

# System Suitability and Validation for Chiral Purity Assays of Drug Substances



Measurement of chiral purity is a necessary means of quality control for drug substances that exhibit chiral centers. This article describes a simple and practical approach to setting up system suitability and validation for chiral purity assays.

**T**he measurement of chiral purity is a necessary means of quality control for drug substances that exhibit chiral centers or regions of axial asymmetry. A compound having a single chiral center is likely to exist in two enantiomeric forms, that is, species that appear as mirror images of each other. When present in equal abundance, the composite is termed a racemic mixture or racemate. Matters get increasingly complex with more than one chiral center in a molecule, because the potential number of species is  $2^n$ , where  $n$  is the number of chiral centers. Many compounds exhibit their intended pharmacological effects only in one enantiomeric form. This article deals with the simplest case of two enantiomeric forms for a given compound, though the concepts described herein can be extended for the more complicated situations. The principles of chirality have been well documented (1,2).

Most analytical separations for enantiomers are performed using specialized chiral high performance liquid chromatography (HPLC) columns. The common feature for such columns is the unique spatial arrangement of functional groups attached to the stationary phase support that allow for differential recognition and, hence, different retention times of chiral species. Chiral HPLC has been applied successfully on such columns in reverse-phase, normal-phase, and super-

critical fluid modes. Separation also can be achieved on achiral columns following derivatization of enantiomers to diastereomers. Other approaches to chiral separations include use of gas chromatography and capillary electrophoresis. Numerous applications have been published in these areas (3–6).

Chiral purity assays typically are run in the area percent quantitation mode, where the goal is to determine the percent abundance of the undesired enantiomer relative to the total peak area for both enantiomers. Chiral assays are supplementary to the principal purity assay for drug substances. Release analyses on drug substances should minimally include a specification for enantiomeric purity of the desired component. The undesired species must be treated as an impurity with regard to regulatory requirements on reporting, identification, and safety qualification in time for clinical studies and registration. The pharmaceutical industry recognizes that a chiral impurity can be normalized with the main component assay value generated through a separate weight-percent method. One simply has to multiply the weight-percent parent assay value by the area-percent value for the undesired component to generate a value for the abundance of enantiomeric impurity.

The literature is scant on method, system suitability, and validation require-

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**Table I: Validation parameters recommended for chiral purity assays**

Parameter	Major/Desired	Minor/Undesired
Specificity	No blank interferences Acceptable retention time Acceptable resolution	No blank interferences Acceptable retention time Acceptable resolution
Linearity	80–120% of target assay level Visual inspection of plot Report slope, intercept, correlation coefficient, and residual sum of squares	80–120% of specification level Visual inspection of plot Report slope, intercept, correlation coefficient, and residual sum of squares
Sensitivity	Not applicable	Quantitation Limit at 50–100% of specification with $S/N \geq 10$ Detection limit $S/N \geq 3$
Precision	Repeatability at 100% target assay level. $n=6$ replicates Criterion: RSD NMT 5%	Repeatability at specification level. $n=6$ replicates Criterion: RSD NMT 20%
Accuracy	Inferred once linearity, precision, and specificity are established	Inferred once linearity, precision, and specificity are established
Range	Inferred based on acceptable linearity, accuracy, and precision	Inferred based on acceptable linearity, accuracy, and precision
Analyte stability	Verify stability of prepared sample solutions	Verify stability of prepared sample solutions

ments specifically for chiral purity assays. This is probably because chiral assays are performed using established analytical techniques, primarily chromatography and electrophoresis, to yield impurity data and so the regulations for impurities can simply be followed. One generally should heed the compendial and agency requirements for instrumentation, method validation, impurity reporting, impurity identification, and impurity safety qualification.

### System Suitability and Method Validation

Validation of chiral purity methods should generally follow the compendial–regulatory guidelines (7). The desired component as the major peak would require evaluation of specificity, precision, linearity, accuracy, range, and analyte stability. The undesired component as the minor peak would require evaluation of these same parameters plus sensitivity. Robustness can be demonstrated during method development though it can be part of validation. System suitability is implicit during method validation and routine analyses. Each of these parameters is discussed briefly in the following section and summarized in Table I.

### System Suitability

It is best to begin with system suitability because it demonstrates acceptable

method performance in every run, and acts as a mini-validation in itself. The critical pieces of information to evaluate for performance on chiral assays are resolution, sensitivity, and injector precision.

Peaks of interest must exhibit adequate resolution to quantitate each component separately. Equally important is the need to demonstrate quantitation of a low-level limit for the undesired enantiomer. Imagine a situation with an aging detector lamp and getting deceived into believing a test sample has 100% of the desired component because the undesired one cannot be seen. This principle should be applied to any area-percent assay. Finally, monitoring of injection precision is appropriate to assure that column loading is under control throughout the analysis.

The simplest and most efficient way to address system suitability is if key parameters can be assessed with minimal sample preparations and injections. This can be accomplished by preparing a single reference sample containing the desired and undesired components at a ratio corresponding to the specification. The sample can be used to monitor injector precision by injecting it twice during the analysis and calculating percent difference to determine variability. Resolution can be interpreted from these injections as well.

The criterion or target value should be appropriate to the method but one should aim for a minimum of 1.7 to represent baseline resolution. As for sensitivity, the

criterion can be the target area percent of each species plus a tolerance range. An alternate sensitivity criterion is to verify an acceptable signal-to-noise ratio ( $S/N$ ) typically not less than 10 for the minor component peak.

One can track the capacity factor, theoretical plates, and peak tailing as well for system suitability. Sensitivity and resolution are consequences of retention and peak shape quality. If acceptable sensitivity and resolution are demonstrated in the face of a reasonably small retention time shift or peak shape deterioration such as from column wear, the method can still be considered suitable for intended use.

Chromatographers often recognize the delicate nature of most chiral stationary phases and balance that with the relatively high cost of column replacement.

### Specificity

The method should have no significant interferences with quantitation of the relevant peaks. It is recognized that some methods will include resolution and retention time in this category while others will place these parameters under system suitability. Either way is acceptable as long as they get covered.

### Linearity and Sensitivity

Linearity should be demonstrated at an appropriate range, typically 80–120%, centered about the target levels for the desired and undesired components. Lin-

Table II: Sample preparation scenarios for system suitability

Scenario	Approach
The ideal scenario where both markers are available and each are reasonably pure.	One can spike the minor component into a solution of the major component at the appropriate ratio without a problem.
Both markers are available, however, the major enantiomer contains a low but quantifiable level of the minor component.	One can spike the minor component into a solution of the major component, but just have to account for amount of the minor component already present.
Both markers are available, however, the major enantiomer contains the minor component at a level that exceeds the specification.	This is a problem. An appropriate spike cannot be performed. One must resort to using the major component as a surrogate for the minor component in sensitivity evaluation.
Only a racemic mixture is available.	Prepare mixture to achieve the 100% level of each component. A spike cannot be performed, but one can dilute the mixture and focus on sensitivity for the minor component.
The major component and a racemic mixture are available.	Preparation of a spike is possible. Let the racemate serve as the source of the minor component.
The minor component and a racemic mixture are available.	Having only the individual minor component is of no help here, other than for marking of retention time. Proceed as if one only had the racemic mixture.

earity dovetails with sensitivity for the undesired component, and the span can be expanded to include the quantitation limit. One assumes that the response factor is identical for enantiomers.

Sensitivity is critical only for the undesired component. One should target 50–100% of the specification or target value as the quantitation limit. The typical acceptance criterion for the quantitation limit can be expressed as an S/N of not less than 10, which is consistent with industry practice guidelines. It might be appropriate to determine a detection limit as well because this really helps to establish the boundaries of the assay. Similarly, the typical acceptance criterion for the detection limit can be expressed as an S/N of not less than 3. Sensitivity should be confirmed in every analysis as part of system suitability.

### Precision, Accuracy, and Range

Precision is evaluated as variability in the responses for the relevant components at the target assay–specification levels. The extent of validation can be limited to repeatability or can include intermediate precision, depending upon the stage of development and intended use. Considering that chiral columns have relatively poor lot-to-lot reproducibility and that chiral assays are typically performed in an area percent quantitation mode, having a very tight criterion might not be appropriate. A reasonable precision target for

the major component is  $\leq 5\%$  relative standard deviation (RSD), though this is method-dependent. An appropriate target for the minor component is in the range of  $\leq 20\%$  RSD as one approaches the quantitation limit.

Accuracy can be inferred through demonstration of acceptable specificity, linearity, and precision. Range is established through demonstration of acceptable linearity, precision, and accuracy.

### Analyte Stability

Analyte stability should be evaluated for sample solutions. The experimental design can range from a simple run with repetitive injections to a two-point comparison. Degradation can appear as reduction in target peak size and formation of new impurity peaks. Diode array serves a useful tool in this situation to confirm that no degradation products are formed that are coeluted with the key component peaks. It also would be appropriate to confirm lack of significant racemization between the chiral species.

### General Comments

Validation is performed to demonstrate method suitability for intended purpose and done so in step with regulatory requirements and the stage of project development.

Thus, methods are not validated necessarily just once and the work is done. Method validation can be designed for

simplicity at the early stages of drug development with the goal of generating additional and comprehensive validation data over time on the approach to registration filing. This is perhaps most dramatic with the primary purity chromatographic assay, as it can start out as a simple area percent method, but it is highly probable there will be eventual challenges with new impurities, method changes, and additional validation. By contrast, the evolution for validation will be minimal with chiral purity assays because these can always employ area percent quantitation and impurities are known from the start. Factors that can get more stringent during drug development for both chiral and achiral assays include sensitivity and robustness.

### Practical Considerations

Having been in the contract manufacturing business and supporting client projects with chiral purity assays, we have encountered situations where individual markers for reference samples are not available, and yet the customer wants method evaluation and validation performed anyway. The information in Table II summarizes various scenarios and provides recommendations on how to establish a reference sample for system suitability and method validation.

An example case study reflects an assay of a proprietary drug substance bearing a single chiral center and existing as *R*-form

plus *S*-form enantiomers. The only available materials were the racemic mixture and the desired *R*-form enantiomer, the latter being very pure. In this scenario, the racemate was used as the source for spiking the undesired *S*-form into the isolated *R*-form to generate a system suitability reference sample. The math behind preparing the appropriate sample was quite easy and straightforward. The target assay concentration was 1 mg/mL and the specification was not more than 1% *S*-form in the final product. The reference could be prepared to contain a 99/1 ratio of *R/S* components. In an ideal situation where both individual species were available, the analyst would have set up a procedure to target 0.99 mg/mL *R*-form and 0.01 mg/mL *S*-form. However, because the racemate contained both components at equal abundances, it was necessary to double the racemate concentration for providing the target *S*-form, and it was necessary to then subtract the racemate *R*-form contribution from what the analyst would target for the *R*-form. Thus, a solu-

tion was prepared containing 0.98 mg/mL *R*-form and 0.02 mg/mL racemate to achieve the target ratio.

### Conclusion

This article covered two areas pertinent to chiral purity assays, namely, system suitability and method validation. System suitability checks for acceptable method performance every time the assay is run. Critical parameters were discussed and suggestions were offered on how one can get the most system suitability information from a single sample preparation. Validation of chiral methods follows similar principles to those for impurity assays, but merits a relatively simple design because it is supplementary to the primary assay and employs area percent quantitation.

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