Overcoming Quantification Challenges in Ultra-Trace LC-MS/MS analysis of Formulated Drug Products

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Introduction

The class of chemicals known as N-Nitrosamines are a series of impurities that present a serious risk to the health of patients. Brought to public attention in 2018 with the discovery of NDMA a contaminant in the drug valsartan[1]. Due to N-Nitrosamines possessing wide-ranging mutagenic properties in animals, it was determined that they can be mutagenic in humans, affecting different organs depending on length of exposure and by different routes of administration.

They are typically formed from a reaction between nitrites and a secondary or tertiary amines, the main sources of contamination in pharmaceutical products occur in manufacturing processes. Due to the ubiquity of nitrites and the presence of secondary and tertiary amines in many active pharmaceutical ingredients (API), there is a significant risk of nitrosamine formation in pharmaceuticals.

Therefore, pharmaceutical companies perform risk assessments for their products to assess the likelihood of N-Nitrosamine formation and if deemed a suitable risk, the products undergo testing to determine what the content of N-Nitrosamine is in the product relative to acceptable daily intake (ADI). This is typically reported as mass of nitrosamine present in the mass of API administered to the patient in one day.

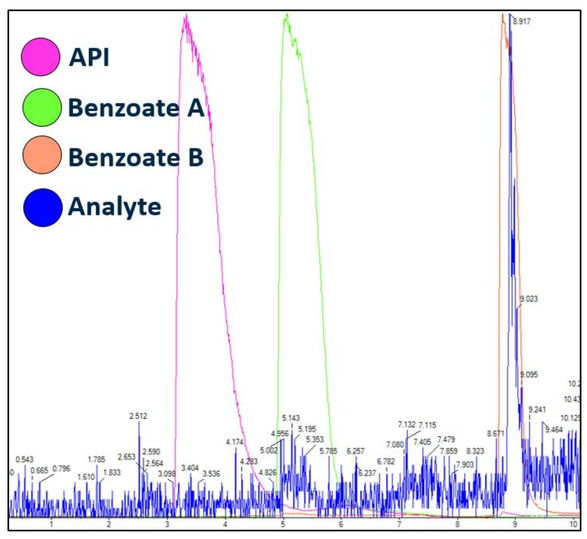
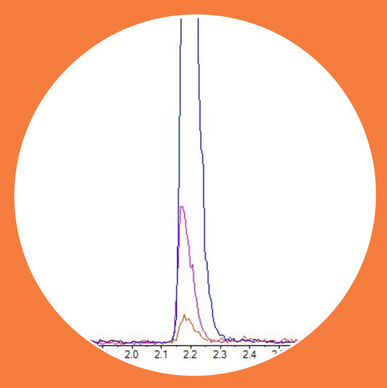
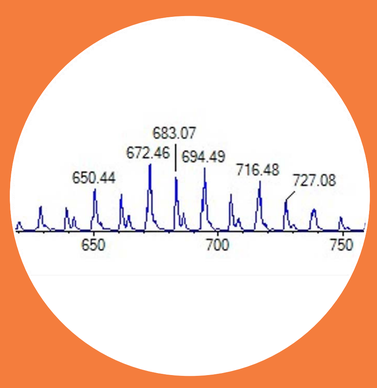


Figure 1: Chromatograms of the API, co-eluting benzoates and analyte



Development Start

- The Analyte response was verified using standards and common LC parameters using a triple quad mass spectrometer
- In standards and spiked samples, by monitoring the API and N-Nitrosamine transition and exchanging the UHPLC column between replicates of the same injections we can determine which column chemistry gives sufficient resolution between the API and N-Nitrosamine.
- Once this is achieved, to meet the required 10% ADI limit, the concentration of sample relative to API is increased, and the API is diverted to waste to prevent contamination to mass spectrometer.

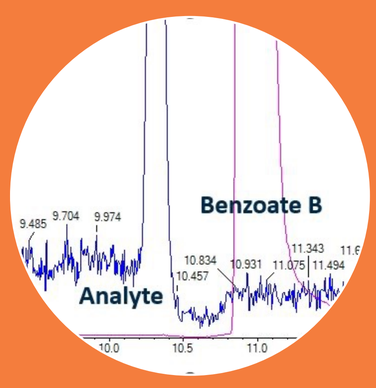


Determining Risks

- Recovery is the value calculated when a known quantity of reference material is spiked into a sample. Then using an un-spiked replicate, the amount of analyte present after subtraction can be calculated and assessed against the known quantity spiked into the sample.
- Using this assessment, it was determined that suppression was present in the sample as recovery was low. Likely caused by co-eluting excipients in the sample matrix, a polysorbate was identified and separated using a different stationary phase.
- This was determined by three different sample concentrations, each at 1 order of magnitude apart from each other were spiked with 1 ng/mL of reference material.

Co-eluting excipients

- Following the separation of the polysorbate, recovery experiments demonstrated that an excipient was still co-eluting. With electrospray ionisation mass spectrometry, co-eluting formulation components can supress[2] the analyte and reduce the sensitivity of the method.
- As a stable labelled internal standard was not available to correct for losses from sample extraction techniques (e.g. Solid Phase Extraction, Liquid-Liquid extraction) chromatographic separation was the only available method to separate the analyte from co-eluting formulation components.
- Therefore, additional mobile phases were screened to determine how to separate the analyte and these interfering excipients. The stationary phase could not be changed as it was still required to separate the polysorbate.



Chromatography

- Categorising the excipients based on solubility and potential retention mechanisms, a short list of components that could co-elute with the analyte was created.
- Monitoring the potential co-eluting excipients using LC-MS/MS, we screened additional mobile phases to get the separation that we required.
- Further work was performed to separate the analyte from these benzoates, the separation proved difficult, as we aimed to elute the analyte in-between the benzoates
- By fine tuning the composition of the organic mobile phase, the analyte was successfully separated from excipients.

Results

- Following the separation of the excipients causing suppression to the analyte, the performance of the method was greatly increased as demonstrated in table 1.
- The suppression was significant as observed in the "Development Start" figure, the overlaid chromatograms are samples that were all spiked to the same analyte concentration, but the API concentration was differed by orders of magnitude.
- The method was greatly improved to give confidence that while the N-Nitrosamine peak in un-spiked samples was below the detection limit, it was not artificially supressed and is a genuine result.
- This method helped ensure the safety of patients using the drug product.

Parameter	Before Development	After Development
Intensity of spiked sample (area)	9,547	74,080
Signal to Noise of spiked sample	16	45
Average Recovery (n=3)	13%	95%
Co-eluting Excipients	1	0
Repeatability of Samples (n=3)	16%	9%

Table 1:
Summary of method performance before and after development

Conclusions

- The method development resulted in a Limit Test Validation in accordance with ICH Q2(R1). The success of the development allowed for specificity and detection limit to be validated. In addition, accuracy and repeatability were validated, which is not normally conducted for limit test validation. This adds increased confidence to the results.
- This research demonstrates the benefits of a tailored method development approach for the ultra-trace detection of N-nitrosamines in formulated drug products.

References
1. https://www.thebts.org/publicstatements/nitrosamines-in-medicines-what-are-the-issues/
2. A. Furey, M. Moriarty, V. Bane, B. Kinsella, M. Lehane, Ion suppression; A critical review on causes, evaluation, prevention and applications, Talanta, Volume 115, 15 Oct 2023, Pages 104-122