Supporting Information (SI)

A simple aptamer-dyes fluorescence sensor for detecting Δ9-tetrahydrocannabinol and its metabolite in urban sewage

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Table of Contents

1. Reagents and materials

Deionized water (DIW, 18 MΩ·cm) produced using a water purification system (Chengdu Ultrapure Technology Co., China) was used throughout this work. Thioflavin T (ThT) was purchased from Sinopharm Chemical Reagent Co. (Shanghai, China) and cresyl violet (CV) was provided by AAT Bioquest (Pleasanton, CA, USA). Thiazole orange, crystal violet, malachite green, and SYBR Green were obtained from Aladdin Reagent Co. (Shanghai, China). $MgCl_2$ and NaCl were bought from Kelong Reagent Factory (Chengdu, China). THC, THC-COOH, and other drugs were permitted and obtained from the Institute of Forensic Science of China and stored at 4 ºC before analysis. The THC1.2 aptamer was cited[1] and synthesized by Shanghai Sangon Biotech Co. Ltd. and purified by HPLC, whose detailed sequence is as follows: 5′-CTTACGACCCAGGGGGGTGGACAGGCGGGGGTTAGGGGGGTCGTAAG-3′.

2. Instrument parameters

The spectra were measured by UV-vis spectrophotometer (PerkinElmer Lambda 365) and obtained with FluoroMax-4 spectrofluorometer (Horiba Jobin Yvon). Circular dichroism spectra were measured on an AVIV MODEL 400 spectropolarimeter (AVIV Biomedical, Lakewood, NJ). Spectra were recorded between 225 and 500 nm with a bandwidth of 1 nm and the scan of buffer was used as a baseline and subtracted from each spectrum.

3. Dyes binding THC1.2 and THC displacement experiment

For dye selection, 200 μL of reaction solution contained buffer (5 mM Tris-HCl, pH 7.4, 20 mM NaCl, 0.5 mM $MgCl_2$), 2 μL of aptamer (final concentration 1 μM), and 2 μL of dye (final concentration 1 μM) was prepared. The fluorescence spectra of ThT (E_x=465 nm, E_m=488 nm), CV (E_x=465 nm, E_m=618 nm), SYBR Green (E_x=495 nm, E_m=525 nm), crystal violet (E_x=585 nm, E_m=643 nm), malachite green (E_x=490 nm, E_m=540 nm), and thiazole orange (E_x=490 nm, E_m=654 nm) together with THC1.2 were measured. After the addition of 1 μM THC and stabilized for 5 min, the fluorescence spectra of the THC displacement experiment were recorded.

4. Construction of the portable fluorescence capture device

A resin holder (72 mm × 72 mm × 100 mm) was designed and printed by a fused deposition modeling (FDM) 3D printer (CR-3040 pro) using a black resin material. An LED with 465 nm excitation (3 W) was fixed on a resin plate and placed on the bottom of the holder, which was in alignment with a customized cuvette (quartz, i.d. 6 mm). A 3.7 V lithium battery pack (6000 mAh, 69 mm × 34 mm × 18 mm) was supplied to the excitation unit. A long-pass emission filter (cut-on 475 nm, 50 mm × 50 mm × 2 mm, Heng Yang Electronic Technology Co.) was embedded right above the cuvette before image capture. A smartphone (HUAWEI P50 Pro) was placed on the top of the holder to acquire a fluorescence image.

A portable fluorescence capture device integrated with a smartphone was thus fabricated, consisting of a cuvette, LED light, battery supply, optical filter, and smartphone. The device could be printed and combined with a smartphone to ensure stable and dark environmental conditions and satisfy the sensing process. The cuvette was customized to pack 200 μL solution and stood with a holder, which was arranged to be perpendicular to the LED, enabling the excitation to be focused on the cuvette efficiently. A long-pass emission filter was used next to the smartphone when acquiring images, avoiding the influence of ambient light and excitation light. A distance of 100 mm was selected between the cuvette and the optical filter to ensure the sharpness of the captured images. The captured images were extracted to the RGB value by a color recognizer and read the concentration of THC.

5. Detection of THC and its metabolite in wastewater and urine samples

To estimate community cannabis use trends, actual wastewater was collected from several Chengdu communities, which were stored at 4 ºC before analysis. The collected samples were filtered by 0.22 μm membrane and subjected to 2-fold dilution with the reaction buffer (5 mM Tris-HCl, pH 7.4, 20 mM NaCl, 0.5 mM $MgCl_2$), which further reacted with the sensor and instantly transferred to the cuvette to image output. Individual wastewater samples spiked with 0.5, 1.8 μM of THC and 1, 2.5 μM of THC-COOH, respectively, were mixed evenly to explore the practicability of this sensor. The practicability of the proposed method was also validated by analysis of urine samples of three healthy and drug-free volunteers, filtered with 0.22 um filter and subjected to 15-fold dilution with the reaction buffer.

6. Fluorescence spectra of dyes

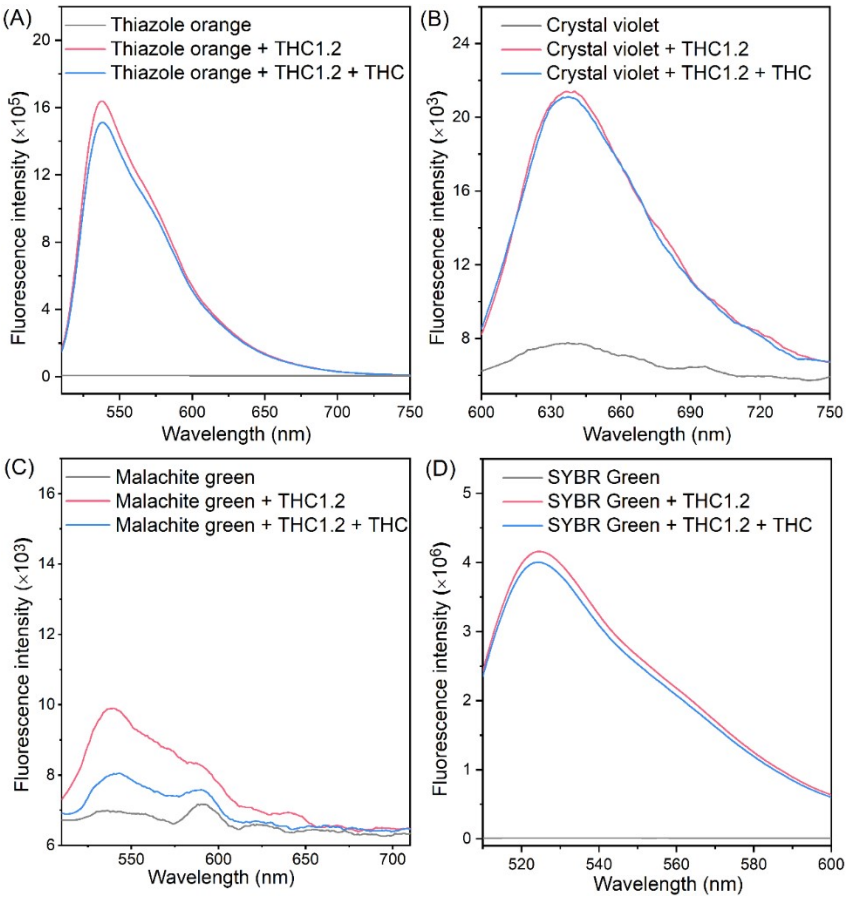

Fig. S1 Fluorescence spectra of thiazole orange (A), crystal violet (B), malachite green (C), and SYBR Green (D) in the presence of THC1.2 and THC.

7. CV mixed with random sequence

Considering the positive charge of CV, there might be electrostatic adsorption between CV and THC1.2 inducing the decreased fluorescence, thus variation fluorescence intensity of CV mixed with random sequences (A15 and T15) were investigated and the results are shown in Fig. S2. It can be observed that the reduced signals were weakened with the addition of random oligonucleotides, revealing that electrostatic adsorption plays a weak role in decreasing CV fluorescence intensity.

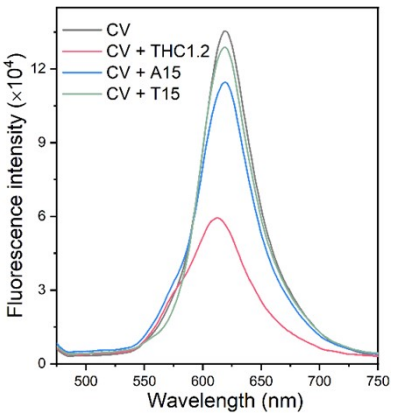

Fig. S2 The changes in fluorescence intensity of CV mixed with THC1.2, A15, and T15.

8. 3D fluorescence spectrums for ThT and CV

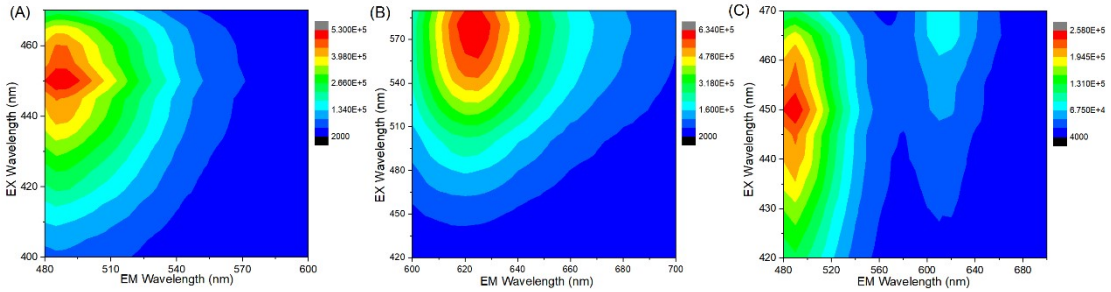

Fig. S3 3D fluorescence spectrum of the THC1.2/ThT (A), CV (B), and THC1.2/ThT/CV (C).

9. Characteristics of circular dichroism spectra and fluorescence spectra

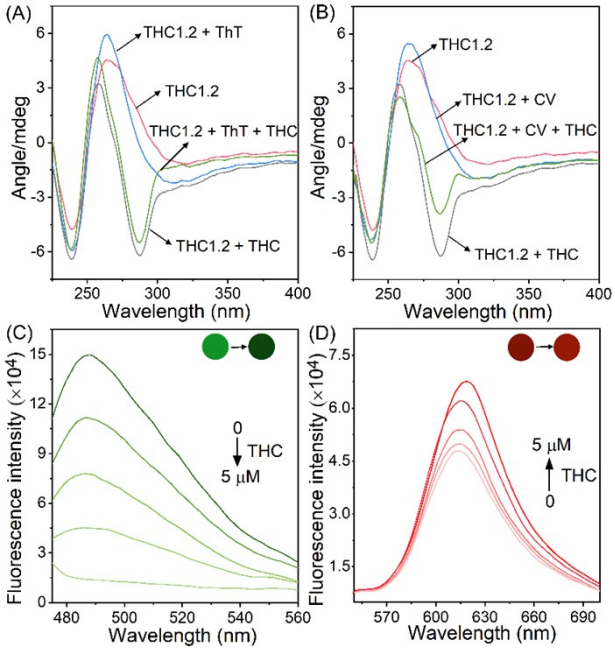

Fig. S4 CD spectra of THC1.2 (5 μM) mixed with (A) ThT (40 μM), (B) CV (40 μM) together with THC (40 μM). Fluorescence spectra of THC1.2/ThT (C) and THC1.2/CV (D) compounds with different concentrations of THC (0, 0.1, 0.4, 1, and 5 μM). Experimental buffer: 5 mM Tris-HCl, pH 7.4, 20 mM NaCl, 0.5 mM $MgCl_2$.

10. Concentration of THC1.2

The THC-responsive THC1.2/ThT/CV sensor from green to red largely depends on the concentration of aptamer, and the selection of aptamer concentration and its effect on the fluorescence visualization was optimized.[2] As shown in revised Fig. S5A, increasing the concentration of THC1.2 in the range of 0.4–1.2 μM resulted in a gradually increasing fluorescence intensity for ThT at 488 nm, and a steady fluorescence emission for CV when the concentration of THC1.2 was 1 μM. Based on that, the responsiveness of the sensor was evaluated in the presence of different concentrations of THC with solutions containing 0.6, 0.8, 1.0, and 1.2 μM THC1.2 and the results are shown in Fig S5B. The sensors with different aptamer concentrations produced linear responses in the range of 0.3–1.5 μM THC and the slope of the linear equation gradually raised, indicating that the sensitivity of the dye displacement assay was affected by the aptamer concentration. It can be observed that a greater and comparable steepness of the THC dose-responsive curve can be achieved when THC1.2 was 1.0 μM or 1.2 μM, showing a better performance for the analysis of THC.

Moreover, the fluorescent images of the aptamer concentration-dependence sensor were also explored. It can be observed from Fig. S5C that the fluorescence image of the sensor gradually turned from dark to bright green with the increasing concentration of THC1.2. Upon the addition of THC, the image output switched from dark green to yellowish-brown, the subtraction of the R/G value as output to achieve noticeable visualization to the naked eye, and 1 μM and 1.2 μM of THC1.2 possessed distinguishable visualization from bright green to brown. In conclusion, 1 μM of THC1.2 was chosen as the final concentration based on the sensitivity and visualization of the ratio sensor toward the target.

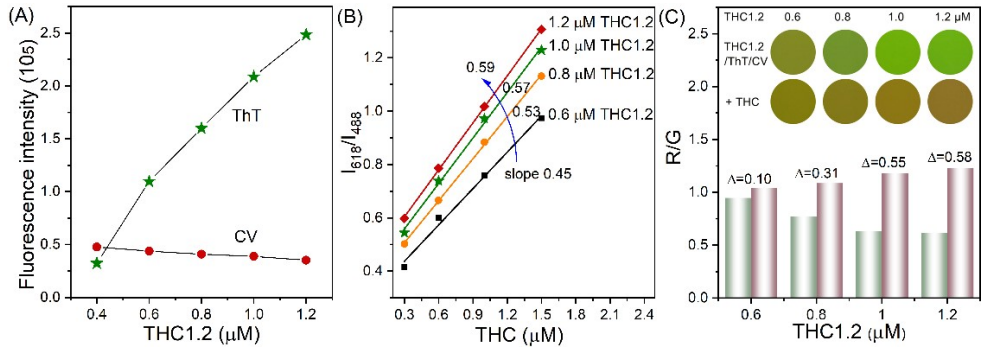

Fig. S5 (A) Fluorescence of ThT and CV with various concentrations of THC1.2. (B) Obtained I_{618}/I_{488} and slope for the direct comparison of sensitivity. (C) The effect of THC1.2 concentration on fluorescence images and the subtraction of the obtained R/G value with the addition of THC. (ThT and CV, 1 μM; Buffer: 5 mM Tris-HCl, pH 7.4, 20 mM NaCl, 0.5 mM $MgCl_2$).

11. THC reaction Time

From Fig. S6, it was observed that there was no significant change in R/G values within 30 min incubation time, indicating that the instant detection of THC was realizable by this proposed sensor.

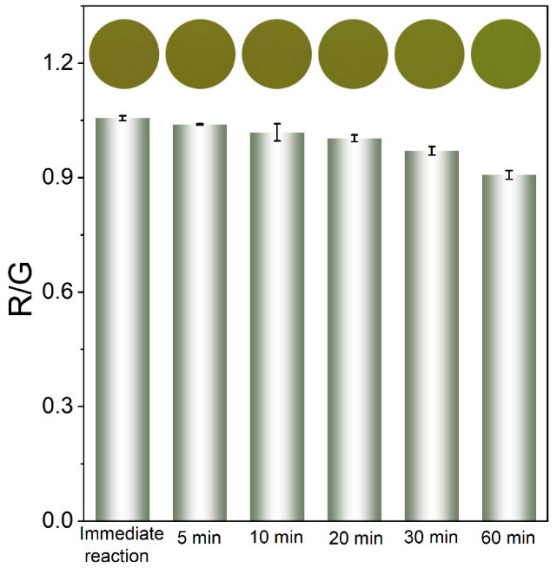

Fig. S6 Effect of THC reaction time on fluorescence images and variation of obtained R/G value over time.

12. Stability of THC and THC-COOH analysis

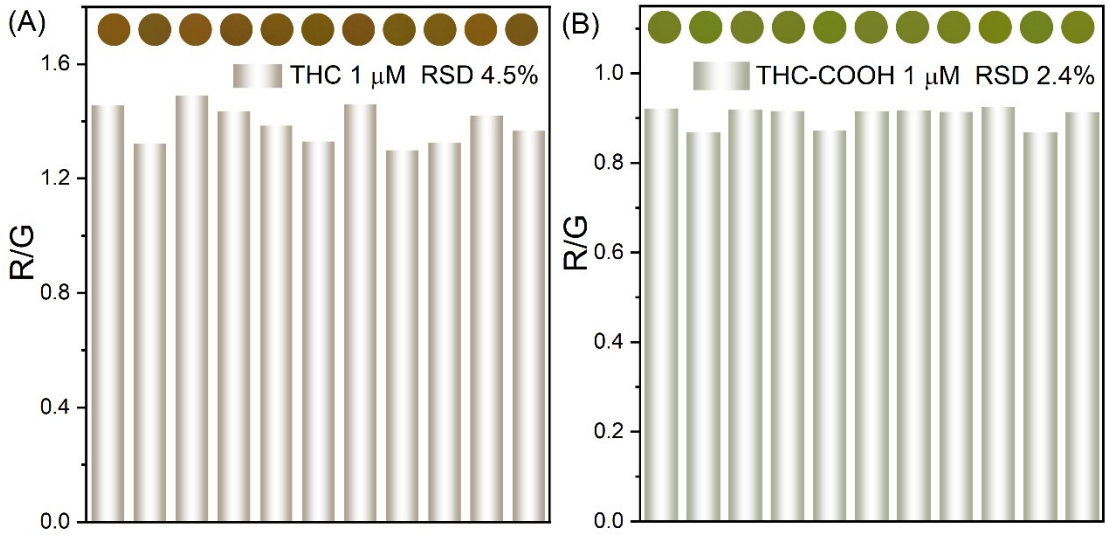

Fig. S7 Obtained signals from 11 measurements of 1 μM THC (A) and 1 μM THC-COOH (B).

13. Obtained LODs by fluorescence spectra method

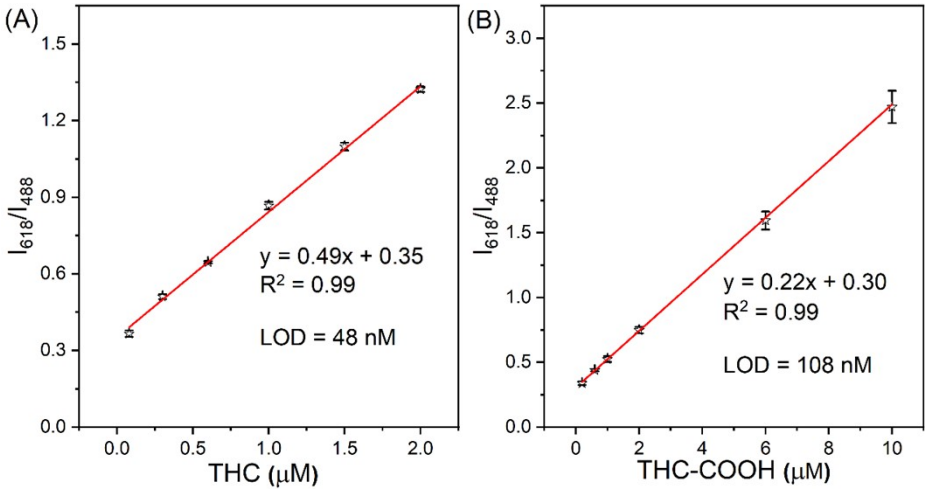

Fig. S8 Calibration curves for this ratiometric fluorescence sensor based on fluorescence spectra and the intensity were obtained by the ratio of 618 nm and 488 nm.

14. Performance comparison

Table S1. Comparison of the performance of this work with available fluorescence and commercialized methods.

Method	Dyes/nanomaterial	LOD / Device cutoff	Time	Portable devices /Devices	Ref
Label fluorescence	FAM	THC 200 nM THC-COOH 200 nM	-	No	1
	DY-481XL	THC 2 ng/mL (6 μM)	10 min	No	3
	Carboxylated polystyrene nanoparticles	THC 0.5 pg/mL (0.002 nM)	10 min	Yes	4
	Phycoerythrin (PE)-fluorescent particles	THC 0.122 ng/mL (4 nM)	< 10 min	Yes	5
	Fluorescent-tagged antibodies	THC 190 pg/fingerprint	< 10 min	Yes	6
Label-free fluorescence	ThT and QDs	THC 97 nM THC-COOH 254 nM	5 min	Yes	7
	Fast blue BB	THC 1590 nM	11–15 min	Yes	8
	ThT and CV	**THC 53 nM THC-COOH 152 nM**	**4 min**	**Yes**	**This work**
Commercialized products	-	THC 99 nM	< 5 min	Cozart DDS 806	9
	-	THC 16–80 nM	< 5 min	DrugTest 5000	
	-	THC 127 nM	5–30 min	Oratect III	

15. Specificity

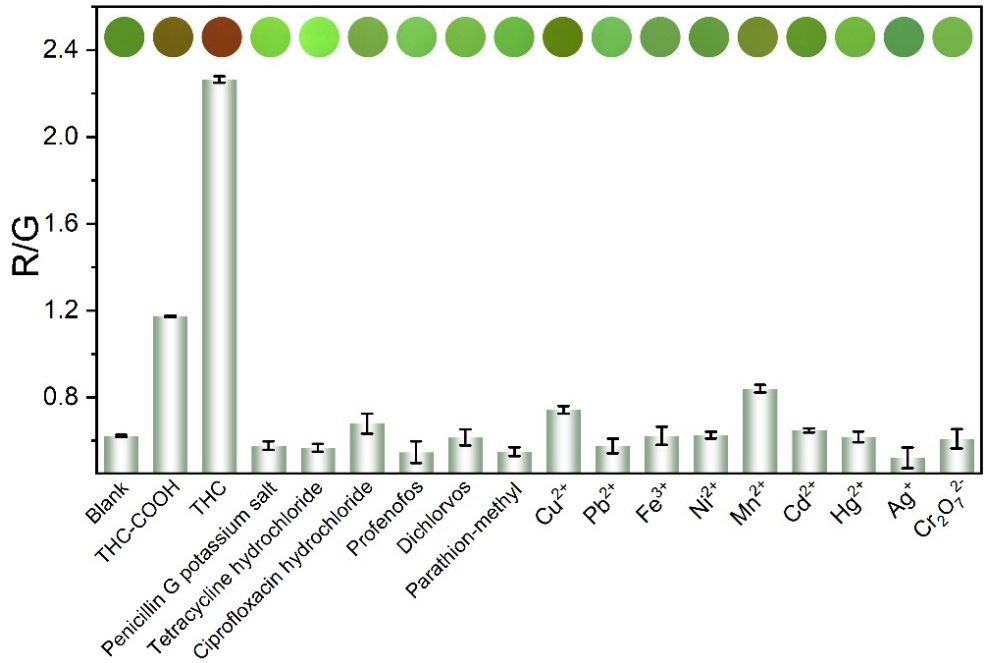

Fig. S9 Specificity tests of the established ratiometric fluorescent sensor for THC and THC-COOH against other common pollutants (antibiotics, 100 μM; other heavy metal ions and organophosphorus pesticides based on the concentration limits in the standard GB/T 31962-2015; organophosphorus pesticides, 0.5 mg/L; Cu^{2+}, 2 mg/L; Pb^{2+}, 0.5 mg/L; Fe^{3+}, 10 mg/L; Ni^{2+}, 1 mg/L; Mn^{2+}, 5 mg/L; Cd^{2+}, 0.05 mg/L; Hg^{2+}, 0.005 mg/L; Ag^{+}, 0.5 mg/L; $Cr_2O_7^{2-}$, 0.5 mg/L).

16. Sewage sample analysis

Table S2. The obtained results in sewage samples by this proposed method.

Sample	Target	Detected (μM)	Added (μM)	Detected (μM)[a]	Recovery (%)
Site 1	THC	ND	0.50	0.53 ± 0.04	106
			1.80	1.86 ± 0.11	103
	THC-COOH	ND	1.00	0.96 ± 0.09	96
			2.50	2.68 ± 0.31	107
Site 2	THC	ND	0.50	0.52 ± 0.03	103
			1.80	1.79 ± 0.10	100
	THC-COOH	ND	1.00	0.97 ± 0.08	97
			2.50	2.71 ± 0.18	108
Site 3	THC	ND	0.50	0.49 ± 0.05	98
			1.80	1.87 ± 0.09	104
	THC-COOH	ND	1.00	0.98 ± 0.12	98
			2.50	2.64 ± 0.24	106
Site 4	THC	ND	0.50	0.52 ± 0.07	105
			1.80	1.90 ± 0.15	106
	THC-COOH	ND	1.00	1.05 ± 0.11	105
			2.50	2.51 ± 0.38	100
Site 5	THC	ND	0.50	0.52 ± 0.05	103
			1.80	1.76 ± 0.09	98
	THC-COOH	ND	1.00	0.93 ± 0.05	93
			2.50	2.59 ± 0.27	104

[a]Mean and standard deviation results (n = 3). ND, not detected.

17. Urine sample analysis

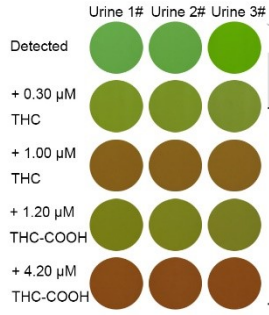

Table S3. The obtained results in urine samples by this proposed method.

Sample	Target	Detected (μM)	Added (μM)	Detected (μM)[a]	Recovery (%)	Added (μM)	Detected (μM)	Recovery (%)
1	THC	ND	0.30	0.32 ± 0.01	108	1.00	1.07 ± 0.01	107
	THC-COOH	ND	1.20	1.17 ± 0.07	98	4.20	4.25 ± 0.02	101
2	THC	ND	0.30	0.32 ± 0.04	106	1.00	1.05 ± 0.01	105
	THC-COOH	ND	1.20	1.20 ± 0.09	100	4.20	4.20 ± 0.07	100
3	THC	ND	0.30	0.33 ± 0.02	109	1.00	1.04 ± 0.02	104
	THC-COOH	ND	1.20	1.19 ± 0.07	99	4.20	4.27 ± 0.02	102

[a]Mean and standard deviation results (n = 3). ND, not detected.

Fig. S10 Fluorescent images of the ratiometric fluorescence assay spiked with various concentrations of THC and THC-COOH in urine samples and their corresponding concentrations.

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