Method Selection and Development

- Initial Considerations
  - What does the method need to do?
    - ✓ What analyte/s need to be assayed?
    - ✓ What range or concentration will be evaluated?
  - How will the data generated from this analysis be used?
    - ✓ Trend data
    - ✓ Identification
    - ✓ Quantification
  - What instruments/methods are currently available?
  - Any special needs/criteria that need to be met
    - ✓ Sample size
    - ✓ Storage/Preservation
  - Utilization of a pilot study may help to answer many of these issues

Analytical Methods

- Qualitative
  - Is a certain analyte present or not?
    - ✓ Confirmation of the presence or absence of impurities
    - ✓ Identification of unknown substances
  - Sensitivity of method is important
- Quantitative
  - What amount of analyte is present?
  - What level of detection is necessary?
    - ✓ Several methods with varying degrees of validation criteria
      - » Validation means that the method has been subjected to evaluation and has been found to provide results which are appropriate for their intended purpose

Analytical Methods (cont.)

- Methods are categorized into the following types:
  - ROUTINE
    - ✓ Screening
      - » High throughput; Low cost
      - » Small number of false positives/negatives
      - » Usually qualitative
    - ✓ Surveillance
      - » Lower throughput
      - » Better sensitivity
      - » Quantitative result
  - REGULATORY
    - ✓ Confirmatory
      - » Positive identification
      - » Routine method with detection system
    - ✓ Reference
      - » Fully validated and tested
      - » Data accuracy and precision
  - ALWAYS remember that the method used must fit the intended utilization of the results

Sources for Methods

- Check the existing methods and QC options currently available (in-house)
- Methods published by scientific literature
  - Journal of Chromatography
  - Journal of Analytical Chemistry
- Methods supplied by trade organizations/suppliers
  - Varian/Shimadzu
- Methods published in books by professional organizations or statutory publications
  - Standard Methods for the Examination of Waste Water (20th Ed.)
  - Environmental Protection Agency EPA
  - U.S. Geological Survey USGS
  - American Public Health Association APHA
  - American Water Works Association AWWA
  - Water Environment Federation WEF

Factors to Consider when Choosing a Method

- Limits of Detection
  - Controversial due to definitions that fall short in explanation and confusion of terms
  - Most analysts agree that the smallest amount that can be detected above the noise in a procedure and within stated confidence limits is the detection limit.
  - Several types of detection limits
    - ✓ **Instrument detection limit (IDL)**
      - » Analyte concentration that produces a signal greater than three standard deviations of the mean noise level.
      - » Instruments produce a signal or noise even when no sample is present or a blank is being analyzed.
        - Large number of blank evaluations helps to well define the mean and standard deviation
        - Useful for determination of the Method Detection Limit (MDL)

Factors to Consider when Choosing a Method (cont.)

Limits of Detection (cont.)

✓ Method Detection Limit (MDL)

» **Defined by EPA as the minimum concentration of a substance that can be measured and reported with 99% confidence that the analyte concentration is greater than zero and is determined from analysis of a sample in a given matrix containing the analyte.** (EPA PT. 136 App. B rev 1.1 pg.305)

» MDL is usually based on 7 to 10 replicate aliquots prepared at a concentration that is 1 to 5 times the estimated detection limit; multiple runs may be required to set MDL

» Ideally the MDL should be at least one-tenth of the concentration to be measured

- EX: Legal limit for lead concentration in tap water is 50 ppb the method used should be capable of detection of lead to 5 ppb level

» Formula for calculation of MDL

- For 7 replicates of a sample
- **MDL= 3.14s**
- 3.14 is the value from the table of one-sided t distribution for t 7-1= 6 degrees of freedom at the 99% level
- s is the standard deviation for the replicates

Factors to Consider when Choosing a Method (cont.)

- **Limits of Detection (cont.)**
  - ✓ Limit of Quantitation (LOQ)
    - » LOQ is the low standard in the calibration curve
    - » Usually 3 to 5 times the MDL
  - Report results below the MDL as “not detected”
  - Report results between the MDL and the LOQ with qualification for quantitation
  - Report results above the LOQ with the value and its associated error
- Accuracy
  - Closeness of measured value to true value
  - Combines bias and precision
  - Developed first with instrument or method; then monitor periodically
- Precision
  - Measure of the degree of agreement among replicate analyses of a sample
  - External source QC; material used to determine reproducibility/consistency for method performance; NOT A STANDARD but similar
    - ✓ Day to day QC result maintains precision

Factors to Consider when Choosing a Method (cont.)

- Speed
  - dependent type of analysis
  - number of samples to be analyzed
  - type of data required
    - ✓ Subset of samples
    - ✓ Assays in combination
      - » Screening method followed up by a confirmation method

- Equipment Required
  - Evaluation of resources available
  - Method may be ideal but without proper equipment or technical support not applicable
    - ✓ Ex: Respiration experiments requiring detection of CO_2

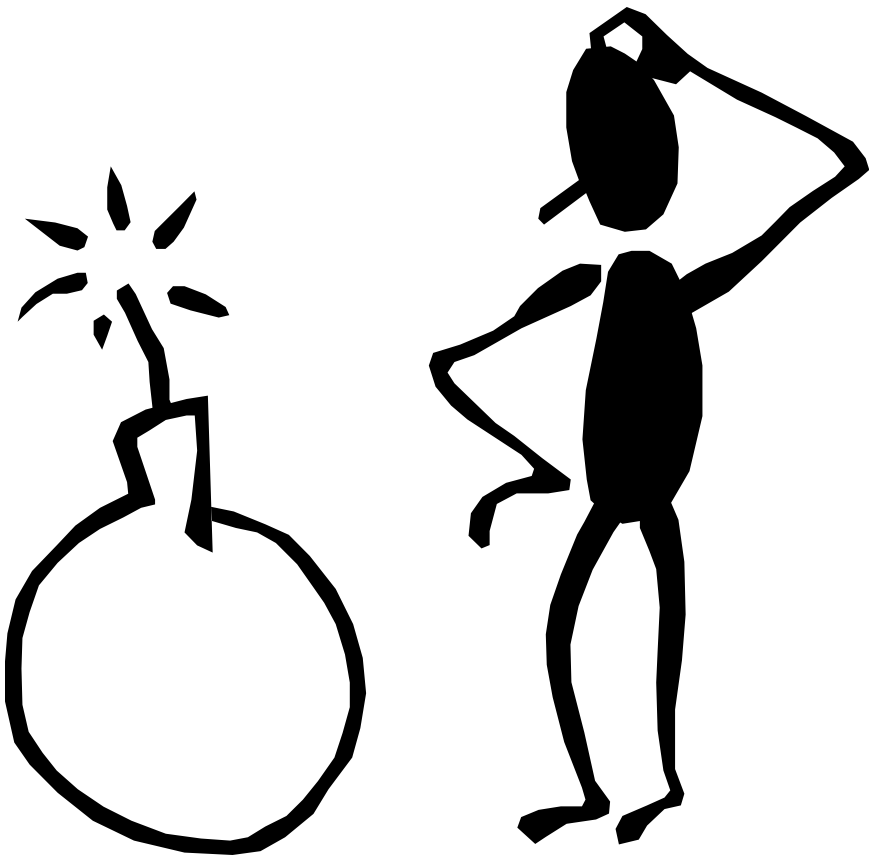
- Sample Size
  - May or may not be a limiting factor in analysis
    - ✓ Ex: Precipitation collectors
      - » Amount of rainfall collected impacts the number and types of analysis that can be completed
  - Linked to limit of detection

Factors to Consider when Choosing a Method (cont.)

- Sample Size (cont.)
  - Linked to limit of detection
    - ✓ Detection levels can sometimes be improved by taking larger weights/volumes of sample
    - ✓ Homogeneity and representative sampling should be considered
- Cost
  - Choice of method may only have small impact on overall cost of analysis
    - ✓ Usually instrumentation and resources have a larger impact
    - ✓ Some methods may require highly specialized training or expensive chemicals
- Specificity
  - Degree of discrimination of the method for the analyte
  - Discrimination of the detection system should also be considered

Factors to Consider when Choosing a Method (cont.)

- Safety
  - Methods that require special facilities or training for safe operation may impact decision
    - ✓ Radioactivity; Toxic or hazardous chemicals
    - ✓ Some statutory methods may leave no alternative choices
      - » Make sure that all personnel associated with method are properly trained and made aware of hazards



Making your choice

- Ultimately the method chosen maybe dependent on one or many of the factors listed
  - Above all chose a method that fits the purpose
    - » Will the method chosen be adequate for the decisions that need to be made when the result is determined?
- Choice of the appropriate method
- Now what?

Method evaluation/Validation

- Precision
  - Within run
    - ✓ Sample or control is run 10x within run
      - » Reproducibility of method
      - » Mean Standard deviation for each value
      - » Meet manufacturer or authors specifications
  - Between runs
    - ✓ 30 to 40 samples on separate days
      - » Method/analyst reproducibility
      - » Sample stability

- Recovery Study (Spike)
  - Linearity check
    - ✓ Adding known quantity of material being assayed for to previously assayed sample
      - » Check recovery % of amount added; Should be + 5%

- Correlation with reference material/laboratory
  - Reference material maybe available to authenticate results
  - Reference laboratory can be utilized to authenticate results

Method evaluation/Validation cont.)

- Sample stability
  - Sample evaluated over a period of time to determine stability
    - ✓ Storage methods
      - » Temperature/Humidity
    - ✓ Preservation
      - » pH adjustment
- Establishment of range
  - Normal range
    - ✓ Suggested reference range listed with instrument from manufacturer
    - ✓ 10-12 “normal” samples (normal population) from published method
- Ongoing demonstration of Capability
  - Some of the above listed items should be run routinely with each analysis to check that method is under control
    - ✓ Blanks
    - ✓ External source QC’s
    - ✓ Recovery checks (spike)

Issues that may impact the method

- What can go wrong?

- Quality of Supplies/Reagents
  - Glassware
    - ✓ Composition
    - ✓ Types
  - Reagents
    - ✓ Chemical grades
      - » Reagent grade
      - » Analytical grade
      - » Chemically Pure
      - » USP and NF
      - » Technical or Commercial grade

- Contamination
    - ✓ Low levels
    - ✓ Solids
    - ✓ moisture
    - ✓ turbidity

Issues that may impact the method (cont.)

- Instrumentation
  - Drift
  - Detector malfunction
  - Column integrity
  - Flow rates
- Analyst
- In Conclusion
  - Consider all the factors that may impact your choice of method
  - Pick a method that suits how you will use the analytical result
  - Set all limits;ranges and QC determinations for method
  - Evaluate the method with a “pilot” study if possible
  - Evaluate possible sources of error
  - Once method is in use, check performance of the method routinely