Assessment of Three Time-of-Flight (TOF) Mass Spectrometry Drug Screening Technologies Using Different Fragmentation Modes

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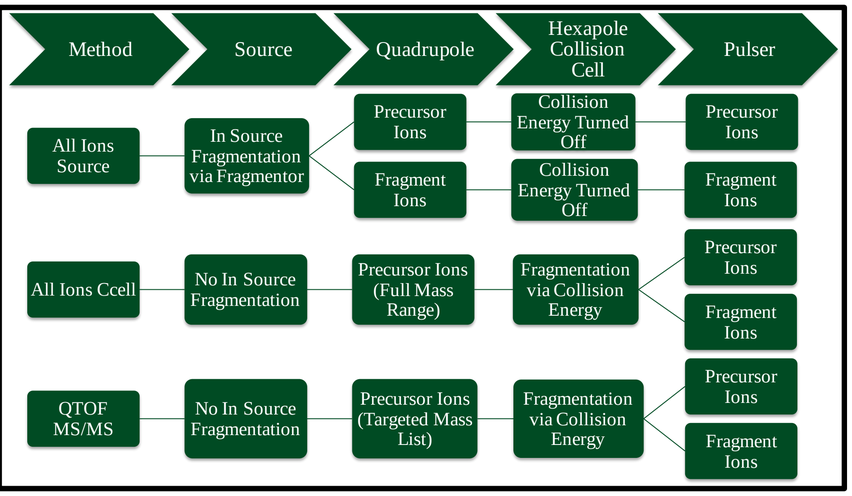
Introduction

- The forensic field is moving toward general screening methods using high resolution accurate mass spectrometry (HRAMS) with LC/TOFMS . The screening via HRAMS LC-TOFMS identifies compounds based on chromatographic retention time, accurate mass and fragmentation. This analytical platform is undergoing constant calibration during acquisition to ensure that accurate masses can be reported.
- HRAMS benefits the screening field in regards to simple sample preparation, and excellent sensitivity. This method of screening can handle a large scope of compounds, and the simple addition of new compounds to databases makes retrospective data mining possible.
- The objective of this research was to evaluate the three different ionization modes: All Ions Source, All Ions Ccell and QTOF MS/MS.
  - In All Ions Source mode fragmentation occurs within the source.
  - In All Ions Ccell mode fragmentation occurs within the collision cell.
  - In QTOF MS/MS mode the quadrupole filtering one precursor mass, and the fragmentation of that mass occurs in the collision cell.

Acquisition Modes



Method Parameters

Instrument: Agilent 1290 Infinity HPLC with an Agilent 6530 Accurate Mass QTOF LC/MS

LC Parameters:

- Column: 3x100mm RRHT EclipsePlus 18, Temperature: 55°C
- Mobile Phase A: 0.05% formic acid in 5mM ammonium formate
- Mobile Phase B: 0.05% formic acid in methanol
- Flow Rate: 0.7 mL/min
- Injection Volume: 5.00µL (when not specified)

MS Parameters:

- Ion Polarity: Positive, Run Time: 11 minutes

Extraction Protocol:

- NMS Labs' Stimulants LC/TOF screening extraction protocol using a 0.5mL blood sample, basic buffer, single step liquid-liquid extraction, evaporate organic layer, and reconstitute in 200µL mobile phase mix (90 MPA:10 MPB).

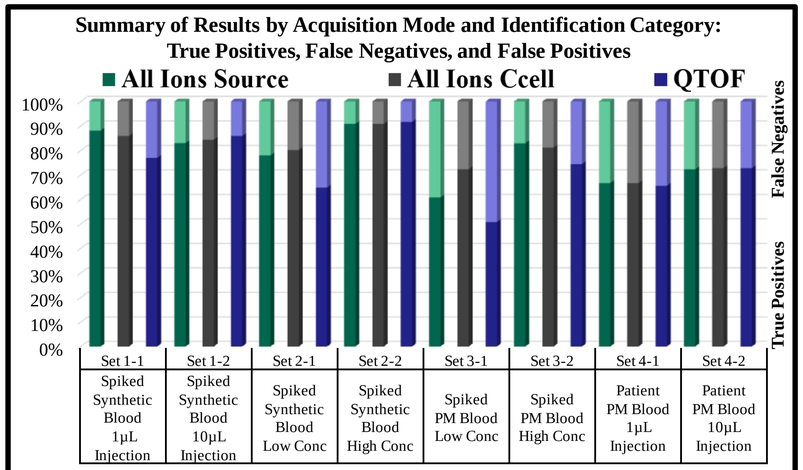
Time (min)	A (%)	B (%)
0	95	5
1	95	5
2	75	25
4	55	45
6	5	95
10	5	95

Table 1: LC gradient conditions

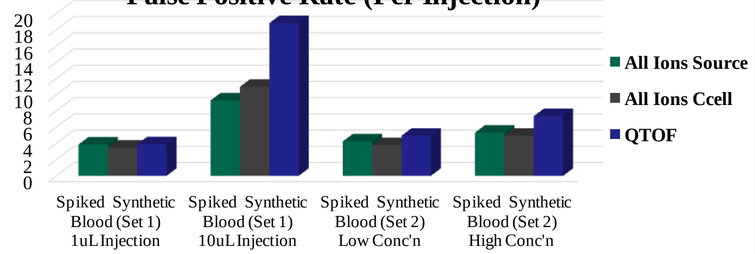
Data Analysis Parameters:

- Qualitative analysis by "Find by Formula" against a Personal Compound Database and Library (PCDL) containing 140 entries with MS/MS and retention time data.
- Probability match score using retention time, mass, and isotopic abundance and spacing generated; enhanced by fragment confirmation requiring the presence and co-elution of at least one fragment ion.

Experiments and Results: Blood Samples



- The three acquisition methods were evaluated in terms of true positives and false negatives in two separate matrices: synthetic blood and post-mortem blood.
- Injection volumes and spiked concentrations were varied to identify method sensitivity.



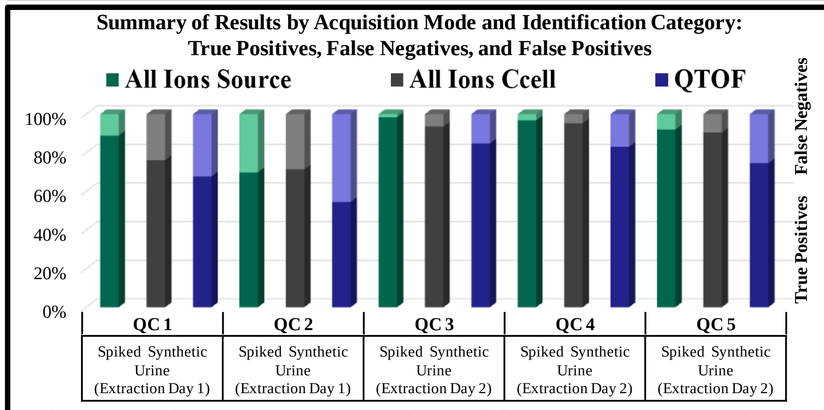
- The false positive rate per injection was only determined for spiked synthetic blood samples. The experiment shows when a larger amount of analyte is put on column, the QTOF acquisition method is most prone to false positives.

Experiment and Results: EDM Urine Samples

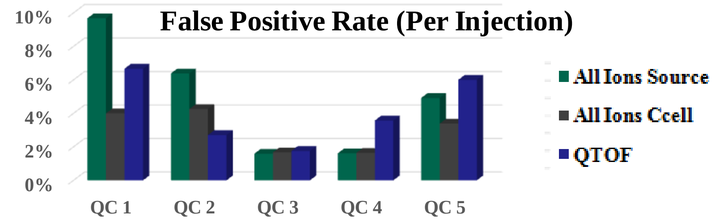
- Samples were collected at an EDM festival in Spring 2014.
- Samples have been analyzed by many platforms including screening by immunoassay, GC/MS, QTOF, and TOF and GC/MS and LC/MS/MS confirmations.

	# Confirmed	All Ions Source	All Ions Ccell	QTOF MS/MS
Methylone	18	16	16	14
α-PVP	10	10	10	9
Ethylone	9	8	9	4
MDA	9	9	9	8
MDMA	8	7	7	8
Amphetamine	5	5	5	4
DXM	4	4	4	4
2-FA/3-FA/4-FA	3	3	3	2
Methamphetamine	3	3	2	2
		93%	**95%**	**76%**

Experiments and Results: Urine Samples



- Spiked synthetic urine controls were run with the EDM samples to give a general idea of true positive/false negative/false positive/true negative rates.
- The chart above shows the percentages of compounds spiked that were identified (true positives) and the percentages that were not identified (false negatives) for each mode.
- The chart below shows the percentage of identifications made that were for compounds not spiked into the synthetic urine.



Discussion

- All Ions methods performed better when detecting spiked drugs using larger injection volumes and higher concentrations.
- General challenges with data interpretation: overloaded and split peaks, fragments co-eluting, and wide retention time windows lead to incorrect peaks being integrated; adducts and losses lead to potential false positives; the lack of MS/MS data in library and co-elution of ions leads to false positive without thorough manual review.
- Fragmentation allows for additional data characteristics to be used for identification thus eliminating some of the false positives caused by matrix effects, artifacts, minor metabolites, degradation products, drug analogs, and isomers.
- The data-dependent nature of the QTOF acquisition mode leads to an increase in analysis time, a decrease in sensitivity, and an overall decrease in the amount of information obtained from a single injection. Issue associated with this mode can be attribute to fewer data points are collected for each ion observed causing poor peak shape and insufficient integration.

Conclusion

- The All Ions Source (equivalent to a TOF) and the All Ions collision cell methods outperformed QTOF method especially at lower concentrations and lower injection volumes.
- All Ions methods of screening by TOF/QTOF provide a powerful, sensitive, and selective tool for broad scope toxicological screening.

Acknowledgments

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