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14. ABSTRACT
This TOP is intended to provide expectations and guidance necessary for laboratories to follow when producing internal documents of operation in relation to purity analysis of chemical agents. These standard practices will provide guidance on equipment and instrumentation that will allow the test laboratory to choose an appropriate methodology for the testing being conducted.

15. SUBJECT TERMS
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U.S. ARMY TEST AND EVALUATION COMMAND
TEST OPERATIONS PROCEDURE

*Test Operations Procedure 08-2-073 18 March 2021
DTIC AD No.

STANDARD PRACTICES FOR DETERMINATION OF NEAT AGENT PURITY

Approved for public release; distribution unlimited.

1. SCOPE.

1.1 Intent.

a. The focus of this Test Operations Procedure (TOP) is to provide expectations and guidance necessary for laboratories to follow when producing internal documents of operation in relation to purity analysis of chemical agents. These standard practices will provide guidance on equipment and instrumentation that will allow the test laboratory to choose an appropriate methodology for the testing being conducted.

b. This TOP is directed toward agent, but there may be specifics in some instances that are necessary to address procedures that differ for particular agents.

c. This document will address neat agents, both traditional and emerging threat. While simulants are not included in this procedure, the best practices described here should be applied. Standard solutions made from neat agent or simulants are not included.

d. For purposes of clarity, references to neat agents in this document apply to liquid, solids, stabilized agents, and thickened agents.

1.2 Purpose.

a. This TOP provides standardized processes for the analysis of neat agents to determine purity.

b. These standard processes provide guidance for chemical agent storage and handling, test preparation, planning, conduct, use of data for comparison, and reporting of results that assess the purity of the neat agent for Test and Evaluation (T&E) purposes.

1.3 Background.

a. Accuracy in the determination of neat agent purity is critical to ensure proper quantification of threat challenges during T&E, and is critical when preparing dilute standards for calibrating referee equipment.

b. Historically, different test facilities conducting purity checks have used internal protocols and processes that lacked consistency. To provide confidence in the test results and comparative data, all laboratories should follow this TOP, which identifies standardized guidance for processes used in the determination of neat chemical agent purity.

c. Historically, the following has not been consistent:

(1) Which testing organizations are required to analyze neat agent for purity.

(2) Which practices/procedures are used to establish neat agent purity.

(3) What data elements are reported to the customer.

(4) How to store and handle neat agents to maintain purity.

(5) Which records to keep regarding purity analysis.

1.4 Limitations.

a. Best practices provided here may not preclude every possible instance where purity could be affected.

b. While common methodologies and instrumentation are described, alternative technologies may become available in the future that are appropriate for use.

c. The purity analysis described in this TOP is not intended to apply to neat agents in their vapor state.

d. This document does not address methods to identify impurities.

2. FACILITIES AND INSTRUMENTATION.

2.1 Facilities.

Facilities used to measure agent purity must be designed and constructed to ensure safe and secure storage, handling, analysis, and decontamination of chemical agents used for research, development, test, and evaluation. Facilities must be equipped and certified to work with chemical agents. The chemical agent laboratory, instruments, and personnel must meet all requirements of Army Regulation (AR) 50-6[1**], AR 385-10[2], AR 190-59[3], the safety requirements of Department of the Army (DA) Pamphlet (PAM) 385-61[4], as well as test site-specific requirements.

2.2 Instrumentation.

a. Instrumentation that can be used for agent purity determination includes the following:

** Superscript numbers correspond to those in Appendix F.

(1) **Nuclear Magnetic Resonance (NMR) Spectroscopy**. NMR spectroscopy uses magnetic fields and radiofrequencies to determine the structures of small molecules in a sample. NMR can be used as a quantitative method when incorporating reference standard materials that allow for determining sample purity. For chemical agents, typical isotopes used for identification include but are not limited to, ^{1}H, ^{13}C, and ^{31}P.

(2) **Gas Chromatography (GC) Separation with Subsequent Detection**. GC is a powerful analytical technique used to separate complex mixtures of volatile and semi-volatile organic compounds into their individual molecular constituents. Modern GC typically uses narrow bore capillary tubes that are internally wall coated with an adsorptive compound, called the stationary phase. Mixtures of molecules in a solvent are volatilized in a heated injection port, and subsequently transported down the capillary column carried by an inert gas, such as helium. Mixtures are separated into groups of like molecules, based on a combination of their vapor pressures and the molecular affinity for the stationary phase. Ideally, the groups of like molecules are well resolved from each other, and elute at the end of the column for subsequent detection and quantification at unique times, termed retention times.

(3) **GC Detectors**. GC detectors are designed to exploit unique characteristics or physical properties of the separated compounds for detection and quantification; different detector options provide different molecular sensitivities and selectivity fit best with specific applications. The following subsections provide a brief overview of some of the GC detectors used for purity analysis:

(a) **Mass Spectrometry Detection**.

1 Mass spectroscopy (MS) is uniquely suited for agent purity analysis as it is both highly sensitive and selective. An additional benefit is it can provide molecular structural information that can assist with identifying the components in the sample. Compounds eluting from the chromatographic column are ionized and the fragments are separated based upon mass-to-charge (m/z) ratios.

2 Mass spectral interpretation can be aided by using computer-based mass spectra data library and search algorithms, or derived using authentic reference standards. Because of the ability to use specific ions for detection of a target analyte, it can correctly quantify the concentration of a compound in the presence of a co-eluting interferent.

(b) **Flame Ionization Detection**. The Flame Ionization Detector (FID) is one of the most universally used GC detectors. The FID combusts organic chemical compounds eluting from a GC in an oxygen enriched hydrogen fueled flame. Organic compounds containing carbon-carbon or carbon-hydrogen bonds form ions when burned in the flame, and the ionized molecular fragments are collected and detected by the current produced. This detector is highly sensitive and has an extremely flat response across the range of different organic compounds that are amenable to the GC separation method. It is not selective, and therefore, compounds must be fully resolved by the chromatographic separation process to prevent interference during detection.

(c) **Flame Photometric Detection**. The Flame Photometric Detector (FPD) combusts compounds eluting from a GC column to form ions in a hydrogen rich flame. The excited state ions formed in the flame emit detectible light as they cool and return to ground state. Different narrow band pass optical filters are used for the phosphorus and sulfur modes, and the emitted photons are collected and detected using a photomultiplier tube. The method is both extremely sensitive and selective for either phosphorus or sulfur containing molecules. As degradation products of chemical agents may still contain either the phosphorus or sulfur atoms, it is still necessary to chromatographically resolve the agent from potential degradation products to prevent the possible interference during quantitation.

(d) **Thermal Conductivity Detection**. A Thermal Conductivity Detector (TCD) is a universal GC detector that compares the thermal conductivity of the column effluent, principally the inert carrier, to a reference stream of the carrier gas. When a compound elutes from the GC, it will usually change the thermal conductivity of the mixture (carrier gas plus the compound) relative to the pure carrier gas, which produces a differentiated quantifiable signal. While not highly sensitive or selective, the TCD is non-destructive, and is often used for permanent gas detection where high sensitivity is often unnecessary. It is not selective, and therefore compounds must be fully resolved by the chromatographic separation process to prevent interference.

(e) **Liquid Chromatography (LC)**. LC operates on many of the same principles as GC but separates molecules in the liquid phase rather than the gas phase, which make LC better suited than GC for application to low volatility or thermally labile molecules. LC separates chemical mixtures into its constituent molecular components using a pressurized liquid mobile phase to carry the mixture through a column packed with solid particles coated with a stationary adsorptive compound. Like the GC methods, a variety of different detectors are coupled with an LC to quantify, and sometimes identify the separated molecules as they elute from the column. As with the GC methods, MS has advantages over other detectors that could be used, as MS is both highly sensitive and selective. Because MS detection operates in the gas phase, a post column interface is required between the LC and MS to remove the bulk of the liquid mobile phase as the molecular components are transitioned into a gas phase (i.e., electrospray ionization) for use in MS. MS not only allows quantification of the eluting compounds, but it can provide additional information to help identify the molecular components in the sample. Compounds eluting from the chromatographic column are ionized and the fragments are separated based upon mass-to-charge ratios. Mass spectral interpretation can be aided by using computer-based mass spectra data library and search algorithms, or derived using authentic reference standards.

b. The advantages and disadvantages of the different instrumentation types with respect to their application to acceptable agent purity methods are summarized in Table 1. NMR is preferred when using an internal standard for determining purity. While NMR has the ability to provide a robust purity measurement with high accuracy, other methods are also acceptable to use in determining purity.

TABLE 1. ANALYTICAL METHODS FOR DETERMINATION OF CHEMICAL AGENT PURITY[a]

Instrumentation	Analytical Method	Description	Advantages	Disadvantage
Nuclear magnetic resonance (NMR) with appropriate nuclei for compound of interest	Internal Standard (IS) Quantitative NMR	Direct method compares active appropriate nuclei in agent to known quantity of appropriate IS.	Robust method that provides high level of certainty. Generally allows observation of interference by impurities.	Requires appropriate IS. May require a high resolution NMR that is in an agent laboratory, providing controlled ventilated space allowing work with neat agents.
Gas chromatography (GC)-mass spectrometry (MS), GC-high resolution MS, Liquid Chromatography (LC)-MS, LC/MS/MS	Stable Isotope Ratio Mass Spectrometry	Direct method that compares response of agent to the known concentration of isotopically labeled agent.	GC is common to most laboratories. May not require complete resolution between agent and impurity, but results are more dependable if separation is achieved.	Not all agents are thermally stable or sufficiently volatile for gas chromatographic analysis. Must obtain stable isotope labeled chemical agents.
GC coupled with MS, flame ionization detector (FID), flame photometric detector (FPD), or thermal conductivity detector (TCD)	GC Determination by the IS Method	The IS method requires developing a response factor relationship between a known purity reference/IS, and a known purity agent. Once response factor relationship is developed, the method can be used routinely with a pure IS and unknown purity agent.	GC is common to most laboratories. Several detector options available, with GC/mass selective detector (MSD) preferred because of the high selectivity ensuring that an impurity does not co-elute with either the IS or the agent.	Not all agents are thermally stable or sufficiently volatile for gas chromatographic analysis. For non-MS detection methods, all impurities must be chromatographically resolvable from the agent and IS, which is difficult to ensure.

a Table is adapted from Duewer, DL; Parris, RM; White, E; May, WE; and Elbaum, H (2004). An Approach to the Metrology Sound Traceable Assessment of the Chemical Purity of Organic Reference Materials[5].

TABLE 1. CONTINUED

Instrumentation	Analytical Method	Description	Advantages	Disadvantage
GC coupled with MS, FID, FPD, or TCD	GC Determination by the External Standard Method	External standard method requires developing a purity adjusted calibration curve using external agent standards of known purity.	GC equipment is common to most laboratories. Several detector options.	Not all agents are thermally stable or sufficiently volatile for gas chromatographic analysis. For non-MS detection methods, all impurity components must be chromatographically resolvable from the agent, which may be difficult to ensure.
LC coupled with MS/MS, MS, Ultraviolet (UV)/Visible (Vis) or fluorescence detectors	LC Determination by the Internal Standard Method	The IS method requires developing a response factor relationship between a known purity reference/IS, and a known purity agent. Once response factor relationship is developed, the method can be used routinely with a pure IS and unknown purity agent.	Operates at lower temperature so thermal stability of agents is not as critical as with GC.	Limited detectors available to couple with the LC. For non-MS detection methods, all impurities must be chromatographically resolvable from the agent and IS, which is difficult to ensure. Large dilution of agent solutions may be needed for highly sensitive MS/MS analysis.
LC coupled with MS/MS, MS, UV/Vis or fluorescence detectors	LC Determination by the External Standard Method	External standard method requires developing a purity adjusted calibration curve using external agent standards of known purity.	Operates at lower temperature so thermal stability of agent is not as critical as with GC.	Limited detectors available to couple with the LC. For non-MS detection methods, all impurities must be chromatographically resolvable from the agent and IS, which is difficult to ensure.

3. REQUIRED TEST CONDITIONS.

3.1 Test Planning.

This TOP provides guidance on agent purity determinations. The agent purity required for testing and the data requirements for reporting should be derived from the Test Plan (TP).

3.2 Documentation.

The following documentation should be available and planning actions taken before agent purity procedures begin:

a. All pertinent current site-specific procedures shall be reviewed before testing.

b. Potential problem areas shall be identified by reviewing previous records, when available.

c. The TP shall specify, through coordination with the customer, the required agent purity. Development of TPs requires familiarization with the applicable test planning and requirements documents such as the Test and Evaluation Master Plan, Operational Test Agency Evaluation Plan, or Capability Development Document. The selection of appropriate purity level will be determined from review of these requirement documents and background information such as references from preceding development and similar studies.

d. Safety and health issues must be given prime consideration in planning. All applicable/available safety documents such as the safety assessment report and health hazard assessments should be reviewed to determine if any safety or health issues require special test protocols.

3.3 Instrumentation Checks and Calibration.

a. Ensure all equipment and instrumentation are functioning properly.

b. Verify that all calibrated items' certificates are current and perform a pretest instrument check to verify that drift has not occurred.

c. Ensure there is traceability between any calibrated items used and their calibration documentation, which can be accomplished by recording the unique calibration number assigned or the instrument's make, model, and serial number as part of the purity analysis record.

3.4 Safety.

a. Operators should develop a risk management worksheet to quantify the risks involved in the operation based on the severity and probability of the hazards for the use of these procedures, as well as the controls implemented to minimize the level of risk based on test site-specific requirements. The composite risk management worksheet may be developed in accordance with

(IAW) AR 385-10, The Army Safety Program, and DA PAM 385-61, Toxic Chemical Agent Safety Standards.

b. Test personnel must be trained in the procedures to be performed. Training should be documented.

3.5 Quality Control (QC) and Quality Assurance (QA).

a. Each test facility's QA program will be designed to ensure that data of the required quality are obtained from each purity analysis. The data quality requirements will be established by the customer as well as by the test facility's QA/QC plan and procedures.

b. The quality of instrument data produced depends on appropriate instrument maintenance, periodic calibration, QC measures, and careful documentation of procedures. Calibration will be conducted IAW the validated calibration protocol of the test facility. In the absence of a validated protocol, calibration will be conducted as recommended by the instrument manufacturer.

c. Examples of QC measures associated with data reporting are agent sample preparation documentation, traceability, evaluation of analytical results, and comparison of results. QC measures will be detailed in the TP and will follow the test facility's QA/QC plan and procedures.

d. Chemical agent sample preparation QC measures will be IAW the test facility's site specific procedures or as specified in the TP. Any problems associated with a particular sample will be noted on the appropriate log sheet or data file. All data collected must be date and time stamped.

e. Data will be independently reviewed and authenticated as required by the test facility.

f. All analysis results and calculations will be peer-reviewed and documented to ensure that random errors in transcribing data or in performing analysis are corrected, as required by the test facility or the test program.

g. The analyte purity will be measured, at a minimum, using single analyses of three separate sample preparations, and the mean and standard deviation reported. For chromatography, a blank should be run between samples.

h. Traceability of primary reference standards to their Certificate of Analysis (COA) will be maintained. Any use of secondary reference standards must be traceable to the primary reference standard and it's COA.

4. TEST PROCEDURES.

During the planning and coordination phase of a project, the customer will be consulted to determine the purity required for testing.

4.1 Receipt of Chemicals.

a. When receiving chemicals, COAs will be thoroughly inspected. The information on the chemical material label will be checked to ensure it matches the information on the COA. Once opened and in use, the purity on the COA is only considered valid for 30 calendar days after the vial is opened.

b. Any chemical planned to be used for testing or purity analysis will be tracked for proper handling IAW the COA. Any required storage conditions for certification of materials to remain valid will be followed and records will be kept. If a COA does not provide information regarding storage, contact the manufacturer for guidance.

4.2 Frequency of Purity Testing.

a. When an unopened sealed vial with a valid COA is opened, its purity should be checked and verified every 30 days thereafter. Under no circumstances shall the testing frequency exceed 45 days from the last purity check.

b. Any stored previously-opened vial shall be checked before reuse and should be checked every 30 days thereafter while still in use. Under no circumstances shall the testing frequency exceed 45 days from the last purity check while in use.

4.3 Preparation of the Laboratory.

a. Before handling neat agent, ensure that the work area inside the hood is clean. Allow the agent to achieve room temperature before opening. Ensure any equipment and glassware being used in the transfer of agent and/or preparation of dilutes is clean. Cover the neat agent with an inert gas at the completion of the agent operation to minimize its degradation. The two inert gases commonly used are argon and nitrogen. Argon's higher density more easily displaces the air covering the agent. Closer attention to technique is required when using nitrogen. Store the neat agent at or below 4 °Celsius (°C) (39 °Fahrenheit (°F)).

b. Source of chemical materials for stocks and working standards:

(1) High purity chemical warfare agents are available from the Chemical Agent Standard Analytical Reference Material (CASARM) program with a COA. A recommended best practice is to purchase CASARM agent in small quantities so that opened vials are used up in the shortest time possible.

(2) Solvents shall be purchased from known and respected certified vendors.

(3) Certified IS chemicals with COAs, other than CASARM, shall be purchased from reputable vendors.

4.4 Procedures for Determination of Agent Purity.

a. There are three common routes for preparing a stock standard from neat agent; volume/volume, mass/volume, and mass/mass:

(1) In a volume/volume preparation, a known volume of neat agent is added to a volumetric flask or a comparable accurate laboratory measuring apparatus containing a known appropriate amount of solvent.

(2) In a mass/volume preparation, a known mass of neat agent is added to a volumetric flask or a comparable accurate laboratory measuring apparatus containing a small amount of solvent.

(3) In a mass/mass preparation, a known mass of neat agent is added to a known mass of solvent in an appropriate container.

b. Any of these routes of preparation are acceptable. Method specific details for neat agent dilution preparation and analysis are addressed in example procedures provided in Appendices A, B, C, and D.

c. NMR with IS.

(1) See Appendix A: Determination of National Institute of Standards and Technology (NIST)-Traceable Quantitative Weight Percentage Purity of Neat Chemical Agent by NMR with Internal Standard.

(2) See Appendix B: Determination of Weight Percentage Purity of Neat Chemical Agent by Proton NMR with Internal Standard.

d. Chromatographic Methods with IS. See Appendix C: Preparation and Analysis of Chemical Agent Sample for Purity Determination by GC/Mass Selective Detector (MSD) with Internal Standard.

e. Chromatographic Methods with External Standard. See Appendix D: Preparation and Analysis of Chemical Agent Sample for Purity Determination by GC/MSD with External Standard.

f. Storage and Handling of Stock and Working Standards for the Preparation of External and Internal Standards.

(1) Compounds used as reference materials in internal or external standard methods should be stored unopened IAW COA requirements until time of use or expiration. After expiration, these reference materials should not be used for purity analysis applications, unless they remain unopened and the purity can still be supported by the organization from which it was received, or analyzed and certified by the internal organization.

(2) Stock standards (high concentration) and working standards (lower level concentration standards produced from stock standards) should be stored at or below 4 °C (39 °F) for a period not to exceed 30 days. After 30 days, stock and working standards should not be used for purity analysis applications.

(3) All stock and working standards shall, at a minimum, be labeled with the agent, lot number, solvent, and date prepared and/or expiration date, if known.

(4) Allow stock and working standards to warm to room temperature before use to prevent solubility issues or degradation via condensation. At the end of use, return standards to cold storage.

(5) Keep organic solvents as water-free as possible.

(6) If preparing a dilution, rinse the syringe or pipette with the solvent of interest three times before obtaining an aliquot of the stock standard. Likewise, draw up some stock standard and discard before transferring an aliquot. Remove any air bubbles in the syringe or pipette before use.

(7) Stocks and working standards shall be containerized using compatible materials to prevent evaporation and diffusion of air and to ensure integrity.

(8) Glassware shall be cleaned IAW site-specific procedures before use.

(9) When possible, open standards in an inert atmosphere.

(10) Cover standards with an inert gas such as argon or nitrogen to prevent degradation (see paragraph 4.3.a).

4.5 Analysis of the Purity Samples.

Single analyses of at least three separate sample preparations of the purity sample will be run on the desired instrument, and, for chromatography methods, with a blank sample in between each analysis.

4.6 Data Handling.

See example methods in Appendices A, B, C, and D, as applicable. The following shall be documented:

a. Chemical receipt data:

(1) COA, including information on chemical purity, storage conditions, and expiration date of the standard.

(2) Chemical storage data, to include temperature logs of the storage location from receipt through use of the vial.

b. Instrumentation data: Analytical results of calibration standards and QC standard(s) as method applicable analytical results of the purity sample.

c. Final purity determination information:

(1) Report sample identification numbers and instrumentation data that were collected.

(2) Report the average purity from the triplicate run with a standard deviation.

5. DATA, DOCUMENTATION, AND RECORD REQUIRED.

a. Record retention should be IAW site-specific procedures.

b. Documentation regarding purity analyses must include:

(1) Lot number/identification number of agent being tested.

(2) Analyst name.

(3) Instrument identification number, type (e.g., GC/MSD, LC/MS/MS, NMR, etc.).

(4) Operating parameters to include column (if applicable) and carrier gas (if applicable).

(5) Date of analysis.

(6) Calibration and/or QC standard lot identification (if used).

(7) Identification or serial number of any calibrated equipment used during the test and the calibration date (e.g., flow meter), if applicable.

(8) Raw data/chromatograms from the instrumentation, a copy of the original chromatogram and the manually integrated chromatogram (if manual integration is performed).

(9) Purity results.

(10) Pretest instrument check results.

(11) Statistical analysis performed for error analysis.

c. Stock solution data for test site retention:

(1) Internal standard used.

(2) Volume of internal standard.

(3) Mass of volumetric flask (calibration and QC flasks).

(4) Volume of neat agent.

(5) Mass of volumetric flask and neat agent (calibration and QC flasks).

(6) Volume of deuterated solvent.

(7) Storage temperature.

d. The final report of the program that requires agent purity checks should have a record of the agent purity results. All other data should be archived. If multiple test sites are conducting testing, these data can be used to determine if the agent purity is a factor in disparate data results.

APPENDIX A. DETERMINATION OF NIST-TRACEABLE QUANTITATIVE WEIGHT PERCENTAGE PURITY OF NEAT CHEMICAL AGENT BY NMR WITH INTERNAL STANDARD.

Use quantitative NMR for purity analysis, when possible, as a best practice.

A.1. The procedure uses an IS 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 IS, and their molecular weights (MW). The weight percent calculations are not negatively affected by the presence of unidentified compounds or undetectable components in the sample (e.g., inorganic salts, insoluble solids), as long as the sample is homogeneous or a thoroughly mixed suspension before it is portioned out from the storage container.

A.2. The IS should be certified or verified as a secondary standard from a certified standard. Sigma Aldrich and other vendors have standards that are certified as NIST-traceable standards. A balance calibrated with NIST-traceable weights can also be used. These modifications make the method NIST-traceable.

A.3. STANDARDS.

The following chemicals are examples that can be used for the procedure. Other chemicals may prove to be more suitable for specific samples:

a. A secondary IS of triethyl phosphate is used, purchased from Sigma Aldrich, Part Number 538728, Chemical Abstracts Services (CAS®) Number (No.) 78-40-0, ReagentPlus® greater than or equal to 99.8 percent purity. This standard is not noticeably hydroscopic and has excellent stability and purity.

b. The NIST-traceable primary IS is dimethyl sulfone, purchased from Sigma Aldrich, Part Number 41867- 1G, CAS® No. 67-71-0, as a TraceCERT® certified reference material standard for quantitative NMR.

c. In cases where triethyl phosphate peaks overlap with the analyte, hexamethylphosphoramide may be used. This material is hygroscopic, and should be handled accordingly. Storage under inert gas and over molecular sieves minimizes the problems with water absorption.

A.4. SAMPLE PREPARATION.

This procedure is performed under proper engineering controls, IAW surety and safety regulations, equipment validations, and approved site-specific procedures. The balance is calibrated using NIST-traceable weights.

APPENDIX A. DETERMINATION OF NIST-TRACEABLE QUANTITATIVE WEIGHT PERCENTAGE PURITY OF NEAT CHEMICAL AGENT BY NMR WITH INTERNAL STANDARD.

a. Tare a screw-cap vial with cap on the balance. Transfer 10-20 mg of neat IS into the vial. Replace the cap and determine the weight of the IS 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 pipette (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 (CDCl3) or an appropriate alternative deuterated solvent.

d. Vortex or mix the sample for at least 15 seconds to dissolve both compounds in the solvent.

e. Transfer the solution into a polytetrafluoroethylene (PTFE; Teflon®) NMR tube insert. (Optional: A glass 4-mm 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 task is performed to doubly contain the agent sample so it can be removed from engineering controls. If regulations permit, masked operation with a singly contained sample will also work.

A-5. 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 percent 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 CDCl3.

c. Shim the magnet to maximize the lock signal.

d. Tune and match the probe.

APPENDIX A. DETERMINATION OF NIST-TRACEABLE QUANTITATIVE WEIGHT PERCENTAGE PURITY OF NEAT CHEMICAL AGENT BY NMR WITH INTERNAL STANDARD.

e. OPTIONAL: Determine the T1 relaxation time of the analytes in the sample solution using an inversion recovery experiment, following the instrument instructions. This procedure to determine the T1 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 one dimensional (1-D) spectrum. For a P-31 spectrum, if the T1 relaxation time is not determined (i.e., step in paragraph A.5.e is not performed), then set the relaxation time to 90 seconds, which is typically 20 times longer than the longest T1 in the solvent. Do not use Nuclear Overhauser Enhancement. Proton decoupling is used.

g. The following parameters are examples used for P-31-containing agents. Actual parameter names and values will vary depending on the make and model of the NMR and the nucleus that is observed. A precision and accuracy study should be under taken to verify that the parameters used are working correctly for a given instrument and nucleus:

(1) Relaxation time: 90 seconds or as determined in step in paragraph A.5.e or f.

(2) Excite pulse: 90 degree pulse (determining the time and amplitude for this pulse that corresponds to a 90 degree excitation should be found in the NMR instrument documentation.)

(3) Number of data points: 64K.

(4) Number of scans: 16 for P-31.

(5) Sweep width: 300 parts per million for P-31.

(6) Center frequency: 15 parts per million for P-31 is typical. (For best results, the center frequency should be equidistant between the IS peak and the analyte peak that will be integrated.)

(7) Automatic gain determination: turned on for the first spectrum, but then the same gain can be used for replicates.

h. Acquire data. A total of seven replicate runs are recommended for statistical determination of the NMR variability, signal to noise ratio, and integration errors. A minimum of three replicate runs are required. Several samples can be prepared to determine the weighing statistical errors, if desired.

APPENDIX A. DETERMINATION OF NIST-TRACEABLE QUANTITATIVE WEIGHT PERCENTAGE PURITY OF NEAT CHEMICAL AGENT BY NMR WITH INTERNAL STANDARD.

A.6. DATA PROCESSING.

a. Apply a window function (exponential multiplication). This task may be performed using a line broadening parameter in the range of 0.5 to 2 Hertz, 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 the data to convert 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 IS.

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 ensure 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 may 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 percent 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 peak can also be split into a multiplet by the protons. All the peaks in the multiplet must be integrated.

A.7. PURITY DETERMINATION OF SECONDARY STANDARD.

a. Because 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 performed using the same procedure as described previously.

b. Several primary standards are commercially available and can be used, but the standard dimethyl sulfone is commonlyused . Proton NMR is used to perform the purity determination.

c. Purity Calculation. The weight percent of the analyte (Wt% A) in the sample is calculated using Equation A-1, where analyte A is the agent and IS the internal standard. The same formula is used for all spectra and IS, but the parameters will change based on the molecule that is being detected.

APPENDIX A. DETERMINATION OF NIST-TRACEABLE QUANTITATIVE WEIGHT PERCENTAGE PURITY OF NEAT CHEMICAL AGENT BY NMR WITH INTERNAL STANDARD.

$$wt\%\ A = \frac{Area\ under\ A\ peak}{Area\ under\ IS\ peak}\ x\ \frac{MW\ of\ A}{MW\ of\ IS}\ x\ \frac{Weight\ of\ IS}{Weight\ of\ A}\ x\ \frac{No\ identical\ P(IS)}{No.P(A)}\ x\ (pur\ IS)\% \quad \textit{(Equation A-1)}$$

where:

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

A.8. CONCLUSION.

By using the NIST-traceable IS, and the balance that is calibrated with NIST-traceable weights, the purity of the chemical warfare agent feedstock agent is determined using a NIST-traceable method with NMR analysis.

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APPENDIX B. DETERMINATION OF WEIGHT PERCENTAGE PURITY OF NEAT CHEMICAL AGENT BY PROTON NMR WITH INTERNAL STANDARD.

B.1. This method provides Chemical Agent (CA) purity analysis using proton NMR. It is intended that the analyst will use NMR for purity analysis **unless** the customer specifically requests a GC-based method. After completing purity analysis, the analyst will provide the customer a certificate of purity analysis. The following method includes descriptions related to a Bruker NMR instrument using Topspin® software. Adapt the suggested procedure to fit the particular NMR instrument and associated software used.

B.2. BACKGROUND.

a. In general terms, the analyte masses for analysis and an added internal standard will be individually determined, mixed to homogeneity, and then placed into an NMR sample tube for analysis. Thereby, the accuracy of this method relies on the following:

(1) The accuracy of reference material used.

(2) The accuracy of an analytical balance.

(3) Homogeneity of the chemical solutions in the vials.

b. Analysts will determine analyte purity by comparing the analyte with the reference to the internal standard integrations on a ^{1}H-NMR spectrum.

c. Analysts will **only** use the minimum amount of analyte necessary to perform the operation, which will be below the surety threshold.

B.3. REQUIRED CHEMICALS FOR THE ANALYSIS.

A deuterated solvent and a known internal standard are required to determine analyte purity. Available deuterated solvents include chloroform-d, dimethyl sulfite-d_6, water-d_2, acetone-d_6, and acetonitrile-d_3 subject to solubility. Known internal standards include dimethyl sulfone, 1,2,4,5-tetramethylbenzene, and trimethyl(phenyl)silane. When necessary, analysts may replace the internal standards with a chemical whose ^{1}H-NMR signals do not interfere with those of the analyte.

NOTE: For compliance with International Organization for Standardization (ISO) 17025 certification, the analyst will purchase the ISO Guide 34 internal standards.

B.4. SAMPLE PREPARATIONS FOR CAs.

a. Preparation of First Dilution of CAs.

APPENDIX B. APPENDIX B. DETERMINATION OF WEIGHT PERCENTAGE PURITY OF NEAT CHEMICAL AGENT BY PROTON NMR WITH INTERNAL STANDARD.

b. In a validated chemical agent hood, use a four-decimal-place analytical balance to measure the masses of CAs and ISs.

NOTE: Hold the antistatic gun inside the balance chamber and click 4 to 5 times **before** placing any item on the balance.

c. Place an empty 10-mL or 25-mL volumetric flask and stopper on the balance. Weigh and record the mass of the empty volumetric flask and the stopper.

d. Remove the empty volumetric flask and stopper from the balance. Use a syringe or an adjustable-volume pipette to transfer the required volume of neat CA into the volumetric flask. Weigh and record the mass of flask, stopper, and neat CA.

e. Remove the volumetric flask (with stopper) containing neat CA from the balance. Use a syringe or an adjustable-volume pipette to transfer 1 to 5 times the volume of internal standard as that from the neat CA volume from paragraph 4.d on Sample Preparations for Chemical Agents to the volumetric flask. Weigh and record the combined mass of flask, stopper, the internal standard, and neat CA.

f. Fill the flask to the mark with the appropriate deuterated solvent and cap with the weighed stopper.

g. Weigh and record the mass of the volumetric flask, stopper, and prepared surety level solution.

h. Ensure the stopper is secure. Mix the solution by inverting the flask a minimum of 20 times.

i. Prepare three first dilution samples for each CA analyte. Perform the steps in paragraphs B.4.a. through g for each sample.

B.5. PREPARATION OF THE STOCK A SOLUTION OR THE BELOW SURETY THRESHOLD STANDARD SOLUTION.

a. In a validated CA hood, use a four-decimal-place analytical balance to measure the mass of the first dilution sample.

b. Zero the Balance.

(1) Weigh and record the mass of the empty container and cap.

(2) Open the first dilution container.

(3) Remove the appropriate amount of parent solution from its container.

APPENDIX B. APPENDIX B. DETERMINATION OF WEIGHT PERCENTAGE PURITY OF NEAT CHEMICAL AGENT BY PROTON NMR WITH INTERNAL STANDARD.

(4) Dispense the parent solution into the dilute solution container allowing the solution to flow out of the pipette near the bottom of the container. Ensure that no solution comes in contact with a ground glass joint (makes cleanup more difficult).

(5) Touch the pipette to the side of the container after dispensing chemical to remove any residual liquid dangling on the end of the pipette tip.

(6) Dispose of the pipette tip in the decontamination solution.

(7) Recap the parent solution container.

(8) Recap the dilute solution container.

(9) Weigh and record the mass of the container, cap, and parent chemical on a spreadsheet.

(10) Remove the cap and fill the container approximately half full with the solvent.

(11) Remove the cap and swirl the container vigorously for at least 30 seconds.

(12) Remove the cap and carefully fill the container. If using a volumetric flask, fill to the mark with the deuterated solvent, adding the last of the solvent with a disposable pipette.

(13) Replace the cap and dispose of the pipette.

(14) Weigh and record the mass of the container and all contents on a spreadsheet.

(15) Ensure the cap is secure and mix the solution by inverting the container a minimum of 20 times.

(16) Transfer each below surety threshold standard solution to a 10-mL conical vial. Wrap the vial with parafilm. Acquire three 10-mL conical vials from the agent storage laboratory and place in the NMR laboratory.

(17) Use Equation B-1 to ensure the solution is below the surety level.

$$C_{DS} = \frac{C_{PC} \times \frac{Wt_{PC}}{D_{PC}}}{V} \qquad \textit{(Equation B-1)}$$

APPENDIX B. APPENDIX B. DETERMINATION OF WEIGHT PERCENTAGE PURITY OF NEAT CHEMICAL AGENT BY PROTON NMR WITH INTERNAL STANDARD.

where:

C_{DS} = concentration of dilution solution (mg/mL)
C_{PC} = concentration of parent chemical (mg/mL)
Wt_{PC} = weight of the parent chemical (g)
D_{PC} = density of the parent chemical (g/mL)
V = volume of the solution (mL)

B.6. PREPARATION OF NMR SAMPLES OF CA SOLUTIONS.

a. Using a glass pipette, transfer 1 mL of vial contents into the 5-mm NMR sample tube.

b. Cap the NMR sample tube with the supplied plastic cap. **NOTE**: The agent is now ready for NMR analysis.

B.7. SAMPLE PREPARATIONS FOR TOXIC INDUSTRIAL COMPOUNDS OR NON-CAs.

a. Use an analytical balance accurate to four decimal places, an antistatic gun, and a 2-mL vial to record the masses of the analyte and an internal standard. Use 50 to 100 mg of material to acquire an accurate mass measurement(s). Using a glass pipette, add 2 mL of an appropriate deuterated solvent to the vial containing the mixture and then cap.

b. Prepare three samples for each analyte. Perform these three steps for each sample:

(1) Mix the contents of the vial to homogeneity using a vortex mixer or inverting the vial at least 20 times.

(2) Using a glass pipette, transfer 1 mL of vial contents into the 5-mm NMR sample tube.

(3) Cap the NMR sample tube with the supplied plastic cap. **NOTE**: The sample is now ready for NMR analysis.

B.8. OPERATIONS, DATA ANALYSIS, AND DATA REPORTING.

a. NMR Operations.

(1) Insert the NMR tube into the NMR spinner and use a depth gauge to adjust the height. Use caution when inserting the tube into the spinner turbine to prevent damaging the NMR tube because it has thin, fragile walls.

(2) Once the spinner is in the correct place on the NMR tube, remove the tube from the sample depth gauge and carefully wipe it with Kimwipes®.

APPENDIX B. APPENDIX B. DETERMINATION OF WEIGHT PERCENTAGE PURITY OF NEAT CHEMICAL AGENT BY PROTON NMR WITH INTERNAL STANDARD.

(3) Place the NMR tube into one of the holders on the carousel, automated sample changer.

(4) Perform all NMR experiments using TopSpin® (instrument) software. Retrieve the NMR tube from the carousel and place the analyzed NMR tube on the designated rack in the engineering controlled fume hood and remove the spinner from the tube.

(5) Use the TopSpin® software for ^{1}H acquisition. The TopSpin® software performs 1-D hydrogen-nucleus (^{1}H, i.e., a proton), carbon-13 (^{13}C), nitrogen-15 (^{15}N), fluorine-19 (^{19}F), and phosphorus-31 (^{31}P) experiments, as well as homonuclear and heteronuclear two-dimensional experiments.

(6) Operators can manually operate the software operation for sample analysis by using the TopSpin® command line or by clicking the Acquire menu and then clicking through each menu item under the Acquire menu [i.e., (1) create dataset, (2) load the sample, (3) lock the solvent, (4) tune and match the probe, (5) spin the sample, (6) shim the sample, (7) load the prosol parameters, (8) set the receiver gain, and(9) start the data acquisition].

(7) Consider the experiment's pre-acquisition delay (experimental Parameter **d1**) because an adequate delay time is required for the integrated areas of the NMR signals to reflect the molar abundance of their corresponding nuclei accurately.

(8) Use a pre-acquisition delay of 40 seconds, **unless** the spin-lattice relaxation times for the ^{1}H signals of the agent and the internal standard have been measured or estimated for a particular sample at a specific temperature and magnetic field strength. For quantitative NMR, the **d1** must be at least **5T1** for accurate purity results.

NOTE: This pre-acquisition delay will be far in excess of five times the longest spin-lattice relaxation time of the sample. Normally, analyte or internal standard nuclei will have considerably different spin-lattice relaxation times when present in different matrices. Relaxation times will change appreciably with experimental temperature and magnetic field strength (i.e., the same analyte nuclei in a particular sample can have different spin-lattice relaxation times when measured with different NMR spectrometers). Therefore, spin-lattice relaxation times are just as much a consequence of sample matrix and experimental conditions as they are the analyte molecule itself. **Do not** assume that the relaxation time measured for a particular analyte nuclei will be the same when the agent is present in a different matrix, even though the two matrices appear very similar.

(9) Create a new experiment file, or open an old file from the TopSpin® browser.

APPENDIX B. APPENDIX B. DETERMINATION OF WEIGHT PERCENTAGE PURITY OF NEAT CHEMICAL AGENT BY PROTON NMR WITH INTERNAL STANDARD.

(a) Enter the command **edc**.

(b) Enter a new filename.

(c) Click the proper experiment from the Bruker defined parameters. The most common experiment is titled PROTON for ^{1}H NMR.

(d) Enter the command **atma** to initiate automatic tuning and matching of probe head.

(e) Enter the command **getprosol** to load solvent-dependent pulses and probe power levels.

(f) Enter the command **ased**, which is a short list for **eda**, to modify the pulse width if necessary.

(g) Enter the command **lock** and select the proper deuterated solvent to lock in the solvent.

(h) Enter the command **topshim** to perform the automatic shimming.

(i) Enter the command **rga** to adjust and identify the receiver gain.

(j) Enter the command **zg** to perform the data acquisition.

NOTE: Perform the auto tune/match, shim and adjust the receive gain **before** using the command **zg** to acquire the spectrum.

(10) After acquiring the spectrum, use the command **ft** or **efp** to transform the time-domain data to frequency domain data (an NMR spectrum).

(11) Manually or automatically phase-correct all the signals of the spectrum.

(12) Data acquisition and processing are now complete.

(13) Use the spectral line integration description from paragraph B.9.d to determine purity.

B.9. DATA ANALYSIS.

a. Perform all spectral data processing using TopSpin® software.

b. Begin quantitative analysis with the integration of all peaks observed in the spectrum to confirm analyte structures and the internal standard.

APPENDIX B. APPENDIX B. DETERMINATION OF WEIGHT PERCENTAGE PURITY OF NEAT CHEMICAL AGENT BY PROTON NMR WITH INTERNAL STANDARD.

c. Select the necessary clean peaks of the analyte and internal standard to perform integrations for an accurate purity determination.

d. Calculate analyte purity, by weight percent, directly from the mass of internal standard, the analyte amount added to the sample, and the integrated area of the spectral lines for the agent and internal standard using Equation B-4. **NOTE**: Equation B-4 was derived from Equations B-2, B-3, and B-4.

e. Determine the calculated ratio of moles between agents and the internal standard from Equation B-2.

$$\frac{mol_{analyte}}{mol_{internal\,standard}} = \frac{M_1}{MW_1} \times \frac{MW_2}{M_2} \qquad \textit{(Equation B-2)}$$

where:

$mol_{analyte}$ = moles of the analyte (mol).
$mol_{internal\ standard}$ = moles of the internal standard (mol).
MW_1 = molecular weight of analyte (g/mol).
MW_2 = molecular weight of the internal standard (g/mol).
M_1 = mass of analyte (mg).
M_2 = mass of the internal standard (mg).

f. Determine the experimented ratio of moles between agents and the internal standard from Equation B-3.

$$\frac{mol_{analyte}}{mol_{standard}} = \frac{I_1}{n_1} \times \frac{n_2}{I_2} \qquad \text{(Equation B-3)}$$

where:

$mol_{analyte}$ = moles of the analyte (mol).
$mol_{standard}$ = moles of the internal standard (mol).
I_1 = values of integration of selected protons or combined protons of analyte.
I_2 = values of integration of protons of the internal standard.
n_1 = number of protons of analyte.
n_2 = number of protons of the internal standard.

APPENDIX B. APPENDIX B. DETERMINATION OF WEIGHT PERCENTAGE PURITY OF NEAT CHEMICAL AGENT BY PROTON NMR WITH INTERNAL STANDARD.

g. Compare the calculated and experimented ratio of moles using Equation B-4.

$$\frac{Experimental}{Calculated} = \frac{I_1}{n_1} \times \frac{n_2}{I_2} \times \frac{MW_1}{M_1} \times \frac{M_2}{MW_2} \qquad \text{(Equation B-4)}$$

where:

I_1 = values of integration of selected protons or combined protons of analyte.
I_2 = values of integration of protons of the internal standard.
n_1 = number of protons of analyte.
n_2 = number of protons of the internal standard.
MW_1 = molecular weight of analyte (g/mol).
MW_2 = molecular weight of the internal standard (g/mol).
M_1 = measured weight of analyte (g).
M_2 = measured weight of the internal standard (g).

NOTES: 1. If unable to acquire a gravimetric measurement, multiply volume (mL) by density (g/mL) and use in place of M_1 and M_2 for analyte and internal standard, respectively.
2. The data are acceptable when the standard deviation for the average three samples is less than 1 percent. If outside this tolerance, prepare another set of samples for reanalysis. Analysis of an additional set of samples is not required when the requestor provides a written acceptance note of the results.

h. Gravimetrically determine analyte purity by weight percent using Equation B-5.

$$P_1 = P_2 \times \frac{I_1}{n_1} \times \frac{n_2}{I_2} \times \frac{MW_1}{MW_2} \times \frac{M_2}{M_1} \times 10 \qquad \textit{(Equation B-5)}$$

where:

P_1 = percent purity of analyte (%).
P_2 = purity of internal standard.
I_1 = values of integration of selected protons or combined protons of analyte.
I_2 = values of integration of protons of the internal standard.
n_1 = number of protons of analyte.
n_2 = number of protons of the internal standard.

APPENDIX B. APPENDIX B. DETERMINATION OF WEIGHT PERCENTAGE PURITY OF NEAT CHEMICAL AGENT BY PROTON NMR WITH INTERNAL STANDARD.

MW_1 = molecular weight of analyte (g/mol).
MW_2 = molecular weight of the internal standard (g/mol).
M_1 = measured mass of analyte (g).
M_2 = measured mass of the internal standard (g).

B.10. DATA REPORTING.

a. Compile a standard data package and write a memorandum summarizing purity results and purity certificate (for CAs **only**).

b. Report the sample identification, lot control number (**only** for CAs), and average percentage purity with a standard deviation. Report the average percentage purity to a minimum of three significant figures.

c. Submit the data package for peer and quality control (QC) review **before** transmitting the report to the customer(s).

d. Archive the data package.

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APPENDIX C. PREPARATION AND ANALYSIS OF CHEMICAL AGENT SAMPLE FOR PURITY DETERMINATION BY GC/MSD OR LC/MS/MS WITH INTERNAL STANDARD.

C.1. PURPOSE AND SCOPE.

a. Purpose. This appendix provides a method to determine the purity of a neat CA in a volume of solvent. Following preparations, purity solutions are analyzed by GC-MS or LC/MS/MS to determine the CA purity as determined by peak area relative to an IS using an established relative response factor (RRF).

b. Scope. This method is designed to be generalized so that it can be applied to both liquid and gas chromatographic instruments coupled with various detectors. Chromatographic separation coupled with MS is the preferred approach for the internal standard RRF method as it minimizes the potential for unresolved compounds to co-elute with the IS being used. While other detection methods may potentially be successful (e.g., flame ionization detection), there are greater opportunities for failure because of their reduced selectivity and should be avoided.

C.2. PRINCIPLES OF THE METHOD.

a. The primary purity analysis instrument used in this method is a gas or liquid chromatograph, coupled with a mass spectrometer (GC/MSD or LC/MS/MS).

b. For the chromatographic method, samples with unknown purity will be analyzed using an IS that has a previously determined RRF with the specific CA being evaluated, and the RRF is determined under identical analysis conditions. Therefore, the accuracy of this method relies on the following:

(1) The availability of known purity CA and IS to develop the RRF on which the method is based.

(2) The accuracy of the solution preparations in terms of the concentration of the IS and CA for both the RRF determination, and for the actual purity analysis.

(3) The accuracy of the instrument.

(4) The ability to fully resolve the CA and IS from any impurities using both the chromatographic separation and selectivity offered by using mass spectrometry for detection.

C.3. MATERIALS.

CASARM of known purity, or chemical agents with a valid COA, and the IS reference material with a valid COA will be used to prepare reference solutions for measurement of the RRF.

APPENDIX C. PREPARATION AND ANALYSIS OF CHEMICAL AGENT SAMPLE FOR PURITY DETERMINATION BY GC/MSD OR LC/MS/MS WITH INTERNAL STANDARD.

C.4. EQUIPMENT AND CALIBRATION.

a. All analytical pipets and analytical balances used for solution preparations will be calibrated annually IAW site-specific procedures. Check the balance with the certified weights before each use, and subsequently confirm the pipet calibration using the balance.

b. Before use, all GCs (LCs) used for this method should have been installed and found operational at the designated location of the operation according to the vendor user's manuals.

c. Chromatographic instrument and detector system will be maintained at a frequencythat is compliant with site-specific procedures, and of no less than annually.

d. All the maintenance performance will be recorded in the instrument logbook.

C.5. ENVIRONMENTAL FACTORS.

The environmental conditions accommodation matrix is shown as Table C-1.

TABLE C-1. ENVIRONMENTAL CONDITIONS ACCOMMODATIONS MATRIX

ENVIRONMENTAL CONDITIONS ACCOMMODATIONS MATRIX		
Requirements, Activities, and Equipment	Environmental Issue	Accommodation
Requirement: Temperature of the laboratory that houses gas chromatography (GC) or liquid chromatography (LC) instrumentation used for purity analysis shall be maintained at 24 ± 5 °C (75.2 ± 3 °F).	If the temperature is too low or too high, the GC oven might not cool down or heat up consistently or it might cause a precipitation or degradation of the GC samples.	The assigned laboratory will be kept at 24 °C (75.2 °F).

C.6. INTERFERENCES.

Impurities may co-elute with the analyte or the IS, which will negatively affect the purity results. Chromatographic methods not coupled with a mass spectrometer should be run on more than one column under different conditions to further ensure that there is adequate separation between the agent, internal standard, and all impurities.

APPENDIX C. PREPARATION AND ANALYSIS OF CHEMICAL AGENT SAMPLE FOR PURITY DETERMINATION BY GC/MSD OR LC/MS/MS WITH INTERNAL STANDARD.

C.7. PROCEDURE.

a. Solution Preparation for Chemical Agent Relative Response Factor Determination (preparation of first-order dilution of CA at known concentration).

(1) First-order dilution agent preparations described here are based on a volume-to-volume dilution. Volume-to-volume, mass-to-mass, and mass-to-volume dilutions are all considered acceptable using the RRF method. Improved accuracy and precision may be offered by preparations using the mass-to-mass method when executed correctly. For solid agents, mass-to-mass and mass-to-volume dilutions are required for obvious reasons.

(2) In a certified chemical agent hood, allow the CA of certified purity to come to room temperature.

(3) Prepare and label clean 60-mL glass vials fitted with Teflon-lined screw caps.

(4) Using appropriate calibrated pipets, pipet the required volume of the selected agent followed by the required volume of the appropriate solvent into the labeled glass vial. Replace cap and mix thoroughly by inverting a minimum of 20 times.

NOTE: Prepared solution concentrations should be below AR 50-6 dilute limits, but high enough to ensure accurate and precise measurement.

(5) Table C-2 provides target concentrations for several common CA.

TABLE C-2. STOCK SOLUTION PREPARATION

Agent	Agent Volume (µL)	Solvent Volume (mL)	Approximate Concentration (µg/mL)
Soman (GD)	50	50	1,000
Sarin (GB)	50	50	1,000
Tabun (GA)	50	50	1,000
Persistent Nerve Agent (VX)	45	50	900
Distilled Mustard (HD)	50	50	1,250

b. Average RRF Determination.

APPENDIX C. PREPARATION AND ANALYSIS OF CHEMICAL AGENT SAMPLE FOR PURITY DETERMINATION BY GC/MSD OR LC/MS/MS WITH INTERNAL STANDARD.

(1) Calculate an average RRF for each CA in each solvent of interest using an IS of known concentration.

(2) Select an IS that does not interfere with the CA response, such as a stable, deuterated chemical (e.g., naphthlene-d8) with a strong molecular ion or primary fragment ion.

NOTE: IS should be purchased from a reputable vendor, with a valid COA, stored at prescribed conditions, and be used within cited expiration date. It is preferable if the IS can be purchased as a solution at a prediluted concentration so that can be used directly. It is also preferable that the IS available in small volume sealed ampules suitable for single use.

(3) Aliquot a known volume of CA solution (e.g., 1.0 mL) into a GC vial.

(4) Add the IS to the CA solution to generate a known concentration of IS. The IS concentration should result in an area response within a factor of 5 of the CA area response.

(5) Analyze the CA solution by full scan GC/MSD or LC/MS/MS using an established instrument method. Analysis should result in acceptable chromatography and response for the CA and IS. The same method must be used for all purity analyses.

(6) Select an m/z value for the CA and IS and measure the area response of each compound.

(7) Calculate the CA RRF as presented in Equation C-1.

$$CA\ RRF = \frac{A_{ref} \times C_{IS}}{A_{IS} \times C_{Ref}} \qquad \textit{(Equation C-1)}$$

where:

CA RRF = CA reference relative response factor
A_{Ref} = area response of CA reference
A_{IS} = area response of IS
C_{IS} = concentration of IS (µg/mL)
C_{Ref} = concentration of CA reference (µg/mL)

APPENDIX C. PREPARATION AND ANALYSIS OF CHEMICAL AGENT SAMPLE FOR PURITY DETERMINATION BY GC/MSD OR LC/MS/MS WITH INTERNAL STANDARD.

(8) To calculate an average RRF (RRF_{Avg}) for each CA, prepare and analyze 11 unique reference solutions[6]. When possible, it is suggested these solutions should include at least three different CA sources.

NOTE: An average RRF should be calculated for each solvent used to generate a CA solution, and in all cases, the determined RRF should only be used in purity determinations for CA prepared in the same solvent, similar ratios of IS to CA, and run using the same analytical method. When possible, a best practice would also be to use the same instrument for determining the RRF and analyzing the purity of a CA.

c. Solution Preparation for CA Purity Determination.

(1) Best practice is to prepare CA solutions in triplicate at the same concentrations used for RRF determination (see Table C-2) to ensure preparations accuracy. Solution preparations can be made by either volume-to-volume, mass-to-mass, or volume-to- mass. Calculate the nominal CA concentration for each preparation using the appropriate equations below (see Equations C-2 (Volume/Volume Basis), C-3 (Weight/Volume Basis), and C-4 (Weight/Weight Basis)).

$$C_{Nom} = \frac{V_{CA}}{V_{Sol}} \times D_{CA} \qquad \textit{(Equation C-2)}$$

where:

C_{Nom} = *nominal CA concentration (µg/mL)*
V_{CA} = *CA volume (mL)*
V_{Solv} = solvent volume (mL)
D_{CA} = CA density (µg/mL) (Should establish list of all CA density values).

$$C_{Nom} = \frac{M_{CA}}{V_{Sol}} \qquad \textit{(Equation C-3)}$$

where:

C_{Nom} = *nominal CA concentration (µg/mL)*
M_{CA} = *CA mass (µg)*
V_{Sol} = solvent volume (mL)

APPENDIX C. PREPARATION AND ANALYSIS OF CHEMICAL AGENT SAMPLE FOR PURITY DETERMINATION BY GC/MSD OR LC/MS/MS WITH INTERNAL STANDARD.

$$C_{Nom} = \frac{M_{CA}}{M_{Sol}} \times D_{Sol} \qquad \textit{(Equation C-4)}$$

where:

C_{Nom} = *nominal CA concentration (µg/mL)*
M_{CA} = *CA mass (µg)*
M_{Sol} = *solvent mass (mL)*
D_{Sol} = solvent density (g/mL)

(2) Aliquot a known volume of CA solution (e.g., 1.0 mL) into a GC vial.

(3) Add IS to the CA solution to generate the same concentration as used to generate RRF_{Avg}.

(4) Analyze CA sample solutions with the same GC/MSD or LC/MS/MS method used to generate the RRF_{Avg} values.

(5) Determine the area response for the CA and IS.

(6) Calculate the measured CA concentration using Equation C-5.

$$C_{CA} = A_{CA} \times \frac{C_{IS}}{A_{IS}} \times \frac{1}{RRF_{Avg}} \qquad \textit{(Equation C-5)}$$

where:

C_{CA} = measured CA sample concentration (µg/mL)
A_{CA} = area response of CA sample
C_{IS} = know concentration of IS (µg/mL)
A_{IS} = area response of IS
RRF_{Avg} = previously determined average relative response factor specific to the CA/solvent solution and analytical method used to determine.

d. Purity Calculation.

APPENDIX C. PREPARATION AND ANALYSIS OF CHEMICAL AGENT SAMPLE FOR PURITY DETERMINATION BY GC/MSD OR LC/MS/MS WITH INTERNAL STANDARD.

(1) Calculate CA sample percent purity using Equation C-6.

$$\%Purity = \frac{C_{CA}}{C_{Nom}} \times 100 \qquad \textit{(Equation C-6)}$$

where:

C_{CA} = measured CA sample concentration (µg/mL) from Equation C-5.
C_{Nom} = nominal CA sample concentration (µg/mL) from Equation C-2, C-3, or C-4.

(2) Report the mean weight percent purity, calculated above, to three significant figures and standard deviation from the three individual preparations (if made) to allow for accurate testing calculations before rounding results.

(3) It is possible that the determined mean weight percent purity is greater than 100 percent. An analysis of the individual replicates, the standard deviation, the solution preparations and IS COA should be conducted. If an assignable cause is not determined, the mean purity value and standard deviation should be reported, and a percent purity value of 100 percent should be used.

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APPENDIX D. PREPARATION AND ANALYSIS OF CHEMICAL AGENT SAMPLE FOR PURITY DETERMINATION BY GC/MSD OR LC/MS/MS WITH EXTERNAL STANDARD.

D.1. PURPOSE.

This appendix provides a method to determine the purity of agents in a liquid state using GC. Purity of the agent is determined by comparing the response against a standard GC calibration curve prepared using standards prepared from a source of known purity.

D.2. SCOPE.

This method is designed to be generalized so that it can be applied to both liquid and gas chromatographic instruments coupled with various detectors. Chromatographic separation coupled with mass spectrometry is the preferred method for external calibration as it minimizes the potential for unresolved compounds to co-elute with the agent that is being evaluated. While other detection methods may potentially be successful, there are greater opportunities for failure because of their reduced selectivity. Additionally, if a laboratory does not have more than one source (e.g., lot) for a known purity standard, then this method is inapplicable for use.

D.3. PRINCIPLES OF THE METHOD.

a. The primary purity analysis instrument used in this method is a gas or liquid chromatograph, preferably coupled with a mass spectrometer (GC/MSD or LC/MS/MS).

b. For the chromatographic method, samples with unknown purity will be analyzed against a standard calibration curve developed using standards prepared from agent reference materials of known purity. Thereby, the accuracy of this method relies on the following:

(1) The accuracy of the solution preparations.

(2) The availability of reliable agent reference materials for preparation of the external calibration.

(3) The accuracy of the instrument.

(4) The ability to fully resolve the analyte from any impurities.

D.4. SAMPLE REQUIREMENTS FOR TEST METHODS.

Sample Preparation for Chemical Agent Purity Analysis. First-order dilution agent preparations described here are based on a mass-to-mass dilution because of the improved accuracy and precision offered by this preparation method when executed correctly. Mass-to-volume and volume-to-volume dilutions are also considered acceptable using the external standard method:

a. In a certified chemical agent hood, use a four-decimal-place analytical balance to measure the masses of chemical agent.

APPENDIX D. PREPARATION AND ANALYSIS OF CHEMICAL AGENT SAMPLE FOR PURITY DETERMINATION BY GC/MSD OR LC/MS/MS WITH EXTERNAL STANDARD.

NOTE: Hold the antistatic gun inside the balance chamber and click four to five times before placing any item on the balance.

b. Place an empty 10-mL or 25-mL volumetric flask and stopper on the balance. Weigh and record the mass of the empty volumetric flask and the stopper. Use the appropriate balance and flasks to provide an accurate resolution for the measurement.

c. Remove the empty volumetric flask and stopper from the balance. Use a syringe or an adjustable-volume pipette to transfer the required volume of neat agent into the volumetric flask. Stopper the flask and reweigh.

d. Remove the volumetric flask (with stopper) containing neat agent from the balance. Use a syringe or an adjustable-volume pipette to transfer and fill the flask to the mark with the appropriate solvent and cap with the weighed stopper.

e. Weigh and record the mass of the volumetric flask, stopper, and prepared surety level solution.

f. Ensure the stopper is secure. Mix the solution by inverting the flask a minimum of 20 times.

g. Using the mass of agent and mass of solvent, calculate the µg/g concentration of the agent in the solution.

h. Prepare three first-order dilution samples for each agent using the procedure described above.

i. If necessary, prepare a suitable dilution of the first-order solution prepared above that is at a suitable target concentration for the standard calibration curve used to calibrate the instrument to be used for analysis. Fill GC (or LC) sample vials and store for subsequent analysis.

D.5. MATERIALS.

CASARM or chemical agents with a valid COA will be used to prepare calibration standard solutions for the calibration curve, Initial Calibration Verifications (ICVs), and Continuous Calibration Verifications (CCVs) samples.

APPENDIX D. PREPARATION AND ANALYSIS OF CHEMICAL AGENT SAMPLE FOR PURITY DETERMINATION BY GC/MSD OR LC/MS/MS WITH EXTERNAL STANDARD.

D.6. EQUIPMENT AND CALIBRATION.

a. An analytical balance will be calibrated annually at a minimum. Check the balance with the certified weights before each use.

b. Before use, all GCs (LCs) used for this method should have been installed and found operational at the designated location of the operation according to the user's manuals.

c. Chromatographic instrument and detector system will be maintained at a frequency that is compliant with site-specific procedures, and of no less than annually.

d. All the maintenance performance will be recorded in the instrument logbook.

D.7. ENVIRONMENTAL FACTORS.

Table D-1 shows the environmental accommodations matrix.

TABLE D-1. ENVIRONMENTAL ACCOMMODATIONS MATRIX.

ENVIRONMENTAL CONDITIONS ACCOMMODATIONS MATRIX		
Requirements, Activities, and Equipment	Environmental Issue	Accommodation
Requirement: Temperature of the laboratory that houses GC or LC instrumentation used for purity analysis shall be maintained at 24 ± 5 °C (75.2 ± 3 °F).	If the temperature is too low or too high, the GC oven might not cool down or heat up consistently or it might cause a precipitation or degradation of the GC samples.	The assigned laboratory will be kept at 24 °C (75.2 °F).

D.8. INTERFERENCES.

CASARM or chemical agents with a valid COA will be used to prepare calibration standard solutions for the calibration curve, ICVs, and CCVs samples.

D.9. PROCEDURE.

a. Solution Preparation.

APPENDIX D. PREPARATION AND ANALYSIS OF CHEMICAL AGENT SAMPLE FOR PURITY DETERMINATION BY GC/MSD OR LC/MS/MS WITH EXTERNAL STANDARD.

(1) Analysts will prepare the purity solutions in triplicate, with the target calculated target concentration for the analyte being about 500 µg/mL.

NOTE: When using an analytical balance to weigh any of the materials, ensure that the smallest measurement weighed has at least two significant figures associated with the determined measurement.

(2) Prepare a set of analysis standards (minimum of three) that bracket the concentration of the agent samples, as well as calibration check CCVs and ICVs IAW with standard site procedures.

(3) The calibration standards must be prepared using a neat agent with a known purity, preferably from a non-expired CASARM, or with material from a chemical laboratory purity certificate.

NOTE: Always use CASARM materials, when available, to prepare the analysis standards.

b. Sample Analysis.

(1) Develop the calibration curve using the prepared standards. Verify the calibration curve using the ICV solution(s). The ICV sample(s) must result in a calculated percent recovery within ±10 percent of the actual concentration and the calibration curve must result in a correlation coefficient (r^2) of 0.99.

NOTE: The established acceptance criteria may be different when the client requests a different tolerance for ICV samples.

(2) Analyze the sample solutions and the CCV solution against the curve. The CCV solution must result in a calculated percent recovery within ±10 percent of the actual concentration.

NOTE: ICV and CCV can be the same solution. The established acceptance criteria may be different when the client requests a different tolerance for CCV samples.

D.10. RECORDING DATA.

a. Compile a standard data package and write a memorandum report/purity certificate listing sample identification, lot control number, weight percent purity, and peak area percent purity.

b. Report the average weight/percent purity to a minimum of three significant figures with a standard deviation.

c. Submit the data package for peer and quality control review before transmitting the report to the requestor(s) IAW site-specific quality control procedures.

d. Archive the data package IAW site-specific document holding and archiving procedures.

D.11. CALCULATIONS.

a. Using the GC/MSD or LC/MS/MS software, determine the measured concentration.

b. Using Equation D-1, determine the percent purity of the agent.

$$P_1 = \frac{C_2}{C_1} \times 100 \qquad \textit{(Equation D-1)}$$

where:

P_1 = percent purity of analyte (percent)
C_1 = calculated concentration from the sample preparation (µg/mL)
C_2 = measure concentration from the analytical result (µg/mL)

D.12. QUALITY CONTROL ASSESSMENT.

The determined ICV and CCV must be within 10 percent of the actual/prepared concentration and the calibration curve must result in an r^2 of 0.99. The established acceptance criteria may be different when the client requests a different tolerance for ICV and CCV samples.

D.13. PERFORMANCE CHARACTERISTICS AND UNCERTAINTY.

Possible causes that might affect the uncertainty of this method are sketched in the fishbone diagram Figure D-1.

APPENDIX D. PREPARATION AND ANALYSIS OF CHEMICAL AGENT SAMPLE FOR PURITY DETERMINATION BY GC/MSD OR LC/MS/MS WITH EXTERNAL STANDARD.

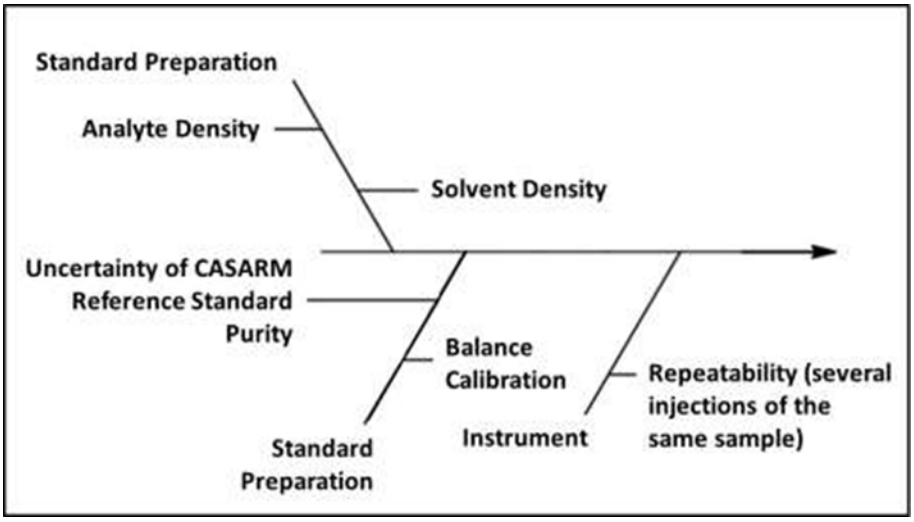

Figure D.1. Uncertainty fishbone chart.

D.14. DATA PACKAGES OR DATA REPORTS.

The data report can be in the form of a purity certificate if requested by the customer. The purity certificate should include the following:

a. Substance: Chemical name.

b. Lot Number: Neat agent vender lot number.

c. Issue Date: Date issued by the site quality manager.

d. Expiration Date: 45 days following the purity determination.

e. Certified Value: The mean percent purity and standard deviation of the three analyzed samples.

D.15. SAFETY.

For chemical agent operations, operator's document shall adhere to the safety, surety, and accountability protocols specified in site-specific standard operating procedures.

APPENDIX E. ABBREVIATIONS.

1-D	one dimensional
^{1}H	hydrogen-nucleus
^{13}C	carbon-13
^{15}N	nitrogen-15
^{19}F	fluorine-19
^{31}P	phophorus-31
AR	Army Regulation
°C	degrees Celsius
CA	Chemical Agent
CAS®	Chemical Abstracts Service®
CASARM	Chemical Agent Standard Analytical Reference Material
CCV	Continuous Calibration Variable
CDC13	deuterated chloroform
COA	Certificate of Analysis
DA	Department of the Army
°F	degrees Fahrenheit
FID	Flame Ionization Detector
FPD	Flame Photometric Detector
GC	Gas Chromatography
IAW	in accordance with
ICV	Initial Calibration Verification
IS	Internal Standard
ISO	International Organization for Standardization
LC	Liquid Chromatography
No.	number
MS	Mass Spectrometry
MSD	Mass Selective Detector
MW	molecular weight
m/z	mass to charge ratio
NIST	National Institute of Standards and Technology
NMR	Nuclear Magnetic Resonance
PAM	Pamphlet
PTFE	polytetrafluoroethylene (Teflon®)
QA	Quality Assurance
QC	Quality Control

APPENDIX E. ABBREVIATIONS.

r^2	coefficient of determination
RRF	relative response factor
T&E	Test and Evaluation
TCD	Thermal Conductivity Detector
TOP	Test Operations Procedure
TP	Test Plan
UV	ultraviolet
Vis	Visible Spectroscopy

APPENDIX F. REFERENCES.

1. AR 50-6, Chemical Surety, 16 April 2018.

2. AR 385-10, The Army Safety Program, 24 February 2017.

3. AR 190-59, Chemical Agent Security Program, 24 February 2017.

4. DA PAM 385-61, Chemical Agent Safety Standards, 1 November 2018.

5. Duewer, DL; Parris, RM; White, E; May, WE; and Elbaum, H (2004). An Approach to the Metrology Sound Traceable Assessment of the Chemical Purity of Organic Reference Materials. National Institute of Standards and Technology Special Publications 1012, 53 pp.

6. NIST/SEMATECH e-Handbook of Statistical Methods, http://www.itl.nist.gov/div898/handbook, accessed on 27 July 2020.

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APPENDIX G. APPROVAL AUTHORITY.

CSTE-CI 18 March 2021

MEMORANDUM FOR

Commander, U.S. Army Operational Test Command
Director, U.S. Army Evaluation Center
Commanders, ATEC Test Centers
Technical Directors, ATEC Test Centers

SUBJECT: Test Operations Procedure 08-2-073, Standard Practices for Determination of Neat Agent Purity, Approved for Publication

1. Test Operations Procedure (TOP) 08-2-073, Standard Practices for Determination of Neat Agent Purity, has been reviewed by the U.S. Army Test and Evaluation Command (ATEC) Test Centers, the U.S. Army Operational Test Command, and the U.S. Army Evaluation Center. All comments received during the formal coordination period have been adjudicated by the preparing agency.

2. Scope of the document. This TOP is intended to provide expectations and guidance necessary for laboratories to follow when producing internal documents of operation in relation to purity analysis of chemical agents. These standard practices will provide guidance on equipment and instrumentation that will allow the test laboratory to choose an appropriate methodology for the testing being conducted.

3. This document is approved for publication and has been posted to the Reference Library of the ATEC Vision Digital Library System (VDLS). The VDLS website can be accessed at https://vdls.atc.army.mil/.

4. Comments, suggestions, or questions on this document should be addressed to U.S. Army Test and Evaluation Command (CSTE-CI), 6617 Aberdeen Boulevard-Third Floor, Aberdeen Proving Ground, MD 21005-5001; or e-mailed to usarmy.apg.atec.mbx.atec-standards@mail.mil.

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Forward comments, recommended changes, or any pertinent data which may be of use in improving this publication to the following address: Policy and Standardization Division (CSTE-CI-P), U.S. Army Test and Evaluation Command, 6617 Aberdeen Boulevard, Aberdeen Proving Ground, Maryland 21005-5001. Technical information may be obtained from the preparing activity: Commander, U.S. Army Dugway Proving Ground (TEDT-DPW-TT), Dugway, Utah 84022-5000. Additional copies can be requested through the following website: https://www.atec.army.mil/publications/documents.html, or through the Defense Technical Information Center, 8725 John J. Kingman Rd., STE 0944, Fort Belvoir, VA 22060-6218. This document is identified by the accession number (AD No.) printed on the first page.