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DETERMINATION OF NIST-TRACEABLE QUANTITATIVE WEIGHT PERCENTAGE PURITY FOR G AGENT STANDARDS

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14. ABSTRACT

Nuclear magnetic resonance (NMR) with phosphorus-31 detection is described to determine the weight percent purity of feedstock samples of agents GA and GD in a way that is National Institute of Standards and Technology (NIST)-traceable. A Precision and Accuracy test is described.

15. SUBJECT TERMS

Tabun (GA)
Soman (GD)
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PREFACE

The work described in this report was authorized under contract number W911SR-10-D-0004. This work was started in December 2013 and completed in May 2018.

This report was published through the Technical Releases Office; however, it was edited by the Technical Information Specialist, Toxicology, Toxicology and Obscurants, Research and Technology Directorate, U. S. Army Edgewood Chemical Biological Center (ECBC).

The use of either trade or manufacturers' names in this report does not constitute an official endorsement of any commercial products. This report may not be cited for purposes of endorsement.

This report has been approved for public release.

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DETERMINATION OF NIST-TRACEABLE QUANTITATIVE WEIGHT PERCENTAGE PURITY FOR G AGENT STANDARDS

1.0 INTRODUCTION

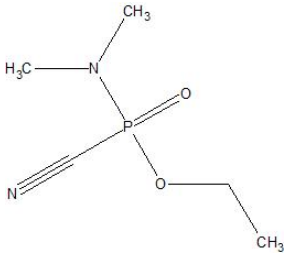
This report is on the procedure to determine the purity by Nuclear Magnetic Resonance (NMR) of the G series agents Tabun (GA) and Soman (GD). This procedure is based on published Technical Report procedures for using NMR instruments for determining the purity of CW agent samples.[1,2,3,4] Previous National Institute of Standards and Technology (NIST)-traceable methods were described for HN-3,[5] HN-1,[6] HD,[7] and T.[8]

The procedure utilizes an internal standard with a known purity to establish an absolute weight percentage for the analyte of interest. Identifying the structures of other components in the mixture is not necessary. All that is necessary is to know the NMR chemical shifts of the major analyte, the internal standard, and the average molecular weights. The weight percent calculations are not negatively affected by the presence of unidentified compounds or undetectable components in the sample (for example, inorganic salts, insoluble solids, etc.), as long as the sample is homogeneous or a thoroughly mixed suspension before it is portioned out from the storage container.

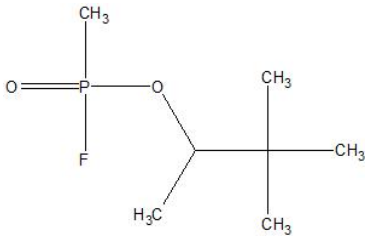
In the previous reports for HD, HN-1, and HN-3, the procedure used a NIST standard material as an internal standard. An internal standard was purchased from Sigma Aldrich that has a NIST-traceable purity. A balance calibrated with NIST traceable weights was also used. These modifications make the method NIST-Traceable.

For the method for Agent T and for this method, a chemical from Sigma Aldrich was used as an internal standard that wasn't NIST certified by the vendor. A secondary step was used to determine the purity of the internal standard referenced to a NIST standard before calculation of the agent purity.

The G series agents contain phosphorus atoms, and phosphorus is a spin ½ nucleus that gives a good NMR signal with good peak resolution. To determine the purity of G series agents, phosphorus-31 (P-31 or ^{31}P) NMR was used for detection. Precision and accuracy testing of the method was done.



GA, MW 162.13



GD, MW 182.17

2.0 PROCEDURE

2.1 Supplies

The following supplies can be used for the procedure. Equivalent supplies may be available from other vendors.

GA and GD neat standards were obtained from the CASARM program, Edgewood Chemical Biological Center, Aberdeen Proving Ground, MD, for this project.

A secondary internal standard of triethyl phosphate is used, purchased from Sigma Aldrich, Part Number 538728, CAS No. 78-40-0, ReagentPlus® ≥99.8% purity. This standard is not noticeably hydroscopic and has excellent stability and purity. The NIST-traceable primary internal standard was dimethyl sulfone, purchased from Sigma Aldrich, Part Number 41867-1G, CAS No. 67-71-0, as a TraceCERT® certified reference material (CRM) standard for quantitative NMR.

The following supplies were purchased from Wilmad (1172 NW Boulevard Vineland, NJ 08360, phone 800-220-5171, http://www.wilmad-labglass.com/ordering/index.jsp):

Item	Part Number
5 mm dia. 8" long NMR tube	WG-1000-8-50
Teflon inserts	6005
pasteur pipets, 9"	C-7095B-9

The following supplies were purchased from Sigma Aldrich (http://www.sigmaaldrich.com/chemistry.html):

Item	Part Number
chloroform, 99.9% D	23,689-6

A JEOL ECS-400 Nuclear Magnetic Resonance spectrometer with a 400 MHz (9.8 T) superconducting magnet and 5 mm liquid analysis probe was used. A Sartorius Cubis microbalance (Model MSA6.6S-000-DM, precision ±1 µg) was used for measuring weights, since it allows small amounts of agent to be measured with good accuracy. It was installed in a fume hood and calibrated using NIST-traceable weights. A Sartorius analytical balance was also used, calibrated with NIST-traceable weights to ±20µg. This balance was less accurate, but it is more commonly available than the microbalance. The microbalance is susceptible to static charges on the sample vials after handling with polymer gloves, which can introduce errors.

NMR systems and balances from other vendors should give comparable results, if the operators have the appropriate training.

Other common laboratory equipment is used, including a vortex mixer, spatulas, and volumetric pipets. This equipment is not critical to the accurate performance of the method.

2.2 Sample Preparation

This procedure was performed under proper engineering controls, in accordance with surety and safety regulations, equipment validations, and SOPs approved by the ECBC Safety and Health Office. The balance was calibrated using NIST-traceable weights.

a. Tare a screw-cap vial with cap on the balance. Transfer 10-20 mg of neat internal standard into the vial. Replace the cap and determine the weight of the internal standard to an accuracy of 0.01 mg, and record the weight. Tare the balance after recording the weight.
b. Add 4-30 mg of feedstock agent sample to the vial. The liquid agent can be measured with a pipet (4 to 30 µl of liquid). (A precision and accuracy test of this method has been done over this range of agent amounts.) Record the weight to an accuracy of 0.01 mg in a laboratory notebook.
c. Add 0.4 ml of reagent-grade deuterated chloroform ($CDCl_3$).
d. Vortex or mix the sample for at least 15 s to dissolve both compounds in the solvent.
e. Transfer the solution into a PTFE NMR tube insert. (Optional: A glass 4mm insert tube may be used and flame sealed, if desired)
f. Place the insert into a 5 mm glass NMR tube and push it to the bottom of the tube. Cap the insert with a PTFE stopper. Cap the NMR tube with a cap, or flame seal the outer tube without damaging the insert. This is done to doubly contain the agent sample so it can be removed from engineering controls.

2.3 Obtaining the NMR Spectrum

Operators of the NMR must have sufficient training to understand the general operational principles and to use the instrument computer control to perform the required tasks. To validate the NMR is functioning correctly, a manufacturer sample such as 0.01% ethylbenzene in deuterated acetone can be analyzed to check the signal response. Detailed QC specifications are not included in this method.

a. Place the NMR tube into the spinner using a depth gauge to orient the tube at the correct depth relative to the detection coils. Lower the sample into the magnet bore. (Note: The doubly-contained NMR tube that contains agent will be outside of engineering controls.)
b. Lock the instrument on the deuterium signal from the $CDCl_3$.
c. Shim the magnet to maximize the lock signal.
d. Tune and match the probe.
e. OPTIONAL: Determine the T_1 relaxation time of the analytes in the sample solution using an inversion recovery experiment, following the instrument instructions. This procedure to determine the T_1 relaxation time should be done if there is an inconsistency in the purity determination, if a new instrument is being used, or if it is necessary to minimize the experiment acquisition time.
f. Load instrument parameters to acquire a 1D spectrum. For a P-31 spectrum, if the T_1 relaxation time is not determined (i.e., step e is not performed), then set the relaxation time to 90 s. (This is typically 20 times longer than the longest T_1 in the solvent.) Do not use Nuclear Overhauser Enhancement (NOE). Proton decoupling is used.
g. Open a new data file on the NMR computer with a unique filename, the sample information, and notebook reference. The following parameters are used. (Actual parameter names will vary depending on the make and model of the NMR and can be found in the NMR documentation.):

    Relaxation time: 90 s or as determined in step e or f.
    Excite pulse: 90º pulse (Determining the time and amplitude for this pulse that corresponds to a 90° excitation should be found in the NMR instrument documentation.)
    Number of data points: 64K
    Number of scans: 16 for P-31
    Sweep width: 300 ppm for P-31
    Center frequency: 15 ppm for P-31. (For best results, the center frequency should be equidistant between the Internal Standard (IS) peak and the analyte peak(s) that will be integrated.)
    Automatic gain determination: on for the first spectrum, but then the same gain can be used for replicates.

h. Acquire data.

i. A total of seven or more replicate runs are acquired for statistical determination of the NMR variability, signal to noise ratio, and integration errors. Several samples can be prepared by weight to determine the weighing statistical errors.

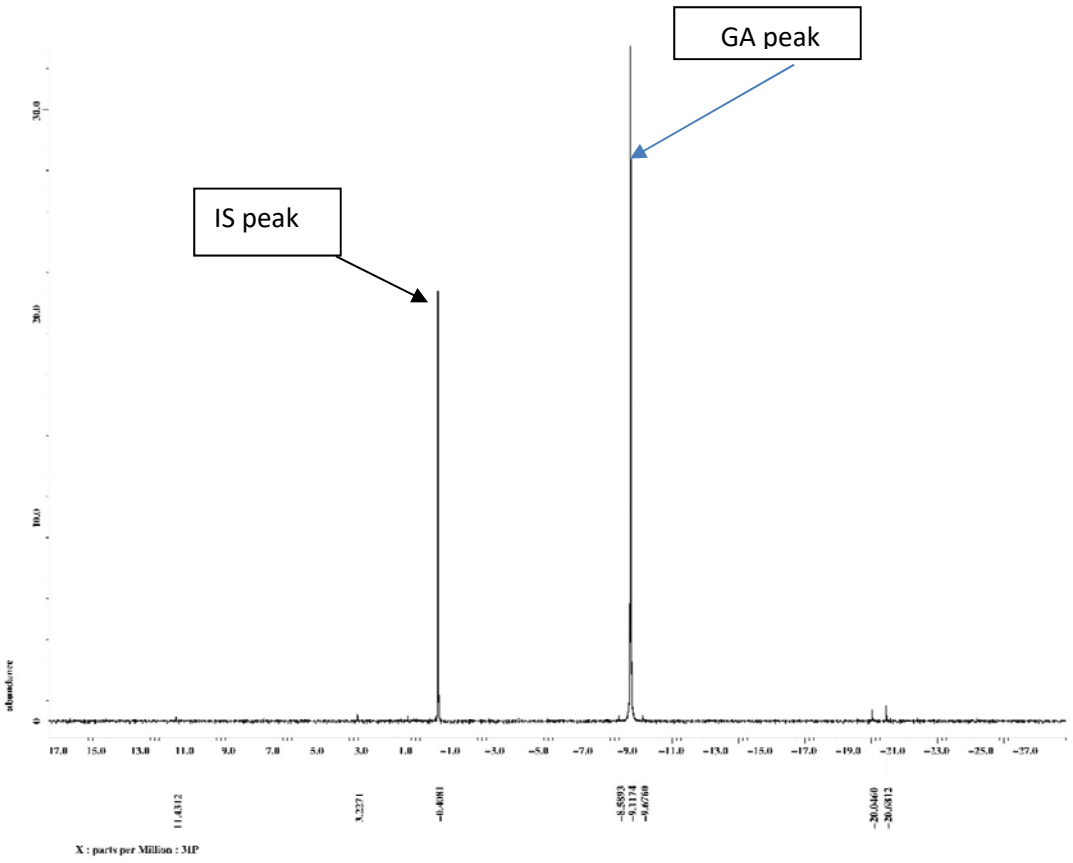


Figure 1. Phosphorus-31NMR spectrum of agent GA and the internal standard TEP.

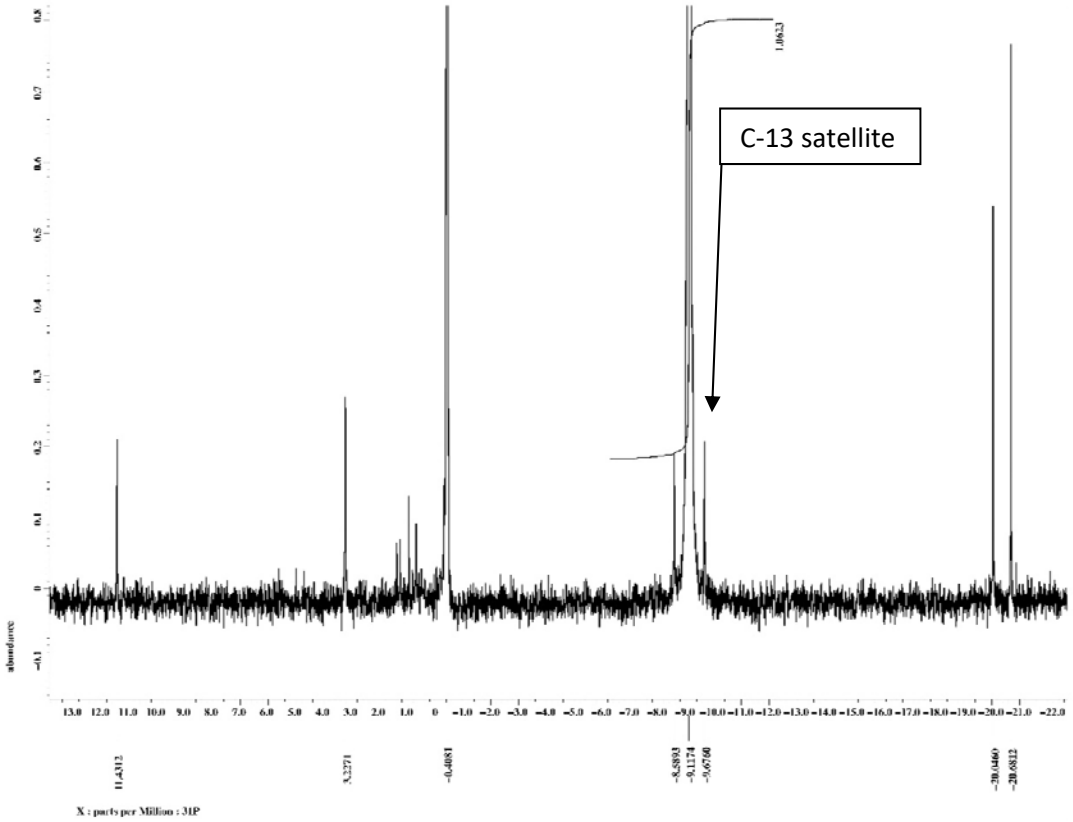


Figure 2. Phosphorus-31 NMR spectrum of agent GA and the internal standard TEP, showing the same spectrum as Figure 1 but with expanded scale and showing the integral trace of the GA peak. Aside from the IS peak, the other peaks are from impurities in the standard, which are common for GA standards.

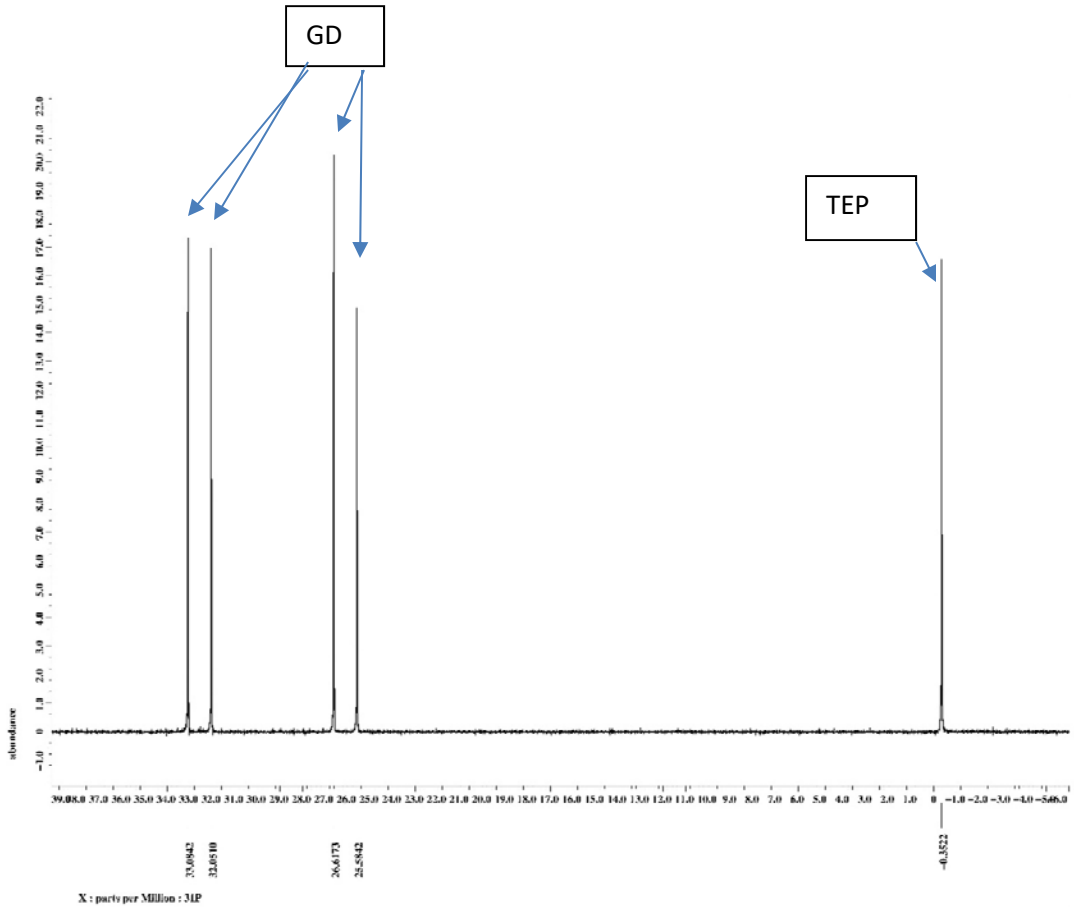


Figure 3. Phosphorus-31 NMR spectrum of agent GD and the internal standard TEP. GD is unusual because the compound has 4 peaks. The wide splitting is caused because the P-31 is bonded to an F atom, and the narrower splitting is caused by the two GD diastereomers, produced because GD has two chiral centers.

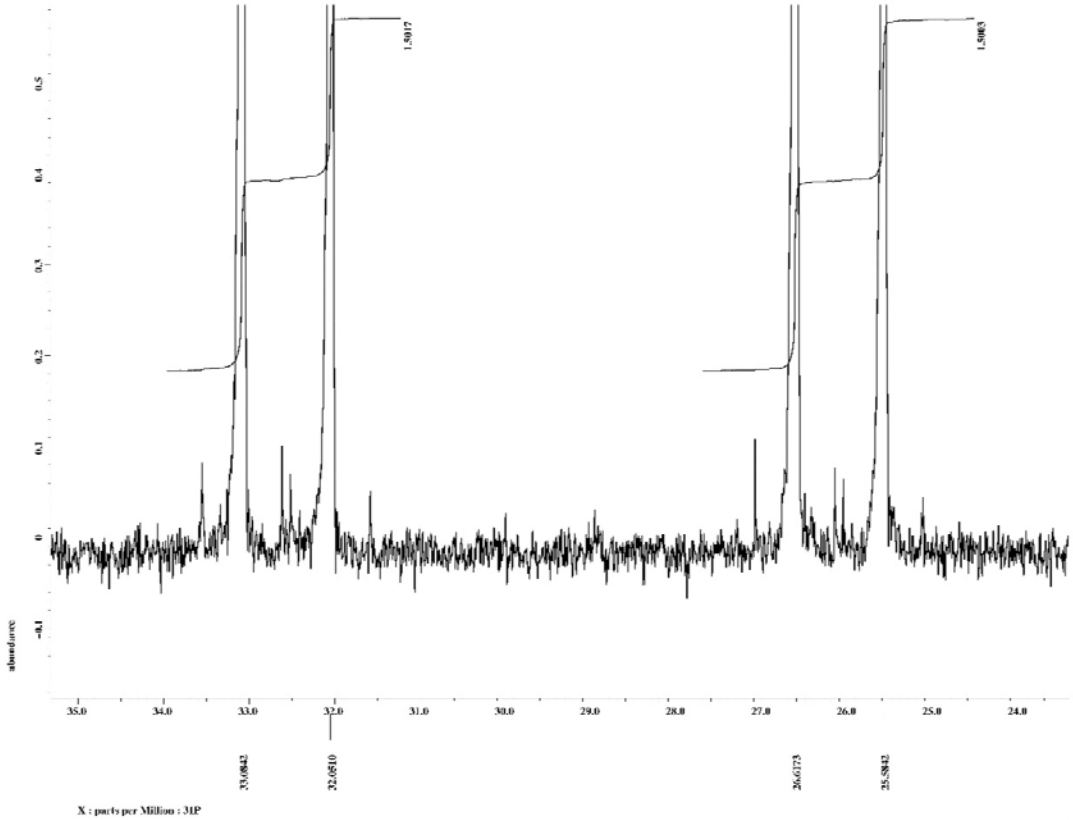


Figure 4. Phosphorus-31 NMR spectrum of the four agent GD peaks, showing the same spectrum as Figure 3 but with expanded scale and showing the integral trace of the GD peaks. C-13 satellite peaks are present for each GD peak, and because they are close to the main peaks, it is often easier to integrate pairs of peaks together.

2.4 Data Processing

a. Apply a window function (exponential multiplication). This may be done using a line broadening parameter in the range of 0.5 to 2 Hz, which can be adjusted to enhance the signal to noise ratio. A larger line broadening produces wider peaks, which can degrade the resolution between peaks. The same value of line broadening must be used for all the data files for the repeat runs.
b. Fourier transform (FFT) to convert data from time to frequency domain and to produce the NMR spectrum.
c. Phase all peaks in the spectrum and correct the baseline if necessary.
d. If necessary for reporting, reference the chemical shift against the internal standard.
e. Integrate the relevant peaks in the spectrum to obtain the relative areas. Some data systems will perform automatic integration of peaks. It is important for the operator to examine the integration to make sure that the correct parts of the peak are included in the integration. If the automatic integration is incorrect, the spectrum can be manually integrated. In particular, compounds with P-C bonds can have satellite peaks on each side of the main peak. These peaks are produced by molecules that have a natural abundance of ^{13}C isotopes, and they each represent 0.55% of the center peak. The satellite peaks should be included in the integration of the central peak. (If the magnet is not well shimmed, the satellite peaks may not be resolved.) If proton decoupling is not used, the P-13 peak can also be split into a multiplet by the protons. All the peaks in the multiplet must be integrated.

2.5 Purity Determination of Secondary Standard

Since the secondary standard, triethyl phosphate, is not a NIST traceable standard, a second purity determination is needed to determine the accurate purity of the standard relative to a primary standard that is NIST traceable. This determination is done using the same procedure as sections 2.2 to 2.4. The determination can be done either before or after the determination with the CW agent, since there isn't any adjustment to the instrument that is required, only a calculation based on the found purity result as discussed in section 2.6.

Since this step doesn't involve CW agent, some of the safety requirements can be relaxed. For example, the sample can be singly contained in a glass NMR tube rather than doubly contained. Several primary standards are commercially available and can be used, but the standard dimethyl sulfone was used. Proton NMR was used to perform the purity determination. For more details about proton NMR, see previous technical reports.[5-8]

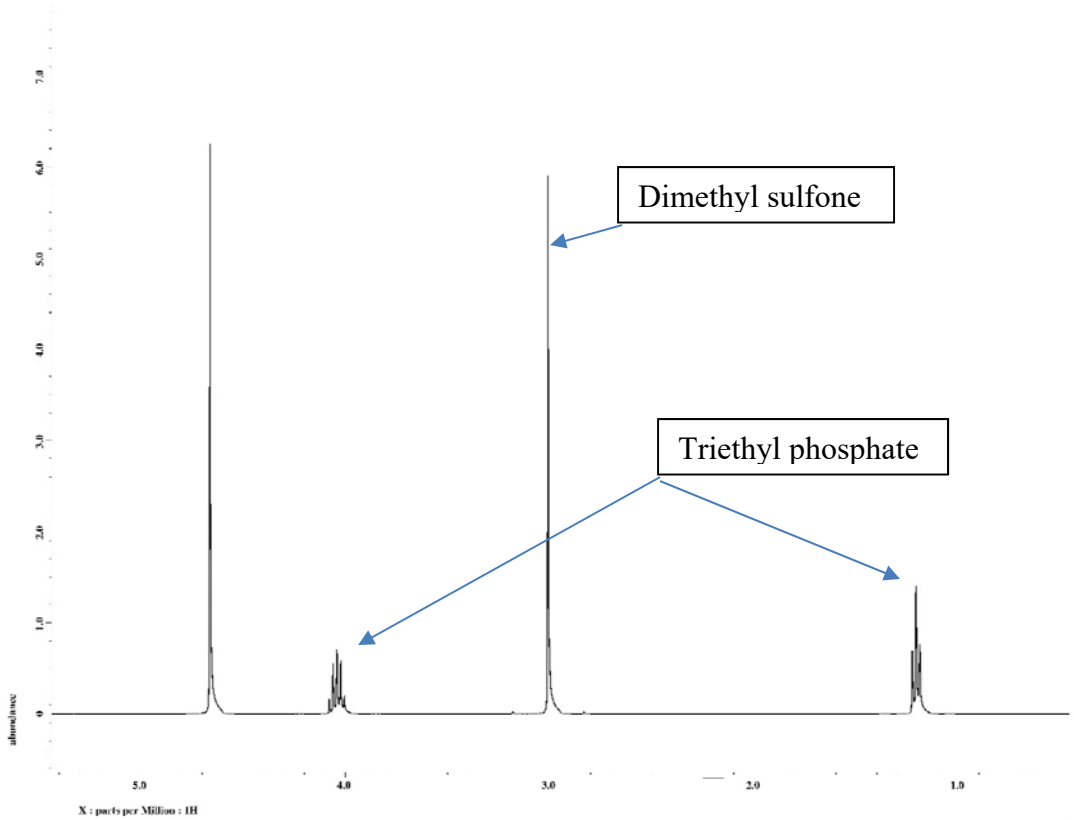


Figure 5. Plot showing the proton spectrum of the internal secondary standard, triethyl phosphate and the primary standard, dimethyl sulfone. All the peaks are baseline resolved, so there is no uncertainty in integrating them.

2.6 Purity Calculation

The weight percent of the analyte (Wt% A) in the sample is calculated using the following formula, where analyte A is the agent and IS is the internal standard. The same formula is used for all spectra and internal standards, but the parameters will change based on the molecule that is being detected.

$$\text{Wt\% A} = \frac{\text{Area under A peak}}{\text{Area under IS peak}} \times \frac{\text{MW of A}}{\text{MW of IS}} \times \frac{\text{Weight IS}}{\text{Weight A}} \times \frac{\text{No. identical P(IS)}}{\text{No. P(A)}} \times (\text{Pur IS})\%$$

The parameters are as follows:
Area under A peak = total sum of the area of the peak and the satellite peaks that are associated with them;
Area under IS peak = total area of the peak and the satellite peaks;
MW of A = average molecular weight of the agent;
MW of IS= average molecular weight of the secondary internal standard;

Weight IS=balance recorded weight of internal standard in the vial;
Weight A=balance recorded weight of agent sample in the vial;
No. identical P(IS)=the number of identical phosphorus atoms in the internal standard;
No. P(A)=the number of phosphorus atoms in the integrated peaks of the analyte;
Pur IS = the purity of the secondary internal standard that is found from the primary purity determination.

Since the compounds and the internal standards only contain one phosphorus atom, the number if identical atoms in the molecules is one. The peaks are commonly well resolved and can be integrated without errors. GD has four peaks, and all four must be integrated and added together for the calculation. GA only has one peak.

If the analytical statistical accuracy is reported, the calculated weight percentages for each replicate run can be averaged to find a mean (average) and standard deviation. For seven replicates, the mean ± 2 × (standard deviation) provides the 95% confidence range.

3.0 PRECISION AND ACCURACY APPROACH

The purity determination method was validated using a modification of the protocol for a Class I Precision and Accuracy (P&A) test.[5-8] This kind of test is typically used for validation of air monitoring methods. The requirements are not exactly applicable to an NMR purity determination test, so it was modified as needed.

A four-day test was used. On each day of the test, 10 samples and two blanks were prepared. The 10 samples were prepared with amounts of agent of 0.2Z, 0.5Z, 0.8Z, 1.0Z, and 1.5Z, each sample in duplicate, where Z = 20 mg. As a result, the purity method was validated for a quantity of agent from 4 mg to 30 mg.

This testing was not in strict accordance with a normal P&A test. First, NMR is not a trace detection method, and the purpose of the method is not to detect low amounts of agent for safety purposes, as it is for air-monitoring applications. For a typical Class I P&A, the amount of agent is measured in nanograms, usually dictated by the need to detect mandatory exposure limits. The NMR method is measured in milligrams, and the Z level is arbitrarily based on detection limits of the current instrument. Since there is no chance of carryover between samples on the NMR, some of the blanks samples weren't reprepared.

The data from a P&A test is typically processed using a program called Certify (latest version is version 6.0). Certify contains statistical criteria for the acceptance of data or the test method within acceptable measurement limits. Certify does not apply to the NMR purity determination very well, however. The target Z levels (where Z is the target amount) are set in the program to be the same for all replicates from the four-day test. For this testing, the approximate target amounts are measured using an adjustable pipet set for the target levels. For

the NMR purity method, the actual amount of agent is determined by the weight of the agent taken from the NIST-traceable balance. The accurate weight is different and more accurately known for each sample of the 4-day test than the target measured with a pipet, even if the nominal target Z is the same. The accurate weight cannot be entered into the Certify program as an x-coordinate, only the target Z level.

The T_1 for the solutions (see Section 2.3 step e) was not determined, and 96 s was used as the P-31 NMR relaxation delay time.

3.1. P&A Results for NMR Analysis of GD

Tables 1 to 4 show the data sets collected on each day of the four-day P&A test. Data are collected using P-31 NMR. The purity of the secondary standard was found using separate analysis runs of proton NMR, and the data for them is shown in Table 5. The found purity was 99.87%, in good agreement with the specification.

Figure 6 shows the data plotted together with the regression lines and correlation coefficients. Correlation coefficients for all the days between the target Z (as a weight) and the found Z are >0.99.

Table 1. P&A Data from Day 1 for GD

Area of Analyte (agent)	Area of Standard (TEP)	Wt. Of Standard	Sample Weight	Z (wt agent/ 20 mg)	Found Z
2.9643	1.0429	10.38	29.69	1.4845	1.4734
3.0991	1.0225	10.01	30.41	1.5205	1.5152
1.9934	1.0315	10.32	20.13	1.0065	0.9960
2.0447	1.0574	10.40	20.06	1.0030	1.0043
1.5385	1.0256	10.68	16.10	0.8050	0.8001
1.6135	1.0363	10.16	16.25	0.8125	0.7900
0.8691	1.0283	11.26	9.59	0.4795	0.4753
0.8575	1.0352	11.65	9.59	0.4795	0.4819
0.3569	1.0444	10.99	3.88	0.1940	0.1876
0.3873	1.0251	13.97	3.90	0.1950	0.2636
0.0000	1	10.00	0.00	0.0000	0.0000
0.0000	1	10.00	0.00	0.0000	0.0000

correlation coefficient	0.998

Table 2. P&A Data from Day 2 for GD

Area of Analyte (agent)	Area of Standard (TEP)	Wt. Of Standard	Sample Weight	Z (wt agent/ 20 mg)	Found Z
2.9690	1.0336	10.24	30.34	1.5170	1.4690
2.9945	1.0376	10.24	30.03	1.5015	1.4759
1.9417	1.0505	10.52	19.54	0.9770	0.9711
1.9538	1.0405	10.50	19.96	0.9980	0.9846
1.5698	1.0471	10.41	15.77	0.7885	0.7794
1.5730	1.0374	10.45	15.94	0.7970	0.7913
0.9394	1.0422	10.42	9.61	0.4805	0.4691
0.9942	1.1147	10.45	9.52	0.4760	0.4655
0.3668	1.0358	10.22	3.93	0.1965	0.1807
0.4004	1.0402	10.31	3.84	0.1920	0.1982
0.0000	1	10.00	0.00	0.0000	0.0000
0.0000	1	10.00	0.00	0.0000	0.0000

correlation coefficient 0.9997

Table 3. P&A Data from Day 3 for GD

Area of Analyte (agent)	Area of Standard (TEP)	Wt. Of Standard	Sample Weight	Z (wt agent/ 20 mg)	Found Z
2.9245	1.014	10.27	29.94	1.4970	1.4792
2.8577	1.0287	10.70	30.04	1.5020	1.4844
2.0146	1.0541	10.20	19.72	0.9860	0.9736
2.0762	1.0912	10.35	19.73	0.9865	0.9835
1.4890	1.0198	10.59	15.76	0.7880	0.7722
1.5902	1.046	10.56	16.10	0.8050	0.8017
0.9993	1.1017	10.42	9.70	0.4850	0.4720
0.9141	1.026	10.63	9.71	0.4855	0.4730
0.3447	1.0312	10.36	3.74	0.1870	0.1729
0.3732	1.0589	10.39	3.95	0.1975	0.1829
0.0000	1	10.00	0.00	0.0000	0.0000
0.0000	1	10.00	0.00	0.0000	0.0000

correlation coefficient 0.9999

Table 4. P&A Data from Day 4 for GD

Area of Analyte (agent)	Area of Standard (TEP)	Wt. Of Standard	Sample Weight	Z (wt agent/ 20 mg)	Found Z
2.7746	1.0128	10.36	30.38	1.5190	1.4174
2.9084	1.0289	10.21	30.01	1.5005	1.4413
1.8145	1.0281	10.61	20.03	1.0015	0.9352
1.8388	1.0266	10.62	19.87	0.9935	0.9500
1.4913	1.0268	10.52	16.16	0.8080	0.7630
1.4006	1.039	10.52	15.81	0.7905	0.7082
0.9337	1.033	10.57	9.51	0.4755	0.4771
0.9985	1.055	10.27	9.98	0.4990	0.4854
0.3768	1.0573	10.33	3.93	0.1965	0.1839
0.4354	1.0352	8.89	3.83	0.1915	0.1867
0.0000	1	10.00	0.00	0.0000	0.0000
0.0000	1	10.00	0.00	0.0000	0.0000

correlation coefficient	0.9990

Table 5. Data for the Purity Determination of the Secondary Standard Triethyl Phosphate (TEP) Relative to the NIST-Traceable Standard Dimethyl Sulfone Using Proton NMR (one sample was prepared and analyzed 7 times)

TEP purity with Dimethyl sulfone, proton data
NB232P08A

Area of Analyte (TEP)	0.445	0.6368	0.6357	0.6356	0.6362	0.4452	0.4455
Area of Standard (DMSO2)	0.65	0.9288	0.929	0.9298	0.9295	0.6493	0.6505
MW of Analyte	182.15	182.15	182.15	182.15	182.15	182.15	182.15
MW of Standard	94.13	94.13	94.13	94.13	94.13	94.13	94.13
Wt. Of Standard	23.24	23.24	23.24	23.24	23.24	23.24	23.24
Sample Weight	20.50	20.50	20.50	20.50	20.50	20.50	20.50
Purity of Standard	99.73	99.73	99.73	99.73	99.73	99.73	99.73
# of nuclei in std	2	2	2	2	2	2	2
# of Nuclei in analyte	3	3	3	3	3	3	3
Weight %	99.85	100.00	99.81	99.70	99.83	100.01	99.89
Average	99.87						
Standard Deviation	0.11						
Confidence Limits	0.22						

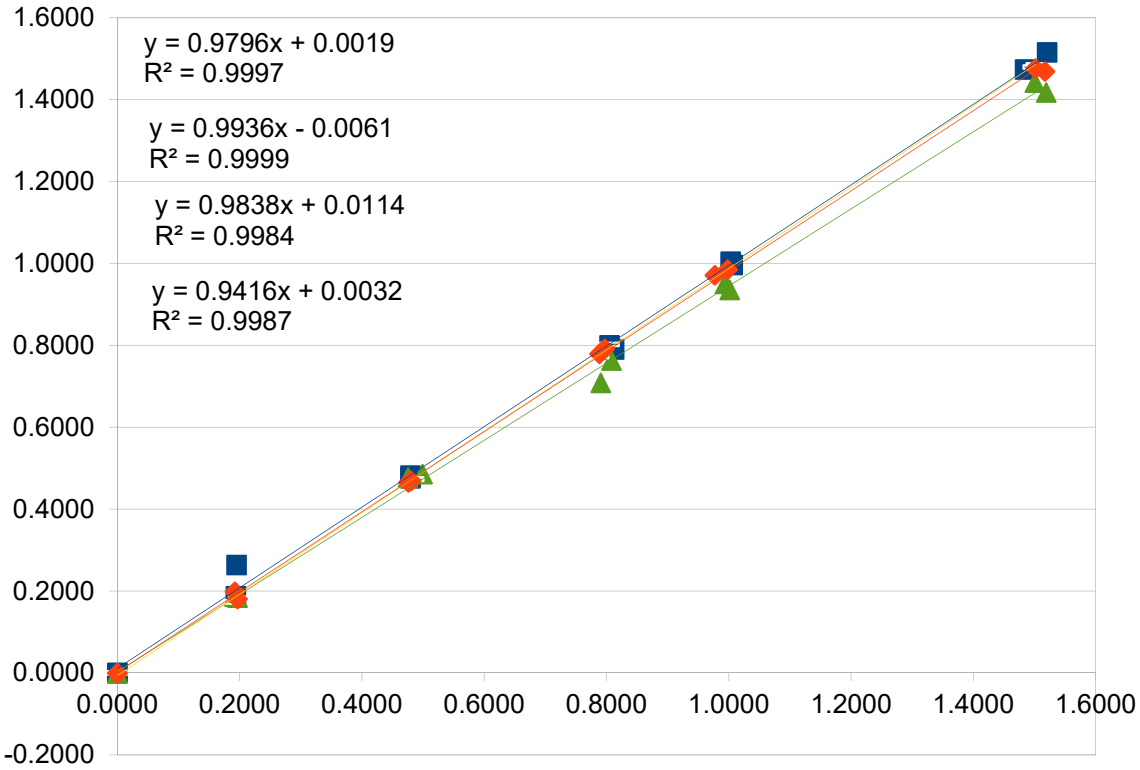


Figure 6. Plot of the data from four days, found Z vs. target Z.

To do the calculation for the found Z, the formula from Section 2.6 was used, except it was normalized to 1Z = 20 mg instead of using the actual Weight A. Using this method, the purity of the GD sample can be determined from the slopes of the curves from Figure 6. Averaging all four slopes gives an average purity of 97.5 wt%.

3.2 Purity Determination Using NMR

The typical way to determine the purity with this method, without an entire P&A study, is simply to calculate purity for each run using the formula in Section 2.6. Table 6 shows the calculations for Day 1 data, excluding the blank runs. The last run was also excluded since it is an outlier. The resulting average purity is 98.99 wt%, with a standard deviation of 1.26%. The 95% confidence limit is 2.52%.

Table 6. Data from Day 1, Used to Calculate Purity for Each Run for GD.

Area of Analyte (agent)	Area of Standard (TEP)	Wt. Of Standard	Sample Weight	Weight %
2.9643	1.0429	10.38	29.69	99.25%
3.0991	1.0225	10.01	30.41	99.65%
1.9934	1.0315	10.32	20.13	98.96%
2.0447	1.0574	10.40	20.06	100.13%
1.5385	1.0256	10.68	16.10	99.39%
1.6135	1.0363	10.16	16.25	97.23%
0.8691	1.0283	11.26	9.59	99.12%
0.8575	1.0352	11.65	9.59	100.51%
0.3569	1.0444	10.99	3.88	96.68%
0.3873	1.0251	13.97	3.90	135.17%

Average purity	98.99 wt%
Std. dev.	1.26%
Confidence limit	2.52%

To minimize the amount of sample preparation, it is possible to prepare only one sample and rerun it multiple times. This approach minimizes the hazard from handling neat agent and minimizes the consumption of agent and generation of waste. However, the repetitions include only the error that is generated by the NMR data acquisition and integration, and not systematic or random errors from weighing. This method wasn't used for this GD sample.

3.3 Certify Results

The results were analyzed by the program Certify 6.0 used for P&A data analysis. The screens that were generated by the program are shown in Figures 7 to 9. Parameters that are calculated by the program are shown on the screens.

The data that is obtained from this test is ±7.4%, which passes the Certify pass/fail criteria of ±25%. This is a higher error result than the accuracy of a purity determination. But because of the way the data is entered into the program, Certify is effectively testing the accuracy of the pipetting, or the correspondence of the target Z with the weight. As shown in Figure 9, there is no scatter in the x-coordinate in the Certify plot, while there is scatter in the x-coordinate for the data shown in Figure 6. The accuracy of the weighing and NMR determination is less than the error from the pipeting. The actual accuracy of the data from weighing and NMR determination is better than the Certify calculations suggest, so using Certify to quantify the P&A results in this case does not accurately indicate the method performance. As

a result, a better way to judge the results is in terms of standard deviations and correlation coefficients of the data. Some of the Certify results were incorrectly labeled as "NMR-GA," although they were for GD data.

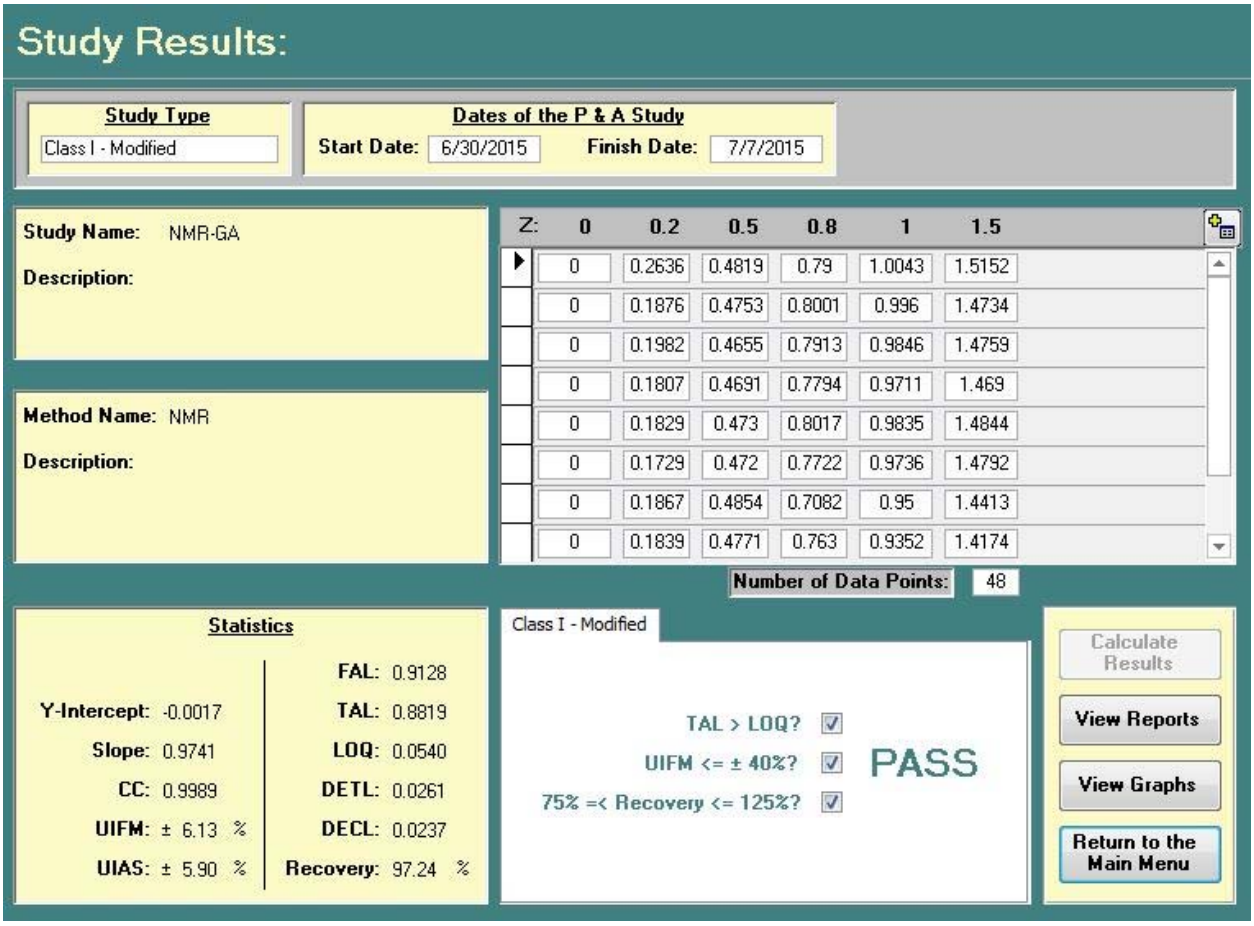


Figure 7. Certify results page for the four-day P&A study.

Report Summary

Class I - Modified

Study Name:	NMR-GA	Start Date:	6/30/2015
Study Description:		Finish Date:	7/7/2015
Method:	NMR		
Laboratory:	Edgewood Chemical, Biological Center		
Agent:	GB		
Environment :	IDLH		
Sample Size:	48		

Target Levels

TC 1 =	0.0000 Z
TC 2 =	0.2000 Z
TC 3 =	0.5000 Z
TC 4 =	0.8000 Z
TC 5 =	1.0000 Z
TC 6 =	1.5000 Z

Target vs. Found Summary

Found Action Level:	0.9128	Z
Target Action Level:	0.8819	Z
Limit of Quantification:	0.0540	Z
Detection Limit:	0.0261	Z
Decision Limit:	0.0237	Z
Percent Recovery:	97.24	%
Uncertainty in Found Mass:	6.13	%
Uncertainty in Air Sample:	5.90	%

Statistical Parameters

Slope:	0.9741
Y-intercept:	-0.0017
Correlation Coefficient:	0.9989
Students-T Statistic:	2.01357

Outliers

Outlier test not performed.

Pass/Fail Results

TAL greater than LOQ:	Passed
UIFM less than or equal to ±25%:	Passed
Recovery within 75% to 125%:	Passed

Figure 8. Certify report summary.

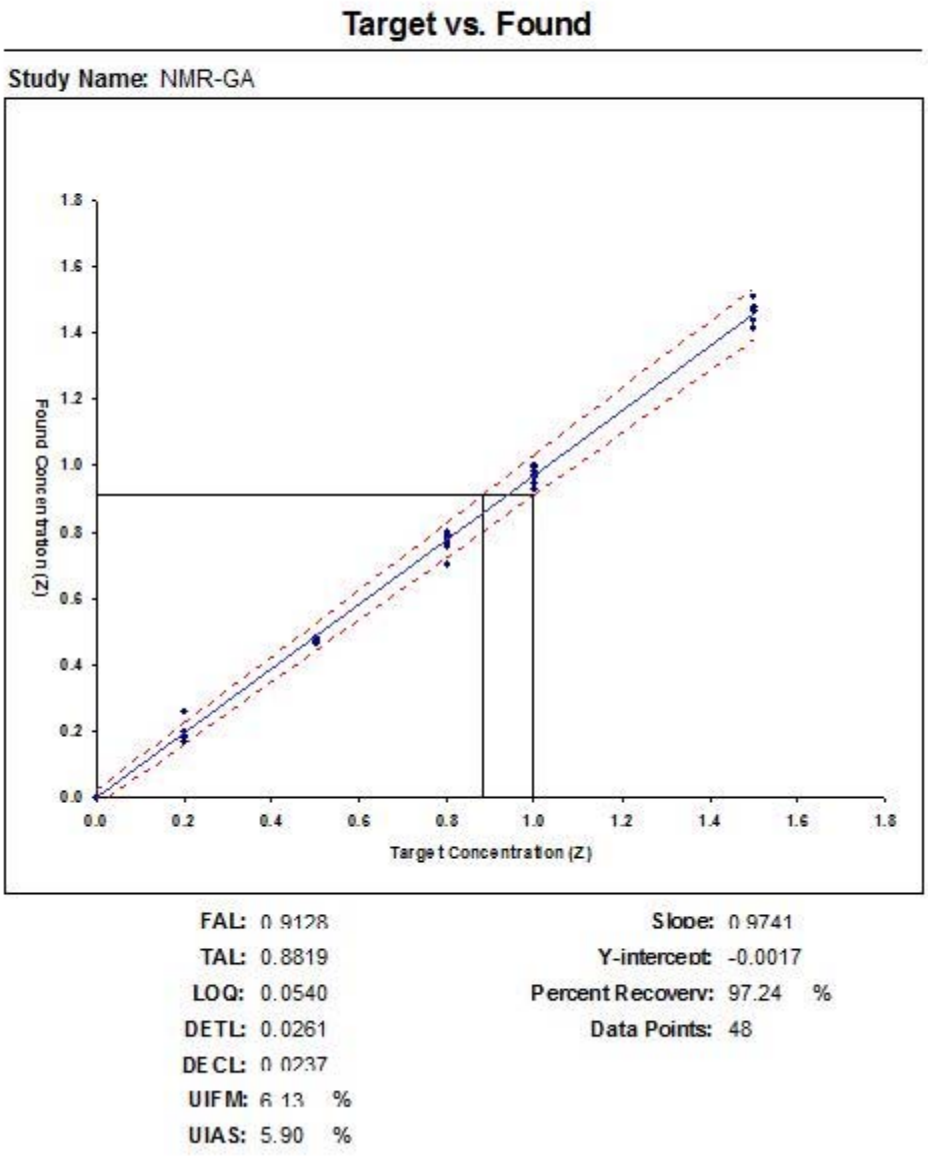


Figure 9. Certify target Z vs. found Z plot screen.

3.4. P&A Results for NMR Analysis of GA

Tables 7 to 10 show the data sets collected on each day of the four-day P&A test for GA. Data are collected using P-31 NMR. The same secondary standard was used with purity of 99.87%.

Figure 10 shows the data plotted together with the regression lines and correlation coefficients. Correlation coefficients for all the days between the target Z (as a weight) and the found Z are >0.99.

Table 7. P&A Data from Day 1 for GA

Area of Analyte (agent)	Area of Standard (TEP)	Wt. Of Standard	Sample Weight	Z (wt agent/ 20 mg)	Found Z
1.0492	0.3576	10.92	32.01	1.6005	1.4240
1.0459	0.3614	11.31	31.95	1.5975	1.4548
1.0584	0.5914	12.23	21.41	1.0705	0.9728
1.0542	0.5147	10.37	20.92	1.0460	0.9440
1.0506	0.6557	11.03	17.37	0.8685	0.7855
1.0601	0.6359	10.52	17.07	0.8535	0.7795
0.9649	1.024	11.86	10.59	0.5295	0.4967
0.9524	1.0231	11.09	10.07	0.5035	0.4589
0.4080	1.038	11.26	4.41	0.2205	0.1967
0.4199	1.0212	10.72	4.13	0.2065	0.1959
0.0000	1	10.00	0.00	0.0000	0.0000
0.0000	1	10.00	0.00	0.0000	0.0000

correlation coefficient	0.9997

Table 8. P&A Data from Day 2

Area of Analyte (agent)	Area of Standard (TEP)	Wt. Of Standard	Sample Weight	Z (wt agent/ 20 mg)	Found Z
1.0455	0.3488	10.68	32.16	1.6080	1.4228
1.0502	0.4098	12.90	32.10	1.6050	1.4694
1.0559	0.5227	10.77	21.32	1.0660	0.9670
1.0472	0.6466	13.47	21.33	1.0665	0.9696
1.0624	0.7046	11.33	16.92	0.8460	0.7593
1.0546	0.7214	11.90	16.97	0.8485	0.7732
1.0310	1.0227	10.80	10.41	0.5205	0.4839
0.8960	1.0369	12.38	10.36	0.5180	0.4755
0.4038	1.0532	11.14	4.14	0.2070	0.1898
0.3688	1.028	12.86	4.24	0.2120	0.2051
0.0000	1	10.00	0.00	0.0000	0.0000
0.0000	1	10.00	0.00	0.0000	0.0000

correlation coefficient 0.9996

Table 9. P&A Data from Day 3

Area of Analyte (agent)	Area of Standard (TEP)	Wt. Of Standard	Sample Weight	Z (wt agent/ 20 mg)	Found Z
1.0482	0.3864	12.39	32.19	1.6095	1.4939
1.0520	0.3704	11.52	31.93	1.5965	1.4542
1.0691	0.5296	11.20	21.41	1.0705	1.0049
1.0602	0.6298	12.87	21.40	1.0700	0.9629
1.0765	0.8992	14.97	17.13	0.8565	0.7966
1.0686	0.6867	11.24	17.10	0.8550	0.7774
1.0158	1.0454	10.83	10.47	0.5235	0.4677
0.8963	1.0303	12.12	10.14	0.5070	0.4686
0.3558	1.0429	12.40	4.15	0.2075	0.1880
0.4176	1.0362	10.70	4.24	0.2120	0.1917
0.0000	1	10.00	0.00	0.0000	0.0000
0.0000	1	10.00	0.00	0.0000	0.0000

correlation coefficient 0.9996

Table 10. P&A Data from Day 4

Area of Analyte (agent)	Area of Standard (TEP)	Wt. Of Standard	Sample Weight	Z (wt agent/ 20 mg)	Found Z
1.0440	0.387	11.76	32.01	1.6005	1.4101
1.0478	0.3914	11.88	32.07	1.6035	1.4136
1.0526	0.5965	12.39	20.67	1.0335	0.9718
1.0517	0.5269	10.54	21.06	1.0530	0.9351
1.0508	0.7489	12.88	16.94	0.8470	0.8033
1.0634	0.6887	11.26	17.13	0.8565	0.7728
1.0042	1.0379	11.65	10.16	0.5080	0.5010
0.9313	1.0213	11.42	10.31	0.5155	0.4629
0.3395	1.035	13.78	4.10	0.2050	0.2009
0.3730	1.0492	11.95	4.00	0.2000	0.1888
0.0000	1	10.00	0.00	0.0000	0.0000
0.0000	1	10.00	0.00	0.0000	0.0000

correlation coefficient 0.9986

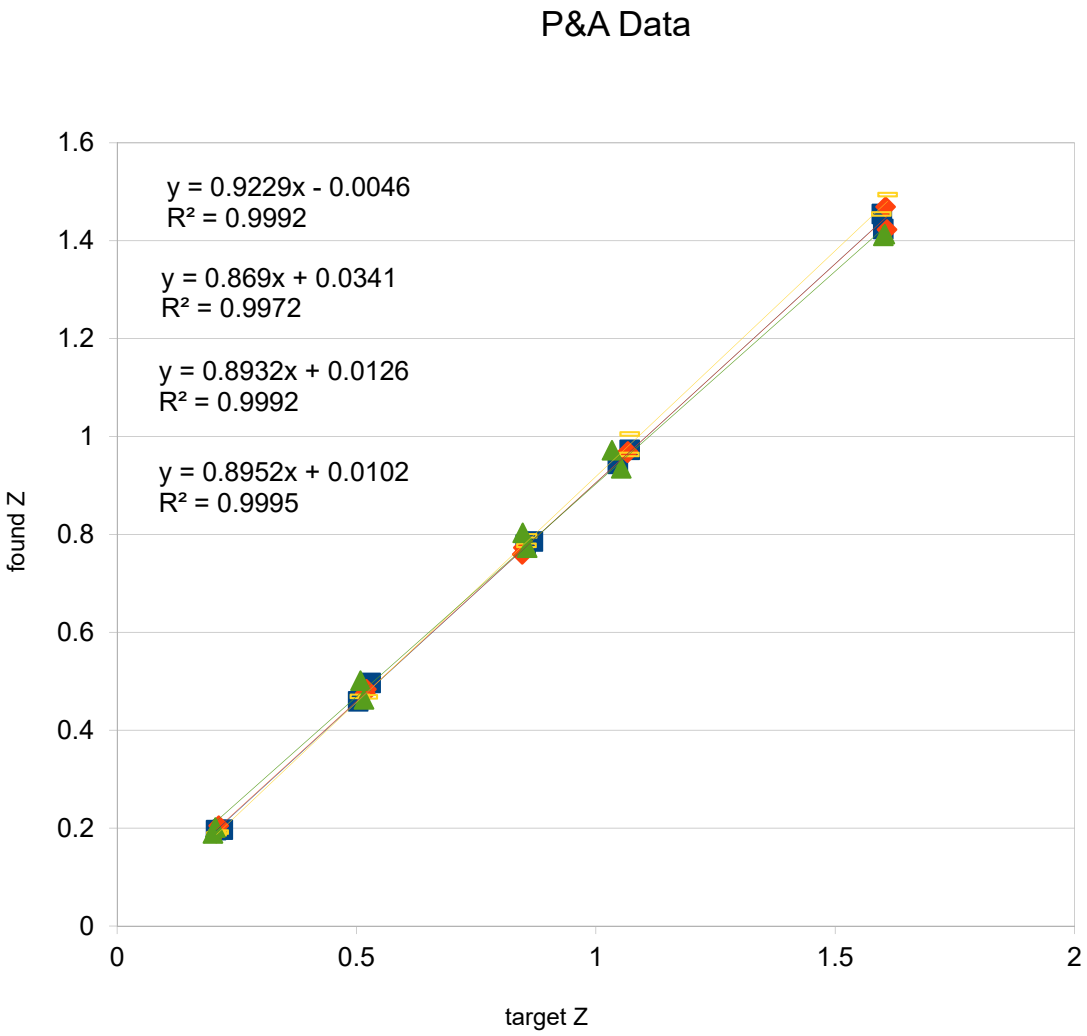


Figure 10. Plot of the data from four days, found Z vs. target Z.

To do the calculation for the found Z, the formula from Section 2.6 was used, except it was normalized to 1Z = 20 mg instead of using the actual Weight A. Using this method, the purity of the GA sample can be determined from the slopes of the curves from Figure 10. Averaging all four slopes gives an average purity of 89.5 wt%.

3.5 Purity Determination Using NMR

The typical way to determine the purity with this method, without an entire P&A study, is simply to calculate purity for each run using the formula in Section 2.6. Table 11 shows the calculations for Day 1 data, excluding the blank runs. The resulting average purity is 91.20 wt%, with a standard deviation of 1.85%.

Table 11. Data from Day 1, Used to Calculate Purity from Each Sample for GA

Area of Analyte (agent)	1.0492	1.0459	1.0584	1.0542	1.0506	1.0601	0.9649	0.9524	0.4080
Area of Standard (TEP)	0.3576	0.3614	0.5914	0.5147	0.6557	0.6359	1.024	1.0231	1.038
MW of Analyte	162.13	162.13	162.13	162.13	162.13	162.13	162.13	162.13	162.13
MW of Standard	182.15	182.15	182.15	182.15	182.15	182.15	182.15	182.15	182.15
Wt. Of Standard	10.92	11.31	12.23	10.37	11.03	10.52	11.86	11.09	11.26
Sample Weight	32.01	31.95	21.41	20.92	17.37	17.07	10.59	10.07	4.41
Purity of Standard	0.9987	0.9987	0.9987	0.9987	0.9987	0.9987	0.9987	0.9987	0.9987
# of nuclei in std	1	1	1	1	1	1	1	1	1
# of Nuclei in analyte	1	1	1	1	1	1	1	1	1
Found Z	88.97%	91.07%	90.88%	90.25%	90.44%	91.33%	93.81%	91.13%	89.21%
Average	91.20%								
Standard Deviation	1.85%								

To minimize the amount of sample preparation, it is possible to prepare only one sample and rerun it multiple times. The repetitions include only the error that is generated by the NMR data acquisition and integration, and not systematic or random errors from weighing and sample preparation. This data is shown in Table 12. The resulting average purity is 90.41 wt%, with a standard deviation of 0.75%.

Table 12. Data from Multiple Repetitions of One Sample from Day 1, Used to Calculate Purity for GA

Area of Analyte (agent)	1.0671	1.0697	1.0726	1.0827	1.0751	1.0661	1.0668
Area of Standard (TEP)	0.3645	0.3583	0.3586	0.3623	0.3594	0.3548	0.3578
MW of Analyte	162.13	162.13	162.13	162.13	162.13	162.13	162.13
MW of Standard	182.15	182.15	182.15	182.15	182.15	182.15	182.15
Wt. Of Standard	10.92	10.92	10.92	10.92	10.92	10.92	10.92
Sample Weight	32.01	32.01	32.01	32.01	32.01	32.01	32.01
Purity of Standard	0.9987	0.9987	0.9987	0.9987	0.9987	0.9987	0.9987
Weight %	88.78%	90.54%	90.71%	90.62%	90.71%	91.12%	90.42%
Average	90.41%						
Standard Deviation	0.75%						
Confidence Limits	1.51%						

3.6 Certify Results

The results were analyzed by the program Certify 6.0 used for P&A data analysis. The screens that were generated by the program are shown in Figures 11 to 13. Parameters that are calculated by the program are shown on the screens.

The data that is obtained from this test is ±7.4%, which passes the Certify pass/fail criteria of ±25%. This is a higher error result than the accuracy of a purity determination. But because of the way the data is entered into the program, Certify is effectively testing the accuracy of the pipetting, or the correspondence of the target Z with the weight. A better way to judge the results is in terms of standard deviations and correlation coefficients of the data.

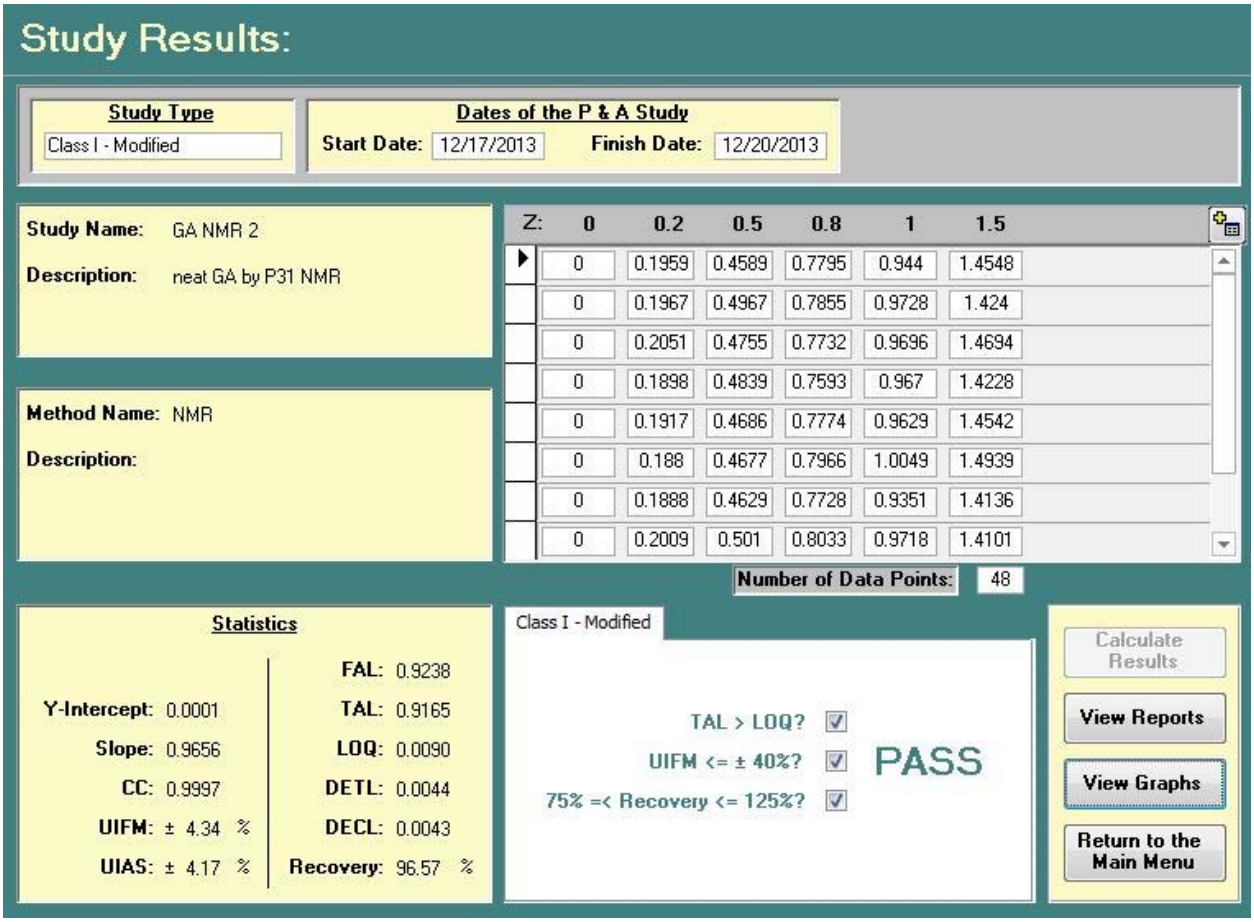


Figure 11. Certify results page for the four-day P&A study.

Report Summary

Class I - Modified

Study Name:	GA NMR 2	Start Date:	12/17/2013
Study Description:	neat GA by P31 NMR	Finish Date:	12/20/2013
Method:	NMR		
Laboratory:	Edgewood Chemical, Biological Center		
Agent:	GB		
Environment :	IDLH		
Sample Size:	48		

Target Levels	
TC 1 =	0.0000 Z
TC 2 =	0.2000 Z
TC 3 =	0.5000 Z
TC 4 =	0.8000 Z
TC 5 =	1.0000 Z
TC 6 =	1.5000 Z

Target vs. Found Summary

Found Action Level:	0.9238	Z
Target Action Level:	0.9165	Z
Limit of Quantification:	0.0090	Z
Detection Limit:	0.0044	Z
Decision Limit:	0.0043	Z
Percent Recovery:	96.57	%
Uncertainty in Found Mass:	4.34	%
Uncertainty in Air Sample:	4.17	%

Statistical Parameters

Slope:	0.9656
Y-intercept:	0.0001
Correlation Coefficient:	0.9997
Students-T Statistic:	2.01357

Outliers

Outlier test not performed.

Pass/Fail Results

TAL greater than LOQ:	Passed
UIFM less than or equal to ±25%:	Passed
Recovery within 75% to 125%:	Passed

Figure 12. Certify report summary.

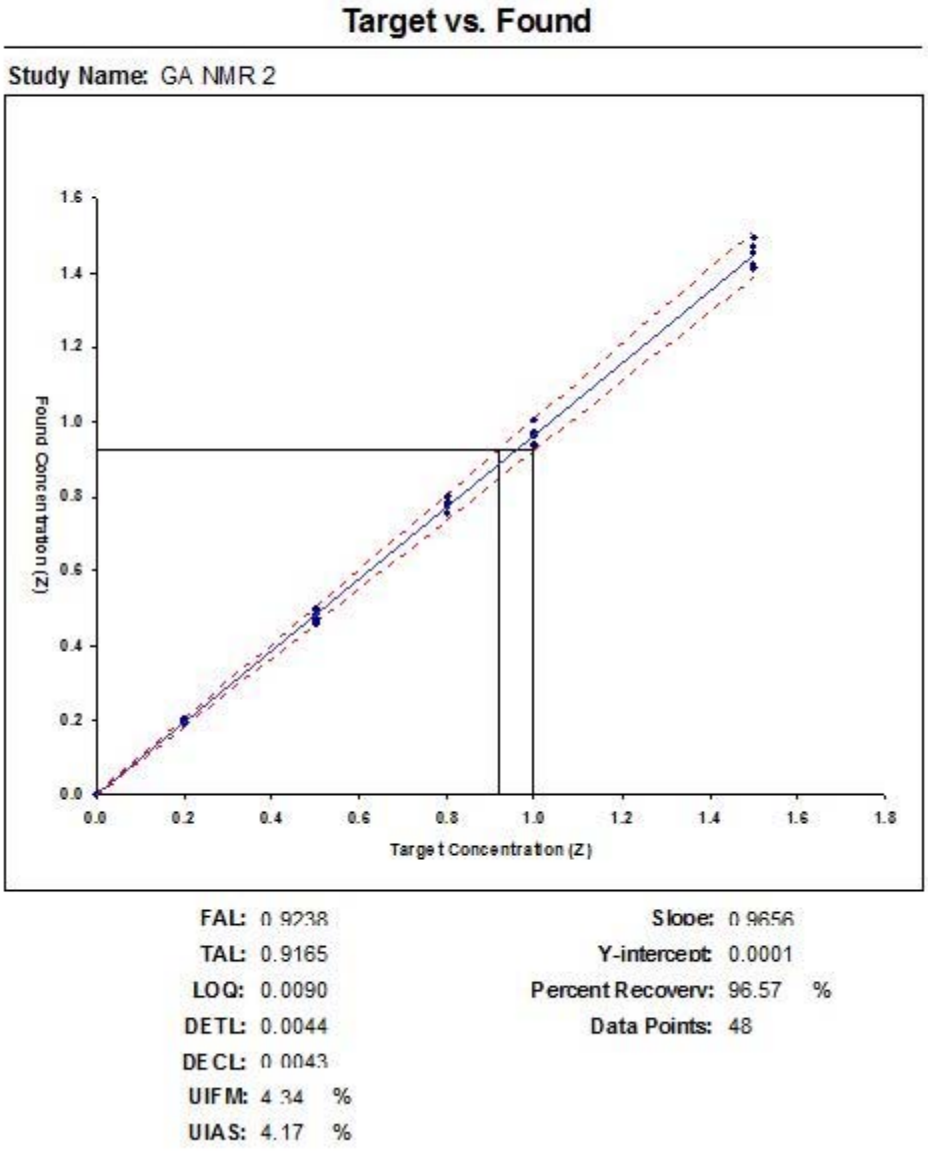


Figure 13. Certify target Z vs. found Z plot screen.

4.0 CONCLUSION

By using the NIST-traceable internal standard, and the balance that is calibrated with NIST-traceable weights, the purity of the CW agent feedstock agent GA and GD are determined using a NIST-Traceable method with P-31 NMR spectra.

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