Design of a LC-MS/MS method for measuring concentrations of cyclosporin A and tacrolimus from dried blood spots

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Introduction:
Patients on immunosuppressive treatment after solid organ transplantation is a patient group subjected to lifelong therapeutic drug monitoring. For these patients, home sampling with dried blood spots will be beneficial.

Whole blood cyclosporin A and tacrolimus are high volume analyses in many clinical laboratories.

Recovery and matrix effects:
Recovery was 77-79% for cyclosporin A and 93-98% for tacrolimus. Quantitative matrix effect was close to 100 % for both analytes.

Effect of spot volumes and different hematocrit:
All hematocrit levels between 20 and 50 % showed to be within acceptable limits for spot volumes between 25-60 µL.

Material and method:

5 mm (~8 µL) spot punched, transferred to filter plate.
150 µL MeOH/H₂O, 80:20, with internal standards.
extraction, shaking during 10 min.

Material and Method parameters:

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<tr>
<th>Internal standards:</th>
<th>cyclosporin-D12, ascomycin</th>
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| Mobile phase:       | A: 2mM ammonium formate w 0.1% FA in MQ water
|                     | B: 2mM ammonium formate w 0.1% FA in methanol |
| Injection volume:   | 20 µL |
| Column:             | HyperSil Gold C18, 20 x 2.1 mm, 1.9 µm
| (precolumn C8 10 x 2.1 mm, 1.9 µm) |
| Column oven temp:   | 60°C |
| Gradient program %B| 0.0-60%, 0.1-60%, 0.3-85%, 0.90-95%, 1.66-60% 2.3 minutes |
| Total runtime:      | 60 µL |
| System:             | Accela pump, Thermo PAL autosampler, Thermo Scientific TSQ Quantum Ultra triple quadrupole mass spectrometer, with electrospray ionisation in positive mode, selected reaction monitoring. |

Analyte | Precursor ion (m/z) | Product ion (m/z) |
---------|---------------------|-------------------|
Tacrolimus | 821.7 | 786.5 |
Ascomycin | 809.5 | 756.4 |
Cyclosporin A | 1220.2 | 1185.3 |
Cyclosporin-D12 | 1232.0 | 1215.0 |

Transitions chosen for the method, precursor ions are ammonium adducts.

Future perspectives:
The method will be used in a clinical study with patients from the department of Transplantation Surgery at the Karolinska University Hospital, aiming at offering dried blood spots as an alternative to venous whole blood sampling.