What does analytical method validation entail for HPLC and LCMS – Primer

The following parameters must be considered to have a validated HPLC method for use with SOP, GMP, GLP and other pharmaceutical, environmental and forensic applications. This is a summary and you should consult your Regulatory Affairs department.

1. **Accuracy**: How close the experimental value is to the real value. The analyst may demonstrate this by comparison to a reference standard, percent **recovery**, and standard addition.

2. **Precision**: According to the International Conference on Harmonization (ICH), **precision** is divided into several sub-sections:

Repeatability: **Precision** under the same operating conditions over a short period of time. This included things like repeated consecutive injections of the same sample and sometimes repeated preparations of the same sample by the same analyst.
Intermediate ***Precision***: Repeated analyses over different days, on instruments, by analysts, and so forth.
Reproducibility: **Precision** of the method over different laboratories. This is typically part of method transfer or collaborative studies.

3.**Linearity**: The accurate correlation of the detector's response to concentration according to a theoretical model of best fit (usually a straight line). Typically the y-intercept and correlation coefficient R^2 are used to assess the model's acceptability.

4. **Range**: The upper and lower bounds for concentrations used in the study.
The **analyte** concentrations must be between these two values.

5. **Limit of Detection (LOD) and Limit of Quantitation (LOQ):** These two are not the same thing but defined as the lowest concentration at which quantitation can be achieved for LOD and the lowest concentration at which the signal can be distinguished from the noise floor for LOD. LOQ is typically defined as a signal-to-noise ratio (SNR) of 10 and LOD as a SNR of 3. The LOD and LOQ can also be defined statistically:

$LOD = 3.3\ S_0/b$

$LOQ = 10\ S_0/b$

where S_0 is the standard deviation of the calibration line and b is the slope. LOD and LOQ are ways of describing sensitivity.

6. **Specificity**: Perhaps the most important attribute of **method validation**, **specificity** describes the

ability of the method to distinguish between the **analyte** and all other matrix components. This may be accomplished by any number of techniques. One way might be chromatographically separating the **analyte** from other matrix components which also give a detector response. Another way is to use a detection method that responds only to the **analyte**, such as in immunological assays. Demonstrating **specificity** in HPLC encompasses studies such as forced degradation, orthogonal separation methods, etc.

7. **Ruggedness**: **Reproducibility** of results when different components are changed such as use of different suppliers of mobile phase solvents, column lots, instruments, analysts, and so on.

8. **Robustness**: The degree to which the method is affected by small but deliberate changes in the method conditions. In an HPLC method, this might include changes to flow rate, column temperature, mobile phase pH, gradient steepness, and so on. The results would then be compared to system suitability criteria such as whether a certain value of resolution is still met or exceeded. **Robustness** should always be kept in mind during method development.

9. **Octanol-Water Partition Coefficient**: The Octanol-Water Partition Coefficient is a physical property used to describe a chemical's lipophilic or hydrophobic properties. It is the ratio of the concentration of your compound in the octanol phase to its concentration in the aqueous phase at equilibrium. Commonly measured and labeled as Log P. Compounds with large non polar structures are usually high values and for compounds with highly polar groups, it is usually very low. The Log P value combined with the pI of the molecule can help predict a compounds retention time on a stationary phase.

10. **Recovery**: This is the amount of your **analyte** that is detected during your method.