K22 Capillary Electrophoresis/Electro- Spray Ionization/Time-of-Flight Mass Spectrometry of Low Dose Benzodiazepines

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The goal of this presentation is to determine the usefulness of capillary electrophoresis electro-spray ionization time-of-flight mass spectrometry (CE-ESI-TOF-MS) for the detection of low dose benzodiazepines.

This presentation will impact the forensic community and/or humanity by providing a new approach to accurately and consistently determine the presence of benzodiazepines at low concentrations. This technique can then be applied to cases where drug facilitated sexual assault is suspected.

Besides being used for therapeutic purposes benzodiazepines are commonly abused at parties, night clubs, and raves because of their ability to cause euphoria and a drunk-like high. Common low dose benzodiazepines include alprazolam, clonazepam, lorazepam, and triazolam. When benzodiazepines are taken in the presence of alcohol, the user experiences heightened effects as well as amnesia and unconsciousness. In such instances the drug is often used as a tool in drug facilitated sexual assault and can be present in such low concentration that it is often difficult to detect. In cases of sexual assault, the type, concentration, and number of drugs taken can aid in the investigation. However, there are few methods capable of fully determining this information.

Recently, capillary electrophoresis has been proposed as an alternative to GC/MS for toxicological analysis, especially when coupled to electrospray mass spectrometry (ESI-MS). CE has several advantages which make it an ideal method to couple with ESI-MS. It has high efficiency, minimal sample requirements, and short analysis time. MS is an analytical technique that can reveal specific, characteristic, and structurally related information about a compound. The analytes must be present in the vapor phase, and this condition is obtained via electrospray analysis.

The present work was performed on an Agilent CE-ESI-TOF-MS which utilizes a sheath flow interface to spray a minimal and regulated amount of the eluted CE sample into the TOF. The TOF provides several advantages when used in combination with CE. The system is very fast compared to trap systems or quadrupoles and has a 3ppm or less mass resolution, allowing extremely accurate empirical formula determination based on high resolution mass determination. The system identifies drugs based on four operational parameters: absolute mass, prediction of related absolute isotopic mass abundances, in-source collisional dissociation, and electrophoretic mobility. The high separation efficiency of CE combined with the high sensitivity and information content of MS makes this instrument a powerful tool for screening and confirmation of drugs. These characteristics make ESI-TOF one of the more suitable mass spectrometric detection methods for CE.

Selected benzodiazepines were analyzed using this system, and extracted ion analysis was performed with high selectivity by using the exact masses of the protonated molecular ions. This capability greatly reduced background noise and improved detection. The effect of buffer pH, concentration, and spray parameters were also examined. The capillary column used had a 75 micron internal diameter and a length of 95mm. The polyimide coating was removed from the end of the capillary and a blunt tip was cut in the fused silica with a sapphire scribe. This was crucial when using the CE-MS to obtain a perfect and precise spray while eliminating adsorption of the sample on the outside of the capillary. The running buffer used was 20mM formic acid at a pH of 2.7. At this low pH the drugs were cationic, and, although a low electro- osmotic flow (EOF) resulted, electrophoretic mobility and induced flow resulting from the sheath liquid during analysis enabled overall run times of under 8 minutes. The results demonstrate CE-ESI-TOF to be a rapid and highly specific detection method for benzodiazepines.

Capillary Electrophoresis, Time-of-Flight Mass Spectrometry, Benzodiazepines

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