Bioanalysis

HT-ADME in a contract research organization laboratory: can you ensure bioanalytical quality in a highly automated environment?

"As with any bioanalytical assay that relies on LC–MS/MS for end point quantification, key parameters need to be established to ensure robust, accurate data are collected."

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Background

It is well documented that between 1991 and 2000, the attrition of new chemical entities in the clinic due to poor PK fell from ~40 to ~10% [1]. This reduction in PK failure was due to the growth of drug metabolism and PK assessments within drug discovery, in particular *in vitro* adsorption, distribution, metabolism and excretion (ADME) assays. These ADME assays provide a quick and efficient manner in which a compound's potential PK properties can be assessed without the need for costly *in vivo* experiments [2].

The rise in complexity and number of available assays [3] resulted in the field of high-throughput ADME (HT-ADME) coming into existence, and with it, a greater demand on the analytical systems supplying end point quantification. LC–MS/MS has long been the gold-standard in compound quantification, with ever increasing throughput demands pushing the technology further and further toward speed of analysis. This desire to decrease turnaround time at the LC–MS/MS bottleneck has been resolved by various means including (u)HPLC, the reduction in chromatographic dead time and through numerous multiplexing interfaces [4–9]. While this move toward speed has undoubtedly allowed a higher volume of compounds to be screened through these assays, it could be argued that increasing throughput by reducing cycle time comes at the expense of quality analytical data.

A good example of this 'quality versus speed' trade-off would be the application of trap and elute systems in support of hepatocyte stability assays. Phase II conjugates, such as glucuronides and sulfates, are known to undergo in-source fragmentation back to the parent compound in atmospheric pressure ionization sources [10–12] and as such, can artificially increase the amount of parent compound quantified. While it is possible to manually identify compounds that might potentially undergo this type of biotransformation, in a contract research organization (CRO), these compounds are frequently assayed without knowledge of their structure and as such cannot be 'flagged' upfront.

So, is it possible to ensure quality in a HT-ADME contract research organization environment?

While 'quality' can cover a wide range of areas, the focus of this article is on how bioanalytical quality can be maintained despite the high volume of compounds, broad chemical space and variety of matrices routinely assayed. Examples of how our laboratories have addressed these issues have been provided to demonstrate that high-quality HT-ADME analytical support can be achieved within a highly automated CRO environment.

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Developing an appropriate LC–MS/MS system

A major goal in high-throughput LC–MS/MS laboratories is to have a generic (u)HPLC system that can support as diverse a chemical space as possible. Not only does this increase productivity (as multiple assays can be run in parallel) but also facilitates efficiency gains, as identical analytical systems allow for 'on the fly' re-arrangement of assay support, should there be any hardware downtime.

As with any bioanalytical assay that relies on LC–MS/MS for end point quantification, key parameters need to be established to ensure robust, accurate data are collected. The most challenging parameter would be the development of a generic u(HPLC) system where regions of ion suppression are well characterized, so the retention time of a compound can used in isolation to determine if the system is likely to provide the highest quality data.

By applying postcolumn infusion and LC–MS/MS experiments on a panel of compounds previously shown to be representative of chemical space exploited in the pharmaceutical industry [13], potential regions of ion suppression/enhancement from common endogenous material [14] can be determined. Due to the wide variety of matrices assayed in a HT-ADME, these experiments should ideally be performed in buffer, cell suspension precipitate (e.g., protein-crashed hepatocyte), plasma and whole blood (rat, mouse and human) and tissue homogenate (liver, brain, lung and heart). Screening these extracts (with the panel of compounds) across different (u)HPLC stationary phases, mobile phases, additives and chromatographic gradients allows for the development of a 'generic' LC–MS/MS method. To maintain bioanalytical quality, there will more than likely be more than one system and at our laboratories we have found a tiered system to be highly successful in supporting >99% of all compounds that have run through HT-ADME assays.

Tiered LC–MS/MS systems

The tiered LC–MS/MS systems we apply at our laboratories are based on three columns, two mobile phase pH and two organic modifiers. Through application of a column select unit on each platform and careful design of the mobile phases, the following four (u)HPLC systems can be screened (and subsequently applied) in an automated manner without the need for system re-plumbing:

- Tier 1: C18-based, polar-embedded (u)HPLC column; low or high pH modified with either MeOH or ACN; 90 s nonlinear gradients; 120 s cycle time;
- Tier 2a: Functionalized HILIC-based (u)HPLC column; low pH with ACN and H_2O mobile phases; 180 s linear gradient; 250 s cycle time;
- Tier 2b: Phenyl–hexyl based (u)HPLC column; low or high pH modified with either MeOH or ACN; 90 s linear gradient; 120 s cycle time;
- Tier 3: C18-based, polar-embedded (u)HPLC column; low or high pH modified with either MeOH or ACN; 180 s nonlinear gradients, gradient chosen linked to retention time on Tier 1 system; 210 s cycle time.

Tiers 1, 2a and 2b are determined 'upfront' before compounds are assayed, while Tier 3 (an on occasion 2b) tend to be applied as a postassay re-analysis option (should the initial analysis prove unsuccessful). A Tier 4 exists however this is akin to standard method development and will follow any unsuccessful analyses on these systems.

To aid throughput in our laboratories, we cassette our samples postassay (≤8 compounds per injection + internal standard). This approach allows us to exploit triple quadrupole MS systems' ability to quantifying numerous multiple reaction monitoring (MRM) channels in (u)HPLC timeframes. While the high specificity of MRM does not usually require chromatographic resolution, metabolic assays have the potential to produce metabolites from one compound that could be detected in the MRM channel from a second – particularly when working within a defined chemical space. Chemical structure would allow for *a priori* cassetting of compounds to minimize this occurrence, however, as this information is not typically unavailable to the CRO, a more resolving chromatographic system has proved to be beneficial in ensuring good quality bioanalytical data.

Software tools to aid bioanalytical quality

LC–MS/MS method(s) to support HT-ADME screening provide the end point measurement, however, prior to this, each individual compound requires optimization and then assessing on the generic method(s). Commercial software exists that facilitate automated compound optimization [15,16] and within these software solutions, parameters can be set to ensure that certain analytical criteria are met. Application of these methods to assays immediately after compound optimization can be factored into the workflow, however, we have found that manual intervention and review is necessary to ensure quality. This is particularly true when assessing a chemical series with close structural similarities as cross-channel talk can still occur, and analyst review ensures that

the LC–MS/MS method is 'fit-for-purpose' and will deliver good quality data.

To aid in these processes, our laboratory has invested resource in building bespoke software solutions that not only facilitate the compound optimization process but also play an important role in building the analytical 'cassettes' that will analyze the HT-ADME assay. When used in conjunction with an analyst's knowledge, these tools use parameters such as precursor ion, retention time, isotopic distribution and common biotransformations to build the LC–MS/MS cassettes. Not only does this help maximize bioanalytical quality but also aids in productivity due to the reduction in re-analyses due to poor analytical data.

Pulling it all together & streamlining the quality

While optimized methods and software tools facilitate bioanalytical quality, manual intervention can still introduce errors. One area in particular that can impact quality is the building of sample lists to support cassette analysis of HT-ADME assays. As mentioned previously, commercial software is capable of supporting assay quantification immediately after compound optimization [15]; however, this removes any determination of whether the LC–MS/MS method is 'fit-for-purpose'. Building sample lists manually can be fraught with pitfalls as each cassette of compounds requires a specific method containing the appropriate MRMs and chromatography method. To remove this potential issue, our laboratories have generated an internal laboratory information system that seamlessly links the assay design stage of HT-ADME to the analytical batch generator. In this way, the ADME scientist can in parallel: plan their assay, prepare robotic scripts that will combine their compounds for analysis, and generate the LC–MS/MS sample lists and methods. This ensures that the correct method is applied to the correct cassette.

Conclusion

In a HT-ADME environment, speed of analysis is often seen as more important than high-quality analytical data. By careful consideration of generic LC–MS/MS systems and application of software, bioanalytical quality can be maintained despite the high volume of samples, broad chemical space and wide variety of matrices assessed. At our laboratories, the tiered LC–MS/MS systems presented here in combination with both commercial and in-house software allow us to deliver up to 4000 data-points/per system/per overnight run. The analytical methods and processes we apply allow for >99% successful method optimization for compounds and >95% success for assay of said compounds (irrespective of matrix being assessed). While developing these methods should focus on ensuring bioanalytical quality, a welcome side-effect observed in our laboratories was increased inefficiencies due to reductions in repeat analysis and faith that the data that have been generated is sound.

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