Research Article

Drug Testing and Analysis

Received: 10 September 2008 Revised: 2 November 2008 Accepted: 3 November 2008 Published online in Wiley Interscience:

(www.drugtestinganalysis.com) DOI 10.1002/dta.11

Identification of the date-rape drug GHB and its precursor GBL by Raman spectroscopy

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Gamma hydroxybutyric acid (GHB), also known as 'liquid ecstasy', has recently become associated with drug-facilitated sexual assaults, known colloquially as 'date rape', due to the ability of the drug to cause loss of consciousness. The drug is commonly found 'spiked' into alcoholic beverages, as alcohol increases its sedative effects.

Gamma hydroxybutyric acid and the corresponding lactone gamma-butyrolactone (GBL) will reach an equilibrium in solution which favours the lactone in basic conditions and GHB in acidic conditions (less than pH 4). Therefore, we have studied both GHB and GBL, as a mildly acidic beverage 'spiked' with GHB will contain both GHB and GBL.

We report the analysis of GHB as a sodium salt and GBL, its precursor, using bench-top and portable Raman spectroscopy. It has been demonstrated that we are able to detect GHB and GBL in a variety of containers including colourless and amber glass vials, plastic vials and polythene bags. We have also demonstrated the ability to detect both GBL and GHB in a range of liquid matrices simulating 'spiked' beverages.

Keywords: Raman spectroscopy; date rape drug; GHB; GBL

Introduction

Gamma-hydroxybutyric acid (GHB) (Fig. 1), known colloquially as 'liquid ecstasy' or 'liquid X'[1] is a Class C drug of abuse[2] that also occurs naturally in mammalian tissues in very small amounts (5 mg/L).[3] The drug has recently become associated with drug-facilitated sexual assaults or 'date rape'.[4] In the 1960s, GHB, a central nervous system (CNS) depressant, first appeared as a medicinal drug to treat sleep disorders, depression and anxiety and has also been used to aid withdrawal from opiate and alcohol addictions.[3] Several applications of the drug exist due to the different effects of high and low doses. At low doses, around 0.5–1 g,[5] the drug has 'desirable' effects, including euphoria, increased sensuality and disinhibition and is therefore becoming increasingly popular recreationally as a 'party drug' and is often taken alongside stimulants such as cocaine and amphetamines.[3] At higher doses, 2.5–4 g,[5] GHB has a sedative effect causing ataxia, lack of awareness of surroundings and loss of consciousness. Hence the drug has been used in drug-facilitated sexual assaults, but also by people with insomnia disorders as a sleeping aid.[3] These depressive effects are amplified when the drug is mixed with alcohol, hence its application as a 'date-rape' drug by 'spiking' alcoholic beverages.

Gamma-hydroxybutyric acid, a short chain carboxylic acid, is capable of extensive hydrogen bonding and will dissociate into an anionic species and a hydrogen ion when in aqueous solution.[6] The anion can then undergo a further reaction, in which it is converted into the corresponding lactone (GBL) by Fischer esterification.[6] An equilibrium is reached between GHB and GBL, which favours the lactone in acidic conditions and the acid in basic conditions.[7] Due to this inter-conversion a beverage spiked with GHB may also contain GBL, as most drinks are mildly acidic. A drink spiked with GBL can also have sedative effects as the basic conditions in the digestive tract can hydrolyse it into the active drug after ingestion.

Previous analysis of GHB and GBL (Fig. 1) in a forensic context has been studied by HPLC,[7] GC-MS,[6] NMR[8] and infrared spectroscopy[8] and often involves removing the drug from the liquid matrix prior to analysis. Raman spectroscopy is a suitable method for this type of analysis as it is a non-destructive technique that can give both qualitative and quantitative information. This, combined with the speed of analysis, typically seconds, and the ability to penetrate containers and analyse materials *in situ* makes Raman spectroscopy an ideal tool for forensic analysis. Raman spectroscopy has been used to identify controlled substances, most commonly opiates, cocaine and amphetamines, both in laboratories[9,10] or *in situ* either at scenes of crimes[11] or at customs inspection points in airports.[12] Raman spectroscopy has also proved useful for the identification of drugs-of-abuse in containers such as glass vials and polythene bags.[12,13]

Initial work centred on the identification and detection of GHB and GBL in a variety of different container types, including glass and plastic vials and a plastic bag, mimicking how a seized sample may be presented to a forensic scientist or government official, and the detection of GHB and GBL in solutions simulating spiked beverages that may be involved with 'date-rape' crimes. A more detailed study was then carried out looking at GHB and GBL in a range of solutions over a wide range of concentrations in order to calculate the detection limits of both drugs in solution.

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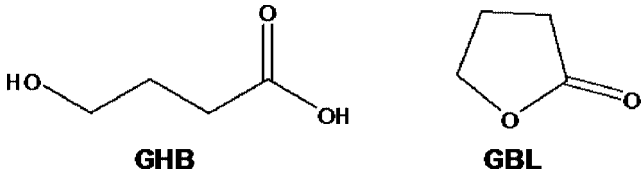

Figure 1. Structure of gamma-hydroxybutyric acid (GHB) and gamma-butrolactone (GBL).

Experimental

Samples

Gamma-hydroxybutyric acid sodium salt (99%) and GBL (99.4%) were obtained from Sigma-Aldrich, Poole Rd, UK. For the detectable limits study beverages were purchased from a local supermarket (see Table 1). For the study of GHB and GBL in containers, 0.5 g of GHB or 2 mL of GBL was measured into each of the container types. For the initial study in solution 25% w/v solutions (0.5 g in 2 mL) of GHB were prepared in water, 15% ethanol, and 40% ethanol, and, 25% v/v solutions of GBL (0.5 mL in 2 mL) in water, 15% ethanol and 40% ethanol.

For the detectable limits study initially a 50% w/v solutions (1 g in 2 mL) was prepared for each beverage matrix. Serial dilutions were then prepared to give the desired range of concentrations for each, summarised in Table 1.

Spectroscopic instrumentation

Raman spectra were obtained using three different spectrometers; one benchtop spectrometer, a Renishaw InVia Reflex dispersive spectrometer and two portable spectrometers, a Renishaw RX210 'Raman-in-a-suitcase' (RIAS) portable Raman analyser, and a Delta Nu Inspector Raman FSX.

Renishaw InVia Reflex spectrometer

Raman spectra were obtained using a Renishaw InVia Reflex spectrometer (Wotton-under-Edge, UK), operating with a high-power NIR diode laser emitting at 785 nm and a thermoelectrically cooled charged-coupled-detector (CCD) (400 × 575 pixels) detector, coupled to a Leica DMLM microscope using 50× (NA 0.75), 20× (NA 0.40), and 5× (NA 0.12) microscope objectives, which provided a spectral footprint of approximately 2–5 microns. The diffraction grating (1200 lines/mm) gives the spectral range 3200–100 cm^{-1} with a spectral resolution of 2 cm^{-1}. Daily calibration of the wavenumber axis is achieved by recording the Raman spectrum of a silicon wafer (1 accumulation, 10 s) in static mode. If necessary, an offset correction is performed to ensure that the position of the silicon band is 520.5 ± 0.1 cm^{-1}. Spectra were recorded with an accumulation of 1 scan, 10 s exposure and 110 mW laser power at the sample. Spectra were not corrected for instrument response. The spectrometer was controlled by a PC with instrument control software (Renishaw WiRE 2 Service Pack 9).

Table 1. Summary of solutions prepared for detectable limits study

Beverage	Percentage alcohol	Concentration range (% w/v or %v/v)
Water	0	50, 40,30,20,10,5,2,1,0.5
15% alcohol[a]	15	50, 40,30,20,10,5,2,1,0.5
40% alcohol[a]	40	50, 40,30,20,10,5,2,1,0.5
Vodka	40	25,15,10,5,2,1,0.5
Gin	37.5	25,15,10,5,2,1,0.5
Bacardi	37.5	25,15,10,5,2,1,0.5
White wine	13	25,15,10,5,2,1,0.5
Vodka and tonic[b]	10	25,15,10,5,2,1,0.5
Vodka and lemonade[b]	10	25,15,10,5,2,1,0.5
Gin and tonic[b]	10	25,15,10,5,2,1,0.5
Gin and lemonade[b]	10	25,15,10,5,2,1,0.5
Bacardi and lemonade[b]	10	25,15,10,5,2,1,0.5

[a] Alcohol solutions were prepared using ethanol and water.
[b] Beverages were mixed using 25 ml of spirit in 100 ml of mixer, hence giving a alcohol content of approximately 10%.

Renishaw Portable Raman Analyser RX210 'RIAS'

The RIAS (Wotton-under-Edge, UK) is equipped with a diode laser emitting at 785 nm and a thermoelectrically cooled (400 × 575 pixels) CCD detector with an attached fibre-optic coupled probe, equipped with a 20× (NA 0.35) Olympus objective lens. The diffraction grating (1000 lines/mm) affords the spectral range ~2100–100 cm^{-1} with a spectral resolution of 10 cm^{-1}. The power of the diode laser is 49 mW at the sample. Daily calibration of the wavenumber axis is achieved by recording the Raman spectrum of a silicon wafer (1 accumulation, 10 s exposure) for static modes. If necessary, an offset correction is performed to ensure that the position of the silicon band is 520.5 ± 0.1 cm^{-1}. Spectra were recorded with an accumulation of one scan, 10 s exposure. Spectra were not corrected for instrument response. The spectrometer was controlled by a portable PC with instrument control software (Renishaw WiRE 2 Service pack 8).

Delta Nu Inspector Raman FSX

The Inspector Raman (Laramie, WY, USA) is equipped with a diode laser emitting at 785 nm, a thermoelectrically cooled (1 × 1024 pixels) CCD detector and a custom 25 mm focal length nose piece. The spectral range is 2000–200 cm^{-1} with a spectral resolution of 8 cm^{-1}. The laser power is 37 mW at the sample. Daily calibration of the wavenumber axis is achieved by recording the Raman spectrum of polystyrene within the calibration routine built into the software. Spectra were recorded with an accumulation of 1 scan, 10 s exposure. Spectra were not corrected for instrument response. The spectrometer was controlled by a portable PC with instrument control software (Nu Spec Version 4.75).

Data comparison

Raman spectra from all instruments were exported to the Galactic *.SPC format. Spectra were then compared using GRAMS AI (Version 8.0, Thermo Electron Corp, Waltham, MA, USA). The Raman spectra were not subjected to any data manipulation or processing techniques and are reported as collected.

Results and Discussion

Detection of GHB and GBL in containers

When samples of drugs are seized by the police or security services they are most often presented in a container of some type. Gamma-hydroxybutyric acid and GBL have been investigated in a number

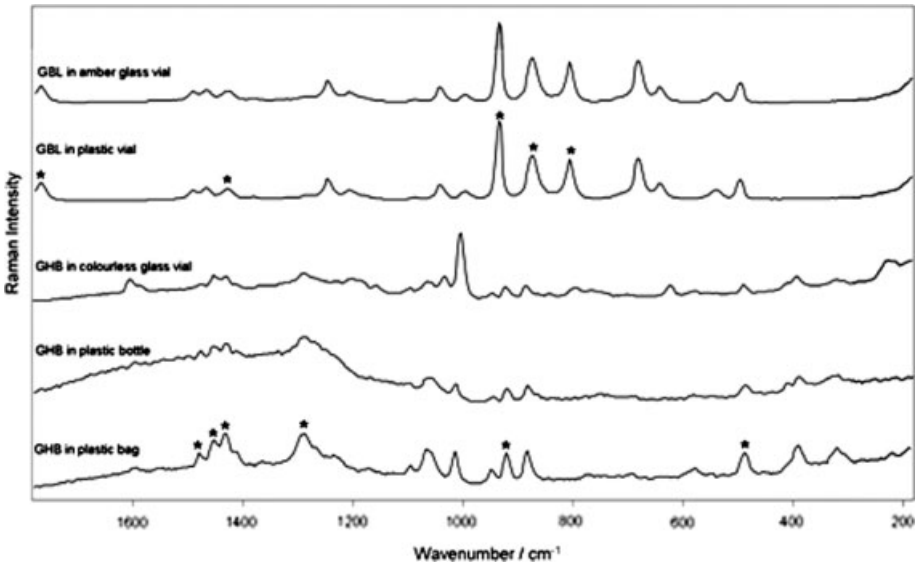

Figure 2. Raman spectra of GHB and GBL in containers obtained on the Renishaw RIAS portable Spectrometer (785 nm, 49 mW, 1 × 10s exposure).

Table 2. Band assignments for GHB and GBL

Band assignments for GHB	
Wavenumber (cm^{-1})	Assignment
486	CO_2 rock
919	CH_2 rock
946	C–C stretch
1285	CH_2 wag
1428-1472	CH_2 bends
Band assignments for GBL	
Wavenumber (cm^{-1})	*Assignment*
810	Ring breathing modes
870	
931	C–O–C symm stretch
1423	C–O–C asym stretch
1761	C═O stretch

of different containers in order to simulate how a seized sample may be presented to a police officer or forensic analyst. Figure 2 shows the Raman spectra of both GHB and GBL in a variety of container types. The key peaks for both GHB and GBL are marked and assignments given in Table 2. Positive identifications of both drugs were made in all container types tested. The average analysis time for these samples was 15 seconds per sample. The ability to identify seized samples whilst still in their original containers is of great forensic value, as the integrity of the sample is preserved by removing the need to open the container. This also provides advantages to the analyst, such as lack of exposure to harmful substances and a reduction of analysis times.

Detection of GHB and GBL in solution

Due to the application of GHB as a 'date-rape' drug by 'spiking' beverages it is essential to determine whether GHB and GBL are detectable in solution. Figure 3 shows the Raman spectra of GHB in water and in a 15% alcohol solution. The spectra exhibit band broadening due to the extensive hydrogen bonding between GHB and water. However, the drug is still identifiable by the peak at 946 cm^{-1} attributed to C-C stretching. Figure 3 also shows the Raman spectra of GBL in water and GBL in a 40% alcohol solution. The GBL is identifiable by the bands at 678, 806 and 929 cm^{-1}.

Detectable limits study of GHB and GBL water and ethanol (model systems)

The average volume of a drink is between 150–200 mL and the common dosage level of GHB to be between 2.5–4 g then the average concentration of the drug in a spiked beverage would range from 1.5 to 3% w/v. The aim of this study was to investigate the lowest amount of GHB and GBL detectable with the three different spectrometers for a variety of solutions simulating spiked beverages.

It has already been demonstrated that we are able to positively identify GHB and GBL in solution using Raman spectroscopy. Figure 4 shows the Raman spectra of GHB in water at a range of the concentrations recorded on the Delta Nu Inspector Raman instrument. The spectra of GHB in water shows two bands, one at 946 cm^{-1} assigned to C-C stretching and the other at 1423 cm^{-1} assigned to C-O-C asymmetric stretch. As mentioned previously, a number of bands are substantially broader than those in the spectrum of the GHB sodium salt, due to the hydrogen bonding that occurs between the salt and the water. However as Fig. 6 shows, GHB bands are clearly visible in water at concentrations as low as 1% w/v, which is lower than the common dosage level. The Raman spectra in Fig. 5 show the results of this study with GBL in a 15% ethanol solution obtained on the Renishaw 'RIAS' spectrometer. The peak at 931 cm^{-1}, attributed to the C-O-C symmetric stretch in GBL, is still visible at 0.25% v/v, much lower than the common dosage level. Table 3 summarises the results for each solution on each spectrometer. Generally GBL has a much lower detection limit than GHB due to the fact that the GHB has a much weaker Raman scattering. The range of detection limits for GHB is between 1–2% w/v, which is towards the lower range of the common dosage level that ranges from 1.5% w/v to 3% w/v. The detection limit of GBL in solution was found to be between 0.25–0.5% v/v, much lower than the expected dosage level. A

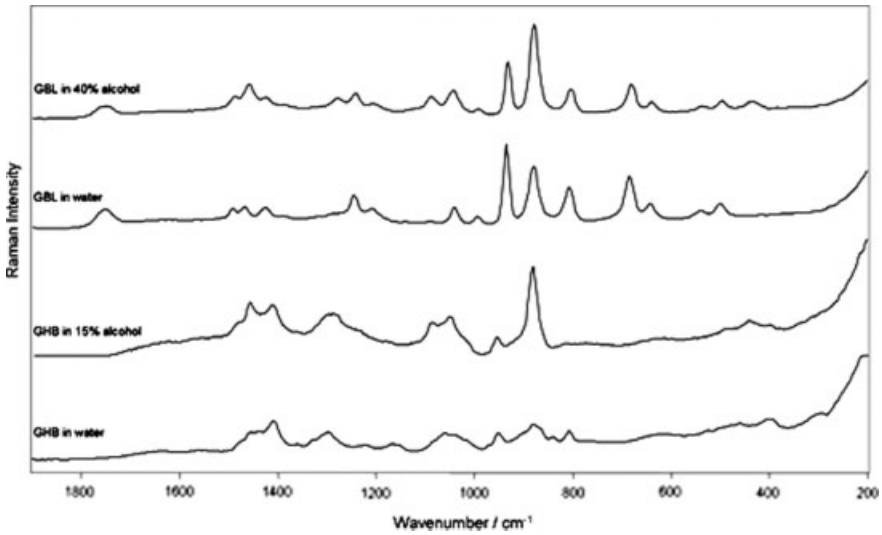

Figure 3. Raman spectra of GHB and GBL in Solution obtained on the Renishaw Invia Reflex spectrometer (785 nm, 110 mW, 1 × 10s exposure).

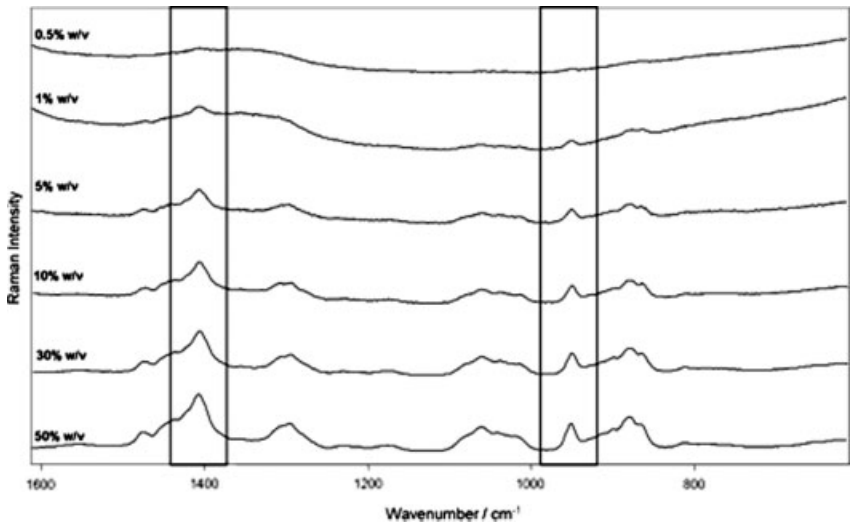

Figure 4. Detectable Limit study of GHB in water obtained on the Delta Nu inspector Raman (785 nm, 35 mW, 1 × 10s exposure).

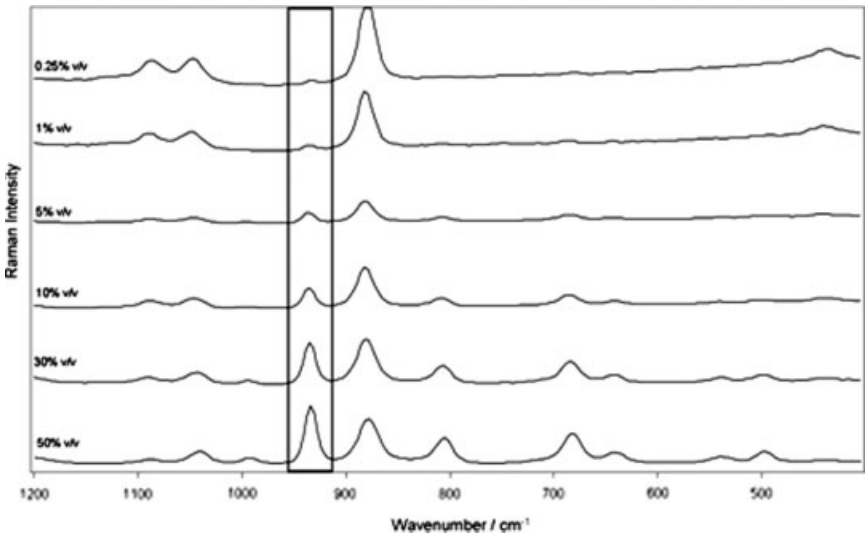

Figure 5. Detectable Limit study of GBL in 15% alcohol obtained on the Renishaw RIAS portable spectrometer (785 nm, 49 mW, 1 × 10s exposure).

Table 3. Results summary for detection limits study in model systems

GHB				GBL			
Beverage/Spectrometer		LOD	DL	Beverage/Spectrometer		LOD	DL
Water	Invia	2.4	1	Water	Invia	1.2	0.25
	Delta Nu	1.9	1		Delta Nu	2.0	0.5
	RIAS	2.2	1		RIAS	1.5	0.25
15% alcohol	Invia	2.5	1	15% alcohol	Invia	2.0	0.25
	Delta Nu	2.3	1		Delta Nu	1.4	0.5
	RIAS	1.9	1		RIAS	1.1	0.25
40% alcohol	Invia	1.8	1	40% alcohol	Invia	1.2	0.25
	Delta Nu	2.2	2		Delta Nu	2.1	0.5
	RIAS	1.7	1		RIAS	1.9	0.5

LOD: Theoretical limit of detection calculated from calibration curve.
DL: Spectrally identifiable detection limit.

key point to be made from the results table is that the portable spectrometers under these conditions gave representative results and compare favourably with the bench-top instrumentation, this is relevant for *in situ* crime scene work.

Figure 6 shows the calibration curves of peak areas and concentration for GHB in water recorded on the Inspector Raman. Each solution in this work was measured in triplicate and processed using GRAMS software to calculate the area of the peak at 946 cm^{-1} for GHB and 931 cm^{-1} for GBL. These measurements were then averaged to give the mean peak area and a calibration curve plotted. The standard deviation (σ) of these measurements was calculated, and from this 3σ values were calculated for each

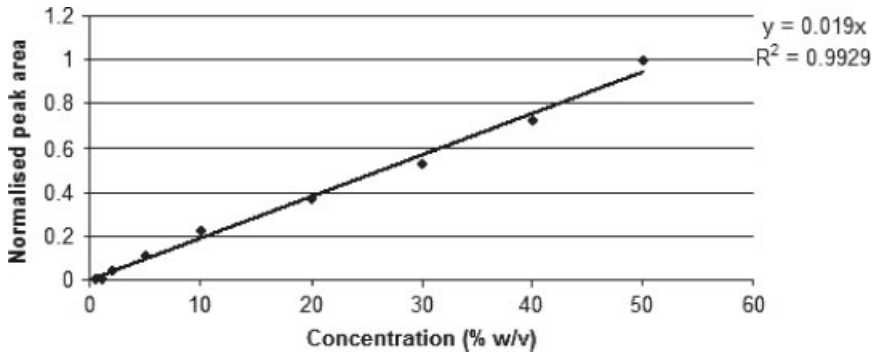

Figure 6. Calibration curve for the LOD of GHB in water (Delta Nu Inspector Raman).

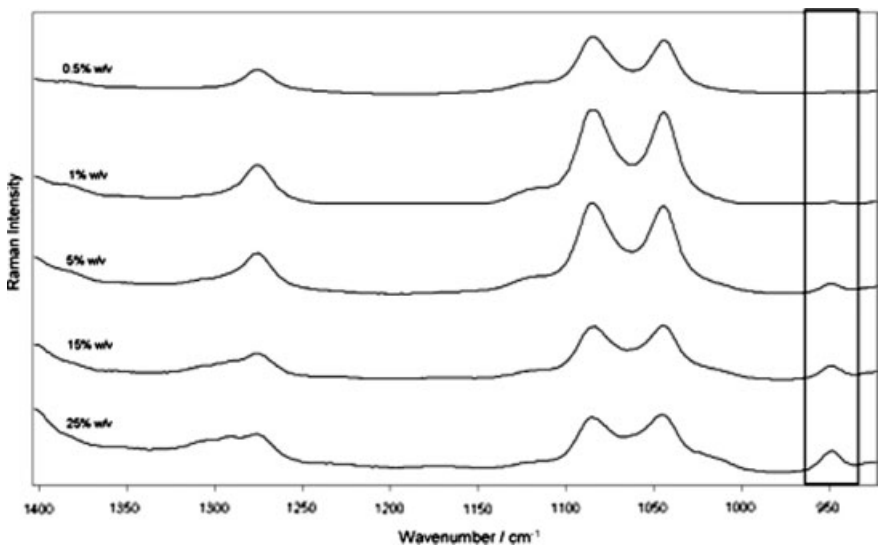

Figure 7. Detectable Limit study of GHB in Vodka obtained on the Renishaw Invia Reflex spectrometer (785 nm, 110 mW, 1 × 10s exposure).

solution. Using the curve the theoretical limit of detection (LOD) was then calculated using the equation below; where σ is the standard deviation and S is gradient of the calibration curve.

$$\mathrm{LOD} = \frac{3\sigma}{S}$$

For GHB in water recorded on the Delta Nu Inspector Raman spectrometer, the LOD was calculated to be 1.9% w/v and 1.1% v/v for GBL in 15% alcohol on the Renishaw RIAS instrument. It should be noted that the intercept of the calibration curve was set to zero, which will affect the slope of the curve and therefore affect the calculated LOD. The limits of detection calculated in this study are shown in Table 3, and range from 1.7–2.5% w/v for GHB and 1.2–2.1% v/v for GBL.

Detectable limits study of GHB and GBL in beverages (real systems)

It was demonstrated in the previous study that it is possible to detect GHB and GBL in solutions simulating spiked beverages (model systems). The next step is to determine whether GHB and GBL are identifiable in real systems.

Figure 7 shows the Raman spectra of GHB in vodka obtained on the Renishaw Invia Reflex benchtop instrument. The spectra show that GHB is still identifiable in real systems, by the peak at 946 cm^{-1}. The peak, which is assigned to the C-C stretching in GHB, is identifiable at 1% w/v, which is lower than the common concentration found in a spiked beverage. A summary of the results in this study are shown in Table 4. The theoretical LOD for this example was calculated to be 1.5% w/v. The Raman spectra of GBL in Fig. 8 show the results for GBL in a gin-and-tonic beverage obtained on the Delta Nu Inspector Raman portable spectrometer.

Table 4. Results summary for detection limits study in real systems

GHB				GBL			
Beverage/Spectrometer		LOD	DL	Beverage/Spectrometer		LOD	DL
Vodka	Invia	1.5	1	Vodka	Invia	1.4	0.25
	Delta Nu	1.5	1		Delta Nu	0.9	0.5
	RIAS	0.7	1		RIAS	1.3	0.5
Gin	Invia	1.1	1	Gin	Invia	1.0	0.25
	Delta Nu	1.6	1		Delta Nu	0.9	0.5
	RIAS	1.3	1		RIAS	1.0	0.5
White wine	Invia	1.8	2	White wine	Invia	1.8	0.25
	Delta Nu	1.9	2		Delta Nu	0.8	0.5
	RIAS	1.2	2		RIAS	0.9	0.5
Vodka and lemonade	Invia	1.4	2	Vodka and lemonade	Invia	0.8	0.25
	Delta Nu	1.3	2		Delta Nu	1.2	0.5
	RIAS	0.9	1		RIAS	0.9	0.5
Gin and tonic	Invia	2.0	1	Gin and tonic	Invia	1.1	0.25
	Delta Nu	2.1	2		Delta Nu	0.9	0.5
	RIAS	1.4	1		RIAS	1.2	0.5

LOD: Theoretical limit of detection calculated from calibration curve.
DL: Spectrally identifiable detection limit.

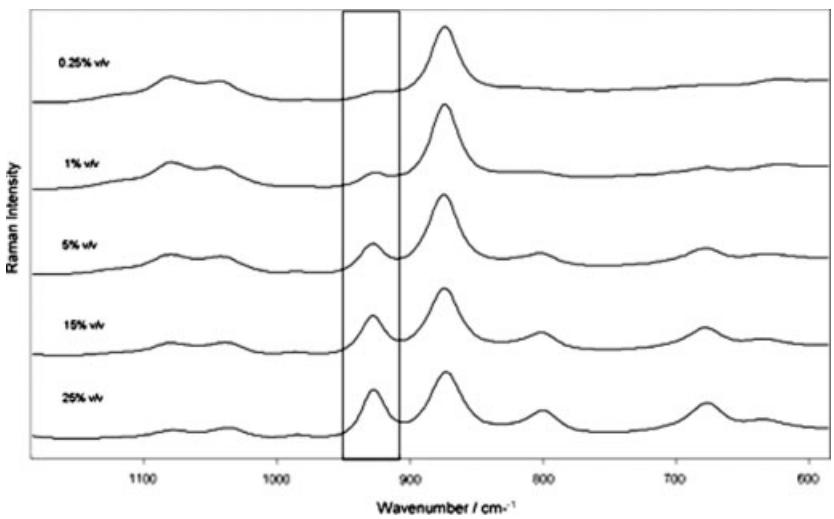

Figure 8. Detectable Limit study of GBL in Gin and Tonic obtained on the Delta Nu Inspector Raman (785 nm, 37 mW, 1 × 10s exposure).

The spectra show that the spectrally detectable limit of GBL in gin-and-tonic beverage is 0.5% v/v on this particular instrument and the LOD calculated from the corresponding calibration curve is 0.9% v/v. The range of detection limits shown in Table 4 indicate that the theoretical and actual detection limits of GHB and GBL in real systems are below the common dosage level range of 1.5–3% w/v. The results in Table 4 also show that the detection limits on the portable instruments are comparable with those from the benchtop instrument, indicating that portable Raman spectroscopy is an ideal tool for the *in situ* interrogation of spiked beverages. All the spectra in this study were recorded through a glass vial, demonstrating the ability to detect both GHB and GBL in spiked beverages while still in their original containers.

Conclusion

We have demonstrated the detection of GHB, a 'date-rape' drug by Raman spectroscopy in a number of different scenarios and have shown that it is possible to detect GHB in a variety of containers. This is particularly advantageous in forensic work as it limits the exposure of the analyst to harmful substances; it also reduces the time needed for each analysis and minimises the risk of contamination of the evidence. It has also been shown that the two portable instruments used in this study are suitable instruments for the rapid first-pass *in situ* identification of GHB. This work clearly demonstrates that portable Raman spectrometers can be used to interrogate samples *in situ*, allowing a more thorough investigation of key samples by Raman and other analytical techniques (LC-MS and GC-MS) in a forensic laboratory where applicable. In addition to this the ability to interrogate various solutions spiked with GHB, simulating spiked alcoholic beverages, and in real systems has been demonstrated. Due to the interconversion of GHB and GBL in solutions, we have also studied the identification of GBL using Raman spectroscopy. It has been shown that the limit of detection of both GHB and GBL is lower than the common dosage level.

References

[1] S. Elliot, V. Burgess, *Forensic Sci. Int.* **2005**, *151*, 289.
[2] Home Office. Available at www.homeoffice.gov.uk/drugs/drugs-law/class-a-b-c/, accessed 12 June 2008.
[3] H. R. Sumnall, K. Woolfall, S. Edwards, J. C. Cole, C. M. Benyon, *Drug Alc. Dep.* **2007**, *1016*, 3090.
[4] Home Office. Available at www.homeoffice.gov.uk/rds/crimstats07.html, accessed 17 May 2008.
[5] G. Galloway, *Addiction* **1997**, *92*, 96.
[6] L. A. Ciolino, M. Z. Mesmer, R. D. Satzger, A. C. Machal, H. A. McCauley, A. S. Mohrhaus, *J. Forensic Sci.* **2001**, *46*(6), 1315.
[7] S. A. Hennessy, S. M. Moane, S. D. McDermott, *J. Forensic Sci.* **2004**, *49*(6), 1.
[8] J. V. DeFrancesco, M. R. Witkowski, L. A. Ciolino, *J. Forensic Sci.* **2006**, *59*, 321.
[9] A. G. Ryder, *J. Forensic Sci.* **2002**, *47*, 275.
[10] E. M. A. Ali, H. G. M. Edwards, M. D. Hargreaves, I. J. Scowen, *Anal. Bioanal. Chem.* **2008**, *390*, 1159.
[11] S. M. Angel, J. C. Carter, D. N. Stratis, B. J. Marquardt, W. E. Brewer, *J. Raman. Spectrosc.* **1999**, *30*, 795.
[12] M. D. Hargreaves, K. Page, T. Munshi, R. Tomsett, G. Lynch, H. G. M. Edwards, *J. Raman Spectrosc.* **2008**, *39*, 873.
[13] M. L. Ramirez, W. Ortiz, R. Orlando, L. Pacheco-Londono, S. P. Hernandez-Rivera. *SPIE,* **2007**, 6538.