Enantiomer Separation of Chiral Acids on CHIRALPAK® AX-QN and CHIRALPAK® AX QD
Chiral Recognition Mechanism

Non-covalent Interactions Stabilizing the Quinine tert-butylcarbamate / 3,5-dinitrobenzoyl leucine complex (X-Ray Crystal Structure)

charge-supported hydrogen bond

face-to-face \( \pi \)-stacking

van der Waals interaction

amide-type hydrogen bond

DNB-(S)-Leu

Cl-tBuCQN

N.M. Maier et al. *Chirality* (1999)
N.M. Maier et al. *JACS* (2001)
Principles of Analyte Retention on CHIRALPAK® AX Columns: Weak Anion Exchange

\[ k \approx K \times \phi \times [SO]_{tot} \times \frac{1}{[X^-]} \times \alpha_{prot,SO} \times \alpha_{diss,SA} \]

- **k**: retention factor
- **K**: association constant
- **\( \phi \)**: phase ratio
- **\([SO]_{tot}\)**: total selector concentration
- **\([X^-]\)**: counterion concentration
- **\(\alpha_{prot,SO}\)**: degree of protonation of selector
- **\(\alpha_{diss,SA}\)**: degree of dissociation of analyte
Impact of pH on Retention Enantioselectivity

![Graph showing the impact of pH on retention enantioselectivity. The graph illustrates the change in %SO<sub>prot</sub> and %SA<sub>diss</sub> as pH changes. The graph includes points for R<sub>3</sub>NH<sup>+</sup>, R<sub>3</sub>N, R-COO<sup>-</sup>, and R-COOH, with pK<sub>s,SO</sub> and pK<sub>s,SA</sub> indicated.]
Impact of Mobile Phase pH on Retention & Enantioselectivity

Retention and enantioselectivity can be optimized by appropriate adjustment of the $\text{pH}_a$ of the mobile phase.

CHIRALPAK AX-QN (150 x 4 mm I.D.);
MeOH-aqu. 0.2 M NH$_4$OAc (80:20, v/v);
1.0 mL/min; 254 nm; $T$ 25 °C
Analyte: N-benzoyl leucine

Impact of Counterion Concentration on Retention & Enantioselectivity

CHIRALPAK AX-QN (150 x 4 mm I.D.);
MeOH-aqu. 0.2 M NH₄OAc (80:20, v/v);
1.0 mL/min; 254 nm; T 25 °C
Analyte: N-benzoyl leucine

Retention adjustable via counterion concentration without significant loss in enantioselectivity.

Method Development Strategy: Optimization Parameter

- **pH** *(pH profile of k and alpha)*

- **Counterions** *(nature, concentration)*

- **Organic modifier** *(nature, relative amounts)*

*Lower flow rates: Strongly electrostatic SO-SA interaction – slow mass transfer.*

*Low temperature: Chiral recognition process enthalpically driven – low temperature favor enantioselectivity.*
Polar Organic Conditions

Preferred due to enhanced enantioselectivity, generality, low viscosity, solubility of most analyte
Little non-specific retention of lipophilic compounds
MS-compatible

Methanol - ammonium acetate - acetic acid (98:2:0.5 to 95:5:2)
Methanol - triethylamine - acetic acid
Methanol - ammonium formate - formic acid

Methanol - acetic acid
Acetonitrile - acetic acid

POM works for most N-acylated amino acids, N-blocked peptides, carboxylic acid and drug compounds with pK_a 3 to 5.
Polar Organic Conditions: Dihydropyrimididine Carboxylic Acid

Ca\(^{2+}\)-Channel Blocker Intermediate

CHIRALPAK AX-QN (150 x 4.6 mm ID), 0.124 % AcOH in acetonitrile; 1.0 mL/min; 250 nm; 25°C.
Polar Organic Conditions: Pyrethroic Acids

cis/trans-Chrysanthemic acid (Pesticide Intermediate)

a) Polar-organic conditions:
0.06% AcOH in acetonitrile-methanol 95:5 (v/v); 0.65 mL/min; 25°C.

b) Reversed-phase conditions:
10mM AcOH in acetonitrile-water 90:10 (v/v), pH 6.0 (NH3 aq.), 0.65 mL/min; 25°C.
Reversed Phase Conditions

Exploits mixed reversed phase/anion exchange mechanisms

For analytes poorly soluble/retained under polar organic conditions:
Phosphoric acids, sulfonic, phosphonic, phosphinic acids, poly-carboxylic acids

Aqueous ammonium acetate – methanol (pH 4.5 to 7.0)
Aqueous sodium phosphate – methanol/acetonitrile (pH 4.5 to 7.0)
Other buffers: Citrate >> phosphate > format > acetate

Column life time may be shortened due to exposure to strong buffers. Column may need regeneration after use of citrate.
Reversed Phase Conditions: Phosphinic Acids

**CHIRALPAK AX-QN** (150 x 4.6 mm ID), methanol-50 mM sodium phosphate buffer (80:20, v/v), pH 5.6; 1.0 mL/min; 250 nm; 40°C.
Reversed Phase Conditions: Phosphinic Acid Derivatives

CHIRALPAK AX-QN (150 x 4.6 mm ID), methanol-50 mM sodium phosphate buffer (80:20, v/v), pH 5.6; 1.0 mL/min; 250 nm; 40°C.

Reversed Phase Conditions: Amino Acid - Thyroxine

Thyroxine (T₄): R₁ = I
Triiodothyronine (T₃): R₁ = H

Chiralpak® QN-AX (150 x 4.6 mm ID), ACN-0.05 NH₄OAc (60:40 v/v); 0.7 mL/min; 240 nm; 25°C
Reversed Phase Conditions: Amino Dicarboxylic Acids

Chiralpak QN-AX (150 x 4 mm ID); MeOH-0.1 M NH₄OAc (80:20, v/v) pHₐ 5.5; 1.0 mL/min; UV 254 nm; 25°C.

Potential mGluR1 Subtype Selective Antagonist
CHIRALPAK® AX-QN and AX-QD: Pseudoenantiomeric CSPs

X-Ray Crystal Structures

CHIRALPAK AX-QN Selector
Co-crystallized with DNB-(S)-leucine

CHIRALPAK AX-QD Selector
Co-Crystallized with DNB-(R)-leucine
Control of Elution Order by Combined Use of CHIRALPAK® AX-QN and AX-QD

Columns (150 x 4 mm ID); 1% (v/v) AcOH in MeOH; 1 mL/min, 25°C; UV 230 nm.

$\alpha = 1.5$
Prep Application

Employing volatile mobile phases to facilitate product recovery

Acetonitrile - Methanol – acetic acid (99:1 to 97:3, v/v)

Acetonitrile / Methanol – formic acid (99:1 to 98:2, v/v)

SFC: CO2 – Methanol

Added acids concentrate upon solvent evaporation – chemical stability and stereochemical integrity of the target compounds need to be established.
Inherently favorable saturation capacity of ion-exchangers

Possibly displacement effects (high affinity enantiomer sharpens front of low affinity enantiomer)

Chiralpak AX-QN (150 x 4.6 mm I.D.); 10 mM ammonium acetate + 30 mM acetic acid in methanol (pHₐ = 6.0); flow rate: 1 mL/min; UV 254 nm, 320 nm; 25°C.
SFC Separation of Diastereomeric Mixture of an Acidic Drug Candidate

MeOH / CO2, no additional acidic additive
CHIRALPAK® AX-QN & CHIRALPAK® AX-QD

Enantioselectivity for a broad range of chiral acidic compounds

Compatibility with all common HPLC solvents and modes (non-polar, polar-organic, reversed-phase and SFC)

Stable in pH range 2 to 8

Control of elution order via pseudoenantiomeric chiral recognition properties

Straightforward method development

Suitable for bio-analytical and MS applications

Manufacture and technical support by DAICEL
Thanks

Michael Lämmerhofer and Wolfgang Lindner
University of Vienna, Austria

All former coworkers at the Department of Analytical Chemistry,
University of Vienna, Austria

Pilar Franco and Tong Zhang
Chiral Technologies Europe, France