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A Workflow Approach for the Identification and Structural Elucidation of Impurities of Quetiapine Hemifumarate Drug Substance

Michael D. Jones, Marian Twohig, Karen Haas, and Robert S. Plumb
Waters Corporation, Milford, MA, USA

INTRODUCTION

The ability to understand the levels of pharmaceutical impurities is not only a regulatory necessity, but a business imperative. Analytical determination of impurities is often time-constraining and resource-consuming. Analysts require a range of mass spectrometry capabilities as well as sophisticated software to facilitate data processing of these complex impurity data sets.

Here, we explore a multidisciplinary approach to impurity analysis using a systematic workflow that is capable of highly specific and highly sensitive detection and determination of impurities that are present in quetiapine hemifumarate active pharmaceutical ingredient (API) drug substance. The designed approach incorporates superior chromatographic resolution, confident impurity identification, and rapid structural elucidation facilitated by intelligent and user-friendly software. This workflow-based methodology improves the ability to evaluate known and unknown impurities in a pharmaceutical drug substance.

Using a variety of software solutions within a central chromatographic data system, results are reported in the MetaboLynx™ XS data browser. The software intelligently processes chromatographic and exact mass data to report retention times, peak area, mass accuracy, and isotope distribution values for *m/z* found. Elemental compositions are confirmed for known impurities and proposed for unknown impurities. The software also performs a fragment analysis, correlating the precursor ion information of the low-energy-collision MS scan to that of the product ion information of the high-energy MS scan. The high-collision-energy MS scan data is imported into the MassFragment™ Software, where structural fragmentation pathways of the impurity compounds are proposed based on the likelihood of breaking certain bonds.

EXPERIMENTAL

LC conditions

LC system:	ACQUITY UPLC®
Column:	ACQUITY UPLC BEH C_{18}, 100 x 2.1 mm, 1.7 µm
Temperature:	65 °C
Injection vol.:	3 µL
Mobile phase A:	20 mM Ammonium Bicarbonate, pH 10
Mobile phase B:	Acetonitrile

Gradient:

	Time (min)	Flow (mL/min)	%A	%B	Curve
1	Initial	0.800	85.0	15.0	Initial
2	1.31	0.800	85.0	15.0	6
3	10.49	0.800	61.0	39.0	6
4	14.40	0.800	57.0	43.0	6
5	18.03	0.800	5.0	95.0	6
6	20.00	0.800	5.0	95.0	6

Detection: ACQUITY UPLC PDA at 250 nm

MS conditions

MS system:	SYNAPT® MS
Source:	ES positive
Capillary:	1.5 kV
Sample cone (V):	40 V for reference 35 V for analyte
Extraction cone:	4.0 V
Desolvation temp.:	450.0 °C
Source temp.:	120.0 °C
Desolvation flow:	900.0 L/Hr
Acquisition range:	100 to 1000 *m/z*
Scan time:	0.095 sec
Interscan delay:	0.02 sec
Lock mass:	300 pg/µL Leucine/Enkephalin at 50 µL/min
MS^E settings:	4 eV low collision energy 20 eV high collision energy

Software

MetaboLynx XS and MassFragment application managers for MassLynx® 4.1 Software

Workflow

The workflow approach shown in Figure 1 may require several iterations to determine the accurate result for the unknown peak of interest. Evaluation of the data can be more involved depending on the complexity of the compound; however, the general workflow remains constant. The benefit of this approach is that it provides a systematic data-driven association to correlate the variety of data acquired by the two scan functions generated by MS^E experiments.

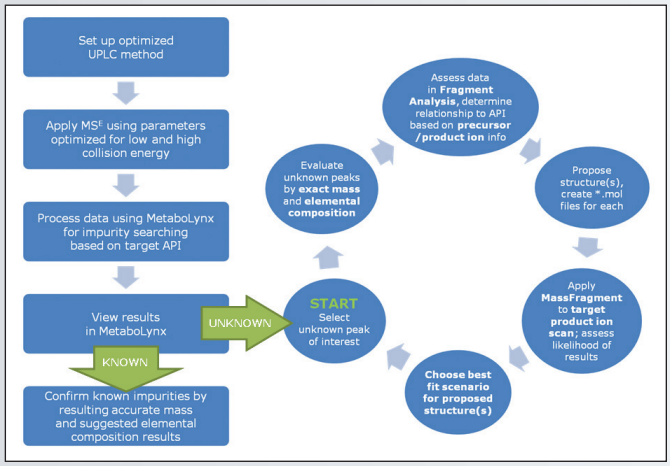

Figure 1. Workflow for impurity identification in an API impurity profile.

RESULTS AND DISCUSSION

The MetaboLynx XS Application Manager provides the flexibility to apply user-defined filters to configure how the reported data is viewed in the browser window. Some useful techniques to apply meaningful data filters were identified by investigating proper integration parameters. Mass defect filters, the dealkylation tool, spectrum intensity thresholding, and selection of components relative to the compound in the elemental composition tab all proved highly useful in displaying more confident data.

For example, to get elemental composition for every peak found in a chromatogram, the analyst would typically have to combine MS scans and perform background subtraction for each peak of interest and then generate individual elemental composition reports. To streamline this process, the MetaboLynx XS browser populates all impurity peaks integrated in the Tof-MS ES+ chromatographic trace with associated elemental compositions, mass accuracy, and isotope pattern scoring using i-FIT,™ and displays the results in a single window (Figure 2).

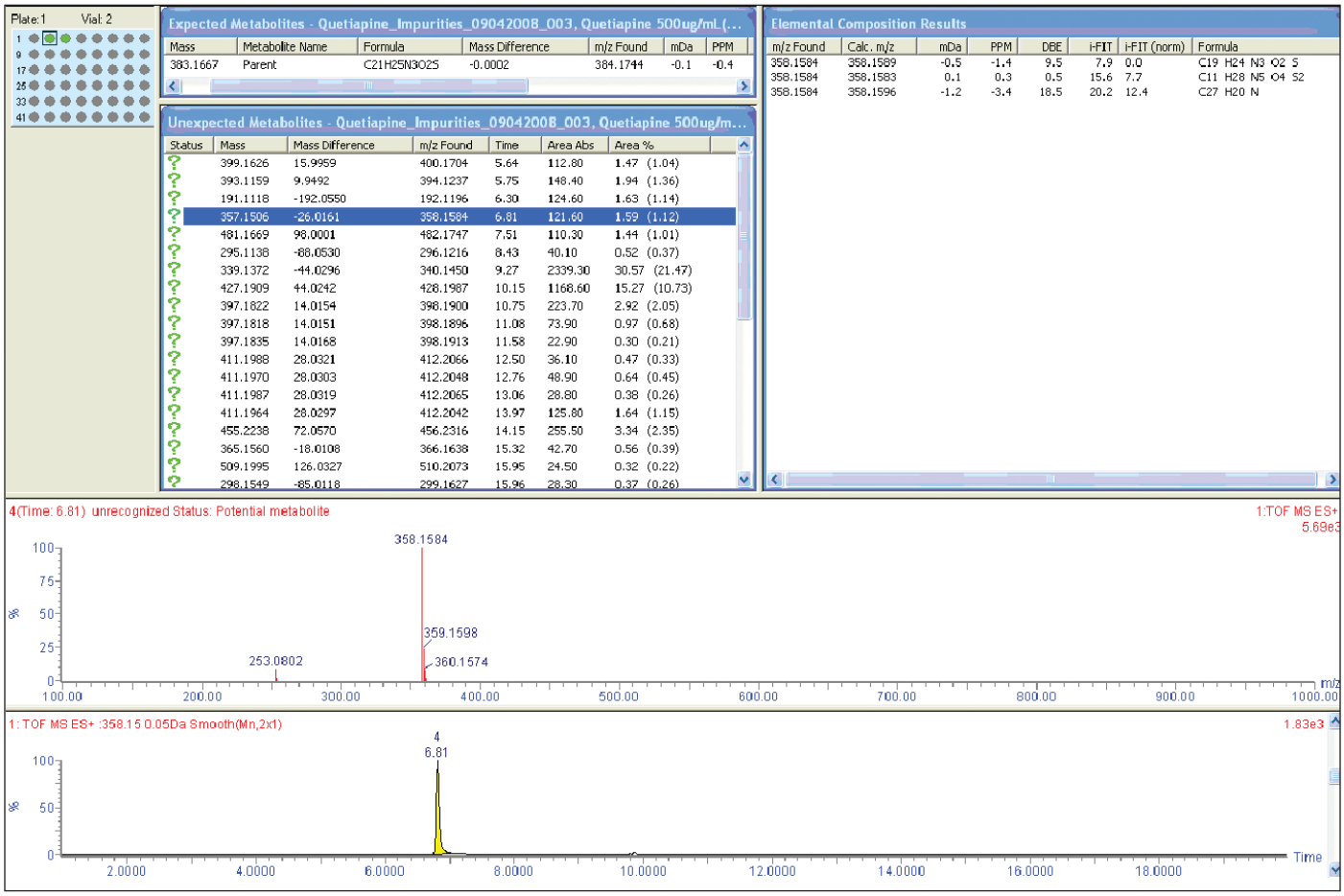

Figure 2. MetaboLynx XS browser window displaying the various chromatographic and MS spectral information generated by the MS^E experiments.

Evaluating known and unknown impurities

Evaluation of the unknown impurity peaks by exact mass and elemental composition of quetiapine hemifumarate using MetaboLynx XS indicated that the mass accuracy of the API quetiapine was reported to be 0.4 ppm. A total of 80 impurity peaks were listed. Upon adjustments to integration and data filtering, 44 peaks were found to be relevant. Non-relevant peaks were observed to be anomalies of initial integration of noise and peaks with extremely low-level response in UV and MS detection.

Ten known impurities were observed with an average mass accuracy of 1.3 ppm. Two known masses, 398.19xx and 412.20xx, had three and four separate retention times listed, respectively. The masses with multiple chromatographic retention times, which indicated possible structural isomers, were:

[M+H] = 398.19xx observed four peaks, three of which met the reporting threshold. The observed [M+H] = 398.1900, 398.1896, 398.1913 at retention times (RT) of 10.75 min., 11.08 min., and 11.58 min., with measured mass accuracies of 0.5 ppm, 1.5 ppm, and 2.8 ppm, respectively, resulted in an identified elemental composition of $C_{22}H_{28}N_3O_2S$

[M+H] = 412.20xx observed five peaks, four of which met the reporting threshold. The observed [M+H] = 412.2066, 412.2048, 412.2065, and 412.2059 at retention times (RT) of 12.50 min, 12.76 min, 13.06 min, and 13.97 min, with measured mass accuracies of 1.7 ppm, 2.7 ppm, 1.5 ppm, and 4.1 ppm, respectively, resulted in an identified elemental composition of $C_{22}H_{29}N_3O_2S$.

In terms of the unknowns that were identified, of 21 entries for 15 chromatographic peaks:

Peaks identified as doubly charged species:

- $[M+2H]2^+$ = 353.1512, [M+H]+ 705.3013 at RT = 17.20 min
- $[M+2H]2^+$ = 309.1256, [M+H]+ 617.2514 at RT = 17.36 min
- $[M+2H]2^+$ = 684.2089 with a large fragment at [M+H] = 382.3485

Peaks with multiple *m/z* ions; which could be possible coelutions, included:

- Peak RT = 15.96 min observed [M+H] = 510.2073, 299.1627, 399.2523 (three intense *m/z* values)
- Peak RT = 17.42 min observed [M+H] = 653.3301, 592.1955 (two intense *m/z* values)

From these data, we can generate and assess the data in the Fragment Analysis function of MetaboLynx XS by determining the relationship to the API based on the MS^E precursor/product ion information.

Fragment analysis

The Fragment Analysis tool aligned the high and low collision energy data that were simultaneously collected during the MS^E acquisition. The resulting information was displayed in a collective window where the precursor and the collision-induced product ions were evaluated spectrally and presented chromatographically. The Fragment Analysis window allowed for numerous iterations by the analyst to assess common fragment ions between peaks of interest (Figure 3). Commonalities were observed between known impurity structures and fragmentation patterns that aided in proposing the structures of other unknown impurity entities.

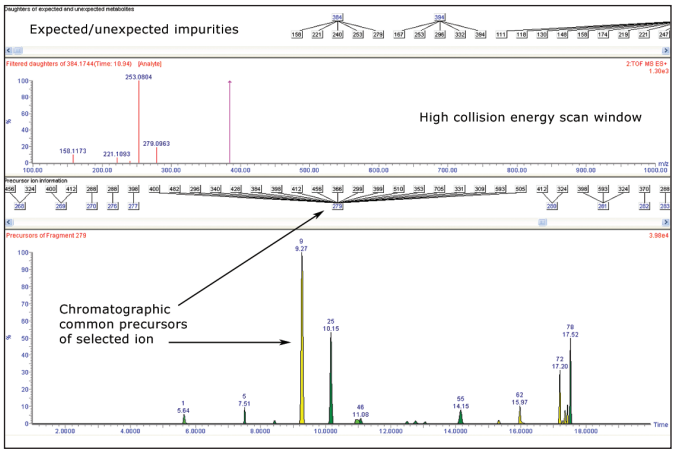

Figure 3. Fragment Analysis window of MetaboLynx XS correlating the low-collision-energy MS scan data with the high-collision-energy MS scan data.

The assessment of the common fragment ions of quetiapine identified the major fragment ions to be *m/z* 279, 253, 221, and 158:

- XIC of precursor 279 was identified in 22 impurity peaks
- XIC of precursor 253 was identified in 25 impurity peaks
- XIC of precursor 221 was identified in 23 impurity peaks
- 14 impurity peaks were deemed not to be directly related to the parent

Structural elucidation

MassFragment is a chemically-intelligent software tool that combines the aligned high and low collision energy data in the MetaboLynx XS Fragment Analysis window with the user's input about a hypothesized structure to facilitate structural elucidation. Prior to performing the elucidation procedure, a proposed parent structure (or structures) is saved as a "*.mol" file.

Upon opening MassFragment, a dialog window prompts the selection of the *.mol file. The fragment ion information from the Fragment Analysis product ion's high-collision-energy scan window of the selected observed impurity mass automatically exports to MassFragment along with the *.mol file. Potential structures are assigned and scored for the precursor ions in the isotopically-filtered spectrum.

Figure 4 shows an example of the report generated by MassFragment for the unknown impurity [M+H] 456.2305. Other conclusions determined by the MassFragment data included:

- Many of the impurities have the common fragment ions *m/z* 279, 253, 221, and 158, as observed in the API quetiapine
- MassFragment confirmed similar fragmentation patterns of the imported structures with excellent mass accuracy generally less than 2.0 mDa
- It was also hypothesized that the structure undergoes a structural rearrangement after the cleavage of the piperazine ring,[1] however this did not seem to affect the mass accuracy of many of the proposed fragmentation pathways of the assumed parent structure of the unknown impurity

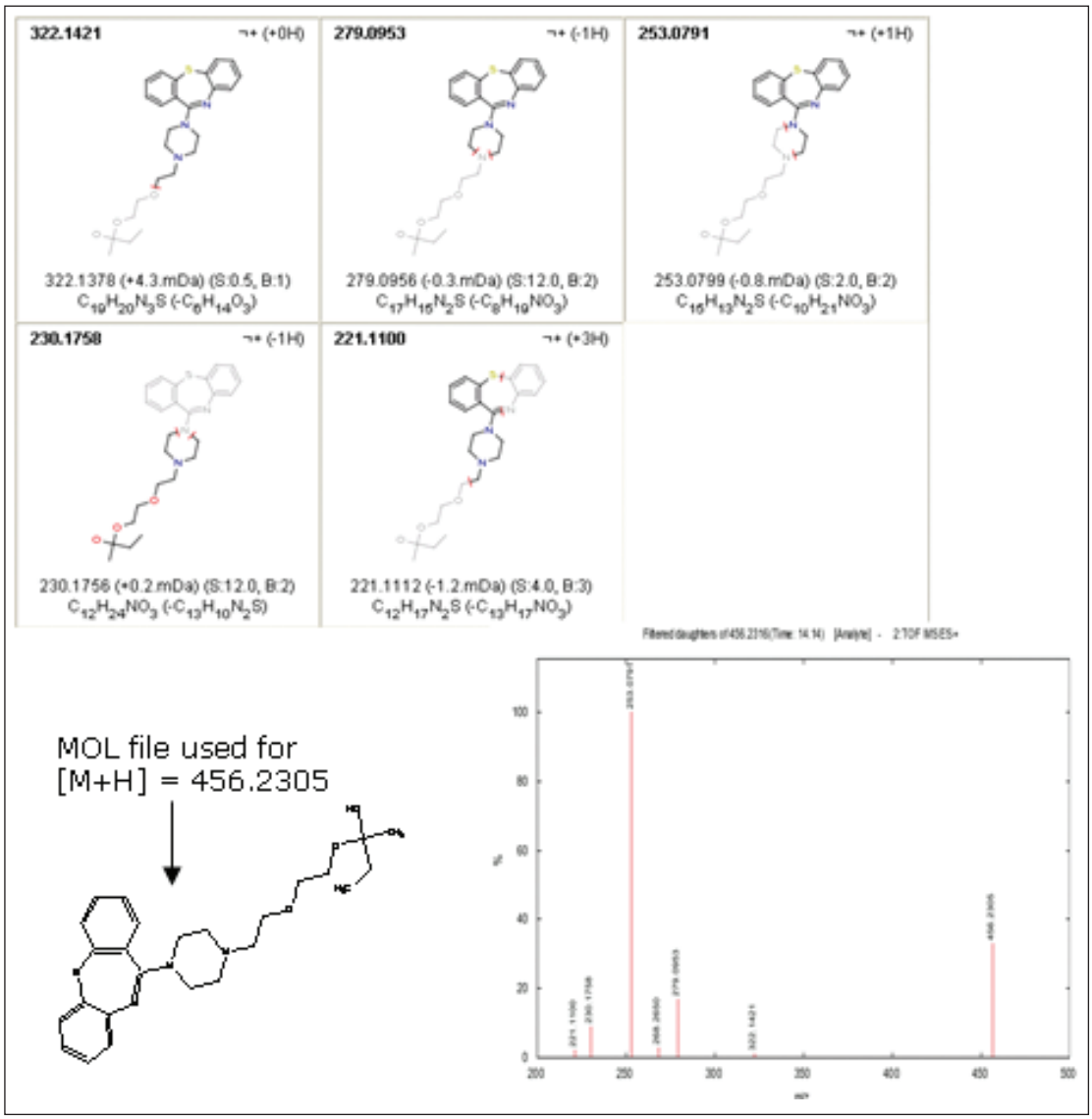

Figure 4. Snapshot of a MassFragment summary report of possible structures for each of the fragment ions in the isotopically-filtered spectrum.

CONCLUSIONS

Data collection using ACQUITY UPLC with the SYNAPT MS provided high chromatographic resolution, ample sensitivity, and superior mass accuracy to identify many of the impurities in the quetiapine hemifumarate drug substance. MS^E provided simultaneous acquisition of both high and low collision energy, maximizing the information gathered from a single injection. This analytical workflow was followed by a deliberate data processing workflow that streamlined the fragment analysis and structural elucidation process and provided greater confidence in the end results.

The MetaboLynx browser provided:

- A comprehensive list of elemental compositions for the known and unknown peaks
- 10 known impurities were rapidly identified with an average mass accuracy <3.0 ppm
- [M+H] = 398 and 412 were observed to have a series of structural isomers

Using MetaboLynx's Fragment Analysis:

- A minimum of 25 impurity peaks were identified as being related to quetiapine utilizing the common fragment ions *m/z* 279, 253, 221, and 158
- 14 integrated impurity peaks were identified with no common fragment ions

Using MassFragment:

- The structures of the 10 known impurities were rapidly confirmed
- Information of the possible structural isomers for [M+H] = 398 and 412 were easily compared to various proposed structural isomers for best-fit correlation to the high collision energy data.

In some cases where the peak identification was more challenging, MetaboLynx was able to help formulate decisions about compound determination. The combination of these three software tools, along with the optimized instrument configurations for impurity analysis and efficient MS^E acquisition, provided a systematic workflow approach that can readily be applied to identify and confirm known and unknown peaks in an impurity profile.

This workflow-based approach delivers the rapid and systematic set of comprehensive results that are needed to identify and confirm impurities in an API impurity profile.

Reference

1. Xu H, et al. J. Pharma. *Biomed. Anal*. 2007; 44: 414–20.

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Waters Corporation
34 Maple Street
Milford, MA 01757 U.S.A.
T: 1 508 478 2000
F: 1 508 872 1990
www.waters.com