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The use of thin layer chromatography combined with surface-enhanced Raman spectroscopy for the identification of controlled substances

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This research evaluates the method of thin layer chromatography coupled with surface-enhanced Raman spectroscopy (TLC-SERS) for the purpose of separating and identifying seized drugs. This technique adheres to the current protocols for the identification of controlled substances that are used by forensic laboratories and detailed in ASTM E2329-17 [1]. TLC is a well-established and commonly used method for the analysis of controlled substances, however it is a presumptive test that requires additional techniques to make a positive identification. SERS is a relatively new technique in forensic science, and is finding increasing acceptance because of notable published research on its capabilities for material identification. SERS corrects for the two main disadvantages of normal Raman spectroscopy: low sensitivity and fluorescence. Alone, each technique has its limitations, but together, they provide a sensitive and selective method for the separation and positive identification of seized drugs. Analyzing illicit drugs mixtures using TLC-SERS involves separating mixtures on a TLC plate then, through the addition of a metallic nanoparticle colloid, identifying the components directly from that TLC plate using Raman spectroscopy. Furthermore, TLC-SERS requires less time, materials, and sample when compared to other methods of drug analysis. In this research, two gold and three silver nanoparticle colloids were evaluated for the identification of ten drugs (amphetamine, caffeine, cocaine, codeine, diazepam, flunitrazepam, lidocaine, methamphetamine, 3,4-methylenedioxymethamphetamine (MDMA), and phenobarbital) by TLC-SERS. One silver colloid preparation was shown to be superior for the identification of controlled substances by TLC-SERS. Ultimately, this research demonstrates that TLC-SERS is a rapid, reliable, and repeatable way to separate and identify a wide range of controlled substances.

Keywords: TLC-SERS; Controlled Substances; Forensic Science.

1 Introduction

The sale, manufacturing, and use of controlled substances continues to rise both globally and in the United States [2]. Apart from marijuana, the use of prescription and non-prescription opioids, tranquilizers, and sedatives, including synthetic and semi-synthetic drug derivatives, have also increased [2]. With drugs of abuse changing, forensic science must adapt its techniques to not only detect the more widely known illegal drugs, but also the new ones and their mixtures. Furthermore, because controlled substances represent a large proportion of forensic casework samples, analytical methods must be rapid, reproducible and reliable. An ideal forensic analytical method would also be nondestructive or minimally destructive, and requires use of a very small amount of sample.

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Illicit drugs are commonly cut with additives for the purposes of either increasing the weight of the product, altering the color or texture, or increasing the intensity of the euphoric effects. These additives are usually common household materials such as baby formula or artificial sweeteners, or other drugs that are not as tightly controlled such as caffeine, ketamine or lidocaine [3]. Although, the landscape of the illicit drug market continues to evolve, the techniques used by forensic science laboratories to optimally separate and identify drugs and/or cutting agents has remained the same for several decades.

The protocol for the analysis of controlled substances in forensic laboratories should adhere to standards set by the American Society for Testing and Materials (ASTM) Standard E2329-17 "Standard Practice for Identification of Seized Drugs" [1], which in 2016 was the first standard to be elevated to the Organization of Scientific Area Committees (OSAC) Registry of Approved Standards. According to this protocol, techniques for analysis are put into three categories based on the discriminating power of each of technique (Table 1). For a positive drug identification, it is recommended 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 [1]. A commonly employed analytical scheme involves the presumptive identification of an unknown sample via color tests followed by a confirmatory test using mass spectrometry after separation of the controlled substance by either gas or liquid chromatography. Although this is rapid, the use of these methods for separation and identification requires a substantial amount of sample and leads to the destruction of the drug after analysis.

Table 1. Analytical technique categories for the identification of seized drugs, according to ASTM E2329-17 [1].

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

To mitigate the current limitations for drug identification in forensic science laboratories, we have employed the combined method of thin-layer chromatography (TLC) with surface-enhanced Raman spectroscopy (SERS) for the fast separation and confirmatory identification of controlled substances. The coupling of TLC and SERS was first reported in 1977 by Henzel [4], however it is an under researched area that has great potential in a range of scientific disciplines. While TLC-SERS has been used in other settings including in industry, cultural heritage, medicine, and pharmaceuticals, to our knowledge it has not been widely applied or robustly researched for the analysis of illicit drugs or other samples of forensic interest [5-10]. The most common technique for TLC-SERS involves applying a colloid containing noble metal nanoparticles directly to the TLC plate, and specifically on top of the separated components. The coupled method of TLC-SERS adheres to ASTM recommendations, because TLC is a category B separation method

and SERS is a specialized method of Raman spectroscopy which is a category A confirmatory technique. Further, TLC-SERS requires smaller sample sizes and reduced sample preparation time compared to other commonly used methods of analysis. TLC-SERS is an emerging technique that delivers rapid, non-destructive analysis that provides forensic laboratories with a novel approach for drug and drug-mixture identification.

TLC is commonly used as a screening tool for controlled substances because it is rapid, inexpensive, and efficient when separating and analyzing components of a mixture. This chromatography technique involves depositing a sample onto a planar stationary phase (often silica gel on glass), and the mixture is separated 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, which are 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 needs to be paired with another confirmatory method of identification.

Raman spectroscopy is a technique that uses inelastic scattering of light to analyze the molecular vibrations of materials that then can be used for identification and differentiation [11,12]. Laser light is focused on a sample (solid or liquid) and the backscattered radiation is collected. There are many advantages of this technique for drug 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, interference due to fluorescence, and subtle instrumental effects that are difficult to understand and control. Additionally, its power of discrimination is decreased when analyzing mixtures due to the resulting complicated spectrum with overlapping peaks [13].

Surface enhanced Raman spectroscopy, SERS, is a variation of Raman spectroscopy that employs metal nanostructures to enhance the signal [12]. The enhancement effect of metal nanostructures was first observed in 1974 on roughened silver electrodes, but its significance was not truly realized until 1977 by Jeanmaire and Van Duyne [14,15]. Metal nanostructures have been seen to enhance the Raman signal by a factor of around 106 but enhancement of as much as 1015 has been reported [16]. Since its discovery, there have been extensive experimental studies but little on the specific enhancement mechanism. Although there continues to be debate on the exact mechanism, it is widely believed that the enhancement is due to three resonances: (1) surface plasmon resonance in the metal nanoparticle, (2) a charge transfer resonance resulting from an electron transfer between the molecule and the metal nanoparticle's conduction band, and (3) the molecule's own resonance [17-18]. SERS is particularly successful for the enhancement of signals from aromatic molecules, thus the application of SERS to the forensic sciences has been focused on the analysis of drugs, both seized and in toxicological samples [19-47], pigments and dyes in fibers and inks [48-57], and explosives [58-94].

Herein, we provide a proof-of-principle analysis for drug mixture separation and spectral identification using TLC-SERS. Further, we constructed four metal nanostructure colloids and compared their ability to enhance the spectra of a diverse range of ten controlled substances: amphetamine, caffeine, cocaine, codeine, diazepam, flunitrazepam, lidocaine, 3,4-methylenedioxymethamphetamine (MDMA), methamphetamine, and phenobarbital.

The Raman spectra, SERS spectra, and TLC-SERS spectra were obtained and the peak height ratio was used to determine spectral enhancement. Together, our data substantiate TLS-SERS to be a viable method of drug separation and identification.

2 Experimental

2.1 Thin-Layer Chromatography

The controlled substances amphetamine, caffeine, cocaine, codeine, diazepam, flunitrazepam, lidocaine, 3,4-methylenedioxymethamphetamine (MDMA), methamphetamine, and phenobarbital were purchased from Sigma Aldrich. 60 µL of 4 mg/mL drug solutions in methanol were spotted on a Whatman LHP-K 10×10 cm glass plates with 200 µm thick silica layer and fluorescent indicator. Drug solutions were allowed to dry for approximately 10 minutes prior to being subjected to the mobile phase (9:1 chloroform: methanol). Plates were in the mobile solution for approximately 20 minutes, removed, and allowed to completely dry. The drug spot separation was visualized on the TLC plate with a Raytech LS5 UV light.

2.2 Raman Spectrometer Analysis

Solid drug sample was first analyzed for reference on a DXR Raman Microscope (Thermo Scientific) equipped with a 780 nm wavelength stabilized single mode diode laser and a 400 lines/mm grating. The aperture used was a 50 µm pinhole resulting in an estimated spectral resolution of 4.7 – 8.7 cm^{-1}. Spectra were collected over a spectral range of 3300 – 150 cm^{-1} at a laser power of 20 mW using the auto exposure mode with a signal to noise ratio (SNR) of 500, and a maximum exposure time of three minutes. Drug solutions were made using solid drug in methanol at 4mg/mL. Dried residues were made by spotting 60 µL of these solutions onto an aluminum foil wrapped microscope slide, thus being consistent with the amount and application of sample used to make the TLC spot, and subsequently analyzed for reference using the aforementioned settings. Three scans, each of three minutes, were determined to provide a standard, optimal spectra and were used for the remainder of the study. For control, drug residues of the 4 mg/mL solutions, and liquid colloids were analyzed five times in three different spot locations to determine variability. A polystyrene reference standard was analyzed prior to analysis to ensure wavelength calibration of the Raman spectrometer.

2.3 Colloid Preparation

Two gold and three silver nanoparticle colloids were prepared and tested in this study. Silver nitrate, gold (III) chloride, hydroxylamine phosphate and the sodium borohydride were purchased from Sigma Aldrich. The sodium citrate and sodium hydroxide both came from J.T. Baker.

2.3.1 Gold Colloids

The first gold colloid (GC1) was prepared according to the published protocol in Cyrankiewicz *et al* [95]. The GC1 gold colloid uses 0.1 ml of the 1 % (w/v) hydrogen tetrachloroaurate solution added to a 40 ml of distilled water while heating and stirring. Then 1 ml of the 1% trisodium citrate solution was added drop wise. The resulting solution was boiled for 5 to 20 minutes or until it turns a light pink color.

The second gold colloid (GC2) was prepared based on a protocol provided by Christopher Palenik, which was adopted for use by Microtrace, LLC, a materials analysis laboratory in Elgin, IL (USA) that specializes in the characterization and identification of small quantities and single small particles of unknown substance. This gold colloid uses 50ml of a 0.5mM gold tetrachloroaurate solution heated to 98 degrees Celsius. 2ml of a 1% (w/v) solution of sodium citrate was added and the solution was kept at 98 degrees Celsius and stirred for half an hour until it turned dark red.

2.3.2 Silver Colloids

The first silver colloid (SC1) was prepared according to the published protocol in Cyrankiewicz *et al* [95]. The SC1 silver colloid was made using 25 ml of the 1mM silver nitrate added in small portions (approximately 2ml) to 75 ml of the 2 mM ice cold sodium borohydride while stirring. The stirring was continued for 10 minutes until the solution had turned a goldish color. It should be noted that the sodium borohydride must be made the day it is to be used, otherwise the color change will not occur.

The second silver colloid (SC2) was prepared based on a protocol provided by Christopher Palenik, which was adopted for use by Microtrace LLC. The SC2 silver colloid was based on the standard Lee and Meisel colloid [96], and was made using 50 ml of the 1mM silver nitrate solution brought to a boil and then 1 ml of the 1% (w/v) sodium citrate was added. A watch glass and a wooden dowel were added to prevent the solution from boiling dry and to prevent bumping. The solution was allowed to boil for a total of 1 hour. The final color was a brownish green and the final volume was between 20 and 40 ml depending on how much the solution was boiled.

The third silver colloid (SC3) was prepared according to 2009 White and Hjortkjaer patented protocol for a stable (>1 year shelf life) aqueous silver colloid solution [97]. The SC3 silver colloid was made by depositing 4.5 ml of a 3.33 mM solution of sodium hydroxide solution in a 7 ml bottle. To this, 100 µl of the 0.077 M hydroxylamine phosphate solution was added. The bottle was then capped and inverted three times. After 30 seconds, 0.5 ml of the 10mM silver nitrate solution was added very quickly and the bottle gently rocked for 1 minute. The final volume was approximately 5 ml and final color was a light grey brown.

SC3 proved to be the fastest and easiest to prepare and effectively enhanced the spectra of the optimization drugs (cocaine, methamphetamine, diazepam and codeine), and, therefore, was selected as the only colloid used with the additional six controlled substances evaluated.

2.4 TLC-SERS Procedure and Calculations

To each developed spot on the TLC plate, 40 µl of colloid was added and then allowed to dry. For each spot on the TLC plate, 5 spectra were collected at distinct locations. The identity of each drug was confirmed by comparing the TLC-SERS spectra to the SERS and normal Raman spectra of the drug.

The amount of spectral enhancement that each colloid provided was the leading criteria in the evaluation of the colloids. The SERS enhancement was calculated from the peak height ratios of the dried drug residues with and without the addition of the metal colloid, but not after TLC separation. During the TLC separation process, as the analytes travel with the carrier solvent up to the plate, they also diffuse outward causing the final spot being larger than the deposited spot. As a result, the density of analyte in the spot decreases, which reduces the amount of Raman scattering. Thus, enhancement was calculated by dividing the area of the highest peak of the SERS spectra by the area of the same peak in the normal Raman spectra of the dried residue of the drug solutions. The peak heights were determined from the averages of the raw spectra without spectral processing (e.g., baseline correction or normalization).

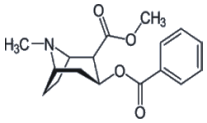
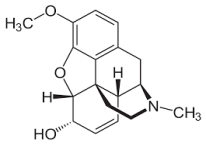
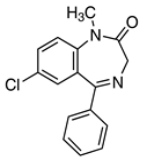
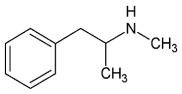
3 Results and Discussion

To determine instrumental parameters and colloid formulation, four drugs (cocaine, codeine, diazepam, and methamphetamine) were used for optimization (termed optimization drugs) of the TLC-SERS technique (Table 2).

These optimization drugs were analyzed using a DXR Raman Microscope to obtain a solid drug reference spectrum (Fig 1A). Normal Raman spectra and SERS spectra of each drug (Fig 1B-C) were collected from the dried residue of the 4 mg/mL drug solutions spotted onto an aluminum foil-wrapped microscope slide in order to replicate the process of deposition onto the TLC plate and also to ensure that dissolution did not alter the drugs' structure (i.e., a polymorphic change) or otherwise interfere with sample identification. The spectra were generated for all optimization drugs (Fig 1; Supplementary Figs 1-3). Moreover, spectra were obtained after application of the GC1, GC2, SC1, SC2 and SC3 colloids (Tables 3-4). The wavenumbers of the peak selected for each optimization drug are 1001 cm^{-1} for cocaine, 628 cm^{-1} for codeine and 1001 cm^{-1} for both diazepam and methamphetamine. The peak heights and ratios were determined for each spectrum to allow for comparison between enhancement methods (Tables 3-4). All colloids showed at least moderate enhancement with the exception of GC2, which exhibited a peak height ratio less than one when used on diazepam (Table 3). The largest enhancement (30× enhancement) was observed using the SC2 on cocaine

(Table 4). Based on the peak height ratios, the SC2 and SC3 colloids showed the highest peak enhancements for optimization drugs.

Table 2. Cocaine, codeine, diazepam, and methamphetamine were used for optimization of the Raman and SERS spectra.

Drug	Classification	Chemical Structure
Cocaine	local anesthetic (ENT)/ stimulant	
Codeine	narcotic	
Diazepam	benzodiazepine	
Methamphetamine	stimulant	

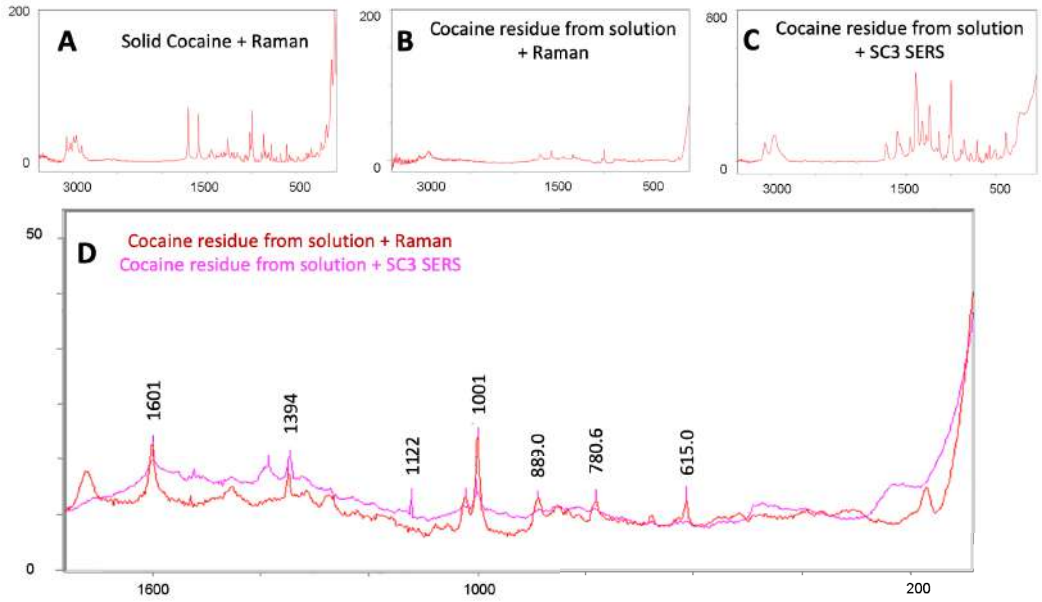


Fig 1. Raman spectra of cocaine solid (A), Raman spectra of cocaine residue from 4 mg/ml solution (B), SERS spectra of cocaine residue from 4 mg/ml solution (C) and spectra of B compared to SERS spectra of cocaine residue separated by TLC with SC3 (D). Note, Figure D is displayed on a full scale to show details of both spectra. Vertical axis is arbitrary units; horizontal axis is wavenumbers (cm^{-1}).

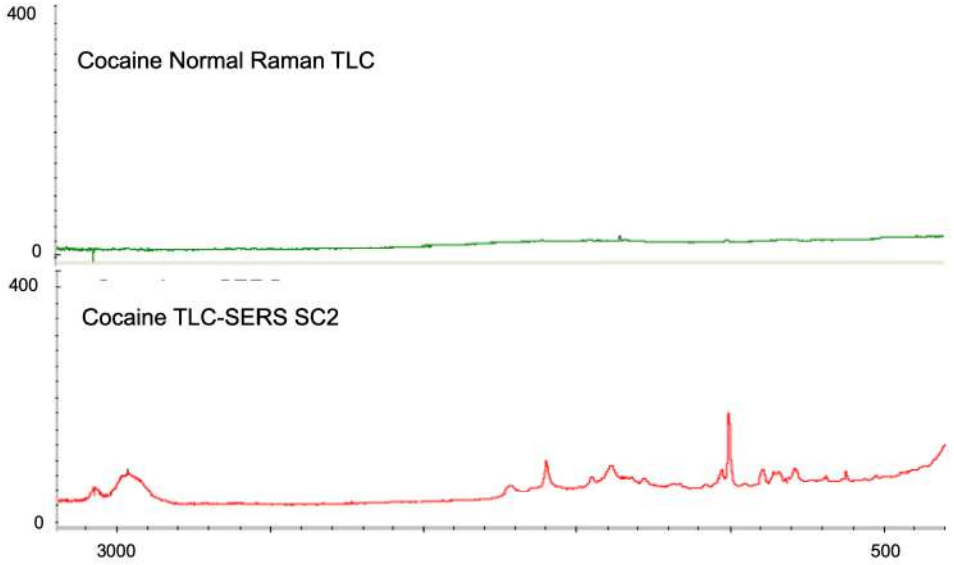


Fig 2. Normal Raman spectra of cocaine solution separated by TLC compared with SERS spectra of cocaine with SC2.Vertical axis is arbitrary units; horizontal axis is wavenumbers (cm^{-1})

Table 3. Peak heights and ratios for the model drugs and the gold colloids.

Drug	Peak Height (Drug solution)	Peak Height (GC1)		Peak Height (GC2)	
		Peak height	Ratio	Peak height	Ratio
Cocaine	17.11	18.99	1.11	31.74	1.85
Codeine	3.61	11.98	3.32	11.38	3.15
Diazepam	28.82	40.27	1.39	11.61	0.402
Methamphetamine	52.53	371.82	7.08	57.85	1.01

Table 4. Peak heights and peak height ratios for the model drugs and the silver colloids. The highest ratio for each drug is in bold.

Drug	Peak Height (Drug solution)	Peak Height (SC1)		Peak Height (SC2)		Peak Height (SC3)	
		Peak height	Ratio	Peak height	Ratio	Peak height	Ratio
Cocaine	17.11	108.03	6.31	513.47	**30.00**	421.98	24.66
Codeine	3.61	34.23	9.49	43.42	**12.03**	7.90	2.10
Diazepam	28.82	42.37	1.47	449.97	**15.61**	184.76	6.41
Methamphetamine	52.53	338.78	6.45	404.56	7.70	557.88	**10.62**

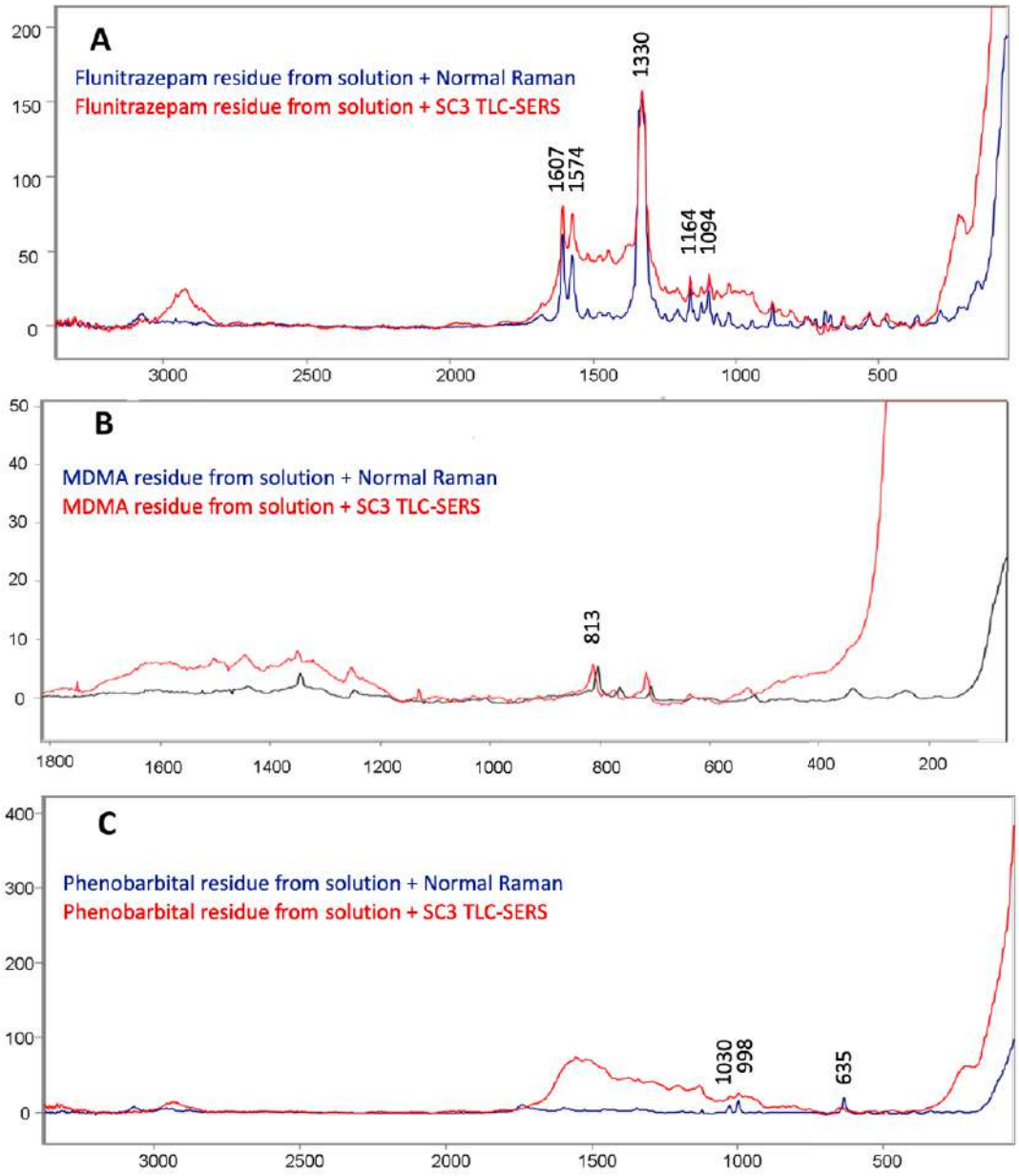


Fig 3. Normal Raman spectra of the residues of 4 mg/ml drug solutions of flunitrazepam (A), MDMA (B), and phenobarbital (C) compared with their respective SERS spectra of the same drug solution separated by TLC with SC3. Vertical axis is arbitrary units; horizontal axis is wavenumbers (cm^{-1}).

Next, we evaluated SERS after TLC separation of the optimization drugs. Prior to analysis of drug samples, a spectrum for the TLC plate alone and with the SC2 colloid was established to account for peak variability (Supplementary Fig 4). There were no substantial peak changes with the application of the colloid onto the TLC plate. Additionally, spectra were taken of a subset of the drugs with no colloid after having been separated using TLC. In this case, no peaks were able to be seen (Fig 2). Similar to the analysis of the drug

samples prior to TLC separation, all four optimization drugs and the five colloids were tested to determine peak height enhancement. When compared to the spectra of the residues of the 4mg/mL drug solution, cocaine, codeine, and methamphetamine subjected to TLC-SERS were undetectable with gold colloids (Tables 5-6). Diazepam spectra was detectable with both gold colloids, however the peak height ratios were less than one (Table 5). With application of the silver colloids, none of the four optimization drugs were detectable using the SC1 colloid (Table 6). Moreover, codeine was undetectable with the SC2 colloid as well, while cocaine, diazepam, and methamphetamine were detectable with a peak height ratio less than one (Table 6). All four optimization drugs exhibited a detectable spectrum when the SC3 colloid was applied, however the peak height ratio was less than one (Table 6). Importantly, while the application of TLC-SERS exhibited a peak height ratio less than one, the reference peak used in the calculation was for the drug solutions prior to TLC, which does decrease the amount of sample due to the separation of the components of the drug. Therefore, it is significant that there are observable peaks with the addition of colloids, in particular with the SC3 colloid, which suggests that enhancement was obtained enough to have identifiable spectral peaks. Since the SC3 colloid offered enhancement for all the optimization drugs, it was selected as the standard colloid used when additional drugs and drug mixtures were evaluated.

Table 5. Peak heights and ratios for the model drugs on TLC plates with gold colloids. A double dash indicates that no peaks were detected.

Drug	Peak Height (Drug solution)	Peak Height (GC1)		Peak Height (GC2)	
		Peak height	Ratio	Peak height	Ratio
Cocaine	17.11	--	--	--	--
Codeine	3.61	--	--	--	--
Diazepam	28.82	2.29	0.079	2.39	0.083
Methamphetamine	52.53	--	--	--	--

Table 6. Peak heights and ratios for the model drugs on TLC plates with silver colloids. A double dash indicates that no peaks were found. The highest ratio for each drug is bold.

Drug	Peak Height (Drug solution)	Peak Height (SC1)		Peak Height (SC2)		Peak Height (SC3)	
		Peak height	Ratio	Peak height	Ratio	Peak height	Ratio
Cocaine	17.11	--	--	2.59	0.151	3.07	**0.179**
Codeine	3.61	--	--	--	--	2.84	**0.795**
Diazepam	28.82	--	--	3.45	0.12	4.84	**0.168**
Methamphetamine	52.53	--	--	9.04	**0.17**	4.74	0.09

Next, our study expanded the number of analyzed drugs to include amphetamine, caffeine, lidocaine, MDMA, flunitrazepam, and phenobarbital (termed expanded drugs). Identifiable peaks were obtained for all of the expanded drugs using both SERS and TLC-SERS (Table 7). Flunitrazepam, MDMA, and phenobarbital all exhibited enhancement with the SC3 colloid (Fig 3). MDMA showed the greatest enhancement with a peak height ratio greater than one (Table 7). Together, these data suggest that TLC-SERS is a viable method and the SC3 colloid formulation could be implemented for optimal enhancement for drug sample mixtures or evidence with limited quantity.

Table 7. Peak heights and ratios for the expanded drugs on TLC plates with the SC3 colloid.

	Selected Peak (cm^{-1})	Peak Height (Drug solution)	Peak Height (SERS)	SERS ratio	Peak Height (TLC-SERS)	TLC-SERS Ratio
Amphetamine	966	33.9	85.4	2.5	5.99	0.2
Caffeine	556	36.7	69.5	1.9	17.8	0.5
Lidocaine	610	4.6	6.5	1.4	1.5	0.3
MDMA	814	10.3	21.2	2.1	11	1.1
Flunitrazepam	1330	136.9	300.3	2.2	127.5	0.9
Phenobarbital	998	16.7	35.8	2.1	5.5	0.3

4 Conclusions

In summary, TLC-SERS is a simple, selective and sensitive method for the separation and identification of controlled substances. After significant experimentation, the SC3 colloid shows the most promise for the analysis of controlled substances by TLC-SERS. SC3 showed good enhancement for the majority of drugs, is inexpensive to produce, easy to make and has a long shelf life (>1 year). Although TLC-SERS using the SC3 colloid was able to identify all separated drugs, continued research on alternative colloids which provide better enhancement is recommended. The construction of the noble metal substrate required to get a SERS signal is a significant area of SERS research in other disciplines, and thus it is not surprising that it would also be an area of future research with respect to this application.

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. Although, the number of controlled substances analyzed in this study is small, the diverse types of drugs analyzed demonstrates potential TLC-SERS has for the identification of the variety of drugs analyzed in a forensic laboratory. Further, TLC-SERS conforms to the currently accepted standards for the identification of seized drugs set forth by ASTM E2329-17 [1]. Ultimately, the obtained results establish the reliability and feasibility of TLC-SERS for the rapid, sensitive and selective analysis of controlled substances.

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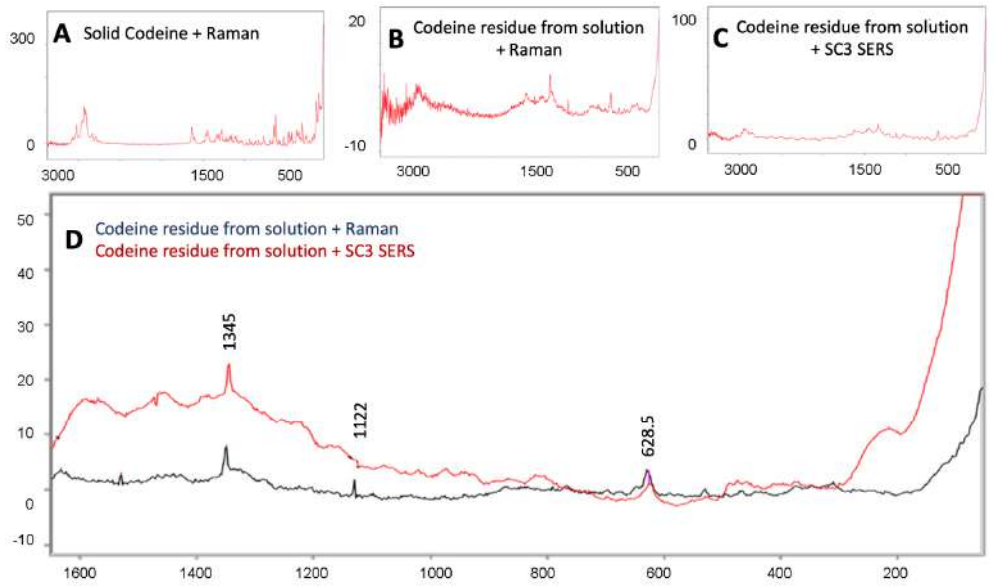
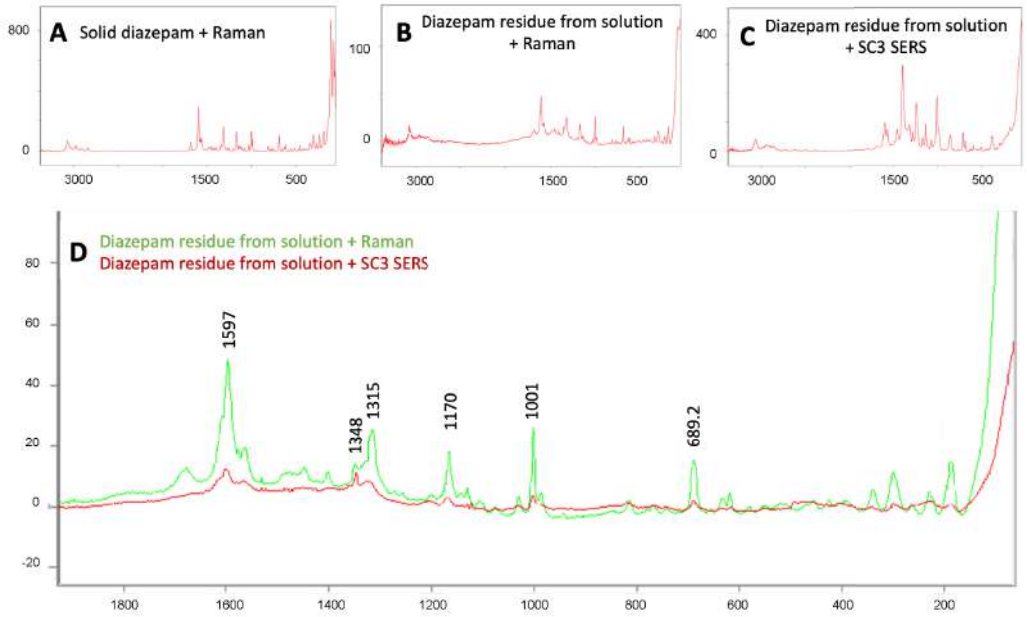
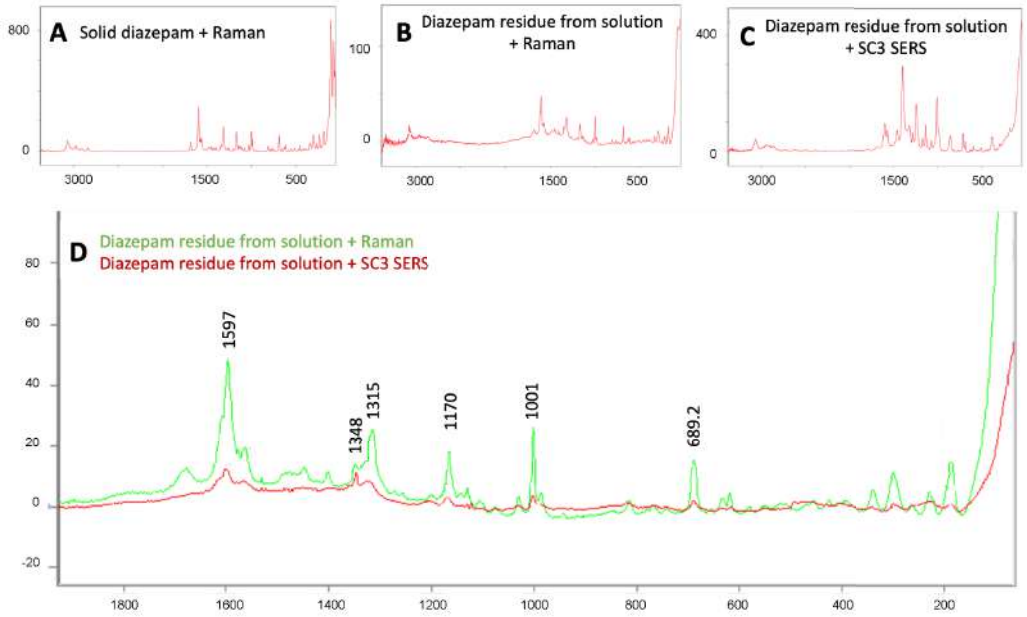
Supplementary Figures

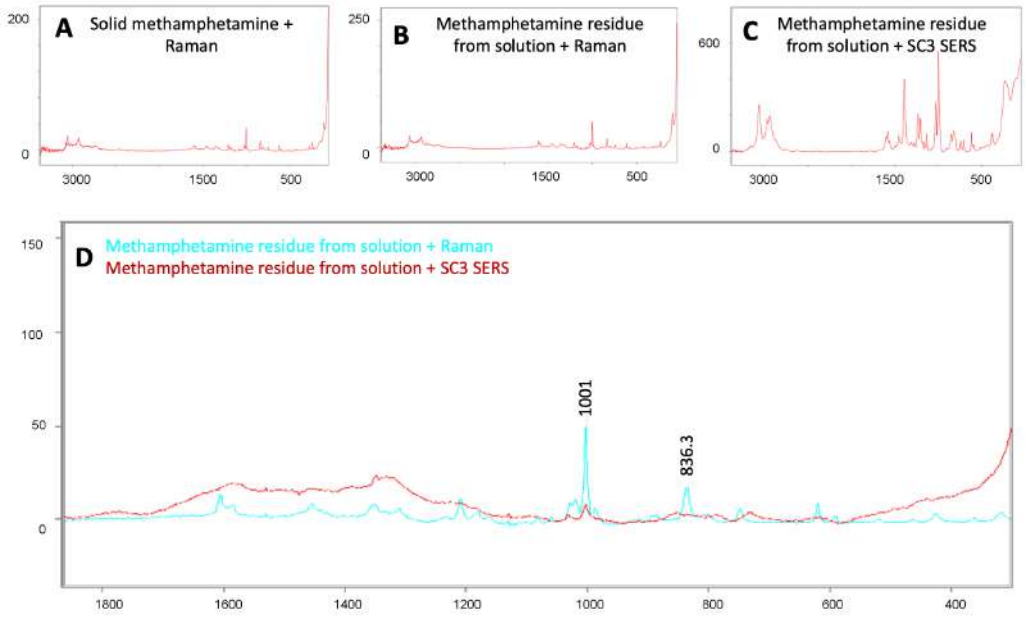


Supplementary Fig 1. Raman spectra of codeine solid (A), Raman spectra of codeine residue from 4 mg/ml solution (B), SERS spectra of codeine residue from 4 mg/ml solution (C) and spectra from B compared to SERS spectra of codeine residue separated by TLC with SC3 (D). Note, Figure D is displayed on a full scale to show details of both spectra. Vertical axis is arbitrary units; horizontal axis is wavenumbers (cm^{-1}).

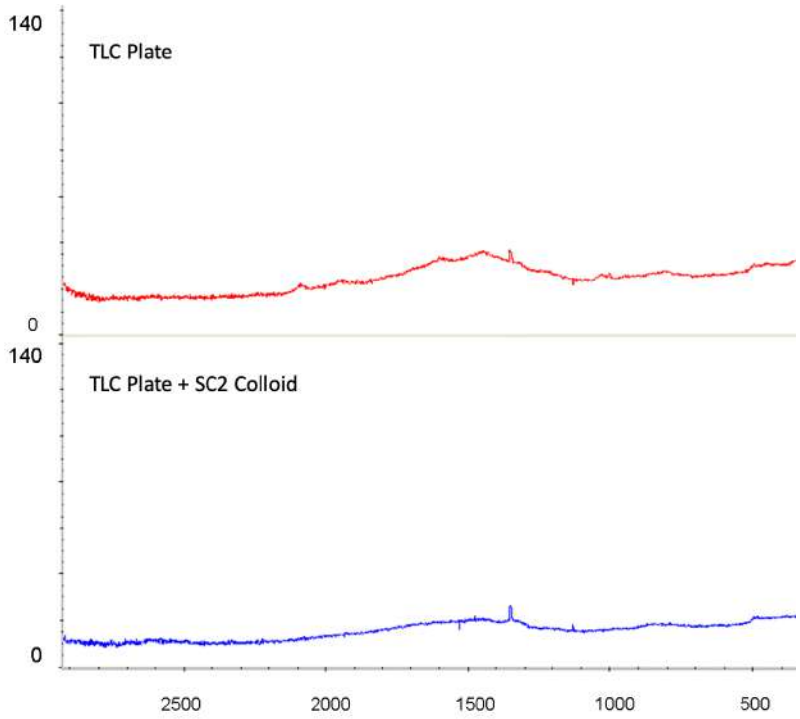




Supplementary Fig 2. Raman spectra of diazepam solid (A), Raman spectra of diazepam residue from 4 mg/ml solution (B), SERS spectra of diazepam residue from 4 mg/ml solution (C) and spectra from B compared to SERS spectra of diazepam residue separated by TLC with SC3 (D). Note, Figure D is displayed on a full scale to show details of both spectra. Vertical axis is arbitrary units; horizontal axis is wavenumbers (cm^{-1}).



Supplementary Fig 3. Raman spectra of methamphetamine solid (A), Raman spectra of methamphetamine residue from 4 mg/ml solution (B), SERS spectra of methamphetamine residue from 4 mg/ml solution (C) and spectra from B compared to SERS spectra of methamphetamine residue separated by TLC with SC3 (D). Note, Figure D is displayed on a full scale to show details of both spectra. Vertical axis is arbitrary units; horizontal axis is wavenumbers (cm^{-1}).



Supplementary Fig 4. Normal Raman spectra of the TLC plate and the TLC plate with SC2 to show that there is no interference from either. Vertical axis is arbitrary units; horizontal axis is wavenumbers (cm^{-1}).

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