

Characterization of Impurities in Synthesized Fine Chemical Products

GOAL

To successfully characterize impurities in synthesized fine chemicals by exact mass MS/MS using *FastDDA* on the Xevo® G2 QTof.

BACKGROUND

Analytical laboratories studying the products of organic synthesis have to consider many things from confirmation of the final product to identification of impurities. Impurity identification, whether expected or not, is an essential part of the manufacture of fine chemicals, as any impurities could adversely affect the properties of the final product. This applies equally to the raw starting materials and the final synthesized product.

Obtaining MS/MS product ion spectra is one useful way of elucidating the structure of impurities, while exact mass provides additional confidence to structural assignments. However, the manual setup of each product ion experiment can be time consuming and prone to human error, so a Data Directed Analysis (DDA) is beneficial with regard to laboratory resources. DDA automatically selects ions for MS/MS acquisitions in real time, as components elute from the column. Recent improvements in the spectral acquisition rate (30 Hz) now enable DDA to be compatible with UPLC® peaks.

***FastDDA* automatically produces MS/MS product ion spectra that can be used to successfully elucidate the structure of trace level impurities.**

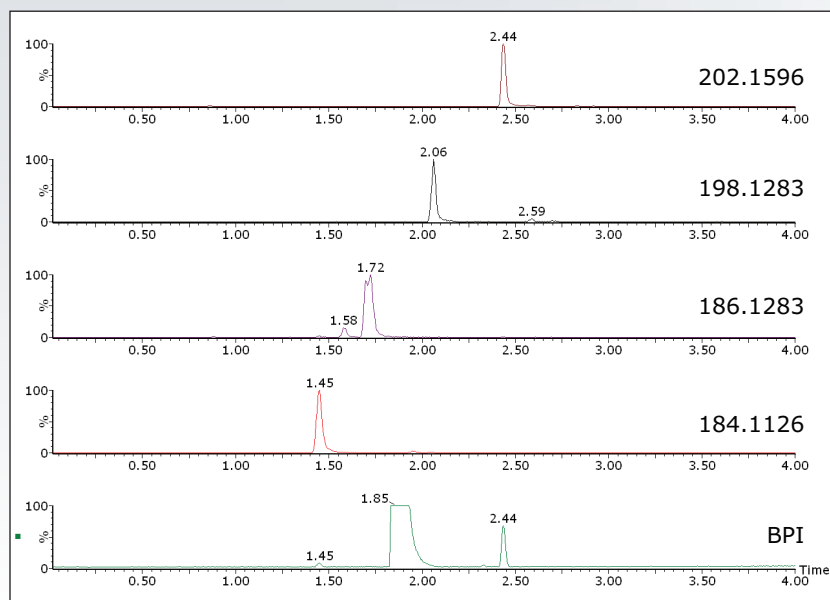


Figure 1. BPI chromatogram for octahydroacridine ($R_t = 1.85$ min) with extracted mass chromatograms for four of the impurities found automatically by *FastDDA*.

Octahydroacridine (>97% purity) was used here for illustration purposes. Base peak intensity (BPI) chromatogram and extracted ion chromatograms of some of the impurities found automatically by *FastDDA* are shown in Figure 1. Octahydroacridine is interesting as it plays an important role in the preparation of alkaloids, dyes, drugs, and other biologically active compounds.

THE SOLUTION

Waters® Xevo G2 QTof, coupled with the ACQUITY UPLC® System and operated in *FastDDA* acquisition mode, rapidly and automatically generated MS/MS product ion spectra of all the impurities, enabling structural elucidation to be performed with MassFragment™ Software.

FastDDA intelligently selects ions for MS/MS acquisitions in real time, as components elute from the chromatographic system. Embedded algorithms rapidly interrogate MS survey spectra and co-eluting precursor ions are selected for MS/MS analysis based on threshold intensity and pre-defined exact mass include/exclude lists.

In this example, octahydroacridine (m/z 188.1439) was excluded from the *FastDDA* setup as the expected compound. This setup allowed seven discrete impurities to be characterized with the automatic generation of high resolution MS/MS product ion spectra, four of which are shown in Figure 2.

MassFragment Software, the automated structural elucidation tool which uses a systematic bond cleavage and ranking algorithm, was employed to rationalize and identify fragment ion structures from potential impurities. By submitting a postulated precursor structure and a MS/MS product ion spectrum, the MassFragment Software tool generates a report of possible fragmentation with exact mass confirmation. An extract from the MassFragment results summary for the impurity, m/z 202.1596, is shown in Figure 3.

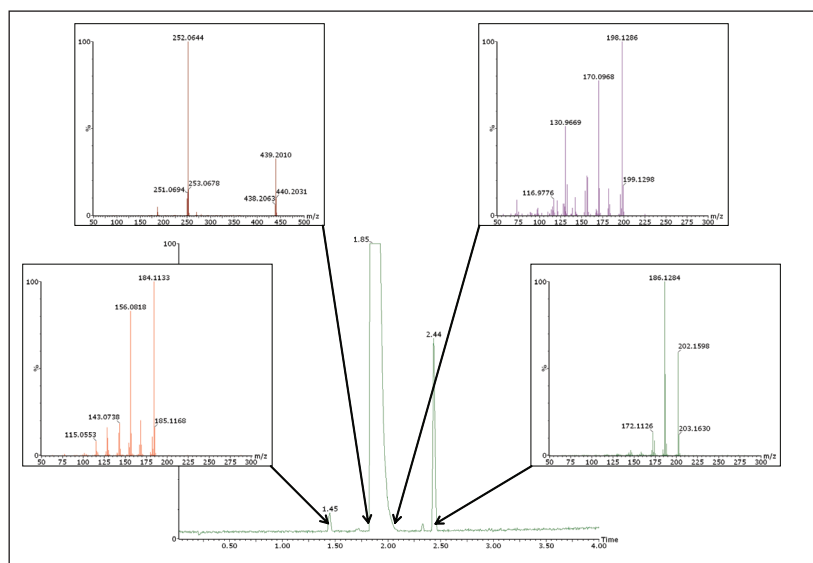


Figure 2. *FastDDA* spectra for some of the impurities of octahydroacridine.






		ID (job)	17			
		Mass (Da)	201.1517			
		Formula	C ₁₄ H ₁₄ N ¹			
		DBE	6			
Product mass(es) (Da)	172.1125-186.1284 187.1340 202.1598 203.1630					
DBE	+/- 0.01 in positive mode, structure filter 1					
Electron count	100					
Maximum m/z	203					
Fragment number of bonds	4					
Scoring	aromatic: 6, multiple: 4, ring: 2, phenyl: 8, other: 1					
Order:	mass					
Daughter (Da) (structure)	Mass (Da)	Mass error (mDa)	Formula	DBE	α-Formula	Structure(s), score & H-deficit
202.1598 (1700002)	202.1596	+0.2	C ₁₄ H ₁₄ N ¹	6.5	none	 0.0000 H+1 <input type="checkbox"/>
187.1340 (1231752)	187.1341	-1.3	C ₁₄ H ₁₄ N ¹	6+	CH ₃	 0.1000 H+1 <input type="checkbox"/>
186.1284 (1700002)	186.1283	+0.1	C ₁₄ H ₁₄ N ¹	6.5	CH ₃	 0.1000 H+1 <input type="checkbox"/>
172.1125 (1400002)	172.1126	-0.1	C ₁₄ H ₁₄ N ¹	6.5	C ₂ H ₅	 0.4000 H+1 <input type="checkbox"/>

Figure 3. MassFragment report summary for m/z 202.1596.

SUMMARY

The synthesized product and trace-level impurities were successfully characterized using the:

- High resolution capabilities of UPLC
- Rapid, automated, and intelligent acquisition rates of *FastDDA*
- Sensitivity, resolution, mass accuracy, and dynamic range of Xevo G2 QTof
- Automation of MassFragment Software

The rapid, automated nature of this solution minimizes the need for manual intervention, which reduces the drain on laboratory resources and maximizes the information obtained from a single analytical experiment.

Waters

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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

