Application Note

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# Analysis of Five Nitrosamine Impurities in Drug Products and Drug Substances Using Agilent GC/MS/MS Instrumentation


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## Abstract

This application note highlights a comprehensive solution for the determination and estimation of five nitrosamine impurities (NDMA, NDEA, NEIPA, NDIPA, and NDBA) in sartan drug products and drug substances at trace levels using an Agilent 7890B or 8890 GC coupled to an Agilent 7010B triple quadrupole GC/MS system. The 7010B triple quadrupole GC/MS is equipped with a high-efficiency source (HES) that offers excellent sensitivity, repeatability, and precision while outperforming regulatory limits. The method allows for LOQs that are 2 to 20 times lower than what is required by current regulations.

## Introduction

Beginning in July 2018, the FDA announced recalls of valsartan due to the presence of N-nitrosodimethylamine (NDMA). Subsequent investigations of API and finished drugs in the angiotensin-receptor blocker (ARB) class from all manufacturers also resulted in additional recalls of valsartan, irbesartan, and losartan. These products were found to contain NDMA and N-nitrosodiethylamine (NDEA), both of which are known animal and suspected human carcinogens.

Subsequently, other impurities, N-nitrosoethylisopropylamine (NEIPA), N-nitrosodiisopropylamine (NDIPA), N-nitrosodibutylamine (NDBA), and N-nitrosomethyl-4-amino-butyric acid (NMBA), were flagged as potential nitrosamine impurities. To date, sartan drugs (valsartan, losartan, and irbesartan) belonging to more than 1,100 different lots have been recalled because they contained these impurities above the interim limits.

Of the several methods released by the FDA Office of Testing and Research (OTR) for the analysis of these impurities, the latest involved analysis using single quadrupole GC/MS with headspace-based injection for four impurities and a liquid injection-based method by triple quadrupole GC/MS/MS for five impurities. Single quadrupole MS often gives ambiguous results and less sensitivity. In contrast, GC/MS/MS is more applicable for attaining specificity in such situations, and is preferred over GC/MS methods.

We performed the OTR method using either the 7890B or 8890 GC coupled to the 7010B GC/MS/MS, and found that both systems provide excellent performance for all five impurities. The HES, with improved ionization efficiency and 20x ion generation characteristics, delivers confident trace analysis. The 8890 GC has a touch screen interface instead of a keypad to control the GC, and offers diagnostic tests, system monitoring alerts, and mobile access.

## Experimental

### Sample preparation

The APIs and drug products tested for this analysis included valsartan, olmesartan, irbesartan, and losartan. A portion of 500 mg of API 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. Some of the sartans (e.g. valsartan and olmesartan) were completely soluble in dichloromethane while some (e.g. irbesartan) formed a cloudy solution or were insoluble. These samples were vortexed for one minute, then placed in the centrifuge and spun at 4,000 rpm for 2.5 minutes. 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.

### 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 either the Agilent 7890B or 8890 GC equipped with an Agilent 7693A automatic liquid sampler coupled to an Agilent 7010B triple quadrupole GC/MS. The GC was configured with a 7697A headspace sampler connected to a multimode inlet (MMI). From the inlet, an Agilent J&W VF-WAXms GC capillary column of dimensions 30 m × 0.25 mm, 1.0 µm was connected to the MS.

Tables 1 and 2 display the GC and MS parameters.

**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 (0.5 min)<br>20 °C/min to 200 °C (0 min)<br>60 °C/min to 250 °C (3 min) |
| Total Run Time | 12.33 min |
| MS Transfer Line Temperature | 250 °C |
| Injection Volume | 2 µL |
| Carrier Gas | Helium, 1 mL/min |

### MS acquisition method

MRMs from the OTR method were used for data acquisition.

## Results and discussion

The compounds were separated sufficiently, and the target peaks were well resolved from solvent and matrix species. Retention times of all five compounds agreed (Figures 1 and 2) with those provided in the FDA regulation.

Calibration curves were generated using a linear fit. The FDA requires the correlation coefficient, $R^2$, to be ≥0.998. Excellent linearities, with $R^2$ >0.999, were obtained in this study for all five impurities, as shown in Figures 3A

**Table 2.** MS parameters.

| Parameter | Value | |
|---|---|---|
| Mode | Electron ionization, 40 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 | |
| Detector Gain | 1 | |
| Quant./Qual. Transitions (FDA method) | Start time: 6.5 min | **NDMA**<br>74 → 44, CE 15, dwell 150 ms<br>74 → 42, CE 20, dwell 50 ms<br>NDMA:C13-$d_6$ 82 →48, CE 20, dwell 100 ms |
| | Start time: 7.60 min | **NDEA**<br>102 →85, CE 10 V, dwell 150 ms<br>102 →56, CE 18 V, dwell 150 ms |
| | Start time: 8.03 min | **NEIPA**<br>116 →99, CE 10 V, dwell 150 ms<br>71 →56, CE 10 V, dwell 150 ms |
| | Start time: 8.25 min | **NDIPA**<br>130 →88, CE 10 V, dwell 150 ms<br>130 →42, CE 10 V, dwell 150 ms |
| | Start time: 8.70 min | **NDBA**<br>158 →99, CE 10 V, dwell 150 ms<br>84 →56, CE 22 V, dwell 150 ms |

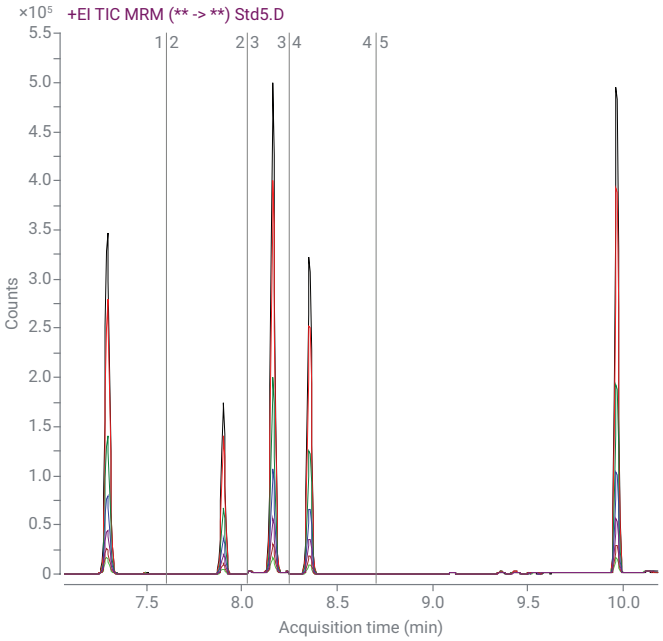


**Figure 1.** MRM TIC chromatogram overlay of seven calibration levels for the five impurities in dichloromethane (Agilent 8890 GC).

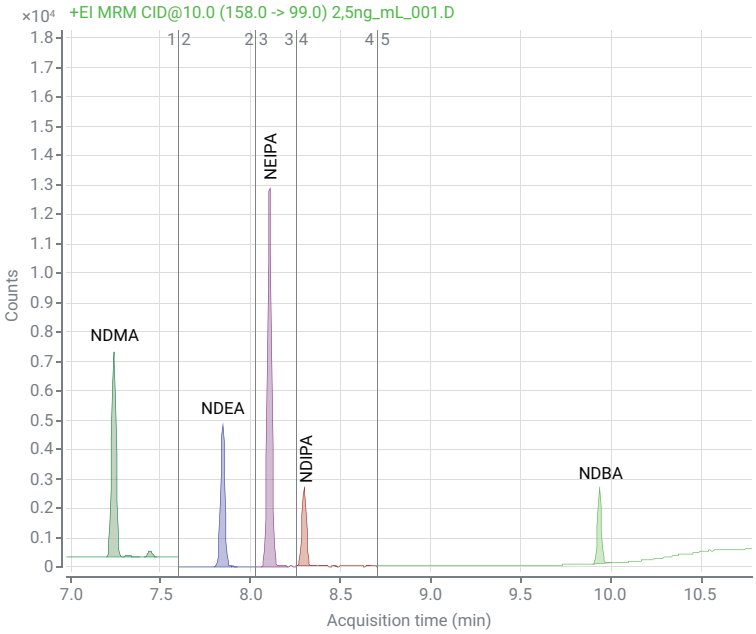


**Figure 2.** Extracted MRM chromatogram (quant transition) of lowest calibration standard at 2.5 ng/mL mix of five impurities in dichloromethane (Agilent 7890B GC).

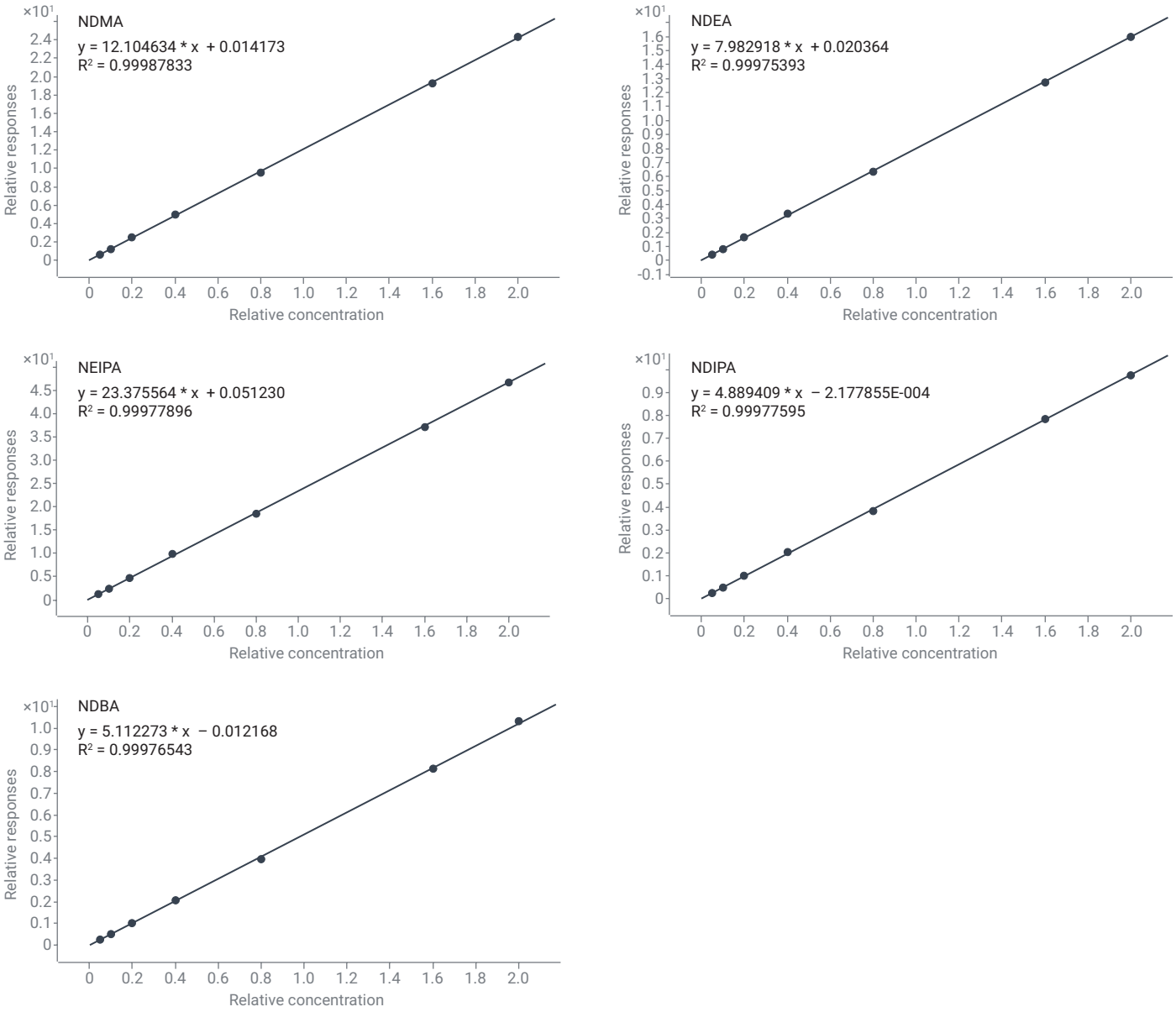


Figure 3A. Calibration curves of five nitrosamine impurities using the Agilent 7890B GC.

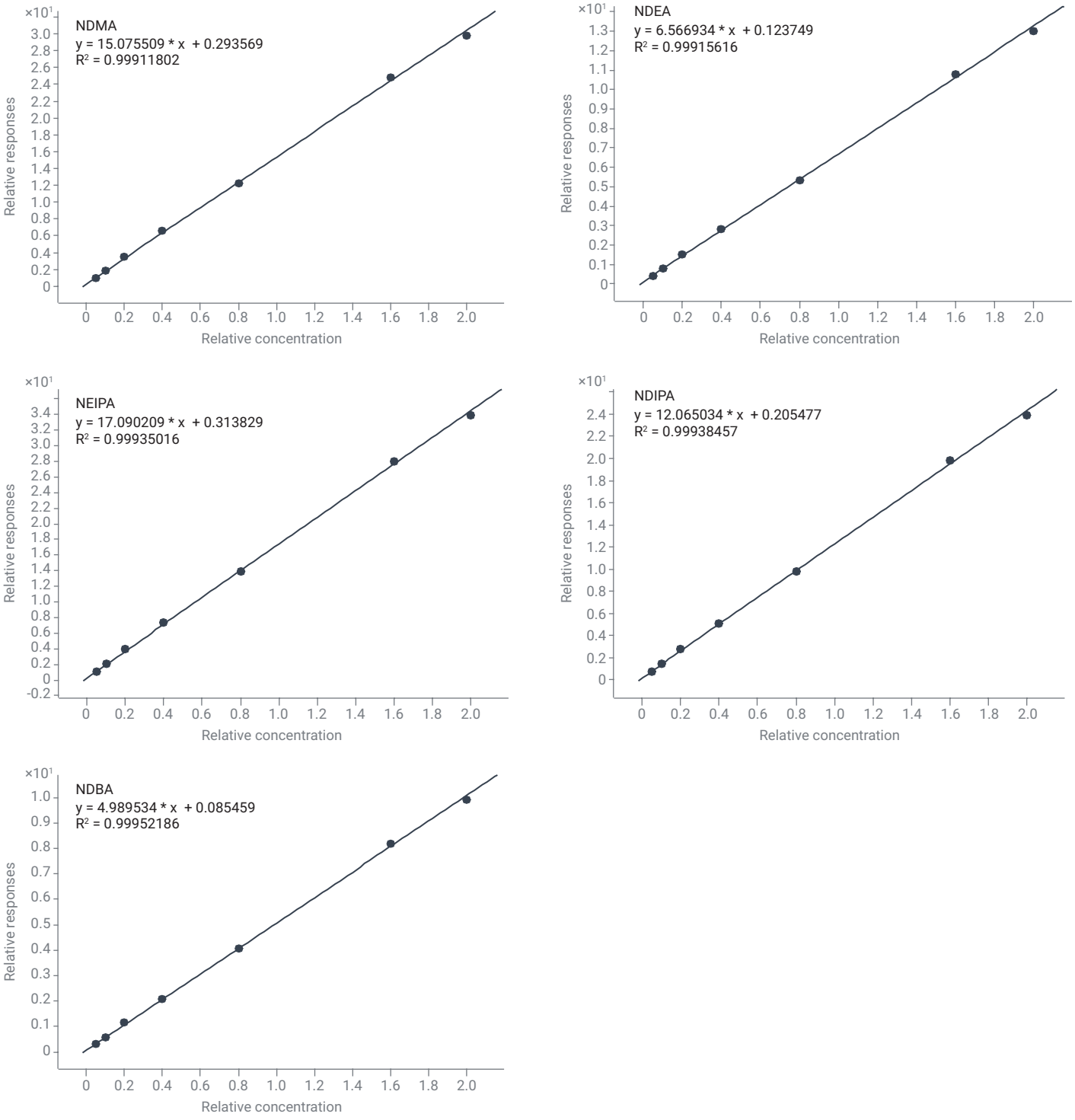


**Figure 3B.** Calibration curves of five nitrosamine impurities using the Agilent 8890 GC.

(7890B GC) and 3B (8890 GC).

The validated FDA-OTR method requires that the %RSD for six replicate injections of the 40 ng/mL standard is ≤5. In our study, the 40 ng/mL standard was checked for repeatability, and %RSDs <2 (six consecutive injections) were obtained for all five impurities, as shown in Table 3.

A signal-to-noise ratio (S/N) of 10 is used as a basis for LOQ determination in the OTR method. In the present study, S/N values for samples spiked at concentrations recommended as per FDA LOQs were found to be much higher than what is required by the method, suggesting that the instrument meets the sensitivity requirements easily and that lower LOQs could be achieved, enabling very trace level detection. Drug product LOQs provided by the FDA along with LOQs obtained in this study are presented in Table 4. Chromatography examples for impurities in samples at the LOQ level along with calculated S/N are

**Table 3.** Peak areas and %RSD values of nitrosamine impurities at 40 ng/mL (Agilent 8890 GC).

| Type | Name | NDMA Area | NDEA Area | NEIPA Area | NDIPA Area | NDBA Area |
|---|---|---|---|---|---|---|
| Std | STD3_001.D | 134044.10 | 57894.56 | 151634.00 | 106545.00 | 43152.93 |
| Std | STD3_002.D | 130975.10 | 57019.84 | 147810.80 | 104067.40 | 42709.92 |
| Std | STD3_003.D | 131357.00 | 56826.24 | 149615.50 | 104264.10 | 42613.02 |
| Std | STD3_004.D | 134631.90 | 57973.19 | 152279.80 | 106116.10 | 43753.81 |
| Std | STD3_005.D | 132140.20 | 57361.97 | 149922.00 | 105469.30 | 43118.18 |
| Std | STD3_006.D | 131370.50 | 57048.14 | 149667.10 | 103762.50 | 42816.16 |
| | RSD (%) | 1.17 | 0.84 | 1.07 | 1.11 | 0.97 |

**Table 4.** FDA method drug product LOQs and LOQs obtained in this study (Agilent 7890B GC).

| Impurity | FDA LOQ (ppm) | LOQ (Obtained, in ppm) | Improvement Factor |
|---|---|---|---|
| NDMA | 0.008 | 0.0025 | >3 |
| NDEA | 0.005 | 0.0005 | 10 |
| NEIPA | 0.005 | 0.00025 | 20 |
| NDIPA | 0.005 | 0.0025 | 2 |
| NDBA | 0.025 | 0.008 | >3 |

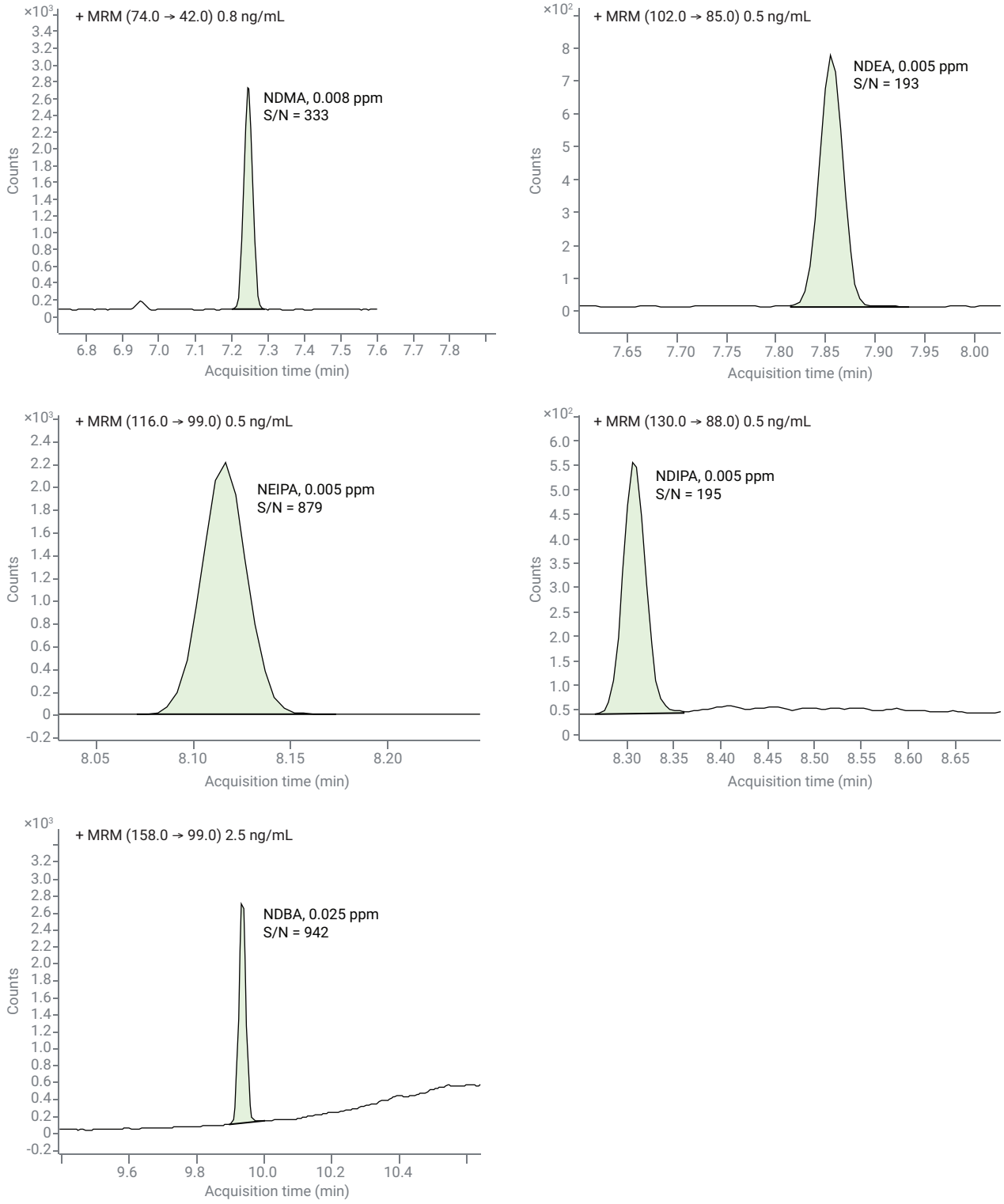


**Figure 4.** S/N of samples spiked at concentrations recommended as per FDA LOQs (Agilent 7890B GC).

shown in Figure 4.

## Conclusion

The 7890B-7010B and 8890-7010B GC/MS/MS systems demonstrated similar, excellent performance for all five nitrosamine drug impurities. The 8890 GC offers diagnostic tests and system monitoring alerts as well as touch screen control and mobile access. 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 7890B and 8890 GCs. These features enabled reliable quantification of all five residues. High-sensitivity analysis with improved LOQs that are 2- to 20-fold lower than recommended levels can be performed without changing method parameters.

## References


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