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Post-Mortem Drug Screening using Paper Spray

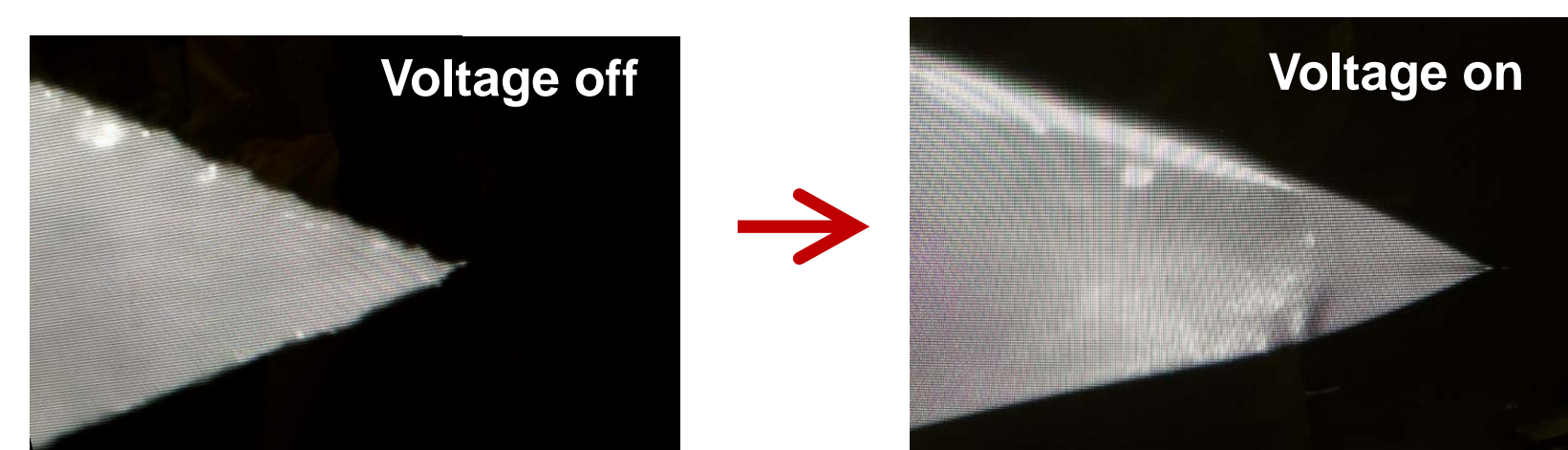
Josiah McKenna, Rachel
Department of Chemistry and Chemical Biology, Indiana University

Overview

- A paper spray drug screening method was developed on a quadrupole-orbitrap mass spectrometer
- 125 target compounds were screened in MS/MS mode using an inclusion list
- 11 internal standards were monitored to perform semi-quantitative analysis
- 30 postmortem blood samples were analyzed by paper spray MS/MS. Results were compared to the screening and confirmatory results from a central toxicology lab.

Introduction

- Blood samples are analyzed as dried spots directly from paper
- Extraction solvent is added to the paper followed by application of a high voltage
- Ionization arises from electrospray from the sharp paper tip
- Screening for the complete drug panel requires about 2 minutes with no sample cleanup



Paper spray cartridge. Blood sample is spotted on paper. Extraction solvent is added in the rear reservoir



Automated paper spray MS interface

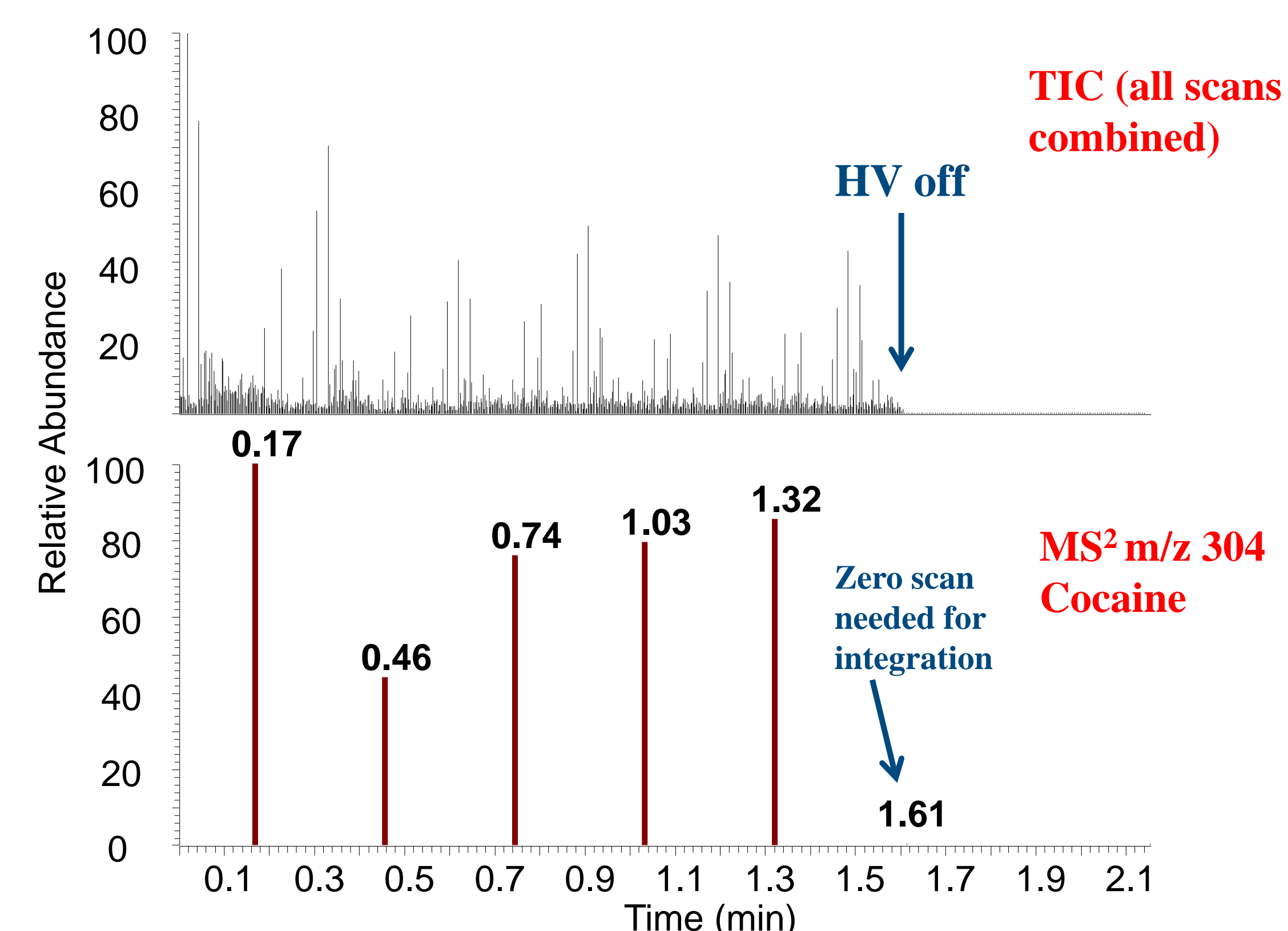
Methods

Paper Spray MS

- **Mass Spec:** Thermo Q-Exactive Focus
- **Paper Spray:** Velox 360 paper spray interface with Velox paper spray sample cartridges
- **Instrument mode:** targeted MS/MS (PRM using an inclusion list)
 - 130 MS/MS scans for 125 targets and 11 internal stds.
- **MS settings:**
 - Isolation width: +/- 0.5 m/z
 - Resolution: 35,000
 - Polarity: positive ion mode
 - Spray voltage: 5000V
 - AGC target: 10⁶
 - Max injection time: 50 ms
- **Detection criterion:** one fragment ion, 5 ppm m/z window
- **Paper spray solvent:** 85:10:5:0.01 ACN:acetone:water:acetic acid

Sample Preparation

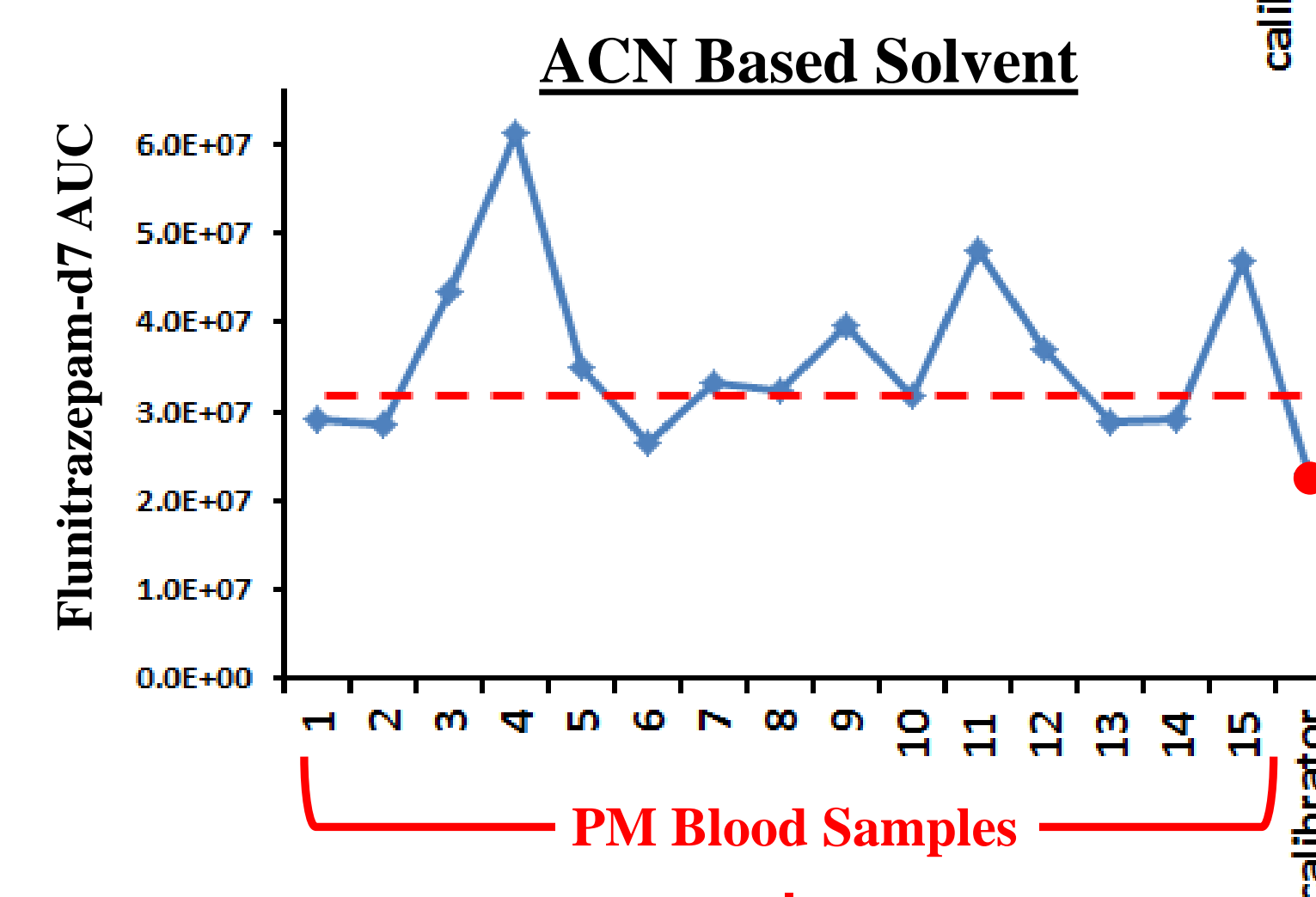
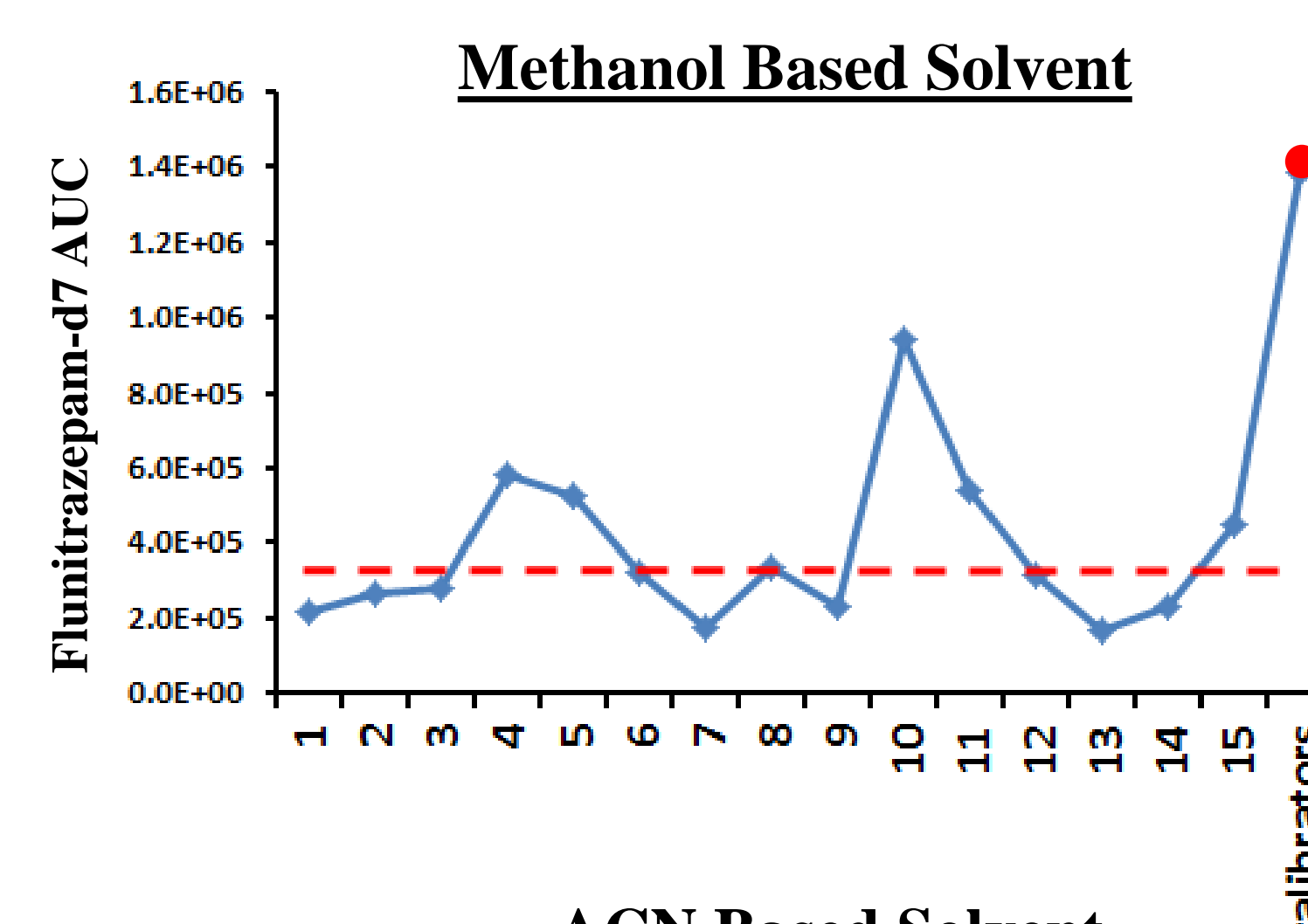
- Blood sample was mixed 1:3 with an aqueous internal standard solution
- 12 μ L of the blood/internal standard mixture was spotted on the cartridge and allowed to dry
- Internal standard solution:
 - 65 ng/mL alprazolam-d5
 - 650 ng/mL benzoylecgonine-d8, cocaine-d3, and methamphetamine-d11
 - 260 ng/mL flunitrazepam-d7, hydrocodone-d3, trimipramine-d3
 - 1300 ng/mL gabapentin-d10
 - 2600 ng/mL metaxalone-d6
 - 325 ng/mL methadone-d3
 - 130 ng/mL zolpidem-d6



- A typical paper spray MS/MS chromatogram is shown to the left
- Cartridge is sprayed for 90 seconds
- 5 scans are obtained for each MS/MS
- Quantitation is performed by integrating the entire 90 second window
- A zero intensity scan is required at the end for automatic peak integration
- Data analysis was performed using TraceFinder 3.3

Results

Solvent Optimization

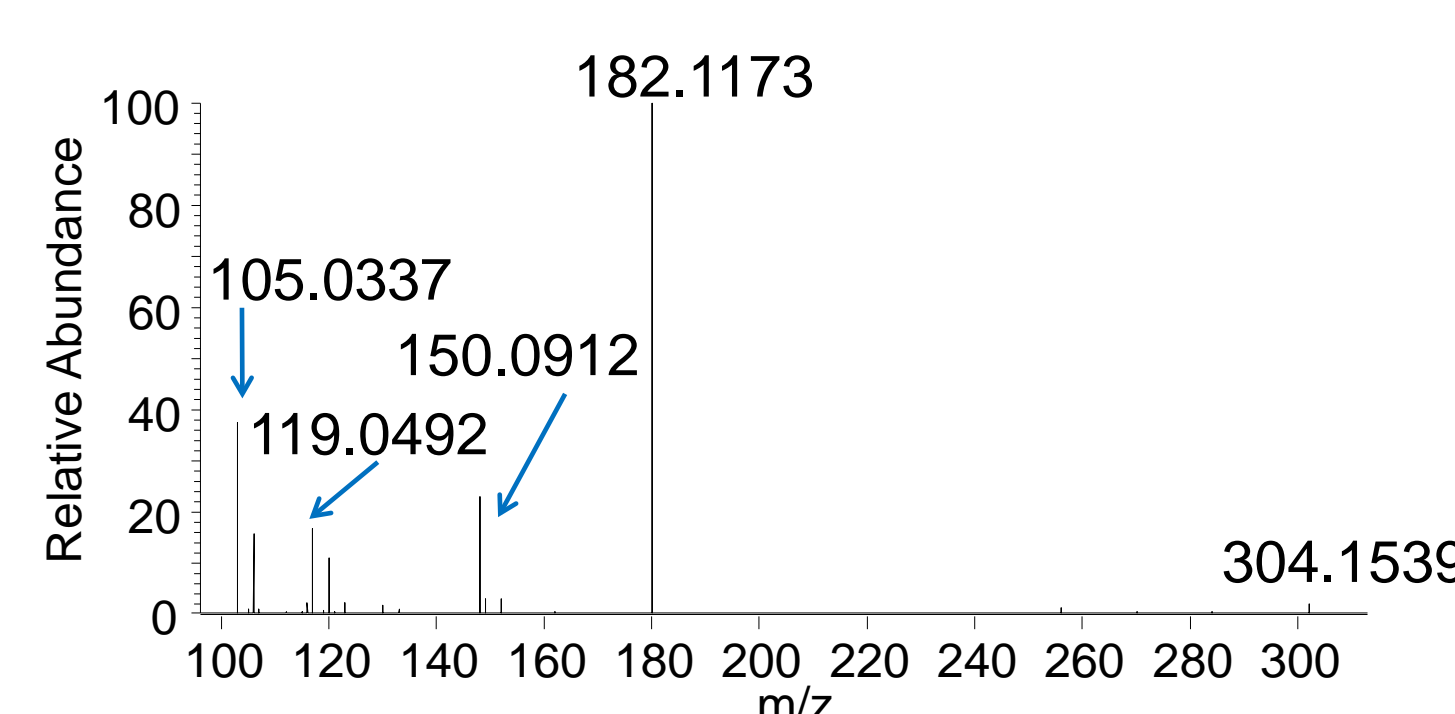


- 95:5 Methanol:water with 0.01% acetic acid showed acceptable results in calibrators
- Significant matrix effects were observed in post-mortem blood samples
- ACN based solvent eliminated relative matrix effects
- Dilution of the blood was required to allow solvent to penetrate into blood spot

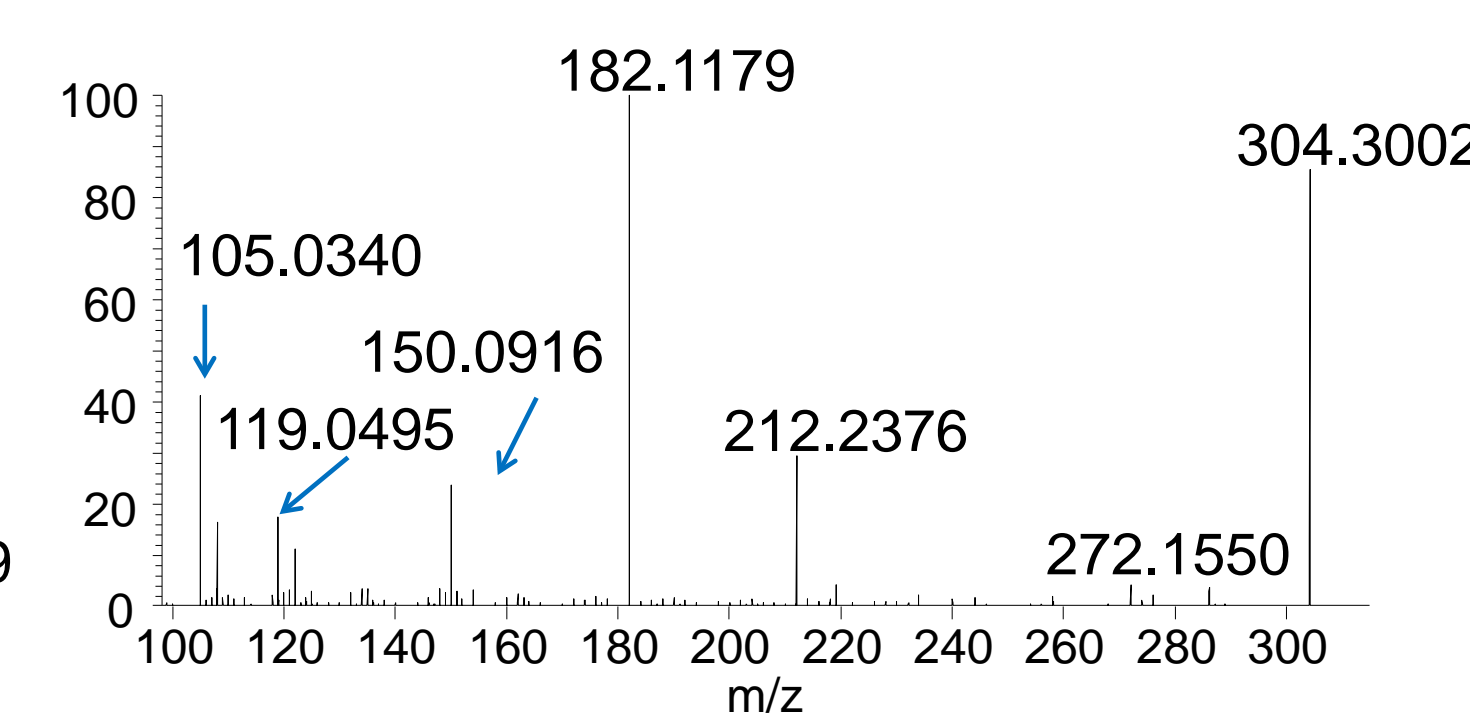
- Average and %RSD of the AUC for each SIL shown in table
- Post-mortem values averaged across 30 different samples
- Calibrators were prepared in single donor fresh blood

Internal Standard	Postmortem Samples	Whole Blood Calibrators
Alprazolam-d5	3.50×10 ⁸ 46%	3.14×10 ⁸ 26%
Benzoylecgonine-d8	3.56×10 ⁸ 28%	3.06×10 ⁸ 27%
Cocaine-d3	6.79×10 ⁸ 28%	5.65×10 ⁸ 24%
Flunitrazepam-d7	4.00×10 ⁷ 32%	2.30×10 ⁷ 27%
Gabapentin-d10	3.52×10 ⁷ 42%	3.59×10 ⁷ 31%
Hydrocodone-d3	6.97×10 ⁷ 30%	8.61×10 ⁷ 28%
Metaxalone-d6	2.41×10 ⁷ 32%	2.96×10 ⁷ 24%
Methadone-d3	9.39×10 ⁸ 50%	8.56×10 ⁸ 32%
Methamphetamine-d11	6.85×10 ⁸ 38%	8.97×10 ⁸ 35%
Trimipramine-d3	6.19×10 ⁸ 48%	5.82×10 ⁸ 27%
Zolpidem-d6	2.26×10 ⁷ 29%	1.88×10 ⁷ 26%

Tandem Mass Spectra Example



ESI Infusion. Neat solvent
Cocaine 200 ng/mL



Paper Spray. Blood Spot
Cocaine 16.6 ng/mL. 3X below cutoff

- Full MS/MS spectra were collected
- Paper spray MS/MS spectra are a composite of target compound and background
- Presence of one fragment ion used for detection
 - More can be used to improve selectivity

Paper Spray MS/MS on a Q-Orbitrap Mass Spectrometer

Michael Potter, and Nicholas E Manicke

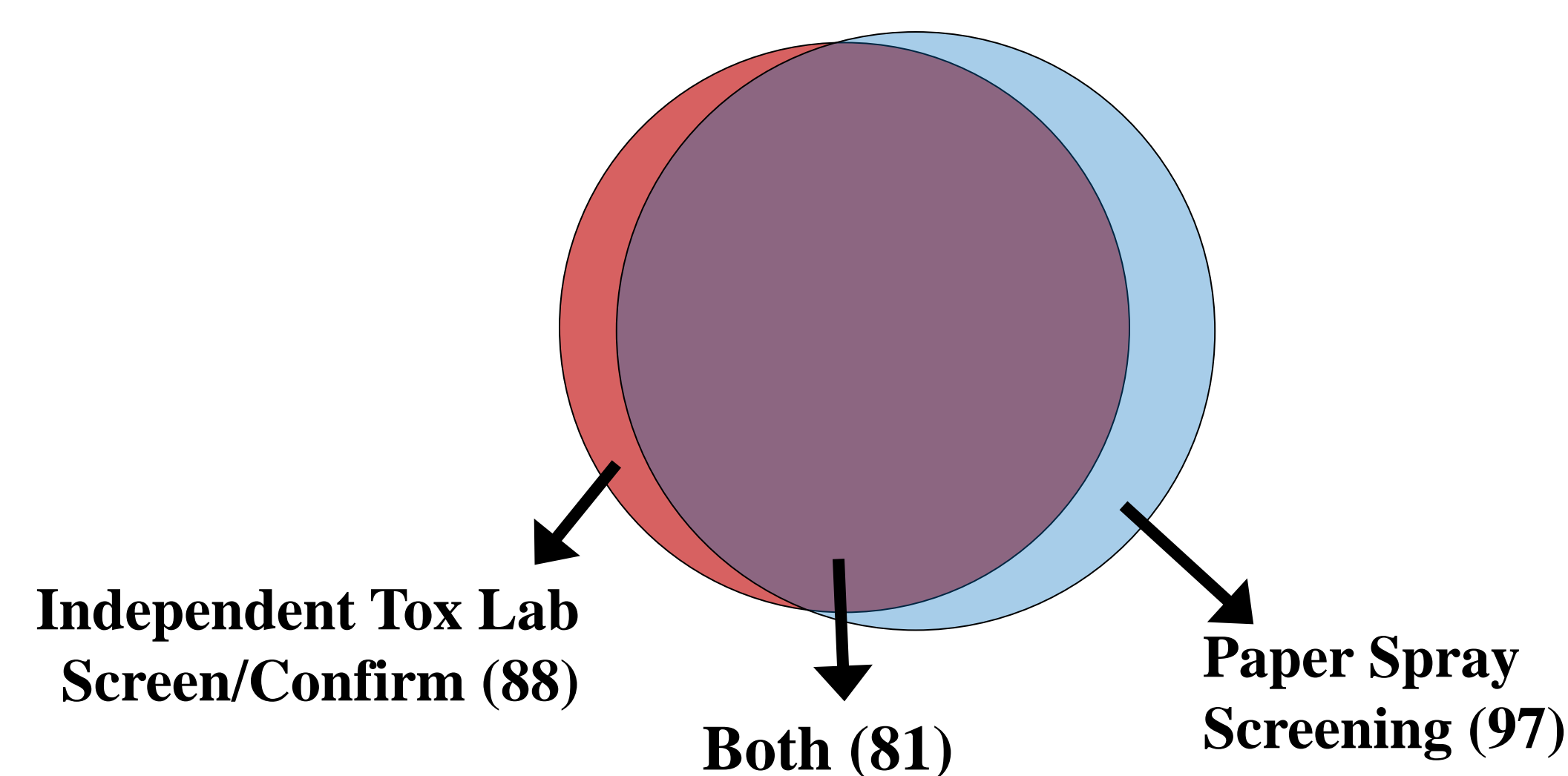
Purdue University Indianapolis, Indianapolis, Indiana 46202, United States

Limits of Reporting and Calibration Curves

Analyte	Limit of Reporting [ng/mL]	Signal to Noise at LOR	Rel. Error in Slope [%]	R ²	Analyte	Limit of Reporting [ng/mL]	Signal to Noise at LOR	Rel. Error in Slope [%]	R ²
6-MAM	20	5	3	0.993	Lorazepam	25	5	6	0.97
7-Aminoclonazepam	25	34	3	0.994	MDA	100	8	4	0.984
7-Aminoflunitrazepam	20	52	3	0.992	MDMA	45	4	3	0.994
9-Hydroxyrisperidone	10	155	7	0.963	MDPV	45	360	4	0.987
Alfentanil	50	257	7	0.961	Meperidine	25	137	5	0.98
Alpha-PVP	50	214	9	0.936	Mephedrone	45	64	5	0.983
Alprazolam	5	3	2	0.996	Meprobamate	1000	3	11	0.91
Amitriptyline	20	72	3	0.994	Mescaline	100	3	6	0.971
Amlodipine	20	2	7	0.959	Metaxalone	1000	37	7	0.966
Amphetamine	800	1	5	0.984	Methadone	15	165	5	0.98
Aripiprazole	50	111	8	0.951	Methamphetamine	45	70	2	0.995
Atenolol	100	48	3	0.993	Methylone	45	17	5	0.983
Benzoylcegonine	50	7	1	0.998	Methylphenidate	20	464	2	0.997
Benzotropine	10	303	6	0.972	Metoclopramide	100	726	5	0.982
Benzylpiperazine	50	2	2	0.997	Metoprolol	45	131	3	0.993
Brompheniramine	25	83	13	0.877	Midazolam	45	65	4	0.987
Bupivacaine	250	4696	5	0.983	Mirtazapine	45	572	4	0.985
Buprenorphine	10	6	4	0.986	Morphine	30	3	5	0.98
Bupropion	50	156	11	0.915	Naproxen	14994	8	14	0.878
Buspirone	6	69	33	0.528	Norbuprenorphine	100	3	13	0.88
Carbamazepine	1000	5925	9	0.94	Norclomipramine	36	276	2	0.998
Chlordiazepoxide	50	120	5	0.981	Norclozapine	45	140	3	0.993
Chlorpheniramine	15	145	5	0.977	Nordiazepam	50	133	5	0.978
Chlorpromazine	50	42	5	0.983	Nordoxepin	20	65	4	0.985
Citalopram	10	63	10	0.926	Norfluooxetine	20	38	2	0.997
Clomipramine	20	35	3	0.993	Norketamine	91	114	5	0.978
Clonazepam	30	2	4	0.984	Normeperidine	25	297	6	0.972
Clozapine	50	542	8	0.951	Norpropoxyphene	50	16	3	0.995
Cocaethylene	50	136	2	0.997	Nortramadol	1500	1	8	0.953
Cocaine	50	36	12	0.903	Nortriptyline	20	66	3	0.994
Codeine	20	7	3	0.992	Norvenlafaxine	25	2	52	0.312
Cyclobenzaprine	10	208	2	0.997	o-/m-CPP	20	89	4	0.989
Demoxepam	50	58	5	0.98	Olanzapine	50	164	7	0.966
Desalkylflurazepam	50	64	6	0.972	Oxazepam	50	41	4	0.987
Desipramine	20	195	4	0.99	Oxycodone	50	18	3	0.992
Dextromethorphan	10	78	8	0.952	Oxymorphone	15	5	5	0.981
Diazepam	50	97	3	0.995	Papaverine	250	2064	3	0.994
Diltiazem	50	129	11	0.91	Paroxetine	15	57	7	0.959
Diphenhydramine	25	7	4	0.986	PCP	25	1	12	0.901
Donepezil	45	47	4	0.989	Pentazocine	50	425	4	0.987
Doxepin	20	128	5	0.977	Pregabalin	250	7	7	0.958
Doxylamine	25	60	6	0.974	Primidone	750	3	11	0.91
Duloxetine	400	1	10	0.963	Promethazine	25	53	5	0.978
EDDP	25	141	6	0.973	Propoxyphene	50	39	3	0.994
Pseudoephedrine	50	25	2	0.996	Propranolol	50	194	2	0.996
Etomidate	100	9	5	0.982	Quetiapine	50	579	5	0.983
Fentanyl	1	10	4	0.988	Ranitidine	250	231	5	0.981
Flecainide	250	549	5	0.981	Risperidone	10	48	7	0.961
Flunitrazepam	20	5	4	0.989	Ropinirole	10	144	5	0.982
Fluoxetine	20	3	3	0.992	Sertraline	100	18	6	0.971
Flurazepam	25	182	5	0.979	Sildenafil	100	14	3	0.994
Fluvoxamine	15	7	9	0.933	Temazepam	50	86	2	0.997
Gabapentin	250	39	7	0.966	TFMPP	50	386	3	0.992
Haloperidol	10	264	7	0.963	Tramadol	100	8	3	0.991
Hydrocodone	20	35	2	0.998	Trazodone	100	539	3	0.995
Hydromorphone	20	11	6	0.973	Triazolam	20	19	5	0.982
Hydroxychloroquine	2000	336	12	0.899	Trimipramine	20	179	3	0.993
Hydroxyzine	10	82	4	0.985	Vardenafil	100	39	4	0.989
Ketamine	100	370	7	0.963	Venlafaxine	50	3	4	0.984
Labetalol	45	50	5	0.983	Verapamil	50	369	4	0.987
Levetiracetam	2000	3	4	0.984	Zaleplon	15	3	4	0.986
Lidocaine	250	4303	3	0.993	Ziprasidone	40	49	9	0.944
					Zolpidem	10	169	1	0.999

Cross-Comparison With Independent Tox Lab Screening

30 post mortem samples were analyzed by both an independent toxicology lab and in-house by paper spray MS. The Tox Lab performed its normal screen and confirm workflow: a combination of HPLC-MS/MS and immunoassay screening followed by HPLC-MS/MS confirmation



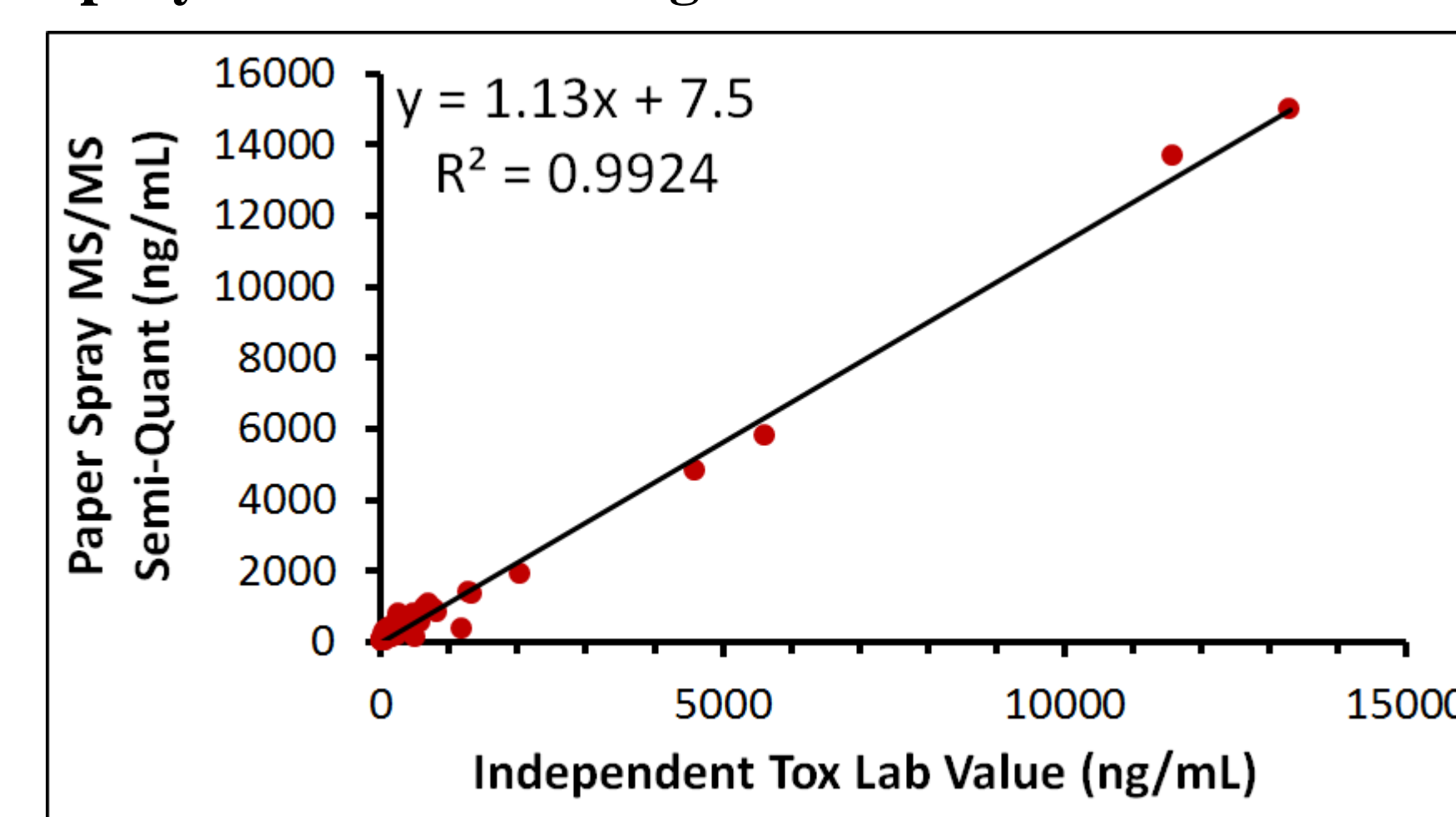
- **81:** Drug and drug metabolite targets detected by both
- **7:** Detected by Tox Lab but not by paper spray MS
 - 5/7 were below paper spray detection limit
 - 2/7 were not quantitated by the Tox Lab
- **16:** Detected by paper spray MS but not Tox Lab
 - 6/16 were not tested by Tox Lab (not ordered by customer)
 - 10/16 are likely false positives by paper spray method
 - 2 FP were opiates in the presence of other opiates
 - 6 were low levels near LOR

Parameter	Result	Calculation
Sensitivity	92.0%	TP/(TP+FN)
Specificity	99.8%	TN/(TN+FP)
positive predictive value	89.0%	TP/(TP+FP)
negative predictive value	99.8%	TN/(TN+FN)

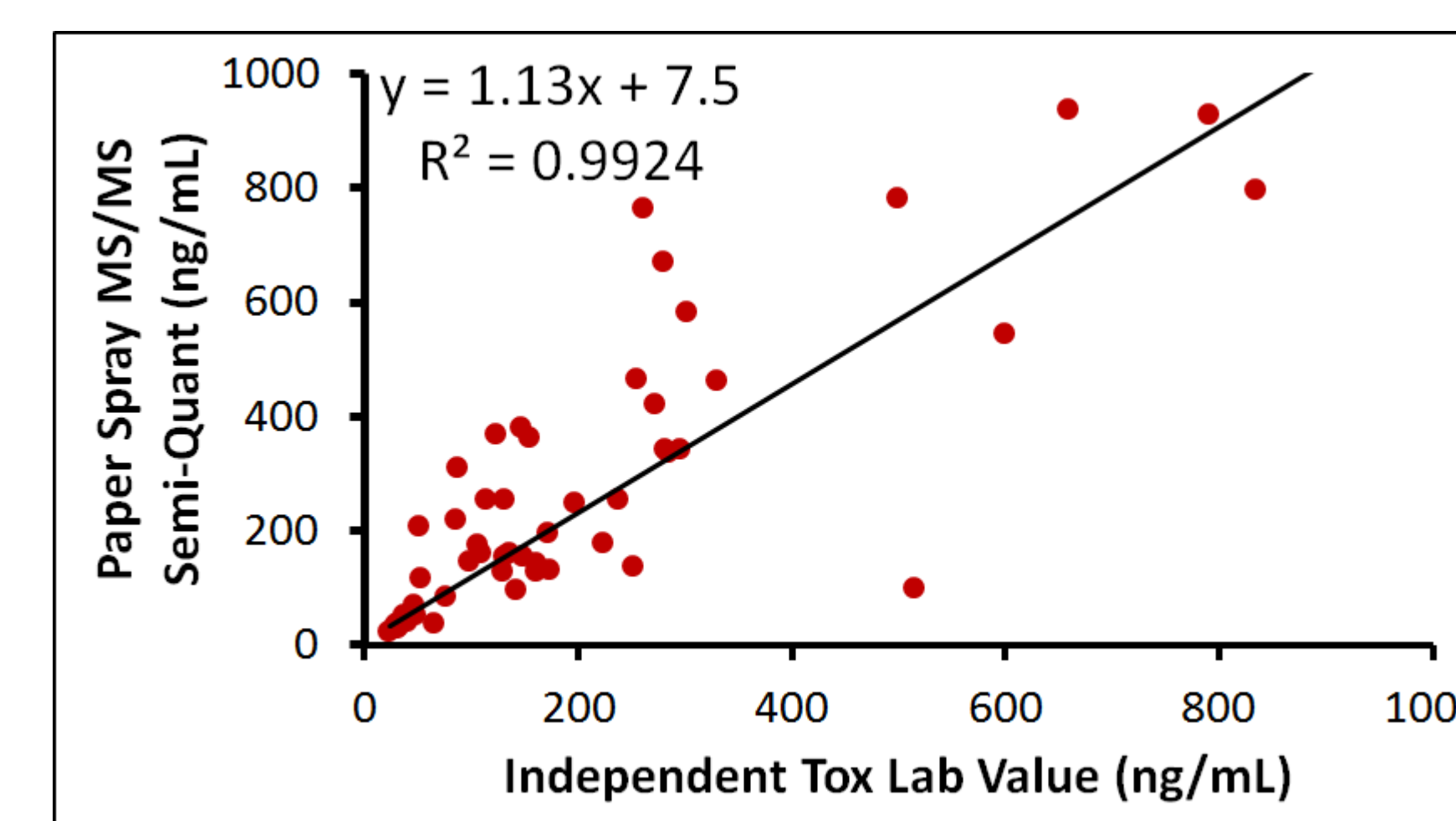
Paper Spray Semi-Quantitative Performance in Post-Mortem Samples

- In 30 post-mortem blood samples, 61 drug concentrations across all targets were obtained by both paper spray and the Independent Tox Lab HPLC-MS/MS confirmation method.
 - Results outside of the paper spray calibration range (<LOR) or above the ULOQ (N=3) were ignored.
- Paper spray correlated well with HPLC-MS/MS confirmation at the Tox Lab (R² > 0.99)
- Paper spray consistently over-estimated the concentration (slope = 1.13). Average deviation was +39%
- Paper spray quantitation could be improved by decreasing the number of targets or increasing the number of isotope labeled internal standards

HPLC-MS/MS Confirmation Compared to Paper Spray MS/MS Screening – All Results



Concentrations < 1000 ng/mL only



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