A206 Development of a Surface-Enhanced Raman Spectroscopy Method for the Detection of Benzodiazepines in Urine

Erika L. Doctor, MS, and Bruce R. McCord, PhD, Florida Int'l Univ, Dept of Chemistry, University Park, Miami, FL 33199*

The goal of this presentation is to show the development of surface-enhanced Raman spectroscopy method for the analysis and detection of trace quantities of benzodiazepines in urine. The optimization of various parameters of this technique as well as the limits of this method will also be discussed.

This presentation will impact the forensic science community by providing information regarding Surface-enhanced Raman spectroscopy (SERS) method and how it has shown the applicability of SERS for the detection of trace quantities of benzodiazepines extracted from toxicological samples 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.

Benzodiazepines are medications for anti-anxiety and anti-depression that are commonly prescribed. These drugs are prominent in the commission of drug-facilitated sexual assaults due to their effects on the central nervous system such as drowsiness, amnesia, confusion, and impaired coordination. 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.

Surface-enhanced Raman spectroscopy (SERS) has previously been shown to detect trace quantities of compounds, such as nicotine, in aqueous solutions. This technique has the advantage 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. Chlorine salts generally provide the greatest enhancement for two reasons. The chlorine ions displace the stabilizing agent to cause aggregation and they affect the ionic strength of the surrounding solution, changing the surface charge of the substrate, therefore increasing 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). A solid phase extraction method specific for benzodiazepines was used. Extraction efficiency was determined by quantitation using direct infusion mass spectrometry. 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 1.67 M 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 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 SERS for the detection of trace quantities of benzodiazepines extracted from toxicological samples 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.

Benzodiazepin, SERS, Drug Analysis

 ** Presenting Author*