Comparing TOF and QTOF for comprehensive drug screening: do you really need fragmentation information?

Jennifer M. Colby[1], Katie L. Thoren[2], Alan H. B. Wu[1], Kara L. Lynch[1]

[1]Department of Laboratory Medicine, University of California San Francisco and San Francisco General Hospital, San Francisco, CA 94110

[2]Department of Laboratory Medicine, Memorial Sloan Kettering Cancer Center, New York, NY 10065

Background: In a typical clinical laboratory, biological samples are tested for the presence of drugs using an initial panel of immunoassay screens, and positive results are confirmed using another technique with increased sensitivity and specificity. Though this approach is widely used, immunoassays are not readily available for emerging or uncommon drugs, and this can limit the scope of the testing. There has been a great deal of interest in alternative drug testing work flows, including using high resolution mass spectrometry (HRMS) based screens. HRMS instruments have the ability to accurately measure the mass of compounds present in a sample. While there are many advantages to using HRMS screens, one of the most widely touted is the ability to identify unknown or unexpected compounds in patient samples.

Many of the HRMS instruments found in clinical laboratories use time of flight (TOF) mass analyzers, which use ion flight time to measure mass. Single stage TOF instruments measure the accurate mass of all ions entering the flight tube. Compounds are identified by a combination of their mass, the measured isotope pattern, and chromatographic retention time. Addition of a quadrupole and a collision cell to a TOF forms a QTOF, a more expensive instrument that is able to isolate and fragment specific ions, as well as measure accurate mass. Molecular fragmentation is largely reproducible, thus the pattern of product ions that is produced in the collision cell can be used as an additional identifying characteristic of the precursor ion.

Liquid chromatography systems coupled to both TOF and QTOF mass spectrometers are used for drug screening, but there is debate in the field about which instrument performs better in routine analyses. Product ion spectra are useful in increasing the confidence of compound identification, but is the ability to select and fragment ions worth the additional cost? In one published study, the authors provide information regarding false positives they observed without fragmentation information for several drugs, but they do not give a complete analysis for all drugs and all samples(1). Another group compared sensitivity and specificity of a comprehensive urine drug screen with and without in-source fragmentation, and found that addition of fragmentation information reduced false positives(2). However, these authors made two injections and collected two different datasets.

In this study, we used data processing tools to turn QTOF data into TOF data, thus making two directly comparable datasets. Our strategy guarantees that the "TOF" and "QTOF" were operated using the same mass accuracy, calibration, and chromatographic conditions and removes a potential confounding variable from the analysis. After we optimized data analysis parameters for TOF and QTOF data, we analyzed 100 patient urine samples using each technique, and compared the results to a gold standard LC-MS/MS assay.

Methods: QTOF data was acquired with an ABSciex TripleTOF® 5600 system operating in positive ion mode, collecting full scan data with IDA triggered acquisition of product ion spectra. Urine samples submitted to our laboratory for routine drug screening were diluted 1:5 into starting chromatographic conditions and 10 µL was injected onto the column. Separations were performed on a Phenomenex Kinetex C18 column (50x3 mm, 2.6 µm) held at 30°C. Mobile phase A consisted of 0.05% formic acid in 5 mM ammonium formate. Mobile phase B consisted of 0.05% formic acid in 50% methanol 50% acetonitrile. The elution gradient was ramped linearly from 2% to 100% B over 10 minutes.

Data was processed using PeakView® (version 2.2, ABSciex) and MasterView® (version 1.1, ABSciex) software. Common parameters included a mass extraction window of ±30 ppm and a retention time window of ±0.5 minutes. For TOF analysis, compounds were considered positive when their combined score was ≥70, using a weighting combination of 75% mass error, 5% retention time error, and 20% isotope pattern error. For QTOF analysis, compounds were considered positive when their combined score was ≥70, using a weighting combination of 10% mass error, 10% retention time error, 10% isotope pattern error and 70% library score. The library we used was home-built, and contained 214 compounds from a variety of drug classes.

The 100 patient urine samples were analyzed using a reference LC-MS/MS method to determine expected drugs for each sample. Samples were injected onto an Agilent HPLC with an ABSciex 3200 QTRAP mass spectrometer in operating positive-ESI mode. Compounds were identified by a combination of RT, one SRM transition and a match between the collected product ion spectrum and our in-house-built spectral library. Prescription information, immunoassay screening results, and gas chromatography mass spectrometry results were also available.

Results: Our screening method was designed to detect 214 unique compounds, from a variety of drug classes. Based on our reference method analysis of the 100 patient urine samples, we expected 770 positive results and 20,630 negative results. Both the TOF and the QTOF methods performed adequately overall, with sensitivities of 77% and 88%, respectively, and nearly identical specificities (Table 1). The main difference came in the high number of false positives that were observed when product ions were excluded from the analysis, which caused the TOF results to have a low positive predictive value (PPV). When the 10 most common false positive drugs were excluded from the analysis, the PPV of the TOF

	True Positives	False Negative	False Positives	True Negatives
TOF	590	180	265	21135
QTOF	677	93	38	21362

	Sensitivity	Specificity	PPV	NPV
TOF	77%	99%	69%	99%
QTOF	88%	100%	95%	100%

Table 1. Predictive characteristics of QTOF and TOF methods.

results increased from 69% to 80%. The QTOF had a PPV of 95%, indicating that most of the positive screen results would be true positives. Both methods had high and comparable negative predictive values of >99%.

Discussion/Conclusion: Based on our analysis, HRMS instruments that do not use fragmentation patterns as identifying features of compounds suffer from a high false positive rate. An increased false positive rate means more work for the laboratory, because positive screens are generally sent to confirmatory testing, and performing unnecessary confirmations costs the lab money in reagents and labor. Whether the cost analysis favors performing the additional confirmation testing or buying an instrument subject to fewer false positives depends on the lab's testing volume.

Provided that all positive screens are confirmed before results are released to providers, TOF instruments that do not generate product ion spectra may be perfectly functional as screening tools. However, if TOF screen results are released to providers before confirmation is performed, there is an increased likelihood that false positives will result in confusion. Cases from our hospital that illustrate this danger will be presented. Compound identification by QTOF, which has a lower false positive rate as well as increased sensitivity relative to single-stage TOF, may be a more robust instrument choice if screening results are released prior to, or without, confirmation.

References

1. Chindarkar NS, Wakefield MR, Stone JA. Liquid chromatography high-resolution TOF analysis: investigation of MSE for broad-spectrum drug screening. Clinical Chemistry. 2014.

2. de Castro A, Gergov M, Östman P, Ojanperä I. Combined drug screening and confirmation by liquid chromatography time-of-flight mass spectrometry with reverse database search. Analytical and Bioanalytical Chemistry. 2012.