Application Note

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Quantification of Nine Nitrosamine Impurities in Sartan Drugs Using an Agilent GC-TQ

Authors

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Abstract

In this application note, a method for the analysis of nine nitrosamine impurities was developed using the Agilent 8890 GC equipped with an Agilent 7693A automatic liquid sampler coupled to an Agilent 7010B triple quadrupole GC/MS/MS. Three different columns were evaluated using two GC programs (12 minutes and 20 minutes) for the nine impurities. Excellent linearities with R^2 >0.997 were obtained in this study. Long-term repeatability studies done by consecutive injections of 150 spiked samples at 30 ng/g of sample yielded <8% RSD, indicating long-term precision of the method. Sample recoveries were calculated by fortifying the drug substance and a homogenized drug product at 30 ng/g, which was found to be satisfactory (within the range of 80 to 120% for all target analytes). A USP signal-to-noise ratio (S/N) of 10 was used as a basis for LOQ determination in the method. In the present study, S/N values for samples spiked at concentrations of less than 0.002 ppm with respect to sample was evaluated, suggesting that the instrument meets the sensitivity requirements easily, and further lower LOQs could be achieved.

Introduction

Nitrosamine impurities have been reported in several drugs and drug substances. Several drugs have been recalled due to the presence of N-nitrosodimethylamine (NDMA). As a result, regulatory agencies have released methods and guidance documents covering a greater number of nitrosamine impurities. All medicines containing chemically synthesized active pharmaceutical ingredients (APIs) are now required to review the product for the possible presence of N-nitrosamines. In earlier application notes, a method for the identification of five nitrosamines was validated in sartan and metformin drugs. The earlier GC methods were developed using a VF-WAXms column within a run time of 12 minutes. Other potential interferences such as dimethylformamide (DMF) may also be present in some drugs. This has previously been reported and can lead to false quantification results for NDMA. This application note expands the scope of the method from five impurities to the nine impurities:

- N-nitrosodimethylamine (NDMA)
- N-nitrosomethylethylamine (NMEA)
- N-nitrosodiethylamine (NDEA)
- N-nitroso-ethylisopropylamine (NEIPA)
- N-nitrosodiisopropylamine (NDIPA)
- N-nitrosodipropylamine (NDPA)
- N-nitrosodi-*n*-butylamine (NDBA)
- N-nitrosopiperidine (NPIP)
- N-nitrosopyrrolidine (NPYR)

This application note also explores the effect of varying DMF concentrations on NDMA peak shapes, and different GC columns on sensitivity of analysis. Two GC programs (resulting in 12 and 20 minute run times) are also compared with respect to precision and sensitivity. As these methods are used for routine analysis of drug substances, long-term precision is also studied.

Experimental

Sample preparation

The APIs and drug products tested for this analysis included valsartan, olmesartan, losartan, irbesartan, and telmisartan. Sample preparation was similar to the process described in application note 5994-1821EN. 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-d_6 in dichloromethane) was added via volumetric pipette. These samples were vortexed for 1 minute, then placed in the centrifuge and spun at 4,000 rpm for 5 minutes. The undissolved 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 the finished drug product, approximately 10 tablets of each drug were separately crushed and homogenized. From the homogenized mixture, a portion equivalent to 500 mg of drug substance was weighed and extraction was carried out in the same manner as described for drug substance.

Standard preparation

The standard stock was diluted appropriately to obtain a calibration solution in the range of 0.06 to 500 ng/mL, each prepared in dichloromethane containing NDMA-d_6 as internal standard.

Instrumentation

Analysis was performed using the Agilent 8890 GC equipped with an Agilent 7693A automatic liquid sampler coupled to an Agilent 7010B triple quadrupole GC/MS/MS. Three different columns were evaluated using two GC programs (12 and 20 minutes) for the nine impurities.

The columns evaluated were:

- Agilent J&W VF-WAXms GC capillary column of dimensions 30 m × 0.25 mm × 0.25 µm (part number CP9205)
- Agilent J&W DB-Select 624 UI GC capillary column of dimensions 30 m × 0.25 mm × 1.4 µm (part number 122-0334UI)
- Agilent J&W DB-1701 GC capillary column of dimensions 30 m × 0.25 mm × 1 µm (part number 122-0733)

Tables 1 and 2 display the GC and MS parameters.

MS acquisition method

Using the Multiple Reaction Monitoring (MRM) optimizer tool, the MRMs for all nine impurities were developed and 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	
Inlet Liner	Ultra Inert, splitless, single taper, glass wool (p/n 5190-2293)	
Oven Temperature Program	Method 1 (12 min) 40 °C (0.5 min) 20 °C/min to 200 °C (0 min) 60 °C/min to 250 °C (3 min)	Method 2 (20 min) 40 °C (0.5 min) 10 °C/min to 145 °C (1 min) 20 °C/min to 190 °C (5 min) 60 °C/min to 250 °C (0 min)
Total Run Time	12.33 min	20.25 min
MS Transfer Line Temperature	250 °C	
Injection Volume	2 µL	
Carrier Gas	Helium, 1 mL/min	

Table 2. MS parameters.

Parameter	Value
Mode	Electron impact, 70 eV
Source Temperature	250 °C
Quadrupole Temperature	Q1 and Q2 = 150 °C
MRM Mode Conditions	
MS1 and MS2 Resolution	All compounds Unit
Collision Gas Flow	Nitrogen at 1.5 mL/min
Quench Gas Flow	Helium at 4 mL/min

Table 3. Quantitative/qualitative transitions (dMRM-based).

	Retention Time (RT in min)							
	Agilent VF-WAXms		Agilent DB-Select 624 UI		Agilent DB-1701		MRM	
Compound	12 min	20 min	12 min	20 min	12 min	20 min	Transition	CE
NDMA-D6	5.387	7.757	5.799	8.122	5.861	8.290	80 → 50	5
NDMA	5.392	7.777	5.821	8.157	5.884	8.313	74 → 44.1	6
							74 → 42.1	24
							43.1 → 42.1	10
NMEA	5.758	8.413	6.688	9.792	6.676	9.819	87.9 → 71	4
							87.9 → 42.1	24
							43.1 → 42.1	10
NDEA	5.974	8.795	7.376	11.109	7.288	10.995	101.9 → 85.1	4
							101.9 → 56	20
							101.9 → 44.1	14
NEIPA	6.221	9.250	7.978	12.386	7.822	12.137	115.9 → 99	6
							115.9 → 44	16
							71 → 56	6
NDIPA	6.406	9.589	8.493	13.381	8.271	13.055	130 → 88	6
							130 → 71	16
							130 → 42.1	12
NDPA	6.930	10.580	8.927	14.190	8.730	13.899	130 → 113.1	2
							101 → 70	2
							70 → 43.1	6
NPYR	8.518	13.668	9.130	14.531	9.165	14.661	99.9 → 70	8
							99.9 → 55	8
							99.9 → 43.1	12
NPIP	8.332	13.304	9.389	15.066	9.329	14.985	113.9 → 97.1	8
							113.9 → 84.1	8
							113.9 → 55	26
							113.9 → 42.1	24
NDBA	8.072	12.951	10.155	17.292	9.902	16.628	158 → 141.1	4
							158 → 99.1	10
							116 → 99.1	4
							84 → 56	22

Results and discussion

The MRMs for this method were developed using the Agilent MassHunter Optimizer for GC/TQ. Automated MRM development was carried out to obtain the optimized MRMs of all nine impurities. The process for automated MRM development is described in 5994-2086EN. The optimized MRMs were saved directly as a dMRM method and used for acquisition of calibration standards and samples.

Two different GC programs were evaluated for each column. Figures 1 and 2 contain chromatograms obtained using method 1 (12 minute run time) and method 2 (20 minute run time), respectively. The compounds were separated sufficiently, and the target peaks (Figures 1 and 2) were well-resolved.

The elution profiles and retention time (RT) in DB-Select 624 UI and DB-1701 were similar. The elution order in VF-WAXms was different from the other two columns with NDBA (12.951 minutes) eluting before NPIP (13.304 minutes) and NPYR (13.668 minutes). With the same GC program, elution of all the compounds was faster in VF-WAXms as compared to the other two columns. Comparison of peak shapes across the columns indicated DB-1701 with overall symmetric peak shapes. The USP tailing factor ranged from 0.9 to 1.2 in DB-1701 as compared to 0.8 to 1.3 and 1.2 to 1.6 in VF-WAXms and DB-Select 624 UI, respectively.

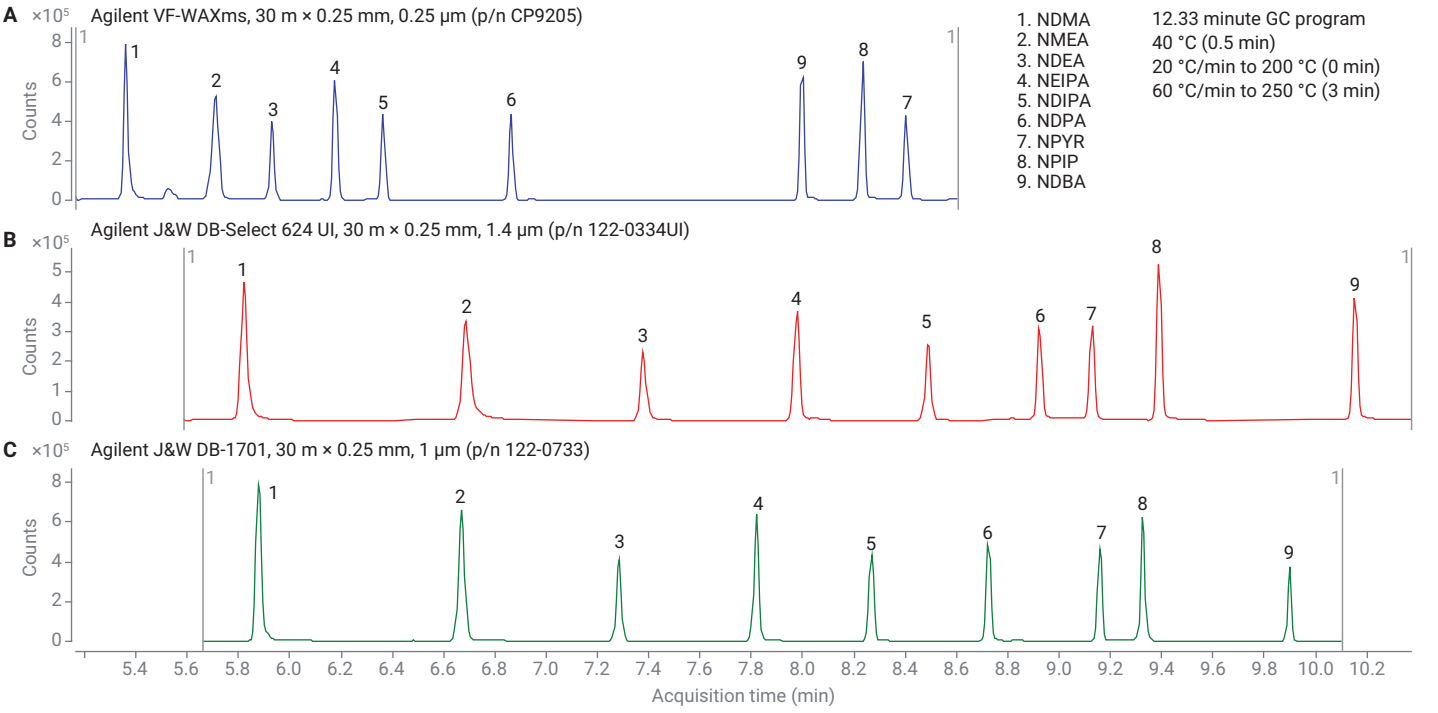

Figure 1. MRM chromatogram of nine impurities N-nitrosodimethylamine (NDMA) (1), N-nitrosomethylethylamine (NMEA) (2), N-nitrosodiethylamine (NDEA) (3), N-nitroso-ethylisopropylamine (NEIPA) (4), N-nitrosodiisopropylamine (NDIPA) (5), N-nitrosodipropylamine (NDPA) (6), N-nitrosopyrrolidine (NPYR) (7), N-nitrosopiperidine (NPIP) (8), N-nitrosodi-*n*-butylamine (NDBA) (9) in three columns: Agilent VF-WAXms (A), Agilent DB-Select 624 UI (B) and Agilent DB-1701 (C) for Method 1 (12 minute run time).

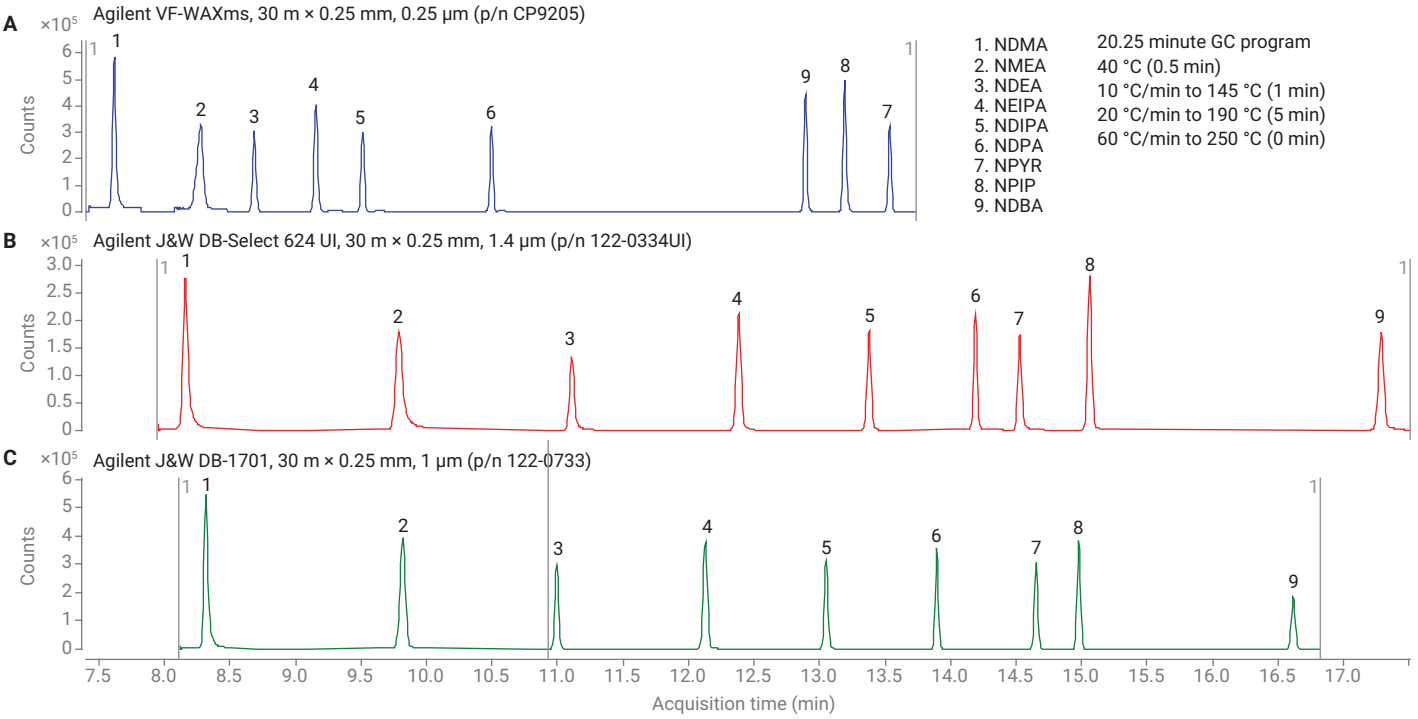

Figure 2. MRM chromatogram of nine impurities N-nitrosodimethylamine (NDMA) (1), N-nitrosomethylethylamine (NMEA) (2), N-nitrosodiethylamine (NDEA) (3), N-nitroso-ethylisopropylamine (NEIPA) (4), N-nitrosodiisopropylamine (NDIPA) (5), N-nitrosodipropylamine (NDPA)(6), N-nitrosopyrrolidine (NPYR) (7), N-nitrosopiperidine (NPIP) (8), N-nitrosodi-*n*-butylamine (NDBA) (9) in three columns: Agilent VF-WAXms (A), Agilent DB-Select 624 UI (B) and Agilent DB-1701 (B) for Method 2 (20 minute run time).

In the VF-WAXms column, peaks of NDMA and DMF elute very close to each other (Figure 3). The DMF concentration was increased from 5 to 100 mg/L and the effect on the NDMA peak was noted. At a DMF concentration greater than 100 mg/L, peak tailing of NDMA was noted. The change in peak shape of NDMA may lead to an error in identification and quantification of NDMA in the presence of DMF.

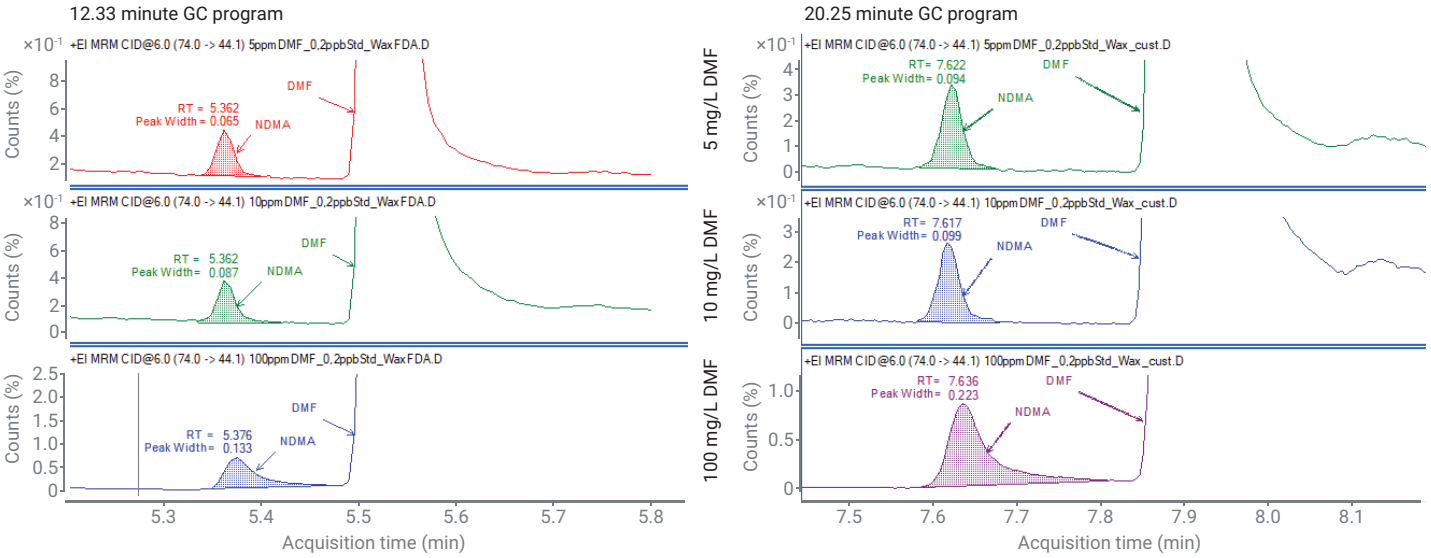

Figure 3. N-nitrosodimethylamine (NDMA) (0.2 ng/mL) and dimethylformamide (DMF) elute very close in both GC programs when analyzed with an Agilent VF-WAXms column. NDMA peaks show tailing at higher concentrations of DMF.

Sufficient separation was observed between DMF and the target analytes when DB-Select 624 UI and DB-1701 were used for chromatographic separation. Peaks shapes of NDMA were not affected with varying concentrations of DMF evaluated from 2 to 100 mg/L (Figure 4).

DMF is a class 2 solvent with a concentration limit of 880 ppm. Considering the possibility of presence of DMF at higher concentrations, DMF was varied up to 100 mg/L or 1,000 ppm with respect to drug substance, while maintaining a trace concentration of NDMA at 0.2 ng/mL or 0.002 ppm with respect to drug substance.

Even at higher DMF concentrations, peak areas of NDMA were comparable to those obtained at lower DMF content (Figure 4). When compared across the DMF concentration range of 2 to 100 mg/L, the variation in peaks area of NDMA (present at 0.2 ng/mL) was within 9.5%. This ensured that despite the presence of DMF in samples, NDMA can be chromatographically separated and quantified, even when present at very low concentrations.

Response in terms of peak area indicated slightly higher response for both slow and fast GC programs in the DB-1701 column. Further studies on repeatability, calibration, and sample analysis were therefore carried out on the DB-1701 column. Repeatability studies were performed by 150 successive injections of a spiked sample at 30 ppb with respect to drug substance. The peak area trend is shown in Figure 5. RSDs calculated with respect to absolute areas were 3.07, 3.96, 4.66, 5.26, 5.94, 4.59, 7.69, 5.66, and 4.72% respectively for NDMA, NMEA, NDEA, NEIPA, NDIPA, NDPA, NDBA, NPIP, and NPYR, while RSDs of calculated concentration (after internal standard

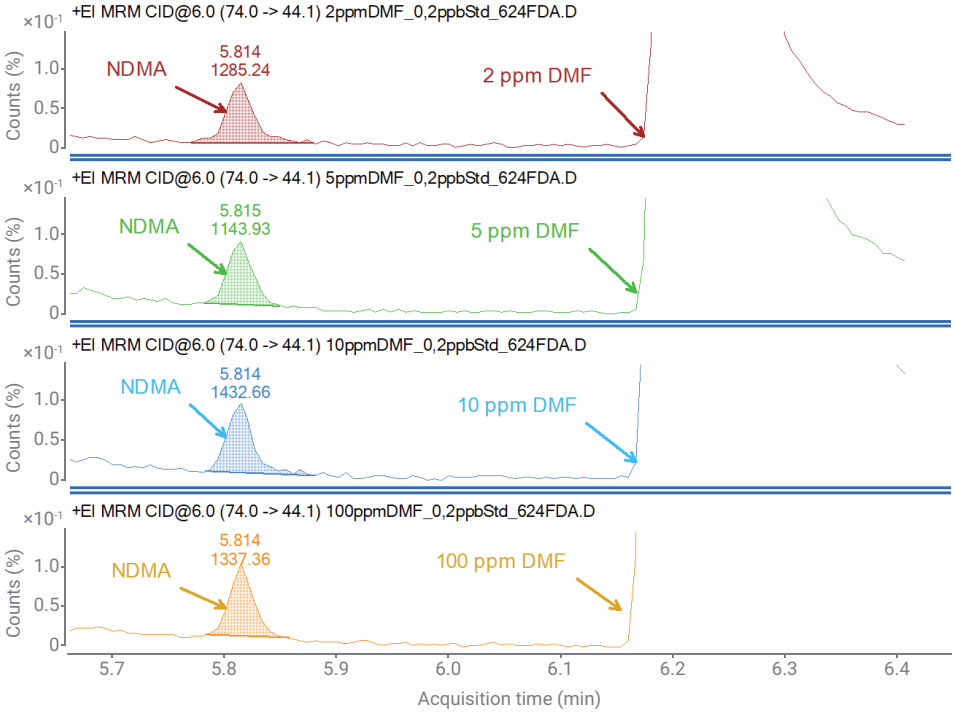

Figure 4. In an Agilent DB-Select 624 UI column, N-nitrosodimethylamine (NDMA) peaks were well separated from dimethylformamide (DMF) (concentration ranging from 2 to 100 ppm).

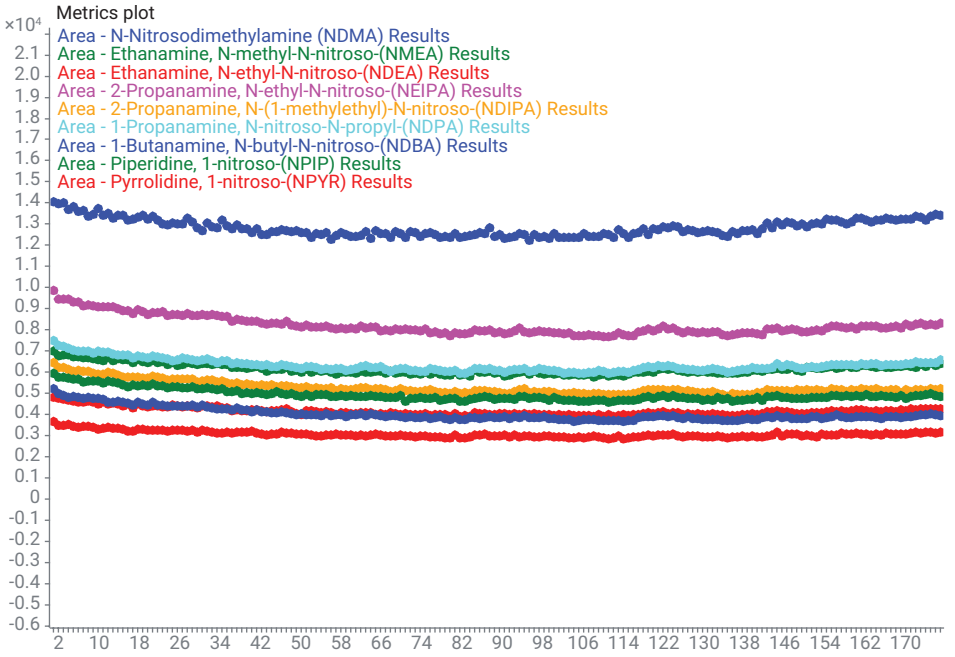

Figure 5. This figure displays the peak area trend, as output by the metric plot feature of Agilent Quantitative Analysis software for nine nitrosamine impurities at 30 ppb with respect to drug substance.

correction) were 1.15, 1.79, 2.71, 3.48, 4.39, 2.49, 5.79, 4.01, and 2.97%, respectively. This indicated long-term stability of response and applicability of method for routine analysis. Calibration curves were plotted for all nine impurities prepared in dichloromethane (Figure 6).

Correlation coefficients were >0.997 for all compounds. Linearity was obtained from 0.06 to 500 ng/mL for NDEA, NEIPA, and NDIPA, 0.1 to 500 ng/mL for NDMA, NDPA, NDBA, and NPIP, and 0.2 to 500 ng/mL for NMEA and NPYR. A separate study done with VF-WAXms and DB-Select 624 UI indicated similar results. Any of the three columns can be used for the analysis. However, in the presence of DMF, identification and quantification of NDMA was affected in the VF-WAXms column.

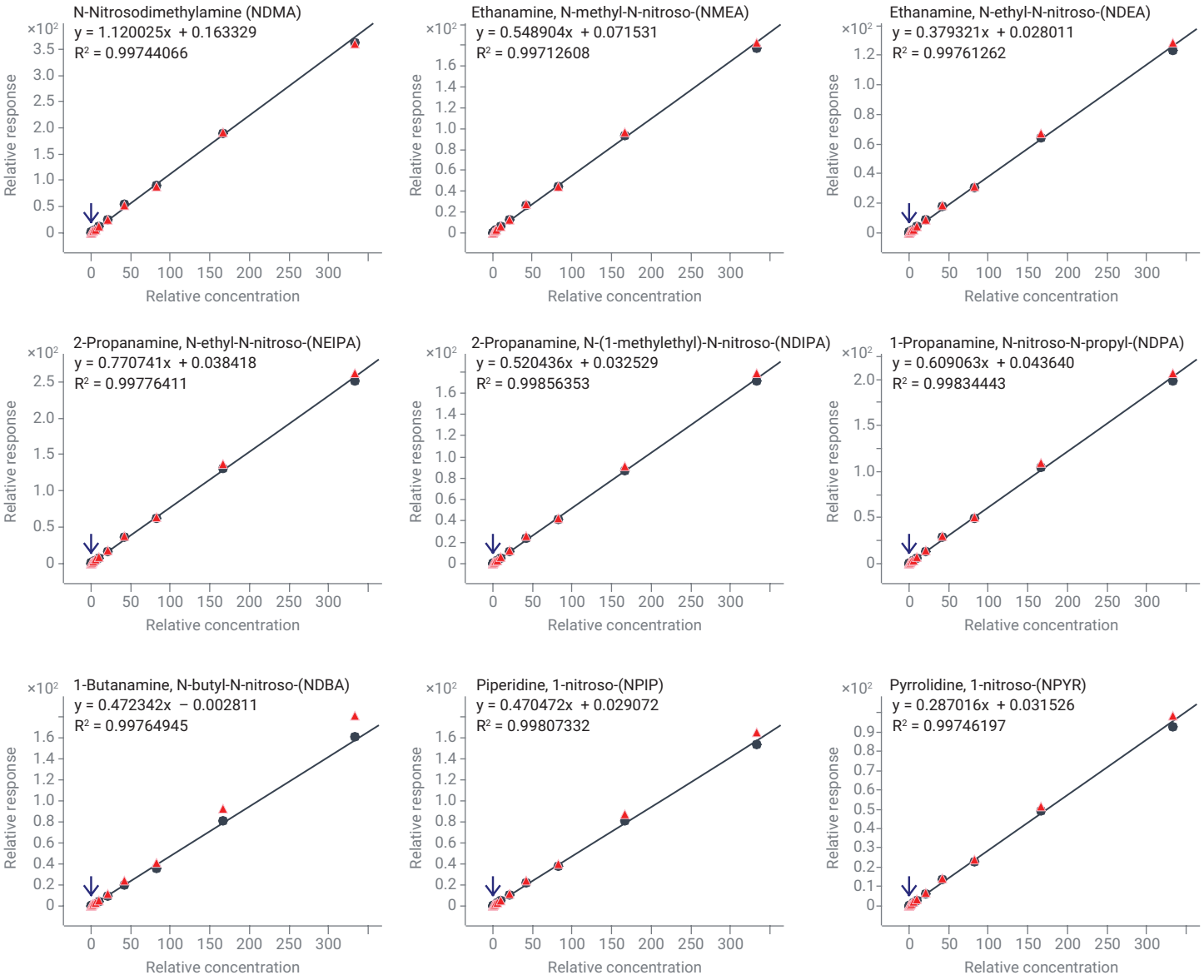

Figure 6. Internal standard-based calibration curve of nine nitrosamine impurities in the range of 0.06 to 500 ng/mL (14-level calibration curve for NDEA, NEIPA, and NDIPA), 0.1 to 500 ng/mL (13-level calibration curve for NDMA, NDPA, NDBA, and NPIP), and 0.2 to 500 ng/mL (12-level calibration curve for NMEA and NPYR). Results were obtained using an Agilent DB-1701 column.

Samples of drug substance and drug product were analyzed using the method described. Sample recoveries were calculated by fortifying the drug substance and drug products of valsartan, irbesartan, losartan, and telmisartan with a homogenized drug product at 0.03 and 0.003 ppm and were found to be satisfactory within the range of 80 to 120% when analyzed using three different columns at both (0.03 and 0.003 ppm) levels.

A USP S/N value of 10 was used as a basis for LOQ determination in the method. In the present study, S/N values for samples spiked at 3 ppb with respect to drug substance level were found to be greater than 10, suggesting that the instrument meets the sensitivity requirements easily, and further lower LOQs could be achieved, enabling trace-level detections. The S/N of the nine impurities using the Agilent DB-1701 column with 20 minute run time is shown in Figure 7. The compounds are present in following concentrations:

- NDEA, NEIPA, and NDIPA (0.0006 ppm)
- NDMA, NDPA, NDBA, and NPIP (0.001 ppm)
- NMEA and NPYR (0.002 ppm)

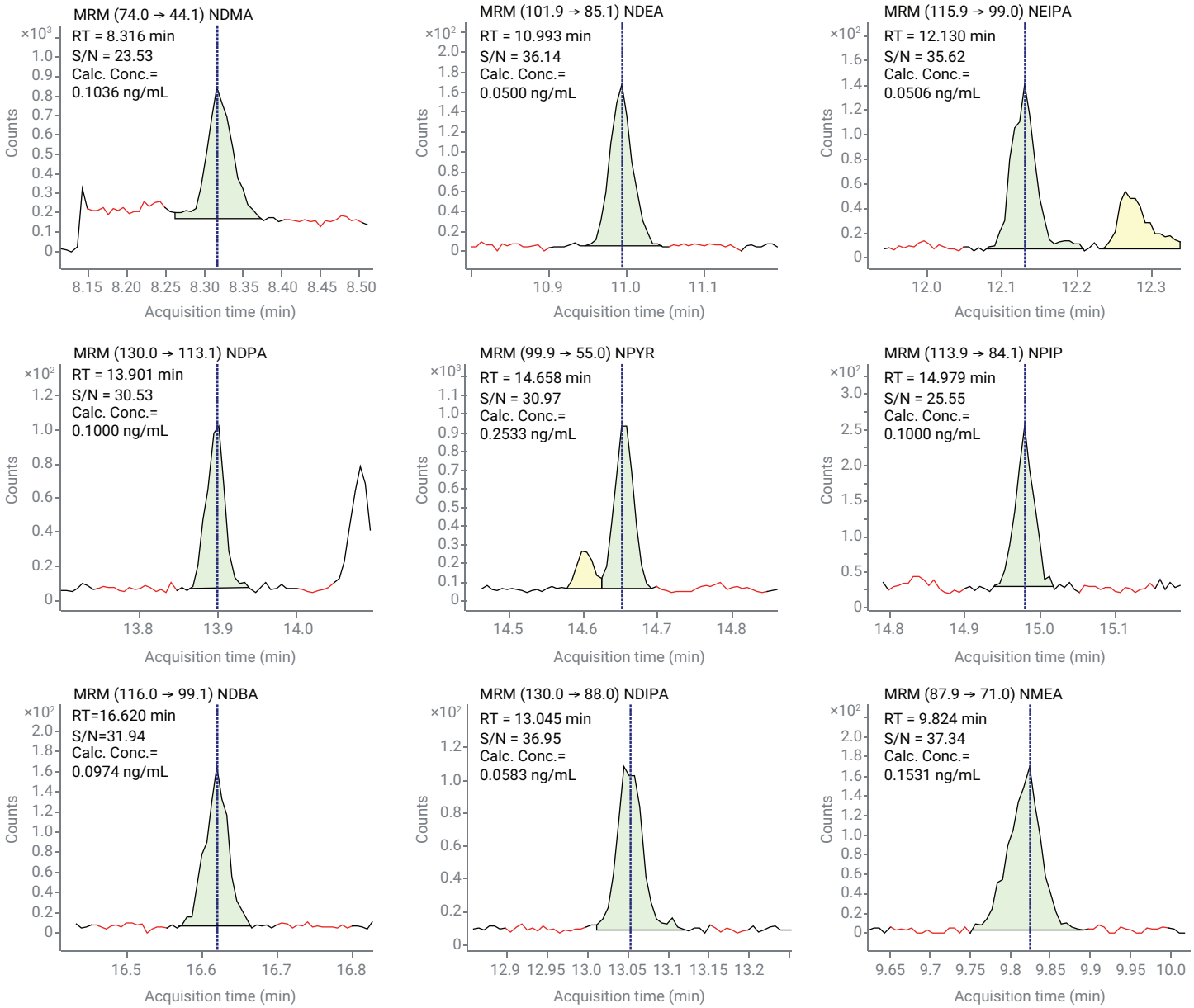

Figure 7. The signal-to-noise ratio of nine nitrosamine impurities was greater than 10 for all nine impurities at the following concentrations: NDEA, NEIPA, and NDIPA (0.0006 ppm); NDMA, NDPA, NDBA, and NPIP (0.001 ppm); NMEA, and NPYR (0.002 ppm) using an Agilent DB-1701 column with a 20-minute run time.

Conclusion

The Agilent 8890 and Agilent 7010B GC/MS/MS system demonstrated excellent performance for determination of nine nitrosamine drug impurities in sartan drug products and substances. The design of the 7010B triple quadrupole GC/MS/MS, which includes the High Efficiency Source (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 nine impurities up to 3 ppb. The S/N of recovery samples spiked at 3 ppb with respect to drug substance indicated that further lower levels could be achieved. The lowest calibration level achieved for the nitrosamine impurities ranged from 0.0006 to 0.001 ppm with respect to drug substance. All three columns could be used for analysis, as satisfactory linearity, recovery, and repeatability could be achieved for all nine impurities. The Agilent DB-1701 was found to be more suitable for the analysis due to separation from DMF and lesser peak tailing.

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