Application Note

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Quantification of Nitrosamine Impurities in Sartan Drugs Using an Agilent GC/TQ with Hydrogen Carrier Gas

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Abstract

In response to helium scarcity, labs are exploring alternative carrier gases for gas chromatography/mass spectrometry (GC/MS) analyses. This application note shows the suitability of hydrogen as a carrier gas and compares it with helium for the analysis of eight nitrosamine impurities in certain sartan drugs using either an Agilent 8890 gas chromatograph system coupled to an Agilent 7010 Series triple quadrupole (TQ) GC/MS system with a high-efficiency ion source (HES), or an Agilent 7000E triple quadrupole GC/MS with an Agilent HydroInert ion source. The spectral matches against the NIST library for the eight nitrosamine impurities analyzed with hydrogen carrier gas ranged from 79 to 97. Excellent calibration linearity was observed over the concentration range of 0.3 to 50 ng/mL with $R^2 > 0.99$. The sensitivity requirements were met at 0.03 ppm following a signal-to-noise ratio (S/N) requirement of 10. The integration of OpenLab ECM XT with MassHunter Acquisition 13.0 streamlines data management, providing analysts with centralized access to instrument-generated data, fostering collaboration, maintaining data integrity, and optimizing workflow processes.

Introduction

Helium availability has been a concern for several years, which has led to a significantly increased interest in transitioning to alternative carrier gases such as hydrogen. While hydrogen is an efficient GC carrier gas, understanding its reactivity with analytes is crucial for obtaining accurate results. Hydrogen has some advantages over helium, including faster run times and better chromatographic resolution. However, hydrogen also has some challenges, such as potential loss of sensitivity and spectral changes arising from hydrogen reactions with sample analytes. Such reactions would alter the mass spectrum of a peak in the total ion chromatogram (TIC), leading to potential misidentification of compounds. Transitioning from helium to hydrogen carrier gas is a significant change that requires careful planning and execution. The "EI GC/MS Instrument Helium to Hydrogen Carrier Gas Conversion Guide[1]" provides comprehensive instructions to facilitate the transition. Also, the introduction of the HydroInert source allowed for the preservation of spectral fidelity even for reactive compounds in the presence of hydrogen. As a result, various applications were successfully performed using hydrogen carrier gas, including the analysis of volatile organic compounds[2], polycyclic aromatic hydrocarbons (PAHs)[3,4], and the target compounds listed in EPA TO-15.[5]

Apart from these in-demand applications, another application of importance is the analysis of nitrosamine impurities in pharmaceutical products. Using helium as a carrier gas has been adopted widely for the analysis of nitrosamine impurities in pharmaceuticals. Nitrosamines may react with hydrogen under certain conditions, undergoing conversion to undesirable amines or hydrazines. Therefore, it is important to establish that spectral quality is not affected when using hydrogen carrier gas. This application note evaluates the use of hydrogen carrier gas with the HES and HydroInert ion sources as part of a method for the analysis of eight nitrosamine impurities: nitrosodimethylamine (NDMA), N-nitrosomethylethylamine (NMEA), N-nitrosodiethylamine (NDEA), N-nitroso-ethylisopropylamine (NEIPA), N-nitrosodiisopropylamine (NDIPA), N-Nitrosodipropylamine (NDPA), N-nitrosodi-*n*-butylamine (NDBA), and N-Nitrosopiperidine (NPIP). The results obtained were evaluated for spectral quality, linearity, repeatability, recovery, and compliance with current regulations for the analysis of nitrosamines in pharmaceutical products.

Experimental

The active pharmaceutical ingredients (APIs) and drug products tested included valsartan, irbesartan, losartan, and olmesartan. A portion of 500 mg of drug substance was accurately weighed 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 using a volumetric pipette. The samples were vortexed for 1 minute, then placed in a 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.

Standard preparation

The standard stock was diluted to obtain a calibration solution in the range of 0.3 to 50 ng/mL and prepared in dichloromethane containing NDMA-d_6 as internal standard.

Instrumentation and analysis

Analyses were performed using an 8890 GC system equipped with an Agilent 7693A automatic liquid sampler (ALS) coupled to a 7010 Series GC/TQ with an HES and a 7000E GC/TQ with the HydroInert ion source. Separation was performed on an Agilent J&W VF-WAXms GC, 60 m × 0.25 mm, 0.25 µm capillary column (part number CP9207). Alternatively, the same parameters can be applied using a midcolumn backflush configuration with two Agilent J&W VF-WAXms GC, 30 m × 0.25 mm, 0.25 µm capillary columns (part number CP9205) at flows of 1 and 1.2 mL/min, respectively. The backflush setup was also evaluated. Tables 1 and 2 provide the GC and MS parameters, respectively.

The GC/TQ was operated in dynamic multiple reaction monitoring (dMRM) mode. The MRM transitions for all nine impurities were developed using the Agilent MassHunter Optimizer for GC/TQ and used for data acquisition (Table 3).

Table 1. GC parameters.

Parameter	Value
GC System	Agilent 8890 GC system
MMI Injection Mode	Pulsed splitless: 15 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	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 min
MS Transfer Line Temperature	250 °C
Injection Volume	2 µL
GC Column	VF-WAXms Helium: 30 m × 0.25 mm, 0.25 µm column (p/n CP9205) Hydrogen: 60 m × 0.25 mm, 0.25 µm column (p/n CP9207) and midcolumn backflushing with two 30 m × 0.25 mm, 0.25 µm columns
Carrier Gas	Hydrogen 1 mL/min (for HES and HydroInert) or Helium 1.2 mL/min (for HES)

Table 2. MS parameters.

Parameter	Value
MS System	Agilent 7010 Series GC/TQ with HES and Agilent 7000E GC/TQ with HydroInert (HI) ion source
Mode	Electron impact, 70 eV (on both HES and HI ion source)
Source Temperature	250 °C
Quadrupole Temperature	Q1 and Q2 = 150 °C
MS1 and MS2 Resolution	All compounds unit
Collision Gas Flow	Nitrogen at 1.5 mL/min
Quench Gas Flow	Helium at 2.25 mL/min when using helium carrier gas; Switched off when using hydrogen carrier gas

Software and data integrity

Agilent MassHunter Workstation, including MassHunter Acquisition 13.0 software for GC/MS and MassHunter Quantitative Analysis 12.1 software, was used for data acquisition and analysis. The OpenLab Electronic Content Management (ECM) XT configuration provides capabilities to facilitate compliance with various national and EU electronic record regulations. The automated tools and processes include the ability to create users with affiliated permissions, generation of audit trails, and remote data storage to minimize the risk of data breach or loss. OpenLab ECM XT, employed with the MassHunter application, provided a flexible data management solution with a single point of access to data generated from instruments, data systems, and laboratory software. With access to data from a storage location, analysts can collaborate without compromising data integrity and can create consistent processes for workflows.

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

Compound	Retention Time (min)	MRM Transition	CE
NDMA-D_6	8.437	80 → 50	5
NDMA	8.448	74 → 44.1	6
		74 → 42.1	24
		43.1 → 42.1	10
NMEA	8.767	87.9 → 71	4
		87.9 → 42.1	24
		43.1 → 42.1	10
NDEA	8.969	101.9 → 85.1	4
		101.9 → 56	20
		101.9 → 44.1	14
NEIPA	9.198	115.9 → 99	6
		115.9 → 44	16
		71 → 56	6
NDIPA	9.366	130 → 88	6
		130 → 71	16
		130 → 42.1	12
NDPA	9.832	130 → 113.1	2
		101 → 70	2
		70 → 43.1	6
NDBA	10.796	158 → 141.1	4
		158 → 99.1	10
		116 → 99.1	4
		84 → 56	22
NPIP	11.088	113.9 → 97.1	8
		113.9 → 84.1	8
		113.9 → 55	26
		113.9 → 42.1	24

Results and discussion

Spectral match quality

Full scan spectra for each of the eight analytes were acquired using helium or hydrogen carrier gas, then compared against the NIST library. Shown in Figure 1, excellent match scores (> 90) were obtained using hydrogen carrier gas with the 7010 Series GC/TQ-HES system. Good match scores (> 80) were obtained when using hydrogen carrier gas with the 7000E GC/TQ-HydroInert source setup. Higher average match scores with the HES could be attributed to higher response due to enhanced sensitivity.

Maintaining the integrity of mass spectra enabled the use of identical MRM transitions for the helium and hydrogen carrier gas methods (Table 3).

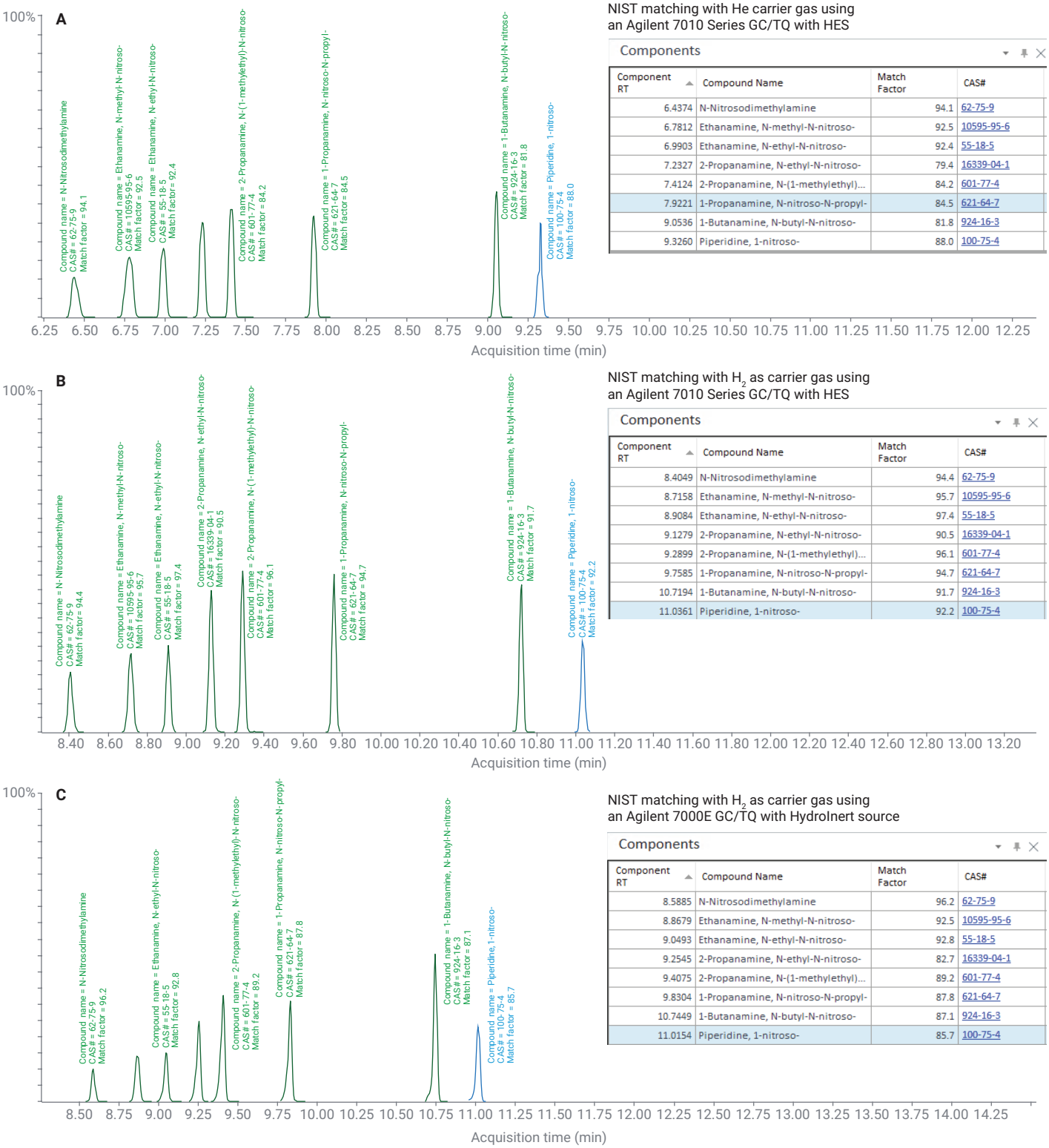

Figure 1. Separation of eight nitrosamine impurities using helium carrier gas with the HES (A), hydrogen carrier gas with the HES (B), and hydrogen carrier gas with the HydroInert source (C). The insets show the associated NIST match scores for the acquired full scan mass spectra.

Linearity

Method calibration performance for the eight impurities was demonstrated over the range of 0.3 to 50 ng/mL with $R^2 > 0.99$ (Table 4). The lowest calibration level was the concentration where the ion ratios for the qualifier ions passed the ion ratio criteria. When using hydrogen carrier gas, detection limits of 3 ng/mL or lower were achieved with both the 7010 Series GC/TQ-HES and 7000E GC/TQ-HydroInert source setups.

Limits of quantification

According to the latest regulatory directives[6,7], the limit of quantification (LOQ) must not exceed the established acceptable limit for the specific nitrosamine impurity measured. When a single analytical method is used to assess various nitrosamines, the method's selectivity at the LOQ for each individual nitrosamine must be validated. The use of methods with LOQs at or below 0.03 ppm is a common pharmaceutical industry requirement for drug substances and drug products.

Sample preparation involves a 10x dilution (500 mg extracted with 5 mL of dichloromethane). Recovery studies that spiked the drug substances at 30 ppb (0.03 ppm) resulted in extracts with a concentration of 3 ng/mL for each nitrosamine impurity measured in this study. This concentration complied with the typical 30 ppb LOQ requirement for each nitrosamine impurity.

With the 7010 Series GC/TQ, the carrier gas was switched between helium and hydrogen over a span of six months and the results were compared across three of those carrier gas changes. Figure 2 illustrates that consistent spectral fidelity, calibration response, ion ratios, and sensitivity were observed. The spectral fidelity remained unaffected over the measurement timeframe and generated consistently high library match scores for all analytes (> 90). The ability to maintain spectral fidelity and produce consistent calibration response, ion ratios, and sensitivity after changing the carrier gas establishes the method's suitability for practical applications.

Table 4. Calibration levels for the different carrier gases and ion sources.

Compound	Calibration Range (ng/mL)			United States Pharmacopeia (USP) S/N of Quantifier Transition at Lowest Calibration Level		
	Agilent 7010 Series GC/TQ with He Carrier Gas	Agilent 7010 Series GC/TQ with H_2 Carrier Gas	Agilent 7000E GC/TQ with HydroInert Source	Agilent 7010 Series GC/TQ with He Carrier Gas	Agilent 7010 Series GC/TQ with H_2 Carrier Gas	Agilent 7000E GC/TQ with HydroInert Source
NDMA	0.1 to 50	0.5 to 50	3 to 50	> 12	> 10	> 10
NMEA	0.2 to 50	0.3 to 50	1 to 50	> 200	> 12	> 40
NDEA	0.05 to 50	0.5 to 50	1 to 50	> 20	> 10	> 100
NEIPA	0.05 to 50	1 to 50	3 to 50	> 50	> 80	> 90
NDIPA	0.05 to 50	0.5 to 50	1 to 50	> 80	> 10	> 60
NDPA	0.1 to 50	0.3 to 50	3 to 50	> 100	> 10	> 10
NDBA	0.1 to 50	1 to 50	3 to 50	> 60	> 10	> 20
NPIP	0.1 to 50	1.25 to 50	3 to 50	> 60	> 10	> 10

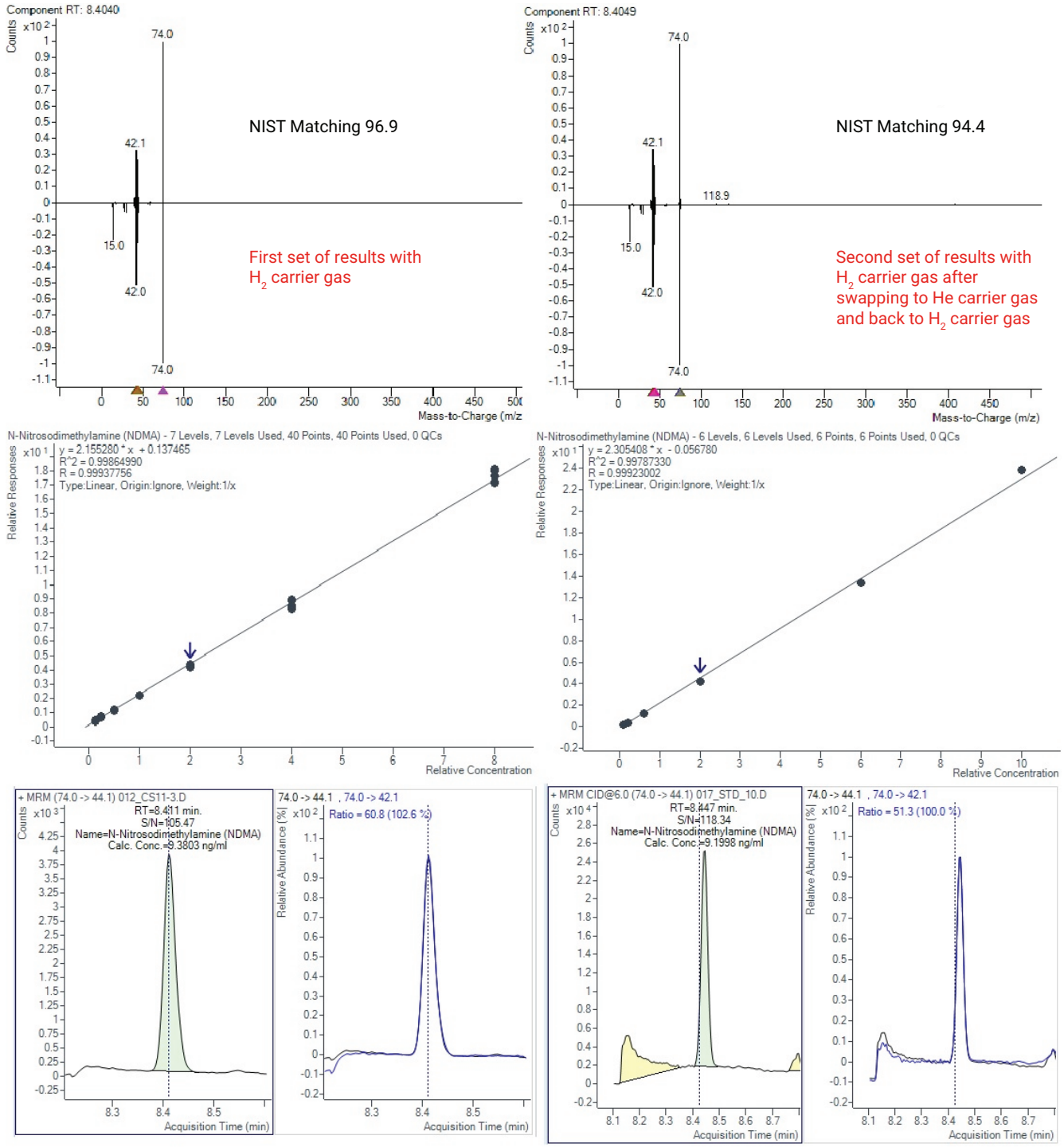

Figure 2. Consistent library match scores, calibration, and ion ratios were obtained using hydrogen carrier gas before and after using helium gas with an Agilent 7010 Series GC/TQ.

Stability

The stability of the results for 150 consecutive injections was previously examined using helium carrier gas (as described in 5994-4618EN[8]). In this work, the same assessment was conducted with hydrogen carrier gas (Figure 3). The RSDs (calculated with respect to absolute areas) were < 10% for all the analytes, and the calculated concentration RSDs (after internal standard correction) were < 7%. This indicated long-term stability of response and applicability of the method for routine analysis.

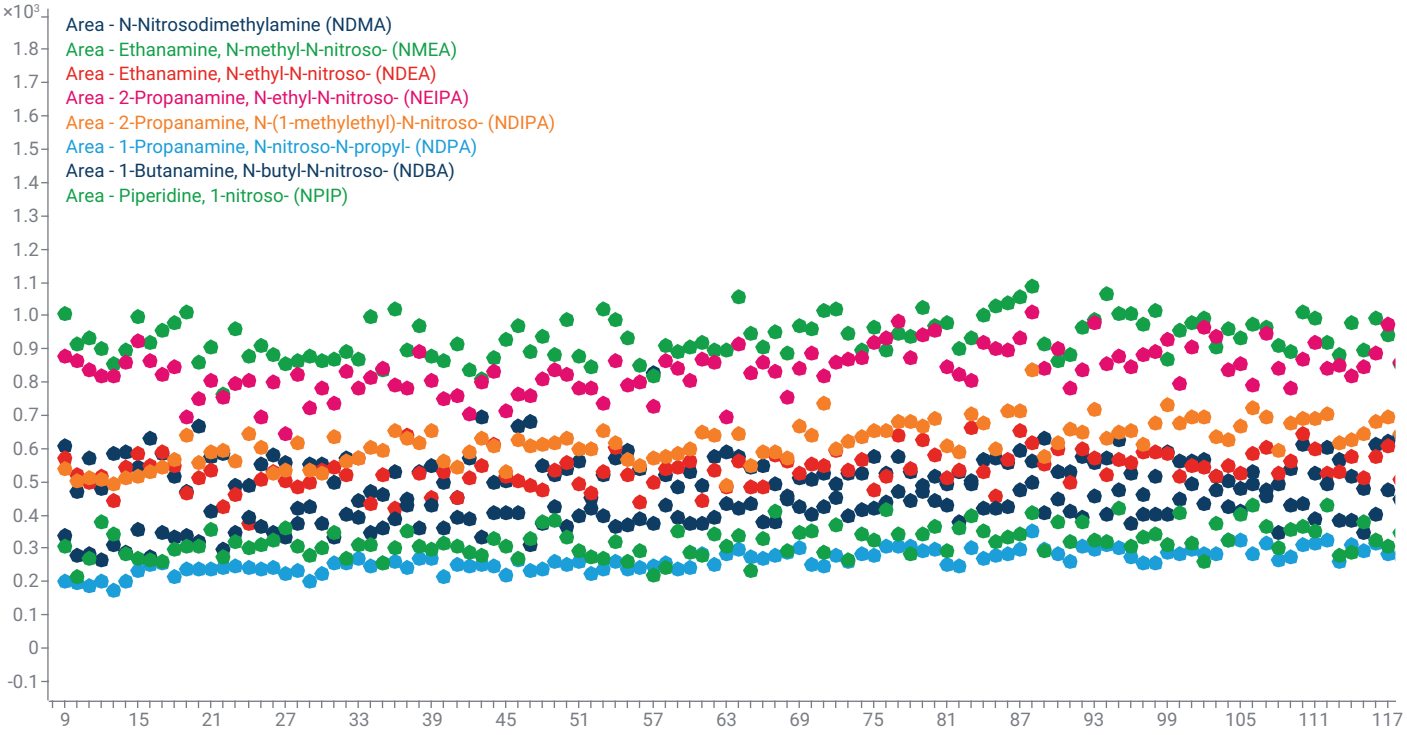

Figure 3. Peak area trend (using an Agilent 7000E GC/TQ with HydroInert source) for a nitrosamine impurity recovery sample at 30 ppb (with respect to the drug substance). The plot was created using the metric plot feature in Agilent MassHunter Quantitative Analysis software.

Midcolumn backflush

With a dilution factor of 10, a considerably high amount of drug substance matrix was introduced into the analytical system. This can result in RT shifts and a gradual decrease in peak response. To overcome this issue, the midcolumn backflush capability can be used. In this configuration, a Purged Ultimate Union (PUU) is installed between two identical 30 m columns (0.25 mm × 0.25 µm). Consistent results, including RTs and peak areas, were obtained when using the midcolumn backflush configuration. Figure 4 shows an example of the RTs and peak areas obtained after 25, 50, and 100 consecutive sample runs.

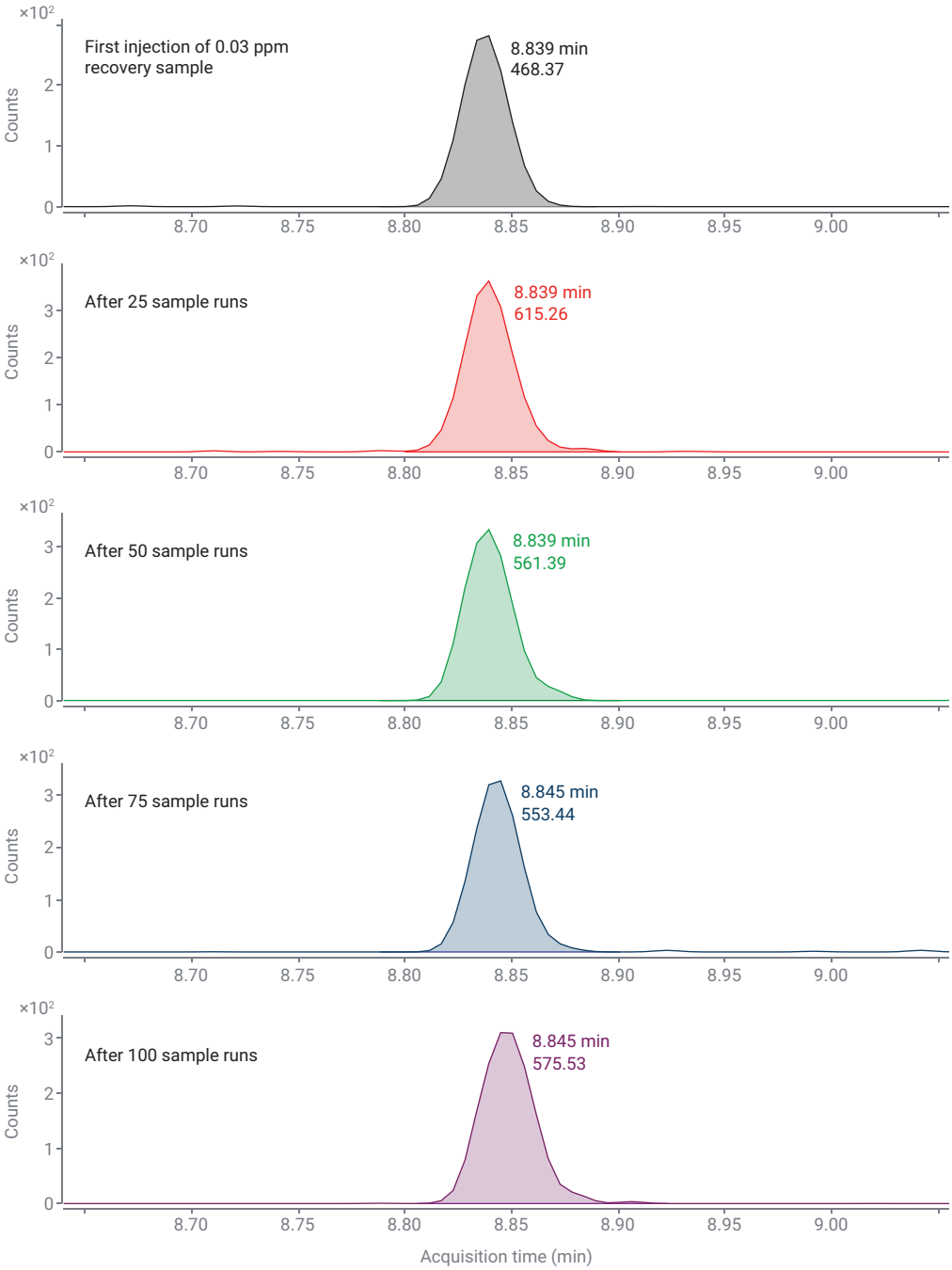

Figure 4. Consistent retention times (RTs) and peak areas were obtained after 25, 50, and 100 consecutive sample runs when the mid-column backflush setup was used.

Recoveries

Sample recoveries were calculated by fortifying the drug substances valsartan, irbesartan, losartan, and olmesartan at 0.03 ppm. Recoveries were satisfactory and ranged from 80 to 120% when using hydrogen carrier gas on both the HES and HydroInert source setups.

Conclusion

Using hydrogen carrier gas on an Agilent 8890 GC system with either an Agilent 7010 Series GC/TQ (HES) or an Agilent 7000E GC/TQ (HydroInert source) demonstrated excellent performance for the determination of eight nitrosamine drug impurities in sartan drug products and substances. Performance was validated at 0.03 ppm with acceptable recovery and long-term repeatability. Both the 7000E GC/TQ with the HydroInert source and the 7010 Series GC/TQ with the HES source facilitated the ability of the system to achieve the required detection limits. The integration of OpenLab ECM XT with MassHunter Acquisition 13.0 streamlines data management, providing analysts with centralized access to instrument-generated data, fostering collaboration, maintaining data integrity, and optimizing workflow processes.

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