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Short communication

Investigation of out-of-specification (OOS) results during content uniformity validation: identification of acid-induced degradation caused by CIP residues

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Introduction

Out-of-specification (OOS) results represent critical deviations within pharmaceutical quality systems, requiring systematic and scientifically justified investigation to determine their origin (FDA, 2006). Such deviations may arise from analytical errors, process variability, or chemical instability of the active pharmaceutical ingredient (API).

Despite well-established regulatory frameworks for OOS investigations, real-case studies demonstrating direct chemical causes remain limited. Therefore, the objective of this study was to investigate the root cause of OOS results observed during validation of the content uniformity test and to evaluate potential degradation mechanisms of the active substance.

Materials and methods

A structured, phase-based OOS investigation was conducted in accordance with Good Manufacturing Practice (GMP) principles, FDA guidance for OOS investigations, and relevant ICH guidelines (FDA, 2006; ICH Q1A(R2), 2003).

Phase I included a detailed evaluation of the analytical procedure, including verification of calculations, sample preparation, instrument performance, and method execution, in order to exclude laboratory error. Phase II involved hypothesis-driven experimental studies aimed at identifying the root cause.

Two hypotheses were evaluated:

(i) moisture-induced degradation; and
(ii) acid-catalyzed degradation due to potential residual contaminants.

Analytical testing was performed using a validated high-performance liquid chromatography (HPLC) method. Additionally, results from forced degradation studies were used to support interpretation of degradation pathways.

Results and discussion

The investigation demonstrated that exposure of the sample to residual cleaning solution (CIP) resulted in a substantial decrease in active substance content to approximately 52%, accompanied by a corresponding increase in impurity C to approximately 51%.

The near mass balance between the loss of active substance and formation of impurity C confirms that the observed OOS result is a consequence of a direct chemical transformation rather than analytical variability. This finding is consistent with the principles of stability-indicating methods, where

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degradation products are quantitatively related to the loss of the parent compound (ICH Q1A(R2), 2003).

The reproducibility of the OOS result, combined with the absence of analytical errors identified during Phase I, indicates the presence of a systematic factor.

Experimental testing of the first hypothesis demonstrated that moisture alone did not induce degradation, as the active substance content remained within acceptable limits and no increase in impurity C was observed. This confirms that water, although a nucleophile, is not sufficient to initiate degradation in the absence of catalytic conditions (Clayden et al., 2012).

In contrast, exposure to acidic conditions resulted in rapid and extensive degradation. These findings are consistent with forced degradation studies, where acidic conditions produced the highest levels of impurity C, confirming that hydrolysis represents the dominant degradation pathway.

The degradation mechanism can be explained by acid-catalyzed hydrolysis. Under acidic conditions, protonation of susceptible functional groups increases the electrophilicity of the molecule, facilitating nucleophilic attack by water and subsequent cleavage of chemical bonds (Carey & Sundberg, 2007). In the case of investigated compound, the sulfonate moiety acts as a favorable leaving group, leading to formation of impurity C as the primary degradation product (Clayden et al., 2012).

The results strongly indicate that residual CIP solution, most likely retained in inadequately rinsed laboratory glassware, represents the root cause of the observed OOS results. Even trace levels of acidic contamination can significantly alter the pH of the system and catalyze hydrolytic degradation.

These findings have direct practical implications, as they demonstrate that inadequate cleaning procedures may lead to false OOS results and misinterpretation of product quality.

Conclusion

The obtained OOS investigation identified residual CIP solution as the root cause of the observed deviation in content uniformity. The results demonstrate that acid-induced hydrolytic degradation leads to significant loss of active substance and formation of impurity C.

This study highlights the critical importance of proper cleaning and rinsing procedures for laboratory glassware. Residual cleaning agents can significantly compromise analytical results, emphasizing the need for strict control and validation of cleaning processes.

The findings are particularly relevant for routine pharmaceutical quality control laboratories, where unnoticed contamination may lead to incorrect conclusions regarding product quality and regulatory compliance.

References

Carey F.A. & Sundberg, R.J., 2007. Advanced Organic Chemistry, Part A: Structure and Mechanisms, 5th ed., Springer, New York. https://doi.org/10.1007/978-0-387-44899-2

Clayden, J., Greeves, N., Warren, S., 2012. Organic Chemistry, 2nd ed. Oxford University Press, Oxford, pp. 209–210.

International Council for Harmonisation (ICH), 2003. Q1A(R2): Stability Testing of New Drug Substances and Products. https://www.ich.org/page/quality-guidelines

U.S. Food and Drug Administration (FDA), 2006. Guidance for Industry: Investigating Out-of-Specification (OOS) Test Results for Pharmaceutical Production. https://www.fda.gov/media/71001/download