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METHODOLOGY

The utility of qNMR to improve accuracy and precision of LC-MS bioanalysis

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In LC-MS bioanalysis, samples and analytes are quantified against calibration solutions and curves which are derived via serial dilution from stock solutions that are prepared from dry reference standards. The analytical errors associated with the mass and volume measurements required for this preparation of stock solutions can culminate in a variance which might affect bioanalytical data accuracy. Especially in the case of extended studies with intermittent sample analysis, the multiple preparation of separate stock solutions can also adversely affect bioanalytical data precision. Discussed here is an illustrative case study where a single stock solution was utilized for a longitudinal study with multiple data points by means of an orthogonal and synergistic quality control via quantitative NMR methodology resulting in improved bioanalytical data.

Keywords: LC-MS bioanalysis, quantitative NMR, data accuracy, data precision, quality control of stock solutions of analyte reference standards, quality control of stock solutions of assay reagents.

Introduction

The constantly evolving complexities of the drug discovery and development process necessitate continued advancements of supporting LC-MS bioanalytical assays by improving critical analytical figures of merit such as specificity and sensitivity along with accuracy and precision (**Figure 1**). The principal objective of quantitative assays is to correctly and consistently/repeatedly measure the real values thus affording quality data with high accuracy and high precision. In some cases, when only a change of the levels of endogenous compounds from baseline is monitored, an assay with low accuracy and high precision may be sufficient [1,2]. Occasionally, assays with high accuracy and low precision or even assays with low accuracy and low precision might suitable for fit-for-purpose analysis.

In LC-MS bioanalysis, dry reference standards are used to prepare stock solutions followed by serial dilution to provide calibration solutions and curves against which the samples and analytes are then quantified. The analytical errors interrelated to the mass and volume measurements needed for the preparation of these solutions

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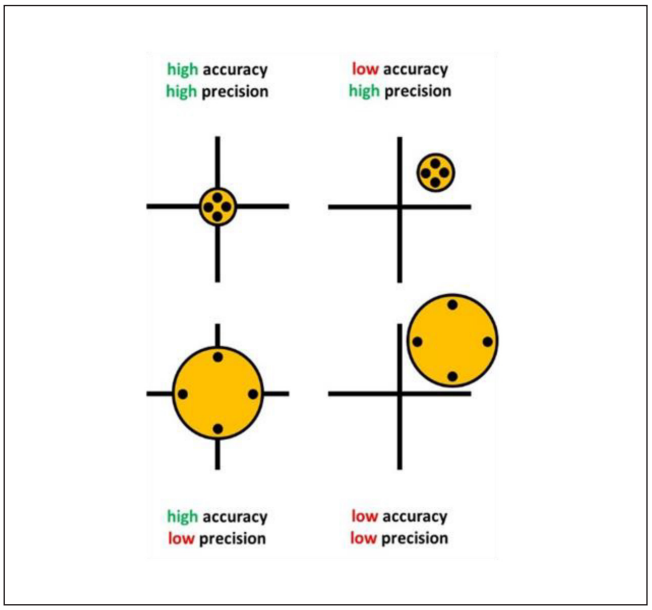

Figure 1. The role of accuracy and precision for the measurements of the real value of the analyte.

can accumulate in a variation which might affect bioanalytical data accuracy. Especially in the case of studies where multiple preparations of separate stock solutions are required, the bioanalytical data precision can also be adversely affected.

Presented here is an illustrative case study where the synergistic utilization of quantitative NMR (qNMR) to evaluate and qualify stock solutions of analyte reference standards afforded superior LC-MS bioanalytical data accuracy and precision as well as noteworthy savings in time and labor.

Design of the case study

A longitudinal *in vivo* study in Neuroscience research on the modulation of several endogenous neurotransmitters in rodents was conceptualized with intermittent sample collections of biofluids at serial time points. Since the investigation planned for the data and results obtained for the analytes at each time point to inform on iterative changes in the ongoing study design (**Figure 2**), an immediate but also specific and sensitive LC-MS bioanalysis of the samples after collection was needed, with analytes expected to be in the picomolar (pM) to nanomolar (nM) concentration range. This time-sensitive requirement thus did not allow for the typical bioanalytical workflow of accumulating all samples during the course of the study for a final batch LC-MS bioanalysis. Furthermore, whereas the inter-animal biological variation within a test population typically results in a wide data range and considerable error margins, this assay design allowed for distinct intra-animal data with anticipated minor changes over time necessitating an immediate but also accurate and precise LC-MS bioanalysis.

Although several endogenous analytes were measured during this study, the workflows will be discussed with the example of just one analyte for simplification and clarification.

LC-MS bioanalysis following routine practices

Once the study was in progress, the samples sets collected at each time point were instantly analyzed by LC-MS bioanalysis. Following routine practices, at each time point, dry analyte reference standard was weighed and dissolved to generate a stock solution followed by serial dilutions to afford the calibration solutions and curves against which the samples were quantified (**Figure 3**). The data and results thus obtained were then applied to the successive study phases and design.

Upon conclusion of the study, a critical review of the workflows and data revealed the issue that the averaging of the individual calibration curves derived from separately prepared stock solutions at the various time points

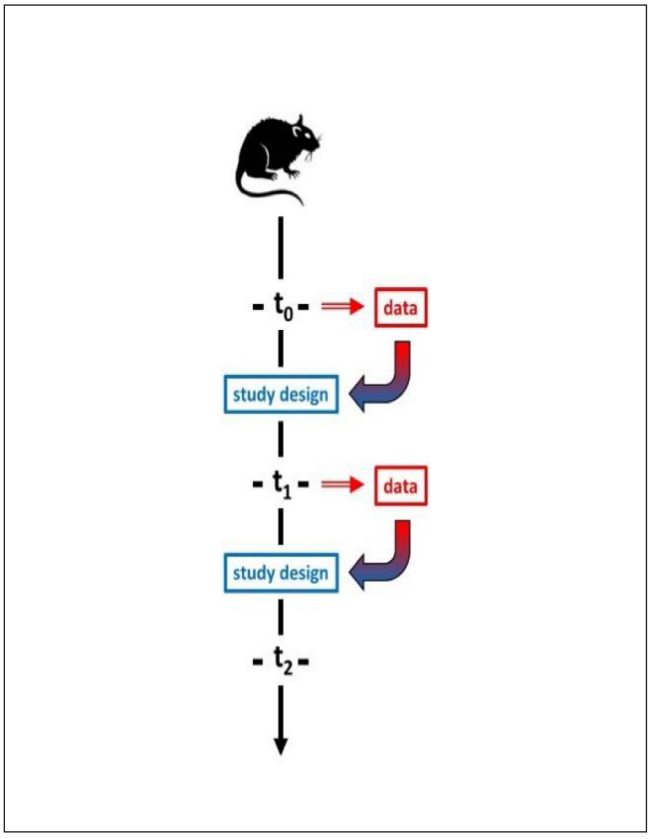

Figure 2. Longitudinal in vivo study with intermittent data points to iteratively update the ongoing study design.

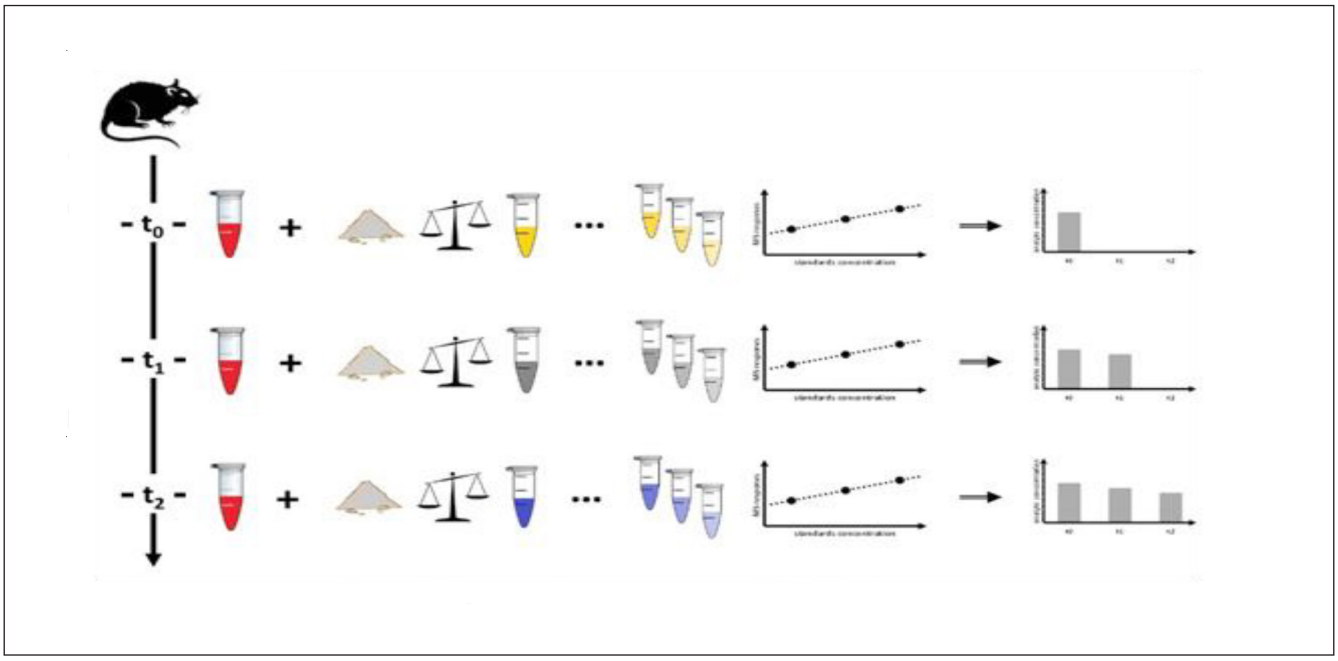

Figure 3. Routine LC-MS bioanalysis with separately prepared stock solutions from dry analyte reference standard at each time point.

resulted in error margins (shown in red, **Figure 4A**) which would exceed the expected changes of the endogenous analyte (indicated with the green line, **Figure 4B**) over the course of the study. Basically, the summation of the analytical errors associated predominantly with the separate preparation of a stock solution and related calibration standards at each time point culminated in a variance which does not allow for the required accurate and precise LC-MS bioanalysis of the analytes. Although utmost care was applied throughout the analytical workflow including the use of a stable-labeled internal standard (IS); it appeared that the mass and volume measurements required for the preparation of the stock solution from dry analyte reference standard at each time point was introducing errors that confounded the accuracy and precision of the LC-MS bioanalysis and data in this traditional workflow.

Utility of qNMR to improve LC-MS bioanalysis

To address the variability introduced by the individual and repetitive preparation of stock solutions from dry analyte standard at each time point, the concept of a single stock solution of analyte reference standard was explored which would cover all study time points by means of preparing a single solution and then freezing aliquots until usage (**Figure 5)**.

However, due to the transient nature and potential instability of the neurotransmitter analyte(s), concerns arose about the validity of this approach warranting an orthogonal and complementary methodology to LC-MS analysis to quantitatively assess the potential degradation of

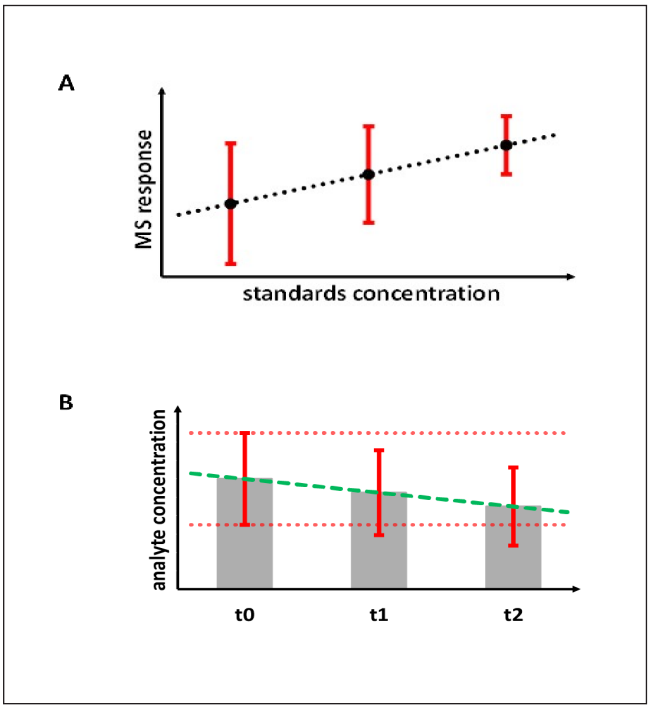

Figure 4. The error margins (red lines, A) of the average of the individual calibration curves derived from separately prepared stock solutions exceed the expected changes of the endogenous analyte (green line, B).

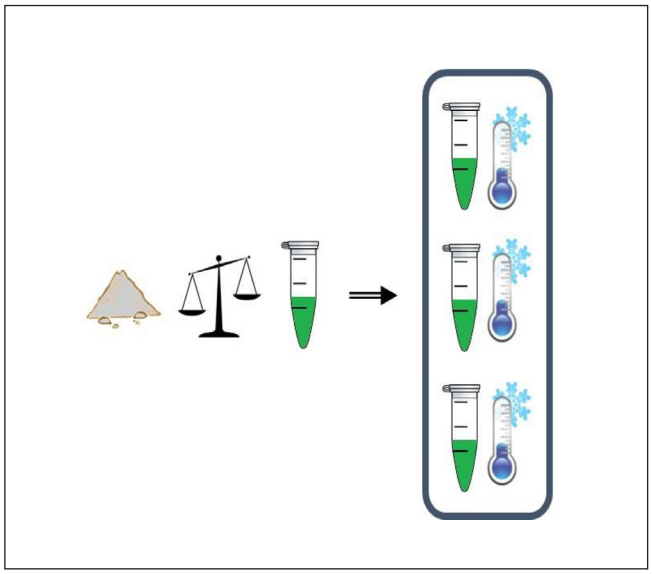

Figure 5. Preparation of a single stock solution from dry analyte reference standard providing identical copies of frozen aliquots of this stock solution.

the stock solution and analytes over time. A technique, quantitative NMR (qNMR), which is exceptionally reproducible (%CV routinely <2%), accurate and precise, has gained increasing attention in the biopharmaceutical industry and is now common practice in many workflows [3-6]. In the example discussed here, adding a small amount of deuterium as a field lock nucleus in the form of D_2O to the stock solution of the analyte reference standard facilitates both the absolute and relative quantitation of analytes and allows for the comparative review of NMR spectra of discrete samples and analytes even over an extended time course.

Prior to the start of the study, the dry analyte standard was weighted and dissolved to produce a stock solution with a final 10% D_2O (v/v) concentration, which was then aliquoted and frozen (**Figure 5**).

Once the study was in progress and samples were collected at the respective time points; an aliquot of the stock solution was retrieved, thawed and subjected to qNMR analysis. The qNMR measurement not only provided both the absolute and relative concentration of the analyte reference standard in the stock solution, but also gave insightful information on the standard's stability by monitoring the NMR spectra for the appearance of new peaks corresponding to degradation products. Notably, in the case of a stability issue, the qNMR data would allow for a nominal correction of the true concentration of the analyte in the stock solution and the study could continue as planned. After the analyte passed the qNMR evaluation and QC criteria, further dilution provided the calibration solutions utilized for calibration curves, sample analysis and quantitation affording data which were applied to the ongoing study design (**Figure 6**).

Again, at the end of the study, a critical review of the

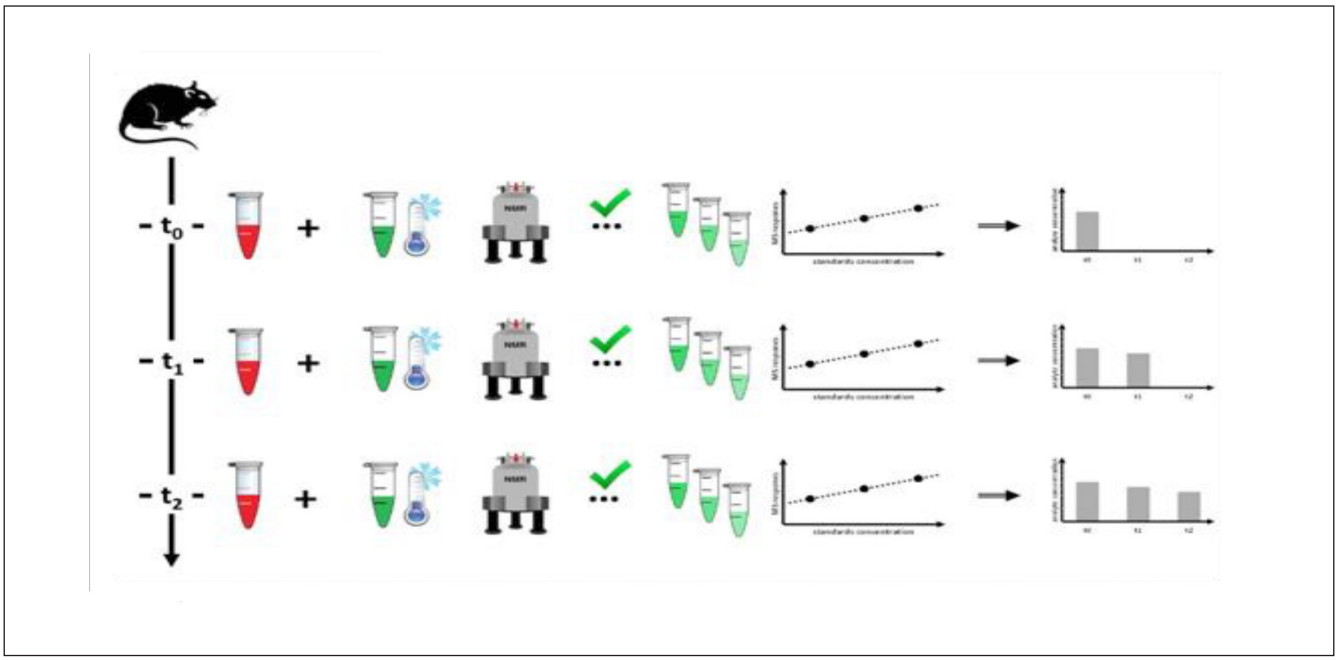

Figure 6. At each time point, qNMR was employed to perform a quality control of aliquots of a single prepared stock solution from dry analyte reference.

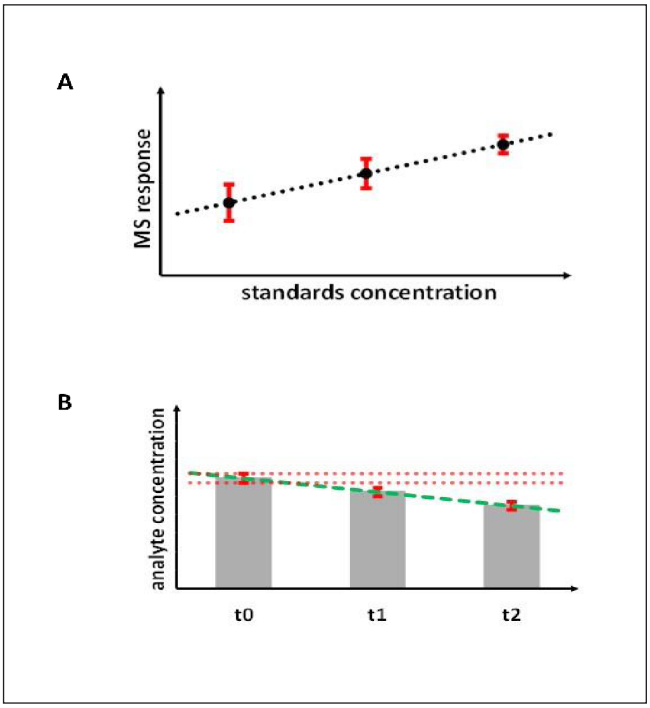

Figure 7. The error margins (red lines, A) of the average of the individual calibration curves derived from aliquots of a single stock solution allow to monitor the expected changes of the endogenous analyte (green line, B).

analytical processes and data was conducted. The average of the individually prepared calibration curves from the aliquoted single stock solution of analyte reference standard afforded error margins (shown in red, **Figure 7A**) which were noticeably smaller compared to the previously discussed error margins resultant of individually prepared stock solutions and associated calibration curves following routine protocols. This novel application of qNMR significantly improved the accuracy and precision of the LC-MS bioanalytical assay thus allowing to monitor the expected changes of the endogenous analyte (depicted with the green line, **Figure 7B**) over the course of the study.

Conclusions

The utility of qNMR to rapidly evaluate and qualify bioanalytical stock solutions of analyte reference standards as an independent orthogonal methodology affords data on both the absolute and relative concentration of analytes along with sample integrity, which ultimately improves the accuracy and precision of LC-MS bioanalysis. This innovative approach supports the design of simplified workflows by allowing facile evaluation of the integrity of stock solutions comprising one or many analytes. This is particularly valuable when studies follow an extended time course and frequent preparation of stock solutions adds additional sources of error.

The methodology of qNMR applied to LC-MS bioanalysis was successfully utilized in a longitudinal in vivo study which design specified the intermittent monitoring of 7 endogenous neurotransmitters over several months. Only a single stock solution containing these 7 analytes was prepared from dry reference standards, then aliquoted and frozen. By submitting these aliquots to qNMR evaluation prior to use, the study was supported with well characterized analytical reagents providing accurate and precise bioanalytical data at multiple interactive time points. Furthermore, this novel approach delivered substantial savings in time and labor compared to the traditional workflow of preparing multiple stock solutions over the course of the study.

As a perspective view, the utility of qNMR can be applied to the quality control of stock solutions of analyte reference standards as well as assay reagents across the drug discovery and development process including regulated assays. Any additional data on the true qualitative and quantitative content of stock solution, which are utilized for the analysis of exogenous and endogenous analytes, assists in the qualification and validation of the obtained assay data. The quality control of reagents is becoming particularly critical for the bioanalysis of biologics modalities and biomarkers employing complex LC-MS methodologies and ELISA.

Summary

- The data accuracy of LC-MS bioanalysis is contingent on the quality of the preparation of the stock solution from dry analyte reference standard. This stock solution is then further serially diluted to afford the calibrations solutions and curves against which the samples are quantified.
- In some cases, longitudinal studies require immediate analysis of intermittent samples thus necessitating the preparation of multiple individual stock solutions from dry analyte reference standard. The summation of the analytical errors associated with this traditional and repetitive workflow might culminate in a variance which can adversely affect the data precision of LC-MS bioanalysis.
- To negotiate the issue of multiple individual stock solutions, a single stock solution can be aliquoted and frozen prior to the start of a study.
- To inform on any concerns about analyte stability and thus validity of the stock solution, the thawed aliquots should be analyzed prior to usage for quality control; however, a methodology should be applied

which is not based on LC-MS bioanalysis.
- An orthogonal and complementary technique to LC-MS bioanalysis is quantitative NMR (qNMR) which provides absolute and relative quantitation of analytes along with sample integrity.
- The detection sensitivity of qNMR supports the quality control analysis of stock solutions of analyte reference standards, dose formulations, and assay reagents which are typically in the micromolar (μM) to millimolar (mM) concentration range.

References

1. Drexler DM, Reily MD, Shipkova PA. Advances in mass spectrometry applied to pharmaceutical metabolomics. Anal Bioanal Chem 399(8), 2645-2653 (2011).
2. Zheng JJ, Shields EE, Snow KJ et al. The utility of stable isotope labeled (SIL) analogues in the bioanalysis of endogenous compounds by LC-MS applied to the study of bile acids in a metabolomics assay. Anal Biochem 503, 71-78 (2016).
3. Barding GA, Salditos R, Larive CK. Quantitative NMR for bioanalysis and metabolomics. Anal Bioanal Chem 404(4), 1165-1179 (2012).
4. Davies SR, Jones K, Goldys A et al. Purity assessment of organic calibration standards using a combination of quantitative NMR and mass balance. Anal Bioanal Chem 407(11), 3103-3113 (2015).
5. Webster GK, Kumar S. Expanding the analytical toolbox: pharmaceutical application of quantitative NMR. Anal Chem 86(23), 11474-11480 (2014).
6. Bharti SK, Roy R. Quantitative 1H NMR spectroscopy. Trends Anal Chem 35 (Supplement C), 5-26 (2012).

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