Trends in Analytical Chemistry 63 (2014) 55–64

Contents lists available at ScienceDirect

Trends in Analytical Chemistry

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

Hydrophilic interaction chromatography of polar and ionizable compounds by UHPLC

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ARTICLE INFO

Keywords:
Dissolution solvent
HILIC
Hydrophilic interaction chromatography
Matrix effect
Mobile phase
Selectivity
Sensitivity
Stationary phase
Ultra-high performance liquid chromatography
UHPLC

ABSTRACT

In recent years, the popularity of hydrophilic interaction chromatography (HILIC) in the analysis of small polar and ionizable molecules increased substantially. A large number of new stationary phases with sub-2-μm and superficially porous particles were developed and used in various applications, including pharmaceuticals, metabolites, biomarkers, amino acids and peptides. Applications of HILIC in ultra-high performance liquid chromatography (UHPLC) mode constitute only about 10% of all current HILIC applications. This review presents an overview of UHPLC in HILIC mode, including the importance of the final sample diluent, a discussion on available stationary phases and approaches to detection, and a comparison of performance with other chromatographic modes.

Contents

1. Introduction

Hydrophilic interaction chromatography (HILIC) has been shown to be most useful for the analysis of small polar and ionizable molecules that are poorly retained in reversed-phase liquid chromatography (RPLC). In many cases, HILIC provides an alternative, highly mass spectrometry (MS)-compatible approach to normal-phase LC (NPLC), ion-exchange chromatography (IEC) or ion-pairing chromatography (IPC) [1–3].

In HILIC, the primary retention mechanism is believed to be partitioning of the analytes between a water-enriched layer of the stagnant eluent partially immobilized on a hydrophilic stationary phase and the relatively hydrophobic eluent of the bulk mobile phase. However, the mechanism is usually more complex, especially when free silanols or other charged functionalities may participate in the interaction [1,3]. The retention mechanism in HILIC has been discussed elsewhere [4–8]. Besides partitioning, electrostatic interactions and hydrogen bonding can also play important roles,

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http://dx.doi.org/10.1016/j.trac.2014.08.004
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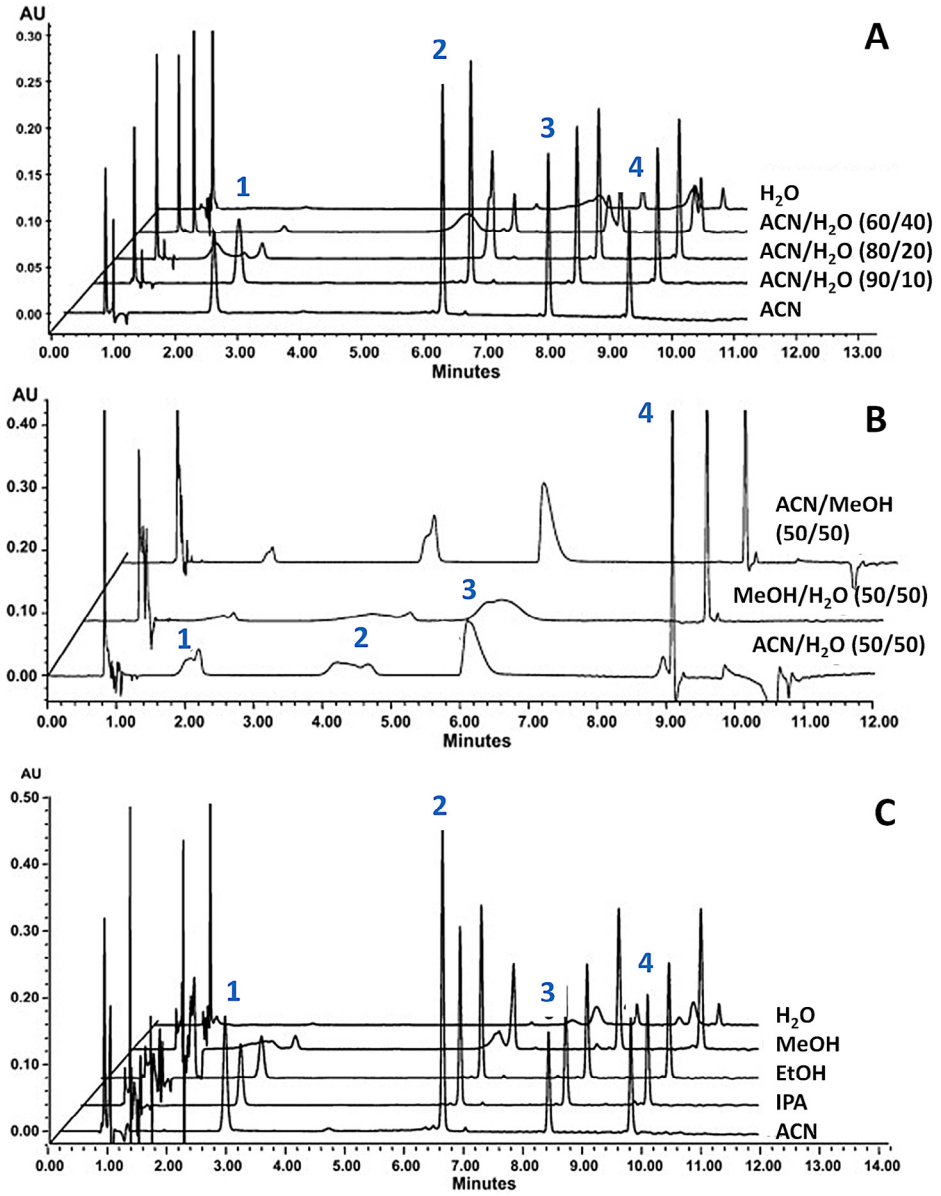

Fig. 1. (A) Effect of the % of water, (B) combination of MeOH, ACN and water in ratio 50/50 and (C) different types of pure organic solvents in the dissolution solvent for the mixture: (1) hypoxanthine (80 μg/mL), (2) cytosine (10 μg/mL), (3) nicotinic acid (30 μg/mL), and (4) procainamide (30 μg/mL). Conditions: column Acquity BEH HILIC (2.1 mm id × 150 mm, 1.7 μm), flow rate = 500 μL/min, λ = 214 nm, volume injected = 5 μL, gradient profile: 95% ACN for 6 min, then 95–75% ACN in 5 min with T = 30°C. {Adapted from [13] with permission}.

depending on the type of stationary phase. The contribution of each interactive mechanism depends on the type of stationary phase, the organic solvent and the aqueous component (pH and ionic strength) in the mobile phase, and the physicochemical properties of the analyte. The stationary phase in HILIC is polar, while the mobile phase is hydrophobic, consisting mostly of an organic solvent (typically acetonitrile, ACN) with 5–40%, of an aqueous component [3,9].

The first commercial UHPLC system introduced in 2004 enabled very fast separations with high efficiencies using columns packed with very small particles. UHPLC separations in the HILIC mode were documented after 2006, after the availability of HILIC stationary phases on with sub-2-μm fully porous particles (FPPs) (bridge-ethyl hybrid sorbent, Waters) and later also on superficially porous particles (SPPs) [10,11]. Because of the lower viscosities of the mostly ACN-based mobile phases, it is generally more feasible to employ columns packed with sub-2-μm or sub-3-μm particles in the HILIC mode, when compared with other modes of chromatography. Using the kinetic plot methods on various HILIC stationary phases, this benefit of higher kinetic performance was experimentally confirmed at working pressures close to 1000 bar [4,12].

This review provides an overview of important operating parameters of HILIC under UHPLC conditions, such as the importance of selection of the final sample diluents, stationary phases, and approaches to detection in comparison with other modes of chromatography. As HILIC operating with UHPLC has been practiced for a short period of time by few researchers, we believe that a review of the principles, selected applications and recent advances would be helpful to other practitioners less familiar with this mode of chromatography.

2. The importance of the sample diluent in HILIC

An important parameter for acceptable peak shapes in HILIC is the right choice of dissolution solvent for the sample (the final