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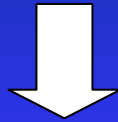


Immunosuppressive and Antiretroviral Drugs

*Dr. Therese Koal
Prof. Volkhard Kaefer*

Therapeutic Drug Monitoring (TDM)

quantification of known agents:



individualized therapy

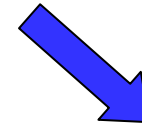
- *side of action, e.g. tissue, cytosol*
- *plasma / serum / whole blood*
- *protein-free plasma*
- *saliva*
- *urine*

concentration



too low

loss of effect



too high

toxicity

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



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)

Clin Chem Lab Med 40:285 (2002)

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

J Chromatogr B 805:215 (2004)



How to optimize patient sample extraction ?

normal procedures:

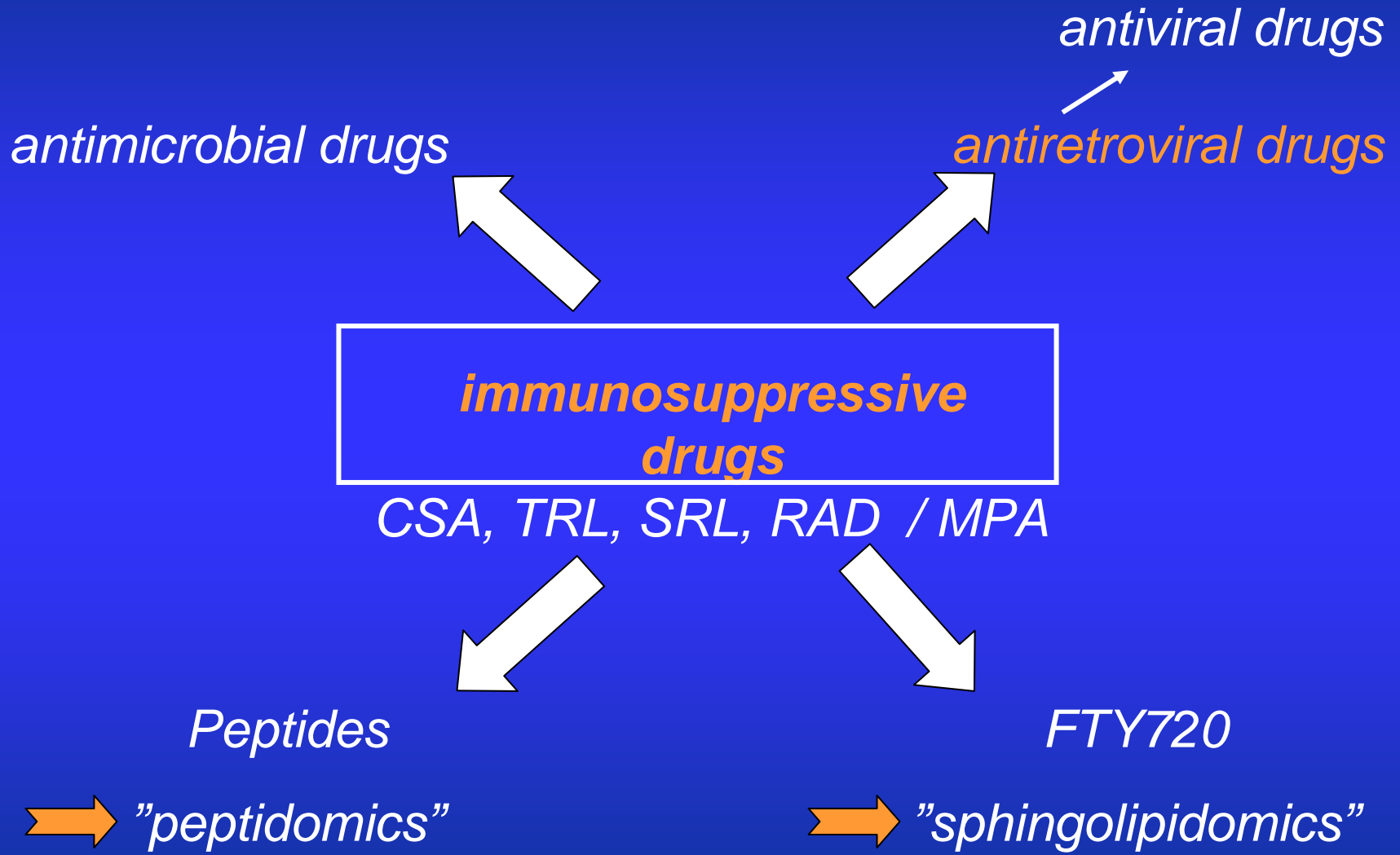
- *protein precipitation (off-line)*
- *LLE or SPE (off-line / on-line)*
- *HPLC (UV / MS / MSMS)*

alternative procedures:

- "dilute & inject"*** (*plasma / serum / urine*)
- "sorbent sampling techniques"*** (*whole blood*)
DBS (dried blood samples)



Quantification by LC-ESI-MS/MS



Immunosuppressive Drugs

(Application Note in cooperation with Spark Holland)

Sirolimus (SRL)

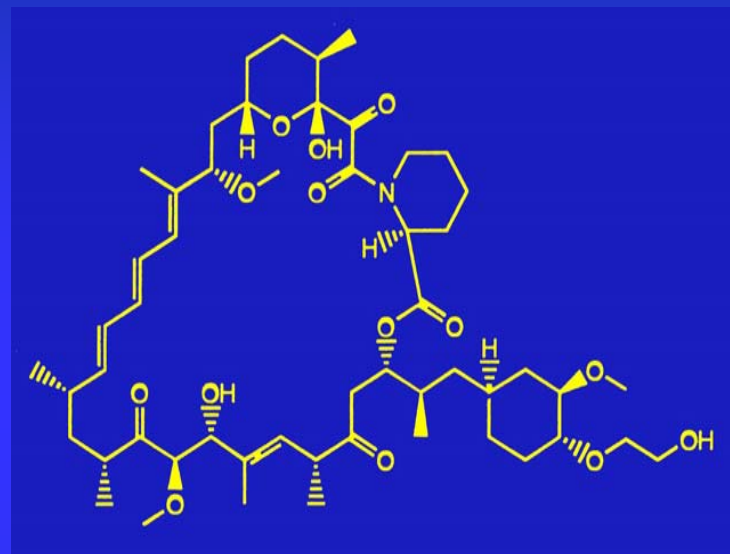
Tacrolimus (TRL)

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**

LC-Eluent

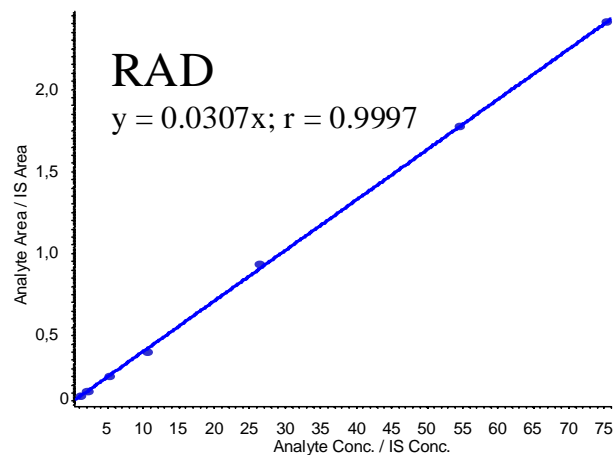
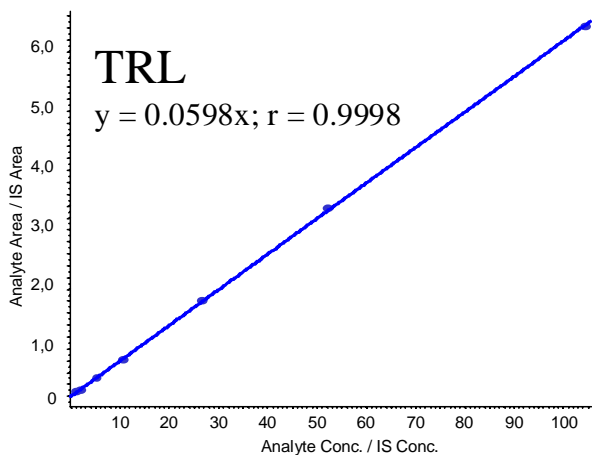
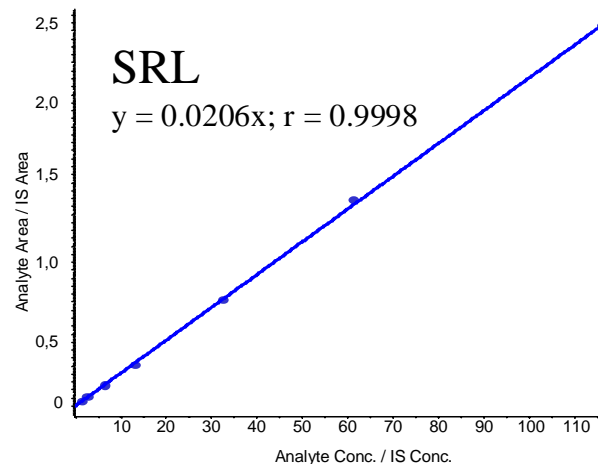
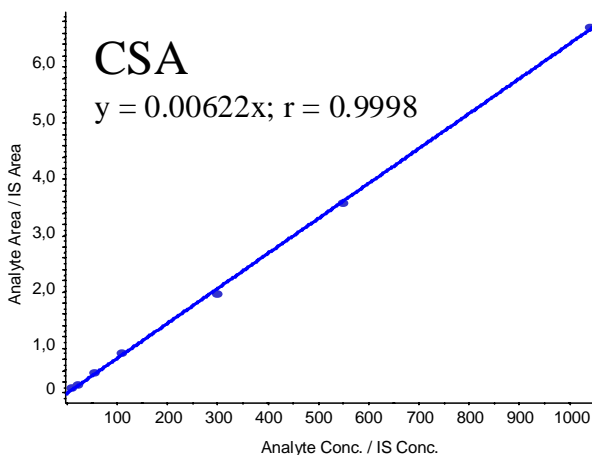


Column oven @ 60°C

API 3000



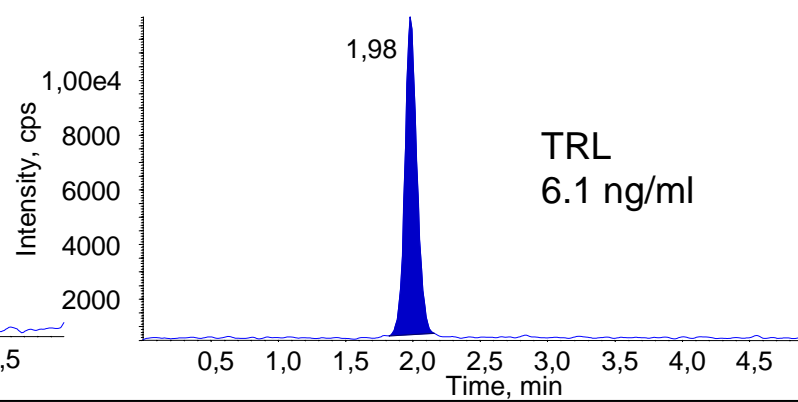
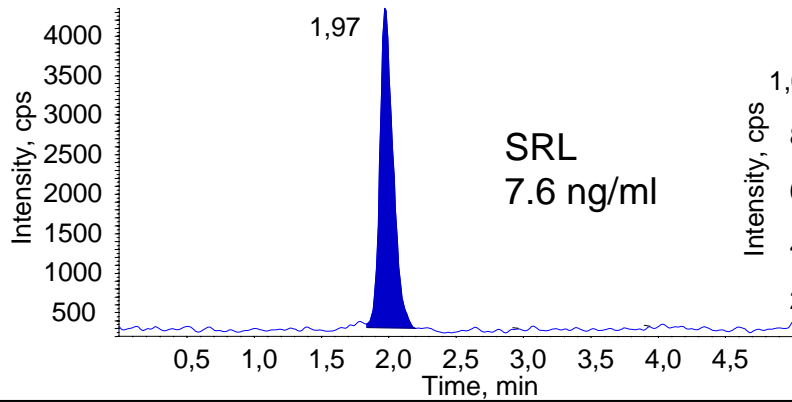
Immunosuppressive Drugs



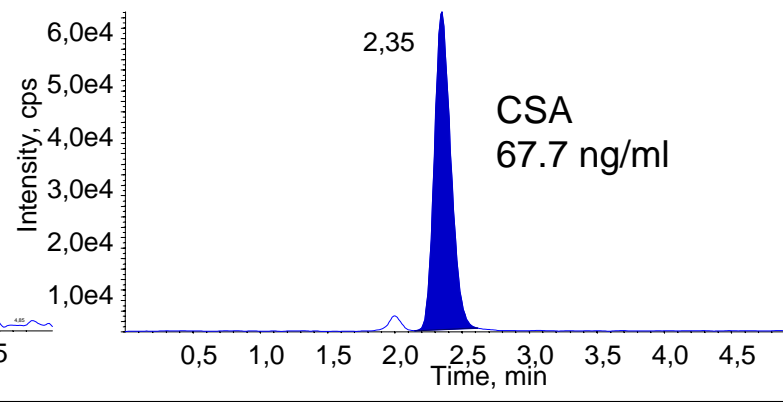
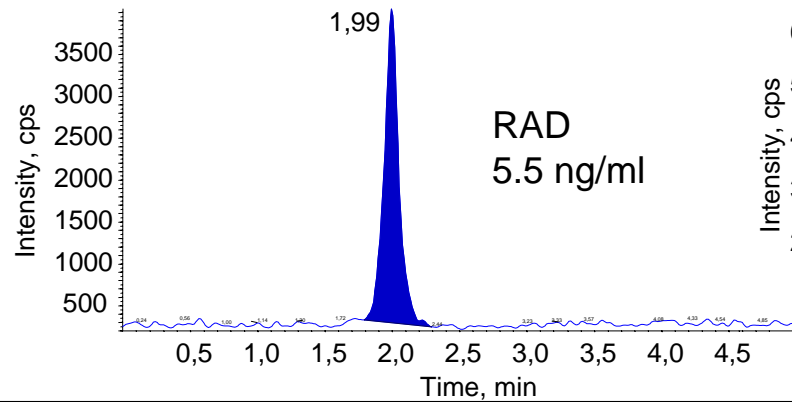
Immunosuppressive Drugs

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

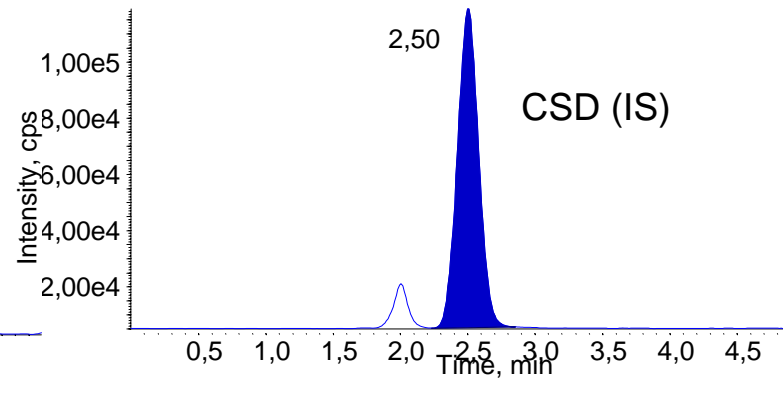
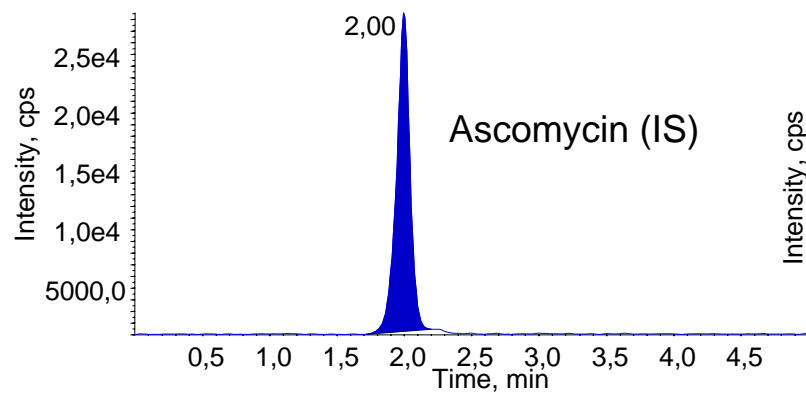




Patient I



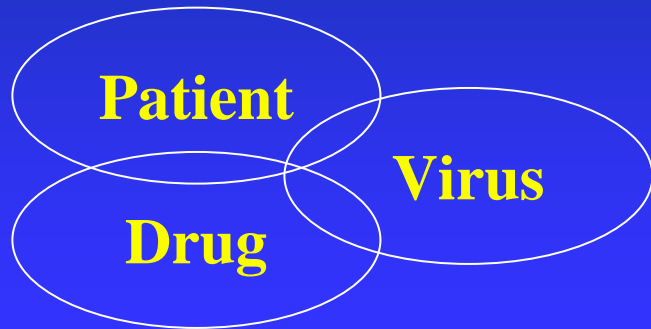
Patient II



IS

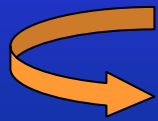


TDM of antiretroviral Drugs /HIV-AIDS



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

- **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)*



LC-ESI-MS/MS

2. *Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTI)*



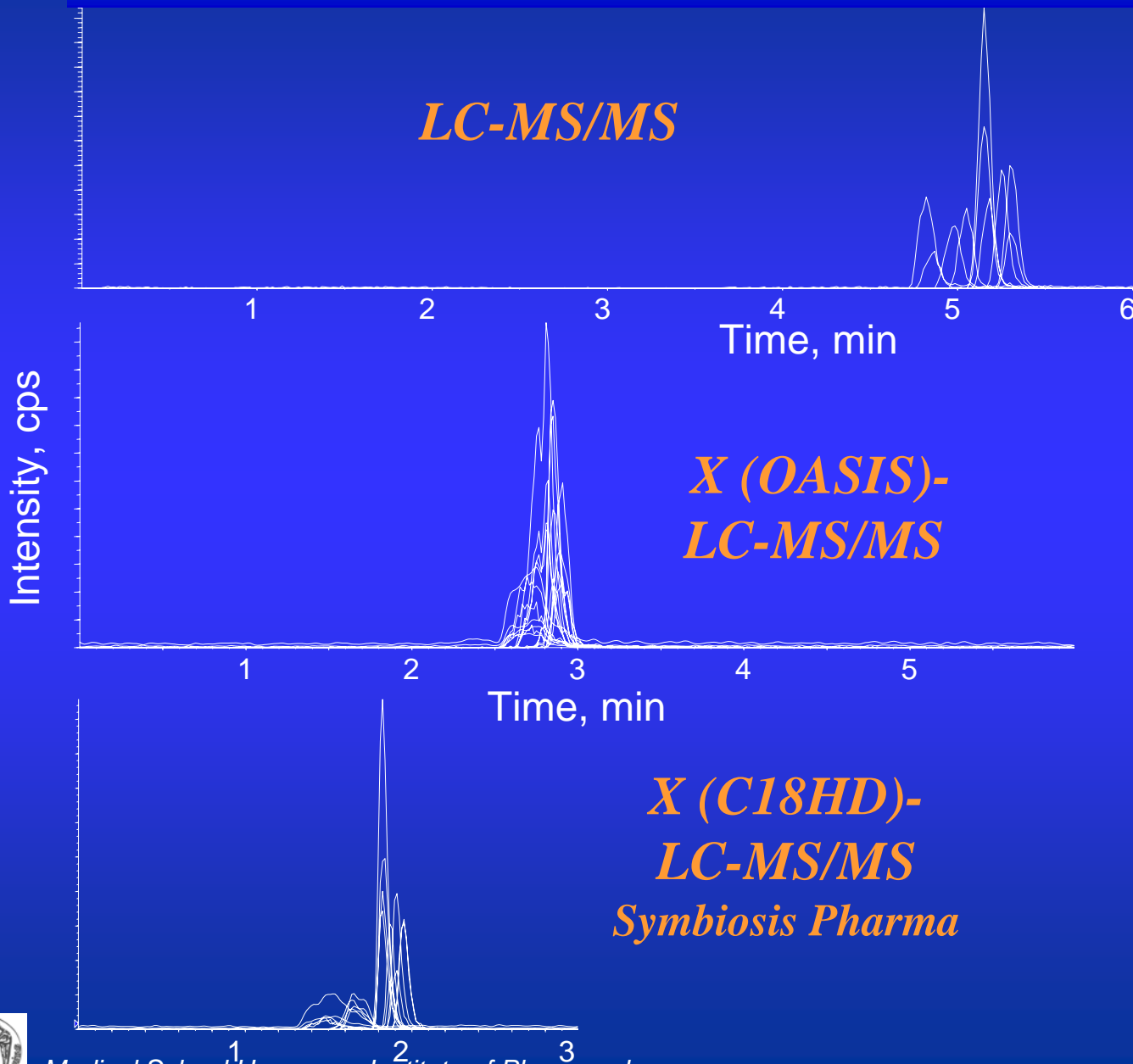
LC-ESI-MS/MS

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)



Total

8 min

6 min

3 min



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)*

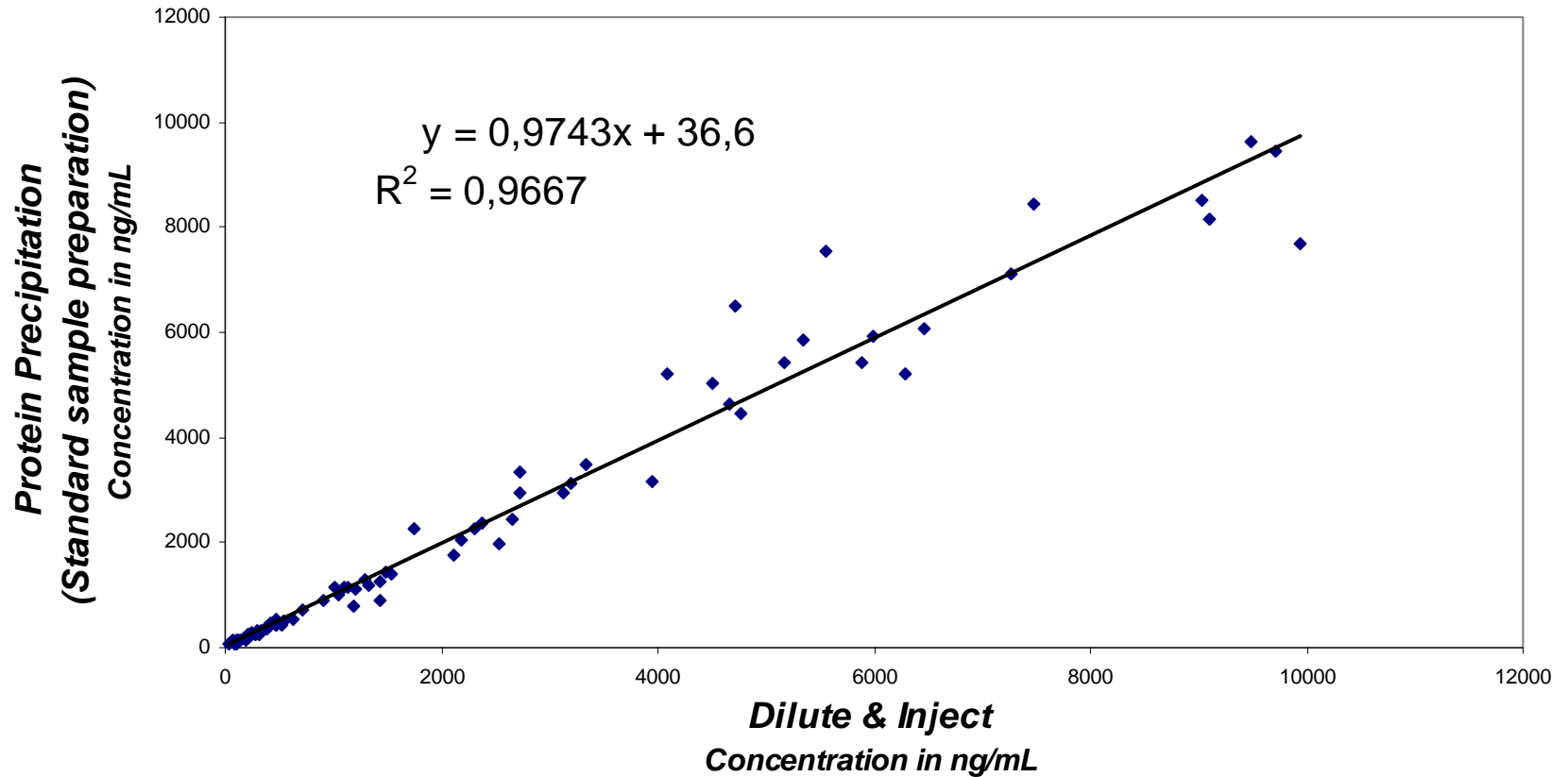


*Dried Blood Spots (whole blood)
(Koal et al., RCM, in press)*

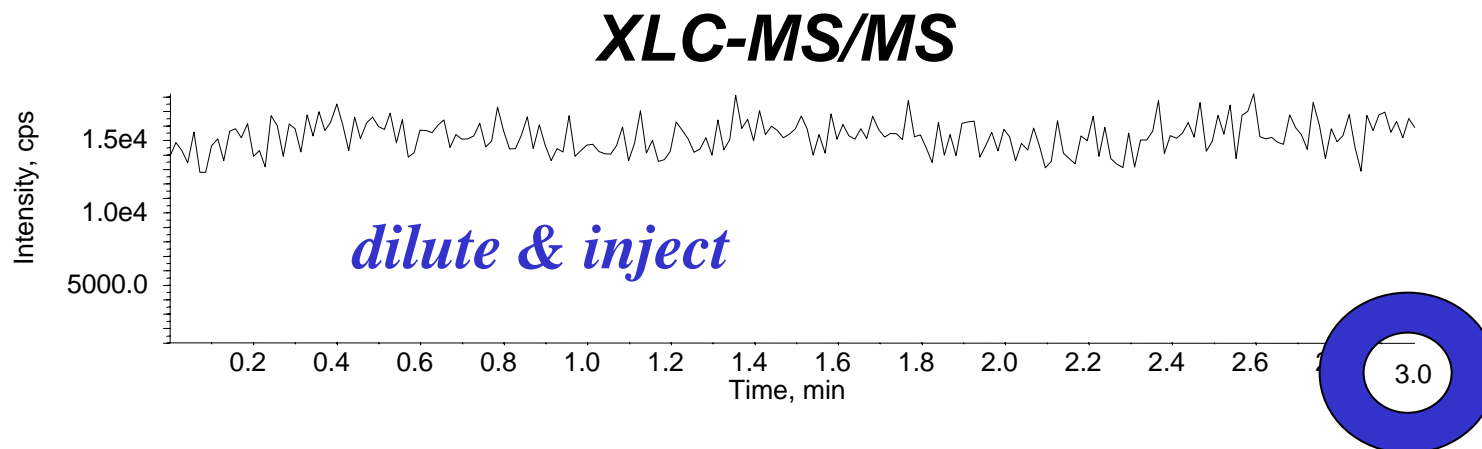
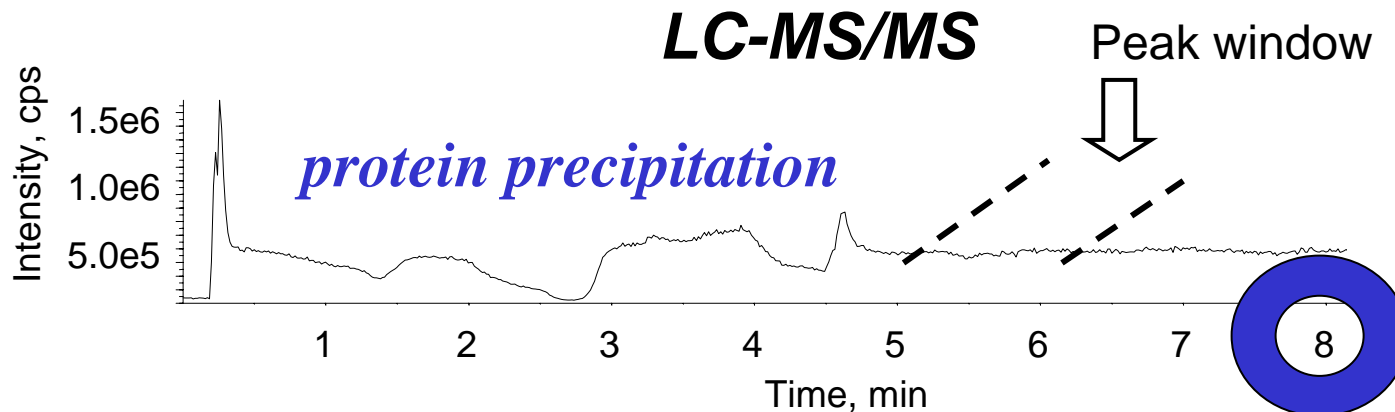


Antiretroviral Drugs (dilute & inject)

Method Comparison
n=50 Patients



Antiretroviral Drugs (matrix effects)



Short analysis time !

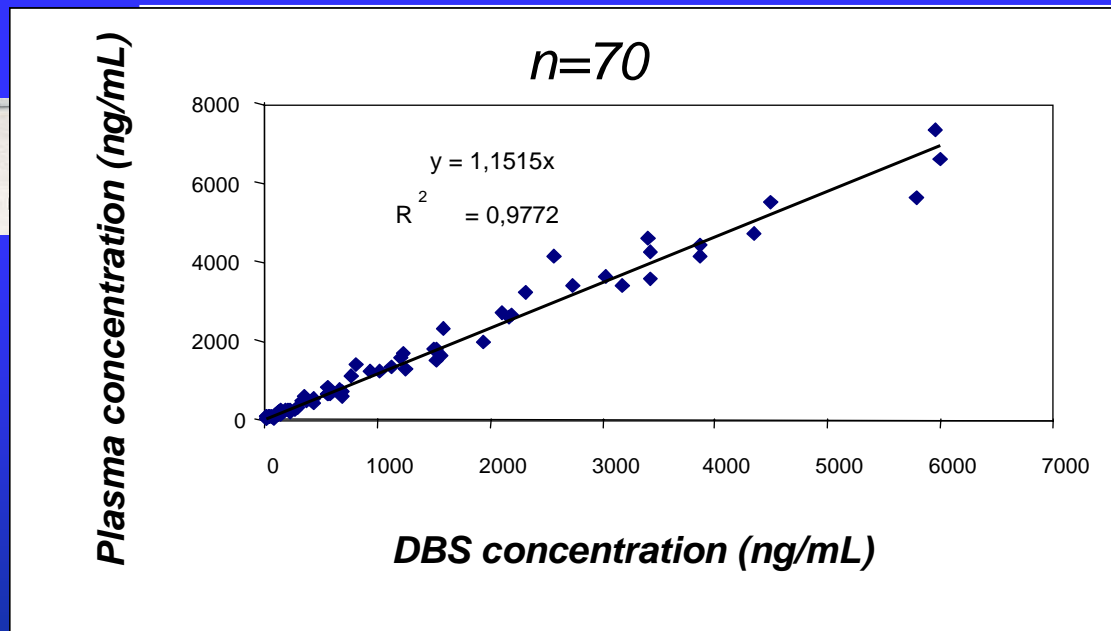
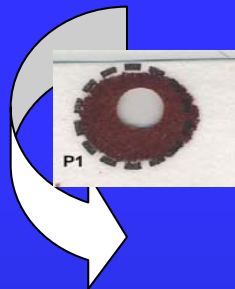
matrix elimination !

only sample preparation: dilution !



Antiretroviral Drugs (Dried Blood Spots)

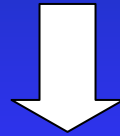
- ★ sample mailing & decrease of the infection risk
- ★ **5 μ l** EDTA-whole blood; 5mm Spot



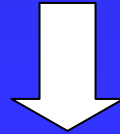
tribution !

Antiretroviral Drugs (carry-over effect)

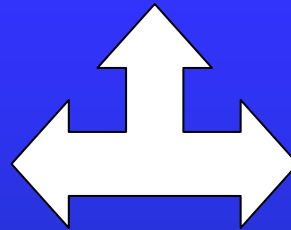
high target concentrations for PI and NNRTI



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 !



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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NIH Aids Reagent Reference Program

*Spark Holland
Applied Biosystems*

