Supplementary information.

Rapid through-container detection of fake spirits and methanol quantification with handheld Raman spectroscopy

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Materials and methods

Sample preparation

Included in a preliminary sample set were simulated counterfeit products, where a number of compounds were added to vodka, whisky, gin and rum. These compounds (Table S1) include:

i) flavourings, and a sweetener (sucrose), commonly found added to counterfeit whisky samples such as vanillin, limonene and trans-anethole;
ii) methanol due to the health implications of its consumption; and
iii) denaturants that were commonly used in Europe prior to Commission Implementing Regulation (EU) 2016/1867.

The vodka, whisky, gin and rum used as diluents were created by homogenising a bulk (3 L volume) of each spirit category, in a beaker on a magnetic stirrer plate for 1 hour.

The initial recipe for the harmonised European formula for completely denatured alcohol, prior to Commission Implementing Regulation (EU) 2016/1867, was 3 L of isopropyl alcohol (IPA), 3 L of methyl ethyl ketone (MEK) and 1 g of denatonium benzoate per 100 L of pure ethyl alcohol (Commission Implementing Regulation (EU) 162/2013). At the time, there was also a popular German formulation that contained 1 L of a ketone mixture with 1 g of denatonium benzoate per 100 L of alcohol, the ketone mixture consisting of 95-96% by weight methyl ethyl ketone, 2.5-3% by weight methyl isopropyl ketone and 1.5-2% by weight ethyl sec-amyl ketone (Commission Implementing Regulation (EU) 162/2013). This information was used to prepare an appropriate sample test set: this set was analysed without knowledge of the sample contents and this blind coding was only broken after analysis.

The simulated counterfeit spirits were created such that concentration levels of the flavourings, methanol and denaturant compounds were present at levels which are relevant detection concentrations. For example, denatonium benzoate is a denaturant used at 1 g/100L of pure ethyl alcohol in the harmonised formula for the complete denaturing of alcohol. If this is reduced to 40% alcohol, the strength typically seen in spirit drinks, the concentration would be 4 mg/L. Due to the bitter taste of denatonium benzoate it is likely that a counterfeiter would attempt to remove the denaturant or dilute it further with genuine spirit. For these experiments the further dilution or attempted removal is represented by diluting the samples to 1/20th of the concentration seen in 40% spirit, giving 0.2 mg/L of denatonium benzoate in the test samples. All of the denaturants and flavourings were prepared individually in vodka (a simple spirit) and whisky (a complex spirit); trans-anethole and limonene were not prepared in rum or gin as they may be present in the genuine base spirits used in this experiment.

Sample preparation for the simulant samples used parent standards in 100% pure ethyl alcohol (Rathburn Chemicals Ltd., Walkerburn, Scotland), where the chemical was accurately weighed into a volumetric flask and made up to volume with the ethanol. Working standards in 40% ethanol/ultra high quality water (UHQ) were then created; these were prepared so that the same volume of working standard was diluted into each diluent for all chemicals used. In order to avoid dilution effects, the same volume of 'blank' 40% ethanol/UHQ water was then added to the blank samples. All samples were filled directly into sample vials using a Pasteur pipette.

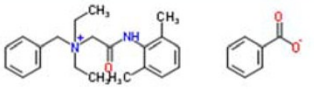
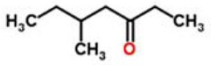
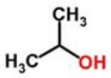
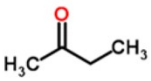
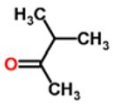
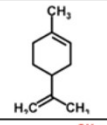
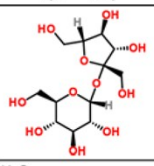
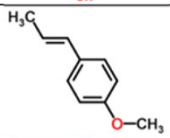
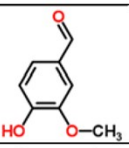
Multiblock-PCA in discriminating different simulated counterfeit spirits

Similar to our previous study [1], multi-block PCA (MB-PCA) [2] was applied to the Raman spectra of the four types of spirit samples to highlight the differences between genuine spirit samples and those with various denaturants added. The Raman spectra collected from four types of spirit samples were first partitioned into four blocks: one for each type of spirit drink. Between these blocks, the rows are matched upon the type of denaturant added; i.e., across the four blocks the same row has the samples added with the same type of denaturant.

PLSR for methanol quantification and determination of limit of detections

Partial least squares regression (PLSR) was used to build models in order to predict the concentrations of methanol in the four spirits. Four sets of PLSR models were built, one for each type of spirit. The models were trained using the Raman spectra of spirit samples spiked with different concentrations of methanol and validated using 1,000 bootstrapping re-sampling, and we generated prediction plots of *only* the test samples from these 1,000 bootstraps. These models were then applied to the blind test samples and the predictive accuracies were reported in term of squared coefficient of determination (Q^2), which gives an estimate of model accuracy (the closer to 1 the better). The limit of detection of methanol was also estimated based on the predictions of these blind test samples using the procedure described in [3].

Table S1. Details of the 10 compounds used to produce counterfeit alcohol . These include six denaturants (red) and four flavourings (blue). This table also details the minimum concentrations detected with handheld Raman spectroscopy.

Name	Synonym(s)	Chemical Formula	Concentration (ppm)	Structural Formula
Denatonium benzoate	Bitrex	$C_{28}H_{34}N_2O_3$	0.2	
Ethyl sec-amyl ketone	5-Methyl-3-heptanon	$C_8H_{16}O$	4	
Iso-propyl alcohol	Isopropanol	C_3H_8O	600	
Methanol	Carbinol/ Wood alcohol	CH_4O	250	
Methyl ethyl ketone	Butanone	C_4H_8O	190	
Methyl isopropyl ketone	3-Methylbutan-2-one	$C_5H_{10}O$	6	
Limonene	1-Methyl-4-methylethenylcyclohexene	$C_{10}H_{16}$	100	
Sucrose	Table sugar	$C_{12}H_{22}O_{11}$	100	
Trans-anethole	(E)-1-(4-Methoxyphenyl) propene	$C_{10}H_{12}O$	10	
Vanillin	4-hydroxy-3-methoxybenzaldehyde	$C_8H_8O_3$	10	

Potable ethanol can be denatured with a range of chemicals that make it unfit for human consumption and exempt from excise taxes. The exemption status of so-called denatured alcohol provides the economic incentive for its fraudulent use in counterfeit spirit products.

Figure S1. Mean Raman spectra of all sample classes analysed in this study. Solid lines represent genuine samples, and dashed lines represent samples containing denaturants.

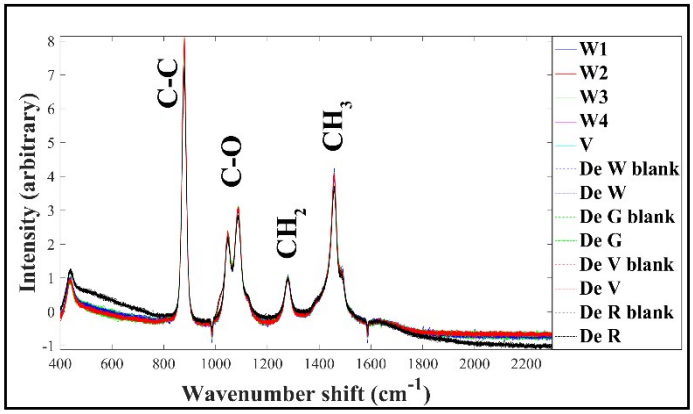


Figure S2. Raman spectra of ethanol and methanol collected with the CBEx 1064 nm handheld spectrometer.

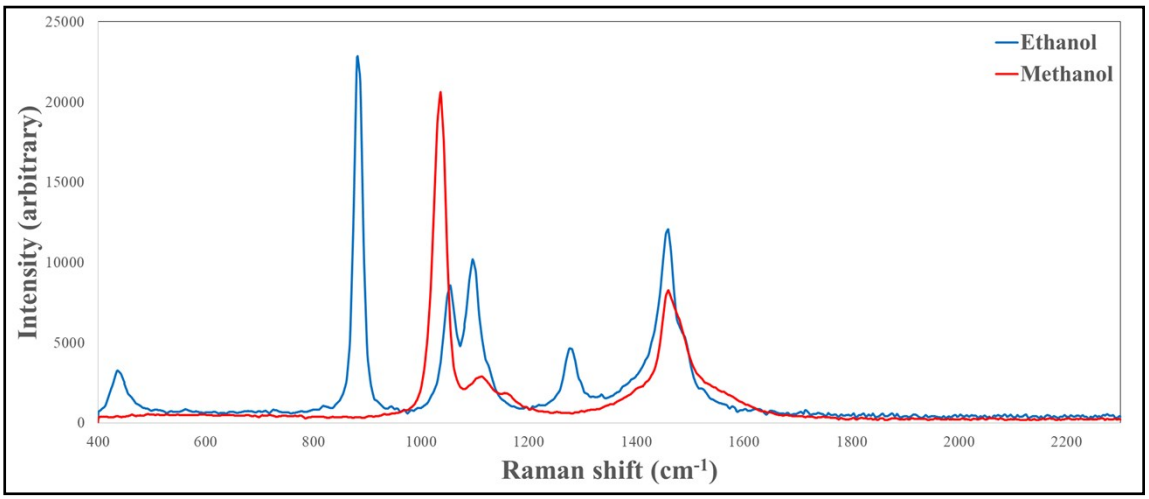


Figure S3. (A) PCA scores plots of all Raman spectral data collected. Different samples are presented by different symbols and colours (see legend), and denaturant containing samples are presented as empty symbols. (B) PCA scores plots of the same samples following the removal of rum samples. With rum samples removed, any separation between these non-rum samples can be more readily observed. TEV = total explained variance.

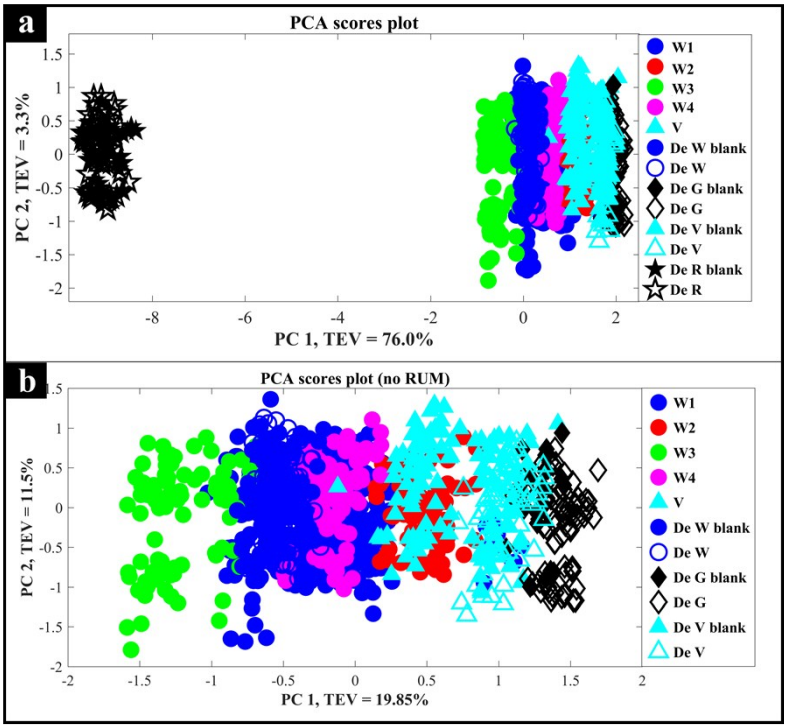


Figure S4. MB-PCA super scores plot of all Raman spectral data collected from whisky samples adulterated with different compounds, which are found in counterfeit whisky as denaturants or added flavourings. TEV = total explained variance.

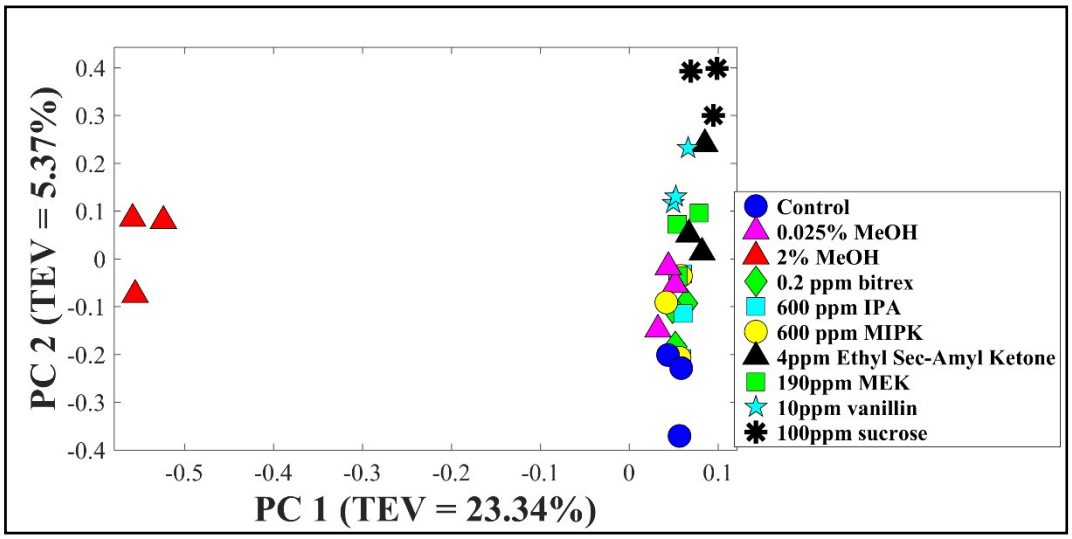


Figure S5. MB-PCA super scores plot of Raman spectral data collected from adulterated whisky samples, after removing Raman spectra from the methanol-containing samples. TEV = total explained variance.

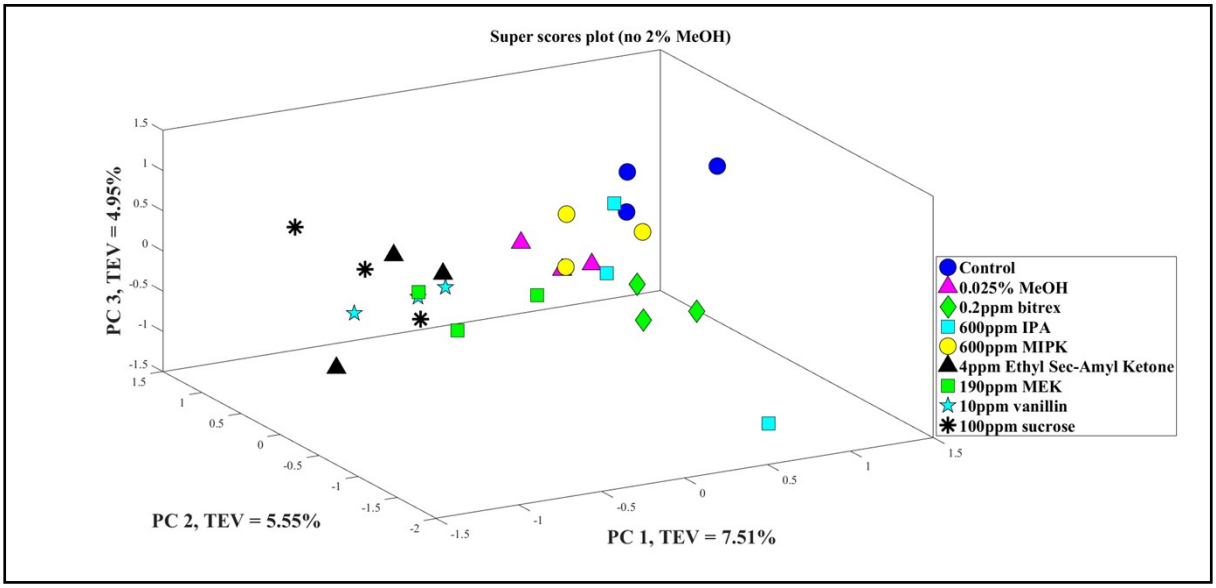
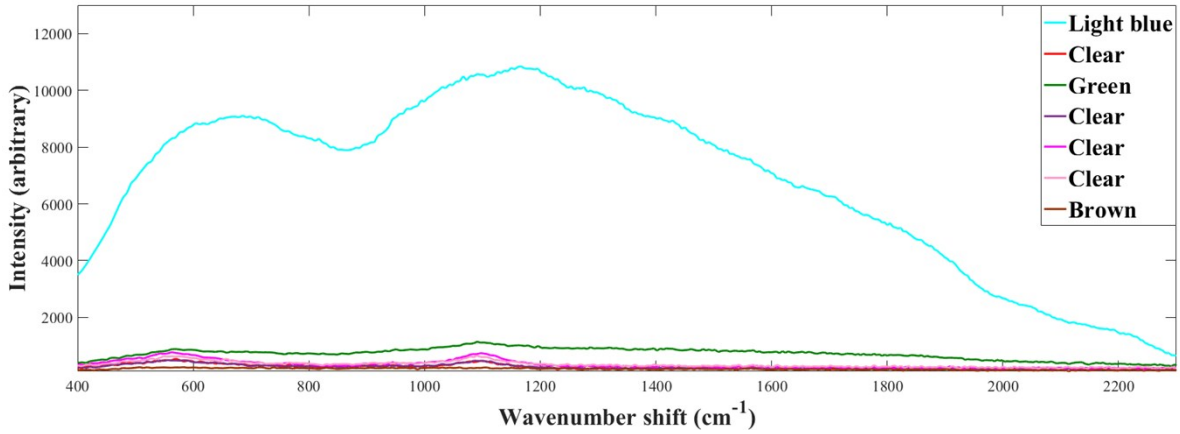


Figure S6. Image of the commercial glass bottles of a range of spirits drinks used in this study.

Figure S7. Averaged Raman spectra of three measurements collected from empty glass spirit bottles, with fluorescence from the light blue-coloured bottle clearly visible.



References

1. D. I. Ellis, R. Eccles, Y. Xu, J. Griffen, H. Muhamadali, P. Matousek, I. Goodall and R. Goodacre, *Scientific Reports*, 2017, **7**.
2. A. K. Smilde, J. A. Westerhuis and S. de Jong, *J. Chemometr.*, 2003, **17**, 323-337.
3. M. Blanco, M. Castillo, A. Peinado and R. Beneyto, *Anal. Chim. Acta*, 2007, **581**, 318-323.