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Note d'application

Suggested Approaches for Minimizing Background Chemical Noise in Low Mass MRM Transitions for Trace Level Quantification of N-Nitrosamines

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Ce document est une note d'application et ne contient pas de section détaillée concernant l'expérimentation.

Abstract

Since the detection of N-nitroso-dimethylamine (NDMA) in a valsartan active pharmaceutical ingredient (API) in 2018, and the subsequent nitrosamine discoveries in several classes of pharmaceuticals, health authorities have required that Pharmaceutical Market Authorization Holders (MAH) employ approaches to control and mitigate for the presence of nitrosamines in new, in development, and marketed medicines. Nitrosamines are mutagenic and considered probable human carcinogens.

Trace analysis of nitrosamines at the regulatory set Acceptable Intake threshold levels (AI) requires sensitive and selective analytical methods. Tandem quadrupole mass analyzers offer the best performance characteristics for quantitative measurements in complex matrices due to their ease of use, robustness, sensitivity, and the assay selectivity afforded through selection (acquisition) of precursor and product ions in multiple reaction monitoring (MRM) experiments.

Despite the selectivity of LC-MS/MS experiments, it is still possible to observe noise in MRM acquisitions, which can decrease the detection sensitivity of the method. The trace analysis of low molecular weight analytes can be challenging due to the chemical interference often present in this mass range. The following work describes the analysis of nitrosamines using Ultra-Performance Liquid Chromatography (UPLC) with atmospheric pressure chemical ionization (APCI) and a tandem quadrupole mass spectrometer. Background noise encountered during the analysis of NDMA, and additional nitrosamines was mitigated using a combination of strategies including evaluation of the cone gas, cone voltage, and the use of high purity mobile phases.

Benefits

- Trace level detection of nitrosamines using the Xevo™ TQ Absolute Tandem Quadrupole Mass Spectrometer in MRM acquisition mode
- Increased confidence in the selectivity of impurity detection using multiple confirmation ions
- Noise mitigation tools to aid in improving analyte signal-to-noise ratios
- Retention and chromatographic resolution of seven nitrosamine impurities including isobaric NDPA and NDIPA (*m/z* 130*)* using the premier HSS T3 Column and the ACQUITY™ Premier UPLC System

Introduction

Since the detection of N-nitroso-dimethylamine (NDMA) in a valsartan active pharmaceutical ingredient (API) in 2018, and the subsequent nitrosamine discoveries in several classes of pharmaceuticals, there have been concerted efforts to mitigate the presence of nitrosamines in all marketed medicines. As nitrosamines are mutagenic, and considered probable human carcinogens, medicine manufactures, and market authorization holders were required to carefully monitor and control the presence of nitrosamines to ensure continued safe access to critical medications. Health authorities have guided manufacturers in following a three-step process to mitigate and control the presence of nitrosamines in all marketed and new medicines, which includes risk assessment, confirmatory analytical testing where risk is identified, and reporting.

When risk assessment determines that nitrosamines could be formed and present in the final drug product then quantification through analytical testing is required.[1–3] Due to the very low AI of some nitrosamines, the trace analysis of nitrosamines at the regulatory permitted threshold levels requires sensitive and selective analytical

methods. Furthermore, to provide manufacturers the ability to mitigate the need for ongoing release batch testing, sensitive assays that allow for quantification to 10% of the regulatory set AI threshold for the impurity, are necessary. Tandem quadrupole mass analyzers offer excellent performance characteristics for trace level quantitation of analytes in complex matrices through the use of MS/MS. MRM acquisitions, in particular, achieve high selectivity and sensitivity by associating a structurally relevant product ion with a specific precursor. Both LC-MS/MS and GC-MS/MS methods have been used for the trace level quantitative measurement of various N-nitrosamines.[4–8] LC-MS/MS is particularly suited to the analysis of polar and nonpolar compounds, in addition to those that are thermally unstable.[7] Electrospray (ESI), a solution based ionization technique, and APCI where ionization takes place in the gas-phase have been used for the analysis of nitrosamines in a variety of matrices.[4,5,9,10–12] APCI has been reported to be less susceptible to matrix interferences.[7,11,12]

Despite the selectivity of LC-MS/MS experiments a common challenge in the trace analysis of very low-level mass analytes such as nitrosamines is the observed level of background noise in MRM acquisitions which can interfere with and decrease the detection sensitivity. To maintain the trace level detection capabilities in LC-MS/MS assays, it is essential to take steps to preserve system cleanliness and to avoid contamination that may be introduced to the system during the analysis. The following work describes approaches taken to reduce the impact of background chemical noise on the analysis of nitrosamines using Ultra-Performance Liquid Chromatography (UPLC™) with APCI positive ionization and a tandem quadrupole mass spectrometer (Figure 1). Noise mitigation strategies for quantitation and confirmatory nitrosamine MRM transitions will be described which include the evaluation of cone voltage, cone gas flow rate and mobile phases. The optimized method was applied to the analysis of seven N-nitrosamines authentic standards (Table 1 and Figure 2) to ultra-low levels of detection.

Experimental

Figure 1. The Xevo TQ Absolute Tandem Quadrupole Mass Spectrometer and the ACQUITY Premier System.

Compound	Name	Formula	$[M+H]^+$	CAS number
NDMA	*N*-Nitrosodimethylamine	$C_2H_6N_2O$	75.05	62-75-9
NDEA	*N*-Nitrosodiethylamine	$C_4H_{10}N_2O$	103.08	55-18-5
NEIPA	*N*-Nitrosoethylisopropylamine	$C_5H_{12}N_2O$	117.10	16339-04-1
NDPA	*N*-Nitrosodipropylamine	$C_6H_{14}N_2O$	131.11	621-64-7
NDIPA	*N*-Nitrosodiisopropylamine	$C_6H_{14}N_2O$	131.11	601-77-4
NMBA	*N*-Nitroso-N-methyl-4-aminobutyric acid	$C_5H_{10}N_2O_3$	147.07	61445-55-4
NDBA	*N*-Nitroso-dibutylamine	$C_8H_{18}N_2O$	159.14	924-16-3

Table 1. List of N-nitrosamines, chemical formulae, observed m/z, and CAS numbers.

Compound	Precursor ion (*m/z*)	Product ion (*m/z*)	Cone voltage (V)	Collison energy (eV)
NDMA	75.10	58.00 (quantifier) 43.00 (confirmation 1) 44.00 (confirmation 2)	30 50 30	10 8 8
NDEA	103.10	75.00 (quantifier) 47.00 (confirmation 1) 29.00 (confirmation 2)	25 25 25	10 14 10
NEIPA	117.10	75.00 (quantifier) 47.00 (confirmation 1) 43.05 (confirmation 2)	20 20 20	9 14 14
NDPA	131.10	89.10 (quantifier) 47.05 (confirmation 1) 43.05 (confirmation 2)	16 16 16	10 12 12
NDIPA	131.10	89.10 (quantifier) 47.05 (confirmation 1) 43.10 (confirmation 2)	20 20 20	8 12 12
NMBA	147.10	117.00 (quantifier) 44.10 (confirmation 1)	15 15	5 12
NDBA	159.20	57.10 (quantifier) 103.10 (confirmation 1) 41.10 (confirmation 2)	30 30 30	12 10 12

Table 2. Quantification and confirmatory MRM transitions, cone voltage and collision energy settings used for the seven nitrosamines analyzed.

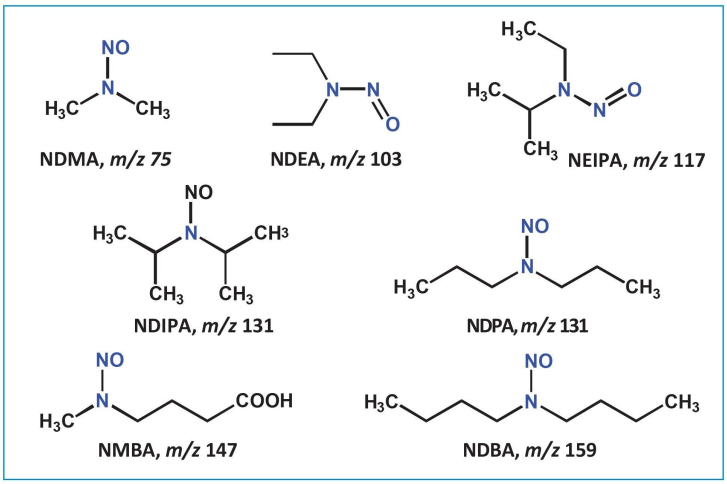

Figure 2. Structures of nitrosamines used in the study.

Results and Discussion

Optimizing the Cone Gas

The use of confirmatory ions and their ratios to the quantification ion (Table 2) can greatly help in increasing confidence in the correct identification of a suspected impurity. This is particularly helpful for the detection and quantification of low-level low mass impurities in the presence of high chemical background or interferences, for example high concentrations of an active pharmaceutical ingredient (API). The chromatograms showing the primary and two confirmatory MRM transitions of an authentic standard of NDMA at a retention time (t_R) of 2.09 minutes are displayed in Figure 3A. During method development a cone gas flow rate study was performed to aid in reducing the presence of background noise observed in the 75.1>43 NDMA confirmatory transition. The cone gas is a useful parameter that can be optimized to help to reduce the presence of solvent clusters and other interfering ions, which results in improvements to the signal-to-noise (S/N). Figure 3B shows the effect of increasing the cone gas flow rate on the background noise observed in the 75.1>43 MRM transition for NDMA. As the cone gas flow rate is increased from 150 L/hr to 500 L/hr the noise decreases. The parameter can be optimized to give the best S/N. It is important to ensure that the increased cone gas does not adversely affect the S/N of other analytes or MRM transitions.

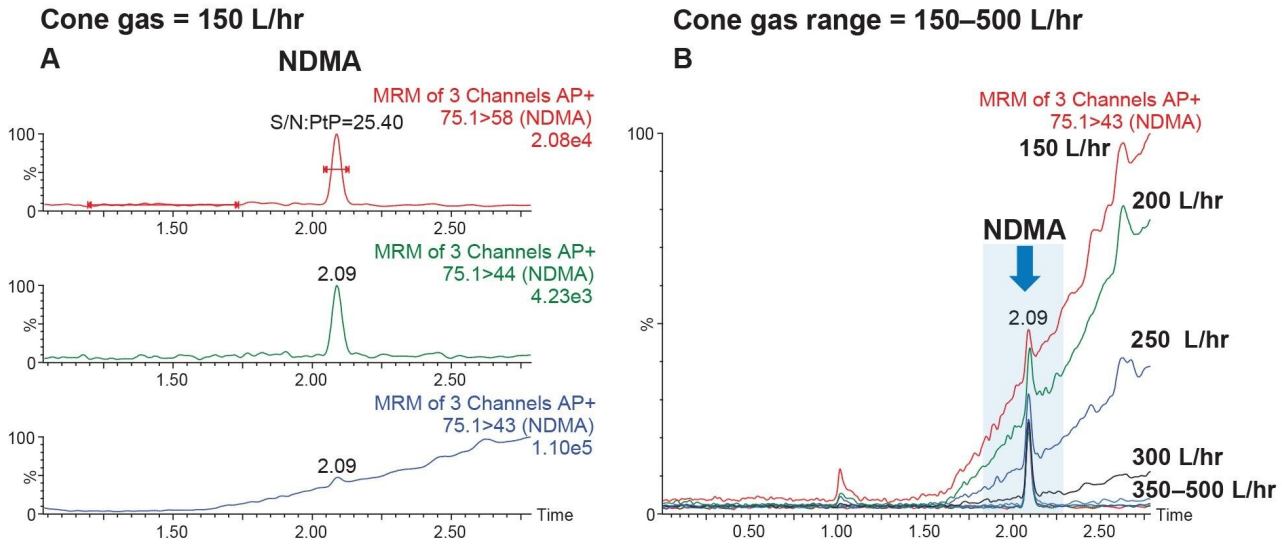

Figure 3. Chromatograms from the analysis of an authentic standard of NDMA, (0.5 ng/mL, 10 µL). Elevated background noise in the confirmatory MRM transition (75.1>43) is shown in 3A (lower left). The effect of increasing the cone gas flow rate from 150 L/hr to 500 L/hr on the noise level is observed for the 75.1>43 MRM transition in the superimposed chromatograms (Figure 3B, right).

The optimum cone gas flow rate was determined as 350 L/hr (Figure 4A), as it provided the best S/N without adversely affecting the S/N of the other NDMA MRM transitions (Figure 4B). The background noise in the 75.1>43 transition was significantly improved using a cone gas setting of 350 L/hr enabling improved visibility of this confirmatory transition. In addition, signal enhancements were also observed for the other NDMA transitions 75.1>58 and 75.1>44 resulting in improvements in S/N for these transitions (Figure 4B).

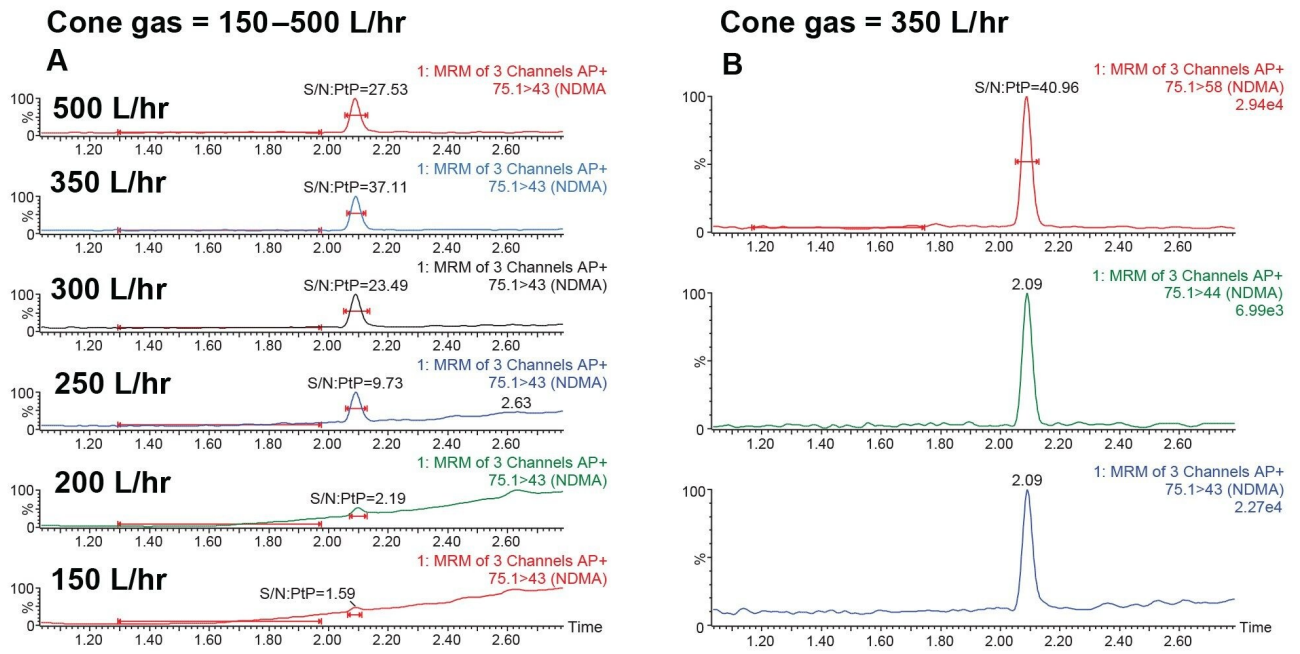

Figure 4. A) Chromatograms showing the S/N for cone gas flow rates ranging from 150–500 L/hr. B) MRM Chromatograms from the analysis of NDMA (NDMA 0.5 ng/mL, 10 µL) showing the optimized cone gas flow rate of 350 L/hr and the significant noise reduction achieved.

Optimizing Cone Voltage in Noise Affected MRM Transitions

The cone voltage is optimized to maximise ion transmission from the source to the next stage of mass analysis. Compound specific cone voltage optimization can be done automatically using Intellistart autotune in the MassLynx software. The cone voltage can also be used in situations where elevated baseline noise is observed. As described in the previous section elevated background noise was observed in one of the confirmatory MRM transitions of NDMA. Performing a cone voltage optimization study for this transition enabled the reduction in noise for this MRM channel and was used in combination with the cone gas.

Using a cone gas setting of 350 L/hr, the cone voltage was varied for the 75.1>43 MRM transition from 25–70 V. Figure 5A shows the S/N for each cone voltage setting. The optimum cone voltage, when the noise is measured in the post peak elution region, where the baseline noise increases, was determined to be 50 V. Even though the signal is highest at a cone voltage of 25 V, the increased noise in this region decreases the overall S/N observed at this cone voltage (Figure 5B). It is important to balance the gain in S/N with any decrease in signal intensity

observed. The cone voltage can be independently applied to the affected MRM transition without impacting the intensity of the quantifier or other MRM transitions that may not be impacted by background noise.

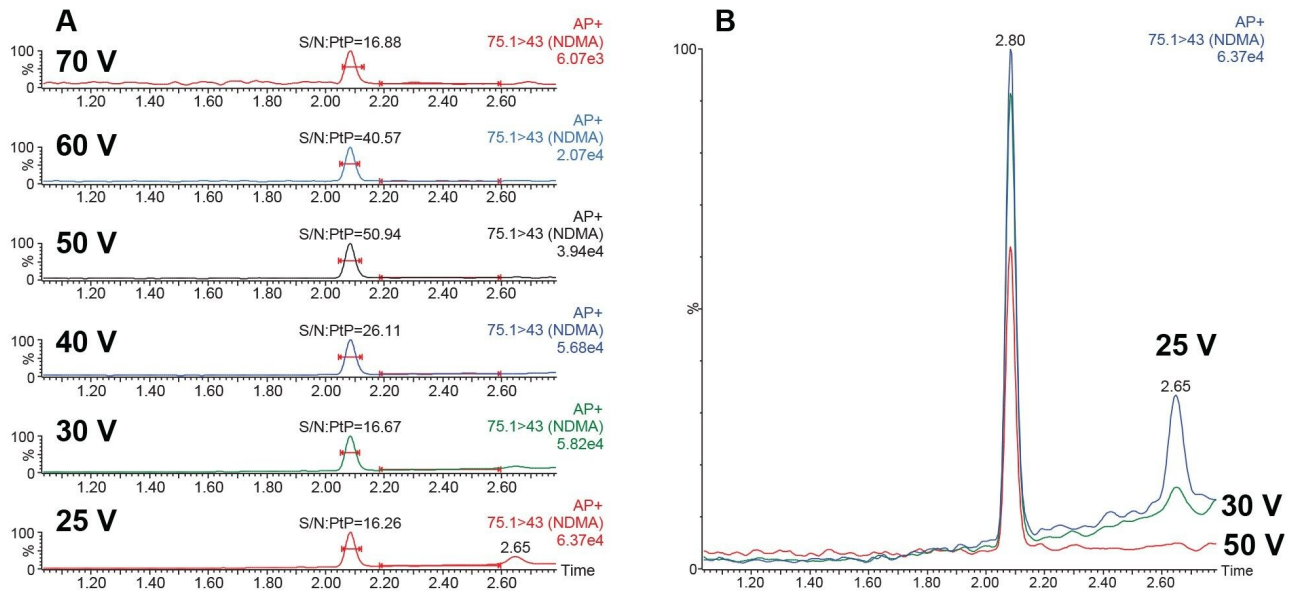

Figure 5. A) Chromatograms from the analysis of an authentic standard of NDMA (1.0 ng/mL, 10 µL) for the confirmatory transition 75.1>43 showing the S/N at cone voltages ranging from 25–70 V. B) Superimposed chromatograms displaying the effect of cone voltage on the signal intensity and baseline noise at 25 V, 30 V, and 50 V.

Noise Contributions From Mobile Phases

Trace level quantitative analysis requires care in the setup of the entire analytical system, avoiding contamination of mobile phase reservoirs and solvents, that could lead to increased background noise and a degradation in assay sensitivity. The use of high purity mobile phases and additives is critical to maintain the detection sensitivity needed to reach trace level concentrations. LC-MS grade formic acid and ammonium formate were used in this study along with LC-MS grade solvents. A comparison of two different brands of LC-MS grade methanol was performed. Figure 6A shows the chromatograms from the analysis of an authentic standard of NEIPA (0.1 ng/mL, 10 µl injection) using methanol as the strong elution solvent. The standard was injected five times using methanol from each solvent brand and the chromatograms were superimposed with the intensity axis linked. Elevated background noise in the primary transition of NEIPA (117.1>75) was observed when

methanol from brand, A was used which significantly impacted the S/N observed (Figure 6B). Increased noise was also observed for the confirmatory transitions of NEIPA, as well as the quantitation and confirmatory transitions of NDPA and NDIPA (data not shown). A decrease in the background noise and an increased S/N value were observed in these transitions when methanol brand B was used as the strong elution solvent.

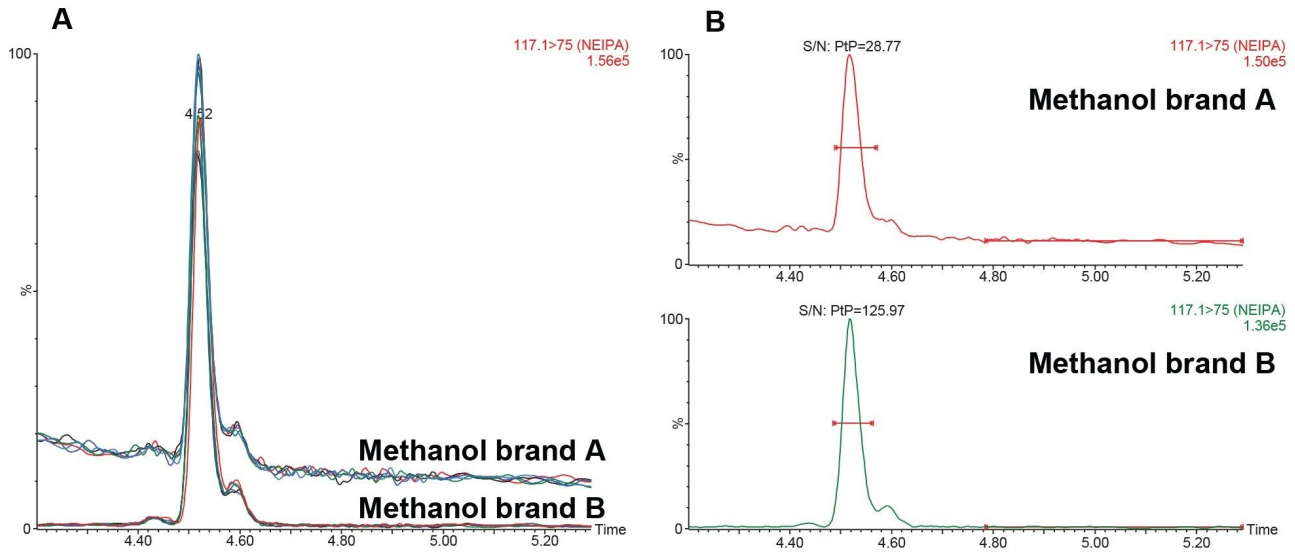

Figure 6. A) Superimposed chromatograms for the primary transition (117.1>75) of NEIPA analyzed using methanol from solvent brand A (n=5) and methanol from solvent brand B (n=5) showing the increased noise visible in this MRM transition when solvent brand A is used (0.1 ng/mL, 10 µL). B) Comparison of the S/N observed with each methanol brand.

Trace Level Quantitation of N-Nitrosamines

After method optimization and background noise minimization, the sensitivity and linear range of the mass spectrometer was assessed using authentic nitrosamine solvent standards injected in triplicate. The limit of detection (LOD) and limit of quantitation (LOQ) for each nitrosamine was determined using the S/N criteria of 3:1 and 10:1, respectively. The signal at the retention time of the nitrosamine standards along with a representative region of baseline noise was used to calculate the S/N at the LOD and LOQ for each compound as is demonstrated for NDMA using the Peak-to-Peak algorithm in Figure 7A.

The response for NDMA was linear over the range 0.02–100 ng/mL (Figure 7B). The results for the additional nitrosamines are summarized in Table 3.

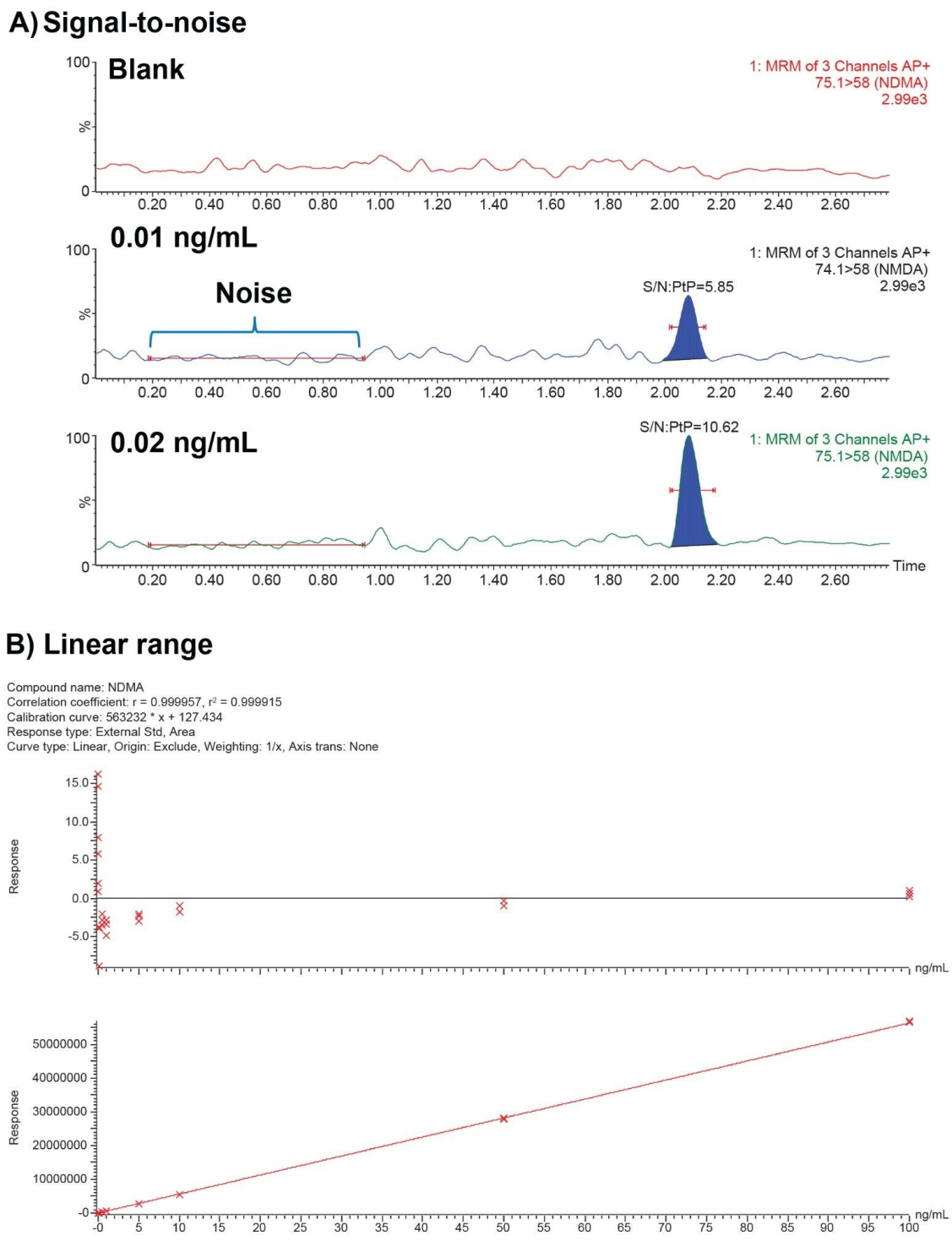

Figure 7. A) Chromatograms from the analysis of authentic standards of NDMA at the LOD (0.01 ng/mL) and LOQ

(0.02 ng/mL) and a blank injection (30 µL injection). S/N at each level is shown. B) Linear range for NDMA 0.02–100 ng/mL, and residuals <16% at the LOQ and <+/- 9% across the calibration range tested, injected in triplicate (top).

Nitrosamine	LOD[1] ng/mL	LOQ ng/mL	Linear range[2]	R^2
NDMA	0.01	0.02	0.02–100 ng/mL	0.9999
NDEA	0.005	0.01	0.01–100 ng/mL	0.9999
NEIPA	0.005	0.01	0.01–100 ng/mL	0.9999
NDPA	0.005	0.01	0.01–100 ng/mL	0.9997
NDIPA	0.005	0.01	0.01–100 ng/mL	0.9996
NMBA	0.005	0.01	0.01–10 ng/mL	0.9995
NDBA	0.005	0.01	0.01–100 ng/mL	0.9997

Table 3. The LOD and LOQ were determined for the authentic nitrosamine standards analyzed, linear range and R^2 values are also shown.

Chromatograms for the quantitation (75.1>58) and two confirmatory MRM transitions (75.1>43 and 75.1>44) for NDMA at the LOD and LOQ are shown in Figure 8 along with the blank injection.

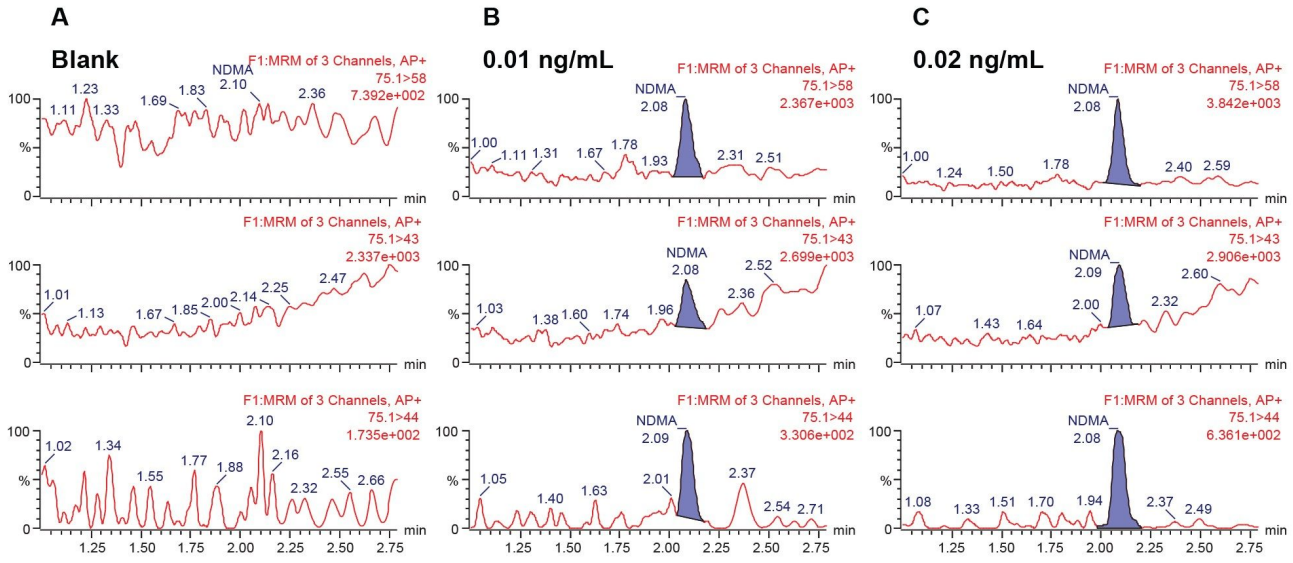

Figure 8. Chromatograms from the analysis of an authentic standard of NDMA (0.01 ng/mL and 0.02 ng/mL, 30 µL injection) and solvent blank.

Conclusion

To maintain the ultimate detection sensitivity in trace nitrosamines analysis, the background noise in the entire LC-MS/MS system needs to be managed so that optimum S/N ratios can be obtained. The use of LC-MS/MS grade solvents and additives that do not add additional noise to the target MRM background are desirable. The cone gas flow rate can be optimized in the event of elevated baseline noise helping to reduce interfering ions which can improve ionization efficiency and increase S/N for quantitation and confirmation ions. The use of confirmatory ions is critical to increase confidence in impurity identification in the presence of high concentrations of API. The cone voltage is used to optimize analyte sensitivity; however, it may also be useful in noise affected MRM transitions to improve S/N. An optimization study needs to be performed to balance the reduction in peak intensity with the S/N gains.

The observations included in this document are intended to aid in situations where elevated background noise is observed or suspected during method development for quantitative nitrosamines analysis. Further method optimization including sample preparation or chromatographic separation may be needed to complement the

optimization parameters above in the analysis of samples containing API or drug product.

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