Validation and Implementation of In-Process Control HPLC Assays for Active Pharmaceutical Ingredients

An in-process control (IPC) assay is a monitor of reaction progress applied to process validation and commercial production of an active pharmaceutical ingredient, and it signals whether to proceed with a subsequent unit operation. High performance liquid chromatography (HPLC) is used commonly for quantitative IPC assays, and a current industry practice is to validate such methods. This paper describes a sensible approach for validation and implementation of HPLC IPC assays.

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An in-process control (IPC) is a monitor of reaction progress in the synthesis of an active pharmaceutical ingredient and signals the production chemist to proceed with a subsequent unit operation. IPCs are applied to process validation and commercial production. The decision about whether to use an IPC in a synthesis is established during process development and based on scientific judgment. The IPC assay is a quantitative test employed to monitor reaction progress.

The most common types of IPC testing include chromatographic assay, moisture determination, residual solvents assay, loss on drying, titration, and pH measurement. Although not specifically cited as a regulatory requirement, it is a pharmaceutical industry best practice to validate chromatographic IPC assays. The objective of this paper is to describe a sensible approach for validation and implementation of high performance liquid chromatography (HPLC) IPC assays employed in active pharmaceutical ingredient synthesis. The tactic described here reminds one of the appropriate simplicity of validation and the assay itself based on the intended method purpose.

Method Development

Although it is not the objective of this paper to cover method development, a few comments on this topic are warranted. When the IPC calls for HPLC as the measurement technique, it is usually because there is a need to monitor disappearance and formation of reaction components. Such an assay would require adequate resolution of the reaction species, sufficient sensitivity to quantitate the dwindling component, and typically, the ability to accommodate various sample loads. It might not be necessary to start from scratch and set out on a comprehensive method development project, as one might be able to modify an existing release method. Emphasis should be placed on shortening run time, resolution of key components, optimizing sensitivity, and understanding potential interferences by reaction components. Robustness evaluation should be performed as appropriate.

An important facet of IPC testing is speed, as the production chemist requires a fast data turnaround. The strategy is to develop an IPC assay that can be performed with just several injections that include blank, standard, and sample. System suitability checks in the assay should be employed to support the expected variability in the IPC sample concentration, and acceptance limits should be based on appropriate method development experiments.

Method Validation

The validation exercise can be kept simple for IPC assays. As with any validation of a quantitative assay, one must consider key parameters such as accuracy, precision, linearity, sensitivity, and specificity required to demonstrate that the method is suitable for intended use. Documents published by the

Table I: Validation parameters and results summary from case study

Parameter	Criterion	Result
Specificity	No interferences by blank with quantitation of RA and CB peaks.	Blank injections exhibited no interferences with RA and CB peaks.
Linearity	Plot of signal versus concentration shows linear response for RA and CB in the range 20–200% of their target standard concentrations.	Correlation coefficients for CB and RA components exceeded 0.999 using either least-squares or origin-force models.
Sensitivity	Quantitation limit defined as lowest RA concentration where signal/noise ratio is not less than 10.	Using the linearity sample set, the quantitation limit was defined as 20% of the RA standard concentration.
Precision	RSD of response factors from triplicate CB/RA preparations at the standard concentration not more than 5%.	RSD for CB = 0.5%. RSD for RA = 0.9%.
Accuracy	Inferred once criteria for specificity, linearity, and precision are met.	Criteria for specificity, linearity, and precision met.
System Suitability	Signal/noise ratio for RA peak in standard should be not less than 20. Resolution not less than 1.7 for CB/RA pair.	Signal/noise ratio for RA > 60. Resolution for CB/RA pair > 3.

Validation parameters are described in the text. The terms RA and CB refer to the reactant and product components, respectively. RSD = relative standard deviation

International Conference on Harmonization (1,2) provide a framework for method validation of commercial drug substance and drug product release assays, but there is no formal requirement to follow these guidelines for IPC assays. Our recommended approach to validating a chromatographic IPC assay for reaction progress is illustrated by the following case study of a two-component reaction mixture.

Case Study

This case study involves the two-component reaction mixture containing the proprietary reactant RA and crude product CB, where the IPC required a 0.5% limit of RA present in the reaction mixture before the next filtration step could proceed. The IPC assay was developed by refining an existing release method to shorten run time and optimize target assay concentrations, while maintaining resolution and sensitivity of the key reaction components. Analysis of reaction mixtures and examination of spectral data confirmed that reaction mixture components and sample diluent would not interfere with quantitation of the RA and CB peaks.

The acceptance limit of this IPC was to have no more than 0.5% of RA by area percent. Knowing the differential response factor values, a standard preparation was established containing CB and RA in proportions that gave an approximate 99.5/0.5 ratio by area percent. The column load for the standard and samples was targeted at 10 μg with respect to the sum of the CB and RA components, and this provided adequate sensitivity for the minor RA peak while keeping the major CB peak response within detector range. With this information, the standard concentration was set at 1 mg/mL CB and 0.0025 mg/mL RA. Because the anticipated concentration of IPC mixtures was 100 ± 20 mg/mL, samples would be diluted 100-fold for analysis in order to achieve a column load similar to that for the standard. The chromatographic conditions and a profile of the standard are shown in Figure 1.

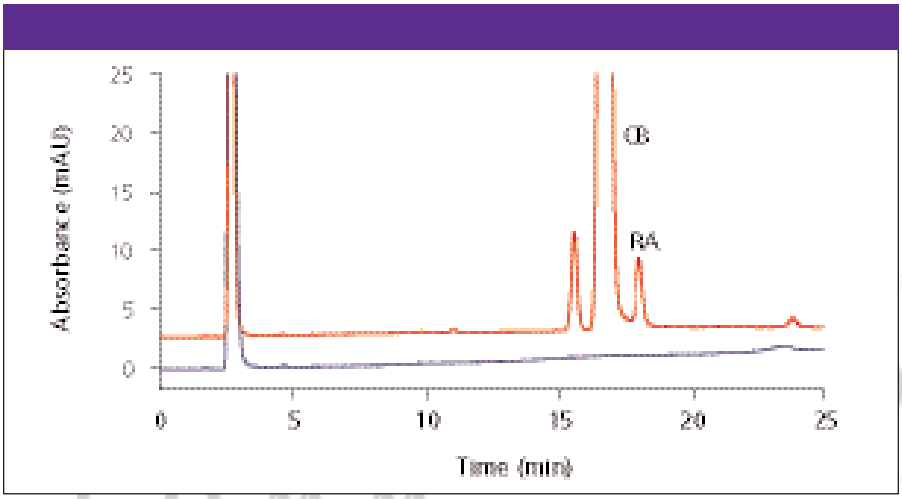

Figure 1: Chromatograms of (top) the assay standard containing reactant RA and product CB at area percent proportions that correspond to the acceptance limit of reaction progress and (bottom) a diluent blank injection. Column: 250 mm × 4.6 mm, 5-μm d_p C18; gradient: 55–80% acetonitrile over 25 min; flow rate: 1.2 mL/min; temperature: 25 °C; target load: 10 μg (RA plus CB); detection: UV absorbance at 257 nm.

This validation was conducted using a one-analyst/one-run design for specificity, linearity, sensitivity, precision, and accuracy. The protocol requirements and results are summarized in Table I. Specificity was demonstrated as the lack of interference with CB and RA quantitation by the sample diluent and reaction mixture components. Linearity and sensitivity were evaluated by analyzing CB–RA mixtures at 20–200% of the target concentrations, holding constant the relative proportions of the two components. Regression analysis data were consistent with a linear response for both CB and RA. The quantitation limit was defined at 20% of the RA target standard concentration, which was the lowest level where the signal-to-noise ratio (S/N) remained above 10. Because this IPC measures disappearance of RA, and because the submitted sample is likely to show reaction near completion, the quantitation limit is not relevant for CB. Precision was confirmed by preparing the standard in triplicate and calculating the variability. Using relative standard deviation as the metric, the acceptance criterion was met for both CB and RA. Accuracy was inferred because the criteria for specificity, linearity, and precision were met.

Discussion

The IPC assay for a two-component reaction mixture can be accomplished with single injections of blank, standard, and sample. Depending upon the run time per sample and the set-up in anticipation of the

pending sample submission, results can be turned around typically within an hour. Execution of appropriate validation experiments sets the stage for reliable and rapid analysis.

Linearity and sensitivity: The linearity–sensitivity determination allows one to assess whether the detector responses for the reaction components are proportional to their concentration within a practical range dictated by realistic variability in sample concentration. Because one reaction component might be present in significant excess of the other, such as the 200-fold difference in the case study, it is important to establish the range at which linearity is preserved for both components.

The single standard injection serves as an indicator of sensitivity. Given that IPC samples submitted for analysis typically will have variable concentrations, there might be a concern about sensitivity for a minor reaction component being monitored should the IPC sample concentration be lower than the standard concentration, and the question of whether the IPC sample must be prepared using a different dilution scheme might arise. By setting a minimum S/N criterion for the minor component in the standard at 20 for system suitability, and knowing that a minimum value of 10 is appropriate for a quantitation limit, the IPC sample load can be 50% relative to the standard without the need to reprepare the sample, and assay sensitivity is assured. Application of this concept is extremely useful in early stages of process development, when reaction component concentrations can be highly variable. For situations in which it is not possible to isolate a transient reaction component in sufficient quantity or of adequate purity to evaluate assay sensitivity, a diluted standard of the major component can be prepared as a surrogate of the minor component using the assumption that both reaction species have similar response factors.

Specificity, precision, and accuracy: Confirmation of specificity assures that the diluent blank and ancillary reaction components do not interfere with the analytes of interest. The precision validation addresses reproducibility of the sample preparation at concentrations that correspond to the acceptance limits in the IPC assay. Evaluation of accuracy ideally would be performed with matrix-spiking experiments. However, the spiking of a reaction cocktail lacking the key reactant and product components would very likely result in an unstable sample preparation, given the probability of reaction occurring in the sample. For this reason, it is appropriate to infer accuracy based upon successful demonstration of specificity, linearity, and precision.

Summary

Validation experiments for IPC assays should follow a simple protocol consistent with the intended purpose of the method. Appropriately short analysis sequences and efficient use of system suitability checks should be incorporated into the assay for the benefit of fast data turnaround with assurance of method performance.

References

(1) International Conference on Harmonization Guideline for Industry, "Q2A Text on Validation of Analytical Procedures," October 1994.
(2) International Conference on Harmonization Guideline for Industry, "Q2B Validation of Analytical Procedures: Methodology," November 1996.

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