Targeted Screening for Drugs of Abuse in Postmortem Blood using LC-MS/MS

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Overview

- Targeted screen for 42 drugs in blood with LC-MS/MS

Introduction

Currently the majority of forensic toxicology laboratories are using immunoassay for the screening of drugs of abuse in blood. This research aims to replace immunoassay testing with a targeted LC-MS/MS method for screening of drugs of abuse in postmortem blood. This method will be less time consuming and less expensive than immunoassay testing. In addition, a decrease in false positives caused by cross reactivity of immunoassay technology is expected (1, 2). Targeted LC-MS/MS screening will also allow for the easy addition of targets to the current immunoassay screen which is presently limited by the kits available from the vendor. This method utilizes a triple quadrupole which is already utilized by many forensic laboratories and therefore doesn't require the purchase of additional instrumentation.

Method Development

This method utilizes a Thermo TSQ Vantage LC-MS/MS (Fig. 1) with Tracefinder and Xcalibur software. Method parameters were taken from an existing opioid quantitation method. The column is Thermo Hypersil Gold PFP (100mm x 2.1mm; 3µm particle size). The column temperature is held at 30°C with a flow of 0.3 mL/min. Initial conditions are 95% A (0.25 g/L ammonium formate/0.1% formic acid in water) and 5% B (0.25 g/L ammonium formate/0.1% formic acid in methanol) followed by a gradient to 95% B over 7 minutes and returning to starting conditions for a total run time of 10.3 minutes. Retention times are consistent indicating adequate equilibration time. Two MRM transitions are monitored for each analyte. Scan width is 0.500 m/z and scan time is 0.01 sec. Collision energy and S-lens voltages were optimized for each compound (Fig. 2 & 3). The majority of drugs are analyzed in positive mode. The remaining are analyzed in negative mode.

Figure 1 – Thermo TSQ Vantage

Method Development

Figure 2 – S-lens and Collision Energy Optimization

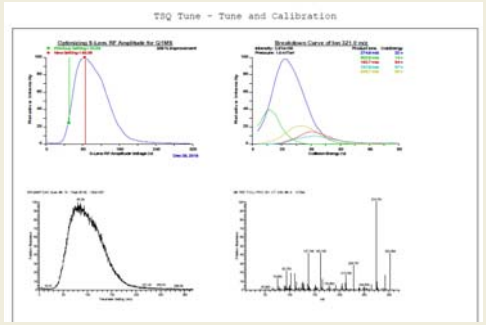

Figure 3 – S-lens and Collision Energy Optimization

Sample Preparation

- 50 µL of each stock solution spiked into 250 µL blank blood to give desired limit of detection (LOD) (Table 1).
- 50 µL of 10 µg/mL methapyrilene and 100 µL of 1 µg/mL 11-Carboxy-THC-d3 added as internal standards
- 1 mL acetonitrile added (Fig. 4), vortexed and centrifuged for 10 min at 3000 rpm (Fig. 5)
- Decanted (Fig. 6) and dried down under nitrogen at ambient temperature
- Reconstituted in 200 µL mobile phase A

Figure 4 – After vortex

Figure 5 – After centrifuge

Figure 6 – After decant

Method

Mix 1	Amount stock (µL) in 10 mL	Stock Sol'n Conc.	Final Concentration µg/mL	Cutoff Concentration (LOD) mg/L
Amphetamines	250	10 µg/mL	0.25	0.05
Oxazepam	5	1 mg/mL	0.5	0.10
Temazepam	5	1 mg/mL	0.5	0.10
Diazepam	5	1 mg/mL	0.5	0.10
Chlordiazepoxide	5	1 mg/mL	0.5	0.10
Nordiazepam	5	1 mg/mL	0.5	0.10
Butalbital	50	1 mg/mL	5	1.0
Phenobarbital	50	1 mg/mL	5	1.0
Salicylic Acid	2500	1 mg/mL	250	50
Levetiracetam	50	1 mg/mL	5	1.0
Risperidone	5	1 mg/mL	0.5	0.10
Topiramate	50	1 mg/mL	5	1.0
Phenytoin	50	1 mg/mL	5	1.0
Gabapentin	50	1 mg/mL	5	1.0
Lamotrigine	50	1 mg/mL	5	1.0
Mix 2				
Cocaine Mix	10	100 µg/mL	0.1	0.02
Opiate Mix	10	100 µg/mL	0.1	0.02
Benzodiazepine A	200	5 ug/mL	0.1	0.02
THC Mix	100	1 µg/mL	0.01	0.002
Carisoprodol	100	1 mg/mL	10	2.00
Meprobamate	100	1 mg/mL	10	2.00
Fentanyl	100	1 µg/mL	0.01	0.002
4-ANPP	100	1 µg/mL	0.01	0.002
Buprenorphine	100	1 µg/mL	0.01	0.002
Acetaminophen	1000	1 mg/mL	100	20

Table 1 – Solution preparation and LOD

Drug	Retention Time	Parent	Product 1	Collision Energy	Product 2	Collision Energy	S-lens Voltage
Levetiracetam	3.88	171.1	125.95	16	68.94	31	41
Morphine	4.13	286.084	157.04	40	185.05	29	96
Oxymorphone	4.40	302.089	227.09	28	284.15	19	73
Hydromorphone	4.56	286.1	152.03	59	165.05	38	106
Gabapentin	4.86	172.2	154.01	13	136.99	16	54
Codeine	5.00	300.096	152.05	63	215.11	24	84
6MAM	5.23	328.095	193.05	26	211.07	26	101
Benzoylecgonine	5.39	290.3	167.86	19	104.81	31	73
Oxycodone	5.41	316.094	241.1	28	298.16	17	84
Hydrocodone	5.48	300.111	128.02	57	199.073	29	102
Meprobamate	5.53	219	96.89	15	158.2	15	42
7-Amino-Flunitrazeapm	5.69	284.1	134.86	27	147.83	26	99
MDA	5.91	180.2	162.95	10	76.93	36	40
Methamphetamine	5.93	150.1	90.97	19	64.98	39	52
MDMA	6.10	193.8	162.94	12	134.9	20	53
Carisoprodol	6.40	261.2	54.93	29	96.91	17	51
Lorazepam	6.43	321	274.77	22	302.82	14	85
Lamotrigine	6.47	256	158.74	29	165.8	26	90
Oxazepam	6.54	287.1	240.85	21	268.85	14	88
Triazolam	6.64	343	164.73	31	110.73	49	96
Clonazepam	6.70	316.2	269.84	25	213.8	37	50
Temazepam	6.73	301.25	254.86	23	282.87	13	57
Chlordiazepoxide	6.74	300.1	226.82	27	240.83	18	56
Alprazolam	6.77	309.3	204.84	40	280.87	25	104
Nordiazepam	6.79	271.2	139.81	27	164.79	27	109
Cocaine	6.86	304.2	181.88	19	81.87	31	73
Diazepam	7.00	285.1	153.82	27	192.87	32	86
Methapyrilene	7.02	262.4	96.86	31	216.91	13	66
Midazolam	7.23	326.1	290.91	26	243.81	24	103
4-ANPP	7.34	281.1	104.86	32	187.92	17	74
Buprenorphine	7.44	468.2	414.09	33	396.06	36	115
Risperidone	7.46	411.16	190.76	29	109.73	46	96
Fentanyl	7.48	337.2	104.84	35	187.87	24	113
Dextromethorphan	7.48	272.85	121.02	32	215.16	23	70
THC	7.78	315.2	192.85	21	122.78	32	88
Methadone	8.01	310.28	90.99	38	265.2	18	79

Table 2 – Optimized Positive mode MRM transitions

Method

Drug	Retention Time	Parent	Product 1	Collision Energy	Product 2	Collision Energy	S-lens Voltage
Butalbital	5.95	223.09	180	13	41.96	17	41
Phenobarbital	5.36	231	41.96	19	187.95	12	56
Phenytoin	6.06	251	207.93	15	101.92	22	44
Topiramate	5.87	338	95.73	23	77.75	40	53
11-Carboxy THC	7.40	343	299.04	22	190.88	33	78
11-Carboxy THC-d3	7.39	346	248.19	33	302.34	23	74
Acetaminophen	3.80	150	106.97	19	134.96	15	49
Salicylic Acid	5.63	137	92.98	16	65	33	33

Table 3 – Optimized Negative mode MRM transitions

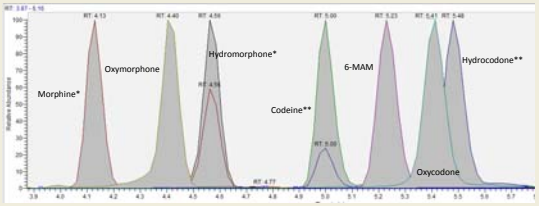

Figure 7 – Opiate compounds at LOD (* and ** denote isobaric compounds)

Discussion

The collision energy and S-lens voltage were optimized for each compound successfully. Adequate separation was achieved for all compounds including the isobaric opioid compounds utilizing the given gradient. The desired LOD was achieved using 250 µL of sample for all compounds except amphetamine. Amphetamine is extremely volatile and may be lost during the dry down step. Future work will be done to try and minimize the loss of amphetamine.

Future Works

- Validate method following SWGTOX guidelines
- Analyze real postmortem blood samples
- Compare samples run using ELISA and LC-MS/MS

References

(1) Saitman, A., Park H., Fitzgerald R. *Journal of Analytical Toxicology*, **2014**, 38 (7), 387-396.
(2) Verplaetse, R., Decabooter, S., Cuypers, E., Tytgat, J. Journal of Forensic Toxicology & Pharmacology, 2013, 2:2.

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