The Determination of Response Factors for Reaction Monitoring by Gas Chromatography

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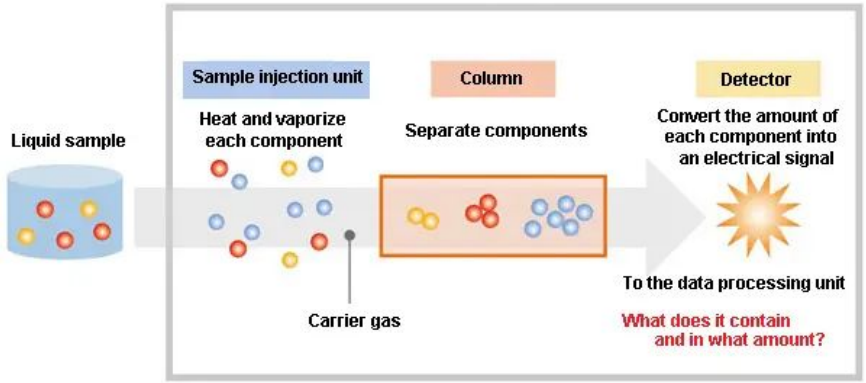
Gas Chromatography (GC)

Modern GC was developed in 1952 by A. T. James and A. J. P. Martin based on the principles of boiling point. There are three steps in any GC experiment; injecting (at the inlet), separating (inside the column in the oven) and detecting (done in the detector and displayed on the processing unit). There are two different phases within the column, the stationary phase and the mobile phase. The stationary phase is a liquid (coating the inside of the column) and the mobile phase is a gas (an inert carrier gas). The compounds will separate in the column and reach the detector at different times depending on their physical properties. The compounds will then appear on the chromatogram.

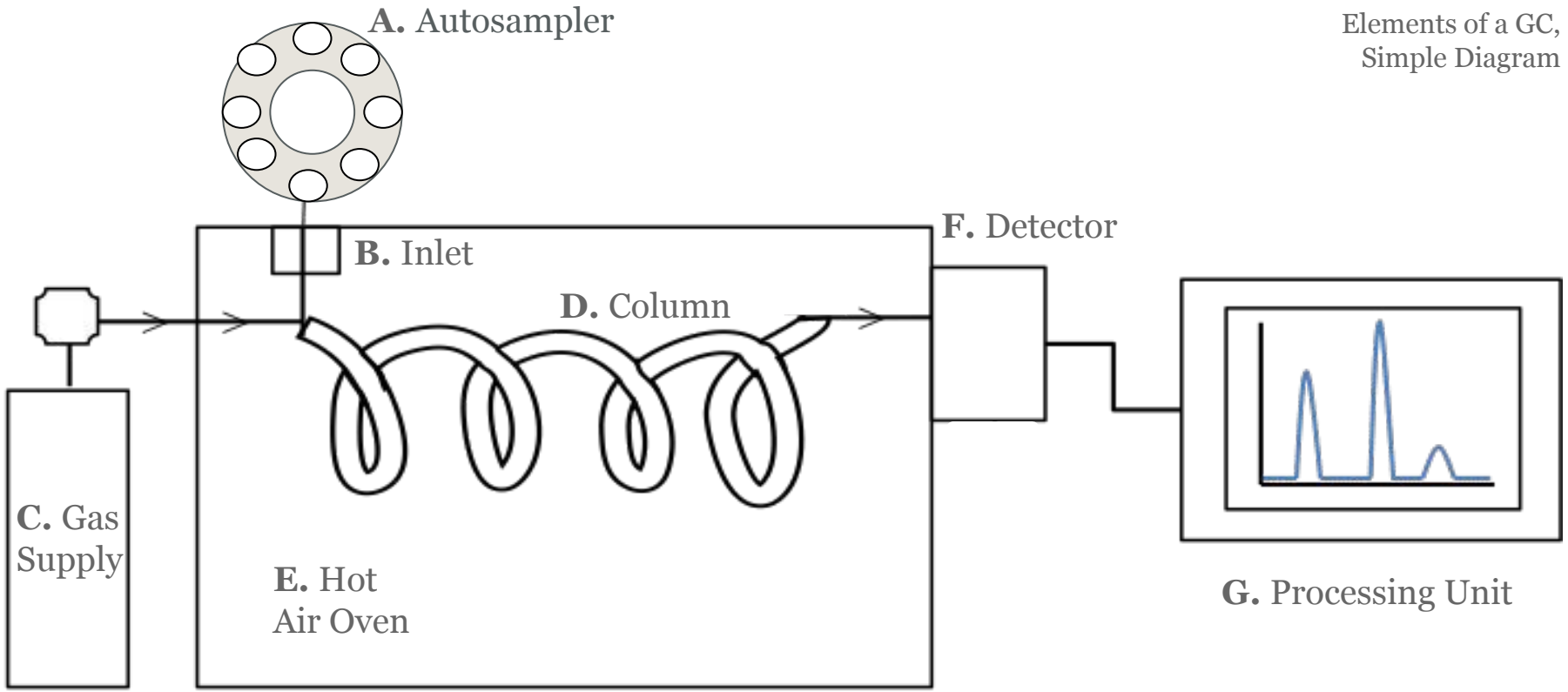


Agilent Technologies. (2011). *7820A Gas Chromatograph: Operating Guide.* Retrieved from https://www.agilent.com/cs/library/usermanuals/public/g4350-90012.pdf (accessed 2021-08-07)

Bartle, Keith D. et al. History of gas chromatography. *TrAC Trends in Analytical Chemistry*, 2002 Vol. 21, 547-557.

Shimadzu, What is Gas Chromatography, *Gas Chromatography Separation.* https://www.ssi.shimadzu.com/products/gas-chromatography/fundamental-guide-to-gas-chromatography/what-is-gas-chromatography.html (accessed 2021-08-09)

Elements of a GC,
Simple Diagram

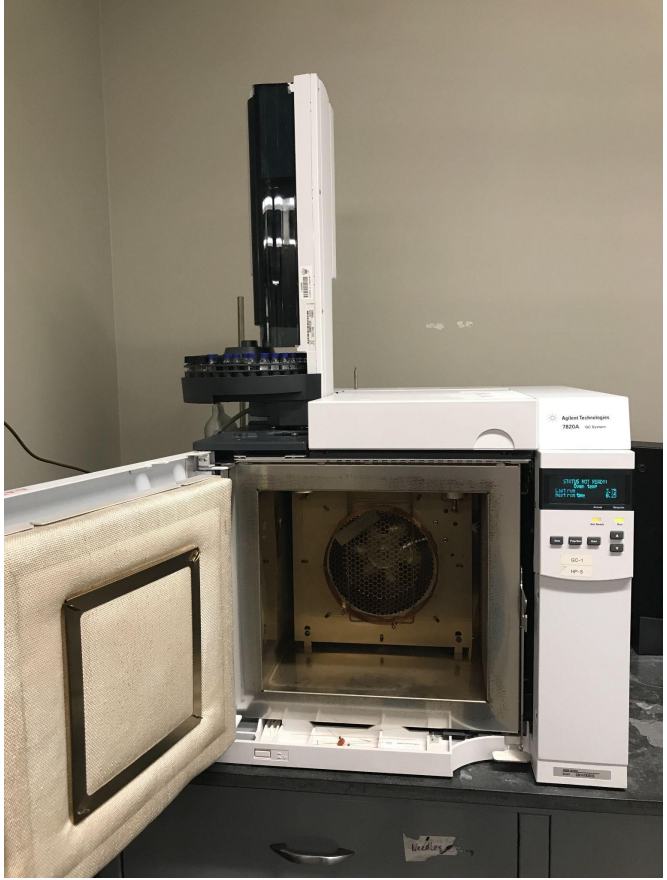


Torres, Jessica Carrying You Through Gas Chromatography. *BiteSizeBio*, 2016. https://bitesizebio.com/28687/carrying-gas-chromatography/ (accessed 2021-08-11)

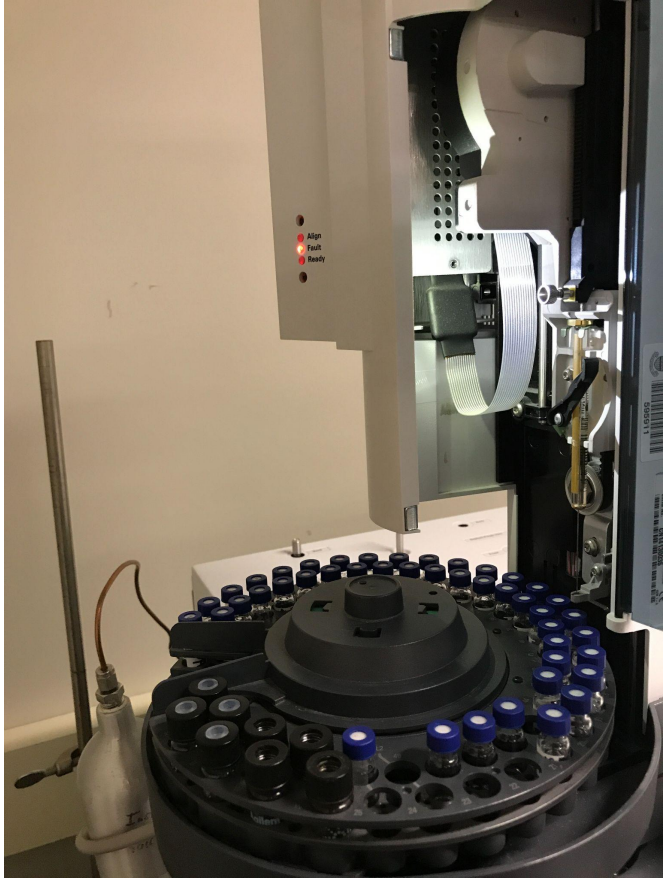
Elements of a GC

A. Automatic Sampling Device (Autosampler): a mechanical device that introduces a sample to the inlet via computer direction (software like ChemStation).
B. Inlet: the inlet used is selected based on the type of sample.
C. Gas Supply: several gas supply connections are on the machine, the types of gasses used will vary depending on the detector type (an inert carrier gas is always required).
D. Column: one end is attached to the inlet and the other to the detector. Columns vary in length, diameter and coating, select one based on type of sample.
E. Hot air oven: the oven can heat anywhere from 0℃ to 425℃.
F. Detector: there are several types of detectors (selected based on type of analysis).
G. Processing Unit: the electrical signals are digitized and received on a computer and can be viewed using ChemStation.

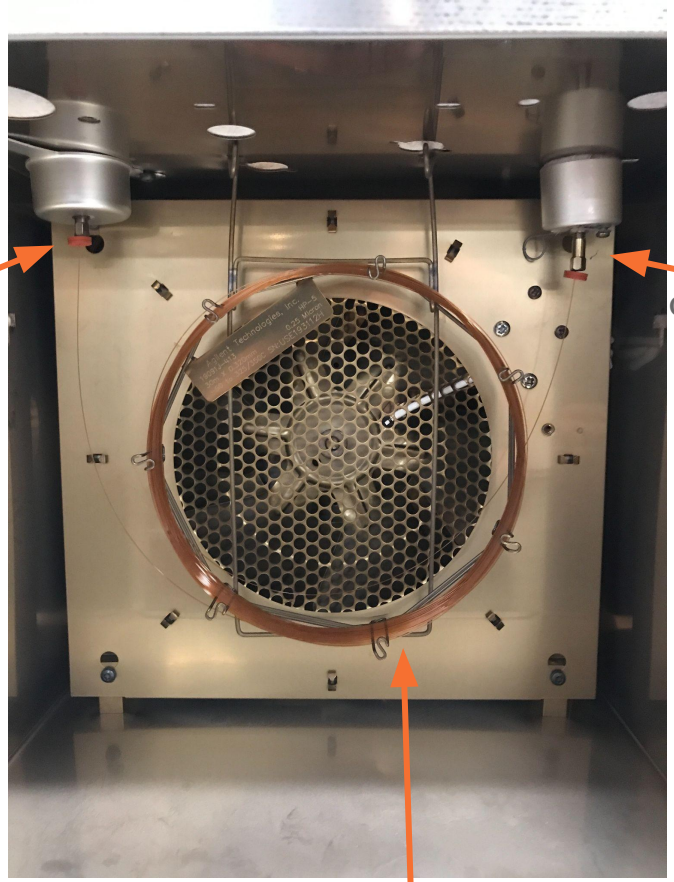
Agilent Technologies. (2011). *7820A Gas Chromatograph: Operating Guide.* Retrieved from https://www.agilent.com/cs/library/usermanuals/public/g4350-90012.pdf (accessed 2021-08-07)



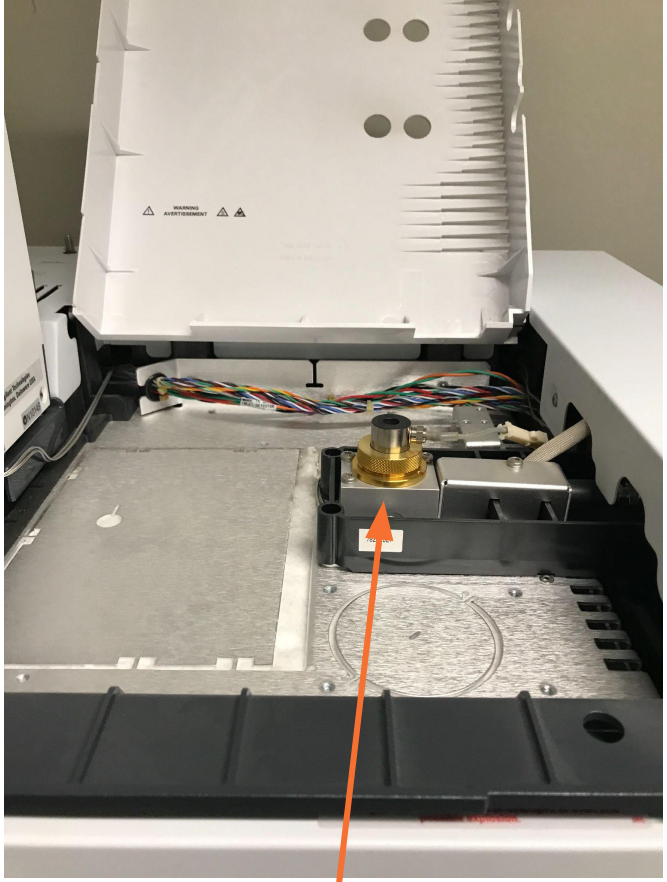
Agilent 7820A GC unit



Autosampler (robot that samples and injects to inlet)



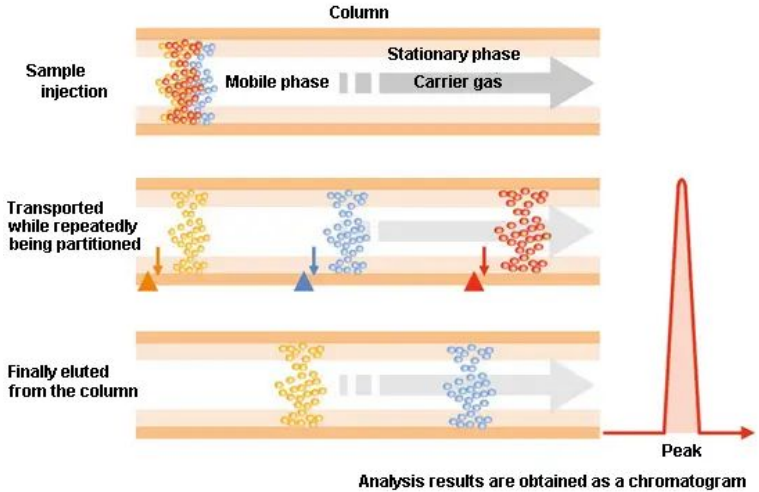
Inside oven, can see column and base of inlet/detector sites



The Flame Ionization Detector (FID)

Theory and Mechanics of a GC-FID

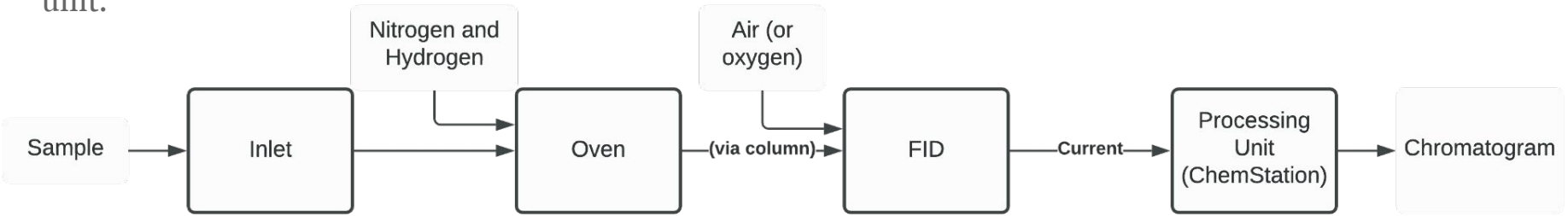
GC works based on the physical properties of the compounds within the solution. The run will start with the oven temperature at 100℃ and will ideally heat up enough to vaporize all substances. The heat will increase as the run progresses and cool back down to 100℃ between runs. This means that the compounds with lower boiling points and densities will travel through the column faster and appear on the chromatogram sooner.



Shimadzu, What is Gas Chromatography, Gas Chromatography Separation. https://www.ssi.shimadzu.com/products/gas-chromatography/fundamental-guide-to-gas-chromatography/what-is-gas-chromatography.html (accessed 2021-08-09)

Theory and Mechanics of a GC-FID

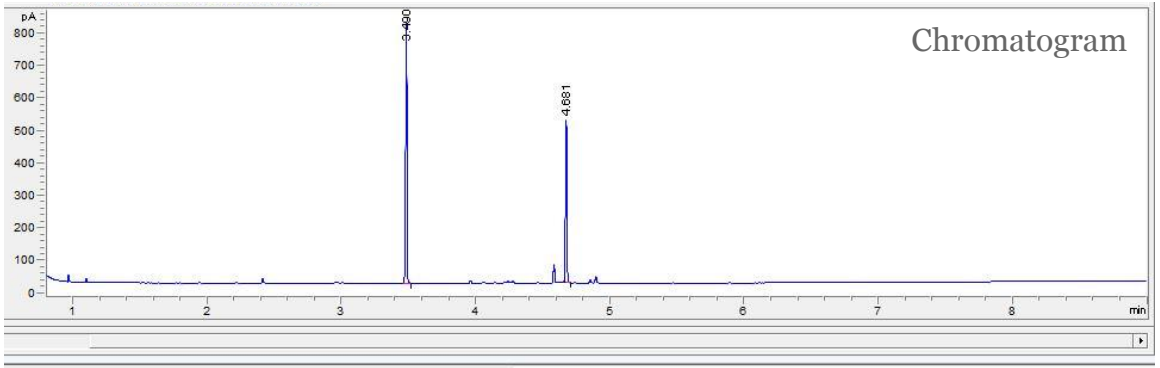
FID is simply the type of detector being used. In a FID detector, sample and carrier gas will move from the column through a hydrogen-air flame. The hydrogen-air flame alone creates few ions, but burning an organic compound increases the number of ions produced. A polarizing voltage attracts these ions to a collector located near the flame. The current produced is proportional to the amount of sample being burned. This current is sensed by an electrometer, converted to digital form, and sent to a processing unit.



Agilent Technologies. (2011). *7820A Gas Chromatograph:* Advanced *User Guide.* Retrieved from https://www.agilent.com/cs/library/usermanuals/public/G4350-90020.pdf

What is a Chromatogram?

Each peak on a chromatogram represents a different compound reaching the detector. This means that if the same sample is run several times, each compound will appear at the same time. The x-axis is the time (min) of the trial and the y-axis is the current sensed by the detector. The area under the peaks is often used when calculating the response factor (the height can be used as well, but this is less common). The table under the chromatogram summarizes this data in a clear concise way.

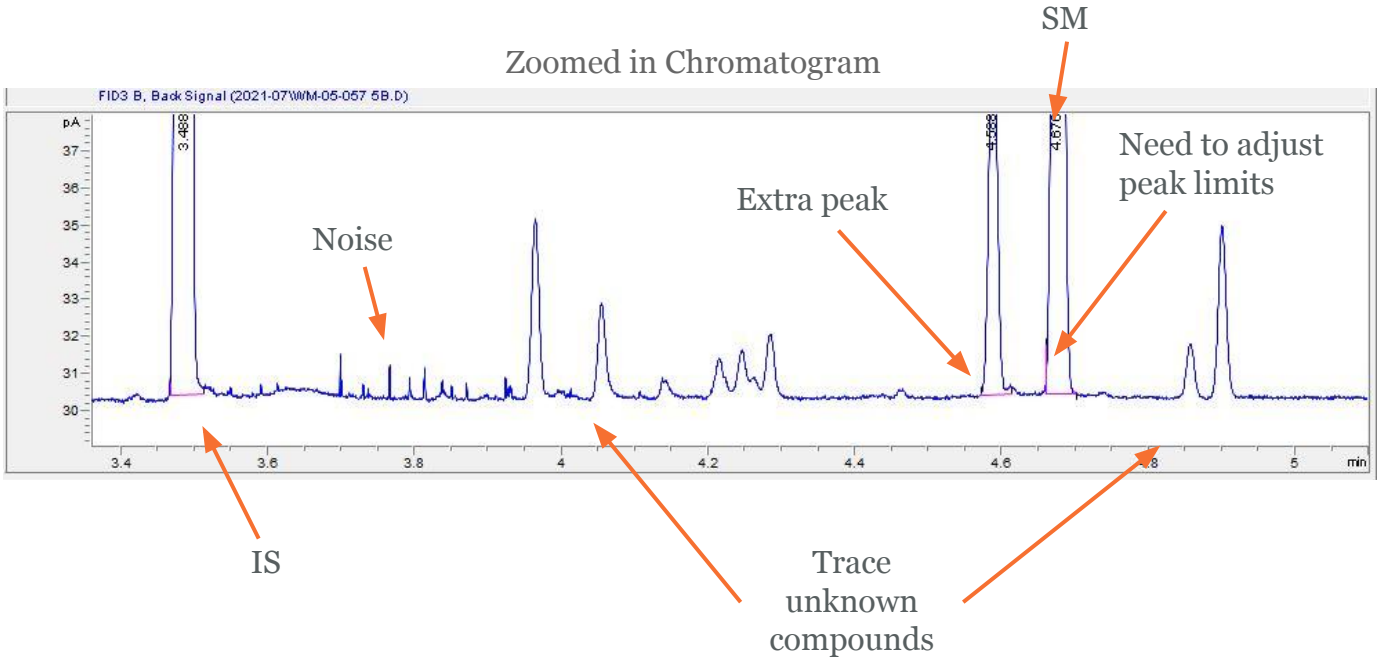


File Information	
GC-File	WM-05-057 3B.D
File Path	C:\CHEM32\1\DATA\2021-07
Date	30-Jul-21, 12:13:03
Sample	WM-05-057 3B
Sample Info	
Barcode	
Operator	SYSTEM
Method	100_300_9MIN_10-TO-1_SPLIT.M

#	Time	Area	Height	Width	Area%	Symmetry
1	3.49	565.1	784.7	0.0116	60.133	1.116
2	4.681	374.7	496.5	0.012	39.867	1.136

How to Analyze a Chromatogram

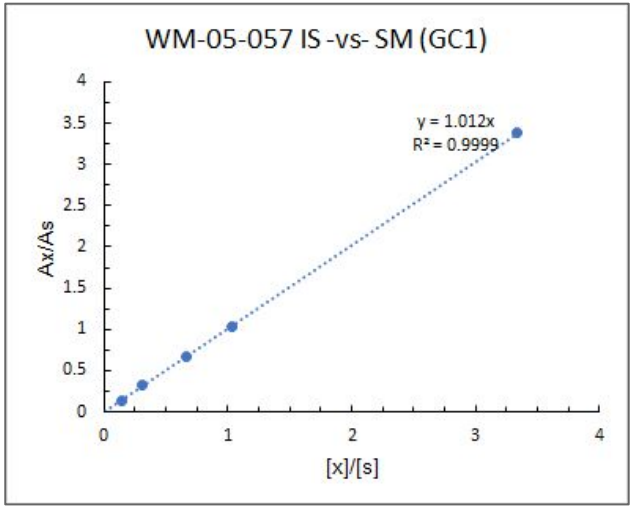
- ➔ Identify peaks (IS, SM, etc.).
- ➔ Ensure signal to noise ratio is acceptable.
- ➔ Adjust integration of detected peaks if necessary.



Calibrating a GC-FID

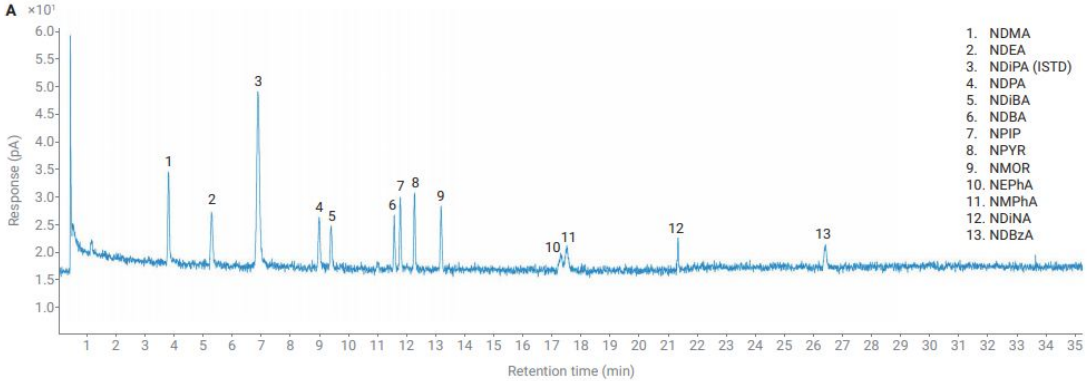
In order to achieve meaningful values from a GC experiment and to combat human error or variances between different GCs, methods such as a calibration curves are used. A calibration curve is generated using the formula below and plotted on a graph. The x-axis is the quotient of the concentrations, the y-axis is the quotient of the signals, and the slope is equal to the response factor (RF). The RF is a ratio of the unknown and the known substances, in this case SM and IS respectively. The R^2 value represents how linear the line is (can data be accurately interpolated and extrapolated); ideally it would be 1.

$$\frac{A_x}{A_s} = R_F \left(\frac{[x]}{[s]} \right)$$



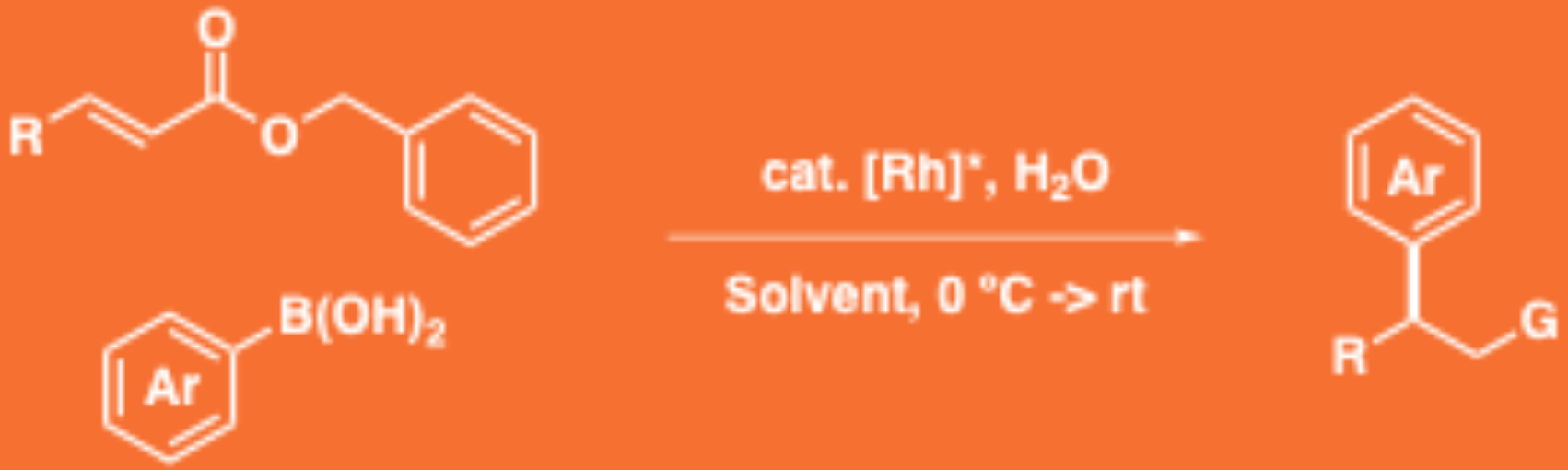
Uses of GC

GC is used to analyze liquid samples and their chemical makeup. GC can be used to separate and quantify compounds within a solution, test the purity of a substance, and can aid in the identification of an unknown solution. These uses can be applied in industries like pharmaceuticals, quality assurance, food and agriculture safety, forensics and environmental analysis.



GC chromatogram from trials on the release of N-nitrosamines from rubber treats and soothers. It is a calibration solution at a concentration of 25 µg/L.

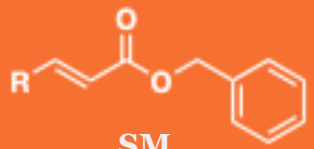
Zhang, Y. et al; *Analysis of the Release of N-Nitrosamines from Rubber Teats and Soothers*; Agilent Technologies, 2021. https://gcms.cz/labrulez-bucket-strapi-h3hsga3/application_nitrosamines_rubber_8890_gc_5994_3031en_agilent_0d04338b29/application-nitrosamines-rubber-8890-gc-5994-3031en-agilent.pdf



My Work

Procedure

Since I participated in the program virtually, all of my samples were prepared for me, but I will include the process here.



SM

IS

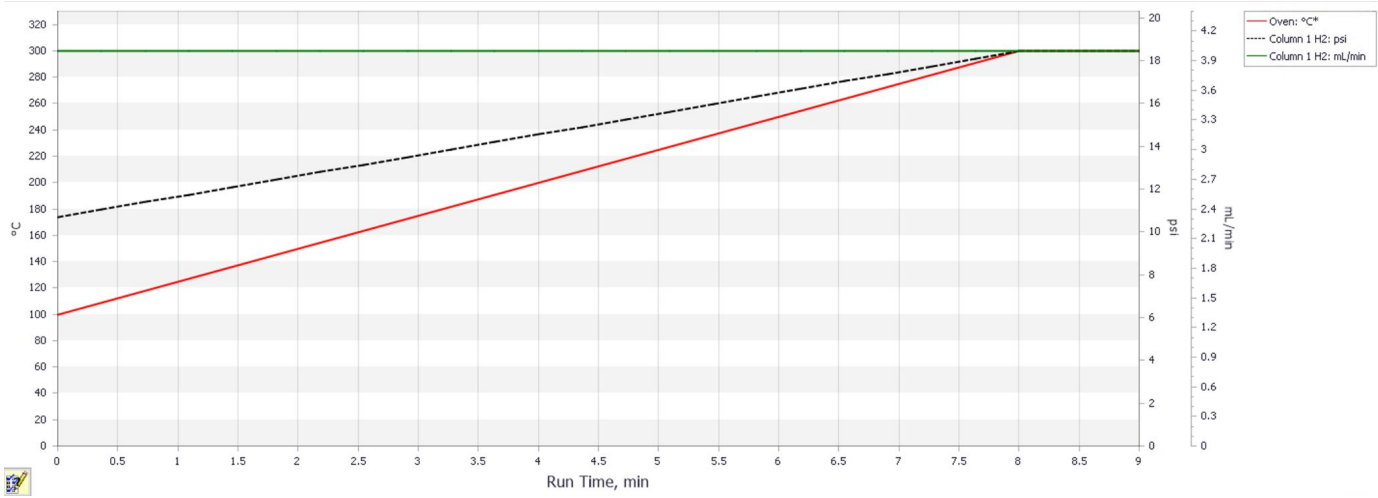
My experiments were analyzed using an internal standard (IS). This ensures that if any human error occurs with the sample, the concentration and area under peak (signal) of the IS will be proportionate to that of the starting material (SM). Five solutions were made and three samples of each solution were tested. Samples were weighed using an analytical balance and recorded as the below data. EtOAc was added last and 10µL of each was dispensed into ~1mL of EtOAc. Samples were stored in a freezer before they were used.

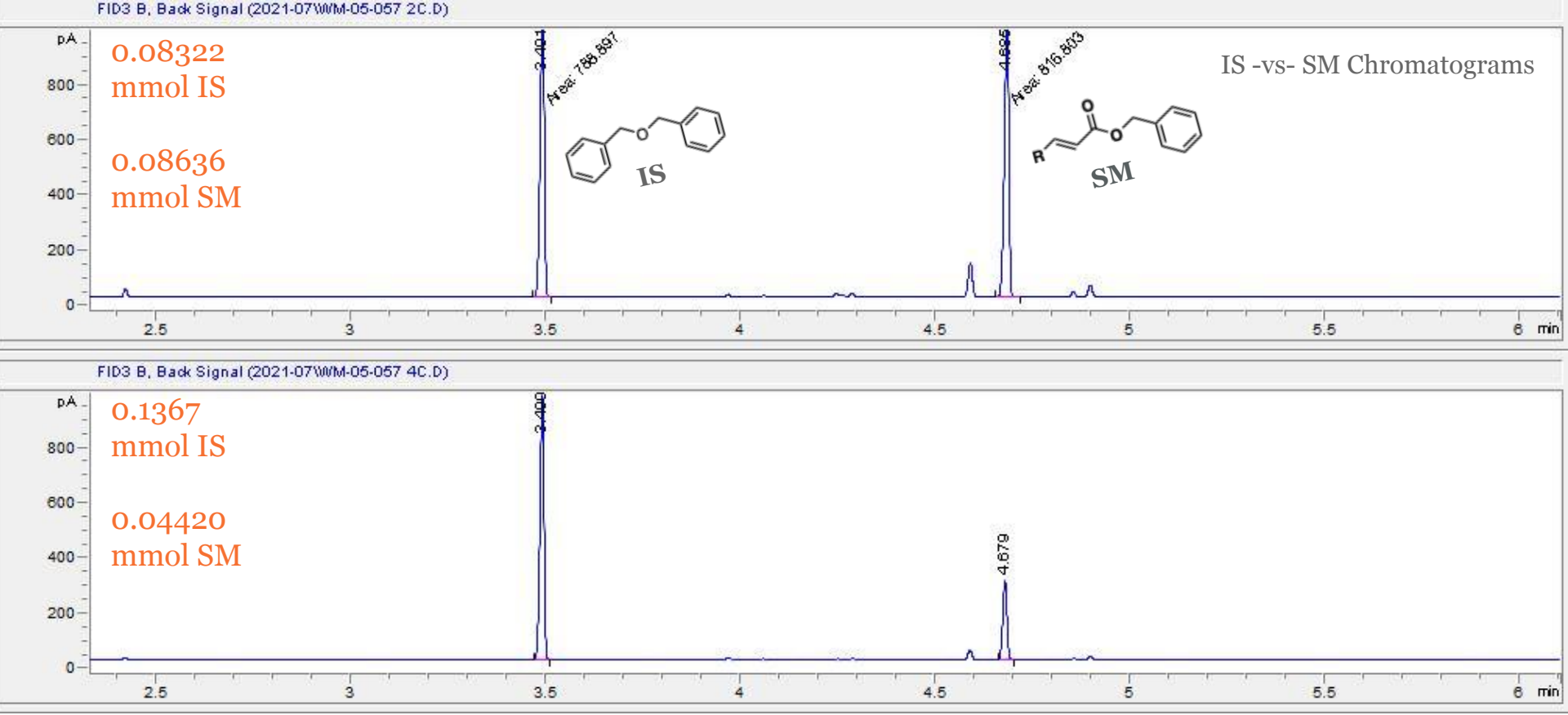
Sample Name:	1	2	3	4	5
Mass SM (mg)	35.8	21.1	22	10.8	7.4
Mass Bn_2O (mg)	8.6	16.5	26.5	27.1	42.9
EtOAc Added (mL)	0.25	0.25	0.5	0.5	1

Method

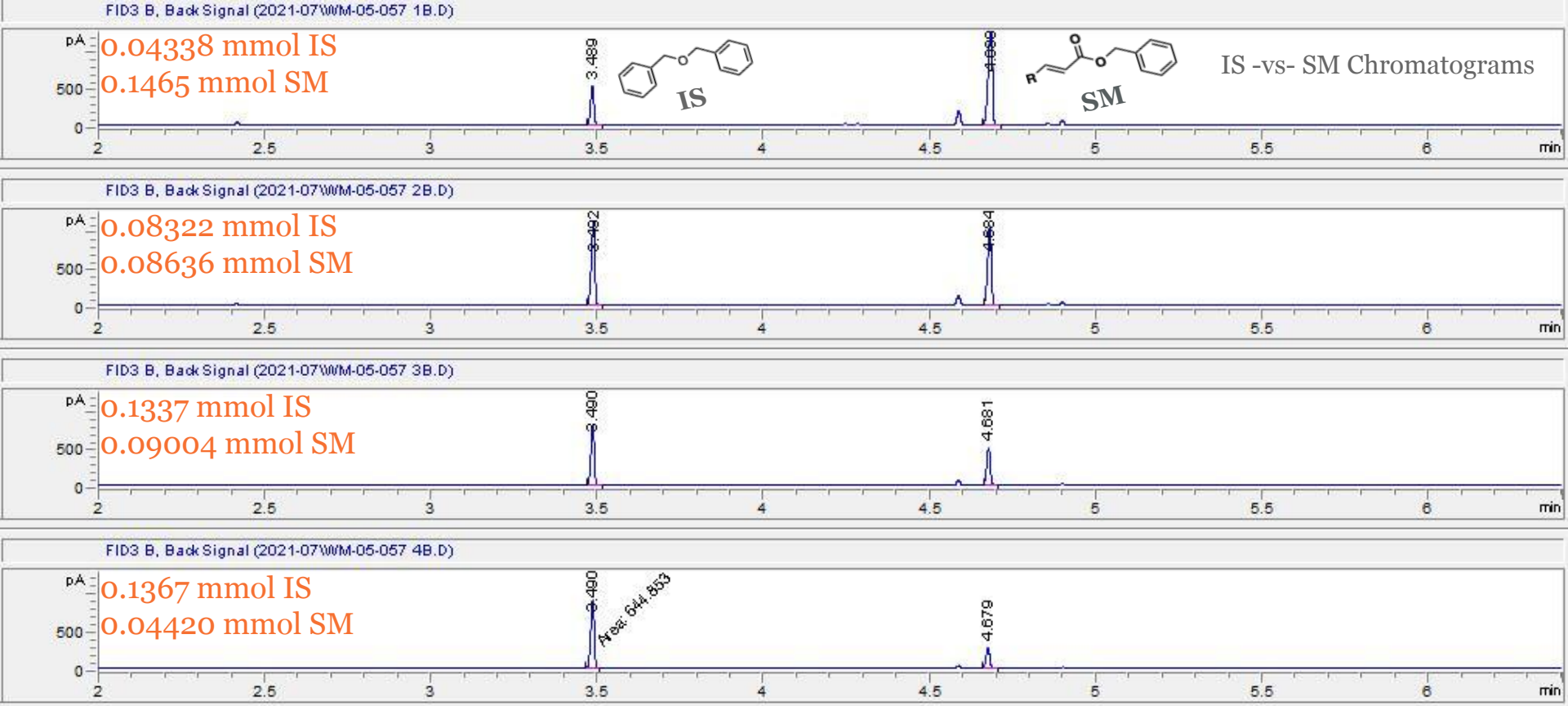
- 1µL liquid samples
- Back inlet
- Silica column coating
- Hydrogen and dry air
- ~175-300 psi
- 100-300℃
- FID

GC Method





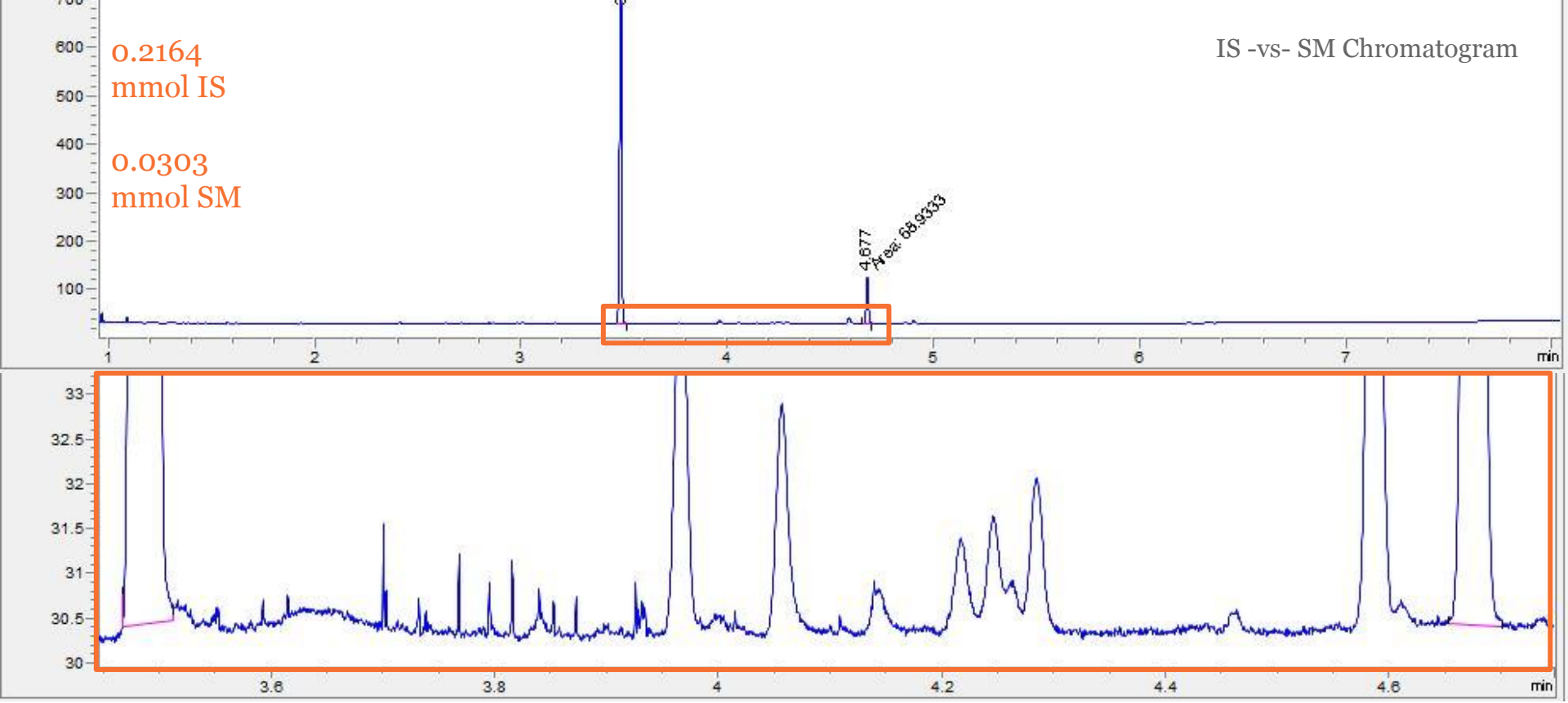
This is a comparison between trial C for solutions 2 and 4. You can see that the timestamps of both compounds is the same, but the area under the peaks vary. Solution 2 has a higher concentration of SM than solution 4.



Here is a comparison of the B trials of sample solutions 1-4. Again, you see that each compound appears at the same time, though the area under the peaks vary depending on concentration of each compound.

My Approach

The simple solutions were placed in the Autosampler for me and I connected to the GC computer through the VPN so that I could run my own samples. When the computer program receives the data, the system automatically detects the peaks and lists the information in the table at the bottom, so once my data was collected, I used ChemStation to remove irrelevant peaks and sometimes adjusted the integration of the detected peaks so that the area was most accurate for my RF calculations. This makes the data clearer and easier to analyze. I also had to watch the baseline and ensure that the signal to noise ratio was acceptable. Since my samples weren't complex and all particles were gaseous, I didn't have to run blanks between trials (clears residue from columns ensuring data is clear and accurate).



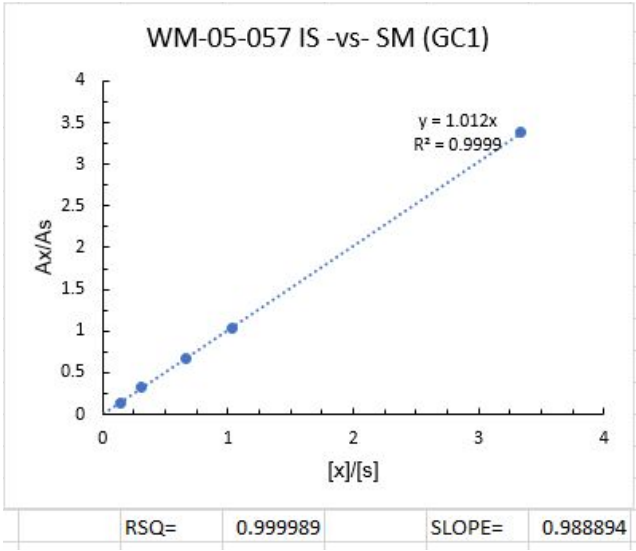
Both images are of trial 5B (also on slide 11), the second photo has been zoomed in on the baseline. The peaks underlined in pink are the signals of the IS and SM, and everything else is the noise. I also manipulated the start and end points of the area for this experiment.

Results & Conclusion

The samples I used and data I collected resulted in me achieving an R^2 value of 0.9999 and a RF of 0.9889. I also calculated the average single point RF in the three trails for solution 1 (0.98791), 2 (0.99416), 3 (0.98544), 4 (0.97008), and 5 (0.97338).

	IS -vs- SM			
	SM mmol	IS mmol	A SM	A IS
1	0.146523	0.043375	1182.933	354.4667
2	0.086359	0.08322	786.6667	762.5
3	0.090042	0.133656	394.6	594.3667
4	0.044203	0.136682	200.4	638.7
5	0.030287	0.216372	72.66667	533.3

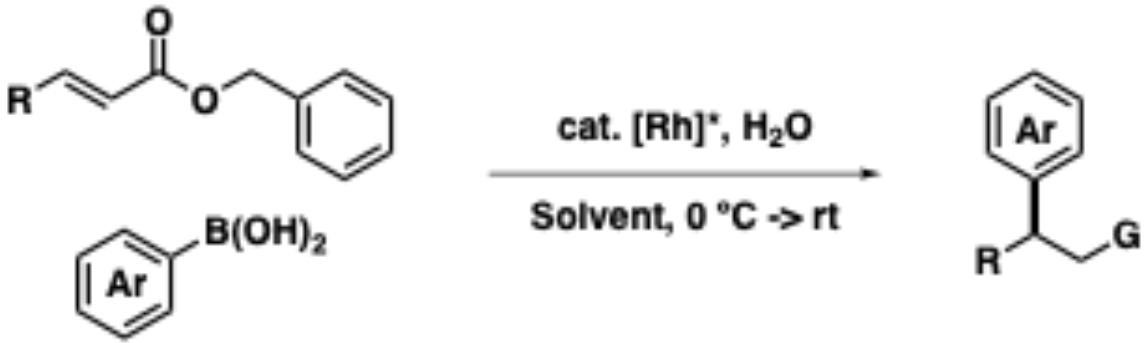
	IS -vs- SM	
	[x]/[s]	Ax/As
1	3.37804	3.337209
2	1.037717	1.031659
3	0.673685	0.663873
4	0.323396	0.31372
5	0.139976	0.136251



	RSQ=	0.999989	SLOPE=	0.988894

Application of my work to the Lab Group

- I created an instructional document for developing RFs that will be useful to future SRP students
- I helped with reaction monitoring during optimization of GC experiments when trying to move away from Nuclear Magnetic Resonance (NMR) testing



Application of Lab Group's work to Science

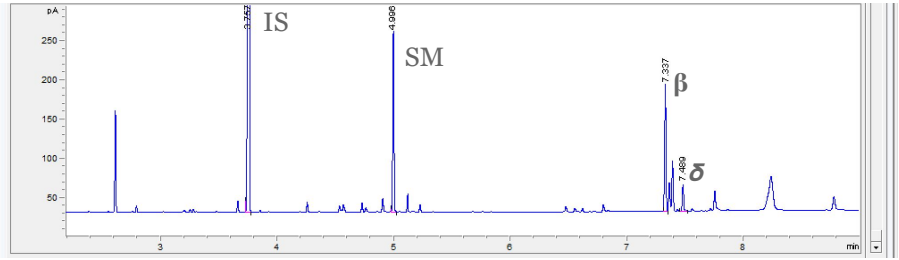
The Lundgren lab focuses primarily on methodology development. This means that they work to improve or invent organic chemistry reactions, generally for the preparation of small molecules. Two examples of why their work is important are:

- ➔ Advancing the field (with reference to understanding) of chemistry.
- ➔ In the synthesis/preparation of valuable molecules (either a new molecule or improving a previous method), new reactions need to be invented.

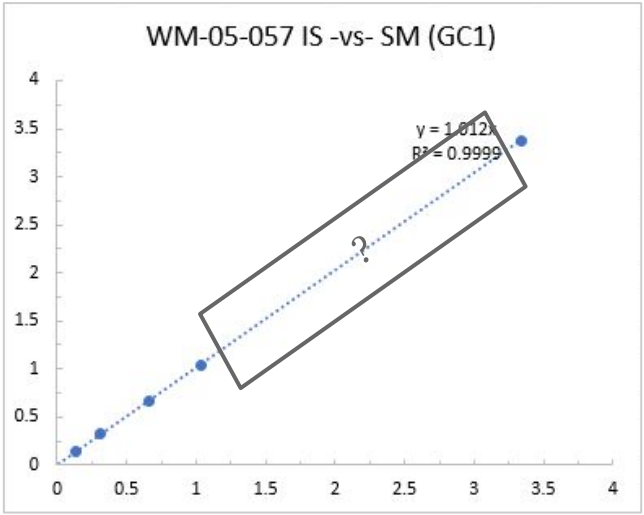
McNutt, W. Faculty of Science - Chemistry, University of Alberta. Personal communication August 2021.

Recommendations for Future Work

- Retest the same concentrations of SM to ensure accuracy and estimate method error.
- Run concentrations between points 4 and 5 to get an even more accurate RF (image A).
- Do GC testing for main and side products (image B).



B



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Acknowledgements

I would like to extend a huge thank you to: WISEST for accepting me into the program and putting on incredible PD sessions, to my sponsors for making my placement in the program possible, to the Lundgren lab group for having me in their lab this summer, and especially my supervisor for answering all of my questions and making sure I got to do exciting things despite the virtual setting!