Steam Volatile Oil
(Modified Clevenger Method)

Purpose: To determine the amount of steam volatile oil (See Notes 1 and 8).

A. Apparatus:

1. Boiling flask, shortneck, 1 or 2 L with T.S. 24/40 ground joint.
2. Suitable electric heating mantle or oil bath.
3. Variable voltage transformer to control heat.
4. Magnetic stirrer with Teflon-covered stirring bar.
5. Volatile oil traps, Clevenger with T.S. 24/40 ground joints (see Fig. 1 & Note 2).
   a. For lighter than water oils.
   b. For heavier than water oils.
6. West condenser, 400 mm. length with drip tip and T.S. 24/40 ground joints.

B. Reagents:

1. Xylene, ACS grade.
2. Antifoam agent, Dow Corning Antifoam, (or equivalent containing no volatile oil).
3. Sodium alkylbenzene sulfonate

C. Preparation of Sample:

1. Use Method 1.0.

D. Procedure:

1. Weigh accurately sufficient size sample to yield 2 to 5 mL of oil and transfer quantitatively to flask -- using water if necessary.
2. Add about 500 mL. of water. If magnetic stirring is to be used, insert stirring bar.
3. Assemble apparatus as shown in Fig. 2, selecting the trap depending upon the density of the oil to be trapped. (Note 3)
4. Heat the flask to boiling and maintain a reflux rate of 1 to 2 drops per second. (Note 4)

5. Reflux until two consecutive readings taken at 1 hour intervals show no change of oil volume in the trap. (Note 5, 6 & 7)

6. Cool to 20°C either by allowing to stand in air or immersing trap in a suitable water bath. If the trap is read at some temperature other than 20°C, this fact should be noted in the report. If the calculated volume of oil is below 2 mL or above 5 mL, the test should be repeated with appropriate adjustment to the amount of sample used (see Step 1 above).

E. Calculation:

$$\text{Volatile oil \% (v/w)} = \frac{\text{Vol. of oil (mL) 20°C}}{\text{Wt. of sample (g)}} \text{ X } 100$$

When xylene is used (Note 3), calculate the volatile oil by:

$$\frac{\text{Vol. of oil (mL) - vol. of xylene in blank}}{\text{Wt. of sample (g)}} \text{ X } 100$$

F. Statistics:

Interlaboratory measurements from Jan 1997 through Aug 2005 through the ASTA Check Sample Program have established a coefficient of variation or relative standard deviation of ±14% for this method for percent volatile oil in black pepper. The coefficient of variance is an important factor to consider when setting tolerance limits for product acceptability.

G. Notes:

1. Use method 16.0 for the determination of steam volatile oil in cassia.

2. Traps should be calibrated for volumetric accuracy by adding water to the trap and then adding xylene from a class A buret. Alkaline laboratory detergents that are designed to remove organic material and decontaminate glassware may be used to clean the traps. It is recommended to rinse the trap with acetone immediately after use and then soak in the detergent solution for at least 12 hours followed by water rinsing. Make sure the trap is clean by adding some distilled water into the trap to see that the water flows down the wall of the trap smoothly. If not, repeat the cleaning until it does.

3. If the density of the volatile oil is nearly one, as for example, clove oil, add 1.0 mL. of xylene to the trap before connecting the condenser. Calibrate each trap for the retention of xylene during distillation by running three blanks and taking the average volume. Repeat the procedure one time per year. This volume must be subtracted

from the volume of volatile oil collected from the distillation as shown in the calculations.

4. If foaming occurs, add a non-steam distillable anti-foaming agent. (Dow Corning Antifoam B or equivalent). The water used to cool the condenser should be cold enough to keep the reflux ring lower than 1/3 of the full condenser height.

5. If the separation of oil is not satisfactory, agitate the liquid in the trap with a wire inserted through the condenser. If this fails, carefully add a few mL of a saturated sodium chloride solution to the trap.

6. If a significant amount of refluxing occurs in the neck of the trap, wrap this area with suitable heat insulating material.

7. If difficulty is encountered in holding oil in the trap, add a concentrated aqueous solution of sodium alkylbenzene sulfonate dropwise through the condenser tube. 3 to 5 drops are usually sufficient. Continue distillation an additional 10 min. after the final addition of detergent.

8. Percent VO calculated with this method is based upon <u>total</u> mass of sample and, therefore, will be theoretically lower than values obtained by ISO method 6571 ("Spices, condiments and herbs – determination of volatile oil content"), which expresses percent VO as the volume of oil per <u>dry</u> mass of herb or spice. Other small differences in results between these methods could result from differences in trap design, sample grinding, distillation time and conditions for reading volumetric results.

H. References:

AOAC Official Methods of Analysis 962.17.

I. Revision History

3/22/10 Added instruction to repeat measurement if result falls outside of specified test range (Proc. Step 6). Added formula to to explain how to correct for use of xylene. Added coefficient of variation for %VO in black pepper to Statistics section. Clarified the importance of the coefficient of variation. Revised Note 2 to specify trap volume calibration and cleaning procedure (replaced use of chromic acid with alkaline detergent). Revised Note 3 to include trap xylene calibration. Revised Note 4 with instruction to keep condenser from overheating. Added Note 8 to explain differences between this method and ISO method 6571.

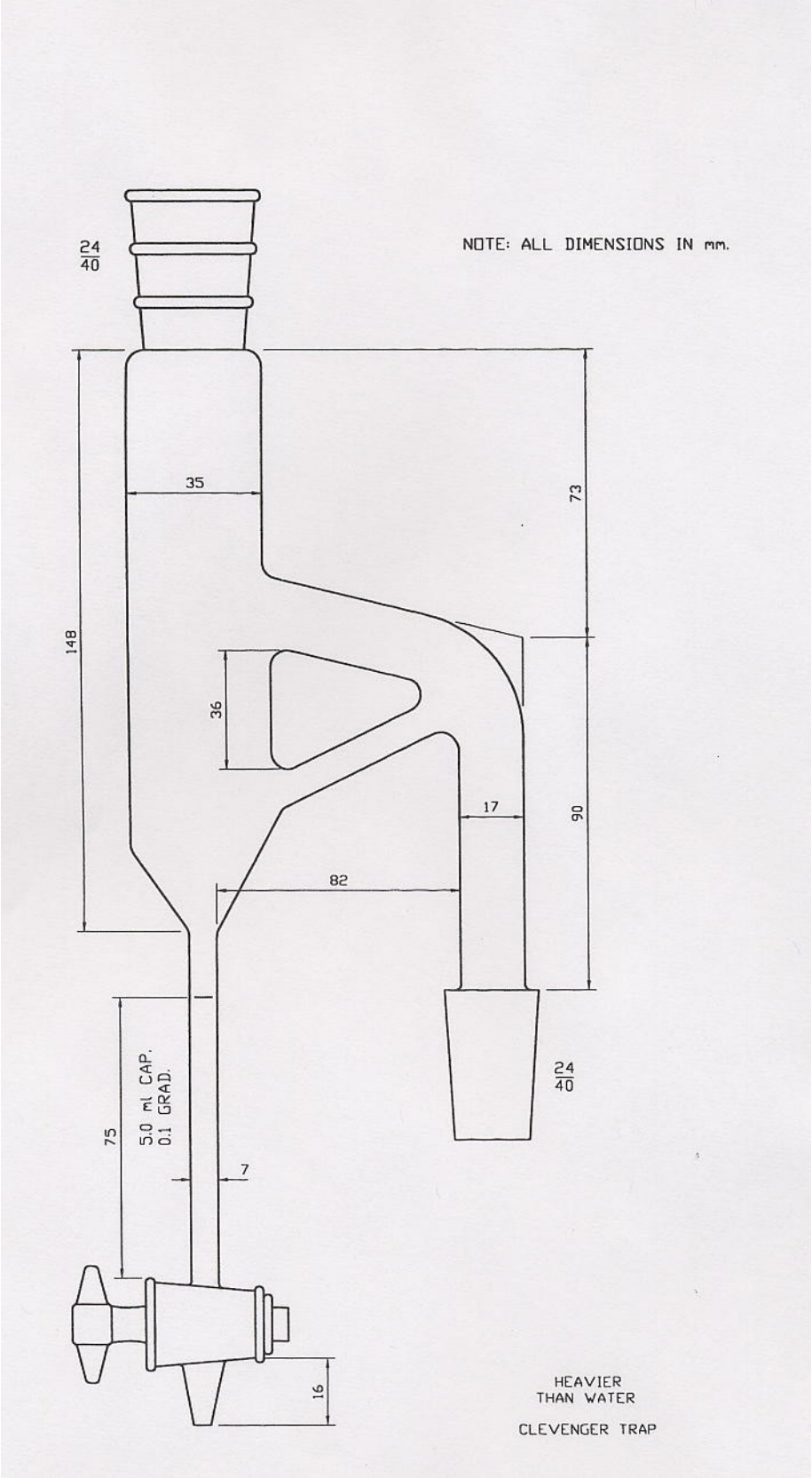

Figure 1a. Heavier than water Clevenger trap.

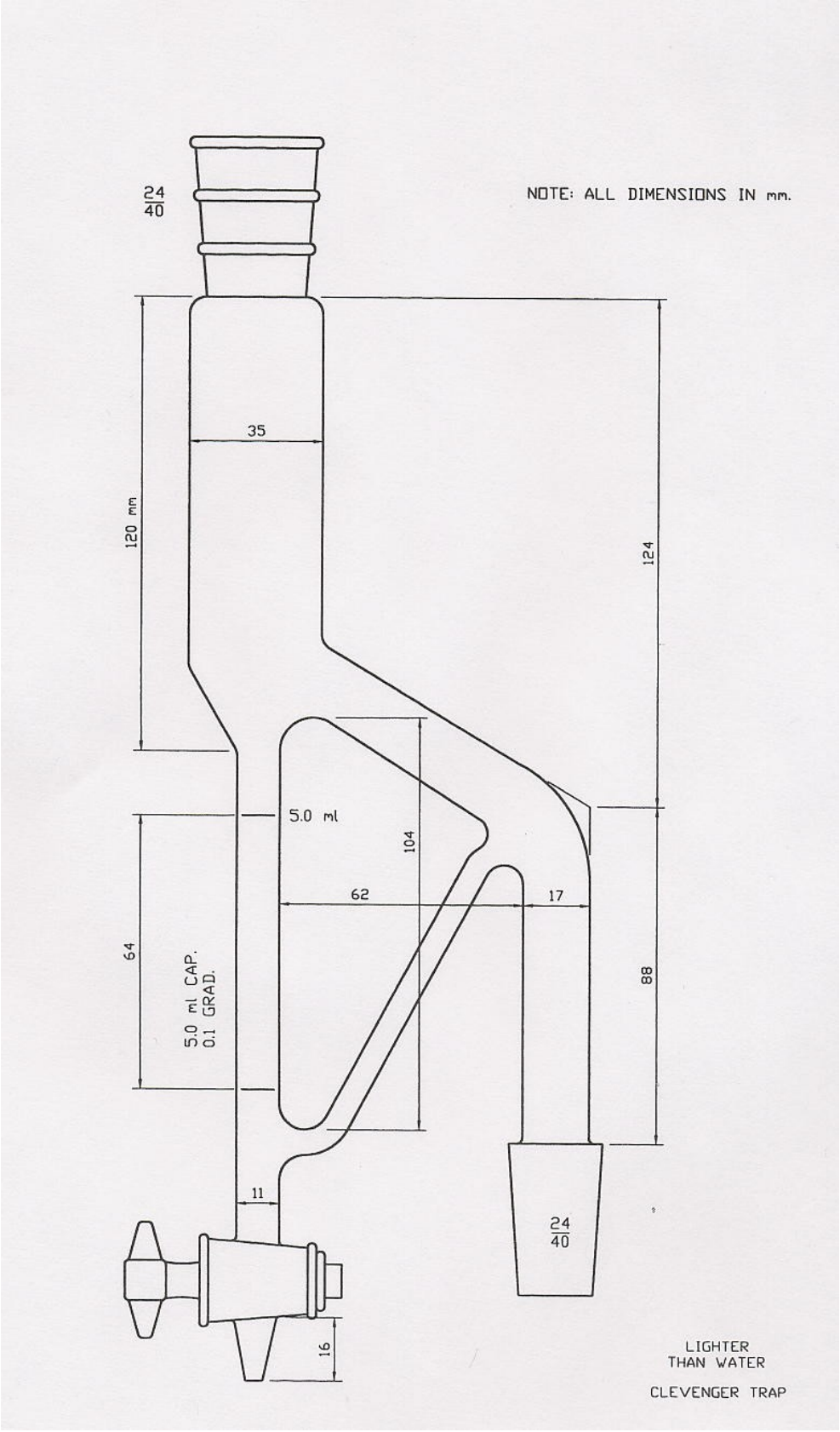

Figure 1b. Lighter than water Clevenger trap.

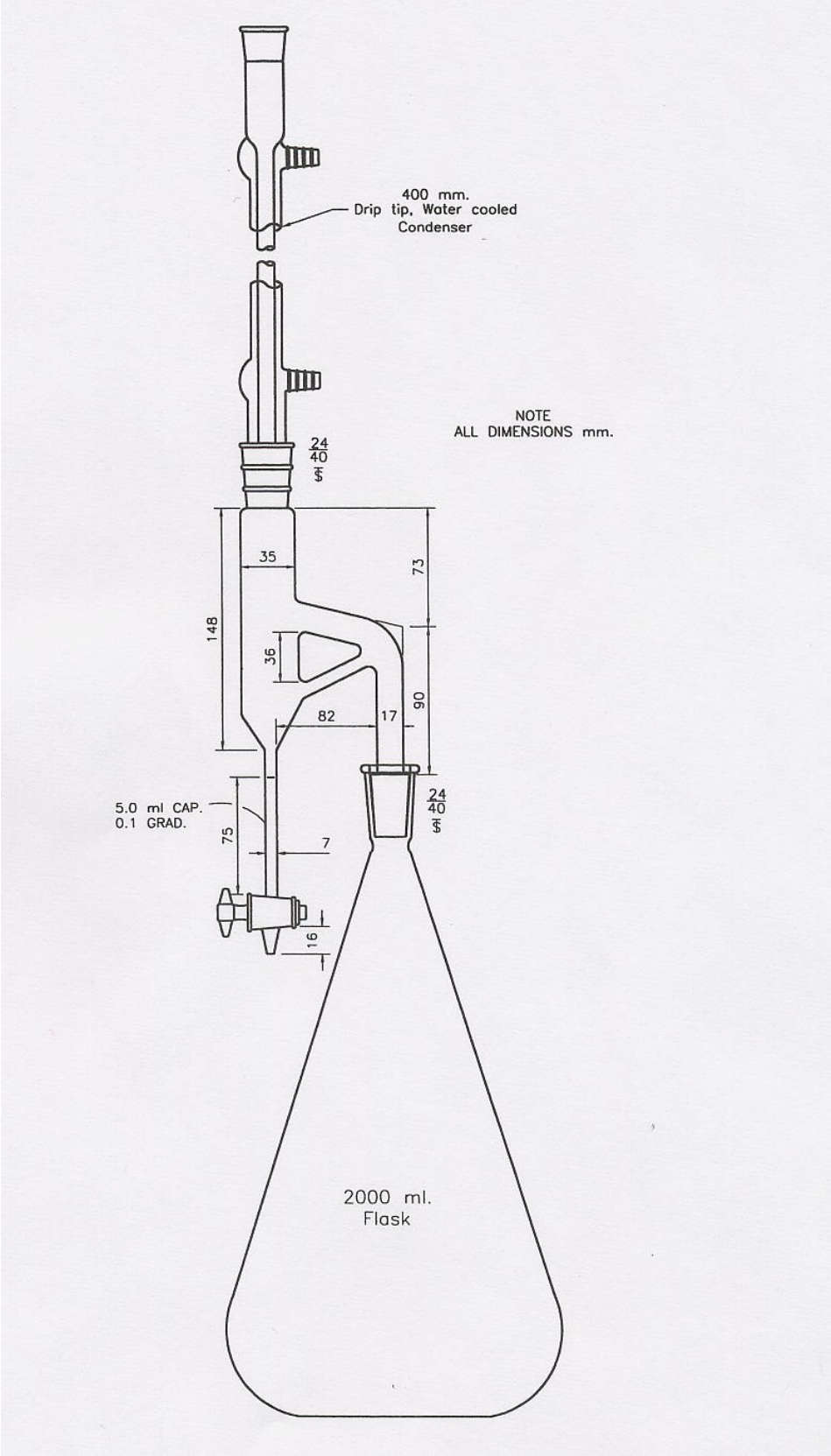

Figure 2. Apparatus assembled for steam distillation (ASTA method 5.0).