Summary
This application note shows results from multiple analytical techniques, comparing the results of a decaf coffee to a caffeinated brand.

Background
Coffee is prepared by steeping roasted ground coffee beans in hot water, and then removing the grounds. Caffeine, a naturally occurring stimulant found in coffee, can be removed from the coffee bean by a variety of methods. Benzene was originally used to extract caffeine from coffee in the early 1900s, but its toxicity resulted in this process being abandoned. Water extraction, or the Swiss Water Process, is sometimes used, whereby the water is infused with desirable oils found in the coffee to prevent their extraction, and the unroasted beans (green coffee beans) are repeatedly extracted until the desired level of caffeine is achieved. Dichloromethane or ethyl acetate are also sometimes used to extract the caffeine from the beans, as well as super critical carbon dioxide. Caffeine levels in coffee vary according to the bean and the decaffeination process. Decaf will typically contain around 20 ppm of caffeine, while regular coffee may contain around 800 ppm. The decaffeination process may remove or alter desirable aromatics in the coffee that impart its flavor and aroma, hence processors are concerned not only with caffeine levels, but also other properties of the coffee following decaffeination.

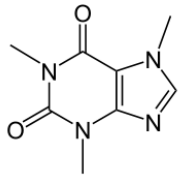


Figure 1: Chemical structure of caffeine

Samples
Testing was performed on Starbucks French Roast and Starbucks Decaf House Blend. Both samples were purchased in ground form, and were stored at ambient conditions prior to testing.

Procedure and Results

ATR-FTIR
FTIR analysis was conducted on the ground beans of both a caffeinated and decaffeinated coffee. A small amount of each coffee was placed on a plastic sample holder under the Varian 610-IR microscope portion

of the 640-IR spectrometer. A Germanium ATR crystal attachment was used to lightly contact each sample. The resulting spectra were compared to a Biorad Know-It-All database. A comparison of the two spectra is shown in Figure 2. A spectrum of caffeine is shown in Figure 3. Both samples show the presence of caffeine, with the French roast (caffeinated) showing a higher absorption peak at the caffeine absorption peak. Each coffee sample was compared to a spectral database (Biorad Know-It-All) with the best match, an emulsifier for both samples, shown in Figure 4 and Figure 5.

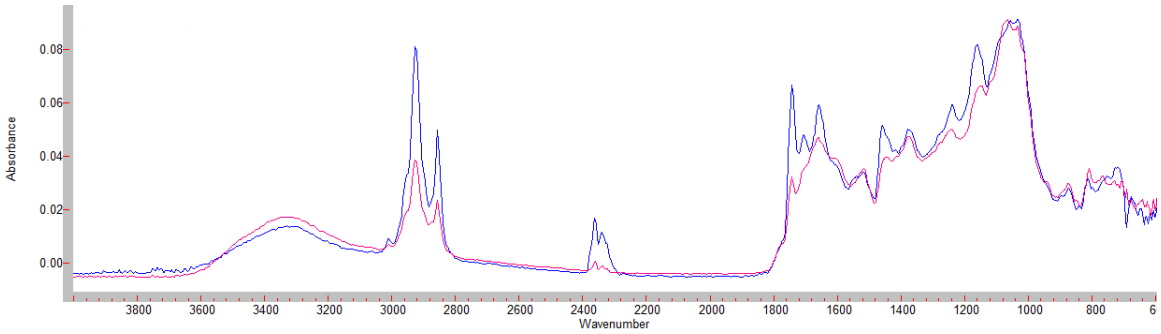


Figure 2: Comparison of caffeinated (blue line) and decaffeinated (red line) coffee.

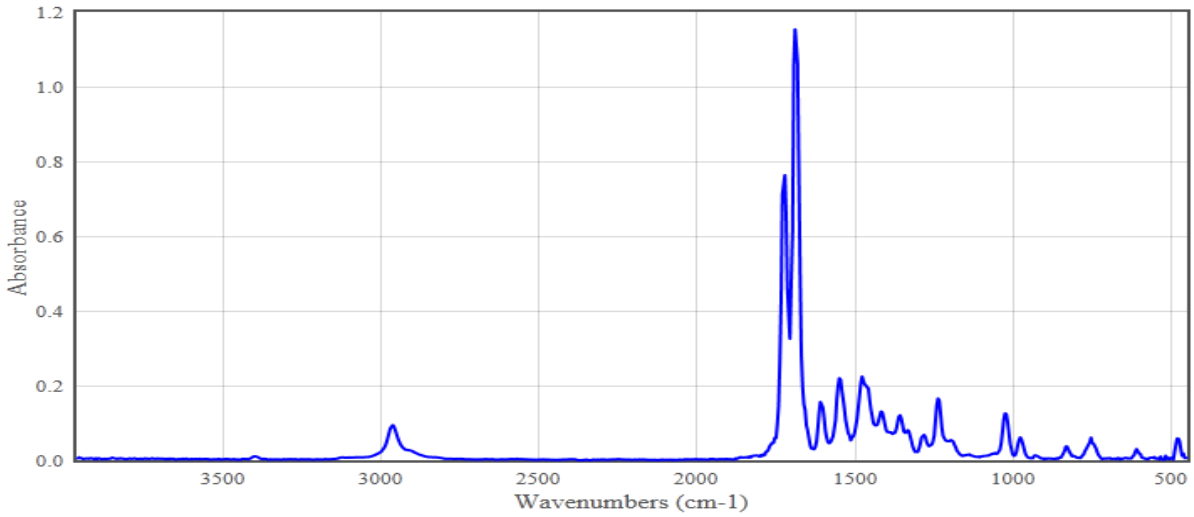


Figure 3: Spectrum of caffeine.[1]

[1] NIST Chemistry WebBook

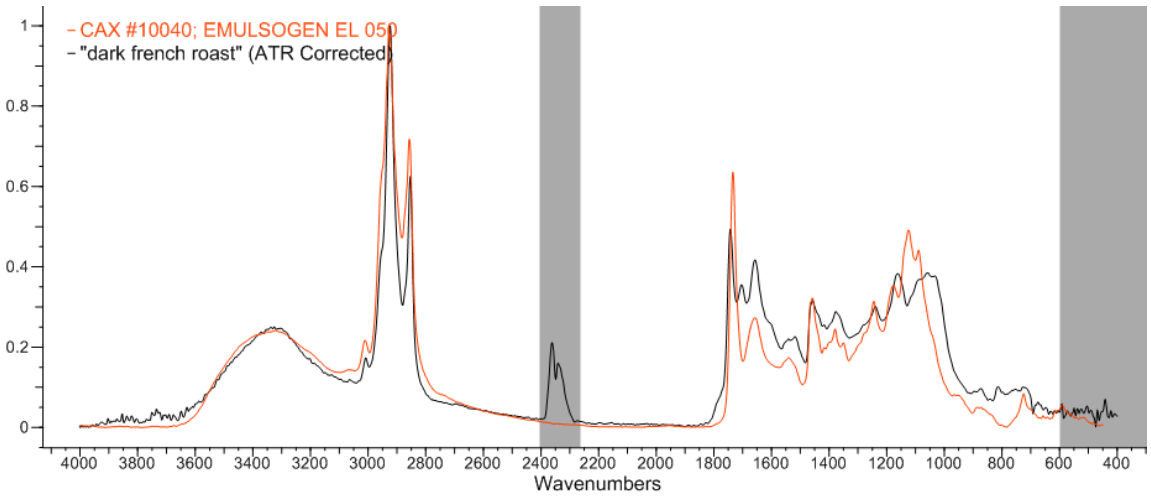


Figure 4: Comparison of caffeinated coffee (black line) with the best match from the spectral database (orange line). The match is to an emulsifier.

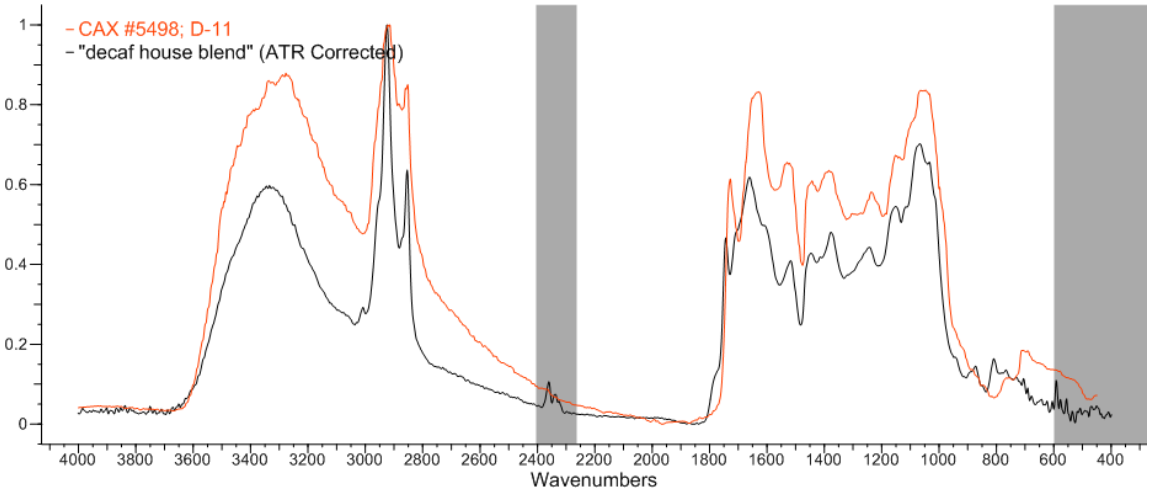


Figure 5: Comparison of decaffeinated coffee (black line) with the best match from the spectral database (orange line). The match is to an emulsifier.

TGA-FTIR

TGA-FTIR was run on the two samples using a TA Instruments Q500 TGA couple to a Biorad Excalibur FTS3000 FTIR bench. The TGA was fitted with the TA Instruments Evolved Gas Accessory (EGA) and the evolved gases were transported to the FTIR bench using a Pike Scientific TGA/FTIR accessory. The TGA was run in a constant heating rate mode from ambient to 600 °C at 10 °C/min. The transport line and flow-cell on the FTIR bench were set to 260 °C. Both samples, with a caffeine reference were initially run in the TGA's "high resolution" mode, where the heating rate is modified to optimize signal separation. The same samples were then run under conventional linear heating at 10 °C/min. A comparison of the two modes is shown in Figure 6. It is evident that the high resolution mode does indeed provide extra separation in the materials, and in fact picks up additional peaks on the leading edge of the major mass-loss transitions. However, this varying heating rate means that the FTIR signal may detect off-gas before the control loop on the TGA has slowed the heating rate down substantially, as can be seen in Figure 7.

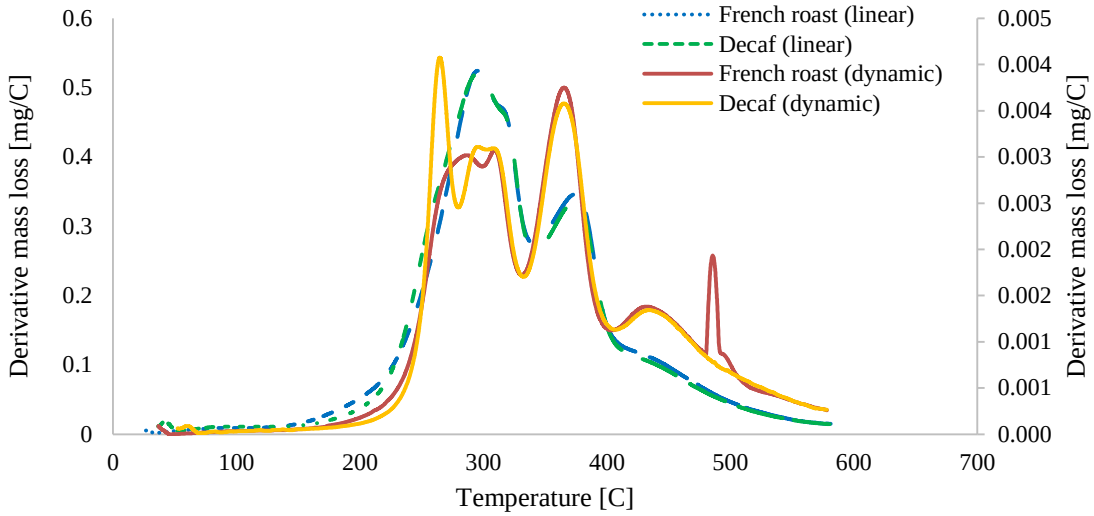


Figure 6: High resolution ("dynamic") TGA data versus linear heating for French roast, decaf house blend and caffeine

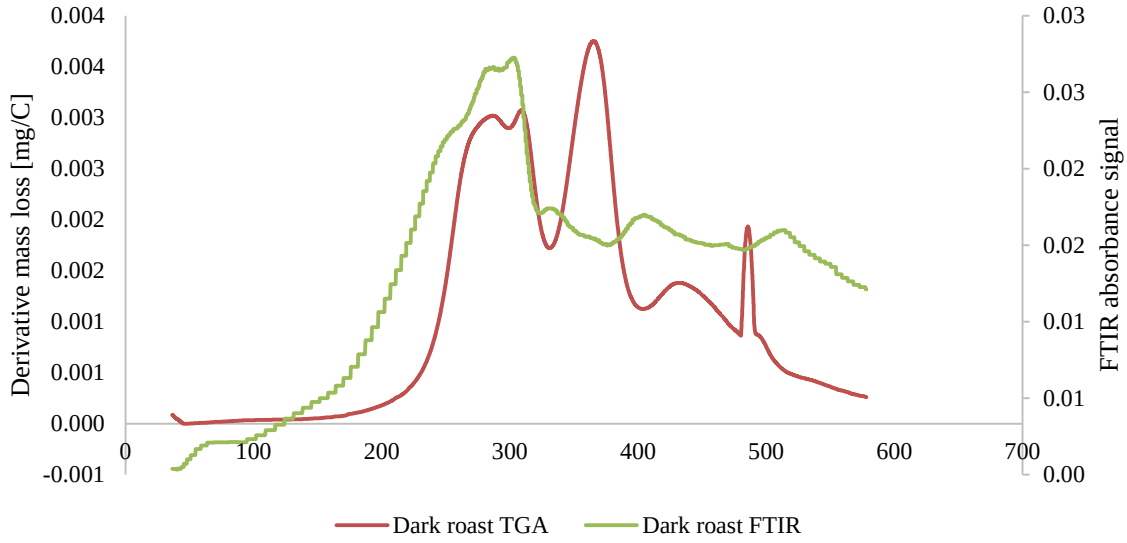


Figure 7: FTIR and TGA using the high resolution mode (Dark roast sample)

To provide accurate synchronization of the FTIR and TGA signal the experiment was run in linear mode (see Figure 8). In all cases, the FTIR traces largely match the TGA response, with no distinguishing features between the two coffees. The caffeine standard shows a sharp and pronounced peak in the TGA mass loss rate at approximately 200 °C, although this sample emphasizes that the FTIR is sensitive to the species under consideration earlier than the TGA data.

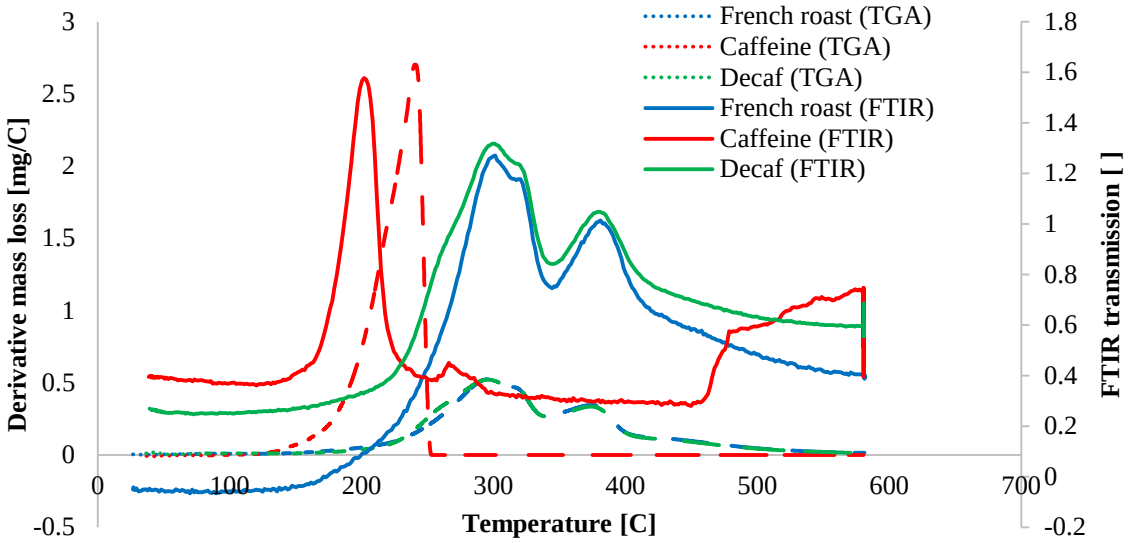


Figure 8: FTIR and TGA using linear mode (caffeine, dark roast and decaf)

The FTIR accessory has the ability to track individual peaks of interest in the FTIR spectra. In the previous figures the average (Gram-Schmidt) absorption is reported. But each "functional group" can be used to track individual species. The data for the functional groups in the coffees are provided in Figure 9. It is clear that in the coffee there are two major peaks detected at ~2320 cm^{-1} and 2926 cm^{-1}. Neither of these transitions are visible in the caffeine sample which presents only one primary peak, at 1746 cm^{-1}. This peak is weakly visible in both the coffees, but at a higher temperature than the caffeine alone would predict.

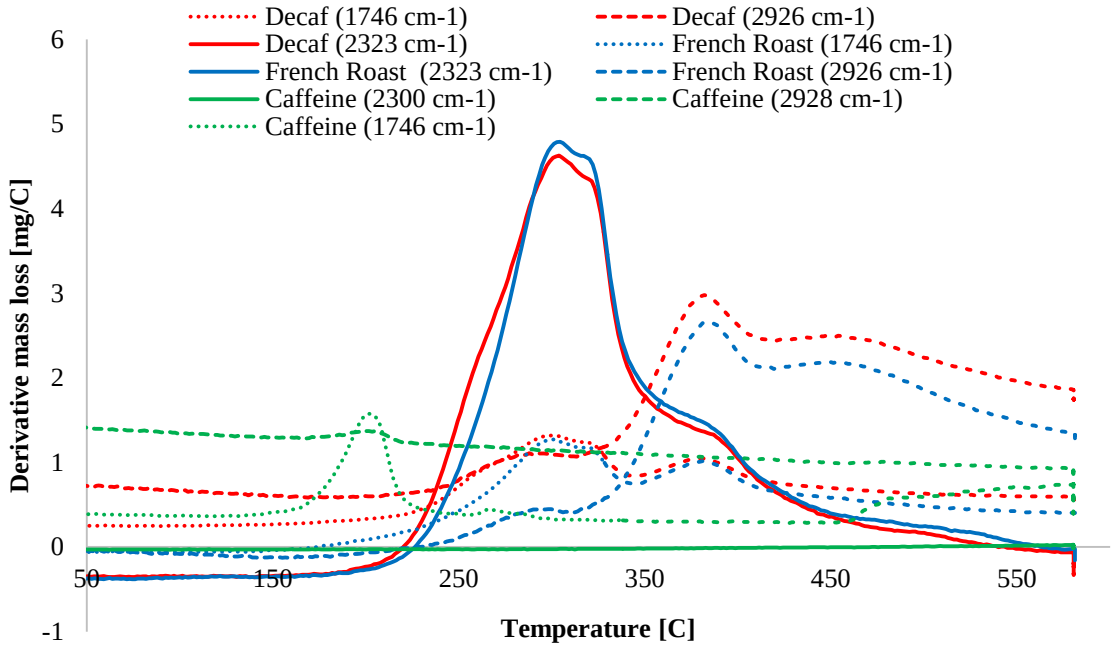


Figure 9: FTIR and TGA using the high resolution mode (Dark roast sample)

Although the coffee traces are very complex, these data highlight that TGA coupled to FTIR is a strong technique that has value in deformulation and root-cause analysis.

Head Space GC-MS

Approximately 1.5 g of each sample was weighed on an AND GR-202 analytical balance and then placed into a 10 mL headspace vial equipped with a PTFE/silicone septum. The headspace vial was heated to 100 °C and allowed to equilibrate for 45 minutes. After this time, a gas-tight syringe was used to manually sample 1.0 mL of headspace from the vials and inject it into the injection port of an Agilent 6890 gas chromatograph equipped with an Agilent 5973 mass spectrometer. The instrument parameters were:

Column: Phenomenex ZB-5ms, 30 m x 0.25 mm ID x 0.25 µm film thickness
Injection parameters: Manual Injection, Split (split ratio 5:1)
Injection volume: 1 mL
Inlet temperature: 250 °C
Helium flow: 1.0 mL/min
Oven temperature program: Start at 50 °C, ramp 10 °C/minute to 300 °C, hold for 10 minutes
Solvent delay: none
EI scan mode: scan (m/z 25 – 500)

A method blank, consisting of an empty headspace vial, was tested as a control using the same experimental conditions. The syringe was washed with methanol after each injection.

For each peak present in the samples, the mass spectrum was measured using the Automated Mass Spectral Deconvolution and Identification System (AMDIS) software v2.70. The resulting spectrum was screened against the 2011 NIST/EPA/NIH mass spectral library using the NIST Mass Spectral Search Program v2.0g. The best library match is reported for each peak along with the Match Factor for the unknown and library spectrum. A perfect match results in a value of 999; spectra with no peaks in common result in a value of 0. As a general guide, 900 or greater is an excellent match; 800–900, a good match; 700–800, a fair match. Less than 600 is a very poor match. However, unknown spectra with many peaks will tend to yield lower Match Factors than similar spectra with fewer peaks. Also, even an excellent match should not be considered as a definitive identification of the compound—true confirmation should be done by testing the sample alongside an analytical standard of the compound in question.

The total ion chromatogram of the French Roast and Decaf samples is shown in Figure 10. Forty-nine peaks were detected in the headspace analysis, and are tabulated in Table 1, sorted according to their relative peak size in the decaf sample. The best library match for the measured mass spectrum of each peak is shown with its CAS number, match factor, and chemical structure. The majority of the identified compounds consist of pyridines, pyrazines, furans, and other compounds that are known to be influential to coffee aroma.

Overall, the chromatograms for the two samples are very similar, contain no mutually exclusive peaks, and in most cases the relative intensity of each peak was found to be approximately equal between the two samples. However, some peaks were observed to be present in substantially higher intensities in the French Roast sample. These peaks were identified as Pyridine, Pyrazine, and methyl Pyrazine—commonly known aroma/flavor compounds in roasted coffees. The probable reason for the decreased concentration of these compounds in the decaf sample is that the extraction process used to remove caffeine also partially removes these compounds. The use of GC/MS as a quality control tool could be used to refine the extraction process to better preserve these characteristic coffee aroma constituents while selectively removing caffeine.

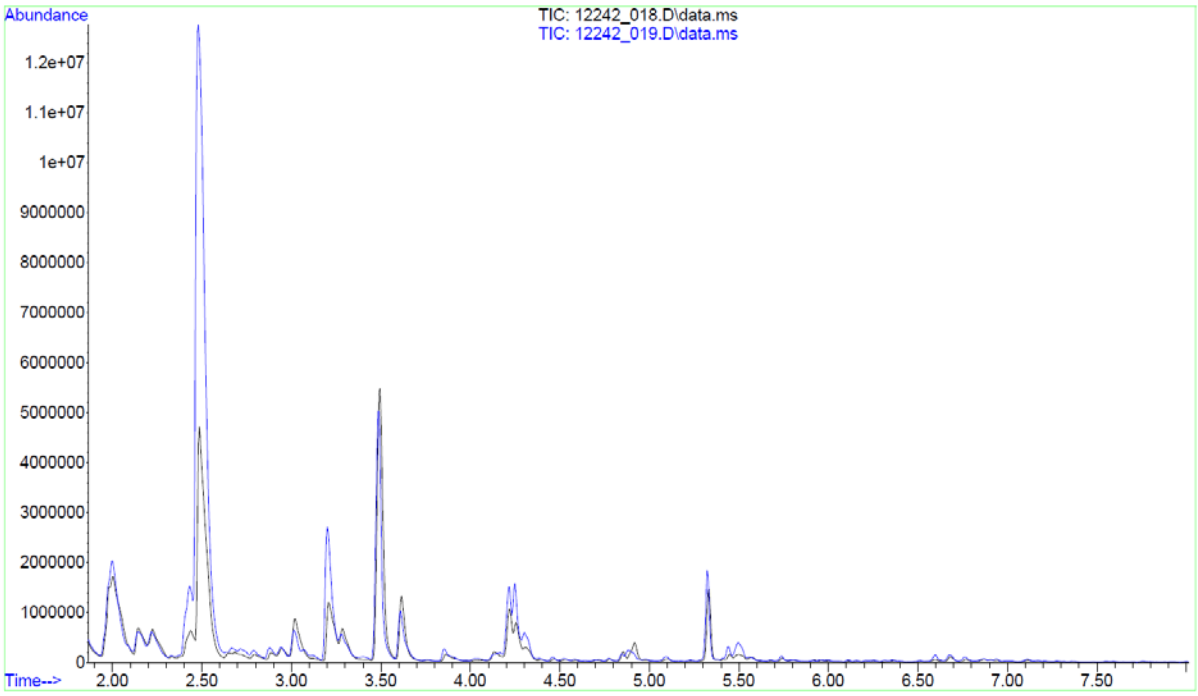
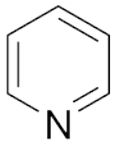
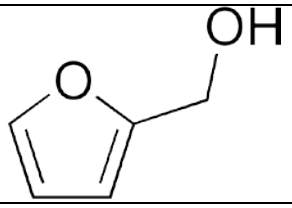
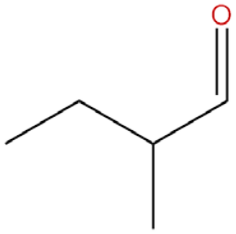
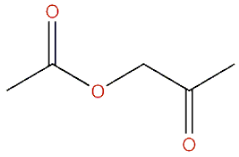
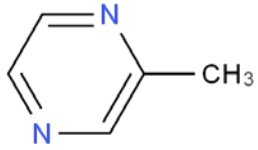
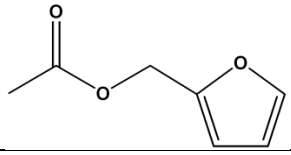
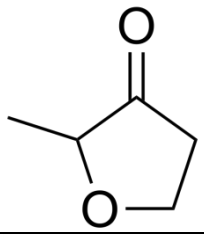
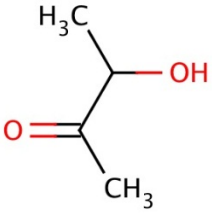
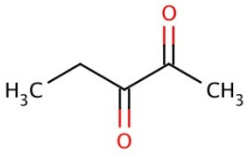
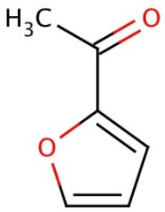
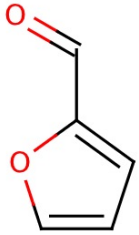
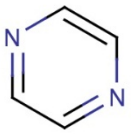
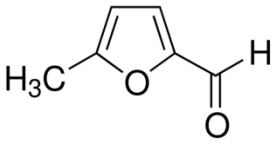
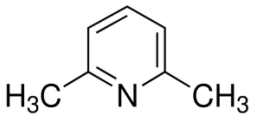
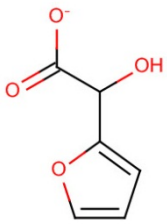
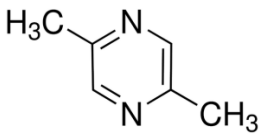
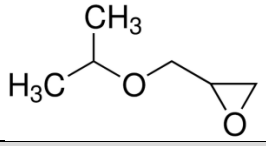


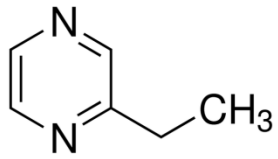
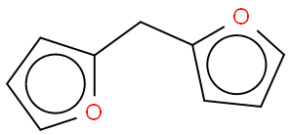
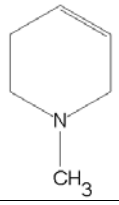
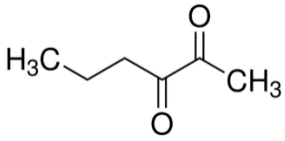
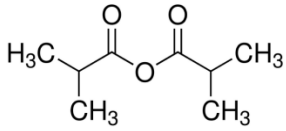
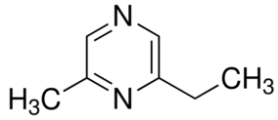
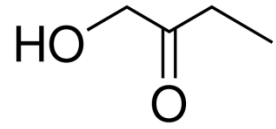
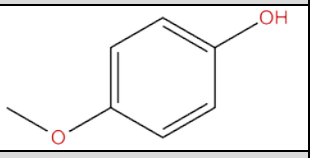
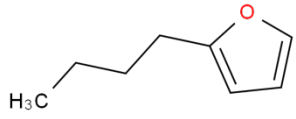
Figure 10: Overlay showing the total ion chromatogram of the French Roast (blue trace) and Decaf (black trace) samples.

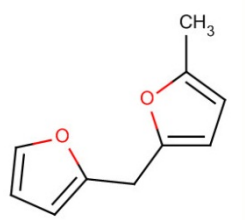
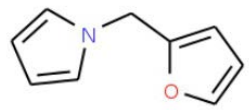
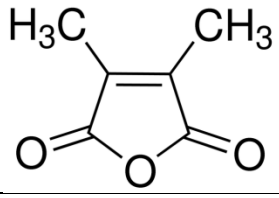
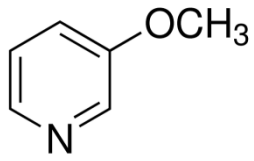
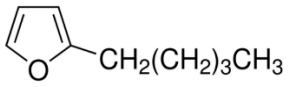
Table 1: Headspace GC/MS results of the french roast and decaf samples. Only peaks representing more than 0.01% of the total peak area are reported. The table is sorted according to the relative peak size in the decaf sample, with the largest peaks at the top of the table. The best library match for each peak is listed with the compound's CAS number, spectral match quality, and chemical structure. Rows highlighted in gray indicate that no library match was found with a match quality of >650. Compounds with a match quality of 600-700 are indicated in red text—the actual compounds for these peaks are likely to be structurally similar to the reported library match, but different than the exact compound reported.

Retention Time [min]	% of total	Best Library Match	CAS#	Match Quality	Comments	Chemical Structure
2.486	25.79%	Pyridine	110-86-1	962	Produced in coffee roasting process. Aroma constituent.	
3.495	24.30%	2-Furanmethanol	98-00-0	955	Occurs naturally in arabica coffee.	

2.001	14.68%	Butyraldehyde, 2-methyl-	96-17-3	890	Flavor/Odor constituent. Naturally occurring compound in coffee.	
3.615	4.99%	2-Propanone, 1-(acetyloxy)-	592-20-1	906	Found in roasted arabica coffee.	
3.206	4.32%	Pyrazine, methyl-	109-08-0	905	Aromatic compound found in roasted coffee	
5.334	4.10%	2-Furanmethanol, acetate	623-17-6	953	Found in roasted coffee	
3.021	3.70%	(2-methyltetrahydrofuran-3-one)	3188-00-9	915	Also known as "Coffee Furanone" Volatile constituent of the aroma complex of roasted coffee	
2.224	2.26%	Acetoin	513-86-0	861	Natural Coffee Flavor. Commercially produced by coffee fermentation	
2.142	1.99%	2,3-Pentanedione	600-14-6	874	Flavor/Odor constituent. Naturally occurring compound in coffee.	

4.215	1.88%	2-Acetylfuran	1192-62-7	931	Occurs naturally in coffee	
3.283	1.73%	Furfural	98-01-1	873	Occurs naturally in coffee, but is toxic. LD50 = 65 mg/kg (oral, rat)	
2.437	1.58%	Pyrazine	290-37-9	823	Contributes to the roasted, walnut, cereal, cracker, or toast-like flavors in coffee	
4.919	1.16%	2-Furancarboxaldehyde, 5-methyl-	620-02-0	927	Coffee aroma constituent	
3.861	0.64%	Pyridine, 2,6-dimethyl-	108-48-5	951	Occurs naturally in coffee. Has a nutty, coffee, cocoa, musty, bread, and meaty flavor	
4.134	0.63%	Furfuryl formate	13493-97-5	827	Occurs in roasted arabica coffee	
4.254	0.62%	Pyrazine, 2,5-dimethyl-	123-32-0	819	Natural coffee aroma Nutty/roasted odor	
2.95	0.57%	Oxirane, [(1-methylethoxy)methyl]-	4016-14-2	709		
4.854	0.42%	No match				
5.497	0.41%	No match				

4.314	0.41%	Pyrazine, ethyl-	13925-00-3	722		
6.681	0.37%	Furan, 2,2'-methylenebis-	1197-40-6	881	Minor constituent of coffee	
2.682	0.35%	No match				
2.884	0.33%	1-Methyl-1,2,3,6-tetrahydropyridine	694-55-3	762		
2.797	0.29%	2,3-Hexanedione	3848-24-6	861	Occurs naturally in coffee	
5.099	0.23%	No match				
5.568	0.19%	Propanoic acid, 2-methyl-, anhydride	97-72-3	809		
5.448	0.19%	Pyrazine, 2-ethyl-6-methyl-	13925-03-6	896		
2.65	0.18%	1-Hydroxy-2-butanone	5077-67-8	841	Natural Coffee odor	
5.743	0.16%	No match				
6.769	0.16%	Hydroquinone methyl ether	150-76-5	846	Hydroquinone is naturally found in coffee	
4.466	0.14%	No match				
4.783	0.13%	2-n-Butyl furan	4466-24-4	759	Found in roasted coffee	

6.594	0.12%	No match				
4.706	0.12%	No match				
7.118	0.12%	No match				
4.532	0.11%	No match				
6.872	0.09%	No match				
8.083	0.08%	5-Methyl-2-furfurylfuran	13678-51-8	875	Found in roasted coffee	
8.138	0.08%	1H-Pyrrole, 1-(2-furanylmethyl)-	1438-94-4	909	Naturally found in coffee	
5.923	0.07%	2,5-Furandione, 3,4-dimethyl-	766-39-2	782	Found in roasted coffee	
6.37	0.06%	Pyridine, 3-methoxy-	7295-76-3	721	?	
6.905	0.04%	No match				
6.518	0.04%	Furan, 2-pentyl-	3777-69-3	688		
6.114	0.04%	No match				
6.168	0.04%	No match				
5.961	0.03%	No match				
6.218	0.03%	No match				
6.25	0.03%	No match				
6.949	0.02%	No match				

Conventional GC-MS on brewed coffee

Brewed coffee was prepared by extracting approximately 0.040g of ground coffee in 50.0 mL of water at 90 °C for 5 minutes. After this time, the mixture was filtered to separate the liquid extract from the coffee grounds.

The liquid extract was transferred to an autosampler vial and injected into the injection port of an Agilent 6890 gas chromatograph equipped with an Agilent 5973 mass spectrometer. The instrument parameters were:

Column: Phenomenex ZB-5ms, 30 m x 0.25 mm ID x 0.25 µm film thickness
Injection parameters: Split (split ratio 5:1)
Injection volume: 1 µL
Inlet temperature: 250 °C
Helium flow: 1.0 mL/min
Oven temperature program: Start at 50 °C, ramp 10 °C/minute to 300 °C, hold for 10 minutes
Solvent delay: none
EI scan mode: scan (m/z 25 – 550)

The total ion chromatogram of the French Roast and Decaf samples is shown in Figure 11. The two samples are found to be very similar, except for the common peak present at 16.4 minutes in both samples. The peak area in the Decaf sample was found to be 1.4% of the peak area of the French Roast sample. The measured mass spectrum of this peak was compared against the 2011 NIST/EPA/NIH mass spectral library, and the best spectral match was found to be caffeine, as shown in Figure 12. Therefore, even decaffeinated coffee still contains a trace amount of caffeine (as shown in Figure 13), suggesting that the extraction technique used by the manufacturer is not 100% effective.

A caffeine calibration curve was prepared to quantify the concentration of caffeine in the French Roast sample, and is shown in Figure 14. The concentration of caffeine in the brewed coffee was found to be 129.8 ppm, or 6.49 mg of caffeine in 50 mL of brewed coffee. A comparison of the relative size of the caffeine peak in the French Roast sample to the caffeine peak in a 100 ppm caffeine stock solution is shown in Figure 15.

Assuming that the brewing process is able to extract 100% of the caffeine in the ground coffee, this corresponds to a wt./wt. concentration of 1.61% of caffeine in the coffee grounds. Literature reports suggest that most roasted coffees contain between 0.8% and 4.0% caffeine by weight, with darker roasts containing relatively less.

Another compound identified in the brewed coffee extract was acetic acid, partly responsible for coffee's acidic taste, and produced during the post-harvest fermentation and roasting processes.

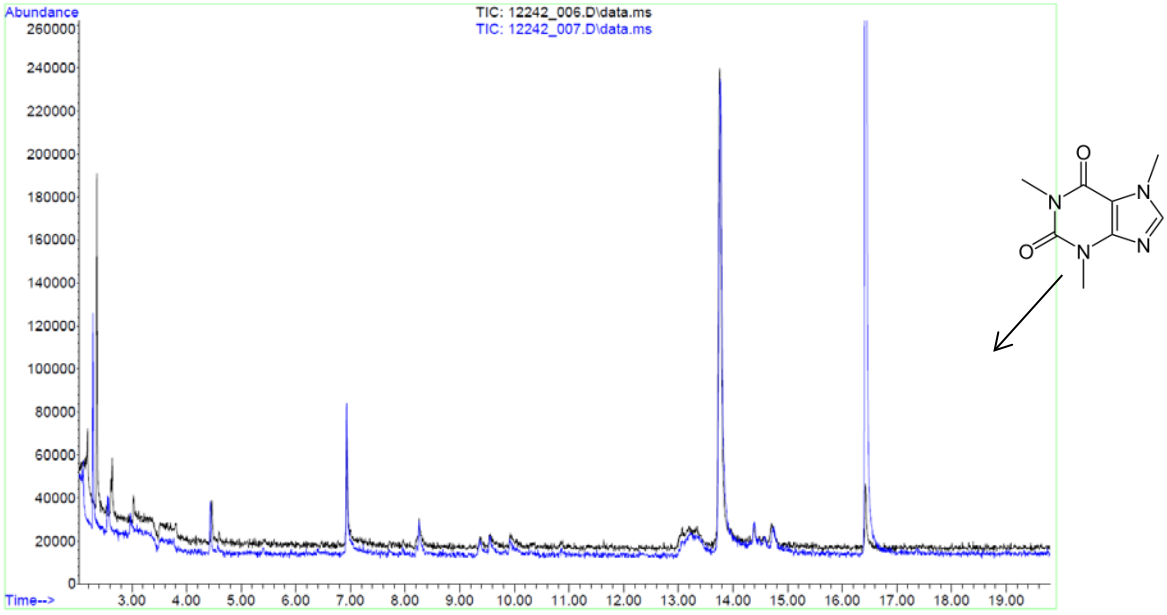


Figure 11: Overlay showing the total ion chromatogram of the French Roast (blue trace) and Decaf (black trace) samples. Caffeine elutes from the GC column at 16.4 minutes.

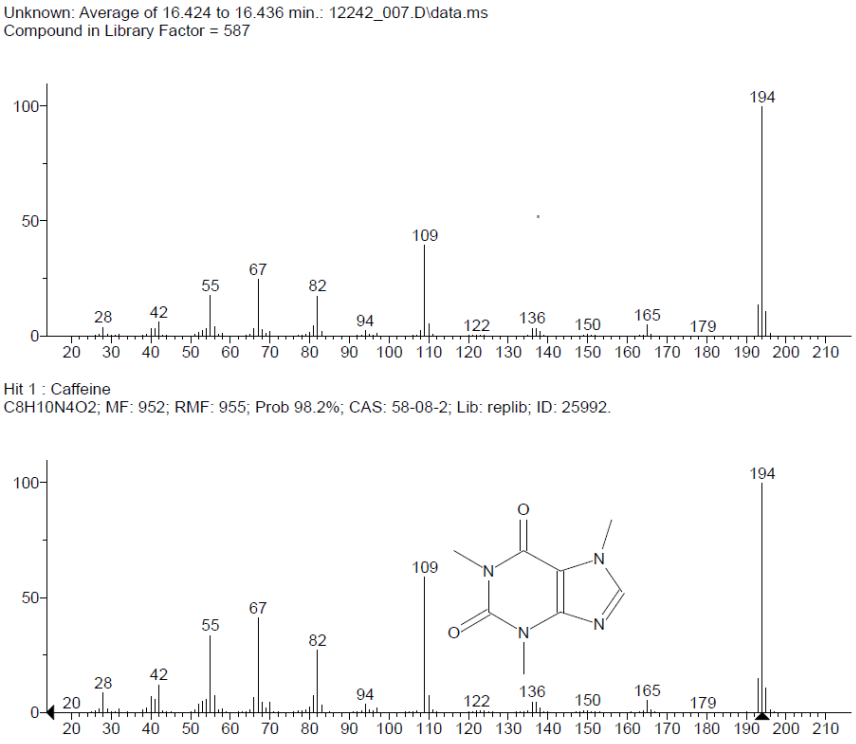


Figure 12: Best spectral library match for the peak located at retention time 16.4 minutes in the French Roast sample. The top chart is the measured mass spectrum of the unknown peak, and the bottom chart is the library mass spectrum of the closest spectral match, caffeine.

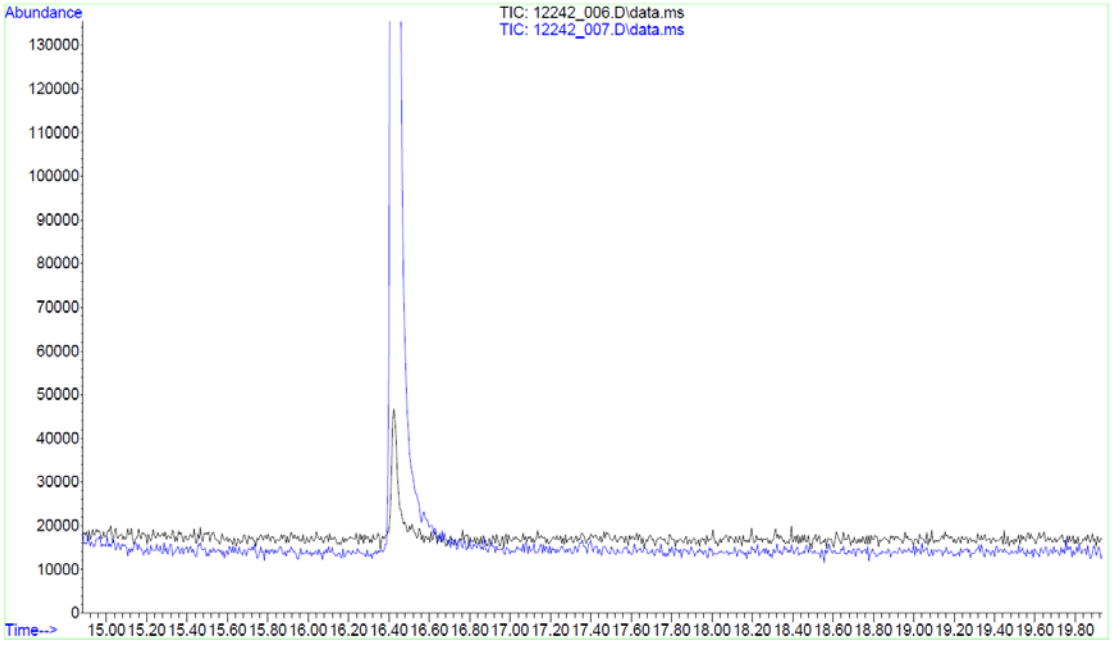


Figure 13: Total ion chromatogram showing the caffeine peak in the Decaf sample (black trace) relative to the French Roast sample (blue trace).

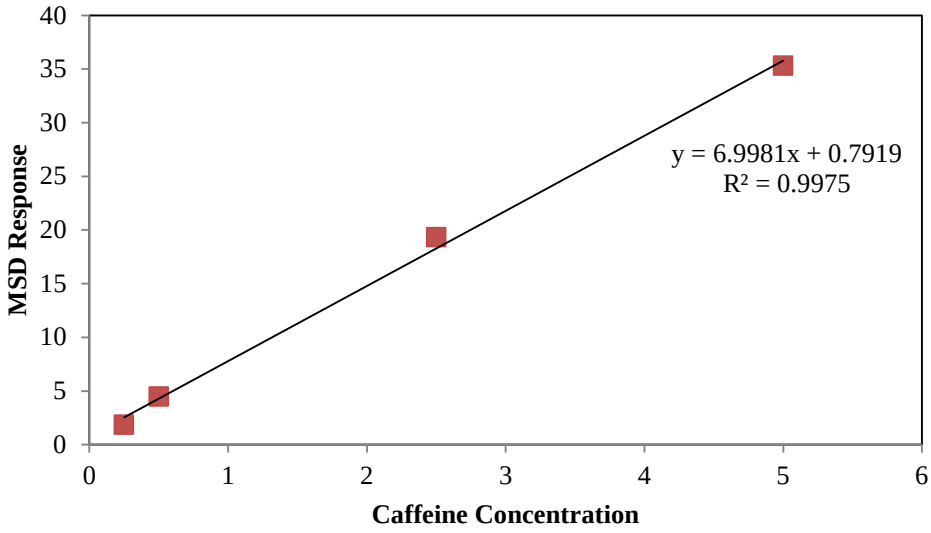


Figure 14: Caffeine calibration curve used for quantitative analysis. MSD response and caffeine concentration are calculated relative to internal standard isopropyl alcohol.

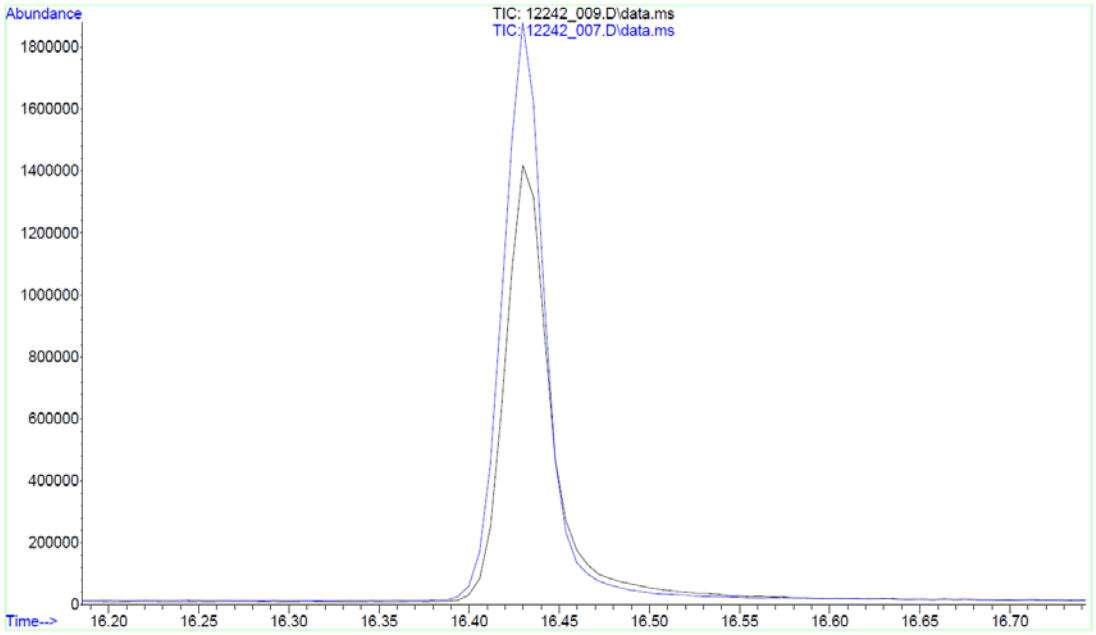


Figure 15: Total ion chromatogram comparing the relative size of the caffeine peak in the French Roast sample (blue trace) to the caffeine peak in a 100 ppm caffeine stock solution.

UV-Vis of brewed coffee

Ground roasted coffee (Starbucks French Roast and Starbucks Decaf House Blend) was dispensed into a beaker filled with 30 mL distilled water (DW) at 90 °C. After stirring for 5 minutes at 90 °C, the mixture was filtered using a filter paper (VWR, Catalog # 28310-026). After cooling the filtrate was adjusted to 50 mL using (DW) in preparation for analysis.

A Cary 50 UV-VIS spectrometer was used for collection of UV-VIS spectra of coffee extracts and standard solutions in a scan mode at 200-800 nm range, using DW as a background. Caffeine (Aldrich-Sigma) at a concentration of 20 µg/mL was used for preparation of standards. A linear calibration curve using absorbance at 275 nm, shown in Figure 16, was used for caffeine quantitation.

The UV-VIS spectra extract of French Roast and Decaf coffee is presented in Figure 17, showing sufficient absorption for Decaf at 275 nm. This signal is due to additional components in coffee that have absorption at 275 nm and overlapping with caffeine signal, as well as residual caffeine. To compensate for this interference, a calculation of caffeine content in French Roast coffee was based on its absorption after subtraction of Decaf coffee absorption at 275 nm. The caffeine content for French Roast coffee extract, using adjusted absorption, constituted 90.9 µg/mL that when re-calculated on a solid coffee weight constituted 11.3 ppm or 1.13 % of the solid coffee weight.

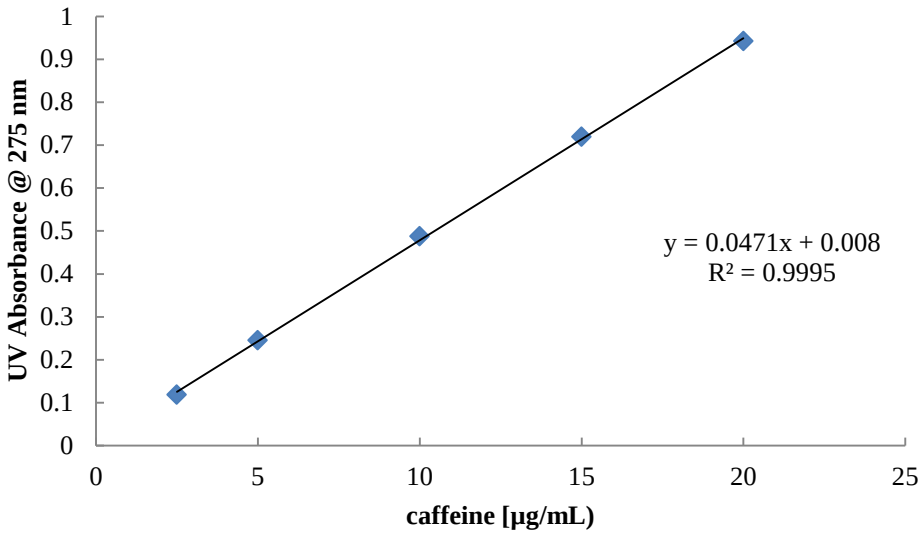


Figure 16: Linear calibration of caffeine standards at absorption 275 nm.

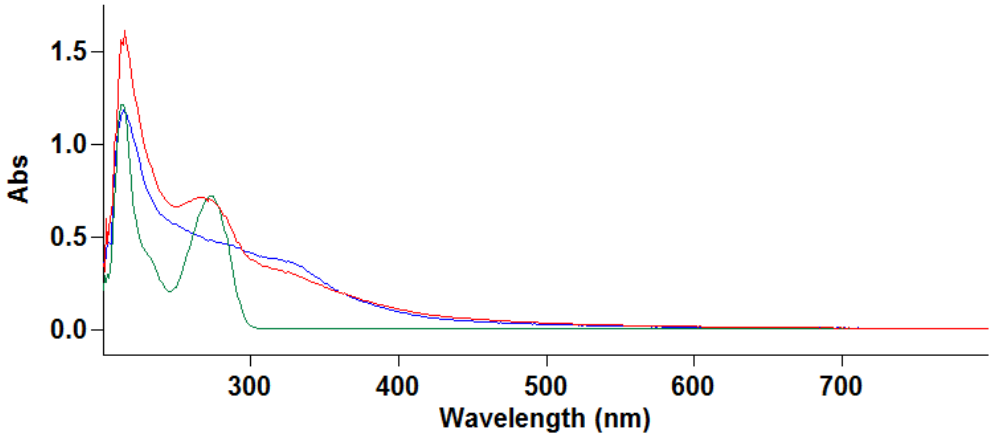


Figure 17: UV-VIS spectra of coffee extract for French Roast (red color), Decaf (blue color) and caffeine standard at 15 µg/mL (green color).

OIT of coffee

Oxidation induction time (OIT) was determined using a TA Instruments Q2000 differential scanning calorimeter (DSC). The OIT indicates the propensity of a material to oxidize, and can be used to infer shelf-life. A small amount of both the caffeinated and decaffeinated coffee was placed in a standard aluminum pan bottom and placed in the cell for analysis. After the isothermal step at 150.00 ºC for 5 minutes, the nitrogen purge was switched to oxygen. The testing parameters are listed below:

Equilibrate at 40.00 ºC
Ramp 20.00 ºC/min to 150.00 ºC
Isothermal for 5.00 min
Select Gas: Oxygen at 50 mL/min
Isothermal for 60.00 min

The tangent analysis method, as outlined in ASTM D3895-07 was used to determine OIT. The OIT according to the tangent method was determined by intersection of the extended baseline with the steepest linear slope of the oxidative exotherm.

A comparison of the two samples is shown in Figure 18. The OIT for the two samples is similar, 0.19 min for the caffeinated coffee and 0.18 min for the decaffeinated coffee. Although the OIT is similar for the samples, there is a difference in the shape of the oxidative curve. The caffeinated coffee has a larger peak, indicating that there is more material to oxidize, although it all oxidizes at a similar rate to the decaf coffee. These results suggest that the decaf and regular coffee should have similar shelf-storage lives.

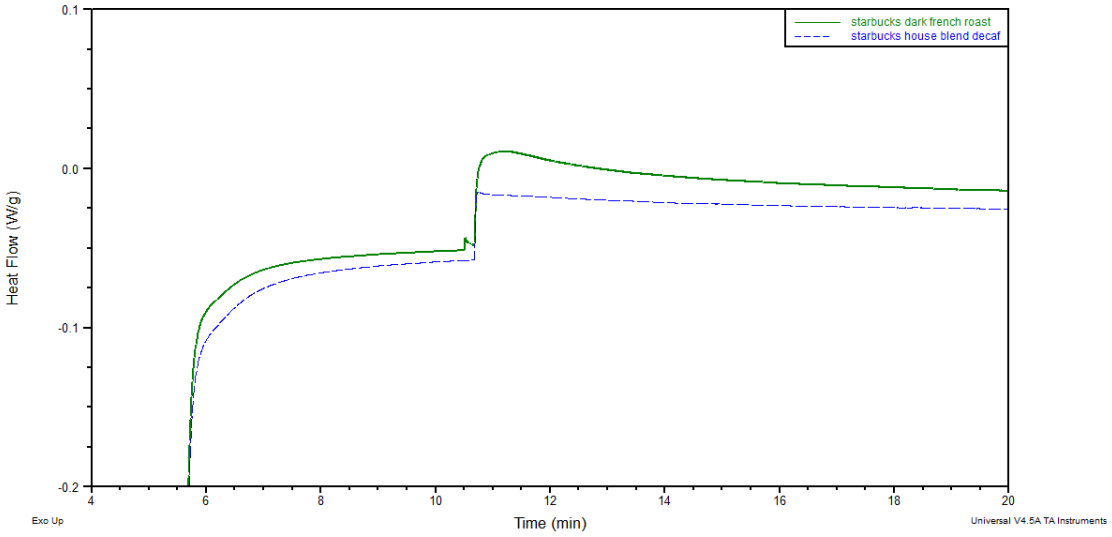


Figure 18: Comparison of OIT traces for caffeinated (green line) and decaffeinated (blue line) samples.

Rheology of brewed coffee

The shear viscosity of coffee extracts (Starbucks French Roast and Starbucks Decaf House Blend) was measured using a TA Instruments AR-G2 rheometer and compared with the viscosity of distilled water (DW) and Trader Joe's Half &Half Cream. Viscosity was measured under continuous torque ramp from 10 to 0.01 µN.m using 60 mm cone geometry at 25 °C. Viscosity as a function of shear rate is presented in Figure 19, showing no difference between the coffee extracts and water, as anticipated, while the viscosity of cream was higher for all shear rates with a modest thinning effect at high shear rates, which is typical for emulsions like cream.

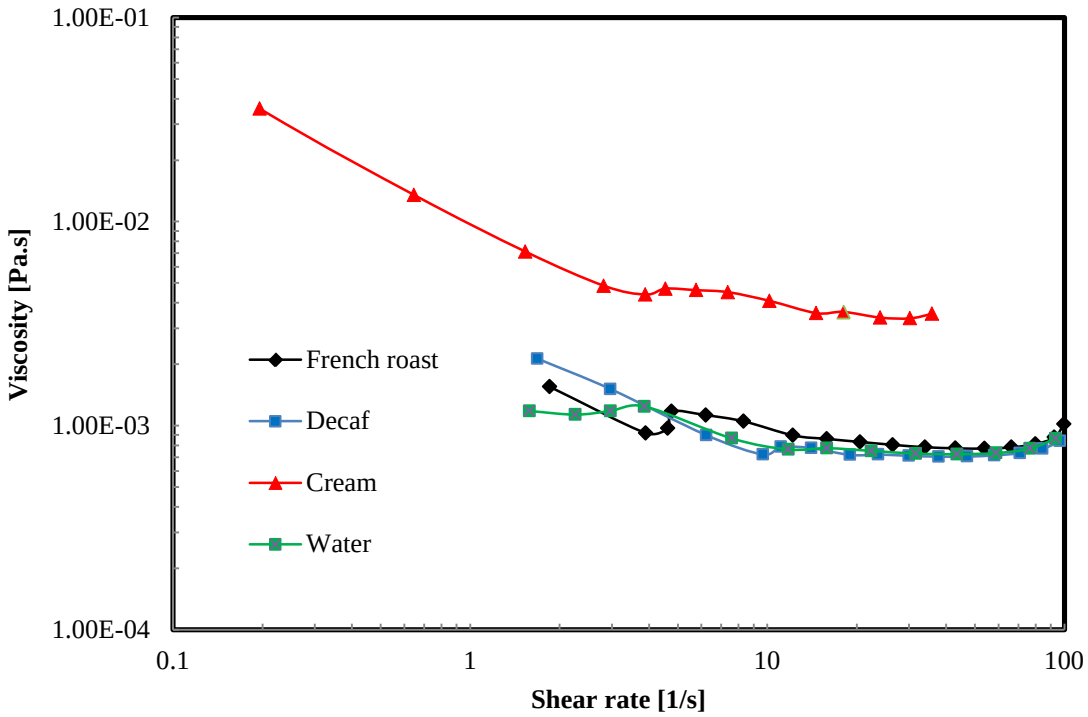


Figure 19: Viscosity as a function of shear rate for French Roast (blue color), Decaf (blue), distilled water (green) and cream (red).

Sol/Gel on grounds

The mass loss during brewing was determined for the caffeinated and decaffeinated coffees following a procedure based on ASTM D2765 in hot water (Method A). Specimen holders, prepared using 120 mesh stainless steel, were used to contain approximately 0.3 g of coffee. The samples were immersed in approximately 80 mL of distilled water heated to 90 ºC for five minutes under gentle agitation. After five minutes, the samples were removed from the water and left to dry in a 50 ºC oven. The change in mass of the coffee samples, weighed on an AND GR-202 analytical balance, is shown in Table 2. More mass is lost from the caffeinated sample than the decaf sample, which may indicate more extractable species, such as caffeine and other aromatics removed in the decaffeination process, is present in the French Roast.

Table 2: Mass measurements from sol-gel study of coffee.

Sample	Initial Mass [g]	Final Mass [g]	Mass Loss [g]	% Mass Loss
Dark French Roast	0.3020	0.2360	0.0660	21.9
Decaf House Blend	0.3001	0.2458	0.0543	18.1

Free-radical concentration

Free radicals are important pre-cursors to oxidation and degradation. In polymers in particular free-radicals often result from processing steps such as irradiation to induce crosslinking in polyethylene. In these cases it becomes critical to understand the concentration and time-evolution of the radicals. In this experiment a Bruker escanM was used to rank the relative number of free radicals in two coffee samples. Conventional parameters were used, with a scan width of 170 G, centered at 3469 G. The radicals detected (see Figure 20) were simple OH radicals, with the two samples exhibiting essentially the same radical concentration. These results suggest a similar propensity of the two coffees to oxidation during shelf-storage, which is corroborated with the previously presented OIT measurements.

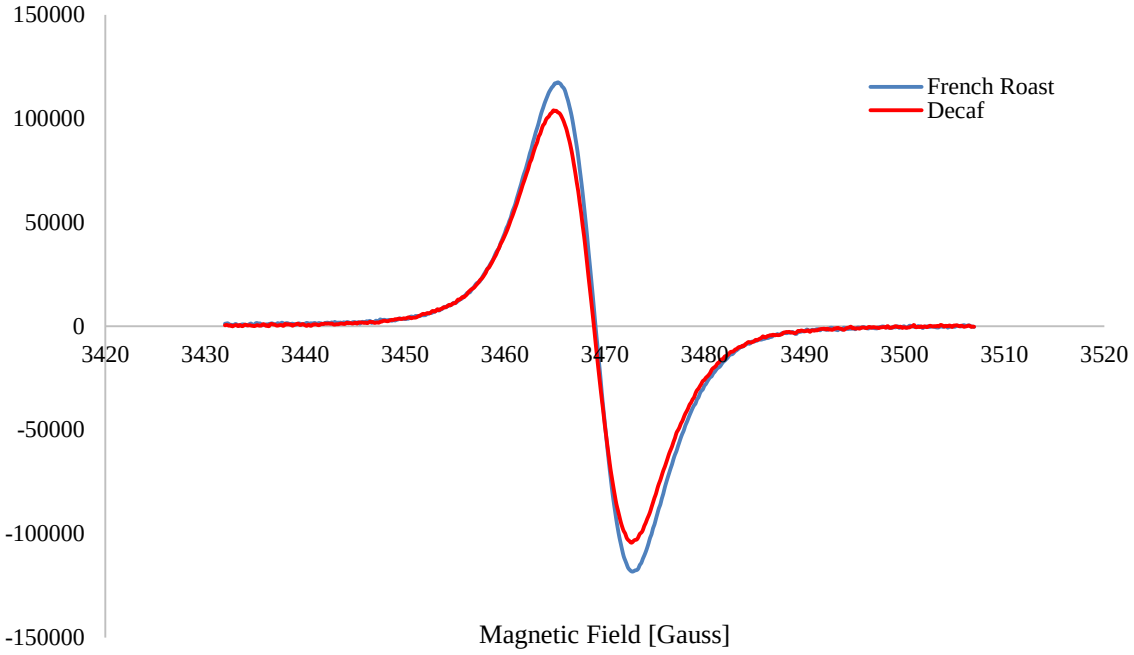


Figure 20: ESR spectra of French roast and decaf samples