Supplementary Information (SI)

AQbD driven stability indicating HPLC method for simultaneous estimation of lamivudine, tenofovir disoproxil fumarate and efavirenz in plasma

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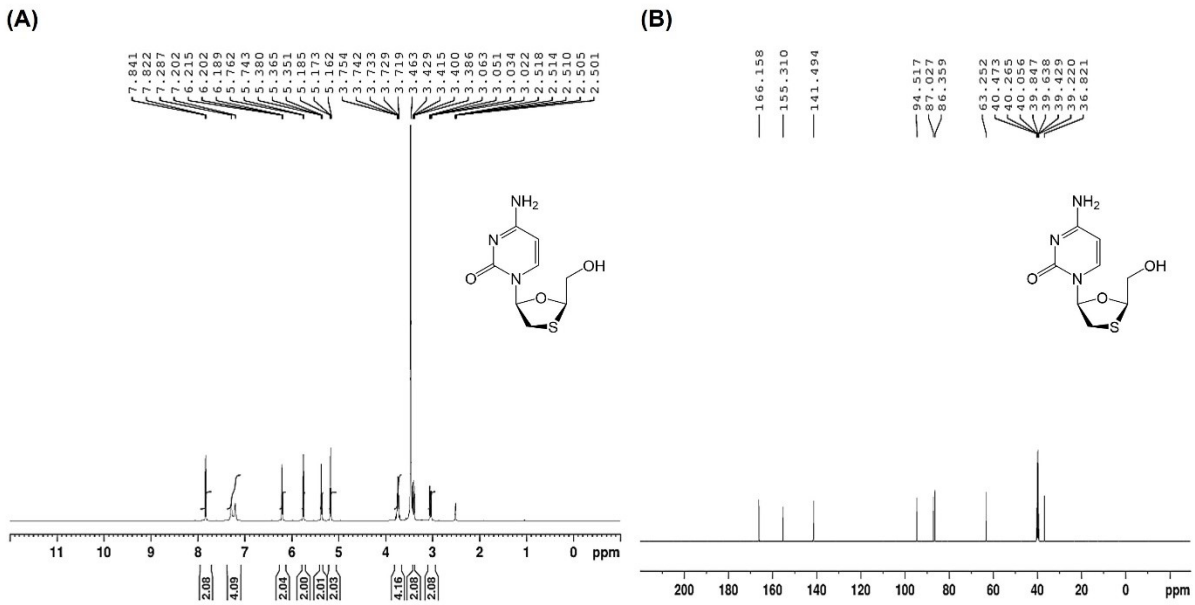

Fig. S1- The ^{1}H NMR (A) and ^{13}C NMR (B) spectra of the pure drug Lamivudine (LVD). (7.84-7.82)-d, 1H 6th position of pyridine ring; (7.28-7.20) – 2 S, 2 H of –NH2 ;(6.21-6.18) – t, 1 H of 5th position 5" of oxathiolane ring; (5.76-5.74) – d, 1 H of 5th position of pyridine ring; (5.38-5.35) – t, 1 H of -OH; (5.18-5.16) – t, 2nd position of oxathiolane ring; (3.75-3.71) – m, 2 H of –CH2 attached at 2" of oxathiolane ring; (3.06-3.02) – dd, 2 H of 4th position 4" of oxathiolane ring. 166.158 (1C, 4th position of pyridine ring – C4); 155.310 (1C, -C=O carbonyl carbon at 2nd position of pyridine ring – C2); 141.494 (1C, 6th position of pyridine ring - C6); 94.517 (1C, 5th position of oxathiolane ring – 5"); 87.027 (1C, 5th position of pyridine ring – C5); 86.359 (1C, 2nd position of oxathiolane ring – 2"); 63.252 (1C, -CH2OH attached at 2nd position of oxathiolane ring); 36.821 (1C, 4th position of oxathiolane ring – 4")

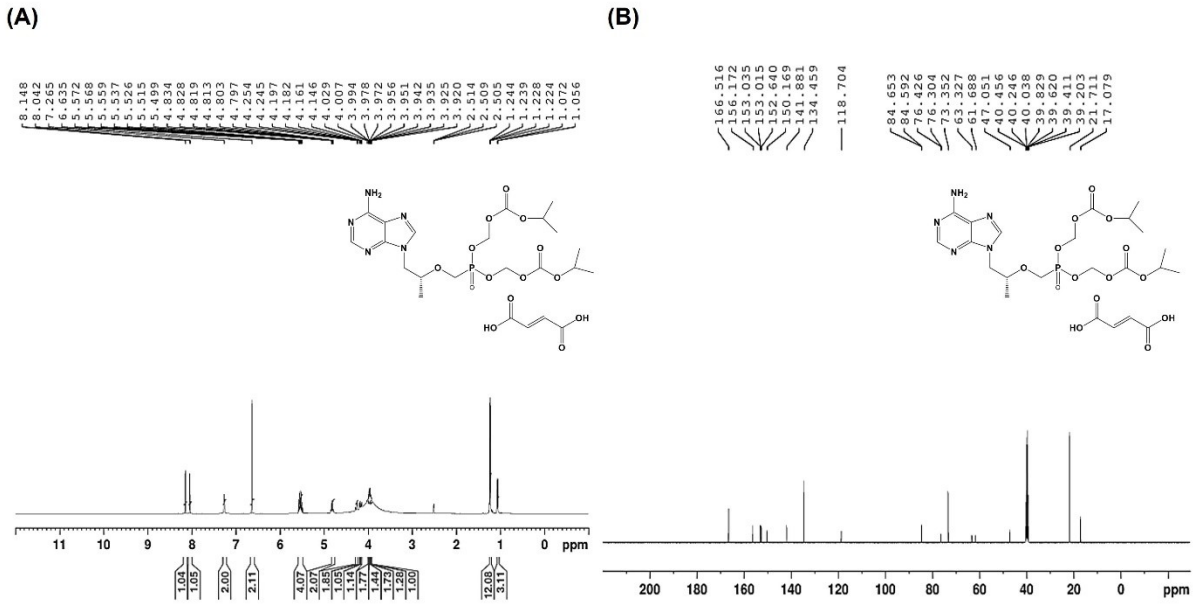

Fig. S2- The ^{1}H NMR (A) and ^{13}C NMR (B) spectra of the pure drug Tenofovir Disoproxil Fumarate (TDF).

^{1}H NMR: 8.14 (s, 2nd position of purine ring); 8.04 (s, 8th position of purine ring); 7.265 (s, 2H of –NH2); 6.635 (s, 4H of methylene of two side chains – 5”); 5.499 (m, 2H of methine 6” position); 4.83-4.79 (td, 2H of methylene attached to N at 9th position of purine ring – 1”); 4.25-4.14 (qd, 2H of –OCH2 attached to P – 4”); 4.02-3.92 (m, 1H of –CH of methine at 2” position); 1.244-1.22 (d, 12 H of four terminal methyl groups); 1.07-1.05 (d, 3 H of –CH3 at 3” position).

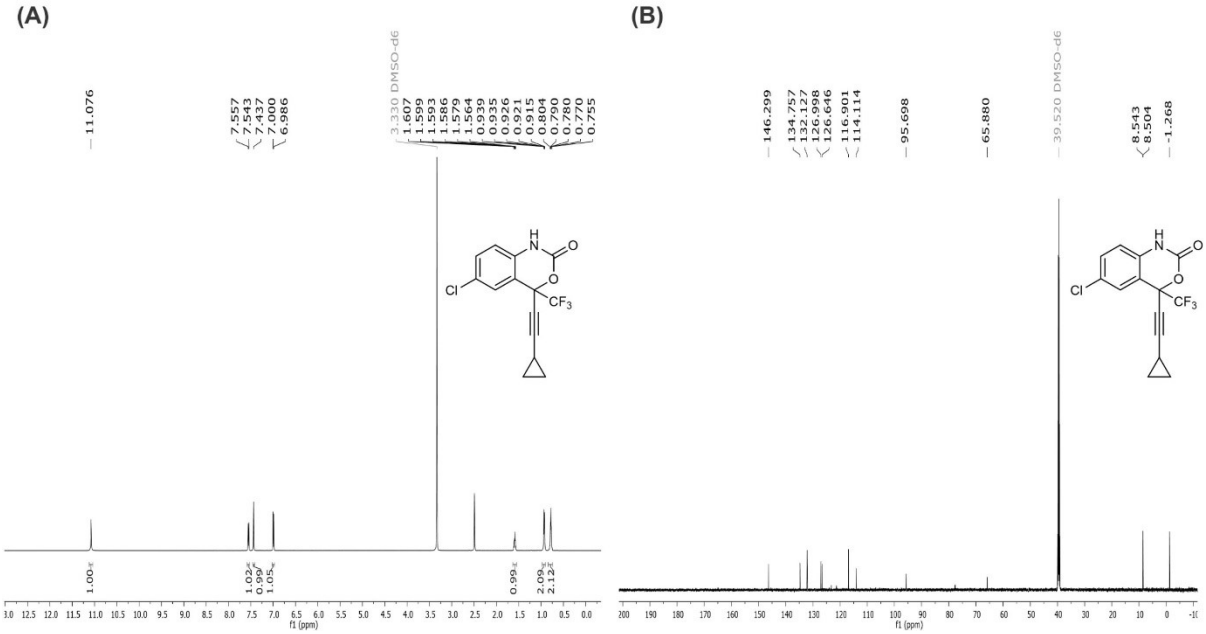

Fig. S3- The ^{1}H NMR (A) and ^{13}C NMR (B) spectra of the pure drug Efavirenz (EFZ). 11.076 (s, 1H, -NH); 7.557 (d, 1H, Ar-H); 7.437 (s, 1H, Ar-H); 1.607, 1.599, 1.593, 1.586, 1.579, 1.564(m, 1H, cyclopropyl ring; 0.939, 0.935, 0.926, 0.921, 0.915, 0.804, 0.790, 0.780, 0.770, 0.755 (m, 4H, cyclopropyl ring); -1.26 (1C, cyclopropyl ring); 8.5, 8.5 (2C, cyclopropyl ring); 65.8 (1C, 4th C of benzoxazin-2-one ring); 95.69 (2C, -C triple bond -C); 116.9 (1C, -CF3); 126.6 (1C, -C-Ar); 126.99 (2C, 5th & 7th position of benzoxazine-2-one ring); 132.1 (1C, -C-Cl); 134.75 (1C, -C-Ar); 146.2 (1C, -C=O at 2nd position of benzoin-2-one ring).

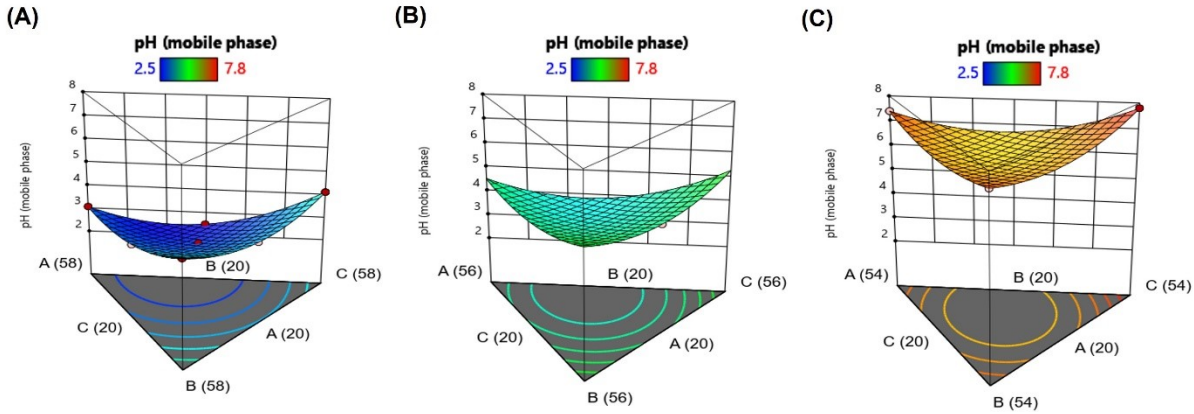

Fig. S4- 3D surface graphs showing the final pH changes after incorporating KOH at concentrations of 2% (A), 4% (B), and 6% (C) with acetonitrile, methanol, and buffer. The graphs are color-coded, with blue representing low values (2.5±0.1) and red indicating high values (7.8±0.3).

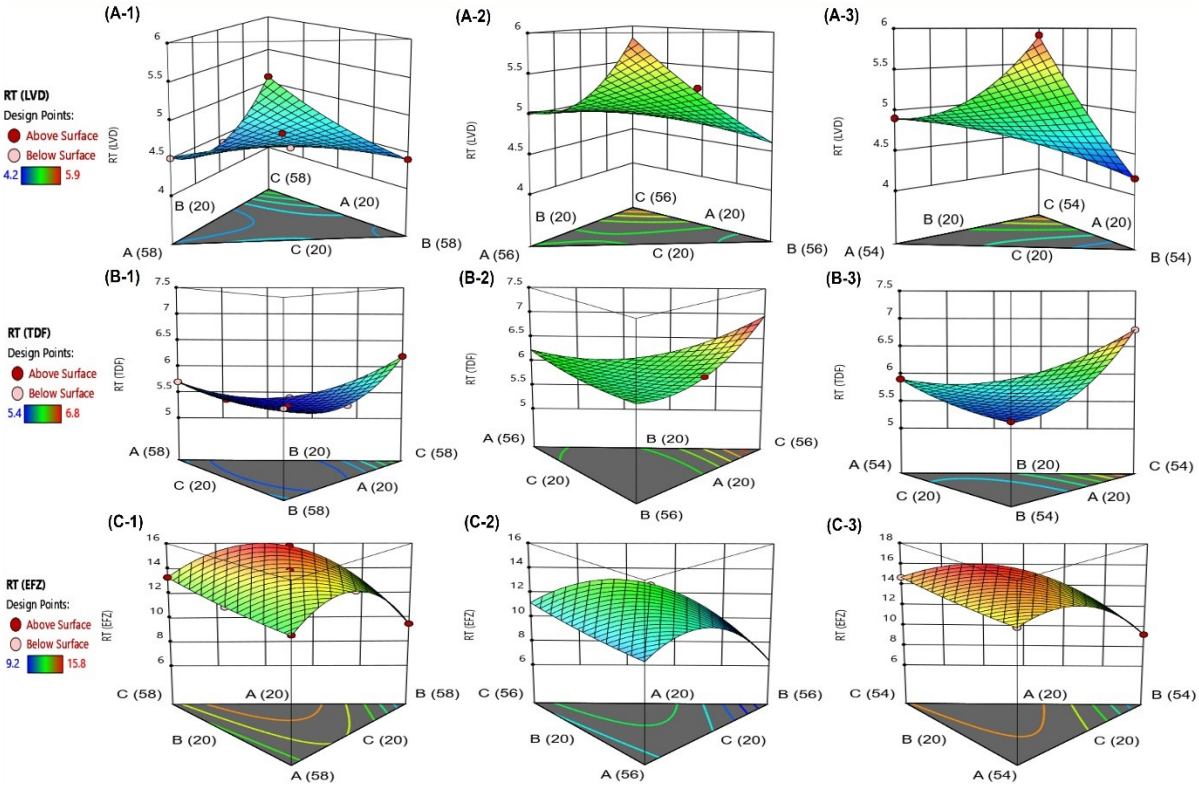

Fig. S5- 3D surface graphs showing the effect of organic phase on RT for Lamivudine (LVD) (A-1, A-2, A-3), Tenofovir Disoproxil Fumarate (TDF) (B-1, B-2, B-3) and Efavirenz (EFZ) (C-1, C-2, C-3) respectively. The graphs are color-coded, with blue representing low values and red indicating high values.

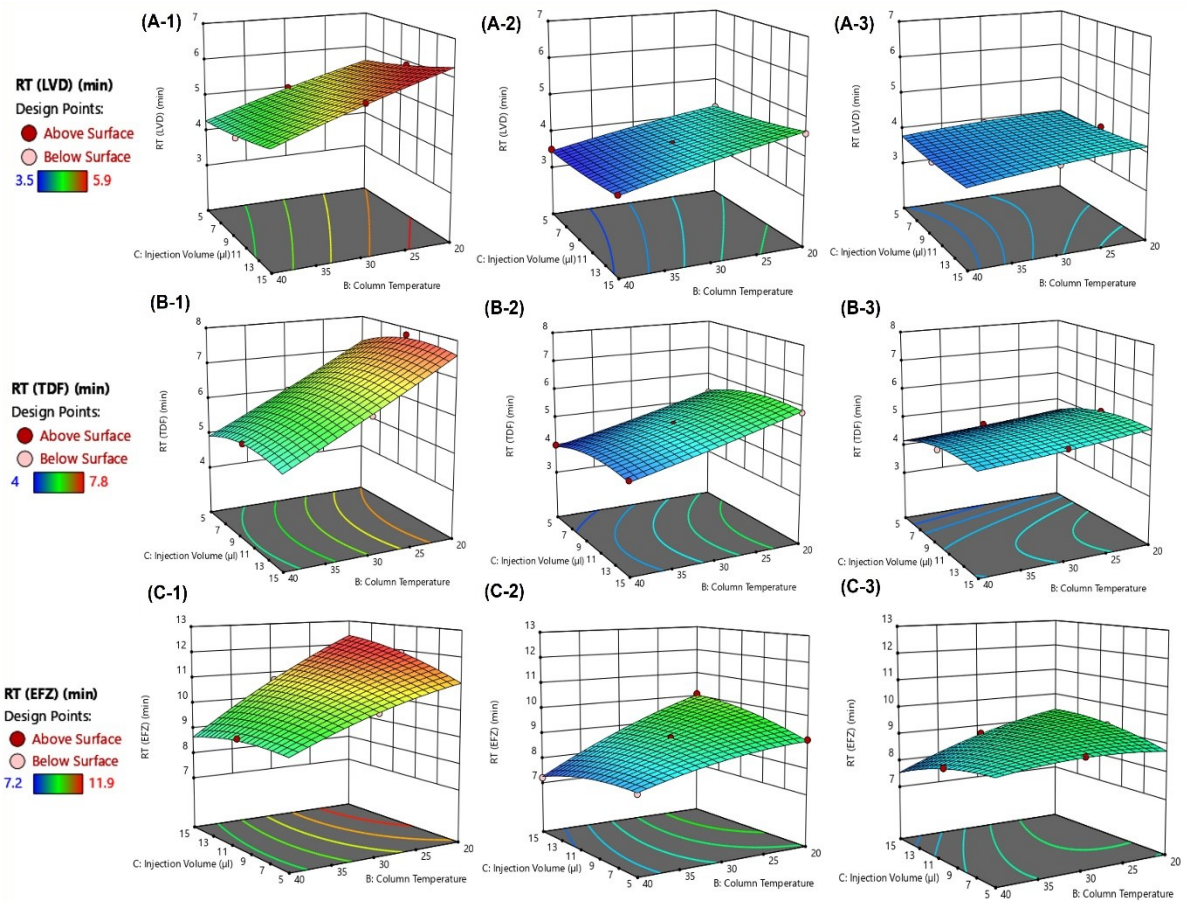

Fig. S6- 3D surface graphs showing the effect of flow rate on RT for Lamivudine (LVD) (A-1, A-2, A-3), Tenofovir Disoproxil Fumarate (TDF) (B-1, B-2, B-3) and Efavirenz (EFZ) (C-1, C-2, C-3) respectively. The graphs are color-coded, with blue representing low values and red indicating high values.

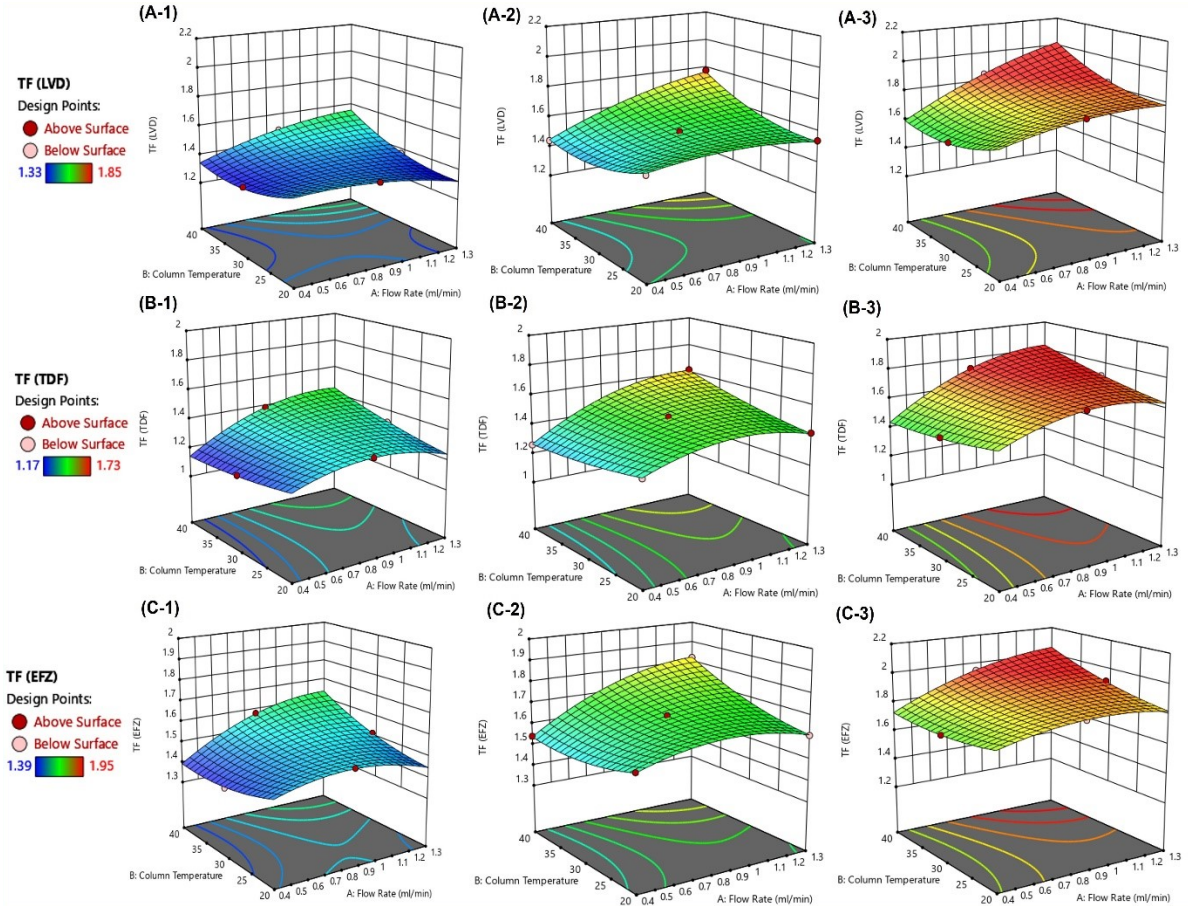

Fig. S7- 3D surface graphs showing the effect of injection volume on TF for Lamivudine (LVD) (A-1, A-2, A-3), Tenofovir Disoproxil Fumarate (TDF) (B-1, B-2, B-3) and Efavirenz (EFZ) (C-1, C-2, C-3) respectively. The graphs are color-coded, with blue representing low values and red indicating high values.

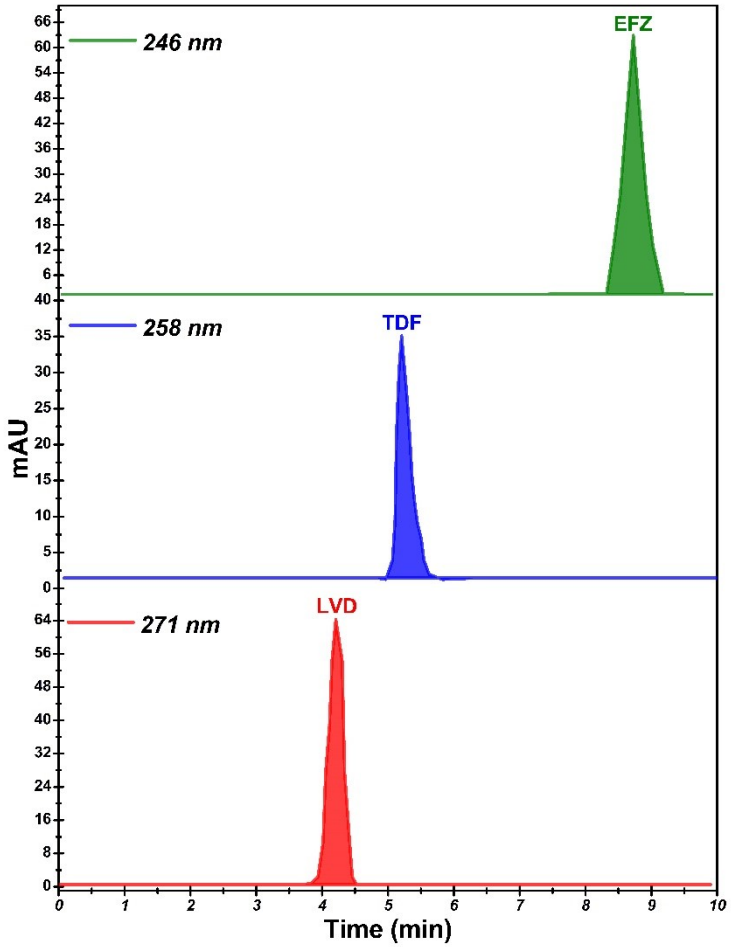

Fig. S8- The individual HPLC chromatograms of the developed method are shown for Lamivudine (LVD) at 4.1 ± 0.2 min, Tenofovir Disoproxil Fumarate (TDF) at 5.1 ± 0.3 min, and Efavirenz (EFZ) at 8.6 ± 0.4 min.

Table S1: The details of the linearity data for the antiretroviral agents Lamivudine (LVD), Tenofovir Disoproxil Fumarate (TDF), and Efavirenz (EFZ).

Sample	Concentration (µg/ml)	Peak Area	
		Mean	S.D.
LVD	0.25	6219	345
	1.25	10946	478
	2.5	24278	539
	5	48768	594
	10	98935	791
	20	203627	1691
	40	393276	11815
	80	758154	14853
TDF	0.25	13986	445
	1.25	24282	678
	2.5	68264	739
	5	120658	1494
	10	246064	2791
	20	418635	3991
	40	927446	16815
	80	1779196	29853
EFZ	0.25	20688	345
	1.25	43501	478
	2.5	92065	539
	5	183933	1584
	10	321305	2978
	20	745908	6691
	40	1425802	46815
	80	2799721	49853

Table S2: The details of the robustness data for the antiretroviral agents Lamivudine (LVD), Tenofovir Disoproxil Fumarate (TDF), and Efavirenz (EFZ).

Parameter		LVD			TDF			EFZ		
		Mean Area	SD	%RSD	Mean Area	SD	%RSD	Mean Area	SD	%RSD
Column Temperature (°C)	41.95	99314	748	0.75	248917	1648	0.66	339527	2617	0.77
	36.95	98921	630	0.64	232564	1791	0.77	323465	2596	0.80
	31.95	97584	647	0.66	217953	1627	0.75	318051	2307	0.73
Flow Rate (ml/min)	0.817	96752	629	0.65	225961	1539	0.68	319426	2285	0.72
	0.617	98426	598	0.60	245867	1934	0.78	327547	2751	0.84
	0.417	99854	574	0.57	251977	1978	0.79	341859	2563	0.74
Mobile Phase (ACN : Methanol : Buffer : KOH)	27 : 48 : 23 : 2	97452	755	0.77	237896	1603	0.67	308316	2347	0.76
	25 : 46 : 25 : 4	98217	612	0.62	243771	1749	0.72	317481	2726	0.89
	23 : 44 : 27 : 6	98783	671	0.67	263816	2165	0.82	327609	2297	0.70

Table S3: The details of the accuracy data for the antiretroviral agents Lamivudine (LVD), Tenofovir Disoproxil Fumarate (TDF), and Efavirenz (EFZ).

Sample	Concentration (µg/ml)	Mean Recovered Concentration (µg/ml)	Accuracy (% w/w)
LVD	8 (80%)	7.96	99.58
	10 (100%)	9.93	99.35
	12 (120%)	12.14	101.22
TDF	8 (80%)	7.97	99.72
	10 (100%)	9.98	99.85
	12 (120%)	12.16	101.37
EFZ	8 (80%)	7.99	99.91
	10 (100%)	9.96	99.64
	12 (120%)	12.22	101.83