Supplementary information

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Quality by Design based development of a selective stability-indicating UPLC method of dolutegravir and characterization of its degradation products by UPLC-QTOF-MS/MS

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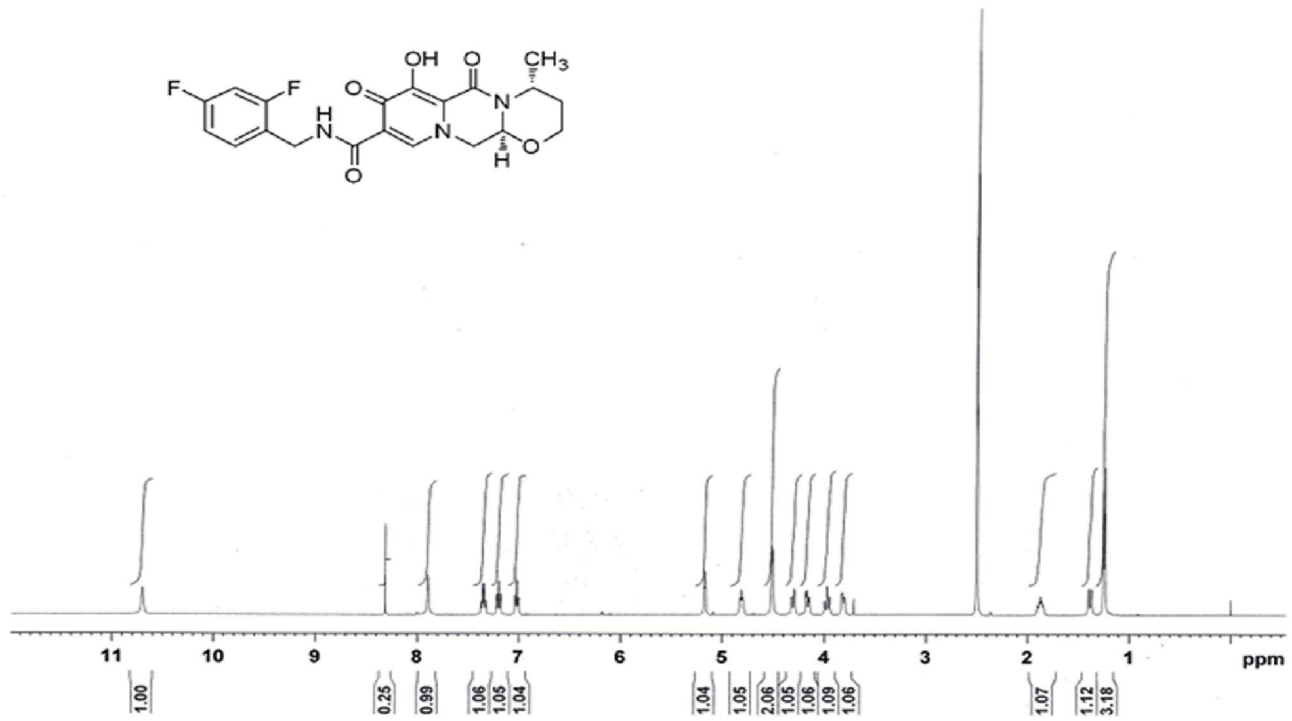

Fig. S1.1 ^{1}H-NMR spectra of dolutegravir [1H-NMR (DMSO-d6) δ: 1.27 (3H, d), 1.45-1.50 (1H, m), 1.89-1.90 (1H, m), 3.82-3.86 (1H, m), 4.04 (1H, t), 4.20-4.23 (1H, m), 4.30-4.34 (1H, m), 4.5 (2H, d), 4.81-4.83 (1H, m), 5.20-5.23 (1H, m), 7.00-7.03 (1H, m), 7.20-7.23 (1H, m), 7.33 (1H, dd), 7.93 (1H, s), 8.3 (1H, s), 10.73 (1H, s)]

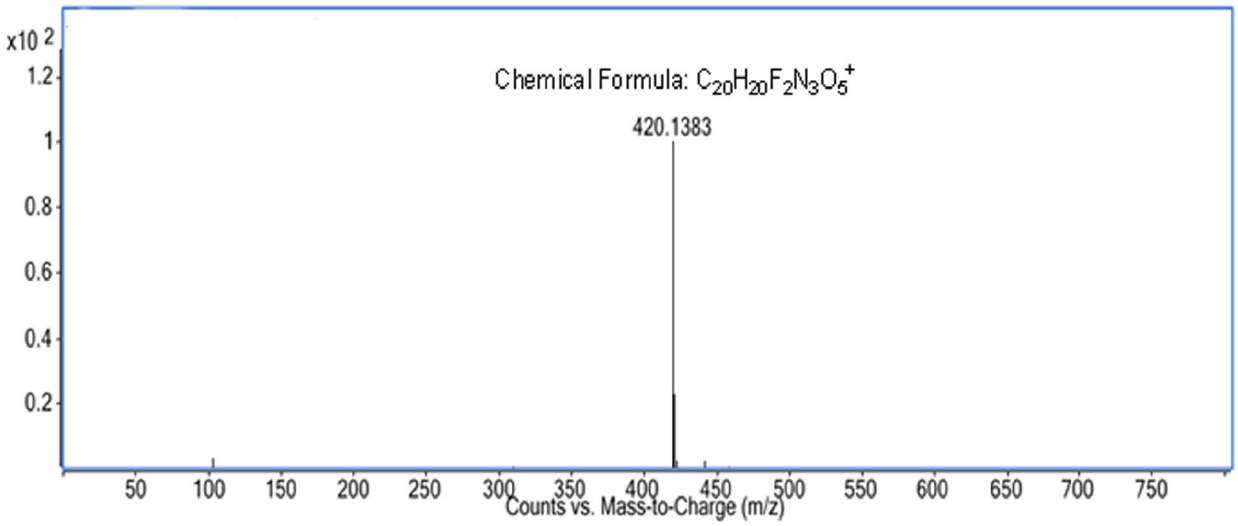

Fig. S1.2 MS-QTOF spectra of dolutegravir

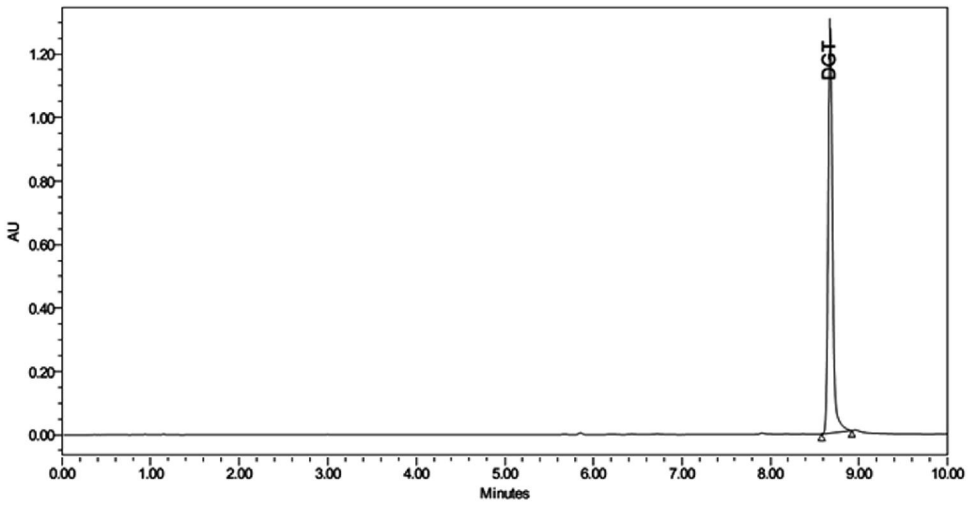

Fig. S 1.3 LC-PDA chromatogram of dolutegravir

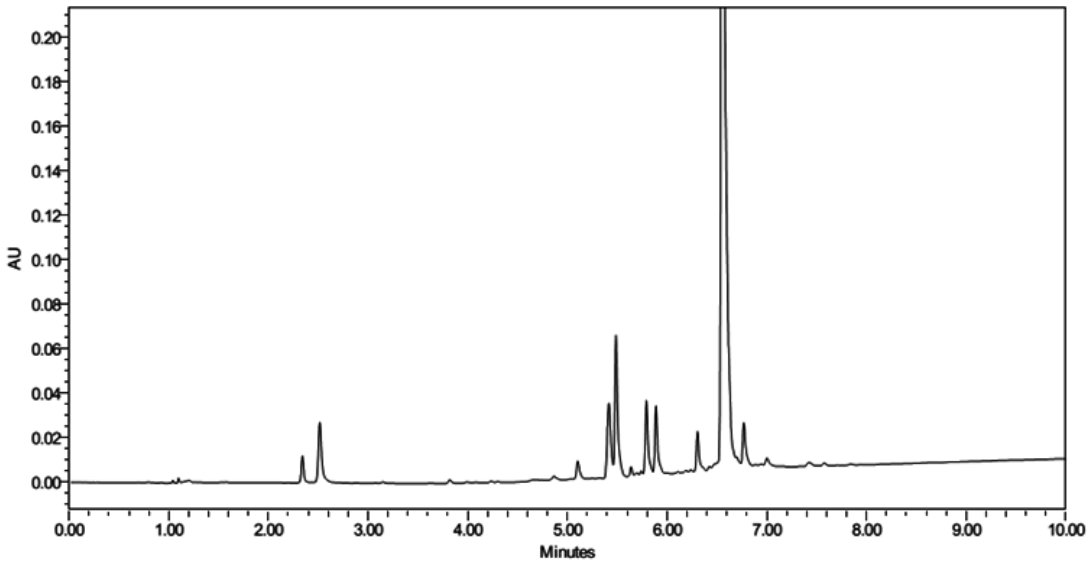

Fig S2. Chromatogram recorded for dolutegravir and its stress degradation samples using the CSH Phenyl hexyl (100 × 2.1 mm I.D., 1.7 µm) stationary phase, in presence of MeOH at pH 4. A generic gradient from 10 to 90% of MeOH was performed in 5 min at 30°C and Flow = 0.3 ml/min.

Table S1. Experiment Design Matrix

Run No	Organic modifier	pH	Columns	No of peaks integrated	No of peaks >= Rs 1.5
1	ACN	3	CSH Phenyl hexyl	12	9
2	ACN	4	CSH Phenyl hexyl	12	9
3	ACN	5	CSH Phenyl hexyl	13	12
4	MeOH	3	CSH Phenyl hexyl	11	11
5	**MeOH**	**4**	**CSH Phenyl hexyl**	**14**	**13**
6	MeOH	5	CSH Phenyl hexyl	12	11
7	ACN	3	CSH C18	12	11
8	ACN	4	CSH C18	12	11
9	ACN	5	CSH C18	13	12
10	MeOH	3	CSH C18	12	12
11	MeOH	4	CSH C18	13	12
12	MeOH	5	CSH C18	13	12
13	ACN	3	HSS Cyano	13	10
14	ACN	4	HSS Cyano	10	10
15	ACN	5	HSS Cyano	11	9
16	MeOH	3	HSS Cyano	11	9
17	MeOH	4	HSS Cyano	10	8
18	MeOH	5	HSS Cyano	13	10

Table S2 High resolution mass spectrometry (HRMS) data of product ions of protonated dolutegravir (DTG) and its degradation products

DTG and its DPs	Molecular formula $[M+H]^+$	Calculated *m/z*	Observed *m/z*	Error (ppm)
DTG	$C_{20}H_{20}F_2N_3O_5^+$	420.1366	420.1374	-1.90
	$C_{19}H_{19}F_2N_2O_4^+$	377.1307	377.1325	-4.77
	$C_{13}H_{13}N_2O_5^+$	277.0819	277.0823	-1.44
	$C_7H_5F_2^+$	127.0354	127.0352	1.57
H1	$C_{20}H_{22}F_2N_3O_6^+$	438.1471	438.1473	1.30
	$C_{19}H_{21}F_2N_2O_5^+$	395.1413	395.1418	-1.27
	$C_{13}H_{15}N_2O_6^+$	295.0925	295.0928	-1.02
	$C_9H_7N_2O_5^+$	223.0349	223.0351	-0.90
	$C_7H_5F_2^+$	127.0354	127.0355	-0.79
H2	$C_{20}H_{22}F_2N_3O_6^+$	438.1471	438.1476	-1.14
	$C_{19}H_{21}F_2N_2O_5^+$	395.1413	395.1415	-0.51
	$C_{13}H_{15}N_2O_6^+$	295.0925	295.0931	-2.03
	$C_9H_7N_2O_5^+$	223.0349	223.0351	-0.90
	$C_7H_5F_2^+$	127.0354	127.0353	0.79
H3	$C_{20}H_{20}F_2N_3O_5^+$	420.1366	420.1359	1.67
	$C_{16}H_{12}F_2N_3O_4^+$	348.079	348.0787	0.86
	$C_{15}H_{11}F_2N_2O_3^+$	305.0732	305.0739	-2.29
	$C_{13}H_{13}N_2O_5^+$	277.0819	277.0814	1.80
	$C_9H_5N_2O_4^+$	205.0244	205.0242	0.98
	$C_7H_5F_2^+$	127.0354	127.0351	2.36
H4	$C_{20}H_{20}F_2N_3O_5^+$	420.1366	420.1368	-0.48
	$C_{19}H_{19}F_2N_2O_4^+$	377.1307	377.1318	-2.92
	$C_{13}H_{13}N_2O_5^+$	277.0819	277.0814	1.80
	$C_7H_5F_2^+$	127.0354	127.0356	-1.57
P1	$C_8H_{13}N_2O_3^+$	185.0921	185.0918	1.62
	$C_4H_5N_2O_2^+$	113.0346	113.0341	4.42
	$C_3H_5N_2O^+$	85.0396	85.0398	-2.35
	$C_2H_4NO^+$	58.0287	58.0290	-5.17
	$C_2H_5N_2^+$	57.0447	57.0450	-5.26
	$C_4H_7^+$	55.0542	55.0545	-5.45
P2	$C_8H_{11}N_2O_3^+$	183.0764	183.0761	1.64
	$C_4H_3N_2O_2^+$	111.0189	111.0187	1.80

	$C_3H_3N_2O^+$	83.024	83.0241	-1.20
	$C_4H_7^+$	55.0542	55.0543	-1.82
P3	$C_{10}H_8F_2NO_3^+$	228.0467	228.0469	-0.88
	$C_7H_5F_2^+$	127.0354	127.0357	-2.36
	$C_7H_4F^+$	107.0292	107.0290	1.87
	$C_5H_3F_2^+$	101.0197	101.0198	-0.99
P4	$C_{13}H_{11}F_2N_2O_3^+$	281.0732	281.0730	0.71
	$C_7H_6F_2N^+$	142.0463	142.0460	2.11
	$C_7H_5F_2^+$	127.0354	127.0355	-0.79
	$C_5H_6NO_2^+$	112.0393	112.0391	1.79
	$C_7H_4F^+$	107.0292	107.0290	1.87
P5	$C_{20}H_{22}F_2N_3O_8^+$	470.1369	470.1365	0.85
	$C_{12}H_{15}N_2O_7^+$	299.0874	299.0871	-2.34
	$C_{11}H_{10}F_2NO_4^+$	258.0572	258.0571	0.39
	$C_{11}H_{15}N_2O_5^+$	255.0975	255.0974	0.39
	$C_8H_{13}N_2O_2^+$	169.0972	169.0974	-1.18
	$C_7H_8F_2N^+$	144.0619	144.0624	-3.47
	$C_7H_5F_2^+$	127.0354	127.0352	1.57
	$C_5H_{10}NO^+$	100.0757	100.0755	2.00
P6	$C_{19}H_{20}F_2N_3O_6^+$	424.1315	424.1310	1.18
	$C_{19}H_{18}F_2N_3O_5^+$	406.1209	406.1202	1.72
	$C_{18}H_{18}F_2N_3O_4^+$	378.126	378.1262	-0.53
	$C_{17}H_{18}F_2N_3O_3^+$	350.1311	350.1312	-0.29
	$C_{12}H_{13}N_2O_6^+$	281.0768	281.0763	1.78
	$C_{11}H_{13}N_2O_4^+$	237.087	237.0863	2.95
	$C_7H_5F_2^+$	127.0354	127.0348	4.72
P7	$C_{19}H_{20}F_2N_3O_6^+$	424.1315	424.1312	0.71
	$C_{19}H_{18}F_2N_3O_5^+$	406.1209	406.1225	-3.94
	$C_{12}H_{13}N_2O_6^+$	281.0768	281.0789	-7.47
	$C_{12}H_{11}N_2O_5^+$	263.0662	263.0660	0.76
	$C_{11}H_{11}N_2O_4^+$	235.0713	235.0719	-2.55
	$C_8H_{13}N_2O_3^+$	185.0921	185.0929	-4.32
	$C_7H_5F_2^+$	127.0354	127.0346	6.30
	$C_4H_5N_2O_2^+$	113.0346	113.0348	-1.77
P8	$C_{20}H_{20}F_2N_3O_7^+$	452.1264	452.1265	-0.22
	$C_{19}H_{20}F_2N_3O_5^+$	408.1366	408.1361	1.23
	$C_{19}H_{18}F_2N_3O_4^+$	390.126	390.1251	2.31

	$C_{18}H_{20}F_2N_3O_4^+$	380.1416	380.1411	1.32
	$C_8H_{13}N_2O_2^+$	169.0972	169.0970	1.18
	$C_7H_{12}NO_2^+$	142.0863	142.0860	2.11
	$C_6H_{13}N_2O^+$	129.1022	129.1027	-3.87
	$C_7H_5F_2^+$	127.0354	127.0350	3.15
	$C_5H_{10}NO^+$	100.0757	100.0755	2.00
P9	$C_{20}H_{20}F_2N_3O_7^+$	452.1264	452.1271	-1.55
	$C_8H_{12}N_2O_3^+$	185.0921	185.0923	-1.08
	$C_7H_5F_2^+$	127.0354	127.0351	2.36
	$C_4H_5N_2O_2^+$	113.0346	113.0348	-1.77
P10	$C_9H_{11}F_2N_2O_6^+$	281.0580	281.0579	0.36
	$C_8H_9F_2N_2O_4^+$	235.0525	235.0517	3.40
	$C_8H_6F_2NO_4^+$	218.0259	218.0256	1.38
	$C_7H_8F_2NO_3^+$	192.0467	192.0464	1.56
	$C_7H_6F_2NO_3^+$	190.0310	190.0312	-1.05
	$C_6H_8F_2NO_2^+$	164.0518	164.0514	2.44

Table S3 Chromatographic parameters of the developed method

Analytes	Purity Angle	Purity Threshold	USP Resolution	USP Tailing
P1	7.016	10.101	--	1.23
P2	0.936	1.121	5.33	1.24
P3	10.210	13.144	4.46	1.20
P4	29.102	38.129	11.08	1.18
P5	0.700	1.008	6.03	1.29
P6	9.226	12.033	10.02	1.22
P7	0.211	1.092	8.46	1.19
P8	0.172	1.037	11.63	1.30
P9	0.919	1.294	2.37	1.25
H1	6.110	8.014	7.04	1.38
P10	10.200	12.113	2.12	1.24
H2	7.010	8.221	2.07	1.40
H3	0.311	0.492	10.74	1.59
DTG	3.012	3.901	7.15	1.55
H4	18.291	36.782	2.24	1.51

Table S4. Intra-day and inter-day precision study

	Amount added (µg/mL)		
	50	150	250
Intra-day precision			
Mean concentration	49.52	149.06	241.56
SD	0.14	2.96	2.35
%RSD	0.28	1.98	0.97
Inter-day precision			
Day-1 (n=3)			
Mean concentration	49.94	150.98	239.09
SD	0.57	2.81	0.60
%RSD	1.15	1.86	0.25
Day-2 (n=3)			
Mean concentration	49.93	149.87	239.53
SD	0.69	2.50	1.01
%RSD	1.38	1.67	0.42
Day-3 (n=3)			
Mean concentration	49.90	149.10	239.53
SD	0.81	2.10	1.98
%RSD	1.62	1.41	0.82
Analyst-I			
Mean concentration	49.89	151.03	240.01
SD	0.68	2.83	1.61
%RSD	1.36	1.87	0.67
Analyst-II			
Mean concentration	50.00	150.98	240.11
SD	0.61	2.82	2.46
%RSD	1.22	1.87	1.03
Column -I			
Mean concentration	49.89	150.74	240.46
SD	0.68	2.56	2.13
%RSD	1.36	1.70	0.89

Column -II			
Mean concentration	49.96	148.60	240.43
SD	0.65	1.18	2.22
%RSD	1.30	0.79	0.92

Table S5. Accuracy study

Amount added (µg/ml)	**Mean amount found (µg/ml) ± SD**	**Recovery (%)**
50	49.94 ± 0.66	99.88
100	98.40 ± 1.73	98.40
150	151.03 ± 2.82	100.69