

Supplementary Information

**Detection and quantification of the opioid tramadol in urine using surface enhanced Raman scattering.**

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**Synthesis and Elimination of Tramadol**

Tramadol hydrochloride (1 RS, 2 RS)-2-[(dimethylamino-methyl]-1-(3-methoxyphenyl)-cyclohexanol HCL, was previously synthesised by Cheme.G by exploiting coupling reactions between aminoketones with aryl derivatives using Grignard's reaction. Recently Alvarado *et al.* (2005) <sup>1</sup> use the same approach of the coupling reaction with organolithium derivatives. The general scheme of the reactions used is given below in Figure S1.

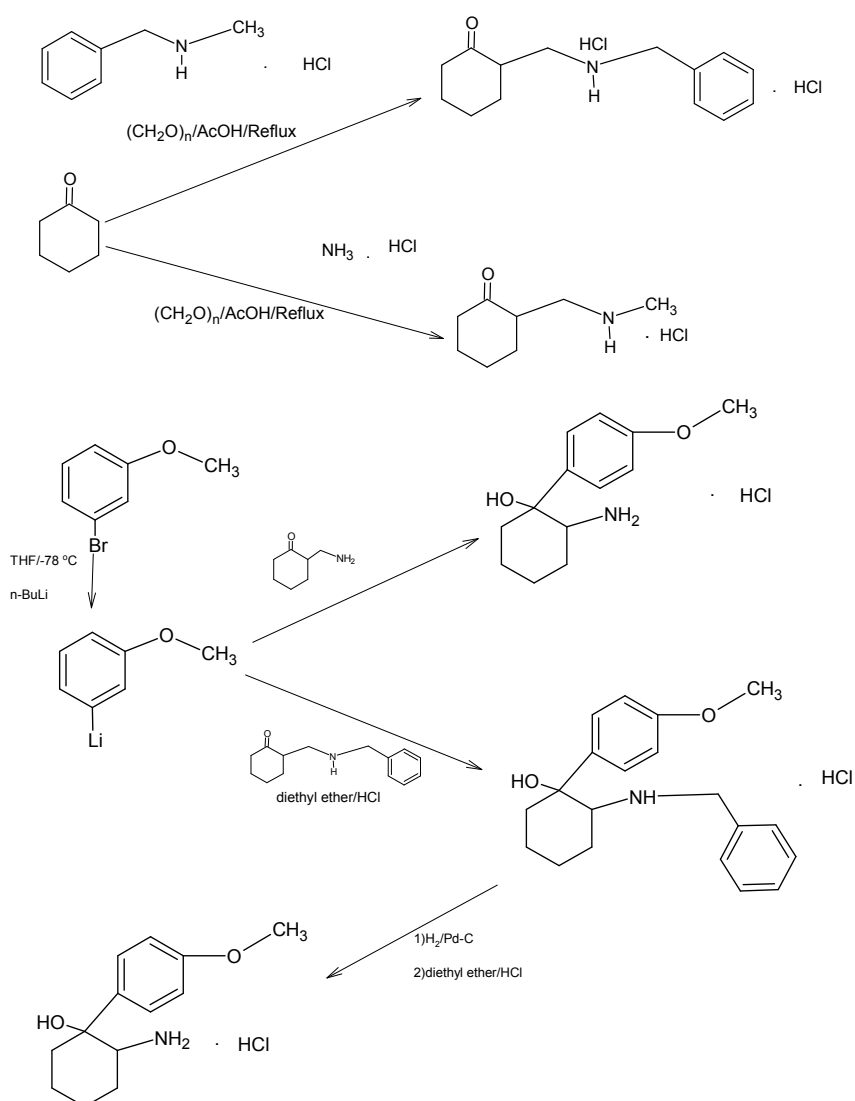


Figure S1: Schematic diagrams for (A) the synthesis of aminoketones as the first step in (B) tramadol synthesis.

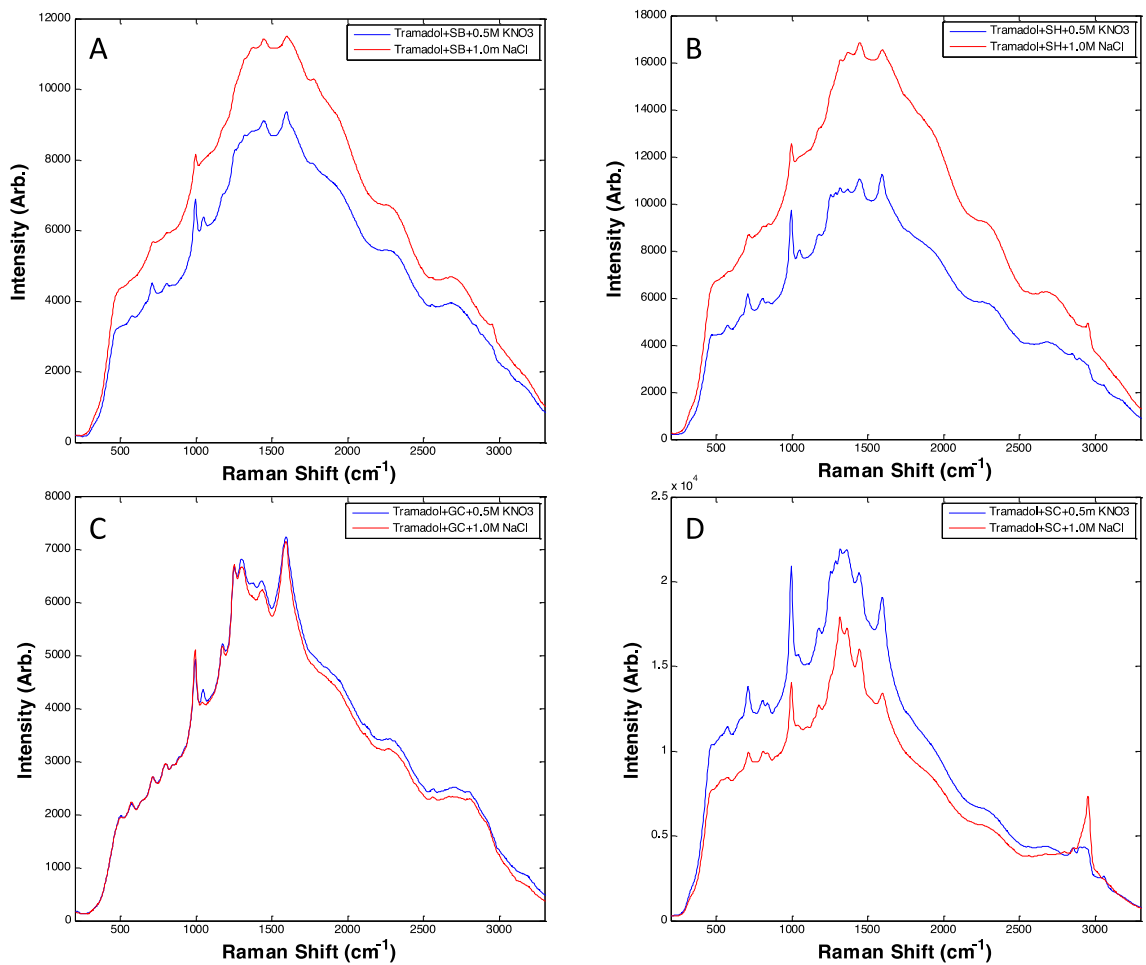


Figure S2: Typical SERS spectra using the four different colloids – silver borohydride (A), silver hydroxylamine (B) gold citrate (C), and silver citrate (D) – with either 0.5 M KNO<sub>3</sub> or 1.0 M NaCl as the aggregating agent.

## Conventional Raman spectroscopy

Due to the small amount of tramadol available we used a Raman microscope for analysis. Raman data were collected using a Renishaw 2000 Raman microscope (Renishaw Plc., Old Town, Wotton-under-Edge, Gloucestershire, U.K.) with a low-power (27 mW) 633 nm diode laser, with the power on the sample being 2-4 mW, and a spectral resolution 6  $\text{cm}^{-1}$ . The instrument was wavelength calibrated with a silicon wafer focused under a 50x objective lens used for analysis. Data were collected as static spectra centered at 520  $\text{cm}^{-1}$  for 1 s. The GRAMS WiRE software package (Galactic Industries Corp., 395 Main St., Salem, NH, USA) running under Windows 95 was used for instrument control and data capture. Raman spectra of tramadol (Figure S2) were recorded between 200 and 3500  $\text{cm}^{-1}$  for 1 min. Table S1 details the assignments for these Raman vibrations.

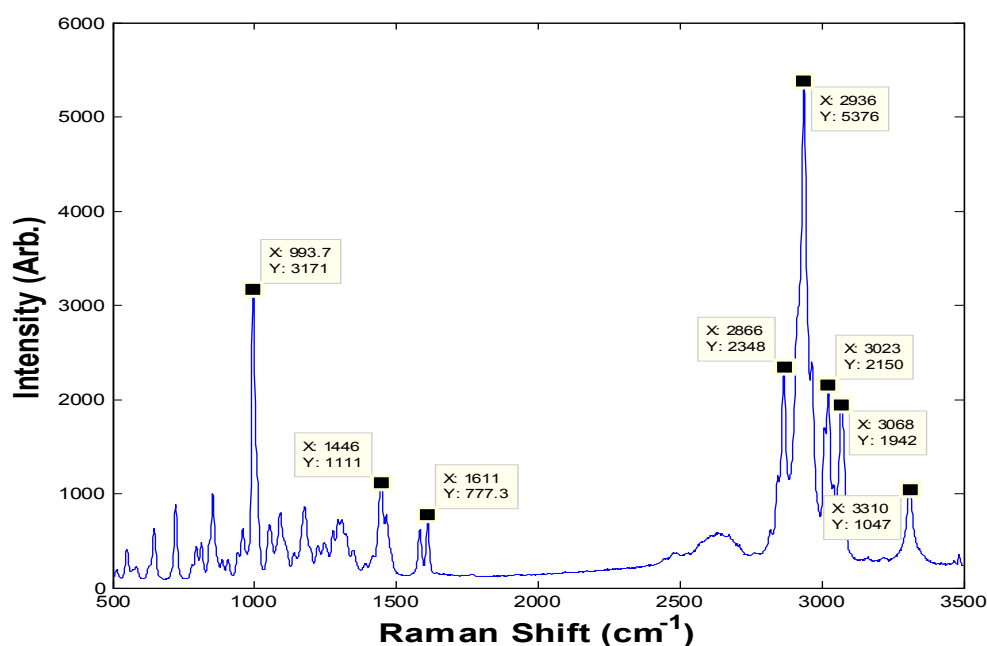


Figure S3: Raw Raman spectrum of powdered tramadol

Table S1: Tentative band assignments for Raman spectra of tramadol hydrochloride

Raman shift $\text{cm}^{-1}$	Assignment
3310	Alcohol (O-H) stretch
3023	Aromatic (C-H) stretch
2936, 2866	Aliphatic (C-H) stretch
2630 (broad)	Ammonium (N-H) stretch
1611, 1576	Aromatic ring skeleton stretch
993	Ring breathing
847	(C-N) symmetric stretch
717, 641	(C-H) out of plane stretch

## Colloidal preparations and characterisation

The reagents used in this investigation used for colloids preparation included: silver nitrate (>99.99%), trisodium citrate (99%), sodium borohydride NaBH<sub>4</sub> (98+%), hydroxylamine hydrochloride 99% and chloroauric acid (HAuCl<sub>4</sub>, 99.9%) all purchased from Sigma-Aldrich (Sigma-Aldrich Dorset, UK). Potassium nitrate and sodium chloride were used as aggregating agents and were also purchased from Sigma-Aldrich. Acetic acid (analytical reagent grade) was purchased from Fisher Scientific Ltd. (Loughborough, U.K.), sodium hydroxide standard solution 0.1 mol/L was obtained from Riedel de-Haen Company (Seelze, Germany).

Four colloids were prepared as detailed below and each colloid type was prepared in triplicate to assess SERS reproducibility.

All colloids were stored in glass flasks covered in foil. These were stable when stored at room temperature in the dark.

### *Silver citrate colloid*

Silver citrate nanoparticles were prepared using the Lee and Meisel method.<sup>2</sup> Briefly, 90 mg of silver nitrate was dissolved in 500 mL of deionised water, after which the solution was heated to boiling point. 10 mL of 1% aqueous solution of tri-sodium citrate was added to the boiling silver nitrate solution drop-by-drop while the solution was vigorously stirred. The mixed solution was kept boiling for a further 10 min. A green-grey silver colloid was obtained, which proved to be stable for several weeks.

### *Silver borohydride colloid*

Silver nanoparticles were prepared by the reduction of AgNO<sub>3</sub> by using NaBH<sub>4</sub> aqueous solution as a reducing agent, following the method of Lee and Meisel.<sup>2</sup> AgNO<sub>3</sub> (1×10<sup>-3</sup> M, 25 mL) was added to an ice-cold solution of vigorously stirred NaBH<sub>4</sub> (2×10<sup>-3</sup> M, 75 mL) to form a yellow colloid of Ag nanoparticles.<sup>2</sup>

### *Silver hydroxylamine colloid*

The silver hydroxylamine colloid was obtained by the method described by Leopold and Lendl.<sup>3</sup> A total of 4.5 mL of a sodium hydroxide solution (0.1 M) was added to 5 mL of a 6×10<sup>-2</sup> M hydroxylamine hydrochloride solution. Then the mixture was added rapidly to 90 mL of a 1.11×10<sup>-3</sup> M silver nitrate aqueous solution, and the resulting mixture was shaken until a homogeneous mixture was obtained. The resulting colloid showed a milky grey colour.

### *Gold citrate colloid*

Gold nanoparticles were synthesised according to a protocol described by Turkevich *et al.*<sup>4</sup> 100 mL of HAuCl<sub>4</sub> solution (50 mg) was added to 850 mL of boiling water under vigorous

stirring. Once the solution had returned to a boil, 50 mL of 1% trisodium citrate was added. After 30 min of continuous boiling and stirring the gold nanoparticle solution was left to cool.

### *Colloidal Characterisation*

UV-visible spectra of all colloids (three batches of each of the four colloid types) were collected using a BioMate 5 Spectrophotometer, wavelength range 190 to 1100 nm, spectral bandwidth 2 nm, optical system double-beam; quartz-coated and lamp source is deuterium lamp; tungsten lamp. All colloids were assessed using UV-visible spectroscopy.

All UV-visible spectra of the colloids provided evidence that nanoparticles of silver or gold had been produced and were very similar to that synthesised by us previously.<sup>5-8</sup>

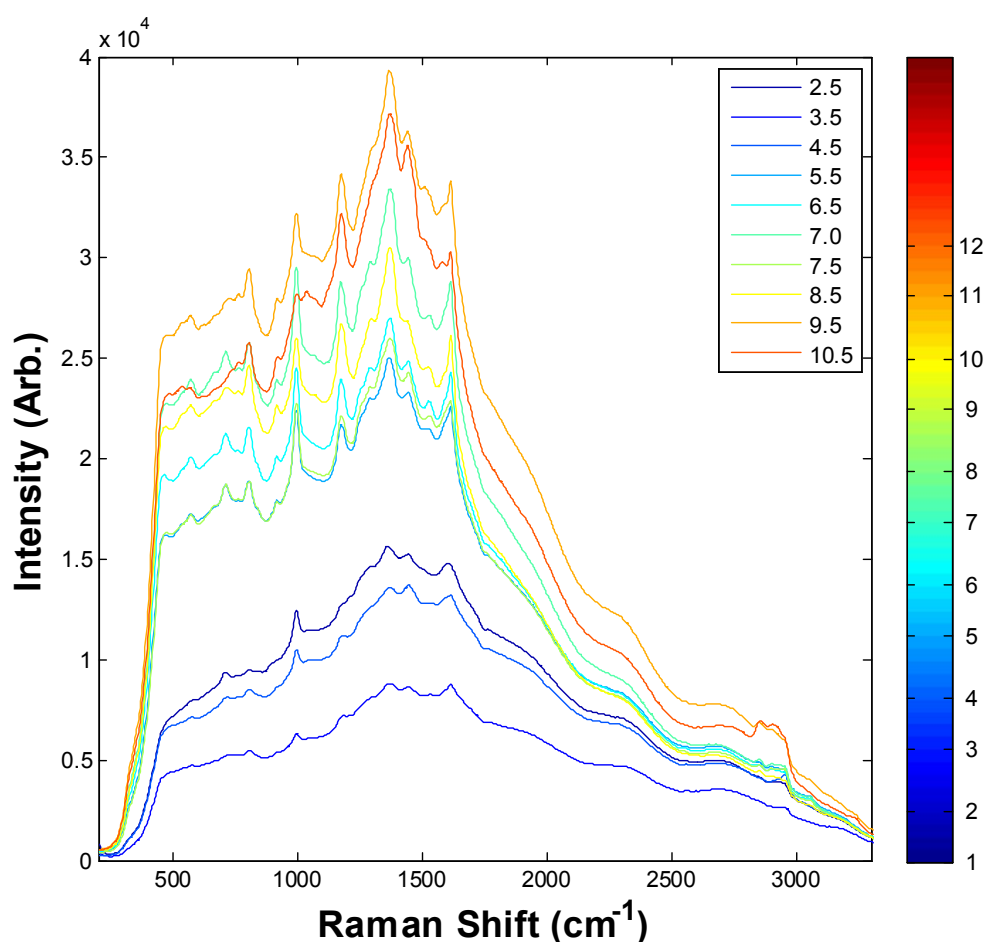


Figure S4: SERS spectra of 0.01 M tramadol from pH 2.5 – 10.5. The SERS substrate used was silver hydroxylamine colloid and 0.5 M NaCl was used as the aggregating agent.

Table S2: Sample preparation for tramadol in artificial urine (AU)

Sample	AU/mL	Tramadol ( $1 \times 10^{-2}$ M) /mL	Colloid /mL	Final concentration
1	25	175	200	$8.75 \times 10^{-3}$ M
2	50	150	200	$7.50 \times 10^{-3}$ M
3	75	125	200	$6.25 \times 10^{-3}$ M
4	100	100	200	$5.00 \times 10^{-4}$ M
5	125	75	200	$3.75 \times 10^{-4}$ M
6	150	50	200	$2.50 \times 10^{-4}$ M
7	175	25	200	$1.25 \times 10^{-4}$ M
8	180	20	200	$1.00 \times 10^{-4}$ M
9	185	15	200	$7.50 \times 10^{-5}$ M
10	190	10	200	$5.00 \times 10^{-5}$ M
11	195	5	200	$2.50 \times 10^{-5}$ M
12	197.5	2.5	200	$1.25 \times 10^{-5}$ M
13	198.5	1.5	200	$6.25 \times 10^{-6}$ M
14	199.5	0.5	200	$2.50 \times 10^{-6}$ M
15	199.75	0.25	200	$1.25 \times 10^{-6}$ M

## References

- (1) Alvarado, C.; Guzmán, Á.; Díaz, E.; Patiño, R. *J Mex Chem Soc* **2005**, *49*, 324-327.
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