A132 Development of a Supported Liquid Extraction Method for Benzodiazepine in Urine With Surface-Enhanced Raman Spectroscopy Detection

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The goal of this presentation is to show the development of a Supported Liquid Extraction (SLE) method for the extraction of trace quantities of benzodiazepines in urine with Surface Enhanced Raman Spectroscopy (SERS) detection. The optimization of this technique as well as the limits of this method will be discussed.

This presentation will impact the forensic science community by detailing how this method has shown the applicability of SLE for the efficient extraction of trace quantities of benzodiazepines from toxicological samples with SERS detection and the use of the technique over a wide range of compounds. SERS is more specific than currently used immunoassays because it provides spectral information for the compounds present.

Benzodiazepines are among the most frequently prescribed compounds for anti-anxiety and anti-depression and are commonly present in many toxicological screens. These drugs are also prominent in the commission of drug-facilitated sexual assaults due to their effects on the central nervous system. Due to their potency, a low dose of these compounds is often administered to victims; therefore, the target detection limit for these compounds in biological samples is 50ng/mL, which is well below therapeutic concentrations. Currently, these compounds are predominantly analyzed using immunoassay techniques; however, more specific screening methods are needed.

SERS has previously been shown to be able to detect trace quantities of benzodiazepines in aqueous solutions. This technique has the advantages of overcoming the low sensitivity and quenching the unwanted fluorescence effects seen with conventional Raman spectroscopy. SERS spectra are obtained by applying a compound of interest onto a SERS-active metal substrate such as colloidal metal particles or metal films. In this case, the colloidal particles are spherical gold nanoparticles in aqueous solution. SERS signals can be further increased with the addition of aggregate solutions. These agents are salt solutions which cause the nanoparticles to amass and form hot-spots which increase the signal intensity.

Spiked urine samples were prepared by adding diluted benzodiazepine and metabolite samples (prepared in 10% methanol) to drug-free urine at a range of benzodiazepine concentrations (1ng/mL-500ng/mL). An SLE method specific for benzodiazepines was used. Compared to the standard liquid-liquid extraction, SLE provides cleaner extracts and is less time consuming. A number of different method parameters were examined, including buffer concentration and pH as well as elution solvent. This extraction method has been shown to provide efficient extraction for the benzodiazepines in this study. Extraction efficiency was also determined. Aqueous colloidal dispersions of gold spherical nanoparticles were prepared using a modified Lee Meisel 1% sodium citrate reduction method. Particle size and shape were confirmed with an average size of approximately 30nm. Previous work has shown that for benzodiazepines, an aggregate solution made of $MgCl_2$ prepared at a concentration of 1.67M provided the highest signal intensity at the lowest drug concentration and was used in this study. Aggregate solutions were added to colloidal dispersions followed by the addition of extracted benzodiazepine samples and SERS spectra were obtained.

Overall, this method allows for the extraction and detection of a wide variety of benzodiazepines and their metabolites. The presence of individualizing spectral peaks provides a high degree of specificity for sample determination. The technique is sensitive with a limit of detection of 2.5ng/mL and linear over several orders of magnitude for the drugs chosen. This method has shown the applicability of SLE for the efficient extraction of trace quantities of benzodiazepines from toxicological samples with SERS detection and the use of the technique over a wide range of compounds. SERS is more specific than currently used immunoassays as it provides spectral information for the compounds present. Also, this technique has higher sensitivity and permits detection of drugs such as lorazepam, which have poor cross reactivity when using standard immunoassays.

Benzodiazepine, SERS, Drug Analysis

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