Laboratory Manager Approval: Mary K. Bowman / 08/19/2021

QA Manager Approval: Jeffrey Moore / 08/19/2021

SOP for Diazomethane Generation

Access to this SOP shall be available within the laboratory for reference purposes; the official copy of this SOP resides on the official Georgia EPD website at https://epd.georgia.gov/about-us/epd-laboratory-operations. Printed copies of this SOP will contain a watermark indicating the copy is an uncontrolled copy.

1 Scope and Application

1.1 This is a procedure used to generate Diazomethane reagent for the methylation of herbicide extracts from drinking water, surface water, soil and waste samples. Due to the final product, Diazomethane, being an explosive agent and Ether being a very flammable solvent, the procedure must be performed under a hood. To avoid an explosion, all flammable solvents must be kept away from the hotplate and none of the glassware may contain ground glass joints. At no point in the procedure should the distillation process be left unattended. All other normal safety precautions should also be taken.

2 Definitions

2.1 Refer to Section 3 and Section 4 of the Georgia EPD Laboratory Quality Assurance Manual for quality control definitions.

3 Interferences

3.1 Method interferences may be caused by contaminants in solvents, reagents, glassware and other sample processing apparatus that may lead to discrete artifacts or elevated baselines on the GC chromatograms.

3.2 Glassware must be scrupulously cleaned according to laboratory protocol.

3.3 Use high purity reagents and solvents to minimize interference problems.

4 Safety

4.1 Refer to the Laboratory Chemical Hygiene Plan, online revision.

4.2 The Ethyl Ether solvent bottles must have the Peroxides measured with peroxide test strips before use. A peroxide value of >20 PPM indicates the solvent bottle must be disposed of in the hazardous waste mixed solvent drum.

5 Apparatus and Equipment

5.1 Diazomethane generator kit or Diazald kit
5.2 Heavy-duty beakers: 250mL, 1000mL
5.3 Graduated cylinders, Class A: 25mL, 100mL
5.4 Hot plate with magnetic stirring
5.5 Magnetic stirrer, second without hotplate
5.6 Magnetic stir bars
5.7 Balance: capable of precision weighing up to 0.01g
5.8 Collection flask with Teflon stopper: 250-300mL
5.9 Aluminum weigh boat
5.10 Spatulas
5.11 Aluminum foil
5.12 Thermometer: 0-100°C or higher
5.13 Tap water source
5.14 Peroxide Test Strips: Baker, part number 4416-01, or equivalent

6 Reagents and Standards

6.1 Potassium Hydroxide pellets, reagent grade
6.2 Reagent water: Purified water which does not contain any measureable quantities of target analytes or interfering compounds for each compound of interest (deionized, HPLC, Milli-Q or equivalent). Milli-Q water has a resistivity of 18MΩ·cm or greater at 25°C and a TOC of 50µg/L or less.)
6.3 Carbitol (diethyl glycol monoethyl ether)
6.4 Ethyl ether, pesticide grade, 1 Liter Bottles
6.5 Diazald reagent (n-methyl-n-nitroso-p-toluene sulfonamide)
6.6 Methanol, pesticide grade
6.7 Ice cubes

7 Sample Collection

7.1 Not Applicable

8 Calibration

8.1 Not Applicable

9 Quality Control

9.1 Not Applicable

10 Procedure

10.1 Weigh out 12g of KOH then dissolve in 20mL of reagent water in the distilling flask without heat. This will result in heat being generated. Allow the solution to cool.
10.2 Add 70mL of Carbitol (diethyl glycol monoethyl ether), 20mL of ethyl ether and a small stirring bar to the distilling flask containing the dissolved KOH.
10.3 Weigh out 21.5g of Diazald in two separate 250mL beakers.

10.4 **Note:** Ethyl Ether must be tested for peroxides before use. This is performed by using peroxides strips. The peroxide value must be <20 PPM. (See section 4.2 Safety).

10.5 Add 140mL of ethyl ether and a stirring bar to each beaker and cover each beaker with aluminum foil to minimize moisture absorption.

10.6 Dissolve one of these Diazald solutions using a magnetic stirrer away from the hotplate. *Note: place the other beaker aside with it covered with aluminum foil.*

10.7 Connect the diazomethane generation unit similar to Figure 10.1. *Note: The laboratory's kit may differ slightly from the diagram in Figure 10.1.* See Figure 10.2 for an illustration of an in-house set-up. Secure the joints with glassware clamps. *Note: the diazomethane collection flask at the end of the unit must be on ice.*

10.8 Connect the lower water hose (in) to a tap water source and the higher water hose (out) to a water drain.

10.9 Warm up approximately 400-450mL of tap water in a 1000mL beaker to a temperature no higher than 60°C on a hotplate with magnetic stirrer.

10.10 Immerse the distilling flask with the KOH/Carbitol/Ether solution in the beaker with warm water and attach it to the rest of the diazomethane generation unit, securing the joint with a glassware clamp.

10.11 Gently turn on the water.

10.12 Add the Diazald solution into the dripping funnel attached to the generation unit 20-25mL at a time and distill at a rate of one batch per 20 minutes. *Note: keep the remainder of the Diazald solution under constant stirring.*

10.13 Place a Teflon stopper at the top of the diazomethane generation unit after about 1 minute of starting the distillation process.

10.14 Dissolve the other Diazald solution on the second magnetic stirrer with no hotplate when the volume of the first one is getting low and repeat Section 10.12.

10.15 After the distillation is complete, add another 20-25mL of ethyl ether to the vessels that contained the Diazald solution and continue to distill until the distillate runs out as colorless.

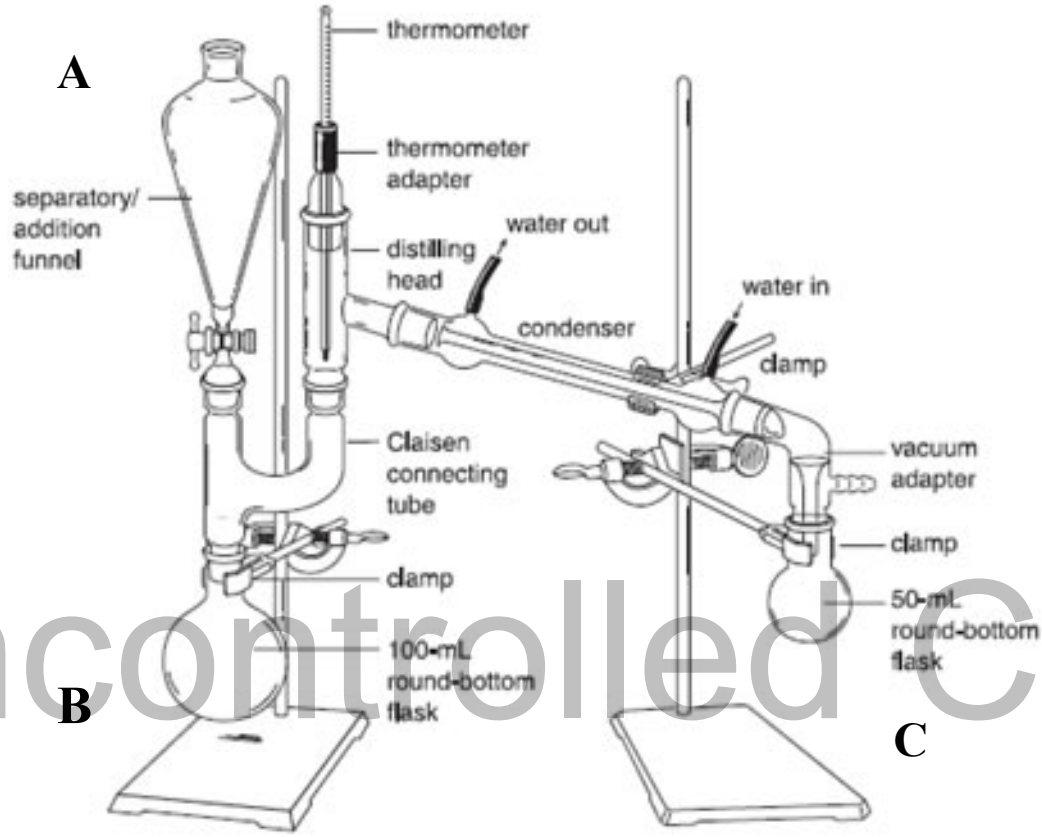
10.16 Stop distillation and stopper the collection flask with a Teflon stopper.

10.17 Properly label the diazomethane collection flask and store in the freezer in a 500 ml rubber bottle carrier for safely transporting and storing.

10.18 Add 50-80mL of methanol to the distillation flask to neutralize the KOH/Carbitol solution then pour it down the drain.

10.19 Rinse all the glassware thoroughly with methanol then thoroughly rinse again with ethyl ether. *Note: do not wash any of the glassware with soap or detergent.*

Figure 10.1

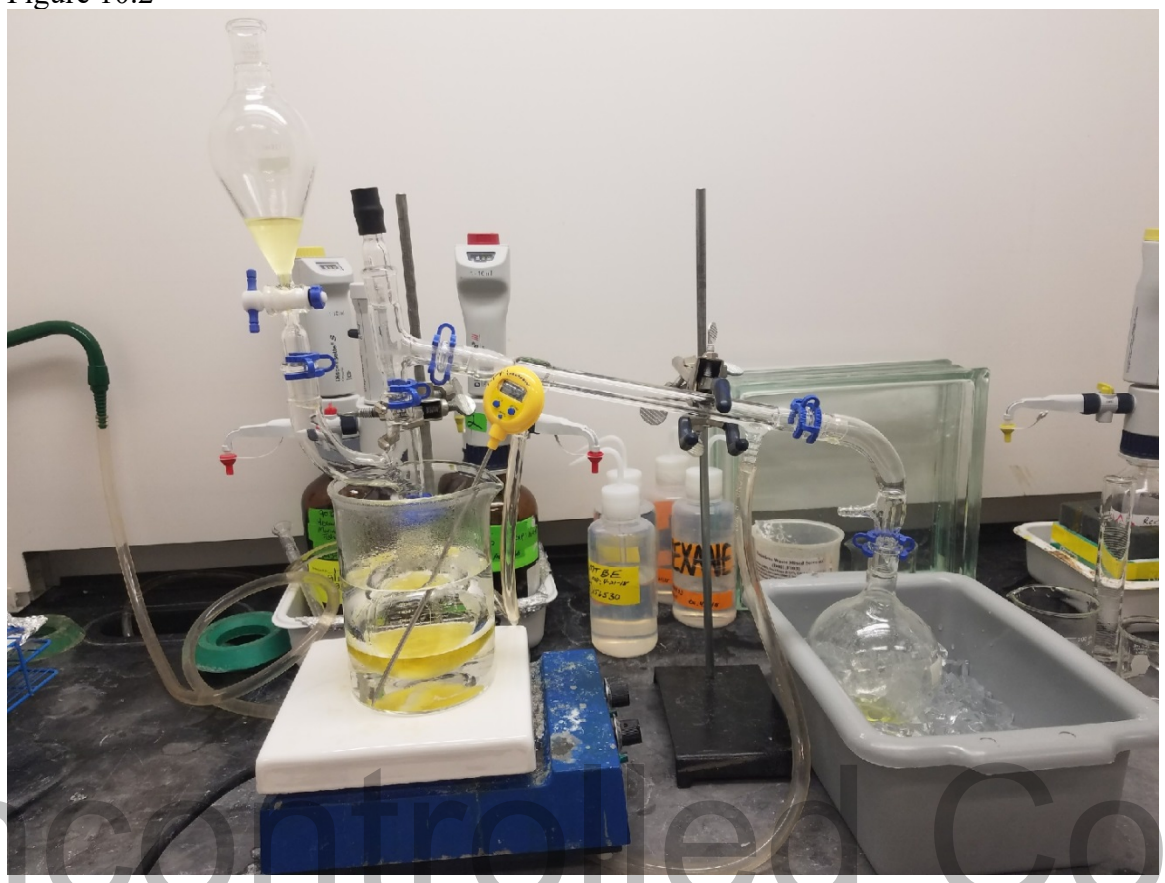


A – Dropping Funnel

B – Distilling Flask (Placed in hot water on hot plate with a magnetic stirrer and thermometer. Water must be < 60°C.)

C – Collection Flask (Placed on ice.)

Figure 10.2



11 Calculations

11.1 Not Applicable

12 Waste Management

12.1 See GA EPD Laboratory SOP – EPD Laboratory Waste Management Standard Operating Procedures, SOP 6-015, online revision.

13 References

13.1 EPA Method 8151A, Rev. 1, December 1996, SW 846 Test Methods for Evaluating Solid Waste Physical/Chemical Methods.
13.2 EPA Method 515.4, Rev. 1.0, 2000, EPA/600/4-88-039.
13.3 Diazomethane generator kit
13.4 Figure 10.1 and Figure 10.2 in Section 10

14 Reporting Limits (RLs), Precision and Accuracy Criteria and Quality Control Approach

14.1 Not Applicable

15 Associated LabWorks Test Codes

15.1 Not Applicable