Quantification of B1 FD&C dye in a Marshmallow Peep Using Y6 Dye As an Internal Standard

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Introduction:

Internal standard calibration is an analytical chemistry technique that allows the quantification of an unknown analyte concentration. The benefits of this calibration method are that the sample containing the analyte can be inhomogeneous, and the exact volume of the sample is not needed to determine analyte concentration.[1] These are especially beneficial characteristics when a sample is comprised of several compounds and when the sample itself is too large to fit into appropriate glassware for analysis.

For example, a study monitoring gene expression patterns must test the genes in a wide variety of physiological states, which involves many different proteins and compounds. However, if only one protein is of particular interest on the effect of gene expression, an internal standard method may be employed in order to quantify transcription. [2] An internal standard, a chemical substance, added in constant amount to different samples can be used for calibration by plotting the ratio of the analyte signal to the internal standard signal versus the analyte standard concentration. Therefore, two important notable characteristics of internal standards are (1) the volume of the sample is not necessary for unknown concentration calculations because the calibration curve is prepared based on the ratio of absorbance of analyte and internal standard, and (2) an internal standard must show similar behavior to the analyte.

In this experiment, we demonstrated the accuracy of the internal standard technique by determining the amount of blue FD&C dye in a marshmallow peep.[3] Additionally, we tested the theory that an internal calibration curve is not dependent on volume by treating two identical peep solutions with different volumes of internal standard. For our purposes, the marshmallow peep was too large to fit into a volumetric flask and needed to be dissolved in a beaker of water; the boiled solution also evenly distributed the blue dye from the peep throughout the sample and separated the dye from physical contact with the marshmallow and sugar compounds.

The blue, B1, dye of the marshmallow peep has less than 0.1 absorbance around 400-420nm and a maximum absorbance between 550-650nm; based on absorbance spectra, the internal standard chosen to determine the amount of B1 dye in the peep was yellow, Y5. The Y5 internal standard has a maximum absorbance at $\lambda_{max}{\sim}430nm$. This internal standard was chosen because it does not have a signal in the region of B1 signals, and similarly, B1 does not have any signals within the Y5 signal range. Therefore, the signals are readily distinguished by the Spectronic 20 Genesys spectrometer used to measure absorbance of the solutions. Two samples of the marshmallow peep solution were prepared, one receiving 39.9mL of Y5 and the other receiving 60.07mL.

Based on the known concentrations of the stock Y5 solutions ($4.608 \times 10^{-5}M$), the number of moles of B1 and Y5 added to each internal standard solution could be determined. Plotting the ratio of absorbance for the analyte B1 and internal standard Y5 versus the moles of B1 versus moles of Y5

presented an internal standard calibration curve. According to Beer's Law, absorbance A (unitless) is related to the molar concentration c (with units M) of a sample by the formula:

$$A = b\varepsilon c \quad (1)$$

Where b is the sample pathlength (cm), and ε is the molar absorptivity ($M^{-1}cm^{-1}$). We can therefore use linear regression analysis of the internal calibration curve to determine an unknown mass (µg) of B1 in the original Peep.

This experiment was performed for two reasons. The first purpose of this experiment was to quantify the unknown mass (µM) of B1 dye in the original marshmallow Peep, with an aim of demonstrating the usefulness and accuracy of internal standard calibration in determining the amount of an analyte in an inhomogeneous sample with unknown volume. The second purpose was to illustrate that internal standard curves are dependent on the ratio of moles of analyte to moles of internal standard present in the sample. This was accomplished by preparing two dissolved Peep solutions and adding different, known amounts of Y5 internal standard to each sample; despite the different volumes added, the moles of B1 dye originally present in the Peep was unchanged. We therefore expected the amount of B1 in both solutions to be relatively equal.

References

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