Impurity Profiling of Amiodarone Stability Study Samples using PDA and Accurate Mass Analysis with Automated Software Processing

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ABSTRACT

In this study stability studies of amiodarone were performed under acidic, basic and oxidative stress conditions and sampled at various time points. The SCIEX X500 Series QTOF high-resolution, accurate mass system coupled to a SCIEX ExionLC™ UPLC with photodiode array detection (PDA) and MetabolitePilot™ 2.0 software were used to measure stability and investigate the samples more extensively for impurity detection and identification. Samples were chromatographed following the USP method using a 100 mm column and a 20 minute isocratic gradient and data were acquired using full spectrum PDA, TOF MS and information dependent (IDA) acquisition to collect MSMS information. The data were processed using a combination of SCIEX OS Analytics and MetabolitePilot™ 2.0 software to speed impurity identification and assignment.

INTRODUCTION

Pharmaceuticals impurities are found in starting and intermediate materials used in the manufacturing of active pharmaceutical ingredients (API) and are also the undesired material that remains with the API. The impurities may be unreacted starting materials or reaction by-products arising during synthesis. Impurities may also be formed as a result of the instability of drug substances or reaction with added excipients or packaging materials. The amount of impurities found in drug substances will determine the safety and potency of the final product. Therefore, the identification, quantitation, qualification, and control of impurities are a critical part of the drug development process.

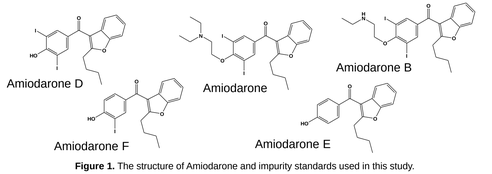

Figure 1. The structure of Amiodarone and impurity standards used in this study.

METHODS

Degradation Conditions:

1. Incubations of amiodarone (500 µg/mL) were performed at room temperature under the following conditions:
   1. Oxidation – 1:1, acetonitrile:30% H_2O_2
   2. Basic Hydrolysis - 1:1, acetonitrile:1N NH_4OH
   3. Acid Hydrolysis - 1:1, acetonitrile:1N HCl
   4. Control - 1:1, acetonitrile:H_2O
2. At 2 hr intervals a 5 µL aliquot was injected for analysis.

MS and DAD Data Collection:

- SCIEX X500R QTOF System with SCIEX OS 1.3
- IDA: Small molecule criteria, threshold 100 cps, with DBS; Top 5 ions
- DAD: 100 to 700 nm, 4.17 Hz

The SCIEX X500R QTOF System and Exion AD LC.

LC Conditions:

- SCIEX ExionLC™ AD system
- Phenomenex Luna Omega 1.6um C18 100A 100 x 2.1 mm, 40 ° C
- Elution was performed using an isocratic gradient of 75%B at 0.20 mL/min. A – H_2O, B – ACN 0.1% CH_2O_2

RESULTS

Figure 2 shows the UV (240 nm) and MS chromatograms of amiodarone and the impurity standards. The peak purity of amiodarone was assessed the TOFMS scans and found to contain only one mass matching the theoretical isotope pattern of amiodarone.

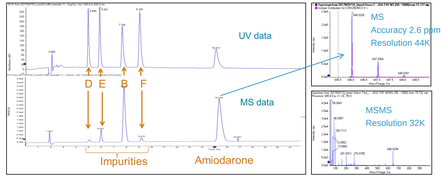

Figure 2. LC-MS and UV chromatogram of a mixture of amiodarone and the impurity standards B, D, E and F. The panel on the top right shows the TOF MS chromatogram of amiodarone and below that the MSMS chromatogram, both were collected with > 30K resolution and <3 ppm mass accuracy.

SCIEX OS Analytics was used to assay the amount of amiodarone present. Figure 3 shows the UV (240 nm) calibration curve for amiodarone in 50% acetonitrile. A linear response was observed from 0.50 µg/mL to 500 µg/mL. The calibration curve was used to: assay the amount of amiodarone remaining after 48 hours of stress testing, estimate the amount of impurities present at the end point and to calculate a mass balance for the reaction.

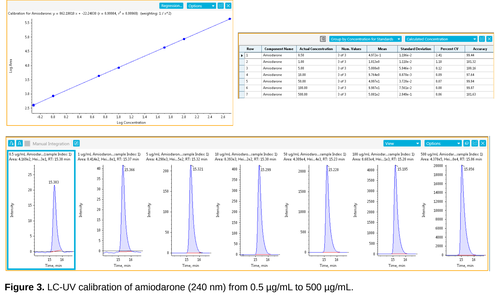

Figure 3. LC-UV calibration of amiodarone (240 nm) from 0.5 µg/mL to 500 µg/mL.

The stability of amiodarone under acidic, basic and oxidative conditions was monitored by both UV and TOFMS over 30 hours. After 30 hours there was 70% of amiodarone remaining under oxidative conditions, 73% under basic conditions and 98.5% under acidic conditions.

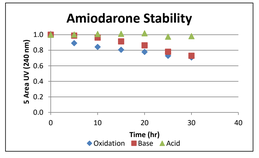

Figure 4. Amiodarone stability profile.

At each stability time point the LC-UV chromatogram at 240 nm ± 4 nm was extracted and an area % of each peak was calculated after baseline subtraction (1.0 min half-window). All peaks above 0.05% total peak area were reported. An example chromatogram from the 30 hour oxidation sample is shown in Figure 5. Figure 6 shows the impurity profile from the oxidation and basic conditions for all reportable peaks above 0.05%.

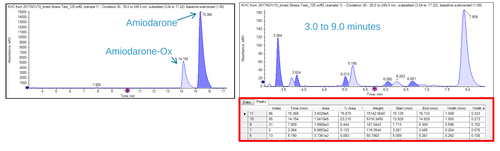

Figure 5. An example chromatogram from the 30 hour oxidation time point. The panel on the right is a zoom from 3.0 to 9.0 minutes.

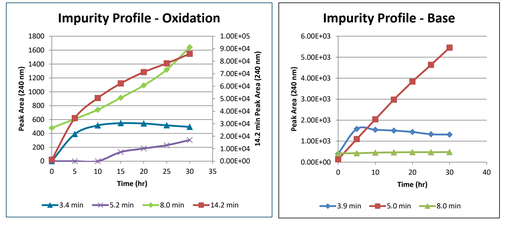

Figure 6. The impurity profile from the oxidative and basic incubation conditions for all peaks above 0.05% area/area. One peak at 8.0 minutes is common to both profiles.

Once the reportable impurities were calculated several tools were used to determine their identity. SCIEX OS Analytics has both quantitative and qualitative (targeted and non-targeted screening) functionality built-in. The screening workflow can take advantage of library searching and includes a connection to ChemSpider for compound look-up. In addition to ChemSpider, the NIST library was used.

Matches were found for the 4.8 minute and 8.0 minute peaks from the base incubations in the ChemSpider database and NIST library. The peaks were identified as amiodarone impurity D and amiodarone impurity B. These were then confirmed by spiking with the authentic impurity standards.

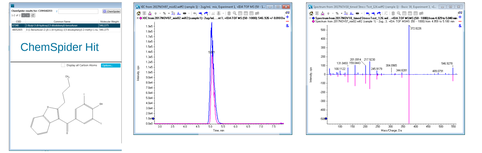

Figure 7. The peak at retention time 5.0 minutes from the basic impurity profile (m/z 546.926) was identified as Amiodarone Impurity D from a ChemSpider library hit. This was confirmed by spiking and analysis of the impurity standard. The middle pane is the TOFMS XIC for 546.926 ±0.01, blue is the 30 hour basic sample and pink is the impurity reference standard. The pane on the right is the high resolution MSMS spectra of the 30 hour base impurity sample (blue) and the impurity reference standard (pink).

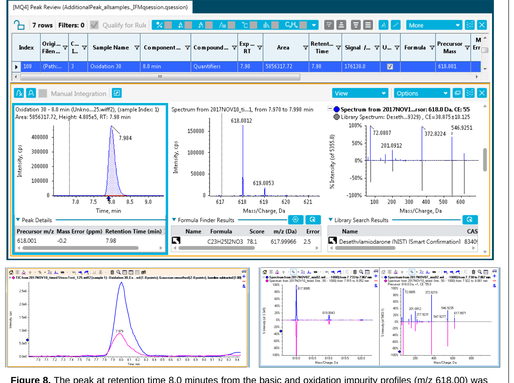

Figure 8. The peak at retention time 8.0 minutes from the basic and oxidation impurity profiles (m/z 618.00) was identified as Amiodarone Impurity B from a ChemSpider library hit. This was confirmed by spiking and analysis of the impurity standard. The top pane is the SCIEX OS Analytics view showing XIC, TOFMS and MSMS of the impurity and the library hit (grey). The bottom left pane is the TOFMS XIC for 618.00 ±0.01, blue is the 30 hour oxidation sample and pink is the impurity reference standard. The bottom right panes on the right are the high resolution TOFMS and MSMS spectra of the 30 hour oxidation impurity sample (blue) and the impurity reference standard (pink).

The peak at 14.2 minutes in the oxidation samples with m/z 662.0239 had four ChemSpider matches. The top hit is shown in the SCIEX OS Analytics window and the other three shown beside in Figure 9.

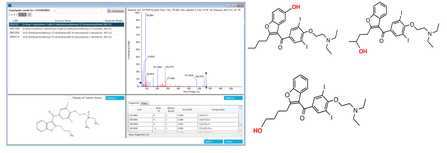

Figure 9. The four ChemSpider hits for the 14.2 minute peak in the oxidation incubation.

The four possibilities have unique sites of oxidation and the MSMS information can be used to distinguish between them. To aid in the MSMS interpretation MetabolitePilot™ 2.0 software was used to process the data. MetabolitePilot™ 2.0 software has an automated structural proposal tool and features both targeted (for cleavages and transformations like oxidations) and untargeted peak finding capabilities. Figure 10 shows the interpretation workspace of MetabolitePilot™ 2.0 software for the 14.2 minute peak. The top proposal has the site of oxidation on the terminal carbon of the N-diethyl group. The MSMS fragment at 201.0911 (Bolded) helps eliminate the 3 ChemSpider proposals shown in figure 9 that are oxidized on the butyl-benzofuran moiety.

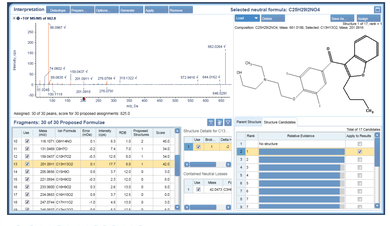

Figure 10. The interpretation workspace of MetabolitePilot 2.0 software displaying results of the 14.2 min amiodarone-ox peak, and the top structural proposal. The MSMS fragment at 201.09 shows there is no oxidation on the butyl-benzofuran moiety and establishes that oxidation is occurring on the other side of the molecule .

CONCLUSIONS

The SCIEX X500R QTOF System with SCIEX OS and MetabolitePilot 2.0 software was used to study the forced degradation of Amiodarone. Two known impurities (B and D) of amiodarone were found under basic and oxidative conditions through library matching and confirmed with authentic standards. An oxidized metabolite of amiodarone was found and four possible matches were found in ChemSpider, then the MSMS interpretation tools of MetabolitePilot 2.0 software were used eliminate three of these possibilities and to narrow down the site of oxidation to the N-diethyl moiety.

TRADEMARKS/LICENSING

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