Comparison of the ALCOHOL ("Ethanol") Content of Different Mouthwash products by "Gas-Less" Gas Chromatography

INTRODUCTION:

The World of Analytical CHROMATOGRAPHY involves the ***Separation*** of the individual components in a Sample, THEN the measurement of their optical / spectral / physical or chemical properties. In the realm of Organic Chemicals, there are VOLATILE Compounds (low boiling points, easily evaporated, like most Solvents) and NON-VOLATILE Compounds (high BP / MP, usually solid materials, like many drug & plastic chemicals). If materials can be VOLATILIZED, or turned into a GAS; then they can be analyzed by GAS CHROMATOGRAPHY ("GC"), otherwise the next logical technique is LIQUID CHROMATOGRAPHY.

To perform a GC analysis, a combination of Carrier Gas Flow (compressed Air) and Temperature help to "push" a "blob" of an injected Sample along a Packed Column (a metal tube filled with special "sand" packing material) where the individual compounds present in the Sample are separated in time based on the properties of Boiling Point temperature (goes from Liquid to Gas), Vapor Pressure (makes a Gas with a little or lot of "pressure" based on its evaporation) and molecular Polarity (Non-Polar, like Hexane or Polar, like Acetic Acid). The separated "fractions" of the Sample pass by a Detector, where they are detected by some physical or chemical property. The "position" of the Peak in time is called the Retention Time, and is the characteristic used to IDENTIFY the compound (What is it?). The "size" or integration of the Peak's Area is used to prepare a Calibration Curve for QUANTITATIVE analysis of the material (How much?).

PURPOSE:

Using the AIR-GC system optimized for the separation of Alcohols, and a 3-part Standard of Methanol (C1, BP ~65^{o}C), Ethanol (C2, BP ~79^{o}C) and 2-propanol or isopropyl alcohol (C3, branched, BP ~82^{o}C), 25% each in Water; we can IDENTIFY (based on RETENTION TIME) the specific ALCOHOLS and their CONCENTRATION (based on PEAK AREA) present in the STANDARD (known material). Using this information, the common consumer materials (ie: Mouthwash, etc) can be "chromatogrammed" to give us BOTH the Qualitative information on the SEPARATED components present in the Sample, and the Quantitative concentration of those materials. The Test Sample include different varieties of Mouthwash containing "Ethanol".

INSTRUMENT:

Model 310 Air-GC system w/ CCD Detector and internal Air Pump (for CARRIER Gas)
Computer w/ Peak-Simple Software
10 uL Syringe

SUPPLIES:

Mixed Methanol / Ethanol / 2-Propanol (~25% each, for QUALITATIVE Identification)
10% Ethanol in Water & 50% Ethanol in Water (Calibration Standard for QUANTITATIVE Analysis)
-=> *Available with the FUN-SCI™ Experiment Kits*

SAMPLES:

Vials of Test Samples = Mouthwash (Ethanol), various brands of mouthwash available

EXTRA CREDIT = Have students bring is other "OTC" materials; like Witch Hazel, or even Alcoholic Beverages (with parental permission); such as a liquor, whisky, rum, vodka, etc.; to check the relative amounts of Ethyl Alcohol present and calculate the "proof" of the beverage.

SAFETY!:

Be sure to wear Safety Glasses. Most alcohols are irritating AND flammable! Wipe up spills with paper towels immediately. Please observe basic Good Laboratory Practices when working with Solvents and in performing ANY Lab experiment!

PROCEDURES:

SEPARATING the ALCOHOL Peaks ("Qualitative") =

[1] Make sure the 310-GC is ON and the PEAK-SIMPLE program has been loaded from Windows.
The ALC-1.CON Control File is loaded… which sets all the correct instrument parameters, and the OVEN Temp is stabilized at 125°C to 165°C for ISOTHERMAL runs (varies from GC to GC).
-=> ***For this experiment, the TEMPERATURE, INTEGRATION, COMPONENT & CALIBRATION files have been pre-set on each of the GC systems. Do NOT edit or delete… otherwise the Separation and Quantitative measurement may not work!***

[2] To check the RETENTION TIMES (quality of the Separation) for the 3-part Alcohol Standard, carefully fill the Syringe with **1.0uL** of the Mixed Alcohol Standard, quickly wipe the tip of the needle off with dry fingers, gently insert the needle into the Septum Nut and twirl as you insert it all the way up to the syringe barrel. Hold the Plunger so the Air pressure from the pump does not blow the syringe out at you!

[3] Simultaneously, push the Plunger completely into the Syringe Body AND press the Spacebar on the Computer to begin the data collection. Try to be within a second or two of the injection, since this is what defines the Retention Time used to identify the Peaks. ***REMOVE*** the Syringe at 0.15 minutes on the timer!

[4] Observe the screen as the THREE (3) Peaks come off the Column. They should line up with the light Blue **"retention window"** bars seen in the middle of the screen; the first one being Methanol, the second is Ethanol and the last is 2-propanol. **This is just to confirm the CORRECT times for these components!**
-=> ***NOTE: If anything does NOT fall within these "windows", contact the Workshop Instructor!***

CALIBRATING the GC ("Quantitative") =

[5] Make sure the 310-GC is ON and the PEAK-SIMPLE program has been loaded from Windows.
Make sure the Temperature file, Events file and Post-Run file are set w/ "ALC-1" file names, and the Oven Temperature should be stabilized at 125°C to 165°C for ISOTHERMAL running (varies from GC to GC).

[6] To check the STANDARD Calibration, carefully fill the Syringe with **1.0uL** of the 10% ETHANOL STANDARD, quickly wipe the tip of the needle off with dry fingers, gently insert the needle into the Septum Nut and twirl as you insert it all the way up to the syringe barrel. Hold the Plunger so the Air pressure from the pump does not blow the syringe out at you!

[7] Simultaneously, push the Plunger completely into the Syringe Body AND press the Spacebar on the Computer to begin the data collection. Try to be within a second or two of the injection, since this is what defines the Retention Time used to identify the Peaks. ***REMOVE*** the Syringe at 0.15 minutes on the timer!

[8] Observe the screen as the ETHANOL Peak comes off the Column… and it should line up with the light Blue "retention window" bar for Ethanol seen in the middle of the screen.

[9] When the chromatogram is finished and the screen re-sets with little Red circles over the ETHANOL Peak; click on "FILE" from the top toolbar, select "PRINT" and "PRINT" again to get a hard-copy with the Integrated AREA for that Peak.

[10] Repeat with the 50% ETHANOL STANDARD using a 1uL injection, and print out the result as well.

EVALUATING SAMPLES =

[11] After the TWO (2) Standards have been run and printed, fill the syringe with **1.0uL** of one of the Mouthwash Samples and make the injection as noted above, trying to keep the injection time and the start time as close as possible.

[12] Let the chromatogram run until it is finished, and the screen re-sets with little Red circles over the ETHANOL Peak; click on "FILE" from the top toolbar, select "PRINT" and "PRINT" again to get a hard-copy

with the Integrated AREA for that Peak.
[13] Fill in the enclosed Results chart to establish a Calibration Curve to evaluate the SAMPLE.

RESULTS: [fill in the following Results Chart below with the exact Peak AREA Integration (A_i) values & Retention TIME (R_t) data generated above]

Based on the principles of Chromatographic Separation technology over the past 50+ years, any components that have the SAME Retention Times under the SAME Analytical Conditions (Column, Temperature, Gas Flow) ***ARE the SAME!*** Make careful note of the Retention Times of the STANDARD and the Samples to make an accurate QUALITATIVE analysis, and make careful note of the Integrated Peak AREA for precise QUANTITATIVE data.

MATERIAL	***Methanol***	***Ethanol***	***i-Propanol***
Mixed STANDARD Peaks (QUAL Info)	R_t_____	_____	_____
	A_i____X____	____X____	____X____
10% Ethanol STD Peak (QUANT Info)	R_t____X____	_____	____X____
	A_i____X____	_____	____X____
50% Ethanol STD Peak (QUANT Info)	R_t____X____	_____	____X____
	A_i____X____	_____	____X____
Mouthwash #1 SAMPLE	**R_t____X____**	_____	**____X____**
ID:_____	**A_i____X____**	_____	**____X____**
Mouthwash #2 SAMPLE	**R_t____X____**	_____	**____X____**
ID:_____	**A_i____X____**	_____	**____X____**
Mouthwash #3 SAMPLE	**R_t____X____**	_____	**____X____**
ID:_____	**A_i____X____**	_____	**____X____**
Mouthwash #4 SAMPLE	**R_t____X____**	_____	**____X____**
ID:_____	**A_i____X____**	_____	**____X____**

CALCULATIONS:

Most GC Software has the ability to generate a CONCENTRATION value based on the internal Calibration Curve, but for this Workshop, we will perform our OWN calculations to verify the DATA for the Samples. We will use a simple ratio of the Integrated Peak Area ($\mathbf{A_i}$) for each component and the pure Standard to get the Concentration of Alcohols in the Samples (SMP).

EXAMPLE = The ETHANOL Standard at 50% has an $\mathbf{A_i}$ of 2000
The LAVORIS sample has an ETHANOL Peak with an $\mathbf{A_i}$ of 850
What is the Percent ETHANOL in this Sample?

[(50% EtOH in STD) * (850 $\mathbf{A_i}$ in SMP)] = [(2000 $\mathbf{A_i}$ in STD) * (X% EtOH in SMP)]
X = 11% EtOH present in LAVORIS Mouthwash Sample

-=> NOTE: For simplicity, use the STANDARD with the CLOSEST Peak Area ($\mathbf{A_i}$) to the SAMPLE.

-=> NOTE-2: For accuracy, prepare a LINEAR / 1^o or Quadratic / 2^o Calibration with a Calculator or Excel and INTEPOLATE the SAMPLE Are to the STANDARD Calibration Curve.

MATERIAL	***Methanol***	***Ethanol***	***i-Propanol***
Mouthwash #1 SAMPLE	**____X_____**	**_____%**	**_____X_____**
ID:_____			
Mouthwash #2 SAMPLE	**____X_____**	**_____%**	**_____X_____**
ID:_____			
Mouthwash #3 SAMPLE	**____X_____**	**_____%**	**_____X_____**
ID:_____			
Mouthwash #4 SAMPLE	**____X_____**	**_____%**	**_____X_____**
ID:_____			