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Enantioselective Separations Based on High-performance Liquid Chromatography

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1. Introduction

Natural compounds can exist in different forms. Those having the same formula but different structure are called isomers. We now know that one form of asymmetry, the phenomenon of chirality, is universal, and at the molecular level chirality is of paramount importance. It is therefore no coincidence that the pharmaceutical industry pays special attention to chiral compounds in the research of biologically active substances. Mapping the different behaviors of enantiomers in drug molecules is of immense importance, while one member of the pair of enantiomers plays a positive role (eutomer) with the expected biological activity, the other enantiomer (distomer) can often cause inefficiency or, at worst, cause undesirable side effects. It is understandable that the US Food and Drug Administration, the FDA, has required pharmaceutical manufacturers to market a racemic product for any drug containing a chiral active ingredient if both enantiomers of the active ingredient were prepared, the pharmacological activity of each enantiomer separately and of the racemate mixture was investigated [1].

One of the important tasks of modern analytical chemistry today is to separate enantiomers of chiral compounds, especially those of biological and / or pharmaceutical importance. High-performance liquid chromatography (HPLC) is the best solution for these conditions, but of course gas chromatography, supercritical fluid chromatography or capillary electrophoresis can often be a good solution.

2. Chiral stationary phases

Chiral stationary phases have undergone very significant development over the last two decades. It can be clearly stated that, after initial difficulties, thanks to continuous improvements, robust, in many cases, multimodal, widely applicable stationary phases are now available which produce well reproducible results. Below we describe the three families of the most frequently employed chiral stationary phases.

2.1. Chiral stationary phases based on macrocyclic antibiotics

The concept of using macrocyclic antibiotics as CSPs was initiated by Armstrong in 1994 [2]. Macrocyclic antibiotics possess several characteristics that allow them to interact with analytes and serve as chiral selectors. There are hundreds of these compounds and, unlike other classes of chiral selectors, they comprise a large variety of structural types. The macrocyclic antibiotics used for chiral separations in HPLC include the ansamycins (rifamycins), the glycopeptides (avoparcin, teicoplanin, ristocetin A, vancomycin and their analogs), and the polypeptide antibiotic thiostrepton. In the past two decades, they have had a rapid and significant impact on the field of enantioseparation. They have unique structural features and functionalities that allow various chiral interactive sites and interactions (i.e. electrostatic, hydrophobic, H-bonding, steric repulsion, dipole stacking, π-π-interactions, etc.) between the analyte and the stationary phase. One of the most characteristic features of the antibiotic-based selectors is their chiral ionic character. The commercially availbale Chirobiotic phases possess analogous ionizable groups which have been proven to play important role in the chiral recognition. Their specific structures and the variety of functionalities give them the power of efficient resolution of almost all types of neutral, acidic and basic racemates [3].

2.2. Chiral stationary phases based on modified polysaccharides

The utilization of the ability of polysaccharides to resolve racemic mixtures dates back to 1951, when Kotake et al. reported the resolution of some amino acids by paper chromatography [4] using cellulose as stationary phase. Among the various polysaccharides, cellulose and amylose have been used for the preparation of commercialized CSPs. The enantiorecognition ability of the polysaccharide-based CSPs is generally assumed to be based on hydrogen bonding and dipole-dipole interactions. For these interactions to take place the presence of water as a strongly competing species should be avoided. Thus applying alkanes (e.g. hexane, heptane) and alcohol (e.g. propan-2-ol) in NP mode can be a good choice as starting mobile phase for a polysaccharide-based CSP [5]. Polysaccharide-based columns recently commercialized can also be applied under reversed phase conditions, but it is important to note that varying the chromatographic modes may result in extended equilibration times upon change of mobile phases and reduction of the observed efficiency.

2.3. Chiral stationary phases based on ion-exchangers

In case of CSPs based on ion-exchange procedure retention relies on ionic interactions forming between ionic solutes and the ionic functional group(s) of the chiral selector. It should be noted that the ionic sites are always solvated, and the size of the solvation shell depends on the type of solvent components. Cinchona alkaloids are one of the most important chiral selectors of ion-exchangers. Over the last years, a number of variants of quinine- and quinidine-based chiral selectros have been developed. Nowadays the most popular ones are the zwitterionic selectors, based on the chemical fusion of a weak positively charged moiety related to the protonated quinuclidine-containing site with a moiety bearing a deprotonated thus negatively charged site related to a sulfonic acid residue. Because of the ampholytic character of the zwitterionic selectors and the somewhat independent property and spatial position of the positively and negatively charged sites, these ampholytic selectors can, in principle, also act individually as chiral anion-exchanger or chiral cation-exchanger. Nonaqueous polar organic solvents (e.g. methanol, acetonitrile) in combination with acid and/or base modifiers proved to be the preferential mobile phases when employing Cinchona alkaloid-based CSPs. The use of methanol as a protic solvent and acetonitrile as a polar, but aprotic solvent (which can strengthen ionic interactions, but interferes with π-π interactions) seems to be the best combination allowing the suppression of nonspecific hydrophobic interactions with enhanced enantioselectivity. Varying the nature and concentration of the organic solvents is the primary choice to tune the overall chromatographic performance [6].

3. Conclusions

For the early 2000's chromatographic methods applying chiral stationary phases became the most effective techniques for the resolution of chiral compounds on both analytical and preparative scales. Nowadays HPLC employing various type of chiral selectors covalently bonded on silica-based supports offers a state-of-the-art methodology for "chiral analysis". Since no universal column exist it is obvious that in practice one needs a good portfolio of different columns to face the challenging task of enantiomeric resolutions.

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