Development of a Green Spectrofluorimetric Method for Mefenamic Acid Determination Using Rhodamine 6G with Mechanistic Investigation and Central Composite Design Optimization

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Table S1: Central composite design matrix showing experimental runs with actual values for pH (Factor A), Rhodamine 6G concentration (Factor B), and reaction time (Factor C).

		Factor 1	**Factor 2**	**Factor 3**
Std	Run	A:pH	B:Reagent Conc.	C:Reaction time
			µg/mL	min
10	1	8.4	10	6.5
18	2	6	10	6.5
20	3	6	10	6.5
9	4	3.6	10	6.5
19	5	6	10	6.5
16	6	6	10	6.5
11	7	6	4	6.5
3	8	4	15	3
5	9	4	5	10
12	10	6	16	6.5
13	11	6	10	2.3
1	12	4	5	3
17	13	6	10	6.5
15	14	6	10	6.5
2	15	8	5	3
4	16	8	15	3
6	17	8	5	10
7	18	4	15	10
8	19	8	15	10
14	20	6	10	10.7

Table S2: . Model validation statistics for the central composite design model.

Std. Dev.	2.44	**R^2**	0.9897
Mean	51.31	**Adjusted R^2**	0.9838
C.V. %	4.76	**Predicted R^2**	0.9607
		Adeq Precision	35.5757

Table S3: Recovery study results for spiked human plasma samples at four concentration levels

Spiked (µg/mL)	*Found (µg/mL)*	*Recovery (%)*	*RSD (n = 3, %)*
0.2	0.199	99.562	3.416
0.5	0.511	102.208	3.135
1	0.963	96.299	2.119
3	2.95	98.348	1.719

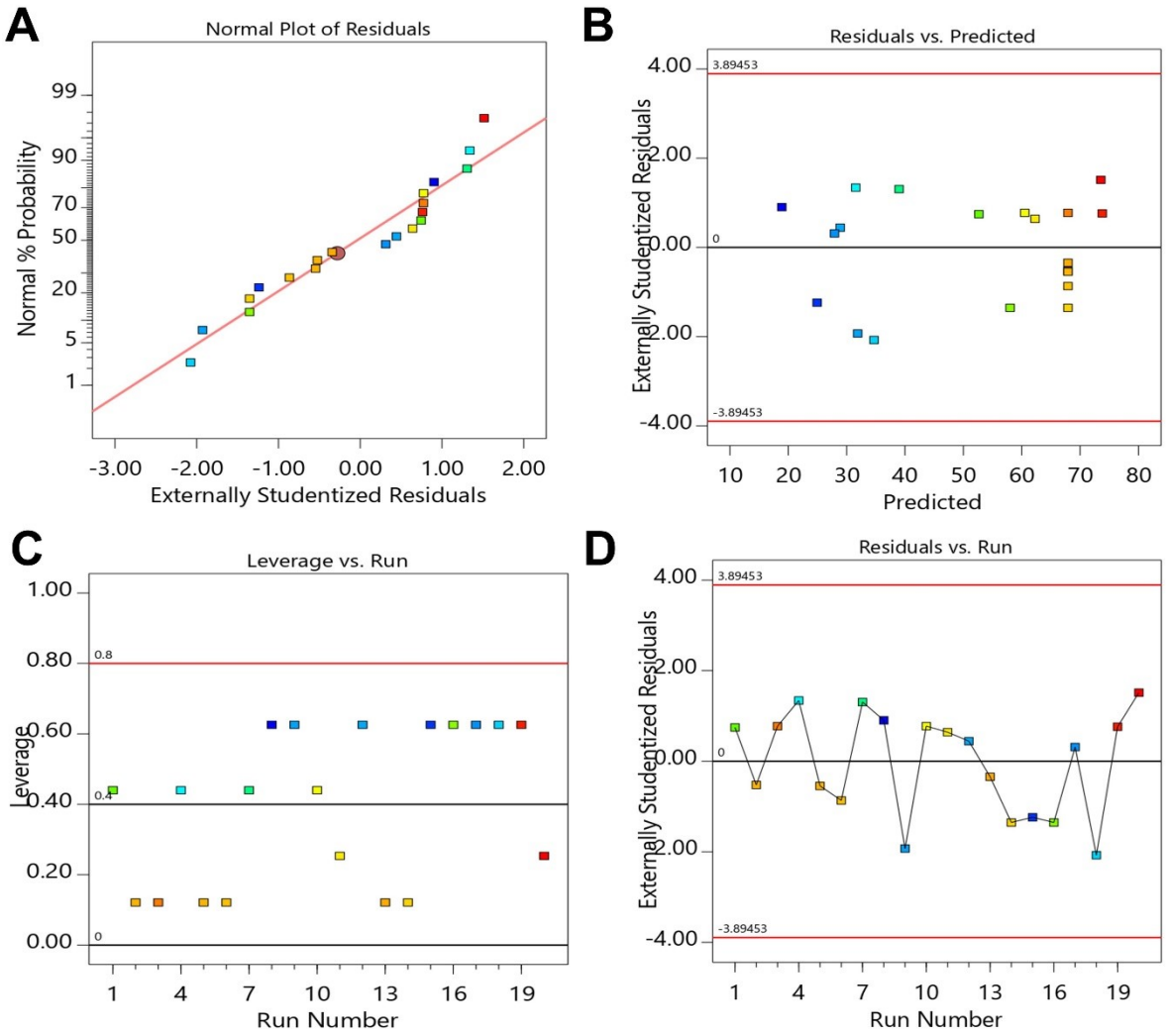

Fig. S1: Model validation diagnostic plots including (A) normal probability plot of residuals, (B) residuals versus predicted values, (C) leverage versus run number, and (D) residuals versus run order demonstrating model adequacy and absence of systematic errors.

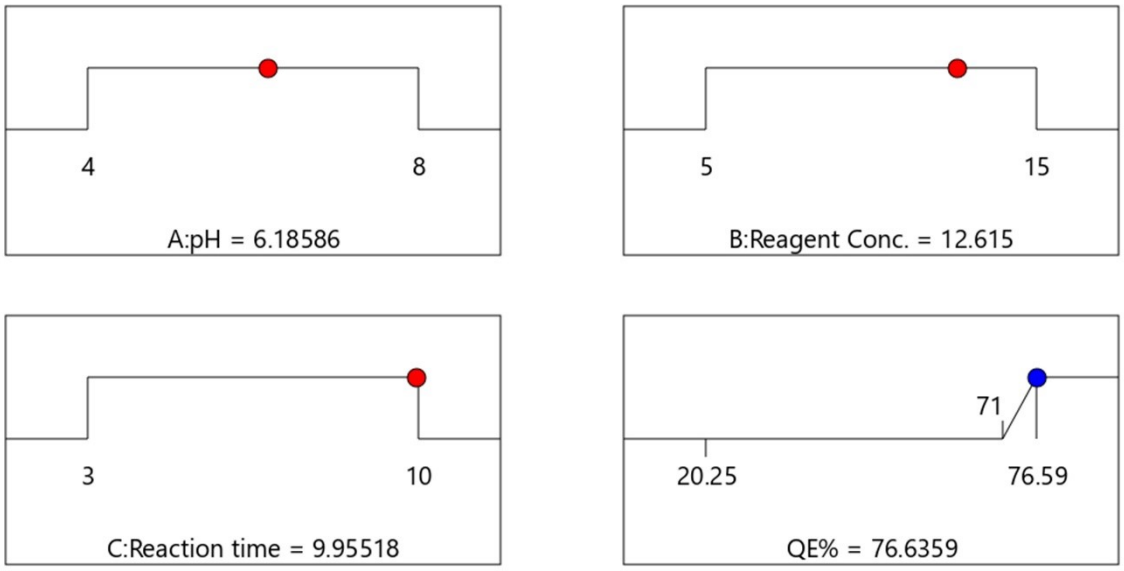

Fig. S2: Desirability function optimization plot showing the identification of optimal experimental conditions for maximum quenching efficiency.

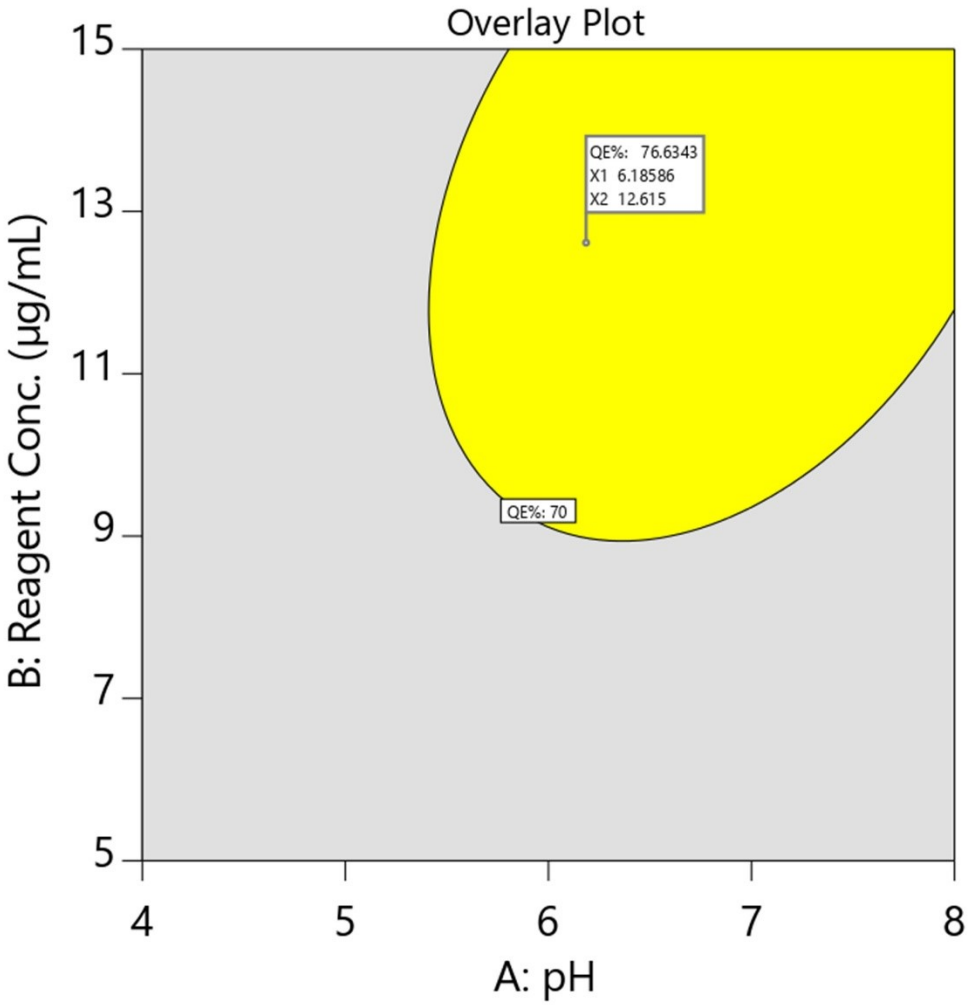

Fig. S3: Design space analysis (overlay plot) showing the operational region where quenching efficiency exceeds 70%, providing robust conditions for routine analytical applications within the pH and Rhodamine 6G concentration ranges.