Ultra fast cyclosporin A quantitation in whole blood by Laser Diode Thermal Desorption – Tandem Mass Spectrometry

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OVERVIEW

• Liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) has been the gold-standard analytical method for the therapeutic drug monitoring of immunosuppressive drugs for the last decade: higher selectivity, higher sensitivity, lower costs.

• The Laser Diode Thermal Desorption (LDTD) represents a new technological breakthrough that removes the chromatographic separation step.
LDTD significantly increases the analytical throughput for the quantitation of cyclosporin A for clinical diagnostics.

OBJECTIVE

To validate a new method for ultra fast quantitation of cyclosporin A in whole blood by LDTD-MS/MS and to compare it with the reference LC-MS/MS method.

INTRODUCTION

Since the last 10 years the quantitation of immunosuppressive drugs has seen vast improvements in analytical methods to optimize cost, time and accuracy of analysis. The transfer of immunoassays to LC-MS/MS has significantly improved these three performance criteria but has yet to achieve an analytical runtime below a minute. The Laser Diode Thermal Desorption (LDTD) represents a technological breakthrough that removes the chromatographic separation step and thereby significantly increases the analytical throughput for the quantitation of cyclosporin A in the field of clinical diagnostics.

LDTD (Figure 1)
– Plug-and-play ionization source interfacable to most popular mass spectrometer.
– Thermal desorption induced by a laser diode.
– The sample is carried by air to a corona discharge region for APCI.
– Loader accomodates up to 10 96-well plates (LazWell™).

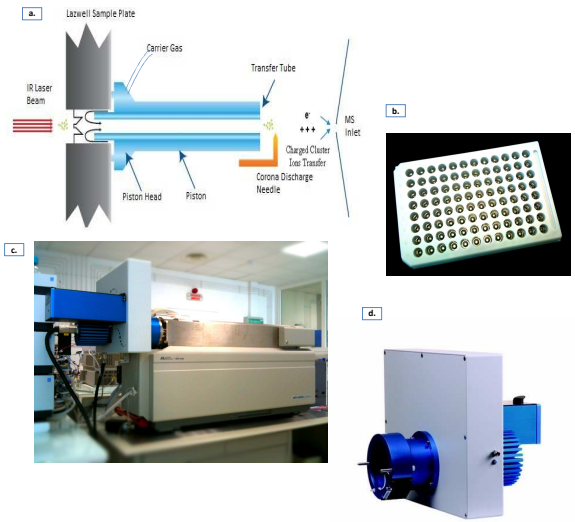


Figure1. a. Schematic of the LDTD ionization source. **b.** LazWell™ sample plate. **c.** LDTD-MS/MS analytical system. **d.** LDTD S-960 model compatible with AB Sciex interfaces.

METHODS

• Sample preparation:
100 µL of EDTA–treated Whole blood samples were treated with 200 µL precipitation reagent (methanol/0.2M $ZnSO_4$ (50/20, *v/v*) including 200 ng/mL Cyclosporine D used as Internal Standard (IS). After 30 seconds vortex and 15 minutes centrifugation (20800xg at room temperature), 150 µL of supernatant were evaporated to dryness prior to reconstitution step with 50 µL of water and 10 µL of ethyl acetate.

• Lazwell plates preparation and analytical conditions:
5 µL of the extract was spotted on a LazWell™ 96-well plate and laid 5 minutes at room temperature for complete evaporation

• LDTD parameters:
The lazer pattern was operated with a 20 W diode and the heating rate was set as follows (figure 2.).
The run time was 7.2 s and *lasted only* 9 s from sample to sample.

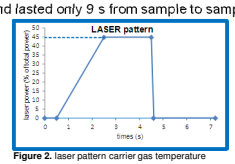
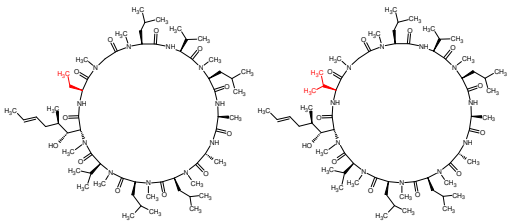


Figure 2. laser pattern carrier gas temperature (30°C) and air flow rate (3 L/min).

• MS/MS parameters:
- Tandem mass spectrometer: API 4000 (ABSciex),
- Negative APCI ionization mode (-4200 V) and negative needle voltage (-2µA)
- Compound-dependent parameters (table 1.)

Analyte	Q1 m/z	Q3 m/z	Dwell time (ms)	DP (V)	EP (V)	CE (V)°	CXP (V)
Cyclosporin A	1201.1	1088.9	25	-140	-10	-40	-9
Cyclosporin D	1214.8	1102.8	25	-140	-10	-40	-9

Table 1. MS/MS Compound -dependent parameters



Cyclosporin A: $C_{62}H_{111}N_{11}O_{12}$; $C_{62}H_{111}N_{11}O_{12}$: exact mw=1201.84
Cyclosporin D (SI); $C_{63}H_{113}N_{11}O_{12}$; exact mw=1215.86

Figure 3. Chemical structures, formulas and molecular weights of the target compounds

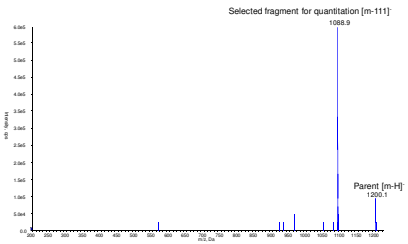


Figure 4. Cyclosporin A fragment spectrum [m-H]⁻

RESULTS

• Intra-day and inter-day accuracy and precision:
Accuracy data lay within the acceptance interval of 15% of the nominal value and the precision values expressed as coefficient of variation (CV) were smaller than 15% for the four levels of quality controls (table 2.) .

Nominal concentration (ng/mL)	Intra-day precision and accuracy (n=6)			Inter-day precision and accuracy (n=6)		
	Mean Concentration (ng/mL)	CV(%)	Biais(%)	Mean Concentration (ng/mL)	CV(%)	Biais(%)
52.8	50.4	4.3	95.5	50.2	5.3	95.0
260	243.0	6.0	93.5	241.8	6.4	93.0
476	452.2	3.4	95.0	448.0	8.1	94.1
1050	1004.5	13.1	95.7	1132.0	5.3	107.8

Table 2. Intra-day and inter-day accuracies and precisions . Precisions, expressed as repeatability (CV%) and accuracies, expressed as bias%.

• Extraction recovery:
Extraction recovery was calculated by spiking samples before and after the protein precipitation step.
These values indicated a limited and reproducible loss of compounds during the sample pre-treatment (table 3.).

	Nominal concentration	Extraction recovery (%) (n=6)	CV (%) (n=6)
Cyclosporin A	200	92.0	9.4

Table 3. extraction recovery., expressed as calculated as the ratio of absolute peak area responses

• Linearity, selectivity and desorption profile:
The calibration curves were evaluated over a nominal range of 23.5 ng/mL to 896 ng/mL with a linear regression model weighted 1/x (Figure 5. a.). The acceptable precision and accuracy obtained for the highest quality control point allowed to fix the upper limit of linearity up to 1050 ng/mL.
Blank analysis (Figure 5. b.) revealed a signal (peak surface) 10 times lower than the first point of the calibration curve fixed as the lower limit of quantitation (Figure 5. c.) i.e. interference percentage = 12.1%: selectivity of the method was in accordance with the expected level of concentration of cyclosporin A measured in patient samples (figure. 5. d.).

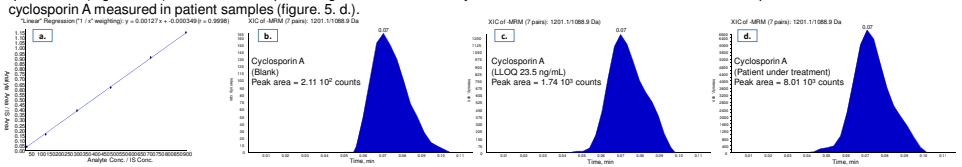


Figure 5. a. Calibration curve. **b.** desorption profile of a blank sample. **c.** desorption profile of the lower limit of quantitation. **d.** desorption profile for a patient under cyclosporin A treatment (283 ng/mL)

• Method comparison:
120 whole blood samples were analyzed for method comparison between LDTD-MS/MS and the reference LC-MS/MS method. Both methods agreed, with concordance correlation coefficient of 0.99 (95% confidence interval 0.982 – 0.991) and Person p ≥0.99. The passing-Bablok regression (figure 6. a.) revealed no significant deviation from linearity (Cusum test, P=0.11). Bland and Altman plot (figure 6. b.) showed that the mean bias of the two methods was +0.9 (1.96 SD, -19.7 to 21.6) ng/mL.

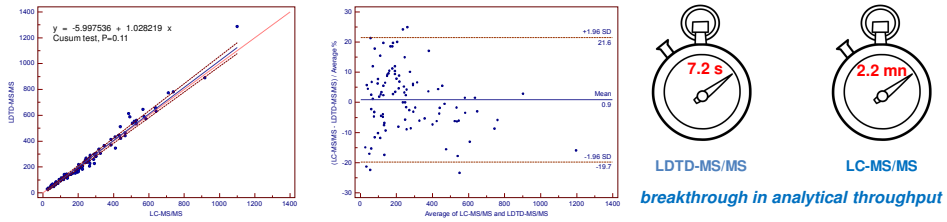


Figure 6. a. Passing-Bablok regression. **b.** Bland and Altman plot.

CONCLUSIONS

LDTD-MS/MS :
– allows an ultra fast cyclosporin A quantification in whole blood (8 times faster than the most rapid LC-MS/MS method described),
– provides good signal linearity over 23.5 to 1050 ng/mL with precision and accuracy based on bias and CV analysis,
– and is suitable for supplying the reference LC-MSMS method.

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