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ULTRAVIOLET RESONANCE RAMAN SPECTROSCOPY FOR THE DETECTION OF COCAINE IN ORAL FLUID

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ABSTRACT

Detecting and quantifying cocaine (COC) in oral fluid (OF) is of significant importance for practical forensics. Up to date, mainly destructive methods or biochemical tests have been used, while spectroscopic methods were only applied to pretreated samples. In this work, the possibility of using resonance Raman spectroscopy (RRS) to detect COC in OF without pretreating samples was tested. It was found that ultraviolet resonance Raman spectroscopy (UVRRS) with 239-nm excitation allows to detect COC in OF at a concentration as low as 10 μg/mL. Further method development will be needed for reaching the practically useful levels of COC detection.

Introduction

The detection of COC in OF is of great importance for practical forensics due to a high worldwide COC consumption and to the ease of obtaining OF samples. Up to date, the majority of the methods used to detect COC in OF are based on immunological procedures or chromatographic techniques coupled with mass spectrometry or tandem mass spectrometry (GC-MS or LC-MS). However, enzyme immunoassays (EIAs) involve a significant probability of false negative and false positive results. As a consequence, these methods are only used for preliminary screening followed by further analyses to confirm the results. Hyphenated chromatographic techniques are used as confirmatory tests, but they are destructive and require complex sample pretreatment, which makes the analysis cost- and time-consuming [1-6]. Most recently, spectroscopic techniques have been utilized to carry out rapid and confirmatory analyses using small sample quantities. However, due to the strong interference from OF, a prior extraction and/or preconcentration pretreatment was needed before the spectroscopic analysis could be conducted [7].

Raman spectroscopy (RS) allows for confirmatory identification of COC and other illegal drugs of abuse for forensic purposes [8]. Typically, near-IR light is used for exciting Raman scattering to combat fluorescence interference and sample damage. Sands et al. [9] demonstrated that COC as well as other narcotics and explosives could be efficiently identified using RRS with an ultraviolet (UV) laser emitting at 244 nm. They reported RS and RRS spectra of pure COC powder as well as that of COC mixed with a "scouring" compound. Resonance excitation allowed to avoid the fluorescence interference and identify COC in both samples [9].

In this preliminary study, instead, it was evaluated the capability of RRS with UV excitation to detect COC in liquid OF, without any sample pretreatment. The UV-visible absorption spectrum of COC exhibits two electronic transitions at ~200 nm and ~230 nm. UVRRS with 239 nm excitation was utilized for characterizing liquid OF samples doped with various concentrations of COC and the detection limit (LOD) was estimated.

Material and methods

Chemicals and sample preparation

Standard COC used to prepare samples was purchased from Cerilliant (Round rock, TX, USA) as 1 mg/mL acetonitrile solution. OF samples were purchased from Bioreclamation Inc. (Hicksville, NY, USA). All aqueous solutions were prepared in distilled water.

Since standard COC was initially dissolved in acetonitrile, various amounts of the standard COC solution were evaporated under vacuum for 30 min at 25 °C to remove the organic solvent, and then redissolved in the same volumes of OF. The concentration of COC in the prepared samples of OF varied from 1 mg/mL to 1 µg/mL. A reference solution of COC in water was also prepared by using the same procedure. Liquid samples were then transferred in quartz NMR tubes and directly analyzed.

Instrumental

Resonance Raman (RR) spectra were recorded using a homebuilt Raman spectrograph equipped with a liquid nitrogen cooled CCD camera (Roper Scientific, Inc., Sarasota, FL, USA) [10]. Pulsed laser radiation (pulse duration 6-7 ns, repetition rate 50Hz) at 239 nm (the first anti-Stokes

component of the Stimulated Raman Scattering of the fourth harmonic of the Nd:YAG laser) was used for excitation. The radiation power on the sample was 10 mW. The spectra were recorded using the WinSpec 32 software (Roper Scientific, Inc., Sarasota, FL, USA) in the range of 500-1900 cm-1. Each spectrum was obtained by averaging 20 accumulations with 30 s acquisition time for every accumulation.

Spectral treatment

The collected Raman spectra were processed using Thermo Scientific OMNICTM for dispersive Raman 8.3.103 software (Waltham, MA, USA): the fluorescence contribution was corrected using a 3rd order polynomial baseline correction and the noise was reduced by an 11-point smoothing. In order to estimate the LOD, the reference spectrum of pure OF was subtracted from the spectra of COC-doped OF samples using the automatic subtraction algorithm of the software employed.

Results and discussions

Liquid samples of OF doped with COC concentrations between 1 mg/mL and 1 µg/mL were analyzed in NMR quartz tubes at 239-nm excitation. An aqueous solution of pure COC and a sample of pure OF were also analyzed for comparison. A liquid sample was constantly stirred with a magnetic bar to avoid photodamage. Each UV Raman spectrum represented an average of short (30 s) accumulations. The individually accumulated spectra were compared to check for possible photodamage prior averaging. No apparent photodegradation was found under the used experimental conditions.

In Figure 1, the normalized RR spectra are shown for an aqueous solution of 1 mg/mL COC, a pure OF sample, and an OF sample doped with 100 µg/mL of COC. The spectrum of pure OF was subtracted from that of a 100 µg/mL COC-doped OF sample, and the resulting difference spectrum is also shown in figure 1. One can appreciate that both COC and OF have characteristic Raman bands, which allow for their differentiation. Although, some bands (998 cm-1 band in particular; circled in Fig. 1) are present in both spectra.

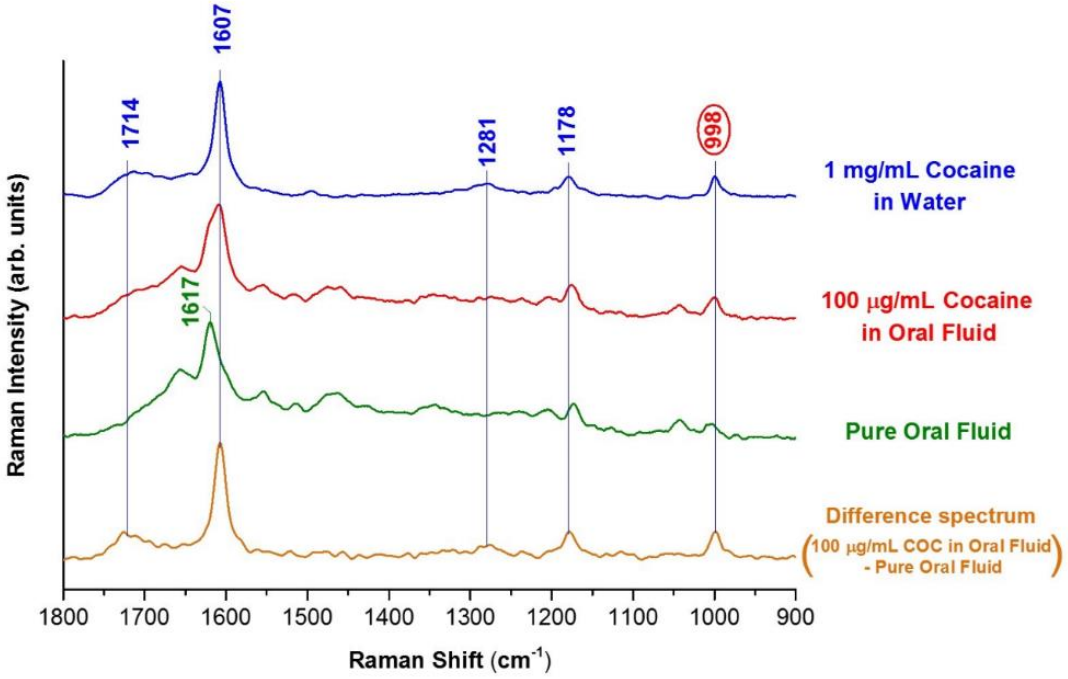

Figure 1. Resonance Raman spectra of 1 mg/mL COC in water, 100 µg/mL COC in OF, pure OF, and a difference spectrum between those of COC in OF and the pure OF. Excitation wavelength was 239 nm and excitation power was 10 mW.

The spectra of COC in aqueous solution and in OF (difference spectrum in figure 1) are close to each other and resemble well the Raman spectrum of solid COC hydrochloride obtained at 244-nm excitation [9]. Raman band at 998 cm-1 can be tentatively attributed to the symmetric breathing mode of the phenyl ring in COC molecule. Raman peak at 1607 cm-1 and a broad band centered at 1714 cm-1 can be tentatively assigned to the trigonal phenyl ring breathing mode and to the ester carbonyl C=O stretch, respectively [9, 11]. Bands at 1281 and 1178 cm-1 could be assigned to the vibrational modes of the benzoate group in the COC molecule, in particular to the C=O stretching and the CH in-plane bending, respectively [12]. Finally, the OF band at 1617 cm-1 could be attributed to vibrational modes of proteins commonly present in that matrix [13].

In order to determine the LOD of COC in OF, OF samples doped with lower concentrations of COC, between 50 µg/mL and 1 µg/mL, were analyzed. Figure 2 shows the spectra obtained after the subtraction of the pure OF spectrum from the spectra of the samples doped with the different concentrations of COC.

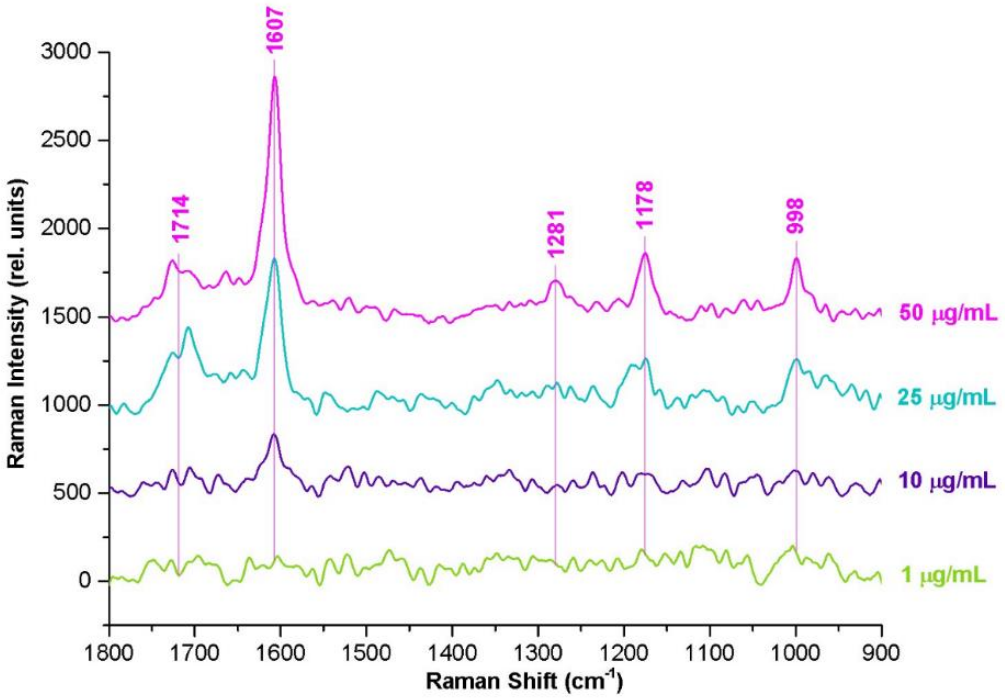

Figure 2. Difference Raman spectra obtained after the subtraction of the OF spectrum from the spectra of the OF samples doped with COC at the indicated concentrations. Excitation wavelength was 239 nm and the excitation power was 10 mW.

The LOD could be estimated by monitoring the gradual disappearance of the characteristic bands of COC in the difference spectra until the strongest band (1607 cm-1) reached a signal-to-noise ratio of approximately 3. As evident from figure 2, COC was sufficiently detectable until a concentration of 10 μg/mL. In fact, for the sample doped with a COC concentration of 1 μg/mL, no bands were observed in the difference spectrum.

Conclusions and future trends

In this work, the potential of UVRRS for the detection of COC in OF was investigated. The current methods used to detect COC in OF require typically a complex pretreatment of the sample, which complicates its practical application for in situ analyses. In this study, instead, untreated COC-doped OF samples were directly analyzed by UVRRS employing a laser excitation at 239 nm. At this wavelength, the absorption of OF is almost insignificant, so that only the Raman signal of COC could be resonantly enhanced, and the direct analysis of doped OF samples proved to be possible.

The subtraction of pure OF spectrum from the COC-doped OF spectra allowed to estimate a LOD of 10 μg/mL that, however, is not yet enough for immediate practical application, if compared with the currently established cut-off value of 8 ng/mL [14].

Further improvement in the LOD is then necessary for practical forensic applications of this methodology. In particular, the excitation with 200-nm light could result in more significant enhancement of COC Raman signal due to the presence of a shorter wavelength electronic transition. This study is currently underway in our laboratory.

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