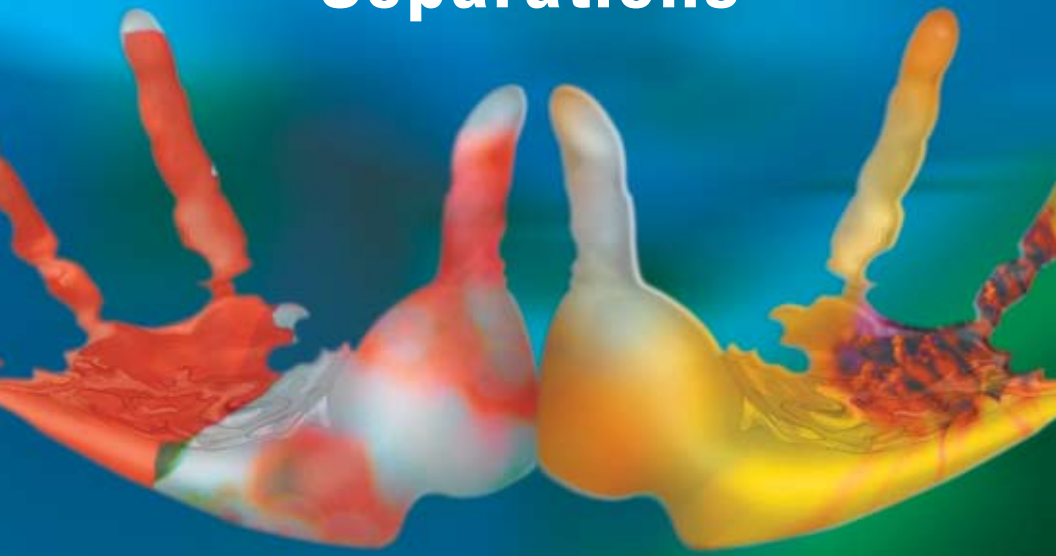
Technical Guide To

Chiral HPLC Separations



Review of

Chiral Phases

and

Method Development Strategies

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Technical Guide To Chiral HPLC Separations

Column Selection and Method Development Techniques

INTRODUCTION AND OBJECTIVES

Effective separations of enantiomers have become increasingly important to the pharmaceutical and agrochemical industry, as well as to biotechnology and most other areas of natural products chemistry. The rapid introduction of optically active medicinal drugs and pesticides, along with increasing government regulation, necessitates that rapid, sensitive and reliable stereochemical methods be devised for their analysis. Chromatography is by far the most powerful and sensitive analytical technique for resolving enantiomers, and chiral HPLC is probably the most versatile and important tool employed today.

This guidebook introduces the concepts of chirality and chiral separations by HPLC, and explores in detail the approach to column selection and method development. It summarizes the techniques used to develop and optimize chiral separations, and teaches by example and illustration. Data have been extracted from a large number of applications developed by both Phenomenex and our customers.

A systematized approach to column selection is presented, and is highly dependent on the structure of your compound. Ultimately, however, column selection becomes an empirical process, based on what has already been shown to work, or by exploring column utility for related compounds, or more simply, by trial and error. Once one or more columns are screened for their utility, optimization of the separation on a given column is a more methodical and straightforward process. In this guidebook our intention is to suggest by illustration general separation strategies you can use to successfully resolve and purify enantiomers directly and without difficulty. There are few hard and fast rules to follow. But in view of the applicability and versatility of the chiral stationary phases described here, you should have confidence that you are working with the most sophisticated tools available today for chiral HPLC.

Phenomenex maintains a dedicated chiral separation services laboratory which can assist you in developing or improving your chiral separation. Please call on us for help. We can assist you in column selection, method development, scale up or any other aspect of chiral chromatography. Phenomenex is the leader in advanced chiral separations.

Guidebook Outline

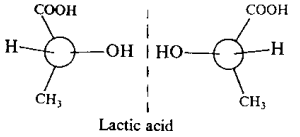
Chiral HPLC Separations of Racemic Compounds

- 🕮 Chirality
- 🕮 The Chirotechnology Revolution
- 🕮 HPLC Separation Techniques
- 🕮 Pirkle-Concept Separations
- 🕮 Method Development Techniques
- 🕮 Pharmaceutical Applications

Chirality and Molecular Structure

Causes of Molecular Asymmetry due to the arrangement of atoms in space

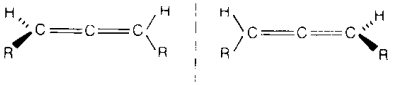
Chiral Center



Lactic acid

C, N, P, S or B

Chiral Axis

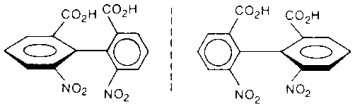


Allene substitution

Chiral Plane

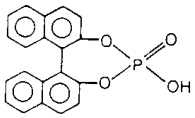
Helicenes,
Natural polymers

Atropisomerism



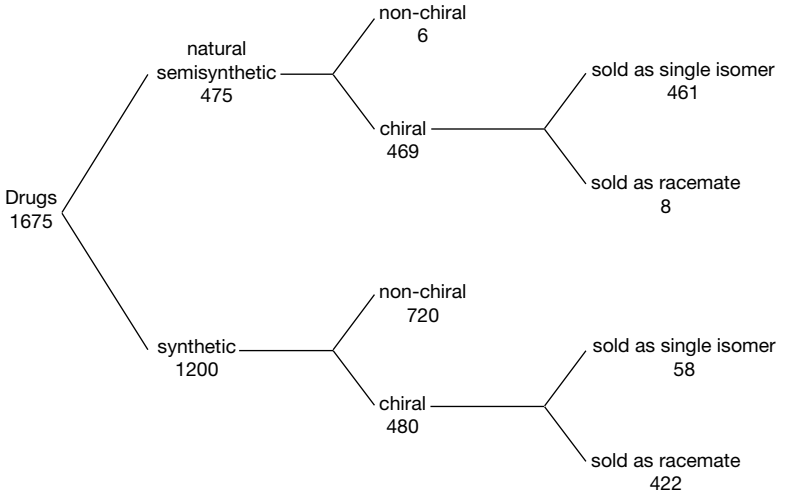
Substituted Biaryls

Molecular Strain



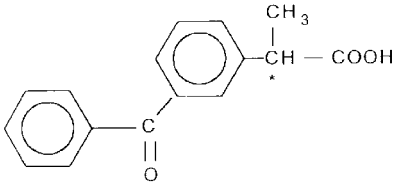
Ortho-bridging of
Biaryls

Chiral Drugs and their Applications as Single Isomers or Racemic Mixtures

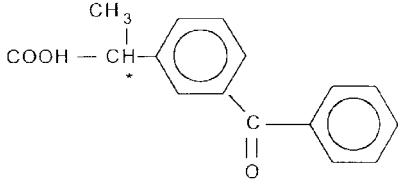


- ☞ 40% of all man-made synthetic drugs are chiral
- ☞ 60% of all pharmaceuticals are chiral
- ☞ 45% of all chiral drugs are sold as racemates

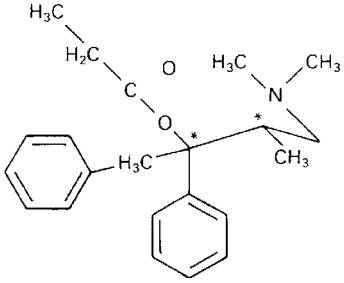
Stereospecific Activity



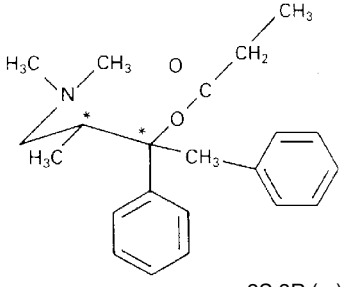
S - (+) - KETOPROFEN
(analgesic, antiinflammatory)



R - (-) - KETOPROFEN
(slows periodontal bone loss)



2R.3S (+)
dextropropoxyphene
analgesic



2S.3R (-)
levopropoxyphene
antitussive

DARVON NOVRAD

Enantiomers with different types of action. The analgesic action is incompatible with the antitussive, respiration depressant action. Both enantiomers are marketed, one as an analgesic, the other as an antitussive (anti-cough) drug. Their chemical mirror-type relationship is reflected in the names Darvon and Novrad [9].

Enantiomers May Confer Benefits Over Racemates for Therapeutic Uses

Properties of racemate	Potential benefits of enantiomer
One enantiomer is exclusively active	Reduced dose and load on metabolism
The other enantiomer is toxic	Increased latitude in dose and broader use of the drug
Enantiomers have different pharmacokinetics	Better control of kinetics and dose
Enantiomers metabolized at different rates in the same person	Wider latitude in setting the dose; reduction in variability of patients' responses
Enantiomers metabolized at different rates in the population	Reduction in variability of patients' responses; greater confidence in setting a single dose
One enantiomer prone to interaction with key detoxification pathways	Reduced interactions with other common drugs
One enantiomer is agonist, the other antagonist	Enhanced activity and reduction of dose
Enantiomers vary in spectra of pharmacological action and tissue specificity	Increased specificity and reduced side effects for one enantiomer; use of other enantiomer for different indication

Source: Chiros

These drugs are Best Candidates for Racemic Switches

Cardiovascular

- Acebutolol
- Alprenolol
- Atenolol
- Betaxolol
- Bisprolol
- Bopindolol
- Bucumolol
- Bufetolol
- Bufuralol
- Bunitrolol
- Bupranolol
- Butofililol
- Carazolol
- Carvedilol
- Curteolol
- Disopyramide
- Dobutamine
- Indenolol
- Mepindolol
- Metipranolol
- Metoprolol
- Nadolol
- Nicardipine
- Oxpranolol
- Pindolol
- Propanolol
- Sotalol
- Toliprolol
- Verapamil
- Xibenolol

Central nervous system

- Dobutamine
- Fluoxetine
- Ketamine
- Lorazepam
- Meclizine
- Oxaprotiline
- Phenylpropanolamine
- Thioridazine
- Polycloramphetamine
- Tomoxetine
- Toloxaton
- Viloxazin

Respiratory

- Albuterol/salbutamol
- Metaproteranol
- Terbutaline

Anti-inflammatory and analgesics

- Cicloprofen
- Corticosteroids
- Dihydroxythebaine
- Fenbuphen
- Fenoprofen
- Flurbiprofen
- Ibuprofen
- Indoprofen
- Ketoprofen
- Minoxiprofen
- Pirprofen
- Steroids
- Suprofen
- Triamcinolone

Anticancer

- Cytarabine

Antibiotics, antiinfectives, antivirals

- Ciprofloxacin
- Norfloxacin
- Ofloxacin

Hormones, genitourinary

- Benzyl glutamate
- Butoconazole
- Calcitonin
- Estradiol
- Fluorogesterone
- Gonadorelin
- Ketodesogestrel/ estrogen
- Norgestrel
- Progestin nitanol
- Testosterone

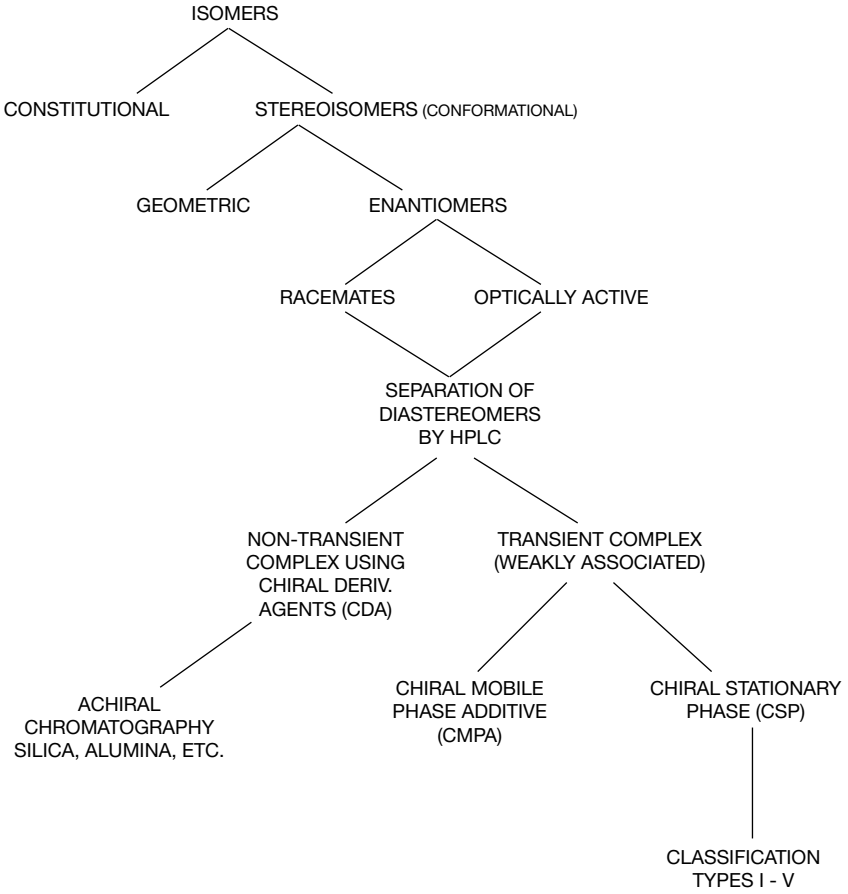
Antihistamine and cough/cold

- Astemizole
- Terfenadine

Source: Technology Catalysts Interational

A **Racemic Switch** is the redevelopment of a drug now marketed as a racemate to the pure enantiomer.

Isomer Separation Flow Chart

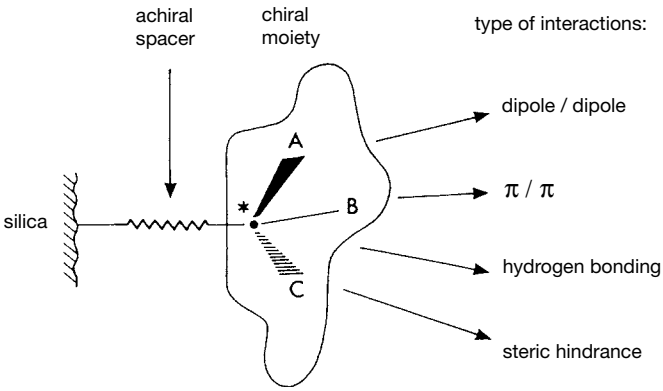


Classification of Chiral Stationary Phases (CSPs)

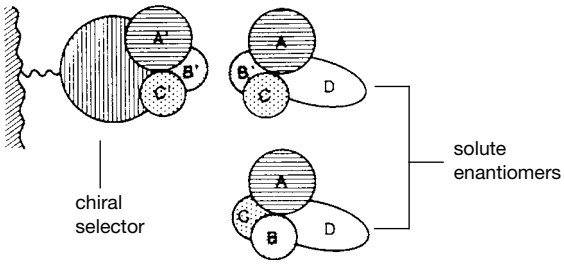
Type	Description	Chemistry	Mechanism
I	BRUSH	Various chiral selectors	Attractive interactions
(Pirkle)	Ionic or covalent bonding	Hydrogen Bonding	
	Charge transfer (π-π interaction)		
Dipole stacking			
II	HELICAL POLYMERS	Cellulose derivatives	Attractive interactives
	Poly methacrylates	Insertion complexes	
Poly acrylamides			
III	CAVITY	Cyclodextrins	Inclusion complexes
	Crown ethers		
IV	LIGAND EXCHANGE	Amino acid-metal complex	Diastereomeric metal complex
V	PROTEIN	α-acid glycoprotein	Hydrophobic interactions
	Bovine Serum Albumin	Polar interactions	
VI	MACROCYCLIC	Antibiotic	Attractive interactions
GLYCOPEPTIDE			Hydrogen Bonding
			Inclusion complexes
			Polar interactions
			Charge transfer (π-π interaction)

Pirkle-Concept CSPs
Mechanism of Interaction

Enantiorecognition involves hydrogen bonding, dipolar and charge transfer interactions and steric hindrance



Class-I CSPs and types of interactions that can be involved during the chiral recognition process.

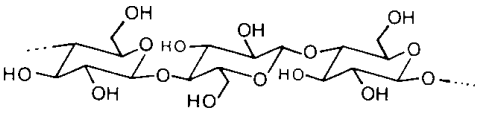


The three-point interaction model advanced by Dalgliesh

Chiral Stationary Phases (CSPs)

CSP TYPE II. INSERTION COMPLEX
HELICAL POLYMERS (Natural CELLULOSES and DERIVATIVES and VARIOUS SYNTHETIC POLYMERS)

Natural:



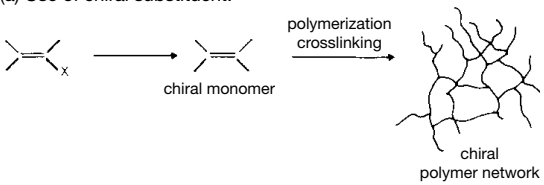
linear poly[1 - 4 - β - D -glucose]

Cellulose
Amylose
Chitosan
Dextran

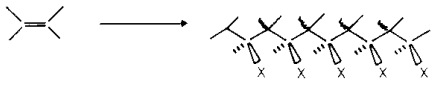
Derivatives:
Acetates
Esters
Carbamates

Synthetic:

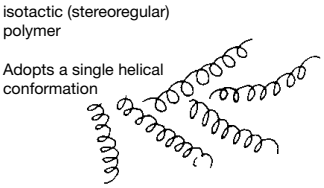
(a) Use of chiral substituent:



(b) Use of chiral catalyst:

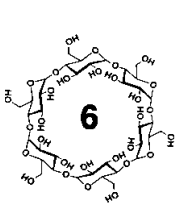


Polymethacrylates
Polyacrylamides

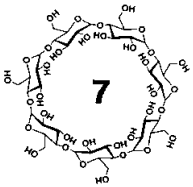


Chiral Stationary Phases (CSPs)

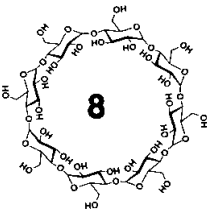
CSP TYPE III. INCLUSION (CAVITY) COMPLEX
CYCLODEXTRINS and DERIVATIVES



α-Cyclodextrin

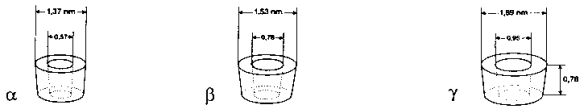


β-Cyclodextrin

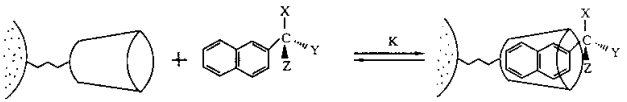


γ-Cyclodextrin

Derivatives:
Acetates
Esters
Carbamates



The structure of α, β and γ cyclodextrin.



Schematic showing the reversible inclusion complex formation typical of cyclodextrin bonded stationary phase.

CSP TYPE IV. LIGAND EXCHANGE
DIASTEREOMERIC METAL COMPLEX

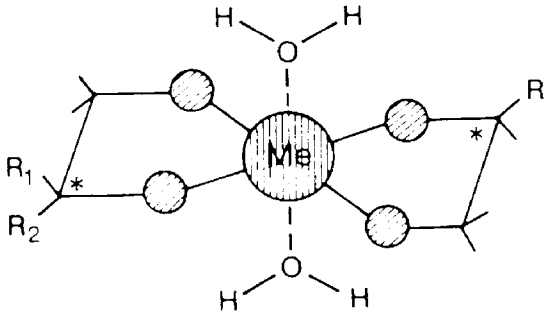
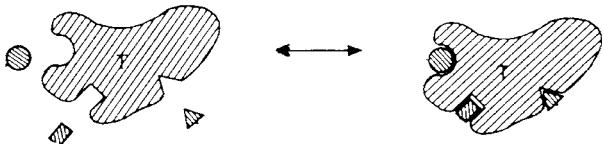


Illustration of the principle of chiral ligand exchange used for chromatographic optical resolution. Reversible formation of diastereomeric metal complexes is obtained in a reaction between a chiral selector (left-hand side) and the respective enantiomers (right-hand side).

Chiral Stationary Phases (CSPs)

CSP TYPE V. PROTEIN

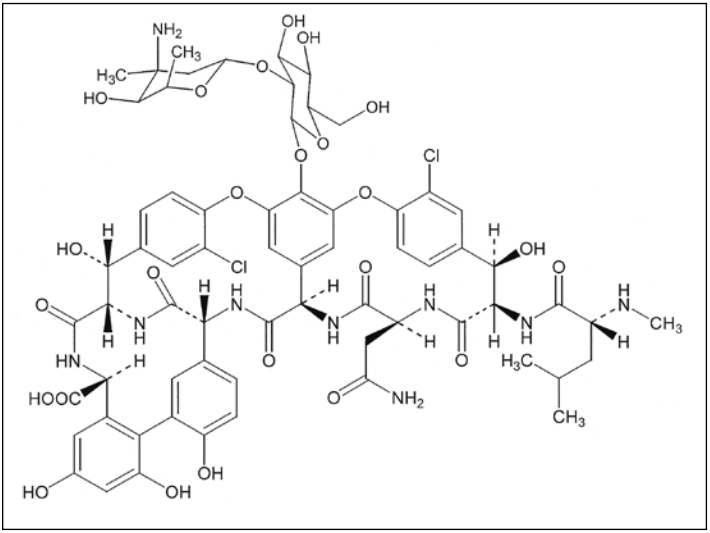
AGP, BSA, HSA, OVOMUCOID



CSP TYPE VI

MACROCYCLIC/ANTIBIOTIC

VANCOMYCIN, TEICOPLANIN, RISTOCETIN



The Chirex Advantage

- Wide variety of supports
- High enantioselectivity
- High efficiency, covalently bound columns
- Chemically stable and rigid supports
- Choice of elution order (opposite CSPs available)
- Packed columns from minibore to preparative

Chiral Stationary Phases

Stationary phases in **Chirex** columns contain various chiral components. They are classified into three types: **amide, urea and ligand exchange types**. In the amide type, asymmetric carbon atoms are bonded directly with the **CONH** group, and in the urea type, asymmetric carbon atoms with the **NHCONH** group, and they are chemically (covalently or ionically) bonded to silica gel. In the ligand exchange type, the chiral components are coated hydrophobically on ODS.

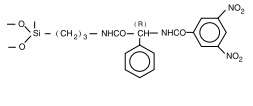
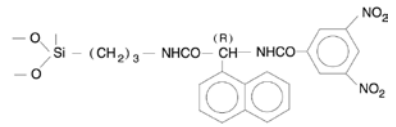
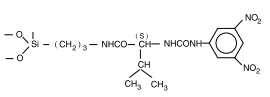
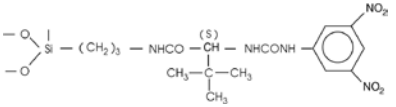
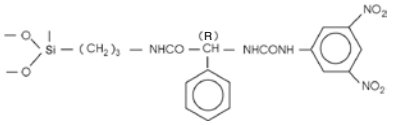
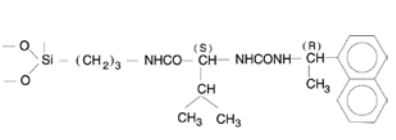
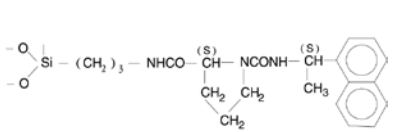
CHIREX	Chiral Component	Mode*	Application
Amide type			
3001	(R)-phenylglycine	NP	esters, amides, carboxylic acids, alcohols, ketones
3005	(R)-l-naphthylglycine	RP	
Urea type			
3010	(S)-valine	RP	carboxylic acids (including Z-amino acids, BOC-amino acids, benzoylamino acids)
3011	(S)-tert-leucine	RP	
3012	(S)-phenylglycine	RP	
3014	(S)-valine (R)-l-(α-naphthyl)ethylamine	NP	amines, amides, amino alcohols, alcohols, esters, phosphoric amides
3017	(S)-proline, (S)-l-(α-naphthyl)ethylamine	NP	
3018	(S)-proline (R)-l-(α-naphthyl)ethylamine	NP	
3019	(S)-tert-leucine (S)-l-(α-naphthyl)ethylamine	NP	
3020	(S)-tert-leucine (R)-l-(α-naphthyl)ethylamine	NP	
3022	(S)-indoline-2-carboxylic acid (R)-l-(α-naphthyl)ethylamine	NP	
Ligand-exchange type			
3126	(D)-penicillamine	RP	α-amino acids, α-hydroxy acids

*NP : Normal phase mode

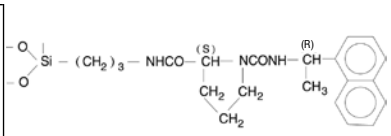
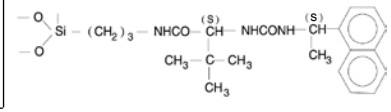
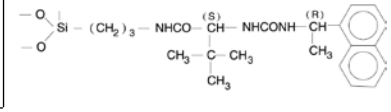
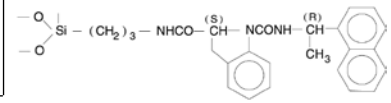
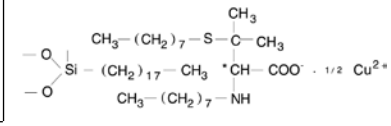
RP : Reverse phase mode, typically consisting of ammonium acetate in methanol

Enantiomeric stationary phases are available except for 3022, and on this phase the elution order of enantioners are inverted.

Chirex Chiral Stationary Phases (CSPs)

Phase	Description	Structure
3001	(R)-phenylglycine and 3,5-dinitrobenzoic acid amide linkage, electron acceptor designed for the separation of carboxylic acids, alcohols, esters sulphoxides	
3005	(R)-1-naphthylglycine and 3,5-dinitrobenzoic acid amide linkage, electron acceptor designed for the separation of: carboxylic acids, alcohols, esters non-steroidal anti-inflammatory agents	
3010	(S)-valine and 3,5-dinitroaniline urea linkage, electron acceptor designed for the separation of: carboxylic acids, amino acid derivatives hydroxy acids Dabsyl and Dansyl derivatives of amino acids	
3011	(S)-tert-leucine and 3,5-dinitroaniline urea linkage, electron acceptor designed for the separation of: carboxylic acids, amino acid derivatives	
3012	(R)-phenylglycine and 3,5-dinitroaniline urea linkage, electron acceptor designed for the separation of: carboxylic acids, amino acid derivatives	
3014	(S)-valine and (R)-1-(α-naphthyl)ethylamine urea linkage, electron donor designed for the separation of: π-acceptor derivatives of amines, carboxylic acids and amino acids the esters and amides of these acids underivatized alcohols	
3017	(S)-proline and (S)-1-(α-naphthyl)ethylamine urea linkage, electron donor designed for the separation of: amines, alcohols and amino acids underivatized β-blockers aromatic amines pesticides	

Chirex Chiral Stationary Phases (CSPs)

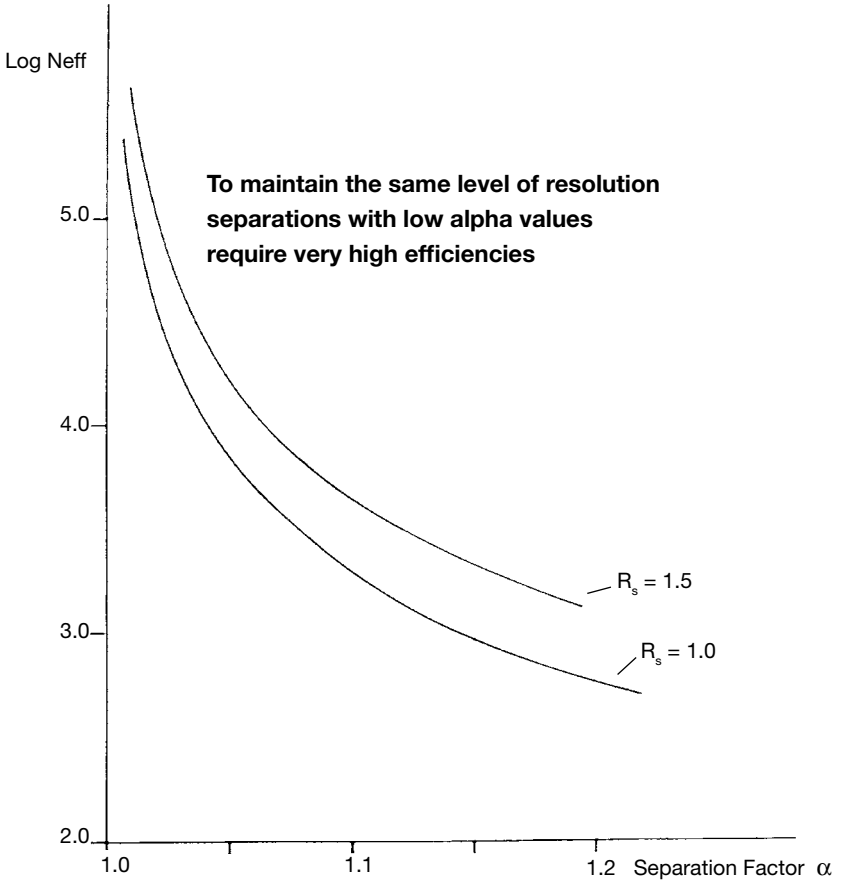
Phase	Description	Structure
3018	(S)-proline and (R)-1-(α-naphthyl)ethylamine urea linkage, electron donor designed for the separation of: amines, alcohols and amino acids underivatized ß-blockers aromatic amines pesticides	
3019	(S)-tert-leucine and (S)-1-(α-naphthyl)ethylamine urea linkage, electron donor designed for the separation of: esters, amino alcohols underivatized ß-blockers aromatic amines cyano alcohols pesticides	
3020	(S)-tert-leucine and (R)-1-(α-naphthyl)ethylamine urea linkage, electron donor designed for the separation of: amines, amino alcohols, alcohols underivatized β-blockers aromatic amines cyano alcohols pesticides	
3022	(S)-indoline-2-carboxylic acid and (R)-1-(α-naphthyl)ethylamine urea linkage, electron donor designed for the separation of: amines, amino alcohols, alcohols	
3126	(D)-penicillamine ligand exchange, electron acceptor designed for the separation of: α-amino acids, their derivatives α-hydroxy acids, amino alcohols	

Chirex Column Selection Guide

Class	Aromatic	—N—	—COOH	—OH	OTHER	Comment
Group 1	Y	Y	Y			Aromatic α-amino acids, α-hydroxy acids
Group 2	Y	Y		Y		
Group 3	Y	Y			Y	
Group 4	Y		Y			
Group 5	Y			Y		
Group 6	Y					Y
Group 7		Y	Y			Aliphatic α-amino acids, α-hydroxy acids and their derivatives
Group 8			Y			
Group 9					Y	
Group 10					Y	Asymmetric other than carbon. Chiral center at N,S,P,B, etc.

Class	First Choice	Second Choice	Third Choice
Group 1	3126	3005 or 3001	3011 or 3012
Group 2	3022 or 3020	3014	3017 or 3018
Group 3	3014 or 3020	3022	3017 or 3018
Group 4	3005	3010	3001
Group 5	3001 or 3014	3017 or 3005	3020 or 3022
Group 6	3001	3005	3019 or 3020
Group 7	3126		
Group 8	3126	3010	3001
Group 9	3014	3019 or 3020	3001
Group 10	3014	3010	3005

Importance of Separation Factor α

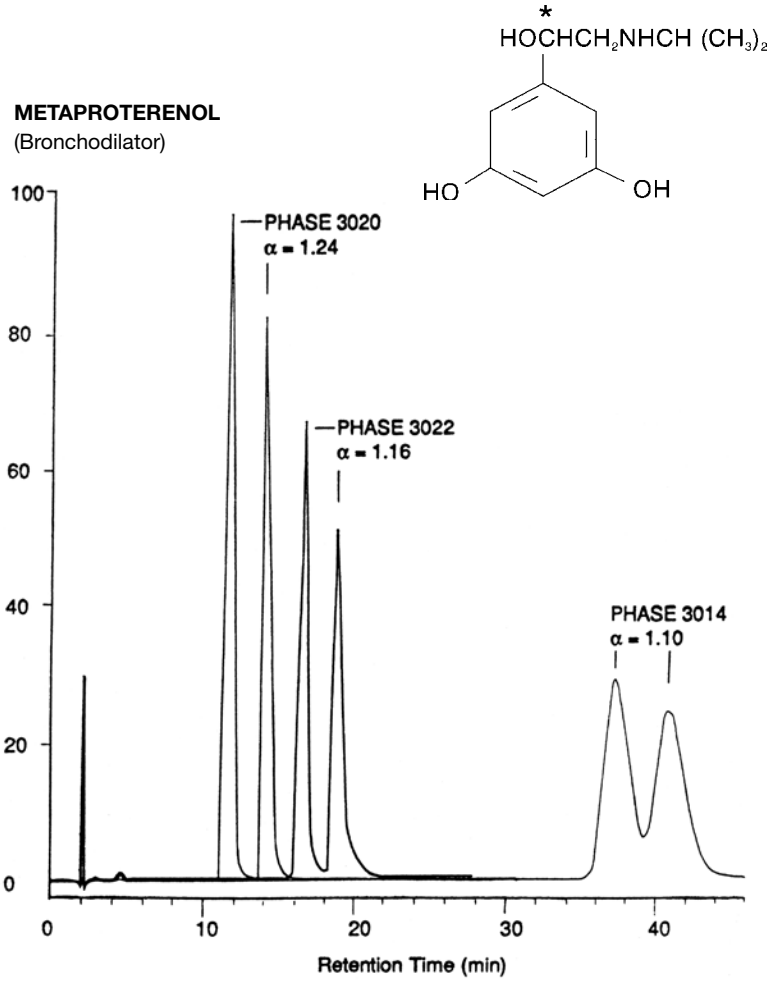


Plots of plate numbers vs. α-values at different constant resolution values. Note the drastic increase in plate numbers needed to maintain resolution at very low α-values

Enantioselectivity

Column Comparison

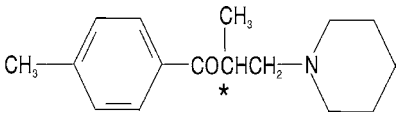
Optimized Separations of Metaproterenol



Chiral Stationary Phases

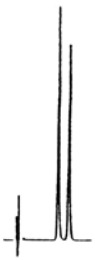
Differences in Enantioselectivity

TOLPERISONE
(Bronchodilator)



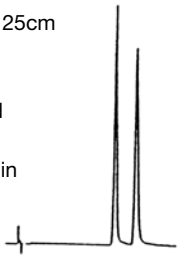
CHIREX 3017 α = 1.25

Column: 4.0mmID x 25cm
Mobile Phase:
Hexane/ethanol/
trifluoroacetic acid
(200:40:0.6)
Flow Rate: 1.0mL/min
Detector: UV254nm



CHIREX 3019 α = 1.22

Column: 4.0mmID x 25cm
Mobile Phase:
Hexane/ethanol/
trifluoroacetic acid
(200:40:0.6)
Flow Rate: 1.0mL/min
Detector: UV254nm

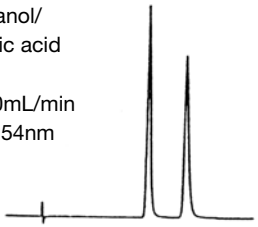


CHIREX 3018 α = 2.13

Column: 4.0mmID x 25cm
Mobile Phase:
Hexane/ethanol/
trifluoroacetic acid
(200:40:0.6)
Flow Rate: 1.0mL/min
Detector: UV254nm

CHIREX 3020 α = 1.35

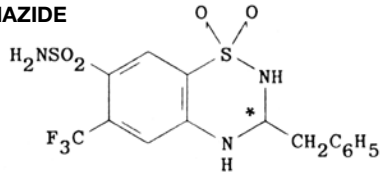
Column: 4.0mmID x 25cm
Mobile Phase:
Hexane/ethanol/
trifluoroacetic acid
(230:20:1)
Flow Rate: 1.0mL/min
Detector: UV254nm



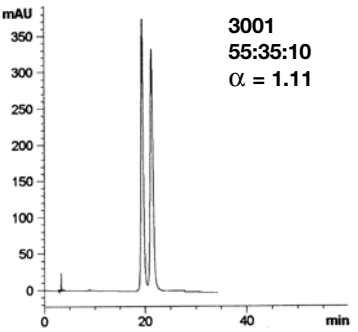
Chiral Stationary Phases

Differences in Enantioselectivity

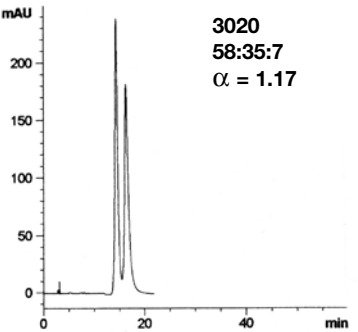
BENDROFLUMETHAZIDE
(Diuretic)



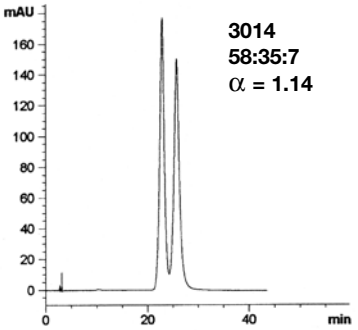
Dimensions: 250 x 4.6mm
Mobile Phase:
Hex/DCE/EtOH-TFA
(as indicated)
Flow Rate: 1.0mL/min
Detector: UV @ 272nm



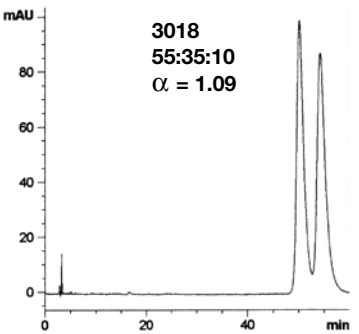
3001
55:35:10
α = 1.11



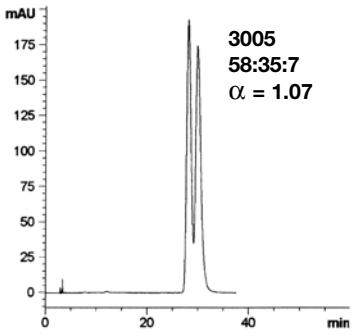
3020
58:35:7
α = 1.17



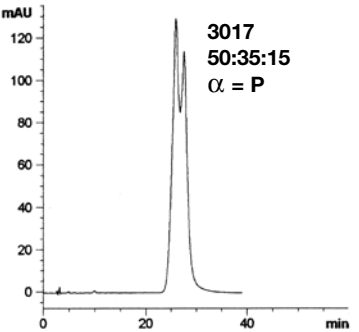
3014
58:35:7
α = 1.14



3018
55:35:10
α = 1.09



3005
58:35:7
α = 1.07



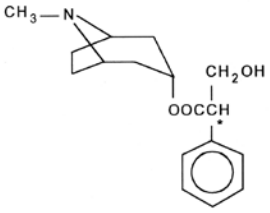
3017
50:35:15
α = P

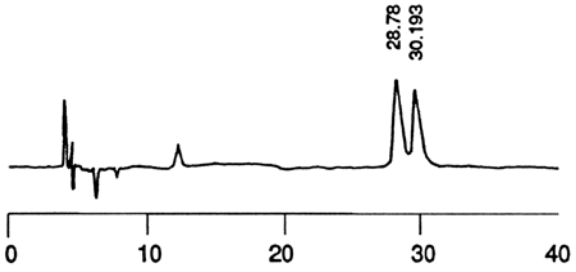
Chiral Stationary Phases

Improve Resolution by Increasing Column Length

ATROPINE (3-TROPANYL TROPATE)

Column: Chirex 3018
Dimensions: 250 x 4.6mm (2x)
Mobile Phase:
Hexane/1,2-Dichloroethane/
Methanol/Trifluoroacetic Acid
(250:140:10:1)
Flow Rate: 1.0mL/min
Detector: UV @ 254nm



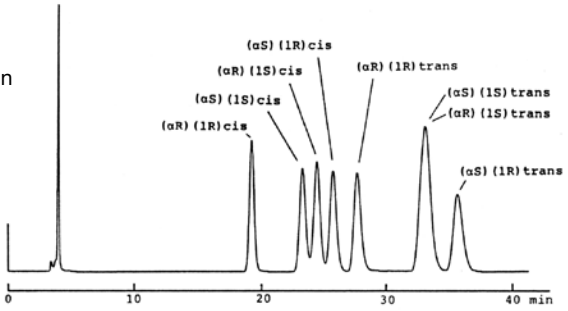


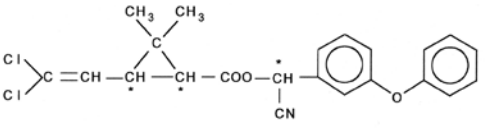
Chiral Stationary Phases

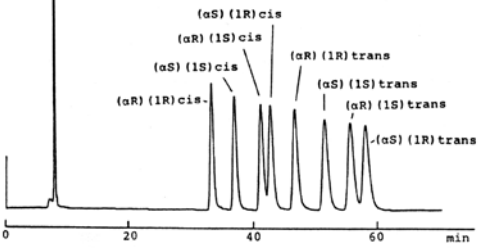
Improve Resolution by Combining Different Column Selectivities

Mobile Phase:
Hexane/1,2-Dichloroethane/
Ethanol (500:10:0.05)
Flow Rate: 1mL/min
Temperature: Ambient
Detector: UV @ 230nm
Sample: Racemic Cypermethrin

Chirex 3019
250 x 4.0mm



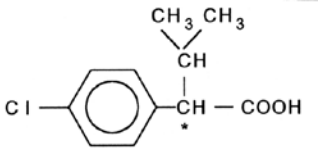




CYPERMETHRIN

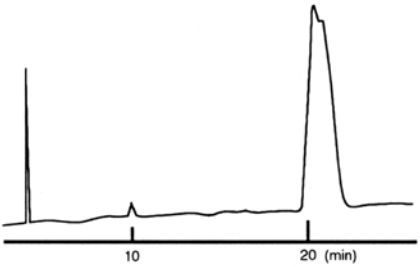
Chirex 3019 + 3020
250 x 4.0mm (2X)

Effect of Mobile Phase on the Separation of 2- (4-Chlorophenyl) -3-Methyl Butyric Acid

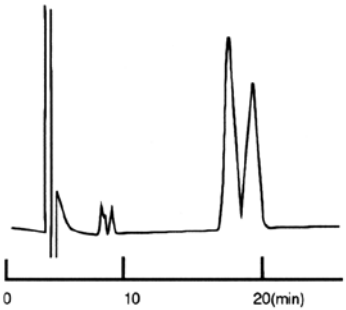


Mobile Phase: 0.1M Ammonium Acetate in:

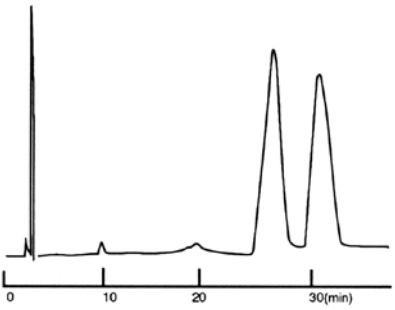
Water/Methanol
(60:40)



Water/Acetonitrile
(60:40)



Water/Tetrahydrofuran
(60:40)

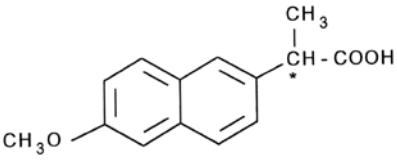


CONDITIONS:

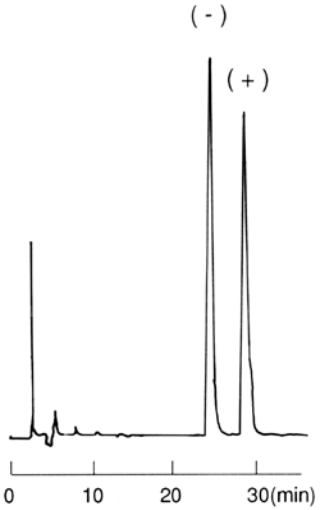
Column: Chirex Phase 3012
(250 x 4.6mm)
Flow Rate: 1.0 mL/min
Detector: UV @ 254nm

Normal vs. Reverse Phase on 3005

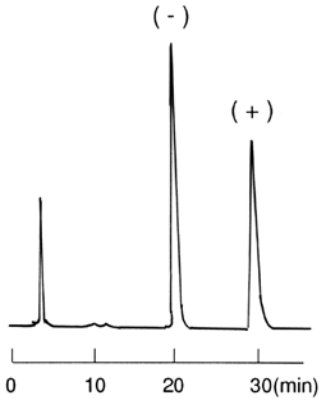
NAPROXEN
(Antiinflammatory,
Analgesic)



Hexane/1,2-dichloroethane/ ethanol/acetic acid (190:9:1:1)



0.05M ammonium acetate in methanol



CONDITIONS:

Column: Chirex Phase 3005
(250 x 4.6mm)
Flow Rate: 1.0 mL/min
Detector: UV @ 254nm

Enantiomer Separations of Propranolol by HPLC Effect of Mobile Phase

Column: Chirex Phase 3014 (250 x 4.0mm ID)
Mobile Phase: containing 0.25% trifluoroacetic acid
Flow Rate: 1.0mL/min
Detector: UV@254nm

TEST SYSTEM 1

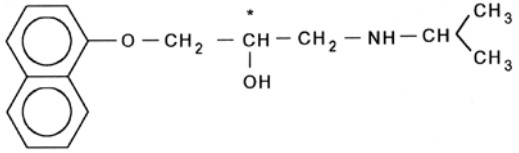
Hexane/ethanol	k_1	α
100 : 0	11.8	1.00
97.5 : 2.5	23.0	1.07
95 : 5	8.4	1.07
92.5 : 7.5	4.8	1.06

TEST SYSTEM 2

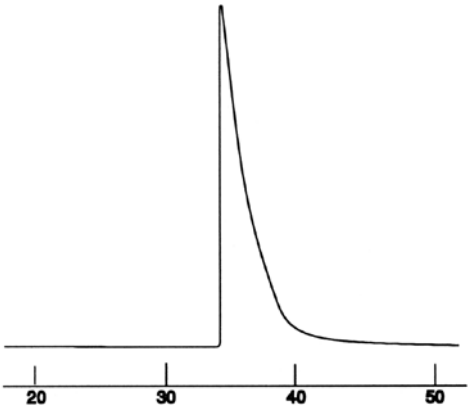
1,2-dichloroethane/ethanol	k_1	α
100 : 0	0.8	1.00
99.5 : 0.5	5.5	1.00
97.5 : 2.5	4.6	1.07
95 : 5	1.6	1.08

Effect of Mobile Phase on Propranolol

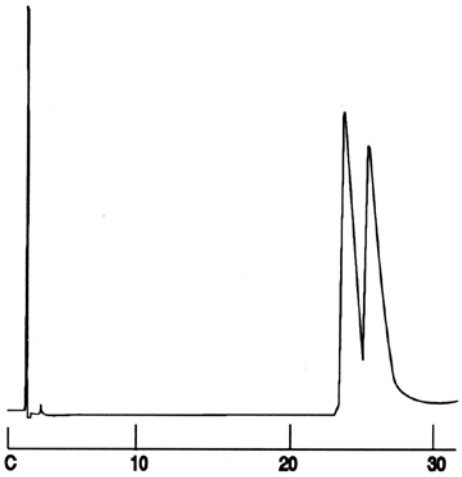
PROPRANOLOL
(Antiarrhythmic,
Antianginal)



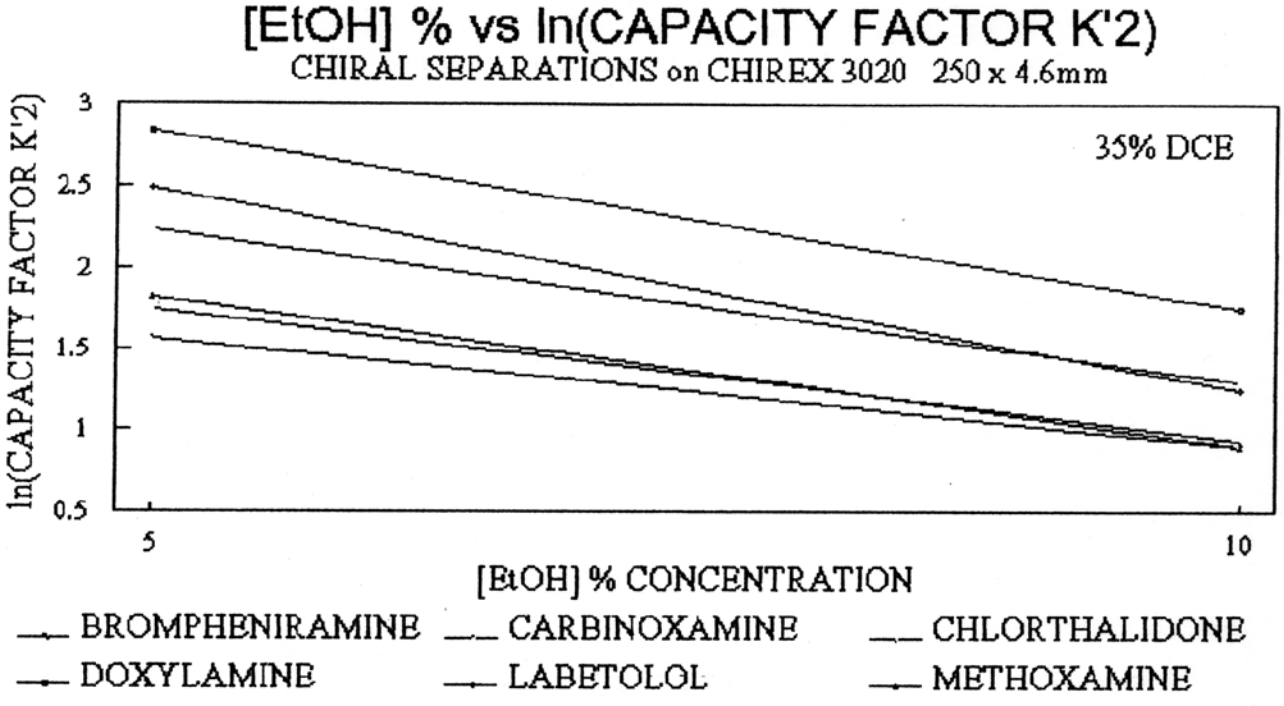
Hexane



Hexane/ethanol
(95:5)

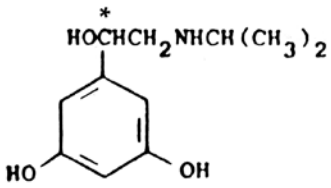


Column: Chirex Phase 3014
(250 x 4.6mm)
Mobile Phase: containing 0.25% trifluoroacetic acid
Flow Rate: 1.0 mL/min
Detector: UV @ 254nm


[EtOH] % vs ln(CAPACITY FACTOR K'2)
CHIRAL SEPARATIONS on CHIREX 3020 250 x 4.6mm
35% DCE
ln(CAPACITY FACTOR K'2)
3
2.5
2
1.5
1
0.5
5
10
[EtOH] % CONCENTRATION
BROMPHENIRAMINE
CARBINOXAMINE
CHLORTHALIDONE
DOXYLAMINE
LABETOLOL
METHOXAMINE

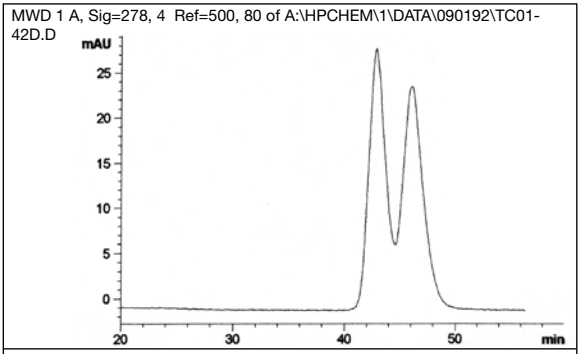
Effect of Dichloroethane

METAPROTERENOL
(Bronchodilator)

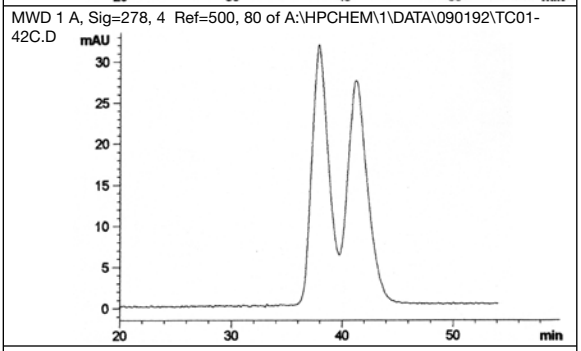


Column: Chirex Phase 3014
(250 x 4.6mm)
Mobile Phase: Hex/DCE/EtOH-TFA
Flow Rate: 0.7 mL/min
Detector: UV @ 278nm

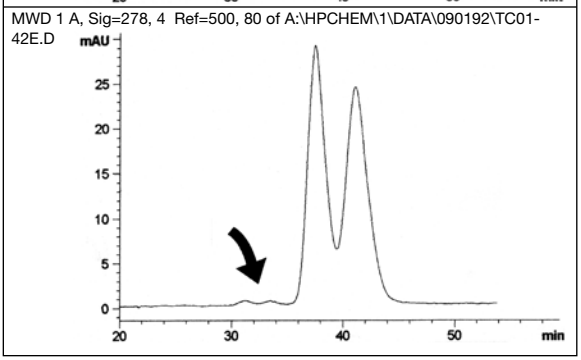
73:20:7



58:35:7

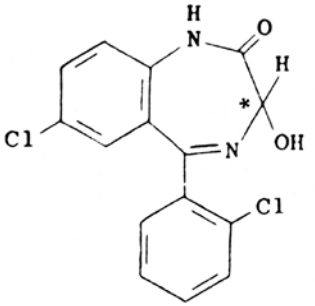


43:50:7

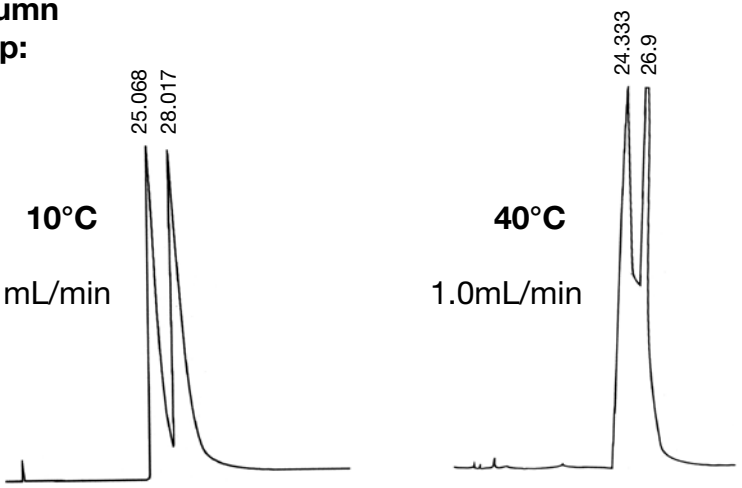


Effect of Temperature on the Separation of Lorazepam

LORAZEPAM
(Anxiolytic)



Column temp:



Column: Chirex Phase 3010
Mobile Phase: hexane/dichloroethane/ethanol (73:20:7,V/V/V)
Flow Rate: (as indicated)
Detector: UV @ 254nm

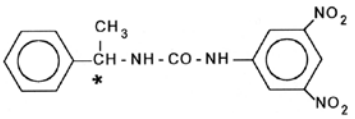
Source: Journal of Liquid Chromatography, 16 (4), 879-891 (1993)

Reversal of Elution Order

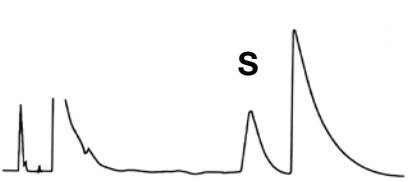
UREIDES of α-METHYLBENZYLAMINE

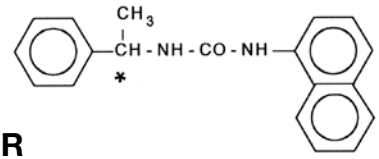
CHIREX PHASE 3005

Heptane-Isopropanol (90:10)

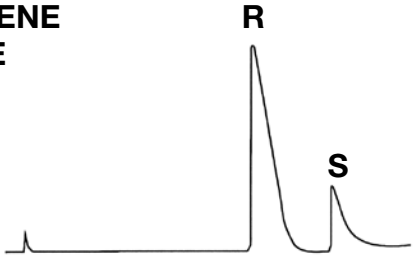


DINITROBENZENE DERIVATIVE



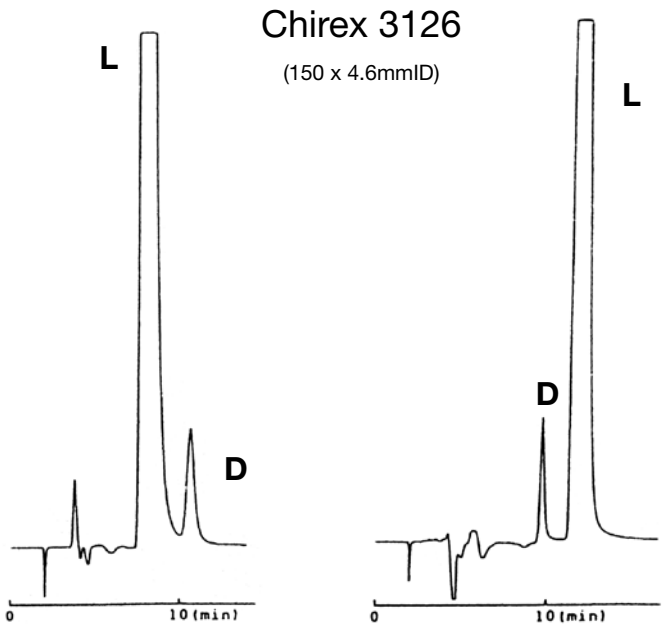


NAPHTHYLENE DERIVATIVE



Reversal in Elution Order
Enantiomeric Stationary Phases

LACTIC ACID

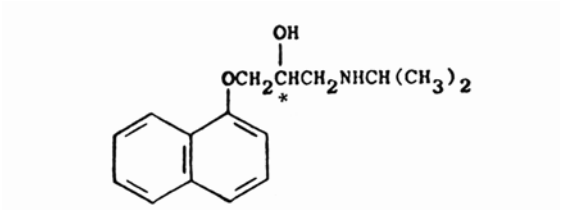


D-pennicillamine LEC **L-penicillinamine LEC**

Column temp: Room temperature
Mobile Phase: 2mM copper (II) sulfate in water/acetonitrile (95:5)
Flow Rate: 1.0 mL/min
Detector: UV @ 254nm

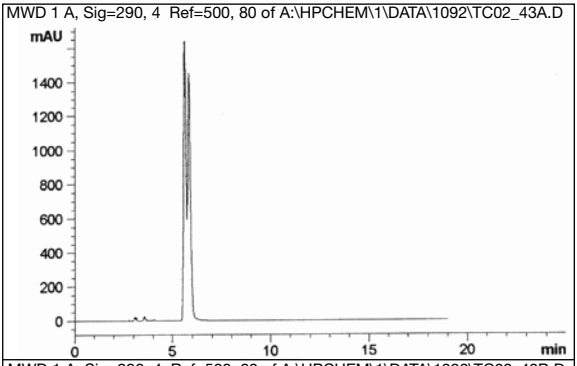
Method Development

PROPRANOLOL
(Antiarrhythmic,
Antihypertensive)

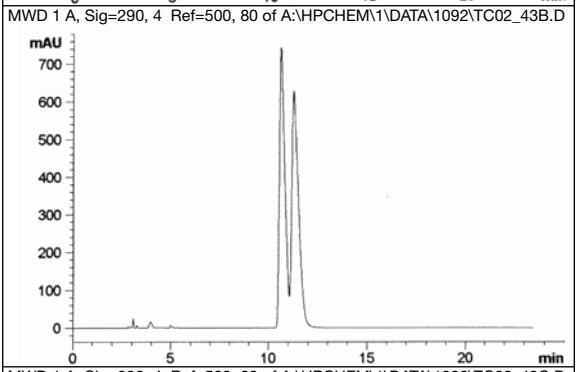


Column: Chirex 3020
(250 x 4.6mm)
Mobile Phase: Hex/DCE/
EtOH-TFA (as indicated)
Flow Rate: 1.0 mL/min
Detector: UV @ 254nm

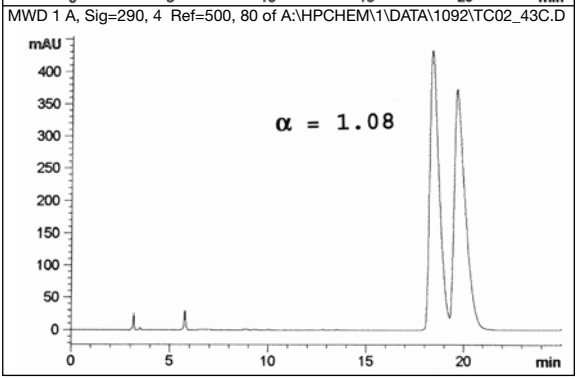
55:35:10



60:35:5

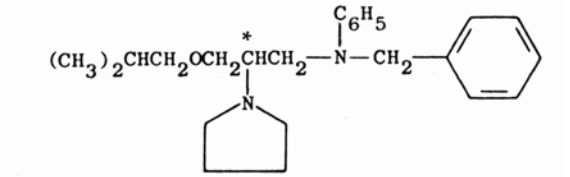


76:20:4



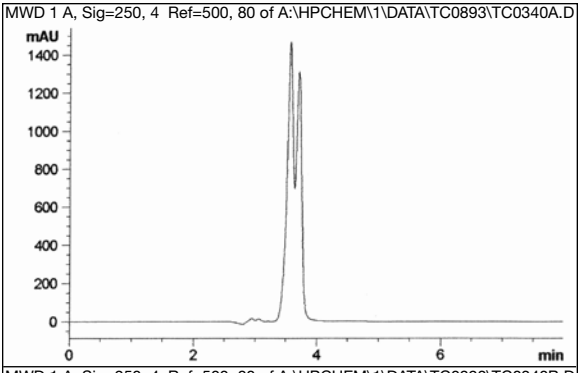
Method Development

BEPRIDIL
(Antianginal)

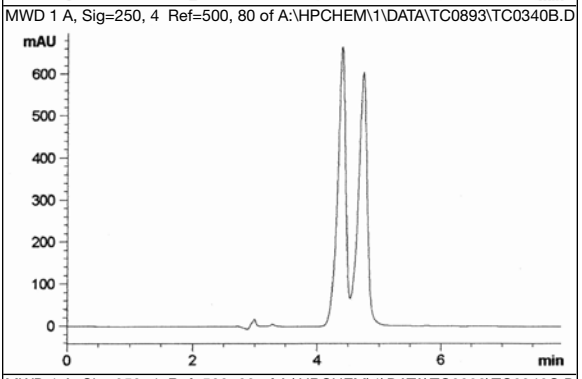


Column: Chirex 3014
Mobile Phase: Hex/DCE/ EtOH-TFA (as indicated)
Flow Rate: 1.0 mL/min
Detector: UV @ 250nm

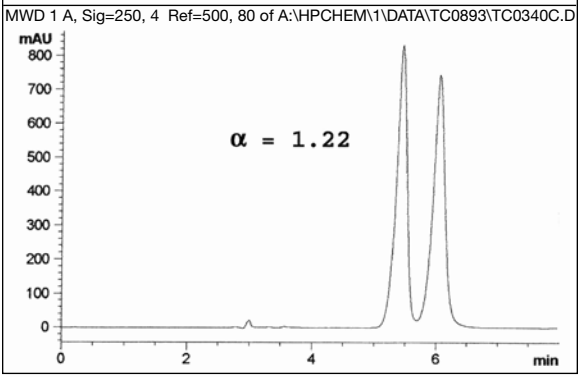
55:35:10



60:35:5

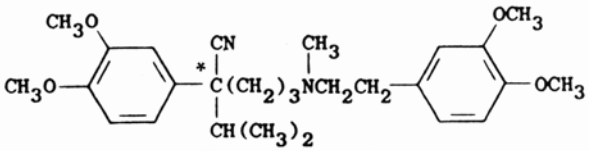


62:53:3



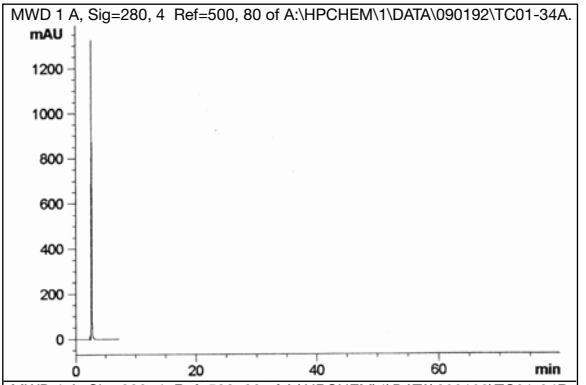
Method Development

VERAPAMIL
(Antiaarrhythmic)

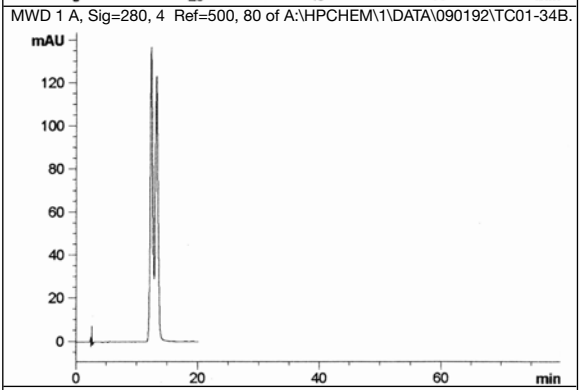


Column: Chirex 3014 (250 x 4.6mm)
Mobile Phase: Hex/DCE/EtOH-TFA (as indicated)
Flow Rate: 1.0 mL/min
Detector: UV @ 280nm

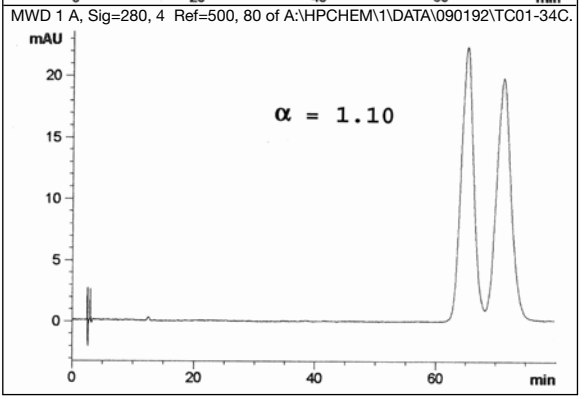
40:35:25



75:20:5



87:10:3

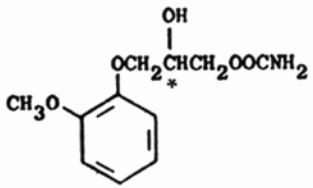


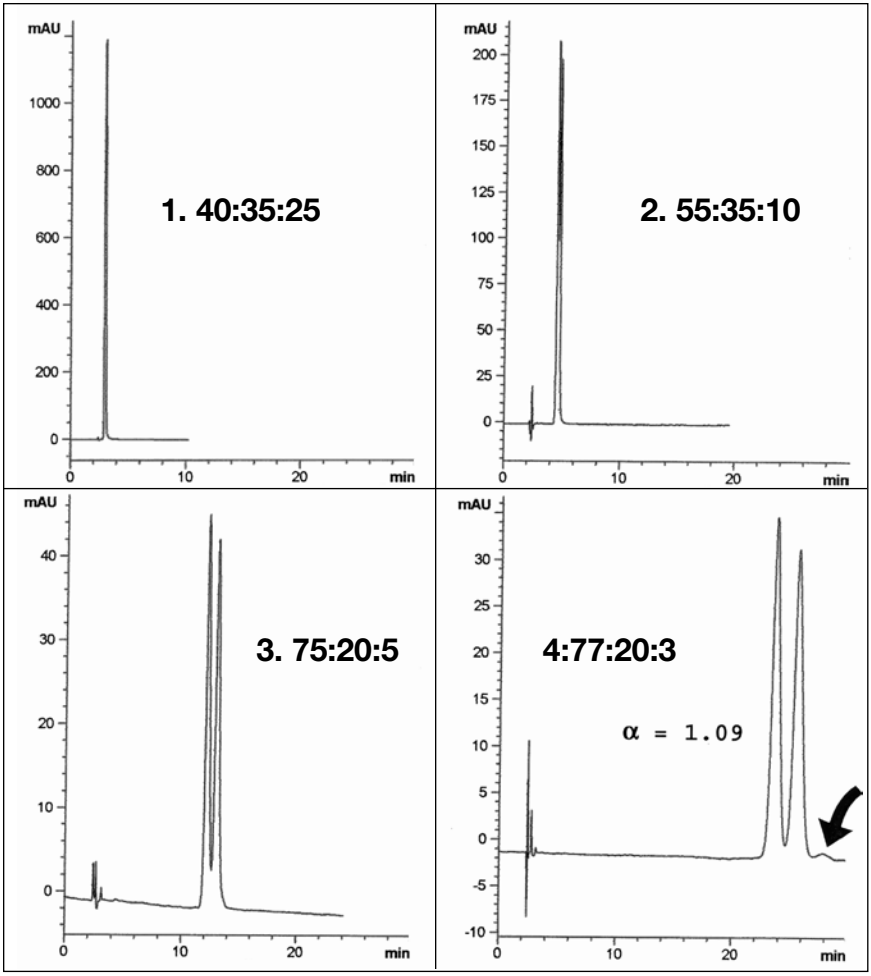
Method Development

METHOCARBAMOL

(Skeletal Muscle Relaxant)

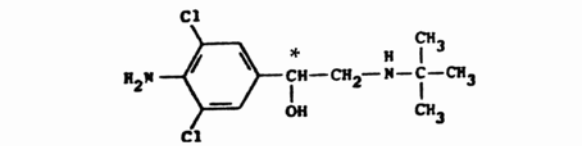
Column: Chirex 3014
(250 x 4.6mm)
Mobile Phase: Hex/DCE/EtOH-TFA
(as indicated)
Flow Rate: 0.7 mL/min
Detector: UV @ 260nm





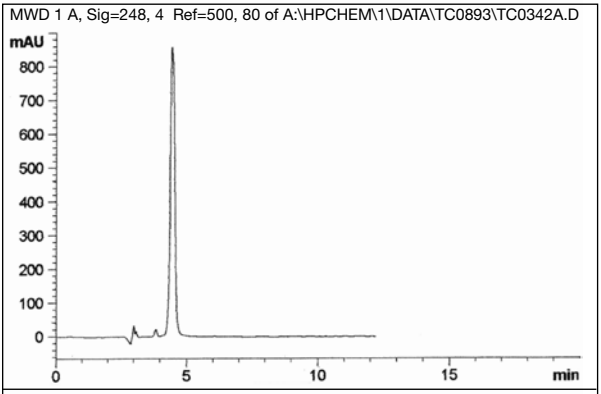
Method Development

CLENBUTEROL
(Bronchodilator)

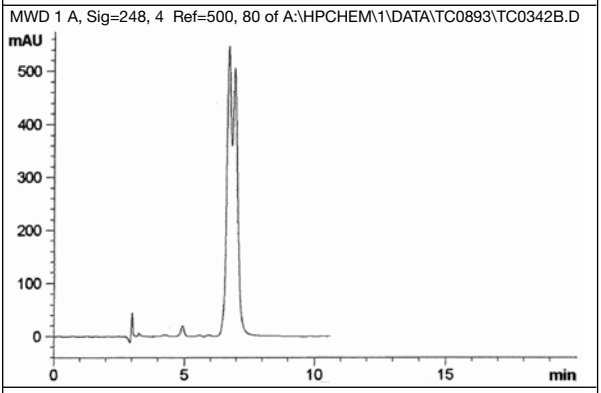


Column: Chirex 3020
Mobile Phase: Hex/DCE/EtOH-TFA (as indicated)
Flow Rate: 1.0 mL/min
Detector: UV @ 248nm

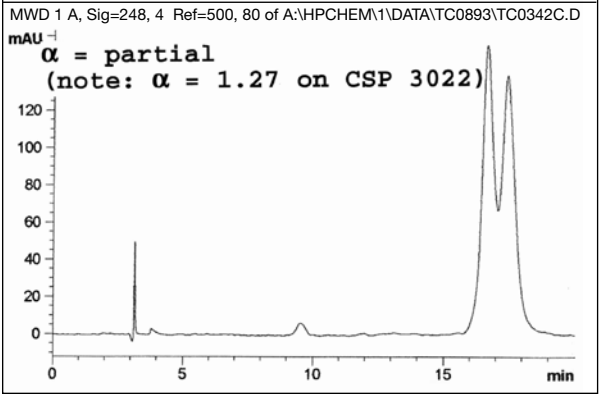
55:35:10



60:35:5

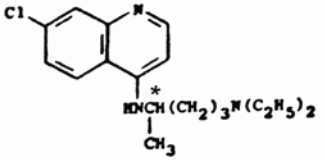


77:20:3



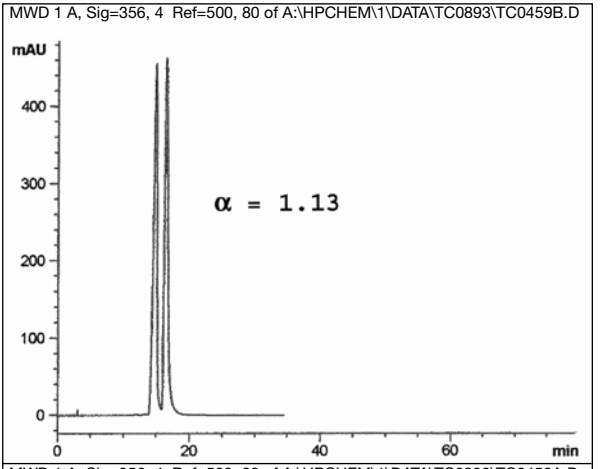
Method Development

CHLOROQUINE
(Antimalarial)

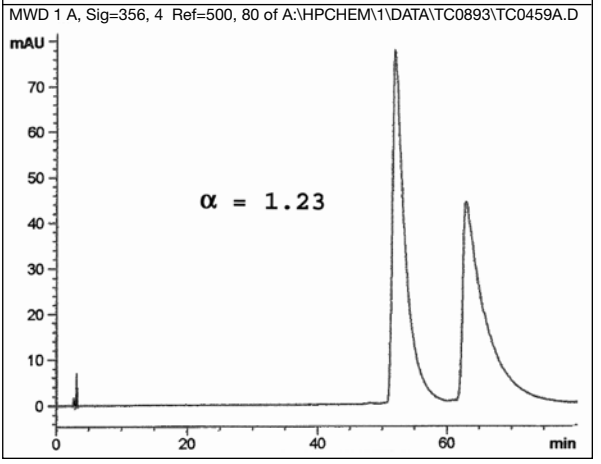


Column: Chirex 3022
Mobile Phase: Hex/DCE/ EtOH-TFA (as indicated)
Flow Rate: 1.0 mL/min
Detector: UV @ 346nm

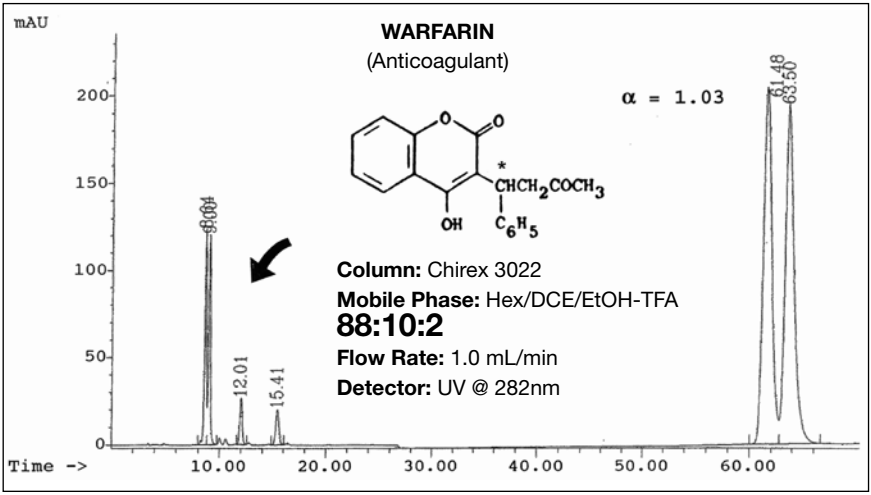
40:35:25



50:35:15



Method Development



Area Percent Report

MWD A, Sig=282, 4 Ref=500,80

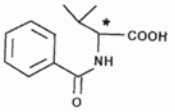
PK#	Ret Time	Area	Height	Type	Width	Area%
1	8.641	1480.076	123.785	BV	0.199	5.5719
2	9.003	1507.973	120.679	VV	0.198	5.6769
3	12.008	415.730	26.497	BV	0.249	1.5651
4	15.409	414.812	20.118	BB	0.323	1.5616
5	61.476	11175.077	204.599	BV	0.829	42.0696
6	63.504	11569.626	194.712	VB	0.893	43.5549

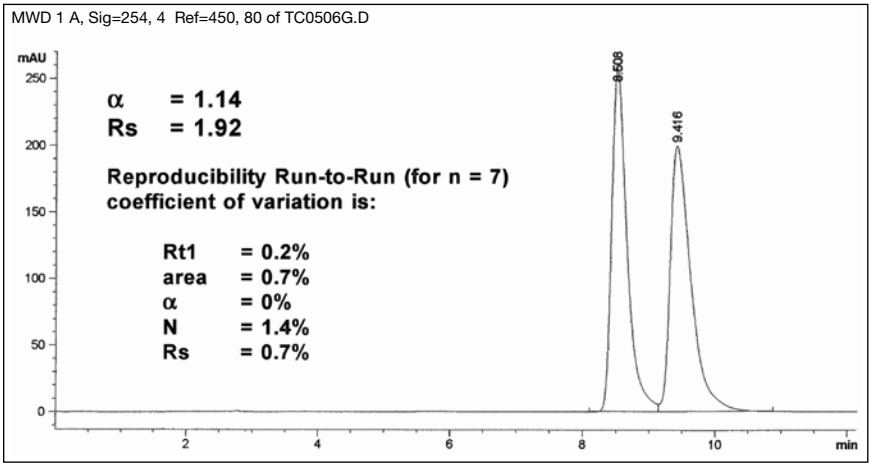
Total area = 26563

Reproducibility

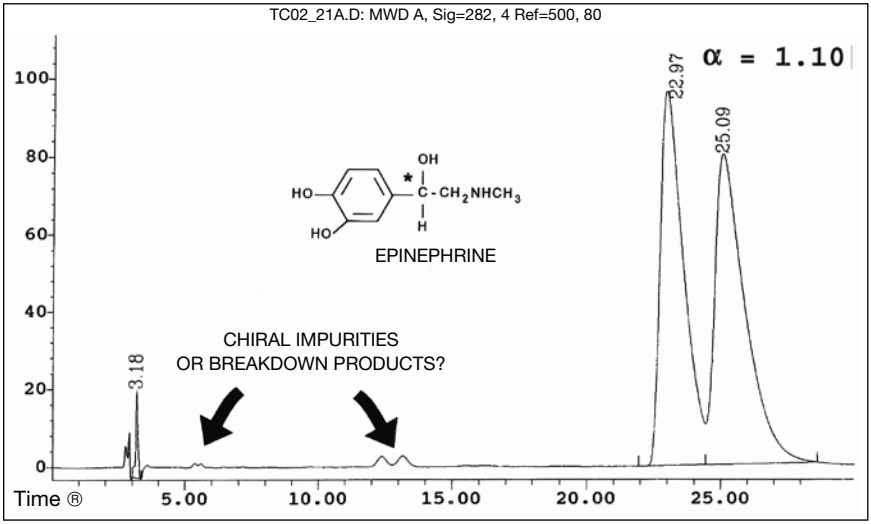
OPTIMIZED SEPARATION OF N-BENZOYLVALINE

Column: Chirex 3012 (250 x 4.0mm)
Mobile Phase: 10mM ammonium acetate in methanol
Flow Rate: 1.0 mL/min
Detector: UV @ 254nm
Injection: 10µL of 3.4mg/2.0mL (17µg on-column)





Retention Time (min)



Area Percent Report

Sample Name : EPINEPHRINE
Injection Number :
Run Time Bar Code :
Sequence Line :
Instrument Method : CRX_06.M
Analysis Method : CRX_06.M
EPINEPHRINE 4.6MG/4ML IN HEX/EtOH 75:25 FLOW: 0.5mL/min 595psi M P:HEX/DCE/EtOH-TFA 55:35:10 EtOh-TFA 20:1 CRX 3022 MID-BORE 250 x 3 .2 s/n 40644.
MDW A, Sig=282, 4 Ref=500,80

PK#	Ret Time	Area	Height	Type	Width	Area%
1	8.641	1480.076	123.785	BV	0.199	5.5719
2	9.003	1507.973	120.679	VV	0.198	5.6769
3	12.008	415.730	26.497	BV	0.249	1.5651
4	15.409	414.812	20.118	BB	0.323	1.5616
5	61.476	11175.077	204.599	BV	0.829	42.0696
6	63.504	11569.626	194.712	VB	0.893	43.5549

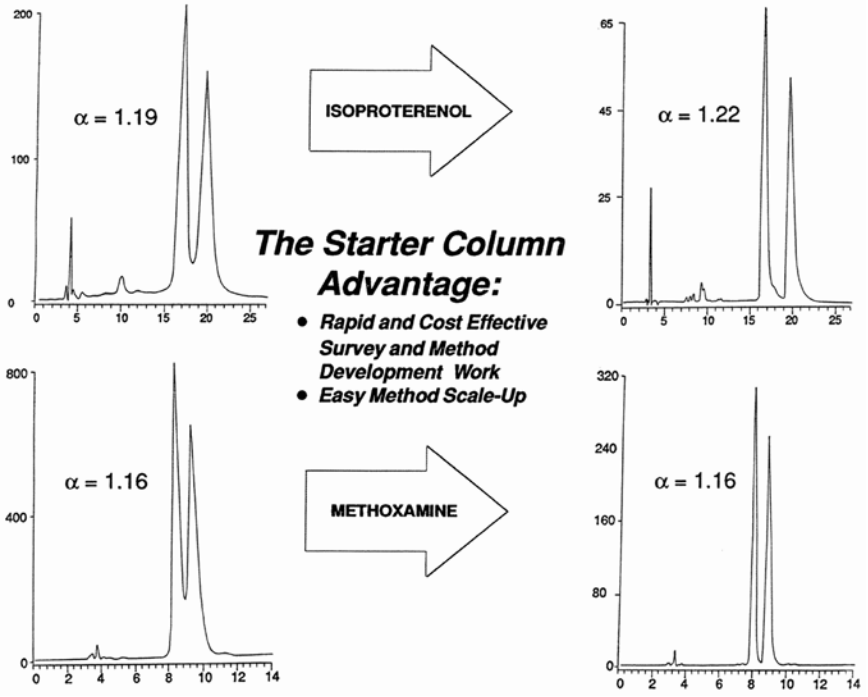
Total area = 26563

Starter Columns for Method Development

STARTER COLUMN
Column Length: 50mm
Flow Rate: 0.2 mL/min

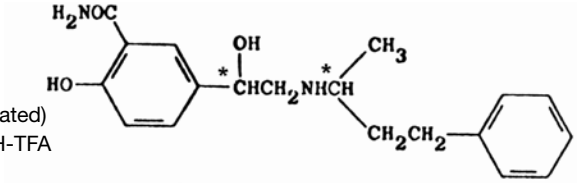
Separation conditions were the same for both columns.

STANDARD ANALYTICAL
Column Length: 250mm
Flow Rate: 1.0 mL/min



Column Hardware Comparison

LABETOLOL
(Antihypertensive)

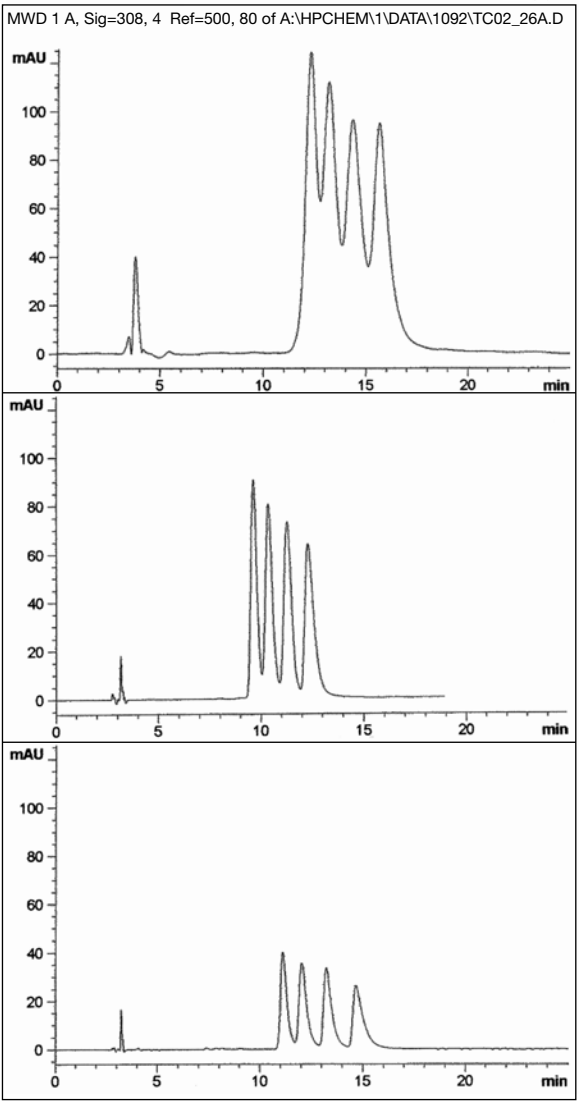


Column: Chirex 3022
(dimensions as indicated)
Mobile Phase: Hex/DCE/EtOH-TFA
(55:35:10)
Flow Rate: as indicated
Detector: UV @ 308nm

50 x 4.6mm
"Starter"
0.2mL/min

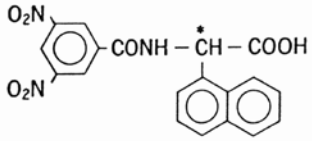
250 x 3.2mm
Midbore ID
0.5mL/min

250 x 4.6mm
Standard Analytical
1.0 mL/min

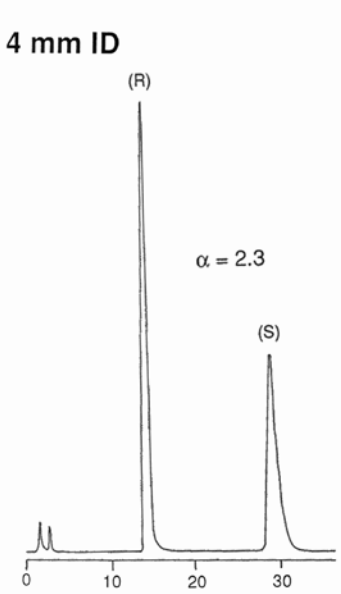


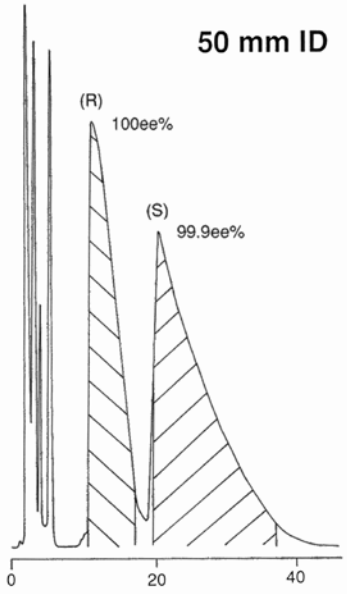
Preparative Separations

Dinitrobenzoyl Naphthylglycine



Chirex 3014

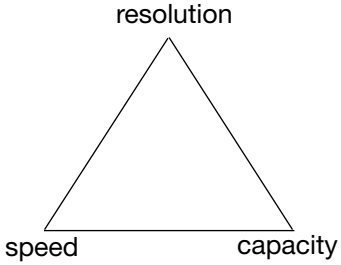




Mobile Phase: 2% acetic acid in methanol/ 1.2-dichloroethane (70:30)
Flow Rate: 1.0mL/min
Detector: UV @ 254nm
Sample amount: 5µg

Mobile Phase: 2% acetic acid in methanol/ 1.2-dichloroethane (70:30)
Flow Rate: 150mL/min
Detector: UV @ 300nm
Sample amount: 2.3µg

Preparative Separations



Vel2 = Vel1 $(R2/R1)^2$

Load2 = Load 1$(L2/L1)(R2/R1)^2$

Examples of load capacity of columns used for various preparative purposes

Column type	Inner diameter (mm)	Use	Amount of stationary phase (g)	Approximate maximum load (mg)
Analytical	1-5	Isolation of pure samples for MS or IR (0.001-0.1mg)	0.2-3	0.2-3
6-11				
Analytical to semi-preparative	6-11 (0.1-25mg)	Isolation of pure samples for NMR or elemental analysis	3-25	3-25
Semipreparative to preparative	10-30	Small scale synthesis (0.1-1g)	25-100	20-1000
Preparative	20-100	Large scale synthesis (1-100g)	100-10^4	100-10^4
Industrial	100-1000	Industrial scale synthesis	10^3-10^5	10^3-10^5

Operating conditions for preparative scale chromatography according to the analytical value of selectivity α

Operating conditions	Selectivity		
	1.05< α<1.25	**1.25< α<2**	**α>2**
Behavior	linear (analytical)	intermediate (mass overload)	non-linear
dp (µm)	5-7 µm	7, 10, and 15-40 µm	15-40µm and >40 µm
Loading rate g/g of CSP	10^{-4}-10^{-3} g	>10^{-3} g	>10^{-2} g
Comments	possibility of recycling	semipreparative scale	“flash chromatography”

Separation Utility of 7 Chirex CSPs

	3001	3005	3014	3017	3018	3020	3022	ALL
S	3	4	24	3	7	29	29	46
P	0	1	11	3	5	6	13	10
T	31	24	66	26	28	64	65	70
%S	10	17	36	12	25	45	45	60
%S + P	10	21	53	23	43	55	65	80

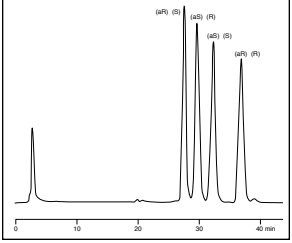
S = Separated enantiomeric pair (baseline or valley height between the pair is less than 25% of the height of peak one).

P =Partial separation of the enantiomeric pair (valley height is greater than 25% of the height of peak one).

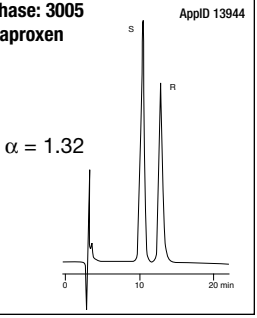
T =Total number of compounds tested.

Selected Chirex Applications

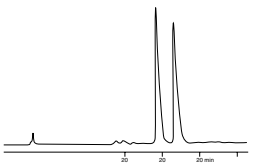
Phase: 3001
Fenvalerate
AppID 13995



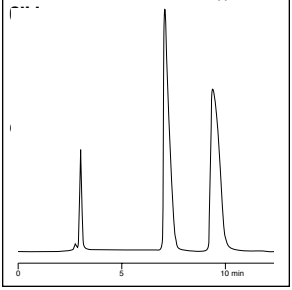
Phase: 3005
Naproxen
AppID 13944



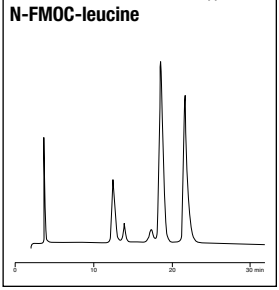
Phase: 3005
Pranoprofen
AppID 5244



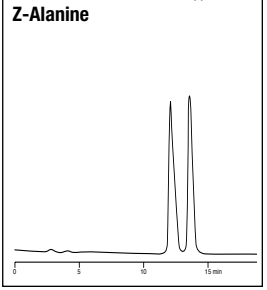
Phase: 3010
AppID 13930



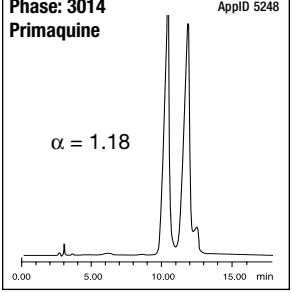
Phase: 3011
N-FMOC-leucine
AppID 13800



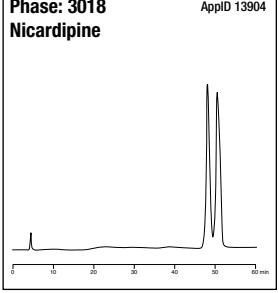
Phase: 3011
Z-Alanine
AppID 13729



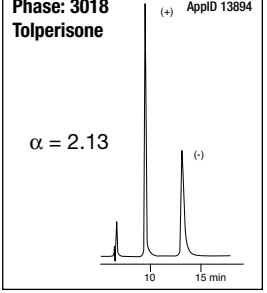
Phase: 3014
Primaquine
AppID 5248



Phase: 3018
Nicardipine
AppID 13904

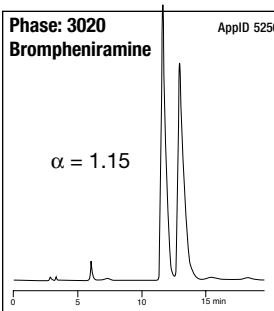


Phase: 3018
Tolperisone
AppID 13894

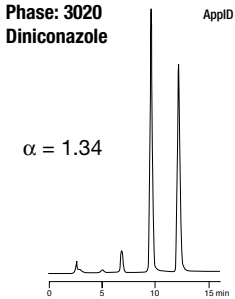


Selected Chirex Applications

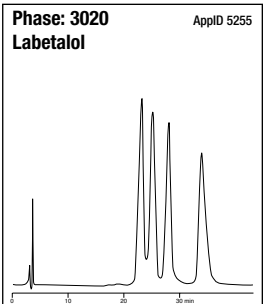
Phase: 3020
Brompheniramine
AppID 5256



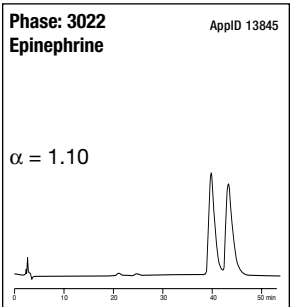
Phase: 3020
Diniconazole
AppID 5261



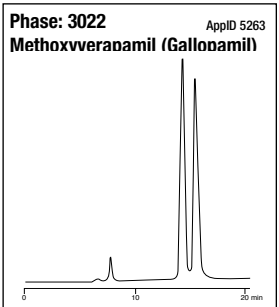
Phase: 3020
Labetalol
AppID 5255



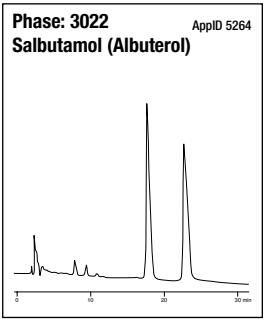
Phase: 3022
Epinephrine
AppID 13845



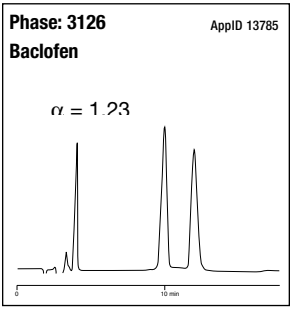
Phase: 3022
Methoxyverapamil (Gallopamil)
AppID 5263



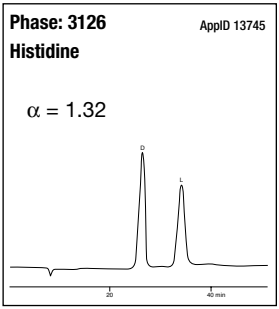
Phase: 3022
Salbutamol (Albuterol)
AppID 5264



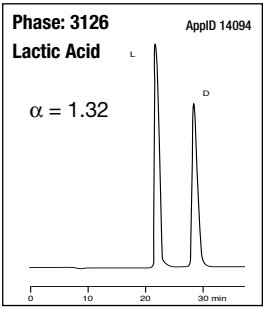
Phase: 3126
Baclofen
AppID 13785



Phase: 3126
Histidine
AppID 13745

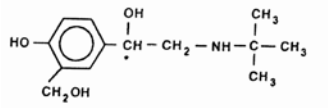


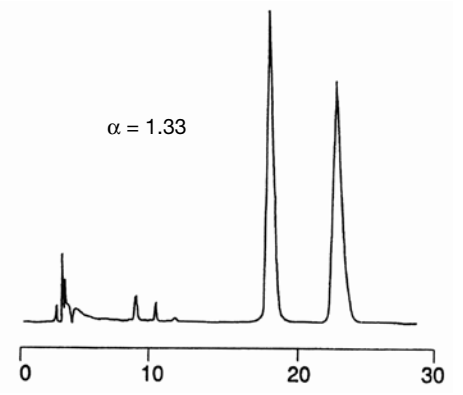
Phase: 3126
Lactic Acid
AppID 14094



Pharmaceuticals

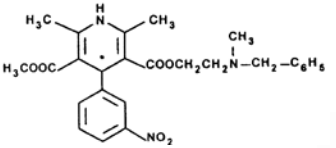
SALBUTAMOL
(Bronchodilator)

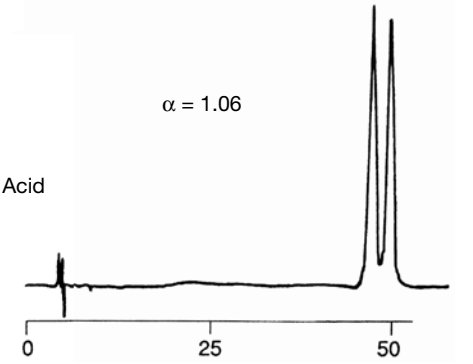




Column: Chirex 3022
Size: 250 x 4.6mm ID
Mobile Phase: Hexane/
1,2-Dichloroethane/
Methanol/Trifluoroacetic Acid
(240:140:20:1)
Flow Rate: 1.0 mL/min
Detector: UV @ 248nm

NICARDIPINE
(Antianginal)



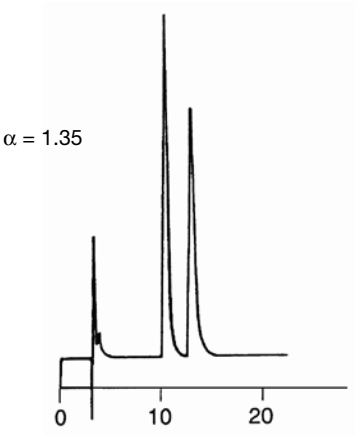


Column: Chirex 3018
Size: 250 x 4.0mm ID
Mobile Phase: Hexane/
1,2-Dichloroethane/
Methanol/Trifluoroacetic Acid
(240:140:10:1)
Flow Rate: 1.0 mL/min
Detector: UV @ 254nm

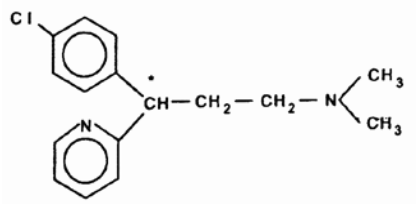
Pharmaceuticals

NAPROXEN
(Antiinflammatory)

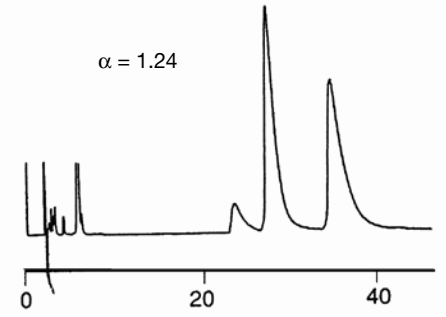
Column: Chirex 3005
Size: 250 x 4.0mm ID
Mobile Phase: 0.03 Ammonium Acetate in Methanol
Flow Rate: 1.0 mL/min
Detector: UV @ 254nm



CHLORPHENIRAMINE
(Antihistaminic)

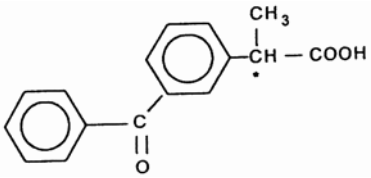


Column: Chirex 3014
Size: 250 x 4.0mm ID
Mobile Phase: Hexane/Ethanol/ Trifluoroacetic Acid (200:40:0:6)
Flow Rate: 1.0 mL/min
Detector: UV @ 254nm

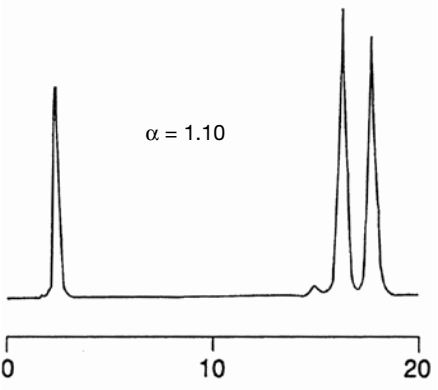


NSAIDs

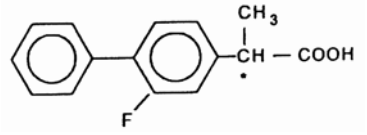
KETOPROFEN



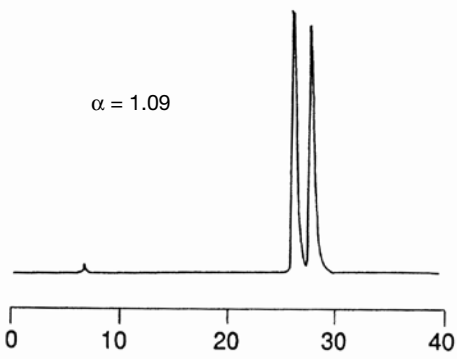
Column: Chirex 3005
Size: 250 x 4.6mm ID
Mobile Phase: 0.02 Ammonium Acetate in Methanol
Flow Rate: 0.8 mL/min
Detector: UV @ 254nm



FLURBIPROFEN

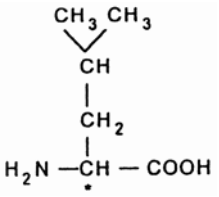


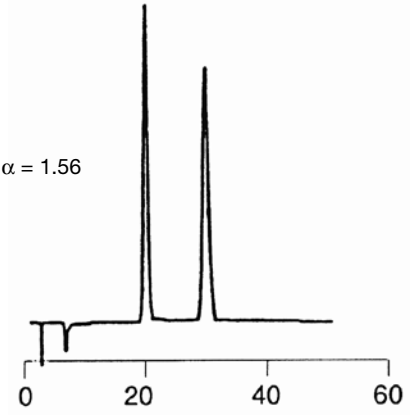
Column: Chirex 3005
Size: 250 x 4.6mm ID
Mobile Phase: 0.02 Ammonium Acetate in Methanol
Flow Rate: 0.5 mL/min
Detector: UV @ 254nm



Amino Acids

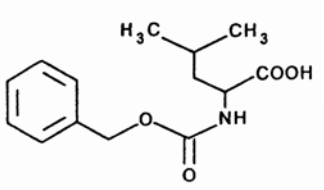
LEUCINE

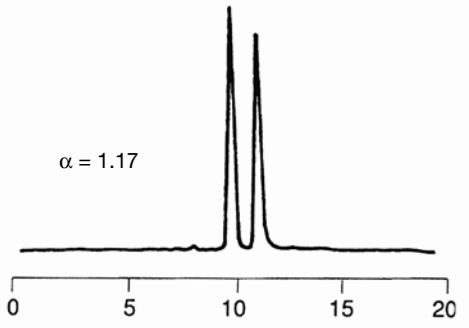




Column: Chirex 3126
Size: 150 x 4.6mm ID
Mobile Phase: 2mM Copper (II) Sulfate in Water/Methanol (85:15)
Flow Rate: 1.0 mL/min
Detector: UV @ 254nm

Z-LEUCINE





Column: Chirex 3011
Size: 250 x 4.0mm ID
Mobile Phase: 0.01 Ammonium Acetate in Methanol
Flow Rate: 1.0 mL/min
Detector: UV @ 254nm

Conclusions

CHIREX column line has WIDE UTILITY for the separation of enantiomers

WIDE VARIETY of CSPs to choose from

ENANTIOMER SEPARATION is often possible on TWO or MORE COLUMNS

50mm “STARTER” COLUMNS permit RAPID and ECONOMICAL METHOD DEVELOPMENT

MIDBORE column dimensions SAVE SOLVENT and INCREASE SENSITIVITY

Chirex Chiral HPLC Applications

Chirex Phase	Separaton Factor(α)	Compound	APP ID No.
3020	1.05	Abscisic acid	14115
3022	1.09	Acebutolol	13584
3020	1.06	o-Acetylmandelic acid	13946
3126	1.66	Alanine	14004
3126	2.26	Alanylglycine	14080
3126	1.62	Alanylglycyl-glycine	14082
3019	ó	Allethrin	5251
3020	1.12	Allethrolone	14129
3126	1.67	Alloisoleucine	14038
3126	1.19	Allothreonine	14046
3126	1.30	1-Aminoethylphosphonic acid	14089
3126	1.80	2-Amino-n-butyric acid	14034
3126	1.31	2-Amino-4-phosphonobutyric acid	14087
3126	1.30	3-Amino-e-caprolactam	14070
3011	1.28	o-Anthracene-1-carbonyl lactic acid	13960
3011	1.57	o-Anthracene-1-carbonyl mandelic acid	13963
3011	1.55	o-Anthracene-9-carbonyl lactic acid	13965
3126	2.15	Arginine	14027
3126	1.10	Asparagine	14049
3126	1.42	Aspartic acid	14019
3022	1.10	Atenolol	13824
3018	1.06	Atropine	13839
3126	1.23	Baclofen	13785
3001	1.11	Bendroflumethiazide	5243
3011	1.10	1-Benzocyclobutenecarboxylic acid	13967
3022	1.07	Benzoin	13978
3014	1.22	Bepridil	13593
3010	1.56	1,1'-Bi-2-naphthol	14143
3010	2.66	1,1'-Binaphthyl-2,2'- diylhydrogen phosphate	14152
3012	1.09	t-BOC-leucine	14065
3012	1.09	t-BOC-phenylalanine	13784
3012	1.10	t-BOC-valine	14063
3126	1.36	p-Boronophenylalanine	13790
3005	1.11	α-Bromophenylacetic acid	13969
3126	1.05	2-Bromopropionic acid	14116
3020	1.15	Brompheniramine	5256
3018	1.19	Carbinoxamine	13905
3010	1.06	2-(4-Chlorophenyl)-3-methylbutyric acid	13934
3020	1.21	Chloroquine	13604
3014	1.24	Chlorpheniramine	13889
3020	1.08	Chlorthalidone	13608
3010	ó	Chrysanthemic acid	5246
3022	1.27	Clenbuterol	13615
3010	1.48	Clidanac	13930
3017	1.10	α-Cyano-3-phenoxybenzyl alcohol	13979
3014	1.09	Cyclopentolate	13840
3019	ó	Cypermethrin	5252
3020	ó	Cypermethrin	5252

Chirex Phase	Separaton Factor(α)	Compound	APP ID No.
3126	2.47	Cystine	14085
3126	2.77	2,6-Diaminopimelic acid	14066
3022	1.23	Dichloroisoproterenol	13621
3017	1.14	3-(3,4-Dichlorophenyl)-2-dimethylamino -2-methylpropane-1-ol	
3010	ó	3-(2,2-Dichlorovinyl)-2,2-dimethyl-cyclopropanecarboxylic acid	14095
3005	1.09	cis-3-(2,2-Dichlorovinyl)-2,2-dimethyl-cyclopropanecarboxylic acid	
3005	1.10	trans-3-(2,2-Dichlorovinyl)-2,2-dimethyl-cyclopropanecarboxylic acid	
3019	1.06	2,3-Dihydroflavone	
3011	1.07	3,4-Dihydro-2H-pyran-2-carboxylic acid	14114
3126	1.09	3,4-Dihydro-2H-pyran-2-carboxylic acid	14113
3020	1.34	Diniconazole	5261
3010	1.79	o-(3,5-Dinitrophenyl) aminocarbonyl lactic acid	13791
3011	1.77	o-(3,5-Dinitrophenyl) aminocarbonyl lactic acid	13792
3010	1.36	o-(3,5-Dinitrophenyl) aminocarbonyl mandelic acid	13794
3011	1.35	o-(3,5-Dinitrophenyl) aminocarbonyl mandelic acid	13795
3014	1.20	Diperodon	5249
3020	1.06	1,2-Diphenylethylamine	13882
3126	1.09	1,2-Diphenylethylene diamine	13916
3126	1.15	dl-Threo-3-phenylserine	13787
3126	1.22	DOPA [3-(3,4-Dihydroxyphenyl) alanine]	13750
3020	1.07	Doxylamine	13627
3022	1.10	Epinephrine	5260
3020	1.25	o-Ethyl o-2,4-dichlorophenyl-isopropylphosphoramidothioate	5258
3007	1.06	Ethyl 3-Hydroxybutyrate	14139
3007	1.14	Ethyl lactate	14140
3022	1.11	Etilefrine hydrochloride	13867
3005	1.10	Fenoprofen	13923
3022	1.12	Fenoterol	13848
3019	1.15	Fenpropathrin	13988
3001	ó	Fenvalerate	13995
3019	1.06	Flavanone (2,3-Dihydroflavone)	14002
3014	1.09	Floctafenine	13874
3005	1.09	Flurbiprofen	13925
3001	ó	Furamethrin	13998
3126	1.11	Glutamic acid	14047
3126	1.71	Glutamine	14022
3126	1.78	Glycylalanine	14079
3126	1.69	Glycylvaline	14081
3020	1.08	Hexobarbital	14051
3126	1.32	Histidine	13745
3022	1.08	Homochlorcyclizine	13909
3126	1.14	Homocysteine thiolactone	14072
3126	1.61	2-Hydroxybutric acid	14098
3126	1.53	2-Hydroxyhexanoic acid	14108
3126	1.43	p-Hydroxynorephedrine	13862

Chirex Chiral HPLC Applications

Chirex Phase	Separaton Factor(α)	Compound	APP ID No.
3020	1.11	Hydroxy-phenyl-5-phenyl-hydantoin	13635
3005	1.05	Ibuprofen	13928
3022	1.08	Indapamide	13638
3005	1.08	Indoprofen	13639
3020	1.21	Isoetharine	13640
3022	1.17	Isoproterenol	5259
3020	1.37	Isoxsuprine	13646
3022	1.12	Ketamine	5262
3005	1.15	Ketoprofen	5245
3020	ó	Labetalol	5255
3126	1.32	Lactic acid	14094
3126	1.56	Leucine	14009
3126	1.36	Leucylglycyl-glycine	14083
3010	1.11	Lorazepam	
3126	1.83	Lysine	14018
3126	3.60	Malic acid	14099
3126	1.24	Mandelic acid	14942
3022	1.12	Metanephrine	13651
3020	1.24	Metaproterenol	5257
3022	1.13	Metaproterenol	13873
3005	1.11	α-Methamphetamine-3,3-dinitrobenzoyl amide	
3005	1.19	α-Methamphetamine-2-naphthyl amide	
3005	1.03	α-Methamphetamine-2-naphthyl amide	
3005	1.16	α-Methamphetamine-1-naphthyl uride	
126	1.42	Methionine	14024
3020	1.09	Methocarbamol	13655
3022	1.10	Methoxamine	13657
3005	1.15	α-Methoxyphenylacetic acid	13947
3017	1.10	Methoxyphenamine	13903
3022	1.15	Methoxyverapamil (Gallopamil)	5263
3005	1.27	α-Methylbenzylamine-2-naphthyl amide	
3005	1.24	α-Methylbenzylamine-3,5-dinitrophenyl uride	
3005	1.30	α-Methylbenzylamine-1-naphthyl uride	
3007	1.10	Methyl lactate	14138
3126	1.59	α-Methyl leucine	
3001	1.06	Methylmandelate	13984
3020	1.10	2-Methyl-4-oxo-3-(2-propynyl) - cyclopent-2-enyl alcohol	14134
3126	1.18	α-Methyltryptophan	
3014	1.06	Metoprolol	13663
3001	1.09	Midodrine	13664
3126	1.17	N-Acetylalanine	14052
3126	1.39	N-Acetylleucine	14058
3126	1.27	N-Acetylmethionine	13728
3020	1.37	N-Acetylphenylalanine	13804
3126	1.50	N-Acetylvaline	14055
3007	1.09	1-(1-Naphthyl)ethyl alcohol	13972
3020	1.16	1-(1-Naphthyl)ethylamine	13878
3126	1.42	Naphthylglycine	13789
3012	1.09	Naproxen	13945
3012	1.14	N-Benzoylglutamic acid	13782
3012	1.11	N-Benzoylleucine	
3012	1.17	N-Benzoylphenylalanine	13730
3012	1.13	N-Benzoylphenylglycine	
3012	1.19	N-Benzoylvaline	13778
3011	1.24	N-Dansylnorvaline	13732
3011	1.27	N-Dansylphenylalanine	13771
3012	1.18	N-Dansylthreonine	13734
3010	1.16	N-Dansyltryptophan	13774
3011	1.28	N-Dansylvaline	13763
3126	1.25	N-Dansylmethionine	
3018	1.14	Nefopam	5254
3011	1.19	N-FMOC-Alanine	
3011	1.20	N-FMOC-Leucine	13800
3011	1.10	N-FMOC-Phenylalanine	13796
3011	1.12	N-FMOC-Valine	13798
3126	1.37	N-Formylvaline	13721
3126	1.25	N-Formylmethionine	13722
3020	1.12	N-Formylphenylalanine	13802
3018	1.05	Nicardipine	13904
3014	1.10	Nicotine	13673
3017	1.07	Norepinephrine	13868
3126	1.23	Normetanephrine	14160
3126	1.95	Norvaline	14029
3126	1.28	Octopamine	13857
3126	1.38	Ornithine	14041
3014	1.08	Oxprenolol	13826
3017	1.50	Oxyphencyclimine	13678
3126	1.63	Pantoic acid	14117
3014	1.11	Pantolactone	14118
3007	ó	Permethrin	
3012	1.13	2-Phenylbutyric acid	13949
3126	1.26	3-Phenyllactic acid	13943
3001	ó	Phenothrin	13992
3010	1.08	2-Phenoxypropionic acid	13939
3126	1.26	Phenylalanine	13740
3001	1.12	2-Phenylbutyrophenone	13999
3001	1.14	2-Phenylcyclohexanone	13986
Chirex Phase	Separaton Factor(α)	Compound	APP ID No.

NOTE: This is only a partial list of applications. Many other applications available.
Some compounds have been separated on more than one chiral stationary phase.

Chirex Phase	Separaton Factor(α)	Compound	APP ID No.
3005	1.08	trans-2-Phenyl-1-cyclopropanecarboxylic acid	
3014	1.08	1-Phenylethylamine	13875
3126	1.78	Phenylglycine	13748
3126	1.26	Phenyllactic acid	
3001	1.05	2-Phenylpropionic acid	13933
3014	1.06	1-Phenyl-2-(p-tolyl)ethylamine	13888
3022	1.12	Pindolol	13831
3126	1.80	Pipecolic acid	14031
3017	1.22	Piperoxan	5253
3019	ó	Prallethrin	14127
3005	1.13	Pranoprofen	5244
3020	1.17	Prilocaine	13680
3014	1.18	Primaquine	5248
3005	1.45	Proglumide	13685
3126	2.50	Proline	14011
3020	1.12	Promethazine	13688
3017	1.12	Propafenone	13832
3022	1.11	Propranolol	13813
3011	1.52	o-Pyrene-1-carbonyl lactic acid	13952
3011	1.97	o-Pyrene-1-carbonyl mandelic acid	13955
3011	1.72	o-Pyrene-1-carbonyl 2-hydroxybutyric acid	13958
3011	1.55	o-Pyrene-1-carbonyl leucic acid	13959
3014	1.12	PTH-valine	13733
3022	1.33	Quinacrine	13695
3001	ó	Resmethrin	13992
3022	1.33	Salbutamol (Albuterol)	5264
3126	1.17	Serine	14016
3007	1.09	Styrene oxide	13987
3022	1.10	Synephrine	13700
3126	1.19	Synephrine	13871
3126	6.44	Tartartic acid	14100
3019	1.18	Terallethrin	14123
3020	1.16	Terbutaline	13852
3126	1.81	tert-Leucine	14074
3126	1.06	Tetrahydro-2-furancarboxylic acid	14110
3017	1.28	Tetrahydrozoline	13706
3001	ó	Tetramethrin	13852
3022	1.10	Thioridazine	13711
3126	1.20	Threonine	14043
3018	2.13	Tolperisone	13894
3126	1.10	trans-1,2-Diaminocyclohexane	14067
3001	1.41	2,2,2-Trifluoro-1-(9-anthryl)ethanol	13973
3007	1.06	α-(Trifluoromethyl)benzyl alcohol	13985
3020	1.11	Trimepramine	
3020	1.17	Trimeprazine	
3022	1.10	Tropicamide	13715
3126	1.11	Tryptophan	13737
3126	1.34	Tyrosine	13743
3020	1.29	Uniconazole	5242
3126	1.91	Valine	14006
3022	1.15	Verapamil	13910
3022	1.03	Warfarin	13720
3012	1.16	Z-Alanine	13752
3010	1.12	Z-Asparagine	13759
3011	1.17	Z-Leucine	13731
3011	1.13	Z-Norvaline	13755
3012	1.08	Z-Phenylalanine	13761
3011	1.09	Z-Serine	13758
3012	1.07	Z-Valine	13754

NOTE: A dash ("—") under separation factor indicates the compound has more than one chiral center. Separation involves more than one enantiomeric pair

Chiral HPLC Method Development Kits

Phenomenex offers two very economical Chiral method development column kits. Each kit is composed of five short-length columns which maintain resolving power while offering the most flexible approach to column selection and general method development.

CHIRAL METHOD DEVELOPMENT KIT A.

FOR PHARMACEUTICALS, AGROCHEMICALS

The broad range of Pirkle-concept (Type I, or brush type) columns carefully chosen for this Kit will enable the chiral chromatographer to quickly and easily survey stationary phase utility and separation conditions for a wide variety of enantiomeric compounds. Used with normal phase solvent systems, these latest generation chiral columns are highly efficient tools particularly well-suited for enantiomeric separations of pharmaceuticals and agrochemicals.

This kit contains five columns, 50 x 4.6mm:

Column 1: Chirex 3001; 3,5-dinitrobenzoic acid derivative of (R)-phenylglycine
Column 2: Chirex 3005; 3,5-dinitrobenzoic acid derivative of (R)-1 naphthylphenylglycine
Column 3: Chirex 3010; 3,5-dinitroaniline derivative of (S)-valine
Column 4: Chirex 3014; (R)-1-(α-naphthyl) ethylamine derivative of (S)-valine
Column 5: Chirex 3020; (R)-1-(α-naphthyl) ethylamine derivative of (S)-tert-leucine

ORDERING INFORMATION

Order No.	Description
KH0-1892	Chiral Method Development (Kit A)

CHIRAL METHOD DEVELOPMENT KIT B.

FOR α-AMINO ACIDS, α-HYDROXY ACIDS, CARBOXYLIC ACIDS, AROMATIC AMINES, ALCOHOLS AND AMINO ALCOHOLS

This Kit contains a collection of chiral columns for method development of enantiomeric α-amino acids, α-hydroxy acids, and aromatic amines, alcohols and amino alcohols. The kit includes four Pirkle-concept columns and one ligand-exchange type column. The Pirkle-concept columns are chosen for their high utility/versatility in separating derivatized amino acids, whereas the ligand-exchange column confers excellent enantioselectivity for underivatized α-amino acids, and α-hydroxy acids.

This kit contains five columns, 50 x 4.6mm:

Column 1: Chirex 3010; 3,5-dinitroaniline derivative of (S)-valine
Column 2: Chirex 3011; 3,5-dinitroaniline derivative of (S)-tert-leucine
Column 3: Chirex 3012; 3,5-dinitroaniline derivative of (R) phenylglycine
Column 4: Chirex 3014; (R)-1-(α-naphthyl) ethylamine derivative of (S)-valine
Column 5: Chirex 3126; Ligand-Exchange type based on (N,S) dioctyl-(D)-penicillamine complexed with copper(II)

ORDERING INFORMATION

Order No.	Description
KH0-1893	Chiral Method Development (Kit B)

Chiral HPLC Columns

ORDERING INFORMATION

5μ Starter™ Columns (mm)

Phase	Chirex Phase Description	Bond Type	Linkage Type	50 x 4.6
3001	(R)-PGLY and DNB	Covalent	Amide	00B-3001-E0
3010	(S)-VAL and DNAn	Covalent	Urea	00B-3010-E0
3011	(S)-LEU and DNAn	Covalent	Urea	00B-3011-E0
3012	(R)-PGLY and DNAn	Covalent	Urea	00B-3012-E0
3014	(S)-VAL and (R)-NEA	Covalent	Urea	00B-3014-E0
3017	(S)-PRO and (S)-NEA	Covalent	Urea	00B-3017-E0
3018	(S)-PRO and (R)-NEA	Covalent	Urea	00B-3018-E0
3019	(S)-LEU and (S)-NEA	Covalent	Urea	00B-3019-E0
3020	(S)-LEU and (R)-NEA	Covalent	Urea	00B-3020-E0
3022	(S)-ICA and (R)-NEA	Covalent	Urea	00B-3022-E0
3005	(R)-NGLY and DNB	Covalent	Amide	00B-3005-E0
3126	(D)-Penicillamine	Ion-Metal	Lig Exchange	00B-3126-E0

5μ Analytical Columns (mm)

				Analytical			
Phase	Chirex Phase Description	Bond Type	Linkage Type	150 x 2.0	250 x 2.0	150 x 4.6	250 x 4.6
3001	(R)-PGLY and DNB	Covalent	Amide	—	00G-3001-B0	00F-3001-E0	00G-3001-E0
3010	(S)-VAL and DNAn	Covalent	Urea	—	—	00F-3010-E0	00G-3010-E0
3011	(S)-LEU and DNAn	Covalent	Urea	—	—	00F-3011-E0	00G-3011-E0
3012	(R)-PGLY and DNAn	Covalent	Urea	—	—	00F-3012-E0	00G-3012-E0
3014	(S)-VAL and (R)-NEA	Covalent	Urea	—	00G-3014-B0	—	00G-3014-E0
3017	(S)-PRO and (S)-NEA	Covalent	Urea	—	—	00F-3017-E0	—
3018	(S)-PRO and (R)-NEA	Covalent	Urea	—	—	00F-3018-E0	00G-3018-E0
3019	(S)-LEU and (S)-NEA	Covalent	Urea	—	—	00F-3019-E0	00G-3019-E0
3020	(S)-LEU and (R)-NEA	Covalent	Urea	00F-3020-B0	—	00F-3020-E0	00G-3020-E0
3022	(S)-ICA and (R)-NEA	Covalent	Urea	00F-3022-B0	00G-3022-B0	00F-3022-E0	00G-3022-E0
3005	(R)-NGLY and DNB	Covalent	Amide	—	00G-3005-B0	00F-3005-E0	00G-3005-E0
3126	(D)-Penicillamine	Ion-Metal	Lig Ex	00F-3126-B0	00G-3126-B0	00F-3126-E0	00G-3126-E0

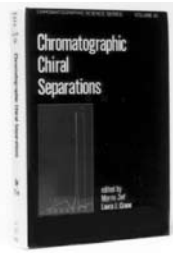
Chirex columns are available in all sizes from microbore to preparative. Included with each column are detailed notes on column care and use, test procedures and the test chromatogram for your column.

For more information on any application shown or listed in this booklet, or for assistance in developing or improving chiral separations, please call, fax or write Phenomenex.

Ask for our FREE comprehensive brochure, designed to guide you in the selection of the right column for your application.

CHROMATOGRAPHIC CHIRAL SEPARATIONS

M. Zief and L. Crane, eds. 1988, 410pp.



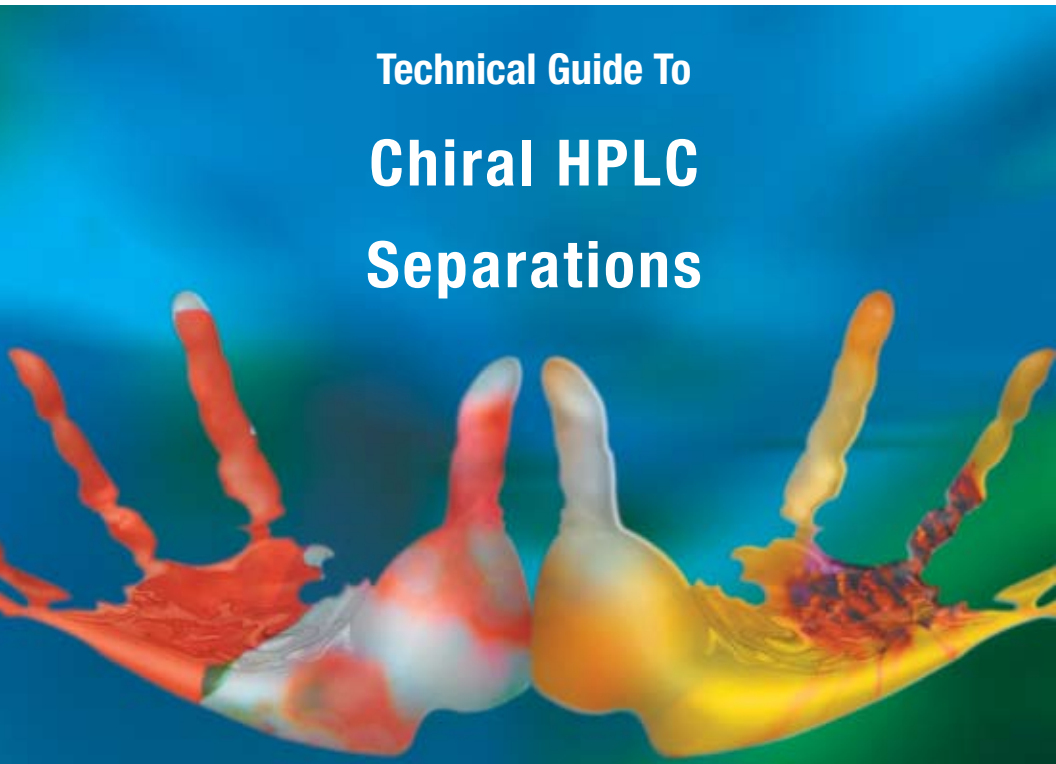
This outstanding book affords a complete evaluation of the "state of the art" in enantiomeric separation techniques. The book explains direct separation of enantiomers by available chiral stationary phases synthesized from chiral amino acids as well as synthetic and natural polymers. It also covers indirect separation by the formation of diastereomers and includes discussions of preparative as well as analytical separations.

Chromatographic Chiral Separations delineates the scope of the technique and the mechanisms responsible for effecting separation. Furthermore, the book enumerates practical applications of these systems in the development and production of pharmaceuticals, agricultural products, and synthetic organic chemicals. This book will provide an invaluable resource for analytical and organic chemists, biochemists and chromatographers in all areas concerned with chiral compounds.

Order No.: AA0-2902

Technical Guide To

Chiral HPLC Separations



Review of
Chiral Phases
and
Method Development Strategies

2716_L