Cross validation of Immunosuppressant quantification in whole blood by LDTD-MS/MS and LC-MS/MS using Triple Ion Source

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OVERVIEW

Purpose
• Develop a single quantification method for immunosuppressants using LDTD-MS/MS and cross-validate it using LC-MS/MS

Method
• Protein crash
• Solid phase extraction on OFX
• Fast LDTD-MS/MS analysis
• LC-MS/MS analysis cross validation

INTRODUCTION

Everolimus (EVE), sirolimus (SIR), tacrolimus (TAC) and cyclosporine-A (Cyc) are widely used to prevent the rejection of solid organ transplants in recipients. However, these immunosuppressants have small therapeutic windows and large intra- and inter-patient variability in their pharmacokinetics. Immunoassays and liquid chromatography (LC) mass spectrometry (MS) are commonly used to perform their quantification but these methods are not perfect: they either lack specificity, are costly, are tedious or cannot be multiplexed.

We propose to perform the quantification of immunosuppressants by Laser Diode Thermal Desorption Mass Spectrometry (LDTD-MS), an ultra-fast technique that does not rely on LC separation or immunoassay.

METHOD

Standard Curve and Sample Preparation
Mix:
- 180 µL blood (sample or clean matrix)
- 20 µL immunosuppressant standards in acetonitrile (only acetonitrile for samples and blanks)
- 200 µL H₂O
- 400 µL of IS solution (fresh):
  - 280 µL IS stock solution
  - 2920 µL Methanol
  - 800 µL ZnSO₄ (1N)
Vortex and centrifuge (14 000 RPM, 2 min)

SPE extraction
Cartridge: OFX (SPEware)
1. Pre-condition:
   - 1 mL Methanol
   - 1 mL H₂O
2. Load (simultaneously):
   - 750 µL H₂O
   - 400 µL sample upper-layer
3. Wash:
   - 1 mL H₂O
4. Dry
5. Elute:
   - 375 µL Methanol

LDTD Mix:
- 75 µL of elution
- 25 µL of desorption solution (in H₂O):
  - EDTA 40 µg/mL
  - HEPES 200 µg/mL
  - BSA 400 µg/mL
Spot 5 µL on LAzWell Evaporate until dry at room temperature

LC Mix:
- 100 µL of elution
- 100 µL of mobile phase A
Injection volume: 50 µL

RESULTS:

Linearity
The calibration curve for each immunosuppressant is linear over a wide range: 2.25 ng/mL to 50 ng/mL for tacrolimus, everolimus and sirolimus and 25 ng/mL to 1000 ng/mL for cyclosporine-A. Correlation coefficients are greater than 0.995 for the quantification curve of each molecule. The LLOQ is greater than the required blood concentration in the case of organ transplant which is the most common use of the drugs.

Intra-Day Reproducibility
Comparison of intra-day reproducibility using relative standard deviation (%) on technical replicates of QC samples (n=6)

CONCLUSIONS

• Accurate quantification of Everolimus, Sirolimus, Tacrolimus and Cyclosporine-A in whole blood is achieved at 8 seconds per sample using LDTD-MS/MS
• Passing-Bablok regression revealed no significant deviation from linearity between the 2 methods
• LDTD-MS/MS results are equivalent to LC-MS/MS in a faster analysis (8 seconds vs 5 minutes)
• Complete method cross-validation on a single instrument set-up