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Commentary

Theme: Best Practices for Bioanalytical Methods: Recommendations from the Global Bioanalysis Consortium
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New Frontiers—Accelerator Mass Spectrometry (AMS): Recommendation for Best Practices and Harmonization from Global Bioanalysis Consortium Harmonization Team

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Abstract. The technique of accelerator mass spectrometry (AMS) is applicable to the analysis of a wide range of trace elemental isotopes. However, in the context of the pharmaceutical industry, it is invariably used to measure radiocarbon (^{14}C). There are two broad modes of application: analysis of total ^{14}C sometimes termed "direct AMS" and analysis of specific ^{14}C-labelled analytes in a variety of matrices following some method of isolation. It is the latter application which is within the remit of the GBC team, and the team has made efforts to propose harmonized recommendations for the validation of AMS when used in a regulatory bioanalytical mode, *i.e.* the quantification of specific analyte(s) using liquid chromatography with off-line detection by AMS now known as "LC + AMS". The GBC team has reached a position where they have agreed to many aspects, but also differ on some aspects of what constitutes a bioanalytical assay validation in support of clinical studies using this technology. The detail of most of this will be covered under separate publication(s), but for the purposes of this paper, we have outlined the points of consensus. The purpose of this article is not to provide a roadmap for validation of LC + AMS assays, but to highlight agreements amongst the industry representative experts and the practitioners, as well as identifying specific areas essential for establishing assay quality but where additional discussion is required to reach agreement.

KEY WORDS: accelerator mass spectrometry; bioanalysis; LC + AMS.

Leading industry practitioners, representatives of the wider bioanalytical community and representatives of the three main commercial providers of accelerator mass spectrometry (AMS) services to the pharmaceutical industry were brought together under the GBC A10 New Frontiers AMS sub-team. The technique of AMS is applicable to the analysis of a wide range of trace elemental isotopes. However, in the context of the pharmaceutical industry, it is invariably used to measure radiocarbon (^{14}C). There are two broad modes of application: analysis of total ^{14}C sometimes termed "direct AMS", which involves very limited sample preparation prior to analysis usually in support of A(D)ME studies, and analysis of specific ^{14}C-labelled analytes in a variety of matrices following some method of isolation. It is the latter application which is within the remit of the GBC team and the team has made efforts to propose harmonized recommendations for the validation of AMS when used in a regulatory bioanalytical mode, *i.e.* the quantification of specific analyte(s) using liquid chromatography with off-line detection by AMS now known as "LC + AMS". Agreeing on the use of this terminology was one of the first harmonization achievements for the team. This terminology appropriately highlights the off-line approach used to couple analyte isolation to detection by AMS and brings clarification to a variety of terms used by the community (such as LC/AMS or LC-AMS) which imply on-line coupling. The latter is currently not in use for any routine AMS operation. The EBF white paper on the scientific validation of quantitative AMS methods [1] formed the

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basis for initial discussions and provided a platform on which to build, although not all aspects of the recommendations in that paper are universally applied by AMS providers at this time nor indeed will all of them be applied in the future.

To this point, the team is comfortable with the recommendations below. This partial list brings to the fore areas where LC + AMS practices and approaches will substantially differ from those covered by regulated bioanalytical method validation (BMV) guidance documents (e.g. for LC/MS). These points will serve as the basis for future discussion documents. It is recognized that these consensus points are broad in scope and there could be exceptions to any seemingly conclusive statement.

- Instrument performance: Performance of AMS instruments is independent of the assay since the matrix analyzed by the instruments is always the same and, therefore, does not require assay-specific validation *per se*. The linearity, accuracy and precision of the AMS instrument should be provided in an OQ/PQ testing phase that applies universally to any compound converted to elemental graphite or gaseous CO_2 (not common practice yet, but soon to be available) [2]. In contrast, the sample preparation including LC analysis portions of the assay procedure have the potential to introduce variability into the method and require assessment during validation.
- Assay qualification/validation: The concept of a tiered approach [3] to assay qualification/validation may be as appropriate to LC + AMS as it is deemed to be for LC/MS assays, but the details of the form that these approaches would take are yet to be agreed upon and will be the subject of future communications.
- Accuracy and precision: The overall LC + AMS assay procedure does require assessment of accuracy and precision due to potential for recovery losses in the sample preparation procedure (pre-graphitization).
- Analyte recovery: The principle of single point recovery normalization is commonly used, as normal practice is to spike the non-labelled analyte (sometimes performing all of the LC + AMS assay relevant functions of an internal standard) into each and every sample, standard and QC at relatively high concentrations, such that total analyte mass is very similar in every instance. With proper verification of equivalent recovery across the full ^{14}C concentration range, single point normalization of recovery is scientifically valid. AMS is thus measuring the change in the specific activity of the analyte, but the total drug concentration is relatively constant; non-linearities in recovery and instrument response are negligible (or can be addressed at the instrument level). This important point will have the effect of streamlining AMS-based studies without compromising data quality. Future communications will provide more detail around this concept.
- QC samples: The use of control matrix samples spiked with ^{14}C-labelled analyte to provide QC samples and the use of pre-defined acceptance criteria as an objective measure of assay performance is an appropriate approach.
- Preparation of standards and QCs: Independent weighings in the preparation of matrix standards and QCs are not relevant to the LC + AMS assay process since they are prepared from high ^{14}C spiked matrix (which are verified by direct measurement, *e.g.* by liquid scintillation counting).
- Analyte stability: Prior stability data from other validated assays should be extended to the LC + AMS assay since stability is independent of the analytical instrumentation. This will streamline validation without compromising the analytical dataset (more on this later in this paper).
- Assay robustness: The total radioactivity (TRA) determinations where performed (often by direct AMS) of, *e.g.* plasma samples are valuable supporting data assessments and can be used as a reference measurement for the pharmacokinetic determinations for individual analytes. For example, the LC + AMS concentration of a specific analyte cannot exceed the TRA value, minor analytical variations notwithstanding. If this did occur, a flaw in either the LC + AMS assay or the total radioactivity measurement would be indicated. This information provides robustness to the overall dataset.

The discussions of the team often centred on the more general application of direct AMS for total ^{14}C determination, as applied to investigations such as mass balance and metabolite profiling. Whilst these digressions were not the central intent of the meetings, the TRA methodology (graphite production) serves as the basis for all determinations by AMS. This TRA analysis thus underpins the accuracy of standards and QCs in the LC + AMS methods. Best scientific practices have been published individually by several of the team members, but there are distinct differences in the routes to graphite formation amongst the representatives and it cannot be assumed that all routes are equivalent in output [4]. The discussions thus were enlightening and fruitful. Indeed, one of the main outcomes of these deliberations under the GBC harmonization team umbrella is that wider and continuing discussions than just those around the direct bioanalytical applications of the technology are warranted.

Focusing on the remit of the GBC as applied to LC + AMS, in the absence of any applicable guidance, the team carried out a comprehensive assessment of the approaches currently taken to "validation" of LC + AMS assays. This term in itself is somewhat contentious as validation implies a direct tie to specific regulatory guidance documents issued by, for example, the FDA and EMEA for LC/MS and ligand-binding assays. Whilst a consensus term was not decided upon, all were in agreement that validation without some qualifying term should be avoided. Other terms that have been in circulation include "technique-appropriate validation" [5],

"qualification" or "science-based validation" [1]. "Fit for purpose" validation was frequently referred to in the team discussions and is in line with new technologies that have not fully entered the purview of the regulatory space [6]. However, importantly, it was agreed by all in the team that reluctance to use the term validation was not due to limitations of the methodology, rather it is an attempt to avoid possible confusion with future regulatory reviews that could categorize the MS in AMS as a technique that is simply another form of mass spectrometry and should therefore be judged against BMV guidance documents.

It is clear from this process that there are differences, most subtle though some more substantial, in the approaches taken but the team agreed that, dependent on the purpose to which the assay is being applied, the assay does need to undergo an assessment of its scientific rigour and this should be documented. Much of that, at least at the highest level will look familiar to those from the world of regulated bioanalysis. However, there will necessarily be some fundamental differences, addressing the inherent dissimilarities between the techniques of LC/MS and LC + AMS. The team agreed that as indicated above, LC + AMS assays can be developed to a suitably high quality, but as there are fundamental differences between the technology compared to, e.g. LC/MS ; detailed elsewhere [7], it is not appropriate to measure them against guidances developed for LC/MS.

To address the need for scientific rigour, it was agreed that the assay must be proven to be accurate, precise and reliable. Regardless of the term used, validation will precede bioanalysis of clinical samples. The scope and content of these validation experiments, however, must be judged in the context of how the study data will be used. LC + AMS assays are often used in support of a single clinical study which is designed to provide information of an investigative nature (e.g. absolute bioavailability study) rather than primary safety or efficacy endpoint support. These studies often are conducted after extensive validation by traditional methods have been completed and usually with years of preclinical and early clinical bioanalysis experience. As stated in the consensus recommendations above, it would therefore be prudent to have the option to rely on existing work, particularly in the areas of matrix stability, frozen matrix stability, freeze thaw stability and stock solution stability rather than reaffirming this for support of a single clinical study that does not centre around safety endpoints.

In summary, there is an existing body of publications that cover AMS validations, best practices and method-specific acceptance criteria. Until recently, however, these individual publications have not been brought together by a globally based consensus. The recent EBF recommendation paper [1] is seen as an excellent start of the discussions to lead to harmonization of appropriate working practices. However, individual differences exist between the AMS providers, and it is the team's intention to continue to work together to develop a comprehensive, scientifically justified framework for the conduct of analysis of biomedical samples by AMS. Future work by the GBC team will be to more tightly define what is essential to producing a "valid" assay and what practices in the context of existing regulatory BMV guidance are of little relevance. Equally, there is a need for experts in the field to continue to discuss the wider uses of the AMS technology as applied to areas beyond isolated analyte measurement for bioanalysis.

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