Advances in Mass Spectrometry for targeted and untargeted drug screening

Kara L. Lynch
University of California San Francisco
United States

Scope of the lecture:

High-resolution mass spectrometry (HRMS) using quadrupole time-of-flight (QqTOF) or Orbitrap technology has gained recognition as a valuable tool for broad spectrum drug screening in a variety of biological matrices. In contrast to LC-MS/MS methods, which collect nominal mass data primarily in a targeted manner, HRMS instruments collect untargeted, accurate mass data for precursor and product ions. With the adoption of this technology there are many data acquisition approaches and data analysis parameters to evaluate and optimize prior to implementation in a routine clinical laboratory. This abstract/presentation will describe the work our laboratory has done to evaluate and optimize HRMS for routine drug testing.

Learning objectives:

1. Recognize the advantages and disadvantages of using high resolution mass spectrometry for drug screening compared to traditional approaches such as tandem mass spectrometry
2. Describe the different approaches for the analysis of data acquired using high resolution mass spectrometry
3. Compare and contrast data-dependent and data-independent strategies for the collection of data using high resolution mass spectrometry

Extended abstract:

High-resolution mass spectrometry (HRMS) using quadrupole time-of-flight (QqTOF) or Orbitrap technology has gained recognition as a valuable tool for broad spectrum drug screening in a variety of biological matrices. In contrast to LC-MS/MS methods, which collect nominal mass data primarily in a targeted manner, HRMS instruments collect untargeted, accurate mass data for precursor and product ions. With the adoption of this technology there are many data acquisition approaches and data analysis parameters to evaluate and optimize prior to implementation in a routine clinical laboratory. This abstract/presentation will describe the work our laboratory has done to evaluate and optimize HRMS for routine drug testing.

First, we developed a broad-spectrum drug screen on a QqTOF that collected data in an untargeted manner and compared its performance to a nominal mass instrument [triple quadrupole linear ion trap (QqLIT)] that collected data in a targeted manner. Both methods used information-dependent acquisition (IDA) of product ion spectra. We evaluated the lower limits of detection and matrix effects for each method and compared their ability to identify drugs in 100 routine clinical urine samples. Additional information (patient prescription history, drug screening results, etc.) was used to confirm discordant results. QqLIT was slightly more analytically sensitive than QqTOF; however, this difference did not significantly affect compound identification in patient samples. QqLIT identified 596 drugs in the urine samples, of which 531 (89%) were confirmed. QqTOF identified 515 drugs, of

which 500 (97%) were confirmed. There were 562 instances of a confirmed drug (68 unique drugs) in the 100 urine samples; the methods were concordant in 469 of these instances. Overall, QqTOF performed similarly to QqLIT and could serve as an alternative method for general unknown drug screening.

Second, one of the most challenging aspects of implementing an HRMS drug screen is establishing appropriate data analysis parameters for identifying compounds. Unlike other types of mass spectrometry data, guidelines for HRMS data analysis and acceptability criteria have not been established. Although many laboratories have published on the utility of HRMS for drug screening, few have included details on how they determined allowable errors and set positivity criteria. We developed a detailed procedure that we used to determine appropriate positivity criteria for our screening procedure. Our approach was empirical; we collected data and analyzed it with commonly available software. We found that a combined scoring approach using a threshold of 70, with 70% weight given to library match and 10% weight given to each of mass error, retention time error and isotope pattern difference provided optimum drug identification efficiency of 99.2%. Our results demonstrate the importance of library matching in accurately identifying compounds, and underscore the utility of robust product ion spectra that contain information on the lineage, mass and relative abundance of fragments. We determined that with careful selection of error limits and positivity criteria, HRMS instruments are capable of producing high-quality, high-confidence results that may reduce the need for confirmatory testing.

Third, untargeted data collection using HRMS methods allows for expanded drug detection capabilities, since the data can be retrospectively analyzed using several techniques. Most laboratories using HRMS analyze data in a targeted manner. To perform targeted analysis, a laboratory must first analyze a reference standard to determine the expected characteristics of a given compound. In an alternate technique known as suspect screening, compounds can be tentatively identified without the use of reference standards. Instead, predicted and/or intrinsic characteristics of a compound, such as the accurate mass, isotope pattern, and product ion spectrum are used to determine its presence in a sample. The fact that reference standards are not required *a priori* makes this data analysis approach very attractive, especially for the ever-changing landscape of novel psychoactive substances. We compared the performance of four data analysis workflows (targeted and three suspect screens) for a panel of 170 drugs and metabolites, detected by LC-QqTOF. We found that retention time was not required for drug identification; the suspect screen using accurate mass, isotope pattern, and product ion library matching was able to identify more than 80% of the drugs that were present in human urine samples. We showed that the inclusion of product ion spectral matching produced the largest decrease in false discovery and false negative rates, as compared to suspect screening using mass alone or using just mass and isotope pattern. Our results demonstrate the promise that suspect screening holds for building large, economical drug screens, which may be a key tool to monitor the use of emerging drugs of abuse, including novel psychoactive substances.

Fourth, we compared data-dependent and data-independent HRMS acquisition approaches for drug screening. Our primary QqTOF method (described above) utilizes information-dependent acquisition (IDA) of product ion spectra which triggers the collection of spectra for the 20 most abundant precursor ions at any given time in the chromatographic run. Low abundance ions can be missed using IDA due to the limited number of triggered product ion scans. We developed two data-independent acquisition (DIA) methods using Sequential Windowed Acquisition of all Theoretical fragment-ion spectra (SWATH) and

compared them to our IDA method. One method had fixed isolation windows (fSWATH) and the other had optimized variable isolation windows (vSWATH). SWATH performs data-independent fragmentation of all precursor ions entering the mass spectrometer in specified isolation windows covering the specified mass range. This allows multiple repeat analyses of each window during the elution of a single chromatographic peak. With IDA, the Q1 mass isolation window for collection of product ion spectra is typically less than 1 Da, however with DIA the window is larger (~20 Da). Product ions are collected for all ions within the window resulting in "less-pure" spectra compared to IDA spectra; however, data is collected for all ions. The limit of detection for the drugs and metabolites evaluated were significantly lower for the vSWATH method compared to fSWATH. When comparing IDA to vSWATH, vSWATH had a lower LOD for 43% of the drugs/metabolites, DDA had a lower LOD for 22%, and the LOD was equal for 35%. Overall the vSWATH method was slightly more sensitive, but in many cases, this was a difference of only 5 or 15 ng/mL. Matrix effects were observed and similar for the three methods. The detection capabilities in the 50 remnant samples were similar for IDA and vSWATH, with each method detecting 275 and 274 drugs/metabolites, respectively. Of the drugs/metabolites detected 90% were confirmed for the IDA method, and 92% for vSWATH. The results suggest that vSWATH is a viable alternative to IDA methods and in many cases resulted in more sensitive detection of low abundance ions. However, vSWATH has a few limitations for production laboratories; 1) the data review process is more time consuming compared to IDA and 2) the collection of spectra using a larger Q1 isolation window limits the ability to detect unknown drugs and metabolites using data analysis approaches other than targeted.

With careful development, evaluation and validation, HRMS has the potential to transform how clinical and forensic laboratories identify commonly encountered illicit and pharmaceutical drugs as well as emerging drugs of abuse and novel psychoactive substances. Our studies to date highlight our systematic approach to the adoption of HRMS for routine clinical toxicology drug screening.