A134 Electrophoretic Separation of Drugs of Abuse Using Laser-Induced Fluorescence Detection

Bruce R. McCord, PhD, Department of Chemistry, Florida International University, University Park, Miami, FL 33199; and Britt E. Turnquest, BSc, Florida International University, 11200 SW 8th Street, Miami FL 33199*

After attending this presentation, attendees will have a better understanding of how amphetamine-related drugs of abuse can be derivatized, separated and detected using the fluorescent tag 5-(4,6-dichloro-s-triazin-2-ylamino) fluorescein (5-DTAF) and capillary electrophoresis system with laser-induced fluorescence detection. Also, the effects of various organic modifiers and surfactants on elution and resolution will be discussed.

This presentation will impact the forensic science community by providing an excellent screening method for trace amounts of phenethylamines and other related compounds which are efficacious at low doses due to their readily being absorbed, distributed and metabolized within the human body.

In capillary electrophoresis, a voltage is applied at the distal ends of capillaries filled with an electrolytic solution. These results in compounds being separated based on their mass-to-charge ratios. This method uses sample volumes in the nanoliter range and is capable of detection in the ng/mL range. For this study five commonly encountered drugs and precursors (used in illicit preparations) were investigated: amphetamine; methamphetamine; norephedrine; ephedrine; and, methylenedioxyamphetamine (MDMA). These compounds represent both primary and secondary amine moieties as well as three variations to the phenethylamine parent structure. Laser-induced fluorescence is a commonly utilized detection method in electrophoretic separations. This is due to its high sensitivity and specificity despite the short optical path length necessary when using capillary columns. Because compounds which natively fluoresce are rare, in order to utilize this detection method, the analytes of interest must first undergo derivatization. Fluorescence derivatization is the process whereby non-fluorescent analytes are coupled to an additional compound in order to produce an overall fluorescent molecule.

Due to their structure phenethylamine related compounds all have a pKa approximately within the range of 9.0 to 10.0 and migrate at a fairly similar rate. To compensate for this, modifiers in the form of surfactants and/or cyclodextrins were added to the run buffer to provide a pseudo-stationary phase. Organic solvents are also added to affect the equilibrium between the sample and this pseudo-phase. As a result, the individual drugs separate into distinct zones which are then excited by the laser as they pass the detection window on the way to the cathode. The signal from this excitation and subsequent emission is then collected by the detector and converted into an electropherogram for interpretation.

Drug standards were obtained from the International Forensic Research Institute at Florida International University and dissolved in analytical-reagent grade methanol for storage at 4°C. Prior to analysis, samples were diluted to appropriate concentrations using deionized water. For this method a micellar running buffer comprised of 50mM borate, pH 9.5/30mM Brij-35 was used for the separation of the analytes. A background electrolyte of 50mM borate, pH 9.5, and a derivatization buffer of 0.5M $NaHCO_3/Na_2CO_3$, pH 9.5 were also utilized. Experiments were conducted using a Beckman P/ACE MDQ unit interfaced with a computer utilizing Karat 32 software (version 7.0). The fused-silica capillary was 60.5cm (effective length 50cm) with an internal diameter of 50µm. An argon ion laser was used as an excitation source (488nm) and electropherograms were recorded by monitoring the emission intensity at 520nm. New capillaries were conditioned by thorough rinsing with 0.1M sodium hydroxide, deionized water, and micellar running buffer in series.

Amphetamines, Capillary Electrophoresis, 5-DTAF

 ** Presenting Author*