K25 Inline Derivatization and Detection of Primary and Secondary Amine Containing Drugs Via CE-LIF

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After attending this presentation, attendees will be able to understand the mechanism by which drugs containing primary and secondary amine groups can be derivatized on-capillary using 4-fluoro- 7-nitro-2,1,3-benzoxadiazole (NBD-F) for the purpose of detection using capillary electrophoresis with laser-induced fluorescence.

This presentation will impact the forensic science community by providing a method that can be an excellent screening procedure for trace levels of amphetamines and other drugs in body fluids.

In forensics capillary electrophoresis has become an increasingly common analytical method due to its ability to be coupled to a variety of detection systems. One such method of detection is through laser-induced fluorescence which can provide high sensitivity and specificity in spite of the short path length used. This is particularly useful for the detection of compounds that undergo extensive first-pass metabolism and thus are present at trace levels in biological matrices. Unfortunately, compounds which fluoresce naturally are few and in order for them to be detected by fluorescence derivatization is necessary. This presentation will permit attendees to understand the mechanism by which drugs containing primary and secondary amine groups can be derivatized on- capillary using 4-fluoro-7-nitro-2,1,3-benzoxadiazole (NBD-F) for the purpose of detection using CE-LIF. NBD-F is a non-fluorescent compound which reacts to primary and secondary amines through a nucleophilic reaction whereby the fluorine attached at the benzene ring is lost and the amine group of the analyte loses a hydrogen atom and subsequently binds at that site. The resulting derivative is strongly fluorescent and has an emission wavelength around 530 nm.

Many drugs commonly feature amine groups within their structures which can be primary, secondary or tertiary in nature. The authors have focused on four specific compounds (3, 4-methylenedioxyamphetamine, 3, 4-methylenedioxymethamphetamine, norephedrine and ephedrine) that represent primary and secondary amines as well as two distinct chemical structures. It is to be noted that these compounds are either pre-cursors to or commonly encountered illicit “designer” drugs. To determine the capability of the selected fluorescent tag derivatize the analytes of interest in this study and optimize the reaction kinetics, an offline procedure was used based on work previously done by Lurie. These kinetic studies looked at derivatization temperature and time, drug concentration, molar ratio of tag to analyte, tag concentration and buffer pH.

Drug standards for each drug were obtained from Cerilliant and diluted to concentrations of 1 µg/mL. 75 µL aliquots of the drug, 20 mM NBD-F freshly prepared in ethanol and 50 mM sodium tetraborate buffer at pH 6.5 were combined in a 1:1:1 ratio and placed in a thermocycler for 10 minutes at 60°C. Samples were then hydrodynamically injected at 0.3 psi for 5 seconds into a fused silica capillary of 50 µm inner diameter, 40 cm length, 30 cm effective length. Separation took place using 50 mM $Na_2B_4O_7$ with 10 mM sodium dodecyl sulfate buffer at pH 8.5 at an applied potential of -15kV for 5 minutes. All steps in the CE method used reverse flow and polarity in order to shorten the effective length of the capillary to 10 cm. Fluorescence is then induced using an argon laser at 488 nm and separation is done using the Beckman Coulter P/ACE MDQ system. This procedure produced detection limits in the pg/µL range for each of the mentioned analytes.

Given the lack of fluorescence of NBD-F prior to derivatization, the detected fluorescence intensity can be used to quantify the amount of the analyte present. Differences in mobility between compounds and analyte(s) in question. The method proposed by the authors would incorporate an electrokinetic mixing step to facilitate a fast reaction between the analyte(s) and the tag after a hydrodynamic sandwich injection. As this method is geared towards urine samples where analytes of interest are in trace quantities the drug samples would be isolated via a liquid-liquid extraction procedure and reconstituted in run buffer prior to injection into the instrument.

On-Capillary derivatization, Amines, Drugs

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