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Original Article

Calibration Curve of Amoxicillin Trihydrate in Different Solvents

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Abstract

In analytical chemistry, a calibration curve is a general method for determining the concentration of a substance in an unknown sample by comparing the unknown to a set of standard samples of known concentration. Chemical methods are developed to fit particular business, regulatory and research purposes. The objective of this research paper is to set out principles which should be followed when documenting methods of chemical testing. The calibration curve of Amoxicillin trihydrate was plotted by using different solvents like distilled water, methanol, ethanol and dilute sodium hydroxide. Coefficient correlation (R^2) of amoxicillin was found to be 0.999 in ethanol and less than 0.999 in methanol, distilled water and dil. sodium hydroxide.

Keywords: Calibration curve, Amoxicillin trihydrate, Coefficient correlation.

1. Introduction

The calibration curve is a plot of how the instrumental response, the so-called analytical signal, changes with the concentration of the analyte (the substance to be measured). The operator prepares a series of standards across a range of concentrations near the expected concentration of analyte in the unknown. [1] The concentrations of the standards must lie within the working range of the technique (instrumentation) they are using (figure01). Analyzing each of these standards using the chosen technique will produce a series of measurements. For most analyses a plot of instrument response vs. analyte concentration will show a linear relationship. The operator can measure the response of the unknown and, using the calibration curve, can interpolate to find the concentration of analyte. [2, 3, 4] A general method for analysis of concentration involves the creation of a calibration curve.

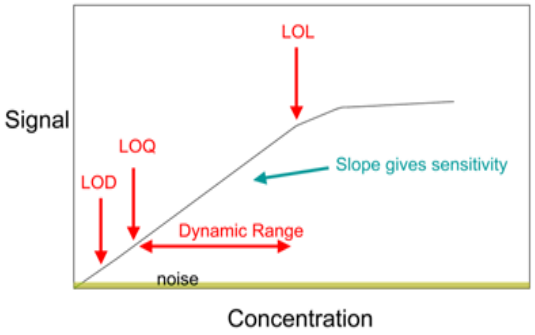

Fig. 1. Calibration curve.

This allows for determination of the amount of a chemical in a material by comparing the results of unknown sample to those of a series known standards. If the concentration of element or compound in a sample is too high for the detection range of the technique, it can simply be diluted in a pure solvent. If the amount in the sample is below an instrument's range of measurement, the method of addition can be used. In this method a known quantity of the element or compound under study is added, and the difference between the concentration added, and the concentration observed is the amount actually in the sample. [5]

Amoxycillin Trihydrate is a broad spectrum antibiotic, chemically (2S,5R,6R)-6-{[(2R)-2-

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amino-2-(4-hydroxyphenyl)-acetyl]amino}-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0] heptanes -2- carboxylic acid [2],Which acts on both gram positive and gram-negative bacteria. Amoxicillin acts by inhibiting the synthesis of bacterial cell wall. It inhibits cross-linkage between the linear peptidoglycan polymer chains that make up a major component of the cell walls of both Gram-positive and Gram negetive bacteria [6,7].

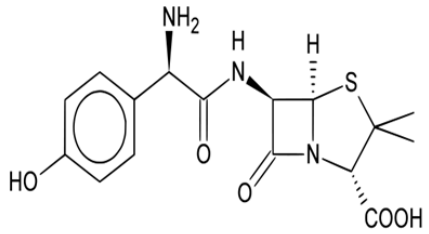

Fig. 2. Structure of Amoxicillin trihydrate.

Molecular Formula: $C_{16}H_{19}N_3O_5S, 3H_2O$
Molecular Weight: 419.45
Category: Antibacterial.
Description: White or almost white, crystalline powder

2. Materials and methods

2.1 Materials

Amoxicillin trihydrate was procured as a gift sample from Sunrise pharmaceutical Ltd Mahisal , Maharashtra and all other chemicals of AR grade were obtained from Research lab fine Mumbai Maharashtra, India. The instrument used were JASCO- V- 550 ,UV/VIS double beam spectrophotometer, Digital electronic balance (Shimadzu-Japan)

2.2 Method

2.2.1 Solubility and solvent effect:

A most suitable solvent is one that does not itself absorb in the region under investigation. Most commonly used solvent is 95% ethanol. Ethanol is a best solvent as it is cheap and is transparent down to 210 mμ. hexane and other hydrocarbons can be used because these are less polar and have least interaction with the molecule under investigation.

2.2.2 Choice of solvent:

1. It should not itself absorb radiations in the region under investigation.
2. It should be less polar so that it has minimum interaction with the solute molecules.

2.2.3 Calibration curve of Amoxicillin trihydrate in different solvents:** [8]

1. Amoxicillin in Distilled Water

Drug : Amoxicillin
Solvent: Distilled Water

Procedure:

100 mg Amoxicillin was weighed accurately and dissolved in 100 ml of distilled water in volumetric flask. Flask was shaken for 5 minutes to dissolve drug properly. Flask was labeled as Stock Solution. 1 ml of stock solution was further diluted into 100 ml of distilled water. λ max was determined by scanning on UV – Visible spectrophotometer. Further dilutions were prepared by diluting 1 ml stock solution in 100 ml, 2ml stock solution in 100 ml, and so on.

2. Amoxicillin in dil. NaOH

Drug : Amoxicillin
Solvent: dil. NaOH

Procedure:

100 mg Amoxicillin was weighed accurately and dissolved in 100 ml of dil. NaOH in volumetric flask. Flask was shaken for 5 minutes to dissolve drug properly. Flask was labeled as Stock Solution. 1 ml of stock solution was further diluted into 100 ml of dil. NaOH. λ max was determined by scanning on UV – Visible spectrophotometer. Further dilutions were prepared by diluting 1 ml stock solution in 100 ml, 2ml stock solution in 100 ml, and so on.

3. Amoxicillin in Ethanol

Drug : Amoxicillin
Solvent: Ethanol

Procedure:

100 mg Amoxicillin was weighed accurately and dissolved in 100 ml of ethanol in volumetric flask. Flask was shaken for 5 minutes to dissolve drug properly. Flask was labeled as Stock Solution. 1 ml of stock solution was further diluted into 100 ml of ethanol. λ max was determined by scanning on UV – Visible spectrophotometer. Further dilutions were prepared by diluting 1 ml stock solution in 100 ml, 2ml stock solution in 100 ml, and so on.

4. Amoxicillin in Methanol

Drug : Amoxicillin
Solvent: Methanol

Procedure:

100 mg Amoxicillin was weighed accurately and dissolved in 100 ml of methanol in volumetric flask. Flask was shaken for 5 minutes to dissolve drug properly. Flask was labeled as Stock Solution. 1 ml of stock solution was further diluted into 100 ml of methanol. λ max was determined by scanning on UV – Visible spectrophotometer. Further dilutions were prepared by diluting 1 ml stock solution in 100 ml, 2ml stock solution in 100 ml, and so on.

Result and Discussion

3.1 Calibration curve of Amoxicillin trihydrate in distilled water

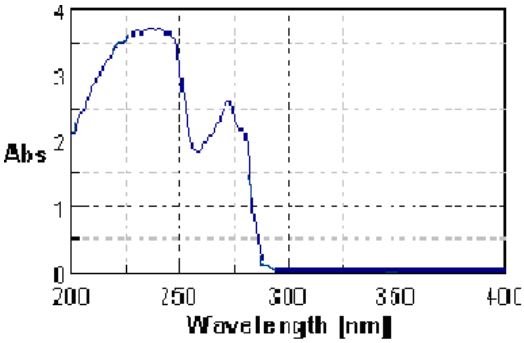

Fig. 3: Scanned λ max value.

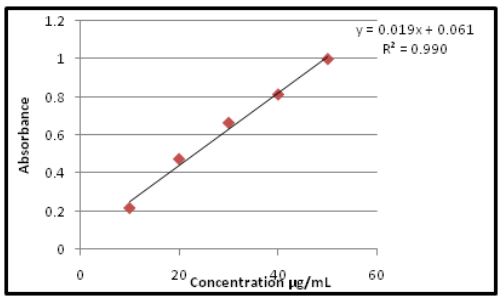

Fig. 4. Calibration curve of Amoxicillin trihydrate in distilled water.

3.2 Amoxicillin in dil. NaOH:

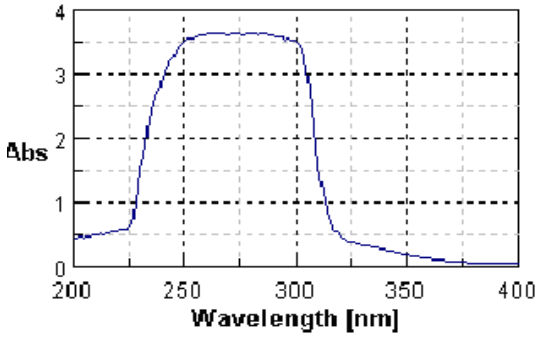

Fig. 5. Scanned λ max value.

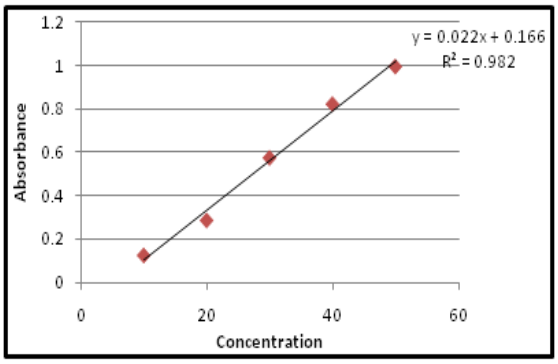

Fig. 6. Calibration curve of Amoxicillin trihydrate in dilute sodium hydroxide.

3.3 Amoxicillin in Ethanol

Scanned λ max value: 223.4 nm.

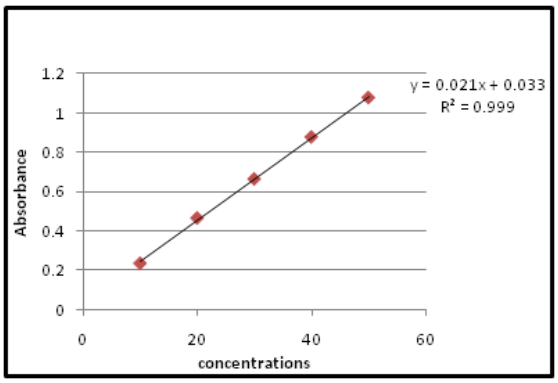

Fig. 7. Calibration curve of Amoxicillin trihydrate in ethanol.

3.4 Amoxicillin in Methanol:
Scanned λ max value: 227 nm

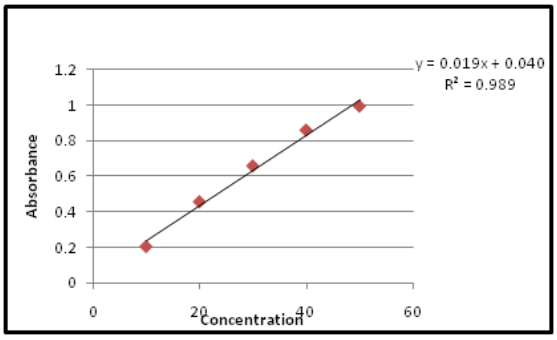

Fig. 8. Calibration curve of Amoxicillin trihydrate in methanol.

Conclusion

The calibration curve provides a reliable way to calculate the uncertainty of the concentration calculated from the calibration curve (using the statistics of the least squares line fit to the data). [5] The calibration curve provides data on an empirical relationship. The mechanism for the instrument's response to the analyte may be predicted or understood according to some theoretical model, but most such models have limited value for real samples. (Instrumental response is usually highly dependent on the condition of the analyte, solvents used and impurities it may contain; it could also be affected by external factors such as pressure and temperature). [6] Calibration curve of amoxicillin trihydrate was performed in different solvents viz. distilled water, dil. Sodium hydroxide, ethanol and methanol. Coefficient correlation (R^2) of amoxicillin was found to be 0.999 in ethanol and less than 0.999 in methanol, distilled water and dilutes sodium hydroxide. Hence this result can further be used in various research related to amoxicillin trihydrate.

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Table 1. Solubility of Amoxicillin trihydrate.

Solvent	Solubility
Distilled Water	Slightly soluble
Dil. NaOH	Soluble
Ethanol	Slightly soluble
Methanol	Slightly soluble

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