Application Note

Keywords

- Denatured alcohol
- Methyl ethyl ketone (MEK)
- Spirit Sampler system

Techniques

- Absorbance spectroscopy
- Chemometric analysis

Applications

- Authentication
- Quality assurance

Detecting Denaturants in Alcohol with UV-Vis Absorbance

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In many countries, alcoholic spirits are heavily taxed to generate government revenue and as a matter of public health policy, while ethanol intended for fuel or industrial use is not. To ensure that the two are not intermingled for profit by criminals, denaturing materials are added to the less expensive grade of alcohol to make it unfit for human consumption, after which it is known as denatured alcohol. More than 100 denaturants are authorized for use in the U.S. alone[1], often chosen because they are poisonous, bitter-tasting, foul smelling, or brightly colored, thus highlighting to the recreational drinker that the product they are about to consume does not meet food-grade standards.

The types of denaturants added can depend on the country of manufacture, and the use for which the denaturant is intended. Specially Denatured Alcohol (SDA), also known as partially denatured alcohol, contains a lower concentration of denaturants, and can be used by permit only. It is often used in cosmetic products, pharmaceuticals, chemical manufacturing, or as a solvent or reactant in other products. Completely Denatured Alcohol (CDA) has much higher concentrations of denaturants that are nearly inseparable from the alcohol, making it completely unfit for beverage use and eliminating the need for permit restrictions.

Methanol has been a popular denaturant historically, but can result in illness, blindness or even death if consumed. This has led to increased use of additives like denatonium benzoate (a highly bitter substance, sometimes sold under trade name Bitrex®), as well as chemicals like

isopropanol, methyl ethyl ketone, methyl isobutyl ketone, pyridine, benzene, diethyl phthalate and naphtha, and dyes like methyl violet.

In an effort to standardize the process for denaturing alcohol, in 2013 the European Union adopted a formula for denatured alcohol consisting of 3% isopropyl alcohol and 3% methyl ethyl ketone by volume, supplemented by 10 ppm denatonium benzoate. The denatonium benzoate prevents accidental ingestion due to its taste, methyl ethyl ketone (MEK) is a smelling agent, and the isopropyl alcohol (IPA) is a good chemical marker that is difficult to remove by distillation. Together, they serve to deter criminals from using denatured alcohol as a substitute or dilutant in spirits sold for consumption, and make such use easier to identify.

Detection of denaturants in alcohol is key to preventing fraud. Having tackled the issue of spirit authentication with our Spirit Sampler system, we decided to see whether UV-Vis spectroscopy could be a useful tool for detection of some common denaturants at the concentrations typically used in denatured alcohol.

Overview

A set of denaturants was diluted in an artificial 40% spirit and a vodka stock and measured using UV-Vis spectroscopy with a view toward detecting those denaturants in spirits. Chemometrics analysis was used to determine a very rough limit of detection to establish proof of concept. The denaturants tested included:

- denatonium benzoate (B)
- methyl-ethyl-ketone (MEK)
- methyl-isopropyl-ketone (MI)
- ethyl-secondary-amyl-ketone (ES)

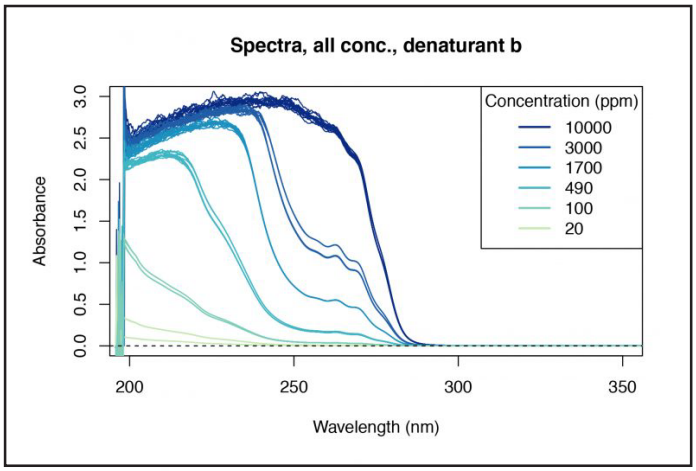
Measurement Setup

The experiments were performed using the Spirit Sampler application-ready system, introducing samples into the "LIGHT" beverage sample port. This customized system can be approximated with a modular system consisting of a xenon light source, a cuvette holder with a 1 mm pathlength cuvette, and a Flame-S-UV-VIS spectrometer. Measurements were taken with a 245 ms integration time, 10 averages, and with electrical dark and nonlinearity correction enabled.

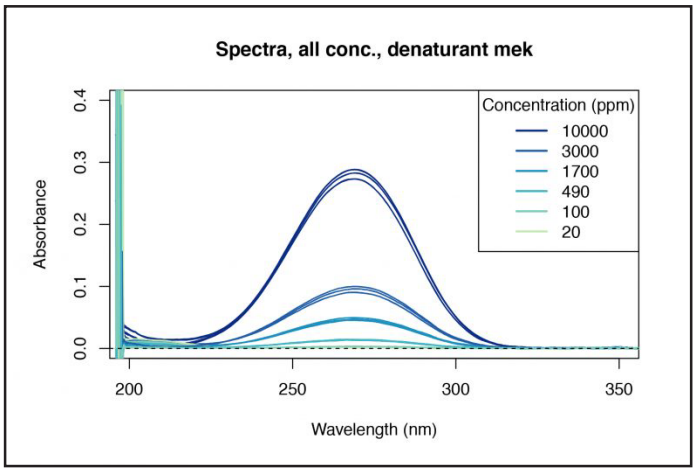
Method

We began with stock solutions of the denaturants prepared at 10,000 ppm concentration in a 40% ethanol/water solution. These stock solutions were diluted, yielding solutions with concentrations from 3300 ppm down to 20 ppm. The plain 40% ethanol/water solution and the vodka solution were used as blanks (references) in this absorbance method.

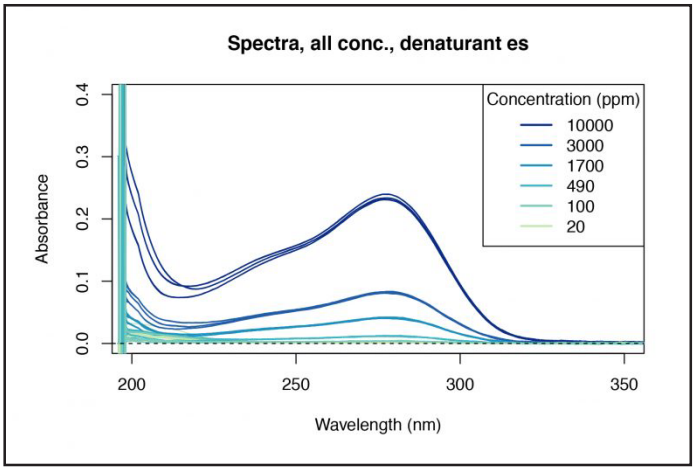
Multiple measurements were made in absorbance mode for each denaturant at concentrations from 20 ppm to 10,000 ppm to serve as the raw data for chemometric analysis, the goal being to determine a prediction error as an estimate for the limit of detection. Looking at the spectra for Bitrex, methyl-ethyl-ketone, and ethyl-secondary-amyl-ketone, it can be seen that each has characteristic absorbance curves in the 200-350 nm range. For all denaturants we see a nice concentration dependence and little to no influence of the solvent.



Denatonium benzoate (B) UV-Vis absorbance spectra



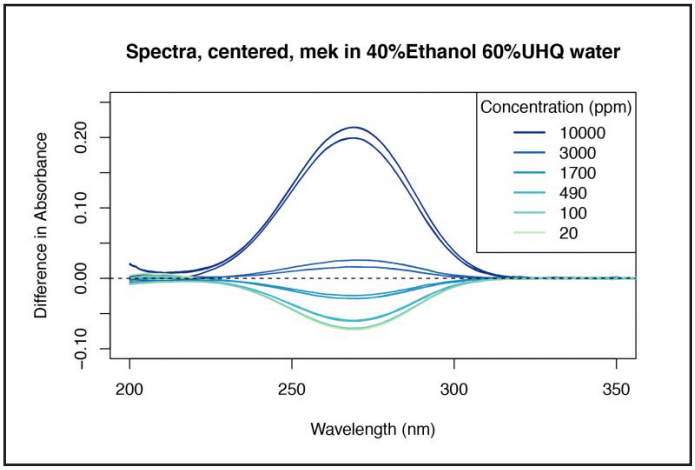
Methyl-ethyl-ketone (MEK) UV-Vis absorbance spectra



Ethyl-secondary-amyl-ketone (ES) UV-Vis absorbance spectra

Chemometric Analysis of a Single Denaturant

To demonstrate the process of chemometric analysis to determine limit of detection, we will focus on a single denaturant in a single solvent. Here we will use methyl-ethyl-ketone (MEK) in a 40% alcohol/60% water solution. As a first step, mean centering is performed to draw out the differences between the spectra. This involves subtracting the mean spectrum for a given denaturant and solvent from the spectrum at each individual concentration. Liquid spectra in a cuvette do not require scaling so we can mean-center immediately to look at the differences.



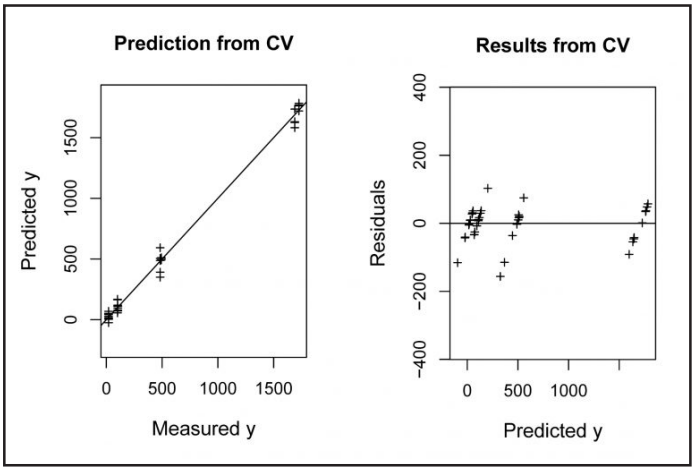
Methyl-ethyl-ketone (MEK), mean-centered data

We can then look for correlations between the spectra and the concentration — what regions of the spectra show the best correlation with the concentration, or which wavelength regions are the most sensitive indicators of the concentration? For MEK, we saw a perfect correlation value of 1.0 for the 225-300 nm spectral range, which bodes well for chemometric prediction.

A Partial Least Square (PLS) optimization can then be applied for up to 10 different components. By using repeated double cross-validation to find the distribution of error (mean standard error of prediction) for each model complexity (number of components in the PLS model), we can pick the model that produces the lowest error with the smallest number of components.

In our initial analysis of all concentrations of MEK in 40% alcohol/60% water, we found that a three component model was the optimum model complexity. The standard error of prediction (SEP) analysis indicated a prediction error of 100 ppm when all concentrations are considered. Though this model might have an error of 100 ppm at the highest concentration, the error might be better for the lower concentrations. In other words, the variation of the high concentration spectra might dictate the prediction error, but the limit of detection could be lower.

To assess this, we repeated the analysis with concentrations only up to 2000 ppm, finding that a seven component model offered better standard error of prediction (SEP). Comparing the predicted concentration with the measured concentration, we found a very linear fit, as shown below. Looking at how the variance changes with concentration (the residuals), we can assess the error in cross validation, using it as a simple estimate for the prediction error. For lower concentrations, we find an error of prediction from cross validation of 60 ppm for the denaturant methyl-ethyl-ketone (MEK) in 40% alcohol/60% water.



Limit of Detection for all Denaturants

By repeating this method of analysis for all denaturants diluted in vodka as the solvent, we were able to find the prediction error for each denaturant, which can be considered the limit of detection. In comparing these to typical concentrations, we find our prediction error to be well below the typical concentrations that would be seen in denatured alcohol for all four denaturants, establishing proof of concept for this method.

Denaturant	Prediction Error	Typical Concentration
Denatonium benzoate (B)	2.3 ppm	4 ppm
Ethyl-secondary-amyl-ketone (ES)	6.3 ppm	80 ppm
Methyl-ethyl-ketone (MEK)	28.1 ppm	3800 ppm
Methyl-isopropyl-ketone (MI)	6.2 ppm	120 ppm

Conclusions

A basic proof-of-concept test of UV-Vis absorbance spectroscopy and chemometric analysis for the presence of common denaturants in clear spirits shows that typical concentrations easily can be detected using a PLS model. Through additional work at lower concentrations and a much larger sample set, as well as multiple solvents, the detection limit of this method could be further improved. If used in combination with portable systems such as the Spirit Sampler, this method shows strong potential for use in field testing of spirits for the presence of denaturants.

References

1. "27 CFR 21.151 – List of Denaturants Authorized for Denatured Spirits." *LII / Legal Information Institute*. N.p., n.d. Web. 21 Sept. 2016.

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