K28 Fluorescent Derivatization for Trace Detection of Opiates and Other Drugs of Abuse by Capillary Electrophoresis

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Attendees will learn a number of new procedures for the fluorescent derivatization and detection of tertiary amines such as opiates and other drugs of abuse using capillary electrophoresis with laser induced fluorescence.

This presentation will impact the forensic community and/or humanity by providing improved methods for the trace detection of drugs of abuse by capillary electrophoresis.

The described procedure involves a facile demethylation followed by a fluorescent derivatization reaction that can be used by forensic practitioners to determine 6-monoacetyl morphine and other tertiary amines at ultra trace levels. Highly selective conditions are then described for the separation and detection of these compounds using capillary electrophoresis with laser induced fluorescence. In addition, other fluorescent derivatization reactions are utilized for the analysis of primary and secondary amines such as benzyl piperazine using diode lasers with capillary electrophoresis.

Capillary electrophoresis (CE) methods are becoming increasingly popular as screening tools for forensic drug analysis. However, most separations using CE involve UV detection with relatively short detection window pathlengths when compared to HPLC. This limits sensitivity. While a number of useful techniques have been developed for sample preconcentration based on field amplified sample stacking, (especially for basic drugs) there still is a need for improved detection for toxicological samples. One of the best and most successful ways to improve CE detection limits is with laser-induced fluorescence. Because native fluorescence is limited to only a few compounds, most drugs of abuse need to be derivatized. This derivative should be fairly polar for best compatibility with CE. Unfortunately most fluorescent derivatization reactions involve reactive dyes that interact mainly with primary and secondary amines. Compounds such as opiates and cocaine that contain tertiary amines will not react with these dyes. In this project researchers explore methods for generation of secondary amines from these compounds and examine a variety of derivatization reactions for compatibility with capillary electrophoresis separation methods.

Spiked urine samples were extracted using Bond Elute Certify SPE (Varian) columns following manufacturer's suggested protocols. Samples were then diluted in dichloroethane and reacted with 50 microliters of 1chloroethyl chloroformate by heating to reflux for 2-4 hours. The solvent was then removed, and the sample was pH adjusted to 8.5 with bicarbonate and reacted for 30 minutes with fluorescein isothiocyanate. The resulting compounds produced a fluorescent emission at wavelengths above 520 nm that was compatible with commonly used 488nm argon-ion lasers. Alternatively, samples were reacted with the dye Cy5 NHS ester in a mixture of triethyl amine and DMSO. These samples were analyzed using an inexpensive diode laser operating at 635nm with emission at 665nm.

Separation of derivatized samples such as opiates, which have very similar structures, can be particularly challenging by any method. To perform these separations by capillary electrophoresis, beta-cyclodextrins were added to the buffer in order to form highly specific inclusion complexes with the derivatized drugs. In addition, altering the formation constants of these complexes using a mixture of organic solvents further optimized separations. Sample analysis was performed using a Beckman P/ACE MDQ capillary electrophoresis system with LIF detector, and the method was developed to be compatible with microfluidic devices. The results provided a highly sensitive screening tool for specific drugs of abuse with detection limits as low as 50pg/mL.

Capillary Electrophoresis, Drug Analysis, Laser Induced Fluorescence

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