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Lab Automation Insights in Sample Preparation for LC/MS Analysis

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This week in San Diego, USA the Society for Lab Automation and Screening (SLAS) annual meeting and expo is taking place. This conference features 132 word class podium presentations and 400 poster presentations attracting experts from industry and academia, students, engineers and business leaders from around the World. This is only the third annual meeting, representing a relatively young and rapidly growing and developing field of automation for different laboratory workflows and processes, which is steadily replacing semi-automatic or manual methods. Initially, the complete automation of manufacturing processes was achieved in industry for various production lines. Next, automation has brought benefits to sterile environments, e.g. vaccine production and blood banks in order to minimize employee exposure to biological hazards, minimizing human error, and ensuring product purity and integrity. The next breakthrough of automation was in routine laboratory operations, such as immunoassays, crystallography, DNA screening/sequencing, cell culture and stem cells assays. Automation of immunoassays has allowed multiplexing (multiple parallel assays) using single source serum samples significantly speeding up analysis while enhancing sample integrity by eliminating multiple freeze thaw cycles, and/or reducing sample requirements by reformatting from a single source sample. ELISA and other plate based immunoassay are the most frequently used assays in clinical diagnostics. They allow fast, relatively specific and sensitive assays of many analytes or metabolites. A workflow bottleneck for immunoassays is a typical incubation time of several hours, that makes the processing throughput of manual processing relatively slow - only a few plates per day per lab technician. Automation of plate based assays, which has matured over the past few years has increased the productivity of clinical laboratories, decreased operational costs, minimized (human) errors, while enhancing workplace safety for laboratory personnel.

Recent progress in automation allowed various state-of-the-art technological solutions, offered by leading vendors such as Agilent Technologies, Hamilton, Perkin Elmer, and Tecan. A major impediment in the past to developing automated processes was the limited numbers, and significant cost of third party devices (such as readers, centrifuges, shakers, bar code readers) that can be loaded on the deck and interfaced (controlled) by the liquid handler operating software. This limitation has been steadily overcome, and as a result, lab automation is now capable of facilitating progress in high throughput technologies in the fields of drug discovery, drug target biology, informatics, biomarker research, molecular diagnostics, bio-analytical chemistry and high throughput screening.

Currently the most challenging area in applying automation processes in bio-analytical chemistry is in the area of liquid chromatography combined with mass spectrometry analysis (LC/MS). Unlike immunoassay where there is no need for sample preparation due to analyte(s) of interest binding specifically with their targeted antibody within the sample matrix, LC/MS analysis of biological fluids, such as serum, plasma or urine typically require extensive sample preparation followed by chromatographic separation. Sample preparation (plasma protein precipitation, solid phase extraction or liquid-liquid extraction) and chromatographic separation are time-consuming processes. While it is possible to accelerate sample preparation by processing samples in 96 well plate format (manually or semi-automatically), chromatographic separation prior to mass spectrometry detection remains one sample at a time. Increasing LC/MS throughput is one of the greatest challenges in the field of modern chromatography. This has been partially solved by implementing smaller (sub 2 micron particle) UHPLC columns and fused-core materials with modern, low dead volume chromatographic equipment, that allows maintaining appropriate resolution of chromatographic separations at higher flow rate. However, the main bottleneck in LC/MS analysis has been the classical design for data acquisition: one chromatographic column, single injection–single data file. This approach cannot support multiple sequential injections into several chromatographic columns under control of a single LC/MS system. While this limitation cannot be resolved by lab automation, the second bottleneck–automated optimization of sample preparation prior to chromatographic separation, -has not attracted the necessary attention and real software solutions. To date, the main goal of automation development has been direct translation of the steps in a manual workflow into the robot's software, requiring precise and detailed specification of the sequence of commands while taking into account the imprecision inherent to manual workflow vs that of robotic devices. Again, the goal of this method transfer is to obtain a single method for a robot. Automation of sample preparation using a liquid handler is very important and time-saving task, however it is typically reported as an off-line process. For example in the development of a liquid-liquid extraction process, it is necessary optimize the following variables on one 96 well plate: volumes of sample and extraction solvent, type of solvent (hexane, MTBE, ethyl acetate, etc), proportions (if mixtures are used) and pH. Assuming preparation in triplicate to assess reproducibility, it would anticipate defining 32 different liquid handler methods. For duplicate analysis, creation of 48 different methods sequence respectively! For highly complex samples, a second stage of liquid-liquid extraction might be beneficial further complicating method development and optimization. It should be noted that sample plates when prepared via manual vs automated methods, may quite possibly yield different results due to different imprecisions in the tools used. Therefore, it would be highly beneficial to do all screening of sample preparation conditions using the same liquid handler, if further workflow will be automated. Unfortunately, no general routine software tool has been developed which allows custom-based conditions for screening using one generic liquid handler method.

In conclusion, fully automated method development software

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tools in support of robotic sample preparation for LC/MS analysis has been overlooked in the past (perhaps, due to higher costs of LC/MS and robotic equipment and limited availability of on-deck sample preparation tools). For example, a robotic version of a positive pressure manifold for SPE and filtration plates has only just been introduced by Hamilton at the current SLAS meeting, while the stand alone (manually controlled) devices have been used in bioanalytical laboratories for at least 5 years.

10 years ago, liquid–liquid extraction (LLE) was considered difficult to automate, and a less efficient approach to the reduction of matrix effects, compared to solid-phase extraction. The recent recognition of phospholipids as the major contributor to matrix effects in LC/MS analysis, and appropriate actions during the LLE method development, such as monitoring analyte and phospholipids yield during extraction, have promises to provide more efficient sample clean up and should lead to more efficient LLE-based and fully automated sample preparation, comparable to SPE.