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Short communication

Risk-based assessment and targeted LC–MS evaluation of small nitrosamine impurities in anticoagulant finished drug product

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Introduction

The presence of small nitrosamine impurities in pharmaceutical products has become a critical quality and safety concern due to their recognized carcinogenic potency and the low acceptable limits established for their control (EMA, 2020).

Their formation is commonly associated with amines originating from reagents used in the synthesis of active pharmaceutical ingredients (APIs), which may react with nitrosating agents. Therefore, assessment of nitrosamine risk requires detailed knowledge of the API synthetic process (López-Rodríguez et al., 2020; Schlingemann et al., 2023). These compounds also present analytical challenges due to their low molecular weight and structural similarity, requiring detection at trace levels and adequate separation in complex matrices (James and Edge, 2021).

In this paper, risk-based assessment was applied to anticoagulant finished drug product, focusing on the API synthetic route and amine-containing materials as potential sources of small nitrosamines. This enables targeted selection of relevant nitrosamines, followed by development of a selective and sensitive LC–MS method for their determination in the finished drug product.

Materials and methods

A risk-based assessment evaluated the API synthesis route as one of the potential pathways for nitrosamine formation, alongside other contributing factors in the finished product such as product composition, packaging and the manufacturing process. Based on this review, specific small nitrosamines were selected for analytical testing in the finished drug product.

Quantitative analysis of the selected nitrosamines was performed on a Thermo Scientific Vanquish UHPLC system equipped with an Orbitrap Exploris 120 mass spectrometer. Chromatographic separation was achieved on a C18 column (Thermo Hypersil Gold, 150mm×4.6mm,3.0µm), using 0.1% formic acid in water and methanol as mobile phases under gradient elution ranging from 17% to 95% methanol. The flow rate was 0.5 mL/min, column temperature was set to 40 °C and injection volume to 25 µL. All nitrosamine impurities were analyzed using selected ion monitoring (SIM) scan and a heated electrospray ionization (HESI) source operated in positive ionization mode.

Nitrosamine impurity standards were obtained from the EDQM-Strasbourg, France. Standard solution was prepared using a water–methanol

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mixture (20:80, *v/v*) as diluent. Finished drug product samples were prepared by dissolving of finely ground tablets in the same diluent using magnetic stirrer, followed by centrifugation and filtration prior to analysis.The analytical method was evaluated for suitability in terms of sensitivity, precision, and accuracy at trace levels relevant to current regulatory expectations.

Results and discussion

The risk assessment identified the API synthetic route as the primary potential source of small nitrosamine impurities. Evaluation of process materials revealed the presence of amine-containing reagents and solvents, including N,N-dimethylformamide (DMF), triethylamine (TEA), tetrabutylammonium bromide (TBAB), and morpholine (MOR), as potential nitrosamine precursors. Focus was placed on DMF and TEA, specified as residual solvents, due to their potential persistence beyond synthesis and their association with N-Nitroso-dimethylamine (NDMA) and N-Nitroso-diethylamine (NDEA) formation, respectively. MOR and TBAB, although limited to the synthetic stage, were included to ensure a complete evaluation of potential sources, with MOR linked to N-Nitroso-morpholine (NMOR) formation and TBAB linked to N-nitroso-dibutylamine (NDBA) formation.

In addition, nitrosamine formation requires both amine precursors and nitrosating conditions, which may be present as trace impurities or process-related factors. As these cannot be fully excluded, the assessment focused on plausible formation pathways.

Method development was guided by differences in polarity and retention behavior among target nitrosamines, API and its related impurities. A balanced gradient chromatographic approach enabled adequate retention and separation, while optimization of the initial gradient composition improved peak shape and selectivity of early-eluting nitrosamines. Optimization of sample preparation was also required due to limited API solubility and the need for high sample concentrations for this kind of determinations. A water–methanol mixture (20:80, *v/v*) was selected as the most appropriate diluent, providing adequate solubility and consistent analyte recovery.

The final method achieved complete separation of structurally similar nitrosamines with appropriate peak shape and signal response. High sensitivity was demonstrated, with LOQs below 10% of the acceptable intake limits, together with acceptable precision and accuracy.

Analysis of the finished drug product with the developed method showed no detectable levels of the targeted nitrosamines. This was further confirmed in samples subjected to accelerated stability conditions, indicating the absence of nitrosamine formation during storage of drug product.

Conclusion

This study demonstrates that a risk-based approach enables targeted identification of relevant nitrosamine impurities and supports focused and efficient evaluation beyond routine screening.

A selective and sensitive LC–MS method was developed for the determination of targeted small nitrosamines at trace levels. None of the targeted nitrosamines were detected in the analyzed samples, however, their evaluation was justified by the presence of amine-containing materials in the API synthesis. Overall, the study demonstrates that the applied risk-based approach, combined with targeted LC–MS analysis, is suitable for the assessment and control of nitrosamine impurities, ensuring the quality and safety of the anticoagulant finished drug product.

References

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