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An Overview – Hydrophilic Interaction Liquid Chromatography

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ABSTRACT
Hydrophilic interaction liquid chromatography (HILIC) is an alternate liquid chromatography method for separating hydrophilic and polar ionic substances. The separation process involves the combination of mobile phases from the reverse phase liquid chromatography with stationary phases from the normal phase chromatography. It separates polar analytes by partitioning them into a water rich layer on to the stationary phase. HILIC is effective for a various type of chromatographic techniques which include active pharmaceutical substances, small molecules, metabolites, toxins, carbohydrates, oligosaccharides, peptides, amino acids, and proteins. It involves various processes, including ion exchange interactions. There is a growing demand for HILIC columns, In HILIC mode; chemicals may be separated based on many variables such as column temperature, mobile phase ratio's, pH, concentrations of buffer and its types. This overall review summarizes the recent breakthroughs in stationary phases, mobile phases used and advantages of hydrophilic interaction liquid chromatography.
Keywords: *Hydrophilic interaction liquid chromatography, ion exchange interactions, columns, stationary phase, mobile phase.*

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INTRODUCTION

Hydrophilic interaction liquid chromatography (HILIC) is a term that was first suggested in 1990 by Alpert [1]. He utilized the column for the separation of various peptides, amino acids and polar substances. In liquid chromatography, molecules are separated using differential equilibrium between a mobile phase and a stationary phase, which is determined by their solubility or by the degree of contact with the mobile and stationary phase. Initially analytes interact with the particles in the stationary phase before being eluted if they have a greater contact with the solvent flowing through the chromatographic column than the stationary phase. The optimal characteristics of the solvent and the stationary phase depends upon the analysis performed [2]. The HILIC methodology provides an alternative approach for effectively separating small polar molecules by employing a stationary phase with higher polarity. Hydrophilic interaction liquid chromatography is a combination of polar stationary phases used in normal phase chromatography (NP-HPLC) and a mobile phase that is more appropriate for a reversed phase liquid chromatography (RP-HPLC). Furthermore, it is ideal for highly hydrophilic and amphiphilic analytes that are highly polar to be retained in RP-HPLC, but their charge is adequate to allow the conditions for ion exchange chromatography (IEC). One significant advantage of HILIC it may overcome the disadvantage of many analytes having low water solubility, which is common in NP-HPLC. The most frequent eluent used in HILIC is acetonitrile, which is highly polar in nature. It is illustrated that HILIC is not only restricted to polar compounds and metabolites, but rather comparatively non-polar substances, peptides and biomarkers may also be detected [3]. The HILIC separation technique is gaining popularity because it resolves previously difficult separation problems, such as those of small organic acids, basic drugs, cations, anions and many other neutral and charged compounds. This is the main reason for employing HILIC in the recent times. [6]. HILIC separates small and large hydrophilic and high polar molecules such as carbohydrates, amino acids, peptides, proteins, glycoprotein's, nucleosides, vitamins, phenols, pesticides, toxins and a variety of other hydrophilic metabolites found in food, water, body fluids and tissue extracts. HILIC is becoming a routine technique in proteomic, glycomic and metabolomics research, and it is increasingly contributing in the fields of pharmaceuticals, environmental and food chemistry [7].

STATIONARY PHASE

Approximately at the same time when Alpert introduced the HILIC technique, Huber, et.al; noted that correct selection of suitable adsorbent is the crucial step for the success of so called "solvent-generated" liquid chromatography the category to which basic HILIC mechanism is attributed [8]. The basic types of HILIC columns include plain silica, neutral polar chemically bond, ion exchange and zwitterion stationary phase [9]. The various types of stationary phases are as follows:

Silica gel:

Type A silica, crafted through the precipitation of alkali silicate solutions, stands as a flexible chromatographic material. However, it often carries metal impurities, rendering it unsuitable for HILIC applications. Conversely, type B silica forms spherical particles when silica sols aggregate in the air[10]. They have exceptionally low metal contamination concentrations and are typically stable at intermediate and higher pH values, even pH 9. During the hydrolysis process, a significant fraction of the Si-OH silanol surface groups are replaced by Si-H groups in Type C, the "Hydride silica". Besides traditional silica materials primarily designed for non-aqueous applications, newer alternatives like Atlantis HILIC silica emerged, boasting reduced surface silanol content tailored for use with aqueous-organic mobile phases. These represent perhaps the most prevalent stationary phases in modern HILIC applications. The purity of commercially available silica gel materials varies based on preparation techniques. Consequently, bare silica columns sourced from different manufacturers may yield notable distinctions in retention, efficiency, and peak morphology. Silica hydride material was chemically modified by using low-polarity bonded groups to increase its hydrophobicity, which provides some new selectivity properties for HILIC separations of less polar compounds[12]. Hydrophobic interactions possess the capability to alter retention properties through a mixed-mode mechanism, particularly noticeable at lower concentrations of acetonitrile within the mobile phases. The diamond hydride stationary phase contains 2.5% carbon, designated as CCA. Chemically modified commercially available columns include cholesterol and bidentate C18 silica hydride columns. In their study, Matyska et al.[11] modified the silica hydride surface with undecanoic acid (UDA silica) and compared it with diamond hydride as materials for HILIC separation of nucleotides. They employed gradients of increasing concentrations of ammonium formate buffer in acetonitrile. UDA silica exhibited superior separation selectivity for mono-, di-, and triphosphate nucleotides due to enhanced ionic interactions compared to the diamond hydride stationary phase. Silica hydride columns modified with non-polar moieties show some features of dual reversed phase/normal phase retention mechanism and can be used in highly aqueous mobile phase in RP-HPLC or for separations in aqueous normal phase (HILIC) mode in buffered mobile phases containing more than 50% - 70% acetonitrile [12], unlike the unmodified silica hydride column, which shows low hydrophobic selectivity and retention under RP-HPLC conditions. The research investigates the HILIC separation of eight phenolic acids using both unmodified silica hydride columns and hydrosilated silica columns modified with cholesterol and bidentate C18 ligands. The separation is conducted in a solvent comprising 95% acetonitrile and a 10 mmol L-1 ammonium acetate buffer. While the elution sequence remains consistent, the silica hydride column exhibits higher retention and separation selectivity for vanillic and ferulic acids under identical HILIC conditions. Conversely, the hydrosilated column with bidentate C18 ligands demonstrates inferior retention and selectivity. Columns packed with completely porous sub - 2 m silica silica gel particles, or superficially porous particles of 2.7 m size, enable for faster and more effective HILIC separations of a larger number of sample compounds than silica columns filled with porous 5 m size[15]. Due to the low viscosity of acetonitrile-rich mobile phases, bare silica columns packed with sub-2 m particles can be operated at regular HPLC pressures and flow rates comparable to those used in UPLC on reversed phase columns. This allows for fast (<1 min) separations and quantification of strongly and moderately polar compounds.

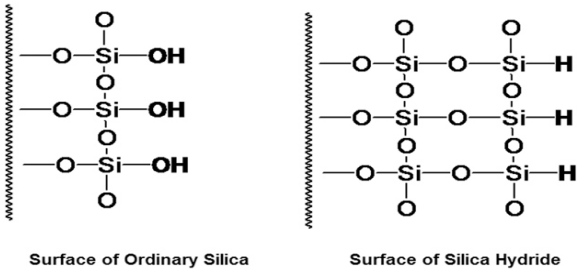

Figure 1: Silica Hydride

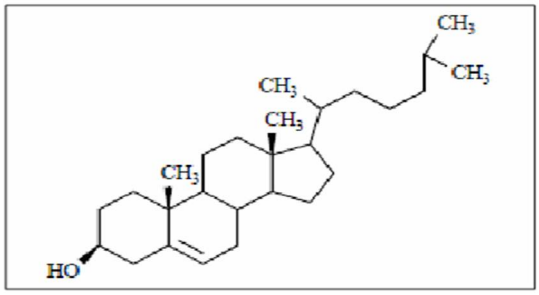

Figure 2: Cholesterol hydride

Polar chemically bonded stationary phase:
Silica-based polar bonded phases for HILIC separations are typically generated by chemically altering the silica gel surface using trialkoxysilanes containing polar and alkyl groups. Moderately polar stationary phases with chemically bonded cyano-, diol-, amino-, cyclodextrin-, polyethylene glycol (PEG), pentafluorophenylpropyl, alkyls with embedded amide or carbamate groups, and some other functionalities were originally designed for reversed-phase applications in water-rich mobile phases. In general, the retention of polar analytes tends to increase in the following sequence: cyanopropyl < diol < aminopropyl << silica stationary phases. However, the selectivity of separation can be significantly influenced by selective interactions. This illustration showcases the structures of several silica-based stationary phases employed in HILIC separations.

Amide Stationary phase:
Amide stationary phases have a carbamoyl or amide group linked to the silica gel surface via a short alkyl spacer and, unlike amino-silica columns, do not have basic characteristics, hence the retention of ionizable analytes is not significantly impacted by ion-exchange interactions. Irreversible sample adsorption is less likely to occur, resulting in greater stability over time. Amide columns often operate effectively without the need for ionic mobile phases, resulting in lesser delivery of salts into a mass spectrometer, thus avoiding potential hindrances to the sample ionization process. Primarily employed for peptide separation, amide stationary phases demonstrate stronger retention for hydrophilic peptides compared to hydrophobic ones. Additionally, utilizing HILIC on amide silica columns proves to be an efficient technique for separating various hydrophilic or amphiphilic polymer materials, including oligosaccharides, glycoprotein's and glycosides.

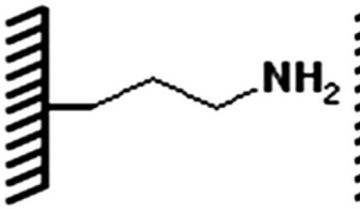

Figure 3: Amino group linked to silica gel surface

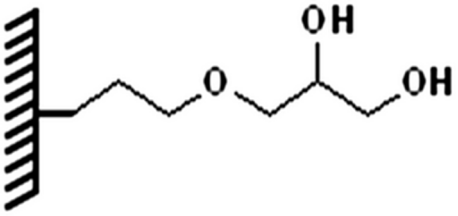

Figure 4: Diol group linked to silica gel surface

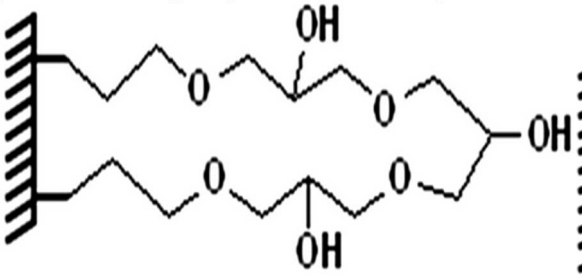

Figure 5: Cross-linked group linked to silica gel

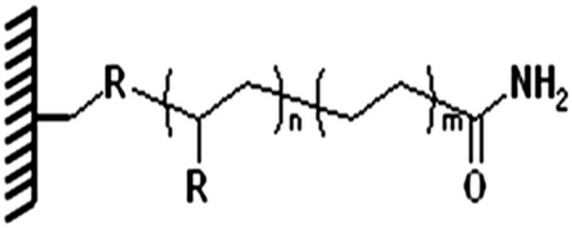

Figure 6: TSK-Gel Amide-80 linked to silica gel

Diol, Polyethylene glycol and Cyano-propyl Stationary Phases:
Chemically bonded diol phases usually contain neutral hydrophilic 2,3-dihydroxypropyl ligands and are prepared by bonding glycidoxypropyltrimethoxy silane to the silica gel surface, followed by hydrolysis of epoxy groups. These phases, like bare silica, exhibit strong polarity and hydrogen bonding characteristics and lack ionizable groups other than non-reacted leftover silanols, which can be partly blocked by a silylating reagent to avoid irreversible adsorption of polar substances. Diol silica columns have found application in protein HILIC separations, as well as in the separation of low molecular weight phenolic compounds using both low organic solvent RPLC and high organic solvent HILIC modes. These columns consist of a stationary phase composed of undecyl-1,2-diol ligands attached to silica gel, featuring a long hydrophobic alkyl chain terminated with polar diol functionality[20]. This design enables a dual retention mechanism encompassing both RP and HILIC interactions. Typically, such columns can be utilized in both RP LC mode employing low-organic mobile phases and HILIC mode with elevated organic solvent concentrations in acidic conditions, diol columns may undergo gradual release of the bonded phase. These columns excel in resolving anomers and other cyclic forms of monosaccharides, a capability lacking in aminopropyl-silica phases. This feature allows for the observation of the transformation rate of individual forms. For the quantitative analysis of polar active pharmaceutical ingredients in drug formulations, HILIC on a diol column using aqueous acetonitrile with 10 mmol L−1 ammonium chloride as the mobile phase has been recommended. This separation mode complements gradient reversed-phase chromatography on a C18 column.

Zwitterionic stationary phases:

Stationary phases with zwitterionic functionalities were originally intended for ion-exchange separations. Irgum's group developed sufoalkylbetaine stationary phases for the HILIC separation of inorganic salts, small organic ionic molecules, and proteins. The active layer attached to wide-pore silica gel or polymer support contains both strongly acidic sulfonic acid groups and strongly basic quaternary ammonium groups, separated by a brief alkyl spacer. This configuration enables the simultaneous separation of anionic and cationic chemicals. The equal presence of these oppositely charged groups in a 1:1 molar ratio results in a bonded layer with a markedly low net negative surface charge, primarily attributed to the greater distance of the sulfonic groups from the silica gel surface. The leftover silanol groups are well insulated by the self-association of functional groups with opposing charges. Water is significantly adsorbed by the sulfoalkylbetaine-bonded phases. Polar (hydrogen-bonding and dipole–dipole) interactions in the stationary phase are of primary importance, even though weak electrostatic interactions affect the separation of analytes carrying either positive or negative charges. Zwitterionic columns are marketed under the trademarks ZIC-HILIC (silica gel support) and ZIC-pHILIC (polymer support). Zwitterionic materials have considerably different chromatographic characteristics than other HILIC phases. ZIC-HILIC columns are commonly used to separate tiny polar molecules, metabolomes, glucosinolates, aminoglycosides, peptides, glycopeptides, and other substances. The HILIC separation of peptides on ZIC-HILIC columns is substantially pH dependent. At pH 3, it is comparable to separations on strong cation exchangers (SCX). In compared to SCX separations, it exhibits greater chromatographic resolution at higher pH (7-8), particularly for prominent +2 and +3 charged peptides. The highest resolution was obtained at pH 6.8, although the orthogonality versus a C18 phase was improved at pH 3HILIC was coupled with mass spectrometry using electrospray ionization (ESI-MS) on a capillary zwitterionic ZIC-HILIC column in a single run, enabling the detection and identification of over 100 N-glycopeptides and O-glycopeptides.

Other Types of Silica-Based Chemically Bonded Stationary Phases:

Several different polar silica-based stationary phases were employed for separations under HILIC conditions. Cosmosil HILIC, a basic column having bound 1,2,4-triazol groups, has been shown to separate water soluble vitamins, carboxylic acids, amino acids, peptides, and polar medicines with excellent efficiency. Fluorinated silica-bonded stationary phases can be used for HILIC separations of aromatic amines, halogenated chemicals, and other polar aromatics.2-mercaptoethanol and 1-thioglycerol groups were bonded to vinylised silica, followed by on-phase oxidation with excess hydrogen peroxide in an aqueous solution to produce 2-mercaptoethanol and 1-thioglycerol packing's that can be employed in both the HILIC and RP modes. The separation selectivity, along with the elution order, of various sample types such as vitamins, nucleosides, and bases on these columns exhibited significant differences compared to the diol bonded phase.

Table 1: Column Manufacturers

MANUFACTURER	NAME	PORE SIZE	MODIFICATION	pH-RANGE	SURFACE	HILIC-COLUMN TYPE
ACE	HILIC N	100A	Polyhydroxy	2.0-7.0	Neutral	1
GL Sciences	Intersil HILIC	100A	Diol	2.0-7.5	neutral	1
AMT Halo	Penta hydroxy	90 A	Pentahydrooxy	2.0-9.0	neutral	1
Kromasil	HILIC-D	60 A	Diol	2.0-8.0	Neutral	1
Chromanik	Sunshell HILIC-Amide	90 A	Amide+hydrophilic group	2.0-8.0	neutral	1
Merck	Sequant	100 A,200A	sulfobentaine	2.0-8.0	neutral	1
Tosoh Bioscience	TSK Gel Amide-80	100 A	Carbamoyl	2.0-7.5	neutral	1/3

Table 1 shows the different types of column manufacturers and their modifications with respective to pore size, pH range and surface.

MOBILE PHASE

Once an appropriate stationary phase and column dimension have been determined, the mobile phase composition should be modified. HILIC on polar stationary phases uses organic-rich mobile phases, which typically comprise 5-40% water or a buffer (usually volatile ammonium formate or ammonium acetate if used in combination with MS or charged aerosol detection, CAD). In order to ensure that all sample chemicals are eluted, it is advised that first tests be performed at a reasonably high concentration (for example, 40%) of aqueous buffer in acetonitrile. The elution intensity is regulated by incrementally increasing the acetonitrile concentration until satisfactory sample retention is achieved. Alternatively, a scouting gradient involving a decrease in acetonitrile concentration can be employed. Successful HILIC

separations of diverse biological samples, particularly with bare silica columns, have been achieved using gradients starting with a high initial concentration of organic modifier and progressively increasing water content. Typically, the gradient initiates with 95% acetonitrile mixed with 5% aqueous ammonium acetate or ammonium formate buffer, with a gradual decrease in acetonitrile content. Running the gradient with a high-water concentration (up to 90%) may assist in eliminating sample interferences that are highly retained. Mixed aqueous-organic mobile water is highly adsorbed on the surfaces of polar adsorbents or bonded phases, whether charged or uncharged. In mobile phases with more than a few hundred ppm of water, preferential adsorption results in the creation of a water-rich adsorbed diffuse liquid multilayer. In aqueous-organic NP having more than 0.5-1% water, the layer of adsorbed water is thick enough to cause liquid-liquid partition between the bulk mobile phase and the adsorbed aqueous liquid layer. However, sample adsorption on polar surface centers may have a substantial role in retention. The choice of organic solvent has a significant impact on HILIC retention. The elution strength of organic solvents in the HILIC mode usually rises with increased solvent polarity and capacity to engage in proton-donor/proton-acceptor interactions.

Methanol > Ethanol > 2-Propanol > Tetrahydrofuran > Acetonitrile

Acetonitrile is the most commonly used organic solvent in HILIC applications, as mobile phases containing other solvents frequently give insufficient sample retention and wide or non-symmetrical peak morphologies. This is especially true for methanol, which is rarely Methanol's effectiveness may be attributed to its similarities to water: both methanol and water are protic solvents that compete to solvate the surface of silica or other polar stationary phases and offer strong hydrogen bonding interactions with one another. This is true to a lesser extent for other alcohols such as ethanol, 2-propanol, and so on, but not for acetonitrile, which lacks proton-donor interactions and hence produces greater disparities with regard to the aqueous-rich liquid stationary phase occluded on the surface of polar adsorbents. Several attempts to replace acetonitrile with a less hazardous solvent have been documented, but these efforts have been largely unsuccessful, with the exception of a few situations where acetonitrile can be substituted with tetrahydrofuran. Acetone has a comparable polarity to acetonitrile, but has lesser retention under HILIC conditions and considerable selectivity variations; also, it absorbs in the UV region and produces lower intensity MS signals, hence it is not suggested as a straight substitute for acetonitrile. The introduction of carbon dioxide into the acetonitrile–water and ethanol–water mobile phases influences the retention and separation selectivity of nucleic bases, cortisol, flurbiprofen, theophylline, and caffeine in HILIC on bare silica. It has been observed that at elevated concentrations of carbon dioxide in ethanol, separations resembling those achieved with acetonitrile-water mobile phases have been reported. During separation, it's crucial for weak acids and weak bases to be present in either ionized or non-ionic forms; the presence of mixed forms can lead to band broadening, irregular peak shape, or even peak splitting. In reversed-phase LC, ionization is often minimized because maintaining ionic forms is rare. HILIC mobile phases typically incorporate a buffer, where the pH and ionic strength significantly influence the retention and separation selectivity of ionizable substances. Unlike RP LC, the buffer's pH in HILIC can be adjusted to facilitate the ionization of sample molecules (pH > 7 for acids and pH < 7 for bases), especially when the HILIC stationary phases contain ionic functionalities. While pH-dependent changes in selectivity can be exploited in HILIC, the differences in retention between high and low pH are generally not as pronounced as in reversed-phase HPLC, owing to reduced sample ionization at high acetonitrile concentrations. In HILIC systems, retention on non-ionic polar stationary phases typically rises with escalating salt (buffer) concentrations, owing to heightened hydrogen-bonding interactions between the analyte and the stationary phase. This is facilitated by an increase in the population of solvated salt ions in the mobile phase (known as the salting-out effect). However, an increase in ionic strength might diminish HILIC retention in systems utilizing bonded amino phases and stationary phases with ionic functionalities. This occurs because salt counter ions compete with sample ions for oppositely charged sites in the stationary phase via ion-exchange interactions, displacing ionized sample molecules from the stationary phase. In HILIC, tailing peaks are more common compared to RP operation due to the quicker overloading of HILIC columns. Enhancing the peak shape can be achieved by increasing the ionic strength, with some samples necessitating buffers as concentrated as 100 mmol L−1. The inclusion of trifluoroacetic acid (TFA) in the mobile phase enhances the peak shapes of basic compounds on silica-based columns by forming ion associates. However, it's important to note that high buffer concentrations or the presence of TFA are not recommended in HILIC/MS applications, as they may hinder electrospray ionization.. TFA should be avoided whenever feasible in favor of formic acid. As with non-aqueous NP, retention in the HILIC mode rises proportionately with solute polarity and is greater on more polar stationary phases and less polar mobile phases. Aside from selecting an appropriate stationary phase, the percentage of organic solvent in the mobile phase is the most important component in determining separation results. Augmenting the proportion of organic solvent (acetonitrile) in the mobile phase enhances the retention of polar compounds across various stationary phases. Adjusting the water

content (or aqueous buffer) in the mobile phase could lead to alterations in separation selectivity and potentially elution order.

DETECTION

HILIC varies from standard reversed-phase chromatography in terms of detectors employed. Mass spectrometry is the optimum detector for HILIC, whereas UV-Vis is less suitable than in reversed-phase mode. Finally, universal detectors such as the refractive index detector (RI) and evaporative light scattering detector (ELSD) are more popular in polar studies.

UV-Visible Detection:

Sugars, amino acids, and inorganic ions are all examples of polar molecules that lack chromophores. This is because many UV-active groups (phenyl rings, esters, amides, and C=C bonds) are nonpolar, whereas polar groups (-OH, -NH2, and C-O-C) have absorbances that overlap with common buffers and solvents. Nonetheless, UV remains a low-cost and sensitive choice for molecules containing a polar group with a high UV sensitivity, such as organic acids.

MS Detection:

Mass spectrometry is an effective approach for detecting polar analytes due to its high ionization efficiency and sensitivity. Furthermore, HILIC functions at high organic concentrations and handles volatile buffers well, making electrospray ionization (ESI) extremely efficient.

Evaporative Light Scattering Detection:

Evaporative light scattering detectors provide high sensitivity and responsiveness for nonvolatile substances including sugars, metals, and big molecules. ELSD, like MS, requires volatile buffers but may sustain larger buffer concentrations and the demanding circumstances of sugar analysis (high pH or high temperature). Volatile chemicals, such as tiny organic acids or amines, are difficult to test with ELSD and may necessitate an alternate detector.

Refractive Index Detection:

Refractive index (RI) detectors can detect almost any substance; however, they are less sensitive and do not support gradient elution. RI tolerates high buffer concentrations and can identify volatile substances that would otherwise be missed on an ELSD. This makes them the ideal detector for mixes containing volatile substances like alcohols or small organic acids.

Other Detection Techniques:

Polar analytes are also tested using additional detection technologies that Agilent does not presently offer. These include conductivity detectors for inorganic ions and pulsed amperometric detectors (PAD) for sugars and amines. • Charged aerosol detector (CAD) for universal detection, comparable to ELSD.

ADVANTAGES OF HILIC

In view of the changes in solute elution order and the high retention of hydrophilic species, HILIC might be considered a supplementary approach to reversed-phase HPLC. Additionally, it offers the following advantages:

- When combined with mass spectrometry (MS), the detection sensitivity improves. The high organic content of the mobile phase in HILIC enables for effective spraying and desolvation in electrospray MS; a sensitivity enhancement of up to three orders of magnitude was reported for the study of the bronchodilator salbutamol in comparison with reversed-phase HPLC-MS.
- Extracts may be injected straight from C18 solid-phase extraction (SPE) columns, which typically elute solutes with high organic content. The use of a different HPLC mechanism than that used for sample preparation brings some orthogonality into the whole approach.
- Considering the low viscosity of mobile phases with high organic content, higher flow rates may be employed; the viscosity of 90% acetonitrile at 20 °C is 0.43 cP, which is almost half the 0.90-0.86 cP for 10- 30% acetonitrile, which is generally used for separation of hydrophilic solutes by reversed-phase HPLC. Furthermore, solute diffusivity is enhanced in HILIC mobile phases. Thus, at high flow rate, considerably larger efficiencies may be attained compared to reversed-phase HPLC because the C term area of the van Deemter plots is flatter.
- Alternatively, low-viscosity mobile phases enable the employment of columns that are roughly twice as long as identical reversed-phase columns while retaining a comparable back pressure. Commercially accessible silica phases with tiny particle size (dp = 2.7 µm, thickness of porous shell 0.5 µm) include Halo (Advanced Materials Technology, Wilmington, Delaware). C18 particles have reached very high efficiency while maintaining minimal backpressure; this advantage can be paired with the low viscosity of HILIC mobile phases.

APPLICATIONS

HILIC columns work effectively for the separation of hydrophilic and polar molecules even in complex mixtures, and they have been employed in a variety of applications including pharmaceuticals, food and beverage, biological, and environmental. Some examples of applications are shown below:

In formulation analysis: A HILIC technique was developed using bare silica gel to detect dextromethorphan, pseudoephedrine, and diphenylhydramine in a cough formulation. The mobile phase was a combination of methanol and an ammonium-triethylamine buffer. All three components were separated, and the approach was verified for quantitative evaluation [32].

Analysis of drug impurities: Drug impurities are trace quantities of chemicals that can be formed by degrading the primary API or manufacturing contaminants. The HILIC approach works effectively for detecting and separating contaminants. Al-Tannak isolated oxprenolol and its impurities using a cyanopropyl column used in HILIC mode, and the identities of the impurities were validated using mass spectrometry [33].

Bioanalysis and Drug Metabolism: Grumbatch *et. al* [34] found that HILIC provides an excellent approach for separating numerous polar chemicals, including choline and acetylcholine, by employing bare silica as an HILIC phase. HILIC has also been used to conduct pharmacokinetic research. For example, Hsieh identified nicotinic acid molecules in their metabolites. The approach was developed utilizing a bare silica HILIC column connected to a mass spectrophotometer.

Many antibiotics are polar which makes them difficult to separate by using reversed phase chromatography. Oertel *et.al* [56] developed a method for the separation and analysis of neomycin in human plasma using mass spectrometry as the detection method. Neomycin is a very polar compound with at least six amino groups in its structure and a HILIC method was developed for its analysis based on a ZIC-HILIC column [35].

Miscellaneous Applications: HILIC is potentially useful for pharmaceutical development and drug discovery. For example Koh et al [37] developed a method for the separation of polar compounds such as dencichine, which is a hemostatic agent, from a traditional Chinese medicine.

They also developed an HILIC technique for determining a neurotoxic chemical found in Lathyrus sativus seed, often known as grass pea. The procedures were carried out utilizing unmodified silica in HILIC mode with mass spectrophotometry detection; they were extensively verified. HILIC can be used to separate polar molecules in food and drink. For example, in parmesan cheese Schlichtherle-Cerny et.,al[36] showed unique substances including glutamic acid, arginine and many polar dipeptides by separation on an aminopropyl stationary phase under HILIC conditions and with detection by mass spectrometry. A carbohydrate rich drink was analysed by using HILIC for the separation and detection of methionine and taurine. The method was very simple without the need for an extraction method other than a dilution process. The method showed very good precision, accuracy, linearity and low limit of detection [37] Many toxins are very polar and produced within living cells in environment [38].

CONCLUSION

Hydrophilic interaction liquid chromatography, an additional liquid chromatography mode, is particularly useful for distinguishing hydrophilic and polar ionic chemicals. Hydrophilic interaction liquid chromatography has several advantages over traditional liquid chromatography techniques, including a green and water-soluble mobile phase that enhances the solubility of hydrophilic and polar ionic compounds, no need for ion-pairing reagents, and high organic solvent content for mass spectrometry detection. Additionally, it has high orthogonality to reverse-phase liquid chromatography. As a result, hydrophilic interaction liquid chromatography has advanced fast in a variety of applications during the last few decades. HILIC is used in a variety of biomedical applications, including chemical engineering, biochemistry, pharmacology, agriculture, and the food industry. It can analyze polar components in plant extracts, such as phenolic acids, carbohydrates, peptides, and flavonoids, to determine protein function and stability.

CONFLICT OF INTEREST

The authors whose names are listed above declared no Conflict of Interest.

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