Application note | 003215

Pharma

Highly sensitive method for the determination of 12 nitrosamine impurities in multiple ARBs, the class of sartan drug formulations

Authors

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Keywords

Angiotensin receptor blockers, ARB, angiotensin II inhibitors, TSQ Altis Plus triple quadrupole MS, API, active pharmaceutical ingredient, Vanquish Flex UHPLC, drug substance, nitrosamine, Nitrosamine Drug Substance-Related Impurities (NDSRI), olmesartan, irbesartan, valsartan, PVDF, APCI, high throughput screening

Application benefits

- Single method applicable for highly sensitive quantitation of 12 nitrosamines in three ARB drug substances.
- A robust gradient method with significant chromatographic separation between nitrosamines and ARB drug substances.
- Better retentivity and resolution of nitrosamines and APIs with comparatively shorter runtime, ideal for high throughput screening.

Goal

Development of a sensitive and robust LC-MS/MS method to quantitate the following 12 nitrosamine impurities in three ARB drug substances: *N*-nitrosodimethylamine (NDMA), *N*-nitroso-diethylamine (NDEA), *N*-ethyl-*N*-nitroso-2-propanamine (NEIPA), *N*-nitroso-diisopropylamine (NDIPA), *N*-nitroso-di-*n*-propylamine (NDPA), *N*-nitroso-methylphenylamine (NMPA), *N*-nitroso-di-*n*-butylamine (NDBA), *N*-nitroso-*N*-methyl-4-aminobutyric acid (NMBA), *N*-nitrosomorpholine (NMOR), *N*-nitrosopyrrolidine (NPYR), *N*-nitrosomethylethylamine (NMEA), and *N*-nitrosopiperidine (NPIP).

Introduction

Angiotensin receptor blockers (also called ARBs or angiotensin II inhibitors) are medicines that dilate blood vessels and are used in the treatment of high blood pressure (hypertension), heart failure, or kidney disease in people with diabetes.[1] These ARBs, also known as sartan medicines, have a specific tetrazole ring-containing structure whose synthesis could potentially lead to the formation of nitrosamine impurities.[2]

If the content of nitrosamine impurities is found to be greater than the acceptable limit, then regulatory authorities need to be informed, and that may ultimately lead to the drug substance product being recalled. There have been numerous drug recalls associated with nitrosamine impurity levels exceeding the allowable limits in drug substances of various pharmacological categories, including ARBs.[3] In 2018 and in 2019, the U.S. Food and Drug Administration (FDA) found traces of NDMA and NDEA impurities in the ARB drug products valsartan, losartan, and irbesartan. Since July 2018, more than two dozen specific ARB products have been recalled owing to the unacceptable presence of potentially carcinogenic nitrosamine impurities.[4]

In 2018, the FDA issued guidance to the industry on how to assess and control impurities. EMA finalized a review under Article 5(3) of Regulation (EC) No 726/2004 in June 2020 to provide guidance to marketing authorization holders on how to avoid the presence of nitrosamine impurities in human medicines.

The CHMP asked marketing authorization holders to review all chemical and biological human medicines for the possible presence of nitrosamines and test products at risk. Regulators across the globe have been vigilant on nitrosamine testing and subsequent modification of guidelines or testing procedures to ensure the best quality products reach the market. Recently, the FDA[5] and EMEA[6] updated guidelines with a more comprehensive and categorized approach towards nitrosamine testing.

In this application note, the goal was to develop an LC-MS/MS solution to the increased frequency of demands for higher sensitivity and quantification of an extensive array of nitrosamine impurities in pharmaceutical formulations with a one-method approach for multiple products. From the multiple techniques available today, liquid chromatography-tandem mass spectrometry is one of the best for highly sensitive and reproducible quantification of nitrosamines. It not only benefits sensitivity in sub-nanogram concentrations but also provides a more robust, linear, and reproducible solution for nitrosamine detection. The guidelines followed in this application are from the ICH Q2(R2)[7], FDA[5], and EMEA[6].

As demonstrated by the excellent results observed in this study, the Thermo Scientific™ TSQ Altis™ Plus Triple Quadrupole mass spectrometer connected to the Thermo Scientific™ Vanquish™ Flex UHPLC system makes a suitable configuration for the highly sensitive analysis of multiple nitrosamines in a single method applicable for 3 ARBs—olmesartan, irbesartan, and valsartan. For data processing and reporting, Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) provides a complete solution, which fulfils all compliance requirements and ensures data integrity and security requirements are met, with a detailed workflow that includes user management along with instrument and data audit trails.

Setup

Instrumentation

Vanquish Flex UHPLC system consisting of:

- System Base Vanquish Horizon/Flex (P/N VF-S01-A-02)
- Binary Pump F (P/N VF-P10-A)
- Split Sampler FT (P/N VF-A10-A)
- Column Compartment H (P/N VH-C10-A)
- Diode Array Detector FG (P/N VF-D11-A-01) with 10 mm, SST 13 µL flow cell (P/N 6083.0510)
- TSQ Altis Plus triple quadrupole mass spectrometer (P/N TSQ03-10002)
- Chemyx Infusion pump, model F100T2, and Rheodyne 6 port divert valve
- PEAK Scientific Genius XE nitrogen generator (P/N 3300252)
- SCAT HPLC supply and waste set – safety caps with air valves for reagent bottles (P/N 307447)

Software

Chromeleon CDS, version 7.3.2

Reagents and consumables

- Reference standards procured from Cleanchem Laboratories:
  - *N*-nitroso-dimethylamine (NDMA)
  - *N*-nitroso-diethylamine (NDEA)
  - *N*-ethyl-*N*-nitroso-2-propanamine (NEIPA)
  - *N*-nitroso-diisopropylamine (NDIPA)
  - N-nitroso-di-*n*-propylamine (NDPA)
  - *N*-nitroso-methylphenylamine (NMPA)
  - *N*-nitroso-di-*n*-butylamine (NDBA)
  - *N*-nitroso-N-methyl-4-aminobutyric acid (NMBA)
  - *N*-nitroso-morpholine (NMOR)
  - *N*-nitroso-pyrrolidine (NPYR)
  - *N*-nitroso-methylethylamine (NMEA)
  - *N*-nitroso-piperidine (NPIP)
- Fisher Scientific™ Formic acid, Optima™ LC/MS grade (Fisher Scientific P/N A117-50 or equivalent)
- Fisher Scientific™ Methanol, Optima™ LC/MS grade (Fisher Scientific P/N A456-4 or equivalent)
- Fisher Scientific™ Water, Optima™ LC/MS grade (Fisher Scientific P/N W64 or equivalent)
- Invitrogen™ 2 mL microcentrifuge tubes (P/N AM12475)
- Thermo Scientific™ Titan3™ 0.2 µm PVDF syringe filters (P/N 42213-PV)
- Thermo Scientific™ SureSTART™ 2 mL GOLD-grade glass screw top vials (P/N 6PSV9-1PG)
- Thermo Scientific™ SureSTART™ 9 mm screw caps (P/N 6PSC9TST)
- Thermo Scientific™ SureSTART™ 1.5 mL CLR SCR TR Vials with caps (P/N 6PCK993)
- Thermo Scientific™ Acclaim™ 120 C18 column, 4.6 mm × 250 mm, 5 µm (P/N 059149)

Solutions and sample preparation

Diluent solution and blank preparation

For the diluent solution and blank, 60 mL of methanol and 40 mL of water were mixed.

Stock standard solution preparation

Individual stock standard solution of 100 µg/mL in methanol was prepared for each nitrosamine impurity.

Standard preparation of 1.5 ng/mL (0.030 ppm with respect to drug substance sample concentration)

For each impurity, 10 µL of stock standard solution was transferred into a 10 mL volumetric flask and diluted to volume using diluent solution to prepare the mixed intermediate dilution. Then, a 150 µL aliquot of the mixed intermediate dilution was transferred into a 10 mL volumetric flask and diluted to volume with diluent solution.

Preparation of linearity standards, LOQ of 15 µL/mL (0.003 ppm with respect to drug substance sample concentration) and LOD

A mixed standard solution (Intermediate solution-1) of all 12 nitrosamines was prepared at a concentration of 20 ng/mL by taking a suitable volume from the individual stock standard solution (100 µg/mL) and diluting with diluent solution. This mixed standard solution served as the linearity standard of the highest concentration. From this solution, a suitable volume was taken and serially diluted to achieve five more linearity standards. It was diluted further to prepare the LOQ solution (150 pg for all 12 nitrosamines). The LOQ solution serves as the calibration curve standard of the lowest concentration in linearity.

For the LOD solution, a mixed standard solution (Intermediate solution-2) was prepared containing a concentration of 20 ng/mL for NDMA, NMBA, NPYR, NEIPA, and NDIPA and 10 ng/mL for other nitrosamines. Then, 15 µL of this mixed standard solution was transferred into a 20 mL volumetric flask and diluted to volume using diluent solution to achieve a concentration of 75 pg/mL of NDMA, NMBA, NPYR, NEIPA, and NDIPA and 37.5 pg/mL of other nitrosamines.

Drug substance sample preparation

For the drug substance samples, 100 mg of API was weighed and transfered into a 15 mL centrifuge tube. Then, 2 mL of diluent solution was added to prepare a solution of 50 mg/mL of API. The solution was vortexed briefly and sonicated in an ultrasonicate bath for 15 minutes. It was then vortexed again for 2 minutes and the entire solution was transferred into 2.0 mL microcentrifuge tubes. The tubes were centrifuged @12,000 rpm at 5 °C for 25 minutes. The supernatant was filtered using a 0.22 µm PVDF syringe filter, the first 0.2 mL was discarded, and the filtered sample was transferred into total recovery HPLC vials for LC/MS analysis.

Experimental design

Sample analysis, data processing, and reporting were performed using Chromeleon CDS, version 7.3.2.

Chromatographic conditions

Table 1. HPLC conditions

Parameter	Value		
HPLC column	Acclaim 120 C18 5 µm, 4.6 mm × 250 mm (P/N 059149)		
Column temp.	45 °C		
Flow rate	0.500 mL/min		
Mobile phase A	0.1% formic acid in water		
Mobile phase B	0.1% formic acid in methanol		
Gradient	**Time (min)**	**A%**	**B%**
	0	75	25
	1	75	25
	3	55	45
	6	55	45
	9	50	50
	10	45	55
	16	45	55
	17	35	65
	25	35	65
	26	33	67
	27	15	85
	30	5	95
	33	5	95
	33.5	75	25
	40	75	25
Injection volume	40 µL		
Autosampler temp.	20 °C		
Needle wash	80:20, methanol:water		
Static mixer	150 µL		

Table 2. Divert valve settings

Time (min)	Position	Flow direction
0	1-2	LC → MS
15.7	1-6	LC → UV
16.3	1-2	LC → MS
21.8	1-6	LC → UV
28.4	1-2	LC → MS
31.25	1-6	LC → UV
39.5	1-2	LC → MS

Mass spectrometer parameter settings

Table 3. Ion source settings

Parameter	Value
Ion source type	APCI
Sheath gas flow rate	45 arbitrary units
Aux gas flow rate	7 arbitrary units
Sweep gas flow rate	1 arbitrary unit
Corona discharge voltage	6 µA
Capillary temperature	325 °C
Aux gas heater temperature	300 °C
Polarity	Positive

Table 4. SRM settings

Compound	Start time (min)	End time (min)	Precursor (*m/z*)	Product (*m/z*)	Collision energy (V)	RF lens (V)	Source fragmentation (V)	Q1 resolution (FWHM)	Q3 resolution (FWHM)
NDMA	0	40	75.02	58	14	38	25	0.7	1.2
				*43	18				
NMEA	0	40	89	60.88	13	45	20.4	0.4	1.2
				*43	16				
NPYR	0	40	100.98	41	29	48	18	0.4	1.2
				*55	17				
NDEA	0	40	102.98	29	18	44	24.5	0.4	1.2
				*75	14				
NPIP	0	40	115.05	69	16	54	30	0.4	1.2
				*41	22				
NMOR	0	40	116.97	87	12	55	20	0.4	1.2
				*45	19				
NEIPA	0	40	117.12	43	19	34	16	0.4	1.2
				*75	11				
NDIPA	0	40	130.97	89	10	35	5	0.7	1.2
				*43	15				
NDPA	0	40	130.98	89	10	43	10	0.4	1.2
				*43	15				
NMPA	0	40	136.97	66	22	52	24.5	0.4	1.2
				*107	13				
NMBA	0	40	147.1	117	7	33	0	0.7	1.2
				*44	15				
NDBA	0	40	159.05	103	12	50	20.4	0.4	0.7
				*57	15				

*Qualifier ion

Injection order

A. LOD, LOQ, linearity, and reproducibility

1. Diluent blank injection at beginning of the sequence
2. Six replicate injections of standard solution (1.5 ng/mL) followed by one injection of diluent blank
3. Three replicate injections of LOD
4. Six replicate injections of LOQ
5. Single injection each of seven linearity standards in order of increasing concentration from 0.15 ng/mL to 20 ng/mL followed by one injection of diluent blank

B. Recovery evaluation

1. Diluent blank injection at beginning of the sequence
2. Six replicate injections of LOQ (0.15 ng/mL)
3. Six replicate injections of specification level concentration (1.5 ng/mL)
4. Six replicate injections of highest concentration (20.0 ng/mL)
5. Diluent blank injection
6. As such sample of first API in triplicate
7. LOQ spiked sample of first API in triplicate
8. Specification level spiked sample of first API in triplicate
9. Highest concentration spiked sample of first API in triplicate
10. Diluent blank injection
11. Bracketing standard (1.5 ng/mL)
12. Repeat step 6 to 11 for second API followed by third API

System suitability requirements[8]

- The area of an interference peak for nitrosamine impurities in the blank injection, if present, should be no more than 5% of the peak area in the standard solution.
- The % RSD of the peak area for each nitrosamine impurity for the first six injections of standard solution should be no more than 10%.
- The cumulative % RSD of the peak area for each nitrosamine impurity should be no more than 15%. (Cumulative % RSD of the peak area is calculated by combining the initial six replicate injections of the standard solution and each subsequent bracketing standard.)

Observation shared by the FDA method[8]

- NMBA and NEIPA exist as syn and anti-conformers due to the restricted rotation of the N-N bond, and these conformers can be partially separated by the method's chromatographic conditions.
- The NMBA peak is observed as a doublet at a ratio of approximately 3:1.
- Integrate both peaks and use the combined peak area for NMBA.
- Depending on the column and concentration of the sample, the NEIPA peak may appear as a doublet or a single peak with a tailing shoulder. Include the resolved second peak or the tailing of the main peak when integrating the NEIPA peak(s).
- The retention time difference of any impurity in the analyzed samples should not be more than 2% of the retention time of the corresponding standard in the standard solution.
- Report the nitrosamine impurity content in ppm with three significant figures if the value is ≥ LOD
- Report "not detected" if no nitrosamine impurity is detected or if the value is < LOD

Calculation as per the FDA method[8]

Drug substance:

$$\text{Nitrosamine impurity (ppm)} = \frac{A_{spl}}{A_s} \times C_s \times \frac{1\ \text{mg}}{1 \times 10^6\ \text{ng}} \times \frac{V}{W} \times 10^6$$

Nitrosamine impurity (ppm) = Each individual impurity

A_{spl} = Area of the nitrosamine impurity peak in the sample solution

A_s = Average area (n = 6) of the nitrosamine impurity peak from the first six consecutive injections of the standard solution

C_s = Concentration of the nitrosamine impurity in the standard solution (3.0 ng/mL)

W = Weight of drug substance (mg)

V = Volume of the diluent in the sample solution (mL)

Results and discussion

System suitability was performed by injecting six replicate injections from the standard solution of 1.5 ng/mL. Table 5 shows the results of % RSD calculated for retention time and peak area response of the nitrosamine impurities.

Table 5. System suitability

System suitability (%RSD) for nitrosamine impurities										
		NDMA			NMOR			NMBA		
Sr. no.	Injection name	Name	Ret. time (min)	Area (counts*s)	Name	Ret. time (min)	Area (counts*s)	Name	Ret. time (min)	Area (counts*s)
1	Standard-100%-1	NDMA	7.31	217032	NMOR	8.07	128124	NMBA	8.14	61470
2	Standard-100%-2	NDMA	7.31	228278	NMOR	8.07	132512	NMBA	8.14	53505
3	Standard-100%-3	NDMA	7.31	223288	NMOR	8.07	125759	NMBA	8.14	58709
4	Standard-100%-4	NDMA	7.31	229099	NMOR	8.07	131623	NMBA	8.14	59882
5	Standard-100%-5	NDMA	7.31	231981	NMOR	8.07	131415	NMBA	8.14	56968
6	Standard-100%-6	NDMA	7.31	228093	NMOR	8.07	126887	NMBA	8.14	57776
		MEAN	7.313	226295.2	MEAN	8.071	129386.7	MEAN	8.139	58051.7
		SD	0.0000	5332.98	SD	0.0000	2824.38	SD	0.0000	2734.58
		%RSD	0.0	2.4	%RSD	0.0	2.2	%RSD	0.0	4.7
		NPYR			NMEA			NDEA		
Sr. no.	Injection name	Name	Ret. time (min)	Area (counts*s)	Name	Ret. time (min)	Area (counts*s)	Name	Ret. time (min)	Area (counts*s)
1	Standard-100%-1	NPYR	8.94	142709	NMEA	9.03	391686	NDEA	11.06	301379
2	Standard-100%-2	NPYR	8.94	135755	NMEA	9.03	390775	NDEA	11.06	290680
3	Standard-100%-3	NPYR	8.94	136564	NMEA	9.03	387142	NDEA	11.06	298463
4	Standard-100%-4	NPYR	8.94	139272	NMEA	9.03	385863	NDEA	11.06	296608
5	Standard-100%-5	NPYR	8.94	135903	NMEA	9.03	384992	NDEA	11.06	294041
6	Standard-100%-6	NPYR	8.89	141504	NMEA	9.03	393417	NDEA	11.06	290965
		MEAN	8.931	138617.8	MEAN	9.034	388979.2	MEAN	11.056	295356.0
		SD	0.0200	3009.32	SD	0.0000	3441.79	SD	0.0000	4251.41
		%RSD	0.2	2.2	%RSD	0.0	0.9	%RSD	0.0	1.4
		NPIP			NEIPA			NDIPA		
Sr. no.	Injection name	Name	Ret. time (min)	Area (counts*s)	Name	Ret. time (min)	Area (counts*s)	Name	Ret. time (min)	Area (counts*s)
1	Standard-100%-1	NPIP	11.99	657611	NEIPA	14.02	114683	NDIPA	17.31	141268
2	Standard-100%-2	NPIP	11.99	653776	NEIPA	14.02	118453	NDIPA	17.31	143676
3	Standard-100%-3	NPIP	11.99	644290	NEIPA	14.02	116311	NDIPA	17.31	143844
4	Standard-100%-4	NPIP	11.99	646677	NEIPA	14.02	116621	NDIPA	17.31	142460
5	Standard-100%-5	NPIP	11.99	658575	NEIPA	14.02	118235	NDIPA	17.31	142261
6	Standard-100%-6	NPIP	11.99	657818	NEIPA	14.02	118349	NDIPA	17.31	145968
		MEAN	11.992	653124.5	MEAN	14.015	117108.7	MEAN	17.306	143246.2
		SD	0.0000	6195.30	SD	0.0000	1508.13	SD	0.0000	1640.26
		%RSD	0.0	0.9	%RSD	0.0	1.3	%RSD	0.0	1.1
		NMPA			NDPA			NDBA		
Sr. no.	Injection name	Name	Ret. time (min)	Area (counts*s)	Name	Ret. time (min)	Area (counts*s)	Name	Ret. time (min)	Area (counts*s)
1	Standard-100%-1	NMPA	18.39	398976	NDPA	19.22	228284	NDBA	30.28	174859
2	Standard-100%-2	NMPA	18.39	405930	NDPA	19.22	238640	NDBA	30.23	175560
3	Standard-100%-3	NMPA	18.39	400824	NDPA	19.22	231068	NDBA	30.23	177117
4	Standard-100%-4	NMPA	18.39	406072	NDPA	19.22	228473	NDBA	30.23	181679
5	Standard-100%-5	NMPA	18.39	408127	NDPA	19.22	233357	NDBA	30.23	168794
6	Standard-100%-6	NMPA	18.39	402222	NDPA	19.22	232827	NDBA	30.23	183031
		MEAN	18.393	403691.8	MEAN	19.224	232108.2	MEAN	30.236	176840.0
		SD	0.0000	3548.78	SD	0.0000	3838.28	SD	0.0200	5140.47
		%RSD	0.0	0.9	%RSD	0.0	1.7	%RSD	0.1	2.9

Reproducibility, including bracketing standards, can be observed in Figure 1 that gives confidence in the robustness of the system during analysis of samples for longer sequence durations. This robustness is one of the most significant factors that supports method reproducibility and consistency of the analytical results.

During method development, it was found that APCI best suits the desired level of sensitivity for all twelve nitrosamine impurities, whereas ESI was found suitable only for a few impurities.

Excellent linearity, as shown in Figure 2, was observed over a concentration range of 0.150 ng/mL to 20 ng/mL. R^2 value was found to be >0.999 for all 12 nitrosamines.

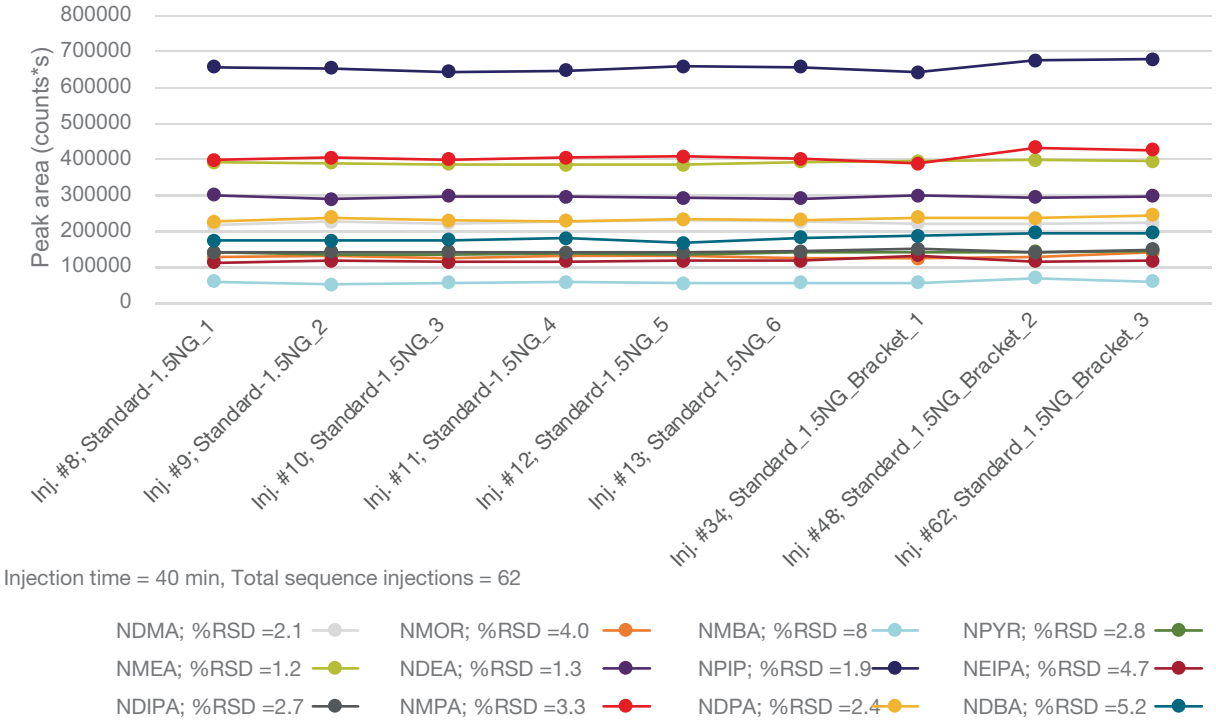

Figure 1. Robustness results

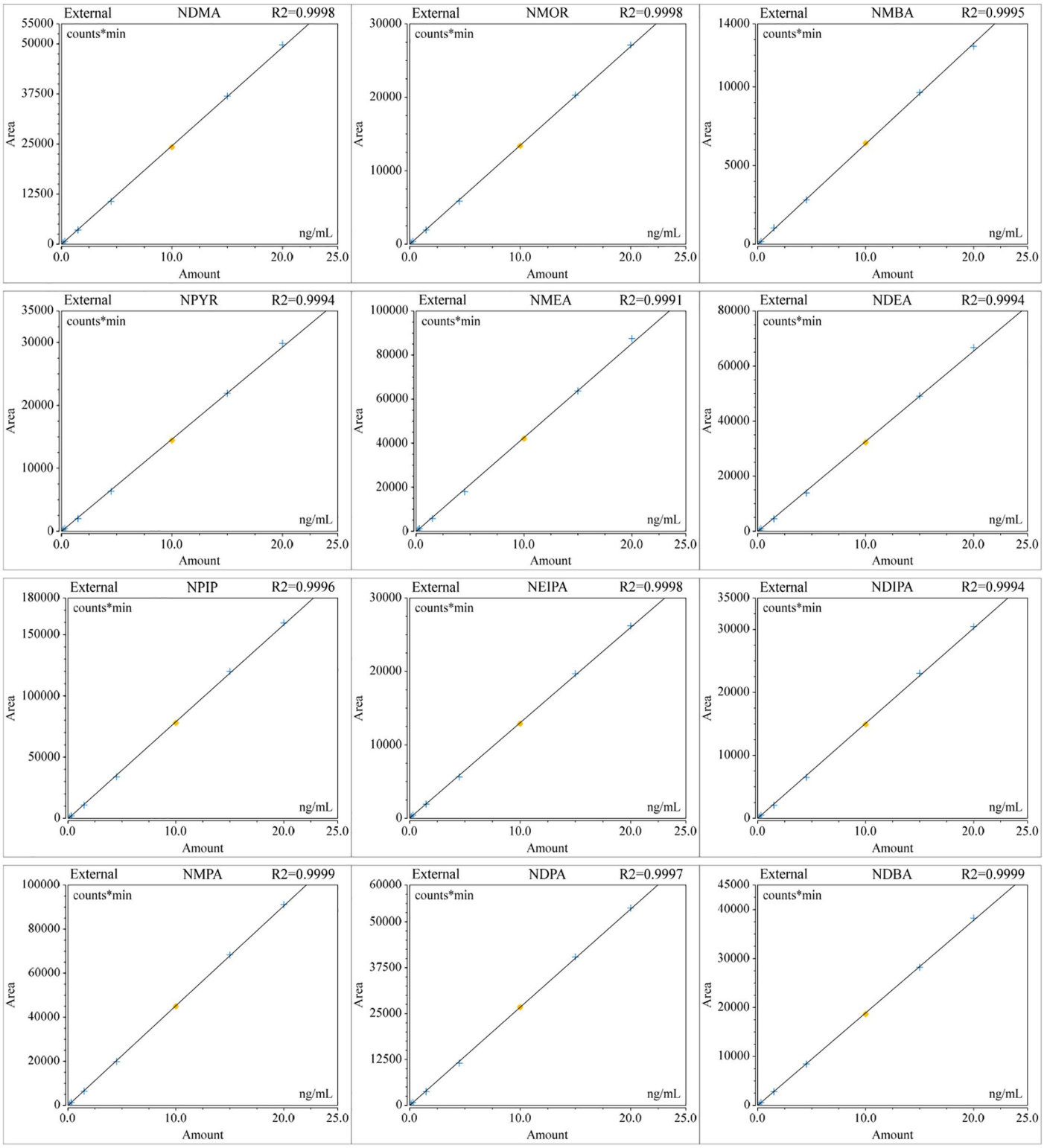

Figure 2. Linearity

Chromatographic data is shown in Figures 3, 4, and 5 to observe retention time and peak area response of 12 nitrosamine impurities corresponding to injections of diluent blank, LOQ (0.150 ng/mL), and standard (1.5 ng/mL).

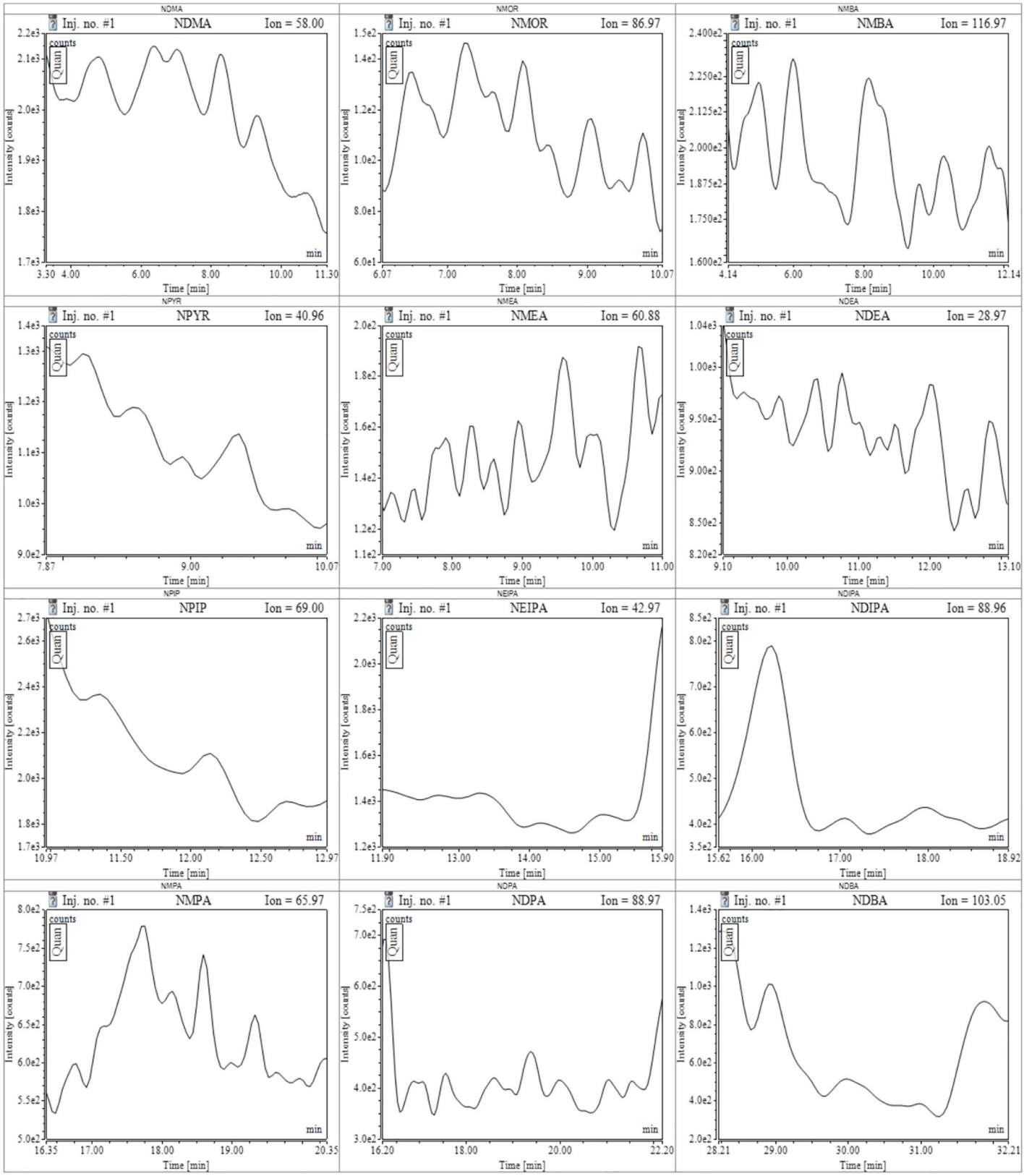

Figure 3. Chromatogram of diluent blank

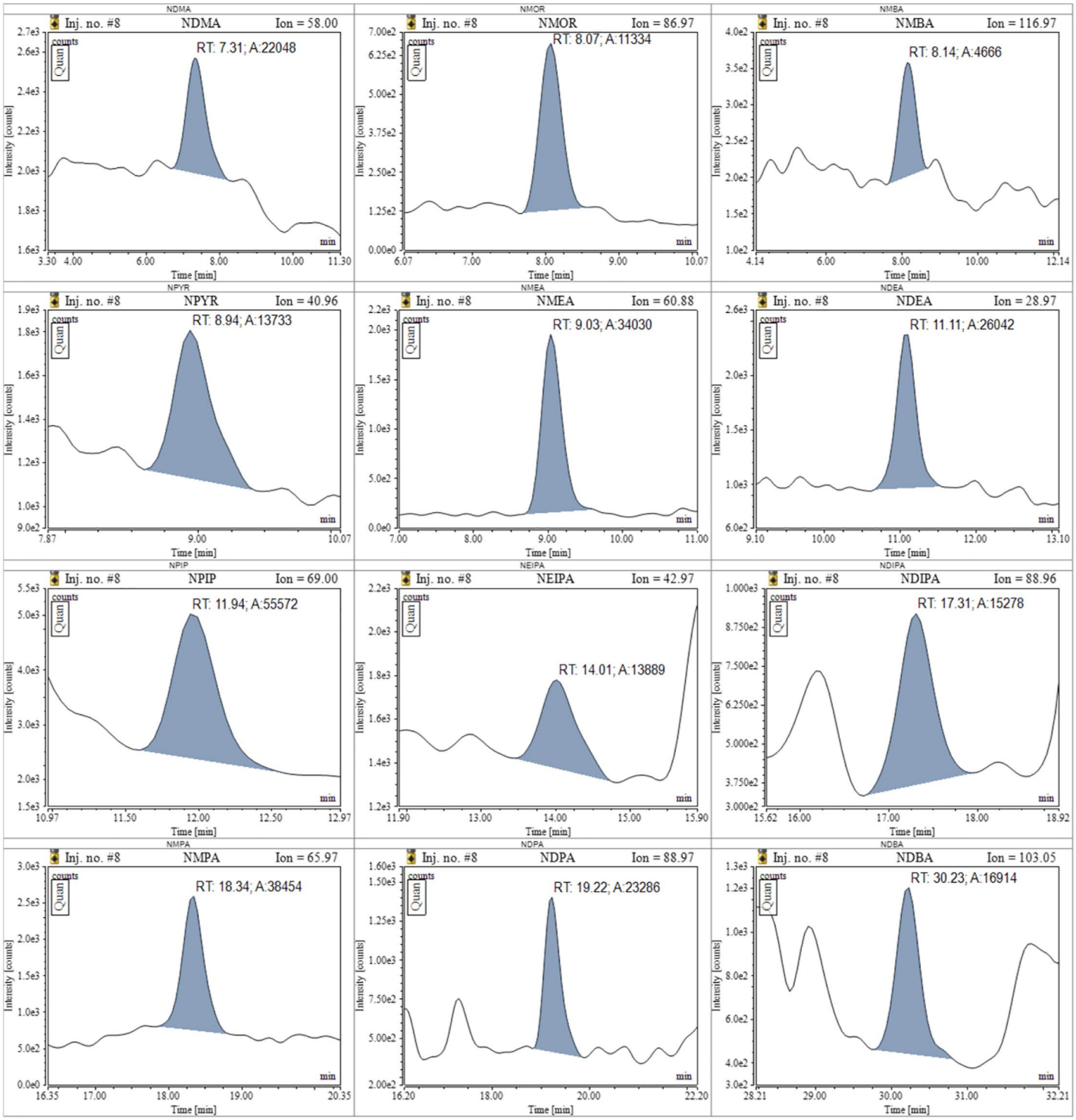

Figure 4. Chromatogram of LOQ (0.150 ng/mL)

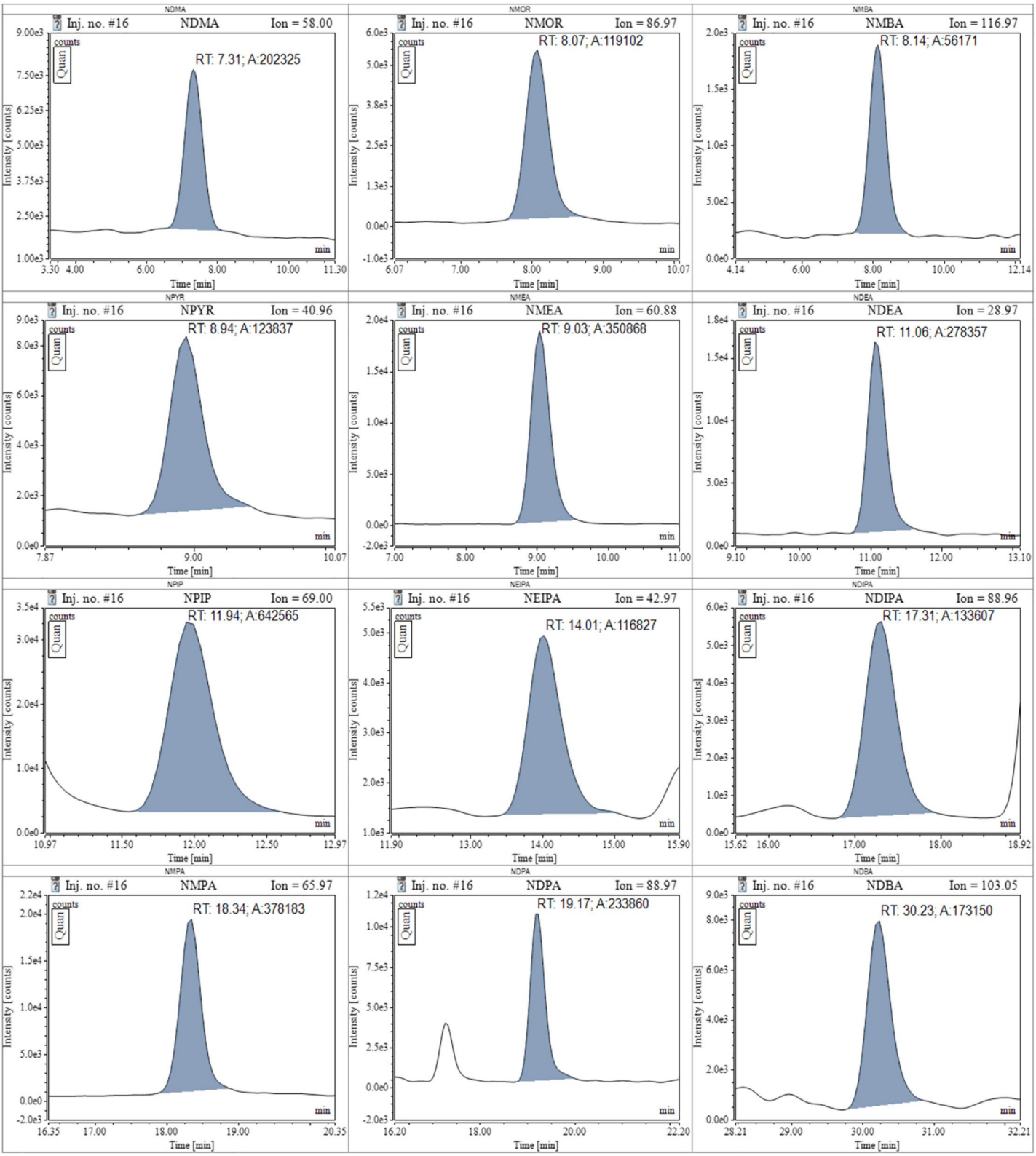

Figure 5. Chromatogram of standard solution (1.5 ng/mL)

Excellent chromatographic separation between 12 nitrosamines and API peaks, as shown in Figure 6, was achieved by using very robust reversed phase column chemistry—the Acclaim 120 C18 column, 4.6 mm × 250 mm, 5 µm. By using this column, the key benefit observed was unmatched retentivity for nitrosamines and high-throughput analysis with desired chromatographic separation from API peaks with applicability towards three different ARB drug substances.

A recovery study was performed with spiked samples for all three ARBs (olmesartan, irbesartan and valsartan) at three different concentration levels in triplicate and was found to be well within the acceptance criteria as shown in Table 6. The results summary with LOD and LOQ values, linearity range, and respective S/N ratio obtained at the LOQ level for 12 nitrosamines is shown in Table 7.

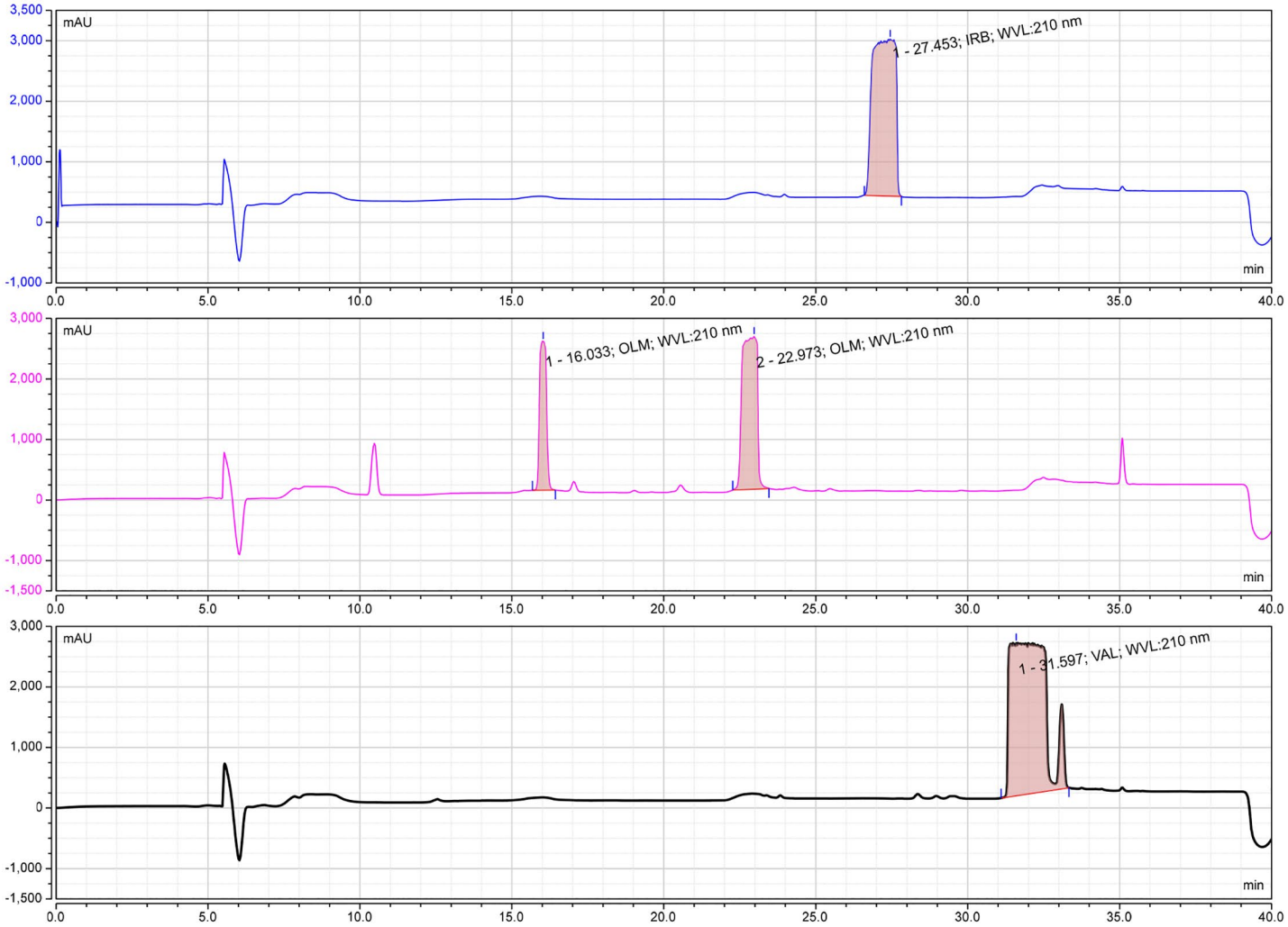

Figure 6. Chromatogram of UV for 3 ARBs (IRB = Irbesartan, OLM = Olmesartan, VAL = Valsartan)

Table 6. Recovery results

Olmesartan												
Recovery at low concentration level (0.150 ng/mL)												
Impurity	NDMA	NMOR	NMBA	NPYR	NMEA	NDEA	NPIP	NEIPA	NDIPA	NMPA	NDPA	NDBA
% Recovery*	106.5%	97.1%	110.5%	111.2%	104.7%	80.0%	98.4%	119.3%	102.2%	106.0%	88.5%	80.9%
Recovery at mid concentration level (1.500 ng/mL)												
Impurity	NDMA	NMOR	NMBA	NPYR	NMEA	NDEA	NPIP	NEIPA	NDIPA	NMPA	NDPA	NDBA
% Recovery*	100.3%	97.8%	103.4%	100.0%	102.7%	98.1%	103.0%	89.1%	102.2%	108.8%	106.8%	94.4%
Recovery at high concentration level (20 ng/mL)												
Impurity	NDMA	NMOR	NMBA	NPYR	NMEA	NDEA	NPIP	NEIPA	NDIPA	NMPA	NDPA	NDBA
% Recovery*	100.9%	103.1%	105.1%	100.4%	102.1%	105.5%	101.7%	106.9%	104.3%	113.2%	114.6%	107.8%
Irbesartan												
Recovery at low concentration level (0.150 ng/mL)												
Impurity	NDMA	NMOR	NMBA	NPYR	NMEA	NDEA	NPIP	NEIPA	NDIPA	NMPA	NDPA	NDBA
% Recovery*	81.5%	92.5%	100.7%	120.2%	104.0%	121.5%	91.5%	89.0%	97.5%	92.3%	110.2%	116.3%
Recovery at mid concentration level (1.500 ng/mL)												
Impurity	NDMA	NMOR	NMBA	NPYR	NMEA	NDEA	NPIP	NEIPA	NDIPA	NMPA	NDPA	NDBA
% Recovery*	100.4%	103.6%	112.4%	100.6%	103.0%	113.3%	103.6%	86.9%	97.3%	94.3%	106.8%	96.3%
Recovery at high concentration level (20 ng/mL)												
Impurity	NDMA	NMOR	NMBA	NPYR	NMEA	NDEA	NPIP	NEIPA	NDIPA	NMPA	NDPA	NDBA
% Recovery*	100.3%	103.1%	117.8%	103.0%	101.6%	103.8%	103.3%	102.7%	98.1%	98.8%	105.5%	99.5%
Valsartan												
Recovery at low concentration level (0.150 ng/mL)												
Impurity	NDMA	NMOR	NMBA	NPYR	NMEA	NDEA	NPIP	NEIPA	NDIPA	NMPA	NDPA	NDBA
% Recovery*	92.8%	88.8%	81.1%	78.1%	103.8%	106.2%	78.9%	90.0%	85.3%	83.0%	84.6%	97.7%
Recovery at mid concentration level (1.500 ng/mL)												
Impurity	NDMA	NMOR	NMBA	NPYR	NMEA	NDEA	NPIP	NEIPA	NDIPA	NMPA	NDPA	NDBA
% Recovery*	93.6%	96.4%	99.8%	93.6%	96.3%	92.1%	90.0%	75.5%	88.9%	86.6%	96.7%	87.5%
Recovery at high concentration level (20 ng/mL)												
Impurity	NDMA	NMOR	NMBA	NPYR	NMEA	NDEA	NPIP	NEIPA	NDIPA	NMPA	NDPA	NDBA
% Recovery*	96.9%	97.5%	95.0%	96.1%	96.1%	96.7%	93.1%	94.0%	89.8%	84.9%	96.9%	89.4%

Table 7. Results summary

S. no.	Impurity name	Limit of detection (LOD) nominal conc. (ng/mL)	Limit of quantification (LOQ) nominal conc. (ng/mL)	S/N at LOQ	Linearity range
1	NDMA	0.0750	0.1500	11.5	0.15–20 ng/mL
2	NMOR	0.0375	0.1500	22.7	
3	NMBA	0.0750	0.1500	11.2	
4	NPYR	0.0750	0.1500	13.2	
5	NMEA	0.0375	0.1500	69.4	
6	NDEA	0.0375	0.1500	31.2	
7	NPIP	0.0375	0.1500	22.5	
8	NEIPA	0.0750	0.1500	17.0	
9	NDIPA	0.0750	0.1500	26.3	
10	NMPA	0.0375	0.1500	22.0	
11	NDPA	0.0375	0.1500	20.9	
12	NDBA	0.0375	0.1500	14.3	

Conclusion

An LC-MS/MS method for the analysis of 12 nitrosamine impurities in 3 ARB drug substances (olmesartan, irbesartan and valsartan) has been successfully developed with excellent reproducibility, linearity, and recovery. The method showed capabilities to meet expectations even lower than currently required concentrations.

1. The R^2 reported for all compounds is >0.999.
2. The LOQ for all twelve nitrosamine impurities was established lower than currently required by regulatory expectations. The % RSD at desired absolute standard concentration (1.5 ng/mL) in system suitability was less than 5% for all compounds, which meets reproducibility requirements.
3. % Recovery was determined at specification level 0.03 ppm and found to be within the permissible limit (70 to 130%). Recovery was determined at two additional concentration levels (LOQ level and highest linearity standard concentration), and the results were found to be within 70–130%.
4. Cumulative % RSD (including system suitability standard and bracketing standards) was found to be within 8% for all twelve impurities when the entire sequence was analyzed for 41 hours continuously, which reflects the robustness of the system and method suitability.
5. The TSQ Altis Plus MS system is capable of successfully achieving the desired concentration limits for the nitrosamine impurities. The sensitivity and reproducibility of the instrument and method meets expectations for the determination of 12 nitrosamine impurities in three ARB drug substances.
6. This method has an excellent high-throughput benefit of quantifying 12 nitrosmaines in 3 drug substances namely olmesartan, irbesartan, and valsartan within a runtime of 40 minutes.

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