White Paper
November 2015

Performing method transfer and validation for a LC+AMS assay

Introduction

Accelerator Mass Spectrometry (AMS) measures levels of drug-related material 1,000 to 10,000 times lower than LC-MS/MS. This white paper sheds light on critical aspects of method transfer and validation for a LC+AMS assay.

The linearity and freedom from matrix interference nature of the AMS detector has been well established. However the robustness of the entire LC+AMS assay must be assessed. Losses or inconsistency may arise during multiple pre-detector processing steps such as extraction, LC separation, transfer of LC fraction, and graphitization. These must be fully characterized if a quantitative bioanalytical assay is to be considered fit to support clinical development. The transfer of an assay to LC+AMS from LC-MS/MS entails significant changes and thus requires care followed by a full validation of the LC+AMS assay. Limited aspects of a previously validated LC-MS/MS may be directly transferrable but insufficient testing of an LC+AMS method may introduce significant risk to the clinical study objectives and developmental timelines.

Method transfer

LC+AMS assays use sources of ^{12}C plus ^{14}C-labelled drug material and normally performs at lower amounts than those typically used by LC-MS/MS. It is important that a method intended for LC+AMS use is assessed during transfer for the following:

- Absence of ^{14}C in unlabeled drug standards
- Non-specific binding and dilution integrity over time at very low concentrations
- Column handling integrity at high volume loads
- Extraction efficiency and reproducibility
- Confirmation of baseline chromatographic separation of available known metabolites and parent analyte

Method validation

The following LC+AMS assay-specific critical characteristics must be accounted for.

- Use of a ^{14}C (tracer) analyte at mass concentrations not used during LC-MS/MS
- Use of unlabeled ^{12}C analyte as an Internal Standard
- Low assay concentration range
- Lower mass concentration in spiking solutions

Xceleron uses a three batch approach to ensure that intra-day and inter-day accuracy and precision and other aspects of reproducibility are fully understood.

Post Validation

Once clinical samples arrive the analytical method selectivity is confirmed using a post-dose pooled plasma sample. The clinical matrix is spiked with parent and known metabolite reference standards to assure baseline chromatographic separation and alignment of ^{14}C radiochromatogram and ^{12}C UV response.

Xceleron Quantitative Analytical Methodology Checklist

Method Transfer	Comment	☑ Checked?
Unlabelled analytical standards	Must be free of ^{14}C	
Non-specific binding	Combined with dilution integrity screen over time at a range of concentrations	
Column handling integrity	Ensure that high (100 µL) loads can be sustained, no deterioration in chromatographic performance	
Extraction efficiency	Is reproducible and ideally high (for maximum sensitivity)	
Analytical selectivity	• Mixture of available known metabolites and parent reference standards for baseline chromatographic separation • Alignment of ^{14}C radiochromatogram and ^{12}C UV response	

Three Batch Validation	Comment	☑ Checked?
Calibration Standards	• At least six concentrations (n=2) • ± 20% (25% at Low calibration standard)	
QC Standards	• Four concentrations – LLQC, LQC, MQC, HQC • ± 20% (25% at LLQC)	
Zero	Control matrix with IS added. To assess ^{14}C carryover, run after each replicate of High Cal	
Blank	Control matrix, without IS added, to assess for carryover of the cold IS or endogenous peaks that would interfere with the integration of the IS peak or selection of the peak for fractionation.	
Carryover	• Criteria 1 - pMC of Zero should be less than three times the mean SB value • Criteria 2 - pMC of Zero should be less than 20% of LLOQ • Non-labelled carryover can be assessed by injecting blank after zero	
Stability	If specific stability information not available from the sponsor, stability can be investigated for spiking solution, matrix, room temperature, post extraction, autosampler to ensure the reproducibility of the assay.	
Internal Standard	Utilizing the internal standard approach to account for the sample loss that may occur at various steps of the LC+AMS assay.	

Post Validation	Comment	☑ Checked?
Matrix selectivity confirmation	• Post dose plasma pool of clinical samples • Mixture of available known metabolites and parent reference standards for baseline chromatographic separation • Alignment of ^{14}C radiochromatogram and ^{12}C UV response	

Find out more

Get in touch to discover how we can help design an investigation that will improve time- and cost-efficiency and help you achieve your commercial objectives.

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