A user's perspective on UPLC-Q-Exactive™ high resolution mass spectrometry: application to comprehensive drug screening in clinical laboratory

Cristiana Stefan and Ernest Wong

Centre for Addiction and Mental Health, Toronto, ON, M6J 1H4

In the past decades the strategies for comprehensive drug screening adopted by the clinical laboratories have been to a large extent vendor driven. With the discontinuation of the REMEDI instrument employing HPLC-UV technology (Bio-Rad), mass spectrometry has been the methodology of choice, regardless of its advantages and/or disadvantages. The switch involved not only a steep learning curve of new analytical concepts, but also considerable efforts towards in-house developments, changes in the testing workflow, and not surprisingly increased costs. Thus, the UV absorption and UV spectra concepts were replaced by concepts of mass measurement, ionization, mass to charge ratio, fragmentation, mass spectrum, solid phase extraction, derivatization; knowledge of the various mass spectrometers was needed, being GC or LC-based; single-quad, triple-quad or ion-trap type.

Having dealt for a couple of years with a complex and expensive workflow for comprehensive drug screening that involved combined GC-MS and LC/MS/MS protocols, our laboratory was definitely interested in exploring not only more powerful technology, yet affordable and with high throughput capabilities, but also more straightforward one; certainly moving away from gas chromatography and focusing on liquid chromatography; avoiding complex specimen preparation, and just dilute-and-shoot non-hdyrolyzed specimens (in as much as possible), while improving both the analytical sensitivity and specificity.

We have therefore set for adopting LC-high resolution mass spectrometry (LC-HR/MS/MS) principles and instrumentations and for pioneering this strategy for the clinical laboratory. Q-Exactive™ is a high resolution mass spectrometer. The high resolution principle is closely linked to the concept of mass accuracy, and, thus, a new learning curve has started for our laboratory. More precisely we had to expand on the mass concept to understand the various mass definitions: from nominal or integer to monoisotopic/exact mass (calculated), accurate mass

(experimental), and mass error. In high resolution mass spectrometry strategies we are not talking at the element level, but at the isotope level and/or its abundancy. We are not talking at the nominal (average) mass, but at the exact (monoisotopic) mass level. The atomic weight of the ^{1}H isotope is not 1 a.m.u anylonger, but 1.0078 (using 4 decimals). The nominal mass of 286 for morphine becomes 286.1438 in exact mass terms, leading to a mass defect of 0.1438, which can be an important parameter for accurate identification. In high resolution strategies one would also like to see that isomers with the same elemental formula, such as morphine, nor-codeine, nor-hydrocodone and hydromorphone ($C_{17}H_{19}NO_3$), hence same exact mass, are well separated and identified with high level of confidence.

Using a reverse-phase column consisting of core silica and PFP (pentafluorophenyl) shell technology we achieved complete separation of isomers, as indicated in the table below, in urine specimens for which dilution was the only handling prior to chromatographic separation.

Drug	**Elemental Formula**	**M+H**	**RT (min)**	**Mode**
(nor)Codeine	$C_{17}H_{19}NO_3$	286.1438	8.22	+
(nor)Hydrocodone	$C_{17}H_{19}NO_3$	286.1438	9.03	+
Hydromorphone	$C_{17}H_{19}NO_3$	286.1438	7.44	+
Morphine	$C_{17}H_{19}NO_3$	286.1438	6.77	+

Identification of compounds cannot rely only on exact (accurate mass) and retention time exclusively, as more than one chemical structure may share these analytical parameters. To achieve further specificity additional ‘fingerprint’ is necessary and this is the mass fragmentation spectrum. Using three optimized collision energy levels we developed an in-house MS^2 library that currently has more than 800 entries covering a large number of drug classes that include opiates, opioids, benzodiazepines, barbiburates, antipsychotics, antidepressants; newer drugs of abuse such as synthetic cannabinoids, bath salts and herbals active principles. The compounds are either parents or phase I metabolites such as nor- or desalkyl-, hydroxy-, carboxy-, N-oxides, or phase II metabolites such as glucuronides and sulfates. All these can be technically identified in just one run. Running data-dependent experiments, we collected data with mass accuracy of 5 ppm, and used polarity switch so that both positively and negatively ionized compounds were

analyzed. Compounds were identified based on mass accuracy, retention time, and MS^2 spectra. Validation of results was achieved by method comparison with GC-MS (gold standard) protocols.

The UPLC-Q-Exactive™ methodology has run smoothly at our laboratory since its implementation (6 months ago) showing excellent ruggedness, sensitivity and specificity for our addiction and mental health client population and for the matrices of interest (primarily urine, swabs, and/or herbal products). A couple of cases will be included in the presentation outlining the advantages of LC-HR/MS/MS strategy. We believe that our development is valuable for the Clinical Laboratory field, having the potential of becoming a gold standard for drug screening.