AVANTES
MEMBER OF THE NYNOMIC GROUP

SPECTRA OF THE MONTH

# REGULAR VS DECAF: USING UV ABSORBANCE SPECTROSCOPY TO MEASURE CAFFEINE PRESENCE

CONDUCTED BY: INCLUDE NAME

## INTRO

# BACKGROUND OF APPLICATION

Every spring, about a third of all countries participate in a ritual known as Daylight Savings Time, a practice in which people set their clocks ahead one hour. This is intended to add an extra hour of daylight, as this time is when the majority of people are awake.

This tradition has roots in increased evening productivity, saving energy, and even as a business tactic,  the extra hour of sunlight promoting more people to shop after a day of work. In the United States, this "spring forward" occurs on the second Sunday of March. As such, many workers in the U.S. report increased lethargy and reduced productivity the following days. To combat this effect of the time shift, individuals often turn to increased consumption of caffeinated beverages such as coffee and tea. Over time, individuals adjusting to the new schedule may either cut back on the consumption of caffeinated beverages or swap them out for less caffeinated beverages such as decaf coffee. While decaf coffee contains about 97% less caffeine than regular coffee, there is still a common misconception that it contains much more caffeine, with some believing it contains almost as much caffeine as regular coffee. In this study, we will determine the truth of this assumption through spectroscopic techniques.

This experiment aims to demonstrate the ability of UV absorbance spectroscopy to detect caffeine in coffee samples. Both regular and decaf coffee samples were measured and compared to see if an absorbance peak for caffeine was present. Additionally, a third sample of fruit juice with added caffeine from black and green tea was measured to confirm that and absorbance peak seen could be attributed to caffeine and not the result of the color of the coffee (Figure 1).

**FIGURE #1:** Caffeine samples used for this experiment (from left to right: dark roast coffee, medium roast decaf coffee, and fruit juice with added caffeine from black and green tea).

# DESCRIPTION OF SPECTROSCOPY SETUP

The setup for this experiment (Figure 2) utilized our AvaSpec-ULS2048x64-EVO. Alongside our cooled AvaSpec-ULS2048x64TEC-EVO, the ULS2048x64-EVO offers the same 2048x64 back-thinned CCD detector in an uncooled device for less demanding applications in the UV and NIR range. While cooling offers a more stable signal, this is often unnecessary for applications that use integration times shorter than 2 seconds. Like all of our EVO instruments, the ULS2048x64-EVO uses the AS-7010 electronics board, offering USB3.0 and gigabit ethernet communication along with better signal processing. Additional options include an order-sorting filter to reduce second-order effects and purge ports for deep-UV measurements. This unit comes with a wide range of slit sizes and gratings and can be configured with SMA or FC/PC fiber optic entrance connectors. The unit used in this experiment was optimized for the UV range with a 200-450 nm wavelength range.

**FIGURE #2** Spectroscopy Setup. The caffeine sample is placed inside a 1 mm path length cuvette, which is measured in-line using our cuvette holder and cuvette cover. The light source is controlled by the spectrometer via the interface cable.

The light source used for this experiment was the AvaLight-XE-HP, a high-powered pulsed xenon light source. This light source comes in a compact housing, making it well-suited for integration into customer systems. Compared to the AvaLight-XE, which has a maximum power of 2 W, the AvaLight-XE-HP provides significantly more power (6 W). When connected to an AvaSpec spectrometer via our custom interface cable, the number of flashes per scan can be set in our AvaSoft software, and  the flashes are synchronized with the data collected by the spectrometer.

Other accessories used for this experiment included our CUV-FL-UV/VIS in-line cuvette holder and cover, a 1 mm path length cuvette, two custom 0.3-meter, 100-micron core fiber optic cables to connect the spectrometer and light source to the cuvette holder, and a custom interface cable to connect the AvaLight-XE-HP to the AvaSpec-ULS2048x64-EVO to control flashes per scan and to power the light source. This interface cable was designed by our engineering team and highlights some of the custom build options that can be assembled for our customers

## EXPLORE MORE APPLICATIONS AND INDUSTRIES SERVED BY AVANTES

**Agriculture & Food**
Food Sorting
Precision Agriculture

**Semiconductor**
Thin Film Coating
End-Point Detection

**Environmental**
Contamination &
Pollution Monitoring

**(Bio)Medical**
Blood Analysis
Cancer Detection

**System Integration**
Integrate into your
product or system

# DESCRIPTION OF METHODOLOGY

The caffeine samples used for this experiment were a dark roast coffee, a medium roast decaf coffee, and a fruit juice with added caffeine from black and green tea. The coffee samples were prepared in a single-serve coffee maker, while the caffeinated fruit juice was stored in an aluminum can. Both coffee samples were allowed to cool for one hour after brewing to minimize potential damage to the 1 mm path length cuvette due to excessive heat. The caffeinated fruit juice was stored at room temperature before measurements were taken. Additional preparation included diluting each sample to a 1:4 ratio using deionized water, as measurements of the samples with no dilution showed no discernible peaks, which was attributed to light scattering from the opacity of the coffee samples.

For data analysis, we used the Absorbance mode in AvaSoft, our exclusive custom software package. This mode is specifically designed for absorbance applications, where the reference measurement will report 0 A.U. (absorbance units) and 5 A.U. when the light source is turned off. In this experiment, the reference measurement was the 1 mm path length cuvette was filled with water. We used an integration time of 10 milliseconds, which can be adjusted to increase or decrease the amount of light being measured at one time and affects the overall magnitude of the reported spectrum. With a 10 ms integration time, the number of flashes per scan for the light source was set to 1, the maximum amount for this integration time. Averaging was set to 100, meaning 100 measurements were averaged together to account for the pulse-to-pulse variation of the xenon light source and provide more consistent results in the spectra.



# TEST DATA AND RESULTS

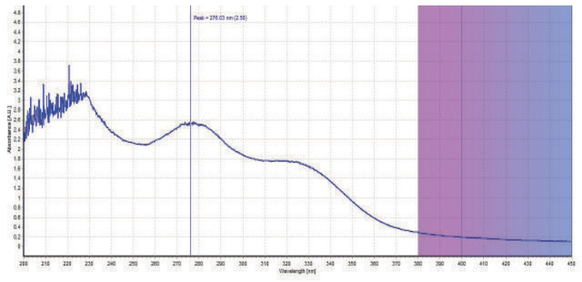

**FIGURE #3:** Absorbance spectrum of regular coffee sample (caffeine absorbance peak at 276.03 nm).

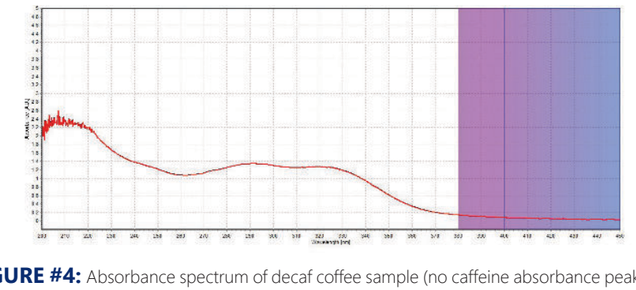

**FIGURE #4:** Absorbance spectrum of decaf coffee sample (no caffeine absorbance peak present).

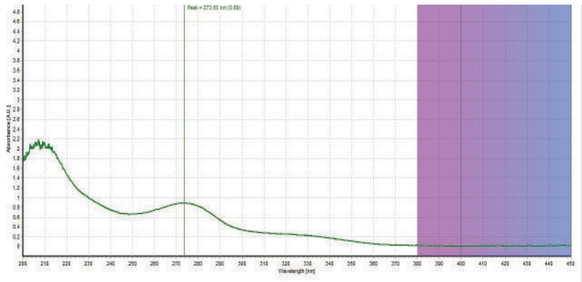

**FIGURE #5:** Absorbance spectrum of caffeinated juice sample (caffeine absorbance peak at 273.50 nm).

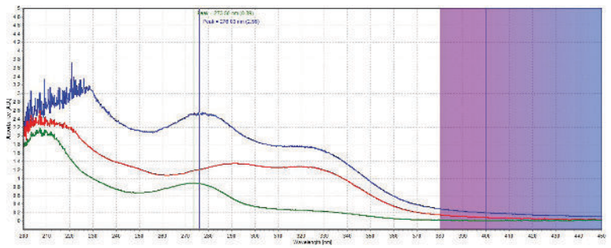

**FIGURE #6:** Spectra of regular coffee (blue), decaf coffee (red), and caffeinated juice (green) samples, shown together for comparison.

# ANALYSIS

As expected, the two caffeinated samples showed absorbance peaks in the UV region that match a previously reported absorbance peak for caffeine (around 273 nm). The regular coffee sample demonstrated a significant peak in this area at 276.03 nm (Figure 3). The decaf coffee sample did not show this peak and instead showed shallow peaks around 290 and 320 nm, neither of which correspond to caffeine (Figure 4). The caffeinated juice sample also reported an absorbance peak near the known caffeine absorbance peak at 273.50 nm (Figure 5). A graph of all the spectra together is included for comparison of all samples (Figure 6).

Comparing the three samples, it is clear that the known caffeinated samples show absorbance peaks that correspond to a known caffeine peak. Interestingly, the regular coffee sample absorbance peak had a significantly higher peak than the caffeinated juice sample, despite the juice advertising itself as having nearly as much caffeine (80 mg per 8 fl oz serving) as an average cup of coffee (90 mg per 8 fl oz serving). There is a chance that the coffee used in this experiment contained more than the reported average caffeine, or that the dilution process did not thoroughly mix the caffeine samples and water. Also of note is the location of the absorbance peak in the regular coffee sample, which is at a slightly higher wavelength (276.03 nm) than the wavelength of the reported caffeine absorbance peak (around 273 nm). This slight shift could be attributed to the shallow peak at 290 nm seen in the decaf coffee sample influencing the caffeine absorbance peak.

# CONCLUSION

In conclusion, the present experiment highlights the use of spectroscopic analysis in detecting caffeine in coffee samples or other fluid samples that contain caffeine. Comparison of the regular and decaf coffee samples illustrated clearly that decaf coffee has no measurable traces of caffeine, at least in terms of UV absorbance peaks. While the data presented here only detects the presence of caffeine based on the presence of an absorbance peak, further quantification could be utilized to link the magnitude of the absorbance peak to the amount of caffeine present in the measured sample.

The AvaSpec-ULS2048x64 provides the same high-performance capabilities as its thermally-cooled counterpart in applications where shorter integration times do not require the added stabilization of a cooled instrument. The AvaLight-XE-HP is a xenon light source that is well-suited for applications where high power is needed or compact form factor is a must. The custom interface cable highlights the capabilities of our engineering team to provide custom assemblies and solutions for customer needs. Please contact Avantes for more information on the configuration that is best suited for your data collection.

AVANTES
MEMBER OF THE NYNOMIC GROUP

## CONTACT

# WE'RE HAPPY TO HELP

Curious how spectroscopy can help you reveal answers by measuring all kinds of material in-line, at your production facility, in a lab, or even in the field? Visit our website or contact one of our technical experts. We are happy to help you.


**Avantes Headquarters**
Phone: +31 (0) 313 670 170
Email: info@avantes.com
Website: www.avantes.com

**Avantes Inc.**
Phone: +1 (303) 410 866 8
Email: infousa@avantes.com
Website: www.avantesUSA.com

**Avantes China**
Phone: +86 (0) 108 457 404 5
Email: info@avantes.com.cn
Website: www.avantes.cn


**Follow us on social media:**