



Department of the Treasury  
Alcohol and Tobacco Tax and Trade Bureau

# *Laboratory Analysis* for *Small Wineries*



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*(Note: This guide was originally developed to accompany a seminar conducted by the Bureau of Alcohol Tobacco and Firearms, TTB's predecessor agency.)*

# SULFUR DIOXIDE

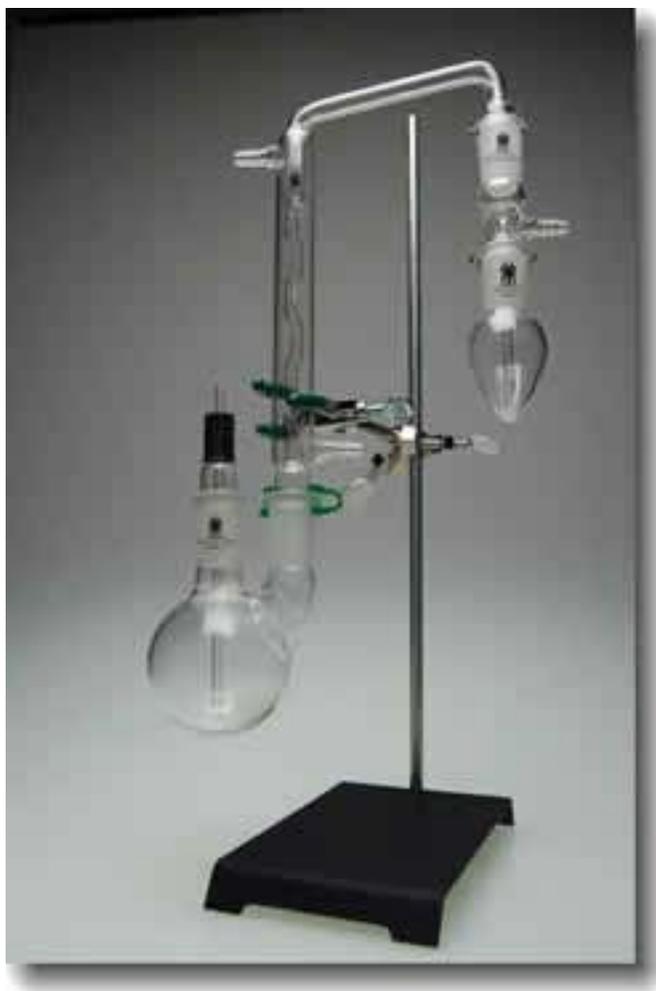
Federal regulations for the Labeling and Advertising of Wine, 27 CFR Part 4, limit the amount of total sulfur dioxide in finished wine to 350 mg/L (27 CFR 4.22(b)(1)). Also, section 4.32(e) requires that a sulfite declaration appear on the labels for wine and other beverage alcohol products containing 10 or more mg/L total sulfur dioxide, regardless of whether any sulfiting agents were added to the grapes, juice or wine.

Furthermore, the federal Wine Regulation 27 CFR 24.309(m) requires that when wine is transferred in bond to another bonded wine premises that the transfer in bond record (typically a bill of lading) contain information about any substance that requires a label declaration on the finished product.

## DETERMINATION OF SULFUR DIOXIDE IN WINES BY AERATION/OXIDATION

### Equipment:

1. Distillation-scrubber apparatus (all 14/20 standard taper pieces, referenced as Kontes part numbers) or equivalent
  - A. 250 mL round-bottom flask, K 294000-0250
  - B. Claisen adapter K 273750
  - C. Pasteur pipette through rubber stopper
  - D. 300 mm Graham condenser K 439000-2430
  - E. Connecting adapter K 275050
  - F. Vacuum Adapter K 276750
  - G. Two 50 mL pear flask K 294250-0050
  - H. Inlet adapter, size 21 K 1798000-2114
  - I. Connecting adapter, 24/40 – 14/20 K 274750-0263
  - J. Bushing adapter, 24/40 – 14/35 K 150750-0242
2. 10 mL/100 mL Burette
3. Vacuum source/water aspirator
4. 5, 10 and 20 mL volumetric pipettes
5. Heat shrink tubing to connect to water and vacuum source



Assemble as shown. Connect Pasteur pipette inlet as described above. Connect a recirculating ice bath to the Graham condenser and place a micro-burner below the round flask.

**Reagents:**

1. 0.01 N sodium hydroxide solution: purchase pre-mixed or prepare by dissolving 0.41 g NaOH in 500 mL distilled water and diluting to 1 L.
2. Standardized 0.01 N sulfuric acid (for calibrating the sodium hydroxide solution).
3. 0.3% hydrogen peroxide solution: prepare just prior to use by diluting 10 mL reagent grade 3% hydrogen peroxide solution to 100 mL with distilled water. (Keep the 3% hydrogen peroxide refrigerated.)

4. Indicator: dissolve 0.100 g methyl red and 0.05 g methylene blue in 50% aqueous ethanol and make to 100 mL.
5. 25% phosphoric acid solution: add 280 mL 90% phosphoric acid to 600 mL distilled water; cool; make to 1 L.

**Standardization Procedure:**

1. Add 10 mL distilled water and 3 drops of indicator to the pear flask; adjust the purple color of the solution to turquoise green by adding small drops of sodium hydroxide solution; remember the color.
2. Pipette 5 mL of standard sulfuric acid solution into the flask; swirl.
3. Fill the burette with 0.01N Sodium Hydroxide to the zero mark and titrate the solution to the same green color as noted in Step 1; record the titer.
4. Repeat the standardization until 3 titers agree within 0.05 mL. This is also excellent practice in recognizing the endpoints.
5. The base normality is  $0.01 \times 5 / (\text{average mL base used in standardization})$ .

### **Sample Determination:**

Pass a continuous stream of cold water through the condenser during sample testing. Draw air through the vacuum adapter at 200 mL/min

1. Add 10 mL hydrogen peroxide solution and 3 drops of indicator to the pear flask; adjust the purple color of the solution to turquoise green by adding small drops of sodium hydroxide solution and attach the flask to the end of the vacuum adapter.
2. a) Total SO<sub>2</sub>: Pipette 10 mL wine and 10 mL phosphoric acid solution into the 50 mL pear shaped flask. If the wine is gassy or foamy, add a drop of anti-foam. Attach the flask to the end of the Claisen adapter.  
  
b) Free SO<sub>2</sub>: Pipette 100 mL wine + 20 mL H<sub>2</sub>PO<sub>4</sub> into 250 mL round bottom flask. Draw air through vacuum adapter at 200 mL/min for 15 minutes.
3. For total sulfur dioxide: draw air through the vacuum adapter at 200 mL/min while gently boiling the pear flask with a micro-burner for 15 minutes.
4. Carefully remove the pear flask, rinsing the tip of the vacuum adapter into the flask.
5. Fill the burette to the zero mark with 0.01N sodium hydroxide and titrate the solution to a turquoise green color; record the titer.

### **Calculation:**

$$\text{SO}_2 \text{ (free/total) mg/L} = \frac{(\text{mL base titer}) \times 32000 \times (\text{normality base})}{10 \text{ (for total) or } 100 \text{ (for free)}}$$

### **Reporting Results:**

Report values as mg/L (ppm) with no decimals, i.e., XXX mg/L (ppm).

## DETERMINATION OF SULFUR DIOXIDE IN WINE BY THE RIPPER METHOD

Reaction Equation:  $\text{H}_2\text{SO}_3 + \text{I}_2 + \text{H}_2\text{O} \rightarrow \text{H}_2\text{SO}_4 + 2 \text{HI}$

### Equipment:

1. 250 mL Erlenmeyer Flask
2. 25/10 mL Burettes and holders
3. 20/50 mL volumetric pipettes for sample
4. 5, 10, and 25 mL pipettes for starch, NaOH and  $\text{H}_2\text{SO}_4$
5. Stopper or parafilm
6. Titration Light Source

### Reagents:

1. Sulfuric acid solution: 1 + 3 (10 mL (concentrated) sulfuric acid plus 30 mL water) (Remember, always add acid to water, slowly and with stirring.)
2. 1 N sodium hydroxide solution (40 grams NaOH per liter water): for total  $\text{SO}_2$
3. 2% starch indicator solution
4. Iodine: 0.020 N, standardized against sodium thiosulfate solution.  
Note: Iodine solution should be stored in dark-colored glass containers.  
Also, iodine oxidizes easily and should be standardized frequently.
5. 0.1N Standard sodium thiosulfate solution

### **Procedure: (Free)[Total] Sulfur Dioxide**

Note: Quantities used for free sulfur dioxide are in( ) and for Total in[ ]

1. Pipette (50)[20] mL wine into a 250 mL Erlenmeyer flask. For total sulfur dioxide only: [Add 25 mL of sodium hydroxide solution; mix, stopper, and let stand for 10 minutes].
2. Add 5 mL starch indicator solution; add (5)[10] mL sulfuric acid solution.
3. Titrate rapidly with 0.020 N iodine solution; endpoint is the first bluish color persisting for about 30 seconds.

**Calculations:**

$$\text{SO}_2 \text{ [free/total]}(\text{mg/L}) = \frac{(\text{mL iodine}) \times (\text{N iodine}) \times (32000)}{\text{mL sample}}$$

Example: 20 mL sample uses 4.6 mL of 0.020 N iodine, then  
 $\text{SO}_2 = (4.6) \times (0.020) \times (32000) / 20 = 147 \text{ mg/L}$

**References:**

Ough, C.S. and Amerine, M.A., Methods for Analysis of Musts and Wines, J. Wiley and Son, New York (1988)

Zoecklin, S.W., Fugelsang, K.C., Gump, B.H. and Nury, F.S., Production Wine Analysis, Van Nostrand Reinhold, New York (1990)

## WINE ALCOHOL

Following water, ethyl alcohol is the second major constituent of wine. Alcohol is very important because it makes significant contributions to both the sensory properties and the stability of wine.

Wine is defined in the Federal Alcohol Administration Act as containing not less than 7 percent and not more than 24 percent alcohol by volume (27 U.S.C. 211(a)(6)). Internal Revenue Code regulations define wine as containing not less than 0.5 and not more than 24 percent alcohol by volume (27 CFR 24.10). Federal labeling regulations require that wines be labeled with an alcohol representation within a +/- 1.5 percent tolerance for wines between 7 and 14 percent alcohol by volume, and a tolerance of +/- 1.0 percent for wines over 14 percent alcohol by volume (27 CFR 4.36(b)(1)). There is a tolerance of +/- 0.75 percent for wines with less than 7 percent alcohol by volume stated on the label (27 CFR 24.257(a)(3)).

Still wine products containing between 0.5 and 14 percent alcohol by volume are taxed at the rate of \$1.07 per gallon. Wine products above 14 percent and not over 21 percent alcohol by volume are taxed at the rate of \$1.57 per gallon (26 U.S.C. 5041). Wineries producing not more than 250,000 gallons in a calendar year may receive a tax credit of up to \$.90 per gallon on the first 100,000 gallons of wine removed annually for consumption or sale (27 CFR 24.278).

Since still wine is taxed on alcohol content and wine labels must accurately reflect the alcohol content of wine, accurate alcohol determinations are important. Additionally, Federal regulations require wineries that produce or process bulk wine to have ready access to a suitable means of determining the alcohol content of wine (27 CFR 24.170). Section 24.255(e) requires wineries to conduct alcohol tests of wine bottled or packed, and Section 24.308(c) requires wineries to maintain records of these alcohol tests.

## **DETERMINATION OF ALCOHOL IN WINE BY DISTILLATION WITH HYDROMETRY OR DENSITOMETRY**

Distillation converts wine into an alcohol-water solution of the same concentration as the original wine. The alcohol concentration of this distillate may be determined by density (hydrometer or density meter), refractometer, or, if the original wine solids were too high, with an ebulliometer.

### **Equipment:**

One of the following hydrometers of appropriate range with calibrated corrections:

Indicating specific gravity with divisions of 0.0001

Indicating proof with divisions of 0.2°

Indicating percent alcohol with divisions of 0.1%

Thermometer with calibrated corrections with divisions of 0.2°F

Conversion Table (depending upon the hydrometer or densitometer used):

Table 1 of the Gauging Manual, 27 CFR Part 30

[http://www.ttb.gov/foia/Gauging\\_Manual\\_Tables/Table\\_1.pdf](http://www.ttb.gov/foia/Gauging_Manual_Tables/Table_1.pdf)

Table 913.02 of the Official Methods of Analysis of

AOAC International (18<sup>th</sup> ed.) (available on-line at [aoc.org](http://www.aoc.org))

Alcohol Correction Table from Bureau of Internal

Revenue Bulletin 7 (1937) (attached)

Hydrometer cylinder – clear glass 2.5” diameter

Or Densitometer (ATF Ruling 91-2)

### **Distillation:**

For hydrometry use a 250 mL volumetric flask with stopper.

For Densitometry use a 100 mL volumetric flask with stopper.

Distillation apparatus – electric or gas operated – with:

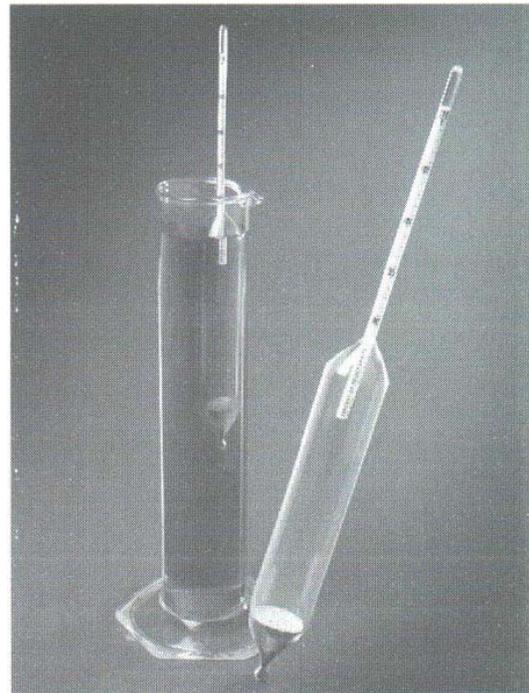
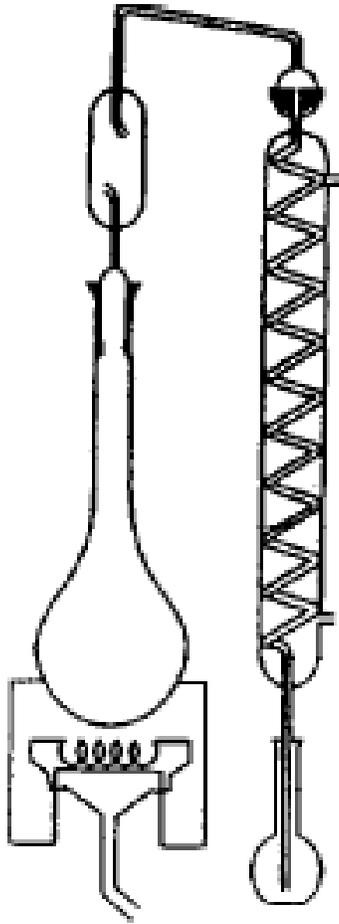
Distillation flask, Condenser and Kjeldahl Connector

Constant temperature water bath (maintained at 20°C  
(68°F) +/- 0.1°C)

Boiling chips

**Reagents:**

Antifoam agent, distilled water, ice  
2 N aqueous sodium hydroxide (NaOH) solution



### **Procedure:**

1. Fill the 250 mL volumetric flask to above the graduation mark with the wine sample and stopper.
2. Temper at 20°C for one hour, adjust wine volume to graduation mark.
3. Quantitatively transfer the entire contents of the flask to a distillation flask. Rinse the volumetric flask at least 3 times using a total of 50 to 60 mL of distilled water, adding all of the rinse water to the distillation flask.
4. Newly fermented, gassy, or wines in excess of 5 percent sugar may require the addition of a drop of antifoam agent to the contents of the distillation flask to prevent excessive foaming.
5. Wines containing in excess of 200 mg/L of sulfur dioxide or 0.1 g/100 mL volatile acidity must be neutralized with two drops of 2 N NaOH prior to distillation to avoid interferences resulting from the presence of these materials.
6. Position and connect the distillation flask ensuring a tight seal between the flask and the trap, and the trap and the condenser.
7. Place the rinsed receiving flask in a beaker of ice water so that it will be immersed nearly to the stem during the distillation period. The outlet of the condenser should extend into the neck of the flask, but should not touch the surface of the distillate.
8. Pass a continuous stream of cold (<25°C) water rapidly through the water jacket of the condenser.
9. Heat the flask rapidly until the liquid reaches its boiling point, then distill at a uniform rate, completing the distillation in from 30 to 60 minutes, depending upon the alcohol content of the wine sample.
10. Collect approximately 225 mL of distillate, stopper, and again temper for one hour at 20°C.
11. Bring volumetric flask up to volume using tempered (20°C) distilled water. Stopper and mix thoroughly.
12. You may read the alcohol content directly on the densitometer now.
13. For hydrometry, temper flask at room temperature for one hour.

**Determination:**

13. Transfer distillate to dry hydrometer cylinder containing thermometer, and immerse a dry hydrometer of appropriate range.

14. After hydrometer reaches equilibrium, read hydrometer to nearest:

0.02° for proof indicating hydrometers

0.01% for % alcohol indicating hydrometers

0.0002 units for specific gravity indicating hydrometers

Record temperature of distillate to nearest 0.02°F. Correct the hydrometer and thermometer readings.

Densitometer: Follow manufacturer's instructions. Most will be calibrated to read alcohol % by volume at 60 degrees F directly.

**Calculation:**

Percent alcohol-indicating hydrometers: convert the % alcohol at the measured temperature to 60°F via the table in Bulletin 7.

Proof-indicating hydrometers: convert the proof at the measured temperature to 60°F via the Gauging Manual and divide the resulting proof by 2.

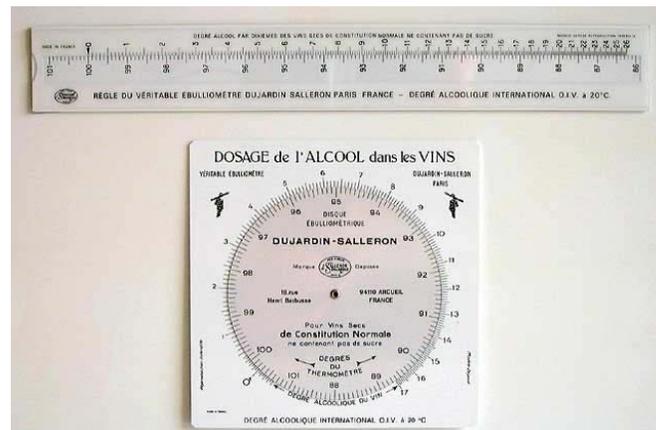
Specific gravity-indicating hydrometers: convert the specific gravity at the measured temperature to 60°F via A.O.A.C. Table 52.003. (Some interpolation may be necessary to use this table.)

This method is accurate to +/- 0.1% Report results to 1 decimal place.

# DETERMINATION OF WINE ALCOHOL BY EBULLIOMETER

## Equipment:

Ebulliometer  
Slide rule/conversion table  
Alcohol/micro Bunsen burner  
200 mL volumetric flask with stopper  
50 mL pipette  
100 mL graduated cylinder  
Narrow range ebulliometer thermometer



**Reagents:** antifoam solution and distilled water

**Procedure:**

1. Bring the sample and a supply of distilled dilution water to a temperature of 20°C (68°F).
2. Using a graduated cylinder, transfer the required amount of distilled water to the boiler. Insert the thermometer. No water is added to the condenser for the determination of the boiling point of water.
3. Protect the ebulliometer from drafts.
4. Ignite micro-burner/alcohol lamp. Adjust flame (which may require trial runs) to start the temperature rise within 8-9 minutes.
5. Observe temperature rise, noting (writing) temperatures every 15 seconds; continue reading temperature until 3 successive readings are the same. (Note that the smallest graduation is 0.05°C; estimate readings to 0.01°.)
6. Remove flame. Remove thermometer. Drain the hot water carefully.
7. Transfer 50 mL of the sample to a 200 mL volumetric flask (by pipette) and make up to volume with distilled water, stopper and mix.
8. Rinse boiler 3 times with approximately 25 mL of diluted sample. Transfer by graduated cylinder the required amount of diluted sample to boiler. Attach condenser firmly. Insert thermometer. Add cold (ice) water to condenser jacket. Put finger over condenser vent tube to prevent water from entering the boiler.
9. Light flame; adjust flame to attain temperature rise within 8-10 minutes. Observe and record every 15 seconds until temperature stabilizes (three consecutive readings are the same). Repeat the determination if condenser gets warm.
10. Remove flame; remove thermometer, drain sample; drain condenser.
11. Repeat steps 7-10 to replicate analysis or for new sample.
12. Calculate the alcohol from the slide rule/conversion table that comes with the instrument; multiply the alcohol found by the dilution factor.

## Notes:

- The ebulliometer and glassware should be “squeaky” clean internally as well as externally, and the work area should be orderly and free of miscellaneous equipment.
- Clean the boiler by boiling dilute alkaline solution every 25-30 determinations.
- If you have access to a known alcohol standard solution (such as 80 proof [40% alcohol] gin) you will be better able to evaluate the performance of your equipment and techniques.
- To prevent loss of alcohol, reflux condensers must be securely tightened into the lower housing and the thermometer must also be sealed at the housing.
- Cooling water in the condenser must be as cold as possible.
- In newly fermented wines, excessive foaming may be prevented by the addition of antifoaming agents.
- The marked tubes for diluting samples frequently furnished with the instrument are adequate for approximate work; for best precision use class A pipettes calibrated to deliver (T.D.), and class A volumetric flasks calibrated to contain (T.C.); both at 20°C (68°F).
- Do not blow out pipettes that are calibrated to deliver. As the last bit of sample flows from the tip, touch the end of the pipette briefly against an interior surface for proper drainage.
- Do not exceed a temperature difference of 4°C between the boiling points of water and the sample. Wine should be diluted to 4 or 5% alcohol; 1:4 dilution is even better (50 mL wine aliquot diluted to 200 mL with distilled water).
- Sugar levels in excess of 2 percent as well as aldehydes and esters depress the boiling point of the sample resulting in higher apparent alcohol levels. To minimize these errors, sweet wines can be quantitatively diluted with distilled water to less than 2% sugar or distilled.

## References:

1. Ough, C.S. and Amerine, M.A., Methods for Analysis of Malts and Wines, J. Wiley and Son, New York (1988).
2. Official Methods of Analysis of the A.O.A.C. International, Arlington, VA.

3. Zoecklin, S.W., Fugelsang, K.C., Gump, B.H., and Nury, F.S., Production Wine Analysis, Van Nostrand Reinhold, New York, (1990).

## FILL OF WINE CONTAINERS

Section 27 CFR 24.255(b) of the Federal Wine Regulations requires, in part, that bottles and other containers be filled as nearly as possible to conform to the net contents as shown on the label, but in no event may the amount of wine contained in any individual container, due to lack of uniformity of containers, vary from the amount stated on the label more than:

- 1.0% for 15.0 liters and above
- 1.5% for 1.0 to 14.9 liters
- 2.0% for 750 mL
- 2.5% for 500 mL\*
- 3.0% for 375 mL
- 4.5% for 187 mL and 100 mL
- 9.0% for 50 mL

(\*Acceptable tolerance, not listed in regulation)

There will be substantially as many bottles overfilled as there are bottles underfilled for each lot of wine bottled. A "lot of wine bottled" is defined as the same type of wine bottled or packed on the same date into containers (27 CFR 24.10).

27 CFR 24.255(d) requires that fill tests be conducted at representative intervals of wine bottled or packed during the bottling or packing operations for each bottling or packing line to determine if the wine contained in the bottle is in agreement with that stated on the label.

27 CFR 24.308(c) requires, in part, that records of fill tests be maintained for each lot of wine bottled or packed, or for each bottling or packing line operated each day, showing the date, type of test, item tested and results.

27 CFR 24.170(b) requires, in part, that bottling or packing wineries have ready access to equipment for determining the net contents of bottled or packed wine. Fill tests may be conducted either volumetrically or by weight.

## DETERMINATION OF FILL OF CONTAINERS BY WEIGHT

### Equipment:

1. Top loading balance with 0.1 g readability
2. Specific gravity hydrometer=

### Procedure:

For containers which have already been filled:

1. Weigh full bottle ( $W_o$ ), noting the labeled capacity of the container ( $V_o$ ). If the sample is highly carbonated, carefully open the container and let it de-gas for 20 minutes.
2. Empty the contents and save a portion to determine density. Let the container thoroughly drain.
3. Reweigh the drained container ( $W_e$ ), including the closure.
4. Determine the density ( $D$ ) of the contents, noting the temperature. Be sure that carbonated products are thoroughly degassed.

For containers on the filling line:

1. Weigh a marked container ( $W_e$ ), noting the label capacity of the container ( $V_o$ ); a group of containers may be run at the same time.
2. Obtain a bulk sample of the product from the bottling tank and determine the density ( $D$ ), noting the temperature. Be sure carbonated products are thoroughly degassed.
3. Run the container(s) through the filler and recover them. Determine full weight of the container ( $W_o$ ), noting the labeled capacity of the container ( $V_o$ ). If the sample is highly carbonated, let it de-gas for 20 minutes before weighing.

### Calculations:

$$\text{Fill (as \% of label volume)} = \left( \frac{(W_o - W_e) - V_o}{D} \right) \times \frac{100}{V_o}$$

[Fill is reported as + or - % of label contents with one decimal place, i.e., + or - X.X%].

## WINE ACIDITIES

Wine acid is very important as it contributes to the color, stability and sensory properties of wine. Tartaric and malic acid are the two major acids in grape wines and account for about 90% of a grape wine's total titratable acidity. Occasionally, winemakers add acids to must or wine to compensate for natural deficiencies and also for stabilization purposes.

The Federal Wine Regulations, 27 CFR Part 24, provide for the addition of tartaric and/or malic acids to grape must prior to fermentation and any one or a combination of the following acids to grape wine after fermentation: Tartaric, malic, citric, lactic and fumaric to compensate for naturally occurring deficiencies (27 CFR 24.182(b)). However, this same section of the regulations states that the use of these acids, either prior to, during or after fermentation, may not increase the fixed acidity (fixed being the difference between the total [titratable] acidity and volatile acidity) of the finished wine (calculated as tartaric) above 9.0 grams per liter.

In cases where the wine contains 8.0 or more grams of total solids per 100 mL of wine, acids may be added to the extent that the finished wine does not contain more than 11.0 grams per liter of fixed acid (calculated as tartaric).

In fruit (other than grape) wine production, 27 CFR 24.182(c) provides that only citric acid may be added to citrus fruit, juice or wine, only malic acid may be added to apple fruit, juice or wine, and only citric acid or malic acid can be added to other fruit or to juice or to wine derived from other fruit (including berries) to correct for natural deficiencies to 9.0 grams per liter of finished wine; however, if the wine contains 8.0 or more grams of total solids per 100 mL of wine, acids may be added to correct for natural deficiencies to the extent that the finished wine does not contain more than 11.0 grams per liter of fixed acid (calculated as malic acid for apples and citric acid for other fruit, including berries).

27 CFR 24.244 provides for the addition of up to 0.7 grams per liter of citric acid, regardless of the fixed acidity level, to stabilize wine. Citric acid chelates iron present in wine and helps prevent iron instabilities. The same section of the regulations provides for the addition of up to 3.0 grams per liter of fumaric acid, regardless of the fixed acidity level, to impede malolactic fermentation.

27 CFR 24.318 requires that wineries which add acid to wine shall maintain records showing the date of use, the kinds and volume of wine in which used, and, when used to correct natural deficiencies, the fixed acid level of the juice or wine before and after the addition. The record will account for all acids received and will be supported by purchase invoices.

Volatile acid at high levels is often regarded as an indication of spoilage. Federal regulations provide limitations on the amount of volatile acidity in standard wine. Section 27 CFR 4.21(a)(1)(iv) of the Federal Labeling and Advertising of Wine regulations provides a maximum volatile acidity limitation, calculated as acetic acid exclusive of sulfur dioxide, of 0.14 g/100 mL for natural red wine and 0.12 g/100 mL for other grape wine. However, the maximum volatile acidity for wines produced from unameliorated juice of 28 or more degrees Brix is 0.17 g/100 mL for red wine and 0.15 g/100 mL for white wine.

# DETERMINATION OF TITRATABLE (TOTAL) ACIDITY

## Equipment:

10 mL burette (marked in 0.1 mL)  
1, 5 and 10 mL pipettes  
(pH meter with combination reference/pH electrode – optional)  
Magnetic stirring table and stir bar  
250 mL beaker

## Reagents:

0.1N NaOH  
1% Phenolphthalein indicator (if used)

## **Procedure**

1. Fill the burette with 0.1N NaOH
2. Standardize the pH meter using pH 10.0 and pH 7.0 buffers.
3. Rinse electrodes with distilled water
4. Add enough distilled water to a 250 mL beaker so that electrode end will be sufficiently submerged when lowered into the beaker.
5. Place electrode into the beaker and adjust to pH 8.2 using 0.1 N or less NaOH. This neutralizes any acid which may be present in the distilled water and need not be a quantitative addition. Place electrode away from the magnetic stir bar to prevent possible damage to electrode. If a phenolphthalein indicator end point is used in lieu of a pH meter, add 2 or 3 drops of a 1% phenolphthalein solution to the distilled water. Titrate with 0.1 N or less NaOH to a faint pink color.
6. Pipette exactly 5.0 mL of degassed wine sample into the same beaker using a T. D. (to deliver) pipette. Do not blow out or otherwise attempt to remove any of the wine residue from the pipette as these pipettes are calibrated “to deliver” their rated capacity.
7. Titrate the water-wine solution using 0.1 N NaOH, slowly allowing the titrant and the sample to mix. As the pH approaches 8.2, add the titrant very slowly as to not over shoot the pH 8.2 end point. If a phenolphthalein indicator is used, add about 1 mL of phenolphthalein solution to the distilled water-wine solution and titrate using 0.1 N NaOH to a faint pink color end-point. Three or four

samples may be analyzed using the same buffering solution prepared in steps 3 and 4 above.

1. Record the quantity of NaOH used in the titration.

**Calculation:**

Report results in g/L using the following formula:

Total Acid: (as appropriate acid in g/L)

Grape wines:  $\text{g/L tartaric} = \text{mL NaOH} \times \underline{N} \text{ NaOH} \times 0.075 \times 200$

Apple wines:  $\text{g/L malic} = \text{mL NaOH} \times \underline{N} \text{ NaOH} \times 0.067 \times 200$

Citrus wines:  $\text{g/L citric} = \text{mL NaOH} \times \underline{N} \text{ NaOH} \times 0.064 \times 200$

**Note:** The above calculations are only valid when a 5.0 mL wine sample is used.

## DETERMINATION OF VOLATILE ACIDITY

### **Equipment:**

10 mL burette (marked in 0.1 mL)  
10 mL pipettes  
Low vacuum source or water aspirator  
A source of condenser water  
Cash still  
(pH meter with combination reference/pH electrode – optional)  
Magnetic stirring table and stir bar  
250 mL beaker

### **Reagents:**

1% Phenolphthalein indicator  
0.1N NaOH

### **Procedure:**

1. Fill the outer chamber of the Cash still about half full of water; make sure that the condenser water supply is on.
2. Turn the stopcock then to the inner chamber filling position. Pipette 10 mL freshly prepared sample into the inner chamber (most wines will require a drop of antifoam) and rinse the sample funnel with about 5 mL distilled water into the inner chamber.
3. Turn the stopcock to the vent position, turn on the heater and allow the outer chamber water to boil.
4. When steam is venting freely, turn the stopcock to the closed position and distill about 100 mL into a beaker.
5. Remove the distillate container; turn the heater off; turn the stopcock to the inner chamber filling position; empty the still by opening the drain tube. While the still is draining, quickly add two 15 mL portions of distilled water through the sample funnel; the draining action will pull these rinses out and clean out the inner chamber for the next sample.



### **Titration of Volatile Acid:**

1. Sample preparation (volatile acid): To the distillate from the volatile acid distillation, add 3 drops phenolphthalein indicator solution.
2. Titrate the sample to a phenolphthalein end point of faint pink with 0.1 N NaOH; record the volume of base used.

Alternatively, a pH meter can be used to titrate each sample to pH 8.2 as described above for total acidity

### **Calculation:**

Formula 1: Volatile acidity (g/100 mL) = mL NaOH x N NaOH x 0.6

**NOTE:** The above calculations are only valid when a 10.0 mL wine sample is used.

Wines with high levels of sulfur dioxide or volatile acidity approaching or exceeding the legal limit should be corrected for sulfur dioxide using one of the following procedures:

- a) Add about 0.5 mL of 0.3% H<sub>2</sub>O<sub>2</sub> solution after the sample addition. The hydrogen peroxide solution oxidizes sulfur dioxide and sulfites to nonvolatile sulfate ions, **or**
- b) Immediately after titration is complete, cool distillate by holding flask under cold water, add 2-3 mL of starch solution and 1 mL of 1+3 sulfuric acid solution and one crystal of potassium iodide. Rapidly titrate with a 0.002 N iodine solution to a faint blue end point. The quantity of iodine used corresponds to the free sulfurous acid present in the distillate. Record the amount of titrant used to determine free sulfurous acid. For bound sulfurous acid, add 10 mL of 1 N NaOH and boiling beads to the same flask and boil the solution for about 3 minutes. Stopper and cool. Add 2 or 3 mL of starch and acidify with 5 mL of 1+3 sulfuric acid solution and titrate with standardized iodine solution to faint blue end point. The quantity of iodine used corresponds to the amount of bound sulfurous acid. Record the amount of titrant used to determine bound sulfurous acid.

**To calculate the total sulfurous acid as acetic acid, use the following:**

Total the quantities of iodine titrant used in both of the above titrations (free and bound sulfurous acid) in paragraph b) above and calculate the total sulfurous acid, expressed as g/L of acetic acid, using the following formula:

Formula 2: SO<sub>2</sub> correction = g/100mL acetic acid = mL of 1<sub>2</sub> x N of 1<sub>2</sub> x 6

**NOTE:** The above calculations are only valid when a 10.0 mL wine sample is used.

The quantity of sulfurous acid, calculated as acetic acid, (Formula 2) is then subtracted from the quantity of acetic acid as determined after steam distillation (Formula 1, above).

The results of the analysis should be noted as “volatile acid exclusive of sulfur dioxide.”

## FIXED ACIDITY

Fixed acidity is simply the difference between the total (titratable) acidity and the volatile acidity. As total acidity is expressed in g/L of the principal acid of the fruit from which the wine was made, (e.g., tartaric acid in the case of grape wine) and volatile acid is expressed in g/100mL as acetic acid, the following calculations are necessary to determine the fixed acidity of the wine:

### Grape wines:

Fixed acidity (as tartaric) =

Total acid g/L as tartaric – (volatile acid g/100mL as acetic acid x 10 x 1.25)

### Apple wines:

Fixed acidity (as malic) =

Total acid g/L as malic – (volatile acid g/100mL as acetic acid x 10 x 1.12)

### Citrus wines:

Fixed acidity (as citric) =

Total acid g/L as citric – (volatile acid g/100mL as acetic acid x 10 x 1.17)

Total and fixed acidities are reported in g/L with one decimal place, i.e., X.X g/L, while volatile acid is reported in g/100mL with two decimal places, i.e., 0.XX g/100mL.

### References:

1. Ough, C.S. and Amerine, M.A., Methods for Analysis of Musts and Wines, J. Wiley and Son, New York (1988).
2. Official Methods of Analysis of the A.O.A.C. International, Arlington, VA.
3. Zoecklin, B.W., Fugelsang, K.C., Gump, B.H., and Nury, F.S., Production Wine Analysis, Van Nostrand Reinhold, New York, (1990).

## OTHER INFORMATION

### COMMON CONVERSION FACTORS

#### Mass

1,000 kilograms = 1.10 tons

1 kilogram = 1,000 grams = 2.21 pounds

1 pound = 0.454 kilograms = 454 grams

#### Volume

1 gallon = 3.785 liters = 0.134 cubic feet = 231 cubic inches

1 liter = 0.26417 gallons

1 hectoliter = 100 liters = 26.4 gallons

1 cubic foot = 7.48 gallons = 28.3 liters

1 cubic meter = 10 hectoliters = 35.3 cubic feet = 1,000 liters

#### Concentration

1 part per million =

1 milligram per liter

1 gram/1,000 liters

0.00833 pound/1,000 gallons

1 milligram/kilogram

1,000 parts per million =

0.1 percent

1,000 milligram/liter

1.0 gram/liter

1.0 part per thousand

1 pound per 1,000 gallons =

120 parts per million

120 grams/1,000 liters

0.12 gram/liter

120 mg/liter

0.012 gram/100 mL

0.012 percent

## COMMONLY USED WINE TREATMENT MATERIALS AND CORRESPONDING THEORETICAL LEVEL INCREASES

**Potassium Sorbate** (Regulatory limit = 300 ppm as sorbic acid; 27 CFR 24.246)

$$\frac{\text{Grams of Potassium Sorbate}}{(.005092 \times \text{gals wine})} = \text{sorbate level (ppm)}$$

Example:

22.43 lbs. of potassium sorbate added to 10,000 gals of wine

$$(454 \text{ grams per lb.}) \times 22.43 \text{ lbs.} = 10,183 \text{ grams}$$

$$\frac{10,183}{(.005092 \times 10,000 \text{ gals})}$$

$$\frac{10,183}{50.92} = 199.7 \text{ ppm (sorbate level)}$$

### **Sulfite Calculations for Potassium Metabisulfite (KMBS) Additions**

#### A. Weight of Fruit

$$\frac{\text{pounds of fruit}}{2.2 \text{ lbs. per kilogram}} \times \frac{\text{## ppm SO}_2}{1,000} \times 1.74 = \text{grams KMBS to add}$$

↓  
 Convert pounds to kilograms

↓  
 Factor: KMBS is 57.5% SO<sub>2</sub>  
 (1/0.575 = 1.74)

↓  
 Desired addition level of SO<sub>2</sub> in mg/L (ppm). Must convert mg to grams since equation determines the number of grams of KMBS to add.

Example:

How many grams of KMBS are needed to add 50 ppm SO<sub>2</sub> to 750 pounds of grapes?

$$\frac{750 \text{ pounds}}{2.2} \times \frac{50}{1,000} \times 1.74 = 30 \text{ grams KMBS}$$

## B. Volume of Wine or Juice

$$3.786 \times \text{gallons} \times \frac{\text{increase in SO}_2 \text{ (mg/L)}}{1,000} \times 1.74 = \text{grams of KMBS}$$

Factor: KMBS 57.5% SO<sub>2</sub>

Desired increase in SO<sub>2</sub> in mg/L (ppm). Must convert mg to grams (divide by 1,000) since equation determines the number of grams of KMBS to add.

Convert gallons to liters

Example:

How many grams of KMBS are required to increase the SO<sub>2</sub> level in 400 gallons of wine from 10 ppm to 30 ppm?

$$3.785 \times 400 \text{ gals} \times \frac{(30-10)}{1,000} \times 1.74 = 53 \text{ grams KMBS}$$

### **Sulfur Dioxide** (Regulatory limit = 350 ppm as sulfur dioxide; 27 CFR 4.22(b)(1))

#### 1. Wine

1 lb. potassium metabisulfite / 1,000 gals wine = 69 ppm SO<sub>2</sub>

1 lb. liquid sulfur dioxide / 1,000 gals wine = 120 ppm SO<sub>2</sub>

#### 2. Tons of grapes

1 oz of potassium metabisulfite / ton = 18 ppm SO<sub>2</sub>

1 oz of tank (liquid) sulfur dioxide / ton = 30 ppm SO<sub>2</sub>

### **Acidulants** (27 CFR 24.182)

1 lb citric acid / 1,000 gals wine = TA increase of 0.13 g/L

1 lb malic acid / 1,000 gals wine = TA increase of 0.125 g/L

1 lb tartaric acid / 1,000 gals wine = TA increase of 0.11 g/L

**Laboratories Certified for the Analysis of Wine for the EU**

[http://www.ttb.gov/ssd/pdf/wine\\_certlist.pdf](http://www.ttb.gov/ssd/pdf/wine_certlist.pdf)

**Laboratories Certified for the Analysis of Distilled Spirits for the EU**

[http://www.ttb.gov/ssd/pdf/ds\\_certlist.pdf](http://www.ttb.gov/ssd/pdf/ds_certlist.pdf)

Alcohol Correction Table: Bureau of Internal Revenue, Bulletin 7 (1937)

% Read	At 57° F	At 58° F	At 59° F	At 61° F	At 62° F	At 63° F	At 64° F	At 65° F	At 66° F	At 67° F	At 68° F	At 69° F	At 70° F	At 72° F	At 74° F	At 76° F	At 78° F	At 80° F
1	0.14	0.10	0.05	-0.05	-0.10	-0.16	-0.22	-0.28	-0.34	-0.41	-0.48	-0.55	-0.62	-0.77	-0.93	-----	-----	-----
2	0.14	0.10	0.05	-0.05	-0.11	-0.17	-0.23	-0.29	-0.35	-0.42	-0.48	-0.56	-0.63	-0.78	-0.94	-1.10	-1.28	-1.46
3	0.14	0.10	0.05	-0.06	-0.12	-0.18	-0.24	-0.30	-0.36	-0.43	-0.50	-0.57	-0.64	-0.80	-0.96	-1.13	-1.31	-1.50
4	0.14	0.10	0.05	-0.06	-0.12	-0.19	-0.25	-0.32	-0.38	-0.45	-0.52	-0.59	-0.67	-0.83	-1.00	-1.17	-1.35	-1.54
5	0.15	0.10	0.05	-0.07	-0.13	-0.20	-0.26	-0.33	-0.40	-0.47	-0.54	-0.62	-0.70	-0.86	-1.03	-1.21	-1.40	-1.60
6	0.17	0.11	0.06	-0.07	-0.14	-0.20	-0.27	-0.34	-0.42	-0.50	-0.57	-0.66	-0.74	-0.90	-1.09	-1.27	-1.46	-1.66
7	0.18	0.12	0.06	-0.07	-0.14	-0.21	-0.29	-0.36	-0.44	-0.52	-0.60	-0.68	-0.77	-0.94	-1.13	-1.32	-1.52	-1.73
8	0.19	0.13	0.06	-0.08	-0.16	-0.23	-0.31	-0.39	-0.47	-0.55	-0.64	-0.73	-0.81	-0.99	-1.18	-1.38	-1.59	-1.80
9	0.21	0.14	0.07	-0.08	-0.16	-0.24	-0.32	-0.41	-0.50	-0.58	-0.67	-0.76	-0.86	-1.04	-1.25	-1.46	-1.67	-1.89
10	0.23	0.16	0.08	-0.08	-0.17	-0.25	-0.34	-0.43	-0.52	-0.61	-0.71	-0.80	-0.90	-1.10	-1.32	-1.54	-1.76	-1.99
11	0.25	0.16	0.08	-0.09	-0.18	-0.27	-0.37	-0.46	-0.56	-0.65	-0.75	-0.85	-0.96	-1.16	-1.39	-1.61	-1.84	-2.09
12	0.27	0.18	0.09	-0.10	-0.20	-0.29	-0.39	-0.49	-0.59	-0.70	-0.80	-0.91	-1.02	-1.23	-1.46	-1.70	-1.94	-2.20
13	0.29	0.19	0.10	-0.10	-0.21	-0.31	-0.42	-0.52	-0.63	-0.74	-0.85	-0.97	-1.08	-1.31	-1.55	-1.80	-2.05	-2.31
14	0.32	0.21	0.11	-0.11	-0.22	-0.32	-0.44	-0.55	-0.66	-0.78	-0.91	-1.02	-1.14	-1.39	-1.65	-1.91	-2.17	-2.44
15	0.35	0.23	0.12	-0.12	-0.24	-0.35	-0.48	-0.60	-0.71	-0.84	-0.97	-1.10	-1.23	-1.50	-1.76	-2.03	-2.30	-2.58
16	0.37	0.24	0.12	-0.13	-0.26	-0.38	-0.52	-0.65	-0.77	-0.90	-1.03	-1.17	-1.31	-1.60	-1.88	-2.16	-2.44	-2.72
17	0.40	0.26	0.13	-0.14	-0.27	-0.41	-0.54	-0.68	-0.82	-0.96	-1.10	-1.25	-1.40	-1.70	-1.99	-2.28	-2.58	-2.87
18	0.44	0.29	0.14	-0.14	-0.29	-0.44	-0.58	-0.73	-0.88	-1.03	-1.18	-1.33	-1.49	-1.80	-2.10	-2.41	-2.72	-3.02
19	0.47	0.32	0.16	-0.15	-0.30	-0.46	-0.62	-0.78	-0.94	-1.10	-1.26	-1.42	-1.58	-1.90	-2.22	-2.54	-2.86	-3.17
20	0.51	0.34	0.17	-0.16	-0.32	-0.49	-0.66	-0.82	-0.98	-1.15	-1.33	-1.48	-1.65	-2.00	-2.32	-2.65	-2.98	-3.33
21	0.53	0.35	0.18	-0.17	-0.34	-0.51	-0.68	-0.85	-1.02	-1.20	-1.38	-1.54	-1.72	-2.06	-2.41	-2.76	-3.10	-3.45
22	0.56	0.38	0.19	-0.17	-0.36	-0.53	-0.71	-0.90	-1.07	-1.25	-1.44	-1.61	-1.78	-2.13	-2.48	-2.84	-3.20	-3.56
23	0.58	0.40	0.20	-0.18	-0.37	-0.55	-0.74	-0.92	-1.11	-1.30	-1.49	-1.66	-1.84	-2.20	-2.56	-2.93	-3.30	-3.67
24	0.60	0.40	0.20	-0.18	-0.38	-0.56	-0.77	-0.96	-1.16	-1.35	-1.54	-1.72	-1.91	-2.27	-2.65	-3.03	-3.40	-3.78