Online Mixed-Mode SPE for Quantitative LC-MS/MS analysis of a N-Nitrosamine Drug Substance-Related Impurity in an Allergy Relief Drug Product

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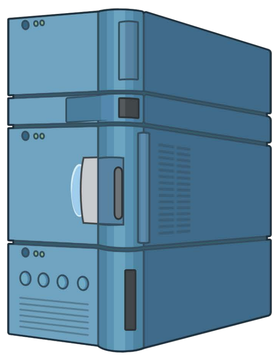
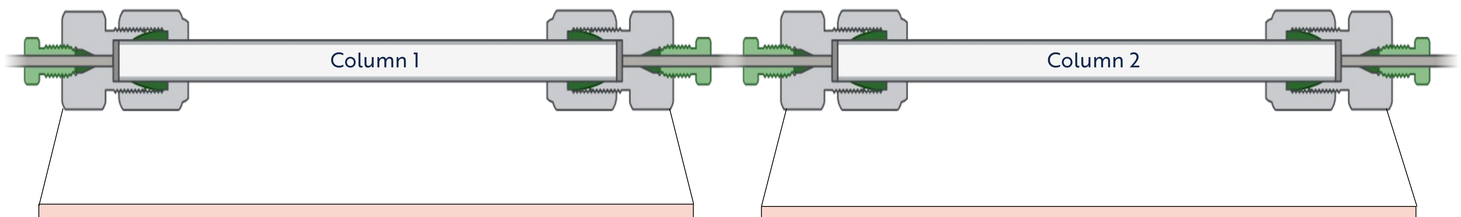
Introduction

- N-nitrosamines are a class of impurities with suspected genotoxic and carcinogenic effects on humans.[1] When present in pharmaceutical products, they represent a significant health risk to patients.
- Many Active Pharmaceutical Ingredients (API) contain nitrosatable amine functional groups and are therefore at risk of forming N-nitrosamine drug substance-related impurities (NDSRIs).
- Regulatory authorities require pharmaceutical manufacturers to risk assess all pharmaceutical products for the presence of N-nitrosamines, and where a risk is identified, develop methods to analyze them.[2]
- A major pharmaceutical company requested the development and validation of a quantitative GMP method for the analysis of a NDSRI in a drug product for allergy relief.
- Several issues had to be overcome as part of the development including a requirement for sub ng/mL quantitation limit (QL), a complex sample matrix, and the lack of a commercially available deuterated internal standard.

Online Solid Phase Extraction Setup

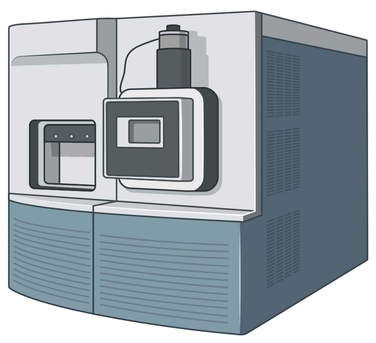
- An online mixed-mode Solid Phase Extraction (SPE) approach during LC-MS/MS analysis was developed to provide the necessary separation of compounds from the poorly retained NDSRI.





Reverse Phase C18
- N-Nitrosamine is poorly retained.
- Retains API and other excipients.
- 4.6 x 150 mm dimensions chosen to maximise column capacity for high sample loading.
- Larger internal diameters chosen to reduce system backpressure from increased column length.

Ion Exchange Z-HILIC
- N-Nitrosamine is poorly retained.
- Retains salts and prevent them from enhancing/suppressing the poorly retained NDSRI
- 4.6 x 100 mm dimensions chosen to maximise column capacity for high sample loading.
- Larger internal diameters chosen to reduce system backpressure from increased column length.



Offline Solid Phase Extraction Results

- An offline Solid Phase Extraction (SPE) for the removal of API was found to be unsuitable due to the presence of a water sensitive excipient in the allergy relief drug product that would block SPE cartridges upon contact..
- Additional offline extractions to remove the excipient were attempted but made the overall preparation procedure complex and time consuming.
- The lack of a commercially available deuterated internal standard meant that any matrix effects and recovery loss during extractions could not be compensated for.
- This led to significant negative impacts on analyte recovery, reproducibility, and sensitivity of the method.

Assessment	Result
Recovery	< 70%
Reproducibility (%RSD)	> 20%
Sensitivity	Sponsor criteria not met

Online Solid Phase Extraction Results

- The developed method provided an effective separation between the N-Nitrosamine and other sample components.
- An organic diluent was used for a “dilute and shoot” approach to avoid addition of water.
- The use of online SPE reduced the amount of sample preparation steps required prior to analysis. This increased sample throughput and the sustainability of the method by reducing the use of single use plastics.
- The automation of the SPE process reduced the variability of the method and the risk of human error and likelihood of repeating analysis.

Assessment	Result
Recovery	70 - 130%
Reproducibility (%RSD)	≤ 20%
Sensitivity	Sponsor criteria met

Conclusion

- The online SPE enabled an effective and reproducible separation and sample cleanup despite the lack of an internal standard and the complexity of the sample matrix.
- The application of online SPE using a mixed mode approach resulted in the successful GMP validation of a quantitative method for NDSRI analysis in an allergy relief drug product.
- The method achieved the required criteria for the sponsor and demonstrated the benefits of online SPE for the analysis of NDSRIs in a drug formulation with complex matrices.

References

1. https://www.fda.gov/news-events/press-announcements/statement-alerting-patients-and-health-care-professionals-ndma-found-samples-ranitidine
2. https://www.fda.gov/regulatory-information/search-fda-guidance-documents/control-nitrosamine-impurities-human-drugs