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A review of Recent Advances in The Estimation of Pharmaceutical Products Using Hydrophilic Interaction Chromatography (HILIC) Technology

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Abstract

Liquid Chromatography as a separation tool has made significant strides in a systematic study. The most widely used chromatographic process is reverse-phase liquid chromatography (RPLC), which uses polar mobile and hydrophobic stationary phases. However, specific polar composites are challenging to study using this approach. Normal-phase liquid chromatography (NPLC) is another separation process that uses polar stationary phase and organic eluents. When analyzing polar compounds, NPLC produces asymmetric chromatographic peak forms and low-efficiency separations. HILIC is a promising alternative approach for polar compound analysis. HILIC is a separation process that blends the stationary phases of the NPLC method with the mobile phase of the RPLC technique. Hydrophilic interaction liquid chromatography (HILIC) was used to separate small molecules, medicinal compounds, metabolites, contaminants, sugars, peptides, oligosaccharides, proteins, and amino acids, among other liquid chromatographic separations. Tiny polar composites can be efficiently separated on polar stationary phases using HILIC. The target of the study was to look at the different ways to characterize HILIC stationary phases and how they can be used to separate polar compounds in complex matrices. Since processes other than hydrophilic partitioning may exist, the features of the hydrophilic stationary phase may influence in some situations, restricting the selections of ion intensity, mobile phase construction, and buffer pH value available. Increasing our knowledge of HILIC retention activity broadens the range of liquid chromatography applications. HILIC systems bio uses are also discussed. This paper gives a broad description of how HILIC has been used in pharmaceutical testing in various sample matrices, including plasma, pharmaceutical dosage forms, environmental samples, serum, plant origin samples, and animal origin samples. This study also reflects on the most modern and a selection of papers in pharmaceutical science from 1991 to the suggestion deadline in 2020, which deal with examining various mechanisms using HILIC.

Keywords: Pharmaceutical products, zwitterionic phases, HILIC stationary phases, mobile phase

1. Introduction

In 1990, Alpert coined the phrase "hydrophilic interaction chromatography." He omitted the abbreviation HIC to evade the mistake with a method of hydrophobic contact chromatography. The above is an extension of the reverse-phase (RP) process, in which large biomolecules are eluted from the stationary phase using decreasing salt concentrations [1]. However, it's conceivable that "HILIC" periods back to the early times of LC, when Synge & Martin isolated amino acids using H_2O drenched $CHCl_3$ as the mobile phase on a silica column. According to these authors, the separation mechanism separates solute between $CHCl_3$ and H_2O layer retained on the column surface [2]. HILIC is a method that is becoming more common for hydrophilic, isolating ionizable and compounds polar that are tough to

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extract using reversed-phase chromatography due to deprived preservation. HILIC usually employs a stationary phase, such as bare silica, a polar bonded phase, as well as an solvent containing at minimum 2.5 % water and more 60% organic mobile phase, such as ,ethanol, methanol and acetonitrile (ACN) [1]. According to the Web of Information, the quantity of journals on HILIC among 1990 (when the term was first used) and 2017 using the search terms "HILIC". In the early 2000s, the HILIC approach gained widespread adoption (Fig.1).

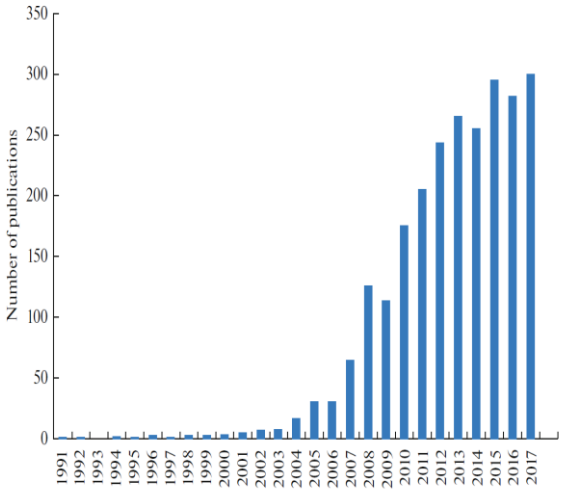

Figure 1: Permitting to Scopus (search through term mixtures "HILIC" and "hydrophilic liquid chromatography"), the number of publications in HILIC increased from 1991 to 2017[3-9].

About the fact that HILIC has exceptional retention properties for hydrophilic materials, the increased interest represents the benefits of HILIC over reverse-phase in circumstances where both techniques are acceptable. The high organic content of traditional mobile phases and the high volatility and low viscosity account for many advantages. The mobile phase is further quickly desolvated in identical electrospray interfaces using HILIC and mass spectrometry (MS), resulting in higher sensitivity than RP approaches. Coworkers and Grumbach discovered sensitivity upturns of 3 to 4 orders of greatness when compared HILIC's study of the drugs bamethan and salbutamol on a bare silica column by a gradient investigation beginning at ninety percent acetonitrile (ACN) with that on a C18 reverse-phase column by a gradient starting at zero percent acetonitrile (ACN) [5]. The viscosity of eighty to ninety percent acetonitrile (ACN) mixtures with H_2O, as used in HILIC, is just around half that of twenty to thirty percent acetonitrile (ACN) mixtures used in reverse phase separation [6].

On the other hand, Lengthier columns can be used to achieve high efficiencies at pressures similar to those used in RP analysis [7]. Low viscosity in the mobile process facilitates solute diffusion, resulting in increased mass transfer and broader van Deemter C terms and the ability to run columns in height flow rates with more minor performance sufferers for accelerated testing [8]. Lindon and Lawhead used a micro-Bondapak carbohydrate column to isolate sugars such as glucose, fructose, melibiose, raffinose, and sucrose (a 10 m particle scale amino silica column) [9]. HILIC separations have become increasingly common in recent years, especially in the last 14 years. When it comes to distinguishing polar, hydrophilic, or ionized molecules, HILIC outperforms RP-LC. Though the method is not as recognized as reverse-phase liquid chromatography, it is gaining popularity as researchers understand how these separations work.

2. Mechanisms of Separation in HILIC

"HILIC" is a standard method for isolating hydrophilic, ionizable, and polar compounds that are difficult to extract using reversed-phase chromatography owing to their poor preservation. HILIC usually employs a stationary phase, such as bare silica or a polar fused phase, and eluent. To separation organic amines on a silica column, Bidlingmeyer and colleagues used "reversed-phase eluents," which were usually acetonitrile–H_2O (60:40, v/v) comprising ammonium phosphate buffer, pH 7.5 [10]. They discovered that cumulative salt concentration reduced the retention of ionized basic composites, implying that ionic retention is essential in the overall process. Flanagan and Jane's previous work on bare SiO_2 columns displayed the separation of basic preparations, but this period with non-aqueous ionic eluents [9,10]. Nearby and colleagues isolated a diversity of basic analytes on bare silica columns with variable metallic contented by buffered acetonitrile mobile phases and methanol with minor organic concentrations (usually 20–40%) [12]. Cox and Stout looked at how nitrogenous bases like thiamine and morphine were retained [13]. In general, HILIC theoretical models were used to gain information on leading developments in the retention estimate, retention method, and quantitative

descriptions of the effect of mobile phase associated influences on analyte selectivity and retention on various forms of stationary phases [14]. Much before the term was invented, the HILIC mode of isolation was used to distinguish amino acids, sugars, organic amines, essential medicines, and nitrogenous bases [15]. In the biological, clinical, and medicinal fields, HILIC has been extensively used. Tiny molecules, peptides, and even intact proteins can be separated using this method [16]. In 2019, Liu and colleagues developed the 2-D RPLC / HILIC method it provides a solution to purify polar composites from Caulis Multifloral polygon. This technique talked about the problem of poor retention. It showed the orthogonality of the superior separation [17]. Via the submission of pulsated elution variation technique, the planned RPLC × HILIC method permits the chromatographic separation to be improved autonomously in together dimensions [18]. A new strategy focused on the synergistic influence of their precursor and carbon dots was introduced to increase the selectivity of HILIC [19]. In hydrophilic contact chromatography, the H_2O rich liquid coating powerless on the surface of the polar phases is essential for polar compound preservation HILIC [20]. In the isolation of glycopeptide enrichment and hydrophilic compounds, HILIC has intrinsic advantages. Traditional HILIC has an unbiased binding with hydrophilic solutes, resulting in poor glycopeptide enrichment selectivity [21]. Since hydrophilic interaction chromatography incorporates characteristics of normal phase (polar stationary phase), reversed-phase (polar mobile phase), and ion-exchange chromatography, the procedure of analyte separation in HILIC is more diverse than in RP-HPLC.

3. Mobile Phase for HILIC

The organic mobile phase used influences the selectivity of analyte separation in HILIC. The value of pH has the most significant impact on the selectivity of differentiation among the various parameters of the mobile phase. The mobile phase comprises an organic mobile phase (seventy–ninety-eight %) and H_2O. When switching from RP-HPLC mode to HILIC mode, the great concentration of mobile phase in the eluent conformation favoritisms an improvement in electrospray sensitivity and the efficiency of observed indications in mass spectrometric exposure. Chemical solvents have greater elution potential as their polarity grows, and there is a tendency toward proton acceptor/proton donor interactions. In HILIC, ACN is the greatest commonly used mobile phase.

On the other hand, acetone is a weakly aprotic solvent used to vary separation selectivity [22]. Expert's usage alcohols that form hydrogen bonds with functional assemblages of adsorbent and are accomplished of a rival with H_2O particles for active locations on the SiO_2 surface if the analyte is poorly dissolved in acetonitrile. In this study, the role of small molecular alcohols (ethanol, isopropanol, methanol) as mobile phase modernizers on retention of hydrazines on a zwitter ionic column was observed [23]; It was discovered that as the distance of the hydrocarbon chain in the alcohol molecule improved, so did the holding periods of analyte. In one study, the nature of an organic mobile phase affects the isolation of hydrophilic vitamins on diol column [24]. Methanol and isopropanol as organic mobile phase yielded the lowest selectivity, while acetonitrile provided the highest separation. The concentration and structure of the buffer solution affect separation selectivity in HILIC, allowing for control of the grade of ionization of purposeful groups on the adsorbent surface and analyte separation factors [8]. Since one of the processes of analyte preservation is ion exchange, a variation in the composition of the buffer solution influences the extraction of polar analyte in hydrophilic interaction liquid chromatography to a much greater degree than in reverse phase hydrophilic liquid chromatography. Since their significant solubility in organic solvents with high ACN concentrations, salts (ammonium acetate or formate) are generally used to make HILIC buffer solutions.

Furthermore, they can be detected using mass spectrometry. An upsurge in the concentration of a buffer solution or (salt) in the organic mobile phase favoritisms an escalation in the aqueous organic level on the adsorbent surface, which indicates to increase in retention times of polar materials [23-26]**.** By diol columns, Karatapans et al., examined the influence of mobile phase form on the differentiation of 6 hydrophilic vitamins. Isopropanol and methanol-based separations were less selective, while ACN-based separations were more selective (Fig.2).

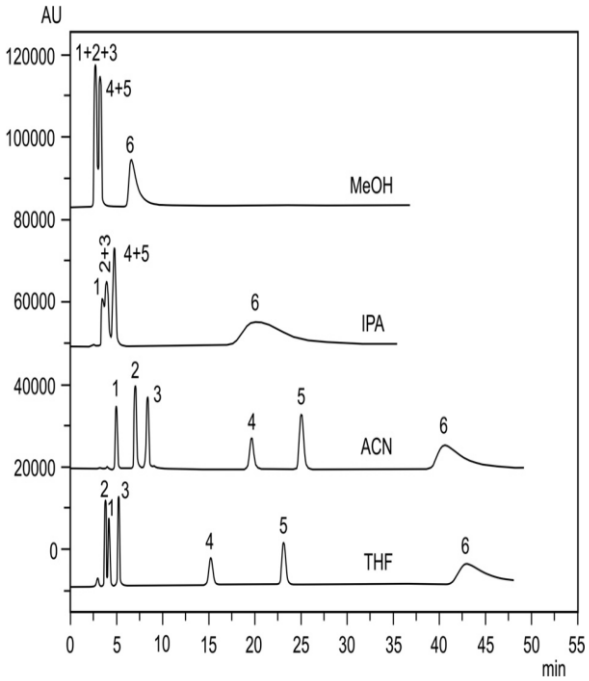

Figure 2: Influence of mobile phase on vitamins separation. Circumstances: column Inertsil-Diol (150 mm . 4.6 mm, 5µm); mobile phase:90:1 v / v mobile phase: ammonium acetate (10 mmol^{-}, pH 5.0). Composites: 1-pyridine-3- carboxamide, 2- vitamin B_6, 3- vitamin B_2, 4- Niacin, 5- Vitamin C, 6- vitamin B1. Mobile phase: methanol (CH_3OH), isopropanol, acetonitrile, tetrahydrofuran[24].

To date, however, there has been no comprehensive assessment of the efficacy and selectivity of glycopeptide enrichment using HILIC mobile phases. We present BDrop-HILIC, a novel, condensed HILIC enrichment technique used to methodically test the effect of the organic solvent on ZICHILIC glycopeptide enrichment [27]. In HILIC, a method for estimating the various step volumes is discussed. A Linear Free Energy Relationships (LFER) homologous categorization technique, including phenones, n-alkyl-benzenes, and ketones, are used to estimate the organic solvent volume (dead volume) for a ZIC-HILIC column in several ACN- and CH_3OH-H_2O preparations. We establish that the column is a HILIC column when the organic solvents comprise medium and high proportions of ACN or CH_3OH[28]. The effects of several eluent-H_2O organic mobile phases on retention performance of polar rebaudioside A (RA) and its equivalents on a sulfonic acid functionalized cation exchange column was studied above a broad variation of mobile phase contents to study the separation and retention of stevioside polar composites on the stationary phases [29].

4. Stationary Phases for HILIC

In recent years, the study and literature on (HILIC) have expanded. It has been followed via a similar upsurge in the number of HILIC stationary phases produced. In general, all polar stationary phases can be used in HILIC, which can be classified into three groups: charged (strong electrostatic-interactions with analytes), zwitterionic (weak electrostatic-interactions with analytes), and neutral (no electrostatic interactions with analytes). Since the forming of an adsorbed H_2O layer on the adsorbent surface, which is requisite for applying the hydrophilic interaction chromatography method, is influenced by specific functional groups of stationary phase, the existence of the stationary phase has a significant effect on analyte holding[30]. Standard HILIC stationary phases have silica modified with diol, amino or amide, and zwitterionic groups. The most used silica is still unmodified silica, used in around 35% of the studies (Fig.3).

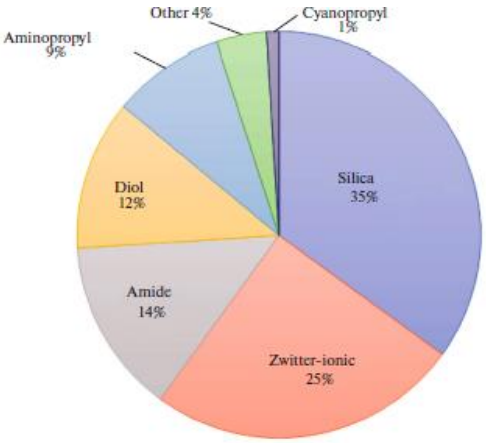

Figure 3: Stationary phases in hydrophilic interaction chromatography were given to Scifinder Academic from 2003 to 2017[4].

In hydrophilic interaction liquid chromatography(HILC)[30], stages with attached sulfo-alkyl betaines sturdily adsorb H_2O through hydrogen bonds and form a bulky H_2O layer on the adsorbent surface, regulating the separation of polar substances. In the sequence cyanopropyl(N≡C-CH_2-CH_2-CH_2-), diol, aminopropyl, SiO_2, the polarity of polar stationary phases in HILIC increases [31]. As

polar ionogenic composites were separated on unmodified SiO_2, silica with attached zwitterionic[32], diol, amide groups, and SiO_2 altered by a long-chain alkyl radical with a polar group, the unmodified SiO_2 had the maximum retention influences for polar analytes (Fig.4) [33]. Powerful electrostatic interactions, which are common in such phases, often decrease the diffusion and efficiency of the chromatographic regions in this condition [34].

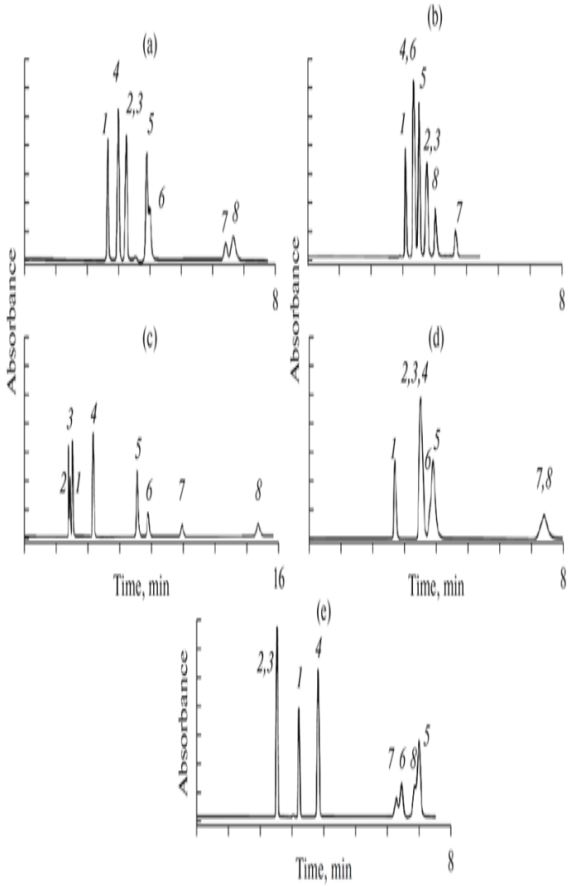

Figure 4: Separation of polar composites on dissimilar stationary phases HILIC situations. Stationary phases: (a) zwitterionic, (b) di-ol, (c) SiO_2, (d) amide (RC(=O)NR′R), (e) mixed type phase. Organic mobile phase: ACN− NH_4HCO_2 (85:15,v/v) 5mM, pH=3. (1)(C_6H_5OH); (2) naphthalene-1-sulfonic acid; (3) *p*-xylene-1-sulfonic acid; (4) caffeine ($C_8H_{10}N_4O_2$);(5)nortriptylin($C_{19}H_{21}N$); (6) diphenyl-hydramine ($C_{17}H_{21}NO$); (7) benzyl-amine ($C_6H_5CH_2NH_2$); (8) procainamide ($C_{13}H_{21}N_3O$) [32].

The utility of HILIC for the analytical characterization of protein biopharmaceuticals has been investigated using a novel stationary step based on extensive pore hybrid SiO_2 bonded with an amide ligand [35]**.** In deep eutectic solvents (DESs), a stationary phase established on poly (itaconic acid) grafted SiO_2 (Sil-PIA) was characterized and produced in detail. Deep eutectic solvents (DESs) as a new green solvent were used to homopolymerize itaconic acid on SiO_2 using a surface radical chain transfer response [36]. Via assembling porous graphene on SiO_2(Sil-PG) microspheres, a novel SiO_2-porous graphene stationary phase was effectively produced [35]. In 2020, a new stationary phase established on glucose derivative carbon dots modified on SiO_2 (SiO_2- Glc - CDs) was developed and used in HILIC. Compared to a homemade industrial glucose modified SiO2 (Sil - Glc) column and HILIC column, the Sil-Glc-CDs column displayed greater retaining separation and capacity selectivity against polar analytes such as saccharides and amino acids, nucleobases, nucleosides, antibiotics, and ginsenosides [37]. A basic physical approach was used to create a new stationary step of hydro-phobically associating polyacrylamide (HAPM) covered SiO_2, which showed to be a great separation substantial HILIC. It also had a high level of repeatability and stability [38]. If additional stationary phases for HILIC isolation of polar composites become usable, it develops increasingly significant to offer a more profound acceptance of their selectivity and retention features. Subsequently is impossible to justice based on the confirmation of the functional group; relative retentivity of the stationary phase must be determined experimentally. The relative retentivity can be different by the electrostatic interaction between charged solute and stationary phase[39]. Despite broad variations in functional classes, the experimental results show that most stationary phases have comparable retentivity against neutral solute; however, the comparative retentivity for charged solute on charged stationary phase may change. For different solutes, the selectivity of a stationary phase can vary greatly[40]. It gives you a chance to discover the right stationary step to get the separation you want.

5. The zwitterionic stationary phases

For HILIC, Irgum and colleagues developed a community of zwitterionic silica-based stationary phases [41,42]. Attaching a lively layer comprehending sulfoalkylbetaine groups onto extensive pore silica ZIC-HILIC or a polymeric support product this zwitterionic phase (ZIC-pHILIC). In contrast, these phases comprehend both

negative & positive charges. Their ion-exchange properties are weak. Since the oppositely charged groups are in a 1:1 molar ratio, their net charge is close to 0. The low surface areas of these phases can explain their weak ion exchange properties [43]and the oppositely charged functionalities shielding free silanols [44]. Such phases are ideal for HILIC because zwitterions are heavy osmolytes [45]; they make it easier for water to adhere to their surfaces. Sulfoalkylbetaine silica phases, on the other hand, have a very slight negative charge due to the distal sulfonic-acid ($HS(=O)_2(OH)$) groups. This extreme negative charge is unaffected by pH. B. Buszewski and S. Noga discovered that the pH of the HILIC columns tested has the least effect on retention on sulfoalkylbetaine silica columns[46]. HILIC separations of nucleobases [47], peptides [48-50] metabolites [51,52], ions [53], and other polar analytes [54,55] have been registered, although these phases were originally planned for the determination of inorganic cations and anions. Zwitterionic micellar coated the stationary phase with uncontaminated H_2O as an organic solvent, separation of zwitterionic ions and inorganic solutes were accomplished [52]. By adding several functional groups such as hydrophilic macromolecules, ionic liquids, and zwitterionic groups, several new stationary phases have been created to make available an extensive range of applications and selectivity. Many types and orientations of zwitterionic stationary phases were advanced with several charge spatial arrangements. Phosphorylcholine moieties or sulfobetaine were bonded on the surface of polymeric materials or SiO_2 to yield traditional zwitterionic stationary phases (ZIC-pHILIC, ZIC-cHILIC, and ZIC-HILIC) [55]. HILIC on bonded amide, urea, and bare SiO_2, cursorily porous phases were used to separate numerous simple, neutral antibiotic, antiretroviral, and zwitterionic composites [56].

6. HILIC Method Development

In fields including pharmaceuticals, drug research, bioanalysis, food chemistry, agricultural and medicinal chemistry, method production of LC is one of the essential areas of separation technology. Developing liquid chromatography methods aims to find the best-employed situations for successful analyte separation [57]. HILIC is an actual separation type of modern LC that is increasingly used to solve systematic agricultural, pharmaceutical, and biomedical problems [58]. Over the last period, HILIC investigation has yielded a wealth of information that has helped us better comprehend the fundamentals of hydrophilic interaction liquid chromatography, such as stationary phase properties, retention methods, and the results of different chromatographic factors. Developing an analytical protocol for quantitative and qualitative determination of the analyte of attention by the chosen chromatographic method is known as technique growth. Numerous process growth strategies have been developed for engrained chromatographic systems such as RPLC [59-61]. However, systematic process production for HILIC has not been widely explored or documented. Dejaegher et al. examined several HILIC-based biological and pharmaceutical assays and discovered that most methods were developed by trial and error [62,63]. Chiritta et al. suggested a choice tree focused on the impact of different chromatographic variables on the hydrophilic interaction liquid chromatography separation of neuro-transmitters in addition to a selectivity evaluation of hydrophilic interaction liquid chromatography phases focused on principal-component-analysis (PCA) to aid process growth and optimization [64]. HILIC techniques can be used to resolve a variety of investigative problems in a variety of fields, including the pharmaceutical and bioanalytical uses mentioned below:

1. Analyze lively medication or metabolite in active pharmaceutical ingredient (API) in drug substance and dose preparations, biological matrices, counterion in drugs produces, and examine different impurities as samples of single constituent assays.
2. Many compounds of attention are investigated, including production impurities in drugs components, degradation yields in drug produces, and drugs metabolite in organic matrice.
3. Techniques for determining the stability of a drug ingredient or a drug product.

According to the literature review, HILIC applications in pharmaceutical science are growing every year. It was discovered that HILIC allows for the simultaneous study of several substances with various detectors.

7. Optimization of Method Parameters

7.1. Column Selection for HILIC

Aimed at ultra-high pressure liquid chromatography (UHPLC) uses, (HILIC) columns

with sub-2μm particle (e.g. 1.7μm) have recently become available. HILIC columns usually contain 3- or 5μm particles widely used in analytical separations. A HILIC approach using sub-2 m particles allow for faster separation without sacrificing separation performance, making it ideal for applications requiring quick analysis. In addition to particle size, the length of column needed must be chosen to accomplish desired resolution and adequate efficiency, whereas complementary run time (especially for isocratic systems) and back-pressure. Owing to the reduced viscosity of the mobile phase comprising a high organic solvent, the back-pressure in HILIC is usually lower than in RPLC. The systematic flow rate range (0.5-3 mL/min) is best served by 4.6 mm ID column, whereas 2.1 mm ID column is more suitable to UHPLC and HILIC-MS uses[65- 67].

7.2. Organic Solvents for HILIC

Water is an integral organic solvent constituent (at least 3 to 5 % in organic solvent). It keeps the stationary phase's surface aqueous layer stagnant, needed for HILIC separation. Weaker mobile phases are often required to reduce the mobile phase's absolute polarity, allowing the solutes to partition into the aqueous layer and be retained. The most popular mobile phase in HILIC is acetonitrile. Acetonitrile's aprotic structure promotes greater hydrogen bonding among the stationary phases and analytes, improving retention. Alternative solvents for HILIC include CH_3OH, CH_3CH_2OH, and isopropyl alcohol (IPA), with retention times increasing with carbon number in the order of MeOH, EtOH, and IPA [41]. To ensure proper hydrophilic interaction, HILIC mobile phases must usually comprehend at least 60percent organic solvents. When the ACN content in the eluent process approaches 86 percent, even a slight improvement in acetonitrile content will induce a large change in retention. As a result, the final organic material chosen in the mobile process may hugely affect the HILIC method's reproducibility and robustness. Other organic solvents, such as tetrahydrofuran (THF) and acetone, can also be employed. The generally solvent- strength increase in the order of CH_3COCH_3 < ACN < IPA < EtOH < MeOH < H_2O [41]. The organic solvent can also be employed in several compositions to regulate selectivity & retention.

7.3. Mobile Phase pH for HILIC

In HILIC, changing the ionization state-run of stationary phases and analytes significantly influences selectivity and retention [59]. However meanwhile, utmost HILIC SiO_2-based columns are unstable at extreme pHs; caution must be exercised (less than two or greater than 8). Due to improved hydrophilic interactions, ionized analytes (e.g., primary and acidic composites) usually retain better. Not only will the eluent pH influence the stationary phase's ionizable functional groups (e.g., triazole phase and amino), but then it can also have a significant effect on the bare SiO_2 and SiO_2-based neutral phases via ionizing surface silanol groups. The pH of the mobile process can also affect selectivity, but it has a more significant effect on acidic composites than on primary compounds. Fig.5. shows one example of the influence of organic mobile phase pH on the selectivity of selected composites [67].

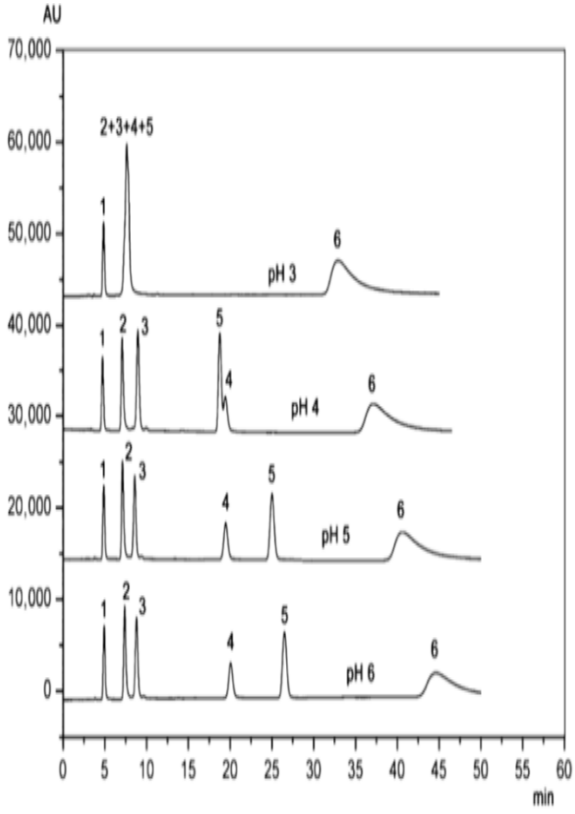

Figure 5. Influence pH value on selected separation H_2O-soluble vitamin. Condition's: column, inertsil diol (particle size 5μm,150 mm×4.6mm): column heat=25°C; flow rate equal 0.6 mL/min wavelength of detection = 272nm, mobile phase equal ACN-H_2O 90-10v/v comprising CH_3COONH_3 10mM, with aqueous buffer adjusted at numerous pH value. Peak

project: 1- vitamin B_3 2- vitamin B_6, 3- vitamin B_2, 4- NiacinB_3, 5- vitamin C, and 6- vitamin B_1 [67].

7.4. Concentration and Buffer Types for HILIC

In chromatographic methods, buffers are widely used to change the buffer capability, and mobile phase pH can avoid pH uncertainty in the mobile phase, subsequent in more stable and repeatable techniques. To achieve the desired pH and ionic strength in hydrophilic interaction liquid chromatography, buffer salts that are solvable in the eluent and have a high organic contented are required. Formate or ammonium acetate is often used for acidic pHs, while carbonate and NH_4OH are suitable substitutes for high pHs. Because of their poor solubility in the organic solvent process often used in hydrophilic interaction liquid chromatography and their unsuitability with mass (MS) or charged aerosol detector (CAD) spectrometry detectors, phosphate buffers should be avoided. As soon as UV detection at a low wavelength is required, phosphate buffer at a low concentration may be suitable. The essence of the analysis relationship with the stationary phase in HILIC determines the influence of buffer concentration on selectivity and retention [46]. To maintain a good peak shape, an opposite buffer concentration (5-100mM) is usually suggested for HILIC. The nature of analyte interaction with stationary phase in HILIC limits the effect of buffer concentration on selectivity and retention. The primary factor influencing analyte retention for non-ionizable materials are separating the column's hydrophobic mobile phase and stagnant aqueous layer. When the buffer salt concentration is increased, the retention time increases expressively. Higher salt concentrations are thought to increase the amount of the stagnant aqueous layer, allowing for more analyte partitioning and more extended retention periods. For example, increasing the ammonium acetate concentration (10-20 mM) dramatically changed the elution pattern of a set of acidic chemicals on the silica column, as seen in Fig.6 [67].

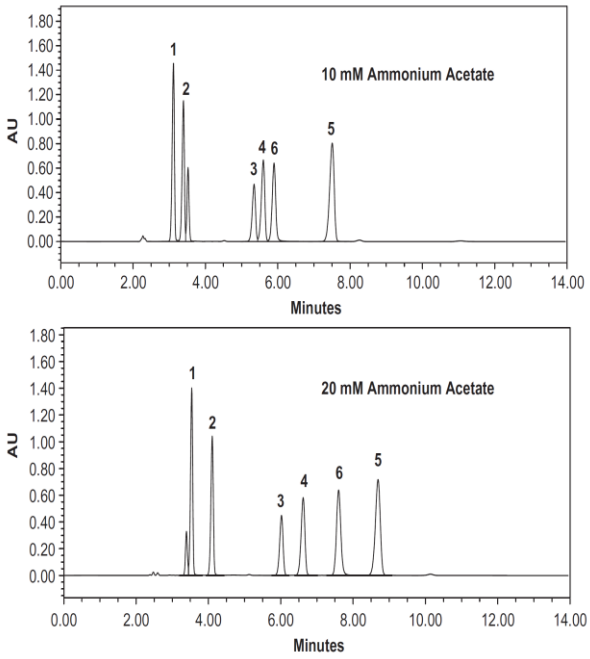

Figure 6. The typical acidic composites are separated on the SiO_2 phase. ACN/H_2O (85/15,v/v) mobile phase comprising 10 &20 mM ammonium acetate Dimensions of the column are 250mm x4.6mm ID, with a particle size of 5m. Column temperature of is30°C. 1.0mL/min flow rate at 228nm, UV detection. (1) Salicylic-acid ($C_7H_6O_3$), (2) Gentisic-acid($C_7H_6O_4$), (3) Acetylsalicylic-acid($C_9H_8O_4$), (4) Salicyluric-acid($C_9H_9NO_4$), (5) Hippuric-acid($C_9H_9NO_3$), and (6) Hydroxyhippuric-acid ($C_{10}H_{11}NO_5$) [42].

7.5. Column Temperature

In chromatographic techniques, the temperature of the column is often employed to adjust selectivity and preservation of target analyzes. The temperature of the column has an effect on the retention time of polar molecules in HILIC separation. In RPLC, the vant- Hoff equation is frequently employed to describe connection among factor of capacity (k) and temperature of column (T):

$$\ln k = -\frac{\Delta Ho}{RT} + \frac{\Delta So}{R} + \ln\varphi$$

where ΔS^o and ΔH^o are retention entropy and enthalpy, R constant of gas and φ phase ratio[68-70]. As soon as the hydrophilic activity is the primary retention mechanism for most polar composites in HILIC, an increase in column temperature typically decreases retention. While another retention process takes precedence, divergence from this pattern can occur. For instance, on the amino phase, the retention of acetylsalicylic acid (Aspirin) upsurges as the

temperature of the column rises [42]. In HILIC, it was discovered that the column temperature has less effect on the preservation of acidic composites than the contented of buffer/salt or organic solvent concentration[70]. While the column temperature was raised from 20°C to 50°C, Marrubinis work on nucleosides and nucleic acid bases found a comparatively minor difference in retention. And while the column temperature reached 80°C were, significant improvements were found [71]. The influence of temperature is estimated by ΔH0 of the interaction between the eluted molecule and the stationary phase. Enthalpy can be determined founded on the slope of the graph, well-defined as vant Hoff's equation (relating the retention factor (logarithm log k) to the reciprocal of absolute temperature (1/T))[68].

7.6. Sample Solvents

ACN is always the first select sample solvent, although other organic solvents (e.g., EtOH, IPA, &methanol) may also be employed in HILIC. When the solubility of sample in pure ACN is an issue, a (50:50) combination of IPA and ACN (e.g., 50:50, v/v) should be used as a sample solvent. Because of its capacity to dissolve a broad range of organic composites at elevated concentrations, dimethyl sulfoxide $(CH_3)_2SO$ (DMSO) can be used in some instances. DMSO may well be considered in some cases because it can dissolve a wide range of organic composites at high concentrations. Though the volume of H_2O in sample diluent must be warily controlled, subsequently, too much H_2O might cause the peak form to degrade (split or broadened) [69-71].

8. Detection for HILIC

In the HPLC method, detection is composition answerable for revolving a chemical and physical characteristic into a determinate signal corresponding to identity or concentration. Identification was typically made during the development days of HPLC by examining them offline and assembling fractions. The first online detection for HPLC was not developed until the 1940 & the 1950s. Undoubtedly a development over offline methods, sensitivity was an issue. Exploration for more sensitive common detections of HPLC led academics to adjust Gas Chromatography (GC) detector for usage in HPLC, but the elimination of the HPLC mobile phase eventually limited applicability. In 1960 the first UV detections for HPLC was presented, and significant developments in a project led to enhanced enhancements and sensitivity such as mutable diode array and wavelength detectors. For analyte with one or more chromospheres, UV detectors are probably most extensively employed exposure technique in LC caused by its relatively broad linear range, use ease, low cost, and fact that it is well-matched with most solvent employed in the eluent in gradient elution or isocratic method[72,73] As substitutes to (UV-Vis) detectors[74,75], predominantly for composites missing strong charged aerosol detection (CAD) [76], MS[77, 94], and UV absorbance are beneficial. Further detections established on evaporation (evaporative light scattering detectors (ELSD)) [78,79], are also selections. Less universally employed detections such as chemiluminescent nitrogen (CLND), electrochemical [80,81], fluorescence [82], and refractive index (RID) [83,84] may also be well-suited with HILIC circumstances and may be applicable for exact uses. Conversely, RID & ELSD detection has positive dynamic range, sensitivity, and precision limitations. RID is too not well-suited with gradient elution because of the presence of high organic content in the organic mobile phase; CAD and MS detection have a special benefit in HILIC.

9. Some applications of HILIC

Several pharmaceutical applications for (HILIC) review. The "applications" contain not only the study of drugs products and drugs substances, on the other hand as well the study of raw reagents, constituents, and intermediates used in the drugs substance's production. Since these precursors are mostly lesser, more polar particles than the drug product, they are amenable to HILIC examination. HILIC analysis could be possible for excipients used in medication formulations[85]. When it is decided that HILIC is an effective model for the study, choosing between mobile and stationary phases for the HILIC study of pharmaceuticals is a simple judgment. HILIC column manufacturers may provide helpful information about process construction and case studies, but much of the data is exclusive to the manufacturer's brand of the column. HILIC applications for pharmaceutical research have been mentioned in many publications. They have concentrated on opioid detection in biological fluids. Some articles have discussed process production in general or impurity determination in particular [85]. HILIC has been used

to analyze mixes of lesser polar particles, such as nucleosides, biomarkers, nucleotides, saccharides, amino acids, oligosaccharides, glycosides, hydrophilic drugs, sugars, alkaloids, between further ionized or polar substances. Biomedical and pharmaceutical chemistry, metabolomics, proteomics, food and agricultural chemistry are all areas where it plays a role. Peptides and proteins, as well as certain inorganic compounds, are commonly separated using the HILIC mode[86-88]. Because of the strong "compatibility" of the mobile phase used in HILIC by the ionization process of LC mass-spectrometry, many related studies have exploded in the last decade. For the study of gabapentin in medicinal formulations, Jia et al. tested 2 aerosol established detectors (evaporative light scattering detector (ELSD**)** and CAD) [89]. They tested four HILIC columns in this study: (ZIC-pHILIC) zwitterionic polymer, ZIC-HILIC (zwitterionic), Atlantis HILIC (cross-linked diol) (silica), and Luna HILIC (cross-linked diol). Pyrimidines, nucleosides, and purines were separated on ZIC-HILIC columns and TSKgel Amide-80 [90]. Using aqueous buffer/acetonitrile as a mobile process, adsorption and partition mechanisms were together active. Silanols Ionization and analyzes on TSKgel Amide-80 balanced retention of the compounds tested, while ZIC-HILIC was less affected in the pH 3–5 range. Olsen showed the evolution of 5-fluoro-uracil in 5-fluoro-cytosine on together amino and silica columns in early work with HILIC [91].

Thus, the HILIC process can be used to isolate uncharged strongly polar and amphiphilic compounds that are difficult to separate using RP-HPLC or/and ion-exchange chromatography, such as simple carbohydrate, polar pharmaceutical formulations and peptides [92,93]. While HILIC is unlikely to supplant RP-HPLC as the workhorse technique for pharmacological research, it has been exposed to be useful for polar composites. HILIC research applies to polar drugs, medication product polar excipients, salt counterions, and excipient or drug impurities. With an acceptable CSP, enantiomer separation may also be done in the HILIC mode. Several blends of HILIC mobile and stationary phases have been presented to be appropriate for specific uses. In other situations, the analytical targets and the analyte and\or sample properties were used to direct analytical and condition selection process formulation. The stationary phase in most applications is a polar moiety bonded to silica. A buffered mobile phase is suggested where ionic interactions between the analyzes and stationary phase are present. As more familiarity with HILIC implementations is obtained, general standards for method implementation will continue to evolve. The table.1 shows the most important modern applications of HILIC technology.

10. Advantages and disadvantages of HILIC

HILIC separations have several benefits that are viable alternatives to RPLC and NPLC evaluation. Its feasibility is also endorsed from an economic standpoint and scientific. The effective determination of ionized and polar composites, which is trying to accomplish by the reverse-phase liquid chromatography method, is one of the critical advantages of HILIC[94]. The elution order of polar substances in "HILIC" evaluations is characteristically inverse of that seen in reverse phase liquid chromatography evaluations, implying that the "HILIC" mode can be used to help composite individuality validations[8]. The HILIC determination mechanisms reduce problems, including peak asymmetry and irreversible retention, common in polar analyte evaluations using the normal phase liquid chromatography method [95]. The usage of mobile phases comprehending commonly low viscosity organic solvents in HILIC enables separations to be performed at lower pressures than in reverse-phase liquid chromatography, resulting in shorter separation times and higher flow rates [96,97]. HILIC-mode processing needs no changes to the chromatographic separation equipment used in NPLC and RPLC and uses the same mobile and stationary phases together. As a result, the running expenses are very close to NPLC and RPLC. HILIC, though favourable in several ways, has some drawbacks compared to RP-LC. Since the determination process of HILIC is less well understood than that of the reverse phase, predicting the impact of changing separation conditions is challenging [98]. HILIC is often unfitting for hydrophobic particles since they lack adequate interactions to achieve the same isolation as RP. Another drawback mentioned in the literature is the difficulty of unravelling a mix with a large amount of basically related analyses due to their HILIC being similar. Some scientific findings dispute this statement[99].

Table1. The modern applications of HILIC technology

11. Conclusion

"C18 column (75 mm x4.6 mm; 3.5 µ)"	"ammonium acetate buffer (10 mM; pH 4.0) and acetonitrile in a ratio 40:60 v/v"	(UV) 210	1-16 µgmL^{-} R^2 = 0.999 % Rec = 99.91 %RSD = 0.15 LOD = 0.189 µgmL^{-} LOQ = 0.8µgmL^{-}	Telmisartan	(100)
"Purospher Start C18 (250 mm x 4.6 mm, 5 µm)"	"methanol: water (70:30 v/v) pH 3.0"	(UV) 225	5-25 µgmL^{-1} R^2 = 0.999 % Rec = 101.11 %RSD = 0.0073 LOD = 0.4 µgmL^{-1} LOQ = 2.3 µgmL^{-1}	Captopril	(101)
"C18 column (2.1 mm × 50 mm,1.7 µm particle size) and inline 0.2 µm"	"(0.1% formicacid in water) and solvent B (acetonitrile) as follows: 35–35%B (0–0.5 min), 35–80%"	(UV) 250	5–3000 ngmL^{-} R^2 = 1.000 % Rec = 90.7 %RSD = 4.3 LOD = 5 ngmL^{-} LOQ =15 ngmL^{-}	Irbesartan	(102)
"C8, Kromasil KR 100 ,C8 column"	"acetonitrile, 15 mM orthophosphoric acid (37:63), and 0.25% v/v of triethylamine' (pH 2.5)	(UV) 238	5–500 ng ml^{-1} R^2 = 0.9979 % Rec = 99.3 %RSD = 0.0620 LOD = 1–80 ng mL^{-1} LOQ = 1 ngmL^{-1}	Carvedilol	(103)
ZIC2-HILIC ZIC4-HILIC	acetic acid (HAc), Sodium Acetate (NaOAc), Acetonitrile (ACN)	(UV) 240	8-1200ngmL^{-1} R^2 = 0.9998 % Rec = 100.86 ± 0.68% %RSD = 0.31-1.02 LOD =2.33 and 1.40ngmL^{-1} LOQ = 7.07 and 4.25ngmL^{-1}	Ointments	(104)
ZIC-HILIC and ZIC-pHILIC	acetic acid (HAc،(Sodium Acetate (NaOAc), Acetonitrile (ACN(	(UV) 275	0.01 - 0.9 µgmL^{-1} R^2 = 0.9998 % Rec = 98.50-99.25% %RSD = 0.48 ± 0.12 and 0.49 ± 0.22 LOD =0.058 and 0.04 µgmL^{-1} LOQ = 0.203 and 0.14 µgmL^{-1}	pharmaceutical preparations	(105)
ZIC1 and ZIC5	acetic acid (HAc،(Sodium Acetate (NaOAc), Acetonitrile (ACN(	(UV) 275	0. 1 - 1.2 µgmL^{-1} R^2 = 0.9998,0.9983, % Rec = 98.50-99.25% %RSD = 1.54and 1.61 LOD =0.009 and 0.007 µgmL^{-1} LOQ = 0.0315and 0.0245µgmL^{-1}	human serum	(106)
ZIC2 and ZIC3	mixing buffer (20% sodium acetate-40 mM, pH 5.5), 80% acetonitrile	(UV) 270	0.01-4 µgmL^{-1} R^2 = 0.9998 % Rec= 99.70%, 99.58% %RSD = 1.26 ± 0.06 LOD =0.13,0.19 µgmL^{-1} LOQ =0.45,0.66 µgmL^{-1}	pure form and in pharmaceutical dosage	(107)
HALO-HILIC column	NaOAc/HAc buffer (40 mM-pH 4.75) with acetonitrile (10:90) (v/v)	(UV) 280	0.01-3 ppm R^2 = 0.9999 % Rec= 99-102.5% %RSD = 0.23-0.76 LOD = 0.0242 ppm LOQ = 0.008 ppm	pharmaceutical formulations	(108)

HILIC has been increasingly common in current years. Isolation and polar compounds difficult to study using RP-HPLC or different methods may be achieved using HILIC. Metabolites and Drugs in biochemicals, biological fluids, pharmaceuticals, environmental, and diets applications have all benefitted from HILIC. Many HILIC stationary phases have been advanced, and two-dimensional separation systems that use mass spectrometric detection or HILIC are becoming more widespread. Many researchers lack comprehensive experience and knowledge with HILIC, principally when reversing phase-HPLC. There are many well-known sources for data on RP-HPLC processes, theory, and technique growth. Despite the modern increase in the usage of HILIC, there is a scarcity of knowledge to help future practitioners understand and improve robust HILIC separations. Due to a lack of experience with HILIC, method implementation can be trial-and-error, resulting in less-than-optimal outcomes for a given application. The popularity of HILIC separations has exploded in recent years, particularly in the last 20 years. HILIC outperforms reverse-phase liquid chromatography when unravelling polar, hydrophilic, or ionized compounds. While the procedure is not quite as well-known as reverse-phase liquid chromatography, a more excellent thought of the mechanism of these evaluations is emergent. The complicated dynamics separations in the HILIC method are much more complex than those suggested via Alpert at the outset. According to the literature, all-composite has a particular appliance for preservation in various chromatographic circumstances. Therefore, to choose the most acceptable chromatographic situations for determination, attention should be paid to both the physical and chemical properties of the matrix and the analyte before choosing the HILIC separation mode. While HILIC has a lot of potential for polar compound separations, it has a lot of obstacles to overcome (e.g., exchange of evaluation column employed in reverse phase method, accountable for more than seventy percent of the implementation of high-performance liquid chromatography). HILIC, on the other hand, is an intriguing solution for evaluations that are difficult to achieve with RP and NP, or also by ion-pair or ion-exchange chromatography. We believe the production of HILIC to be based on miniaturization to minimize waste and cost generation and speed up investigations, in line with the general trend in separation techniques. Microdevices are still being used in RPLC and HILIC, and further studies in this area should include them. The use of monolithic phases in other separation approaches is gaining popularity, but the study of modern stationary phases, mostly novel monoliths and bonded phases, offer a short term viewpoint.

12. Conflicts of interest

There are no conflicts to declare.

13. Formatting of funding sources

There are no formatting sources.

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