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Rapid detection and quantification of paracetamol and its major metabolites using surface enhanced Raman scattering

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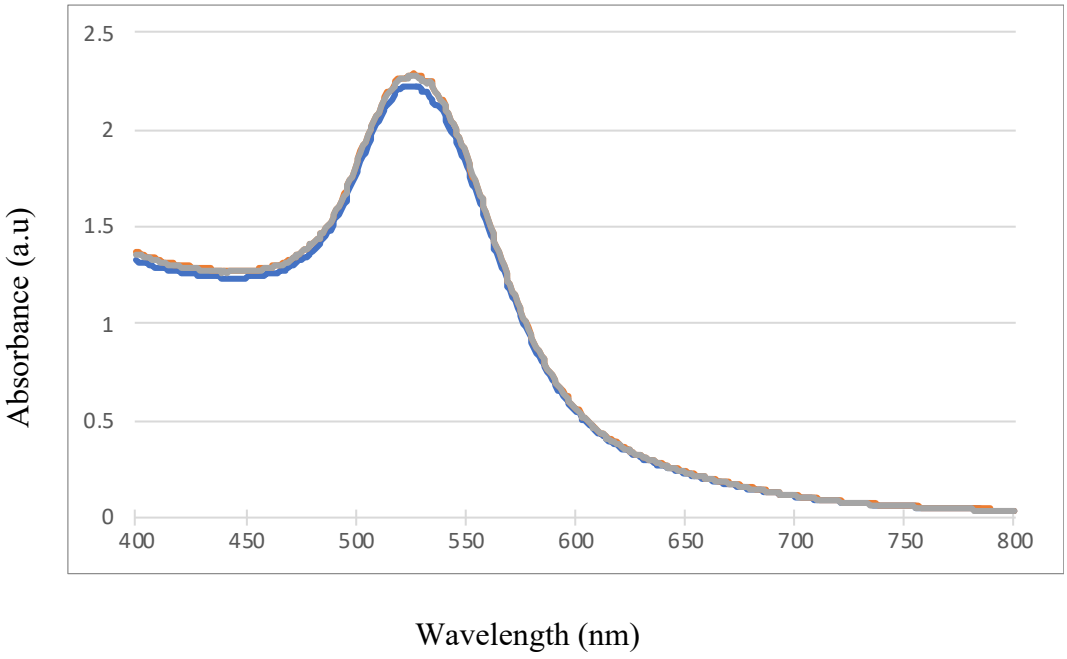

Fig. S1 Replicate (n = 3) UV-visible absorption spectra of gold nanoparticles made by citrate reduction of chloroauric acid.

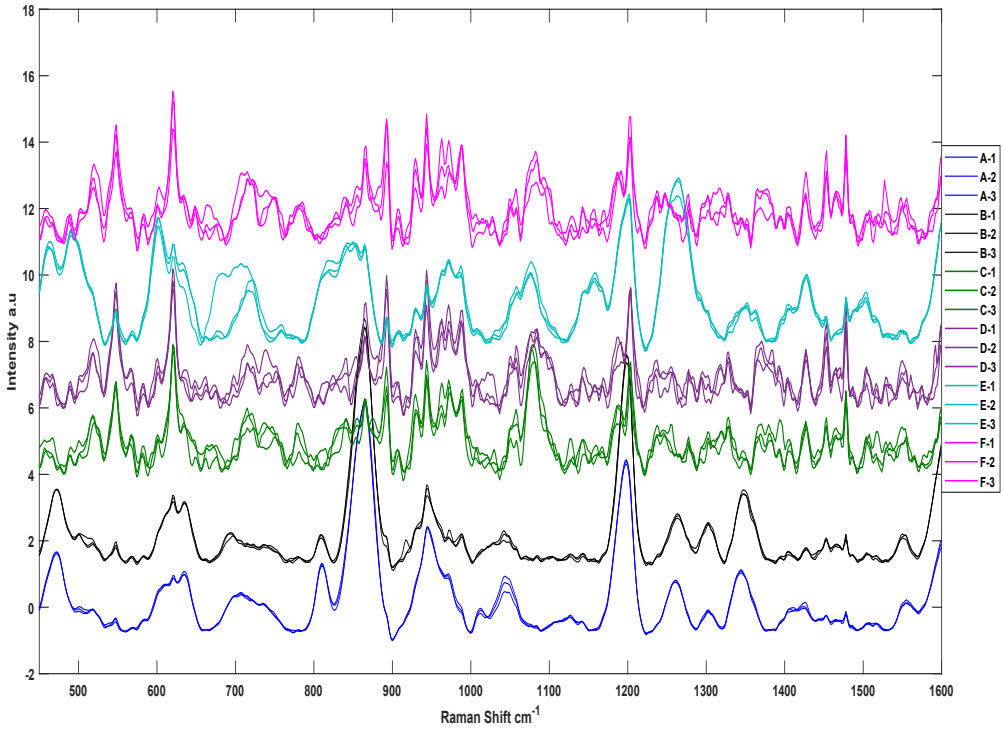

Fig. S2 Replicate SERS spectra of paracetamol using the following different colloids and aggregating agents:
(A) gold citrate with KNO_3 or (B) NaCl,
(C) silver hydroxylamine with KNO_3 or (D) NaCl,
(E) silver borohydride with KNO_3 or (F) NaCl.
The concentration of all of the aggregating agents used in this study was 0.045 M.
The *y*-axis is offset for clarity.

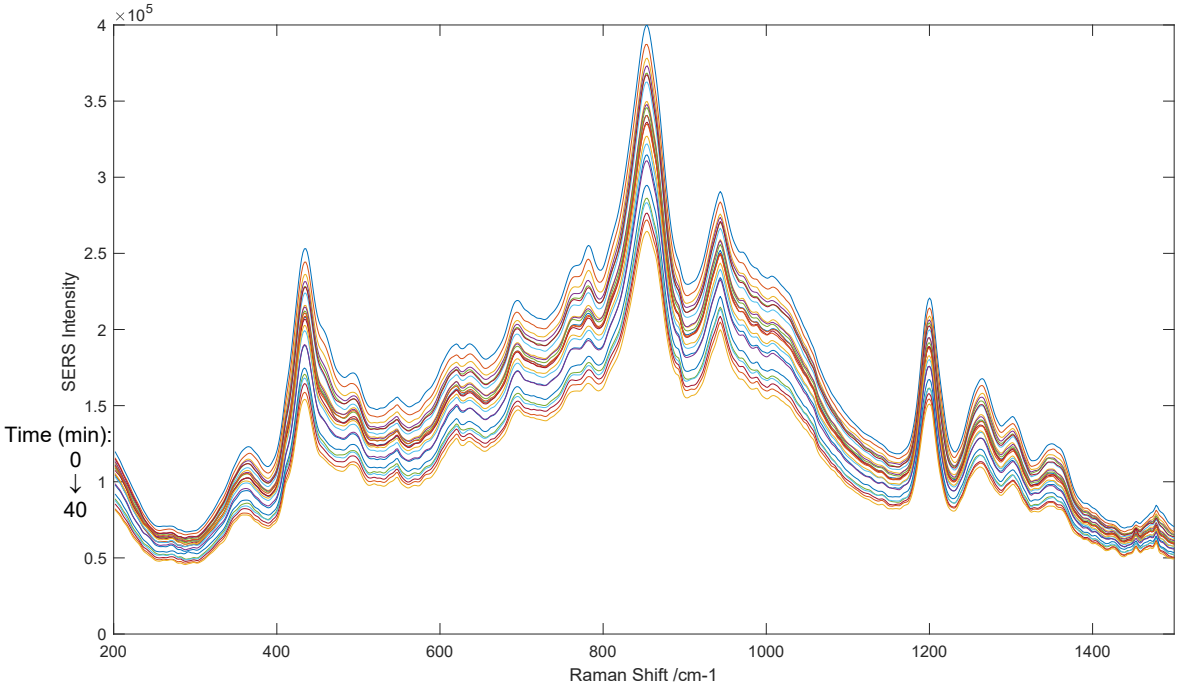

Fig. S3 Raw SERS spectra of paracetamol where paracetamol was first mixed with gold colloid and then 50 µL of 0.5 M NaCl was used as the aggregating agent. The spectra were collected after aggregation for 0 to 40 minutes in intervals of 5 min. Although not labelled in the first 10 min the signal remained constant and then there was a slight reduction on intensity.

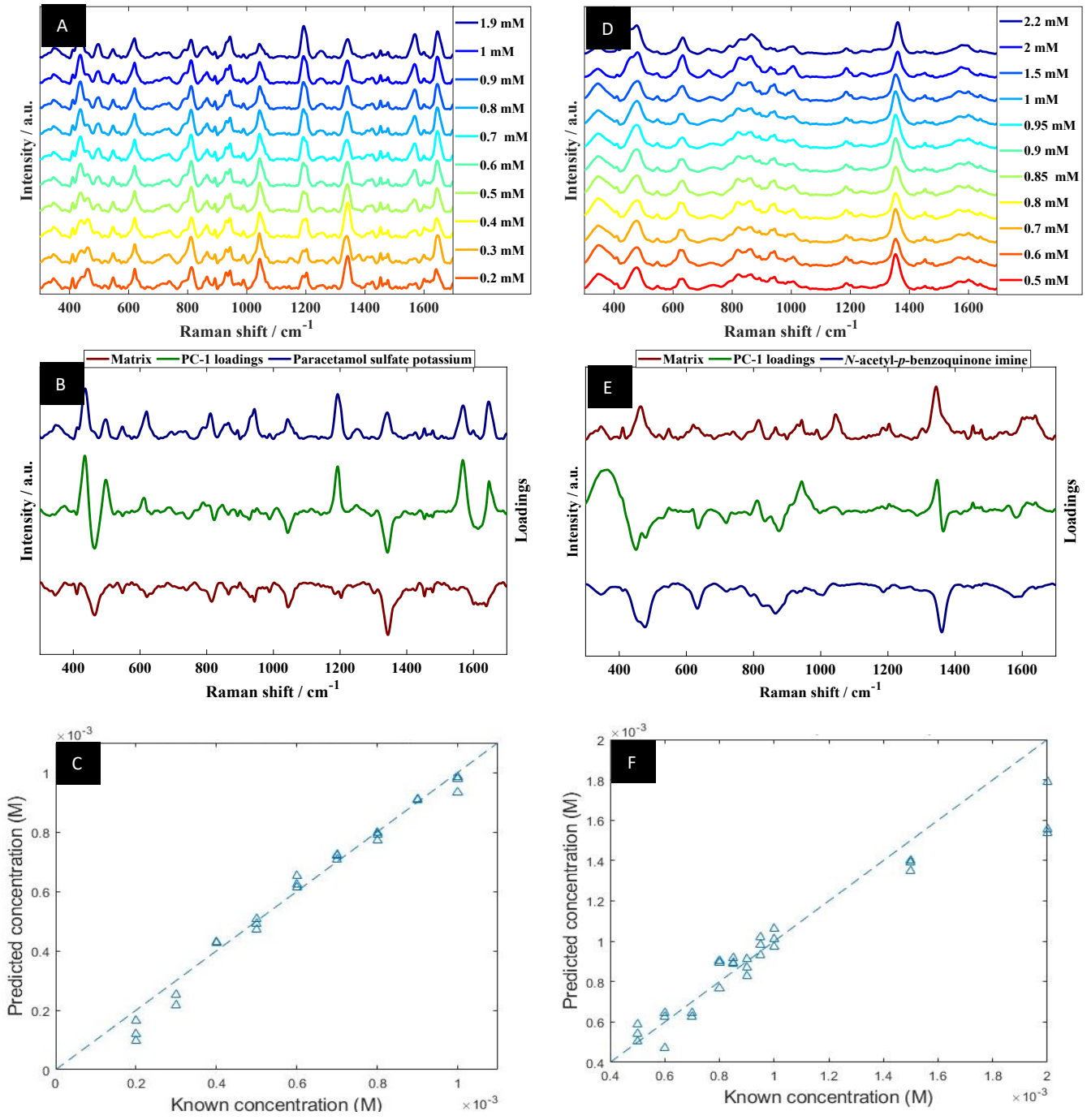

Fig. S4 Baseline-corrected SERS spectra showing quantification (A) paracetamol sulfate and (D) NAPQI. PCA (not shown) illustrated trends in increasing analyte concentrations in PC1, and the corresponding PC1 loadings plots show the significant molecular vibrations that contribute toward the separation of the paracetamol sulfate (B) and NAPQI (E) where the pure spectra of matrix (in brown) or analyte (paracetamol in blue or NAPQI in blue) are also shown associated with these trends in PCA. PLSR prediction plots generated using SERS data from paracetamol sulfate (C) and NAPQI (F).

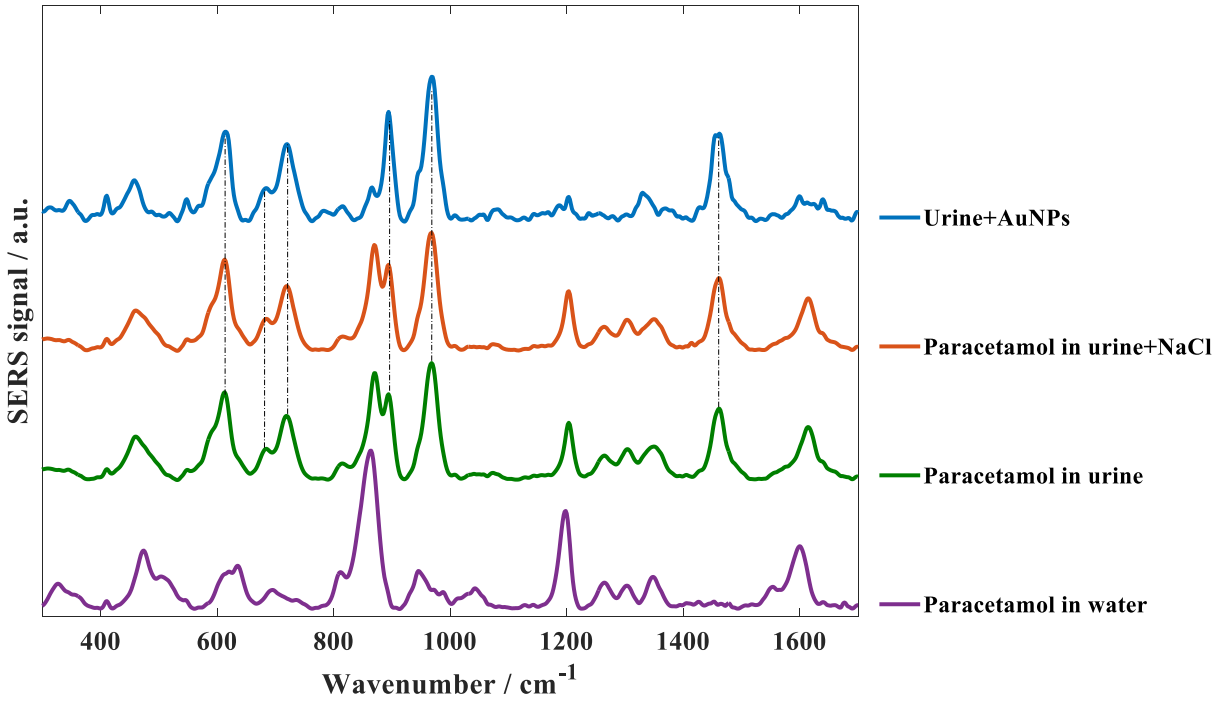

Fig. S5 A series of SERS spectra illustrating that urine-related SERS peaks are present when paracetamol is analysed in urine. The dotted line illustrates peaks that are related to urine alone and can be assigned to biochemical components such as uric acid (~640 cm^{-1}: =O–N deformation; ~890 cm^{-1}: N−H bending), hypoxanthine (~720 cm^{-1}: C–H stretching) and tryptophan (~680 cm^{-1} and ~1360 cm^{-1} (also ascribed to guanine)) that are found in urine (as described in the text, and see citations therein).

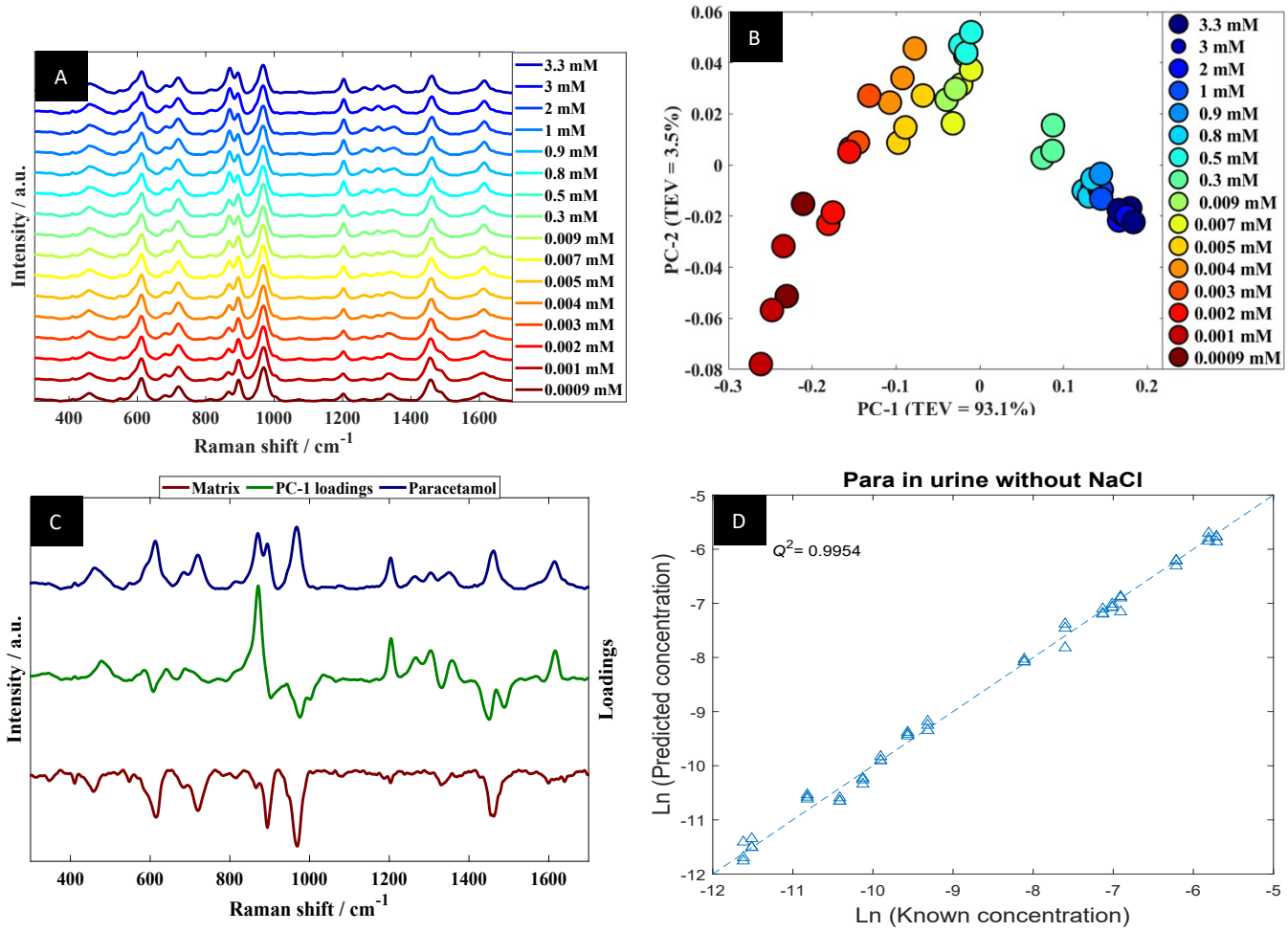

Fig. S6 (A) SERS spectra of paracetamol in artificial urine at different concentrations without the addition of any aggregating agent, spectra are offset for clarity. (B) PCA scores plot of SERS data acquired from paracetamol in artificial urine at different concentrations. (C) PC1 loadings plot (green line), paracetamol in water (blue), SERS spectra of matrix (brown line) that has been multiplied by (-1) and offset for clarity. (D) PLSR quantitative output generated using SERS data from paracetamol in artificial urine.

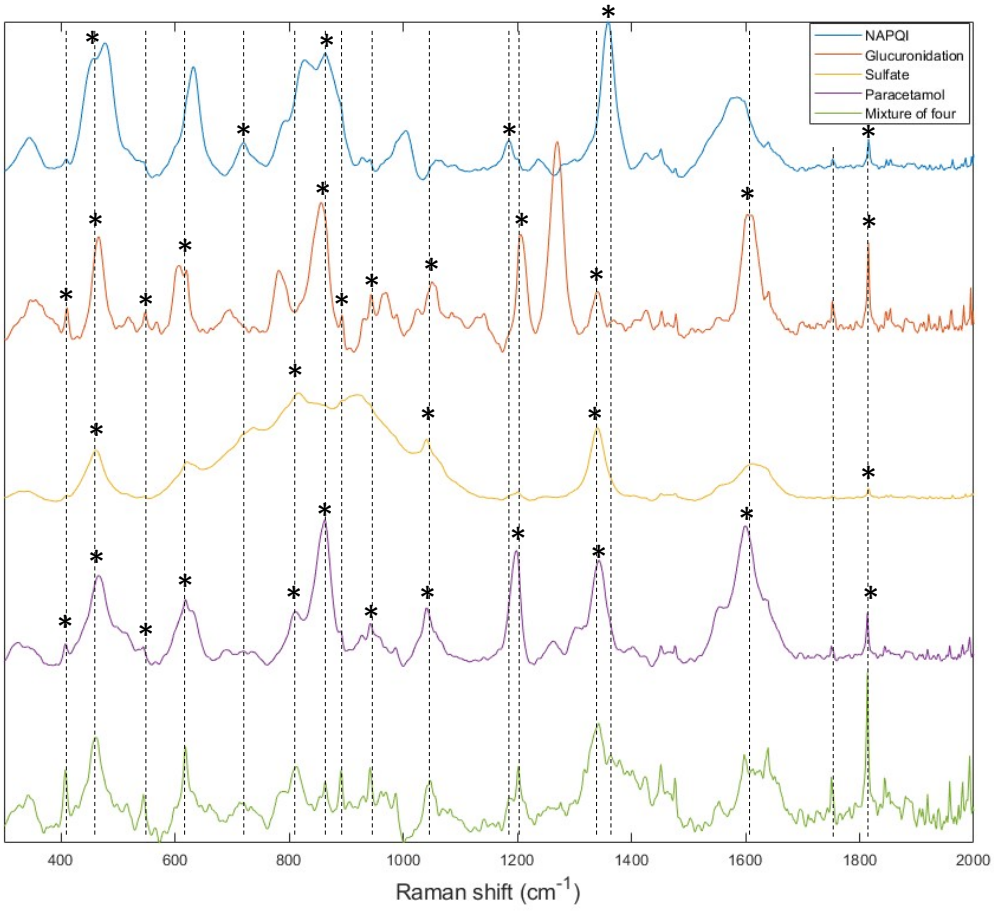

Fig. S7 SERS spectra of *N*-acetyl-*p*-benzoquinone imine (NAPQI; in blue), *p*-acetamidophenyl β-d-glucuronide (in orange), paracetamol sulfate potassium (in yellow) and paracetamol (in purple) along with a mixture containing all for analytes (in green) at ratio of approximately 2:3:3:2 for NAPQI : glucuronide : sulfate : paracetamol. For clarity these spectra have been offset. Asterisks highlight peaks/features that are found in the mixture spectrum that are also present in the spectra from the four pure compounds.

Table S1: Although not specified by Sigma-Aldrich the following components have been reported in artificial urine

Component	g per 1 L
Peptone L37	1
Yeast extract	0.005
Lactic acid	0.1
Citric acid	0.4
Sodium bicarbonate	2.1
Urea	10
Uric acid	0.07
Creatine	0.8
$CaCl_2 \cdot 2H_2O$	0.37
NaCl	5.2
$FeSO_4 \cdot 7H_2O$	0.0012
$MgSO_4 \cdot 7H_2O$	0.49
$Na_2SO_4 \cdot 10H_2O$	3.2
KH_2PO_4	0.95
K_2HPO_4	1.2
NH_4Cl	1.3

This recipe is made in 1 L of distilled water
Source: T. Brooks and C. W. Keevil, *Lett. Appl. Microbiol.*, 1997, **24**, 203-206.