Supporting Information for:

Separation and detection of trace fentanyl from complex mixtures using gradient elution moving boundary electrophoresis

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Experimental Methods

Resolution between two analytes. The resolution, *R*, between two analytes with differing peak/step widths for a GEMBE separation has been discussed previously, and was calculated by the following equation:

$$R = \frac{\Delta t}{2(\sigma_1 + \sigma_2)} \quad (1)$$

where Δt is the step spacing between the two analytes; σ_1 and σ_2 are the step widths or the standard deviations of the temporal derivative of the step shape for each analyte.[1]

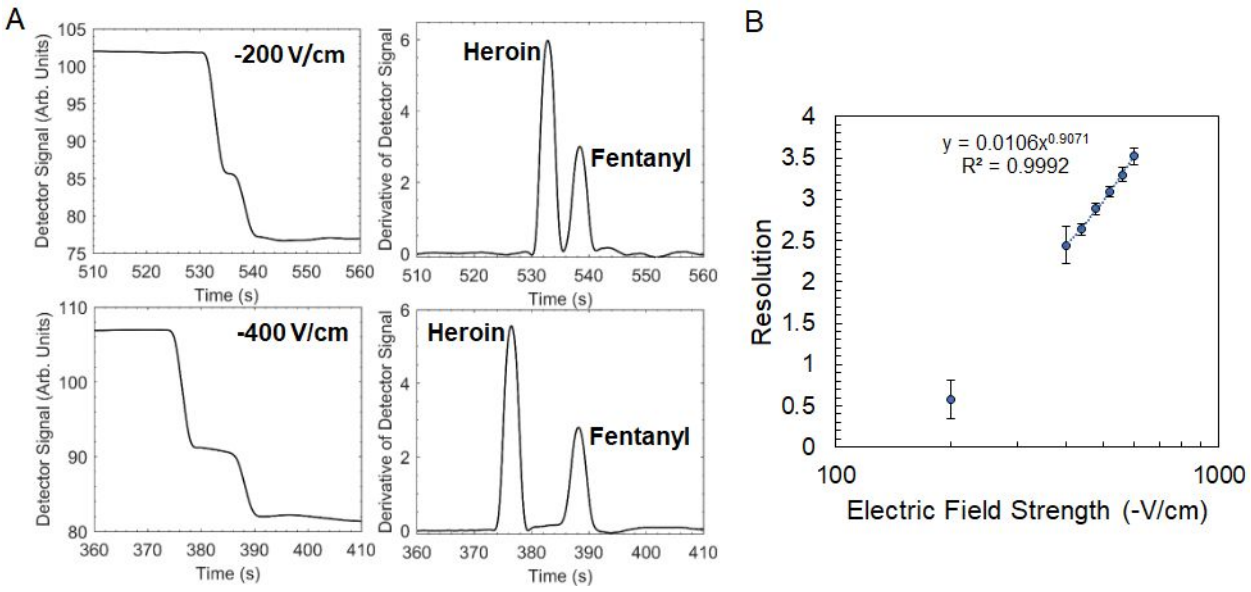

Figure S1. GEMBE separation of binary heroin and fentanyl mixture at varied electric field strengths. (A) Representative raw data and derivative plots showing stepwise decreases in conductivity from the elution of 50 µmol/L heroin and fentanyl at two different electric field strengths. (B) Plot showing increased resolution of heroin and fentanyl steps as the electric field strength increased. Separation conditions included pressure starting at 14.5 kPa and decreasing at -25 Pa/s. Average and standard deviations represented for 8 replicates.

[1] Ross, D. *Electrophoresis* **2010**, *31*, 3650-3657.

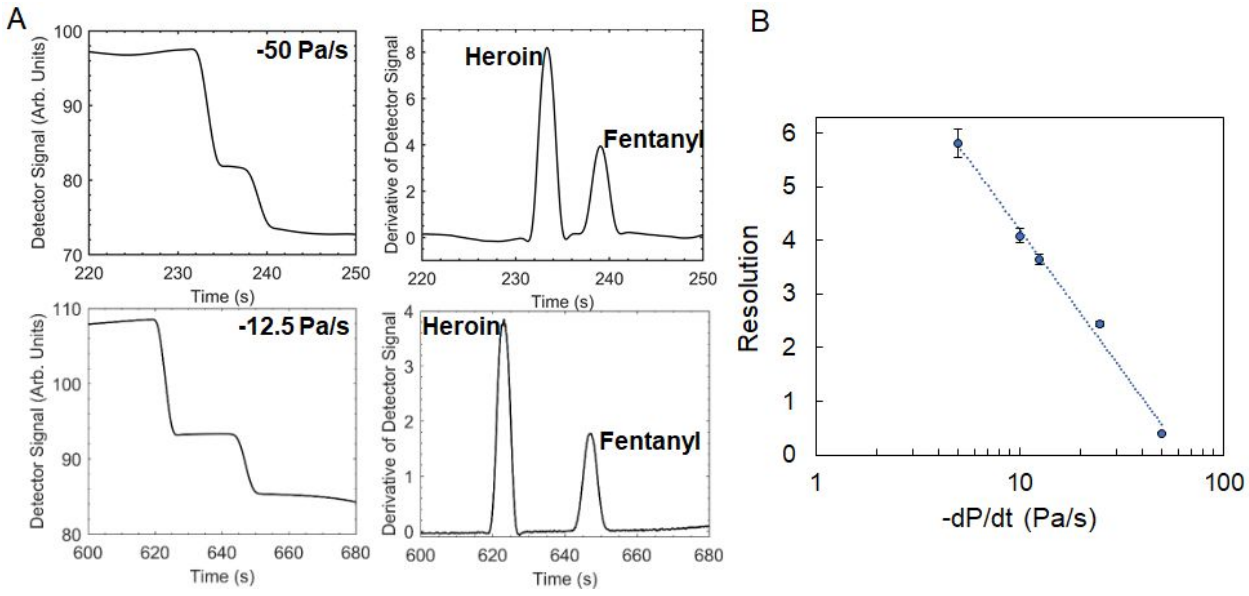

Figure S2. GEMBE separation of binary heroin and fentanyl mixture at varied pressure ramp rates (counterflow acceleration). (A) Representative raw data and derivative plots showing stepwise decreases in conductivity from the elution of 50 μmol/L heroin and fentanyl at two different pressure ramp rates. (B) Plot showing decreased resolution of heroin and fentanyl steps as the pressure ramp rate increased. Separation conditions included a -400 V/cm electric field and pressure starting at 14.5 kPa. Average and standard deviations represented for 9 replicates.

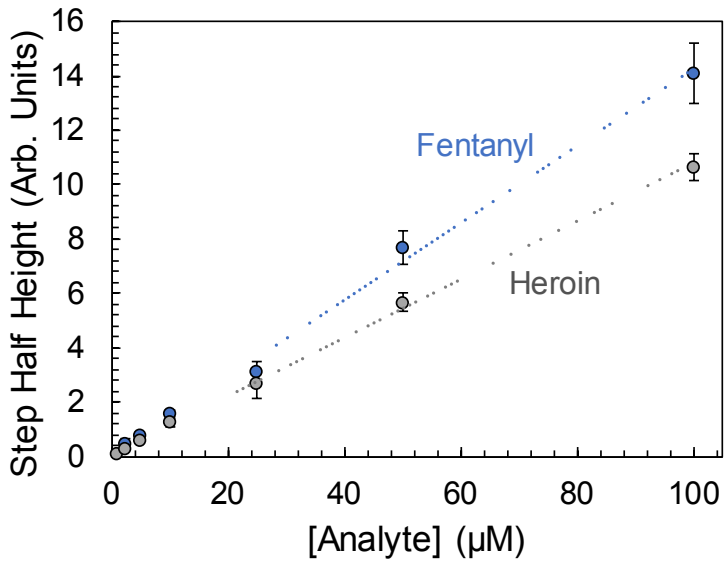

Figure S3. Fentanyl and heroin standard curve of the measured step half height signal at varied concentrations to empirically determine the limits of detection. Separation conditions included a -560 V/cm electric field and pressure starting at 14.5 kPa and decreasing at -25 Pa/s. Average and standard deviations represented for 7 to 10 replicates.

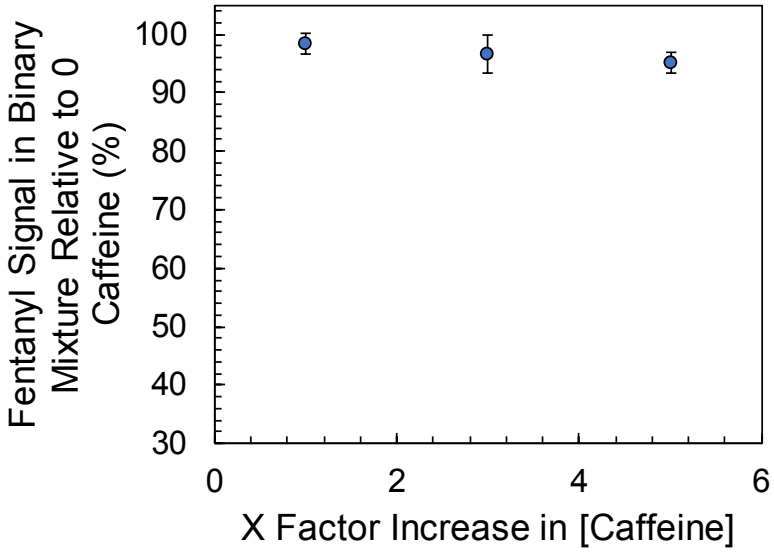

Figure S4. Fentanyl signal suppression as a function of binary mixture ratios for caffeine. Concentration ratio given as (caffeine : fentanyl) with fentanyl a constant 10 µmol/L. Separation conditions included a -560 V/cm electric field and pressure starting at 14.5 kPa and decreasing at -25 Pa/s. Average and standard deviations represented for 6 replicates.

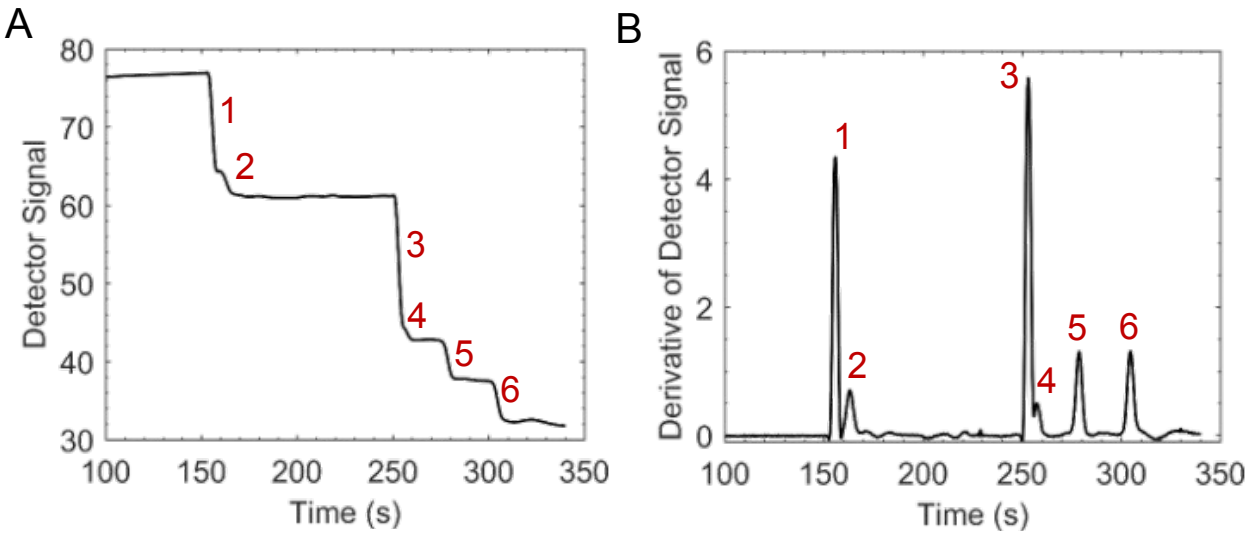

Figure S5. (A) Representative GEMBE separation of narcotics mixture containing (1) amphetamine, (2) methamphetamine, (3) cocaine, (4) PCP, (5) heroin, and (6) fentanyl at 50 µmol/L showing stepwise decreases in detector signal. (B) Peak representation obtained by differentiation of raw detector signal with respect to time. Separation conditions included a -480 V/cm electric field and pressure starting at 14.5 kPa and decreasing at -25 Pa/s.

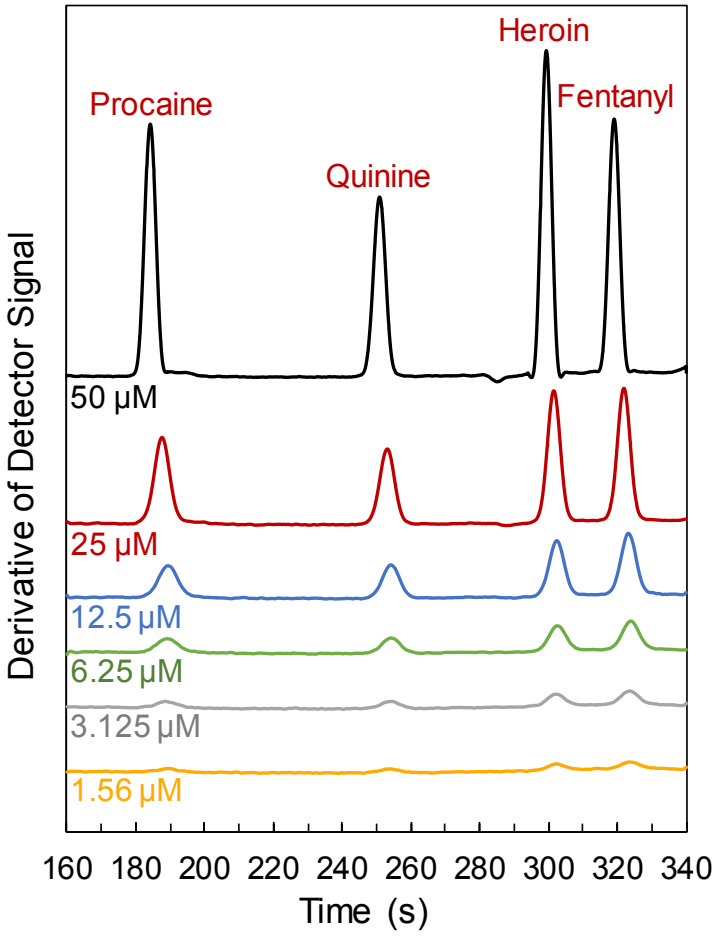

Figure S6. GEMBE separation of demonstrative narcotic mixture containing excipients: procaine and quinine, and opioids: heroin and fentanyl at concentrations of (1.56, 3.125, 6.25, 12.5, 25, and 50) µmol/L. Separation conditions included a -480 V/cm electric field and pressure starting at 14.5 kPa and decreasing at -25 Pa/s. Data shifted vertically for visualization.

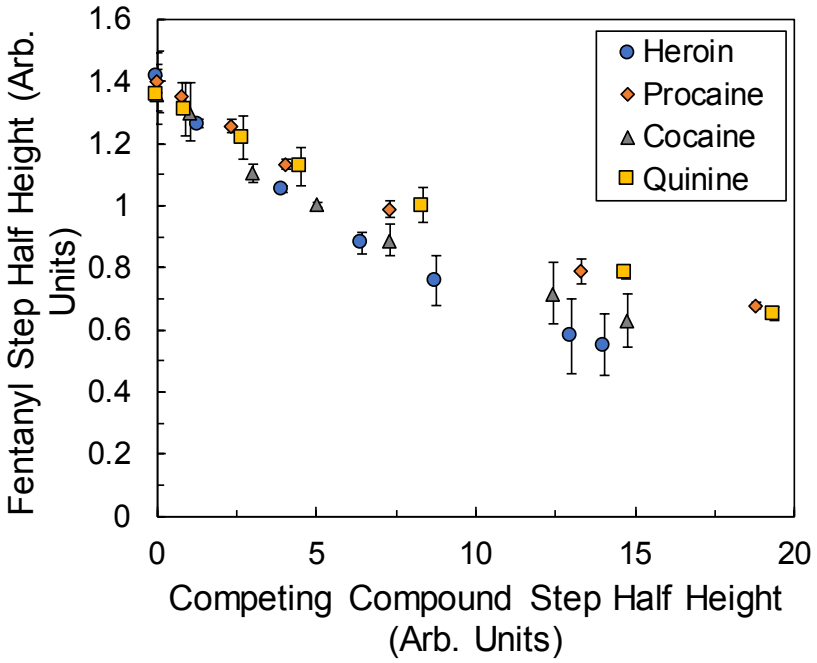

Figure S7. Fentanyl signal suppression as a function of competing compound signal for the absolute value of each step half height at binary mixture ratios of 0:1, 1:1, 3:1, 5:1, 10:1, 20:1, and 30:1. Various competing compounds were evaluated, including heroin, cocaine, quinine, and procaine, with fentanyl at constant 10 μmol/L. Average and standard deviations represented for 7 to 10 replicates.

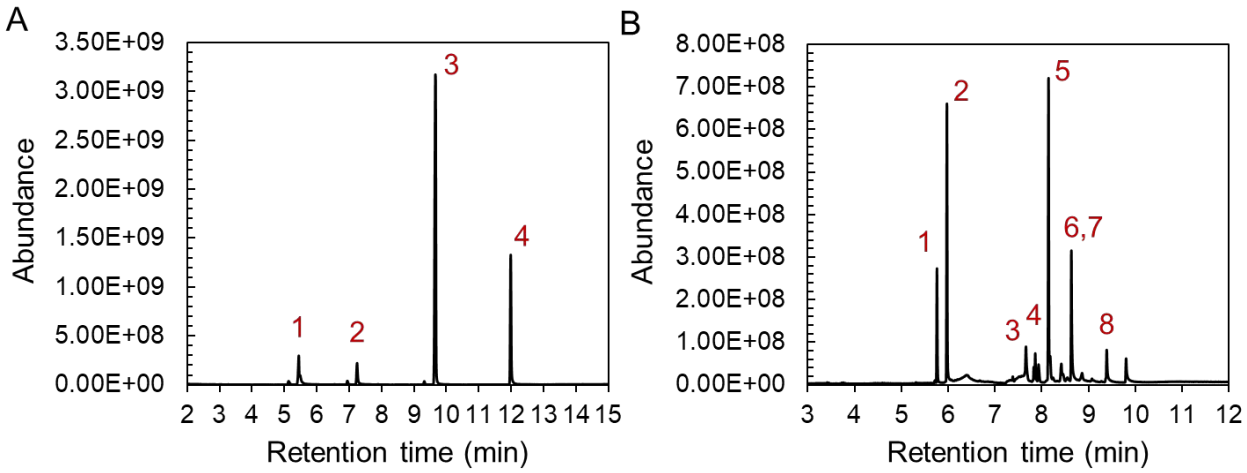

Figure S8. Representative gas chromatography separations for complex drug mixtures containing fentanyl related species. (A-B) Adjudicated case samples that contained fentanyl and several additional compounds provided by the Maryland State Police, Forensic Science Division. (A) Labeled chromatograms from a blue-colored suspension with undissolved particulate that contained unidentified cutting agent (1,2), synthetic opioid U-47700 (3), and fentanyl (4). (B) Labeled chromatograms from a clear solution with nonuniform, undissolved white particulate containing caffeine (1), lidocaine (2), codeine (3), 6-monoacetylmorphine (4), heroin (5), coelution of methacryl fentanyl (6) and methoxyacetyl fentanyl (7), and phenyl fentanyl (8).