How to Select the Right Chiral HPLC Column

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This is the first of three Technical Notes covering general separation strategies you can use to successfully resolve and purify chiral compounds directly without difficulty.

Introduction

Chiral HPLC is by far one of the most powerful and sensitive analytical techniques used today for resolving enantiomers. However, even experienced chromatographers may find the choice of column along with the most appropriate mobile phase to be problematic and a bit overwhelming. With the Chirex series of Pirkle-type chiral stationary phases (CSPs) from Phenomenex this is a much less arduous task.

Chirex™ columns are ideally suited for the separation of typical pharmaceutical and agrochemical compounds containing aromatic group(s) and one or more proton-donor and/or acceptor groups. There are ten Pirkle or "brush"-type columns from which to choose. All are rugged, versatile and highly stable. Typically run under normal phase conditions, these columns make scale up to preparative and the recovery of purified materials easier and more efficient than other CSPs where bleed may be a problem, or where residual buffer salts used in reversed phase must be removed from the recovered material.

A second type of CSP, the ligand exchange type, is also available in the Chirex line. Phase 3126 runs under reversed phase conditions and is highly selective for the separation of α-amino acids, α-hydroxy acids, and dipeptides. The features and applications of this phase were covered in detail in Technical Note TN-1005.

In this Technical Note we present a systematic approach to helping you select the right chiral column for your analysis. Although there are few hard and fast rules to follow, our guidelines offer an easy starting point for exploring the potential of various Chirex CSPs. Their overall applicability and versatility should give you the confidence that you are working with the most sophisticated tools available today for chiral HPLC.

Instrumentation & Equipment

Analyses were performed using an HP 1100 LC system (Agilent Technologies, Palo Alto, CA, USA) equipped with a quaternary pump, in-line degasser, multi-wavelength detector, and autosampler. HP Chemstation software was used for the data analysis. The HPLC columns used for the analysis are Chirex™ brand (Phenomenex, Torrance, CA, USA, see Ordering Information). Standards were purchased from Sigma (St. Louis, MO), Aldrich (Milwaukee, WI), or Fluka (Ronkonkoma, NY), depending on availability.

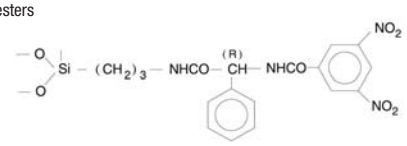
Table 1. Chirex chiral stationary phases and their descriptions

CHIREX™ Chiral Stationary Phase Descriptions

Amide type — 3001

(R)-phenylglycine and 3,5-dinitrobenzoic acid amide linkage, electron acceptor designed for the separation of:

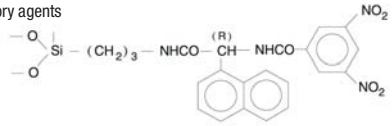
- carboxylic acids, alcohols, esters
- sulphoxides



Amide type — 3005

(R)-1-naphthylglycine and 3,5-dinitrobenzoic acid amide linkage, electron acceptor designed for the separation of:

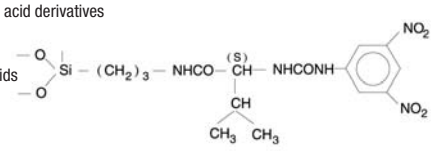
- carboxylic acids, alcohols, esters
- non-steroidal anti-inflammatory agents



Urea type — 3010

(S)-valine and 3,5-dinitroaniline urea linkage, electron acceptor designed for the separation of:

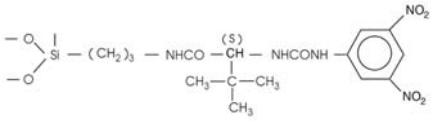
- carboxylic acids, amino acid derivatives
- hydroxy acids
- Dabsyl and Dansyl derivatives of amino acids



Urea type — 3011

(S)-tert-leucine and 3,5-dinitroaniline urea linkage, electron acceptor designed for the separation of:

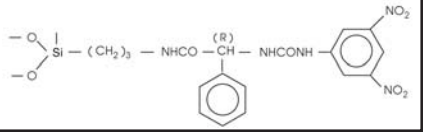
- carboxylic acids, amino acid derivatives



Urea type — 3012

(R)-phenylglycine and 3,5-dinitroaniline urea linkage, electron acceptor designed for the separation of:

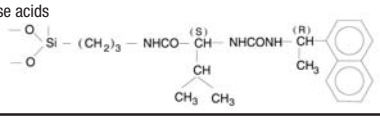
- carboxylic acids, amino acid derivatives



Urea type — 3014

(S)-valine and (R)-1-(α-naphthyl)ethylamine urea linkage, electron donor designed for the separation of:

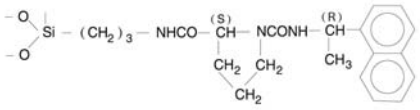
- π-acceptor derivatives of amines, carboxylic acids and amino acids
- the esters and amides of these acids
- underivatized alcohols



Urea type — 3018

(S)-proline and (R)-1-(α-naphthyl)ethylamine urea linkage, electron donor designed for the separation of:

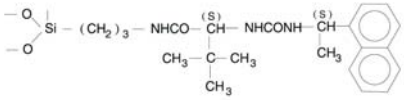
- amines, alcohols and amino acids
- underivatized β-blockers
- aromatic amines
- pesticides



Urea type — 3019

(S)-tert-leucine and (S)-1-(α-naphthyl)ethylamine urea linkage, electron donor designed for the separation of:

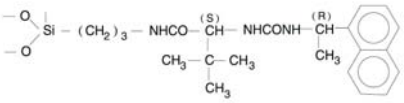
- esters, amino alcohols
- underivatized β-blockers
- aromatic amines
- cyano alcohols
- pesticides



Urea type — 3020

(S)-tert-leucine and (R)-1-(α-naphthyl)ethylamine urea linkage, electron donor designed for the separation of:

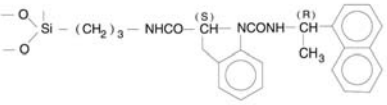
- amines, amino alcohols, alcohols
- underivatized β-blockers
- aromatic amines
- cyano alcohols
- pesticides



Urea type — 3022

(S)-indoline-2-carboxylic acid and (R)-1-(α-naphthyl)ethylamine urea linkage, electron donor designed for the separation of:

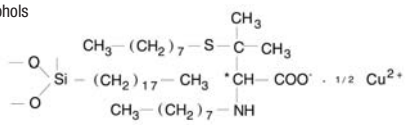
- amines, amino alcohols, alcohols



Ligand Exchange type — 3126

(D)-penicillamine ligand exchange, electron acceptor designed for the separation of:

- α-amino acids, their derivatives
- α-hydroxy acids, amino alcohols



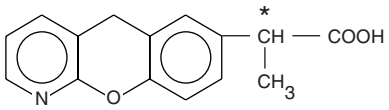
Based on the chemical groupings present in your compound it is possible to narrow your choice of chiral stationary phases (CSPs) to just a few. Just follow the flow chart below and read off the candidate phases to try.

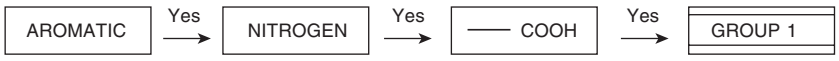
Chirex™ Column Selection Guide

Presence of Chemical Groupings in Chiral Solute							Recommended Columns		
Class	**Aromatic**	**—N—**	**—COOH**	**—OH**	**OTHER**	**Comment**	**First Choice**	**Second Choice**	**Third Choice**
Group 1	Y	Y	Y			Aromatic α-amino acids, α-hydroxy acids (3126 only)	3126 *	3005 or 3001	3011 or 3012
Group 2	Y	Y		Y			3022 or 3020	3014	3018
Group 3	Y	Y			Y		3014 or 3020	3022	3018
Group 4	Y		Y				3005	3010	3001
Group 5	Y			Y			3001 or 3014	3005	3020 or 3022
Group 6	Y				Y		3001	3005	3019 or 3020
Group 7		Y	Y			Aliphatic α-amino acids, α-hydroxy acids and their derivatives	3126		
Group 8			Y				3126	3010	3001
Group 9					Y		3014	3019 or 3020	3001
Group 10					Y	Asymmetric other than carbon. Chiral center at N,S,P,B, etc.	3014	3010	3005

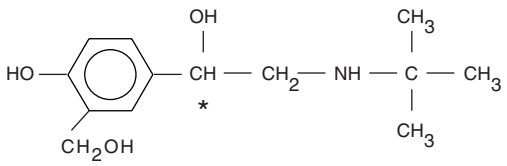
* Note: Chirex phase 3126 is a ligand-exchange column with special utility for analyzing chiral α-amino acids, α-hydroxy acids and dipeptides only.
For more information please ask for a copy of Technical Note TN-1005.

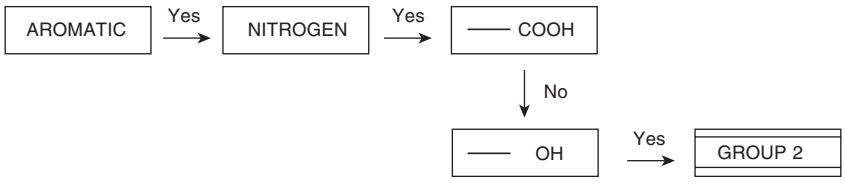
To illustrate the flow chart, suppose your compound is a carboxylic acid, such as this profen compound below. If we follow the flow chart and classify the chiral compound according to the presence or absence of various functional groups, we see this compound belongs in Group 1. Since this compound is not suited for phase 3126, the initial two choices are phases 3005 and 3001.





In the same way we can place this next compound, an amino alcohol, into Group 2. Multiple CSPs should be evaluated for their selectivity, run time and overall resolution.

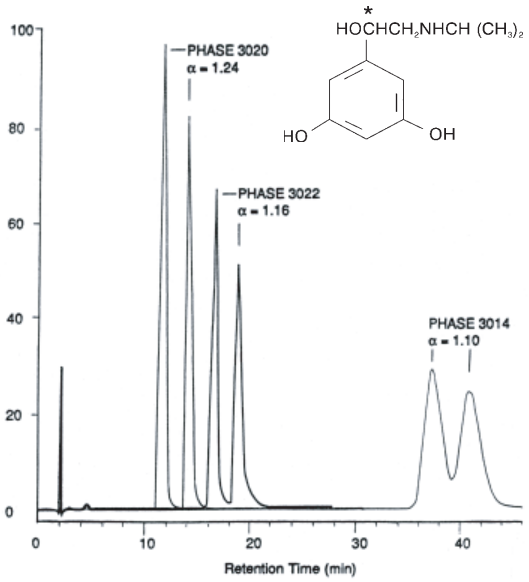




One or more of the suggested CSPs will often confer a high probability of success as the overlaid chromatograms in Figure 1 illustrate. Three of the recommended columns successfully resolved the enantiomers of metaproterenol. It is typical that one or more Chirex CSP will resolve the enantiomers, but one will usually show superior resolution over the others, as Chirex CSP 3020 shows in this example.

Fig. 1. Enantioselectivity of metaproterenol on three Chirex CSPs

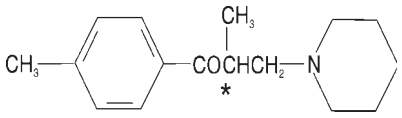
Metaproterenol
(Bronchodilator)



In another example, the utility of different phases for the separation of the bronchodilator Tolperisone is compared in Figure 2. Certainly, resolution is sufficient in all of the separations to be useful analytically, but if scale up to preparative HPLC is the goal, then Chirex phases 3018 or 3020 would be obvious choices to enable recovery of the maximum amount of purified material in a single run.

Fig. 2. Enantioselectivity of Tolperisone on four Chirex CSPs

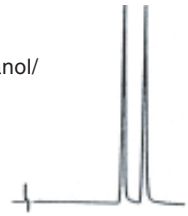
TOLPERISONE
(Bronchodilator)



CHIREX 3017
Column: 250 x 4.0mm
Mobile Phase: Hexane/ethanol/ trifluoroacetic acid (200:40:0.6)
Flow Rate: 1.0mL/min
Detector: UV254nm

α = **1.25**

CHIREX 3019
Column: 250 x 4.0mm
Mobile Phase: Hexane/ethanol/ trifluoroacetic acid (200:40:0.6)
Flow Rate: 1.0mL/min
Detector: UV254nm

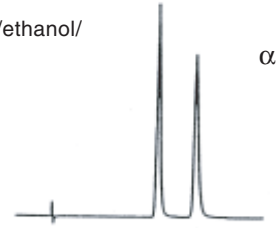


α = **1.22**

CHIREX 3018
Column: 250 x 4.0mm
Mobile Phase: Hexane/ethanol/ trifluoroacetic acid (200:40:0.6)
Flow Rate: 1.0mL/min
Detector: UV254nm

α = **2.13**

CHIREX 3020
Column: 250 x 4.0mm
Mobile Phase: Hexane/ethanol/ trifluoroacetic acid (230:20:1)
Flow Rate: 1.0mL/min
Detector: UV254nm



α = **1.35**

A summary of the most popular Chirex phases and their utility is provided below

- 3001 General workhorse, good screening column
- 3005 Excellent for NSAIDs (e.g., Ibuprofen, Naproxen)
- 3011, 3012 Derivatized (N-protected) amino acids
- 3014 Derivatives of amines and carboxylic acid
- 3020, 3022 Broadest utility for typical pharmaceuticals (aromatic amines, amino alcohols, alcohols)
- 3126 α-amino acids, α-hydroxy acids, and dipeptides (see Technical Note TN-1005)

Results & Discussion:

Enantioselectivity of the CSP is highly dependent on the structure of your compound. In this Technical Note we present a proven approach to column selection from among 11 versatile Chirex phases. Based on the presence or absence of various functional groups in the target compound, initial column recommendations can be made. For typical pharmaceutical and agrochemical compounds, different degrees of resolution using one or more of the recommended columns can be expected. The best column for the application is selected after having compared the resolution and run time for several phases.

To enable chiral chromatographers to quickly and efficiently survey the utility of a variety of CSPs, Phenomenex offers two different method development kits. Each kit contains five short 50mm "Starter" columns carefully chosen for rapid and cost effective method development. Kit A (Order No. KH0-1892) is especially designed for the method development of pharmaceuticals and agrochemicals, while Kit B (Order No. KH0-1893) offers a collection of chiral columns for separating enantiomeric α-amino acids, α-hydroxy acids, dipeptides, aromatic amines, alcohols and derivatized amino alcohols.

Chiral Method Development Kits

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.

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

Chiral Method Development Kit A

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

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 offers excellent enantioselectivity for underivatized α-amino acids, and α-hydroxy acids.

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)

Chiral Method Development Kit B

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

Once one or more columns have been screened for their utility, optimization of the separation on a given column is methodical and straightforward with Chirex materials. The approach to method optimization, including tips and traps, is discussed in detail in our next Technical Note in the series, TN-1016.

References

1. Cleveland, T., J. Liq. Chromatogr. 18(4): 649-671, **1995**.

If you would like more information on these chiral columns or any of the applications listed, please contact Phenomenex. Also, if you are new to chiral HPLC or are doing method development work call us today to reserve your ***FREE*** copy of our 70-page ***Guidebook to Chiral HPLC Method Development***.

CHIREX™

Ordering Information:
Chirex is available in a wide range of phases and column sizes, from analytical to preparative. All phases are also available in bulk (15 and 30µ particle size).

The columns discussed in this Note are listed below.

5µ Analytical Columns (mm)

Chirex Phase and Bond Linkage, 250 x 4.6mm ID

Phase	Description	Order No.
3001	(R)-PGLY and DNB Covalent Amide	00G-3001-E0-TN
3005	(R)-NGLY and DNB Covalent Amide	00G-3005-E0-TN
3014	(S)-VAL and (R) -NEA Covalent Amide	00G-3014-E0-TN
3018	(S)-PRO and (R)-NEA Covalent Amide	00G-3018-E0-TN
3019	(S)-LEU and (S)-NEA Covalent Amide	00G-3019-E0-TN
3020	(S)-LEU and (R)-NEA Covalent Amide	00G-3020-E0-TN
3022	(S)-ICA and (R)-NEA Covalent Amide	00G-3022-E0-TN

Chiral Method Development Kit A

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

Chiral Method Development Kit B

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

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