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## Surface-Enhanced Raman Spectroscopy for Cocaine Detection

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# **Surface-Enhanced Raman Spectroscopy for Cocaine Detection**

An honors thesis presented to the  
Department of Chemistry,  
University at Albany, State University of New York  
in partial fulfillment of the requirements  
for graduation with Honors in Chemistry  
and  
graduation from The Honors College

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Research Advisor: Igor Lednev, Ph.D.

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## **Abstract**

The detection of drugs of abuse using Raman spectroscopy is of particular interest to forensic researchers at the moment. Raman spectroscopy is highly specific, fast, non-destructive, and can be adapted for in-situ measurements, making it the ideal forensic technique. Researchers working under the guidance of Doctor Igor Lednev have been able to use UV resonance Raman spectroscopy to detect cocaine in oral fluid without the need for sample pretreatment. They were however unable to detect the cocaine at forensically relevant levels. To overcome this limitation, surface-enhanced Raman spectroscopy (SERS) was used in this proof of concept study for the detection of trace amounts of cocaine. Solutions of 1 mg/mL- 5 ng/mL, which are forensically relevant concentrations of this drug, were tested using homemade SERS substrates. Raman peak identification was performed as well as additional analysis using specialized statistical software. It was concluded that forensically relevant levels of cocaine could be detected using SERS. The future of this project is the detection of cocaine in saliva.

## Acknowledgments

I would first like to thank my family for always supporting me and giving me all the encouragement I needed to learn and grow. I would like to thank all of my friends for reminding me I am not alone. I would especially like to acknowledge all that Jalissa Thomas has done for me, I could not have done this without her help and I would not have wanted to. I would like to thank Mrs. Lewis for inspiring me to be a chemist, and helping me get to university. I would also like to thank Professor Paul Toscano who has always been a great help, and has some of the best stories about his days as a researcher. Another thanks to Dean Chang for all the encouragement and allowing me the space to talk through my problems with someone who really cares. I have to thank everyone at Lednev Lab who has taught by example how to be a good scientist and an even better person. A very special thank you to Lamyaa Almehmadi, who is a guiding light to me, and will make a truly wonderful professor one day. Without her patience and careful instruction none of this would be possible. And lastly thank you to Dr. Lednev, for allowing me to be a part of the most wonderful lab. I must thank him for all that he has taught me, and all the time he has given. And thank you for giving me the coolest project in the world.

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## **Introduction:**

The detection of cocaine is of vital importance for forensic purposes. According to the Centers for Disease Control, cocaine is an ever-growing problem as deaths caused by overdoses of cocaine and its derivatives have been on the rise since 2012<sup>5</sup>. More recently, deaths involving cocaine and a mixture of opiates and other drugs have surged, causing the problem to worsen<sup>9</sup>. In order to put a stop to this, forensic scientists need to be able to rapidly and confidently test for cocaine. When testing for cocaine, the method should be accurate and specific which is a challenge because scientists analyze evidence in trace amounts. New methods have to meet certain standards set by SAMHSA, or the Substance Abuse and Mental Health Services Administration. SAMHSA defines the cut-off values acceptable for a method's limit of detection (LOD). The government publishes an updated list of cut-off values every year, where each level is specific to the test used, the compound of interest, and the sample matrix, oftentimes a body fluid like blood. The cut-off level for cocaine in oral fluid is 20 ng/mL of saliva for a presumptive test, and 8 ng/mL for a confirmatory tests. As of now the most common techniques for detection are chromatographic and enzyme immunoassay techniques. These current techniques can be time-consuming and do not offer the sensitivity of surface-enhanced Raman spectroscopy (SERS). Additionally due to the fact that the power of SERS comes from the substrates and not the instrument this method could easily be adapted for a portable Raman spectrometer and allow for in-situ sample analysis at either crime scenes or road-side stops<sup>10</sup>.

The enhancement of Raman signal achieved with SERS happens when molecules interact with nanoparticles of gold or silver. This occurs due to chemical and electromagnetic factors<sup>7</sup>. It has been found that gold increases cocaine's signal the most<sup>6</sup>. The substrates were made by electrochemical deposition onto a solid surface. Then the solutions of cocaine dissolved in

acetonitrile were deposited on the solid substrate and measured using typical Raman settings for cocaine. SERS depends on the analyte being in contact with a hot-spot. Hot-spots are the spaces in-between where two of the gold nanoparticles are close to each other<sup>1</sup>. It's in these spots that the strongest signal from cocaine can be detected.

Already the power of Raman spectroscopy (RS) was demonstrated for detecting cocaine in oral fluid without the need for sample preparation<sup>4</sup>. However, due to the enhancement of the signal SERS is capable of detecting cocaine at below the cut-off levels. Currently, the cut-off levels for cocaine in oral fluid are 20 ng/mL for preliminary tests, and 8 ng/mL for confirmation. Using SERS substrates cocaine can be detected at 5 ng/mL.

## **Methods:**

### *SERS Substrate Components*

Substrates were created using a silicon substrate with a coat of Cr/Au and a cyanide-gold solution "Pure Gold SG-10". The following reagents were used for cleaning the substrate, the glass petri dish that housed them for transportation and the tweezers used for manufacturing; ethanol, DI water, acetone, and Nanopurified DI water created by SUNY Polytechnic.

The cocaine (COC) was obtained from Cerilliant (Round Rock, TX, USA) and diluted in acetonitrile (ACN). All spectral measurements were obtained using a Renishaw inVia Raman Microscope; 785 nm excitation, laser at 50% power, wavenumber range 400 – 2200  $\text{cm}^{-1}$ . Mapping of the substrate surfaces was done from 0-80 microns along the x axis and 0-40 microns along the y axis with 10 micron steps each. A total area of 4500 microns was measured and 45 spectra were taken.

### *Rationale*

The experimentation began by initially testing and confirming that SERS substrates could be used to detect 5 ng/mL of COC. The study originally was undertaken after researchers showed that RS was capable of detecting COC in oral fluid without costly sample pretreatment<sup>4</sup>, and after solid electroplated gold substrates were shown to be able to detect a single protein<sup>1</sup>. The solid substrate was chosen due to its stability and the ease at which it could possibly be used *in situ*<sup>10</sup>.

### *Substrate Manufacturing*

The substrates were manufactured by electroplating a solid silicon substrate with gold from the cyanide-gold solution. Before the deposition of gold the silicon substrate was cut into rectangles measuring 1 by 3 cm. A 1 by 1 cm area of this substrate was attached to an electrode and submerged in the cyanide-gold solution. A constant potential of 4.9 V was applied for 30 seconds. The substrate was cleaned with Nanopurified DI water and ethanol and acetone. Substrates were checked for contamination and only those that were uncontaminated were used.

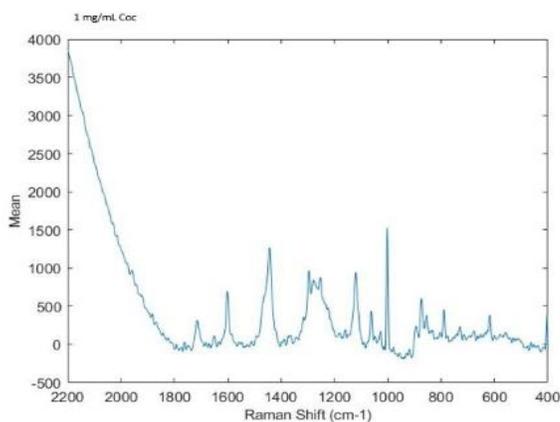
### *Cocaine Solutions*

The cocaine solutions of 20, 8, and 5 ng/mL were made by dilution of a 3.3  $\mu$ M solution of COC. Once the solutions and substrates were made 4  $\mu$ L of each sample was placed on a substrate and the acetonitrile was given five minutes to evaporate. This same procedure was used every time, including for measurements made on aluminum substrates.

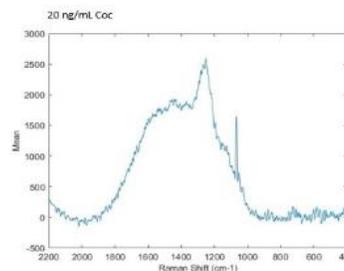
## Results and Discussion:

### *Preliminary Analysis*

Initial testing was done under standard conditions, the sample was deposited on an aluminum substrate all other parameters were kept the same. The rationale for this was two-fold. One to compare the spectra obtained for the standard COC on aluminum and the SERS spectra in order to be certain that any interaction with the SERS substrate did not change the identifying peaks. Two this was used as a control, showing that without the use of SERS substrates traces of COC at the relevant levels are undetectable. The standard, 1 mg/mL COC, was measured on aluminum along with 20 ng/mL, 8 ng/mL, and 5 ng/mL. On the prepared aluminum slide, in marked sections, 4  $\mu$ L of each solution were deposited and then measured.



**Figure 1:** Standard COC at 1 mg/mL in acetonitrile measured on aluminum using 20x objective, 785 nm excitation, 50% laser power, 20-sec acquisition, wavenumber range 400 – 2200  $\text{cm}^{-1}$ . Preprocessing done in Matlab, Baseline (Automatic Weighted Least Squares), SavGol smoothing



**Figure 2:** 20 ng/mL COC measured on an aluminum substrate under standard conditions. Preprocessing done in Matlab. Only one peak is detectable at 1060  $\text{cm}^{-1}$ .

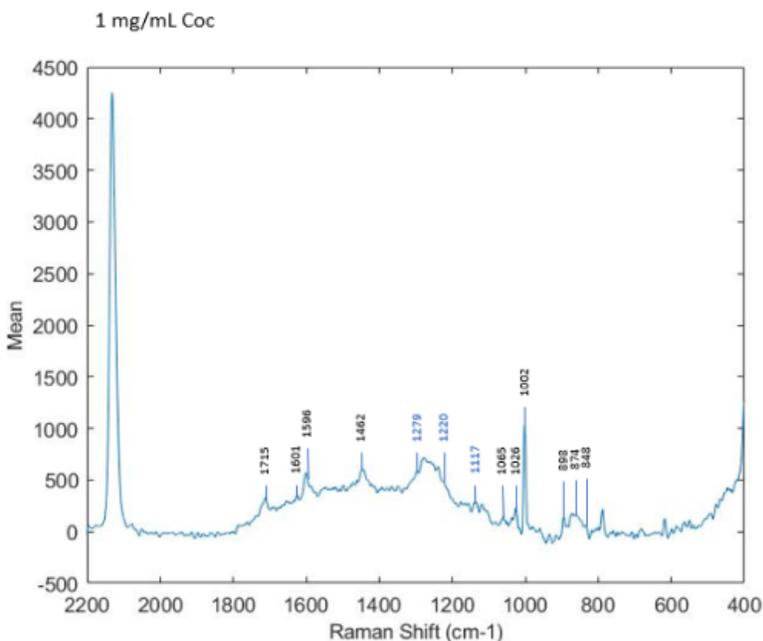
Of the four concentrations, only the standard and 20 ng/mL had measurable peaks.

However, the spectrum obtained for 20 ng/mL was not identifiable as COC.

Another control was tested, acetonitrile on the SERS substrate, to determine if there was any contribution from the solvent in the spectra. The acetonitrile was given five minutes to

evaporate, the same amount of time the COC solutions were given. There is nothing to report for this control, as no peaks were found.

### Standard Solution Analysis



**Figure 3:** Spectrum of standard COC at 1 mg/mL in acetonitrile measured on a SERS substrate. All peaks attributed to COC are visible. The peaks labeled in black have tentative assignments. The peak at 2100  $\text{cm}^{-1}$  is attributed to the manufacturing process of the substrates.

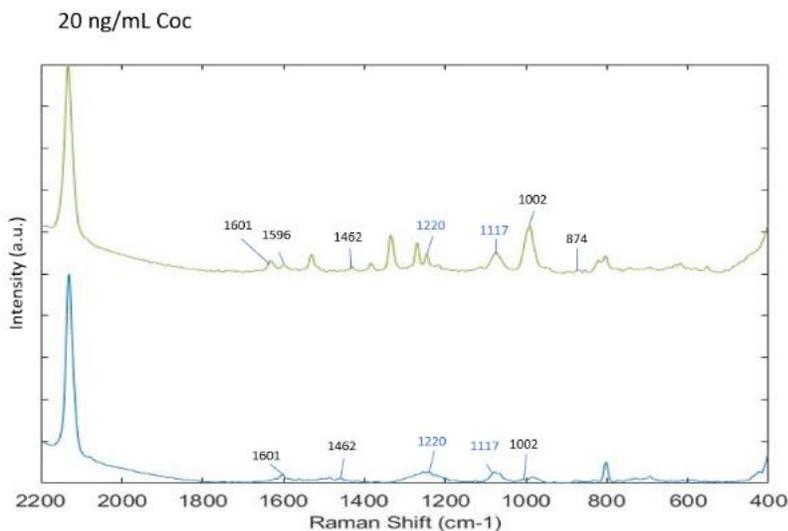
Following the confirmation that acetonitrile would not interfere with the signal, and that enhancement was needed to detect trace concentrations, 4  $\mu\text{L}$  of the standard solution was measured using SERS. The signal was easily detectable, as enhancement was not

needed, but mapping was done to provide plenty of spectra, should some be contaminated. All of the peaks that were characteristic of cocaine were present, and many of them had tentative assignments. The peaks at 848, 874, and 898  $\text{cm}^{-1}$  are from stretching of the C-C bond in the tropane ring. The highest peak at 1002  $\text{cm}^{-1}$  is symmetric stretching occurring in the aromatic ring. The two most common peaks to find are at 1462  $\text{cm}^{-1}$  and 1601  $\text{cm}^{-1}$ , respectively those peaks represent asymmetric  $\text{CH}_3$  deformation and C=C stretching in the aromatic ring. The two unassigned peaks occur in COC spectra often as well and despite not having a tentative assignment in literature were clearly attributed to the cocaine<sup>3</sup>. The identification of COC in the spectra taken was done by comparing the peaks to the spectrum above. As there was often

interference from contaminants on the substrates and lower peak intensities a minimum of four peaks was used for positive confirmation of the presence of COC. In another study of the application of SERS for detecting trace amounts of COC, a minimum of two peaks was used. The four peak minimum was chosen for this study due to the fact that the contaminants in the SERS substrates often occurred around 990, and 1250  $\text{cm}^{-1}$ . At first glance, those peaks could appear to be COC therefore as a safeguard four peaks were chosen as the minimum.

### *Cut-off Concentration Analysis*

The next step was to collect spectra of the diluted solutions on SERS substrates. The



**Figure 4:** COC at 20 ng/mL in acetonitrile measured on a SERS substrate. Many spectra were identified as containing COC, the two with the most peaks were selected as examples to be displayed for clarity.

three concentrations measured were 20 ng/mL, 8 ng/mL, and 5 ng/mL. For each of these concentrations over 90 individual spectra were taken. The spectra were evaluated individually and peaks were found using the automated option in PLS Toolbox, as well

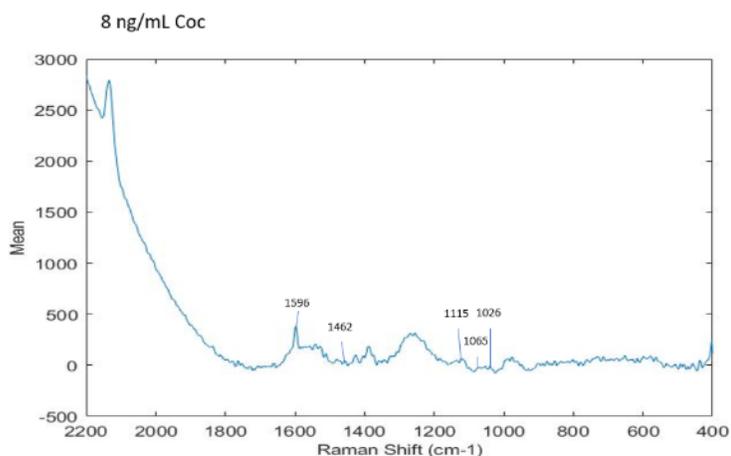
as using the cursor. In Figure 4 it is clear that where an aluminum substrate could not measure COC at this level it is entirely possible with the SERS substrate. Multiple peaks were measured, many of them attributable to specific movement within COC. In the spectra for 20 ng/mL the peaks at 1002, 1462, and 1601  $\text{cm}^{-1}$  are clearly visible, as is the peak at 1596  $\text{cm}^{-1}$ . This peak only occurs in COC HCl and along with the peak at 1601  $\text{cm}^{-1}$  represent the C=C stretching in the aromatic ring<sup>3</sup>.

After it was confirmed that the SERS substrates could be used to measure COC at the first cut-off level the 8 ng/mL concentration was tested. Again multiple peaks were found, though identification proved more difficult as the strongest peak at 1002cm<sup>-1</sup> did not appear in many spectra. When it did it was often alone, or with one or two other peaks, not enough for identification. Despite not being able to use the peak at 1002 cm<sup>-1</sup> alone for identification COC was measured at 8 ng/mL. In

Figure 5 several peaks are seen, including the peak at 1026 cm<sup>-1</sup> which is attributed to asymmetric stretching in the aromatic ring in COC HCl<sub>3</sub>. The concentration of 8 ng/mL is the lowest cut-off level a forensic test must be able

to achieve if it is to be used to

measure COC in oral fluids, however, a lower concentration was tested as well in order to prove that SERS has the sensitivity required of forensics and beyond.

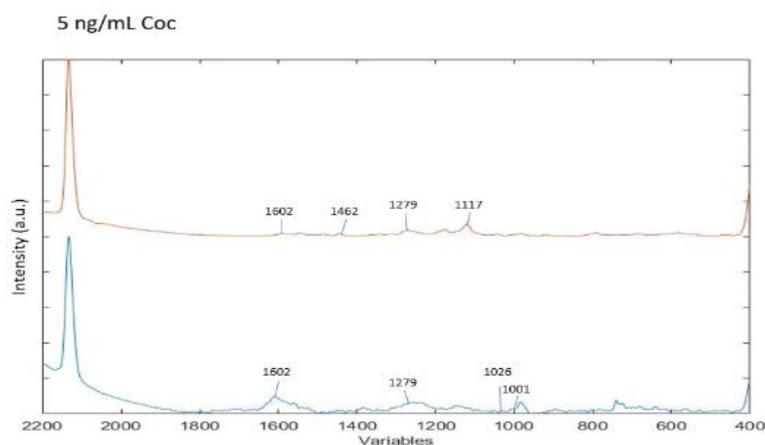


**Figure 5:** COC at 8 ng/mL in acetonitrile measured on a SERS substrate. This spectrum was selected as an example due to the distinct peaks.

level a forensic test must be able

to achieve if it is to be used to

measure COC in oral fluids, however, a lower concentration was tested as well in order to prove that SERS has the sensitivity required of forensics and beyond.



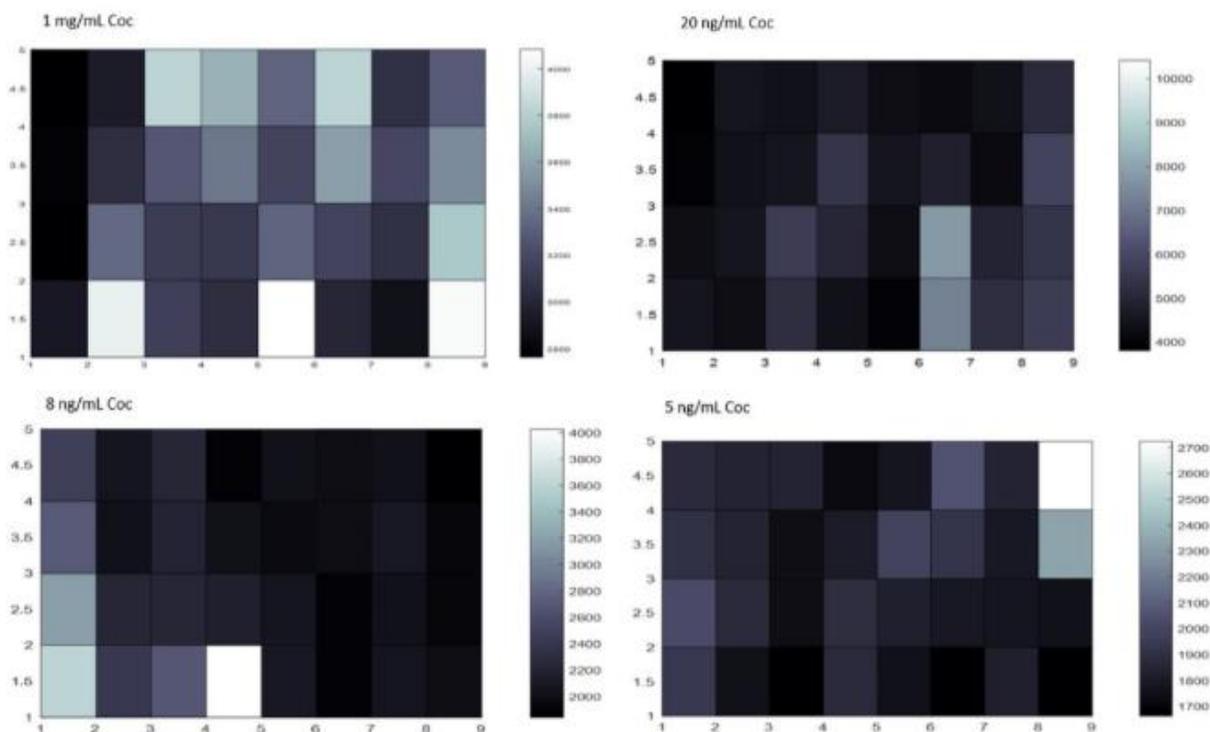
**Figure 6:** COC at 5 ng/mL in acetonitrile measured on a SERS substrate. Peak intensity is minimal, but, several peaks are visible. 135 spectra were collected, 5 contained COC.

A solution of 5 ng/mL COC in acetonitrile was tested on the SERS substrate. One of the peaks that appear is 1279 cm<sup>-1</sup> this is attributed to C-N stretching, which may also occur due to the process of creating the substrate,

which is why it is important to have at least 4 peaks to confirm the presence of COC. The intensity of the peaks from spectra varied due to the changes in enhancement that occur when molecules are not directly in a hot spot. Spectra that were collected at a hot spot had more intense peaks like the bottom spectrum in Figure 6. These peaks were of equal or comparable intensity to 8 and 20 ng/mL.

### *Substrate Mapping Analysis*

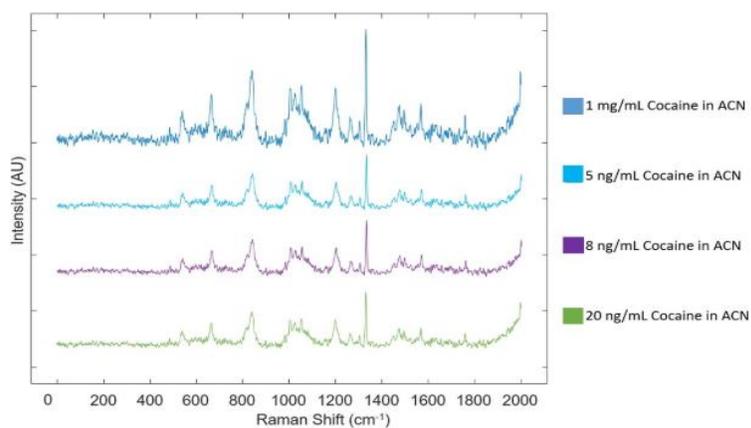
After COC had been measured and identified a map of the substrate was created to show the distribution of the COC across the surface. This was done in order to visualize occupied hot spots and to see how much concentration affects the spread of hot spots. Figure 7 below depicts four “heat maps”. Each rectangle depicts an area of 10 by 10 microns in which a spectrum was collected. The colors represent the intensity of the point  $1000\text{ cm}^{-1}$  of each spectrum, the lighter the color the higher the intensity. Places that are white, or light blue, are points where a COC signal was found. These hot spots are where the signal enhancement happens. The difference between the three cut-off and below concentrations is negligible, and hot spots were not needed to measure the standard COC solution. These figures show the relatively low density of hot spots on the substrate, but they also show that there is not a large difference in enhancement between 20 and 5 ng/mL. This would suggest that the LOD of this method could be significantly lower than 5 ng/mL.



**Figure 7:** Heat maps of the intensity of the point at  $1000\text{ cm}^{-1}$ . This point was chosen as the peak at  $1002\text{ cm}^{-1}$  was very strong, but did not appear as sharp or as often at  $8\text{ ng/mL}$ , and broadened significantly when at low concentrations.

### HAMAND Analysis

The final analysis of the spectra was done using a software program called HAMAND.



**Figure 8:** The spectra of cocaine after processing in HAMAND all the peaks for cocaine are present and very clear. Visual identification is possible and intensity is relatively similar between  $20$ ,  $8$ , and  $5\text{ ng/mL}$ .

This stands for hypothetical addition multivariate analysis with numerical differentiation. The

true applications and potential faults of this software are still being tested. HAMAND separates known spectral components from a spectrum that may contain interferences<sup>2</sup>. When asked to find the spectral components of COC, using the spectrum generated on an aluminum substrate, HAMAND found COC in spectra with only one visible peak. HAMAND was not used as a primary tool for the identification of COC due to the fact that its limits and drawbacks are still being looked into. However, it does suggest that the spectral components of COC are indeed there; even when less than 4 peaks can be seen.

### **Conclusion:**

In conclusion, the research presented here proves that Surface Enhanced Raman Spectroscopy is a viable technique for detecting trace amounts of cocaine. SERS offers the sensitivity and specificity that make it a promising tool for the future of forensic science. This is possible due to the enhancement that occurs in hot spots of the SERS substrates.

The goal of this experiment was to explore the possibility of using SERS to detect forensically relevant levels of cocaine. This was accomplished and using a four peak minimum for positive identification cocaine was detected at a concentration as low as 5 ng/mL. Mapping of the substrate's surface is used to clearly visualize the hot spots and to make inferences in regards to how the solution dispersed along the substrate surface. Analysis using HAMAND indicated the presence of all spectral components of cocaine.

With further development and testing the LOD of COC in saliva can be found for this method, and as indicated by the results above this will be a forensically viable test.

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