APPLICATION NOTE

No. AN54312

Forensic analysis of drug residue with Raman spectroscopy

Authors: R. Narizzano,[1] F. Risso,[1] A. Bazzano,[1] A. Magherini,[1] F. Femminella,[1] P. Legnani,[1] R. Berolotto,[1] Barbara Bravo,[2] Valerio Pasini,[2] and Daniele Benzono[2]

[1]Regional Laboratory Department, Chemical and Physical Analysis Division, Regional Agency for Environmental Protection–Liguria (ARPAL), Italy | [2]Thermo Fisher Scientific

Introduction

Drug residue is a common finding in forensic investigations. In the absence of biological fluids, it may be the only indicator of a subject's intoxication. In these situations, Raman spectroscopy, a form of vibrational spectroscopy, is an effective technology. It produces fast and accurate results without destroying the sample itself.

This analysis used a drug dilution if that was the likely state if found at a crime scene. The study of hypnotic sedatives was based on tablets since there is no literature about oral solutions or dilutions in beverages.

Included in the analysis were:

- Effortil (Boehringer Ingelheim): Active ingredient: Etilefrine clorhyrate; used to treat heart failure and as a doping substance
- Sonirem: Active ingredient: zolpidem tartrate; sleep aid
- Benzodiazepines: Lorazepam (Ativan), Bromazepam (Lexotan), Alprazolam (Xanax); Used to treat anxiety
- Cocaine: Derived from the plant *Erythroxylum coca*; narcotic

Methods

Analyses were performed using the Thermo Scientific™ DXR2 Raman Microscope.

For diluted compounds, a drop was first dried on a clean microscope slide. In some cases, a drop of the dilution was instead added to a closed glass microvial to prevent the rapid evaporation of the solvent.

Spectra were compared to and confirmed using spectra obtained from known samples. Figure 2 shows the experimental and reference spectra results.

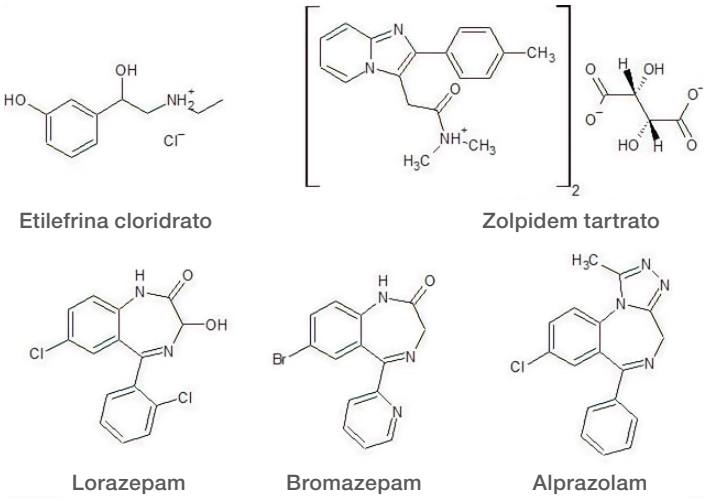

Figure 1: Active ingredients analyzed with Raman spectroscopy.

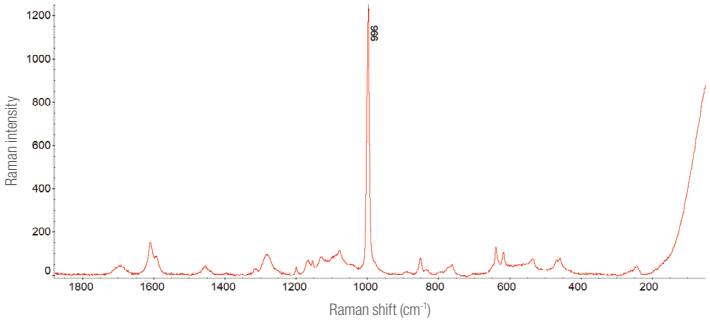

Figure 2: Raman spectrum of ethylephrine hydrochloride, collected with a 10 mW laser at 532 nm, using 300 1-second exposures.

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Results

Effortil

The initial spectra indicated the presence of more than just the active substance. (See Figure 3.) Using reference library data, this compound was identified as propyl para-hydroxybenzoate, one of the main excipients found in the oral solution.

By reserving the spectrum of the active substance it is easy to notice that the peak present at about 996 cm^{-1} is much more intense of everyone else. It was therefore decided of keep it as a reference for the analysis On Samples Diluted evaluating it of Time In Time the area. For “complicate”

To simulate an athlete’s dissolution of ethylmorphine hydrochloride in a stimulating drink, dilutions were prepared using fruit juices. Corrections were made for the interferences caused by the juices. See Figure 4.

The area under the peak of interest can be accurately measured and is proportional to the concentration of the active substance. The linear instrumental response allows quantitative analysis to be performed using calibration curve. See Figure 5 and Table 1.

µL effortil	10	20	30
Peak Area (996 cm^{-1})	37,80	79,93	124,5

Table 1: Data used to generate Figure 5.

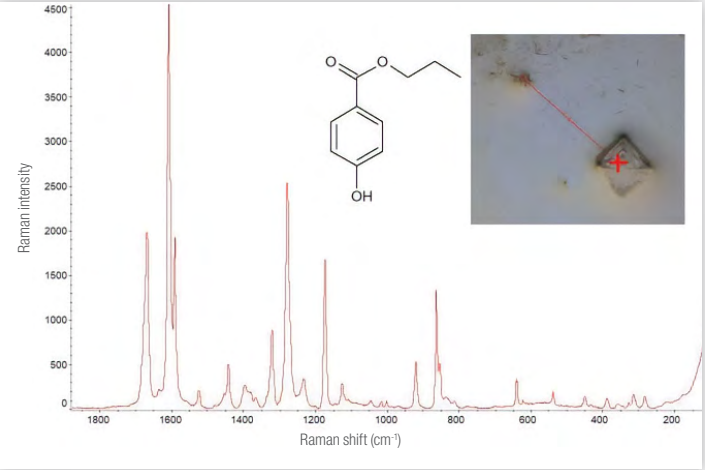

Figure 3: Propyl para-hydroxybenzoate. Raman spectrum (532 nm laser, 10mW – 50 1s exposures), molecule and image of the point on the map where it was detected (10x lens).

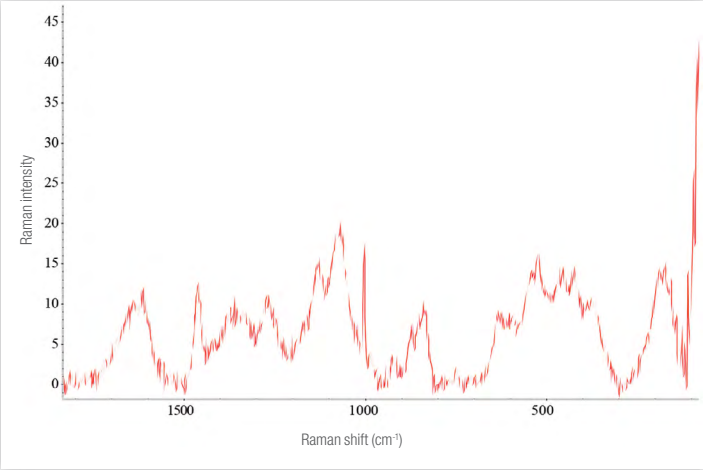

Figure 4: Raman spectrum of Effortil diluted in a matrix of pear juice (laser 532 nm - 10 mW, 8 acquisitions of 60 s).

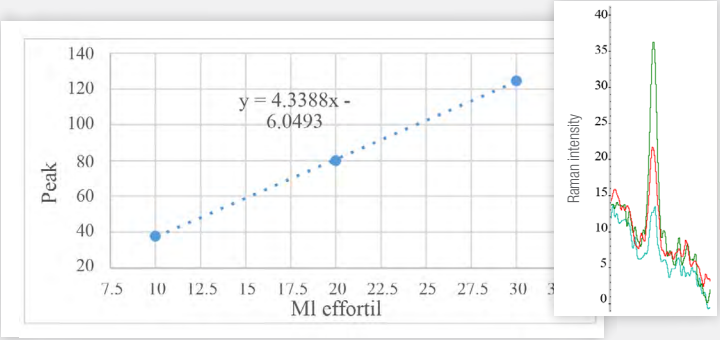

Figure 5: Area of the most intense peak of the Raman spectrum of samples of 10 (blue), 20 (red), 30 (green) µL of Effortil in the cranberry juice.

Sonirem

The Raman spectrum for zolpidem tartrate was obtained in a similar manner to that of Effortil. See Figure 6.

Using the identical instrument parameters, the spectra and the level of interference varied by the dilution in water. See Figure 7. Amounts equal to a half, full, double, and triple the recommended dose of 10 mg) were analyzed.

The results (see Table 2 and Figure 8) show the linearity of the peak area at 1623 cm^{-1} as a function of the dilutions. The ratios with adjacent peaks (1570 cm^{-1}) does not vary significantly across samples.

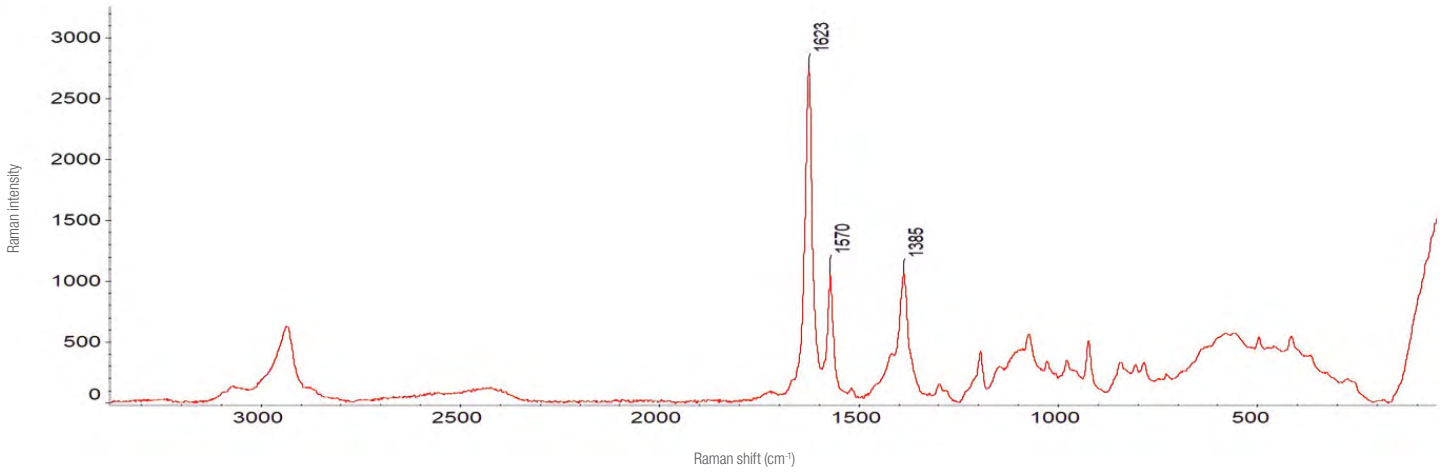

Figure 6: Spectrum of zolpidem tartrate. Obtained with the following instrumental parameters: laser 532 nm – 10 mW,90 acquisitions of 1 s each.

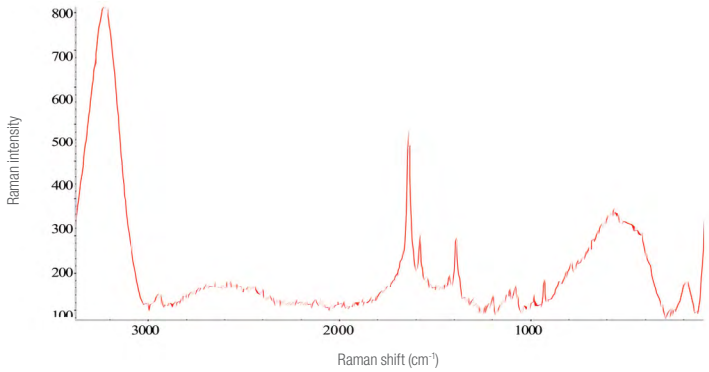

Figure 7: Raman spectrum of four times the recommended dose of zolpidem tartrate diluted in water.

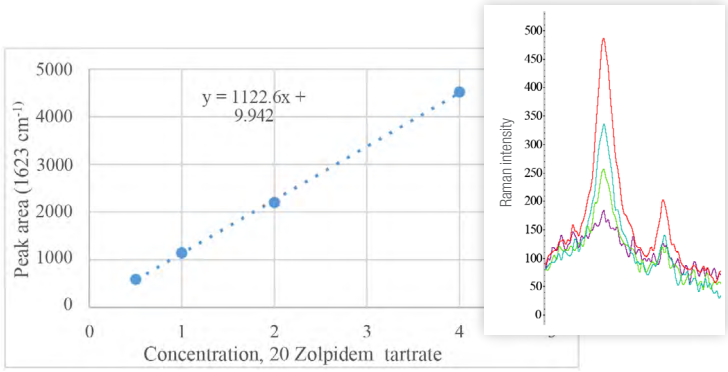

Figure 8: Graph of peak area at 1623 cm^{-1} (highest in Raman spectra on the right, purple to red with increasing doses) as a function of the concentration of zolpidem tartato.

Dose		V_i [µL]	V_f [µL]	C_i [mg/mL]	C_f [mg/mL]	Peak area (1623 cm^{-1})	Peak area (1570 cm^{-1})	Area ratio
Half	0.5	10	200	10	0.5	587.3	94.7	6.20
Single	1	10	100		1	1146.4	187.3	6.12
Double	2	20	100		2	2206.0	347.3	6.35
Quadruple	4	40	100		4	4519.2	696.6	6.49

Table 2: Zolpidem tartrate samples, diluted to final concentration (Cf) with water. Initial (Vi) and final (Vf) volume, as well as critical peak areas, are also shown.

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Lorazepam

The hydroalcolic oral solution (Lorazepam Dorom) evaporates very quickly in air, which makes the active ingredient difficult to detect. A better Raman spectrum was obtained by adding a drop of the drug to a closed glass microvial and letting the solvent evaporate slowly.

The differences are evident in the Raman spectra (Figure 9).

Bromazepam and Alprazolam

The galenic formulations of these two products differ only slightly, and both have an excipient (propylene glycol, PEG) that obscures the active substance. Although we tried to separate the PEG using a basifining agent and chromatographic techniques (thin layer and column), the Raman spectra produced showed only propylene glycol (1,2-propandiol). See Figure 10.

Cocaine

Erythroxylum coca, the source of cocaine, is a native Latin American plant. An alkaloid narcotic substance, it acts as a central nervous system stimulant. It is typically injected (as a hydrochloride salt) or inhaled as a free base.

The spectrum of cocaine hydrochloride from the sample appears in Figure 11.

Conclusions

The results demonstrate the potential for using Raman spectroscopy for qualitative analysis in the forensic field. Results are available in a short period of time, minimal samples are required, and little if any sample preparation is needed.

Liquid sample testing should be performed with low laser power to avoid solvent evaporation and sample crystallization. If the solvent does not evaporate, which would concentrate both the analyte and interferants, quantitation can be measured using the area under the peak.

Both the active ingredients and excipients for various drugs can be readily detected using Raman spectroscopy. However, preliminary separative treatments may be needed if results demonstrate only the presence of the known excipients.

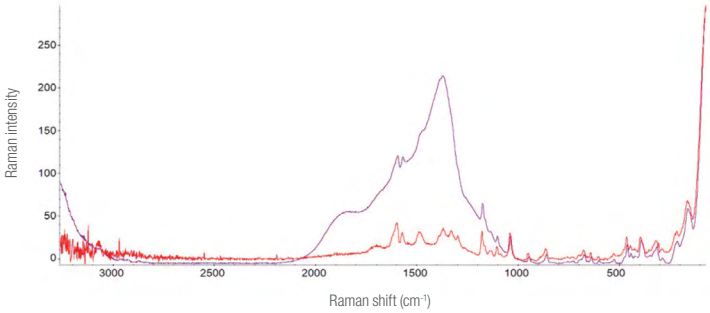

Figure 9: Raman spectra of Lorazepam. In purple is reported the one evidently interfered (laser 785 nm – 30mW, 32 acquisitions from 5s). In red the one obtained using the "microvial" and acquiring the Raman spectrum through it (laser 785 nm – 14mW, 40 acquisitions of 2s).

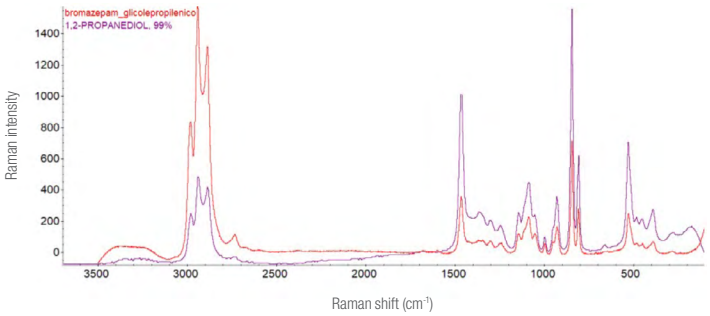

Figure 10: Raman spectrum and molecular structure of propylene glycol. In red the experimental spectrum (laser 532 nm, 4 mW – 45 acquisitions from 1s), in purple the theoretical one assigned by the software recognition system.

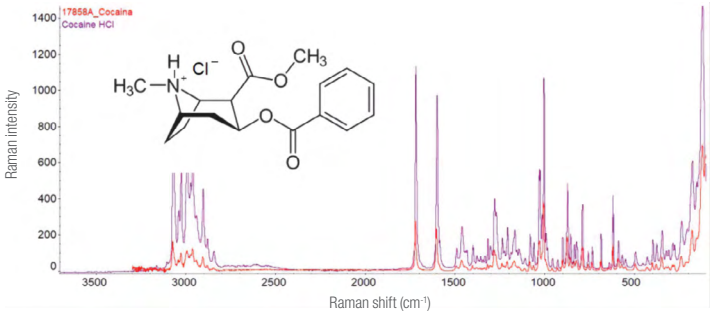

Figure 11: Experimental Raman spectrum (in red, laser 785 nm – 25 mW, 5 exposures of 8 s each) and library (purple) of cocaine hydrochloride and its structure formula.

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