Supporting Information

Continuous and Automated Slug Flow Nanoextraction for Rapid Partition Coefficient Measurement

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Table of Contents:

Supplementary Methods.

HPLC-MS of Eserine Samples.

To determine if eserine was degrading due to ambient conditions, HPLC-MS was performed to analyze changes in analyte and degradation product signals. An eserine (Sigma Aldrich) stock was dissolved to 50 uM in 10 mM Tris buffer at pH 7.4. Samples were analyzed before and after a two-hour shake time at 22 ºC under ambient light conditions. The samples were analyzed with an Agilent 1290 Infinity II outfitted with a Phenomenex Kinetex C18 column (2.1 x 100 mm, 1.7 μm) and an Agilent 6410 mass spectrometer. The mobile phase was ramped from 5% to 50% acetonitrile in water with 0.1% formic acid over 7 minutes. The column was re-equilibrated at 5% acetonitrile for 1.5 minutes. Full mass spectra were collected, and the extracted ion chromatograms were integrated.

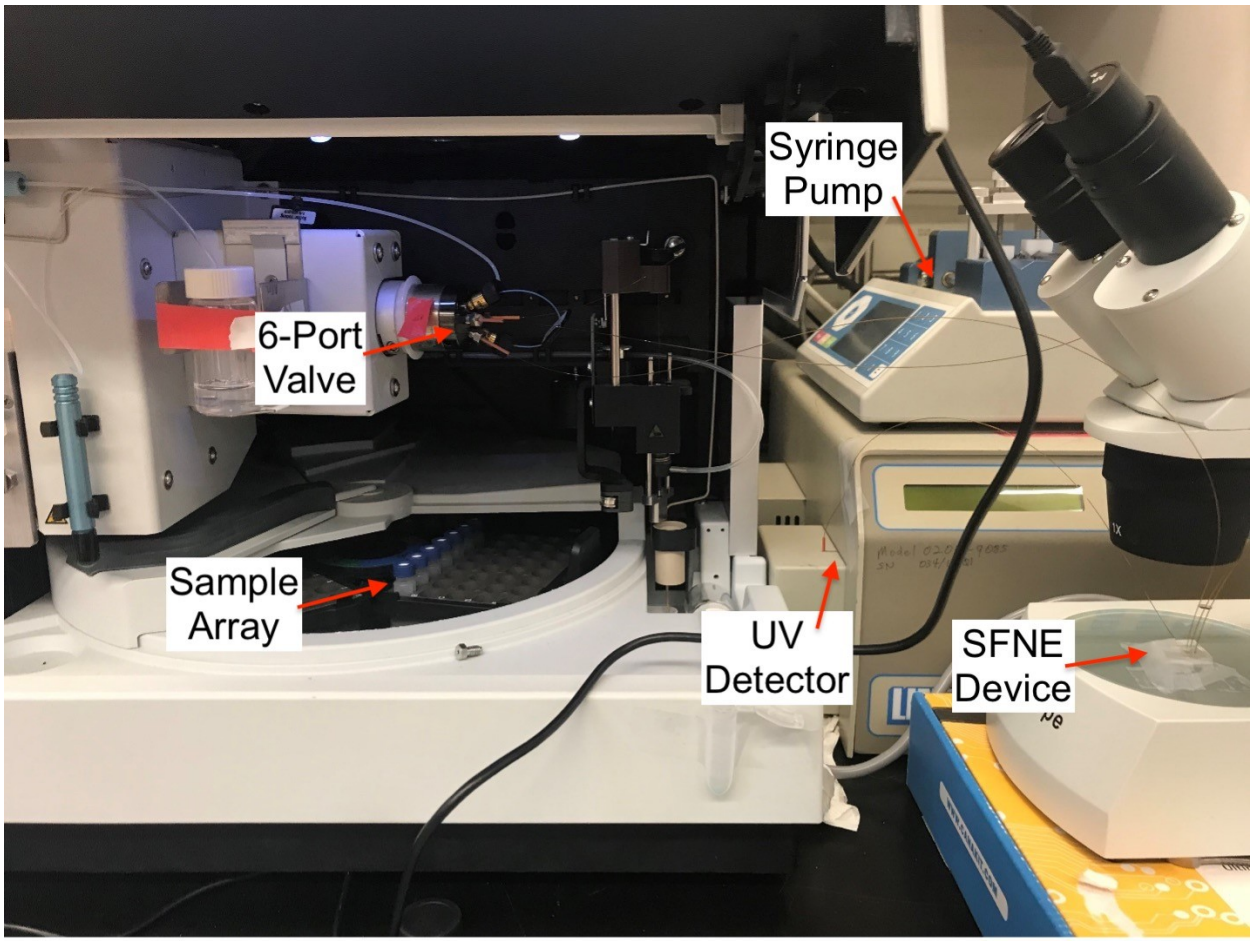


Figure S1. Complete online, automated system for SFNE. The autosampler can be loaded with a vial holder or a microwell plate from which calibration and extraction standards can be injected (through the 6-port valve). This allows for the device to be connected to the 6-port valve with continually flowing aqueous phase and various samples/standards to be introduced into the continuous aqueous phase. The device is then connected to the UV detector, where calibration standards and unknown extraction equilibrium concentrations can be quantified.

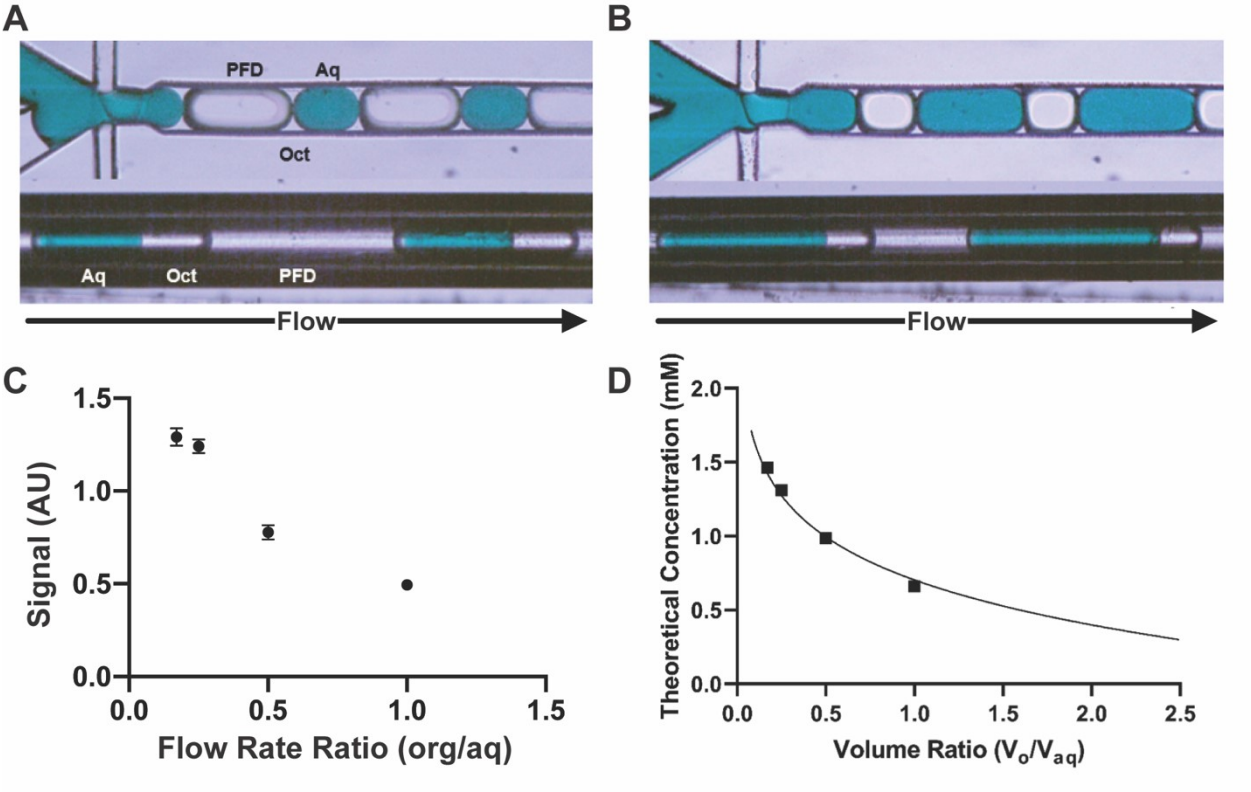
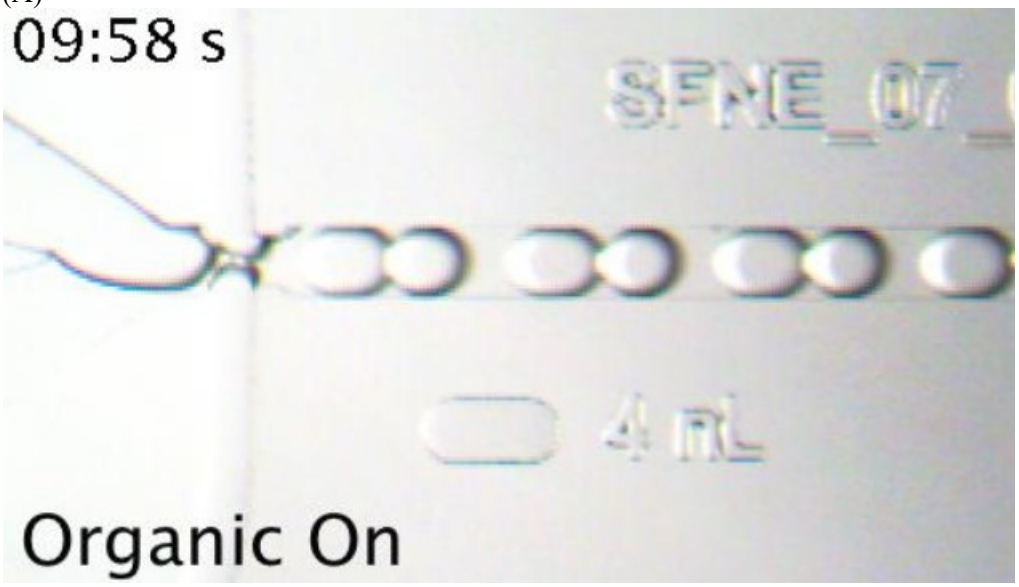


Figure S2. Volume manipulation via flow rate changes. Relative flow rates into the generation device control the organic to aqueous volume ratio ($V_R = V_{org}/V_{aq}$ where V_{org} is volume of organic phase and V_{aq} is volume of aqueous phase). [A] When using flow rates of 1.5, 1.0, and 0.8 µL/min for PFD, octanol, and aqueous, respectively, a V_R of ~0.75 was observed. [B] By adjusting the PFD, octanol, and aqueous flow rates to 0.6, 0.5, and 1.0 µL/min, respectively, the V_R was reduced by a factor of 2.2 to ~0.34 [C] Using 1 mM ACP in water, flow rate ratio was varied from 1.0 to 0.17 and signal intensity in octanol are reported. Signal increases as the flow ratio decreases, showing the effective preconcentration. At least 400 replicates (equilibrium octanol plugs) were measured for each flow ratio. [D] Theoretical concentrations for acetaminophen (initial concentration in aqueous of 1 mM) as volume ratio is adjusted to achieve preconcentration. ACP equilibrium concentration in octanol should increase by 220% as V_R is decreased from 1.0 to 0.17. Theoretical values were obtained using the equation

$$C_{org,eq} = KC_{aq,eq} = \frac{KC_{aq,initial}}{1 + KV_o/V_{aq}}$$

where the octanol-water partition coefficient, K, is equal to 1.95 and the initial concentration in water ($C_{aq,initial}$) is equal to 1 mM. The theoretical curve is represented by the solid line. Experimental values are shown by squares; the signal intensity increased by 260% ± 20% as F_R was decreased from 1.0 to 0.17. Though the signal intensity change does not perfectly reflect concentration change, this comparison demonstrates F_R modulation can achieve similar preconcentration effects as expected by V_R modulation.

(A)



(B)

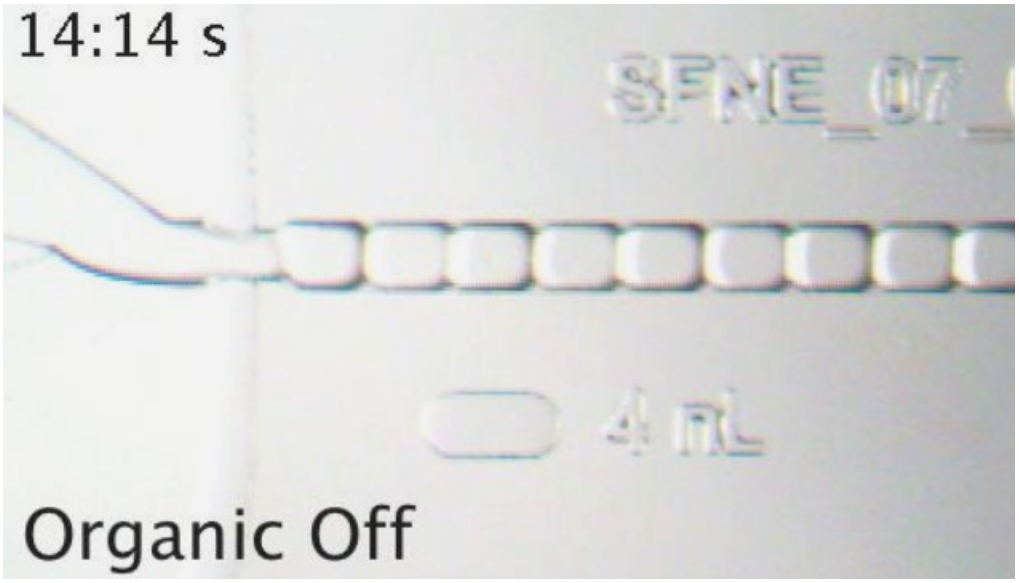


Figure S3. Images are a placeholder for their corresponding video. The video shows how rapidly and reliably the microfluidic device can toggle between generating three phase "phase pairs" and two phase droplets when the organic phase is toggled on or off. (A) Snapshot of the device just before the organic (octanol) flow is toggled off. Phase pairs are being reliable generated here. (B) Snapshot of the device ~four seconds after the organic has been toggled off, with reliable two-phase droplet generation.

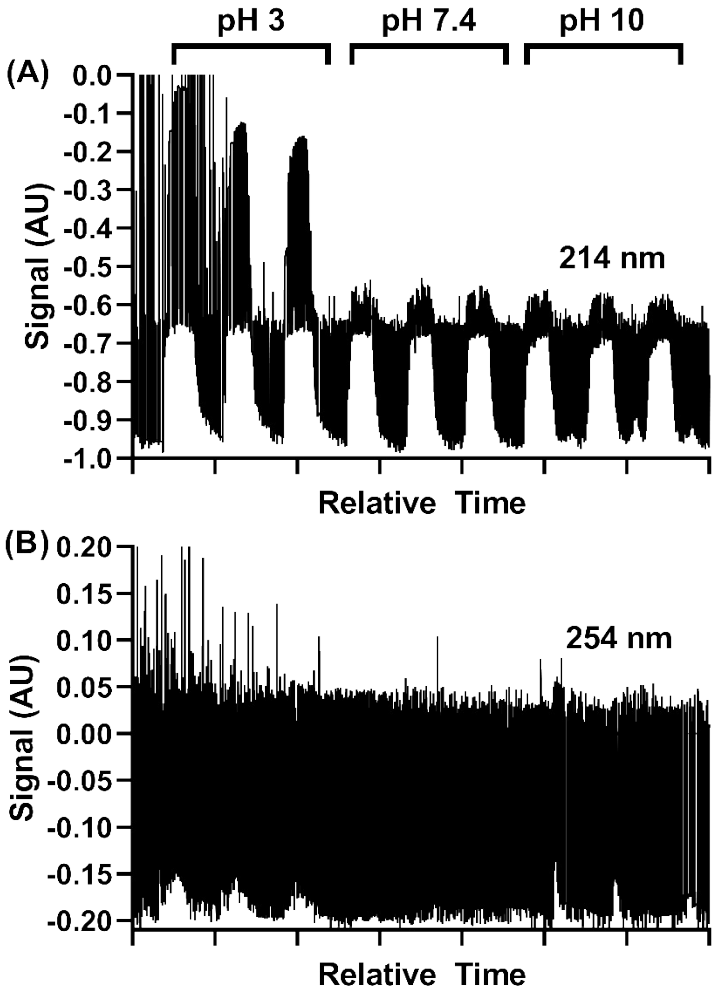


Figure S4. Triplicate injections of blank buffer used for extractions standards at each pH during log K_{ow} determination at (A) 214 nm and (B) 254 nm. The average of the signal intensity for each pH was subtracted from the corresponding extraction standards when measuring $C_{aq,eq}$.

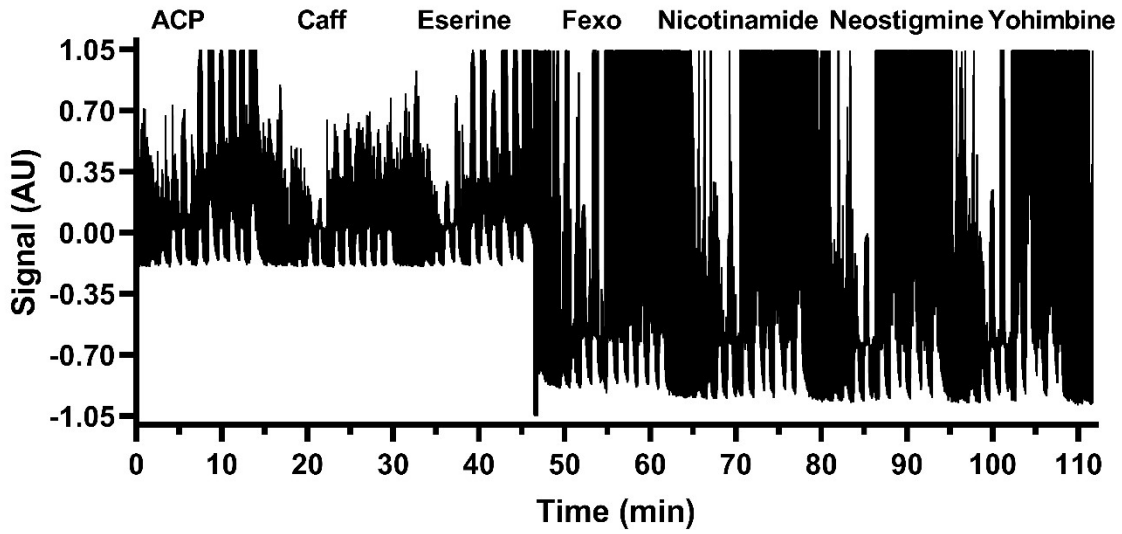


Figure S5. Raw trace of entire 7 compound screen where each compound has Log K_{ow} determined at 3 different biological pH's (3, 7.4, and 10) with a 5 point aqueous calibration curve before extraction for quantification. All 21 K_{ow}s were measured in under 2 h of analysis using automated sample introduction and pumps (including the toggle of octanol phase).

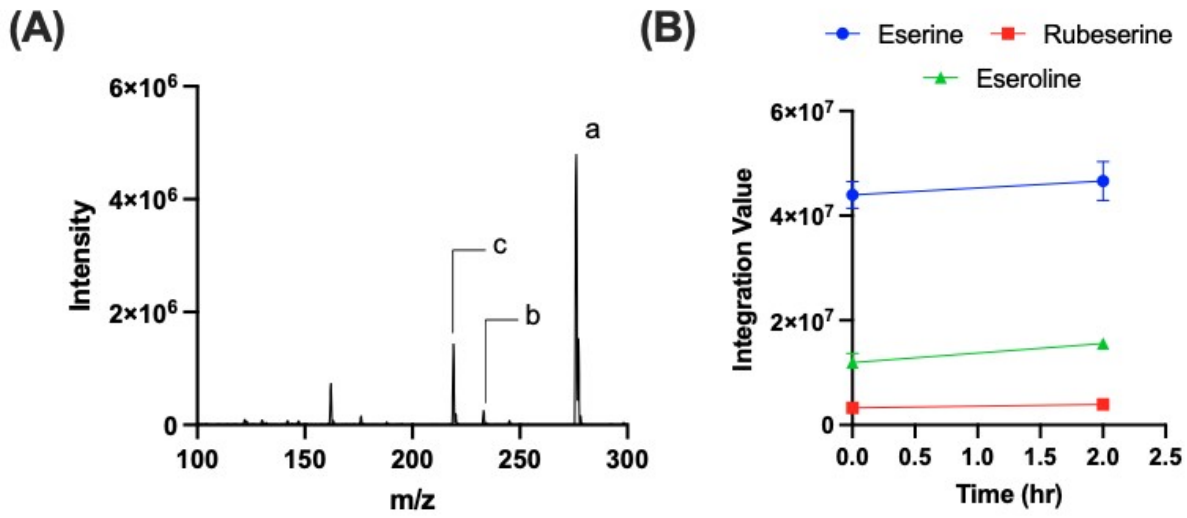


Figure S6. Investigation into possible degradation of eserine. (**A**) Mass spectrum of a 50 uM eserine solution. Three peaks labelled correspond to **a**: eserine (M+H, 276 m/z), **b**: rubeserine (M+H, 233 m/z), and **c**: eseroline (M+H, 219 m/z). The signal of eseroline (c) may be artificially high due to an in-source fragment of eserine (57 m/z corresponding to losses of both C_2H_3NO and C_3H_7N). **(B)** Signal integration values from LC-MS of the selected ions in (A) before and after two-hours shaking at ambient conditions. Error bars represent standard error with n = 3. No significant changes were measured, suggesting no substantial degradation of eserine over the two-hour shake times.

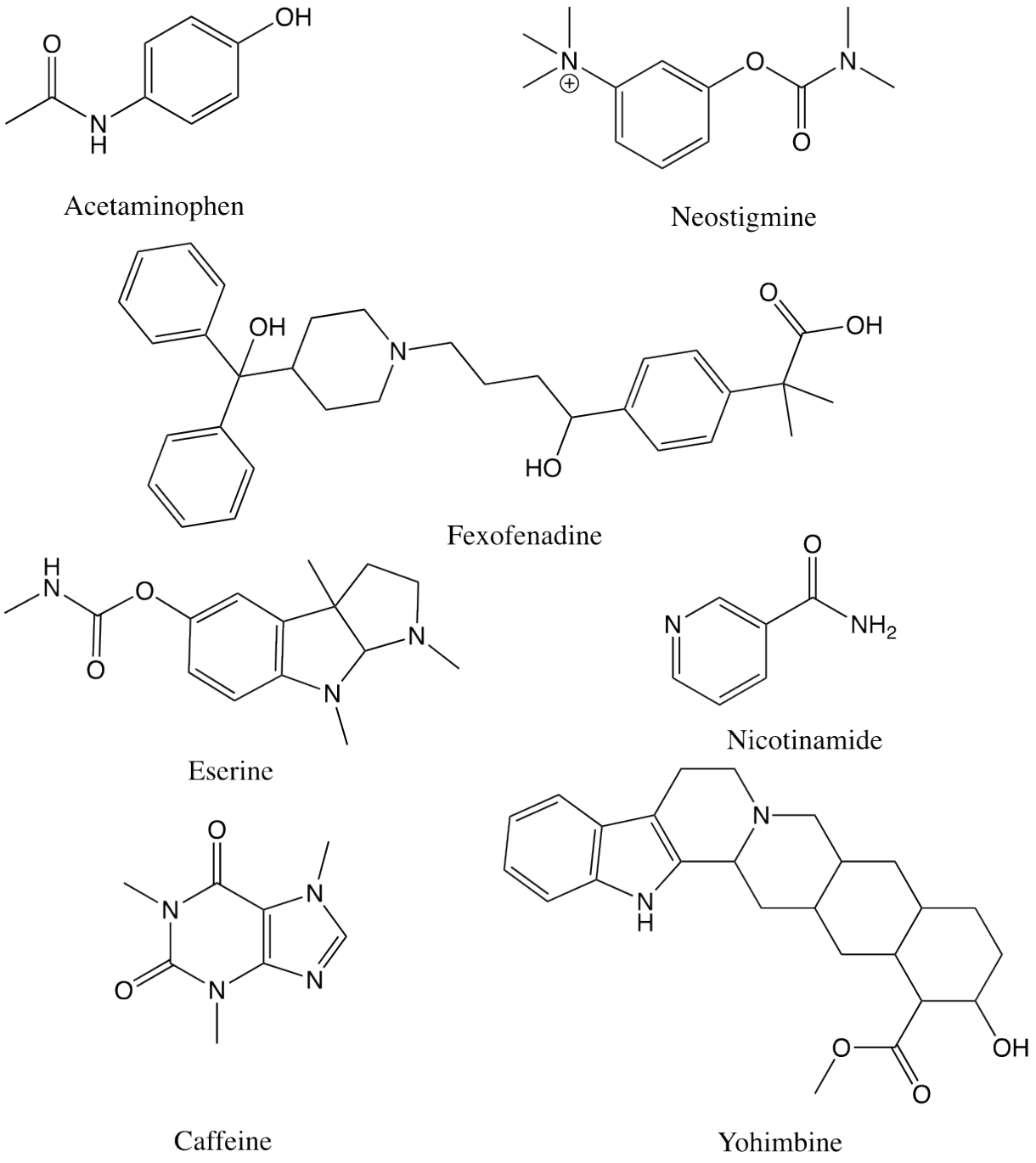


Figure S7. Structures of pharmaceutical compounds screened for log K_{ow}

Table S1. Mean absolute errors and SD of errors from references

Reference	Mean absolute error (log units)	SD of error (log units)
Wells, Payne, Kennedy	0.17	0.22
S. Han, J. et al., *Chemosphere*, 2011 [c]	0.11	0.08
I. V. Tetko and P. Bruneau, *J Pharm Sci*, 2004	0.50	N/A
L. Ayouni, G. et al., *Chroma*, 2005 [a]	0.10	0.19
J. T. Smith and D. V. Vinjamoori, *J Chromatogr B Biomed Appl*, 1995 [a]	0.26	0.32
H. Mo, K. M. Balko and D. A. Colby, *Bioorg Med Chem Lett*, 2010 [a]	0.12	0.21
A. Paschke, P. L. Neitzel, W. Walther and G. Schüürmann, *J Chem Eng*, 2004 [b]	0.42	0.52
	0.34	0.23

[a] Calculated from reference Table 1

[b] Calculated from reference Table 2

[c] Calculated from reference Table 3