VALIDATION SUMMARY

Multi-drug Screen in Blood and Urine by LC/MS/MS Using SLE+ Extraction Plates

Overview

Idaho State Police Forensic Services (ISPFS) has established methods for the screening and confirmation of over 100 drugs utilizing an SLE+ extraction and running on an LC/MS/MS or LC/Q-TOF. This validation was performed to determine the suitability of adding new compounds and making modifications to the methods to increase efficiency.

Methods

Analytical methods #25 and #29 are approved, published methods for the testing and detection of multiple drugs by LC/MS/MS and LC/Q-TOF, respectively. These methods use a sample volume of 250µl blood or urine, which is added to an analytical plate, extracted, and injected on the instrument. One modification tested within the validation was a reduction of the sample volume from the required 250µl to 50µl of blood or urine. This reduction in sample volume affected the method performance as there was a reduction in abundances of the compounds of interest and the internal standards. To compensate for the reduction in sample volume, modifications were made to the acquisition parameters of the LC/MS/MS instrument that reduced the number of transitions being monitored for each compound from two to one to potentially increase the response.

The second modification tested was to eliminate the 15-minute plate shaking/incubation step that takes place after adding sample to the analytical plate and prior to adding ammonium hydroxide. The purpose of removing this step was to reduce the extraction time. Comparison runs were performed where the extraction was run as currently written versus run with eliminating the 15-minute shaking/incubation step. The data showed that this change did not cause any issues with sample recovery or chromatography and as such, the analytical methods will be modified to remove this unnecessary step. Since the extraction for AM #29 is identical to AM #25 and AM #28, this modification will also be implemented for it as well.

The other portion of the validation was to determine if additional compounds could be added to the method without affecting the already existing compounds. While some of the compounds worked well, there were a few that did not. Overall, there appeared to be a loss in sensitivity with all the compounds, including those that were already validated and part of the existing methods. This decrease in sensitivity is likely due to lowering the sample volume rather than adding in the new compounds. As such, the addition of these compounds will be evaluated by adding the compounds to the analytical plate and evaluating them over the course of several runs (the addition of compounds to an existing run is detailed in AM #24).

Results/Conclusions

The validation was completed in the Idaho State Police Pocatello and Coeur d'Alene forensic laboratories using the following instruments: Agilent 1290 HPLC with Agilent 6470 MS/MS (Pocatello property #: 069910, Coeur d'Alene property #: 69679). A total of eight validation runs were completed. The extractions were done by Anne Nord, Britany Wylie, Celena Shrum, Sarah Collins, Sophia Jackson, and Tamara Salazar. The original plan included running

the samples on both the LC/MS/MS and the LC/Q-TOF. Once the evaluation of the LC/MS/MS data was completed we did not proceed with the Q-TOF portion.

Due to the reduction in sensitivity with the lower volume and only having one transition to evaluate for each compound rather than two, we have concluded those two updates to the method will not be employed.

All of the data for the runs as well as the case comparisons and summaries for each run can be found in the following folder: I:\TOXICOLOGY\Validations_Studies_Projects\Coroner Validation

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01/28/2022

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