Quantification of nitrosamines and related impurities in multiple drug products by LC-MS

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ABSTRACT

A method for the analysis of impurity 5-(4′-(azidomethyl)-[1,1′-biphenyl]-2yl)-1H-tetrazole (AZBT) in a sartan API and drug product matrix delivered a limit of quantification (LOQ) value of 0.5 ng/mL in solution. Quantification limits of AZBT were far below the 1.5 µg/day limit outlined in the ICH M7 guidelines.

The quantification of 6 nitrosamine impurities was tested in a pioglitazone tablet matrix and this method achieved detection limits below the recommended limit of 30 ng/g.

An ultra-high sensitivity analysis of nitrosamines in a losartan API was achieved with LOQ values as low as 0.01 ng/mL in solution, far below the current limit of 30 ng/g in sample stipulated by regulatory authorities, such as the European Medicines Agency (EMA) and the US Food and Drug Administration (FDA).

INTRODUCTION

Nitrosamines have been a major point of concern in the pharmaceutical industry since they were detected in sartan medications. These compounds continue to be found in drug products, resulting in an analytical challenge to develop new methods or alter existing methods to suit changing needs.

The EMA recently released guidance permitting skip testing to be performed if sufficiently low LOQ values are achieved. This emphasizes a need for highly sensitive methods to detect nitrosamines, in addition to further chromatographic optimization.

In addition to nitrosamines, the sartan impurity known as AZBT provided positive Ames tests, suggesting it may be genotoxic. Therefore, a sensitive and robust method must be established to analyze this impurity.

MATERIALS AND METHODS

QTRAP 4500 system (AZBT quantification in a sartan API and drug product matrix)

Standard preparation: A stock solution of 100 µg/mL AZBT in methanol was diluted into 50:50 water/methanol to construct a calibration curve between 0.1 ng/mL and 100 ng/mL.
Sample preparation: A sample solution (irbesartan API or candesartan tablet) at a concentration of 2 mg/mL in 50:50 water/methanol was prepared.
Spiked sample preparation: A sample solution at a concentration of 2 mg/mL, at a spike concentration of 10 ng/mL (irbesartan API) or 0.5 ng/mL (candesartan tablet) was prepared in 50:50 water/methanol. Note: spikes were prepared at different concentrations due to the amount observed in the unspiked irbesartan API.
Chromatography: The ExionLC AD system from SCIEX was used with a Phenomenex Kinetex analytical (XB-C18, 2.6 µm, 50 x 2.1 mm) column.
Mass spectrometry: The QTRAP 4500 System, operating in positive ion mode and using electrospray ionization (ESI), was used for analysis.

X500R QTOF system (nitrosamine quantification in a pioglitazone tablet matrix)

Standard preparation: A mixed nitrosamine standard solution at 1,000 µg/mL in methanol was diluted to provide multiple standard solutions between 0.1 ng/mL–100 ng/mL.
Sample preparation: A sample solution at a concentration of 40 mg/mL in water was prepared.
Spiked sample preparation: A sample solution at a concentration of 40 mg/mL, at a spike concentration of 1 ng/mL (equivalent to a 25 ng/g spike concentration in relation to sample), was prepared in water.
Chromatography: Separation was performed using a Phenomenex Biphenyl (150 x 4.6, 2.6 µm) column and an ExionLC AD system.
Mass spectrometry: The X500R QTOF system from SCIEX was operated in positive atmospheric pressure chemical ionization (APCI) mode using MRM^{HR} acquisition.

SCIEX 7500 system (nitrosamine quantification in a losartan API)

Sample preparation: Losartan API was weighed out and diluted to a final concentration of 20 mg/mL in 10% methanol in water.
Standards preparation: Six nitrosamine compounds - NDMA, NDEA, NMBA, plus N-nitrosodibutylamine (NDBA), N-nitrosoethylisopropylamine (NEIPA) and N-nitrosodiisopropyl- amine (NDIPA) - were dissolved and diluted in 10% methanol in water to calibration points covering a range of 0.01 ng/mL to 100 ng/mL of each analyte.
Chromatography: An ExionLC AD System with a Phenomenex Kinetex F5 (2.6µm 100 x 4.6 mm) column (Phenomenex part # 00D-4723-E0) was used for separation.
Mass spectrometry: Data was collected using the SCIEX 7500 system using positive polarity and APCI.

For more information, see the associated SCIEX application notes[1-3].

RESULTS

QTRAP 4500 system (AZBT quantification in a sartan API and drug product matrix)

Here, an assay has been developed for the sensitive detection of AZBT in sartan drug substances and products using the QTRAP 4500 system. This has been achieved with a total run time of 8 minutes and comfortably meets the limits recommended by ICH M7 guidelines.

A number of criteria have been assessed - including specificity, accuracy, precision, limit of detection (LOD), LOQ and linearity - with all criteria exceeding specifications for a low-level impurity analysis. In addition, ion ratio values have been used to improve the specificity of the analysis. Figure 1 below shows both unspiked and spiked extracted ion chromatograms (XICs) of an irbesartan API and candesartan drug product, with a spiked accuracy of 85 to 115% achieved for both matrices.

For more information, refer to the referenced application note[1].

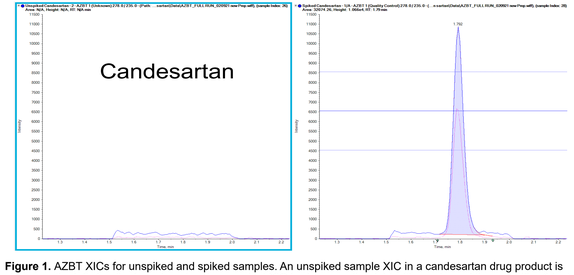

Figure 1. AZBT XICs for unspiked and spiked samples. An unspiked sample XIC in a candesartan drug product is shown on the left. A spiked candesartan drug product XIC is shown on the right with overlays of the quantifier (blue) and qualifier (pink) ions. Ion ratio lines highlight the tolerance (±20%), which has been allocated for the ratio between the quantifier and qualifier fragments.

X500R QTOF system (nitrosamine quantification in a pioglitazone tablet matrix)

Here, an assay has been developed for six nitrosamines in pioglitazone - including NDMA, which is currently the only nitrosamine that has been found in pioglitazone - using the X500R QTOF system.

The current recommended limit for most drug products for total nitrosamines is 30 ng/g, which is derived from a maximum daily dose below 880 mg/day. The 45 mg maximum daily dose of pioglitazone (in the UK) and so falls well below this threshold, meaning that a 30 ng/g limit can be implemented.

As with the AZBT analysis, a range of criteria were assessed, with all criteria meeting generic specifications for a low-level impurity analysis. In addition, the mass accuracy of all measured precursor and fragment ions was assessed, with all ions having a mass error below 0.3 mDa. All ions that were above 80 m/z achieved a mass error below 3 ppm.

Figure 2 shows an unspiked and spiked (1 ng/mL spike) pioglitazone tablet sample, with spiked recovery values being between 70 and 130% for all compounds analyzed.

For more information, refer to the referenced application note[2].

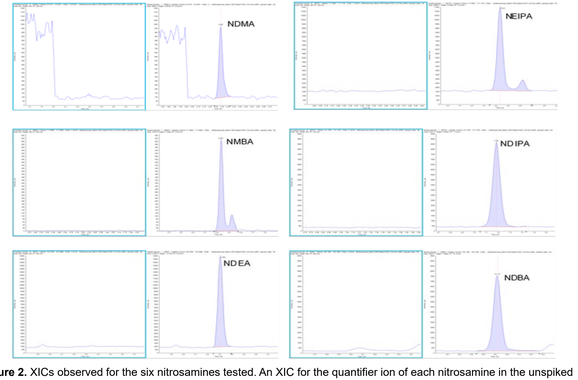

Figure 2. XICs observed for the six nitrosamines tested. An XIC for the quantifier ion of each nitrosamine in the unspiked sample is shown on the left side of each pane, highlighting that unspiked sample is free from nitrosamine contamination. Each spike was performed at 1 ng/mL (25 µg/g), and the right side of each pane shows a clear peak at this level.

SCIEX 7500 system (nitrosamine quantification in a losartan API)

The method presented shows the utility of the highly sensitive SCIEX 7500 system for the analysis of six commonly monitored nitrosamines in losartan. LOQ's of between 0.01 ng/mL and 0.10 ng/mL, depending on the analyte, were reported for the nitrosamine compounds in this study, far exceeding current regulatory limits when analyzing sartan medications (30 ng/g for medications with a maximum daily dose below 880 mg/day). Several other criteria were also accessed, and all specifications were sufficiently achieved. Table 1 highlights the sensitive and is possible for each nitrosamine impurity analyzed and includes spike information (%recovery and %CV) and ng/day exposure limits based on the LLOQ.

For more information, refer to the referenced application note[3].

Table 1. Matrix spike recoveries and LLOQs.

Compound ID	1 ng/mL spike %recovery average / %CV	LLOQ ng/mL	ng/day exposure equivalent*
NDMA *N-nitrosodimethylamine*	*103.4 / 2.5*	*0.01*	*0.05*
NDEA *N-nitrosodiethylamine*	*104.9 / 3.2*	*0.02*	*0.10*
NMBA *N-nitroso-N-methyl-4-aminobutyric acid*	*96.1 / 12.1*	*0.05*	*0.25*
NDBA *N-nitrosodibutylamine*	*113.2 / 4.6*	*0.10*	*0.50*
NEIPA *N-nitrosoethylisopropylamine*	*104.1 / 5.3*	*0.01*	*0.05*
NDIPA *N-nitrosodiisopropylamine*	*104.0 / 5.3*	*0.01*	*0.05*

**Based on a typical maximum daily dose for losartan of 100 mg/day*

CONCLUSIONS

QTRAP 4500 system (AZBT quantification in a sartan API and drug product matrix)

- A method suitable for the analysis of AZBT in both an irbesartan API and candesartan tablet was developed
- An LOD value of 0.1 ng/mL and an LOQ value of 0.5 ng/mL highlight the excellent sensitivity provided by the QTRAP 4500 system
- A linearity spanning from 0.5 ng/mL and 100 ng/mL with an r value >0.99 demonstrates accurate quantification across this range
- Spike recovery was reliable for both irbesartan and candesartan samples, with accuracy values between ±15% of the expected amount
- The use of ion ratio values increased specificity and ensured accurate quantification in matrix
- Custom calculations created in SCIEX OS software supported all data processing on a single platform

X500R QTOF system (nitrosamine quantification in a pioglitazone tablet matrix)

- The X500R QTOF system provides excellent sensitivity, reproducibility and linearity when quantifying nitrosamine impurities in pioglitazone
- Quantification from spiked samples demonstrated that the current method could easily detect the impurities below the current recommended limit (30 ng/g) in a pioglitazone drug product
- High levels of mass accuracy in tandem with the use of ion ratio values allows for improved accuracy and specificity, which are increasingly important when quantifying low molecular weight analytes, and help ensure that any potential false positive results are captured

SCIEX 7500 system (nitrosamine quantification in a losartan API)

The method presented demonstrated good chromatographic separation of the six analyzed nitrosamine compounds from the losartan API, as well as good linearity over the concentration range monitored. The lower limits of quantification for each compound translate to daily intake limits significantly lower than those currently specified by the FDA and EMA. These results demonstrate that the method is suitable for lot release in the event of future increased restrictions and lower required LOQs.

REFERENCES

1. 5-(4′-(azidomethyl)-[1,1′-biphenyl]-2yl)-1H-tetrazole (AZBT) quantification in a sartan drug substance and drug product. SCIEX technical note, RUO-MKT-02-13939-A.
2. Nitrosamine analysis in a pioglitazone drug product. SCIEX technical note, RUO-MKT-02-13355-A.
3. Analysis of genotoxic nitrosamines in losartan using the SCIEX Triple Quad 7500 LC-MS/MS ─ QTRAP Ready System. SCIEX technical note, RUO-MKT-02-13005-A.

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