TDM of Immunosuppressant in Whole Blood in 8 Seconds Using LDTD-MS/MS

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OVERVIEW

Purpose

- Develop a rapid assessment method of immunosuppressants for therapeutic drug monitoring.

Method

- Red blood cell crash and liquid-liquid extraction are used to extract immunosuppressants from the blood sample.
- Samples analyzed by LDTD-MS/MS (Axino Ion Source).

Quantification

- Accuracy results were lower than 17,8% from the nominal value.
- Samples analyzed with a runtime of 10 seconds using LDTD-MS/MS technique.

INTRODUCTION

Everolimus, sirolimus, tacrolimus, and cyclosporine-A are frequently used to prevent solid organ transplant rejection in recipients. However, these immunosuppressants have narrow therapeutic concentration windows and display considerable variability in their pharmacokinetics both within and between patients. As a result, prompt feedback of blood sample results is essential. We suggest employing the Axino Ion Source, utilizing LDTD (Laser Diode Thermal Desorption) process, in combination with a mass spectrometer, to enable ultra-fast quantification. This approach would facilitate accurate measurement through a reference calibration curve.

Axino Ionization Source:

The Axino Ion Source (**Figure 1**) is the new generation of sample introduction and ionization source based on the LDTD technology for mass spectrometry. The Axino Ion Source uses a Laser Diode to obtain unmatchable thermal uniformity giving more precision, accuracy and speed.

LDTD coupled to the Uplyft flow process begins by shaping a cone out of the well of a Domino LazWell plate. The dry samples are then rapidly evaporated using indirect heat. The cone shape improves the flow of the neutral molecules that were thermally desorbed from the surface into the corona discharge region. High-efficiency protonation and strong resistance to ionic suppression characterize this type of ionization and is the result of the absence of solvent and mobile phase. This thermal desorption process yields high-intensity molecular ion signal in a few seconds sample-to-sample and allows for very small volumes to be used.

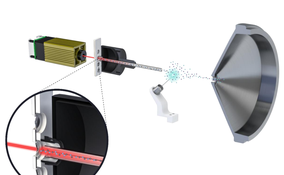

Figure 1 *Axino Ion Source Process*

METHOD

Sample Preparation

- A 100 µL sample of whole blood (with EDTA-K2) is combined with 100 µL of the internal standard solution.
- To precipitate the red blood cells, 40 µL of ZnSO4 (1N) and 145 µL of methanol are added.
- Mix for 10 seconds.
- Centrifuge at 14,000 rpm for 2 minutes.
- Transfer 200 µL of the upper layer in borosilicate tube.
- Add 67 µL of water.
- Add 167 µL of MTBE.
- Mix for 30 seconds.
- Collect the organic layer and combine with the desorption solution.
- Spot 5 µL onto a Domino plate and allow to dry.

Figure 2 *Domino Plate*

Immunosuppressant molecules size implies a need for higher energy to achieve vaporization. The desorption buffer is optimized to reduce to a minimum the binding energy of those molecules.

Instrumentation

- Ion source: Phytronix Axino Ion Source
- Mass spectrometer: Sciex, Q-Trap System 5500

Figure 3 *Axino Ion Source*

Axino Parameters

- Laser power pattern:
- Increase laser power to 85% in 6s.
- Hold 2 s. at 65%.
- Carrier gas flow: 6 L/min (Air)
- Infusion in carrier gas: Ammonia 5,6 % (1 uL/min)

MS Parameters

- APCI (+)
- IonSray Voltage: 6000
- DP: 80,0
- EP: 10,0
- CXP: 13,0
- Curtain (CUR): 10
- CAD: 7
- Time: 10 msec

Table 1 *MRM transitions parameters*

Drugs	Transition	CE
Tacrolimus	821.4 → 768.5	25
Ascomycin (ISTD for Tacrolimus)	809.4 → 756.5	25
Sirolimus	931.5 → 864.6	25
Sirolimus-d_3	934.5 → 864.6	25
Everolimus	975.6 → 908.6	25
Everolimus-d_4	979.6 → 912.6	25
Cyclosporin A	1219.9 → 1184.9	20
Cyclosporin D (ISTD for Cyclosporin A)	1233.9 → 1198.9	20

RESULTS

Validation

Since the test was developed on the Axino Ion Source for rapid screening and results, one 3-point calibration line and one quality control sample are analyzed per Domino plate (4 wells only). The following acceptance criteria are used:

- Linearity with R ≥ 0.995
- Each QC concentration must be ±20% of the nominal value (%Bias).
- Inter-run results on the QCs must be lower that 20%CV.

Linearity

Three-point calibration curves (ranging between 2.25 ng/mL and 50 ng/mL for Everolimus, Sirolimus, and Tacrolimus and between 25 and 1000 ng/mL for Cyclosporin A) are used to validate the method.

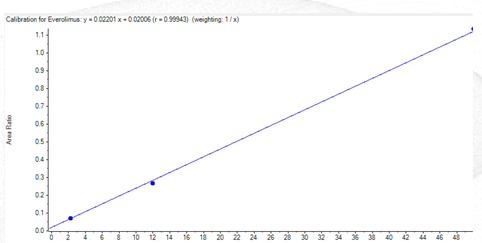

Figure 4 *Calibration curve for Everolimus*

Table 2 *Linearity results (correlation coefficient (R))*

	Cyclosporin A	Everolimus	Sirolimus	Tacrolimus
Run 1	0.99668	0.99943	0.99917	1.00000
Run 2	0.99712	0.99969	0.99999	0.99993
Run 3	0.99976	1.00000	0.99943	0.99758
Run 4	0.99804	0.99766	0.99907	0.99906
Run 5	0.99511	0.99972	0.99915	0.99908
Run 6	0.99835	0.99990	0.99765	0.99998

Precision and Accuracy

The precision of the method was assessed on the inter-run data of the analyzed quality control samples. 6 samples (therefore 6 runs) allowed us to obtain a %CV lower than 16%CV for the four analyzed immunosuppressants.

To ensure method accuracy, this parameter can be determined for both intra-run and inter-run analysis. For intra-run analysis, all QCs are within 18% of the nominal value, while for inter-run analysis, QCs are within 5% of the nominal value.

Table 3 *Inter-run precision and accuracy results*

	Cyclosporin A	Everolimus	Sirolimus	Tacrolimus
Conc (ng/g)	500	20	20	20
N	6	6	6	6
Mean (ng/g)	508.1	20.7	21.0	20.4
%CV	15.1	10.3	6.9	9.6
%Bias	1.6	3.4	4.9	2.0

Dry Stability

The dry stability (extracted sample spotted on a Lazwell plate, and dried) has been assessed. Quality control samples (at 125 ng/mL, and 800 ng/mL for Cyclosporin A and at 6 ng/mL, and 25 ng/mL for Tacrolimus, Everolimus, and Sirolimus) were used to evaluate the dry stability. After one hour at room temperature, the %CV remains below 15%, and the accuracy falls between 92.4% and 105.2% for all analytes.

CONCLUSION

- **LLE extraction followed by sample deposition on a Domino LazWell plate can be successfully used for immunosuppressant analysis.**
- **High-throughput analysis with AXINO-MS/MS.**
- **Accuracy meets acceptance criteria.**
- **Sample-to-sample analysis in 10 seconds.**

COI Disclosure: I have a financial relationship with Phytronix Technologies as a salaried employee.