Current practice and future vision on metabolite profiling and quantification in Drug development

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PHARMACEUTICAL COMPANIES
OF Johnson&Johnson

Outline

- Tiered Approach
- Technologies
- Current Trend & Future vision

Janssen strategy for metabolite Prof & Quan

Tier A Pre-MAD

- Estimation of abundance or quantification of metabolites before completion of the clinical MAD study using a qualified bioanalytical research method (QRM*).

Tier B MAD

- Identify and estimate the abundance of circulating human metabolites in **MAD study**
- Which metabolites can be of pharmacological or toxicological relevance in humans
- Quantify these metabolites in a selection of samples from the MAD study and in a selection of samples from the main toxicology species.

Tier C Post-MAD

- Conduct a method validation for metabolites that need to be quantified in studies after Tier B.

Leclercq L. et al. Chem. Res. Toxicol. 2009, 22, 280–293

**Currently referred to as Scientific validation, Timmerman P., Bioanalysis 2014, 6 (5), 599-604*

Tier A: Pre-MAD

- Disconnect biological effect – exposure in animals (LO).
- High amount of a human (HLM e.g.) in vitro metabolite with high in vitro potency.
- In vitro metabolism in HLM or hepatocytes indicate potential for a major metabolite in humans relative to the intended toxicology species.

Tier B

- Tier B1: **Identification** of circulating metabolites
  - Unbiased LC/MS analysis (control-analyte, software tools,….)
  - Prior knowledge of in vitro and in vivo preclinical metabolism work
  - High dose SAD vs MAD

- Tier B2: **Estimation** of metabolite abundance
  - MAD profile (T_{max}, 2x T_{max}, 4x T_{max})
  - Authentic Standard > Radiometric > UV > MS

- Tier B3: **Quantification** to document coverage of human metabolite(s) in animal species
  - Discuss sample selection
  - Discuss relative quantification or QRM (need for authentic standard synthesis)

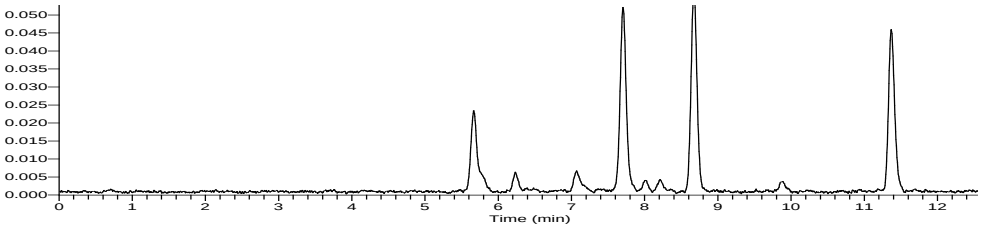
Cave: dose and time dependent changes

$$PAI = \frac{\text{AUCM } x \text{ in vitro potency P}}{\text{AUCP } x \text{ in vitro potency M}}$$

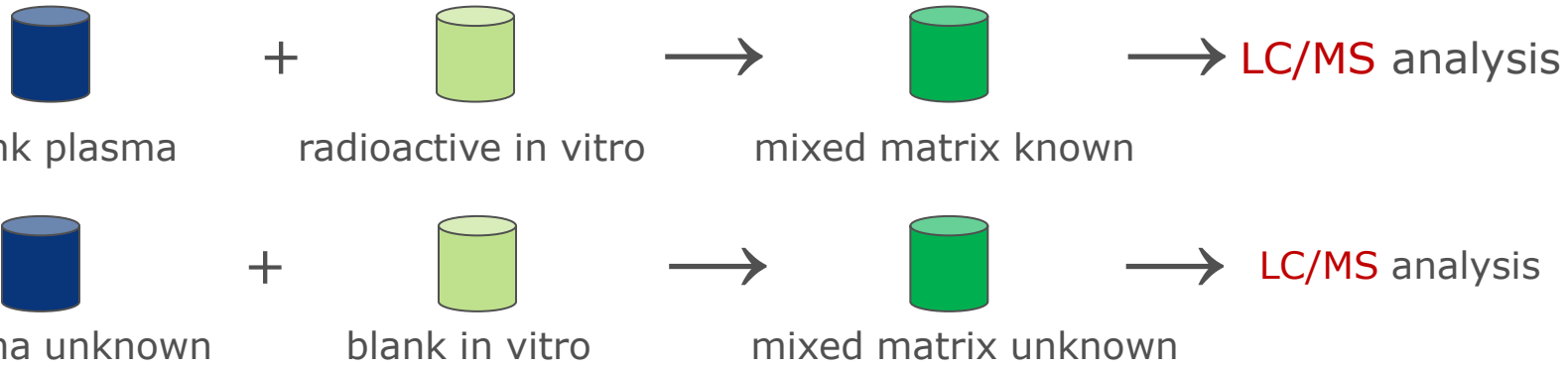
MS in combination with RAD + matrix mixing

Radioactive sample as reference + matrix matching

1. LC-RAD detection of ^{14}C or ^{3}H preclinical sample containing the same metabolites *(in vitro* preferred over *in vivo* sample because of less complex matrix)



2. Reduce ion suppression effects by matrix mixing:

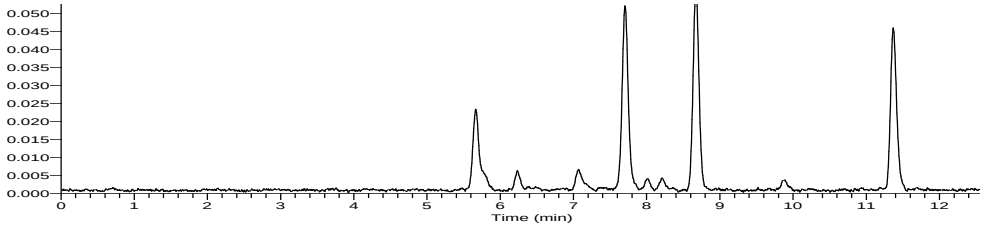


3. $C_{UNK} = C_{RAD} \times (MS_{UNK}/MS_{REF})$

C.P. Yu et al. Rapid Commun. Mass Spectrom. 21: 497 (2007)

Isotope dilution with ^{14}C sample + analysis with ESI-MS

1. LC-RAD detection of ^{14}C preclinical sample *or a mix of samples* containing the same metabolites

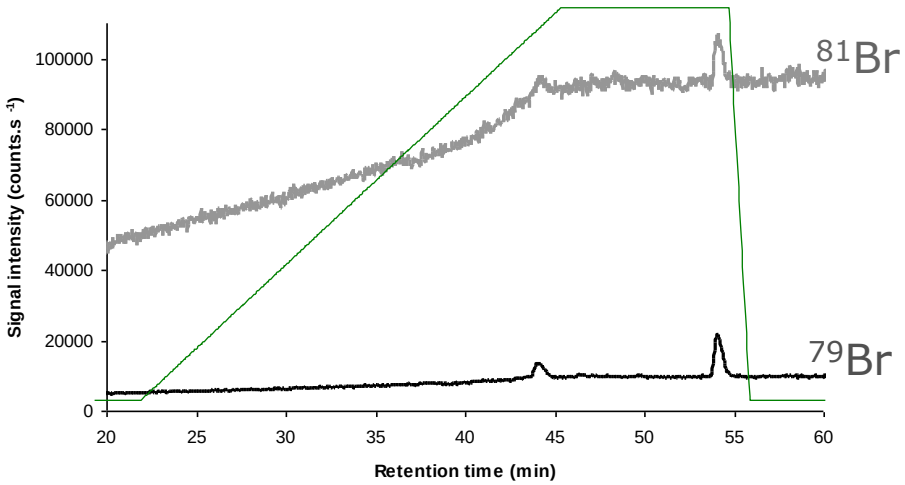


2. sample mixing (*no blank matrices or matrix mixing involved*):

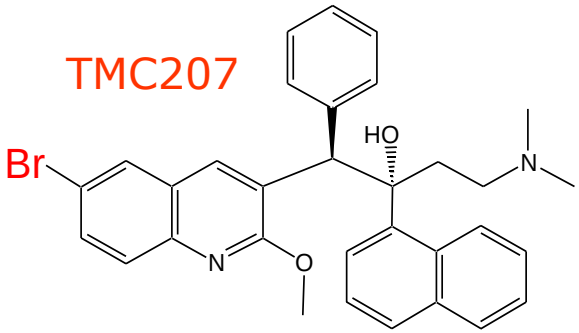
3. $C_{UNK} = C_{RAD} \times (^{12}C/^{14}C)$

F. Cuyckens et al. Bioanalysis 4(2): 143 (2012)

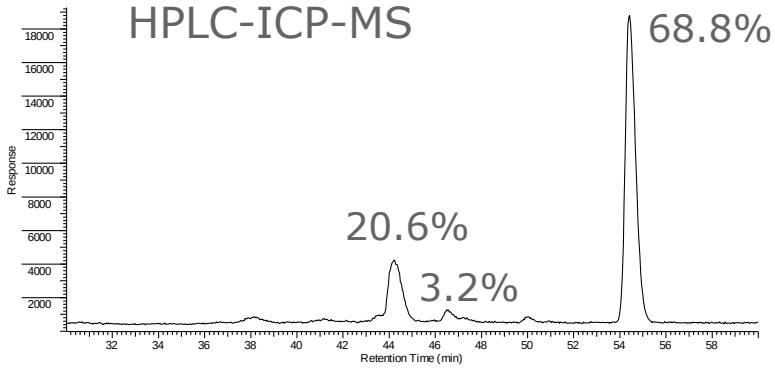
HPLC-Isotope Dilution ICP-MS

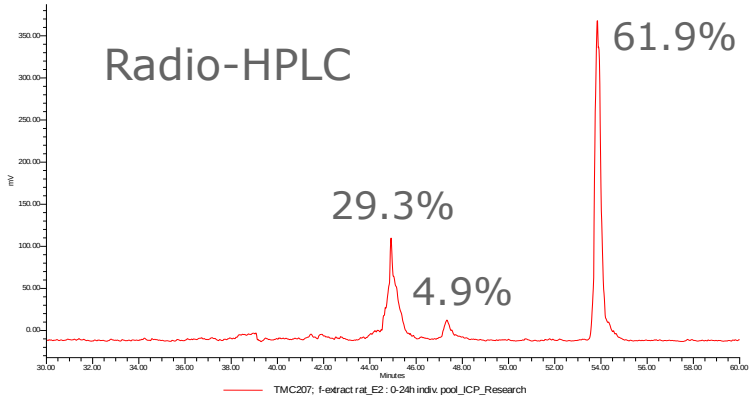


LC gradient: 5 - 95% CH_3OH/CH_3CN (1/1)



Bedaquiline rat faeces 0-24 h

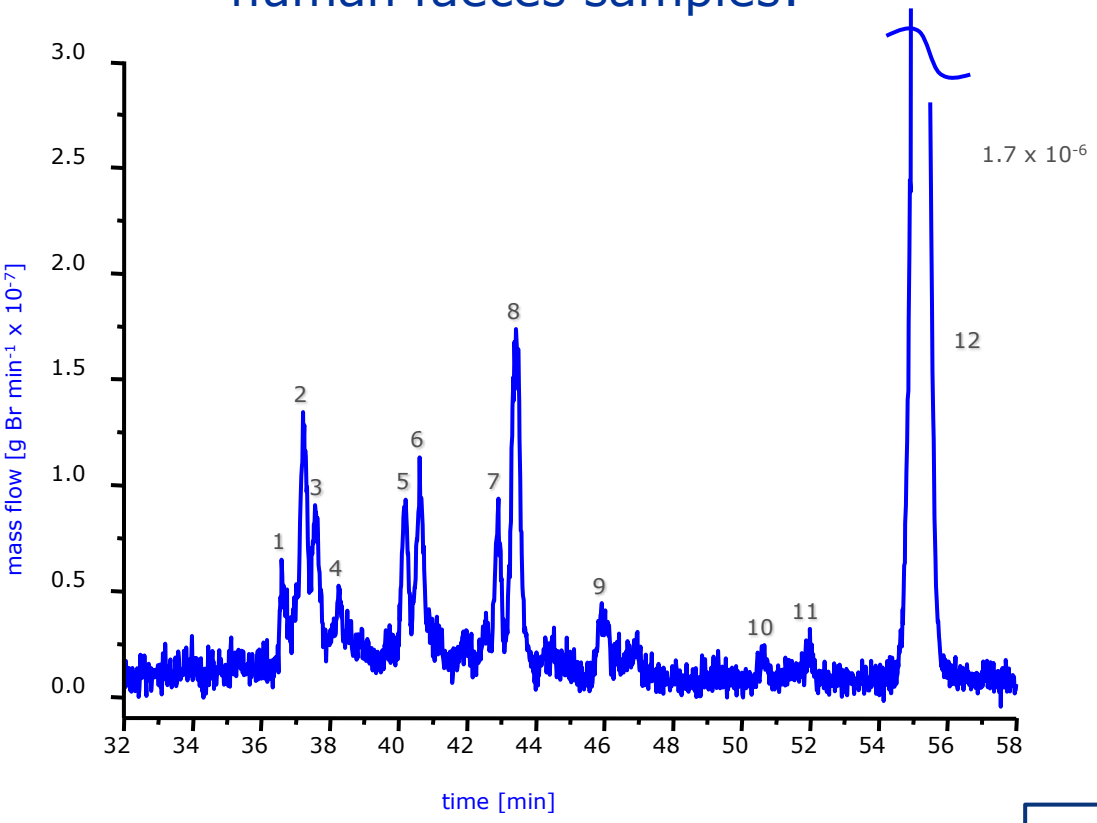




F. Cuyckens et al. Anal. Bioanal. Chem. 390: 1717 (2008)

ICP-MS analyses of patients treated with Bedaquiline

"bromatogram" of one of the human faeces samples:



LOD ~ 0.5 mg Br L^{-1}

Peak	Conc. [mg Br L^{-1}]
1	2.74
2	1.09×10^1
3	6.49
4	1.31
5	6.67
6	8.31
7	5.21
8	1.71×10^1
9	3.69
10	2.13
11	2.43
12 - unmet. drug	2.49×10^2
Sum	3.16×10^2
Total quant. FI	3.26×10^2

Flow injection measurement of total Br in the sample

column recovery: 97%

B. Meermann et al. Anal. Bioanal. Chem. 402: 439 (2012)

Tier C: strategy after Tier B

- Based on results obtained in Tier B, decide which metabolites need quantification in further studies.
- Examples
  - human metabolite >10% of TDRM AUCss and exposure is covered
  - active human metabolite of which *in vitro* activity contributes to <50% of the *in vivo* target pharmacological effect based on unbound systemic exposure

    If there are active metabolites contributing to the efficacy and safety of the drug, the exposure to these metabolites should be evaluated in the interaction studies. Moreover, ***if there are pharmacologically active metabolites which do not contribute significantly to in vivo effects of an investigational drug during normal conditions, the need for determining the exposure of these metabolites should be considered as a marked increase in exposure resulting from the interaction could be clinically relevant****.*

  limit quantification to a few relevant clinical studies
    - patient populations
    - selection of DDI studies

Tier C: strategy after Tier B

When does a metabolite qualify for regulatory validation in BA?

- **Clinical**:
  after full MIST/activity assessment, the metabolite significantly contributes to (≥ 25% PAI) the activity (including all PK and PD data, not only based on the AUC) : continued measurement in clinical studies using a regulatory validated method.

- **Preclinical**:
  a disproportionate metabolite in humans is dosed to animals to ensure MIST coverage in a GLP study *(rare situation)*.
  Once coverage is obtained, no further measurement in (pre)clinical studies is required.

  Prior to full MIST/activity assessment, there is no need for a regulatory validated method. In practice, the situations to set up these methods would be relatively limited.

$$PAI = \frac{\text{AUCM}_x \text{ in vitro potency P}}{\text{AUCP}_x \text{ in vitro potency M}}$$

Current Trend & Future vision

- 80/20 radiolabel yes/no
- More ^{3}H-labelled compounds
- Metabolite characterization (activity) screening
  - pro-active (bioassay) instead of case-by-case (isolated metabolites)
  - P450 inhibition screen
- Pharmacological dose + Microtracer (AEM + PK)
  Microdose (PK) (+ microtracer AEM + PK) in FIH
- Other matrices
  - Enterotest for bile sampling in ^{14}C or cold clinical studies
- MetQuan Advisory Board

MetQuan Advisory Board

Governance body for strategic discussions around metabolite quantification across portfolio, across disciplines (PDM, BA, ClinPharm, TOX) at important milestones of a project.

- Metabolite synthesis
- Review SAD-MAD data
- Very potent parent compound, metabolites of “concern” present at very low concentrations
- Highly metabolized parent compound, which represents a negligible percentage of TDRM in circulation.