



### **B178 The Separation of Fentalogs From Complex and Confounding Mixtures Using Gradient Elution Moving Boundary Electrophoresis (GEMBE)**

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**Learning Overview:** After attending this presentation, attendees will understand how Gradient Elution Moving Boundary Electrophoresis (GEMBE) can be utilized for the separation and detection of fentanyl and various fentanyl analogues from complex mixtures including heroin and other excipients and cutting agents.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by introducing the advantages of GEMBE over traditional electrophoretic separations, as well as demonstrating its capabilities for the separation of fentalogs from complex mixtures. The research will present the technique's potential as an analytical tool for the forensic practitioner.

The analysis of fentanyl and fentanyl analogues from complex mixtures remains a challenging and increasingly frequent endeavor for forensic examiners. While their direct detection and identification is imperative, these species are often found as a low abundance component in a mixture containing other narcotics (e.g., heroin, as well as excipients, adulterants, diluents, and bulking agents). To address these complex mixtures, a range of traditional separations are available, including, capillary electrophoresis (CE), gas chromatography (GC), liquid chromatography (LC), and ion mobility spectrometry (IMS). These separations represent Category B analytical techniques for chemical analysis based on SWGDRUG classifications.

Here, gradient elution moving boundary electrophoresis (GEMBE) was employed and its utility characterized for addressing this increasingly prevalent class of samples. GEMBE is a simple microfluidic separation technique consisting of run buffer and sample reservoirs connected by a relatively short capillary (5 cm) and the application of an electric field. Unlike other electrophoretic separations, GEMBE does not require a defined injection. This simplification readily enables easy multiplexing or array formatting to scale up throughput. Separation in GEMBE is achieved by applying pressure to the run buffer reservoir sufficient to drive buffer through the capillary, holding target analytes in the sample reservoir under the application of an electric field. The applied pressure is then ramped down, allowing analytes to sequentially enter the capillary for detection as their electrophoretic velocity overcomes the pressure driven counter flow. The counterglow nature of GEMBE also enables particulates, fibers, and other problematic components in the sample to be excluded from the microfluidic capillary, reducing sample preparation requirements and capillary fouling. Further, the separation resolution for GEMBE can easily be manipulated through the control of the pressure ramp and applied electric field as opposed to the need for increasing channel length or altering the electroosmotic mobility of the channel, typical of traditional capillary electrophoresis.

This study introduces a preliminary analysis of the capabilities and limitations of GEMBE for the separation of fentanyl and a range of fentanyl analogues from complex mixtures. Parametric optimization and analytical performance metrics will be presented for representative cases using predefined mixtures of chemical standards. Methods for enhancing resolution, potential quantitative capabilities, and limits of detection will be discussed.

#### **Fentanyl, Electrophoretic Separation, Complex Mixtures**