Electronic Supplementary Material (ESI) for Analyst.

Electronic Supplementary Information

Plyhydric polymer-loaded pyrene composites as the powerful adsorbents and fluorescent probes: Highly efficient adsorption and test strips-based fluorimetric analysis for curcumin in urine and plant extracts

Zhiqiang Duan[a,b], Mengyuan Yin[a], Chuanxian Zhang[a], Guilin Song[a], Shengying Zhao[a], Fan Yang[a], Luping Feng[a], Chuan Fan[a], Shuyun Zhu[a], Hua Wang[a]*

[a] Institute of Medicine and Materials Applied Technologies, College of Chemistry and Chemical Engineering, Qufu Normal University, Qufu City 273165, P. R. China;

[b] College of Physics, Qufu Normal University, Qufu City 273165, P. R. China.

* E-mail: huawangqfnu@126.com; Tel: (+86) 5374456306; Web: http://wang.qfnu.edu.cn.

Abstract

Supporting information includes the experimental details and data. The experimental section includes the materials and instruments, the synthesis of PVA-Pyr composites, the preparation of test strips, the fluorescence imaging for curcumin (Cur), the optimization of fluorimetric detection conditions, and the Cur sample extraction. Data are provided for the microscopy images of PVA-Pyr in the absence and presence of Cur, the fluorimetric selectivity in sensing Cur, optimization of analysis conditions, the calibration curve for the detection of Cur, the robustness and variability of the developed test strips, the comparison for the determination of Cur, the sample analysis results for Cur in human urine, and the detection recoveries of Cur in plant extracts.

EXPERIMENTAL SECTION

Experimental section

Materials and Instruments

Poly (vinyl alcohol) (PVA, MW89 000), dimethyl sulfoxide (DMSO), p-toluenesulfonic acid, 1-pyrenecarboxyaldehyde (Pyr), curcumin (Cur) and Whatman filters used for test strips were purchased from Sigma-Aldrich (Beijing, China). Hexadecyltrimethoxysilane (HDS) and aminopropyltriethoxysilane (APS) were bought from Sinopharm Chemical Reagent Co. (China). All other reagents were of analytical grade. Deionized water (>18 MΩ) was supplied from an Ultra-pure water system (Pall, USA).

The fluorescence measurements were conducted using the fluorescence spectrophotometer (F-7000, Hitachi, Japan) operated at an excitation wavelength at 345 nm, with both excitation and emission slit widths of 5.0 nm. The photographs of different materials and reaction products were recorded under UV light at 365 nm. Fourier transform infrared (FTIR) spectra were obtained by FTIR spectrophotometer (Thermo Nicolet Nexus 470FT, USA). UV-vis spectrophotometer (Shimadzu, UV-3600, Japan), inverted fluorescence microscope (Olympus, IX73-DP80, Japan) and scanning electronic microscopy (SEM, Hitachi E-1010, Japan) were separately applied for the characterization of different materials or products. HPLC were recorded on an Agilent 1200 liquid chromatography system fitted with analytical column C18 (Agilent SB, 2.1 mm 50 mm 1.8 mm). An Agilent 1260 series VWD detector was used for detection at a wavelength of 420 nm.

Synthesis of polymer-loaded pyrene composites

An aliquot of PVA (40.0 mg mL^{-1}) and Pyr (1.0 mg mL^{-1}) were mixed in DMSO of 50.0 mL. Then, p-toluenesulfonic acid (1.0 mg mL^{-1}) was dropwise added into the mixture to react at 80 ℃ for 6 h. After being cooled down to room temperature, the mixture was poured into CH_3OH (100.0 mL) to be precipitated and separated out. The resulted products of the fluorescent PVA-Pyr probes were further washed three times with CH_3OH (50.0 mL) to be dried under a vacuum (yield of 77%).

Preparation of polymer-based fluorescent test strips

The preparation procedure of the PVA-Pyr composites test strips for probing Cur were conducted

simply by following the procedure reported previously in our group. Typically, the filters papers were first cut into the slices of test strips (10 mm x 10 mm) and then soaked into the above PVA-Pyr composites (5.0 mg L^{-1}) for 10 min. After that, the test strips were immediately placed onto the HDS-modified super-hydrophobic patterns to be dried in vacuum for 20 min. Furthermore, the resulted test strips were immerged into APS solutions for 30 s for the hydrophilic treatments. Subsequently, the APS-treated test strips were again placed onto the super-hydrophobic patterns to be dried in vacuum for 10 min to be dried and stored in dark for future usage.

Fluorescence imaging for Cur using the PVA-Pyr composites

Optical fluorescent microscopy was employed to characterize the feasibility of fluorescence imaging of the PVA-Pyr probes. An aliquot of probes (1.0 mg mL^{-1}) in the presence and absence of Cur (0.1 mM) was dropped on a glass slide. After being dried in the room temperature, the fluorescent images were taken by using optical filters of UV light (λem = 340-380 nm) for the excitation of photoluminescence.

Fluorimetric measurements of Cur

The optimization of analysis conditions for the fluorimetric detections of Cur were conducted separately using different amounts of PVA-Pyr, pH values, ion strengths, and reaction time. Typically, an aliquot of PVA-Pyr probes was introduced into the Cur solutions. The Cur - quenched fluorescence intensities of the probes on test strips were recorded by a fluorescence spectrometer with a holder for the measurements solid-state fluorescence. The quenching efficiencies of PVA-Pyr probes by ions were calculated according to the following equation: quenching efficiencies = $(F_0 - F)/F_0$, where F_0 and F refer to the fluorescence intensities of PVA-Pyr (λex = 345 nm) in the absence and presence of ions and compounds. The selective fluorimetric analysis with centrifuge tubes was performed for Cur (10.0 μM), together with the possibly interferential substances (30.0 μM) as the control tests, including Fe^{3+}, Fe^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Ag^{+}, Hg^{2+}, Cd^{2+}, Pb^{2+}, Sn^{2+}, Cr^{3+}, Ca^{2+}, Al^{3+}, F^{-}, Br^{-}, S^{2-}, CO_3^{2-}, CH_3COO^{-}, PO_4^{3-} ions and fructose (Fru), sucrose (Suc), glucose (Gluc), urea, uric acid (UA), ascorbic acid (AA), L-glutamate (Glu) , L-glycine (Gly), L-phenylalanine (Phe), L-cysteine (Cys), L-Valine (Val), L-lysine (Lys), L-leucine (Leu), L-alanine (Ala), L-tyrosine (Tyr), L-tryptophan (Try), L-serine (Ser), L-Threonine (Thr), Catechol, and Dopamine (DA). Under the optimized conditions, the

developed test strips-based fluorimetric method with PVA-Pyr was subsequently applied for the detection of Cur of different concentrations spiked in urine samples (0, 0.005, 0.01, 0.025, 0.05, 0.10, 0.5, 1.0, 2.0, 5.0, and 10.0 μM). The calibration curves of the fluorimetric detections for Cur were obtained, with the analysis results further compared with those obtained by the classic HPLC method. Herein, all experiments were performed in compliance with the relevant laws and institutional guidelines, and the institutional committees have approved the experiments.

Sample extraction

Extractions of curcumin separately from the plants and spices were done according to the previously reported procedures[S1]: After being dried (100 °C for 24 h), the samples of Cur-containing plants like mustard or spices like curry were carefully ground in an agate mortar to obtain a fine intimately mixed powder. Then, 2.0 g of the powder was dissolved in a minimum volume of acetonitrile. Undissolved particles were removed by centrifugation. The clear centrifugate and combined acetonitrile washings were transferred into a 50.0 mL volumetric flask and diluted to the mark with acetonitrile. An appropriate volume of this solution was pipetted into a 10.0 mL colorimetric tube and its Cur was determined according to the procedure described above. The Cur content was calculated according to the linear regression equation.

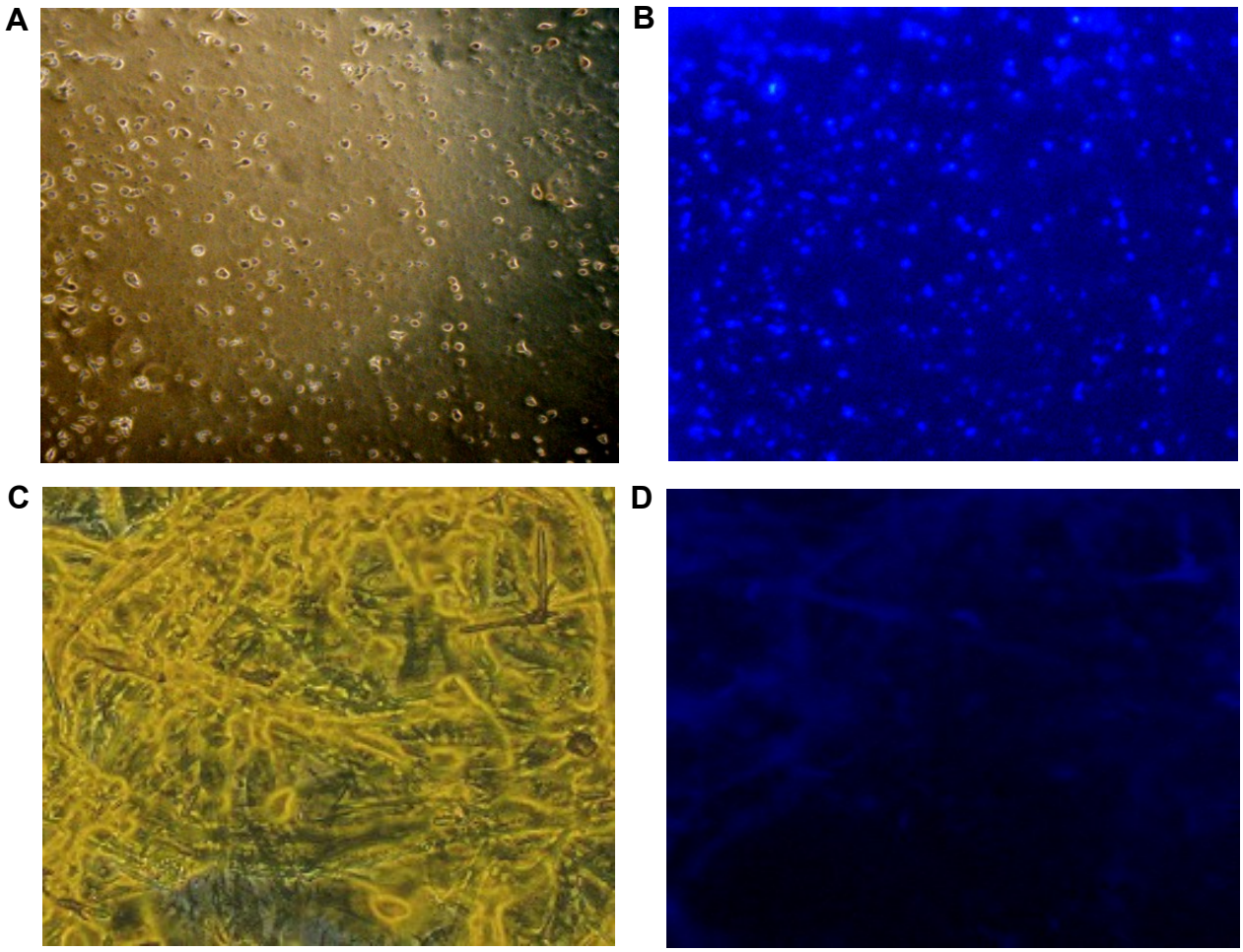

Figure S1. Microscopy images of the PVA-Pyr composites (1.0 mg mL^{-1}) in the (**A** and **B**) absence and (**C** and **D**) presence of Cur (0.10 mM). (**A** and **C**) Bright-field images; The fluorescence images (**B** and **D**) were taken by using optical filter of blue light for the photoluminescence excitation, where the samples were dropped on the glass slides and further dried at the room temperature.

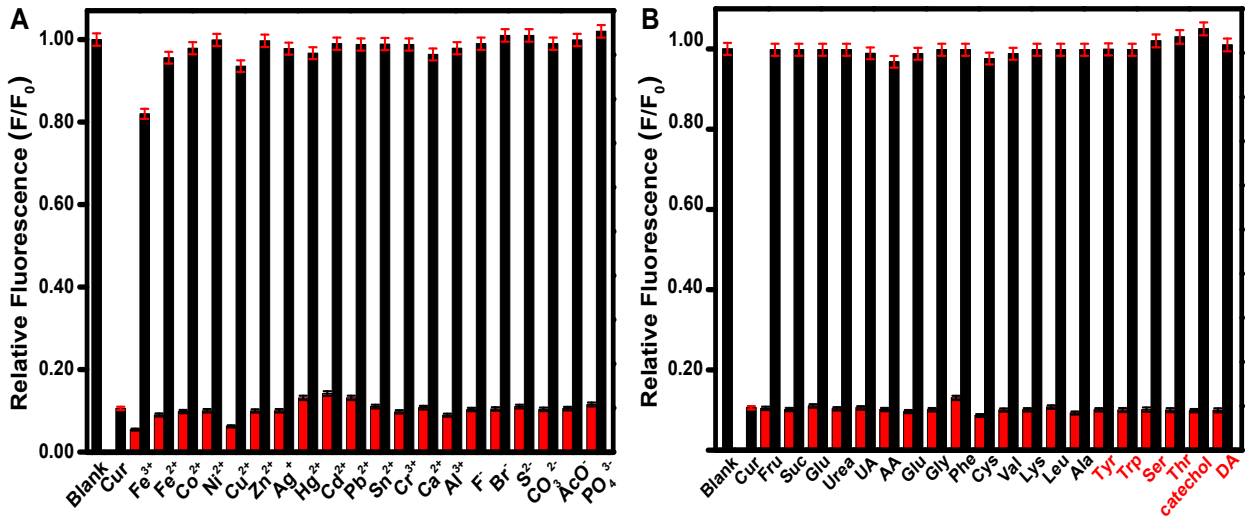

Figure S2. Fluorescence intensity responses of PVA-Pyr (1.0 mg L^{-1}) to (**A**) different ions; (**B**) compounds alone and separately mixed with Cur (10.0 μM) using various ions and compounds of 30.0 μM indicated, except for urea of 10.0 mM and UA of 1.0 mM. The error bars represent the standard deviations of three replicated measurements.

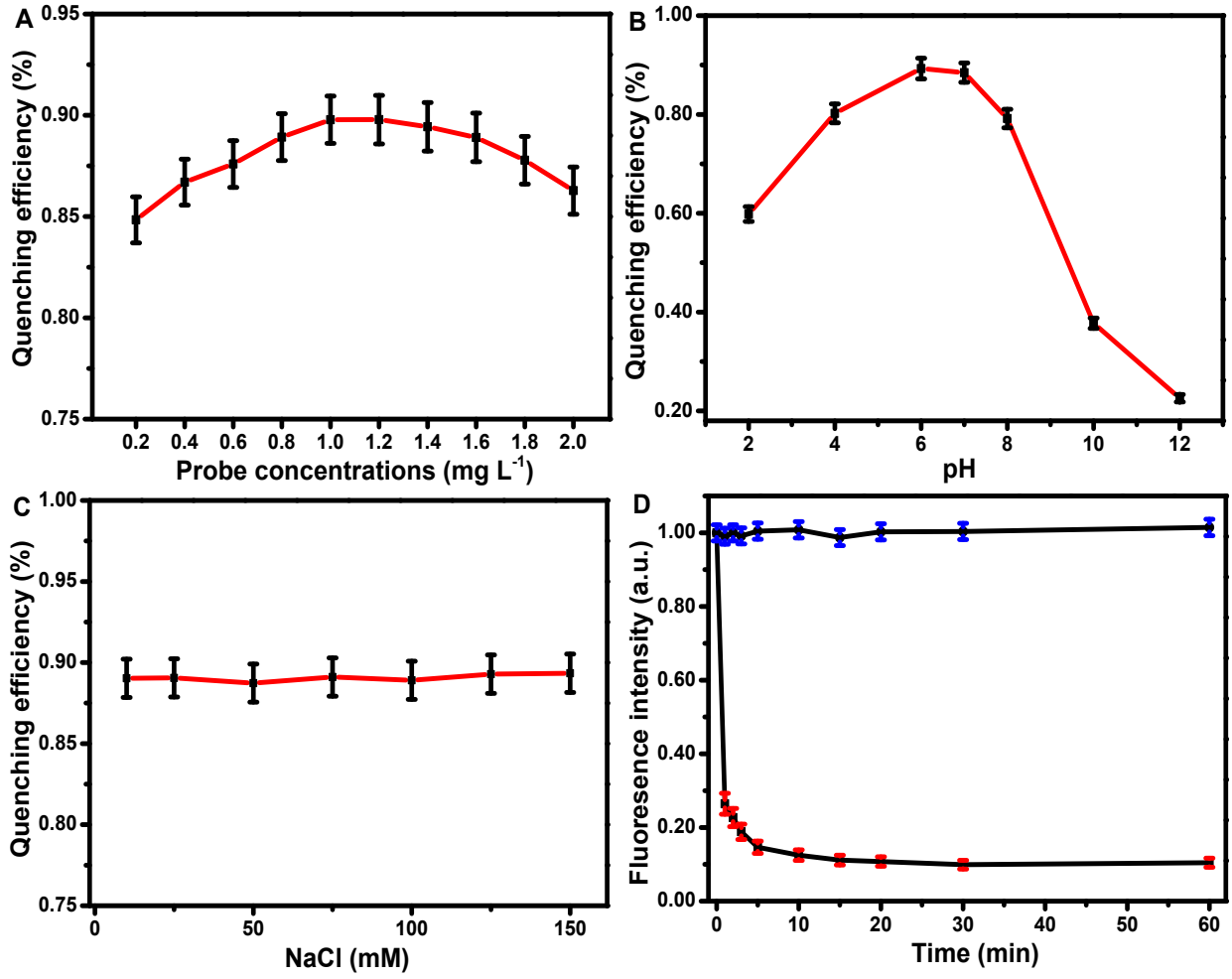

Figure S3. Optimization of the detection conditions for the fluorometric method with the fluorescence intensities depending on (**A**) the PVA-Pyr amounts; (**B**) pH values; (**C**) ion strengths of different NaCl concentrations; (**D**) reaction time of which the fluorescence intensities of PVA-Pyr probes were recorded in (**a**) absence and (**b**) presence of Cur (10.0 μM).

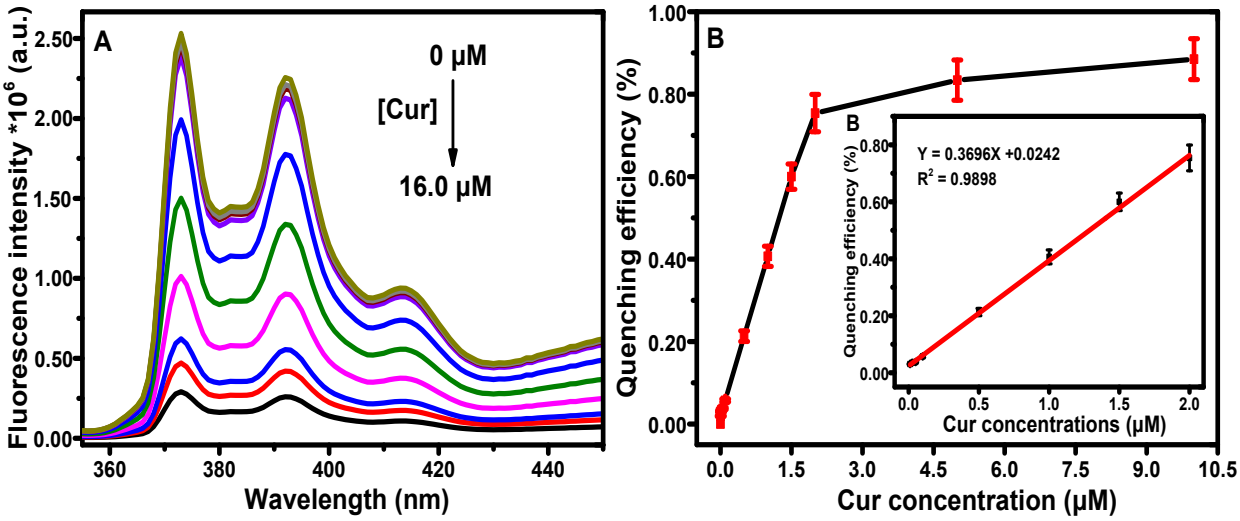

Figure S4 (**A**) Fluorescence spectra of PVA-Pyr (1.0 mg L^{-1}) with Cur solutions of different concentrations (0, 0.005, 0.01, 0.025, 0.05, 0.10, 0.5, 1.0, 2.0, 5.0, and 10.0 μM); (**B**) the relationship between the fluorescence quenching efficiencies versus the Cur concentrations.

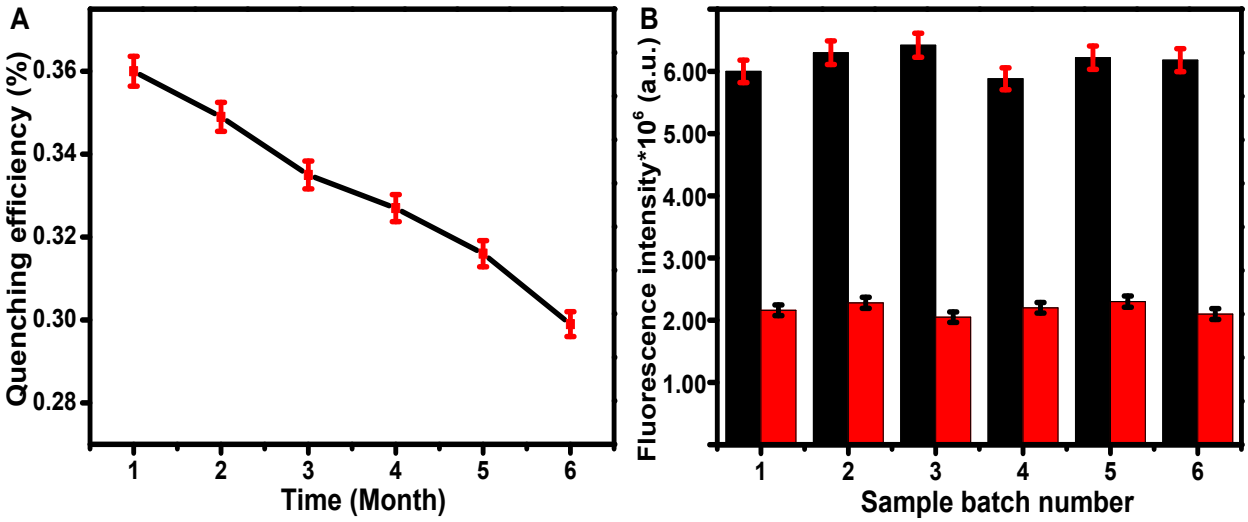

Figure S5 (A) Quenching efficiency of the test strips-based fluorimetric responses to Cur samples (2.0 μM) by stored over different time intervals; (**B**) Fluorescence intensities of the test strips-based fluorimetric responses to Cur samples (2.0 μM) prepared by different batch productions.

Table S1 Comparison of the Cur analysis results among the developed fluorimetric method and other analysis techniques.

Analysis methods	Detection limit (μM)	Linear range (μM)	References
Spectrophotometry	0.20	0.0 - 40.6	[1]
Fluorimetry with quantum dots	0.037	0.16 - 16.9	[2]
Liquid chromatography	0.17	0.59- 271.1	[3]
Fluorimetry with carbon dots	0.0848	0.20 - 10.0	[4]
Fluorimetry with PVA-Pyr	0.0083	0.05 - 4.0	This work

References:

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2 X. Zhao, F. Li, Q. Zhang, Z. Li, Y. Zhou, J. Yang, C. Dong, J. Wang and S. Shuang, *RSC Adv.*, 2015, **5**, 21504-21510.

3 M. Rahimi, P. Hashemi and F. Nazari, *Anal. Chim. Acta*, 2014, **826**, 35-42.

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Table S2 Comparison of analysis results for Cur in human urine samples with HPLC analysis methods (n = 5, confidence limit = 95%).

Urine samples	Added (μM)	Found (μM)		Recovery (%)	
		Proposed strips	HPLC	Proposed strips	HPLC
1	0.10	0.095	0.096	95.00	96.00
	1.00	0.930	0.950	93.00	95.00
2	0.10	0.105	0.106	105.00	106.00
	1.00	0.970	0.950	97.00	95.00
3	0.10	0.094	0.095	94.00	95.00
	1.00	1.060	1.050	106.00	105.00

Table S3 Results for the recoveries of Cur in the plant extracts samples (n = 5, confidence limit = 95%).

Plants samples	Sample contents (μM)	Cur added (μM)	Cur found (μM)	Recovery (%)
Curry	1.85	1.00	2.88±0.21	103
	2.20	1.00	3.16±0.28	96
	2.70	0.50	3.22±0.30	105
Mustard	1.95	1.00	2.99±0.25	104
	2.42	1.00	3.39±0.32	97
	2.80	0.50	3.28±0.35	96