technique: combines HPLC & CE



# Aim

Is it possible to define generic screening and optimization strategies by means of CEC?

- Applicable on large sets of structurally diverse molecules
- Idea of enantioselectivity in a relatively few number of experiments
- Achieve baseline separations ( $R_s = 1,5$ ) for most components

# Methodology

Used CSP:

– Cellulose derivatives

Chiralcel® OD-RH

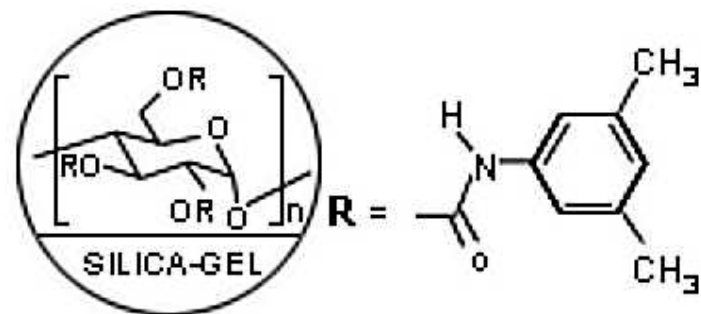
Chiralcel® OJ-RH

– Amylose derivatives

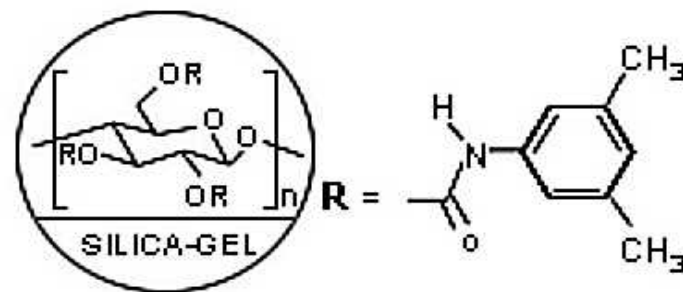
Chiralpak® AD-RH

Chiralpak® AS-RH

– Commercial HPLC phases used – 5  $\mu\text{m}$



Chiralpak AD-RH



Chiralcel OD-RH

# Methodology

- Definition of strategies based on:
  - Literature + preliminary results
  - Experimental design: effects [ACN], pH, applied voltage, [buffer], temperature

↓

Set of limited number (3-5) of components
- Evaluation of the strategies on their applicability by means of large test sets

# Methodology

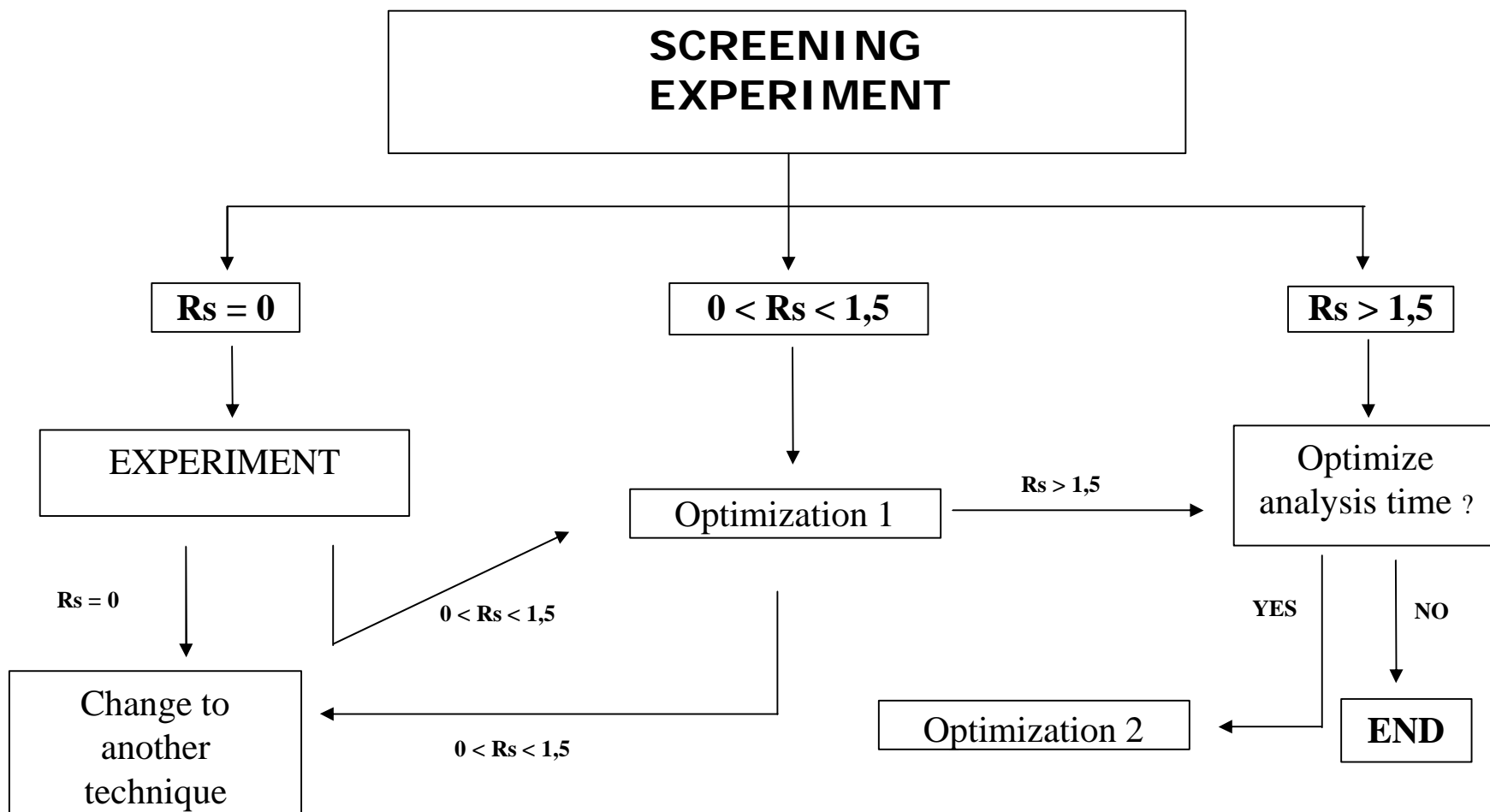
- Neutral chiral selectors
  - Substances must be uncharged
  - Two separate strategies, combined into one



Acidic  
molecules

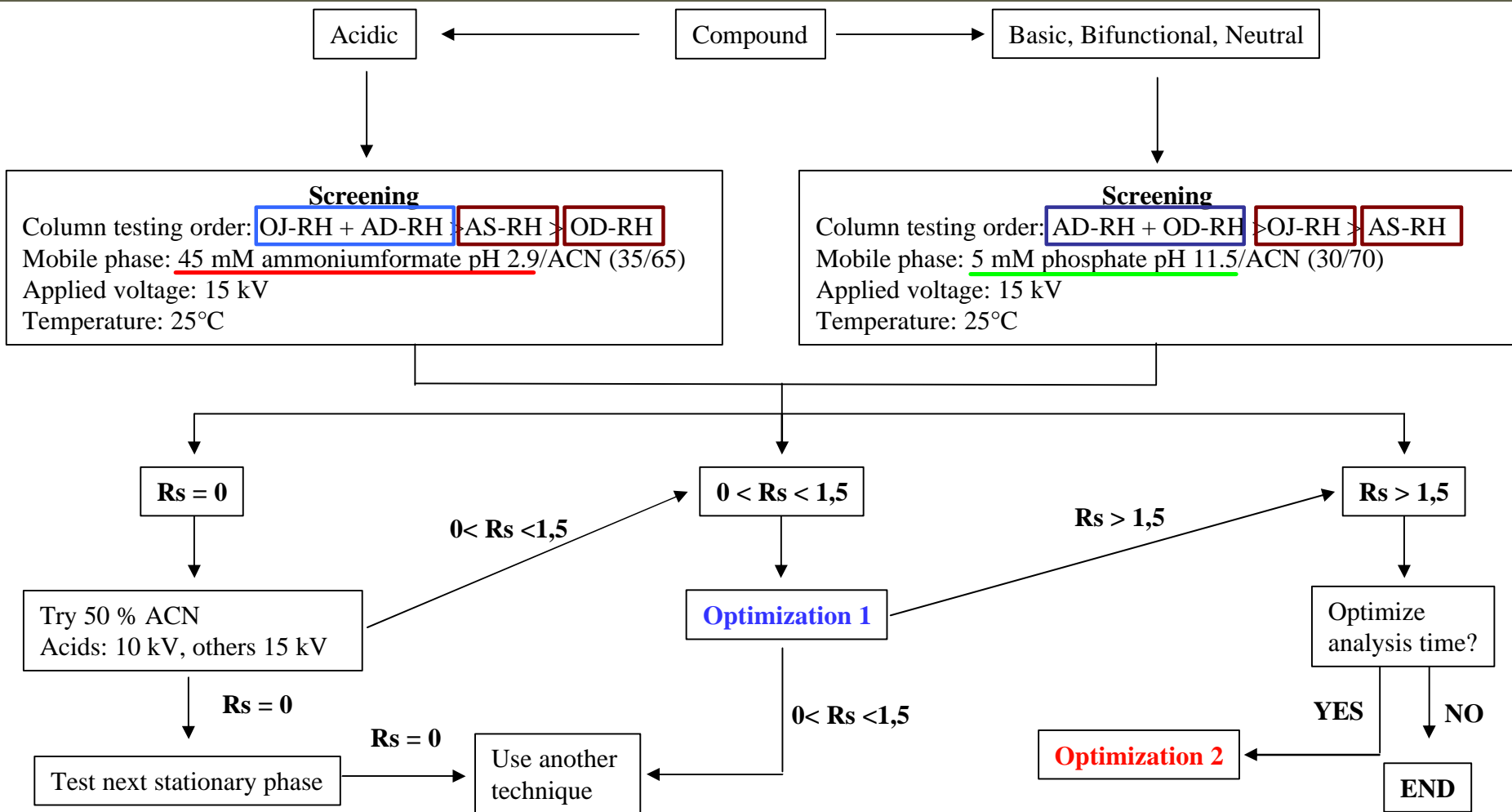
Basic  
Bifunctional  
Neutral

# Chiral Separation Strategy



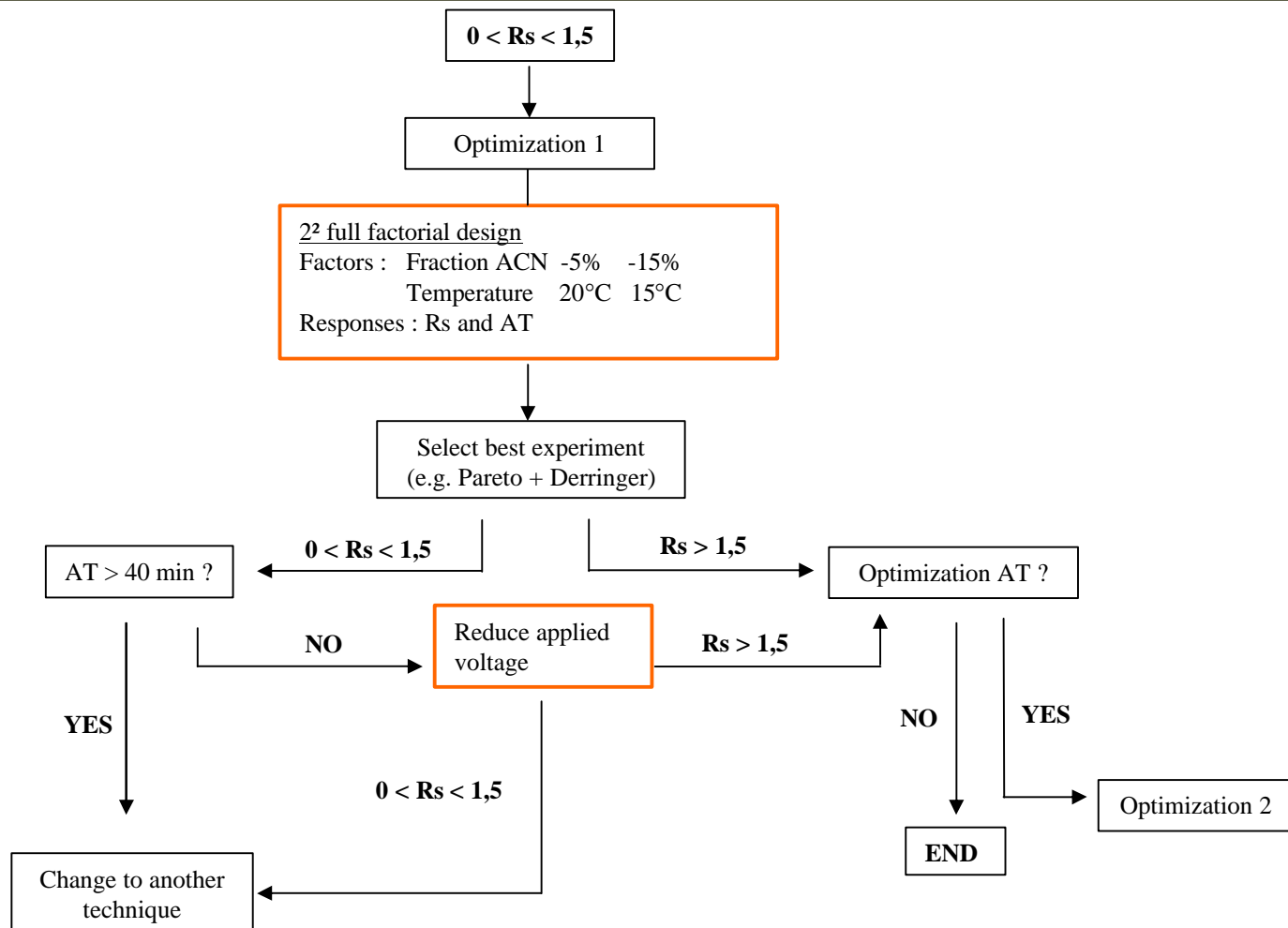
# Chiral Separation Strategy

## General structure



# Acidic compounds

## Optimization 1 ( $0 < R_s < 1,5$ )

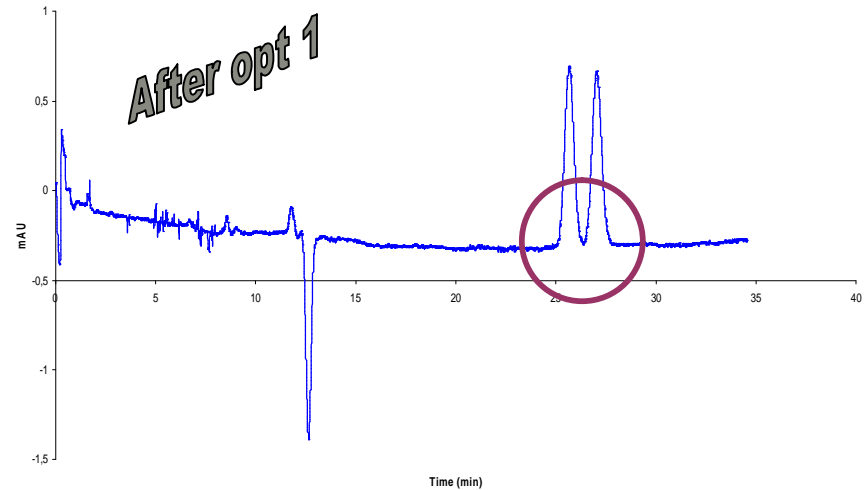
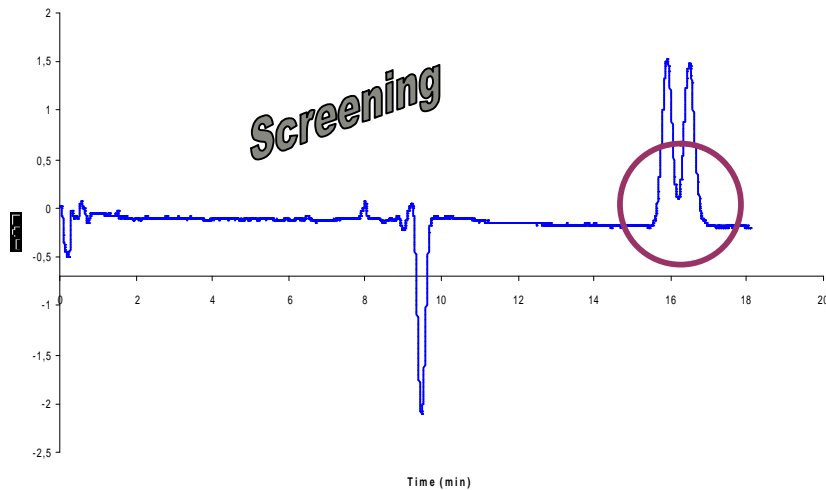




# Acidic compounds

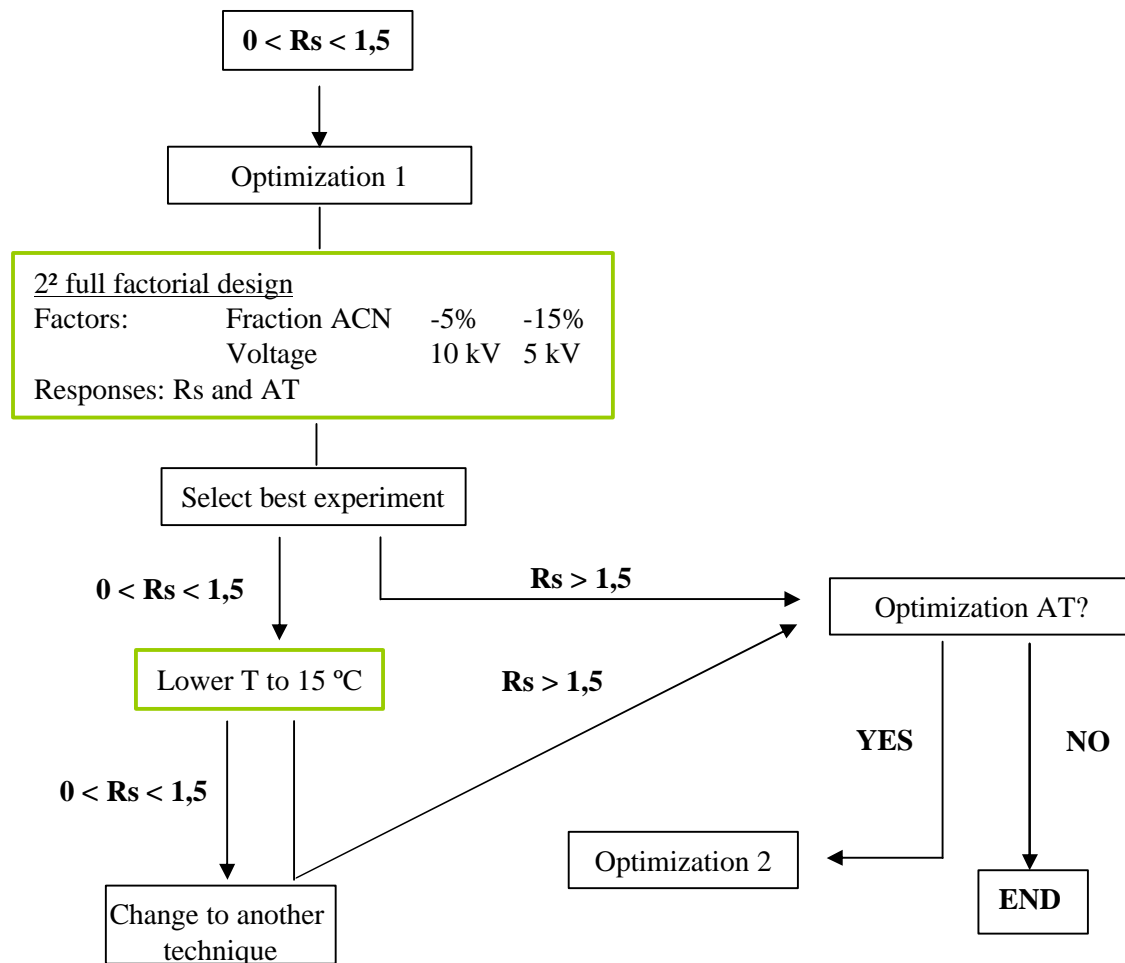
## Optimization 1 ( $0 < R_s < 1,5$ )

Fenoprofen



# Non-acidic compounds

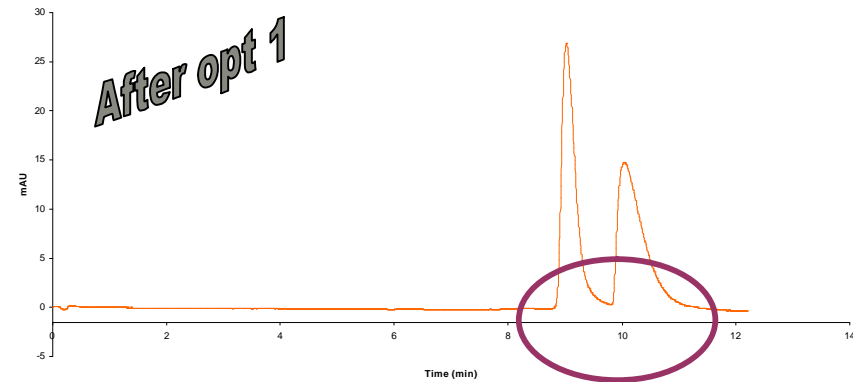
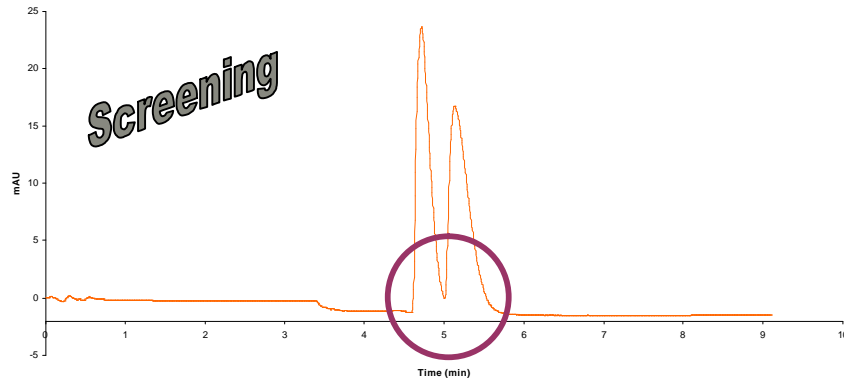
## Optimization 1 ( $0 < R_s < 1,5$ )



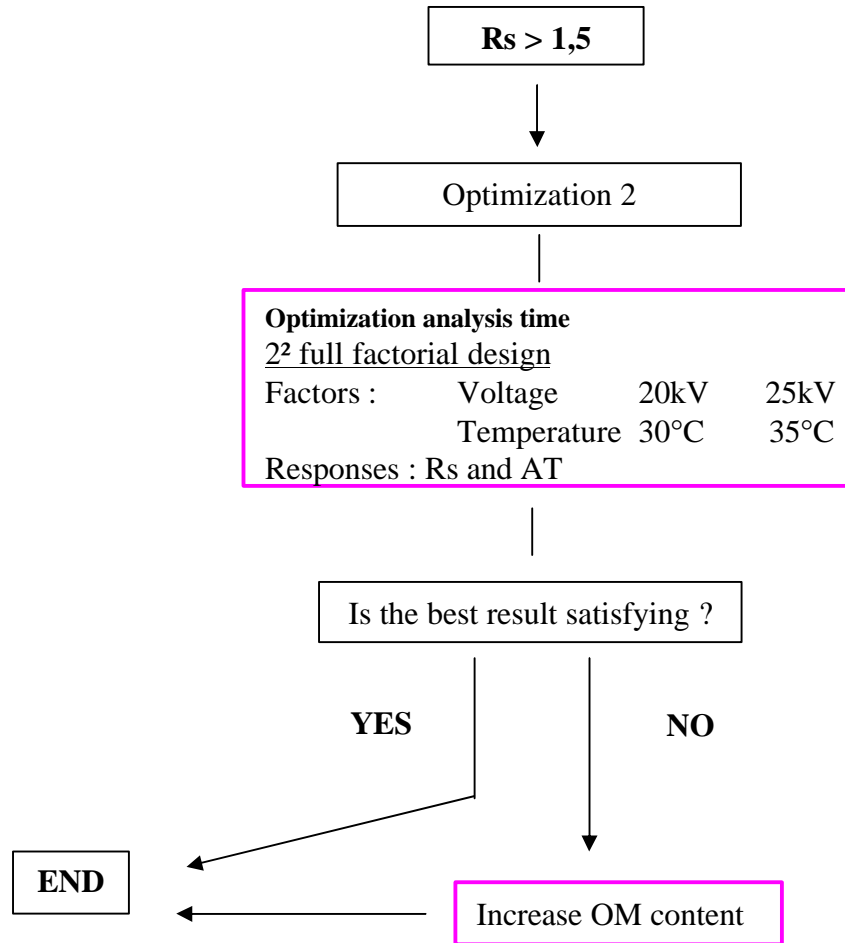
# Non-acidic compounds

## Optimization 1 ( $0 < R_s < 1,5$ )

Toliprolol

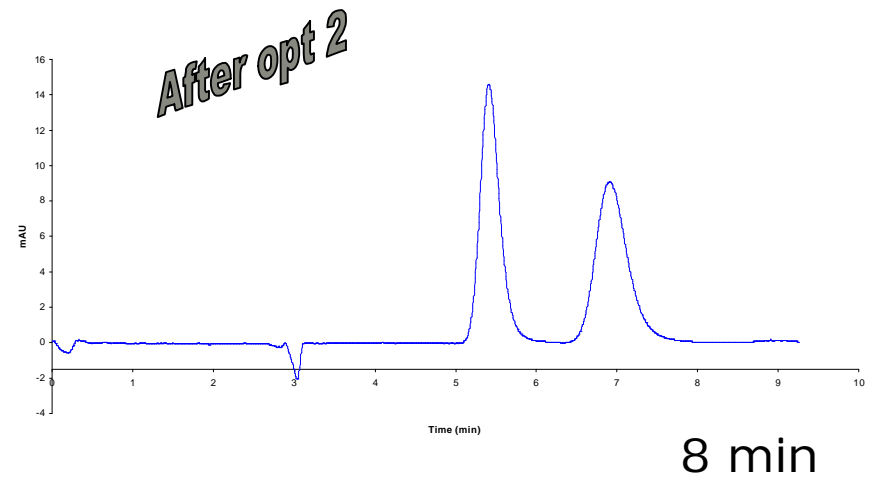
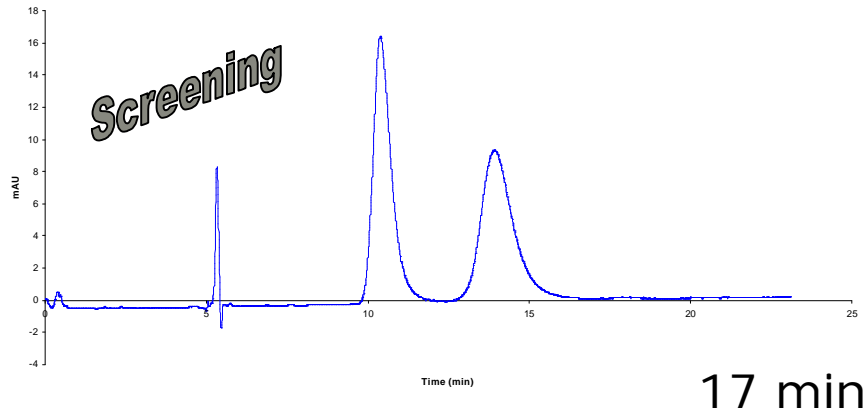


# Acidic & Non-acidic compounds Optimization 2 ( $R_s > 1,5$ )



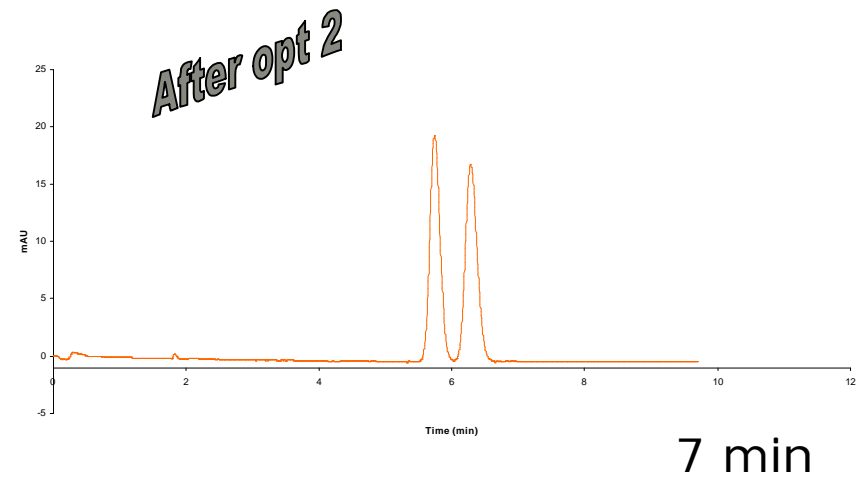
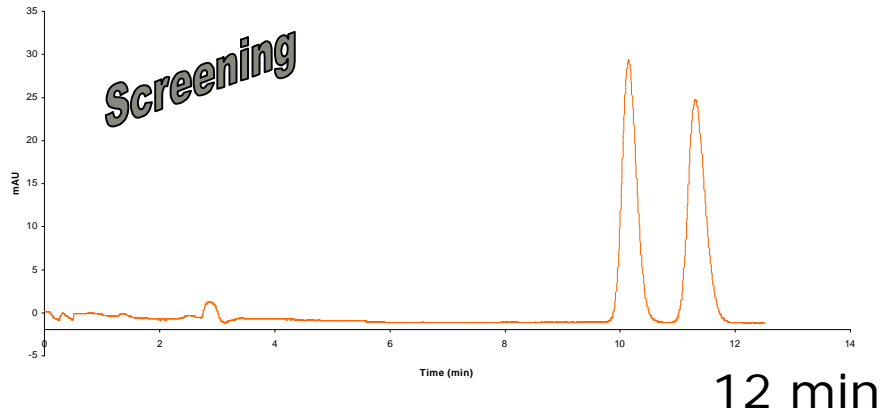
# Acidic compounds Optimization 2 ( $R_s > 1,5$ )

Coumachlor



# Non-acidic compounds Optimization 2 ( $R_s > 1,5$ )

Meberevine



# Evaluation of strategies

## Acidic

### – Screening

- 5/15 baseline separated
- 6/15 partially separated
- 4/15 not separated

### – After optimization steps

- 10/15 baseline separated
- 1/15 partially separated
- 4/15 not separated

# Evaluation strategies

## Non - acidic

### – Screening

- 16/48 baseline separated
- 15/48 partially separated
- 17/48 not separated

### – After optimization steps

- 21/48 baseline separated
- 20/48 partially separated
- 7/48 not separated

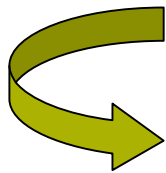


# Conclusions

- CEC has potential for chiral separations
- Generic separation strategies can be defined using polysaccharide CSP
- Good results can be obtained with the proposed strategy
  - ✓ Over 80 % of test compounds showed enantioselectivity after execution
  - ✓ More than 65 % of the substances were partially/baseline separated at screening conditions
  - ✗ Only 49 % resulted in a  $R_s = 1.50$  (optimization)

# Conclusions

- Several drawbacks CEC
  - No robust columns
  - Frits in the columns cause fragility
  - Lack of CEC instruments
  - No loop injection
  - Between-column variability
- Researchers still continue to work with CEC and on these drawbacks



Future CEC not secure, not gone either

# Future perspectives

- Monolithic stationary phases: absence of frits
  - Silica based
  - Polymer based
- Sub-micronized particle stationary phases
  - High efficiencies
- Instrument for CEC allows:
  - CE/CEC/p-CEC/CLC
  - Loop injection, high pressurization

# Acknowledgements

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Thank you for your attention