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Short communication

Strategies for mass balance evaluation in stability-indicating method development

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Introduction

Mass balance (MB) has been recognized as critical component of pharmaceutical development, providing a quantitative assessment of the relationship between the loss of active pharmaceutical ingredient (API) and the formation of degradation products while considering analytical variability. Although conceptually simple, MB determination is influenced by numerous analytical and experimental factors that can significantly affect the final result (ICH, 2003). Therefore, minimizing the variability associated with critical factors is essential to ensure the reliability and accuracy of MB calculations.

Despite extensive research on forced degradation and stability testing, relatively few studies have adopted a systematic and scientifically justified approach for evaluating the factors that govern MB outcomes. Most investigations focus on regulatory expectations and degradation pathways, with limited emphasis on how stress conditions, analytical methods, and impurity characterization collectively influence MB results (Baertschi et al., 2013; Hokanson, 2006).

This study proposes a risk-based approach for MB determination in forced degradation studies, emphasizing the identification, assessment, and control of critical variables affecting the final result.

Simvastatin (SIM) was selected as the model compound due to its widespread use and well-documented instability, making it a suitable candidate for evaluating the proposed methodology.

Materials and methods

SIM *CRS* and SIM for peak identification were provided by the EDQM (Strasbourg, France). All reagents used were of analytical grade. SIM samples were received from Teva Pharmaceutical Industries Ltd., Israel and Ranbaxy Research Laboratory (Gurgaon, India). SIM samples were subjected to forced degradation under acidic, alkaline, oxidative, thermal and photolytic stress conditions. All stress studies were conducted at an initial concentration of 1 mg/mL, using amber glassware to minimize the risk of photodegradation.

Determination of the MB was performed by determination of the API content using a validated HPLC method (Hadzieva Gigovska et al., 2018a), determination of water content by Karl Fischer titration, and characterization of impurities before and after forced degradation studies. Enantiomeric purity was assessed using the HPLC method described in the European Pharmacopoeia

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monograph for SIM. Residual solvents were determined by headspace gas chromatography with flame ionization detection (HS-GC-FID) according to Ph. Eur. 2.4.24, while inorganic impurities were quantified by inductively coupled plasma (ICP) analysis. Organic impurities were quantified using a previously reported HPLC method (Hadzieva Gigovska et al., 2018b).

Results and discussion

To establish a scientifically sound and multifactorial strategy aligned with the principles of a risk-based approach, mass balance (MB) was considered a complex analytical process rather than a single numerical outcome. The first stage involved identifying potential risk factors using a cause-and-effect (Ishikawa) diagram. Sources of variability were grouped into six categories: Equipment, Method, Measurements and Calculations, Materials, Environment, and Personnel enabling systematic identification of variables that could significantly impact MB determination.

Following risk identification, a structured risk assessment was performed using Failure Mode and Effects Analysis (FMEA). Each variable was evaluated according to its likelihood of occurrence, potential impact on the analytical result, and detectability. Risk evaluation was based on the calculation of the Risk Priority Number (RPN), allowing all identified factors to be quantitatively ranked. Subsequently, the risks were classified into low, medium and high-risk categories using a traffic-light ranking system, providing a clear visualization of their relative significance. To further support the prioritization process, Pareto analysis was applied, enabling the identification of the limited number of critical parameters responsible for the majority of the overall risk.

The parameters associated with the highest risk (like sample preparation process, method for assay of API and method for organic impurities) were selected for targeted experimental investigation. Experimental studies were conducted to determine their actual influence on MB determination and to establish appropriate control strategies. In addition, measurement uncertainty was estimated for all critical parameters and incorporated into a secondary risk assessment.

The reassessment demonstrated a significant reduction in the risk associated with the previously identified critical variables, confirming the effectiveness of the implemented controls. As a result, the impact of these factors on the final MB calculation was minimized to an acceptable level with the combined expanded uncertainty remaining within ±2%. The overall approach provided a robust and scientifically justified framework for MB determination, increasing confidence in the analytical results and ensuring their reliability, accuracy, and suitability for regulatory and scientific purposes.

Conclusion

In conclusion, the proposed strategy establishes a systematic and scientifically sound framework for MB evaluation in FDS. By integrating scientific and practical knowledge, this approach successfully mitigates the risks associated with data variability. This framework ensures the validity and reproducibility of the analytical data and confirms the stability-indicating capability of the method. Moreover, the proposed analytical–statistical approach, demonstrated using simvastatin as a model compound, is broadly applicable to other pharmaceutical molecules.

References

Baertschi, S.W., et al. 2013. Assessing mass balance in pharmaceutical drug products: New insights into an old topic. TrAC Trends Anal Chem. 49, 126–136. https://doi.org/10.1016/j.trac.2013.04.008

Hadzieva Gigovska, M., et al. 2018a. Chemometrically assisted optimization, development and validation of UPLC method for the analysis of SIM. Maced. Pharm. Bull. 64(1), 25–38. https://doi.org/10.33320/maced.pharm.bull.2018.64.01.003

Hadzieva Gigovska, M., et al. 2018b. Comprehensive Assessment of Degradation Behavior of SIM by UHPLC/MS Method, Employing Experimental Design Methodology. Int. J. Anal. Chem. 2018. https://doi.org/10.1155/2018/8254891

Hokanson, G.C. 2006. Reconciling Mass Balance in FDS. Pharm Technol. 30(10), 86–95.

ICH, 2003. Q1A(R2): Stability Testing of New Drug Substances and Products. International Council for Harmonisation