

Determination of Riboflavin (Vitamin B2) and Caffeine in Energy Drinks Using HPLC with Fluorescence detection/UV-VIS

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Background

Name	REC. DAILY INTAKE	AMOUNT IN CAN	RISKS
Riboflavin	1.3 mg	5.8 ppm	<ul style="list-style-type: none">• Diarrhea• Increased urine• Bright yellow urine.
Caffeine	400 mg	351 ppm	<ul style="list-style-type: none">• Irregular heartbeat• Seizures.

Question:

Can riboflavin and caffeine be quantitatively determined in a can of Monster Energy?

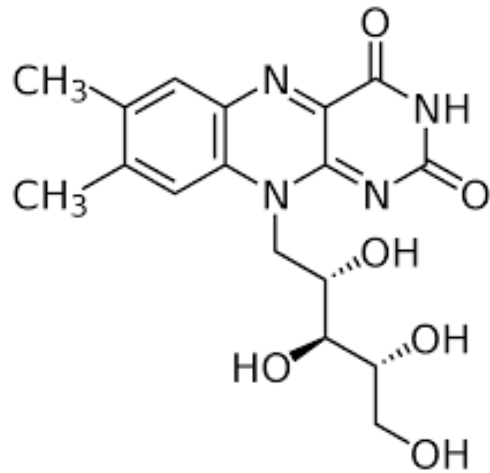


Figure 1. Structure of Riboflavin $C_{17}H_{20}N_4O_6$



Figure 2. Monster Energy Assault (Real Sample)

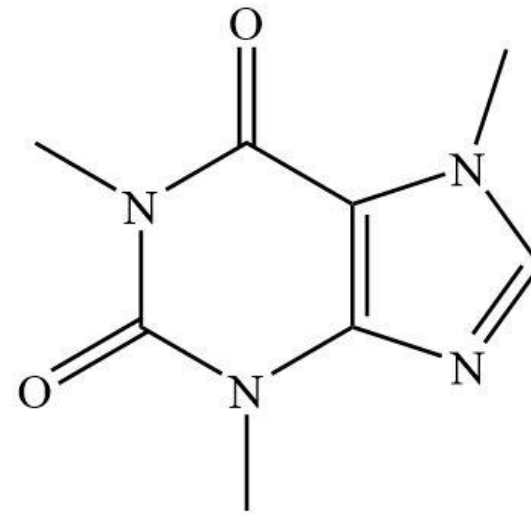


Figure 3. Structure of Caffeine $C_8H_{10}N_4O_2$



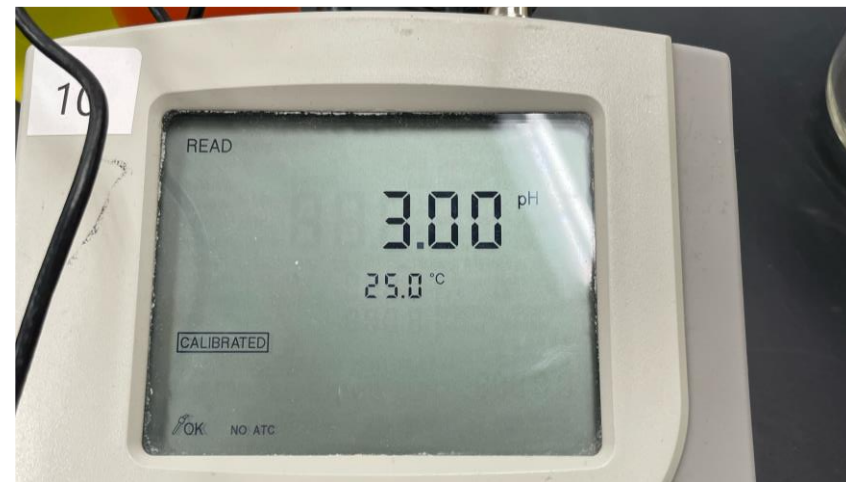
Instrument

	Riboflavin	Caffeine
HPLC Detector(s)	Fluorescent and UV/Vis	UV/Vis
Flow Rate	0.5 mL/min	0.5 mL/min
Column	Kinetex 2.6 μ m Polar C18 100 A	Kinetex 2.6mm EVO C18 100 A LC column
Mobile phase	Acetonitrile-phosphate buffer (50mM, pH 3.00) 20:80 (v/v)	0.5% Acetic acid-Methanol 75:25 (v/v)
Wavelength	Excitation/Emission: 450 nm/530 nm	273 nm



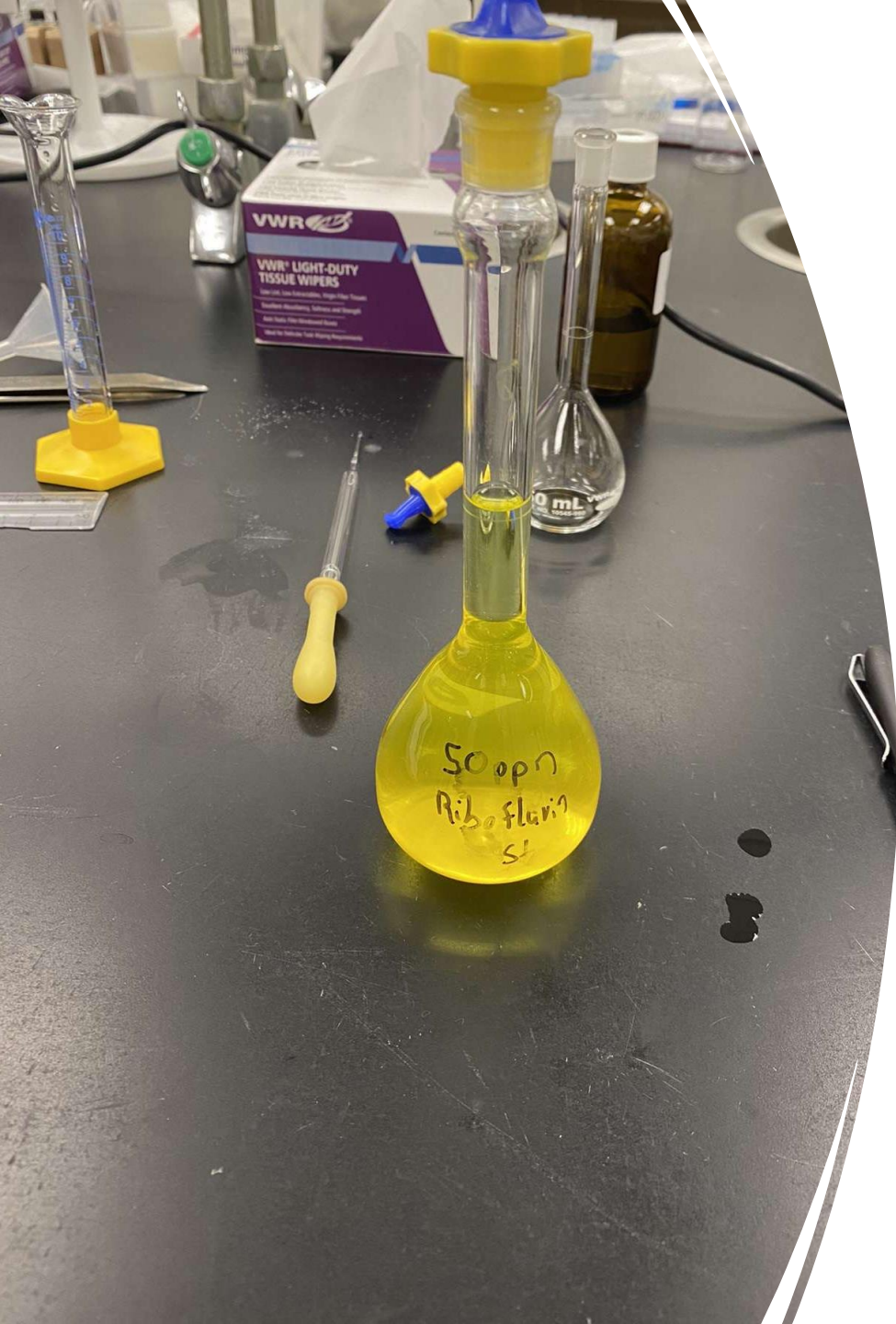
Phosphate Buffer Solution

- ▶ 50mM
- ▶ 3.00 pH
- ▶ 1.00 L





Real Sample Prep



Stock Solutions

- ▶ 54 ppm riboflavin stock solution in 100 mL volumetric flask

Creating Caffeine Standards

	Concentration Caffeine	Volume Stock solution used	RT	Peak Area	Peak Height
	(ppm)	(uL)	(min)	(mAU*s)	(mAU)
Blank	0.0	0	-	0	0
Standard 1	250	1249.0	2.599	7339.916020	1106.75879
Standard 2	30.x10 ¹	1499.0	2.596	9300.900390	1335.48743
Standard 3	350	1748.0	2.594	10894.300000	1504.44092
Standard 4	40.x10 ¹	1998.0	2.589	12361.200000	1642.1908
Standard 5	450	2248.0	2.615	14033.100000	1769.52966

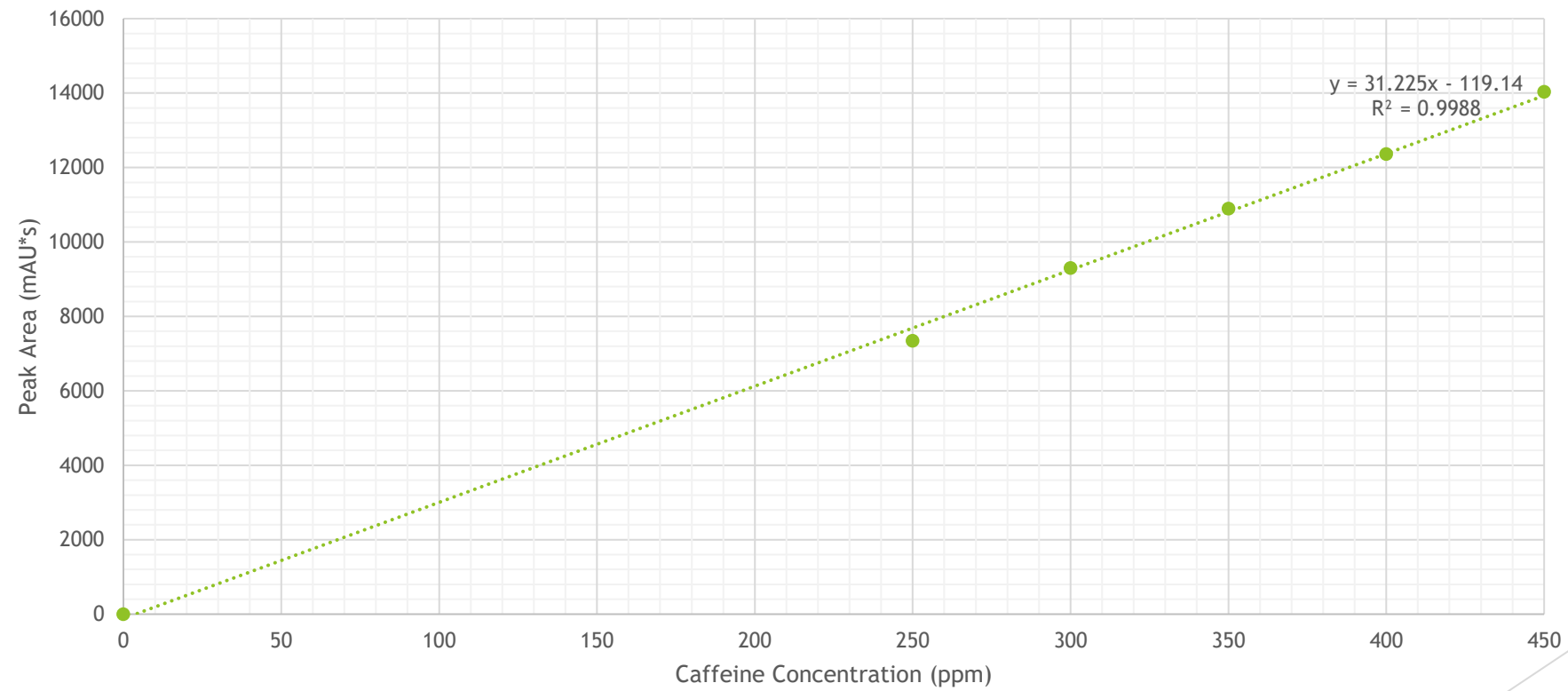
- Created using 100-1000 uL micropipettes into 5.0 mL volumetric flasks

Creating Riboflavin Standards

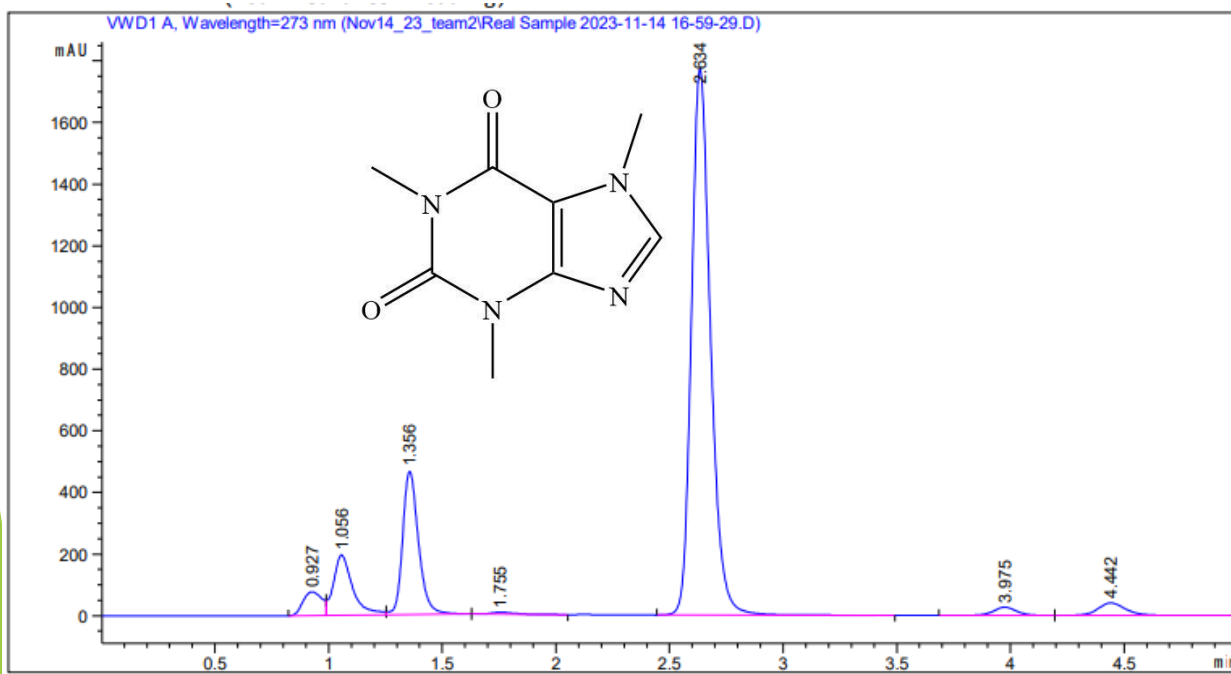
	Concentration Riboflavin (ppm)	Volume Stock Solution used (uL)
Blank	0.0	0
Standard 1	2.0	185.0
Standard 2	4.0	370.0
Standard 3	6.0	556.0
Standard 4	8.0	741.0
Standard 5	10	926.0

- Created using 100-1000 uL micropipettes into 5.0 mL volumetric flasks

Data and results



Data and Results



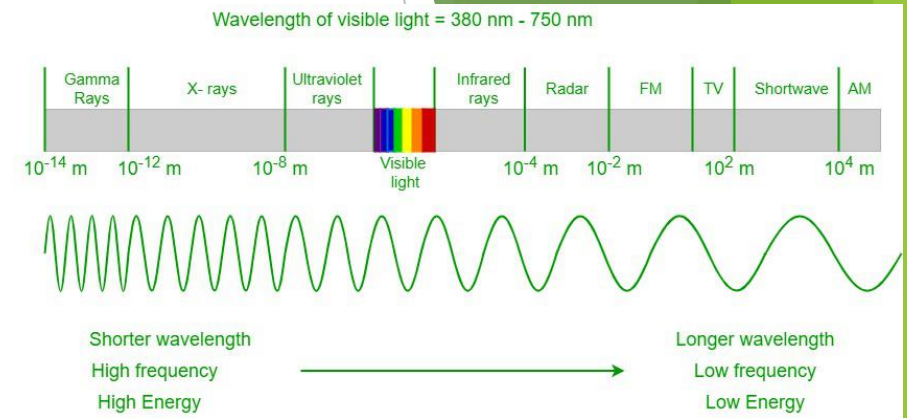
- ▶ Caffeine concentration determined to be 335 ppm
- ▶ Caffeine content listed to be 351. ppm

Challenges

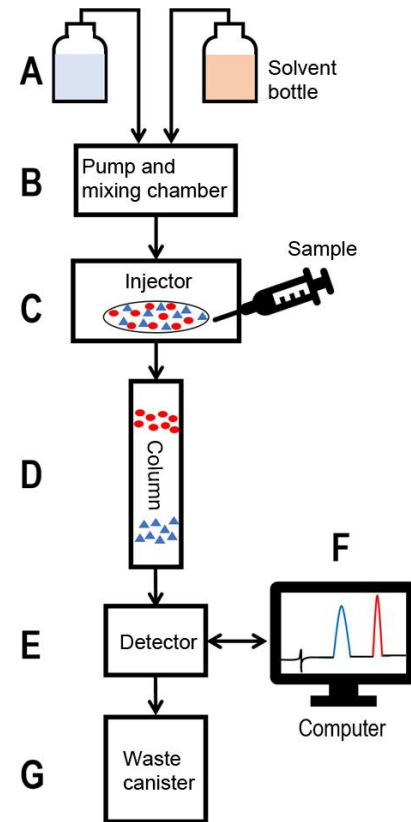
- ▶ Riboflavin stock solution preparation
 - ▶ 200ppm not dissolving
- ▶ Riboflavin detection
 - ▶ No significant peaks

Lessons

- ▶ Relearned buffer solution preparation
- ▶ Column and Wavelength matters



Future Work

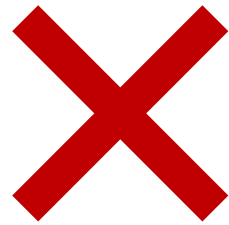


► Parameters

► Multiple cans, multiple flavours

Conclusion

▶ Riboflavin



▶ Caffeine



References

- ▶ Brewer, S. Chemistry 3120 Instrumental Analysis Laboratory Manual; Thompson Rivers University: Kamloops, BC, 2023.
- ▶ Health Canada, Caffeine in foods, Government of Canada, 2022. <https://www.canada.ca/en/health-canada/services/food-nutrition/food-safety/foodadditives/caffeine-foods.html> (accessed November 20, 2023)
- ▶ Marti-Andres, P. L.; Escuder-Gila, L. Y.; Martin-Biosca, S. Y.; Sagrado, S.; Medina-Hernandez, M. J. Simultaneous Determination of Pyridoxine and Riboflavin in Energy Drinks by High-Performance Liquid Chromatography with Fluorescence Detection. *J. Chem. Educ.* **2015**, *92*, 5, 903–906. <https://doi.org/10.1021/ed500544h>
- ▶ Harvard T. H. Chan School of Public Health, Vitamin B2 The Nutrition Source. 2023. <https://www.hsph.harvard.edu/nutritionsource/riboflavin-vitamin-b2/#:~:text=RDA%3A%20The%20Recommended%20Dietary%20Allowance,and%201.6%20mg%20daily%2C%20respectively>. (Accessed November 20, 2023)