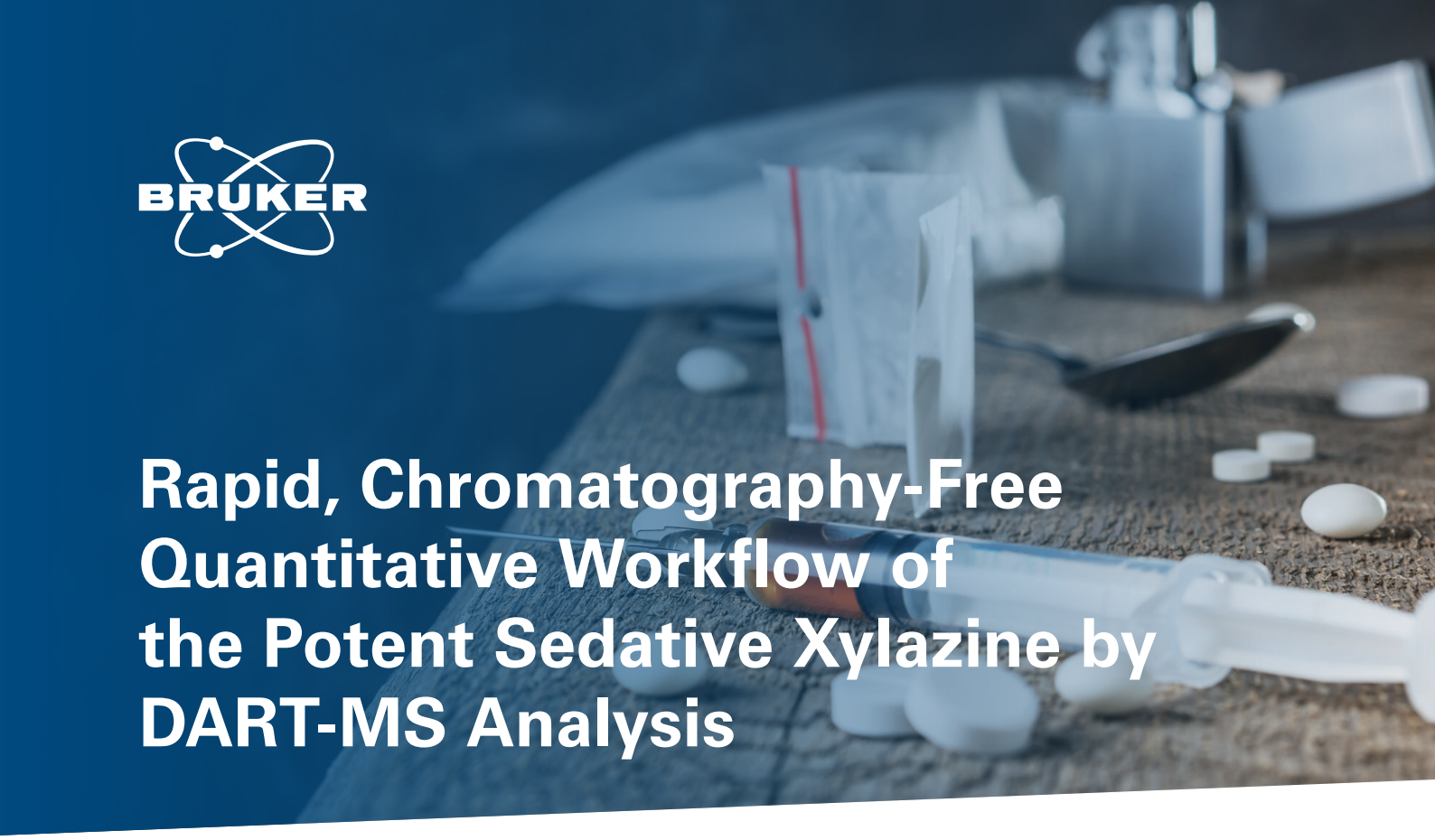

Rapid, Chromatography-Free Quantitative Workflow of the Potent Sedative Xylazine by DART-MS Analysis

The increasing threat of common illicit drugs laced with xylazine requires a quick and easy workflow for the analysis of this potent adulterant and public health threat.

Abstract

Today, the numbers of opioid overdoses continue to be significant and the detection of xylazine as an adulterant in illicit drugs is increasing each year. Thus, interest to be able to measure this drug rapidly and effectively in mixed lab samples is escalating. Current methods to quantitate the potent tranquilizer xylazine include LC-MS/MS and GC-MS/MS which are effective techniques but are time consuming and also require significant sample preparation that can be costly. An alternative technique utilizing a chromatography-free workflow by Direct Analysis in Real Time ionization coupled with mass spectrometry (DART-MS) offers simple, quick identification of xylazine. For this study, a mixture containing xylazine, fentanyl, and chlorpromazine spiked into drug-free urine was used to demonstrate the effectiveness of DART-MS as a powerful and successful tool for measuring the drug xylazine as part of the measures needed to combat this emerging public health threat.

Introduction

Xylazine ($C_{12}H_{16}N_2S$) is a synthetic compound that has escalated concern within United States health and law enforcement agencies due to its increased use as an adulterant in the illicit street drug fentanyl. Its contribution to rising drug overdoses, as it cannot be reversed using naloxone [Narcan] because it is not an opioid[1], is also worrisome. Xylazine was first synthesized by Bayer Pharmaceuticals and investigated as an antihypertensive drug. Clinically it was found to decrease heart rate and blood pressure to dangerously low levels and therefore has not been approved for human use.[2,3] Consuming drugs tainted with xylazine can also lead to severe wounds which could develop into necrosis and lead to amputations.

Keywords:
Chromatography-free; DART; TQ; forensics

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Table 1
EVOQ Elite mass spectrometer method transitions, and collision energies

Compound	Transition (m/z)	Collision Energy (eV)
Xylazine	221 > 164	23
Fentanyl	337.5 > 188	17.9
Fentanyl-d5	342.5 > 105	44
Chlorpromazine	319 > 86	13.4
Chlorpromazine-d3	322 > 89	15

Results

Analytes were quantified in both aqueous solution (Figures 1–3) and in urine extracts (Figures 4–6). A summary of the linearity for each sample is shown in the following table.

The high level of linearity (Table 2) and demonstrated levels of detection can be observed in both aqueous and urine-based samples.

Table 2
Table 2. Analyte calibration curve linearity (R^2)

Compound	Matrix	R^2 Correlation Scores
Fentanyl	Aqueous	0.99277
Xylazine	Aqueous	0.94416
Chlorpromazine	Aqueous	0.99351
Fentanyl	Urine	0.99595
Xylazine	Urine	0.98042
Chlorpromazine	Urine	0.99282

Analysis in aqueous solution

Figure 1.
Fentanyl: 6 point calibration curve in aqueous solvent

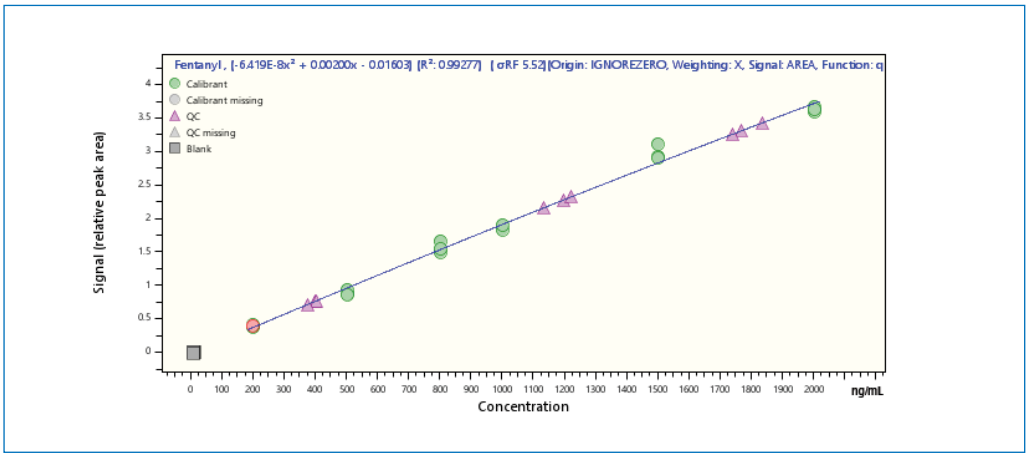


Figure 2.
Xylazine: 6 point calibration curve in aqueous solvent

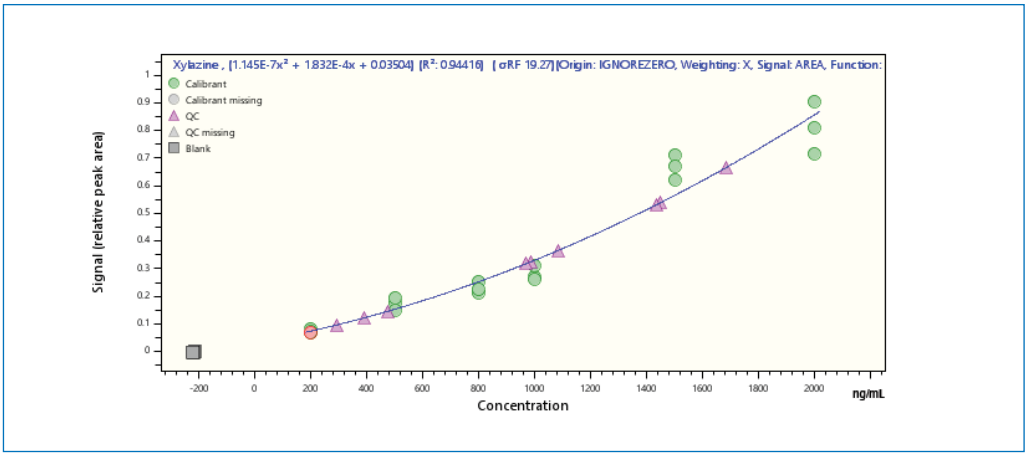
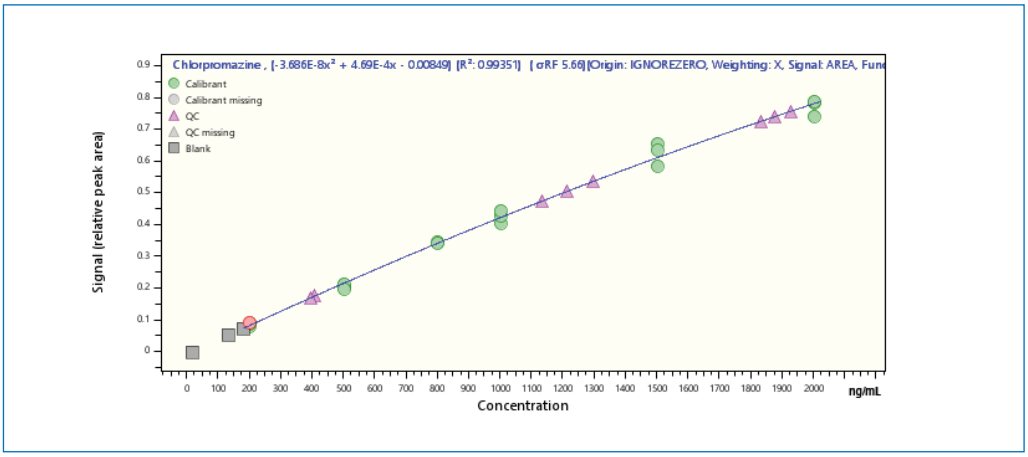


Figure 3.
Chlorpromazine: 6 point calibration curve in aqueous solvent



Analysis in urine extracts

Figure 3.
Fentanyl: 6 point calibration curve in urine

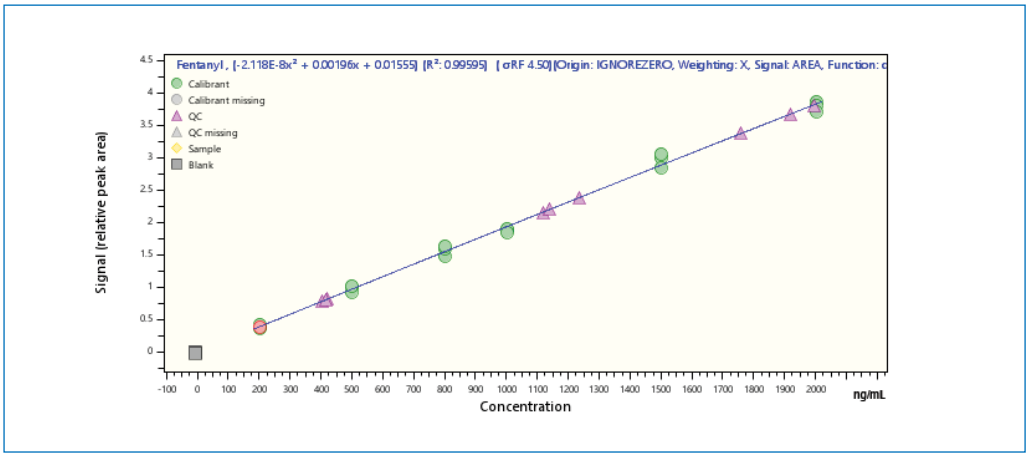


Figure 5.
Xylazine: 6 point calibration curve in urine

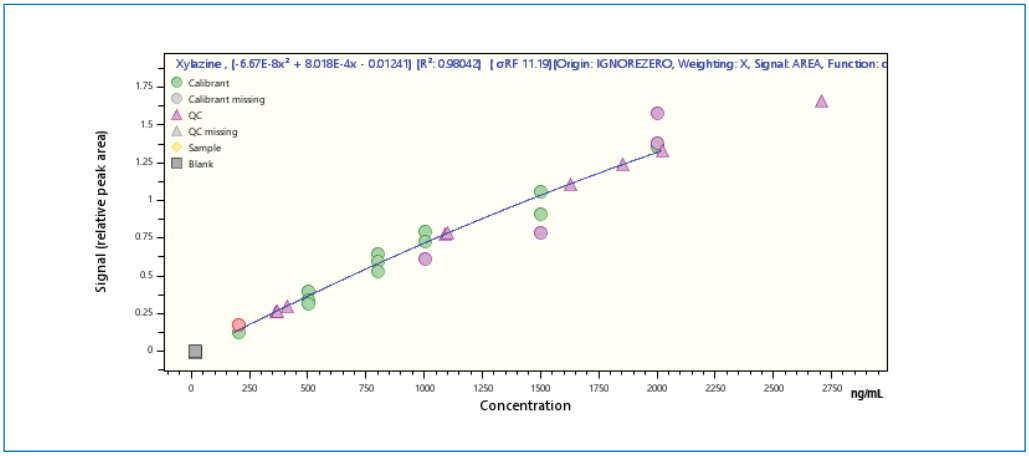
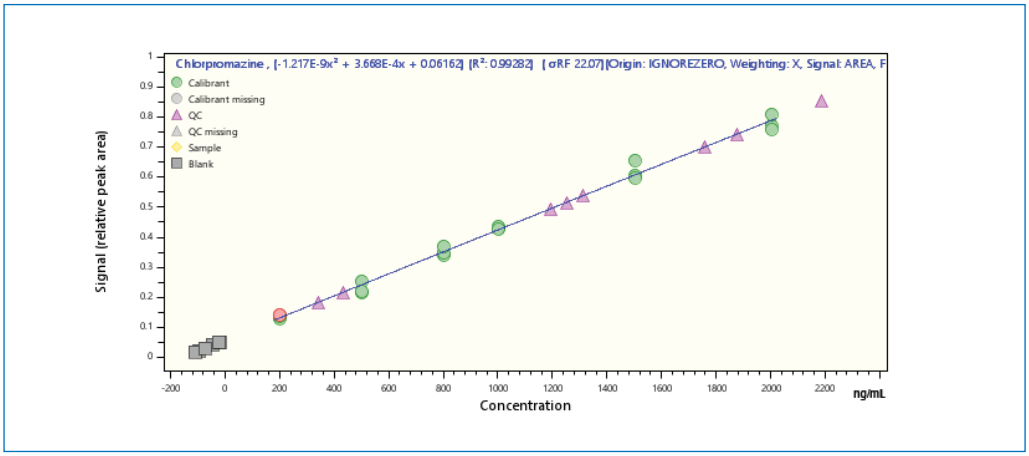


Figure 6.
Chlorpromazine: 6 point calibration curve in urine



In April of 2023, the current US government administration designated the combination of fentanyl with xylazine as an emergent public health threat, the first such designation issued of its kind.[4]

In addition to being detected in seized fentanyl samples, xylazine is also found as an adulterant in other illicit drugs such as heroin and cocaine[5], and it has raised concern as a potential residue in animal-derived food products[6]. Because of its increased detection in these cases, the need to measure this compound quickly and accurately is obvious.

In an effort to avoid using time-consuming LC-MS/MS analysis of urine samples containing fentanyl and xylazine, a chromatography-free DART-MS workflow was employed to demonstrate confident, reliable identification of xylazine and other drugs of concern.

Methods

A mixture of xylazine, fentanyl, and chlorpromazine was prepared in an aqueous solution. The mixture was analyzed in both neat matrix and in drug-free urine. A liquid-liquid extraction was performed utilizing the PinPoint Testing ToxBox® custom drug panel protocol, with the samples in urine and aqueous phase. A range of 200 – 2000 ng/mL was used for analysis. All analytes and their corresponding deuterium-labeled equivalents were purchased from Millipore-Sigma

DART-MS analysis was performed using an EVOQ® Elite (Bruker Daltonics) triple

quadrupole mass spectrometer fitted with a DART ionization source (Bruker Daltonics). Samples were vaporized using pulse sampling a duration of 4 s at 400°C in positive ion mode. Each run was 0.32 min in duration with a 0.5 min equilibration time followed by a 25 ms scan time per transition. The cone temperature was set to 250°C with a pressure of 20 psi, and CID gas was set at 1.5 mtorr. Table 1 below contains the MRM transitions and collision energies for each analyte.

Conclusions

The results of this chromatography-free workflow using DART-MS show that this new technique is successful and proficient as a rapid and accurate screening method for the detection of the illicit drug xylazine in mixed drug samples. The linear responses of the drugs with respect to the QC samples and across a large detection range ensure that this chromatography-free workflow will be able to detect samples at referenced levels. DART-MS provides a novel way to analyze drugs of concern in a simple, reliable, and reproducible way. The ability to conduct rapid analysis of such drugs allows for quicker and more confident treatment decisions to overdose victims, who may not fully respond to current overdose treatments.

References

1 *DEA Reports Widespread Threat of Fentanyl Mixed with Xylazine*. https://www.dea.gov/alert/dea-reports-widespread-threat-fentanyl-mixed-xylazine

2 *US Department of Justice Drug Enforcement Administration*, **2022**. The Growing Threat of Xylazine and its Mixture with Illicit Drugs https://www.dea.gov/sites/default/files/2022-12/The%20Growing%20Threat%20of%20Xylazine%20and%20its%20Mixture%20with%20Illicit%20Drugs.

3 *EMEA Report*. **1999**. Committee for veterinary medicinal products xylazine hydrochloride, summary report (1). The European Agency for the Evaluation of Medicinal Products, EMEA/MRL/611/99-Final-corrigendum.

4 *White House.gov*, **2023**. Biden-Harris Administration Designates Fentanyl Combined with Xylazine as an Emerging Threat to the United States. https://www.whitehouse.gov/ondcp/briefing-room/2023/04/12/biden-harris-administration-designates-fentanyl-combined-with-xylazine-as-an-emerging-threat-to-the-united-states/

5 Reyes, JC., Negron, JL., Colon, HM., Padilla, AM., Millan, MY., Matos, TD., Robles. The Emerging of Xylazine as a New Drug of Abuse and its Health Consequences among Drug Users in Puerto Rico. *J Urban Health* **2012**; 89(3): 519-526.

6 Zheng, X., Mi, X., Li, S., Chen, G. Determination of Xylazine and 2,6-Xylidine in Animal Tissues by Liquid Chromatography – Tandem Mass Spectrometry. *J. Food Sci* **2013**;78(6): T955-9

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