LC-MS/MS Method for the Determination of Tenofovir from Plasma

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Key Words
SPE, SOLA CX, Hypersil GOLD, tenofovir

Abstract
A liquid chromatography-tandem mass spectrometry method for tenofovir from plasma has been developed. Using Thermo Scientific™ SOLA™ CX plates, sample preparation is fast and efficient, giving excellent recovery levels for the compound. Separation was carried out on a Thermo Scientific™ Hypersil GOLD™ UHPLC column with a cycle time of 3 minutes. Good chromatographic peak shape and linearity over the dynamic range 1 to 1000 ng/mL was achieved with excellent recovery and reproducibility.

Introduction
Tenofovir (Figure 1) belongs to a class of antiretroviral drugs known as nucleotide analogue reverse transcriptase inhibitors (NRTIs), which block reverse transcriptase, an enzyme crucial to viral production. Tenofovir is marketed under the name Viread®.

SOLA products are revolutionary solid phase extraction (SPE) devices. This first-in-class SPE product range introduces next generation, innovative technological advancements, giving unparalleled performance characteristics compared to conventional SPE, phospholipid, and protein precipitation products.

This includes:

- Higher levels of reproducibility
- Higher levels of extract cleanliness
- Reduced solvent requirements
- Increased sensitivity

SOLA SPE plates or cartridges have significant advantages when analyzing compounds in complex matrices, particularly in high-throughput bioanalytical and clinical research laboratories where reduced failure rates, higher analysis speed, and lower solvent requirements are critical. SOLA products' superior performance gives higher confidence in analytical results and lowers cost without compromising ease-of-use or requiring complex method development.

Hypersil GOLD columns offer excellent peak shape. Based on highly pure silica, Hypersil GOLD columns provide very symmetrical peaks, even when analyzing compounds that give notoriously poor peak shape on traditional silica-based chemistries. Hypersil GOLD media provides a stationary phase with C18 selectivity and a predictable elution order but can provide new capabilities such as improved peak shape, increased peak capacity, and greater sensitivity, especially for trace compound analysis. Obtaining symmetrical peak shapes is critical to ensuring that optimum resolution and sensitivity are

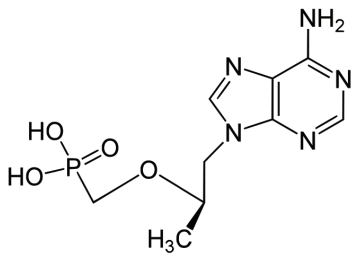

Figure 1: Tenofovir

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achieved for basic pharmaceutical compounds. Hypersil GOLD columns use proprietary bonding technology to make certain that symmetrical peaks are obtained, producing data with the highest confidence in the accuracy and quality of results.

Experimental Details

Consumables	Part Number
Fisher Scientific™ LC-MS grade water	W/011217
Fisher Scientific LC-MS grade methanol	M/4062/17
Fisher Scientific LC-MS grade acetonitrile	A/0626/17
Fisher Scientific Analytical grade acetic acid	A/0360/25
Fisher Scientific™ Optima™ formic acid	A117-50
Fisher Scientific Analytical grade ammonia solution	A/3295/PB05
Fisher Scientific Reagent grade ammonium acetate	A/3440/50
Mass Spec Certified Target DP 2 mL clear screw thread vial, ID patch, blue cap with bonded PTFE/silicone septum	MSCERT4000-34W

Sample Handling Equipment	Part Number
Thermo Scientifc 96 well plate vacuum manifold	60103-351
Thermo Scientifc™ UltraVap™	CLS-229070

Sample Pretreatment

A standard spiking solution (tenofovir) was prepared in methanol / water (50:50 v/v) for each concentration of calibrator and quality control needed.
200 µL of blank plasma was taken.
For standards and quality control (QC) samples, 10 µL of relevant standard spiking solution was added. For blank samples, 10 µL of methanol / water (50:50 v/v) was added.
200 µL of 1% formic acid in water was then added and mixed well.

Sample Preparation		Part Number
Compound:	Tenofovir	
Matrix:	Plasma	
Cartridge type:	SOLA CX 96 well plate 10 mg/2 mL	60309-002
Condition:	Apply 0.5 mL methanol then 0.5 mL water under gentle vacuum	
Application:	Apply all of the spiked sample to the SOLA CX plate under gentle vacuum	
Wash 1:	Apply 500 µL 1% formic acid in water under gentle vacuum	
Wash 2:	Apply 500 µL 1% formic acid in methanol under gentle vacuum	
Elution:	Apply 2 x 250 µL 20% ammonia solution in methanol. Evaporate to dryness under nitrogen at 40 °C and reconstitute in 200 µL water / methanol (90:10 v/v). Mix well.	

Separation Conditions		Part Number
Instrumentation:	Thermo Scientific™ Accela™ 600	
Column:	Hypersil GOLD, 1.9 µm, 50 × 2.1 mm	25002-052130
Mobile phase A:	25 mM ammonium acetate (pH 5)	
Mobile phase B:	methanol	
Gradient:	0%–100% B in 1 minute	
Flow rate:	0.8 mL/min	
Column temperature:	40 °C	

Injection details:	10 µL
Injection wash solvent 1:	water / acetonitrile (80:20 v/v)
Injection wash solvent 2:	IPA / acetonitrile / acetone (45:45:10 v/v/v)

MS Conditions	
Instrumentation:	Thermo Scientific™ TSQ Vantage™ MS
Ionization conditions:	HESI
Polarity:	Positive
Spray Voltage (V):	3000
Vaporizer temp (°C):	350
Sheath gas pressure (Arb):	60
Aux gas pressure (Arb):	40
Capillary temp (°C):	300
Collision pressure (mTorr):	1.5
Scan time (s):	0.02
Q1 (FWHM) (Da):	0.7
Q3 (FWHM) (Da):	0.7

Compound:	Tenofovir
Parent (*m/z*):	288.1
Products (*m/z*):	176.2
Collision energy:	24
S-lens:	115

Data Processing	
Software:	Thermo Scientific™ LCQUAN™

Results

Chromatography

The Hypersil GOLD UHPLC column gave excellent peak shape. The chromatography of the QC at 500 ng/mL is shown in Figure 2. The chromatography of the LOQ at 1 ng/mL is shown in Figure 3. The tenofovir peak at 0.8 minutes is well separated from the interference peak at 1.05 minutes.

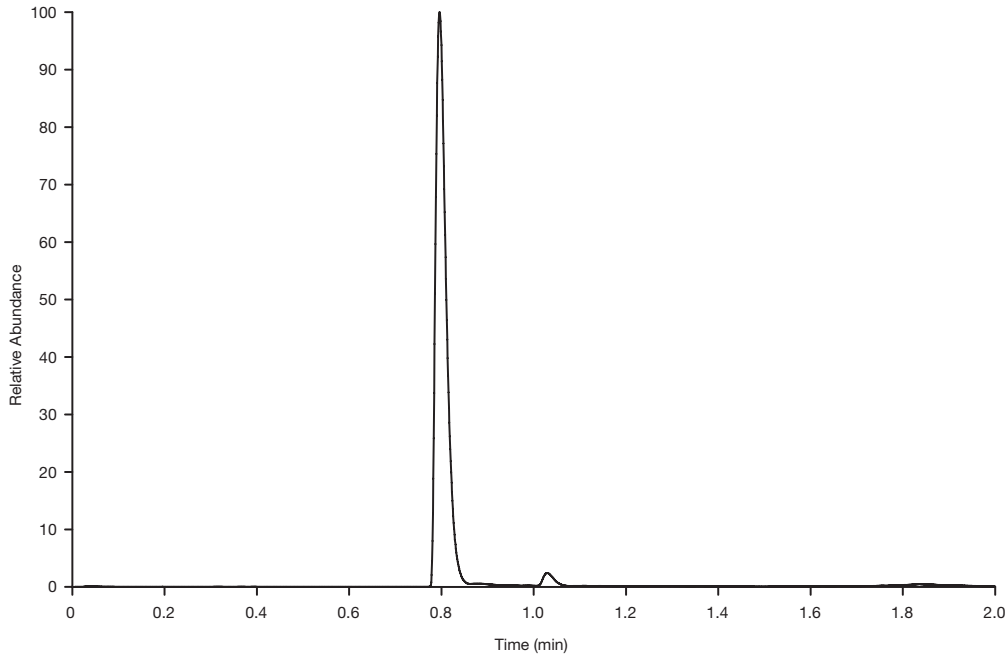

Figure 2: Representative chromatogram of tenofovir SRM, extracted from plasma at 500 ng/mL

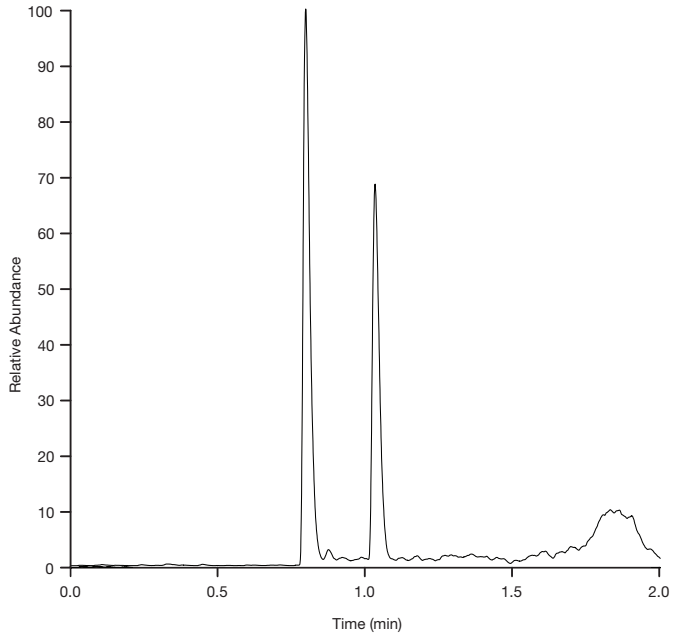

Figure 3: Chromatogram of tenofovir SRM, extracted from plasma at 1 ng/mL

Linearity

Extracted tenofovir standards from plasma gave a linear calibration curve over the dynamic range of 1 to 1000 ng/mL with an r^2 coefficient of 0.998 (Figure 4 and Table 1).

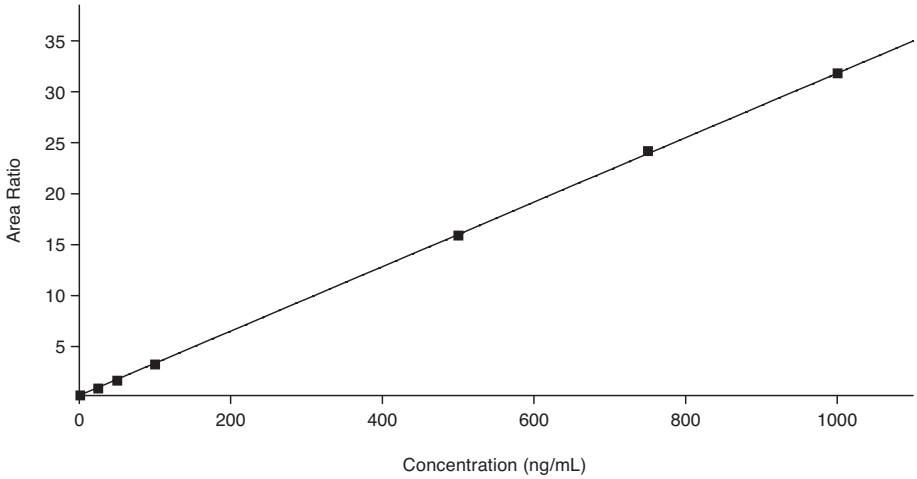

Figure 4: Tenofovir linearity over the dynamic range 1 to 1000 ng/mL

Standard	Specified Concentration (ng/mL)	Calculated Concentration (ng/mL)	%Diff
S1	1	0.848	-15
S2	50	56.0	12
S3	100	107	7
S4	500	487	-3
S5	750	725	-3
S6	1000	1030	3

Table 1: Accuracy data for 6 extracted tenofovir standards over the linear range 1 to 1000 ng/mL

Accuracy and Precision

QC samples were run in replicates of six at a concentration of 500 ng/mL. The precision of the QC level was < 3.7% CV (Table 2).

Standard	Concentration (ng/mL)	Average Calculated Concentration (n=6)	%CV
QC	500	451	3.7

Table 2: Average precision data for six replicate QCs for tenofovir

Recovery

Overspikes (post extraction fortified blank extracts) were run in triplicate at a concentration of 500 ng/mL and used to calculate the percentage recovery level for tenofovir of 88.5% (Table 3).

Standard	Response (n=3)	% Recovery
Average QC area response	3275534	88.5
Average overspike area response	3702982	

Table 3. Recovery data for tenofovir

Conclusion

- SOLA CX SPE and Hypersil GOLD products allow for a simple extraction and quantification of tenofovir from plasma.
- A LOQ of 1 ng/mL for tenofovir in plasma was achieved.
- Extraction recovery was high >88%.
- The method showed excellent precision with %RSD (n=6) <4% at 500 ng/mL.
- The method on SOLA CX SPE was extremely reproducible. There was no need for an internal standard that would have been essential when using a conventional loose-packed SPE product.

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