

Quantitative detection of codeine in human plasma using surface enhanced Raman scattering via adaptation of the isotopic labelling principle

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Conventional Raman spectroscopy

Raman spectra were collected using a Renishaw inVia Raman microscope (Renishaw PLC., Wotton-under-Edge, Gloucestershire, UK) coupled with a high powered 633 nm diode laser. The laser power was set to 100% (~30 mW on the sample) for all Raman data that were acquired. The instrument was calibrated at the start of each experiment using an internal silicon standard. The wavenumbers were also calibrated on the microscope stage through focusing x50 objective onto a silicon wafer, where static spectra centered at 520 cm⁻¹ for 1 s at 10% power was collected. Data collection was achieved using Wire 4 software. Raman spectra of codeine and codeine-*d*₆ (Figure S1) were recorded between 400 to 3200 cm⁻¹ for 10 s.

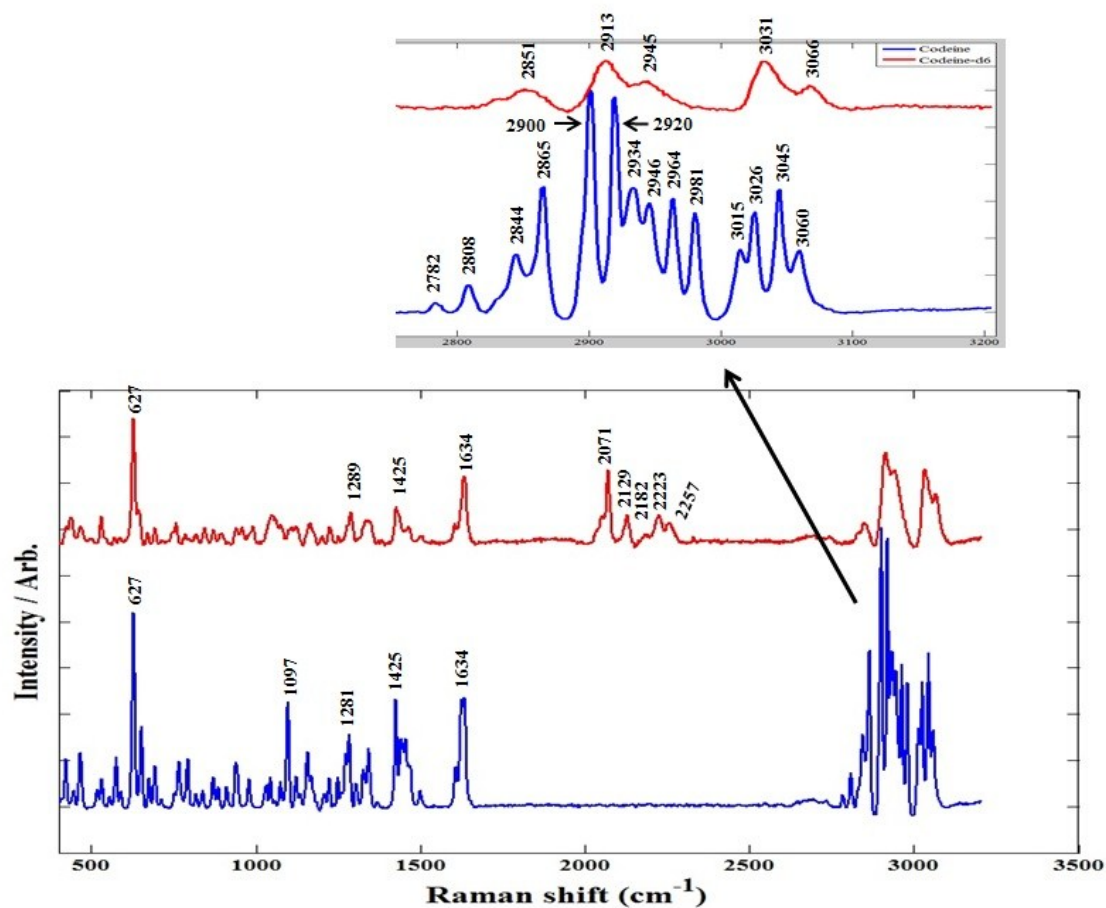


Figure S1. Raman spectra of codeine (blue) and codeine- d_6 (red); the insert is a zoom of the region from ca. 2800-3100 cm^{-1} .

Density function theory calculations

All density functional theory (DFT) calculations were carried out using Gaussian09.¹ Geometry optimisation, vibrational frequency, and Raman intensity calculations were performed at the B3LYP/cc-pVTZ level of theory²⁻⁴. The assignments of Raman bands for codeine and codeine- d_6 were based on calculation in the gas phase.⁵ Generation of Raman spectra and visualisation of vibrational modes were carried out using Gaussview 5.⁶

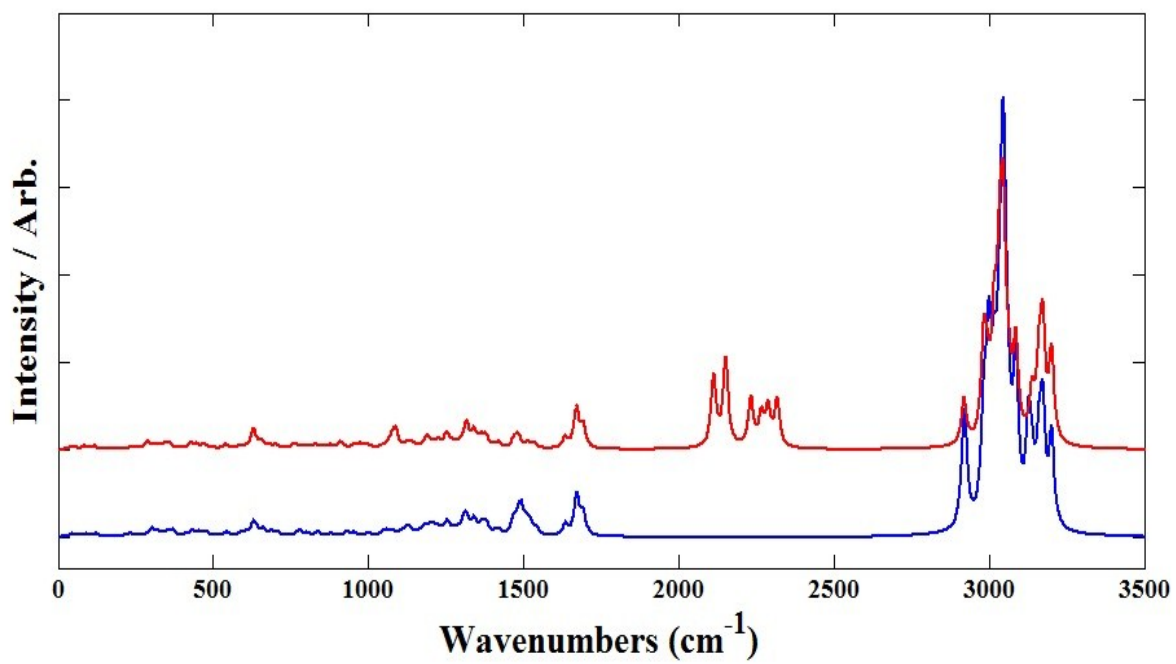


Figure S2. Calculated gas phase spectra of codeine (blue) and codeine-*d*₆(red)

Table S1. Vibrational assignments of codeine and codeine- d_6 from experimental and calculated Raman bands (from DFT), along with SERS. Red font is used to highlight any vibrations involving deuterium.

Raman Codeine	DFT Calculated	Raman Codeine	DFT Calculated	SERS Codeine	SERS Codeine	Vibrational assignment
627	629	630	630	637	637	Ring breathing
779	780	780	773	771	—	Ring deformation
930	931	909	908	—	—	C-O stretch in 5-membered ring + CH ₃ Bound to N CD₃ rocking bonded to O
972	997	975	974	—	—	C-C stretches in 6-membered ring (plane with the N containing ring)
1073	1078	1077	1085	—	—	C-C stretches in 6-membered ring (plane with the N containing ring)
1123	1127	1121	1127	1131	1131	Quaternary carbon stretch with C on N containing ring
1284	1293	1289	1293	1281	1288	C-N stretching + C-H wagging + ring breathing
1313	1317	1316	1317	—	—	C-H wag on 6-membered ring
1635	1672	1635	1672	1684	1684	C-C ring stretches on 6-membered
2921	2920	2071	2113	2953	2075	Sym C-H stretch on CH ₃ bonded to N Sym C-D stretch on CD₃ bonded to N
3001	3001	2129	2152	3028	2128	Sym C-H stretch on CH ₃ bonded to O Sym C-D stretch on CD₃ bonded to O
3026	3019	2223	2233	3031	2225	Asy C-H stretch (on CH ₂ two atoms way from N on 6-ring) Asy C-D stretch on CD₃ bonded to N
3045	3045	2257	2267	3059	2275	Asy C-H stretches around N on 6-membered ring Asy C-D stretch on CD₃ on O-CD₃
3060	3086	—	2289	—	—	Asy C-H stretch on CH ₃ bonded to N Asy C-D stretch on CD₃ bonded to N
—	3130	—	2318	—	—	Asy C-H stretch on CH ₃ bonded to O Asy C-D stretch on CD₃ bonded to O

Table S2. Raman band assignments from pure plasma⁷⁻⁹

Band (cm⁻¹)	Vibrational mode	Assignment
491	Ring vibration	Cellulose, guanine, L-arginine
590	—	Ascorbic acid, amide-VI
644	C-S	L-Tyrosine,
725	C-H	Adenine
810	C-C-O	L-Serine, glutathione
885	C-O-H	Glutathione, D-(C)-galactosamine
1012	C-C	Phenylalanine
1074	C-N	Collagen
1131	C-N	D-Mannose
1209	Ring vibration	L-Tryptophan, phenylalanine
1366	—	Tryptophan, adenine, guanine
1448	CH ₂	Collagen, phospholipids
1580	C-C	Phenylalanine, acetoacetate, riboflavin
1684	C=O	α -Helix, collagen

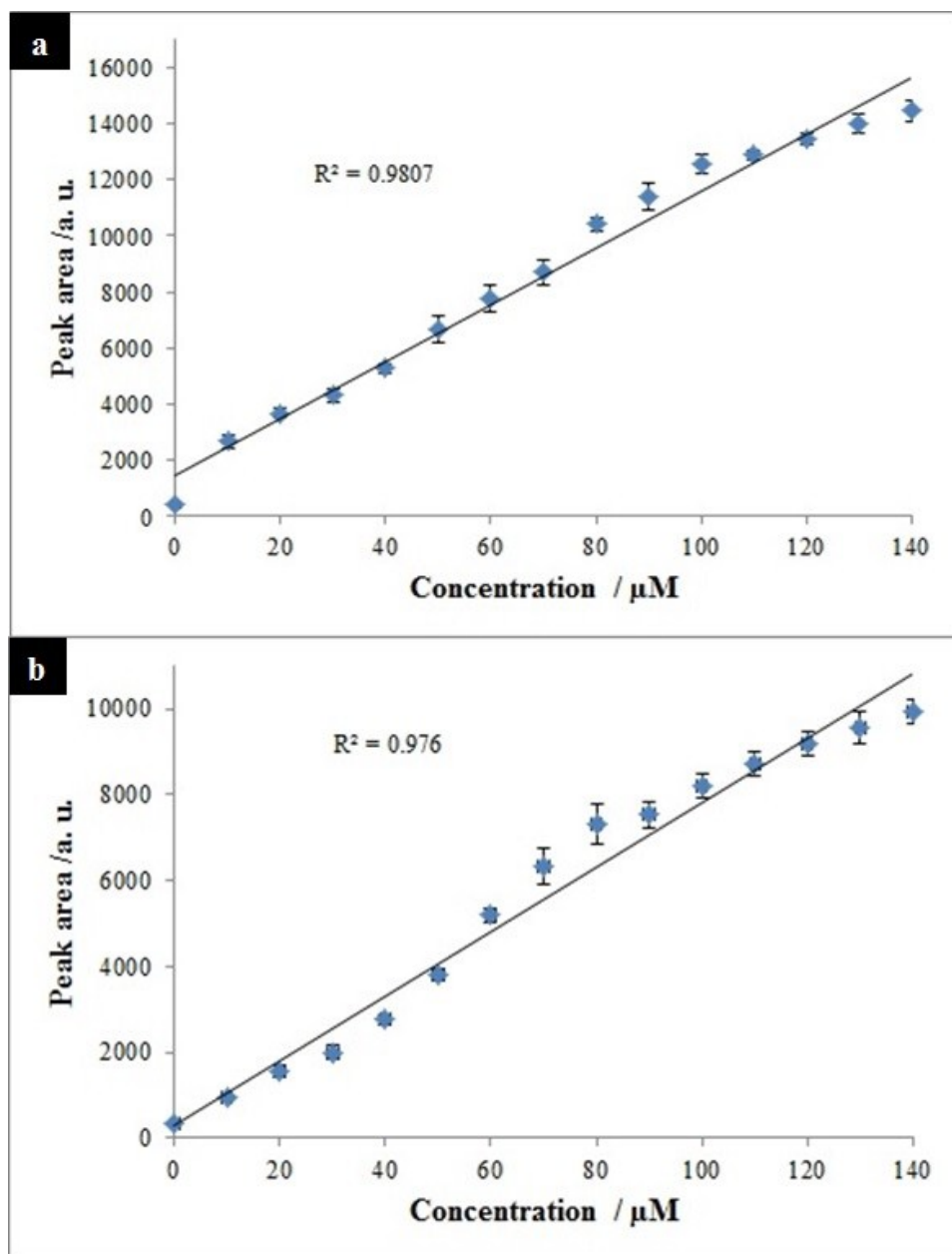


Figure S3. Calibration curves of codeine peak area at 1281 cm⁻¹ versus concentrations (a) water (b) plasma. The error bars denote standard deviation of the mean of ten measurements.

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