Comprehensive identification and quantification of nitrosamine impurities by LC-MS/MS

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ABSTRACT

Due to the increasing need for various strategies in order to identify and quantify various nitrosamine impurities, multiple LC-MS/MS methods have been utilized which cover a range of instrumentation (SCIEX QTRAP® 4500, 5500+ and 6500+ LC-MS/MS Systems) and matrices which have been identified to be at risk from nitrosamine contamination.

INTRODUCTION

Nitrosamines are a group of compounds that are proven to be potent carcinogens in animal models. Therefore, genotoxicity is assumed in human models as well. It is then paramount that individuals limit their contact with these compounds to ensure that a negative impact on health is not observed.
In 2018, it was found that a number of angiotensin II receptor blockers (ARBs) which includes various 'sartan' products were possibly contaminated with nitrosamine impurities. In addition, nitrosamines were found in other products such as ranitidine (a treatment for heart burn and stomach ulcers), suggesting the problem was larger than first envisioned. As a result, numerous drug products were recalled, with analytical testing now needing to be performed before medications are released.

MATERIALS AND METHODS

SCIEX QTRAP 4500 LC-MS/MS System (losartan and ranitidine)

Sample preparation: Ranitidine: 100 mg of the powdered API was dissolved in 2 mL of 10% methanol in water. For analysis of the drug product, four 150 mg tablets were crushed and dissolved in the same diluent as the API to a concentration of 50 mg/mL. The mixture was vortexed for 15 minutes and sonicated for another 10 minutes. The mixture was then centrifuged at 4000 rpm for 5 minutes and the supernatant was filtered through a PVDF filter.

Losartan: API powder was weighed out and diluted to a final concentration of 40 mg/mL in 10% methanol in water.

LC conditions: Separations were performed using the ExionLC™ AD System with a UV detector. See Supplementary Information for details on the chromatographic conditions for both methods.[1]

SCIEX QTRAP 5500+ LC-MS/MS System (valsartan)

MS/MS conditions: The QTRAP 4500 System was operated in positive polarity using the atmospheric pressure chemical ionization (APCI) probe on the Turbo V™ Ion Source for both assays. For ranitidine, only NDMA was assayed. For losartan, the suite of nitrosamines was assayed. Please see Supplementary Information for instrument settings, MRM transitions and source conditions for both methods.[1]

Sample preparation: API was weighed and completely dissolved by vortexing in methanol to a concentration of 100 mg/mL.

LC conditions: The gradient was developed to separate all of the analytes in the panel from the valsartan API to prevent any ionization suppression from occurring, and includes a divert step to prevent large amounts of the API from contaminating the mass spectrometer source. Please see the Supplementary Information for details of the chromatography, including gradient profile, column and mobile phases.[2]

MS/MS conditions: The SCIEX Triple Quad™ 5500+ System - QTRAP Ready was operated in positive atmospheric pressure chemical ionization (APCI) mode using Analyst® Software 1.7.1. The MRM compound parameters (DP, EP, CE, CXP) were optimized for nitrosamines, the source parameters were also optimized. Details can be found in the Supplementary Information.[2]

SCIEX QTRAP 6500+ LC-MS/MS System

Standard preparation: The nitrosamine standard mixture was obtained from LGC. The standard was dissolved and diluted in water to obtain standards covering a range of 2.5 – 5000 pg/mL.

Chromatography: Chromatographic separation was performed using the ExionLC AD System which provides very low carryover and full ULC capabilities. The column used was a Phenomenex Luna Omega C18 1.6µm, 100 x 2.1mm. Details of the chromatography are outlined in the Supplementary Information.[3]

Mass spectrometry: These experiments were performed using the SCIEX QTRAP 6500+ LC-MS/MS System. The System was operated in positive ionization mode using the atmospheric pressure chemical ionization (APCI) probe on the IonDrive™ Turbo V Ion Source. Data was acquired using Analyst® Software. Details for MS conditions are outlined in the Supplementary Information.[3]

RESULTS

SCIEX QTRAP 4500 LC-MS/MS System (losartan and ranitidine)

An example of the separation achieved for nitrosamine compounds in the presence of losartan API is shown in Figure 1. Standard curves were run from 0.2 ng/mL to 153.6 ng/mL for all of the nitrosamine compounds in this assay, which corresponds to .005 to 3.84 ppm with regard to losartan. The LOD for all six compounds was evaluated to be 0.2 ng/mL, with the LLOQ being 0.4 ng/mL.

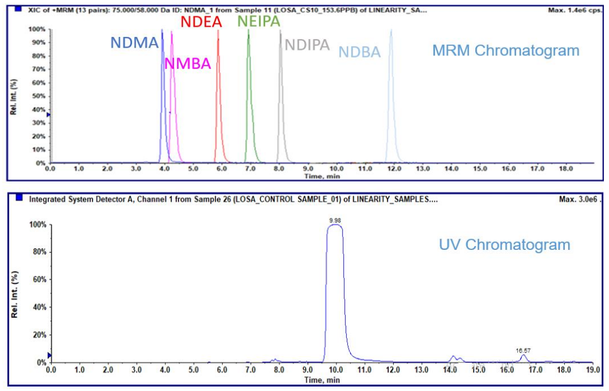

Figure 1. Chromatography for losartan method. (Top) MRM signals from the six nitrosamine compounds in the assay. (Bottom) UV chromatogram showing losartan elution.

Example chromatography showing NDMA and ranitidine elution is shown in Figure 2. A 10 point standard curve was run from 0.5 ng/mL to 500 ng/mL, which corresponds to 0.01 to 10 ppm in 50 mg of the API. The LLOQ of NDMA was evaluated to be 1.5 ng/mL, which corresponds to 0.03 ppm in 50 mg of the API. A linear calibration curve was observed over the range. Precision and accuracy was evaluated at three concentrations levels: the LOD, LLOQ and specification limit level. The recoveries at all three levels were within 80-120% of nominal with good precision.

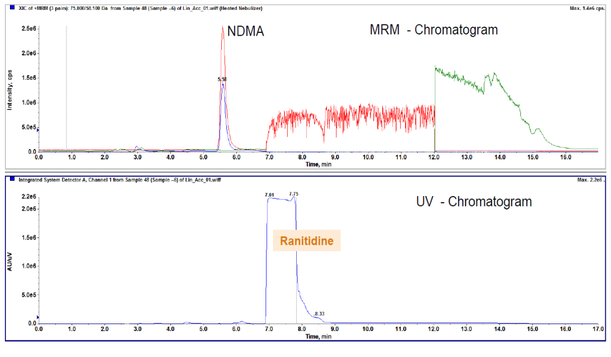

Figure 2. Chromatography for ranitidine method. (Top) MRM signals of NDMA, two MRM transitions were monitored (blue and red). The signal in green is the MRM for ranitidine. (Bottom) UV chromatogram showing ranitidine elution during the valve divert time between 7.01 and 8.7 minutes.

SCIEX QTRAP 5500+ LC-MS/MS System (valsartan)

Time was spent on optimizing the chromatography such that the valsartan API is separated from all 6 nitrosamines. Because of the high level of API, it is critical to achieve separation from the impurities to reduce risk of ion suppression.

Linearity, precision and accuracy were all evaluated as part of the method verification. Recovery in matrix was evaluated at the limit of detection, limit of quantification and at the FDA specified limit of daily exposure (0.03 ppm in API).

Linearity for all six compounds was good over the nine point range of 0.5 to 500 ng/mL, with all compounds showing >0.99 R^2 correlation values.

It is worth noting that even though reagent grade API was used for this analysis, five of the six nitrosamine compounds in this panel were found in the API. Because of this, standard addition quantification was used to calculate the recovery.

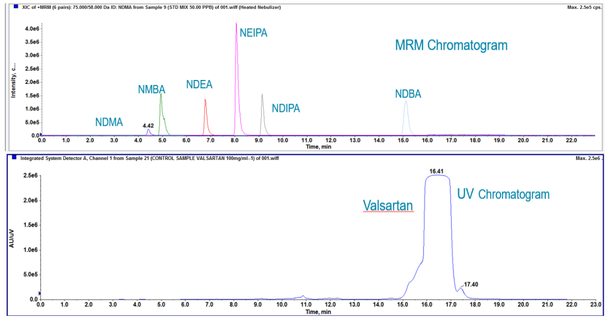

Figure 3. Example chromatography. Extracted ion chromatograms of all 6 nitrosamines standards (top) at 50ng/mL along with UV chromatogram showing elution of valsartan (bottom).

SCIEX QTRAP 6500+ LC-MS/MS System

Impressive sensitivity was obtained using the optimized assay on the QTRAP 6500+ LC-MS/MS System. The majority of compounds were able to achieve an LLOQ of 10.0 pg/mL and an LLOD of 2.50 pg/mL, with the demonstrated sensitivity being much lower than necessary for the analysis based on current regulations. All eight nitrosamine impurities (N-Nitrosodimethylamine (NDMA), N-Nitrosodibutylamine (NDBA), N-Nitrosodi-n-propylamine (NDIPA), N-Nitrosomethylethylamine (NMEA), N-Nitrosodiethylamine (NDEA), 1-Nitrosopyrrolidine (NPYR), 1-Nitrosopiperidine (NPIP), 4-Nitrosomorpholine (NMOR)) provided a calibration curve with an r value >0.99. In addition to this, all impurities were able to achieve %CV values <7.50 at LLOQ level and mean accuracies between 80 and 120% which are both well within the levels of acceptability for an ultra-trace level analysis.

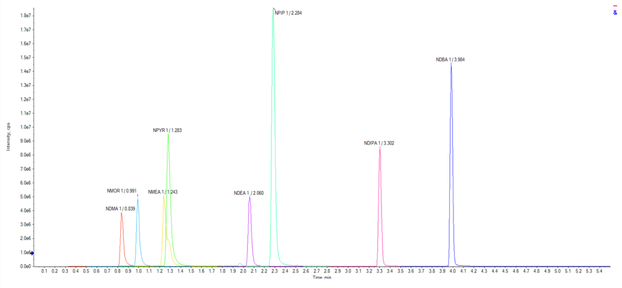

Figure 3. Chromatography of all eight nitrosamine impurities tested. The data above illustrates a 5000 pg/mL mixed standard injection, clearly showing the excellent separation obtained using a Phenomenex Luna Omega C18 1.6 µm column.

Compound confirmation with QTRAP System technology

QTRAP System technology allows the acquisition of full scan MS/MS simultaneously with an MRM experiment mode. This MS/MS can be searched against a compound library for an orthogonal confirmation of identity beyond the detection of an MRM signal. For this assay, a library of relevant nitrosamine impurities was created using SCIEX OS and LibraryView™ Software based on data from nitrosamine standards. This library was then used in subsequent assays to confirm the identity of the nitrosamines detected by the MRM quantitative assay. This ensures that the correct analyte is being measured and helps rule out any artefact peaks that can occur.

CONCLUSIONS

It is noted that all three methods have been optimized independently and were performed using different chromatographic conditions due to the individual challenges each sample type presents. This is to ensure that when injecting very large concentrations of API, no co-elution or interference is observed with the analytes of interest as this could cause ionization suppression which in turn can lead to reduced sensitivity and inaccurate quantitation.

SCIEX QTRAP 4500 LC-MS/MS System (losartan and ranitidine)

Selective, sensitive and reproducible methods for the detection and quantification of nitrosamine compounds in both losartan and ranitidine are described. The sensitivity of the SCIEX QTRAP 4500 System allows quantification below current established limits for these compounds in the respective active pharmaceutical ingredients, without extensive sample preparation. These methods are suitable for incorporation into a quality control environment to support lot release analysis for these important medicines.

SCIEX QTRAP 5500+ LC-MS/MS System (valsartan)

The method described here is capable of accurately detecting and quantifying the six nitrosamines in valsartan API at levels well below the current FDA specifications. Implementation of this method on the SCIEX Triple Quad 5500+ System – QTRAP Ready will enable the monitoring of the six listed nitrosamine compounds to help ensure fewer costly product recalls, and unnecessary patient exposure to these genotoxic compounds.

SCIEX QTRAP 6500+ LC-MS/MS System

Here a highly sensitivity assay for the detection of nitrosamines has been demonstrated using the QTRAP 6500+ LC-MS/MS System. The lower limits of quantification were 10 pg/mL for most compounds, with excellent reproducibility and accuracy. The calibration curves were evaluated across a range of 10 to 5000 pg/mL and demonstrated very good linearity. Note the assay has been tested here in buffer, next steps are to obtain active pharmaceutical ingredients (APIs) and test detection limits in matrix.

MRM signal is often sufficient for detection of compounds but higher confidence that the correct compound has been detected can be achieved through the acquisition of full scan MS/MS on the compound. This is easily done in a single injection using the QTRAP System's unique configuration. MRM triggered MS/MS acquires high quality MS/MS that can be used post-acquisition for compound confirmation. This simultaneous quantitative and qualitative analysis was demonstrated and has been shown to be highly successful. Using the integrated data processing power of SCIEX OS Software, both the quantitative MRM data and the qualitative MS/MS data can be processed within a single software.

The AutoPeak integration algorithm was used for automatic MRM integration with minimal need for re-integration before statistical analysis was then performed on the results. Library searching against a curated nitrosamine library confirmed the identity of all quantified nitrosamines.

REFERENCES

1 Download Supplementary Information. SCIEX QTRAP ® 4500 LC-MS/MS System (losartan and ranitidine)
2 Download Supplementary Information. SCIEX QTRAP® 5500+ LC-MS/MS System (valsartan)
3 Download Supplementary Information. SCIEX QTRAP® 6500+ LC-MS/MS System
4 American Chemical Society, C&EN news, "A side reaction may have led to impurities found in valsartan heart drugs", Published online, Feb 19, 2019.
5 Food and Drug Administration, FDA announces voluntary recall of several medicines containing Valsartan following detection of an impurity, 13th July 2018.
6 European Medicines Agency, EMA advises companies on steps to take to avoid nitrosamines in human medicines, 26th September 2019.
7 Next Generation Quality Control in Pharma Applications. SCIEX technical note RUO-MKT-02-10464-A.

TRADEMARKS/LICENSING