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Automation in new frontiers of bioanalysis: a key for quality and efficiency

"To achieve high efficiency and quality in these new frontiers, automation is a key component and needs to be explored, evaluated and exploited."

Keywords: automation ■ dried blood spot ■ protein therapeutics ■ quality

Bioanalysis, the quantitative determination of drugs and their metabolites in biological matrices, plays an important role in drug discovery and development. The most commonly used techniques in bioanalysis are LC–MS/MS for small-molecule drugs and ligand-binding assays for large-molecule biological drugs. A typical LC–MS/MS assay involves the following steps:

- Preparation of calibration standard (STD) and QC samples;
- Sample identity confirmation, sorting and organization;
- Sample aliquoting and dilution;
- Sample extraction (including liquid–liquid extraction [LLE], SPE, protein precipitation and so on);
- Sample analysis by LC–MS/MS;
- Data collection and processing;
- Report generation.

The implementation of automation in this process is expected to provide increased throughput with improved consistency and quality. In addition, automation can improve safety compliance, for example, freeing analysts from repetitive motion to avoid ergonomic problems. With these advantages, automation has been widely applied to bioanalysis [1,2] and has made significant progress over recent years [3]. Actually, automation is so successfully used in some parts of bioanalysis that people may rarely realize its existence, such as automatic data acquisition/processing, or automatic sample injection made possible by autosamplers.

New developments in bioanalysis include the adoption of new technologies in existing fields as well as the application of mature technologies to new scientific fields. Two recent examples are the introduction of dried blood spot (DBS)

technology and the application of LC–MS/MS for protein therapeutics. To achieve high efficiency and quality in these new frontiers, automation is a key component and needs to be explored, evaluated and exploited. In addition to developing and applying new automation technologies, it is also important to think outside the box to find an easier alternative way to facilitate automation. In this article, we present a brief summary of a few significant advances of automation in bioanalysis. We would like to share our view of applying automation to DBS techniques, and our assay development work, which could facilitate implementation of automation for both DBS bioanalysis and LC–MS/MS quantitative bioanalysis of protein therapeutics. We would also like to present our view of the future perspectives of automation in the field.

Automation for improving efficiency & quality

One significant achievement of automation in bioanalysis has been the introduction of the 96-well plate format in sample extraction (SPE, LLE and so on) and its combination with robotic liquid handling systems in the 1990s [4,5]. The parallel preparation of samples in 96-well plate coupled with automatic liquid transfer significantly improved throughput and efficiency. The accuracy and precision of the automated approach was found to be comparable to manual operation [6]. This semi-automated or fully-automated approach is now routinely used in bioanalytical laboratories in both drug discovery and drug development.

Quality, a critical component of bioanalysis, primarily refers to the accuracy, reproducibility and robustness of the assay. However, in addition to the performance of the assay itself, quality also results from the execution of the assay. The efforts and precautions put into assay development and optimization to ensure quality

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will be less meaningful without accurate and reproducible operation of the assay in production [7]. Inconsistency and error are difficult to completely eliminate in manual operation. Automation can provide consistent execution of the assay and ultimately improve the quality of bioanalysis. Traditionally, automation was mainly applied to increase throughput and efficiency without compromising data quality. More recently, the effect of automation on improving quality has been increasingly realized. For example, it was reported that manual pipetting among different analysts caused differences and bias in final immunoassay results, while using robotic liquid handlers for automated pipetting minimized the variability and improved the quality of the assay [8]. Another example is the implementation of the automated 2D barcode processing in sample preparation. This process completely eliminated sample identity errors, and significantly improved efficiency by reducing the time required for sample identity confirmation, sorting and organization [9,10]. Automation will continue to be increasingly applied to improve quality in bioanalysis, especially for more complicated processes/assays.

Automation in DBS bioanalysis

DBS, a microsampling technology to collect blood samples on a filter paper, has recently gained interest in the bioanalytical community [11]. Compared with conventional plasma sampling, DBS requires much smaller blood volumes and offers simpler storage and transportation, which makes it a very promising alternative sample collection procedure. A typical DBS bioanalysis assay includes multiple steps: DBS sample collection, preparation of STD and QC samples in blood, spotting of blood samples on DBS cards, punching out the spots, and sample extraction. Manual operation of these steps is very labor intensive and time consuming. Consequently, integrating automation into DBS bioanalysis is very desirable. Many different automation approaches have been evaluated for different steps in DBS bioanalysis. For example, the use of an automated blood sampler was successfully applied for blood collection and DBS sampling in discovery PK studies [12]. Their results obtained from automation were consistent with those from manual operations. Besides improved efficiency, automated DBS sampling eliminated human error and improved quality during this process. We recently reported automated DBS

STD and QC preparation using a robotic liquid handler [13]. The robotic liquid handler was used to prepare whole blood STD and QC samples and then spot them onto DBS cards. This fully automated process enabled an efficient and hands-free operation. It also provided improved quality and safety, especially for the preparation of a large batch of samples.

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For DBS punching, there are many automated or semi-automated instruments commercially available. Typically, DBS cards are fed in manually or by a robotic gripper, and then punched into 96-well plates automatically. A barcode reader can also be incorporated into the system to enable accurate and automatic sample identification. These instruments greatly improve the efficiency of the DBS punching step, as well as quality and safety. From our experience, once analysts have mastered the operation of a DBS punching instrument, they never go back to using the manual puncher. For alternative sample extraction methods (other than regular LLE, protein precipitation or SPE) there are also other automation options available, such as automated flow-through online DBS extraction technology [14]. In this technology, DBS cards are extracted by flowing the extraction solvent through the DBS samples without punching out a disc. This technology can be coupled with online SPE cleanup and/or LC separation, and enable automation of the whole DBS bioanalysis process. Overall, with the improvement of automation, the efficiency and quality of DBS bioanalysis will be greatly and continuously improved. It is very likely that the progress of automation will help DBS bioanalysis to reach the same level of convenience and quality as conventional plasma analysis.

Facilitation of automation in new frontiers of bioanalysis: examples in DBS & quantitative analysis of protein therapeutics

Unlike plasma samples that can be easily diluted with blank plasma, the commonly used approach for the dilution of DBS samples is to use

extracted blank DBS samples containing only the internal standard (IS). This approach is very inconvenient and hard to automate, especially for a large dilution factor (e.g., >tenfold). Liu *et al.* reported a novel 'IS tracked dilution' methodology for DBS samples [15]. In this method, instead of using a single concentration of IS working solution, dilution factor-adjusted IS working solutions were added to the samples requiring dilution (e.g., for a tenfold dilution, a ten-times concentrated IS solution was used). The samples were then extracted and approximately diluted into the assay's linear response range for LC-MS/MS analysis. This approach significantly simplifies the dilution of DBS samples and enables easier implementation of automation, especially when a large number of samples require dilution and/or a large dilution factor is needed.

If dilutions can be eliminated, then inconvenient sample dilution processes will not be an issue. A broad calibration curve range would eliminate the need for dilutions, thus greatly improving efficiency and avoiding the trouble of automating a complicated dilution process in a DBS assay. Yuan *et al.* achieved a linear calibration curve range of five orders of magnitude by monitoring two SRM channels of different intensities simultaneously [16]. They proposed that this approach could be particularly useful for DBS assays, which are hindered by complex sample dilution. For a typical toxicokinetic or PK study, this wide range is enough to cover the concentration range of all the samples without dilution.

Recently, LC-MS/MS combined with enzymatic digestion has emerged as a promising alternative approach for the bioanalysis of protein therapeutics [17]. In this approach, one or more surrogate peptides from proteolytic digests are analyzed by LC-MS/MS. A consistent and efficient digestion is very critical to achieve accurate and reproducible measurements. Pretreatment procedures, such as sequential denaturation, reduction and alkylation, are commonly applied in protein digestion, especially for large proteins [17]. However, these pretreatment procedures are very time consuming, making protein digestion the bottleneck step of the assay. Recently reported pellet digestion methodology eliminates the tedious pretreatment procedures, and offers simple yet efficient digestion for large proteins [18]. It also provides efficient digestion for hard-to-digest proteins, such as a monoclonal antibodies with multiple disulfide bonds [19]. This is a very promising technique in LC-MS/MS

bioanalysis of large proteins since it is simple, fast, efficient and, most importantly, easy to automate.

Future perspective

Automation greatly improves efficiency and productivity, can eliminate human error, and enhances overall quality in bioanalysis. Once an automated system is set up correctly, calibrated, and appropriately maintained, it will provide consistent, accurate and predictable operation. In addition, with most automated systems, every operation can be recorded in the software's instrument logs, which makes it easy to track and fix errors. With audit trails and error logs, automation also ensures better compliance with GLP regulations. However, it may not be practical to automate every step in an assay. The recommended strategy is to automate steps that are labor intensive and time consuming, critical steps for which human intervention may lack accuracy and consistency, and steps that involve a safety concern.

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Delivering high-quality data with high efficiency is always a goal for analysts. Automation will continue to play an important role in achieving this goal, and will be a key for improving quality and throughput in the new frontiers of bioanalysis. The implementation of automation may greatly facilitate the development and application of these new frontiers in bioanalysis, whereas the lack of automation may actually hinder progress. It is important to take automation into consideration from the early stages when developing new technologies and new applications in bioanalysis.

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