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ORIGINAL PAPER

Raman spectroscopic investigation of cocaine hydrochloride on human nail in a forensic context

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Abstract This study describes the application of Raman spectroscopy to the detection of drugs of abuse and noncontrolled substances used in the adulteration of drugs of abuse on human nail. Contamination of the nail may result from handling or abusing these substances. Raman spectra of pure cocaine hydrochloride, a seized street sample of cocaine hydrochloride (77%), and paracetamol could be acquired from drug crystals on the surface of the nail. An added difficulty in the analytical procedure is afforded by the presence of a nail varnish coating the nail fragment. By using confocal Raman spectroscopy, spectra of the drugs under nail varnish could be acquired. Spectra of the drugs could be readily obtained nondestructively within three minutes with little or no sample preparation. Raman spectra could be acquired from drug particles with an average size of 5–20 μm. Acquisition of Raman point maps of crystals from both pure and street samples of cocaine hydrochloride under nail varnish is also reported.

Keywords Cocaine hydrochloride · Nail · Forensics · Raman spectroscopy

Introduction

In recent years, keratinized matrices such as hair and nail have received considerable attention as unconventional matrices for the detection of drugs of abuse. Advances in sensitive analytical instrumentation have enabled the analysis of drugs in these unconventional biological samples to be accomplished [1–3]. Advantages of analyzing hair and nail samples over traditional media, like urine and blood, include their easy and noninvasive collection, the small sample size required for analysis, and their ease of storage at room temperature. Moreover, the temporal window of drug detection is dramatically extended to weeks, months or even years [4]. Nails provide a readily accessible matrix that can be employed in post-mortem detection of drugs of abuse. Methamphetamine, amphetamine, cocaine, opiates and cannabis have been successfully detected in the nail clippings of drug abusers [5–11].

The collection of trace evidence from beneath the fingernails of victims of violent crimes is one part of routine forensic examination. However, the collection of fingernail fragments from a crime scene is much less common. Fingernail clippings of victims have been examined for the identification of the DNA of assailants in cases where the victims struggled to defend themselves [12–14]. Also, it was found to be possible to undertake forensic DNA typing of human nails at various stages of decomposition [15].

Raman spectroscopy is increasingly being used for the identification of drugs of abuse. Raman spectroscopy is a nondestructive, noncontact technique that requires minimal or no sample preparation; this offers a special role for Raman spectroscopy in the first-pass evaluation screening of potential material of forensic relevance. It has previously been applied for the identification of ecstasy [16], cocaine [17, 18], barbiturates [19], and benzodiazepines [20]. It has also been used for the quantitative analysis of drugs of abuse in mixtures with caffeine, anhydrous D-glucose, mannitol, lactose, maltose, talc powder, flour, and baby formula [21–23].

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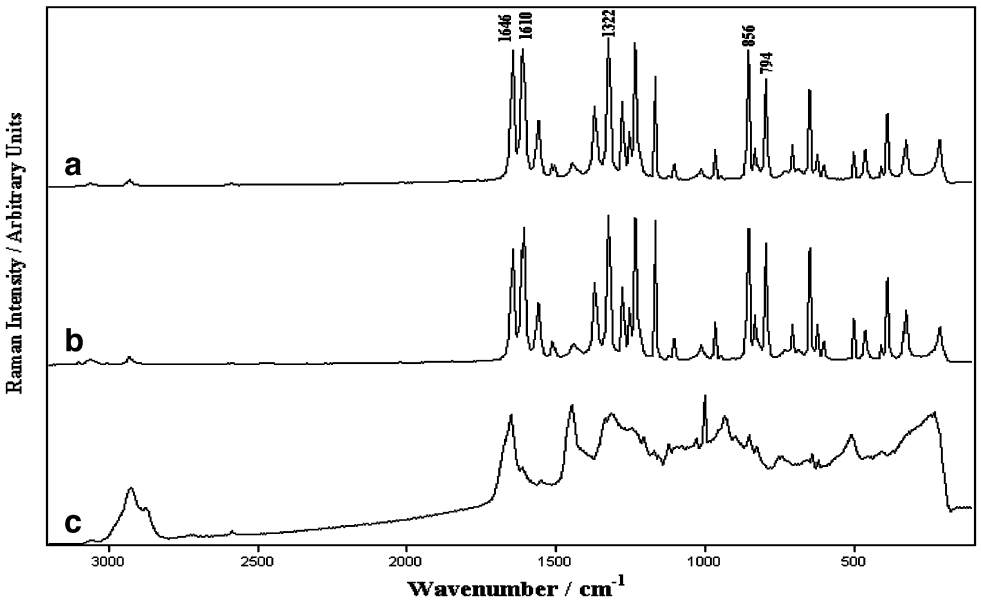

Fig. 1 Raman spectra of (**a**) paracetamol crystal on nail, (**b**) reference paracetamol, (**c**) human nail. All spectra: 785 nm excitation, 10 s exposure. One accumulation for **a** and **b**, five accumulations for **c**

Raman spectroscopy has been used for the identification of drugs in polymeric drug delivery systems [24, 25], and drugs of abuse could be successfully detected in cyanoacrylate-fumed fingerprints [26].

Several reports have demonstrated the use of Raman spectroscopy for the characterisation of the molecular structure of human nail. Williams et al. [27] used Raman spectroscopy to study the molecular structure of human keratotic biopolymers, such as skin, callus, hair and nails. A comparative study of the FT-Raman spectra of mammalian and avian keratotic biopolymers (stratum corneum, human nail, feather, and bull's horn) was carried out by Akhtar and Edwards [28] .The use of Raman spectroscopy and multivariate classification techniques for the differentiation of fingernails and toenails has been investigated by Widjaja and Seah [29].

The ability to identify drug particles unambiguously in situations where the material is obscured from visual identification affords significant advantages to the investigator. This study describes the application of Raman spectroscopy to the detection of drugs of abuse and noncontrolled substances used in the adulteration of drugs of abuse on human nail. Contamination of the nail may result from handling or abusing these drugs. An added difficulty in the analytical procedure is afforded by the presence of nail varnish coating the nail fragment; here, we shall demonstrate the discrimination of confocal Raman spectroscopy for the detection of drugs on uncoated nail

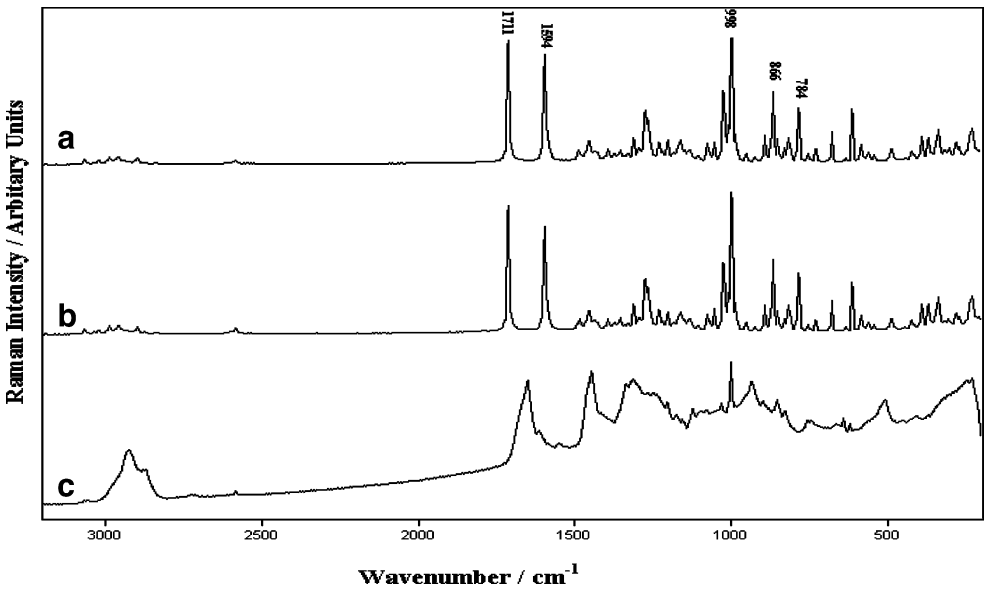

Fig. 2 Raman spectra of (**a**) pure cocaine.HCl on nail, (**b**) reference cocaine.HCl, (**c**) human nail. All spectra: 785 nm excitation, 10 s exposure. One accumulation for **a** and **b**, five accumulations for **c**

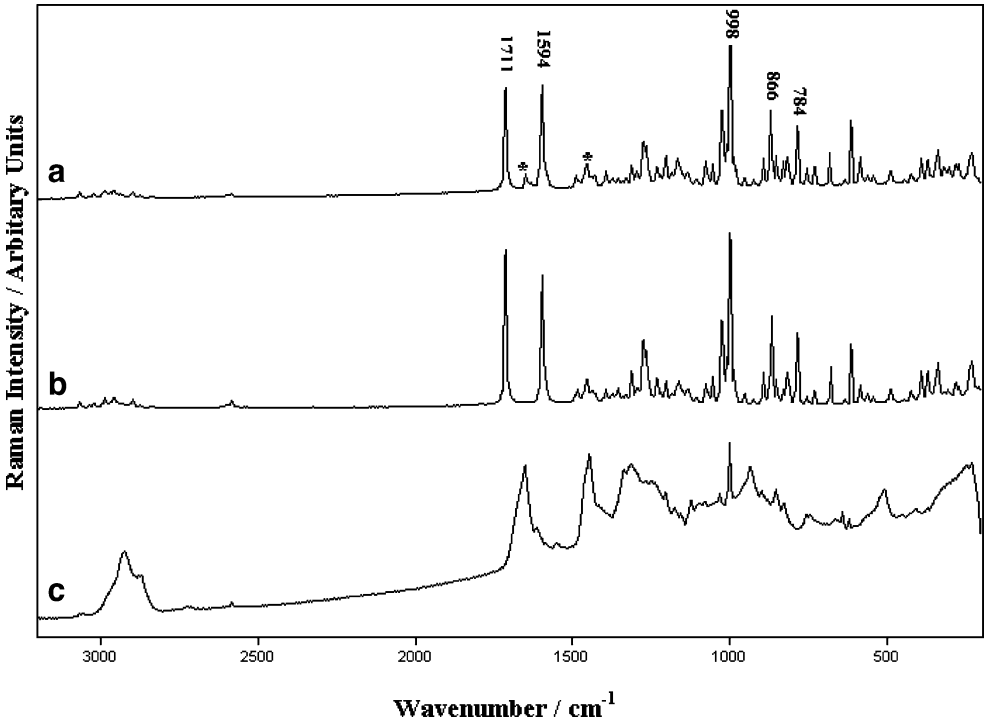

Fig. 3 Raman spectra of (**a**) street cocaine.HCl on nail (*asterisks* indicate peaks from excipients), (**b**) reference cocaine. HCl, (**c**) human nail. All spectra: 785 nm excitation, 10 s exposure. One accumulation for **a** and **b**, five accumulations for **c**

and also under a coating of nail varnish. Furthermore, the application of Raman mapping techniques allows the particle morphology to be visualised in obscured settings. This investigation establishes the utility of these Raman spectroscopic techniques in this context for the first time and establishes the technique of in-situ Raman mapping as a useful tool for forensic investigation.

Experimental

Samples

Cocaine hydrochloride and paracetamol used in this study were supplied by Sigma-Aldrich Company Ltd. (Gillingham, UK). Paracetamol was selected due to its similar crystal appearance to controlled substances, which may lead to it being mistaken for drugs of abuse or used as an adulterant. A seized street sample of cocaine hydrochloride (77%) was also used (supplied by FSS).

Fingernail clipping samples were donated from the authors. A red nail varnish (GC, Procter & Gamble, "Nail Slicks Well Red 141, Rouge Expert") was purchased from a local store.

Reference Raman spectra of the drug samples, nail varnish and human nail were obtained from samples on an aluminium slide. The nail clippings were doped with a few crystals of the three drugs. Raman spectra were collected from individual crystals on the surface of the nail. Raman spectra were collected from drug particles with an average size of 5–20 μm. A single application of the nail varnish was applied to the doped nails and spectra of the drug crystals under nail varnish were collected. Point Raman maps were acquired from crystals of both the pure and street samples of cocaine hydrochloride under the nail varnish coating.

Fig. 4 Pure cocaine.HCl crystal embedded into the surface of the nail

Raman spectroscopy

Raman spectra of the drugs on an aluminium slide, drugs on nail, drugs under nail varnish, nail, and nail varnish were collected using a Renishaw (Wotton-under-Edge, UK) InVia dispersive Raman microscope. The Raman scattering was excited with a 785-nm near-infrared diode laser. A 50× objective lens was used, giving a laser spot diameter of

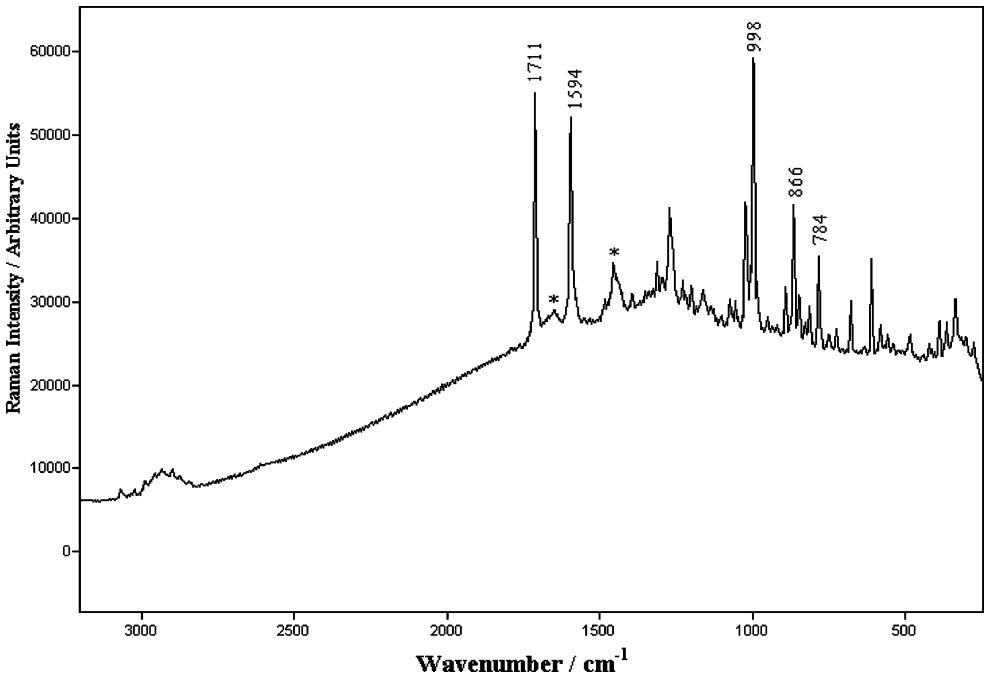

Fig. 5 Raman spectrum of pure cocaine.HCl crystal embedded into the surface of the nail (nail bands marked with *asterisks*); 785 nm excitation, 10 s exposure, one accumulation

5 μm. Spectra were obtained for a 10-s exposure of the CCD detector in the region 100–3200 cm^{-1} using the extended scanning mode of the instrument. With 100% laser power, one accumulation was collected for the drugs and five accumulations for both the nail and nail varnish. The total acquisition time was between three and eight minutes. Raman spectra of pure cocaine hydrochloride and paracetamol crystals on aluminium slide were obtained for use as reference spectra when comparing with the spectra of the drugs on uncoated nail and under nail varnish. Spectral acquisition, presentation, and analysis were performed with the Renishaw WIRE and GRAM© AI version 8 (Galactic Industries, Salem, NH, USA) software.

Raman point maps (~40×40 μm map area) were acquired for crystals of pure and street cocaine hydrochloride under the nail varnish. Using a 50× objective, Raman maps were obtained by collecting spectra with 10 s exposure time and subsequently moving the sample in a raster pattern with a step size of 3 μm. The laser intensity at the sample was reduced to 25% to avoid burning the sample. Data acquisition covered the spectral range 1800–100 cm^{-1} with a spectral resolution of 4 cm^{-1} with each exposure of the CCD detector. The data acquisition time for a typical Raman mapping experiment described here was about 12 h.

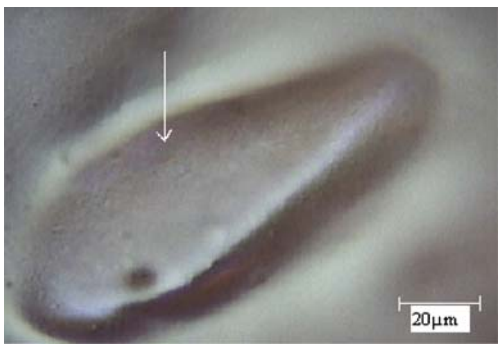

Fig. 6 Paracetamol crystal covered by nail varnish showing the sampling point

Results and discussion

The spectra obtained from paracetamol, pure cocaine hydrochloride and street cocaine hydrochloride (77%) crystals on human nail are shown in Figs. 1, 2 and 3, respectively. Comparison of these spectra with the reference spectra obtained for the drugs on an aluminium slide showed that the drugs could be easily identified using their Raman spectra. The Raman spectrum of paracetamol contains characteristic group frequencies assigned to the amide I (C=O) stretch at 1646 cm^{-1}, aromatic ring stretches at 1610 cm^{-1} and 794 cm^{-1}, amide III at 1322 cm^{-1}, the phenol group (C–OH) bend at 1233 cm^{-1}, and the aromatic ring bend at 856 cm^{-1}. Similarly, the Raman spectra of pure cocaine hydrochloride has several characteristic features that can be used to identify the drug, such as the benzoate ester (C=O) stretch at 1711 cm^{-1}, the aromatic ring (C=C) stretch at 1594 cm^{-1}, the ring breathing mode at 998 cm^{-1}, the pyrrolidine ring (C–C) stretch at 866 cm^{-1}, and the piperidine ring (C–C) stretch at 784 cm^{-1}, respectively. Importantly, these characteristic signatures are not obscured

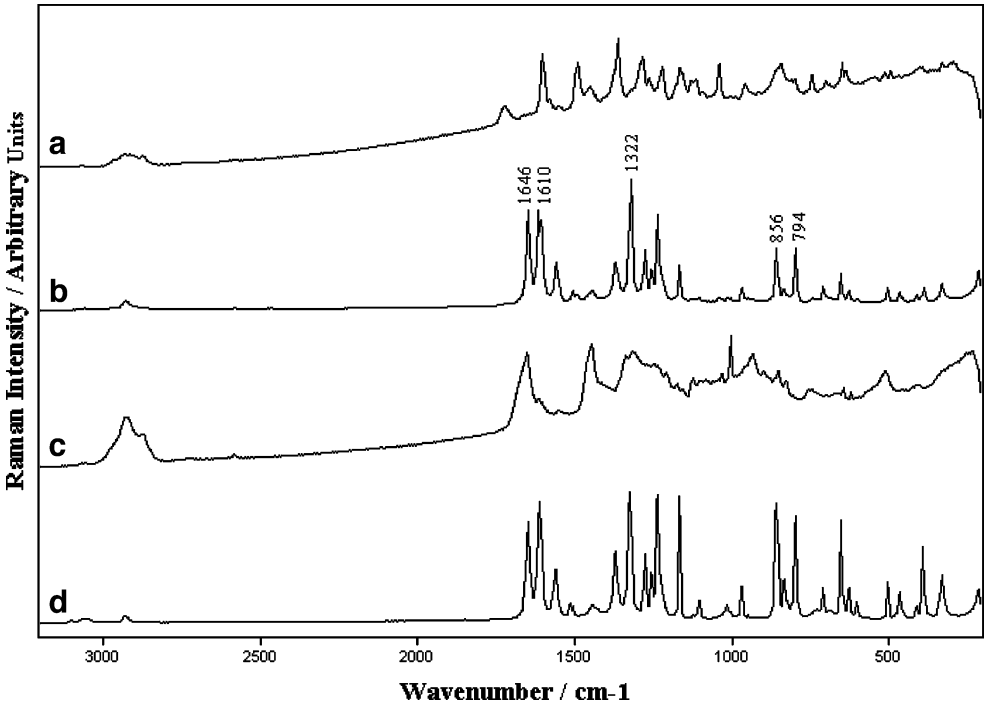

Fig. 7 Raman spectra of (**a**) nail varnish over paracetamol crystal, (**b**) paracetamol crystal under nail varnish, (**c**) nail under paracetamol crystal, (**d**) reference paracetamol. All spectra: 785 nm excitation, 10 s exposure. Five accumulations for **a** and **c**, one accumulation for **b** and **d**.

by spectral bands due to excipients in the street samples (Fig. 3).

Figure 4 shows an image of a crystal of pure cocaine hydrochloride several microns in size embedded into the surface of the nail (due to rubbing of finely powdered drug particles into the nail surface). The Raman spectrum of this is shown in Fig. 5. Although there is slight background fluorescence and some nail bands in the spectrum of the drug, the characteristic features of cocaine hydrochloride are still observable.

Raman spectra could be acquired from drug particles on the surface of the nail with an average size of 5–20 μm. The spectra are of a high quality with a good signal/noise ratio and no appreciable background due to fluorescence. Confocal Raman microscopy was applied to focus the laser beam and collect the Raman scattering from the drug crystals on the surface of the nail. The NIR laser at 785 nm gave excellent spectra for the drugs and there was no detectable background fluorescence. Interference from the nail, including background fluorescence, was overcome by careful focusing of the confocal beam, and the resulting spectra allow ready differentiation from interference from the nail substrate bands.

Figure 6 shows an image of a paracetamol crystal obscured by nail varnish. By changing the focus of the microscope objective, a series of Raman spectra were

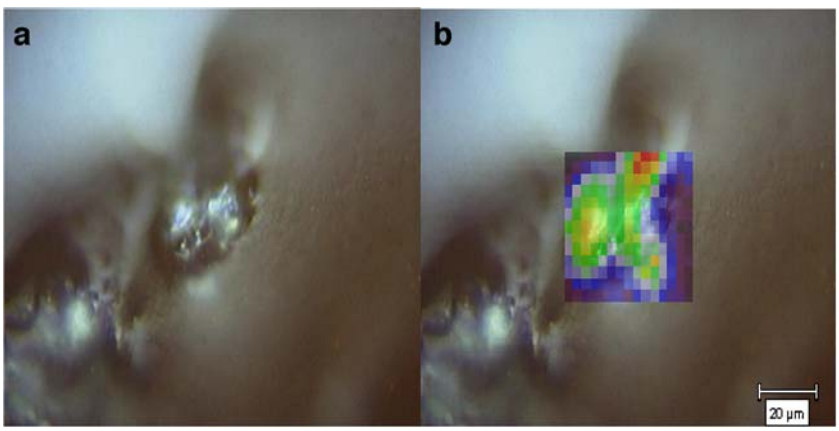

Fig. 8 **a** Video image of a pure cocaine.HCl crystal under nail varnish. **b** Raman map of a pure cocaine.HCl crystal under nail varnish, acquired using 998 cm^{-1} peak area; low peak area in *blue*, high peak area in *red*

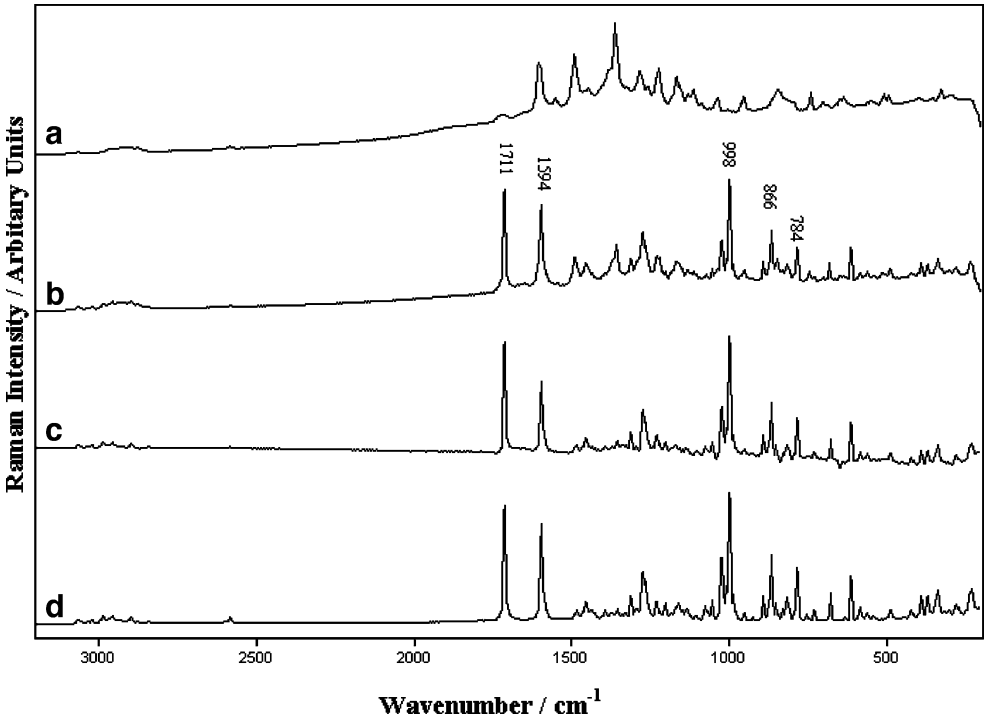

Fig. 9 Raman spectra of (**a**) nail varnish above pure cocaine.HCl crystal, (**b**) pure cocaine.HCl crystal under nail varnish, (**c**) subtracted spectrum (**b**–**a**), (**d**) reference cocaine. HCl. All spectra: 785 nm excitation, 10 s exposure. Five accumulations for **a**, one accumulation for **b** and **d**

collected from three different regions: the nail varnish covering the drug crystal; the drug crystal under the nail varnish; and the nail under the drug crystal. These spectra are shown in Fig. 7. Upon comparing the spectrum of the drug under the nail varnish with the reference drug spectrum, it is clear that the drug can be identified and no significant peaks in the spectrum are clearly attributable to either the nail or nail varnish. This provides a strong demonstration of the major advantage of confocal Raman microscopy, namely the ability to focus the incident laser radiation onto and collect Raman scattering from a small point within the interior of a larger sample. The presence of nail varnish had no effect on the Raman spectrum of the drug, which could be attributed to paracetamol; it had a good Raman scattering cross-section and a limited confocal scattering volume.

Figure 8a shows an image of a pure cocaine hydrochloride crystal under nail varnish. Raman spectra collected from the nail varnish covering the drug crystal and from the same drug crystal under nail varnish are shown in Fig. 9a and b, respectively. Although the spectrum of the drug under nail varnish contains three bands which could be assigned to the nail varnish (bands at 1489, 1358, and 1164 cm^{-1}), the characteristic cocaine hydrochloride bands mentioned above are still observable. The presence of these bands in the drug spectrum did not interfere with the identification of the drug and could be successfully removed by spectral subtraction. Figure 9c shows the

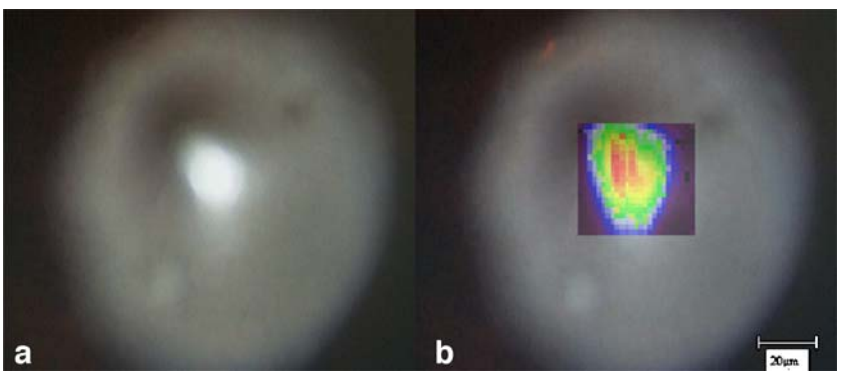

Fig. 10 **a** Video image of a street cocaine.HCl crystal under nail varnish. **b** Raman map of a street cocaine.HCl crystal under nail varnish, acquired using 998 cm^{-1} peak area; low peak area in *blue*, high peak area in *red*

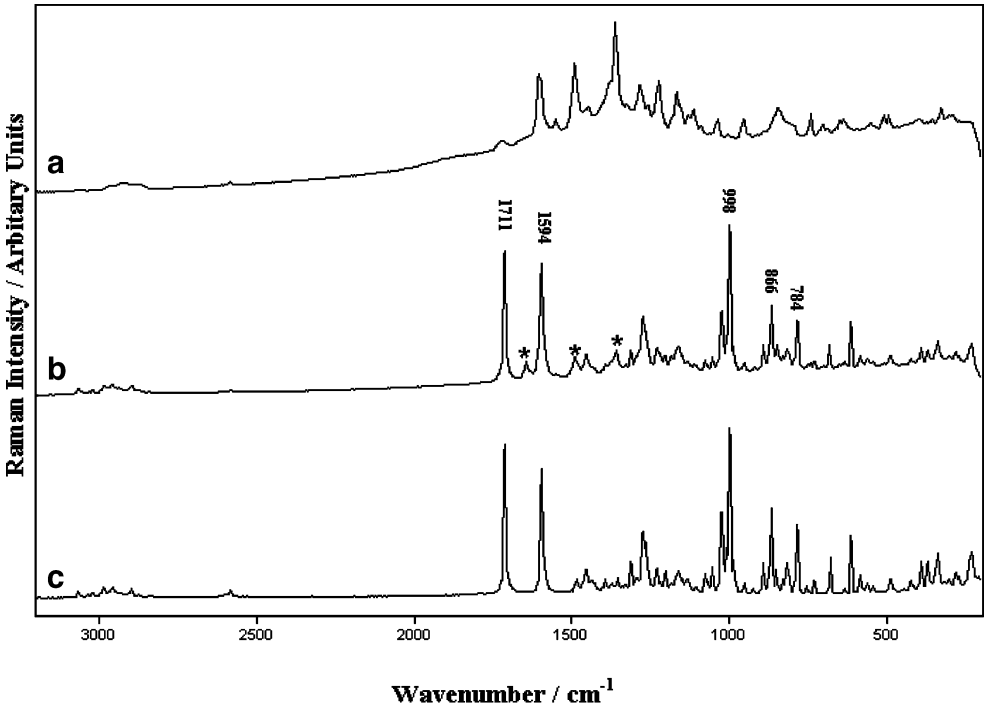

Fig. 11 Raman spectra of (**a**) nail varnish above a street cocaine.HCl crystal, (**b**) street cocaine.HCl crystal under nail varnish (*asterisks* indicate peaks from excipients and nail varnish), (**c**) reference cocaine.HCl crystal. All spectra: 785 nm excitation, 10 s exposure. Five accumulations for **a**, one accumulation for **b** and **c**

difference spectrum achieved by subtracting the nail varnish spectrum from the spectrum of the drug under the nail varnish. The difference spectrum agrees well with the reference spectrum of pure cocaine hydrochloride depicted in Fig. 9d.

Figure 8b shows a Raman point map of a pure cocaine hydrochloride crystal under nail varnish acquired using 998 cm^{-1} peak area. The map shows that the drug crystal could be clearly located under the nail varnish.

Figure 10a shows a video image of a street cocaine hydrochloride crystal (77%) under nail varnish. A stack-plot of the spectra collected from the nail varnish coating the drug crystal, the drug crystal under nail varnish, and a reference pure cocaine hydrochloride crystal on an aluminium slide is shown in Fig. 11. The spectrum of the drug contains peaks attributed to both the excipients and nail varnish, which are marked by asterisks in Fig. 11b. The presence of these peaks did not prevent identification of the drug, which can be clearly identified by its strong bands at 1711, 1594, 998, 866, and 784 cm^{-1}.

Figure 10b shows a Raman point map of a street cocaine hydrochloride crystal under nail varnish acquired using 998 cm^{-1} peak area. The map shows that the drug crystal could be clearly located under nail varnish.

These results demonstrate the discrimination of confocal Raman spectroscopy for drugs of abuse and selected adulterants on nails and under nail varnish. Raman spectra could be obtained from drug particles with an average size of 5–20 μm. The presence of nail varnish coating the drug crystals did not prevent identification of the drugs. This discrimination ability can be attributed to the ability of the confocal system to focus the incident laser radiation to obtain data nondestructively from the drug crystals under the nail varnish coating. Consequently, the resulting Raman spectrum contains Raman signal almost exclusively from the focal point of the laser. Also, the likelihood of generating fluorescence is reduced due to the use of the near-infrared laser as an excitation source. These results confirm that the detection of drug particles on nail and other articles related to an individual, such as hair or clothing, could be of evidential value when identifying this individual as a drug user or drug dealer.

Conclusion

Confocal Raman spectroscopy can be an invaluable tool for the detection and identification of drugs of abuse in obscured situations. Interference-free Raman spectra as well as two-dimensional Raman maps could be acquired from drug crystals on the surface of the nail and under the a nail varnish coating. The use of near-infrared lasers to excite samples is an added advantage of the technique, because the likelihood of generating fluorescence is much reduced compared to when visible lasers are used. Little or no sample preparation was required, and drug spectra could be acquired within three minutes without affecting the original state of the sample.

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