Sections in This Document

1. Purpose

To determine the tritium content in food samples by liquid scintillation counting of the free water obtainable by the reduced pressure distillation of a sample composite.

2. Scope

This method applies to analysis of Tritium in foods performed at the Winchester Engineering and Analytical Center (WEAC). The method has been validated for a variety of fish, milk and agricultural samples. The method is not intended for highly viscous samples, like honey.

3. Responsibility

A. Supervisor

1. Ensures the proper implementation of this procedure.
2. Ensures that the appropriate personnel are trained to perform the analysis using this SOP.

B. Method Monitor

1. Document and provide accessibility to method related information, including method validation, calibration and QC specification information.
2. Provide method support as needed (e.g. training and issue resolution).

C. Analyst

1. Adheres to this SOP.
3. Performs the required function verification and preventive maintenance on the spectrometer used for analysis.
4. Properly disposes of chemical and radioactive wastes resulting from sample analysis.

4. Background

Tritium (H-3) has become more abundant in the environment due to nuclear power plants and weapons testing. The EPA has set a limit for tritium in drinking water of 20,000 pCi/L (740 Bq/L). This SOP describes a method for determining the tritium content in free water obtained from food samples by reduced pressure distillation. The tritium content of the water is evaluated by observing its beta emissions (0-18.6 keV) through liquid scintillation counting (LSC).

This method assumes that tritium will primarily be present in the food sample in the water form.

5. References

A. LIB 1913, “Dehydration of Food Samples for Assay of Tritium in Free Water” 1976, Emilio J. Troianello.

B. LIB 1913 (Revised), “Dehydration of Food Samples for Assay of Tritium in Free Water” 1978, Emilio J. Troianello.

C. Food Composition and Nutrition Tables (Souci, Fachman, and Kraut, 1994, CRC Press, Boca Raton, Ann Arbor, London, Tokyo).

D. Standard Methods for the Analysis of Water and Wastewater 20th Edition, (Greenberg, Clesceri, and Eaton, 1992, American Public Health Association, Washington, D.C.)

E. ThermoScientific Coolant Bath Manual

6. Procedure

6.1. Instrumentation, Equipment, and Supplies

A. Liquid Scintillation Counter (LSC)

B. Top Loading Balance: Mettler XP8001S or equivalent

C. Analytical Balance: Mettler XP205 or equivalent

D. LSC sample plastic counting vial

E. Cooling bath capable of freezing the distillate and an associated heating system

F. A distillation apparatus, consisting of glass sample tube, condensate tube, vacuum manifold, O-rings, and connecting clamps

G. Vacuum grease

H. A vacuum pump capable of evacuating the empty distillation apparatus down to ≤ 200 mTorr

I. Thermocouple vacuum gauge

J. A fluorescing agent (Ultima Gold LLT, or equivalent)

K. A NIST-traceable tritiated water standard

L. Transfer pipettes (1 mL and 10 mL)

6.2. Sample Assignment in Database

A. Create an assignment using the first sub-form in the "New Assignment" tab in the startup menu (which is equivalent to the "Regulatory Analyses" tab and "Create Assignment" button) of the Analysis Database, Complete the information in the first sub-form.

Note: FACTS # may be an unofficial, unique, alpha numeric, concise identifier for samples not in FACTS.

Note: Successful completion of a regulatory worksheet for tritium requires that a gamma analyst be identified, but tritium calculations and attachments can be generated.

B. Enter the specific sample portions being analyzed for tritium into the second sub-form with the same ("New Assignment") tab.

C. Return to the startup menu. Future changes to the method assignments are made using the "Update Assignment" tab. (**Note**: the "Update Assignment" tab found within the "Regulatory Analyses" tab is equivalent.) Future changes to the tritium sample types analyzed are made using the "New Assignment" tab.

D. Return to the startup menu and select the "Regulatory Analyses" tab. Click "Create Batch ID", enter initials and batch number and click "Create H-3 Batch ID". If an error message occurs, that Batch ID may already exist, and you may need to use a higher batch number.

E. Return to the "Regulatory Analysis Menu" and click on the "H-3 Assignments" tab and enter the name of the analyst assigned tritium. Go to the "Batch ID" field for each record and assign a batch identification. This batch identification will include all samples with a common liquid scintillation count and samples need not be distilled simultaneously.

F. Return to the "Regulatory Analysis Menu" and click on the "H-3 Raw Data" tab and select the batch identification from the pulldown menu. Print the raw data sheet.

6.3. Sample Preparation

A. For regulatory samples, ensure that all sample portions are properly secured and tracked on the sample worksheet.

B. Ensure that all balances meet WEAC-LAB.6.0 Laboratory Balances QC requirements and enter balance data into associated WEAC-TMPL.112 FV/PM Sheet.

C. Composite a portion of the edible part of the food sample.

D. Identify and weigh the empty sample and condensate tubes with the associated, identified cork. Record the weights on the tritium raw data sheet.

E. Select an amount of the homogenized sample such that the distillation process will yield more than 10 g of water. Samples of about 20 g are usually appropriate. If an approximate moisture content of the sample is known the calculation (15 g/fractional moisture) may be used to determine an approximate sample portion. Add the aliquot of sample to the sample tube and cover with the associated and identified cork. Record the weight.

6.4. QC Sample Spike Preparation

A. If a valid QC sample spike has been analyzed and reported within the last seven days by the same analyst, a QC sample spike is not required.

B. Identify and weigh the empty QC sample and condensate tubes with the associated and identified cork. Record the weights on the tritium raw data sheet.

C. Take a portion of composite from a food sample, which has also been prepared for distillation in its unspiked form. Sample size of ~20 g is usually appropriate. Add the portion of food sample to the QC sample tube and weigh with the associated and identified cork and record the weights on the tritium raw data sheet.

D. Transfer a portion of a NIST-traceable standard of tritiated water to a glass liquid scintillation vial (or equivalent), close the vial and weigh on an analytical balance. Then, transfer ~1 mL of this standard solution to the QC sample tube, cover the vial and reweigh on the analytical balance.

6.5. Distillation Procedure

A. The cooling bath must be brought to a stable low temperature, before distillation can begin, so that freezing of the distillate will take place. Measure the temperature of the bath with a calibrated thermometer prior to starting the distillation.

B. Assemble the distillation apparatus, condensate tube, sample tube, and vacuum manifold, with O-rings, vacuum clamps, and vacuum grease. Assemble the apparatus in such a way that the condensate tube may be evacuated with the vacuum pump directly, while the sample tube can be evacuated only through its manifold connection with the condensate tube (See Attachment A).

C. Position the distillation apparatus so that the bottom of the condensate tube is submerged in the cold bath and the sample tube is contained within the heating well. The heating well remains off until pump down is completed.

D. Position the manifold valve so the condensate tube is opened to the vacuum pump and evacuate the tube to the point where the pressure within is ≤ 500 mTorr.

E. Turn the valve so that the condensate tube is opened to the sample tube, which causes the pressure in both tubes to be the same (approximately one-half atmosphere). Repeat this process until the pressure reading from the condensate tube remains less than 1 Torr immediately following equilibration with the sample tube.

F. Adjust the temperature of the heating system (approximately 50 °C) and allow the distillation to continue to dryness (typically overnight). Heat is used to accelerate distillation and may be decreased or delayed for samples that are vigorously evaporating and distilling prior to the addition of heat. It is important to maintain a good vacuum seal, so that the mean free path of the water vapor is sufficient to allow the gaseous water molecules to work their way to the condensate tube. If there is a leak, condensate will appear in the manifold just above the sample tube. If a leak is detected, you will have start over at the sample preparation step (section 6.2).

G. Allow the distillation to continue to dryness (typically overnight).

H. Once the sample is dry, remove the distillation apparatus from the bath and heater.

I. Allow ambient air into both tubes to allow them to attain room air pressure. Remove the topmost glassware bearing the valve and the O-rings.

J. Thoroughly wipe the coolant liquid off the condensate tube and thoroughly wipe the grease from both tubes.

K. Cover both tubes with the associated and identified cork and allow them to reach room temperature. The condensate will be frozen; if not, a contaminant is present which is depressing the freezing point of the water and the condensate must be redistilled.

L. Weigh the dry sample and condensate tubes and record each weight on the tritium raw data sheet.

M. The condensate must be clear and colorless; otherwise, quenching of the sample will interfere with the liquid scintillation count.

N. Go to the “Regulatory Analyses” tab of the startup menu of the Analysis Database and select the “H-3” tab on the left. Enter the data for sample analysis in both the “Tritium Assignment” and the “Distillation” tabs. Verify that distillation QC has met mass recovery specification for each distillation. If the distillation does not meet specification, redistill another portion if necessary.

6.6. Counting Procedure

A. Transfer 10 mL of sample condensate to a tared, identified, opaque plastic scintillation vial and reweigh (use an analytical balance).

B. Add 10 mL of scintillating agent, “Ultima Gold LLT” or equivalent, to the vial. Cap the vial and mix gently by repeated inversion. Weigh the vial and record the weight on the raw data sheet.

C. Make an efficiency-standard by transferring 10 mL of a NIST traceable tritium standard to a tared, identified, opaque plastic scintillation vial and reweigh (use an analytical balance). Then add 10 mL of the scintillating agent, cap the vial, mix gently by repeated inversion, weigh the vial and record the weight on the raw data sheet. The amount of standard used must be enough to produce at least 10,000 counts over the course of the count.

D. Prepare a method blank by adding 10 mL of distilled water to a tared, identified, opaque plastic scintillation vial and reweigh (use an analytical balance). Then add 10 mL of scintillating cocktail to a vial. Cap the vial, mix it by repeated inversion, weight the vial and record the weight on the raw data sheet.

E. Count the blank, efficiency standard, QC spike sample and sample(s) using the tritium protocol on the LSC instrument for 100 minutes each.

F. Allow a few minutes for darkening prior to initiating the sample count.

G. Enter Reagent/Equipment information, Calibration Information and Tritium Counting Data (in Regulatory Analysis Menu –H3) as prompted in the corresponding tabs. Once data is complete, review the worksheet using the final tab to review the worksheet and add initials to equipment information.

H. Calculation attachments and worksheets are created using the “Worksheets/Attachments” tab.

6.7. Quality Control

A. **Batch**: A batch is defined as all samples counted on a particular detector and by a particular analyst within 7 days of the initial batch

containing an LCS count and may potentially contain samples distilled separately.

B. **Blank**: A portion of distilled water is analyzed using a liquid scintillator. The counts of the blank are subtracted from the counts of the sample.

C. **QC Spike Sample**: An aliquot of one of the samples from the batch, spiked with a known amount of tritiated water before distillation, is run as a sample. The results of the spiked sample are recorded in the LCS Control Chart. Results are acceptable if the measured result falls into the 95% confidence range of the true value.

D. **Sample Distillation:** The starting weight of sample and the sum of the weights of condensate and dry sample should yield a % recovery of original sample weight of not less than 95%. Note that a failure to meet this specification does not indicate a sample failure and distillation may be repeated without initiating a non-conformance report.

6.8. Calculations

6.8.1. Sample Calculations

The tritium activity concentration of a food sample in Bq/kg is determined by:

$$A_{smp} = \frac{(R_s) \times M_s}{W_s \times E \times D_s \times 60 \times DF_{smp} \times k}$$

Where,

R_s = the net sample count rate, in cpm; Rgs – Rb
Rgs = the gross sample count rate, in cpm
Rb= the method blank (background) count rate, in cpm
Ms = the mass of total distillate recovered; (Mgs-Mts)
W_S = the mass, in kg, of the food that produced M; (Wgs – Wts)
Ds = the mass of distillate counted; (Dgs - Dts)

E = the counting efficiency (cpm/dpm), which is determined by:

$$E = \frac{R_e / 60}{C_{Std} \times W_e \times DF_{Std}}$$

Where,

Re = Count rate, in cpm of the efficiency std
Cstd = Activity concentration of the H-3 efficiency standard in Bq/g
We = Weight of standard counted in grams; Wge – Wte

$DF_{std(e)}$ = Standard decay factor for the efficiency standard
k = A factor representing the fraction of free-water contained within the sample that is obtained by the distillation process. This value is 1.
60 = Conversion factor, dpm to Bq
DF = Decay factor, exp((-ln2) x ΔT/ $T_{1/2}$)
For the DFsmp, Δtsmp = Time in years between the collection date and the assay date
For the DFstd(e), ∆tstd = Time in years between the efficiency standard reference date and the assay date

The MDC in Bq/kg is determined by the Currie "paired observation" method:

$$MDC = \frac{2.71 + 4.65 \times \sqrt{R_b \times T}}{E \times W_s \times \frac{D_s}{M_s} \times (T \times 60) \times DF_{smp}}$$

R_B = Blank count rate in cpm
T = Count time in minutes

The LOQ in Bq/kg is determined by the Currie "paired observation" method:

$$LOQ = \frac{\left[50 \times (1 + \sqrt{(1 + \frac{(R_b \times T)}{12.5}}\right]}{E \times W_s \times \frac{D_s}{M_s} \times (T \times 60) \times DF_{smp}}$$

6.8.2. QC Sample Calculations

The measured tritium activity concentration of a QC sample in Bq/kg on the assay date is determined by:

$$A_{qs} = \frac{(R_{qs}) \times M_{qs}}{(P_{qs})(E)(D_{qs})(DF_{qs})(k)(60)}$$

Where,
Rqs = Net sample count rate, in cpm; Rgs – Rb
Rgqs = Gross sample count rate, in cpm
Rb = Method blank (background) count rate, in cpm
Mqs =Mass of total distillate recovered; (Mgqs-Mtqs)

Pqs =Mass, in g, of the spiked reference standard
E = Counting efficiency (cpm/dpm), calculated as previously described

Dqs = Mass of distillate counted; (Dgqs - Dtqs)
DFqs = Decay factor for H-3 from count to the reference time of the std
k = A factor representing the fraction of free-water contained within the sample that is obtained by the distillation process. This value is 1.
60 = Conversion factor, dpm to Bq

6.8.3. Uncertainty Calculations

The absolute uncertainty of the sample activity concentration, σA, is culled from an examination of the uncertainties associated with mass of sample, count rate of sample, and instrument efficiency.

$$\sigma A_s = A_s \times \sqrt{\left(\frac{\sigma R_s}{R_s}\right)^2 + \left(\frac{\sigma M_s}{M_s}\right)^2 + \left(\frac{\sigma E}{E}\right)^2 + \left(\frac{\sigma W_s}{W_s}\right)^2 + \left(\frac{\sigma D_s}{D_s}\right)^2 + \left(\frac{\sigma k}{k}\right)^2}$$

To avoid an error when the net counts (Rs) is zero, the following equation is used (refer to validation document for derivation):

$$uA_{smp} = \sqrt{\left(\frac{M_s}{W_s E D_s DF_{smp} k 60}\sqrt{\left(\frac{uM_s}{M_s}\right)^2 + \left(\frac{uW_s}{W}\right)^2 + \left(\frac{uE}{E}\right)^2 + \left(\frac{uD_s}{D_s}\right)^2 + \left(\frac{uDF_{smp}}{DF_{smp}}\right)^2 + \left(\frac{uk}{k}\right)^2}\right)^2 + \left(\frac{M_s}{W_s E D_s DF_{smp} k 60} dR_s\right)^2}$$

The uncertainty in the counting rate is calculated as follows,

$$\sigma R_s = \sqrt{\left(\frac{\sqrt{R_{gs} \times T}}{T}\right)^2 + \left(\frac{\sqrt{R_b \times T}}{T}\right)^2}$$

The uncertainty of the distillate recovered (M) is calculated as follows,

$$\sigma M_s = \sqrt{\sigma M_{gs}{}^2 + \sigma M_{ts}{}^2}$$

The uncertainty in the efficiency is calculated as follows,

$$\sigma E = E \times \sqrt{\left(\frac{\sigma R_e}{R_e}\right)^2 + \left(\frac{\sigma W_e}{W_e}\right)^2 + \left(\frac{\sigma C_{Std}}{C_{Std}}\right)^2}$$

The uncertainty in the sample composite weight is calculated as follows:

$$\sigma W_s = \sqrt{\sigma W_{gs}{}^2 + \sigma W_{ts}{}^2}$$

The uncertainty of the distillate counted (m) is calculated as follows:

$$\sigma D_s = \sqrt{\sigma D_{gs}{}^2 + \sigma D_{ts}{}^2}$$

σk is 5%, this value is based on the observed agreement between duplicate sample % moisture content.

The absolute uncertainty of the QC sample measured activity concentration, σAqs, is calculated as follows:

$$\sigma A_{qs} = A_{qs} \times \sqrt{\left(\frac{\sigma R_{qs}}{R_{qs}}\right)^2 + \left(\frac{\sigma M_{qs}}{M_{qs}}\right)^2 + \left(\frac{\sigma E}{E}\right)^2 + \left(\frac{\sigma P_{qs}}{P_{qs}}\right)^2 + \left(\frac{\sigma D_{qs}}{D_{qs}}\right)^2 + \left(\frac{\sigma k}{k}\right)^2}$$

The uncertainty in the QC counting rate is calculated as follows,

$$\sigma R_{qs} = \sqrt{\left(\frac{\sqrt{R_{gqs} \times T}}{T}\right)^2 + \left(\frac{\sqrt{R_b \times T}}{T}\right)^2}$$

The uncertainty of the QC distillate recovered (M) is calculated as follows:

$$\sigma M_{qs} = \sqrt{\sigma M_{gqs}{}^2 + \sigma M_{tqs}{}^2}$$

The uncertainty in the sample composite weight is calculated as follows:

$$\sigma P_{qs} = \sqrt{\sigma P_{gqs}{}^2 + \sigma P_{tqs}{}^2}$$

The uncertainty of the distillate counted is calculated as follows:

$$\sigma D_{qs} = \sqrt{\sigma D_{gqs}{}^2 + \sigma D_{tqs}{}^2}$$

The absolute uncertainty in the spiked activity concentration is determined as follows:

$$\sigma A_{spike} = A_{spike} \times \sqrt{\left(\frac{\sigma P_{qs}}{P_{qs}}\right)^2 \times \left(\frac{\sigma C_{std}}{C_{std}}\right)^2 \times \left(\frac{\sigma W_{qs}}{W_{qs}}\right)^2}$$

Table 1: Uncertainty Budget - At LOQ level

Variable Name	Symbol	Value	Relative Standard Uncertainty (%)	Sensitivity Factor
Sample Composite Wgt. (g)	W_{ST}	20.2	**3.47%**	**1.0**
Counting efficiency, fractional	E	0.213	**5.16%**	**1.0**
Condensate Wgt. (g)	W_C	18.9	**3.70%**	
Sample Condensate Assayed (g)	W_{SC}	10.0628	**0.00%**	
Decay during counting correction	DF_E	0.554	**0.00%**	
Net Count Rate of Sample	R_S	3.01	**10.30%**	
Fraction distilled	k	1.0	**5.0%**	

Activity concentration of H-3, Bq/kg	21.90
Combined standard uncertainty, Bq/kg	2.76
Expanded uncertainty (2-σ), Bq/kg	5.51
Expanded uncertainty (2-σ)), %	25.18%

An uncertainty of up to 5% is introduced by matrix differences and incomplete distillation (for sample in which all QC parameters have been met)

6.9. Safety and Waste Management

A. Refer to the Chemical Hygiene Plan (WEAC-LAB.12.0) and Hazardous Waste Management Program (WEAC-LAB.14.0) and the WEAC Radiation Safety Manual (WEAC-LAB-RS-002) and Radioactive Waste Handling Procedure (WEAC-LAB-RS.004) for general guidance in handling and disposing of radioactive standards.

1. The coolant bath fluid is a skin and eye irritant, appropriate precautions should be taken.
2. The scintillator cocktail is a skin and eye irritant and a potential inhalation hazard, appropriate precautions should be taken.

B. Tritium standard use is tracked in the Analysis Database automatically when standard weights are entered for samples. Waste tracking reports are also available using the waste tab.

C. The following waste types are possible:

1. Residual distillate without detectable tritium may be poured down the drain.
2. Vials with Ultima Gold LLT and distillate without tritium should be placed in a waste accumulation tray, identified as Ultima Gold LLT and water.
3. Vials with Ultima Gold LLT and tritiated water and vials containing tritiated water should be placed in an accumulation tray, identified as Ultima Gold LLT and tritiated water. The tray must be accompanied by a Radiological Waste Record correlating the vial (by label and/or position) to its activity and the source information of the tritium (e.g. standard identification, sample number, PT number). It's the analyst's responsibility to record accurate activity and source information.

   Once accumulation trays are full and all relevant radiological waste information has been entered, contact the Industrial Hygienist for removal of the non-radioactive waste and contact the Radiation Safety Officer for removal of the radioactive waste.

7. Glossary/Definitions

A. **Laboratory Control Sample (LCS)** – A sample matrix free from tritium with known amounts of tritium added used to establish on-going precision and bias and evaluate method performance. In this procedure, a QC sample spike that meets QC criteria is an LCS.

B. **QC sample spike** – A sample with unknown levels tritium that is additionally analyzed after adding a known quantity of tritium.

8. Records

A. Access (Analysis Database) produced sample worksheet

B. Access (Analysis Database) produced sample calculation sheet

C. Access (Analysis Database) produced QC sample calculation sheet

D. Access database (Analysis Database) QA tracking for tritium method

9. Supporting Documents

A. WEAC-LAB.6.0 Laboratory Balances

B. WEAC-AB-RN.13.0 PerkinElmer Tri-Carb 3170 TR/SL Liquid Scintillation Counter

C. WEAC-AB-RN.15.0 PerkinElmer Quantulus 1220 Liquid Scintillation Counter

D. SOP-000281 PerkinElmer Quantulus GCT 6220 Liquid Scintillation Counter

10. Document History

Revision #	Status* (D, I, R)	Date	Author Name and Title	Approving Official Name and Title
1.0	I	4/20/2015	KELLY GARNICK, CHEMIST	PAT REGAN, ANALYTICAL BRANCH DIRECTOR
2.0	R	2/4/2016	KELLY GARNICK, CHEMIST	CONG WEI, ACTING ANALYTICAL BRANCH DIRECTOR
2.1	R	5/20/2016	MARGARET BLEAU, CHEMIST KELLY GARNICK, CHEMIST	PATRICK REGAN, ANALYTICAL BRANCH DIRECTOR
03	R	4/29/2019	KELLY GARNICK, CHEMIST	PATRICK REGAN, ANALYTICAL BRANCH DIRECTOR
04	R	11/8/2019	KELLY GARNICK, CHEMIST	PATRICK REGAN, ANALYTICAL BRANCH DIRECTOR
05	R	SEE INFOCARD	KELLY GARNICK, CHEMIST	PATRICK REGAN, ANALYTICAL BRANCH DIRECTOR

* - D: Draft, I: Initial, R: Revision

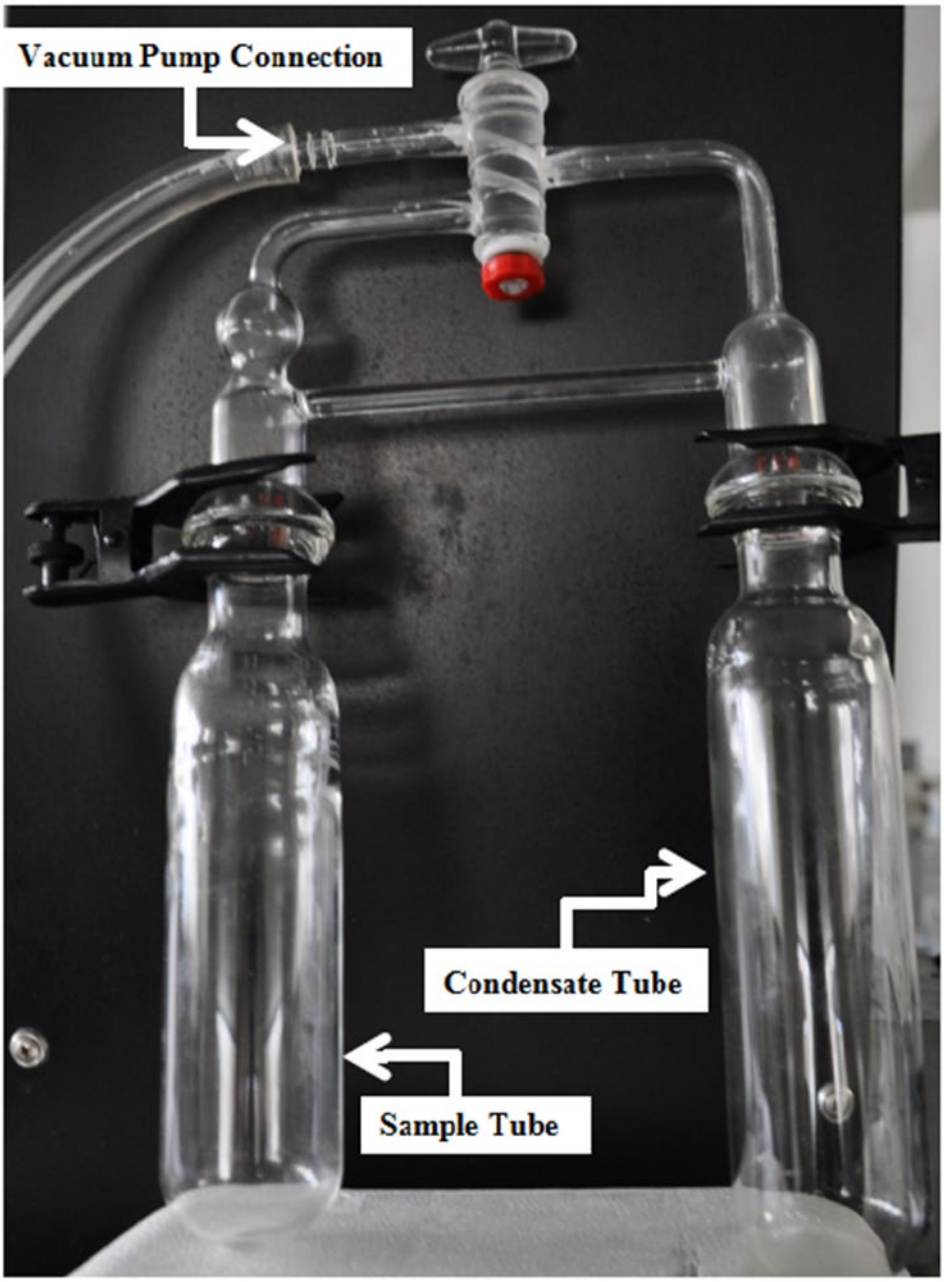
11. Change History

Revision #	Change
05	Added 3.B method monitor responsibilities, changed waste handling in section 6.9.C, and added 9.D.

12. Attachments

List of Attachments

Attachment A - Distillation Glassware and Equipment



Configuration of distillation glassware while evacuating condensate tube

Attachment A: Distillation Glassware and Equipment (Cont.)