SOFT 2022
Abstract Submission Form

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*****Do not exceed 600 words including tables and charts.*****

TITLE: *Quantitative Analysis of Anticoagulant in Human Blood by UPLC coupling with Triple Quadrupole Mass Spectrometry*

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ABSTRACT: *Structure the abstract using the following headers.*

Background/Introduction:
Anticoagulant agents within the warfarin and superwarfarin class are commonly used in commercial rodenticides. Currently, these drugs are unregulated and commercially available for purchase. As such, these drugs have been implicated in forensic casework including suicides, homicides, and accidental poisonings. Toxic clinical effects of anticoagulant exposure include spontaneous internal and external bleeding, which can result in death. Recently, anticoagulant drugs have emerged as toxic adulterants within the illicit drug supply. Two major anticoagulant outbreaks, Chicago (2018) and Tampa (2021), have been reported, where synthetic cannabinoids were laced with anticoagulant drugs, which led to five deaths and numerous hospitalizations. The detection and quantification of these compounds in forensic casework is challenging due to the small amount of drug in samples and the analytical properties of the drugs, which routine toxicology testing might not detect.

Objectives:
The objective of this project was to develop and validate a quantitative, targeted liquid-liquid extraction to isolate ten anticoagulants from human blood with subsequent analysis by liquid chromatography coupled with triple quadrupole mass spectrometry.

Methods:
Analysis was performed using a Water ACQUITY UPLC coupled with a Waters Xevo TQ-S micro mass spectrometer. A reverse phase gradient using 0.02% ammonium hydroxide in water (MPA) and 0.02% ammonium hydroxide in methanol (MPB) on ACQUITY UPLC BEH C18 analytical column was used for chromatographic separation with a total run time of four minutes. The mass spectrometer operated in

negative electrospray ionization mode for ten anticoagulant compounds: brodifacoum, bromadiolone, chlorophacinone, coumatetralyl, difenacoum, difethialone, diphacinone, flocoumafen, pindone, and warfarin. The analytes and internal standards were monitored and analyzed in multiple reaction monitoring (MRM), with cone voltages and collision energies varied among analytes.

Results:

This method was validated in accordance with standards set by Academy Standards Broads in Forensic Toxicology (ANSI/ASB Standard 036). Parameters of method validation included calibration, bias/precision, limit of quantification (LOQ), limit of detection (LOD), carryover, interferences, and ionization suppression/enhancement. Additional parameters evaluated included recovery, auto-sampler stability, and matrix matching. The calibration model was constructed with seven-point calibrators ranging 5 to 250 ng/mL with quality control set at three different concentrations: 15, 100 and 200 ng/mL. The LOD was administratively set at 1 ng/mL. All parameters of validation were met.

Following validation, authentic deidentified samples (n=79) from the recent outbreak in Florida were provided by NMS Labs for research purposes. In 74 samples, brodifacoum was detected and quantitated across three different matrices (blood, serum, and plasma). In six serum samples, difenacoum was detected with three of those samples being quantitated.

Drug	Matrix	Mean concentration (ng/mL)	Median (ng/mL)	Range (ng/mL)
Brodifacoum	Blood(n=13) Serum(n=45) Plasma(n=16)	159.5(±131) 575.9(±432) 134.3(±133)	110 476.3 53.2	48.8-429.8 86.2-1995 11.5-365.3
Difenacoum	Serum(n=6)	7.4(±2.2)*	7.4*	5.3-9.6*

*Quantified in three samples only.

Conclusion/Discussion:

The developed liquid-liquid extraction was highly efficient for the extraction of anticoagulant drugs, which are highly lipophilic. The analytical method proved to be very sensitive, selective, and suitable for the detection and quantitation of ten anticoagulant drugs in human blood or other matrices. With respect to authentic cases, brodifacoum was identified in several cases in three different matrices. Median concentrations were greatest in serum, almost five-fold higher than in blood, however, paired samples were not samples. These results also demonstrate the utility of serum as a specimen screening and confirmation. Additionally, some serum samples were also found difenacoum, which was detected at much lower concentrations relative to brodifacoum. Because of this, laboratories should consider including additional anticoagulant drugs into their panels. As the number of incidents involving anticoagulant drugs adulterating seized material increases, laboratories should be aware of the challenges associated with the detection of anticoagulant drugs and consider incorporating these drugs into their panels.