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3.1 Preliminary Drug Screening by LC-MS/MS

3.1.1 Analyte(s)

See toxicology scope of analysis in Toxicology Procedure Manual 1.14

3.1.2 Specimen Requirements

250 µL blood

3.1.3 General Description of Method

A preliminary analysis for drugs in blood using liquid chromatography/tandem mass spectrometry

3.1.4 Equipment and Reagents

- QTRAP 4500 or LC-MS/MS 4500 equipped with validated column
- 1.7 mL micro-centrifuge tube
- 12x75 mm culture tube
- 11 mm autosampler vial with conical insert and appropriate caps
- The usual assortment of laboratory glassware, pipettes, vortexers, centrifuges and turbovaps
- LC-MS grade methanol
- HPLC grade, or higher, acetonitrile
- High Purity formic acid
- LCMS grade water
- Certified reference materials
- Mobile phase A, 0.1% formic acid in water
- Mobile phase B, 0.1% formic acid in methanol

3.1.5 Sample Preparation

3.1.5.1 Internal Standards

- Pipette 600 µL of acetonitrile containing D5-PCP and D9-THC-COOH internal standards to all micro-centrifuge tubes

3.1.5.2 Standards and Controls

- Pipette 250 µL of blank blood and 20 µL of the following standards and controls into micro-centrifuge tubes
 - Control - to be run as standard

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- Control - to be run as control
- 0.5x control A - run at beginning of analytical batch
- 0.5x control B - run at end of analytical batch
- negative control

3.1.5.2 Unknowns

- Pipette 250 µL of each sample into the appropriate labeled micro-centrifuge tube

3.1.5.3 Additional Sample Preparation

- Close tubes and vortex briefly for 5-10 seconds
- Centrifuge samples at 13,000 RPM for 10 minutes
- Remove samples and transfer 550 µL of supernatant to 12x75 culture tube
- Evaporate samples to dryness under nitrogen (~ 15 minutes at 8-10 psi)
- Reconstitute samples in culture tubes with 100 µL 85/15 mobile phase A/mobile phase B
- Transfer to autosampler vials described in 3.1.4 and crimp caps
- Tap vials or vortex to remove any existing air bubbles at bottom of vial

3.1.6 Instrument Preparation

3.1.6.1 Maintenance

- Check to ensure that the following maintenance has been completed
 - Curtain plate has been cleaned within the last 7 days
 - Guard column has been changed within the last 2 months
 - Mobile phases have been prepared within the last 30 days and are of sufficient volume to complete the analytical run
- HPLC is connected to MS
- Correct mobile phases are being used
- Correct column is in place
- Waste container is not full
- Pump valves are closed

3.1.6.2 Equilibrate

- Equilibrate until operating temperature (40°C) is obtained
- Ensure that operating pressures are stable and record in log book
- Run at least two blanks to warm up the system

3.1.6.3 Retention Time Update

- Run appropriate standard and update retention times in acquisition and quantitation methods

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3.1.7 Run Samples

- Subsequent to all steps in 3.1.6, samples may be analyzed on the QTRAP 4500 or LC-MS/MS 4500

3.1.7.2 Controls

- In addition to casework samples, each analytical batch shall contain a standard, control, 0.5x control A, 0.5x control B and negative control.
- If one control is out of range, no action is necessary.
- If two controls are out of range, the following workflow shall occur:
 - Any positives shall be confirmed
 - Any below cutoff (BC) results that have other positive analytes in the same confirmatory panel will be confirmed
 - Any below cutoff (BC) results that do not have other positive analytes in the same confirmatory panel will be reanalyzed
 - Negative results can be reported

Analyte	Standard	Control	0.5x Control
6-acetylmorphine	0.7	0.7	0.35
acetyl fentanyl	0.7	0.7	0.35
alprazolam	10	10	5
amitriptyline	20	20	10
amphetamine	10	10	5
benzoylecgonine	100	100	50
buprenorphine	0.7	0.7	0.35
butalbital	500	500	250
carfentanil	0.7	0.7	0.35
carisoprodol	500	500	250
chlordiazepoxide	20	20	10
clonazepam	10	10	5
cocaine	5	5	2.5
codeine	10	10	5
cyclobenzaprine	10	10	5



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diazepam	10	10	5
(es)citalopram	10	10	5
etizolam	50	50	25
fentanyl	0.7	0.7	0.35
flubromazolam	20	20	10
flubromazepam	20	20	10
flunitrazepam	10	10	5
gabapentin	1000	1000	500
hydrocodone	10	10	5
hydromorphone	5	5	2.5
ketamine	10	10	5
lamotrigine	500	500	250
lorazepam	10	10	5
MDA	20	20	10
MDMA	20	20	10
meprobamate	500	500	250
methadone	20	20	10
methamphetamine	10	10	5
midazolam	20	20	10
morphine	10	10	5
naloxone	10	10	5
N-desmethyldiazepam	10	10	5
norbuprenorphine	1	1	0.5
oxazepam	20	20	10
oxycodone	10	10	5
oxymorphone	5	5	2.5
phencyclidine	10	10	5

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phenobarbital	500	500	250
phentermine	10	10	5
sertraline	10	10	5
temazepam	20	20	10
THC-COOH	10	10	5
tramadol	10	10	5
trazodone	500	500	250
venlafaxine	50	50	25
zolpidem	10	10	5

3.1.7.3 Control values within $\pm 30\%$ of the target value are acceptable.

3.1.8 Data Analysis

3.1.8.1 Cutoff levels were developed using the following as guidance:

- National Safety Council Recommended Scope and Cutoffs for Toxicological Investigation of Drug-Impaired Driving and Motor Vehicle Fatalities
- Disposition of Toxic Drugs and Chemicals in Man, Randall C. Baselt
- Winek's Drug and Chemical Blood-Level Data 2001
- Therapeutic ("normal"), toxic, and comatose-fatal blood-plasma concentrations (mg/L) in man, Schulz 2012
- Confirmatory instrument capabilities
- Cutoff levels may be adjusted to ensure analyte detection is suitable and fit for purpose.

3.1.8.2 Cutoff levels

Analyte	Cutoff +	Cutoff ++
6-acetylfentanyl	0.4	3
acetylfentanyl	0.4	3
alprazolam	7	20
amitriptyline	15	40
amphetamine	5	20
benzoylecgonine	70	200

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buprenorphine	0.4	3
butalbital	300	1000
carfentanil	0.4	3
carisoprodol	300	1000
chlordiazepoxide	18	100
clonazepam	7	20
cocaine	3.5	10
codeine	7	20
cyclobenzaprine	7	20
diazepam	8	50
(es)citalopram	7	20
etizolam	40	100
fentanyl	0.4	3
flubromazepam	18	100
flubromazolam	18	100
flunitrazepam	7	20
gabapentin	700	1400
hydrocodone	7	20
hydromorphone	3.5	10
ketamine	7	20
lamotrigine	300	1000
lorazepam	7	20
MDA	15	40
MDMA	15	40
meprobamate	300	1000
methadone	10	50
methamphetamine	5	20

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midazolam	18	100
morphine	7	20
naloxone	7	20
N-desmethyldiazepam	8	50
norbuprenorphine	1	3
oxazepam	18	100
oxycodone	7	20
oxymorphone	3.5	10
phencyclidine	7	20
phenobarbital	300	1000
phentermine	7	20
sertraline	7	50
temazepam	18	100
THC-COOH	5	10
tramadol	7	20
trazodone	300	1000
venlafaxine	30	100
zolpidem	7	20

3.1.8.3 Unknowns

- Precursor and product ions have been validated and shall not be changed
- Transition ion 1 is used for quantitation, transition ion 2 is used as a qualifier ion
- Retention times listed are intended to aid the analyst in data interpretation, however may need to be adjusted from batch to batch
- Each unknown shall be monitored for the following analyte product ions

Analyte	Precursor Ion	Product Ion 1	Product Ion 2	RT
6-acetylmorphine	328	165	211	2.76
Acetylfentanyl	323	105.1	188.1	4.53

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alprazolam	309.1	281	205	6.41
amitriptyline	278	233.2	91.1	5.91
amphetamine	136.1	91	65.1	2.54
benzoylecgonine	290.1	168	77.1	3.61
buprenorphine	468.1	55.2	414.2	5.20
butalbital	223.1	180.1	85	5.22
carfentanil	395.1	335.2	363.1	5.15
carisoprodol	261.2	176	97.1	6.09
chlordiazepoxide	300	227	241	4.77
clonazepam	316	270	214	5.95
cocaine	304	182.1	77.1	3.95
codeine	300.1	215	165.2	2.10
cyclobenzaprine	276.1	215.1	231.1	5.74
diazepam	285.1	193	154	6.85
(es)citalopram	325	109.1	262	5.1
etizolam	343.1	314	289.1	6.43
fentanyl	337.2	188	105	4.90
flubromazepam	333	226	184	6.53
flubromazolam	371	343	292	6.19
flunitrazepam	314	268	239	5.95
gabapentin	172	137.1	154	2.53
hydrocodone	300	199	171	2.68
hydromorphone	286.1	185	157	1.49
ketamine	238.1	125.1	207	3.54
lamotrigine	256	211	43.2	3.83
lorazepam	321	275	229	6.22
MDA	180.1	133	105.1	2.71

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MDMA	194.1	163	105.1	2.81
meprobamate	219.1	158	97.2	4.68
methadone	310.1	265	105	5.93
methamphetamine	150.1	91	119	2.69
midazolam	326.1	291.1	249	5.07
morphine	286	165	152.1	1.10
naloxone	328	268.2	212.1	2.34
N-desmethyldiazepam	271.1	140	208	6.45
norbuprenorphine	414.1	152.2	165.2	4.76
oxazepam	287.1	269	241	6.23
oxycodone	316.1	241	256.1	2.54
oxymorphone	302	227	198	1.28
phencyclidine	244.1	86.1	159	4.79
phenobarbital	231	188	85	4.53
phentermine	150.1	91.1	105.1	3.20
sertraline	306	275	159	6.27
temazepam	301	255	177.1	6.50
THC-COOH	343	245	299.1	8.40
tramadol	264	58.1	42.2	3.76
trazodone	372	176	148.1	4.36
venlafaxine	278	58.2	121.1	4.68
zolpidem	308.1	235	263.1	4.47
D5-PCP	249.1	86.1		4.78
D9-THC-COOH	352.2	308		8.41

3.1.8.4 MultiQuant

- All data analysis shall occur in the MultiQuant software

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- Prior to Printing reports, a competent scientist shall review all peak integrations