SpectraPlasmonics

# Enhancing Drug Response: Innovations in Identifying Xylazine in Drug Mixtures

**Keywords**
Xylazine, Fentanyl, Overdose, Surface Enhanced Raman Spectroscopy (SERS), On-site Testing.

Use of xylazine with opioids can lead to severe side effects. Surface Enhanced Raman Spectroscopy (SERS) offers a fast, accurate method to detect xylazine, improving drug analysis and public safety.

WHITE PAPER

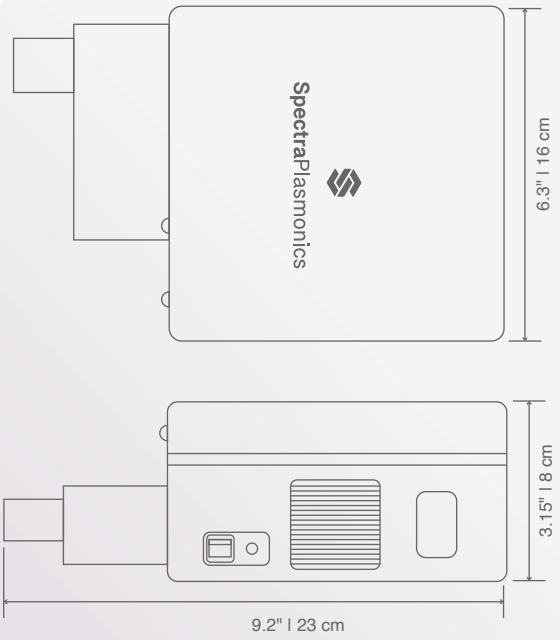
## Introduction

Xylazine is an alpha-2 adrenergic agonist, a chemical compound that has gained significant attention for its role outside its traditional use[1]. Originally developed for veterinary purposes, xylazine acts primarily as a sedative, analgesic, and muscle relaxant, aiding in the management and treatment of animals by reducing anxiety, pain, and involuntary muscle movements. When combined with potent opioids like fentanyl, xylazine can amplify the sedative and analgesic effects, resulting in a deeper and longer-lasting state of sedation. This synergistic effect has been noted in unregulated drug circles, enhancing the 'high' experienced through consumption. However, human ingestion of xylazine comes with severe health consequences, including pronounced respiratory depression, which can dangerously slow or even stop breathing, bradycardia or a decrease in heart rate, hypotension, leading to a drastic drop in blood pressure, and with prolonged or severe use, it has also been linked to skin ulcerations and severe wound formation. The risk of overdose increases significantly when xylazine, a non-opioid sedative, is mixed with narcotics such as fentanyl. Narcan (naloxone), effective against opioid overdoses, cannot reverse xylazine's effects due to its different action mechanism. This leaves individuals at risk even after Narcan administration.

While xylazine's dangers are now well-known, its widespread prevalence and accessibility on the streets have surged since its introduction, frequently finding its way into drug mixes without the knowledge of consumers. In 2023, the White House designated fentanyl and xylazine an emerging threat[2] and called for better access to community drug testing and data monitoring solutions in their response plan[3]. As such, vigilant monitoring and prompt detection have become indispensable. While instruments like gas chromatography-mass spectrometry (GC-MS) and liquid chromatography-mass spectrometry (LC-MS) are highly effective, they are often costly, time-consuming, and inaccessible, making them less practical for immediate needs. This has led to an urgent call for advanced tools that facilitate immediate, on-site testing. Such tools would offer a promising avenue for the swift and precise detection of xylazine, enhancing the capabilities of drug analysis and contributing to the broader efforts in ensuring public safety and integrity. To this end, a new method using Surface Enhanced Raman Spectroscopy (SERS)[4] is outlined and evaluated to determine its accuracy and efficacy in detecting xylazine.

## Amplifi ID™ Drug Analysis Spectrometer

Amplifi ID™ ensures agencies can effectively combat the ever-evolving landscape of illicit substances. Designed for ease of use, our portable device provides fast and easily interpretable results, enabling personnel to make informed decisions and collect data swiftly. Leveraging SERS and state of the art spectral processing algorithms, Amplifi ID™ provides detailed detection of a wide range of substances, including the ability to detect fentanyl analogues and xylazine at trace levels.




Device Dimensions -
Highly Portable and Transportable

## Amplifi ID™ Benefits

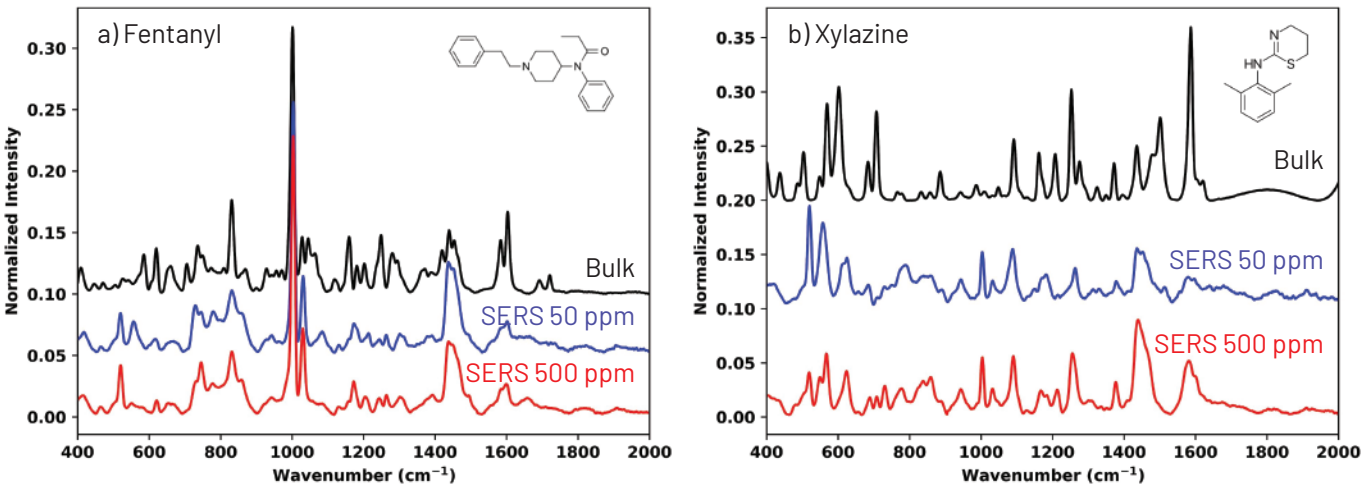
- Compact design offering lab-grade Raman Spectroscopy performance.
- Bulk and Trace Scan modes within a single device.
- Detect trace substances less than 5 wt.%.
- User-friendly software mastered in under 10 minutes.
- Detailed historical trends for surveillance and risk management.
- Analytical Chemists available on-demand for results review.


The Amplifi ID™ drug analysis solution includes:

**a.** Raman Spectroscopy instrument.
**b.** Chemical Detection Analytics Package and Dashboard.
**c.** Bulk Scan and Trace Scan attachments.
**d.** Trace Scan Kits.

## Fentanyl and Xylazine Spectra Using Amplifi ID™ Bulk and Trace Scan Modes

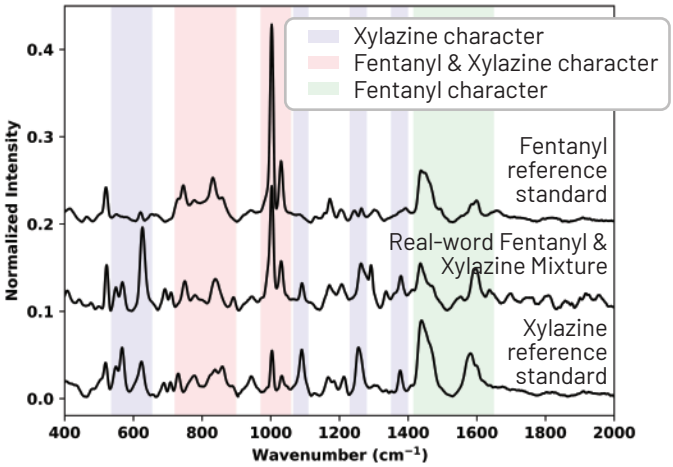
Spectra of pure fentanyl and pure xylazine were captured using Amplifi ID's Bulk (Raman) and Trace (SERS) Scan modes. The bulk and trace spectra of fentanyl exhibit peaks arising from its aromatic rings (~820 $cm^{-1}$, 1000 $cm^{-1}$, 1450 $cm^{-1}$) and amide group (~1600 $cm^{-1}$). The bulk and trace spectra of xylazine exhibit peaks arising from its C-S (~580 $cm^{-1}$), C-N (~1280 $cm^{-1}$), C=N (~1600 $cm^{-1}$) groups. The measurement conditions for all trace spectra were the same for all samples, however, for Bulk Scan a parameter sweep is performed to ensure maximum signal intensity is captured without resulting in signal saturation. The spectra show the relationship between bulk and trace and concentration sensitivity. It is evident that even though the two concentration 50 ppm and 500 ppm are an order of magnitude different, the spectra have high agreement in character and Raman band location. This highlights the capability of the Amplifi ID SERS substrate to enhance the signal even at low concentrations with negligible loss in resolution in the relevant drug concentration range.

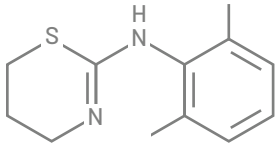

Raman spectra obtained with the Amplifi ID using both Bulk Scan and Trace Scan modes for **a)** pure fentanyl powder and **b)** pure xylazine powder. Surface Enhanced Raman Spectroscopy (SERS) substrates were tested by dissolving the pure powders in methanol to create 50 ppm and 500 ppm solutions.

## Spectral Region Analysis

The ability for SERS and Raman to collect information-rich spectral signals allows for specificity in detection and mixture analysis. In this analysis, we compare a real-world sample, which contains a mixture of fentanyl and xylazine and is measured using Trace Scan mode, against pure reference standards of fentanyl and xylazine to identify the unique spectral regions characteristic of each substance. We use blue to highlight areas exclusive to xylazine's distinct chemical signature, red to denote regions unique to fentanyl, and green to mark overlapping spectral regions between the two substances. This visual representation helps in comprehending the intricate relationship between the spectral characteristics of these compounds, essential for their precise identification and analysis.




## Limit of Detection for Xylazine




To determine the limit of detection of Amplifi ID's Trace Scan mode, xylazine powder, sourced from Millipore Sigma, was dissolved in pure methanol to create different concentrations. Each concentration was tested across four replicates on three different Amplifi ID instruments. A positive (+) result indicates the detection of xylazine, whereas a negative (-) result indicates no detection of xylazine. We observed that the Amplifi ID system consistently detected xylazine at concentrations of approximately 10 ppm and higher.

**+ Xylazine ID** **- No Xylazine ID**

| Xylazine (ppm) | Amplifi ID A | Amplif ID B | Amplifi ID C |
|---|---|---|---|
| 100 ppm | ++++ | ++++ | ++++ |
| 50 ppm | ++++ | ++++ | ++++ |
| 10 ppm | ++++ | ++-- | ++++ |
| 5 ppm | ++-- | ++-- | +--- |

## Detecting Xylazine with Cutting Agents Using Trace Scan Mode

To evaluate the sensitivity of the Amplifi ID system in the presence of cutting agents, we prepared solutions of pure cutting agents in methanol, alongside solutions containing a 95 wt.% cutting agent to 5 wt.% xylazine ratio in methanol. Both types of solutions were then loaded into Amplifi ID Trace Scan cartridges and subjected to testing across three different instruments, each in sets of five replicates. The solutions containing xylazine consistently reported a positive ID for xylazine, with no false positives, demonstrating the system's reliability and accuracy in identifying substances.

**+ Xylazine ID** **- No Xylazine ID** ** MDMA Detected as well

| | 100% Cutting Agent | | | 95 wt.% Cutting Agent + 5 wt.% Xylazine | | |
|---|---|---|---|---|---|---|
| Cutting Agent | Amplifi ID A | Amplifi ID B | Amplifi ID C | Amplifi ID A | Amplifi ID B | Amplifi ID C |
| Sodium Bicarbonate | ----- | ----- | ----- | +++++ | +++++ | +++++ |
| Caffeine | ----- | ----- | ----- | +++++ | +++++ | +++++ |
| MDMA | -----** | -----** | -----** | +++++** | +++++** | +++++** |
| Phenacetin | ----- | ----- | ----- | +++++ | +++++ | ++++- |
| Mannitol | ----- | ----- | ----- | +++++ | +++++ | +++++ |

## Trace Scan Mode Tested Using Laboratory-Prepared Samples

In this study, we explore the efficacy of the Amplifi ID system in detecting the presence of fentanyl and xylazine in laboratory prepared samples made from reference standards. Samples 1 and 2 do not contain any fentanyl and xylazine and serve as negative controls within the context of predicting fentanyl and xylazine. Samples 3, 4, and 5 contain increasing concentrations of xylazine (5 wt.%, 10 wt.%, 15 wt.%) and decreasing concentrations of fentanyl (50 wt.%, 45 wt.%, 40 wt.%). Across all replicates, the algorithm accurately identified both fentanyl and xylazine. Sample 6 contains 1 wt.% fentanyl and 50 wt.% xylazine and in this instance, the algorithm failed to identify the fentanyl. This lack of detection can be attributed to the high concentration of xylazine, which, being a strong scatterer, likely masked the fentanyl's signal. Later analysis will demonstrate that the algorithm accurately predicts several street samples with fentanyl concentrations below 5 wt.%. Sample 7 included an extra 5 wt.% fluorofentanyl, and in 6 out of 9 replicates, there was a positive detection of both fentanyl and xylazine.

| | |
|---|---|
| +/- **Fentanyl ID/ No Xylazine ID** | +/+ **Fentanyl ID/ Xylazine ID** |
| -/+ **No Fentanyl ID/ Xylazine ID** | -/- **No Fentanyl ID/No Xylazine ID** |

| # | Sample Contents (wt.%) | Test Results | | |
|---|---|---|---|---|
| 1 | Cocaine (30%), Lidocaine (50%), Dimethyl sulfone (2%), Caffeine (18%) | -/- | -/- | -/- |
| | | -/- | -/- | -/- |
| | | -/- | -/- | -/- |
| 2 | Cocaine (30%), Lidocaine (5%), Dimethyl sulfone (2%), Caffeine (63%) | -/- | -/- | -/- |
| | | -/- | -/- | -/- |
| | | -/- | -/- | -/- |
| 3 | 4-ANPP (1%), **Fentanyl (50%)**, **Xylazine (5%)**, Caffeine (44%) | +/+ | +/+ | +/+ |
| | | +/+ | +/+ | +/+ |
| | | +/+ | +/+ | +/+ |
| 4 | 4-ANPP (1%), **Fentanyl (45%)**, **Xylazine (10%)**, Caffeine (44%) | +/+ | +/+ | +/+ |
| | | +/+ | +/+ | +/+ |
| | | +/+ | +/+ | +/+ |
| 5 | 4-ANPP (1%), **Fentanyl (40%)**, **Xylazine (15%)**, Caffeine (44%) | +/+ | +/+ | +/+ |
| | | +/+ | +/+ | +/+ |
| | | +/+ | +/+ | +/+ |
| 6 | 4-ANPP (1%), **Fentanyl (1%)**, **Xylazine (50%)**, Caffeine (48%) | -/+ | -/+ | -/+ |
| | | -/+ | -/+ | -/+ |
| | | -/+ | -/+ | -/+ |
| 7 | 4-ANPP (1%), **Fentanyl (5%)**, Fluorofentanyl (5%), **Xylazine (50%)**, Caffeine (39%) | +/+ | +/+ | +/+ |
| | | +/+ | +/+ | +/+ |
| | | -/+ | -/+ | -/+ |

## Real-World Street Samples Tested for Xylazine and Fentanyl

The dataset shown below was gathered in partnership with Substance Drug Checking (Victoria, BC), a drug analysis service that operates a community drug checking program on Vancouver Island. The testing procedure was conducted by a drug testing technician who took a small sample, typically 2-5 milligrams, and placed it into a new bag for non-destructive Bulk Scan analysis using the Amplifi ID, which can scan through the bag. This same sample was then dissolved in methanol for further analysis in the Amplifi ID Trace Scan mode. Additionally, the sample underwent testing with an FTIR instrument and confirmatory analysis using Paper Spray Mass Spectrometry (PS-MS). The data presented below exclusively reflects the findings from the Trace Scan mode used to detect fentanyl and xylazine.

Using PS-MS, it was determined that xylazine was absent in samples 13-18, while its concentration in samples 1-12 ranged from 0.08 wt.% to 8.47 wt.%, with a median concentration of 1.05 wt.%. Fentanyl and xylazine detection results are detailed in the table below, using PS-MS as the benchmark tool for comparison.

| | |
|---|---|
| +/- **Fentanyl ID/ No Xylazine ID** | +/+ **Fentanyl ID/ Xylazine ID** |
| -/+ **No Fentanyl ID/ Xylazine ID** | -/- **No Fentanyl ID/No Xylazine ID** |

| # | Sample Contents (% purity) | Test Results | |
|---|---|---|---|
| | | Amplifi ID | FTIR |
| 1 | Fentanyl (0.23%), Xylazine (0.19%), Sucrose (no data) | +/+ | +/- |
| 2 | Caffeine (no data), Fentanyl (4.46%), Fluorofentanyl (1.04%), **Xylazine (1.14%)** | +/+ | +/- |
| 3 | Caffeine (no data), Fentanyl (8.39%), Fluorofentanyl (1.07%), **Xylazine (4.45%)** | +/+ | +/+ |
| 4 | Caffeine (no data), Bromazolam (1.65%), Fentanyl (3.66%), Fluorofentanyl (0.20%), **Xylazine (1.12%)** | +/+ | +/- |
| 5 | Caffeine (no data), Bromazolam (4.32%), Fentanyl (4.40%), Fluorofentanyl (0.49%), **Xylazine (0.98%)** | +/+ | +/- |
| 6 | Caffeine (no data), Fentanyl (15.54%), Etizolam (1.06%), **Xylazine (4.57%),** Flubromazepam (2.80%) | +/+ | +/- |
| 7 | Fentanyl (0.14%), **Xylazine (0.08%)**, Sucrose (no data) | +/+ | -/- |
| 8 | Caffeine (no data), Bromazolam (0.82%), Erythritol (no data), Fentanyl (0.16%), Fluorofentanyl (12.13%), **Xylazine (8.47%)** | +/+ | +/- |
| 9 | Caffeine (no data), Erythritol (no data), Bromazolam (0.54%), Fentanyl (0.14%), Fluorofentanyl (10.70%), **Xylazine (8.10%)** | +/+ | +/+ |
| 10 | Fentanyl (6.95%), Etizolam (1.07%), Flubromazepam (0.73%), **Xylazine (0.50%),** Fluorofentanyl (0.46%), Caffeine (no data) | +/- | +/- |
| 11 | Fentanyl (9.94%), Fluorofentanyl (0.34%), Etizolam (1.37%), Flubromazepam (0.58%), **Xylazine (0.25%),** Caffeine (no data) | +/- | +/- |
| 12 | Fentanyl (13.20%), Fluorofentanyl (0.51%), Etizolam (1.42%), Flubromazepam (0.65%), **Xylazine (0.19%),** Caffeine (no data) | +/- | +/- |
| 13 | Fentanyl (no data) | +/- | +/- |

| # | Sample Contents (% purity) | Test Results | |
|---|---|---|---|
| | | Amplifi ID | FTIR |
| 14 | Heroin (2.07%), Acetylfentanyl (3.22%), Fentanyl (9.32%), Acetylcodeine (0.72%), Acetylmorphine (0.46%), Bromazolam (no data), Caffeine (no data) | +/- | +/- |
| 15 | Fentanyl (17.59%), Bromazolam (3.60%), Caffeine (no data), Erythritol (no data) | +/- | +/- |
| 16 | Cocaine (no data) | -/- | -/- |
| 17 | Cocaine (no data) | -/- | -/- |
| 18 | Methamphetamine (no data) | -/- | -/- |

## Conclusions

The rise in xylazine's availability and its incorporation into street drugs with critical side effects underscores the urgent need for improved detection and monitoring to mitigate its impact and safeguard public health. The deployment of the Amplifi ID system, which employs Surface Enhanced Raman Spectroscopy (SERS), provides a promising avenue for rapid, in-field and accurate detection, thus enhancing drug analysis and bolstering public safety measures. The effectiveness of the Amplifi ID Trace Scan in distinguishing and identifying substances such as fentanyl and xylazine, even at low concentrations, has been demonstrated, significantly enhancing the management and understanding of these compounds across different settings.

## References

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