Application Note

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Quantification of Nitrosamine Impurities in Metformin Using Agilent GC/MS/MS Instrumentation

Authors

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Abstract

Since June 2018, several drugs were recalled due to the presence of nitrosamine impurities. The impacted molecules belonged mostly to sartans, followed by ranitidine and metformin. Regulatory authorities have allowed a transition period to make these changes in manufacturing processes for manufacturers to minimize nitrosamine impurities in finished products. During this transition period, interim limits have been applied to products. The low levels at which the nitrosamine impurities are expected to be analyzed creates challenges for testing. This application note highlights a complete solution for the determination and estimation of five nitrosamine impurities N-nitrosodimethylamine (NDMA), N-nitrosodiethylamine (NDEA), N-nitrosoethylisopropylamine (NEIPA), N-nitrosodiisopropylamine (NDIPA), and N-nitrosodibutylamine (NDBA) in metformin drug substances and drug products at trace levels. An Agilent 8890 GC coupled to an Agilent 7010B triple quadrupole GC/MS system was used to perform this. The limits of quantification (LOQs) achieved for NDMA, NDEA, NEIPA, and NDIPA impurities were <0.001 ppm while LOQ for NDBA was <0.0025 ppm with respect to drug substance. At LOQ, recoveries ranged within 80 to 120% with <5% RSD.

Introduction

Detection of nitrosamine impurities in sartans led to investigations into other medicines for the possible presence of nitrosamines.[1,2] All products that were identified as having a risk of nitrosamine formation were mandated for further testing to confirm the absence of nitrosamines. One such identified drug was metformin, available as immediate-release (IR) and extended-release (ER) forms.

Testing by the U.S. Food and Drug Administration (USFDA) has indicated the presence of NDMA at unacceptable levels in several lots of the ER formulation of metformin.[3] Currently, the elevated levels of NDMA have been detected only in some tablets of the ER formulation but have not been detected in drug substance.

The European Network of Official Medicines Control Laboratories (OMCLs) has released a few methods for the analysis of NDMA and NDEA in metformin drug substances and drug products.[4] These methods are based on the extraction of the drug substance or the finished product with dichloromethane alone or with an additional partitioning step with water. This is followed by analysis using a single quadrupole GC/MS or a triple quadrupole GC/MS/MS. Interfering peaks have been observed while analyzing drug products by single quadrupole MS. At lower concentrations of the impurities, this may lead to false positives. The relative intensity of the qualifier is much lower than the quantifier and hence it is difficult to confirm the presence or absence of the impurities. The higher baseline also results in reduced sensitivity. In contrast, GC/MS/MS methodology is better equipped for attaining specificity and sensitivity, and as such is preferred over GC/MS methods.

Although the official method from the OMCL network specifies only two impurities, it is still worthwhile to monitor other impurities such as NEIPA, NDIPA, and NDBA, all of which may arise unintentionally in the final product as carryover from the use of contaminated solvents.

We tested the previously developed method for nitrosamines in sartans for testing metformin using the 8890 GC coupled to the 7010B GC/MS/MS. The 7010B GC/MS/MS is equipped with the High Efficiency Source (HES) that has an improved ionization efficiency and 20x ion generation characteristics. This delivered confident trace analysis and helped to attain the very low detection limits required for the analysis. The 8890 GC has a touch screen interface, instead of a keypad, to control the GC and offers instrument operation diagnostic tests, system monitoring alerts, and mobile access.

Experimental

Sample preparation

The APIs and drug products tested for this analysis included metformin, metformin ER (500 mg, 750 mg, and 1,000 mg). Three different methods of sample preparation were evaluated.

Method 1: For drug substance: A portion of 500 mg of drug substance was weighed accurately into a disposable 15 mL glass centrifuge tube, and 5 mL of internal standard solution (~50 ng/mL NDMA:C13-d_6 in dichloromethane) was added via volumetric pipette. The sample was vortexed for one minute, then placed in the centrifuge and spun at 4,000 rpm for five minutes. The undissolved metformin drug substance settled at the bottom. Using a disposable pipette, approximately 2 mL of the dichloromethane layer was filtered through a 0.45 µm nylon filter and transferred to a GC vial for analysis.

For finished drug product: Approximately 10 tablets were crushed and homogenized. From the homogenized mixture, a portion equivalent to 500 mg of drug substance was weighed and processed for extraction as above.

Method 2: For drug substance: A portion of 500 mg of drug substance was weighed accurately into a disposable 15 mL glass centrifuge tube, and 5 mL of internal standard solution (~50 ng/mL NDMA:C13-d_6 in water) was added via volumetric pipette. The sample was vortexed for one minute followed by an addition of 5 mL of dichloromethane. The tubes were again vortexed for two minutes, then placed in the centrifuge and spun at 4,000 rpm for five minutes. This resulted in the formation of a lighter aqueous layer and a heavier organic layer. Using a disposable pipette, approximately 2 mL of the dichloromethane layer at the bottom was withdrawn and filtered through a 0.45 µm nylon filter and transferred to a GC vial for analysis.

For finished drug product: Approximately 10 tablets were crushed and homogenized. From the homogenized mixture, a portion equivalent to 500 mg of drug substance was weighed and processed for extraction as above.

Method 3: For drug substance: A portion of 500 mg of drug substance was weighed accurately into a disposable 50 mL glass centrifuge tube, and 5 mL of internal standard solution (~50 ng/mL NDMA:C13-d_6 in 1 N HCl) was added via volumetric pipette. The sample was vortexed for one minute followed by an addition of 5 mL of dichloromethane. The tubes were again vortexed for two minutes, then placed in the centrifuge and spun at 4,000 rpm for five minutes. This resulted in the formation of a lighter aqueous layer and a heavier organic layer. Using a disposable pipette, approximately 2 mL of the dichloromethane layer at the bottom was

withdrawn and filtered through a 0.45 µm nylon filter and transferred to a GC vial for analysis.

For finished drug product: Approximately 10 tablets were crushed and homogenized. From the homogenized mixture, a portion equivalent to 500 mg of drug substance was weighed and processed for extraction as above.

Standard preparation

The standard stock was diluted appropriately to obtain calibration solutions of the following concentrations: 100, 80, 40, 20, 10, 5, and 2.5 ng/mL, each prepared in dichloromethane containing NDMA:C13-d_6 as internal standard.

Instrumentation

Analysis was performed using the 8890 GC equipped with the 7693A automatic liquid sampler coupled to the 7010B triple quadrupole GC/MS/MS. From the inlet, an Agilent J&W DB-WAX GC capillary column (30 m × 0.25 mm, 0.5 µm) was connected to the MS.

Tables 1 and 2 display the GC and MS parameters.

MS acquisition method

Using the MRM optimizer tool, the MRMs for all five impurities were developed and were used for data acquisition.

Table 1. GC parameters.

Parameter	Value
MMI Injection Mode	Pulsed splitless: 12.285 psi until 0.5 min
Inlet Temperature	250 °C
Oven Temperature Program	40 °C (1.5 min) 20 °C/min to 200 °C (0 min) 60 °C/min to 250 °C (3 min)
Total Run Time	13.33 minutes
MS Transfer Line Temperature	250 °C
Injection Volume	2 µL
Carrier Gas	Helium, 1 mL/min

Table 2. MS parameters.

Parameter	Value	
Mode	Electron ionization, 70 eV	
Source Temperature	250 °C	
Quadrupole Temperature	Q1 and Q2 = 150 °C	
MRM Mode Conditions		
MS1 Resolution	All compounds unit	
MS2 Resolution	All compounds unit	
Collision Gas Flow	Nitrogen at 1.5 mL/min	
Quenching Gas Flow	Helium at 4 mL/min	
Quantifier/Qualifier Transitions	Start time: 6.5 min	**NDMA** 74 → 44.1, CE 6, dwell 150 ms 74 → 42.1, CE 22, dwell 50 ms NDMA:C13-d_6 82 → 48, CE 20, dwell 100 ms
	Start time: 7.60 min	**NDEA** 102 → 85, CE 4 V, dwell 80 ms 102 → 56.1, CE 18 V, dwell 80 ms 102 → 44.1, CE 14 V, dwell 80 ms
	Start time: 8.03 min	**NEIPA** 116 → 99.1, CE 4 V, dwell 80 ms 71 → 56.1, CE 4 V, dwell 80 ms 116 → 44.1, CE 14 V, dwell 80 ms
	Start time: 8.25 min	**NDIPA** 130 → 88, CE 4 V, dwell 150 ms 130 → 42, CE 10 V, dwell 150 ms
	Start time: 8.70 min	**NDBA** 158 → 99.1, CE 8 V, dwell 75 ms 84 → 56.1, CE 20 V, dwell 75 ms 84 → 42.1, CE 14 V, dwell 75 ms 158 → 141.2, CE 2 V, dwell 75 ms

Results and discussion

The MRMs for this method were developed using the TQ optimizer. Automated MRM development was carried out to obtain the optimized MRMs of all five impurities. The process for automated MRM development is described in another application note.[5]

The optimized MRMs were exported to the method and sample acquisition was carried out. Figure 1 describes the optimized MRMs and the chromatogram obtained is displayed in Figures 2 and 3.

The compounds were separated sufficiently, and the target peaks (Figure 2) were well resolved from solvent and matrix species.

Calibration curves were generated using a linear fit. Excellent linearities with R^2 >0.999 were obtained in this study for all five impurities, as shown in Figure 4.

The repeatability of injections was tested by consecutive injection of a 40 ng/mL standard. Six consecutive injections resulted in <2% RSDs for all five impurities.

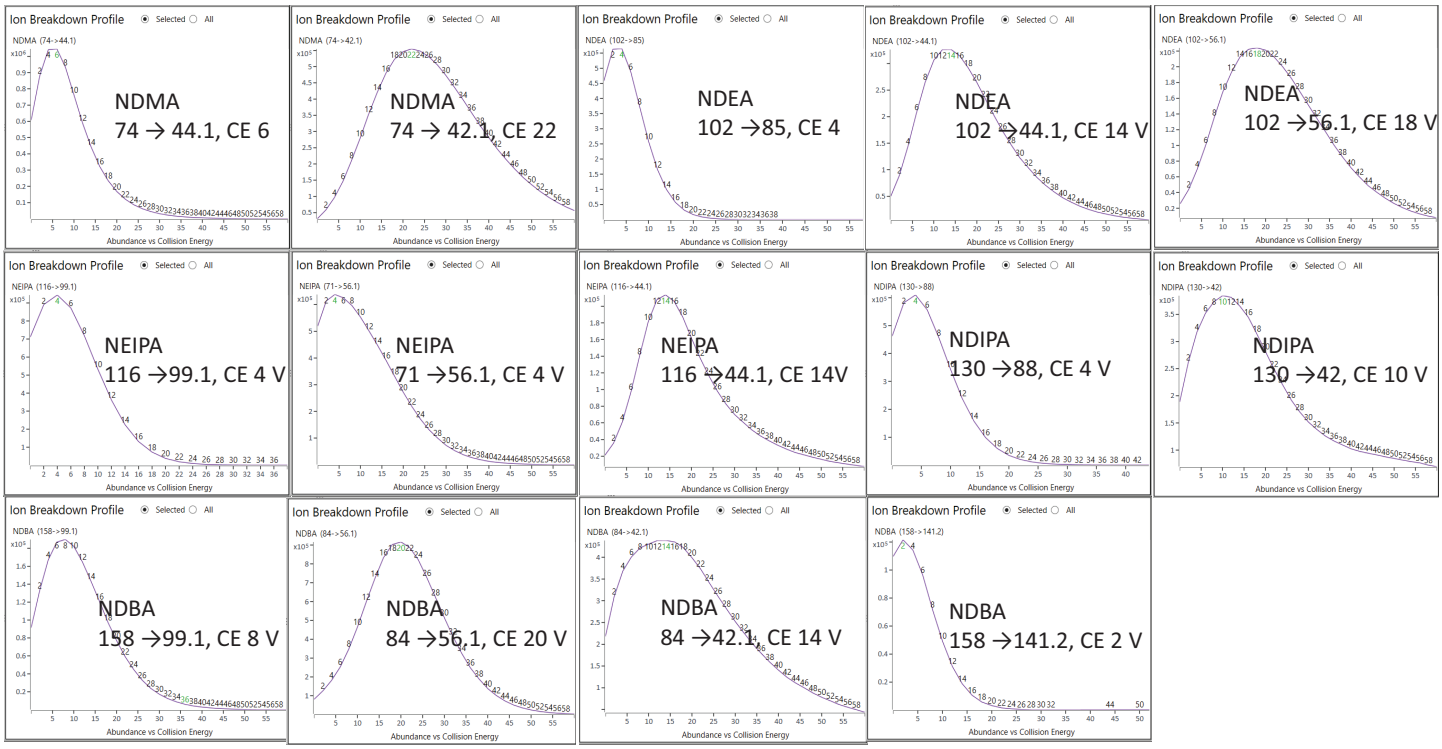

Figure 1. Optimized MRM transitions of five nitrosamine impurities using the TQ optimizer.

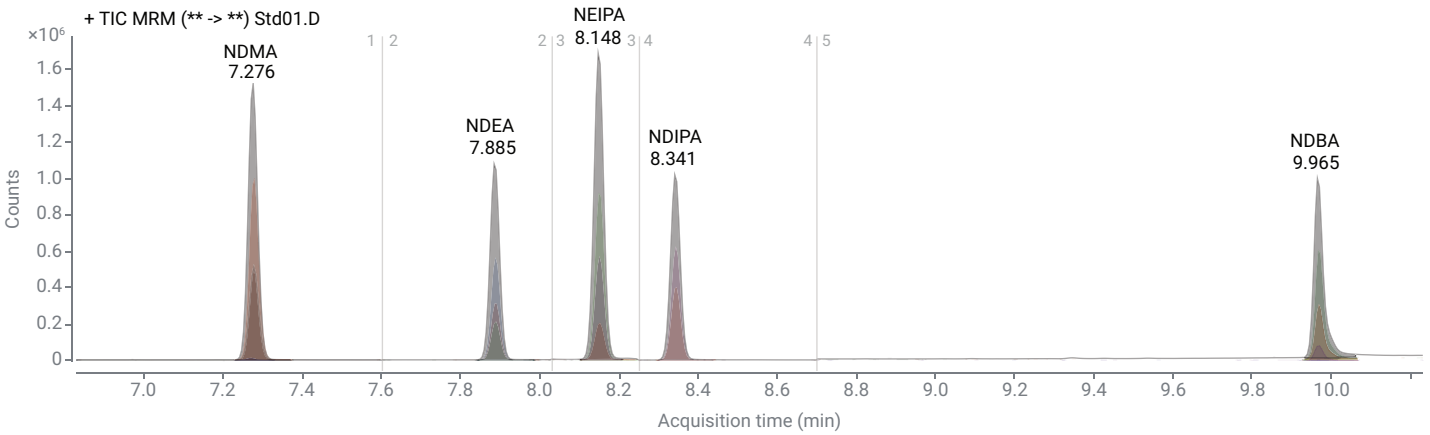

Figure 2. TIC chromatogram overlay in MRM mode of 100 ng/mL of five impurities in dichloromethane.

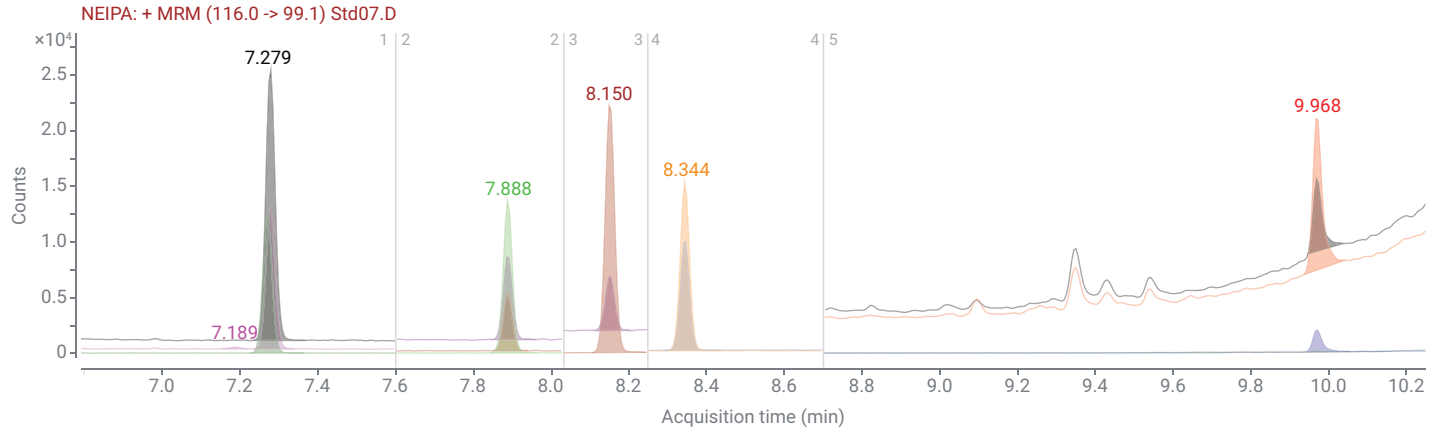

Figure 3. Extracted MRM chromatogram (quantifier and qualifier transition) of lowest calibration standard at 2.5 ng/mL mix of five impurities in dichloromethane.

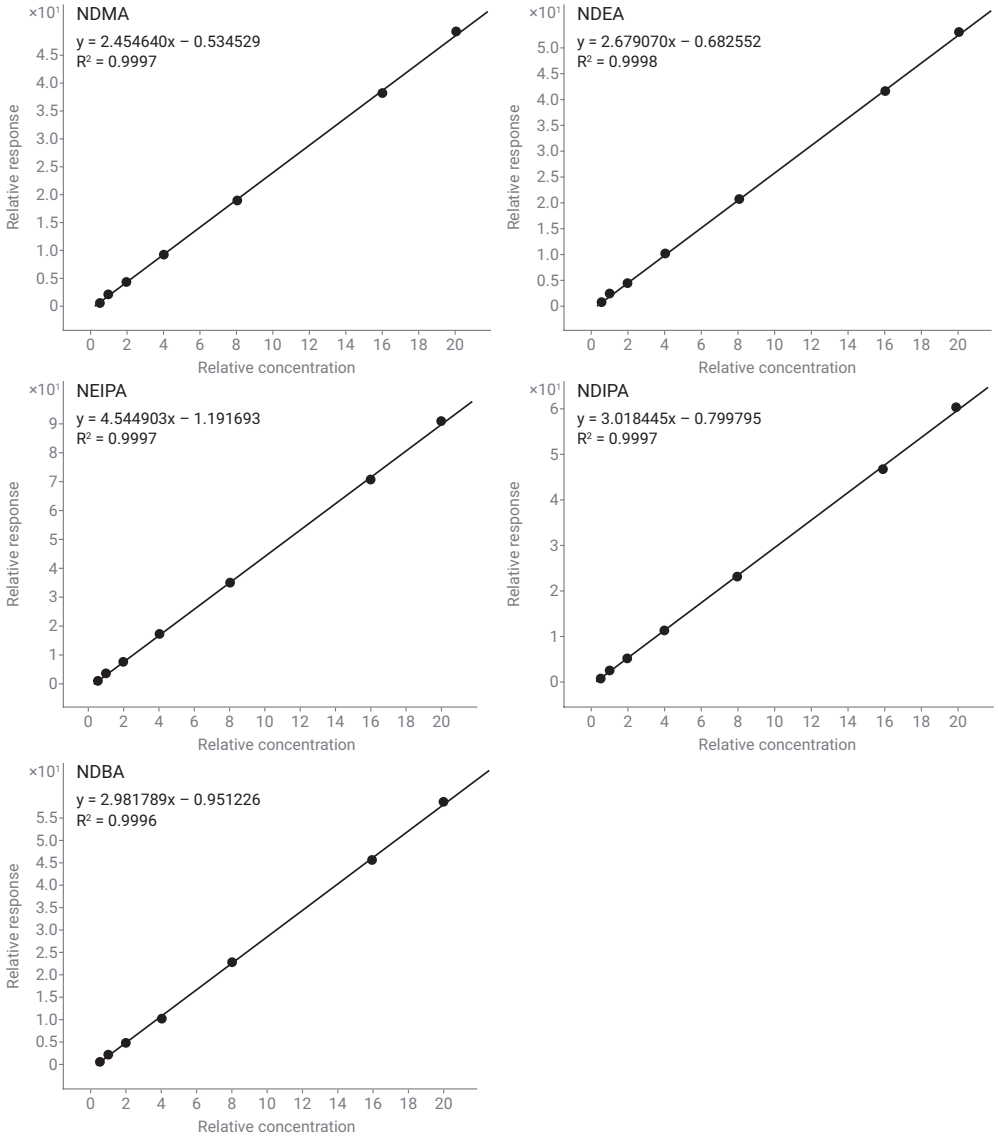

Figure 4. Calibration curves of NDMA, NDEA, NEIPA, NDIPA, and NDBA (2.5 to 100 ng/mL).

Samples of drug substances and drug products were analyzed using all three methods as described in previous sections. When a drug substance was analyzed using methods 1 and 3, relatively higher peak areas were noted. Method 2 resulted in comparatively lower peak areas; however, the calculated values were similar owing to internal standard correction (Figure 5). Similar observations were noted for drug product as well (Figure 6).

Method 1 could be used efficiently for all samples tested for drug substance. When the same method was used for drug products, the presence of certain excipients resulted in the formation of a viscous solution. Some of the excipients (e.g., povidone K-30, povidone K-90) present in the final drug product were freely soluble in dichloromethane. As a result, it was impossible to obtain a clear extract through centrifugation or filtration. Therefore method 1 was unsuitable for most of the drug products.

Methods 2 and 3 were advantageous in terms of phase separation where the gel formation was limited to the aqueous layer and it was relatively easier to separate the clear organic layer after centrifugation. No specific advantages in terms of reduction in interfering peaks or coelutions were noted in the samples tested. Method 2 resulted in lesser peak areas, and therefore was associated with higher LOQ. Methods 1 and 3 resulted in lower method LOQs.

Table 3. Peak areas and %RSD values of nitrosamine impurities at 40 ng/mL.

Standard Injections	NDMA (Area)	NDEA (Area)	NEIPA (Area)	NDIPA (Area)	NDBA (Area)
Std3_RepCheck_001.D	381642	403839	676849	445496	412430
Std3_RepCheck_002.D	379964	402289	669489	445593	413609
Std3_RepCheck_003.D	383455	404972	684527	451592	403516
Std3_RepCheck_004.D	386450	407865	685165	457072	398952
Std3_RepCheck_005.D	389766	409850	694079	460929	408615
Std3_RepCheck_006.D	392361	418186	704485	466412	405082
	1.25	1.41	1.80	1.86	1.37

Method 1 is simplest in terms of sample preparation but unsuitable for formulations that can result in viscous extracts of dichloromethane. Method 2 can be used to analyze both drug substances and drug products. Lower absolute areas indicate that recoveries are reduced. However, quantification based on internal standard correction results in satisfactory recoveries. Method 3 can be used to analyze both drug substances and formulation.

Sample recoveries were calculated by fortifying the drug substance and a homogenized drug product at 0.005 ppm. The recoveries were found satisfactory within the range of 80 to 120% for all three methods.

A United States Pharmacopeia (USP) signal-to-noise ratio (S/N) of 10 was used as a basis for LOQ determination in the methods. In the present study, S/N values for samples spiked at concentrations of 0.001 ppm were evaluated. This demonstrates that the instrument meets the sensitivity requirements easily and further lower LOQs could be achieved, enabling very trace level detections (Figure 7).

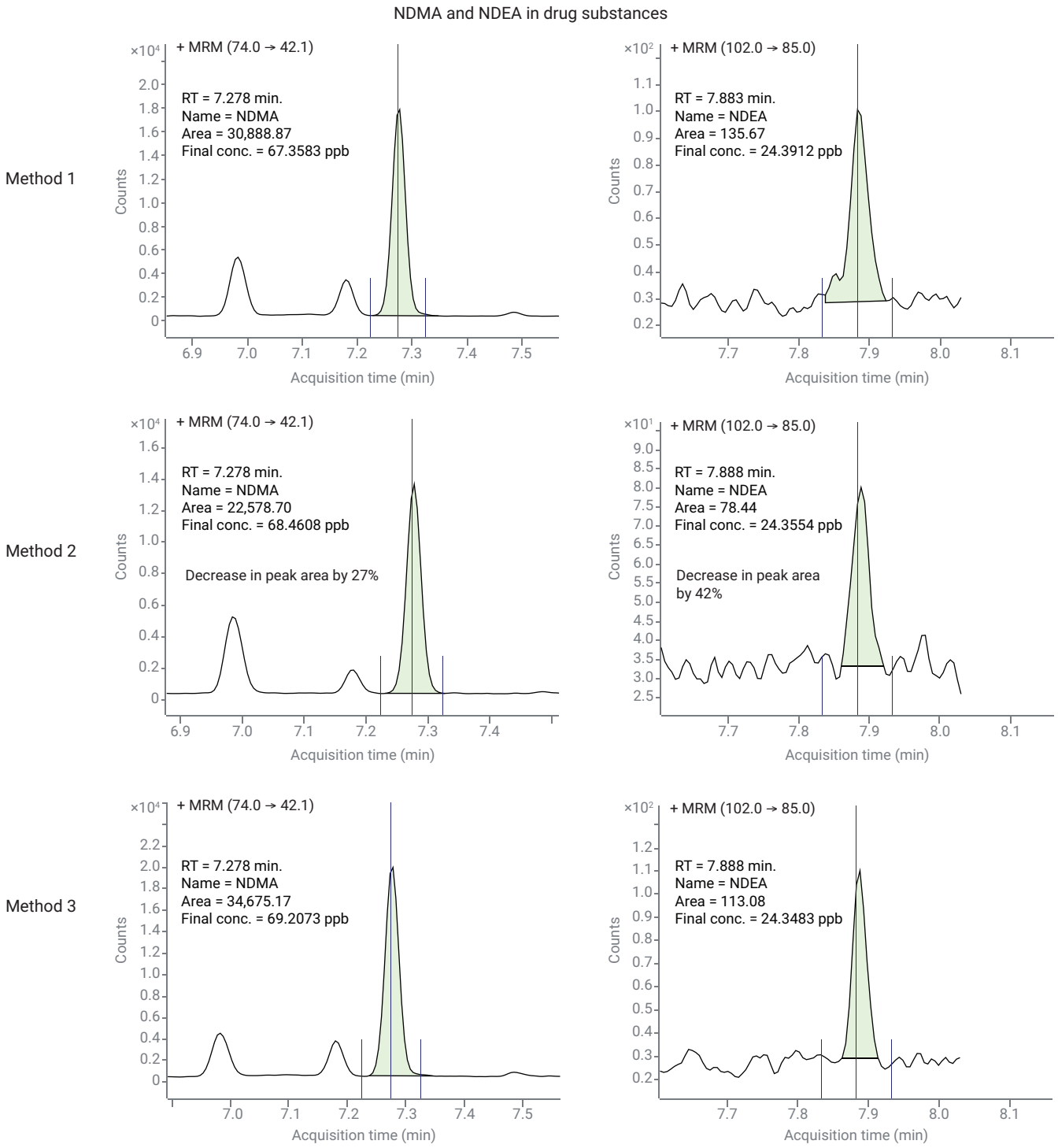

Figure 5. NDMA and NDEA in a metformin sample using three different extraction methods.

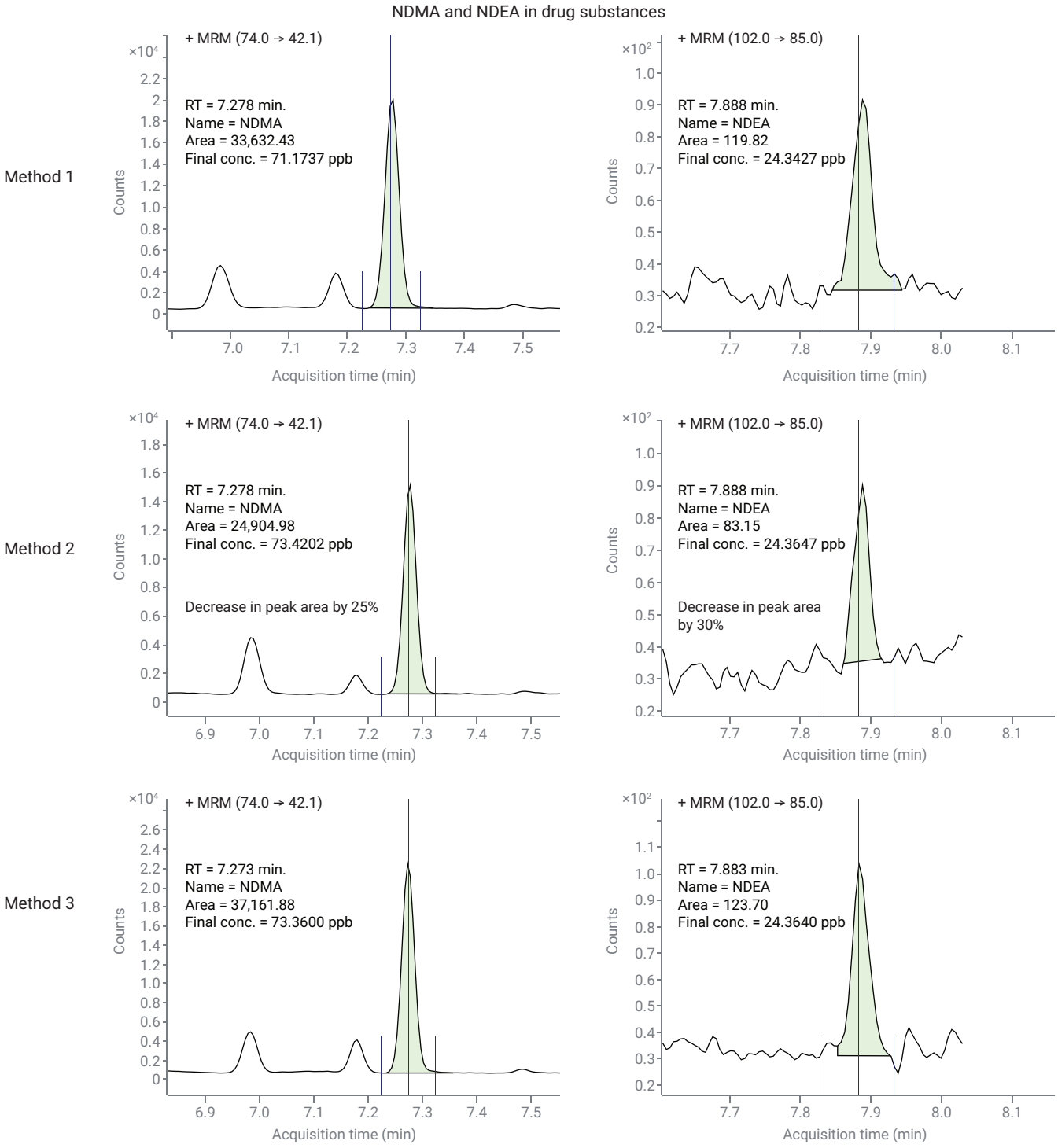

Figure 6. NDMA and NDEA in a metformin finished product using three different extraction methods.

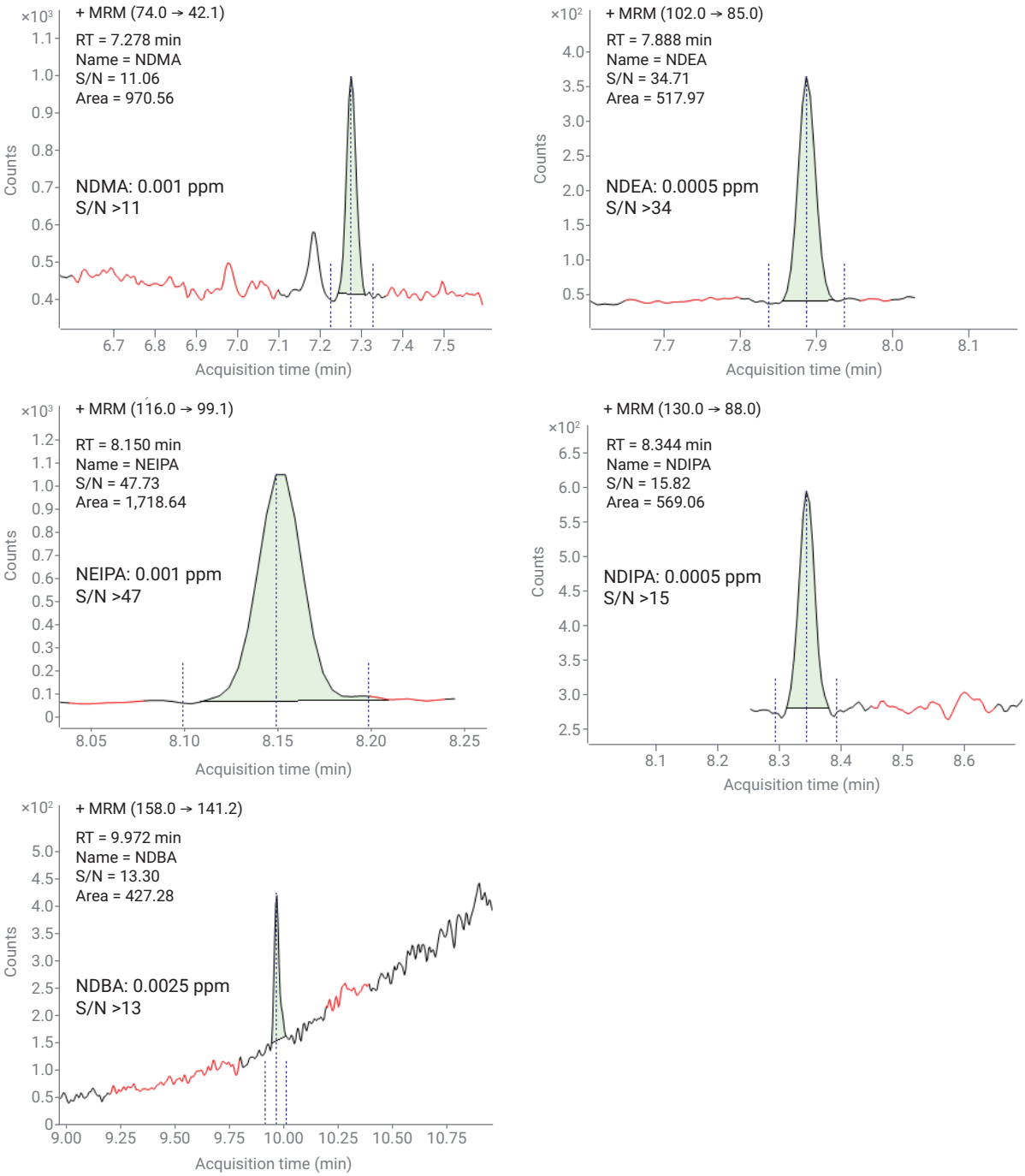

Figure 7. S/N of samples spiked at trace levels for LOQ determination.

Conclusion

The Agilent 8890 GC and the Agilent 7010B GC/MS/MS systems demonstrated excellent performance for the determination of all five nitrosamine drug impurities in metformin. The 8890 GC offers instrument operation diagnostic testing and system monitoring alerts as well as touch screen control and mobile access. The mobile access option enables operators and managers to securely monitor instrument status and function while away from the lab, ensuring minimum downtime along with unassisted troubleshooting. These features are useful for continued operation of the system. The design of the 7010B triple quadrupole GC/MS, which includes the HES, enables lower detection limits for trace-level impurities when combined with the inert sample path provided by the 8890 GC. These features enabled reliable quantification of all five residues.

References

1. https://www.ema.europa.eu/en/human-regulatory/post-authorisation/referral-procedures/nitrosamine-impurities
2. https://www.ema.europa.eu/en/news/ema-update-metformin-diabetes-medicines
3. https://www.fda.gov/drugs/drug-safety-and-availability/fda-updates-and-press-announcements-ndma-metformin
4. https://www.edqm.eu/en/ad-hoc-projects-omcl-network#Methods%20for%20determination%20of%20nitrosamines%20in%20metformin
5. Andrianova, A.; Quimby, B.; Churley, M.; Automated MRM Method Development for US EPA Method 8270 with the Agilent MassHunter Optimizer for GC/TQ. *Agilent Technologies application note*, publication number 5994-2086EN, **2020**.

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