Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 5, Suppl 3, 2013

Review Article

DEVELOPMENT OF FUSED-CORE SILICA HPLC COLUMNS AND THEIR RECENT PHARMACEUTICAL AND BIOLOGICAL APPLICATIONS: A REVIEW

RUWIDA KAMOUR[1]*, AMAL AMMAR[1], MOHAMED EL-ATTUG[1], TARIQ ALMOG[1]

[1]Department of Medicinal and Pharmaceutical Chemistry, Faculty of Pharmacy, University of Tripoli, Tripoli, Libya.
Email: ruwidakamour@yahoo.co.uk

Received: 22 Jun 2013, Revised and Accepted: 03 Aug 2013

ABSTRACT

In many analytical procedures fast and reliable analysis technique is required. The development of HPLC stationary phases were in line with these goals. To achieve these goals of fast and efficient analysis, work was focused on reducing particle size of packing materials. Therefore, the development of superficially porous silica particles provided a new approach with overwhelming operating results to insure fast and reliable analysis at decreased system back pressure values.

This work presents features and development aspects of fused core technology. Also it describes their chromatographic properties in comparison with monolithic and sub-2 µm particle columns. In addition, there is a discussion of its applicability to be used with conventional HPLC instruments. Finally, it outlines some of its recent applications in biological and pharmaceutical field.

Keywords: High pressure liquid chromatography, Core-shell particle columns, Sub-2 µm particle columns, Monolithic columns, Back pressure.

INTRODUCTION

HPLC methods have been used for the analysis of many products for various purposes. The advantages of HPLC allowed the possibility of analysing wide variety of compounds. A specific analytical procedure is usually designed to match a certain goal such as selectivity, sensitivity, LOD, quick analysis time and reproducibility which are basically accustomed by the properties of the compound being analyzed. Therefore, a single method for a particular compound should be of high sensitivity and selectivity and short analysis time. The packing materials of HPLC columns are the key function of the separation technique. Therefore, researchers have been pushed to use new column technologies to compromise the demand for high sample throughput with fast analysis. Recently, there have been many silica stationary phases developed and commercially available such as non-porous, porous, superficially porous and monolithic silica. These columns are widely applied for analysis of pharmaceutical and biological samples [1].

The porosity of the packing material has a direct relation with the pressure of the elution system, the size of molecules separated as well as speed of analysis. Lately, approaches of HPLC technologies have interested in increasing the speed of analysis [2, 3, 4]. This approach should be run in balance with low back pressure and maximum column efficiencies.

The development of fused-core silica technology has brought advantages over monolithic and sub-2 µm particle columns. Their principle work aims to speed analysis time preserving column efficiency with comparatively low back pressure [5, 6].

This review is driven in an attempt to provide an overview about different facts related to fused-core silica columns regarding the development of this technology and their chromatographic features compared to conventional and monolithic columns. Also there will be a review of their application in the field of pharmaceutical and biological analysis.

Structure and development

The fused-core particle technology, also named superficially porous or shell particles, was first developed by Jack Kirkland where it was intended to achieve faster separation with higher sample throughput with maintaining column reliability [7, 8].

Commercially, there are 3 new brands of shell particles available; Poroshell (Agilant, Little River, DE, USA), Halo (Advanced Material Technology, DE, USA), and Kinetex (Phenomenex, Torrance, CA, USA) [9, 10, 11, 12]. Poroshell have particle size of 5 µm and shell thickness of 0.25 µm. It was reported that they have 50% larger peak capacity than Zorbax column which packed with fully porous silica of same average particle size and of same silica material as that of the shell of the Poroshell [12].

The Advanced Material Technology has made this approach commercially available which is known as HALO HPLC column which consists of 2.7 µm particles are manufactured by fusing small silica particles as a porous layer (0.5µm in thickness) onto 1.7 µm solid silica particle . The porous layer has 9 nm pores, Fig. 1. These columns are available as different bonded reversed phase chemistry [13]. The steps of forming the solid core and adhering the porous shell into it are described in details by Kirkland [14]. The particle size distribution of the packed particles is very narrow which results in narrow diffusion path length and thus sharper peaks, Fig. 2. The porous layer designed to permit faster penetration of the mobile phase and therefore faster separation with modest loss in column efficiency and at low back pressure [15, 16].

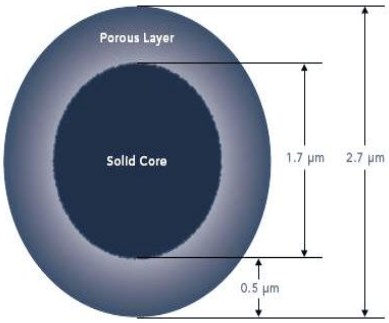

Fig. 1: graphical illustration of the structure of the fused-core partile. HALO particle from Advanced Materials Technology

Fig. 2: Scanning Electron Microscope (SEM) photo of HALO particles. It can be seen clearly the very narrow size distribution of particles in addition to the porous layer of particles.

The kinetex was first made commercially available in 2009. They have particle size average of 2.5 µm (close to that of Halo) however; they demonstrated better efficiency than Halo-packed columns [11, 17]. Gratti and his colleagues [18] conducted a study to compare the physical properties of these three packing materials in relation to chromatographic column performances. They studied the differences between Kinetex and Halo as well. The latter two were found to have five different physical properties that have lead to differences in their performances.

Separation using a column packed with such particles occurs in less than one minute for low molecular weight analytes generating approximately 300 bar column pressure [5]. This approach, superficially porous silica columns, is also applicable for separating macromolecules at higher velocities. In Kirkland and colleagues study [14], the resulted very fast separation (< 2min) showed good resolution; sharp narrow peaks and low system back pressure suggesting excellent kinetic properties for a gradient flow-HPLC system.

In 2010, Kirkland and co-authors [19] described a newly designed Halo particle packed columns with 160A° mesopore size compared to classical 90A°. These columns were released by Advanced Material technology as Halo-ES peptide which was designed particularly for rapid separation of peptides and some small proteins. Gritti and Guiochon [20] tested the kinetic properties of these columns compared to standard Halo 90A° by using protein insulin, β-lipoprotein in addition to other small molecules like uracil and toluene (MW < 150). They found a substantial decrease in transfer resistance which owed to the larger pour size of the porous shell. These improvements were noticed for large molecules but not markedly seen for small molecules even though the increase in mesopore size was accompanied by increase in the fraction of the surface area of the particles of the Halo-ES.

Chromatographic properties of fused-core silica columns

The high efficiency of superficially porous particles has made it one of considerable interests for fast and reliable HPLC separation.

One of the features of this technology is the very fast elution times which are attributed to the fused/solid core of the particles and thin porous layer. This structure creates shorter elution baths and permits faster permeation of un-retained molecules compared to fully porous particles [21, 22].

An experiment carried out by DeStefano and co-authors [9] were a comparison of performance of fused-core column with sub-2 µm particle column. It was found that these columns perform at high efficiencies that are comparable to that of sub-2 µm particle columns but with much lower back pressure, Fig. 3. In addition, they demonstrated a very fast separation of mixtures of pesticides in less than one minute, Fig. 4, and explosives (less than 4min). Also the study approved that these columns reduced plate height up to 1.2 compared to 2 for totally porous silica columns with lowest pressure value patterns as mobile phase velocity increased.

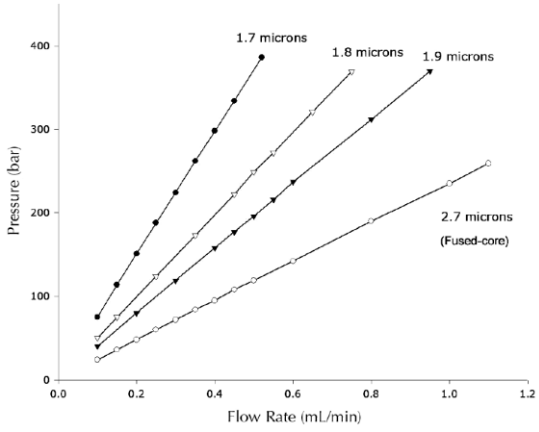

Fig. 3: low pressure values at high flow rate velocities provided by fused-core columns compared to other particle sizes [9].

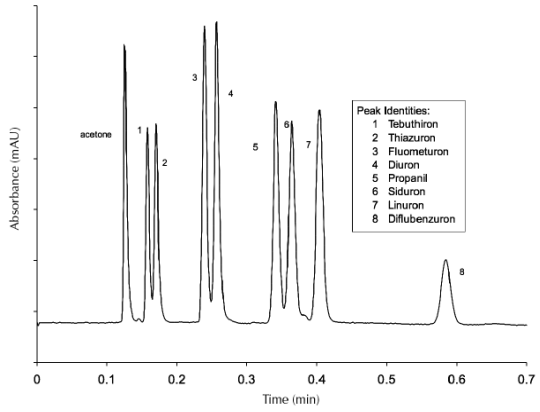

Fig. 4: Isocratic separation of pesticides using fused-core silica columns in less than one minute [9].

Studies have suggested that columns packed with silica beads of very narrow particle size distribution would have higher efficiencies [23, 24], Fig. 5. By comparing effect of increased mobile phase velocity on efficiency of column, fused-core showed a better stability of homogenous packed bed. These features of fused core particles resulted in ability to operate at higher flow rates with lower back pressure.

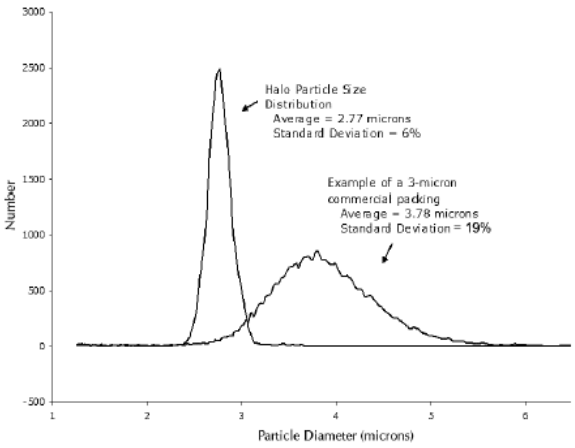

Fig. 5: Particle size distribution of 2.7µm particles (very narrow distribution) compared to 3 µm totally porous particles [9].

Another feature of fused core that it has improvement in mass transfer at even higher mobile phase velocities which seems to be an appealing approach for fast separation of large molecular weight compounds [25, 26].

Regarding sample capacity, for some analytes fused-core columns showed to be as highly stable as totally porous silica columns in terms of peak symmetry as concentration of analyte increased. This indicates stability of fused-core columns in situations where minor and major analytes are targeted for analysis [9].

Under pressure, fused-core columns showed to have a remarkable stability over extensive use in terms of retention times, theoretical plates, reproducibility, peak tailing and resolution. This again can be contributed to by the very narrow particle size distribution of fused-core silica particles [27, 7].

Porous, monolithic and fused-core – A comparison

The approach of using small porous particles (sub-2 µm) provides fast and efficient separation over conventional 3-5 µm particles [28, 29]. However, these efficiencies are countered by increased column back pressure [2].

Monolithic columns are bi-porous in structure, large macropores for high flow rates and small mesopores for high surface area to increase efficiency. There are two types of monolithic columns: silica

monoliths for separating small molecules and polymer monoliths for large molecules [30]. In packed columns, mechanism of mass transfer is diffusion while in monoliths molecules transferred by convective flow which enables increased separation speed [31].

Monolithic columns were first introduced to achieve separation on conventional LC at high flow rates [32] where these properties are due to high porosity and small skeleton size of these columns. Monolithic columns have disadvantages such as limited availability of stationary phases in industry and its unsatisfactory retention [33]. Fused-core columns, on the other hand, have many advantages such as fast elution, excellent mass transfer kinetics, and better performance at high mobile phase velocities [9, 27]. Another feature is the narrow particle size distribution which makes column with remarkable ruggedness [7].

A study carried out by Gritti et al. [34] to compare adsorption isotherm of some low-molecular weight compounds on RP-HPLC using a monolithic and classical packed column. The findings indicated closeness of adsorption energies using the two columns. However, adsorption capacities of the monolithic column were slightly better than packed column. This can be explained by the fact that accessibility surface area of monolithic column is greater than that of the packed column which is related to the high connectivity between large macropores and mesopores of the monolithic column structures.

Similarly, Kirkland and co-authors [7, 9] suggested that fused-core particles revealed comparable efficiencies to that of sub-2 µm porous particles but with modest back pressure which may be accounted for by narrow particle size distribution and higher density of fused-core particles. Moreover, decreased diffusion path for analytes may lead to reduction in hindrance to mass transfer permitting use of higher flow rates with minimum loss in efficiency [35].

Abrahim et al. [33] compared efficiency of fused-core to that of sub-2 µm particles column in terms of retention, selectivity and loading capacity. Also they used impurities profiling to assess fast separation without loss of efficiency. The retention time results of fused-core were comparable to those of the sub-2 µm particles. These similarities were previously reported by Cunliffe and Maloney [6]. For selectivity, fused-core column noted to have comparable selectivity but with favourable values over sub-2 µm particles. Sample capacity for fused-core column, however, was 25% lower than that of sub-2 µm particles but cannot be considered significant for such analytical applications. Efficiency in terms of retention factor and theoretical plate heights were lower in case of 1.7 µm particles compared to fused-core columns suggesting that sub-2 µm particles have better efficiency. This study also pointed out that fused-core had 55% lower back pressure than that produced by 1.8 µm porous silica column of same length. In addition, better resolution for tested compounds and their impurities was achieved on fused-core column.

Dioszegi and Raynie [36] have extensively studied a comparison of kinetic properties of monolithic and fused-core capillary C18 columns using Heptanophone as the test compound. Although results were comparable for both columns, the results for monolithic column were better than the fused-core column for <100,000 plates.

Fused-core for conventional HPLC

Conventional instruments with 400bar pressure limits can be used for fast separation using fused-core columns at high mobile phase velocities [9]. Some references assured that fused-core columns have substantial advantage of using with conventional LC but with minor modifications of column parts or accessories (like connecting tubes, detector cell and needle seat capillary) [37, 38]. They operate at approximately half back pressure making it possible to use with conventional HPLC where 2 µm porous inlet frit can be used which is used for conventional 3-5 µm particles. This in turn reduces problem of plugging which arises with sub-2 µm particles column especially for pharmaceutical samples with complex matrix [33].

Wei Song et al. [39] compared fused-core and conventional analytical C18 column at the same operating conditions. The fused-core column showed a reduction in separation times and up to 3-folds increase in efficiency compared to the conventional column. Also back-pressure of the HPLC system using fused-core did not exceed 3400psi making it a possible approach for standard HPLC systems. Although UPLC using sub-2 µm particles can improve traditional HPLC methods for pharmaceutical analysis [40, 41, 42], UPHPLC new instruments are needed to handle pressure exceeding 6000psi. This means UPHPLC are limited and new alternatives are needed for use with current traditional HPLC systems.

Salisbury [43] studies the possibility of using fused-core columns as an alternative to sub-2 µm particle columns for conventional HPLC and at its pressure limits. This study was carried out in an attempt to assess robustness, reproducibility, resolution on extensive use of a fused-core column (≈500 injections). It was found that fused-core particles achieved 80% of efficiency compared to sub-2 µm particles with half observed back pressure. The method proved to be robust by measuring the reproducibility of results over 500 injections. They found that %RSD for retention times, theoretical plates, peak asymmetry and resolution were observed to be less than 1%. Consequently, these findings assume the capability of these particles to concur the commercial needs of pharmaceutical applications.

Recent applications for pharmaceutical analysis

The advantages provided by fused-core technology have made it an attempting approach for high-speed pharmaceutical analysis as well as biological and environmental applications.

Alexander and Ma [44] Described the applicability of fused-core columns for 2-D LC for pharmaceutical analysis of similarly co-eluted samples. The samples were separated by using two independent flow rates, gradient profiles and mobile phase compositions. The technique showed to be very simple and elegant. However, the appropriate sensitivity of the method was limited by base-line noise.

Alexander et al., [45] studied the possibility of using the fused-core column with conventional HPLC. The method approved to progressively decrease Extra-column dispersion ECD with simultaneous decrease in extra-column volume ECV.

DeGrsse et al., [15] developed a fast method for analyzing shellfish toxins using fused-core column technology. Resolution was enhanced and reproducible retention times and peak areas were obtained. Faster elution times suggest the possibility for using this method for routine paralytic shellfish toxins PST monitoring.

Wagner and colleagues [46] studied particle size, pore size, shell thickness of fused-core to obtain optimum parameters for biomacromolecules separation. Particles with this porous shell structures showed to have better mass transfer kinetics compared to conventional porous particles.

Yang and co-authors [47] developed a method for DNA analysis by LC-MS. The method run time was in less than 1 min with good efficiency and reproducibility along with good sensitivity (low LOD values).

Suleman et al. [48] developed a stability indicating HPLC method for routine analysis of β-artemether and lumefantrine combination anti-malarial products. They used fused-core/reversed-phase amide stationary phase with an isocratic mobile phase (acetonitrile/phosphate buffer, pH 3). The method was fast (run time of 4 min), accurate and precise making it suitable method for quality control of these compounds in the presence of their related degradation products.

The availability of shell particle columns in different chemistries such as C8, C18, RP amide and HILIC has encouraged researchers to utilize it for many analytical procedures. Table.1, lists some pharmaceutical applications which are fast, economic and reproducible.

Table 1: Some pharmaceutical and biological applications of fused-core columns

API	MP	column	FR	detection	Ref
Antibiotics + antifungal	Potassium phosphate buffer, pH=3 , Acetonitrile	HALO RP-Amide	2mL/min	UV 230 nm	AMT note
B-blockers	Ammonium formate, pH=3 , Acetonitrile	HALO 2.7 µm	3 mL/min	UV 254 nm	AMT note
Cephalosporines	Phosphate buffer (pH=2.7) and Methanol	HALO ES-CN	2 mL/min	UV 254 nm	AMT note
Lipids	Methanol/water/acetic acid	Halo C8	gradient	CAD	Dionex note
posaconazole	Acetone/tetrahydrofuran/ methanol/water/ammonium hydroxide	Halo C18	Gradient	MS/MS	Ref. [49]
Proteomics	Formic acid in water/ ACN	Halo C18	different	MS	AMT note

CONCLUSION

HPLC approaches would usually target fast, economic and reliable technology. These requirements are fulfilled by the features of fused core technology. These columns have approved through many studies that are capable of performing very fast, inexpensive and efficient analysis.

As they are available in different chemistries, these technologies can be applied for different compounds of low or high molecular weights. Moreover, the moderate back pressure generated at high mobile phase velocities made it possible to use with conventional HPLC systems. Therefore, all the above mentioned properties have made this technology an appealing approach for routine biological and pharmaceutical applications.

REFERENCES

1. Zheng J, Patel D, Tang Q, Markovich R, Rustum A: Comparison study of porous, fused-core and monolithic silica-based C18 HPLC columns for Celestoderm-V Ointment® analysis. Journal of Pharmaceutical and Biomedical Analysis 2009; 50: 815-822.
2. Wu N, Clausen A: Fundamental and practical aspects of ultrahigh pressure liquid chromatography for fast separations. Journal of Separation Science 2007; 30(8): 1167–1182.
3. Swartz M.E. UPLC: an introduction and review. J. Liq. Chromatogr. Relat. Technol. 2005; 28: 1253–1263.
4. Wu N: Grushka, N. Grinberg, Advances in Chromatography, Schmidth Periodicals GmbH, Germany, 2007; 46: 213.
5. Unger KK, Skudas R, and. Schulte MM: Particle Packed Columns and Monolithic Columns in HPLC – a Comparison and Critical Appraisal. J Chromatogr A. 2008; A 1184: 393- 415.
6. Cunliffe JM, Maloney TD: Fused-core particle technology as an alternative to sub-2-µm particles to achieve high separation efficiency with low backpressure. Journal of Separation Science 2007; 30(18):3104–3109.
7. Kirkland JJ, Langlois TJ, and DeStefano JJ: Fused-core particles for HPLC Columns. Am. Lab.*2007;* 39: 18–21.
8. Kirkland JJ. Superficially porous silica microspheres for the fast high-performance liquid chromatography of macro- molecules. Anal. Chem. 1992; 64: 1239–1245.
9. DeStefano JJ, Langlois TJ, and Kirkland JJ: Characteristics of Superficially-Porous Silica Particles for Fast HPLC: Some Performance Comparisons with Sub-2-µm Particles. Journal of Chromatographic Science 2008; 46: 254-260.
10. Gritti F; Cavazzini A; Marchetti N; Georges Guiochon G: Comparison between the efficiencies of columns packed with fully and partially porous C18-bonded silica materials. J Chromatogr A. 2007; 1157(1-2):289-303.
11. Gritti F, Leonardis I, Shock D, Stevenson P, Shalliker A, Guiochon G: Performance of columns packed with the new shell particles, Kinetex-C18. J Chromatogr A. 2010a:1217(10):1589-603.
12. Wang X, Barber WE, and Carr PW: A practical approach to maximizing peak capacity by using long columns packed with pellicular stationary phases for proteomic research. J Chromatogr A. 2006; 1107: 139–151.
13. Unger KK: Scientific achievements of Jack Kirkland to the development of HPLC and in particular to HPLC silica packings--a personal perspective. J Chromatogr A. 2004; 1060(1-2):1-7.
14. Kirkland JJ, Truszkowski FA, Dilks Jr. CH, Enge GS: Superficially porous silica microspheres for fast high-performance liquid chromatography of macromolecules. J Chromatogr A. 2000; 890:3–13.
15. DeGrasse SL , DeGrasse JA, Reuter K: Solid core column technology applied to HPLC-FD of paralytic shellfish Toxins. Toxicon 2011; 57:179–182.
16. Ali I, AL-Othman ZA and Al-Za'abi M: Superficially porous particles columns for super fast HPLC separations. Biomed. Chromatogr. 2012; 26:1001–1008.
17. Gritti F; Guiochon G: Performance of columns packed with the new shell Kinetex-C18 particles in gradient elution chromatography. J Chromatogr A.2010a;1217(10):1604-15.
18. Gritti F, Leonardis I, Abia J, Guiochon G: Physical properties and structure of fine core–shell particles used as packing materials for chromatography Relationships between particle characteristics and column performance. J Chromatogr A., 2010c; 1217:3819–3843.
19. Schuster SA, Wagner BM, Boyes BE, Kirkland JJ: Wider pore superficially porous particles for peptide separations by HPLC. J Chromatogr Sci. 2010; 48(7):566-71.
20. Gritti F, Guiochon G: The mass transfer kinetics in columns packed with Halo-ES shell particles. J Chromatogr A. 2011; 1218:907–921.
21. Ali I, Gaitonde VD and Grahn A: Halo columns: new generation technology for high speed liquid chromatography. J Chromatogr Sci. 2010; 48: 386–394.
22. Berger TA: Characterization of a 2.6 mm Kinetex porous shell hydrophilic interaction liquid chromatography column in supercritical fluid chromatography with a comparison to 3 mm totally porous silica. J Chromatogr A. 2011; 1218: 4559–4568.
23. Billen J, P. Gzil, Lynen F, Sandra P, van der Meeren P, and Desmet G: Poster Presentation. *HPLC 2006*, San Francisco, CA, June 2006.
24. Unger KK and Weber E: *A Guide to Practical HPLC.* Git Verlag GMBH, Darmstadt, 1999, p. 45.
25. Cavazzini A, Gritti F, Kaczmarski K, Marchetti N and Guiochon G: Mass-transfer kinetics in a shell packing material for chromatography. Anal. Chem. 2007; 79: 5972–5979.
26. Kaczmarski K and Guiochon G: Modelling of the mass-transfer kinetics in chromatographic columns packed with shell and pellicular particles. Anal. Chem. 2007; 79: 4648–4656.
27. Gritti F, Cavazzini A, Marchetti N and Guiochon G: Comparison between the efficiencies of columns packed with fully and partially porous C18 bonded silica materials. J Chromatogr A. 2007a; 1157: 289–303.
28. Anspach JA, Maloney TD, Colón LA: Ultrahigh-pressure liquid chromatography using a 1-mm id column packed with 1.5-microm porous particles. J Sep Sci. 2007; 30(8):1207-13.
29. Jerkovich AD, Mellors JS, Jorgenson JW: The use of micrometer-sized particles in ultrahigh pressure liquid chromatography, LC–GC N. Am. 2003; 21:600–610.
30. Poppe H: Some reflections on speed and efficiency of modern chromatographic methods Review Article. J Chromatogr A. 1997; 778(1–2): 3-21.
31. Giddings JC: Comparison of theoretical limit of separating speed in gas and liquid chromatography. Anal. Chem. 1965; 37: 60–63.
32. Kirkland JJ, Destefano JJ: The art and science of forming packed analytical high-performance liquid chromatography columns. J Chromatogr A. 2006; 1126(1-2):50-7.
33. Abrahim A, Al-Sayah M, Skrdla P, Bereznitski Y, Chen Y, Wu N: Practical comparison of 2.7_m fused-core silica particles and porous sub-2_m particles for fast separations in pharmaceutical process development. Journal of Pharmaceutical and Biomedical Analysis 2010; 51:131–137.

34. Gritti F, Piatkowski W, Guiochon G: Comparison of the adsorption equilibrium of a few low-molecular mass compounds on a monolithic and a packed column in reversed-phase liquid chromatography. J Chromatogr A. 2002; 978:81–107.
35. Hseih Y, Duncan CJG, and Brisson J: Fused-core silica column high-performance liquid chromatography/tandem mass spectrometric determination of rimonabant in mouse plasma. Anal. Chem. 2007; 79: 5668–73.
36. Diószegi TA and Raynie DE: Kinetic performance comparison of a capillary monolithic and a fused-core column in micro-scale liquid chromatography. J Chromatogr A. 2012; 1261:107– 112.
37. Gritti F, Sanchez CA, Farkas T, Guiochon G: Achieving the full performance of highly efficient columns by optimizing conventional benchmark high-performance liquid chromatography instruments. J Chromatogr A. 2010b; 1217(18):3000-12.
38. Gritti F; Georges Guiochon G: On the extra-column band-broadening contributions of modern, very high pressure liquid chromatographs using 2.1 mm I.D. columns packed with sub-2 µm particles. J Chromatogr A. 2010b; 1217(49):7677-89.
39. Song W, Pabbisetty D, Groeber EA, Steenwyk RC: Fast DM: Comparison of fused-core and conventional particle size columns by LC-MS/MS and UV: application to pharmacokinetic study. J Pharm Biomed Anal. 2009; 50(3):491-500.
40. MacNair JE, Lewis KC, and Jorgenson JW: Ultrahigh-pressure reversed-phase liquid chromatography in packed capillary columns. Anal. Chem. 1997; 69: 983–89.
41. MacNair JE, Patel KD, and Jorgenson JW: Ultrahigh-pressure reversed-phase capillary liquid chromatography: isocratic and gradient elution using columns packed with 1.0-µm particles. Anal. Chem.1999; 71: 700–708.
42. Wu N, Lippert JA, and Lee ML: Practical aspects of ultrahigh pressure capillary liquid chromatography. J Chromatogr A. 2001; 911:1–12.
43. Salisbury JJ: Fused-Core Particles: A Practical Alternative to Sub-2 Micron Particles. J Chromatogr Sci. 2008; 46: 883-886.
44. Alexander AJ, Ma L: Comprehensive two-dimensional liquid chromatography separations of pharmaceutical samples using dual Fused-Core columns in the 2nd dimension. J Chromatogr A. 2009; 1216:1338–1345.
45. Alexander AJ, Waeghe TJ, Himes KW, Tomasella FP, Hooker TF: Modifying conventional high-performance liquid chromatography systems to achieve fast separations with Fused-Core columns: A case study. J Chromatogr A. 2011; 1218:5456– 5469.
46. Wagner BM, Schuster SA, Boyes BE, Kirkland JJ: Superficially porous silica particles with wide pores for biomacromolecular separations, J Chromatogr A. 2012; 1264:22-30.
47. Yang I, Fortin MC, Richardson JR, Buckley B: Fused-core silica column ultra-performance liquid chromatography–ion trap tandem mass spectrometry for determination of global DNA methylation status. Analytical Biochemistry 2011; 409:138–143.
48. Suleman S , Vandercruyssen K, Wynendaele E, D'Hondt M, Bracke N, Duchateau L, Burvenich C, Peremans K and De Spiegeleer B: A rapid stability-indicating, fused-core HPLC method for simultaneous determination of β-artemether and lumefantrine in anti-malarial fixed dose combination products. Malaria Journal 2013; 12:145.
49. Cunliffe JM, Noren CF, Hayes RN, Clement RP, Shen JX: A high-throughput LC-MS/MS method for the quantitation of posaconazole in human plasma: Implementing fused core silica liquid chromatography. J Pharm Biomed Anal. 2009; 50(1):46-52.