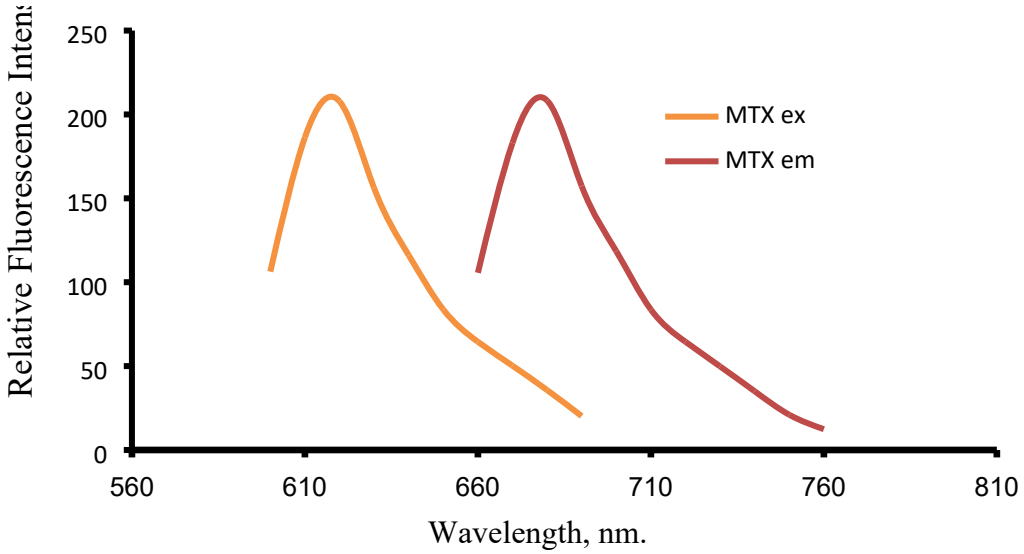
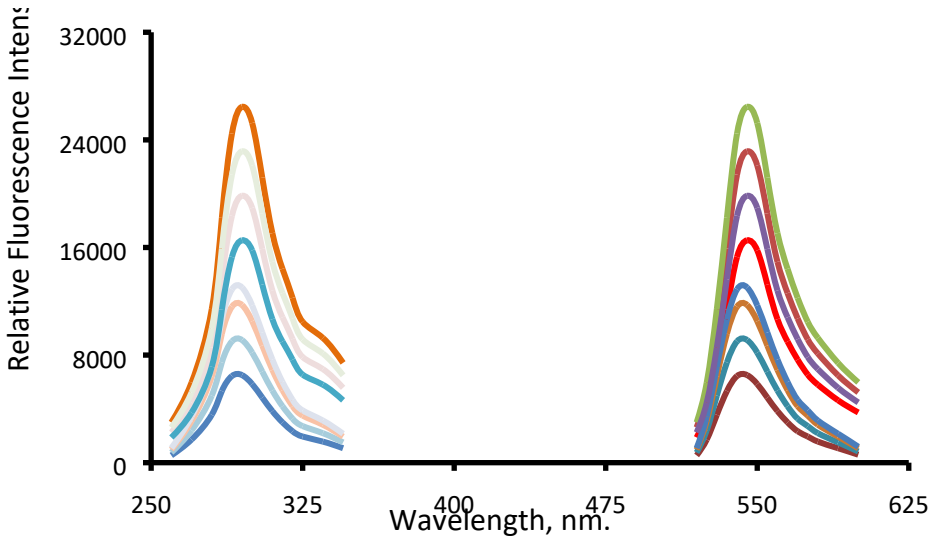
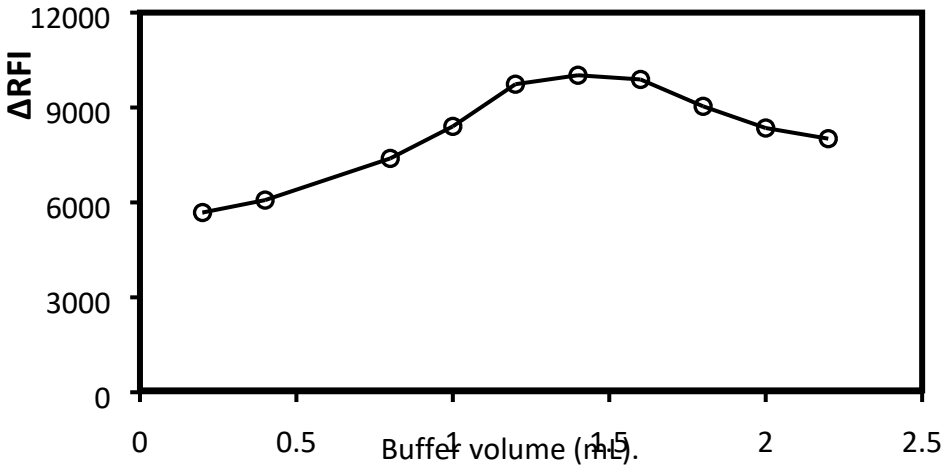


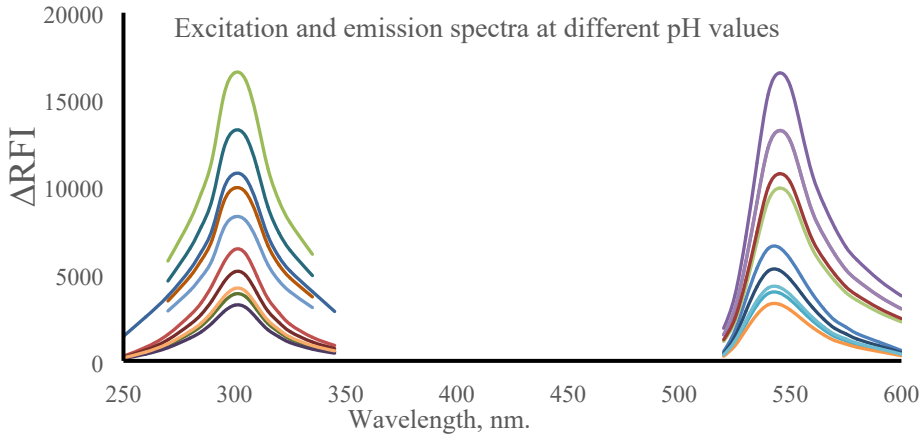
SI 1.1: Pure MTX (1.0 µg mL^{-1}) spectra (excitation and emission) in aqueous buffered solution (pH 4).



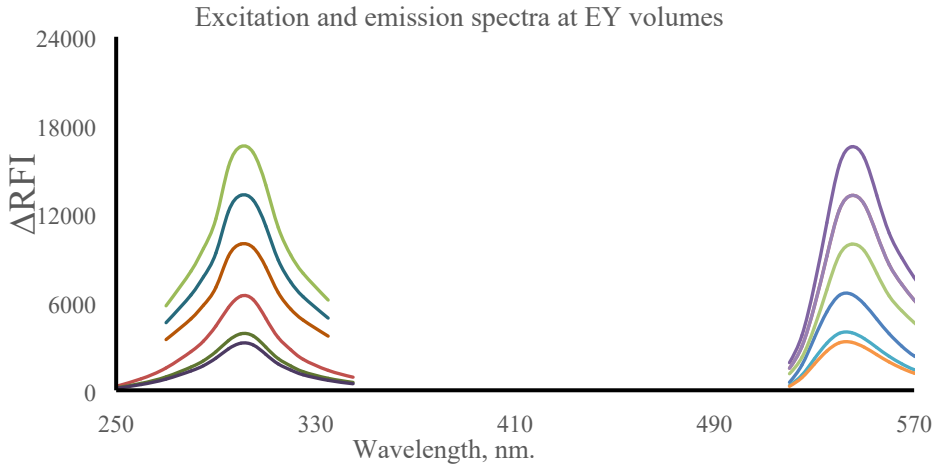
SI 1.2: Full spectra (excitation and emission) of the fully optimized MTX-EosinY system conditions at different MTX concentrations within the operating range.



SI 2.1: Effect of buffer volume on the fluorescence quenching of the formed association complex between MTX (1.0 µg mL^{-1}) and Eosin Y.

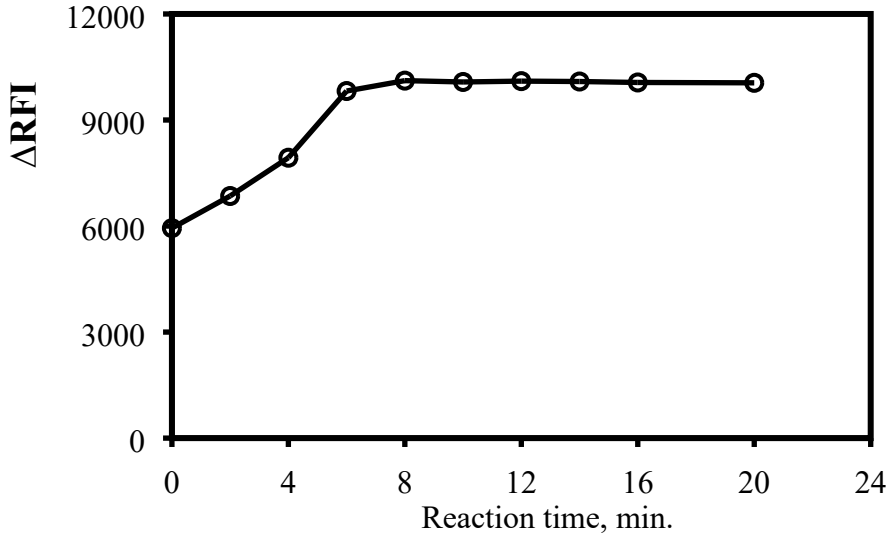


SI 2.2: Excitation and emission spectra at different pH values and buffer volumes for the formed association complex between MTX (1.0 µg mL^{-1}) and Eosin Y.

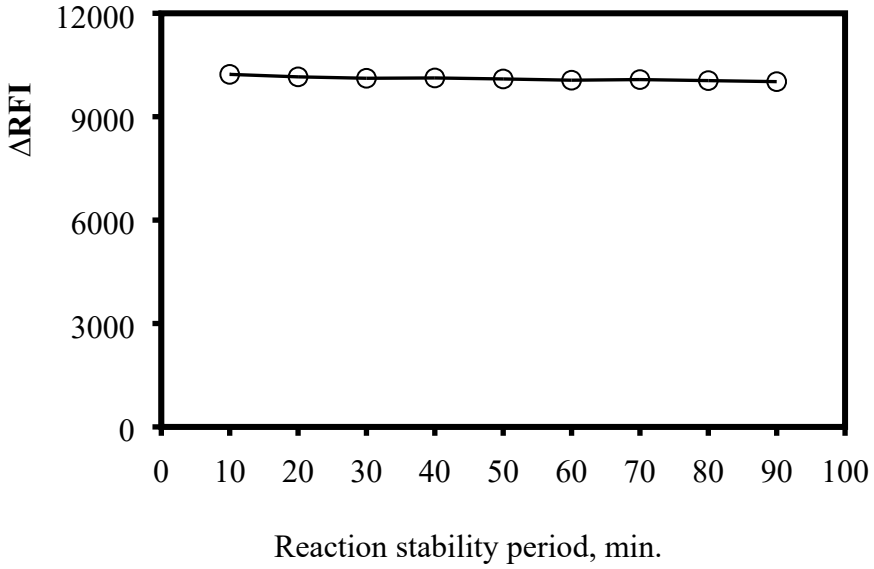


SI 2.3: Excitation and emission spectra at different volumes of EY (0.02% w/v) for the formed association complex between MTX (1.0 µg mL^{-1}) and Eosin Y.

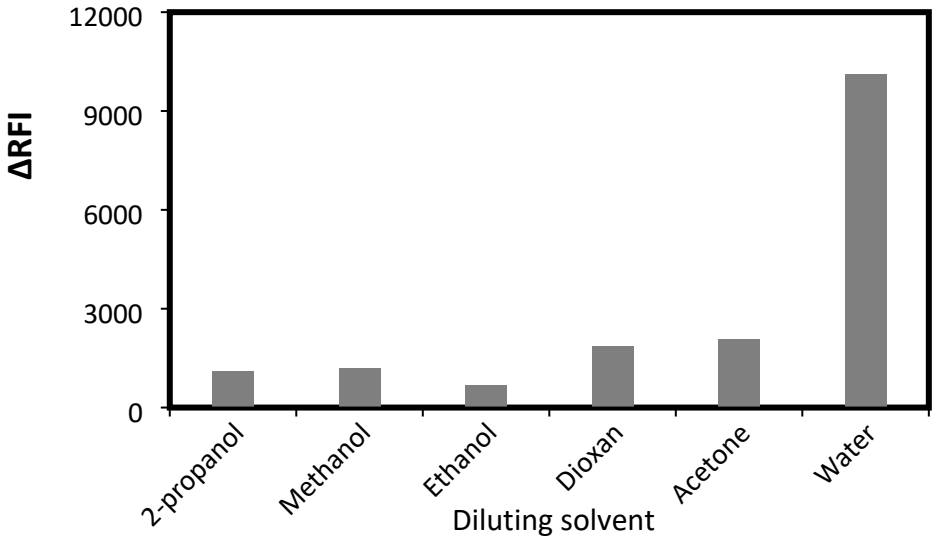
SI 3: Effect of buffer type on the fluorescence quenching of the formed association complex between MTX (1.0 µg mL^{-1}) and Eosin Y.



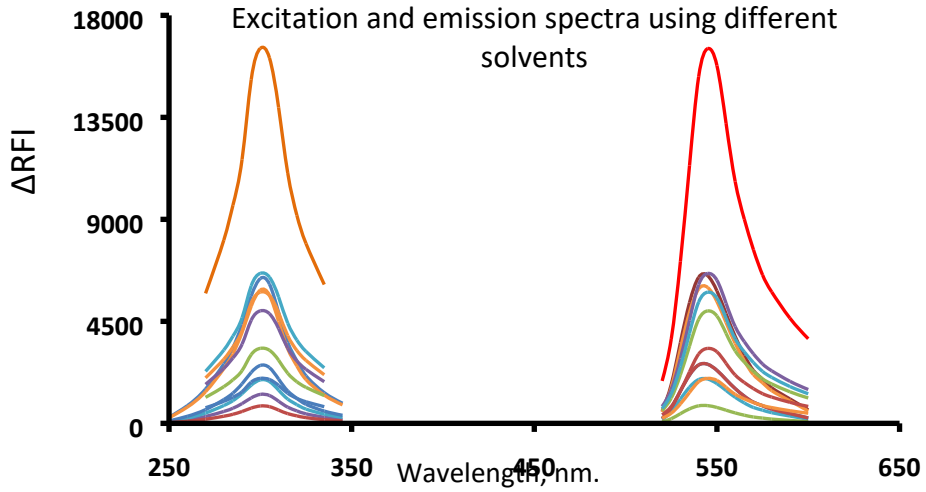
SI 4: Effect of reaction time on the fluorescence quenching of the formed association complex between MTX (1.0 µg mL^{-1}) and Eosin Y.



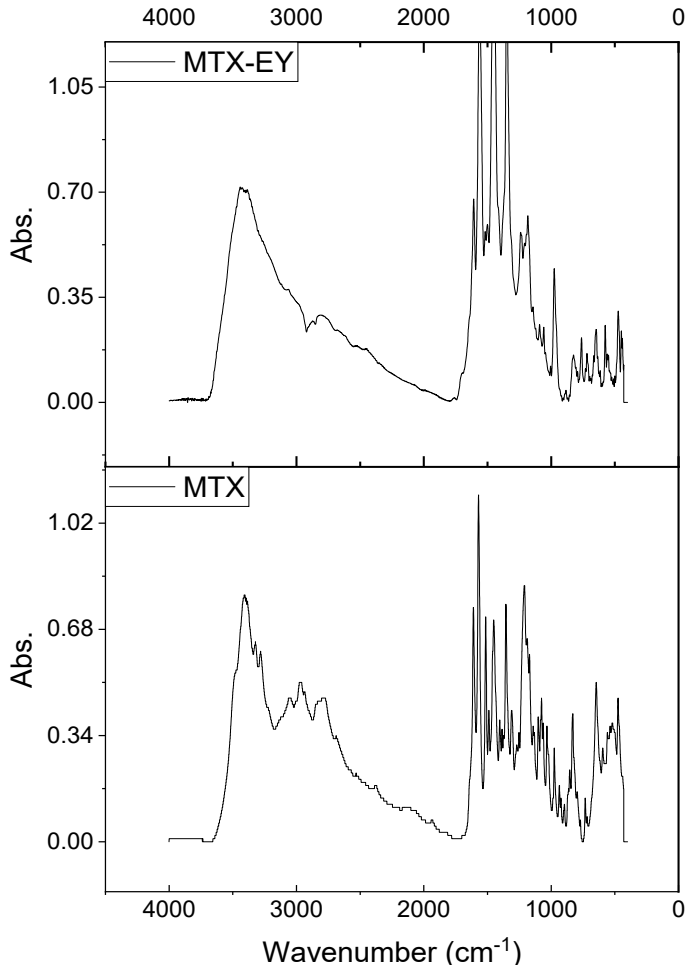
SI 5: Effect of stability time on the fluorescence quenching of the formed association complex between MTX (1.0 μg mL^{-1}) and Eosin Y.



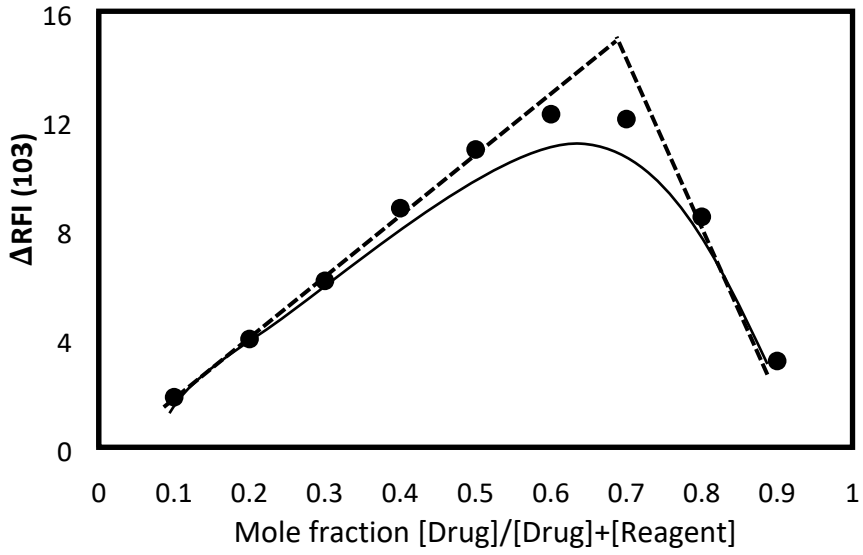
SI 6.1: Effect of diluting solvent on the fluorescence quenching of the formed association complex between MTX (1.0 μg mL^{-1}) and Eosin Y.



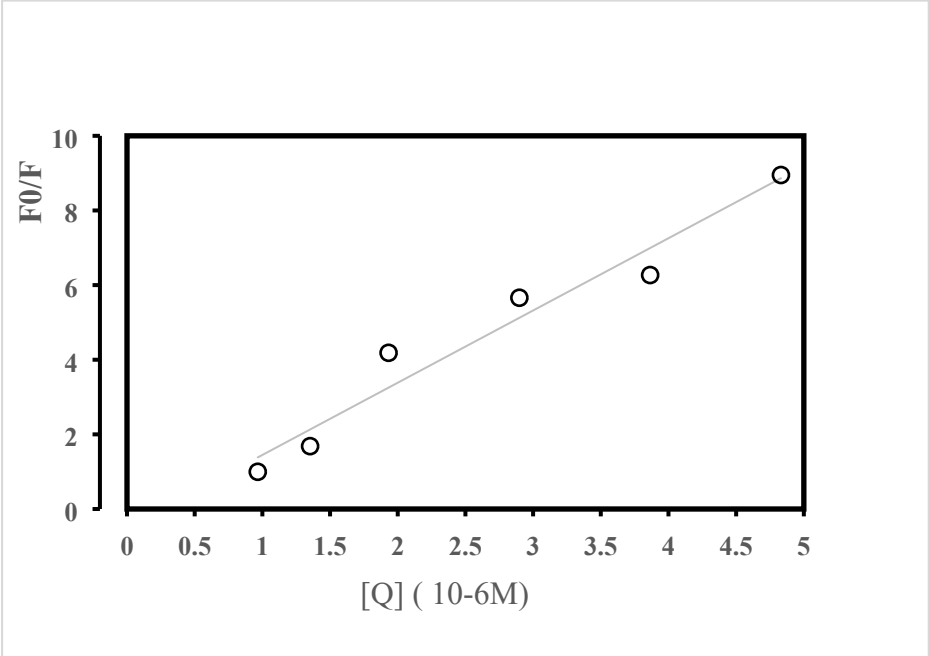
SI 6.2: Full excitation and emission spectra showing the effect of different diluting solvents on the formed association complex between MTX (1.0 µg mL^{-1}) and Eosin Y.



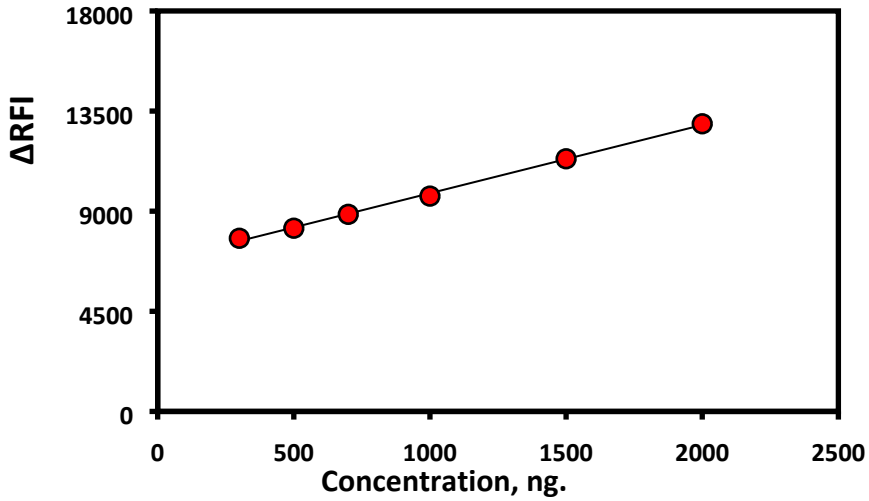
SI 7: FT-IR spectra for MTX and formed MTX-EY complex



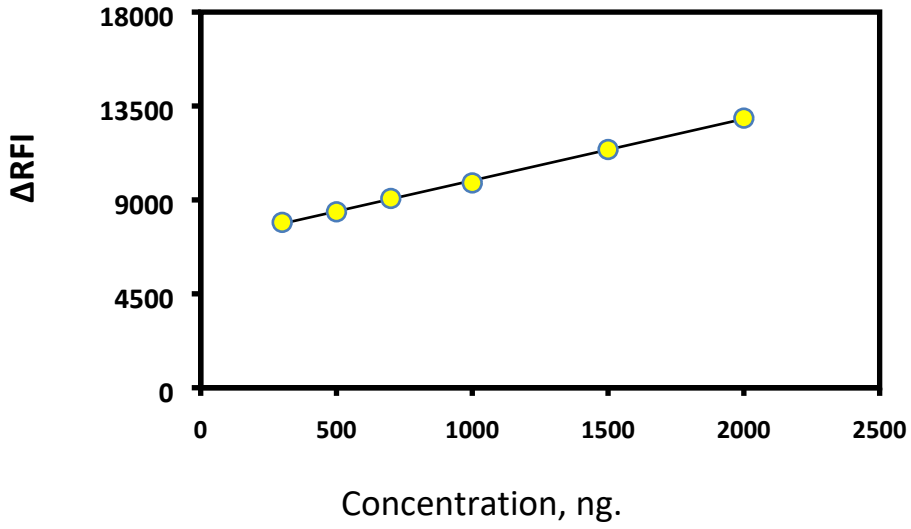
SI 8: Job's plot for association complex formation between MTX and eosin using an equimolar concentration (2.8×10^{-4} M) of master solutions.



SI 9: Stern–Volmer plot describing Eosin Y quenching caused MTX.



SI 10: Calibration curves for MTX in plasma.



SI 11: Calibration curves for MTX in urine

SI Table 1: Comparable cases showing quenching of fluorescence upon association complex formation with MTX and other compounds with Eosin Y and other fluorescence active dye.

drug	Dye or reagent	method	mechanism	application	λ_{ex}/ λ_{em} (nm)	Ref.
MTX	MSA-CdT-QDs as fluorescence probe	Fluorimetry	Fluorescence quenching	Pure form and human serum sample	365/ 500-700	[1]
MTX	AuNPs probe by nanometal surface energy transfer.	Fluorimetry	quenching nature of the MTX–	Live cells biological samples	633	[2]
MTX	molybdate	RRS	RRS based on an ion-association complex between MTX and molybdate	serum and urine	365/365	[3]
ABZ, FBZ & MBZ	Erythrosine B	Fluorimetry	Fluorescence quenching	Bulk powder, tables, suspension and human plasma	517/544	[4-6]
Mebeverine	Eosin y	Fluorimetry	Fluorescence quenching	commercial tablets	390/540	[7]
Fluoxetine and paroxetine	Eosin Y	Fluorimetry	Fluorescence quenching	Bulk powder and pharmaceutical formulations	301/545	[8]
Amlodipine and nicardipine	Eosin Y	fluorimetry	Fluorescence quenching	Powder and tablets	549	[9]

SI Table 2: polarity index and dielectric constant for solvents used.

Solvent	**Polarity index**	**Dielectric constant**
Water	9.0	80.2
Methanol	6.6	32
Acetone	5.4	20.7
Ethanol	5.2	24.8
Dioxane	4.8	2.2
2-propanol	4.2	19.9

SI Table 3: Comparison between the sensitivity of the current fluorescence method and the other techniques in utilized in the determination of MTX.

Technique	**Range**	**Ref**
HPLC	1-2000 ng mL^{-1} (serum)	[10]
HPLC	5-1000 ng ml^{-1} (plasma) 25-2000 ng ml^{-1} (mouse tissue)	[11]
HPLC	5–1000 $ngml^{-1}$ (plasm) 25–2500 $ngml^{-1}$ (liver) 12.5–2500 ng ml^{-1} (other tissue)	[12]
CE	7.0×10^{-8} –1.0×10^{-6} M	[13]
CL	5.0×10^{-9} –1.0×10^{-7} g/ml	[14]
Polarography	5 x 10^{-7}–2.5 x 10^{-5} M	[15]
Voltammetry	2.0×10^{-7}–6.0×10^{-6} mol dm^{-3}	[16]
ELISA	0.25-50 ng ml^{-1}	[17]
Spectrophotometry	4-10 mg L^{-1}	[18]
Spectrofluorimetry	0.0675–0.337 µM	[1]
SERRS	2.5×10^{-9}–1×10^{-6} M.	[19]
Spectrofluorimetry	70–2500 ng ml^{-1} 0.3–2 µg ml^{-1} (biofluids)	Current method

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