Application Note

Pharmaceuticals

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Simultaneous Estimation of Eleven Nitrosamine Impurities in Metformin Drug Product Using an Agilent 6495D LC/TQ

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Abstract

This application note presents a comprehensive analytical method for the simultaneous detection and quantification of 11 nitrosamine impurities—NDMA, NMOR, NMBA, NPYR, NTHP, NDEA, NEIPA, NDIPA, NMPA, NMPEA, and NDBA—in metformin drug product. The method uses an Agilent 1290 Infinity II LC system coupled with an Agilent 6495D triple quadrupole mass spectrometer (LC/TQ), employing multiple reaction monitoring (MRM). The enhanced sensitivity of the 6495D LC/TQ system, with robust sample preparation techniques and optimized chromatographic conditions, helps ensure high accuracy, specificity, and reproducibility. This method demonstrates exceptional linearity within a concentration range of 10 to 1,000 pg/mL, which is the equivalent of 1 to 100 ppb for an active pharmaceutical ingredient (API) load of 10 mg. Furthermore, the coefficient of regression (R^2) values exceed 0.999, and the method achieves a limit of detection (LOD) of 5 pg/mL and limit of quantification (LOQ) of 10 pg/mL. Recovery is between 80 and 120% with relative standard deviations (RSDs) below 5.0% at LOD, LOQ, and 100 pg/mL. Signal-to-noise ratios (S/N) meet or exceed the specified limits. This work underscores the critical role of LC/MS/MS-based methods in ensuring compliance with updated nitrosamine guidelines, safeguarding drug safety, and supporting pharmaceutical manufacturers in meeting global regulatory standards.

Introduction

Nitrosamines are a class of chemical compounds characterized by the presence of a nitroso group (–NO) bonded to an amine group. These compounds are classified as probable mutagenic (Group 2A) by the International Agency for Research on Cancer (IARC). This classification is based on strong evidence from animal studies and limited evidence from human data linking nitrosamines to mutagenicity.[1] Nitrosamines can form during manufacturing processes, storage, or even through chemical reactions involving excipients and active pharmaceutical ingredients (APIs). Their presence in pharmaceutical products has raised significant patient safety concerns, prompting regulatory agencies to establish stringent limits to mitigate exposure.

Regulatory bodies, including the U.S. Food and Drug Administration (FDA) and the European Medicines Agency (EMA), have implemented strict guidance on nitrosamine impurities in pharmaceuticals. Acceptable intake (AI) limits, expressed in nanograms per day, have been established for specific nitrosamines such as N-Nitrosodimethylamine (NDMA) and N-Nitrosodiethylamine (NDEA). These limits are based on a lifetime mutagenic risk threshold of 1 in 100,000, ensuring that exposure remains minimal and within acceptable safety margins. For instance, the FDA's AI limit for NDMA is 96 ng per day, while for NDEA it is 26.5 ng per day. These thresholds are critical for protecting patient safety, as even trace levels of nitrosamines in long-term drug consumption could pose significant risks.[2] In September 2024, the FDA issued a revised guidance titled "Control of Nitrosamine Impurities in Human Drugs," which includes updated AI limits for various nitrosamine impurities. This guidance provides drug manufacturers with a framework for risk-based safety assessments of nitrosamines that may be present in both approved and marketed formulations. Establishing and updating AI limits for nitrosamines is crucial for ensuring patient safety. By adhering to these limits, manufacturers can minimize the risk of long-term exposure to potentially carcinogenic impurities, thereby safeguarding health.[3]

The concept of interim limits has also been introduced to address immediate concerns while manufacturers develop more robust methods for detecting and controlling nitrosamine impurities. Interim limits are higher than AI limits and are applied during the transition period, allowing companies time to reformulate or optimize manufacturing processes. However, the goal remains to bring nitrosamine levels below AI thresholds as quickly as possible. These interim measures balance the need for immediate access to essential medications with the imperative of minimizing carcinogenic risk.[4]

The FDA guidance recommends that manufacturers use validated LC/MS methods, implementing robust LC/MS analytical procedures to confirm the presence and concentration of nitrosamines, ensuring that they remain within the acceptable intake limits.[4] In this application note, quantification of targeted impurities was done using multiple reaction monitoring (MRM) mode. MRM mode selectively filters the precursor and product ions of the compound of interest, thus increasing the sensitivity and specificity of the analysis.

The atmospheric pressure chemical ionization (APCI) source used in this application works for ionizing the low-mass compounds. APCI, a gas phase ionization technique, is preferred for ionizing the volatile nitrosamine compounds.[5] APCI helps to increase the sensitivity of the instrument, reduce the contamination of the interface region, and reach lower detection limits with consistent results over multiple long batches. This method allows manufacturers to accurately monitor nitrosamine levels, ensuring compliance with regulatory standards and maintaining the safety and efficacy of their formulations.

Experimental

Chemicals and reagents

All nitrosamine standards used in this study (Table 1) were procured locally from pharmaceutical companies in India. LC/MS-grade solvents (methanol, water) were purchased from Biosolve (Valkenswaard, Netherlands) and formic acid was purchased from Sigma-Aldrich (Kansas, USA).

Table 1. All nitrosamine standards used in this study.

Nitrosamine Standards	
NDMA	N-Nitrosodimethylamine
NMOR	N-Nitrosomorpholine
NMBA	N-Nitrosomethylbutylamine
NPYR	N-Nitrosopyrrolidine
NTHP	N-Nitrosotetrahydropyridine
NDEA	N-Nitrosodiethylamine
NEIPA	N-Nitrosoethylisopropylamine
NDIPA	N-Nitrosodiisopropylamine
NMPA	N-Nitrosomethylphenylamine
NMPEA	N-Nitrosomethylphenethylamine
NDBA	N-Nitrosodibutylamine

Instrument configuration

Configurations and parameters used for the LC are displayed in Table 2. Triple quadrupole MS parameters are shown in Table 3. Compound-specific parameters are shown in Table 5.

Table 2. LC configurations and parameters.

Parameter	Value
Instrument	Agilent 1290 Infinity II High Speed Pump (G7120A) Agilent 1290 Infinity II Multisampler (G7167B) Agilent 1290 Infinity II Multicolumn Thermostat (G7116B) Agilent 1290 Infinity II Variable Wavelength Detector (G7114B) Agilent 6495D Triple Quadrupole system (G6495D)
Needle Wash	S1: MeOH:water (80:20) S2: Water:MeOH (80:20)
Sample Diluent	Water:MeOH (95:5 v:v)
Multisampler Temperature	10 °C
Injection Volume	50 µL
Analytical Column	Agilent Infinity Lab Poroshell Phenylhexyl, 150 × 3.0 mm, 2.7 µm (p/n 693975-912 T)
Column Temperature	40 °C
Mobile Phase A	0.1% formic acid in water
Mobile Phase B	0.1% formic acid in water
Flow Rate	0.4 mL/min
Run Time	22 min
Gradient	Time (min) %A %B 0 100 0 4 100 0 7 70 30 12 45 55 15 10 90 19 10 90 19.1 100 0 22 100 0

Table 3. Triple quadrupole APCI source parameters.

Sr. No.	Parameter	Positive Value
1	Gas Temperature (°C)	250
2	Gas Flow (L/min)	11
3	Nebulizer (psi)	25
4	Capillary Voltage (V)	2,000
5	Corona Current (µA)	8
6	Vaporizer (°C)	350

Table 4. Diverter valve program settings.

Sr. No.	Start Time (min)	Timetable Value	Timetable Type
1	0	To waste	Diverter valve
2	3	To MS	Diverter valve
3	20	To waste	Diverter valve

Table 5. Compound-related parameters in dMRM mode.

Sr. No.	Compound Name	Precursor (*m/z*)	Product (*m/z*)	RT (min)	RT Window (min)	Fragmentor (V)	CE (V)	CAV (V)	iFunnel Mode	Polarity
1	NDMA	75.1	58.1	3.91	1	166	12	4	Standard	+
	NDMA	75.1	43.2	3.91	1	166	16	4	Standard	+
2	NMOR	117.1	87	7.23	1	166	11	4	Standard	+
	NMOR	117.1	45	7.23	1	166	21	4	Standard	+
3	NMBA	147.1	117	8.39	1	166	2	4	Standard	+
	NMBA	147.1	44	8.39	1	166	10	4	Standard	+
4	NPYR	101.1	55	8.46	1	166	16	4	Standard	+
	NPYR	101.1	41	8.46	1	166	28	4	Standard	+
5	NDEA	103.1	75	10.18	1	166	8	4	Standard	+
	NDEA	103.1	47	10.18	1	166	15	4	Standard	+
6	NTHP	113.1	65	10.76	1	166	24	4	Standard	+
	NTHP	113.1	41	10.76	1	166	12	4	Standard	+
7	NEIPA	117.1	75	11.94	1	166	8	4	Standard	+
	NEIPA	117.1	47	11.94	1	166	16	4	Standard	+
8	NDIPA	131.2	89	13.6	1	166	8	4	Standard	+
	NDIPA	131.2	43	13.6	1	166	12	4	Standard	+
9	NMPA	137.1	107	14.72	1	166	8	4	Standard	+
	NMPA	137.1	66	14.72	1	166	20	4	Standard	+
10	NMPEA	165.2	105	15.11	1	166	8	4	Standard	+
	NMPEA	165.2	79	15.11	1	166	28	4	Standard	+
11	NDBA	159.1	57	16.59	1	166	12	4	Standard	+
	NDBA	159.1	41	16.59	1	166	23	4	Standard	+

Sample preparation

Specification limit: The following calculation was used to determine the specification (spec) limit, where MDD represents the maximum daily dose in milligrams, and AI represents acceptable intake:

Specification limit = AI ÷ MDD

The AI for NDEA is 26.5 ng/day. Considering the MDD of an immediate-release metformin tablet is 1,000 mg, the permissible limit for NDEA was calculated as 0.026 ng/mg (ppm) using the formula:

26.5 ng/day ÷ 1,000 mg/day = 0.0265 ng/mg (ppm)

To ensure sufficient sensitivity, the target limit of quantification (LOQ) was set at 10 times below this limit, equating to 0.00265 ppm (2.65 ppb).

The achieved LOQ for nitrosamines was 10 pg/mL, demonstrating the method's capability to detect impurities at trace levels. The impurity levels in the sample were reported in terms of pg/mg (ppb).

Preparation of working standard solution: Working standards were prepared using the diluent specified in Table 2 to achieve a final concentration range of 10 to 1,000 pg/mL for the calibration plot.

Drug product sample preparation: The appropriate number of Metformin IR tablets was crushed and weighed to the 100 mg equivalent of the metformin API. The sample was then transferred to a 10 mL volumetric flask, and 500 µL of methanol was added; the sample was then sonicated for 15 minutes. LC/MS-grade Millipore Sigma Milli-Q water was added to make up the volume such that the final target concentration was 10 mg/mL. The sample was sonicated again for 15 minutes, followed by 10 minutes of shaking using a shaker. After extraction, the sample was centrifuged for 15 minutes at 4,500 rpm. The supernatant was filtered using a 0.22 µm polyvinylidene fluoride (PVDF) syringe filter, with the first 1 mL discarded. The filtered sample was transferred into an HPLC vial for LC/MS analysis.

Recovery sample preparation: Impurity standard solutions, at concentrations of 10 and 100 pg/mL, were spiked into the samples, following the same extraction protocol specified for drug product sample preparation. The supernatant was filtered using a 0.22 µm PVDF syringe filter, with the first 1 mL discarded. The filtered sample was transferred into an HPLC vial for LC/MS analysis.

Data acquisition and data analysis

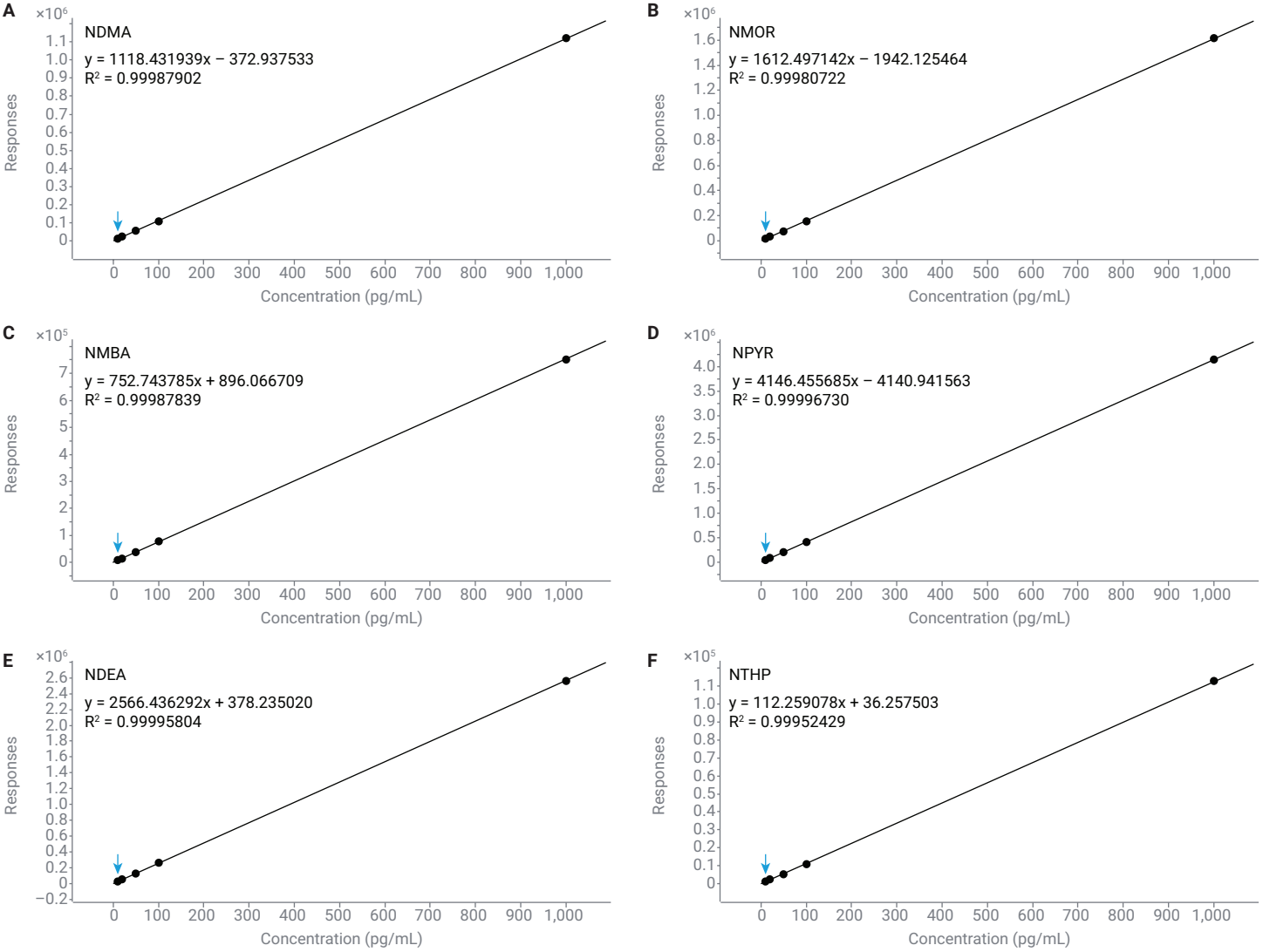
All samples were acquired using the Agilent MassHunter acquisition software for GC/MS systems (version 12.0). MRM transitions and compound parameters were optimized using the MassHunter Optimizer tool integrated within the MassHunter acquisition software for GC/MS systems. A 100 ng/mL standard solution was introduced into the MS using Flow Injection Analysis (FIA) mode, employing an injection volume of 5 µL. Method development used the positive ionization mode with an APCI ionization source, optimizing compound parameters such as precursor ions, product ions, and collision energies for all 11 nitrosamines.

Through the automated workflow, two product ions with the highest intensities were selected for MRM method development. Parameters such as gas temperatures, gas flows, and dwell times were fine-tuned to achieve optimal sensitivity and specificity. Instrument MRM parameters (Table 5) and source settings (Table 3) were further optimized to enhance sensitivity while ensuring method robustness for large batch analyses. Chromatograms were analyzed using Agilent MassHunter Qualitative Analysis software, while batch quantitation was performed using Agilent MassHunter Quantitative Analysis software (version 12.0).

Results and discussion

Metformin and NDMA separation were critical. Therefore, the method development process involved extensive evaluation of various chromatographic columns and gradient conditions to optimize the separation between metformin and NDMA. This optimization was crucial to minimizing matrix effects caused by the API, which can interfere with the accurate quantification of targeted nitrosamine impurities. The inbuilt diverter valve program was used to avoid the entry of a large amount of API into the MS, eliminating the chances of contamination, as described in Table 4. The MS/MS instrument parameters were rigorously optimized to maximize sensitivity and ensure robust detection of the nitrosamine impurities at trace levels.

Key performance characteristics of the method were systematically characterized, including linearity, which demonstrates a proportional response over a wide concentration range for all nitrosamine impurities specified. Linear regression (weighting factor 1/x) was applied (Figure 1). The regression coefficient (R^2) was > 0.999, which shows a linear response throughout the concentration range of 10 to 1,000 pg/mL (1.0 to 100 ppb with respect to a drug load of 10 mg).



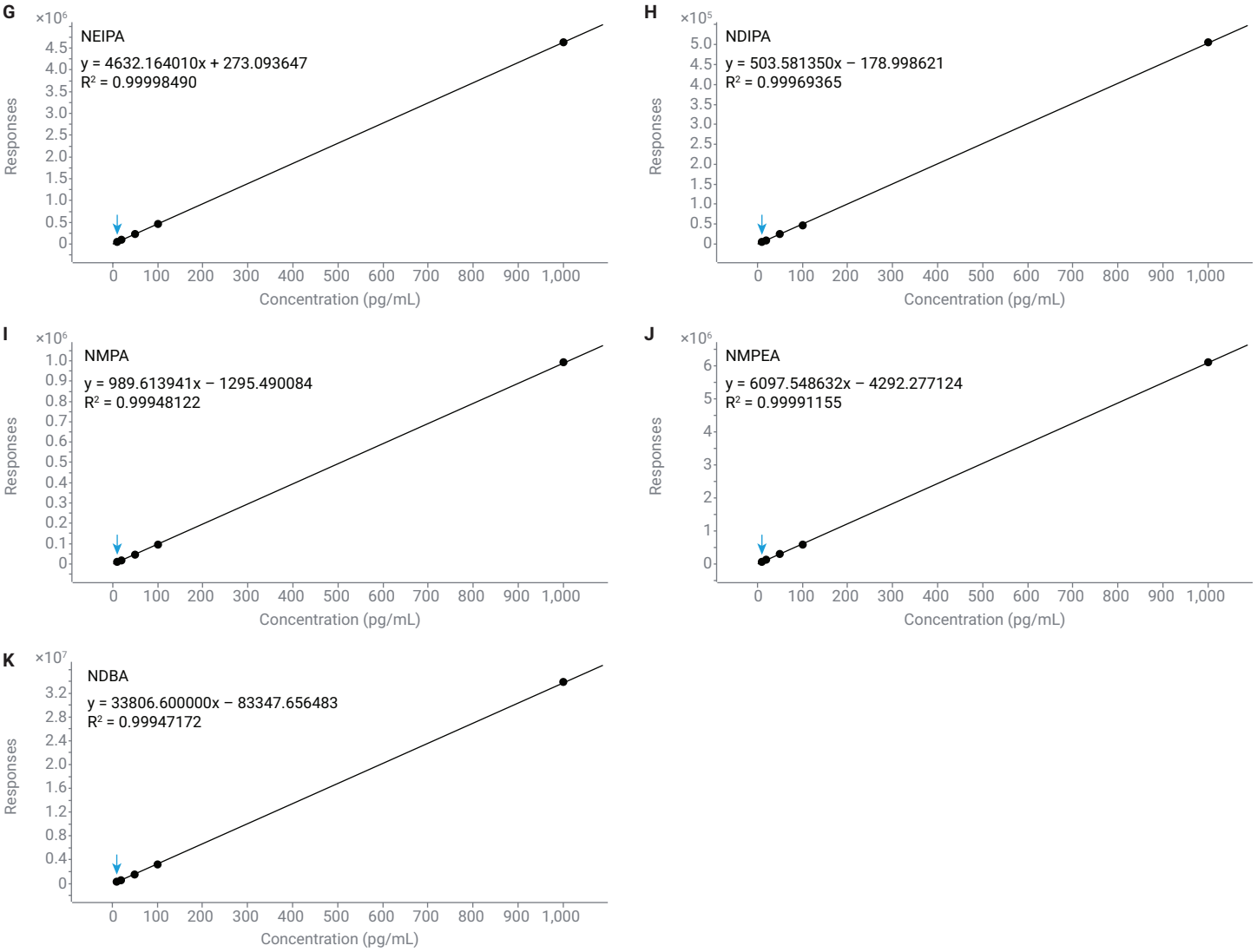


Figure 1. Linear regression plots (weighting factor 1/x) for (A) NDMA, (B) NMOR, (C) NMBA, (D) NPYR, (E) NDEA, (F) NTHP, (G) NEIPA, (H) NDIPA, (I) NMPA, (J) NMPEA, and (K) NDBA.

The S/N was calculated at the LOD and LOQ level using a root mean square (RMS) algorithm. The noise reference was taken from the standard chromatogram, as shown in Figures 2 and 3 (LOD) and Figures 4 and 5 (LOQ).

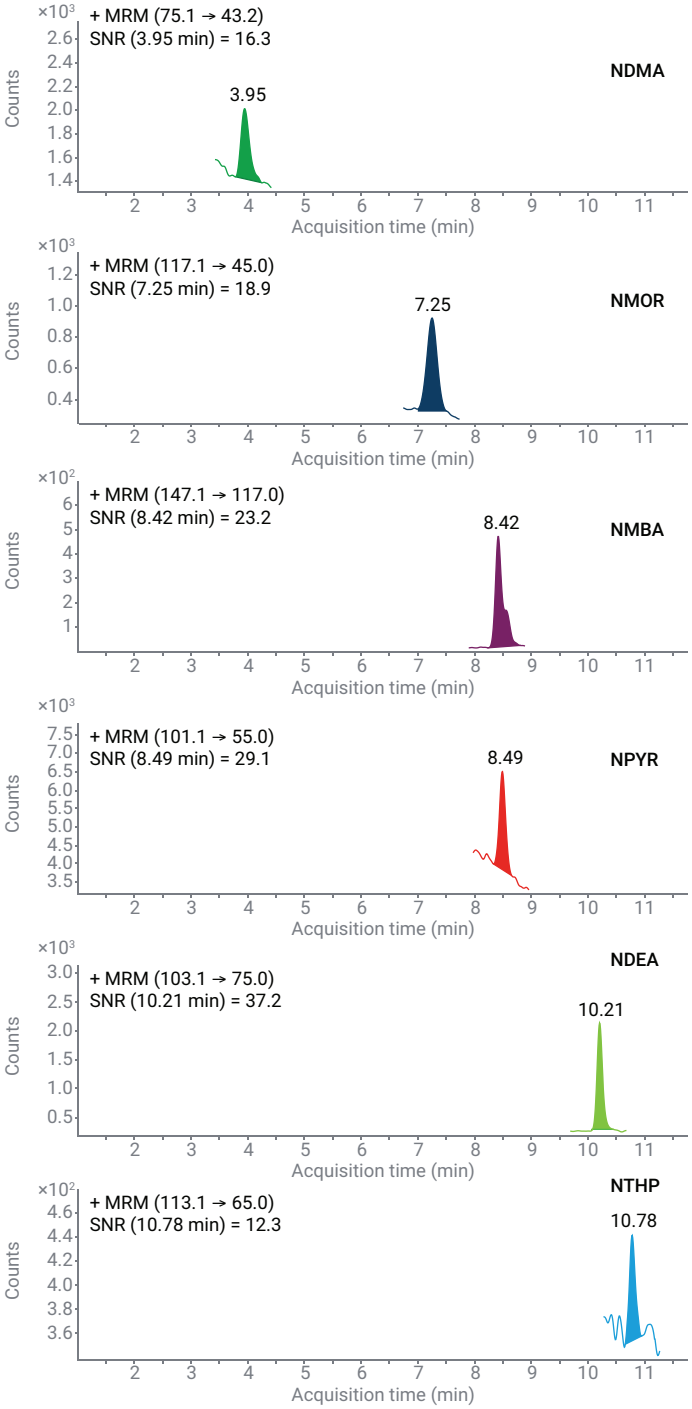


Figure 2. LOD chromatograms for NDMA, NMOR, NMBA, NPYR, NDEA, and NTHP.

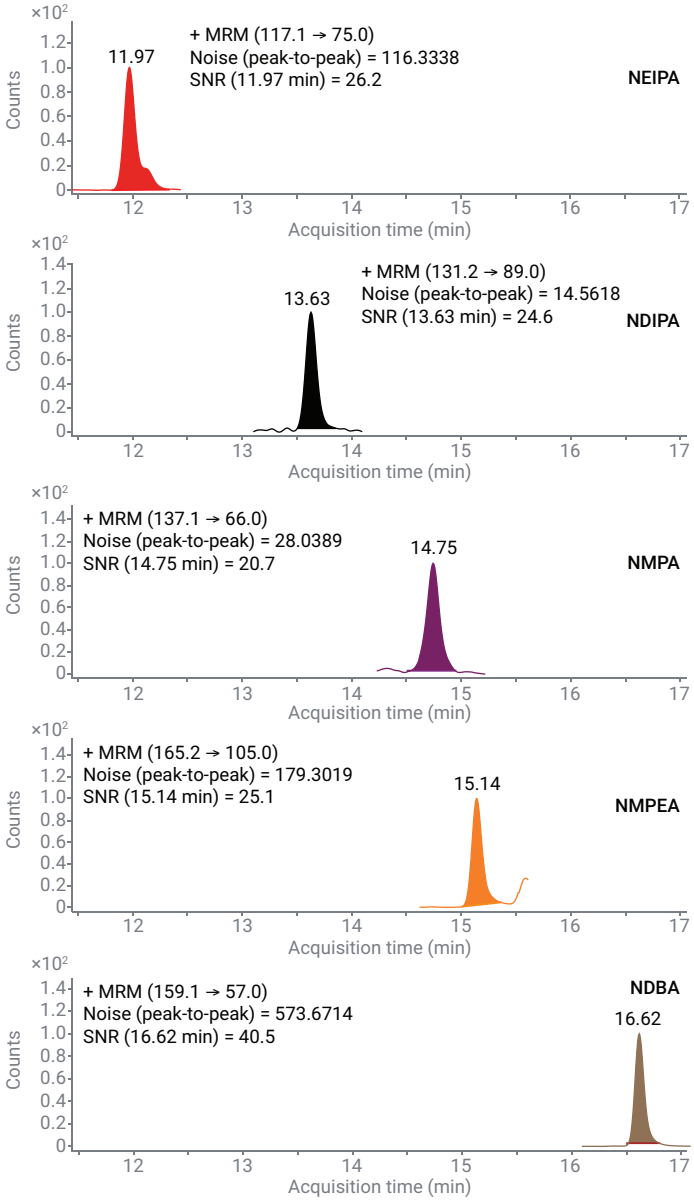


Figure 3. LOD chromatograms for NEIPA, NDIPA, NMPA, NMPEA, and NDBA.

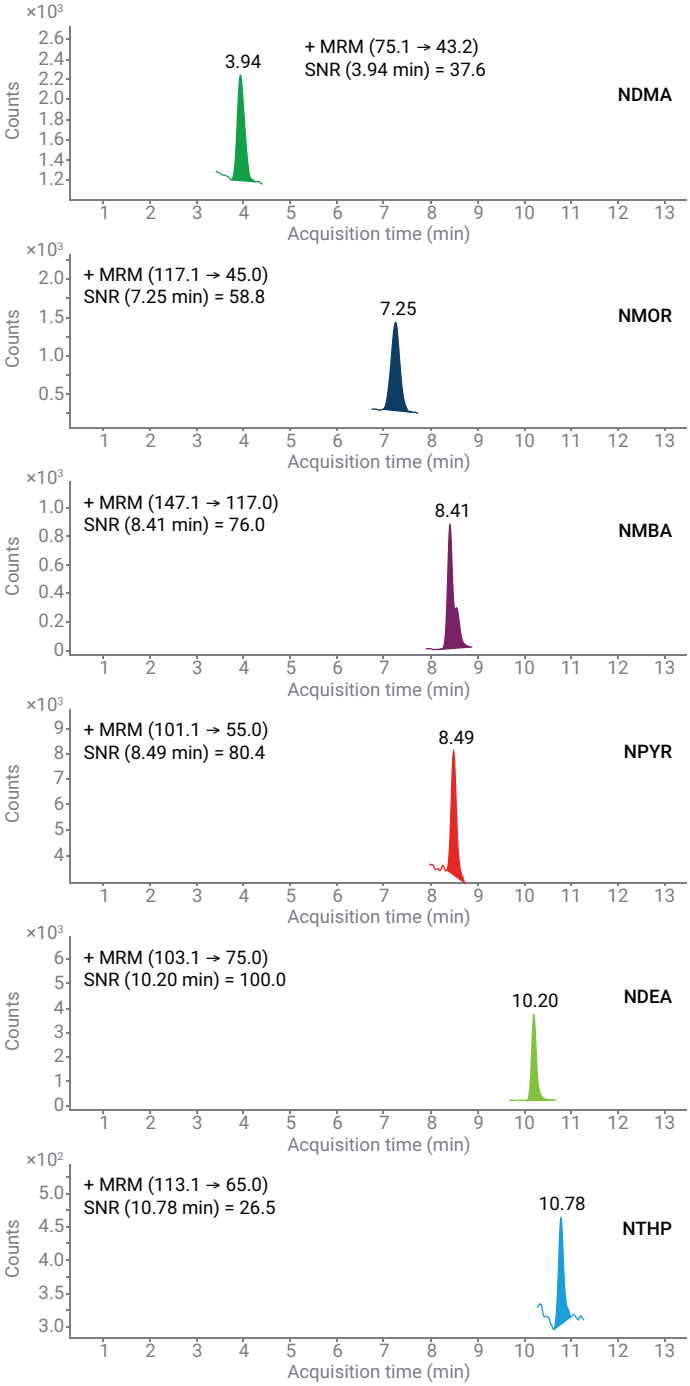


Figure 4. LOQ chromatograms for NDMA, NMOR, NMBA, NPYR, NDEAs, and NTHP.

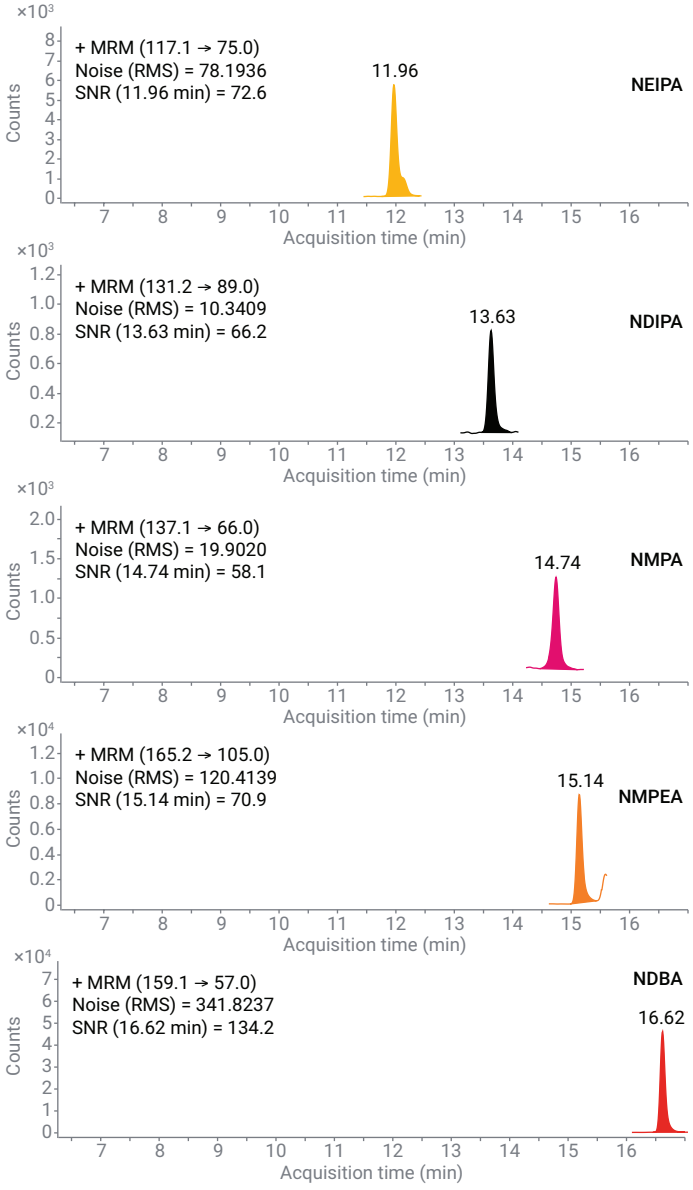


Figure 5. LOQ chromatograms for NEIPA, NDIPA, NMPA, NMPEA, and NDBA.

Table 6 shows that sensitivity was achieved with calibration plot data, a limit of detection (LOD) of 5 pg/mL (S/N > 10:1), and an LOQ of 10 pg/mL (S/N > 20:1).

Figure 6 highlights the selectivity and chromatographic separation between metformin API and all 11 nitrosamine impurities with optimized column, gradient, and LC method conditions.

Table 6. Result summary data includes signal-to-noise (S/N), calculated LOD/ LOQ, coefficient of regression, and calibration curve fit. All standards used 1/x weighted calibration curve.

Compound	LOD		LOD (S/N)	LOQ		LOQ (S/N)		Linearity Range	
	pg/mL	*ppb		pg/mL	ppb		R^2	pg/mL	ppb
NDMA	5	0.5	16.3	10	1	37.6	0.9999	10 to 1,000	1 to 100
NMOR	5	0.5	18.9	10	1	58.8	0.9998	10 to 1,000	1 to 100
NMBA	5	0.5	23.2	10	1	76.0	0.9998	10 to 1,000	1 to 100
NPYR	5	0.5	29.1	10	1	80.0	0.9999	10 to 1,000	1 to 100
NDEA	5	0.5	37.2	10	1	26.5	0.9999	10 to 1,000	1 to 100
NTHP	5	0.5	12.3	10	1	100.0	0.9995	10 to 1,000	1 to 100
NEIPA	5	0.5	26.2	10	1	72.6	0.9999	10 to 1,000	1 to 100
NDIPA	5	0.5	24.6	10	1	66.2	0.9996	10 to 1,000	1 to 100
NMPA	5	0.5	20.7	10	1	58.1	0.9994	10 to 1,000	1 to 100
NMPEA	5	0.5	25.1	10	1	70.9	0.9999	10 to 1,000	1 to 100
NDBA	5	0.5	40.5	10	1	134.2	0.9994	10 to 1,000	1 to 100

S/N was calculated with the RMS algorithm, using Agilent MassHunter Qualitative Analysis 12 software.
* ppb concentration is the actual concentration with respect to a test concentration of 10 mg/mL.

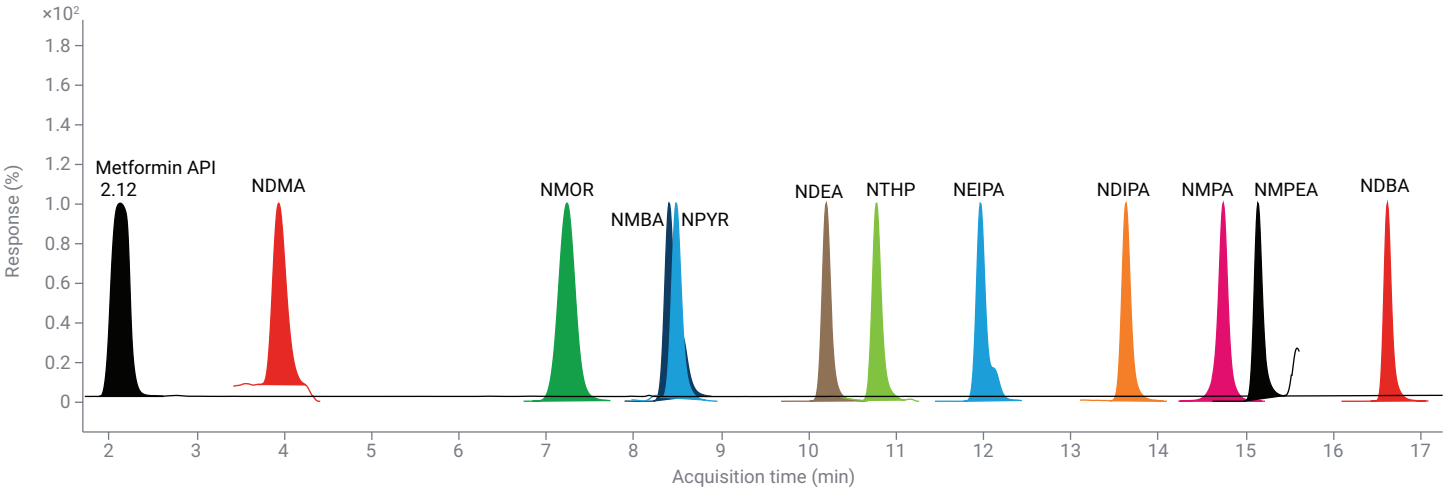


Figure 6. Overlapping normalized chromatograms of metformin API and 11 nitrosamine impurities.

The repeatability experiment was performed, as illustrated in Table 7, at three different concentration levels with six replicates in an aqueous standard. Results show an LOD of 5 pg/mL, an LOQ of 10 pg/mL, an STD-05 of 100 pg/mL (six replicates), and relative standard deviations (RSDs) of < 5% (Figure 7).

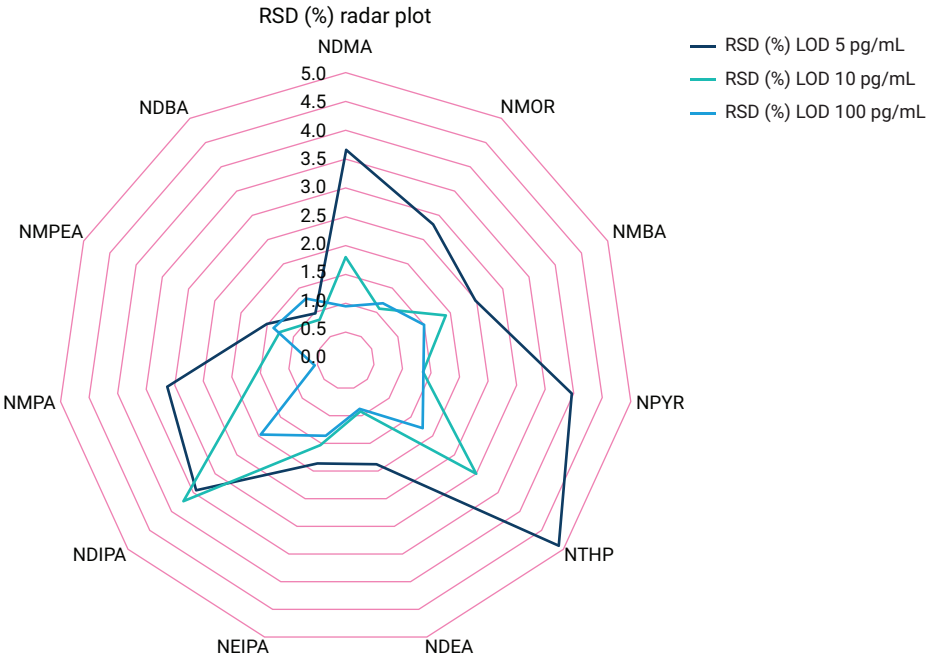


Figure 7. Radar plot representing the reproducibility at 10 pg/mL for 11 nitrosamine impurities.

Table 7. Representative data for reproducibility of the method at 10 pg/mL.

Replicates	NDMA	NMOR	NMBA	NPYR	NDEA	NTHP	NEIPA	NDIPA	NMPA	NMPEA	NDBA
R1	10,210	13,399	6,667	33,087	21,585	940	37,585	4,245	9,297	48,801	260,985
R2	10,071	13,663	6,408	33,708	21,691	998	36,493	3,902	9,166	49,156	257,668
R3	10,507	13,823	6,540	33,510	21,186	1,023	36,528	3,856	9,415	50,084	256,022
R4	10,452	13,559	6,710	33,314	21,276	1,014	37,216	3,980	9,009	49,353	257,004
R5	10,220	13,681	6,419	34,371	21,500	1,011	36,064	4,018	9,333	48,322	255,531
R6	10,074	13,541	6,505	33,307	21,346	1,006	36,547	3,848	9,223	48,742	255,085
Mean	10,256	13,611	6,542	33,549.5	21,431	999	36,738.83	3,974.83	9,240.50	49,076.33	257,049.17
SD	185.57	144.98	125.14	453.81	193.57	30.00	555.12	148.59	142.58	609.08	2,149.12
RSD (%)	**1.81**	**1.07**	**1.91**	**1.35**	**0.90**	**3.00**	**1.51**	**3.74**	**1.54**	**1.24**	**0.84**

Table 8. Representative data for reproducibility of the method at 100 pg/mL.

Replicates	NDMA	NMOR	NMBA	NPYR	NDEA	NTHP	NEIPA	NDIPA	NMPA	NMPEA	NDBA
R1	98,130	143,737	65,487	367,514	224,451	9,935	390,898	41,090	86,780	524,791	2,905,818
R2	98,259	143,990	65,719	377,714	228,878	9,781	381,016	40,085	86,273	526,283	2,843,270
R3	97,858	139,443	63,593	375,787	225,460	9,871	384,377	39,535	85,584	518,949	2,939,481
R4	97,016	141,836	63,731	372,193	223,950	9,849	381,357	39,790	86,038	521,002	2,910,840
R5	95,866	141,332	63,551	368,391	224,313	9,754	376,239	39,407	86,802	518,708	2,890,150
R6	96,878	142,477	63,970	364,764	223,643	9,445	378,064	38,759	86,368	506,066	2,851,147
Mean	97,335	142,136	64,342	371,061	225,116	9,773	381,992	39,778	86,308	519,300	2,890,117
SD	919.55	1,679.61	990.51	5,044.63	1,943.57	172.99	5,193.98	781.13	462.32	7,175.27	36,948.82
RSD (%)	**0.94**	**1.18**	**1.54**	**1.36**	**0.86**	**1.77**	**1.36**	**1.96**	**0.54**	**1.38**	**1.28**

Due to the presence of both quantifier and qualifier ions with an ion ratio of ± 20%, MRM-based selectivity was introduced, and confirmatory analysis was performed (Figure 8).

Note: The response in the system may vary depending on external conditions. However, RSD will remain consistent if sensitivity, in terms of S/N, and chromatography reproducibility are maintained.

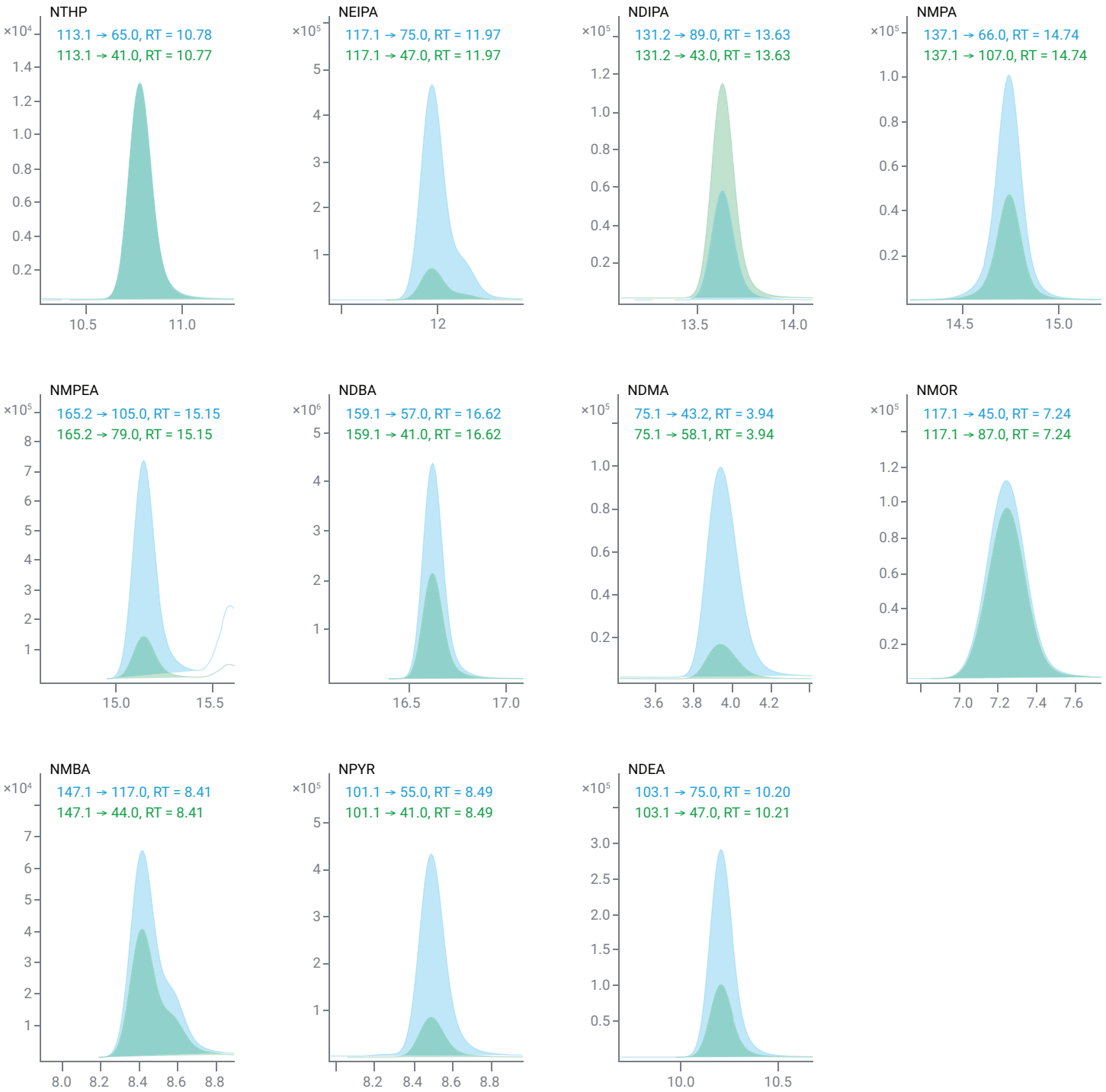


Figure 8. Chromatograms representing the qualifier ion and quantifier ion for all 11 nitrosamine impurities.

Accuracy and specificity were evaluated by performing recovery tests, ensuring clear resolution of each impurity in the standard mix. The chromatogram and acquisition data show no interference from other matrix components in the spiked sample chromatogram (Figures 9 and 10). The recovery was observed to be optimal, ranging from 80 to 120%.

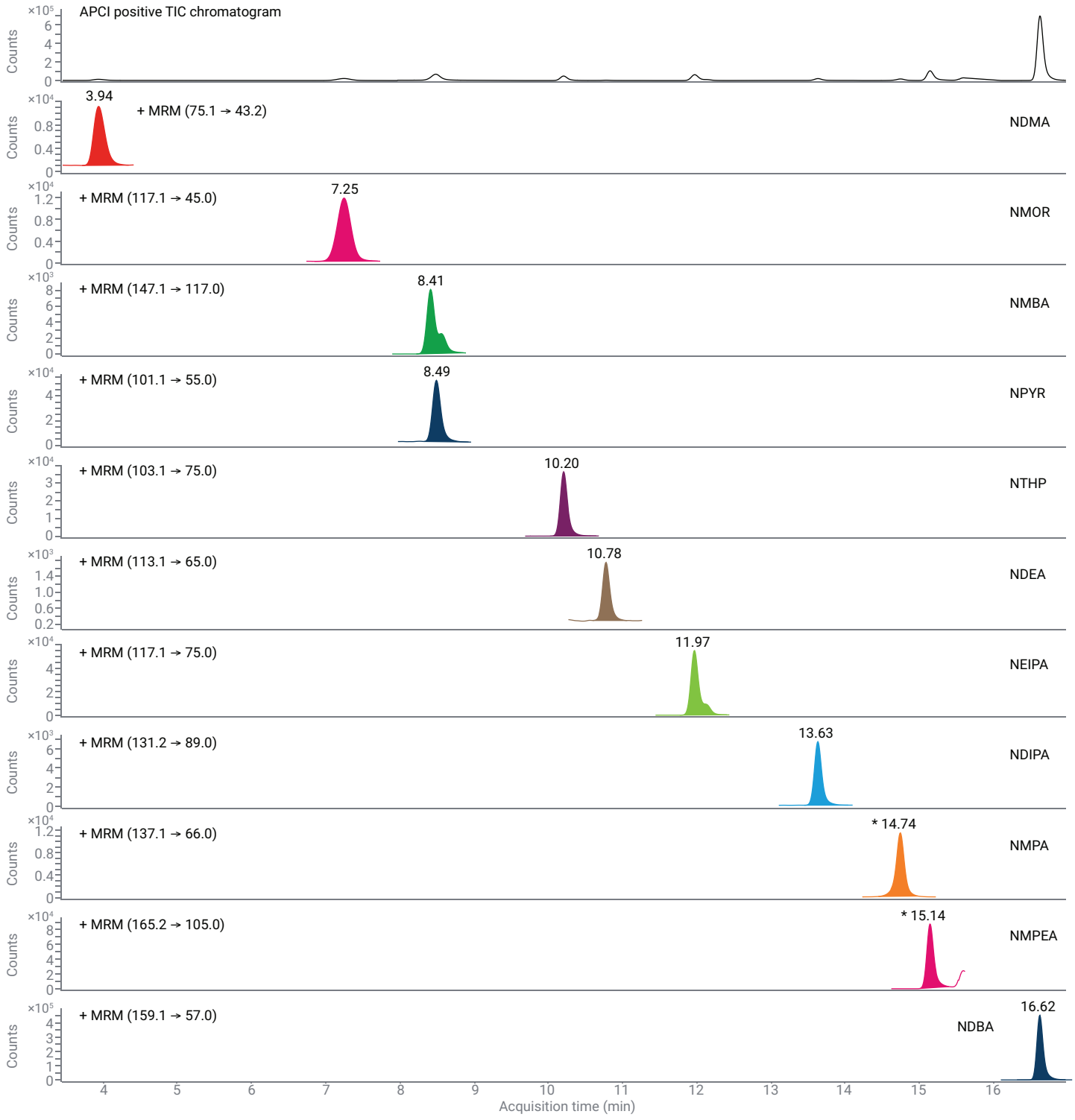


Figure 9. Chromatogram representing the recovery at LOQ level (100 pg/mL) spiked into metformin drug product.

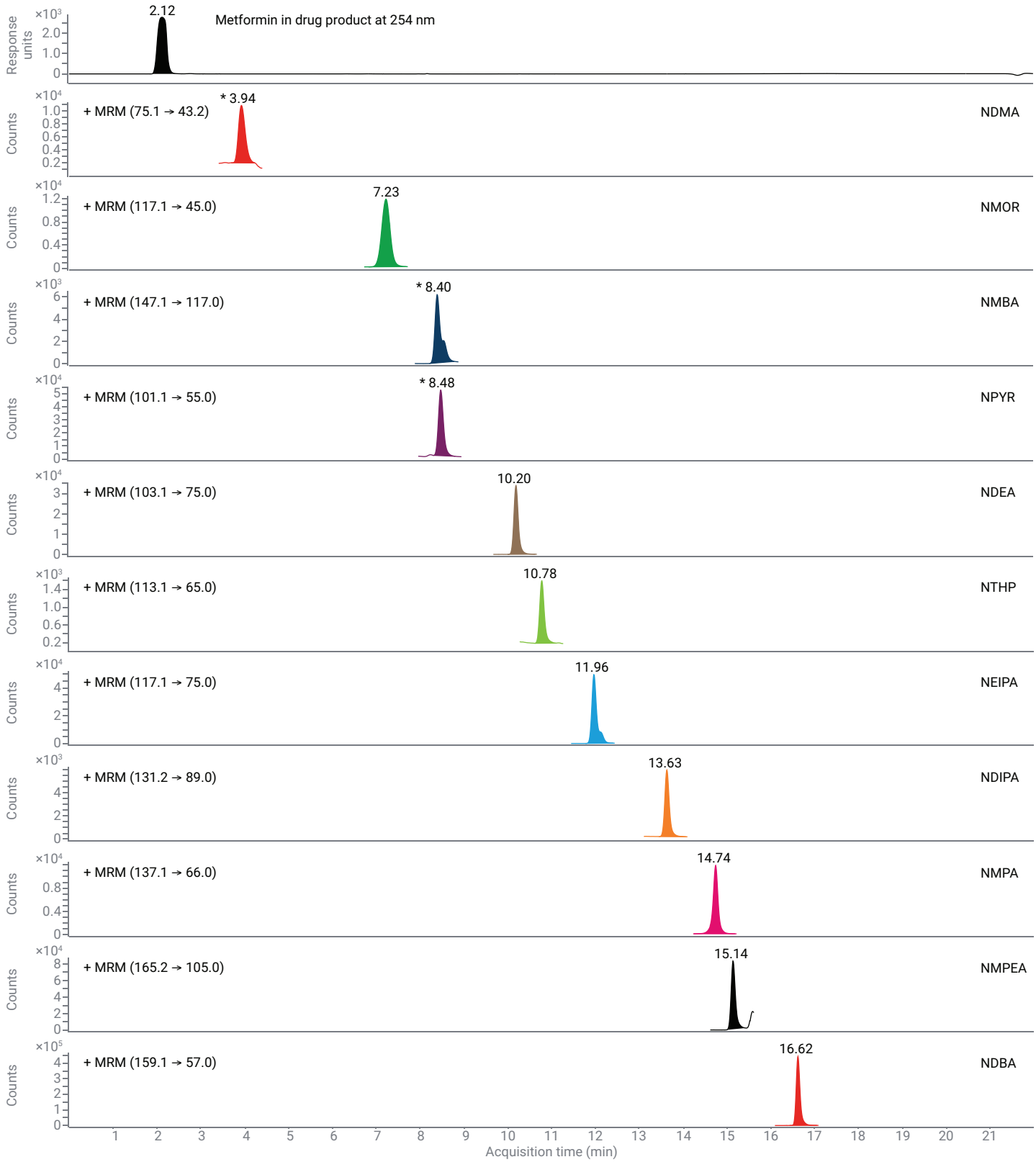


Figure 10. Chromatogram representing the recovery at standard level (100 pg/mL) spiked into metformin drug product.

Note:

1. The recovery experiment was performed with triplicate injections.
2. Since the Agilent 6495D LC/TQ is capable of very low limits of detection and estimation, a sample concentration of 10 mg/mL of metformin in formulation used.

Recovery was calculated using Equation 1.

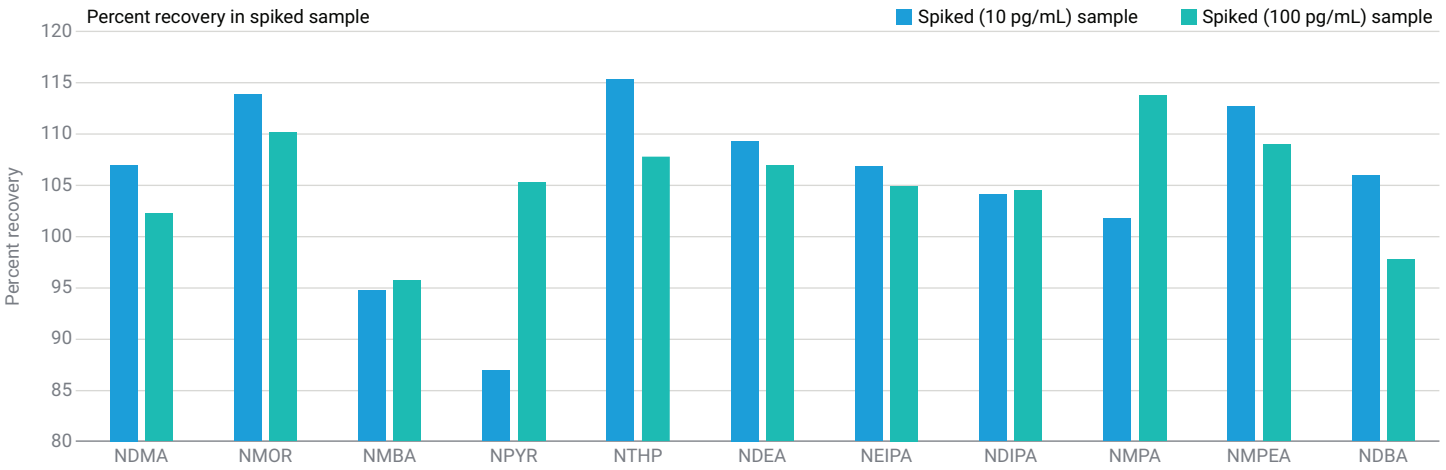
Method accuracy and specificity were evaluated by performing recovery testing at the LOQ level using two different preparations (n = 2) in a sample matrix, with each preparation injected in triplicate. Each impurity exhibited a recovery between 80 and 120%, as shown in Figure 11.

Table 9. Summary of recovery experiments in metformin drug product.

Nitrosamine Impurities	10 pg/mL of Standard Stock Spiked in Metformin Drug Product for Sample Size of 10 mg/mL (1 ppb)	100 pg/mL of Standard Stock Spiked in Metformin Drug Product for Sample Size of 10 mg/mL (10 ppb)
NDMA	106.99	102.30
NMOR	113.87	110.16
NMBA	94.92	95.61
NPYR	86.94	105.39
NDEA	109.34	106.97
NTHP	115.38	107.83
NEIPA	106.86	105.07
NDIPA	104.10	104.51
NMPA	101.75	113.75
NMPEA	112.70	109.01
NDBA	106.02	97.77

$$\text{Recovery \%} = \frac{\text{Response in spiked sample} - \text{Response in control sample}}{\text{Response in prepared standard}} \times 100$$

Equation 1.



Nitrosamine Impurities	NDMA	NMOR	NMBA	NPYR	NTHP	NDEA	NEIPA	NDIPA	NMPA	NMPEA	NDBA
Spiked (10 pg/mL) Sample	106.99	113.87	94.92	86.94	115.38	109.34	106.86	104.10	101.75	112.70	106.02
Spiked (100 pg/mL) Sample	102.30	110.16	95.61	105.39	107.83	106.97	105.07	104.51	113.75	109.01	97.77

Figure 11. Percent recovery calculated in sample matrix (drug product) spiked at 10 and 100 pg/mL.

Conclusion

The analytical method described in this application note enables the simultaneous quantification of 11 nitrosamine impurities at trace levels. The advanced sample preparation techniques and optimized column chemistries meet stringent regulatory requirements, ensuring high precision and reliability. Results show that this method achieves a remarkable limit of detection (LOD) of 5 pg/mL with a signal-to-noise ratio (S/N) of 10, and a limit of quantification (LOQ) of 10 pg/mL with an S/N of 20. Recovery studies confirm the method's accuracy, with recovery rates between 80 and 120%, demonstrating robustness in quantifying trace impurities in metformin formulations. Reproducibility is also demonstrated by RSD values consistently ≤ 5% at the LOD, LOQ, and 100 pg/mL levels.

Integrated with an Agilent 6495D LC/TQ system, automated workflows optimize method performance and enhance throughput, streamlining impurity screening processes. The method can be validated for diverse pharmaceutical formulations, and with slight tweaks, can expand its applicability across various drug delivery systems and product types. The high sensitivity, precision, and adaptability of this method make it an invaluable tool for supporting diverse analytical needs in pharmaceutical quality control.

References

1. Kruhlak, N.; Schmidt, M.; Frötschl, R.; Graber, S.; Haas, B.; Horne, I.; Horne, S.; King, S.; Koval, I.; Kumaran, G.; *et al.* Determining Recommended Acceptable Intake Limits for N-Nitrosamine Impurities in Pharmaceuticals: Development and Application of the Carcinogenic Potency Categorization Approach (CPCA). *Regul. Toxicol. Pharmacol*. **2024**, *150*, 105640. DOI: 10.1016/j.yrtph.2024.105640.
2. U.S. Department of Health and Human Services Food and Drug Administration Center for Drug Evaluation and Research (CDER). Recommended Acceptable Intake Limits for Nitrosamine Drug Substance-Related Impurities (NDSRIs) Guidance for Industry. *Pharmacology/Toxicology* **2023**. [Online]: https://www.fda.gov/media/170794/download.
3. European Medicines Agency (EMA). Questions and Answers for Marketing Authorization Holders/Applicants on the CHMP Opinion for the Article 5(3) of Regulation (EC) No 726/2004 Referral on Nitrosamine Impurities in Human Medicinal Products. EMA/409815/2020 **2020**.
4. U.S. Food and Drug Administration (FDA). Guidance for Industry for Control of Nitrosamine Impurities in Human Drugs. FDA **2024**. [Online]: https://www.fda.gov/regulatory-information/search-fda-guidance-documents/control-nitrosamine-impurities-human-drugs.
5. United States Pharmacopeia (USP). Quantitative Analysis of Nitrosamine Impurities by LC-MS Methods from the United States Pharmacopeia General Chapter. USP General Chapter 2024.
6. International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH). Q2(R2) Validation of Analytical Procedures. ICH **2023**. [Online]: https://database.ich.org/sites/default/files/ICH_Q2%28R2%29_Guideline_2023_1130.pdf.
7. U.S. Food and Drug Administration (FDA). Development and Validation of a RapidFire-MS/MS Method for Screening of Nitrosamine Carcinogen Impurities N-Nitrosodimethylamine (NDMA), N-Nitrosodiethylamine (NDEA), N-Nitrosoethylisopropylamine (NEIPA), N-Nitrosodiisopropylamine (NDIPA), N-Nitrosodibutylamine (NDBA) and N-Nitroso-N-methyl-4-Aminobutyric Acid (NMBA) in ARB Drugs. FDA **2019**. [Online]: https://www.fda.gov/media/125477/download.
8. U.S. Food and Drug Administration (FDA). Liquid Chromatography-High Resolution Mass Spectrometry (LC-HRMS) Method for the Determination of NDMA in Ranitidine Drug Substance and Drug Product. FDA **2019**. [Online]: https://www.fda.gov/media/130801/download.
9. U.S. Food and Drug Administration (FDA). Liquid Chromatography-High Resolution Mass Spectrometry (LC-HRMS) Method for the Determination of Six Nitrosamine Impurities in ARB Drugs. FDA **2019**. [Online]: https://www.fda.gov/media/125478/download.

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