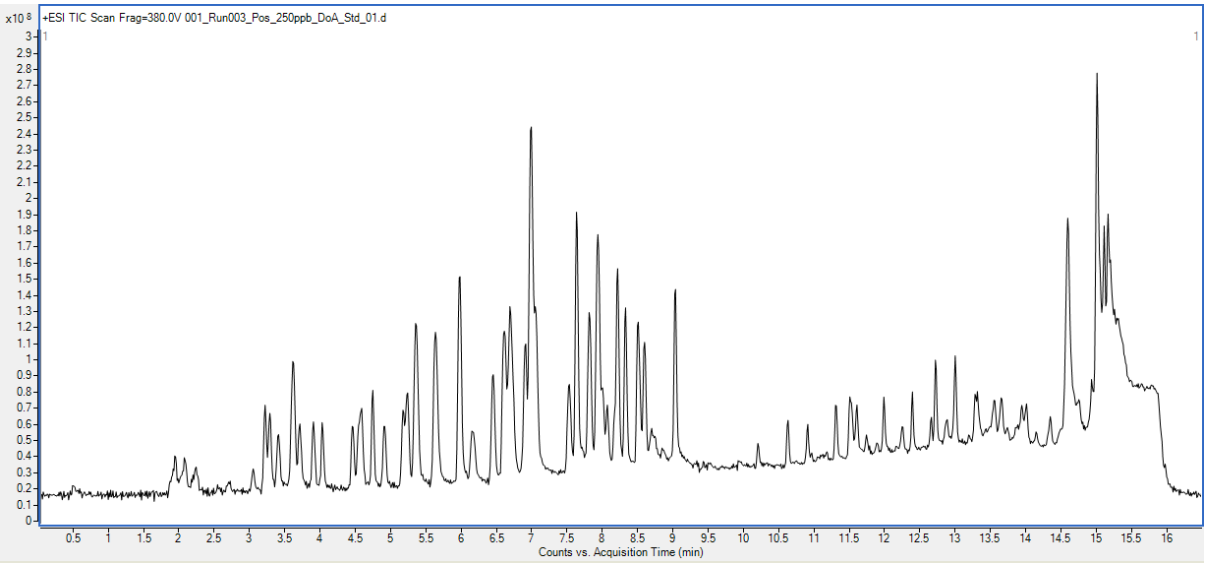
HPLC-QTOF Demonstration – 26th Mar 2021:

To demonstrate the system was performing as expect a 250 ppb drugs of abuse standard containing a mix of 59 drugs and their metabolites was analysed.

The total ion chromatogram (TIC) is shown below, this is a composite spectrum of all data collected during the chromatographic run:

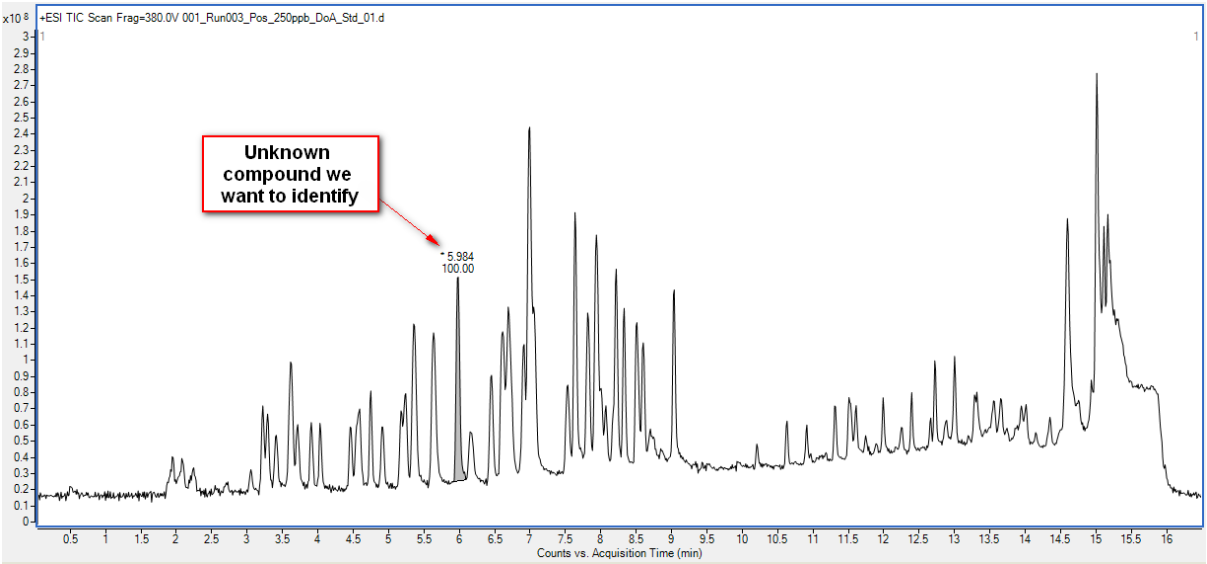


We can use this data to set to demonstrate two common reasons to use a QTOF over other forms of HPLC detection.

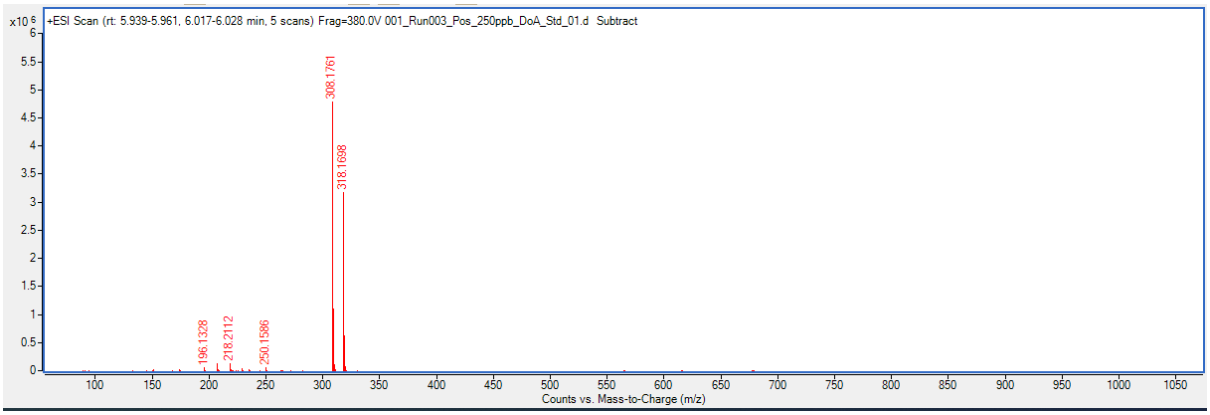
1. Unknown Identification:

LC-QTOF offers a number of advantages over other techniques when it comes to identification of unknown peaks in a chromatogram.

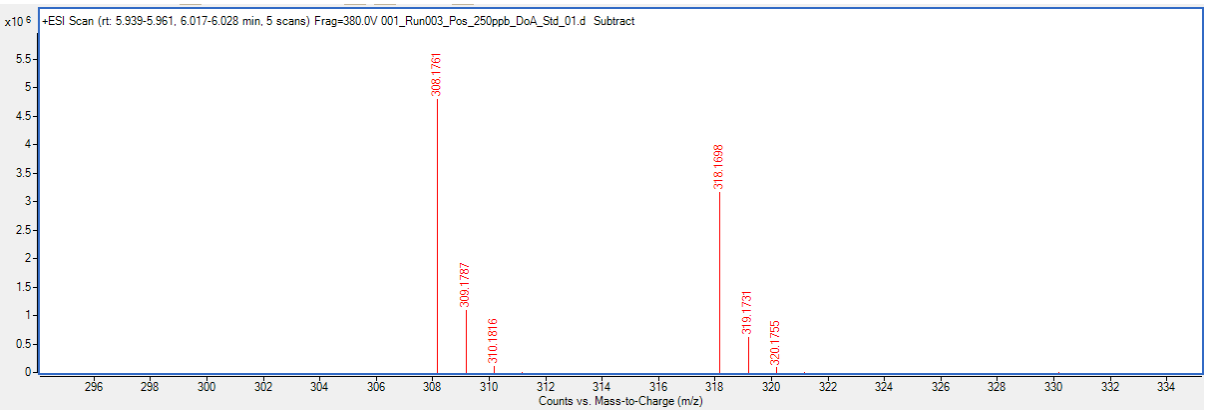
If instead of being a mixed standard, we imagine the chromatogram below to be a sample that contains an unknown peak that we wish to identify. The peak at 5.98 mins has been arbitrarily selected to demonstrate this:



As QTOF is a form a mass spectrometric detection we can extract the mass spectrum for this peak:



Expansion:



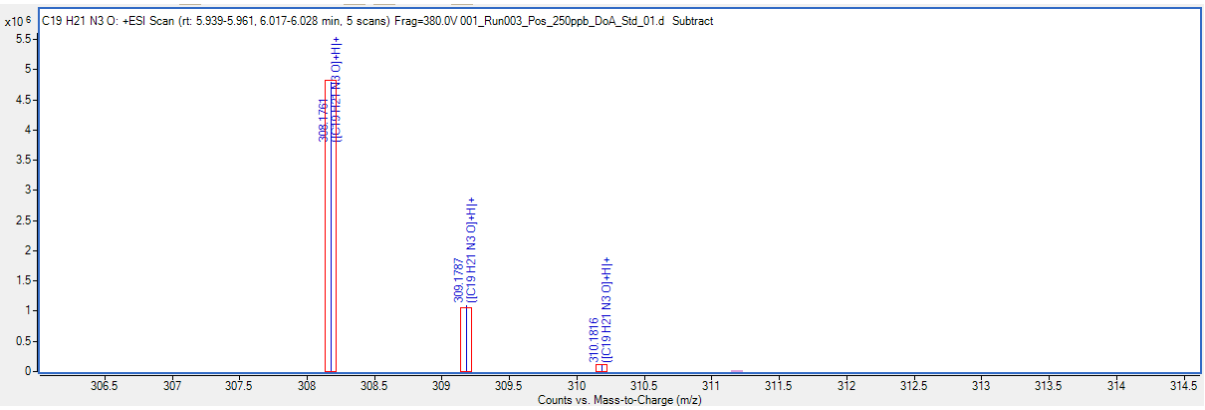
The first thing to notice is that although the peak at 5.98 mins appears on the TIC as a single, symmetrical peak, it is actually two co-eluting species, a non-selective detection technique, such as UV/Vis would not have been able to differentiate these. The second thing to note is that the mass is reported accurately to 4 d.p., rather than unit mass that would be obtained using a less accurate mass spectrometer such as a quadrupole.

The two molecular ions, 308.1761 m/z and 318.1698 m/z, can then be used to elucidate the molecular formulae responsible for the peaks by asking the software to determine what combinations of common elements (usually C, H, N and O, although any element can be included) would produce the detected accurate masses. The table reported for each detected molecular ion is shown below:

<u>308.1761 m/z:</u>

Best	Formula	Species	m/z	Score	Diff (ppm)	Score (MFG)
◉	C19 H21 N3 O	(M+H)+	308.1761	99.21	0.83	99.21
○	C21 H23 O2	(M+H)+	308.1761	94.64	-3.76	94.64
○	C17 H19 N6	(M+H)+	308.1761	90.34	5.45	90.34

Three formulae match the detected mass, these are assigned a score which is based on the closeness of the detected mass to the theoretical mass and the closeness of the observed isotope pattern with the theoretical isotope pattern. The top formula of $C_{19}H_{21}N_3O$ gives a score of 99.21% with a mass difference of only 0.83 ppm compared to the theoretical mass, the isotope match is shown below, the red boxes are the theoretical isotope intensities and the blue lines are the observed intensities:



318.1698 m/z:

The matching formulae for this peak are shown below:

Best	Formula	Species	m/z	Score	Diff (ppm)	Score (MFG)
◉	C18 H23 N O4	(M+H)+	318.1698	99.75	-0.47	99.75
○	C16 H21 N4 O3	(M+H)+	318.1698	94.74	3.98	94.74
○	C19 H19 N5	(M+H)+	318.1698	91.29	-4.47	91.29

The top match is $C_{18}H_{23}NO_4$, which has a score of 99.75% and mass difference of only -0.47 ppm.

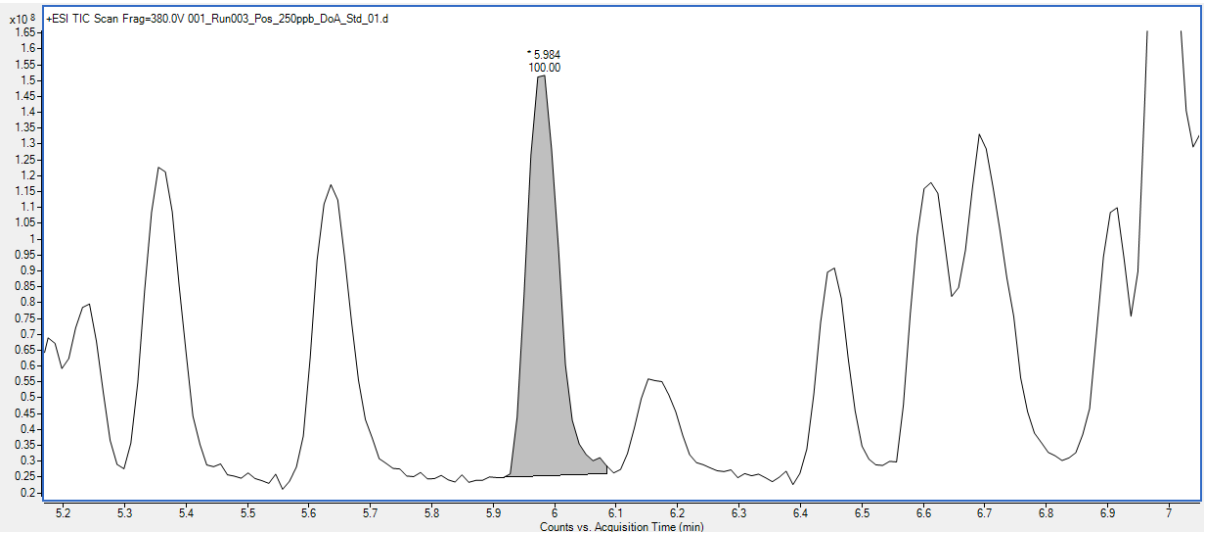
These two formula match Zolpidem ($C_{19}H_{21}N_3O$) and Cocaethylene ($C_{18}H_{23}NO_4$), which are two of the compounds within the drug mix. The accurate mass detection allowed these two formula to be assigned with much greater certainty than would be obtain from a less accurate mass spectrometer. In addition, as the instrument is a QTOF this could be further extended to apply a collision energy to the molecular ion, inducing fragmentation. The accurate mass of the fragments can then be used to generate formulae for these fragments and help identify the constituent components of the compound.

2. Extraction of Known Compounds from the Composite Chromatogram:

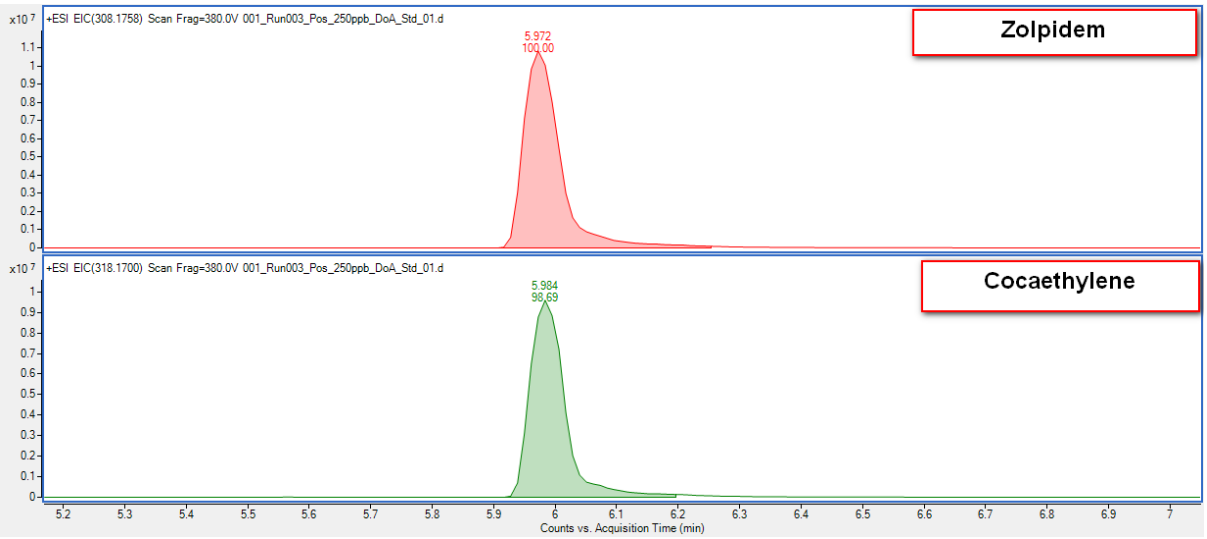
With traditional detection methods such as UV/Vis, it is necessary to completely resolve the peaks of interest to accurately quantify them, this is not necessary with QTOF as we can use the accurate mass of each compound to selectively extract them from the data.

If we take the example above, zolpidem and cocaethylene are found to co-elute, however, if we extract the molecular ion mass for each (318.1700 m/z for cocaethylene and 308.1758 m/z for zolpidem) for the total ion chromatogram we can independently quantify each, despite the fact they co-elute:

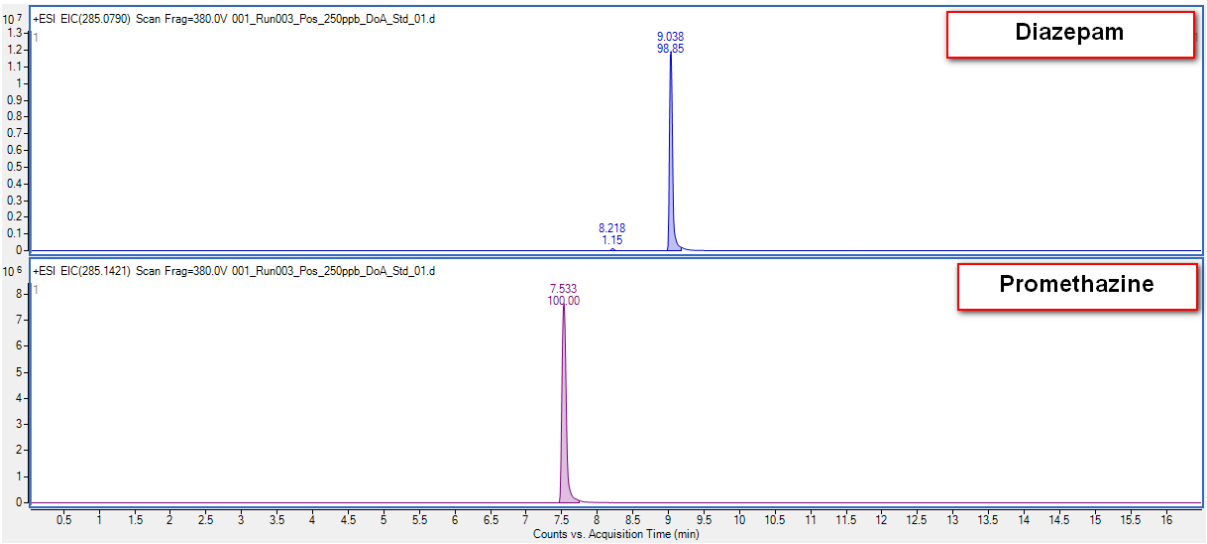
Expanded TIC:



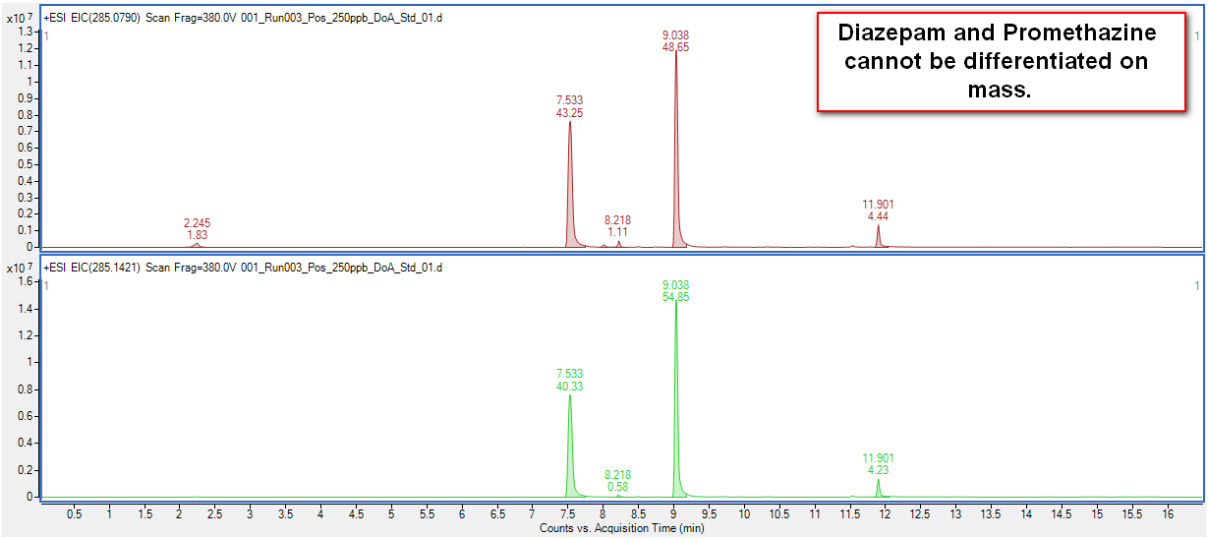
Extracted Ion Chromatograms for Cocaethylene, 318.1700 m/z and Zolpidem, 308.1758 m/z:



Another example is shown below using Diazepam and Promethazine. These two compounds elute relatively close together on this method and have almost identical molecular ion masses with the molecular ion for Diazepam being 285.0790 m/z and Promethazine being 285.1421 m/z. A single quadrupole mass spectrometer could not tell these two compounds apart as the mass difference between them is only 0.0631 m/z, however, this mass difference is easily differentiated using the QTOF. The screenshot below shows the extracted ion chromatograms for each:



As can be seen, the accurate mass detection allows the retention time of the two compounds to be unambiguously assigned despite them having almost identical molecular weights. The screenshot below shows the same data analysis but this time with the mass resolution dumbed down to the level of a single quadrupole detector:



The screenshot demonstrates that if a quadruploe mass spectrometer had been used both extracted ion chromatograms show two peaks and we would not be able to differentiate the two compounds without running individual standards, in addition, if these two compounds co-eluted, a single quadrupole would not be able to resolve them whereas the QTOF can do so with ease.