Immunosuppressive and Antiretroviral Drugs

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Prof. Volkhard Kaever
Therapeutic Drug Monitoring (TDM)

quantification of known agents:

- side of action, e.g. tissue, cytosol
- plasma / serum / whole blood
- protein-free plasma
- saliva
- urine

individualized therapy

concentration

too low

loss of effect

reasons: non-compliant patient
no normal PK or PD
drug interactions

too high

toxicity
Determination of immunosuppressive drugs and their metabolites by HPLC techniques

Christians U et al.
Measurement of cyclosporine A and four metabolites in whole blood by high-performance liquid chromatography
J Chromatogr 413:121 (1987)

Kirchner GI et al.
Fast quantification method for sirolimus and its major metabolites
Transplant Proc 33:1091 (2001)

Deters M et al.
Simultaneous quantification of sirolimus, everolimus, tacrolimus and cyclosporine by liquid chromatography-mass spectrometry (LC-MS)

Koal T et al.
Simultaneous determination of four immunosuppressants by means of high speed and robust on-line solid phase extraction-high performance liquid chromatography-tandem mass spectrometry
How to optimize patient sample extraction?

normal procedures:
- protein precipitation (off-line)
- LLE or **SPE** (off-line / **on-line**)
- HPLC (UV / MS / MSMS)

alternative procedures:
"**dilute & inject**" (plasma / serum / urine)
"**sorbent sampling techniques**" (whole blood)
DBS (dried blood samples)
Quantification by LC-ESI-MS/MS

**Immunosuppressive Drugs**
- CSA, TRL, SRL, RAD / MPA

- Peptides: "peptidomics"
- FTY720: "sphingolipidomics"

**Antimicrobial Drugs**

**Antiretroviral Drugs**

**Antiviral Drugs**

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Immunosuppressive Drugs

(Application Note in cooperation with Spark Holland)

Sirolimus (SRL)
Tacrolimus (TRL)
Everolimus (RAD)
Cyclosporine A (CSA)

Mycophenolic acid
FTY720

simultaneous quantification by online XLC-ESI-MS/MS
XLC: Symbiosis Pharma (Spark Holland)
MS/MS: API 3000 (Applied Biosystems)

matrix: human EDTA blood
sample preparation: protein precipitation (offline)
**Immunosuppressive Drugs**

**Eluents for SPE**

SPE enrichment:
90/10 H₂O/MeOH (v/v)  
2 min

SPE elution & LC-MS/MS:
3/97 H₂O/MeOH,  
10mM NH₄Oac, 0.1% Hac  
5 min

**Simultaneous analysis of two samples**

**Column oven @ 60°C**

**API 3000**
Immunosuppressive Drugs

**CSA**
\[ y = 0.00622x; \quad r = 0.9998 \]

**SRL**
\[ y = 0.0206x; \quad r = 0.9998 \]

**TRL**
\[ y = 0.0598x; \quad r = 0.9998 \]

**RAD**
\[ y = 0.0307x; \quad r = 0.9997 \]

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## Immunosuppressive Drugs

<table>
<thead>
<tr>
<th></th>
<th>Cyclosporin A (CSA)</th>
<th>Tacrolimus (TRL)</th>
<th>Sirolimus (SRL)</th>
<th>Everolimus (RAD)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>LOD [ng/mL]</strong></td>
<td>1.0</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
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<tr>
<td><strong>LLOQ [ng/mL]</strong></td>
<td>10.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
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<tr>
<td><strong>Linearity [R²]</strong></td>
<td>0.9998</td>
<td>0.9998</td>
<td>0.9998</td>
<td>0.9997</td>
</tr>
<tr>
<td><strong>Recovery [%]</strong></td>
<td>&gt;90%</td>
<td>&gt;90%</td>
<td>&gt;90%</td>
<td>&gt;90%</td>
</tr>
<tr>
<td><strong>RSD low level [%]</strong></td>
<td>1.8</td>
<td>3.5</td>
<td>9.2</td>
<td>5.6</td>
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<tr>
<td><strong>Intra-day</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>RSD high level [%]</strong></td>
<td>1.5</td>
<td>3.4</td>
<td>4.6</td>
<td>3.8</td>
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<tr>
<td><strong>Intra-day</strong></td>
<td></td>
<td></td>
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<tr>
<td><strong>RSD low level [%]</strong></td>
<td>6.4</td>
<td>5.7</td>
<td>7.6</td>
<td>12.2</td>
</tr>
<tr>
<td><strong>Inter-day</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>RSD high level [%]</strong></td>
<td>4.2</td>
<td>3.5</td>
<td>5.0</td>
<td>4.8</td>
</tr>
<tr>
<td><strong>Inter-day</strong></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td><strong>Accuracy [%]</strong></td>
<td>95.0 ± 5.0</td>
<td>96.2 ± 2.8</td>
<td>100.0 ± 4.8</td>
<td>84.6 ± 8.8</td>
</tr>
</tbody>
</table>

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Patient I

- SRL 7.6 ng/ml
- TRL 6.1 ng/ml

Patient II

- RAD 5.5 ng/ml
- CSA 67.7 ng/ml

IS

- Ascomycin (IS)
- CSD (IS)

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TDM of antiretroviral Drugs /HIV-AIDS

- *always* combination therapy
- *lifelong* therapy
- *too low* drug levels: *virus resistance*
- *too high* levels: *unwanted side effects*
- *drug interactions*

**Patient**

**Drug**

**Virus**

**PD-TDM:** Virus load / CD4 positive cells

**PK-TDM:** LC-MS/MS

*decrease of unwanted side effects?*  
*lipodystrophy, hepatotoxicity*
Antiretroviral Drugs

HAART = combination of

1. Protease Inhibitors (PI)

2. Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTI)

3. Nucleoside/Nucleotide Reverse Transcriptase Inhibitors (NRTI)

- no correlation between drug concentration & antiretroviral effect
- prodrugs
- analysis of intracellular triphosphorylated metabolites!
Antiretroviral Drugs (PI and NNRTI) (pos. MRM)

**LC-MS/MS**

**X (OASIS)-LC-MS/MS**

**X (C18HD)-LC-MS/MS**

*Symbiosis Pharma*

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<table>
<thead>
<tr>
<th>Total</th>
<th>8 min</th>
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<tr>
<td></td>
<td>6 min</td>
</tr>
<tr>
<td></td>
<td>3 min</td>
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</table>
Antiretroviral Drugs

*PI (7)*
*NNRTI (2)*

simultaneously by validated XLC-MS/MS

analysis time: 3 min / sample

protein precipitation of patient plasma (offline)

alternative techniques:

*directly in plasma ("dilute & inject")*
(Koal et al., submitted)

*Dried Blood Spots (whole blood)*
(Koal et al., RCM, in press)
**Antiretroviral Drugs (dilute & inject)**

**Method Comparison**

\[ n=50 \text{ Patients} \]

\[ y = 0.9743x + 36.6 \]

\[ R^2 = 0.9667 \]

![Graph showing method comparison with equation and R^2 value.](image-url)
Antiretroviral Drugs (matrix effects)

**LC-MS/MS**
- Protein precipitation
- Peak window

**XLC-MS/MS**
- Dilute & inject

**Short analysis time!**
**Matrix elimination!**
**Only sample preparation: dilution!**
Antiretroviral Drugs (Dried Blood Spots)

★ sample mailing & decrease of the infection risk
★ 5µl EDTA-whole blood; 5mm Spot

I. DBS-drying time (up to 5 days)
II. DBS-extraction time (20 sec - 75 min)

\[ y = 1,1515x \]
\[ R^2 = 0,9772 \]

Plasma concentration (ng/mL) vs. DBS concentration (ng/mL)

\( n=70 \)

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Antiretroviral Drugs (carry-over effect)

high target concentrations for PI and NNRTI

high concentrations in samples (>10,000 ng/mL)

carry-over effects

online SPE @
1 SPE-column/1000 analyses
Oasis

MeOH blanks!

online SPE @
1 SPE-cartridge/1-30 analyses
Symbiosis (Spark Holland)

no MeOH blanks!

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Résumé

★ XLC-MS/MS for TDM of patient samples

- short analyses times (two samples simultaneously)

- efficient matrix elimination

- similar method and set-up for different applications

- simple method integration of new drugs

★ alternative techniques:

- Plasma direct ("dilute & inject")

- Dried Blood Spots
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Heike
Patrick

Prof. Klaus Resch

NIH Aids Reagent Reference Program

Spark Holland
Applied Biosystems