I have spent the past several days looking at the data you sent to me. I’m not sure I know what is going on. If you did not told me that the twenty sample scans were of the same sample I would have told you that the Production unit reporting of 54.xx OHV was either a different sample or the calibration was in need of bias correction.

I thought I would first look at the predicted values for each instrument to get a sense of the mean and standardization for each group of ten replicates.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | QC run 1 | QC run 2 | Difference | Production run1 | Production run 2 | Difference |
| Ave OHV value | 47.14 | 46.54 | 0.60 | 54.60 | 48.71 | 5.89 |
| Std Dev. | 0.57 | 0.14 |  | 0.27 | 0.32 |  |

I would have thought the replicate precision for each instrument would have been tighter. Assuming the samples are at the same temperature, this variability points to the uniformity of the vial and possible changes in sample scatter from the vial.

Next I calculated the average for all 20 replicates measure on each of the two different instruments. Again the data appears to be biased.

|  |  |  |  |
| --- | --- | --- | --- |
|  | QC all measurements | Production all measurements | Difference |
| Ave OHV value | 46.84 | 51.65 | -4.81 |

I did a number of PLS1 calibrations using a combination of QC and Production spectra that you sent. Each time I did a prediction on any production sample (samples that were not included in the calibration) the prediction results reported both a (very) high M-Distance and high Residual Ratio values. These two statics are very helpful diagnostics tools. The M-distance defines the boundary for the calibration standards. The average distance for the calibration samples from the boundary edge to the center is set to be 1.0. Values exceeding 1.0 are general related to concentration effects, that being samples beyond the calibration range. The second statistic is the Residual Ratio; a high value here indicates that there are features in the spectra that exceed the average residual for the model. Often times, but not limited to some type of carry over blending issue.

|  |  |  |  |
| --- | --- | --- | --- |
| Sample Id | OHV predicted | M-Distance | Residual Ratio |
| 472sksm | 55.39 | 107 | 511 |
| 472\_pr | 48.86 | 7.4 | 52 |
| 472\_qc  (not used in calibration) | 46.68 | 0.09 | 0.42 |

One of the things the software can do is produce a “prediction spectrum” (this is what the quant method thinks the spectrum should look like) and then compared it to the actual spectrum. One then can produce a residual spectrum for the features not modeled by the calibration for that predicted sample.

Here is what this residual spectrum looks like. The black spectra are all from production measurements while the red spectrum is from a QC instrument.



This is a huge difference. Now the question is where does this come from? If the sample had and air bubble in it would have shown up in the measure spectrum and would look more like the glass vials. Temperature effects are likely to move band positions but not band intensities. If I did not know the same tube was used I would have you confirm that the sample was measured using the same sample path length. To be frank, I am not sure what the error source is.

Not sure how much help this is.