

A Sustainable Spectrofluorimetric Method for Ivabradine Quantification Based on Erythrosin B Quenching with Quantum Mechanical Modeling and Box-Behnken Design Optimization

Arwa Sultan Alqahtani^a, **Maram H Abduljabbar**^b, **Reem M. Alnemari**^c, **Musaad M Althobaiti**^b, **Mohammed F. Aldawsari**^d, **Aamal A. Al-Mutairi**^a, **Ahmed Serag**^{e,*}, **Atiah H. Almalki**^{f, g}

^a Department of Chemistry, College of Science, Imam Mohammad Ibn Saud Islamic University (IMSIU), P.O. Box 90950, Riyadh 11623, Saudi Arabia

^b Department of Pharmacology and Toxicology, College of Pharmacy, Taif University, P.O. Box 11099, Taif 21944, Saudi Arabia

^c Department of Pharmaceutics and Industrial Pharmacy, College of Pharmacy, Taif University, P.O. Box 11099, Taif 21944, Saudi Arabia

^d Department of Pharmaceutics, College of Pharmacy, Prince Sattam Bin Abdulaziz University, Al-kharj 11942, Saudi Arabia

^e Pharmaceutical Analytical Chemistry Department, Faculty of Pharmacy, Al-Azhar University, Nasr City 11751, Cairo, Egypt

^f Addiction and Neuroscience Research Unit, Health Science Campus, Taif University, P.O. Box 11099, Taif 21944, Saudi Arabia

^g Department of Pharmaceutical Chemistry, College of Pharmacy, Taif University, P.O. Box 11099, Taif 21944, Saudi Arabia

*Corresponding author email address: (Ahmed Serag) Ahmedserag777@hotmail.com

Table S1: Box-Behnken design matrix with experimental runs and factor levels.

		Factor 1	Factor 2	Factor 3	Factor 4
Std	Run	A:pH	B:Buffer volume	C:Reagent concentration	D:Reaction time
			mL	µg/mL	min
17	1	3	1	10	6.5
16	2	5.5	1.5	30	6.5
6	3	5.5	1	30	3
11	4	3	1	20	10
24	5	5.5	1.5	20	10
7	6	5.5	1	10	10
3	7	3	1.5	20	6.5
15	8	5.5	0.5	30	6.5
23	9	5.5	0.5	20	10
1	10	3	0.5	20	6.5
27	11	5.5	1	20	6.5
18	12	8	1	10	6.5
4	13	8	1.5	20	6.5
9	14	3	1	20	3
5	15	5.5	1	10	3
25	16	5.5	1	20	6.5
14	17	5.5	1.5	10	6.5
21	18	5.5	0.5	20	3
26	19	5.5	1	20	6.5
8	20	5.5	1	30	10
12	21	8	1	20	10
28	22	5.5	1	20	6.5
29	23	5.5	1	20	6.5
13	24	5.5	0.5	10	6.5
20	25	8	1	30	6.5
19	26	3	1	30	6.5
2	27	8	0.5	20	6.5
22	28	5.5	1.5	20	3
10	29	8	1	20	3

Table S2: Model adequacy statistics for Box-Behnken design.

Std. Dev.	3.24	R²	0.9727
Mean	50.81	Adjusted R²	0.9598
C.V. %	6.38	Predicted R²	0.9150
		Adeq Precision	29.2476

Table S3: Recovery studies for spiked human plasma samples at different concentration levels

<i>Spiked ($\mu\text{g/mL}$)</i>	<i>Found ($\mu\text{g/mL}$)</i>	<i>Recovery (%)</i>	<i>RSD (n = 3, %)</i>
0.025	0.026	104.59	4.72
0.05	0.052	104.27	3.73
1	1.032	103.16	2.36
1.5	1.520	101.34	1.56

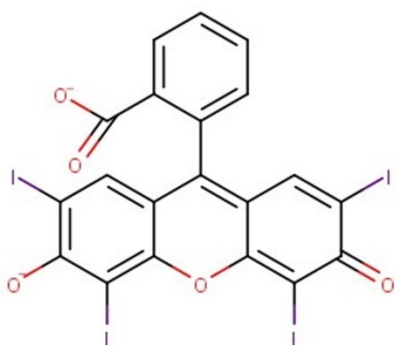
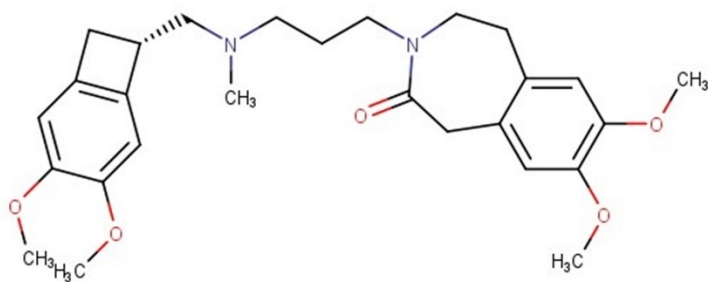
A**B**

Fig. S1: Chemical structures of **(A)** Erythrosin B, showing the planar xanthene framework with four peripheral iodine substituents and anionic carboxylate group, and **(B)** ivabradine hydrochloride, showing the benzofuran-benzazepine tetracyclic framework with the tertiary amine group responsible for its cationic character at the optimized pH.

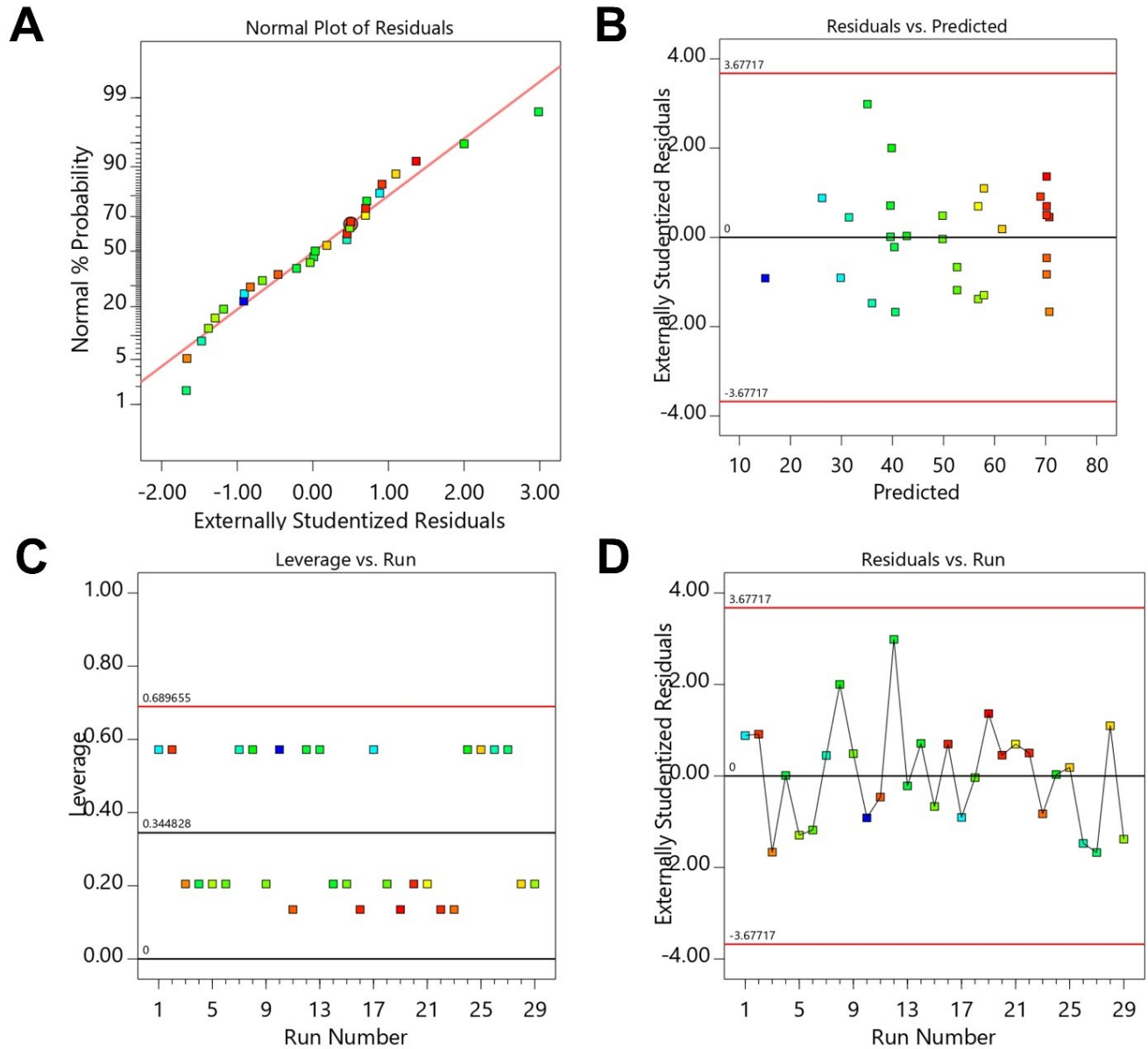


Fig. S2: Model diagnostic plots for Box-Behnken design validation and assumption verification: (A) Normal probability plot of residuals demonstrating normal distribution along the reference line; (B) Residuals versus predicted values plot showing random scatter without systematic patterns; (C) Leverage versus run number plot identifying potential outliers and influential points; (D) Residuals versus run order plot confirming absence of time-related systematic errors.

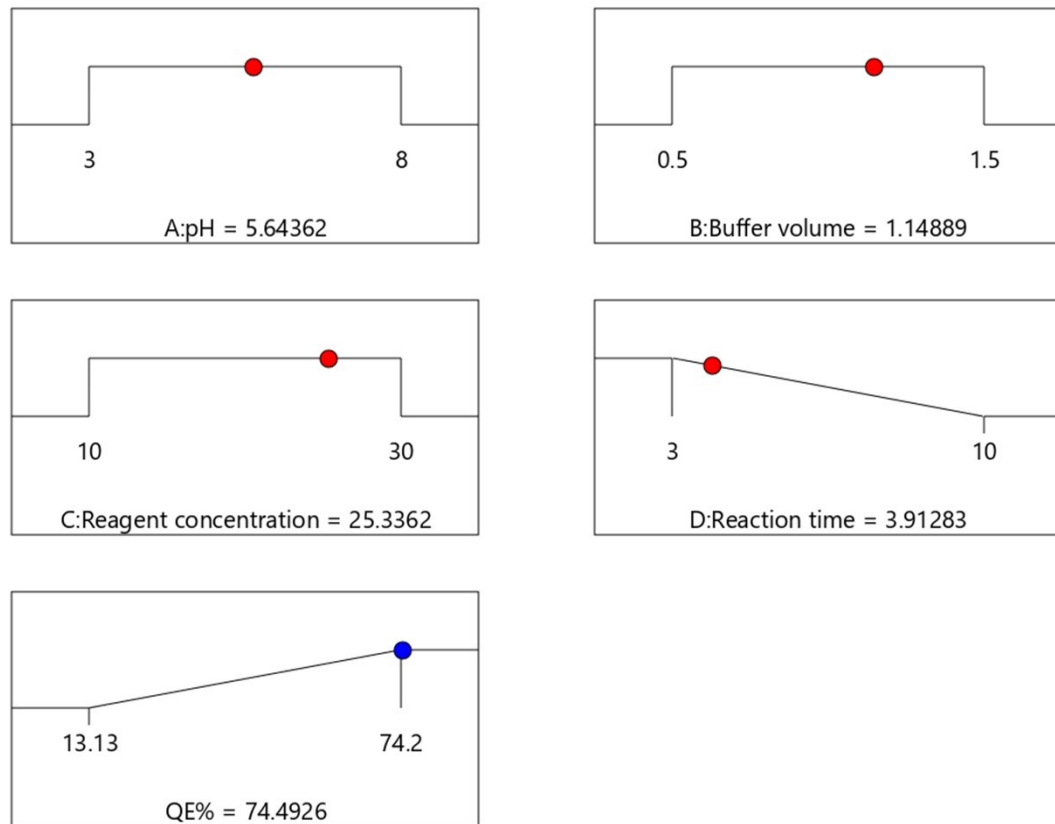


Fig. S3: Desirability function optimization ramp plots displaying individual factor settings and overall desirability profile for achieving maximum fluorescence quenching efficiency, showing optimal values for pH, buffer volume, reagent concentration, and reaction time with corresponding predicted response.

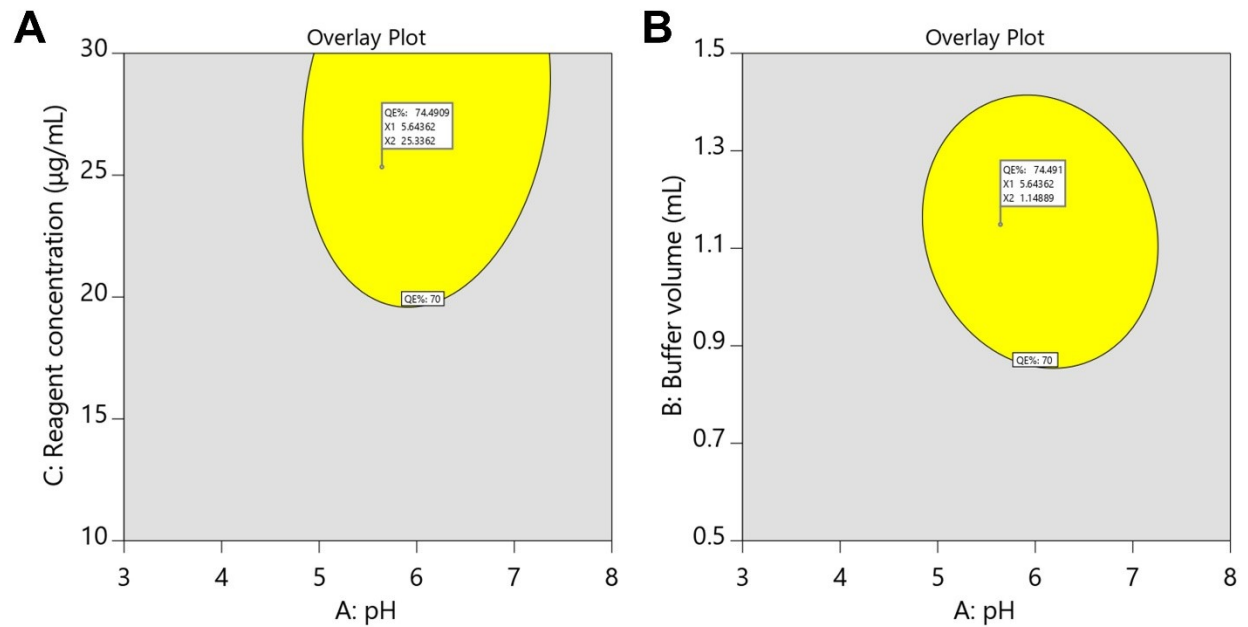


Fig. S4: Overlay contour plots illustrating feasible optimization regions (yellow areas) where multiple response criteria are simultaneously satisfied, providing operational flexibility while maintaining analytical performance specifications across different factor combinations.

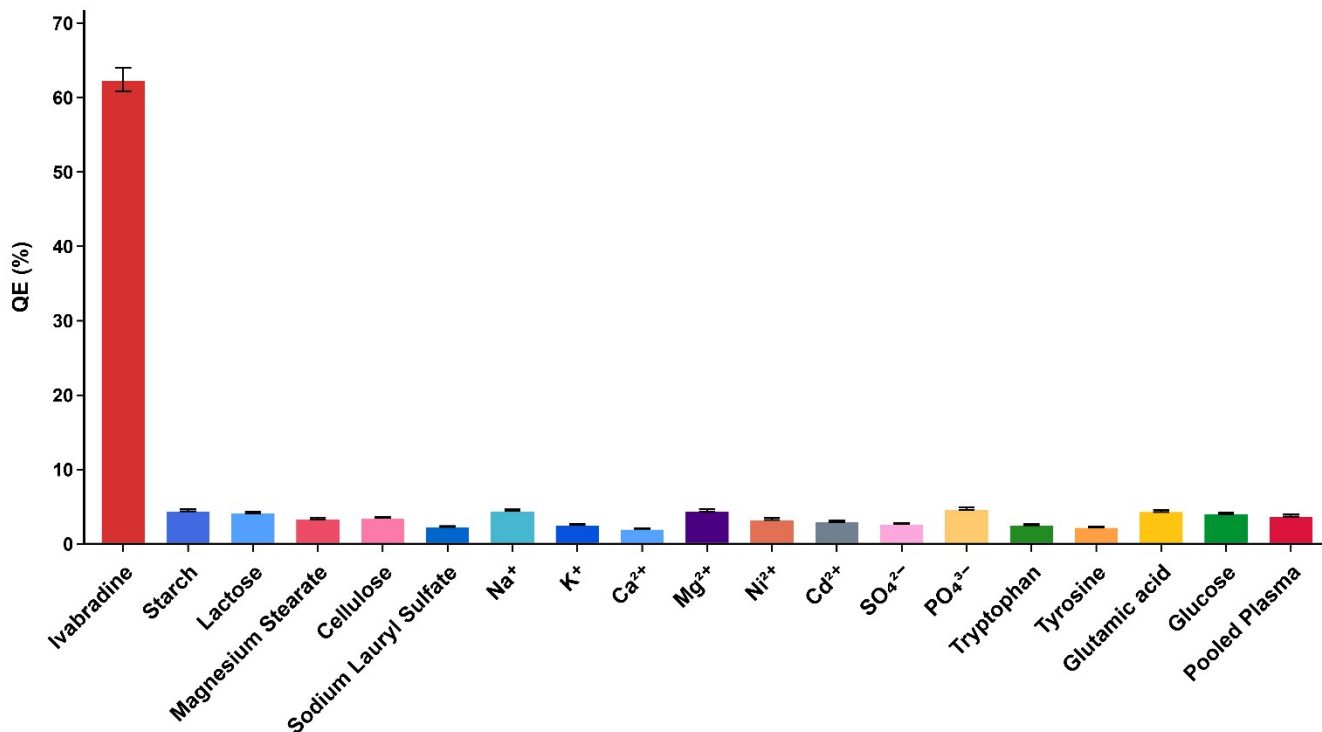


Fig. S5: Method selectivity evaluation showing fluorescence quenching efficiency comparison between ivabradine and various potential interferents including pharmaceutical excipients, electrolytes, and biological components tested at 10-fold excess concentration relative to 1 $\mu\text{g/mL}$ ivabradine.