

## ANALYTICAL ARTIFACTS IN GB ANALYSIS

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### Abstract

*Analysis of GB hydrolysates have previously shown low level (ng/mL) backgrounds of GB, despite kinetic data supporting an expectation to the contrary. While a number of explanations have been proposed, one of the most widely accepted involves reformation of GB during analysis. Application of cool on-column sample injection with GC-MS to the analysis of GB in extracts of caustic hydrolysate demonstrates the absence of the artifact detected in a traditional heated hot GC injector traditionally used in gas chromatography. Hydrolysate extracts containing low-level GB spikes produced detectable levels using the cool-on-column injector, confirming that intact GB in the extracts would be detectable. The absence of GB in extract analysis using a cool on-column injector and presence of GB in a hot injector provides unequivocal evidence that GB is an analytical artifact arising from hot injectors. These results led to significantly improved analytical methods for GB in caustic hydrolysate, with method detection limits below 5 ng/mL determined for the original hydrolysate.*

### 1 Introduction

The United States is committed to the timely disposal of its stored chemical warfare agents. The U.S. Army's Chemical Materials Agency (CMA) is charged with the responsibility to store, treat, and dispose of chemical weapons safely and effectively. In 1997 Congress established the Assembled Chemical Weapons Alternatives (ACWA) Program to develop and validate alternative technologies to the baseline incineration process for the demilitarization of assembled chemical weapons. The alternative technology to destroy chemical agents in munitions selected for the Blue Grass Chemical Agent Destruction Pilot Plant (BGCAPP) was neutralization and supercritical water oxidation (SCWO). A preliminary technical risk reduction survey performed as part of the original plant design identified a number of Technical Risk Reduction Projects (TRRPs). These projects corresponded to data gaps that required study and testing to mitigate a perceived risk to an acceptable level.

Included among these risks was the effective and efficient Neutralization of GB and the capability to definitively and accurately quantitate aqueous GB concentration at the low levels required to clear the hydrolysate for transfer to the supercritical water oxidizing (SCWO) treatment. While 30 plus years of experience of neutralization of GB with caustic hydrolysis demonstrates its promise in conventional demilitarization activities, low levels of GB (several  $\mu\text{g/mL}$ ) have previously been observed during analysis<sup>1,2</sup>

Subsequent testing by Edgewood Chemical Biological Center (ECBC) and during the ACWA program suggested that these problems could be overcome<sup>3,4</sup>. However, the origin of the GB artifact was not definitively reported, and it was recommended that additional testing be conducted prior to designing a larger scale system to minimize operating costs and production time. Critical to this testing was characterization and validation of the GB analysis method in accordance with 40 CFR Part 136 Appendix B<sup>5</sup>.

In 2004, the Bluegrass Chemical Agent Pilot Plant (BGCAPP) team sought to characterize the ECBC method using hydrolysate generated from GB recovered from an M55 rocket at Anniston Army Depot (“Anniston” GB). In the course of performing the analyses, the ECBC/CAMDS method for GB analysis in Anniston GB hydrolysate was found to provide unacceptably noisy GC-MSD chromatograms. Method variables were adjusted to obtain optimal analytical performance (chromatogram “cleanliness”, GB spike recovery in hydrolysate, method detection limit), but in all cases, low levels of GB were detected in the extracted hydrolysate. The BGCAPP team sought to unequivocally identify the origin of the GB artifact and eliminate it.

## 2 Experimental.

There are two primary steps in the analysis of GB in a caustic hydrolysate matrix: solvent extraction with pH adjustment at ambient temperature and instrumental analysis by gas chromatography. In the extraction of the native caustic hydrolysate, 5 mL of the hydrolysate is placed in a 50 mL conical centrifuge tube. 2mL of dichloromethane solvent is added without agitation to prevent extraction of GB prior to pH adjustment. A 3 mL portion of phosphate buffer (60 g KH<sub>2</sub>PO<sub>4</sub> and 30 g NaCl in 200 mL Milli-Q water) is then added with a Luer lock syringe. The tube is agitated vigorously using a vortex mixer and then centrifuged. The dichloromethane layer is withdrawn and submitted for GC-MS analysis.

Characterization of the method by 40 CFR Part 136 Appendix B requires determination of recovery efficiency for spiked matrices<sup>5</sup>. For the extraction of the spike caustic hydrolysate, the same steps as the extraction of the native caustic hydrolysate were followed except for the addition of the GB spike to the buffer before adding the buffer into the hydrolysate contained within the 50 mL conical tube. Spiking in this manner prevents unnecessary modification of the caustic hydrolysate and degradation of GB before extraction is undertaken. The level of spike is determined in accordance with 40 CFR Part 136 Appendix B.

The instrumental analysis is conducted on an Agilent GC-MS instrument equipped with an analytical column (Rtx-5Sil MS, 30 m X 0.25 mm X 1 µm) and a cool on-column injection inlet. Quantitation of GB is determined with the instrument operating in single-ion monitoring (SIM) mode, and collection of ion abundances corresponding to the three fingerprint ions of GB at m/z 125, 99, and 81.

GB calibration solutions are analyzed first before any of the extracts. The GB calibration curve is plotted using linear regression, forcing the y-intercept to zero. The level of GB in the extracts is determined through comparison to the calibration curve. At various points in the method development and characterization, low levels of GB were introduced (“spiked”) into the hydrolysate and subsequently extracted and analyzed to characterize the capability to recover GB from hydrolysate.

## 3 Results and Discussion

### 3.1 Characterization of GB Source Material Composition

Analyses of Anniston and ECBC GB source materials are provide in Table 1.

**Table 1. NMR Analysis of Neat GB Source Materials**

Compound Name/ Abbreviation	Formula / Molecular Weight	ECBC GB Feed (SH) GB-S- 0290-CTF-N1	Anniston M55 Liquid GB (Jan 2004)
Isopropyl methylphosphono-fluoridate / GB	(iC <sub>3</sub> H <sub>7</sub> O)CH <sub>3</sub> P(O)F /140	92.6	81.8 *

Compound Name/ Abbreviation	Formula / Molecular Weight	ECBC GB Feed (SH) GB-S- 0290-CTF-N1	Anniston M55 Liquid GB (Jan 2004)
Diisopropyl methylphosphonate / DIMP	(iC <sub>3</sub> H <sub>7</sub> O) <sub>2</sub> CH <sub>3</sub> P(O) /180	0.62	11.3
Difluoromethylphosphonate/DF	F <sub>2</sub> CH <sub>3</sub> P(O) /100	2.04	0.18
Methylphosphonofluoridic acid / FA (fluoracid)	FCH <sub>3</sub> P(O)OH /98	0.028	1.97
Isopropyl methylphosphonic acid / IMPA	(iC <sub>3</sub> H <sub>7</sub> O)CH <sub>3</sub> P(O)OH /138	n.d.	0.54
Dimethylphosphinic Acid / Me <sub>2</sub> P(O)OH	(CH <sub>3</sub> ) <sub>2</sub> P(O)OH /94	n.d.	0.028
Diisopropyl fluorophosphate / (iPrO) <sub>2</sub> P(O)F, DFP	(iC <sub>3</sub> H <sub>7</sub> O) <sub>2</sub> P(O)F /184	0.019	0.18
Hydrofluoric acid / HF/F <sup>-</sup>	HF /20	0.90	0.22
Hexafluorophosphoric acid/ HPF <sub>6</sub>	HPF <sub>6</sub> / 146	0.012	0.040
Isopropanol / iPrOH	iC <sub>3</sub> H <sub>7</sub> OH /60	0.425	0.48
Tri-n-butylamine (TBA) -HF	(nC <sub>4</sub> H <sub>9</sub> ) <sub>3</sub> N -HF /205	2.94	2.71
Diisopropylamine –HF /iPr <sub>2</sub> NH-HF (tentative)	(iC <sub>3</sub> H <sub>7</sub> ) <sub>2</sub> NH-HF /122	0.40	0.28
N,N'-diisopropylcarbodiimide / DICDI	iC <sub>3</sub> H <sub>7</sub> -N=C=N-iC <sub>3</sub> H <sub>7</sub> /126	n.d.	n.d.
N,N'-diisopropylurea / DIPU	iC <sub>3</sub> H <sub>7</sub> -NH-C(O)-NH-iC <sub>3</sub> H <sub>7</sub> /144	n.d.	0.78

Historically, GB was stabilized with one or both of the following materials: Tri-n-butylamine (TBA) CAS Number 50-31-7 and N,N'-diisopropylcarbodiimide (DICDI) CAS Number 693-13-0<sup>1</sup>. Unstabilized GB also exists, but not in stockpile materials. ECBC GB was obtained from the Chemical Transfer Facility at Aberdeen Proving Ground and Anniston GB from a drained rocket at Anniston Army Depot. NMR analyses determined GB purity to be greater than 80% GB and similar levels of TBA (approximately 3% of the total weight)<sup>6</sup>. While DICDI was not detected in either GB source materials, the presence of diisopropylurea (DIPU) in the Anniston GB source is indicative of the use of TBA/DICDI stabilization.

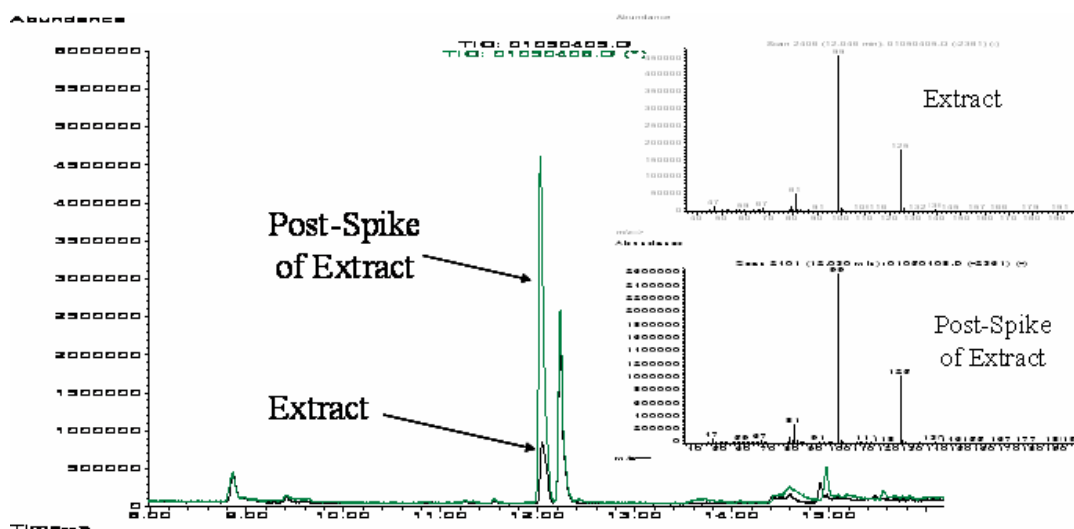
### 3.2 Authentication of GB Artifact in Hydrolysate

Figure 2 shows chromatograms collected from analysis of GB hydrolysate at Battelle's Hazardous Materials Research Center (HMRC) using hot injection GC-MS. The black trace demonstrates a peak in the GB window, and analysis of a spiked sample of GB (green trace). The similar retention times are suggestive of the presence of GB, and confirmed with the mass spectra taken in the GB retention window and presented as an inset to the figure. A subsequent analysis of this extract several months later also demonstrated apparent GB in the hydrolysate, inconsistent with an expectation of complete caustic neutralization. In both analyses, a hot injector GC-MS inlet was used. These results posed two questions: authenticity of the detected GB, and potential reversal in the destruction of GB.

**Table 2. GB Hydrolysate Extracts Analytical Results**

Date		September 2004	January 2005
Laboratory		HMRC	Columbus
Extracting Solvent	Sample Identity	GB, µg/mL	GB, µg/mL
CH <sub>2</sub> Cl <sub>2</sub>	50311-97-10	0.159	10.942
	50311-97-12	0.163	10.841
	50311-97-14	0.164	10.552
CHCl <sub>3</sub>	50311-76-15	0.083	0.550
	50311-75-18	0.793	2.453
	50311-75-07	0.885	2.373

In order to answer the two questions, experiments were devised to search for the answers sequentially. To authenticate the artifact, we analyzed the extracts again followed with an analysis of a solvent blank, and then with the GB post spiked aliquots of the extracts. The solvent blank was necessary to ensure the GB detected was not due to carryover from previous samples. The post spiking made at a significantly higher level would confirm the retention time of GB and the mass spectral fingerprints of the agent.

**Figure 1. Authentication of GB Detected in Hydrolysate Extract**

The post spiked extract in Figure 1 shows an increase in response of GB and that the GB peak in the extract is congruent to the GB in the post spiked extract. The mass spectral fingerprints for both samples are comparable. The GB in the chromatograms is real.

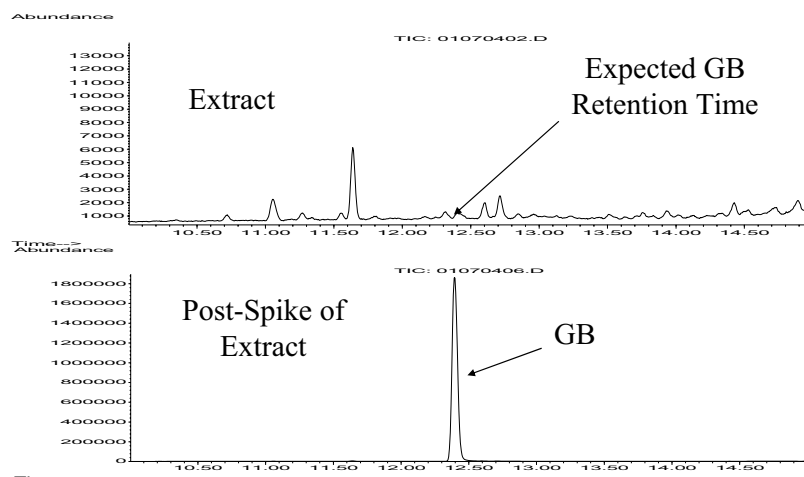
Investigating the possibility of the reversal of GB destruction and determining whether aging of extracts accelerates reformation of GB would require an enormous amount of resources that were not available. In addition, it was considered more likely that analytical artifacts were the cause of the GB in the chromatograms. Therefore, a literature search to identify alternate analytical techniques was conducted. The search identified four candidate analytical techniques:

- Purge and Trap Gas Chromatography Mass Spectrometry (PTD-GC-MS)
- Solid Phase Microextraction Gas Chromatography Mass Spectrometry (SPME- GC-MS)
- Cool on-column GC-MS
- Enzymatic detection

Evaluation of the first three of the four analytical techniques had hardly commenced when the cool on-column results showed the absence of GB in the hydrolysate extracts.

### 3.3 Cool-on-Column Analysis of Select Hydrolysates

To make certain that the cold injector result was not due to signal suppression as a result of a low injector temperature (ambient), a post-spiked sample of an extract was also performed (Figure 2).



**Figure 2. Cool on-Column Analysis of Hydrolysate**

Since analysis of spiked hydrolysate extract provides a detectable GB response and no such response for the simple extracted hydrolysate, a compelling argument is made that the artifact is attributed to the GC heated inlet for Anniston GB hydrolysate.

### 3.4 GC-COC-MS Precision and Accuracy (P&A) Study

A precision and accuracy study was used to certify the cool-on-column GC-MSD method. A modified Class I P&A was conducted for the method to determine how well the analytical method, instrument, and operator perform. Results were evaluated by pooling all 4 days of generated data into a single group and a linear regression analysis of the target concentration (TC) versus the found concentration (FC) for the data population performed.

**Table 3. P&A Method Types and Certification Requirements**

Type of Certification	Number of Operators	Number of Instruments	Number of Days <sup>a</sup>	Target Concentrations <sup>b</sup>	Total Number of Points <sup>c</sup>	Criteria
Class I	2 or more	2 or more	4	8 each at 0.0, 0.5, 0.75, 1.0, 1.5, and 2.0 times the monitoring level	48	<ul style="list-style-type: none"> <li>▪ Target action level (TAL) is greater than the statistically calculated limit of quantification (LOQ).</li> <li>▪ Uncertainty in found mass (UIFM) is less than or equal to <math>\pm 25</math> percent. Recovery at the monitoring level is within 75 to 125 percent.</li> <li>▪ For MSD methods, UIFM is less than or equal to <math>\pm 40</math> percent.</li> </ul>
Modified Class I	1 or more	1 or more	4			
Class A	1	1	1	6 each at 0.1, 0.5, 1.0, and 2.0Z	24	<ul style="list-style-type: none"> <li>▪ Estimated analytical recovery must demonstrate accuracy within <math>\pm 25</math> percent with 95 percent confidence at the monitoring level.</li> </ul>



operator prior to initiation of testing, in this case 1Z) to intersect with the lower confidence boundary. The corresponding found concentration is the found action limit (FAL), the concentration at which there is 95 percent certainty that the concentration detected is the at the hazard level or greater. If a horizontal line is generated from the intersection of the hazard limit and the lower confidence boundary to the upper confidence boundary, the intersection of this line and the upper confidence boundary represents the target action limit (TAL). The TAL is the target or true analyte concentration that can be distinguished as lower than the hazard level with confidence.

**Table 4. CERTIFY 1.0 Results**

	TAL	LOQ	Decision limit	UIFM (<40%) <sup>1</sup>	% Percent Recovery (75> <125%) <sup>1</sup>	TAL>LOQ
<b>ECBC GB</b>	0.57	0.057	0.024	16.25	93.78	yes
<b>Anniston GB</b>	0.587	0.031	0.015	22.06	82.81	yes

<sup>1</sup> Requirements as outlined in U.S. Army CMA Laboratory and Monitoring Quality Assurance Plan

Precision and accuracy studies performed using both ECBC and Anniston GB passed the standards set by CMA in the LMQAP for a modified Class I P&A (Table 3). For both data sets (Table 4), the TAL is greater than the LOQ, %UIFM is less than 40%, and the percent recovered is between 75 and 125 percent.

#### 4 Conclusion

The GC-COC-MS method demonstrated that the GB detected in extracts of caustic hydrolysate is an analytical artifact. The method shows consistent and reproducible data for both the ECBC and Anniston GB hydrolysates.

#### 5 Acknowledgments

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#### 6 References

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