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 CO₂

- **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