

SCREENING AND CONFIRMATORY TESTING OF A COUNTERFEIT M-30 PILL ADULTERATED WITH XYLAZINE

Michael Wakefield,¹ Emily Lee,² Jane Cooper,² Alexandra Harvey,³ Jeff Salamat.³

¹ Waters Corporation, Pleasanton, CA, USA.

² Forensics and Toxicology R&D, Waters Corporation, Wilmslow, UK.

³ San Diego County Sheriff's Regional Crime Laboratory, San Diego, CA, USA.

INTRODUCTION

- Xylazine, a veterinary tranquilizer, is reportedly being used as an adulterant in an increasing number of illicit drug mixtures and linked to a growing number of overdose deaths.¹
- Fake pills are being mass-produced and marketed as legitimate prescription pills.
 - These are easily accessible and often sold on social media and e-commerce platforms
 - Many are made to look like prescription pills such as oxycodone (Oxycontin® and Percocet®)
- Xylazine has been found to be present in counterfeit M-30 pills across the United States.
- A real counterfeit M-30 pill extract was provided to Waters™ for screening and confirmatory analysis by the San Diego County Sheriff's Department - Controlled Substance Unit.
- Here we illustrate the use of the ACQUITY RDa™ Detector for seized drug screening utilizing a dissolve, filter, dilute and shoot sample preparation of a counterfeit M-30 pill. An LC-MS/MS system operating in MRM detection mode was used for confirmation and semi-quantitative analysis of the identified components

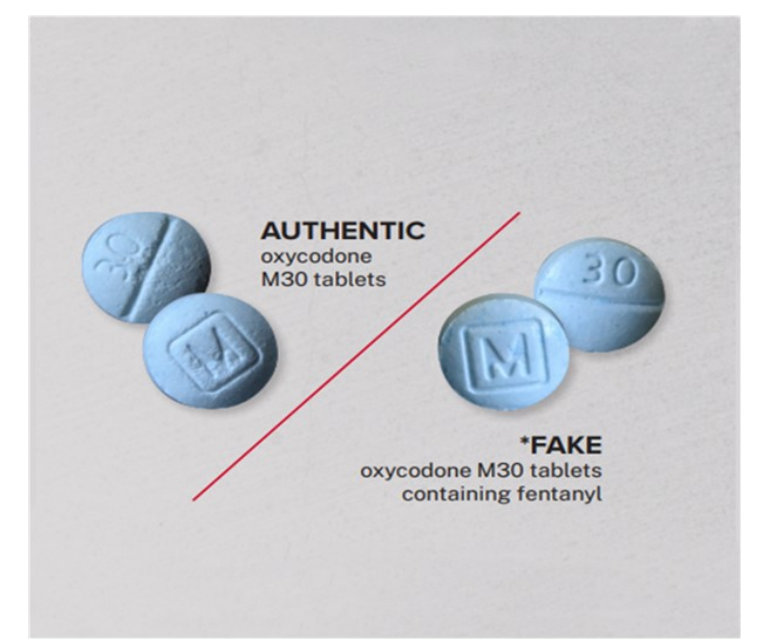


Figure 1. Fake M-30 pills made to look like prescription drugs such as Oxycodone²

SAMPLE PREPARATION

- The counterfeit M-30 pill was shaved to produce a powder.
- Approximately 1 mg of powder was dissolved in 1 mL of ethanol.
- The solution was filtered with a 0.2 µm PTFE Acrodisc® Syringe Filter.
- Three drops of the filtered solution were diluted in 20% aqueous methanol for screening analysis.
- The screening solution was further diluted 1 in 100 using 80/20 (v/v) water:methanol for confirmation analysis.

SCREENING ANALYSIS

- Screening analysis was performed using an ACQUITY RDa Detector (Figure 2) in combination with an ACQUITY UPLC™ I-Class PLUS FTN System.
- Data was acquired using the high-resolution mass spectrometer using:
 - positive ionization mode
 - full scan accurate mass (50-2000 m/z) with fragmentation
 - 9.5 minute gradient elution
 - 10 µ injection volume
- Data was processed using waters_connect™ Software:
 - a custom target list (library) of 400 compounds was used for screening analysis
 - compounds were positively identified based on accurate mass, retention time and compound fragmentation
 - included the ability to elucidate and identify unknowns

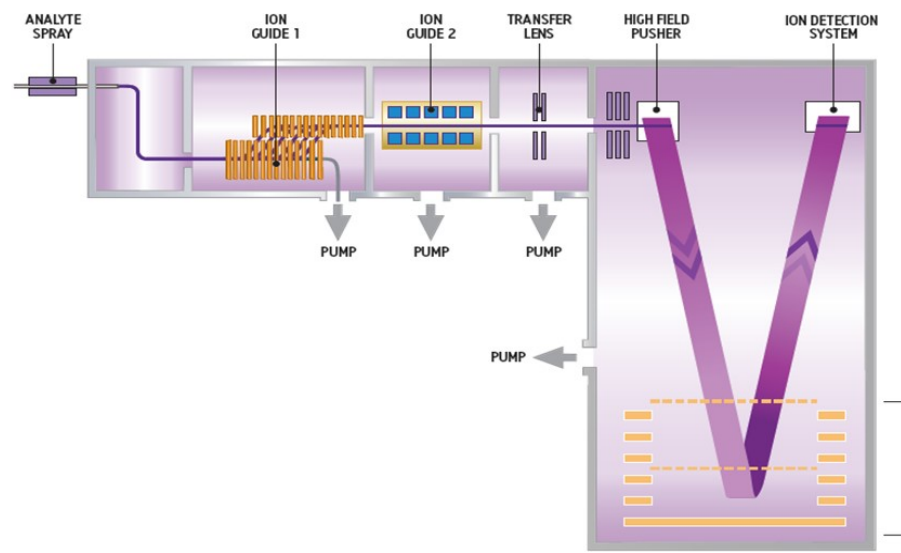


Figure 2. Schematic diagram of the ACQUITY RDa Detector

CONFIRMATION AND SEMI-QUANTITATIVE ANALYSIS

- Confirmation and semi-quantitative analysis was performed using a Xevo™ TQ-Absolute mass spectrometer in combination with an ACQUITY UPLC I-Class PLUS FTN System.
- Data acquisition using the Xevo TQ-Absolute Mass Spectrometer:
 - positive ionisation mode
 - MRM detection mode using both a quantitative and qualitative transitions for all compounds
 - 4.5 minute run time
 - 1 µL injection volume
 - for 11 of the compounds detected in screening analysis, a five-point calibration curve was prepared at either 0.5 ng/mL - 100 ng/mL or 1 ng/mL - 250 ng/mL
 - external standard quantification was employed
- Data was processed using TargetLynx™ XS Software:
 - compounds were positively identified using MRM and retention time



SCREENING RESULTS

- Screening analysis of the counterfeit M-30 pill identified fourteen presumptive positive compounds
 - Acetaminophen, 4-ANPP, 4-methylamino antipyrine (European analgesic), acetylfentanyl, caffeine, despropionyl para fluorofentanyl, fentanyl, lidocaine, O(p)-fluorofentanyl, para-fluoro phenethyl 4-ANPP, phenethyl 4-ANPP, proadifen (GCMS internal standard), procaine, tramadol, xylazine were all presumptively identified.
 - The observed mass for xylazine was m/z 221.1110 with a mass error of 1.3 ppm (0.3 mDa), and the observed retention time was 2.30 min compared to the expected 2.38 min (Figure 3).
 - The observed mass for fentanyl was m/z 337.2272 with a mass error of -0.8 ppm (-0.3 mDa), and the observed retention time was 3.06 min compared to the expected 3.10 min (Figure 4).

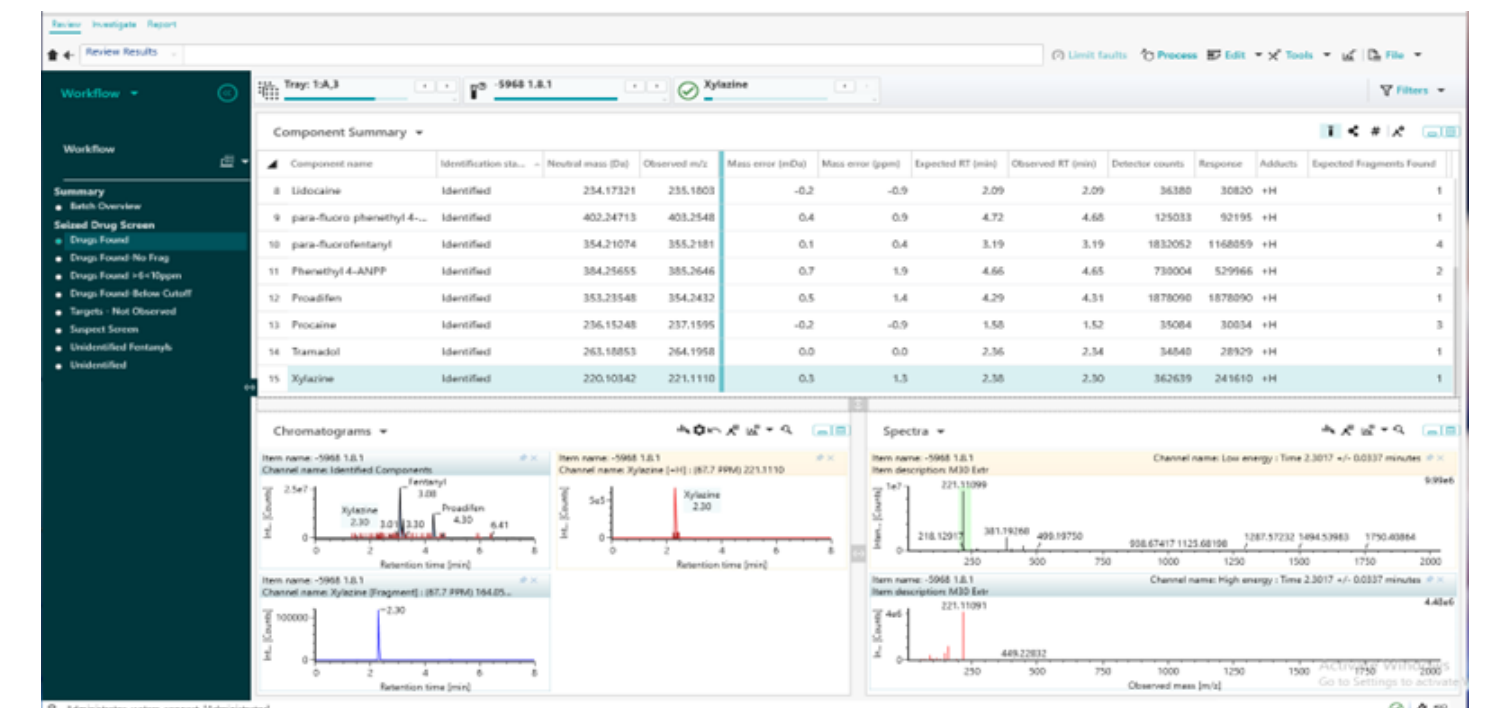


Figure 3. Waters_connect software results for seized drug screening analysis for xylazine from the M-30 pill analysis.

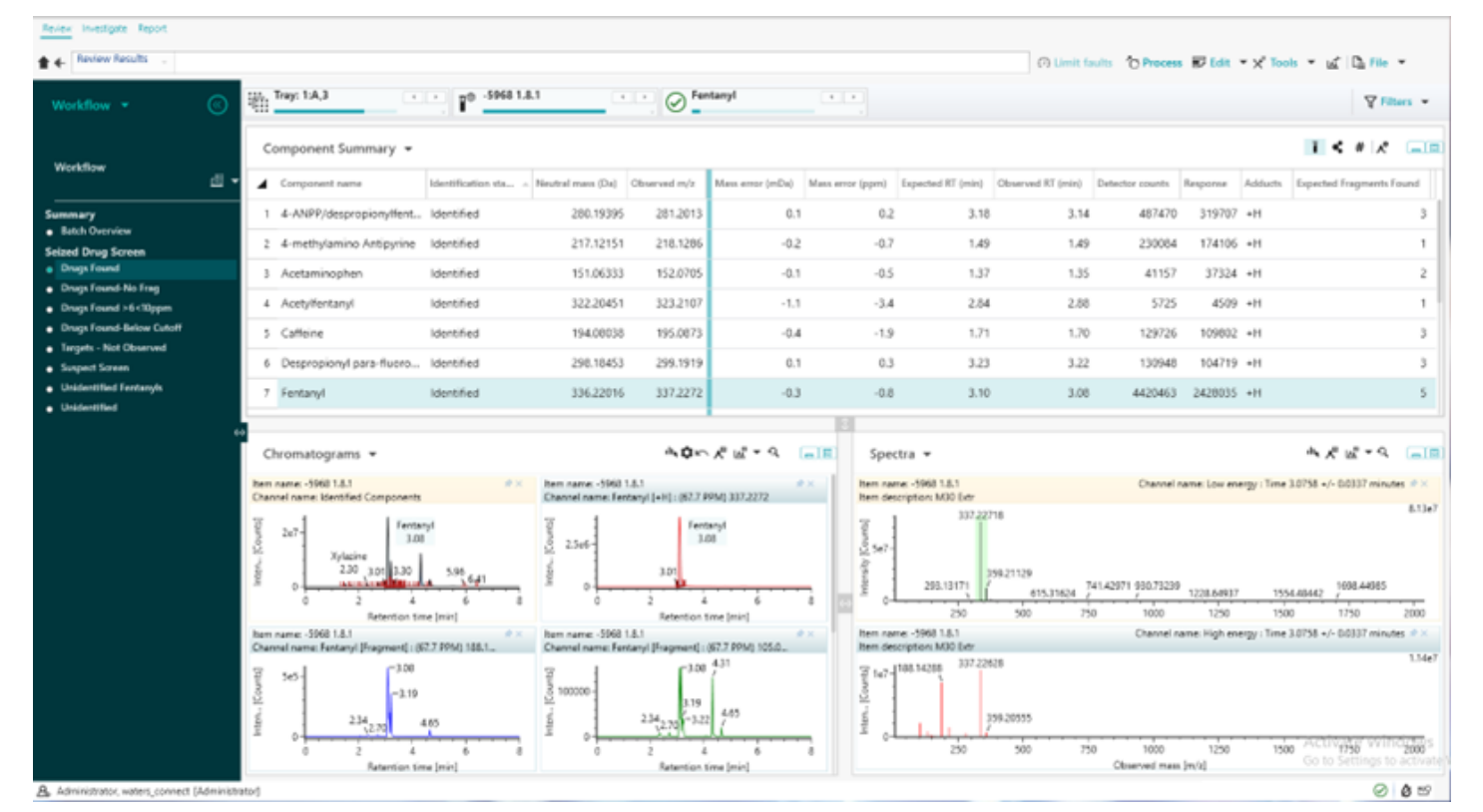


Figure 4. Waters_connect software results for seized drug screening analysis for fentanyl from the M-30 pill analysis.

RESULTS AND DISCUSSION

CONFIRMATION RESULTS

- Confirmation and semi-quantitation was performed for 11 of these identified compounds using tandem mass spectrometry (Figure 5).
 - Each compound provided a linear response with R² > 0.995 (Figure 6 and 7).
 - Xylazine was detected at a concentration of 913 ng/mL (Figure 6).
 - Fentanyl was detected at a concentration of 7444 ng/mL (Figure 7).
 - 4-methylamino antipyrine, 4-ANPP, O(p)-fluorofentanyl, acetaminophen and caffeine were detected at levels above 500 ng/mL.
 - Lidocaine, tramadol, procaine and acetylfentanyl detected at levels below 50 ng/mL.

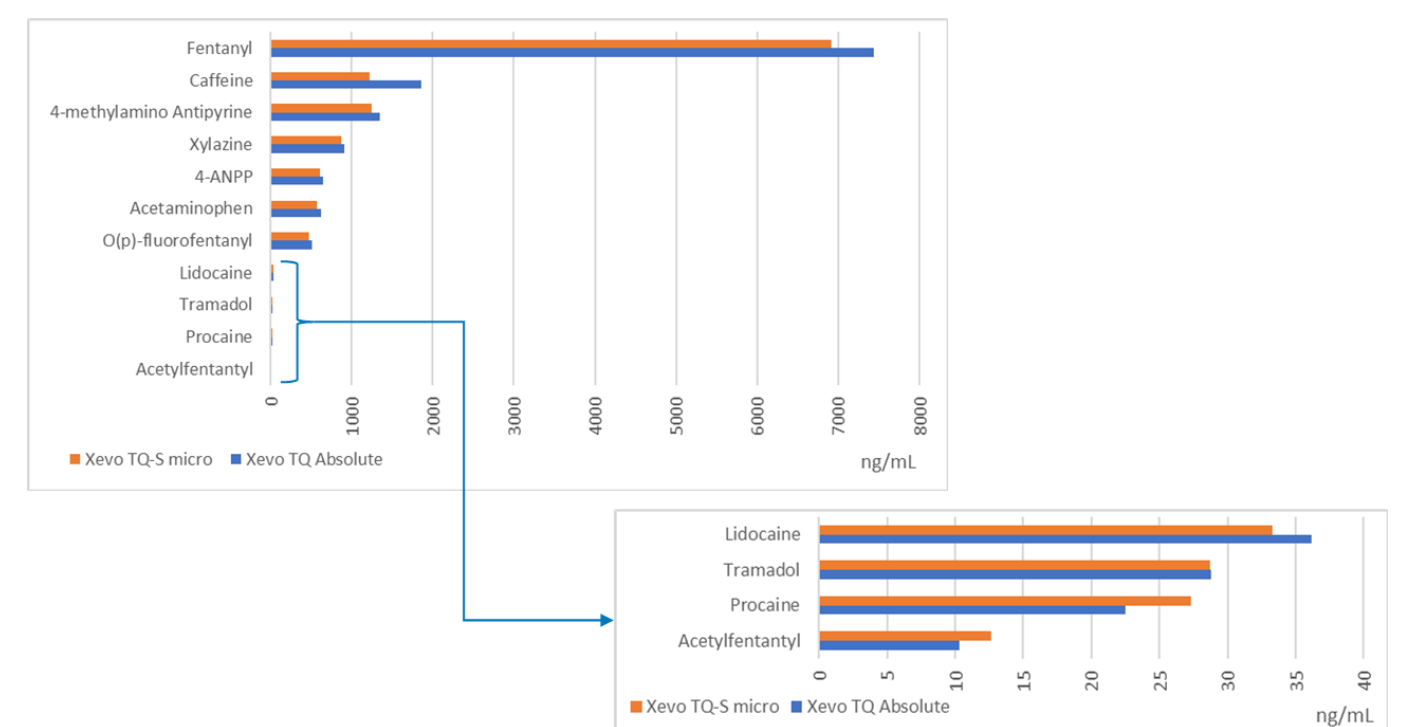


Figure 5. Summary of the semi-quantitative results obtained from the Xevo TQ-Absolute and Xevo TQ-S-micro analysis of the M-30 pill extract.

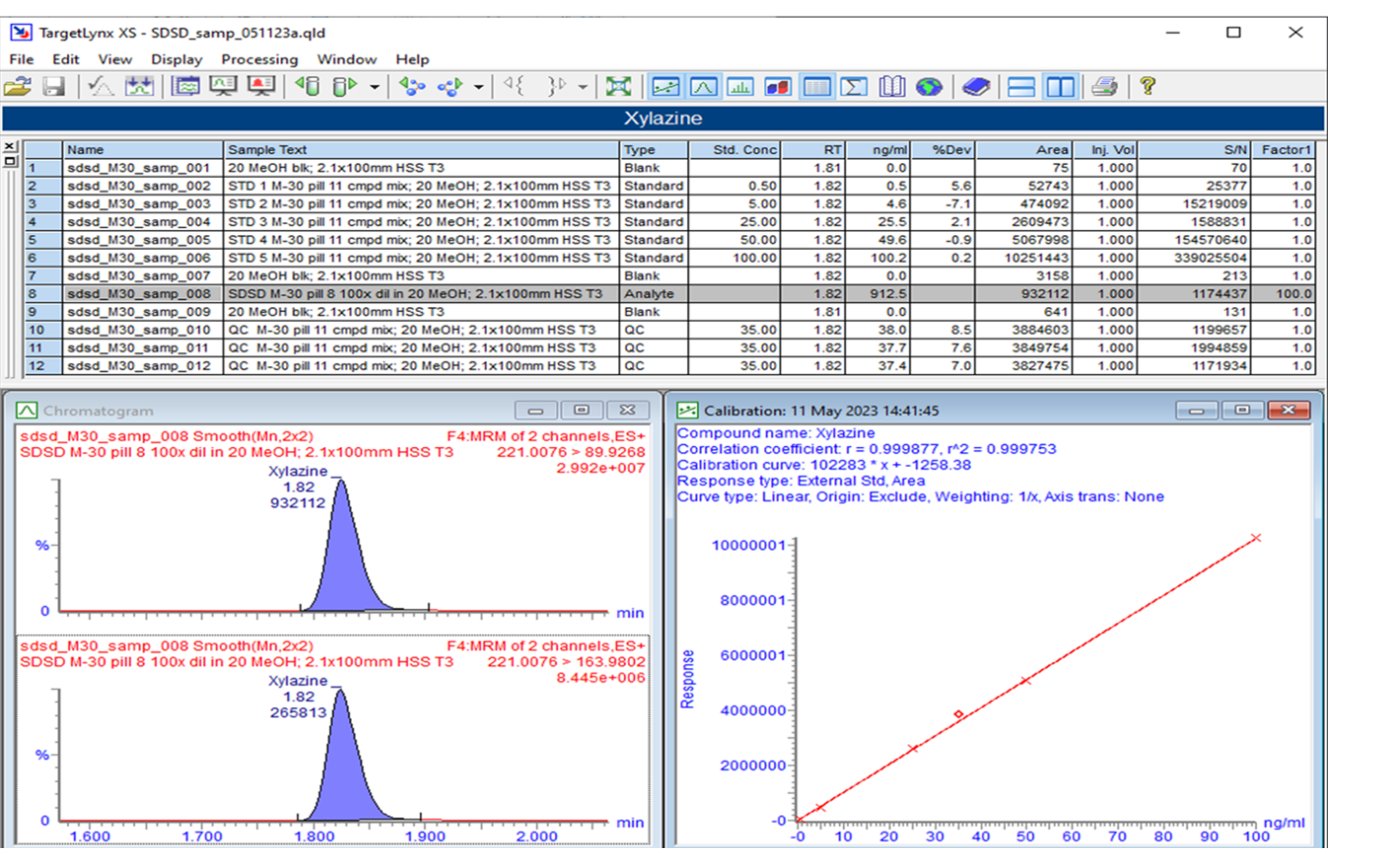


Figure 6. TargetLynx XS results of the M-30 pill analysis for xylazine on the Xevo TQ-Absolute.

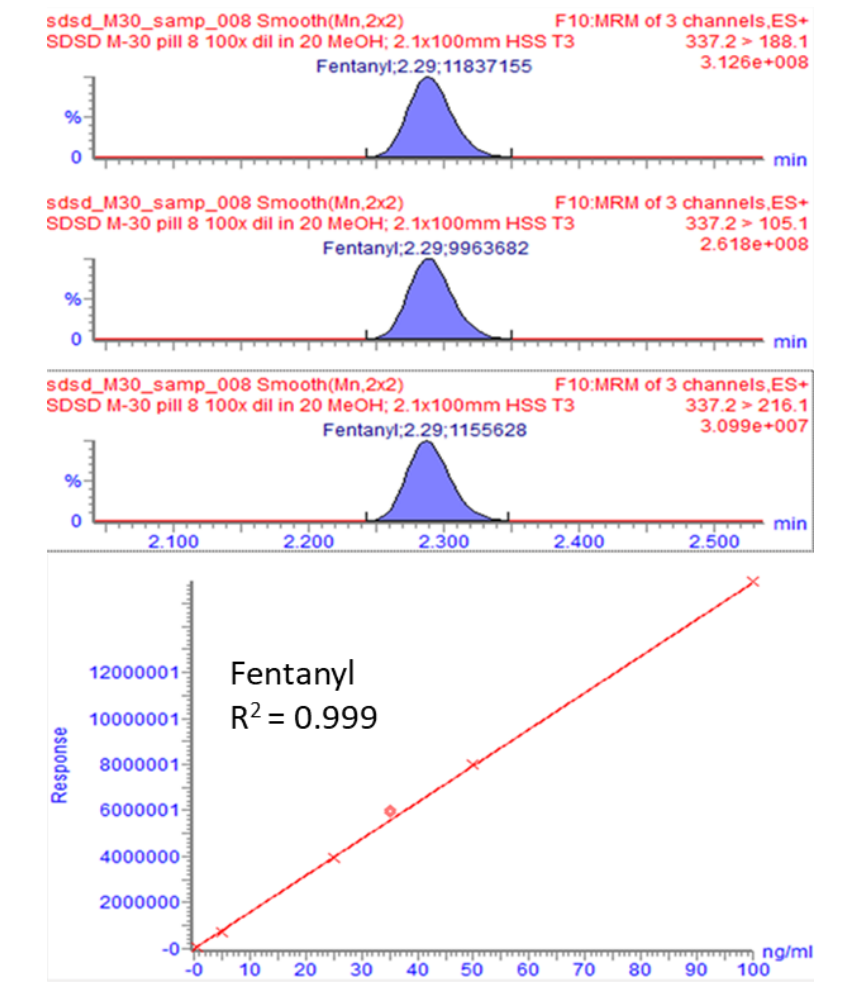


Figure 7. Fentanyl quantifier and qualifier chromatograms from the confirmatory analysis for the counterfeit M-30 pill analysis, and the calibration curve prepared for the semi-quantitative analysis (0.5 ng/mL - 100 ng/mL).

CONCLUSION

- An example of a screening and confirmation workflow has been demonstrated for the analysis of a counterfeit M-30 pill that was found to be adulterated with xylazine.
- LC-ToF analysis using the ACQUITY RDa Detector provides a quick and efficient method to perform a comprehensive screen on seized drug samples.
- In this example, a complex mixture of fourteen compounds were presumptively identified, in a pill that should contain one active ingredient.
- The combination of accurate mass, a customizable and expandable library, and full scan data acquisition with fragmentation provides a high confidence result for seized drug screening.
- Confirmation testing using Xevo TQ Absolute Mass Spectrometer provided retention time and MRM semi-quantitative data for 11 of the compounds identified.
- For the counterfeit M-30 pill analyzed, xylazine was detected and quantified at a concentration of 913 ng/mL.

References

- <https://www.dea.gov/sites/default/files/2022-12/The%20Growing%20Threat%20of%20Xylazine%20and%20its%20Mixture%20with%20Illicit%20Drugs.pdf> Accessed 31 May 2023
- <https://www.dea.gov/onepl1>