Impurity Profiling of Amiodarone Stability Samples using Accurate Mass Analysis and Automated Data Processing

Using the X500R QTOF System, UV Optical Data, SCIEX OS Analytics, and MetabolitePilot™ Software

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In order to increase the safety and efficacy of drug products, impurity profiling is routinely performed to identify and quantify any residual impurities. These impurities may result from the instability of drug substances or reaction with added excipients or packaging materials, or they may arise from starting and intermediate materials used in the manufacturing of active pharmaceutical ingredients (API). Since the amount of impurities will determine the safety and potency of the final product, a key challenge for drug development researchers is to identify, quantify, and control any impurities as early and efficiently as possible.

Because there can potentially be many impurities, some of which can be toxic and of low abundance, data processing can be onerous and time consuming if the software, and the quality and type of data itself, are not up to the task. Thus, an automated, high performance solution for detecting and identifying impurities is essential for relieving the burden and efficiently finding any stability or degradation issues.

In this study, the X500R QTOF system coupled to a ExionLC™ UPLC system with photodiode array detection (PDA), SCIEX OS Analytics, and MetabolitePilot™ 2.0 software were used to measure stability and detect impurities in amiodarone samples. Amiodarone is an anti-arrhythmic medication used to treat and prevent a number of types of irregular heartbeats. This integrated hardware and software solution compounds from MS and MS/MS data and reduces the need for manual review.

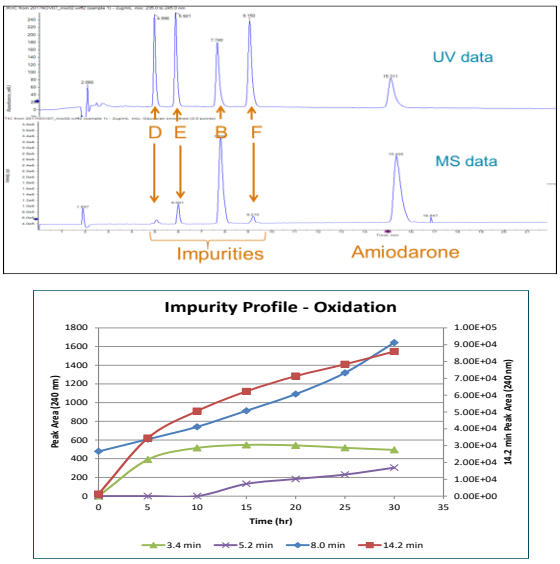


Figure 1. Impurity Analysis of Amiodarone. The forced degradation of amiodarone under oxidative conditions is shown. Top: Integrated UV and MS data showing alignment of peaks for easier identification. Bottom: Impurity profiles for several Amiodarone degradation compounds over the course of 30 minutes.

Key Features of Profiling Workflow

- X500R QTOF System - high-resolution, accurate mass system
  - Fast scan speeds and high sensitivity ensure even low level impurities are identified.
  - High resolution, accurate mass MS/MS data gives the highest confidence in compound identification
- SCIEX OS and MetabolitePilot Software
  - Easy to learn and use, well integrated software simplifies the experience for new users
  - Batch analysis ensures streamlined data processing
  - Non-targeted peak detection starting from a defined structure narrows the field for compound identification
  - Automated search for compound ID from various sources (e.g., Chemspider, NIST) provides flexibility
- ExionLC system with photodiode array (PDA)
  - Optical data integrated into the MS data file in a single injection ensure key compounds aren't missed
  - UV with MS data are aligned to assign IDs to UV peaks

Methods

Sample Preparation: Amiodarone and impurity standards were obtained from Sigma-Aldrich. Degradation studies were performed by incubating amiodarone (500 µg/mL) 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

At 5 hour intervals, a 5 µL aliquot was injected for analysis.

Chromatography: A SCIEX ExionLC™ AD system equipped with a Phenomenex Luna Omega 1.6um C18 100A 100 x 2.1 mm column, held at 40 °C was used. Elution was performed using an isocratic gradient of 75%B at 0.20 mL/min. Buffer A – H2O, Buffer B – ACN 0.1% CH_2O_2

Mass Spectrometry: A SCIEX X500R QTOF System controlled by SCIEX OS Software 1.3 was used. IDA Experimental conditions: threshold 100 cps, with DBS (Dynamic Background Subtraction) enabled; Top 5 ions. PDA conditions: 100 to 700 nm, 4.17 Hz

Data Processing: SCIEX OS 1.3 Analytics was used for non-targeted identification and screening in addition to ChemSpider and NIST library search. MetabolitePilot Software 2.0 was used for confirmation of amiodarone oxidation product from MS/MS data.

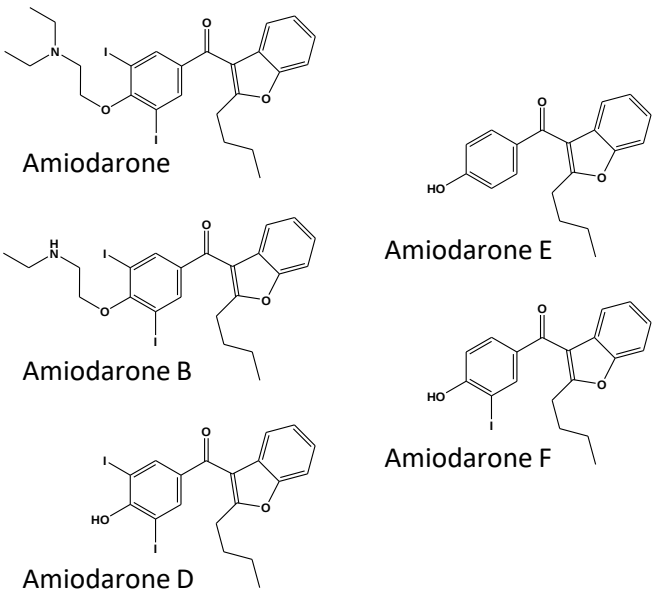


Figure 2. Chemical Structures of Amiodarone. The structure of amiodarone and impurity standards used in this study are shown above.

Identification and Quantitation of Amiodarone and Impurity Standards

Figure 1, top, shows the UV (240 nm) and MS chromatograms of a mixture of amiodarone and the impurity standards B, D, E, and F (Figure 2). The MS and optical data are collected within a single injection and integrated into a single data file. This ensures that key compounds aren't missed. Alignment of the UV with MS data enables assignment of ID's to the UV peaks.

Figure 3 shows the TOF MS and MS/MS spectrum of amiodarone. Both were collected with >30K resolution and <3 ppm mass accuracy. The X500 QTOF system enables fast MS/MS data collection with high resolution especially at low m/z, and high mass accuracy, while still maintaining excellent sensitivity. The high resolution, accurate mass MS/MS data provide more confident compound ID while the fast scan speeds ensure nothing is missed. This high sensitivity, high quality data is obtained even for low abundant species. In this case, the peak purity of amiodarone was assessed using the TOF MS scans and found to contain only one mass matching the theoretical isotope pattern of amiodarone using SCIEX OS Analytics.

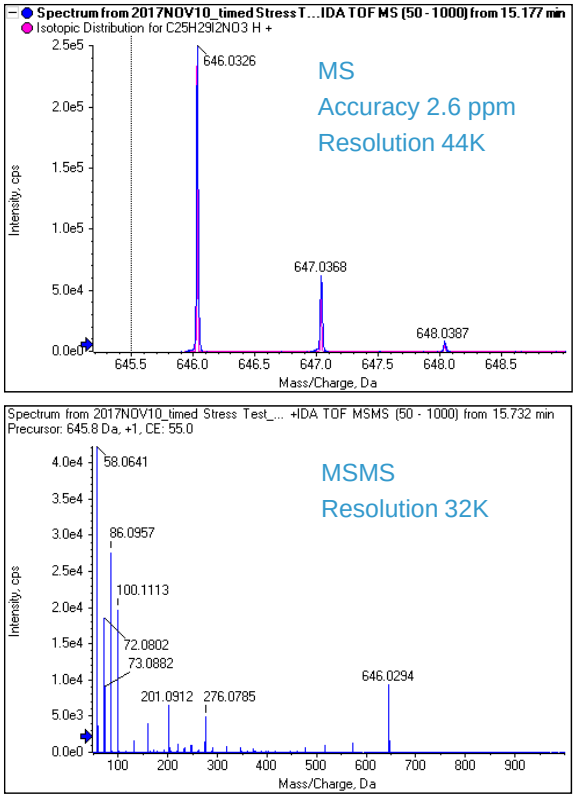
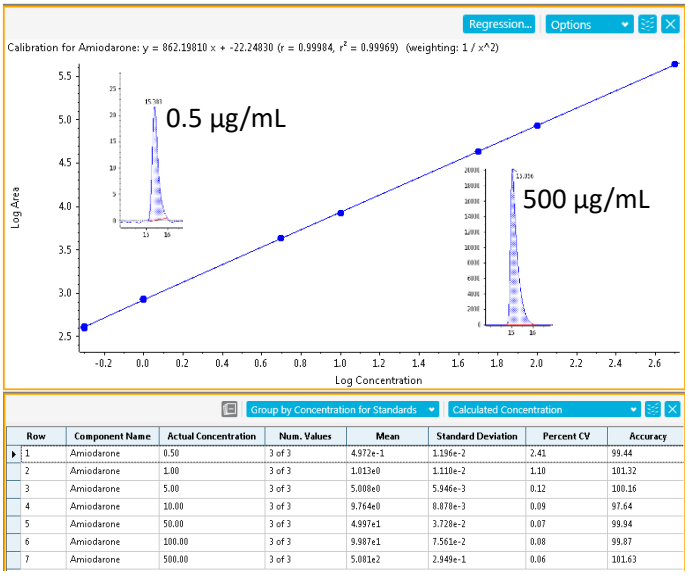


Figure 3. MS and MS/MS Spectra of Amiodarone. The TOF MS (top) and MS/MS (bottom) spectra of amiodarone from the peak at 15.4 min in Figure 1 shows the resolution and mass accuracy typical of the X500 QTOF System. Both were collected with >30K resolution and <3ppm mass accuracy.

SCIEX OS Analytics was used to assay the amount of amiodarone present. Figure 4 shows the UV (240 nm) calibration curve for amiodarone in 50% acetonitrile. A linear response was observed from 0.5 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.



Row	Component Name	Actual Concentration	Num. Values	Mean	Standard Deviation	Percent CV	Accuracy
1	Amiodarone	0.50	3 of 3	4.972e-1	1.196e-2	2.41	99.44
2	Amiodarone	1.00	3 of 3	1.013e0	1.110e-2	1.10	101.32
3	Amiodarone	5.00	3 of 3	5.008e0	5.946e-3	0.12	100.16
4	Amiodarone	10.00	3 of 3	9.764e0	8.878e-3	0.09	97.64
5	Amiodarone	50.00	3 of 3	4.997e1	3.728e-2	0.07	99.94
6	Amiodarone	100.00	3 of 3	9.987e1	7.561e-2	0.08	99.87
7	Amiodarone	500.00	3 of 3	5.081e2	2.949e-1	0.06	101.63

Figure 4. LC-UV Calibration of Amiodarone (240 nm) from 0.5 µg/mL to 500 µg/mL. Linearity is achieved over three orders of magnitude with $r^2 = 0.999$ using $1/x^2$ weighting.

Stability Profile of Amiodarone under Oxidative, Basic, and Acidic Conditions

The stability of amiodarone under oxidative, basic, and acidic conditions was monitored by both UV and TOF MS over 30 hours. After 30 hours, 70% of amiodarone remained under oxidative conditions, 73% under basic conditions, and 98.5% under acidic conditions (Figure 5).

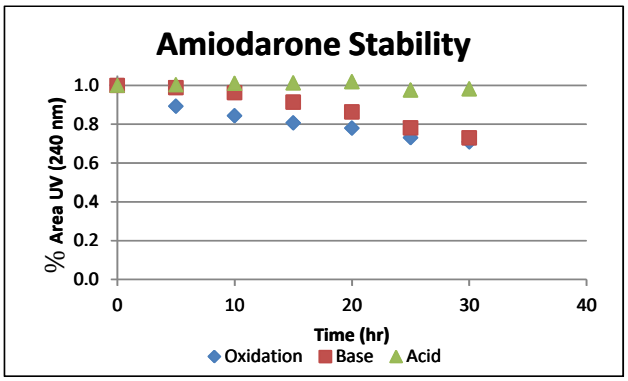
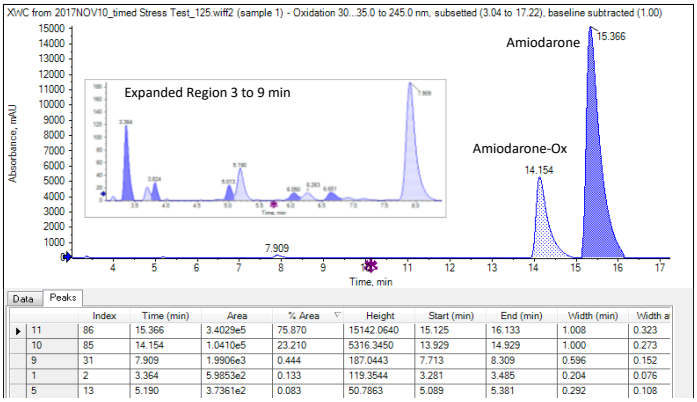


Figure 5. Stability of Amiodarone Under Different Stress Conditions. The UV profile of Amiodarone is shown under oxidative (blue), basic (red), and acidic (green) conditions over the course of 30 hours with time points every 5 hours.

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 6. Figure 7 shows the impurity profile from the oxidation and basic conditions for all reportable peaks above 0.05%.



	Index	Time (min)	Area	% Area	Height	Start (min)	End (min)	Width (min)	Width a
11	86	15.366	3.4029e5	75.870	15142.0640	15.125	16.133	1.008	0.323
10	85	14.154	1.0410e5	23.210	5316.3450	13.929	14.929	1.000	0.273
9	31	7.909	1.9906e3	0.444	187.0443	7.713	8.309	0.596	0.152
1	2	3.364	5.9853e2	0.133	119.3544	3.281	3.485	0.204	0.076
5	13	5.190	3.7361e2	0.083	50.7863	5.089	5.381	0.292	0.108

Figure 6. LC-UV Chromatogram from the 30 Hour Oxidation Time Point. Peaks above 0.05% total peak area are reported in the table below. The insert is an expanded region from 3.0 to 9.0 minutes.

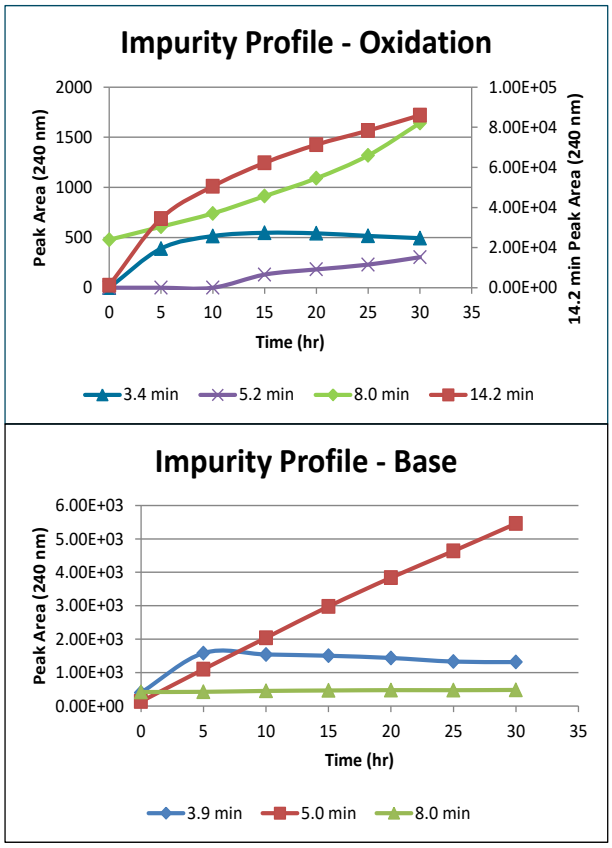


Figure 7. Impurity Profiles of Amiodarone Impurities under Oxidative and Basic Conditions. Profiles for all peaks above 0.05% area/area are shown. One peak at 8.0 minutes is common to both profiles.

Identification of Amiodarone Impurities

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 searched. Matches were found for the 5.0 minute and 8.0 minute peaks from the basic incubations in the ChemSpider database and NIST library. The peaks were identified as amiodarone impurity D (Figure 8) and amiodarone impurity B (Figure 9). These were then confirmed by spiking with the authentic impurity standards.

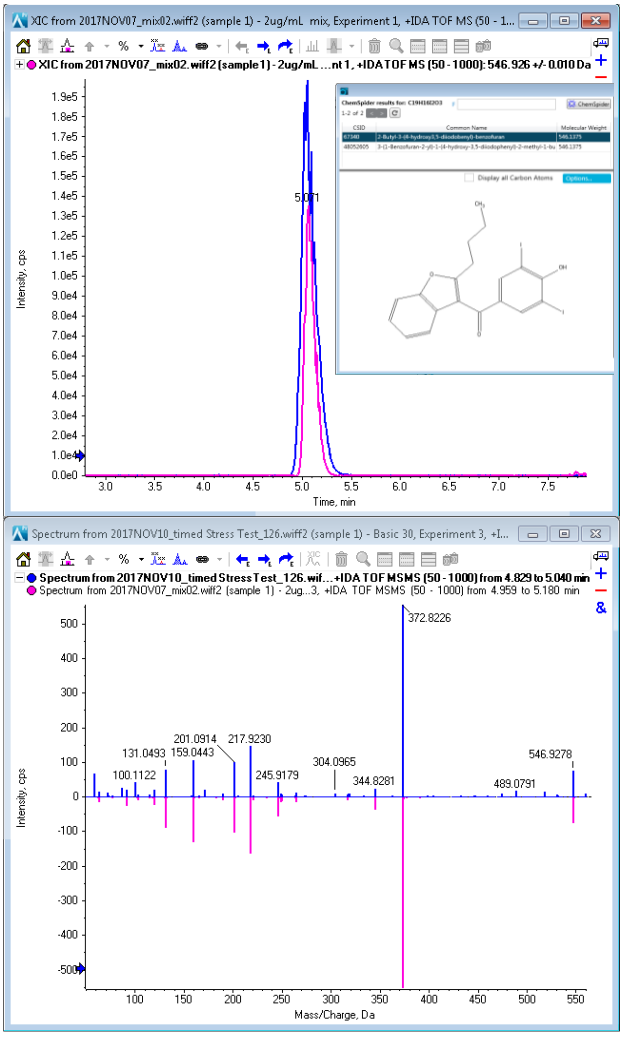


Figure 8. Identification of Amiodarone Impurity D. The peak at retention time 5.0 minutes from the basic impurity profile (m/z 546.926) was identified as Amiodarone Impurity D from a ChemSpider library hit and confirmed by spiking and analysis of the impurity standard. Top: TOF MS XIC for 546.926 ±0.01, blue is the 30 hour basic impurity sample and pink is the Amiodarone D reference standard. Bottom: High resolution MS/MS spectra of the 30 hour basic impurity sample (blue) and the Amiodarone D reference standard (pink).

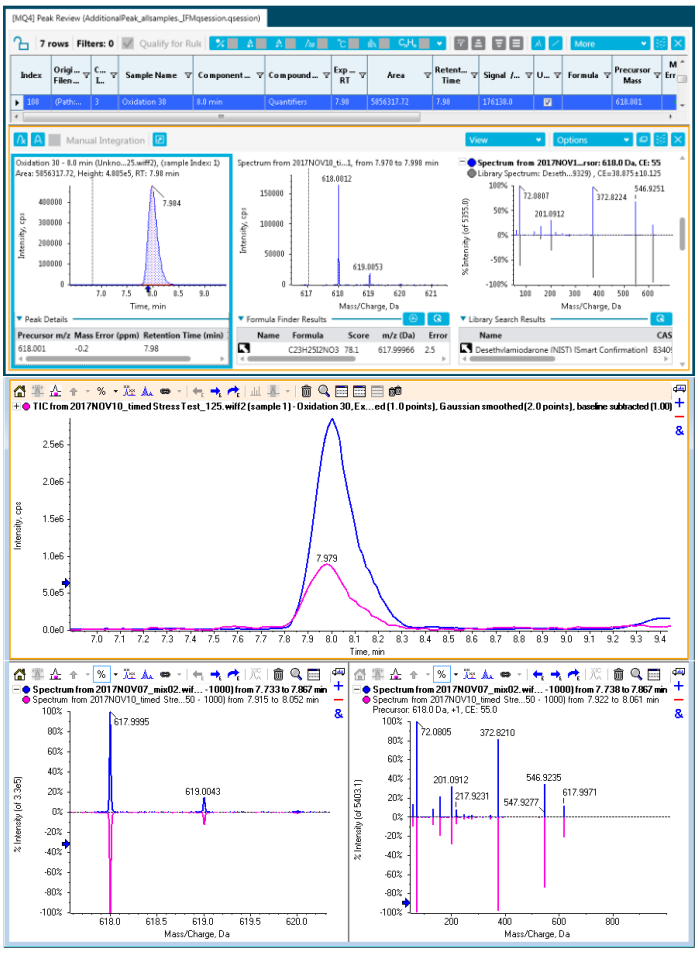
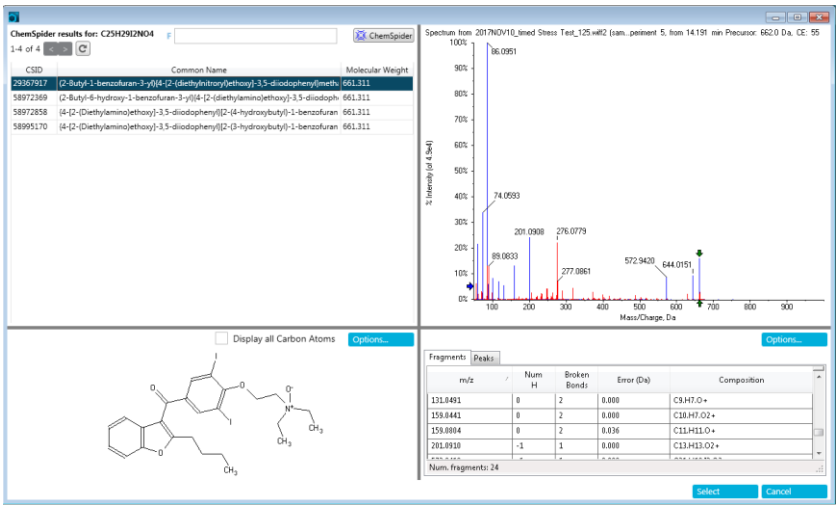


Figure 9. Identification of Amiodarone Impurity B. The peak at retention time 8.0 minutes from the basic and oxidation impurity profiles (m/z 618.00) was identified as Amiodarone Impurity B from a NIST library hit. This was confirmed by spiking and analysis of the impurity standard. Top: SCIEX OS Analytics view showing XIC, TOF MS and MS/MS of the impurity and the library hit (grey). Middle: TOF MS XIC for 618.00 ±0.01, blue is the 30 hour oxidation sample and pink is the Amiodarone B reference standard. Bottom: High resolution TOF MS (left) and MS/MS (right) spectra of the 30 hour oxidation impurity sample (blue) and the Amiodarone B reference standard (pink).

MS/MS Data for Identification of Amiodarone Oxidation Isomers

The peak at 14.2 minutes in the oxidation samples with m/z 662.0239 had four ChemSpider matches with identical chemical formulas and mass. Figure 10 shows the top hit and additional hits for this impurity. The four possibilities have unique sites of oxidation and the MS/MS information can be used to distinguish between them. To aid in the MS/MS interpretation MetabolitePilot software 2.0 was used to process the data. MetabolitePilot software has an automated structural proposal tool and features both targeted (for cleavages and transformations like oxidations) and untargeted peak finding capabilities.



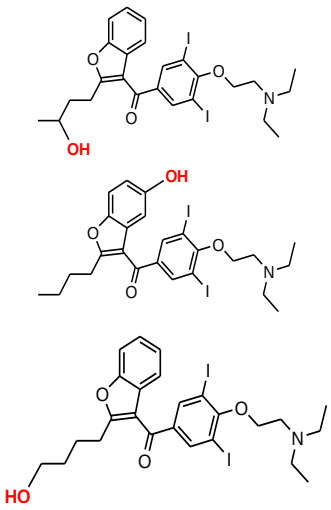


Figure 10. ChemSpider Hits for Oxidation Incubation. The peak at 14.2 minutes in the oxidation samples with m/z 662.0239 had four ChemSpider matches. The top hit is shown in the SCIEX OS Analytics window and the other three are shown to the right.

Figure 11 shows the interpretation workspace of MetabolitePilot software for the peak at 14.2 minutes. The top proposal has the site of oxidation on the terminal carbon of the N-diethyl group. The MS/MS fragment at 201.0911 (bolded) helps eliminate the 3 ChemSpider proposals shown in Figure 10 that are oxidized on the butyl-benzofuran moiety.

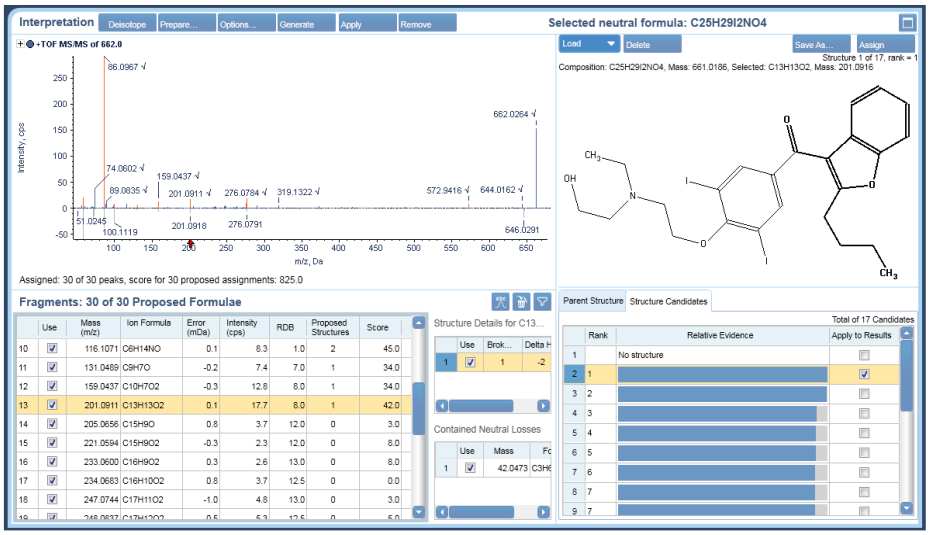


Figure 11. MetabolitePilot Software Interpretation of Amiodarone Oxidation Impurity Isomers. The interpretation workspace of MetabolitePilot software displaying results of the 14.2 min amiodarone-oxidation peak, and the top structural proposal. The MS/MS fragment at 201.09 shows there is no oxidation on the butyl-benzofuran moiety and establishes that oxidation is occurring on the other side of the molecule

Conclusions

The X500R QTOF System equipped with the ExionLC system, SCIEX OS and MetabolitePilot 2.0 software provides an integrated solution for the routine and automated identification of drug impurities. The speed of the data acquisition ensures nothing is missed, while the sensitivity, high resolution, and high mass accuracy in both MS and MS/MS mode provide high quality data critical for the definitive identification of impurities. In this study, Amiodarone was degraded under different stress conditions. The high mass accuracy of the MS data facilitated the straightforward identification of two known impurities (B and D) under basic and oxidative conditions through library matching, which were then confirmed with authentic standards. An oxidized metabolite of amiodarone was found and four possible matches of identical mass were found in ChemSpider. Further analysis of the MS/MS data using MetabolitePilot software 2.0 interpretation tools eliminated three of these possibilities and confirmed the site of oxidation on the N-diethyl moiety.

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