A smart and rapid colorimetric method for detection of codeine sulphate, using unmodified gold nanoprobe

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Materials and methods:

All chemicals used were of analytical grade or of the highest purity available. Each solution was prepared in double-distilled water. All glassware was thoroughly cleaned with freshly prepared 3:1 HCl/HNO_3 (aqua regia) and rinsed thoroughly with Milli-Q water prior to use. $HAuCl_4$ was purchased from Sigma-Aldrich. Drug standards codeine sulphate, morphine, phenylephrine, phenobarbital, diazepam and lorazepam were obtained in powder form which were dissolved in deionized double distilled water as per requirement. The stock solution of codeine sulphate (0.01 M) was prepared by dissolving 0.69681 g codeine sulphate in 100 ml deionized water and was diluted to 1 mM, 500 µM, 100 µM, 80 µM, 60 µM, 40 µM, 20 µM, 10 µM, 9 µM, 8 µM, 7 µM, 6 µM, 5 µM, 4 µM, 3 µM, 2 µM, 1 µM, and 0.1 µM and was detected by a 0.07 mM solution of AuNPs. To study the effect of pH, 0.025M potassium dihydrogen orthophosphate (KH_2PO_4) buffer solution was added into 0.2% orthophosphoric acid to get a pH from 3.5 to 7.5 and to make basic pH, 0.025 M potassium dihydrogen orthophosphate (KH_2PO_4) buffer solution was added into 0.2% Na_2CO_3 to maintain the pH (8–12). UV–Vis absorption spectra was acquired on a Jasco V-570 UV–Vis spectrophotometer, IR spectra was recorded with a Bruker Tensor 27 FT-IR spectrometer, Transmission electron micrograph (TEM) was recorded by JEOL, JEM-2100 (200kV), DLS measurements were done using Nanotrac instrument and ESI Mass spectra were taken on a Shimadzu GCMS-QP 2000 A.

Synthesis of citrate modified AuNP:

The AuNPs synthesis procedure was essentially the same as that developed by Menon et al[15]. The synthesis was carried out in a modified CEM Discover microwave using single mode with continuous power at 2.45GHz. The reactions were carried out in a sealed reaction vessel containing 3ml of 0.4 mM $HAuCl_4$ solution and 2 mL of 13 mM sodium citrate. This mixture was heated at 75^0C at a power up to 300 W for 4 min. The color of solution changed from pale yellow to burgundy to yield Au nanoparticles of ~44 nm. Typically, 10 mL of 0.40 mM AuNPs suspensions was diluted to 60 mL as 0.07 mM stock solution.

Animals used for drug administration:

The animals used were housed at the Animal Care Facility of Department of Zoology, Gujarat University. The Experimental Animals, Adult male Wistar rats (Rattus norvegicus) and Albino mice (Mus musculus) of Swiss strain, weighing

between 250-350 g & 25-45 g respectively were procured from Zydus-Cadila Health Care, Ahmedabad under the Animal Maintenance and Registration No. 167/1999/CPCSEA from the Ministry of Social Justice and Empowerment, Govt. of India. Upon arrival at the animal care facility, the animals were given 7-15 days to acclimatize to their conditions. The animals were housed under standard temperature (26±2^{o}C), operating on a 12 h dark/light condition and relative humidity of 30-70%. Animals of experiment were caged separately and maximum of two rat per cage and five mice per cage were maintained on a standard animal food obtained from Pranav Agro Industries, containing wheat - 70%, gram - 20%, fish meat - 5% and yeast powder - 5% and distilled water was given *ad libitum*. Adult Mice and adult rats received oral dose of codeine sulphate in water at the maximum tolerated acute and chronic doses. Adult male Wistar rats (Rattus norvegicus) and Albino mice were given 400 mg/kg and 350 mg/kg acute dose of codeine sulphate respectively and sacrificed under light ether anaesthesia. The delay periods between drug administration and killing were chosen on the basis of the half-life of codeine sulphate as per respective animal and its weight. For chronic dose, adult Wistar rats (Rattus norvegicus) and Albino mice (Mus musculus) received the codeine sulphate dose on the basis of their weight for 7 days in increasing order started with half dose of LD50 and then scarified animal was buried in sterile and non-sterile soil.

Marrow Preparation

The bone marrows were isolated from the bone of the drug administrated animals after the formation of skeletal remain of animal body. Then it weighed 1g and dissolved in 5 mL 0.225 M NaOH with ultra-sonication.

Bone Preparation

After the transformation of animal body to skeletal remains the remaining bone samples were collected. To remove the tissues and other soil debris adhering to the surface of the bones, it was socked under hot alkaline water (0.1M NaOH around 90^{0}C) for 2 h and then it was scraped with a scalpel.

Soil preparation

Soil samples were collected from bottom of the grave by spatula and stored at room temperature in airtight container after the air dry.

Extraction from marrow:

Added saturated NaOH to maintain between 8–9 pH range and extracted with a mixture of 20 ml CH_2Cl_2-Isopropanol (9:1 v/v). The organic phase was filtered through anhydrous sodium sulphate, washed with organic phase. The solvent was immediately evaporated to dryness under stream of N_2. Bone marrow contains the high amount of lipids and fat that may interfere in process. To prevail over this problem, it is re-extracted with 5 ml of 0.5 M HCl. The organic phase was discarded and maintained the pH between 8.5-9 and evaporated to dryness.

Extraction from Bone:

The bone pieces were cleaned from muscle tissues and grounded in mortar. 10 ml of 2 M HNO_3 was added to 1 g of bone powder and the mixtures were demineralized at room temperature (37^0C), overnight. Then it was extracted for the drug as described above, maintains the pH between 8.5-9 with NaOH.

Extraction from Soil

Added 10 ml of 1 M HCl in 1g of soil and incubated at room temperature (37^0C), overnight. After the incubation period it was filtered out and discarded the soil debris, taken out the filtrate for further process. Added the saturated NaOH solution in the filtrate to maintain the pH between 8.5-9 and then was extracted by a mixture of hexane:ethanol (7:3 v/v) and hexane layer is discarded. The remaining ethanol-water layer is re-extracted by hexane:ethanol (7:3 v/v). The remaining ethanol-water layer was evaporated to dryness for further examination.

Fig.S1

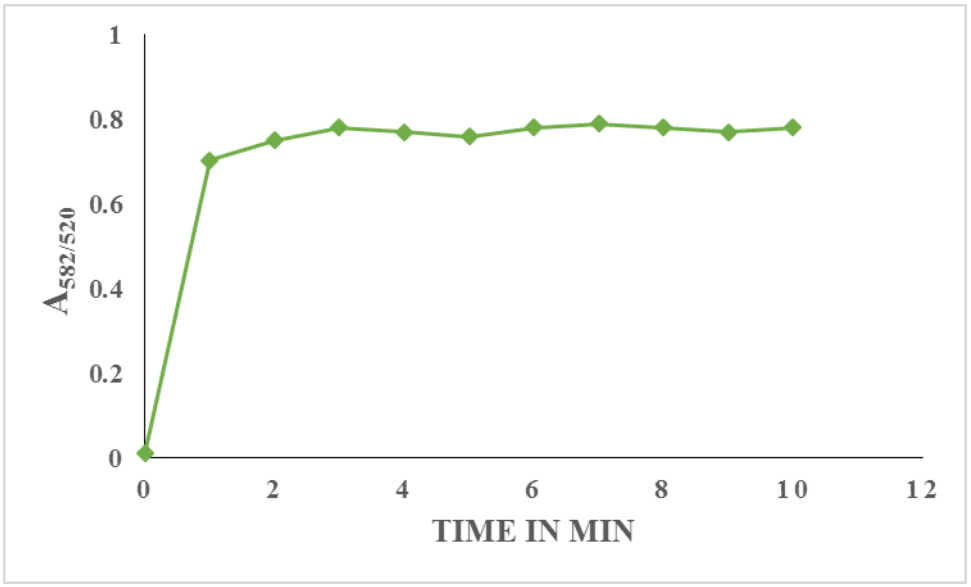

Fig S1 shows the response behaviour after addition of 0.1 mM codeine sulphate in 0.07 mM AuNp

Fig.S2

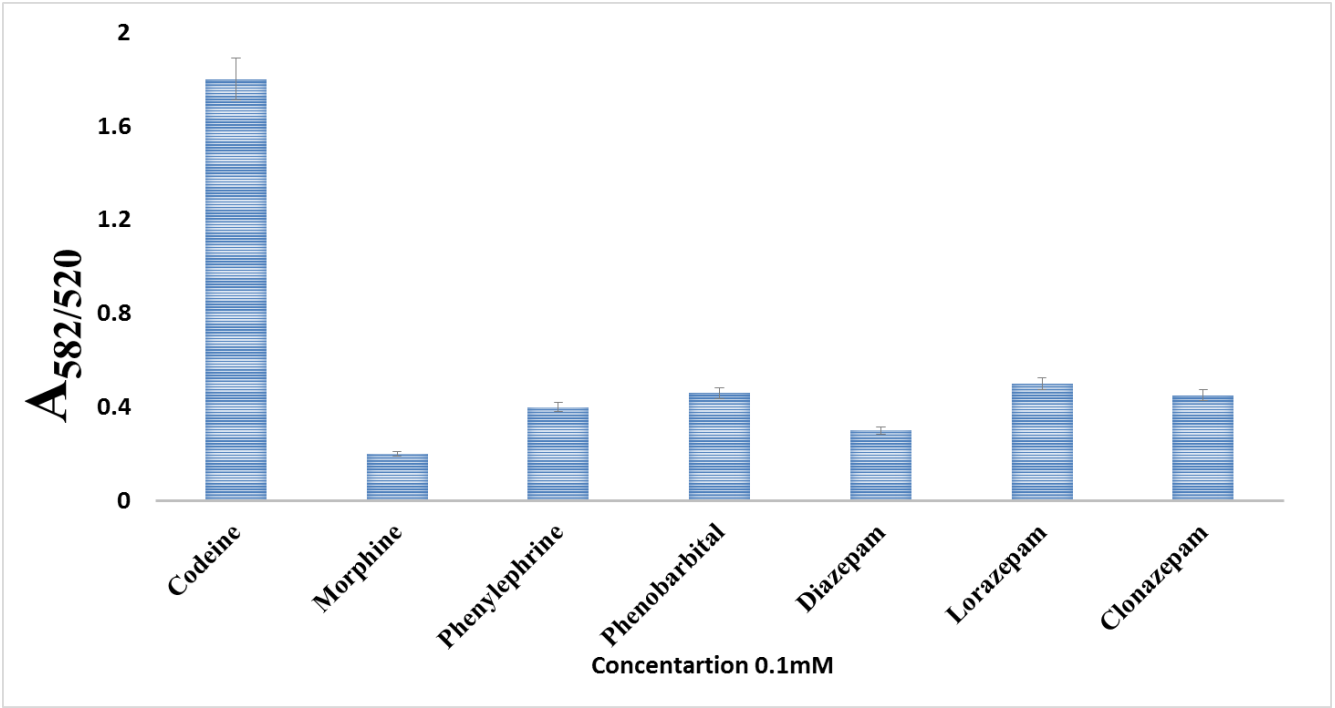

Fig. S2 The relative absorbance change of AuNPs at 520 nm in the presence of 0.1 mM codeine sulphate, morphine, phynylphrine, phenobarbital, diazepam, lorazepam and clonazepam.

Fig.S3

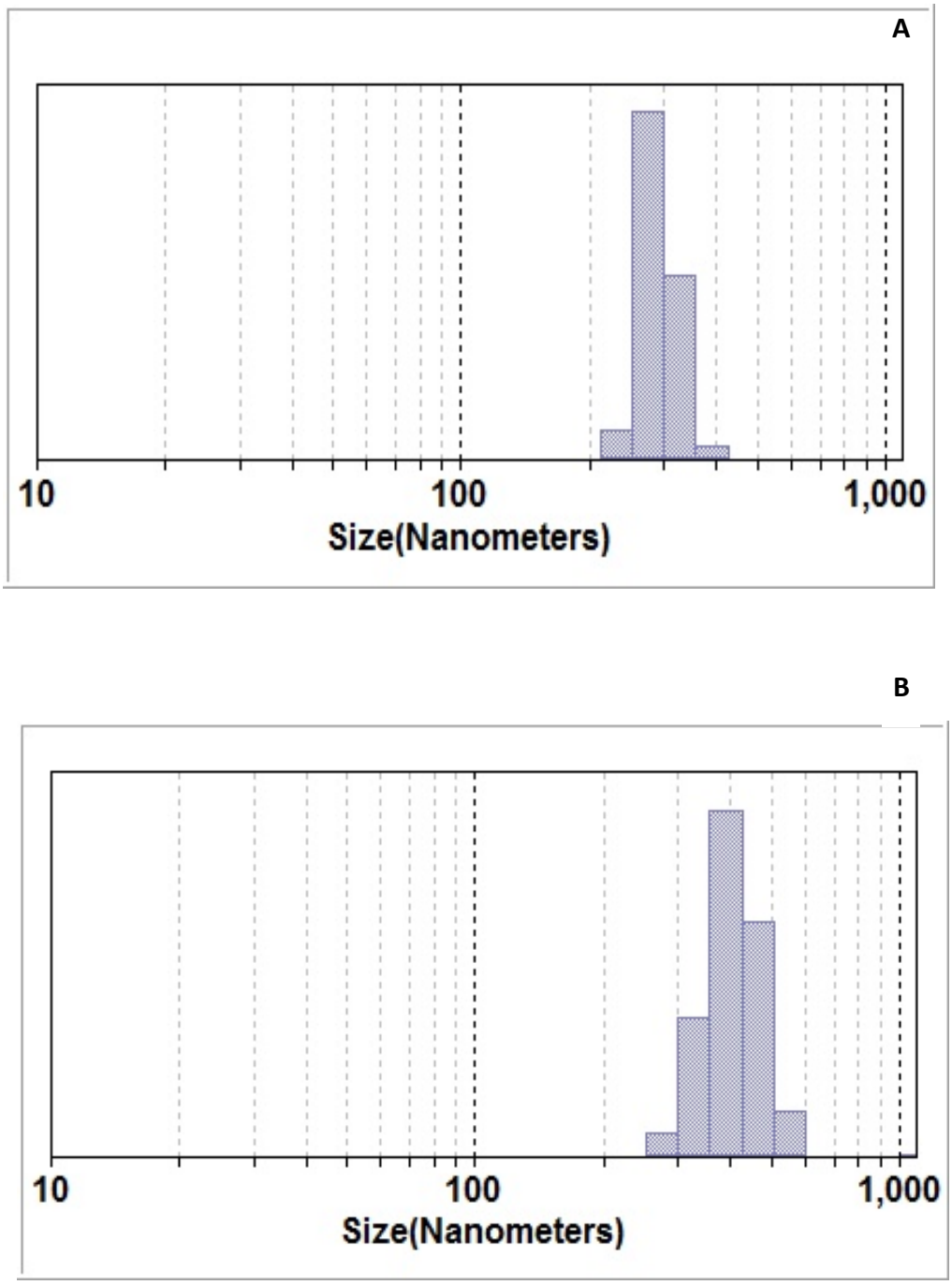

Fig S3 Size distribution of AuNPs measured by DLS (A) In the presence of 1 µM codeine sulphate (B) in the presence of 10 µM codeine sulphate

Fig.S4

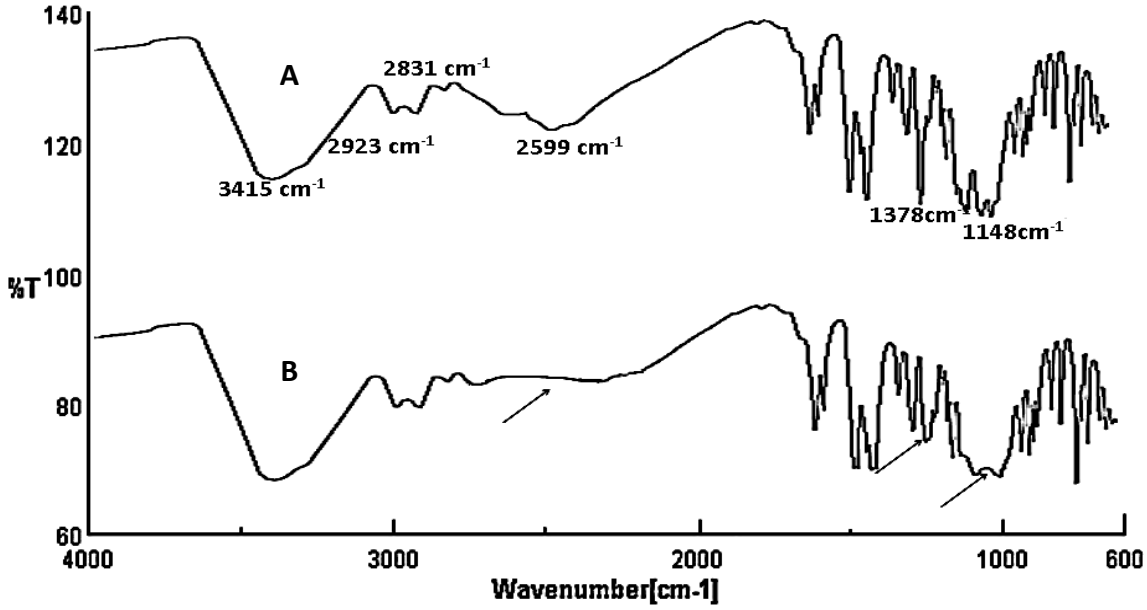

Fig.S4 Transmission IR spectra of (A) codeine sulphate (B) AuNPs-Cod complex formation

Fig.S5

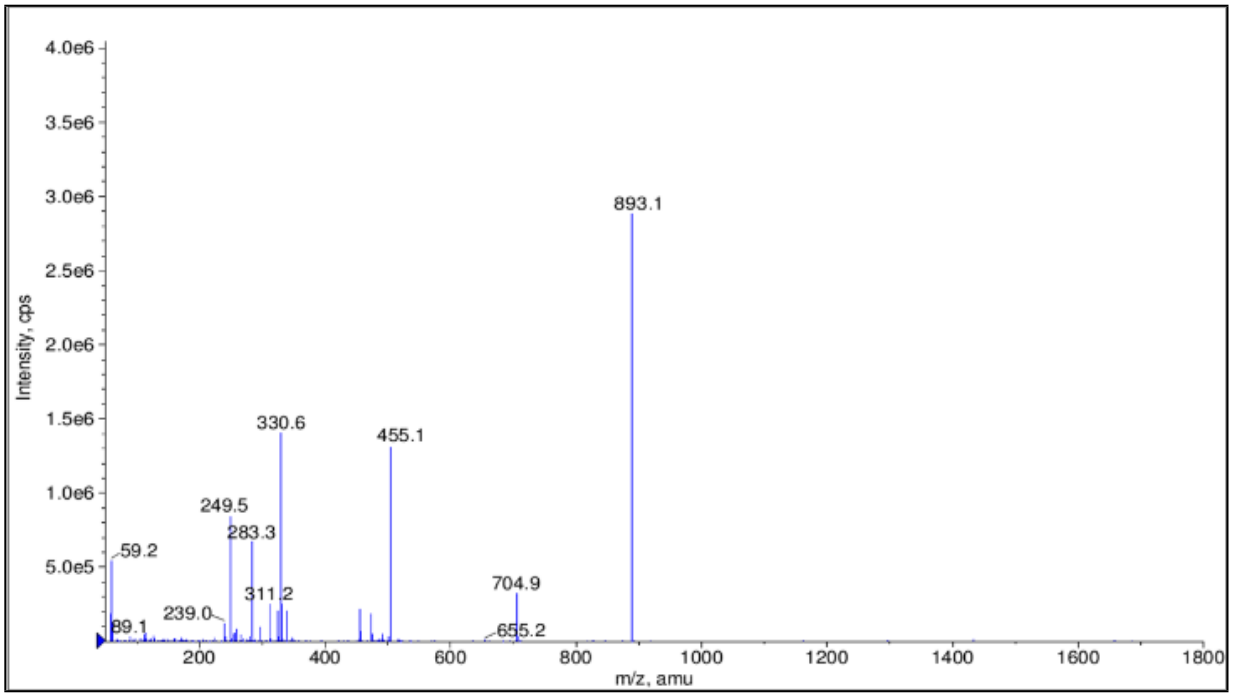

Fig. S5 ESI Mass spectra of AuNPs and after addition of codeine sulphate

Fig.S6

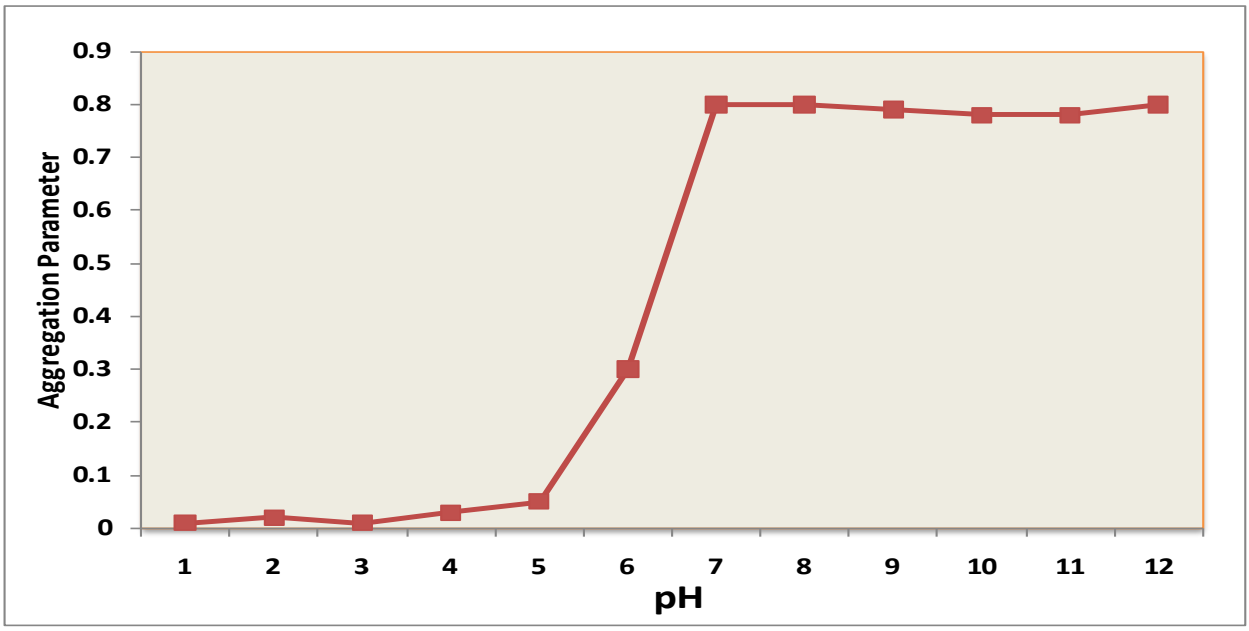

Fig.S6 Shows the plot after addition of codeine sulphate concentration into the AuNPs at different pH

Table S1

Parameter	Linear range (µM)	Calibration equation	Linearity (R2)
Red	1-10	y = -3.107x + 98.74	R^2 = 0.9905
Green	1-10	y = -4.314x + 106.95	R^2 = 0.9945
Blue	1-10	y = -4.0016x + 108.03	R^2 = 0.9932
RGB	1-10	y = -3.8075x + 104.57	R^2 = 0.9994

Table S1 Linear ranges and calibration equations of methods using iPhone 5S for capture product images.

Table S2

Solution of pH	**2**	**4**	**6**	**8**	**10**
Stability of AuNPs	2 h	4h	Weeks	Months	Months

Table S2 Stability of 0.07 mM citrate capped AuNPs at different pH conditions

Table S3

Samples	Added(µM)	Found(µM)	Recovery (%)	RSD(n=3)
Bone	0	1.3	-	1.21
	10	10.9	96.4	0.98
	50	48.7	94.9	0.97
Bone marrow	0	15.4	-	1.34
	10	25.1	98.8	1.42
	50	64.6	98.7	1.22
Soil	0	11.3	-	1.53
	10	20.4	95.7	1.01
	50	62.8	102.4	1.14

Table S3 Results of determination of codeine sulphate in bone, bone marrow and soil

Table S4

Sr. No	Method	Sample	Lower detection limit	Reference
1.	Aptamer based Electrochemical Au-mesoporous silica nanoparticles biosensor	PBS Buffer	3 pM	18[a]
2.	Citrate modified Gold Nanoparticles	PBS buffer, Bone, Bone marrow and Soil	0.9 µM	Present method
3.	Cyclic electrochemical voltammetry-RNA aptamer	PBS Buffer	1.0 µM	18[b]
4.	Device Oratect® III	Blood	1.67 µM	18[c]

Table S4 Comparison of present method with other methods