Macedonian Pharmaceutical Bulletin, 72 (Suppl 1) 221 - 222 (2026)
Online ISSN: 1857-8969
DOI: 10.33320/maced.pharm.bull.2026.72.03.105
Short communication

Comprehensive approach in Out-of-Trend investigation in stability studies of medicinal products

Aleksandra Petrovska*, Tatjana Spanchevska Kaeva, Viktorija Jakimovska Pokupec, Marina Chachorovska, Ornela Kuzmanovska, Elizabeta Karadzinska

Alkaloid AD Skopje, blvd. Aleksandar Makedonski 12, 1000 Skopje, Republic of North Macedonia

Introduction

According to the ICH Q1E guideline (Evaluation for stability data, 2003), annually conducted stability studies on medicinal products and proper assessment are essential to support product or process modifications, thereby ensuring continuous quality in production batches in established product shelf life. In order to identify the patterns that indicate change over time and to be proactive in the identification of potential Out-of-Specification (OOS) results, trend analyses are used (ECA Analytical Quality Control Working Group, 2016).

In the stability study of Alkaloid's in-house medicinal product containing an active substance from the benzodiazepine class of drugs, results for "Any unspecified impurity" with an RRT~0.27 were obtained in a range from 0.25% to 0.29%, which are within the shelf life specification limit of "Not more than 0.30%", identified as OOT according to applied statistics and observed adverse trend compared to other historical stability batches.

This study aimed to conduct a thorough investigation by performing a forced degradation study and applying an integrated HPLC-DAD/HRMS analytical method in order to identify and prove the root cause for OOT results and provide a more accurate estimation of impurities during the quality evaluation of the medicinal product.

Materials and methods

HPLC-DAD/HRMS method

Analyses were carried out on a Thermo Scientific Vanquish HPLC system, equipped with a DAD detector. Separation was achieved on column: Synergi Polar, 150 mm x 4,6 mm, i.d 4 µm, at temperature 25 °C. The mobile phase consisted of mobile phase A: 50mM ammonium acetate buffer pH 4,2 and methanol in a ratio 44:56 *%V/V*, and mobile phase B: 50mM ammonium acetate buffer pH 4,2 and methanol in a ratio 5:95 *%V/V*. Flow rate is 0.9 mL/min, gradient elution and run time of 50 minutes. Detection UV at 254 nm and injection volume 25 µL (Rocha Gonsalves et al., 2010).

Mass spectrometer condition

The MS analysis was performed on a Thermo Scientific Orbitrap Exploris 120 High Resolution mass spectrometer, equipped with heated electrospray ionization (HESI) in positive ionization mode with source voltage 4000V, sheath gas 15 (Arb), aux gas 5 (Arb), sweep gas 0 (Arb), capillary temperature 275 °C and source heater temperature 300 °C. MS data were acquired in positive ionization mode, with a full scan covered mass range of *m/z* 100-1000, and with data depended additional MS^2 scan events.

*apetrovska@alkaloid.com.mk

Materials

Active substance from the manufacturer Centaur, belonging to the benzodiazepine class of drugs. Secondary reference standard prepared in Alkaloid AD Skopje. Ammonium acetate (p.a.), Glacial acetic acid (p.a.), Methanol (HPLC grade), Purified water. The batch named as placebo batch consisted of Lactose monohydrate from Meggle GmbH & Co., Starch 1500 from Colorcon, Magnesium stearate from FACI S.p.a., Polyplasdone from Ashland, Tween 80 from Croda, and Povidone K25 from BTC Europe GmbH. Medicinal product batch consisted of the same excipients in the same quantity as the placebo batch and active substance in a concentration of 0.25*% w/w.*

Results and discussion

In a review of the impact of the quality of the active substance, raw materials, and manufacturing process of a concerned OOT medicinal product batches, no deviation or nonconformity has been observed. Review of the analytical performance demonstrates that, during product testing throughout the lifecycle, the present analytical method HPLC-DAD is specific and selective in the identification of peaks from the diluent, excipients and API related impurities. Declared OOT impurity with RRT~0.27 exhibits a UV spectrum with a maximum absorbance λ_{max} = 248 ± 2 nm. Within the OOT investigation, two batches for investigation purposes only have been manufactured using the same conditions regarding raw materials and equipment. The first, placebo batch and the second batch, an medicinal product batch containing an active ingredient, were exposed to stress conditions at 40 °C and 75% RH for 30 days. In testing after 30 days, the same unspecified impurity with an RRT~0.27 was increased in both batches to a level of 0.30%. Since the impurity was observed in the formulation without API, it has been concluded that it may be an excipient generated impurity, initiated by increased temperature and humidity.

A suitable analytical technique, such as HPLC-DAD/HRMS, was applied for the identification and characterization of the unspecified impurity, to confirm that the impurity originates exclusively from the excipients present in the formulation. By comparison between the obtained MS spectrum of the active component and the obtained MS spectra from the peak of interest, an evident difference in spectra has been observed. Namely, the presence of a great amount of noise and many fragments atypical for API fragmentation was observed, indicating a structure of a more complex and specific nature, such as a component of the placebo. With the use of Orbitrap Exploris 120, as a high resolution mass spectrometer, additional fragments detected at mass ranges of 848 *m/z* and 757 *m/z* supported the notion that placebo would be origin of the unknown peak, as such large *m/z* values corresponded to larger molecular weights (typical for components of the placebo).

An identical MS profile of the peak of interest was observed in all analyzed samples in which it was detected. This confirms that the peak originates from the placebo in product formulation and, as such, it can be disregarded and further structure elucidation is not necessary.

Conclusion

Since the origin of the peak is confirmed as a placebo, the analytical method for related and degradation products has been revised as follows: unspecified impurity with an RRT of about 0.27 is excluded from quantification under the disregard criterion. The proposed change was justified as having no impact on the efficacy or safety of Alkaloid's in-house medicinal product, which will remain fit for its intended use in terms of quality.

References

ICH Q1E: Evaluation for stability data, February 2003.

Laboratory Data Management Guidance, Out of Expectation (OOE) and Out of Trend (OOT) results, 2016. ECA Analytical Quality Control Working Group. https://analytical.gmp-compliance.org/

Rocha Gonsalves, A. et al., 2010. Identification of Alprazolam and its degradation products using LC-MS-MS. ARKIVOC 2010 (v). 128-141. https://doi.org/10.3998/ark.5550190.0011.513