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Method development and optimization on cinchona and chiral sulfonic acid–based zwitterionic stationary phases for enantiomer separations of free amino acids by high-performance liquid chromatography

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ABSTRACT

CHIRALPAK ZWIX(+) and ZWIX(−) are cinchona alkaloid-derived zwitterionic chiral stationary phases (CSPs) containing a chiral sulfonic acid motif which serves as negatively charged interaction site. They are versatile for direct enantiomer resolution of amino acids and many other ampholytic compounds by HPLC. The synergistic double ion-pairing between the zwittrionic chiral selector and the ampholyte is the basis for interaction and chiral recognition mechanisms. ZWIX(+) and ZWIX(−) type CSPs or columns behave pseudo-enantiomerically and provide the feature of reversing enantiomer elution order by column switching. In the current study, extensive experimental work was carried out with the aim of developing schemes for an efficient generic screening and proposing straightforward approaches for method optimization on these ZWIX columns. Various chromatographic parameters were investigated using a large series of diverse amino acids and analogues for the purpose. The role of methanol (MeOH) as the protic solvent in the mobile phase is confirmed to be essential. The presence of water in a low percentage is beneficial for peak shape, resolution, analysis speed, sample solubility and MS detection performance. The involvement of acetonitrile (ACN) or tetrahydrofuran (THF) can help for adjusting retention time and selectivity. Incorporation of a suitable pair of acidic-basic additives at a right ratio in the mobile phase is determinant as well for the double ion-pairing mechanism. 50 mM formic acid + 25 mM diethylamine (or ammonium hydroxide) in MeOH/ACN/H_2O and in MeOH/THF/H_2O at 49:49:2 (by volume) are recommended as the starting mobile phases for method development. Some other parameters are also considered in the proposed scheme to achieve successful enantioselective or stereoselective separation of the ampholytes.

1. Introduction

Direct enantiomer or stereoisomer analysis of chiral amino acids, small peptides and related compounds by HPLC has been a challenge for researchers and process engineers working in the fields of research, development, in-process production monitoring and final product quality control. Despite diverse applications in various areas such as proteomics, biochemical research, drug development, clinical diagnosis and food analysis, the exigency for the analytical method remains common. That is, the method in use should be of high efficiency, reliability and accuracy in addition to the easy control of analysis conditions, sample solubility, the appropriate detection method and a reasonable run-time.

The advancement of chiral analysis of common and uncommon amino acids by HPLC has been directly related to the development of dedicated chiral stationary phases (CSPs) [1,2], accompanied by the understanding of the enantioselective recognition mechanisms. The specificity of enantiomer separations of amino acids lies in the intrinsic ionic properties with opposite charges in a single compound. Such features must be addressed and handled in searching for most appropriate chiral separation methodologies. In the last decades, several types of CSPs based on very different enantiorecognition mechanisms (e.g. ligand-exchange, inclusion complex, ion-exchange or ion-pairing) have been proposed, including notably the dynamically coated or chemically bonded chiral ligands [3–7], coated or immobilized chiral crown ethers [8–12],

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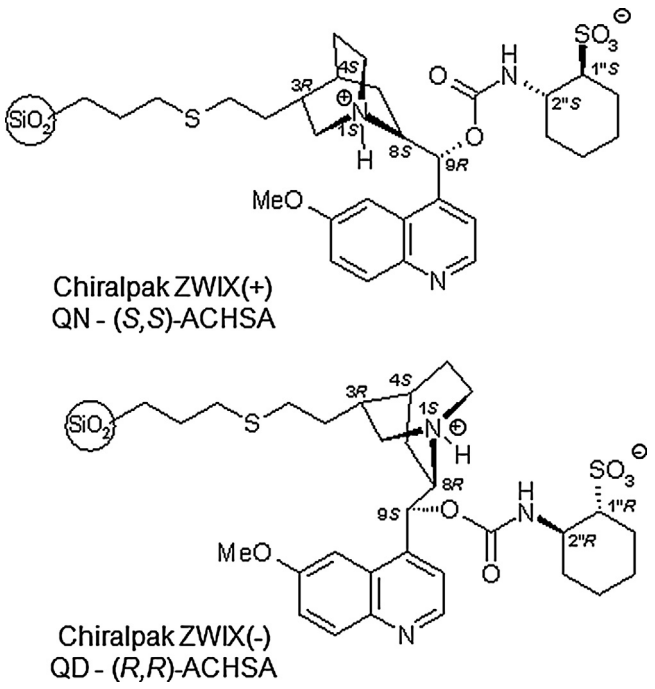

Fig. 1. Structures of the zwitterionic chiral selectors in the study.

macrocyclic glycopeptides-based supports [13–16] and cinchona alkaloid-derived ion-exchangers [17–22]. Among them, the most recently developed zwitterionic CSPs based on cinchona alkaloids and sulfonic acid units, currently under the product names of CHIRALPAK ZWIX(+) and CHIRALPAK ZWIX(−), have emerged as effective chiral supports for direct enantiomer separation of underivatized amino acids and many other ampholytic compounds.

ZWIX(+) and ZWIX(−) columns have in their individual chiral selector structures a cationic and an anionic functional group. They are the products of the chemical fusions between quinine (QN) and (*S,S*)-*trans*-2-aminocyclohexanesulfonic acid (*S,S*-ACHSA) for ZWIX(+) and between quinidine (QD) and (*R,R*)-ACHSA for ZWIX(−), respectively, as depicted in Fig. 1. These two chiral selectors are diastereoisomers, having identical configurations at N1, C3 and C4 but opposite configurations at C8 and C9 in the cinchona scaffold and at C1″ and C2″ in the ACHSA ring. Experimentally, these two selectors behave as pseudo-enantiomers, enabling the reversal of enantiomer elution order in a quasi-systematic way. Both chiral selectors are chemically bonded onto 3 μm silica gel by thiol–ene click chemistry via a mercaptopropyl spacer [18]. The use of such a small particle size as the matrix renders the packed analytical columns high efficiency, befitting the modern LC instruments and benefiting the method performance.

Since the first reporting in 2008 by Lindner and co-workers about the CSPs designed upon the zwitterionic concept [18], the chiral supports based QN-(*S,S*-ACHSA) and QD-(*R,R*-ACHSA) (corresponding to CHIRALPAK ZWIX(+) and ZWIX(−), respectively) have captured great interests in the scientific world for chiral analysis of diverse common and unnatural amino acids by HPLC without precolumn derivatization. Thus far they have been successfully used for direct enantioselective and stereoselective separation of various ampholytic compounds including α-, β- and γ-amino acids, primary and secondary amino acids, aliphatic and aromatic amino acids, cyclic and acyclic amino acids, amino acids with apolar, polar or charged side chains, amino acids containing one or two chiral centers, amino carboxylic acids, amino sulfonic acids, amino phosphonic and phosphinic acids, as well as small peptides [20–28].

In our previous research work carried out with ZWIX(+) and ZWIX(−), we thoroughly investigated their potential performance in chiral separation using a large number of ampholytics with deductive reasoning and confirmation of the zwitterionic stereorecognition mechanism [28]. Another important consideration would be the elucidation on the chromatographic conditions to be used for generic sample screening and for specific separations. In the present study, we focus on the experimental approaches for efficient method development and optimization on ZWIX(+) and ZWIX(−) columns.

2. Experimental

2.1. Chemicals

Mobile phases for chromatography were prepared from HPLC grade solvents. Methanol (MeOH), acetonitrile (ACN), tetrahydrofuran (THF) and water (H_2O) were purchased from Carlo Erba Reagents (Val de Reuil, France). Formic acid (FA), acetic acid (HOAc), diethylamine (DEA), ammonium hydroxide (NH_4OH, 27–28%), formic acid ammonium salt ($HCOONH_4$) and most of the racemic samples or pure enantiomers were supplied by Sigma-Aldrich Chimie S.a.r.l. (Saint Quentin Fallavier, France). Compressed nitrogen (5.0, Messer France SAS, Puteaux France) was used as the nebulizing gas for Evaporative Light Scattering Detector (ELSD).

2.2. Instrumentation and chromatographic conditions

The HPLC system in use was an Agilent apparatus composed of a 1200 series G1322A degasser, a 1100 series G1311A QuatPump, a 1100 series G1313 automatic liquid sampler with a seat assy G1329-87012 of 0.12 mm i.d., a 1200 series G1316A thermostated column compartment, a 1100 series G1315B diode-array detector (DAD). The system had been optimized according to an Agilent Application Note [29] to minimize its void volume. The ELSD (ELSD 2000ES, Alltech, France) was hyphenated to Agilent DAD via an interface 35900E for detection of analytes with no chromophores or being weakly UV absorbing.

The columns used in this study were CHIRALPAK ZWIX(+) and ZWIX(−) (3 μm particle size, 150 × 3 mm, 250 × 3 mm, and 250 × 4 mm i.d.) commercialized by Daicel Corporation (Tokyo, Japan) and manufactured at Chiral Technologies Europe (Illkirch, France). Unless otherwise specified, the flow rate was set at 0.5 mL/min for 3 mm i.d. columns; 1.0 mL/min for 4 mm i.d. columns, the column temperature at 25 °C and the injection volume at 10 μL. The samples were dissolved in MeOH (or in a mixture of MeOH/H_2O (8:2 v/v), if not soluble enough in MeOH) at 0.5 mg/mL. Various mobile phases were examined in the current study. For mobile phase (MP), we mainly varied the composition of the bulk solvents and kept the concentration of additives (50 mM FA + 25 mM DEA) constant: MP-*a* ACN/H_2O 90:10; MP-*b* MeOH/ACN/H2O 49:49:2; MP-*c* MeOH/THF/H2O 49:49:2; MP-*d* MeOH/H2O 98:2. The bulk solvents were always measured by volume. The generic parameters of ELSD were: gas flow, 1.7 L/min; drift tube temperature, 70 °C; gain, 1; impactor, OFF.

3. Results and discussion

In an appropriate mobile phase medium, the chiral selector in ZWIX(+) or ZWIX(−) is in a solvated zwitterionic state bearing a positively charged site at the nitrogen atom of the bicyclic quinuclidine and a negatively charged site at the level of sulfonic acid in the ACHSA ring (see Fig. 1). By the same principle, the ampholytic analyte undergoes a similar ionization process in the same mobile phase system. In these circumstances, strong coulombic attraction