Journal of Chromatography A, 1562 (2018) 128–133

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Short communication

Tunable normal phase enantioselectivity of amino acid esters *via* mobile phase composition

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ARTICLE INFO

Article history:
Received 5 April 2018
Received in revised form 25 May 2018
Accepted 26 May 2018
Available online 28 May 2018

Keywords:
Mobile phase additives
Amino acid ester
Chiral HPLC
Enantiomeric separation
Immobilized polysaccharide-based chiral stationary phase

ABSTRACT

The ability to tune chiral selectivity through mobile phase modifiers is a powerful tool in chiral separations. Beyond improving efficiency and/or resolution, some mobile phase systems can even invert elution order, a highly desirable result for trace analyses or preparative scale isolations. Previous work has demonstrated that acidic modifiers, such as ethanesulfonic acid (ESA), can greatly impact separations of enantiomers. However, prior studies were primarily performed on coated chiral stationary phases (CSPs), which limited the selection of the bulk mobile phase component.

In this work, the effect of ESA modifier was studied for the enantioseparation of six pairs of amino acid esters on a CHIRALPAK® IA column, an immobilized amylose-based CSP, with different combinations of standard solvents (hexane and ethanol) as well as "non-standard" solvents, such as methyl t-butyl ether, ethyl acetate, tetrahydrofuran, acetone, or 1,4-dioxane. ESA generally improved selectivity, and multiple instances of elution order reversal were observed. A Van Deemter plot study reveals that ESA exerts its effect by pulling the enantiomer deeper into the chiral cavity of the chiral polymer to increase the interactions between the analytes and the stationary phase, which is the main reason for the increased enantioselectivity.

1. Introduction

Currently, polysaccharide-based chiral stationary phases (CSPs), developed by Okamoto and co-workers, are the most widely used chiral columns (see Reference [1] for a thorough recent review of CSPs). These types of phases have proven to be among the most useful CSPs because of their versatility and durability, as well as high loadability for preparative scale chromatography. More than dozens of derivatized amylose- or cellulose-based CSPs are commercially available. First generation polysaccharide CSPs are prepared by physically coating the polysaccharide polymer onto a silica support, and second generation CSPs are prepared by immobilizing chiral polymers to the silica surface. A limitation of the coated CSPs is their incompatibility with many common organic solvents because they could be swollen or partially dissolved. Immobilized CSPs are more resistant to solvents such as methyl t-butyl ether (MTBE), ethyl acetate (EtOAc), tetrahydrofuran (THF), acetone, 1,4-dioxane or chlorinated solvents and offer more flexibility for mobile phase optimization even at the preparative scale [2].

The effect of acidic and basic mobile phase additives has been investigated intensively on coated polysaccharide-based CSPs, with significant impacts on retention and selectivity being observed [3–14]. All the published additive investigations were performed using mainly hexane, ethanol and/or isopropanol. Organic eluents with intermediate polarities, such as methyl t-butyl ether, ethyl acetate, tetrahydrofuran, acetone, or 1,4-dioxane, were not studied due to the nature of these coated CSPs. Therefore, the combination effect of acidic and basic additives and "non-standard" solvents remains unknown.

It has been reported that nearly 90% of analytical chiral separations can be performed on four types of coated CSPs (CHIRALPAK® AD, CHIRALPAK OD, CHIRALPAK AS, and CHIRALPAK OJ) [15]. Understanding the role of mobile phase additives when combined with solvents other than alcohols is important to expanding the application of these types of CSPs and will help to provide a detailed understanding of the mechanisms of enantiodiscrimination [12]. CHIRALPAK IA is an immobilized CSP based on a 3,5-dimethylphenylcarbamate derivative of amylose, which is also used to prepare coated CHIRALPAK AD columns. The key roles that bulk mobile phases played and solvent effects on the enantioseparations of a broad variety of chiral compounds on CHIRALPAK IA column were reported previously [15].

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https://doi.org/10.1016/j.chroma.2018.05.054
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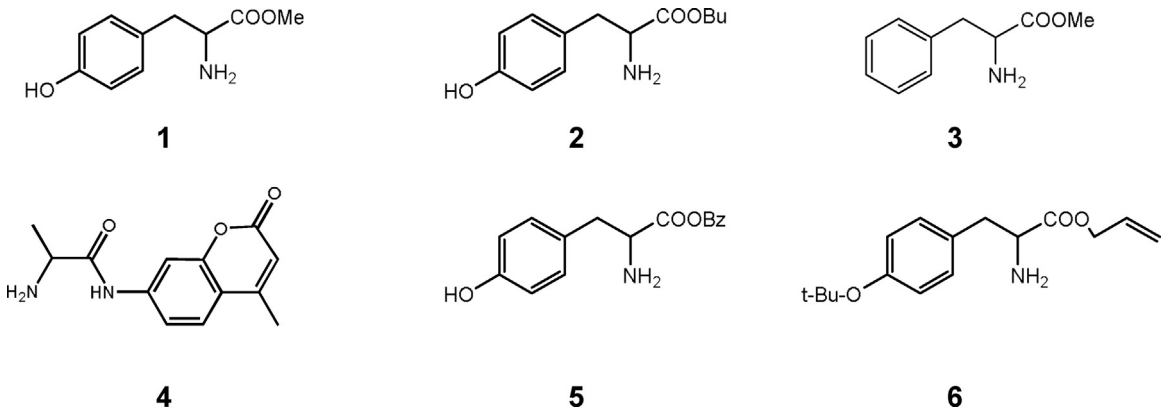

Fig. 1. Structures of the probe molecules studied.

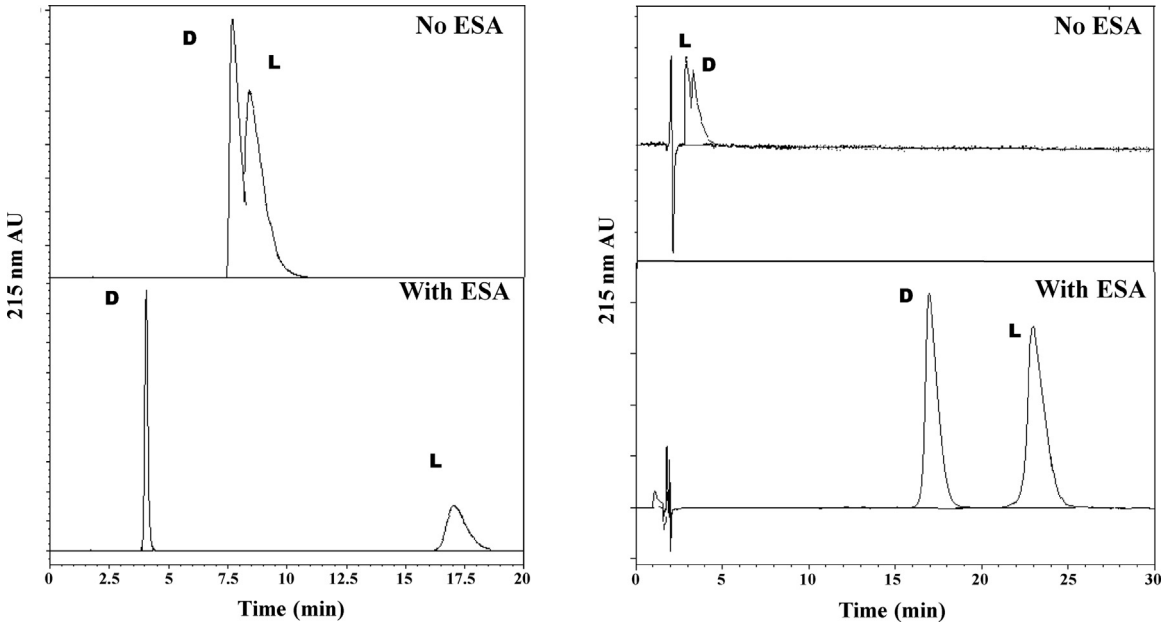

Fig. 2. Typical separations. Left: Compound **1** in (top) hexane: ethanol (85: 15) without ESA and (bottom) in hexane: ethanol (80: 20) with 0.2% ESA. Right: Compound **2** separations obtained in (top) 100% MTBE without ESA and (bottom) in MTBE: THF (60: 40) with 0.2% ESA.

Previous work on coated CSPs has shown that ethanesulfonic acid (ESA), an acidic additive, can have a significant impact on chiral amine enantioseparation using conventional normal phase solvents such as hexane with either ethanol or isopropanol [3–10]. In some cases, more than ten times improvement in selectivity improvement was observed with ESA addition [5]. In this study, the role of ESA in combination with different organic solvents, including ethanol, hexane, MTBE, EtOAc, THF, acetone, 1,4-dioxane, and acetonitrile (ACN) were studied on the CHIRALPAK IA CSP. Six pairs of amino acid esters probe compounds were used to investigate the effects of ESA on enantioseparation in the different organic solvent combinations, many of which cannot be studied on coated polysaccharide-based CSPs. The retention behavior and enantioselectivity of the probe molecules with or without ESA are compared and summarized. The ability to tune enantioselectivity *via* a wide range of compatible solvents with and without modifiers greatly increases the chances for a successful separation.

2. Experimental

All reagents used in this study were reagent grade or better. HPLC grade hexane and isopropanol were purchased from EM Sciences (Gibbstown, NJ). Absolute ethanol was obtained from Aaper Alcohol and Chemical Co. (Shelbyville, KY). All other reagents were obtained from Sigma-Aldrich (St. Louis, MO). The individual enantiomer and racemic compounds were obtained from Sigma-Aldrich (St. Louis, MO) and Bachem (King of Prussia, PA), and used without further purification. The structures of the studied compounds are shown in Fig. 1. Sample solutions were prepared in ~10% ethanol in hexane with a final concentration of ~1 mg/mL.

Chromatographic studies were performed on an HP 1100 liquid chromatograph (Agilent, Palo Alto, CA) equipped with a vacuum degasser, quaternary pump, autosampler, thermostatted-column oven, and a variable-wavelength UV detector. The chromatographic data were acquired and processed with Agilent Chemstation software. The CHIRALPAK IA column (150 × 4.6 mm, 5 μm particle) was purchased from Chiral Technologies, Inc. (Exton, PA) and was used as received. Unless otherwise noted, chromatographic studies were performed at 40 °C with a flow rate of 1.0 mL/min. The mobile phases consisted of combinations of different organic solvents with or without 0.2% ESA, as described in Results and Discussion. Triethylamine (TEA) was used as a basic additive for the hexane/EtOAc and all MTBE-based mobile phases due to poor peak shapes. After column equilibrium had been achieved, 5 μL of the sample solution