



CHIRAL
TECHNOLOGIES

DAICEL GROUP



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Immobilised Zwitterionic Stationary Phases

CHIRALPAK® ZWIX(+) | CHIRALPAK® ZWIX(-)

move easily
move reliably
move quickly

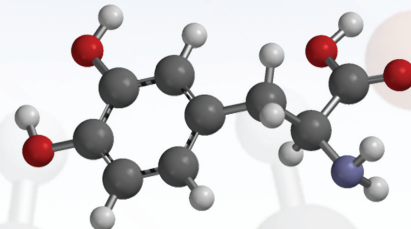
move ahead

DAICEL

Zwitterionic Stationary Phases for Separation of Free Amino Acids

Our zwitterionic stationary phases (ZWIX™) are chiral supports that incorporate both anion- and cation-exchange functional groups. These novel ZWIX selectors exhibit enantioseparation capabilities toward zwitterionic molecules such as amino acids and peptides.

Amino acids combine to form proteins – the building blocks of life. Most amino acids are chiral, containing a stereogenic carbon, to which the amino group is attached. Amino acids play a vital role in human nutrition and serve as important tools in drug discovery and API manufacturing. Therefore, analysis of **underivatised** amino acids, in terms of their composition and enantiomeric purity, is essential in pharmaceutical and food industries. We have recently developed CHIRALPAK ZWIX – chiral selectors specifically designed to meet the growing demand for direct separation, detection, and identification of amino acids.



ZWIX Chiral Stationary Phases | Features and Benefits

Features

Separation of underivatised amino acids and zwitterionic compounds of diverse structural functionalities



Benefits

Save time by maximising operational flexibility

Reversal of enantiomer elution order



Expand knowledge base by accurately determining D-enantiomers

Use of polar mobile phases



Increase productivity with the use of LC/MS detection and identification

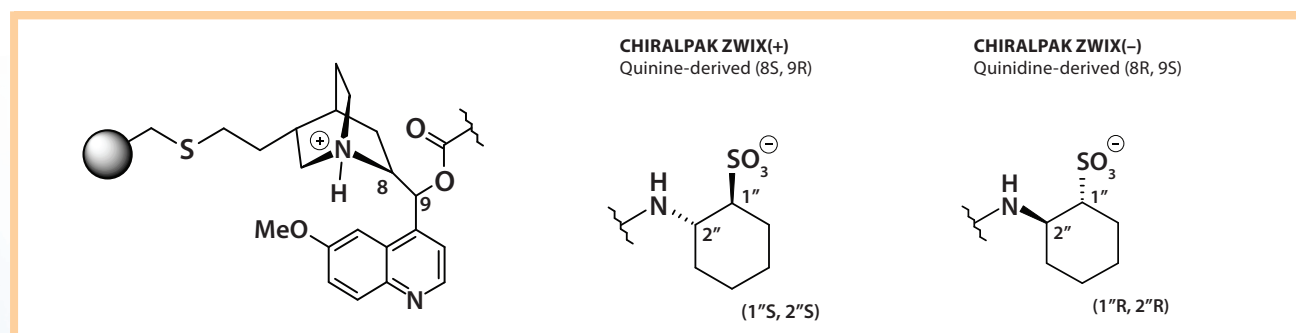
High column efficiency



Gain complete confidence in results

ZWIX Chiral Selectors

The zwitterionic chiral stationary phases (CSPs), CHIRALPAK ZWIX(+) and CHIRALPAK ZWIX(-), are synthesised by the fusion of the cinchona alkaloids with trans-2-aminocyclohexanesulfonic acid (ACHSA) at the C-9 position via carbamate linkage.



In CHIRALPAK ZWIX(+), quinine is fused with (S,S)-ACHSA, while the chiral selector in CHIRALPAK ZWIX(-) is formed by the fusion of quinidine with (R,R)-ACHSA. Both chiral selectors are chemically bonded onto 3- μ m silica gel. The chiral selectors separate zwitterionic molecules on the basis of a double ion-pairing mechanism.

Analyses of Underivatised Amino Acids

In biological systems, most amino acids are L-isomers. The origin of this homochirality is not clear and is still the subject of debate. However, it is known that L- and D-amino acids may have different biological functions. The role of D-amino acids in the onset and development of a number of diseases is an area of intensive and active research. Therefore, the availability of effective test methods is essential for separation and quantitation of D-amino acids.

Control of Elution Order

The two ZWIX™ chiral selectors are pseudo enantiomers. Therefore, the elution order of amino acid enantiomers can be reversed by using either the ZWIX(+) selector or ZWIX(-) selector. Typically, L-enantiomers elute first from a CHIRALPAK ZWIX(+) column, as shown in Figure 1a. The elution order is reversed by simply switching to a CHIRALPAK ZWIX(-) column (Figure 1b). The control of the elution order is an important feature of these new selectors providing easy impurity identification and quantitation of trace levels.

Figure 1a

CHIRALPAK ZWIX(+)
Quinine-derived (8S, 9R)

trans-4-Hydroxy-DL-Proline

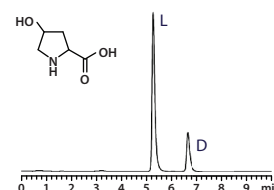
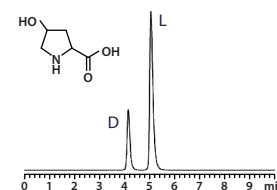


Figure 1b

CHIRALPAK ZWIX(-)
Quinidine-derived (8R, 9S)

trans-4-Hydroxy-DL-Proline



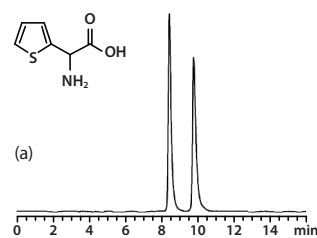
Separation of α -, β -, γ -Amino Acids

Unnatural amino acids, in particular α - and β -amino acids, are important compounds in the area of peptide research. Due to their structural diversity and functional versatility, these amino acids are widely used as chiral blocks and components in drug development processes. Separation examples are provided in Figure 2.

Figure 2

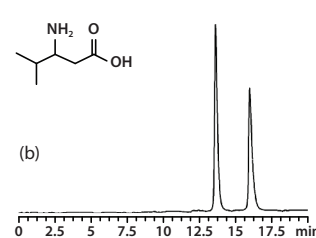
Column: CHIRALPAK ZWIX(+); 3 mm i.d. x 250 mm long | Flow rate: 0.5 mL/min | Detection: ELSD

DL- α -Amino-2-thiopheneacetic acid



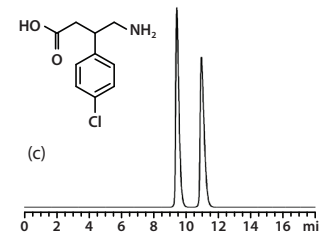
Mobile phase: (a)(b) MeOH/ACN/H₂O 49/49/2; (c) MeOH/HOAc/H₂O 96/2/2

DL- β -Leucine



Additives: 50mM FA + 25mM DEA

Baclofen



Approaches to Method Development

Method development strategies are simple and straightforward. As a protic solvent, methanol is an essential component of mobile phases employed with the zwitterionic CSPs and is typically used in a mixture with acetonitrile or tetrahydrofuran. Adding a low percentage of water is beneficial for developing LC/MS separations. Due to the intramolecular counter ion effect of the chiral selectors, use of acidic and basic additives, such as formic acid and diethylamine, is necessary.

To learn more about ZWIX CSPs, visit www.chiral.fr.