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Electronic Supplementary Material

Derivative matrix-isopotential synchronous spectrofluorimetry: A solution for the direct determination of urinary δ-aminolevulinic acid

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1. Scheme of derivatization.

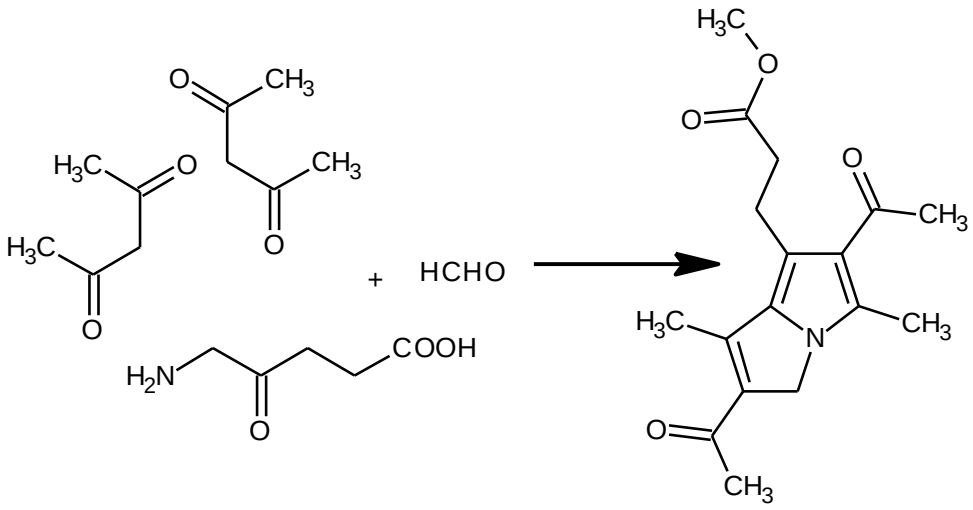

Fig. S1 Scheme of derivatization reaction[1]

2. Experimental details on fluorescence study.

Firstly, the excitation and emission spectra of the derivatized ALA were obtained in the form of ASCII files and then characterized as excitation and emission peaks. These spectra (of derivatized ALA and the urine) were then used for the generation of theoretical three-dimensional (3D) spectra by employing a home-made data-processing program.[2] For the selection of the suitable path, this three-dimensional spectra were subsequently transformed into two-dimensional plots by joining points of equal fluorescence intensity. These two-dimensional plots are used to select the best possible trajectory for the matrix-isopotential synchronous fluorescence (MISFS). The total fluorescence spectra for derivatized ALA and urine as contour lines, highlighted the substantial fluorescence background (spectral overlap) exhibited by urine at the point of fluorescence maxima of derivatized ALA.

The matrix-isopotential trajectory was selected from the contour plots of derivatized ALA and the urine. This trajectory passed through the excitation and emission maxima of derivatized ALA. Therefore, the sensitivity achieved in this way, was the same as that obtained in the direct determination (in the absence of urinary fluorescence) of derivatized ALA. Hence, the problem of severe background interference was overcome. However, at this point, it was not possible to measure the fluorescence intensity regarding the ends of the spectrum. The complication of the

unknown matrix constant value was overcome by applying the first derivative technique. Moreover, the negative signal in the DMISF spectra were obtained directly through the automated electronic differential device attached with the spectrofluorometer, using the matrix isopotential trajectory obtained earlier[3]. In case of the isopotential trajectory, the value for the first derivative of the spectrum of urine sample was zero. Consequently, the value of the first derivative of the spectrum of derivatized ALA in urine sample coincided with the first derivative of the spectrum of derivatized ALA.

3. HPLC Study.

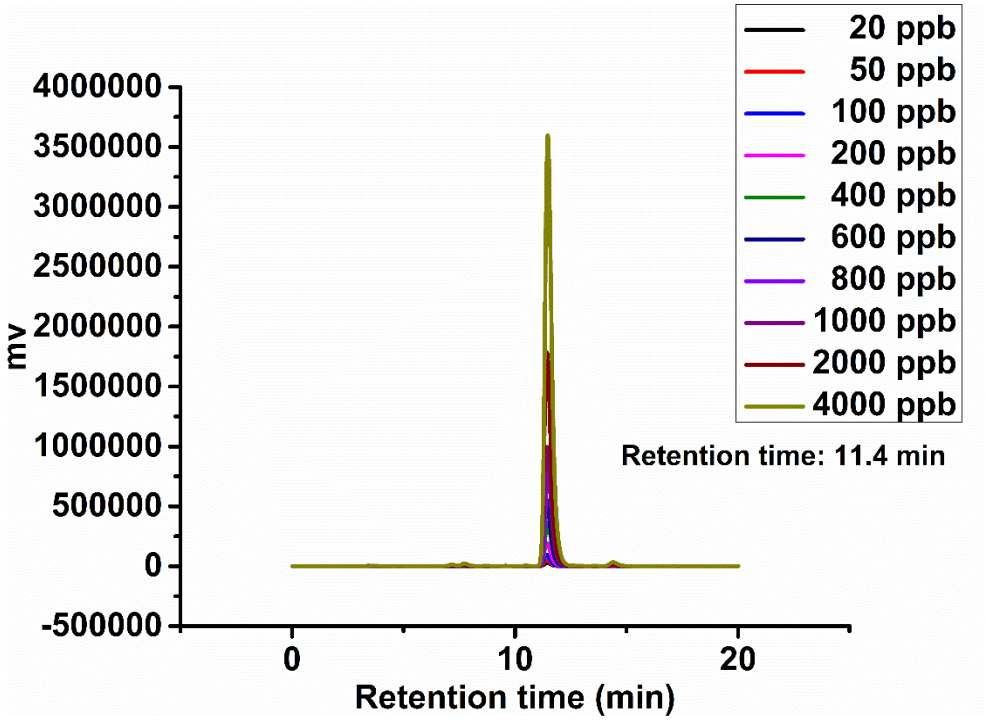

Fig. S2 HPLC results for different standard concentrations added in urine samples (20ppb-4000ppb)

The mobile phase used in the system consisted of methanol, acetic acid and water with a flow rate of 1 mL min^{-1}. The injection volume was 20 µL and the temperature of the column was kept constant at 40 ^{0}C in order to maintain the reproducibility of retention times. The peak corresponding to derivatized ALA eluted at 11.4 min.

4. Linear fits for fluorescence and HPLC studies.

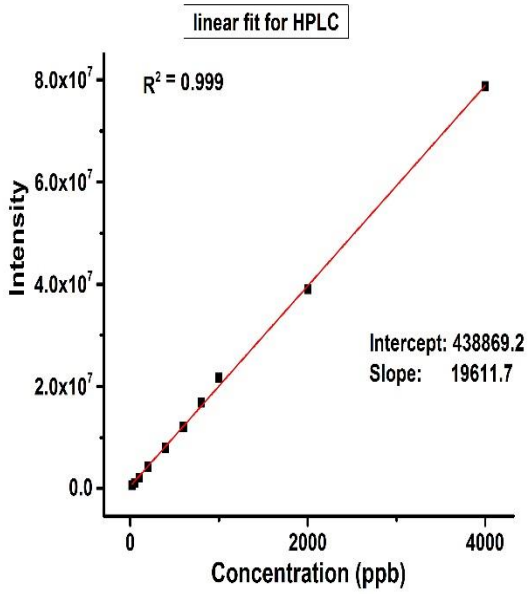

Fig. S3 (A) Linear fit for HPLC

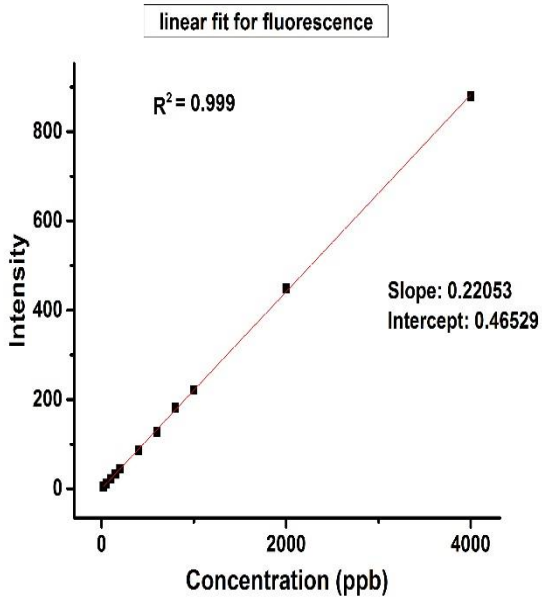

Fig. S3 (B) Linear fir for DMISFS

5. Precision test.

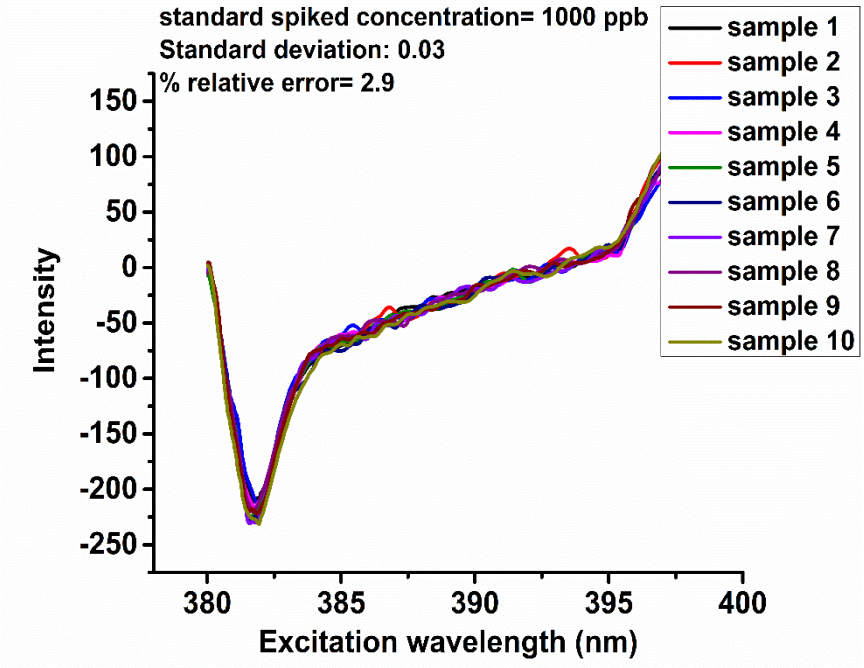

Fig. S4 Ten spiked samples with same concentration for repeatability experiment

The precision was assessed by preparing a series of ten replicates containing 1000 ppb of the δ-ALA in urine samples under conditions similar to the calibration standards. Having applied the IUPAC definition, the relative error was found as 2.9%.

6. %age recovery test.

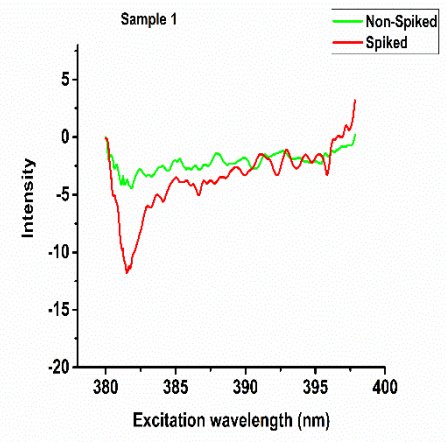

Fig. S5 (A)

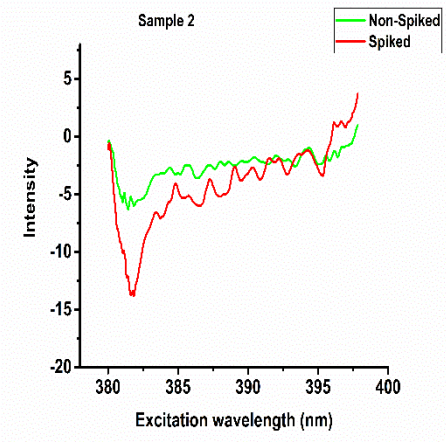

Fig. S5 (B)

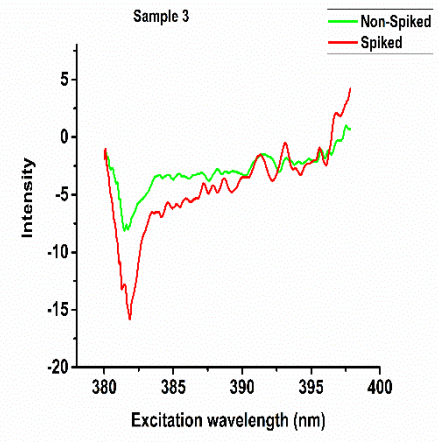

Fig. S5 (C)

The accuracy and reliability of the given method were further validated by undertaking recovery tests. For this purpose, three different urine samples were spiked with the same concentration (35 ppb) of standard and the average recoveries were obtained.

References

1 M. Kajiwara, K. I. Hara, K. Takatori, and K. Matsumoto, *Clin. Chem*., 1993, **39**, 1867-1871.
2 W. U. Chu, S. Wei, Y. Q. Li, *Chem. J. Chin. Univ*., 1999, **20** (Sup), 422
3 Y. H. Liu, P. P. Wu, Q. Liu, H. D. Luo, S. H. Cao, G. C. Lin, D. S. Fu, X. D. Zhong and Y. Q. Li, *Food Anal. Method.*, 2016, **9**, 3209-3217.