Supplementary Information

Harnessing Zinc (II) Chelation for Fluorescence Determination of Besifloxacin in Ophthalmic and Biological Matrices

Islam M. Mostafa[a,b*], Demiana W. Fakhry [c], Mohamed A. Abdelshakour [c], Deena A. M. Nour El-Deen[a,b]

[a] Analytical Chemistry Department, Faculty of Pharmacy, Minia University, Minia, 61511, Egypt

[b] Analytical Chemistry Department, Faculty of Pharmacy, Minia National University, New Minia, 61511, Egypt

[c] Department of Pharmaceutical Analytical Chemistry, Faculty of Pharmacy, Sohag University, Sohag 82524, Egypt.

List of content

Table S1. Comparison between the analytical performance of the developed method and other reported methods.

Method	Linear range	LOD	LOQ	Applications	Remarks	Ref.
HPLC	0.3 – 2.3 µg/mL	0.07 µg/mL	0.3 µg/mL	Dosage form	- Low sensitivity -Hazardous solvents	[1]
UHPLC	0.75-3.75 µg/mL	0.1 µg/mL	0.5 µg/mL	Dosage form	-Expensive instrumentation -Moderate sensitivity -Hazardous solvents	[2]
HPLC	0.9-7.5 µg/mL	0.30 µg/mL	0.90 µg/mL	Dosage form	- Low sensitivity -Hazardous solvents	[3]
Electrochemical method	2.2×10^{-6} mol/L to 5.5×10^{-5} mol/L	9.12×10^{-7} mol/L	3.04×10^{-6} mol/L	Dosage form	-Electrode modification	[4]
Spectrophotometry	3-30 µg/ml	0.62, 0.72 and 0.88 µg/ml	1.88, 2.10, 2.60 µg/ml	Dosage form and simulated tears	- Low sensitivity	[5]
Spectrofluorimetry	200-1000 ng/mL	8.47 ng/mL	28.24 ng/mL	Dosage form and simulated tears	- Required derivatization -Expensive reagent	[6]
Spectrofluorimetry (based on Hantzsch condensation reaction and measuring fluorescence at λ_{ex} of 400 nm (λ_{em}= 485nm)	0.15-1.0 (µg/mL)	0.02 µg/mL	0.05 µg/ml	Dosage form and aqueous humor	- Required derivatization -Moderate sensitivity - Long heating time	[7]
Spectrofluorimetry (based on the formation of micelle and measuring fluorescence at λ_{ex} of 274 nm (λ_{em}= 446nm)	5.0 - 100 ng/mL	0.64 ng/mL	1.93 ng/ml	Dosage form and artificial aqueous humor	-Dependency on precise surfactant concentration - Green - Simple	[8]
Spectrofluorimetry	10 – 150 ng/mL	2.0 ng/mL (4.64 nM)	6.08 ng/mL	Dosage form and aqueous	- Simple - No derivatization	Present work

			(14.1 nM)	humor	- Eco-friendly	

Figure S1. Stoichiometry of the reaction between zinc(II) and BFN using Job's method.

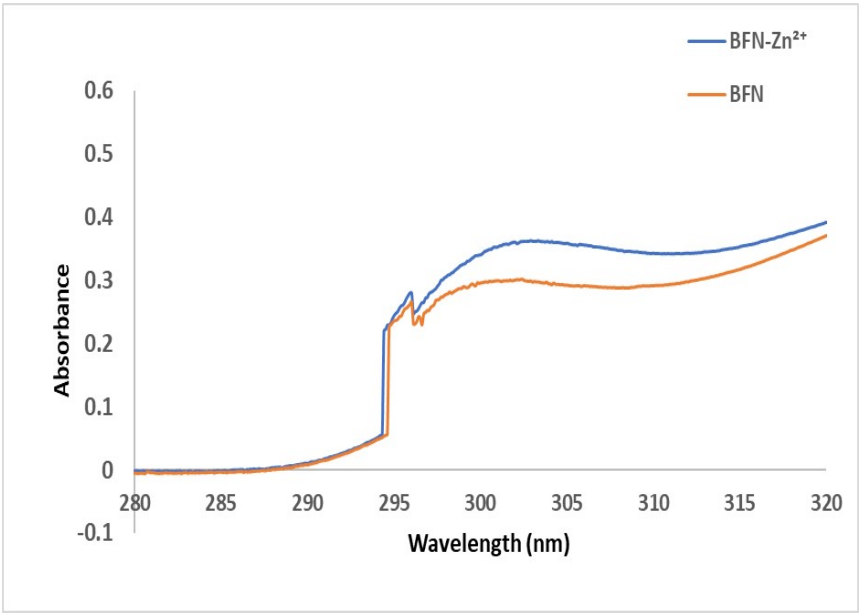

Figure S2. UV spectrum of BFN (20 µg/mL) in the presence and absence of zinc(II).

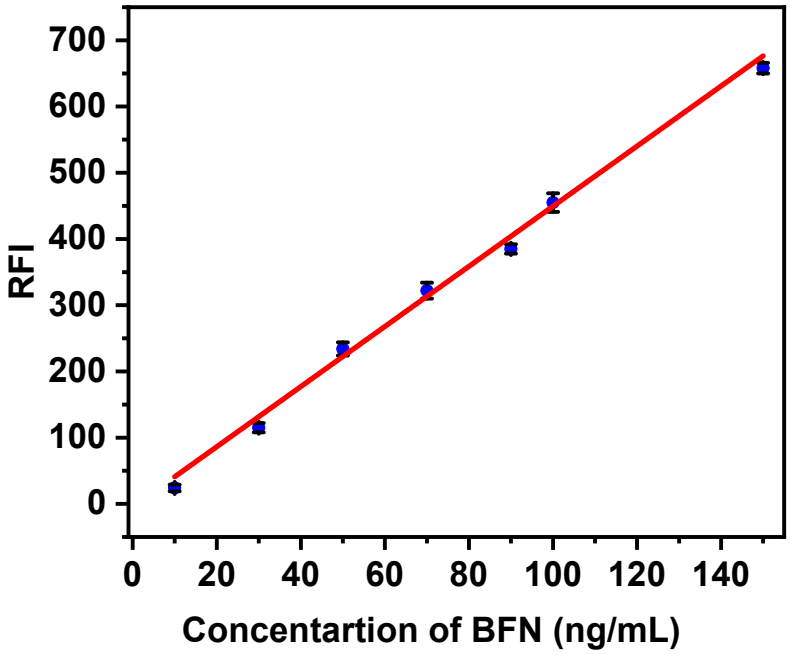

Figure S3. Calibration curve for the determination of BFN by the proposed method.

Table S2. Robustness of the proposed method for determination of BFN (100 ng mL^{-1})

Parameters	**% Recovery ± RSD**
pH of buffer	
5.8	97.32 ± 0.43
6.0	100.16 ± 0.45
6.2	98.63 ± 0.44
Zn^{2+} volume	
0.8	97.72 ± 1.26
1.0	99.99 ± 0.33
1.2	100.89 ± 0.47

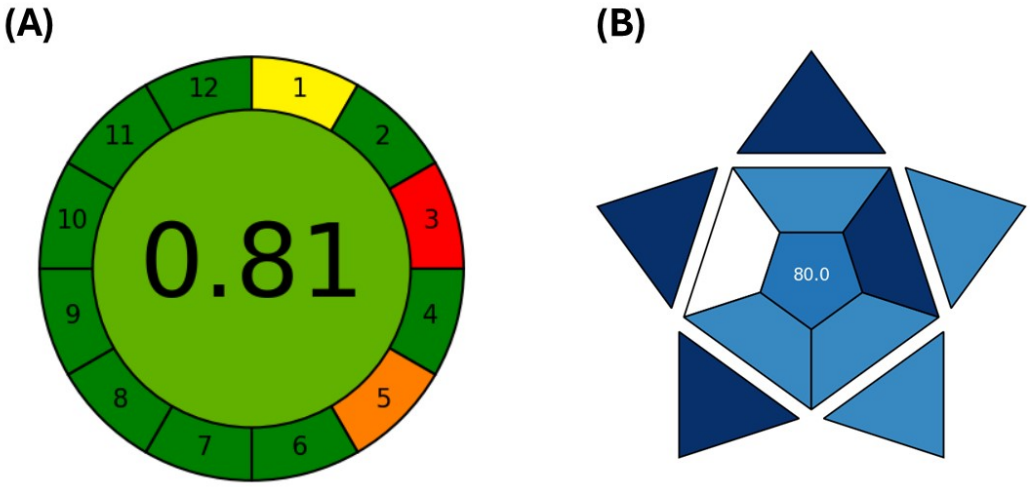

Figure S 4. Greenness and blueness evaluation of the developed method using (A) AGREE metric, and (B) BAGI metric, respectively.

References

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