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The detection of flunitrazepam in beverages using portable Raman spectroscopy

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Portable Raman spectroscopy has been used for the detection of the date-rape drug flunitrazepam in spiked beverages that may be involved in cases of drug-facilitated sexual assault. Solutions of flunitrazepam with different concentrations were prepared in water and for each beverage type. Although some bands attributable to the beverage matrix are present, they did not interfere with the identification of the drug. Definitive evidence for contamination of the spiked drink concerned can be acquired within 10 s. The data can be acquired *in situ* and sample extraction and/or preparation steps are unnecessary. The ability of portable Raman spectrometers to interrogate spiked alcoholic beverages with flunitrazepam has been demonstrated. Copyright © 2016 John Wiley & Sons, Ltd.

Keywords: forensic; flunitrazepam; beverages; portable Raman spectroscopy

Introduction

Flunitrazepam is a well known date rape drug commonly known as Rohypnol, roofies, or the 'forget pill'. Flunitrazepam is clinically indicated for cases of severe and debilitating sleep disorders. Other indications are premedication for anaesthesia and for induction of general anaesthesia.[1,2] The effects of the drug vary depending on the dosage given, but they range from sedation to stage four coma. After consuming the drug, it takes 20–30 min for its sedative effects to begin, and the effects can last from 8–24 h. As well as being a strong sedative, flunitrazepam also causes anterograde amnesia, which may preclude the victim from remembering events for several hours following initial ingestion.[3–5] Flunitrazepam actions result from the potentiation of inhibitory central nervous system (CNS) effects of gamma-aminobutyric acid (GABA). When used as a date rape drug, most commonly flunitrazepam is dissolved in the victim's drink or beverage by the rape perpetrator. Since the drug dissolves readily and is colourless, odourless, and tasteless, it often goes undetected by the unsuspecting victim. When flunitrazepam is combined with an alcoholic beverage, the CNS effects may be significantly enhanced.[6]

The Forensic Science Service (FSS) encouraged investigating police officers to submit blood and urine samples in all cases of alleged drug-facilitated sexual assaults (DFSA) and to look for any other items of possible relevance (e.g. drink residues in empty glasses/bottles, vomit at the scene).[7] The main laboratory procedures used for identifying the drugs of abuse in these cases included initial screening for drugs of abuse by means of enzyme immunoassay (EIA)[8,9] followed by confirmatory analysis using gas chromatography-mass spectrometry (GC-MS) and, where applicable, quantification either by GC-MS or high performance liquid chromatography (HPLC).[7,10–12] These analytical techniques require preparation steps in which the sample to be analyzed is extracted into an organic solvent before injection into GC. Also, each of these approaches requires isolation and/or destruction of the analyte and these techniques therefore alter or destroy the evidential material during analysis.

Raman spectroscopy has recently been shown to be an effective technique for several forensic applications[13–16] and technological advances in commercial Raman spectrometers have broadened the use of Raman spectroscopy in forensic applications. Raman spectroscopy produces molecular-specific spectra and, in most cases, sample preparation is minimal or unnecessary, allowing for the non-destructive *in situ* analysis of tablets, powders, and liquids.[17,18] This is particularly important with regard to the speed of analysis, prevention of sample contamination, and preservation of evidential material. Recent advances have allowed the production of compact and field portable Raman systems that are commercially available; the principal developments allowing this technological advancement are the advent of compact, powerful, stable, and reliable near-infrared solid-state laser sources along with the use of high-resolution charge coupled device (CCD) detectors.[19]

The detection of drugs of abuse in drinks residue has a high evidential value in cases of alleged DFSA. Portable Raman spectroscopy can be applied in these cases as a screening technique for the detection of drugs of abuse in spiked drinks. In these instances, the non-destructive and non-contact character of the technique offers a special role for portable Raman spectroscopy in the first-pass evaluation screening of materials of forensic relevance. This work describes the application of portable Raman spectroscopy for the detection of the date-rape drug flunitrazepam in spiked beverages that may be involved in cases of DFSA. Definitive evidence for contamination of the spiked drink concerned can be acquired within

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10 s. The data can be acquired *in situ* and sample extraction and/or preparation steps are unnecessary.

Experimental

Samples

Pure flunitrazepam used in this study was supplied by the Sigma-Aldrich Company Ltd (Dorset, England, United Kingdom). A range of proprietary beverages was purchased from a local supermarket including Bacardi rum (37.5% alcohol), gin (37.5% alcohol), dark rum (37.5% alcohol), and vodka (40% alcohol). Solutions of flunitrazepam with different concentrations were prepared in water and for each beverage type as shown in Table 1. For the limit of detection (LOD) study, water and vodka were chosen and the serial concentrations of flunitrazepam in water and vodka are shown in Table 1.

Spectroscopic instrumentation

The Raman spectra of the drug solutions were recorded using a Delta Nu Inspector Raman FSX(Laramie, WY, USA). The Inspector Raman instrument is equipped with a diode laser emitting at a wavelength of 785 nm , a thermoelectrically cooled (1 x 1024 pixels) CCD detector, and a custom 25 mm focal length lens in a nose piece. The spectral wavenumber range is 2000–200 cm^{-1} with a spectral resolution of 8 cm^{-1}. The laser power at the sample was 37 mW. Daily calibration of the wavenumber axis is achieved by recording the Raman spectrum of polystyrene within the calibration routine built into the software. The spectra were collected directly from the solutions by focusing the laser on the solution surface. This approach was adopted to avoid fluorescence from the glass containers or weakening of the Raman signal by the glass barrier. The small calibre nose piece (about 1 cm in diameter) facilitated the collection of the spectral data directly from the opened glass containers. A reference Raman spectrum of flunitrazepam was recorded for comparison with the spectra collected from the drug solutions in water or proprietary alcohol. Spectra were recorded with the accumulation of 1 scan, 10 s exposure, and were not corrected for instrument response. The spectrometer was controlled by a portable PC with instrument control software (Nu Spec Version 4.75).

Results and discussion

Raman spectra were obtained from flunitrazepam solutions (concentrations from 0.01 to 0.04% *w/v*) in spiked beverages. In each case the spectra from each drink were compared with the reference spectra of flunitrazepam to evaluate the identification of the drug spectral band signatures. Figure 1 shows the Raman spectra obtained from flunitrazepam solutions in water. Comparison of these spectra with the reference spectrum of flunitrazepan indicates that

Table 1. Beverages used in the study and serial concentrations of flunitrazepam in each beverage type

Beverage	Alcohol Percentage	Concentration (*w/v* %)
Water	0	0.01,0.02,0.03,0.04
Bacardi	37.5%	0.01,0.02,0.03,0.04
Gin	37.5%	0.01,0.02,0.03,0.04
Rum	37.5%	0.01,0.02,0.03,0.04
Vodka	40%	0.01,0.02,0.03,0.04

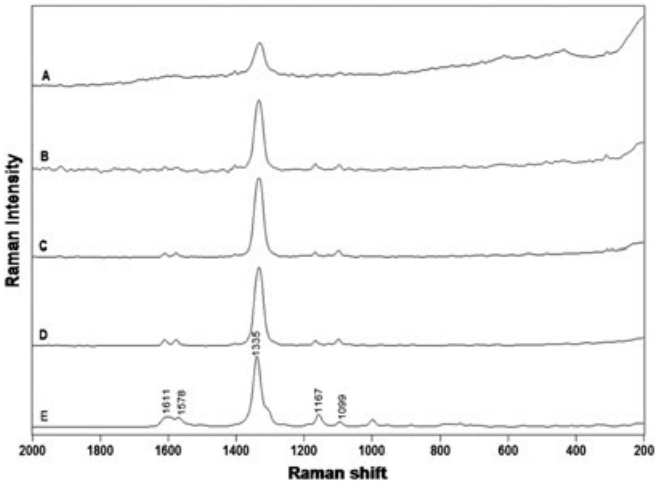

Figure 1. Raman spectra of flunitrazepam in water (A) 0.01% concentration (*w/v* %) (B) 0.02 % concentration (*w/v* %) (C) 0.03 % concentration (*w/v* %) (D) 0.04 % concentration (*w/v* %) (E) Reference flunitrazepam.

the drug can be clearly identified. The Raman spectrum of flunitrazepam has several characteristic features that can be used to identify the drug (Figure 2), such as the C = N stretch at 1611 cm^{-1}, the C = C stretch at 1578 cm^{-1}, the symmetric NO_2 stretch at 1335 cm^{-1}, the C-C-N stretch (diazepine ring) at 1167 cm^{-1}, and the aromatic in-plane CH deformation at 1099 cm^{-1}.[20]These bands can be clearly identified in the spectra of the different drug solutions. Figure 3 shows some exemplar spectra acquired from the drug solutions in white Bacardi rum. Several signature bands of

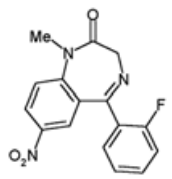

Figure 2. Chemical structure of Flunitrazepam.

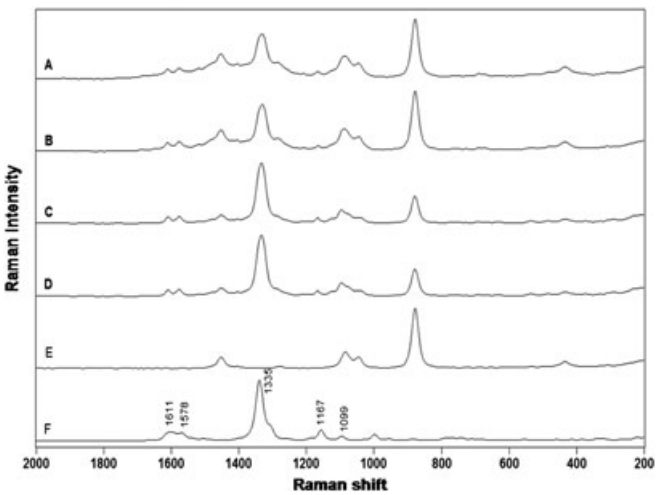

Figure 3. Raman spectra of flunitrazepam in white Bacardi rum (A) 0.01% concentration (*w/v* %) (B) 0.02 % concentration (*w/v* %) (C) 0.03 % concentration (*w/v* %) (D) 0.04 % concentration (*w/v* %) (E) Bacardi rum (F) Reference flunitrazepam.

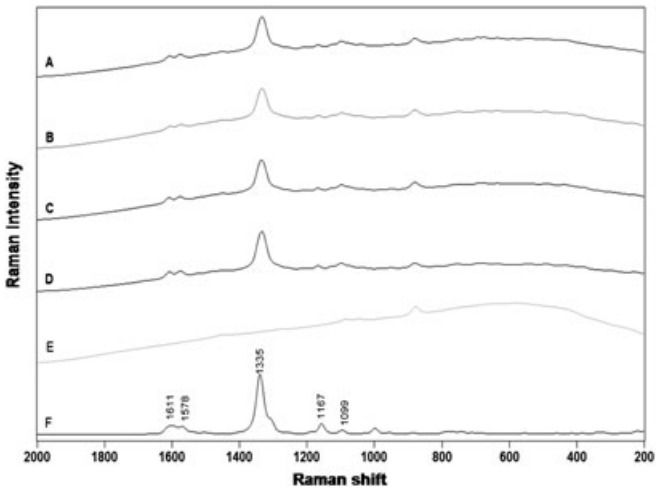

Figure 4. Raman spectra of flunitrazepam in dark rum (A) 0.01% concentration (*w/v* %) (B) 0.02 % concentration (*w/v* %) (C) 0.03 % concentration (*w/v* %) (D) 0.04 % concentration (*w/v* %) (E) Rum (F) Reference flunitrazepam.

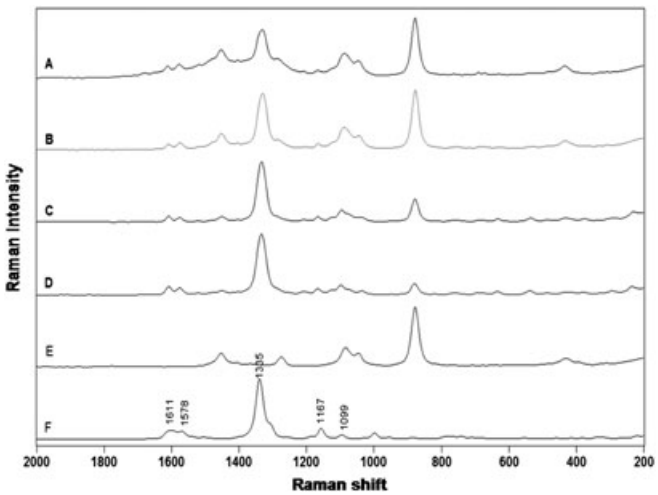

Figure 5. Raman spectra of flunitrazepam in gin (A) 0.01% concentration (*w/v* %) (B) 0.02 % concentration (*w/v* %) (C) 0.03 % concentration (*w/v* %) (D) 0.04 % concentration (*w/v* %) (E) Gin (F) Reference flunitrazepam.

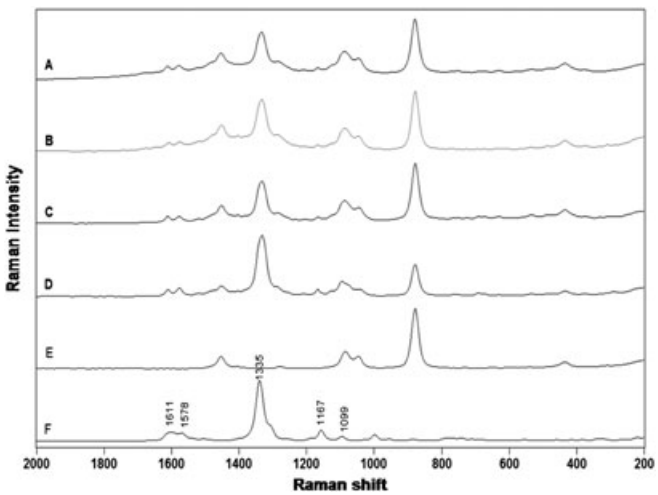

Figure 6. Raman spectra of flunitrazepam in vodka (A) 0.01% concentration (*w/v* %) (B) 0.02 % concentration (*w/v* %) (C) 0.03 % concentration (*w/v* %) (D) 0.04 % concentration (*w/v* %) (E) Vodka (F) Reference flunitrazepam.

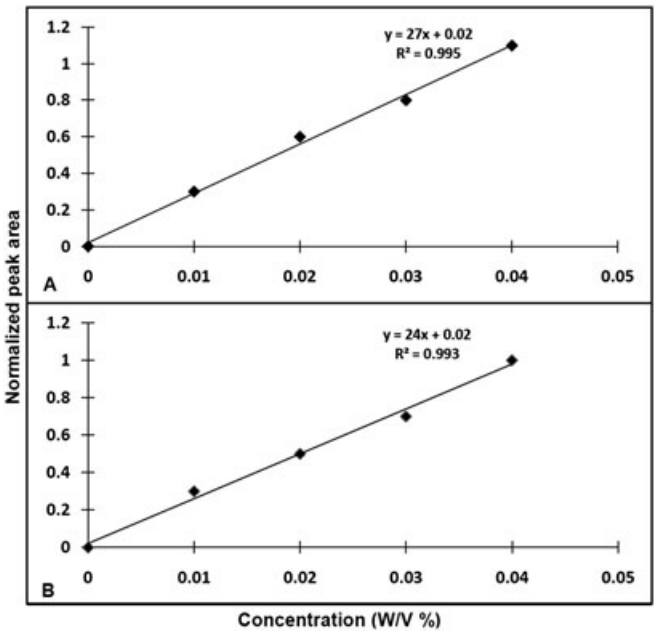

Figure 7. Calibration curve for the limit of detection (LOD) of flunitrazepam in water (A) and vodka (B).

the drug can be identified in these spectra down to the lowest 0.01% concentration studied. Although the presence of some bands in the spectrum is attributable to the beverage matrix, such as those at 1453, 1085, 1046, and 877 cm^{-1}, these bands did not prevent the identification of the drug. The Raman spectra collected from flunitrazepam solutions in dark rum are shown in Figure 4. It is clear that the Raman spectrum of the dark rum now contains a few bands assigned to the ethanol content superimposed on a significant fluorescence background (Figure 4E) which could possibly swamp Raman signals from the drug. This fluorescence background is ascribed to the additives which are present in the beverage. It is observed that a broad fluorescence background can be seen in the spectra but the characteristic Raman bands of the drug are still clearly observed.

Another example of a spiked spirit is shown in Figure 5 in which the Raman spectra collected from the drug solutions in gin are displayed. All the characteristic Raman bands of the drug can be clearly identified in all samples in the range of concentrations studied here. The last example is shown in Figure 6 in which the Raman spectra of flunitrazepam spiked vodka are displayed. The vodka used in these studies contains the highest ethanol concentrations (about 40%) and this could have interfered with the identification of flunitrazepam. In all the spectra the signature Raman bands of flunitrazepam such as those at1611, 1578, 1335 and 1167 cm^{-1} are clearly observed even in the presence of the higher alcohol concentration.

Figure 7A and 7B show the calibration curves of peak areas and concentrations for flunitrazepam in water and vodka, respectively. Each solution in this work was measured in triplicate and processed using GRAMS software to calculate the area of the peak at 1335 cm^{-1}. These measurements were then averaged to give the mean peak area and a calibration curve plotted. These curves show that flunitrazepam can be detected down to the lowest concentration of the drug studied here (0.01% *w/v*), demonstrating the ability of portable Raman spectroscopic instrumentation to detect the drug in spiked beverages *in situ*.

Conclusions

Portable Raman spectroscopy can be applied efficiently as a screening technique for the *in situ* identification of the date rape drug flunitrazepam spiked in water and in several alcoholic beverages. Raman spectra of the drug in the spiked beverages can be acquired within 10 s. The potential for application of this technique in real-life situations as a rapid preliminary, forensic screening procedure for law enforcement is obvious and attractive to non-specialist operators as it does not involve prior chemical pretreatment or extraction of the analyte from the samples.

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