Novel quantitative approach for biodistribution of drug-related compounds in tissues using LESA-µLC-MS

Presenter: Walid Elbast
Contributors: Jim Glick, Jimmy Flarakos and Franck Picard
EBF 8th Open Meeting, Barcelona
18-20 November 2015
Outline

- **Introduction**
  - MS based quantitative methods for tissue analysis
  - Evolution of Surface & Imaging methodology
  - LESA-µLC-MS principal and workflow

- **Applications**
  - Supporting early discovery
  - Supporting topical formulation selection

- **Ongoing and future directions**
  - Refine the quantitative analysis: iMatrixSpray
  - Improve spacial resolution: laser height sensing
## Introduction

**MS based quantitative methods for tissue analysis**

<table>
<thead>
<tr>
<th>Method</th>
<th>Resolution</th>
<th>Sensitivity</th>
<th>Molecules</th>
<th>Quant.</th>
<th>Maturity</th>
<th>comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>MALDI MS</td>
<td>50 µm</td>
<td>100 nM</td>
<td>Nearly all</td>
<td>++</td>
<td>+++</td>
<td>no Phase II metabolites, coating matrices, matrix effect, no isomeric separation</td>
</tr>
<tr>
<td>ICP MS</td>
<td>?</td>
<td>nM - µM</td>
<td>Compound, metabolites, peptides</td>
<td>+</td>
<td>++</td>
<td>need special component (Pt, Al, Au, Br.) or derivatization</td>
</tr>
<tr>
<td>LESA Direct</td>
<td>1 mm</td>
<td>1 nM</td>
<td>Compounds, metabolites, peptides</td>
<td>-/+</td>
<td>++</td>
<td>LESA Clarity, +/- mode, semi-quantification, imaging. no isomeric separation</td>
</tr>
<tr>
<td>LESA-LC</td>
<td>1 mm</td>
<td>1 nM</td>
<td>Compound, metabolites, peptides</td>
<td>+++</td>
<td>+</td>
<td>Automated micro-flow LC/MS. Integration with current Sciex software platform</td>
</tr>
<tr>
<td>LAESI-MS</td>
<td>10 µm</td>
<td>?</td>
<td>LMW</td>
<td>-</td>
<td>+</td>
<td>Sensitivity seems poor (issue related to the laser use and no coupling to a LC), no isomeric separation</td>
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Introduction

Evolution of Surface & Imaging methodology

2009: LESA
- Automated sampling
- 1 mm spot size
- Requires flat surface
- No separation

2012: LMJ-SSP/HPLC-MS (v2)
- Automated sampling
- Analyst integration
- 1 mm precision
- Heat maps
- No hardware

2010: LMJ-SSP/HPLC-MS
- Manual sampling
- Hardware modification
- 1 mm spot size
- Separation
- Requires flat surface

2014: dropletProbe (v3)
- 0.7 mm spot size
- Improved speed
- Heights / Custom sample holders
- 0.1 mm precision
Introduction

LESA-μLC-MS principal and workflow
Introduction

**LESATyLC-MS principal and workflow** *(Courtesy of Dr. Gary Van Berkel, ORNL)*
Introduction

**LESA-µLC-MS principal and workflow**

- Whole body, organ(s) or tissue biopsies frozen
- Cryo-section prepared
Applications

Supporting early discovery

ABC111 distribution in tissues (ng/ml) at 4h (100 mg/kg p.o)

<table>
<thead>
<tr>
<th>Tissue</th>
<th>ABC111 Distribution (ng/ml)</th>
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<tbody>
<tr>
<td>under skin</td>
<td>381</td>
</tr>
<tr>
<td>fat/muscles?</td>
<td>401</td>
</tr>
<tr>
<td>lung</td>
<td>234</td>
</tr>
<tr>
<td>heart</td>
<td>245</td>
</tr>
<tr>
<td>thymus</td>
<td>213</td>
</tr>
<tr>
<td>liver</td>
<td>660</td>
</tr>
<tr>
<td>kidney</td>
<td>120</td>
</tr>
<tr>
<td>GI</td>
<td>156593</td>
</tr>
<tr>
<td>large intestine</td>
<td>89686</td>
</tr>
<tr>
<td>fat around intestine</td>
<td>1116</td>
</tr>
<tr>
<td>adrenal</td>
<td>285</td>
</tr>
</tbody>
</table>

ABC111 metabolite distribution in tissues (ng/ml) at 4h (100 mg/kg p.o)

<table>
<thead>
<tr>
<th>Tissue</th>
<th>ABC111 Metabolite Distribution (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>under skin</td>
<td>199</td>
</tr>
<tr>
<td>fat/muscles?</td>
<td>53</td>
</tr>
<tr>
<td>lung</td>
<td>33</td>
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<tr>
<td>heart</td>
<td>53</td>
</tr>
<tr>
<td>thymus</td>
<td>74</td>
</tr>
<tr>
<td>liver</td>
<td>178</td>
</tr>
<tr>
<td>kidney</td>
<td>167</td>
</tr>
<tr>
<td>GI</td>
<td>35254</td>
</tr>
<tr>
<td>large intestine</td>
<td>33620</td>
</tr>
<tr>
<td>fat around intestine</td>
<td>95</td>
</tr>
<tr>
<td>adrenal</td>
<td>69</td>
</tr>
</tbody>
</table>

✔ Excellent agreement between QWBA data and LESA-µLC-MS data
Applications
Supporting early discovery

ABC222 distribution in tissues at 0.5h (100 mg/kg p.o)

ABC222 metabolites distribution in tissues at 0.5h (100 mg/kg p.o)

✓ Excellent agreement between QWBA data and LESA-µLC-MS data
Applications

Supporting topical formulation selection

- Excellent agreement between LC-MS/MS and LESA-μLC-MS
- “Form A” appears to have greater depth of penetration when compared to the “Form B”
Applications

Supporting topical formulation selection

- Excellent agreement between LC-MS/MS and LESA-µLC-MS
- Similar skin concentrations from Cream and Liquid Crystal and confirmation of lowest concentrations using Emulgel
Ongoing and future directions

Refine the quantitative analysis: iMatrixSprayer

Cs and QCs prepared in blood, plasma or tissue homogenate, embedded in CMC and cutted (40 µm)

Evaluate the iMatrixSpray device for deposition of Cs and QCs directly on blank tissue sections
Ongoing and future directions

*Improve spatial resolution: laser height sensing*

Improve the spot size to improve spatial resolution from 1-2mm to ~0.5mm
Acknowledgements

- Gary Van Berkel
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- Brett Peterson

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- Steve Martin
- Vijay Bhargava
- Markus Stoeckli
- Novartis

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Back-up slides

Atomic
Structural biology
Biophysics
Structure/activity relationships

Cell membrane
Permeability
Transport mechanisms
Receptor occupancy
PKPD relationships

Tissue/Organ
Toxicity
Support in silico PBPK modeling
PKPD relationships
Drug delivery

Genetic
Chromatin remodeling
Epigenetic regulation of gene expression
DNA damage and repair
Transcriptional regulation

Cellular
Cell-type specific
drug/metabolite distribution
Biomarkers of efficacy/toxicity