Determination of lidocaine in urine samples using gc analysis

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Objective:

To purpose of this experiment is to quantify lidocaine present in a urine sample. This is achieved by running gas chromatography. There are a number of standards made from a stock solution.

Introduction:

[1] Lidocaine is a local anaesthetic. It is typically combined with other medications to treat soreness and irritation on skins such as eczema and is used to numb haemorrhoids around the anal area. Lidocaine is also used to relieve post-shingle patients. It helps sooth the nerve pain felt. Lidocaine is also used for controlling contractions in the heart (cardiac arrhythmias). [2] It works as it blocks the nerve signal pathways. [3] When pain occurs in the body, it happens in the pain nerve endings. It prevents sodium from entering the nerve ending. This stops the pain. Lidocaine has a molecular weight of 232. 343g/mol and a molecular formula of $C_{14} H_{22} N_2 O$.

To analyse the amount of lidocaine, standards must be made. Lidocaine is a liquid and must undergo ‘ liquid/liquid’ extraction. [4] Liquid/liquid extraction is the separation of one solute in one solvent to another. It is also known as ‘’partitioning’’. The solvents must be immiscible. This means they should not mix and if they do, only a small amount should mix. The liquid/liquid extraction depends on the polarity of the solute. For example, water and oil. Water is more polar and is also denser than oil. The oil remains at the top of the solute in the organic layer. The reason they don’t mix is due to the repulsion between the polar and non-polar molecules. There is poor attraction between polar and non-polar molecules due to the lack of dipoles

present. Dipoles are intermolecular forces that are formed. They happen due to an unequal sharing of electrons. Due to the lack of dipoles, the polar compound cannot form bonds with the non-polar. This causes the polar compound to form intermolecular forces and repel the non-polar compounds. Non-polar compounds are known as ‘ organic layers’ and polar compounds are known as ‘ aqueous layers’. These layers separating/partitioning is achieved by shaking the solute. [5] The distribution coefficient can be measured when equilibrium has happened in the solute. It is calculated by

1= 10ml (stated as per manual)

C_2 = 10mg/ml (highest concentration)

V_2 = 10ml (total volume of volumetric flask)

Firstly, From the chromatogram of the unknown standard,

Peak area= 91636

To calculate,

91636 = 22709 x + 793 . 5

Rearrange,

91636 – 793 . 5 = 22709 x

Rearrange to isolate x,

90842 . 5 22709 = x

This gives x as,

4 . 11 g ml = x

Discussion:

Graph 1. 1 represents the relationship between the two variables: content of lidocaine in standards and their peak areas. The graph above is a calibration curve. Calibration curves are used as they can determine unknown concentrations regarding the standards. There is a directly proportional relationship between the lidocaine concentration and their peak area. This means that when one of the variables is increased or decreased, the other variable follows. The calibration curve was created using Microsoft excel. As seen in Graph 1. 1, an equation of the line is formed with an R^2 value. This equation was used to find the actual concentration from the lidocaine sample. R^2 represents regression. Regression measures the relationship between the variables. It measures their closeness to one another. The regression value was calculated above using excel. The R^2 value is 0. 9289. This shows a positive correlation above. It is positively correlated as the value is close to +1. If the R^2 was closer to -1, it would be negatively correlated. A curve would not form. Since the R^2 is close to +1, it can be definite there is a relationship between the variables.

The concentration of lidocaine present in the unknown sample was calculated using graph 1. 1. The result achieved was good. It was within the range that was used in the graph. There appears to be little error. However, in table 1. 1, it is seen that there were 5 standards made. The fifth standard

was not used in the calibration curve. This was because it showed as an outlier on the graph. It decreased the regression value. This would have shown a poor correlation between the two variables. The result of the unknown lidocaine standard is good because its concentration did not lie in the ‘’gap’’ of the outlier. If the concentration of the unknown fell in the ‘’gap’’ of the outlier, it would show error in the results. In this practical, there is no definite literature value for the lidocaine concentration present in a urine sample. According to the calibration curve and the concentration calculated, it can be estimated the results achieved were of good standard. Potentially, errors can still exist.

Errors can occur easily in this practical. One of these errors is due to lack of internal standards used in the GC analysis. As discussed in the introduction, internal standards are very important and useful to use. Without using an internal standard, it may be difficult to differentiate between the peak of interest and the other peaks. This leads to more errors occurring in the analysis. In this practical, an internal standard was not used. Without internal standards, it is difficult to handle and predict any errors in the volume of standard. It is easier to control peak errors with internal standards and identify them on chromatograms. The reason an internal standard was not used in this practical is because they are expensive to purchase.

Another source of error happens in the liquid/liquid extraction. There are various ways to optimise liquid/liquid extraction. One of them is using the correct solvent. The solvent used for extraction must be suitable for the sample. The solvent’s structure must be compatible with the analyte’s

structure. By re-extracting the sample, it can increase the yield of the product. Re-extraction can remove any remainder analyte left in the solute.

A third source of error is caused by human error. There were standards made in this practical. By using inaccurate measuring techniques such as pipetting. By following proper measuring standards, like reading at eye level and filling flask until it sits at the bottom of the meniscus, this will improve accuracy of the practical. There was also manual injection used in the GC. If there was poor injection technique or a dirty needle used, this can lead to issues also. By cleaning the needle between each injection, it prevents cross contamination. By injecting the needle correctly into the port, the risk of the sample volatilising before it reaches the detector is minimised.

Conclusion:

Overall, this experiment was successful. The unknown standard of lidocaine was successfully analysed using GC analysis. A calibration curve was made and could quantify the lidocaine standard. To improve results, internal standards should be used, and accurate measuring techniques should be followed.

References

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