Turn-off fluorescence sensing of raloxifene using erythrosine B with detailed spectroscopic and quantum mechanical studies for pharmaceutical and environmental applications

2.5. Method Validation

Linearity was assessed by analyzing a series of raloxifene standard solutions with concentrations ranging from 0.1 to 3.0 μg/mL under optimized conditions. The ratio of fluorescence intensity (F0/F) was plotted against the corresponding concentrations, where F0 and F are the fluorescence intensities of erythrosine B in the absence and presence of raloxifene, respectively. Six replicates were performed for each concentration level.

LOD and LOQ were calculated using the formulas:

$$LOD = 3.3\sigma/S \text{ and } LOQ = 10\sigma/S$$

where σ is the standard deviation of the blank measurements (n = 10) and S is the slope of the calibration curve.

Accuracy of the method was evaluated by performing recovery studies at three different concentrations of raloxifene (0.5, 1.5, and 2.5 μg/mL), analyzing each concentration in triplicate. The percentage recovery was calculated using the formula:

$$\text{Recovery (\%)} = (\text{Measured concentration} / \text{Spiked concentration}) \times 100.$$

Precision was determined by assessing both repeatability (intra-day precision) and intermediate precision (inter-day precision). For repeatability, nine determinations covering the specified range (three concentrations: 0.5, 1.5, and 2.5 μg/mL, three replicates each) were performed on the same day. For intermediate precision, the same procedure was repeated on three consecutive days. The results were expressed as percent relative standard deviation (%RSD).

Robustness was evaluated by deliberately varying critical method parameters (pH: 3.9, 4.0, 4.1; buffer volume: 1.4, 1.5, 1.6 mL; erythrosine B volume: 0.9, 1.0, 1.1 mL) and assessing their impact on the fluorescence signal using a raloxifene concentration of 1.5 μg/mL. Selectivity was thoroughly assessed by evaluating potential interference from common pharmaceutical excipients (starch, lactose, stearate, cellulose, sodium lauryl sulfate), inorganic ions (Na^+, K^+, Ca^{2+}, Mg^{2+}, Ni^{2+}, Cd^{2+}, SO_4^{2-}, PO_4^{2-}), and biological components (tryptophan, tyrosine, glutamic acid, albumin, glucose, and pooled plasma). The quenching efficiency percentage (QE%) was compared with that of pure raloxifene solution (1.5 μg/mL).

Table S1: Comparison of the proposed method with previously reported methods for raloxifene determination.

Analytical Method	Detection System/Material	Linear Range	LOD	LOQ	Analysis Time	Sample Matrix	Reference
Fluorescence (quenching)	Erythrosine B	0.1–3.0 µg/mL	31.2 ng/mL	93.7 ng/mL	3 min	Pharmaceutical formulations, plasma, water	Current work
RP-HPLC	C18 column (290 nm) / Acetonitrile buffer (30:70)	0.5–50 µg/mL	Not reported	Not reported	10.6 min	Tablets	Reddy et al. (2006)
RP-HPLC	C18 column (289 nm) / Acetonitrile acetate (33:67)	0.2–75.0 µg/mL	0.014 µg/mL	0.2 µg/mL	5.32 min	Rat plasma	Yang et al. (2007)
RP-HPLC	C8 column (287 nm) / Acetonitrile water pH 3 (40:60)	0.1–3.2 µg/mL	25.41 ng/mL	77.01 ng/mL	2.258 min	Mice plasma	Johnson et al. (2024)
LC-MS/MS	C18 column (MRM m/z 474→112) / Formic acid in water and acetonitrile	1.01–260 nM	0.017	1.01 nM	2.1 min	Human urine	Trdan et al. (2011)
LC-MS/MS	C18 column (MRM m/z 474.2→112.2) / Acetonitrile acetate (70:30)	0.5–100 ng/mL	Not reported	0.5 ng/mL	2.5 min	Human urine	Chen et al. (2013)
Electrochemical	reduced graphene oxide-carbon nanotube nano-composite /linear sweep voltammetry	0.006–15.0 µM	2 nM	Not reported	5 min	Pharmaceutical formulations, plasma	Ghalkhani et al. (2021)
Fluorescence (enhancement)	SDS micelles (λex 290 nm / λem 583 nm)	0.1–1.5 µg/mL	0.01 µg/mL	0.05 µg/mL	Not reported	Tablets	Ibrahim et al. (2018)

Fluorescence (enhancement)	Al^{3+} complexation (λex 296 nm / λem 594 nm)	0.1–2.0 μg/mL	0.02 μg/mL	0.06 μg/mL	Not reported	Tablets	Ibrahim et al. (2018)
Fluorescence (quenching)	Gold nanoparticles (λex 320 nm / λem 400 nm)	0.5–50 μM	0.34 μM	1.1 μM	8 min	Urine, sea water	Wu et al. (2022)
Fluorescence (quenching)	Zn-MOF (λex 310 nm / λem 404 nm)	0.7–350 nM	0.485 nM	1.619 nM	9 min	Urine, tap water	Madvar & Taher (2024)

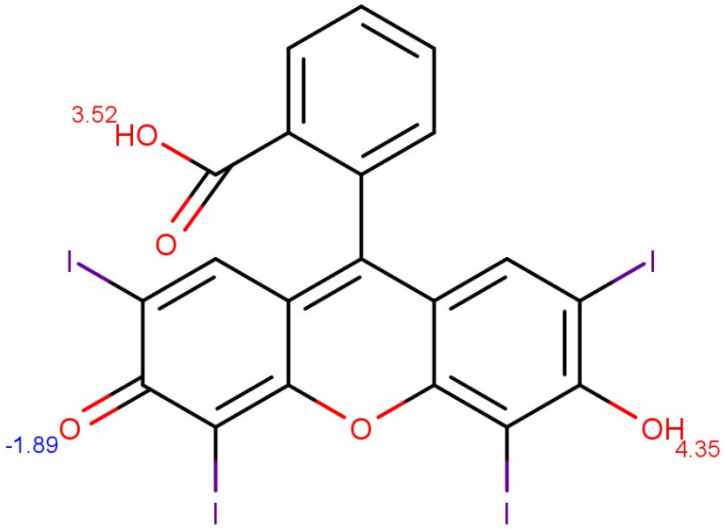

Fig. S1: Chemical structure of erythrosine B showing ionizable groups with calculated pKa values, illustrating the pH-dependent ionization state critical for complex formation with raloxifene.

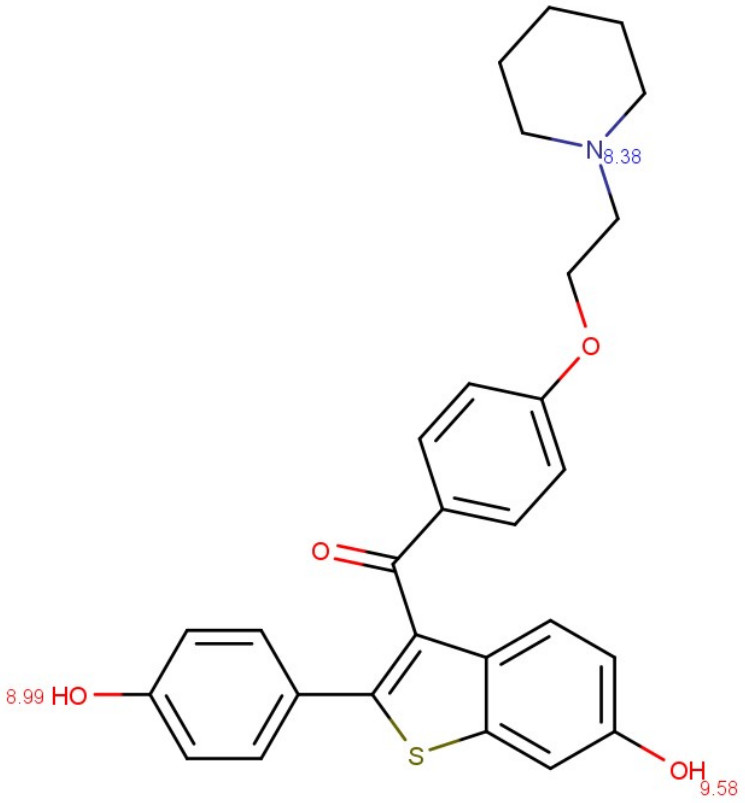

Fig. S2: Chemical structure of raloxifene highlighting ionizable functional groups with calculated pKa values, demonstrating the basis for pH-dependent interaction with erythrosine B.