Ensuring Pharmaceutical Safety: Mass Spectrometry Approaches to Nitrosamine Analysis

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Background:

Nitrosamines, a class of potent carcinogenic and mutagenic compounds, have been reportedly associated with food, cosmetics and pharmaceutical industry. Their detection in pharmaceutical products sparked global regulatory concern due to their ability to form unintended by-products during drug synthesis and formulation. The contamination of common medications—including ranitidine, metformin and pioglitazone—prompted widespread investigations and recalls, underscoring the urgent need for robust detection and mitigation strategies.

Regulatory agencies, including the Food and Drug Administration (FDA) and European Medicines Agency (EMA), have established strict permissible limits, particularly for the “Cohort of Concern” nitrosamines, where thresholds can be as low as 18 ng/day as per EMA’s description. Identifying and eliminating these impurities demands ultra-sensitive analytical methodologies, particularly mass spectrometric techniques that offer unparalleled precision in trace-level detection. Almac Sciences plays a critical role in ensuring pharmaceutical safety by developing and optimising LC-MS methodologies to monitor harmful nitrosamines and thus allowing purging strategies to be put in place. A case study of the development and validation of a LC-MS method for the limit test of nitrosamine is presented. Almac Sciences can employ a variety of instruments (LC-QQQ-MS, GC-MS, Orbitrap) depending on the requirements of the methodology.

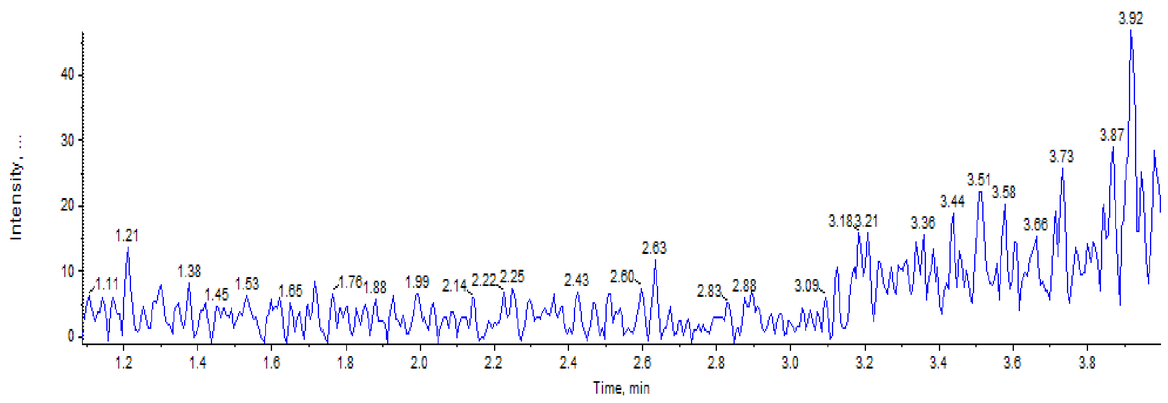
Method:

- A validated method to determine nitrosamine content in 1 mg and 3 mg Drug Product formulations by LC-QQQ-MS was established. The method was validated for specificity, linearity, accuracy, precision (including repeatability), sensitivity (limit of quantitation (QL) and limit of detection (DL)) and solution stability.
- Detection performed on a Waters UPLC H-Class Plus system with Sciex API-4000 Triple Quadrupole Mass Spectrometry. This instrument facilitates very low levels of detection using multiple reaction monitoring (MRM) which is unique to the compound of interest and offers excellent sensitivity and specificity.
- Each sample was dissolved in diluent, inverted and subjected to sonication to achieve homogeneity. The resulting solution was syringe filtered before being transferred into an HPLC vial for analysis.
- During method development UV detection was utilised to ensure both the excipient and API peaks were adequately resolved from the impurity peak of interest and did not enter the MS detector where it could potentially cause ionisation suppression.

Results:

No interference was observed in the filtered and unfiltered blank diluent chromatograms at the retention time of the nitrosamine.

Figure 1: Chromatogram of a Typical Diluent Blank



The nitrosamine peak in the chromatograms for the 100% spiked samples was observed at ~3.4 minutes and was within ± 0.5 minutes of the corresponding peak in the 100% Working Standard.

Figure 2: Chromatogram of a Typical QL Solution

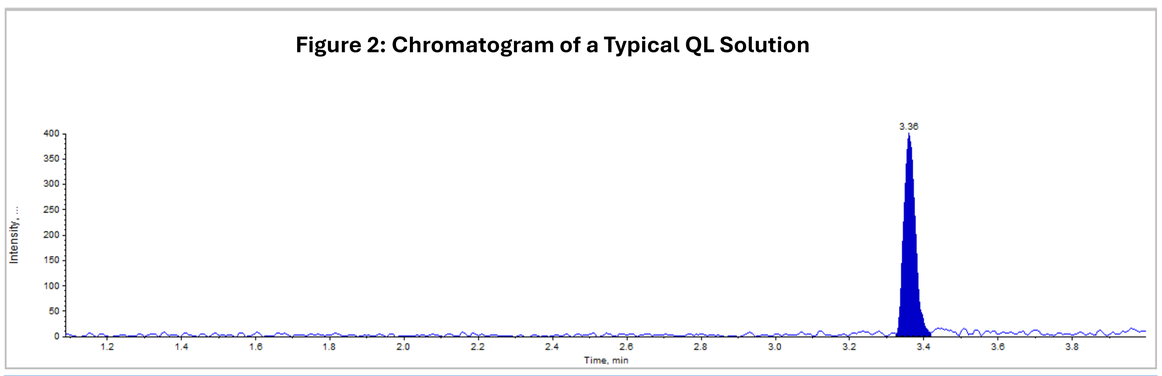


Figure 3: Chromatogram of a Typical 100% Working Standard

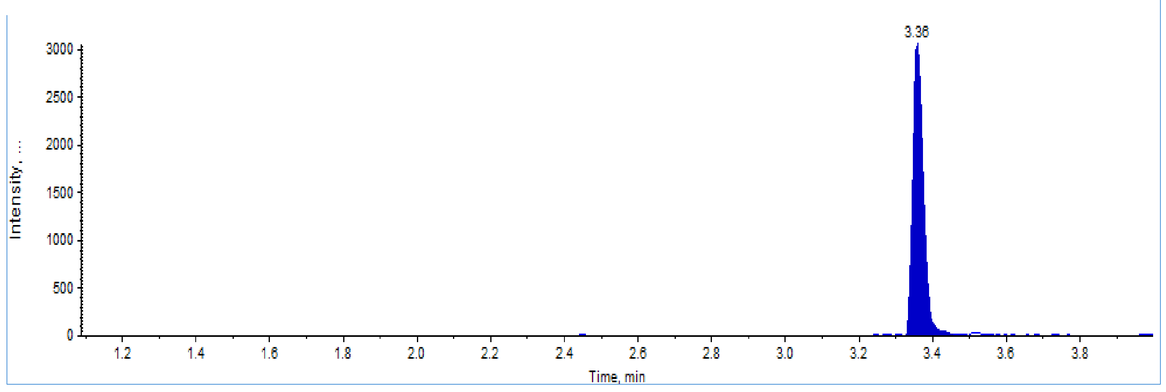
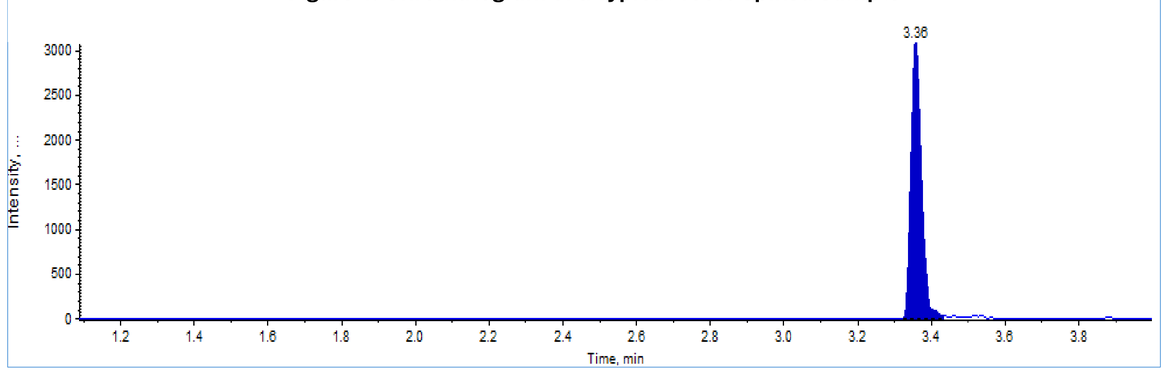


Figure 4: Chromatogram of a Typical 100% Spiked Sample



For accuracy, the mean recovery for the samples at the 10% (QL) level (n=3) and at 100% level (n=6) were both within 80-120%.

Table 1: Recovery of Nitrosamine for Samples Spiked at 10% (n=3) and 100% (n=6) Levels

Recovery		
Statistics	(n=3)	(n=6)
Overall Mean	97.5	89.0
SD	5.78	3.96
Overall %RSD	5.9	4.5

For the validation of sensitivity, a QL standard (~0.167 ng/mL, n=6) and a DL solution (~0.0557 ng/mL, n=3) were prepared and analysed. The signal to noise value of each injection at QL and DL was >10:1 and >3:1, respectively.

Table 2: Limit of Quantification Results

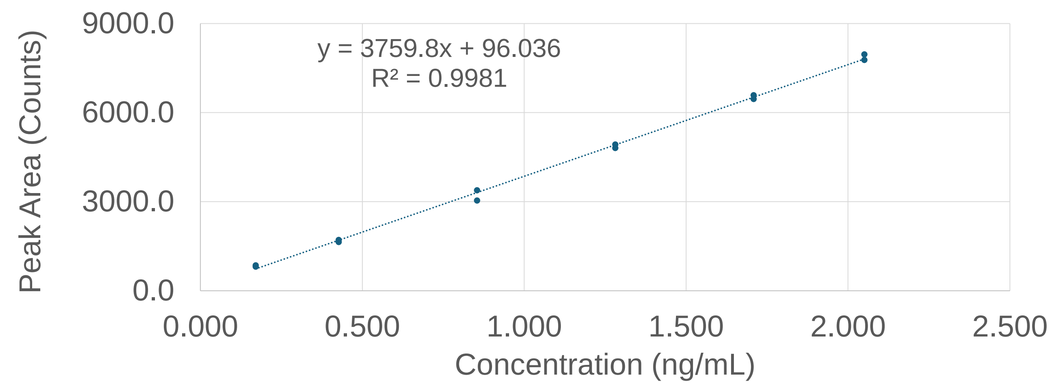
Sample Name	Area (counts)	S/N
Sens. QL Sol. Inj.1	766.9	237
Sens. QL Sol. Inj.2	768.5	209
Sens. QL Sol. Inj.3	761.0	180
Sens. QL Sol. Inj.4	777.9	207
Sens. QL Sol. Inj.5	742.6	220
Sens. QL Sol. Inj.6	828.7	262
Mean	774.3	219

Table 3: Limit of Detection Results

Sample Name	Peak Area (counts)	S/N
DL Sol. Inj.1	311.7	88
DL Sol. Inj.2	346.4	78
DL Sol. Inj.3	279.1	96
Mean	312.4	87

Linearity was assessed over the range at levels corresponding to 10% (QL), 25%, 50%, 75%, 100% and 120% of the nominal limit for the nitrosamine. The correlation co-efficient was >0.99 and the plot demonstrates a linear response.

Figure 5: Nitrosamine Linearity Plot



Conclusion

A method for the low-level quantitation of a nitrosamine by LC-MS was successfully developed and validated. In the current study, Almac has employed a scrutinous GMP compliant approach which demonstrates that it is possible to accurately monitor nitrosamines down to levels of ~0.167ng/mL or lower with a robust and repeatable method using LC-QQQ-MS.