Supporting Information

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Establishment of HPLC method for evaluation of process related impurities of letermovir and LCMS/MS characterization of forced degradation compounds

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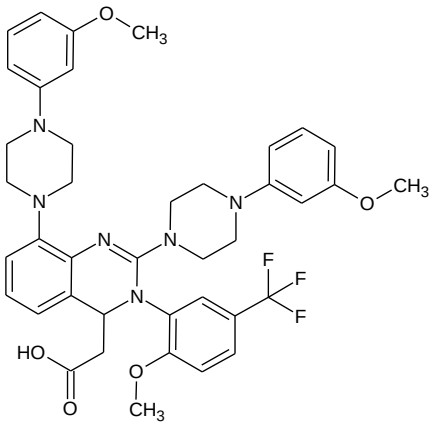
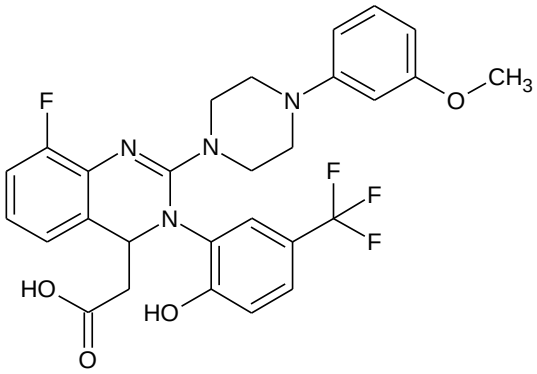


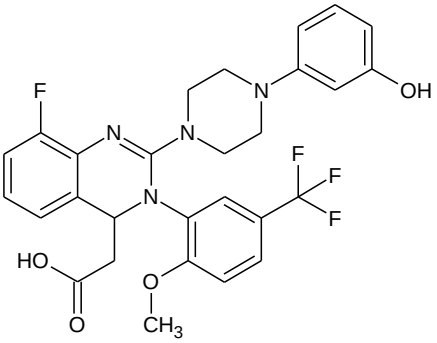
Impurity 1

Name: *(S)-2-(3-(2-Methoxy-5-(trifluoromethyl)phenyl)-2,8-bis(4-(3-methoxyphenyl)piperazin-1-yl)-3,4-dihydroquinazolin-4-yl)acetic acid*;
Formula: $C_{40}H_{43}F_3N_6O_5$; **Mass**: 744.8 g/mol



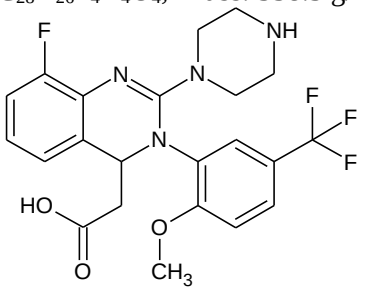
Impurity 2

Name: *(S)-2-(8-Fluoro-3-(2-hydroxy-5-(trifluoromethyl)phenyl)-2-(4-(3-methoxyphenyl)piperazin-1-yl)-3,4-dihydroquinazolin-4-yl)acetic acid*;
Formula: $C_{28}H_{26}F_4N_4O_4$; **Mass**: 558.5 g/mol



Impurity 3

Name: *(S)-2-(8-Fluoro-2-(4-(3-hydroxyphenyl)piperazin-1-yl)-3-(2-methoxy-5-(trifluoromethyl)phenyl)-3,4-dihydroquinazolin-4-yl)acetic acid*;
Formula: $C_{28}H_{26}F_4N_4O_4$; **Mass**: 558.5 g/mol



Impurity 4

Name: *(S)-2-(8-Fluoro-3-(2-methoxy-5-(trifluoromethyl)phenyl)-2-(piperazin-1-yl)-3,4-dihydroquinazolin-4-yl)acetic acid*;
Formula: $C_{22}H_{22}F_4N_4O_3$; **Mass**: 466.4 g/mol

Figure S1: Details of impurities in the study

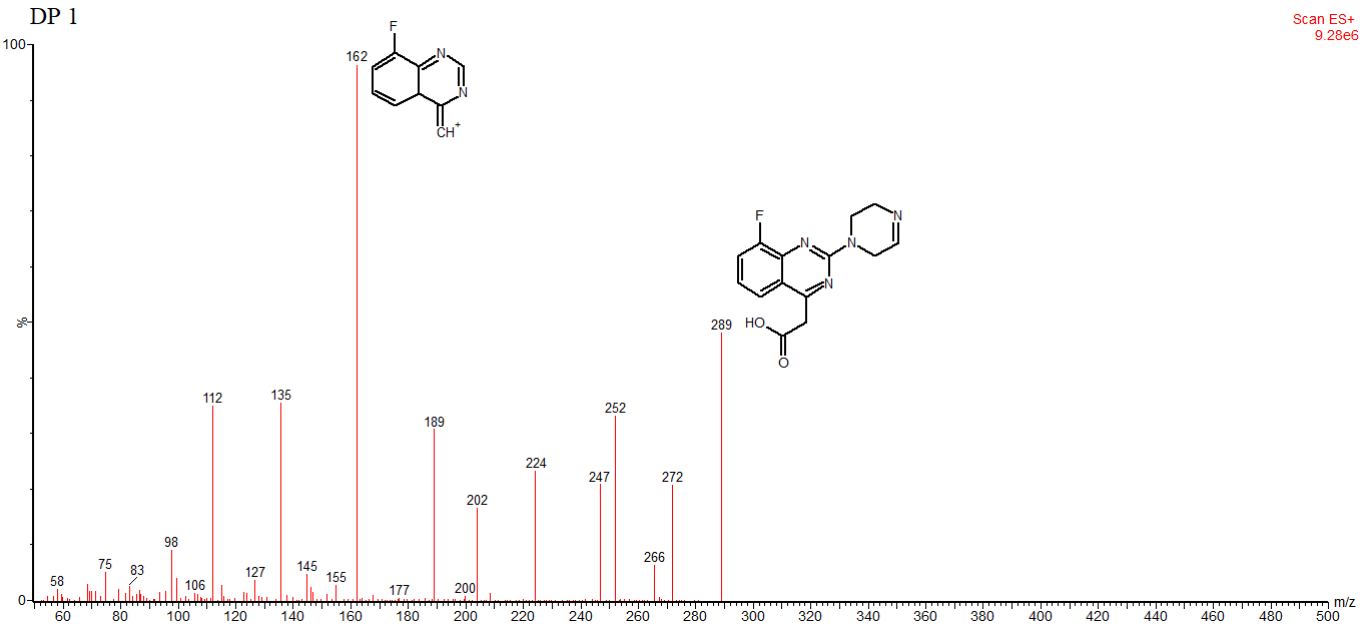


Figure S2A: Mass spectrum of DP 1

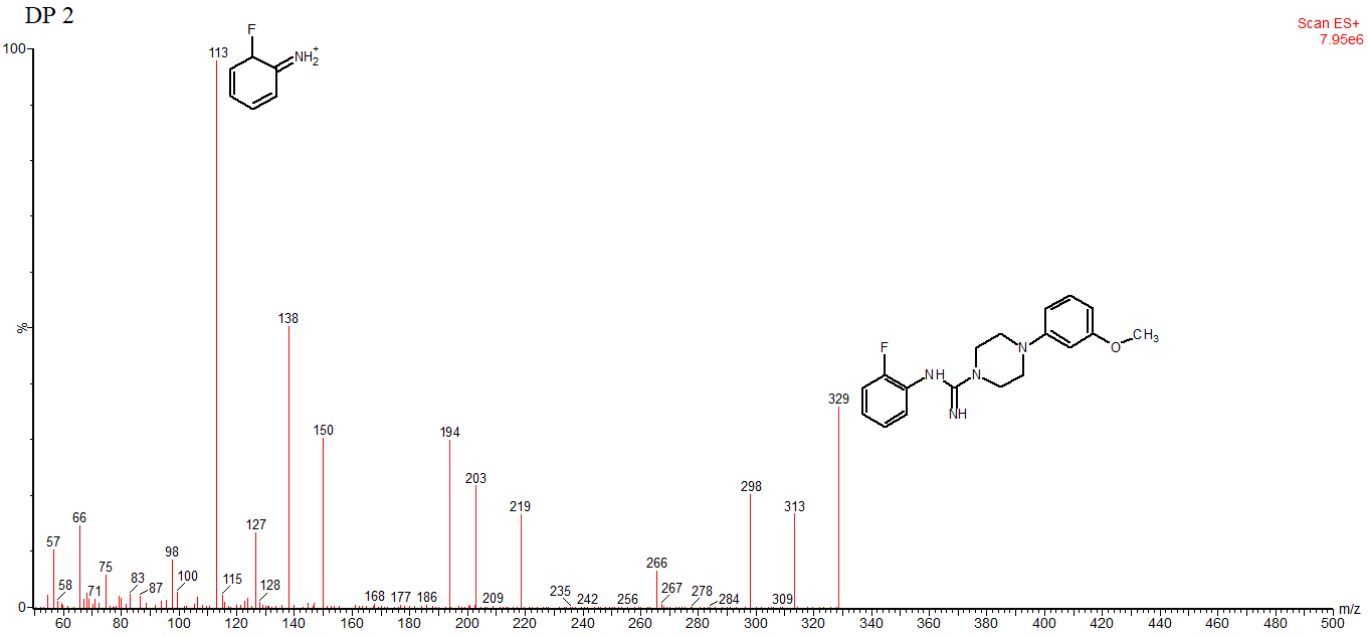


Figure S2B: Mass spectrum of DP 2

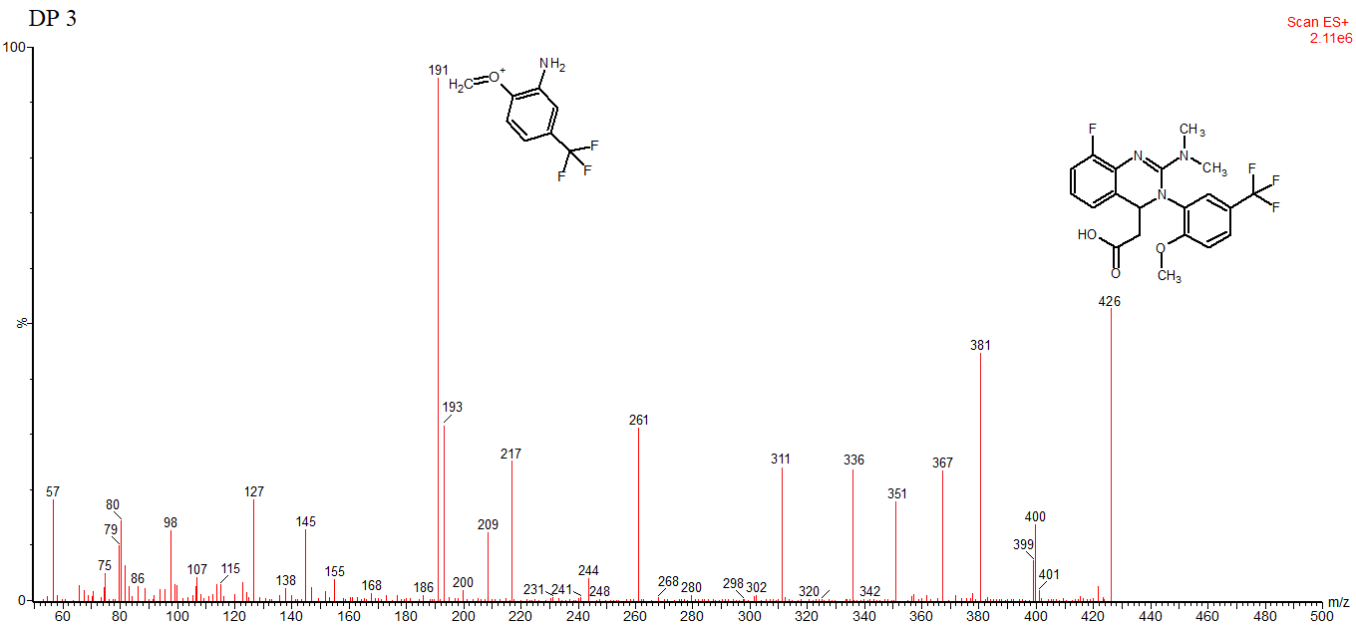


Figure S2C: Mass spectrum of DP 3

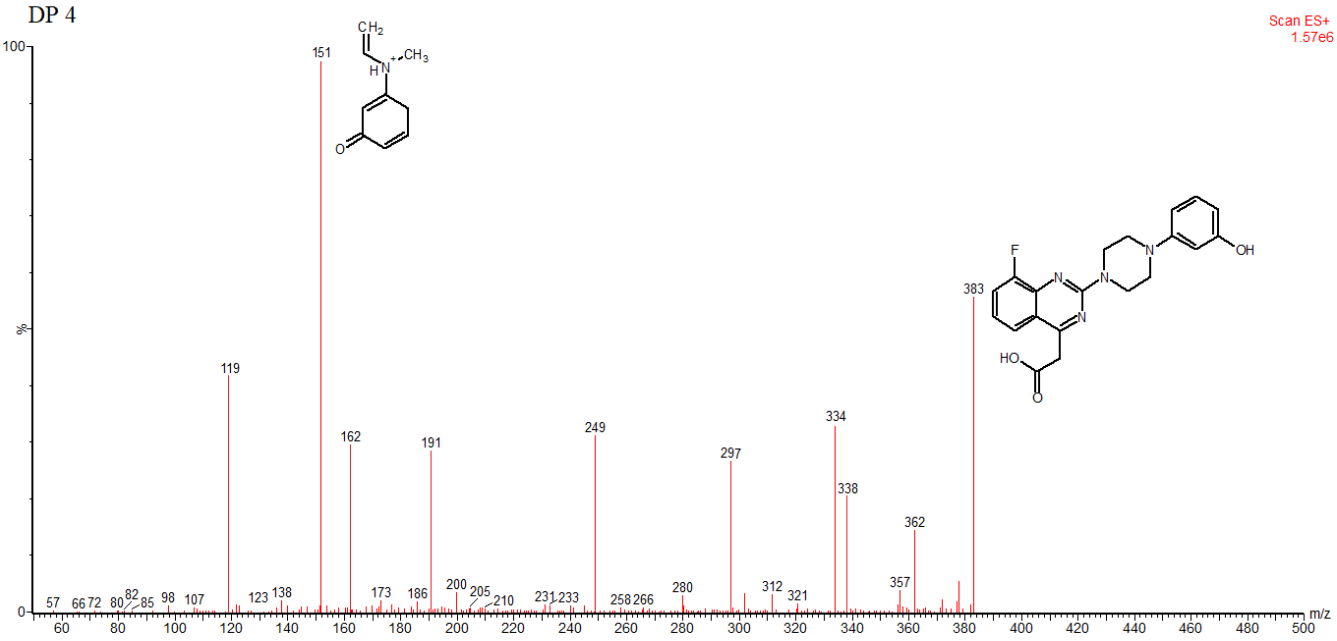


Figure S2D: Mass spectrum of DP 4

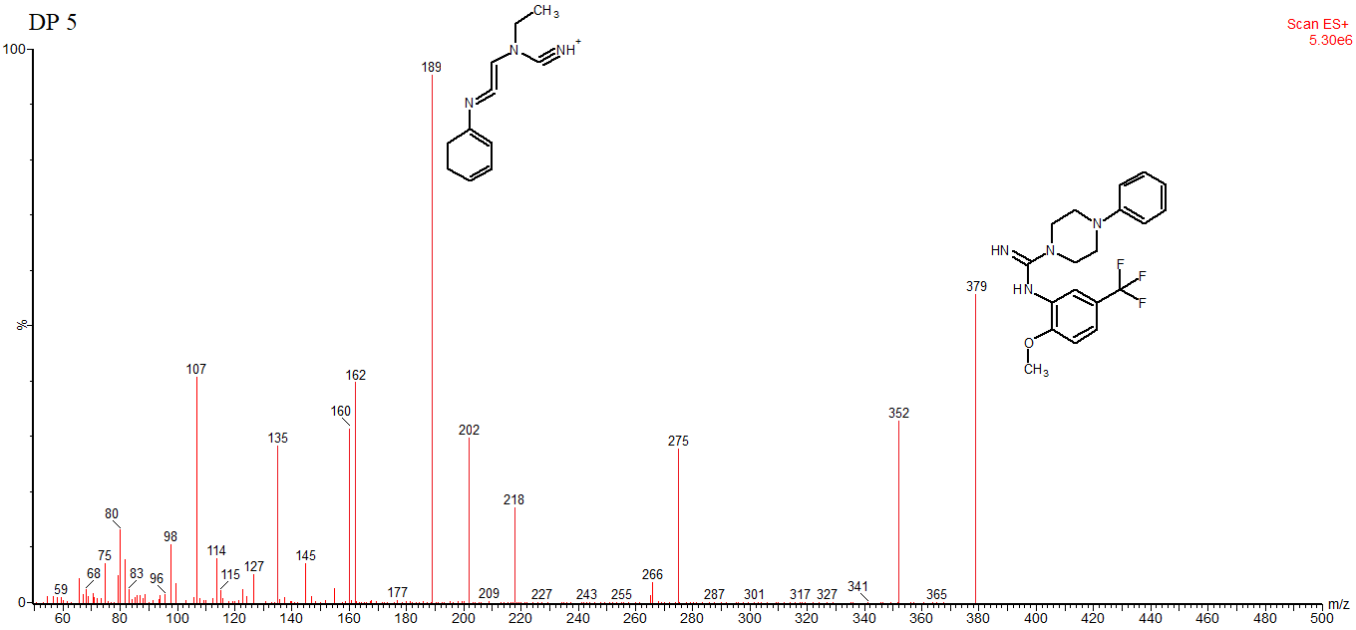


Figure S2E: Mass spectrum of DP 5

Table S1: Uncertainty Assessment of letermovir

Uncertainty in standard sample		X	Xi	N	$U_{standard}$ Formula	$U_{standard}$
		100	98.75	3	$\frac{X - Xi}{\sqrt{n}}$	0.722
Uncertainty in Slope of Calibration Plot		SD	n	Standard Error	$U_{Calibration}$ Formula	$U_{Calibration}$
Slope 1	9837.3	7.65	3	4.419	$\frac{Standard\ Error\ of\ Slope\ X\ 100}{Slope}$	0.045
Slope 2	9826.8					
Slope 3	9841.7					
Uncertainty in Recovery of Sample		SD	n	Standard Error	$U_{Recovery}$ Formula	$U_{Recovery}$
Recovery 1	98.63	0.597	6	0.244	$\frac{Standard\ Error\ of\ Recovery\ X\ 100}{Recovery}$	0.247
Recovery 2	98.14					
Recovery 3	99.47					
Recovery 4	98.59					
Recovery 5	99.03					
Recovery 6	99.74					
Uncertainty associated with repeatability		SD	n	Standard Error	$U_{Repeatability}$ Formula	$U_{Repeatability}$
Repeatability 1	149.58	0.496	6	0.202	$\frac{Standard\ Error\ of\ Repeatability\ X\ 100}{Repeatability}$	0.136
Repeatability 2	149.63					
Repeatability 3	148.99					
Repeatability 4	150.52					
Repeatability 5	149.57					
Repeatability 6	149.85					
					$U_{Combined}$ Formula	$U_{Combined}$
					$\sqrt{(U_{standard})^2+(U_{Calibration})^2+(U_{Recovery})^2+(U_{Repeatability})^2}$	0.776
					$U_{Expanded}$ Formula	$U_{Expanded}$
					$U_{Combined}$ X 1.96	1.521

Table S2: Uncertainty Assessment of Impurity 1

Uncertainty in standard sample		X	Xi	N	$U_{standard}$ Formula	$U_{standard}$
		100	99.32	3	$\frac{X - Xi}{\sqrt{n}}$	0.393
Uncertainty in Slope of Calibration Plot		SD	n	Standard Error	$U_{Calibration}$ Formula	$U_{Calibration}$
Slope 1	801787	131.50	3	75.93	$\frac{Standard\ Error\ of\ Slope\ X\ 100}{Slope}$	0.009
Slope 2	801524					
Slope 3	801658					
Uncertainty in Recovery of Sample		SD	n	Standard Error	$U_{Recovery}$ Formula	$U_{Recovery}$
Recovery 1	98.74	1.186	6	0.484	$\frac{Standard\ Error\ of\ Recovery\ X\ 100}{Recovery}$	0.488
Recovery 2	98.34					
Recovery 3	98.45					
Recovery 4	100.58					
Recovery 5	100.95					
Recovery 6	98.44					
Uncertainty associated with repeatability		SD	n	Standard Error	$U_{Repeatability}$ Formula	$U_{Repeatability}$
Repeatability 1	0.1498	0.0019	6	0.00078	$\frac{Standard\ Error\ of\ Repeatability\ X\ 100}{Repeatability}$	0.531
Repeatability 2	0.1463					
Repeatability 3	0.1488					
Repeatability 4	0.1479					
Repeatability 5	0.1465					
Repeatability 6	0.1445					
					$U_{Combined}$ Formula	$U_{Combined}$
					$\sqrt{(U_{standard})^2+(U_{Calibration})^2+(U_{Recovery})^2+(U_{Repeatability})^2}$	0.821
					$U_{Expanded}$ Formula	$U_{Expanded}$
					$U_{Combined}$ X 1.96	1.609

Table S3: Uncertainty Assessment of Impurity 2

Uncertainty in standard sample		X	Xi	N	$U_{standard}$ Formula	$U_{standard}$
		100	98.75	3	$\frac{X - Xi}{\sqrt{n}}$	0.722
Uncertainty in Slope of Calibration Plot		SD	n	Standard Error	$U_{Calibration}$ Formula	$U_{Calibration}$
Slope 1	370263	416.34	3	240.37	$\frac{Standard\ Error\ of\ Slope\ X\ 100}{Slope}$	0.065
Slope 2	370114					
Slope 3	370898					
Uncertainty in Recovery of Sample		SD	n	Standard Error	$U_{Recovery}$ Formula	$U_{Recovery}$
Recovery 1	99.36	0.494	6	0.201	$\frac{Standard\ Error\ of\ Recovery\ X\ 100}{Recovery}$	0.202
Recovery 2	100.41					
Recovery 3	100.32					
Recovery 4	99.75					
Recovery 5	99.82					
Recovery 6	99.18					
Uncertainty associated with repeatability		SD	n	Standard Error	$U_{Repeatability}$ Formula	$U_{Repeatability}$
Repeatability 1	0.1485	0.00076	6	0.00031	$\frac{Standard\ Error\ of\ Repeatability\ X\ 100}{Repeatability}$	0.209
Repeatability 2	0.1479					
Repeatability 3	0.1474					
Repeatability 4	0.1489					
Repeatability 5	0.1494					
Repeatability 6	0.1491					
					$U_{Combined}$ Formula	$U_{Combined}$
					$\sqrt{(U_{standard})^2+(U_{Calibration})^2+(U_{Recovery})^2+(U_{Repeatability})^2}$	0.781
					$U_{Expanded}$ Formula	$U_{Expanded}$
					$U_{Combined}$ X 1.96	1.530

Table S4: Uncertainty Assessment of Impurity 3

Uncertainty in standard sample		X	Xi	N	$U_{standard}$ Formula	$U_{standard}$
		100	99.25	3	$\frac{X - Xi}{\sqrt{n}}$	0.433
Uncertainty in Slope of Calibration Plot		SD	n	Standard Error	$U_{Calibration}$ Formula	$U_{Calibration}$
Slope 1	664903	324.84	3	187.55	$\frac{Standard\ Error\ of\ Slope\ X\ 100}{Slope}$	0.028
Slope 2	664258					
Slope 3	664513					
Uncertainty in Recovery of Sample		SD	n	Standard Error	$U_{Recovery}$ Formula	$U_{Recovery}$
Recovery 1	99.63	0.367	6	0.150	$\frac{Standard\ Error\ of\ Recovery\ X\ 100}{Recovery}$	0.151
Recovery 2	99.74					
Recovery 3	99.37					
Recovery 4	98.85					
Recovery 5	98.91					
Recovery 6	99.43					
Uncertainty associated with repeatability		SD	n	Standard Error	$U_{Repeatability}$ Formula	$U_{Repeatability}$
Repeatability 1	0.1496	0.00082	6	0.00033	$\frac{Standard\ Error\ of\ Repeatability\ X\ 100}{Repeatability}$	0.224
Repeatability 2	0.1491					
Repeatability 3	0.1498					
Repeatability 4	0.1486					
Repeatability 5	0.1503					
Repeatability 6	0.1509					
					$U_{Combined}$ Formula	$U_{Combined}$
					$\sqrt{(U_{standard})^2+(U_{Calibration})^2+(U_{Recovery})^2+(U_{Repeatability})^2}$	0.511
					$U_{Expanded}$ Formula	$U_{Expanded}$
					$U_{Combined}$ X 1.96	1.002

Table S5: Uncertainty Assessment of Impurity 4

Uncertainty in standard sample		X	Xi	N	$U_{standard}$ Formula	$U_{standard}$
		100	99.65	3	$\frac{X - Xi}{\sqrt{n}}$	0.202
Uncertainty in Slope of Calibration Plot		SD	n	Standard Error	$U_{Calibration}$ Formula	$U_{Calibration}$
Slope 1	474396	1626.67	3	939.16	$\frac{Standard\ Error\ of\ Slope\ X\ 100}{Slope}$	0.198
Slope 2	474029					
Slope 3	471413					
Uncertainty in Recovery of Sample		SD	n	Standard Error	$U_{Recovery}$ Formula	$U_{Recovery}$
Recovery 1	99.44	0.397	6	0.162	$\frac{Standard\ Error\ of\ Recovery\ X\ 100}{Recovery}$	0.162
Recovery 2	100.59					
Recovery 3	100.12					
Recovery 4	100.24					
Recovery 5	99.88					
Recovery 6	99.79					
Uncertainty associated with repeatability		SD	n	Standard Error	$U_{Repeatability}$ Formula	$U_{Repeatability}$
Repeatability 1	0.1487	0.00139	6	0.00057	$\frac{Standard\ Error\ of\ Repeatability\ X\ 100}{Repeatability}$	0.381
Repeatability 2	0.1493					
Repeatability 3	0.1476					
Repeatability 4	0.1509					
Repeatability 5	0.1512					
Repeatability 6	0.1504					
					$U_{Combined}$ Formula	$U_{Combined}$
					$\sqrt{(U_{standard})^2+(U_{Calibration})^2+(U_{Recovery})^2+(U_{Repeatability})^2}$	0.502
					$U_{Expanded}$ Formula	$U_{Expanded}$
					$U_{Combined}$ X 1.96	0.983