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Editorial

The Importance of Recent Advances in Liquid Chromatography Techniques to the Biomedical Field

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Editorial

The versatility and applicability of Liquid Chromatography (LC) techniques have long been recognized and consequently used for numerous analytical problems in fields ranging from bioanalysis, environmental analysis to materials analysis. Recent advances in the field of chromatography including innovative supports and instrumentation lead to a higher separation efficiency and faster analysis time, which have further expanded its applicability [1]. Especially for pharmaceutics and biomedical research, the multitude of chromatographic techniques plays an increasingly important role.

Since natural compounds with medicinal applications can be separated based on many different properties, an array of different separation techniques will allow highly purified analytes, which are useful for detecting and characterizing proteins and small organic molecules, which has not been previously described in quest of new therapeutics [2-5]. One example is the purification of the Blue Tiger King Lectin (BTKL) from the *Phaseolus vulgaris* plant using a multistep purification protocol involving ion exchange chromatography, affinity chromatography and gel filtration [6]. For subsequent characterization, a Fast Protein Liquid Chromatography (FPLC) gel-filtration method was applied to determine the molecular mass under native conditions, and the N-terminal amino acid sequence was analyzed with HP 1000A Edman degradation and HP 1000 HPLC system. BTKL showed hemagglutinating antiproliferative activity against Hep G2 cells, a perpetual cell line derived from well-differentiated hepatocellular carcinoma tissue, and yet no toxicity is found against the normal human cell line WRL 68. Thus, it may provide a new therapeutic candidate against hepatocellular carcinoma, a globally common cancer with currently limited options of treatment and poor prognosis.

A widely used technique is High-Performance Liquid Chromatography (HPLC), which utilizes pumps to drive a pressurized liquid sample through the column. Introduction of sub 2μm particles offers simultaneously higher resolution, better sensitivity and faster analysis time due to the increased surface area and the ensuing requirement of higher backpressure. The latter has given rise to the name Ultra-High Performance Liquid Chromatography (UHPLC). Another innovation of this UHPLC is the fused core consisting of a solid core, most commonly made of silica, coated by a porous material, which determines the mode of interaction with the sample components. This shortens the diffusion path, leading to decreased resistance to mass transfer and thereby minimizing peak broadening [1]. The advantages of UHPLC to conventional HPLC enables analyses of much more complex samples with a growing number of analytes, which have implicated its use in fields such as toxicology, forensics and doping control.

The analysis of Traditional Chinese Medicines (TCM) is one interesting application [7]. These compounds have been used for centuries in China and are typically composed of a cocktail of herbal ingredients with many active constituents believed to act synergistically. The concentrations of the active substances are dependent on breed, growing conditions and processing techniques inter alia, making the individual samples highly complex and difficult to perform quality control on. UHPLC with a variation of detecting methods including Diode Array Detector (DAD), Mass Spectrometry (MS) and Quadrupole Time-of-Flight MS (QTOF-MS) has allowed many quantitative studies determining the active ingredients in a multitude of TCM concoctions, providing an analysis time of 2-8 min and separation of some eighty constituents. Purification and characterization of these active ingredients in the so-called fingerprint analyses allow better controlled use of TCM and have been accepted by the WHO for the assessment of herbal medicines. Furthermore, it may lead to the discovery of substances that may be introduced as new therapeutics in western medicine.

The higher separation efficiency of UHPLC compared to conventional HPLC is also utilized in many emerging omic fields, such as genomics, proteomics and metabolomics. Such studies involve very complex samples and they are driving the demand for increasingly more efficient and high-throughput separation techniques. Being fast and accurate UHPLC-MS has become the predominant analytical technology in global metabolite profiling. This holistic approach holds especially great promise for identifying new biomarkers for early disease detection as well as disease progression. Drug efficacy prediction is another useful application [8]. Much of the metabolomics research has focused on finding biomarkers for cancer [9]. Attaining better diagnostic tools is expected to have a significant impact on the prognoses in particular for those cancer forms in which the majority of cases have already progressed to advanced stages at the time of diagnosis. The greatest challenge for untargeted metabolite profiling continues to be, however, whether instrumentation can provide a high enough resolution and sensitivity [10]. It will be interesting to follow the development and new applications for UHPLC and other

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chromatographic techniques in the biomedical field as improvements in methods and instrumentation are constantly being achieved.

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