CHIRALPAK® QD-AX / QN-AX

NEW from CHIRAL TECHNOLOGIES EUROPE

For Acids
and Amino Acid Derivatives

Expert Technical Support
Immediate Delivery
DAICEL Quality

CHIRAL
TECHNOLOGIES EUROPE
SUBSIDIARY OF DAICEL CHEMICAL INDUSTRIES, LTD.

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CHIRAL
TECHNOLOGIES EUROPE
Pure simplicity

Application and Features

These new Daicel products are able to separate an extensive variety of chiral pharmaceutical intermediates including:

N-derivatised amino acids

aminosulfonic acids

aminophosphonic acids

aryloxycarboxylic acids

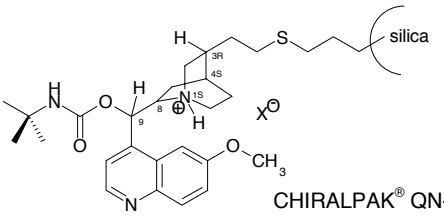
N-derivatised peptides

The following features make CHIRALPAK® QN-AX and CHIRALPAK® QD-AX an ideal choice for the enantioseparations of chiral acidic compounds:

- Demonstrated separations for many chiral acidic compounds
- Stability against all common HPLC solvents
- Stable in pH range 2 to 8
- Logical method development
- Manufacture and technical support by Daicel
- Suitable for use in bio-analytical and LC-MS with compatible mobile phases and buffers

Mechanism and History

Quinine (QN) and quinidine (QD)-derived carbamates immobilized onto silica represent one of the most stereoselective chiral stationary phases (CSPs) for the direct resolution of chiral acids by HPLC. These CSPs can be classified as weak *chiral anion exchangers* (AX) due to the tertiary nitrogen within the quinuclidine substituent that is protonated at the usual working pH of the mobile phase.



CHIRALPAK® QN-AX: (8S,9R)

CHIRALPAK® QD-AX: (8R,9S)

Prof. Lindner's research group (University of Vienna) has undertaken exhaustive investigation of these phases for several years[1-3]. Their research results demonstrate the benefit of these chiral phases in pharmaceutical development and quality control. As a result, Daicel Chemical Industries Ltd. has undertaken a collaboration which has resulted in the market introduction of the *tert*-butylcarbamates of QN and QD immobilized on 5 µm silica gel: **CHIRALPAK® QN-AX and CHIRALPAK® QD-AX.**

1 M. Lämmerhofer, W. Lindner, *J. Chromatogr. A* **741** (1996) 33-48.
2 M. Lämmerhofer, N. M. Maier, W. Lindner, *Am. Lab.* **30** (1998) 71-78.
3 N. M. Maier, L. Nicoletti, M. Lämmerhofer, W. Lindner, *Chirality* **10** (1999) 522-52

Method Development on CHIRALPAK® QN-AX and CHIRALPAK® QD-AX

The method development is based on *the type of acidic compound* to be separated.

- The majority of all acidic compounds will be separated in polar organic (PO) mode.

- RP conditions (RP) should be used for chiral *sulfonic*, *phosphonic*, *phosphoric* and *di-* or *multicarboxylic acids*, as well as *amphoteric compounds*.

We recommend that method development be undertaken on CHIRALPAK® QD-AX only as both columns work in an identical manner with only the elution order being inverted when changing column.

The polar organic (PO) mode

Method development on CHIRALPAK® QD-AX and QN-AX requires optimisation of three key parameters:

1. **Organic solvent**
2. **Counter ion Concentration**
3. **Apparent pH**

A suitable mobile phase to start the screening would be methanol/acetic acid/ammonium acetate (98:2:0.5, v/v/w).

In *Figure 1* the separation of racemic FMOC-Leucine on CHIRALPAK® QN-AX and QD-AX with this solvent mixture is shown. This solvent combination leads to excellent resolution between the two enantiomers and also for a wide range of **N-derivatised amino acids.**

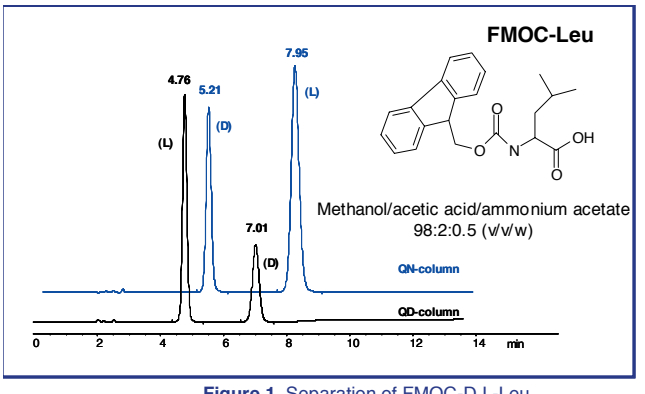


Figure 1. Separation of FMOC-D,L-Leu on CHIRALPAK® QN-AX and QD-AX (150 x 4.6 mm, 25°C, 1 mL/min).

Selection of Organic Solvent

CHIRALPAK® QN-AX and QD-AX are stable to all miscible chromatographic solvent combinations. In practice acetonitrile and methanol will give acceptable solubility and enantioselectivity.

To modulate enantioselectivity, acetonitrile and methanol, pure or combined, can be tested.

- For *N-derivatized amino acids* methanol has been found to be the better choice,
- For *arylcarboxylic acids* acetonitrile produced higher enantioselectivity values.
- For *hydroxycarboxylic acids* a mixture of acetonitrile/methanol may be the preferred solvent.

Due to the organic solvent stability of these columns, there is a wide choice of solvents possible for sample injection (increased solubility). The only limitations will be high-pressure drop (max. pressure 180 bar) or extreme pH ranges, which can damage the silica gel.

Optimisation of Counter Ion Concentration

If retention times are extended, an increase in the counter-ion concentration will decrease retention.

A change of the concentration of the competing acid (counter-ion) in the mobile phase at identical acid-basic ratio usually has a negligible effect on the enantioselectivity. However, it will considerably affect retention time by modulation of the ion-exchange capacity (see Figure 2).

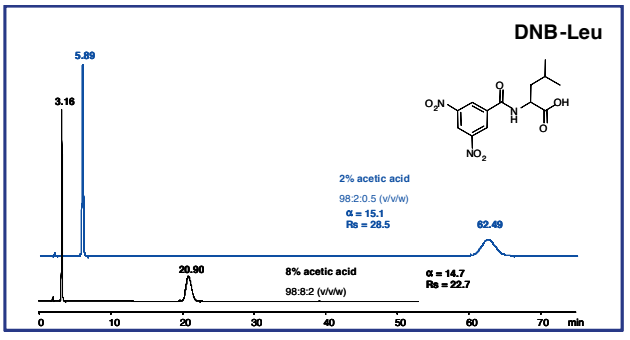


Figure 2. Separations of DNB-D,L-Leu on CHIRALPAK® QN-AX with methanol/acetic acid/ammonium acetate mixtures (150 x 4.6 mm, 25°C, 1 mL/min).

Optimisation of apparent pH

By variation of the acid-base ratio (e.g. the acetic acid to TEA ratio), the apparent pH can be altered.

This will have a significant effect on retention and enantioselectivity. With increasing excess of acid the retention will increase. The optimal concentration in terms of selectivity will depend on the solute structure.

Alternatively, if retention is too long, the acetate buffer may easily be replaced by the formate buffer, which has higher elution strength.

Table 1 has examples of typical acid-base ratios and the apparent pH (pHa).

Other chromatographic parameters that can modulate resolution and retention are the flow rate and the temperature.

Column Dimensions

CHIRALPAK® QN-AX and QD-AX columns are both available from Chiral Technologies Europe in the following sizes:

Microbore	2.1 mm (i.d.)	150 mm (length)
Analytical	4.6 mm	150 mm
Semi-Prep	20 mm	150 mm

Table 1. Examples of acid-base ratios

	pHa < 6	pHa ≃ 6	pHa ≃ 6.5	pHa > 7
Acetic acid/TEA	2 : 0.2	3 : 1 2 : 0.6 1 : 0.3	2 : 1.6 1 : 0.8	2 : 3 1 : 1.5
Acetic acid/NH_3	2 : 0.2	3 : 0.7 2 : 0.5 1 : 0.2	2 : 1.2 1 : 0.5	2 : 2 1 : 1
Formic acid/TEA	0.5 : 0.5	1 : 3 0.5 : 1.5	1 : 3.5	1 : 4 0.5 : 1.8
Formic acid/NH_3	0.5 : 0.3	1 : 1.5 0.5 : 0.8	0.5 : 1	0.5 : 1.5

⇩ Starting conditions

Reverse Phase (RP) Mode

If a partial or no separation are found in the polar organic mode, it would be advised to evaluate the RP-mode. The addition of some aqueous component in the mobile phase may lead, for certain acidic compounds, to their baseline resolution (Figure 3).

Typical mobile phase combinations will contain methanol or acetonitrile with an organic buffer (acetate, formate, phosphate, etc.) and the pHa of the final mixture can be adjusted with TEA or NH_3.

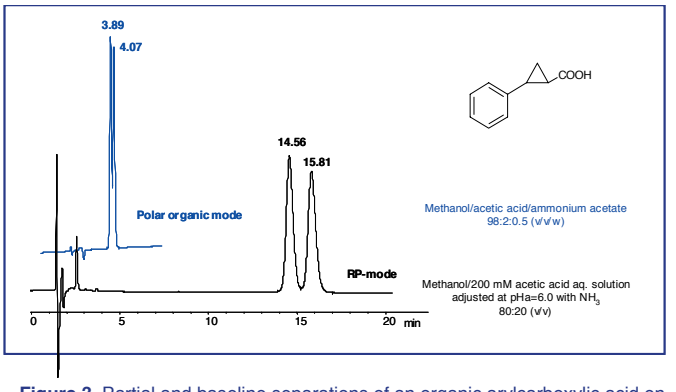


Figure 3. Partial and baseline separations of an organic arylcarboxylic acid on CHIRALPAK® QN-AX under PO- and RP-mode (150 x 4.6 mm, 25°C, 1 mL/min).

Inverse Elution

The separation of enantiomers can be inverted by running the same method on CHIRALPAK® QD-AX and then switching to QN-AX.

A table of known amino acid derivative elution orders is available upon request.

Technical Support from Daicel

Technical support from Chiral Technologies Europe is available for CHIRALPAK® QN-AX and QD-AX columns. Please call for method development, application information and general technical enquiries.

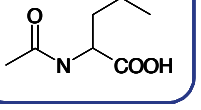
The columns are available from stock in Europe and are available for same day despatch to any European location.

CHIRALPAK® QN-AX and QD-AX are manufactured by Daicel and come with Daicel quality as standard.

CHIRALPAK® QN-AX and CHIRALPAK® QD-AX

Analytical HPLC applications

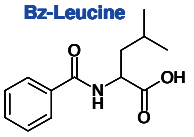
Ac-Leucine



methanol / acetic acid
100:1 (v/v)
k_1 = 1.77
α = 1.46
Rs = 6.00

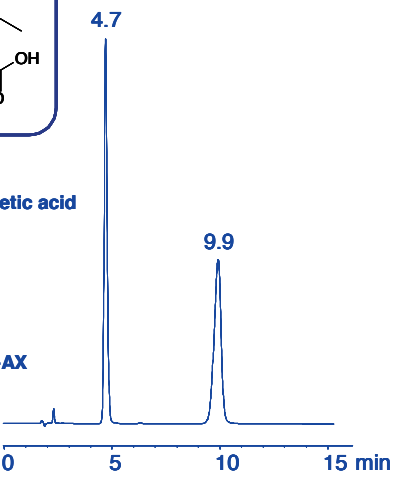
CHIRALPAK® QD-AX

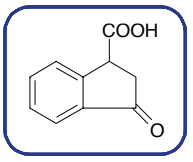
Bz-Leucine



methanol / 0.2 M aq. acetic acid
pH = 7
90:10 (v/v)
k_1 = 1.58
α = 2.82
Rs = 12.43

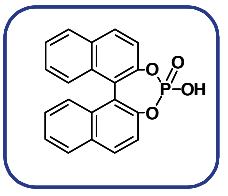
CHIRALPAK® QD-AX





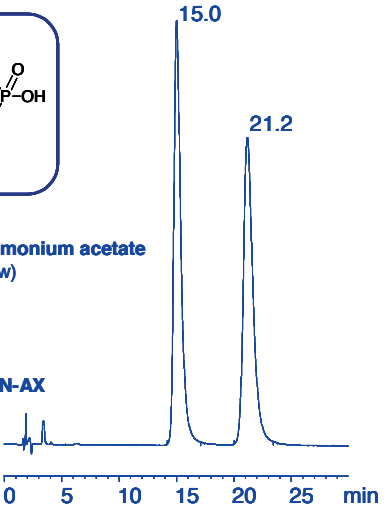
acetonitrile / acetic acid
97:3 (v/v)
k_1 = 7.31
α = 1.09
Rs = 1.95

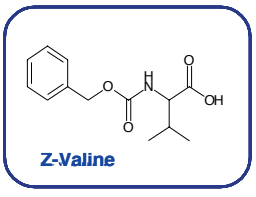
CHIRALPAK® QD-AX



methanol / acetic acid / ammonium acetate
98:2:0.5 (v/v/w)
k_1 = 7.07
α = 1.47
Rs = 5.04

CHIRALPAK® QN-AX

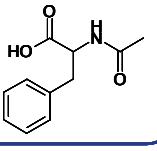




methanol / acetonitrile / acetic acid
48.5:48.5:3 (v/v/v)
k_1 = 1.85
α = 1.40
Rs = 5.43

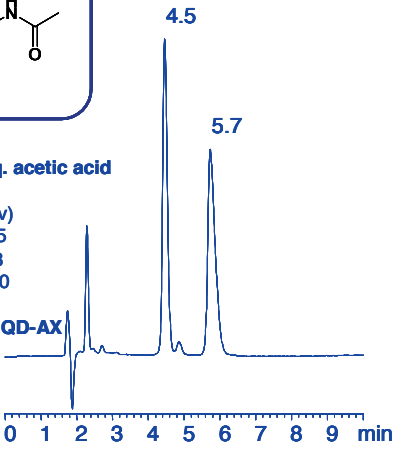
CHIRALPAK® QN-AX

Ac-Phenylalanine



methanol / 0.2 M aq. acetic acid
pH = 7
90:10 (v/v)
k_1 = 1.45
α = 1.48
Rs = 4.20

CHIRALPAK® QD-AX



General conditions: CHIRALPAK® QN-AX or CHIRALPAK® QD-AX (150 x 4.6 mm, Flow rate: 1 mL/min, 25°C)

For more detailed information about this column and other Daicel supports, refer to our catalogue also available on our website: http://www.chiral.fr or contact **CHIRAL TECHNOLOGIES EUROPE**

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