EXCELLENT LINEARITY BETWEEN AUTOMATED CO₂ COMBUSTION AMS AND LIQUID SCINTILLATION COUNTING FOR PLASMA, BLOOD, URINE AND FECES SAMPLES

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REASONS FOR HIGH ATTRITION RATES DURING DRUG DEVELOPMENT

- Undesirable pharmacokinetics
- Insufficient efficacy
- Safety concerns
- Drug targets become more complicated

Ideal situation: Early availability of human data
- PK
- Metabolism
- Absolute bio-availability
- Mass balance data
ADVANTAGES OF HUMAN DATA GENERATION EARLY DURING DRUG DEVELOPMENT

- Minimize late stage surprises
- Resources spent on promising therapeutics
- Significant decrease of toxicity studies in animals
- Early optimization of formulation (limited absolute bioavailability)
- Shorten development time (benefit for patients and industry)

- By selecting the right study at the right time crucial studies are not longer on the critical path
MICRODOSING AND MICROTRACING RESEARCH

- Regulatory approval for application ICH M3 (R2) guideline. Into operation: December 2009
- To allow in humans: Single dose ≤ 100 µg and (≤ 1/100th NOAEL and ≤ 1/100th pharmacologically active dose)
- Requirement: Extended 14 day single dose, one species (rodent), intended route or i.v.; genotoxicity testing not advised, but SAR required

- Phase 0 – Microdose: PK, metabolite
- Phase 1 – Microtrace: AB, MIST, DDI, MB
HUMAN STUDIES INCLUDING A 14C LABEL

› Traditional label study
  › Typical dose 50-200 μCi
  › Dosimetry calculations (QWBA, rat ADME)
  › Analysis by LSC
  › Generally accepted by the authorities

› Microdose/trace study
  › Extreme low dose (100 nCi)
  › Considered safe both in terms of toxicology as radioactivity
  › 14 day rodents tox test only
  › GMP material required, although non-GMP materials are acceptable in some EU states and the USA

Misconceptions AMS
  › Slow
  › Expensive
  › Difficult
  › Not linear to LSC
  › Not dose linear
1 MV AMS AT TNO

Analysis in the fg/mL range (extreme sensitivity)
SAMPLE PREPARATION

CO$_2$ (50 µg carbon)

Pipetting
Evaporate to dryness
Place in carousel

Graphite (1 mg carbon)

Evaporate to dryness
Add CuO, flame seal
Combustion
Cryogenic trap
Convert to graphite (Fe and Zn)
Press into target

TNO AUTOMATED SAMPLE INTRODUCTION

METHODOLOGY

- Gaseous CO₂ is used instead of graphite
- No sample processing
- Only 50 µg of carbon
- As little as 2 µL of plasma
- Currently ~72 samples/day
- >140 samples day (Jan 2016)

Esther van Duijn
EBF 2015 Barcelona
## Instrument Qualification, Total $^{14}$C Qualification

<table>
<thead>
<tr>
<th>Matrix</th>
<th>Volume (µL)</th>
<th>Dynamic range (mBq/mL)</th>
<th>Absolute amount of activity (µBq)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine</td>
<td>15</td>
<td>0.41-102.5</td>
<td>0.75-1537.5</td>
</tr>
<tr>
<td>Whole blood</td>
<td>5</td>
<td>1.90-950.9</td>
<td>9.50-4754.5</td>
</tr>
<tr>
<td>Feces</td>
<td>30</td>
<td>2.64-528.0</td>
<td>79.2-15840</td>
</tr>
<tr>
<td>Plasma</td>
<td>1.8</td>
<td>0.65-821.2</td>
<td>1.2-1478.2</td>
</tr>
</tbody>
</table>

*van Duijn E et al., Anal.Chem. 2014, 86, 7635-7641*
To measure above the LLOQ within a reasonable time window the preferred method for analysis may differ
CONCENTRATION LINEARITY AMS – LSC (WHOLE BLOOD)
CONCENTRATION LINEARITY AMS – LSC (PLASMA)

Nominal or measured concentration (dpm/mL) by Novartis

R² = 0.9997
VOLUME LINEARITY CAMS

- The ability to process larger volumes is especially valuable for feces samples (to ensure homogeneity of the analyzed aliquot)
CLINICAL STUDY SAMPLES

70 samples from a clinical study (human mass balance)

cAMS results are within 15% of previous numbers (2 outliers)
SUMMARY

- LSC, LLSC, cAMS and gAMS are linear
- Use multiple methods without compromising on data quality
- Apply microdosing/tracer research only when required

- Knowledge on human PK, AB, metabolites, MB
- Sample throughput is not limiting, requires small sample volumes (pediatric)
- Place the costs in perspective as it may save a complete clinical study

- Allows innovation in drug discovery and improved clinical development plans
THANK YOU FOR YOUR ATTENTION

TNO
DIMITRI GROSSOUW
HUGO SANDMAN
RIANNE DE LIGT
STEVEN ERPELINCK
WOUTER VAES

NOVARTIS
PIET SWART
ARNOLD DEMAILLY
FREDERIC LOZAC’H
GIAN CAMENISCH
MARJORIE SIMON
POSTERS; NOT TO MISS
BOOTH A8

TNO (Esther van Duijn, Steven Erpelinck)

› Microdosing of biologicals in healthy volunteers: a safe and fast tool to predict clinical pharmacokinetics

TNO Triskelion (Brigitte Buscher, Jasja Wolthoorn, Luuk Renfurm, Schelto Korf)

› Comparison of triple quadrupole and orbitrap mass spectrometry for quantitative bioanalysis of intact proteins
› Bioanalysis of Infliximab (Remicade) in rat serum using conventional and ionKey UPLC-MS/MS
› A tiered bioanalytical approach to support the assessment of human oral bioaccessibility of oral drugs using in vitro dynamic gastrointestinal models (TIM)
› Optimization of LC and MS settings for the detection of signature peptides using IonKey-MS
› Development of a biosensor-based assay for characterization of anti-drug antibodies in human serum
WORKSHOP PEDIATRIC MICRODOSING
ADDED VALUE IN PIP

Thursday December 10th 2015
Location: Exchange Avenue, Schiphol Airport, The Netherlands

PRELIMINARY PROGRAM
09.00 - 09.30 Reception
09.30 - 12.30 Presentations
12.30 - 13.30 Lunch
13.30 - 16.00 Plenary discussion
16.00 - 17.00 Closing remarks and networking

CHAIRMAN
Daniel Bar Shalom  Associate professor, Department of Pharmacy, University of Copenhagen

SPEAKERS LIST
- Wouter H.J. Vaas  TNO. Lead Human Biology (Member of Pamper + Pedmic)
- Microdosing and related microtracer technology in early clinical development
- Francis P. Crawley  Good Clinical Practice Alliance - Europe (Member of Pamper + Ethics Working Group, European Paediatrics Academy, Brussels
- The Ethics of Microdosing in Children: Scientific, Regulatory, and Societal Considerations in the Context of Practical Experience
- Saskia N. de Wildt  Erasmus MC/Sophia Childrens Hospital, Rotterdam,
- Pediatric intensivist (Member of Pedmic, Chair of Dutch MCIR)
- Microdosing in children: a feasible and valuable tool for pediatric drug development
- Mark A. Turner  Department of Womens and Children’s Health, Institute of Translational Medicine, University of Liverpool, Liverpool, UK (Member of Pamper)
- Preliminary title: Pediatric microdosing in Europe. The Pamper programme and beyond