Revolutionary monolithic HPLC columns technology for excellent separations in liquid chromatography and column selection guide-fields of application

P. Ravisankar*, M. Nithya Satya, M. M. Eswarudu, A. Bhavani Sailu,
P. Srinivasa Babu, P. Sai Sreeja
Department of Pharmaceutical Analysis, Vignan Pharmacy College, Vadlamudi,
Guntur-522 213, Andhra Pradesh,India
Corresponding author: Prof. P. Ravisankar & id: banuman35@gmail.com

Abstract

Silica based monolithic HPLC column technology creates high porous rods of silica with a revolutionary bimodal pore structure. Macroporous (allow rapid flow up to 9 ml/minute and Mesoporous (creates large surface area) enables monolithic HPLC columns to provide excellent separations in a fraction of the time compared to a standard particulate column. Infact fast efficient separation and it always remains a big challenge in high-performance liquid chromatography (HPLC). Columns developed are rapidly moving towards having smaller particle sizes and internal diameters. Different monolith derivatization and the end-capping process will be elaborate and followed by the highlights of the performance such as monolithic columns. The stationary phase of monolithic columns contains a continuous porous material, sealed against the wall of a tube instead of beads. Decrease in improved sensitivity, less backpressure, sample usage, reusable frit less columns, chemical usage monolithic columns are very popular in the field of capillary electrochromatography.
Keywords:Monolithic HPLC columns, High-performance liquid chromatography, stationary phase, frit less columns, capillary electrochromatography, liquid chromatography columns.

I.INTRODUCTION

To truly accelerate chromatographic separation, there's no better choice than monolithic HPLC Columns [1-7]. Due to their revolutionary monolithic technology, columns provide excellent separation in a fraction of the time required by conventional particulate columns. The secret to the speed of monolithic HPLC columns is their exceptionally low backpressure. Produced from a continuous piece of porous silica using a sol-gel process, monolithic HPLC columns possess a defined bimodal pore structure with macropores (average 2 µm in diameter) and mesopores form the fine porous structure (130 Å) of the column interior and create a very large surface area on which adsorption of the target compounds can occur. The high permeability and porosity of the silica skeleton and the resulting low back pressure allow for more flexible flow rates than the particle-packed columns. As a result, HPLC columns enable high-throughput analysis without loss of separation efficiency or peak capacity. Fig. 1 represents electron microscopy photographs of monolithic silica inside a capillary. Fig. 2 represents mesoporous and macroporous revolutionary bimodal pore structures in monolithic HPLC columns. a. Silica monolithic column, b. Silica monolithic layer deposited on REP column, c. Polymer monolithic column, d. Silica monolithic layer deposited on the capillary column for use in open-tubular liquid chromatography, e. Silica monolithic column synthesized in pillar array column f. 3D printed monolithic column are

shown in Fig 3[8-10]. Fig 4 shows the Manufacturing process of the monolithic column.[11-13].

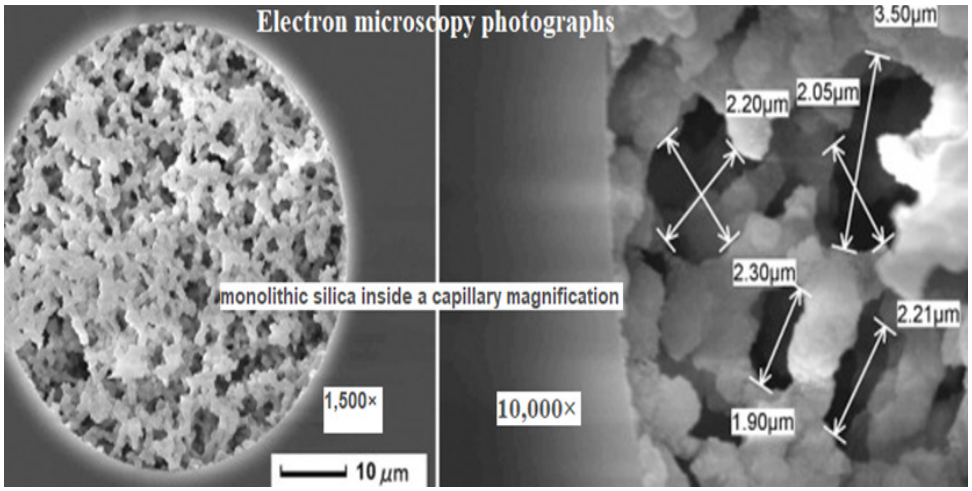

Fig. 1. Electron microscopy photographs of monolithic silica inside a capillary

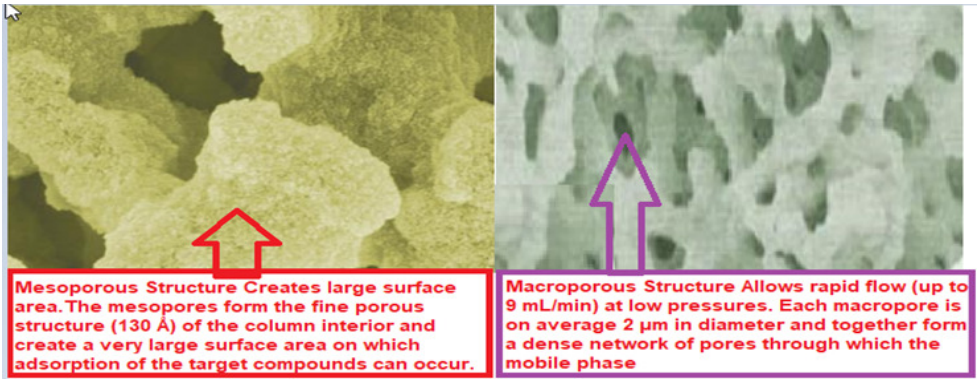

Fig. 2. Mesoporous and Macroporous revolutionary bimodal pore structures in monolithic HPLC column.

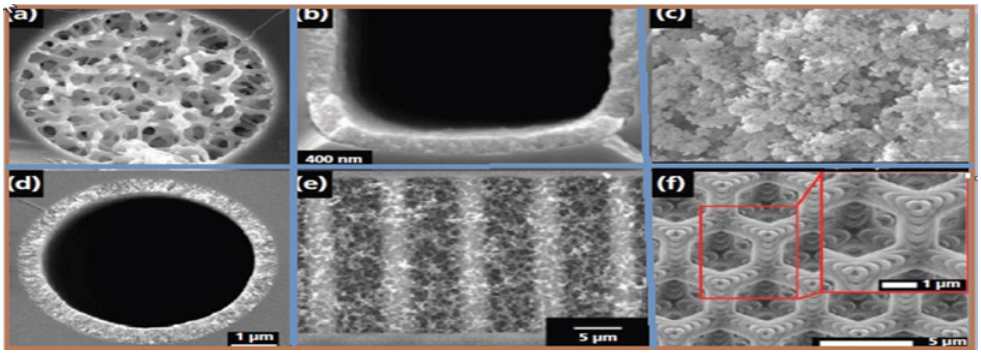

Fig. 3. a. Silica monolithic column, b. Silica monolithic layer deposited on REP column, c. Polymer monolithic column, d. Silica monolithic layer deposited on the capillarycolumn for use in open-tubular liquid chromatography, e. Silica monolithic column synthesized in pillar array column f. 3D printed monolithic column.

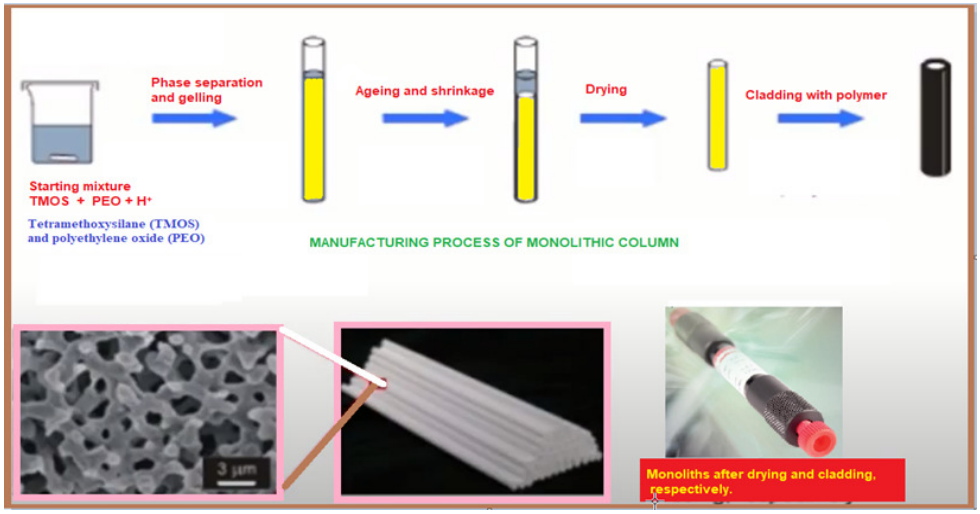

Fig. 4. The manufacturing process of the monolithic column.

II. Benefits andFields of application of monolithic columns

Benefits of monolithic HPLC columns:

- Cutting run times by more than half would be a significant benefit.
- Suitable use for standard HPLC instruments.
- Dramatically increase throughput.
- Rapid screening.
- Compatible with all mass spectrometers.
- Improved HPLC system security- robustness, versatility, reliability, etc.,
- High flow rates at low pressure.
- Available in various sizes and modifications.
- Compatible with various organic solvents-ethanol, isopropanol, methanol, etc.,
- Substantially longer column lifetime.
- Higher porosity allows fast adsorption and desorption kinetics.
- Fast Van Deemter curve allows flow rate flexibility and flows gradients.
- Compatible with various organic solvents, e.g., Ethanol, methanol, Isopropyl alcohol.
- Column length no longer pressures limited.
- Cost savings from higher sample throughput offset the expense of method revalidation in one month.
- Monolithic porous silica rod columns allow high flow rates due to high porosity.
- Low backpressure so less stress on system and column.
- Increased throughput that's why significantly shorter run time.
- No inlet bed setting so increased reliability, reproducibility, and lifetime also.
- Monolithic silica technology is the solution for reduced sample loading time and column equilibration time; more analysis time means more proteins identified in complex mixtures.

Methods should be validated to include consideration of characteristics included in the International Conference on Harmonization (ICH) guidelines [14-23]. Linearity, precision, accuracy, selectivity, limits of detection and quantification, and robustness were used as the validation parameters.

Table 1. Fields of application of monolithic columns (column selection guide)

Monolithic silica HPLC columns	Field of applications	Columns with some successful separation cases
RP-18 end-capped columns	Aflatoxin, aldehydes, alkaloids, antibiotics, explosives, esters, fat-soluble vitamins, lipids, fatty acids, flavonoids, ketones, nitrosamines, PAH, PCB, peptides, pesticides, phenols, phthalates, preservatives, steroids, metabolized steroids, sulfonamides, sweeteners, water-soluble vitamins.	Alcohols, aliphatic amines, amino acids, aromatic amines, carotenoids, oils, nucleotides, phospholipids, proteins.
High-resolution RP-18 end-capped columns	Aflatoxin, aldehydes, alkaloids, antibiotics, explosives, esters, fat-soluble vitamins, lipids, fatty acids, flavonoids, ketones, nitrosamines, PAH, PCB, peptides, pesticides, phenols, phthalates, preservatives, steroids, metabolized steroids, sulfonamides, sweeteners, water-soluble vitamins.	Alcohols, aliphatic amines, amino acids, aromatic amines, carotenoids, oils, nucleotides, phospholipids, proteins.
RP-8 end-capped columns	Amino acids, esters, proteins.	Aflatoxins, aldehydes, alkaloids, antibiotics, fat-soluble vitamins, ketones, nucleotides, PCB, steroids, metabolized steroids.
Phenyl columns	Aromatic amines, flavonoids, PAH, peptides, preservatives.	Aldehydes, alkaloids, antibiotics, fat-soluble vitamins, ketones, nucleotides, PCB, metabolized steroids, sweeteners.
Cyano columns (CN)	Flavonoids, phenols, phthalates, steroids.	Alcohols, oils, glycols, sulfonamides.
DIOL columns	Amino acids, carotenoids, preservatives, proteins, sugars, water-soluble vitamins.	Alcohols, oils, glycols, phospholipids, sulfonamides.
Silica columns (Si)	Carotenoids, oils, fat-soluble vitamins, lipids, fatty acids.	Aflatoxins, flavonoids, nucleotides, phospholipids, glycols,steroids.And Ketones.
Amino columns (NH_2)	Carboxylic acids, esters, nucleotides, steroids, sugars, sugar alcohols, sweeteners, water-soluble vitamins.	Alcohols.

The most suitable column modification can be easily selected and the application areas include drug abuse, vitamins, porphyrins, proteins, sulphonamides, aflatoxins, nucleotides, antibiotics, phospholipids, glycols, carotenoids,therapeutic drug monitoring, and also differentfields of application of monolithic columns are presented in table 1.[24-35].

III. Characterization of monolithic HPLC columns

The monolithic HPLC column comprises a single rod of high purity polymeric silica gel with a bimodal pore structure of macropores and mesopores. This unique construction enables highly efficient separations at high speeds.

3.1. Analysis speed

Monolithic HPLC columns own their rapid separation speed to their unique bimodal pore structure of macropores and mesopores. The macropores reduce column back pressure and allow the use of faster flow rates, thereby considerably dropping analysis time. The mesopores form a fine porous structure, which creates a very huge active surface area for high-efficiency separations.

3.2. Flow programming

Monolithic HPLC columns respond very quickly to changes in flow rate, giving you the maximum flexibility in flow programming. Rates can be adjusted in mid-flow either to augment the peak definition of the target compound or to shorten the total separation time once the compound has fruitfully eluted. This enables clear separation of two closely eluting peaks, without significantly affecting the total run time. A mid-flow change in rate can also decrease the total run time when certain compounds elute much later than others. High separation efficiency. The traditional plate-count method of measuring quality shows that the separation efficiency of Monolithic HPLC columns is better than standard 5 µm particulate columns and just as good as 3.5µm columns, but with the ability to continue up to 9 mL/min without reaching HPLC system pressure limits. The van Deemter plot of the HPLC column demonstrates that separation efficiency does not decrease significantly when the flow rate is increased, as is the case with particulate columns. It is therefore possible to operate HPLC columns at high flow rates with minimal loss of peak resolution.

3.3. Long-term stability

Moreover lower back pressure and greater flow rate flexibility, HPLC columns also achieve faster equilibration after gradient elution than particle-packed columns of alike dimensions. These features allow high-throughput analysis – without loss of separation efficiency or peak capacity.

3.4. Column robustness

Monolithic HPLC columns offer exceptional robustness and unsurpassed column lifetime. This not only ensures maximum reliability and versatility but also minimizes maintenance on the HPLC system. As a result, HPLC columns reduce costs per analysis while enhancing data integrity.

3.5. Monolithic Capillary columns

Monolithic capillary columns have become increasingly important in the separation of biomolecules, especially in combination with mass spectrometry. In contrast to particulate columns, monolithic capillaries do not require frits and have a much lower tendency to clog. This allows higher flow rates, improving the speed and quality of biomolecule characterization.

3.5.1. Benefits of capillary columns

- Robust and easy handling.
- Higher flow rates than particle-packed capillary columns at low pressure.
- Long column lifetime and ideal compatibility with LC/MS.
- Able to accommodate large sample capacities and provide higher sensitivity.
- Useful for trace component analysis or purity screening using direct injections.

3.6. Monolithic Analytical columns

Standard HPLC columns with 3 or 5 µm silica particles often suffer from high backpressure. HPLC columns are not packed with small particles. Instead, each column consists of a single monolithic rod of high-purity polymeric silica gel with a revolutionary bimodal pore structure. This allows excellent separations in the fraction of time that the standard particulate column takes.

3.6.1. Benefits of monolithic analytical columns

- Possibility of flow gradients.
- Compatible with all low dead volume LC instruments (UHPLC, UPLC, HPLC).
- Cost savings from higher sample throughput and column durability.
- Added column performance by column coupling.
- Substantially longer column lifetime.
- High resistance to column blockage.
- Very fast, high-performance results.

3. 7. Monolithic RP-18 end-capped HPLC columns

The chemical basis of monolithic HPLC RP-18 end-capped columns – from starting materials to surface modifications – is the same as high-end particulate columns. This allows the use of standard methods when developing new protocols. The columns are based on high-purity silica, hence they minimize the negative effect of trace metals. they are chemically modified with n-alkyl chains that possess a high ligand density, and fully end-capped to reduce the effect of unmodified silanol groups.Ultra-high performance and extremely low operating pressure make HPLC 2 mm columns truly unique. Excellent, ultra-fast results are obtained, not only in the new UHPLC and UPLC instruments. HPLC 2 mm columns have macropores of 1.5 µm in diameter, resulting in a column efficiency that exceeds 100,000 plates/meter. The mesopores are 13 nm (130 A) in diameter, and the surface modification is octa decylilane with full end-capping.

3.7.1. Monolithic HPLC high resolution i.e. RP-18 end-capped columns:

The faster way to trouble-free separations HPLC 4.6 mm i.d. columns represents the most commonly used column dimension. They are compatible with all standard HPLC instruments, and allow a wide range of flow rates, from 0.6 to 4.5 mL/min. These columns are available in two versions: standard columns with 2 µm macropores, and High-Resolution columns with 1.15 µm macropores. Standard HPLC columns have 2 µm macropores, and

efficiency equal to 4.5 μm particulate columns. They allow very high flow rates, extreme throughputs, and the analysis of relatively dirty samples. The lifetime of these columns is particularly long. In contrast, HPLC High-resolution (HR) columns possess 1.15 μm macropores.

3.7.2. Monolithic RP-8 end HPLC capped columns

It offers less retention and slightly different selectivity than HPLC RP-18 end-capped. Thus, it is possible to achieve a baseline separation on the RP-8 end-capped bonded column, HPLC RP-8 end-capped HPLC columns offer all the benefits of monolithic silica technology for reversed-phase chromatography.

3.8. Monolithic Phenyl HPLC columns

Due to their π-π interactions, HPLC Phenyl HPLC columns offer greater selectivity towards aromatic ring-containing compounds than standard alkyl phases. These columns are ideal for the separation of aromatic compounds, flavonoids, fatty acids, PAH, preservatives, purines, and pyrimidines.

3.9. Monolithic CN HPLC columns

Cyano columns are generally more polar than traditional alkyl silica columns. The modification also allows cation exchange activity, which is higher at neutral pH than in acidic conditions. HPLC CN columns are suitable for the separation of alkaloids, oils, flavonoids, glycols, phenols, phthalates, steroids, and sulfonamides.

3.10. MonolithicDiol HPLC columns

HPLC Diol columns are more versatile than bare silica columns, and often offer improved reproducibility. The bonded phase's hydroxyl groups offer good selectivity without excessive retention. This is due to weaker hydrogen bonding with diol groups. Columns are commonly used for the separation of steroids, sterols under normal-phase conditions and suitable for the separation of alcohols, amino acids, carotenoids, oils, glycols, preservatives, proteins, sugars, sulfonamides, water-soluble vitamins.

3.11. Monolithic NH_2 HPLC columns

HPLC amino propyl-modified columns possess medium polarity. Therefore, they display hydrophilic as well as hydrophobic properties and can be used under both reversed-phase and normal-phase conditions. In acidic solutions, the amino groups are protonated (-NH3 +X–) Hence, the columns can also be used as ion exchangers. HPLC amino columns offer high matrix tolerance and analysis speed, as well as an extended lifetime within the pH range of 2.5 to 7.5. These columns are suitable for the separation of anions, organic acids, and carbohydrates.

3.12. Monolithic Preparative columns

Monolithic HPLC Preparative and Semi Preparative HPLC columns are ideal The excellent accessibility of the mesopores (total porosity > 80 %), and the short diffusion length inside the pores ensure fast adsorption and desorption kinetics. This leads to faster separations and higher productivity. Column reliability, reproducibility, and extended lifetime are ensured.Higher productivity and greater efficiency than particulate sorbents.

3.13. Monolithic HPLC Semi Preparative preparativecolumns

Perfect scale-up from analytical to preparative LC Optimum separation at flow rates exceeding 40 mL/min Semi Preparative HPLC columns are ideally suited for direct scale-up from analytical to semi-prep. This is because they offer faster sample throughput at a lower operating pressure compared to semi-prep columns packed with 5 µm particles. They have the same bimodal porous silica rod structure as analytical columns with an internal diameter. Preparative HPLC involves much higher sample volumes than analytical chromatography. The combination of macro and mesopores maximizes separation efficiency and flow rate while minimizing resistance. Analytical separations can be easily transferred to HPLC Semi Preparative and Preparative columns by linear transfer methods.

3.14. Monolithic HPLC guard cartridges and kits

Monolithic columns are well known for their robustness. The guard columns are chemically modified with hydrophobic n-octadecyl (C_{18}) groups on the surface of the monolithic silica rod, making them suitable for reversed-phase chromatography.

3.15. Guard cartridges

HPLC guard cartridges are extremely easy to use. They are simply added directly in front of the main column to protect it from chemical or mechanical contamination. Moreover, guard columns can be used as trap columns when large sample volumes are to be injected. Guard columns should be changed frequently to avoid excessive accumulation of impurities.

3.16. Guard cartridge starter kit

The HPLC guard cartridge kit includes everything needed to significantly enhance the lifetime of monolithic columns: a guard cartridge holder, and three guard cartridges.

3.17. Monolithic HPLC Column coupler

The HPLC column coupler is intended for linking several monolithic columns together to further increase separation efficiency and column performance. The combination results in a theoretical plate count that is significantly higher than any particulate column available. This makes column coupling perfect for chromatographic separations of typically non-separatable.

IV. CONCLUSION

In the current situation, the development of faster separation processes is one of the most important issues in the HPLC. Chiefly in industry, chromatographers wish to speed up separations and analyze lots of samples with the limited financial and human resources available. So in current condition, the monolithic silica column is very promising. The key reason for monolithic column's popularity in an analytical arena is because of their principle and minimize backpressure to maximize speed. It delivers a systematic approach to modify and optimize the sizes of the dissimilar geometrical elements separately, which is essential to do chromatographic separations, the through pores, the mesopores, the domains, and the porons. Indeed traditional silica particulate columns are having high flow resistance, high backpressure, reduced throughput, and bed splitting possible. Infact monolithic columns are comparable concerning selectivity, reproducibility, and performance. Some authors have claimed that monolithic silica columns are more stable than packed ones due to their rigid silica structure. One of the most important features of a monolithic column is its high permeability, so it can be operated at a high flow rate of up to 10 mL/min, thus allowing fast separations of various mixtures. Therefore, monolithic silica columns appear to show a great possibility for the near future as further advances may lead to heightened efficiency which will be needed in the remarkable field of high throughput and bioanalytical analysis.

References

1. Khoo HT, Leow CH. Advancements in the preparation and application of monolithic silica columns for efficient separation in liquid chromatography. Talanta. 2020;224:121777.
2. Gaurav Sharma, Anjali Tara, Vishnu Dutt Sharma. Advances in monolithic silica columns for high-performance liquid chromatography. Journal of Analytical Science and Technology. 2017, 8:16.
3. Al-Bokari M, Cherrak D, Guiochon G. Determination of the porosities of monolithic columns by inverse size-exclusion chromatography. J Chromatogr A. 2002;975(2) :275–84.
4. Vuignier K, Fekete S, Carrupt P, Veuthey J,Guillarme D. Comparison of various silica-based monoliths for the analysis of large biomolecules? J. Sep. Sci. 2013, 36, 2231–2243.
5. Aggarwal P, Tolley H.D, Lee M.L. Monolithic bed structure for capillary liquid chromatography. J. Chromatogr. A 2012, 1219, 1–14.
6. Cabrera K, Lubda D, Eggenweiler H,Minakuchi H, Nakanishi K. A new monolithic-type HPLC column for fast separations. J. High Res. Chrom. 2000, 23, 93–99.

7. Cabooter D, Broeckhoven K, Sterken R, Vanmessen A, Vandendael I, Nakanishi K, Deridder S, Desmet G. Detailed characterization of the kinetic performance of first and second generation silica monolithic columns for reversed-phase chromatography separations. J. Chromatogr. A 2014, 1325, 72–82.

8. Detobel F, Eghbali H, De Bruyne S, Terryn H, Gardeniers H, and Desmet G, J. Chromatogr. A 2009, 1216, 7360–7367.

9. Futagami S, Hara T, Ottevaere H, Baron G.V, De MalscheW., J. Chromatogr. A 2017,1523, 234–241.

10. Hara T, Futagami S, Eeltink S, De Malsche W, Baron G. V, and Desmet G, Anal. Chem. 2016,88, 10158–10166.

11. Núñez O, Nakanishi K, Tanaka N. Preparation of monolithic silica columns for high-performance liquid chromatography. J. Chromatogr. A 2008, 1191, 231–252.

12. Svec, F. Porous polymer monoliths: Amazingly wide variety of techniques enabling their preparation. J. Chromatogr. A 2010, 1217(6),902–924.

13. Nováková L, Vlčková H. A review of current trends and advances in modern bio-analytical methods: Chromatography and sample preparation. Anal. Chim. Acta 2009, 656, 8–35.

14. Lavanya Chowdary G, Ravisankar P, Akhil Kumar G, Mounika K, Srinivasa Babu P, Analytical Method Validation Parameters: An Updated Review, Int. J. Pharm. Sci. Rev. Res., 2020, 61(2), 1-7.

15. P. Ravisankar, Abhinav Pentyala, Ch. Baladatta Sai, P.Hemasri,P. Srinivasa Babu,Validation characteristics and statistics inanalytical method development, High technology letters, 2021,21(7),76-88.

16. International Conference on Harmonization (ICH), Q2A: Text on Validation of Analytical Procedures, March 1995.

17. International Conference on Harmonization (ICH), Q2B: Validation of Analytical Procedures: Methodology, May 1997.

18. Validation of Compendial Methods, , U.S. Pharmacopoeia 26-National Formulary 21, United States Pharmacopeial Convention, Rockville MD, 2003.

19. Q2 (R1), Validation of analytical procedures, Text, and methodology, International Conference on Harmonization, Geneva, 2005,1-17.

20. Ravisankar P, Naga Navya Ch, Pravallika D, Navya Sri D, A review on step-by-step analytical method validation, IOSR Journal of Pharmacy, 2015;5:7-19.

21. Ravisankar P, Sai Geethika A, Racgana G, Srinivasa Babu P, Bhargavi J, Bioanalytical method validation: A comprehensive review, Int.J. Pharm. Sci. Rev. Res, 2019;56(1),50-58.

22. Ravisankar P, Gowthami S, Devala Rao G, A review on analytical method development, Indian journal of research in pharmacy and Biotechnology, 2014;2:1183-1195.

23. Ravisankar Panchumarthy, Anusha S, Supriya K, Ajith Kumar U, Fundamental chromatographic parameters, Int. J. Pharm. Sci. Rev,Res.2019;55(2):46-50

24. Ravi Sankar P, Sai Snehalatha K, TabassumFirdoseShaik, Srinivasa Babu P, Applications of HPLC in Pharmaceutical Analysis, Int.J.Pharm.Sci.Rev.Res. 2019;59(1):117-124.

25. Cabrera K. Applications of silica-based monolithic HPLC columns. J Sep Sci. 2004;27(10–11):843–52.

26. Chen J, Zhang P, Jia L. Ionic liquids-assisted fabrication of silica-based monolithic columns. J Chromatogr A. 2011;1218(23):3699–703.

27. Cabrera, K. Applications of silica-based monolithic HPLC columns. J. Sep. Sci. 2004, 27, 843–852.

28. Deeb SE, Preu L, Wätzig H. Evaluation of monolithic HPLC columns for various pharmaceutical separations: method transfer from conventional phases and batch to batch repeatability. J Pharm Biomed Anal. 2007;44(1):85–95.

29. Svec, F, Kurganov A.A. Less common applications of monoliths: III. Gas chromatography. J. Chromatogr. A 2008, 1184, 281–295.

30. Jandera, P. Advances in the development of organic polymer monolithic columns and their applications in food analysis—A review. J. Chromatogr. A 2013, 1313, 37–53.

31. Núñez O, Gallart-Ayala H, Martins C.P.B, Lucci P. New trends in fast liquid chromatography for food and environmental analysis. J. Chromatogr. A 2012, 1228, 298–323.

32. Samanidou, V.F.; Karageorgou, E.G. An overview of the use of monoliths in sample preparation and analysis of milk. J. Sep. Sci. 2011, 34, 2013–2025.

33. Krenkova J, Svec F. Less common applications of monoliths: IV. Recent developments in immobilized enzyme reactors for proteomics and biotechnology. J. Sep. Sci. 2009, 32, 706–718.

34. Ma C, Chen H, Sun N, Ye Y, Chen H. Preparation of molecularly imprinted polymer monolith with an analogue of thiamphenicol and application to selective solid-phase microextraction. Food Anal. Method. 2012, 5, 1267–1275.

35. Chen, Y, Wang, K, Yang, H, Liu Y, Yao S, Chen B, Xu G. Synthesis of sulfo/vinyl biphasic silica hybrid monolithic capillary column and its application to on-column preconcentration for capillary electrochromatography. Journal of chromatography a, 2012,1233, 91-99.