Basics of Stereochemistry, Major Techniques for Separation of Enantiomers and Overview of Chiral Separation Systems

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Basics of stereochemistry, production of enantiomerically pure compounds and enantioselective analysis

Analytical and preparative-scale liquid-phase separation of enantiomers

Scientific background behind the development of Lux series of chiral columns for HPLC enantioseparations

Overview of currently available columns of Lux series for chiral HPLC separations
Basics of Stereochemistry

- Definition of chirality
- Stereoisomers (enantiomers and diastereomers)
- Stereogenic center
- Axial, planar and helical chirality
- Topological chirality/Chiral objects

Relevance of chirality to biological activity of chemical compounds

Chiral drugs

Comparative description of methods for preparation of enantiomerically pure chiral compounds

Analytical methods for evaluation of enantiomeric purity of chiral compounds
Types of isomers

Constitutional isomers

Cis-trans/geometric isomers

Stereoisomers
  Diastereoisomers
  Meso isomers
  Enantiomers
A chiral molecule is a type of molecule that lacks an internal plane of symmetry and has a non-superimposable mirror image.

The feature that is most often the cause of chirality in molecules is the presence of an asymmetric center.
Enantiomers and Diastereomers

- Stereoisomers contain the same atoms with the same connectivity but with different orientation in space.
- Stereoisomers which relate to each other as the mirror images are enantiomers.
- Stereoisomers which do not relate to each other as the mirror images are diastereomers.
- Diastereomers can be resolved by using achiral stationary phases while for separation of enantiomers a chiral stationary phase (CSP) must be used.

- The sign of optical rotation depends on the solvent used, sample concentration, wavelength, etc. and does not directly relate to the absolute stereochemical configuration of a chiral compound.
Cahn-Ingold-Prelog (CIP) Nomenclature

R  *rectus*  right    S  *sinister*  left

Br > Cl > F > H

R  Fluorochlorobromoethane  S
imaginary mirror
(S)-alanine  |  (R)-alanine

imaginary mirror
Carbon atom as a center of chirality
Nitrogen atom as a center of chirality

Troger’s Base
Sulfur atom as a center of chirality

2-(Benzylsulfinyl)benzamide

2-(Phenylsulfinyl)ethylamide

Benzyl 2-(benzylsulfinyl)benzoic acid benzyl ester

Omeprazole
Phosphor atom as a center of chirality

Cyclophosphamide

Trofosfamide

Iphosphamide
Axial Chirality

- Atropisomers were discovered in 1922
- a Greek for not, *tropos* rotation
Helicenes

Hexahelicene
 Spirocompounds

6,6´-Dimethyl-2,2´-spirobichromane

Di(naphthylcyclopentylketone)

Spira Latin for *twist* or *whorl*
Planar Chirality

- Cyclophans, Ansacompounds, Metallocens
Topological Chirality

- Catenanes, Rotaxanes, Knots
- Macromolecular chirality
- Chirality on the level of objects
Helix

Right-handed (右手－右巻き)

Left-handed (左手－左巻き)
Macromolecular Helicity Induction

S-Enantiomer  =  \[ \text{Functional group} \]  =  R-Enantiomer

Left - handed  \[ H \]  \[ \rightarrow \]  Right - handed

\[ \text{H} \]

\[ H \]

\[ H \]

\[ H \]
Chirality of Objects

Powers of Ten - Helix in Nature

Right-handed
（右手—右巻き）

Left-handed
（左手—左巻き）
Chirality of Objects
Ketotifen

Systematic (IUPAC) name

4-(1-methylpiperidin-4-ylidene)-4,9-dihydro-10H-benzo[4,5]cyclohepta[1,2-b]thiophen-10-one

Lux Cellulose-1
n-Hex/EtOH/DEA=80/20/0.1 v/v/v, 1 ml/min
Basics of Stereochemistry

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Stereogenic center
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Topological chirality (chiral objects)

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Different Biological Effects of Enantiomers

- Taste
- Smell
- Toxicity
- Pharmacology (Thalidomide)
Capillary electrophoresis (CE) is very powerful technique for analytical-scale enantioseparations.

5-Hydroxythalidomide [1,2]

trans-5’-Hydroxythalidomide [3,4]
cis-5’-Hydroxythalidomide [5,6]

Thalidomide [7,8]
Reflections of stereochemistry in the taste of compounds
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Chiral Drugs
“Stereochemistry of the molecule must be carefully considered in all fields of pharmaceutical sciences ranging from discovery to the bedside. An understanding of the role of chirality in the properties of the molecule of interest is undoubtedly a main key to the rational process of developing as well as clinical use of chiral drugs. Keeping in mind the issue of “racemate versus single enantiomer” during various steps of drug discovery and development may save money and time”.

“Driven by needs of the drug industry and fueled by the ingenuity of chemists, sales of single-enantiomer chiral compounds keep accelerating”

Single enantiomers are 20% of best selling drugs

Total number of drugs = 95

Note: Figures are for 95 best selling drugs according to U.S. drugstore sales, excluding hospitals and international sales. Sources are biotechnology, fermentation and extraction. Source: DSM Fine Chemicals
Two-thirds of drugs in development are chiral

- Developed as single isomer: 51%
- Achiral: 32%
- Other chiral*: 17%

Developmental drugs worldwide = 1,200

*a Developed as racemates or no decision made on development

Source: Technology Catalysts International
Chiral Components
Sales of enantiomeric intermediates and single-enantiomer drugs are up

<table>
<thead>
<tr>
<th></th>
<th>ENANTIOMERIC INTERMEDIATES</th>
<th>BULK ENANTIOMERIC DRUGS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-inflammatory/analgesics</td>
<td>$150</td>
<td>$156</td>
</tr>
<tr>
<td>Antiviral</td>
<td>794</td>
<td>830</td>
</tr>
<tr>
<td>Cancer</td>
<td>892</td>
<td>1.073</td>
</tr>
<tr>
<td>Cardiovascular</td>
<td>1.133</td>
<td>2.281</td>
</tr>
<tr>
<td>Central nervous system</td>
<td>1.038</td>
<td>1.142</td>
</tr>
<tr>
<td>Dermatology</td>
<td>82</td>
<td>85</td>
</tr>
<tr>
<td>Gastrointestinal</td>
<td>251</td>
<td>331</td>
</tr>
<tr>
<td>Ophthalmic</td>
<td>238</td>
<td>284</td>
</tr>
<tr>
<td>Respiratory</td>
<td>576</td>
<td>656</td>
</tr>
<tr>
<td>Other</td>
<td>140</td>
<td>170</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td>$5.294</td>
<td>$7.008</td>
</tr>
</tbody>
</table>

SOURCE: Technology Catalysts International
Levalbuterol (1999)  
S-Cetirizine (2000)  
Levalbuterol  
S-Cetirizine  

S-Zopiclone (2002)  
S-Zopiclone  
R,R´-Formoterol  

S-Oxybutinin
Ticalopride

(S)-Doxazosin (2004)

SEP-174559\textsuperscript{d} (2004)

(S)-Sibutramine metabolite\textsuperscript{e} (2004)
(R)-Ondansetron

(S)-Amlodipine

(R)-Norfluoxetine
Structure of Protease Inhibitors for the Treatment of HIV Infection

Saquinavir

Indinavir

Amprenavir

Ritonavir

Lopinavir/Ritonavir

Nelfinavir

L. A. Sorbera, L. Martin, J. Castañer, R. M. Castañer, Drugs of the Future, 2001, 26, 224
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## Strategy and need of enantioseparation techniques in chiral drug development

<table>
<thead>
<tr>
<th>Drug Development step</th>
<th>Required enantiomer</th>
<th>Amount</th>
<th>Cost importance</th>
<th>Importance of scale-up feasibility</th>
</tr>
</thead>
<tbody>
<tr>
<td>Discovery</td>
<td>both</td>
<td>mg-50 g</td>
<td>minor</td>
<td>minor</td>
</tr>
<tr>
<td>Early Development</td>
<td>both</td>
<td>100g-10kg</td>
<td>minor</td>
<td>middle</td>
</tr>
<tr>
<td>Full Development</td>
<td>active enantiomer</td>
<td>5-100 kg</td>
<td>middle</td>
<td>major</td>
</tr>
<tr>
<td>Production</td>
<td>active enantiomer</td>
<td>tons</td>
<td>major</td>
<td>prerequisite</td>
</tr>
</tbody>
</table>
Preparation methods for enantiomers

- Racemates
  - Crystallization
  - Kinetic resolution
- Chiral pool
- Prochiral compounds
  - Synthesis
  - Asymmetric synthesis
Scope and Limitations of Major Production Methods for Enantiomerically Pure or Enriched Molecules

<table>
<thead>
<tr>
<th></th>
<th>Catalysis</th>
<th>Biocatalysis</th>
<th>Chiral pool</th>
<th>Resolutions HPLC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enantioselectivity</td>
<td>1-2</td>
<td>1</td>
<td>1</td>
<td>1-2</td>
</tr>
<tr>
<td>Productivity</td>
<td>1-2</td>
<td>2-3</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Availability, diversity</td>
<td>1-2</td>
<td>2-3</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Substrate specificity</td>
<td>2</td>
<td>3</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Work-up, ecology</td>
<td>1-2</td>
<td>2-3</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Development time, effort</td>
<td>2</td>
<td>3</td>
<td>1</td>
<td>1-2</td>
</tr>
<tr>
<td>Application in the lab</td>
<td>2</td>
<td>3</td>
<td>1</td>
<td>1-2</td>
</tr>
<tr>
<td>Application in development</td>
<td>1-2</td>
<td>2</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Small scale production</td>
<td>1-2</td>
<td>1-2</td>
<td>1</td>
<td>1-2</td>
</tr>
<tr>
<td>Large scale production</td>
<td>1</td>
<td>2-3</td>
<td>2</td>
<td>1-2</td>
</tr>
</tbody>
</table>

Rating: 1-high; 2-medium/some problems; 3-low/problematic.
“For products in development, the time to develop a catalytic asymmetric process may not meet the need to get materials out rapidly for testing”

A.M. Rouhi, C & EN, June 14, 2004
Chiral Pool

L-\((+)-\text{Tartaric acid}\)

\[\text{(S,S)-Ethambutol}\]
Enantioselective Synthesis

Esomeprazole Sodium, 99.5% ee

Esomeprazole, 94.5% ee
Preparation methods for enantiomers

- **Racemates**
  - Crystallization
  - Kinetic resolution

- **Chiral pool**
  - Synthesis

- **Prochiral compounds**
  - Asymmetric synthesis
Methods for the Resolution of Racemates

Racemates

- Preferential crystallization
- Kinetic resolution
- Diastereomeric crystallization

Chemical

Enzymatic
Examples of Pharmaceuticals Resolved using Diastereomeric Crystallization in the Process

<table>
<thead>
<tr>
<th>Pharmaceutical</th>
<th>Resolving agent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin</td>
<td>D-Camphorsulphonic acid</td>
</tr>
<tr>
<td>Ethambutol</td>
<td>L-(+)Tartaric acid</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>D-Camphorsulphonic acid</td>
</tr>
<tr>
<td>Dextropropoxyphene</td>
<td>D-Camphorsulphonic acid</td>
</tr>
<tr>
<td>Fosfomycin</td>
<td>R-(+)Phenethylamine</td>
</tr>
<tr>
<td>Thiamphenicol</td>
<td>D(-)-Tartaric acid</td>
</tr>
<tr>
<td>Naproxen</td>
<td>Cinchonidine</td>
</tr>
<tr>
<td><em>cis</em>Diltiazem</td>
<td>R-(+)Phenethylamine</td>
</tr>
</tbody>
</table>
The diagram illustrates the synthesis of EMD-53998, starting from (I) and (II), followed by various chemical reactions involving reagents like AlCl₃, HCl, Et₃N, and heat. The processes include the chromatographic separation of diastereomers and enantiomers, resulting in the racemic form rac-(VIII) and the enantiomers (+)-EMD-53998 and (-)-EMD-53998.
“Despite the unrelenting pace of research in catalytic asymmetric chemistry, relatively few catalytic enantioselective processes are currently operated on a commercial scale. Until more bio- and chemocatalytic chiral routes are developed that are robust and cost-effective for large-scale production, the bulk of optically pure compounds will have to be prepared through traditional chemistry, including conventional syntheses based on chiral substances or stoichiometric chiral induction and separations, such as chromatographic resolutions.”

A.M. Rouhi, C & EN, June 14, 2004
Preparative-scale Liquid-chromatographic Separation of Enantiomers
Chromatographic Separation Methods useful for Preparation of Enantiomers

- Column Chromatography
- Recycling Loop Chromatography
- Simulating-Moving Bed Chromatography
- VARICOL
Schematics of SMB Unit

SMB is a continuous separation process
Selected examples of applications of SMB-chromatography for the production of enantiomerically pure compounds.

<table>
<thead>
<tr>
<th>Chiral compound</th>
<th>CSP</th>
<th>Capacity</th>
<th>Consumption of the mobile phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aminoglutethimide</td>
<td>Chiralcel OJ</td>
<td>7.5 g/h·kg</td>
<td>0.740 ml</td>
</tr>
<tr>
<td>1,1’Binaphthyl-2,2’-diol</td>
<td>Pirkle-type 3,5-DNBPG</td>
<td>2000 g was resolved per day</td>
<td></td>
</tr>
<tr>
<td>2000 g was resolved per day</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chiral drug candidate (potent partial agonist at muscarinic receptors)</td>
<td>Chiralpak AD</td>
<td>ca. 30-60 g/h·kg</td>
<td>ca. 0.190-0.380 ml</td>
</tr>
<tr>
<td>CGS 26 214</td>
<td>Chiralcel OJ</td>
<td>47 g/d·kg</td>
<td>4.561</td>
</tr>
<tr>
<td>Cycloalkanone</td>
<td>Chiralcel OC</td>
<td>1082 g/d·kg</td>
<td>0.280</td>
</tr>
<tr>
<td>DOLE</td>
<td>Chiralcel OF</td>
<td>272 g/d·kg</td>
<td>0.440</td>
</tr>
<tr>
<td>EMD 53 986</td>
<td>Polyacrylamide</td>
<td>319 g/d·kg</td>
<td>2.540</td>
</tr>
<tr>
<td>EMD 77 697</td>
<td>Chiralpak AD</td>
<td>432 g/d·kg</td>
<td>2.600</td>
</tr>
<tr>
<td>EMD 122 347</td>
<td>Chiralcel OD</td>
<td>451 g/d·kg</td>
<td>1.640</td>
</tr>
<tr>
<td>Formoterol</td>
<td>Chiralcel OJ</td>
<td>1.2 g/h·kg</td>
<td>0.515</td>
</tr>
<tr>
<td>Hetrazepine</td>
<td>Cellulose triacetate (CTA)</td>
<td>119 g/h·kg</td>
<td>0.094</td>
</tr>
<tr>
<td>Guaiifenesin</td>
<td>Chiralcel OD</td>
<td>10.0 g/h·kg</td>
<td>0.380</td>
</tr>
<tr>
<td>Oxo-oxazolidine</td>
<td>L-Chiraspher</td>
<td>6.8 g/h·kg</td>
<td>0.900</td>
</tr>
<tr>
<td>Phenylethylalcohol</td>
<td>Chiralcel OD</td>
<td>0.98 g/L</td>
<td>5.300</td>
</tr>
<tr>
<td>Praziquantel</td>
<td>CTA</td>
<td>4.7 g/h·kg</td>
<td>0.056</td>
</tr>
<tr>
<td>Propranolol</td>
<td>Chiralcel OD</td>
<td>3.83 g/h·kg</td>
<td>0.200</td>
</tr>
<tr>
<td>Sandoz-epoxide</td>
<td>CTA</td>
<td>59 g/d·kg</td>
<td>0.800</td>
</tr>
<tr>
<td>Sandoz-epoxide</td>
<td>Chiralcel OD</td>
<td>37 g/d·kg</td>
<td>0.500</td>
</tr>
<tr>
<td>1a, 2, 7, 7a-Tetrahydro-3-methoxy-naphth(2,3-6) oxirane</td>
<td>CTA</td>
<td>1.45 g/h·kg</td>
<td>0.400</td>
</tr>
<tr>
<td>Threonine</td>
<td>Chirosolve L-proline</td>
<td>30 g was resolved</td>
<td></td>
</tr>
<tr>
<td>Tramadol</td>
<td>Chiralpak AD</td>
<td>50 g/h·kg</td>
<td>0.500</td>
</tr>
<tr>
<td>Wieland-Mieschler Ketone</td>
<td>Chiralpak AD</td>
<td>84 g/h·kg</td>
<td>0.490</td>
</tr>
</tbody>
</table>
Loadability of Chiral Stationary Phases

- Protein-based CSPs
  - Vancomycin CSP
- Cyclodextrin-based CSPs
- Tetraamide CSPs (Kromasil)
- Polyacrylamide (Chiraspher)
- Brush-type CSPs (Pirkle)
- Polysaccharide-based CSPs
Basics of Stereochemistry

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“Good analytics are crucial with any project. If they are not reliable or are slower than the throughput of screening systems, they are useless”

Major Techniques Available for Separation of Enantiomers

Gas Chromatography (GC)
High Performance Liquid Chromatography (HPLC)
Capillary Electrophoresis (CE)
Capillary Electrochromatography (CEC)
Supercritical Fluid Chromatography (SFC)
Gas Chromatography

Advantages:
Relatively high peak efficiency

Disadvantages:
Applicable only for thermostable and volatile compounds
Limited variety of available stationary phases
No flexibility from the viewpoint of selectivity adjustment based on the mobile phase
Not easy to be applied for preparative separations
High-Performance Liquid Chromatography (HPLC)

Advantages:

Applicable for thermolabile and nonvolatile compounds

Wide variety of stationary phases are available

Selectivity can be adjusted based on the mobile phase

Applicable for preparative scale separations

Disadvantage

Lower plate numbers than in other separation techniques (GC, SFC and CE)
Stationary Phases Available for HPLC Enantioseparations

Pirkle (Brush) type chiral selectors

Proteins

Cyclodextrines (Cyclofructans)

Synthetic chiral polymers (polyacrilamides, polymethacrylates)

Macrocyclic antibiotics

Cinchona alkaloids

Polysaccharides
Why shall we use polysaccharide derivatives as chiral stationary phases (CSP) in liquid phase separation techniques?

• Polysaccharide based materials are rather universal CSPs

• These materials can be used with normal-, reversed- and polar organic mobile phases

• Applicable for both pressure and voltage-driven separations

• Extremely high enantioselectivity can be achieved using these CSPs for various group of analytes

• A large family of polysaccharide-based CSPs are available

• Polysaccharide-based CSPs are very useful for preparative and product scale enantioseparations
Loadability of Chiral Stationary Phases

Protein-based CSPs
- Vancomycin CSP

Cyclodextrin-based CSPs

Tetraamide CSPs (Kromasil)

Polyacrylamide (Chiraspher)

Brush-type CSPs (Pirkle)

Polysaccharide-based CSPs
Super/Sub-critical Fluid Chromatography (SFC)

Advantages:

Higher plate numbers than in HPLC (but lower than in GC and CE)
Applicable for thermolabile and nonvolatile compounds
Applicable for preparative separations
Uses HPLC stationary phases
Green(er) technology

Disadvantages:

No alternative (its own) separation principle
Lower flexibility from the viewpoint of mobile phases
Capillary Electrophoresis

Advantages:

Alternative separation mechanism

Very high plate numbers

Various chiral selectors available

High flexibility from the viewpoint of adjustment of selectivity (and enantiomer migration order)

Miniaturized technique

Disadvantages:

May require special skills on order to achieve required reproducibility/repeatability

Not applicable for gaseous molecules

Not applicable for preparative separations
Separation Technology

Chiral Selector

Carrier

Mobile Phase
Major Liquid-phase Instrumental Techniques Available for Separation of Enantiomers

High Performance Liquid Chromatography (HPLC)

Supercritical Fluid Chromatography (SFC)

Capillary Electrophoresis (CE)

Capillary Electrochromatography (CEC)

(Microfabricated devices)
Electrical Isolation

- Fused silica capillary
- Pt electrodes
- High voltage supply
- Buffer reservoirs
- Detector
CZE

\[ \mu = \frac{v}{E} = \frac{Q}{6\pi \gamma \eta} \]

\[ E = \frac{V}{d} \]
Flow profiles in chromatography and CE

- Parabolic flow
  - Chromatography
  - Δp

- Plug-like flow
  - Electrodriven techniques
  - ΔE

Narrow peaks
Capillary electrophoresis (CE) is a very powerful technique for analytical-scale enantioseparations.
Enantioseparation of meptazinol with β-CD column in HPLC and simultaneous enantioseparation of meptazinol and metabolites in CE using β-CD as a chiral selector.
Capillary electrophoresis is very powerful technique not only for analytical scale enantioseparations but also for investigation of fine mechanisms of enantioselective intermolecular interactions.
Advantages of CE for Separation of Enantiomers

CE allows very fast screening of selector-select and interactions in order to prevail the most promising chiral selector. There is no other instrumental separation or non-separation technique that may compete with CE in this respect.

The high peak efficiency in CE permits to observe (enantio)selective features in selector-selectand interactions which are invisible by other (separation) techniques.

A small thermodynamic selectivity of recognition can be transformed into a high separation factor in CE.

CE is more flexible than chromatographic techniques in order to adjust the (enantio)separation factor

Combination of chiral selectors is much easier in CE than in HPLC.
Techiques Available for Mechanistic Studies Related to Chiral CE:

1. X-ray crystallography
   (Provides information about most likely stoichiometry and structure of selector-selectand complex but in the solid state)

2. NMR spectroscopy
   (Provides information about the stoichiometry, binding constants and structure of selector-selectand associates in solution but is not suitable for mixed complexes)

3. Mass Spectrometry
   (Provides information about the stoichiometry and the binding constants of selector-selectand complexes and is also suitable for mixed complexes)

4. Molecular Modeling/Mechanics Calculations
   (May allow to calculate major forces involved in intermolecular interactions and in chiral recognition)
X-Ray Structure of the Complexes of brompheniramine maleate with β-CD (a) and heptakis-(2,3,6-tri-O-methyl)-β-CD (b)
Affinity of Aminoglutethimide Enantiomers Towards Native Cyclodextrins (α, β, γ)
H$^1$-NMR spectra of (±)-aminoglutethamide complexes with α-, β-, and γ-CDs
β-CD/aminogluthethimide Complex
β-Cyclodextrin/aminogluthethimide Complex
γ- CD/aminoglutethimide Complex

H(3) H(6) H(4)

H(2) H(5)

CH₂ CH₃

CH₂-CH₃

ppm 6.4 4.8 3.3 1.6 0.6
\( \gamma \)-Cyclodextrin/aminogluthethimide Complex
CE Enantioseparation and the Structure of Complexes Between aminoglutethimide and β- and γ-cyclodextrins

Aminoglutethimide
1D-ROESY Spectrum of (+/-)-clenbuterol Complex with β-CD
1D-ROESY spectrum of (+/-)-clenbuterol complex with HDA-β-CD
1D-ROESY spectrum of (+/-)-clenbuterol complex with HDA-β-CD
Enantioseparation of clenbuterol with β-CD and heptakis-(2,3-diacetyl-β-CD) and structure of intermolecular complexes in solution.
MM+ Optimized Structure of clenbuterol/β-CD Complex
MM+ Optimized Structure of clenbuterol/β-CD Complex
MM+ optimized structure of clenbuterol/HDA-β-CD complex
MM+ optimized structure of clenbuterol/HDA-β-CD complex
Electrophoresis, 2010, 31, 1667-1674
J. Sep. Sci., 2010, 33, 1617-1624
Correlations Between Structure and Binding

(Nonaqueous medium)

R-Propranolol/HDAS-β-CD

S-Propranolol/HDAS-β-CD
Major Liquid-phase Instrumental Techniques Available for Separation of Enantiomers

- High Performance Liquid Chromatography (HPLC)
- Supercritical Fluid Chromatography (SFC)
- Capillary Electrophoresis (CE)
- Capillary Electrochromatography (CEC)

(Microfabricated devices)
Capillary Electrochromatography

- o-CEC (open tubular capillary electrochromatography)
- p-CEC (packed capillary electrochromatography)
- rod-CEC (monolithic capillary electrochromatography)
p-CEC

- Combines selectivity of LC and efficiency of CE
- Applicable for neutral compounds
Pore structure and particle size of packing material
Scanning electron microscopy (SEM)
Monolithic columns for CEC

- Inorganic (silica-based) monoliths
- Organic Monoliths
- Hybrid Organic-inorganic monoliths
100 µm ID monolithic silica capillary before and after modification with polysaccharide derivative
CLC vs. CEC

2.5mM ammonium acetate in MeOH
Native silica- 2000 Å, 5µm, 5% CDCPC

♦ At the optimal flow rate peak efficiency in CEC is at least two times higher compared to capillary LC

♦ CEC tolerates high linear flow velocities much better than capillary LC
Does CEC offer higher peak efficiency compared to CLC under identical conditions?

a) Capillary LC: 12 bar inlet vial

b) CEC: -5 kV
   \( N_1 : 160061/m \)
   \( N_2 : 154852/m \)
   \( R_S : 1.65 \)

2-Benzylsulfinylbenzamide

The diagram shows the comparison of peak efficiency between CLC and CEC.

CLC

CEC
CLC vs. CEC

At the optimal flow rate peak efficiency in CEC is at least two times higher compared to capillary LC

♦ CEC tolerates high linear flow velocities much better than capillary LC

2.5mM ammonium acetate in MeOH
Native silica- 2000 Å, 5µm, 5% CDCPC
Fast Enantioseparation of (±)-piprozolin in CEC

5% CDCPC, CSP: Aminopropylsilanized silica gel (2000 Å, 5µm), 8.5 cm packed bed.
Buffer: 10 mM ammonium acetate; Applied voltage: 25 kV
Enantioseparation of thalidomide and its metabolites in CLC and CEC

CLC (13 bar)  CEC (20 kV)

Separation conditions: 25 % (OD+AD), native silica 2000 Å, 5 µm. Buffer: 10 mM ammonium acetate in (MeOH+EtOH (55/45).