



K46 Sensitive Detection of Amphetamines and Other Basic Drugs Using Eosin Isothiocyanate

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After attending this presentation, attendees will understand the growing applications of microfluidic systems such as how they can be used to solve crimes as well as diagnosis health issues.

This presentation will impact the forensic community and/or humanity by demonstrating that microfluidic systems can perform extremely rapid analyses of compounds utilized in crimes such as Drug Facilitated Sexual Assaults and DUIs.

The application of microfluidic systems to toxicological screening and clinical diagnostics is growing rapidly. Rapid analysis of small molecules is essential in the detection of drugs for the prosecution of crimes such as drug-facilitated sexual assault and DUIs. Detection of biogenic amines for identifying health disorders and diseases is also of

interest. Therefore, these systems are of interest to both criminal investigators and medical personnel. Microfluidic systems utilize very small quantities of samples (μLs) and perform rapid separations (2 min. or less). Their small size makes them potentially portable for use at crime scenes. In addition microchips can be inexpensively, and the designs are simple to minimize user interaction. Quick analysis (preferably on-site) and high sensitivity are crucial to detection because some drugs can be metabolized and rapidly eliminated.

Although liquid phase drugs are traditionally detected by UV, its sensitivity is limited in microfluidic analysis by short path lengths (approximately 50 μm or less). Fluorescence is by far the most common detection method utilized by microfluidic systems due to its high sensitivity. However, most drugs are not naturally fluorescent so analysis must be derivatized. Biogenic amines and many drugs of abuse which are primary or secondary amines can easily be derivatized by amine reactive dyes.

Alnajjar, et al presented a method for the derivatization of opiates and their derivation with fluorescein isothiocyanate (FITC) and detection by CE-LIF with a 488nm argon-ion laser.¹ The method presented is the detection of several phenethylamines, primary and secondary, by microchip CE-LIF. The drugs are derivatized by eosin isothiocyanate (EITC) and detected on a Micralyne microfluidic Tool Kit (μTK) equipped with a 532nm frequency-doubled laser. Tertiary amines can also be derivatized following a demethylation procedure.²

One of the advantages of microfluidic systems is their potential to perform simultaneous sample preparation, separation, and detection. This growing trend in microfluidics to create micro total analysis systems (μTAS) greatly improves the overall analysis speed. It has been shown that several phenethylamines³ and biogenic amines⁴ can be fluorescently derivatized in a few seconds making on-chip reactions feasible. A method for performing rapid derivatization of phenethylamines by EITC on-chip is proposed. Several chip layouts were tested to promote mixing of reagents, and very narrow reaction chambers improved mixing. The reactions were optimized by placing the mixed reagents in the dark at room temperature for 48 hours and measuring the product yield relative to an internal standard every few hours. Although it took about 24 hours for the reaction to go to completion, products formed at a detectable level in less than 10 minutes.

Derivatized amphetamine, methamphetamine, and ephedrine could be separated by CE with baseline resolution using a basic buffer, pH = 9.8, containing cyclodextrins and a separation voltage of 10 kV in a 40 cm long capillary. In addition microchip separation could be simulated on a traditional CE by injecting the sample on the short end of the capillary. The combination of the rapid microchip separation and the on-line sample preparation resulted in a μTAS which could perform a screening test in a matter of minutes.

References:

- 1 Alnajjar, A. et al, *Electrophoresis* 2004, 25, 1592-1600.
- 2 Olofson, R.A., *Pure appl. Chem.* 1988, 60, 1715-1724.
- 3 Wallenborg, S. R., *Electrophoresis* 2000, 21, 3257-3263. Ro, K. W., *Electrophoresis* 2002, 23, 1129-1137.
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DFSA, Microfluidic, Fluorescence