The Identification of Controlled Substances by TLC-SERS

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Abstract

This research evaluated the method of thin layer chromatography combined with surface-enhanced Raman spectroscopy (TLC-SERS) for the purpose of separating and identifying controlled substances. This technique adheres to the current standards set forth by the Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG) and requires less time, money, and sample when compared to other methods of drug analysis. Analyzing illicit drugs and drug mixtures using TLC-SERS involves separating mixtures on a TLC plate then, through the addition of a metallic colloid, identifying the components directly from that TLC plate using Raman spectroscopy. SERS corrects for the two main disadvantages of normal Raman spectroscopy: low sensitivity and fluorescence. The controlled substances cocaine, methamphetamine, 3,4-methylenedioxymethamphetamine (MDMA), and codeine were analyzed using TLC-SERS, which proved to be a successful method of separation and identification.

Introduction

Thin layer chromatography (TLC) and surface-enhanced Raman spectroscopy (SERS) proved to be an ideal method for the separation and identification of controlled substances. This method has the potential to benefit the forensic science community because it required smaller sample sizes and reduced sample preparation compared to other commonly used methods for illicit drug analysis and identification. This combined technique is also a rapid, reliable, and repeatable way to analyze controlled substances and controlled substance mixtures.

Forensic laboratories often encounter cases involving drugs and controlled substances. A drug is any substance that produces physiological or psychological changes within the body after ingestion. A controlled substance is any drug that is deemed illegal unless prescribed by a physician. Because controlled substances are commonly analyzed in forensic laboratories, a rapid and reliable method of analysis is a necessity. A technique that requires minimal amounts of drug sample is also important because criminal cases do not always have large quantities of evidence to test.

The Drug Enforcement Administration (DEA) divides controlled substances into five schedules based on their potential for abuse, the accepted medical use of a substance, and the safety associated with use of a substance. These five controlled drug schedules are compared in Figure 1. The controlled substances used for this research were chosen because they are commonly encountered in crime laboratories, and have various levels of dependency and abuse risk. The most dangerous and regulated drug analyzed in this study is 3,4-methylenedioxy-N-methylamphetamine (MDMA). MDMA, commonly known as ecstasy or Molly, is a schedule I controlled substance that has a high potential for abuse, severe psychological and/or physical dependency, and has no accepted medical use. Schedule II controlled substances, such as cocaine and methamphetamine, have a high potential for abuse and severe dependency but have accepted medical applications. Codeine is characterized as a schedule III controlled substance, which has a moderate potential for abuse and dependency.

Figure 1: Schedule of controlled substances

Schedule	Accepted for Medicinal Use?	Potential For Abuse & Physical or Psychological Dependency	Examples
I	No; research use only	High potential	Marijuana, mescaline, LSD, heroin, ecstasy, methaqualone
II	Yes	High potential; severe dependency	Cocaine, amphetamine, morphine, methadone, PCP, most barbiturates
III	Yes	Moderate potential; moderate dependency	Anabolic steroids, codeine preparations, pseudoephedrine
IV	Yes	Low potential; moderate to limited dependency	Phenobarbital (anti-seizure) and diazepam (Valium).
V	Yes	Low potential; low or limited dependency	Some are sold in over-the-counter preparations

The protocol for the analysis of controlled substances in forensic laboratories should adhere to standards set by the Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG). Techniques for analysis are put into three categories based on the discriminating power of each of technique (see Figure 2). For a positive drug identification, SWGDRUG recommends that a technique from category A be paired with a technique from either category B or C. When a Category A technique is not used, at least three different validated methods from category B or C should be performed

Figure 2: SWGDRUG's analytical technique categorization

Category A	Category B	Category C
Infrared Spectroscopy	Capillary Electrophoresis	Color Tests
Mass Spectrometry	Gas Chromatography	Fluorescence Spectroscopy
Near Infrared Spectroscopy	Ion Mobility Spectrometry	Immunoassay
Nuclear Magnetic Resonance Spectroscopy	Liquid Chromatography	Melting Point
Raman Spectroscopy	Microcrystalline Tests	Ultraviolet Spectroscopy
	Pharmaceutical Identifiers	
	Thin Layer Chromatography	
	Cannabis only: Macroscopic Examination Microscopic Examination (Counts as one each)	

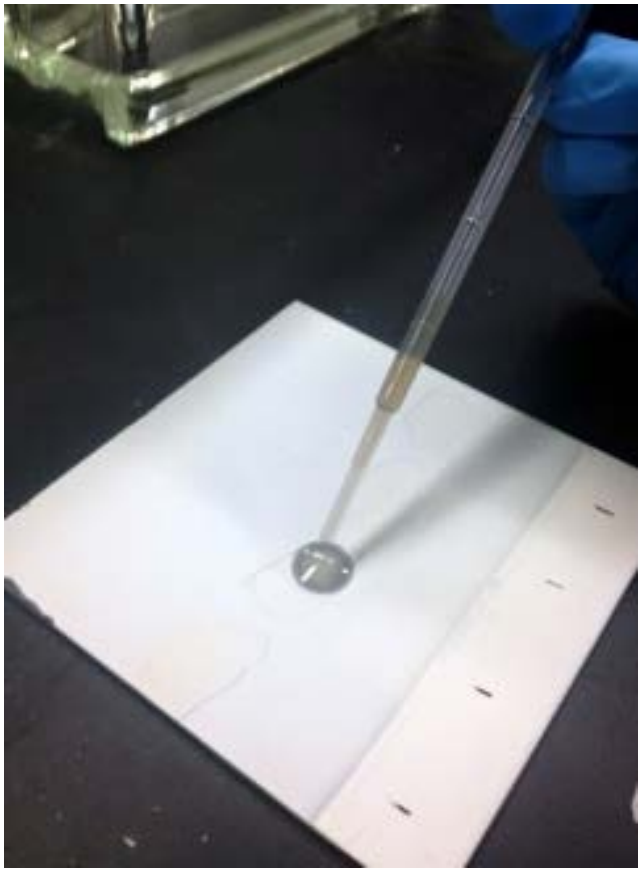
The methods used in this research follow these SWGDRUG recommendations. Thin layer chromatography (TLC) from category B and Raman spectroscopy from category A were used to separate and identify the drug samples.

TLC is commonly used as a screening tool in forensic science because it is rapid, inexpensive, and efficient when separating and analyzing components of a mixture. TLC is categorized as a category B technique by SWGDRUG, and is commonly used in forensic laboratories as a screening tool in the examination of controlled substances. This chromatography technique involves depositing the sample onto a planar stationary phase (often silica gel on glass), and using a liquid mobile phase that travels up the stationary phase by capillary action. The components of the sample move at different rates depending on the component's size and affinity for the mobile phase. The ending result is a plate of spots (separated components of the mixture) that have travelled various distances. The retention factor of each component of the mixture is then calculated by dividing the distance the component travelled by the distance the solvent travelled. Retention factors are used as a quick way to make a preliminary identification of a substance, but are not specific to a single compound. Thus positive identification is not possible with TLC alone, which is why it must be paired with a category A confirmation method of identification.

Raman spectroscopy is an identification method from SWGDRUG category A that looks at the frequency change of a light source due to its interaction with the sample. A spectrum is obtained when the instrument shines a laser light source onto a sample then detects the inelastic scattering of light. Inelastic scattering occurs when the frequency of photons in the laser change after the photons interact with the sample. This scattering allows low-frequency changes, in this case vibration, to be detected in the sample's molecules. The vibrational frequency depends upon the molecular structure of the sample being tested. There are many advantages to this technique for identification such as minimal sample preparation, accurate identification analysis, and the non-destructive nature of the technique. The major limitations to Raman spectroscopy are the weak signal produced with many samples and the interference due to fluorescence.

Surface-enhanced Raman spectroscopy, SERS, essentially mirrors the procedure for Raman spectroscopy and is a technique that enhances the scattering procedure, which essentially corrects for the two disadvantages of normal Raman spectroscopy: low sensitivity and fluorescence. The main difference between Raman spectroscopy and SERS is the addition of a metallic colloid, which allows for enhancements up to 10^6 in scattering efficiency, thus improving the sensitivity (see Figure 3). The addition of a metal colloid boosts the Raman scattering signal of the molecules and allows for spectra to be collected from samples that cannot be detected using normal Raman spectroscopy. A SERS spectrum can be acquired quickly and still includes all the advantages of normal Raman spectroscopy. Spectra are unique to single compounds and therefore allow this method to be used as a technique for identification. These advantages make it one of the most sensitive methods for identification.

Figure 3: TLC-SERS analytical procedure, showing the deposition of the silver colloid directly onto the visualized and marked TLC spots. Following this addition, the spots were analyzed with the Raman microspectrophotomer equipped with a 780 nm frequency-stabilized single mode diode laser



Materials and Methods

Four common controlled substances were analyzed using TLC-SERS: cocaine, methamphetamine, MDMA, and codeine. Caffeine was used as an adulterant in drug mixture analysis. Pure samples of these drugs were analyzed with Raman spectroscopy and SERS in order to identify the spectra associated with each drug and method. The Raman spectrometer used was a dispersive Raman equipped with a 780 nm frequency-stabilized single mode diode laser (see Figure 4). The parameters were set to collect a spectrum with a target signal to noise ratio of 500 and a three minute maximum collection time. To ensure the Raman microspectrophotometer was working properly, a polystyrene reference standard was analyzed daily to check the wavelength calibration.

Figure 4: The dispersive Raman microspectrophotometer that was used in this research.

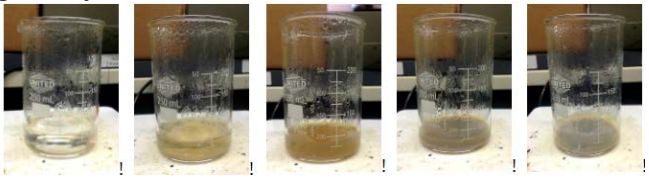
The same procedure was carried out for all four drug analyses. A drug sample of 0.100g was weighed out and placed into a vial. From that vial, 0.0100g was combined with 2.5mL of methanol to make a liquid solution with a concentration of 4 mg/mL of each drug. Before TLC-SERS was conducted, preliminary analysis was done using the solid drug samples and drug solutions. First, a small amount of controlled substance was placed onto an aluminum slide and focused on the Raman microscope. Once the sample was focused, it was analyzed using normal Raman spectroscopy. For the drug solutions, a small amount of liquid was placed onto an aluminum slide and allowed to evaporate. A thin film of drug would develop on the slide. That film was focused on the Raman microscope and then analyzed using normal Raman. Next, both the solid drug and drug solutions were analyzed in the same fashion except with the addition of one drop of silver colloid onto the sample. This was completed in order to compare the normal Raman and the SERS spectra.

Before separation, the silica gel TLC plates were spotted using the drug solutions. A capillary was used to transfer the drug solution onto the TLC plate. Once a sufficient amount of solution was transferred onto the TLC plate, the plate was placed into a TLC bath tank. The bath was composed of a 9:1 solution of chloroform to methanol. The plate took approximately 20-25 minutes to completely separate. After the plate was separated, it was removed from the tank then allowed to dry. The spots were then visually identified using a short-wave ultraviolet (UV) light source. To recognize the placement of the spots, a circle was drawn around the spots using pencil.

Once the TLC plates of the various drug spots were separated and visually identified, they were analyzed using normal Raman spectroscopy. This involved placing the TLC plate where the spots were known to be under the Raman microscope. Each spot was analyzed using normal Raman and a spectrum was obtained. The process was repeated using SERS by adding a drop of silver colloid directly to the TLC plate then analyzing that plate with the Raman.

Various colloids and colloid preparations were evaluated for use in this research. In addition to the prepared silver colloids, two commercially available silver colloids were purchased. After comparative analysis of the enhancement of the prepared and purchased silver colloids, it was determined that the following method below for the preparation of silver colloids produced the most significant SERS enhancement. The colloid preparation used for this research first involved making two solutions. The first solution was composed of 0.170g silver nitrate and 1.00L deionized water. The second solution was composed of 1.00g sodium citrate and 0.100L deionized water. To make the silver colloid, 50.0mL of silver nitrate in a 100-mL beaker was brought to a boil. Once that solution was boiling, 1.00mL of sodium citrate was added to the silver nitrate solution. The colloid underwent various stages of color transformation as seen in Figure 5.

Figure 5: Photographs showing the preparation of the silver colloid, taken in 10 minute increments. The colloid started as a clear, boiling liquid. As heating progressed, the solution slowly turned into a light brown solution. The colloid gradually darkened until it reached a dark silver color.

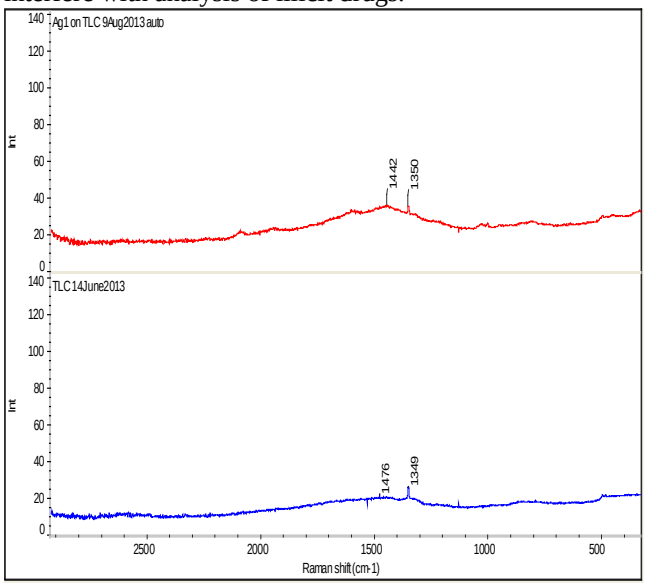


Results and Discussion

The controlled substances were not detectable on the TLC plates using normal Raman spectroscopy. However, when the TLC spots of the drugs were analyzed using SERS, the spectrum was greatly enhanced. The same results were found when analyzing the drug solutions mixed with a caffeine solution. The enhancement provided by the colloid enables direct drug identifications on the TLC plate without significant additional sample preparation or analysis time.

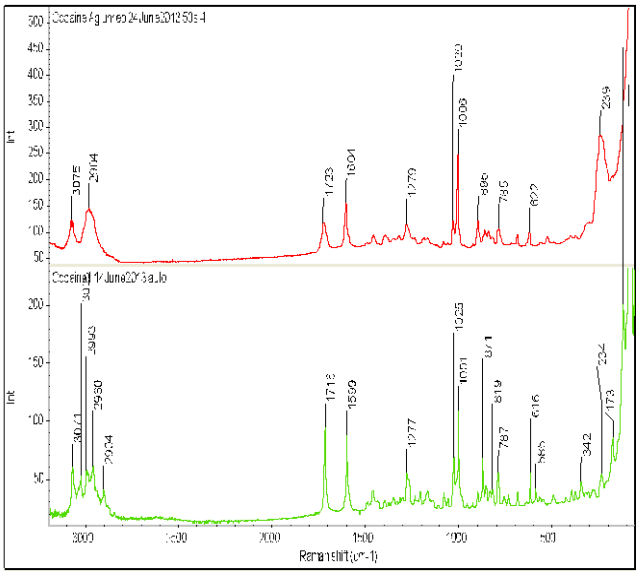
The TLC plates used in this research are made of silica gel with a 254 nm fluorescent indicator and a gypsum/polymeric binder. These are the common TLC plates used in forensic science laboratories for the analysis of illicit drugs, thus it was imperative to determine if there would be any inherent interference from the gel or indicator that would hinder the Raman and/or SERS analysis. As seen in Figure 6, there were no significant peaks present in the Raman or SERS spectra of the TLC plates.

Figure 6: Normal Raman (red) and SERS (blue) spectra of a TLC plate showing no significantly intense peaks that could interfere with analysis of illicit drugs.



As expected, the SERS spectra for each of the drugs showed significant similarities to their normal Raman counterparts. Figure 7 demonstrates this consistency between the normal Raman and SERS spectra for cocaine. However, some differences can be expected because not all vibrational modes experience the same enhancement with the silver colloid, thus it is recommended that SERS reference spectra be used when doing a spectral identification.

Figure 7: Normal Raman (red) and SERS (green) spectra of cocaine. The normal Raman spectrum was collected from a solid cocaine sample mounted on an aluminum microscope slide; the SERS spectrum was collected on the same sample with the addition of a drop of the silver colloid.



All illicit drugs analyzed in this research proved that identification was not possible using TLC-normal Raman spectrum due to the low concentration of the drugs after TLC analysis and separation. However, the enhancement provided by the silver colloid enabled direct drug identification on the TLC plate via SERS. In addition, the TLC-SERS spectra were consistent with those of SERS alone, which demonstrated that the process of TLC does not affect a drug's spectrum. These results are shown in Figures 8, 9 and 10 for the illicit drugs cocaine, MDMA and methamphetamine, respectively.

Figure 8: TLC- Raman (green), TLC-SERS (blue) and SERS (red) spectra of cocaine.

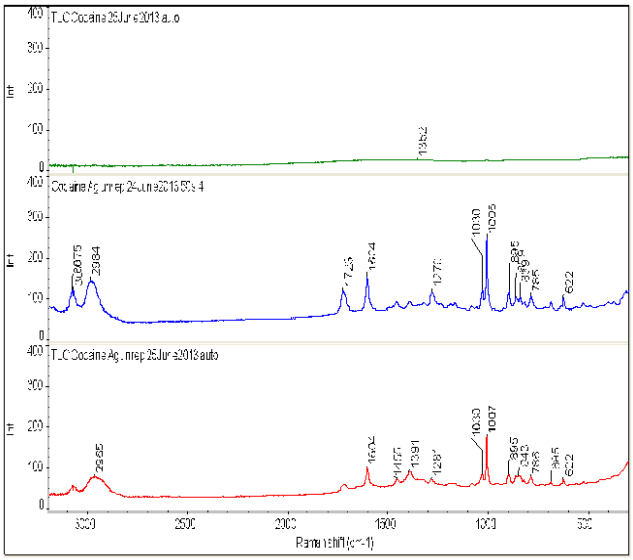


Figure 9: TLC- Raman (green), TLC-SERS (purple) and SERS (red) spectra of MDMA.

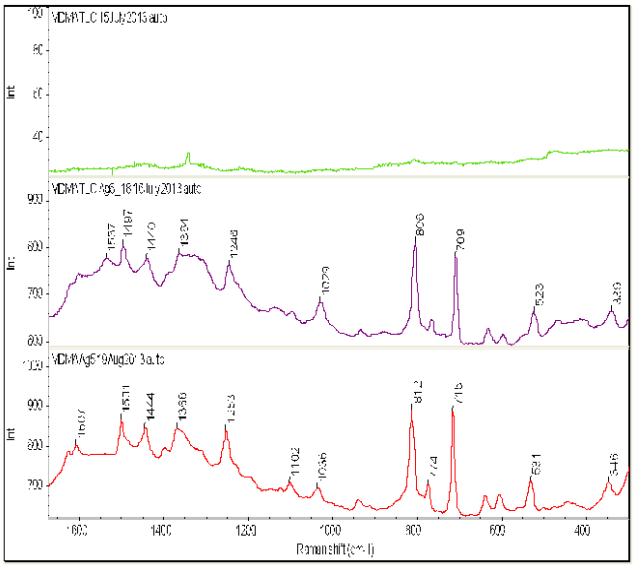
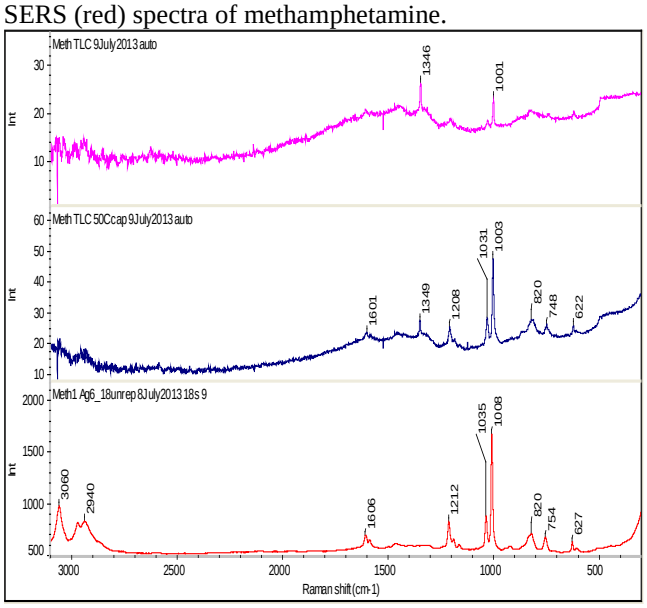


Figure 10: TLC- Raman (purple), TLC-SERS (blue) and SERS (red) spectra of methamphetamine.



In conclusion, illicit drug identification can be accomplished using the novel method of TLC-SERS. Both SERS and TLC-SERS spectra are reproducible and interpretable, thus this research proved that TLC-SERS is a successful method for the separation and identification of drugs and drug mixtures. Coupling TLC with SERS is a convenient way to reduce the amount of material, equipment and time needed for controlled substance analysis when compared to current methods, and conforms to the standards set forth by SWGDRUG.

This research was a preliminary study that evaluated the effectiveness of TLC-SERS in the separation and identification of controlled substances and controlled substance mixtures. In the future, additional colloids, such as those made with gold nanoparticles, will be evaluated in comparison to the silver colloid used in this research. Also, the limits of detection will be evaluated for various illicit drugs. Last, additional controlled substances and mixtures will be analyzed using both silver and gold SERS colloids.

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Biography

Kasey Cargill, originally from Blairsville, Georgia, is a junior at the University of New Haven. She is majoring in Forensic Science, Biology Pre-Medical, and Biotechnology and minoring in Chemistry. In addition to her studies, she is a member of the Forensic Science and Chemistry club, the Biology club, and the Honor Society for Experiential Education. She is also a general chemistry and organic chemistry laboratory teaching assistant and a genetics and molecular biology tutor for both undergraduate and graduate students. Upon graduation, Kasey plans to attend graduate school to study genetics and molecular biology.