Journal of Visualized Experiments

Quantitative Detection of Trace Explosive Vapors by Programmed Temperature Desorption Gas Chromatography-Electron Capture Detector

--Manuscript Draft--

Manuscript Number:	JoVE51938R2
Full Title:	Quantitative Detection of Trace Explosive Vapors by Programmed Temperature Desorption Gas Chromatography-Electron Capture Detector
Article Type:	Invited Methods Article - JoVE Produced Video
Keywords:	Gas Chromatography; Electron Capture Detector; Explosives; Quantitation; Thermal Desorption; TNT; RDX; Vapor
Manuscript Classifications:	4.2.640: Nitro Compounds; 5.5.196: Chemistry Techniques, Analytical; 5.5.196.181.349: Chromatography, Gas; 5.5.196.59: Analytic Sample Preparation Methods; 92.25.2: analytical chemistry; 92.28.19: explosives
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Abstract:	The direct liquid deposition of solution standards onto sorbent-filled thermal desorption tubes is used for the quantitative analysis of trace explosive vapor samples. The direct liquid deposition method yields a higher fidelity between the analysis of vapor samples and the analysis of solution standards than using separate injection methods for vapors and solutions, i.e. samples collected on vapor collection tubes and standards prepared in solution vials. Additionally, the method can account for instrumentation losses, which makes it ideal for minimizing variability and quantitative trace chemical detection. Gas chromatography with an electron capture detector is an instrumentation configuration sensitive to nitro-energetics, such as TNT and RDX, due to their relatively high electron affinity. However, vapor quantitation of these compounds is difficult without viable vapor standards. Thus, we eliminate the requirement for vapor standards by combining the sensitivity of the instrumentation with a direct liquid deposition protocol to analyze trace explosive vapor samples.
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Editorial Board
Journal of Visualized Experiments

Dear Editorial Board,

Attached is the submission of the manuscript "Quantitative Detection of Trace Explosive Vapors by Programmed Temperature Desorption Gas Chromatography-Electron Capture Detector" for review and sole publication as an invited, full length article in the *Journal of Visualized Experiments*. This manuscript presents a remarkably simple method using thermal desorption tubes to establish calibration curves for explosives vapors. We believe this work to be of interest to your readers because we focus on universally applicable sample preparation procedures and utilizing commercial instrumentation for reproducible quantitation of traces explosive vapors, whereas other research has focused on particle detection from wipes, analysis in solution samples, or novel, prototype instrumentation for application-specific analysis. This work is well-suited for JoVE's unique multimedia format because procedures involving instrument maintenance, sampling methods, and sample analysis are generally provided as text, but these activities are inherently visual, especially when describing location of instrument components and proper, quantitative sampling protocols.

We have been in close communication with JoVE Editor Elizabeth Sheeley during the writing and submission of this manuscript. She has greatly assisted us during the submission process and with handling corresponding author changes.

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    - Revised, directed, and integrated the Protocol section into the manuscript
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    - Established the work flow from sample injection to quantitative result
    - Created the TDS-CIS-GC-ECD optimized, quantitative method
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Sincerely,
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Title:

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Keywords: Gas Chromatography (GC), Electron Capture Detector, Explosives, Quantitation, Thermal Desorption, TNT, RDX

Short Abstract:

Trace explosive vapors of TNT and RDX collected on sorbent-filled thermal desorption tubes were analyzed using a programmed temperature desorption system coupled to GC with an electron capture detector. The instrumental analysis is combined with direct liquid deposition method to reduce sample variability and account for instrumentation drift and losses.

Long Abstract:

The direct liquid deposition of solution standards onto sorbent-filled thermal desorption tubes is used for the quantitative analysis of trace explosive vapor samples. The direct liquid deposition method yields a higher fidelity between the analysis of vapor samples and the analysis of solution standards than using separate injection methods for vapors and solutions, *i.e.* samples collected on vapor collection tubes and standards prepared in solution vials. Additionally, the method can account for instrumentation losses, which makes it ideal for minimizing variability and quantitative trace chemical detection. Gas chromatography with an electron capture detector is an instrumentation configuration sensitive to nitro-energetics, such as TNT and RDX, due to their relatively high electron affinity. However, vapor quantitation of these compounds is difficult without viable vapor standards. Thus, we eliminate the requirement for vapor standards by combining the sensitivity of the instrumentation with a direct liquid deposition protocol to analyze trace explosive vapor samples.

Introduction:

Gas Chromatography (GC) is a core instrumental analysis technique of Analytical Chemistry and is arguably as ubiquitous as a hot plate or balance in a chemistry laboratory. GC instrumentation can be used for the preparation, identification, and quantitation of a multitude of chemical compounds and can be coupled to a variety of detectors, such as flame ionization detectors (FIDs), photo-ionization detectors (PIDs), thermal conductivity detectors (TCDs), electron capture detectors (ECDs), and mass spectrometers (MS), depending on the analytes,

methodology, and application. Samples can be introduced through a standard split/splitless inlet when working with small sample solutions, specialized headspace analysis inlets, solid phase micro-extraction (SPME) syringes, or thermal desorption systems. GC-MS is often the standard technique used in validation and verification applications of alternative or emerging, detection techniques because of its utility, flexibility, and identification power with established chemical databases and libraries.[1–7] GC and its related sampling and detecting components is ideal for routine chemical analysis and more specialized, challenging analytical applications.

An analytical application of increasing interest to military, homeland security, and commercial enterprises is trace explosive vapor detection, with detection including identification and quantitation. Trace explosive vapor detection is an unique analytical chemistry challenge because the analytes, such as 2,4,6-trinitrotoluene (TNT) and cylcotrimethylenetrinitramine (RDX) have physical properties that make them especially difficult to handle and separate using broader, more generic chemical analysis methodologies. The relatively low vapor pressures and sub parts-per-million by volume (ppm_v) saturated vapor concentration, combined with relatively high sticking coefficients, necessitate special sampling protocols, instrumentation, and quantitation methods.[8–12] A GC coupled to an Electron Capture Detector (ECD) or mass spectrometer (MS) is an effective method for quantitating explosive analytes, specifically dinitrotoluene (DNT), TNT, and RDX.[6,13–17] GC-ECD is particularly useful for nitro-energetic compounds because of their relatively high electron affinity. The U.S. Environmental Protection Agency (EPA) has created standard methods for explosive analyte detection using GC-ECD and GC-MS, but these methods have focused on samples in solution, such as ground water, and not samples collected in the vapor phase.[2,18–23] In order to detect explosive vapors, alternative sampling protocols must be used, such as vapor collection with sorbent-filled thermal desorption sample tubes, but quantitative detection remains difficult due to lack of vapor standards and calibration methods that do not account for sample tube and instrumentation losses.

Recently, quantitation methods using thermal desorption systems with a cooled inlet system (TDS-CIS), coupled to a GC-ECD have been developed for TNT and RDX vapors.[24,25] The losses associated with the TDS-CIS-GC-ECD instrumentation for trace explosive vapors were characterized and accounted for in example calibration curves using a direct liquid deposition method onto sorbent-filled thermal desorption sample tubes. However, the literature focused on instrumentation characterization and method development but never actually sampled, analyzed, or quantitated explosive vapors, only solution standards. Herein, the focus is on the protocol for sampling and quantitating explosive vapors. The protocol and methodology can be expanded to other analytes and trace explosive vapors, such as Pentaerythritol tetranitrate (PETN).

Protocol:

1.) Instrument Preparation

1.1) Ensure the instrument, oven, and detector are at room temperature. Turn off gas flow to the inlet and detector.

1.2) Remove the TDS from the GC. Consult the manufacturer's user manual for the instrument-specific procedure.

1.3) Remove the TDS adaptor from the CIS inlet and remove the liner from the CIS.

1.4) Inspect the CIS inlet for particles and debris while the liner is removed. Clean any visible debris with compressed air, or preferably nitrogen.

1.5) Attach a new graphite ferrule to a new CIS liner using the manufacturer provided tool and instructions for ferrule-to-liner binding.

1.6) Insert the liner with the attached graphite ferrule into the CIS. Replace the TDS adaptor and re-mount the TDS.

1.7) Remove a new column from its packaging and remove the silicone protection from the ends of the column.

1.8) Insert a nut and ferrule onto each end of the column. Use an ECD detector nut and ferrule for one end of the column and a CIS ferrule for the opposite end of the column.

1.9) Using a ceramic column cutting tool, remove approximately 10 cm from each end of the column. Ensure the nuts and ferrules remain on the column but away from the end of the column to avoid clogging and debris.

1.10) Secure the column into the oven using the instrument manufacturer guidelines. Insert the column into the inlet. Connect the other end of the column to the detector port. The depth of insertion is specific to instrument, inlet, and detector manufacturer. See the user manual and specifications for the exact column insertion depth.

Note: A pre-bake may be required for the column before connecting the opposite end of the column to the detector ports. Consult the column and instrument manufacturer documentation to determine if a pre-bake is required.

1.11) Gently hand-tighten nuts and ferrules onto their respective ports for the inlet and detector. Using a wrench, tighten with approximately a quarter turn of rotation the nuts and ferrules. Too much force or over-tightening will damage the ferrules causing leaks or the column to break and clog.

1.12) Bake out the TDS, inlet, column, and detector. A typical bake out consists of setting the temperature for all zones to just below the maximum operating temperature (typically 300 ^{o}C) while flowing carrier gas for at least two hours.

1.13) Cool all zones and retighten all nuts and ferrules to ensure leak-free operation. Heating and cooling during the bake out will cause the nuts and ferrules to loosen, which can introduce leaks.

1.14) Load, or reload, the instrument method using the software interface. Verify correct temperatures and flow rates have been achieved. Instrumentation is ready for analysis.

2.) Preparation of Standards

2.1) Remove 1,000 ng µL^{-1} 3,4-DNT, 10,000 ng µL^{-1} TNT, and 10,000 ng µL^{-1} RDX from the freezer or refrigerator and allow the three stock solutions to reach room temperature.

2.2) Dispense 100 µL of stock 1,000 ng µL^{-1} 3,4-DNT and add 900 µL of acetonitrile into an amber sample vial.

2.3) Dispense 100 µL of the 100 ng µL^{-1} 3,4-DNT solution from Step 2.2 and add 900 µL of acetonitrile into an amber sample vial.

2.4) Dispense 150 µL of the 10 ng µL^{-1} 3,4-DNT solution from Step 2.3 and 4,850 µL of acetonitrile into an amber sample vial. This is the internal standard for direct liquid deposition.

2.5) Dispense 100 µL of stock 10,000 ng µL^{-1} TNT solution, 100 µL of stock 10,000 ng µL^{-1} RDX solution, and 800 µL of acetonitrile into an amber sample vial.

2.6) Dispense 100 µL of the 1,000 ng µL^{-1} TNT and RDX solution in Step 2.5 and 900 µL of acetonitrile into an amber sample vial.

2.7) Dispense 100 µL of the 100 ng μL^{-1} TNT and RDX solution from Step 2.6 and 900 µL of acetonitrile into an amber sample vial.

2.8) Dispense 100 µL of the 10 ng μL^{-1} TNT and RDX solution from Step 2.7 and 900 µL of acetonitrile into an amber sample vial. This creates the 1.0 TNT/1.0 RDX ng μL^{-1} solution standard ready for direct liquid deposition onto sample tubes.

2.9) Dispense 60 µL of the 10 ng μL^{-1} solution in Step 2.7 and 940 µL of acetonitrile into an amber sample vial. This creates the 0.6 TNT/0.6 RDX ng μL^{-1} solution standard ready for direct liquid deposition onto sample tubes.

2.10) Dispense 40 µL of the 10 ng μL^{-1} solution in Step 2.7 and 960 µL of acetonitrile into an amber sample vial. This creates the 0.4 TNT/0.4 RDX ng μL^{-1} solution standard ready for direct liquid deposition onto sample tubes.

2.11) Dispense 20 µL of the 10 ng μL^{-1} solution in Step 2.7 and 980 µL of acetonitrile into an amber sample vial. This creates the 0.2 TNT/0.2 RDX ng μL^{-1} solution standard ready for direct liquid deposition onto sample tubes.

2.12) Dispense 100 µL of the 1.0 ng μL^{-1} solution in Step 2.8 and 900 µL of acetonitrile into an amber sample vial. This creates the 0.1 TNT/0.1 RDX ng μL^{-1} solution standard ready for direct liquid deposition onto sample tubes.

3.) Sample Collection

3.1) Connect one sorbent-filled thermal desorption sample tube to a sample pump or similar equipment using a small piece of flexible silicone tubing. A red arrow is provided on the sample tubes indicating the air flow direction for sample adsorption, and it should be pointing in the direction of the silicone tubing and sample pump.

3.2) Attach a piston flow meter to the sample tube at the opposite end from the sample pump attached in Step 3.1. Adjust the flow rate on the sample pump, or similar equipment, such that the flow rate is approximately 100 mL min^{-1} through the sample tube according to the readings from the piston flow meter. The flow rate should be set to ±5.0 mL min^{-1} of the 100 mL min^{-1} desired set point.

3.3) Disconnect the piston flow meter from the sample tube and temporarily shut off the sample pump but leave the sample tube connected to the pump. The sample pump will be reactivated to begin sample collection. The sample tube is ready for collection.

3.4) Place the sample tube with the still connected sample pump in the explosives vapor stream. The vapor source could be the headspace above a solid sample, an open environment, or a variety of analyte vaporization systems.

3.5) Set a timer based on the approximate sampling times listed in Table 2. The sampling times are listed as a general guideline based on the suspected concentration of material in the vapor phase. These sampling times, with a flow rate of 100 mL min^{-1}, will generally yield a mass in the center of the calibration curve, which is ideal for quantitation.

3.6) Activate the sample pump and start the timer. Wait until the timer has stopped and shut off the sample pump. Disconnect the sample tube from the pump and place it in the packaging provided with the sample tube. Cap the tube and store for analysis.

3.7) Record the unique serial number stamped onto each sample tube, the sample time, and the flow rate for the sample tube in a laboratory notebook. These values will be important for quantitation.

4.) Calibration Curve Generation

4.1) Pipet 5.0 µL of the solution standard directly on the glass frit of an unused, conditioned sample tube. Hold the sample tube and pipet upright with a gloved hand during deposition.

4.2) Repeat Step 4.1 for each of the six calibration standards onto three different sample tubes.

4.3) Deposit 5 µL of the 0.3 ng $µL^{-1}$ 3,4-DNT on each of the tubes as well.

4.4) Allow the eighteen sample tubes (three per solution concentration, six solution concentrations) to sit at room temperature for at least 30 minutes to evaporate the solvent.

4.5) Use the twenty tube autosampler and the previously described TNT and RDX TDS-CIS-GC-ECD method to run and analyze all eighteen tubes overnight.[24,25] A summary of the TDS-CIS-GC-ECD parameters for the method is provided in Table 1.

4.6) Integrate the peaks associated with 3,4-DNT, TNT, and RDX in the chromatogram for each of the eighteen sample tubes. The 3,4-DNT, TNT and RDX peaks will occur at approximately 4.16, 4.49 and 4.95 minutes, respectively.

4.7) Note the 3,4-DNT, TNT and RDX peak areas for each of the eighteen tubes along with the corresponding mass of TNT and RDX that was deposited on the sample tube in a spreadsheet and laboratory notebook.

4.8) Normalize the peak areas for both TNT and RDX by dividing each peak area by the peak area for 3,4-DNT. Do this for all eighteen tubes.

4.9) Calculate the average and standard deviation of the normalized TNT and RDX peak areas for the six standard concentrations.

4.10) Plot the average normalized peak area versus mass of analyte present on the tubes for both TNT and RDX.

4.11) Add a linear trend line for both the TNT and RDX data points. Identify the slope and y-intercept for each analyte. Record the slope, intercept, and R^2 value in a spreadsheet and laboratory notebook.

4.12) Place used sample tubes in a tube conditioner for three hours at 300 ^{o}C and 500 mL min^{-1} nitrogen flow.

5.) Sample Analysis

5.1) Deposit 5.0 µL of the 0.3 ng µL^{-1} 3,4-DNT on each of the sample tubes.

5.2) Allow the tubes to sit at room temperature for at least 30 minutes to evaporate the solvent from the internal standard.

5.3) Use the twenty tube autosampler and the previously described TNT and RDX method to run the tubes overnight on the TDS-CIS-GC-ECD.[24,25] A summary of the instrumentation parameters for the analysis method is provided in Table 1.

5.4) Integrate the peaks associated with 3,4-DNT, TNT, and RDX in the chromatogram for each of the eighteen sample tubes. The 3,4-DNT, TNT and RDX peaks will occur at approximately 4.16, 4.49 and 4.95 minutes, respectively.

5.5) Note the 3,4-DNT, TNT and RDX peak areas for each of the sample tubes in a spreadsheet and laboratory notebook.

5.6) Use the peak areas and calibration curve to calculate the vapor concentration in parts-per-billion by volume (ppb_v) for each analyte. See Equations 1-4.

5.7) Place used sample tubes in a tube conditioner for three hours at 300 ^{o}C and 500 mL min^{-1} nitrogen air flow.

Representative Results:

Obtaining quantitative results for trace explosive vapor samples begins with establishing a calibration curve for the TDS-CIS-GC-ECD instrumentation using the direct liquid deposition method of solution standards onto sample tubes to account for instrument losses and differences between solution standards and vapor samples. The TDS-CIS-GC-ECD instrumentation and method for TNT and RDX trace analysis has been previously described in detail elsewhere, but the instrument parameters are summarized in Table 1.[24,25] Here, Figure 1 shows a series of chromatograms obtained using the published method and parameters in Table 1. Peaks for 3,4-DNT, TNT, and RDX are observed at 4.16, 4.49, and 4.95 minutes, respectively. The peak height and area for the internal standard is constant for all masses of TNT and RDX, while the peak height and area increases with mass of the analyte. The peak areas for TNT and RDX for each mass are normalized by the peak area for 3,4-DNT to account for irreproducibility and losses associated with sample tube injection. The normalized peak areas for each analyte are then plotted versus mass on sample tube to establish a calibration curve. A linear regression is conducted to obtain the slope, intercept, and coefficient of determination (R^2). The slope and intercept are used for converting the normalized peak area for vapor sample to mass, or ultimately concentration. Figure 2 shows an example calibration curve generated from the chromatograms shown in Figure 1. The error bars indicate one standard deviation with three replicate measurements per mass of analyte (N=3). An ideal calibration curve with no instrument or sampling losses and linear detector response would have a R^2 value near unity. A R^2 value that significantly deviates from unity, approximately less than 0.98, is typically an indicator the instrument needs servicing, the solution standards were not properly prepared, or solution standards and the internal standard were not properly deposited onto the glass frit of the sample tubes.

The calibration curve, the plot, and related raw data, are saved in the same spreadsheet as the sample information so the calibration used for quantitation is easily accessed and tracked with the analyzed samples. The calibration curve and peak areas from a sample can be used to calculate a vapor concentration using the following set of equations:

$$Actual \;\; ng \;\; = \frac{\frac{A_a}{A_S}+b}{S} \qquad (1)$$

$$Actual\ \ mol\ = \frac{Actual\ \ ng}{C \times M} = \frac{\frac{A_a}{A_s}+b}{C \times M \times S} \quad (2)$$

$$Air\ \ mol\ = \frac{Q_s \times t \times P}{L \times R \times T} \quad (3)$$

$$Actual\ \ ppb_v\ = \frac{Actual\ \ mol \times C}{Air\ \ mol} = \frac{\frac{A_a}{A_s}+b\ \times L \times R \times T}{M \times S \times Q_s \times t \times P} \quad (4)$$

where A_a is the analyte peak area, A_s is the internal standard peak area, b is the calibration curve Y-intercept for the analyte, S is the calibration curve slope for the analyte, C is a conversion factor to parts-per-billion by volume (ppb_v, 10^9), M is the molecular weight for the analyte (g mol^{-1}), Q_s is the sample flow rate (mL min^{-1}), L is a conversion factor from milliliters to liters (10^3), R is the ideal gas constant (8.314 L kPA K^{-1} mol^{-1}), T is the temperature (K), t is the sample time (min), and P is the pressure (kPA). These series of equations can be embedded into a spreadsheet for automatic calculation of quantitation values. Importantly, these equations assume an ideal gas, so the concentrations have a reduction in accuracy because none of the analytes are ideal gases.

Figure 3 shows an example of a chromatogram that indicates the instrument is in need of service or new solution standards should be prepared. Additional peaks other than those identified as 3,4-DNT, TNT, and RDX appear in the chromatogram. Additional peaks are always present when using sorbent-filled thermal desorption sample tubes because the sorbent material degrades over time with repeated usage and does not selectively adsorb just DNT, TNT, and RDX. However, the degradation products do not co-elute with 3,4-DNT, TNT, and RDX with a properly maintained instrument.[26] A blank tube should be run before and after each calibration series to identify peaks that are present from either sorbent material degradation or impurities captured during the vapor sampling collection. This is easily achieved with the use of a twenty sample tube autosampler, where eighteen calibration standard sample tubes are used for the calibration curve and two additional positions are free for blank tubes at the start and end of the sequence. Additional peaks not observed in the blank, but observed in sample tubes deposited with solution standards to generate a calibration curve, typically indicates solution analyte degradation and new solution standards should be prepared and deposited on a new set of sample tubes. Additional peaks have also been observed if sample tubes are left in the tube conditioner for greater than three hours.

Furthermore, the peak shapes deviate greatly from a Gaussian shape, specifically for the peaks at approximately 4.6 and 4.825 min. Some instrumentation and data analysis software packages provide a “Symmetry” calculation for each peak in a chromatogram that attempts to quantitate the deviation from a Gaussian shape. This value can be used as an indicator to replace the column and inlet liner of the instrument when it significantly deviates from unity, where unity indicates a perfect Gaussian peak shape. The ECD is very sensitive to nitro-aromatics such as DNT and TNT, but has a limited dynamic range. This results in peaks becoming clipped at the upper bounds of the dynamic range, as seen for the peak at approximately 4.825 min in Figure 3. If peaks become clipped, then it might be necessary to reduce the sampling time for vapor samples during sample collection. Running a new

calibration curve before each sample collection series or on a repeated schedule, such as every other night, is a good way to catalog instrument performance and determine when an instrument requires maintenance or service before analyzing valuable samples.

Tables and Figures:

Figure 1: An example chromatogram of the separation of 3,4-DNT (internal standard), TNT, and RDX using the TDS-CIS-GC-ECD instrumentation with the direct liquid deposition method for generating calibration curves of vapor samples. The chromatogram has been trimmed to the relevant portion, but the total run is 8 minutes long. The 3,4-DNT peak area is relatively constant (1.5 ng) while the TNT and RDX peak areas, and heights, increase with mass of analyte on sample tube: (black) 0.1 ng, (red) 0.5 ng, (green) 1.0 ng, (blue) 2.0 ng, and (orange) 3.0 ng.

Figure 2: An example calibration curve for (■) TNT and (●) RDX using the direct liquid deposition method with solution standards and a TDS-CIS-GC-ECD instrumentation. The normalized peak area on the Y-axis is obtained from dividing the TNT and RDX peak areas in a chromatogram by the peak area for 3,4-DNT, the internal standard. The error bars represent one standard deviation for the average of three replicate sample tubes per solution standard, or analyte mass.

Figure 3: A chromatogram resulting from poor instrument maintenance, column degradation, and sample tube sorbent material degradation. Additional peaks other than the 3,4-DNT, TNT, and RDX peaks are observed. The peak at approximately 4.825 min is clipped because the analyte mass is at the upper limit of the dynamic range of the detector. A shoulder appears at 4.850 min, indicating poor separation. The baseline, or lower limit, response is elevated causing baseline drift and increase in noise.

Figure 4: A conceptual diagram illustrating the correct procedure for depositing solution onto a sorbent-filled thermal desorption sample tube for the direct liquid deposition method. The micropipette tip should be touching the glass frit and not the side walls of the sample tube. A new tip should also be used for each deposition between analytes and sample tubes.

Figure 5: An alternative to using personal sample pumps for collecting explosive vapors on sorbent-filled thermal desorption sample tubes. Flexible tubing is used to connect the sample tubes to a mass flow controller (MFC) which allows for electronic input of a desired flow rate. The mass flow controllers, when combined with a pump, automatically adjust for flow rate through the sample tubes to a desired set point regardless of variations between sample tubes. A six MFC configuration is shown with a manifold to connect all MFCs to a common pump, but configurations with different numbers of MFCs are possible.

Table 1: The TDS-CIS-GC-ECD instrumentation parameters for quantitation of TNT and RDX vapors using the direct liquid deposition method.

Table 2: The approximate sampling time for collecting explosive vapors for three solution concentration of TNT and RDX. Actual sampling times may need to be adjusted to yield peaks in a chromatogram suitable for quantitation.

Discussion:

Reproducibility is a critical attribute for the quantitation of trace explosive vapors using the direct liquid deposition method with TDS-CIS-GC-ECD instrumentation, and Relative Standard Deviation (RSD) is often used as a metric for reproducibility. We have experienced RSDs for inter- and intra-sample reproducibility of approximately 5% for TNT and 10% for RDX. Any RSD above 15% is used as an indicator to check common sources of variation that reduce the effectiveness of the protocol. Sources of variation that have led to unacceptable RSDs in the past are highlighted in the following discussion.

A common source of variation that can lead to relatively large standard deviations for replicate measurements of solution standards and significant deviation from unity for R^2 is consistent deposition of solution standards and the internal standard onto sample tubes. We have found an electronic micropipette is ideal for minimizing variation during deposition, as opposed to a manual micropipette. During several recent projects, where multiple personnel were involved in quantitating explosive vapors over several days of sample collections, the source of variation in the results was largely dependent on the individual and his/her usage of the manual micropipette. At first glance, the usage of a manual micropipette appears relatively straightforward, but small variations in plunger depression and release between users yielded a significant source of variation in the quantitative analysis of explosive vapors. When the manual micropipette was switched for an electronic micropipette, the variation between users could not be distinguished between instrument variation and sampling noise. It is also important to hold the micropipette upright during solution uptake and deposition. The solution should be deposited directly onto the glass frit of the sample tube, *i.e.* the micropipette tip should contact the glass frit and not the glass side walls. A new micropipette tip should also be used for each deposition and sample tube. Figure 4 shows a conceptual drawing of the procedure for depositing the internal standard or a solution standard onto the glass frit of a sample tube.

Another source of variation that can diminish reproducibility with the quantitation of explosive vapors is the vapor sampling procedure. In the protocol, a commercial sample pump is connected to the sample tube and calibrated with a piston flow meter using a small set screw and screwdriver. The flow rate must be adjusted for every sample tube to account for packing differences of the sorbent material between sample tubes and pump performance. This procedure can be cumbersome and error prone, especially when attempting to collect multiple sample tubes in parallel. Similar to the replacement of a manual micropipette with an

electronic micropipette to reduce variation, we have also implemented an electronic sample tube system that uses a vacuum pump and mass flow controllers (MFC). Figure 5 shows a conceptual diagram of a six sample tube vapor collection manifold. The MFCs automatically correct for variations in the packing between sample tubes and automatically adjust the flow rate to 100 mL min^{-1} without user intervention. The flow rates should still be routinely checked and calibrated with a piston flow meter, but the flow rate can be adjusted electronically rather than manually with a screwdriver. It is possible to create a single MFC sample tube collection manifold, but the MFC-based configuration seen in Figure 5 is meant to demonstrate the scalability of the alternative method. Notably, the individual commercial sample pumps are less expensive than the MFC-based configuration and the MFC-based configuration is a custom assembly, but the MFC-based configuration can reduce variation, improve reproducibility, and be easier to use.

Variation is also present in the TDS-CIS-GC-ECD instrumentation. Over time, as the various internal components of the instrument heat and cool during analysis, parts will expand and contract causing fatigue of consumables, such as ferrules, nuts, columns, and liners. The gradual fatigue of components is unavoidable and a source of variation over time. When performing quantitation of trace (sub parts-per-million by volume, ppm_v) explosive vapors, the gradual variation in instrument performance becomes amplified. Thus, it is important to establish calibration curves for quantitation in a timely fashion, typically before analysis of samples. If possible, calibration curves should be generated the same day as the sample analysis to be conducted. This is not always possible due to time constraints and instrument workload. Furthermore, typically at least five replicates are used per mass, or concentration, for a calibration curve because more replicates yield a more robust calibration curve for quantitation. However, the calibration curve in Figure 2 was established with three replicates. The number of replicates was reduced so a full calibration curve over the entire dynamic range of the detector could be established overnight in a single autosampler tray (two blanks, eighteen solution standard sample tubes, and twenty sample tube capacity). To compensate for the reduced number of replicates per analyte mass, a new calibration curve was established overnight with samples run immediately the following day to account for variation associated with instrument drift and prevent a back log of sample tubes for quantitation of explosives vapors with TDS-CIS-GC-ECD, which is approximately twenty minutes per sample tube.

Determining the peak areas for 3,4-DNT, TNT, and RDX in a chromatogram, such as the example seen in Figure 1, can be a subjective process that can introduce irreproducibility to the quantitation of explosive vapors with direct liquid deposition method and TDS-CIS-GC-ECD instrumentation. Many data analysis software packages supplied with GC-ECD instrumentation include manual and automatic peak detection and integration methods. The field of chromatographic data analysis and its related techniques for automatic peak detection and integration is long and extensive,[27–31] with many of the algorithms provided in data analysis software. A complete review of the various characteristics and procedures for integrating peak areas is beyond the scope of this work. It is more important for a research group to standardize, document, and use the same procedure for the calibration curve as the samples to minimize

variation in quantitation of explosive vapors from more subjective peak area integration processes.

Finally, degradation of sample tubes and solution standards can affect quantitation of trace explosive vapors. Similar to component fatigue from use and thermal cycling of the TDS-CIS-GC-ECD instrumentation, the sorbent material in the sample tubes can degrade over time with repeated sampling and thermal desorption. New sample tubes are tightly packed and white in color. Over time as the sample air is flowed in one direction and carrier gas, typically helium, flowed in the opposite direction during thermal desorption, the packing of the sample tubes becomes loose and yellow in color. The yellow color indicates degradation of the sorbent material from repeated thermal cycling within the instrumentation and tube conditioner. Additional peaks in blanks and sample tubes are also indicators of sorbent material degradation products.[26] After each analysis, sample vapors are conditioned in a tube conditioner for a maximum of three hours. This is to desorb any remaining material from the sorbent material and effectively clean the sample tubes. However, leaving a sample tube in the conditioner at relatively high temperature (300^{o}C) longer than three hours can significantly shorten the lifetime of the sample tube and introduce variation in quantitation. Similarly, solution standards will degrade over time, which will artificially reduce the mass, or concentration, of analyte in each standard for a calibration curve. To minimize solution degradation, the solution standards should be stored in an amber glass vial in a freezer or refrigerator and the solution standards should be periodically analyzed using the GC-ECD without the TDS-CIS and a standard split/splitless inlet to identify additional peaks or degradation products. A complementary quantitation method, such as gas chromatography mass spectrometry or high performance liquid chromatography, can also be used to ensure the solution standards have not degraded and are suitable for the direct liquid deposition method for quantitating trace explosive vapors.

Acknowledgements:

Financial support was provided by the Department of Homeland Security Science and Technology Directorate.

Disclosures:

We have nothing to disclose.

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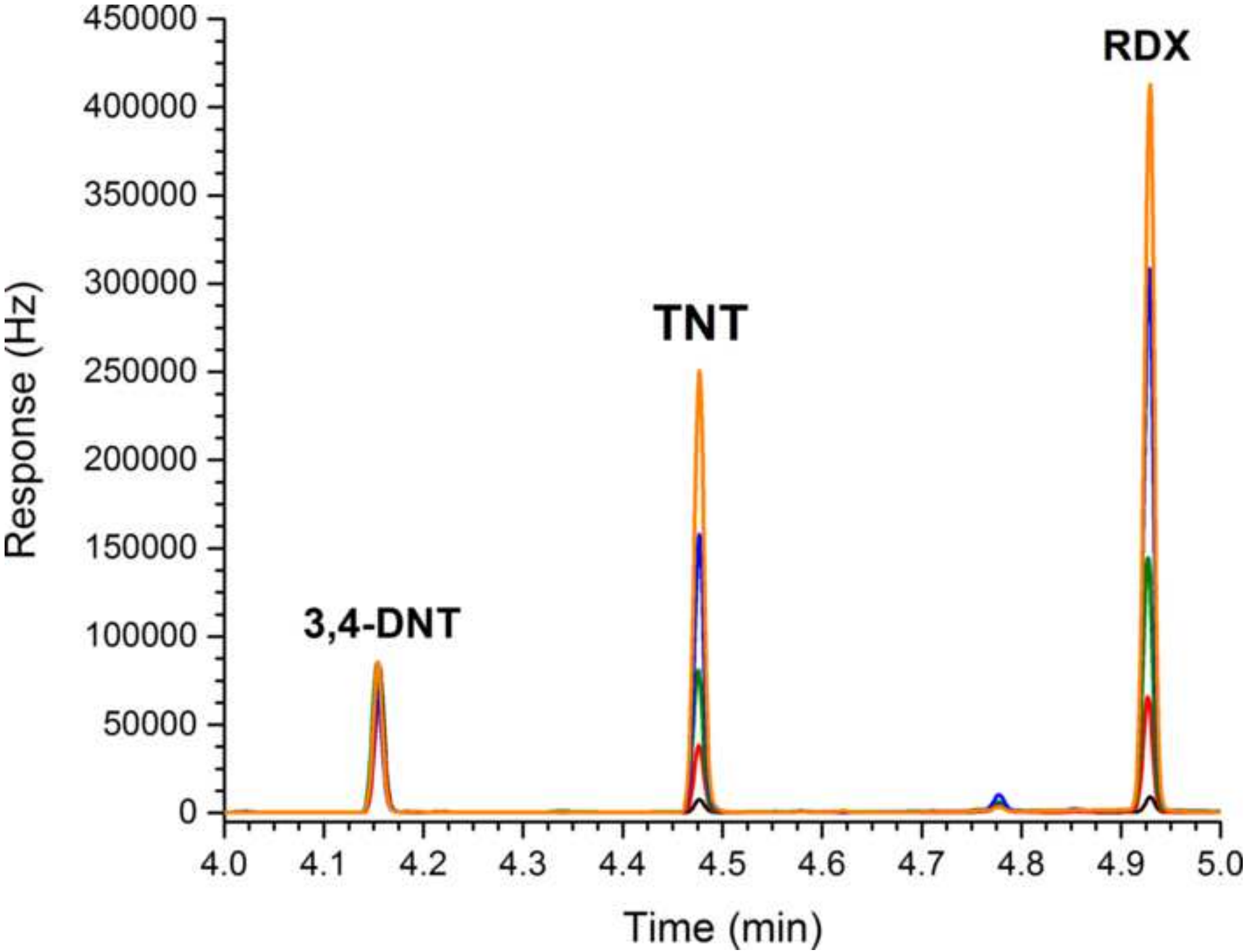

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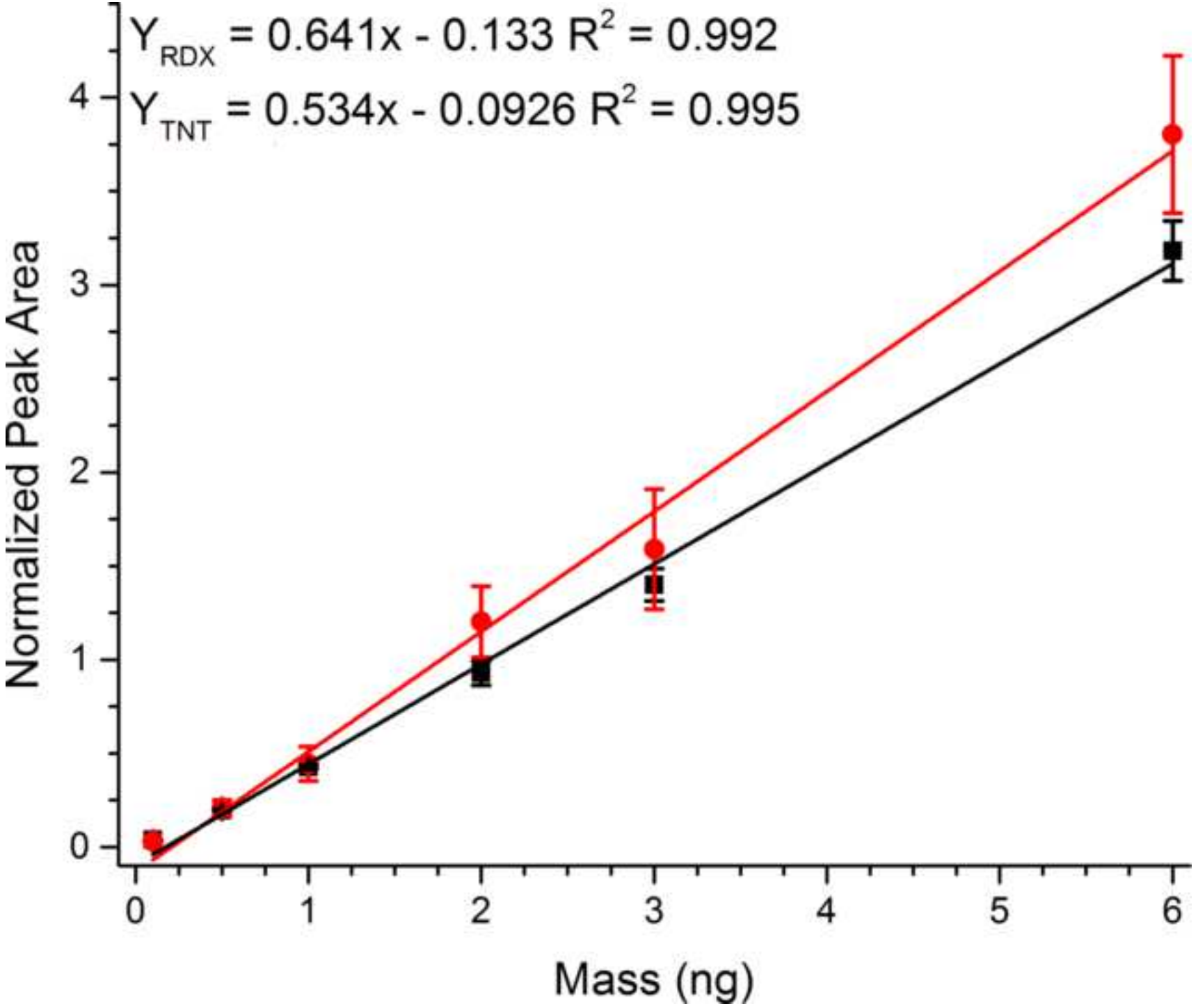

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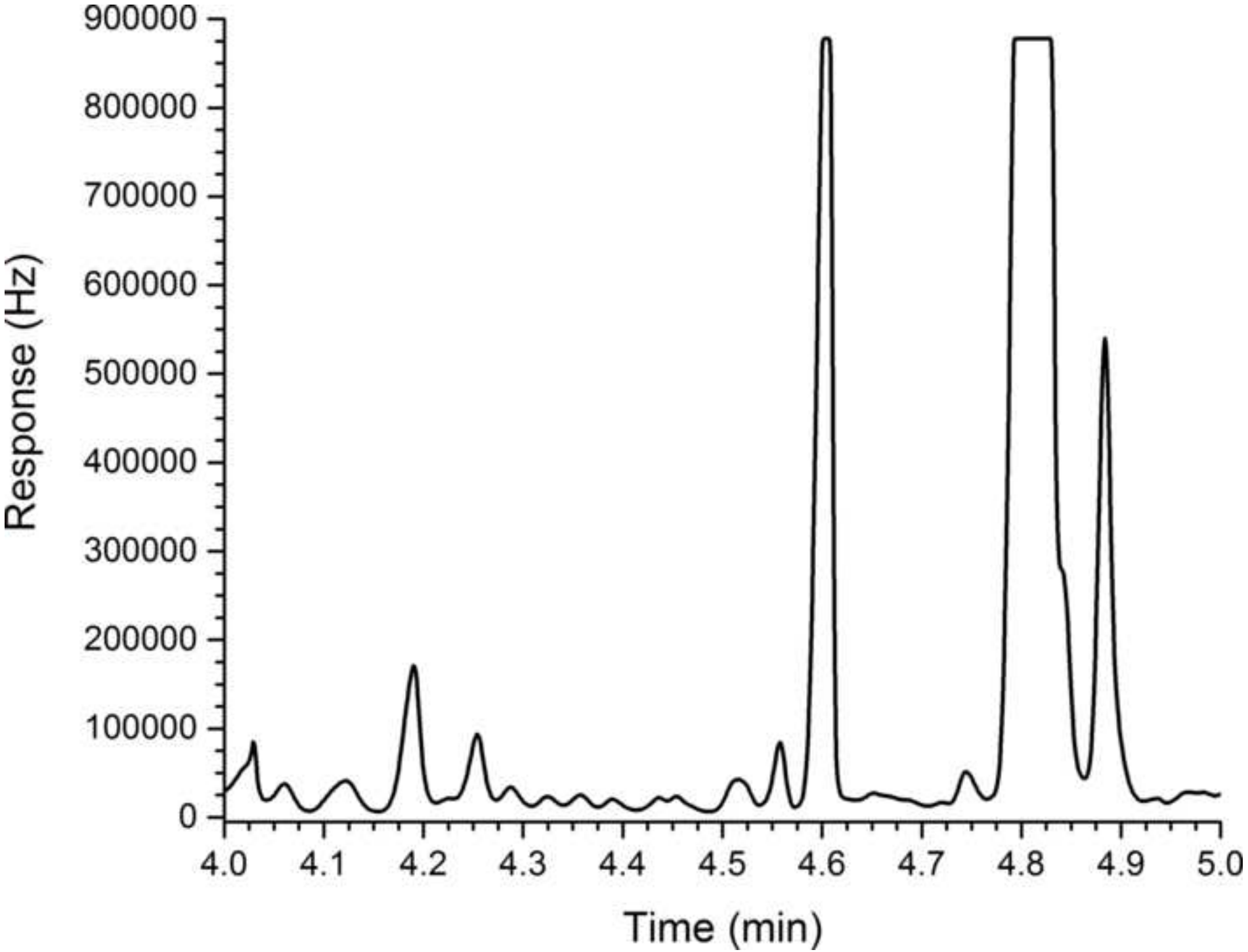

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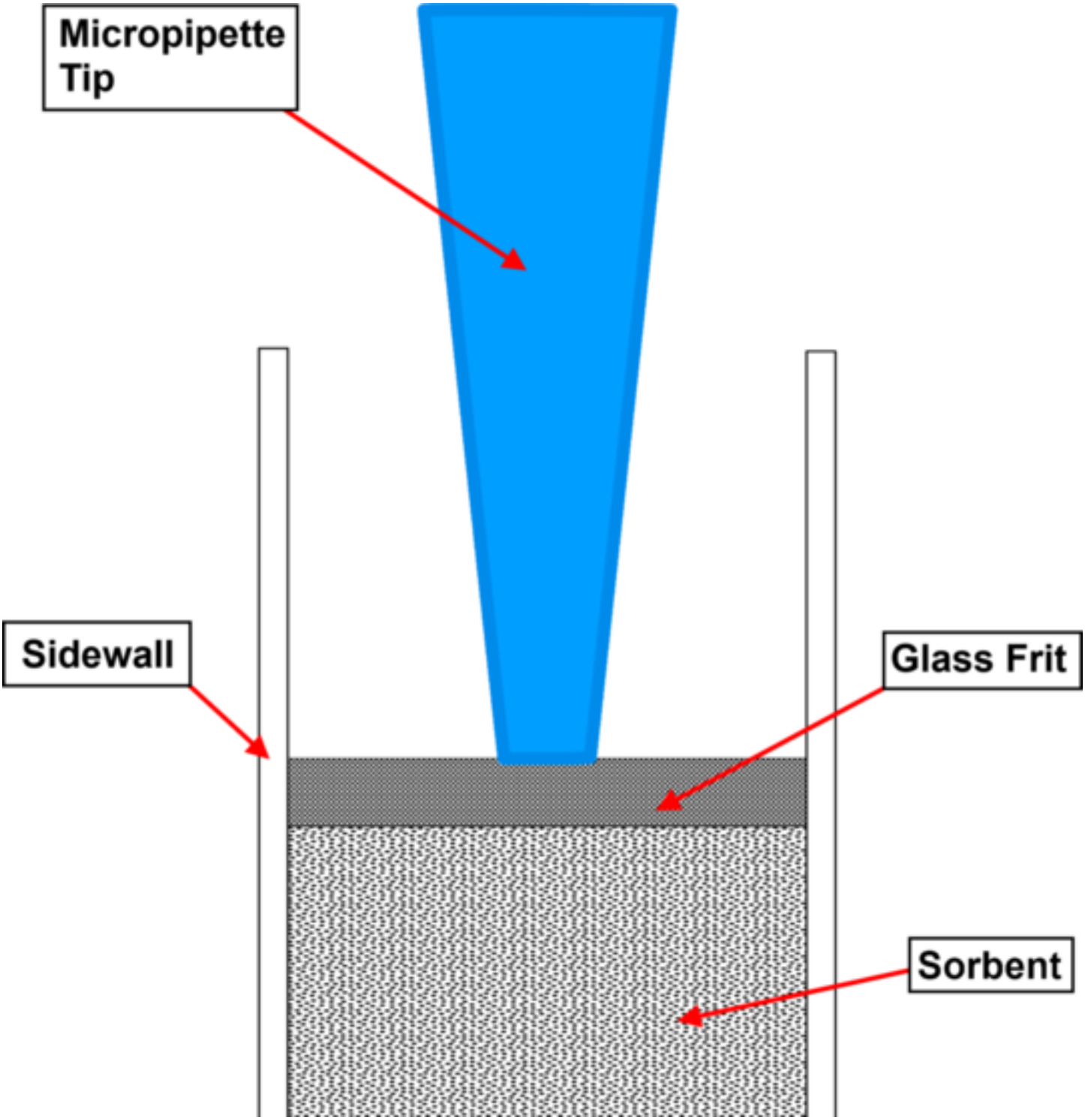

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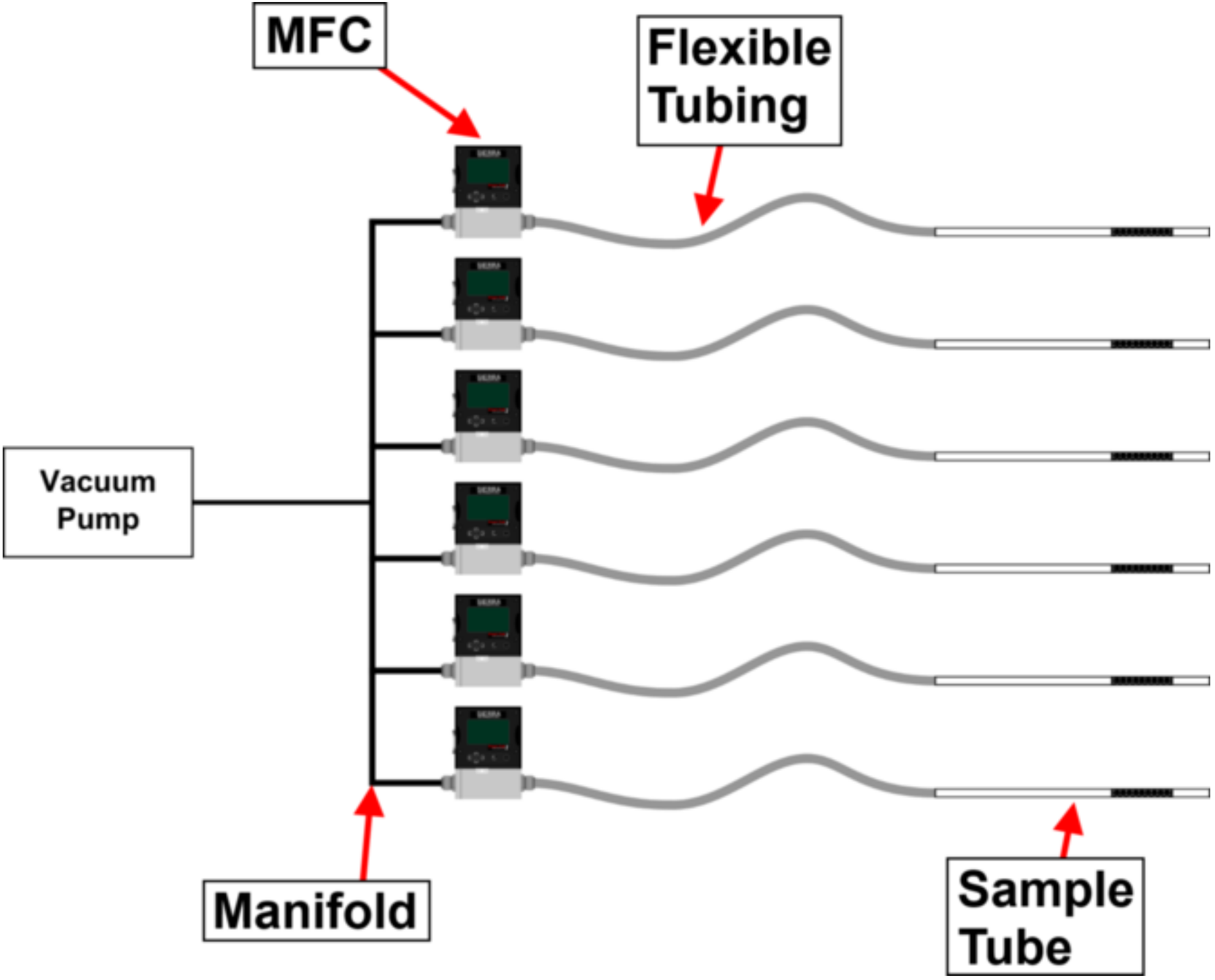

Table 1

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Parameter Name	Value	Units
TDS Initial Temperature	25	^{o}C
TDS Final Temperature	250	^{o}C
TDS Temperature Ramp	40	$^{o}C\ min^{-1}$
TDS Hold Time	2	min
TDS Flow Rate	455	$mL\ min^{-1}$
TDS Mode	PTV Solvent Vent	N/A
TDS Transfer Line Temperature	300	^{o}C
CIS Initial Temperature	0	^{o}C
CIS Final Temperature	250	^{o}C
CIS Temperature Ramp	12	$^{o}C\ sec^{-1}$
CIS Hold Time	3	min
CIS Flow Rate	108	$mL\ min^{-1}$
CIS Mode	PTV Solvent Vent	N/A
Oven Initial Temperature	30	^{o}C
Oven Initial Hold Time	0.5	min
Oven Final Temperature	250	^{o}C
Oven Temperature Ramp 1	40	$^{o}C\ min^{-1}$
Oven Temperature Hold 1	210	^{o}C
Oven Temperature Ramp 2	40	$^{o}C\ min^{-1}$
Oven Temperature Hold 1	250	^{o}C
Oven Hold Time	1	min
Column Carrier Gas	Helium	N/A
Column Flow Rate	5.6	$mL\ min^{-1}$
Column Pressure	23.642	PSI
Column Coating	5% polysilioxane (DB5-MS)	N/A
Column Length	15	meters
Column Inner Diameter (ID)	0.25	mm
Column Outer Diameter (OD)	250	mm
ECD Temperature	275	^{o}C
ECD Flow Rate	60	$mL\ min^{-1}$
ECD Carrier Gas	Nitrogen	N/A

Table 2

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Solution TNT and RDX Concentration ($ng\ \mu L^{-1}$)	Approximate Vapor Concentration (ppb_v)	Sampling Time (min)
0.1 TNT/0.25 RDX	0.050 TNT/0.125 RDX	120
0.4 TNT/1.0 RDX	0.200 TNT/0.500 RDX	30
2.0 TNT/5.0 RDX	1.00 TNT/2.50 RDX	6

Excel Spreadsheet- Table of Equipment

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Name of Material/ Equipment	Company	Catalog Number	Comments/Description
Gas Chromatograph	Agilent	7890A	
Electron Capture Detector (ECD)	Agilent	µECD	
Thermal Desorption System (TDS)	Gerstel	015-710-084-02	
Cooled Inlet System (CIS4)	Gerstel	015-710-084-02	
TDS A2 Autosampler	Gerstel	013200-000-02	
Tube Conditioner	Gerstel	012892-000-02	
Sample Pump	SKC	AirChek 2000	
Piston Flow Meter	Brandt Instruments	Defender 510	
Electronic Micropipette	Eppendorf	4861000015	
Mass Flow Controller	Sierra Instruments, Inc.	M100 Smart Trak 2	

Revised Excel Spreadsheet- Table of Materials/Equipment

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Name of Material/ Equipment	Company	Catalog Number	Comments/Description
2,4,6-Trinitrotoluene (TNT)	Accu-Standard	M-8330-11-A-10X	10,000 ng μL^{-1}
Cyclotrimethylenetrinitramine (RDX)	Accu-Standard	M-8330-05-A-10X	10,000 ng μL^{-1}
3,4-Dinitrotoluene (3,4-DNT)	Accu-Standard	S-22988-01	1000 ng μL^{-1}
Tenax® TA Vapor Sample Tubes	Gerstel	009947-000-00	Tenax® 60/80
CIS4 Liner	Gerstel	014652-005-00	
Transfer Line Ferrule	Gerstel	001805-008-00	
Inlet Liner Ferrule	Gerstel	001805-040-00	
CIS4 Ferrule	Gerstel	007541-010-00	
ECD Detector Ferrule	Aglient	5181-3323	
DB5-MS Column	Res-Tek	12620	

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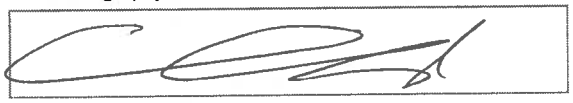
Signature:	 Date: 1/7/2014

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Jaydev Upponi
Elizabeth Sheeley
Journal of Visualized Experiments

Dear Jaydev Upponi & Elizabeth Sheeley:

The authors would like to thank the editors and reviewers for their time and advice. We greatly appreciate everyone's efforts and expertise to construct a great publication.

A summary of our revisions and responses can be found in the table below. The "Track Changes" feature was used to indicate changes in the manuscript.

Comments	**Response**
The editor highlighted of the headings of steps 1, 3, 4, and 5.	The authors are confused by this comment. Is the Editor asking the authors to highlight the headings or did the Editor highlight the headings on behalf of the authors? Regardless, the Headings 1, 3, 4, and 5 of the Protocol section have been highlighted in the revised manuscript and the changes are noted with "Track Changes".
The Short Abstract is a giant run-on sentence that should be broken up.	The Short Abstract has been broken up into separate sentences. Changes are highlighted with "Track Changes" in the revised manuscript
Step 3.7 should begin in active voice.	Step 3.7 has been changed to active voice. Changes are noted with "Track Changes" in the revised manuscript
The protocol section is just within the page limit when short steps are combined. Any increase following peer review may put it over the page limit.	The authors appreciate the helpful warning about the page limit. No major revisions were added to the Protocol section that would change the length and violate the page limit. None of the reviewers' comments asked for additional details on the specific steps of the method. In fact, the reviewers generally wanted less protocol description and more results and analysis.
Please take this opportunity to thoroughly proofread your manuscript to ensure that there are no spelling or grammar issues. Your JoVE editor will not copy-edit your manuscript and any errors in your submitted revision may be present in the published version.	The authors have thoroughly proofread the manuscript in our best effort to minimize spelling and grammar issues.

Often reviewers request the addition of a large amount of details or explanations. We realize that, especially in the protocol section, brevity and clarity are important for a JoVE publication and expect the focus to be on providing a framework for the method presented rather than a comprehensive review of the research field. Please address each comment in your rebuttal and note if you choose not to include the requested information in the text and the reasoning behind this decision.	Acceptance or rejection of a suggestion or revision has been noted in this letter along with justification for decisions with regard to omission of a revision. All accepted changes have been noted using the "Track Changes" feature except in Excel files where the feature is not available.
We do not require in depth or novel results for publication in JoVE, only representative results that demonstrate the efficacy of the protocol. However, please ensure that all claims made throughout the manuscript are supported by either results or references to published works.	The authors have made sure all results are supported with figures or references. The results presented in the manuscript are representative results another user should expect when executing the method. However, in-depth sample analysis and results would violate length requirements and are also difficult to publish due to clearance issues with the authors' institution.
Add actual data along with the quantitative analysis of an actual sample (or samples) using this protocol or use a blind test sample (or samples).	The authors greatly appreciate the time and general curiosity from the reviewer on this subject. We are not sure how numbers and results for specific samples would help other readers understand and accurately execute the described method. Large tables of numeric data is generally consider bad form in scientific publications and reduces effective communication of presented ideas. Due to the nature of the analysis, the results of quantitative analysis is largely numeric and more appropriately presented as calibration curves and chromatograms, and the authors have provided chromatograms and calibration curves. The authors believe this meets the requirements and scope of the journal without providing excessive details.
Reproducibility is mentioned; however, the authors do not attempt to quantify this. We would be interested in both inter and intra-sample reproducibility. In addition, describe in the text what the authors feel is an adequate target precision value for this protocol. This	The authors appreciate the suggestion and the desire for additional details about the described method. We typically target a relative standard deviation of 5% for TNT and 10% for RDX with any RSD above 15% signaling a problem with method execution,

could be done quite simply by adding a precision analysis to the data.	instrumentation maintenance, or samples. A full precision analysis using a large sample set is beyond the scope of this manuscript as the focus is on the protocol and the methodology, not necessarily specific results and analysis. Please see the previous response for additional justification as these two comments are related. However, the first paragraph of the Discussion section has been revised to include this information about RSDs and summarize typical results we have experienced in the past. This provides a nice lead-in to the discussion on sources of variation that can affect the reproducibility.
Change or correct all ppb or ppm terms as appropriate to either 'by weight' or 'by volume', i.e. ppbv or ppmw	All parts-per-million and parts-per-billion units have been designated as either “by weight” or “by volume” with either a subscript “w” or “v” respectively. Additional text has been added and noted with “Track Changes” to clarify the measurements as either “by weight” or “by volume”.
Long Abstract Line 52: add 'the' reads for the quantitative Line 59: … high electron affinity. However, vapor quantitation … Line 61: with a direct	All of these line edits have been added to the revised manuscript and noted using the “Track Changes” feature.
Introduction Line 64: remove comma Line 65: remove 'it' Line 67: remove comma Line 73: change 'instrumentation' to 'technique' Line 74: remove comma after alternative Line 75: remove comma after GC Line 76: remove comma after 'components' Line 82: remove a.k.a and add (RDX) Line 84: add comma after 'concentration' Line 85: add comma after 'coefficients' Line 94: change to sample tubes. Unfortunately, quantitative … Line 98: add comma after (TDS-CIS) Line 102: remove remove second 'instrumentation' Line 102: after development add , but never	The majority of these line edits have been added to the revised manuscript and noted using the “Track Changes” feature. The edits recommended for Lines 463 and 507 could not be found and were not added to the revised manuscript.

(remove and) Line 103: end sentence after 'vapors.' Line 107: change uppercase E T and N to lowercase Line 146: change 'ferrules on to their'…. Line 277: remove 'air' Line 311: change to 'each analyte' Line 351: change to 'RDX. However, the degradation … Line 433: change his or her to his/her Line 463: change be to is Line 507: change vapors to tubes	
Table of Materials Superscript the inverse microliter (-1) Add column type: DB5-MS	The inverse microliter has been superscripted for both occurrences. The column type has been added as well. Excel does not provide a "Track Changes" option to note the changes.
Table 2 Clarify column: Approximate Vapor Concentration - are these TNT/RDX as in the first column?	A "TNT" and "RDX" has been added to the concentrations similar to Column 1 of Table 2 to indicate the concentrations relative to the analyte. Excel does not provide a "Track Changes" option to note the changes.
Again: everywhere the authors have a ppm or ppb they need to specify whether this is by weight or by volume	As previously addressed, additional text has been added to clarify measurements "by weight" or "by volume" and noted with "Track Changes" within the revised manuscript.
The operation procedures were described too detailly, it should be breifly decipted.	While the operation procedure section is close to the page limit for the journal, the journal is focused on the methodology and not necessarily the results. Therefore, the operation procedure is expected to be more detailed and extensive than in other publications. No changes were made to the manuscript.
Too much narration, less analysis of the results.	Again, the journal is focused on methodology with the intention to make it easier for a potential reader to execute the method. The focus is not on analysis or results and only representative results are supposed to be included. The authors have written the manuscript based on these thoughts outlined in the guidelines for submission. No changes were made to address this comment.
There are neither captions on Figures nor any	The authors believe the reviewer may have

details relating to specific instruments/types of flow meters etc.	missed the captions and information in the tables or a technical error has occurred in the distribution of the submission to the reviewer. According to the documentation and material received by the authors during submission, all figures and tables have captions located at the end of the “Representative Results” section as required by the journal. Instrumentation details are provided in the required “Table of Equipment” and “Table of Materials” files. No changes were made by the authors to address this comment.
If this manuscript is intended to be a script for video component it is suggested that some sort of schematic would be helpful.	The authors are confused by this comment. There is a video component to the final publication and the “Protocol” section with highlighting service as a template for a script and storyboard to be generated by the journal after accepting the submission. The reviewer is requesting a schematic, but it is unclear of context and content the schematic should include. No changes were made by the authors to address this comment.
There should also be an emphasis on verification of accuracy and validation of results.	The journal emphasizes methodology and the ability to reproduce the method by readers. Results are representative of proper execution of the method or guides to include when a problem has arisen in the execution of the method. Detailed analysis of samples and results, as required by results-focused publications, is considered beyond the scope of this manuscript by the authors. No changes were made by the authors to address this comment.

Thank you very much.

Sincerely,
Dr. Christopher Field