Low-level quantification of 10 mutagenic nitrosamine impurities in Pioglitazone hydrochloride using accurate mass spectrometry

Featuring excellent sensitivity for the quantification of nitrosamine impurities using the X500R QTOF system

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This technical note presents an accurate mass spectrometry method for quantifying 10 mutagenic nitrosamines in Pioglitazone hydrochloride, including NDMA. Excellent chromatographic separation was achieved for all 10 nitrosamines and the Pioglitazone hydrochloride active pharmaceutical ingredient (API) (Figure 1). Statistically significant quantitative performance and linearity were achieved using accurate mass spectrometry at low concentration levels.

Pioglitazone is used to treat type 2 diabetes because it increases the effectiveness of insulin produced by the body to help maintain blood sugar levels and alleviate symptoms.[5] It is essential to ensure that drug products used to treat disease are free from contamination and safe to use. As a result, medicines such as Pioglitazone have been scrutinized since the nitrosamine crisis began in 2018.[4]

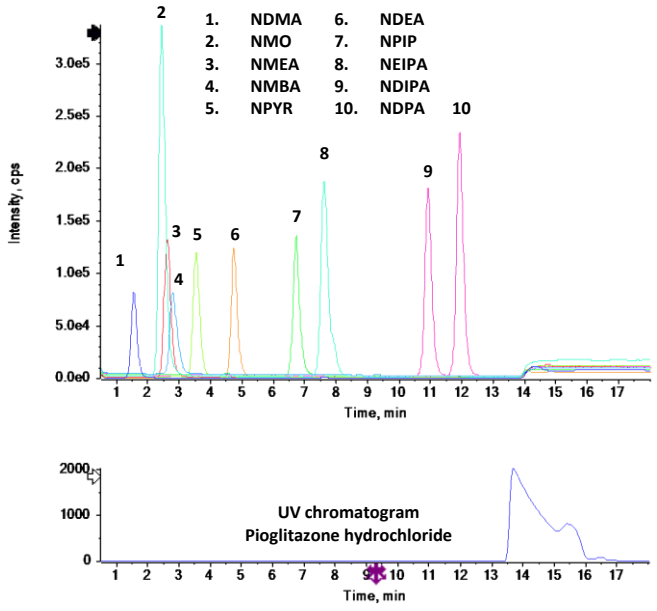

Figure 1. Representative extracted ion chromatogram (XIC) for 10 nitrosamines and UV chromatogram for Pioglitazone hydrochloride. Excellent separation was achieved for the 10 nitrosamines and the Pioglitazone API.

Figure 2. X500R QTOF system.

The recommended limit for total nitrosamines in most drug products is currently 30 ng/g, which is derived from a maximum daily dose of less than 880 mg/day. Pioglitazone hydrochloride has a maximum daily dose of 45 mg and falls well below this threshold where a 30 ng/g limit can be implemented.[4]

Key features for the analysis of nitrosamines using the X500R QTOF system

- **Excellent mass accuracy and selectivity:** Achieve low-level quantification of 10 nitrosamines in Pioglitazone hydrochloride API with excellent mass accuracy (<1 ppm) and selectivity
- **Rapid acquisition speed:** The fast acquisition speed enables the measurement of 10 different nitrosamines simultaneously with precursor ions in full scan TOF MS and MRM^{HR} mode using the X500R QTOF system (Figure 2)
- **Statistically significant quantitative performance:** Achieve excellent quantitative performance with both precursor ion and MRM^{HR} experiments in terms of linearity, accuracy and precision
- **Streamlined data management:** Data acquisition and processing are simplified with SCIEX OS software, a 21 CFR Part 11-compliant platform.

Methods

Standard preparation: A stock solution containing 10 µg/mL of each nitrosamine was prepared in water from standard solutions. Serial dilutions in water were performed to generate calibration solutions with concentrations of 100, 50, 25, 5, 1, 0.4, 0.2, 0.1, 0.050, 0.025 and 0.010 ng/mL.

Spiked sample preparation: A 200 mg sample of Pioglitazone hydrochloride API was weighed into a suitable vessel. A 5 mL aliquot of a 1 ng/mL nitrosamine mixed standard solution was added and vortexed for 30 seconds. The solution was sonicated for 15 minutes and then centrifuged at 4500 rpm for 5 minutes. The supernatant was removed and filtered through a 0.2 µm PTFE filter and transferred to a HPLC vial for analysis. The resulting solution had a sample concentration of 40 mg/mL with a spike concentration of 1 ng/mL of nitrosamine mix. This was equivalent to the 25 ng/g spike concentration of the sample.[1,2]

Chromatography: An ExionLC system with a Phenomenex Kinetex Biphenyl column (2.1 x 100 mm, 2.6 µm, 100 Å) was used for chromatographic separation at a flow rate of 0.4 mL/min. The column was operated at 30°C. Mobile phase A was 0.1% formic acid in water and mobile phase B was 0.1% formic acid in methanol. The injection volume was 25 µL. Table 1 summarizes the gradient conditions.

Table 1. LC gradient.

Time (min)	Mobile phase A (%)	Mobile phase B (%)
0.0	*95*	*5*
0.5	*90*	*10*
14.0	*55*	*45*
16.0	*5*	*95*
20.0	*5*	*95*
20.1	*95*	*5*
22.0	*95*	*5*

Mass spectrometry: The X500R QTOF system was operated in positive ion mode using APCI ionization. The data were collected using full scan TOF MS and MRM^{HR} methods simultaneously. Table 2 outlines the source and MS parameters and Table 3 summarizes the compound-specific TOF MS and MRM^{HR} parameters.[1]

Table 2. Source and gas parameters.

Parameter	Value
Curtain gas	*30 psi*
CAD gas	*7*
Nebulizing current	*5 µA*
Temperature	*300°C*
Ion source gas 1	*55 psi*

Table 3. MRM conditions and compound parameters.

	TOF MS	MRM^{HR}		
Compound	Precursor ion (*m/z*)	Q1 (*m/z*)	Q3 (*m/z*)	CE (V)
N-Nitrosodimethylamine (NDMA)	75.0552	75.0	43.0297	22
N-Nitrosodiisopropylamine (NDIPA)	131.1178	131.1	43.0543	14
N-Nitrosomethylethylamine (NMEA)	89.0709	89.0	61.0403	14
N-Nitrosodiethylamine (NDEA)	103.0865	103.0	75.0555	14
1-Nitrosopyrrolidine (NPYR)	101.0709	101.1	55.0546	24
1-Nitrosopiperidine (NPIP)	115.0865	115.1	41.0386	30
4-Nitrosomorpholine (NMO)	117.0658	117.1	87.068	14
Ethyl(nitroso)(propan-2-yl) amine (NEIPA)	117.1022	117.0	75.0557	12
4-[methyl(nitroso) amino] butanoic acid (NMBA)	147.0764	147.0	117.079	8
N-Nitrosodi-n-propylamine (NDPA)	131.1178	131.1	43.0543	16

Data processing: All data were processed using SCIEX OS software. The MQ4 algorithm was used for quantification.

Quantitative performance

The accuracy of mass measurement is crucial when using an accurate mass spectrometer. This is increasingly important for compounds that have low molecular weights, such as nitrosamines. Consequently, Table 5 shows the high levels of mass accuracy that the X500R QTOF system can achieve with precursor and fragment ions that are used for the quantification

of nitrosamines in spiked samples at 1 ng/mL, equivalent to 25 ng/g of Pioglitazone hydrochloride API.

The calibration curves for 10 nitrosamines were plotted across a concentration range of 0.010-100 ng/mL (Figures 3 and 4). A linear dynamic range of 3 orders of magnitude was achieved for most nitrosamines. No carryover was observed within the blank injection following the highest concentration.

A high level of accuracy was achieved across the calibration range, meeting the requirements for nitrosamine impurities in Pioglitazone hydrochloride. For both precursor ion and MRM^{HR} based quantification of nitrosamines, the r^2 value was >0.98 (Table 4).

With accurate mass spectrometry, users can choose a workflow that best meets their needs. With TOF MS, method setup is straightforward and requires minimal method development. The MRM^{HR} workflow adds another layer of selectivity with the flexibility to choose the most sensitive and selective fragments for quantification.

The XICs at the LOQ levels for 2 representative nitrosamines using TOF MS and MRMHR quantification are shown in Figure 5. Based on the experimental observations, the relative sensitivity of the MS method versus the targeted MS/MS method depends on the nature of the interference from the matrix. For example, an 8-fold improvement in the LOQ was observed using TOF MS for NDEA. However, in the case of NMBA, a 50-fold improvement in LOQ was reached with MRM^{HR}.

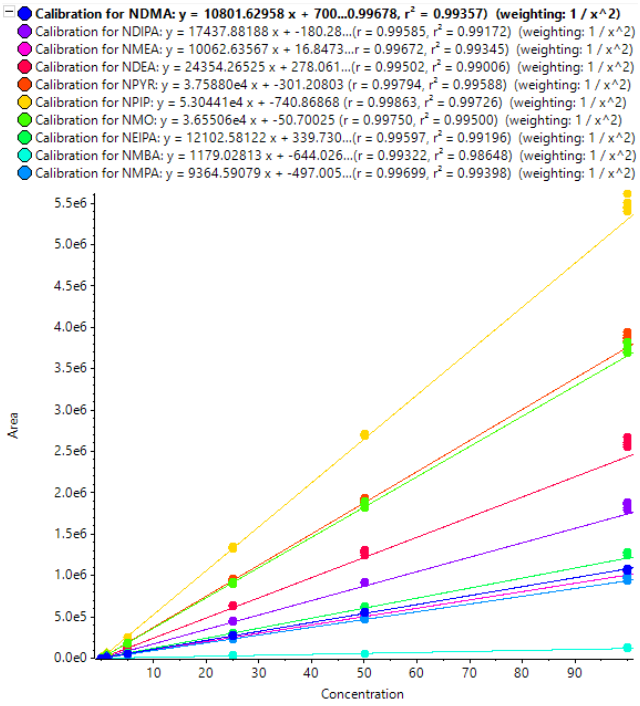

Figure 3. Calibration curves representing the quantitative results from 10 nitrosamines using precursor ions in full scan TOF MS mode. Correlation coefficients (r^2) of >0.98 were observed for the 10 nitrosamines quantified.

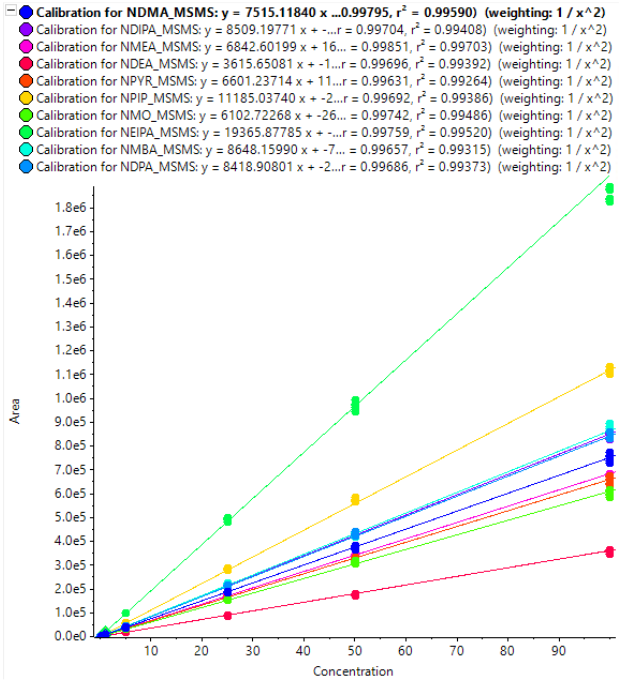

Figure 4. Calibration curves representing the quantitative results from 10 nitrosamines using MRM^{HR}. A correlation coefficient (r^2) of >0.99 was observed for the 10 nitrosamines.

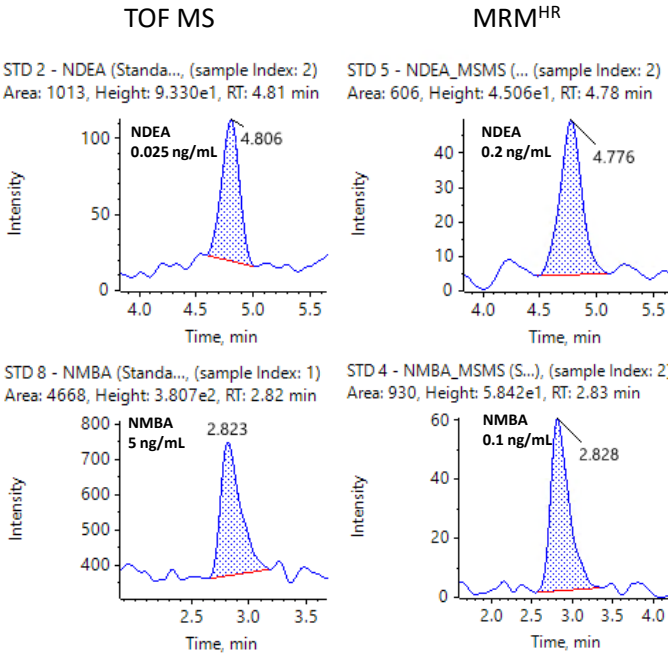

Figure 5. XICs at the LOQs of 2 nitrosamines using precursor ion (TOF MS) and MRM^{HR} based quantification.

Table 4. Mass error, %CV and %accuracy for spiked samples. Each concentration was evaluated in 6 replicates. Nitrosamines that were below the limit of quantification (BLQ) were also indicated.

	TOF MS			MRM^{HR}		
Component name	Mass error (PPM)	%CV	%Accuracy	Mass error (PPM)	%CV	%Accuracy
N-Nitrosodimethylamine (NDMA)	-0.679	13.5	85.9	-0.538	3.40	101
N-Nitrosodiisopropylamine (NDIPA)	-0.258	1.80	112	-0.093	5.70	114
N-Nitrosomethylethylamine (NMEA)	0.537	7.60	85.2	0.560	4.40	110
N-Nitrosodiethylamine (NDEA)	0.040	3.40	109	0.543	9.70	110
1-Nitrosopyrrolidine (NPYR)	-0.384	5.80	112	0.622	13.3	105
1-Nitrosopiperidine (NPIP)	0.396	3.00	100	0.679	11.1	102
4-Nitrosomorpholine (NMO)	0.301	5.40	112	-0.045	3.30	107
Ethyl(nitroso)(propan-2-yl) amine (NEIPA)	-0.971	4.10	99.1	0.508	3.10	107
4-[methyl(nitroso) amino] butanoic acid (NMBA)	BLQ	BLQ	BLQ	-0.452	6.10	98.9
N-Nitrosodi-n-propylamine (NDPA)	-0.326	1.80	95.6	0.176	5.60	101

Accurate and precise quantification in spiked samples

Accuracy and precision metrics were evaluated in standard solutions and spiked samples. A 1 ng/mL concentration in spiked solution (equivalent to 25 ng/g in sample concentration) was used for the assessment. The acceptable criteria for accuracy and precision at this concentration level were ±30% and <25% of the nominal concentration, respectively.

The spiked Pioglitazone hydrochloride API sample met the specified requirements for all nitrosamine impurities (Table 5).

Overall, the %CV was <13.5% and <13.3% for precursor ion and MRM^{HR} quantification experiments, respectively. The percent accuracy was within ±15% of the nominal concentration for both quantification workflows. The mass error was <1 ppm in spiked samples, demonstrating high mass accuracy for nitrosamine impurity analysis in the API. The LOQ for NMBA was 5 ng/mL using the precursor ion TOF MS workflow which was found to be BLQ in spiked samples. Figures 6 and 7 show representative chromatograms of 10 nitrosamines in 1 ng/mL spiked samples using MRM^{HR} and 9 nitrosamines using TOF MS experiments for quantification.

Table 5. Linearity range, %CV and correlation coefficient (r^2) for 10 nitrosamines.

	TOF MS			MRM^{HR}		
Component name	Linearity range (ng/mL)	%CV at LOQ (n=6)	Correlation coefficient (r^2)	Linearity range (ng/mL)	%CV at LOQ (n=6)	Correlation coefficient (r^2)
N-Nitrosodimethylamine (NDMA)	0.1-100	10.5	0.993	0.2-100	8.90	0.995
N-Nitrosodiisopropylamine (NDIPA)	0.05-100	11.7	0.991	0.1-100	9.30	0.994
N-Nitrosomethylethylamine (NMEA)	0.1-100	13.1	0.993	0.2-100	6.40	0.997
N-Nitrosodiethylamine (NDEA)	0.025-100	8.60	0.990	0.2-100	11.0	0.993
1-Nitrosopyrrolidine (NPYR)	0.1-100	8.70	0.995	0.4-100	16.1	0.992
1-Nitrosopiperidine (NPIP)	0.1-100	8.80	0.997	0.4-100	11.8	0.993
4-Nitrosomorpholine (NMO)	0.05-100	17.1	0.995	0.2-100	8.60	0.994
Ethyl(nitroso)(propan-2-yl) amine (NEIPA)	0.2-100	12.3	0.991	0.1-100	13.6	0.995
4-[methyl(nitroso) amino] butanoic acid (NMBA)	5-100	16.7	0.986	0.1-100	14.0	0.993
N-Nitrosodi-n-propylamine (NDPA)	0.05-100	11.7	0.991	0.1-100	13.4	0.993

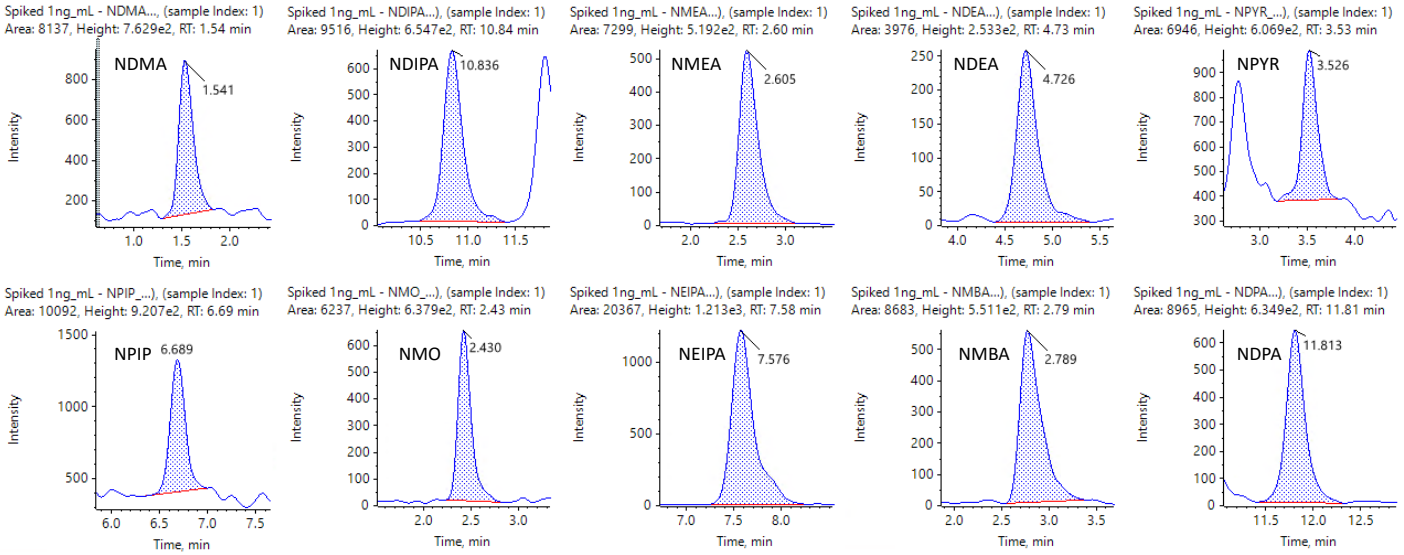

Figure 6. Representative chromatograms for 10 nitrosamines in spiked samples using MRM^{HR}. XICs for 10 nitrosamines at a level of 1 ng/mL in spiked samples.

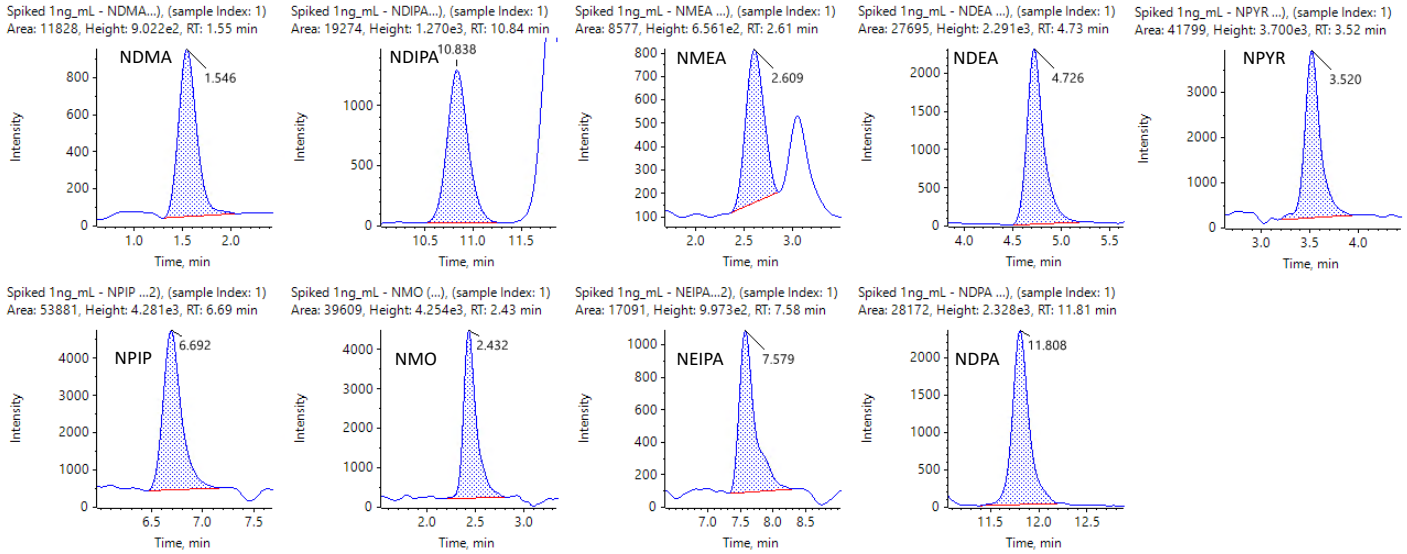

Figure 7. Representative chromatograms for 9 nitrosamines in spiked samples using the precursor ion in full scan TOF MS mode. XICs for 9 nitrosamines at a level of 1 ng/mL in spiked samples.

Compliance-ready SCIEX OS software

To meet the regulation outlined in 21 CFR Part 11, SCIEX OS software is designed as a closed system, and includes the requirement for the records and signatures to be stored electronically. SCIEX OS software can open raw data files from any visible storage location, which enables the flexibility to work within a closed network using designated processing workstations. Figure 8 illustrates 3 types of controls that are required for 21 CFR Part 11 compliance. The workflow presented here is fully compliant with these guidelines, as SCIEX provides 1) technical controls over hardware and software configuration, 2) network security and secure operating systems and policies and 3) procedures and user training (Figure 8).

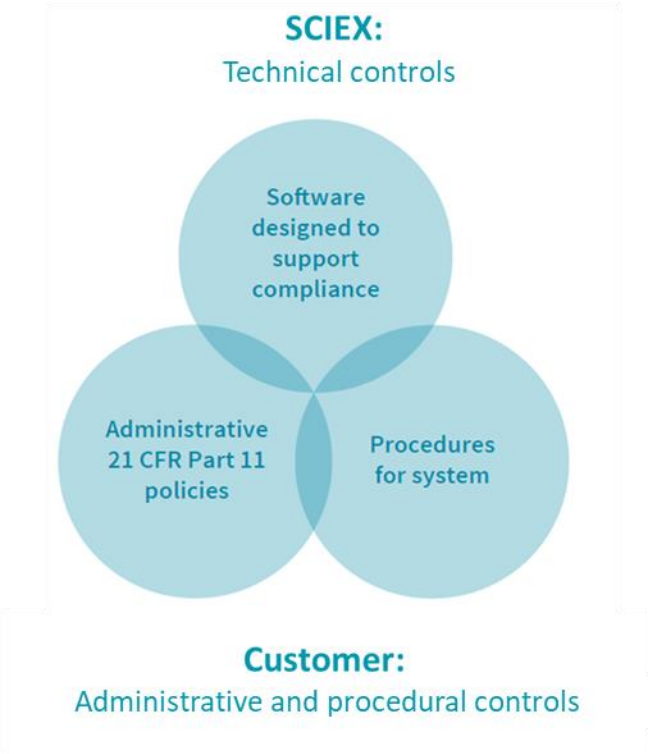

Figure 8. Controls required for 21 CFR Part 11 compliance.

Conclusions

- Low-level quantification was achieved for 10 nitrosamines in spiked samples using the X500R QTOF system
- Excellent linearity and precision were reached for the analysis of nitrosamines, demonstrating exceptional quantitative performance
- High mass accuracy (<1 ppm) for low molecular weight nitrosamines was accomplished, minimizing false positive results
- Utilizing the fast-scanning speed of the X500R QTOF system, simultaneous monitoring was performed on 10 nitrosamines in precursor ion in full scan TOF MS mode and MRM^{HR} experiments
- The method demonstrated the quantification of nitrosamine impurities below the current recommended limit (30 ng/g) in the Pioglitazone hydrochloride drug product
- A single platform for streamlined data acquisition, processing and management with SCIEX OS software was presented

References

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