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Short communication

Scientific approach and implementation of a measurement uncertainty in mass balance determination

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Introduction

Mass balance (MB) is a concept used in the pharmaceutical industry to determine the possible loss of the active pharmaceutical ingredient (API) and increase in degradation products, due consideration of the margin of analytical error (ICH, 2003). Although it is a simple concept, its determination is a real challenge because there are many critical factors that have significant influence on the results.

Forced degradation studies (FDS) are performed in order to understand the degradation pathways of API. During these studies, MB determination is performed to evaluate the correlation between the degradation of the API and the measured increase in the amount of degradation products (Hokanson et al., 2006).

Based on the literature review and the findings so far (Baertschi et al. 2013; Hokanson et al., 2006), it could be said that there is a limited number of studies presenting a systematic approach for assessing the critical factors that influence the results of the MB study (especially in FDS). Far from our knowledge, no report exists on application of measurement uncertainty (MU) in them (Baertschi et al. 2013; Hokanson et al., 2006). Therefore need of scientific approach for determination of MB in FDS that will include measurement uncertainty is recognized.

The purpose of this study is to propose a scientific approach for estimation of the MU for the MB determination in FDS, and to highlight the advantage of its application in order to obtain a valid analytical result. Statin molecule was taken as a model substance, because this API is important not only from the pharmaceutical, but also from the analytical point of view due to its proven inherent instability.

Materials and methods

Statin samples were received from Teva Pharmaceutical Industries Ltd., Israel and Ranbaxy Research Laboratory (Gurgaon, India). Reference standards were provided by the EDQM (Strasbourg, France). All reagents used were of analytical grade.

Samples were subjected to stress under acidic, alkaline, oxidative, thermal and photolytic conditions. All stress studies were performed at an initial concentration of 1 mg/mL in amber color glassware in order to protect the solutions from light degradation.

Determination of the MB was performed by: determination of API content by HPLC method (Hadzieva Gigovska et al., 2018a); determination of the water content (by Karl Fisher titration); determination of the impurities (by various techniques) before and after the forced degradation studies. The enantiomers were quantified by high performance liquid chromatography (HPLC) method proposed in the pharmacopeia monograph for the API. For residual solvent analysis, headspace gas chromatography – flame ionization detector (GC-FID) analysis was carried out as stated in Ph. Eur. 2.4.24 (European Pharmacopoeia 10.0). In this research, inductive coupled plasma (ICP) was performed to analyze inorganic impurities. Organic impurities were quantified using HPLC method (Hadzieva Gigovska et al., 2018b).

Results and discussion

The importance of the estimation of MU of the results of MB determination is seen from the definition

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itself, where it is stated that the determination of the MB is performed taking into account the possibility of analytical error. MU is related to the result of the performed measurement and characterizes the dispersion of the values of the measured quantity.

In order to build a scientific multifactorial strategy following the principles of risk-based approach, during the evaluation of MB, all variables that could have an impact on the result were taken into account, ranked by the intensity and severity and the most risky variables were selected for further study. Based on the risk assessment analysis, several parameters were identified as having the largest impact to mass balance like the sample preparation, problems with quantification and relative responses. Special attention was dedicated to the development, optimization and validation of the HPLC methods used for quantification. All of them include computer-assisted approach which has involved the adaptation of diverse chemometric techniques. In addition, it can be assumed that all impurities have been detected and quantified and that results of the individual techniques have been appropriately combined. Also, chromatographic peak purity, as one of the tools mentioned by ICH to demonstrate specificity, was used (ICH, 2006). Obtained results indicated absence of co-eluting peaks with the main peaks. As additional proof for the separation power of the methods flow injection technique was used comparing the total integrated area of bolus peak with and without the column in place. Differences in relative response factor (RRfs) are perhaps the most common contributor to analytical mass imbalance, so the RRfs were determinate using the ratio of the slope of the calibration curve of each impurity to that of the API. The GC analysis of residual solvents showed that they are present in small quantities (ppm) with a low impact of the final results and are not evaluated further. The obtained results showed that contribution of the results for nonvolatile inorganic, as well as the result for the volatile organic impurities, was minimal and therefore, can be ignored in the final calculation of the MB and MU. To increase the reliability of the information obtained from the measurement results, the MU was determined according to the Eurachem/Citac Guide. In calculation of MU for assay of API "bottom up" approach and for related and degradation products "top down" approach were used. When reporting the results obtained from determining the MB with MU included, an interval (result) is obtained in which the values with a higher degree of confidence (95%) are assumed to be found. All obtained results for MB meet the generally accepted criterion and accordingly the obtained values for combined MU are within ± 2%. According to these results, the uncertainties associated with accuracy and precision were the most significant, contributing to 57% of the overall uncertainty. On the other hand, repeatability of standard peak areas was almost insignificant (less than 5% of overall uncertainty).

The analysis of MU proved to be useful in development of a well-characterized methodology, suitable for determining the MB that integrates scientific and practical knowledge. The ultimate result is an understanding of the risks, and step that need to be followed when evaluating MB, reducing the variability of the critical factors and ensuring the validity of the obtained results.

Conclusion

Implementing MU is one of the approaches that devoutly make scientist to understand the process closely. The study showed that MU can be successfully implemented in MB calculation. Analyst also gains confidence in the obtained results as this approach provides understanding between the variables and performance. The overall advantage of the approach is improved proficiency, reduced variability and gained knowledge.

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