Determination and quantification of related substances and degradation products in Bictegravir by Full Factorial Design evaluated HPLC and Mass Spectrometry.

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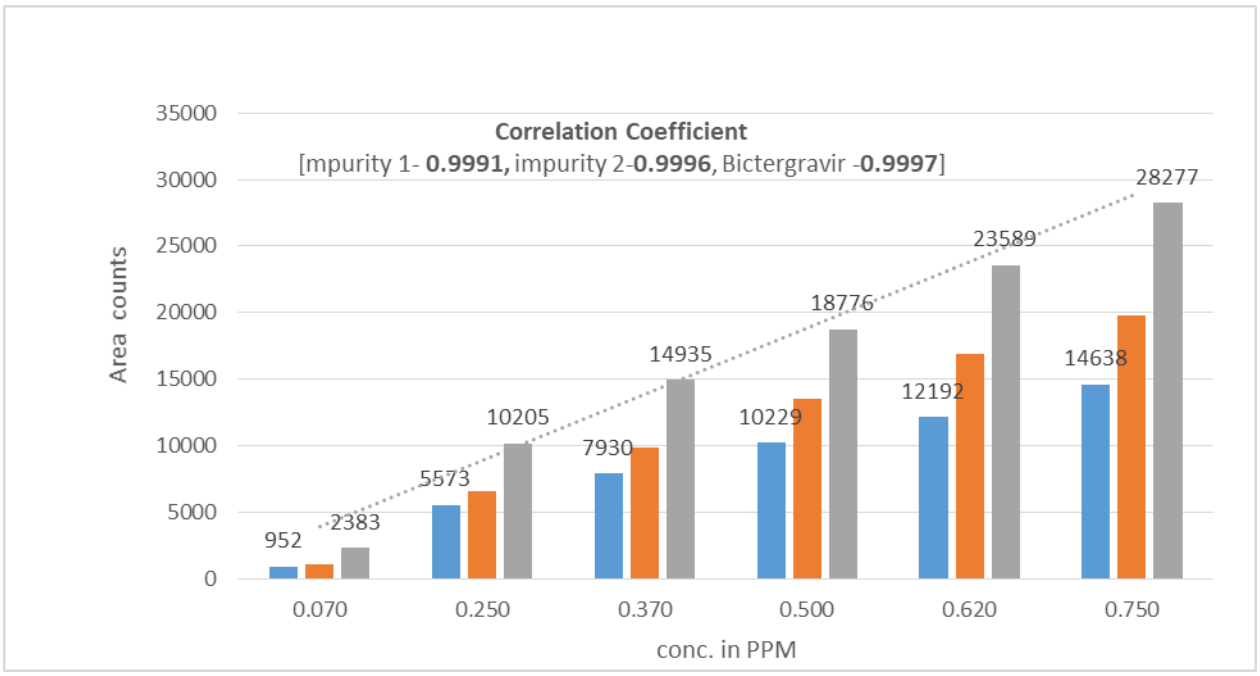

Fig. S1. Linearity plot of Bictegravir (gray bar), Impurity-I (blue bar) and Impurity-II (orange bar)

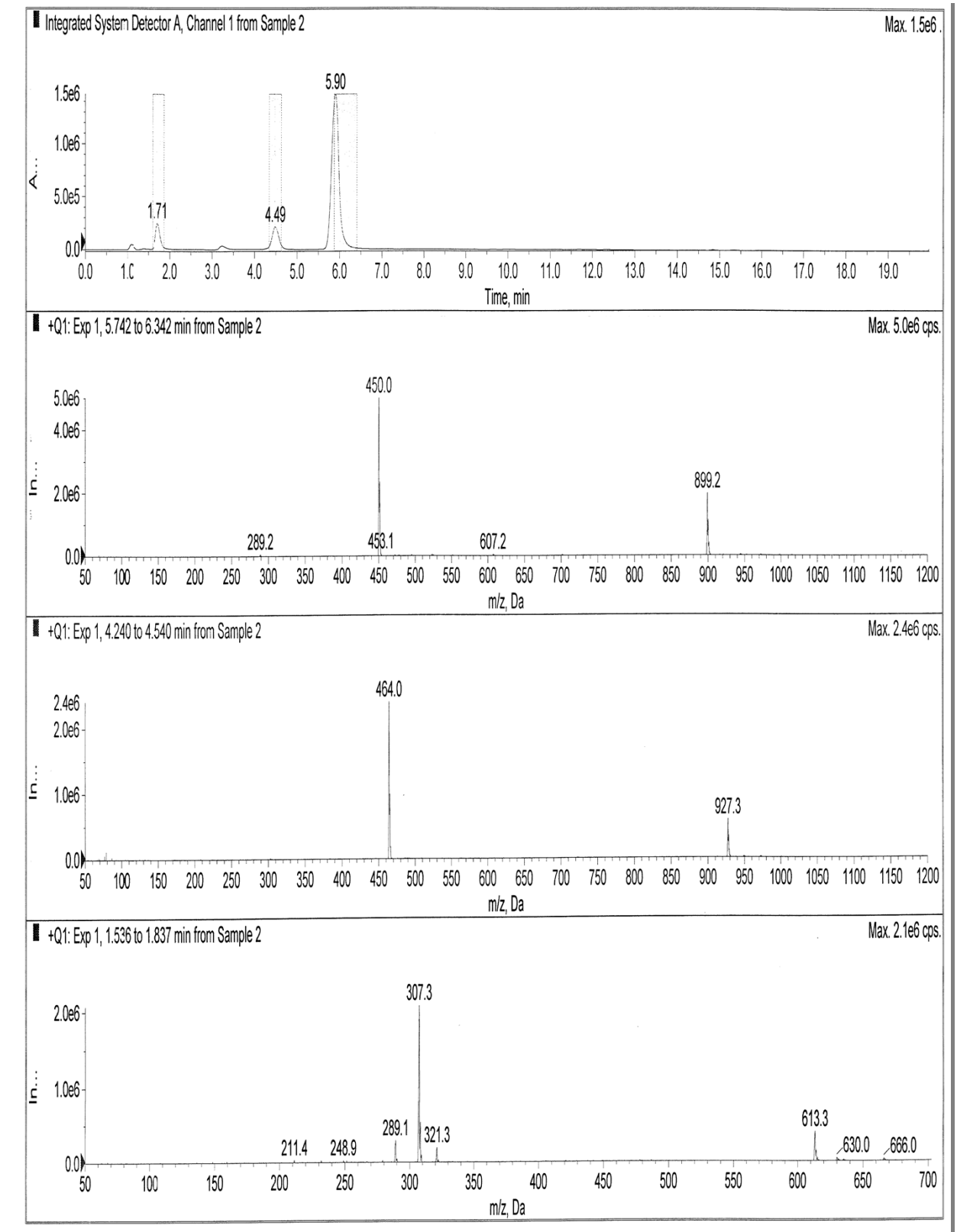

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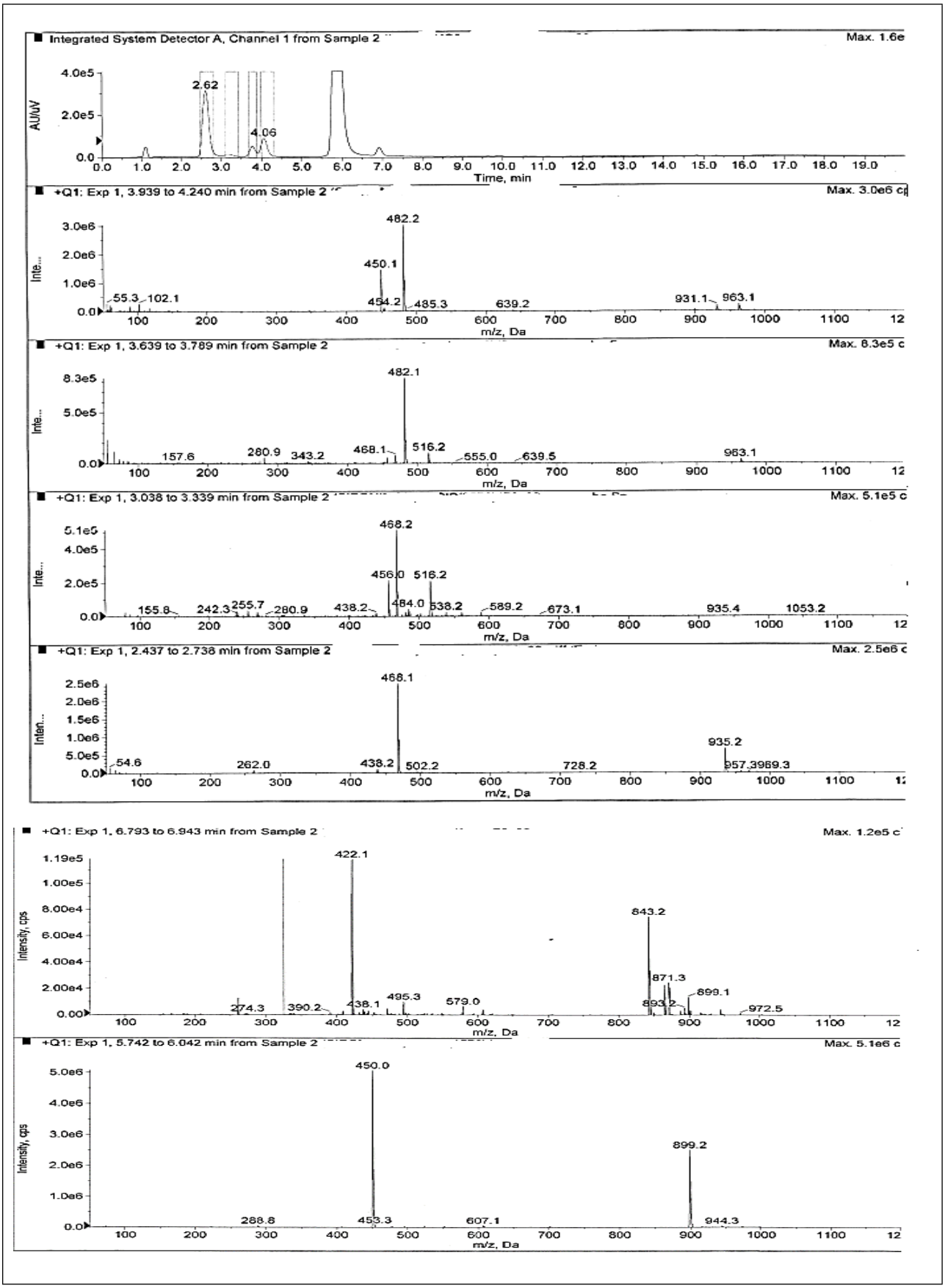
Fig. S2. Mass Spectra of determined impurities and Bictegravir



41 Fig. S3. LCMS Mass Spectra of degraded Bictegravir in acid

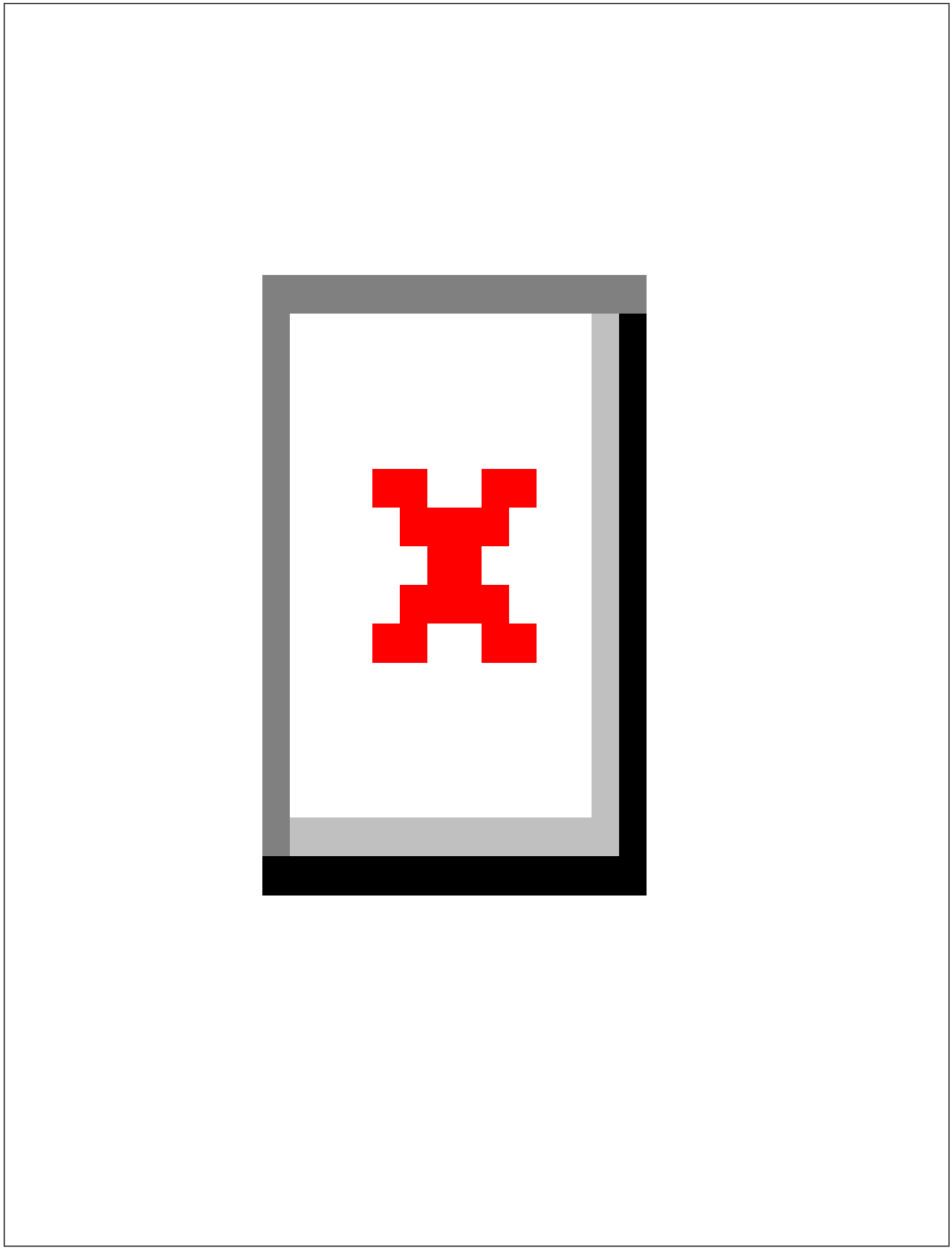


Fig. S4. LCMS/MS fragmentation pattern of degraded Bictegravir in acid

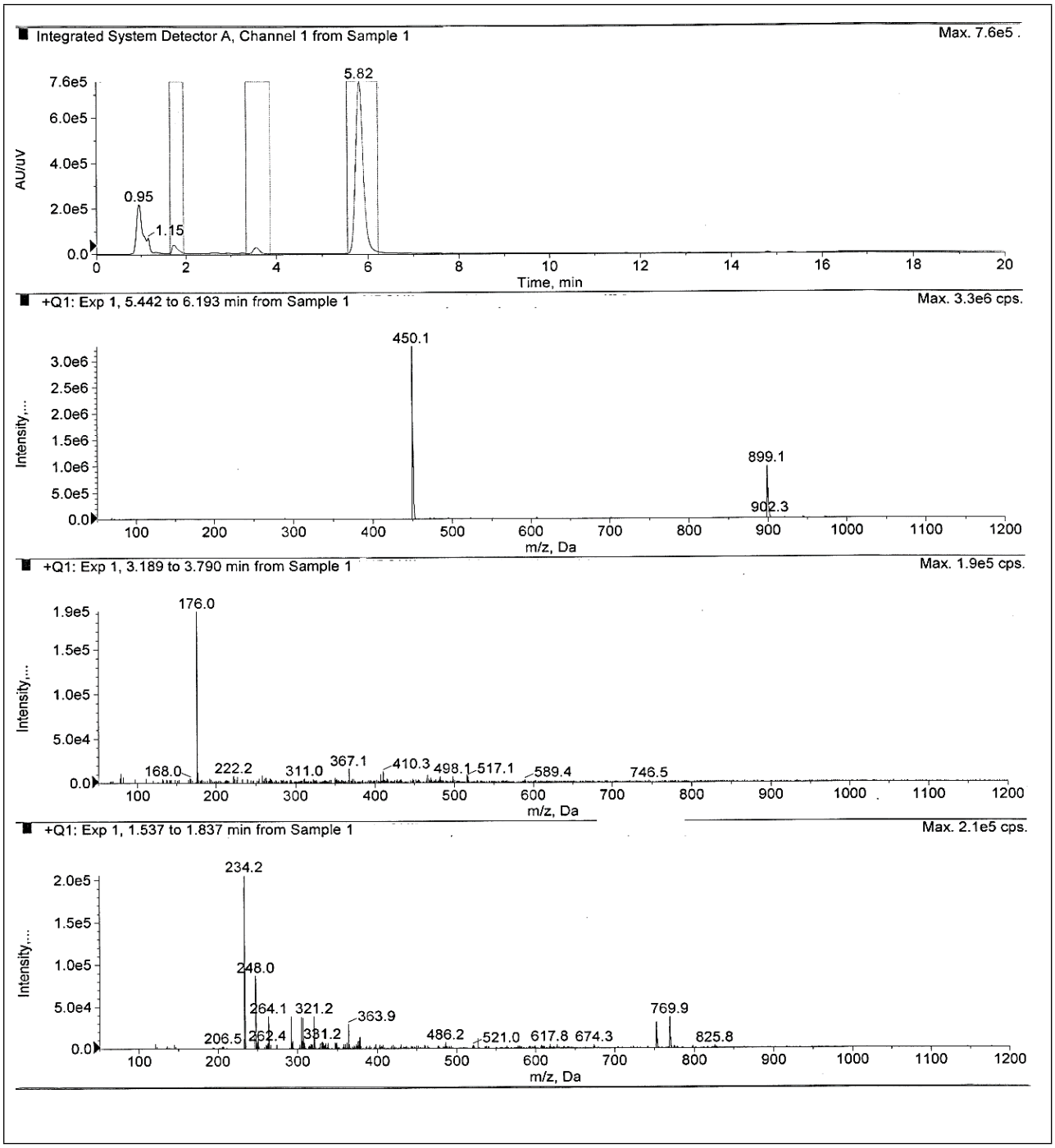


Fig. S5. LCMS Mass Spectra of degraded Bictegravir in peroxide

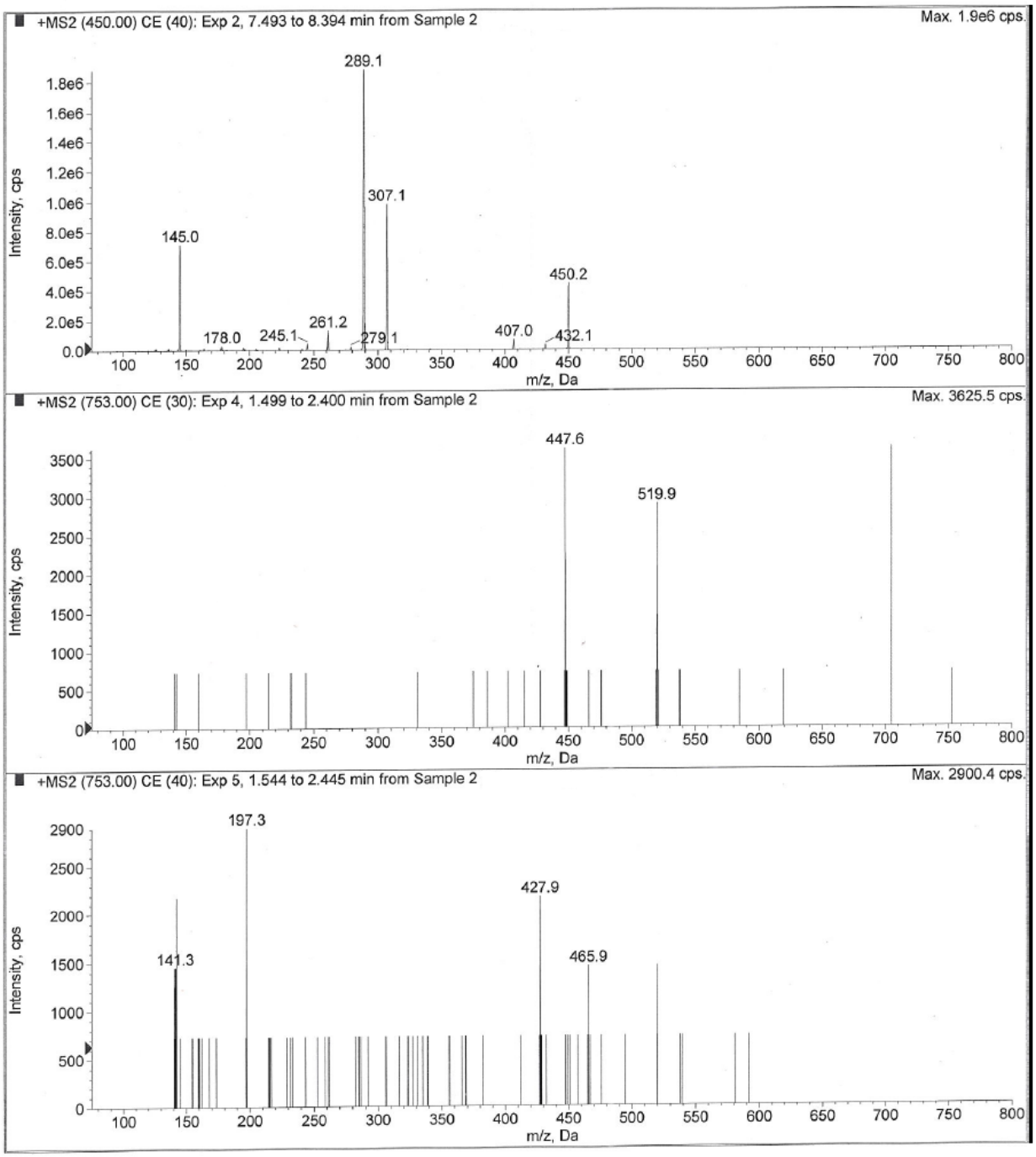


Fig. S6. LCMS/MS fragmentation pattern of degraded Bictegravir in peroxide

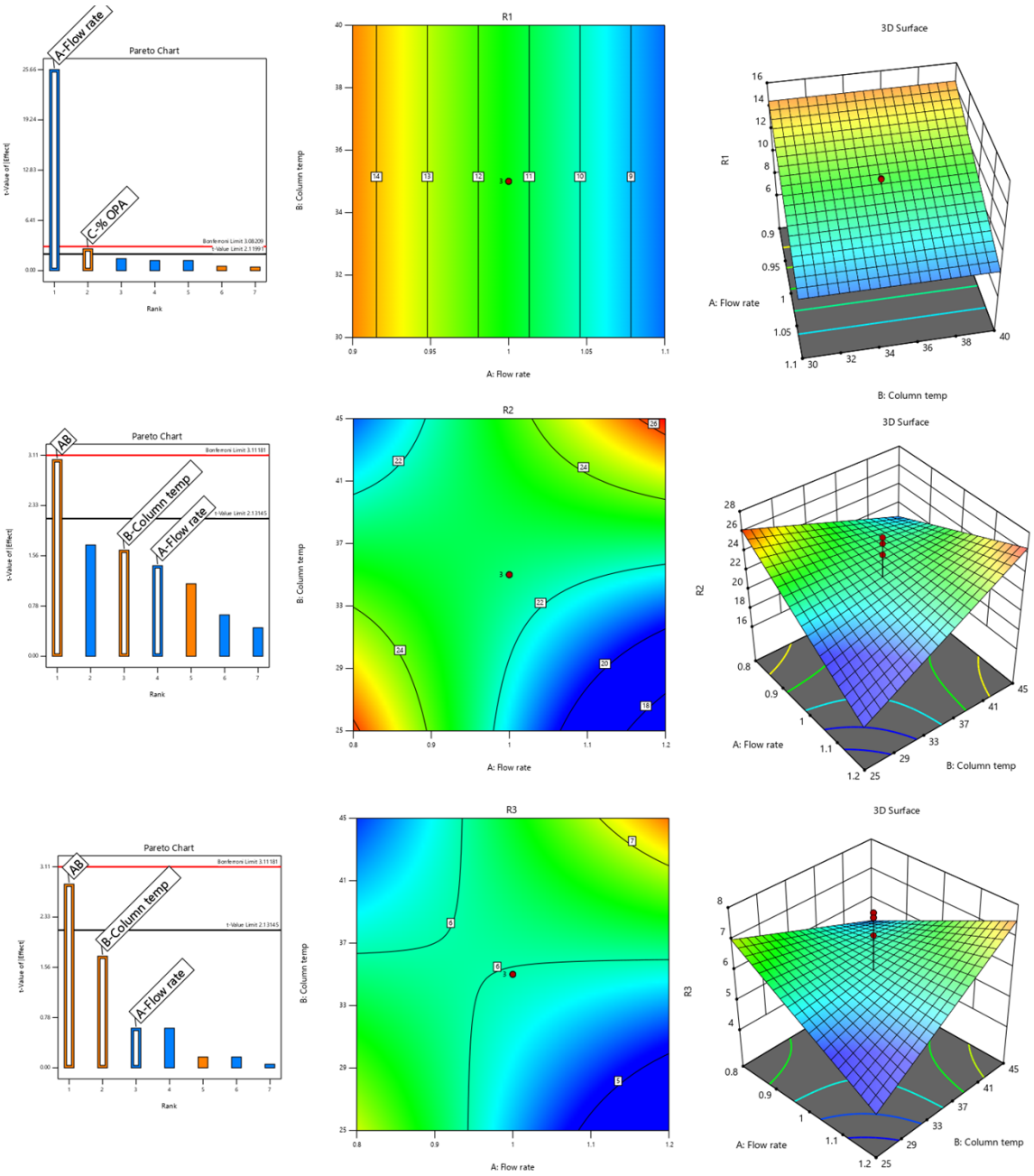


Fig. S7. Pareto charts, 2D half normal plots and 3D response surface plots

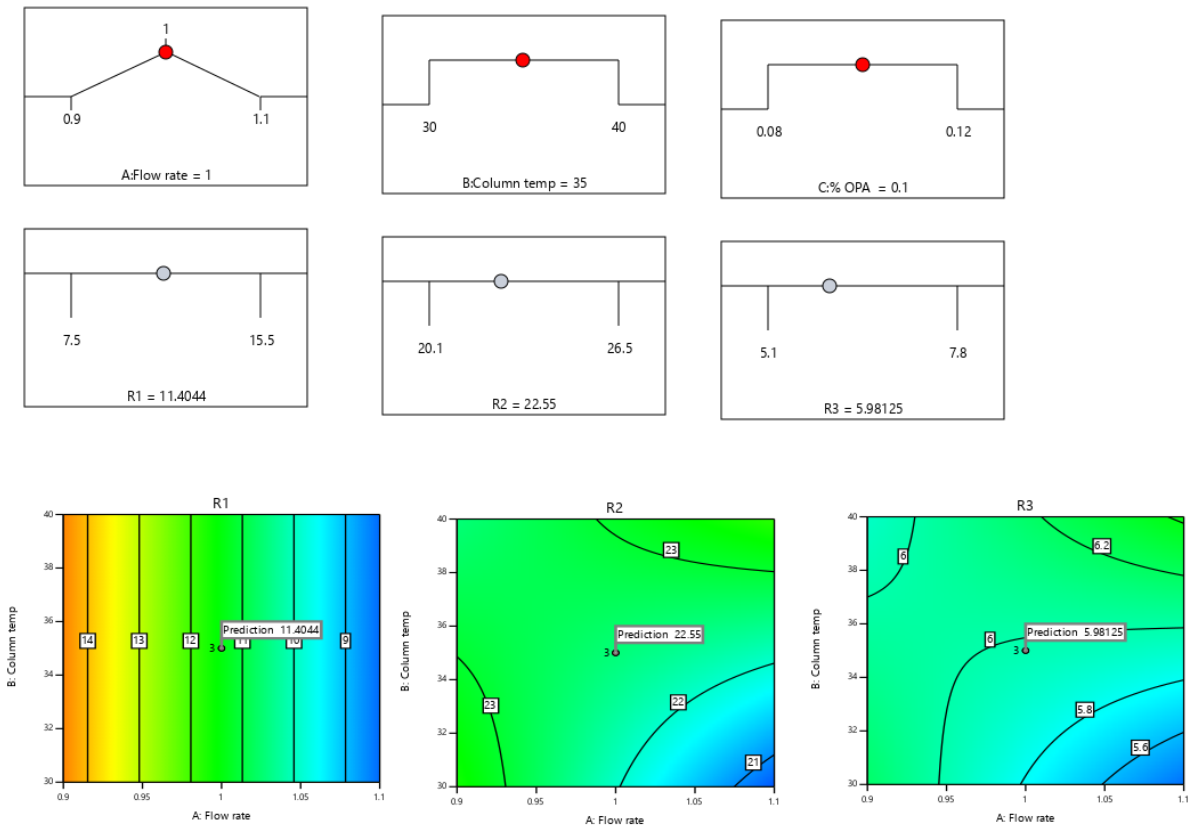


Fig. S8. The numerical solutions plots for all responses

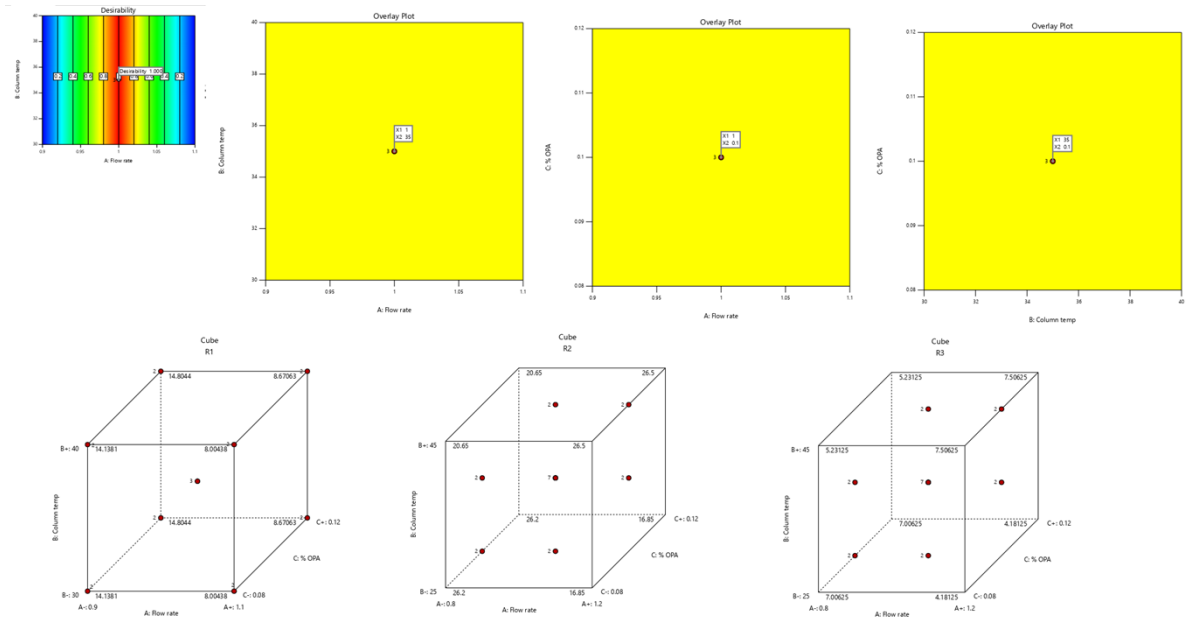


Fig. S9. 3D cube, overlay plots and desirability plots for the responses R1-R3

Table S1 Linearity plot of related novel Impurities in Bictegravir

S.No.	Results					
	Conc. (mg/ml)	Impurity-I Area (Bictegravir acid)	Conc. (mg/ml)	Impurity-II Area (Methyl Bictegravir)	Conc. (mg/ml)	Bictegravir Area
1	0.00007	952	0.00007	1073	0.00007	2383
2	0.00025	5573	0.00025	6597	0.00025	10205
3	0.00037	7930	0.00037	9894	0.00037	14935
4	0.00050	10229	0.00050	13507	0.00050	18776
5	0.00062	12192	0.00062	16870	0.00062	23589
6	0.00075	14638	0.00075	19836	0.00075	28277
Correlation coefficient	-	0.9991	-	0.9996	-	0.9997

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168 Table S2 Comparison of available literature methods with the currently developed method

Name of the source	Mobile phase	Condition	Column	Observation
Prathipati, P. K., Mandal, S., & Destache, C. J. (2018. Biomedical Chromatography, e4379	Mobile phase A consisted of 0.1% formic acid in water and Mobile phase B consisted of 0.1% formic acid in acetonitrile (80: 20 % v/v)	Flow: 0.250 mL/min Column temperature: 4°C - 30°C Injection Volume: 2µl Elution: Isocratic	Kinetex EVO C18 column, 50 × 3.0 mm, 5 µm	1. In this method LC-MS/MS is used to quantify the Bictegravir content along with other antiretroviral drug in Human plasma. 2. No related impurities of Bictegravir or other impurities were determined in this method.
Kokkirala, T. K., & Suryakala, D. (2019). Journal of Taibah University for Science, 13(1), 1137–1146.	Mobile phase A consisted of 0.1% Orthophosphoric acid buffer and Mobile phase B consisted of Acetonitrile (50:50 % v/v)	Flow: 1 mL/min Column temperature: 30°C Injection Volume: 10µl Elution: Isocratic	Denali C18 column (150 mm × 4.6 mm, 5 µm)	1. This is an assay method in which RP-HPLC is used to quantify the Bictegravir content in bulk and pharmaceutical dosage form. This is a cost reducing method but it cannot be used for the determination of related substances. 2. No related impurities of Bictegravir or other impurities determination were addressed in this method.
Gouget, H., Noé, G., Barrail-Tran, A., & Furlan, V. (2019). j.jpba.2019.113057	Mobile phase A consisted of 0.1% (v/v)formic acid in water and phase B consisted of and acetonitrile	Flow: 0.45 mL/min Column temperature: 10°C -50°C Injection Volume: 40µl Elution: Gradient	Acclaim TM RSLC 120 C18 column (2.1 × 100 mm, 2.2 m)	This UPLC-MS/MS method is used to quantify the Bictegravir content in Human plasma. 2. No related impurities of Bictegravir or other impurities were determined in this method.

Attaluri Tanuja*, Seru Ganapaty (2022). IndJPhaEdRes-56-4-1190	Mobile phase A consisted of Acetonitrile and Mobile phase B consisted of 0.1% Formic acid in Water (70:30 % v/v)	Flow: 0.15 mL/min Column temperature: 30°C Injection Volume: 10µl Elution: Isocratic	Zorbax XDB C18 Column (2.1 X 50 mm, 5µm)	1. In this Bio-analytical assay method UPLC-MS is used to quantify the Bictegravir content in Human plasma. 2. No related impurities of Bictegravir or other impurities were determined in this method.
Current method	Mobile phase A- 0.1% Orthophosphoric acid in water. Mobile phase B- Acetonitrile	Flow: 1.0ml/min Column temperature: Ambient Injection Volume: 10µl Elution: Gradient	Xterra RP18 150mm×4.6, 5um	Determined the related impurities in Bictegravir drug substance in a simple and cost reducing RP-HPLC method and the method was evaluated by application of QbD(Design expert).

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173 Table S3 Application of the developed method in real samples analysis

Test by HPLC % area Chromatographic purity	In-process batch sample	Holding time Initial sample	Holding time 3rd Month sample
Bictegravir	95.78	99.81	99.78
Impurity-I (Bictegravir acid)	2.06	ND	ND
Impurity-II Area (Methyl Bictegravir)	2.12	ND	Below LOQ (LOQ = 0.07)
Unknown	0.08	0.01	0.03

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