An analytical method (UHPLC-MS/MS) to determine the pharmacodynamic behavior of the topically applied antiviral drug acyclovir

Reingard Raml[1]*, Denise Schimek,[1] Anton Mautner[1], Denise Kollmann[1], Maria Edeling[1], Tiffner Katrin[1], Bodenlenz Manfred[1], Christoph Magnes[1],Sinner Frank[2]

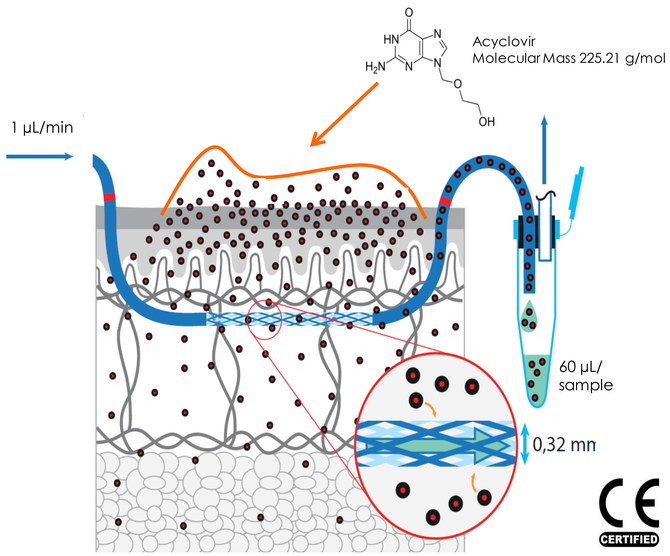
[1]Joanneum Research Forschungsgesellschaft mbH, HEALTH-Institute for Biomedicine and Health Sciences, Graz, Austria

Reingard Raml

[2] Division of Endocrinology and Metabolism, Dept. of Internal Medicine, Medical University of Graz, Graz, Austria

Introduction

Assessing the pharmacokinetics (PK) of topical drugs at their site-of-action in skin is a challenging task. Usually only small amounts of the applied drug cross the first effective skin barrier, the stratum corneum. To investigate PK in human dermis in vivo, we have developed a minimally invasive, continuous sampling method called open flow microperfusion (OFM) that provides continuous access to the dermal interstitium.



Analytical methods optimized for OFM have to be optimized for

- Small sample volumes (60 µL)
- High sensitivity and selectivity
- High sample throughput
- High regulatory demands (GLP, GCP)

Aim

The development of an analytical method that is able to investigate bioequivalence of different acyclovir formulations with OFM in a clinical study.

Results

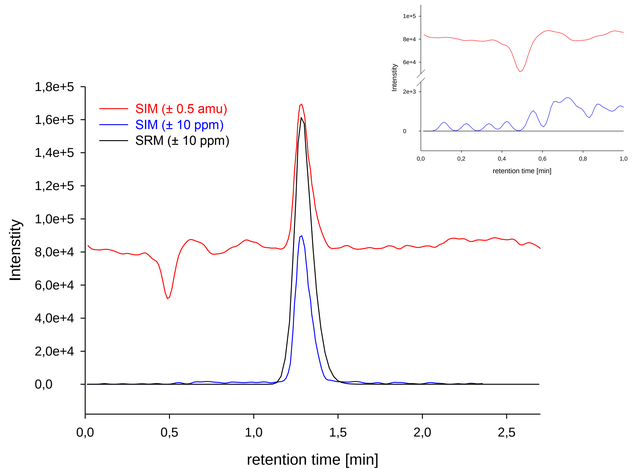
Small sample volumes

SPE (solid phase extraction) is a efficient sample preparation method. The use of µ-Elution plates (2 mg sorbent) in 96 well plate format allow convenient handling of small sample volumes (10 µl).

High selectivity and sensitivity

The Thermo Q Exactive combines a quadrupole mass filter with high resolution mass detection. Using SRM mode with high accuracy detection of the fragment ion we could significantly decrease background signal and enhance sensitivity compared to published methods .

Comparison of 10 ng/mL standard in
SIM (± 0.5 amu)
SIM (± 10 ppm)
SRM (± 10 ppm)



High sample throughput

Automatisised steps with a Hamilton MICROLAB® STARlet Liquid handling workstation :

- Preparation of calibration standards and QCs
- Transfer of standards and samples in 96 well plate
- Addition of internal standard

Enables preparation of 304 samples in 8 hours!

Validation Results:

Parameter	Value
LLOQ	0.1 ng/mL
ULOQ	100 ng/mL
Accuracy	99% (91 – 110% ;n = 60)
Precision Within-run	<6 % (n = 6)
Precision Intra-batch	<12% (n = 60)
Recovery	108 ± 2 %
Short term stability	24 hours @ room temperature
Long term stability	2 months @ -80 °C
Stock solution stability	6 months @ 4 °C
Freeze thaw cycles	4 cycles
Processed sample stability	3 days @ 2-8 °C

Experimental

Sample preparation:
Waters µElution SPE strong cationic exchange MCX SPE protocol

- Apply sample 20 µL
- Wash 1: 0.1 N HCl
- Wash 2: water
- Wash 3: methanol
- Elution: 2.5% NH_4OH in methanol (2x 25 µL)

Chromatographic conditions:
Acquity UPLC BEH C18 column (1.0 mm x 50 mm, 1.7 µm) & water + 0.1% formic acid 10% and methanol + 0.1% formic acid (isocratic conditions; 3.7 min). Flow rate was 400 µL/min; injection volume: 7 µL; 30°C
MS conditions:
Targeted-MS^2 polarity positive resolution 35.000, AGC target 3e5, Maximum inject time 500 ms, Isolation window 1. m/z, NCE 10.0

References

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Acknowledgement

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