

Table S1: ANOVA for quadratic model of retention time

Source	Sum of squares	df	Mean square	F-value	p-value
Model	24.90	9	2.77	1479.05	<0.0001
A-Flow Rate (mL/min)	6.81	1	6.81	3639.35	<0.0001
B-Oven temperature (°C)	0.0023	1	0.0023	1.22	0.3063
C-Concentration of ACN (%)	16.94	1	16.94	9058.48	<0.0001
AB	0.0003	1	0.0003	0.1485	0.7114
AC	0.1777	1	0.1777	94.98	<0.0001
BC	2.500E-07	1	2.500E-07	0.0001	0.9911
A ²	0.1327	1	0.1327	70.93	<0.0001
B ²	0.0009	1	0.0009	0.5054	0.5002
C ²	0.7965	1	0.7965	425.82	<0.0001

Table S2: ANOVA for quadratic model of peak area

Source	Sum of squares	df	Mean square	F-value	p-value
Model	1.155E+10	9	1.284E+09	666.19	<0.0001
A-Flow Rate (mL/min)	1.129E+10	1	1.129E+10	5859.12	<0.0001
B-Oven temperature (°C)	49088.89	1	49088.89	0.0255	0.8777
C-Concentration of ACN (%)	1.033E+05	1	1.033E+05	0.0536	0.8235
AB	5.110E+05	1	5.110E+05	0.2652	0.6224
AC	1.913E+07	1	1.913E+07	9.93	0.0161
BC	5.278E+05	1	5.278E+05	0.2739	0.6169
A ²	2.397E+08	1	2.397E+08	124.42	<0.0001
B ²	2.523E+06	1	2.523E+06	1.31	0.2901
C ²	6.513E+05	1	6.513E+05	0.3380	0.5792

Table S3: ANOVA for quadratic model of NTP

Source	Sum of squares	df	mean square	F-value	p-value
Model	2.668E+07	9	2.965E+06	605.88	<0.0001
A-Flow Rate (mL/min)	1.114E+07	1	1.114E+07	2276.31	<0.0001
B-Oven temperature (°C)	5.441E+05	1	5.441E+05	111.19	<0.0001
C-Concentration of ACN (%)	1.471E+07	1	1.471E+07	3006.91	<0.0001
AB	6642.25	1	6642.25	1.36	0.2822
AC	10.03	1	10.03	0.0020	0.9652
BC	24440.11	1	24440.11	4.99	0.0605
A ²	80553.69	1	80553.69	16.46	0.0048
B ²	11502.33	1	11502.33	2.35	0.1691
C ²	1.575E+05	1	1.575E+05	32.18	0.0008

Table S4: ANOVA for quadratic model of tailing factor

Source	Sum of squares	df	Mean square	F-value	p-value
Model	0.0024	9	0.0003	52.35	<0.0001
A-Flow Rate (ml/min)	0.0004	1	0.0004	81.52	<0.0001
B-Oven temperature (°C)	0.0007	1	0.0007	137.89	<0.0001
C-Concentration of ACN (%)	0.0013	1	0.0013	245.14	<0.0001
AB	2.250E-06	1	2.250E-06	0.4412	0.5278
AC	1.111E-07	1	1.111E-07	0.0218	0.8868
BC	4.000E-06	1	4.000E-06	0.7844	0.4052
A ²	0.0000	1	0.0000	4.34	0.0758
B ²	6.148E-06	1	6.148E-06	1.21	0.3085
C ²	6.579E-08	1	6.579E-08	0.0129	0.9128

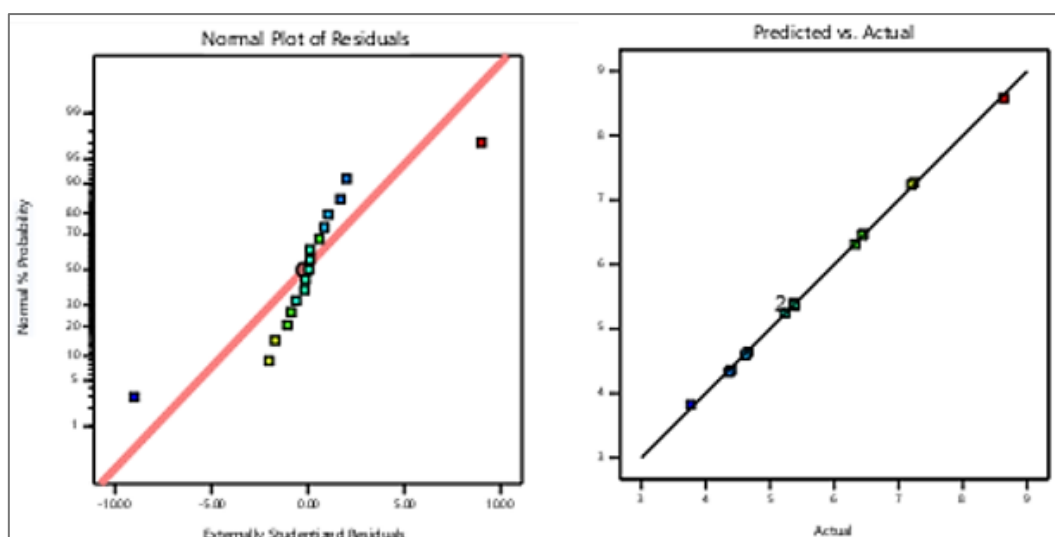


Fig. S1: Residual plots of retention time

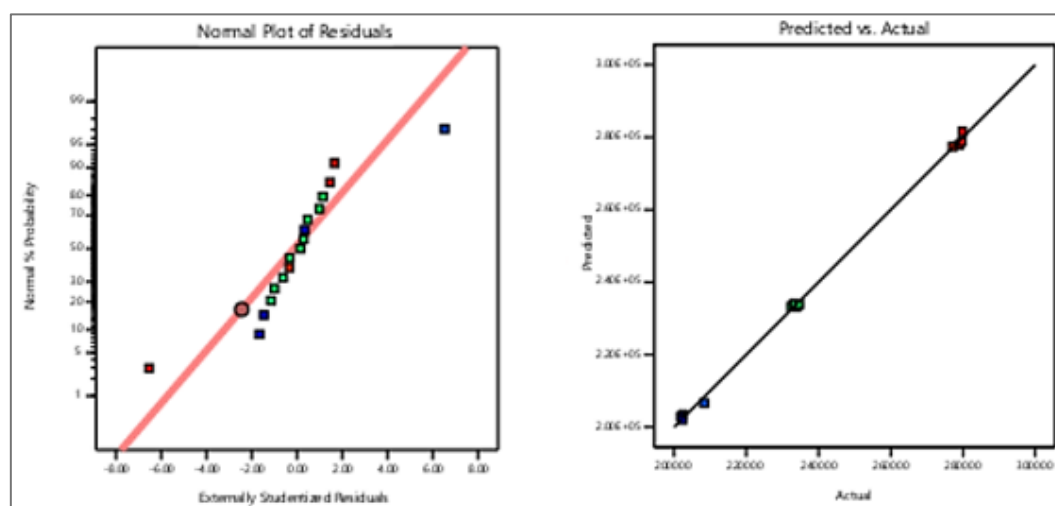


Fig. S2: Residual plots of peak area

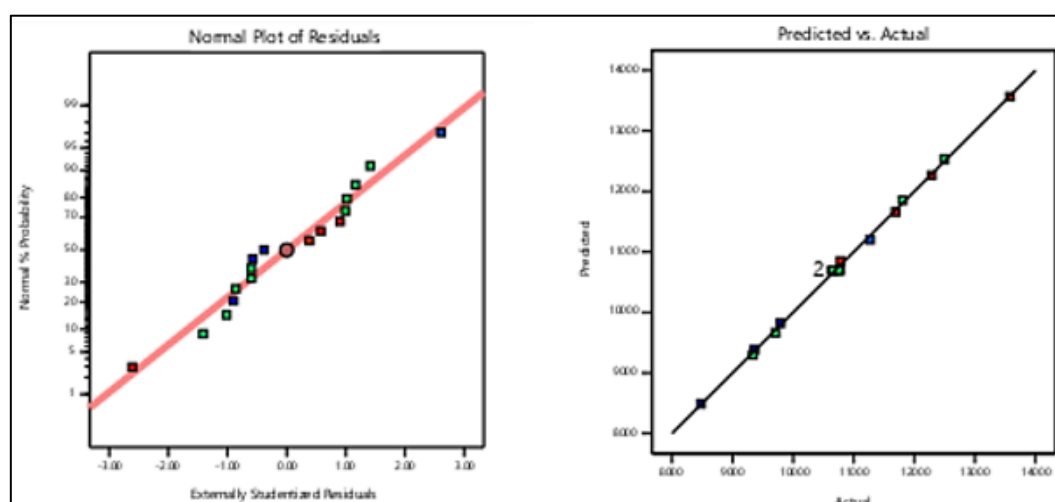


Fig. S3: Residual plots of NTP

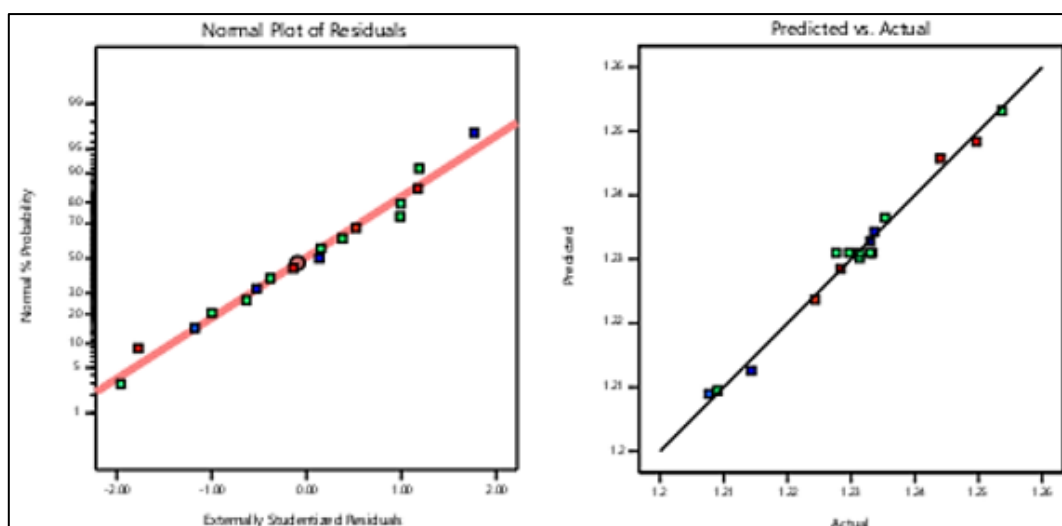


Fig. S4: Residual plots of tailing factor

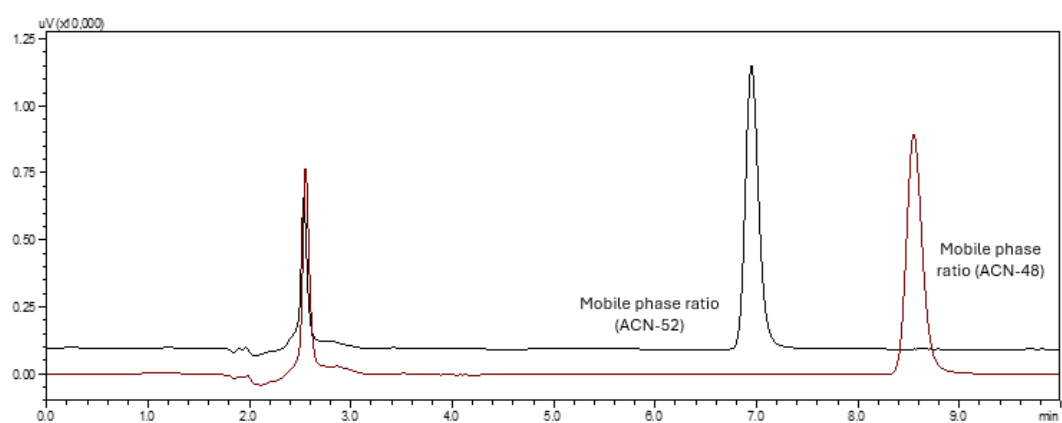


Fig. S5: Overlay of chromatogram for robustness by changing mobile phase ratio

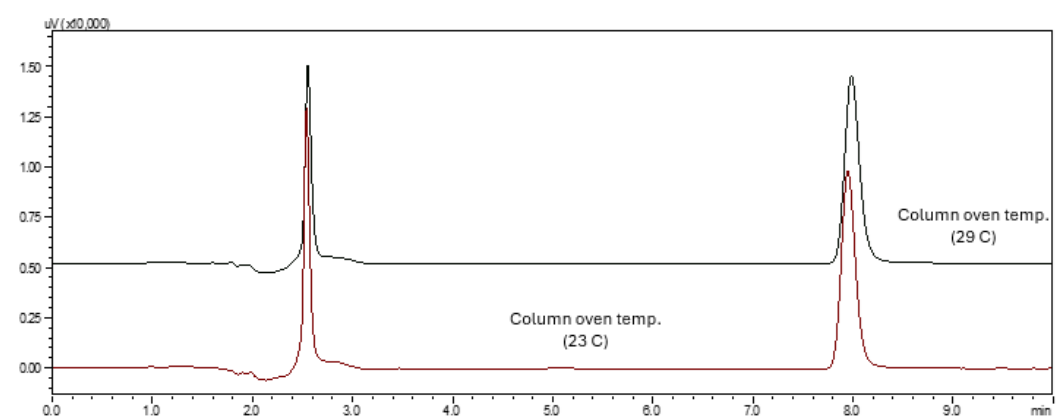


Fig. S6: Overlay of chromatogram for robustness by changing column oven temperature

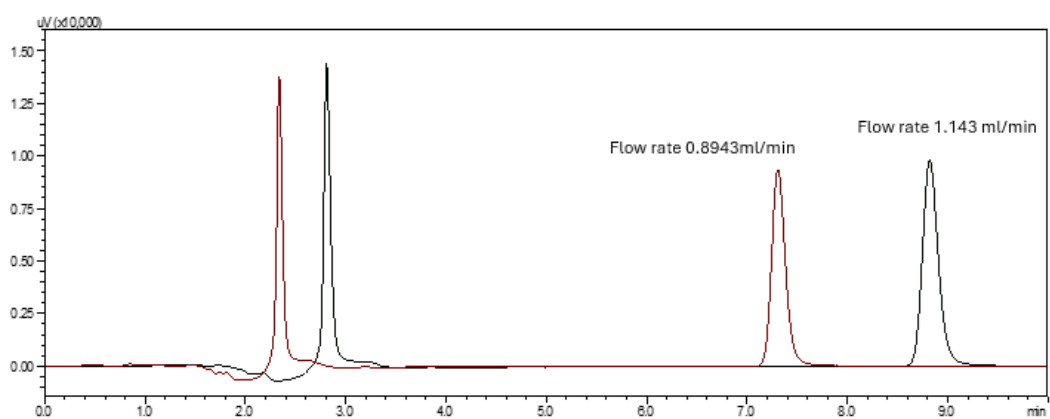


Fig. S7: Overlay of chromatogram for robustness by changing the flow rate

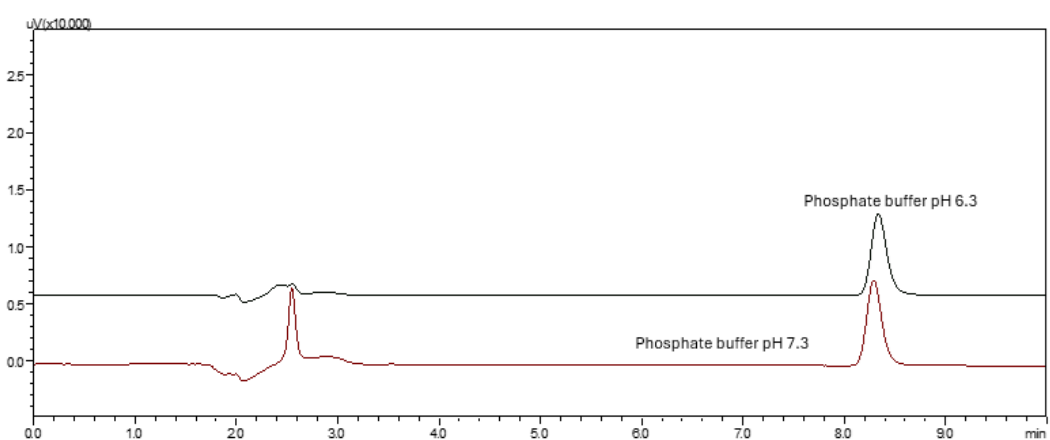


Fig. S8: Overlay of chromatogram for robustness by changing the pH

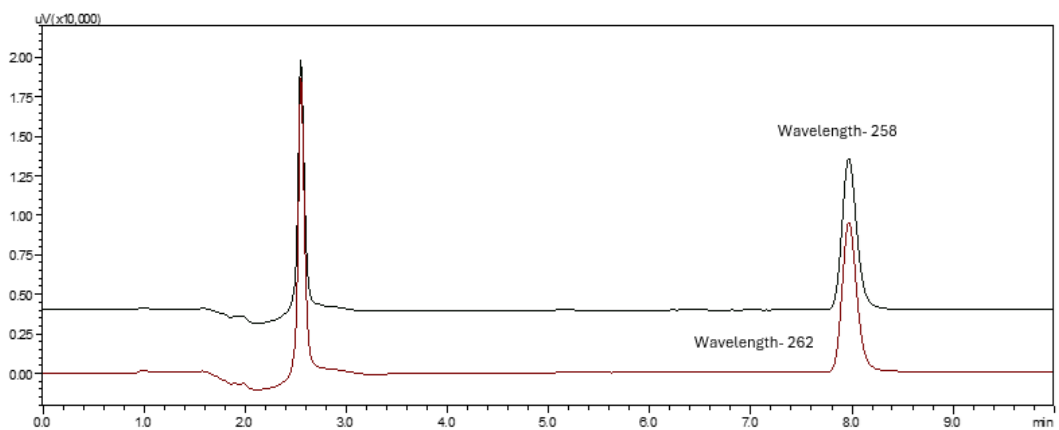


Fig. S9: Overlay of chromatogram for robustness by changing the wavelength

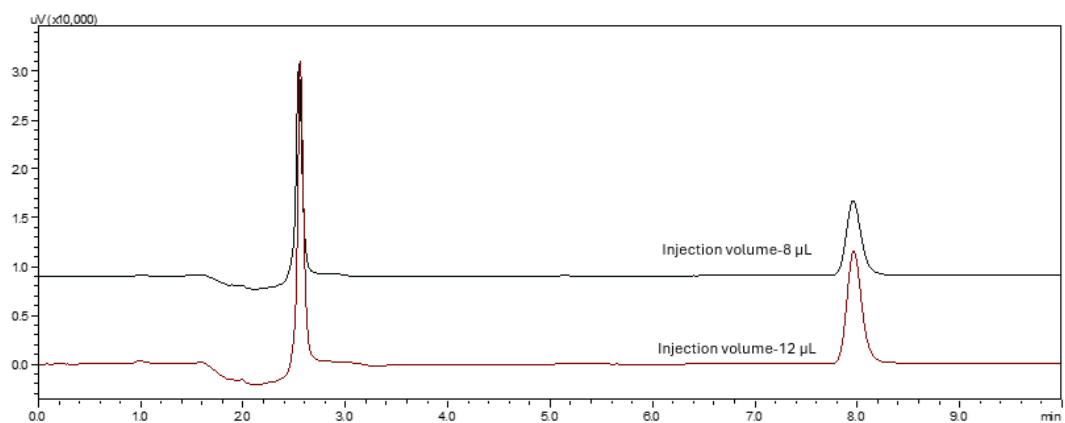


Fig. S10: Overlay of chromatogram for robustness by changing the injection volume

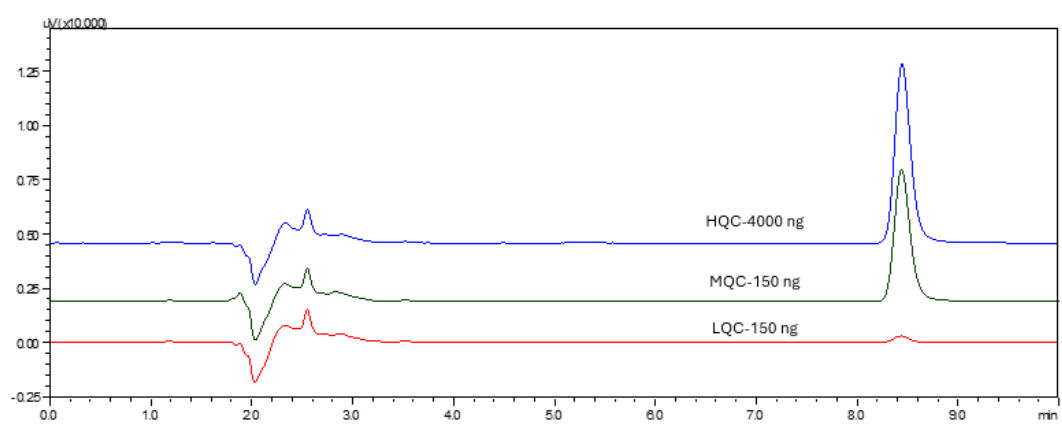


Fig. S11: Overlay of chromatogram for precision

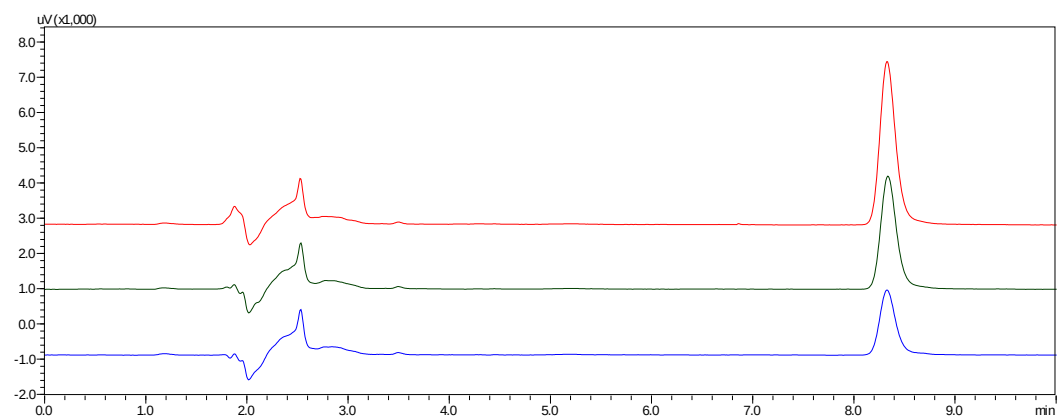


Fig. S12: Overlay of chromatogram for accuracy