

Improving the Transmission of Nitrosamine Impurities with the Waters Xevo G3 QTof Mass Spectrometer

Lisa Reid, Lee A. Gethings, Jayne Kirk

Waters Corporation

Abstract

Demonstrating improved transmission of nitrosamines when utilizing the soft ionization transmission mode on a Waters Xevo™ G3 QTof mass spectrometer compared to the default StepWave values. The method outlined in this document demonstrates enhanced sensitivity for the nitrosamine contaminants NDMA, NMPA, NMBA, and NDBA when soft ionization StepWave settings are used.

Benefits

- A QTof platform offering superior sensitivity in Tof-MRM acquisition mode
 - Robust, reproducible, and flexible workflow
 - A versatile system offering a choice from multiple acquisition methods allowing for both targeted and screening type acquisitions
 - An instrument that provides specificity and increases confidence in analyte identification through visualization of spectra and providing accurate mass information
 - Providing a complete workflow from data acquisition to report generation in a GXP compliant-ready software
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Introduction

Within recent years regulatory bodies have issued guidance requesting pharmaceutical manufacturers to risk assess, quantify, and reduce the amount of nitrosamine contaminants found within their products.¹ Initially some prescription medications were found to contain levels of nitrosamines deemed as being above a safe limit and therefore posed a cancer risk to patients receiving the medication. At the time, batches were recalled, and subsequently pharmaceutical manufacturers are recommended to test products to ensure nitrosamine contaminants are below a level of 26 ng/day (total) at the maximum daily dosing level of the API, or varying ng/day limits for individual compounds found in isolation. As such, monitoring the level of these harmful compounds at ng/mL concentrations or lower is of high priority and relies on highly sensitive and selective analytical methods.

The Xevo G3 QTof offers excellent sensitivity and selectivity and provides the added advantage of being a flexible platform offering multiple different acquisition modes giving it the ability to both characterize and quantify analytes of interest depending upon application goal. Additionally, the Xevo G3 QTof is equipped with a StepWave™ XS design allowing the user to optimize transmission of ions into the mass spectrometer and reduce any potential fragmentation of labile precursor analytes.

For this analysis both MS^E and Tof-MRM acquisition methods were employed: MS^E a screening acquisition where all precursor and product ions within a defined mass range (*e.g.* 50–1200 Da) are recorded, is useful for method development and looking for unknowns. Tof-MRM is a targeted mode of acquisition where pre-determined precursor masses are selected within the quadrupole of the mass spectrometer prior to being fragmented. Pre-determined fragment ions are then target enhanced allowing for improved sensitivity when compared to screening acquisition modes. Screening acquisition modes such as MS^E are typically employed when the operator requires the identification of unknowns and targeted analysis such as Tof-MRM, can be used when only known analytes are being analysed. For more details of different acquisition modes available on this instrument please reference the application note: [720008021](#).² Being a QTof instrument, regardless of analysis mode, spectra can be viewed providing the user with accurate mass measurements and information regarding potential interfering isomeric compounds.

Experimental

Sample Preparation

Standards of NDMA, NMBA, NMPA, and NDBA were purchased from Merck (Poole, UK). Stocks were prepared as 1 mg/mL solutions in methanol, from which a working solution containing 10 µg/mL of each standard was prepared in methanol and a stock was prepared by serially diluting to 100 ng/mL in ultrapure water.

The stock solution was diluted using ultrapure water to prepare the standard curve.

A standard of metformin was purchased from Merck and prepared in ultrapure water at a concentration of 100 mg/mL. QC samples were prepared by combining the metformin, a suitable standard solution and water to dilute, providing a final metformin concentration of 20 mg/mL.

LC Conditions

LC system:	ACQUITY Premier (FTN)
Column(s):	Atlantis™ Premier BEH C ₁₈ AX (2.1 x 100, 1.7 µm), p/n: 186009368
Column temperature:	40°C
Injection volume:	30 µL
Flow rate:	0.4 mL/min
Mobile phase A:	Water 0.1% formic acid, 5 mM ammonium formate
Mobile phase B:	Methanol 0.1% formic acid, 5 mM ammonium formate
Gradient:	98% A hold 1.5 minutes, 98%–5% A 1.5–6.5 minutes, 5% A hold 6.5–7 minutes, re-equilibrate

initial conditions 7–9 minutes.

MS Conditions

MS system:	Xevo G3 QTof
Source type:	APCI+
Corona mode:	Current
Corona current (μA):	2.5
Cone voltage (V):	25
Source temperature ($^{\circ}\text{C}$):	150
Probe temperature ($^{\circ}\text{C}$):	325
Cone gas flow (L/hr):	300
Desolvation flow (L/hr):	450
StepWave mode:	Soft Transmission ³
Detector auto gain:	Off
Analyzer mode:	Sensitivity
Acquisition events:	Initial: divert to waste, 1 minute flow state LC, 8 minutes divert to waste

Lockspray flow ($\mu\text{l}/\text{min}$):	10
Lockspray settings:	Dual Point, leucine enkephalin (duration 2 seconds and sampled every 60 seconds)
IDC:	Off

MSE Settings

Acquisition range:	50–600 Da
Scanning speed:	2 Hz (0.5 second scan speed)
Collision energy low (eV):	2
Collision energy high (eV):	10

Tof-MRM Settings

Acquisition range:	Individual transitions (Figure 1)
Spectrum:	Narrow (<i>C₁₂ information only for reduced background signal</i>)
Scanning speed:	2 Hz (0.5 second scan speed)
Collision energy low (eV):	2
Collision energy high (eV):	Individual – Optimized per compound

MRM Experiment Experiment Type

Transitions Radar

Add Copy Paste Import Delete Delete All Spectrum: **Narrow** Scan time: s

	Name	Precursor (m/z)	Product (m/z)	Collision energy ramp start (V)	Collision energy ramp end (V)	Start time (min)	End time (min)	Run time (min)
1	NDMA	75.0550	75.0550	2	2	0.00	2.00	0.00 to 2.00
2	NMBA	147.0760	117.1000	5	5	2.00	3.80	2.00 to 3.80
3	NMPA	137.0710	66.1000	15	15	3.80	5.80	3.80 to 5.80
4	NDBA	159.1490	57.1000	10	10	5.80	8.50	5.80 to 8.50

Figure 1. ToF-MRM transitions displayed in the UNIFI app within waters_connect.

Data Processing

A complete workflow, from data acquisition to report generation was performed within the GXP compliant-ready software package: UNIFI app v 3.1.0.16. within waters_connect version 2.2.0.

Results and Discussion

Chromatographic separation of NDMA, NMBA, NMPA, and NDBA was performed using an ACQUITY Premier System and data were acquired on the Xevo G3 QToF fitted with an APCI source. Data acquisition, processing and review was carried out using the UNIFI application within the waters_connect software. Figure 2 shows a typical extracted ion chromatograph (XIC) of the nitrosamine contaminants and a metformin peak (typically diverted to waste to avoid MS saturation).

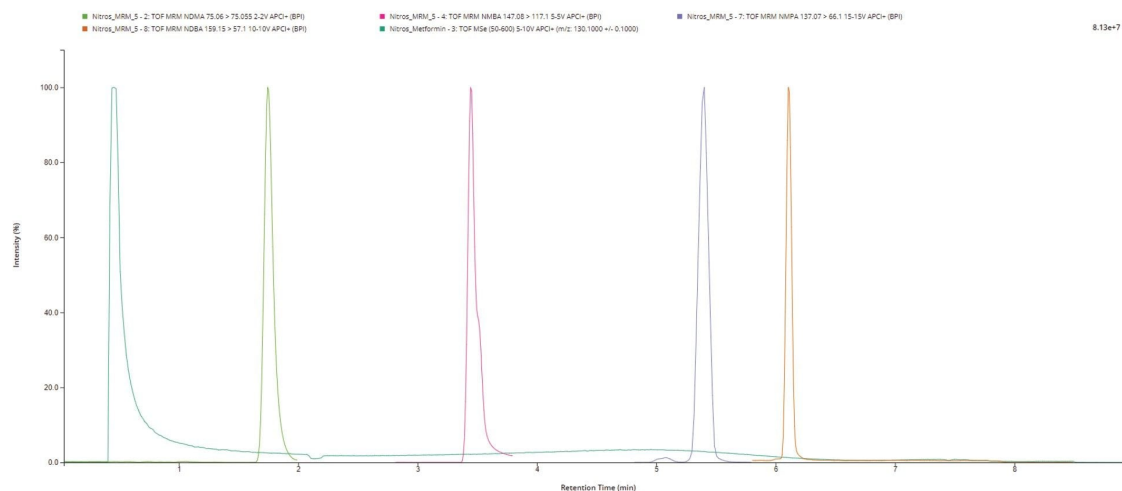


Figure 2. Overlaid XIC's of (in order left to right) Metformin, NDMA, NMBA, NMPA, and NDBA. The metformin peak displayed is from a 1 μ g column loading, all nitrosamine signals shown are with a 15 μ g column loading (note: axes are not linked).

The MS^E acquisition mode was utilized to optimize source conditions and ion transmission settings on a mix of standards with a 3 μ g column loading. During this optimization it was noted that utilizing the soft transmission StepWave settings³ (detailed in application note: [720007794](#)) improved the signal observed for all four precursor ions. Figure 3 shows each of the four compounds analyzed by MS^E with both default and soft transmission settings. Once soft transmission was shown to improve sensitivity the prepared curve and QC samples were analyzed using the ToF-MRM acquisition mode to establish limits of detection and robustness of the method settings.

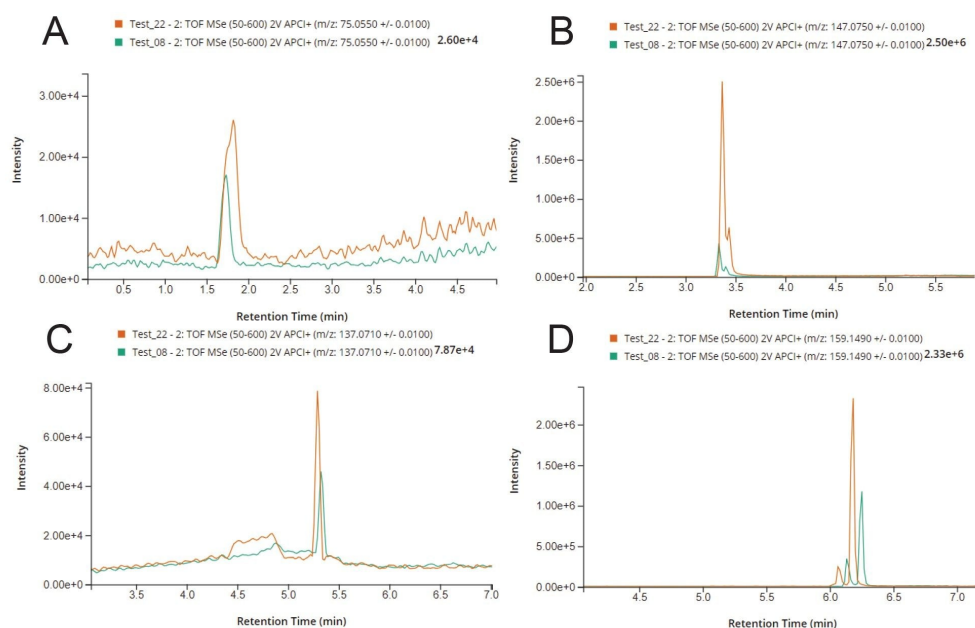


Figure 3. Overlaid extracted ion chromatograms for NDMA (A), NMBA (B), NMPA (C), and NDBA (D), showing signal improvement between soft ionisation StepWave settings (orange) and default StepWave settings (green).

Tof-MRM operates by time-segmented selection of the specified precursor mass, which is isolated in the quadrupole before entering the collision cell where either ion transmission or collision induced dissociation (CID) occurs. Then, user specified m/z value(s) are subjected to targeted signal enhancement through pusher synchronization in the Tof region.⁴ For the nitrosamine compounds: NMBA, NMPA, and NDBA were analyzed using a precursor to product transition and target enhancement was applied to the selected product ion m/z . For NDMA, a pseudo-MRM transition was performed and therefore targeted enhancement was performed for the precursor ion m/z .

Sensitivity and linear performance of the Xevo G3 QToF was assessed using a dilution series ranging from 0.001–10 ng/mL, with triplicate injections for each point. The resulting lower limits of detection and quantification (LLOD/Q) for the Tof-MRM acquisitions can be seen in Table 1. LLOD was determined as the lowest point on the calibration curve with a S/N ratio >3, LLOQ was determined as the lowest point on the calibration curve with an average % deviation from the curve of less than 20%, a %RSD of <10% and a S/N ratio >10, LLOD ranged from 0.005 ng/mL (5 pg/mL) for NMBA to 0.01 ng/mL (100 pg/mL) for the remaining three compounds. The LLOQ

ranged from 0.005 ng/mL (5 pg/mL) for NMBA to 0.05 ng/mL (50 pg/mL) for NDMA and NDPA, resulting in a 0.25 to 2.5 ppb sensitivity for quantification within a 20 mg/mL API solution. Figure 4 demonstrates the signal to noise values for each LLOQ observed.

Compound	Formula	Precursor (m/z)	Product (m/z)	RT (mins)	LLOD (ng/mL)	LLOQ (ng/mL)	LLOD (on column)	LLOQ (on column)	LLOQ (ppb*)	LLOQ (%RSD)
NDMA	C2H6N2O	75.06	75.1	1.7	0.01	0.05	30 ag	150 ag	2.5	1.9
NMBA	C5H10N2O3	147.08	117.1	3.3	0.005	0.005	15 ag	15 ag	0.25	2.3
NMPA	C7H8N2O	137.07	66.1	5.3	0.01	0.02	30 ag	60 ag	1.0	3.1
NDBA	C8H18N2O	159.15	57.1	6.2	0.01	0.05	30 ag	150 ag	2.5	6.4

*ppb equivalent of each standard calculated based upon a 20 mg/mL solution of API.

Table 1. Nitrosamine standards overview. Detailing ToF-MRM LLOD/Q for each standard compound and expressed as both the solution concentration in ng/mL and as column loading with a 30 μ L injection volume.

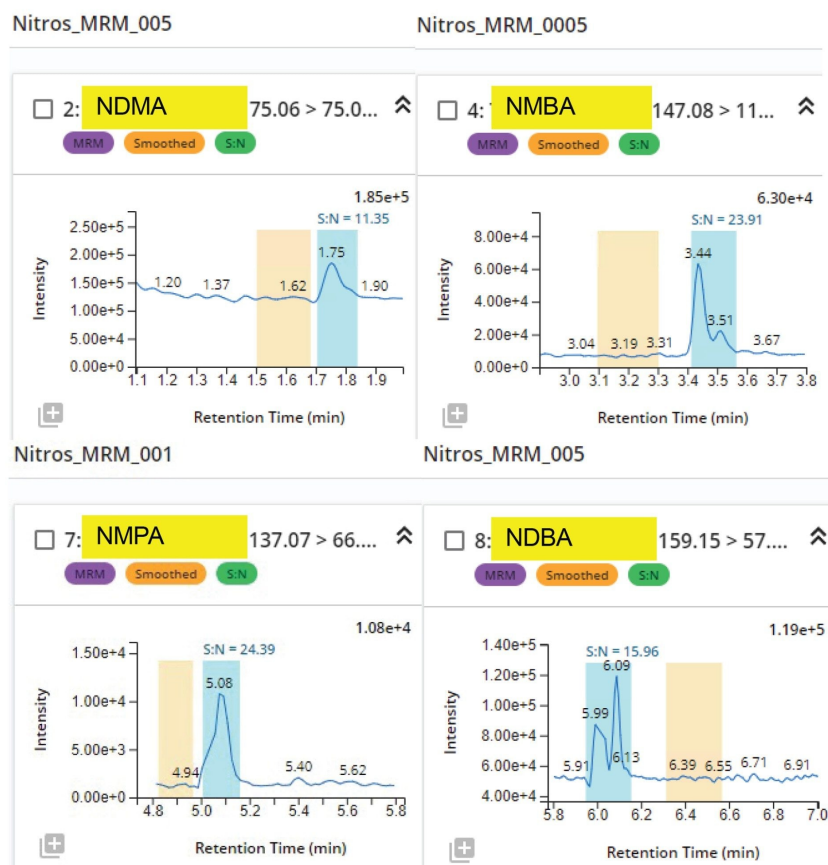


Figure 4. LLOQ Signal to noise (peak to peak S/N) calculation of NDMA, NMBA, NMPA, and NDBA. Smoothing performed is Stavitsky Golay with a width of +/- 2 datapoints and 2 iterations.

Tof-MRM linear performance for each compound can be seen in Figure 5, displayed as UNIFI produced (weighting 1/X) graphs which were used to determine linearity and deviation from the curve for LLOQ determination purposes. All four compounds showed a linear response demonstrating R^2 values of >0.998 within both processing software platforms. The maximum concentration analyzed was 10 ng/mL resulting in 300 pg on-column equivalent per compound. This loading did not exceed the linear dynamic range of the system for any of the compounds analyzed.

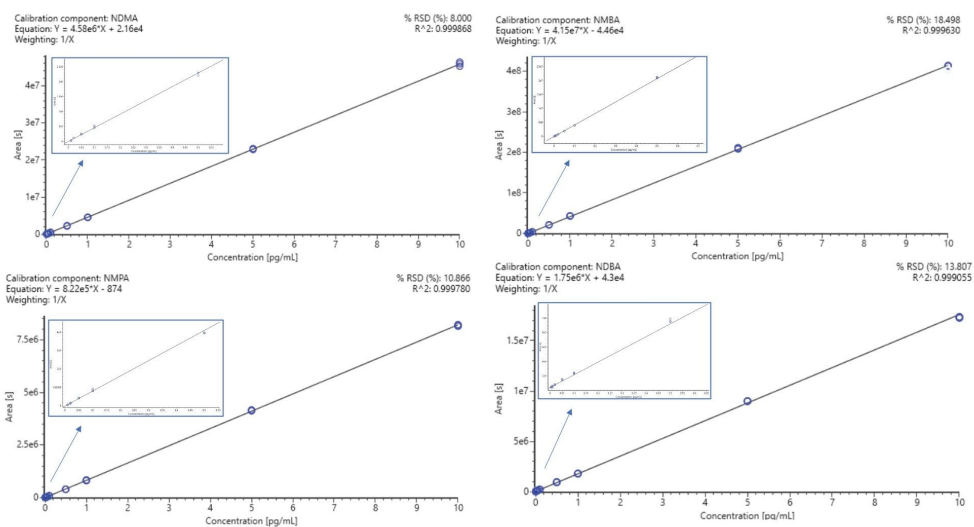


Figure 5. UNIFI generated linearity curves for each of the four nitrosamine standards. The linearity curves were utilized to assess the data these curves are shown with a 1/X weighting and were used to assess linearity ratings and to calculate % deviation from the curve for LLOQ determination.

For demonstration of quantification accuracy, nitrosamine standards were spiked into an API sample at two QC levels (0.5 ng/mL and 5 ng/mL) in a 20 mg/mL solution of metformin API. Calculated concentrations were averaged across 3 replicate injections at each level, and values calculated are within 15% of the spiked concentration. Figure 6 shows an example QC view from the UNIFI processing.

Injection reproducibility was shown to be excellent across all compounds and was assessed by calculating the standard deviation for the triplicate QC injections before taking an average for each compound. The highest average relative standard deviation (RSD) was for NMBA, with an average of 2.4% and the lowest average RSD being for NDMA with an average of 0.6%. When reproducibility was assessed using the standard deviation for the triplicate injections over the whole analysis - curve up to and including the LLOQ and QCs - the highest average RSD was NMBA with an average of 2.1% and the lowest average RSD being observed for NDMA with an average of 1.1%.

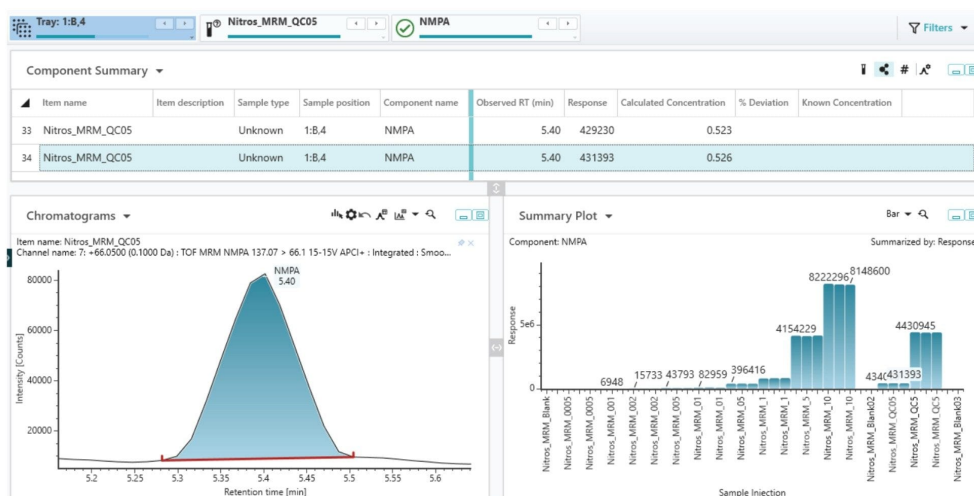


Figure 6. Representative UNIFI processing view, showing the summary plot of NMPA across all injections. The highlighted injection corresponds to a 0.5 ng/mL spiked QC injection.

Conclusion

The Xevo G3 QToF platform offers a flexible solution for nitrosamine analysis, benefiting from the ability to perform more routine quantification workflows utilizing Tof-MRM or full screening assays looking for unknowns utilizing MS^E. Being a QToF instrument, regardless of analysis mode, spectra can be viewed providing the user with accurate mass measurements and information regarding potential interfering isomeric compounds.

The flexibility of software platform allows for a tailored experience based upon laboratory environment, application or user requirements. The StepWave design enables transmission optimization based on fragility of compounds of interest, ensuring improved sensitivity for target analytes. Being a QToF mass spectrometer this instrument will also provide the user with accurate mass information for their analytes of interest, helping give confidence regarding compound identity and for identification of unknowns.

Providing a complete workflow from data acquisition to report generation in a fully compliant software platform: UNIFI app within waters_connect.

Sensitivity and linear performance of the Xevo G3 QTof was assessed using a dilution series ranging from 0.001–10 ng/mL, with triplicate injections for each point resulting in a 0.25 to 2.5 ppb sensitivity for quantification within a 20 mg/mL API solution. The system was shown to be linear up to a concentration of 10 ng/mL (300 pg on-column equivalent) for all four compounds analyzed, with R^2 values >0.998 and demonstrated an average RSD of $<2.4\%$.

References

1. FDA Guidance for industry: Control of Nitrosamine Impurities in Human Drugs, Revision 1, February 2021.
 2. Natural Products Solution on the Xevo G3 QTof Mass Spectrometer. Waters Application Note. [720008021](#). August 2023.
 3. Improved Transmission of Labile Species on the Xevo™ G3 QTof Mass Spectrometer with the StepWave™ XS. Waters Application Brief. [720007794](#). November 2022.
 4. Targeted High-Resolution Quantification with ToF-MRM and HD-MRM. Waters Application Note. [720004728](#). June 2013.
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