High Throughput LDTD-MS/MS Quantification of 4 immunosuppressive drugs in whole blood

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Introduction

Over the last 10 years the quantitation of immunosuppressive drugs has been subject to continuous improvements in analytical methods to optimize cost, time, and accuracy of results. The transition from immunoassays to liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) has significantly improved all these criteria but has not reduced the analytical time below a minute. The Laser Diode Thermal Desorption (LDTD) represents a technological breakthrough that removes the chromatographic step and significantly increases the analytical throughput for the quantitation of Tacrolimus, Sirolimus, Everolimus and Cyclosporin A in the field of clinical analysis.

LDTD-MS/MS System

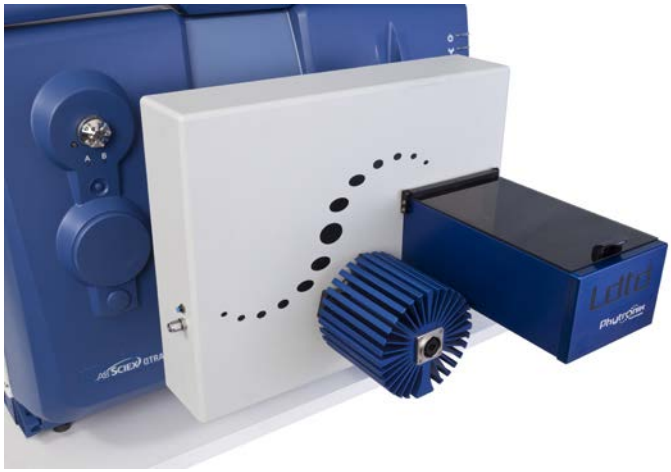

Figure 1: LDTD system on AB SCIEX 5500 Qtrap Mass Spectrometer

Sample Method

Extraction Procedure

- 62.5 µL of $ZnSO_4$ crashed solution (contains IS)
- 25 µL whole blood
- Vortex and centrifuge
- Add 50 µL water and 125 µL MTBE
- Vortex and leave 1 min for phase separation
- Mix 90 µL of organic phase with EDTA solution
- Spot 2 µL on 96 LazWell™ Plate

LDTD-MS/MS Parameters

Laser power pattern:

- Increase power from 0% to 55% in 6 sec
- Hold at 55% for 1 sec then back to 0%

Carrier gas flow: 3.0 L/min Air with trace NH_3

MS/MS transitions – Positive APCI:

Analyte	Q1	Q3	Collision Energy
Sirolimus	931.4	864.3	25
Sirolimus-d3	934.4	864.3	25
Everolimus	975.4	908.4	25
Everolimus-d4	979.4	912.4	25
Tacrolimus	821.4	718.3	25
Ascomycin	809.5	756.3	25
Cyclosporin-A	1219.8	1184.6	20
Cyclosporin-A-d4	1223.8	1188.6	20

Table 1: MS/MS transitions monitored

Results and Discussion

The analysis time was achieved in only 9 seconds from sample to sample as shown in **Figure 2**.

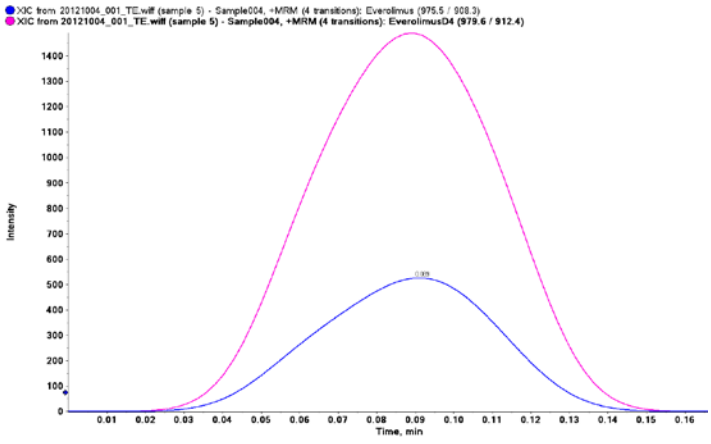

Figure 2 : Everolimus (blue) and Everolimus-d4 (pink) Desorption profile from an extracted sample. Other compound traces are hidden but simultaneously monitored.

Linearity Results

The extraction procedure yielded high recovery (88-92%; RSD=8.9%, n=6). Lower limits of quantification (LLOQ) are fixed at the first level of calibration from the Chromsystem kit, ranging from 2-50 ng/mL for Sirolimus, Everolimus, Tacrolimus and from 25-1870 ng/mL for Cyclosporine A. **Figure 3** shows typical calibration curves obtained with triplicate measure at every point to assess reproducibility.

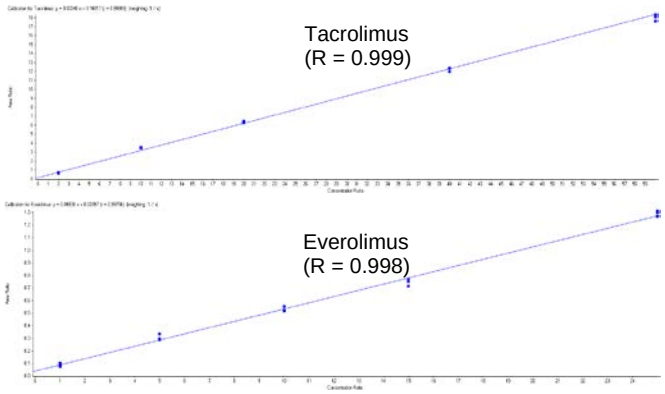

Figure 3 : Linear calibration curves for Tacrolimus and Everolimus

Operating without chromatographic separation in APCI and no liquid mobile phase, possible interferences need to be evaluated. After further studies, no interferences were observed, either endogenous or between the compounds themselves.

Method comparisons

A set of samples containing the 4 immunosuppressant drugs show the correlation between the LC-MS/MS and LDTD-MS/MS. The concordances of results show correlation coefficients superior than 0.97 as shown on figure 4.

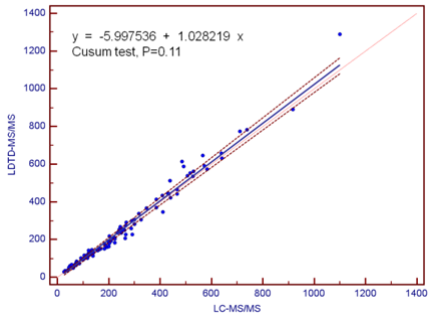

Figure 4 : Correlation between LC-MS/MS and LDTD-MS/MS results for all 4 immunosuppressant drugs in a set of clinical samples

Another set of 120 whole blood samples containing Cyclosporine A were analyzed in a second study for method comparison. Both methods agree, with concordance correlation coefficient of 0.99 (95% confidence interval 0.982 – 0.991) and Person p ≥ 0.99. The passing-Bablok regression revealed no significant deviation from linearity (Cusum test, P=0.11), Figure 5. Bland and Altman plot showed that the mean bias of the two methods was +0.9 (1.96 SD, -19.7 to 21.6) ng/mL.

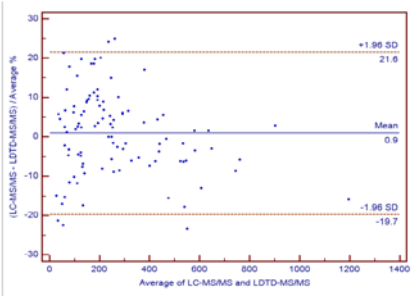

Figure 5 : Bland and Altman plot

Conclusions

LDTD-MS/MS system performs with an incredible sample-to-sample analysis time of **9 seconds** per sample for the simultaneous quantification of 4 immunosuppressant drugs in whole blood. The analytical speed provided by the LDTD increases the throughput without compromising the accuracy of the results.