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Development and Application of Liquid Chromatography in Life Sciences

Bo Zhu* **and Ying Y Chen**

Faculty of Health Sciences, University of Macau, Taipa, Macao SAR, China

***Corresponding author:** Bo Zhu, Faculty of Health Sciences, University of Macau, Taipa, Macao SAR, China, Tel: 8822 2734; E-mail: bozhu@umac.mo

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Abstract

Liquid chromatographic approaches cover a wide range of applications today. It is closely relevant in scientific studies, which provides a powerful identification of certain components that will be effective for curing specific diseases. Especially important, LC methods can assist us for isolation and purification of therapeutic drugs that benefit for the development of medical industry. As the development of liquid chromatography, the single effective component within a drug can be separated and purified under extreme purity requirements from a biological system with great complexity and abundance. However, improvement of LC methods is still urgently needed for better application of this method to benefit our lives.

Keywords: Analytical methods; Application; Development; Life sciences; Liquid chromatography

Introduction

This review focuses on liquid chromatography development history, current state of development, in particular, its application in the life sciences.

Currently, liquid chromatography is developing into a widely applied analytical method. LC is becoming an increasingly important tool that helps us discover the underlying of specific phenomenon in life sciences. Actually, LC is the method essential for the development of biomedicine industry, especially for purification of drug components, recombinant proteins and antibodies, which cannot be used for application without help from LC.

This review aims to provide information that covers the history of LC development, especially, to highlight its applications in the study of life sciences. Commonly, the "life sciences" has broader definition that sciences study live organisms, including microbes, plants, animals, and humans, and it can also be grouped by application differences, including diagnostics and therapeutic in clinic, contaminants and pollutants analysis, and other aspects. Since the 1950's, it has continuous improvements for the discovery, development, and production of drug for the following decades. As the coupling of LC to mass spectrometry, it supports us in advanced analytical platform which facilitates peptide and proteins identification and purification, and separation of other small molecules.

Development history of liquid chromatography

Back to 19th century, the accurate separation of small size chemicals seems impossible although adsorptive separations were already applied in the 19th century [1]. The term "chromatography" was first adopted in 1903 for isolation of chlorophyll constituents [2]. The key difference between "chromatography" and previous methods is the use of suitable and sufficiently well characterized adsorbents. The finding of suitable adsorbents provides a possibility for invention of novel methods, liquid chromatography, which needs huge background knowledge for the selection of materials and liquid-solid interactions.

Two decades later, Kuhn and Brockmann, the two German scientists recognized that adsorbents are reproducible and selective adsorbents are needed. Therefore, the improved requirements of stationary phases trigger the production of materials with different adsorption strengths, which is the first attempt to reproduce separations for individual components.

After this early liquid chromatography, between 1960 and 1970, the application of LC appeared faster due to the development of high-performance liquid chromatography (HPLC) [3]. The benefits of improved-column LC method over the other common column LC due to several factors as follows, firstly particle size of the adsorbent reduced, then the small particles were packed into pressure-stable columns, and high and constant velocities were required for operation of the separation [4]. Soon after the manufacture of mechanically stable adsorbents like microparticulate silica which was packed into stainless-steel column densely, further improved the efficiency of HPLC [5]. The combination of improvements in the adsorbents, proper column, and corresponding system development became typical innovations during the past 50 years for exploration phase of HPLC.

The modern LC methods are based on three foundations, including process LC, analytical HPLC, and bio-separation, and they have been discussed one by one as follows.

Process LC

As the continuous progress of chromatography, the invention of stimulated moving-bed (SMB) technology, together with materials optimization for specific separation tasks contributed to high efficiency for application in petrochemical area [6]. Actually, the concept of SMB chromatography was not adapted until 1990s; further the technology was transferred to the production of pharmaceuticals [7]. It is great important to develop several types of highly selective adsorbents in 1980s, the resolution is for analytical HPLC application [8], which allows precisely separation of components from complex products. In particular, the combination of HPLC methods and SMB technology with the assist of dynamic axial compression (DAC) [9], facilitates the production of large amounts of pure products.

LC is considered as a great powerful method for analysis, currently, it acts as the only technique that deals with the separation and identification of compounds from complex biofliud sample at the level of femtomolar amounts. Furthermore, LC is also applied for the purification and isolation of industrial products at high amount level.

Analytical HPLC

Analytical HPLC was widely applied for analysis of composition for drugs and chemicals. The most revolutionized developments for HPLC in the 1970s were the application of small particle silica as adsorbent, with its diameters smaller than 10 μm, which was packed into stainless-steel columns [10]. The past several decades, the diameters of adsorbents for packing is significantly reduced from 10 μm to 5 μm or 3 μm, and further to less than 2 μm to improve the efficiency of column, reduce time for analysis, and enhance the sensitivity [11].

The breakthrough of column development occured around 1980, it is the first application of fused-silica capillary columns with a coat of polyethyleneimine. Special micro- and nano- LC instruments are required to operate these capillary columns which results in generation of flow rates in the range of μl min^{-1} to nl min^{-1} [12]. Furthermore, the efficiency for analysis is dramatically improved by capillary columns. For example, the capillaries packed with microparticulate silica can analyze plate over the speed of 100000 plate min^{-1} [13].

The great development of LC triggers it as a common tool of analysis in life sciences research for several benefits. The most obvious advantages include extremely low amount of sample required, low consumption of solvent, and extremely high level of sensitivity for reduced sample dilution required. However, the operating of HPLC systems is more difficult than conventional LC and high demand of knowledge and skills are needed for the operator.

The most important breakthrough in the development of HPLC was its coupling to mass spectrometry (MS). Thus, MS coupled HPLC can act as both the detector and separator; this great advancement occured during the 1990s [14]. It is well known that mass spectrometry can be divided two classes, electrospray ionization (ESI) and matrix-assisted laser-desorption/ionization (MALDI) techniques. The advantages of LC-MS used for analysis of environmental pollutants, drug components, etc. are improvement of accuracy for detection of in low-mass molecules, improvements of sensitivity level, reduced limits for detection, etc. Furthermore, the low rates of flow can be produced after coupling micro- and nano-LC systems to MS.

All in all, analytical HPLC provides a wide range of opinions for selection of various efficient silica columns, and the columns provide us an effective platform for precise analysis in the industry of pharmaceuticals, such as the discovery and development for drugs.

Bio-separations

Bio-separations originate the application of cross-linked dextrans which are used as packing materials for size-exclusion by researchers around 1950 [15]. The defects of the packing gels were not stable as rigid silicas to pressure. Since the hydrophilic polymers showed swelling properties, they were named as soft gels, for example, agarose gel was classified as one of soft gels.

Since the disadvantage of LC packing materials were intrinsic, it is difficult for diffusion in the pores, therefore, around 1980, small size of silicas that with relative large pores were used as packing materials to achieve more efficient and faster separation than before [16]. Other methods to overcome pore-diffusion effects trigger the introduction of hydrophobic interaction chromatography (HIC) and ion-exchange and affinity chromatography [17] due to retention, which occurs not inside of microparticles but at the outer surface. Actually, the development of robust adsorbents for process-scale chromatography has been increased fast from the late 1960s. However, the challenge was still to overcome diffusion-induced mass-transfer limitation. Many factors can contribute this limitation, including time of retention, size and porosity of bead, stationary phases, and matrix morphology.

Since 2000, Protein A adsorbents have been widely applied for affinity chromatography, especially for the dominant application for purification of biological components from biofluid mixture, such as monoclonal antibodies. The benefits of this Protein A adsorbents-based affinity chromatography were improved stability to alkaline cleaning, and high specificity of binding capacities against individual antibody. The bottleneck for biopharmaceutical development mainly locates at the downstream processing [18]. The cost for processing chromatography is the most expensive part among downstream technologies [19]. However, it is still the dominant powerful tool for biopharmaceutical industry, since affinity chromatography showed a unique advantage over other technologies, its selectivity. Recently, the techniques for purification of biological products, especially for recombinant proteins, have been improved dramatically, such as continuous multicolumn counter-current solvent gradient purification processes [20].

In summary, the methods for bioseparation are comprised of several different modes according to selectivity differences, including hydrophobic interaction chromatography (HIC), hydrophilic interaction chromatography (HILIC), ion-exchange chromatography (IEC), the variants of affinity chromatography (AC), and size-exclusion chromatography (SEC) [21], which are widely applied either for scientific research or product purification in industry.

LC in the new era

After the completion of human genome project in April, 2003, it was found that human proteome with higher level of complexity than genome consisted of a protein system that randomly combined 20 different amino acids. The dynamic protein system has a wide range of posttranslational modifications, huge concentration difference of abundance. Therefore, proteomics has a much higher level of complexity than genomics.

The traditional 2D gel-based followed by MS analysis has its limitation as follows:

The major defect is the missing for detection of small proteins and peptides, and few low-abundance proteins and peptides can be identified due to the loading limitation. Furthermore, the capacity for separation of proteins and peptides is also limited. Moreover, the procedure for accurate identification is time consuming since various posttranslational modification of the same protein might disperse over the entire gel.

Considering the limitations of gel-based proteomics for protein identification, liquid chromatography provides the possibility for separation with high flexibility and selectivity. Different substances can be monitored and separated according to differences of quantities by adjusting the column size. The sensitivity can be improved by using column with reduced internal diameter. Furthermore, high separation capacities can be achieved by using multidimensional LC. It is difficult

to quantify the abundance of proteins with extreme abundance differences by LC-based approaches [22]. Therefore, the combination of suitable columns chosen and processing of multidimensional LC, allows us to trace a number of components in a complex sample.

Normally, some well-known proteins in biofluids that have high abundance, such as albumin, increase the difficulty for detection of low-abundance proteins and peptides. Therefore, it is necessary to kick these proteins out before digestion of samples followed by separation. One of the most highly specific approaches is to load the total protein sample into affinity column that has specific adsorption of these high abundance proteins, such as albumin. However, the specificity of the affinity column requires highly concentrated samples. Furthermore, antibody-based affinity column also can be applied for reducing the abundance of albumin in biofluids. However, the affinity chromatography can be only used to reduce the amount of known high-abundance proteins, and is not useful for handling low-abundance targeted proteins.

It is well known that sample clean-up is essential for further analysis by LC system. To obtain highly reproducible data, on-line sample clean-up procedures are mandatory. The development of column with restricted access materials (RAMs) packed that has hydrophobic interior but coated by some hydrophilic material [23]. Therefore, only the small molecules can pass the column, however, the large molecules have to be excluded from pore by a size-excluding mechanism. Furthermore, RAM columns that have strong cation-exchange groups on surface, which facilitate extraction of positively charged peptides from biofluids. At present, two dimensional LC system is dominantly applied in biological research, especially for separation of endogenous peptides from biofluids. Normally, strong cation-exchange chromatography can increase mass load capacity, and it is considered as a primary separation technique. Normally, the reversed-phase LC is applied as secondary separation technique due to its function of salts-clean. More importantly, it is compatible to couple to mass spectrometry equipment. Actually, the particular benefits of this multidimensional LC system lay on its high reproducibility and high separation capability.

In summary, LC in new era has some features that are essential for application in life sciences. Firstly, LC system can do the cleanup for selective sample; even quantify its content in an automated mode. Secondary, LC system has a number of phases for selection to analyze individual samples, such as HIC, HILIC, SEC, AC. Thirdly, multidimensional process can be integrated into LC system, especially valuable for high concentrated samples.

Application of Liquid Chromatography in Life Science

Pharmaceutical analysis

Pharmaceutical analysis is important for the discovery and development of drugs, also essential for drug quality control. Such analysis cannot be performed without the development of LC, especially HPLC technique. There are high requirements of pharmaceutical analysis, including higher efficiency and sensitivity, and faster analysis. Furthermore, the biggest difficulty for pharmaceutical analysis is low abundance of partial active components in a complex mixture. Therefore, LC is a better choice than gel-base proteomics for detection of low abundance components which can also relatively reduce the complexity of mixtures. After separation of different components in biofluids by LC, mass spectrometry usually acts as equipment for identification, while liquid chromatography is considered as a device for sample clean-up. Currently, LC with reversed-phase silica columns is dominantly preferred for pharmaceutical analysis [24].

Metabolomics analysis

Metabolomics aims in identification and quantitation of metabolites smaller than 1kDa in the metabolome [25]. Metabolomics is also considered as the process of detecting and measuring metabolites changes responding to different status, such as diseases, environmental or genetic perturbation etc. It was reported that at least 200000 metabolic compounds in plants, which acted as a pool for searching new products for medical and nutritional usage [26]. Importantly, the development of human comparative metabolomics triggers greatly improved diagnostic power and enables individualized treatment for disease [27].

Traditional approaches for metabolomics investigation have their limitations due to the large differences of abundance for different metabolites. LC system is compatible for analysis, for it's multiple selectivity. The improvements of mass spectrometry equipment further promote the development of metabolomics. As the advancements of highly selective columns and MS technique, coupled LC to MS is becoming the dominant technique for quantitative and qualitative analysis in metabolomics.

LC in clinical analysis

The objective of clinical analysis is mainly for detection, extraction, enrichment of targeted components in biofluids. Removing target pathogens and toxins out of biofluids is the major goal of therapy. Although the wide application of LC technique for analytical purposes is still a long way for its selective cleans of pathogenic and toxic compounds in biofluids. There are several important parameters which need to be considered for application of LC columns, especially for packing materials, including parameters which refer to the specificity, capacity, particle size, toxicity, ligand stability etc. Safety and efficiency are the priority for LC clinical application. Furthermore, the characteristics of column surface should be biocompatible to *in vivo* cellular environment to reduce cellular response to minimal, either for acute or chronic treatment.

Although it is still a long distance for LC technique to be widely applied for clinical application, highly selective chromatographic packing materials can be used for treatment of a number of diseases. We have confidence to believe that LC-based technology shows great potential for clinical applications as more and more scientists equally have both medical skills and skills in separation sciences.

Proteomics analysis

Liquid chromatography which is currently a widespread application in proteomics study is normally coupled to MS detection after protein digestion. The sample cleanup is priority that can affect the data quality. At present, various clean-up methods prior to sample separation have been rapidly developed with the assist of multidimensional LC technique, however, it still needs further development for application. LC technique is powerful for proteomics research, its application in proteomics can be considered as milestone due to the skip of gel-based digestion, which also boost the perspective of biological industry. Many biological companies have achieved

benefits by using LC in proteomics application, for example, diagnostic and pharmaceutical companies.

Conclusion and Perspectives

The history of LC development is over a century since 1903, LC technique shows tremendous improvements during the past century. Meanwhile, the advantages of LC mentioned above cannot be easily replaced by other methods for sample isolation and purification. Especially, the benefit of analytical LC for small molecules analysis is very significant, including applications involved in analysis of pharmaceutical, food, environmental materials etc. At present, it is well known that the application of HPLC for small molecules analysis in pharmaceutical and metabolomics has reached a mature level.

However, LC chromatography technique in life sciences still needs to keep developing. Sometimes, LC deals with some unstable target molecules, worse still, these unstable target molecules might have extreme wide range of concentration. Therefore, the progress in materials and system design of LC can never stop to handle some tough tasks.

Fortunately, the development LC techniques facilities the processes of biomarker discovery, antibody purification and pharmaceutical products isolation, which must be a breakthrough, in a way for obtaining a high quality live using biotechnology, especially for the development of life sciences.

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