EXPERIMENT 1:

GAS CHROMATOGRAPHY – MEASURING THE ALCOHOL CONTENT OF WINE

AIM:

Aim of this experiment is to determine the concentration of wine by gas chromatography using the quantification techniques, calibration and internal standard methods and also to determine the chromatographic elution parameters such as resolution and capacity factor.

OUTLINE OF METHOD:

Calibration method: Standard solutions of ethanol in water with concentrations of 5, 10, 15 and 20% (v/v) ethanol were prepared. Measurements for peak area were obtained by injecting standards and wine samples to the Gas-Liquid chromatography (GLC) instrument using a syringe.

Internal Standard method: Measurements for peak area of15%ethanol-15%acetone sample and the wine sample that was prepared by adding acetone to make it up to 15% (v/v) concentration was obtained using GLC instrument. Finally, a calibration curve of peak area against concentration of standards was plotted. Using the regression equation concentration of wine was determined.

SUMMARY OF RESULTS:

Peak elution data

Table 1: Peak elution data for tm, t_R, w and calculated values for capacity factor (k') and resolution (R)

t_M = (1.10 + 0.94 + 1.02)/3 = 1.02 mins		
t_R (ethanol) = (1.67 + 1.61 + 1.46)/3 = 1.58 mins	t'_R (ethanol) = (1.58 – 1.02) = 0.56 mins	k' (ethanol) = 0.55
t_R (acetone) = (1.31 + 1.23 + 1.15)/3 = 1.23 mins	t'_R (acetone) = (1.23 – 1.02) = 0.21 mins	k' (acetone) = 0.21
w (ethanol) = (0.20 + 0.35 + 0.32)/ 3 = 0.29 mins	w (acetone) = (0.20 + 0.35 + 0.28)/ 3 = 0.28 mins	R = 2[(1.58 – 1.23)/ (0.29+0.28)] = 1.23

Calibration data

Table 2: Measurements for peak area obtained from GLC instrument and calculated mean area, standard deviation, %RSD for the corresponding standard concentrations

Ethanol concentration %(v/v)	Peak area 1	Peak area 2	Peak area 3	Mean area	Standard deviation	% RSD
10	4.207×10^6	4.394×10^6	3.484×10^6	4.028×10^6	480589.568	11.931%
15	5.697×10^6	5.423×10^6	4.899×10^6	5.340×10^6	405474.208	7.593%
20	5.304×10^6	6.130×10^6	5.89×10^6	5.775×10^6	424906.264	7.358%

Calibration Graph

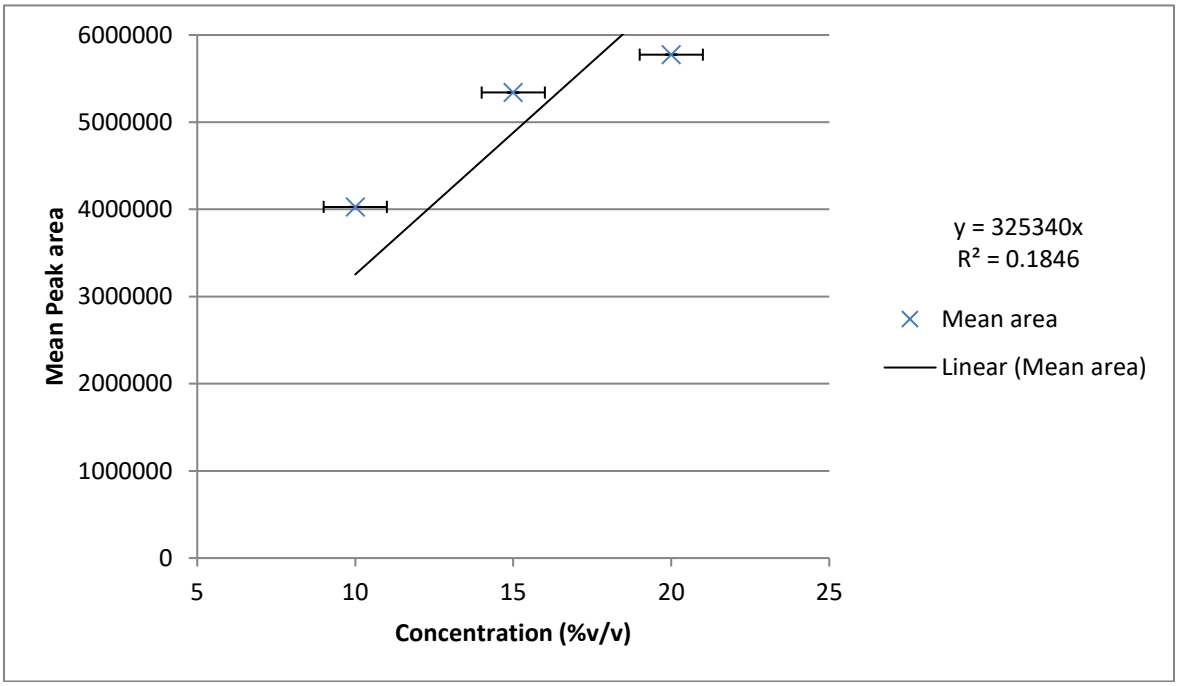

Figure 1: Standard curve of mean peak area against concentration of standard solutions in the range 10 - 15% (v/v)

Calibration method results

Table 3: Calculated ethanol concentration values, standard deviation and %RSD for the ethanol samples

Sample run number	Peak area (Ethanol)	Calculated Ethanol Concentration - %(v/v)
1	3.696×10^6	11.360%
2	4.200×10^6	12.909%
3	4.062×10^6	12.485%
	Average concentration - %(v/v)	12.250%
	Standard deviation	0.802
	Relative standard deviation (%)	6.547%

Internal standard method results

Table 4: Measured peak areas of Acetone and ethanol present in standard solutions and wine samples using the GLC instrument

Internal standard number	Peak area (Acetone) **A**	Peak area (Ethanol) **B**	
1	6.675×10^6	5.832×10^6	
2	6.468×10^6	5.583×10^6	
3	6.237×10^6	5.654×10^6	
Average area	6.460×10^6	5.689×10^6	
Standard Deviation	219109.562	128274.445	

Spiked wine sample	Peak area (Acetone) **C**	Peak area (Ethanol) **D**	Calculated Ethanol Concentration
1	5.590×10^6	3.696×10^6	11.351%
2	5.839×10^6	4.200×10^6	12.499%
3	5.964×10^6	4.062×10^6	11.269%
		Average concentration (% v/v)	11.706%
		Standard deviation	0.688
		Dilution factor	0.85
		Ethanol concentration in wine - corrected for dilution (% v/v)	9.950%
		Relative standard deviation (%)	5.877%

CALCULATIONS

t'_R (ethanol):

t'_R (ethanol) = t_R (ethanol) – t_M

t'_R (ethanol) = (1.58 – 1.02) = <u>0.56</u> mins

Capacity factor (k'):

$k' = t'_R / t_M$

Hence, the k' for ethanol = (1.58 – 1.02)/ 1.02 = <u>0.55</u>

** These calculations were applied to acetone*

Resolution:

Using equation:

$R = \frac{2\,(tRb - tRa)}{Wa + Wb}$

Therefore, R = 2[(1.58 – 1.23)/ (0.29+0.28)] = <u>1.23</u>

Mean Peak area for 10% concentration

= [(4.207x10^6) + (4.394x10^6) + (3.484x10^6)]/3 = <u>4.028x10^6</u>

Standard deviation for 10% concentration**:**

10% concentration:

$$= \sqrt{\frac{\Sigma X^2 - (\Sigma X - Mean)^2}{N-1}}$$

$$= \sqrt{\frac{[(4207000-4028333)^2 + (5697000-4028333)^2 + (5304000-4028333)^2]}{3-1}}$$

<u>=480589.568</u>

% Relative Standard Deviation:

RSD for 10% concentration = $\frac{\text{standard deviation (10\%)}}{\text{mean peak area (10\%)}} \times 100\%$ = (480589.568/4.028x10^6)x100%

= 11.931%

** These calculation was applied to other standards and wine sample.*

Concentration of ethanol in wine sample:

From the equation in Figure 1, y = 325340x where y = peak area and *x* = concentration [%(v/v)],

Concentration of ethanol of pure wine:

Peak area = 325340xconcentration

In sample run number 1, peak area = 3.696x10^6

Concentration = (3.696x10^6)/ 325340 = <u>11.360%</u>

The average concentration of the ethanol = $\frac{11.360+12.909+12.485}{3}$ = <u>12.250% (v/v)</u>

Mean peak area (acetone internal standard solution):

= $(6.675 \times 10^6) + (6.468 \times 10^6) + (6.237 \times 10^6)$

= 6.460×10^6

** This calculation was applied to ethanol (internal standard) and spiked wine sample.*

Concentration of ethanol in the mixture of wine/acetone:

Using the equation,

$$\frac{(\text{peak ethanol in sample/acetone peak in sample})}{(\text{peak of ethanol in standard/peak of acetone in standard})} = \frac{Sample\ concentration}{15}$$

$[(3.696 \times 10^6)/ (5.590 \times 10^6)] / [(5.832 \times 10^6)/ (6.675 \times 10^6)]$ = Sample concentration / 15

Ethanol concentration = 11.351% (v/v)

** This calculation was applied to other calculated ethanol concentration.*

Dilution Factor:

8.5 mL of wine was added into the 10 mL volumetric flask,

Dilution factor = 8.5/10 = 0.85

Ethanol concentration in wine- corrected for dilution:

Mean × dilution factor = 11.351× 0.85

= 9.950% (% v/v)

DISCUSSION AND CONCLUSIONS:

According to the results tabulated in table 1, adjusted retention times of acetone and ethanol were calculated to be 0.21 and 0.56 minutes respectively. Retention time is the time solvent takes to travel through the solvent. As acetone has a shorter retention time, it is visible that acetone will be eluted first and ethanol will be eluted last. The stationery phase used is BP20 which is a very polar phase. According to the theory 'like dissolves like', ethanol which is more polar than acetone binds to the stationery phase, whereas acetone which is less polar is eluted. Retention time of acetone is smaller as it is detected first before ethanol and it showed a maximum peak height for acetone. Retention time varies according to the compound analysed. It may also vary depending on composition of solvent, temperature, particle size of stationery phase and polarity of the solvent and stationery phase both (Sarafraz-Yazdi *et al*. 2011).

Capacity factor (k') of ethanol and acetone were found to be 0.55 and 0.21. The separation of peaks depends on the interaction of the components with the stationary phase is calculated using capacity factor and ideally k' should be within the range 2-5 whereas in this experiment it is less than 2 (out of the range) (Sarafraz-Yazdi *et al*. 2011). This indicates that there was no or little separation from injection peak. Resolution which measures the degree of separation should ideally be more than or equal to 1.5. From results obtained, R is 1.23 which is less than 1.5 suggesting poor separation of peaks.

Precision is the closeness of agreement between replicated measurements or results obtained under the same prescribed conditions. The results obtained in this experiment can be considered highly precise as it has a low percentage of relative standard deviation (RSD) and small values for standard deviation. The %RSD of 10, 15, 20% standard solutions were 11.931%, 7.593% and 7.358% respectively. %RSD of ethanol obtained from calibration method results is 6.547% whereas that obtained from internal standard method is 5.877%. As internal standard method %RSD value is smaller than that of calibration method, internal standard method values can be considered more precise but both the values are still within the theoretical value of 10%. It is also considered accurate as the %RSD of all values except that of 10% standard solution falls within the threshold value of 10%. The %RSD value of 11.931% could do higher due to random errors like sample mass transferred might be slightly lower than the actual 1 or the dilution is made might slightly over the line will affect the final results (Huang *et al*. 2002). Overall, the results obtained by this experiment are highly precise and reliable.

Accuracy is the closeness of an experimental measurement or result to the true or accepted value. The more accurate data is, higher the reliability of the data or information. In order to determine how accurate the data is, its percentage error should be calculated (Huang *et al*. 2002). As the concentration of the Standard Reference Material is already known, it can be used to calculate the Percentage Error of the measurements.

% Relative Error = $\left[\frac{\bar{x}-Xt}{Xt}\right] \times 100\%$

$\bar{X}$= Measured value of ethanol concentration = 11.706%

X_t = Known value of interntal standard of ethanol concentration in wine = 12.5

% Relative Error = [(12.5-11.706)/12.5] x100% = 6.352%

The Percentage Error of 6.352% is considered as a low percentage of error as it falls within the minimal acceptable baseline of error (threshold value) which is 10%. Hence the data obtained by carrying out this experiment can be considered accurate.

Calibration technique involves preparation of a calibration graph using the measurements of standard method to determine unknown concentrations of sample. This method is prone to error as injection technique is not reproducible and fluctuation in flow rate may occur as well.

In internal standard method a compound of known concentration is added to sample and standards both to be used as a reference. Substance added should be of similar composition to analyte and elute around the same time. This addition of internal standard method to both samples and standards compensates for instrumental errors like temperature variations, fluctuations in temperature, carrier flow rate and injection technique(Vandenheuvel & Horning 1962).

Deviations in results could be due to random errors like pipetting the wrong amount of solution, using the same pipette to transfer two solutions and weighing out wrong volumes of wine initially. Another error could be vaporization of wine.

Though internal standard method is more precise, in labs calibration technique is much easier to be carried out.

R^2 value of the calibration graph is less than 1 indicating a poor correlation between peak area and concentration.

Conclusion

Concentration of ethanol in wine samples using calibration graph and internal standard method was found to be 12.250% and 11.706% respectively.

QUESTIONS:

1 What effect would increasing the oven temperature have had on the retention time of ethanol and acetone?

Retention time is the amount of time elapsed from the injection of a sample into the chromatographic system to the recording of the peak (band) maximum of the component in the chromatogram. Oven temperature is a factor that affects retention time of the solvent. With increased temperature, rate of progression through column is faster whereas slower with lower temperatures resulting in shorter or longer temperatures respectively. Acetone is more volatile than ethanol as alcohols like ethanol have strong hydrogen bonding between molecules, requiring more energy to break the bonds thereby making it harder to vaporize whereas acetone has only weak van der Waal forces making acetone to be vaporized easily (Vandenheuvel & Horning 1962). Hence, increasing temperature would have increased the velocity of molecules thereby increasing rate of progression through the column and decreasing the retention time of acetone and ethanol. Other factors that affect retention time are type of compound, Column (dimension or stationery phase), flow rate, column pressure, temperature, carrier gas and column age. As ethanol is eluted last (retention time = 0.56minutes) and acetone is eluted first (retention time = 0.21 minutes), increasing the temperature allows acetone and ethanol both to be eluted much faster.

2 Suggest why a column with a BP20 stationary phase was specified for this analysis?

BP20 stationery phase is a Polysiloxane phase in which the bonded phase is made of polyethylene glycol and is used at a temperature between 20 - 260°C. The polyethylene glycol phase shows unique selectivity hydrogen bonding type molecules and is useful in the analysis of complex oxygenated samples but is at risk for oxygen degradation. It is not suitable for analysing mixtures that contain silylating reagents. As it is a very polar phase it is appropriate for analysis of aldehydes, alcohols (example: ethanol) and ketones (example: acetone). It also allows efficient separation of aromatic isomers like Xylene isomers and are cross- linked for stability and washing. As the stationery phase is more polar and ethanol is more polar than acetone, ethanol would remain bonded to stationery phase whereas acetone would be eluted suggesting acetone to have a shorter retention time than ethanol (Sarafraz-Yazdi *et al*. 2011). When adjusted retention times considered, this phenomenon can be observed. Adjusted retention time of ethanol is 0.56 minutes whereas that of acetone of 0.21 minutes depicting ethanol will be eluted last as expected.

3 Are the acetone and ethanol peaks sufficiently resolved to enable the proper use of the *internal standard* method?

Resolution is a measurement used to quantify peak spacing in a liquid chromatography (LC) separation. R should be greater or equal to 1 for baseline resolution of perfectly triangular peaks but as the peaks are more towards Gaussian in shape, Resolutions should be approximately equal to or greater than 1.5 (it corresponds to a <2% overlap). Resolution of the results obtained is 1.23 which is less than 1.5 indicating that the peaks are not resolved. Equation for measuring resolution is given below where t1 and t2 are retention times of peaks of interest and w1 and w2 is the peak widths measured at the baseline between tangents drawn to the peak sides (Hurrell & Perry 1962)

$$R = 2(t2 - t1) / (w1 + w2)$$

As it is better, little extra baseline between peaks to tolerate some deterioration in the separation, mostly a value of 1.75 to 2.0 is selected as the minimum acceptable resolution. When resolution is too small, tailing effect occurs which does not allow peaks to be symmetrical. This could be due to overloading of column and poor solvent selection. Similarly, when the peaks are too broad, broadening effect occurs. In this case, resolution is less than 1.5 which means tailing effect occurs suggesting acetone and ethanol peaks are not sufficiently resolved to enable the proper use of the *internal standard* method (Hurrell & Perry 1962).

4 There have been cases where wine has been mistakenly adulterated with ethylene glycol (1,2-ethanediol, bp_{760} 197.6 ˚C). As a forensic chemist, suggest how would you use GLC to prove conclusively that ethylene glycol was actually present?

Standards with a solvent with a polarity similar to ethylene glycol should be prepared and the retention time of wine samples should be obtained from the GLC instrument. The stationery phase should be changed to a polarity less than that of ethylene glycol so that it would be eluted first. Polyethylene glycol stationery phase works better between 20 - 260°C hence, the oven temperature should be adjusted to match the boiling point of ethylene glycol (197.6°C) (Huang *et al*. 2002).

REFERENCES:

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