**An ultra-high resolution accurate mass LC/MS solution to Forensic Toxicology screening in serum**

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### Introduction

LC-MS/MS has fast become the technology of choice for the screening of illicit drugs. Two main approaches for tandem MS have been used in this area. The first one is called MTS™ Multi-Target-Screening, and the second one is GLUE™ General Unknown Screening. In both cases, these two approaches are limited by the number of entries available in the MS2 library. In this work, we will present a completely new approach based on accurate mass confirmation. Mass confirmation is made using accurate mass detection of the analyte (below 5 ppm) and retention time. Data obtained from real samples (information in Figure 1) will be presented and extra parameters used for confirmation of the results will be discussed.

### Methods

**HPLC**

Chromatographic analyses were performed using the Thermo Scientific Acuity UHPLC system. The chromatographic conditions were as follows:

- Column: Thermo Scientific Hyperion GOLD RP18 5 µm, 150 x 2.1 mm,
- Flow rate: 0.4 ml/min,
- Mobile phase A: water containing 10 mM ammonium acetate and 0.1% formic acid,
- Mobile phase B: MeCN containing 0.1% formic acid,
- Gradient: (A) 100% to (B) 100% in 10 minutes.

**Mass Spectrometry**

MS analysis was carried out on the Exactive™ mass spectrometer with an electrospray ionization (ESI) source. The MS conditions were as follows:

- ESI Source: Positive and negative,
- Capillary voltage: 3: 5 kV (A) 10 kV (B),
- Sheath Gas: N2 (A) 10 kPa (B),
- IS: 100 (A), 40 (B),
- Fragmentation: HCD 40-60 after every MS scan.

### Results

A serum sample was spiked with a mixture of 40 different molecules (see Figure 1). The concentration for each of the analytes was 1.25 µg/ml. Then successive dilutions were made in 40/60 A/B (see Methods for A and B) down to 0.4 µg/ml to evaluate the sensitivity of the instrument. Most of the selected molecules are isobars or isomers. As an example, Am Ally1 and Amarylline and DDD are isobars. They have exactly the same mass. Bromopyram and Clonazepam are isobars. Their masses differs by few milli-amu.

Mass accuracy was evaluated at different concentrations and at different resolution settings. On average, when using external calibration the mass accuracy for all the molecules was ± 2-3 ppm and with internal calibration it was ± 1 ppm. Figure 2 reports the percentage of molecules that were identified at different concentrations and at different resolution settings (10K and 100K). Identification was confirmed for a mass accuracy below 5 ppm. When going to 0.4 µg/ml, 65% of the compounds are still identified at a resolution of 100K and 62.5% at 10K resolution. Overall, the percentage of molecules that have been identified is higher at high resolution. In low resolution conditions, some molecules coming from the matrix may interfere with the analyte peaks and therefore increase the mass accuracy of the analyte above the threshold of 5 ppm.

### HCD Fragmentation

When isomers elute at very close retention times, another criteria has to be selected to differentiate properly the analytes. Fragment ions generated under gas collision dissociation in the HCD collision cell can be used to fulfill this criteria. Figure 3 A shows an example with maprolamine and amyltriptiline. Both have the same mass as they are isomers (Formulas: C_{21}H_{26}N_{3}O_{2} and C_{21}H_{25}N_{3}O_{2}) and they have very similar retention times under our LC conditions. The only difference is that maprolamine generates a fragment ion at 250.158 m/z that is not seen with amyltriptiline. Using fragment ions, is, in general, mandatory to confirm the presence of an analyte.

### Resolution Settings

The signal-to-noise ratio for the fragment ion is much higher under high resolution conditions (614 versus 19). At 100K resolution, the instrument is able to separate the fragment of the analyte from other compounds available in the matrix or the mobile phase. This is not the case at 10K resolution where the trace of the fragment being monitored is contaminated by another molecule coming from the mobile phase (probably a phthalate). For this reason, this background in this lower resolution setting is high, which results in lower signal-to-noise.

### Ultra-High resolution is necessary in some cases to differentiate two analytes having the same retention time or from interfering matrix ions. Figure 4 shows the example of Qnine and Bisproisol, two compounds that have the same retention time (Fig. 4A). Their molecular weight differ by 1 amu; thus the 14C isotope peak of qnine is not resolved from the 14C isotope peak of bisproisol at 10K resolution (Fig. 4C). Using ultra-high resolution, the 14C isotope of qnine and 14C isotope of bisproisol are clearly separated, and thus, allows easy identification of bisproisol with 3 ppm mass accuracy.

### Ultra-high resolution can also be used to further confirm and identify an analyte’s presence. In Figure 5A a spectrum of olanzapine has a region around one of the isotope peaks highlighted in pink. At 10K (Fig. 5B) the peak provides no additional information than its accurate mass, however, when 10K is utilised (Fig. 5B) the isotope pattern of the sulfur compound within Olanzapine is visible. This isotope pattern information further aids unique identification of this analyte in this sample.

### Data Processing

All data acquired was reprocessed using ToXID™ software. ToXID automatically generates reports that contain the list of molecules that have been identified, and also the mass accuracy and the present or absence of fragment ions. The retention time is also used as a criteria for confirmation.

### Conclusions

- Limits of Detection (LODs) obtained for most drugs in the analyte panel were below 1 µg/L.
- Ultra high resolution (100K) was utilised to solve a number of screening issues such as co-eluting and isobaric ions. Additional isotope pattern information can be obtained using this setting too.
- HCD fragmentation can be utilised to provide additional ions for analytic confirmation.
- ToXID software is ideally suited for library searching and reporting of results.

### References


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