Towards improved quantitative analysis using surface-enhanced Raman scattering incorporating internal isotope labelling

Abdu Subaihi[1], Yun Xu[1], Howbeer Muhamadali[1], Shaun T Mutter[1,2], Ewan W. Blanch[3], David I. Ellis[1] and Royston Goodacre[1*]

[1]School of Chemistry, Manchester Institute of Biotechnology, University of Manchester, 131 Princess Street, Manchester, M1 7DN, UK

[2]Department of Chemistry, Cardiff University, Cardiff, Wales

[3]School of Science, RMIT, GPO Box 2476, Melbourne, Victoria 3001, Australia

[*]**Corresponding author:**
Royston Goodacre, E-mail: roy.goodacre@manchester.ac.uk, Tel: 0161 306-4480

Keywords: Tryptophan, caffeine**,** Raman spectroscopy, SERS and isotopic labelling.

Instrumentation

UV-visible (UV-vis) absorption/extinction characterisation of nanoparticles

UV-vis characterisation was carried out as follows: 1 mL of the diluted nanoparticle solution was placed into a quartz cuvette and inserted into the sample holder of a Thermo Biomate 5 (Thermo Fisher Scientific Inc., Massachusetts, USA). An absorbance spectrum was collected over a range of 300 to 800 nm.

Scanning electron microscopy (SEM)

SEM analysis of the gold and silver nanoparticles was carried out using a field-emission scanning electron microscope with a beam size of 1 nm operating down to 100 eV (Carl-Zeiss Straße 56, 73447 Oberkochen, Germany). 10 μL of the nanoparticles were spotted onto a silicon slide and left to dry.

Raman spectroscopy

All 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 acquisitions. 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 were collected. The data collection was achieved using Wire 4 software. Samples were spotted (5 µL) onto calcium fluoride slides and allowed to air dry at room temperature for ~ 45 min. The acquisition time for each measurement was 10 s with a single accumulation.

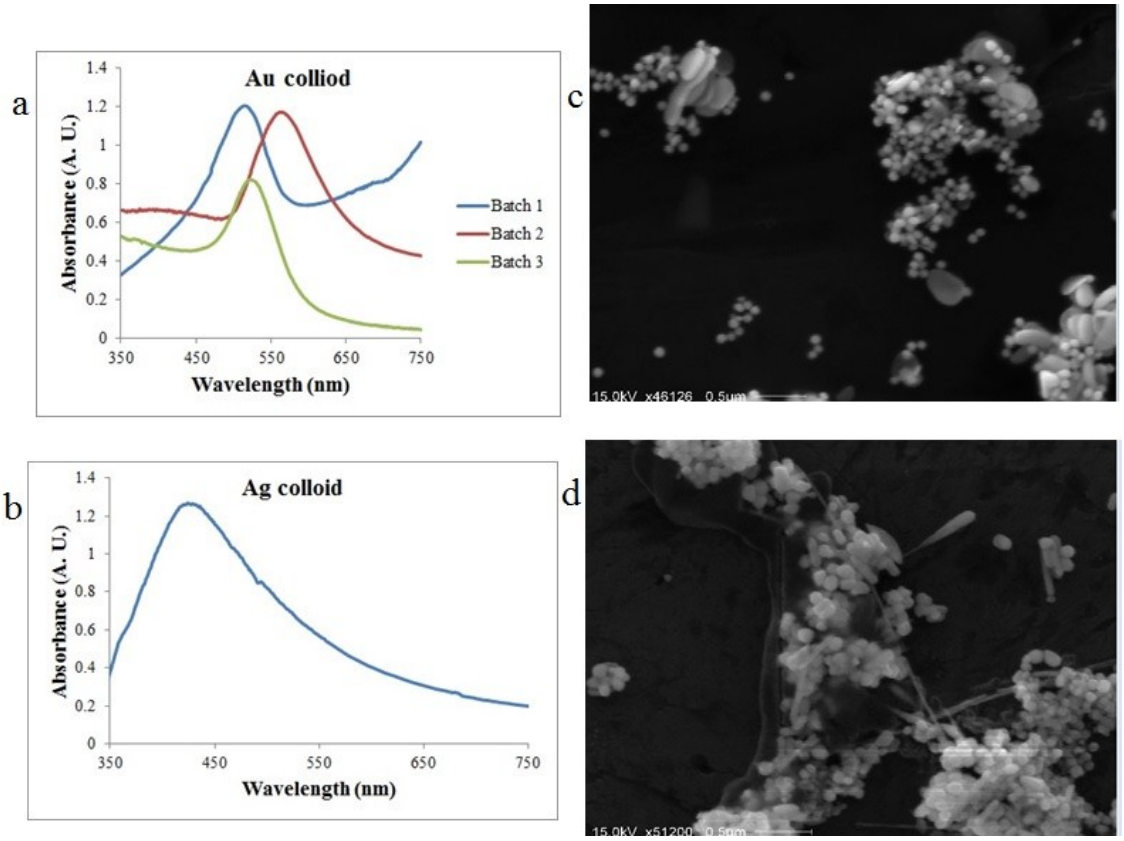

Figure S1. UV-vis absorbance of (a) gold batches 1-3 and (b) silver nanoparticles showing that the surface plasmon band λ_{max} were at 534, 563 and 527 nm for gold and 429 nm for silver respectively. The SEM images show the morphology of (c) gold colloid from the first batch (as an illustration) and (d) silver colloid, the magnification is inset in each image, showing an average size of 16±12 nm and 83±26 nm respectively.

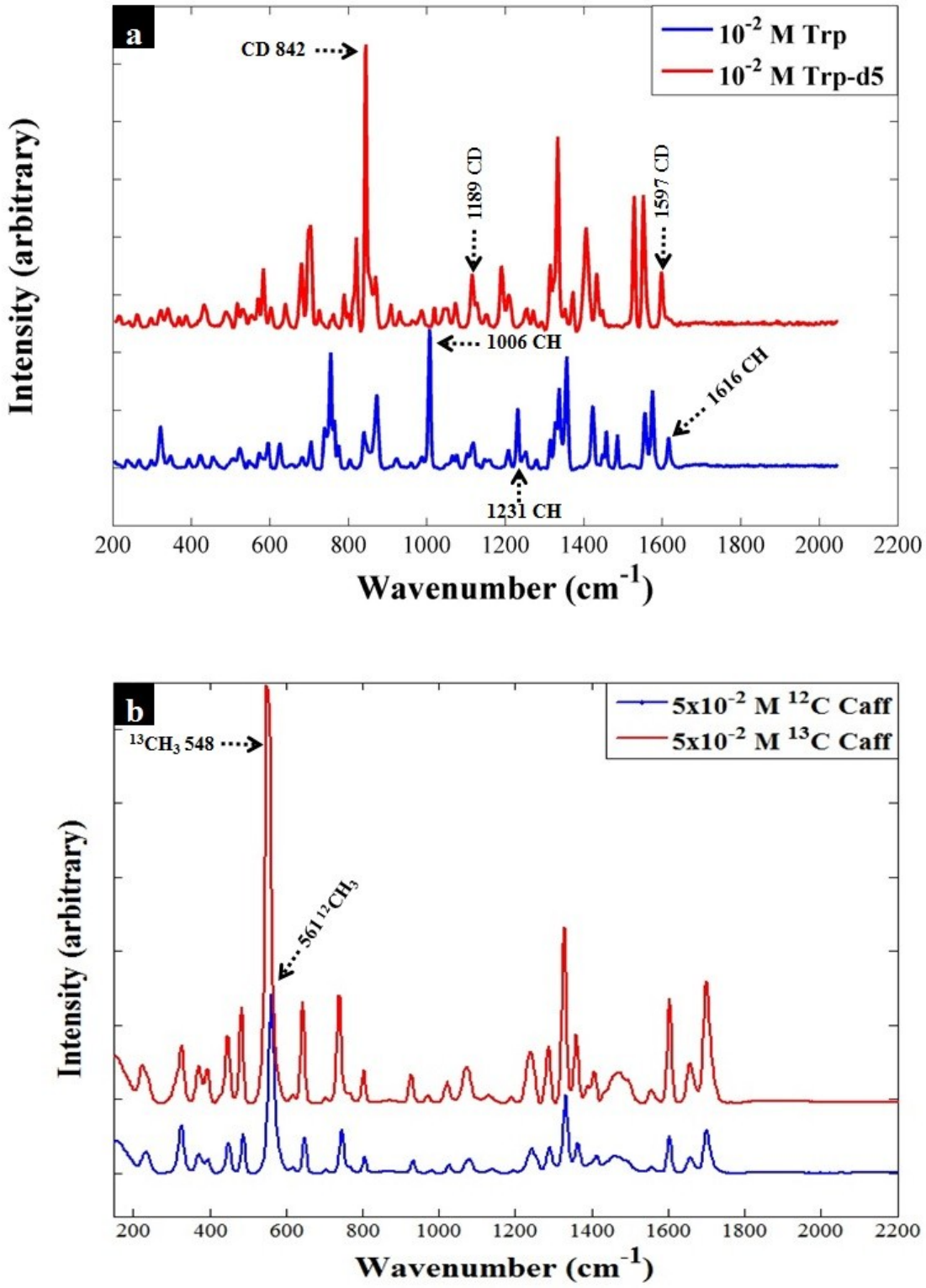

Figure S2. Raman spectra of (a) equimolar normal Trp (blue) and deuterated Trp (red). While (b) shows unlabelled caffeine (blue) and ^{13}C labelled caffeine (red) spectra. All Raman spectra were obtained on a Renishaw Raman microscope (633 nm, 10 s and single accumulation).

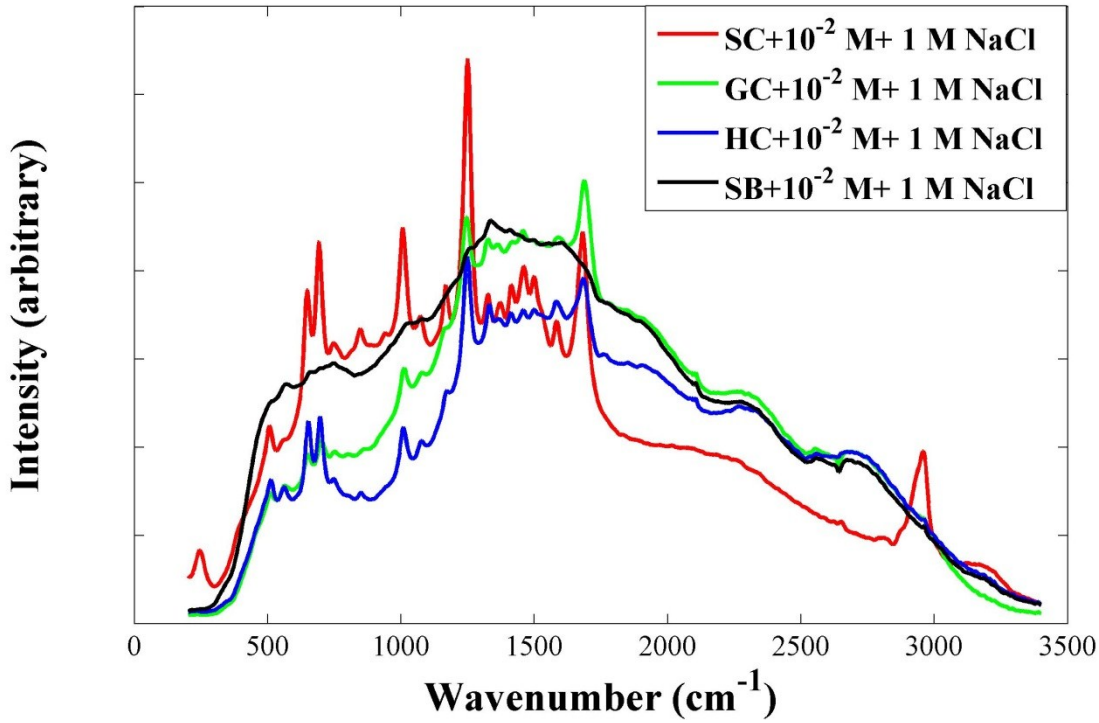

Figure S3. SERS spectra of 10^{-2} M caffeine with various colloids, including: silver citrate (red), gold citrate (green), hydroxylamine silver citrate (blue) and silver borohydride (black).

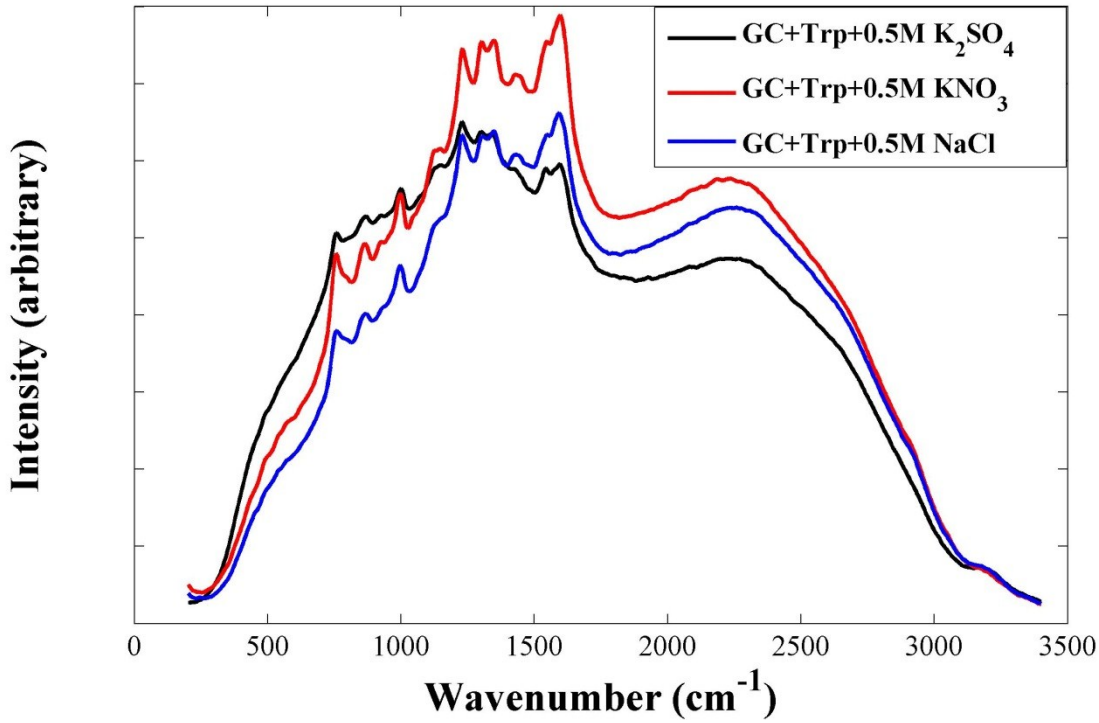

Figure S4. SERS spectra of 10^{-3} M Trp using different aggregation agents including: potassium nitrate (red), sodium chloride (blue) and potassium sulfate (black).

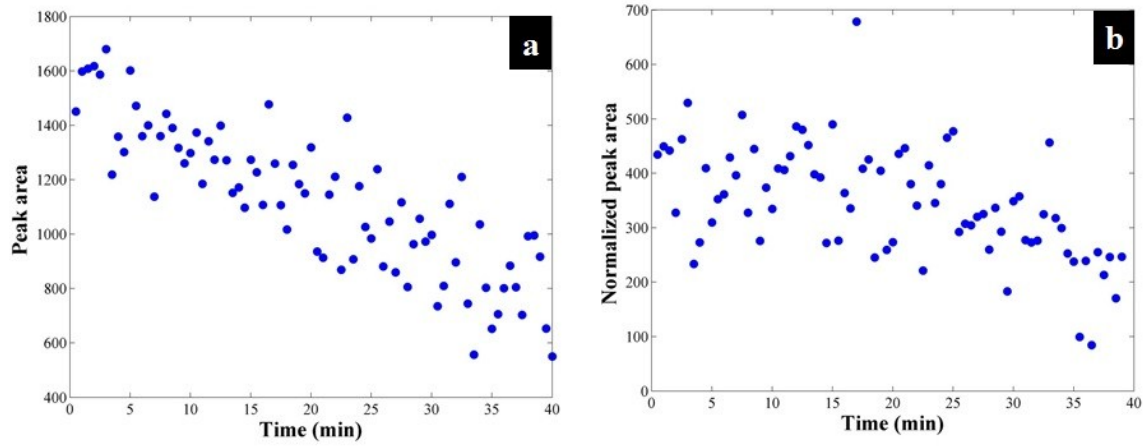

Figure S5. Time related plot showing the effect of 0.5 M KNO_3 aggregation agent of (a) unlabelled Trp peak at 1122 cm^{-1} (b) a mixture of unlabelled Trp at 1122 cm^{-1} and the IL internal standard at 1047 cm^{-1}.

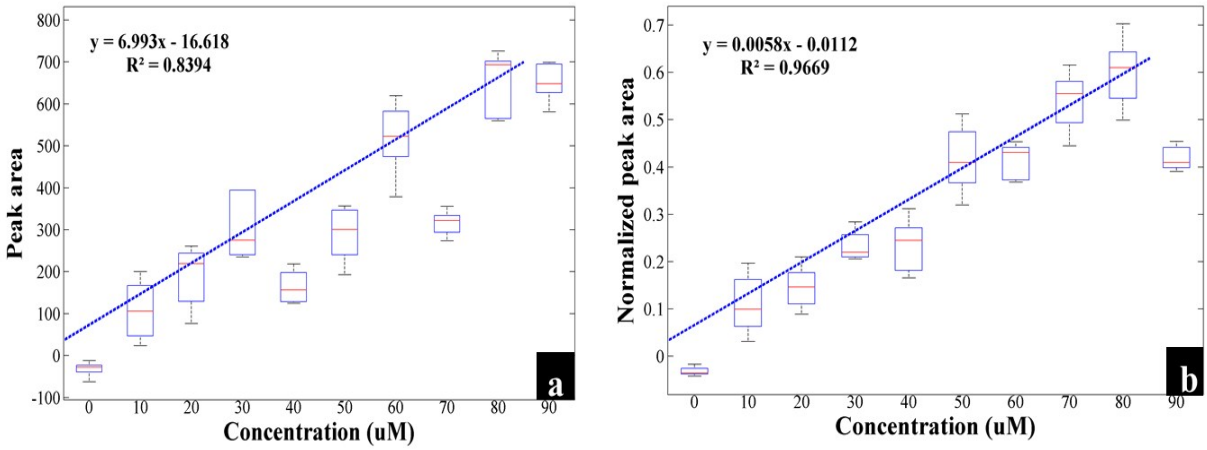

Figure S6. Plots of characteristic peak for caffeine: (a) represents peak area of caffeine at 1169 cm^{-1} *versus* caffeine concentration; (b) shows normalized peak again using the IL internal standard at 1131 cm^{-1} for correction. The data shown are box-whisker plots from five replicates where the red line represents the median value, the blue box the interquartile range, the error bars the 95% confidence region and the red crosses any outliers (no outliers were seen in these plots).

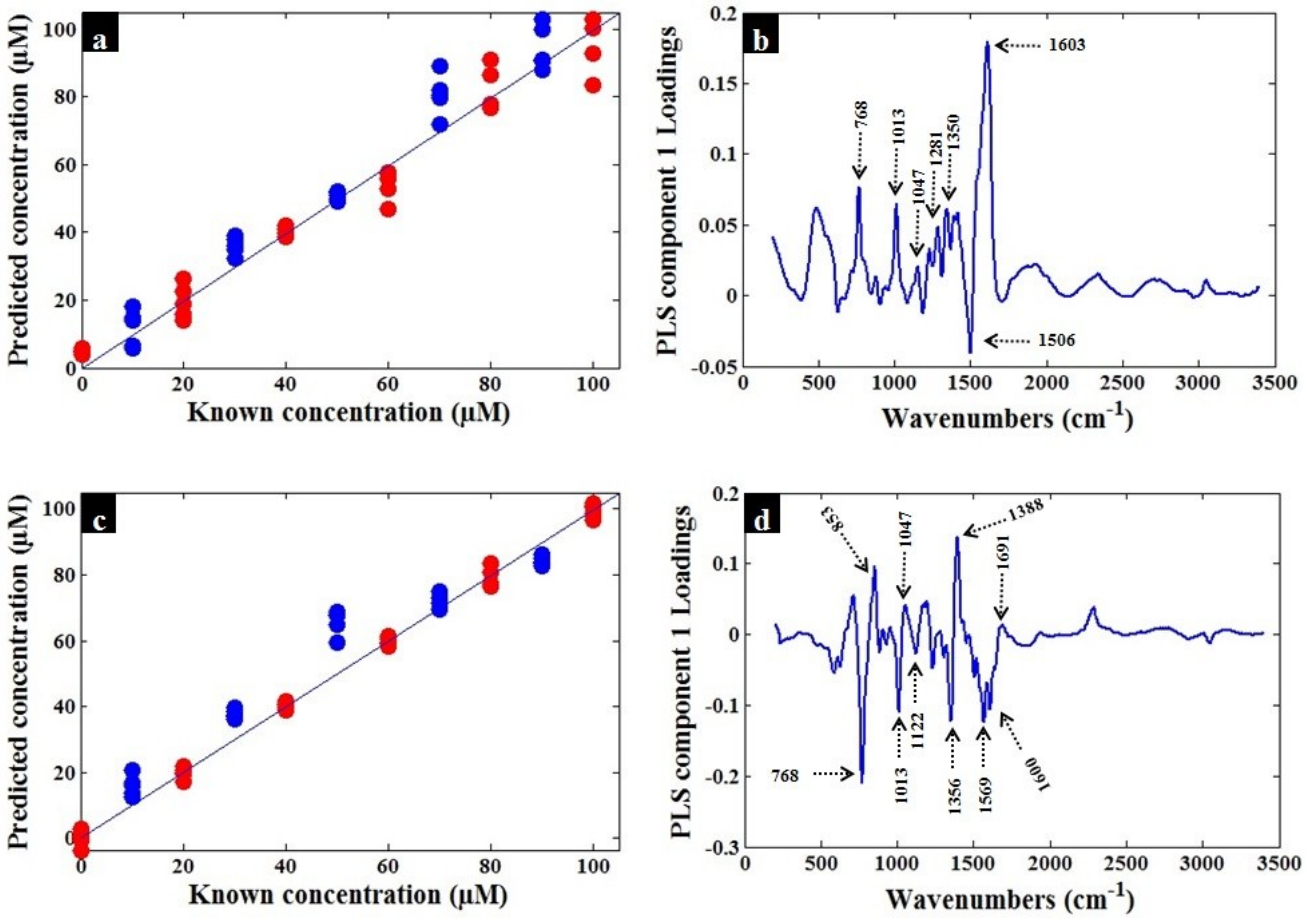

Figure S7. Representative PLSR plots from batch 2 of predicted concentration *versus* known concentrations of (a) Trp and (c) mixtures of Trp + Trp-d_5. Also shown are the corresponding PLSR loading plots for (b) Trp alone and (d) after including the Trp-d_5 isotopic labelled analyte.

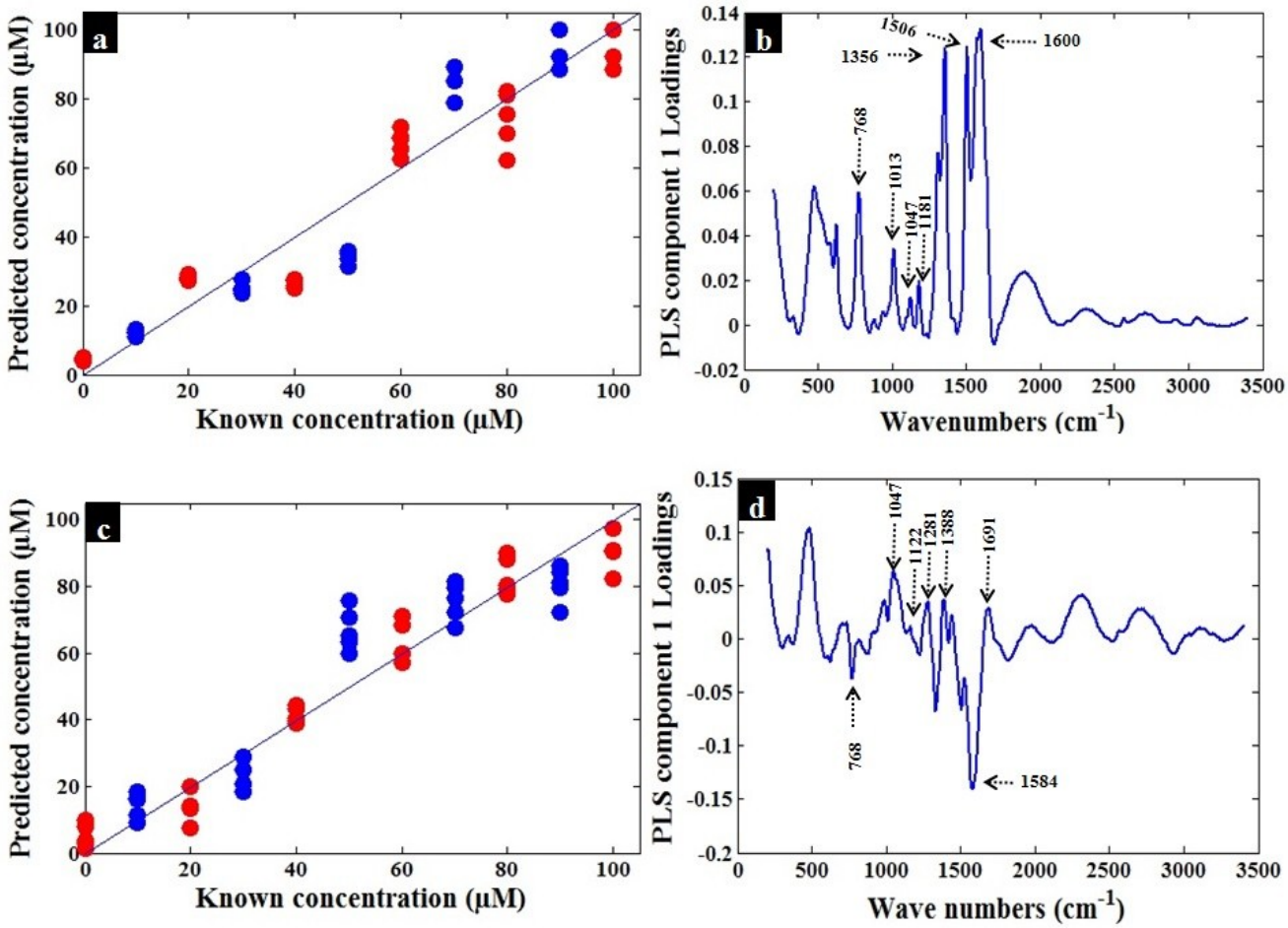

Figure S8. Representative PLSR plots from batch 3 of predicted concentration *versus* known concentrations of (a) Trp and (c) mixtures of Trp + Trp-d_5. Also shown are the corresponding PLSR loading plots for (b) Trp alone and (d) after including the Trp-d_5 isotopic labelled analyte.