Application Note

Characterization of Impurities in the Fungicide Flutriafol Using UNIFI and UPLC-ToF-MS^E

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Abstract

Flutriafol is a triazole fungicide that is used in the treatment of a broad spectrum of diseases in cereal crops. This application note describes the analysis of technical-grade flutriafol material using the ACQUITY UPLC I-Class System coupled to the Xevo G2-XS QTof and PDA Detector

Benefits

- A comprehensive approach to the characterization of structurally related components in technical grade flutriafol.
- A rapid chromatographic separation that provides maximum component resolution.
- Accurate mass precursor and product ion information collected simultaneously increase confidence in the compound assignments.

Introduction

The profiling, identification, and quantification of impurities plays a critical role at all stages of both the pesticide development and manufacturing processes, and are essential requirements for the registration of crop protection products. Impurities in agrochemical products are regulated in order to ensure product safety and compliance. The structure of impurities present at or greater than 0.1% in the technical grade active substance must be identified to ensure that the overall safety of the formulated product is well understood.[1] Impurities of toxicological significance are of particular interest.

During the development process, different routes of synthesis may be evaluated, each producing its own distinct impurity profile. It is critical that these synthesis routes are thoroughly investigated with an aim to identify (or elucidate) relevant impurities alerted by the threshold criteria. Analytical investigation is often performed using a combination of LC-MS and other techniques that provide structural information, such as NMR spectroscopy. Modern, high-sensitivity exact mass instruments with time-of-flight (Tof) technology are capable of extremely fast acquisition rates and offer the opportunity to dramatically simplify the process of impurity data capture and analysis. These high acquisition rates allow mass spectrometry to be coupled together with UltraPerformance Liquid Chromatography (UPLC), thereby greatly facilitating the analysis of complex samples. Additionally, Waters' proprietary Q-Tof technology enables the simultaneous collection of low and high collision energy (CE) data, otherwise referred to as "MS^E",[2] allowing the capture and interpretation of precursor and product ion data from a single analytical injection. As this data is collected with a high degree of mass accuracy, elemental compositions can be obtained for both intact molecular ions (e.g., $M+H^+$) and structurally significant fragments greatly aiding structural elucidation.

In this application note, Waters ACQUITY UPLC I-Class System coupled to the Xevo G2-XS QTof and the ACQUITY UPLC PDA Detector were used to analyze technical-grade flutriafol material. Flutriafol, shown in Figure 1, is a triazole fungicide that is used in the treatment of a broad spectrum of diseases in cereal crops.[3] The Screening Platform Solution with UNIFI was used to screen the sample and expedite the discovery of unknown components of interest, as well as to confirm the presence of the active ingredient (AI). Using the data evaluation tools available in UNIFI, relationships between the unknown components were easily visualized and the impurity relationship to flutriafol was hypothesized. The MS data from the impurity profile can be later be used to further refine the synthetic strategy.

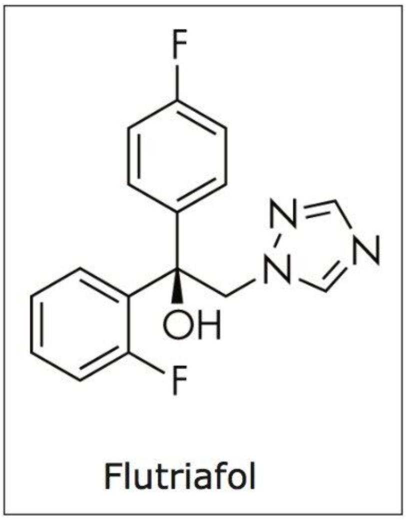


Figure 1. Structure of flutriafol.

Experimental

UPLC conditions

UPLC system: ACQUITY UPLC I-Class

Column:	CORTECS C_{18} 2.1 x 100 mm, 1.6 µm
Mobile phase A:	Water with 0.1% formic acid
Mobile phase B:	Acetonitrile with 0.1% formic acid
Flow rate:	0.6 mL/min
Column temp.:	50 °C
Injection volume:	1 µL
Gradient:	0 min 10% B, 10 min 90% B return to initial conditions

PDA conditions

Detector:	ACQUITY UPLC PDA
Wavelength:	210 to 400 nm
Sampling rate:	20 Hz

MS conditions

MS system:	Xevo G2-S QTof
Ionization mode:	ESI+
Analyzer mode:	MS^E in resolution mode
Capillary voltage:	1.0 kV
Cone voltage:	25 V

Desolvation gas temp.:	450 °C
Source temp.:	150 °C
Cone gas flow:	50 L/hr
Desolvation gas:	900L/hr
MS^E low collision energy (CE):	4 eV
MS^E high collision energy ramp:	15 to 30 eV
MS scan range:	50 to 950 *m/z*
Scan time:	0.15 s

Results and Discussion

UPLC separation using the CORTECS C_{18} Column provided maximum component resolution. Three minor chromatographic peaks (peaks 1–3) and flutriafol (peak 4) were detected in the UV at 220 nm, Figure 2. PDA detection allowed the area% contributions of peaks 1–3 to be determined relative to the AI, where they each exceeded the threshold of >0.1%. MS detection was used to perform the initial structural elucidation of the impurities.

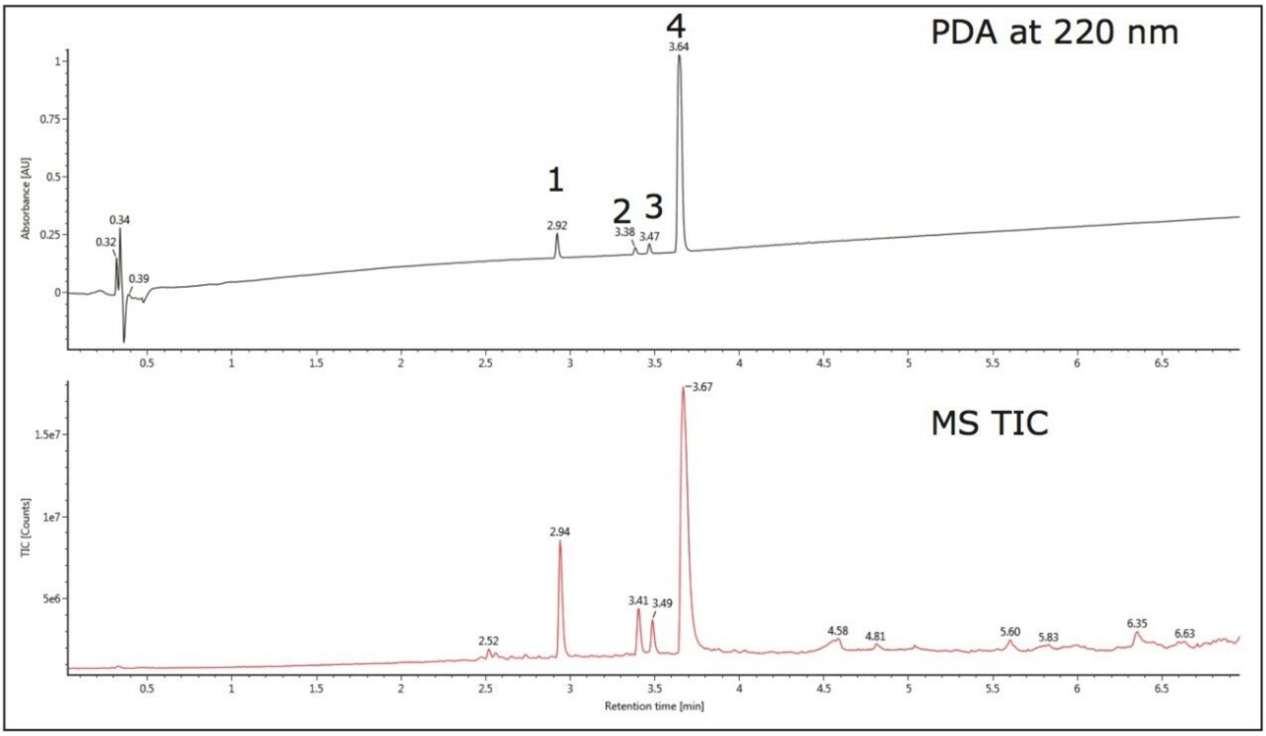
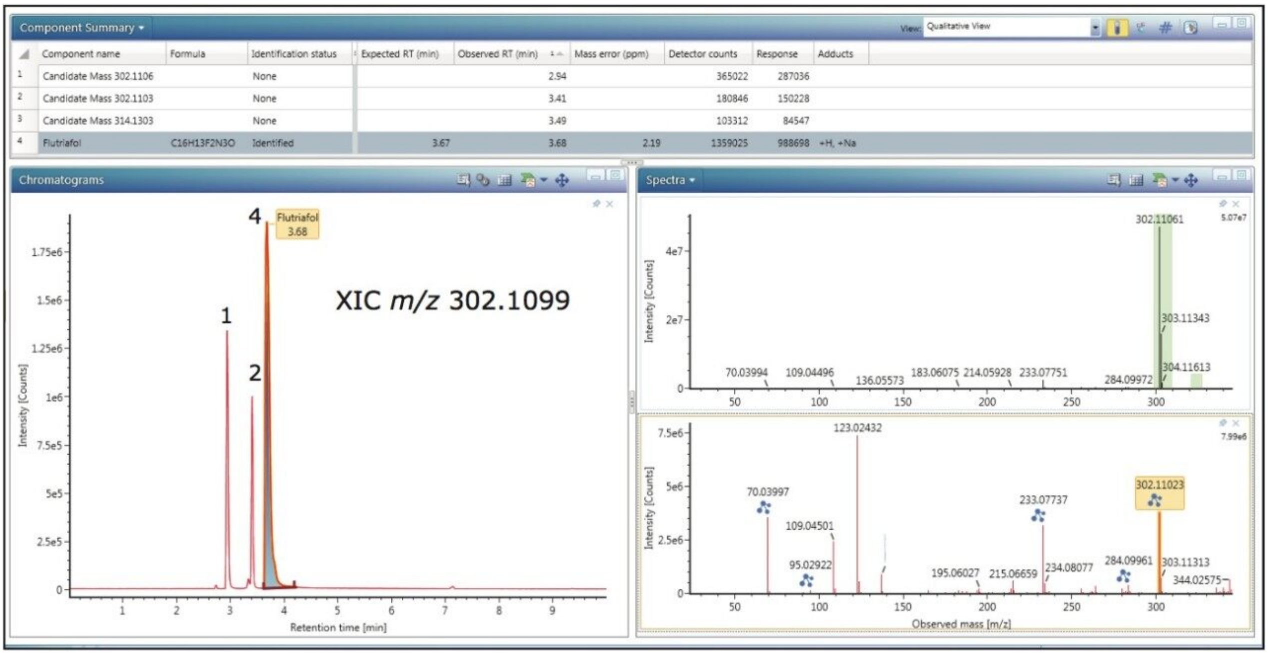


Figure 2. PDA chromatogram at 220 nm (top), showing flutriafol (peak 4) and three minor components (peaks 1–3). MS total ion chromatogram (TIC) is shown beneath.

In the component summary of the UNIFI review window, candidate masses were tabulated (Figure 3) and the identified components are listed.

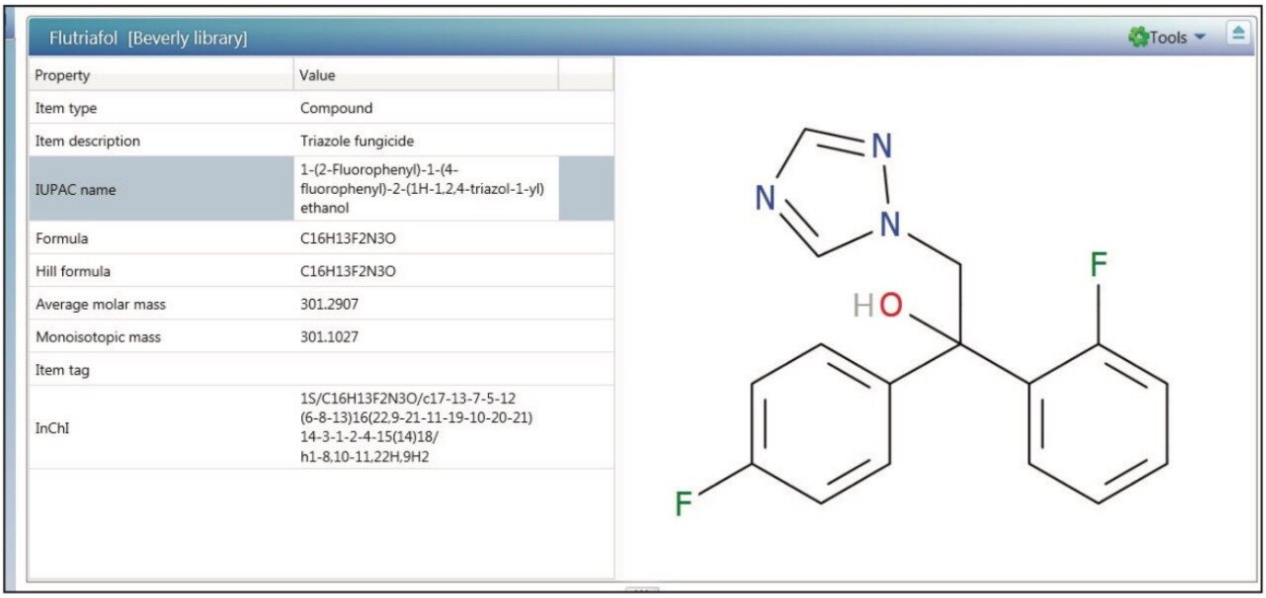
The precursor and product MS^E spectral data for each candidate mass are automatically extracted and can be viewed simultaneously in the spectrum window (Figure 3). If adducts for the precursor ion are present they are automatically assigned. UNIFI also performs a *fragment match* when a structure is available, intelligently assigning likely structures to high CE fragments.



	Component name	Formula	Identification status	Expected RT (min)	Observed RT (min)	Mass error (ppm)	Detector counts	Response	Adducts
1	Candidate Mass 302.1106		None		2.94		365022	287036	
2	Candidate Mass 302.1103		None		3.41		180846	150228	
3	Candidate Mass 314.1303		None		3.49		103312	84547	
4	Flutriafol	C16H13F2N3O	Identified	3.67	3.68	2.19	1359025	988698	+H, +Na

Figure 3. UNIFI review window shows the component summary, extracted ion chromatograms (XIC) for the identified flutriafol (m/z 302.1099), and the isobaric components 1 and 2. MS^E fragmentation and intact precursor spectral information for flutriafol are also shown.

The flutriafol was identified and represented as an extracted ion chromatogram (XIC) using specific information such as compound name, retention time (tR), mol files, and fragment ions that were generated from prior analyses of the fungicide and entered into the UNIFI Scientific Library. This approach can be used to build the analysis method prior to data acquisition (Figure 4). UNIFI's Scientific Library can store structures and begin retaining information associated with the individual library entries. Parent compounds and impurities of interest can be stored in the library for later searching and retrieval.



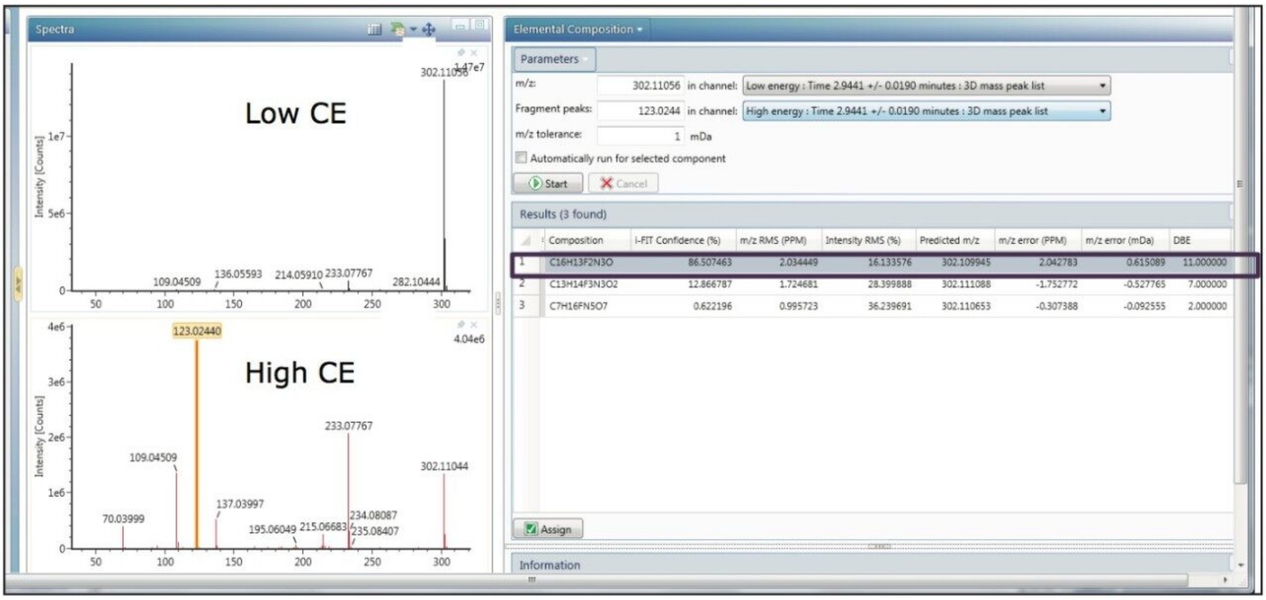
Property	Value
Item type	Compound
Item description	Triazole fungicide
IUPAC name	1-(2-Fluorophenyl)-1-(4-fluorophenyl)-2-(1H-1,2,4-triazol-1-yl) ethanol
Formula	C16H13F2N3O
Hill formula	C16H13F2N3O
Average molar mass	301.2907
Monoisotopic mass	301.1027
Item tag	
InChI	1S/C16H13F2N3O/c17-13-7-5-12(6-8-13)16(22,9-21-11-19-10-20-21)14-3-1-2-4-15(14)18/h1-8,10-11,22H,9H2

Figure 4. UNIFI Scientific Library entry for flutriafol.

The peaks labeled 1 and 2 from Figure 2 eluted at 2.94 min and 3.41 min respectively. Both components were extracted as XICs, as the two peaks have an *m/z* that is within the flutriafol target mass tolerance of 3 ppm set in the processing method (Figure 3).

Candidate masses can be investigated using the elucidation toolset in UNIFI which contains a comprehensive suite of structural elucidation tools that can be utilized by selecting any candidate mass from the component summary list. The elucidation toolset allows precursors and fragment ions to be interrogated more intensively. This includes *ChemSpider* search *(www.chemspider.com, The Royal Society of Chemistry)*, isotope modeling, and elemental composition tools. Discovery tools for related ions using mass defect, common fragment, and neutral losses are also available.

The structural elucidation tools indicated that both components 1 and 2 had identical elemental composition to the flutriafol, $C_{16}H_{13}F_2N_3O$ (Figure 5). In addition the high energy MS^E data indicated that the components had common fragment ions at different ratios compared with the AI (Figure 3). The existence of the high energy product ion data along with the intact precursor increased the confidence in the identifications.



m/z: 302.11056 in channel: Low energy : Time 2.9441 +/- 0.0190 minutes : 3D mass peak list

Fragment peaks: 123.0244 in channel: High energy : Time 2.9441 +/- 0.0190 minutes : 3D mass peak list

m/z tolerance: 1 mDa

☐ Automatically run for selected component

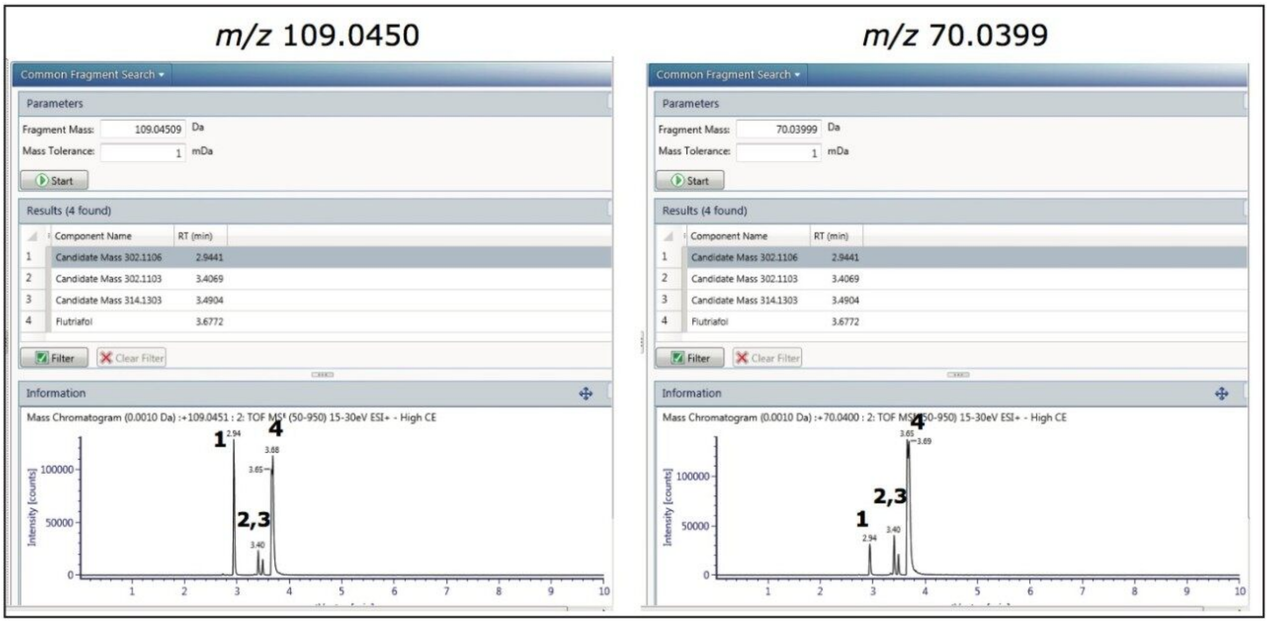
Results (3 found)

	Composition	i-FIT Confidence (%)	m/z RMS (PPM)	Intensity RMS (%)	Predicted m/z	m/z error (PPM)	m/z error (mDa)	DBE
1	C16H13F2N3O	86.507463	2.034449	16.133576	302.109945	2.042783	0.615089	11.000000
2	C13H14F3N3O2	12.866787	1.724681	28.399888	302.111088	-1.752772	-0.527765	7.000000
3	C7H16FN5O7	0.622196	0.995723	36.239691	302.110653	-0.307388	-0.092555	2.000000

Figure 5. The elemental composition $C_{16}H_{13}F_2N_3O$ of the candidate mass m/z 302.1106 (highlighted in the purple rectangle) is determined using the elucidation toolset. The precursor (top) and product ion spectra (beneath) are shown at the left of this screen capture.

Based on these observations it is likely that components 1 and 2 are isomers of the AI.

Using UNIFI's common fragment search function, components that share common structural features can be efficiently extracted from the data. By selecting prominent fragments in the spectrum of component 1, including the signature fragment originating from the triazole ring *m/z* 70.0399 and *m/z* 109.0450, we can see an additional chromatographic peak at t_R 3.49 minutes was extracted using the same product ions, as shown in Figure 6.



m/z 109.0450

Fragment Mass: 109.04509 Da
Mass Tolerance: 1 mDa

	Component Name	RT (min)
1	Candidate Mass 302.1106	2.9441
2	Candidate Mass 302.1103	3.4069
3	Candidate Mass 314.1303	3.4904
4	Flutriafol	3.6772

Mass Chromatogram (0.0010 Da) :+109.0451 : 2: TOF MS^E (50-950) 15-30eV ESI+ - High CE

m/z 70.0399

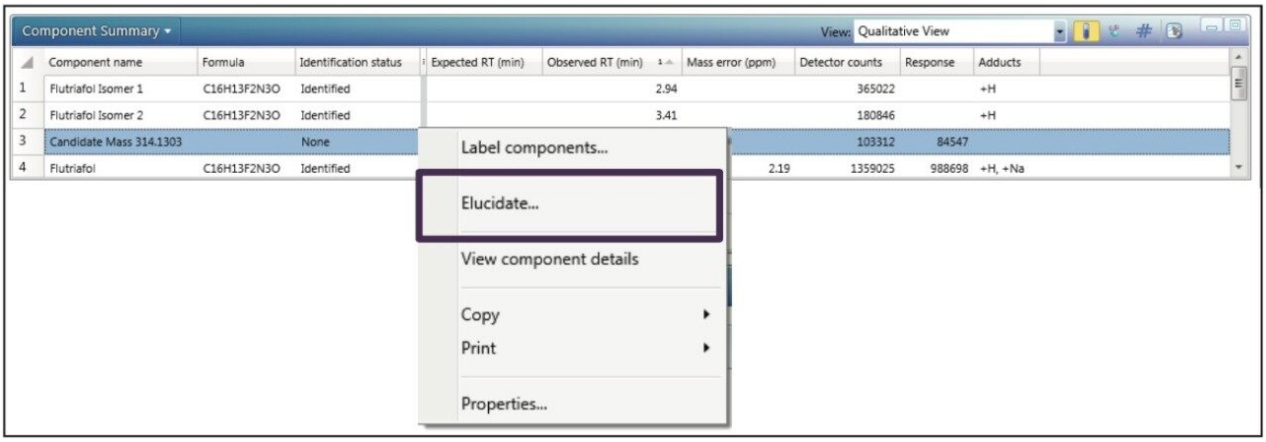
Fragment Mass: 70.03999 Da
Mass Tolerance: 1 mDa

	Component Name	RT (min)
1	Candidate Mass 302.1106	2.9441
2	Candidate Mass 302.1103	3.4069
3	Candidate Mass 314.1303	3.4904
4	Flutriafol	3.6772

Mass Chromatogram (0.0010 Da) :+70.0400 : 2: TOF MS^E (50-950) 15-30eV ESI+ - High CE

Figure 6. Common fragment search for m/z 109.0450 and 70.0399, which was used to efficiently search the technical grade flutriafol for components with common structural features.

This component (peak 3), has an *m/z* of 314.1303 and has been tabulated in the component summary, it was investigated in the same way as the previous example (Figure 7).



	Component name	Formula	Identification status	Expected RT (min)	Observed RT (min)	Mass error (ppm)	Detector counts	Response	Adducts
1	Flutriafol Isomer 1	C16H13F2N3O	Identified		2.94		365022		+H
2	Flutriafol Isomer 2	C16H13F2N3O	Identified		3.41		180846		+H
3	Candidate Mass 314.1303		None				103312	84547	
4	Flutriafol	C16H13F2N3O	Identified			2.19	1359025	988698	+H, +Na

Figure 7. The unknown mass m/z 314.1303 is sent to the elucidation toolset for further investigation.

The elemental composition of the unknown mass was determined to be $C_{17}H_{16}FN_3O_2$. The common fragment search function indicated the component shared common structural characteristics with the flutriafol. The elemental composition of component 3 was searched using the *ChemSpider* Search function, which is embedded in the elucidation toolset.

A suitable match was not made based on the inability to assign all fragments, including the common fragments simultaneously within the same structure proposal. Therefore, an alternative route to assign the structure was carried out, using a combination of elemental composition for the individual fragmentation spectra of component 3, as well as confirmation of the fragments using MS/MS, knowledge of the synthetic route, and a comparison with the elemental composition of the fragmentation spectra for the flutriafol. The proposed structure was then used to assign probable structures to the product ions (Figure 8).

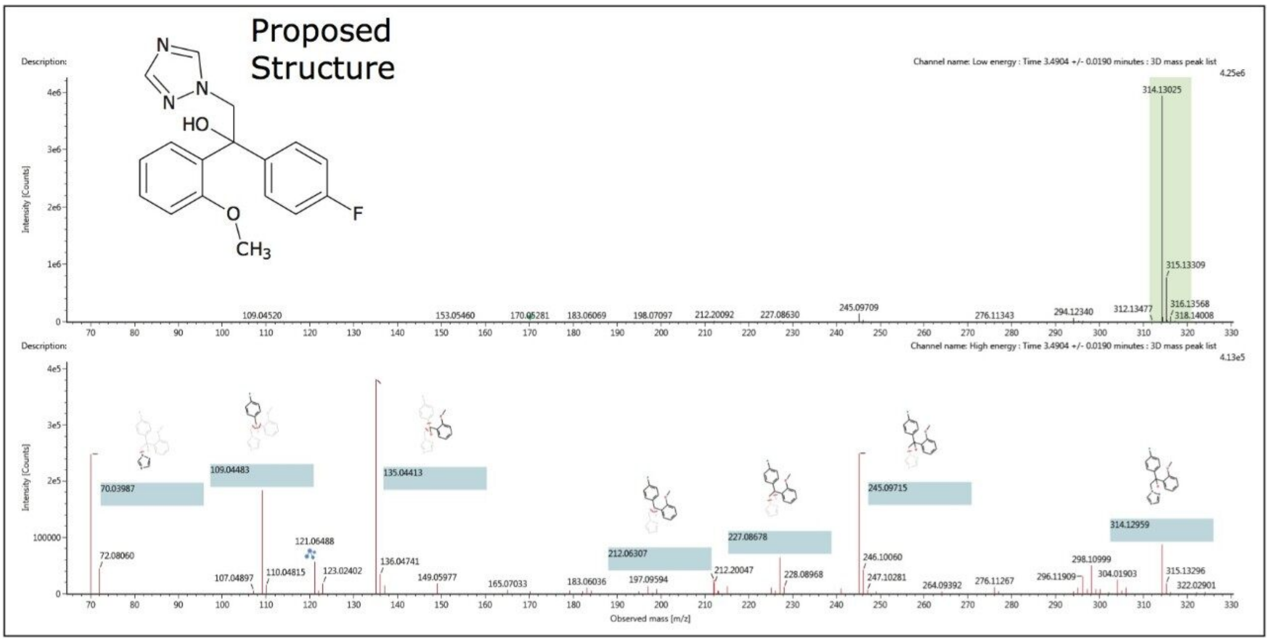


Figure 8. Structure proposal for component 3, MS^E intact precursor and fragmentation spectra with UNIFI's fragment match structural assignments.

Based on the general structural proposal (inset) the fragments were assigned using the UNIFI fragment match tool. Absolute confirmation of the structure would require scale up, trace isolation followed characterization using appropriate techniques, such as NMR spectroscopy.

The components can be added to the UNIFI Scientific Library, including any known information (retention time, known fragments, etc.) so that they can be tracked across further syntheses.

Conclusion

During chemical synthesis residual starting products, intermediates, or reaction by-products can lead to impurities in the final technical grade materials. Identification of the components can help to optimize the synthetic process. Minor components present in the technical grade flutriafol were detected in the PDA at 220 nm. The unidentified components were characterized using UPLC coupled with ToF MS^E and the UNIFI Screening Platform Solution. Two components were tentatively identified as isomers of the active ingredient, and a third unknown component was also observed. Using the elucidation toolset within UNIFI, the unknown component was found to have structural features that were common to flutriafol. Based on the elemental composition of the fragments, knowledge of the synthetic route, and the structure of the active ingredient, a structure for the unknown component having *m/z* 314.1303 was tentatively proposed. This tentative structure is in agreement with all of the data that iscurrently available. Future impurity isolation studies will allow for the definitive determination of the structures of these impurities.

References

1. Commission Regulation (EU) No 544/2011. (2011) [cited February 13, 2015]. Available from: http://eur-lex.europa. eu/LexUriServ/LexUriServ.do uri=OJ:L:2011:155:0001: 0066:EN:PDF
2. An overview of the principles of MS^E, The engine that drives MS performance. *Waters White Paper,* p/n 720004036en. October, 2011.
3. Chang, M, Kim, TH, Kim, HD. Stereoselective synthesis of flutriafol. Tetrahedron: *Asymmetry*. 19: 1503–1507, 2008.

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Screening Platform Solution with UNIFI <https://www.waters.com/134682903>

ACQUITY UPLC I-Class PLUS System <https://www.waters.com/134613317>

ACQUITY UPLC PDA Detector <https://www.waters.com/514225>

Xevo G2-XS QTof Quadrupole Time-of-Flight Mass Spectrometry <https://www.waters.com/134798222>

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