

Paper Chromatography

by John Tummon – December 2012

Paper chromatography is generally used as a qualitative measurement of the three most common acids found in wine; tartaric, malic and lactic acids. It provides an economical and relatively easy way to monitor the progression of Malo-lactic fermentation (MLF).

Chromatography paper is spotted with wine samples and when placed in solvent the various acids move up the paper by capillary action; however each of the acids has a different affinity for the paper and the solvent, so they move up a characteristic distance. An indicator in the solvent reacts with the acids to create a yellow spot which appears on the paper after it is removed from the solvent and allowed to dry.

From start to finish, paper chromatography takes about 24 hr. I start in the morning. It takes about an hour to spot the wine on the paper and dry, four or five hours for the paper to absorb the solvent and then I leave it overnight to dry and develop.

- Using a pencil, draw a line 2cm from the bottom of the long edge of the chromatography paper. Mark cross lines at 3cm intervals along this line. This should accommodate 7 samples with most papers. Write the name of the respective samples or wines under these cross lines.
- Most chromatography kits come with 3 acid standards, tartaric, malic and lactic. You may wish to spot these on each chromatography paper for comparison, but tartaric appears near the bottom, lactic near the top with malic in the middle.
- This is where I do things a little different. Put the chromatography paper in the first few pages of your favorite novel with the line and the cross lines sticking out. Place a piece of corrugated cardboard, about 25cm wide, in the last few pages of the book under the paper. The cardboard has little corrugations or holes that will hold your capillary tubes under their respective sample sites or cross lines. (Figure 1)
- Dip the end of each capillary tube in the wine sample. It should fill about 1/3 of the tube. Touch the end of the tube to the respective sample cross line keeping the spot as small as possible (pea size). Spotting should only empty about 1/4 to 1/5 of the wine in the capillary tube. Stick the tube in the cardboard corrugation under the sample and proceed to the next sample.
- Wait for each of the spots to dry, about 5 to 10 minutes. Remove each tube and repeat, spotting each of the samples on their respective cross lines, returning them to the cardboard holder to dry. You will repeat this about 4 or 5 times.
- When the tubes are finally emptied, discard them and allow the paper to thoroughly dry, about 1/2 hour.
- Pour about .5 cm (5mm) of solvent into a chromatography jar or any jar with a lid that will accommodate the paper.

- Curl the chromatography paper into a cylinder and staple the top, bottom and middle to retain this shape. Do not overlap the edges.
- Lower the cylinder into the jar with the sample end immersed in the solvent and the sides not touching the jar. Put the lid on and leave for 3 to 5 hours or until the solvent reaches the upper areas of the cylinder.
- Remove the paper and allow it to dry in a well ventilated area for at least 6 to 8 hours, but it will continue to develop for the next 24 hours.
- Return the solvent to its bottle and wash out the jar in a well ventilated area.

How to Read the Spots (Figure 2)

Once the paper is dry, the staples can be removed. It should have developed yellow spots on a blue-green background. The spots are easier to read if backlit or held up to a light source. The position of each spot corresponds to the position of a specific acid. There are 7 samples in Figure 2 below. To monitor MLF, you should eventually see the complete absence of the spot corresponding to malic and a more intense spot corresponding to lactic acid as in sample #5. A wine that is not going through MLF should show all 3 spots fairly intensely as in sample #6. Samples #1, #2, and #7 are going through MLF and are nearing completion. Samples #3 and #4 are just beginning MLF.

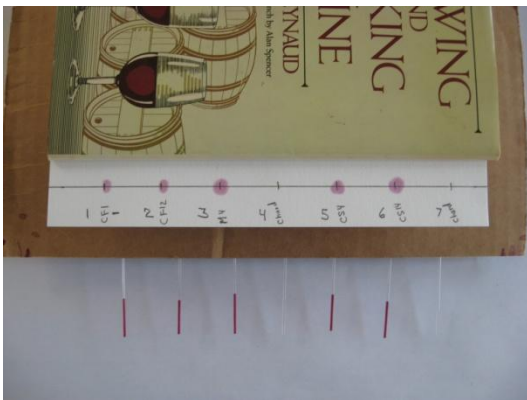


Figure 1

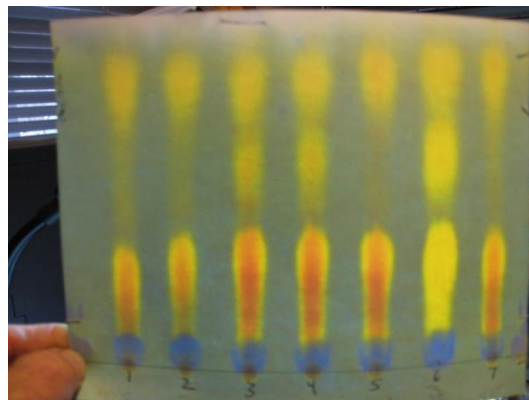


Figure 2

Notes:

- When stapling the tube do not overlap the edges. If possible leave a small space.
- Keep the paper and your hands clean. It doesn't take much to mess up the test.
- Use a pencil never a pen.
- White wine spots disappear when dry so I try to have at least one white wine sample per test paper so I know when the spots have dried..
- Chromatography jars can be purchased, but they can be expensive. However, you can probably find one full of artichoke hearts or sundried tomatoes for less money. You simply

need a jar with a fairly flat bottom and wide and high enough to accommodate the paper cylinder.

- The chromatography solvent can be used many times, however eventually water drops out. You can pour off the solvent and discard the water, but the quality of the solvent will eventually deteriorate. A yellow background or poor separation and development would indicate that it's time to replace it. Typically solvent lasts about one year or less depending on the usage.
- If properly stored, chromatography paper should last several years.
- It's important to keep the spots as small as possible. Like dotting an "i".
- The paper needs to dry in an area of low humidity.
- It may be possible to use ammonia vapor to hasten development, but I find patience works better.
- Over time the spots can fade so you may try scanning or photographing the paper with backlight and no flash.