HYDROPHILLIC INTERACTION LIQUID CHROMATOGRAPHY AS A TOOL FOR QUANTIFICATION OF POLAR ANALYTES

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***Abstract*: Hydrophilic Interaction liquid chromatography (HILIC) is a preferred seperation method for polar analyte as compare to reversed phase liquid chromatography. The HILIC methods were reported to be cost effetive, accurate, precise and robust especially for polar analytes such as glycans. The stationary phases mostly consist of different functional groups and dependng upon the column chemistry, it is further classified into neutral, charged, and zwitterion phases. HILIC mobile phases composition consist of high concentration of acetonitrile (60-97%) and a low concentration of water (3- 40) that make polar analytes to diffuse into surface of stationary phase for the interaction. The review article focued on basic principle, HILIC stationary phases and its application. Small and large hydrophilic and genuinely polar granules, such as glycans, peptides and proteins, nucleosides, vitamins, phenols, fungicides, venoms, and a wide range of polychrome hydrophilic metabolites are separated using the HILIC system from different pharmaceutical products, food samples, water sample and biological matrices.**

***Keywords*: HILIC; Stationary phase; Column; ACN; Water Layer.**

Introduction: In 1990, Andrew Alpert coined the term "Hydrophilic interaction liquid chromatography," which describes the separation of polar analytic solutes on stationary phase using a small constituent of the mobile phase, such as water. [1] Alpert proposed a technique for partitioning solute between bulk mobile phases and partially immobilised on the surface of the stationary phase. [2]HILIC has the potential to hold ionic and polar analytes that elute too rapidly in reversed-phase (RP) analysis, resulting in different selectivity than RP-LC. [3] Due to low viscosity and small back pressures, HILIC mobile phases usually include high percentages of acetonitrile (60-96%) and low concentrations of water (3-40%), even with reasonably long columns [4].
Since the 1970s, when high-performance liquid chromatography (HPLC) was introduced as an analytical separation technique, chromatographic techniques have been applied. Hydrophobic stationary phases with polar mobile phases are used in reverse-phase (RP) separations. There are certain limits to RP HPLC separations, such as studying very polar compounds. Even certain polar compounds are difficult to investigate due to the large aqueous buffer concentrations required. Another separation method, normal phase (NP)-HPLC, which combines a polar stationary phase and organic eluents to analyse polar compounds, syields low-efficiency separations with asymmetric chromatographic peaks. [5]Carbohydrates, amino acids, peptides and proteins, glycoproteins, nucleosides, vitamins, phenols, pesticides, toxins, and a wide range of other hydrophilic metabolites found in food, water, human fluids, and human tissue extracts are all separated using the HILIC approach [6– 13].HILIC is a type of normal phase liquid chromatography, however its separation mechanism is more complex than that of NP-LC. HILIC, like NP-LC, Traditional polar stationary phases such as silica, amino, or cyano are utilised in HILIC [8–12], but the mobile phase is identical to that used in RP-LC [7, 11, 12]. Ion chromatography, for example, uses HILIC to analyse charged compounds (IC).

Materials and methods :

➢ **Stationary Phases used in HILIC**

For separation of polar compound stationary phase is used which mainly retain hydrophilic or polar analytes. The stationary phases comprise a column with diverse chemical compositions such as normal phases column , amino and silica phases, and non-bonded silica containing columns for HILIC separations. For polar compound analysis, many new functionalized stationary phases, have been developed[14]

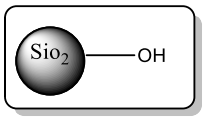
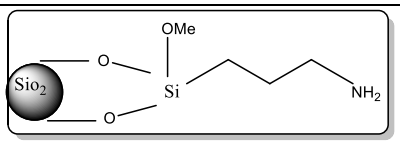
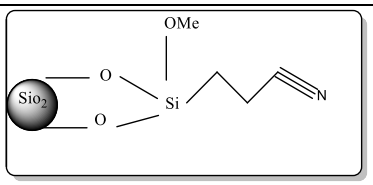
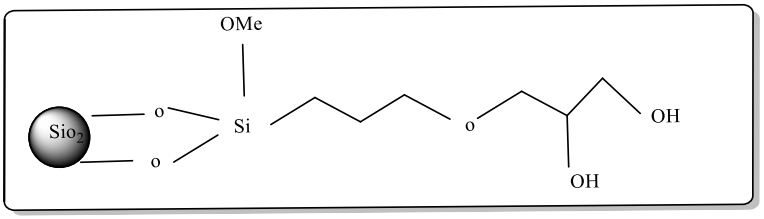
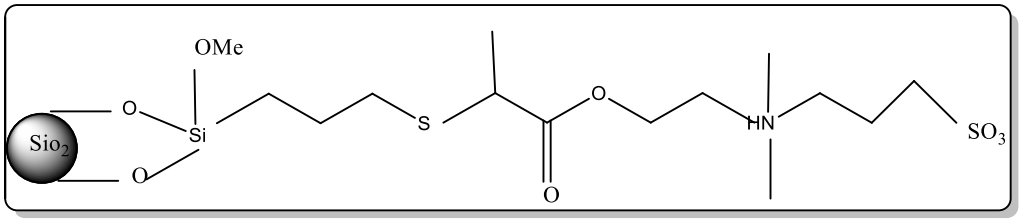
The HILIC stationary phases are classified on the basis of the charged state of the functional groups, and they can be further divided into neutral, charged, and zwitterion phases.

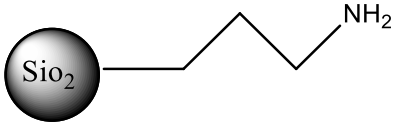
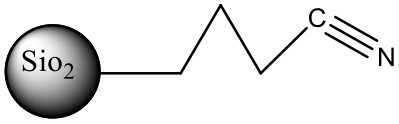
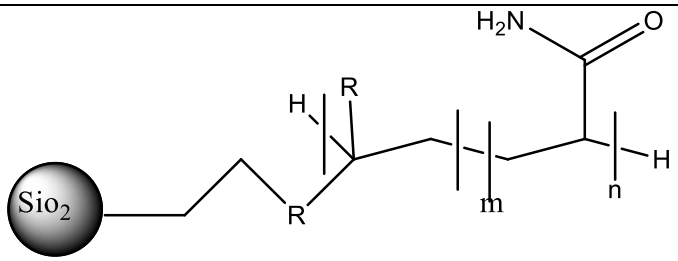
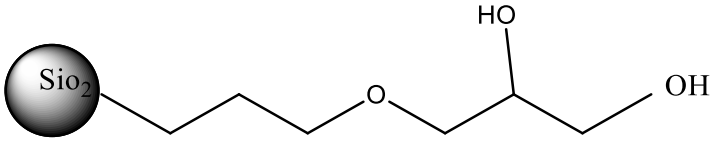
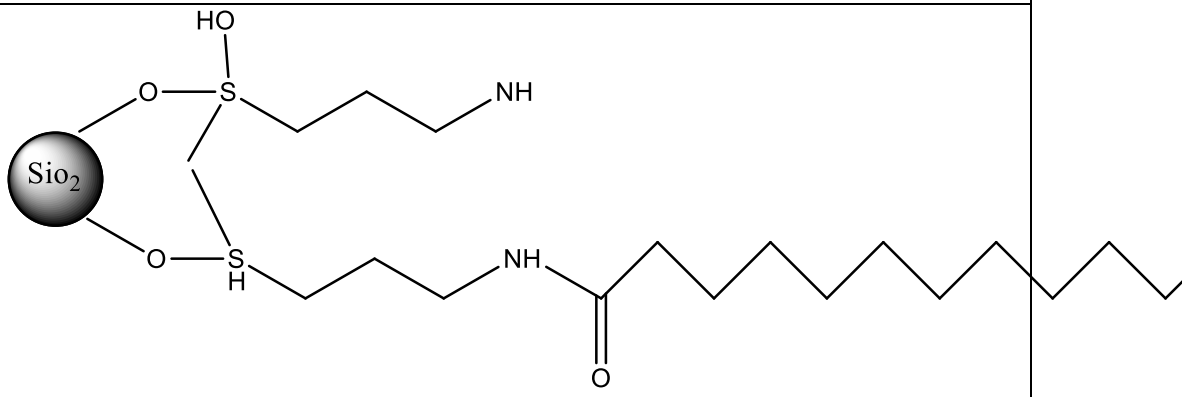
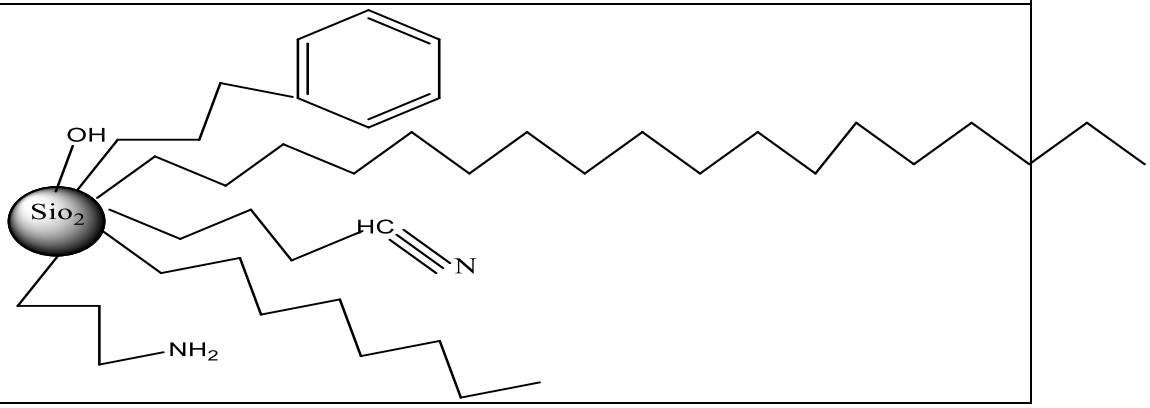
1. Neutral stationary phases : Neutral stationary phases contain functional group having no charge and polar in nature pH in the range of 3-8. There is no ion exchange interaction. Functional group in this type of stationary phases is amide, aspartame, diol, Ciano, cyclodextrine, and saccharides. [15, 16, 17] Amide stationary phases are prepared by functionalization of the silica gel surface with carbonyl or amide groups, linked through an alkyl spacer, the TSK gel 294 Amide-80 is one of the most popular phases extremely hydrophilic for the separation of oligosaccharides and peptides[18-19]

2. Charged stationary phases : charged stationary phases are those which bear positive or negative charge and therefore separation is based on ion exchange mechanism. In this type of polar stationary phases amino phases are used for HILIC. Amino propyl group bonded to silica. The primary amino group is positively charged and shows high affinity for anionic acid compounds [20]; therefore separation takes place by anion exchange mechanism. Amino phases are widely used in proteomic and metabolomics fields. Some applications of the amino phases include the separation of polar metabolites, nucleosides and biomarkers in human and animal samples and natural sweeteners in plant extracts. [21,22]

3. Zwitterionic stationary phases : Zwitterion stationary phases contain equal amounts of oppositely charged groups. The zwitterion ligand comprises generally a strongly acidic and strongly basic functionality. These types of stationary phases developed for separation of inorganic cations and anions in ion-exchange chromatography.

1. Structure of stationary phases used in HILIC

1) Silica Bare	
2) Amino Bonded phases	
3) Cyano Bonded Phases	
4) Diol bonded phases	
5) Zwitteri-onic sufobet-aine bonded phases	

Packing Material	Structure of stationary phases
Amino Bonded phases	
Cyano Bonded phases	
Amide Bonded phases	
Diol Bonded phases	
Alkyl amide	
Mix-mode	

➢ **Advantages**

• Hydrophilic intercourse chromatography(HILIC) is fast growing and also the preferred method for polar and/ or elemental solutes.
• HILIC offer several advantages making the method responsible over Normal and backward phase HPLC.
• The main advantage is that it has capacity to retain hydrophilic combinations without the need for cleansers which are venomous and premium matching as those generally used in normal- phase liquid chromatography, and also without significant quantity of seafarers or ion- pairing reagents, which hardly compatible with MS spotting [23].
• Because of its the strong beneficence of an ion exchange method, the achieved selectivity is generally certifiably different in HILIC as opposed to reversed- phase LC, for exemplification, in the case of peptides separation[24].
• Major benefit of the HILIC mode is the enhanced signal in MS, explained by effective desolvation of extensively organic mobile phases. A delicacy gain of upward to 10 times can be achieved in hydrophilic dealings liquid chromatography – electro spray ionization – mass spectrometry(HILIC – ESI – MS) compared with reversed- phase LC – ESI – MS[25].

➢ **Disadvantages**

1. The main disadvantage is that it depends heavily on aprotic solvent; Acetonitrile will be viewed as the major disadvantage of HILIC from a sourcing perspective.
2.HILIC is a less flexible technique over RP-HPLC methodology as most studies done on RP-HPLC.

- **Applications**

1. HILIC is used in the fields of pharmaceutical chemistry, proteomics, glycomics, metabolomics, agricultural and food chemistry.
2. HILIC mode is used to separate some bio molecules as well as both inorganic and organic molecules.
3. HILIC Using Silica Columns for the Retention of Polar Analytes and increase ESI-MS Sensitivity. It is used for the analysis of polar contaminants in food and environmental samples.
4. HILIC increase sensitivity over RPchromatography because the organic solvent is much more volatile.
5. Hydrophilic interaction chromatography method was used for both identification as well as quantification of glucosinolates. This method applied for the concurrent analysis of sugars and sulphonamide, Genotoxic impurities, including aryl amines and amino pyridines.
6. when combined with several detection techniques it show better separation , such as fluorescense (FL), ultraviolet light absorbance (UV), evaporative light scattering (ELSD), refractive index (RI), and mass spectrometry (MS), charged aerosol (CAD).
8 .Determination of the Stabilizer Sucrose in a Plasma-Derived Antithrombin Process Solution by HILIC with Evaporative Light-Scattering Detection .
10.HILIC is used for for separation of L-fucose, D-galactose, D-mannose, N-acetyl-D-glucosamine, N-acetylneuraminic acid, and D-glucuronic acid, based on an amide silica with evaporative light scattering detection.[26]
12. carbohydrates, peptide separations the first applications of HILIC and it has been shown on practically all commonly used HILIC stationary phases [27-28]. That HILIC has an inverse retention order relative to RP was shown by Alpert [29] for amino acids and for peptides by , using peptides specially designed for testing RP and ion exchange columns [30]
13. Quantification of all metabolites in a biological system, the expectations on separation science must be high [31]. HILIC has been recognized as an important tool in metabolomics, since metabolites are often very polar compounds.[31-32]
14. Due to their amphiphilicity, saponins can be separated both by RPLC [33] and HILIC.[34-35]

- **Conclusion**

HILIC is used for separation of polar fusions, advances in mass spectrometry developed for proteomics and metabolomics, it's now possible to characterize and identify thousands of fusions upon elution in chromatography. Compared to other chromatographic modes, HILIC could offer also several expressive benefits. The extensively organic content provides also lower back- pressure to enable fast separations under improved influx rates or to use columns with small scraps.

- **References**

[1] A.J. Alpert, Hydrophilic-Interaction Chromatography for the Separation of Peptides, Nucleic-Acids and Other Polar Compounds, J. Chromatogr., 499 (1990) 177-196. doi.org/10.1016/S0021-9673(00)96972-3.
[2] J.C. Linden, C.L. Lawhead, Liquid-Chromatography of Saccharides, J. Chromatogr., 105 (1975) 125-133. doi.org/10.1016/S0021-9673(01)81096-7
[3] J. Ruta, S. Rudaz, D.V. McCalley, J.L. Veuthey, D. Guillermo, A systematic investigation of the effect of sample diluent on peak shape in hydrophilic interaction liquid chromatography, J. Chromatogr. A, 1217 (2010) 8230-8240. doi.org/10.1016/j.chroma.2010.10.106
[4] Mahaveer Singh, S.G. Kashkhedikar, Love Soni, Abhinav Garg Tripti Gandhi, Amrish Patel. Development of RP-HPLC Method for Estimation of Carvedilol in Tablet Formulations. Research J. Pharm. and Tech. 1(1): Jan.-Mar. 2008; Page 18-21.
[5]. K. Shukla, S.S. Shukla, V. Jain, R. Pandey, S. Jain, Swarnlata Saraf, S. Saraf. Fingerprinting of Traditional Medicines through RAPD Technology: - A Newer Approach. Research J. Pharm. and Tech. 1(2): April-June. 2008; Page 63-68.
[6]. Alpert, A.J.; Hydrophilic-interaction chromatography for the separation of peptides, nucleic acids and other polar compounds; Journal of Chromatography, (1990); 499: 177– 196. doi.org/10.1016/S0021-9673(00)96972-3
[7]. Marrubini, G., Castillo Mendoza, B.E. and Massolini, G.; Separation of purine and pyrimidine bases and nucleosides by hydrophilic interaction chromatography; Journal of Separation Science, (2010); 33; 803–816. doi.org/10.1002/jssc.200900672
[8]. Karatapanis, A.E., Fiamegos, Y.C. and Stalikas, C.D; A revisit to the retention mechanism of hydrophilic interaction liquid chromatography using model organic 566 compounds; Journal of Chromatography A, (2011); 1218: 2871–2879. doi.org/10.1016/j.chroma.2011.02.069
[9]. Jandera, P. and Hájek, T.; Utilization of dual retention mechanism on columns with bonded PEG and diol stationary phases for adjusting the separation selectivity of phenolic and flavone natural antioxidants; Journal of Separation Science, (2009); 32; 3603–3619. doi.org/10.1002/jssc.200900344
[10]. Sowell, J., Fuqua. M. and Wood, T.; Quantification of total and free carnitine in human plasma by hydrophilic interaction liquid chromatographic tandem mass spectrometry; Journal of Chromatographic Science, (2011); 49: 463–468. doi.org/10.1093/chrsci/49.6.463
[11]. Zazzeroni, R., Homan, A. and Thain, E.; Determination of γ-aminobutyric acid in food matrices by isotope dilution hydrophilic interaction chromatographic coupled to mass spectrometry; Journal of Chromatographic Science, (2009); 47: 564–568. doi.org/10.1093/chromsci/47.7.564
[12]. Nezirević Dernroth, D., Arstrand, K., Greco, G., Panzella, L., Napolitano, A. and Kagedal, B. Pheomelanin-related benzothiazole isomers in the urine of patients with diffuse melanosis of melanoma; Clinica Chimica Acta, (2010); 411: 1195–1203. 29 doi.org/10.1016/j.cca.2010.04.019

[13]. Wang, Y., Wang, T., Shi, X., Wan, D., Zhang, P., He, X. et al. Analysis of acetylcholine, choline and butyrobetaine in human liver tissues by hydrophilic interaction liquid 581 chromatographic-tandem mass spectrometry; Journal of Pharmaceutical and Biomedical Analysis, (2008); 47: 870–875. doi.org/10.1016/j.jpba.2008.02.022
[14]. Jandera, P.; Stationary and mobile phases in hydrophilic interaction chromatography: a review; Analytical Chimica Acta, (2011); 692: 1–25. doi.org/10.1016/j.aca.2011.02.047
[15]. Pravallika, K.E., Ravi, P., Abboodi, A.H., Razzaq, A.H. and Sasivardhan, O., 2017. Development and validation of UV spectrophotometric methods for the estimation of vortioxetine hydrobromide in bulk and pharmaceutical dosage forms. Research Journal of Pharmacy and Technology, 10(11), pp.3928-3932. 10.5958/0974-360X.2017.00713.2
[16]. Sohan S. Chitlange, Imran, Sagar B. Wankhede, Dinesh M. Sakarkar. Simultaneous Estimation of Metoprolol and Amlodipine from Tablet Dosage Form. Research J. Pharm. and Tech. 1(2): April-June. 2008Page 80-82.
[17]. Amit Tapas, Dinesh Sakarkar, Rajendra Kakde. The Chemistry and Biology of Bioflavonoids. Research J. Pharm. and Tech. 1(3): July-Sept. 2008; Page 132-143
[18]. Tolstikov, V.V. and Fiehn, O.; Analysis of highly polar compounds of plant origin: 694 combination of hydrophilic interaction chromatography and electrospray ion trap mass 695 spectrometry; Analytical Biochemistry, (2002); 301: 298–307. 696 doi.org/10.1006/abio.2001.5513
[19] Schlichtherle-Cerny, H., Affolter, M. and Cerny, C.; Hydrophilic interaction liquid 697 chromatography coupled to electrospray mass spectrometry of small polar compounds in 698 food analysis; Analytical Chemistry, (2003); 75: 2349–2354 doi.org/10.1016/B978-0-12-805393-5.00006-3
[20]. Oyler, A.R., Armstrong, B.L., Cha, J.Y., Zhou, M.X., Yang, Q., Robinson, et al.; 716 Hydrophilic interaction chromatography on amino-silica phases complements reversed717 phase high-performance liquid chromatography and capillary electrophoresis for peptide 718 analysis; Journal of Chromatography A, (1996); 724: 378–383. 719 doi.org/10.1016/0021-9673(95)00987-6
[21]. Pucci, V., Giuliano, C., Zhang, R., Koeplinger, K.A., Leone, J.F., Monteagudo, E., et al.; 720 HILIC LC-MS for the determination of 2′-C-methyl-cytidine-triphosphate in rat liver; 721 Journal of Separation Science, (2009); 32: 1275–1283. 722 doi.org/10.1002/jssc.200800722
[22]. Junaedi EC, Lestari K, Muchtaridi M. Recent Quantitative Analysis of Stevioside from Stevia Rebaudiana (Bertoni): A Review. Research Journal of Pharmacy and Technology. 2020 Nov 1;13(11):5503-9.DOI:10.5958/0974-360X.2020.00961.0
[23]. Alpert AJ. Hydrophilic-interaction chromatography for the separation of peptides, nucleic acids and other polar compounds. Journal of chromatography A. 1990 Jan 19;499:177-96. doi.org/10.1016/S0021-9673(00)96972-3
[24]. Hemström P, Irgum K. Hydrophilic interaction chromatography. Journal of separation science. 2006 Aug;29(12):1784-821. doi.org/10.1002/jssc.200600199
[25]. Naidong W. Bioanalytical liquid chromatography tandem mass spectrometry methods on underivatized silica columns with aqueous/organic mobile phases. Journal of Chromatography B. 2003 Nov 5;796(2):209-24. doi.org/10.1016/j.jchromb.2003.08.026
[26] Di Palma S, Hennrich ML, Heck AJ, Mohammed S. Recent advances in peptide separation by multidimensional liquid chromatography for proteome analysis. Journal of proteomics. 2012 Jul 16;75(13):3791-813. doi.org/10.1016/j.jprot.2012.04.033
[27]. Buszewski, B. and Noga, S.; Hydrophilic interaction liquid chromatography (HILIC) – a 593 powerful separation technique; Analytical and Bioanalytical Chemistry, (2012); 402: 594 231–247.
[28]. Fekete S, Fekete J. The impact of extra-column band broadening on the chromatographic efficiency of 5 cm long narrow-bore very efficient columns. J Chromatogr A. 2011;1218(31):5286–5291. doi.org/10.1016/j.chroma.2011.06.045
[29]. Ammerhofer ML, Richter M, Wu J, Nogueira R, Bicker W. Mixed- mode ion-exchangers and their comparative chromatographic characterization in reversed-phase and hydrophilic interaction chromatography elution modes. J Sep Sci. 2008;31(14): 2572–2588. doi.org/10.1002/jssc.200800178
[30]. Dinh NP, Jonsson T, Irgum K. Probing the interaction mode in hydrophilic interaction chromatography. J Chromatogr A. 2011;1218(35): 5880–5891. doi.org/10.1016/j.chroma.2011.06.037
[31]. Srimathi, R., Priya, D. and Kumar, B.S., 2017. Determination of Melamine in Indian Milk and Dairy products by Reverse Phase Liquid Chromatography. Research Journal of Pharmacy and Technology, 10(12), pp.4269-4272. DOI : 10.5958/0974-360X.2017.00782.X
[32] Karlsson G, Winge S, Sandberg H. Separation of monosaccharides by hydrophilic interaction chromatography with evaporative light scattering detection. Journal of Chromatography A. 2005 Oct 28;1092(2):246-9. doi.org/10.1016/j.chroma.2005.08.025
[33] Nalini, C.N., Nithya, A., Afreen, A., Banu, A.A., Hemalatha, P. and Gayathri, E.J., 2018. Analytical methods for determination of lamivudine and their applicability in biological studies. Research Journal of Pharmacy and Technology, 11(11), pp.5166-5172. DOI:10.5958/0974-360X.2018.00944.7..
[34] Prathyusha P, Sundararajan R. UV spectrophotometric method for determination of Bilastine in bulk and pharmaceutical formulation. Research Journal of Pharmacy and Technology. 2020 Feb 15;13(2):933-8. DOI: 10.5958/0974-360X.2020.00176.6
[35]Kirtawade R, Salve P, Kulkarni A, Dhabale P. Hydrophilic Interaction Liquid Chromatography (HILIC). Research Journal of Pharmacy and Technology. 2010 Sep 28;3(3):640-3.