



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

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## Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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Review Article

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## ABSTRACT

**Introduction:** 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.

**Applications:** 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.

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**Advantage:** 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 chromatography is a type of 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 preprogrammed to be 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. 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]. 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

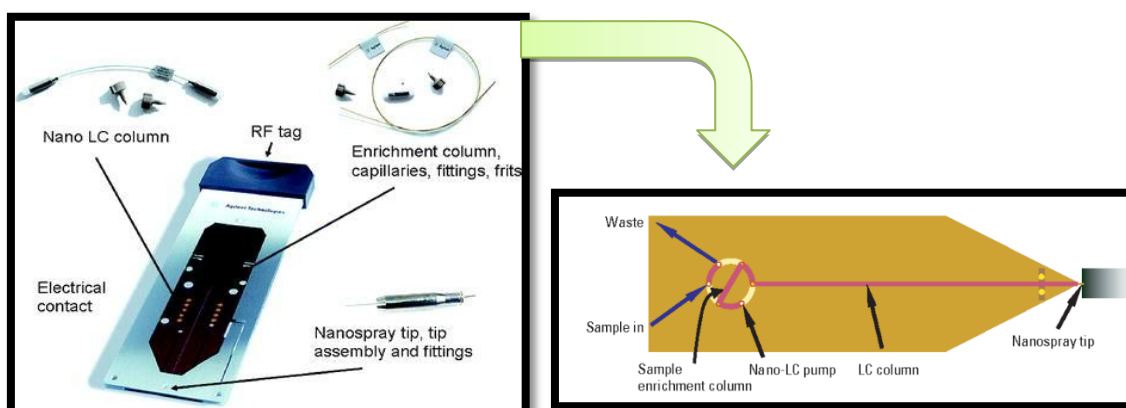
chip liquid chromatography. Additionally, the chip-LC has inherent advantages related to robust operation and usability. Chip-LC has the ability to be used in a wide variety of applications, including pharmaceutical development and production, CombiChem, compound analysis, DMPK (drug metabolism and pharmacokinetics), food safety [8], environmental surveillance, and national security as superior chromatographic performance and significantly enhanced sensitivity is achieved.

## 2. INSTRUMENTATION

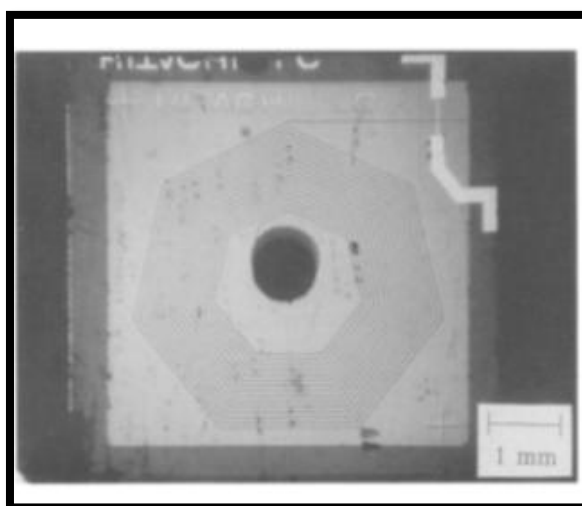
On an inert coated multi-layered poly amide sheet, the chip liquid chromatography device combines a sample enrichment column and analytical column, microvalve connectors, and middle-coated nanoelectrospray technology. The chip-LC's complex design reduces dispersion and integrates all stages from sample 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 connections and nanospray 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.



**Fig. 1. Chip-HPLC instrument**



**Fig. 2. Chip and its components**

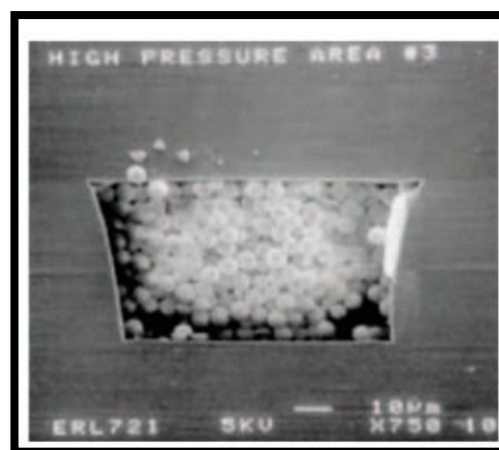


**Fig. 3. Open tubular column**

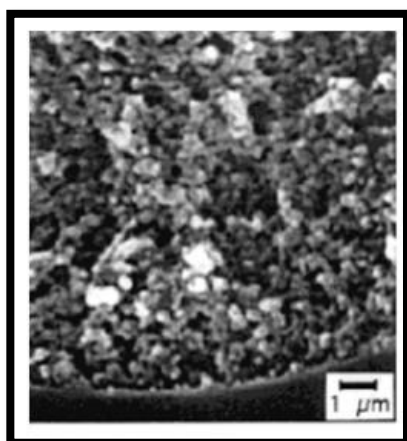
Open-tubular chromatography is the elementary way to carry out chromatography on a chip. This approach was put through as the initial model of Chip-LC, in 1990, Manz et al. published their research that an open tubular column was fabricated however separation was not performed and the channel wall of 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].

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 bed. 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 [16,17].



**Fig. 4. Packed particle column**



**Fig. 5. Monolith 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 [18-22].

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 [23,24,25] LC system by half, considerably reducing the possibility of leaks and inactive volumes while also increasing ease of use, sensitivity, repeatability, and dependability. Chip-IC also includes electrical contacts for nanoelectrospray 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.

An electrospray ionization source with lenses for spray visualization is included in the chip cube. The mass spectroscopy system is used as the detector [21,26].

### **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 [27,28].

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 [29,30,20].
2. The phospho chip sandwiched between reverse phased  $\text{TiO}_2$  trapping column is used for working on post-translational modifications with conventional tools targeted at phosphorylated peptides [31,32].
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 [11,33,34].
4. Small molecule analysis for instance in drug metabolism and pharmacokinetic studies with better sensitivity and much lower sample requirements [7].

## **4. ADVANTAGES OF CHIP-LC OVER CONVENTIONAL COLUMNS**

### **4.1 Column Design**

Special thought has been given to the design of each of the analytical 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 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. 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 [35-39].

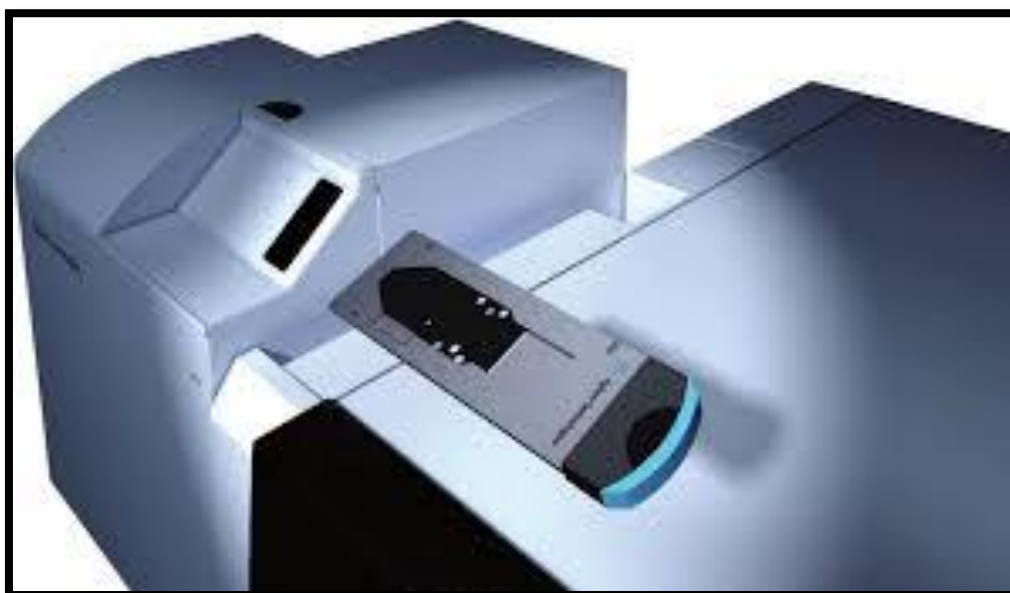


Fig. 6. Chip injection port on the chip-cube

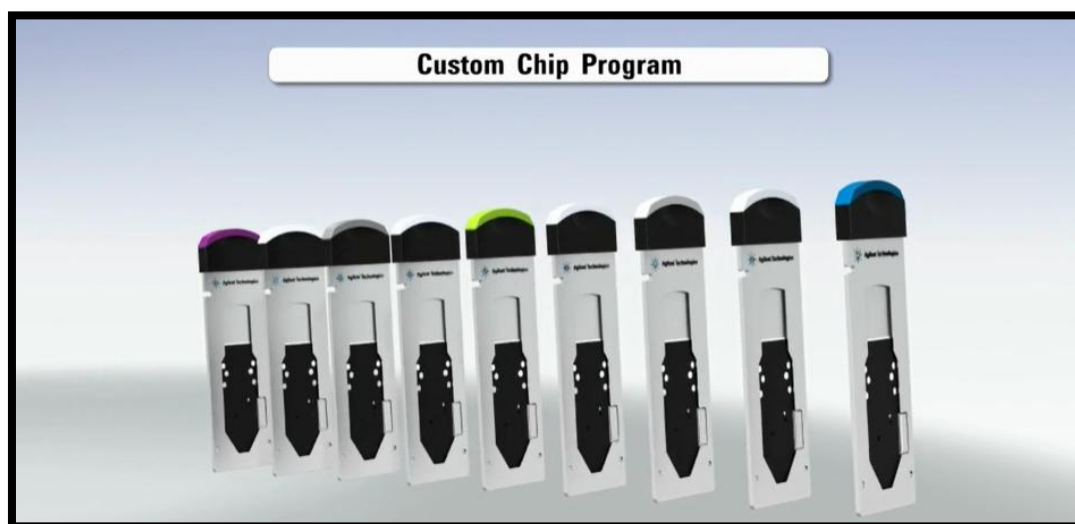


Fig. 7. Chips with various rf tag programming

## 5. PRACTICAL CONSIDERATIONS

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 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 [40].
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 a chiral stationary phase substance, cellulose tris(3,5-dimethyl phenyl carbamate) coated on 5- $\mu$ 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 [39].
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 [41-50].

## 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.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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