Pharmacophore-Targeted Fluorescence Sensing of Ibrutinib via Michael Addition-Induced Electron Transfer on Thiol-Functionalized Carbon Dots

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2.1. Materials and reagents

Ibrutinib analytical standard (≥98.0% purity) was purchased from Tokyo Chemical Industry Co., Ltd. (TCI, Tokyo, Japan). The pharmaceutical dosage form used in this work was Imbruvica® (ibrutinib) film-coated tablets, obtained from Janssen Sciences Ireland (a Johnson & Johnson company), available in strength of 420 mg per tablet. Human serum albumin (HSA), hemoglobin, bilirubin, glucose, and urea were obtained from Alfa Aesar (Thermo Fisher Scientific, Haverhill, MA, USA). Creatinine, uric acid, cholesterol, phosphatidylcholine, and L-cysteine were purchased from Acros Organics (Fair Lawn, NJ, USA). Reduced glutathione (GSH), arginine, NaCl, KCl, $CaCl_2$, $MgCl_2$, and $CaCl_2$ were obtained from Merck KGaA (Darmstadt, Germany). Imatinib, warfarin, apixaban, aspirin, and clopidogrel were purchased from Cayman Chemical Company (Ann Arbor, MI, USA). Clarithromycin, ciprofloxacin, diltiazem, verapamil, and ritonavir were obtained from LGC Standards (Teddington, Middlesex, UK). Venetoclax and rituximab were purchased from MedChemExpress (MCE, Monmouth Junction, NJ, USA). All chemicals and reagents used in this study were of analytical-reagent grade and were used without further purification.

2.2. Instrumentation and characterization

The optical characterization of the synthesized carbon dots was probed through fluorescence (FL) and UV-Vis spectroscopic methods, wherein the FL emission spectra were acquired on a Shimadzu RF-5301PC spectrofluorometer by exciting the samples at 425 nm with 5 nm slit widths, while the absorption spectra were generated using a Shimadzu 1601 PC UV-Vis spectrophotometer. The structural and morphological aspects were elucidated by transmission electron microscopy using a JEOL 2100F instrument operating at 200 kV accelerating voltage to visualize the size and shape of the carbon dots, X-ray diffraction patterns recorded with a Philips PW 1700 diffractometer, and

Fourier transform infrared spectroscopy performed on KBr pellets using a Nicolet 6700 spectrometer. Furthermore, the surface chemical composition and bonding states were evaluated through X-ray photoelectron spectroscopy utilizing an ESCALAB250 spectrometer from Thermo Scientific.

2.4. Quantum yield calculation

The quantum yield (QY) of CDs was calculated according to the following equation using quinine sulfate (QS) as a reference in 0.1 M H_2SO_4 (QY = 54 %). The quantum yield of CDs was calculated using quinine sulfate (QS) in 0.1 M H_2SO_4 (QY = 54%) as a reference standard, where both samples were excited at 360 nm and their absorbance values (maintained below 0.05 to avoid inner filter effects) and integrated fluorescence emission areas (collected over an emission range of 500–650 nm) were substituted into the following formula:

$$\phi_{CDs} = \phi_{QS} \times \frac{F_{CDs}}{F_{QS}} \times \frac{A_{QS}}{A_{CDs}} \times \frac{\eta^2{}_{CDs}}{\eta^2{}_{QS}}$$

Φ_{CDs} represents the quantum yield of CDs, ϕ_{QS} represents the quantum yield of QS, F_{CDs} is the FL intensity of CDs, F_{QS} is the FL intensity of quinine sulphate, A refers to the absorbance value and η refers to the refractive index of the solvent (double distilled water). The synthesized CDs were dissolved in distilled water (η = 1.33) and quinine sulfate was dissolved in 0.1 M H_2SO_4 (η = 1.33).

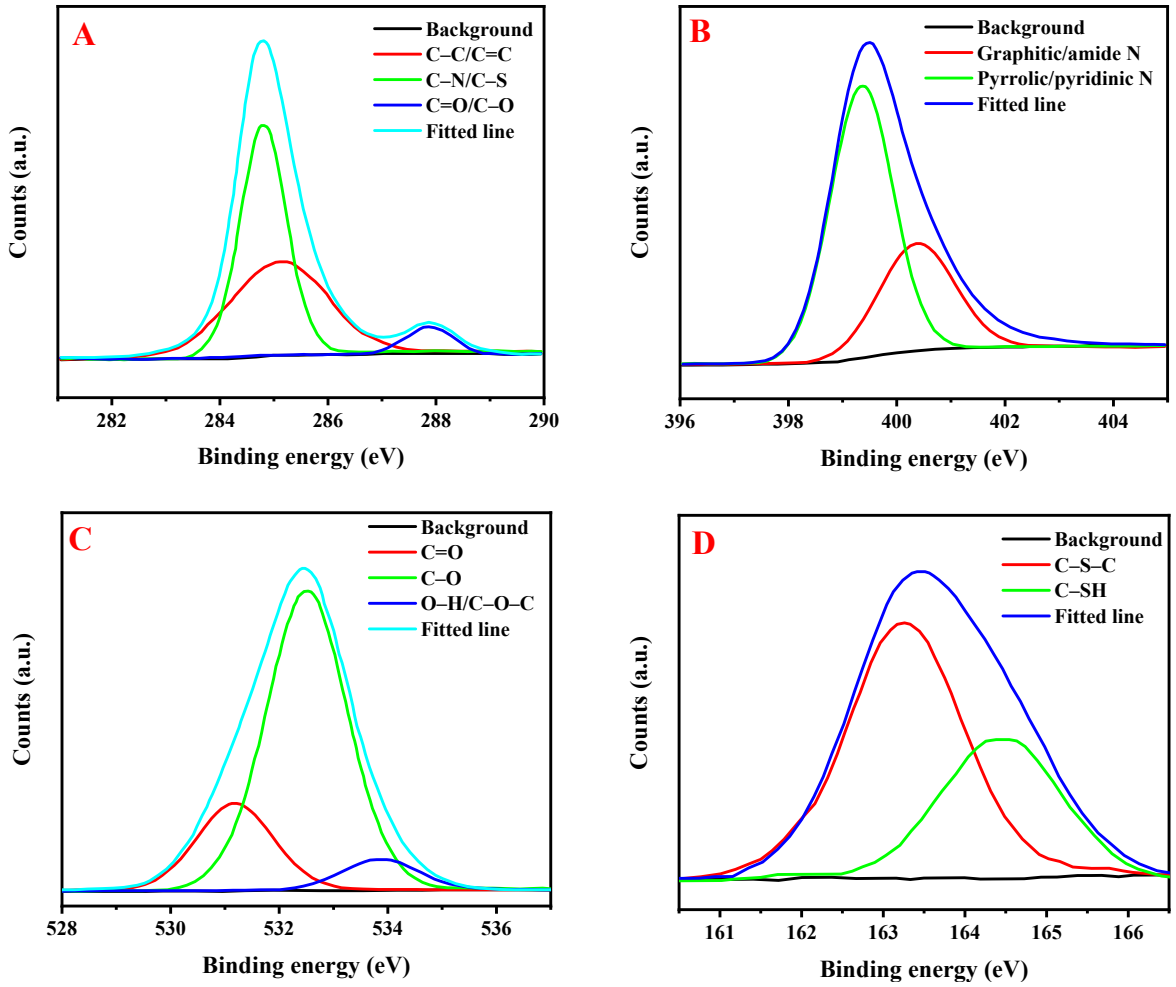

Fig. S1. XPS spectra of HS-CDs showing surface chemical states: **(A)** C 1s, **(B)** N 1s, **(C)** O 1s, and **(D)** S 2p.

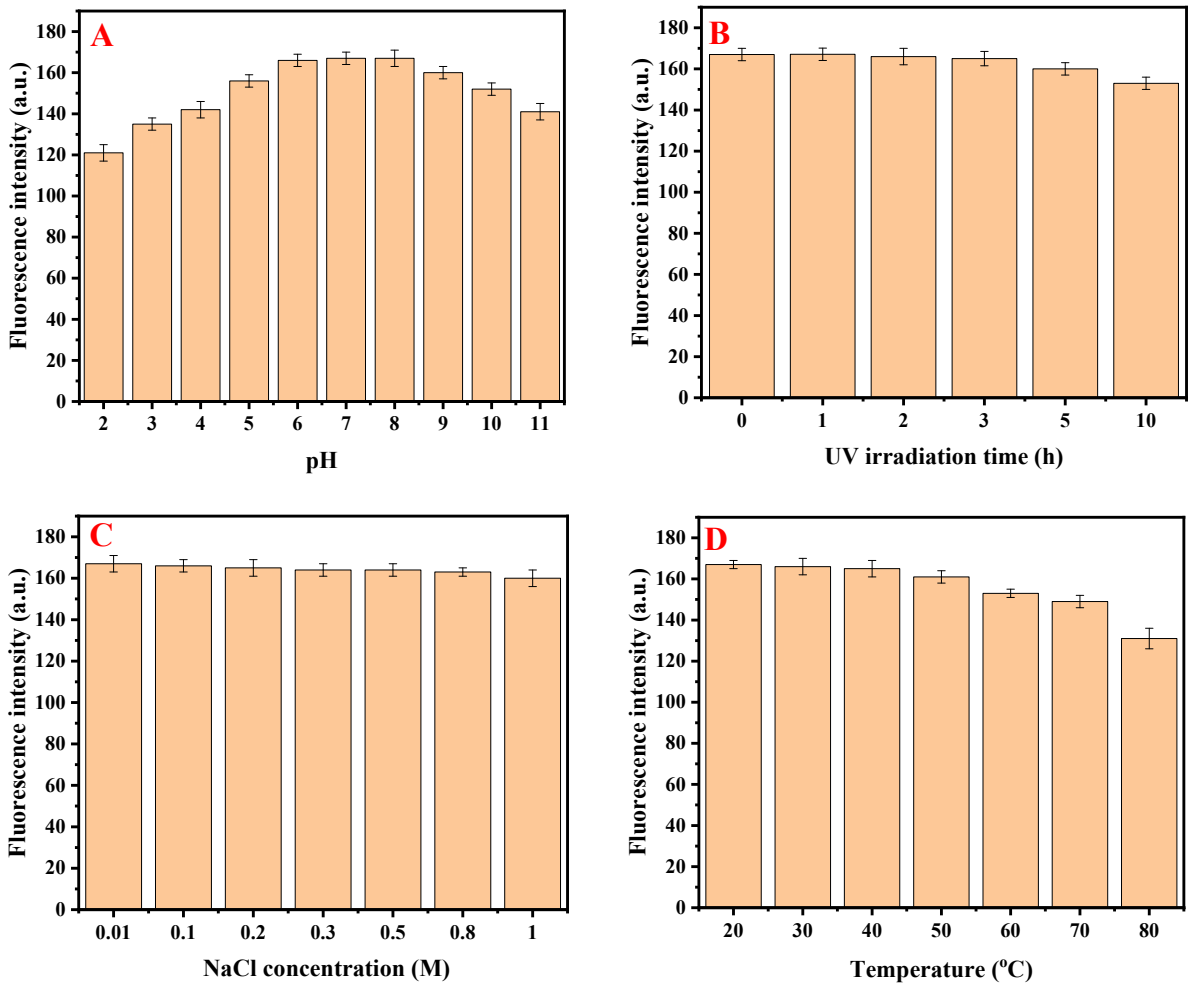

Fig. S2. Stability of HS-CDs under varying conditions: **(A)** FL intensity versus pH (2–11); **(B)** UV irradiation time (0–10 h); **(C)** NaCl concentration (0.01–1.0 M); and **(D)** temperature (20–80 °C).

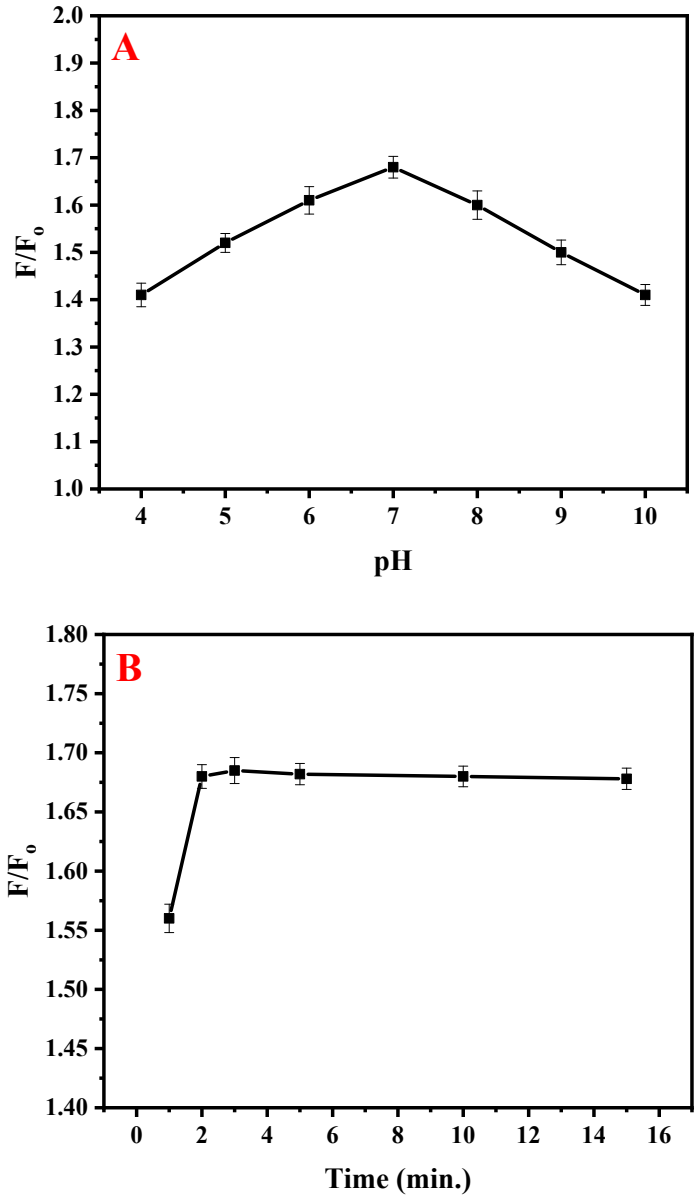

Fig. S3. Optimization of HS-CDs interaction with IBR: **(A)** effect of pH (4–10) on FL enhancement (F/F_0); **(B)** effect of incubation time (0–15 min) on FL.

Table S1. Comparative analysis of the present fluorometric method versus some of the existing methods for IBR detection reported in the literature.

Technique/ sensor	Linearity range (ng/mL)	LOD (ng/mL)	Matrix	Ref.
Native spectrofluorimetric method	100–7500	224	Bulk drug and capsule formulation	[1]
Spectrofluorimetric method (native FL)	20–100	10	Rat plasma	[2]
EEM fluorimetry + chemometrics (ATLD / ANWE / PARAFAC)	20–80	0.11–0.77	Human plasma, urine, HeLa cell lysate, cell culture medium	[3]
HPLC–UV	10–500	*LOQ = 10*	Human plasma	[4]
UHPLC–MS/MS	0.50–30.0 (CSF); 5.0–491.0 (plasma)	*LLOQ = 0.50 in CSF; 5.0 in plasma*	Human cerebrospinal fluid and human plasma	[5]
LC–MS/MS	5–5000	*LLOQ = 5*	Human plasma and mouse plasma	[6]
GO-NH-$B(OH)_2$@AgNPs modified GCE	25–1000	6.0	Pharmaceutical samples and human urine	[7]
4-ABA/IBR@MIP-GCE	0.0004405–0.004405	0.0000270	Biological fluids / commercial serum samples and pharmaceutical dosage forms	[8]
dsDNA biosensor / DPV-based electrochemical DNA biosensor	881–8810	……..	dsDNA biosensor system in acetate buffer	[9]
HS-CDs (spectrofluorimetric method)	1.0 – 30.0	0.21	Human plasma	**This method**

Table S2. Intra-day and inter-day precision of the developed HS-CD fluorometric method for IBR determination (n = 5 for intra-day; n = 3 for inter-day).

Concentration Added (ng/mL)	Intra-day Found (ng/mL)	Intra-day Recovery (%)	Intra-day RSD%	Inter-day Found (ng/mL)	Inter-day Recovery (%)	Inter-day RSD%
1.0	0.98	98.0	2.85	0.97	97.0	3.54
5.0	4.97	99.4	2.14	4.96	99.2	3.21
10.0	10.03	100.3	2.67	10.04	100.4	3.89

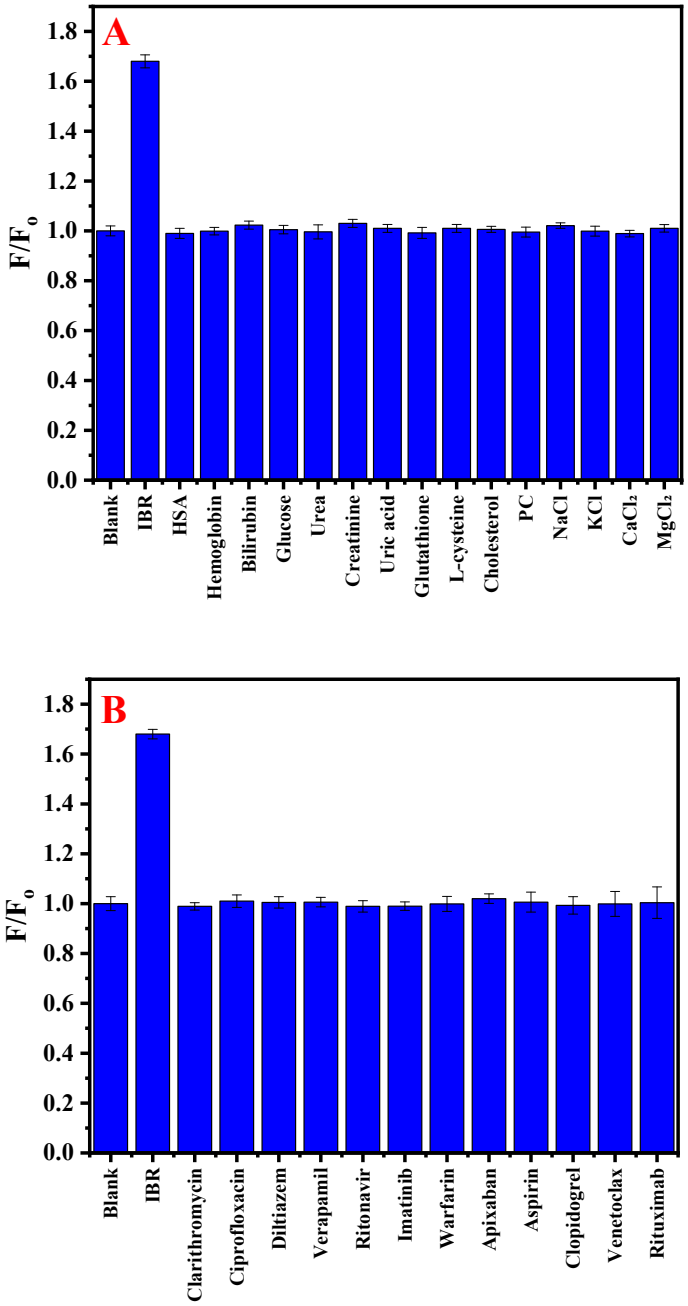

Fig. S4. Selectivity of HS-CDs toward ibrutinib (IBR): **(A)** FL response (F/F_0) in the presence of various potential interferents, including biomolecules and ions; **(B)** FL response in the presence of co-administered drugs.

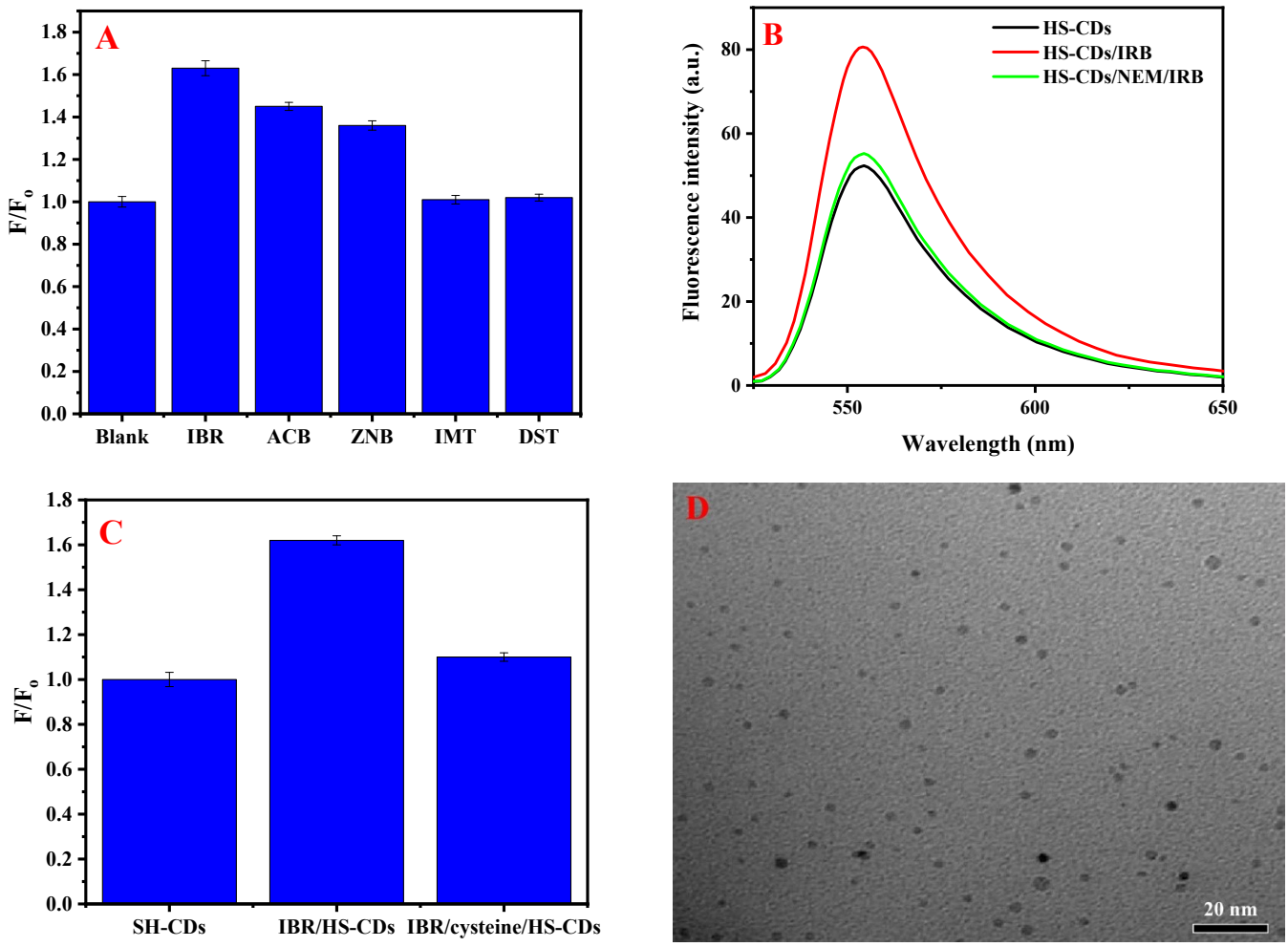

Fig. S5. (A) FL enhancement (F/F_0) of HS-CDs in the presence of IBR and structurally related analogs; (B) FL spectra showing HS-CDs, HS-CDs/IBR, and HS-CDs/IBR after NEM treatment; (C) FL response in competitive assay with cysteine; (D) TEM image of HS-CDs after addition of IBR.

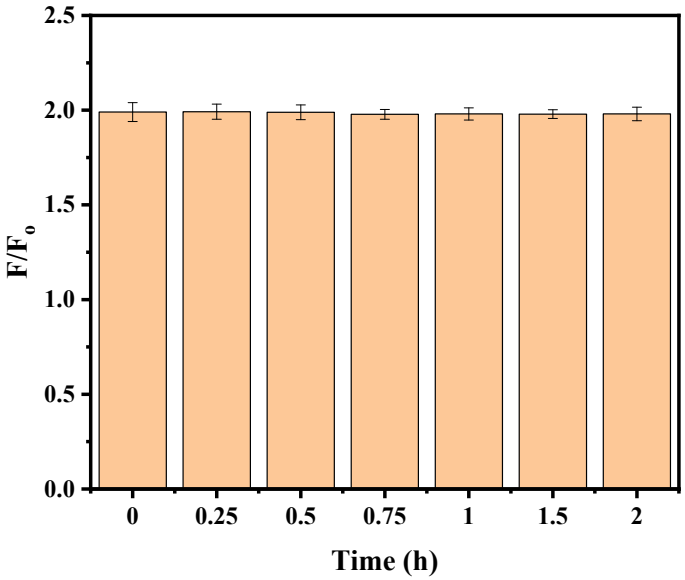

Fig. S6. Fluorescence stability of the IBR–HS-CD complex expressed as F/F₀ ratio measured over 2 hours at room temperature in 10 mM phosphate buffer (pH 7.0), (n = 3).

Table S3. Results of recovery test of IBR in human serum samples (*n = 5*).

Proposed method			**UHPLC–MS/MS method**		***t*-test***
Added (ng/mL)	**Found (ng /mL)**	**Recovery (%) ± RSD%**	**Found (ng /mL)**	**Recovery (%) ± RSD%**	
1.0	0.97	97.0 ± 3.21	0.98	98.0 ± 3.52	0.96
5.0	4.98	99.6 ± 2.36	4.93	98.6 ± 2.99	
10.0	10.02	100.2 ± 3.78	9.75	97.5 ± 3.46	

*Tabulated *t*- value at p = 0.05 is 2.776.

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