

SUPPLEMENTARY DATA

Anwar Z et al.

Research Article

A Kinetic Study for the Estimation of Riboflavin (Vitamin B₂) Sensitized Photooxidation of Pyridoxine HCl (Vitamin B₆) by a Green UV-Visible Spectroscopic and High-Performance Liquid Chromatography (HPLC) Methods

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1. Assay Method Validation

1.1 System suitability

It was performed by the injection of six replicates of the standard solutions of PD (1.00×10^{-4} M), and RF (0.50×10^{-4} M) before analysis of the samples to determine the retention time (t_R) theoretical plates (N) and tailing factor (T).

1.2 Linearity and Range

The linearity of the developed spectrometric and HPLC assay methods for analyzing RF, and PD were carried out in the concentration range of 0.1-1.0 and $0.05-0.5 \times 10^{-4}$ M, respectively. The calibration curve was prepared by plotting the absorbance and peak area (A.U.) versus respective concentrations of AH₂ and RF. The statistical calculation was carried out for the results obtained to estimate the correlation coefficient, slope, intercept, standard deviation of intercept, and standard error of slope and intercept. The range selection was optimized from the results obtained from the linearity.

1.3 Accuracy

Three different concentrations of RF ($0.1, 0.2, 0.3 \times 10^{-4}$ M) and PD ($0.2, 0.4, 0.6 \times 10^{-4}$ M) were selected from the results obtained from the linearity and range studies. Each measurement was carried out in triplicate, and the percentage recoveries were calculated using the following equation.

$$\text{Percent recovery (\%)} = \frac{\text{Amount Added}}{\text{Amount Found}} \times 100 \quad (1.0)$$

where,

$$\text{Amount Found} = \frac{\text{Mean of 3 determinations} - \text{Intercept}}{\text{Slope}} \quad (2.0)$$

The relative accuracy error (%) was also calculated using:

$$\text{Relative accuracy error (\%)} = \frac{\text{Recovery (\%)} - \text{Mean recovery (\%)}}{\text{Mean recovery (\%)}} \times 100 \quad (3.0)$$

1.4 Precision

The precision (repeatability and intermediate) of the developed method was estimated by preparing the six individual concentrations of RF (0.50×10^{-4} M) and PD (0.50×10^{-4} M) were subjected to spectrometric and HPLC analysis. The relative standard deviation (%) was calculated to determine the precision by the following formula.

$$\text{RSD (\%)} = \frac{\text{SD}}{\text{Mean}} \times 100 \quad (4.0)$$

1.5 Sensitivity

The sensitivity of the proposed methods is the minimum quantity of analyte to be detected (LOD) and quantified (LOQ). Linearity data is used to calculate the sensitivity of the proposed methods by following the formula.

$$\text{LOD} = 3.3 \times \frac{\sigma}{S} \quad (5.0)$$

$$\text{LOD} = 10 \times \frac{\sigma}{S} \quad (6.0)$$

where, σ is the standard deviation of the y-intercept and S is the slope of the calibration curve.

1.6 Robustness

Deliberate changes were made in the condition of the proposed methods to determine the robustness. These changes include changes in pH (± 0.1 units), detection wavelength (± 2 nm), buffer concentration (± 0.001 M), and flow rate (± 0.1 ml/min).

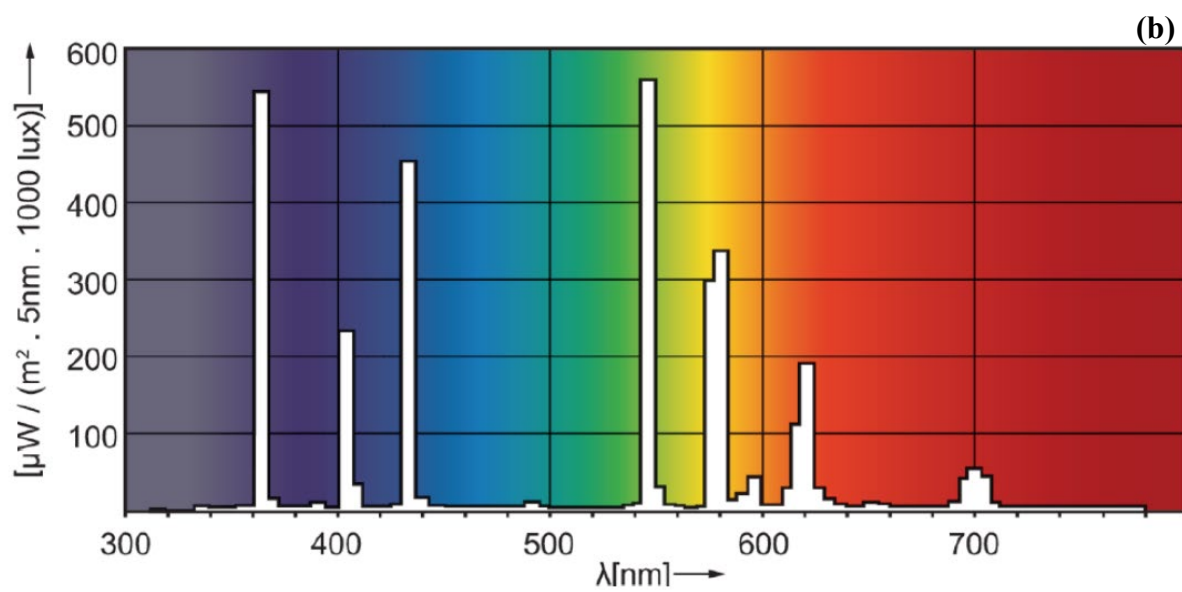
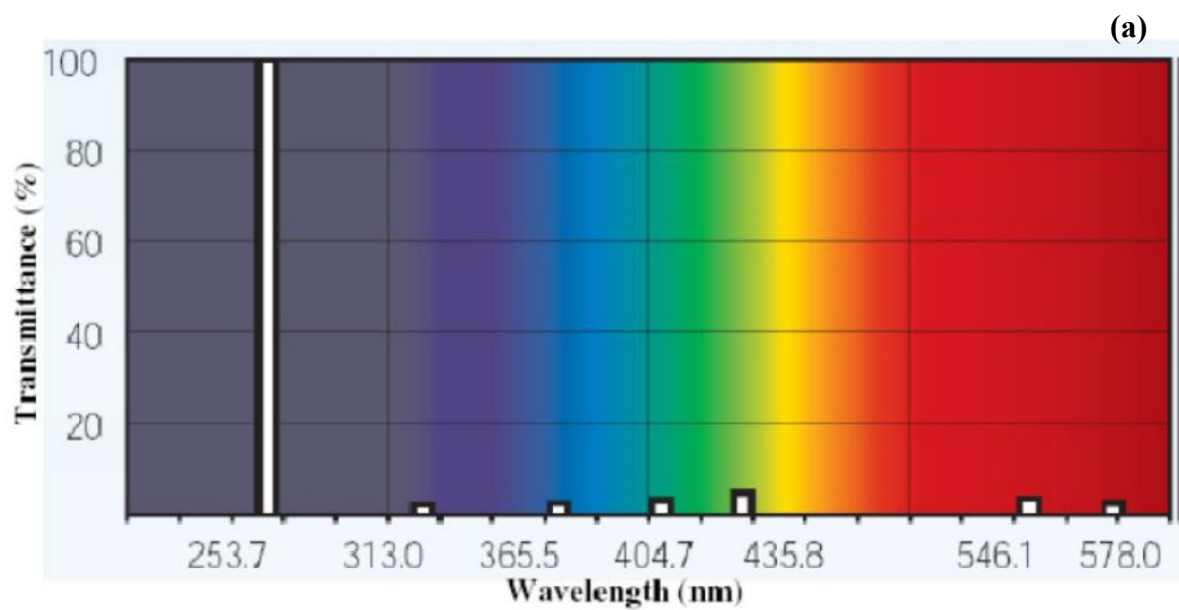


Fig. S1. Spectral emission of UV (a) and visible lamps (b)

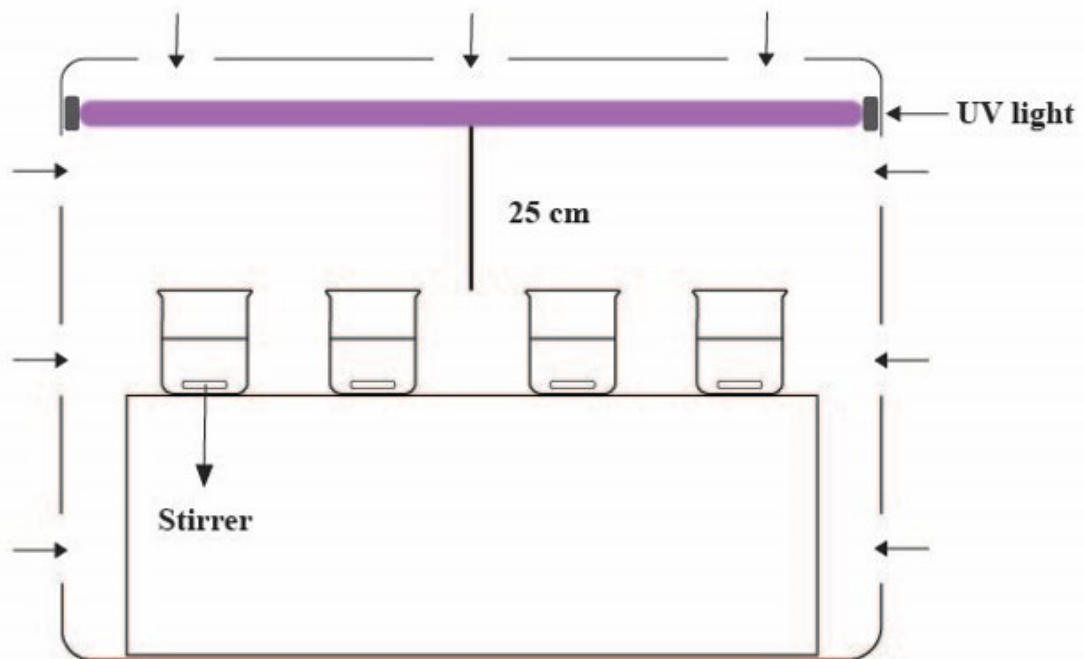


Fig. S2. Irradiation set up for the photolysis of PD in UV irradiation chamber.

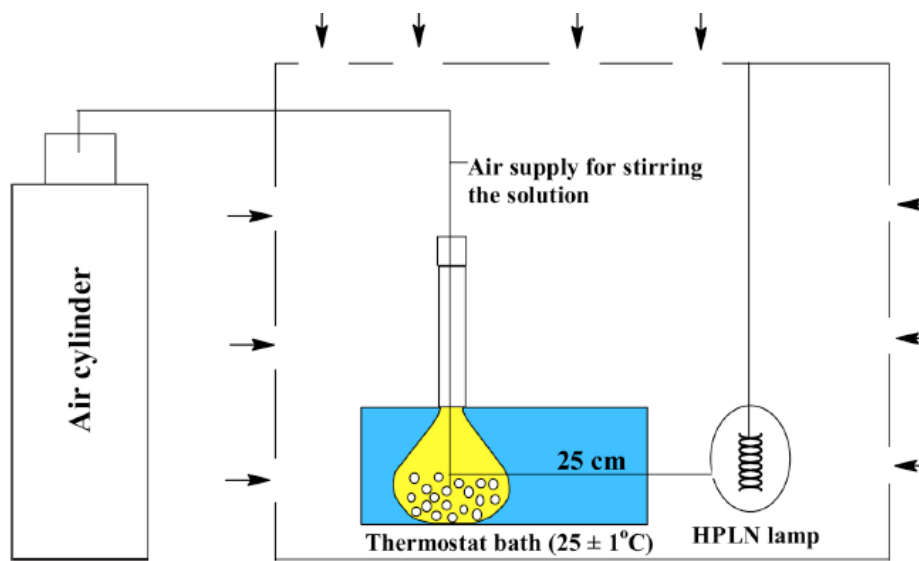


Fig. S3. Irradiation setup for the photolysis of PD in the visible irradiation chamber.

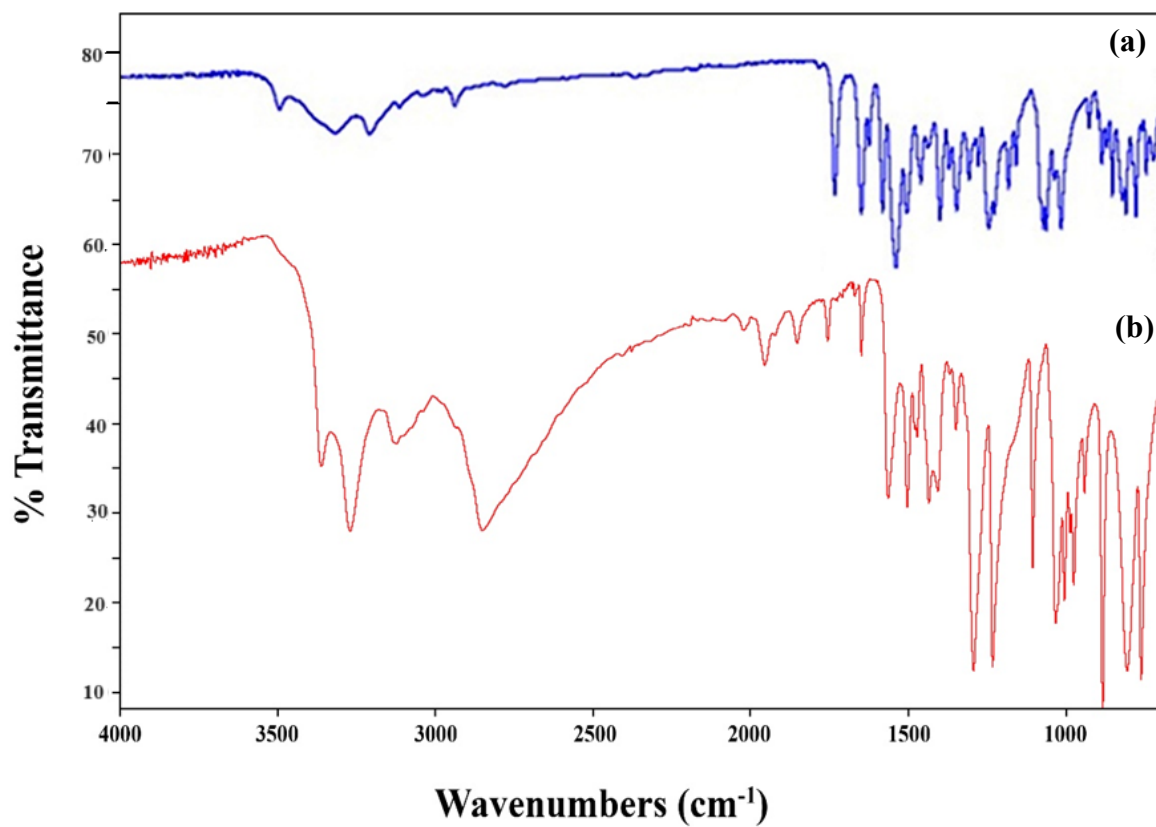


Fig. S4. FTIR spectra of RF (a) and PD (b).

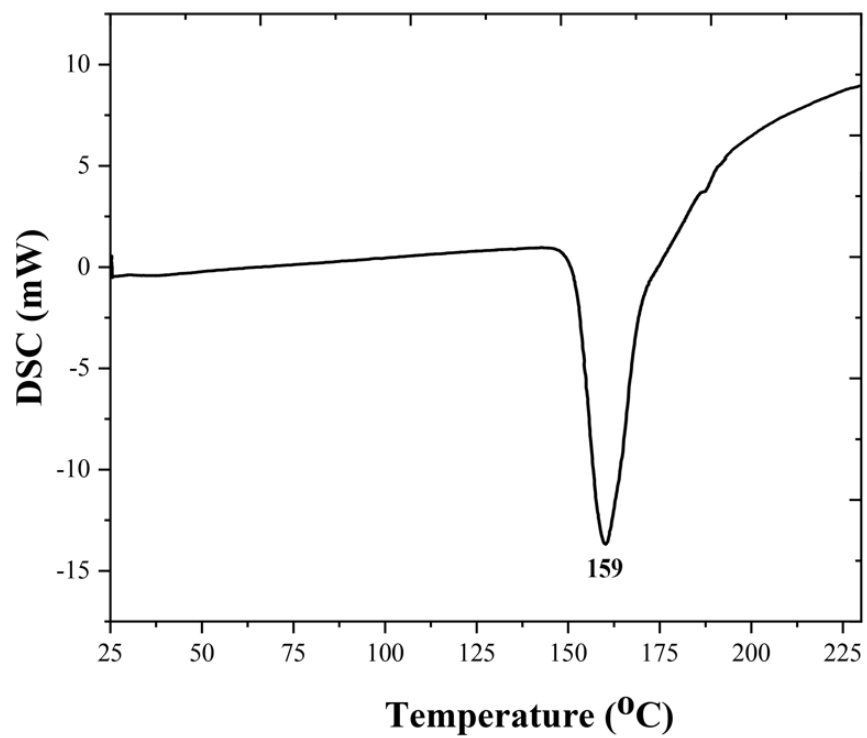


Fig. S5. DSC thermogram of PD.

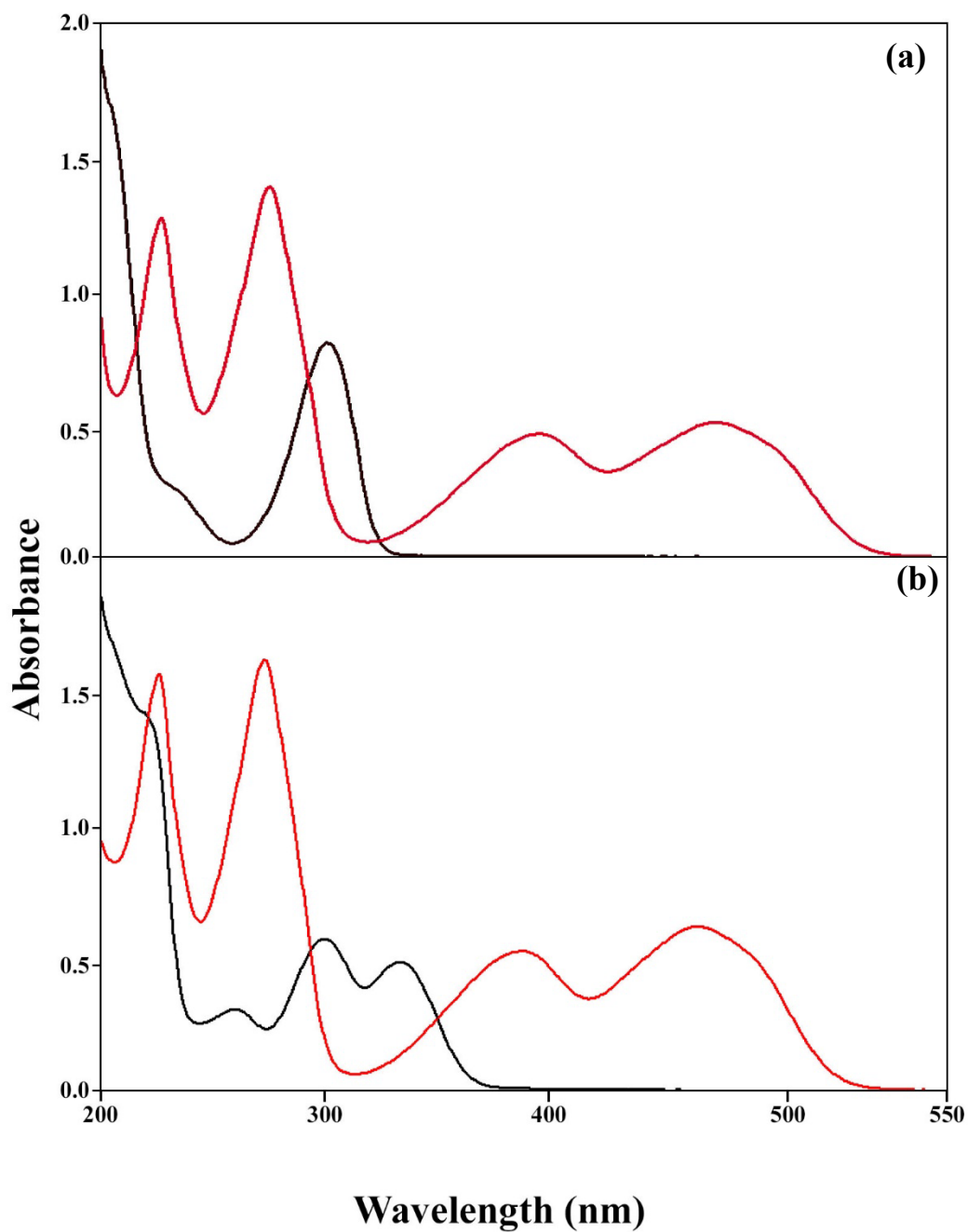


Fig. S6a. Absorption spectra of PD (1.00×10^{-4} M, black line) and RF (0.50×10^{-4} M, red line) in aqueous solution at pH 2.0 (a), and 4.5 (b).

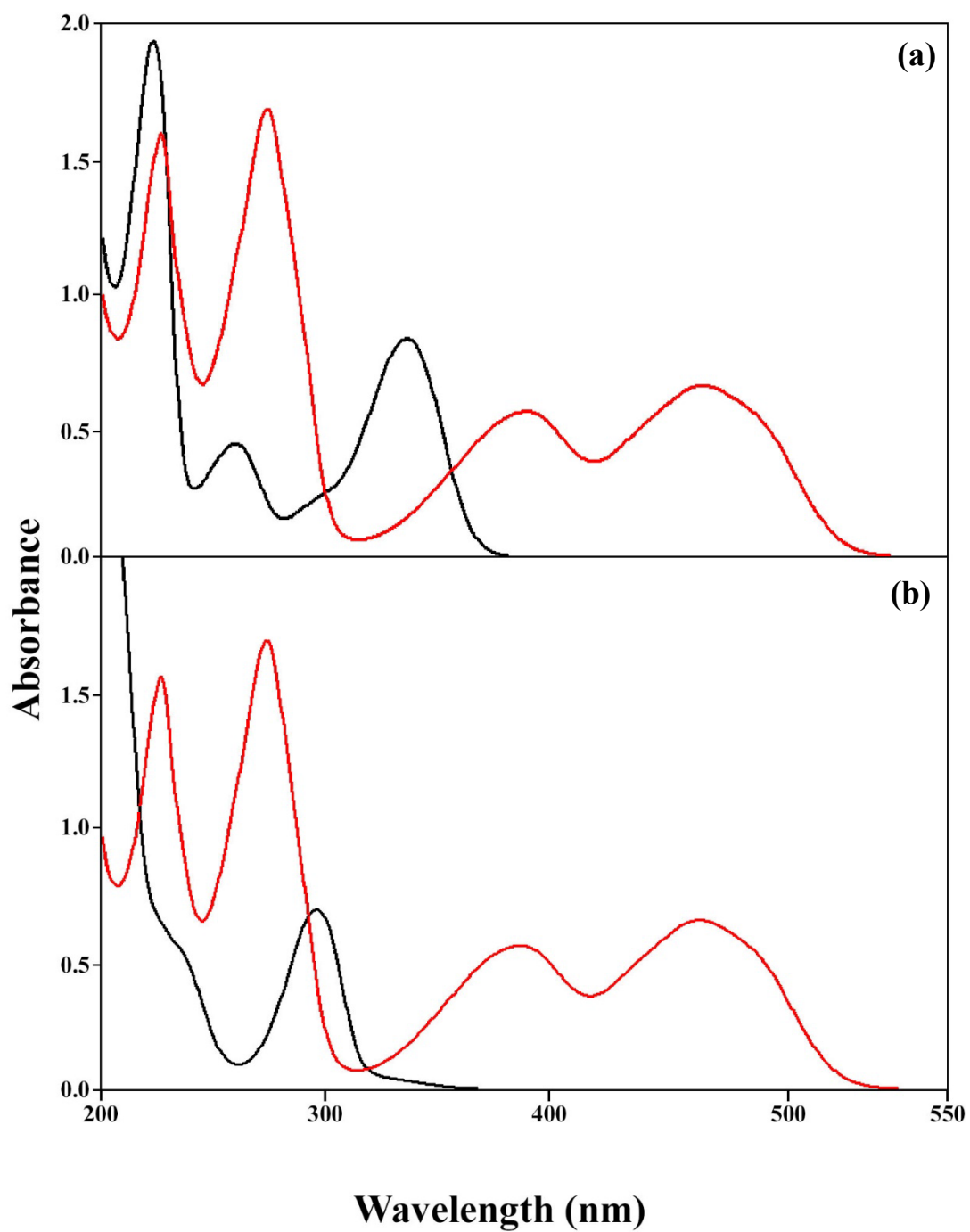
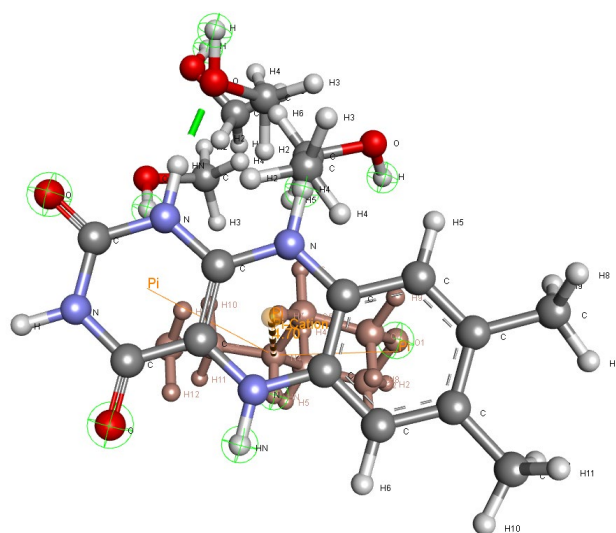
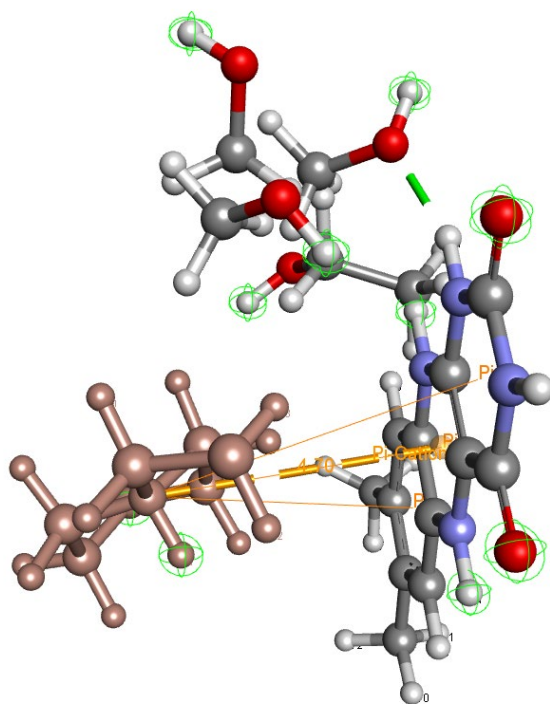


Fig. S6b. Absorption spectra of PD (1.00×10^{-4} M, black line) and RF (0.50×10^{-4} M, red line) in aqueous solution at pH 7.0 (a) and 9.0 (b).



(a)



(b)

Fig. S7. Molecular docking studies of RF (grey structure) and PD (brown structure) indicating the molecular interaction between them

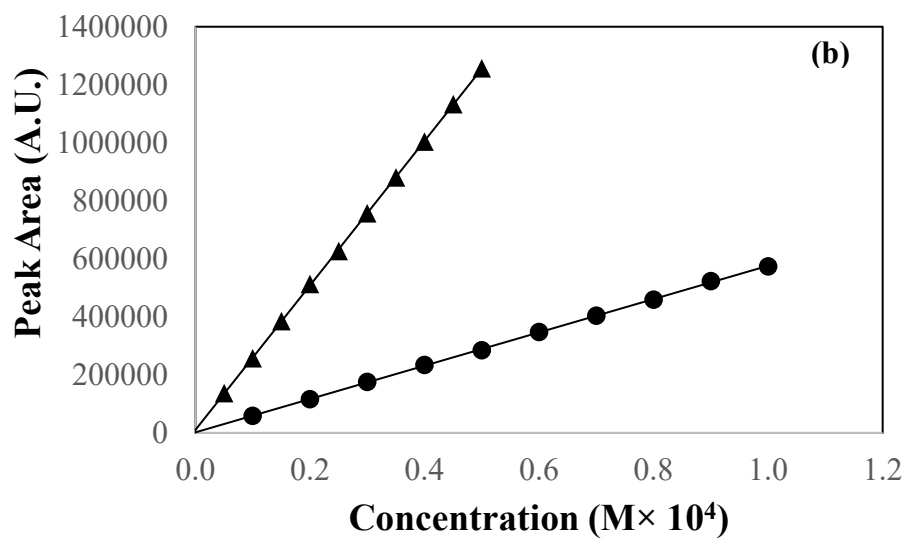
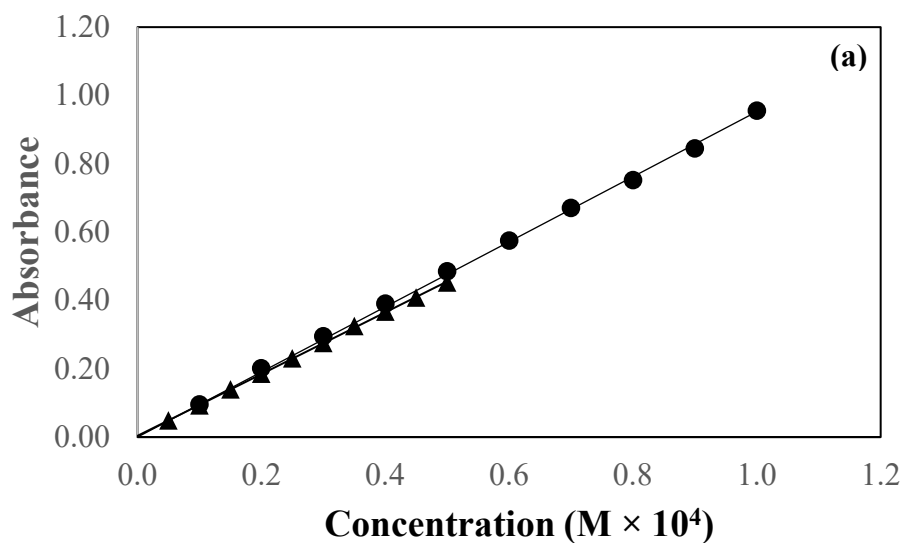


Fig. S8. Calibration curve of PD (●) ($0.10-1.00 \times 10^{-4}$ M) and RF (▲) ($0.05-0.50 \times 10^{-4}$ M) using the proposed two-component spectrometric (a) and HPLC (b) method.

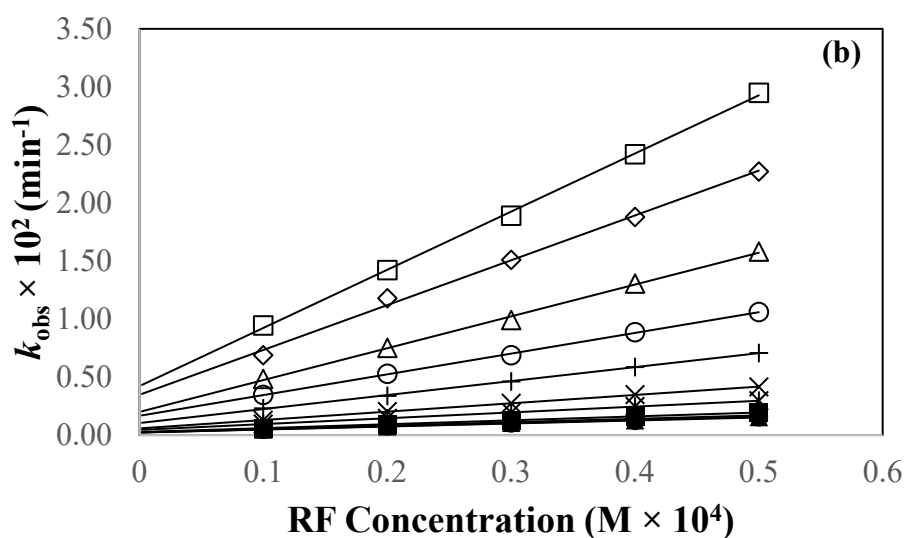
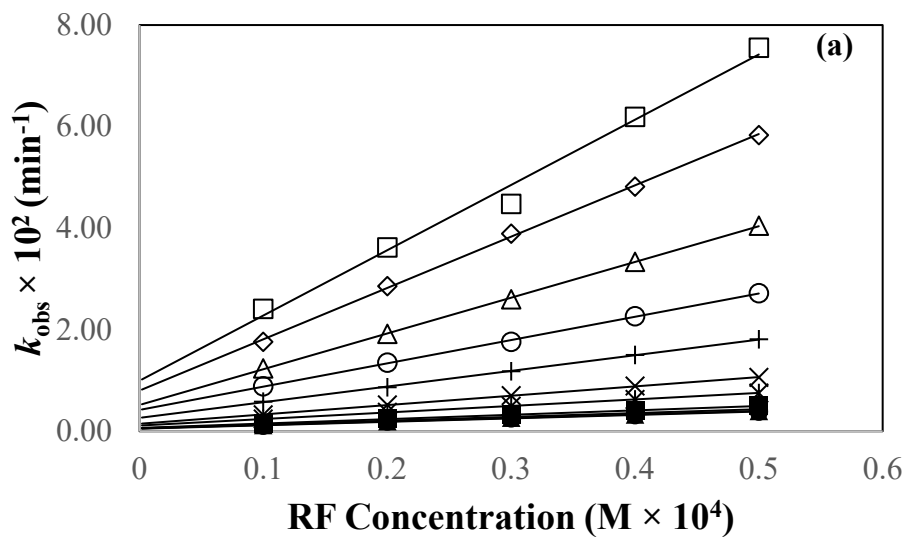


Fig. S9. Second-order plots for the photolysis of PD (1.00×10^{-4} M) in the presence of RF (0.1 - 0.50×10^{-4} M) in aerobic condition using visible (a) and UV (b) irradiation sources at different pH values: 2.0 (\bullet), 3.0 (\blacktriangle), 4.0 (\blacklozenge), 5.0 (\blacksquare), 6.0 ($*$), 7.0 (\times), 8.0 ($+$), 9.0 (\circ), 10.0 (Δ), 11.0 (\diamond), 12.0 (\square).

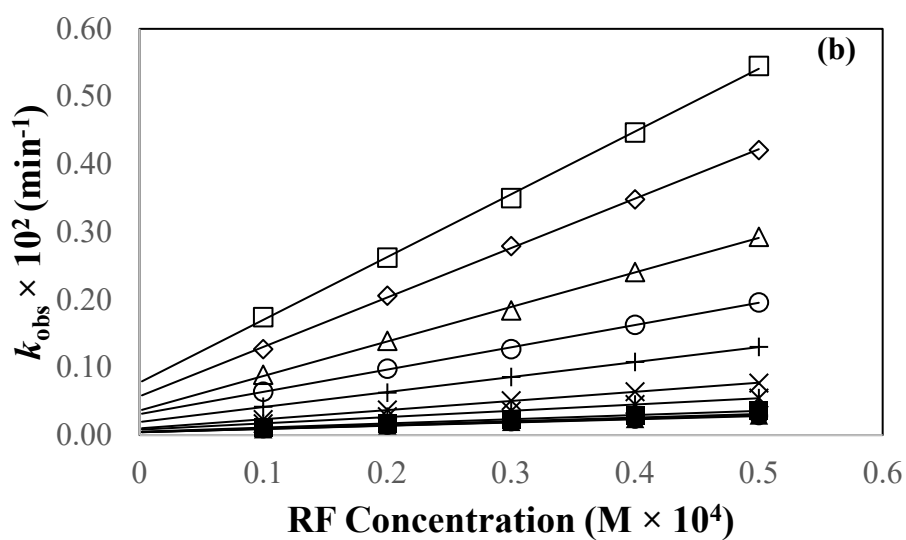
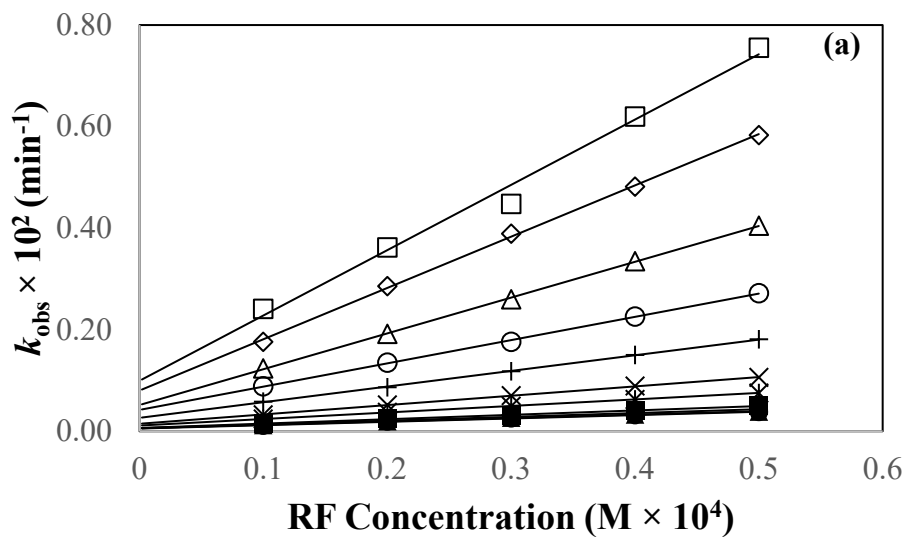


Fig. S10. Second-order plots for the photolysis of PD (1.00×10^{-4} M) in the presence of RF (0.1 - 0.50×10^{-4} M) in anaerobic condition using visible (a) and UV (b) irradiation sources at different pH values: 2.0 (\bullet), 3.0 (\blacktriangle), 4.0 (\blacklozenge), 5.0 (\blacksquare), 6.0 ($*$), 7.0 (\times), 8.0 ($+$), 9.0 (\circ), 10.0 (Δ), 11.0 (\diamond), 12.0 (\square).

Table S1. Accuracy of the proposed spectrometric method for the determination of PD and RF

PD					RF				
Added Conc (M×10 ⁴)	Found Conc (M×10 ⁴)	Recovery (%)	Mean Recovery (%)±SD (%RSD)	Relative Accuracy Error (%)	Added Conc (M×10 ⁴)	Found Conc (M×10 ⁴)	Recovery (%)	Mean Recovery (%)±SD (%RSD)	Relative Accuracy Error (%)
0.200	0.200	100.0	100.5±0.50 (0.49)	-0.498	0.100	0.099	99.0	99.7±0.57 (0.57)	-0.669
	0.201	100.5		0.000		0.100	100.0		0.334
	0.202	101.0		0.498		0.100	100.0		0.334
0.400	0.401	100.3	100.7±0.38 (0.37)	-0.414	0.200	0.200	100.0	100.0±0.50 (0.50)	0.000
	0.403	100.8		0.083		0.199	99.5		-0.500
	0.404	101.0		0.331		0.201	100.5		0.500
0.600	0.601	100.2	100.3±0.19 (0.19)	-0.111	0.300	0.301	100.3	100.2±0.51 (0.51)	0.111
	0.603	100.5		0.222		0.299	99.70		-0.554
	0.601	100.2		-0.111		0.302	100.7		0.443

Table S2. Precision of the developed spectrometric method for the determination of PD and RF

PD					RF				
Added Conc (M×10 ⁴)	Found Conc (M×10 ⁴)	Recovery (%)	Mean Recovery (%)±SD (%RSD)	Relative Accuracy Error (%)	Added Conc (M×10 ⁴)	Found Conc (M×10 ⁴)	Recovery (%)	Mean Recovery (%)±SD (%RSD)	Relative Accuracy Error (%)
Repeatability (Intra-day)									
0.500	0.501	100.2	100.2±0.29 (0.29)	0.040	0.500	0.500	100.0	100.4±0.55 (0.55)	-0.359
	0.499	99.8		-0.361		0.499	99.8		-0.558
	0.503	100.6		0.437		0.501	100.2		-0.159
	0.500	100.0		-0.160		0.503	100.6		0.239
	0.501	100.2		0.040		0.506	101.2		0.837
	Intermediate (Inter-day)								
0.500	0.500	100.0	100.6±0.48 (0.48)	-0.557	0.500	0.500	100.0	100.1±0.30 (0.30)	-0.120
	0.501	100.2		-0.358		0.499	99.8		-0.320
	0.503	100.6		0.040		0.501	100.2		0.080
	0.506	101.2		0.636		0.503	100.6		0.479
	0.504	100.8		0.239		0.500	100.0		-0.120

Table S3. Analysis of a synthetic mixture of PD and RF using the two-component spectrometric method

PD				RF			
Added	Found	Recovery	RSD	Added	Found	Recovery	RSD
(M × 10⁴)	(M × 10⁴)^a	(%)	(%)	(M × 10⁴)	(M × 10⁴)^a	(%)	(%)
0.100	0.099	99.00	0.33	0.450	0.452	100.4	0.41
0.200	0.199	99.50	0.15	0.400	0.406	101.5	0.65
0.300	0.301	100.3	0.63	0.350	0.353	100.8	0.38
0.400	0.405	101.2	0.77	0.300	0.304	101.3	0.66
0.500	0.500	100.0	0.25	0.250	0.252	100.8	0.28
0.600	0.604	100.6	0.41	0.200	0.201	100.5	0.46
0.700	0.703	100.4	0.35	0.150	0.152	101.3	0.64
0.800	0.803	100.3	0.39	0.100	0.101	101.0	0.68
0.900	0.904	100.4	0.41	0.050	0.049	99.80	0.87

^a Values represent the mean of 5 determinations.

Table S3. Accuracy of the proposed HPLC method for the determination of PD and RF

PD					RF					
Added Conc (M×10 ⁴)	Found Conc (M×10 ⁴)	Recovery (%)	Mean Recovery (%)±SD (%RSD)	Relative Accuracy Error (%)	Added Conc (M×10 ⁴)	Found Conc (M×10 ⁴)	Recovery (%)	Mean Recovery (%)±SD (%RSD)	Relative Accuracy Error (%)	
0.200	0.202	101.1		-0.703	0.100	0.098	98.8		-0.065	
			101.8±0.67					98.9±0.14		
	0.205	102.5	(0.66)	0.611			0.099	99.0	(0.14)	0.164
	0.204	101.9		0.092		0.098	98.8		-0.099	
0.400	0.402	100.5		-0.640	0.200	0.201	100.6		-0.376	
			101.1±0.56					101.0±0.35		
	0.406	101.5	(0.56)	0.386			0.202	101.0	(0.35)	0.071
	0.405	101.4		0.254		0.203	101.3		0.305	
0.600	0.603	100.4		0.106	0.300	0.299	99.8		-0.041	
			100.3±0.64					99.8±0.03		
	0.598	99.6	(0.64)	-0.685			0.300	99.8	(0.03)	0.026
	0.605	100.9		0.579		0.300	99.8		0.015	

Table S4. Precision of the developed HPLC method for the determination of PD and RF

PD					RF				
Added Conc (M×10 ⁴)	Found Conc (M×10 ⁴)	Recovery (%)	Mean Recovery (%)±SD (%RSD)	Relative Accuracy Error (%)	Added Conc (M×10 ⁴)	Found Conc (M×10 ⁴)	Recovery (%)	Mean Recovery (%)±SD (%RSD)	Relative Accuracy Error (%)
Repeatability (Intra-day)									
0.500	0.505	101.0	100.9±0.52 (0.52)	0.062	0.500	0.504	100.8	100.9±0.29 (0.29)	0.194
	0.503	100.7		-0.266		0.502	100.4		-0.320
	0.505	101.0		0.082		0.502	100.4		-0.245
	0.509	101.7		0.770		0.503	100.6		-0.005
	0.501	100.3		-0.659		0.505	101.0		0.377
Intermediate (Inter-day)									
0.500	0.508	101.7	101.2±0.53 (0.53)	0.475	0.500	0.502	100.4	101.1±0.93 (0.92)	-0.609
	0.507	101.4		0.148		0.504	100.8		-0.032
	0.509	101.7		0.495		0.507	101.4		0.538
	0.503	100.7		-0.534		0.500	100.0		-1.115
	0.503	100.6		-0.584		0.509	101.8		1.219

Table S6. Robustness of the developed HPLC method for the determination of PD (1.00×10^4 M) and RF (0.50×10^4 M)

Parameters	PD			RF		
	Accuracy(%) ^a ±SD	Precision (%RSD)	<i>t</i> -test ^b	Accuracy(%) ^a ±SD	Precision (%RSD)	<i>t</i> -test ^b
Wavelength (±2 nm)						
278	101.4±0.44	0.44	0.75	100.2±0.61	0.60	0.85
282	100.5±0.21	0.20	0.81	101.3±0.75	0.75	0.63
pH (±0.01 units)						
3.49	101.4±0.38	0.37	0.99	100.2±0.41	0.40	0.97
3.51	100.3±0.11	0.11	0.84	101.4±0.65	0.65	1.01
Flow rate (±0.2 ml/min)						
0.8	100.2±0.34	0.33	1.05	99.85±0.75	0.74	0.82
1.2	100.4±0.11	0.10	0.95	100.3±0.81	0.81	0.94

^a Accuracy is a mean recovery (%), where n = 5

^b At a 95% confidence interval, the tabulated value for two-degree freedom is 2.776 and the values obtained are in the range of 0.63-1.05. Therefore, the $t_{cal} < t_{tab}$, and there is no difference between the applied changes and the proposed method conditions.

Table S7. Analysis of a synthetic mixture of PD and RF using the proposed HPLC method

PD				RF			
Added	Found	Recovery	RSD	Added	Found	Recovery	RSD
(M × 10⁴)	(M × 10⁴)^a	(%)	(%)	(M × 10⁴)	(M × 10⁴)^a	(%)	(%)
0.100	0.101	101.4	0.25	0.450	0.448	99.51	0.62
0.200	0.200	99.86	0.19	0.400	0.402	100.6	0.18
0.300	0.300	100.1	0.34	0.350	0.349	99.61	0.88
0.400	0.399	99.74	0.55	0.300	0.302	100.7	0.47
0.500	0.501	100.1	0.17	0.250	0.252	100.8	0.99
0.600	0.599	99.85	0.19	0.200	0.201	100.5	0.84
0.700	0.702	100.3	0.78	0.150	0.150	100.2	0.25
0.800	0.802	100.2	0.49	0.100	0.100	100.1	0.18
0.900	0.903	100.3	0.87	0.050	0.051	100.1	0.33

^a Values represent the mean of 5 determinations.