



## Technical Note 21: Validation of a NIR Method

Developing a NIR calibration is not the completion of the job when developing a NIR method. The calibration needs to be validated. The following steps are intended as a guide to a Validation Procedure for a NIR Method.

- 1) Accuracy = Standard Deviation of Differences between the predicted (NIR results) vs the reference or lab results for 10 samples across the range of values to be analysed in the future.

Using the calibration model previously developed based on a calibration set, analyse 10 fresh samples in duplicate of the same product using the same sampling device, sample temperature and the same analytical method for determination of the reference results. Record these results in sheet 1 of the attached Excel Worksheet, ie, Validation Document.

All samples should be repacked between analyses. All lab tests should be done in duplicate using different portions of the same sample.

- 2) Precision = Standard Deviation of Differences between duplicate predicted results using the NIR analyser.

The duplicate results in step 1, provide the data used to compute the precision of the method. Record these results into sheet 2 of the worksheet.

- 3) 3 Day Reproducibility = Standard Deviation of Differences between the average of the predicted results over 3 days vs each day results.

Analyse 10 samples used in step 1, in duplicate, on three consecutive days. It is acceptable to use the results from step 1 as Day 1 samples. Record the results into sheet 3 of the worksheet.

- 4) Temperature Stability = the differences between the results obtained at standard temperature and the results obtained at +/-5 and 10C from the standard temperature.

Cool three samples to -10C below the standard temperature used to develop the calibration, ie, room temperature = 22C - 10C = 12C. Analyse these samples and record the results in sheet 4. As the samples warm up to 17C, analyse them again and record the results in sheet 4. Analyse the samples at the standard temperature and record the results. Warm the samples to 27C and then 32C and analyses and record the results.

- 5) Validation of the Reference Methods = Reproducibility

Submit each sample to the laboratory for analyses using the reference methods as BLIND SAMPLES, ie, mark the samples so that the laboratory cannot determine the true sample ID. Record the results of the reference analyses on sheet 5.

By analysing the samples each day using the reference methods, an estimation of the Reproducibility of the reference methods is obtained. By definition, the NIR method cannot be more accurate than the reproducibility of the reference methods.

#### Validation Results:

The data generated by the Validation Procedure will lead to one of two situations.

- 1) Errors are too high and there is a need to go back and further develop the method. By saving all the spectral data used in the validation process, the spectra and lab data can be added to the calibration set.
- 2) Errors are within acceptable limits and therefore the method is accepted and released. The errors are used to define the performance of the method so that future improvements to the calibration set and sampling can be compared. If the improvements reduce the errors then method is updated. If not then, there is a criterion to make decisions.

# Validation Worksheet

## Worksheet 1 Accuracy:

Sample	Con1	Con1	Ave	Lab 1	Diff	Con2	Con2	Ave	Lab2	Diff
1										
2										
3										
4										
5										
6										
7										
8										
9										
10										
				Bias					Bias	
				SEP					SEP	

## Worksheet 2 Precision:

Sample	Con1	Con1	Diff	Con2	Con2	Diff
1						
2						
3						
4						
5						
6						
7						
8						
9						
10						
		SDD			SDD	

## Worksheet 3: 3 Day Reproducibility

Sample	Day1	Day2	Day3	Ave	Diff Ave - Day1	Diff Ave - Day2	Diff Ave - Day3
	Con1	Con1	Con1				
1							
2							
3							
4							
5							
6							
7							
8							
9							
10							
				SDD			

Sample	Day1	Day2	Day3	Ave	Diff Ave - Day1	Diff Ave - Day2	Diff Ave - Day3
	Con2	Con2	Con2				
1							
2							
3							
4							
5							
6							
7							
8							
9							
10							
				SDD			

Worksheet 4 Temperature Stability

Sample	Less 10C	Less 5C	Room Temp	Plus 5C	Plus 10C	Diff
	Con1	Con1	Con1	Con1	Con1	
1						
2						
3						
4						
5						
6						
7						
8						
9						
10						
					SDD	
Sample	Less 10C	Less 5C	Room Temp	Plus 5C	Plus 10C	Diff
	Con2	Con2	Con2	Con2	Con2	
1						
2						
3						
4						
5						
6						
7						
8						
9						
10						
					SDD	

Sheet 5: Reproducibility of Reference Methods

Sample	Ref Day 1	Ref Day 2	Ref Day 3	Average	Range
	Con1	Con1	Con1	Con1	Con1
1					
2					
3					
4					
5					
6					
7					
8					
9					
10					
					SDD

Sample	Ref Day 1	Ref Day 2	Ref Day 3	Average	Range
	Con2	Con2	Con2	Con2	Con2
1					
2					
3					
4					
5					
6					
7					
8					
9					
10					
					SDD