THE CENTER FOR FORENSIC SCIENCE RESEARCH & EDUCATION

Comparison of UPLC-QTOF and GCMS for Detection of Designer Drugs in Urine Samples

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Disclaimer

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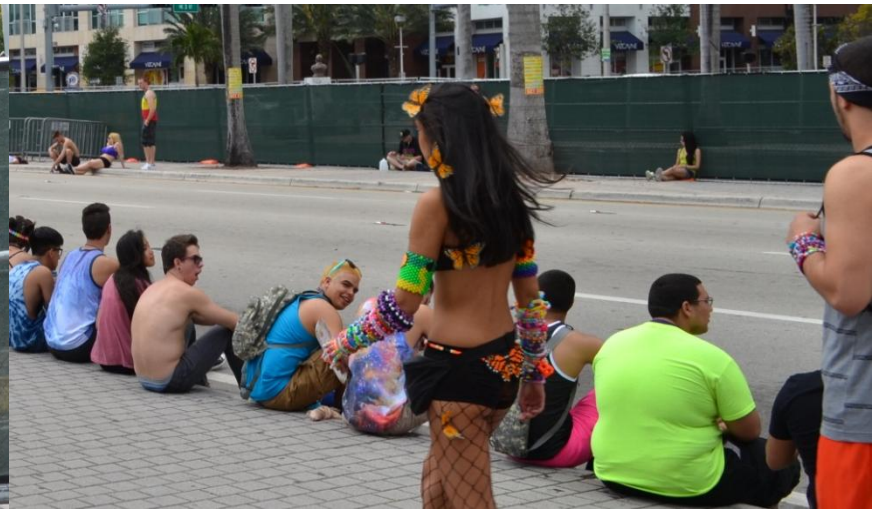
Project Background

- Attendees of electronic dance music festivals (EDM) demonstrate high rates of experimental drug use
- Collection of biological specimens of EDM attendees increases treatment ability for those experiencing adverse reactions and increases ability of toxicology labs to detect compounds



Project Objectives

- Analyze samples to obtain information regarding:
  - New drugs on the market
  - Prevalence of designer drugs
  - Identification of novel designer drugs and metabolites
  - Correlations and comparisons of designer drugs in blood, urine and oral fluid specimens

Sample Collection

- Approached participants on their way to EDM festival
- Location was ~100 yards from entrance gate
- Participants signed consent forms and were asked survey questions
- Samples collected included:
  - Oral Fluid Collection
    - Alere DDS2 Cartridge
    - Quantisal
  - Urine
  - Blood

Disclosure: Participants were not required to donate all 4 samples, and only donated samples based on their comfort level. The gift card incentive was only given if the participant donated a blood sample.

Urine Results

- Total number of urine samples collected: 104
- Samples underwent a battery of screen tests:
  - Immunoassay
  - Volatiles
  - RapidFire-MS/MS
  - **GC/MS**
  - **LC-QTOF**
  - LC-MS/MS

COMPARISON BETWEEN GC/MS AND LC-QTOF AS SCREENING TECHNIQUES

Sample Preparation (GC/MS)

- To 2 mL urine, add internal standard, 100 mM phosphate buffer (pH 6.0)
- To a copolymeric bonded phase extraction column:
  - **Condition**: Methanol, Water, 100 mM phosphate buffer
  - **Apply Sample**
  - **Wash**: Water, 20% Acetonitrile/Water, 100 mM Acetic Acid, then DRY
  - **Wash**: Hexane, Methanol, then DRY
  - **Elute**: Isopropanol, Ammonium Hydroxide, Methylene Chloride
- **Evaporate** (add 10% HCl) and **Reconstitute** with Acetonitrile

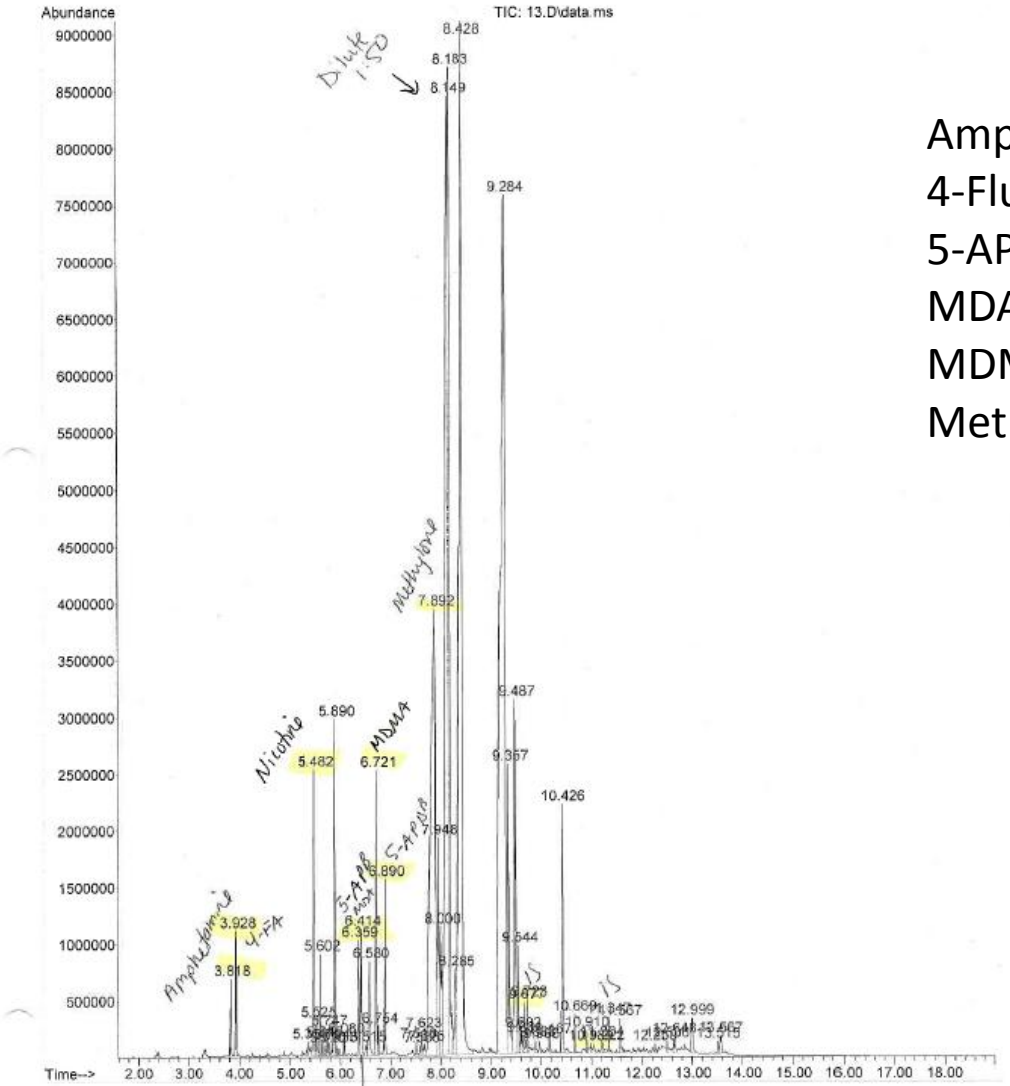
GC/MS Parameters

- Agilent GC (6890)/ MS (5975)
- Column: DB5MS 20m x 0.18mm x 0.18µm
- Split Ratio: 10:1
- Injection Temperature: 250°C
- Injection Volume: 2µL
- GC Oven Programming:
  - Initial 70°C (1 min)
  - Ramp 20°C/min
  - Final 300°C (5.5 min)
- Total Run Time: 17.5 min
- MS Acquisition: 42-550 m/z

Acceptability Criteria (GC/MS)

- Chromatographic peak must be clearly identifiable, as well as internal standard peak
- Chromatographic peak must be within ±2% of analyte in standard
  - If analyte is not present in a standard, standard is analyzed under same conditions to verify retention time
- Mass spectrum minimum confidence of 70% compared to reference library spectrum

Chromatogram of MS124 (GC/MS)



Amphetamine
4-Fluoroamphetamine
5-APB
MDA
MDMA
Methylone

Sample Preparation (LC-QTOF)

- To 0.5 mL urine, add internal standard, water, 100 mM phosphate buffer (pH 6.0)
- To a copolymeric bonded phase extraction column:
  - **Condition**: Methanol, Water, 100 mM phosphate buffer
  - **Apply Sample**
  - **Wash**: Water, 100 mM Acetic Acid, Methanol, then DRY
  - **Elute**: Isopropanol, Ammonium Hydroxide, Methylene Chloride
- **Evaporate** (add 10% HCl) and **Reconstitute** with Mobile Phase

LC-QTOF Parameters

- Waters Acquity I-Class UPLC Conditions:
  - Mobile phase A: 5mM ammonium formate (pH 3.0)
  - Mobile phase B: 0.1% formic acid in acetonitrile
  - Column: Waters Acquity HSS C18 150mm x 2.1mm x 1.8µm
  - Flow rate: 0.4 mL/min
  - Column Temperature: 50°C
  - Injection Volume: 2µL

Time (min)	%A	%B
Initial	87	13
0.5	87	13
10.0	50	50
10.75	5	95
12.25	5	95
12.5	87	13
15.0	87	13

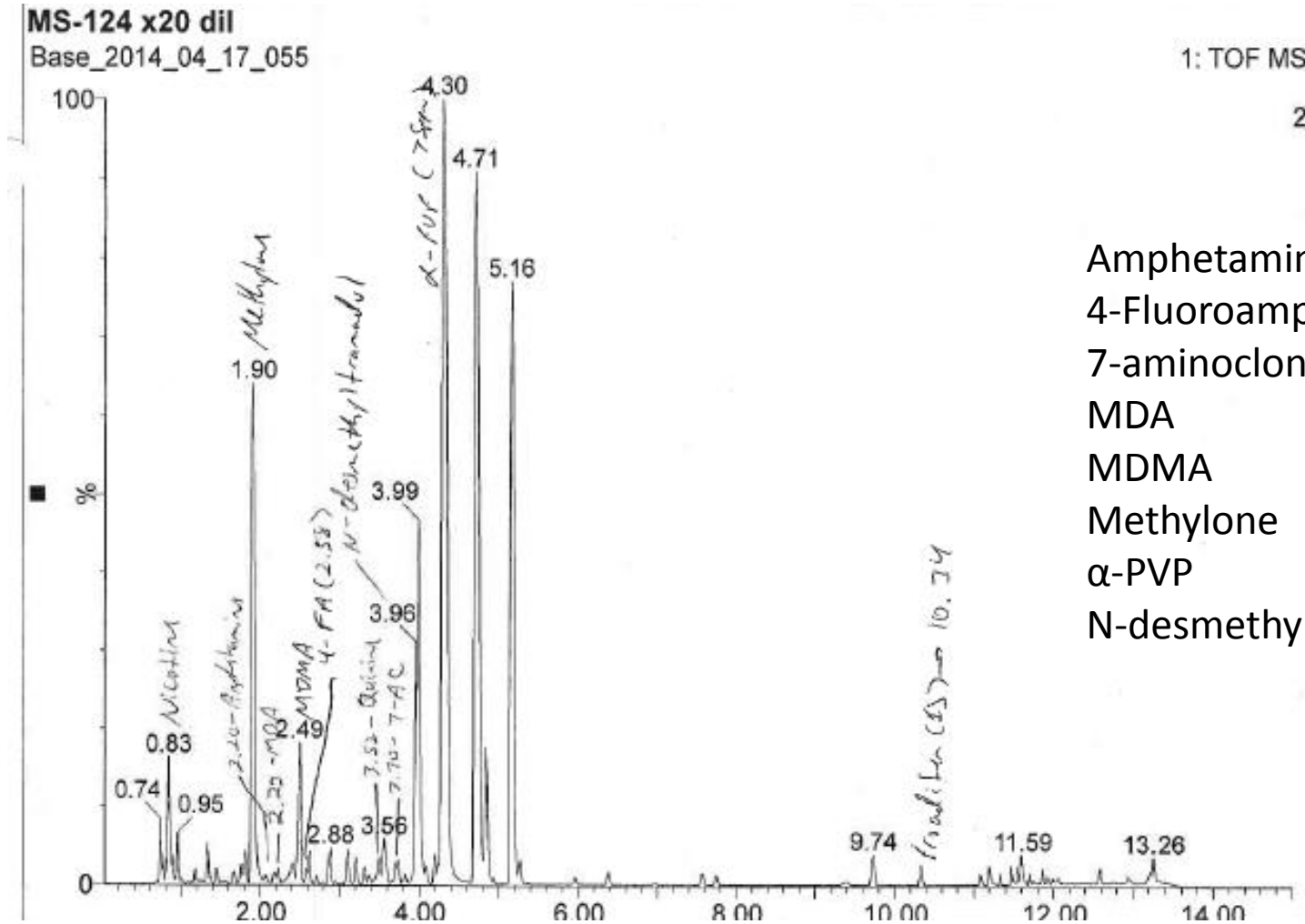
LC-QTOF Parameters

- Xevo G2 QTOF Conditions:
  - Ionization: Positive electrospray
    - Capillary voltage: 0.8 kV
    - Sample Cone Voltage: 20 V
    - Extraction Cone Voltage: 4 V
    - Source Temperature: 140°C
    - Desolvation Temperature/Flow: 500°C/900 L/h
  - Resolution Mode: 50-1000 m/z
    - Collision Energy (Function 1) – 6eV
    - Collision Energy (Function 2) – 10-40eV

Acceptability Criteria (LC-QTOF)

- Chromatographic peak must be clearly identifiable, as well as internal standard peak
- Chromatographic peak must be within ±2% of analyte in standard or within ±0.3 min of analyte in database
  - If analyte is not present in a standard or database, standard is analyzed under same conditions to verify retention time
- Observed mass of molecular ion must be within ± 5ppm of mass in database
- Observed mass of fragment ion must be within ± 5ppm of mass in database

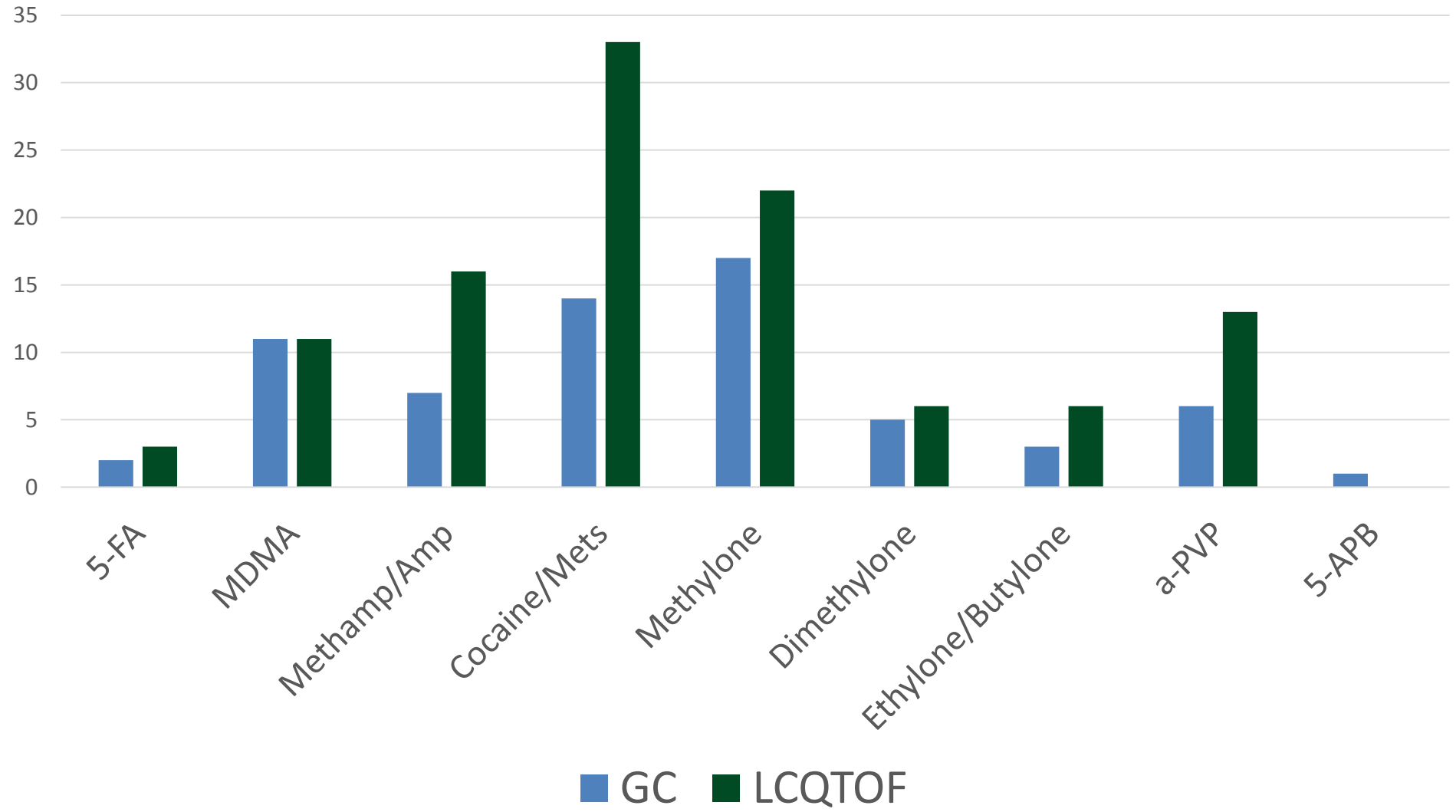
Chromatogram of MS124 (LC-QTOF)



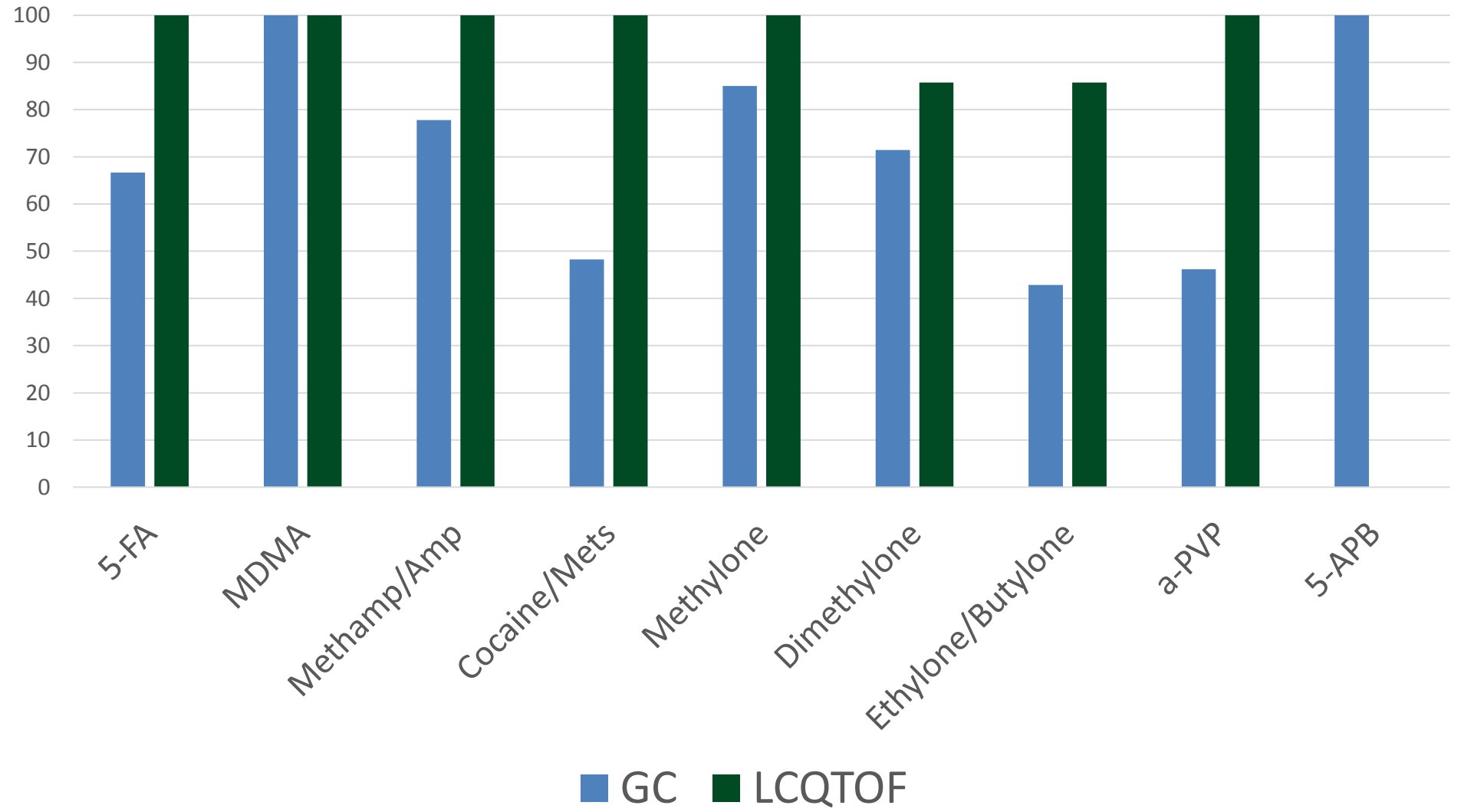
Amphetamine
4-Fluoroamphetamine
7-aminoclonazepam
MDA
MDMA
Methylone
α-PVP
N-desmethyltramadol

GC/MS AND LC-QTOF RESULTS

GC/MS vs. LC-QTOF Positive Screens



GC/MS vs. LC-QTOF Confirmation Rate



Unconfirmed Positives

Analytes	# Positives	# Confirm Positive
Methamp/Amp	16	9
Cocaine/Mets	33	29
Methylone	22	20

GC/MS vs. LC-QTOF

	GC/MS		LC-QTOF	
Rate	**#**	**% of Total**	**#**	**% of Total**
Sample Positivity Rate	49	80.3	63	103.3
False Negative Rate	12	19.6	0	0.0
Total Positive Samples	61	--	61	--

Alcohol Only Positives: 8

THC Only Positives: 16

Total Number of Positive Sample: 85 / 104 = 82%

GC/MS Results

- Missed analytes:
  - Benzoylecgonine, THC, Cyclobenzaprine, DMAA, Alprazolam, Oxazepam, 7-aminoclonazepam, Psilocin, Buprenorphine, Azacyclonol, 3,4,5 Trimethoxy cocaine, PMMA, 2-CB

- Missed analytes due to sensitivity, no derivatization reagents used, poor chromatography on GC

LC-QTOF Results

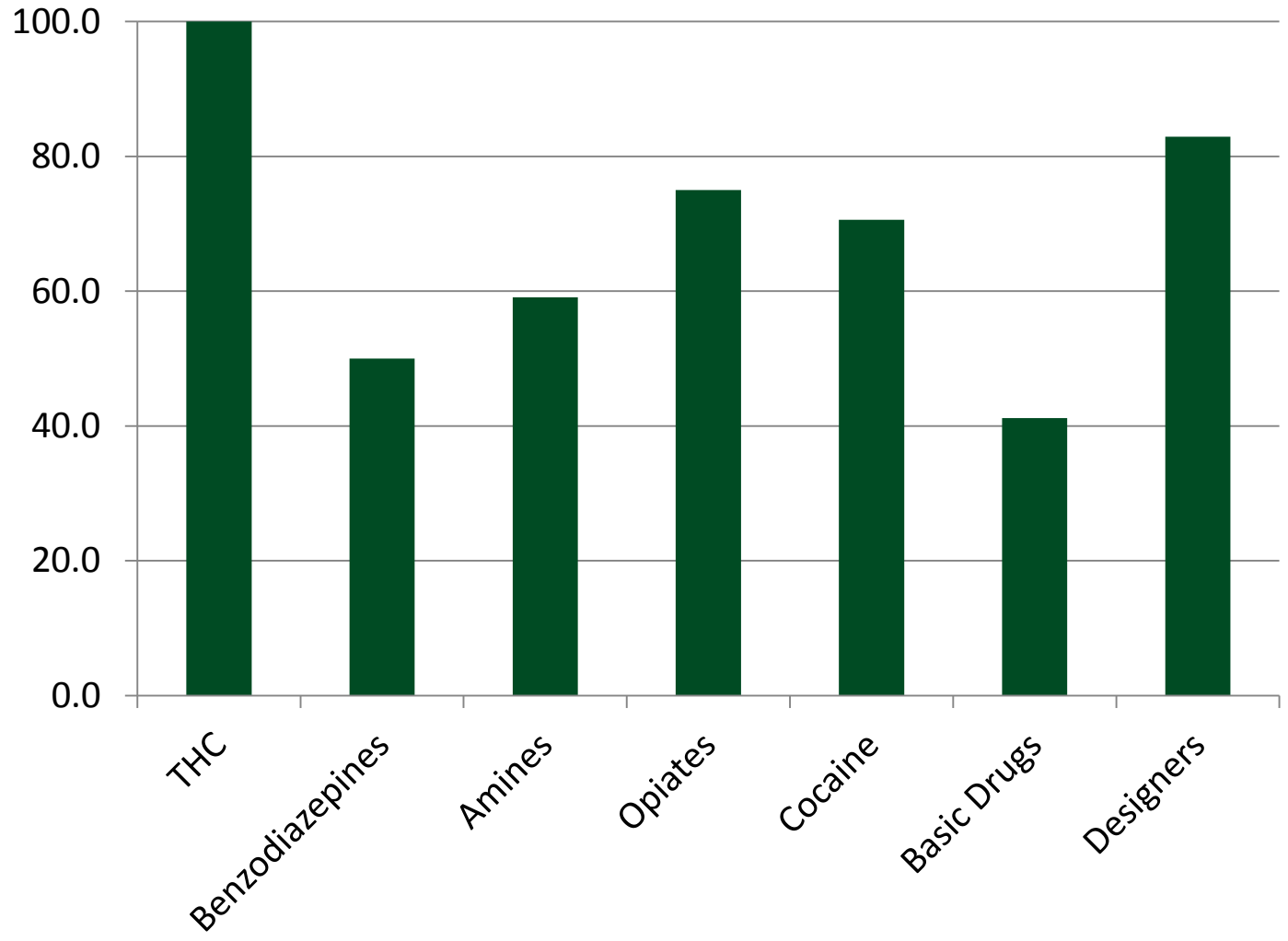
- Missed or poor chromatography analytes:
  - Ecgonine Methyl Ester, THC, 5-APB, Nicotine, Cotinine
- Extra analytes detected due to: increased sensitivity of QTOF vs. confirmation technique, compounds not analyzed for in confirmation technique

Comparison Conclusion

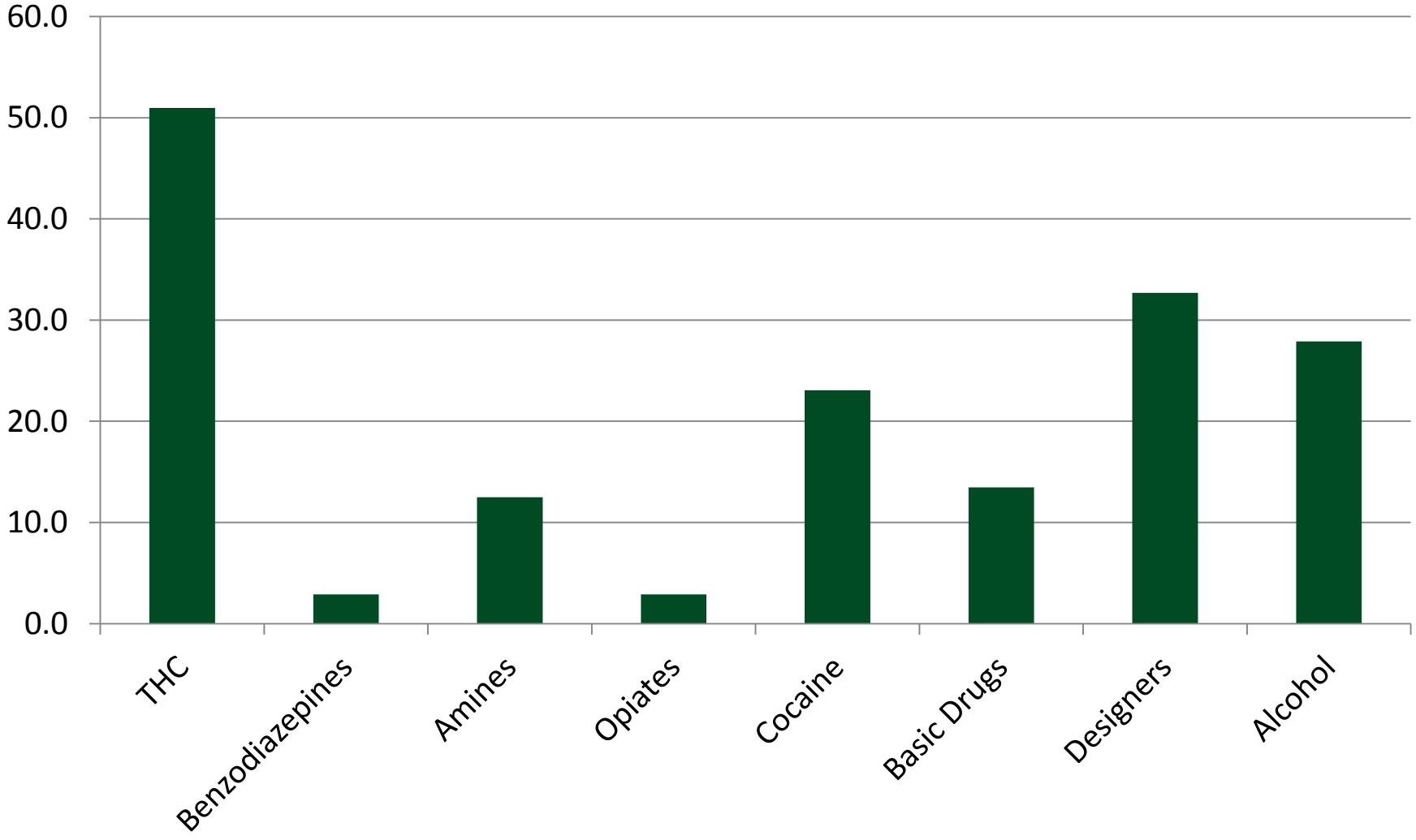
- GC/MS
  - Decreased sensitivity
  - Library search capabilities
  - More false negatives
  - Identified less designer drugs
  - Data interpretation requires less training
- LC-QTOF
  - Increased sensitivity
  - Targeted screen
  - More unconfirmed positives
  - Identified more designer drugs
  - Data interpretation requires increased training

OVERALL RESULTS FOR ANALYTICAL TESTING

Combined % Confirmation Rate



% Positive Rate in Sample Population



“Molly”

- Several participants indicated they had taken “Molly” in the last week
- Samples of subjects (9) who reported taking “Molly” contained:
  - MDMA
  - Methylone
  - Alpha-PVP
- Samples of subjects (15) who reported taking MDMA/Ecstasy contained:
  - MDMA
  - Methylone
  - Dimethylone/Ethylone/Butylone
  - Alpha-PVP

Thank You

- Thank you to everyone at AFMES for helping with all the aliquoting, extractions, data analysis, etc.

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Thank You

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Questions?

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