Highly Sensitive and Robust UPLC-MS/MS Quantification of Nitrosamine Impurities in Sartan and Ranitidine Drug Substances

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Introduction

Ensuring safety and efficacy during drug development and manufacturing is critically important. Genotoxic impurities (GTIs), arising from the synthesis/manufacturing of drug products, specifically carcinogenic N-nitroso compounds, have recently been found in several medications. This has resulted in drug recalls. Due to current and future safety threshold levels recommended by regulatory bodies of 0.3 and 003 ppm, for these impurities, there exists a strong need for sensitive and robust LC-MS methods for their detection and accurate quantification. Developing such methods is challenging due to the chemical diversity of nitrosamines, poor chromatographic retention, MS ionization and fragmentation, often limiting sensitivity and selectivity. This work presents practical considerations for optimization of LC-MS conditions to achieve sensitive and robust simultaneous quantification of several nitrosamine GTIs in ranitidine and 'sartan drug substances.

Results and Discussion

1. Improved MS Performance using IonSABRE APCI vs. ESI probe.

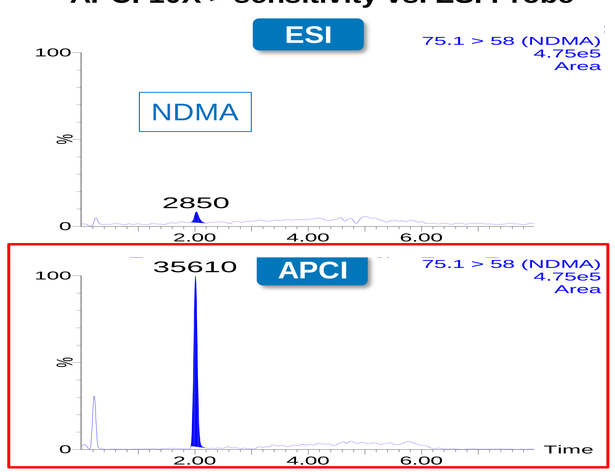

Figure 1: *Comparison of APCI and ESI MS performance for the NDMA nitrosamine impurity, demonstrating a 10X improvement in analyte response using the IonSABRE APCI probe over the ESI probe.*

2. UPLC Chromatographic Separation of Nitrosamine Impurities and Ranitidine/'Sartan API

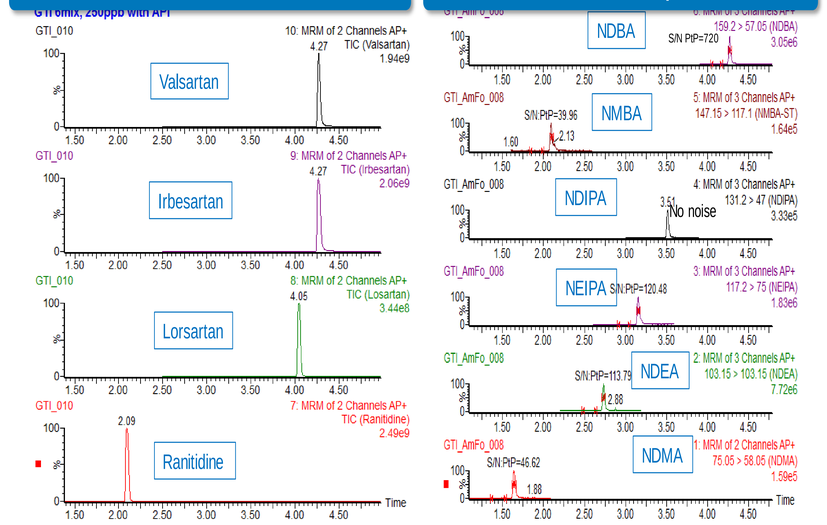

Figure 2. *UPLC Chromatographic Separation of Nitrosamine Impurities and Ranitidine/'Sartan API.* ***System:*** *ACQUITY I Class PLUS LC coupled to Xevo TQ-XS tandem MS.* ***Column:*** *ACUITY UPLC HSS T3 2.1 x 100 mm, 1.6 µm (40 ° C).* ***Mobile Phase:*** *Water (A) and Methanol (B) containing 0.1 % formic acid and 5 mM Ammonium Formate.* ***Flow Rate:*** *0.4 ml/min* ***Gradient:*** *98% A initial (0.25 min hold) to 5% A over 3.75 min (0.6 min hold), return to 98% A in 0.41 minute), hold for 2 min.* ***Analysis Time:*** *7 min.*

3. High Sensitivity LC-MS/MS Analysis of Nitrosamine Impurities LOD/LLOQs between 0.025-0.10 ng/mL

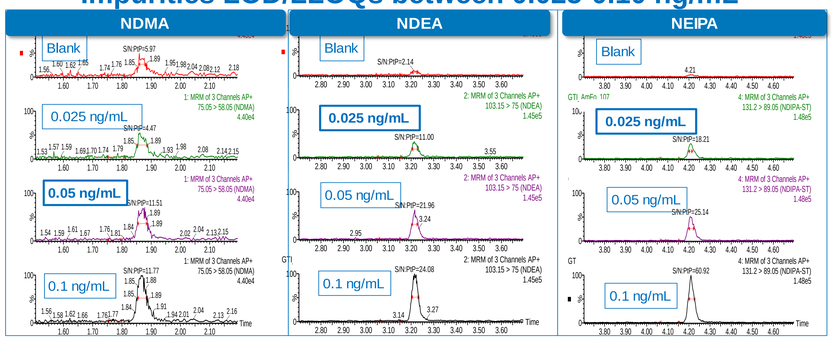

Figure 3. *Representative UPLC-MS/MS performance for the NDMA, NDEA and NEIPA, nitrosamine impurities spiked in a neat solution of water:methanol (80:20) at concentrations of 0.025, 0.05 and 0.1 ng/mL using the ACQUITY UPLC I-Class Plus LC, ACQUITY UPLC HSS T3 Column, and Xevo TQ-XS MS (APCI +).*

4. Detection and Quantification of Nitrosamine Impurities in Drug Product

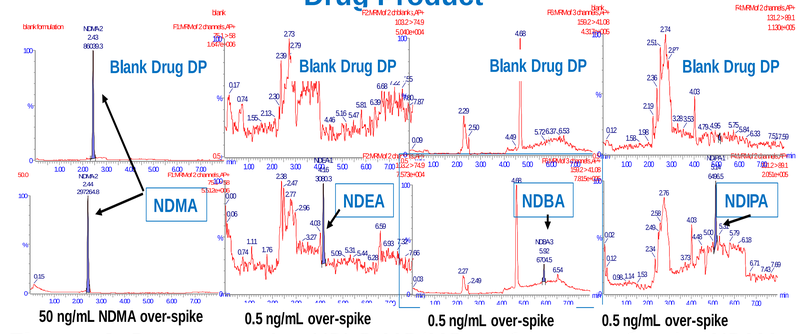

Figure 4. *Representative UPLC-MS/MS performance for the NDMA, NDEA, NDBA, and NDIPA, nitrosamine impurities prepared in DP. Results highlight spiked 0.5 ng/mL (50 ng/mL NDMA) nitrosamine impurities in DP, as compared to Blank (un-spiked) DP. Note the large endogenous NDMA impurity in Blank DP.*

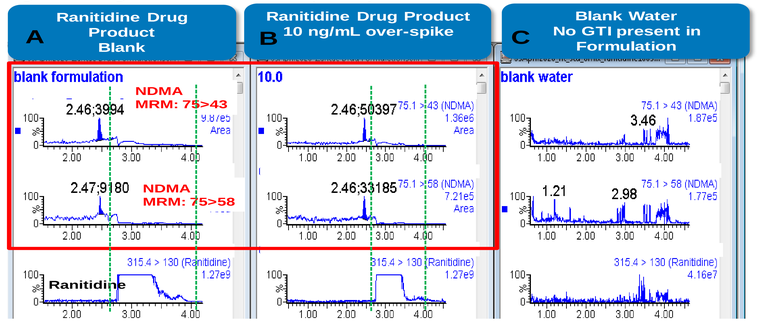

Figure 5. *Confirmation of endogenous NDMA nitrosamine impurity, 29 ng/mL (2 MRM transitions) in ranitidine DP (A), increase in NDMA peak response with 10 ng/mL NDMA over-spike in DP, and absence of the NDMA peak in blank neat solutions before and after injection of DP samples (C). Green line indicates use of divert valve to shuttle API to wasted during MS analysis.*

Conclusions/Summary

- A sensitive LC-MS/MS method, using APCI + MS detection (Fig. 1), was developed for detection and quantification of nitrosamine impurities (Fig. 2).
- LLOQs of 0.025-0.1 ng/mL for nitrosamine impurities were achieved (Fig. 3).
- Quantification of nitrosamines from ranitidine drug product was achieved (Fig. 4) with detection of endogenous NDMA, ~ 30 ng/mL, in DP (Figure 5).