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QUANTIFICATION OF VX NERVE AGENT IN VARIOUS FOOD MATRICES BY SOLID-PHASE EXTRACTION ULTRA-PERFORMANCE LIQUID CHROMATOGRAPHY–TIME-OF-FLIGHT MASS SPECTROMETRY

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14. ABSTRACT:
Liquid chromatography–electrospray ionization mass spectrometry with positive-ion modes of operation were used for the trace-level determination of *O*-ethyl *S*-[2-(diisopropylamino)ethyl] methylphosphonothioate (VX) in various food matrices. The mixed-mode cation exchange (MCX) sorbent and Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS) methods were used for extraction of VX from food samples. The extraction efficiencies obtained with use of the MCX cartridge and QuEChERS methods were compared, and the results revealed that both methods performed similarly. Various VX concentrations, ranging from 5 to 500 ng/mL, were spiked into food samples. The linear range of quantitation for VX was 1 to 330 ng/mL. The total percent recoveries (and percent relative standard deviations) for VX in various food samples are reported.

15. SUBJECT TERMS
Foodstuff
Mixed-mode cation exchange (MCX)
O-ethyl *S*-[2-(diisopropylamino)ethyl] methylphosphonothioate (VX)
Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS)
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PREFACE

The work was started in October 2014 and completed in June 2015.

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CONTENTS

FIGURES

TABLES

QUANTIFICATION OF VX NERVE AGENT IN VARIOUS FOOD MATRICES BY SOLID-PHASE EXTRACTION ULTRA-PERFORMANCE LIQUID CHROMATOGRAPHY–TIME-OF-FLIGHT MASS SPECTROMETRY

1. INTRODUCTION

As recent events in Syria have demonstrated, the continued threat from traditional chemical warfare agents (CWAs) such as (*O*-ethyl *S*-[2-(diisopropylamino)ethyl] methylphosphonothioate (VX; Figure 1) is evident on an almost-daily basis. Issues ranging from food and environmental safety to treaty compliance reinforce the need for low-level VX detection and emphasize its importance. The mere existence of these molecules in either the environment or the food supply could indicate a compliance breach, even if the actual CWA levels were not high enough to cause personal harm.

Although the detection of VX metabolites and adducts from food and biological sample matrices have been reported,[1–9] limited literature exists regarding the direct detection of actual CWAs in food.[10,11] The pesticide literature often includes sample-preparation techniques that are commercially available and affordable, such as solid-phase extraction (SPE) cartridges[12–20] or QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) systems.[21–30] However, the CWA literature seems to focus more on new techniques and specialized equipment that may not be readily accessible to every laboratory.[9,30–35]

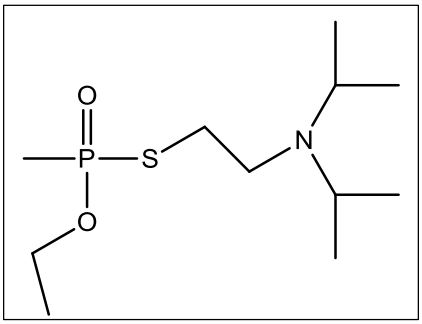

Figure 1. Structure of nerve agent VX.

This document reports the efforts of the Agent Chemistry Branch from the Research and Technology Directorate of the U.S. Army Edgewood Chemical Biological Center (ECBC; Aberdeen Proving Ground, MD) in developing new extraction and analytical detection methodologies using liquid chromatography–mass spectrometry (LC–MS). The objective of this task was to provide development and laboratory support for extraction of V-type agents from various food samples. This includes detection and quantitative and qualitative analysis of complex matrices such as foods with high salt and fat contents. In support of this objective, we have examined five food samples. Apple juice, whole milk, whole egg, tomato sauce (with meat), and hot dogs represented food types commonly associated with school lunch programs. The choice of food types arose from collaborations and conversations with U. S. Department of Agriculture personnel. Foods were tested using commercially available LC columns.

The use of ultra-performance liquid chromatography–time-of-flight mass spectroscopy (UPLC–TOF-MS) or comparable high-resolution LC–MS systems has become more common. From an affordability standpoint, these systems are currently within reach for most laboratories. For this work, extracted agent was analyzed using UPLC–TOF-MS, and percent recovery was calculated from an external calibration curve.

2. EXPERIMENTAL METHODS

2.1 Reagents and Chemicals

The nerve agent VX (>99% purity) was provided by ECBC. All reagents and solvents were LC–MS grade. Acetonitrile, methanol, water, acetic acid, formic acid, and hydrochloric acid (37.0%) were purchased from Sigma-Aldrich (St. Louis, MO). Ammonium hydroxide (28–30%) was obtained from Mallinckrodt (St. Louis, MO). Oasis MCX (mixed-mode cation exchange) sorbent cartridges (60 mg/3 cc) were used with DisQuE dispersive sample preparation AOAC (Association of Analytical Communities) acetate tubes that contained 6 g of magnesium sulfate and 1.5 g of sodium acetate as well as DisQuE cleanup tubes that contained 300 mg of magnesium sulfate and 100 mg of primary secondary amines (PSAs); all were purchased from Waters Corporation (Milford, MA). Apple juice, whole milk, whole egg, tomato sauce, and hot dog food samples were purchased from a local grocery store (Food Lion; Edgewood, MD).

2.2 Instrumentation

All samples were characterized using a Waters Acquity UPLC Synapt G2-S system equipped with an electrospray ionization (ESI) interface. The sampling cone voltage was 20 V. The source and desolvation temperatures were 120 and 500 °C, respectively. The nitrogen desolvation gas flow rate was 800 L/h. The LC–ESI–TOF-multiple reaction monitoring (MRM) and LC–ESI–TOF-MS data were acquired in positive-ion scan mode over a mass range of 50–1200 Da. A leucine–enkephalin solution (1 ng/µL) was used as reference mass with a flow rate of 10 µL/min. The LC separations for all extracted samples were performed on a Waters Acquity UPLC HSS T3 column (100 × 2.1 mm, 1.8 µm). The mobile phase consisted of 0.1% trifluoroacetic acid in water (mobile phase A) and 0.1% trifluoroacetic acid in acetonitrile (mobile phase B) with a 10 µL sample volume. Separation was achieved using an isocratic condition of 20/80 (v/v %) A/B with a flow rate of 0.4 mL/min. The column temperature was maintained at 35 °C using a thermostatted column manager compartment, and the test samples were maintained at 5 °C using a thermostatted sample manager compartment.

2.3 Procedure for VX Extraction from Foodstuffs Using Oasis MCX Cartridges

The extraction procedures were performed in accordance with Waters' generic method for using Oasis MCX cartridges. First, 2 mL of apple juice was spiked with 5 µL of 2 µg/mL VX in acetonitrile standard solution. The pH of the apple juice was 3.5, so no HCl was added to acidify the sample. The 2 mL of apple juice was loaded onto the preconditioned Oasis MCX cartridge, and the cartridge was washed sequentially with 1 mL of 2% formic acid in water and 1 mL of methanol. The cartridge was eluted with 2 mL of 5% ammonium hydroxide in methanol. A 1 mL aliquot of the extract was filtered through a 0.45 mm polytetrafluoroethylene (PTFE) membrane filter, transferred to an autosampler vial, and analyzed by LC–MS/MS. The 2 mL of whole milk was placed in a 50 mL centrifuge tube. The test sample was spiked with 40 µL of 2 µg/mL VX in acetonitrile standard solution, and 20 µL of 37% HCl was added to acidify the milk sample. Ten milliliters of 50/50 v/v % acetonitrile/water was added to the milk sample tube. The sample tube was capped, shaken vigorously by hand for 1 min, and centrifuged at 10,000 rpm for 2 min. The top acetonitrile layer was transferred to a preconditioned Oasis MCX cartridge. Sample analysis was performed in a manner identical to that used for apple juice. Extraction of VX using the generic Oasis MCX method is illustrated in Figure 2.

Extraction and analysis of whole egg, tomato sauce, or homogenized hot dog were performed in a manner identical to that used for apple juice. Ten 3 g samples of apple juice, whole milk, whole egg, tomato sauce, or hot dog were weighed for each matrix. The percent recoveries for VX with the relative standard deviations (RSDs) were obtained by averaging values from 10 analysis runs.

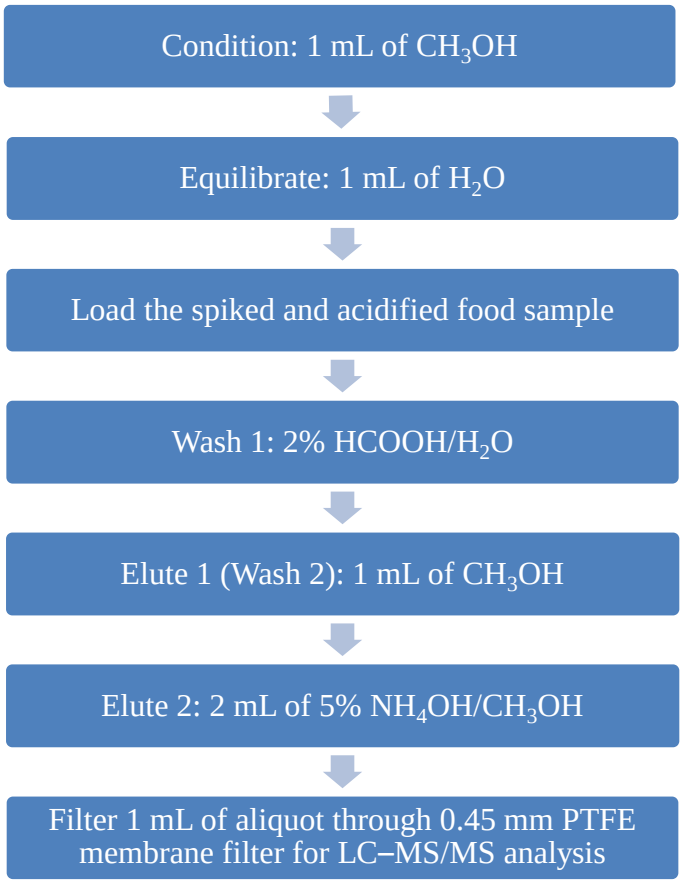

Figure 2. Generic Oasis MCX method.

2.4 Procedure for VX Extraction from Apple Juice and Whole Milk Using the QuEChERS System

In accordance with AOAC Method 2007.01,[36] a 10 g (±0.1 g) sample of apple juice was placed in a 50 mL centrifuge tube. The test sample was spiked with 7 µL of 5 µg/mL VX in acetonitrile standard solution. Next, 10 mL of 1% acetic acid in acetonitrile and a QuEChERS extraction salt packet (6 g of magnesium sulfate and 1.5 g of sodium acetate) from the kit were added to the apple juice. The sample tube was capped and vigorously hand-shaken for 1 min to ensure that the solvent interacted with the entire sample and the crystalline agglomerates were dispersed. The sample tube was centrifuged at 1500 rcf for 1 min. Two milliliters of the top acetonitrile layer was transferred to a Waters QuEChERS dispersive SPE 2 mL tube containing 300 mg of magnesium sulfate and 100 mg of PSA. The tube was tightly capped, vortexed for 30 s, and centrifuged at 1500 rcf for 1 min. A 1 mL aliquot of the extract was filtered through 0.45 µm PTFE membrane filter before it was transferred to an autosampler vial and analyzed by LC–MS/MS. Extraction and analysis of whole-milk samples were performed using procedures identical to those used for apple juice. The sample preparation protocol of the AOAC QuEChERS method is shown in Figure 3. Ten samples of apple juice or whole milk were weighed for each matrix. The percent recoveries for VX (with RSDs) were obtained by averaging values from 10 analysis runs.

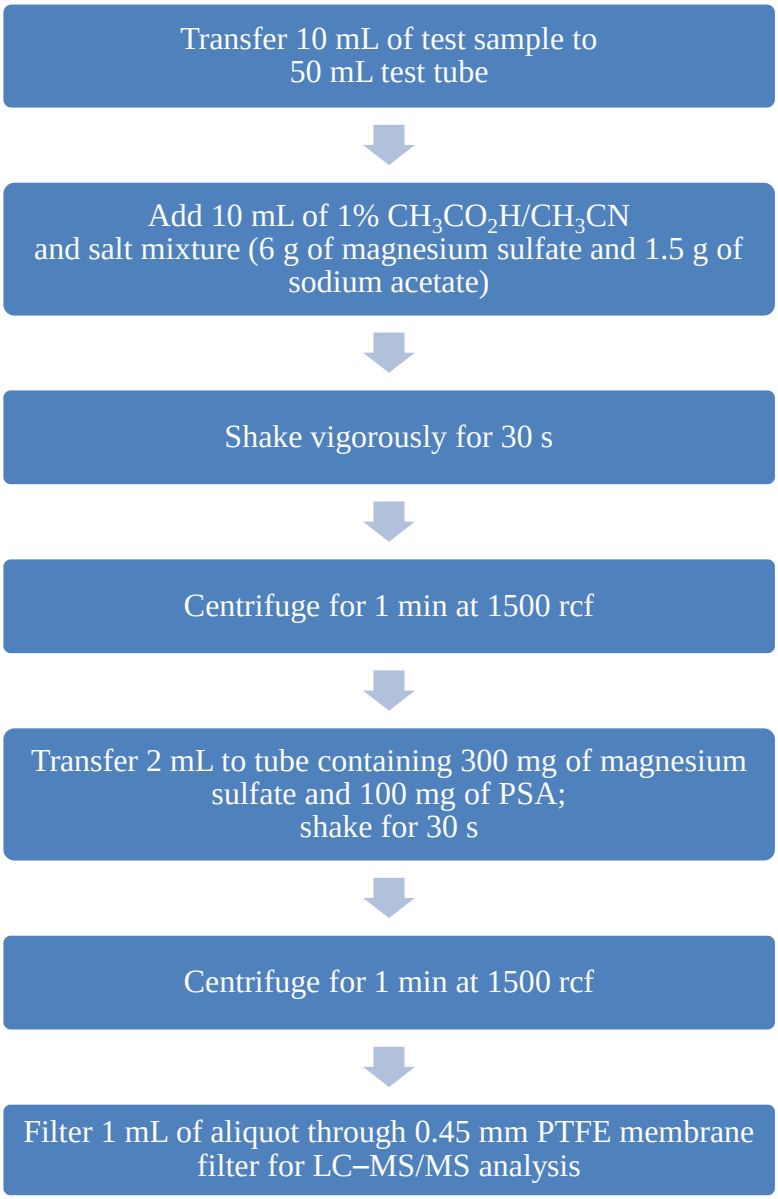

Figure 3. Sample preparation protocol for the AOAC QuEChERS method.

3. RESULTS AND DISCUSSION

3.1 LC Separation and Analytical Figures of Merit

For LC–MS analysis, the MS system was operated in two modes: TOF-MS at a mass-to-charge ratio (*m/z)* of 50–1200 and TOF-MRM at *m/z* 268.1505 → 128.1437 for VX. The TOF-MS mode was used to ensure that we could identify any hydrolysis products. No VX hydrolysis products were found in these extracted samples. TOF-MRM was used to determine the limits of detection (LODs), limits of quantitation (LOQs), and the linear dynamic ranges (LDRs) for VX. The results obtained from TOF-MRM at *m/z* 268.1505 → 128.1437 for VX were used to generate the calibration curves for the LDRs. The VX calibration curve was plotted

over a concentration range of 1–330 ng/mL, with 10 µL injections at each concentration level. The LODs for the nerve agents were calculated using 10 µL injections at concentrations as low as 1 ng/mL, with a signal-to-noise ratio of 3:1. The LOQs for the analyte were also calculated, with a signal-to-noise ratio of 10:1. The linear regression equations were calculated by least-squares analysis using the LDR, LOD, LOQ, equation, and correlation coefficient listed in Table 1.

Table 1. Analytical Figures of Merit for VX

Nerve Agent	LDR (ng/mL)	LOD (ng, on column)	LOQ (ng, on column)	Linear Regression Equation (n = 10)	Correlation Coefficient[a]
VX in 0.5% ammonium hydroxide–methanol	1–330	0.005	0.03	$y = 49.733x - 7.9465$	0.9993

[a]Calculated over the calibration range of 1–12 ng/mL for VX.

3.2 Extraction of VX from Foodstuffs

In this study, we examined the efficiency of VX extraction from five different matrices of food samples. Apple juice, whole milk, whole egg, tomato sauce, and hot dog samples were tested. The extraction solvent was used as designated in the generic Oasis MCX method from Waters as well as AOAC Method 2007.01. No solvent optimization was performed for VX extraction from foodstuffs. The sulfonic acid MCX cartridges enabled greater cleanup selectivity and sensitivity for basic compounds such as VX. When using this SPE cartridge, it is necessary to adjust the pH to maximize analyte retention on the SPE sorbent. Both acetic acid and hydrochloric acid were used in the food samples to adjust the pH to 3.5. The percent recoveries of VX may have been influenced by matrix effects; the extraction efficiency of VX was measured from complex matrices such as high-salt and high-fat content foodstuffs. Apple juice was the only food sample that did not require pH adjustment before it was loaded onto the MCX cartridge.

Whole milk, whole egg, and tomato sauce samples were mixed with acetonitrile solvent to separate the fat layer prior to loading onto the MCX cartridge. VX is soluble in polar and nonpolar solvents, and it might have been buried under the fat layer that we did not analyze. This might explain why the percent recoveries of VX in whole milk, whole egg, and tomato sauce were lower than those of apple juice. The hot dog sample was first spiked with VX and then homogenized with acetonitrile. Once the sample was centrifuged, the eluent was collected for the MCX cartridge. The hot dog sample was the most complex matrix among the tested food samples, and it did exhibit a matrix effect. The LC chromatogram peak was broader, which can generate a higher uncertainty for quantitation.

Representative TOF-MRM chromatograms for VX extracted from various food matrices using the Oasis MCX cartridge and the QuEChERS method are shown in Figures 4 and 5, respectively. The percent recoveries were calculated based on a VX external calibration curve (Figure 6). The results showed that VX recovery from apple juice was >70%, but from the other

matrices, it was <50% (Table 2). As shown in Table 2, the percent recovery of VX from hot dog was three times lower than that from apple juice. A better cleanup process would result in higher percent recoveries of VX from complex matrices.

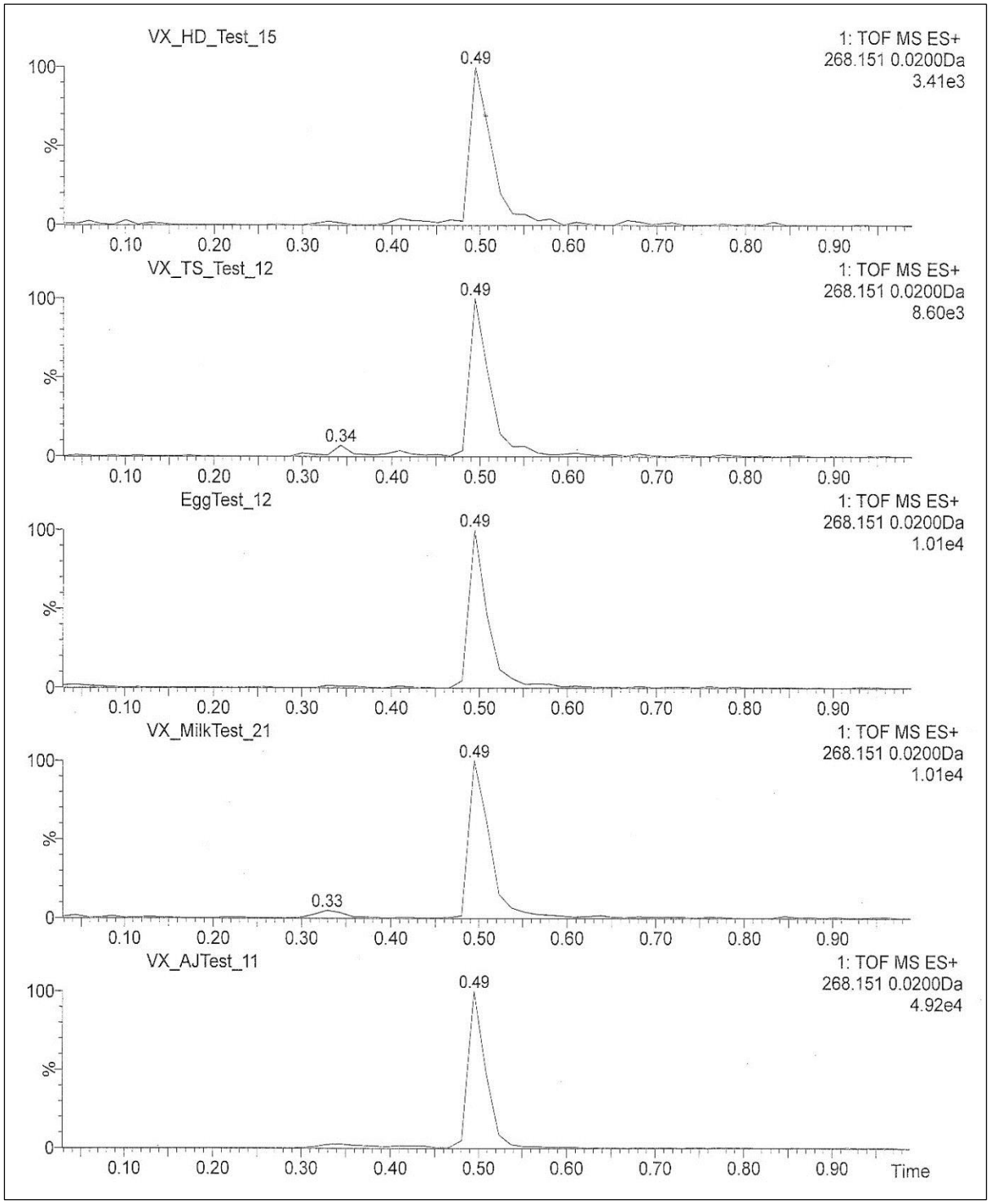

Figure 4. A representative TOF-MRM chromatogram for VX extracted from various food matrices using an Oasis MCX cartridge.

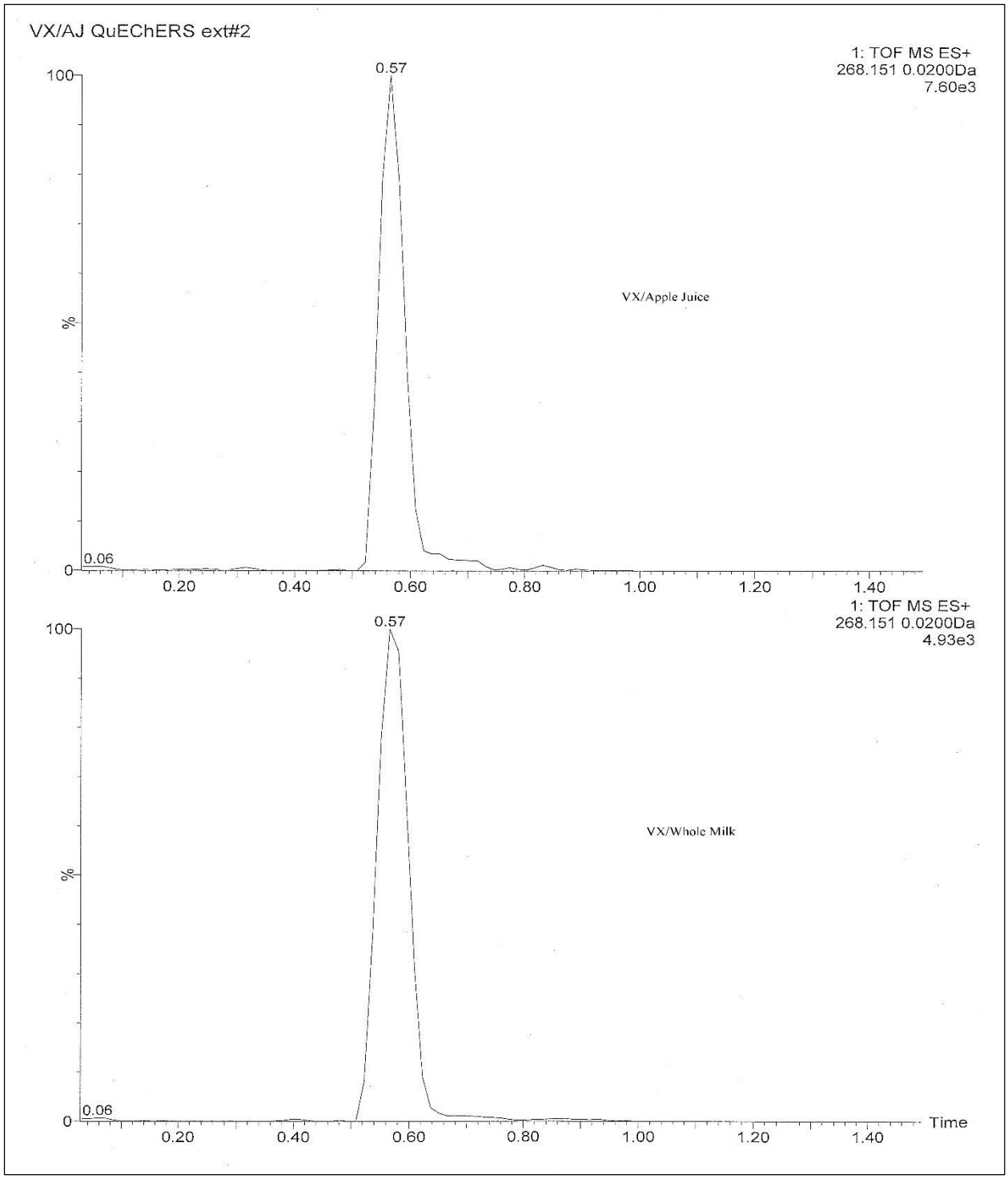

Figure 5. A representative TOF-MRM chromatogram for VX extracted from various food matrices using the QuEChERS method.

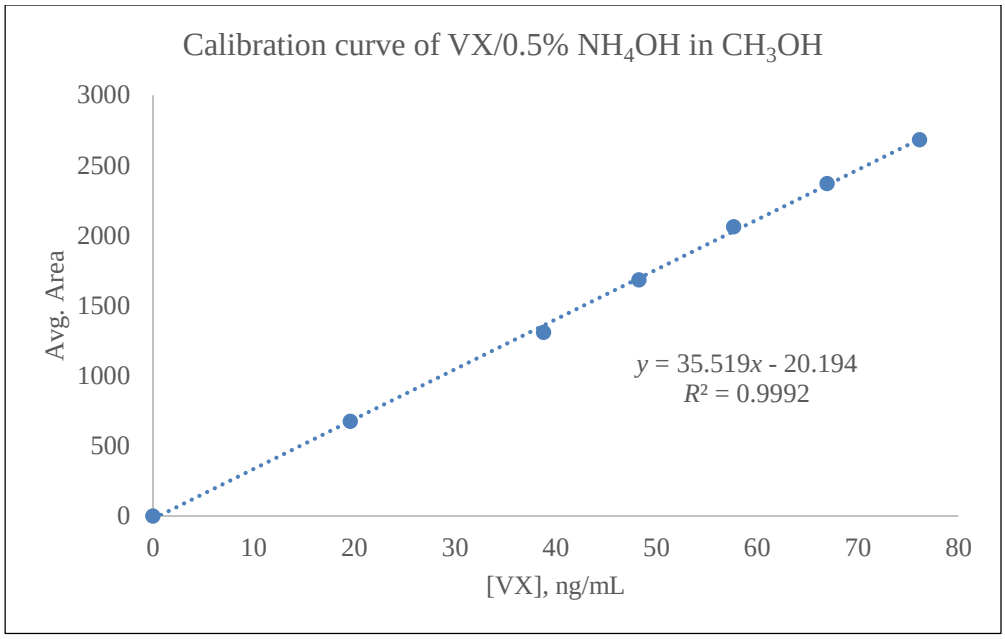

Figure 6. External calibration curve for VX in ammonium hydroxide and methanol.

Table 2. Results of VX Extraction from Various Food Matrices[a]

Food Sample	Recovery ± RSD (%)	
	Oasis MCX Cartridge Sorbent	QuEChERS Method
Apple juice	76 ± 3.3	76 ± 2.9
Whole milk	40 ± 3.6	48 ± 4.3
Whole egg	34 ± 4.1	n/a
Tomato sauce	43 ± 4.2	n/a
Hot dog	20 ± 4.7	n/a

[a]*n* = 10 samples per matrix.

4. CONCLUSION

The extraction techniques developed for VX were accomplished, and the recoveries were >50% for a less-complex food matrix and <50% for higher fat, more-complex food matrices. The extraction analyses and results of the validation study are detailed in this report. The extraction method was easy to use to determine the VX amounts in complex food matrices. During the course of method development, issues of VX extraction efficiency arose in relation to its isolation. This led to a complete solvent oxidation study for VX, which was not originally requested to resolve this issue. Several very important issues regarding oxidation of VX were discovered. Results from completion of that work will determine our future directions.

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ACRONYMS AND ABBREVIATIONS

AOAC	Association of Analytical Communities
CWA	chemical warfare agent
ECBC	U.S. Army Edgewood Chemical Biological Center
ESI	electrospray ionization
LC	liquid chromatography
LDR	linear dynamic range
LOD	limit of detection
LOQ	limit of quantitation
MCX	mixed-mode cation exchange
MRM	multiple reaction monitoring
MS	mass spectroscopy
m/z	mass-to-charge ratio
PSA	primary secondary amine
PTFE	polytetrafluoroethylene
QuEChERS	Quick, Easy, Cheap, Effective, Rugged, and Safe
RSD	relative standard deviation
SPE	solid-phase extraction
TOF	time of flight
UPLC	ultra-performance liquid chromatography
VX	*O*-ethyl *S*-[2-(diisopropylamino)ethyl] methylphosphonothioate

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