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ECBC-TR-509

ANALYTICAL METHOD AND DETECTION LIMIT STUDIES FOR DETECTION OF GB IN GB HYDROLYSATE

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14. ABSTRACT

The work presented here describes the applicability of an extraction/GC/MS analytical method for detection of GB in caustic matrices. The method was designed to minimize GB reformation and spiked GB degradation during workup and analysis. Extractions were performed at pH 9 and 4 °C, using methylene chloride as the solvent. The extraction ratio was 15 mL matrix to 1.5 mL solvent. The GC/MSD in the SIM mode was used for analysis. The method was tested using two distinctly different sources of GB, one containing only TBA as stabilizer, and the other containing TBA and DICDI as stabilizers. Method detection limits (MDL) obtained for NaOH hydrolysate matrices produced from these sources were 4.5 ng/mL (ppb) and 8.4 ng/mL, respectively. The GB spike recoveries were 82.4 and 75.2%, respectively. No GB reformation was observed in the TBA-stabilized unspiked hydrolysate. The GB was detected, presumably via reformation during analysis, in the TBA/DICDI-stabilized unspiked hydrolysate, but was below the MDL.

15. SUBJECT TERMS

Method Detection Limit (MDL)	Nuclear Magnetic Resonance	GB reformation
Selected Ion Monitoring (SIM)	Mass Spectrometry	GB hydrolysate
Gas Chromatography	Stabilizer	

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PREFACE

The work described in this report was authorized by the Program Manager, Assembled Chemical Weapons Alternatives (PM-ACWA), under Sales Order No. 4VFF5R. The work was started in April 2003 and completed in April 2005.

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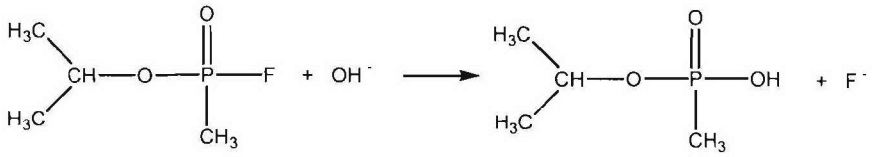
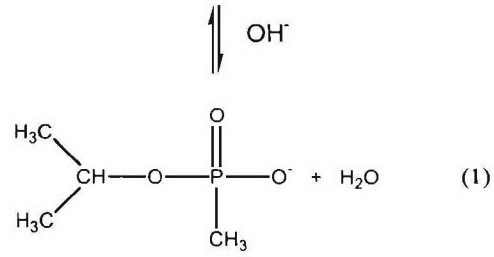
1. INTRODUCTION

Chemical neutralization is currently selected as the technology to destroy GB filled chemical weapons stored at Blue Grass, Kentucky. The neutralization process involves reacting drained liquid GB with aqueous NaOH solution in a stirred batch reactor, in which GB is rapidly and completely converted to the corresponding phosphonic acid and NaF (eq 1). The final reaction mixture, called hydrolysate, is analyzed to demonstrate that 99.9999 % (six 9s) of the initial GB added to the reactor is destroyed before the hydrolysate can be post-processed. The analytical method for detecting trace amounts of GB in the hydrolysate matrix is an indispensable tool to validate the neutralization process.



GB **IMPA**



IMPA anion

The apparent presence of isopropyl methylphosphonofluoridate (GB) in caustic decontamination (brine) solutions has been observed by several laboratories since the 1970s.[1-4] Since GB should be extremely reactive in this matrix, these laboratories postulated that detection of GB was an artifact of the analysis, resulting from GB reformation occurring either in the hydrolysate or the extract during analytical workup. Two factors which were postulated to be particularly detrimental to the analysis were the presence of acidity during analytical workup and the use of chloroform as an extraction solvent.[4] The current analytical method developed at ECBC uses chloroform to extract 50 mL of hydrolysate with 2 x 1 mL of chloroform at pH 8.[5] N,O-Bis(trimethylsilyl)trifluoroacetamide (BSTFA) is added to the extract to tie up free HF and minimize GB reformation. SIM (ions 99, 125, 79, 81 and 140) GC/MSD detection with an injection port temperature of 150 °C is used. The primary concerns with this method are the use of chloroform as extraction solvent (which may not be suitable as evidenced by previous studies) and the use of BSTFA (which can potentially interfere with GB detection). The objective of this

study was to seek an improved method capable of clearing hydrolysate at the drinking water level concentration of 20 ppb GB.[6,7] Two distinctly different sources of munitions grade GB of different purities and impurities were selected to test the method, one containing only tributylamine (TBA) as stabilizer, and the other originally containing TBA and N,N'-diisopropylcarbodiimide (DICDI) as stabilizers. During storage, DICDI hydrolyzes to the urea and was detected in both the GB and hydrolysate as N,N'-diisopropylurea.

2. EXPERIMENTAL PROCEDURES

2.1 Materials.

Methylene chloride used for the methods detection limits' (MDL) study was obtained from Burdick & Jackson (Muskegon, MI) as capillary GC/GC-MS grade, lot CM042. The solvent was received 31 January 2005 and opened 2 February 2005. MDL extraction studies were performed 16-26 March 2005. Isopropyl alcohol (IPA) was purchased from Fisher (Pittsburgh, PA) as optima grade, lot 030746. Concentrated sulfuric acid was purchased from Mallinkrodt Chemicals (Phillipsburg, PA) as 95-98% pure, lot W0PX. Anhydrous sodium sulfate was obtained from Aldrich (Milwaukee, WI) as 99+% ACS grade, lot 07231TQ. Acrodisc® glass fiber syringe filters (25 mm, 1 μm, lot A421912351) were purchased from Gelman Sciences, Ann Arbor, MI.

Other chemicals used for supporting studies were potassium carbonate (Aldrich 99+ ACS lot H7195A), methylene chloride (Burdick&Jackson GC^2, lot CA800), N,N'-diisopropyl-carbodiimide (Aldrich 99%, lot 13811PB) and Bakerbond quaternary amine SPE cartridges (500 mg/3 mL, lot J31562, J.T. Baker, Phillipsburg, NJ). BSTFA containing 1% trichloromethyl-silane (TMCS) was purchased from Supelco (Bellefonte, PA), lot LA73706. Two sources of chloroform were used. The "old" chloroform was purchased from Fisher as Optima grade, lot 033478, and was opened on 21 April 2003. "Fresh" chloroform was purchased from Aldrich as PRA grade, lot 08274PB, and was opened 2 February 2005. Both were stabilized with amylene.

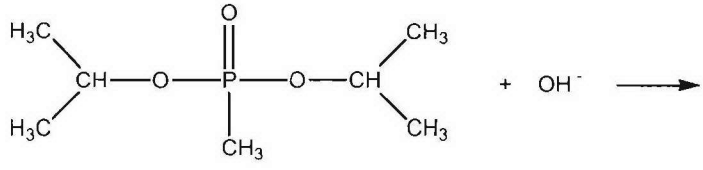
2.2 Spiking Solution and Standards Preparation.

The GB used for preparing standards and spiking solutions was obtained from the Chemical Agent Standard Analytical Reference Material (CASARM) program; lot GB-U-6184-CTF-N, purity 98.7%. A 630 μg/mL stock solution of GB in IPA was prepared. The stock solution was diluted 0.79 mL to 10 mL in IPA to prepare a 50 μg/mL GB solution. This solution was diluted 1.2 mL to 10 mL in IPA to give a 6 μg/mL spiking solution used to spike hydrolysate samples. All solutions were freshly prepared and stored at 4 °C until use.

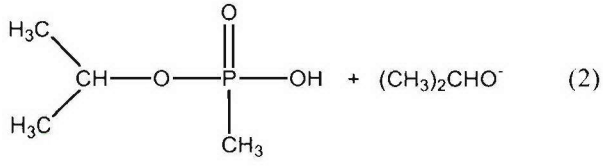
The GB standards were prepared by dilution of the 630 μg/mL IPA stock solution 0.794 mL to 10 mL in methylene chloride to give a 50 μg/mL GB solution. Subsequent dilutions in methylene chloride produced standards at 0.5, 0.25, 0.1, 0.05, 0.025 and 0.01 μg/mL (ppm).

2.3 GB Hydrolysate Samples.

"Anniston Hydrolysate" was prepared at the ECBC Chemical Transfer Facility (CTF) on 21 September 2004 using GB obtained from the M55 rockets (Agent type: PRO-RS) stored at Anniston Army Depot. This GB was identified as GB-10005 and contained TBA and DICDI as stabilizers. The DICDI stabilizer was hydrolyzed to N,N'-diisopropylurea, most of which separated out from the liquid GB as crystalline solids.[8] The hydrolysate was produced by reacting 7.0 vol% GB with 5 wt% NaOH at room temperature. The pH was 14. Multinuclear NMR and GC/MS characterization of the GB-10005 used to prepare the hydrolysate is provided in Tables 1 and 2, respectively. GC/MS and multinuclear NMR characterization of the liquid hydrolysate is shown in Tables 6 and 7, respectively. As shown in Table 6, diisopropyl methylphosphonate (DIMP) is initially a major component but degrades over time via relatively slow hydrolysis (eq 2). DIMP (95 %) and N,N'-diisopropylurea (5%) were the only compounds detected in a methylene chloride extract of fresh Anniston hydrolysate.



DIMP



(2)

The "ECBC Hydrolysate" was prepared at the U.S. Army Edgewood Chemical Biological Center (ECBC), Research and Technology Directorate, on 13 December 2004, using GB lot GB-S-0290-CTF-N-1. This GB contained only tributylamine (TBA) as stabilizer. The hydrolysate was produced by reacting 7.3 vol% GB with 5 wt% NaOH at room temperature. Hydrolysate pH was 14. GC/MS and multinuclear NMR characterization of the GB is provided in Tables 3 and 4, respectively. GC/MS and multinuclear NMR characterization of the hydrolysate is shown in Tables 5 and 7, respectively.

Both hydrolysates contained two layers, as shown in Figures 1 and 2. GC/MS analysis of the upper layer of the ECBC hydrolysate (acetonitrile extraction followed by silylation and GC/MSD analysis) revealed tributylamine as the major organic component. Complete results, obtained 20 days after the hydrolysis reaction was performed, are listed in Table 8. The ECBC hydrolysate contained a dark brown upper layer, which was larger than the Anniston light brown

upper layer. The high concentration of Fe (160 mg/g), detected by ICP in solid isolated from the top layer of the ECBC hydrolysate, is a probable source of the brown color.

Figure 1. Anniston GB Hydrolysate

Figure 2. ECBC Hydrolysate after Shaking (left) and after Settling (right)

Table 1. NMR ($^{1}H/^{19}F/^{13}C/^{31}P$) Characterization of GB-10005 (Anniston)

Mol. Wt.	Name [CAS No]	Structure	Wt %
140	Isopropyl methylphosphonofluoridate [107-44-8] (GB)	MeP(O)(O*i*Pr)F	81.04
100	Methylphosphonic difluoride [676-99-3] (DF)	$MeP(O)F_2$	0.18
180	Diisopropyl methylphosphonate [1445-75-6] (DIMP)	$MeP(O)(O{i}Pr)_2$	11.33
154	Isopropyl ethylphosphonofluoridate [1189-87-3] (Ethyl GB)	EtP(O)(O*i*Pr)F	0.007
98	Methylphosphonofluoridic acid [1511-67-7] (FA)	MeP(O)(F)OH	1.96
184	Diisopropyl phosphorofluoridate [55-91-4]	$(i\text{PrO})_2P(O)F$	0.18
96	Dimethylphosphinic fluoride	$Me_2P(O)F$	0.02
138	Isopropyl methylphosphonic acid [1832-54-8] (IMPA)	MeP(O)(O*i*Pr)OH	0.54
170	Ethyl isopropyl phosphorofluoridate	*i*PrOP(O)(OEt)F (t)	0.03
94	Dimethylphosphinic acid [3283-12-3]	$Me_2P(O)OH$	0.03
20	Hydrofluoric acid / fluoride anion [7664-39-3]	HF/F^-	0.22
146	Hexafluorophosphoric acid [16940-81-1]	HPF_6	0.04
60	Isopropyl alcohol [67-63-0]	*i*PrOH	0.48
62	2-Fluoropropane [420-26-8]	*i*PrF (t)	0.03
126	Ethyl methylphosphonofluoridate	MeP(O)(OEt)F	0.002
205	Tributylamine-HF	Bu_3N-HF	2.71
144*	Diisopropylurea-related unknown	$i\text{Pr}_2\text{NH}$-HF (t)	0.34
118	Chloroform [67-66-3]	$CHCl_3$ (t)	0.07
144	N,N'-diisopropylurea [4128-37-4]	*i*PrNHC(O)NH*i*Pr	0.78
142	Isopropyl phosphorofluoridic acid	*i*PrOP(O)(OH)F (t)	0.005

t-tentative assignment

Table 2. GC/MS Characterization of GB-10005, Vial #2 (Anniston)

Ret. Time (min)	Mol. Wt.	Name [CAS No]	Structure	Area %
1.39	96	Difluorodimethylsilane [353-66-2]	Me_2SiF_2	0.24
1.47	100	Methylphosphonic difluoride [676-99-3] (DF)	$MeP(O)F_2$	0.19
1.47	60	Isopropyl alcohol [67-63-0]	*i*PrOH	0.61
2.43	140	Isopropyl methylphosphonofluoridate [107-44-8] (GB)	MeP(O)(O*i*Pr)F	75.64
3.50		Unknown		0.33
4.46	180	Diisopropyl methylphosphonate [1445-75-6] (DIMP)	$MeP(O)(O{i}Pr)_2$	20.52
5.66	185	Tributylamine (TBA) [102-82-9]	Bu_3N	1.71
6.01	144	N,N'-diisopropylurea [4128-37-4]	*i*PrNHC(O)NH*i*Pr	0.76

Table 3. GC/MS Characterization of GB-S-0290-CTF-N-1

Ret. Time (min)	Mol. Wt.	Name [CAS No]	Structure	Area %
1.65	96	Difluorodimethylsilane [353-66-2]	Me_2SiF_2	0.13
1.72	100	Methylphosphonic difluoride [676-99-3] (DF)	$MeP(O)F_2$	3.00
2.76	140	Isopropyl methylphosphonofluoridate [107-44-8] (GB)	MeP(O)(O*i*Pr)F	93.64
3.50	154	Isopropyl ethylphosphonofluoridate [1189-87-3]	EtP(O)(O*i*Pr)F	0.02
4.92	180	Diisopropyl methylphosphonate [1445-75-6] (DIMP)	$MeP(O)(O{i}Pr)_2$	0.39
6.11	185	Tributylamine [102-82-9] (TBA)	Bu_3N	2.83

Table 4. NMR ($^{1}H/^{19}F/^{13}C/^{31}P$) Characterization of GB-S-0290-CTF-N-1

Mol. Wt.	Name [CAS No]	Structure	Wt %
140	Isopropyl methylphosphonofluoridate [107-44-8] (GB)	MeP(O)(O*i*Pr)F	92.59
100	Methylphosphonic difluoride [676-99-3] (DF)	$MeP(O)F_2$	2.04
180	Diisopropyl methylphosphonate [1445-75-6] (DIMP)	$MeP(O)(O{i}Pr)_2$	0.62
126	Ethyl methylphosphonofluoridate [1189-87-3]	EtP(O)(O*i*Pr)F	0.01
184	Diisopropyl phosphorofluoridate [55-91-4]	$(iPrO)_2P(O)F$	0.02
20	Hydrofluoric acid / fluoride anion [7664-39-3]	HF/F^-	0.90
146	Hexafluorophosphoric acid [16940-81-1]	HPF_6	0.01
60	Isopropyl alcohol [67-63-0]	*i*PrOH	0.42
62	2-Fluoropropane [420-26-8]	*i*PrF (t)	<0.01
185	Tributylamine-HF	Bu_3N-HF	2.94
121	Diisopropylamine - HF	iPr_2NH-HF (t)	0.40
	Other P-F		0.02

t-tentative assignment

Table 5. GC/MS Characterization of ECBC Hydrolysate (Aqueous Layer)

Name [CAS No]	Structure	Relative Area % (Analyzed 3-9-05)
Isopropyl methylphosphonic acid [1832-54-8] (IMPA)	MeP(O)(O*i*Pr)OH*	87.9
Methylphosphonic acid [993-13-5] (MPA)	$MeP(O)(OH)_2$*	12.1

* Detected as trimethylsilyl derivative after BSTFA derivatization

Table 6. GC/MS Characterization of Anniston Hydrolysate (Aqueous Layer)

Name [CAS No]	Structure	Relative Area%	
		9-22-04	3-9-05
Diisopropyl methylphosphonate [1445-75-6] (DIMP)	MeP(O)(O*i*Pr)$_2$	9.8	0.05
Isopropyl methylphosphonic acid [1832-54-8] (IMPA)	MeP(O)(O*i*Pr)OH*	83.5	93.4
Methylphosphonic acid [993-13-5] (MPA)	MeP(O)(OH)$_2$*	5.8	6.1
N,N'-diisopropylurea [4128-37-4]	*i*PrNHC(O)NH*i*Pr	1.0	0.5

* Detected as trimethylsilyl derivative after BSTFA derivatization

2.4 Hydrolysate Extraction Procedure.

- After thorough shaking, a 15 mL representative hydrolysate sample was collected and placed in a 50 mL beaker.
- Hydrolysate sample was adjusted to pH 9.0 ± 0.1 with stirring and drop-wise addition of 4N H_2SO_2. A calibrated pHTestr 3™ (Cole Parmer, Vernon Hills, IL) was used to measure pH.
- The pH 9 hydrolysate was filtered through a 25 mm, 1 µm syringe filter and the filtrate collected in a 20 mL glass centrifuge tube.
- The filtered hydrolysate was refrigerated at 4° C for a minimum of 1 hr. Spiking solution and extraction solvent were also refrigerated until use.
- For spiked samples, 50 µL of IPA containing 6 µg/mL GB was added to the chilled, filtered hydrolysate and the solution mixed 5 s (effective original hydrolysate concentration 20 ng/mL).
- 1.5 mL chilled methylene chloride was added to the chilled hydrolysate, the solution shaken by hand for 30 s and returned to the refrigerator.
- After allowing the layers to settle for about 5 min, the extract was collected using a long disposable pipet and dried by passing through a 2 cm column of anhydrous sodium sulfate in a disposable pipet.
- Extract submitted for analysis.

Table 7. Multinuclear NMR Characterization of GB Hydrolysate (Aqueous Layer)

Name [CAS No]	Structure	Mole %	
		ECBC	Anniston
Isopropyl methylphosphonic acid [1832-54-8] (IMPA)	MeP(O)(O*i*Pr)OH	43.7	39.9
Inorganic Fluoride	F^-	51.0	51.7
Methylphosphonic acid [993-13-5] (MPA)	$MeP(O)(OH)_2$	3.1	2.3
Methylphosphonofluoridic Acid [1511-67-7] (FA)	MeP(O)(OH)F	1.7	nd
Isopropyl alcohol [67-63-0]	*i*PrOH	nd	2.2
Diisopropyl methylphosphonate [1445-75-6] (DIMP)	$MeP(O)(O{i}Pr)_2$	0.18	3.1
N,N'-diisopropylurea [4128-37-4]	*i*PrNHC(O)NH*i*Pr	nd	0.45
Dimethylphosphinic acid [3283-12-3]	$Me_2P(O)OH$	0.04	0.05
Unknown Si-F type		0.04	0.13
Diisopropylphosphoric acid	$(iPrO)_2P(O)OH$	0.01	0.06
Isopropylphosphoric acid	$(iPrO)P(O)(OH)_2$	nd	<0.01
Fluorophosphoric acid [13537-32-1]	$(HO)_2P(O)F$	0.03	0.01
Methylphosphonic acid anhydride (t)	MeP(O)(OH)OP(O)(Me)OH	0.01	<0.01
Hexafluorophosphoric acid [16940-81-1]	HPF_6	<0.01	0.06
0-19 ppm (Phosphates)		0.03	nd
20-32 ppm (Phosphonic acids/esters)		0.02	nd

nd-none detected
t-tentative assignment

Table 8. GC/MS Characterization of ECBC Hydrolysate (Upper Layer)

Name [CAS No]	Structure	Relative Area %
Diisopropyl methylphosphonate [1445-75-6] (DIMP)	$MeP(O)(OiPr)_2$	2.3
Isopropyl methylphosphonic acid [1832-54-8] (IMPA)	MeP(O)(O*i*Pr)OH*	15.5
Tributylamine [102-82-9] (TBA)	Bu_3N	82.1

* Detected as trimethylsilyl derivative after BSTFA derivatization

2.5 Quantitation.

Quantitation was obtained using a six point external calibration curve. Linear regression analysis using GB concentrations at 0.50, 0.25, 0.10, 0.05, 0.025 and 0.01 μg/mL and the area of the m/z 99 extracted ion peak gave linear calibration curves (correlation coefficient > 0.998). Under the spiking and extraction conditions used, 100% recovery yield represents 0.20 μg/mL (ppm) GB in the extract. Calibration curves obtained for the ECBC and Anniston hydrolysate MDL studies are shown in Figures 3 and 4, respectively.

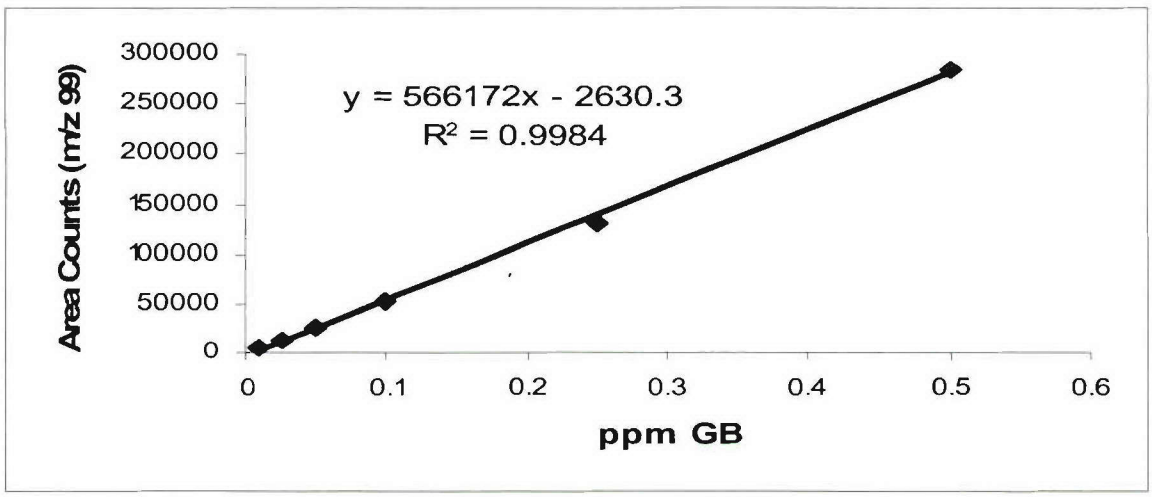


Figure 3. GB Calibration Curve for ECBC Hydrolysate MDL Study

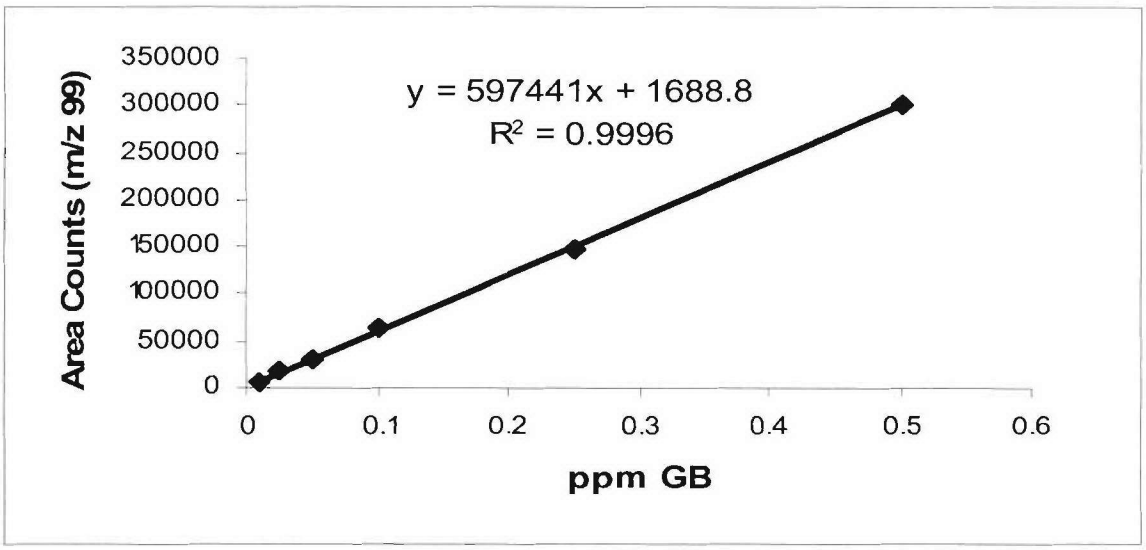


Figure 4. GB Calibration Curve for Anniston Hydrolysate MDL Study

2.6 Method Detection Limit (MDL) Procedure.

Eight replicate extractions were performed for each hydrolysate as in Section 2.4. Each aliquot was spiked with 20 ng/mL (ppb) GB. The order of analysis for each series was calibration curve, solvent blank, MDL series, solvent blank, and 0.25 ppm or 0.1 ppm GB check standard to verify the calibration curve was still valid at the end of the series. Check standards were all within ± 10% of the correct value. The MDLs were obtained according to the Environmental Protection Agency procedure outlined in "Definition and Procedure for the Determination of the Method Detection Limit", Revision 1.11, 40 CFR Chapter 1, Part 136, Appendix B. The MDL was obtained by multiplying the standard deviation by the Students' "t" value appropriate for a 99% confidence.

2.7 Instrumentation.

2.7.1 Gas Chromatography/Mass Spectrometry.

Extracts were analyzed by gas chromatography/mass spectrometry (GC/MS) using a Hewlett Packard 5890 GC / 5972 mass selective detector (MSD) and an Agilent series 6890 auto-injector (Agilent Technologies, Santa Clara, CA). The instrument was equipped with an Rtx-35ms 30 m x 0.25 mm (0.25 µm film) column purchased from Restek Corporation, Bellefonte, PA. GC and MS parameters used for detection of GB in hydrolysate extracts are listed in Tables 9 and 10, respectively. Under these chromatographic conditions GB elutes at 5.9 min.

Table 9. GC Parameters

Column temperature	40 °C (3 min); 15 °C/min to 280 °C (2 min)
Carrier gas	Helium
Flow rate	1 mL/min constant flow
Injection temperature	175 °C
Injection mode	splitless (50:1 split at 0.4 min)
Injection volume	1 µL, autoinjection

Table 10. MS Parameters

Acquisition mode	SIM
Ionization mode	EI
Transfer line temperature	280 °C
Source temperature	150 °C
Solvent delay	3.5 min
Electron energy	70 eV
Multiplier voltage	Autotune + 200 V
Scan ions	79, 81, 99, 125
Ion dwell time	125 msec
Scans/sec	1.77

GC/MS characterization of the GB and GB hydrolysate samples was done using the same conditions except the injection port temperature was 250 °C, samples were analyzed in the split mode (30:1 split), the scan range was from 40-450 amu at 1.86 scans/second with no solvent delay, and the GC oven temperature was programmed from 60-280 °C at 15 °C/min; 5 min at 280 °C. To allow detection of acidic components, GB hydrolysate samples were derivatized prior to analysis by evaporating one drop to dryness under a gentle stream of nitrogen and reacting with 50 µL BSTFA/1% TMCS at 60 °C for 30 min.

2.7.2 Nuclear Magnetic Resonance Spectroscopy.

Multinuclear (^{1}H, ^{13}C, ^{31}P and ^{19}F) NMR characterization of the GB and GB hydrolysate samples was performed using Brucker Biospin Corporation (Billerica, MA) 11.75 Tesla Advance DRX-500 and 7.06 Tesla DRX-300 spectrometers. The DRX-500 was fitted with a 5-mm triple-resonance TXI probehead (inverse-detection configuration) with dedicated ^{1}H, ^{13}C, and ^{31}P channels. The DRX-300 had a dedicated double-resonance 5-mm QNP probehead (inverse-broadband configuration) for ^{1}H, ^{13}C, ^{31}P and ^{19}F observation. ^{1}H and ^{13}C chemical shifts were referenced to external TSP (sodium 3-trimethylsilylpropionate-2,2,3,3-d_4) dissolved in D_2O at δ0.0. ^{19}F and ^{31}P chemical shifts were referenced to external trifluoroacetic acid (δ-76.55, relative to CF_3Cl at δ0.0) and 85% phosphoric acid at δ-0.73, respectively.

3. RESULTS AND DISCUSSION

3.1 Injection Port Studies.

A possible source of GB reformation during analysis is the heated GC injection port. To minimize reformation and select an appropriate injection port temperature, studies were performed to evaluate GB signal intensity as a function of injection temperature (split/splitless injector). Results obtained for an unspiked methylene chloride extract of the Anniston hydrolysate are shown in Table 11. Runs were obtained in the order shown, with standard and extract being analyzed consecutively at each temperature. Results clearly suggest that GB reformation occurs in the injection port for this particular DICDI-stabilized sample. Reference standard sensitivity remains unchanged between 175 and 250 °C, while the extract area counts increase dramatically as the temperature is increased. 175 °C was selected as the optimum temperature for these studies to obtain maximum sensitivity and minimal reformation. Below 175 °C, GB signal response decreases and the likelihood of injection port contamination, which may affect the reproducibility of the analysis, increases. The detection of GB in the blank analyses run at higher temperatures following extract analyses at lower temperatures provide evidence that the components necessary to promote GB reformation may be accumulating in the injection port during the low temperature runs.

Analyses were also performed using ECBC hydrolysate at 250 °C, 225 °C and 175 °C. Data is shown in Table 12. The effect was less pronounced than with the Anniston hydrolysate. No GB was observed in the extract using a 175 °C injection temperature. Blank samples run at 250 °C after a series of hydrolysate analyses at 175 °C were clean with respect to GB, suggesting the precursors necessary to promote reformation are not present in the injection port when using this matrix.

Table 11. Effect of Injection Temperature on GB Detection Using Anniston Hydrolysate

IJ Temp (°C)	GB Area Counts 0.25 ppm GB	GB Area Counts Anniston CH_2Cl_2	Calc. Extract ppm GB	Calc. Hydrolysate ppb GB
250	151,706	281,795	0.46	46.4
225	153,024	69,691	0.11	11.4
200	159,845	4193	0.007	0.66
175	152,344	818	0.001	0.13
150	132,639	110	0.0002	0.02
125	129,741	NONE	NONE	NONE
100	113,308	NONE	NONE	NONE
175	(CH_2Cl_2 Blank) 3914 counts			
175	150,061	17772	0.03	3.0
250	(CH_2Cl_2 Blank) 126,866 counts			
250	148,118	228,925	0.39	38.6

Table 12. Effect of Injection Temperature on GB Detection Using ECBC Hydrolysate

IJ Temp (°C)	Area Counts Anniston CH_2Cl_2	Calc. Extract ppm GB	Calc. Hydrolysate ppb GB
250	16,344[a]	0.028	2.8
225	12,328[b]	0.016	1.6
175	None[b]	None	None

[a] Single extraction and analysis [b] Average of 3 separate extractions and analyses

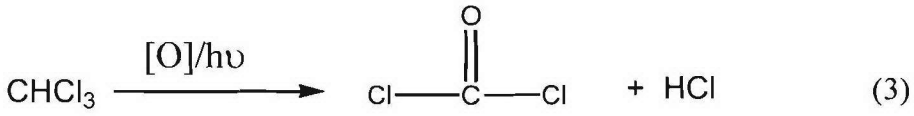
3.2 Selection of Extraction Solvent.

Four solvents were initially screened to determine their effectiveness for extraction of GB from hydrolysate matrices. Extractions were performed at pH 8 and room temperature with a 50:2 hydrolysate:solvent ratio. Extraction recoveries are listed in Table 13. Hexane and isooctane were ineffective extraction solvents. Methylene chloride and chloroform were equally effective.

Table 13. Solvent Extraction Efficiencies (ECBC Hydrolysate)

Solvent	Extraction Efficiency
Chloroform	50 %
Methylene Chloride	49 %
Hexane	5 %
Isooctane	None detected

Previous studies have indicated that GB reformation is especially prevalent when chloroform is used as an extraction solvent.[4] Chloroform is known to decompose spontaneously to phosgene and HCl during storage (see eq 3).[9] An acidic solvent could potentially promote GB



(3)

reformation by either catalyzing the reaction or by increasing the solubility of IMPA and fluoride by converting the salts to the more soluble acid species. To test this theory, pH 8.5 hydrolysate was extracted 15:1.5 mL at room temperature with fresh and aged solvents to examine the extent of GB reformation observed. Results are provided in Table 14. The aged chloroform tested clearly yields an elevated level of GB. GC/MS characterization of this solvent revealed the presence of approximately 0.2% phosgene and 0.8% carbon tetrachloride as impurities. The solvent was also acidic, suggesting the presence of HCl. The aged methylene chloride did not exhibit this behavior.

Table 14. Effect of Solvent Age on GB Reformation (Anniston Hydrolysate)

Extraction Solvent	Date Opened	Extract ppm GB*	Calc. Hydrolysate ppb GB
Chloroform	21 April 2003	442	44,200
Chloroform	2 February 2005	2.0	200
Methylene Chloride	4 October 2001	0.02	2
Methylene Chloride	2 February 2005	0.06	6

* Extractions and analyses performed 2 February 2005

To see if pretreatment of the solvent to reduce the H^+ and Cl^- content would effectively reduce GB reformation, selected studies were performed. Solvents were pretreated by passing through a 2 cm column of potassium carbonate in a Pasteur pipet and/or passing through a 500 mg/3 mL quaternary amine SPE column. Results are provided in Table 15.

Table 15. Effect of Solvent Purification on GB Reformation (Anniston Hydrolysate)

Solvent (open date)	Treatment	Extract ppm GB*	Calc. Hydrolysate ppb GB
Chloroform (4/21/03)	none	442	44,200
Chloroform (4/21/03)	K_2CO_3	273	27,300
Chloroform (4/21/03)	N^+ SPE	59	5900
Chloroform (4/21/03)	N^+ SPE/ K_2CO_3	8.5	850
Chloroform (2/2/05)	none	2.0	200
Chloroform (2/2/05)	N^+ SPE/ K_2CO_3	0.14	14
Methylene Chloride (2/2/05)	none	0.06	6
Methylene Chloride (2/2/05)	N^+ SPE/ K_2CO_3	0.04	4

* Extractions and analyses performed 2 February 2005

Quaternary amine SPE appeared to be more effective than carbonate for reducing GB reformation. The most effective reduction was observed when the two techniques were used in combination. The results suggest that great care must be taken when using chloroform as a solvent for detection of GB. Based upon the above results, methylene chloride was selected as the extraction solvent. Freshly opened, high purity solvent is recommended to avoid potential interference reactions caused by solvent degradation products.

3.3 Selection of Extraction pH and Temperature.

The extraction pH was chosen to balance two characteristics, which are detrimental to the development and validation of a reliable method. Since the hydrolysate matrix is highly reactive toward GB at high pH, the pH must be lowered prior to extraction to be able to recover the GB spike and validate the method. However, lowering the pH also increases the chances for GB reformation during analytical workup. Table 16 shows results obtained from sequential extraction of aliquots of ECBC hydrolysate at pH 8.6, 7.6, 6.0 and 4.5 using chloroform and methylene chloride as extraction solvents. Analysis could not be performed below pH 4.5 because the presence of HF is detrimental to the system. Estimated half-lives for GB in aqueous solution at 25 °C are 5 hr at pH 8, 30 min at pH 9, 3 min at pH 10, and 18 s at pH 11.[10,11] Based on this information, the extraction pH selected for this method was 9.

To test the accuracy of the estimated half-lives, extractions were performed at pH 9 using methylene chloride as extraction solvent. Each hydrolysate aliquot was spiked with 100 ng/mL (ppb) GB. Extractions were performed at 20 °C and -2 °C. All extractions were performed as quickly as possible after adding the spike and mixing the solution except one, which was started 20 min after adding the spike. Spike recoveries obtained are shown in Table 17. As predicted by the estimated half-lives, results confirm that extraction time is of the essence at pH 9 and room temperature. An extraction temperature of 4 °C (refrigerator temperature) was selected for the method MDL studies to maximize spike recovery and increase extraction reproducibility.

Table 16. Effect of GB Hydrolysate pH on GB Detection (ECBC Hydrolysate)

Extraction pH	µg/mL (ppm) GB	
	Chloroform Extract	Methylene Chloride Extract
8.6	5.8	None detected
7.6	6.9	None detected
6.0	9.2	None detected
4.5	18.0	1.4

Table 17. GB Spike Recoveries as a Function of Extraction Temperature and Time*

Hydrolysate Used	Extraction Temperature	Time Between Spike and Start of Extraction	GB Recovery
ECBC	20 °C	None	78 %
ECBC	-2 °C	None	86 %
Anniston	20 °C	None	82 %
Anniston	20 °C	20 min	59 %

* Extraction solvent methylene chloride; pH 9; hydrolysate GB spike 100 ng/mL

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