WHITE PAPER

Infrared/Near-Infrared/UV/Visible Spectroscopy

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Comparison of Molecular Spectroscopic Techniques Applied to Whisky Analysis

Introduction

The size of the counterfeit food market in the UK was estimated at £7 billion per year by the consumer group Which? (2011). It suggested as much as 10% of the food on sale in retail and in the foodservice industry was not the indicated ingredients. This is supported by the range of examples of Foods Standards Agency (FSA) prosecutions for food fraud in the last 10 years. For example, in 2003 the FSA found 46% of basmati rice samples were bulked up with cheaper samples. In 2007, Harrods and Sainsbury's (and others) were caught selling farmed salmon as wild. An FSA investigation found 1 in 10 samples of sea bass and sea bream were in fact farmed, with the level increasing to 1 in 7 for "wild" salmon. The limitations to detection have centered mainly on DNA for these products. An Organization for Economic Co-operation and Development (OECD) report in 2007 identified some of the most "faked" products as alcohol, kiwis, milk powder, and baby food. The European Spirits Organization (CEPS) acknowledges counterfeiting and IPR infringement of spirit brands are a major concern posing a serious health threat by providing inferior or even toxic products. Furthermore, these practices damage not only the spirits industry but also government revenues, such as duties that officially traded products generate. It goes on to estimate that a quarter of products sold in China as imported spirits are actually fakes[1]. Testing for alcohol has been based on laborious laboratory techniques – there has been no effective swift screening test.

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Molecular Spectroscopic-based technologies are particularly well suited to rapid screening testing. They are relatively inexpensive, very easy to operate, and give a fast answer. Molecular spectroscopy techniques are independent of supplies, such as gases and solvents that constrain many analytical approaches to the laboratory. In recent years, the emergence of molecular spectroscopy techniques in mobile or portable forms has enabled screening in labs closer to the point of measurement or in the field itself. For Whisky authenticity, a number of molecular spectroscopy techniques have been launched, each promising to assist in the detection of counterfeiting. This white paper will investigate the potential of molecular spectroscopy for screening a number of whisky authenticity problems and recommend suitable miniaturization approaches for the fight against spirit fraud.

1. Infrared Spectroscopy

The infrared region of the spectrum is generally split into three different regions:

The far-infrared region is primarily used for measuring heavier atoms and inorganic materials, so is not relevant to these types of samples.

- Far-infrared, usually defined as the spectral range below 400 cm^{-1} - 20 cm^{-1}
- Mid-infrared, usually defined as the spectral range 4000 cm^{-1} – 400 cm^{-1}
- Near-infrared, usually defined as the spectral range 14000 cm^{-1} – 4000 cm^{-1}

Mid-infrared spectroscopy is used for observing fundamental vibrations within molecules and will generate spectra that can be used as a fingerprint for different types of materials.

Near-infrared absorptions consist of overtones and combination bands derived from the fundamental vibrations observed in the mid-infrared region of the spectrum. These absorptions in the near-infrared region are considerably weaker than those observed for the fundamental vibrations.

Mid- and Near-infrared spectroscopy has been applied to the whisky samples in this study.

Overview of Sampling Techniques for Liquids in the Mid- and Near-infrared Spectral Regions

There are a variety of sampling techniques available for mid- and near-infrared spectroscopy. They generally fall into two distinct categories: Transmission or Reflectance.

In transmission, the instrument will measure the amount of radiation that is transmitted through the sample. For example, the amount of radiation that has not been absorbed by the sample.

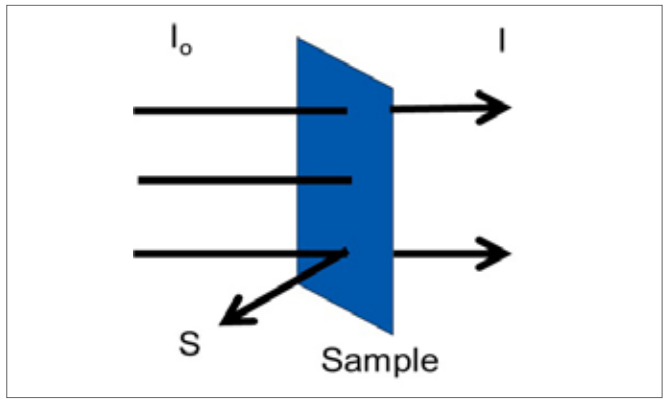

Figure 1. Transmission Sampling Technique.

The liquid sample is placed into a liquid cell with suitable window materials for the infrared region of the spectrum. Typical cell pathlengths for the mid-infrared region range from 0.005 mm -0.1 mm (5 - 100 microns) and for the near-infrared region range from 0.05 mm - 10 mm.

In reflectance, the instrument will measure the amount of radiation that is reflected by the sample. Reflectance techniques, specifically specular or diffuse reflectance, are surface techniques that are used for continuous solids, powders, and pastes. In general, liquids do not reflect very much infrared radiation. The most appropriate reflectance technique for infrared spectroscopy of liquids, such as whiskies, is the technique of Attenuated Total Reflectance (ATR).

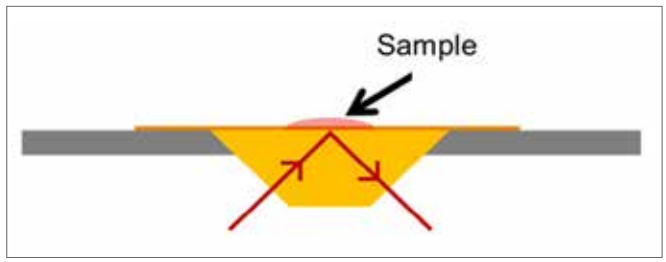

Figure 2. Attenuated Total Reflectance.

In ATR, the sample is placed on top of a suitable crystal material. The infrared beam passes through the crystal and is internally reflected from the top crystal surface. A small evanescent wave will penetrate a small distance from the crystal surface into the sample before it is reflected back in to the crystal and to the infrared detector. The penetration of the infrared beam into the sample is sufficient to generate an infrared spectrum of the material. The typical effective pathlength for a sample in an ATR measurement is in the range 0.5 - 20 microns, depending on the crystal type and the number of reflections in the crystal. This makes the ATR technique suitable for mid-infrared measurements. However, since the near-infrared absorptions are considerably weaker than in the mid-infrared, ATR is not a suitable sampling technique for near-infrared region of the spectrum.

Infrared Transmission Measurements of Whiskies

Whisky samples were injected into a CaF_2 infrared cell with a 0.05 mm pathlength. Spectra were collected spanning the mid- and near-IR region of the spectrum up to 7800 cm^{-1}.

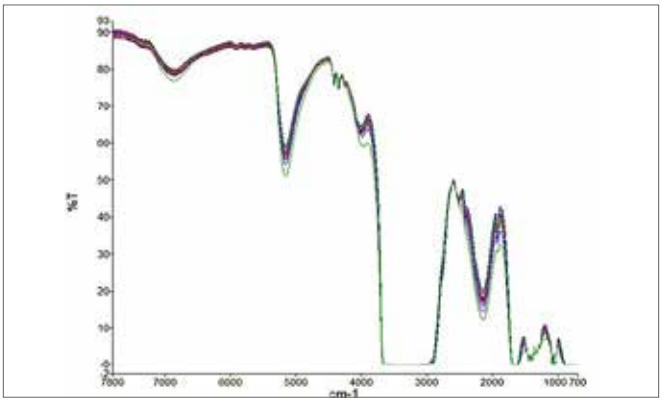

Figure 3. Transmission spectra of all of the samples submitted.

At this pathlength a significant range of the mid-infrared region of the spectrum is obscured by the very strongly absorbing bands for water in the approximate regions of 1640 cm^{-1} and 3400 cm^{-1}. The weaker bands in the near-infrared region of the spectrum are much more suitable for the analysis of aqueous samples. In the near-infrared region of the spectrum, it is possible to observe the combination bands for water at 5167 cm^{-1} and ethanol in the region 4420 cm^{-1} - 4300 cm^{-1}. These ingredients are the major contributors to the overall spectrum of the whisky. The mid-infrared region is the best spectral region for identification of materials. Since this region is greatly obscured at this pathlength it is difficult to use this region for determination of other ingredients. Working with shorter pathlengths would decrease the intensity of the water bands but would also sacrifice the intensity from the other ingredients. Hence, transmission measurements would not be recommended for authenticity studies. However, transmission spectra in the near-IR region offer the possibility to perform quantitative measurements of the alcohol content in alcoholic beverages.

A series of standard mixtures of ethanol and water were prepared over the concentration range of 0 - 0.8 g/mL ethanol. Spectra were collected in a 0.05 mm (50 microns) CaF_2 transmission cell at a spectral resolution of 4 cm^{-1} using four scans.

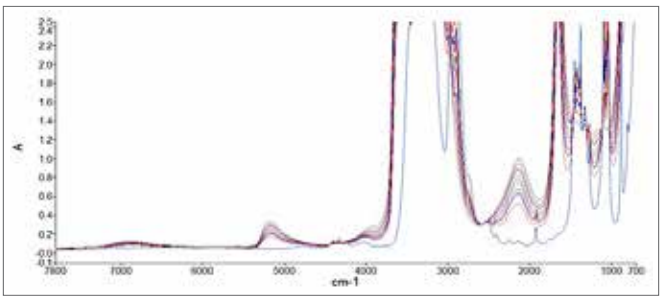

Figure 4. Transmission spectra of ethanol/water standards (mid- and near-IR regions).

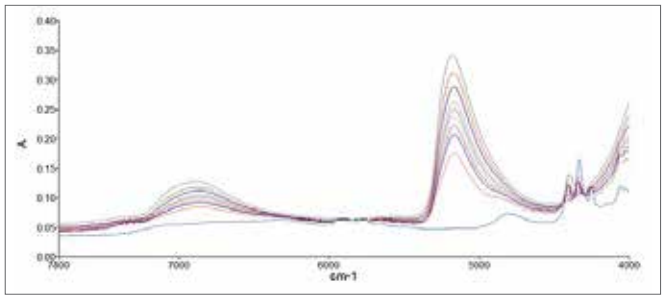

Figure 5. Transmission spectra (Near-IR region) expanded.

A Partial Least Squares (PLS1) algorithm was used to generate a quantitative calibration for these standards. The calibration included data from the spectral range from 7300 - 4000 cm^{-1}, using first derivative data with a noise reduction factor of three. The software calculations show that two Principal Components were required for the model. Cross validation of the model was performed on a "leave-one-out" basis.

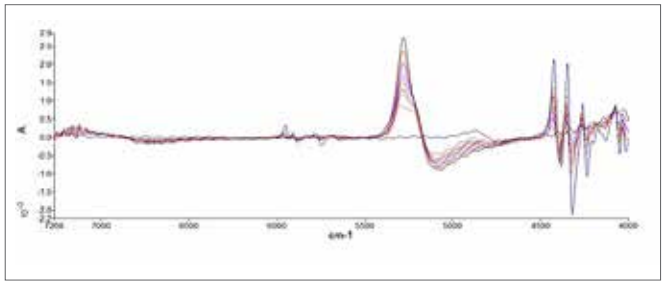

Figure 6. First derivative spectra of the ethanol/water standards.

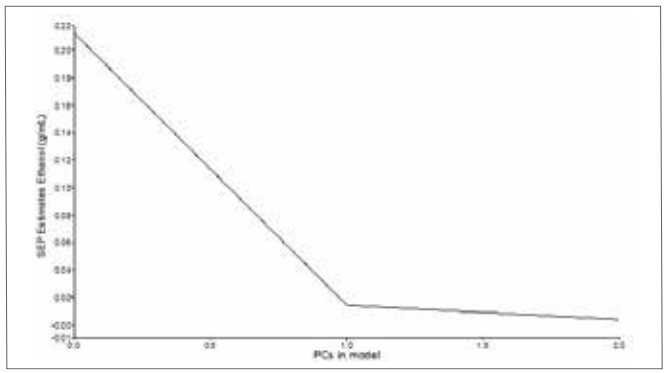

Figure 7. Calibration Model- SEP plot.

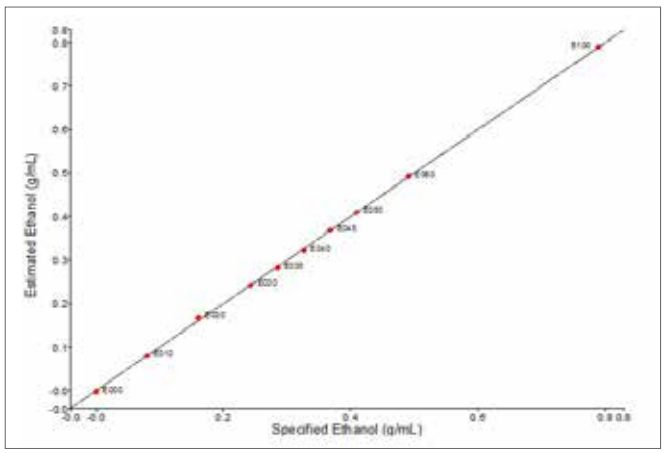

Figure 8. Calibration plot for ethanol concentration.

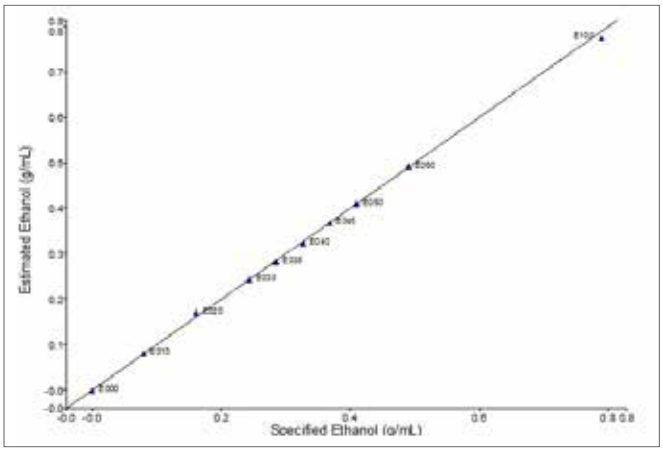

Figure 9. Cross Validation plot for ethanol concentration.

The quantitative model was applied to a series of "adulterated" whisky samples. Sample E0 is a commercially available Scotch whisky. All subsequent samples consisted of sample E0 plus a range of adulterants. The alcohol content of samples E1 and E2 is seen to be particularly low as these samples were diluted with UHQ water. The remainder of the samples were adulterated with ethanol, other Scotch whiskies or non-Scotch alcoholic beverages. Hence, their alcoholic contents were close to the original whisky.

Quantitative Measurements of Alcohol Content

Table 1. Quant Results.

Sample Name	Ethanol (g/mL)
E0	0.3218
E1	0.2396
E2	0.1642
E3	0.3221
E4	0.3224
E5	0.3187
E6	0.3224
E7	0.3221
E8	0.3227
E9	0.3109
E10	0.3220

The quantitative results shown here are capable of detecting dilution of the alcohol content of the whisky, but would not allow for detection of intentional adulteration where the alcohol content is adjusted to match the alcohol content of the original whisky.

Direct ATR Measurements of Whisky

As described previously in this paper, sampling using ATR spectroscopy requires the placing of the sample onto the top of an infrared-suitable crystal material in an ATR sampling accessory. The infrared spectrum is generated from the radiation reflected from the sample after an evanescent wave from the incident radiation has penetrated into the sample. The sampling arrangement makes ATR a very fast and simple means of measuring infrared spectra of materials. However, it does suffer from the drawback that the technique is only looking at the surface of the material, with the depth of penetration of the beam (and hence the effective pathlength) on an ATR being in the region of 0.5 - 2 microns for a single bounce accessory. This is considerably shorter pathlength than typical transmission measurements measured at 25 - 200 microns.

ATR spectra are shown here for a whisky sample, ethanol and water. Since water and ethanol are the major ingredients in whisky, the ATR spectrum of whisky is dominated by their spectral features.

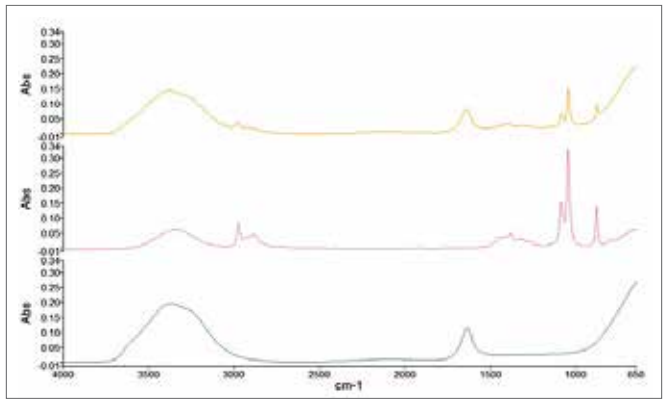

Figure 10. ATR spectra of whisky (top), ethanol (middle) and water (bottom).

Since the spectral features of the other ingredients are very weak, it would be very difficult to check for whisky authenticity using the ATR data obtained from just placing the sample onto the crystal. Utilizing a heated ATR accessory, heated to 65 °C, allows for the evaporation of the water and ethanol to leave behind a residue of the other ingredients within the whisky. Experimental data has determined that after 150 seconds both the ethanol and water have evaporated to leave behind the residue. The process is shown in Figure 11.

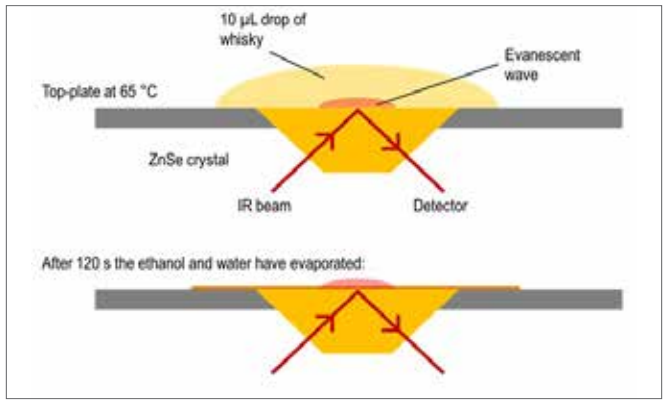

Figure 11. For quantitative consistency, 10 uL of sample was used for all of the samples measured.

After the sample is initially placed on the ATR crystal the spectrum obtained will consist of a mixture of the spectra of all the ingredients. The ethanol will then evaporate leaving a spectrum dominated by the water in the sample. The water will then evaporate to leave a dried residue of the remaining ingredients.

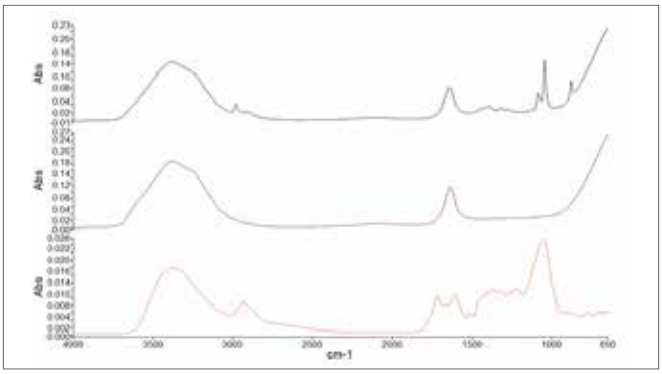

Figure 12. Spectra at different times during the evaporation process (graphic scaling applied).

Top: Time = 0 – whisky spectrum

Middle: Time = 130 seconds - ethanol evaporated leaving remaining ingredients, dominated by water

Bottom: Time = 120 seconds - water evaporated leaving residue

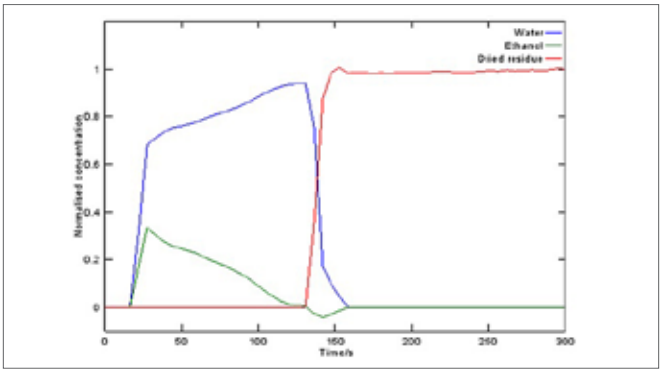

Figure 13. Concentrations of ingredients during the evaporation process.

This evaporation technique generates a high quality and pure spectrum of the residual materials without the presence of any residual solvent.

ATR Measurement on Residues

The residue spectrum obtained appears very similar to the spectrum of the caramel ingredient used in these whiskies as shown in the following plot:

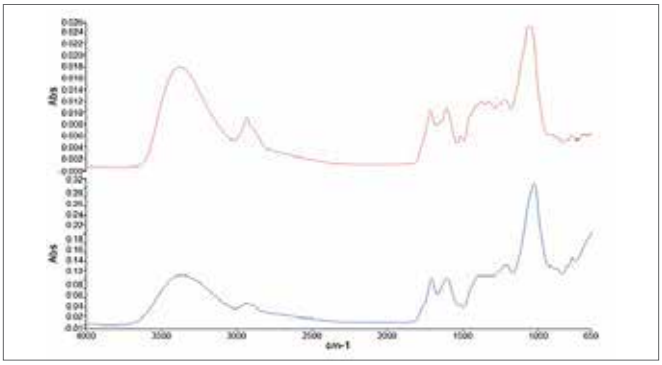

Figure 14. Spectra of whisky residue (top) and caramel sample (bottom).

The addition of E150a caramel is permitted within Whisky legislation to achieve consistency of color within whiskies. The ATR technique should be capable of identifying the type of caramel additive used.

Adulterated Samples

A commercially sourced whisky sample was used as a standard whisky sample and was then adulterated in a variety of ways to determine if the adulteration could be detected. A list of the adulterated samples is shown as Table 2. The adulterations consisted of dilution with UHQ water, ethanol and a variety of other scotch whiskies.

Table 2. Adulterated Samples List

Adulterated Samples		
Standard whisky sample 40 %	BLEND E0	39.99
Standard whisky sample diluted to 30 % with UHQ	BLEND E1 Adulterated Sample 1	29.74
Standard whisky sample diluted to 20 % with UHQ	BLEND E2 Adulterated Sample 2	19.80
Standard whisky sample diluted 50/50 with 40 % ethanol	BLEND E3 Adulterated Sample 3	40.0
Standard whisky sample diluted 50/50 with 40% ethanol colored up with standard DD Williamson E150a (EtOH 40.1 %) Color of 40 % ethanol with caramel to match at 430 nm the value for the base Standard whisky sample	BLEND E4 Adulterated Sample 4	39.96
Standard whisky sample (or alternative) diluted 50/50 with Scotch 1	BLEND E5 Adulterated Sample 5	39.46
Standard whisky sample (or alternative) diluted 50/50 with Scotch 2	BLEND E6 Adulterated Sample 6	39.87
Standard whisky sample (or alternative) diluted 50/50 with Scotch 3	BLEND E7 Adulterated Sample 7	39.81
Standard whisky sample (or alternative) diluted 50/50 with non-Scotch 1	BLEND E8 Adulterated Sample 8	39.91
Standard whisky sample (or alternative) diluted 50/50 with non-Scotch 2	BLEND E9 Adulterated Sample 9	38.32
Standard whisky sample (or alternative) diluted 50/50 with non-Scotch 3	BLEND E10 Adulterated Sample 10	39.88

The ATR spectra of the different adulterated samples, E0 - E10, were measured and are shown here:

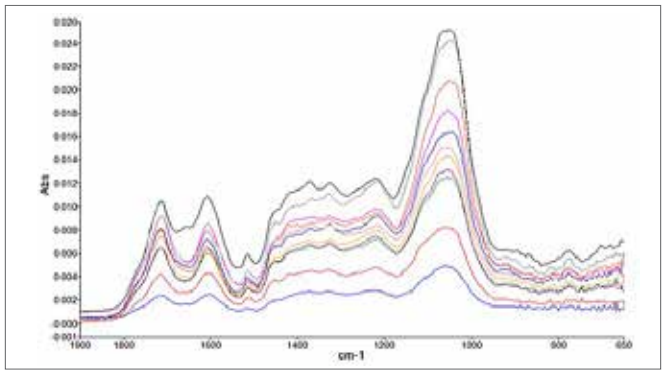

Figure 15. Black- standard whisky sample. Blue and red –diluted samples. Remainder – samples mixed with other scotch or non-scotch.

The spectra of all of the samples look to have similar shapes and spectral features. The overall intensities of the spectra differ slightly, with the exception of the samples diluted with water or ethanol. Their spectra are considerably weaker, as would be expected. The spectrum of sample E4 (diluted with water/ethanol, then color-matched with additional caramel) appeared to be very similar to all of the other samples and does not stand out as being adulterated.

Blends

The ATR spectra of a series of commercially available blends were measured to determine if it would be possible to differentiate the blends using their spectra. The spectra obtained are shown in Figure 16.

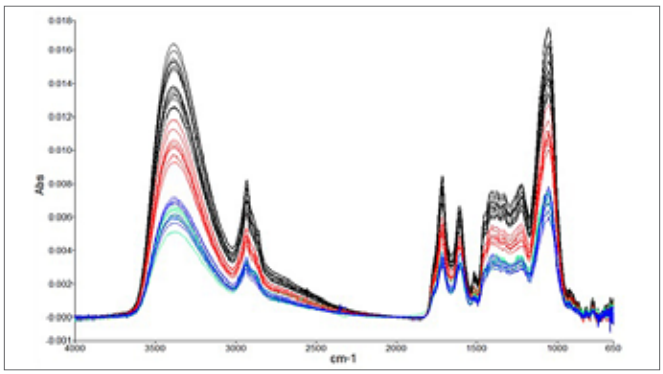

Figure 16. Black- Blend A. Red- Blend B. Green- Blend C. Blue- Blend D.

In the ATR spectra, the spectral shapes are similar, but the overall intensities differ among blends. This suggests different amounts of caramel and/or other dissolved non-volatiles are present in the different blends. Blends A and B differ only by alcohol content. Blend C and Blend D appear the most similar.

Applying chemometrics to the spectral data would allow for qualitative identification of the blends. A Soft Independent Modeling by Class Analogy (SIMCA) algorithm has been applied to this blend data and shows that the different blends separate out into different classes of materials within the model.

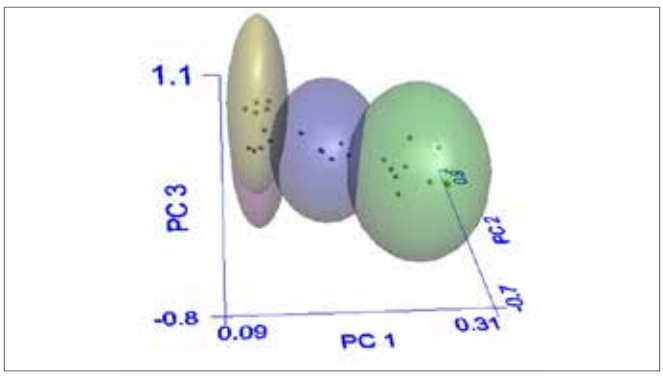

Figure 17. SIMCA plot showing separation of the whisky blend samples.

Non-Scotch

The non-scotch samples in this series of experiments fall into two distinct categories:

a) Non-scotch whisky samples

b) Other non-scotch spirits

The ATR spectra of a series of whisky samples from different geographic origins are shown below:

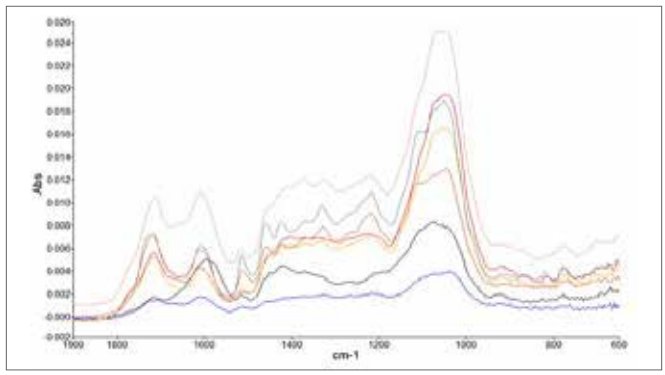

Figure 18. A, B, C, F, H, I and Scotch Blend (all are whisky)Scotch blend is the strongest of these spectra.

The Scotch blend sample is also included in this plot for comparison purposes. The spectra of the residues from the other whiskies are significantly different from the spectrum of the Scotch blend. The spectra are weaker, indicating less residual materials, and also of a different shape, indicating the residual materials are different to those in the Scotch blend. The French whisky (black spectrum) is by far the most different from the other whiskies. The Spanish whisky (blue spectrum) shows the lowest amount of residue.

The spectra of a series of other non-scotch spirits are shown below:

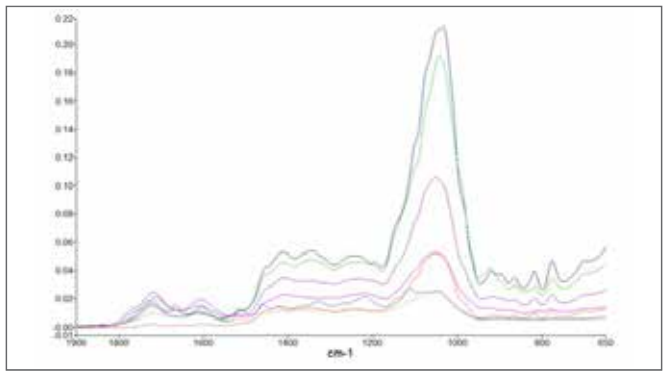

Figure 19. D, E, G, J, K, L and Scotch blend. Scotch blend is the weakest spectrum at about 1060 cm^{-1}. D – Gold Rum, E – Cognac, G- Tequila, J- Bourbon, K- Gold Rum 2, L- Cognac 2.

The Scotch blend spectrum is included in this plot for comparison purposes. The other types of spirits, namely rum, cognac, tequila, and bourbon all have significantly stronger spectra then that of the Scotch blend, indicating the presence of larger amounts of residuals from other ingredients. The bourbon also has a weak spectrum, with the two cognac samples being the strongest of all of these samples.

Generic Scotch

ATR spectra were collected from a series of commercially available Scotch whiskies and are shown in Figure 20.

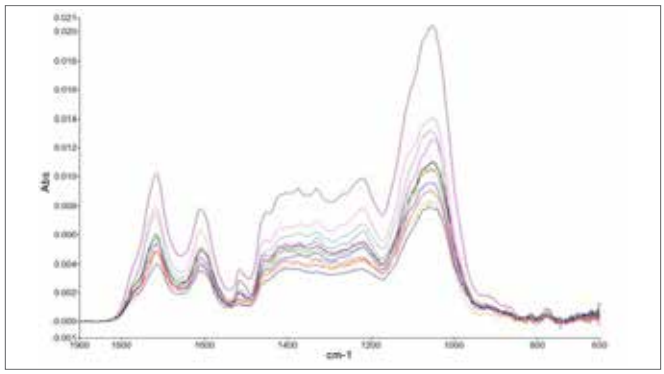

Figure 20. ATR spectra of a series of generic whisky samples.

The spectra are all of similar shape showing that the whiskies have similar ingredients. However, there are subtle differences among the spectra of the different brands, as would be expected. The intensities of the spectra are different, representing the amount of residual ingredients in each of the brands. The most intense spectrum is obtained from a 22-year-old whisky. Two of the samples in this set of data were measured on Malt whisky samples. Overall, the spectra obtained look similar. However, subtle differences can be observed by looking at the derivative spectra of the malt whisky compared to the other whisky samples as in Figure 21.

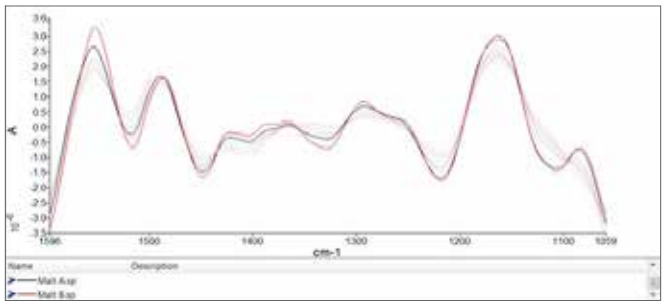

Figure 21. 2nd derivative spectra of the generic whisky samples.

Summary of Infrared Spectroscopy

- Near-IR Transmission measurements allow for accurate quantitative measurements of alcohol concentration in whisky.
- These Near-IR measurements were performed on a Research-Grade laboratory instrument. However, this method could be developed on a portable/handheld Near-IR spectrometer.
- The spectrum obtained from direct ATR measurement of whisky is dominated by spectral bands due to water and ethanol, masking any spectral features due to other ingredients.
- A commercially available heated ATR accessory allows convenient measurement of the non-volatile components of spirits:
  - Required sample volume ~10 µL
  - Drying and measurement time ~5 minutes
- The dried residue spectrum gives useful information about the sample:
  - Semi-quantitative assessment of how much non-volatile material is present
  - Ability to discriminate easily between Scotch and non-whisky spirits
  - Ability to discriminate between Scotch and other whiskies
  - Ability to distinguish blended from single malt whiskies
  - Limited ability to distinguish between different blended Scotch whiskies
- These ATR measurements were performed on a small "portable" benchtop FT-IR that has the option of running from a battery and could be transported and deployed "in the field." However, the heated ATR plate requires mains voltages and would therefore hinder field deployment of this measurement. Future development of a handheld or fully portable instrument would require the implementation of a heated ATR crystal. The instrument would also need to allow for the crystal to be horizontal during the evaporation process.
- Certain types of adulteration can be detected:
  - Dilution with water or water/ethanol
  - Dilution with non-Scotch spirits
  - Substitution of a blended whisky for a single malt
  - But dilution with a water/ethanol appropriately colored with caramel is more difficult to detect.

2. UV-Visible

Ultraviolet-Visible Spectroscopy (UV/Vis) covers the spectral range from 190 nm - 800 nm approximately. The absorptions within this spectral region derive from electronic transitions of the molecules from the ground state to the excited state.

UV/Vis instrumentation is normally dispersive instrumentation, using a monochromator, scanning the wavelengths sequentially. Dual-beam instruments are used with the spectrum of the sample being the ratio of the Sample Beam versus the Reference Beam. Sampling of liquids, such as whisky samples, is performed by placing them into quartz cuvettes with pathlengths in the range 1 mm - 50 mm, most typically 10 mm. Pure water in an equivalent cuvette is placed in the Reference Beam.

Adulterated Samples

A series of adulterated samples, detailed in Table 2, were measured on a UV/Vis instrument by placing the sample into a 10 mm quartz cuvette. The spectra were recorded over the spectral range 230 nm - 850 nm. The spectra collected are shown in Figure 22.

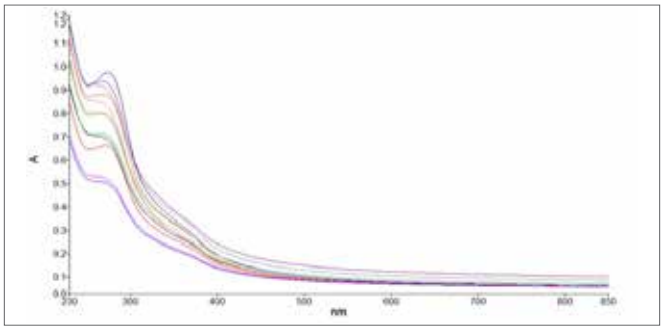

Figure 22. Absorbance spectra of adulterated whisky samples.

The spectral features are quite broad and are contained within the baseline curvature. Therefore, it is normal to apply derivative processing to the data to remove the broad baseline features and to highlight the peaks within the spectra. The second derivative spectra of the same samples are shown in Figure 23 and highlight the differences between the samples.

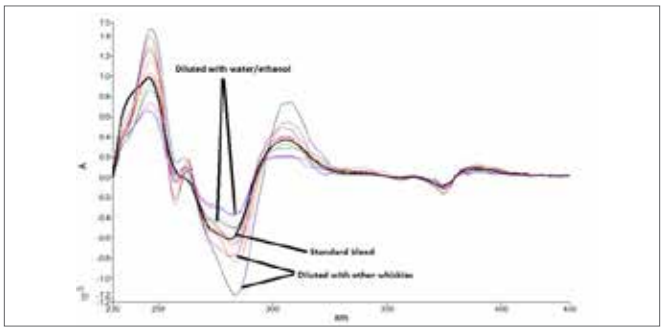

Figure 23. Second derivative spectra of Standard blend (black) and adulterated samples.

It is clear from these spectra that it is possible to observe when the whisky has been diluted with either water or ethanol, since the intensity of the peak at 283 nm is much weaker. When the whisky has been diluted with other whisky types then the peak at 283 nm could be weaker or stronger, depending on the whisky variety used for dilution. However, any mixing of the whisky with other diluents has generated different spectra from a standard blend.

Blends

A series of 35 samples from four different commercial blends were measured to determine whether it is possible to use UV/Vis spectroscopy to differentiate the blends. The UV/Vis spectra obtained are shown in Figure 24.

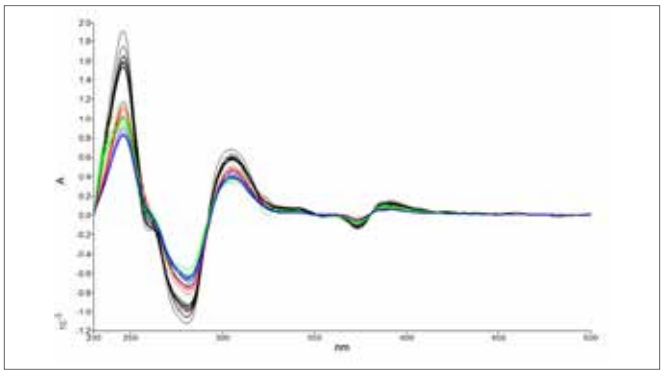

Figure 24. Second derivative spectra of Blends A (black), B (red), C (green), D (blue).

Blends A and B are clearly differentiated from the other blends and there is slight overlap between the spectra of blends C and D. A SIMCA model was generated from the data from all of the blend samples to try to separate the blends. The SIMCA model is shown in Figure 25.

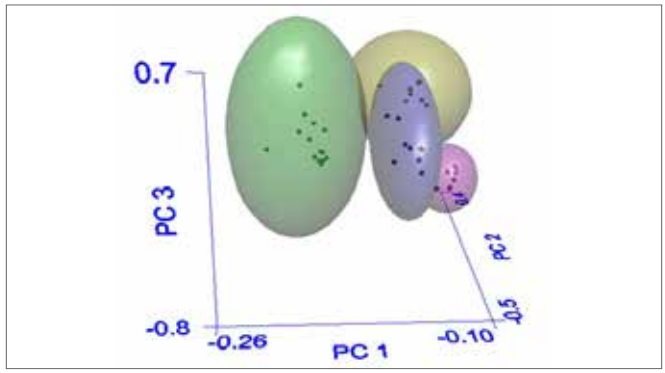

Figure 25. SIMCA model of Blends A-D.

Again, there is clear separation of blends A and B, with very slight overlap between blends C and D. The model suggested that one sample of blend C was overlapping with the blend D samples. Collection of larger numbers of samples for all of the blends would most likely improve the model.

Non-Scotch

The non-scotch samples in this series of experiments fall into two distinct categories:

a) Non-scotch whisky samples from other geographic origins

b) Other non-scotch spirits

The spectra obtained from a series of commercially-available non-scotch whisky samples are shown in Figure 26.

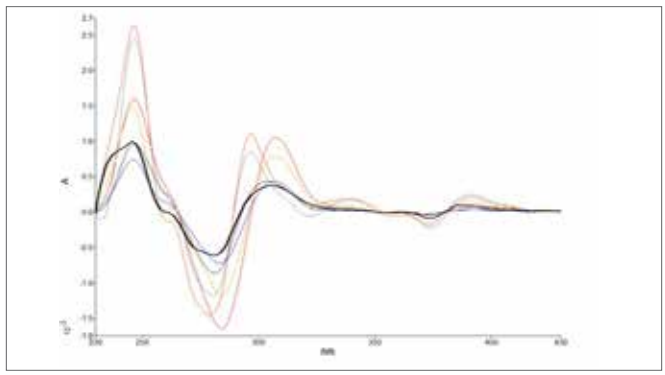

Figure 26. A, B, C, F, H, I (all whisky) Standard blend in black.

The standard blend spectrum is shown as a reference, and it is clear that all of the other whisky samples are different from a standard scotch blend. The spectra show significant band shifts and intensity differences. It would easily be possible to detect a non-scotch whisky based on this data.

The spectra of commercially-available non-whisky spirits are shown in Figure 27, along with a reference spectrum of a standard blend.

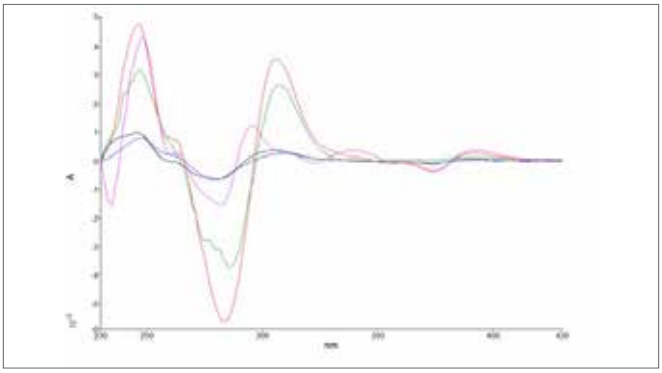

Figure 27. D, E, G, J (non-whisky) Standard blend in black. D – Gold Rum, E – Cognac, G- Tequila, J- Bourbon.

The tequila sample most closely matches the standard blend, but all other samples are significantly different.

Generic Scotch

UV-Visible spectra were measured for a series of commercially-available Scotch whiskies. The samples varied in brand and age and also included two malt whiskies. The spectra are shown in Figure 28.

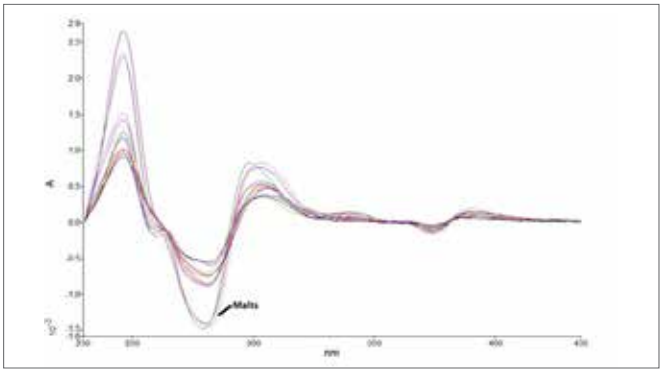

Figure 28. UV-Visible spectra of generic Scotch whiskies.

The spectral profiles are quite similar between the different samples except the malt whiskies and a 22 year old, where the band at 280 nm is very intense.

Summary of UV-Visible Spectroscopy

- UV-Visible instrumentation offers a fast and simple method for sampling of whiskies
- These UV-Visible measurements were performed on a small, benchtop laboratory instrument. However, these measurements could be developed on a portable/handheld UV-Visible spectrometer.
- The UV-Visible spectrum gives useful information about the sample:
  - ♦ Ability to discriminate easily between Scotch and non-whisky spirits
  - ♦ Ability to discriminate between Scotch and other whiskies
  - ♦ Ability to distinguish blended from single malt whiskies
  - ♦ Ability to distinguish between different blended Scotch whiskies with minor overlap
- Certain types of adulteration can be detected:
  - ♦ Dilution with water or water/ethanol
  - ♦ Dilution with non-Scotch spirits
  - ♦ Substitution of a blended whisky for a single malt
  - ♦ Dilution with a water/ethanol appropriately colored with caramel is also possible to detect.

References

1: http://www.europeanspirits.org/thelssues/Counterfeiting.asp

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