

Supplementary Data

Development of an AQbD-Guided Sustainable RP-HPLC Method for Simultaneous Quantification of Saracatinib and Rapamycin in Lipid–Polymer Hybrid Nanocarriers

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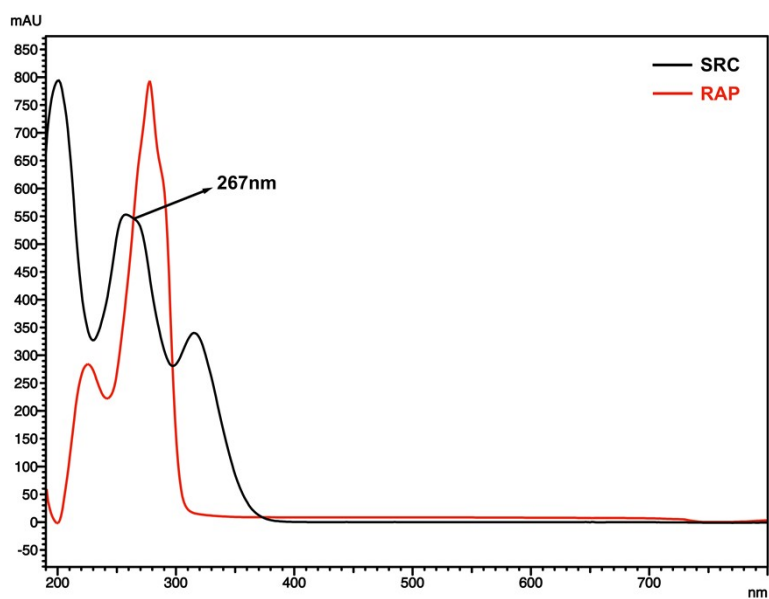


Figure S1. Illustration of the isobestic point in the UV absorption spectra of SRC (black line) and RAP (red line).

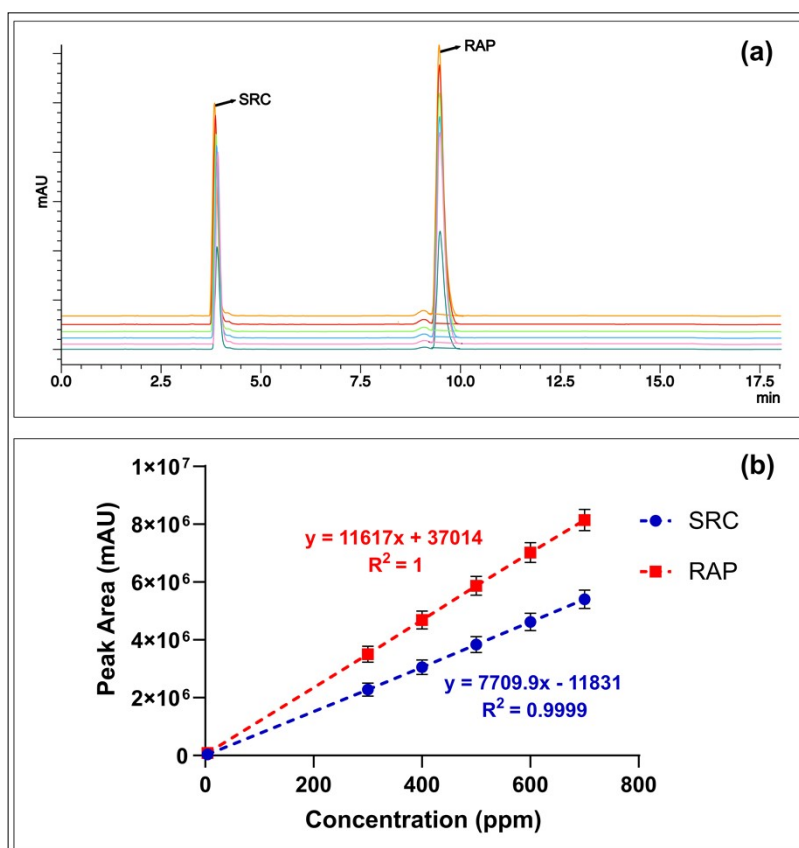


Figure S2. Linearity of SRC and RAP; (a) chromatograms of various concentrations ranging from the LOD to 140% of the desired concentration (i.e. 500 $\mu\text{g}/\text{mL}$) for both drugs, and (b) standard calibration curve for both SRC and RAP with their respective regression equations. The findings are depicted as mean \pm SD, $n = 3$.

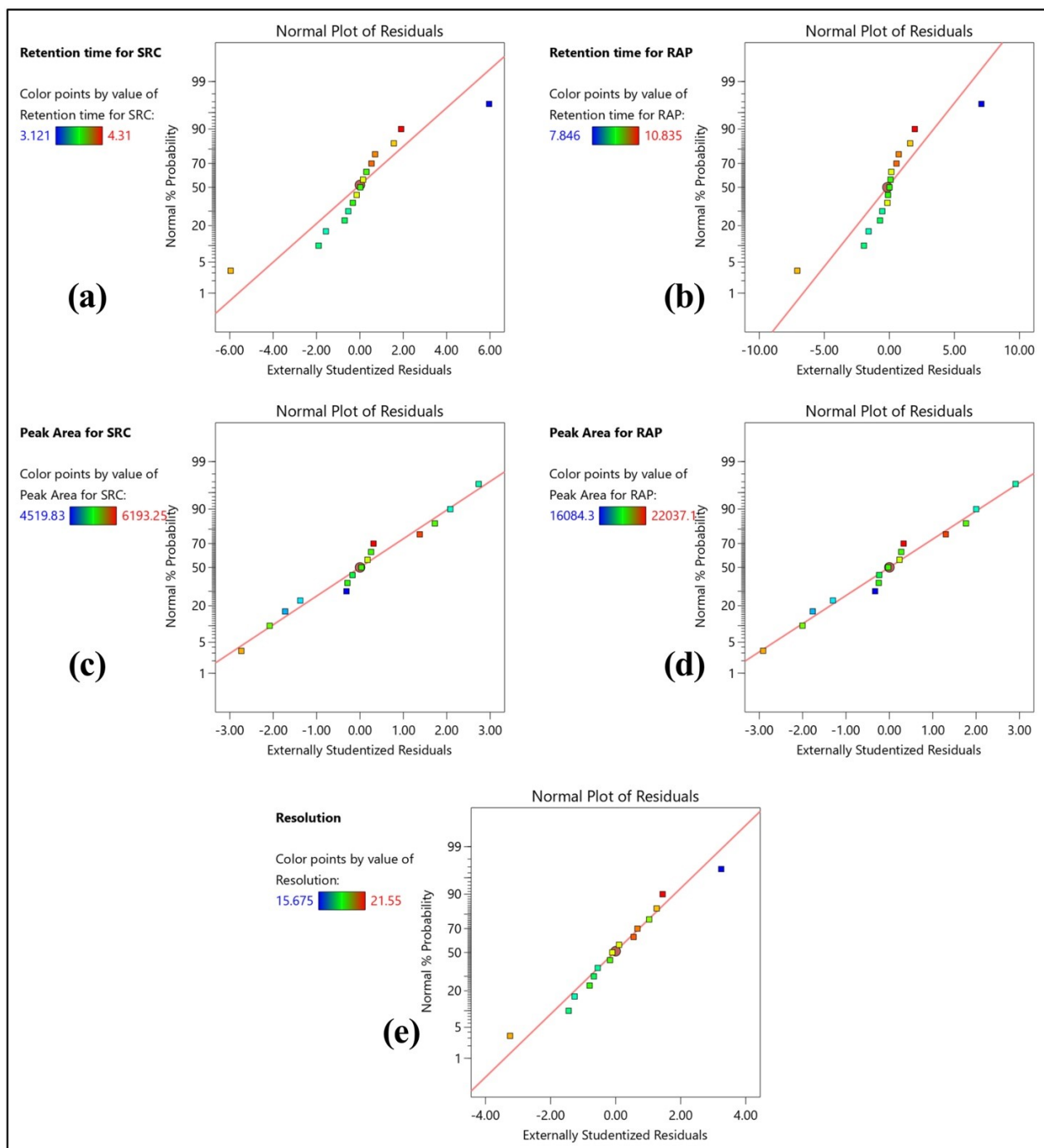


Figure S3. Normal plots of residuals for the responses (a) SRC retention time; (b) RAP retention time; (c) SRC peak area; (d) RAP peak area; and (e) resolution.

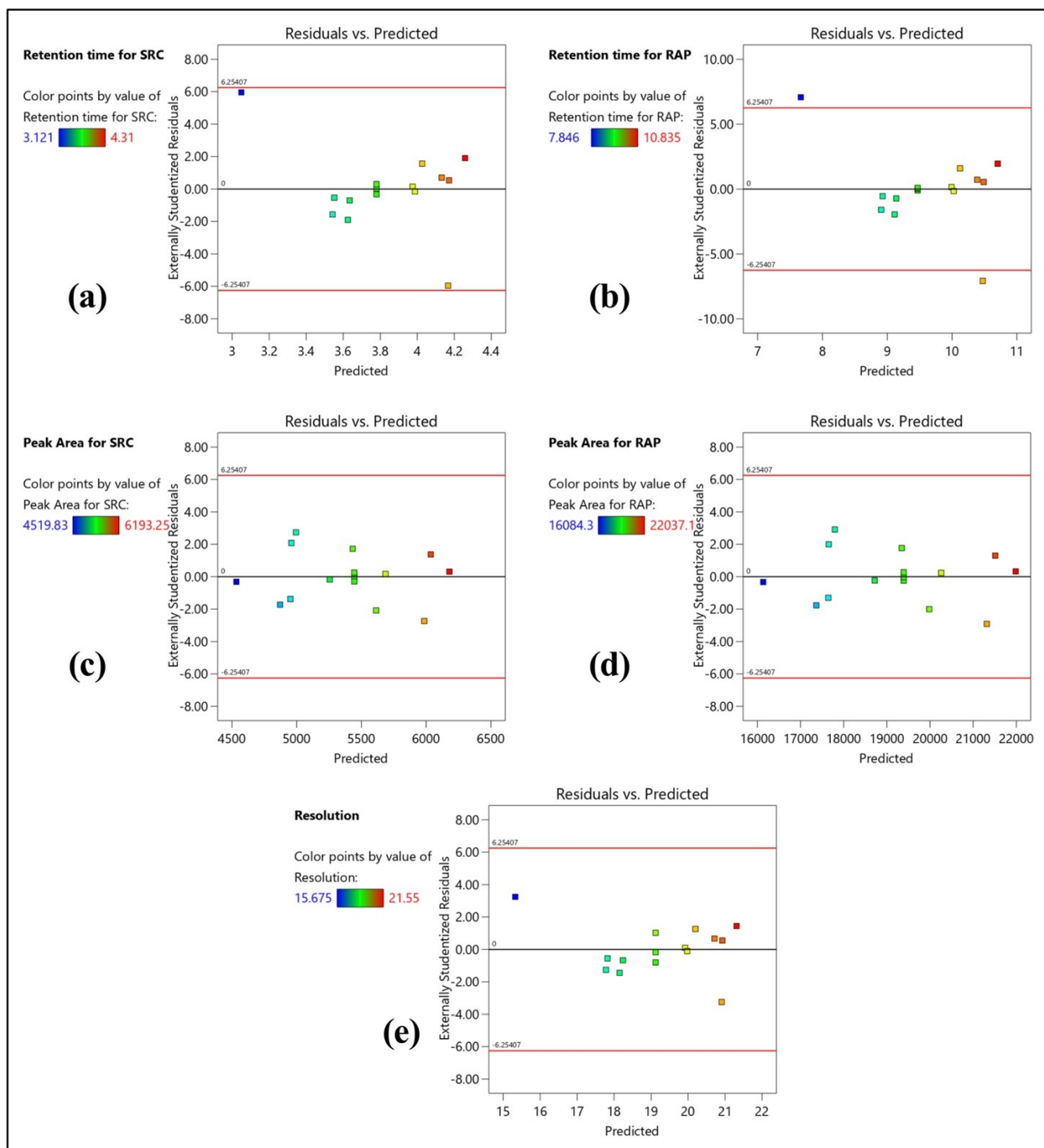


Figure S4. Residual vs Predicted plots for the responses (a) SRC retention time; (b) RAP retention time; (c) SRC peak area; (d) RAP peak area; and (e) resolution.

Table S1. BBD variables for Chromatographic factors and their level.

Factors	Used level, actual (coded)		
	Low	Medium	High
	(-1)	(0)	(+1)
Independent variables			
Mobile phase conc. (% v/v) [A]	10	20	30
Flow rate (mL/min) [B]	0.8	1.0	1.2
Injection volume (μ L) [C]	5	10	15
Dependent responses			
Retention time of SRC (min) [R1]	Minimize		
Retention time of RAP (min) [R2]	Minimize		
Peak area of SRC (AU) [R3]	Maximize		
Peak area of RAP (AU) [R4]	Maximize		
Resolution [R5]	Maximize		

Table S2. Risk assessment for critical method parameters vs HPLC method responses

Input variables (A _n)	Responses (R _n)			Consideration
	Retention time of APIs (t _R)	Peak Area of APIs (mAU)	Resolution	
Mobile Phase concentration	+1	+1	+1	Optimized
Flow rate	+1	+1	+1	Optimized
Injection volume	0	+1	0	Optimized

Column type	+1	+1	+1	Default
Column	0	0	0	Controlled
Temperature				
Column length	0	0	0	Default
Detector	NA	NA	NA	Default
Reagents quality	-1	-1	-1	Controlled

+1 highly considerable; 0 medium considerable; -1 low considerable

Table S3. Regression terms for responses of BBD

Responses	R² value	Predicted R²	Adjusted R²
R1	0.9845	0.7589	0.9566
R2	0.9850	0.7600	0.9579
R3	0.9895	0.8359	0.9707
R4	0.9898	0.8397	0.9714
R5	0.9817	0.7669	0.9488

Table S4. Desirability function parameters for optimization

Response	Goal	Lower Limit	Upper Limit	Importance
R1	Minimize	3.12	4.31	Equal
R2	Minimize	7.84	10.83	Equal
R3	Maximize	4519	6193	Equal
R4	Maximize	16084	22037	Equal
R5	Maximize	15.7	21.6	Equal

Table S5. Robustness data of various factors

Factors	Retention time (min)*	% RSD	Peak Area*	% RSD
Saracatinib				
A: Flow rate (mL/min)				
0.9	3.85 ± 0.03	0.8	3.86 × 10 ⁶ ± 2.1 × 10 ³	0.1
1.0	3.91 ± 0.02	0.5	3.85 × 10 ⁶ ± 3.4 × 10 ³	0.1
1.1	3.85 ± 0.02	0.5	3.87 × 10 ⁶ ± 2.8 × 10 ³	0.1
B: Fluoric acid concentration in mobile phase (% w/v)				
0.09	3.85 ± 0.04	1.0	3.86 × 10 ⁶ ± 4.9 × 10 ³	0.1
0.10	3.89 ± 0.07	1.8	3.84 × 10 ⁶ ± 15.9 × 10 ³	0.4
0.11	3.85 ± 0.05	1.3	3.87 × 10 ⁶ ± 4.1 × 10 ³	0.1
C: Column Temperature (°C)				
23	3.85 ± 0.03	0.8	3.87 × 10 ⁶ ± 16.5 × 10 ³	0.4
25	3.90 ± 0.06	1.5	3.83 × 10 ⁶ ± 2.6 × 10 ³	0.1
27	3.84 ± 0.04	1.0	3.88 × 10 ⁶ ± 28.5 × 10 ³	0.7
Rapamycin				
A: Flow rate (mL/min)				
0.9	9.46 ± 0.03	0.3	5.86 × 10 ⁶ ± 12.3 × 10 ³	0.2
1.0	9.48 ± 0.02	0.2	5.96 × 10 ⁶ ± 5.4 × 10 ³	0.1

1.1	9.47 ± 0.01	0.1	5.87 × 10 ⁶ ± 17.3 × 10 ³	0.3
B: Fluoric acid concentration in mobile phase (% w/v)				
0.09	9.47 ± 0.04	0.4	5.87 × 10 ⁶ ± 16.8 × 10 ³	0.3
0.10	9.48 ± 0.02	0.2	5.94 × 10 ⁶ ± 23.5 × 10 ³	0.4
0.11	9.47 ± 0.03	0.3	5.83 × 10 ⁶ ± 15.3 × 10 ³	0.3
C: Column Temperature (°C)				
23	9.47 ± 0.05	0.5	5.88 × 10 ⁶ ± 19.6 × 10 ³	0.3
25	9.47 ± 0.02	0.2	5.90 × 10 ⁶ ± 7.3 × 10 ³	0.1
27	9.48 ± 0.01	0.1	5.87 × 10 ⁶ ± 48.1 × 10 ³	0.8

*The findings are depicted as mean ± SD; n = 3.