UC Berkeley College of Chemistry

Chemistry 105

Instrumental Methods in Analytical Chemistry

GC/MS Analysis of Caffeine

Author:

Jonathan Melville

Lab Partners:

Jake Precht, Phuong Tran, and Jeremy Hsu

Graduate Student Instructors:

Richard Cooper & Daniel Mortensen

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1 Purpose

In this lab, we utilized a GC/MS to identify the quantity and purity of caffeine in two different samples: a regular cup of joe[1] , and a mixture of deuterated caffeine prepared by a GSI. By adding known quantities of deuterated caffeine to our samples, extracting all the caffeine contained within, and comparing relative caffeine isotope intensities by their mass spectra, we can quantitatively determine the amount of caffeine in our sample.

2 Theory

Gas chromatography (also known as gas-liquid partition chromatography or vapor-phase chromatography) is a form of chromatography that utilizes differences in retention time of gases to separate a sample. To this end, a "mobile-phase" is chosen that will not interact with the sample, usually a relatively inert gas such as helium, argon, or nitrogen. This mobile-phase gas is then laced with sample and flowed through a tube lined with a "stationary-phase" substance, often consisting of a waxy nonpolar liquid or polymer (though differing stationary phases can be used depending on the situation); depending on the amount of intermolecular attraction between the stationary phase and each component of the sample, differing constituents will elute through the column at different times[1]. The more a specific component of the sample is attracted to the stationary phase, the longer it will take to elute through the entire column (thus, for a nonpolar stationary phase, more polar compounds will exit the column first. The detector used in this experiment is a Mass Spectrometer, which ionizes samples at the end of the column to produce molecular ions (possibly fragmenting the molecule in the process), and then measuring the mass-to-charge ratio of the molecular [fragment] ions. The combination of these two methods, also known as GC/MS, allows for the detection of many compounds with good separation and at outstanding sensitivities (as low as a few picograms per second retention[2]). In addition, the multifactored approach that mass spec provides allows us to create several viable data sets and only use ones that have good fits, greatly

[1] coffee[2]

[2] this footnote is satirical, I know you know what a cup of joe is

increasing our precision. However, this precision comes at a price: depending on the specific method of ionization used, the molecules in question may fragment in a difficult-to-predict manner (or worse, fail to ionize altogether). Because of the organic nature of our analyte, it would be plausible to analyze this sample using GC/FID, a similar technique used in a previous lab report. Since deuterated caffeine and regular caffeine have very similar instrumental response factors[3], by picking a point on the calibration curve where the ratio of caffeine to deuterated internal standard caffeine is 1, we can plug that into our calibration regression and find the error of the signal ratio of that value. By knowing the amount of of internal standard we introduced, we can determine the error of the sample as well as its quantity.

3 Experimental

For our unknown sample in this lab report, we mixed 11.56 milligrams of deuterated caffeine (also known as caffeine-d_3, henceforth d_3) in 25.0 mL of coffee, and performed an organic extraction using methanol. We diluted the final organic extract to 100.0 mL, for GC/MS analysis. Standards of caffeine and deuterated caffeine were created using serial dilutions at concentrations of 200 ppm, 100 ppm, and 50 ppm.

4 Results and Discussion

This section contains only tabulated results from the Appendix. Derivations can be found in Appendix A on page 5. Raw data can be found in Appendix B on page 7.

[3]but not identical; the slight difference in molar mass between the two slightly alters the peak height-mass ratio that defines relative instrumental response

4.1 Results

caffeine-d_3 purity	86.0%
σ	±1.9 %
coffee caffeine content	534.22 ppm
σ	±41.23 ppm

4.2 Discussion

We were able to calculate the properties of our two samples, despite an error in which we did not hold the concentration of one of our caffeine samples constant while serially diluting it, causing us to have 3 samples of a constant caffeine:d_3-caffeine ratio instead of 3 samples with a varying ratio, which forced us to use an alternate method of calculation to determine our samples properties. We chose to use the tallest mass spectrometer peaks at 194 and 197 m/z for our analysis, because they corresponded most to the samples we were looking for in unfragmented form (see Figure 1). Perhaps most importantly, however, our predicted coffee caffeine content lines up with experimentally-determined values[4] that claim that most forms of coffee vary between 400 to 600 ppm of caffeine (0.4-0.6 mg/mL).

4.3 Accuracy and Error

Our data is *okay*. Our signal-concentration fit is of reasonable R^2 value ($\tilde{0}.80$), and we were able to calculate the purity of our caffeine product quite accurately (to within 1 part in 50). However, we were not able to calculate our sample's caffeine content very accurately, only to 1 part in 12, mostly due to difficulties resolving the deuterium standard of the calibration curve to the deuterium standard in our internal standard in relation to the caffeine content of the unknown. What's more, due to an improper creation of several of our calibration samples (see the preceding section), we were forced to use an alternate method of utilizing mass spectrometric data to calculate our problems (for which we are grateful for the multidimensional approach GC/MS provides to us, by providing us with many parallel data sets to analyze).

5 Conclusion

Though we are not exceedingly confident in our calculation due to ambiguity in the creation of our standards and some very muddled emails that made things even more confusing, we nevertheless received answers that were plausible, to say the least. We were able to successfully calculate the purity of our deuterated caffeine sample as well as predict the concentration of caffeine in our unknown coffee sample. Though there were some experimental errors that blocked off particular analysis techniques to us, the multidimensional nature of GC/MS allowed us to find another way (comparing all of the mass spectrographs signal ratios to a single calibration standard signal ratio). Because of our lack of confidence in our calculations, our data is probably not particularly precise nor accurate, but we were still able to receive informative data regarding the use of GC/MS as an analysis tool.

References

[1] *Gas Chromatography* [Online]; The Linde Group. http://hiq.linde-gas.com/en/analytical_methods/gas_chromatography/index.html (accessed Mar 19, 2014).

[2] *Theory, Analysis and Methods of Gas Liquid Chromatography* [Online]; Analytical Chemistry Research Foundation. http://www.gas-chromatography.net/gas-chromatography.php (accessed Mar 19, 2014).

[3] *Fragmentation Patterns* [Online]; Carnegie Mellon University. http://svmsl.chem.cmu.edu/vmsl/Caffeine/caffeine_fragment.htm (accessed Mar 19, 2014).

[4] *National Soft Drink Association*, Bunker and McWilliams, J. Am. Diet, 74:28-32, 1979

A Calculations

A.1 Mass Fragments

Our work primarily focused on the mass spectrum of the unfragmented caffeine molecule, the first molecular ion in Figure 1. With a m/z of 194 (or 197 for the d_3), these peaks were by far the most prominent and displayed the most clean trends.

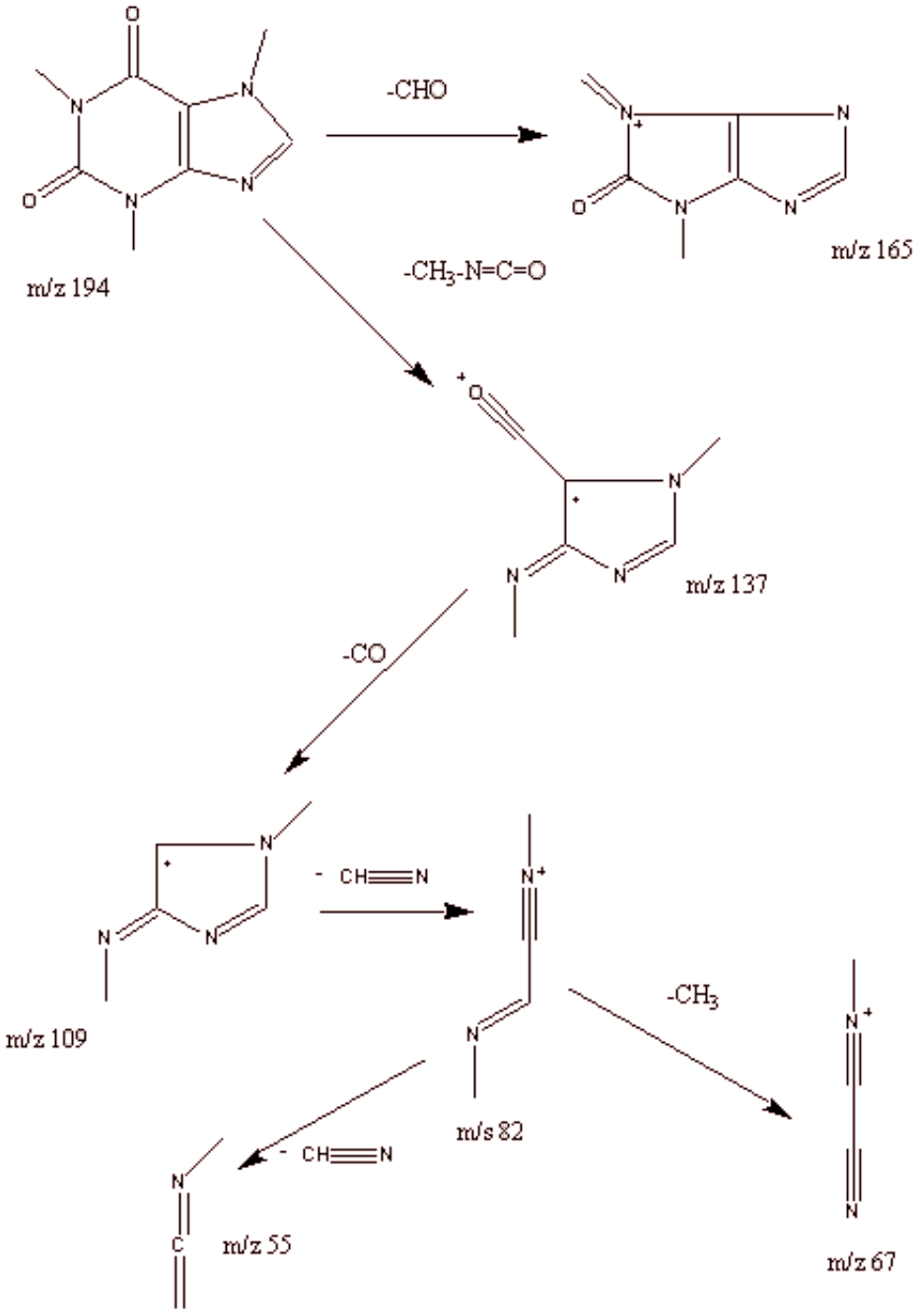

Figure 1: caffeine molecular ion fragments[3]

A.2 Relative Instrumental Response

$$R = \frac{\text{ng caffeine-d}_3}{\text{ng caffeine}} \times \frac{\text{peak height caffeine}}{\text{peak height caffeine-d}_3}$$

$$R = \frac{9.60 \text{ ng}}{9.68 \text{ ng}} \times \frac{493010.14}{419739.674} = 1.16485$$

A.3 Purity Calculation

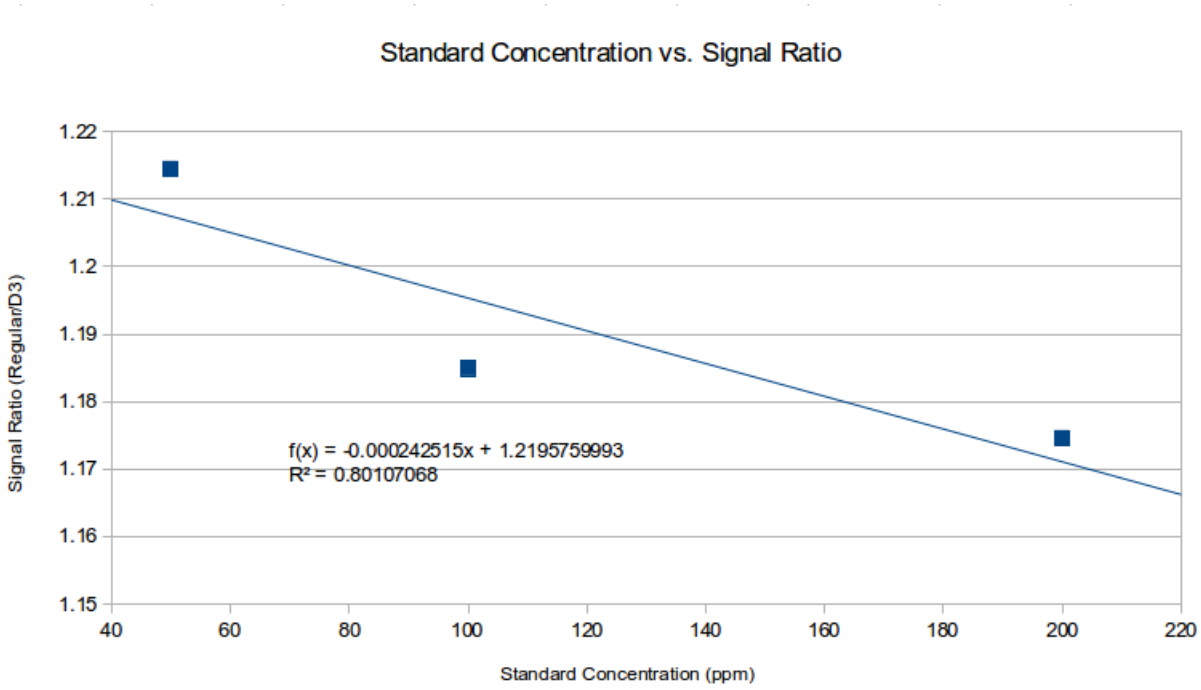

Figure 2: Concentration to signal ratio calibration curve

$$f(x) = -0.000242515x + 1.2195759993$$

Taking signal ratio to be 1:

$0.2195759993 = 0.000242515x$

$x = 905.412$

Plugging into regressions from Figure refcycle2:

Signal ratio $= \mathbb{S} = \frac{1987.66x+27075.80}{2304.5042x+37693.338} = 0.860$

Caffeine-d_3 purity$=\frac{\text{actual}}{\text{expected}} = \frac{0.860}{1} = 0.860$

A.4 Coffee calculation

$$\frac{11.56 \text{ ppm}}{\frac{1987.66(\frac{493010.14}{419739.674})+27075.80}{2304.5042(\frac{493010.14}{419739.674})+37693.338} \times \frac{1}{0.860}} = 534.22 \text{ ppm}$$

B Raw Data

Sample	Regular	Deuterated	Ratio
200 ppm	493010.14	419739.674	1.174561688
100 ppm	284895.872	240447.181	1.1848584409
50 ppm	141750.472	116722.042	1.2144276228
Coffee	901741.167	452609.042	1.9923180567

cal.d_3 mass	9.68 mg
cal. regular mass	9.60 mg
unk. d_3 mass	11.56 mg

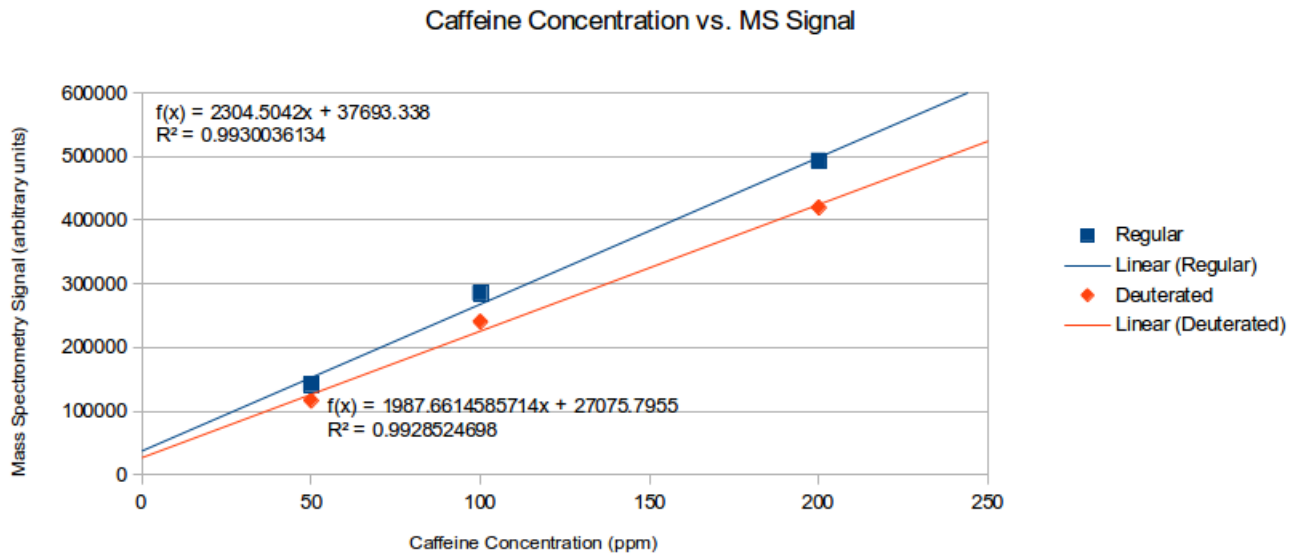

Figure 3: concentration:signal calibration curves for caffeine, deuterated and regular, at 194 and 197 m/z, respectively