A29 Effect of Organic Modifiers on Separation of Fluorescently Labeled Phenethylamines in Capillary Electrophoresis

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After attending this presentation, attendees will gain better insight into the role of organic modifiers in electrophoretic separations of fluorescently-labeled phenethylamines. The relationships between concentration of the fluorescent tag, 5-(4,6-dichloro-s-triazin-2-ylamino)fluorescein (5-DTAF), labeling efficiency, and peak resolution will be discussed.

This presentation will impact the forensic science community by detailing the optimization parameters of a potentially excellent screening method for trace amounts of phenethylamines and other related compounds, which are highly efficacious at low doses.

In electrophoresis, electric potential is applied at the ends of a capillary filled with an electrolytic solution resulting in the generation of an electric field. This field causes the movement of the electrolyte, causing any analytes present to be separated based on their mass-to-charge ratios. Due to the small inner diameter of these capillaries, sample and reagent volumes used are in the nanoliter range making it very cost-effective. Due to the short optical path length required for capillary columns, a commonly utilized detection method for this separation technique is laser-induced fluorescence. This is because of its high sensitivity and specificity, which allows for the detection of compounds 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). Since fluorescence is not a property native to any of these analytes, they must first be coupled to a fluorescent molecule, in this case 5-DTAF, in order to make them compatible with the technique. This process is known as fluorescence derivatization.

Given their small size and overall similarity in structure, the phenethylamines investigated all have similar pKa values and migration rates. Due to this, coelution of peaks is a commonly encountered difficulty. To overcome this and improve the separation between the individual drugs assessed, modifiers were added to the run buffer to alter the electro-osmotic flow and thus the velocity of the bulk solution as well as vary the migration rates of the analytes through their interactions with them. These modifiers included various β-cyclodextrins and organic solvents. As a result, the individual drugs separate into distinct zones. As they pass through the detection window on the way to the cathode, samples are irradiated by the laser. The signal produced from this excitation and subsequent emission is then collected by the detector and converted into an electropherogram for interpretation.

Drug standards were dissolved in analytical-reagent grade methanol for storage at 4°C. Prior to analysis, samples were diluted to appropriate concentrations using deionized water from a ultrapure water purification system and methanol. For this method, a micellar running buffer comprised of 50mM borate, pH 9.5/15mM β-cyclodextrin (β-CD) 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 CE-based analytical system unit interfaced with a computer. 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 (488 nm) 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.

Phenethylamines, Electrophoresis, 5-DTAF

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