Spectrofluorimetric Determination of Brexpiprazole via Quenching of Erythrosine B Fluorescence: Optimization using Box-Behnken Design

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Table S1: Box Behnken experimental design for optimizing the factors affecting the reaction process of erythrosine B and **BRX**.

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

Table S2: Experimental data for robustness testing of the proposed method for **BRX** analysis.

Parameter	Modification	% Recovery ± RSD%
	3.1	98.22 ± 0.573
Buffer (pH)	3.2 (optimum)	99.37 ± 0.371
	3.3	99.23 ± 0.727
	1.1	99.34 ± 0.537
Erythrosine B volume (mL)	1.2 (optimum)	100.71 ± 0.782
	1.3	100.55 ± 0.517
	0.9	99.31 ± 0.844
Buffer volume (mL)	1 (optimum)	100.51 ± 0.551
	1.1	100.40 ±0.776

Table S3: Application of the proposed method for determination of **BRX** in spiked human plasma.

Drug	Amount taken (μg/mL)	Amount found (μg/mL) [a]	%Recovery
BRX	0.4	0.397	99.25
	0.6	0.589	98.17
	0.8	0.791	98.88
	1	0.984	98.40
	1.5	1.487	99.13
mean			**98.77**
SD			**0.467**
RSD%			**0.473**

[a] average of three determinations.

Table S4: Stability study of **BRX** in human plasma.

Conditions	**LQC (0.3 µg/mL)** **% Recovery ± SD**	**HQC (1.8 µg/mL)** **% Recovery ± SD**
Long-term stability	97.66 ± 1.17	97.27 ± 1.14
Short-term stability	98.22 ± 1.06	98.17 ± 0.67
Post-preparative stability	98.57 ± 1.10	98.71 ± 1.47
Stock solution stability	100.31 ± 0.82	100.11 ± 1.12
Three freeze-thaw cycle stability	97.33 ± 1.22	97.28 ± 1.27

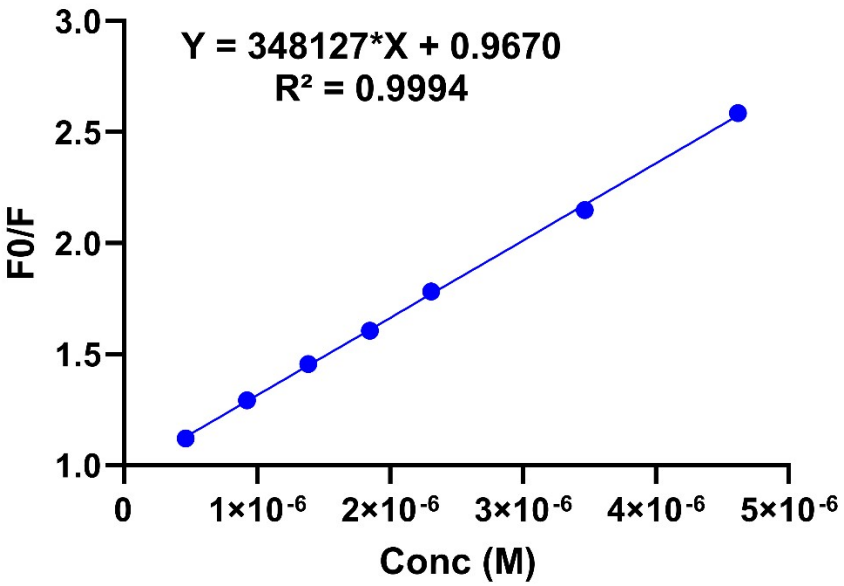

Fig. S1: Stern-Volmer plot for the quenching of erythrosine B fluorescence by brexpiprazole (BRX) showing the linear relationship between F0/F ratio and BRX concentration (M). The linear regression equation F0/F = 348127[BRX] + 0.9670 (R^2 = 0.9994) confirms the static quenching mechanism with a Stern-Volmer constant (KSV) of 3.48×10^5 M^{-1}.

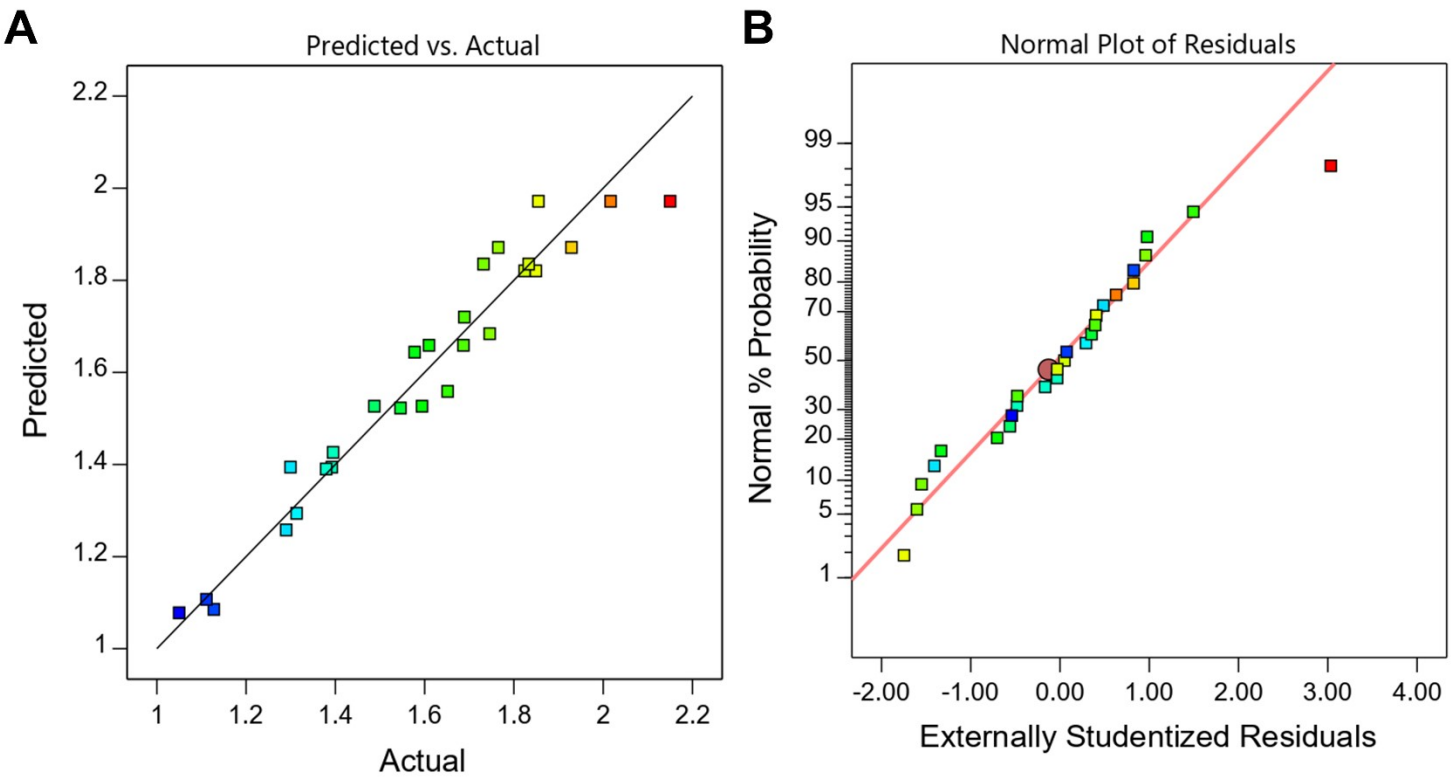

Fig. S2: Model validation plots for the spectrofluorimetric method (A) Predicted vs. actual plot showing a straight line between model predictions and observed values. (B) Normal plot of externally studentized residuals, showing a straight line indicating a normal distribution of the data.

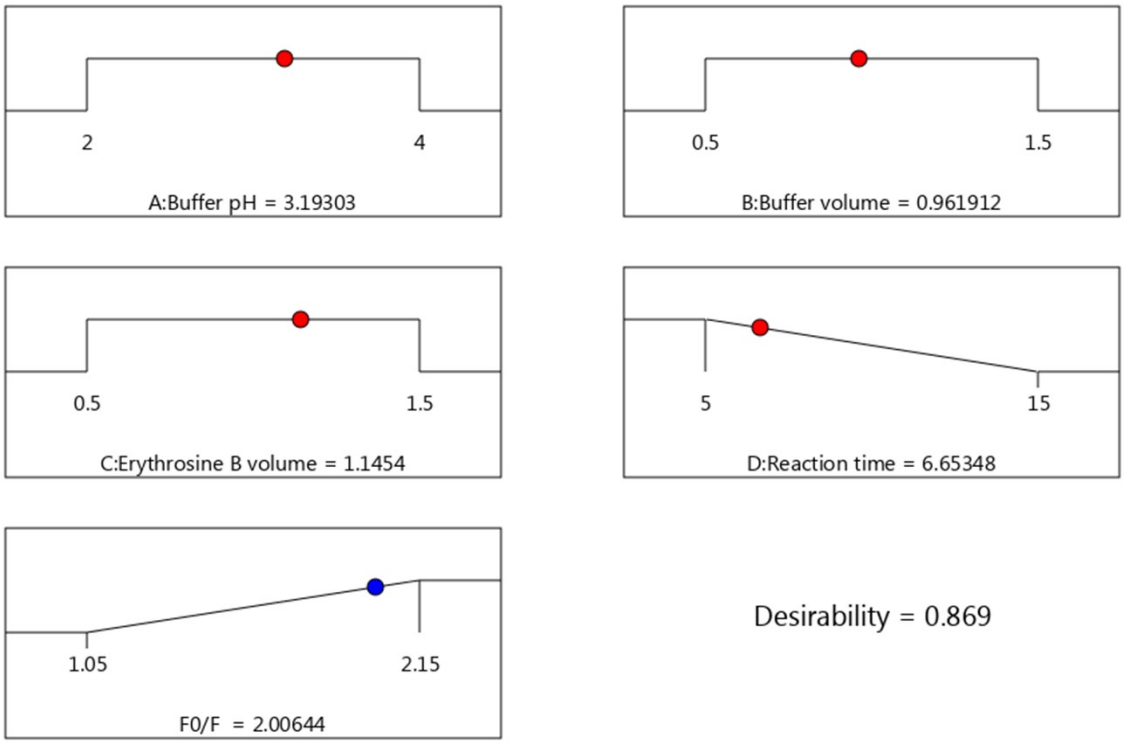

Fig. S3: Numerical optimization of the investigated factors on the reaction process of **BRX** and erythrosine B showing the best conditions for maximum F0/F ratio.