Electronic Supplementary Material (ESI) for Analyst.

Supporting information:

Simultaneous quantification of multiple bacterial metabolites using surface-enhanced Raman scattering

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When applying the PLS model to spiked DCM samples, the analysis of pure components showed that the analyte spectra (purple and blue graphs in Fig. S1) closely resembled SERS fingerprint of pHCA and CA, detected through SERS in EtOH in Fig. 2a. Therefore, the model was able to isolate the contribution of pHCA and CA from the background (black) and enable quantification.

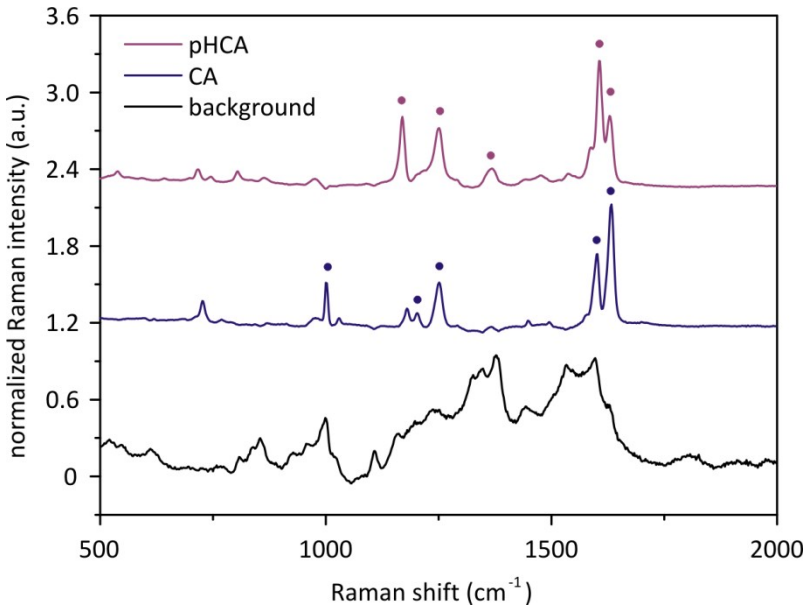

Fig. S1 Graphs of pure components detected in spiked DCM calibration model, with highlighted pHCA and CA characteristic spectral features, normalized between 0 and 1. The spectra were shifted with an arbitrary offset for ease of representation.

The PLS method, applied to control supernatant samples spiked with pHCA and CA, demonstrated the influence of background signal (black graph in Fig. S2). For instance, we observed that in certain regions the characteristic spectral features of CA (e.g. at 1000 cm^{-1}) were covered by the signal recorded from the control. However, significant spectral features were successfully identified, such as the pHCA peak at 1169 cm^{-1}, and the CA peak proportion in the 1580 – 1660 cm^{-1} spectral region, enabling quantification of the target analytes.

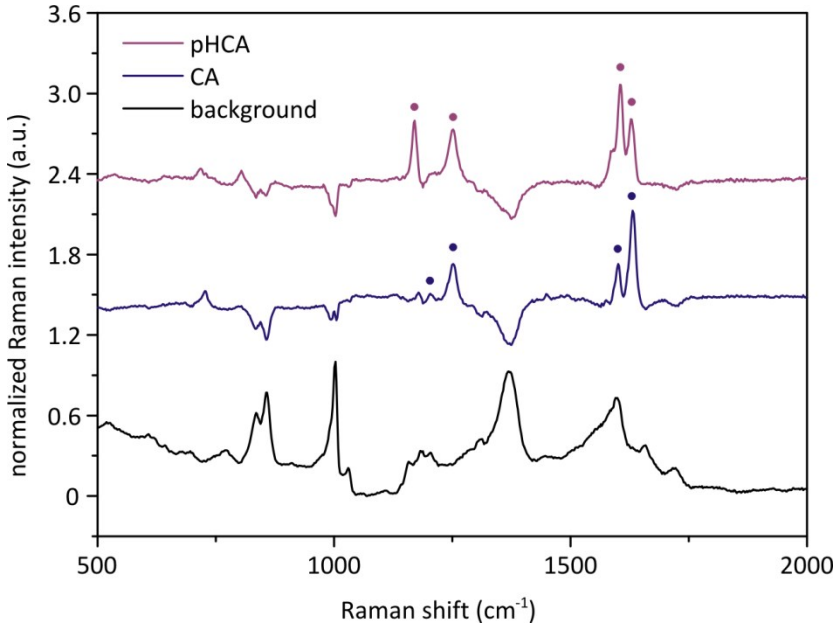

Fig. S2 Graphs of pure components detected in spiked control supernatant (CBJ786) calibration model, with highlighted pHCA and CA characteristic spectral features normalized between 0 and 1. The spectra were shifted with an arbitrary offset for ease of representation.

Fig. S3 shows the mean spectra and standard variations of each bacterial strain in the spectral region used for the PLS model. The spectra share the same peak positions and band intensity variations, therefore all the SERS measurements were considered suitable for pHCA quantification.

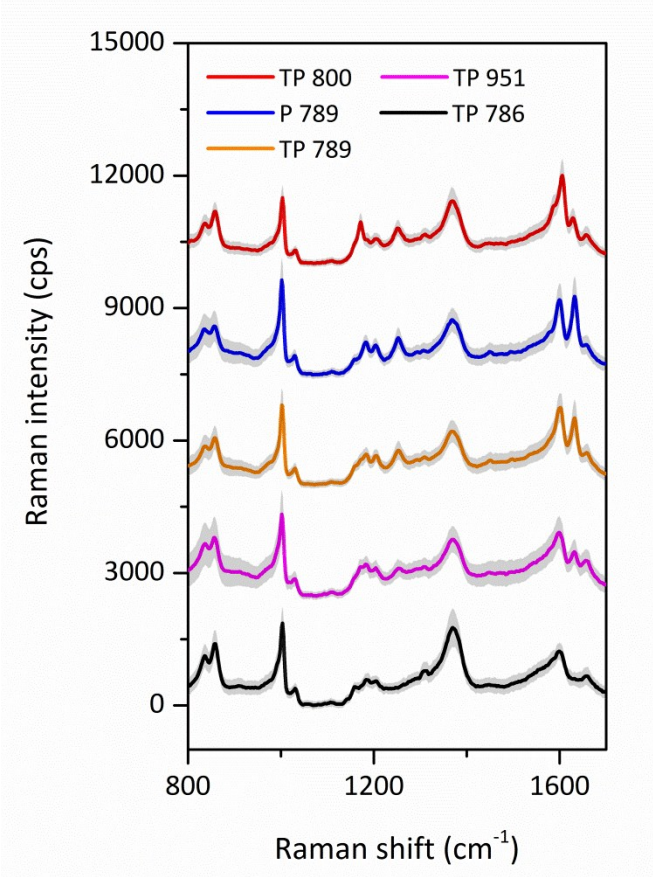

Fig. S3 Mean SERS spectra of *E. coli* supernatant samples after data pre-processing. The grey shade represents the standard deviation of the distribution of all the individual spectra (n = 288).