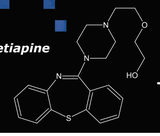
A WORKFLOW APPROACH FOR THE IDENTIFICATION AND STRUCTURAL ELUCIDATION OF IMPURITIES IN QUETIAPINE HEMIFUMARATE DRUG SUBSTANCE



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INTRODUCTION

The analysis and understanding of pharmaceutical impurities are essential for any final product. It is a business objective to understand as many impurities as possible. The purpose of this presentation will be to explore a multidiscipline approach with a workflow concept capable of highly specific and highly sensitive detection and determination of impurities present in quetiapine hemifumarate drug substance. The designed approach incorporates superior chromatographic resolution, confident impurity identification, structural elucidation and ease of use software solutions. The workflow approach demonstrated the ability to evaluate known and unknown impurities in a pharmaceutical drug substance. The use of Ultra Performance Liquid Chromatography (UPLC), inline with oa-ToF Mass Spectrometry, has offered real improvements in resolution of the impurity analysis. Using a variety of software solutions within a central chromatographic data system, results were reported in a data browser whereas chromatographic and exact mass spectroscopic data proposed the elemental compositions and possible structural fragmentation pathways of the impurity compounds. This workflow approach provided a rapid systematic set of comprehensive results needed to identify and confirm impurities in a raw pharmaceutical drug substance impurity profile.

EXPERIMENTAL

Instrument: ACQUITY UPLC
Column: ACQUITY UPLC™ BEH C_{18}
Dimensions: 100 x 2.1mm, 1.7µm
Mobile Phase: A: 20mM Ammonium Bicarbonate, pH10
B: Acetonitrile

Gradient:

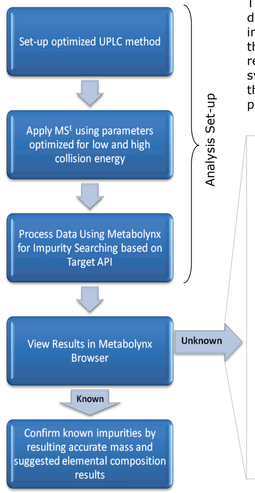
	Time (min)	Flow (mL/min)	%A	%B	Curve
1	initial	0.800	85.0	15.0	initial
2	1.31	0.800	85.0	15.0	6
3	10.49	0.800	61.0	39.0	6
4	14.40	0.800	57.0	43.0	6
5	18.03	0.800	5.0	95.0	6
6	20.00	0.800	5.0	95.0	6

Temperature: 65°C
Inj Volume: 3 µL
Detection: ACQUITY PDA @ 250 nm

MS Conditions
Instrument: Waters® Synapt™
Software: Masslynx™ 4.1

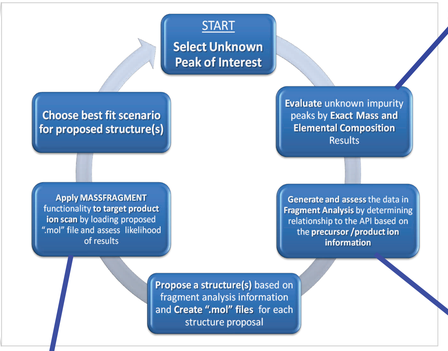
Tune Page Parameters:
Source: ES+
Capillary (kV): 3.2
Sample Cone (V): 40 for reference
35 for analyte
Extraction Cone (V): 4.0
Desolvation Temp (°C): 450.0
Source Temp (°C): 120.0
Desolvation Flow (L/Hr): 900.0
Acquisition Range: 100 - 1000Da
Scan Time: 0.095s
Interscan delay: 0.02s
Lock mass: 300pg/µL Leucine/Enkephalin @ 50µL/min

MS^E settings
Low collision energy: 4eV
High collision energy: 20eV

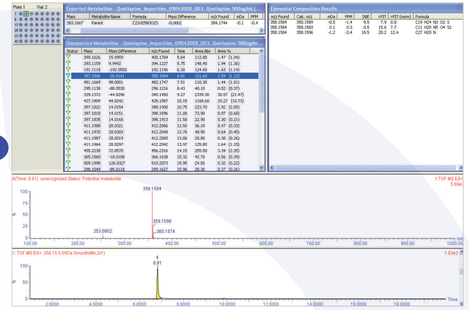


WORKFLOW

The workflow approach illustrated may involve many iterations to determine the appropriate sensible 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 to this approach is that it provides a systematic association to correlate the variety of data acquired from the ToF accurate mass information to the MS/MS fragmentation patterns.



METABOLYNX BROWSER



Notable Browser Results

- Mass accuracy of the API quetiapine was reported to be 0.4ppm
- Upon data filtering, 80 impurity peaks were listed. 44 peaks were relevant after review of integration.

Knowns Identified

- 10 known impurities were observed with an average mass accuracy of 1.3ppm. Two known masses (398.19xx and 412.20xx) had 3 and 4 separate retention times listed, respectively.

Masses with multiple chromatographic retention times (possible structural isomers.)

- [M+H] = 398.19xx observed 4 peaks, 3 of which met reporting threshold. The observed [M+H] = 398.1900, 398.1896, 398.1913 at retention times (RT) = 10.75 min., 11.08 min., and 11.58 min. measuring mass accuracies of 0.5ppm, 1.5ppm, and 2.8ppm, respectively for an identified elemental composition of C22 H28 N3 O2 S.
- [M+H] = 412.20xx observed 5 peaks, 4 of which met reporting threshold. The observed [M+H] = 412.2066, 412.2048, 412.2065, and 412.2059 at retention times (RT) = 12.50min, 12.76min, 13.06min, and 13.97min measuring mass accuracies 1.7ppm, 2.7ppm, 1.5ppm, and *4.1ppm, respectively for an identified elemental composition of C23 H29 N3 O2 S

Unknowns Identified

- 21 entries for 15 chromatographic peaks 3 identified as doubly charged (6 entries)
  - $[M+2H]^{2+}$ = 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.

Peaks with multiple m/z ions (possible co-elutions)

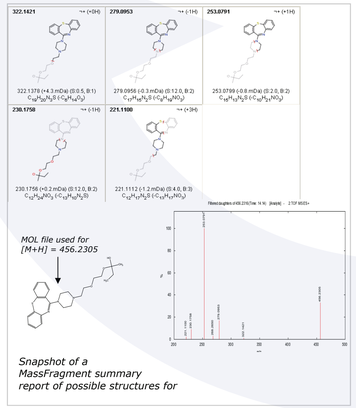
- Peak RT=15.96min observed [M+H] = 510.2073, 299.1627, 399.2523 (3 entries)
- Peak RT= 17.42min observed [M+H] = 653.3301, 592.1955 (2 entries)

The Metabolynx Set-up file allows many approaches to view the data reported in the Metabolynx Browser by applying a variety of user defined filters. Some useful techniques to provide meaningful data filters were identified by spending time investigating proper integration parameters. Mass defect filters, the dealkylation tool, spectrum intensity thresholding and selecting components relative to the compound in the elemental composition tab all proved highly useful to displaying higher confident data. Typically, to get elemental composition for every peak found in a chromatogram, the analyst would have to combine and background subtract MS scans for each peak of interest and generate separate elemental composition reports. To facilitate this process, the Metabolynx browser populated all impurity peaks integrated in the Tof MS ES+ chromatographic trace with associated elemental compositions, mass accuracy in ppm, and isotope pattern (i-Fit) scoring. The Metabolynx browser window enabled the observation of the above information on a batch of drug substance Quetiapine hemifumarate

MASSFRAGMENT

MassFragment is a chemically intelligent software tool that utilizes the aligned low-high CE data in the Fragment Analysis window and the user's input about a hypothesized structure whereas it facilitates structural elucidations. A proposed structure(s) saved as a ".mol" file along with the fragment ion information from the Masslynx Fragment Analysis product ion high-CE scan window of the selected observed impurity mass can be automatically exported to MassFragment. The potential structures can be assigned and scored for the precursor ions in the isotopically filtered spectrum.

- Many of the impurities have common fragment ions 279, 253, 221 and 158 as observed in the API quetiapine.
- MassFragment confirmed similar fragmentation patterns of the imported structures with good mass accuracy.
- It was observed that the mass accuracy of some of the smaller fragment ions (<253 Da) associated with some of proposed fragment structures were not scored appropriately because the default set-up of MassFragment was not optimized for appropriate scoring (ie: easy loss of sulfur in the ring).
- 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 for many of the proposed fragment pathways of the assumed structure of the unknown impurity.



Snapshot of a MassFragment summary report of possible structures for

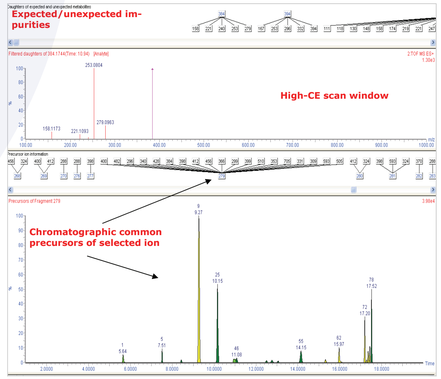
FRAGMENT ANALYSIS

Assessment of the common fragment ions of Quetiapine.

Observed major fragment ions: 279, 253, 221, and 158

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

The Fragment Analysis tool aligned the high and low collision energy data that was 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 chromatographically. The Fragment Analysis window allowed for numerous iterations of assessing common fragment ions between peaks of interest. Commonalities were observed between known impurity structures and fragmentation patterns that aided the proposed structures of the other unknown impurity entities.



CONCLUSIONS

- The collection of the data via UPLC and oa-Tof provided high chromatographic resolution, ample sensitivity, and superior mass accuracy to identify many of the impurities in Quetiapine hemifumarate drug substance.
- MSe provided simultaneous acquisition of low-CE and high-CE maximizing the information gathered for a single injection
- The Metabolynx browser provided information regarding:
  - A comprehensive list of elemental compositions for the known and unknown peaks
  - 10 known impurities were rapidly identified with a mass accuracy < 3.0ppm on average
  - [M+H] = 398 and 412 were observed to have a series of structural isomers
- Using Fragment Analysis
  - A minimum of 25 impurity peaks were identified as related to Quetiapine utilizing the common fragment ions 279, 253, 221, 158
  - 14 impurity peaks integrated 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 iteratively but easily compared to various proposed structural isomers for best fit correlation to the high-CE data.

In some cases where the peak ID was more challenging, Metabolynx was able to steer the compound determination in the decision making process in the right direction. The combination of the three software functionalities within Metabolynx, the optimized instrument configurations for impurity analysis, and efficient MS^E acquisition provided a systematic workflow approach that could be applied to identify and confirm known and unknown peaks in an impurity profile.

References

1. H.Xu et al, J. Pharma. Biomed. Anal. 44(2007)414-420

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