Electronic Supplementary Material (ESI) for Analyst.
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Electronic Supporting Information: Quantitative Online Sheath-flow Surface Enhanced Raman Spectroscopy Detection for Liquid Chromatography

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Summary: Figures S-1 and S-2 show the UV-Vis and SERS calibration curves for thiamine. Figures S-3 and S-4 are the UV-Vis and SERS calibration results for folic acid. Figure S-5 is the control experiment examining the signal from acetonitrile on non-structured silver film.

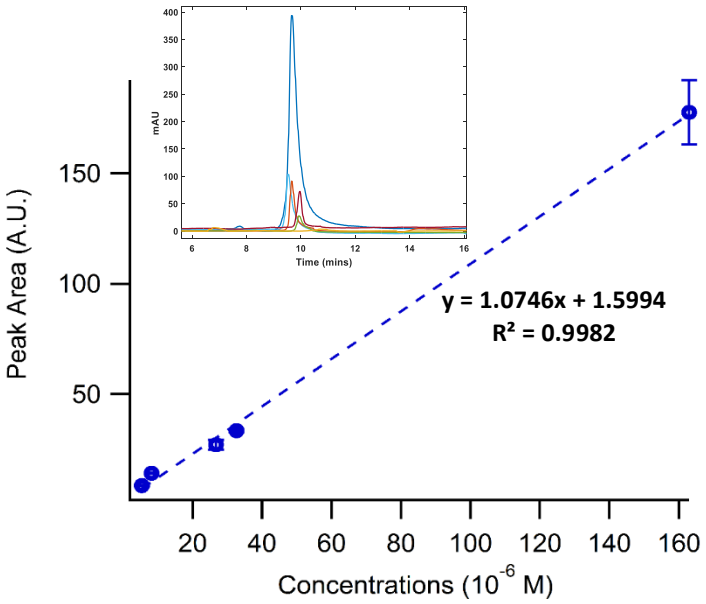

Figure S-1. UV-Vis detection calibration curve obtained by plotting peak area (n=3) as a function of thiamine concentrations in the range from 5.34×10^{-6} M to 163×10^{-6} M. The best fit line was obtained. The average retention time for thiamine is 9.71 ± 0.19 min, determined from LC chromatograms at different concentrations (inset plot).

The limit of detection for thiamine using UV-Vis was determined to be 3.26 µM (3sy/m). The thiamine signal detected from the previously analyzed mixture (Figure 2) was correlated back to the calibration plot. Based on the LC chromatogram, the peak area of thiamine is determined to be 22.101 ± 0.890 mAU which corresponded to 19.08 ± 0.83 µM in concentration with an RMS error of prediction of 0.73 µM

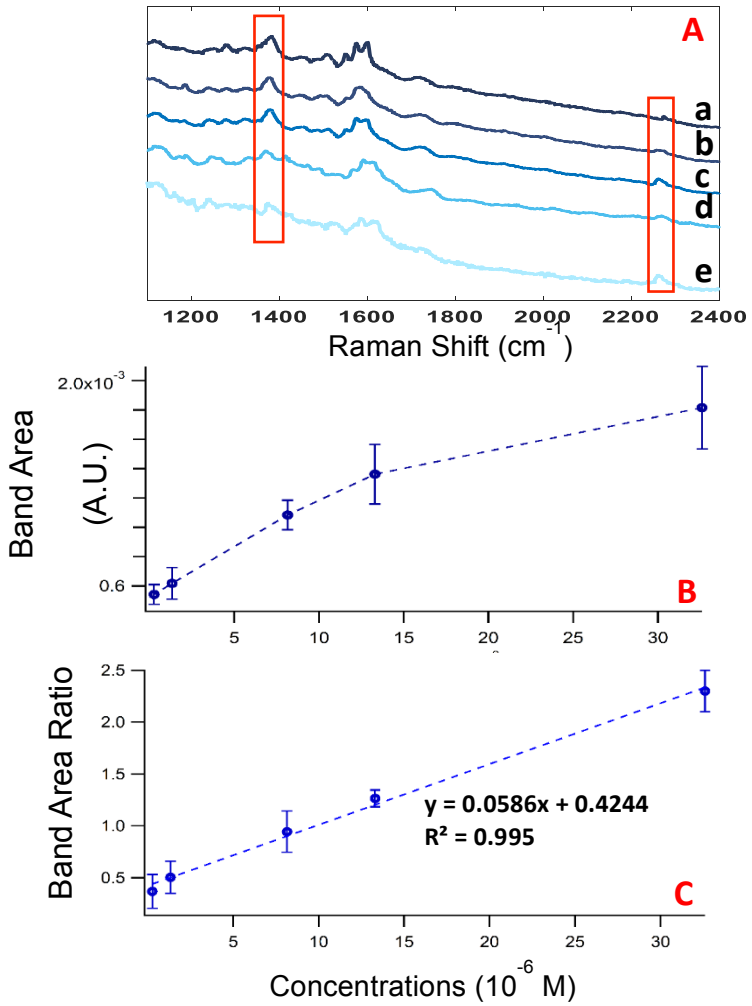

Figure S-2. (A) Average LC-SERS spectra (n=3) of thiamine at different concentrations: (a) 32.6 µM, (b) 13.3 µM, (c) 8.15 µM, (d) 1.33 µM,(e) 0.27 µM . The spectra are offset for clarity. Each SERS spectrum was normalized against its total signal (row-normalization). The band area at 1360 cm^{-1} was plotted as a function of concentrations as shown in (B). Calibration against an internal standard (C) was achieved by plotting band ratio of 1360 cm^{-1} and 2260 cm^{-1} (acetonitrile Raman band) with respect to concentrations. A best fit line was obtained showing a linear thiamine concentrations dependence in SERS band area ratio.

As expected, the plot of band area as a function of concentrations did not show a linear dependence but show a typical Langmuir isotherm at high concentrations. The internal standard plot provides a linear relationship between band area ratios and concentrations with the slope of 0.0586 and y-intercept of 0.4244. SERS limit of detection for thiamine is determined to be 1.02 µM. The concentration of thiamine in the previous analyzed mixture of Figure 2 was calculated, using the internal standard plot, to be 18.3 ± 1.12 µM with the RMS error of prediction of 1.09 µM.

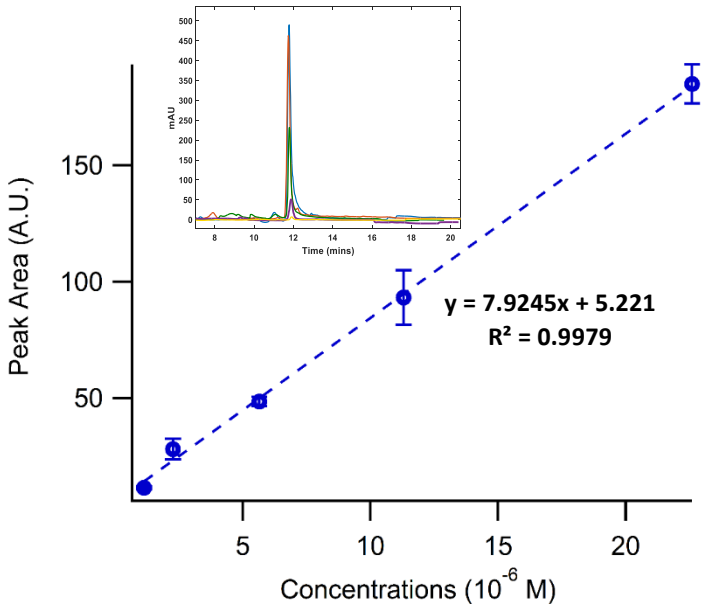

Figure S-3. UV-Vis detection calibration curve obtained by plotting peak area (n=3) as a function of folic acid concentrations in the range from 1.13×10^{-6} M to 22.6×10^{-6} M. The best fit line was obtained. The inset shows overlay LC chromatograms of folic acid solution at different concentrations. The average retention time for riboflavin is 11.82 ± 0.07 min.

The concentration of folic acid in the mixture (Figure 2) was calculated using the UV-Vis calibration curve to be 8.78 ± 0.33 µM with the RMS error of prediction of 0.27 µM, which corresponded to the peak area of 73.30 ± 2.49 mAU. The limit of detection for UV-Vis in the case of folic acid is determined to be 1.33 µM (3sy/m).

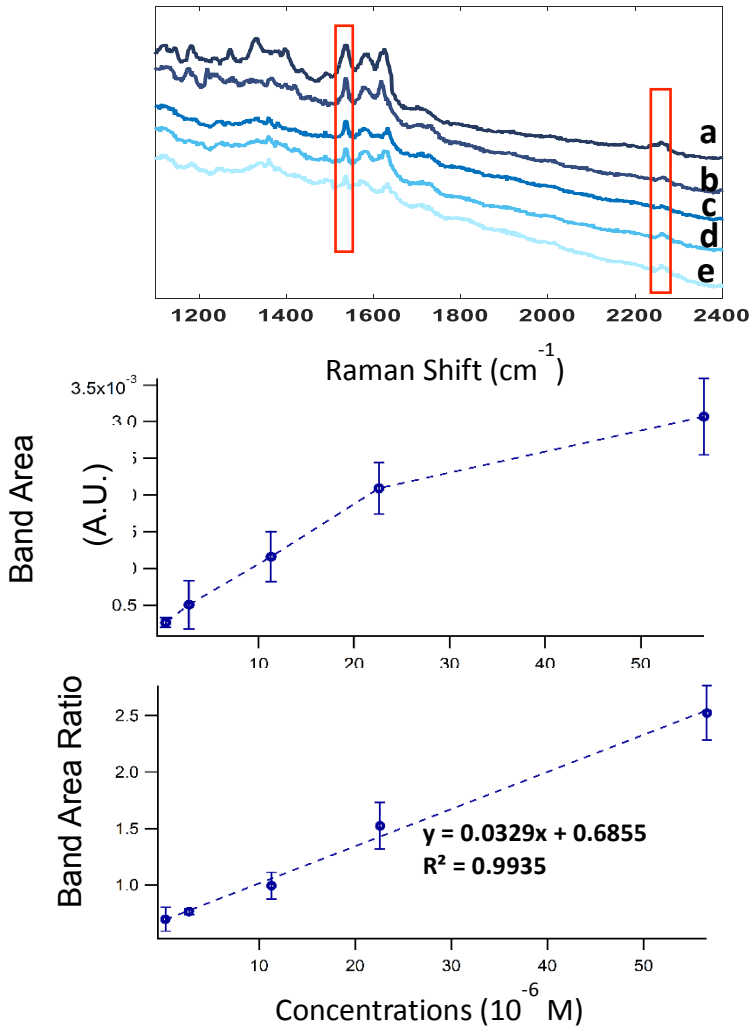

Figure S-4. (A) Average LC-SERS spectra (n=3) of folic acid at different concentrations: (a) 56.6 µM, (b) 22.6 µM, (c) 11.3 µM, (d) 2.72 µM,(e) 0.27 µM . The spectra are offset for clarity. Each SERS spectrum was normalized against its total signal (row-normalization). The band area at 1535 cm^{-1} was plotted as a function of concentrations as shown in (B). Calibration against an internal standard (C) was achieved by plotting band ratio of 1535 cm^{-1} and 2260 cm^{-1} (acetonitrile Raman band) with respect to concentrations. The limit of detection for internal standard plot is 0.94 µM.

Analyzing the SERS spectrum of folic acid (Figure 2-C) provides a band area ratio of 0.99 ± 0.05 which corresponded to the concentration of 8.97 ± 1.15 µM with the RMS error of 0.96 µM.

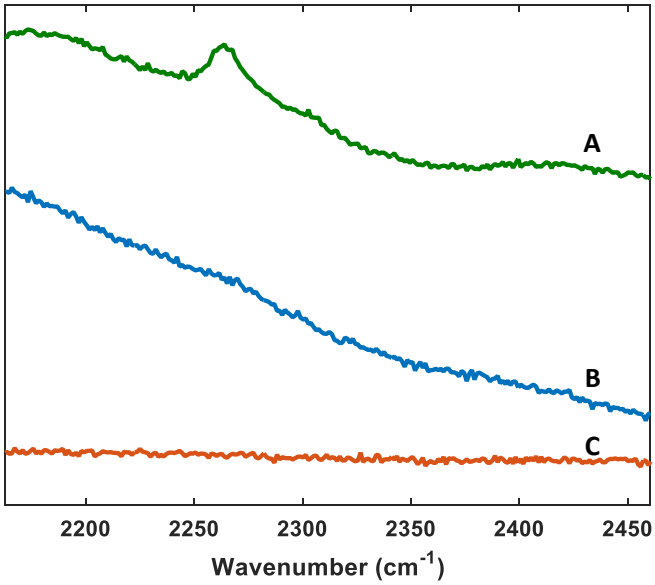

Figure S-5. SERS spectra of (A) blank LC-SERS run with mobile phase only using SERS-active silver substrate, (B) blank LC-SERS run with mobile phase only using silver mirror film and (C) the spectrum observed from a silver mirror film. The nitrile SERS signal (2260 cm^{-1}) only appeared when using the SERS-active substrate, which indicates it is a SERS signal from adsorbed acetonitrile.