Simultaneous quantitation of NDBA and multi-class nitrosamines in IV bag leachables using LC-MS/MS

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This technical note demonstrates a method for the quantitation of N-nitrosodibutylamine (NDBA) and other nitrosamines in intravenous (IV) packaging material, using the SCIEX Triple Quad 6500+ system. A limit of quantitation (LOQ) of 0.035 ng/mL was achieved for NDBA analysis, enabling confident detection at trace levels (Figure 1).[1,2]

FDA's August 2025 emerging scientific statement highlights the risk of NDBA and other nitrosamines leaching from IV bag packaging into drug products and recommends that manufacturers test their products using sensitive methods with ppb-level detection.[1] Given that acceptable intake (AI) limits for nitrosamines such as NDBA can be as low as 26.5 ng/day,[1] highly sensitive and selective methods are essential to ensure patient safety and regulatory compliance. LC-MS/MS has been widely recognized as the method of choice for nitrosamine analysis, offering the sensitivity, selectivity, and quantitative reliability required for trace-level detection.

Here, the developed method is designed to support low level detection consistent with current regulatory expectations[1,2] while maintaining quantitative fidelity for routine pharmaceutical quality and safety assessments.

Key benefits for NDBA analysis using the SCIEX Triple Quad 6500+ system

- **Low level of quantitation:** Reach a limit of quantitation (LOQ) as low as 0.035 ng/mL for the quantitation of nitrosamines in IV bag filtrate.
- **Robust analytical performance:** Achieve accurate and highly reproducible (%CV <10) quantitative performance at all concentration levels.
- **Streamlined data management:** Easily acquire, manage, and process data using SCIEX OS software, a 21 CFR Part 11-compliant platform.

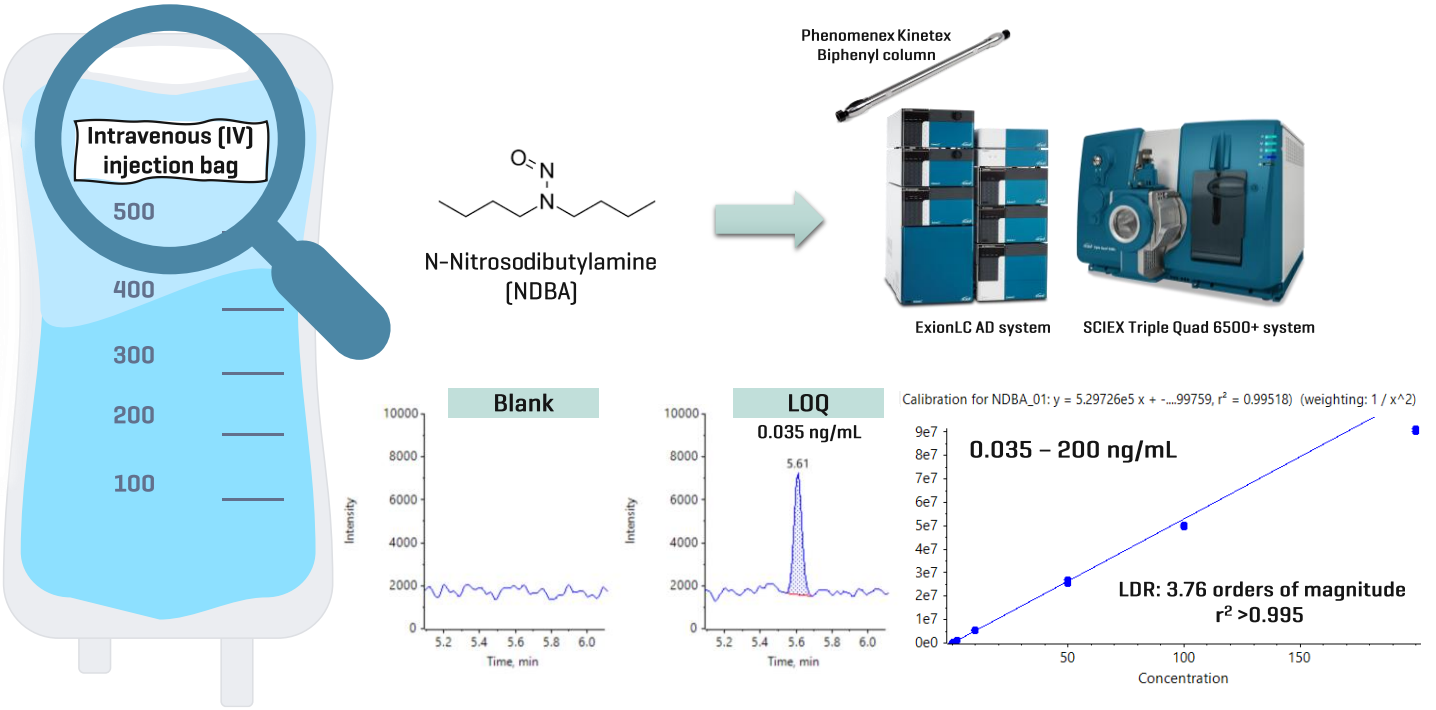

Figure 1. Representative calibration curve and quantitative performance summary for NDBA quantitation workflow on SCIEX Triple Quad 6500+ system. Representative extracted ion chromatograms (XICs) from blank, and LOQ levels were shown. An LOQ of 0.035 ng/mL was achieved for the quantitation of NDBA. No interference was detected in the matrix blank. Linearity was established over 3.76 orders of magnitude, with a coefficient of determination (r^2) value of >0.995.

Introduction

The FDA has recently reported the presence of NDBA in certain drug products stored in IV bags, highlighting the potential for nitrosamine impurities to arise from packaging-related sources.[1] These findings suggest that nitrosamines may form through interactions involving materials such as printed overwraps, adhesives, or packaging components, or migrate into the drug product as leachable contaminants.

Compared to traditionally monitored nitrosamines, NDBA has emerged as a compound of interest due to its potential formation from specific rubber additives, inks, and polymer degradation products used in IV bag systems.[1] As a result, regulatory agencies require comprehensive risk assessment, sensitive analytical testing, and demonstrate sufficient selectivity to confidently quantify NDBA in the presence of complex extractable and leachable matrices.[1,3]

In this study, a panel of 7 nitrosamines (NDBA, NDMA, NDEA, NMBA, NDPA, NDIPA, and NEIPA) was evaluated in IV bag filtrate. Quantitative analysis was performed using the SCIEX Triple Quad 6500+ system, enabling highly sensitive, reproducible, and robust measurement of all 7 nitrosamines.

Methods

Standard preparation: Nitrosamine mix in methanol was purchased from Accustandard (Catalog # FDA-001S). Calibration points ranging from 0.035 to 200 ng/mL were prepared in a 95:5 (v/v) water/methanol mixture.

Sample preparation: A 3 gram piece from the IV bag, including prints such as the warning, brand, and volume indicators, was cut into smaller pieces. The pieces containing prints were extracted in 3 mL of water by sonicating for 1 hour, followed by vortexing for 1 hour. The supernatant was filtered using a 0.22 µm filter. For the recovery experiment, blank and spiked samples (1 ng/mL of nitrosamine mixture) were prepared in IV bag filtrate.

Chromatography: Sample separation was performed using an ExionLC AD system (SCIEX) at a flow rate of 0.6 mL/min on a Phenomenex Kinetex Biphenyl column (2.1 x 100mm, 2.6 µm, 100 Å). The column temperature was maintained at 40°C. A 9-minute gradient was run using 0.1% formic acid in water as mobile phase A and 0.1% formic acid in methanol as mobile phase B (Table 1). An injection volume of 25 µL was used for analysis. A 90:10 (v/v) methanol/water mixture was used as the needle wash solvent.

Table 1. LC gradient conditions.

Time (min)	Mobile phase A (%)	Mobile phase B (%)
0.0	100	0
0.5	100	0
1	90	10
1.5	90	10
1.6	60	40
5	35	65
5.5	35	65
5.6	5	95
7.5	5	95
7.51	100	0
9	100	0

Mass spectrometry: Analysis was performed on the SCIEX Triple Quad 6500+ system. The optimized source and gas parameters are listed in Table 2, and the MRM parameters are discussed in Table 3. For all transitions, the dwell time and EP values were set to 40 msec and 10 V, respectively.

Table 2. Source and gas parameters.

Parameter	Value
Polarity	Positive
Source	APCI
Ion source gas 1	45 psi
Curtain gas	30 psi
Source temperature	375°C
Nebulizer current	1.0 µA
CAD gas	12

Table 3. MRM parameters applied for quantitation.

ID	Precursor ion (m/z)	Fragment ion (m/z)	CE (V)	CXP (V)	DP (V)
NDBA 1	159	103	13	10	45
NDBA 2	159	57	14	10	45
NDMA 1	75.0	58.0	17	6	40
NDMA 2	75.0	43.1	21	7	40
NDEA 1	103	75.1	20	9	40
NDEA 2	103	47.1	22	7	40
NMBA 1	147	117	9	14	40
NMBA 2	147	86.9	15	11	40
NDPA 1	131.1	89.0	12	10	40
NDPA 2	131.1	43.1	20	10	40
NDIPA 1	131.1	89.0	10	10	45
NDIPA 2	131.1	43.1	25	7	45
NEIPA 1	117	75.0	15	12	40
NEIPA 2	117	43.1	19	10	40

Data processing: Data collection and analysis were performed using SCIEX OS software, version 4.0. Peaks were integrated using the MQ4 algorithm, and a weighting of $1/x^2$ was applied for nitrosamine quantitation.

Quantitation of nitrosamines on the SCIEX Triple Quad 6500+ system

The panel includes predominantly dialkyl nitrosamines (NDMA, NDEA, NDBA, NDPA, NDIPA), along with structurally distinct nitrosamines such as NMBA and mixed alkyl nitrosamines like NEIPA. LOQs were determined following Q2(R1) Validation of Analytical Procedures: Text and Methodology Guidance for Industry guidelines.[1,2] Figure 2 shows the XICs of the blank and the LOQ levels for 6 nitrosamines. LOQ of 0.035 ng/mL was reached for NMBA, NDEIPA, and NEIPA, while an LOQ of 0.1 ng/mL was achieved for NDMA, NDPA, and NDEA. The blank XICs shown did not indicate any interferences at the retention time of the nitrosamine analytes.

Additionally, the isobaric nitrosamines, NDPA and NDIPA, were baseline-separated using the Phenomenex Kinetex Biphenyl column with retention times of 4.09 min and 2.39 min, respectively.

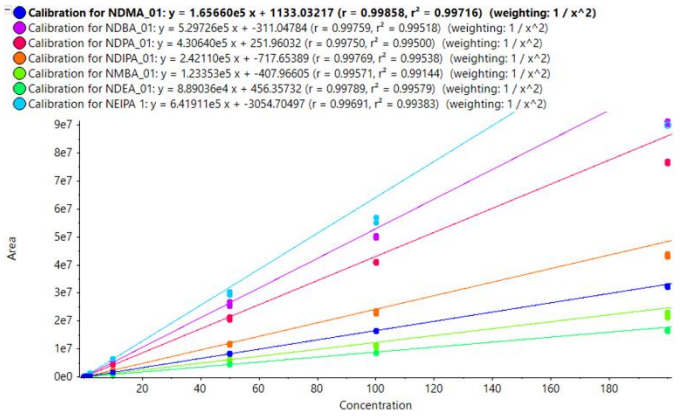

Figure 3. Calibration curves of 7 nitrosamines across a wide range of concentrations using the SCIEX Triple Quad 6500+ system. Good linearity was observed with an r^2 of >0.995 for all measured nitrosamines. A weighing factor of $1/x^2$ was applied for all 7 nitrosamines.

The calibration range for NDBA and NDIPA was from 0.035 to 200 ng/mL (Figures 1 and 3). For NEIPA, the calibration range was between 0.035 and 100 ng/mL. The calibration range for NDMA, NDPA, and NDEA was between 0.1 and 200 ng/mL (Figure 3). Replicate analysis (N=3) was performed at each concentration level.

Excellent linearity was observed across the evaluated ranges, with r^2 values >0.996 using a $1/x^2$ weighting factor (Figures 1 and 3).

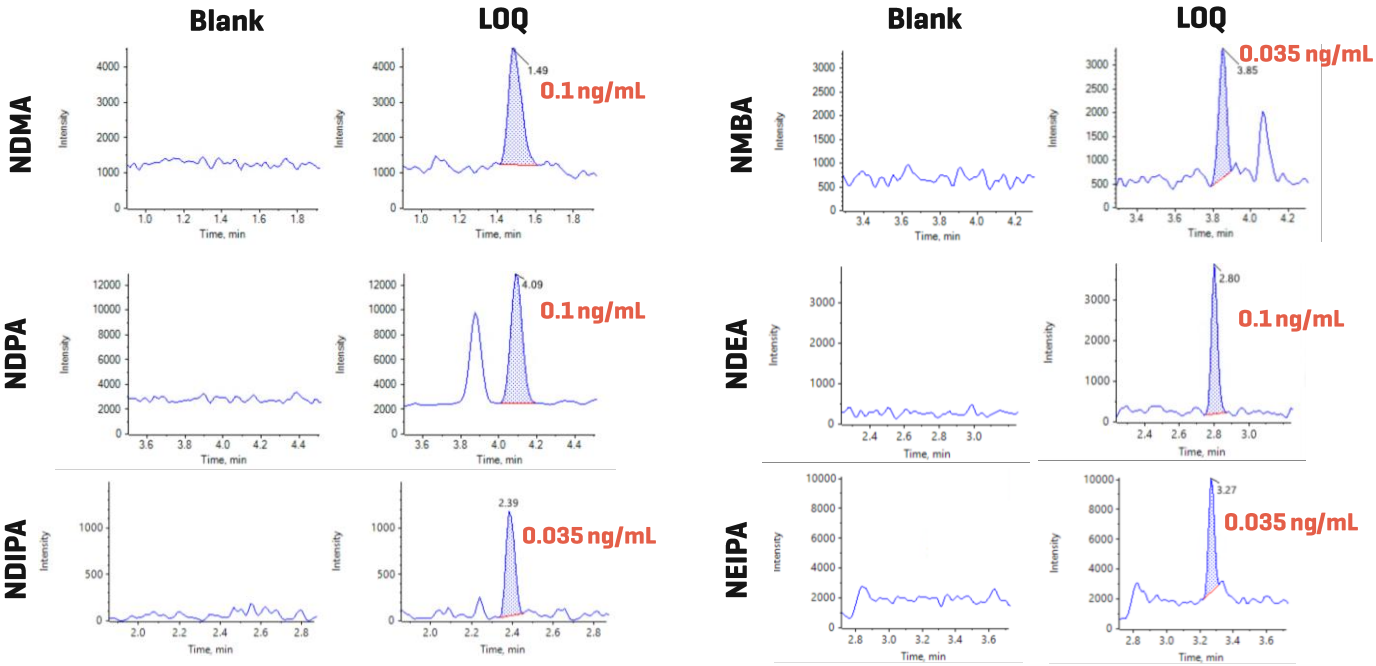

Figure 2. Representative XICs of the blank and LOQ levels for 6 nitrosamines. LOQ of 0.035 ng/mL was reached for NDIPA, NMBA, and NEIPA, while an LOQ of 0.1 ng/mL was achieved for NDMA, NDPA, and NDIPA. No interferences were observed in the blank samples.

Table 4. Average accuracy and precision for each nitrosamine across 3 replicates.

	NDBA		NDMA		NDPA		NDIPA		NMBA		NDEA		NEIPA	
Conc. (ng/mL)	Avg. Acc.	%CV	Avg. Acc.	%CV	Avg. Acc.	%CV	Avg. Acc.	%CV	Avg. Acc.	%CV	Avg. Acc.	%CV	Avg. Acc.	%CV
0.035	98.3	2.16					97.8	5.74	94.4	6.70			93.8	6.44
0.10	103.	3.07	97.5	4.69	105.	5.94	102.	5.76	110.	1.06	99.1	6.88	107.	3.58
0.20	101.	1.87	101.	2.64	102.	4.16	103.	5.43	104.	2.86	103.	9.04	106.	0.466
0.50	102.	3.32	100.	3.61	101.	3.58	104.	4.25	101.	1.31	99.4	1.78	98.8	2.49
1.00	100.	4.78	101.	0.36	99.8	1.89	102.	2.23	107.	1.65	101.	2.54	102.	1.24
2.00	103.	1.00	102.	0.87	100.	0.67	102.	0.83	102.	6.90	104.	3.40	101.	0.583
10	101.	1.39	99.0	0.83	95.1	0.33	98.3	2.06	94.9	4.32	101.	1.34	102.	0.935
50	96.3	3.23	97.9	1.04	93.6	2.53	95.0	1.86	94.5	5.55	96.5	3.29	93.5	2.15
100	92.8	0.94	101.	9.58	103.	0.87	93.1	2.47	88.8	3.57	96.2	1.38	87.8	2.09
200	85.8	0.749	97.6	0.67	89.0	0.65	89.7	1.29	89.8	4.23	93.2	1.87	-	-

Blue font is used to denote LOQ levels (S/N ≥4)

Analytical performance was evaluated for accuracy and precision. The accuracy of the calculated mean was expected to be between 80% and 120% at the LOQ and between 85% and 115% at higher concentrations. The %CV of the calculated mean for each concentration was expected to be <20% at the LOQ.[3]

For all nitrosamines, %CV values at the LOQ levels were ≤7%, and average accuracy was within ±7% (Table 4), demonstrating reproducible and accurate quantitation performance in alignment with ICH Q2(R1) guidance.[2] Assay accuracy for concentrations above the LOQ was within ±15% of the actual concentration with %CV <10. The calculated percentage accuracy and %CV values were within the acceptance criteria for all nitrosamines throughout the concentration range (Table 4).

Recovery was evaluated at 1 ng/mL relative to the blank in the IV bag filtrate using 6 replicates (Table 5). Overall, the recovery across 6 nitrosamines was >86.6% with a %CV <3, indicating a highly reliable and reproducible assay for nitrosamine analysis in IV bag filtrate (Table 5).

Table 5. Recovery was evaluated at 1 ng/mL relative to the blank in IV bag filtrate (N=6).

Compound	Average recovery (%)
NDBA	93.6
NDMA	91.6
NDEA	93.6
NMBA	86.6
NDPA	92.1
NDIPA	92.1
NEIPA	92.2

Compliance-ready SCIEX OS software

Equivalent SCIEX OS software capabilities for nitrosamine analysis can be executed on the SCIEX Triple Quad 6500+ system, ensuring high fidelity when performing method transfers while retaining critical compliance features.

SCIEX OS software is a closed system and requires records and signatures to be stored electronically, meeting the regulations outlined by 21 CFR Part 11. SCIEX OS software can open raw data files from any visible storage location within a closed network by using designated processing workstations.

Figure 4 illustrates the features of SCIEX OS software used to monitor the audit trail, acquire and process data, and configure user access. The audit trail feature enables users to audit critical user actions and locks in data integrity.

The Central Administrator Console (CAC) feature allows users to centralize acquisition and processing using a single platform to maximize efficiency for multi-instrument laboratories, independent of compliance standards. The configuration module allows users to assign roles and access as the administrator, method developer, analyst, and reviewer.

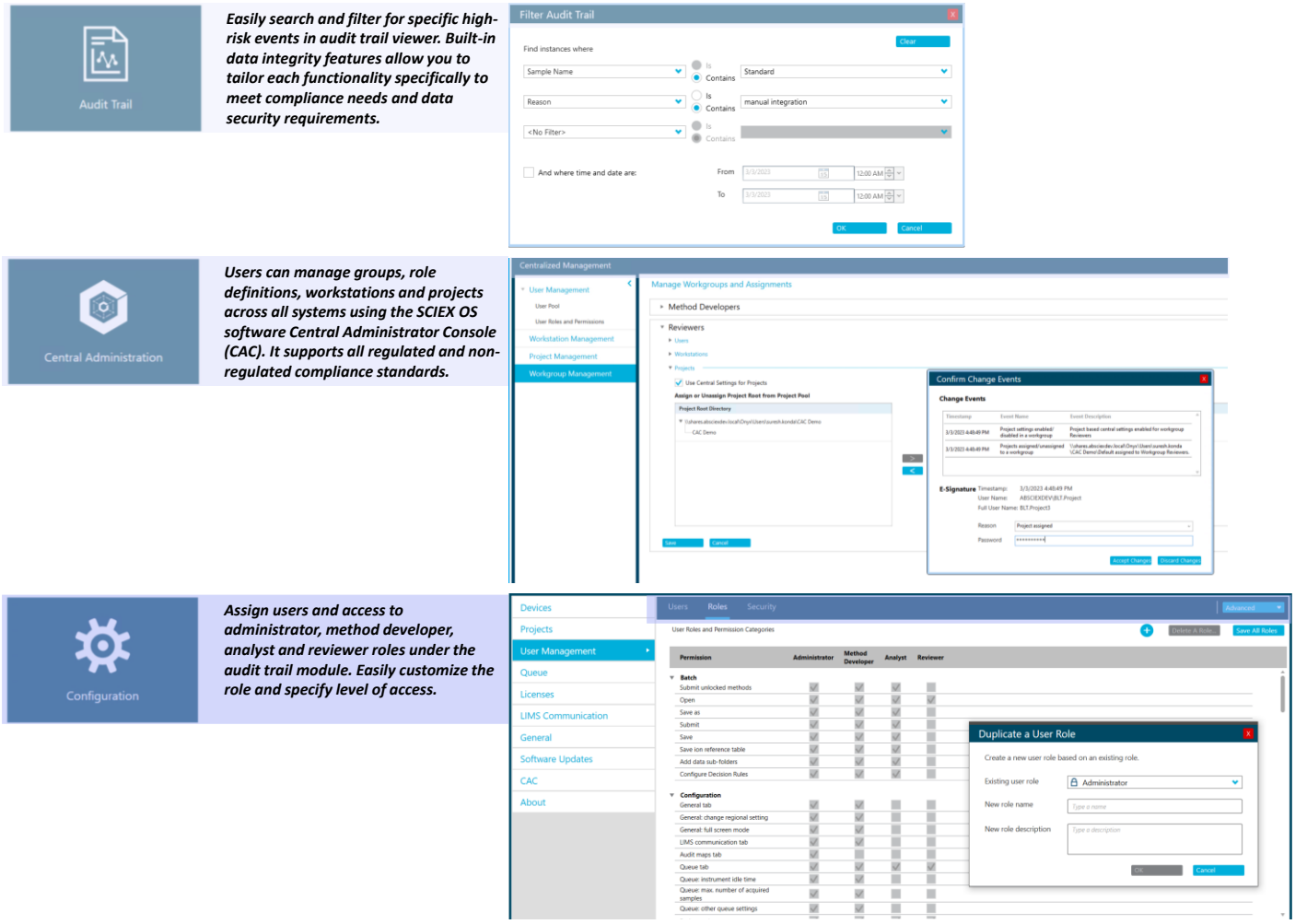

Figure 4. Features of the SCIEX OS software for monitoring user access and evaluating the audit trail. The audit trail view allows users to filter for high-risk events easily and enables data integrity features to meet compliance requirements. The software features a Central Administrator Console (CAC) to manage users and groups, role definitions, workstations, and projects across all systems. The CAC feature supports both regulated and non-regulated compliance standards. The configuration module enables users to quickly set up roles and levels of access for the administrator, method developer, analyst, and reviewer levels.

Conclusions

- Sensitive and reproducible NDBA quantitation was demonstrated in IV bag leachate using the SCIEX Triple Quad 6500+ system, achieving an LOQ of 0.035 ng/mL with excellent linearity over 3.76 orders of magnitude (r^2 >0.995).
- For the additional 6 nitrosamines, an LOQ of 0.035 ng/mL was reached for NDIPA, NMBA, and NEIPA, while an LOQ of 0.1 ng/mL was achieved for NDMA, NDPA, and NDIPA.
- Good quantitative performance was demonstrated with accurate and highly reproducible (%CV <10) results with excellent linearity (r^2 >0.991) on the SCIEX Triple Quad 6500+ system.
- Baseline separation was achieved for isobaric nitrosamines, NDPA and NDIPA using the Phenomenex Biphenyl column.
- Data management and compliance-readiness (21 CFR Part 11) features were shown using the SCIEX OS software to support nitrosamine quantitation on the SCIEX Triple Quad 6500+ system.

References

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2. Q2(R1) Validation of Analytical Procedures: Text and Methodology Guidance for Industry.
3. Control of Nitrosamine Impurities in Human Drugs Guidance for Industry

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