


Low-level quantitation of 10 mutagenic nitrosamine impurities in pioglitazone hydrochloride using accurate mass spectrometry

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ABSTRACT

Pioglitazone hydrochloride is used to treat type 2 diabetes, as it increases the effectiveness of insulin produced by the body and therefore helps maintain blood sugar levels and alleviate symptoms. It is essential to ensure that drug products used to treat disease are free from contamination and are safe to use. As a result, these medicines have been scrutinized since the nitrosamine crisis began in 2018. Therefore, sensitive and selective bioanalytical methods are needed for proper detection and quantitation of nitrosamines. This study presents an accurate mass spectrometry method for quantifying 10 mutagenic nitrosamines, including NDMA, in pioglitazone hydrochloride. Accurate and highly reproducible quantitative performance was achieved with strong linearity for the low-level quantitation of nitrosamines.

INTRODUCTION

Pioglitazone hydrochloride 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.[1] 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 hydrochloride have been scrutinized since the nitrosamine crisis began in 2018.[2] 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.[2]

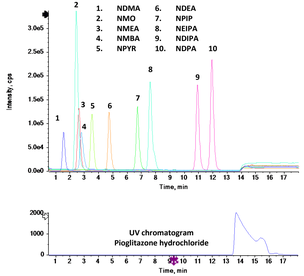

Figure 1. Representative extracted ion chromatogram (XIC) for 10 nitrosamines and UV chromatogram for pioglitazone hydrochloride. Excellent separation was achieved for the 10 nitrosamines and the pioglitazone hydrochloride API.

MATERIALS AND 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.[3,4]

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

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.

Compound	TOF MS Precursor ion (*m/z*)	TOF MS Q1 (*m/z*)	MRM^{HR} Q3 (*m/z*)	MRM^{HR} 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

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.[3]

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

RESULTS

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

Component name	TOF MS Mass error (PPM)	TOF MS %CV	TOF MS %Accuracy	MRM^{HR} Mass error (PPM)	MRM^{HR} %CV	MRM^{HR} %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

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

Component name	TOF MS Linearity range (ng/mL)	TOF MS %CV at LOQ (n=6)	TOF MS Correlation coefficient (r^2)	MRM^{HR} Linearity range (ng/mL)	MRM^{HR} %CV at LOQ (n=6)	MRM^{HR} 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

- A high level of accuracy was achieved across the calibration range, meeting the requirements to detect nitrosamine impurities in pioglitazone hydrochloride
- For both precursor ion and MRM^{HR}-based quantitation of nitrosamines, the r^2 value was >0.98 (Table 4)
- The spiked pioglitazone hydrochloride API sample met the specified requirements for all nitrosamine impurities (Table 5)

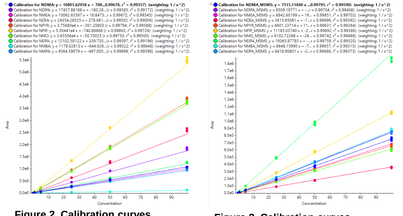

Figure 2. Calibration curves representing the quantitative results from 10 nitrosamines using precursor ions in full scan TOF MS mode. An r^2 of >0.98 was observed for each of the 10 nitrosamines quantified.

Figure 3. Calibration curves representing the quantitative results from 10 nitrosamines using MRM^{HR}. An r^2 of >0.99 was observed for each of the 10 nitrosamines.

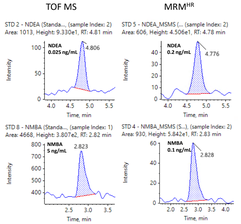

Figure 4. XICs at the LOQs of 2 nitrosamines using precursor ion (TOF MS)- and MRM^{HR}-based quantitation.

- The calibration curves for 10 nitrosamines were plotted across a concentration range of 0.010-100 ng/mL (Figures 2 and 3). 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.
- 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 4).

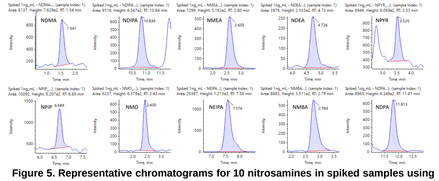

Figure 5. Representative chromatograms for 10 nitrosamines in spiked samples using MRM^{HR}. XICs for 10 nitrosamines at a level of 1 ng/mL in spiked samples.

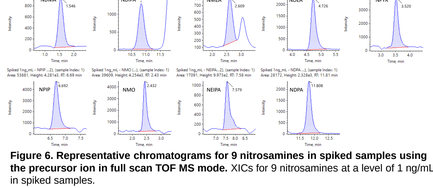

Figure 6. Representative chromatograms for 9 nitrosamines in spiked samples using the precursor ion in full scan TOF MS mode. XICs for 9 nitrosamines at a level of 1 ng/mL in spiked samples.

- Figures 5 and 6 show representative chromatograms of 10 nitrosamines in 1 ng/mL spiked samples using MRM^{HR} and 9 nitrosamines using TOF MS experiments for quantitation

CONCLUSIONS

- Low-level quantitation 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 achieved, 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 quantitation of nitrosamine impurities below the current recommended limit (30 ng/g) in the pioglitazone hydrochloride drug product

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