Validation Summary Toxicology Multi-drug Screen in Blood and Urine by Q-TOF Using SLE+ Extraction Plates

The runs were completed on the following instruments: Agilent 1290 Infinity II HPLCs equipped with an Agilent 6545B Q-TOFs. The property number for the Pocatello instrument is 070060 and the property number for the Coeur d'Alene instrument is 070044.

Extractions were performed by Amy Patton (a contractor from Pinpoint Testing) on 8/5/19, 8/6/19, 8/9/19, and 8/12/19, by Sarah Pickle on 8/7/19, by Celena Shrum on 8/8/19, by Tamara Salazar on 8/19/19, by Anne Nord on 11/21/19, and Britany Wylie on 11/25/19.

For the extractions, toxicology AM #25 was followed with the following exceptions:

*For the multi-drug screen, 450ul of blood + base mixture or urine + base mixture was added to the SLE plates.

*The reconstitution solvent used was 20% MeOH in water (rather than 100% MeOH).

Based on our current methods, and suggestions from PinPoint, the initial criteria for evaluation was set as:

-Retention time criterion for peak identification must be +/- 0.1 minutes of the retention time of the calibrator

-Mass accuracy less than 5

-Signal to noise at greater than 5

With the initial evaluation criteria, some of the calibrators and controls would not be evaluated as positive while some of the negative samples would be evaluated as positive, so this evaluation criteria was re-examined. The criteria was updated so that when it was applied, the calibrators and controls would be evaluated as positive and the negative samples would be evaluated as negative.

The criteria for evaluation was updated to:

-Retention time for peak at +/- 0.1 minutes of the retention time of the calibrator **and**

-Mass accuracy of +/-10 **and/or**

-Mass abundance of 40 or greater

-Samples can also be evaluated as positive (typically based on peak presence and shape) or negative (typically based on peak absence or clear indicators that the "peak" is actually background noise) at analyst's discretion. A sample can be moved forward for confirmation with a retention time that is outside the window if it is due to the sample being at high concentration (which is causing peak widening/shifting).

These compounds passed the evaluation criteria for this method and will be included in the method at the following LOD's :

6-MAM- 1ng/mL
7-aminoclonazepam- 10ng/mL
7-aminoflunitrazepam- 10ng/mL
Acetyl Fentanyl- 1ng/mL
Acetyl Norfentanyl- 1ng/mL
alpha-hydroxyalprazolam- 10ng/mL
alpha-hydroxymidazolam- 10ng/mL
alpha-PVP- 10ng/mL
Alprazolam- 10ng/mL
Amitriptyline- 10ng/mL
Amphetamine- 10ng/mL
Benzoylecgonine- 25ng/mL
Buprenorphine- 10 ng/mL
Bupropion- 10 ng/mL
Carbamazepine- 10 ng/mL
Carisoprodol- 10 ng/mL
Chlordiazepoxide- 10 ng/mL
Chlorpheniramine- 10 ng/mL
Citalopram- 10 ng/mL
Clonazepam- 10 ng/mL
Cocaine- 10 ng/mL
Codeine- 10 ng/mL
Cyclobenzaprine- 10 ng/mL
Desipramine- 10 ng/mL
Dextrorphan- 10 ng/mL
Diazepam- 10 ng/mL
Dihydrocodeine- 10 ng/mL
Diphenhydramine- 10 ng/mL
Doxepin- 10 ng/mL
Doxylamine- 10 ng/mL
Duloxetine- 10 ng/mL
EDDP- 25 ng/mL
Estazolam- 10 ng/mL
Etizolam- 10 ng/mL
Fentanyl- 1 ng/mL
Flunitrazepam- 10 ng/mL
Fluoxetine- 10 ng/mL
Flurazepam- 10 ng/mL
Hydrocodone- 10 ng/mL
Hydromorphone- 10 ng/mL
Imipramine- 10 ng/mL
Ketamine- 10 ng/mL
Lamotrigine- 10 ng/mL
Levamisole- 10 ng/mL

Lorazepam- 10 ng/mL
MDA- 10 ng/mL
MDEA- 10 ng/mL
MDMA- 10 ng/mL
Meperidine- 10 ng/mL
Meprobamate- 10 ng/mL
Methadone- 10 ng/mL
Methamphetamine- 10 ng/mL
Methorphan- 10 ng/mL
Methylphenidate- 10 ng/mL
Metoprolol- 10 ng/mL
Midazolam- 10 ng/mL
Mirtazapine- 10 ng/mL
Mitragynine- 10 ng/mL
Morphine- 10 ng/mL
Norbuprenorphine- 10 ng/mL
Nordiazepam- 10 ng/mL
Norfentanyl- 10 ng/mL
Norhydrocodone- 10 ng/mL
Norpropoxyphene- 10 ng/mL
Nortriptyline- 10 ng/mL
O-desmethyl-tramadol- 10 ng/mL
Olanzapine- 10 ng/mL
Oxazepam- 10 ng/mL
Oxycodone- 10 ng/mL
Oxymorphone- 10 ng/mL
Paroxetine- 10 ng/mL
Phencyclidine- 10 ng/mL
Phentermine- 10 ng/mL
Promethazine- 10 ng/mL
Propoxyphene- 10 ng/mL
Pseudoephedrine- 10 ng/mL
Quetiapine- 10 ng/mL
Sertraline- 10 ng/mL
Sufentanil- 1 ng/mL
Tapentadol- 10 ng/mL
Temazepam- 10 ng/mL
Tramadol- 10 ng/mL
Trazodone- 10 ng/mL
Venlafaxine- 10 ng/mL
Zaleplon- 10 ng/mL
Zolpidem- 10 ng/mL
Zopiclone- 10 ng/mL

Additional compounds that were run that did not meet the criteria were: Methocarbamol, phenazepam, and phenytoin. These compounds did not meet the mass accuracy or mass abundance criteria. These compounds will not be evaluated as part of the method at this time; however, the internal standards and the compounds themselves did not have an effect on the performance of the method for other compounds, so they will continue to be included in the plates.

There were several compounds that did not meet mass accuracy and/or mass abundance criteria during the Coeur d'Alene validation (benzoylecgonine, codeine, dihydrocodeine, hydrocodone, levamisole, MDMA, methocarbamol, norhydrocodone, o-desmethyltramadol, oxycodone, phenazepam, pseudoephedrine, and venlafaxine). As such, the Coeur d'Alene instrument is not approved for use for this method at this time. A performance verification will be completed for this instrument following additional troubleshooting and prior to being used for casework for this method.

A spreadsheet of the overall evaluation for all of the compounds is included in this folder: I:\Toxicology\Validations_Studies_Projects\QTOF Screening

A report with the evaluation for each compound is included in this folder.

An evaluation of samples run using our current methods and samples run using this method is also included in this folder. There were a few inconsistencies between the results run with this method and those obtained with previous methods. All of the inconsistencies could be explained as the compound was not included in the scope or the compound was typically not reported in the previous method(s) (as they were metabolites). For instance, in one sample, ecgonine methyl ester was confirmed using a GC/MS method and benzoylecgonine was not but on the QTOF, the benzoylecgonine was confirmed while the ecgonine methyl ester was not. This difference occurred because benzoylecgonine did not come out well in the GCMS method while the ecgonine methyl ester did and only the benzoylecgonine (not the ecgonine methyl ester) is included in the QTOF method. Another example is one case in which olanzapine was confirmed with the QTOF method but not with the GCMS. This difference is because olanzapine did not come out well in the GCMS method while it does in the QTOF method.

The method is approved for implementation by the Idaho State Police Forensic Services toxicology section. Limitations for reporting will be included in the method to clarify that the results are indicative of the drug being present, but it is not a confirmation of that drug. The Pocatello Lab QTOF instrument is approved for casework and since the analysts that participated in the study demonstrated competence in performing the method, they are approved to perform the method (including the Coeur d'Alene analysts).

Approved By:

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Date: 06/05/2020

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Date: 6/4/2020