Comparison of Data Acquisition Methods for High Resolution Mass Spectrometry Drug Screening

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INTRODUCTION

The proliferation of new psychoactive substances (NPS) continues to challenge forensic toxicology laboratories tasked with the identification of an ever-increasing number of drugs. Structural similarities, isobaric compounds, and structural isomers can further complicate the identification of NPS in biological matrices. Yet, many of these substances are preferred by recreational drug users due to their legal status in some jurisdictions. The rapid emergence and fast-paced drug trends are problematic for laboratories that must develop and validate new screening methods to identify these substances. Conventional immunoassay-based drug testing may no longer be adequate for broad-spectrum screening. As such, interest in high resolution mass spectrometry-based (HRMS) approaches has increased significantly.

HRMS-based instrumentation can offer increased scope of testing, increased specificity using monoisotopic masses and isotopic patterns, increased chemical and structural elucidation with ion fragmentation, and multiple modes of data acquisition. The current study focuses on three modes of data acquisition using the Agilent 6530 liquid chromatograph/quadrupole time-of-flight mass spectrometer (LC/QTOF-MS) **(Table 1).** TOF mode allows drugs to be identified based upon a precursor ion and retention time; "All Ions" mode creates an additional level of drug characterization, fragmenting all precursor ions regardless of abundance by functioning as a data-independent system; Auto MS/MS mode analyzes data according to an established threshold as a data-dependent acquisition system. Only those ions that are abundant above a defined threshold are fragmented using the data dependent approach. As part of ongoing method development to identify >200 drugs in blood in accordance with published standards [1,2,3], optimal modes of data acquisition were investigated.

MATERIALS AND METHODS

The mode of data acquisition was evaluated using an optimized LC system in both positive and negative electrospray ionization (ESI) to accommodate acidic, basic, and neutral drugs. A subset of sixty-four compounds among the 237 drugs were evaluated. These included all Tier 1 drugs recommended for the investigation of drug-impaired driving [1] in addition to other commonly encountered substances in medico-legal death investigations [2]. These included 43 additional drugs considered instrumentally challenging due to concentration, drug class, or accurate mass. The drug data acquisition comparison was conducted in both positive and negative ESI by evaluating mass error, target score, and signal-to-noise ratio (SNR).

Two drug standard mixes were used in methanol; one mix containing all Tier 1 drugs at the recommended cutoffs **(Table 2)** and a second mix comprised of the remaining drugs at concentrations equivalent to 100 ng/mL in blood **(Table 3).** A third mixture of the supplemental drugs was prepared at 1000 ng/mL for analysis in negative ESI. All solvents used were HPLC grade or higher, and reference standards were purchased from Cayman Chemical, Cerilliant Corporation, and Lipomed, Inc.

Drugs were reconstituted in a 90:10 mixture of mobile phase A/B consisting of 5 mM ammonium formate/0.01% formic acid in deionized water and 0.01% formic acid in methanol. Each standard drug mix was analyzed using each mode of acquisition in both positive and negative ESI (n=5). A target-suspect screening workflow was utilized to evaluate each data file with Agilent MassHunter Qualitative Analysis software. A personal compound database library (PCDL) was used for identification purposes. A one-way ANOVA analysis was then completed to determine if the data acquisition modes were significantly different for each parameter tested. Further statistical testing (t-test) was performed, as necessary, to determine which data acquisition provided optimal drug detection and response.

RESULTS

Figure 1: Data Acquisition Comparison (ESI+) - Mass Error

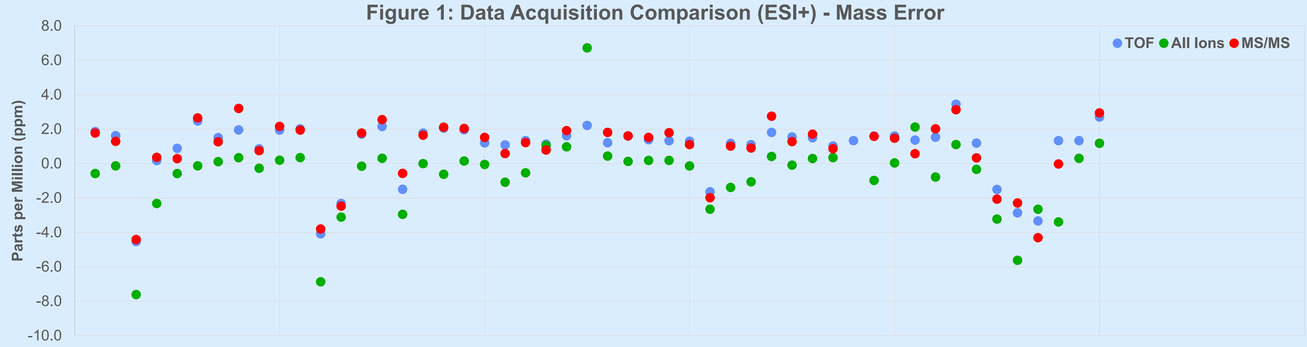


Figure 2: Data Acquisition Comparison (ESI+) - Signal to Noise Ratio (SNR)

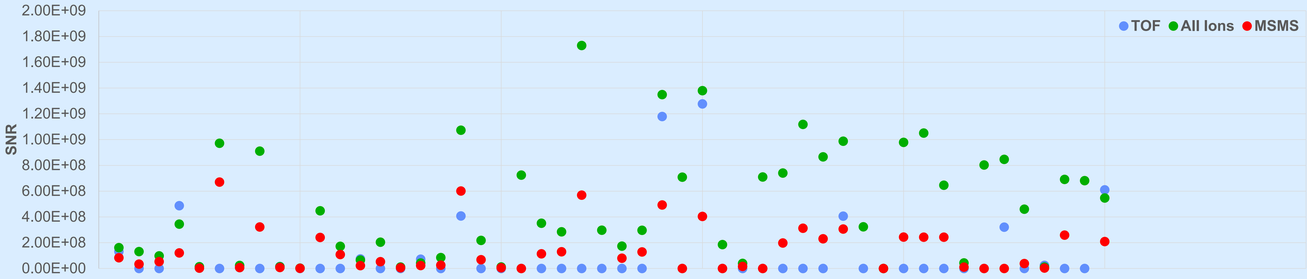


TABLE 2: Tier 1 Drugs

Drug	Cut-Off (ng/mL)
Amphetamine	20
Methamphetamine	20
Buprenorphine	1
7-Aminoclonazepam	10
α-Hydroxyalprazolam	10
Alprazolam	10
Clonazepam	10
Diazepam	50
Lorazepam	10
Nordiazepam	50
Oxazepam	50
Temazepam	50
THC-COOH	10
Fentanyl	1
Zolpidem	10
Carisoprodol	500
Methadone	50
Morphine	10
Oxycodone	10
Tramadol	100
Benzoylecgonine	50

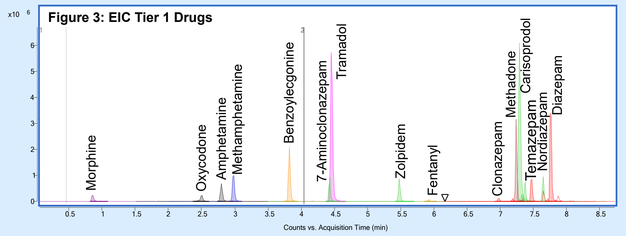
TABLE 3: Supplemental Drugs

11-OH-THC	Isopropyl U-47700
2C-E	Loperamide
2C-T-4	Meclonazepam
2C-T-7	Metaxalone
5F-ADB	Methylclonazepam
5F-AEB	MMB-2201
AB-CHMINACA	MMB-CHMICA
AB-FUBINACA	Naproxen
AB-PINACA	N-Desmethlytramadol
Acetylfentanyl	O-Desmethyltramadol
AH-7921	Pentobarbital
Amobarbital	Phenobarbital
Benzylfentanyl	Phentermine
Butalbital	Phenytoin
Cannabidiol	Psilocybin
Cannabinol	Salicylic Acid
Dibutylone	Secobarbital
DOC	THC-COOH
DOM	U-48800
FUB-AMB	U-51754
GHB	UR-144
Imipramine	Valproic Acid

TABLE 1: Data Acquisition Modes

Instrument Parameters	TOF Mode	All Ions Mode	Auto MS/MS mode
Quadrupole	OFF	OFF	ON
Collision cell	OFF	ON	ON
TOF	ON	ON	ON
Precursor Ions	YES	YES	YES
Product Ions	NO	YES	YES

Figure 3: EIC Tier 1 Drugs



DISCUSSION

No significant difference was identified in mass error, signal to noise ratio (SNR), and target score for each data acquisition mode in negative ESI with p-values of 0.27, 0.32, and 0.42, respectively. Because fewer compounds ionize in this state, data characteristically has diminished chromatographic noise resulting in parallels between each acquisition mode. Drugs acquired using negative ESI are shown in green in **Table 3**. Positive ionization, on the other hand, had increased noise as expected. There were no significant differences identified in target score between data acquisition modes in positive ESI (p-value=0.22). However, significant differences were seen in both mass error and SNR with p-values of 0.0001 and $7.2X10^{-09}$, respectively. Target scores included an evaluation of retention time, isotopic pattern, and mass accuracy. **Figure 1** depicts a scatter plot of the average calculated mass error for each analyte evaluated with each data acquisition in positive ionization. Two-tailed statistical t-testing demonstrated similarities between the TOF and Auto MS/MS acquisition populations. The majority of drugs examined have a mass error ranging from -2 to 2 ppm, respectively. In contrast, the All Ions population proved significantly different from TOF and auto MS/MS acquisition with some outliers ranging from -8 to 8 ppm. The drugs demonstrating higher mass errors included AB-CHMINACA, amphetamine, methamphetamine, and phentermine. Although draft standards recommend a minimum of a single diagnostic ion for screening purposes [4], additional identification criteria can be readily incorporated to improve the sensitivity and specificity of the assay.

DISCUSSION

Meclonazepam and U-51754 were not determined by Auto MS/MS mode due to co-elution with drugs of similar accurate mass and retention time, methylclonazepam and U-48800. As a result of this co-elution, the target/suspect screening algorithm was unable to consistently detect both drugs simultaneously. All Ions mode also encountered this issue with meclonazepam, methylclonazepam, U-48800, and U-51754. However, in contrast to Auto MS/MS, All Ions was able to differentiate each drug based upon its precursor ion when evaluating SNR. **Figure 3** depicts an extracted ion chromatogram (EIC) of Tier I drugs analyzed by All Ions data acquisition at the recommended cutoff.

Statistical testing indicated significant differences between each data acquisition population's SNR results. When determining SNR, the All Ions acquisition had improved discriminating power based upon the precursor ion compared to Auto MS/MS. Average SNR was measured for each of the drugs analyzed in All Ions mode versus Auto MS/MS acquisition mode as shown in **Figure 2.** The TOF mode data acquisition performed well for all parameters including target score, mass error, and SNR in positive ESI. It was able to identify all drugs based upon a precursor ion and retention time. However, the All Ions mode demonstrated the highest overall SNR and comparable results for target score despite a wider range for mass error.

CONCLUSIONS

TOF mode data acquisition was able to identify all compounds based upon precursor ion and retention time. Although All Ions acquisition exhibited higher mass errors for select drugs (compared to TOF and Auto MS/MS) it is advantageous from the standpoint of additional diagnostic ions and the potential for retrospective data analysis. All Ions was the preferred method of acquisition for both positive and negative ionization based upon its mass error, SNR, and target score results. Data-independent acquisition modes such as All Ions broaden the scope of drug detection for forensic toxicology laboratories. It does not rely on an established threshold, and it can retroactively search for newly identified NPS utilizing known chemical formulas and calculated accurate masses. Therefore, this approach was deemed most suitable for screening for a wide variety of conventional drugs and NPS.

REFERENCES

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