FIT-FOR-PURPOSE LC-MS ASSAYS: A CRO PERSPECTIVE

Intelligent Screening Symposium

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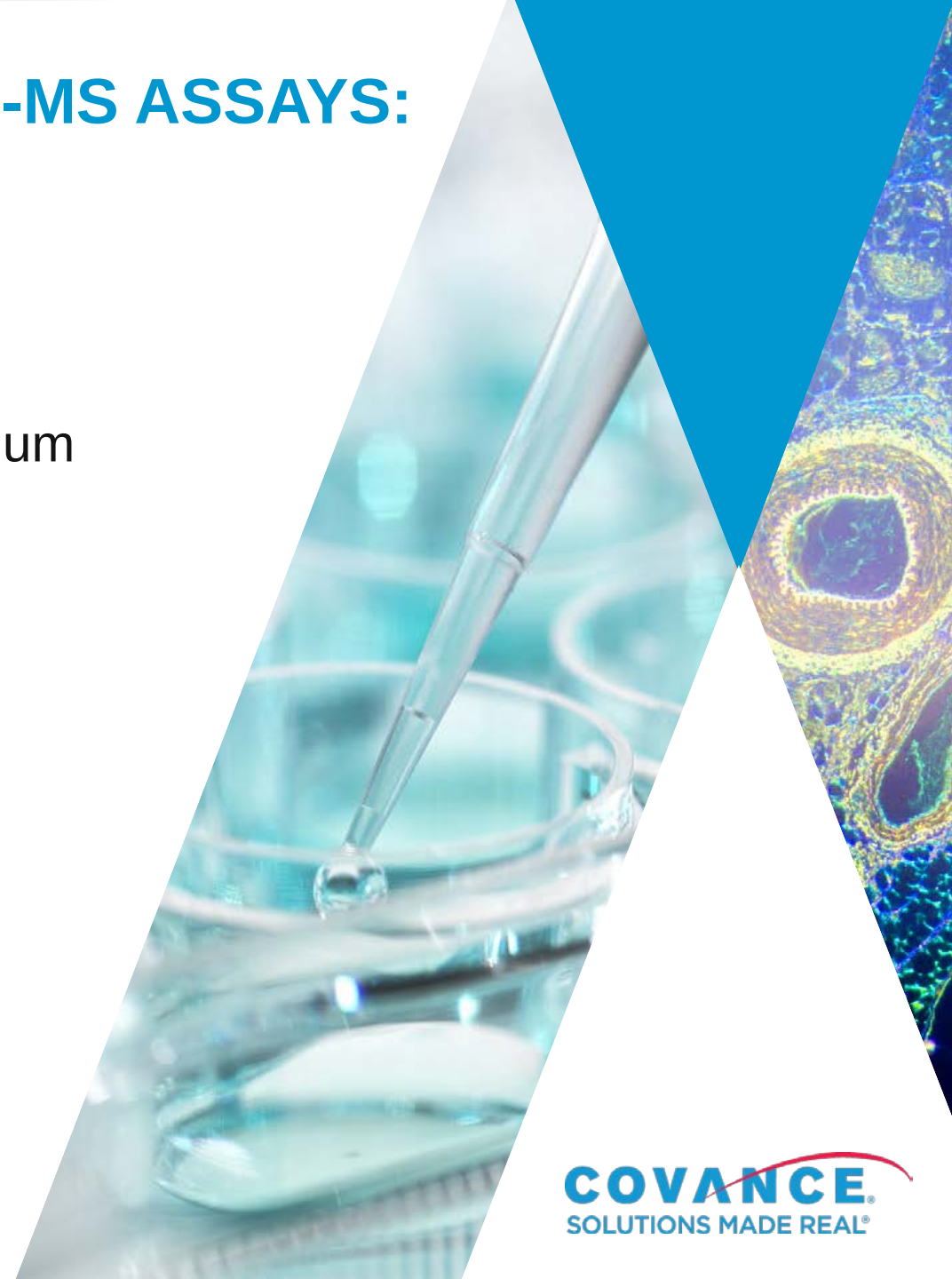
04 October 2018



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Overview

- Quick biography
- Introduction
- Overview of LC-MS assay development tiers
- Tier selection fit-for-screening
- Common pit falls that can happen at any level
- Summary

Biography

- BSc in Biochemistry (University of York)
- Year in Industry (DMPK and Biomarkers, AstraZeneca)
- PhD in Biology (University of York)
- Experimental Officer (Metabolism, Covance)
- Experimental Officer (Discovery BioA, Covance)
- Technical Specialist (Metabolism, Covance)

Introduction

Introduction

The theme of this symposium is “Intelligent Screening” – but what does screening mean to you?

- High throughput screening of 1000s of compounds a month for candidate selection?
- Screening of 10s of compounds a month for *in vitro* or PK parameters?
- Screening 2-3 compounds per month for their major metabolites in different species?

Introduction

All of these scenarios have been described as “screening” – however they are all very obviously on different scales and are used for different purposes.

When analyzing samples we use an assay that is “fit-for-purpose”. But fit for what purpose?

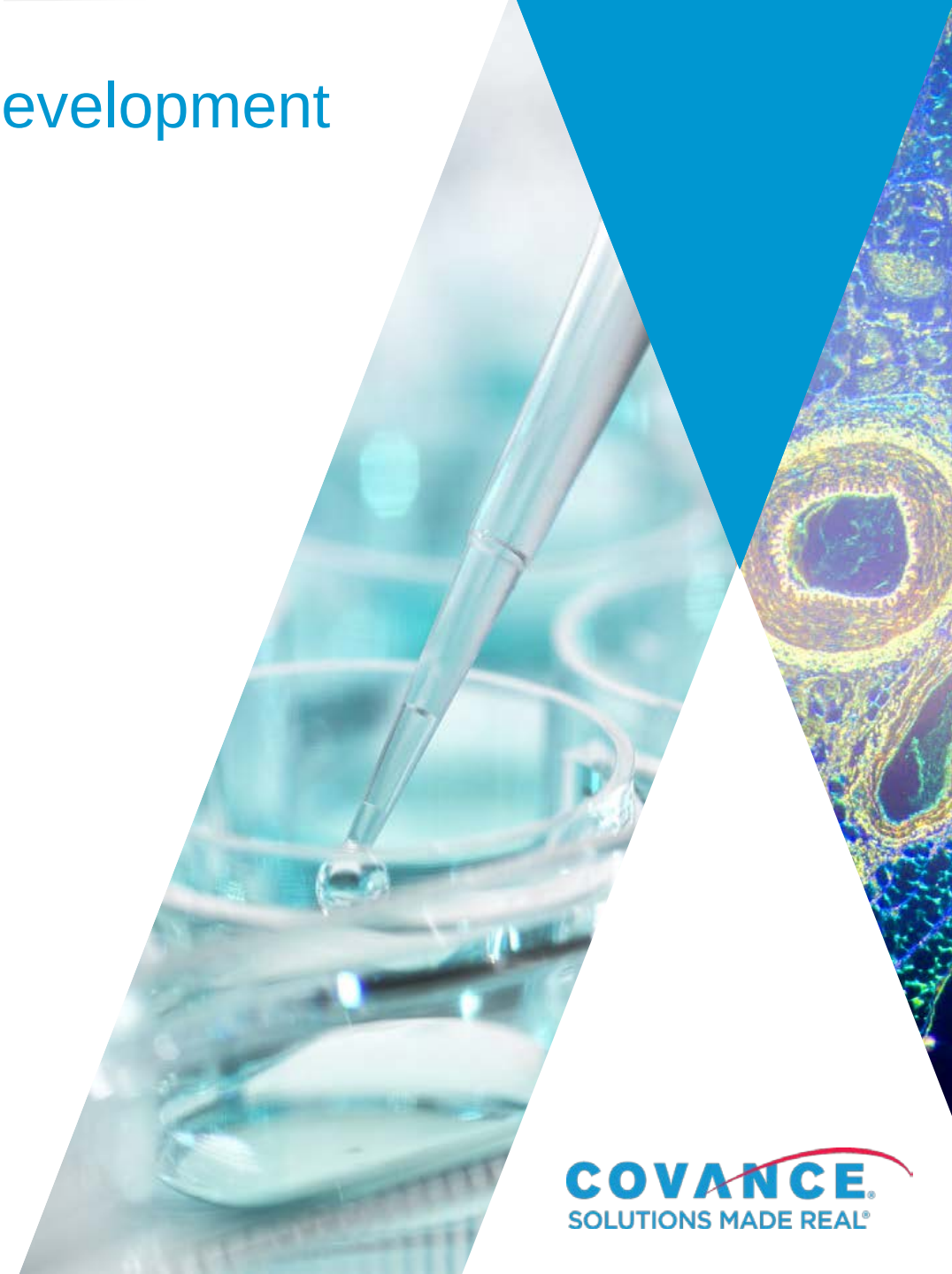
Each of these scenarios would require a different level of confidence in the data generated and therefore would require different amounts of method development and criteria to be applied when deciding if the data can be trusted.

Introduction

This presentation will set out the spectrum of criteria to which LC-MS assays are developed. Examples of traditional studies that are performed to various points along that spectrum will be given.

We will then look at how when some of these examples are performed in a screening context we can move the assay down the spectrum to a simplified set of criteria.

LC-MS Assay Method Development



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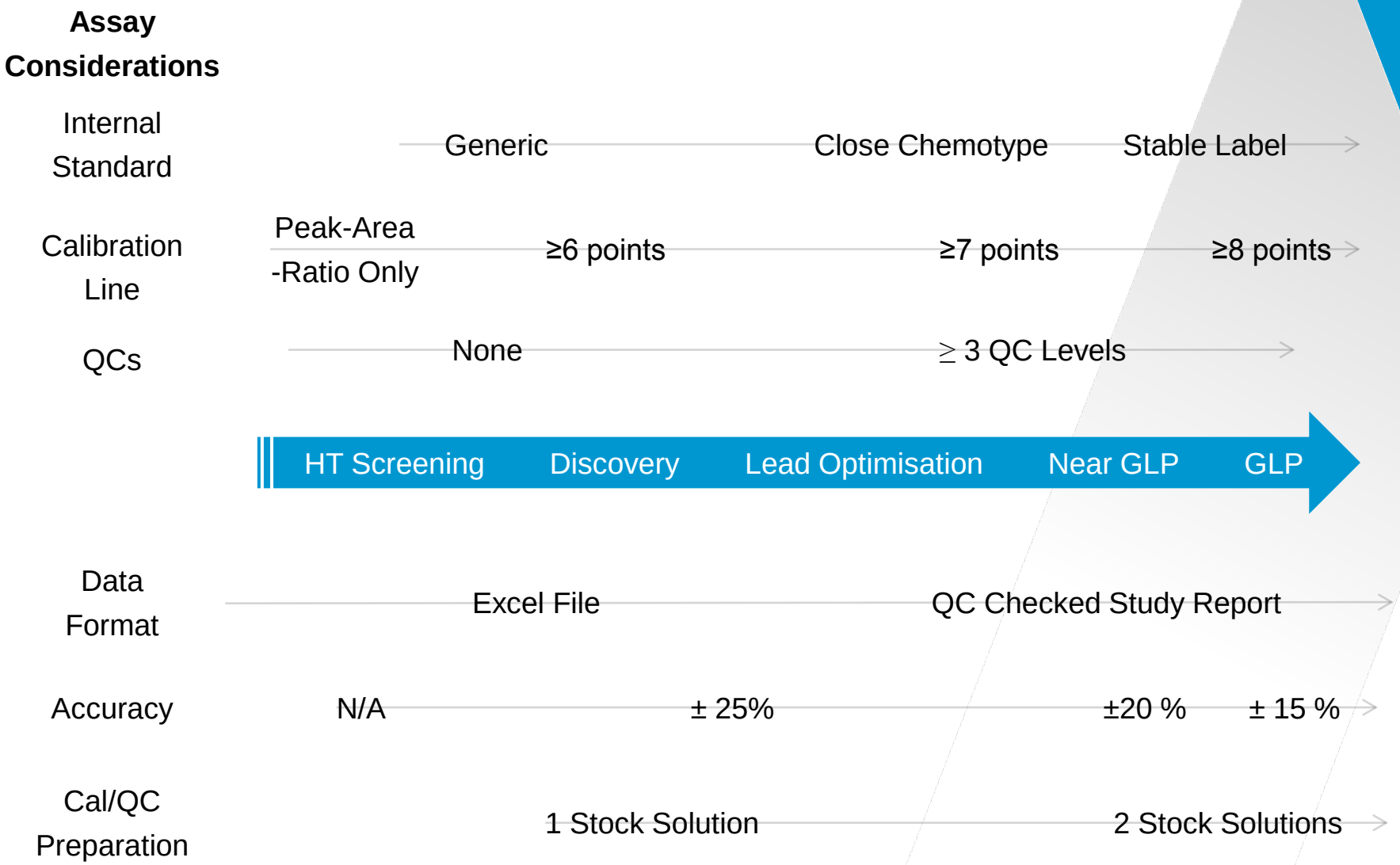
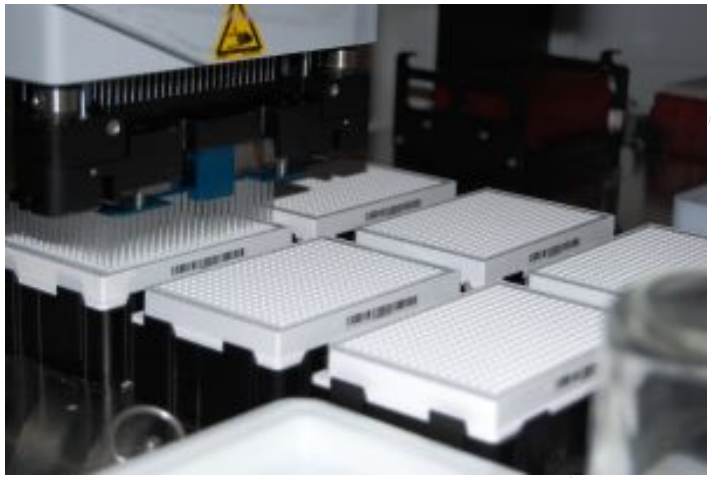
LC-MS Assay Method Development Spectrum



High Throughput Screening

ASSAY FEATURES

- No calibration line or QCs – peak-area-ratio determined only
  - Therefore no % bias/accuracy limits
- Generic internal standard
- Carryover and specificity not assessed
- Designed for very early stage decision making



High Throughput Screening

EXAMPLE USES

- Metabolic stability screening (depletion of parent)
- Permeability screening
- Drug-drug interaction screening

Discovery

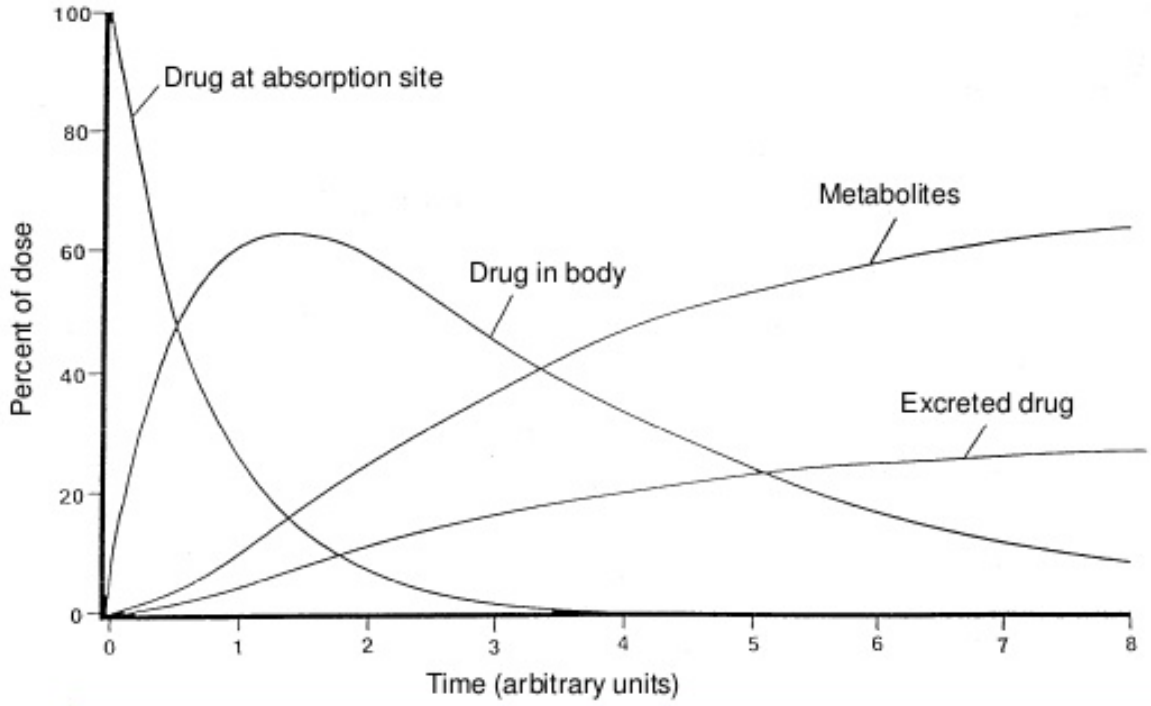
ASSAY FEATURES

- Calibration line, but no QCs
- Generous acceptance criteria (±25%, 30% at LLOQ)
- Generic internal standard
- Carryover and specificity assessed (no criteria)
- Method not qualified prior to sample analysis
- Allows quantitative methods to be developed quickly at the cost of robustness

Discovery

EXAMPLE USES

- Fast-PK screening
- Proof of concept/tissue distribution studies



Lead Optimization

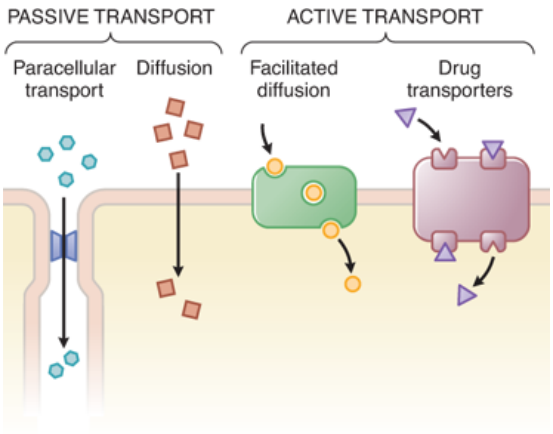
ASSAY FEATURES

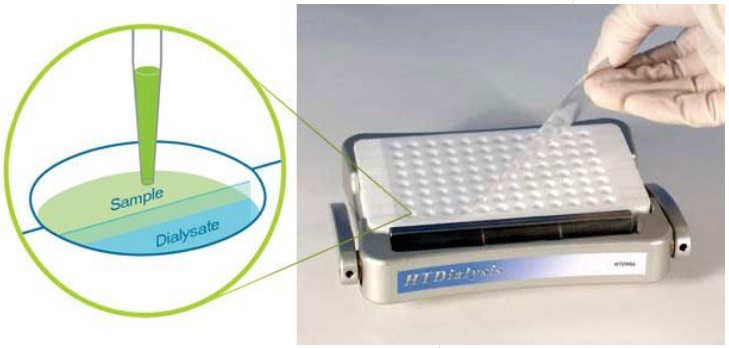
- Calibration line and minimum of 3 QC levels
- Generous acceptance criteria (±25%, 30% at LLOQ)
- Generic internal standard, close chemotype preferred
- Carryover and specificity assessed (< LLOQ)
- Method qualified prior to sample analysis
- Takes longer to develop methods compared to Discovery, but affords a greater degree of confidence in the data

Lead Optimization

EXAMPLE USES

- *In vitro* assays such as plasma protein binding, blood cell partitioning, transporter assays, reaction phenotyping etc.
- Dose range finding/maximum tolerated dose studies
- Standard PK





Near GLP

ASSAY FEATURES

- Calibration line and minimum of 3 QC levels
- More stringent acceptance criteria (±20%, 25% at LLOQ)
- Close chemotype internal standard, stable label pref.
- Carryover and specificity assessed (< 20% of LLOQ)
- Method qualified prior to sample analysis
- Affords a greater degree of confidence in the data over the other tiers, but at the expense of greater still method development time

Near GLP

EXAMPLE USES

- Usually only carried out for studies which don't require a claim of GLP, but for which the client would like a greater degree of certainty in the data
- Sometimes backed up with a QA audit

Deciding Where to Place Your Assay on the Method Development Spectrum

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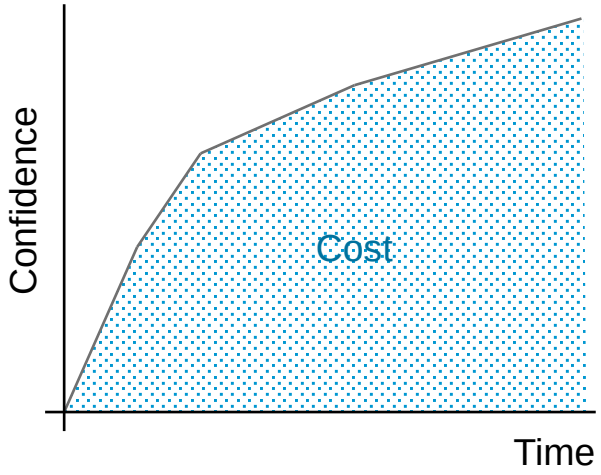
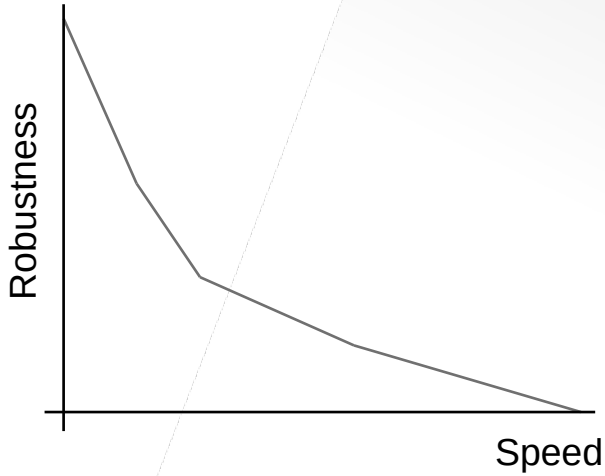
Choosing a Tier

WHY IS IT IMPORTANT TO CHOOSE THE RIGHT TIER

Increasing confidence in the data produced comes at the cost of time and money

Examining how stringent you need the assay to be to produce useful, reliable, meaningful data can help you receive data quicker and more cheaply





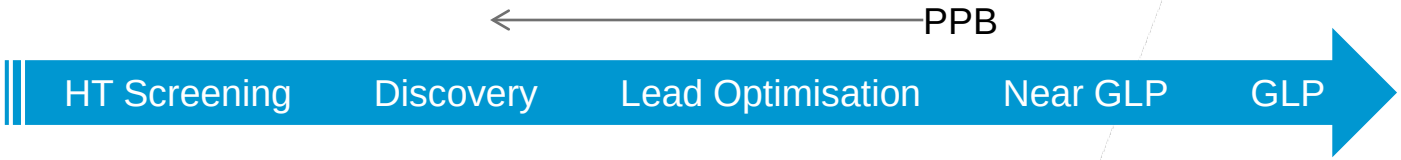
Choosing a Tier – Screening Example 1

PLASMA PROTEIN BINDING

Traditionally performed to lead optimisation/near-GLP criteria with the aim of determining the free drug fraction, and whether this is drug concentration dependant

In screening, compounds would only be assessed at one concentration and would be classified as high, medium or low binding compounds.

Therefore less confidence in the data could be tolerated, allowing the data to be obtained faster and for less cost



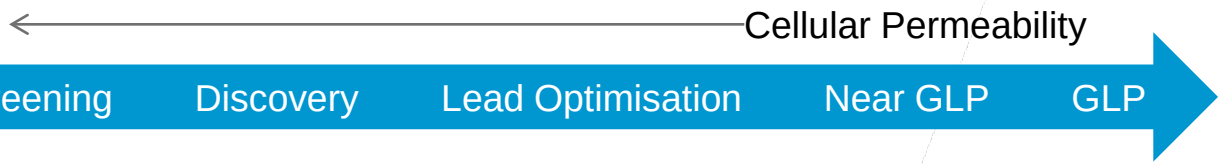
Choosing a Tier – Screening Example 2

CELLULAR PERMEABILITY

The rate at which drug is transported across the intestinal membrane. In lead optimisation/near-GLP: aim to determine the modes of transport involved (e.g. passive trans/paracellular, carrier mediated influx/efflux

In screening it would be suitable to only assess the apparent permeability (Papp) A>B which is the total net transport out of the intestine

Peak-area-ratio only could be used for this.



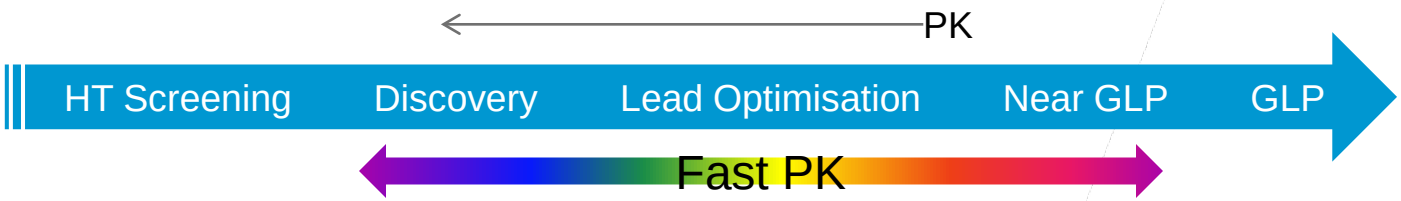
Choosing a Tier – Screening Example 3

FAST-PK (VS TRADITIONAL PK)

Used to determine the amount of exposure an animal has to a test compound, and often the rate and route of clearance of that compound

Two strategies to improving throughput:

- Accept lower robustness in the data and move to a “Discovery” level approach
- Pick and choose the features which are important to you for robustness, streamline process as much as possible with automation, accept that not every compound will work as expected and will require more effort



Where Problems Arise

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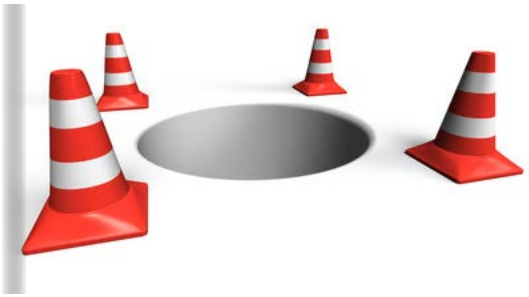
Common Pit Falls…



The most common reason that LC-MS assay development can fail to progress in a timely manner or meet the requirements of the study is

- A missing piece of critical information (e.g. pH or temperature stability, solubility, chemical moiety, existing in-house method)
  - Client may be unable to share these details (proprietary knowledge)
  - They might not know these details yet
  - Simply don’t know that the CRO needs to know these details
  - The CRO might not know that this information was critical

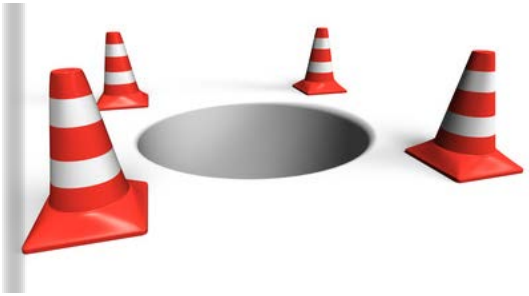
…and How to Avoid Them

The easiest way to avoid this issue is to establish an effective dialogue between the client and CRO

If we have a Bioanalysis contact with whom we can openly discuss the compound it can save a great deal of wasted time and effort

This means we don't have to ask for more method development hours (£$€) and are able to progress your project sooner

Common Pit Falls…



Occasionally, we qualify a method without issue; however when we move to sample analysis we discover anomalies

- Sometimes this is due to the nature of the matrix (old vs. fresh, manner of sample collection) producing a matrix effect in samples only
- Differences in approach between bioanalysis and the sample generating experiment (formulation and spiking, sampling tubes)

…and How to Avoid Them

Most of these issues cannot be foreseen and some cannot be corrected for:

- Rapid *in vivo* metabolism making it impossible to obtain a reliable measurement for a parent compound
  - Metabolite structure unknown and no standard available to allow it's quantification

Where they can be corrected for, this usually requires investigation and further experiments. This takes extra time and make take the study beyond it's initial scope, as a result timelines may be missed.

Open dialogue with the client may help mitigate the delay.

Summary

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Summary

- LC-MS assays can be developed to deliver data with varying degrees of confidence in their accuracy
- The more confidence you require in the data the longer it will take to develop the assay, and as a result the more money it will cost to develop
- Carefully considering what degree of confidence will be “fit-for-purpose” could help you reduce lead times for data and save cost
- LC-MS assay development runs most smoothly when there are good channels of communication between the client and the CRO
- We can work with you to select appropriate criteria to help take time and cost out of your assays

Thank You

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