VOLUMETRIC ABSORPTIVE MICROSAMPLING AS AN ALTERNATIVE SAMPLING STRATEGY FOR CEREBROSPINAL FLUID

Lisa Delahaye / 11th EBF Open Symposium / November 2018

CSF MICROSAMPLING WITH VAMS

- Low sampling volume
  - Especially interesting in pediatric patient population

- No need for micropipette to obtain volumetric samples
  - Allows minimal sample handling, even in hospital setting

- Easy sample storage and transportation

- Possibly automated workflow in the future

- Same workflow for blood and CSF samples
QUANTIFICATION OF PARACETAMOL

Instrument set-up and parameters:

LC: Waters UPLC®
Column: ACQUITY UPLC® HSS T3 1.8 µm (2.1 x 100 mm) – 45 °C

MP A: 95/5 H₂O/MeOH + 0.01% FA
MP B: 95/5 MeOH/H₂O + 0.01% FA

MS: SCIEX API 4000
+ MRM
- Quantifier ion: 152.04 → 110.00 m/z
- Qualifier ion: 152.04 → 93.00 m/z

Preparing the VAMS + Dry for 2 hours
Remove VAMS tip

Extraction solvent:
300 µL 80/20 MeOH/H₂O + IS* + 0.01% FA

Thermomixer:
10 min; 22 °C; 1400 rpm
Centrifugation:
10 min; 10 000 g

250 µL supernatant + 750 µL H₂O (0.01% FA)

LC-MS/MS
No blank CSF available for generation of calibrators and QCs
→ Alternative matrix needed: can *water* be used?
- Calibration line with spiked water (= alternative matrix) → generate VAMS samples
- Real-life liquid CSF patient samples (6) → generate VAMS samples
- Dilute same CSF patient samples (6) 1/10 with water (6) → generate VAMS samples

<table>
<thead>
<tr>
<th>Concentration undiluted samples</th>
<th>=</th>
<th>Concentration diluted samples (after recalculation)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average difference = 3.33 %</td>
<td></td>
<td>Demonstrates equality of process efficiency between two matrices</td>
</tr>
<tr>
<td>%RSD of difference = 7.71%</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
VALIDATION

- No blank CSF available for generation of calibrators and QCs
  - Evaluation of selectivity? Evaluation of matrix effect?
    - Preparation of artificial CSF
    - Comparison of transition ratio's of calibrators/QCs ($n=16$) vs. patient samples ($n=55$)
    - Evaluation of IS area in calibrators/QCs vs. patient samples

No interfering peaks detected in artificial CSF
Transition ratios samples within $\pm 4\%$ of the ratio in calibrators/QCs
  - No issues with interferences for this assay

IS had an overall an %RSD of 7.3% in calibrators, QCs and patient samples
  - No significant ion suppression/enhancement in patient samples
VALIDATION

Calibration:
Range: 100 ng/mL to 40 µg/mL
Calibration model: linear model with 1/x² weighting

Accuracy and precision:

<table>
<thead>
<tr>
<th>QC Level</th>
<th>Accuracy (%bias)</th>
<th>Intra-day precision (%RSD)</th>
<th>Total precision (%RSD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LLOQ</td>
<td>16.8</td>
<td>6.9</td>
<td>6.9</td>
</tr>
<tr>
<td>Low</td>
<td>2.5</td>
<td>5.3</td>
<td>5.3</td>
</tr>
<tr>
<td>Medium</td>
<td>6.0</td>
<td>4.5</td>
<td>4.5</td>
</tr>
<tr>
<td>High</td>
<td>-0.43</td>
<td>3.9</td>
<td>7.6</td>
</tr>
</tbody>
</table>

Dilution integrity:

<table>
<thead>
<tr>
<th>Dilution factor</th>
<th>Accuracy (% bias)</th>
<th>Precision (%RSD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>1.0</td>
<td>5.4</td>
</tr>
<tr>
<td>5</td>
<td>7.5</td>
<td>5.9</td>
</tr>
</tbody>
</table>

Recovery:

<table>
<thead>
<tr>
<th>QC level</th>
<th>Recovery*</th>
<th>%RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>70 %</td>
<td>9</td>
</tr>
<tr>
<td>High</td>
<td>78 %</td>
<td>3</td>
</tr>
</tbody>
</table>

Selectivity:
No interferences detected
No carry over detected

Meets acceptance criteria ✅

* ANOVA did not reveal any statistically significant difference between the groups

Meets acceptance criteria ✅
Aqueous VAMS samples are stable for at least **9 months** when stored at **-80°C and 4°C**.

Aqueous VAMS samples are stable for at least **1 month** when stored at **RT**.

Aqueous VAMS samples stored at **50°C** and for more than **1 month** at RT show **decreased concentrations**.

? Stability data for **blood** VAMS samples extracted following the same protocol are within the acceptance criteria for all tested conditions:

→ no problem with chemical stability of paracetamol

Apparent stability issue is most likely **recovery related**

→ Cause of decreased extractability?

**Extractability-mediated recovery bias can be matrix dependent!**
ANALYSIS OF EXTERNAL QUALITY CONTROL MATERIAL

No CSF-based QC material available
→ Start from serum-based QC material

- Dilute 1/10 with water
- Generate VAMS samples

Serum-based
Diluted (aqueous)

- 14/18 individual measurements are within the acceptance limits
- Mean values per QC level are within the acceptance limits
- Concentrations of the QCs are within the therapeutically relevant concentrations
→ Supports validity of the method
ANALYSIS OF PATIENT SAMPLES

- Venous blood and CSF VAMS samples collected from pediatric patients treated with IV paracetamol

Concentrations of patient samples were within the calibration range of the method

No unforeseen issues encountered with the analysis of patient samples

Visual inspection of CSF VAMS samples = difficult → Need for good sample quality!
CONCLUSION

- Development of methods for the quantitative determination of paracetamol in blood and CSF using VAMS

- Successful validation of these methods based on international guidelines

- The methods were successfully applied on external QC material and authentic patient samples

- An extractability-mediated recovery bias can be matrix dependent
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