DoE Optimisation of Xevo TQ-S StepWave Parameters for LC-MS Analysis of 1,4-Dinitrosopiperazine in a Pharmaceutical Drug Product

M. Knox
Resolian, Newmarket Road, Fordham, Cambridgeshire, CB7 5WW
MKnox@Resolian.com

Introduction

- The recent discovery of carcinogenic N-nitrosamine impurities in pharmaceutical products present a significant health risk to patients.[1]
- As a result, regulatory agencies around the world have published guidance for allowable limits of N-nitrosamine in pharmaceutical formulations. Pharmaceutical companies must risk assess drug products for the presence of N-nitrosamines. When a risk is identified, an analytical method must be developed to test for the genotoxic impurities.
- A major pharmaceutical company requested the development of a Liquid Chromatography - Mass Spectrometry (LC-MS) analytical method for 1,4-Dinitrosopiperazine (DNPZ) in a pharmaceutical drug product.
- Based on an acceptable daily intake of 400 ng/day for DNPZ, the sponsor requested a Quantitation Limit (QL) of 26.7 ng/g relative to the drug product's active pharmaceutical ingredient (API).[2]
- This study describes the work performed to optimise the StepWave ion guide parameters in a Waters Xevo TQ-S to increase sensitivity and reach the required QL concentration for DNPZ in a major pharmaceutical company's drug product.

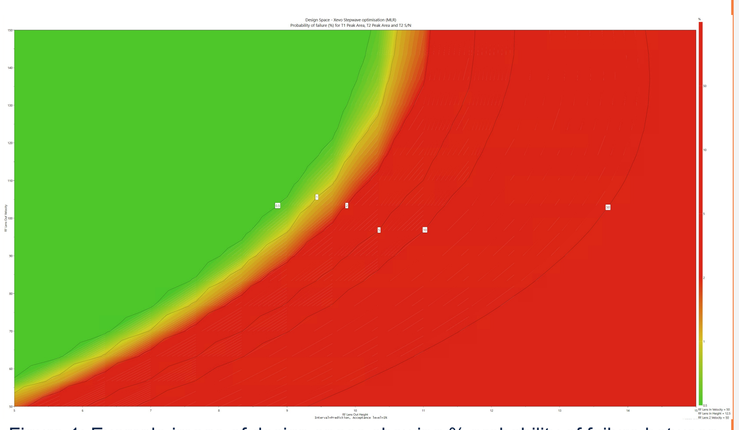

Figure 1: Example image of design space showing % probability of failure between two StepWave parameters

Sciex 6500+ Unoptimised

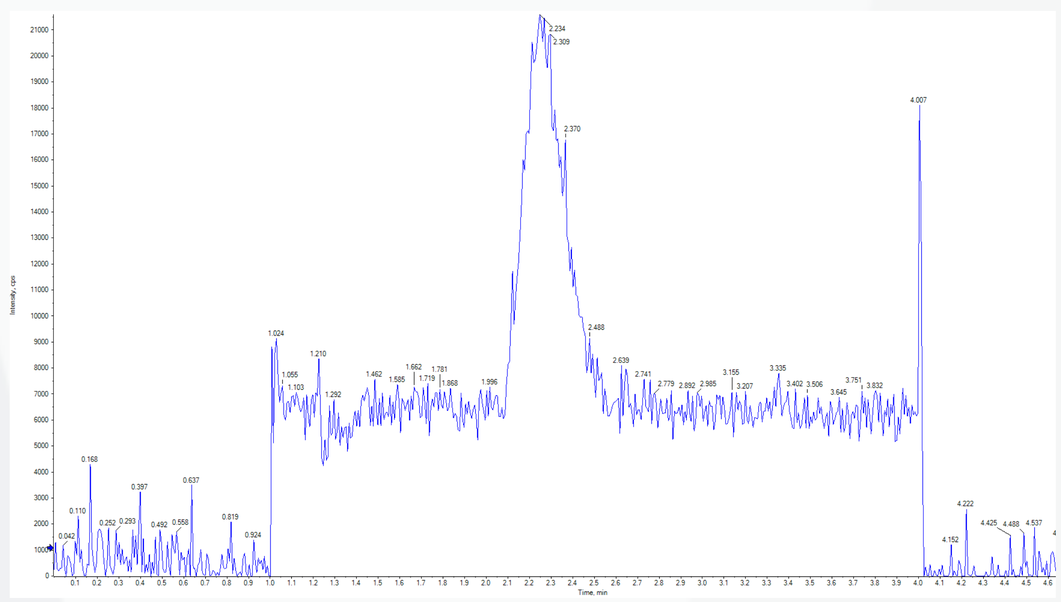

Figure 2: Chromatogram of a 4 ng/mL DNPZ standard injection performed on a Sciex 6500+.

4 ng/mL Standard - Signal to Noise: 16

Waters Xevo TQ-S StepWave Optimised

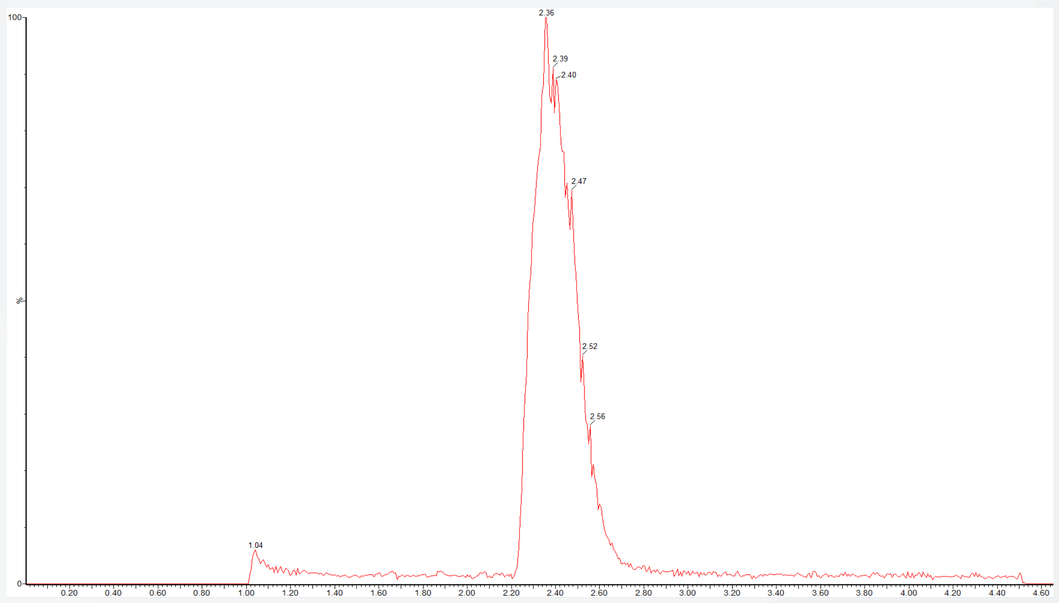

Figure 3: Chromatogram of a 4 ng/mL DNPZ standard injection performed on a Waters Xevo TQ-S.

4 ng/mL Standard - Signal to Noise: 86

Method

- 1,4-Dinitrosopiperazine standards were prepared at a range of concentrations in water for optimisation experiments.
- Experiments were performed on an Acquity UPLC coupled to a Sciex 6500+ or a Waters Xevo TQ-S using mobile phases consisting of 0.1% formic acid in water and 0.1% formic acid in methanol and a reverse phase C18 column.
- Modde Pro Design of Experiments software was used for experiment planning and optimisation.

Discussion

- The initial development of the method suffered a combination of poor analyte MS response, poor chromatographic retention on many columns, and incompatibility with common concentration procedures during sample preparation which led to an achievable QL concentration of 4 ng/mL (400 ng/g relative to the drug product's API) using a Sciex 6500+ Mass Spectrometer. This was not suitable for sponsor requirements.
- The Xevo TQ-S contains the StepWave, an ion guide designed to increase ion transmission to the mass analyser by actively removing neutral contaminants. While often left at default parameters, they can be tuned for more efficient ion transport to the mass analyzer for low m/z compounds or labile species.[3]
- Design of Experiments (DoE) was utilised to optimise StepWave and source parameters accurately, simultaneously and in an automated way using a reduced number of injections.
- A significant increase in sensitivity for 1,4-Dinitrosopiperazine was observed.

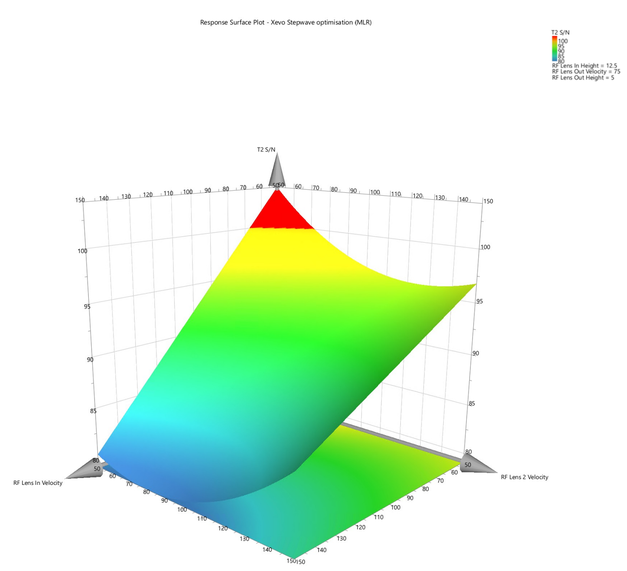

Figure 4: Example response surface plot showing change in signal to noise between two StepWave parameters.

- This met the sponsors requirements of a QL of 0.267 ng/mL (26.7 ng/g relative to the drug product's API).
- The work demonstrated the benefits of evaluating the StepWave for other small N-nitrosamines such as N-nitrosodimethylamine (NDMA) that typically suffer from poor sensitivity by LC-MS.
- Additionally, there is an increasing industry focus on green and sustainable practices in analytical chemistry.[4] Full optimisation of established equipment to achieve trace level sensitivity, even for poorly responding N-nitrosamines, is an environmentally friendly and cost-effective alternative to purchasing new instruments.

Conclusion

- Very low quantitation limits are often requested by pharmaceutical companies for N-nitrosamine analysis to meet the regulatory agency guidelines.
- Optimising the StepWave ion guide in a Waters Xevo TQ-S enabled a significant increase in sensitivity for 1,4-Dinitrosopiperazine, and DoE was used to evaluate all parameters simultaneously in an accurate and time effective fashion.
- As a result of this work, we were able to meet the quantitation limits requested by the sponsor and validate a selective, sensitive and robust analytical method.
- This study has demonstrated the potential sensitivity benefits for other similar N-nitrosamines and environmental benefits by using existing instruments to their fullest extent.

References

1. https://www.fda.gov/news-events/press-announcements/statement-alerting-patients-and-health-care-professionals-ndma-found-samples-ranitidine
2. https://www.canada.ca/en/health-canada/services/drugs-health-products/compliance-enforcement/information-health-product/drugs/nitrosamine-impurities/established-acceptable-intake-limits.html
3. https://www.waters.com/nextgen/gb/en/library/application-notes/2022/improved-transmission-of-labile-species-on-the-xevo-g3-qtof-mass-spectrometer-with-the-stepwave-xs.html
4. Naccarato, A. Development and Application of Green or Sustainable Strategies in Analytical Chemistry. Separations 2023, 10, 32. https://doi.org/10.3390/separations10010032