Analytical Method Validation: 5-Hydroxytryptophan

A reverse phase HPLC method with UV detection was developed and validated for 5-Hydroxytryptophan. The validation was designed to fit the method's intended purpose and based on requirements set forth in the USP <1225> Validation of Compendial Procedures and USP <621> Chromatography, as well as FDA and ICH guidelines.

Summary of Validation Data

Test performed	Criteria	Results	Pass/Fail
Specificity	No interference between the drug peak and any other peaks	No Interference	**Pass**
System Suitability	The peak areas for the 5 reference standard injections have a Relative Standard Deviation (RSD) of ≤ 2.0% and No interference between the drug peak and any other peaks	% RSD = 0.2 and No Interference Observed	**Pass**
Accuracy	The test results for the drug tested at 3 concentrations must be within 5.0% of the expected result	Low = 100.5% Med = 100.5% High = 100.1%	**Pass**
Filter Qualification	The test results of a filtered sample must be within 2.0% of the test results for an unfiltered sample	Difference PVDF = 0.2% PTFE = 0.8%	**Pass**
Precision	The RSD for triplicate test results at 3 concentrations is ≤ 2.0%	% RSD = 0.3	**Pass**
Linearity	The coefficient of determination (R^2) of all test results is ≥ 0.99 and The Y-Intercept is ≤ 5.0% of the response at the nominal concentration	R^2 = 0.9999 and Y-Intercept = 0.3%	**Pass**

Validation performed according to ARL QUP-027-V1.
Experimental data recorded under ARL 571443-01.
Future analysis of 5-Hydroxytryptophan will follow the guidelines set forth in AMIF-1943.