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## Introduction

Leaf (Barnyard Grass) samples exposed to known amounts of VX were evaluated for both free and bound concentrations. Free VX methodology extracts the compound in isopropanol, and is analyzed via Liquid Chromatography-Tandem Mass Spectroscopy. Bound VX can be determined by generating the nerve agent as G-analogue analyte in the presence of excess fluoride, analyzed via Gas Chromatography-Tandem Mass Spectroscopy.

## Leaf Extraction Preparation

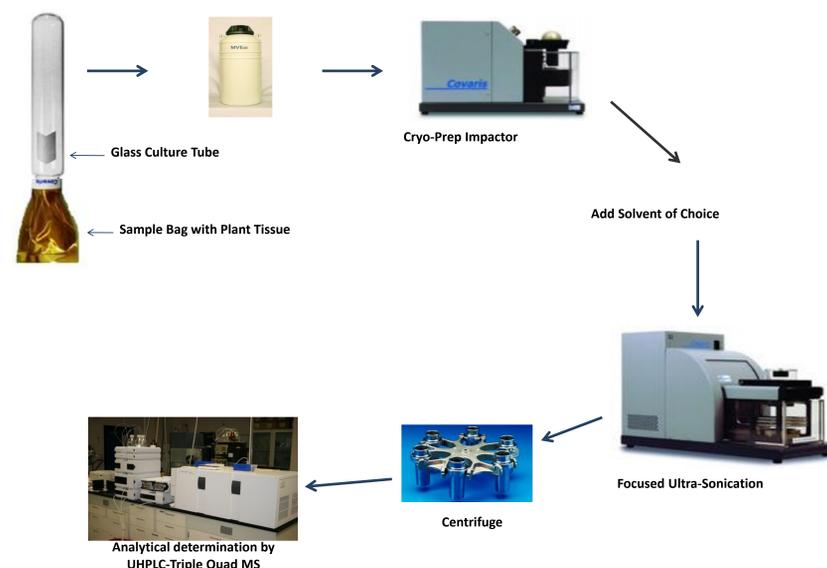
Exposed leaf samples were harvested, placed into Cryo-bags, then flash frozen in a dewar of liquid nitrogen. Samples were then cryogenically pulverized and weighed, before adding 10mL Isopropanol Alcohol(IPA). The solution was then vortexed, acoustically homogenized, and centrifuged. The supernatant was then removed and set aside for free VX analysis. The resulting leaf pellet was washed two times, by adding 10mL IPA, vortex and centrifuge as above, removing supernatant and setting each wash aside for free VX analysis.

## Equipment

S2 Focused Acoustic Ultrasonicator, Covaris Inc.  
 CryoPrep Pulverizer, Covaris Inc.  
 Vortex Genie2, Scientific Industries  
 Centrifuge 5804R, Eppendorf  
 Sample Concentrator, Techne Inc.  
 7000A GC/QQQ, Agilent Technologies  
 RTx-1701 (30m x 0.25mm x 25uM) column, Restek  
 Oasis HLB (3cc, 60mg) SPE Cartridge, Waters

## Fluoride Ion Regeneration Method

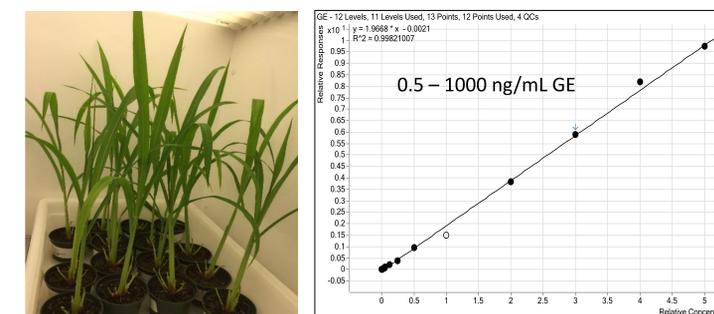
To the remaining leaf pellet, add Acetate Buffer pH 4.0 and 6M potassium fluoride. Vortex, acoustically homogenize, and centrifuge, before removing supernatant. Repeat procedure, and combine supernatants (2500uL total). Vortex supernatant before adding a 25uL aliquot to a conditioned HLB Solid Phase Extraction (SPE) cartridge. Spike 1uL Internal Standard directly onto cartridge bed, drain, rinse, and elute with 1mL ethyl acetate. Remove the moisture from the ethyl acetate with sodium sulfate, then concentrate sample to 50uL.



## Conclusions

This method was developed to support the Agent Plant Interaction Study. The fluoride regeneration method developed to detect the presence of chemical agents in biological matrices, was modified to facilitate the high concentrations bound in the leaf samples.

## Results and Discussion



*Echinocloacrus-galli*  
(Barnyard Grass)

Figure 1. Calibration curve

QC Type	Replicates	Spike Level	AVG Result (ng/mL)	Recovery %
Method Blank	4	0	<0.5	*
Solvent Spike	2	100	82.3750	82
Method Spike	2	100	104.9124	105
Matrix Spike	1	100	79.8679	80

## References

C.E. Byers, J.M. McGuire, S.W. Hulet, D.C. Burnett, B.I. Gaviola, E.M. Jakubowski, S.A. Thomson. Gas Chromatography-Tandem Mass Spectroscopy Analysis of Red Blood Cells from Minipig following Whole-Body Vapor Exposure to VX. *J. Anal. Toxicol.* **32**: 57-63 (2008)

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