

SUPPLEMENTARY DATA

Cause of death determination using Paper based microfluidic device as colorimetric probe

Niha Ansari^a, Anand Lodha^a, Alok Pandya^b, S. K. Menon^{*a}

^a Department of Forensic Science, School of Sciences, Gujarat University, Ahmedabad, Gujarat-380009, India.

E-mail: shobhanamenon07@gmail.com; n.ansari3@gmail.com; forensicwithanand@gmail.com

^b Department of Chemistry Center of Engineering and Enterprise University and Institute of Advanced Research Gandhinagar, Gujarat-382007, India.

Synthesis of silver nanoparticles

In the present study, silver nanoparticles were synthesized in CEM Discover Benchmate microwave using single mode having continuous power at 2.45 GHz. In brief, 20 ml of ethylene glycol was taken into a 50 ml reaction flask containing 1 mM silver nitrate. In this solution, (1M) tri-sodium citrate solution was added dropwise until the solution color changes to yellow, indicating the formation of silver nanoparticles. These synthesized silver nanoparticles were characterized by UV-Vis spectrophotometer, FT-IR, TEM and DLS.

Alprazolam extraction from body fluids

For the extraction of ALP from VH and blood was spiked with 10 ng/ml ALP, 500 µl sample was mixed with 500 µl of deionized water, followed by addition of 1 ml aqueous 0.1 M zinc sulphate solution. The mixture was then vortex-mixed for 1 min and kept at room temperature (37°C) for 15 min. The resultant solution was then centrifuged at 12,500 rpm for 5 min. After centrifugation, organic phase was collected, filtered and evaporated to dryness. The residue was reconstituted by adding 1 ml of methanol and the extract was further used for analysis.

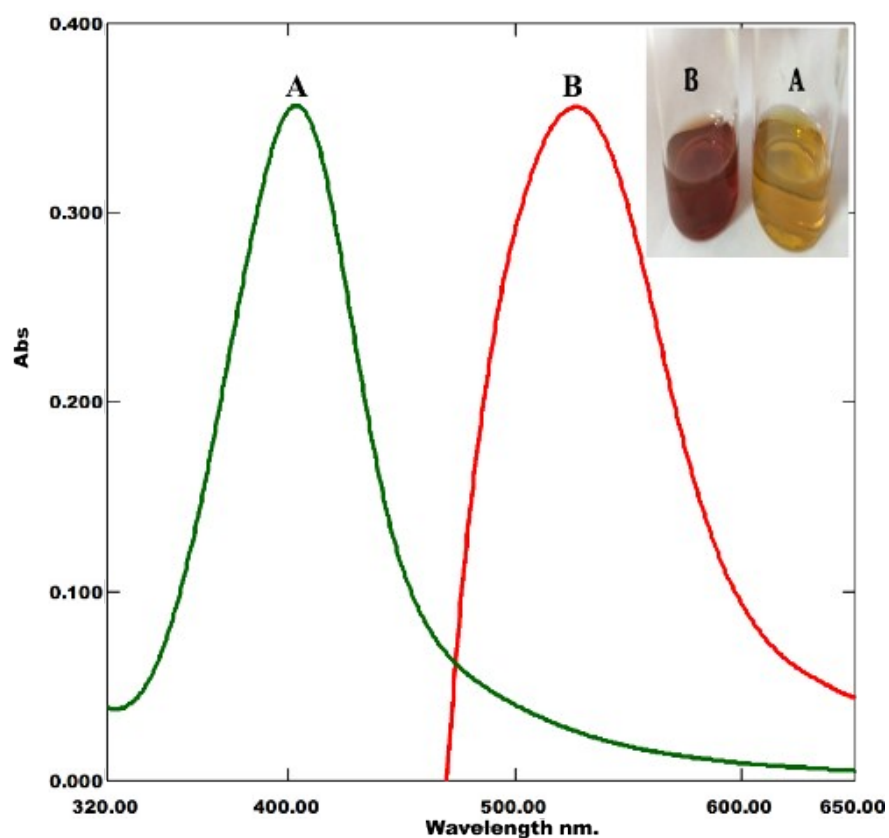


Figure S1 Showing the UV-Vis spectra of the

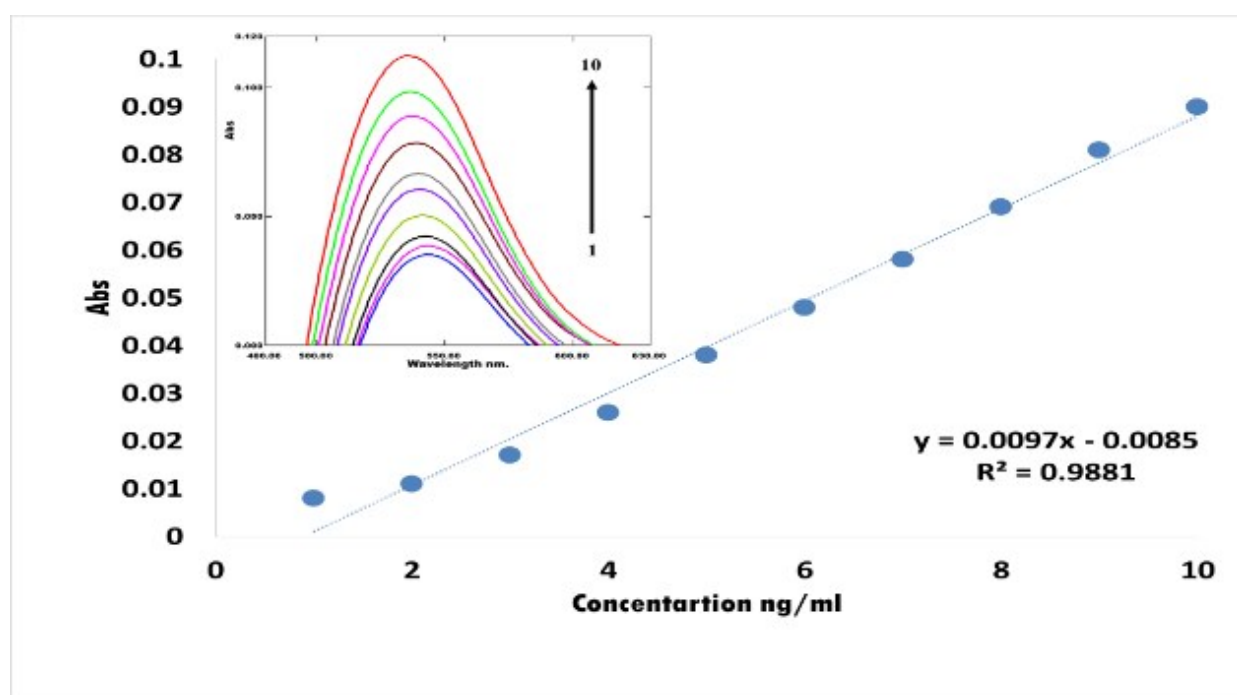


Figure S2 Linearity of ALP (1-10 ng/ml) with AgNPs having $R^2=0.9881$ and inset UV-Visible spectra of AgNPs in presence of ALP concentrations (1 to 10 ng/ml).

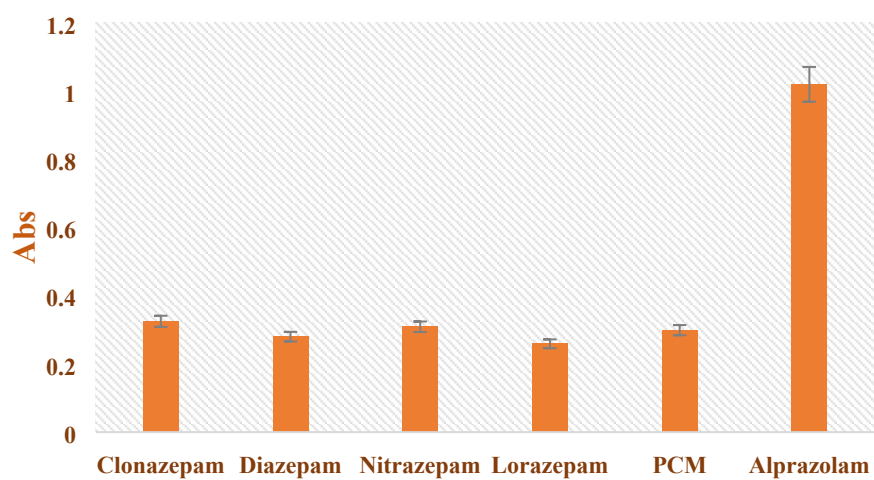


Figure S3 Relative absorbance change of AgNPs at 520 nm in the presence of clonazepam, diazepam, nitrazepam, lorazepam, paracetamol (PCM) and ALP.

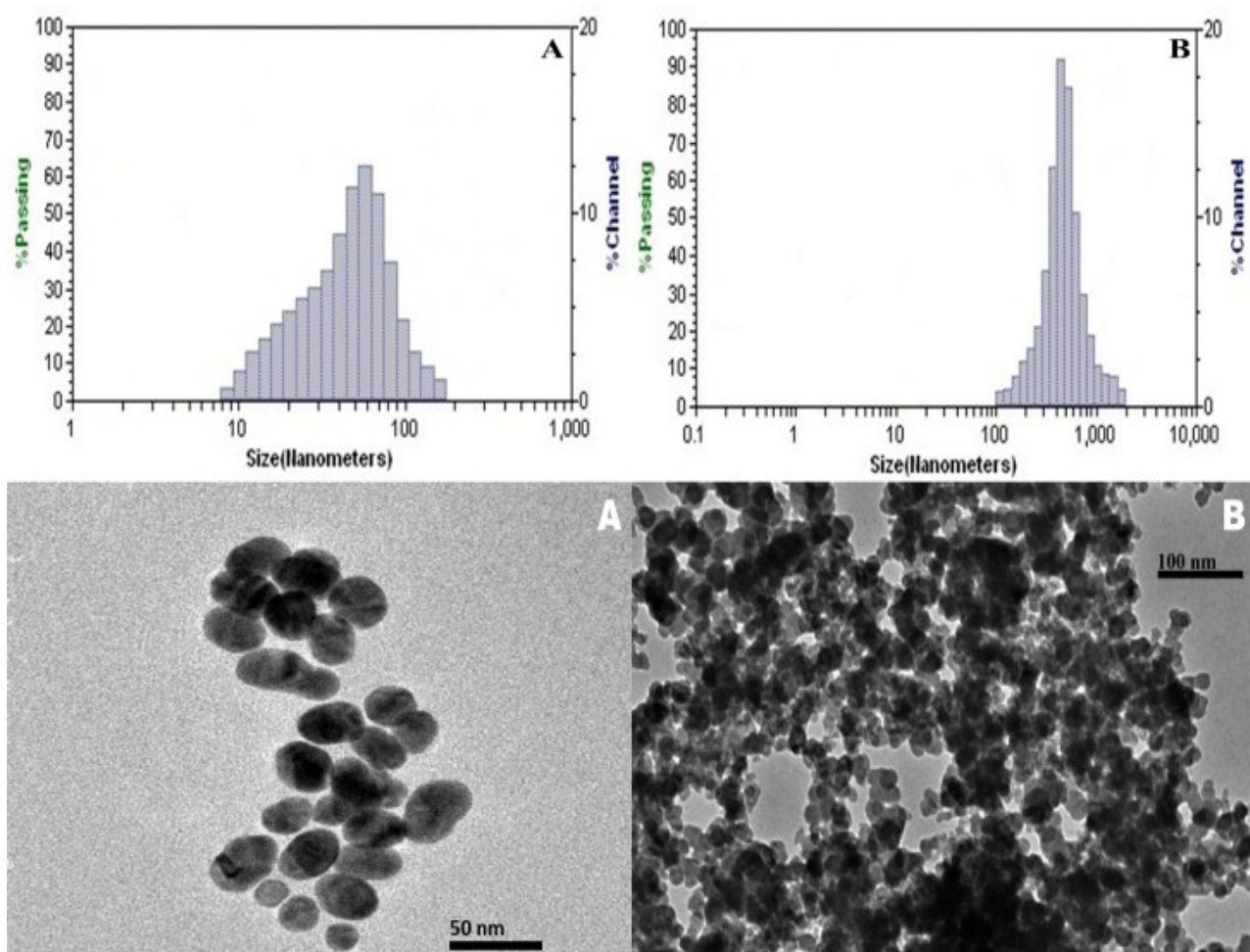


Figure S4 Size distribution of silver nanoparticle (A) in the presence of ALP (B).

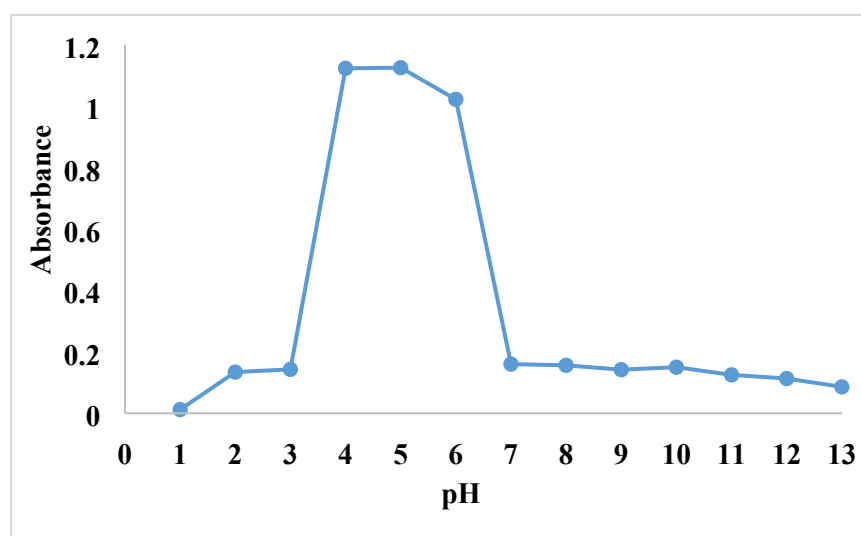


Figure S5 shows the plot of ALP - AgNPs complex at different pH

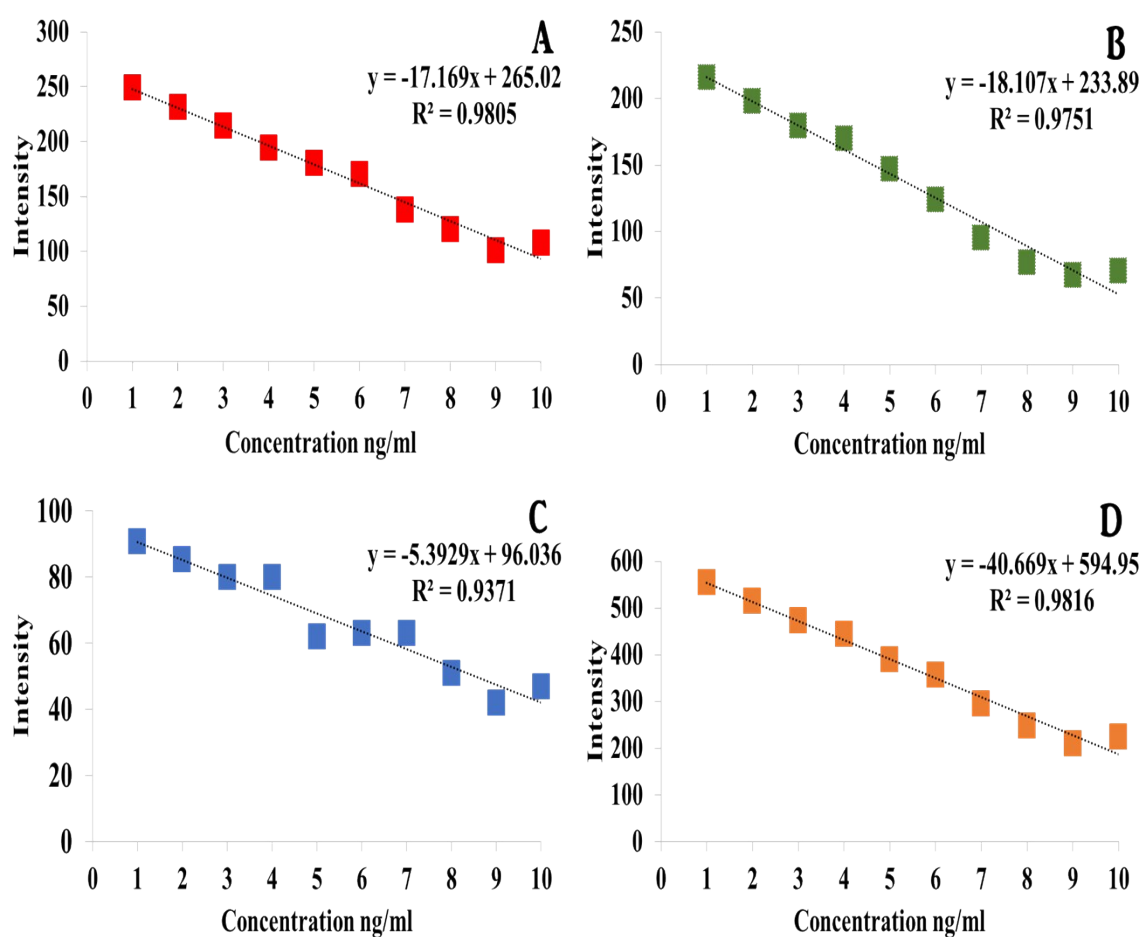


Figure S6 shows Relationship between intensity of (A) red light, (B) green light, (C) blue light, (D) intensity of total RGB and ALP concentration (1-10 ng/ml)

Table S1 Showing limit of detection of the present method

Method	Linear range (ng/ml)	Regression equation	Correlation coefficient (R ²)	LOD (ng/ml)
UV-Vis Spectrophotometry	1 - 10	$y = 0.0097x - 0.0085$	0.9881	0.8
Smart phone ananlysis of reaction	1 - 10	$y = -40.669x + 594.95$	0.9816	1
Smart phone ananlysis of μ PAD device	10 - 180	$y = -0.5746 + 494.87$	0.9798	10

Table S2 Comparison with other reported work for detection of ALP

Sr. No.	Method	Detection Limit (ng/ml)	Ref
1.	AuNPs	3.3	[1]
2.	GC and immunoassay	40	[2]
3.	HPLC	150	[3]
4.	HPLC	6.7	[4]
5.	EI-GC-MS	50	[5]
6.	HPLC Photodiode-Array Detection	3	[6]
7.	Present method	0.8	--

- [1] E. L. Doctor and B. McCord, Comparison of aggregating agents for the surface enhanced, Raman analysis of benzodiazepines, *Analyst* 138 (2013) 5926-5932.
- [2] I. Rasanena, M. Neuvonena, I. Ojanpera and E. Vuoria, Benzodiazepine findings in blood and urine by gas chromatography and immunoassay, *Forensic Science International* 112 (2000) 191–200.

- [3] M. N. Uddin, V. F. Samanidou and I.N. Papadoyannis, Stability Study of Six 1,4-Benzodiazepines in Bio-fluids Stored at -20°C, *Chiang Mai J. Sci.* 37 (2010) 451-463.
- [4] M. N. Uddin, V.F. Samanidou and I. N. Papadoyannis, Development and Validation of an HPLC Method for the Determination of Six 1,4- Benzodiazepines in Pharmaceuticals and Human Biological Fluids, *Journal of Liquid Chromatography & Related Technologies* 31 (2008) 1258–1282.
- [5] N. B. Tiscione, X. Shan, I.Alford and D. Tate Yeatman, Quantitation of Benzodiazepines inWhole Blood by Electron Impact-Gas Chromatography– Mass Spectrometry, *Journal of Analytical Toxicology* 32 (2008) 644-652.
- [6] P. Cabarcos, M.J. Tabernero, I. Álvarez, P. López, P. Fernández, and A.M. Bermejo, Analysis of Six Benzodiazepines in Vitreous Humor by High- Performance Liquid Chromatography–Photodiode-Array Detection, *Journal of Analytical Toxicology* 34 (2010) 539-542.