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Electronic Supplementary Information

Inner filter effect based sensing system for the determination of caffeine in beverages samples.

Natalia González[a], Sara P. Lantmann Corral[a], Graciela Zanini[a], Hernán Montejano[b,c], Carolina C. Acebal[a]*

[a] *INQUISUR, Departamento de Química, Universidad Nacional del Sur (UNS-CONICET), Av. Alem 1253, 8000 Bahía Blanca, Argentina.*

[b] *Departamento de Química, Facultad de Ciencias Exactas, Físico-Químicas y Naturales, Universidad Nacional de Río Cuarto, Ruta Nacional 36 Km 601, X5804BYA Río Cuarto, Córdoba, Argentina.*

[c] *Instituto de Investigaciones en Tecnologías Energéticas y Materiales Avanzados (IITEMA); CONICET – UNRC*

Section S.1. HPLC method

To evaluate the accuracy of the proposed method, the method suggested by Pearson was followed[1]. Thus, the determination of CF in the samples of coffee and tea infusions, and energy drinks was performed by HPLC with UV detection (280 nm). The samples were previously extracted performing a solid-phase extraction procedure using a C18 packed column.

S.1.1 SPE procedure

The SPE column was packed into a cylindrical glass tube (3.2 cm length, 0.4 cm i.d.) with 100 mg of C18. Cotton was used as frits at both sides of the column in order to avoid losses of sorbent. The C18 column was coupled to a flow system as is shown in

Fig. S1. To perform the extraction and preconcentration of the CF, the peristaltic pump (PP) was switched on and the sorbent was activated with 5.0 mL of methanol that passed through the extraction column (EC) and finally was directed to waste. Once the column was conditioned, it was washed with 5.0 mL of water. After this, 10.0 mL of the standard solution or sample was introduced to perform the extraction of the analyte. Once the standard solution/sample passed through the EC, it was washed with water to remove the sample residuals that may have remained in the column. Then 5.00 mL of mobile phase ($5.0x10^{-3}$ mol L^{-1} sodium acetate/ tetrahydrofuran, 95: 5 at pH 5) passed through the EC to elute the CF.

The eluate was collected, filtered with a 0.22

µm syringe filter and injected in the HPLC system for the analysis. The extraction blank was performed in a similar way.

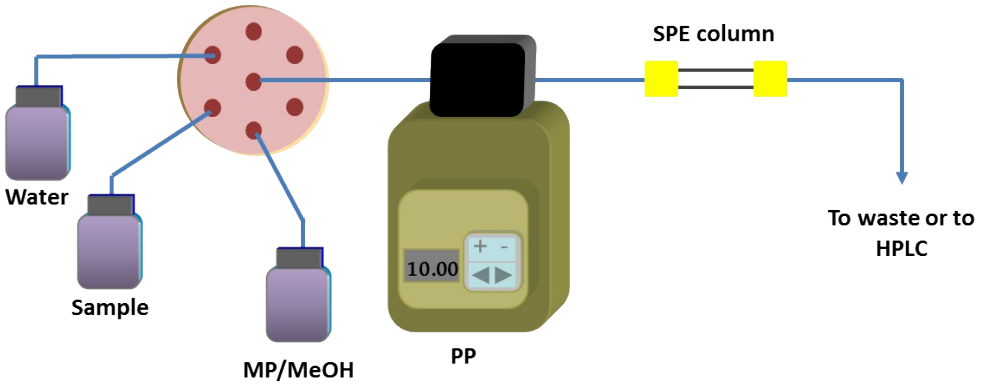


Fig. S1. Flow manifold used to perform SPE extraction of CF from caffeinated beverages samples. MP: mobile phase; PP: peristaltic pump; SPE: solid phase extraction.

S.1.2 Sample preparation

Coffee and tea infusions were prepared according to the manufacturer instructions (200 mL of hot water, 5 min). Then, 40.0 mL of this solution were diluted with water up

to 100.0 mL. Thereafter, 10.00mL of the diluted sample was filtered with a 0.45µm syringe filter and passed through the C18 column.

For energy drinks, 10.00 mL of each sample was taken, placed in a 100.0 mL volumetric flask and brought to volume with water. Then, 10.00 mL was filtered with a 0.45µm filter and introduced into the SPE column.

Section S.2 Concentrations of the potential interfering compounds in beverages samples

The effect on the GLB fluorescence signal of the components that were in higher concentration in the selected samples was studied. In the case of energy drinks, the information declared on the label by the manufacturers was taken into account. Thus, sucrose was in a concentration of 27-28 g per 250 mL of beverage, and the concentration of taurine varied according to the brand between 100 mg and 1000 mg per 250 mL. In this case, the experiments were performed taking into account the highest concentration. Only one energy drink sample contained HFCS in a concentration of 28 g per 250 mL of beverage. On the other hand, the sucrose content declared in the coffee label was 7g per 100 g of coffee.

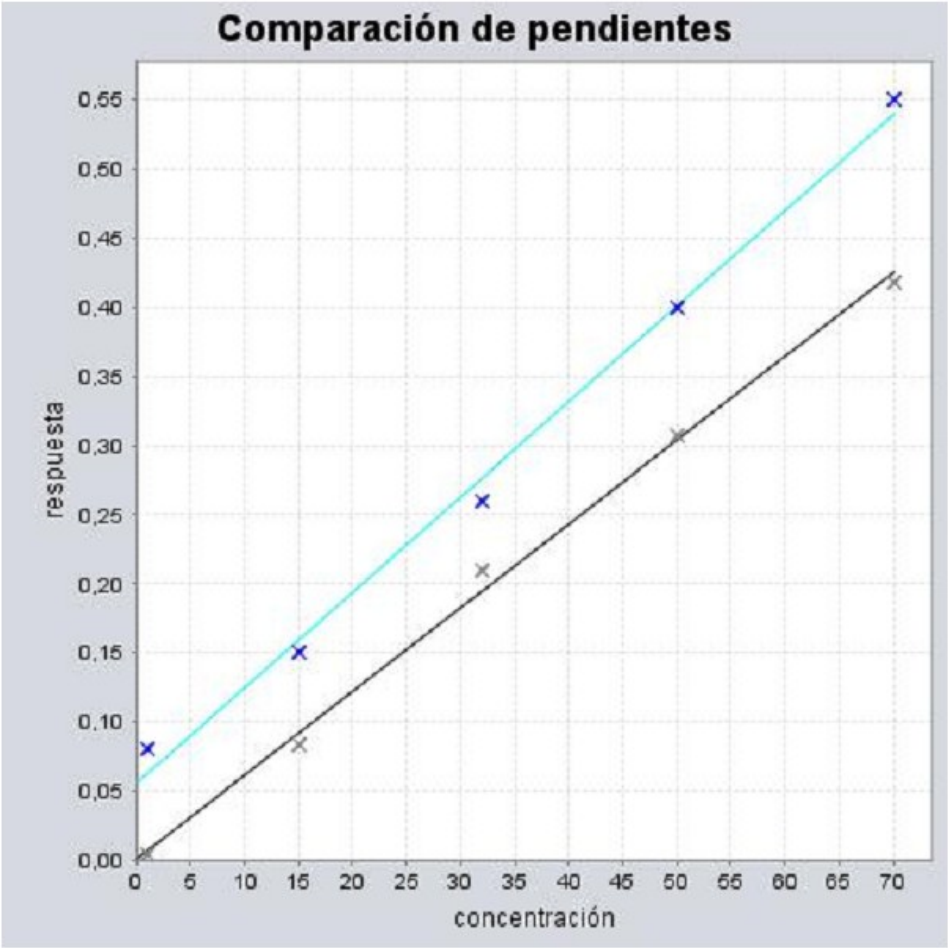
In the case of tea, the study was performed with gallic acid that was considered as the representative compound of total polyphenols and the concentration tested was obtained from the literature according to Dias Diniz and co-workers[2].

Section S.3. Results of the statistical tests for sample matrix effect

The matrix effect was evaluated by comparing the slope of the calibration plot and the slope from three spiked samples representative of the different matrices (tea, coffee and energy drink) randomly selected, at the same concentration levels. The t calculated values for coffee, tea and energy samples were 2.33, 0.40 and 2.24 respectively, corresponding to them a t critical value of 2.44 (α=0.05).

S.3.1 Comparison of the slopes for a coffee sample

Comparison of the slopes of two regression lines

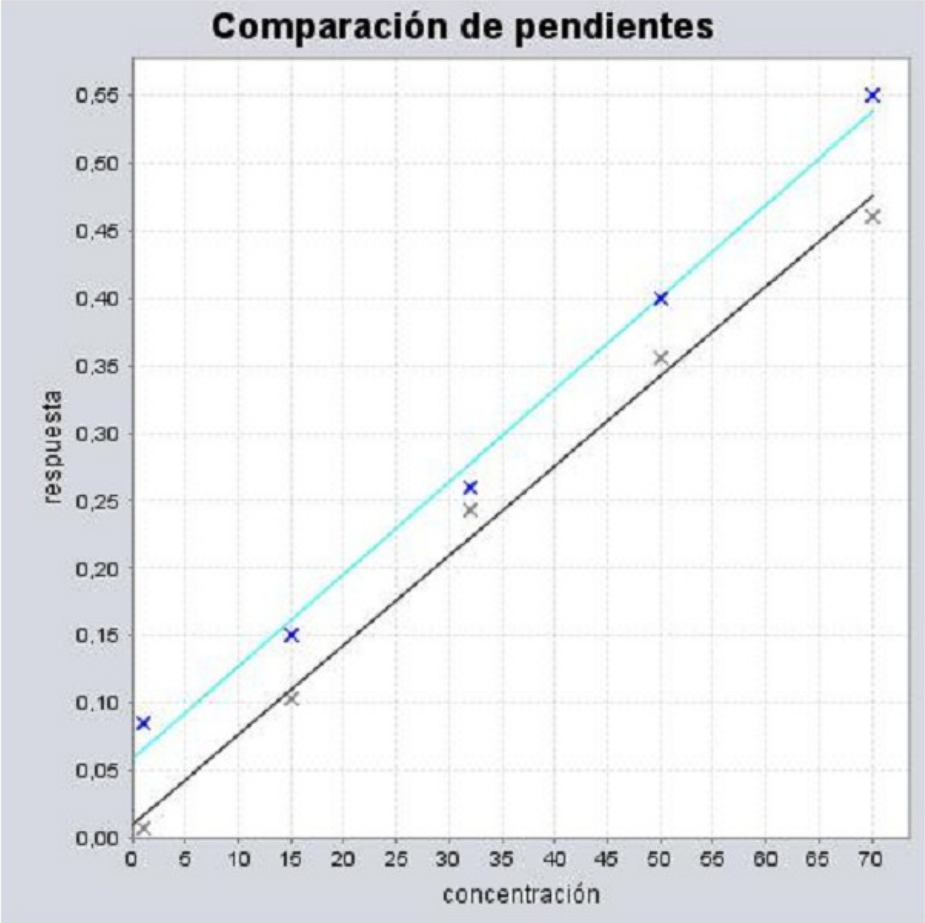


Inputs	
α variance	0.05
α slope	0.05

Results	**Line 1**	**Line 2**
Regression equation	ŷ = b0 + b1·x	ŷ = b0 + b1·x
Slope	0.007	0.006
Intercept	0.056	0.001
Slope std. dev.	0	0
Intercept std. dev.	0.012	0.008
Residual std. dev.	0.016	0.011
Correlation coefficient	0.997	0.998
Determination coefficient	0.995	0.997
Quality coefficient	5.638	5.467

S.3.2 Comparison of the slopes for a tea sample

Comparison of the slopes of two regression lines

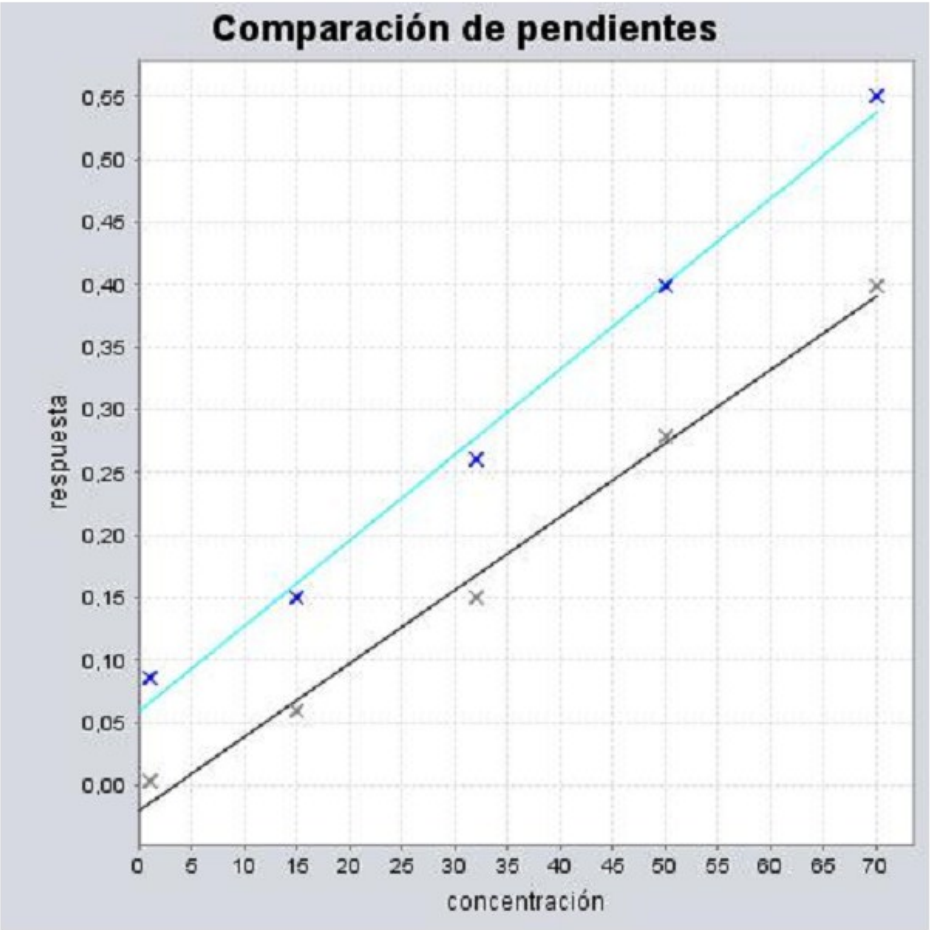


Inputs	
α variance	0.05
α slope	0.05

Results	**Line 1**	**Line 2**
Regression equation	ŷ = b0 + b1·x	ŷ = b0 + b1·x
Slope	0.007	0.007
Intercept	0.059	0.01
Slope std. dev.	0	0
Intercept std. dev.	0.014	0.014
Residual std. dev.	0.018	0.018
Correlation coefficient	0.997	0.996
Determination coefficient	0.993	0.993
Quality coefficient	6.239	7.64

S.3.3 Comparison of the slopes for an energy drink sample

Comparison of the slopes of two regression lines



Inputs	
α variance	0.05
α slope	0.05

Results	**Line 1**	**Line 2**
Regression equation	ŷ = b0 + b1·x	ŷ = b0 + b1·x
Slope	0.007	0.006
Intercept	0.059	-0.019
Slope std. dev.	0	0
Intercept std. dev.	0.014	0.012
Residual std. dev.	0.018	0.016
Correlation coefficient	0.997	0.996
Determination coefficient	0.993	0.993
Quality coefficient	6.239	8.938

Table S.1. Relative fluorescence quantum yields, taking as reference the value determined at pH 3.2.

$\Phi_F^{pH\ 3.2}/\Phi_F^{pH\ 3.2}$	$\Phi_F^{pH\ 7.0}/\Phi_F^{pH\ 3.2}$	$\Phi_F^{pH\ 10.0}/\Phi_F^{pH\ 3.2}$
$\mathbf{1.00 \pm 0.05}$	$\mathbf{0.72 \pm 0.05}$	$\mathbf{0.69 \pm 0.05}$

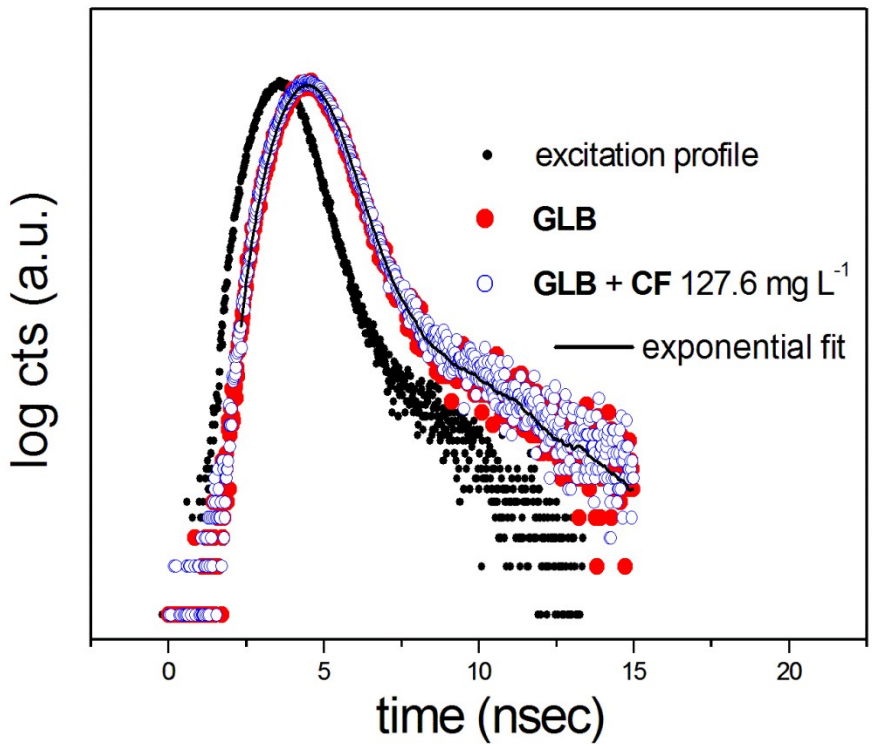


Fig. S2. Time-resolved fluorescence decays of GLB (16 mg L^{-1}) in the presence and absence of CF.

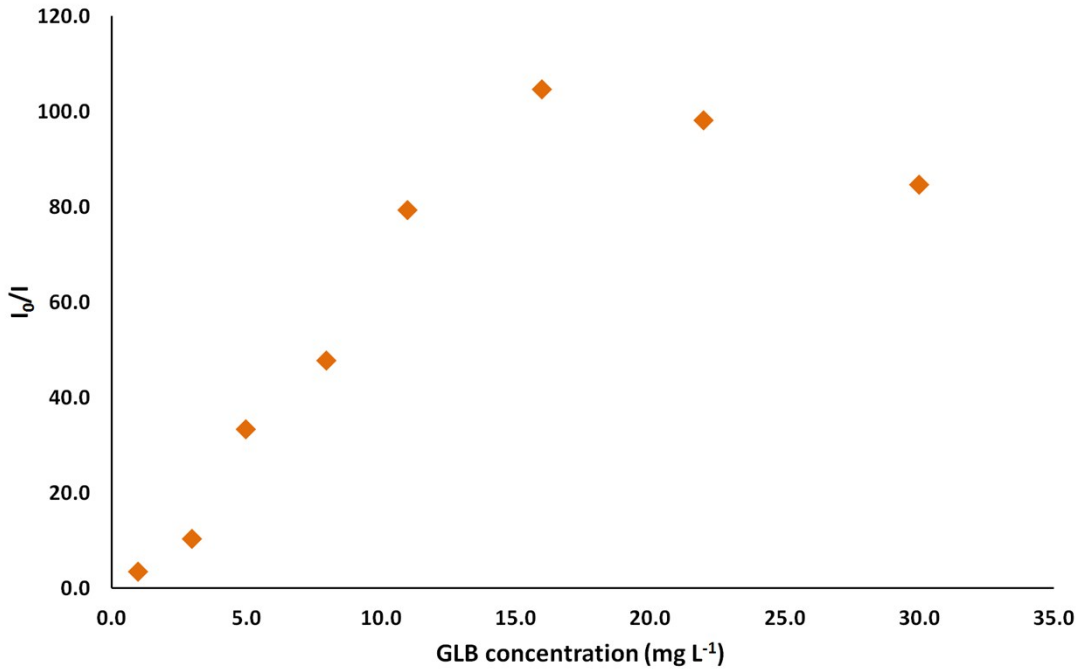


Fig.S3. Study of the optimal concentration of the GLB at a constant concentration of CF (30 mg L^{-1}).

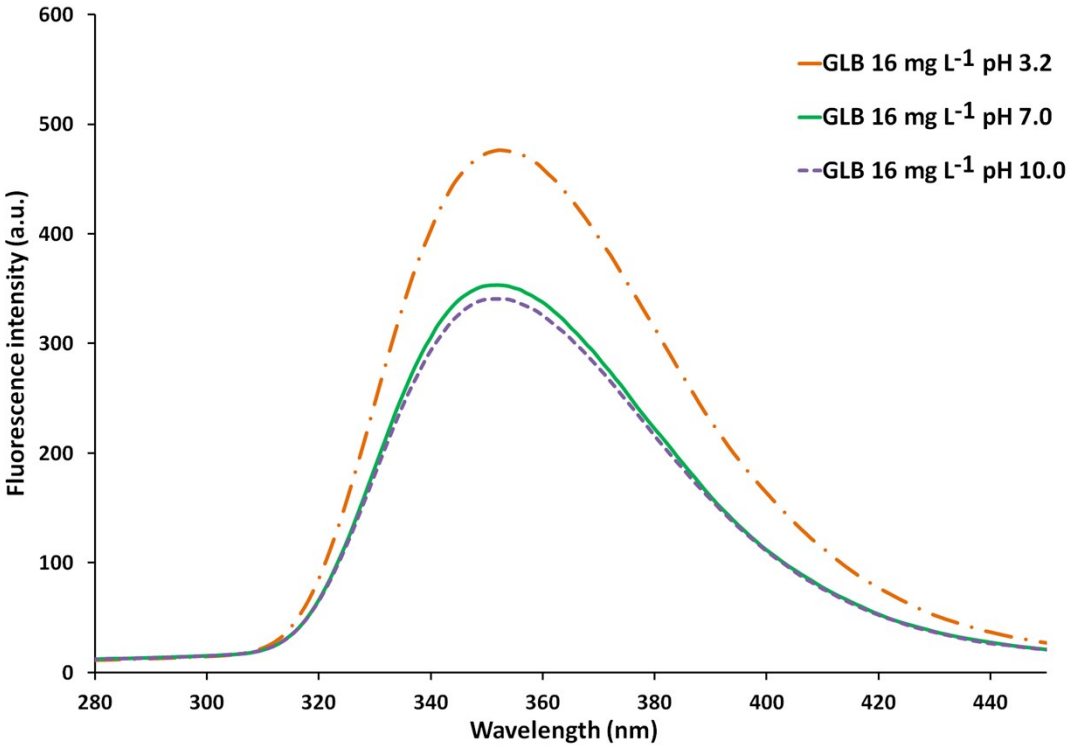


Fig. S4. Fluorescence spectra of GLB (16 mg L^{-1}) at different pH values (λ_{ex} = 234 nm; λ_{em} = 350nm).

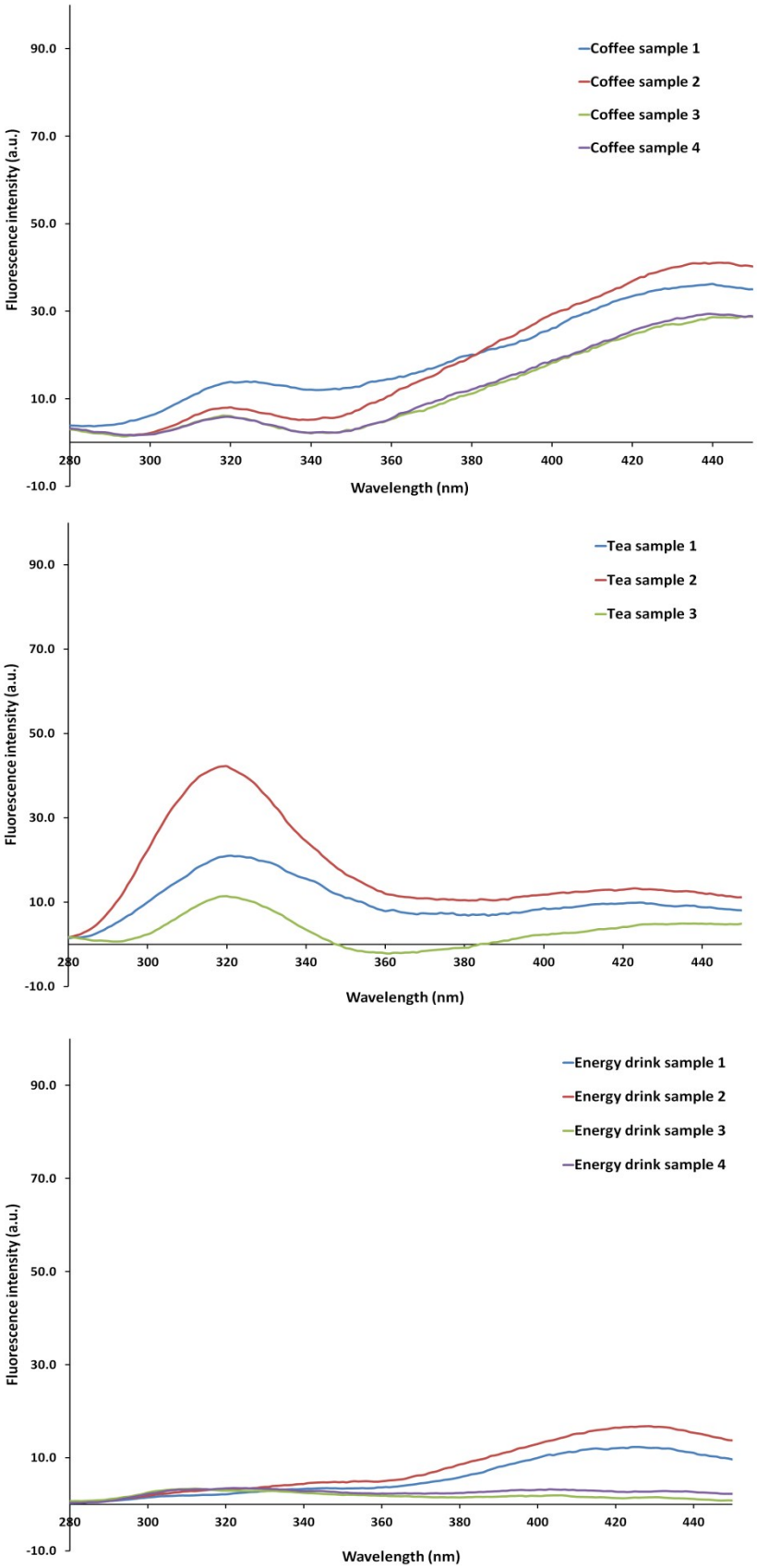


Fig. S5. Fluorescence spectra of the coffee, tea and energy drink samples after the appropriate dilution (λ_{ex} = 234 nm; λ_{em} = 350nm).

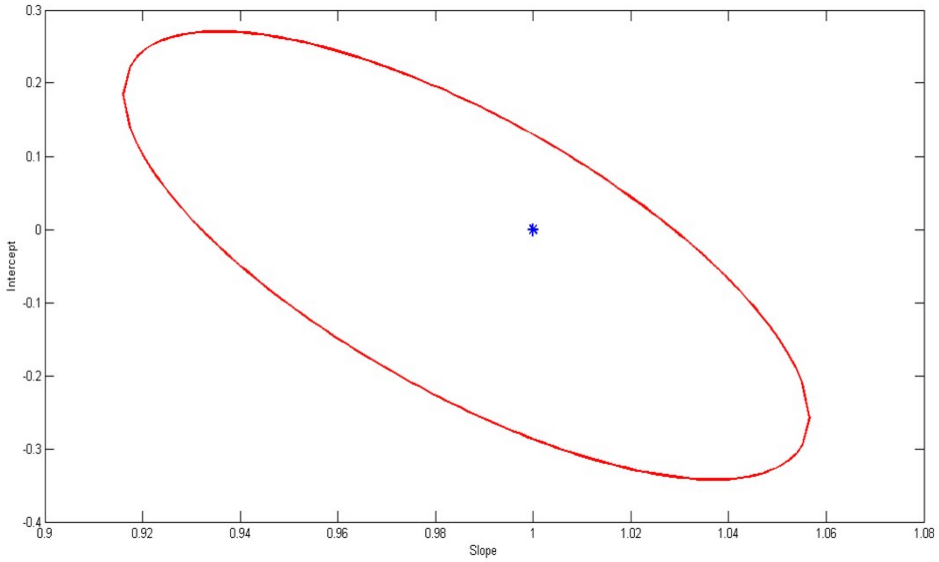


Fig. S6. Elliptical joint confidence region (EJCR) plot at 95% confidence limit, derived from the regression between the CF concentration obtained by the proposed method and the values obtained by the reference method. The asterisk marks the ideal point (slope=1, intercept=0).

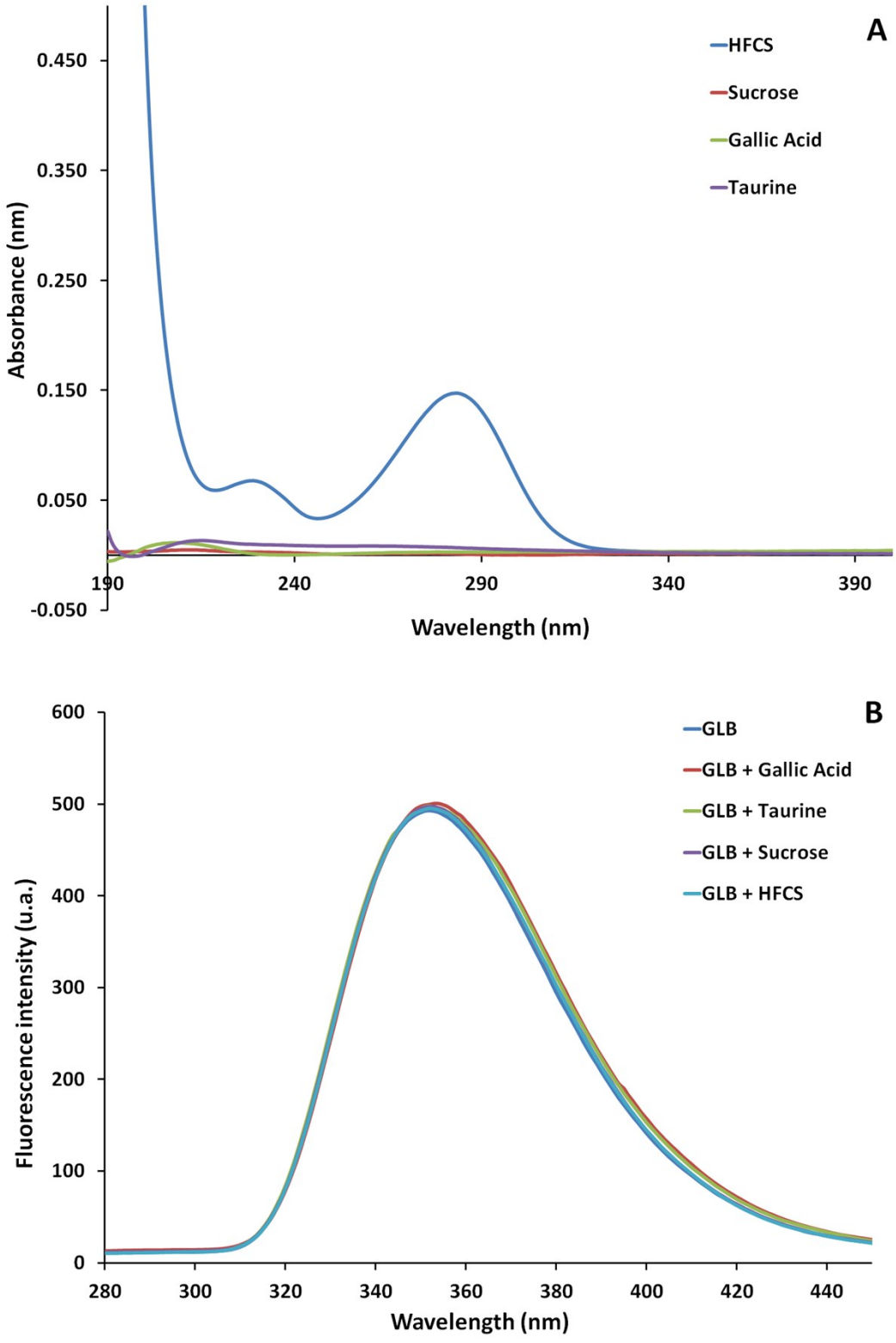


Fig. S7. (A) Absorption spectra and (B) fluorescence emission spectra of GLB in absence and presence of different potential interferents at the concentrations found in the samples (λ_{ex} = 234 nm; λ_{em} = 350nm).

References

[1] S.K. Ronald, S. Ronald and E. Harold, Pearson's Composition and Analysis of Foods, New York, Longman, 1991, 356.

[2] P. Goncalves Dias Diniz, M. Pistonesi, M. Alvarez, B. Fernández Band, M. Ugulino de Araújo, J. Food Compos. Anal, 2015, 39, 103–110.