Low Level Analytes and Reducing the Time to Detection in Forensic Toxicology Analysis

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Low Level Analytes / Limited Sample

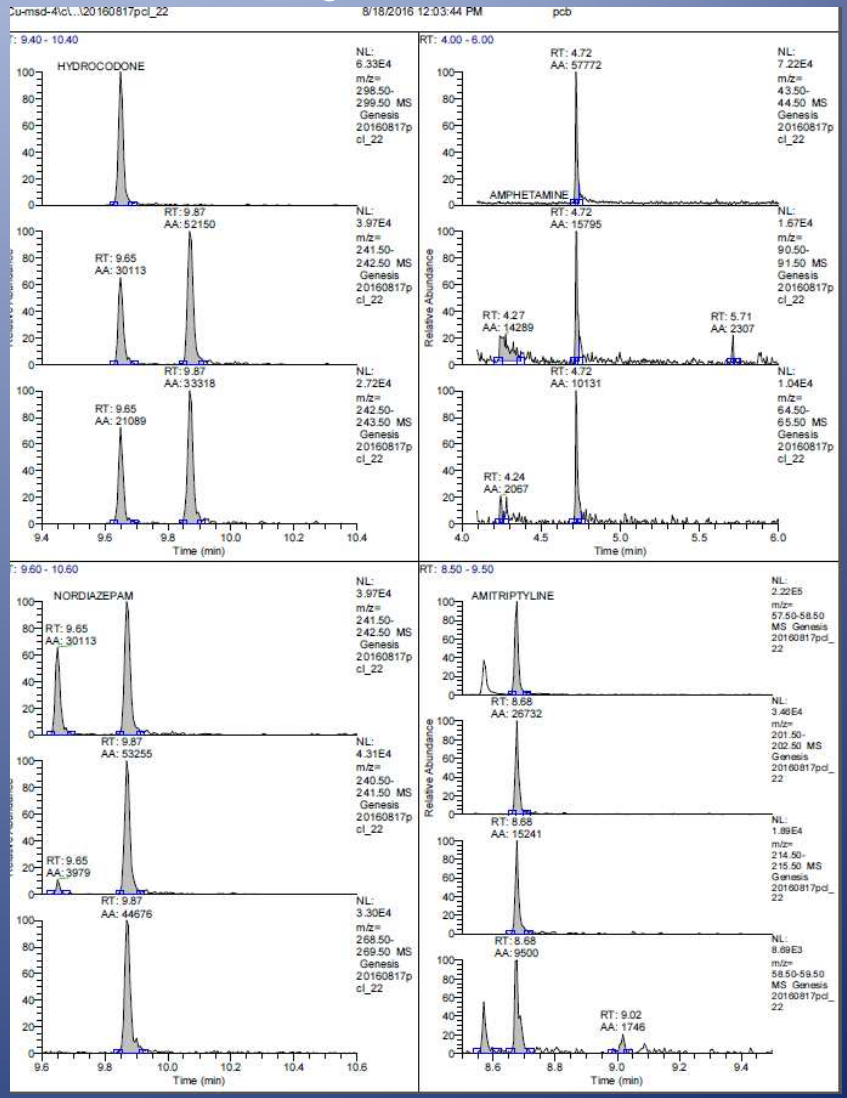
Screening for Basic Drugs

- Diverse
- Important
- Complex

- Analgesics
- Antidepressants
- Antihistamines
- Antipsychotics
- Benzodiazepines
- Cardiac Drugs
- Hallucinogens
- Hypnotics
- Stimulants
- Sympathomimetic Amines

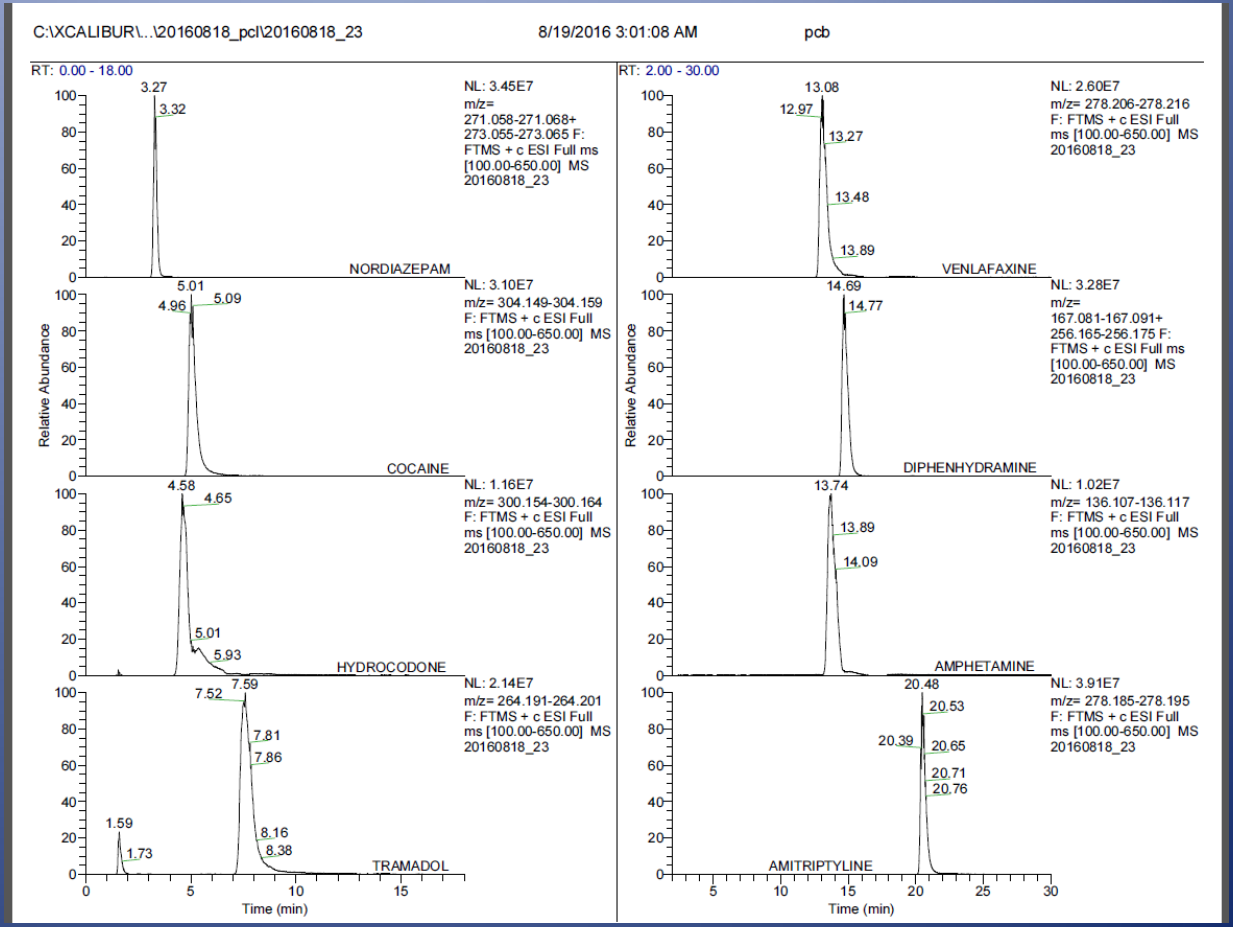
Historical Analysis

- LLE or SPE
  - LLE analyst variability
  - SPE relatively fast
  - 1 mL sample – LODs ~10 ng/mL for many
- GC/MS (35 min runtime)
  - Advantage – EI library matching
  - Disadvantage – not amenable to all drugs and metabolites



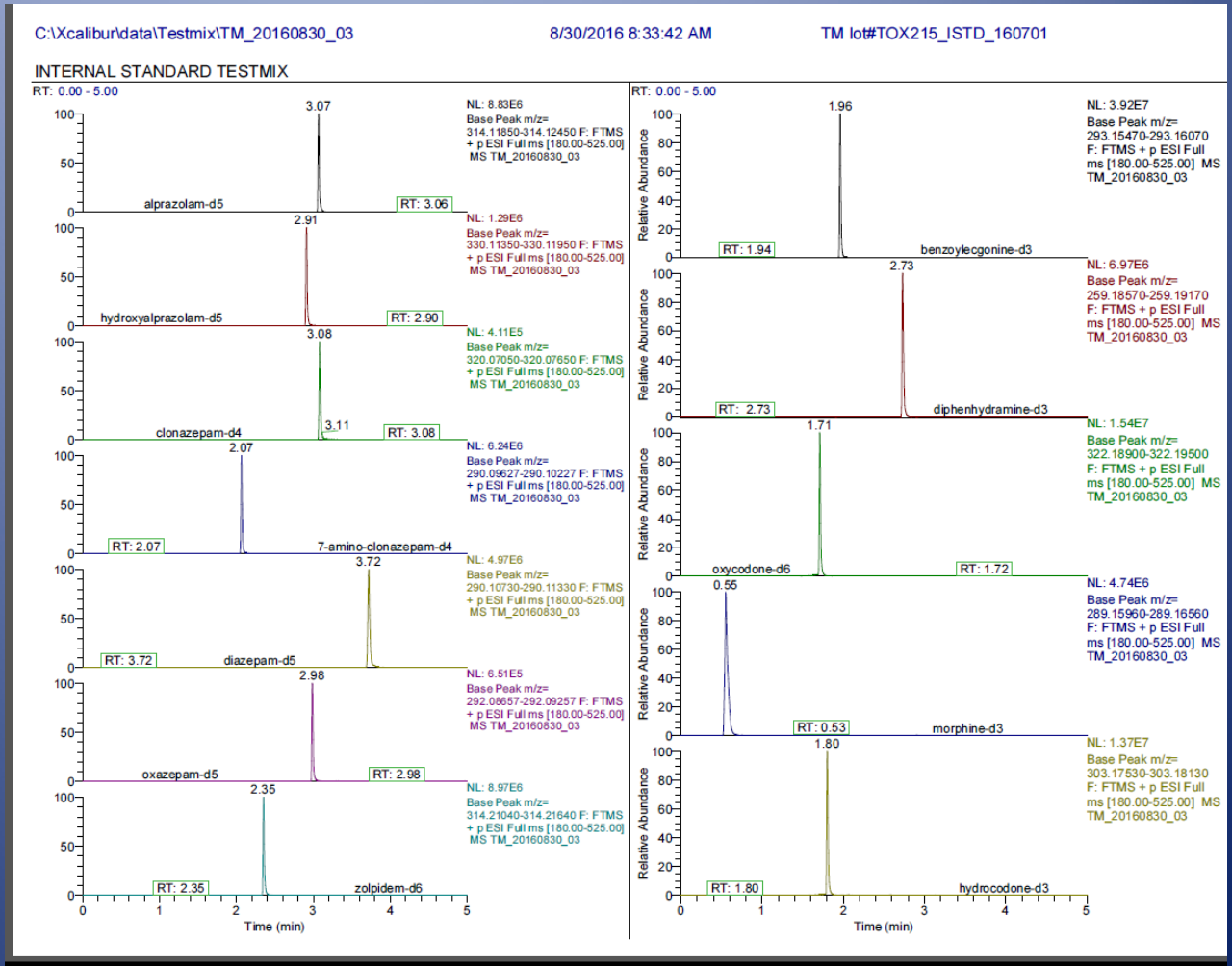
Historical Analysis

- LC/MS (45 min runtime)
  - Advantage: increased sensitivity and scope; HRMS
  - Disadvantage: long runtime; poor chromatography for some classes
  - Full Scan Plus Data Dependent MSMS Scanning
    - reinjection
    - labor intensive data analysis



New and Improved…SLE with UPLC-MS/(MS)

- SLE – fast
  - 0.3 mL sample size
- UPLC – fast
  - Ten minute run time



UPLC Parameters

- Column
  - C18 (1.6µ, 2.1 x 50 mm)
  - Solid core particle technology claims to increase chromatographic efficiency
- Mobile Phases
  - 5 mM Ammonium formate in 0.1% formic acid
  - 0.1% formic acid in acetonitrile
- Flow rate: 0.5 mL/min

Time (min)	Aqueous (%)	Organic (%)
0	95	5
0.9	95	5
2.67	60	40
4.67	60	40
5.56	0	100
7	0	100
7.25	95	5
10	95	5

New and Improved…SLE with UPLC-MS/(MS)

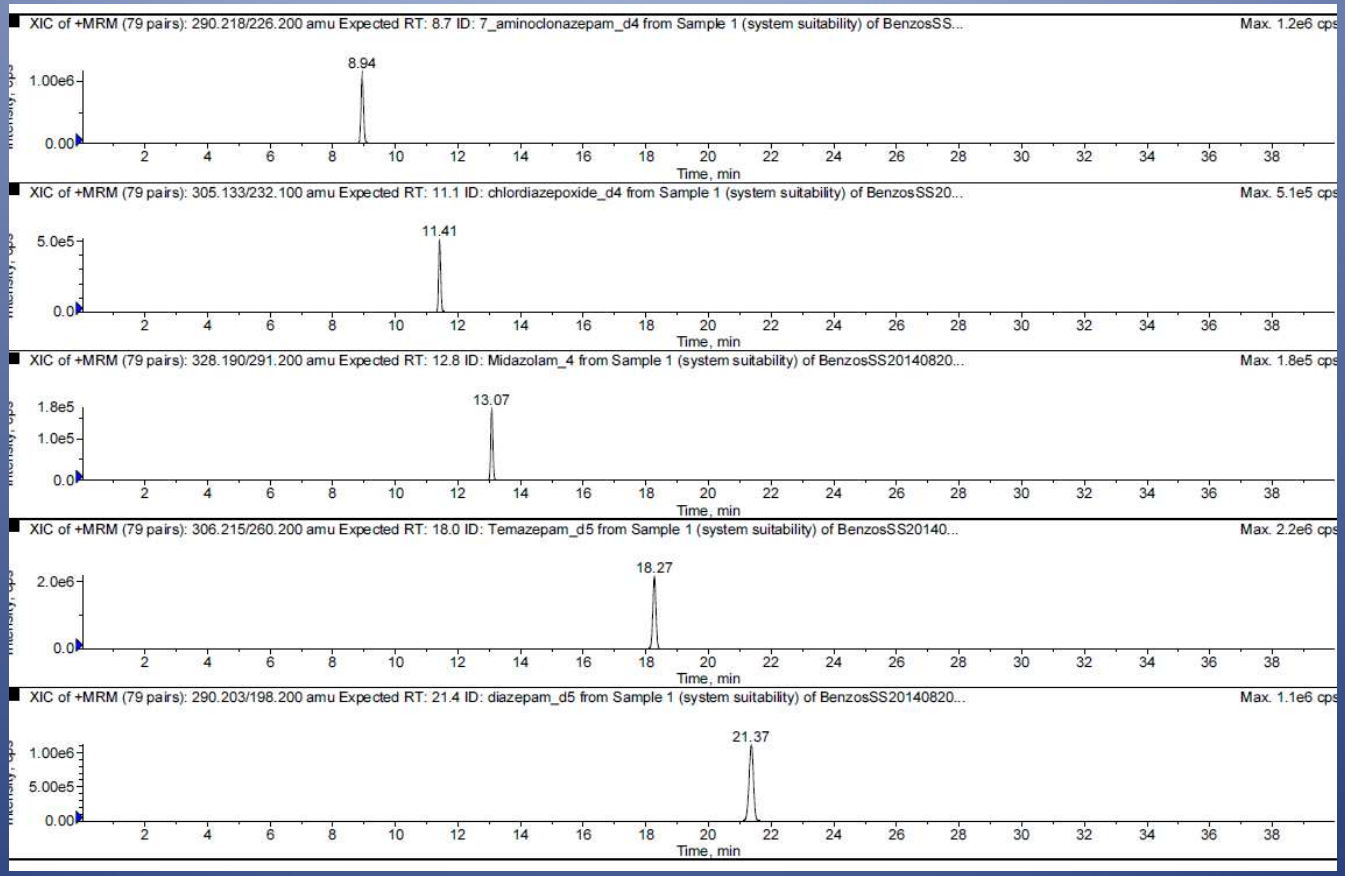
Component	Final Concentration in Specimen (ng/mL)
benzoylecgonine-d_3	10
morphine-d_3	10
hydrocodone-d_3	5
oxycodone-d_6	5
clonazepam-d_4	5
7-aminoclonazepam-d_4	3
α-hydroxyalprazolam-d_5	3
alprazolam-d_5	3
oxazepam-d_5	3
diazepam-d_5	3
diphenhydramine-d_3	3
zolpidem-d_6	2

New and Improved…SLE with UPLC-MS/(MS)

Analyte	Molecular Ion (M+1)	Analyte	Molecular Ion (M+1)
α-hydroxyalprazolam	325.0851	**flunitrazepam**	314.0936
α-hydroxymidazolam	342.0804	**flurazepam**	388.1586
α-hydroxytriazolam	359.0461	**lorazepam**	321.0192
7-aminoclonazepam	286.0742	**lormetazepam**	335.0348
7-aminoflunitrazepam	284.1194	**medazepam**	271.0996
alprazolam	309.0902	**midazolam**	326.0855
bromazepam	316.0080	**nordiazepam**	271.0633
chlordiazepoxide	300.0898	**oxazepam**	287.0582
clonazepam	316.0484	**phenazepam**	348.9738
desalkylflurazepam	289.0539	**prazepam**	325.1102
desmethylflunitrazepam	300.0779	**temazepam**	301.0738
diazepam	285.0789	**tetrazepam**	289.1102
estazolam	295.0745	**triazolam**	343.0511
etizolam	343.0778		

27 compounds; 10 minutes

In Contrast – LC Quant Separation for Benzos



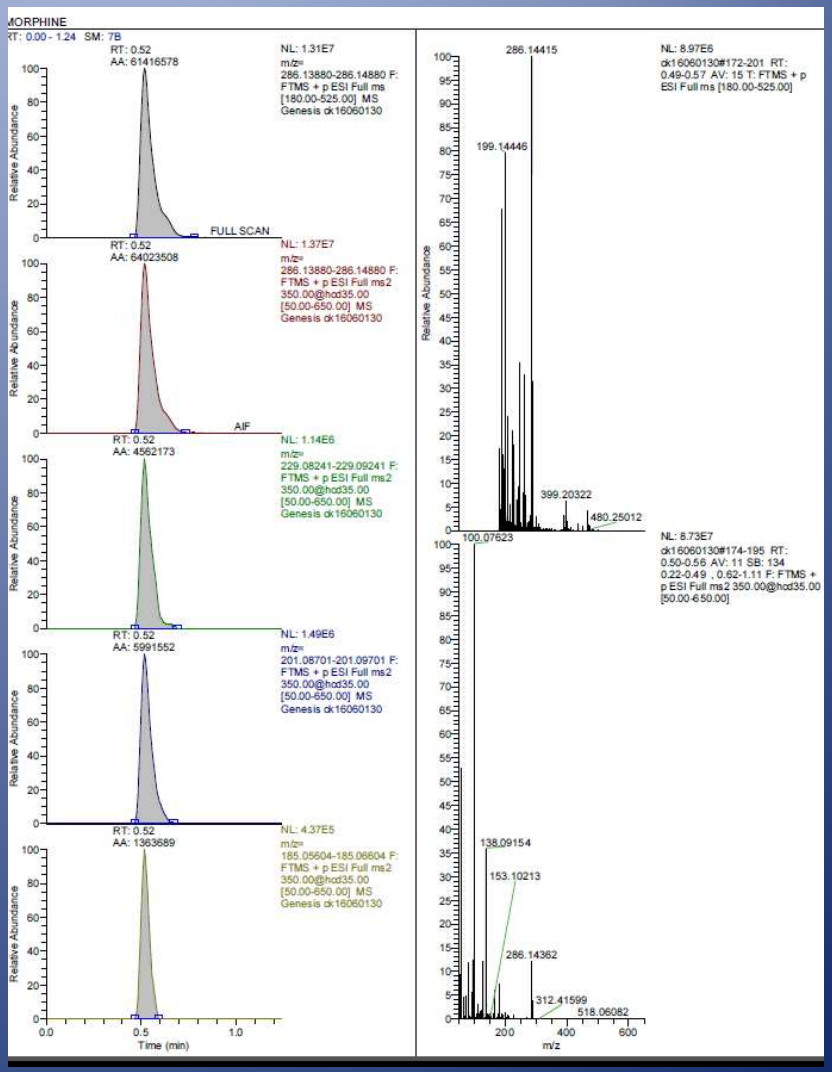
19 compounds; 40 minutes

New and Improved…SLE with UPLC-MS/(MS)

- Using MS as a screening tool
  - Advantage: high resolution data
  - Ruling out is usually easy
  - Ruling in requires MSMS analysis
    - All-ion fragmentation analysis

All-Ion Fragmentation

- All ions fragmented
- Not precursor specific
- Automated data analysis possible



Conclusions

- UPLC and HRMS can reduce sample analysis time
- Data analysis is the next hurdle
- How much data do we need to make a decision to go or no-go?