DAICEL CHIRAL TECHNOLOGY

Principal of Chiral HPLC Column

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DAICEL
Sustainable Value Together

Applied Chemical & Instrument Co.,Ltd.

DAICEL CHIRAL TECHNOLOGIES
(INDIA) PRIVATE LIMITED

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Overview of Chiral Separation

www.chiraltech.com

CHIRAL CHROMATOGRAPHY
Analytical | Preparative | Commercial
www. Chiraltech.com

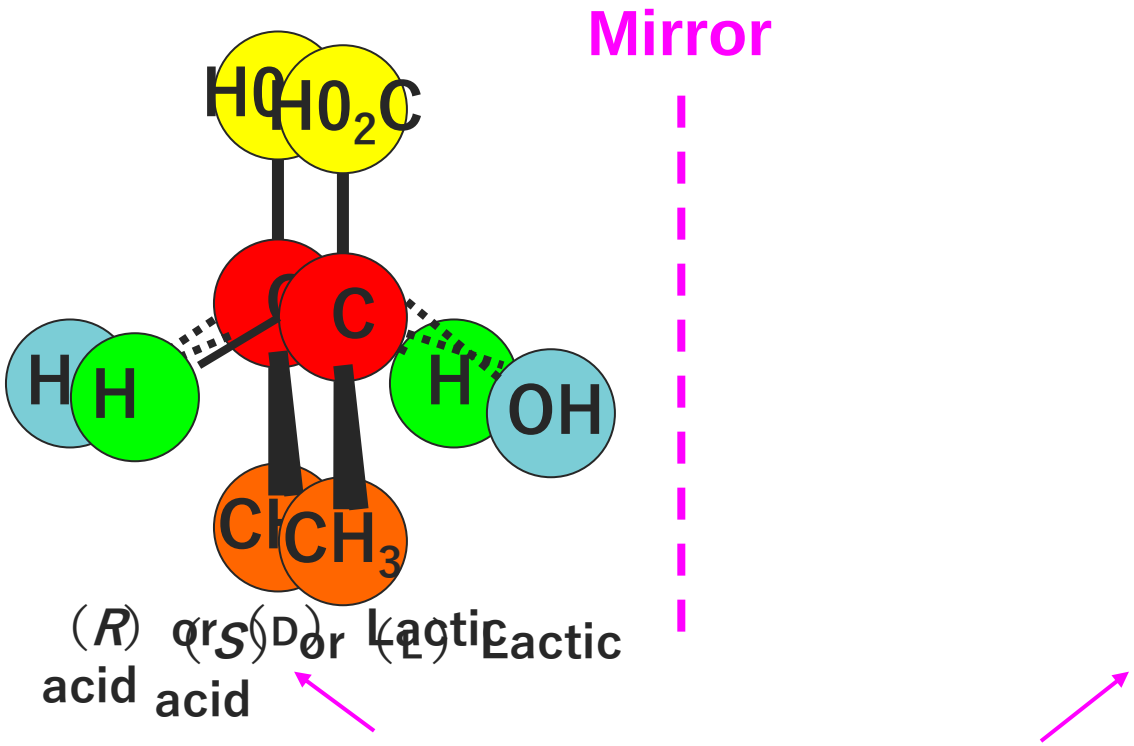
PHARMA SERVICES
Analytical | Purification | Synthesis
www.daicelpharmaservices.com

PHARMA STANDARDS
Impurity | Labeled | Peptide
www.daicelpharmastandards.com

1. Overview of chiral separation “What is chiral?”
2.Purpose of using chiral column(Difference between ODS column and Chiral column)
3.International guideline of enantiomeric purity analysis
4.Application of chiral generic drug (USP, new method)
5.Q&A

Overview of chiral separation
“What is chiral?”

Optical isomer (Enantiomer)

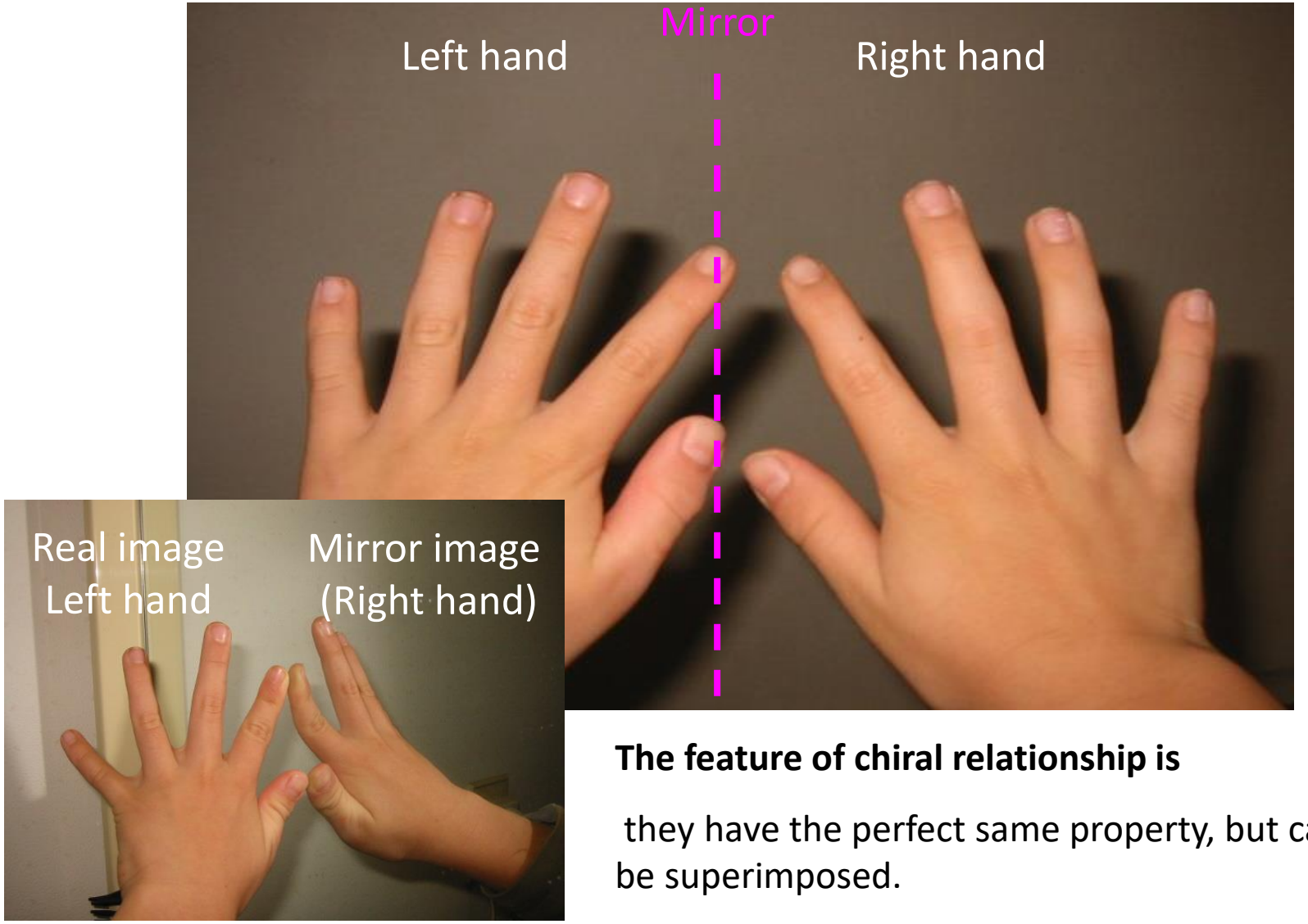


The important features;

・Physical and chemical properties, such as boiling point, melting point and solubility into solvents are perfectly identical except for direction of rotation of plane polarized light.

・Sometimes these isomers show different bioactivities to animals and plants.

Chirality : Right and left hands



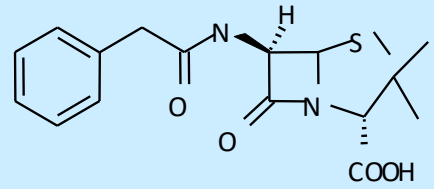
The feature of chiral relationship is

they have the perfect same property, but can never be superimposed.

Differences of Bioactivities between Enantiomers （drug)

A. Only one enantiomer is active

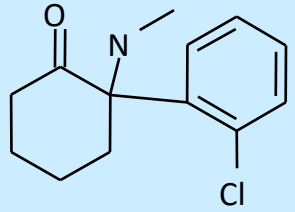
(Penicillin G)



（＋）: antibacterial
（－）: NOT antibacterial

B. Another enantiomer has side-effect

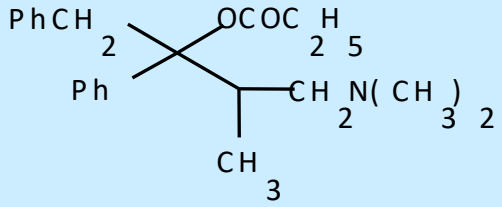
(Ketamine)



（＋）: anesthetically-active
（－）: anxiogenic effect

C. Both enantiomers have different activity

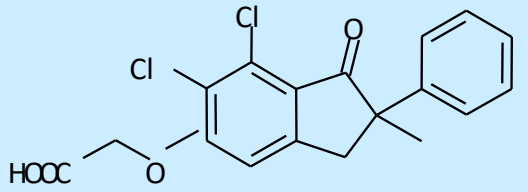
(Propoxyphene)



（＋）: Analgesic action **Darvon**
（－）: Antitussive action **Novrad**

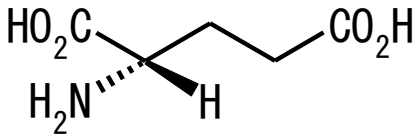
D. Inhibit another side-effect by combination use

(Indacrinone)



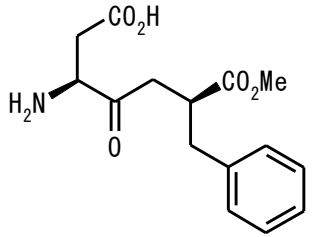
（＋）: Diuretic action, accumulated action of uric acid
（－）: Inhibit accumulated action of uric acid of（＋）

Differences of Bioactivities between Enantiomers
(food・fragrance・pesticide)



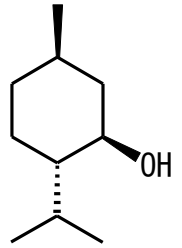
Glutamic acid

(S) Umami-taste
(R) Umami-free



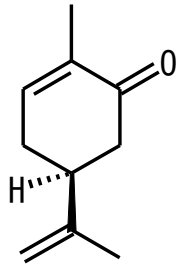
Aspartame

(S,S) 200 Times as sweet as sugar
(R,R) Bitter



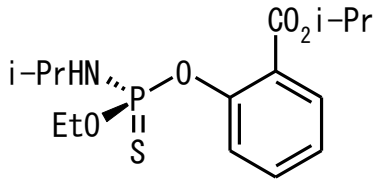
Menthol

(-) Feeling of coolness
(+) Small effect



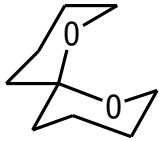
Carvone

(+) Caraway odor
(-) Spearmint odor



Isofenphos

(+) Insecticidal effect：greater
(-) Insecticidal effect：minimally



Pheromone of Olive fruit fly

(S) To female Olive fruit fly
(R) To male Olive fruit fly

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Purpose of using chiral column

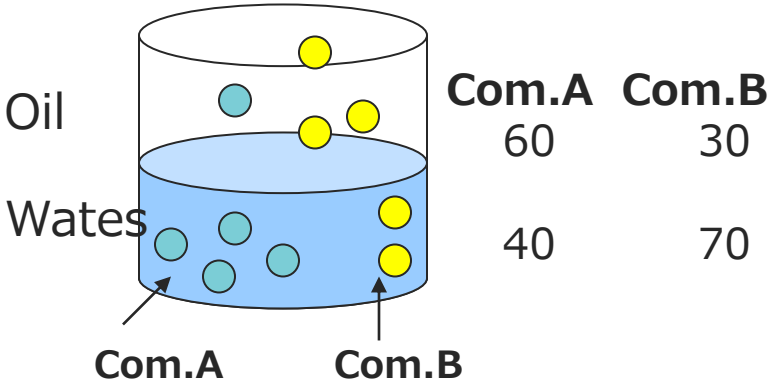
(Difference between ODS column and Chiral column)

Principle of chromatographic separation

Separation can be performed based on physical property,
such **as (1) Solubility, (2) Adsorption property, (3) Vaporization……**

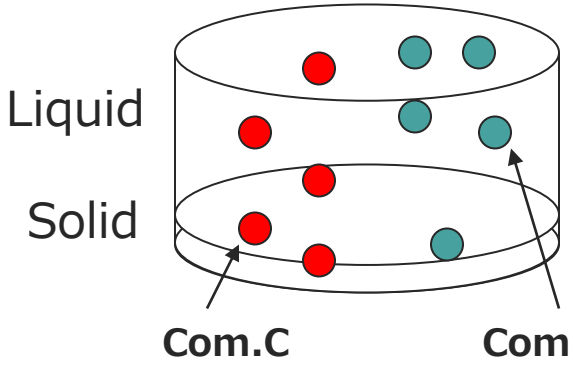
In chromatographic separation compounds are separated based on the difference of several physical property under more than 2kinds of environments.

Distribution based on solubility



	Com.A	Com.B
Oil	60	30
Wates	40	70

Distribution based on solubility and adsorption



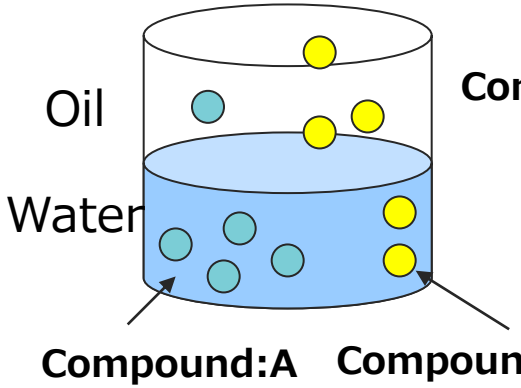
	Com.C	Com,D
Liquid	40	80
Solid	60	20

In almost cases, 2kinds of compounds never show the perfect same physical property under multi conditions.

→ Even if subtle differences, chromatographic method can success separation by amplification of such subtle differences.
For example, the difference between 49.9999999:50.0000001

Principle of ODS column separation

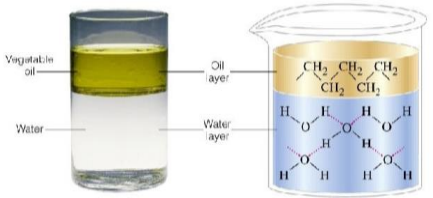
Solubility into water and oil

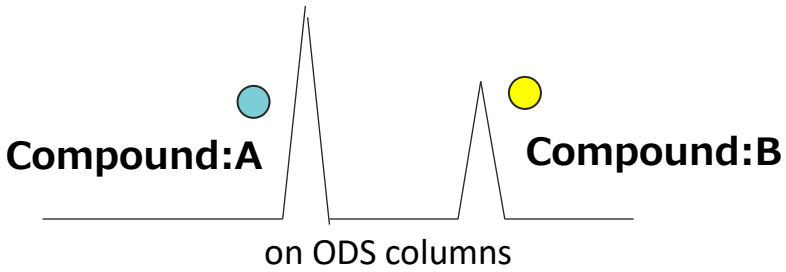


	Comp.A	Comp.B
Oil	20	60
Water	80	40

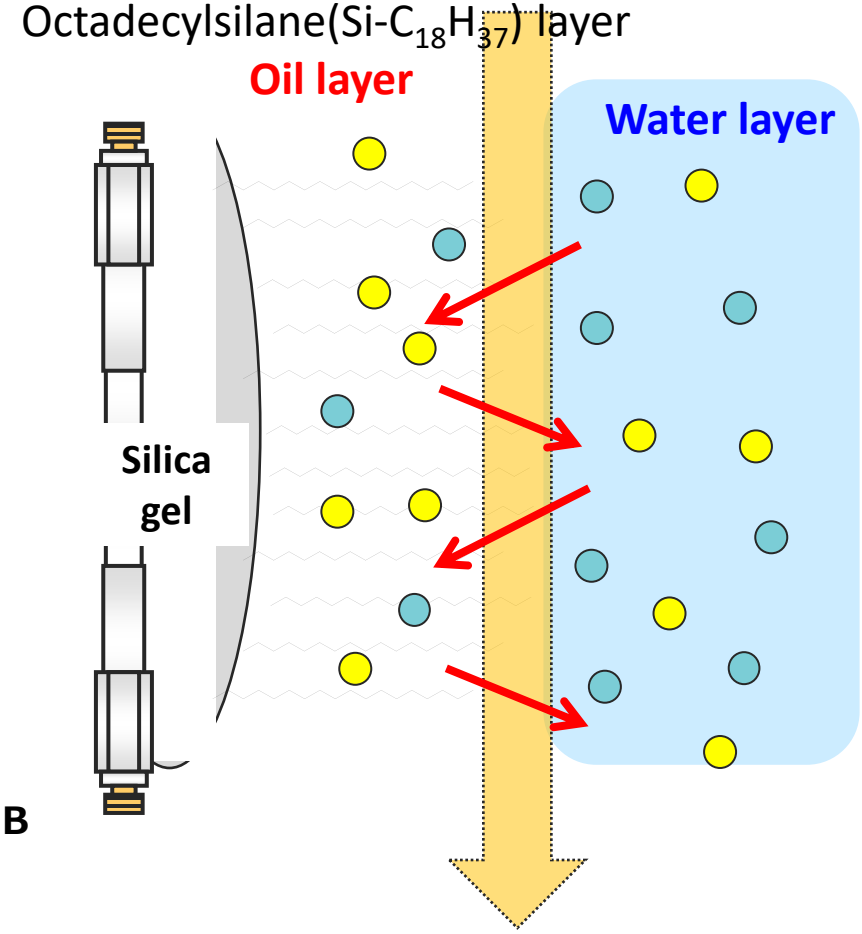
Difference of solubility of each compounds into both water and oil is based upon separation principle of ODS columns.

Almost compounds has smaller or larger different property, like as solubility and so on.



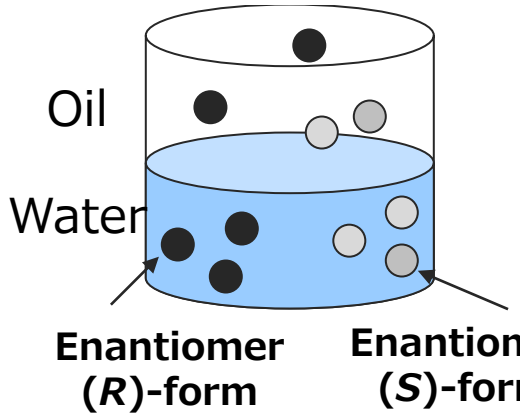


Principle of ODS column separation



Enantiomers never be separated on ODS column

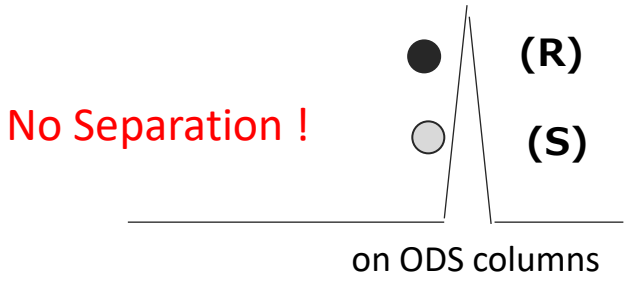
Solubility into water and oil



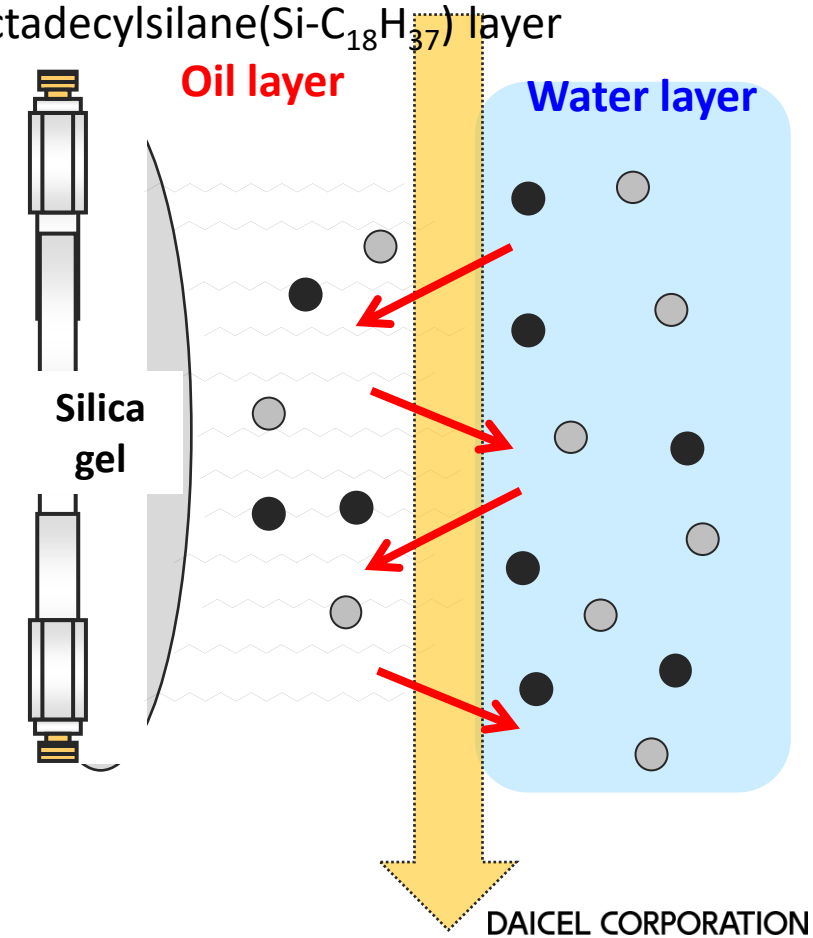
	(R)	(S)
Oil	40	40
Water	60	60

There is **no difference of solubility** of each enantiomer into both water and oil

Enantiomers never be separated on ODS column because of the perfect same property (solubility)



Principle of ODS column separation



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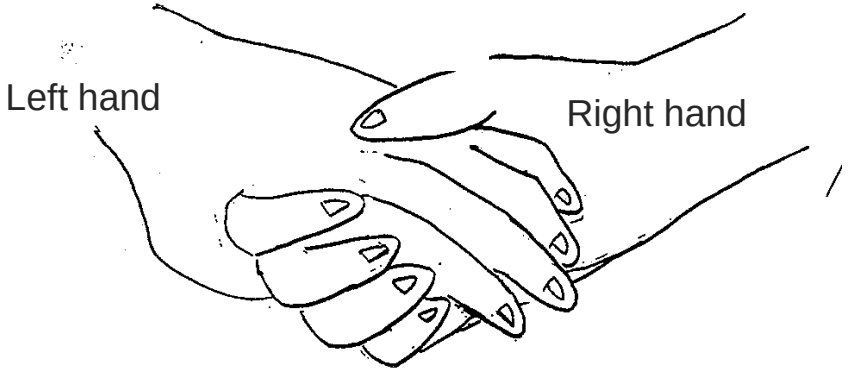
Why can right and left hands be recognized ?

Nice to meet you!

=> More retained



Nice? to meet you……

=> Less retained

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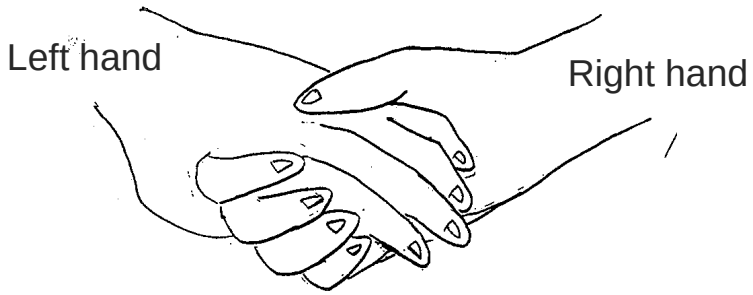
Why can enantiomers be separated on chiral columns?

Nice to meet you!

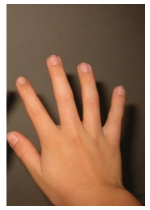
=> More retained

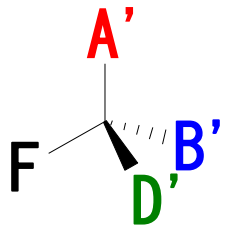


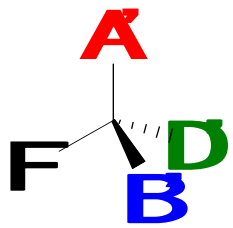
Nice? to meet you……

=> Less retained

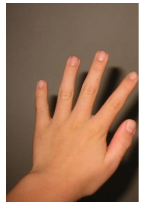
Right hand







Left hand



Optical isomers
(Racemate)
II
Analytes

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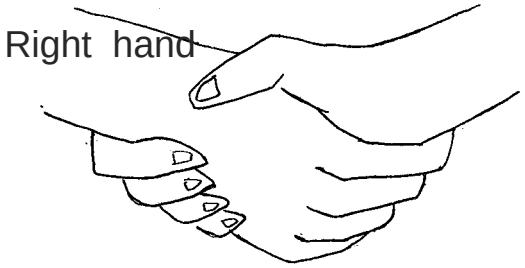
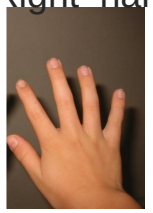
Why can enantiomers be separated on chiral columns?

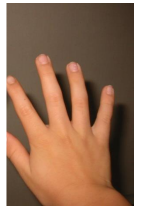


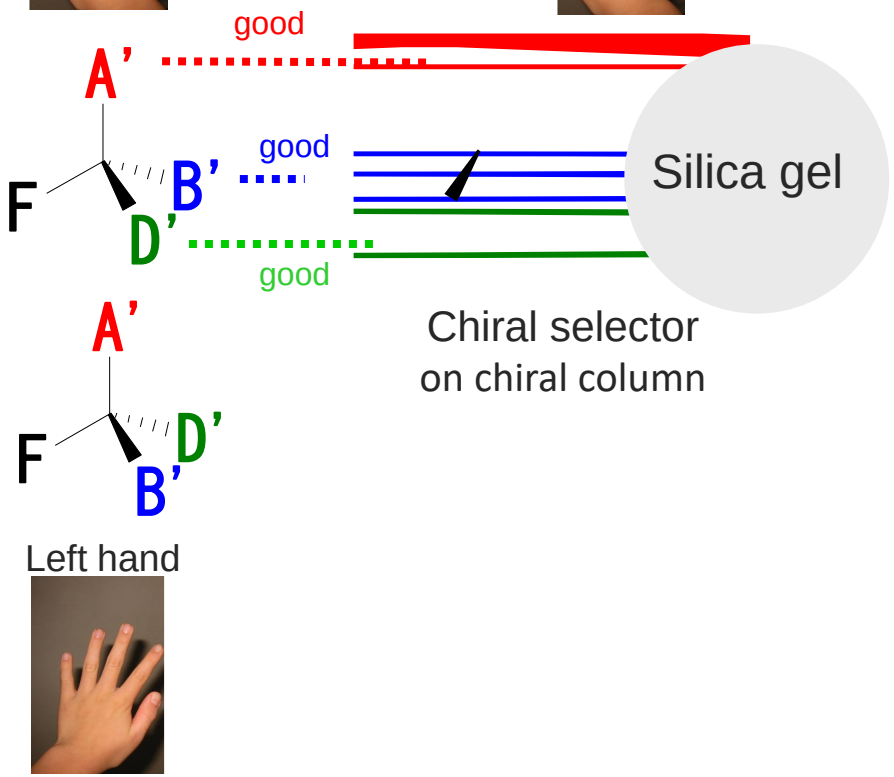
Nice to meet you!

=> More retained

Silica gel







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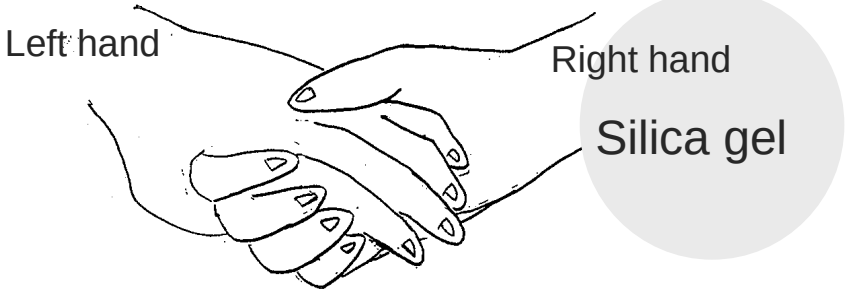
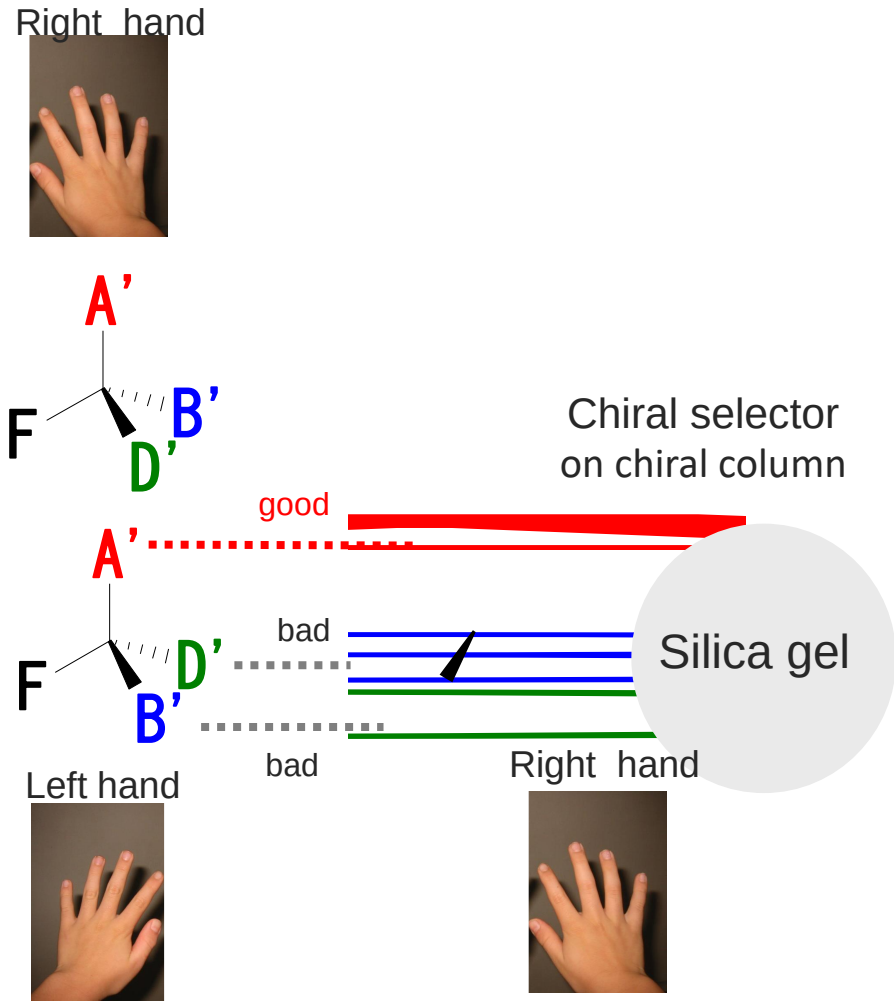
Why can enantiomers be separated on chiral columns?

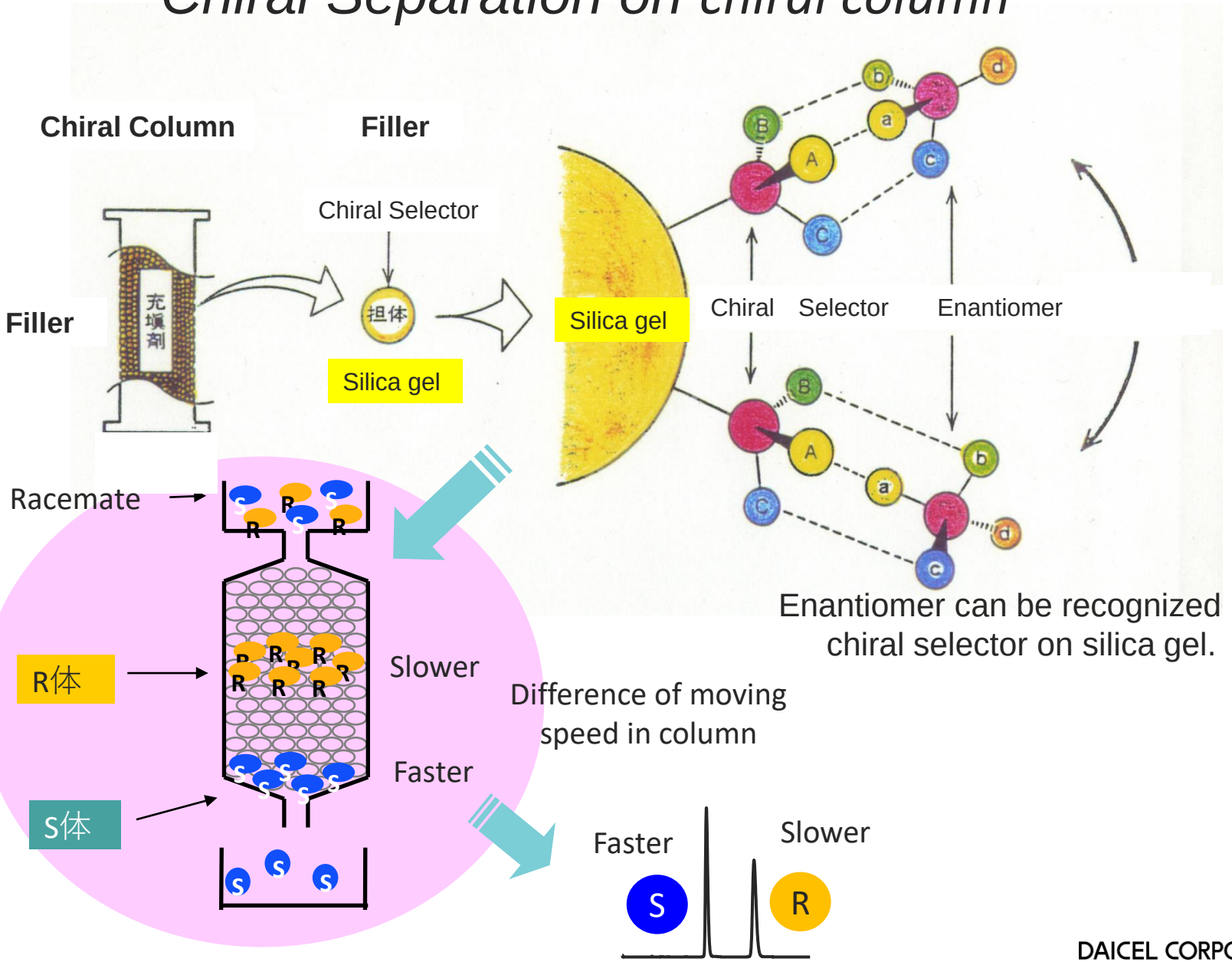


Nice? to meet you……

=> Less retained

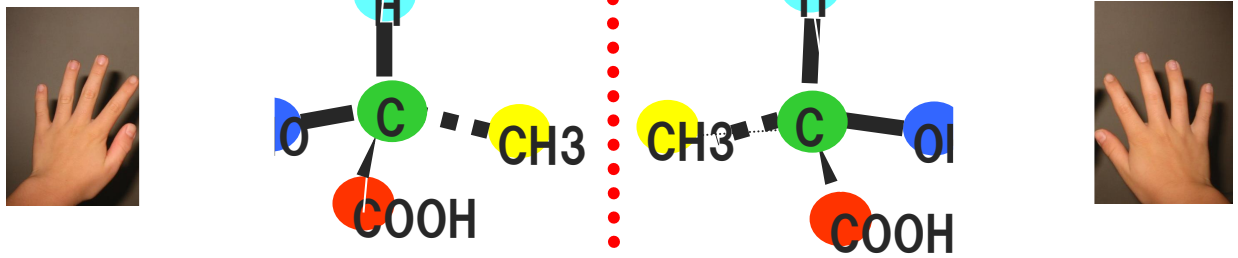


Chiral Separation on chiral column

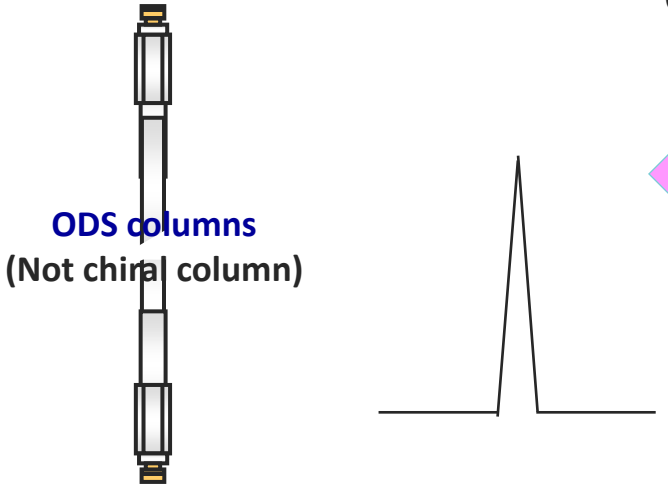


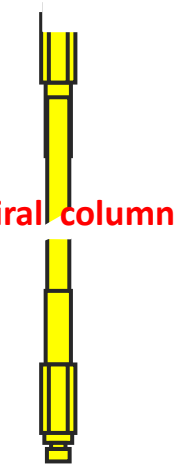
Enantiomer can be recognized by chiral selector on silica gel.

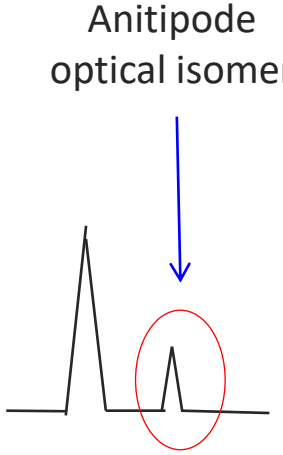
Summary for Purpose of using chiral column



Optical isomers





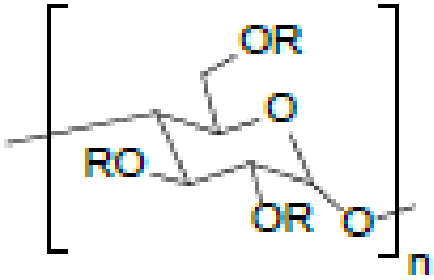
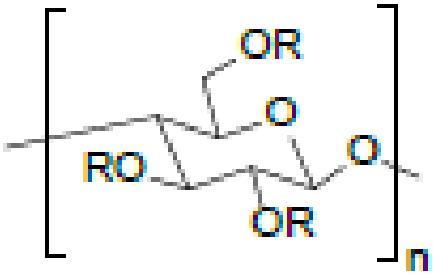


ODS column cannot separate mixture of optical isomers and cannot analyze their optical purity.

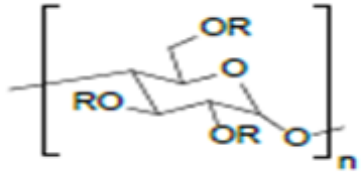
Only chiral column can separate mixture of optical isomers into each single enantiomer and can determine optical purity.

Polysaccharide Based CSPs

Back Bones of Chiral selectors

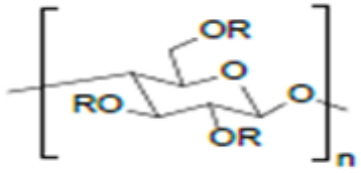
AMYLOSE-BASED	CELLULOSE-BASED
	
Immobilized on 3 µm silica gel	Immobilized on 3 µm silica gel

AMYLOSE-BASED



Immobilized on 3 µm silica gel

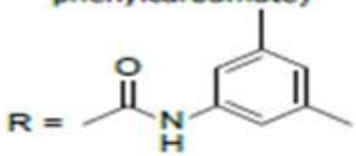
CELLULOSE-BASED



Immobilized on 3 µm silica gel

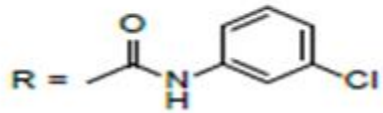
CHIRALPAK® IA-3

Amylose tris(3,5-dimethyl-phenylcarbamate)



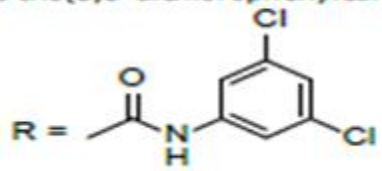
CHIRALPAK® ID-3

Amylose tris(3-chloro-phenylcarbamate)



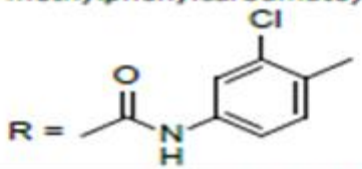
CHIRALPAK® IE-3

Amylose tris(3,5-dichlorophenylcarbamate)



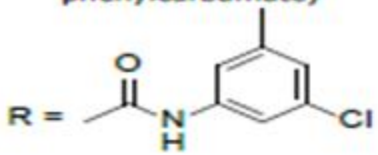
CHIRALPAK® IF-3

Amylose tris(3-chloro-4-methylphenylcarbamate)



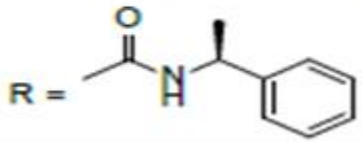
CHIRALPAK® IG-3

Amylose tris(3-chloro-5-methyl-phenylcarbamate)



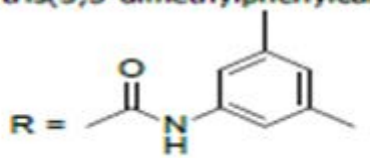
CHIRALPAK® IH-3

Amylose tris[(S)- α-methylbenzylcarbamate]



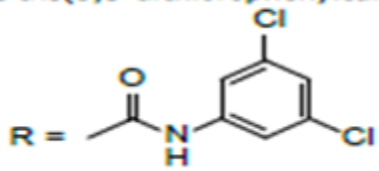
CHIRALPAK® IB-3
CHIRALPAK® IB N-3

Cellulose tris(3,5-dimethylphenylcarbamate)



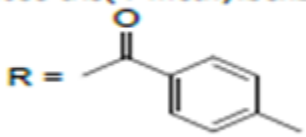
CHIRALPAK® IC-3

Cellulose tris(3,5-dichlorophenylcarbamate)



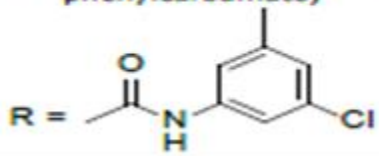
CHIRALPAK® IJ-3

Cellulose tris(4-methylbenzoate)



CHIRALPAK® IK-3

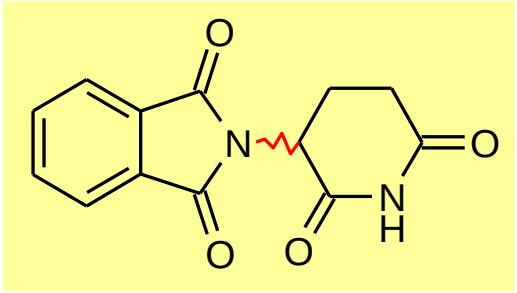
Cellulose tris(3-chloro-5-methyl-phenylcarbamate)



21

International guideline of enantiomeric purity analysis

Tragedy of Thalidomide (1)



Thalidomide (1956-)

Hypnosis sedative as racemate

with very low toxicity

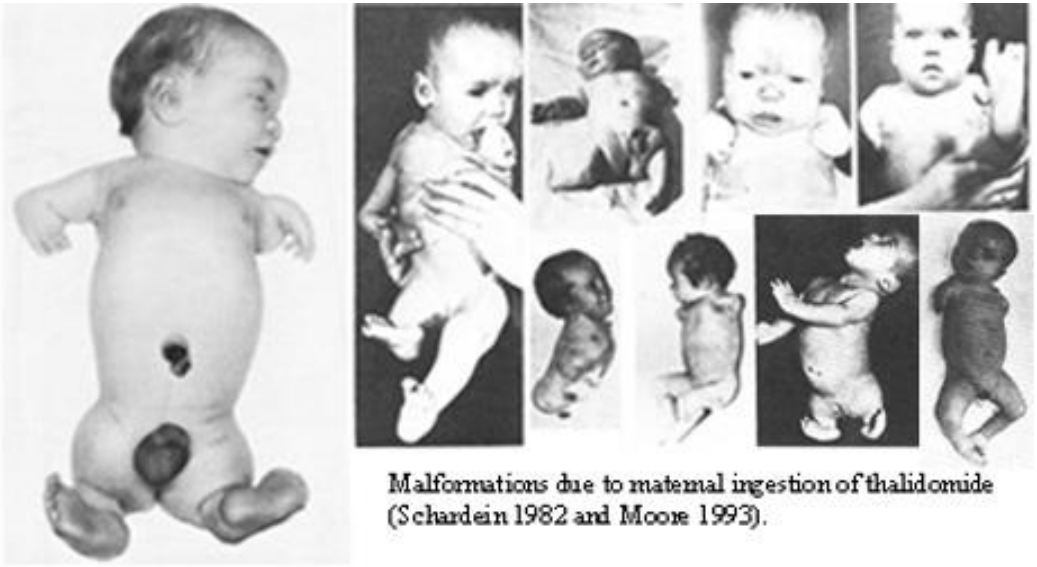
Commercial name ”Contergan”

↓

1962-

teratogenic

caused phocomelia etc.

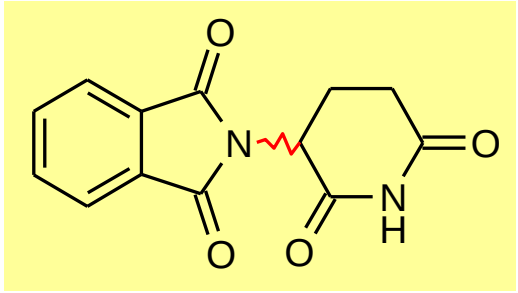


Malformations due to maternal ingestion of thalidomide (Schardein 1982 and Moore 1993).

http://en.wikipedia.org/wiki/Phocomelia

Throughout the world, about 10,000 cases were reported of infants with phocomelia due to thalidomide

Tragedy of Thalidomide(2)



Thalidomide (1956-)

Hypnosis sedative as racemate

with very low toxicity

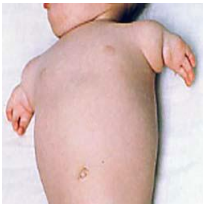
Commercial name ”contergan”

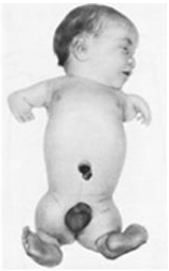
↓

1962-

teratogenic

caused phocomelia etc.

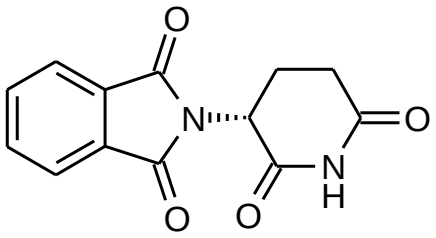




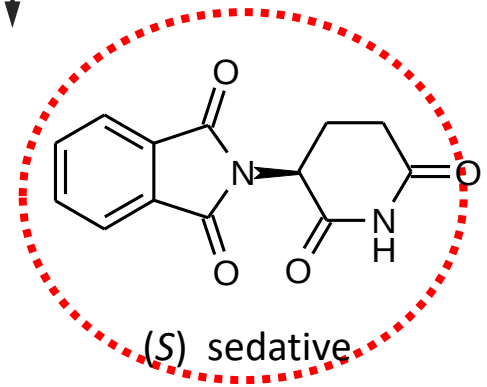
G. Blaschke,1979

Only (S)-form has teratogenic activity.

(Arzneim.-Forsh., **29**, 1640-1642 (1979))



(*R*) sedative



(*S*) sedative

teratogen

G. Blaschke 1994

In-vitro racemization is occurred.

(J. Chromatography A,

666, 235-340 (1994))

Reproducibility for bioactivity of each enantiomer has not been clear yet.

This was obvious opportunity and driving force for movement of single enantiomer drugs development from racemic drugs.

Merit of single enantiomer drugs

Drug safety	Undesired enantiomer can be toxic, may cause side-effects.
Drug efficacy	Enantiomers have different bioactivity.
Minimized dosing	Single enantiomer drugs can decrease dosage by up to 50%.

Comply with regulations	**FDA, ICH, EEC require full study and disclosures of bio-activities of each enantiomer in drugs; proof of enantiomeric purity using validated methods.**

International guideline of chiral compound in drugs

1992 : US FDA published a policy statement of new stereoisomeric drugs
1994 : EEC “Investigation of chiral active substances”
1999 : ICH Topic Q6A : Specifications
2000 : Canada, “Stereochemical issues in Chiral drug Development”

Now, many countries are preparing the regulation to check the enantiomeric purity of single enantiomer drugs in the world.

Importance of chiral purity analysis has been increased.

DRUG APPROVAL AND LICENSING PROCEDURES IN JAPAN 1989
(extraction)

B-2 Physicochemical Properties

a It is essential to include results of elementary analysis and ultraviolet, visible and infrared spectrum tests. If necessary, data based on the results of nuclear magnetic resonance spectrum, mass spectrum, optical rotatory dispersion, crystalline polymorphism and other tests must also be included.
The structural determination may also be verified by means of data related to the method of synthesis.

For mixtures of optical isomers, it is recommended to perform chromatographic tests in addition to optical rotatory dispersion tests.

(F) Test Data Concerning Absorption, Distribution, Metabolism and Excretion
F-1 Absorption, F-2 Distribution, F-3 Metabolism, F-4 Excretion

A careful investigation of the absorption, distribution, metabolism and excretion of the crude drug and the various dose forms of the final product by means of different administration routes other than the clinical administration routes of the drugs is necessary not only to predict th effects .....

When the drug concerned is a racemic body, it is recommended to investigate the absorption, distribution, metabolism and excretion of each optical isomer.

US FDA: New policy for chiral drug development (after Thalidomide tragedy)

Racemic drug means containing 50% other compounds

Development of New Stereoisomeric Drugs
Publication Date: 5/1/1992

II. POLICY IN GENERAL

The stereoisomeric composition of a drug with a chiral center should be known and the quantitative isomeric composition of the material used in pharmacologic, toxicologic, and clinical studies known.

1) When the drug product is a racemate and the pharmacokinetic profiles of the isomers are different, manufacturers should monitor the enantiomers individually to determine such properties as dose linearity and the effects of altered metabolic or excretory (ADBE) function and drug-drug interactions

2) If the pharmacokinetic profile is the same for both isomers or a fixed ratio between the plasma levels of enantiomers is demonstrated in the target population, an achiral assay or an assay that monitors one of the stereoisomers should suffice for later evaluation

↓

Single enantiomer drug development

Enantiomeric purity (1) “ICH”

ICH Topic Q6A Decision tree #5 :

Establishing identity, assay and enantiomeric impurity procedures for chiral new drug substances and new drug products containing chiral drug substances

- If the new drug substance is chiral and one enantiomer,
  - Needed for drug substance specification;
    - ☛ Chiral identity, Chiral assay, and Enatiomeric impurity
  - Needed for drug product specification;
    - ☛ Chiral assay, Enantiomeric impurity
- A chiral assay or an enantiomeric impurity procedure may be acceptable in lieu of a chiral identity procedure.
- An achiral assay combined with a method for controlling the opposite enantiomer is acceptable in lieu of chiral assay.
- The level of the opposite enantiomer of the drug substance may be derived from chiral assay data or from a separate procedure.

Enantiomeric purity (2) “EEC, Canada”

EEC 3CC29a in 1994 :

6.6 Generic applications of chiral medicinal products

Bioequivalence studies supporting generic applications of chiral medicinal products should be based upon enantiospecific bi-analytical methods, unless:

1) Both products contain the same, stable single enantiomers as the active sustance, or
2) Both products contain the racemate and both enantiomers show linear pharmacokinetics.

Health Canada in 2000 :

2.1.2 Drug Product

The enantiomeric purity of the drug substance in the drug product should be investigated using a validated enantioselective method prior to and during the stability studies conducted to determine the shelf life. Results from the primary stability studies may be considered sufficient. However, a test for enantiomeric purity should be incorporated into the drug product specification if results of these investigations warrant.

Enantiomeric purity analysis is included in international pharmacopoeia, such as, USP, EP, etc.

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World trend of chiral drugs

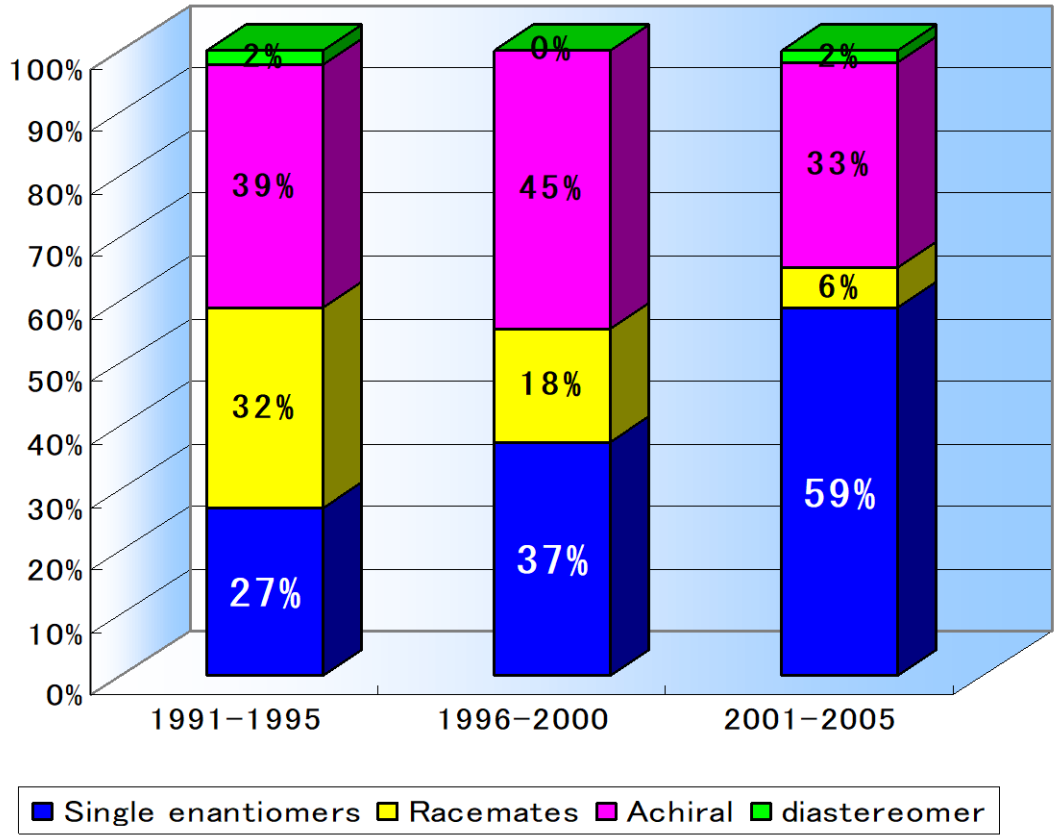
Trend of worldwide-approved drugs

Single enantiomer drug development has increased from that guideline.

ACS Med. Chem. Lett. 2023, 14, 7, 875–878
Publication Date:June 12, 2023

Trend of worldwide-approved drugs

Single enantiomer drug development has increased from USFDA guideline.



Finechemical, vol. 37, No. 5, 94-97 (2008)

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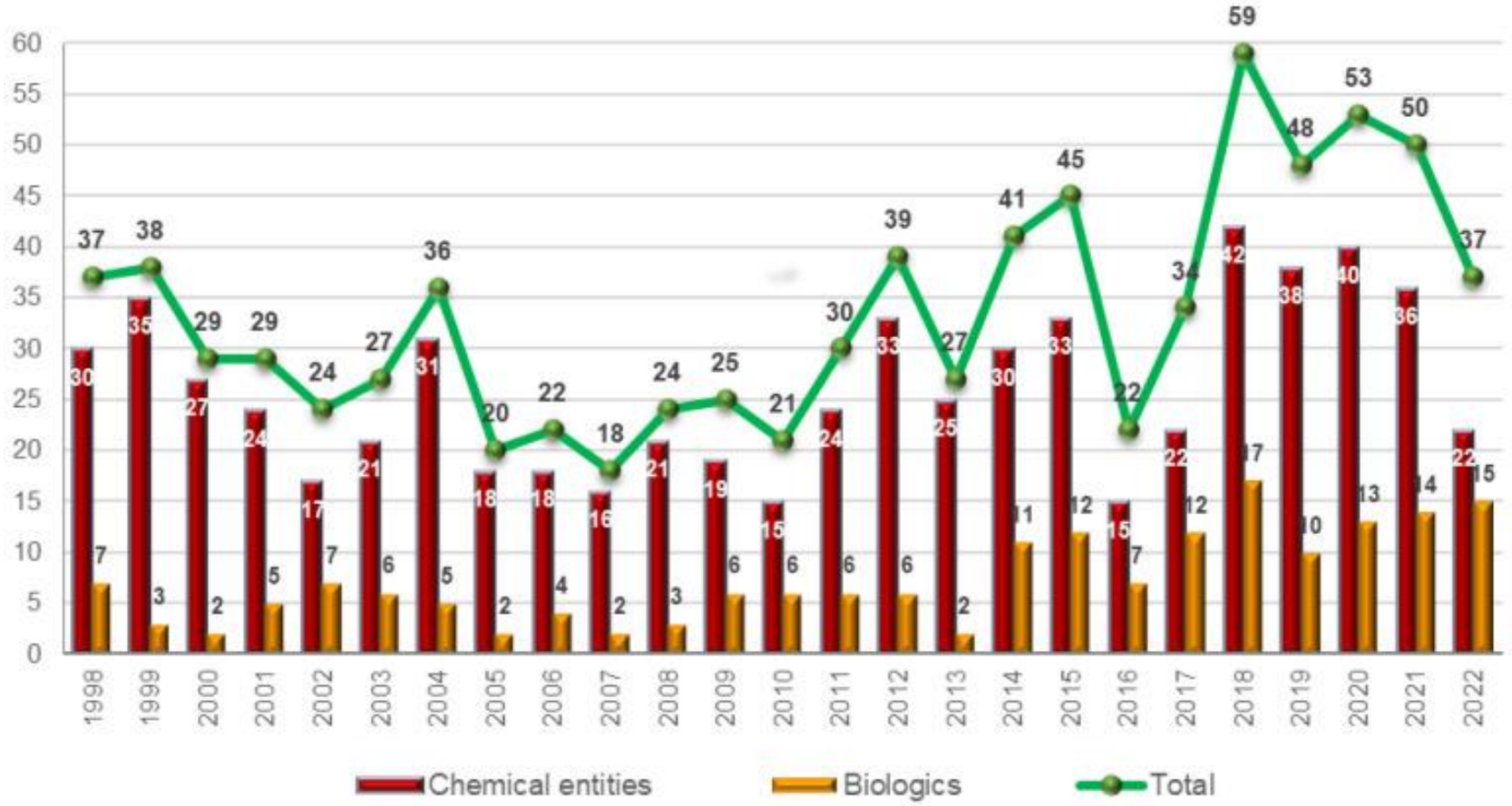
USFDA approved new drug trend

Recently, biomolecule drugs are focused extensively, but small molecule drugs development has been still continued energetically.



The following table lists pharmaceuticals that have been available in both racemic and single-enantiomer form. These single-enantiomer drug switched from the respective racemic drug are referred to as chiral switch.

Racemic mixture	Single-enantiomer
Amlodipine (Norvasc)	Levamlodipine (Conjupri)
Amphetamine (Benzedrine)	Dextroamphetamine (Dexedrine)
Bupivacaine (Marcain)	Levobupivacaine (Chirocaine)
Cetirizine (Zyrtec / Reactine)	Levocetirizine (Xyzal)
Chlorphenamine (INN) Chlorpheniramine (USAN) (Chlor-Trimeton)	Dexchlorpheniramine (Polaramine)
Citalopram (Celexa / Cipramil)	Escitalopram (Lexapro / Cipralex)
Fenfluramine (Pondimin)	Dexfenfluramine (Redux)
Formoterol (Foradil)	Arformoterol (Brovana)
Ibuprofen (Advil / Motrin)	Dexibuprofen (Seractil)
Ketamine (Ketalar)	Esketamine (Ketanest S)
Ketoprofen (Actron)	Dexketoprofen (Keral)
Methylphenidate (Ritalin)	Dexmethylphenidate (Focalin)
Milnacipran (Ixel / Savella)	Levomilnacipran (Fetzima)
Modafinil (Provigil)	Armodafinil (Nuvigil)
Ofloxacin (Floxin)	Levofloxacin (Levaquin)
Omeprazole (Prilosec)	Esomeprazole (Nexium)
Salbutamol (Ventolin)	Levalbuterol (Xopenex)
Zopiclone (Imovane / Zimovane)	Eszopiclone (Lunesta)

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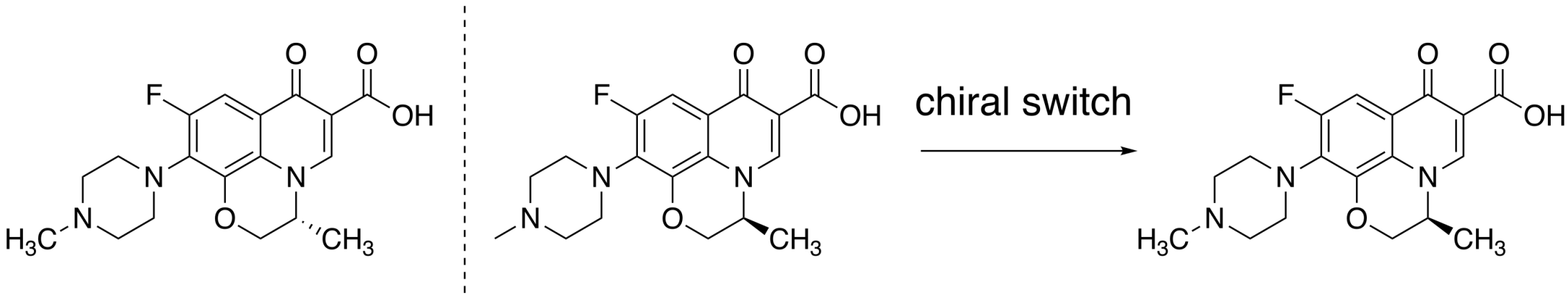


racemic ofloxacin

chiral levofloxacin

Worldwide Top 20 drugs in Sales

Brand Name	Generic Name	Chirality	Patent expires (in US)	Worldwide Sales in US$MM
Lipitor	**atorvastatin Ca**	**chiral**		**10,862**
Zocor	**simvastatin**	**chiral**	**2005**	**5,197**
Seretide/Advair	**salmeterol/fluticasone**	**chiral**	**2003**	**4,504**
Norvasc	amlodipine	r	2007	4,463
Zyprexa	olanzapine	N		4,420
Nexium	**esomeprazole**	**chiral**		**3,883**
Takepron/Prevacid	lansoprasol	r	2005	3,454
Pravachor	**pravastatin**	**chiral**	**2006**	**3,398**
Zoloft	**sertraline**	**chiral**		**3,361**
Effexor XR	venlafaxine	r	2007	3,347
Plavix	**clopidogrel**	**chiral**	**2003**	**3,327**
Celebrex	celecoxib	N		3,302
Neurontin	gabapentin	N	2004	2,723
Lovenox/Clexane	**enoxaparin Na**	**chiral**	**2004**	**2,366**
Plavix	**clopidogrel**	**chiral**	**2003**	**2,105**
Avandia	rosiglitazone	r		2,042
Seroquel	quetiapine	N		2,027
Losec/Prilosec	omeprazole	r	2002	1,947
Paxil/Seroxat	**paroxetine**	**chiral**	**2003**	**1,945**
Allegra/Telfast	fexofenadine HCl	r	<2002	1,866

r:racemic; N:achiral

10 Single enantiomer drugs per 20 drugs

TYPE OF CHIRAL STATIONARY PHASE

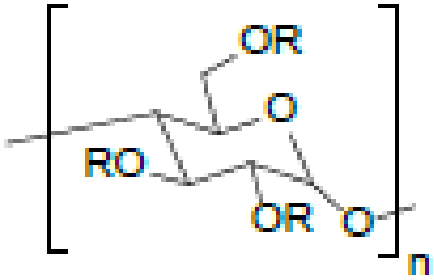
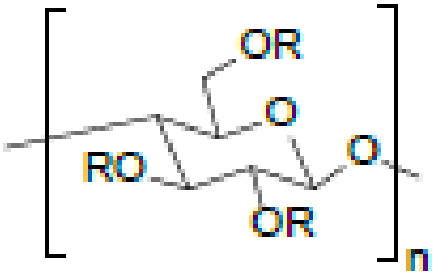
DAICEL
Sustainable Value Together

Types of CSPs and their loading capacities

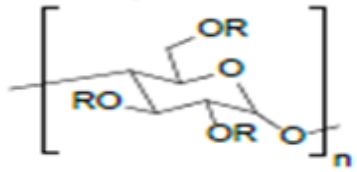
Type	CSPs	Loading capacity (mg solute / g CSP)
I	Pirkle type (Brush type)	1-50
II	Polysaccharide derivatives	**5-150**
III	Macrocyclic type	
	Cyclodextrins	0.1-5
	Glycopeptides	0.1-5
	Chiral Crown ether	0.1-5
IV	Ligand exchange	0.1-1
V	Protein type	0.1-0.2

Polysaccharide Based CSPs

Back Bones of Chiral selectors

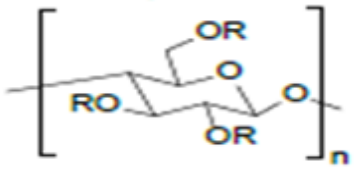
AMYLOSE-BASED	CELLULOSE-BASED
	
Immobilized on 3 µm silica gel	Immobilized on 3 µm silica gel

AMYLOSE-BASED



Immobilized on 3 µm silica gel

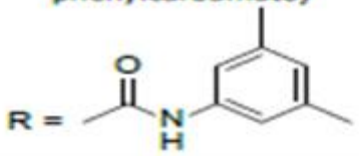
CELLULOSE-BASED



Immobilized on 3 µm silica gel

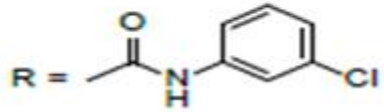
CHIRALPAK® IA-3

Amylose tris(3,5-dimethyl-phenylcarbamate)



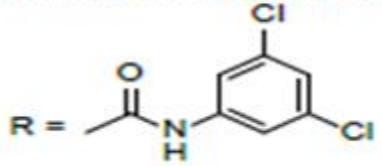
CHIRALPAK® ID-3

Amylose tris(3-chloro-phenylcarbamate)



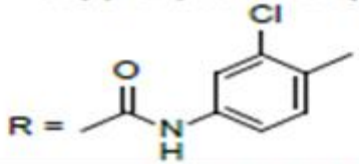
CHIRALPAK® IE-3

Amylose tris(3,5-dichlorophenylcarbamate)



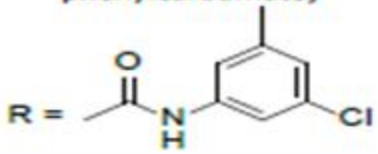
CHIRALPAK® IF-3

Amylose tris(3-chloro-4-methylphenylcarbamate)



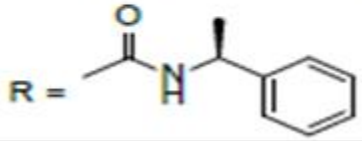
CHIRALPAK® IG-3

Amylose tris(3-chloro-5-methyl-phenylcarbamate)



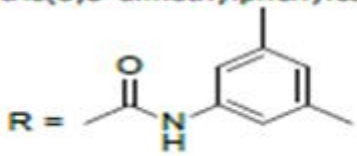
CHIRALPAK® IH-3

Amylose tris[(S)- α-methylbenzylcarbamate]



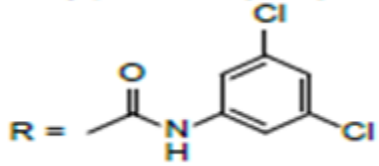
CHIRALPAK® IB-3
CHIRALPAK® IB N-3

Cellulose tris(3,5-dimethylphenylcarbamate)



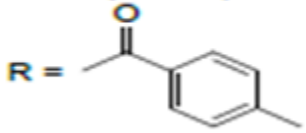
CHIRALPAK® IC-3

Cellulose tris(3,5-dichlorophenylcarbamate)



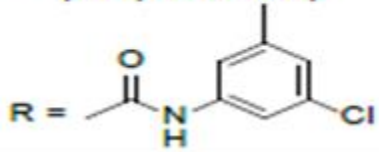
CHIRALPAK® IJ-3

Cellulose tris(4-methylbenzoate)

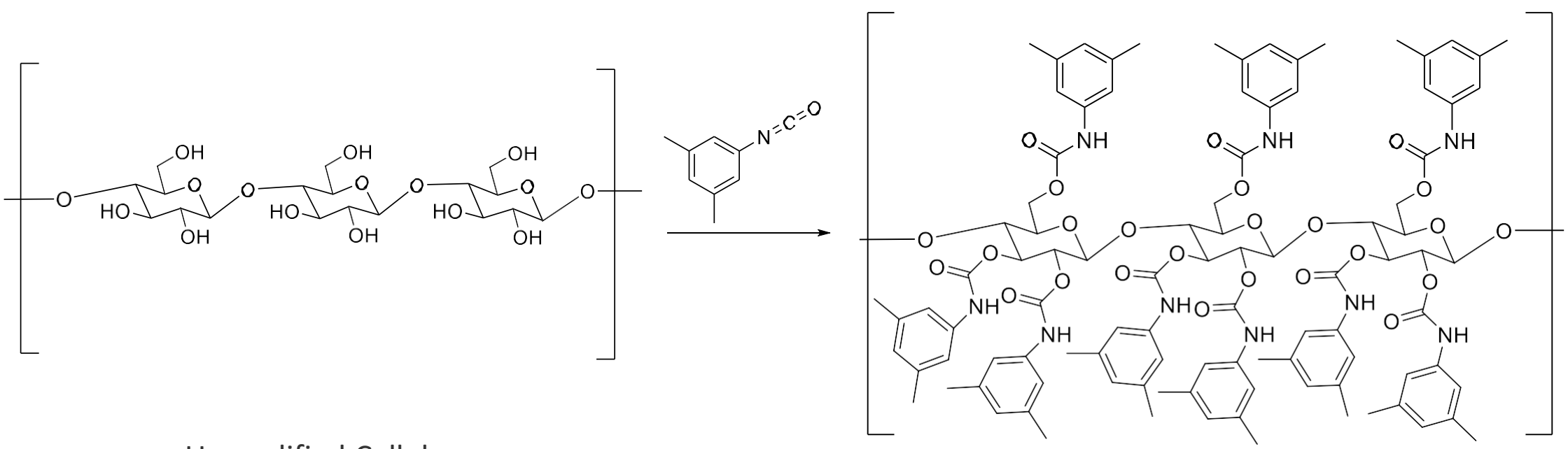


CHIRALPAK® IK-3

Cellulose tris(3-chloro-5-methyl-phenylcarbamate)



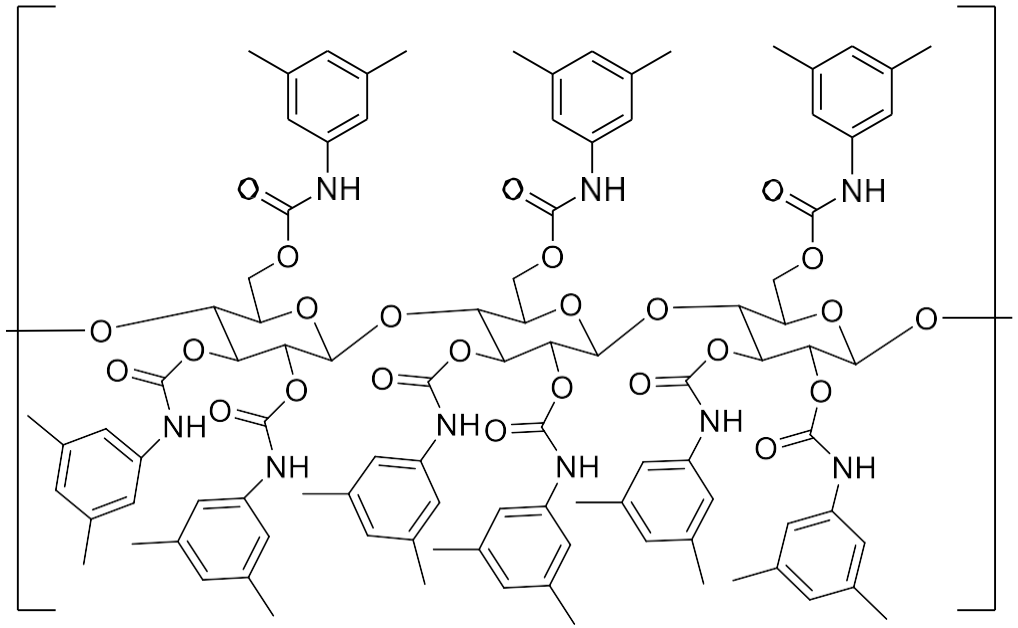
Chiral Stationary Phase Synthesis



Unmodified Cellulose Polymer

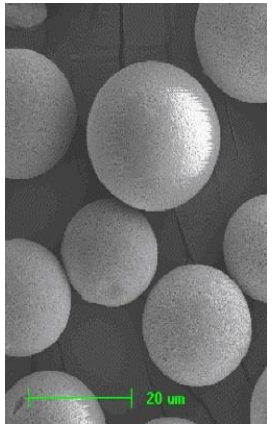
Tris(3,5-dimethylphenylcarbamate) Cellulose Polymer

Chiral Stationary Phase Synthesis



Tris(3,5-dimethylphenylcarbamate)
Cellulose Polymer
Dissolved in Strong Solvent (DCM/THF)

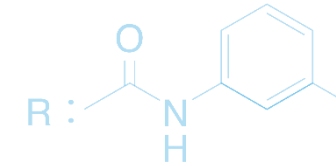
Coat → Dry



Porous Silica Gel

Coat → Dry → Immobilize

Coated CSP

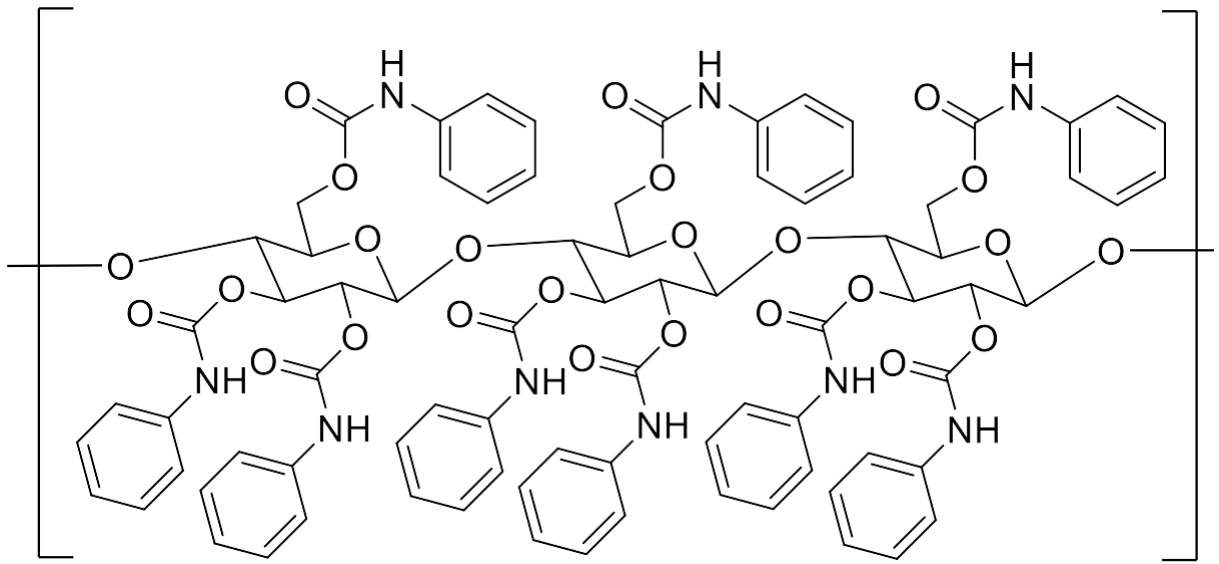


CHIRALCEL® OD
Tris(3,5-dimethylphenylcarbamate)
Cellulose
USP designation L40

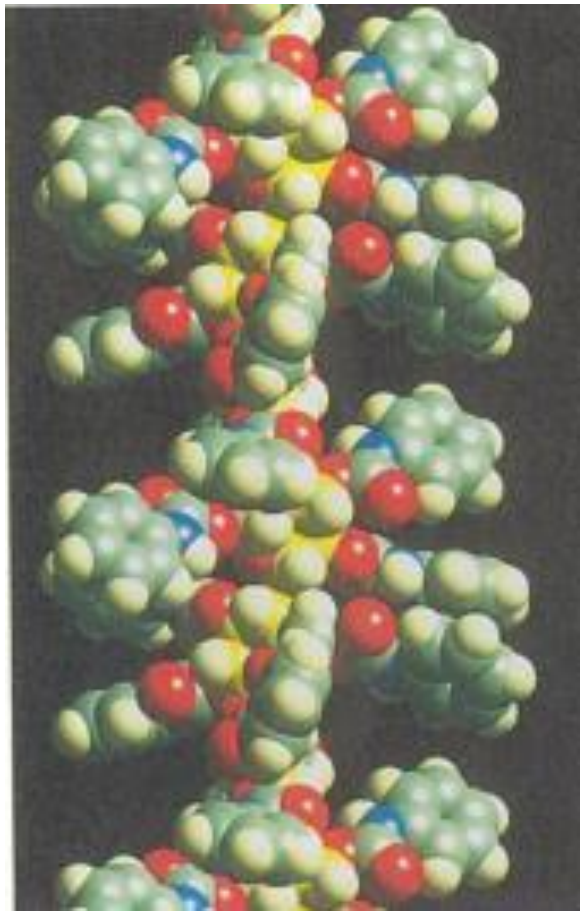
Immobilize CSP

CHIRALPAK® IB or IB-N
Tris(3,5-dimethylphenylcarbamate)
Cellulose

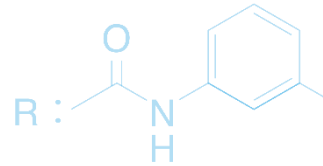
Chiral Recognition



CHIRALCEL® OC
Tris(phenylcarbamate) Cellulose



Chiral Recognition

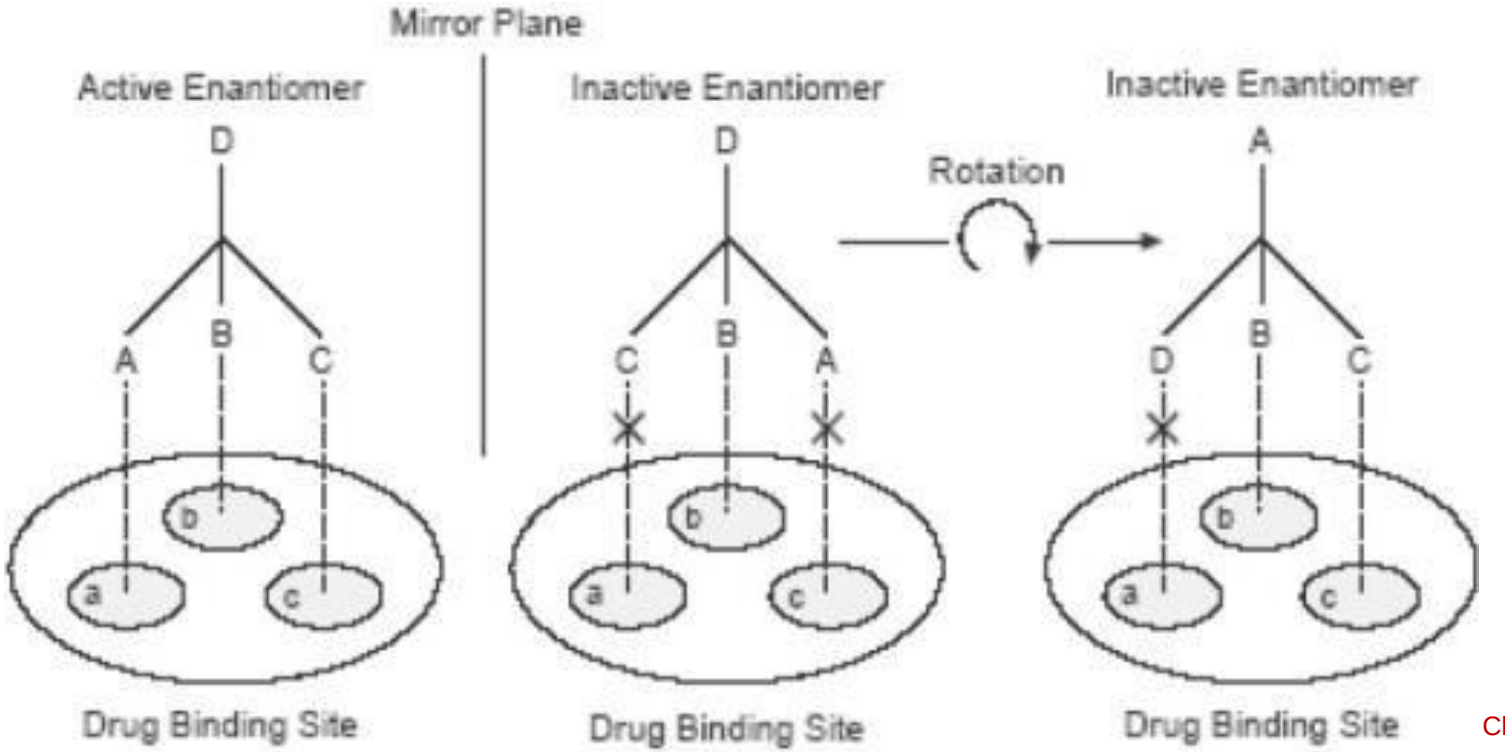


Type of interaction	Strength	Direction	Working distance
Coulomb or electric	Very strong	Attractive (+/–) or repulsive (same charges)	Medium range ($1/d^2$)
Hydrogen bond	Very strong	Attractive	Long range
Steric hindrance	From weak to very strong	Repulsive	Short range
$\pi-\pi$ interaction	Strong	Attractive (donor/acceptor)	Medium range
Ion–dipole	Strong	Attractive	Short range
Dipole–dipole	Intermediate	Attractive	Short range ($1/d^3$)
Dipole-induced dipole	Weak	Attractive	Very short range ($1/d^6$)
London dispersion or van der Waals forces	Very weak	Attractive	Very short range ($1/d^6$)

Chiral Recognition in Separation Methods, A Berthod, 2010

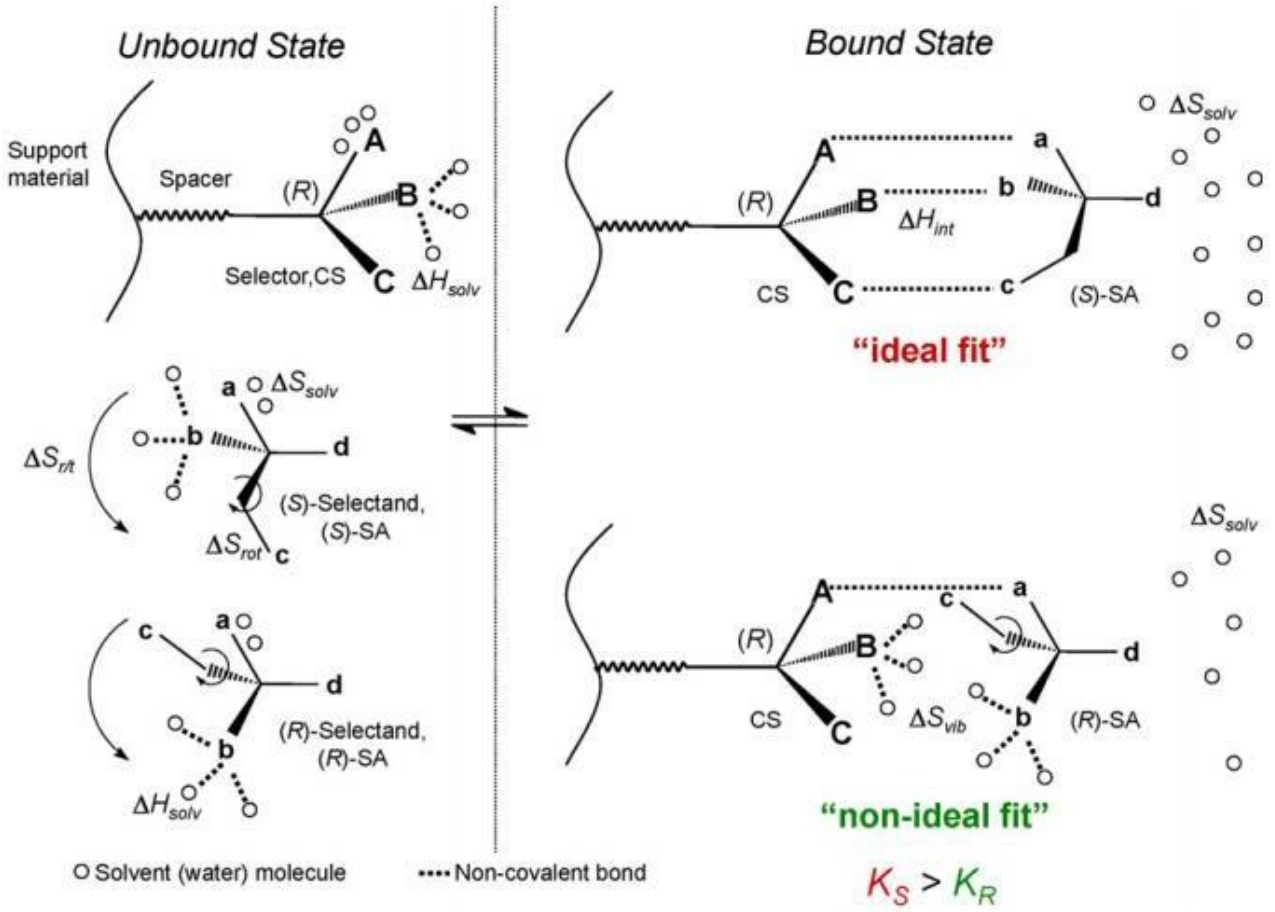
Chiral Recognition

- Easson-Stedman Model



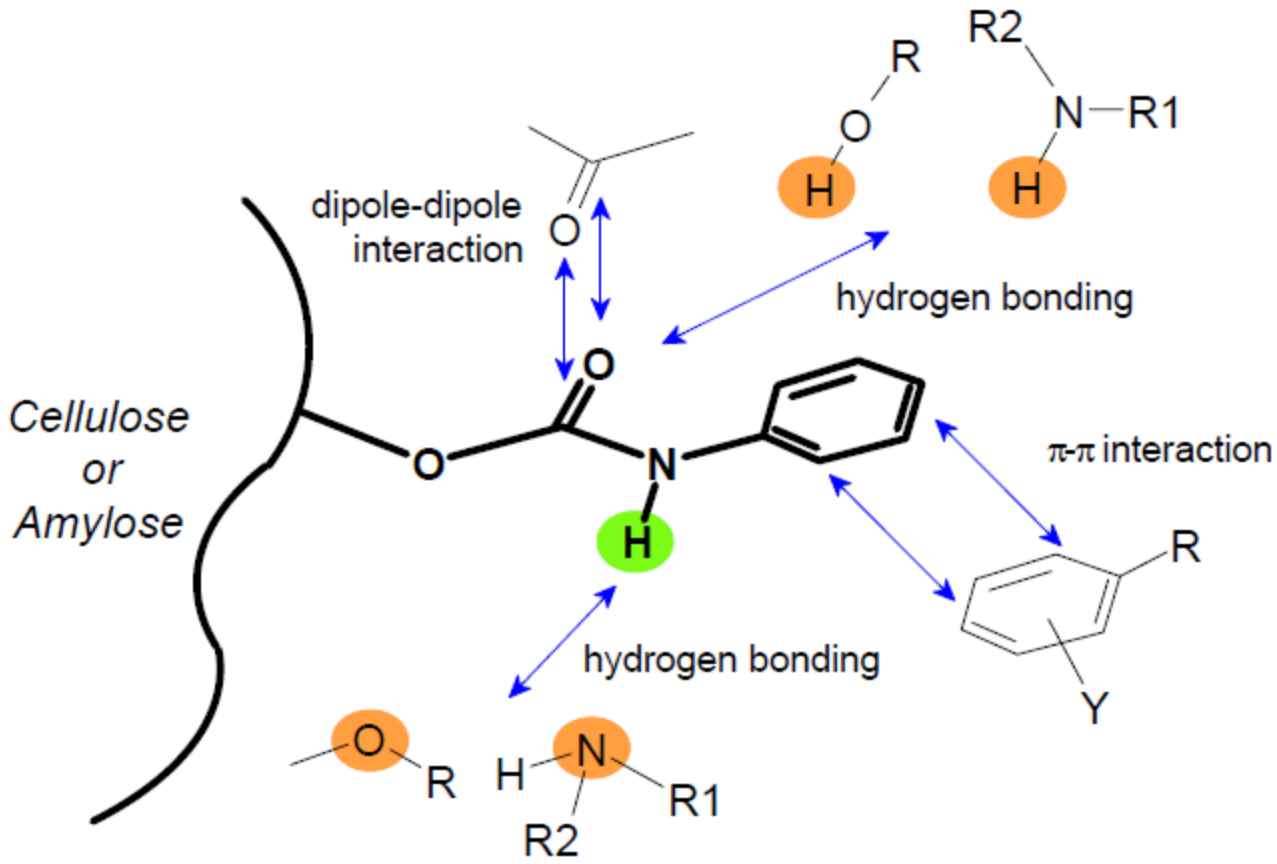
Chiral Drugs: An Overview, L Nguyen, 2006

Chiral Recognition

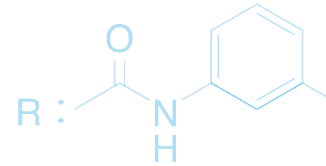


Chiral Recognition by Enantioselective Liquid Chromatography, Lammerhofer, JoCA, 2009

Chiral Stationary Phase Mechanicsm



Chiral Selectors



Polysaccharides: CHIRALPAK® (Coated Amylose and Immobilized Columns) and CHIRALCEL® (Coated Cellulose)

Normal, Polar Organic, and Reversed Phases, and SFC

✓First Generation COATED Columns

AD, AS, AY, AZ, OA, OB, OC, OD, OF, OG, OJ, OK, OX, OZ

✓Second Generation IMMOBILIZED COLUMNS **(Compatible with forbidden normal phase solvents)**

IA, IB, IB-N, IC, ID, IE, IF, IG, IH, IJ, IK

Specialty Selectors

✓Protein-based Phases -- CHIRALPAK AGP, HSA and CBH

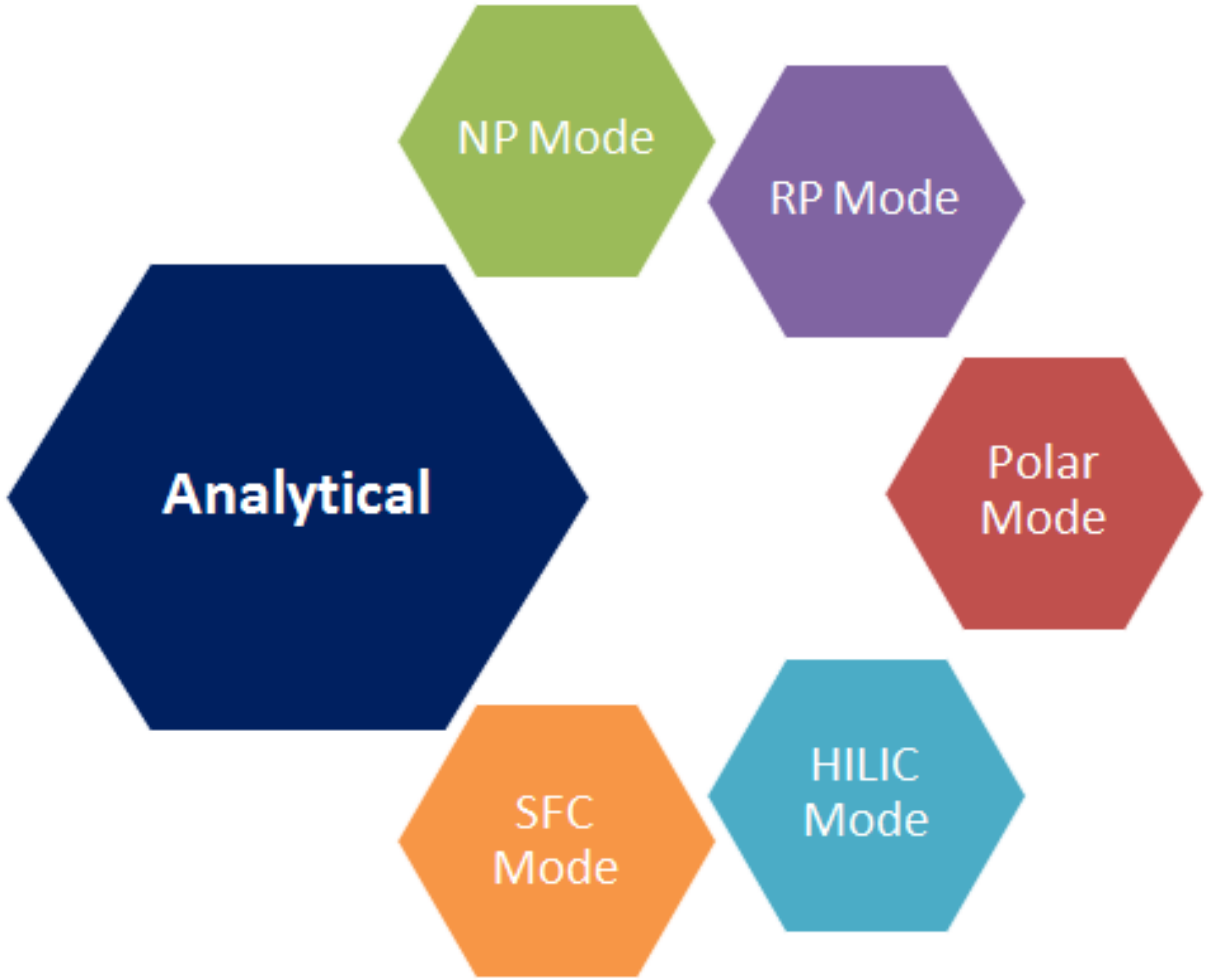
✓Ligand Exchange: MA+, WH

✓Chiral Crown Ethers: CROWNPAK CR, CR+, CR-

✓Lindner Anion Exchange Phases: QD-AX, QN-AX

✓ZWIX™ (*Zwitterionic Stationary Phases*)

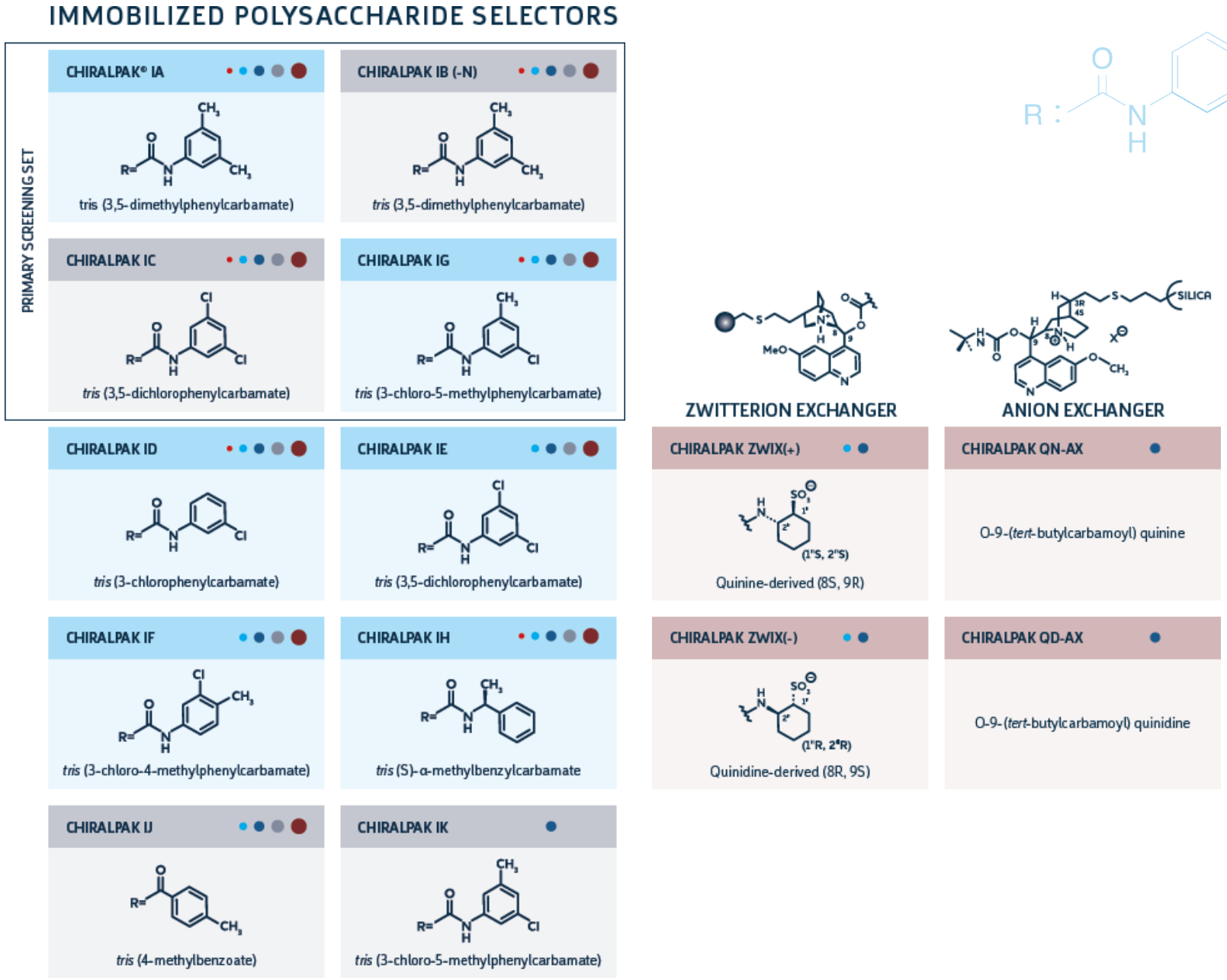
Chiral chromatography Elution Modes



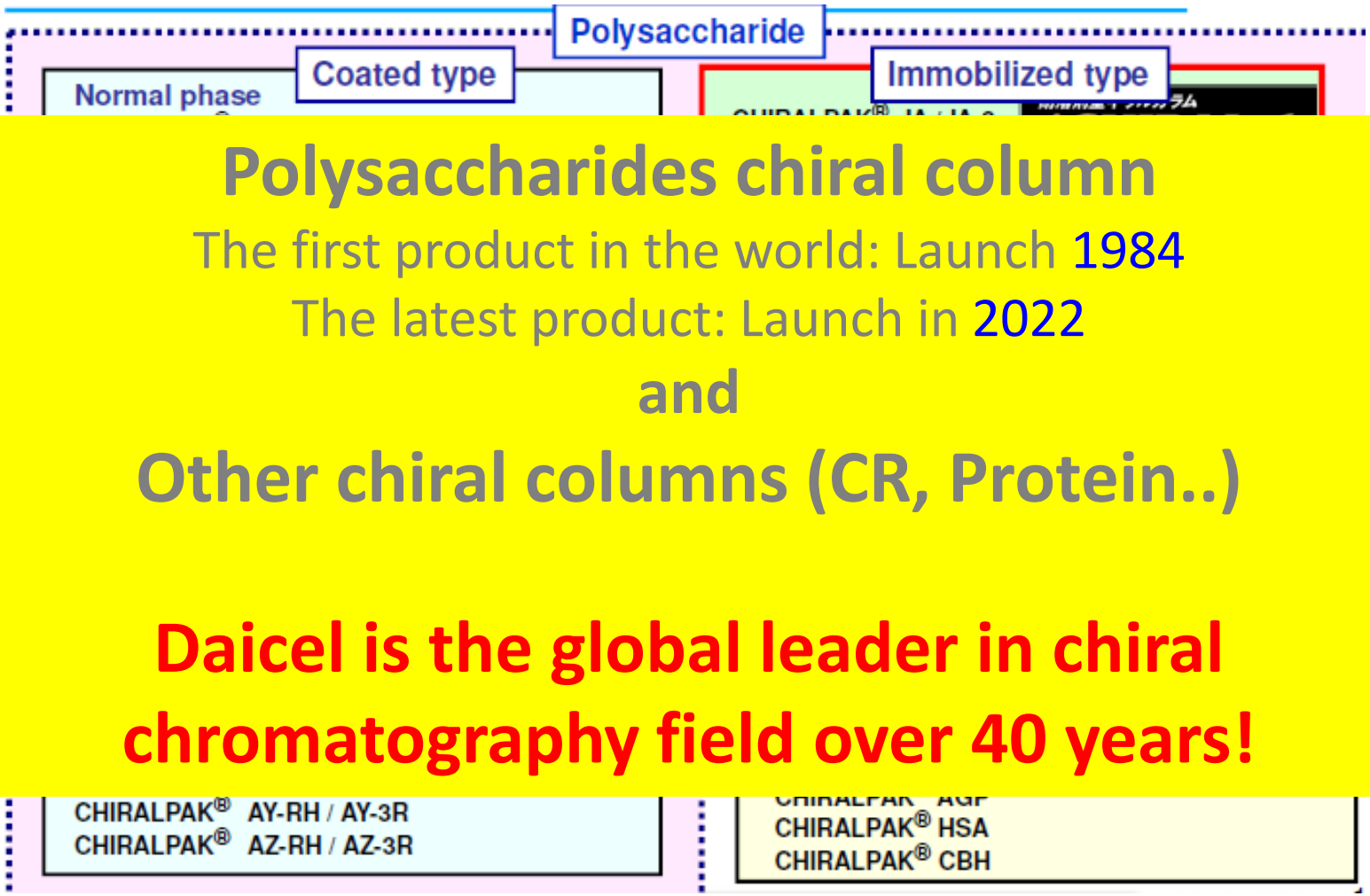
DAICEL
Chiral Technologies

Chiral Selectors

- 43 Chiral Stationary Phases
- 35 unique selectors
  - 8 selectors are available coated and immobilized
    - IA=AD, IB/IB N=OD, IF=AZ, IH=AS, IJ=OJ, CR-I (+/-) = CR (+/-)
- Amylose or cellulose
  - AD and OD, IG and IK



Daicel chiral columns portfolio



Polysaccharides chiral column

The first product in the world: Launch 1984

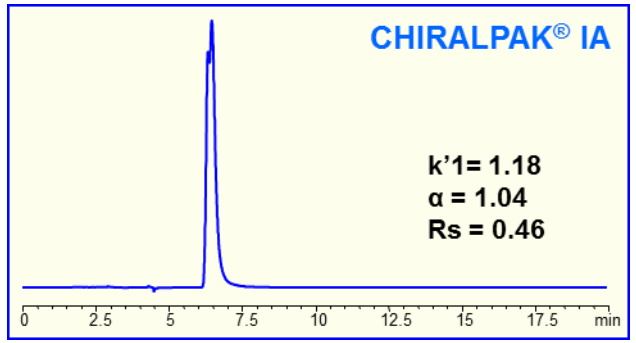
The latest product: Launch in 2022

and

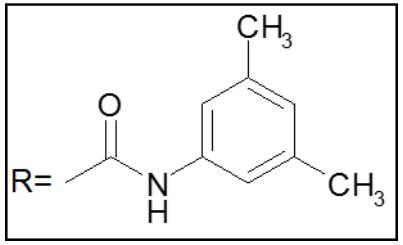
Other chiral columns (CR, Protein..)

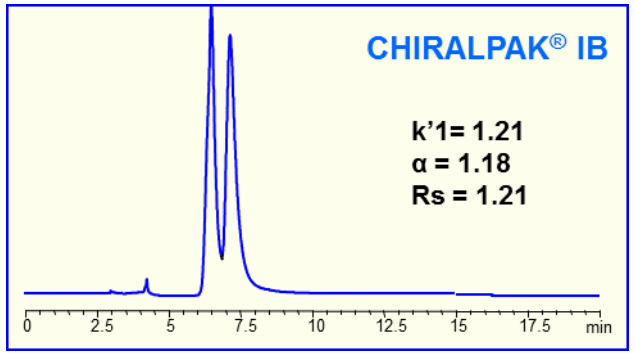
Daicel is the global leader in chiral chromatography field over 40 years!

Chiral Recognition

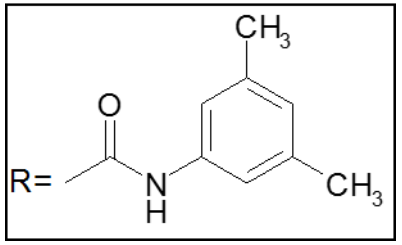


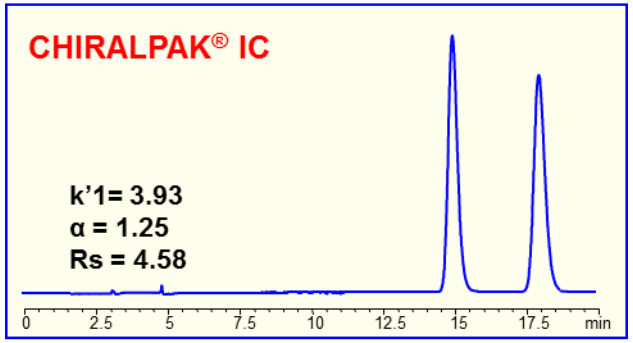
Amylose



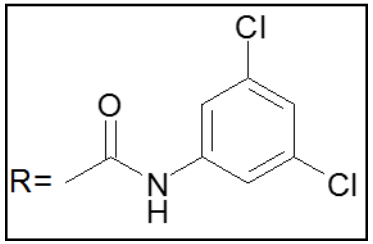


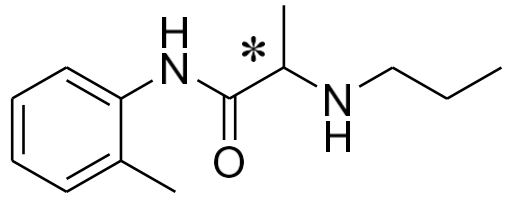
Cellulose





Cellulose





Prilocaine

Hexane-IPA-DEA (90:10:0.1 v/v/v)

-Hydrogen bonding
-π-π stacking
-dipole-dipole stacking
-steric inclusion

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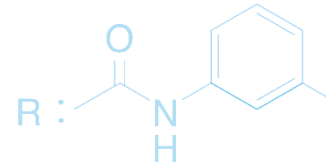
What is different Properties on Coated & Immobilize

Mobile Phases for Coated CSPs



Normal phase conditions:

- Alkane/2-propanol
- Alkane/Ethanol

Polar mode:

- Acetonitrile
- Ethanol
- Methanol
- Other alcohols

Reversed phase conditions (for -3R / RH-versions):

- Water/alcohol or acetonitrile
- Phosphate buffer (pH 2-8)/alcohol or acetonitrile
- KPF_6 pH 2/acetonitrile
- Borate buffer / bicarbonate buffer (pH 9)/alcohol or acetonitrile

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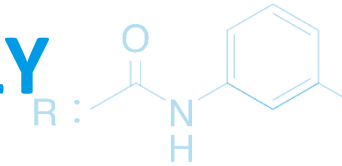
Mobile Phases for coated CSPs TO AVOID ABSOLUTELY



Coated CSPs are not stable with all solvents

NEVER USE

(even as a sample solvent)

- Chloroform
- Methylene chloride
- Ethyl acetate
- Acetone
- THF
- DMF
- DMSO

These will irreversibly destroy the coated CSP

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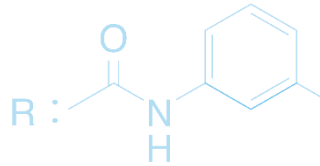
Most of common trouble in coated type columns : Use of the wrong solvent type



- ✓ The coating type column can be easily destroyed, if wrong solvents are contacted to the columns.
- ✓ When a wrong solvent is contacted, back pressure of the column goes up in polysaccharides columns..
- ✓ When a customer reports sudden increase of pressure and/or sudden loss of separation, the most common case is wrong solvent use.
- ✓ Once wrong solvents are used, the chances are very low to recover the initial performance.
- ✓ When you encounter such troubles, check the column performance according to the QC chromatogram, which attached with each column.

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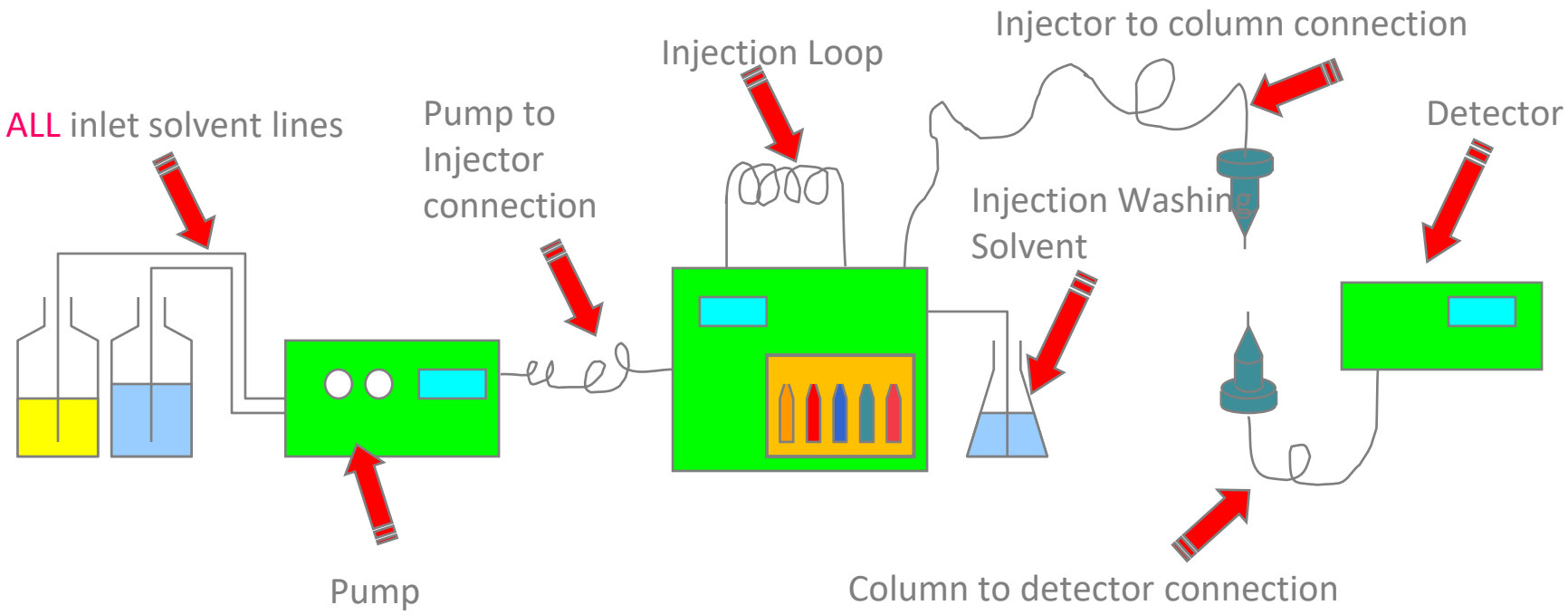
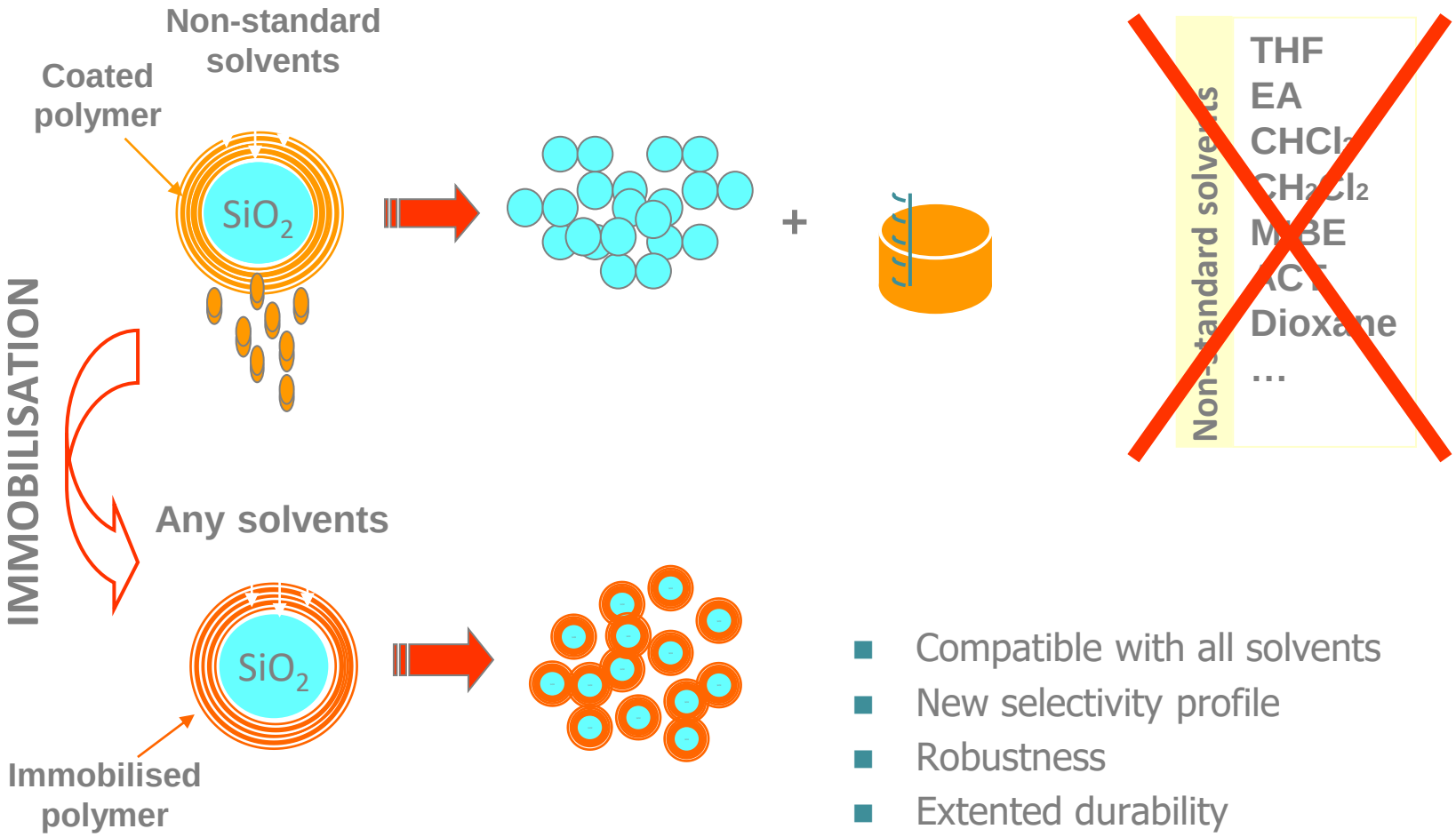
Before connecting the column to the system:

1. Flush all *the HPLC unit* with a **compatible solvent – preferably 2-propanol.**
2. Flush the *entire unit* with **the column storage mobile phase.**



Coated and Immobilized Polysaccharide-Derived CSPs
Solvent Compatibility



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

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Immobilized polysaccharide-derived CSPs Background

Coated Polysaccharide-derived
Chiral Stationary Phases (CSPs)

- Highly selective
- Broad application domain
- High loading capacity

The immobilisation of the chiral polymer
on the silica support

- Makes the CSP resistant to a broader range of solvents
- Enlarges the application domain of polysaccharide-derived CSPs, when the appropriate immobilisation process is applied

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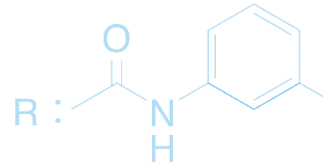
New Generation Chiral Stationary Phases
Immobilized CSP Advantage



- High sucess rate and Broad application domain
- Highly durable and can be regenerated
- Compatible with all miscible solvents
- Rugged phases hence carefree operation
- High Preparative potential
- Compatibility with RP, NP and Polar mode

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Method Development

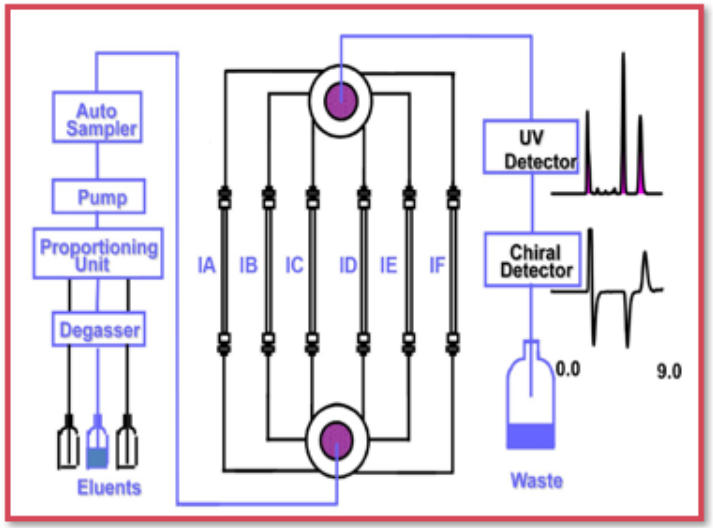
Screening approach: 2

Normal Phase

Compound
- n-Hexane : EtOH : DEA (or TFA) (80/20/0.1, v/v/v)
- n-Hexane : 2-PrOH : DEA (or TFA) (80/20/0.1, v/v/v)

Normal Phase-Non standard

Compound
- MtBE: MeOH: DEA (or TFA) (95/05/0.1, v/v)
- n-Hexane:EtOAc: DEA (or TFA) (50/50/0.1, v/v)



New Generation Chiral Columns

- CHIRALPAK® IA
- CHIRALPAK® IB
- CHIRALPAK® IC
- CHIRALPAK® ID
- CHIRALPAK® IE
- CHIRALPAK® IF
- CHIRALPAK® IG
- CHIRALPAK® IH
- CHIRALPAK® IJ

Polar Organic

Compound
- MeOH: DEA (or TFA) (100/0.1, v/v)
- EtOH: DEA (or TFA) (100/0.1, v/v)
- ACN: DEA (or TFA) (100/0.1, v/v)

Reverse Phase

Compound
- Acidic: aq. HCOOH (pH=2.0) : ACN (60/40, v/v)
- Basic: aq. NH_4HCO_3 : ACN (60/40, v/v)
- Neutral: aq. NH_4OAc : ACN (60/40, v/v)

Daicel Chiral Technologies (India) Pvt Ltd Survery No.542, IKP Knowledge Park,
Koltur Village, Shamirpet Mandal, Medchal-Malkajgiri District, Hyderabad-01. India.

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International pharmacopoeia(USP/EP)

Chiral drug list in the USP/EP/JP

		Pharmacopoeia		
Drug chemical name	**Drug brand name**	**USP**	**EP**	**JP**
Ropivacaine	Naropin	L41(AGP)		
Galantamine Hydrobromide	Razadyne	L41(AGP)		
Montelukast Sodium	Singulair	L41(AGP)	AGP	AGP
Efavirenz	Sustiva	L51(AD,AD-H)		
Escitalopram Oxalate	Lexapro	L51(AD,AD-H)		
Abacavir Sulfate	Ziagen	L51(AD,AD-H)		
Emtricitabine	Emitriva	L51(AD,AD-H)		
Levetiracetam	Keppra	L51(AD,AD-H)		
Zolmitriptan	Zomig	L51(AD,AD-H)		
Tolterodine Tartrate	Detrol	L51(AD,AD-H)		
Atrovastatin Calcium	Lipitor	L51(AD,AD-H)		
Fulvestrant	Faslodex	L51(AD,AD-H)		
Paroxetine hydrochloride	Paxil	L51(AD,AD-H)	AGP	AGP
Brinzoamide	Azopt	L51(AD,AD-H)		
Atomoxetine Hydrochloride	Strattera	L40(OD,OD-H)		
Valsartan	Diovan	L40(OD,OD-H)	OD	AGP
Irinitecan Hydrochloride	Camptosar	L40(OD,OD-H)		
Eszopiclone	Lunesta	L80 (OJ, OJ-H)		
Clopidogrel Bisulfate	Plavix	L80 (OJ, OJ-H)	OJ	OJ
Oxaliplatin	Eloxatin	L70(OC-H)		
Valacyclovir Hydrochloride	Valtrex	L66(CR(+))		CR(+)
Rosuvastatin	Crestor	L##(OJ-RH)	OJ-RH	
Levodropropizine			OD	
Sertraline Hydrochloride	Zoloft		AD	
Silodosin				OJ

International phamacopoeia : USP/EP (Valsartan)

Chemical purity

Chiral purity

Exmaple : Valsartan

	Item	USP	Item	EP
1	Diffinition			
2	Identfication	A. Infrared absorption. B. The retention time of the major peak of the sample solution corresponds to that of the standard solution, as obtained in the Assay.	Idetification	A. Infrared absorption spectrophotometry Comparison: valsartan CRS B. Enantiomeric purity C. Secific optial rotation
3	Assay	Chromatographic system Column : L1 System suitability (relative standard deviation)	Test	Related substances (Liquid chromatography) Column :-stationary phase: end-capped octadecylsilyl silica gel for chromatography R (5 μm)
4	Impurities	Limit of Valsartan related compound A Chromatographic system Column : L40 (CHIRALCEL® OD) System suitability (Resolution, relative standard deviation)	Test	Enantiomeric purity (Liquid chromatography) Column :-stationary phase: silica gel OD for chiral separation R (CHIRALCEL® OD) System suitability (resolution)
5	Impurities	Limit of Valsartan related compound B, C, and other related compounds, as directed in the Assay.		
6	Specific test	Water determination, absorbance	Others	Heavy metals, Water, Sulfated ash
7			Assay	Specified impurties : A, B, C

International phamacopoeia USP/EP (Clopidogrel)

Exmaple：Clopidogrel Bisulfate

	Item	USP	Item	EP
1	Definition			
2	Identfication	A. Infrared absorption. B. The retention time of the major peak of the sample solution corresponds to that of the standard solution, as obtained in the assay. C. Identification tests -general	Idetification	A. Specific optical rotation B. Infrared absorption spectrophotometry C. Enantiomeric purity D. It gives reaction of sulfates
3	Assay	Chromatographic system Column ：L57 System suitability (Resolution)	Test	Appearance of solution
4	Impurities	Chromatographic system Column：L1 System suitability (Peak to valley ratio)	Test	Enantiomeric purity (Liquid chromatography) Column：-stationary phase: silica gel O J for chiral separation R (10 μm) System suitability (resolution, signal to noise ratio)
5	Impurities	Limit of Clopidogrel related compound C Chromatographic system Column：L80 (CHIRALCEL® OJ) System suitability (Resolution, signal to noise ratio)	Test	Related substances (Liquid chromatography) Column：-stationary phase: end-capped octadecylsilyl silica gel for chromatography R (5 μm)
6	Specific test	Loss on drying	Others	Heavy metals, Water, Sulfated ash
7			Assay	Specified impurties：A,B,C

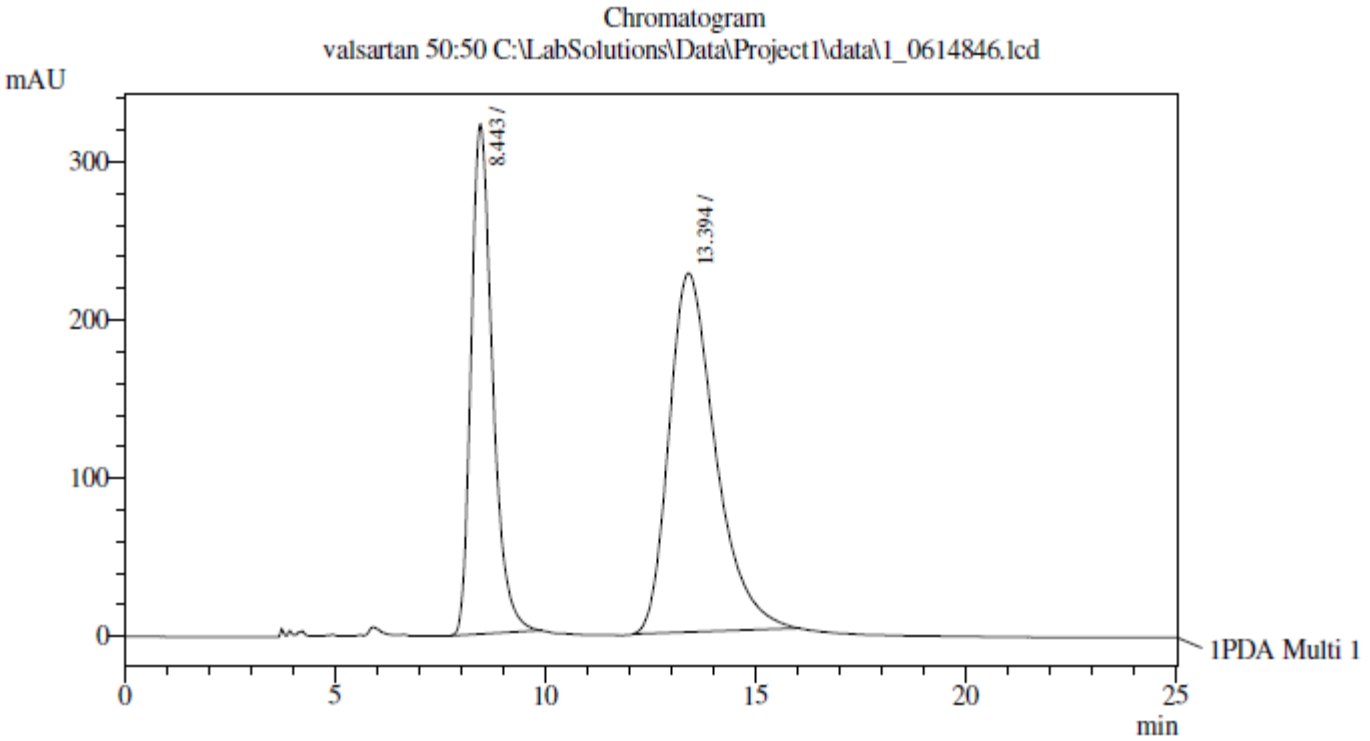
CORPORATION

Application of chiral generic drugs (USP, new method)

Chromatogram (USP) - Valsartan

USP method
Column ID: CHIRALCEL OD-H (4.6 x 250 mm, 5µm)
M.P:n-hexane:IPA:TFA (85/15/0.1);
Flow: 0.8mL/min,Temperature:25°C
UV at 230nm
Sample Conc: 0.5mg/---
Diluent: MP

CHIRALCEL® OD-H
4.6x250mm, 5,micron



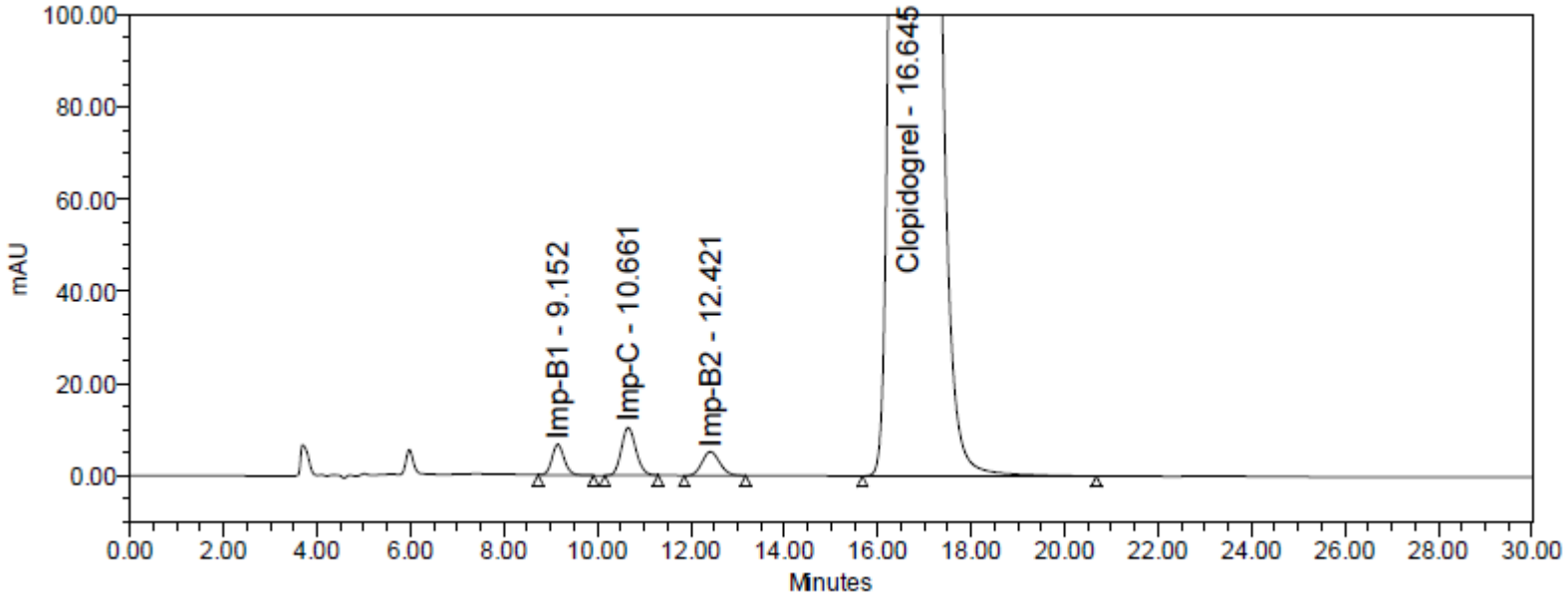
PDA Ch1 230nm 4nm

Peak#	Ret. Time	Area	Area %	heoretical Plat	Tailing Factor	Resolution
1	8.443	11451349	39.649	1258.519	1.395	0.000
2	13.394	17430138	60.351	725.117	1.420	3.366
Total		28881487	100.000			

Chromatogram (USP) - Clopidogrel

Column: CHIRALCEL OJ (4.6 x 250) mm
Mobile Phase: n-Heptane:Ethanol (85:15; v/v)
Flow rate: 0.8 mL/min; UV:220 nm; COT: 25°C

CHIRALCEL® OJ
4.6x250mm, 10micron



	Peak Name	RT	Height	Area	% Area
1	Imp-B1	9.152	6626	120716	0.26
2	Imp-C	10.661	10364	224339	0.49
3	Imp-B2	12.421	5197	136003	0.30
4	Clopidogrel	16.645	1147705	45276741	98.95

Clopidogrel for LC/MS condition

Method Information :

Column: CHIRALCEL OJ-RH (4.6 x 150) mm, 5µ

Mobile Phase: 2 mM NH4OAc in 100% MeOH

Flow Rate: 1.0 mL/min

Injection volume: 5 µL

Detection: By UV at 240 nm

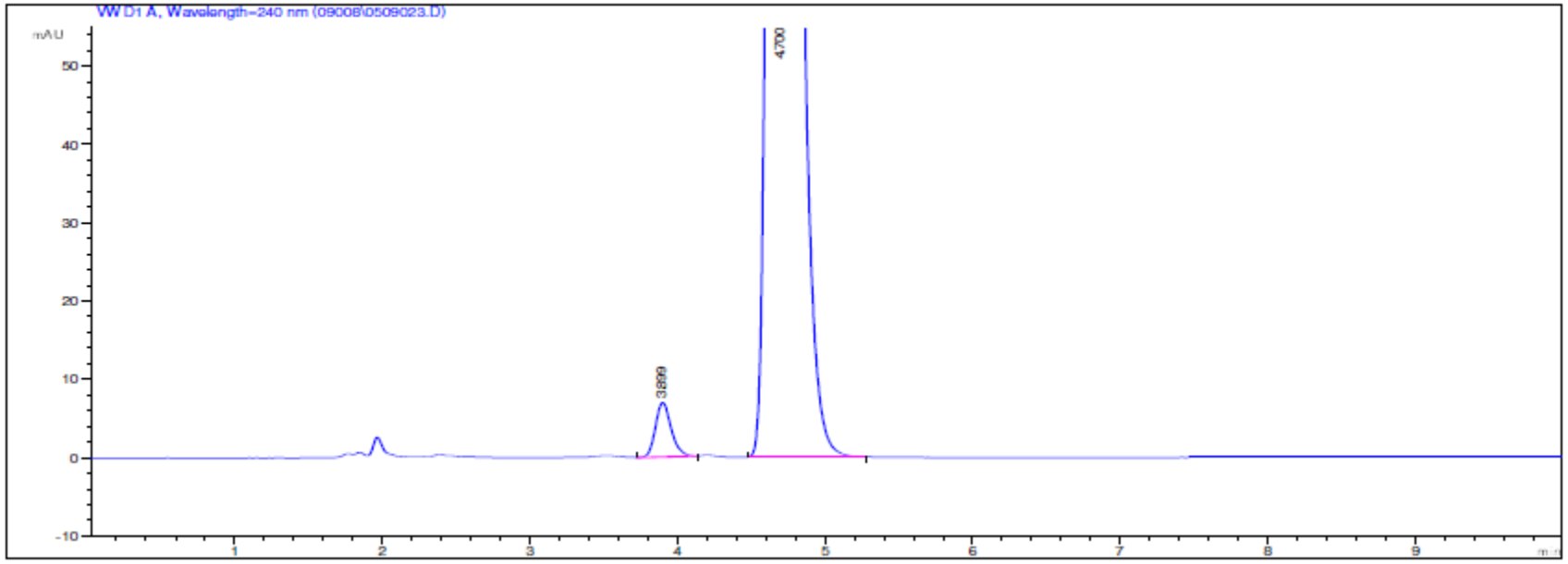
Temperature: 25° C

CHIRALCEL® OJ-RH
4.6x250mm, 5micron
<Reverse phase>

Results:

Ret. Time	N	Rs	T
3.9	6561	--	1.3
4.7	6063	3.5	1.2

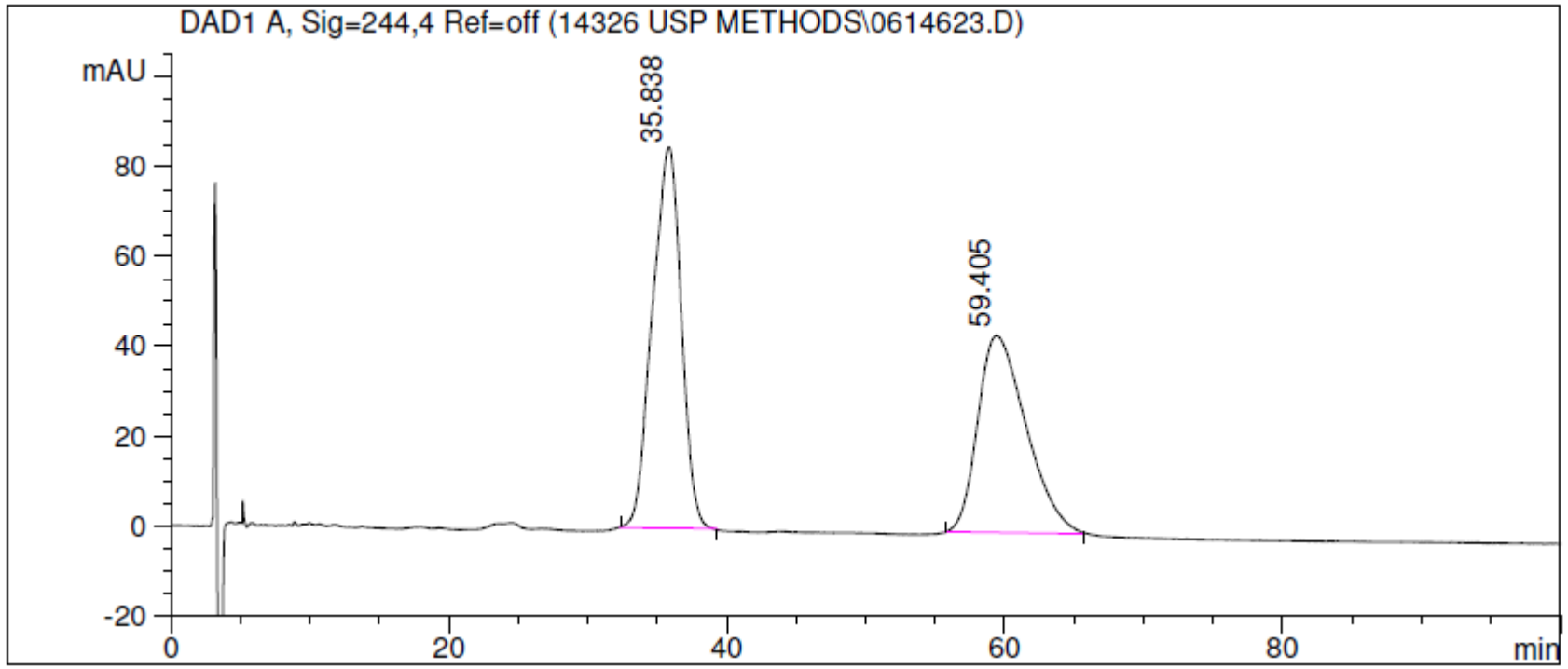
Chromatogram representing distomer at 1%:



Chromatogram (USP) - Atrovastatin

USP method: Column:CHIRALPAK AD-H (4.6*250mm,5um)
MP:n-Hexane:EtOH:TFA(94/06//0.1, v/v/v)
Flow:1.0mL, COT@25C; UV:244nm

CHIRALPAK® AD-H
4.6x250mm, 5micron

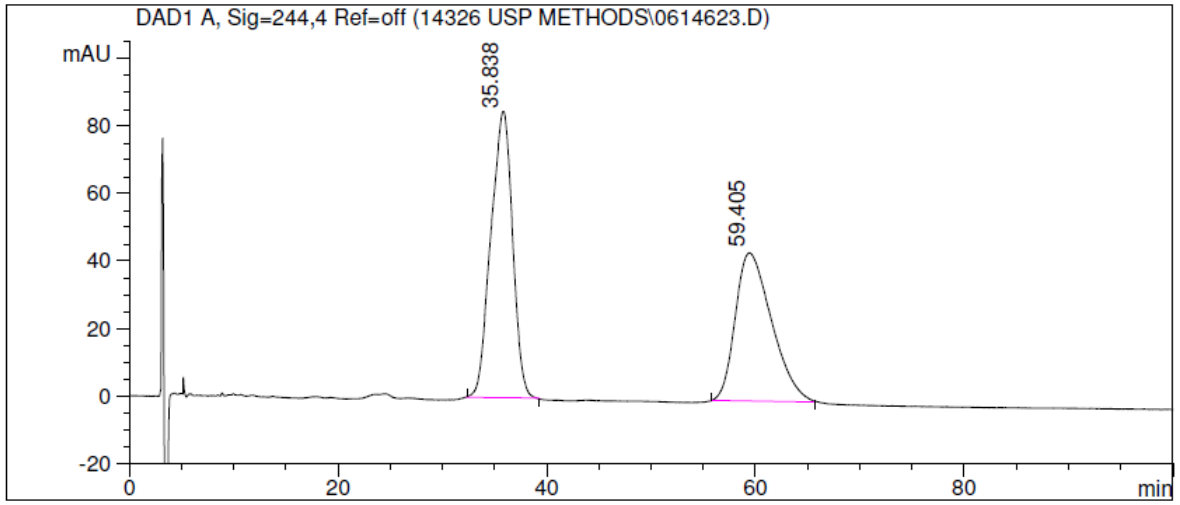


RetTime [min]	k`	Area [mAU*s]	Halfh. Width [min]	Symm.	USP Tail.	Plates	Resolution
35.->		12255.396	2.340	1.284	0.917	1299	
59.->		10622.604	3.760	0.612	1.383	1383	4.540

Chromatogram (USP vs New) - Atrovastatin

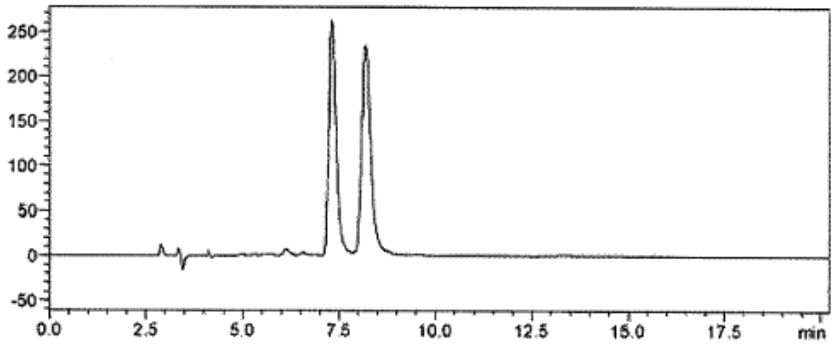
USP method

It takes more than 60 minutes.



Daicel new method

It takes less than 10 minutes.



ANALYTICAL REPORT

Column Trade Name	CHIRALPAK® IA-3		
Mobile Phase Composition	n-hexane/ ethanol / TFA 85/15/0.1		
Chromatographic Mode	Normal Phase		
Column Size	250 x 4.6 mm	Particle Size	3 µm
Flow rate (ml/min)	1	Injection amount (mg)	
Temperature (°C)	25	Rt1 & Rt2 (min)	7.31 / 8.18
Pressure (Bars)		k'1 & k'2	1.44 / 1.73
Detection	UV 245 nm	Alpha value	1.2
Resolution	2.34		

Atorvastatin: the (R,R)-enantiomer

CHIRALPAK IA-3 4.6x250mm, 3micron

New Analytical Method for Generic Drugs (1)

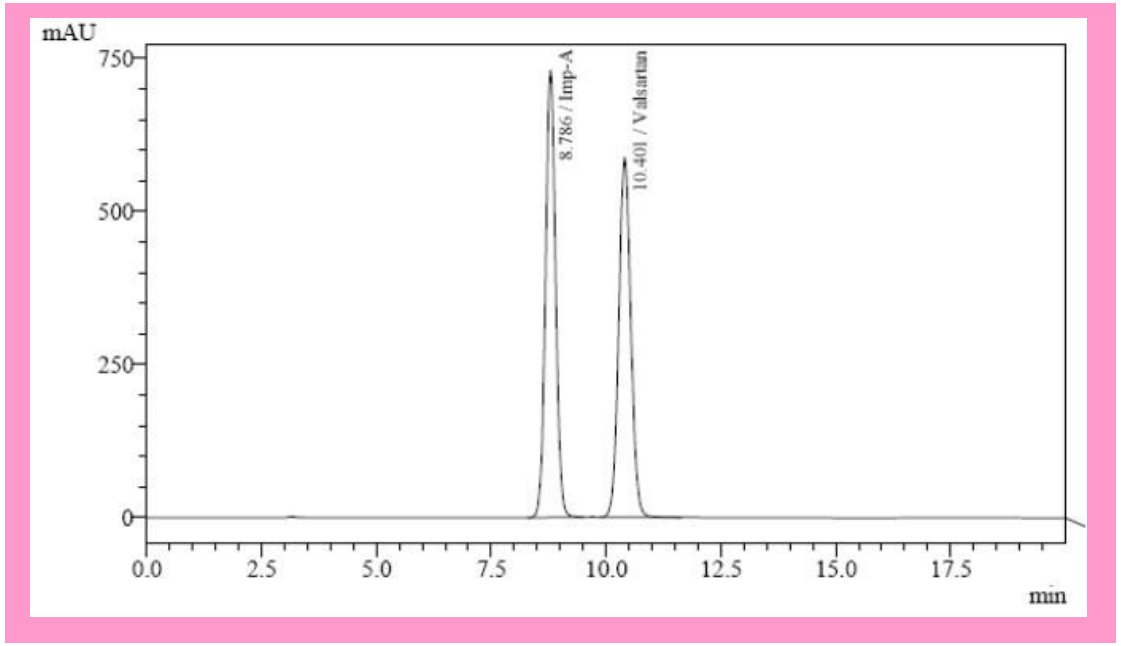
Our first generation chiral column were chose as analytical method column for various drugs.
Now we would like to introduce our newly developped method by our new generation column, such as immobilized column, IA, IB, IC, ID, IE and IF.

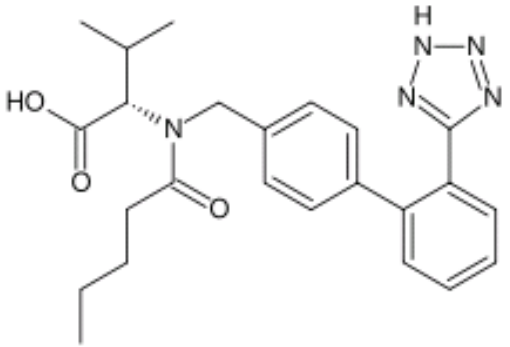
Drug	Patent expire date	**DAICEL's choice**	Listed column (USP/EP)	Note
Valsartan	2012.9	**IC**	OD-H(L40):Daicel	Better separation
Clopidgrel bisulphate	2011.11	**OJ-3, OJ-H**	OJ (10micron)/EP	3micron and 5miron are better than 10 micron.
Levofloxacin	2010.12	**AS-H**	Chiral mobile phase using L1(C18) column	USP method is used very expensive mobile phase.
Montelukast sodium	2012	**IA**	AGP(L41):Daicel	Protein based column is not enough for durability and lot-to-lot reproducibility.
Rosuvastatine calcium	2012.11	**IB**	OJ-RJ(L##): Daicel	
Escitalopram Oxalate	2012.3	**IC**	AD-H(L51):Daicel	Immobilized column is better and easier to be handled.

New Analytical Method for Generic Drugs (2)

Drug	Patent expire date	**DAICEL's choice**	Listed column	Note
Atrovastatin Calcium	2011.11	**IA-3**	AD-H(L51):Daicel	Faster and better separation, and immobilized column is better and easier to be handled.
Fulvestrant	2007	**IA**	AD(L51):Daicel	Much more simple mobile phase
Lamivudine	2012	**IC**	L45	Superior method. The separation of USP method seems to be not good and stable.
Levocetirizine	2013	**IC**		
Oxaliplatin	2008	**IC**	OC-H(L70)	Better separation.
Valacyclovir	2009.6	**IA-3**	CR(L66)	IA-3 method is more stable and good for the quantification of enatiomer but not other achiral impurities.

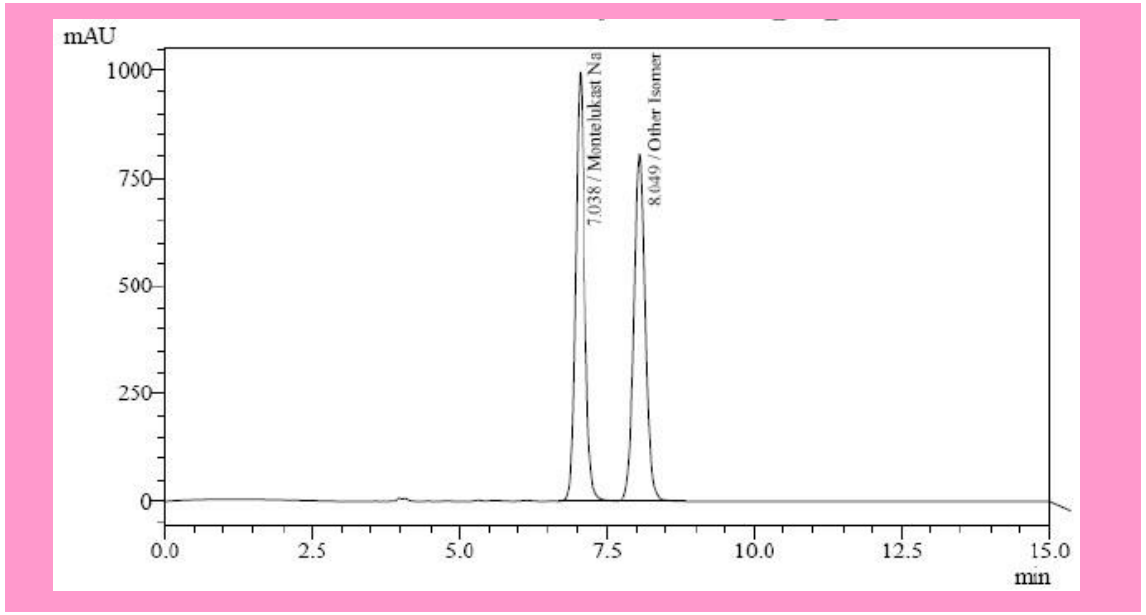
Valsartan

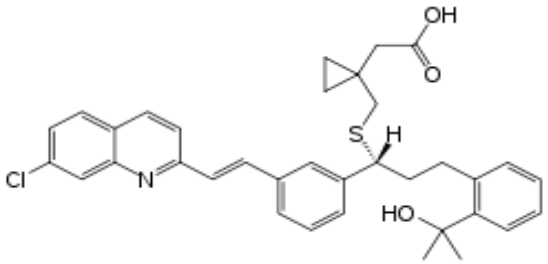




Column:CHIRALPAK® IC(4.6×250mm)
Mobile phase:n-Hex./EtOH/TFA=85/15/0.1
Flow rate:1.0mL/min.
Detect:UV 230nm
Temp:25℃
Concentration:0.5mg/mL
Injection:10uL

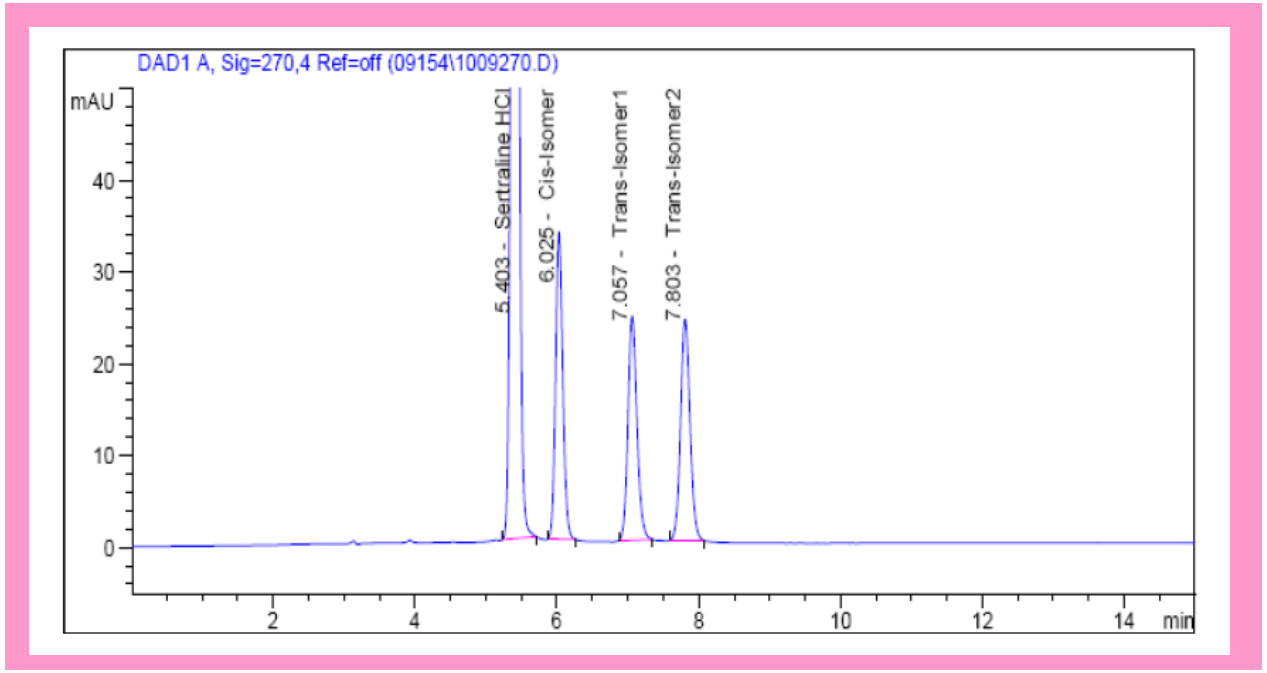
Montelukast sodium

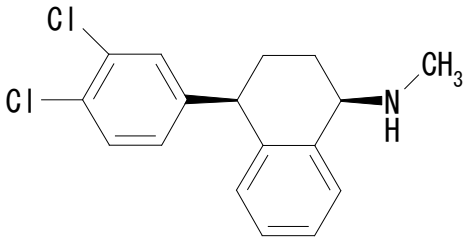




Column:CHIRALPAK® IA(4.6×250mm) with guard column
Mobile phase:n-Hex./EtOH/1,4-Dioxane/TFA/DEA=65/25/10/0.3/0.05
Flow rate:1.0mL/min.
Detect:UV 280nm
Temp:25℃
Concentration:1.0mg/mL
Injection:10uL

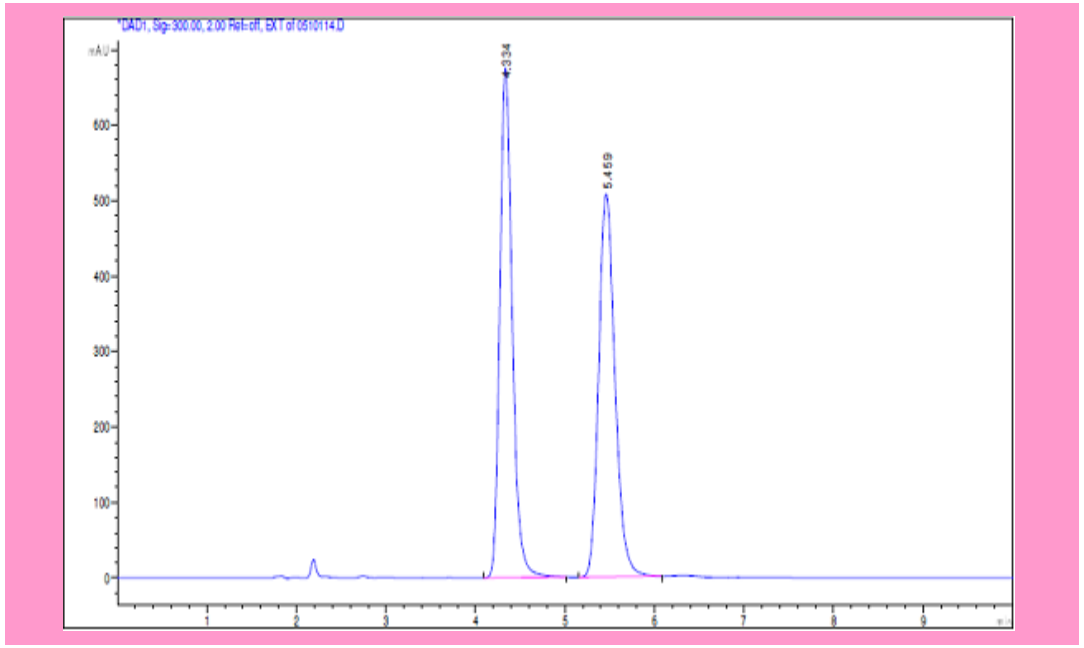
Sertraline



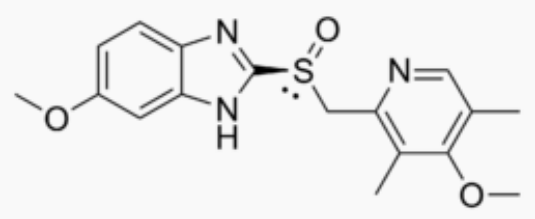


Column:CHIRALPAK® IA(4.6×250mm)
Mobile phase: n-Hexane/Ethanol/MeOH/DEA (98/1/1/0.1, v/v/v/v)
Flow rate:1.0mL/min.
Detect:UV 270nm
Temp:25℃
Concentration:0.5mg/mL

Esomeprazole



t1: 4.3
t2: 5.5
Rs: 3.5



Column:CHIRALPAK® IC-3(4.6×150mm)
Mobile phase:5 mM NH4HCO3 in H2O / MeOH (10/90, v/v)
Flow rate:1.0mL/min.
Detect:UV 300nm
Temp:25℃

The Chiral Separation of the (+) and (-) Enantiomers of Cannabidiol

Figure 9: Separation of (+) and (-) CBD on IG-U with Hex-EtOH = 95:5 (v/v).

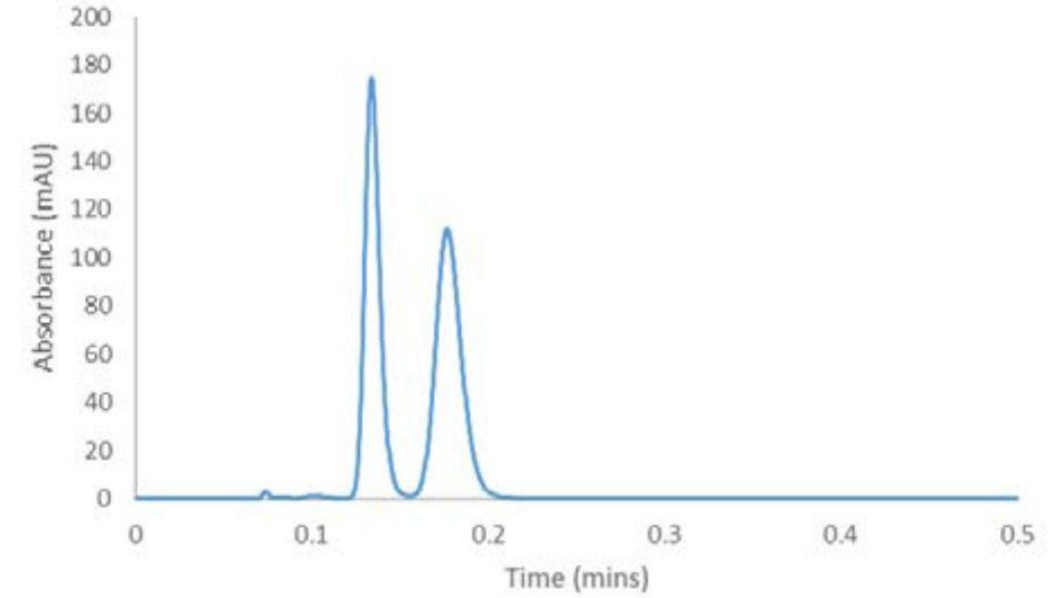
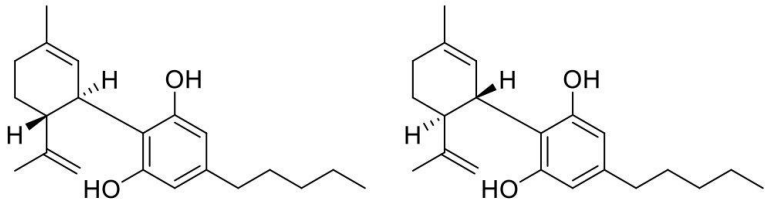


Figure 1: Enantiomers of cannabidiol (- CBD, left; + CBD, right).



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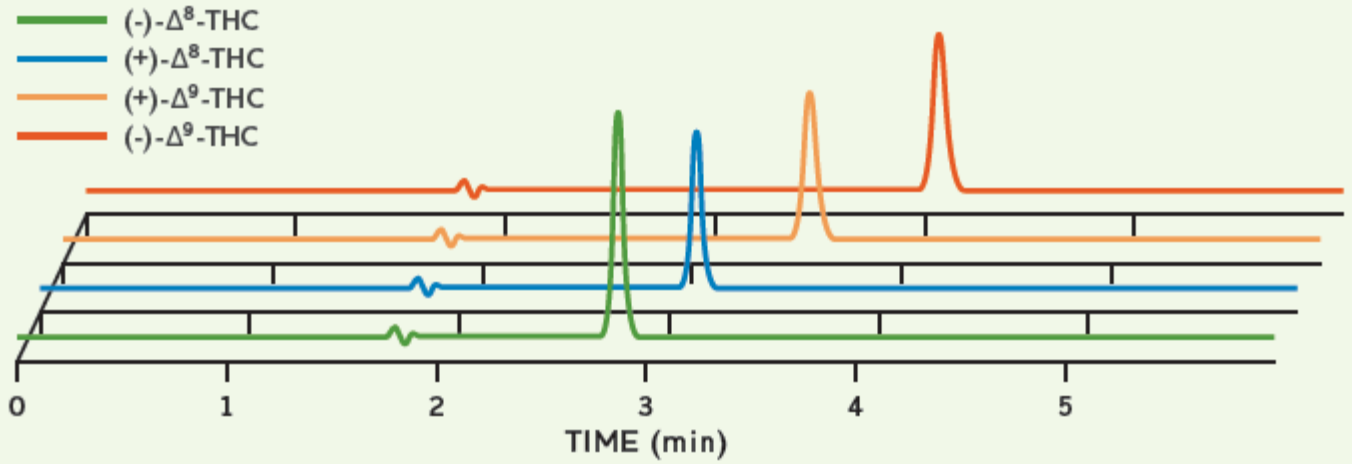
SEPARATION OF THE ENANTIOMERS OF (+/-) DELTA8-THC AND (+/-) DELTA9-THC

MIXTURE OF FOUR ISOMERS

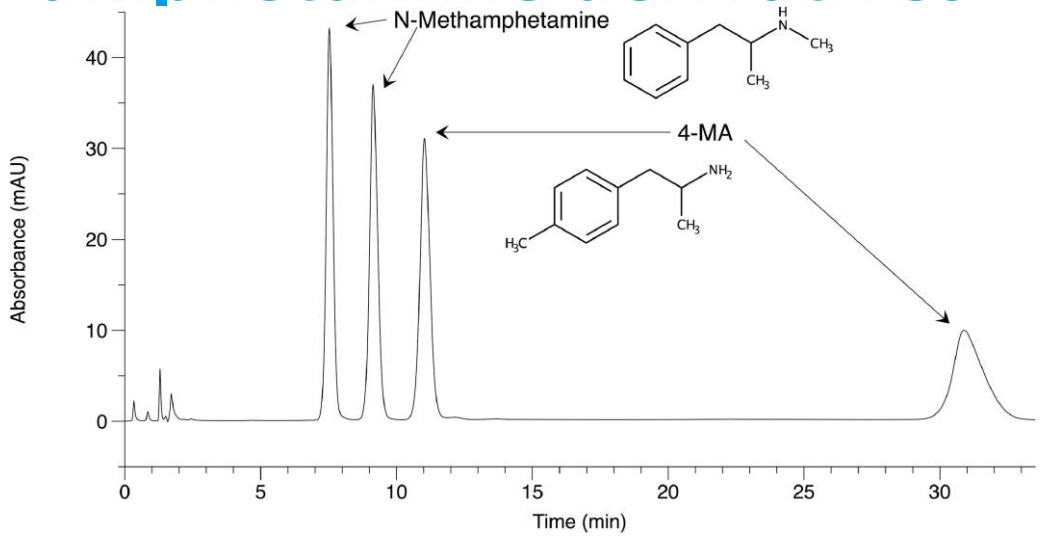


CHROMATOGRAPHIC CONDITIONS

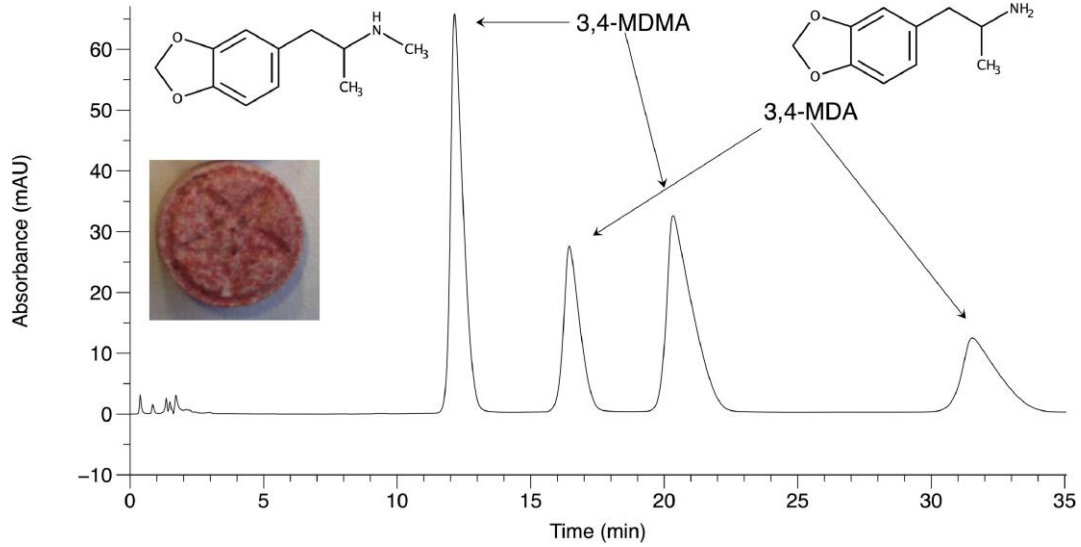
Column: CHIRALPAK® IF-3
Column Size: 4.6 mm i.d. x 150 mm long
Mobile Phase: n-Hexane/Isopropanol (95:5) v/v
Flow rate: 1.0 mL/min.
Temperature: 25° C
Sample Mixture: 1.0 mg/ml in heptane, Single isomer: Mobile Phase
Inject. Vol.: Mixture of 4 isomers: 5.0 µl, Mixture of 2 isomers: 2.5 µl, Single isomer: 0.5 µl

DAICEL Chiral Technologies

A simple and isocratic protein-based high performance liquid chromatography method for the enantioseparation of amphetamine derivatives



Simultaneous enantioseparation of N-methamphetamine and 4-MA.
Conditions:
Column: Chiralpak® CBH 150×3.0 mm (5 µm), mobile phase: 5 mM sodium dihydrogen phosphate buffer (pH=6.9) / methanol = 85 : 15, 25±1 °C, flow: 0.5 mL/min, UV: 210 nm, injection volume: 1 µL.



Simultaneous separation of 3,4-MDA and 3,4-MDMA.
Conditions: Column: Chiralpak® CBH 150×3.0 mm (5 µm), mobile phase: 5 mM sodium dihydrogen phosphate buffer (pH=6.9) / methanol = 85 : 15, 25±1 °C, flow: 0.5 mL/min, UV: 210 nm, injection volume: 1 µL.

Concerning the tested real-life sample representing a pink colored Ecstasy tablet seized by Austrian police in 2014, the enantiomers of both active components of the tablet, MDA and MDMA could clearly be distinguished as displayed in Fig. 4.

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Major drugs expiring schedule (based on IMS)

Major protection expiries by country and year

Protection expiry year	US		Japan	UK	France	Germany
2011	Lipitor® Advair® Diskus® Zyprexa®	Levaquin® Xalatan® Femara®	Actos®	Lipitor® Zyprexa® Clexane® Xalatan® Femara®	Zyprexa® Xalatan® Femara®	Clexane® Zyprexa® Xalatan® Femara®
2012	Plavix® Seroquel® Singulair®	Actos® Lexapro® Diovan®	Seroquel®	Seroquel® Singulair®	Singulair®	Seroquel®
2013	Oxycontin® Aciphex®	Zometa® Xeloda®	Aricept® Diovan® Plavix®	Seretide® Xeloda®	Seretide® Xeloda®	Xeloda®
2014	Nexium® Cymbalta®	Copaxone® Celebrex®	Abilify®	Abilify® Celebrex®	Abilify® Celebrex®	Abilify® Celebrex®
2015	Abilify® Gleevec®	Namenda®	Alimta® Spiriva®	Spiriva® Alimta®	Alimta® Spiriva®	Spiriva® Alimta®

Method of column selection

1. Looking for our application data / past data

- Literature（Exact structure）
- Application Guide 4th Edition (CD / WEB)

Daicel Web https://search.daicelchiral.com/
E-mail:chiral@jp.daicel.com

- CHIRBASE （Commercial database for chiral HPLC）

B.Koppenhoefer, R.Graf, H.Holzschuh, A.Nothdurft, U.Trettin, P.Piras and C.Roussel, *J.Chromatogr., A*, **666**, 557 (1994).

- Speculation based on similar structures

2. Assumption on basis of the separation trends

- Select columns from the data shown in the previous slides＋Experience
- Analogy of internal data on past separation examples

3. Ask us.

4. Try and error with some columns (with special inspiration !)
   In such case, automatic screening system is useful.

Our service "**Global Generic Drug Application Center**"

We can help your research and development, through our method research service.

Pharmacopoeia (USP, EP etc.)	✓ We can check USP, EP and provide the chromatogram. (in progress) ✓ We can check for the reproducibility
Our database (WEB service)	✓ Search the analytical condition through our database. ✓ Check the latest method.
Analytical service at our **GGDAC** in India	✓ We can find the method condition for you at **GGDAC, "Global Generic Drug Application Center" in India.** ✓ We can provide Chiral **LC-MS method development** for bio-studies of generic drugs
Screening service	✓ We can find the suitable column and condition through our free column screening service.
Market information	✓ We can exchange the market information through our global network.

Customer advantage through our service

1．If you tell us the compound name, we can check the analytical method, which include USP/EP and better method.

→ You can save the time!

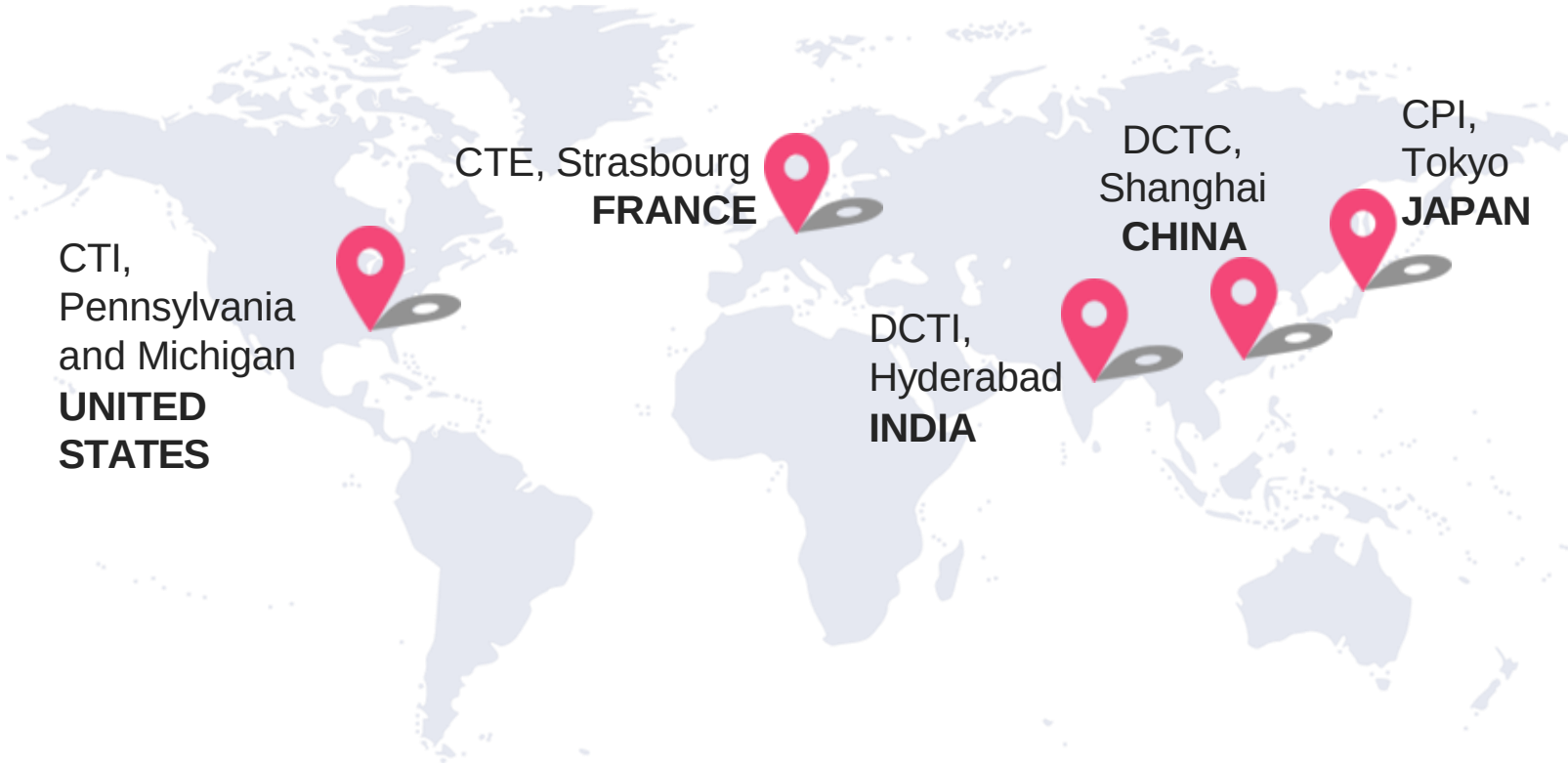
2．Sometimes, the original method is difficult to be reproduced. We can recommend a better method to avoid the future problem.

→ You can check the reproducible problem in advance.

3．We can provide the latest condition by using our new generation column.

→ In most case, the original method is established with our first generation column. Now, we developed the new products, so that faster, more stable, more durable condition can be provided.
Due to this, you can save time and cost.

Daicel Chiral Technologies Global Network



Thank you for kind attendance !

Sustainable Value Together

DAICEL CORPORATION

THANK YOU

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