Lowering the Bar for Mass Spec: A Comparison between Immunoassay and Rapid LC-TOF-MS for Presumptive Analytical Screening of Drugs in the Clinical Research Lab

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Introduction

Immunoassay-based techniques have historically been the analytical method of choice for drug screening in clinical research. Presumptive detection of the analyte of interest in a biological specimen is most often reflexed to more specific, confirmatory testing that typically uses gas or liquid chromatography coupled to tandem mass spectrometry. Incorrect presumptive immunoassay results requiring additional testing are common problems that may have substantial down-stream consequences for laboratory operations and total costs. Therefore, an analytical LC-TOF-MS method has been developed for drug screening to combat these common problems seen with immunoassay-based techniques, improving overall data quality.

Experimental

A simple "dilute and shoot" sample preparation was utilized, which included a rapid enzymatic hydrolysis.

- 1:10 in starting mobile phase conditions for drug analysis
- 1:50 for creatinine analysis

Analytical testing was completed using an Agilent 1290 liquid chromatograph coupled to an Agilent 6550 quadrupole time-of-flight mass spectrometer operated in TOF mode, using separate positive and negative mode analytical methods.

Chromatographic separation was achieved on a 2.1x50mm, 2.7um Agilent Poroshell SB-C8 column heated to 75°C.

Positive Mode LC Conditions

- 5mM ammonium formate, pH 3.5
- 0.1% formic acid in ACN
- 2uL injection
- 5%-95% B in 1.25 minutes 0.5 minute re-equilibration
- 1 mL/minute flow rate

Negative mode LC conditions

- 0.1% acetic acid in water
- Methanol
- 5 uL injection
- 15%-95% B in 1.25 minutes, 0.5 minute re-equilibration
- 1 mL/minute flow rate

	Positive Mode	Negative Mode
Gas Temp	250ºC	250ºC
Drying gas	15 L/min	15 L/min
Nebulizer	60 psig	60 psig
Sheath Gas Temp	400ºC	400ºC
Sheath Gas Flow	12 L/min	12 L/min
Vcap	3500 V	3500 V
Nozzle Voltage	0 V	0 V, 500 V at 0.8 m
Fragmentor	125 V	125 V
Scan Speed	4 spectra/sec	4 spectra/sec

Figure 1. Mass spectrometer analytical method conditions.

Experimental

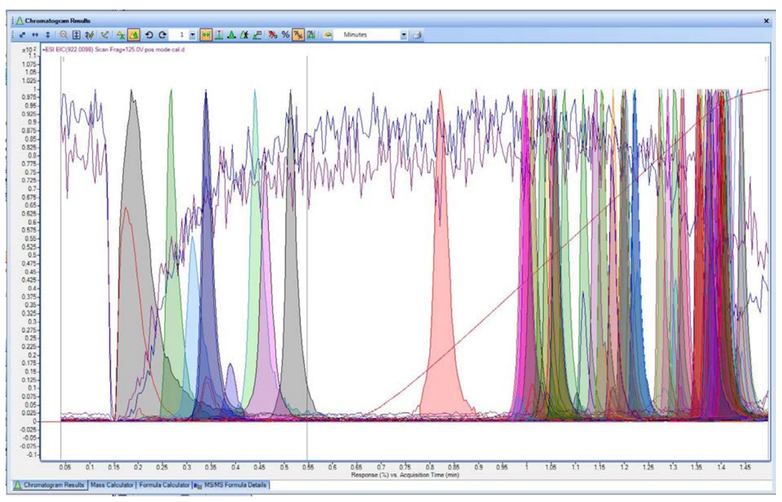

Figure 2. Positive mode analytes and internal standards, mobile phase B gradient, and reference mass solution response.

Eighty-four drugs and metabolites were included and reported qualitatively using eleven isotopically labeled internal standards selected to represent compound classes, retention time, and expected abundances to control for method inefficiencies and matrix suppression/enhancement. A one-point calibration at the cutoff and forced through the origin was used to determine an analyte's presence. QC materials representing each drug class were at 50% (Negative Control) and 125% (Positive Control) of the cutoffs. Data analysis was completed using Mass Hunter Quantitative Analysis software.

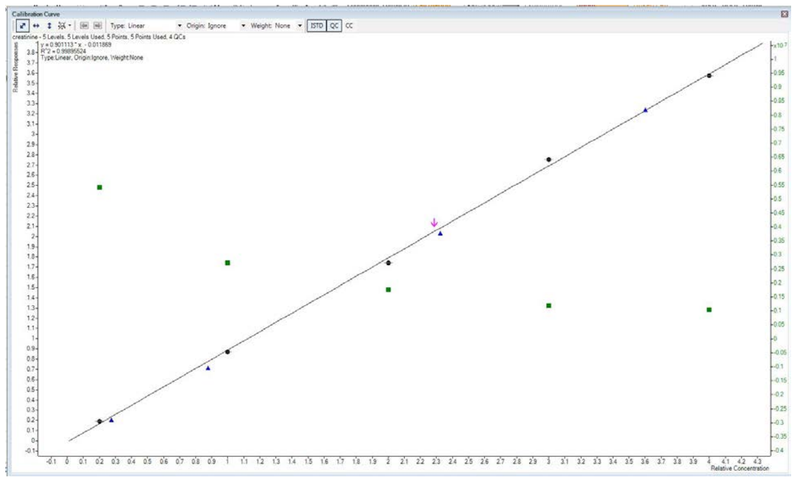

Figure 3. Creatinine calibration curve showing four QC levels and internal standard response.

For creatinine, a 5-point calibration curve was used. Four levels of quality control materials were run to verify calibration. Because of high creatinine concentration in the biological specimen and the high analytical sensitivity of the instrument, the first carbon isotope of creatinine was used to determine concentration in each sample.

To test method performance, 420 individual biological specimens originally screened by immunoassay with reflex to confirmation by mass spectrometry were analyzed.

Results and Discussion

Drug Class	Immunoassay TP	TN	FP	FN	LC-TOF-MS TP	TN	FP	FN
Amphetamines	50	350	20	20	50	370	0	0
Barbiturates	7	711	1	2	9	411	0	0
Benzodiazepines	66	328	4	22	87	333	0	0
Buprenorphine	12	203	1	0	12	203	0	1
Cannabinoids	90	326	0	4	94	326	0	0
Carisoprodol	3	212	1	0	3	213	0	0
Cocaine	18	399	1	2	20	400	0	0
Ethanol Glucuronide	35	380	0	5	41	378	0	1
Fentanyl	38	175	3	0	38	178	0	0
MDMA	0	370	50	0	0	420	0	0
Meperidine	0	209	7	0	0	216	0	0
Methadone	24	395	1	0	24	396	0	0
Opiates	171	240	3	6	177	243	0	0
Oxycodone	92	321	4	3	93	326	0	1
PCP	0	420	0	0	0	420	0	0
Tapentadol	1	205	9	0	1	215	0	0
Tramadol	18	198	0	0	18	198	0	0
Zolpidem	3	201	12	0	3	213	0	0

Figure 3. Comparison of immunoassay vs. TOF results. TP-True Present TN-True Not Present FP-False Present FN- False Not Present.

Of the 420 biological specimens with analytes showing as present by immunoassay, 117 failed to confirm by more specific mass spectrometry methods, indicating false present responses by the immunoassay technique. None of these 117 sample results were above the cutoff calibrator on the LC-TOF-MS assay, suggesting no false present results. The LC-TOF-MS method failed to detect the presence of analyte in each of the following classes: buprenorphine, ethanol markers, and opiates due to concentrations below the established cutoffs.

275 out of 579 specimens (47.4%) screened positive by LC-TOF-MS for nicotine and at least two of its metabolites, though this was not part of the original immunoassay screen.

Creatinine quantitation showed inter- and intra-assay imprecision of less than 3% at two QC levels. Carryover was calculated to be 0.02%. Comparison of biological specimens to the original results via the Jaffe method yielded a slope of 0.91 and correlation coefficient of 0.96. To the authors' knowledge, this is the first time the first carbon isotope of creatinine was used to quantitate it.

Conclusions

Investigation into the replacement of immunoassay-based drug screening and creatinine quantitation by a rapid, LC-TOF-MS screen with higher specificity and accuracy than existing analytical methods was shown. The LC-TOF-MS method was found to be a sensitive and more specific way to screen for drugs providing creatinine quantitation. Further research is needed before implementing in a clinical setting.

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