Review Article

Ultrasensitive Chip-Liquid Chromatography Analytical Tool for Quantification-A Review

ABSTRACT:

Chip liquid chromatography (LC) is a form of liquid chromatography (LC) that has been scaled down to suit a microchip for quick, high-throughput analysis with tiny sample volumes and minimal reagent usage. The term also goes by To date, four distinct on-chip LC strategies have been created; these strategies make use of columns that are open-tubular, packed-particle, monolithic, and pillar array. This technique is used to analyze small compounds and medications in a variety of biological materials, as well as for proteomics. High quantities of other endogenous chemicals that may cause interference are present alongside these biological compounds, which are only present in tiny levels.LC miniaturization offers a solution to such problems.On-a-chip operations like sampling, sample transfer, separation, and identification are carried out. Sample preparation is the most challenging task at the micro level and is an important component of most analytical techniques, particularly in bioanalysis.One of the crucial steps in liquid chromatography is separation. Sensitive detection methods such as laser-induced fluorescence microscopy and mass spectrometry are being used.

Keywords: chip liquid chromatography, open-tubular columns, packed particle column, monolith columns, pillar array columns, separation column and chip.

1. INTRODUCTION:

Chip liquid ~~chromatographyis~~ chromatography isa typeof liquid chromatography technology that has been miniaturized to accommodate a chip. The chip carries out nano flow to obtain maximum sensitivity with minimal sample size. It is smaller than a credit card [1,2,]. In chip LC, sampling, sample transport, and chromatographic separations and detection are ~~preprogrammedto~~ preprogrammed tobe carried out on a chip. The chip-LC is a microfluidics-based technology for nanoflow LC systems.It may also be referred to as lab-on-a-chip.The benefits of the chip liquid chromatographic system are Smaller sample sizes, reduced dead volumes, very low solvent consumption, quicker and higher throughput analysis, mobility of the analytical system, and on-site and distant analysis ~~are all benefits of the chip liquid chromatographic system~~[3-6].~~ThisChip-LC~~This Chip-LC technology terminates 50% of the traditional fittings and connections over conventional LC systems. For extremely sensitive and precise mass determination in proteomics and metabolomics investigations, LC coupled with MS is the widely chosen analytical approach [7]. It is still challenging to achieve a satisfactory separation because some crucial biological compounds (metabolites) are present in small amounts along with high concentrations of other endogenous compounds that may act as interferences, such challenges are overcome by chip liquid chromatography.Additionally, the chip-LC has inherent advantages related to robust operation and usability. Chip-LC has the ability to be used in a wide variety of applications, including pharmaceutical development and production, Combi Chem, compound analysis, DMPK (drug metabolism and pharmacokinetics), food safety [8], environmental surveillance, and national security as superior chromatographic performance and significantly enhanced sensitivity is achieved.

2. INSTRUMENTATION:

Fig1: chip-HPLC instrument

Comment [tb1]: Please add site of figures in main text

On an inert coated multi-layered poly amide sheet, the chip liquid chromatography device combines a sample enrichment column and analytical column, microvalve connectors, and middle-coated ~~nanoelectrospray~~nano electrospray technology. The chip-LC's complex design reduces dispersion and integrates all stages from sample loading to ionization of the compound for smooth operation. On the chip, the sample enrichment column and separation columns of the nanoflow LC system are desegregated with detailed ~~connectionsand~~connections and~~nanospray~~nano spray technology for compounding ionization in mass spectrometry [9,10].For sample separation, various kinds of columns were created and incorporated into the microfiber chip. Reverse phase gradient separation is typically performed on chip-LC.

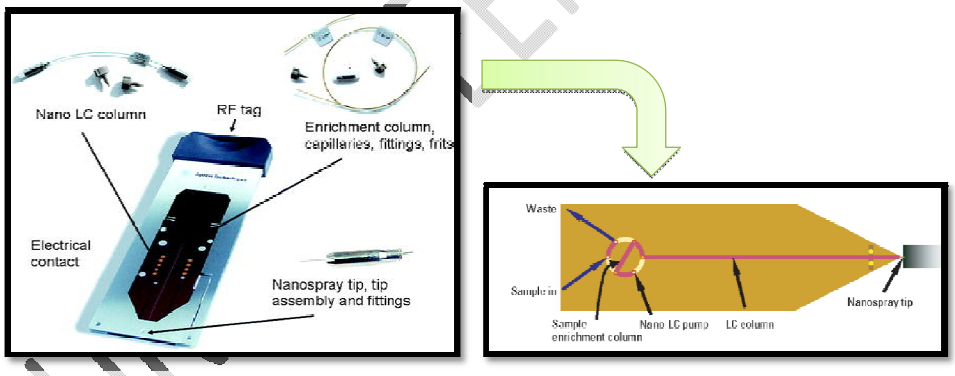

Fig2: Chip and its components

Open-tubular chromatography is ~~theelementary~~ the elementaryway to carry out chromatography on a chip. This approach was put through as the initial model of Chip-LC, in 1990, Manz et al.published their research that an open tubular ~~columnwas~~ column wasfabricated however separation was not performed and the channel wall of the chip is taken as the stationary phase after direct modification to yield the required ability. Though back pressure is low, the main disadvantage is the low capacity of the column due to the low surface-to-volume ratio [11-15].Fig3:

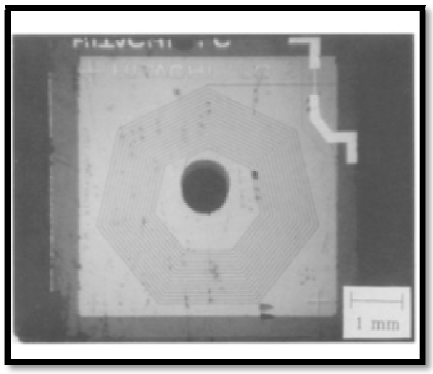

Fig3: Open tubular column

Packed particle columns are commonly used in conventional lc systems. These pre-prepared particles can be launched into a microchannel on a chip, resulting in the creation of a packed be. C8, C18, ion exchange, hydrophilic interaction chromatography (HILIC), and a variety of other particle kinds were used with various changes to create particle-packed chips. They are, however, typically used in conjunction with mass spectrometry. The particle packaging circumstances influence the efficiency of the particle-packed chip-LC device [16-18]. Fig4

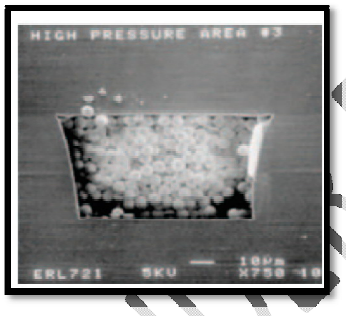

Fig4: Packed particle column

Monoliths are made up of a single cylinder of porous substance that is created in situ through a polymerization process. Unlike open-tubular column capacity, these have a larger specific area and column capacity. By filling the channels with poly (methyl acrylate) monoliths, a monolith nano-porous semiconductor was created [19-23].Fig5

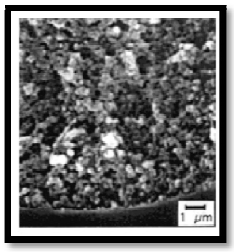

Fig5: Monolith column

The autosampler and loading pump are connected directly to the chip cube. By sandwiching the chip in between both the stator and rotor of the built-in microvalve, leak-tight connections are immediately created. The rotor and stator duct onto the chip, establish flow paths from nano LC to the surface. This lowers the number of fittings and connectors needed in a nanoflow [24-27] LC system by half, considerably reducing the possibility of leaks and inactive volumes while also increasing ease of use, sensitivity, repeatability, and dependability. Chip-lc also includes electrical contacts for ~~nanoelectrospray~~nano electrospray technology and an embedded radio frequency id tag (rf tag) that monitors the chip's utilization and working settings. The LC chip cube-MS interface houses the LC chip. Fig6

Fig6: Chip injection porton the chip-cube

An electrospray ionization source with lenses for spray visualization is included in the chip cube. The mass spectroscopy system is used as the detector [30,31].

3. ANALYSIS OF DISTINCT TYPES OF COMPOUNDS:

For the analysis of various kinds of substances available, various chips whose basic difference lies in their embedded radio frequency id tag are injected for the desired separation and identification [28,29].

3.1 Protein identification analysis: The digested proteins from the autosampler when enter into the gradient solvent flow moves as peptides from the sample enrichment column and are separated in a separation column [32,33,34].

3.2 The ~~phospho~~ phosphorchip sandwiched between reverse phased TiO_2 trapping column is used ~~forworking~~ for workingon post-translational modifications with conventional tools targeted at phosphorylated peptides [35,36].

3.3 The monoclonal antibodies glycan chip is a specifically designed chip for on-chip d-glycosylation of monoclonal antibodies which provides better and faster sensitivity than conventional systems [37-39].

3.4 Small molecule analysis for instance in drug metabolism and pharmacokinetic studies with better sensitivity and much lower sample requirements [40]. Fig7:

Fig7: chips with various rf tag programming

4. ADVANTAGES OF CHIP-LC OVER CONVENTIONAL COLUMNS:

4.1 **COLUMN DESIGN:** A Special thought has been given to the design of each of the analytical ~~column chips~~column chips and the trap-chips in order to create separations that are equal to or greater to separations obtained using packed capillaries. Using fused silica, cylindrical passageways can be filled with miniature LC columns and traps. In lieu of conventional frits made of fused stationary phase particles, Chip-LC columns use a unique barrier construction to retain stationary phase particulates in the column. Additionally, these kinds of frameworks do not have problems with the absorption of sample components, which can happen with frit material.

4.2 **LEAK-FREE CONNECTION SYSTEM**: To connect to and from each chip, a low ~~hold up~~hold-up volume connection device offering allows for up to seven channels to the external environment through a dead volume of fewer than one nanoliter is used. Because the power that connects the chip is pre-set, whenever the user swaps a chip, a non-leaking connection is accomplished without the need for user modifications.

4.3 **COLUMN-TO-COLUMN REPRODUCIBILITY**: The use of the chip-LC columns improves column-to-column consistency in addition to making it simple to replace a nano-LC column or trap in seconds. The packing method ensures the highest conceivable column-to-column consistency in nano-LC, and all chips are similar. This is critical for applications that demand retention time consistency over extended time spans and across numerous columns. Examples include arranging MRMs for peptide quantitation in biomarker confirmation and using retention time in conjunction with precise mass in peptide/protein identity.

4.4 **EASY OPERATION:**Direct injection, trap-loading, and dual column with direct injection studies can all be switched between with ease using the chip ~~nanoflex~~nano flex. A fluidic jumper chip,

which directs fluid properly for the intended experiment, is used to accomplish this. The column and trap chips and this connector chip can both be replaced with ease [41-47].

5. PRACTICAL CONSIDERATIONS:

5.1 In an article by ~~K.,~~ Brennen *et al*. titled Microfluidic chip for peptide analysis with an integrated HPLC column, sample enrichment column, and ~~nanoelectrospray~~nano electrospray tip. Where the overall performance of the system was exhibited in tryptic protein digests using reversed-phase gradient separations between 100 and 400 nL/min. The microfluidic incorporation of the nano-LC components enabled separations with sub-femtomole detection and minimal carryover, sensitivity, and robustness [48].

5.2 High-Performance Liquid Chromatography for Fast Enantioseparations on a Chip. On a column filled with the particulate chiral stationary phase and incorporated into the microfluidic glass chip, chromatographic separation was carried out~~. As~~ as a chiral stationary phase substance, cellulose tris(3,5-dimethyl phenyl carbamate) coated on 5-m The material used was entirely permeable silica. Several racemic analytes, including medicinal products, were baseline separated into their corresponding enantiomers under reversed-phase, polar organic, and normal-phase conditions to demonstrate the versatility of the glass chip in the area of chiral separations. Enantiomer separations of enantiomers took place in less than 5 s thanks to the use of very short columns with lengths as low as 12 mm [49].

5.3 Conventional methods for cellular metabolomics have shown to be ineffective at simulating the metabolic system of interest and at detecting molecules with low abundance. The pathophysiological processes of different metabolic diseases may be revealed by combining these MFDs with metabolite flux analysis. Once these biochemical alterations are understood, attempts can be made to find indicators and create therapeutic targets to treat these frequently disabling illnesses [50].

6. CONCLUSION:

Chip operations like sampling, sample transfer, separation, and identification are carried out. Sample preparation is the most challenging task at the micro level and is an important component of most analytical techniques, particularly in bioanalysis. One of the crucial steps in liquid chromatography is separation. Sensitive detection. It minimizes time consumption during proteomics, metabolomics, small molecules analysis, and also large molecule analysis.

REFERENCES:

1. McEnery M, Tan A, Alderman J, Patterson J, O'Mathuna SC, Glennon JD. Liquid chromatography on-chip: progression towards a µ-total analysis system presented at the SAC 99 Meeting Dublin, Ireland, July 25–30, 1999. Analyst. 2000;125(1):25-7.
2. Nazario CE, Silva MR, Franco MS, Lanças FM. Evolution in miniaturized column liquid chromatography instrumentation and applications: An overview. Journal of Chromatography a. 2015 Nov 20;1421:18-37.
3. de Mello A. FOCUS On-chip chromatography: the last twenty years. Lab on a Chip. 2002;2(3):48N-54N.
4. Freemantle M. Microscale technology. Chem. Eng. News. 1999 Feb 22;77(8):27-36.

Formatted: Font: Italic

5. O'Neill AP, O'Brien P, Alderman J, Hoffman D, McEnery M, Murrihy J, Glennon JD. On-chip definition of picolitre sample injection plugs for miniaturised liquid chromatography. Journal of Chromatography A. 2001 Jul 27;924(1-2):259-63.
6. Manz A, Graber N, Widmer HÁ. Miniaturized total chemical analysis systems: a novel concept for chemical sensing. Sensors and actuators B: Chemical. 1990 Jan 1;1(1-6):244-8
7. Medina DA, Maciel EV, Lanças FM. Miniaturization of liquid chromatography coupled to mass spectrometry. 3. Achievements on chip-based LC–MS devices. TrAC Trends in Analytical Chemistry. 2020 Oct 1;131:116003.
8. Ríos Á, Zougagh M. Chip-Based Separation Devices Coupled to Mass Spectrometry in Food and Environmental Chemistry. InMass Spectrometry in Food and Environmental Chemistry 2022 May 18 (pp. 359-379). Cham: Springer International Publishing.
9. Maciel EV, de Toffoli AL, Sobieski E, Nazário CE, Lancas FM. Miniaturized liquid chromatography focusing on analytical columns and mass spectrometry: A review. Analytica chimica acta. 2020 Mar 22;1103:11-31.
10. Gao D, Liu H, Jiang Y, Lin JM. Recent advances in microfluidics combined with mass spectrometry: technologies and applications. Lab on a Chip. 2013;13(17):3309-22.
11. Manz A, Miyahara Y, Miura J, Watanabe Y, Miyagi H, Sato K. Design of an open-tubular column liquid chromatograph using silicon chip technology. Sensors and actuators B: Chemical. 1990 Jan 1;1(1-6):249-55.
12. Tsunoda M. On-Chip Liquid Chromatography. Encyclopedia. 2022 Mar 15;2(1):617-24.
13. Tan A, Rodgers K, Murrihy JP, O'Mathuna C, Glennon JD. Rapid fabrication of microfluidic devices in poly (dimethylsiloxane) by photocopyingPresented at the 14th International Symposium on Microscale Separations and Analysis, Boston, January 13–18, 2001. Lab on a Chip. 2001;1(1):7-9.
14. Lee SJ, Lee S. Micro total analysis system (μ-TAS) in biotechnology. Applied microbiology and biotechnology. 2004 Apr;64:289-99.
15. Cheong WJ, Ali F, Kim YS, Lee JW. Comprehensive overview of recent preparation and application trends of various open tubular capillary columns in separation science. Journal of Chromatography A. 2013 Sep 20;1308:1-24.
16. Fonverne A, Ricoul F, Demesmay C, Delattre C, Fournier A, Dijon J, Vinet F. In situ synthesized carbon nanotubes as a new nanostructured stationary phase for microfabricated liquid chromatographic column. Sensors and Actuators B: Chemical. 2008 Feb 22;129(2):510-7.
17. Jung S, Höltzel A, Ehlert S, Mora JA, Kraiczek K, Dittmann M, Rozing GP, Tallarek U. Impact of conduit geometry on the performance of typical particulate microchip packings. Analytical chemistry. 2009 Dec 15;81(24):10193-200.
18. Tan A, Rodgers K, Murrihy JP, O'Mathuna C, Glennon JD. Rapid fabrication of microfluidic devices in poly (dimethylsiloxane) by photocopyingPresented at the 14th International Symposium on Microscale Separations and Analysis, Boston, January 13–18, 2001. Lab on a Chip. 2001;1(1):7-9.
19. He B, Tait N, Regnier F. Fabrication of nanocolumns for liquid chromatography. Analytical Chemistry. 1998 Sep 15;70(18):3790-7.
20. Jung S, Höltzel A, Ehlert S, Mora JA, Kraiczek K, Dittmann M, Rozing GP, Tallarek U. Impact of conduit geometry on the performance of typical particulate microchip packings. Analytical chemistry. 2009 Dec 15;81(24):10193-200.
21. Ericson C, Holm J, Ericson T, Hjerten S. Electroosmosis-and pressure-driven chromatography in chips using continuous beds. Analytical Chemistry. 2000 Jan 1;72(1):81-7.
22. Schlund M, Gilbert SE, Schnydrig S, Renaud P. Continuous sampling and analysis by on-chip liquid/solid chromatography. Sensors and Actuators B: Chemical. 2007 May 21;123(2):1133-41.

23. Collins DA, Nesterenko EP, Brabazon D, Paull B. Controlled ultraviolet (UV) photoinitiated fabrication of monolithic porous layer open tubular (monoPLOT) capillary columns for chromatographic applications. Analytical chemistry. 2012 Apr 3;84(7):3465-72.
24. O'Neill AP, O'Brien P, Alderman J, Hoffman D, McEnery M, Murrihy J, Glennon JD. On-chip definition of picolitre sample injection plugs for miniaturised liquid chromatography. Journal of Chromatography A. 2001 Jul 27;924(1-2):259-63.
25. Li L, Wang X, Pu Q, Liu S. Advancement of electroosmotic pump in microflow analysis: A review. Analytica Chimica Acta. 2019 Jul 4;1060:1-6.
26. Schlund M, Gilbert SE, Schnydrig S, Renaud P. Continuous sampling and analysis by on-chip liquid/solid chromatography. Sensors and Actuators B: Chemical. 2007 May 21;123(2):1133-41.
27. Benvenuto A, Guarnieri V, Lorenzelli L, Collini C, Decarli M, Adami A, Potrich C, Lunelli L, Canteri R, Pederzolli C. Fabrication of a MEMS-based separation module for liquid chromatography. Sensors and Actuators B: Chemical. 2008 Mar 14;130(1):181-6.
28. HPLC-Chip/MS Technology | Agilent [Internet]. www.agilent.com. [cited 2023 Apr 26]. Available from: https://www.agilent.com/en-us/products/liquid-chromatography/low-flow-lc-systems/1260-infinity-hplc-chip-ms-system/gp15400
29. cHiPLC System [Internet]. publish-p93412-e851377.adobeaemcloud.com. [cited 2023 Apr 26]. Available from: https://sciex.com/products/hplc-products/nanolc-products/chiplc-system
30. Šesták J, Moravcová D, Kahle V. Instrument platforms for nano liquid chromatography. Journal of Chromatography a. 2015 Nov 20;1421:2-17.
31. MoravcovÃ D, Kahle V. Instrument platforms for nano liquid chromatography. Journal of chromatography. 2015.
32. Urban J. A review on recent trends in the phosphoproteomics workflow. From sample preparation to data analysis. Analytica Chimica Acta. 2022 Mar 22;1199:338857.
33. Svejdal RR, Sticker D, Sønderby C, Kutter JP, Rand KD. Thiol-ene microfluidic chip for fast on-chip sample clean-up, separation and ESI mass spectrometry of peptides and proteins. Analytica Chimica Acta. 2020 Dec 15;1140:168-77.
34. Laurell T, Nilsson J, Marko-Varga G. Proteomics–protein profiling technology: the trend towards a microfabricated toolbox concept. TrAC Trends in Analytical Chemistry. 2001 May 1;20(5):225-31.
35. Jia X, Yang X, Luo G, Liang Q. Recent progress of microfluidic technology for pharmaceutical analysis. Journal of Pharmaceutical and Biomedical Analysis. 2022 Feb 5;209:114534.
36. Faure M, Sotta B, Gamby J. Investigating the kinetics of paramagnetic-beads linked alkaline phosphatase enzyme through microchannel resistance measurement in dielectric microchip. Biosensors and Bioelectronics. 2014 Aug 15;58:61-7.
37. Li D, Zhang Z, Chang H. Chip-based nanoflow liquid chromatography for on-chip detection of glycated haemoglobin levels. Sensors and Actuators B: Chemical. 2019 Jul 15;291:433-40.
38. Wouters B, De Vos J, Desmet G, Terryn H, Schoenmakers PJ, Eeltink S. Design of a microfluidic device for comprehensive spatial two-dimensional liquid chromatography. Journal of Separation Science. 2015 Apr;38(7):1123-9.
39. Fonverne A, Ricoul F, Demesmay C, Delattre C, Fournier A, Dijon J, Vinet F. In situ synthesized carbon nanotubes as a new nanostructured stationary phase for microfabricated liquid chromatographic column. Sensors and Actuators B: Chemical. 2008 Feb 22;129(2):510-7.
40. Medina DA, Dos Santos NG, da Silva Burato JS, Borsatto JV, Lancas FM. An overview of open tubular liquid chromatography with a focus on the coupling with mass spectrometry for the analysis of small molecules. Journal of Chromatography A. 2021 Mar 29;1641:461989.
41. Nickerson JL, Baghalabadi V, Rajendran SR, Jakubec PJ, Said H, McMillen TS, Dang Z, Doucette AA. Recent advances in top-down proteome sample processing ahead of MS analysis. Mass Spectrometry Reviews. 2023 Mar;42(2):457-95.

42. Müller JB, Geyer PE, Colaco AR, Treit PV, Strauss MT, Oroshi M, Doll S, Virreira Winter S, Bader JM, Köhler N, Theis F. The proteome landscape of the kingdoms of life. Nature. 2020 Jun 25;582(7813):592-6.
43. Thurmann S, Dittmar A, Belder D. A low pressure on-chip injection strategy for high-performance chip-based chromatography. Journal of Chromatography A. 2014 May 2;1340:59-67.
44. Grinias JP, Kennedy RT. Advances in and prospects of microchip liquid chromatography. TrAC Trends in Analytical Chemistry. 2016 Jul 1;81:110-7.
45. Cruz JC, de Souza ID, Lanças FM, Queiroz ME. Current advances and applications of online sample preparation techniques for miniaturized liquid chromatography systems. Journal of Chromatography A. 2022 Mar 1:462925.
46. Davis JJ, Foster SW, Grinias JP. Low-cost and open-source strategies for chemical separations. Journal of Chromatography A. 2021 Feb 8;1638:461820.
47. Medina DA, Maciel EV, de Toffoli AL, Lanças FM. Miniaturization of liquid chromatography coupled to mass spectrometry.: 2. Achievements on modern instrumentation for miniaturized liquid chromatography coupled to mass spectrometry. TrAC Trends in Analytical Chemistry. 2020 Jul 1;128:115910.
48. Yin H, Killeen K, Brennen R, Sobek D, Werlich M, van de Goor T. Microfluidic chip for peptide analysis with an integrated HPLC column, sample enrichment column, and nanoelectrospray tip. Analytical Chemistry. 2005 Jan 15;77(2):527-33.
49. Thurmann S, Lotter C, Heiland JJ, Chankvetadze B, Belder D. Chip-based high-performance liquid chromatography for high-speed enantioseparations. Analytical chemistry. 2015 Jun 2;87(11):5568-76.
50. 1.Edwards JL, Filla LA. Microfluidic chips: Lab-on-a-chip cell culture for metabolomics. European Pharmaceutical Review [Internet]. 2015 [cited 2023 Apr 26];20(3):15–9. Available from: https://www.europeanpharmaceuticalreview.com/article/33095/microfluidic-chips-lab-on-a-chip-cell-culture-for-metabolomics/