**The challenges and innovation in trace analysis of nitrosamines to demonstrate patient safety - the story so far**

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The risk of the presence of trace nitrosamine impurities has been a major issues for the global pharmaceutical industry over the past six years. It has resulted in product recalls and has been high profile within the media. From the early focus on trace nitrosamine levels in the drug substance, the scope expanded to the drug product, associated excipients and the risk of nitrosamine formation in packaging processes. As the scope has expanded, the trace analysis challenge has grown.

Within AstraZeneca, a vast range of activities have been undertaken to meet the technical challenge of nitrosamine trace analysis. This has resulted in the development of robust and transferrable methods, that ensure the supply of safe medicines to patients throughout the globe. In addition, there has been significant scientific progress within the industry to understand the root causes of nitrosamine formation, across numerous drug product formulations.

For trace quantitation of nitrosamines, we have standardised on the Thermo Fisher Orbitrap platform. High resolution LC-MS/MS and GC-MS methods have been developed to meet strict regulatory safety criteria, with requirements for both LOQs and LODs as low as single digit parts per billion (ppb). A number of methods have required analytical technology transfer to internal operations quality control (QC) laboratories or external contract research organisations (CROs). This has delivered the on-going testing of drug products to allow release of the medicines to patients.

During method development and transfer, issues related to robustness, sample preparation, unexpected sources of nitrosamine contamination and the risk of false positives were identified, investigated and controlled. In parallel, opportunities for innovation have been explored. A collaboration with industry partners in a round-robin study has evaluated method robustness and reproducibility across multiple mass spectrometry platforms.1

The quantitation of trace levels of nitrites in a broad range of excipients employed in the formulation of the drug products has been a further area of focus. These measurements are essential to define the risk of nitrosamine formation in a drug product and to inform the development of control strategy.

Though we have successfully developed and implemented trace analysis methods that demonstrate product safety, we have continued to innovate in our, with two key areas of focus:

**(i) Automation**

Automation has been applied to sample preparation for the analysis of metformin extended-release products.2 The automation of the effective but laborious dispersant-first dispersive liquid-liquid microextraction (DF-DLLME) method provided valuable benefits. Sample preparation was reduced from a 7 hours to 2 hours without the need for an analytical scientist’s presence.

**(ii) LC- Exact Matching Isotope Dilution Mass Spectrometry (IDMS)**

Exact matching IDMS is the gold standard method for trace quantitation due to its enhanced accuracy and precision thus low uncertainty. As a result, IDMS is commonly applied for the certification of reference standards and materials. Therefore, we have investigated IDMS to understand the current uncertainty budget associated with trace analysis methods and determine the true variability of results at trace levels. The uncertainty budget derived from the IDMS approach has been compared to both internal standard corrected and external calibration approaches.

**Conclusion**

High resolution LC-MS/MS and GC-MS trace analysis methods capable of single digit ppb LOQs and LODs, have been developed for multiple drug product formulations. These have been successfully transferred and implemented within both QC and external CROs. Significant progress has been made towards an understanding of the root cause of nitrosamine formation, which has been delivered through quantitation of trace levels of nitrites in excipients.. However, the nitrosamine challenge will remain for the foreseeable future, as the next generation of medicines under development will be scrutinised for their nitrosamine risk, on-going innovation in the trace analysis will be required.

**References**

**1)** ***NDMA analytics in metformin products: Comparison of methods and pitfalls***; Matthias Fritzsche, Giorgio Blom, Judith Keitel, Anja Goettsche, Maic Seegel, Stefan Leicht, Brunhilde Guessregen, Sebastian Hickert, Philipp Reifenberg, Alexandra Cimelli, Romane Baranowski, Emmanuel Desmartin, Elodie Barrau, Mark Harrison, Tony Bristow, Nicholas O’Neill, Annette Kirsch, Phillip Krueger, Christoph Saal, Bruno Mouton, Joerg Schlingemann. European Journal of Pharmaceutical Sciences, 2022, Volume 168, 106026

**2)** ***Dispersant-First Dispersive Liquid-Liquid Microextraction (DF-DLLME), a Novel Sample Preparation Procedure for NDMA Determination in Metformin Products***; Caroline Géhin C, Nicholas O'Neill, Amy Moore, Mark Harrison, Stephen Holman , Giorgio Blom. J Pharm Sci. 2023, 112(9), 453-2462.