Validation of Spectroscopy Methods

Tim Herrman
Professor, State Chemist and Director
Office of the Texas State Chemist

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Background

- Near infrared reflectance Technology
- Usage in the food industry
- Advantages
  - Easy to operate, Fast, Accuracy, little sample preparation, environmental friendly, safe, economical.
- Disadvantages

Background cont.

- Principle: Measuring chemical composition
- Instrument: Diffuse reflectance or transmittance measurement in the near infrared wavelength region of 700-2500 nm.
- Sampling

Method Development

- Measure a pool of samples by NIR and a reference method
- Establish calibration from a portion of measured samples.
- Validate the calibration based on the other portion of measured samples.

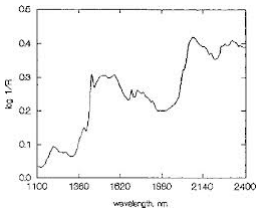
Example

- Protein content measurement
- A pool of samples to be collected and measured for the protein content by a reference method.
- The data collected by the training samples were then used to establish the calibration by modeling.

Reference Method

- The reference samples assayed by wet chemistry should be analyzed by recommended and standardized analytical procedures.
- AOAC methods, AACC methods
- Combustion method

Samples Measured by NIR

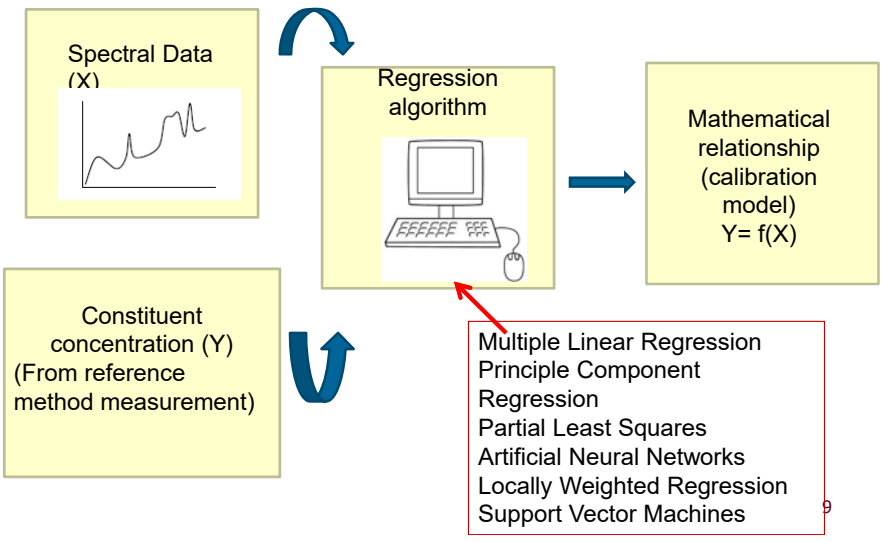


- Measure every sample that have been analyzed by the reference method.
- Software based chemometrics analysis.

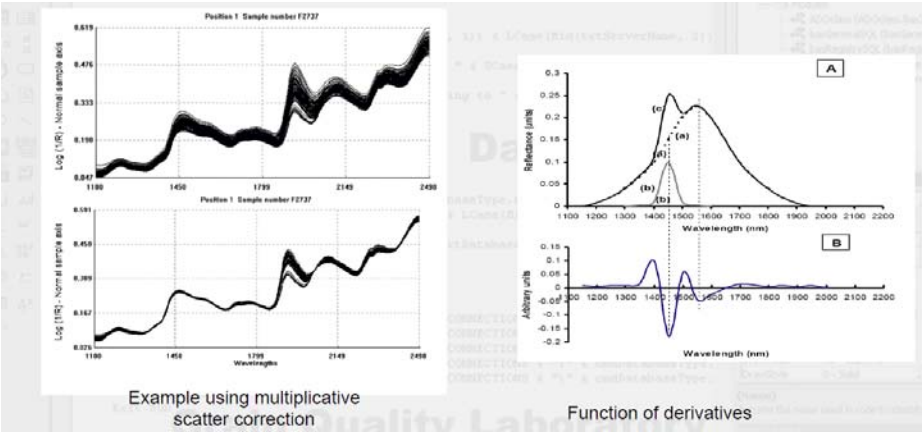
Chemometrics

- Mathematical process: The mathematical calculation to correlate the NIR spectra to reference samples that have been measured by the reference method. The mathematical equations developed from such process is called prediction models, or very often, called “calibrations”.
- Each trait: the prediction model (calibration) is unique.
- Non-destructive: repeated measurements.

NIR Calibration



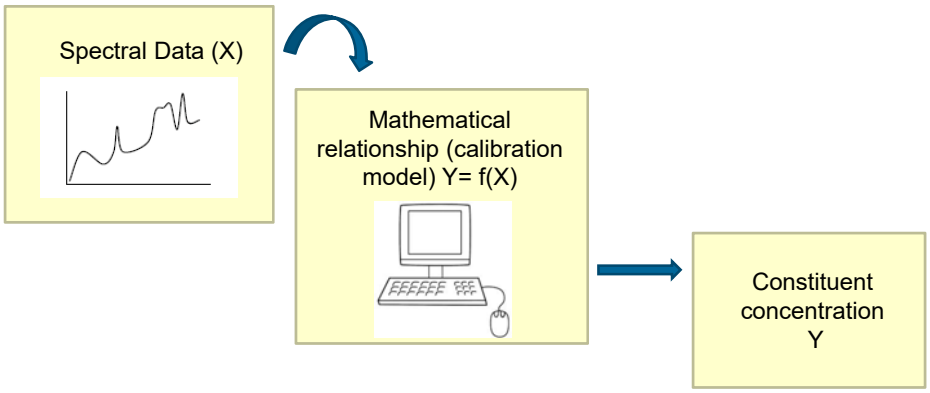
Spectral Preprocessing



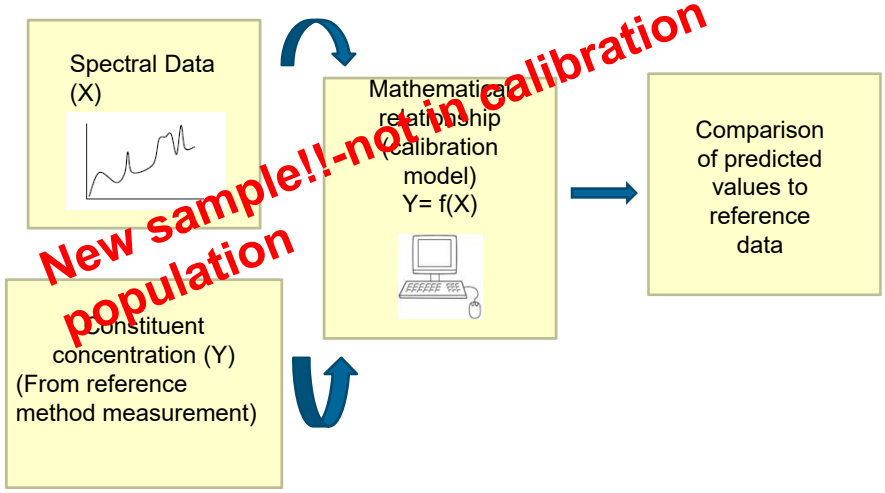
Remove noise

Enhance useful information

NIR Prediction



NIR Calibration Validation



Statistic	Units	Equation
Coefficient of determination (r^2)	Unitless	$r^2 = \frac{(\sum_{i=1}^{n} \hat{y}_i y - \sum_{i=1}^{n} \hat{y}_i \sum_{i=1}^{n} y_i / n)^2}{(\sum_{i=1}^{n} \hat{y}_i^2 - (\sum_{i=1}^{n} \hat{y}_i)^2 / n)(\sum_{i=1}^{n} y_i^2 - (\sum_{i=1}^{n} y_i)^2 / n)}$
Standard error of prediction (SEP)	Same as reference values	$SEP = \sqrt{\frac{\sum_{i=1}^{n} (\hat{y}_i - y_i - bias)^2}{n-1}}$
Root mean square of the error of prediction (RMSEP)	Same as reference values	$RMSEP = \sqrt{\frac{\sum_{i=1}^{n} (\hat{y}_i - y_i)^2}{n}}$
Bias (d)	Same as reference values	$d = \frac{\sum_{i=1}^{n} (\hat{y}_i - y_i)}{n}$
Ratio of performance of deviation (RPD)	Unitless	$RPD = \frac{Sd_y}{SEP}$

$\hat{y}_i = i^{th}$ validation sample predicted value
$y_i = i^{th}$ validation sample reference value
n = number of samples in validation set
Sd_y = standard deviation of reference values from the validation set

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Evaluation of Statistic -RPD

RPD	Class	Application
0.0–2.3	Very poor	Not recommended
2.4–3.0	Poor	Rough screening
3.1–4.9	Fair	Screening
5.0–6.4	Good	Quality control
6.5–8.0	Very good	Process control
8.1+	Excellent	Any application

RPD = (Std. Dev. of Reference Data)/(Std. Error of Prediction)

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For Established Agricultural Commodities

- After the calibration is established and validated, the unknown sample is then measured against the calibration
- The number of samples for running the validation should be sufficient for the statistics used to check the performance.
- At least 20 samples are needed to establish a solid validation.
- Samples selected for calibration purpose should be randomly selected and have a balanced sample distribution over the entire calibration range.

Keep In Mind

- Calibration equation need to be checked for stability
- The more reference samples for calibration, the better

For a Reliable NIR Prediction Model

1. Minimize sources of error in the entire process;
2. Use a precise and accurate reference method
3. Standardize sample preparation and analytical procedures;
4. Use standardized NIR instrument;
5. Use advanced regression methods like partial least squares (PLS) or artificial neural network (ANN) to obtain accurate, predictive spectral information;
6. Perform routine instrument maintenance;
7. Analyze only samples representative of the original population;
8. Obtain routine diagnostics of all associated instruments and undergo yearly prediction model (calibration) updates.

Quality Assurance

- Control sample: At least one control sample should be measured at least once a day to check instrument hardware stability and detect malfunction.
- Evaluation

Method Evaluation

- All the concepts from single lab method validation apply: LOD, LOQ, precision, accuracy
- Fit-for-purpose

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Contact Information:

tjh@otsc.tamu.edu

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