Neoteryx Workshop

Mitra sampling for Immunosuppressants analysis of outpatients

Remco A. Koster PhD
Department of Hospital and Clinical Pharmacy
University Medical Center Groningen / University of Groningen
Mitra sampling
Outpatient monitoring

• Organ transplant patients
• Lifelong medication of tacrolimus, sirolimus, everolimus, cyclosporin A and/or mycophenolic acid
• Therapeutic drug monitoring is mandatory to avoid sub-therapeutic or toxic levels
• Home sampling has many advantages
Extraction procedure

1) Transfer Mitra tip into roundbottom Eppendorf cup
2) Add 100 µL water:methanol (60:40) with IS
3) 30 min of ultrasonication
4) Add 200 µL methanol and vortex for 15 min
5) 15 min of ultrasonication followed by 15 min vortexing
6) Centrifuge for 5 min
7) Transfer to vial with insert and place at -20°C for 10 min
8) Centrifuge for 5 min and inject 20 µL
LC-MS/MS method

Thermo Quantiva with Vanquish UPLC system.

- Accucore C18 column 50 x 2.1 mm 2.6 µm
- Flow 1.0 mL/min
- Runtime 1.5 min
Effect of hematocrit and analyte concentration on recovery of immunosuppressants

DBS full spot

Mitra

Koster et al. Talanta 2013

Koster et al. manuscript in preparation
Effect of hematocrit and analyte concentration on recovery of immunosuppressants

± 48% recovery

DBS full spot

±78% recovery

Whatman 31-ET-CHR paper

Koster et al. Talanta 2013

Koster et al. manuscript in preparation
Effect of hematocrit and analyte concentration on recovery of immunosuppressants

56% recovery

Full spot

Whatman DMPK-C card

60% recovery

Influence of HT and concentration on recovery of everolimus

Koster et al. Bioanalysis 2015

Koster et al. manuscript in preparation
Effect of hematocrit and analyte concentration on recovery of immunosuppressants

63% recovery  60% recovery  56% recovery
Effect of hematocrit and analyte concentration on recovery of immunosuppressants

Influence of HT and concentration on recovery of cyclosporin A

Mitra

Koster et al. manuscript in preparation
The influence of the hematocrit on the analytical bias. Concentrations are 3 ng/mL for tacrolimus, sirolimus, everolimus, 60 ng/mL for cyclosporin A and 0.3 mg/L for mycophenolic acid.
DBS: Total hematocrit effects

**Low and High concentration**

- Five different card types
- Partial spot analysis
The influence of the hematocrit on the analytical bias. Concentrations are 3 ng/mL for tacrolimus, sirolimus, everolimus. 30 ng/mL for cyclosporin A and 0.1 mg/L for mycophenolic acid.
The influence of the hematocrit on the analytical bias. Concentrations are 40 ng/mL for tacrolimus, sirolimus, everolimus. 400 ng/mL for cyclosporin A and 12.5 mg/L for mycophenolic acid.
Linearity, accuracy & Precision

Tacrolimus

\[ Y = 0.0012729 + 0.0623213 \times X \]

\[ R^2 = 0.9971 \]

\[ W: 1/X^2 \]
Linearity, accuracy & Precision

Sirolimus

\[ Y = 0.016435 + 0.0806988X \quad R^2 = 0.9763 \quad W: 1/X^2 \]
Linearity, accuracy & Precision

Everolimus

\[ Y = 0.00415625 + 0.0761048 \times X \quad R^2 = 0.9921 \quad W: 1/X^2 \]
Linearity, accuracy & Precision

Ciclosporine A

\[ Y = -0.0251571 + 0.00786386 \times X \]

\[ R^2 = 0.9888 \]

W: 1/X^2
### Linearity, accuracy & Precision

<table>
<thead>
<tr>
<th>Standard</th>
<th>Mycophenolic acid</th>
<th>Tacrolimus</th>
<th>Sirolimus</th>
<th>Everolimus</th>
<th>Cyclosporin A</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1.8</td>
<td>2.0</td>
<td>3.2</td>
<td>6.2</td>
<td>3.9</td>
</tr>
<tr>
<td>B</td>
<td>3.6</td>
<td>3.6</td>
<td>2.6</td>
<td>5.5</td>
<td>3.4</td>
</tr>
<tr>
<td>C</td>
<td>3.7</td>
<td>4.1</td>
<td>2.3</td>
<td>3.6</td>
<td>3.7</td>
</tr>
<tr>
<td>D</td>
<td>4.9</td>
<td>4.5</td>
<td>5.5</td>
<td>5.0</td>
<td>4.8</td>
</tr>
<tr>
<td>E</td>
<td>4.9</td>
<td>4.6</td>
<td>4.5</td>
<td>4.3</td>
<td>4.7</td>
</tr>
<tr>
<td>F</td>
<td>3.7</td>
<td>3.7</td>
<td>7.4</td>
<td>6.3</td>
<td>4.0</td>
</tr>
<tr>
<td>G</td>
<td>3.4</td>
<td>3.2</td>
<td>5.6</td>
<td>4.7</td>
<td>5.1</td>
</tr>
<tr>
<td>H</td>
<td>3.1</td>
<td>2.7</td>
<td>4.2</td>
<td>4.3</td>
<td>1.9</td>
</tr>
</tbody>
</table>

Linear range for tacrolimus, sirolimus, everolimus: 1.0 – 100 ng/mL, cyclosporin A: 10 – 1000 ng/mL and mycophenolic acid: 0.1 – 5.0 mg/L
Conclusion

• Mitra sampling is a viable alternative for DBS sampling
• Less or practically no HT effects observed for most substances
• Cyclosporin A performs less well than expected
• Longer sample preparation time for Mitra compared to DBS (2.5 h vs 1 h)
• Validation in process
Thank you for your attention

r.koster@umcg.nl

Special thanks to Pascal Niemeijer for performing all the research

Email after 1 may 2017
kosterremco@prahs.nl