Making Reference Materials in Water for Common Winery Lab Procedures

By Pat Howe

To make reference materials, your lab will need to have access to some basic equipment and supplies. These include an analytical balance (which has been recently calibrated), preferably one that is capable of measuring 0.001 grams or better. You will also need volumetric glassware of the highest quality (pipettes and flasks), and a source of distilled and/or deionized water. For very small volumetric measurements, micropipettes are also extremely useful, but also must be calibrated and in good working order. And finally, a calibrated thermometer must be used when making volumetric measurements.

The production of any solution of known concentration requires the use of good analytical chemistry practices. Good techniques must be used when drying, weighing, pipetting, and even mixing. If you are a little rusty on your analytical techniques, and don't have the interest in plowing through an introduction to quantitative chemistry text, try the chapter on "Analytical Techniques" in *Techniques for Chemical Analysis and Quality Monitoring*

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During Winemaking by Iland *et al.* In addition, you should read the instruction manuals that accompany your balance and pipettors.

And, of course, use a pipette bulb. Never mouth pipette!

Many of these standards are not stable for long periods of time, and are best prepared fresh when you are validating or checking your procedures. Many of these water solutions are microbiologically unstable, and spoilage could result in concentrations lower than expected. Although some of these preparations can be made ahead and frozen, many are also affected by low temperatures. None of these are difficult to make, and my advice is to make them fresh when needed.

In addition, nearly all of the solid chemicals used to make standards are hydroscopic, and absorb moisture from the atmosphere. This obviously would affect the weight of the compound, and so most compounds should be dried to a consistent weight before being used to make your standard solutions. Check the compound's MSDS (Material Safety Data Sheets) or the bottle for details on drying procedures, which usually require using an oven and a dessicator. Most chemicals are best dried at about 200° C for a couple of hours, but some, like gallic acid, are actually unstable at this temperature. What would happen if you didn't dry out the absorbed water first? You would make a standard that was proportionally lower in concentration than expected.

If you are not sure about whether your equipment is adequate for preparing your own reference materials, perform a "worst case" test (mathematically) to see what the effect of the limitation would be on your results. For example, in making the Brix standard detailed below, what if your balance only goes to 0.1 grams? If you are off by 0.1 grams your resulting error in your standard should not be more than 0.3%. That is, if you are 0.1 low on your sucrose measurement and 0.1 high on your final measurement, your 30 Brix solution would be 29.9 Brix. If this is acceptable, then go ahead and make your standards.

Brix Standard Solutions in Water

We have discussed the production of Brix standards many times, but here it is once more. The Brix scale is a weight to weight scale, and has nothing to do with volume at all. In other words, to make a standard Brix solution, all you need is a balance. If you find yourself using a volumetric flask, you are probably not making a Brix standard.

Use distilled water and a pure source of sucrose. (See Table 1.)

Table 1

Desired Brix Standard	Grams of Sucrose	Grams of Water
0	0.0	100.0
5	5.0	95.0
10	10.0	90.0
15	15.0	85.0
20	20.0	80.0
25	25.0	75.0
30	30.0	70.0

Ethanol Standard Solutions in Water

The standard solutions for alcohol are completely opposite from the Brix scale. Alcohol is expressed in a volume to volume measurement, and has nothing to do with weight. In other words, to make a standard alcohol solution, you need volumetric glassware. Note that good analytical technique requires paying attention to the temperature of your solutions when dealing with volumetric glassware.

Use distilled water and high purity ethanol. Adjust from the tables below if your percentage ethanol is different. (See Tables 2a and 2b.)

Potassium Acid Tartrate Buffer Standard for pH Measurement

I hope you are all using calibration standards which are certified, available from most chemical supply houses or from your local winemaking supply center. But, in addition to calibrating your equipment, there is another check for your pH meter which uses a reference material which you can make yourself.

Because this is a saturated buffer solution, and not a precise concentration, you do not have to be too worried about your weights or your volumes. To make this buffer, use Potassium Acid Tartrate (KHT), which is also known as potassium hydrogen tartrate, potassium bitartrate, or monopotassium salt of tartaric acid, in case you have trouble finding it in your chemical catalog, and distilled water.

Add approximately 4 grams KHT to about 100 mls of distilled water. Warm and stir the solution for about five minutes (there should be undissolved KHT at the end of the mixing period). Let the solution cool to room temperature, then decant (or filter) off the solids. The pH of this buffer is 3.56 +/ .02 at 25° C.

Standards for SO_2 Measurement in Water (Contains Only Free SO_2)

(This standard contains only unbound SO_2, and can be used to test both Free and Total SO_2 procedures—both tests should give the same results).

Because SO_2 measurement seems to be troublesome, this is a standard which you should make and use on a regular basis, even after validating your procedure. I recommend you make a stock SO_2 solution in water of 1 g SO_2/ L, and dilute this stock to make your standards. You have many options for making this stock:

Sodium Metabisulfite ($Na_2S_2O_5$) with a molecular weight of 190.1 grams per mole, is 0.6744 % SO_2. To make a 1 gram of SO_2 per Liter solution, add 1.4828 grams of Sodium Metabisulfite to a 1 L volumetric flask and bring to volume with distilled water.

Potassium Metabisulfite ($K_2S_2O_5$) with a molecular weight of 222.3 grams per mole, is 0.5767 % SO_2. To make a 1 gram of SO_2 per Liter solution, add 1.7341 grams of Potassium Metabisulfite to a 1 L volumetric flask and bring to volume with distilled water.

Liquid SO_2 solutions: many wineries use a liquid SO_2 solution ranging from 4 to 8 % SO_2. Use the formula below to calculate how many milliliters of solution is needed to make your 1 g SO_2/ L

Table 2a		
Desired Ethanol Standard (%v/v)	mls of 100 % (200 Proof) Ethanol	Final Sample Volume (mls)
0	0.0	100.0
5	5.0	100.0
10	10.0	100.0
15	15.0	100.0
20	20.0	100.0
25	25.0	100.0

Table 2b		
Desired Ethanol Standard- (%v/v)	mls of 95 % (190 Proof) Ethanol	Final Sample Volume (mls)
0	0.0	100.0
5	5.3	100.0
10	10.5	100.0
15	15.8	100.0
20	21.1	100.0
25	26.3	100.0

Table 3				
Desired ppm SO_2	mls of 1 g SO_2/L Stock	Final Sample Volume (mls)	Expected "Free" SO_2 ppm	Expected "Bound" SO_2 ppm
0.0	0.0	100.0	0	0
10.0	1.0	100.0	10	10
25.0	2.5	100.0	25	25
50.0	5.0	100.0	50	50
100.0	10.0	100.0	100	100
150.0	15.0	100.0	150	150
250.0	25.0	100.0	250	250

stock. I don't recommend this method unless you can accurately measure the concentration of your SO_2 solution down to at least 1%; it's probably better to use the metabisulfite solutions otherwise.

$$\frac{(1\text{g } SO_2\text{/L} * 1\text{ L}) \times 100}{(\%\ SO_2 \text{ in solution})} = \text{mls } SO_2 \text{ solution}$$

(add to volumetric and top with DI water)

Once you have your stock solution, it is a good idea to do a complete standard curve, from 0 SO_2 up to at least the highest level of SO_2 you would expect to find. For example, the legal limit for US wines is 250 mg/L; that might be a logical upper limit.

Use your stock solution and distilled water. (See Table 3.)

Standards for SO_2 Measurement in Water (Contains Only Bound SO_2)

(This standard contains only bound SO_2, and can be used to test both Free and Total SO_2 procedures–the Free SO_2 procedure should yield no SO_2, and the Bound SO_2 should be appropriate to the standards).

To bind the SO_2, we will use an acetaldehyde solution. Although we theoretically need 0.684 grams of acetaldehyde to bind 1 gram of SO_2, we want to make sure we have bound all the SO_2, so we will use excess acetaldehyde.

First, make a stock solution of approximately 1 g/L stock solution of acetaldehyde using high purity acetaldehyde and distilled water. Notice that this solution is made by weighing the liquid acetaldehyde; it is easier to weigh it (even though it is a liquid), and then bring it to volume in a volumetric flask. Acetaldehyde is difficult to pipette unless you have positive displacement pipettes. It is also easier to work with when it is cold, and note that it has special storage requirements. (See Table 4.)

Table 4		
Desired Acetaldehyde (1 g/L) Stock Solution	Grams of 100 % Acetaldehyde	Total mLs Sample Volume
Approx 1	Approx 1	1000.0

From this 1 g acetaldehyde/L stock solution and your 1 g SO_2/L stock solution, make your reference standards by pipetting accurate volumes of your SO_2 and your acetaldehyde stock solution and bringing to volume in a volumetric flask. We are using approximately three times the volume of acetaldehyde needed to theoretically bind the SO_2, so there may be a residual aroma of acetaldehyde in these solutions. (See Table 5.)

Table 5					
Desired ppm Free SO_2	mls of 1 g SO_2/L Stock	mls of 1 g Acetaldehyde/L stock	Final Sample Volume (mls)	Expected "Free" SO_2 ppm	Expected "Bound" SO_2 ppm
0.0	0.0	0	100.0	0	0
10.0	1.0	2	100.0	0	10
25.0	2.5	5	100.0	0	25
50.0	5.0	10	100.0	0	50
100.0	10.0	20	100.0	0	100
150.0	15.0	30	100.0	0	150
250.0	25.0	50	100.0	0	250

Standards for VA or Acetic Acid Measurement in Water

Because VA measurement also seems to be troublesome, these are also standards which you might want to run as part of your quality control program even after you have validated your procedure.

Acetic Acid (glacial) can be purchased in purity of greater than 99.8%. As with the SO_2 references, it is a good idea to do a standard curve and to test the upper limits of your procedure. California legal limit for volatile acidity is 1.1 grams acetic per liter for white wines and 1.2 grams acetic per liter for red wines, so go at least that high, preferably somewhat higher.

Make a stock solution of 10 g acetic acid/L first, using high purity glacial acetic acid and distilled water. Notice that this solution is made by weighing the liquid glacial acetic acid; it is easier to weigh the acid (even though it is a liquid), and then bring it to volume in a volumetric flask.

Use high purity glacial acetic acid and distilled water. (See Table 6.)

From this stock 10 gram acetic acid/

Table 6		
Desired Acetic Acid (g/L) Stock Solution	Grams of 100 % Acetic Acid	Total mls Sample Volume
10.0	10.0	1000.0

Liter solution, make your reference standards by pipetting accurate volumes of your stock solution and bringing to volume in a volumetric flask. (See Table 7.)

Standards for Titratable Acidity, Residual Sugar, and Malic and Lactic Acids in Water

These standards are straightforward standards made by accurately weighing out the analyte of interest diluting volumetrically to the appropriate concentrations.

TA

In the United States, we express Titratable Acidity as tartaric acid, so it is easiest to use tartaric acid for your reference standards. You could, of course, use any other organic acid, but you would have to convert grams per equivalent of the acids back into tartaric acid. Make sure you are dealing in the correct units, also (TA is sometimes expressed in grams per Liter and sometimes in grams per 100 mL (or %); this means that the power of 10 error is very easily manifested.

Table 7

Desired Acetic Acid (g/L)	mls of 10g/L Acetic Acid Stock	Total mls Sample Volume
0.00	0.0	100.0
0.20	2.0	100.0
0.40	4.0	100.0
0.80	8.0	100.0
1.00	10.0	100.0
1.20	12.0	100.0
1.60	16.0	100.0

In fact, sometimes it is just easier to memorize this: 1% = 10 g/L. Got it?

Residual Sugar

This is somewhat tricky because you need to select the correct sugars: are you testing for sucrose, glucose, fructose, or some other sugars? Look carefully at your procedure to decide what you are actually testing before making the standards. For example, are you measuring reducing sugars or fermentable sugars? Sucrose is not a reducing sugar, but it is certainly fermentable; and many pentoses from oak barrels are reducing sugars but are not fermentable. Again, as with the Titratable Acidity, beware of the power of 10 error when deciding what units to use.

Malic and Lactic Acids

Making malic and lactic standards is also dependent on what you are actually measuring. For example, some enzymatic tests are specific for either the D- or L- forms of the acid, and so you must use the correct form of the acid.

Why Make and Use Standards?

The purpose of this article is to give you a starting point for making your own reference standards. These standards are needed to help you validate your analytical procedures and give you confidence in your results. Using references and known standard solutions allows you to establish your analytical performance criteria. Is your method linear? Is it accurate?

As usual, I would like to stress that there is not just one right way to make standards. I have suggested a starting point for you, but depending on your individual circumstances there may be better ways for you to achieve the same goals.

References

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