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Analytical Method: Colorado Analytical - 1101 - Chlorpyrifos

Edition: 10/10/88

Submitted By: S.E. Kane, K.K. Fritchman

Approved By:

Subject: Determination of Chlorpyrifos and TCP Residues in Soil.

1.0 SCOPE

This method is used for the determination of residues of Chlorpyrifos and TCP in soil. The limit of detection for the method is 0.05 ppm for each compound.

2.0 PRINCIPLE

Soil samples are extracted at room temperature with acetone using a platform rotary shaker. An aliquot of the extract is diluted with deionized water and the acetone removed by vacuum rotary evaporation. The pH of the residual aqueous phase is adjusted to 11 and the parent chlorpyrifos is partitioned into toluene. The aqueous phase is then adjusted to pH 1 and the TCP is partitioned into toluene. The TCP toluene phase is evaporated to dryness by vacuum rotary evaporation and the residue is treated with diazomethane. The chlorpyrifos toluene partition sample is added to the TCP diazomethane sample and the toluene removed by vacuum rotary evaporation. The sample is reconstituted in toluene and analyzed by capillary gas chromatography using an electron capture detector. The flow diagram for the method is shown in Figure 1.

3.0 APPARATUS

3.1 Bottle, Nalgene, 500-ml.

3.2 Filter paper, Reeve Angel Grade 802, 24-cm.

3.3 Filter paper, Whatman 2V, 24-cm.

3.4 Flask, Erlenmeyer, 500-ml, 250-ml with 24/40 $.

3.5 Flask, round bottom, 250-ml, 500-ml.

3.6 Funnel, separatory, 250-ml with Teflon stopcock.

3.7 Funnel, 12.5-cm size.

3.8 Glass wool.

3.9 Rotary evaporator, Buchi or equivalent.

3.10 Sample vials, GC autosampler.

3.11 Shaker, rotary, platform.

4.0 REAGENTS

4.1 Acetone, distilled-in-glass.

4.2 Chlorpyrifos analytical standard.

4.3 Diazomethane, prepared by Analytical Method: Colorado Analytical-345-Diazomethane.

4.4 Phosphoric acid, concentrated, ACS.

4.5 Sodium carbonate, ACS.

4.6 Sodium sulfate, anhydrous, ACS.

4.7 Toluene, distilled-in-glass.

4.8 3,5,6-Trichloro-2-Pyridinol (TCP), analytical standard.

5.0 PROCEDURE

5.1 Extraction

5.1.1 Weigh 25 grams of a well-homogenized, stone-free soil sample into a 500-ml Nalgene bottle. Add 250 ml of acetone.

5.1.2 Place the bottle on a rotary platform shaker and shake at 250 rpm for 30 minutes.

5.1.3 Filter the extract through a Reeve Angel Grade 802 filter paper inside a Whatman 2V filter paper into a 500-ml Erlenmeyer flask. Cover the funnel with aluminum foil to prevent losses due to evaporation of solvent.

5.2 Chlorpyrifos Partition

5.2.1 Transfer a 100-ml aliquot (10.0 gm of soil) to a 250-ml Erlenmeyer flask. Add 20 ml of deionized water.

5.2.2 Evaporate the aqueous acetone solution to residual water using a rotary evaporator (bath temperature 40°C). NOTE: INSURE THAT ALL ACETONE IS REMOVED BEFORE PROCEEDING TO THE NEXT STEP.

5.2.3 Transfer the aqueous solution from Step 5.2.2 to a 250-ml separatory funnel.

5.2.4 Add 30 ml of a 1% sodium carbonate solution to the residual aqueous sample.

5.2.5 Add 50 ml of toluene and shake the separatory funnel for approximately 45 seconds and allow the layers to separate. Drain the toluene through a bed of anhydrous sodium sulfate.

5.2.6 Repeat Step 5.2.5 two more times.

5.2.7 Wash the sodium sulfate with 25 ml of toluene and set the toluene partition sample aside for further work up.

5.3 TCP Partition

5.3.1 Adjust the pH of the aqueous phase from Step 5.2.5 to 1 with concentrated phosphoric acid (approximately 5.5 ml).

5.3.2 Add 50 ml of toluene and shake the separatory funnel for approximately 45 seconds and allow the layers to separate. Drain the toluene through a bed of anhydrous sodium sulfate into a 500-ml round bottom flask.

5.3.3 Repeat Step 5.3.2 two more times.

5.3.4 Wash the sodium sulfate with 25 ml of toluene.

5.3.5 Evaporate the toluene solution to dryness using a rotary evaporator (bath temperature 40°C).

5.4 TCP Derivatization

5.4.1 Add 4 ml of a diethyl ether diazomethane solution to the residue from Step 5.3.5.

5.4.2 Let the sample sit for 30 minutes. NOTE: THE YELLOW COLOR SHOULD PERSIST FOR THE 30 MINUTE PERIOD. IF NOT, ADD MORE DIAZOMETHANE IN 1 ml INCREMENTS.

5.5 Chlorpyrifos and TCP Analysis

5.5.1 Add the chlorpyrifos toluene solution from Step 5.2.7 to the diazomethane reacted residue from Step 5.4.2.

5.5.2 Evaporate the toluene solution to dryness using a rotary evaporator (bath temperature 40°C).

5.5.3 Bring the sample to volume with 10.0 ml of toluene and analyze by capillary gas chromatography. Dilutions with toluene may be necessary to bring the chlorpyrifos and/or TCP peaks within the range of the standard curves.

6.0 CAPILLARY GAS CHROMATOGRAPHIC ANALYSIS

The sample from Step 5.5.3 is analyzed by capillary gas chromatography using an electron capture detector. The chromatographic conditions are given in Table 1.

6.1 Preparation of Standard Chlorpyrifos

6.1.1 Weigh 10.0 mg of chlorpyrifos into a 100 ml volumetric flask and bring to volume with toluene. The standard solution is 100 micrograms per milliliter. Serial dilutions of this standard solution are made with toluene until a solution containing 1000 nanograms per milliliter is achieved.

6.2 Preparation of Standard TCP (methyl derivative)

6.2.1 Weigh 10.0 mg of TCP into a 100 ml volumetric flask.

6.2.2 Add 5 ml of diazomethane/diethyl ether solution and let the sample stand for thirty minutes. DO NOT evaporate the diethyl ether off.

6.2.3 Bring the sample to volume with toluene. The standard solution is 100 micrograms per milliliter. Serial dilutions of this standard solution are made with toluene until a solution containing 1000 nanograms per milliliter is achieved.

6.3 Preparation of Working Standards of Chloropyrifos and TCP

6.3.1 Combine equal volumes of the chlorpyrifos (Step 6.1.1) and TCP (Step 6.2.3) to yield a solution containing 500 nanograms per milliliter of chlorpyrifos and TCP.

6.3.2 Serial dilutions of this working solution are made with toluene until working solutions containing 250, 100, 50 and 25 nanograms chlorpyrifos and TCP per milliliter of toluene.

6.4 Standardization of Gas Chromatograph

6.4.1 Standardize the gas chromatograph by injecting 2.0 microliter aliquots of the diluted solutions. This represents a working range of 1000, 500, 200, 100 and 50 picograms each of chlorpyrifos and TCP.

6.4.2 Determine the peak area or peak height for the injected standards. Typical chromatograms of standards are shown in Figure 2 and typical standardization data are shown in Table II.

6.4.3 Enter the standardization data into an appropriate computer or electronic calculator.

6.5 Determination of Sample Residues

6.5.1 Inject 2.0 ul aliquots of the samples from Step 5.5.3 into the gas chromatograph. Compare peak area or peak height of unknown samples by entering into the least squares program of the computer or calculator to determine the amount of chlorpyrifos and TCP. Typical chromatograms of check and recovery samples are shown in Figure 3.

6.5.2 Calculate residue results as ppm of chlorpyrifos and/or TCP using the following equations:

$$\text{ppm(wet basis)} = \frac{\text{picograms chlorpyrifos and/or TCP}}{(1000\ \text{pc/ng})(\text{mg injected})(R)(A)}$$

$$\text{ppm(dry basis)} = \frac{\text{picograms chlorpyrifos and/or TCP}}{(1000\ \text{pc/ng})(\text{mg injected})(R)(A)(M)}$$

where R is the recovery factor determined using a fortified control sample carried through the procedure and is expressed as a decimal (100% = 1.00, etc.); where A is the corrected soil weight aliquot based on the soil moisture content and is expressed as a decimal (11.84% moisture from 25-gm soil sample yields 2.96 ml of added water volume. A = 250 ml ÷ 252.96 ml = 0.988); and where M is the dry weight factor for the moisture content of the soil and is expressed as a decimal (11.84% soil moisture yields M = 22.04 ÷ 25.00 = 0.382).

7.0 DISCUSSION

This analytical method was developed with the concept of analyzing both chlorpyrifos and its major metabolite (TCP) in a single analytical run. Since TCP was found not to be amenable to the capillary gas chromatographic analysis used for chlorpyrifos, it was necessary to derivatize the compound with diazomethane. The methoxy compound was amenable to gas chromatographic analysis. Structures of the compounds related to this analytical method appear in Figure 4.

TABLE I

CAPILLARY GAS CHROMATOGRAPHIC CONDITIONS

Instrument: Hewlett-Packard Model 5880A Capillary Gas Chromatograph with Model 7672A Automatic Sampler.

Carrier Gas: Helium, flow adjusted to give 15 psi (1-2 cc per minute).

Makeup Gas: Argon/methane, 30 cc per minute.

Column: J&W DB-1701, 0.25 u, 0.32-mm I.D., 15 meters.

Injection: Splitless.

Detector: Electron capture.

Temperatures:

Injector:	250°C
Detector:	300°C

Oven Program and Run Table

```
PROGRAM:        (ANNOTATION OFF)
10    VALVE 6 ON
20    OVEN TEMP 60
30    OVEN TEMP EQUIB TIME 1
40    OVEN TEMP INITIAL VALUE 60
50    OVEN TEMP INITIAL TIME 1
60    OVEN TEMP PRGM RATE 30
70    OVEN TEMP FINAL VALUE 165
80    OVEN TEMP FINAL TIME 7.5
90    OVEN TEMP POST VALUE 240
100   OVEN TEMP POST TIME 5
110   ATTN 2T15
120   CHART SPEED 0.2
130   %OFFSET 15
140   RUN TIME ANNOTATION OFF
150   RUN TBL ANNOTATION OFF
160   REPORT ANNOTATION OFF
170   REPORT ON
180   DELETE RUN TBL
190   DELETE REPORT TBL
200   PEAK WIDTH 0.11
210   THRESHOLD 7
220   RUN TIME 0 VALVE 6 ON
230   RUN TIME 0.1 INTG OFF
240   RUN TIME 0.5 VALVE 6 OFF
250   RUN TIME 6 ATTN 2T10
260   RUN TIME 5.01 ZERO
270   RUN TIME 5.02 CHART SPEED 1
280   RUN TIME 5.03 INTG ON
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(Continued on the following page)

TABLE 1 (Page 2)

CAPILLARY GAS CHROMATOGRAPHIC CONDITIONS, CONTINUED

290 RUN TIME 5.04 RUN TIME ANNOTATION ON
300 RUN TIME 6.25 INTG OFF
310 RUN TIME 6.26 RUN TIME ANNOTATION OFF
320 RUN TIME 6.31 CHART SPEED 0.2
330 RUN TIME 9 INTG ON
340 RUN TIME 9.01 RUN TIME ANNOTATION ON
350 RUN TIME 9.04 CHART SPEED 0.5
360 RUN TIME 11.3 INTG OFF
370 RUN TIME 11.31 RUN TIME ANNOTATION OFF
380 RUN TIME 11.32 VALVE 6 ON
390 EDIT AUTO SEQ 1,2
400 SIGNAL C DEVICE# 1
410 AREA%
420 REPORT TIME 0 REJECT 0.5

Minimum Detection Limit: 50 picograms

Volume Injected: 2 microliters

Retention Time: TCP: 5.54 minutes ± 0.02 minute
Chlorpyrifos: 10.27 minutes ± 0.02 minute

FIGURE 1

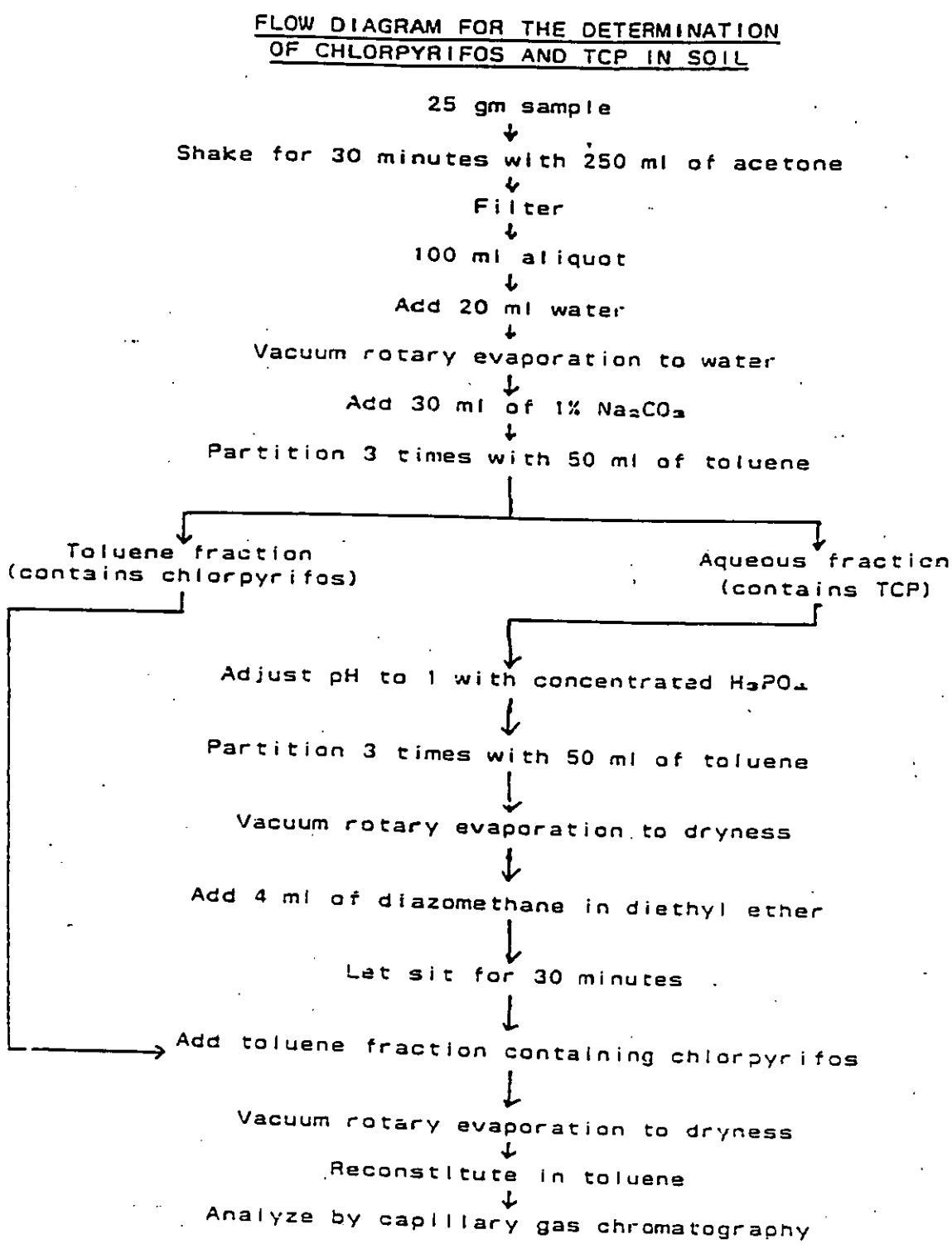
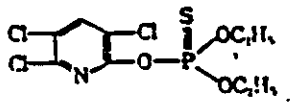

FLOW DIAGRAM FOR THE DETERMINATION OF CHLORPYRIFOS AND TCP IN SOIL
25 gm sample
Shake for 30 minutes with 250 ml of acetone
Filter
100 ml aliquot
Add 20 ml water
Vacuum rotary evaporation to water
Add 30 ml of 1% Na2CO3
Partition 3 times with 50 ml of toluene
Toluene fraction (contains chlorpyrifos)
Aqueous fraction (contains TCP)
Adjust pH to 1 with concentrated H3PO4
Partition 3 times with 50 ml of toluene
Vacuum rotary evaporation to dryness
Add 4 ml of diazomethane in diethyl ether
Let sit for 30 minutes
Add toluene fraction containing chlorpyrifos
Vacuum rotary evaporation to dryness
Reconstitute in toluene
Analyze by capillary gas chromatography

FIGURE 4

CHEMICAL STRUCTURES

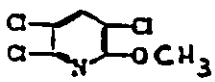


Chlorpyrifos

O,O-Diethyl O-3,5,6-trichloro-
2-pyridyl phosphorothioate

TCP

3,5,6-Trichloro-2-pyridinol



2-Methoxy-3,5,6-Trichloropyridine

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Analytical Method: Colorado Analytical-345-Diazomethane

Edition: 11/12/84

Submitted By: W.D. Rhoads

Approved By: W. D. Rhoads

Subject: Preparation of Diazomethane

1.0 SCOPE

This method describes the preparation of ethyl ether solutions of diazomethane to be used for the methylation of various acidic compounds. The procedures are modifications of those published in Organic Syntheses and the instructions for the Aldrich Chemical Company Diazald kit.

2.0 CAUTIONARY NOTE

Because of the hazardous character of diazomethane, the following statement is reprinted from Organic Syntheses, Coll. Vol. IV, pp. 250-251.

"Caution: Diazomethane is toxic and prone to cause development of specific sensitivity; in addition, it is potentially explosive. Hence one should wear heavy gloves and goggles while performing this experiment and should work behind a safety screen or a hood door with safety glass. Also, it is recommended that ground joints and sharp surfaces be avoided. Thus all glass tubes should be carefully fire-polished, connections should be made with rubber stoppers, and separatory funnels should be avoided, as should etched or scratched flasks. Furthermore, at least one explosion of diazomethane has been observed at the moment crystals (sharp edges!) suddenly separated from a supersaturated solution. Stirring by means of a Teflon-coated magnetic stirrer is greatly to be preferred to swirling the mixture by hand, for there has been at least one case of a chemist whose hand was injured by an explosion during the preparation of diazomethane in a hand-swirled reaction vessel.

"It is imperative that diazomethane solutions not be exposed to direct sunlight or placed near a strong artificial light, because light is thought to have been

responsible for some of the explosions that have been encountered with diazomethane. Particular caution should be exercised when an organic solvent boiling higher than ether is used. Because such a solvent has a lower vapor pressure than ether, the concentration of diazomethane in the vapor above the reaction mixture is greater and an explosion is more apt to occur.

"Most diazomethane explosions take place during its distillation. Hence diazomethane should not be distilled unless the need justifies it. An ether solution of diazomethane satisfactory for many uses can be prepared as described by Arndt, where nitrosomethylurea is added to a mixture of ether and 50% aqueous potassium hydroxide and the ether solution of diazomethane is subsequently decanted from the aqueous layer and dried over potassium hydroxide pellets (not sharp-edged sticks!). When distilled diazomethane is required, the present procedure is particularily good because at no time is much diazomethane present in the distilling flask.

"The hazards associated with diazomethane have been discussed by Gutsche, and LeWinn has reported on a fatal case of diazomethane poisoning."

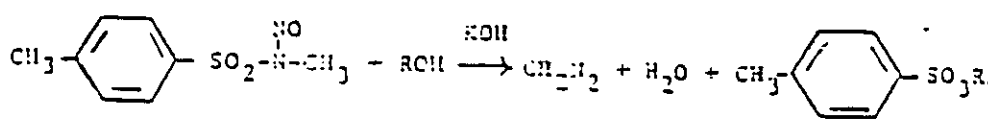
The procedures described in this method are designed to conform with this statement. The method <u>not</u> requiring distillation was not chosen because nitrosomethylurea is a known carcinogen. Clear-Seal joints are used throughout the distillation apparatus instead of ground-glass joints.

It is imperative that extreme care be taken when preparing or using diazomethane. All glassware must be examined and discarded if chips or cracks are found. All operations must be performed in an efficient hood, with the door down as far as possible.

Only experienced personnel may use this method.

3.0 <u>PRINCIPLE</u>

Diazomethane, CH_2N_2, is generated from Diazald, <u>N</u>-methyl-<u>N</u>-nitroso-<u>p</u>-toluenesulfonamide by the action of potassium hydroxide in the presence of Carbitol [2-(2-ethoxyethoxy) ethanol], water and ethyl ether according to the following equation:



The diazomethane is distilled in an ether solution as it is formed.

4.0 APPARATUS

4.1 Diazald Kit (Aldrich Chemical Company, Cat. No. Z10, 025-0) or equivalent glassware as illustrated in Figure 1 with Clear-Seal joints (Available from Wheaton Scientific Co.).

4.2 Combination Stirrer - Hot Plate

4.3 Heating bath: ethylene glycol in a 15-cm crystallizing dish.

4.4 Ice baths.

4.5 Test tube, with rim, 25 x 125 mm.

5.0 REAGENTS

5.1 Diazald (N-methyl-N-nitroso-p-toluenesulfonamide), Aldrich No. D-2800-0.

5.2 Ethyl ether, distilled in glass.

5.3 Diazald Solution: 36g of Diazald in 500 ml of ethyl ether (solution will be cloudy). An ultrasonic bath helps disperse solids.

5.4 Potassium Hydroxide, pellets, reagent.

5.5 Carbitol [2-(2-ethoxyethoxy) ethanol], technical.

6.0 PROCEDURE

6.1 Assemble the reaction-distillation apparatus as shown in Figure 1. An egg-shaped Teflon stirring bar is placed in the distillation flask. An ice-water bath is arranged to be raised and lowered. It should cover the receiving flask during the distillation.

The test tube used as a trap contains about 20 ml of ethyl ether and is immersed in a beaker of ice during the distillation. Run cold water through the condenser.

6.2 Place 24 g of potassium hydroxide and 40 ml of water in the distillation flask and stir to dissolve. Add 140 ml of Carbitol and 80 ml of ethyl ether to this solution. Start heating the bath to 55°C.

6.3 When the bath temperature is near 55°C and ether is distilling, start adding the Diazald-ether solution dropwise through the dropping funnel. Yellow diazomethane in ether will distill into the

receiving flask. Continue the addition at about the same rate as the diazomethane-ether solution is distilled. About 1-1/2 hours is required to add the 500 ml of Diazald solution.

6.4 Whenever the receiving flask is filled, stop the addition of the Diazald solution and transfer the distilled diazomethane solution to an amber storage bottle. Reassemble and continue the reaction.

6.5 When all of the Diazald solution has been added, rinse the Diazald solution flask with 100 ml of ethyl ether and add this wash to the dropping funnel. Add this ether to the distillation flask as before and continue the distillation until the distillate is colorless.

6.6 Combine the distillates with the ether solution in the test tube trap and store in the freezer section of an explosion-proof refrigerator.

7.0 NOTES

7.1 The distilled solution contains about 11 g of diazomethane in 500-600 ml of ether (18-22 mg/ml).

7.2 Diazomethane solutions have been stored for several months with no problems. The yellow color of the solution gives an indication of its concentration.

8.0 REFERENCES

Th. J. deBoer and H. J. Backer in Organic Syntheses, Coll. Vol. IV, N. Rabjohn, ed., pp. 212-253, John Wiley and Sons, New York (1963).

Gutsche, Org. Reactions 8, 391 (1954)

LeWinn, Am. J. Med. Sci. 218, 556 (1949)

Instructions with Aldrich Chemical Co. Diazald Kit.

Figure 1

Diazomethane Distillation Apparatus