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COMMENTARY

Automation of LBA-LC-MS/MS assays

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During a recent visit to a major bioanalytical contract research organization (CRO) in the US, we inquired about the CRO's use of lab automation to help improve wet lab capacity and data quality and integrity for one of our LBA-LC-MS/MS assays placed there, and learned that they only use 96 well aspirator/dispenser type of automation devices to automate a few 96 well plate-wide liquid handling steps. When asked why they don't use lab automation to automate majority of wet lab work, they answered: we don't have the resources for that.

The booming of biotherapeutics and large molecule bioanalysis

Bioanalytical CROs are becoming increasingly important as source for bioanalytical work, especially in large molecule bioanalysis (LMBA) arena. It has recently been estimated that the majority of late stage bioanalysis are in the hands of Bioanalytical CROs. Such CROs range in size from 10-20 staff with unique skill and technologies to those that have thousands of staff with large capacity and capabilities.

Bioanalytical CROs often encounter important challenges in planning and staffing for support of large scale bioanalytical tasks due to the recent booming of biotherapeutics. Bioanalytical CROs may have more challenges in maintaining quality and efficiency in LMBA labs owing to limited manpower of LMBA scientists and nature of large molecule methods, which are prone to analyst to analyst variations. However, these challenges are largely overshadowed by limited resources and headcounts, which in turn fuel demand for innovative approaches to enhance the quality and operational efficiency.

The rise of LBA-LC-MS/MS for large molecule bioanalysis

Today, the bioanalytical technology that's being used to support pharmacokinetic (PK) assessments for large molecule program is ligand binding assay (LBAs) which has been the 'methodology-of-choice' for a number of decades and is now being performed at different CROs. Recently, LMBA is considering switching to innovative liquid chromatography with tandem mass spectroscopy (LC-MS/MS) technology to overcome the varied challenges associated with assay variation, critical reagent unavailability, and difficulties in differentiating binding similar molecules. Despite the obvious needs, LC-MS/MS is still viewed today as being cutting-edge technology for supporting clinical PK of large molecules. Today, the biopharma industry is quickly evolving to a point where LC-MS/MS technology is being viewed as a standard tool in the armamentarium for regulated bioanalysis of biotherapeutics [1,2]. The FDA has certainly been engaged in discussions regarding this evolution in bioanalytical technology. Moreover, a number of their reviewers come from a small molecule background where LC-MS/MS is the standard bioanalytical method used to support PK assays.

The complexities of LBA-LC-MS/MS assays & the need for sample preparation automation

LBA-LC-MS/MS assays are complex in nature. The LC-MS/MS side is itself a self-contained discipline and already demands dozens of experimental and instrumental

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conditions to be optimized and maintained. The good news is that once the conditions are optimized, in routine production, the LC-MS/MS portion is mostly automated. On the sample extraction (preparation) side, LBA-LC-MS/MS assays are more complex than traditional LBA and small molecule assays. A typical LBA-LC-MS/MS assay's sample preparation involves preparation of biotinylated antibody, addition of magnetic beads, washing the beads, elution from the beads, addition of buffer and internal standards, alkylation (if needed), digestion, SPE enrichment (if needed, including reconstitution), with multiple incubation steps employed in between. The absolute number of experimental steps for LBA-LC-MS/MS is higher than LBA and typical small molecule sample preparations such as SPE, PPE, LLE etc.

Each additional sample preparation step presents two fold of challenges: first is an additional (set of) parameter(s) to optimize during sample preparation method development. Second is during manual production, with each additional experimental step, the chance of analyst manual error multiplies and in the end, data integrity and quality suffers. Analyst-to-analyst variation is another dimension of concern. The problem is compounded by the fact that there is currently such a high demand for LBA-LC-MS/MS work that many organizations', especially CROs' LBA-LC-MS/MS related capacity utilization is very high. What comes with high workload is high staff turnover rate as well as the ensuing training of new/junior scientists. All of these are painting a picture of highly volatile landscape where it is very difficult to scale up and sustain the application of LBA-LC-MS/MS. We believe the hope lies with automation.

Suggestions on LBA-LC-MS/MS assay automation

Bioanalytical CROs use a variety of approaches to address these challenges, including the use of lab automations. The bioanalytical industry has long reached the consensus that automation is critical for generating quality bioanalytical data, especially automation for bioanalytical sample preparations [3-7]. Lab automation not only includes the benchtop instrument such as liquid handlers but also includes electronic data query and transfer. It is beyond the scope of this article to provide a comprehensive review of all the automation strategies used by Bioanalytical CROs. Herein we provide an in-depth discussion of several examples, with an emphasis on benchtop automations. In both traditional large molecule LBA and small molecule LC-MS/MS sample preparations, there have been reports of highly integrated automation systems that automate the majority of sample preparation steps [8,9]. Given the complexity of LBA-LC-MS/MS sample preparation and its similarity to the above, we suggest that the majority of its sample preparation steps be automated in the same fashion.

One common key feature of those automated systems is automated calibrator and QC sample preparations. The qualities of calibrator and QC samples preparation directly determines whether the bioanalytical run shall be accepted or rejected, and is a major source for analyst-to-analyst variation. Thus automated, consistent preparation of calibrators and QC samples is a must-have for a good automation system. Another common feature of the above automation systems is the integration of individual automated steps into a system encapsulating the entire workflow in question, regardless of the variations in particular input parameters from assay to assay. A third common feature of the above systems is that they link up with LIMS systems and handles work lists, including dilution factors, very intelligently. Such automation systems would take commitment and effort to develop, but would be well worth the investment [10].

Looking beyond sample preparation production automation, the complexity of LBA-LC-MS/MS sample preparation means there are many more parameters to optimize during method development than traditional large molecule or small molecule sample preparation. To optimize those parameters manually would be much more challenging than before. This offers opportunities for sample preparation method development automation [11]. Method development automation may seem distant to many bioanalysts, but with the right investment, it is entirely feasible and within reach [12].

Conclusions

LBA-LC-MS/MS is enjoying rapid growth in popularity and adoption by the bioanalytical industry due to its unique benefits of both LBA and LC-MS/MS features. LBA-LC-MS/MS sample preparation, by nature, is much more complex than traditional large and small molecule bioanalytical sample preparations. This presents considerable challenges for manual production and manual method development, which dampens its scale-up and rate of adoption. Linearly throwing more resources at the problem, e.g. training more bench scientists may not be the most effective way to tackle the problem. A reallocation of the resources, specifically, dedicating some resources for comprehensive automation development, may turn out to be more effective in terms of overall resource utilization.

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