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Method for Determination of NIST-Traceable Quantitative Weight Percentage Purity for VX Standards

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Nuclear Magnetic Resonance (NMR) with phosphorus-31 detection is described to determine the weight percent purity of feedstock samples of VX in a way that is NIST-traceable. A precision and accuracy test is described.

15. SUBJECT TERMS
VX; Nuclear Magnetic Resonance Spectroscopy; Purity Determination

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PREFACE

The work described in this report was authorized under Contract No. W911SR-10-D-0004. This work was started in September 2019 and completed in October 2019.

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 advertisement.

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1. INTRODUCTION

This report is on the procedure to determine the purity by Nuclear Magnetic Resonance (NMR) of the nerve agent VX. 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] T,[8] and the G agents GA and GD.[9]

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. 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.

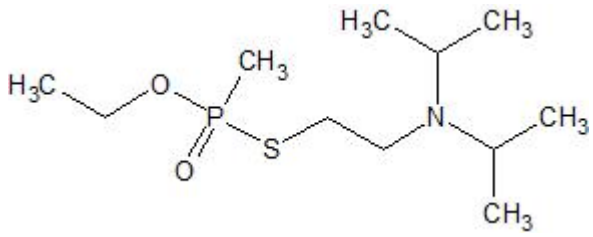
It is necessary is to know the NMR chemical shifts of VX, the internal standard, and the average molecular weights. The chemical shift of VX can depend on pH and solvent,[10] but it is well resolved from other compounds that are likely to be present.

In previous reports, 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 this method, the chemical triethyl phosphate (TEP), purchased from Sigma Aldrich, was used as an internal standard, even though it 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.

VX, like G series nerve agents, contain phosphorus atoms, and phosphorus is a spin ½ nucleus that gives a good NMR signal with good peak resolution, so phosphorus-31 (P-31 or ^{31}P) NMR was used for detection. However, proton NMR isn't useful for determining VX purity with TEP as an internal standard, since both compounds have ethoxy groups that have overlapping proton NMR signals. For purity determination with proton NMR, a different internal standard is required.

Precision and accuracy testing of the method was done to test the reproducibility of the method.



VX, MW 267.2

2. PROCEDURE

2.1 Supplies

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

VX neat standard was obtained from the CASARM program, CCDC 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 standards were:

- For P-31 NMR: Triphenyl phosphate, purchased from Sigma Aldrich, Part Number 05498-1G, CAS No. 115-86-6, MW 326.28 g/mole, certified on June 10, 2016 with expiration date May 2020, a TraceCERT® 31P-qNMR certified reference material (CRM) standard for quantitative NMR with a reported purity of 99.96 wt% and uncertainty of 0.23%.
- For proton NMR: 1,2,4,5-tetrachloro-3-nitrobenzene, purchased from Sigma Aldrich, Part Number 40384-1G, CAS No. 117-18-0, MW 260.89 g/mole, certified on June 15, 2016 with expiration date May 2020, a TraceCERT® certified reference material (CRM) standard for quantitative NMR with a reported purity of 99.86 wt% and uncertainty of 0.21%.

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 Mettler Toledo balance (Model XSR225 Dual Range, precision ±0.01 mg) was used for measuring weights. The balance was installed in a fume hood and calibrated by the CCDC CBC Calibration Branch and also using NIST-traceable weights (Mettler Toledo CarePacs® Calibrated Weights Part No. 11123104, ASTM E617-13 Class 1, calibrated 25 Jul 2019, due 1 Aug. 2020).

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 and personal protective equipment, in accordance with surety and safety regulations, equipment validations, and SOPs approved by the CCDC CBC Safety and Health Office.

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 a precision of 0.01 mg, and record the weight. Tare the balance after recording the weight.
b. Add 4-30 mg of 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 a precision of 0.01 mg in a laboratory notebook.
c. Add 0.4 mL of reagent-grade deuterated chloroform (CDCl3).
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. 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. Signal 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.
    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: 32K-64K
    Number of scans: 16 for P-31
    Sweep width: 300 ppm for P-31
    Center frequency: 30 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.

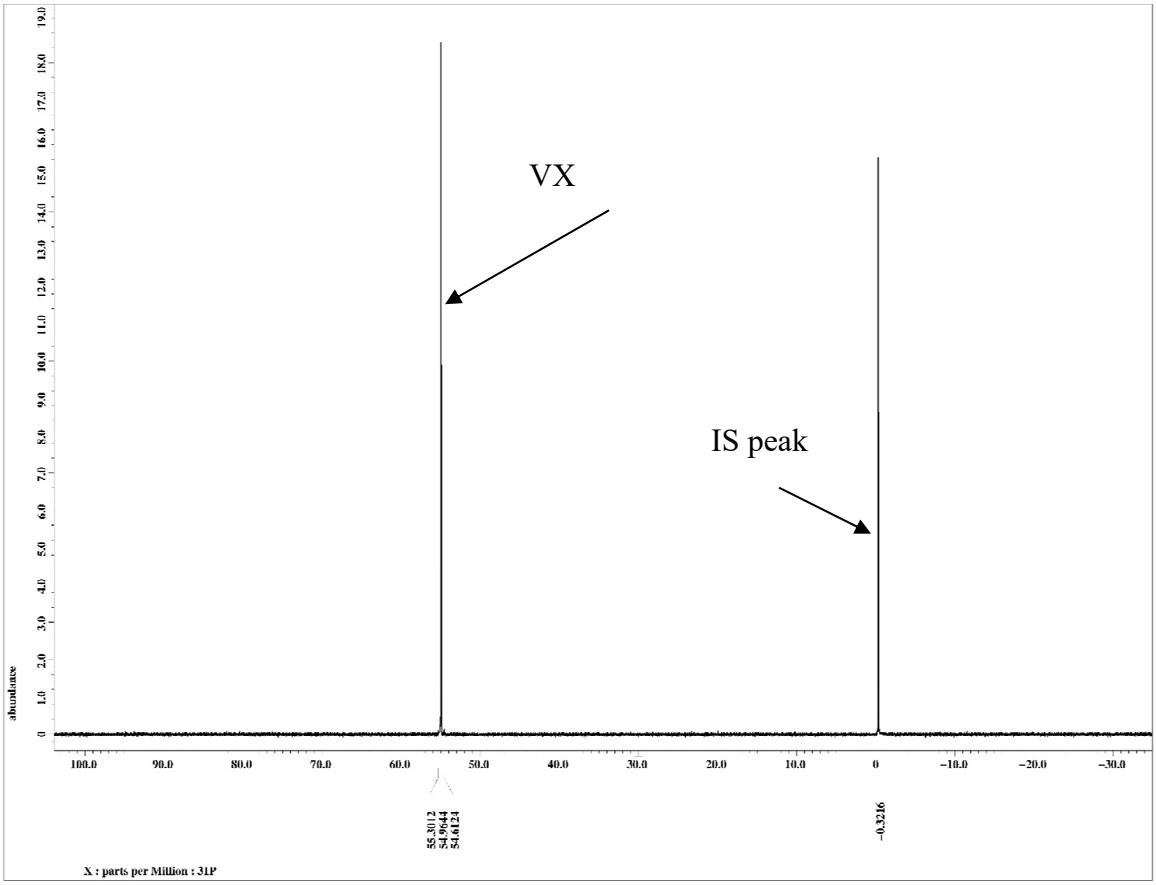


Figure 1: Phosphorus-31 NMR spectrum of agent VX and the internal standard TEP.

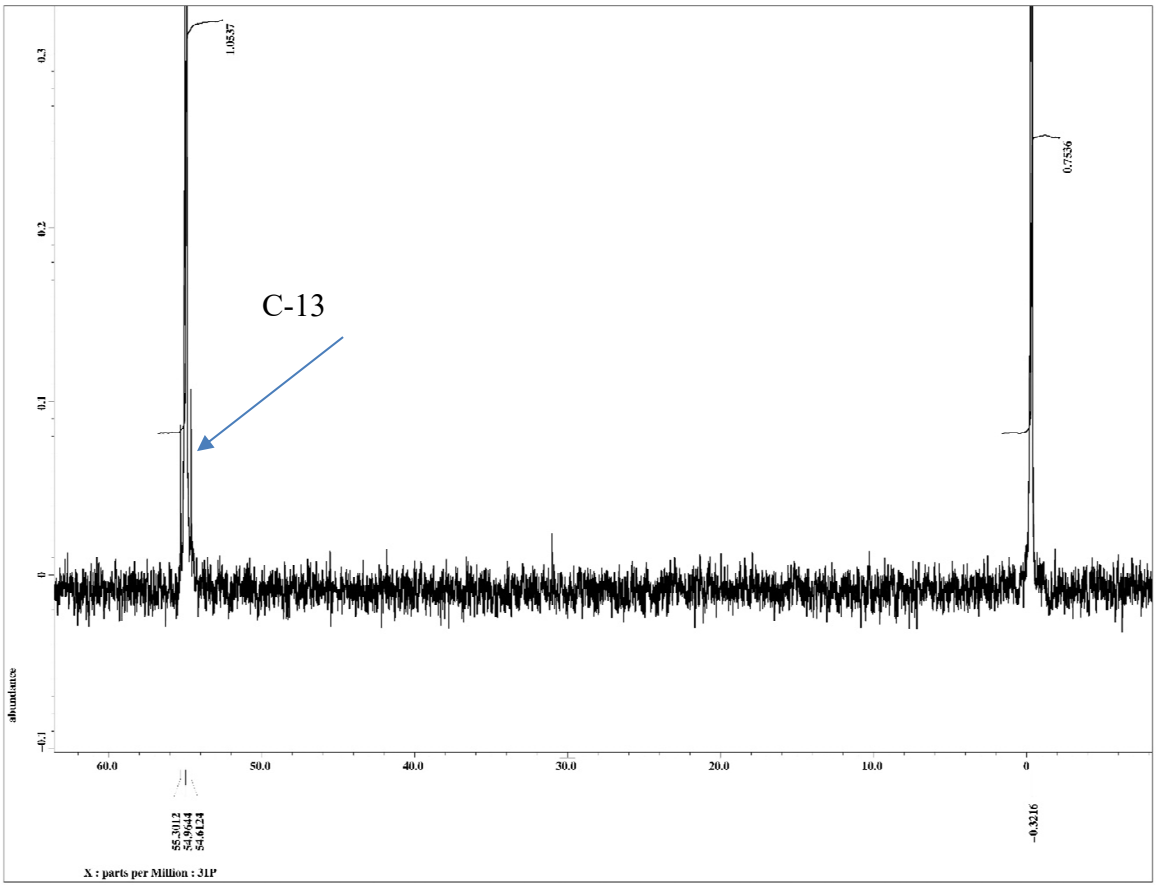


Figure 2: Phosphorus-31 NMR spectrum of agent VX and the internal standard TEP, showing the same spectrum as Figure 1 but with expanded scale and showing the integral trace of the VX peak.

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, adjust the chemical shift (x-axis) of the internal standard to the literature value.

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-31 peak can also be split into a multiplet by the protons. All the peaks in the multiplet must be included in the integration.

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. Two standards were used. Both P-31 and proton NMR were used to perform the purity determination, and the results were consistent. For more details about proton NMR, see previous technical reports.[5-8]

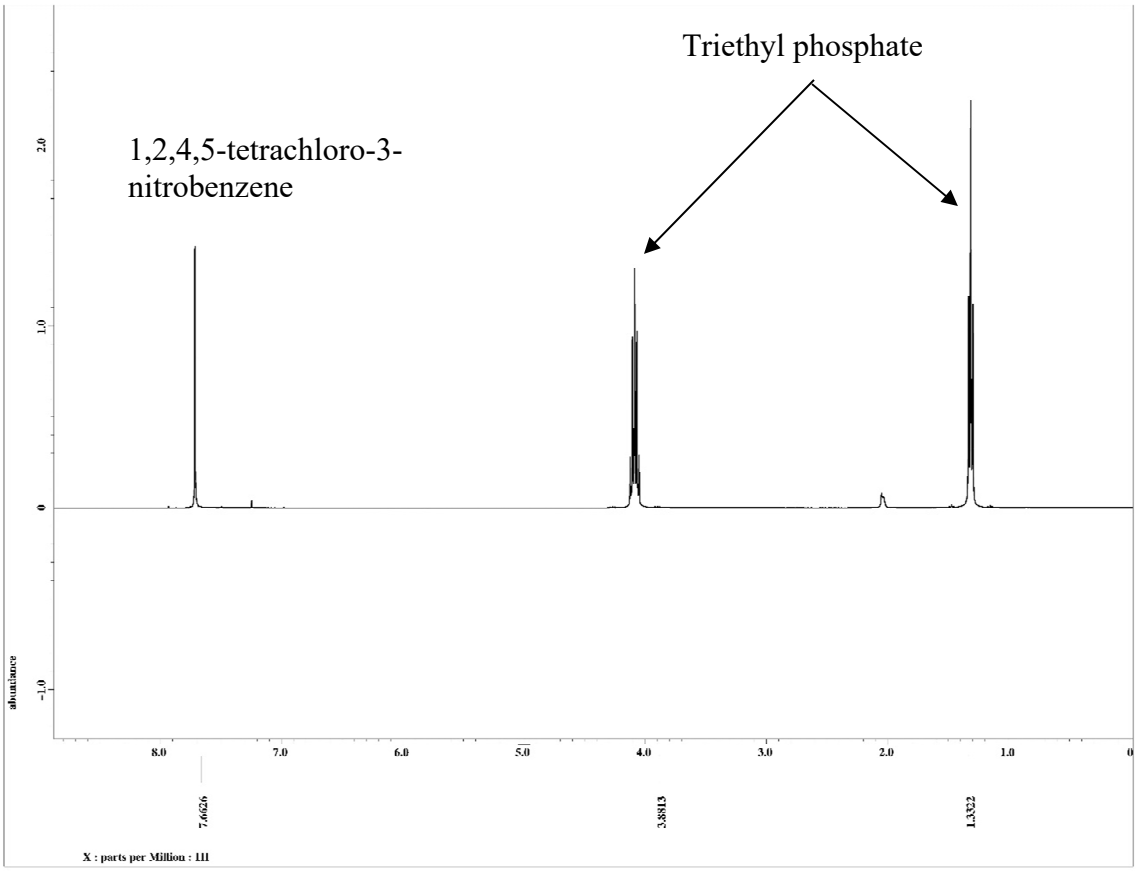


Figure 3: Plot showing the proton NMR spectrum of the internal secondary standard, triethyl phosphate and the primary standard, 1,2,4,5-tetrachloro-3-nitrobenzene. All the peaks are baseline resolved, so there is no uncertainty in integrating them.

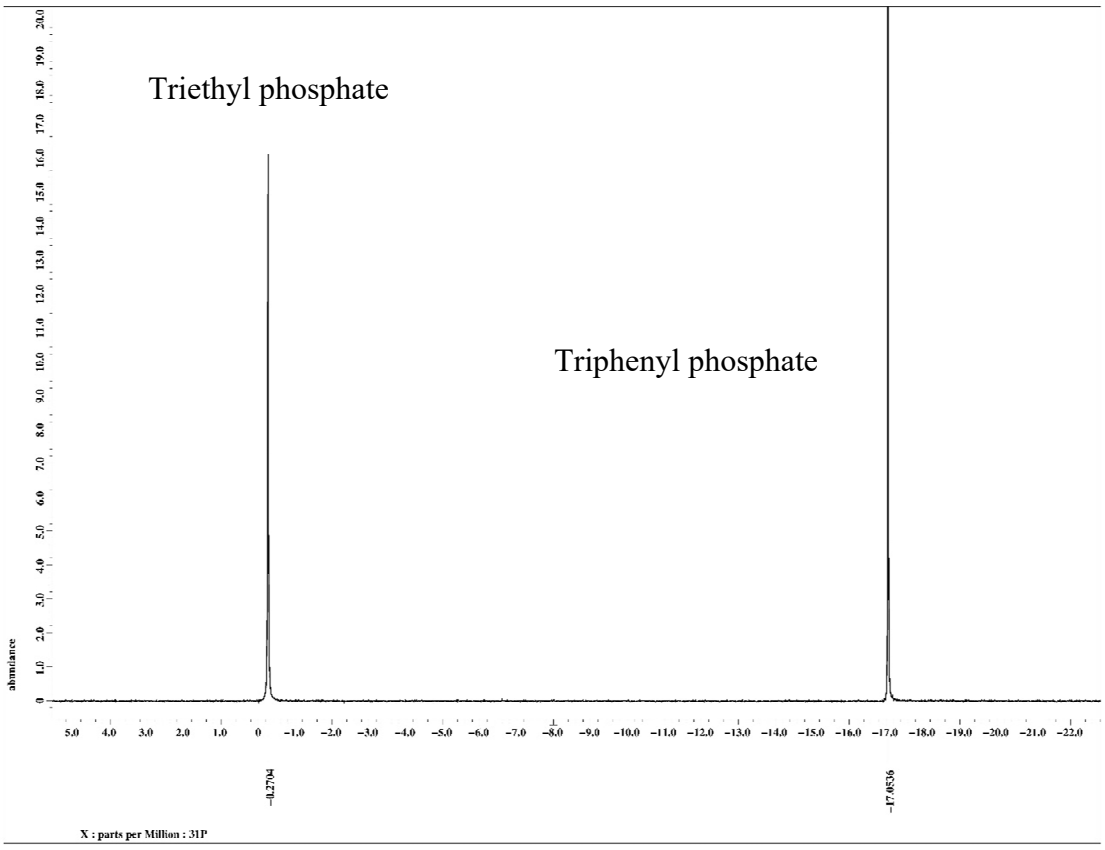


Figure 4: Plot showing the P-31 NMR spectrum of the internal secondary standard, triethyl phosphate and the primary standard, triphenyl phosphate.

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 P-31 (or H-1) atoms corresponding to the integrated peak of the internal standard;
No. P(A)=the number of P-31 (or H-1) atoms corresponding to the integrated peak of the analyte;
Pur IS = the purity of the secondary internal standard that is found from the primary purity determination, or the provided purity of the primary internal standard, in percent.

Since the compounds and the internal standards only contain one phosphorus atom, the number if identical atoms in the molecules is one.

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. PRECISION AND ACCURACY TESTING APPROACH

The purity determination method was validated using a modification of the protocol for a Class I Precision and Accuracy (P&A) test.[5-9] 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 used. 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 the target amount 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 prepared.

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 for analysis) are set in the program to be the same for all replicates from the four-day test. 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 can vary from the exact Z levels but is accurately known for each sample of the 4-day test. The approximate target amounts are

measured using an adjustable pipet set for the target levels by volume to get the nominal target Z. The accurate weight cannot be entered into the Certify program as an x-coordinate, only the target Z level.

The T1 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 VX

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 two separate analyses runs of proton and P-31 NMR, and the data for them is shown in Table 5 and 6. The found purity was 99.57% using P-31 NMR, in good agreement with the specification for TEP and because the standard deviation was smallest. The result for proton NMR was consistent with this result but the standard deviation was higher for the measurement.

Figure 5 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 VX.

Area of Analyte (agent)	Area of Standard (TEP)	Wt. Of Standard	Sample Weight	Z (wt agent/ 20 mg)	Found Z
114.5476	57.1686	9.7700	29.5900	1.4795	1.4296
108.8158	66.7558	12.2900	30.5700	1.5285	1.4631
78.5993	57.1584	10.3000	21.0700	1.0535	1.0344
76.1899	66.0276	12.1900	21.2700	1.0635	1.0273
62.9727	67.788	12.1500	16.7600	0.8380	0.8243
60.4304	67.6622	12.1500	16.2700	0.8135	0.7925
37.7021	67.617	12.0700	10.1200	0.5060	0.4915
37.1969	68.8498	11.9500	9.5800	0.4790	0.4715
13.1781	54.3714	9.6700	3.6400	0.1820	0.1712
17.3465	59.3259	9.8900	4.8600	0.2430	0.2112
0.0000	1	10.3800	0.0000	0.0000	0
0.0000	1	10.3800	0.0000	0.0000	0

slope	0.9688
correlation coefficient	0.99977

Table 2: P&A Data from Day 2 for VX.

Area of Analyte (agent)	Area of Standard (TEP)	Wt. Of Standard	Sample Weight	Z (wt agent/20 mg)	Found Z
118.9210	57.2875	10.1800	31.3200	1.5660	1.5433
107.8156	53.9718	10.1200	29.6800	1.4840	1.4764
73.3602	54.301	10.1400	20.6000	1.0300	1.0005
77.1716	56.6961	10.1400	20.7800	1.0390	1.0080
58.8502	52.2939	10.3800	17.2700	0.8635	0.8531
51.0649	54.0015	10.5900	15.5600	0.7780	0.7313
37.1211	48.461	8.7200	10.1000	0.5050	0.4878
33.2298	55.0709	10.6200	9.6000	0.4800	0.4680
16.6483	56.4875	10.1200	4.3900	0.2195	0.2178
16.1900	58.2761	10.3600	4.2100	0.2105	0.2102
0.0000	1	10.3800	0.0000	0.0000	0
0.0000	1	10.3800	0.0000	0.0000	0

slope	0.9849
correlation coefficient	0.99972

Table 3: P&A Data from Day 3 for VX.

Area of Analyte (agent)	Area of Standard (TEP)	Wt. Of Standard	Sample Weight	Z (wt agent/20 mg)	Found Z
108.4233	53.7689	10.2100	31.3100	1.5655	1.5036
102.9012	57.5561	9.8200	28.1700	1.4085	1.2822
73.2025	61.6107	10.3700	18.3200	0.9160	0.8998
70.7064	54.3045	10.1400	19.8300	0.9915	0.9642
73.0616	60.0154	10.3100	18.9500	0.9475	0.9166
55.1866	52.114	9.0200	14.4700	0.7235	0.6976
33.1105	59.226	10.9900	8.8800	0.4440	0.4487
41.4854	70.21	9.9800	9.1200	0.4560	0.4307
23.6253	102.3111	10.6200	3.4800	0.1740	0.1791
15.6469	67.1714	10.5600	3.9600	0.1980	0.1796
0.0000	1	10.3800	0.0000	0.0000	0.0000
0.0000	1	10.3800	0.0000	0.0000	0.0000

slope	0.9453
correlation coefficient	0.99896

Table 4: P&A Data from Day 4 for VX.

Area of Analyte (agent)	Area of Standard (TEP)	Wt. Of Standard	Sample Weight	Z (wt agent/20 mg)	Found Z
109.4423	57.2192	10.5200	30.8700	1.5435	1.4695
116.6839	65.6369	10.5000	28.8800	1.4440	1.3632
67.0878	54.1588	10.4000	19.3300	0.9665	0.9408
68.2395	56.9512	10.9000	19.2700	0.9635	0.9538
56.4038	56.2788	10.5000	16.1400	0.8070	0.7685
53.2135	52.9958	10.2500	15.6600	0.7830	0.7516
34.3029	56.9047	10.9800	10.2500	0.5125	0.4834
52.5870	81.9205	10.2300	10.0900	0.5045	0.4796
13.5958	55.3468	10.1900	4.3300	0.2165	0.1828
14.4237	56.7954	9.8500	4.1100	0.2055	0.1827
0	1	16.3000	0.0000	0.0000	0
0	1	8.9300	0.0000	0.0000	0

slope	0.9609
correlation coefficient	0.99955

Table 5: Data for the purity determination of the secondary standard triethyl phosphate (TEP) relative to the NIST-traceable standard triphenyl phosphate using P-31 NMR. One sample was prepared and analyzed 7 times.

Area of Analyte (TEP)	Area of Standard (TPP)	Wt. Of Standard	Sample Weight	Weight %
63.3629	73.8167	21.8700	10.4900	99.8662
78.5427	92.323	21.8700	10.4900	98.9770
79.1904	92.0875	21.8700	10.4900	100.0484
78.2499	91.7504	21.8700	10.4900	99.2234
78.1773	91.5225	21.8700	10.4900	99.3782
77.7260	90.9176	21.8700	10.4900	99.4619
78.8230	91.6821	21.8700	10.4900	100.0246
62.2068	182.6567	21.8700	10.4900	99.0559
93.9257	182.6567	21.8700	10.4900	99.7093
62.1562	182.8752	21.8700	10.4900	98.8571
62.3536	183.2776	21.8700	10.4900	98.9533

Average	99.57
Standard Deviation	0.42
Confidence Limits	0.83

Table 6: Data for the purity determination of the secondary standard triethyl phosphate (TEP) relative to the NIST-traceable standard 1,2,4,5-tetrachloro-3-nitrobenzene using proton NMR. One sample was prepared and analyzed 7 times.

Area of Analyte (TEP)	Area of Standard (TPP)	Wt. Of Standard	Sample Weight	Weight %
122.8593	39.7713	30.4800	10.2300	106.9522
164.1971	39.5463	30.4800	10.2300	95.8340
163.9386	39.4057	30.4800	10.2300	96.0246
165.5368	39.7039	30.4800	10.2300	96.2324
130.9717	31.3555	30.4800	10.2300	96.4104
166.6912	40.2428	30.4800	10.2300	95.6059
123.2038	40.1104	30.4800	10.2300	106.3453
183.8805	42.3449	30.4800	10.2300	100.2293

Average	99.06
Standard Deviation	5.20
Confidence Limits	10.39

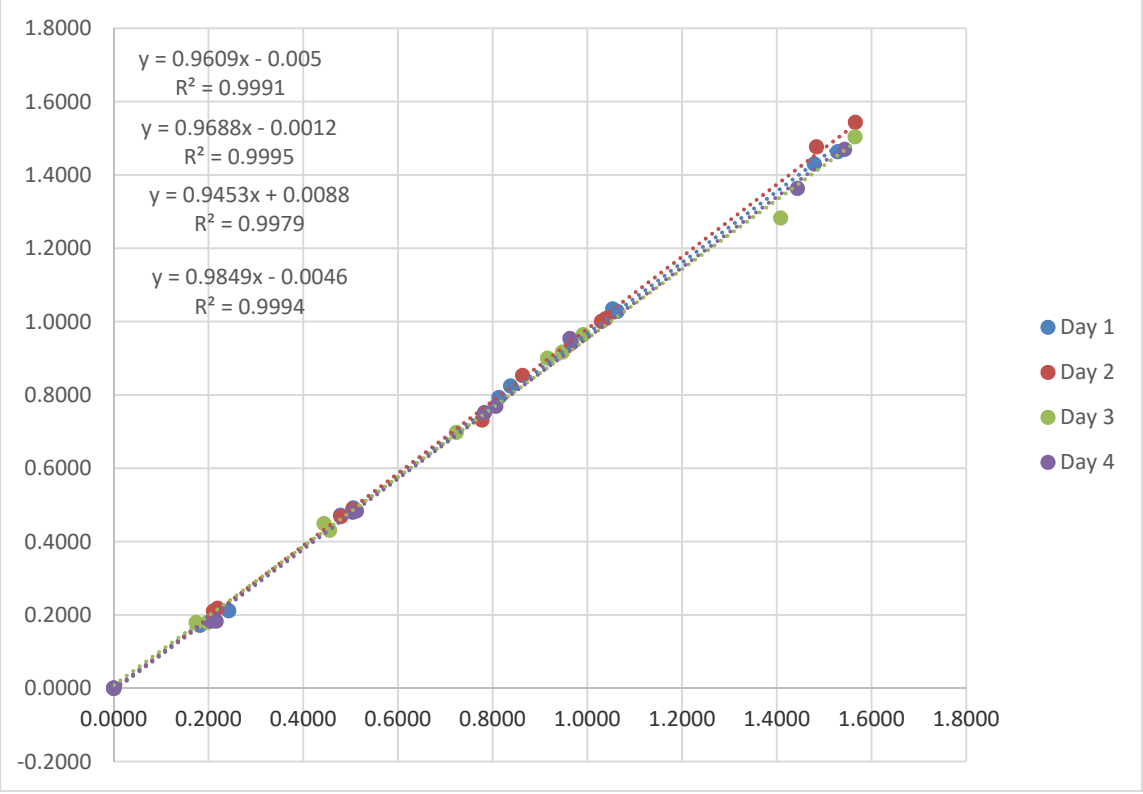


Figure 5: Plot of the data from four days, Found Z vs. Target Z.

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 VX sample can be determined from the slopes of the curves from Figure 6. Averaging all four slopes gives an average purity of 96.5 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 7 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 96.95 wt%, with a standard deviation of 1.42%. The 95% confidence limit is 2.84%.

Table 7: Data from Day 1, used to calculate purity for each run for VX.

Area of Analyte (agent)	Area of Standard (TEP)	Wt. Of Standard	Sample Weight	Weight %
114.5476	57.1686	9.7700	29.5900	96.63
108.8158	66.7558	12.2900	30.5700	95.72
78.5993	57.1584	10.3000	21.0700	98.19
76.1899	66.0276	12.1900	21.2700	96.59
62.9727	67.788	12.1500	16.7600	98.36
60.4304	67.6622	12.1500	16.2700	97.42
37.7021	67.617	12.0700	10.1200	97.13
37.1969	68.8498	11.9500	9.5800	98.43
13.1781	54.3714	9.6700	3.6400	94.05

Average	96.95
Standard Deviation	1.42
Confidence Limits	2.84

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. The calculations for 7 repetitions of a single sample are shown in Table 8. The resulting average purity is 98.72 wt%, with a standard deviation of 0.69%. The 95% confidence limit is 1.37%.

Purity results for this method for the 0.2Z samples are significantly different from the other averages. For example, Table 9 shows results for 7 repetitions of the 0.2Z sample from day 4. This average is only 85.8 wt%. The difference may be due to the lower accuracy of the weight for a smaller amount of agent. However, it appears that more work is required for accurate purity determinations at 0.2Z (4 mg) amounts with the current method.

Table 8: Purity data results for 7 repetitions of one sample from Day 1.

Area of Analyte (agent)	Area of Standard (TEP)	Wt. Of Standard	Sample Weight	Weight %
78.5993	57.1584	10.3000	21.0700	98.19
79.5012	57.2488	10.3000	21.0700	99.155263
79.9756	57.3857	10.3000	21.0700	99.5089853
79.5203	57.3927	10.3000	21.0700	98.9304143
78.9418	57.8125	10.3000	21.0700	97.4975605
79.2122	57.0515	10.3000	21.0700	99.1364772
79.6439	57.6626	10.3000	21.0700	98.6204028

Average	98.72
Standard Deviation	0.69
Confidence Limits	1.37

Table 9: Purity data results for 7 repetitions of 0.2Z sample from Day 4.

Area of Analyte (agent)	Area of Standard (TEP)	Weight %
13.5958	55.3468	84.437
13.9471	56.0633	85.512
13.7328	55.8382	84.538
13.4892	55.217	83.972
14.5339	56.2544	88.807
14.5432	56.2946	88.800
13.8101	55.9579	84.832

Average	85.84
Standard Deviation	2.08
Confidence Limits	4.15

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 6 to 8. Parameters that are calculated by the program are shown on the screens.

The Uncertainty in Found Mass (UIFM) data that is obtained from this test is ±12%, which passes the Certify pass/fail criteria of ±40%. 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 8, the plot doesn't have scatter in the x-coordinate in the

Certify plot, while there is scatter in the x-coordinate for the data shown in Figure 5. 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. The recovery/purity calculated from these results is 96.7%, which happens to be close to the previous purity calculations, but this program is not the best way to calculate the purity of the sample because of the larger error limits.

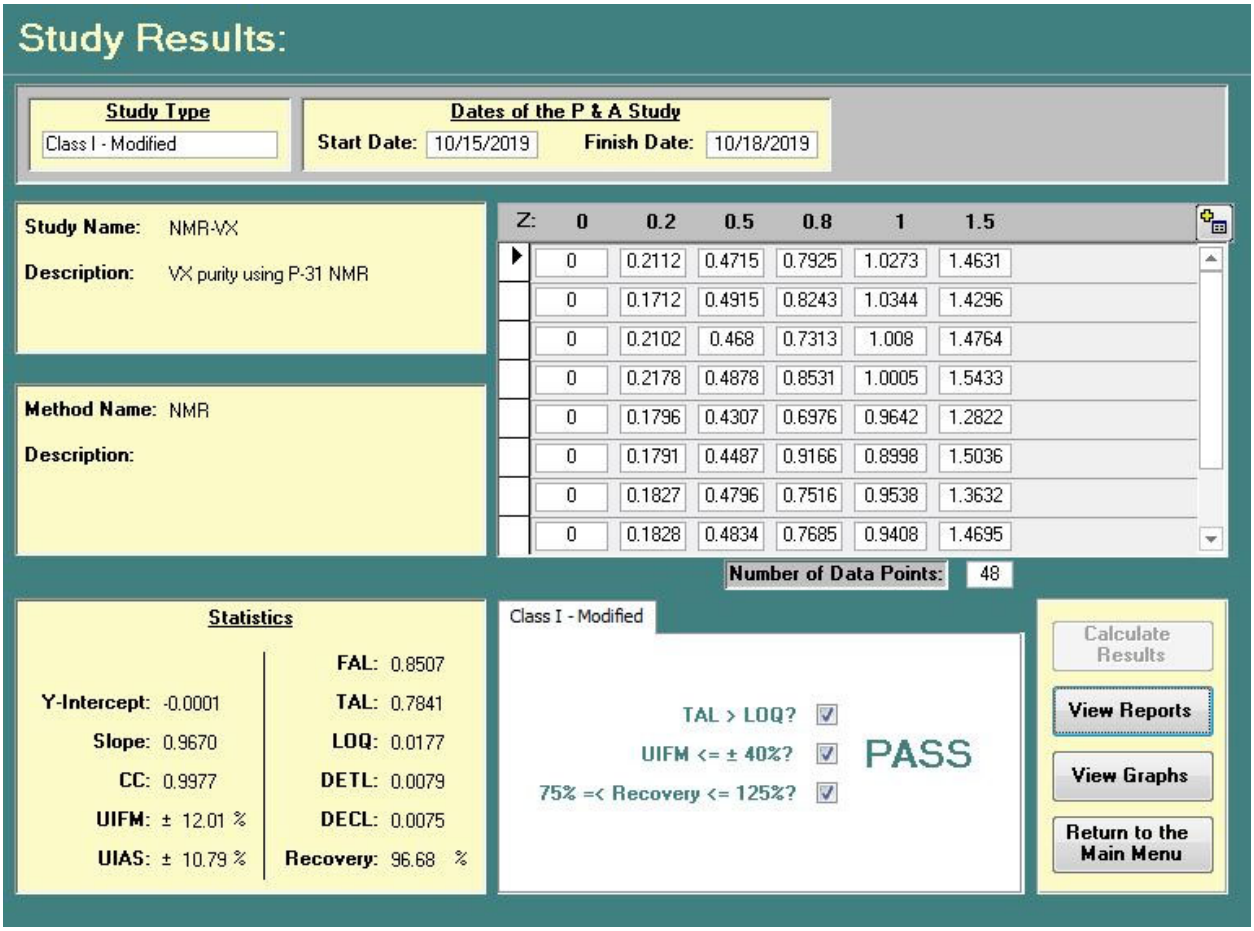


Figure 6: Certify results page for the four-day P&A study.

Report Summary

Class I - Modified

Study Name:	NMR-VX	**Start Date:**	10/15/2019
Study Description:	VX purity using P-31 NMR	**Finish Date:**	10/18/2019
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:	0.8507	Z
Target Action Level:	0.7841	Z
Limit of Quantification:	0.0177	Z
Detection Limit:	0.0079	Z
Decision Limit:	0.0075	Z
Percent Recovery:	96.68	%
Uncertainty in Found Mass:	12.01	%
Uncertainty in Air Sample:	10.79	%

Statistical Parameters

Slope:	0.9670
Y-intercept:	-0.0001
Correlation Coefficient:	0.9977
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

Tuesday, November 05, 2019 Page 1 of 1 CERTIFY 6.0

Figure 7: Certify report summary.

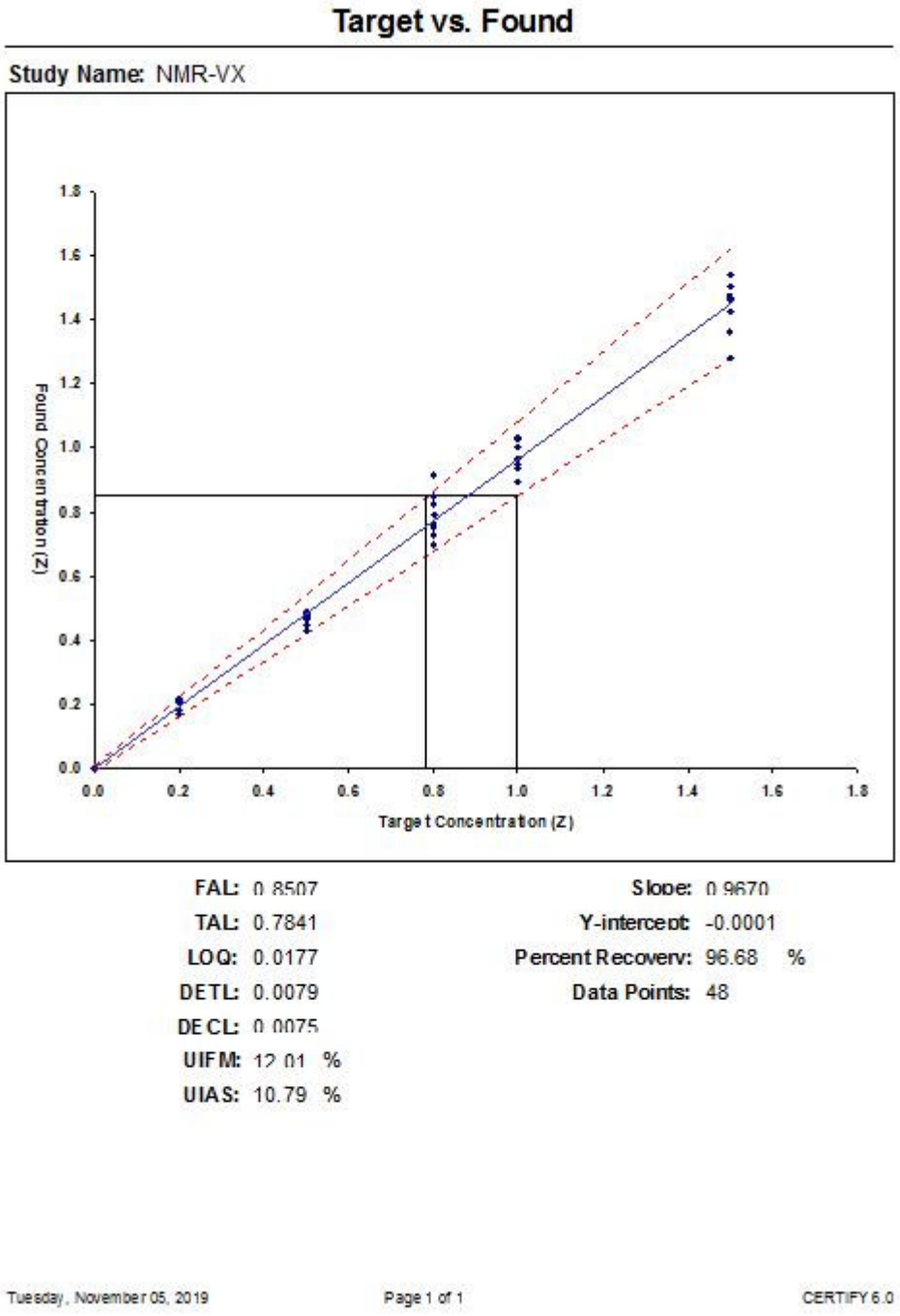


Figure 8: Certify target Z vs. found Z plot screen.

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 VX is determined using a NIST-Traceable method with P-31 NMR spectra.

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ACRONYMS AND ABBREVIATIONS

APG	Aberdeen Proving Ground
CA	chemical agent
CASARM	Chemical Agent Standard Analytical Research Material
CCDC CBC	U.S. Army Combat Capabilities Development Command Chemical Biological Center
CTF	chemical transfer facility
CW	chemical warfare
CWA	chemical warfare agent
ECBC	Edgewood Research Development Center
NIST	National Institute of Standards and Technology
NMR	Nuclear Magetic Resonance Spectroscopy
TEP	Triethyl phosphate
TPP	Triphenyl phosphate
VX	S-[2-(Diisopropylaminoethyl)] ethyl methylphosphonothioate

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