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ECBC-TR-1351

EXTRACTION METHODOLOGICAL CONTRIBUTIONS TOWARD ULTRA-PERFORMANCE LIQUID CHROMATOGRAPHY–TIME-OF-FLIGHT MASS SPECTROMETRY: QUANTIFICATION OF FREE GB FROM VARIOUS FOOD MATRICES

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February 2016

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REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*	**2. REPORT TYPE**	**3. DATES COVERED** *(From - To)*
XX-02-2016	Final	Oct 2014 – Jun 2015

4. TITLE AND SUBTITLE
Extraction Methodological Contributions Toward Ultra-Performance Liquid Chromatography–Time-of-Flight Mass Spectrometry: Quantification of Free GB from Various Food Matrices

5a. CONTRACT NUMBER

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Bae, Sue Y.; and Winemiller, Mark D.

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Director, ECBC, ATTN: RDCB-DRC-C, APG, MD 21010-5424

8. PERFORMING ORGANIZATION REPORT NUMBER
ECBC-TR-1351

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for public release; distribution is unlimited.

13. SUPPLEMENTARY NOTES

14. ABSTRACT:
Liquid chromatography–electrospray ionization mass spectrometry with positive-ion modes of operation were used for the trace-level determination of isopropyl methylphosphonofluoridate (sarin, GB) in various food matrices. The development of a solid-phase extraction method using a normal-phase silica gel column for extraction of GB in foodstuffs is described. GB concentrations ranging from 50 ng/mL to 1.2 µg/mL were spiked into food samples. The linear range of quantitation was 0.3–1.2 µg/mL for GB. The total percent recoveries (and percent relative standard deviations) for GB in various food samples are reported.

15. SUBJECT TERMS
Isopropyl methylphosphonofluoridate (sarin, GB)
Foodstuff
Normal-phase silica gel column
Ultra-performance liquid chromatography–time-of-flight mass spectrometry (UPLC–TOF-MS)

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			Renu B. Rastogi
U	U	U	UU	24	**19b. TELEPHONE NUMBER** *(include area code)* (410) 436-7545

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PREFACE

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

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1. INTRODUCTION

As recent events in Syria have demonstrated, the continued threat from traditional chemical warfare agents (CWAs) such as isopropyl methylphosphonofluoridate (GB or sarin; 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 GB 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.

Numerous reviews have been published that describe low-level pesticide detection,[1–12] and those studies provide the best methods for detecting low levels of organophosphorous pesticides and other molecules in food. By comparison, only limited literature exists regarding actual CWAs, such as sarin or its breakdown products, in food or beverages.[13–15] The pesticide literature often includes sample-preparation techniques that are commercially available and affordable, such as solid-phase extraction cartridges[16–23] or QuEChERS systems (Quick, Easy, Cheap, Effective, Rugged, and Safe).[24–35] However, the CWA literature seems to focus more on new techniques and specialized equipment that may not be readily accessible to every laboratory.

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 GB nerve 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 examined five food samples. Apple juice, whole milk, whole egg, tomato sauce, 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 normal-phase separation 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.

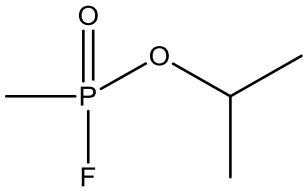

Figure 1. Structure of nerve agent GB.

2. EXPERIMENTAL METHODS

2.1 Reagents and Chemicals

The nerve agent GB (>99% purity) was provided by ECBC. All reagents and solvents were LC–MS grade. Acetonitrile, dichloromethane, dimethyl sulfoxide (DMSO), and diethylmethylamine were purchased from Sigma-Aldrich (St. Louis, MO). 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 an Acquity UPLC Synapt G2-S system (Waters Corp.; Milford, MA) 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. The leucine–enkephalin solution (1 ng/µL) was used as a reference mass with a flow rate 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. A thermostatted sample-manager compartment was used to maintain the temperatures of the column at 35 °C and the test samples at 5 °C.

2.3 Procedure for GB Extraction from Foodstuffs

A packed RediSep Rf normal-phase silica gel column (Teledyne Isco; Lincoln, NE), as shown in Figure 2, was used to extract GB from the various food samples. For spiking, a GB stock solution was prepared that contained 0.535 mg of GB in 5 mL of acetonitrile.

Apple juice samples (2 mL) were placed in glass vials and spiked with 50 µL of GB stock solution. The RediSep Rf column was eluted with 50 mL of 1% diethylmethylamine, 1% DMSO, and 98% dichloromethane. In-house air was used to pass the solution through the column. The GB-spiked apple juice was passed through the column, and the eluent was collected. A 1 mL aliquot of a solution containing 0.1% diethylmethylamine, 1% DMSO, and 98.9% dichloromethane was passed into the column and pushed slightly into the silica gel until

1 mL had just cleared the top of the silica gel. This step was repeated three times. The remaining 47 mL of the solution containing 0.1% diethylmethylamine, 1% DMSO, and 98.9% dichloromethane was added to the column and passed through the bed. A rotary evaporator was used to evaporate the extracted solution to a volume of about 2 mL. A small aliquot of the extracted solution was filtered through a 0.45 µm poly(tetrafluoroethylene) membrane filter, transferred to an autosampler vial, and analyzed using LC–MS.

A 2 mL sample of whole milk was spiked with 50 µL of GB stock solution and diluted with 5 mL of acetonitrile. This mixture was centrifuged for 3 min at 10,000 rpm, and the supernatant was decanted. Another 5 mL of acetonitrile was added, and the mixture was vortexed or sonicated for 1 min and centrifuged for 3 min at 10,000 rpm. The supernatant was removed, and the first and second portions were combined and passed through a RediSep Rf column. The eluents were collected for LC–MS/MS analysis. For extraction of whole egg and tomato sauce, approximately 3 g of each material was spiked with 50 µL of GB stock solution. Sample analysis procedures were then identical to those described for the whole milk analysis.

A 3 g (±0.1 g) sample of hot dog was spiked with 50 mL of GB stock solution and diluted with 5 mL of acetonitrile. The entire sample was homogenized using a Polytron homogenizer (Kinematica; Luzern, Switzerland) at 20,000 rpm for 1–2 min. The mixture was then centrifuged for 3 min at 10,000 rpm, and the supernatant was removed. A second 5 mL portion of acetonitrile was added, and the sample was vortexed or sonicated for 1 min and centrifuged for 3 min at 10,000 rpm. The supernatant was removed, and the first and second portions were combined and passed through a RediSep Rf column. The eluents were evaporated using a rotary evaporator and then collected for LC–MS/MS analysis. A total of 10 food samples were weighed for each matrix, and the percent recoveries for GB with the relative standard deviations (RSDs) were obtained by averaging values from 10 analysis runs.

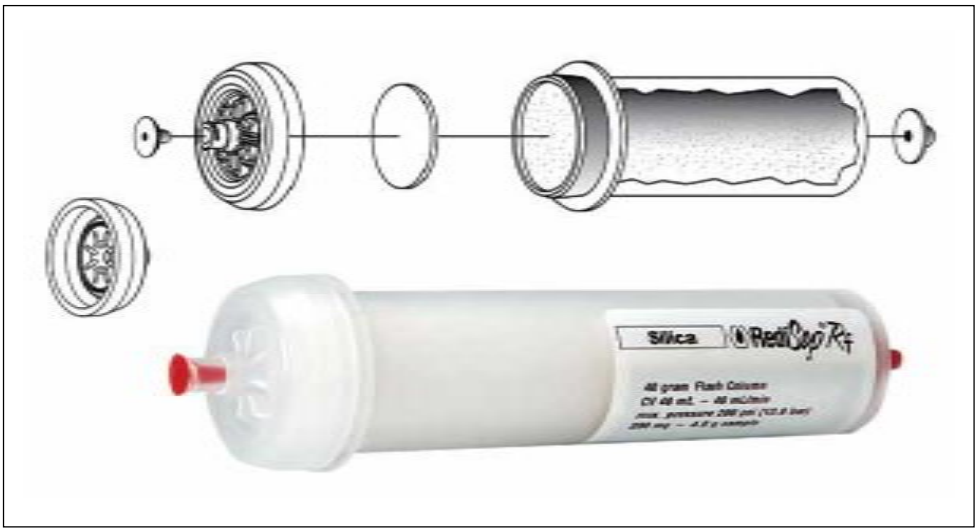

Figure 2. A RediSep Rf normal-phase silica gel column.

3. RESULTS AND DISCUSSION

3.1 LC Separation and Analytical Figures of Merit

For LC–MS analysis of GB, the MS system was operated in two modes: TOF-MS at *m/z* 50–1200 and TOF-MRM at *m/z* 141.1100 → 99.0010. The TOF-MS mode was used to identify the presence of any hydrolysis products. No hydrolysis products of GB were found in these extracted samples. TOF-MRM was used to determine the limits of detection (LODs), limits of quantitation (LOQs), and linear dynamic ranges (LDRs) for GB. The TOF-MRM for GB at *m/z* 141.1100 → 99.0010 was used to generate the calibration curves for the LDRs. The calibration curve for GB (Figure 3) was plotted over a concentration range of 0.3–1.2 µg/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 300 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 with the LDRs, LOD, LOQ, linear regression equation, and correlation coefficient, which are tabulated in Table 1.

Table 1. Analytical Figures of Merit for GB

Nerve Agent	LDRs (ng/mL)	LOD (ng, on column)	LOQ (ng, on column)	Linear Regression Equation (n = 10)	Correlation Coefficient[a]
GB in acetonitrile	50–1200	0.31	1.5	$y = 136.9x + 2.976$	0.9988

[a]Calculated over the calibration range 0.3–1.2 µg/mL for GB.

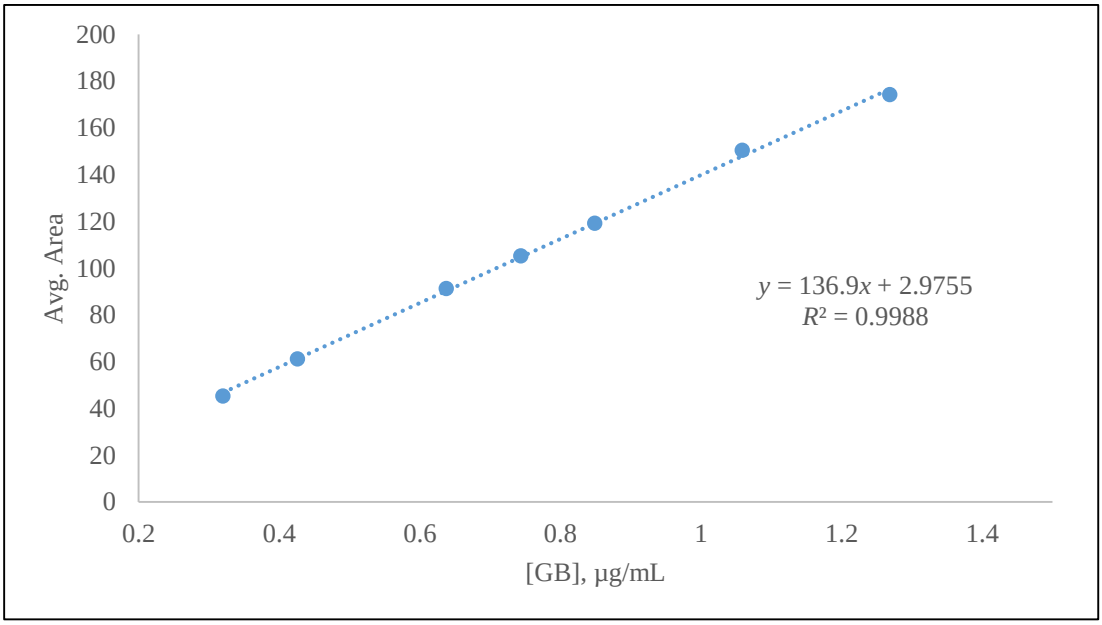

Figure 3. External calibration curve for GB in acetonitrile.

3.2 Extraction of GB from Foodstuffs

A commercially available normal-phase silica gel column was used to extract GB from foodstuffs. Preconditioning of the column was required before the samples were loaded. Various preconditioning solvents were considered, and we found that the solution of 1% diethylmethylamine, 1% DMSO, and 98% dichloromethane was the most suitable. In terms of the extracting solvent, we used acetone, acetonitrile, ethyl acetate, and dichloromethane. Acetone and acetonitrile were miscible in water, and all of the GB analyte came through the silica gel column along with the water and organic layer. However, the process was time-consuming: it took a long time to evaporate the water layer down to 1 mL or less for LC analysis. We therefore eliminated these two solvents because of the insufficient pre-concentration process. From a pre-concentration perspective, the ethyl acetate solvent performed much better than either acetone or acetonitrile; however, the extraction efficiency with ethyl acetate was poor. For these reasons, we started looking at solvent mixtures. Upon considering various mixtures, we determined that the optimal extraction solvent for GB was a solution that contained 0.1% diethylmethylamine, 1% DMSO, and 98.9% dichloromethane. Figure 4 is a typical TOF-MRM chromatogram for GB from various food matrices. No evidence of byproducts or hydrolysis products was present. For each matrix, 10 samples were tested. The results showed a >90% recovery of GB from apple juice, whole milk, and tomato sauce. Lower GB recoveries were measured from whole egg and hot dog matrices (Table 2).

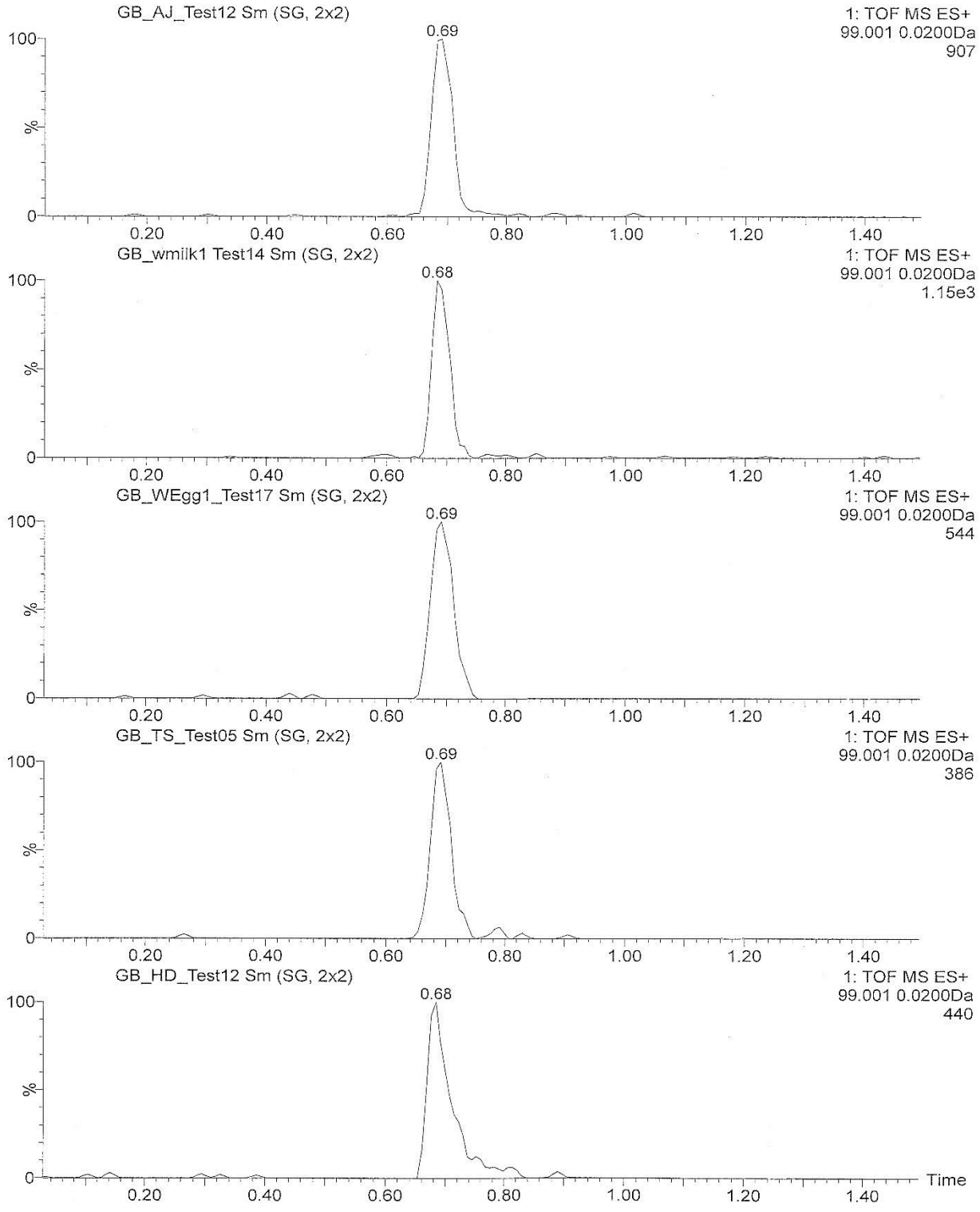

Figure 4. A representative TOF-MRM chromatogram for GB extracted from various food matrices using a normal-phase silica gel column.

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

Foodstuff	Recovery ± RSD (%)
Apple juice	94 ± 3.7
Whole milk	95 ± 4.1
Whole egg	73 ± 4.1
Tomato sauce	90 ± 4.3
Hot dog	56 ± 4.5

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

4. CONCLUSION

An extraction technique for GB was successfully developed, and recoveries were >90% for the less-complex food matrices and 50–75% for the higher-fat, more-complex samples. This report details the extraction analysis and results of the validation study. This easy-to-use extraction method can be used to determine GB amounts in complex food matrices.

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ACRONYMS AND ABBREVIATIONS

CWA	chemical warfare agent
DMSO	dimethyl sulfoxide
ECBC	U.S. Army Edgewood Chemical Biological Center
ESI	electrospray ionization
GB	isopropyl methylphosphonofluoridate, sarin
LC	liquid chromatography
LDR	linear dynamic range
LOD	limit of detection
LOQ	limit of quantitation
MRM	multiple reaction monitoring
MS	mass spectroscopy
QuEChERS	Quick, Easy, Cheap, Effective, Rugged, and Safe
RSD	relative standard deviation
TOF	time-of-flight
UPLC	ultra-performance liquid chromatography

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