

**Supplementary table S1:** Mean observed calibration data for the each analyte. Y is the chromatographic response (Analyte/IS area ratio), while  $x$  is the analyte concentration;  $1/x$  weighing was applied, in order to obtain optimal fitting to the lowest part of the calibration curves.

DRUGs	R <sup>2</sup>	Equation
TFV	0.997	$y = 0.0410 x + 0.0172$
3TC	0.998	$y = -(2.03 \times 10^{-7}) x^2 + 0.0032 x + 0.0060$
FTC	0.997	$y = -(1.94 \times 10^{-7}) x^2 + 0.0037 x + 0.0081$
ABV	0.996	$y = 0.0041 x + 0.0108$
AZT	0.996	$y = 0.00073 x + 0.00112$
TAF	0.997	$y = 0.1810 x + 0.0851$

**Supplementary table S2:** Mean accuracy percentages of the back-calculated concentrations of calibration standards (STDs) during the validation sessions.

DRUGs	STD1 - LLOQ	STD2	STD3	STD4	STD5	STD6	STD7	STD8	STD9 - ULOQ
TFV	116.2	108.1	96.2	98.2	103.6	102.7	98.1	98.2	96.6
3TC	114.5	105.6	102.3	101.5	104.9	102.3	99.8	97.1	97.5
FTC	116.2	109.7	100.2	99.8	101.2	100.6	96.7	97.2	96.3
ABV	84.6	103.7	104.2	105.2	106.7	105.6	103.1	98.2	95.6
AZT	112.2	108.3	107.2	98.7	102.2	106.3	101.7	98.0	96.2
TAF	117.0	106.3	104.8	102.1	98.3	100.5	98.5	97.6	97.4