The Analytical Process

Section 1: The Analytical Chemist's Job

Sampling – this is the process of obtaining a representative small sample to measure

Example – if you want to determine the amount of caffeine in chocolate you would need to analyze many different chocolates from different manufacturers; you may also want to measure multiple samples from the same manufacturer to see if a range of caffeine content exists among the different chocolate bars tested

Homogeneous sample – pure chocolate – any piece from any part of the bar should be the same

Heterogeneous sample – differs in composition from place to place within the bar – an example would be a chocolate bar with nuts in it or with rice krispies in it (Nestle Crunch)

Sampling techniques are different depending upon whether the sample is homogeneous or heterogeneous.

Sample Preparation –

- Weigh out a quantity of chocolate and extract the fat by dissolving the fat in a hydrocarbon solvent
- Fat must be removed due to interference in the chromatography extraction technique later
- To extract the fat effectively, the chocolate would have to be ground up – the chocolate was first frozen, otherwise it would be hard to grind since it is too soft
- Small pieces were added to a centrifuge tube along with petroleum ether (an organic solvent) – caffeine and its precursor, theobromine, are insoluble in the solvent; the fat will dissolve in this solvent

- The tube was centrifuged so that the chocolate could be packed at the bottom; the clear liquid, which contains the dissolved fat, was decanted off
- residual solvent was removed by heating

- calculation of the chocolate residue is found by subtracting the weight of the empty tube
- substances being measured – caffeine and theobromine – are called the analytes
- next, a quantitative transfer (as complete a transfer as possible) of the fat free chocolate is made to an Erlenmeyer flask and the analyte is dissolved in hot water for analysis; a slurry, which is a suspension of a solid in a liquid, was created to ensure complete transfer of the chocolate
- by heating this flask, the caffeine and theobromine would by extracted into the water
- before injecting the analyte into a chromatograph for analysis, the analyte solution needed to be purified further
- a portion of the chocolate residue in hot water was transferred to a centrifuge tube
- after centrifuging, the liquid portion was filtered to further remove any remaining solid residue

The three main steps that needed to be done for sample preparation to make the sample suitable for analysis were:

  - removal of fat from the chocolate
  - extraction of the analyte (caffeine and theobromine) into hot water
  - separation of residual solid from the analyte in the liquid portion

Chemical Analysis

- analyte is injected onto a chromatography column which separates the analyte and measures the quantity
- a chromatogram results with peaks that identify the analyte

- qualitative analysis – identifies what is present
- quantitative analysis – identifies how much of the analyte is present
- in a chromatogram, the area under the peak is proportional to the quantity of the analyte

Calibration Curves

- analytes with equal concentrations will give different detector responses with the instrumentation used
- responses need to be measured for known concentrations of the analyte
- graph which shows detector response as a function of analyte concentration is called a calibration curve or standard curve
- to construct this curve various differing known concentrations of analyte were run on the chromatography column and the peak heights were measured
- a straight line can be fitted through the points of this curve so that an unknown concentration can be determined

Interpretation of Results

- the amount of analyte in the aqueous extract of the chocolate is known, so the amount of theobromine and caffeine could be calculated for the original chocolate
- standard deviation is also computed for three replicate measurements – this statistical calculation is discussed in Chapter 4 – it is a measure of the reproducibility of the results
- if the standard deviation is large, this means that the results are not reproducible

Quality Assurance

- to be sure that analytical results are reliable, a set of practices called quality assurance is followed
- quality assurance gives confidence in the quality of the results
- one method of quality assurance, which is discussed in chapter 5, is to add a known quantity of caffeine, called a spike, to the original chocolate sample
- when analyzed, a quantity of caffeine equal to the original chocolate plus the amount of the spike should be found
- if the expected quantity is found, the analyst can have confidence that their method extracts all the caffeine present and measures it accurately

General Steps in a Chemical Analysis

Formulating the question – what do you want to determine by chemical measurements?

Selecting analytical procedures – search literature to find the procedures required to analyze your sample to find what you are looking for

Sampling – select representative material to analyze – a poorly chosen sample or one that changes between the collection time and analysis time will give meaningless results

Sample preparation – process of converting a representative sample into a suitable form for chemical analysis, which usually means dissolving the sample; if sample has a very low concentration of analyte, the sample may need to be concentrated first; removal of species or masking of species that interfere with the analyte may be necessary

Analysis – measure concentration of analyte in several identical aliquots (portions); replicate measurements assesses the variability or uncertainty in the analysis; uncertainty in measurements is an indicator of the reliability of the data; different analytical methods can be used to see if all methods give the same result; you may also analyze several different bulk samples to see if variations arise from your sampling procedure

Reporting and interpretation – construct a clearly written report of your results – this report can be more technical if written for persons who are specialists in Chemistry; in this class the reports will be the lab write-ups for each experiment and should be written very clearly so that anyone not familiar with what you have done will be able to understand how to interpret your data and perform the necessary calculations

Drawing conclusions – conclusions drawn should be consistent with the data so that anyone wishing to expand on this work will not misinterpret your data