Stationary phases for separation of nucleosides and nucleotides by hydrophilic interaction liquid chromatography

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This review describes recent trends in novel stationary phases for hydrophilic interaction liquid chromatography (HILIC), focusing on separation of nucleosides and nucleotides. The performance of these novel HILIC stationary-phases is discussed in terms of resolution, separation, improved sensitivity and increased speed in order to achieve more efficient, faster chromatographic separations.

Keywords: Column technology; Hydrophilic interaction liquid chromatography (HILIC); Monolith; Nucleobase; Nucleoside; Nucleotide; Resolution; Sensitivity; Separation; Stationary phase

Abbreviations: AAc-co-BA, Acrylic acid-co-n-butylacrylate ester; AAPBA, 3-acrylamidophenylboronic acid; ACN, Acetonitrile; APS, Aminopropylsilica; BACM, 4-aminocyclohexyl)methyl]cyclohexylamine; BVPE, 1,2-bis(p-vinylphenyl)ethane; CMCH, Carboxymethyl chitosan; CNP, Carbon nanoparticle; CTSAP, Click triazole-amino stationary phase; DBN, 1,5-diazabicyclo[4.3.0] non-5-ene; EDMA, Ethylene dimethacrylate; ERLIC, Electrostatic repulsion liquid chromatography; EtOH, Ethanol; $Et(OH)_2$, 1,2-ethanediol; GNP, Gold nanoparticle; HA, Humic acid; HILIC, Hydrophilic interaction liquid chromatography; HMMAA, N-(hydroxymethyl) methacrylamide; HTMA, 2-hydroxyl-3-[4-(hydroxymethyl)-1H-1,2,3-triazol-1-yl]propyl 2-methylacrylate; MAA, Methacrylic acid; ME, 2-mercaptoethanol; MEO, Oxidized 2-mercaptoethanol; MeOH, Methanol; MS, Monolith silica; MSA, [2-(methacryloyloxy)-ethyl]dimethyl-(3-sulfopropyl)ammonium hydroxide; NAHAM, N-acryloyl-tris(hydroxymethyl)-aminomethane; PA, Polyacrylamide; PAA, Polyacrylic acid; PEI, Polyethyleneimine; PETA, Pentaerythritoltriacrylate; PHA, Polyhydroxylethylaspartamide; pNA-SIL, Poly[N-acryoyl-tris(hydroxymethyl)-aminomethane]-coated silica; PSA, Polysulfoethyl; TG, 1-thioglycerol; RAM, Restricted access material; RF, Retention factor; SPE, N,N-dimethyl-N-methacryloxyethyl-N-3(sulfopropyl)-ammonium betaine; TCM, Traditional chinese medicine; TEPIC, Tris(2,3-epoxypropyl) isocyanurate; TGO, Oxidized 1-thioglycerol; ZIC, Zwitterionic interaction chromatography; 6TG, 6-thioguanine

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1. Introduction

In recent years, hydrophilic interaction liquid chromatography (HILIC) has been established as the appropriate chromatographic mode for the retention and the separation of polar and hydrophilic compounds. The combination of the retention of polar compounds and the high organic content in the mobile phase makes this mode of chromatography suited to high-sensitivity, quantitative analysis based on applying liquid chromatography coupled to mass spectrometry (LC-MS).

It has been accepted that partitioning between the aqueous layer associated with the stationary phase and the organic component of the mobile phase is the main retention mechanism. However, the participation of secondary interactions (e.g., dipole-dipole, hydrogen bonding and ion-exchange) can play an important role in separation, leading to changes in selectivity.

As the HILIC mode is becoming more popular for the chromatographic analysis of polar and ionic analytes, a wider range of HILIC stationary phases is available on the market. Novel materials with different types of polar functional groups (including amide, amino, diol, cyano, zwitterionic and other charged and non-charged groups) are continually being developed and assessed as stationary phases under HILIC conditions.

The increasing interest in this chromatographic mode as a viable alternative to reversed-phase LC (RPLC) can be seen

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in recent reviews that have appeared in the literature. In recent years, 26 review articles have been published on the topic, some of them general [1–6]. The applications of HILIC include the analysis of different polar molecules, such as toxic residues – antibiotics [7] and other pharmaceuticals [8] in foods and pesticides in environmental matrices [9]. HILIC also seems to be a promising alternative to more traditional LC methods in bioanalysis. In particular, the analysis of highly polar metabolites, such as nucleosides and nucleobases, involves important difficulties in RPLC, due to the poor retention of these analytes in the stationary phase. Normal-phase LC (NPLC) would afford a better retention but is not desirable, due to the difficulty involved in coupling this chromatographic technique with MS.

Nucleobases, nucleosides and nucleotides are the basic components of all cells, forming the different nucleic acids. Their clinical interest in bioanalysis has expanded considerably in recent decades, so nucleosides are investigated as potential biomarkers of oncological processes, being excreted with or without modifications through the urinary tract [10–13]. Apart from in field of biology, nucleobases, nucleosides and nucleotides are also important in other avenues of enquiry. In foods, monophosphate nucleotides are nutrients of special importance during periods of rapid growth or after injury, hence the importance of the supplementary contribution of such nutrients in neonatal feeding [14–16].

The aim of this article is to discuss current trends and advances in the development and the evaluation of the different approaches recently proposed for new HILIC stationary phases, which may allow selectivity and/or sensitivity to be enhanced, or faster separations to be achieved. It summarizes the information published in more than 75 papers in the literature, mostly in the period 2008–12, with the main focus on recent advances and trends in the HILIC approach for nucleoside and nucleotide analysis.

Also, we consider new trends in column technology (e.g., reduction in particle size down to 1.7 μm, the use of superficially-porous particles, and the use of monolithic columns of different types) as applied to the separation of nucleotides, nucleosides and their bases.

2. Commercial columns

2.1. Unmodified silica

The simplest stationary phase, the first to be used in HILIC, is that formed by unmodified silica (bare silica). The separation mechanism proposed for this stationary phase is based on a partition equilibrium between the aquo-organic phase (usually mixtures of water as the minor component and acetonitrile (ACN) as the major component), and a phase – mainly aqueous – bound to the stationary phase.

The different separations proposed for the compounds studied here have a high percentage of organic components in the mobile phase, which is usual in the stationary phases of bare silica (Table 1). Thus, it has been possible to separate four nucleobases and four nucleosides on Atlantis HILIC Silica, formed by bare silica, in 14 min [17].

The low retention factors (RFs) usually found for these compounds in bare silica columns can be improved by varying the nature of the composition of the mobile phase. Working with an isocratic regime with 90% of ACN, the replacement of the water by $Et(OH)_2$, MeOH and EtOH leads the RFs to increase in the following order: $H_2O < Et(OH)_2 <$ MeOH < EtOH. Thus, an order of elutropic strength of a protic modifier of EtOH < MeOH < $Et(OH)_2 < H_2O$ could be established [18]. This can be explained in terms of the fact that the replacement of water molecules by organic solvent may modify the formation of the semi-stationary layer.

2.2. Hydroxylated stationary phases

The low selectivity and retentions shown by HILIC columns made of bare silica can be improved by introducing functional polar groups. One of the simplest modifications consists of introducing alkyl chains with hydroxyl functional groups. The separation mechanism is based, as in the case of bare silica columns, on partition equilibrium, although the presence of hydroxyl residues allows the appearance of interactions via hydrogen bridges, leading to an increase in selectivity.

Within the columns with hydroxylated stationary phases, Luna-HILIC, formed by a cross-linked diol phase (Fig. 1a), is the one most used for the separation of the compounds of interest, offering a neutral stationary phase that allows a water-rich layer to form readily on the surface [19,20]. For this Luna HILIC column, plots of log k_0 versus log of the percentage of water in the mobile phase resulted in a linear fit in the range 45–55% water in the mobile phase, consistent with an adsorptive mechanism. At around 45% water composition in the mobile phase, a slight curvature of the line was observed, suggesting that a partitioning mechanism was taking hold as the column became saturated. Those authors [19,20] suggested that, during gradient elution at higher amounts of water, the adsorptive mechanism may be more prevalent and the separation will fall under conditions more common in RPLC.

In this phase, the replacement of water by $Et(OH)_2$, MeOH and EtOH has also been studied [18]. The gain in retention upon reducing the polarity of the protic modifier was stronger for nucleobases, although the general trend of increasing retention upon reducing the polarity of the modifier was not so evident for all solutes.

Other columns modified by hydroxylation tested for separation were polyhydroxylethylaspartamide (PHA, Fig. 1b) and Pronto-SIL-DIOL (Fig. 1c). Using PHA, the authors concluded that, in comparison with amine-bonded phases, the functionalization of stationary phases with hydroxy groups resulted in less retentive