Figure2 uv absorbance peak area vs benzoic acid concentration mmol l report examp...

Food & Diet, Coffee

Analysis of Unknown using Standard Solutions and HPLC

Part 1.

The data for the construction of the standard curve for caffeine and benzoic acid are presented below.

The graphing of the Caffeine Standard Curve (figure 1) demonstrates an R2 of 0. 9898 so this is standard curve is satisfactory for our experiment since 0. 9898 approaches 1 closely.

The graphing of the Benzoic Acid Standard Curve shows a better fit as R2 = 0. 9989. Each sample measuerment lies on the standard curve.

Part 2.

5 ml of warm Mello Yello was left to sit until the carbonation had released CO2 into the air (outgassing the cola). Then 2 ml of the outgassed cola was pipeted into a 50 ml volumetric flask and dilted to the mark with 0. 01 M HCl.

The caffeine concentration of the unknown is calculated using the following standard curve

C= 1E-7 * (Peak area of absorbance) = 1E-7 *148007 = 0. 0148 mmol/L

Similarly for Benzoic acid

C= 1E-6 * (Peak area of absorbance) = 1E-6 *52067 = 0. 0521 mmol/L

The dilution calculatons for caffeine and benzoic acid follow.

Dilution Calculation for caffeine

5 ml * x = 50 ml * 0. 014

So x = (50/5)*0. 014 = 0. 114 mMol/L caffeine

Dilution Calculation for Benzoic Acid

5 ml* x = 50 * 0. 0521

So x = (50/5) * 0. 0521 = 0. 521 mMol/L

Part 3.

The pH was controlled by using a 0. 025 M phosphate buffer with a pH = 3. The mobile phase our HPLC must be forced through the short (3 – 25 cm) stationary stage with a pressure of several thousand psi. The column relies on interaction with the solutes in an interaction called “ reversed phase partition.” The time the measurable solute molecules spend in the column are related to their polarity. The retention time of each solute is directly related to the polarity of the solution. The elution of the solutes happens in the order of decreasing polarity. pH needs to be carefully controlled using the appropriate buffer in order for the solutes to leave the column (elute) at reproducible time.

Part 4.

Three main types of erros that can be made in a lab exercise like this follow. In this analysis the caffeine when diluted to a 1 to 10 dilution was not as carefully done as it should have been. This is evident from the concentration not being as close to the middle range of the standard solution curve as it should have been.

Perhaps the glassware wasn’t cleaned properly or too much distilled water was use to prepare the standard curve for the caffeine because R2 was equal

to 0. 9898 and should have been closer to 0. 999 as was the benzoic acid standard solution.

Another error that can be possible when using the HPLC for analysis is the possiblity of residue from a previous analysis being in the column.

References

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