SOURCES OF UNCERTAINTY IN GAS CHROMATOGRAPHY

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ABSTRACT

One of the best analytical technique is Gas chromatography (GC) which suitable to gas, liquid, and solid samples (components must be volatile). When a mixed solution sample is injected into the GC system which consist of carrier gas (mobile phase) which carries the mixture through the stationary phase from the injection port through the column (where the sample is separated into individual components) to the detector resulting a chromatogram which demonstrating the retention time of each component ant the amount of that component. Measuring a number of readings and determining the mean would increase the amount of information (The extra results you use, the closer value to the true mean you get) regularly between 4 and 10 repeats is sufficient to best estimation of the mean and Standard deviation. There are several factors might cause uncertainty for example measuring instrument, the measured sample, Operator skills, Sampling issues or the environment which summarized in Ishikawa diagram. Uncertainty is estimated by two ways: Type A evaluation which estimate uncertainty statistically which can be reduced and type B evaluation which estimate uncertainty from professional judgment. In order to overcome the errors which might occur, all sources of uncertainty must be identified and the range of the uncertainty must be estimated from each source. After that, ultimately combined individual uncertainties to get an overall uncertainty.

Keywords: gas chromatography, mean value, standard deviation, uncertainty.

1. INTRODUCTION

In 1958, the first report had been published about analysing the carbohydrates by using gas chromatography technique while the application of tri-methyl silylation method to carbohydrates was in 1963 by Sweeley and co-workers which proposed the rocky development in this field. Gas Chromatography used to be the primary technique of carbohydrates analysis in foods till the mid1970s when HPLC get going to dominate. However, Gas Chromatography still provides benefits for number of applications for carbohydrates to food technologists. [1, 2]

Most of carbohydrates like mono-saccharides are extremely challenging to be analyzed by GC because of decomposing inside the injector port and "break-down" on the column. The effectiveness of detecting carbohydrates is decreased due to in-volatility and high polarity of sugars. To rise above these problems, sugars must be converted to its derivatives by removing the active group as (OH), for that reason volatility is increased which improve delectability. Cylation reaction is one of The most widely used approaches of derivatizing sugars.[3,4] Gas chromatography compulsory the sample to be in vaporising state and injected onto the head of the chromatographic column. After that, the sample is transferred throughout the column by the flow of gas (mobile phase). The column contains (stationary phase) which is adsorbed on top of the surface of an inert solid. [5, 6]

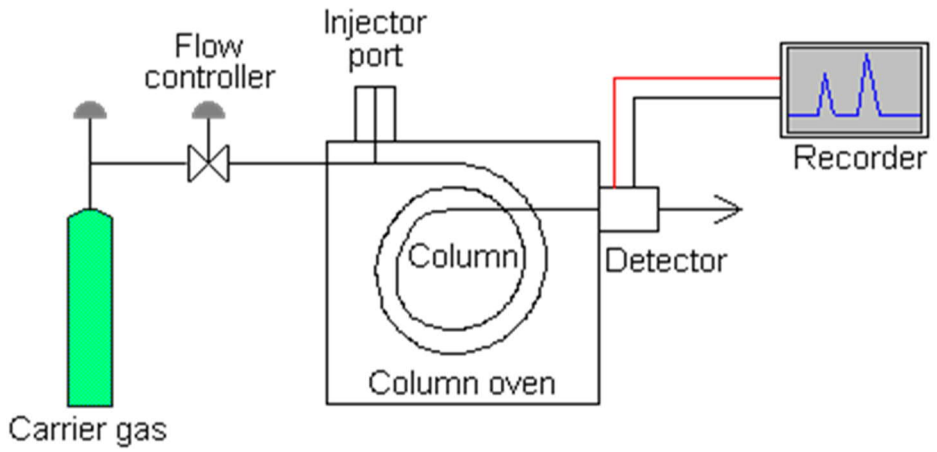

Figure 1 demonstrates the components of gas chromatograph

1.1 Instrumental components of GC

1- Carrier gas: Nitrogen, helium, argon, and carbon dioxide are the commonly used gases

2- Sample injection port: The injection port is a micro syringe which used to inject the sample during a rubber septum toward a flash vaporizer port which placed at the head of the column. The temperature of sample port is typically about 50 C higher than the boiling point of the lowest volatile component of the sample mixture.

3-Columns: There are two primary types of column: packed and capillary (also known as open tubular).

4- Detectors: Three main types of detector are used in GC: 1- A non-selective detector which responds to all compounds apart from the carrier gas. 2- A selective detector which responds to number of compounds with a regular physical or chemical property. 3- A specific detector which responds to a single chemical compound. [7, 8]

2 .MATERIAL AND METHODS

Methane Samples (a non-retained compound), C8, C9 andC10 hydrocarbons, o-xylene, m-Xylene, p-Xylene and an unknown mixture were provided and Methane sample was injected two times into the GC injector where capillary column with a flame ionisation detector have been used to determine the former retention time. After that, methanol solution which containing three hydrocarbons has been injected two times into the GC injector and retention times were determined.

Samples of the three xylene isomers: o-xylene, p-xylene and p-xylene (in methanol) were injected individually two times into the GC injector for determining their retention times. The unknown Sample of xylene has been injected two times into the GC injector in order to determine its retention times. [9, 10]

3. RESULT

TABLE I . RETENTION TIMES HAVE BEEN QUOTED TO TWO DECIMAL PLACES AND S.D TO THREE SIGNIFICANT FIGURES AS IT CAN BE SEEN IN TABLE IN.

	RT 1	RT 2	Average RT
Methane	1.7	1.67	1.685

Compound	RT1	RT 2	Corrected RT1	Corrected RT 2	Log(CRT) 1	Log(CRT) 2	Average log(CRT)
C_8	2.93	2.91	1.245	1.225	0.0951	0.0881	0.0913
C_9	4.18	4.16	2.495	2.475	0.397	0.3935	0.395
C_{10}	6.66	6.63	4.975	4.945	0.696	0.694	0.695
o-xylene	4.2	4.25	2.515	2.565	0.4	0.409	N/A
m-xylene	3.85	3.85	2.165	1.2.165	0.335	0.335	N/A
p-xylene	3.84	3.84	2.155	2.155	0.333	0.333	N/A
Unknown	3.83	3.85	2.145	2.165	0.331	0.335	N/A

TABLE II BELOW ILLUSTRATES THE AVERAGE LOG (CRT) AND (CARBON NUMBER ×100) FOR THREE HYDROCARBON STANDARDS

Compound (carbon number x 100)	Average (corrected retention time)
C8 800	0.0913
C9 900	0.395
C10 1000	0.695

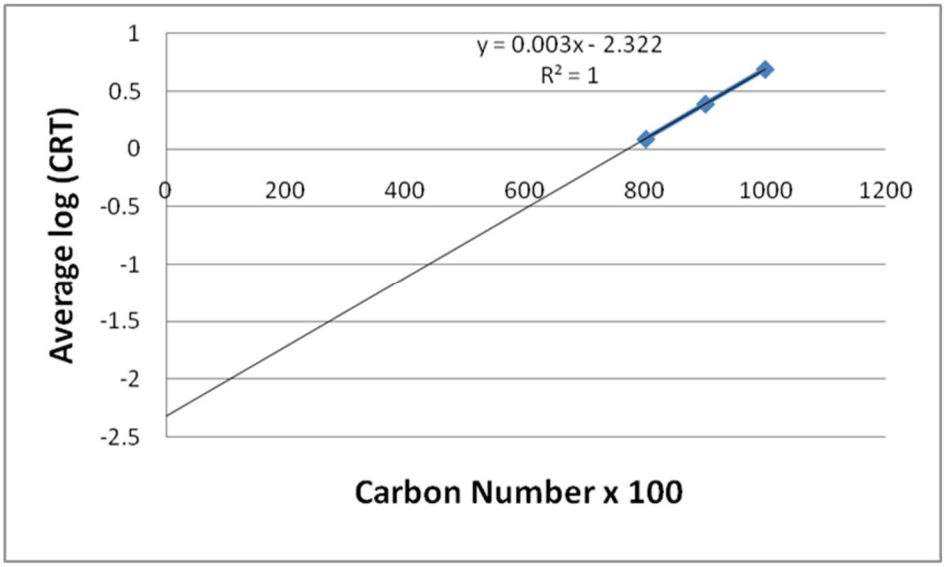

Figure 2. Demonstrates relationship between (average log corrected retention times) and (carbon number x 100)

4. CALCULATION AND DISCUSSION

From Figure 2. The retention index can be calculated by applying the log corrected time for the three isomers of xylene (o, m, and p) and the unknown sample. y = 0.003x - 2.322

Where Y= log (CRT) X = retention index

So,

Retention index for o-xylene:

A- o-xylene (Log [CRT1=0.400]): Y = 0.003x – 2.38

As a result, Retention Index (x)

B- o-xylene (Log [CRT2=0.409]): Y = 0.003x – 2.322

Thus, Retention Index

Retention index for m-xylene:

A- m-xylene (Log [CRT1=0.335]): $Y = 0.003x - 2.322$

So, Retention Index

B- m-xylene (Log [CRT2=0.335]): $Y = 0.003x - 2.322$

Therefore, Retention Index

Retention index for p-xylene:

A- p-xylene (Log [CRT1=0.333]): $Y = 0.003x - 2.322$

Hence, Retention Index

B- p-xylene (Log [CRT2=0.333]): $Y = 0.003x - 2.322$

Thus, Retention Index

Retention index for unknown:

A- Unknown (Log [CRT1=0.335]): $Y = 0.003x - 2.322$

So, Retention Index

B- Unknown (Log [CRT2=0.331]): $Y = 0.003x - 2.322$

So that, Retention Index

THE MEAN AND S.D FROM ABOVE HAVE BEEN DEMONSTRATED IN TABLE III

Compound	Retention index 1	Retention index 2	mean Retention index	SD
o-Xylene	907.33	910.33	908.33	± 2.121
m-Xylene	885.6	885.6	*885.6*	*0*
p-Xylene	885	885	*885*	*0*
Unknown	884.33	885.6	884.965	± 0.898

The average retention index of o-xylene, m-xylene and p-xylene have been calculated (908.33 □0.2.121), 885.6, and 885 respectively. Additionally, the average retention index of unknown sample is 884.965 □0.898. Theoretically, one of the compounds in unknown mixture contains mxylene or p-xylene because their retention index is very close to the retention index of the unknown which is most likely to be. Quite the opposite, it is unexpected to be o-xylene because there is a big different in the average of retention index of o-xylene to the retention index of unknown sample (908.33 to 884.965). GC could be used to determine xylene isomers present in the unknown sample.

4.1 Uncertainty type A

Evaluate uncertainty based on valid statistical method (result which has been obtained in the lab) Error values from repeating the measurement:

- Error value of measuring o-Xylene = $\frac{SD}{mean} x100 = \frac{2.121x100}{908.33} = 0.233\%$

There is no error in the measuring of m-Xylene or p-Xylene due to the value of SD which is zero

- Error value of measuring the unknown sample = $\frac{SD}{Mean} x100 = \frac{0.898}{884.965} = 0.101\%$

4.2 Uncertainty type B

Evaluate uncertainty based on professional judgment or previous knowledge (certificate values)

- Uncertainty of 5 ml volumetric flask:

The value of tolerance for 5 ml volumetric flask is (± 0.02 ml) and the standard deviation is 0.005 ml.

Firstly, convert Cl to SD

SD = $\frac{\text{tolerance value}}{\sqrt{n}}$ SD = $\frac{0.02}{\sqrt{3}}$ = 0.0115 ml

U = $\sqrt{Ua^2 + Ub^2} = \sqrt{(0.0115)^2 + (0.005)^2}$ = ± 0.157x10-3 ml in 5 ml

- Uncertainty of 1 µl injection syringe:

The tolerance value of 1 µl injection syringe is ±3% µl with standard deviation 0.0015 µl

As previous: SD = $\frac{\text{tolerance value}}{\sqrt{n}} = \frac{0.03}{\sqrt{3}}$ = 0.0173 µl

U = $\sqrt{Ua^2 + Ub^2} = \sqrt{(0.0173)^2 + (0.0015)^2}$ = ± 0.010 µl in 1 µl

- Uncertainty of Temperature effect on volumetric flask:

Theoretically, the experiment has been done under room temperature 20 Cº (± 2 Cº)

Assuming that increasing temperature by 1 Cº might cause 0.1% change in the total volume of the flask which leads us to increasing volume by 0.2% of 5ml = 0.01ml.

In 5 ml volume would be ±0.01 ml

Considering that SD of 5 ml volumetric flask = 0.005 ml

U = $\sqrt{Ua^2 + Ub^2} = \sqrt{(\frac{0.01}{\sqrt{3}})^2 + (0.005)^2}$ = 0.0076 ml

± 0.0076 ml in 5 ml flask at room temperature ±2 Cº

- Uncertainty of Temperature effect on 1 µl injection syringe:

As volumetric flask, theoretically, the experiment has been done under room temperature 20 Cº (± 2 Cº) assuming that increasing temperature by 1 Cº cause 0.1% change in the total volume of the 1 µl injection syringe which lead us to increasing volume by 0.2% of 1 µl = 0.002 µl

In 1 µl volume would be ±0.002 µl

Considering that SD of 1 µl injection syringe = 0.0015 µl

U = $\sqrt{Ua^2 + Ub^2} = \sqrt{(\frac{0.002}{\sqrt{3}})^2 + (0.0015)^2}$ = 0.0017 µl

± 0.0017 µl in 1 µl injection syringe at room temperature ±2 Cº

- **Uncertainties**

Volume uncertainty of 5 ml flask =0.157x10-3 ml in 5 ml

Volume uncertainty of 1 µl injection syringe =0.010 µl in 1 µl

Volume uncertainty of (±2 C°) on 5 ml flask =±0.0076 ml in 5 ml

Volume uncertainty of (±2 C°) on 1 µl injection syringe =±0.0017 µl in 1 µl

$U_{result} = 884.965 \times \sqrt{(\frac{0.157x10-3}{5})^2 + (\frac{0.01}{1})^2 + (\frac{0.0076}{5})^2 + (\frac{0.0017}{1})^2 + (0.00101)^2}$ =

884.965 x 0.01 = 9.07 $\Longrightarrow$ $U_{result} = 9.07$

Expanded uncertainty: converting the overall uncertainty into a confidence limit at 95% level

U result = 9.07 x 2 = 18.14 $\Longrightarrow$ U result ± expanded uncertainty = 884.965 ± 18.14 the retention index of the unknown sample = 884.965 ± 18.14

4.3 Comparison of sources of uncertainty in this experiment

By calculating RSD% of each uncertainty contributor we have:

0.101%, 0.233% , $\frac{0.157x10-3}{5}x100$= 0.00314%, $\frac{01}{1}x100$ = 1%

$\frac{0076}{5}x100$ = 0.152%, $\frac{0.0017}{1}x100$= 0.17%

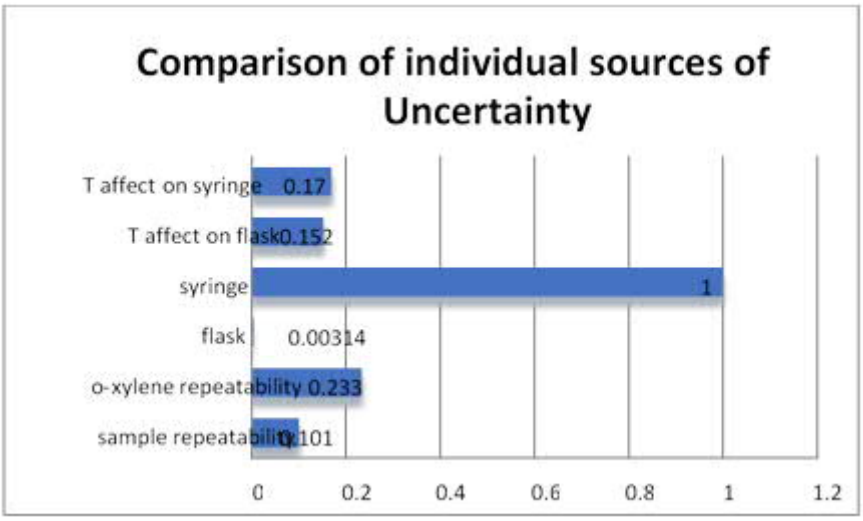

Figure 3. Demonstrates the individual sources of uncertainty

Dominance of major contributor:

$$U_{result} = \sqrt{(0.101)^2 + (0.233)^2 + (0.00314)^2 + (0.152)^2 + (1)^2 + (0.17)^2} = 1.05\%.$$

It can be seen from the **Figure 2.** That the volume of 1 µl injection syringe is the major uncertainty contributor with RSD% = 1%, while the random error from repeating o-xylene is the second with RSD% = 0.233%.

Also, the effect of temperature were almost the same on the volume of the flask and the injection syringe with RSD% = 0.17% and 0.152% in order. On the other hand, the RSD% of the volume of the flask was negligible 0.00314 comparing with RSD% of the injection syringe.

A measurement is assessing the property of something and the Uncertainty of measurement is the range of possible values of the result of any measurement which mean, for all measurement including the most watchful there is a margin of uncertainty.

Why uncertainty is important? There are three answers on this question

1- Calibration uncertainty of measurement has to be stated on the official document.
2- Test uncertainty of measurement is required to decide a pass or not.
3- Tolerance - where uncertainty is needed before deciding whether the tolerance is met or not. [11]

5. CONCLUSION

The amount of information could be increased by running a number of readings and determining the mean, and the standard deviation (The extra results you use, the closer value to that true mean you get) usually between 4 and 10 repeats is sufficient to best estimation of mean and SD. There are several things might cause uncertainty such as (measuring instrument, the measured sample, Operator skill, Sampling issues or the environment).

To work out the uncertainty of a measurement, first of all sources of uncertainty must be identified. After that the range of the uncertainty must be estimated from each source. Ultimately combined individual uncertainties to get an overall uncertainty. Uncertainty is estimated by two ways: Type A evaluation which estimate uncertainty statistically and type B evaluation which estimate uncertainty from professional judgment. Standard uncertainty for type A can be calculated by equation (1) while for type B by equation (2)

SD uncertainty = $\frac{SD}{mean} x100$ (1) , $SD\ uncertanity = \frac{\text{tolerance value}}{\sqrt{n}}$ (2)

Individual standard uncertainties which have been estimated by Type A or Type B evaluations are combined by $U_{\textbf{result}} = \sqrt{Ua^2 + Ub^2}$

Or $U_{\textbf{result}} = result\ x \sqrt{(\frac{a}{a})^2 + (\frac{b}{b})^2}$

Uncertainty contributions should have same units before combining.

It is important to minimize uncertainties of measurement which might be achieved by calibrating the instruments, Making corrections to compensate for any known errors, Measurements should be traceable to national standards, Checking the calculations carefully with the significant numbers, Choosing the best measuring instruments with the lowest uncertainties, Checking the measurements by a different method or by repetition, Using an uncertainty funds to spot the worst uncertainties as well as the addressing, Following the instructions of using instruments, Using knowledgeable staff, and provide preparation for measurement, Checking software to ensure it works correctly And Using the rounding perfectly in calculations.

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