Montgomery County Coroner's Office / Miami Valley Regional Crime Laboratory - Toxicology

Drug Facilitated Crime Enhanced Detection of over 80 Analytes by LC/MS/MS

May 2021

Summary of Validation

The toxicology laboratory validated a LC/MS/MS method for screening and confirming 84 drugs in blood, serum/plasma, and urine. This method uses solid-phase extraction and the analytes are analyzed using a LCMSMS instrument in dynamic mode. Urines are hydrolyzed prior to extraction to convert any conjugated drugs into their unconjugated forms. All calibrators, controls and interference studies were prepared from NIST-Traceable reference standards with certificates of analysis. The drugs included and their cutoff values are listed below:

Analyte	Cutoff, ng/mL	Analyte	Cutoff, ng/mL
7-aminoclonazepam	5	Methadone	10
7-aminoflunitrazepam	5	Methadone Metabolite EDDP	10
8-aminoclonazolam	5	Methamphetamine	10
Alpha-hydroxyalprazolam	5	Midazolam	10
Alpha-hydroxytriazolam	5	Morphine	10
Alprazolam	5	Norbuprenorphine	2
Amitriptyline	10	Norchlorcyclizine	10
Amobarbital/Pentobarbital	25	Nordiazepam	10
Amphetamine	10	Nordoxepin	10
Benzoylecgognine	50	Norfentanyl	1
Brompheniramine	10	Norfluoxetine	10
Brorphine	1	Norketamine	1
Buprenorphine	1	Normeperidine	10
Butalbital	25	Norpropoxyphene	10
Carboxy THC	10	Norsertraline	10
Carisoprodol	50	Nortriptyline	10
Chlorophenylpiperazine (Trazodone met.)	10	O-desmethyltramadol	10
Chlorpheniramine	10	O-desmethylvenlafaxine	10

Citalopram	10	Oxazepam	10
Clonidine	1	Oxycodone	10
Codeine	10	Oxymorphone	10
Cyclobenzaprine	10	Paroxetine	10
Desipramine	10	Phencyclidine (PCP)	10
Dextromethorphan	10	Phenobarbital	25
Diazepam	10	Phenytoin	25
Diphenhydramine	10	Promethazine	10
Doxepin	10	Propoxyphene	10
Doxylamine	10	Quetiapine	10
Estazolam	10	Scopolamine	10
Fentanyl	1	Secobarbital	20
Fluoxetine	10	Sertraline	10
Gabapentin	100	Tapentadol	10
Hydrocodone	10	Temazepam	10
Hydromorphone	10	Tetrahydrozoline	10
Hydroxyzine	10	Tramadol	10
Imipramine	10	Trazodone	10
Ketamine	1	Warfarin	5
Lorazepam	5	Zaleplon	10
MDA	10	Ziprasidone	10
MDMA	10	Zolpidem	10
Meperidine	10	Zolpidem carboxylic	10
Meprobamate	50	Zopiclone	10

The method was validated using calibrators and controls prepared in blood, serum/plasma, and urine. All analytes are qualitative only. The analytical work was done by Treena Wiebe, Kialee Bowles, Elizabeth Kiely, and Quinton Carter and reviewed by Matthew Juhascik.

This validation started on April 30, 2021 and ended on May 7, 2021. LCMSMS2 and LCMSMS3 were used during the method validation and all data was combined. LCMSMS1 is not able to be used for this analysis due to lack of sensitivity required.

The method was determined to be acceptable for the qualitative determination of all drugs listed above and is fit for its intended purpose.

The validated parameters are shown below:

Parameter	Acceptance Criteria	Results
Carryover	A negative sample following a positive sample does not show a response above the cutoff response	No carryover was seen in specimens following positive cases. The potential for carryover must still be monitored during actual casework.
Interference	No interfering signal from matrix, drugs used in assay, internal standards, or other commonly seen drugs	No interferences were seen. Amobarbital and pentobarbital cannot be separated; they will be reported as amo/pentobarbital.
Limit of Detection	As listed in the above table	LOD was achieved for all analytes at the above concentrations on QQQ3 for both the quant and qualifier transitions. On QQQ2, the qualifier transition was not always detected reliably for drugs in negative mode (e.g., barbiturates). An additional negative mode injection is necessary on QQQ2 to achieve reliable detection of all transitions for negative mode drugs. This was added to the SOP.
Matrix Effects, Recovery, Process Efficiency	Determine if any issues with a specific drug are due to these effects	While some drugs had considerable matrix effects or reduced/enhanced recoveries, the targeted LODs were able to be achieved. Additionally, the negative and positive controls demonstrated acceptable responses. If any issues develop in the future, reducing matrix

		effects or increasing recovery can be considered.
Previous Casework	Cases will be analyzed using the new method and evaluated against previous results.	Twenty-six cases were analyzed for 130 drugs previously seen. All 130 drugs were detected; additional drugs were also detected that were not previously tested. All DFC cases currently in-house will be reanalyzed by this method and supplemental reports will be issued for any newly detected drugs.