ALMAC

Partnering to Advance Human Health

Trace Impurity Identification using Q-TOF Mass Spectroscopy

Authors: Dr Alan Thompson and Dr John Malone

Background

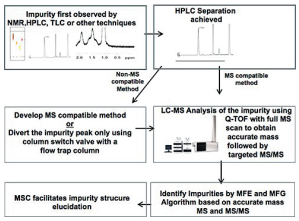
Accurate Mass Spectrometry (MS) techniques are ideally suited to aid in the structural elucidation of trace impurities. A rapid approach used for the isolation and characterisation of trace impurities in drug substances and formulated drug products has been developed. The approach has been successfully employed for the characterisation of impurities from different substances covering a wide variety of structural classes.

FDA and International Conference on Harmonization (ICH) set strict requirements regarding impurities in APIs and formulated drug products. It defines thresholds at which impurities must be identified or adequately tested. Investigations are initiated during the early stages of drug discovery or product development such as the pre-investigational new drug (pre-IND) stage.

Accurate Mass Spectrometry (MS) techniques are ideally suited to aid in the structural elucidation of trace impurities. A rapid approach used for the isolation and characterisation of trace impurities in drug substances and formulated drug products has been developed. The approach has been successfully employed for the characterisation of impurities from different substances covering a wide variety of structural classes. A case study is presented herein.

Outline of the impurity isolation and structure elucidation workflow:

Single sample preparation for Assay and Impurities LOQ ≤ 0.04% Resolution of critical pair ≥ 1.5 Assay and impurities to be determined using calibration curve of 30% -130% of nominal test concentration. Test solution should be stable at least for 6 – 8 hours (Increase of Imp. ≤0.02%)

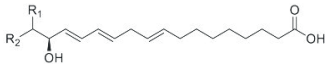


Impurity ID by accurate mass LC-MS/MS (LC-ESI-QTOF)

Almac completed a laboratory feasibility study and manufactured a demonstration batch of API. The work involved necessary analytical and process development to enable safe and reliable process scale up and API stability evaluation conforming to cGMP.

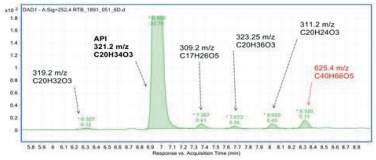
Structure of API

identified the molecular formula of all impurity peaks present in the API chromatography with high score using Molecular Feature Extraction (MFE) and Molecular Formula Generation (MFG) algorithms..



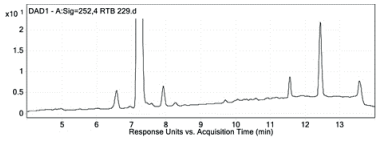
Accurate mass impurity profiling

Almac conducted a stability trial on the API to investigate a range of diluents, stabilisers and conditions. Impurity A concentration increased upon incubation and structural elucidation was necessary.



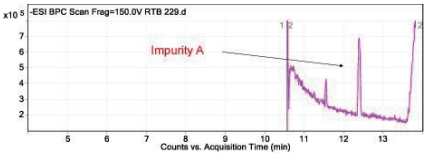
Stability study revealed increases in Impurity A

MS compatible method was developed, and total ion chromatogram was collected (ESI-).



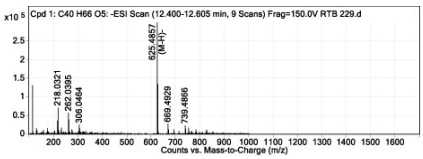
Total ion chromatogram

Blank subtraction and peak extraction of the previous chromatogram yielded the following accurate mass spectrum



Mass spectrum of impurity A

The molecular formula generation (MFG) algorithm successfully identified the formula with high score reflecting accurate-theoretical mass matching and the excellent isotopic distribution matching.



Compound Table

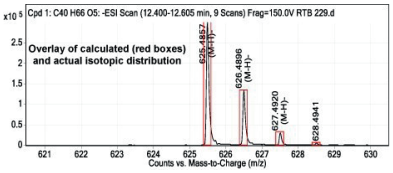
Compound Label	RT	Mass	Formula	MFG Formula	MFG Diff (ppm)	DB formula
Cpd 1: C40 H66 O5	12.498	626.4702	C40 H66 O5	C40 H66 O5	-3.26	C40 H66 O5

Predicted Isotope Match Table

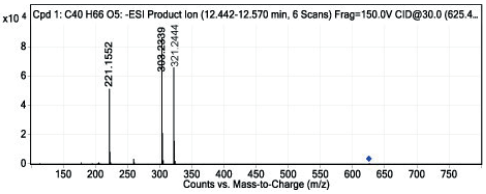
Isotope	m/z	Calc m/z	Diff (ppm)	Abund %	Calc Abund %	Abund Sum %	Calc Abund Sum %
1	625.4857	625.4857	-3.06	100	100	63.66	63.66
2	626.4896	626.4877	-3.56	44.65	44.2	28.53	28.73
3	627.492	627.4909	-1.72	10.26	10.56	6.57	6.73
4	628.4941	628.4933	-1.15	1.65	1.79	1.06	1.14

Predicted isotope match

MS/MS fragmentation pattern @ 30 eV displayed three major daughter ions. The Molecular Formula Generation (MFG) algorithm successfully identified the daughter ions formula enabling the elucidation of the structure of the impurity.



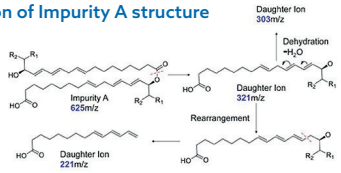
Targeted MS/MS fragmentation



Peak List

m/z		Abund	Formula
221.1552	1	51347.2	C14 H21 O2
222.1586	1	8394	
303.2339	1	87005.1	C20 H31 O2
304.2371	1	21094.2	
321.2444	1	65634.5	C20 H33 O3
322.2475	1	15864.4	

Elucidation of Impurity A structure



Conclusion

The structure of the trace impurity A was successfully determined using Accurate Mass analysis by a Q-TOF mass spectrometer. The targeted MS/MS algorithm successfully identified the general chemical formulas of the impurity molecular and daughter ions. The information was used to elucidate the detailed chemical structure.

For further information:
john.malone@almacgroup.com