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Review Article

A REVIEW ON HYDROPHILIC INTERACTION CHROMATOGRAPHY- A USEFUL TECHNIQUE

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Abstract: Hydrophilic interaction liquid chromatography (HILIC) provides an alternative approach to effectively separate small polar compounds on polar stationary phases. Hydrophilic interaction chromatography (or hydrophilic interaction liquid chromatography, HILIC) is a version of partition chromatography which occupies the opposite end of the partition spectrum from reversed phase liquid chromatography. Both are distinct from normal phase liquid chromatography in that water is part of the mobile phase, and thus not adsorption chromatography. When the mobile phase contains > 60% organic solvent, then hydrophilic interaction becomes significant. The mechanism of HILIC implicates partitioning between a water-enriched layer partially immobilized on the hydrophilic stationary phase and the less polar mobile phase. The purpose of this work is to review the mechanism of behind the hydrophilic interaction chromatography by using the stationary phase and mobile phase for the separation of polar compounds. The working of hydrophilic interaction is explained in this article. The separation mechanisms are given with the intermolecular interactions types. The additives and proper pH is used for the separation of compounds by hydrophilic interaction chromatography. The recent advances related to the hydrophilic interaction chromatography are given this article.
Key**words:** Hydrophilic Interaction Chromatography, Stationary Phases, Mobile Phases, Separation Mechanisms.

INTRODUCTION [1-8]

Hydrophilic interaction chromatography or hydrophilic interaction liquid chromatography, (HILIC) is a version of partition chromatography which occupies the opposite end of the partition spectrum from reversed phase liquid chromatography. Both are distinct from normal phase liquid chromatography in that water is part of the mobile phase, and thus not adsorption chromatography. The name was suggested by Dr. Andrew Alpert in his 1990 paper on the subject [1]. He described the chromatographic mechanism for it as liquid-liquid partition chromatography where analytes elute in order of increasing polarity, a conclusion supported by a review and re-evaluation of published data. Any polar chromatographic surface may be used for Water /Solvent (ACN) mixture separations. Even non-polar bonded silicas have been used with extremely high organic solvent composition, when the silica used for the chromatographic media was particularly polar. To avoid confusion we recommend to use the term HILIC to describe a normal phase separation method for separating some biomolecules including peptides, phosphopeptides, crude extracts, peptide digests, carbohydrates, histones polar lipids oligonucleotides and their antisense analogs nucleic acids and many proteins membrane proteins, by their differences in polarity. HILIC is a novel type of chromatography based on a mixed mode of retention mechanisms. It does not suffer from the problems associated with traditional normal phase chromatography (NPC). Compatible with mass spectrometric detection, HILIC can be used with sample preparation procedures without drying down organic extracts. HILIC is especially suited for the analysis of small polar analytes, but, based on its unique selectivity, the technique may be tried for any compound where traditional approaches have failed.

Hydrophilic interaction liquid chromatography (HILIC) is an alternative high-performance liquid chromatography (HPLC) mode for separating polar compounds. For historical reasons, it has been reported that HILIC is a variant of normal phase liquid chromatography, but the separation mechanism used in HILIC is more complicated than that in NP-LC. While the acronym HILIC was first suggested by Alpert in 1990, the number of publications on HILIC has increased substantially since 2003 (Fig. 1), as outlined in the well-constructed review by Hemström and Irgum.[3]

This paper covers fundamental developments in hydrophilic interaction liquid chromatography. The objective of the present work is to review options for the characterization of HILIC stationary phases and their applications to separations of polar compounds in complex matrices. Gaining a thorough understanding of retention behavior in HILIC enhances the scope of applications of liquid chromatography. The separation mechanism can depend on many factors, such as the physicochemical properties of the stationary phase and hydroorganic mobile phase, and the structures of the samples investigated. Precisely defining which mechanism prevails is currently a difficult and complicated task. This phenomenon is still waiting for theoretical elucidation.

HILIC is a variant of normal phase chromatography that is performed with a very polar

stationary phase and a mostly organic mobile phase. When the mobile phase contains > 60% organic solvent, then hydrophilic interaction becomes significant. With neutral materials such as PolyHYDROXYETHYL A™, this is the only significant force involved. With ion-exchange columns, hydrophilic interaction will be superimposed on the electrostatic effects. See the example of this with Histone H1 phosphorylation variants.

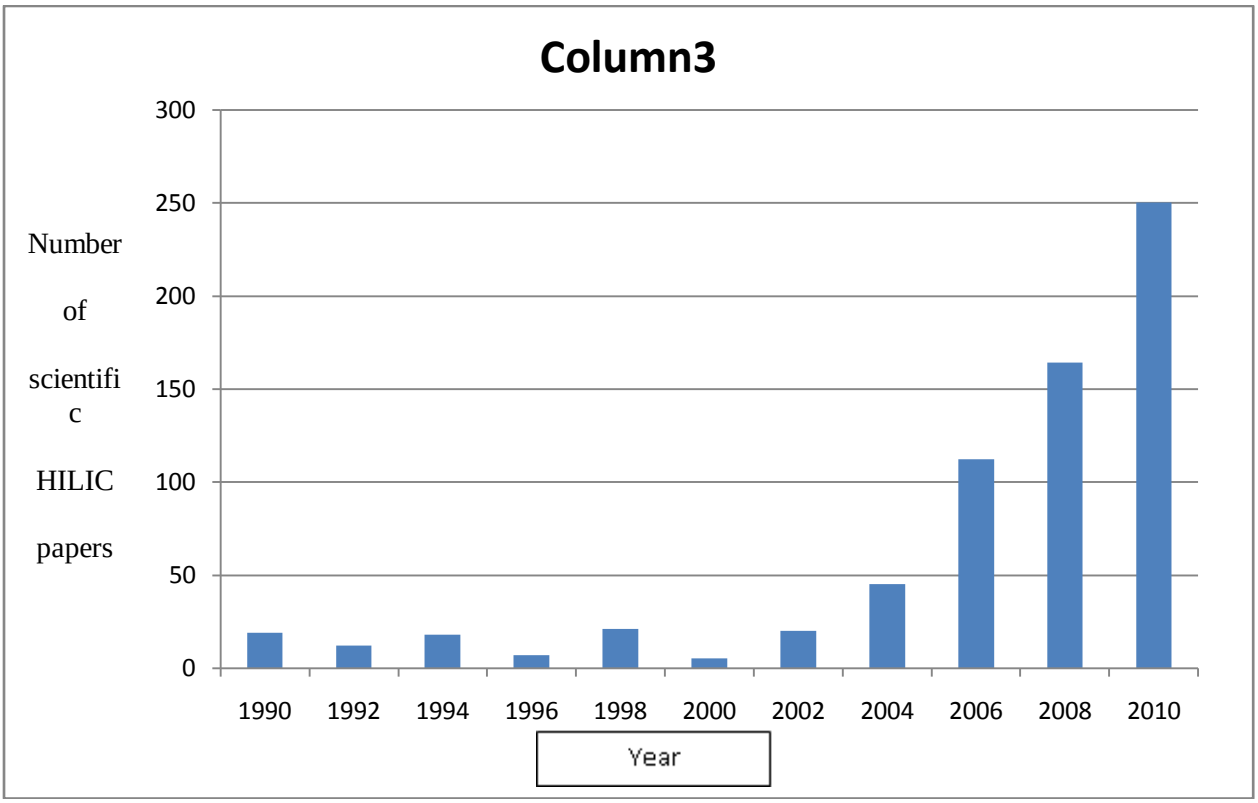

Figure no-1: Number of publications on HILIC

STATIONARY PHASES

The first generation of HILIC mode separated carbohydrates by an amino-silica phase. The next generation of stationary phases for HILIC used DIOL- and amide-silica. The DIOL-silica column has mainly been used for the separation of proteins, and then next is the amide-silica phase soon found common usage in HILIC. Chemically bonded stationary phases with specific structural properties have been prepared, some examples are aminopropyl ligands bonded to silica (SG–NH2); an alkylamide packing phase (SG-AP) and a mixed phase (SG-MIX) containing different types of ligands (–NH2, –CN, –Ph, –C8, –C18) bonded to the support. HILIC has progressed into second and third generation implementations, most of which involve mixed or multiple-interaction solid phases. Unmodified bare silica gel has some advantages for HILIC, in contrast to chemically bonded stationary phases. Type A silica gels, prepared by precipitation from the solutions of silicates, are acidic because they are polluted with certain metals that activate surface silanol groups and form complexes with some chelating solutes, causing strong retention or asymmetric peaks. Type B silica gels are formed by the aggregation of silica sols in air, contain very low amounts of metals, and are more stable at intermediate and higher pH values (up to at least pH 9) than xerogel-type materials. They generally provide better separations, especially for basic samples, because they are highly purified, less acidic "sol-gel" spherical silica particles. At higher pH values, silanol groups are ionized and cation exchange plays an important role in retention, especially for positively charged basic compounds. Suppressing silanol ionization through the addition of TFA may promote the ion-pairing mechanism. Similar effects have also been observed in HILIC on monolithic silica gel columns, which offer higher permeability than the particle-packed HILIC columns. Silica gel type C with a hydrosilated surface populated with nonpolar silicon hydride Si–H groups instead of silanol groups may have up to 95% of its original silanols removed, making it less polar than silica gels with higher populations of silanol groups. It can be used to separate acids or bases in the HILIC mode in buffered mobile phases containing more than 50–70% organic solvent (acetonitrile).

DIOL, amino, amide and other bonded phases used in HILIC are usually prepared by chemically modifying the silica gel surface, like the C18 phases used for RP-LC. Chemically bonded DIOL phases demonstrate high polarity and hydrogen bonding properties, and do not contain ionizable groups other than unreacted residual silanols, meaning that they are appropriate for the HILIC mode. Bonded amino-silica columns are relatively often used in the HILIC mode. While basic analytes are in general strongly retained on silica gel by hydrogen bonding and ion-exchange interactions with silanol groups, acidic compounds show increased affinities to amino-silica columns, which can sometimes even lead to irreversible adsorption.

Chemically bonded phases with other functionalities, such as polyethylene glycol or alkyls with embedded amide or carbamate groups, are generally proposed for RP applications in water-rich mobile phases. On the other hand, when the percentage of organic solvent is high, the retention of many compounds increases with increasing concentration of acetonitrile, showing typical NP behavior. Cyclodextrin-silica stationary phases that possess several linked glucopyranoside units and have chiral recognition properties are useful for HILIC chiral separations.

Zwitterionic sulfoalkylbetaine stationary phases have also been introduced for HILIC separations. The active layer, which is grafted onto wide-pore silica gel or a polymer support, contains both strongly acidic sulfonic acid groups and strongly basic quaternary ammonium groups separated by a short alkyl spacer. Ion-exchange interactions of the zwitterionic stationary phase are assumed. The sulfoalkylbetaine bonded phases strongly adsorb water by hydrogen bonding, and the bulk layer of water, which forms part of the stationary phase, then largely controls the retention mechanism. Zwitterionic columns are commercially available under the tradenames ZIC-HILIC (on a silica gel support) and ZIC-pHILIC (on a polymer support).

The separation of neutral compounds on ion exchangers under typical HILIC conditions has been known about for a very long time. On both cation-exchange and anion-exchange styrene-divinylbenzene resins, only the retentions of some polar compounds (e.g., carbohydrates and related substances) increase with increasing ethanol concentration in the mobile phase. For other compounds, the opposite effects have been observed. Due to the presence of ion-exchange groups, a mixed-mode HILIC/ion-exchange mechanism controls the retention, which may cause specific selectivity effects. The mixed anion-exchange/cation-exchange/HILIC mechanism that occurs on silica-based, small-pore, weak ion-exchange resins was found to be useful for the analysis and purification of compounds from natural products.

MOBILE PHASE SELECTION [3, 9]

Solvents such as acetonitrile with a small amount of water. However, any aprotic solvent that is miscible with water (e.g., tetrahydrofuran, THF, and/or dioxane) can be used. Alcohols can also be adopted, although a higher concentration is needed to achieve the same degree of retention of the analyte relative to an aprotic solvent–water combination.

An eluotropic row is useful for selecting a suitable organic modifier for the mobile phase. This lists solvents according to increasing elution strength. Relative solvent strengths in HILIC can be approximately summarized as follows:

HILIC separations are performed either in isocratic mode with a high percentage of organic solvent or with gradients starting with a high percentage of organic solvent and ending with a high proportion of aqueous solvent.

It is commonly believed that in HILIC, the mobile phase forms a water-rich layer on the surface of the polar stationary phase vs. the water-deficient mobile phase, creating a liquid/liquid extraction system. The analyte is distributed between these two layers.

Because of the reversal of the solvent strengths, the elution order of analytes in HILIC is usually the opposite of that observed in reversed phase chromatography. The most common solvent system is a mixture of water and acetonitrile. Instead of acetonitrile, other water-miscible organic solvents can be used, such as tetrahydrofuran, isopropanol, and methanol. Occasionally, a solvent system is used whereby there is no water at all, e.g. with methanol as the stronger solvent, but this does not seem to confer added selectivity. Because of the pH-dependency of the charge of the stationary phase it is especially important to fix the pH of the mobile phase with a buffer. The components of such buffers need to be soluble in all compositions of the mobile phase, and commonly consist of ammonium acetate or formate salts. The choice of buffering salt may be of importance for the selectivity of the separation, as shown for tetracyclines, which could be separated by a citrate buffer, but not by an acetate buffer.

It is commonly believed that in HILIC, the mobile phase forms a water-rich layer on the surface of the polar stationary phase vs. the water-deficient mobile phase, creating a liquid/liquid extraction system. The analyte is distributed between these two layers. However, HILIC is more than just simple partitioning and includes hydrogen donor interactions between neutral polar species as well as weak electrostatic mechanisms under the high organic solvent conditions used for retention. This distinguishes HILIC as a mechanism distinct from ion exchange chromatography. The more polar compounds will have a stronger interaction with the stationary aqueous layer than the less polar compounds. Thus, a separation based on a compound's polarity and degree of solvation takes place.

ADDITIVES [5, 6]

Ionic additives, such as ammonium acetate and ammonium formate, are usually used to control the mobile phase pH and ion strength. In HILIC they can also contribute to the polarity of the analyte, resulting in differential changes in retention. For extremely polar analytes (e.g. aminoglycoside antibiotics (gentamicin) or Adenosine triphosphate), higher concentrations of buffer (ca. 100mM) are required to assure that the analyte will be in a single ionic form. Otherwise asymmetric peak shape, chromatographic tailing, and/or poor recovery from the stationary phase will be observed. For the separation of neutral polar analytes (e.g. carbohydrates), no buffer is necessary.

Use of other salts such as 100-300mM sodium perchlorate, which are soluble in high-organic solvent mixtures (ca. 70%-90% acetonitrile), can be used to increase the mobile phase polarity to effect elution. These salts are not volatile, so this technique is less useful with a mass spectrometer as the detector. Usually a gradient (to increasing amounts of water) is enough to promote elution.

All ions partitions into the stationary phase to some degree, so an occasional "wash" with water is required to ensure a reproducible stationary phase

USES [5, 6]

The HILIC mode of separation is used extensively for separation of some biomolecules, organic and some inorganic molecules by differences in polarity. Its utility has increased due to the simplified sample preparation for biological samples, when analyzing for metabolites, since the metabolic process generally results in the addition of polar groups to enhance elimination from the cellular tissue. For the detection of polar compounds with the use of electrospray-ionization mass spectrometry as a chromatographic detector, HILIC can offer a tenfold increase in sensitivity over reversed-phase chromatography, because the organic solvent is much more volatile.

CHOICE OF pH [5, 6]

With surface chemistries that are weakly ionic, the choice of pH can affect the ionic nature of the column chemistry. Properly adjusted, the pH can be set to reduce the selectivity toward functional groups with the same charge as the column, or enhance it for oppositely charged functional groups. Similarly, the choice of pH affects the polarity of the solutes. However, for column surface chemistries that are strongly ionic, and thus resistant to pH values in the mid-range of the pH scale (pH 3.5-8.5), these separations will be reflective of the polarity of the analytes alone, and thus might be easier to understand when doing methods development.

WORKING OF HILIC [7, 8]

HILIC separates compounds by passing a hydrophobic or mostly organic mobile phase across a neutral hydrophilic stationary phase, causing solutes to elute in order of increasing hydrophilicity, -the inverse of RPC. With neutral peptides one may use 15mM ammonium formate and reverse organic conditions. Highly charged molecules require low amounts (e.g., 10 mM) of salt for ion suppression, and a slight perchlorate or sulfate gradient (in a high organic solvent concentration) to effect desorption.

It is commonly believed that in HILIC, the mobile phase forms a water-rich layer on the surface of the polar stationary phase vs. the water-deficient mobile phase, creating a liquid/liquid extraction system. The analyte is distributed between these two layers. However, HILIC is more than just simple partitioning and includes hydrogen donor interactions between neutral polar species as well as weak electrostatic mechanisms under the high organic solvent conditions used for retention. This distinguishes HILIC as a mechanism distinct from ion exchange chromatography. The more polar compounds will have a stronger interaction with the stationary aqueous layer than the less polar compounds. Thus, a separation based on a compound's polarity and degree of solvation takes place.

SEPARATION MECHANISMS [8-11]

The mechanism and theoretical description of analyte retention in HPLC has been the subject of many articles. Different research groups and scientific schools still disagree about the most realistic retention mechanism and the best theory to describe and predict it. There are essentially three possible ways to model the separation mechanism. The first is analyte partitioning between the mobile and stationary phases; the second is the adsorption of the analyte onto the surface of the adsorbent; the third assumes the preferential adsorption of the organic mobile phase modifier onto the adsorbent surface, followed by the partitioning of this analyte into the adsorbed layer. The retention phenomenon in HPLC simultaneously depends on various types of intermolecular interactions between the solute and the stationary phase, the solute and the mobile phase, and the stationary and mobile phases.

HILIC OF PEPTIDE AND PROTEINS [5, 6]

A good mobile phase to try is 10 mM TEA, pH 2.8, containing 80% acetonitrile. Run gradients as described above. If retention in inadequate, try 85% acetonitrile.

THE FOLLOWING FACTORS AFFECT RETENTION OF PEPTIDES IN HILIC:

1.Retention is proportional to the hydrophilicity of a peptide: Basic groups are the most hydrophilic, followed by phosphorylated residues. Thereafter, retention follows the opposite trend seen with reversed-phase HPLC: Asn promotes retention the most, followed by Ser-, Gly-, etc., with Phe- and Leu- promoting retention the least.

2. Juxtaposition of an acidic and basic residue: An acidic and a basic residue, or an acidic residue as the N-terminus, largely eliminates the normal retention effects of a basic residue.

3. Change in polarity with a change in pH: At pH 2.8, only basic and phosphorylated groups will be charged. At pH 5.0, both acidic and basic residues will be charged. This factor can be used to manipulate selectivity.

4. Retention proportional to the number of basic residues: In general, at pH 2.8 peptides will elute in order of increasing number of basic residues, as do cation-exchange separations. However, unlike cation-exchange, a particularly hydrophilic peptide can be retained more strongly than a hydrophobic peptide with more basic residues. Thus, the selectivity of the two methods is complementary.

HILIC OF SUGARS AND OLIGOSACCHARIDES:

No salt is necessary unless the carbohydrate is charged. The mobile phase should contain 80-85% acetonitrile (with much lower levels used with amino-sugars). Anomeric forms of reducing sugars are resolved.

HILIC OF OLIGONUCLEOTIDES

Try a salt gradient in 75% acetonitrile. C and G are retained much more than A and T, and may necessitate lower levels of acetonitrile.

HILIC OF PHOSPHOLIPIDS

Try a mobile phase of 15 mM ammonium formate pH 6.5 and 95% acetonitrile decreasing to 50%. Selectivity depends upon the pH and ionic strength.

HILIC OF DRUGS, SMALL MOLECULES AND METABOLITES

Retention will be the opposite of that with reverse-phase HPLC. Initially, try mobile phases with 80% acetonitrile. Some experimentation with the salt level and pH will be necessary in each case. Use of 60Å pore columns for underivatized amino acids and folic acid metabolites gives better retention.

ADVANTAGES

- Hydrophilic interaction chromatography (HILIC) is fast becoming the preferred technique when encountered with polar and/or basic solutes.
- In comparison to reversed-phase, HILIC affords several advantages making the technique in such cases.
- In particular, the kinetic advantages yield much higher solute diffusivity, increased sensitivity with ESI-MS and highly symmetrical peak shapes.
- In normal phase liquid chromatography (NP-LC), the stationary phase is more polar than the mobile phase. The retention increases as the polarity of the mobile phase decreases, and thus polar analytes are more strongly retained than nonpolar ones.
- Enhanced detection sensitivity when used in conjunction with mass spectrometry (MS). The high organic content of the mobile phase in HILIC allows for efficient spraying and desolvation in electrospray MS; a sensitivity increase of as much as three orders of magnitude was demonstrated for the analysis of the bronchodilator salbutamol in comparison with reversed-phase HPLC–MS.
- It might be possible to inject extracts directly from C18 solid-phase extraction (SPE) columns, from which solutes usually are eluted with high organic content. Use of an HPLC mechanism that is different from that of the sample preparation also introduces a degree of orthogonality into the overall procedure

DISADVANTAGES

- Heavy reliance on the aprotic solvent acetonitrile can be viewed as the major disadvantage of HILIC predominantly from a sourcing perspective.
- In light of the highly valued and well understood method development associated with reversed-phase liquid chromatography it could be perceived that HILIC is a less flexible technique.

APPLICATIONS [11-14]

1. Application of electrostatic repulsion hydrophilic interaction chromatography to the characterization of proteome, glycoproteome, and phosphoproteome using nano LC-MS/MS.
2. Application of hydrophilic interaction chromatography for the analysis of polar contaminants in food and environmental samples.
3. HILIC separations are very easy to combine with several detection techniques, such as ultraviolet light absorbance (UV), fluorescence (FL), refractive index (RI), evaporative light scattering (ELSD), charged aerosol (CAD), and mass spectrometry (MS)
4. HILIC–MS - High Resolution and Sensitivity for the Analysis of Very Polar Compounds
5. Alternate Selectivity for Polar Compounds in Hydrophilic Interaction Liquid Chromatography (HILIC) Using a New Amino Type HILIC Column
6. Improved hydrophilic interaction chromatography method for theidentification and quantification of glucosinolates
7. Hydrophilic InteractionChromatography Using Silica Columns for the Retention of Polar Analytes and Enhanced ESI-MS Sensitivity
8. Determination of the Stabilizer Sucrose in a Plasma-Derived Antithrombin Process Solution by Hydrophilic Interaction Chromatography with Evaporative Light-Scattering Detection

RECENT ADVANCES [15, 16]

HILIC WITH MS: Determination of γγ-Aminobutyric Acid in Food Matrices by Isotope Dilution Hydrophilic Interaction Chromatography Coupled to Mass Spectrometry:

The estimation of the dietary intake of γγ-aminobutyric acid (GABA) is dependent upon the knowledge of its concentration values in food matrices. To this end, an isotope dilution liquid chromatography–mass spectrometry method has been developed employing the hydrophilic interaction chromatography technique or analyte separation. This approach enabled accurate quantification of GABA in apple, potato, soybeans, and orange juice without the need of a pre- or post-column derivatization reaction. A selective and precise analytical measurement has been obtained with a triple quadrupole mass spectrometer operating in multiple reaction monitoring using the method of standard additions and GABA-d6 as an internal standard. The concentrations of GABA found in the matrices tested are 7 μg/g of apple, 342 μg/g of potatoes, 211 μg/g of soybeans, and 344 μg/mL of orange juice.

REFERENCES:

1. Alpert and Andrew J. Hydrophilic-interaction chromatography for the separation of peptides, nucleic acids and other polar compounds, *Journal of Chromatography*, **1999**; 499: 177–196.
2. Petrus Hemström and Knut Irgum. Review: Hydrophilic Interaction Chromatography, *J. Sep. Sci*. **1999**; 29: 1784–1821.
3. Hemstrom P and Irgum K. Hydrophilic interaction chromatography, *J Sep Sci*, **2006**; 29(12): 1784-1821.
4. Olsen BA. Hydrophilic interaction chromatography using amino and silica columns for the determination of polar pharmaceuticals and impurities, *J Chromatogr A*, **2001**; 913(1-2): 113-122.

5. Zhu B, Mant C and Hodges R. Mixed-Mode Hydrophilic and Ionic Interaction Chromatography Rivals Reversed-Phase Chromatography for the Separation of Peptides, *J. Chromatog*, **1992**; 594: 75-86.
6. Boutin. HILIC of phosphorylated peptides and tyrosine kinase reaction mixtures, J. Chromatogr, **1992**; 583: 137.
7. Hydrophilic Interaction Chromatography (HILIC) Promo Chrom technologies www.promochrom.com
8. Jaroniec M. *J Chromatogr A*, **1993**; 656: 37–50.
9. Gritti F, Félix G, Achard MF, Hardouin F. J Chromatogr A, **2001**; 922: 51–61.
10. Wolcott RG, Dolan JW, Snyder LR, Bakalyar SR, Arnold MA, Nichols JA. *J Chromatogr A*, **2000**; 869: 211–230.
11. Regina Roemling and Seiji Itoh HILIC–MS - High Resolution and Sensitivity for the Analysis of Very Polar Compounds, Tosoh Bioscience GmbH, Stuttgart, Germany.
12. Regina Roemling, M. Sakata, Y. Kawai,2 H. Yamasaki and H. Moriyama Alternate Selectivity for Polar Compounds in Hydrophilic Interaction Liquid Chromatography (HILIC) Using a New Amino Type HILIC Column, Tosoh Bioscience, Stuttgart, Germany, Tosoh Corporation, Tokyo, Japan.
13. Kristina L. Wade , Ian J. Garrard, Jed W. Fahey Improved hydrophilic interaction chromatography method for the identification and quantification of glucosinolates, *Journal of Chromatography A*, **2007**; 1154: 469–472.
14. Eric S. Grumbach, Diane M. Wagrowski-Diehl, Jeffrey R. Mazzeo, Bonnie Alden, and Pamela C. Iraneta, Hydrophilic Interaction Chromatography Using Silica Columns for the Retention of Polar Analytes and Enhanced ESI-MS Sensitivity, **2004**; 22(10): 1-10.
15. Raniero Zazzeroni, Andrew Homan, and Emma Thain, Determination of γγ-Aminobutyric Acid in Food Matrices by Isotope Dilution Hydrophilic Interaction Chromatography Coupled to Mass Spectrometry, *Journal of Chromatographic Science*, **2009**; 47: 1-12.
16. Joseph Pesek and Maria T. Matyska, A Comparision of Two Separation Modes : HILIC and Aqueous Normal Phase Chromatography, 1-9