Quantification of Tenofovir and Emtricitabine in human plasma using high throughput LDTD-MS/MS

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OVERVIEW

Purpose
- High-throughput quantification of Tenofovir and Emtricitabine in human plasma using LDTD-MS/MS

Method
- SCX SPE cartridges are used in the sample preparation
- Quick derivation using TMPAH
- LazWell plate spotting, evaporate to dryness and LDTD-MS/MS analysis

Quantification
- Linearity: $r^2 > 0.995$ over the calibration range (25 to 5000 ng/mL)
- Inter-run Accuracy ranging from 87.3 to 112.1%
- Inter-run Precision ranging from 3.2 to 14.5%
- **Samples analyzed with a run time of 9 seconds using LDTD-MS/MS system**

INTRODUCTION

Tenofovir (TFV) and emtricitabine (FTC) are prescribed antiretroviral drugs used for the treatment of HIV infection. Both agents are also active against the Hepatitis B virus (HBV). Different LC-MS/MS methods are reported in the literature for the quantification of the individual drugs or together.

Accuracy, turnaround time, and analytical costs are important factors to consider when developing a therapeutic drug monitoring assay. Physicians need a faster turnaround time to help support patient care. Mass spectrometry combined with a high-throughput solution such as the LDTD ion source enhances this turnaround time.

We developed an extraction method using LDTD-MS/MS for a fast quantification of Tenofovir and Emtricitabine in plasma in 9 seconds sample to sample.

LDTD® Ionization Source:

The LDTD uses a Laser Diode to produce and control heat on the sample support **(Figure 1)** which is a 96 wells plate**.** The energy is then transferred through the sample holder to the dry sample which vaporizes prior to being carried by a gas in a 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 allows for very high throughput capabilities of 9 seconds sample-to-sample analysis time, without carry over.

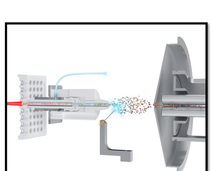

Figure 1 Schematic of the LDTD ionization source

METHOD

Sample preparation:
- 150 µL sample
- 25 µL Internal standard (Acyclovir 5 µg/mL in MeOH)
- 250 µL Formic acid (10% in water)
- Mix

SPE extraction (SiliaPrepX – SCX: 1cc/30mg):
- Activation:
  - 1 mL MeOH
  - 1 mL Formic acid (1% in water)
- Load:
  - All sample preparation mixture
- Wash:
  - 1 mL Formic acid (1% in water)
  - 1 mL MeOH
- Elution:
  - 0.6 mL NH_4OH(10%):MeOH (15:85)
  - Evaporate to dryness
- Derivation:
  - 5 µL TMPAH (0.5M in MeOH) and 60 µL of Methanol
  - React 10 minutes at room temperature
  - Evaporate to dryness
  - Add 400 µL of MeOH:Water (75:25)
- Spot 4 µL elution in a LazWell* plate and evaporate to dryness

*EDTA coated plate

Figure 2 LDTD system on Sciex 5500 QTrap®

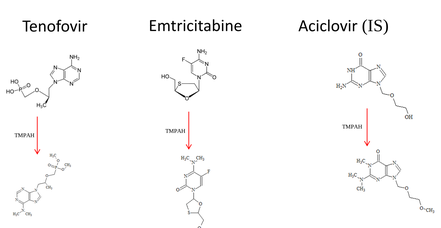

Figure 3 Tenofovir, Emtricitabine and Aciclovir devative reaction

Instrumentation
- LDTD model: S-960
- MS: Sciex 5500 QTrap®

LDTD Parameters
- Laser power pattern :
  - ➢ Increase laser power to 55 % in 6.0 sec
  - ➢ Maintain power 2 sec
  - ➢ Decrease laser power to 0 %
- Carrier gas flow : 3 L/min (Air)

MS Parameters
- APCI (+)
- DP: 100 V
- Dwell: 50 msec
- MRM mode **(Table 1)**

Table 1 MRM transitions

Compound	Q1	Q3	CE
Tenofovir	344.0	204.0	30
Emtricitabine	290.2	158.1	25
Aciclovir (IS)	282.0	89.0	20

RESULT

Linearity results:

A standard calibration curve ranging from 25 to 5000 ng/mL has been prepared in blank plasma matrices and analyzed. All curves have 0.995 coefficients or better. **Figure 4** presents typical calibration curve for Emtricitabine.

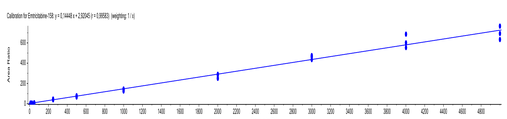

Figure 4 Emtricitabine calibration curve using LDTD-MS/MS system

Inter-run

High, medium and low concentration QCs were added in the analysis. The inter-run accuracy and precision across the calibration curves were between 97.0 to 112.1% and 6.7 to 14.5% for both drugs. **Table 4 and 5** show the Tenofovir and Emtricitabine inter-run results, respectively.

Table 4 Tenofovir inter-run results

	QC-Low	QC-Med	QC-High
Conc. (ng/ml)	250	1000	3000
N	14	14	14
Mean (ng/ml)	242.4	1001.8	3253.4
%RSD	14.2	13.9	14.5
%Nom	97.0	100.2	108.4

Table 5 Emtricitabine inter-run results

	QC-Low	QC-Med	QC-High
Conc. (ng/ml)	250	1000	3000
N	14	14	14
Mean (ng/ml)	276.4	1086.5	3362.3
%RSD	6.7	8.8	8.8
%Nom	110.6	108.7	112.1

Intra-run

ULOQ, LLOQ, High, medium and low concentration QCs were added in the analysis. The inter-run accuracy and precision across the calibration curves were between 87.3 to 112.1% and 3.2 to 14.5% for both drugs. **Table 2 and 3** show the Tenofovir and Emtricitabine intra-run results, respectively.

Table 2 Tenofovir intra-run results

	LLOQ	QC-Low	QC-Med	QC-High	ULOQ
Conc. (ng/ml)	25	250	1000	3000	5000
N	6	6	6	6	6
Mean (ng/ml)	26.5	222.1	876.4	2649.6	5138.3
%RSD	3.5	14.4	11.7	8.9	14.5
%Nom	106.0	88.9	87.6	88.3	102.8

Table 2 Emtricitabine intra-run results

	LLOQ	QC-Low	QC-Med	QC-High	ULOQ
Conc. (ng/ml)	25	250	1000	3000	5000
N	6	6	6	6	6
Mean (ng/ml)	21.8	277.2	1042.0	3363.4	5431.1
%RSD	13.1	6.0	7.8	3.2	6.9
%Nom	87.3	110.9	104.2	112.1	108.6

Wet stability

Following the extraction procedure, all samples were stored at 4°C to evaluate the drugs temporal stability in a wet state. After a waiting period, all samples were re-spotted and analyzed. A wet stability greater than 12 hours was obtained with accuracy between 87.3 and 106.0% and precision between 3.5 and 13.1% for concentrations equivalent to the LLOQ.

Carry over

Carry over was evaluated by analysis of three blank samples after high level calibrator. Peak area detected in blank sample was divided by the mean peak area value of LLOQ sample. Percentages of blank at LLOQ level were reported in **Table 6**.

Table 6 Carry over results

	% Blk interference	
	Tenof.	Emtric.
BLK 1	13.0	5.6
BLK 2	13.7	3.0
BLK 3	9.6	5.8

CONCLUSION

- **Simultaneous quantification of Tenofovir and Emtricitabine drugs in human plasma can be performed in 9 seconds using LDTD-MS/MS.**
- **Good precision and accuracy are obtained.**
- **Good wet stability and No carry over.**
- **Good linearity with coefficients higher than 0.995 for all curves was obtained.**