Application note | 001561

Pharma

Determination of 18 nitrosamine impurities in sartan drug products using gas chromatography coupled with high-resolution Orbitrap mass spectrometry (GC-HRMS)

Authors

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Keywords

Orbitrap Exploris GC mass spectrometer, high-resolution accurate mass (HRAM), genotoxic impurities (GTIs), nitrosamine, sartans, drug product, rapid method, Telmisartan tablets, Losartan tablets, impurity analysis, ICH Q2(R1)

Goal

The aim of this study is to develop a simple and rapid quantitative method for the determination of eighteen nitrosamines impurities in sartan drug products, utilizing the advantage of high-resolution accurate mass measurement of the Thermo Scientific™ Orbitrap Exploris™ GC mass spectrometer.

Introduction

The presence of nitrosamine impurities in sartans, a class of medicinal drugs widely used for the treatment of human blood pressure, heart failure, and in chronic heart diseases, was first reported in 2018. Nitrosamines represent a serious risk to human health, being classified as carcinogenic by the ICH M7 Guideline[1], and are categorized as probable carcinogens by the International Agency for Cancer Research (IARC).[2] The presence of nitrosamine impurities in pharmaceutical products can originate from various sources. Nitrites and secondary/tertiary amines can be present as background contamination in raw materials, reagents and solvents which can result in the formation of nitrosamine impurities.[3,4]

The U.S. Food and Drug Administration (FDA), United States Pharmacopeia, and European Directorate for the Quality of Medicines & HealthCare (EDQM)[5–9] have published several analytical methods for determining nitrosamine contents in active pharmaceutical ingredient (API) and finished drug products. The methods include both gas chromatography (GC) and liquid chromatography (LC) coupled to mass

spectrometry (MS) or high-resolution accurate mass (HRAM) mass spectrometry. Over time, the list of nitrosamines to be monitored has increased due to differing manufacturing processes for production of various medicinal products, resulting in different sets of nitrosamine impurities.

In view of the above scenario, this study was designed to develop a method for quantitation of 18 nitrosamine impurities (Appendix 1). The high-resolution GC-MS approach has become very popular as it offers the advantage of full-scan data acquisition combined with high sensitivity, high resolving power, and accurate mass (<5 ppm). The accurate mass measurement can remove the co-extracted matrix related interferences to provide trace level quantification. This simplifies the analytical workflow through conducting analysis without time-consuming extraction and clean up steps. Moreover, high-resolution full-scan data acquisition allows both targeted and non-targeted analysis to be perfromed. Full scan data aquisition enables identification of unknown peaks that may be of future research interest. The method demonstrates the capability of the Orbitrap Exploris GC-MS for trace analysis of nitrosamines in Telmisartan (40 mg) and Losartan (50 mg) finished drug products.

Experimental

Standard and sample preparation

Sample preparation was carried out as described in European Pharmacopoeia chapter 2.5.42. (*N*-nitrosamines in active substances).[9]

For sample preparation, Telmisartan (40 mg) and Losartan (50 mg) tablets were crushed using mortar and pestle. Telmisartan and Losartan equivalent to 250 mg of API were weighed into separate tubes and extraction was proceeded as in Figure 1.

For the extraction mixture, 40.0 g of sodium hydroxide were dissolved in 500 mL of water. Then, 80 µL of the internal standard solution were added, followed by 50 mL of acetonitrile. The solution was diluted to 1,000 mL with water.[9]

GC-MS analysis

Analysis was performed with a Thermo Scientific™ TRACE™ 1610 GC coupled with an Orbitrap Exploris GC mass spectrometer installed with a Thermo Scientific™ ExtractaBrite™ electron ionization source (Figure 2). Liquid injection was performed by a Thermo Scientific™ AS 1610 Autosampler with data acquisition and evaluation performed using the Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) software. The optimized GC-MS conditions are given in Table 1. If required, the Thermo Scientific™ iConnect™ SSL injector module can be easily adapted for carrier gas saving functionality[10], replacing split and purge flows with Nitrogen (55 mL/min in total), maintaining the method unchanged and with no impact on the analytical performance[11].

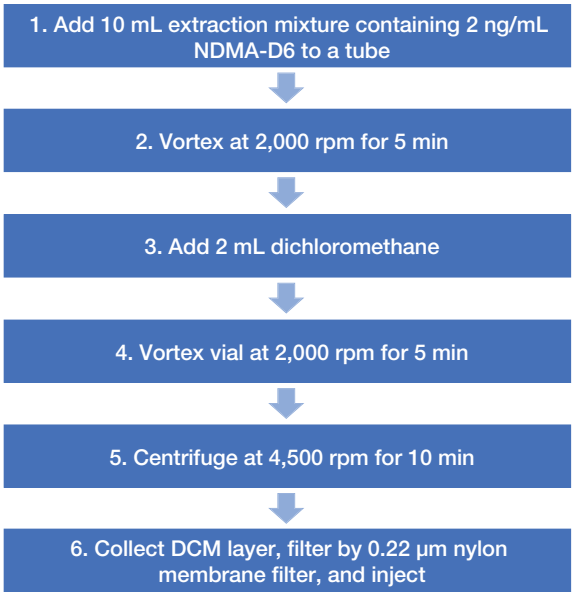

Figure 1. Extraction procedure for sartan drug product

Table 1. GC-MS instrument conditions

Gas chromatography method	
Columns	Thermo Scientific™ TraceGOLD™ TG-624, 30 m × 0.25 mm i.d. × 1.4 µm (P/N 26085-3320)
Injector	Split/Splitless (SSL)
Liner	Single gooseneck with glass wool Thermo Scientific™ LinerGOLD™ (P/N 453A1925-UI)
Injection mode	Splitless with surge
Splitless time	1.0 min
Split flow	50.0 mL/min
Septum purge flow	5.0 mL/min
Surge pressure	200 kPa
Surge time	0.5 min
Injector temperature	240 °C
Carrier gas; column flow	He; 1.3 mL/min
Injection volume	2.0 µL
GC oven program	40 °C, 0.5 min (hold) 60 °C/min to 140 °C, 2 min (hold) 20 °C/min to 180 °C, 0.5 min (hold) 30 °C/min to 240 °C, 5 min (hold)
Orbitrap Exploris GC-MS parameters	
Ion source	ExtractaBrite (EI)
Ion source temperature	240 °C
Transfer line temperature	240 °C
Electron energy	30 eV
Emission current	50 µA
Acquisition mode	Full Scan (FS)
Time	3–15 min
Mass resolution	60000 (FWHM at *m/z* 200, scan speed 7 Hz)
Mass range	40–300 *m/z*

Figure 2. Orbitrap Exploris GC mass spectrometer

Results and discussion

The nitrosamines were separated chromatographically and detected using the advantage of accurate mass measurement of the Orbitrap Exploris GC-MS system (Figures 3–5 and 8). Moreover, the use of 30 eV electron energy provided a significant increase in response compared to 70 eV.[12]

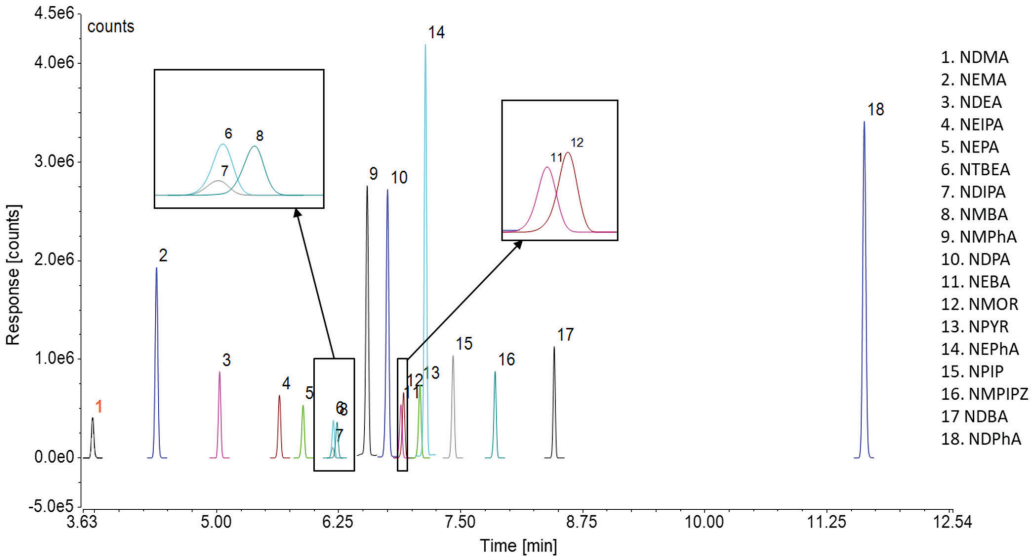

Figure 3. Extracted ion chromatograms (EIC) of 18 nitrosamine impurities in a 10 ng/mL standard

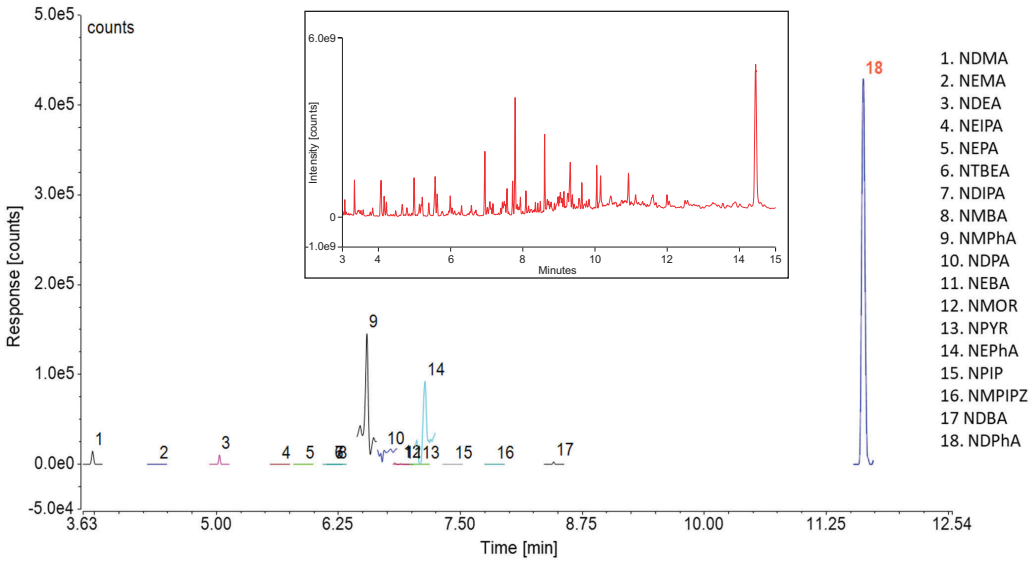

Figure 4. EIC of Telmisartan drug product unspiked extract. Inset: Total ion chromatogram (TIC) of Telmisartan drug product unspiked extract

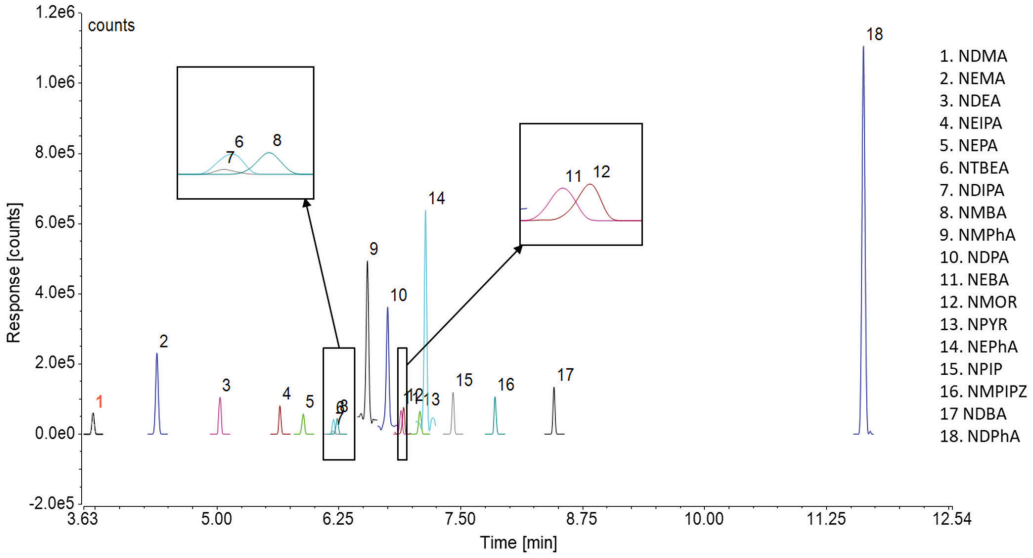

Figure 5. EIC of 18 nitrosamine analytes in Telmisartan sample spiked at 1 ng/mL

Sensitivity

To assess the sensitivity, LOD and LOQ were calculated based on the standard deviation of the calibration curve and the slope, in accordance with ICH guidelines [ICH Q2 (R1)].[13]

$$LOD = \frac{3.3\,\sigma}{S}$$

$$LOQ = \frac{10\,\sigma}{S}$$

Where,

σ = the standard deviation of the response, calculated based on the calibration curve

S = slope of calibration curve

Replicate injections (n=6) of the matrix-matched serially diluted standard (0.25, 0.5, 1.0, 2.5, 5, 10 ng/mL) were used. The LOD were in the range of 0.1 to 0.3 ng/mL (0.8–2.5 ng/g sample) and LOQ were in the range of 0.3 to 1 ng/mL (2.8–7.6 ng/g sample) (Table 2). These results easily meet the FDA regulatory requirements of 30 ppb (ng/g). In addition, three procedural blanks and six unspiked sartan extracts were injected to confirm the presence of nitrosamines (Appendix 2). Negligible concentrations of nitrosamines were detected in the blank and sartan extracts.

Linearity

The response linearity for nitrosamines was evaluated by injecting extracted standard at 0.25, 0.5, 1.0, 2.5, 5.0, and 10 ng/mL. Six replicate injections at each concentration level with internal standard (NDMA-D_6) adjustment was performed. Linear curve fit with 1/x weighting was used for the calibration plot in Chromeleon CDS. The coefficients of determination (R^2) were greater than 0.995 for all the impurities, establishing excellent linear response throughout the range (Table 2). The calibration curve of representative impurities is shown in Figures 6 and 7.

Table 2. LOD, LOQ, precision, and linearity of the nitrosamine impurities

	Compound	RT	Standard deviation of calibration curve (σ)	Slope of calibration curve (S)	LOD (ng/mL)	LOQ (ng/mL)	LOQ (sample) (ng/g)	Peak area %RSD (n=6)	Linearity (R^2)
1	NDMA	3.77	4704	135992	0.1	0.3	2.8	5.6	0.9987
2	NEMA	4.42	23817	650402	0.1	0.4	2.9	4.0	0.9990
3	NDEA	5.06	18500	384659	0.2	0.5	3.8	11.0	0.9992
4	NEIPA	5.67	13810	286594	0.2	0.5	3.9	11.5	0.9950
5	NEPA	5.91	7915	245693	0.1	0.3	2.6	5.7	0.9992
6	NTBEA	6.21	5086	53318	0.3	1.0	7.6	8.6	0.9992
7	NDIPA	6.24	9122	194271	0.2	0.5	3.8	14.3	0.9992
8	NMBA	6.26	9418	134453	0.2	0.7	5.6	9.1	0.9972
9	NMPhA	6.58	42967	950289	0.1	0.5	3.6	7.9	0.9976
10	NDPA	6.78	19174	549158	0.1	0.3	2.8	5.5	0.9995
11	NEBA	6.91	7074	230514	0.1	0.3	2.5	9.3	0.9990
12	NMOR	6.95	12857	261257	0.2	0.5	3.9	12.2	0.9993
13	NPYR	7.10	23153	251073	0.3	0.9	7.4	3.9	0.9986
14	NEPhA	7.16	54016	1297844	0.1	0.4	3.3	7.3	0.9994
15	NPIP	7.46	28431	312355	0.3	0.9	7.3	3.3	0.9996
16	NMPIPZ	7.87	19950	234396	0.3	0.9	6.8	4.0	0.9971
17	NDBA	8.48	18070	387249	0.2	0.5	3.7	7.0	0.9992
18	NDPhA	11.65	148555	1646629	0.3	0.9	7.2	5.3	0.9990

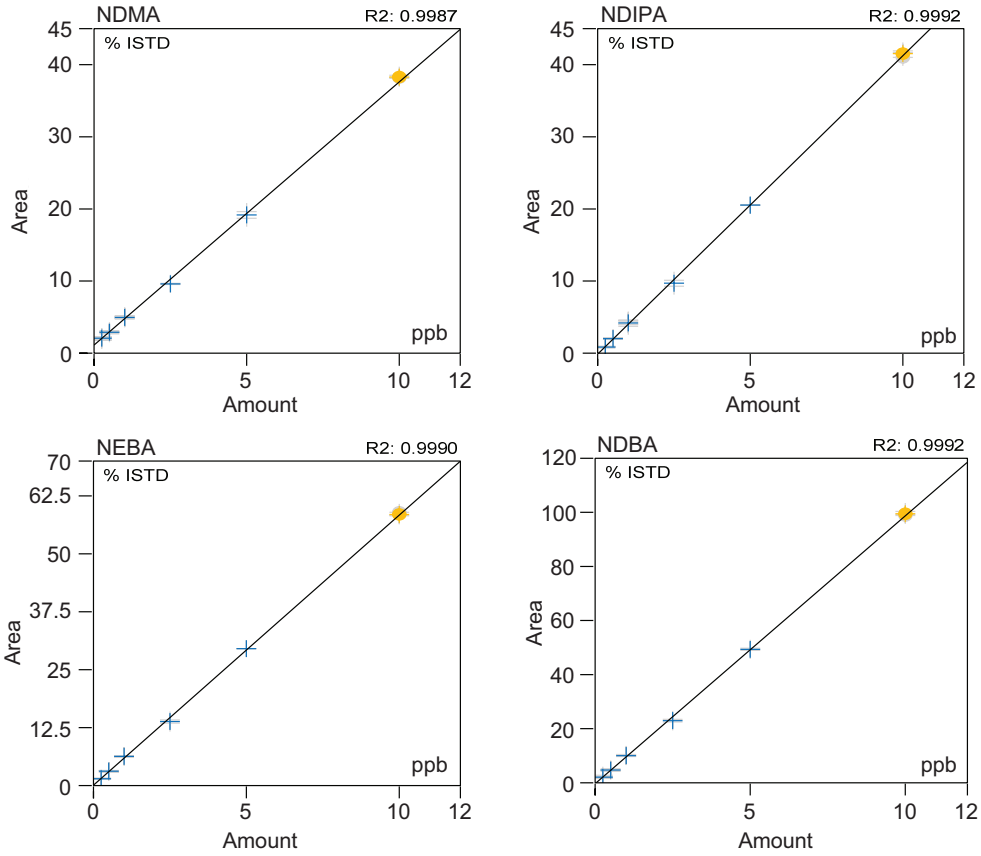

Figure 6. Linearity of some nitrosamine impurities in extracted standards (0.25–10 ng/mL)

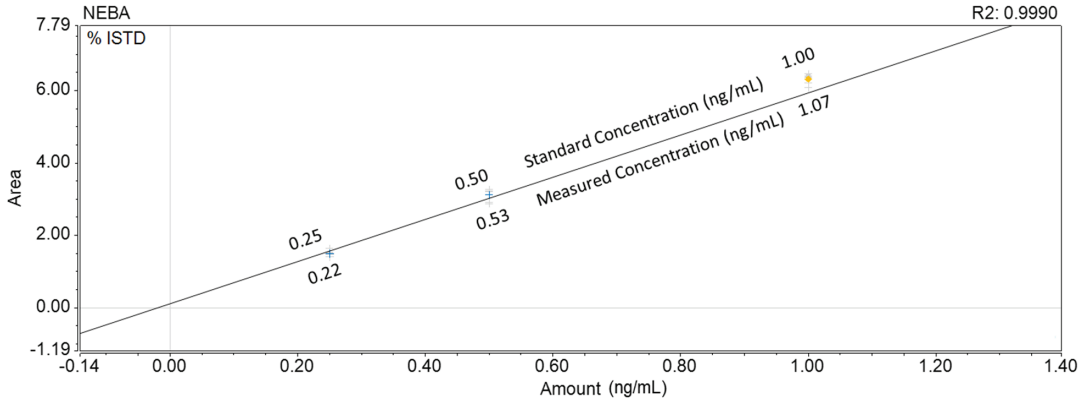

Figure 7. A magnified region of the calibration for NEBA ranging from 0.25 to 1.0 ng/mL (corresponding to 2.0–8.0 ng/g sample)

Accuracy

Accuracy of the method was evaluated by spiking the impurities at 3.0, 4.0, 8.0, and 30 ng/g (w/w) in sartan drug products prior to extraction. Three replicates of each of the spiking levels were assessed for recovery (70–130%) and recovery % RSD (<20%).[3,9,13] Excellent recovery within 70–130% were observed for each of the nitrosamines with recovery % RSD less than 20%. The findings are reported in Table 3.

Table 3. Recovery and recovery %RSD of the nitrosamines

			Telmisartan 40 mg		Losartan 50 mg	
	Compound	Spiked concentration (ng/g)	Mean recovery (%)	Recovery % RSD	Mean recovery (%)	Recovery % RSD
1	NDMA	3.0	115	7.8	117	6.1
2	NEMA	4.0	83	2.6	99	3.0
3	NDEA	4.0	92	6.0	113	2.6
4	NEIPA	4.0	95	18.3	117	10.3
5	NEPA	3.0	92	9.5	111	12.3
6	NDIPA	4.0	93	9.0	112	14.3
7	NTBEA	8.0	84	13.3	88	15.1
8	NMBA	8.0	114	9.5	100	3.2
9	NMPhA	4.0	120	2.2	123	4.9
10	NDPA	3.0	122	4.9	125	11.4
11	NEBA	3.0	98	5.7	108	7.5
12	NMOR	4.0	80	12.7	78	17.3
13	NPYR	8.0	93	8.3	90	14.4
14	NEPhA	4.0	100	8.0	121	8.8
15	NPIP	8.0	104	8.2	104	9.7
16	NMPIPZ	8.0	99	8.0	93	10.5
17	NDBA	4.0	85	7.7	99	9.8
18	NDPhA	4.0	85	9.4	113	8.6

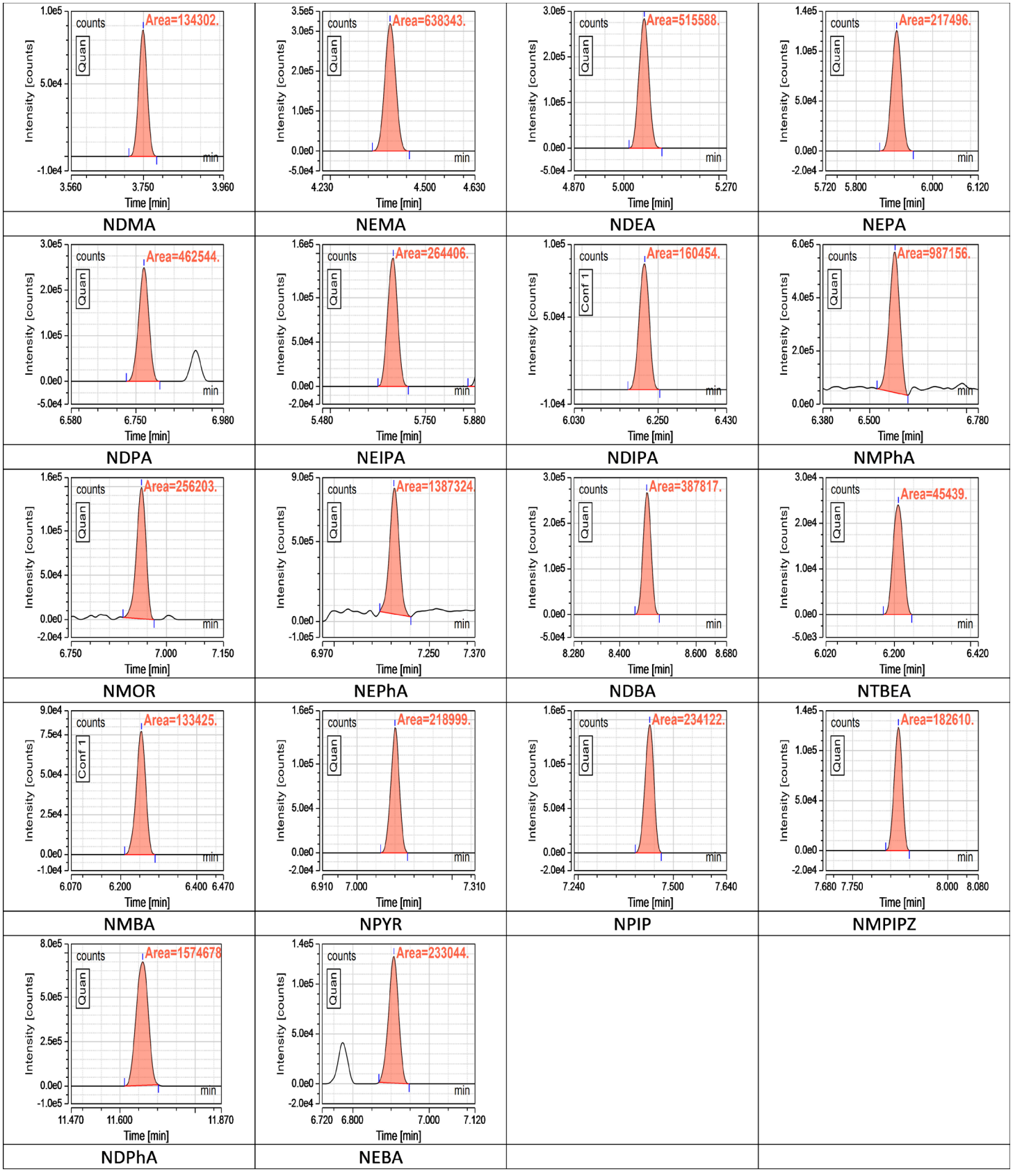

Figure 8. Chromatograms (EIC) of 18 nitrosamine impurities at 1 ng/mL level

Conclusion

The results of these experiments demonstrate:

- Rapid analysis of 18 nitrosamines impurities in sartan drug products was completed within 15 minutes.
- Low quantitation limits were achieved by utilizing the advantage of high resolution and accurate mass measurement in full scan mode. The LOQ were in the range of 0.3 to 1 ng/mL (2.8–7.6 ng/g sample).
- Excellent linearity was observed in the range of 0.25–10 ng/mL (2.0–80.0 ng/g sample).
- Accurate and precise recovery was demonstrated with spiked concentrations within 70–130% recovery and % RSD <20%.

This method highlights the capabilities of the Orbitrap Exploris GC-MS for the detection and quantitation of trace-level nitrosamine impurities in finished medicinal products. Along with quantitative analysis, this method can also be employed effectively using Compound Discoverer software for retrospective analysis of unknown identification.

References

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13. Validation Of Analytical Procedures: Text and Methodology Q2(R1), ICH Harmonised Tripartite Guideline.

Appendix 1. List of Nitrosamines analyzed

Sr. No.	Compounds	Acronym	CAS	MW	Extracted mass
1	*N*-Nitroso dimethylamine-D_6	NDMA-D6	17829-05-9	80.08504	80.08504, 50.08721
2	*N*-Nitroso dimethylamine	NDMA	62-75-9	74.04746	74.04760, 44.04940
3	*N*-Nitroso methylethylamine	NMEA	10595-95-6	88.06311	88.06281, 71.06071
4	*N*-Nitroso diethylamine	NDEA	55-18-5	102.07876	102.07858, 85.07603
5	*N*-Nitroso ethyl isopropyl amine	NEIPA	16339-04-1	116.09441	116.09430, 99.09167
6	*N*-Nitroso ethyl propyl amine	NEPA	25413-61-0	116.09496	99.09153, 116.09436
7	*N*-Nitroso tertiary butylethyl amine	NTBEA	3398-69-4	130.11061	75.05532, 72.08072
8	*N*-Nitroso diisopropylamine	NDIPA	601-77-4	130.11061	88.06304, 113.10721
9	*N*-Nitroso methylbutylamine	NMBA	7068-83-9	116.09496	99.09175, 74.04741
10	*N*-Nitroso *N*-methyl *N*-phenylamine	NMPhA	614-00-6	136.06366	106.06496, 107.07274
11	*N*-Nitroso dipropyl amine	NDPA	621-64-7	130.11061	70.06497, 113.10717
12	*N*-Nitroso ethyl butyl amine	NEBA	17829-05-9	130.11061	88.06308, 113.10719
13	*N*-Nitrosomorpholine	NMOR	59-89-2	116.05803	86.05980, 116.05790
14	*N*-Nitroso pyrrolidine	NPYR	3398-69-4	100.06325	100.06325, 68.04950
15	*N*-Nitroso *N*-ethyl *N*-phenylamine	NEPhA	612-64-6	150.07931	106.06533, 121.08878
16	*N*-Nitroso piperidine	NPIP	100-75-4	114.07876	114.07841, 97.07576
17	*N*-Nitroso methylpiperazine	NMPIPZ	16339-07-4	129.12659	99.09162, 56.04954
18	*N*-Nitroso dibutylamine	NDBA	924-16-3	158.14136	99.09151, 141.13828
19	*N*-Nitroso diphenylamine	NDPhA	86-30-6	198.07931	169.08833, 168.08059

Appendix 2. Average amount of nitrosamines detected

		Procedural blank	Telmisartan 40 mg	Losartan 50 mg
	Compounds	Mean amount detected (ng/g)	Mean amount detected (ng/g)	Mean amount detected (ng/g)
1	NDMA	ND	<2.8	ND
2	NEMA	ND	ND	ND
3	NDEA	ND	ND	ND
4	NEIPA	ND	ND	ND
5	NEPA	ND	ND	ND
6	NTBEA	ND	ND	ND
7	NDIPA	<3.8	<3.8	<3.8
8	NMBA	ND	ND	ND
9	NMPhA	ND	<3.6	<3.6
10	NDPA	<2.8	<2.8	<2.8
11	NEBA	ND	ND	ND
12	NMOR	ND	<3.9	<3.9
13	NPYR	ND	ND	ND
14	NEPhA	ND	<3.3	<3.3
15	NPIP	ND	ND	ND
16	NMPIPZ	ND	ND	ND
17	NDBA	ND	<3.7	ND
18	NDPhA	<7.2	<7.2	<7.2

ND – Not Detected

The instrument configuration used in this application note can be easily visualized and quoted using the on-line GC/GCMS instrument configurator at thermofisher.com/mygc-gcms

Learn more at **thermofisher.com/OrbitrapExplorisGC**