

Nota applicativa

## Fast Updates to the RADIAN™ ASAP Seized Drug Library: Emerging Presence of Xylazine

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Questa relazione è un Application Brief e non contiene una sezione dettagliata sull'esperimento.

### Disclaimer

*Testing for the library entries has been conducted at both 450°C and 600°C. No significant differences were observed in the results between the two temperatures. Therefore, either temperature may be used without impacting the integrity or applicability of the data. Moving forward, the 450°C library will be used as the standard for consistency and efficiency.*

*For forensic use only.*

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### Abstract

Xylazine is a non-opioid sedative, analgesic and muscle relaxant which is used in veterinary medicine which is not approved for human use.<sup>1</sup> Recently there has been an increase in the number of reports that xylazine is being abused in combination with other drugs of abuse, in particular fentanyl.<sup>2</sup> It has also been reported that the number of fatal overdoses where xylazine has been detected is increasing.<sup>3</sup>

Previously we have described the use of RADIAN ASAP for rapid screening for seized materials where spectral data is matched to a seized drug reference library.<sup>4</sup> In addition, we have also described how to add new

compounds to the reference library.<sup>5</sup>

Here we demonstrate expanding and testing the seized drug reference library to include xylazine, enabling drug chemistry laboratories to quickly respond to newly emerging drugs, and update their routine screen to aid in the control of the trafficking, distribution, and use.

## Benefits

Allows forensic drug chemistry laboratories to use the RADIAN ASAP to screen for xylazine in seized drug samples.

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## Introduction

In recent years xylazine, a non-opioid veterinary tranquilizer which is not approved for human use, has been linked to an increasing number of drug overdose deaths in the U.S.A., and has also started to be detected in other geographies.<sup>3,6</sup> Xylazine use in humans can cause hypotension, central nervous system depression, respiratory depression, and bradycardia.<sup>1</sup> It has also been found to cause skin ulcerations, which can lead to complications including amputation.<sup>3,7</sup>

Although xylazine can be abused alone, it has most commonly been detected in polydrug mixes, often used as an adulterant, particularly with opioids such as fentanyl and heroin and also cocaine.<sup>2</sup> Combining the use of opiates, such as fentanyl, and xylazine which are both central nervous system depressants, significantly increases the risk of fatal drug overdose.<sup>6</sup> In Philadelphia between 2010 and 2015, xylazine was detected in 2% of fatal fentanyl and/or heroin overdoses, which increased to 31% in 2019.<sup>1</sup> Therefore, it has been suggested that xylazine should be added to routine forensic toxicology and seized drug testing.<sup>1,6</sup>

Recently we described how to add new compounds to the RADIAN ASAP reference library (Figure 1); the process was illustrated using isotonitazene.<sup>5</sup> Here, we demonstrate expanding the library for the analysis of xylazine, to enable drug chemistry laboratories to routinely screen for this substance.

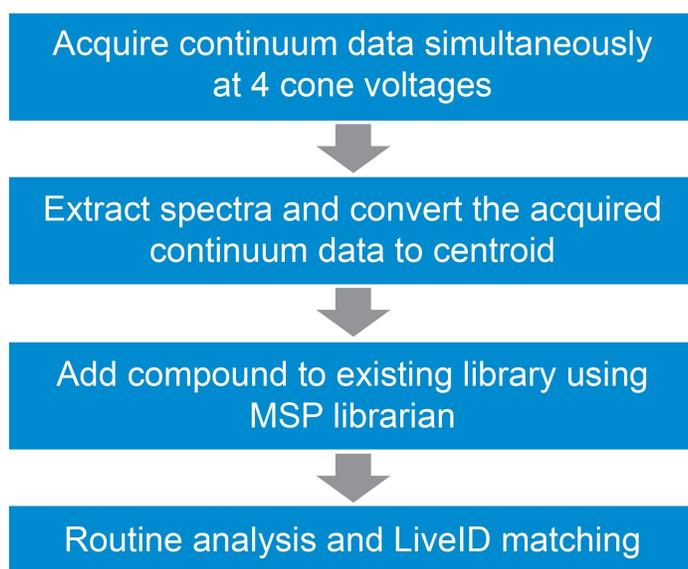
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## Experimental

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Certified reference material (CRM) for fentanyl and xylazine were obtained from Cayman Chemical (Michigan, USA) or Merck (Poole, Dorset, UK). CRM were supplied as 1 mg/mL solutions in methanol/acetonitrile or 1 g of solid material; CRM was dissolved in 1 mL of methanol to obtain individual stock solutions at 1 mg/mL. Prior to analysis, the CRM stock solutions were diluted with methanol to obtain concentrations of 10 µg/mL, 50 µg/mL, and 500 µg/mL. Mixtures of fentanyl and xylazine were made at varying concentrations using these solutions. A counterfeit M-30 pill extract was provided by the San Diego County Sheriff's department- Controlled substance unit, which consisted of approximately 1 mg of an M-30 pill dissolved in 1 mL ethanol and subsequently diluted in 20% aqueous methanol.

Data was matched to a reference library that has been previously updated to include xylazine using the steps in Figure 1.<sup>5</sup>



*Figure 1. Summary of the steps involved in updating RADIAN ASAP reference library with new compounds.*

Data is acquired on the RADIAN ASAP using a “dip and detect” sampling procedure, in positive ionization mode for fentanyl and xylazine CRM solution mixtures and the pill extract, with mass detection performed using full scan MS over the range  $m/z$  50–600 in continuum mode.<sup>4</sup> The data was acquired at four differing cone voltages (15, 25, 35, 50) to generate a spectral fingerprint which includes the precursor and product ions.

LiveID™ library matching software is used for the routine analysis of seized drug materials, performing a spectral match between the acquired spectra and the reference library spectra. The software calculates an average match score (maximum 1000) using a reverse fit model for the data acquired at each of the cone voltages. A match score of 850 was used as the reporting cut off for a sample to be deemed positive.

## Results and Discussion

Data was processed using LiveID in combination with the updated library. Substances analyzed included xylazine at three different concentrations (10 µg/mL, 50 µg/mL, and 500 µg/mL) producing match scores >980 (range 981 to 995). Figure 2 shows an example of the LiveID result obtained for xylazine CRM at a concentration of 10 µg/mL.



Figure 2. LiveID analysis of xylazine CRM at 10 µg/mL. Panel A shows triplicate 'dip and detect' analysis for xylazine reference material and the match score 990 for the first triplicate. Panel B displays the details for the spectral match with the acquired sample and the new library for each cone voltage.

Mixtures of xylazine and fentanyl at various concentrations were also analyzed. The lowest concentration to gain

a positive identification (match score >850) in these mixtures was  $\geq 25 \mu\text{g/ml}$  for xylazine and  $\geq 2.5 \mu\text{g/ml}$  for fentanyl. However, in a mixture with fentanyl (40  $\mu\text{g/mL}$ ), xylazine could be detected at a concentration of 10  $\mu\text{g/mL}$  with a match score >700. Figure 3 shows an example of the LiveID results obtained for a xylazine CRM (25  $\mu\text{g/mL}$ ) and fentanyl CRM (25  $\mu\text{g/mL}$ ) mixture.

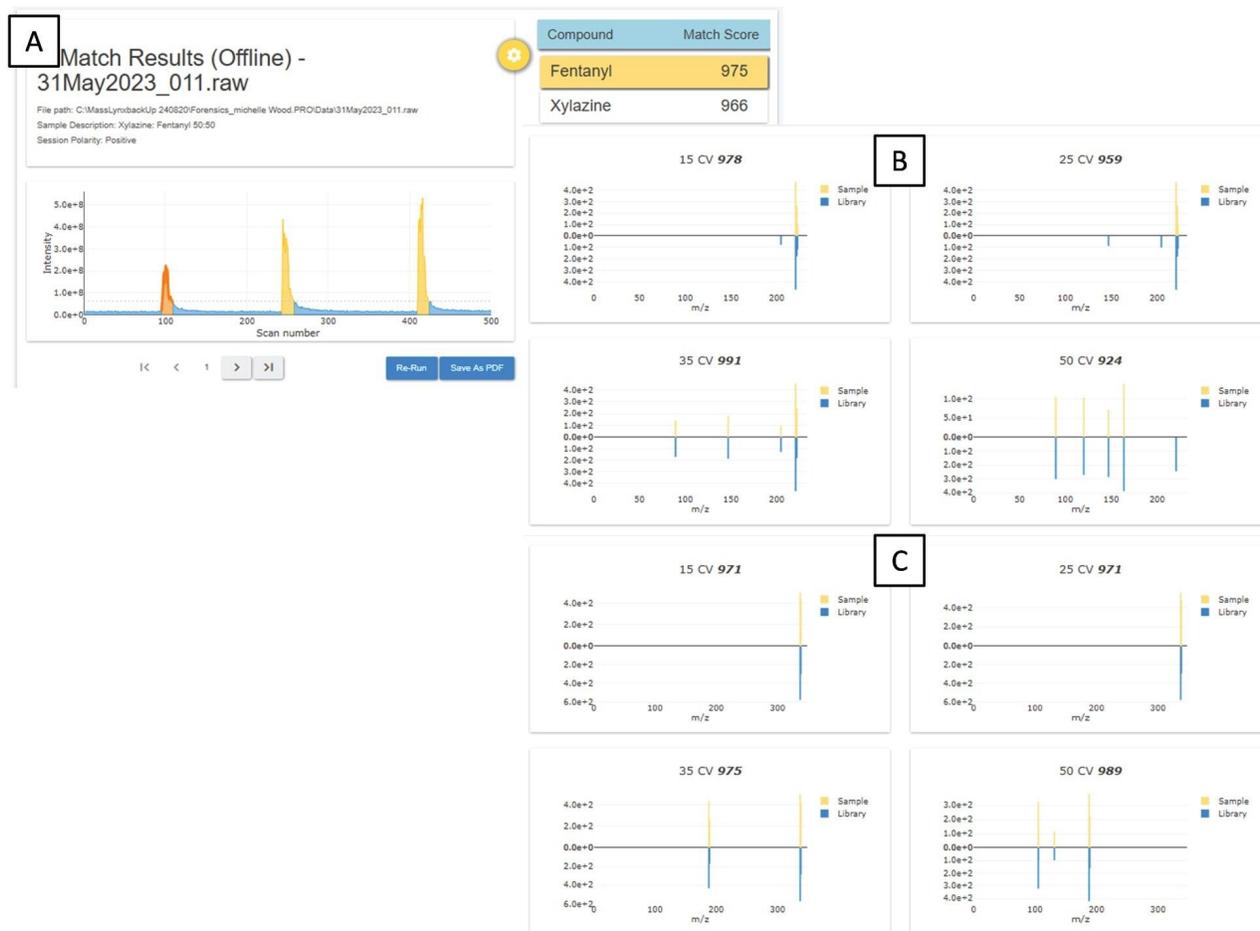


Figure 3. LiveID analysis of xylazine and fentanyl CRM mixture. Panel A shows triplicate analysis for the mixture and the match score 966 (xylazine) and 975 (fentanyl) for the first triplicate. Panel B displays the details for the spectral match for xylazine with the acquired sample and the new library entry. Panel C displays the details for the spectral match for fentanyl with the acquired sample and the library entry.

Analysis of the M-30 pill extract (average of triplicate RADIAN ASAP analysis) resulted in the positive

identification of fentanyl with an average match score of 962.33 and an average match score of 781.67 for xylazine (for the first triplicate analysis, xylazine was detected with a match score of 826). There were detections (match score >800) for additional compounds, including metodesnitazene, paracetamol and caffeine. This showed good agreement with results obtained from the analysis of the M-30 pill, except for metodesnitazene, using confirmatory LC-ToF analysis.<sup>8</sup> Figure 4 shows the LiveID results obtained for the analysis of the M-30 pill extract.

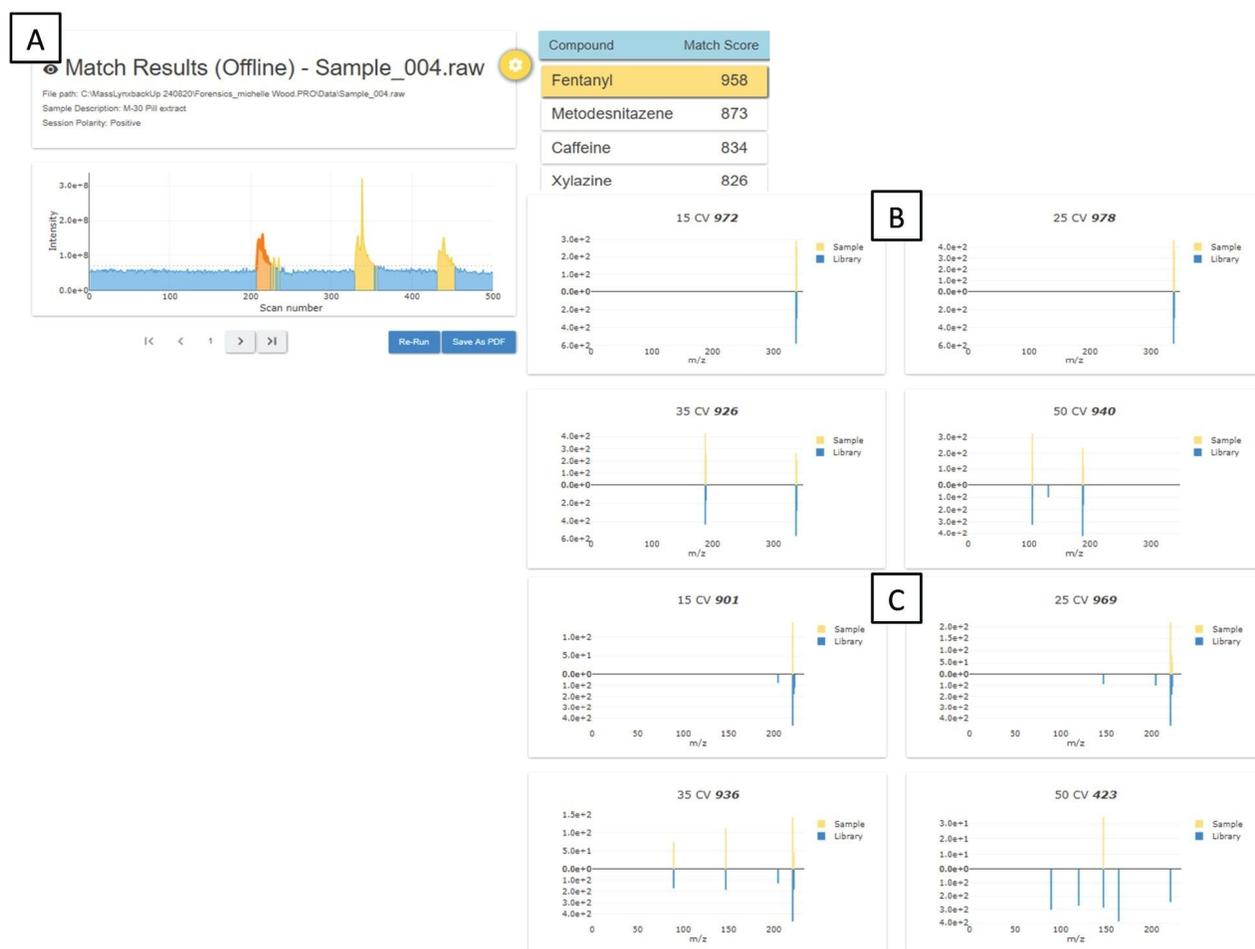


Figure 4. LiveID analysis of a M30 pill extract. Panel A shows triplicate analysis for the pill extract and the match score 958 (fentanyl) and 826 (xylazine) for the first triplicate. Panel B displays the details for the spectral match for fentanyl with the acquired sample and the library entry. Panel C displays the details for the spectral match for xylazine with the acquired sample and the new library entry.

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## Conclusion

The seized drug reference library can be downloaded from Waters™ Marketplace, in the LiveID resources section. (<https://marketplace.waters.com/apps/170156/liveid#!resources> < <https://marketplace.waters.com/apps/170156/liveid#!resources>> ).

This fast update to the library enables users to screen for the newly emerging adulterant, xylazine, in seized drug samples using RADIANT ASAP and LiveID. The screening method in combination with the updated reference library shows promise for screening polydrug mixtures, enabling users to keep their screening methods up to date with trending and emerging drug use patterns.

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## References

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## Featured Products

[RADIAN ASAP Direct Mass Detector <https://www.waters.com/waters/nav.htm?cid=135073413>](https://www.waters.com/waters/nav.htm?cid=135073413)

[LiveID Software <](https://www.waters.com/waters/nav.htm?cid=134939519&alias=AEMredirectForBaseID_134939519)

[https://www.waters.com/waters/nav.htm?cid=134939519&alias=AEMredirectForBaseID\\_134939519>](https://www.waters.com/waters/nav.htm?cid=134939519&alias=AEMredirectForBaseID_134939519)

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