Techniques and solutions for forensic drug screening: an interview with Timothy Fassette

An interview with Timothy Fassette: Senior Forensic Toxicologist at Henderson Forensic Laboratory, Henderson, Nevada, USA

Drug markets are constantly evolving. This together with the need for forensic scientists to identify unprecedented and ever-increasing numbers of novel psychoactive substances (NPS) presents a significant challenge.

Timothy Fassette, is a Senior Forensic Toxicologist at the Henderson Forensic Laboratory in Henderson, Nevada, where he oversees the training of the Laboratory’s scientists, analyzes samples sent for DUID (driving under the influence of drugs) analysis, runs method validation on new analytical techniques and directs the quality control and quality assurance program.

In this interview, Timothy shares his insight into some of the challenges, techniques and solutions for forensic drug screening in his laboratory.

**Q: What types of case sample do you receive in your laboratory? What are the biggest challenges you face with the caseload you process in your laboratory?**

**A:** Our toxicology section receives whole blood samples for DUI and DUI-drug cases for the city of Henderson and a few other surrounding agencies. These samples are analyzed to detect and give a quantitative concentration of any ethanol and other impairing drugs that a driver may have been under the influence of at the time of their arrest. The biggest challenge that our lab faces now, in reference to the samples we analyze in the lab, is the ever-changing nature of what we are looking for. As anyone that has been in this field long enough can tell you, you are constantly chasing your tail when it comes to testing new and emerging drugs. It seems that just as you start to see one

*Figure 1: This unassuming building is the Henderson Forensic Laboratory.*
and attain the ability to test for it, it is gone and replaced by something else that requires a different extraction and analytical technique. It can be very frustrating at times.

Q: What techniques are used in your lab for NPS detection?

A: We currently use our QTRAP® 5500 LC-MS/MS System for most of our NPS detection. Our drug screen starts with targeted multiple reaction monitoring (MRM) selection of certain ions in Q1, fragmentation in Q2 and the linear ion trap being utilized in Q3 to attain a full MS/MS comparison and library matching. This allows us to distinguish between closely eluting analytes with a few, similar ions that in standard LC-MS/MS analysis would lose selectivity due to only scanning for two or three ions at a time. We have a few in-house confirmation techniques for the NPS drugs that we see on a somewhat routine basis utilizing standard LC-MS/MS analysis with MRM acquisition, fragmentation and selective mass filtering of two to three ions. Any NPS drug that we routinely screen for — but do not have an in-house test for — are sent out to third party labs for confirmation and quantitation only after we have identified them in the linear ion trap drug screen.

Q: How successful are these techniques at identifying NPS compounds?

A: The techniques are very successful in identifying NPS drugs in our whole blood samples. It allows us to specifically select out ions that may be clumped in a mass of other analytes and extract them out, fragment the ion and then use the MS/MS library comparison to identify each individual analyte through specific mass fragmentation patterns. This is important in differentiating a number of NPS drugs that elute around the same time, with similar ion masses which recently we have seen in our assessment of a number of fentanyl analogues that we have been analyzing in the lab.

Q: Can you expand on the driving under the influence of drugs (DUID) screening method you have developed and how your QTRAP instrument enables you to perform both screening and quantitative analysis in one, comprehensive workflow?

A: The DUID drug screening method that we employ uses a quick and robust extraction method coupled with our MRM, linear ion trap analysis, and MS/MS library searching technique. This allows us to individually identify over 100 drugs in a 10-minute long method on our QTRAP 5500 System. The extraction utilizes a rapid technique for all of our drugs of interest using the Quechers extraction products. Even though the Quechers products are relatively new to the forensic science field, they have been used in many other fields such as environmental and pharmaceutical chemistry for years. Many extraction methods used for identifying drugs in whole blood DUID samples are specifically optimized for certain classes of drugs. While it is not perfect, this extraction technique is able to readily extract drugs from many different drug classes in a single extraction and does not require a long, drawn out extraction technique. For the instrumental analysis we use the QTRAP (linear ion trap) detection system on the instrument. We run a targeted drug screen using Q1 as a mass selective filter, Q2 as the collision cell for fragmentation and Q3 as the linear ion trap to attain a full scan MS/MS analysis (enhanced product ion scan) on the detected drugs. Then, MS/MS library searching is used for the confirmation of detected compounds in the linear ion trap and only those compounds with a library match of 60% or greater will appear on the final report. For our lab, the combination of a thorough and detailed analytical method coupled with a quick and easy extraction method allowed us to significantly decrease our costs and analysis time while increasing the amount of drugs we could readily identify and the set the specific concentration of each drug we have in the drug screen. For our confirmation method
we are able to use the same QTRAP 5500 System instrument due to the fact that the instrument is a triple quadrupole linear ion trap hybrid mass spectrometer and we use a different extraction technique and analytical method (linear ion trap vs selective mass filtering) for our drug screen and quantitation methods. This falls within the guidelines of using different analytical methods for your drug screening and drug confirmation methods set forth by the society of forensic toxicologists and our laboratory accreditation body.

Q: There are applications for forensic compound screening that use a comprehensive library to obtain retention times and MS/MS spectra, and subsequently perform targeted identification of compounds of interest in DUID samples. What are your thoughts on this type of approach?

A: It is a great approach and very similar to the one we use. We found that the targeted drug screening method — using the MRM data dependent ion survey scans followed by the information dependent acquisition data and enhanced product ion experiments — proved to be a fast, selective and sensitive acquisition method. It allowed us to identify over 100 different drugs in a single analysis.

Q: Speaking of the ion trap DUID drug screening method you have developed; can you expand on the statistics you pulled from the retrospective analysis?

A: Our retrospective analysis that we presented at the Society of Forensic Toxicologists (SOFT) annual meeting in 2019 reported on the extensive DUID data that we have attained over the past two years. Prior to switching to this new method, we only tested our DUI samples for drugs if the blood alcohol concentration (BAC) was below 0.084 g%. After implementing this new method, we tested all DUI samples for drugs regardless of the BAC. We were able to report on the amount of cases above the old threshold of drug testing in our lab and show that approximately 65% of the cases that would not have been tested for drugs under the old testing thresholds actually had drugs in their system. As previously mentioned, this robust drug screening method allowed us to test for many drugs, so we were able to analyze drug trends that we have seen over the past few years and add a number of new and emerging drugs that are not routinely screened for in most parts of the country. This included several synthetic cathinones, synthetic cannabinoids, tryptamines, piperazines, and novel benzodiazepines. This drug screening method did not make us beholden to our drug testing vendors to come out with new testing kits — as was the case previously when our drugs screening was done via enzyme-linked immunosorbent assay (ELISA). With this new technique, once we were able to attain a certified reference standard and optimize that standard on our QTRAP 5500 System, we could perform a method validation following specified validation standards and add the new NPS drug to our routine drug screen.

Q: How often does this lead to prosecution?

A: From this same retrospective analysis, we found that in the last two years there has been a decrease of approximately 31% in the number of cases that were plead down from DUI’s. This is mainly due to the extra drug data being provided in these DUID reports. Instead of pleading down a DUI case with the only results being a 0.09 g% of ethanol, they are now prosecuting these cases because there may also be THC, alprazolam, hydrocodone, etc in the driver’s blood at the time of the crash.

Q: What efforts do you think will be necessary to combat the flux of NPS and in what capacity do you think your laboratory will contribute to this end?

A: In order to combat this influx of NPS drugs you have to stay innovative and flexible. You cannot just rest on the old adage of “this is how we have always done it around here”. You need to talk to your colleagues at other labs in your area and see what they are seeing in their impaired driving cases. You need to talk to your drug analysis section and see what drugs they are seeing on the streets and what NPS drugs officers are finding on individuals that they arrest. Finally, you need to attend professional conferences and see what else is being seen in other parts of the country — and also how these labs are testing for NPS drugs. It all comes down to wanting to stay ahead of the curve and innovative in your analysis; there is no try, you either want to do it or you don’t. As far as our lab goes, we will always try to stay in front of this as much as we can, and will continue to work with other labs to address this issue and also be a resource for labs that want to learn how to begin testing for these NPS drugs in DUID casework.

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