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Introductory Chapter: A Tool for Aided Advanced Diagnostics and Deep View into Biological Sample

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1. Introduction

The mass spectrometry (MS) is a technology enabling the measurement and analysis of multiple analytes with very high sensitivity and selectivity. The MS “use for purpose” includes the analysis of proteins and their modifications using either the top-down or the bottom-up approach, the analysis of multiple parameters for toxicology research, targeted analysis of active pharmaceutical compounds in patients’ samples.

Recent years has brought a significant improvement of technology for ion-trap, the time-of-flight, and triple quadrupole instruments resulting with a surge in applications for the medical, biological, and inorganic field. One of the greatest challenges for the MS was how to solve the problem of the insufficient ion transfer from the ionization source through different mass filters and to the detector. This challenge can be considered essentially solved due to changes in the design of ion-sources and subsequent mass filters such as ion funnels of different design, which enabled hugely improved ion focusing and ion transfer. However, there is still enough space to further improve the design of the interface to enable handling of larger ion currents generated by more powerful and more intense electrospray (ESI) ion sources. That would make bulky, expensive, and complex pumping systems obsolete. Actually, the greatest challenge of improving the MS is the ionization step, particularly when MS is used in combination with the liquid chromatography (LC). The combination of LC and MS (LC–MS) has advanced to the main workhorse in many laboratories, especially in biotechnology and medical laboratories. Since the low LC flow rate significantly increases the efficiency, the sensitivity, and the stability of the ESI, the use of separation columns with small inner diameters is recommended for the hyphenation of the two technologies. In cases with very low flow rates, e.g. 5–50 nl/min, a complete ionization of a substance can be achieved. On the other side, the use of such a low flow rates causes problems with flow’s stability, reproducible flow gradient mixing, and stable ESI performance.

2. Clinical laboratory and mass spectrometry

The laboratory medicine along with medical imaging procedures is one of the pillars of the modern diagnostics. The laboratory medicine has significantly benefitted from technical and technological development of analytical chemistry, miniaturization of instruments, and optimization of analytical methods.

Colorimeter and spectrophotometer were the first modern analytical instruments to be used in a clinical laboratory. Since the time of their introduction, the art of

performing analyses and tests has significantly changed. In the 1950's additional developments were made and, in 1957 and 1959, respectively, the autoanalyzer, which is the precursor to the modern analytical systems, and the first RIA (immunoassay) for analysis of insulin were introduced. The introduction of RIA, which is still widely used, for insulin has significantly improved and changed the art of measuring a large of compounds.

The introduction of the mass spectrometry (MS) into the clinical laboratory had had the same revolutionary impact as the previously mentioned methods. A brief search for "mass spectrometry" and "clinical" from 1950 until 1970 results with only 9 publications! During the next 20 years, until 1990, the number of publications referring to the use of MS for different analyses in clinical laboratory jumped to 798! From the early 1990's until today, the steep rise of MS methods and approaches for analyses in clinical laboratory has steeply raised with more than 5000 publications from January 1st, 2020 – February 1st, 2021.

The significant rise in use of different MS approaches for clinical analyses correlates with improvements in ionization technologies, miniaturization of separation systems, notably of chromatographic systems (HPLC) [1], the significant and exciting improvements in sample preparation even of a single cell [2], and bioinformatic analysis. The mass spectrometry is applied for both "classical" clinical laboratory analyses and for analyzing samples for personalized and precision medicine. Undoubtly, the approaches and methods describe in current book are only a small part of possible applications.

In clinical laboratories, the analysis of clinical samples and monitoring levels of active compounds and their metabolites in e.g. patients' blood and urine samples are the main application fields. It is possible to perform specific detection of target analytes by applying MRM/SRM (multiple-reaction monitoring/selected-reaction monitoring) or SIM (single-ion monitoring) thus significantly enhancing the selectivity and sensitivity of the analytical method and provide targeted and highly specific analytical approach.

3. Separation approaches

Analytes of interest in complex biological samples must be separated prior to mass spectrometric detection and analysis and chromatography is the most widely used separation method for biological samples prior to MS. A number of Companies, e.g. Chromsystems (<https://www.chromsystems.com/>), ThermoFisher Scientific (<https://www.thermofisher.com/at/en/home/clinical/diagnostic-testing/clinical-chemistry-drug-toxicology-testing/therapeutic-drug-monitoring.html>), Biocrates (<https://biocrates.com/>) or BioRad (www.bio-rad.com) developed analytical systems for a broad range of analyses of important clinical parameters. Integrated HPLC–MS clinical systems were also developed but they never really did find broad acceptance. Sciex introduced the Topaz® system (<https://www.businesswire.com/news/home/20170731005050/en/First-Fully-Integrated-LC-MS-System-for-Clinical-Diagnostics-Announce-by-SCIEX>) and Thermo Fisher introduced the Cascadion SM lab analyzer (<https://www.labmedica.com/aacc-2017/articles/294770271/worlds-first-fully-integrated-lc-ms-ms-clinical-analyzer-unveiled.html>) in order to lower the barriers of many laboratories to adopt LC–MS.

The hyphenation of chromatography and mass spectrometry has its primary values in relatively fast detection and analysis of multiple analytes in a single sample with high sensitivity and high selectivity - the key challenge and requirement to detect and quantify low-concentration analytes. Currently, the most widely used separation columns for the HPLC–MS in a clinical laboratory have an inner

diameter of 2 mm. The quality of electrospray is highly dependable on separation conditions, i.e. mobile phase, presence or absence of salts, flow speed, column's inner diameter, etc. In proteomics, the use of separation columns with 50 μm or 75 μm ID is state-of-the-art; however, the columns operated at a low flow rate of several hundreds of nanoliters/minute are still rare in clinical analysis although they can provide a significant increase of sensitivity. Currently, the use of nanoflow separation still cannot cope with the demand for high sample throughput and robustness in clinical applications. Currently, the closest compromise between sensitivity and throughput is the use of the microbore and the capillary columns of 300 μm – 500 μm and 1 mm – 2 mm inner diameter.

A new and exciting application of mass spectrometry in the clinical environment is the use of “live-MS” during surgical operations. Further development of this approach will revolutionize the diagnostics and help surgeons in extracting e.g. tumors with higher accuracy and better prognosis for the patient following the surgery [3–5].

In addition of analyzing small molecules in a targeted approach, the mass spectrometry can be applied in a clinical laboratory for a more widely screening approaches, e.g. screening of the human metabolome. The metabolome shed a light on our biological life story, revealing changes and processes that happened due to our genetics and due to the influence of the environment and the lifestyle. The measurement, detection, and analysis of metabolites is a step towards profiling an individuals' metabolic profile at any given time. That information can help understanding and, eventually, predicting the impact of environmental factors on the health. Therefore, metabolomics, in combination with other omics methods is a potent addition to developing personalized medical approaches.

A number of analytical methods for mass spectrometry in clinical laboratories were developed during the recent past, and the development gains additional momentum as the CoVID-19 pandemics holds the mankind in grip and methods for fast HPLC–MS have been developed and applied [6–15].

4. Clinical proteomics

The use of MS technology for measurement and analysis of clinically important peptides and protein biomarkers will definitively increase with further improving MS technology. It is clear that personalized medicine will become the major field of further development of targeted therapy and MS will be one of the major players in identifying both therapeutically targets and therapeutical agents. It is with great certainty that MS applications for evaluation of protein and peptide-based, or even the mRNA-based therapeutics, will play a crucial part for the quality control of the therapeutics, evaluating drug efficacy, or investigating therapeutic response.

Another issue is the miniaturization of MS and LC–MS systems that can be used portable systems or be applied in smaller field-laboratories. Certain advances were already achieved on developing such devises [16–23] that are being used in a number of analyses.

To conclude, mass spectrometry is a powerful analytical technology that still has not developed her full potential for use in clinical laboratory although its' use is growing. Different mass spectrometry-based devices have been approved for screening newborns, identifying microbes and fungus in cultures of human cells or for detecting measuring the concentrations of drugs (therapeutic and illicit) in body fluids. The development of large and integrated HPLC–MS systems for detecting and measuring peptides or proteins in clinical laboratories is still waiting to happen but mass spectrometry has already gained access to all areas of medical research and diagnostics.

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