

MALOLACTIC CHROMATOGRAPHY

KIT INCLUDES:

- Wide Mouth Jar & Lid
- 2 Plastic Paper Clips
- 100 Capillary Pipettes
- 25 Chromatography Papers
- Chromatography Solvent
- Malic Acid Standard 0.3%
- Tartaric Acid Standard 0.3%
- Lactic Acid Standard 0.3%



OBJECTIVE:

Designed to detect malic, lactic, and tartaric acids in grape juice, must, or wine.

THE SCIENCE:

The principle behind malolactic chromatography involves a solvent being carried up the media (paper) by capillary action. The acids being tested have an affinity for both the solvent and the paper, so they are carried up the paper for a particular distance, which is unique for each acid. The more the acid is attracted to the solvent, the farther up the paper it will move before being deposited.

THE OVERVIEW:

The test consists of spotting standard acid solutions and wine, must or juice samples on chromatography paper, placing the bottom edge of the paper into the solvent solution and waiting until the rising solvent line reaches the top edge. The paper is allowed to dry and, as the formic acid evaporates, yellow acid spots appear on a blue-green background. These spots may be identified by comparing their height on the paper to those of the acid standards.

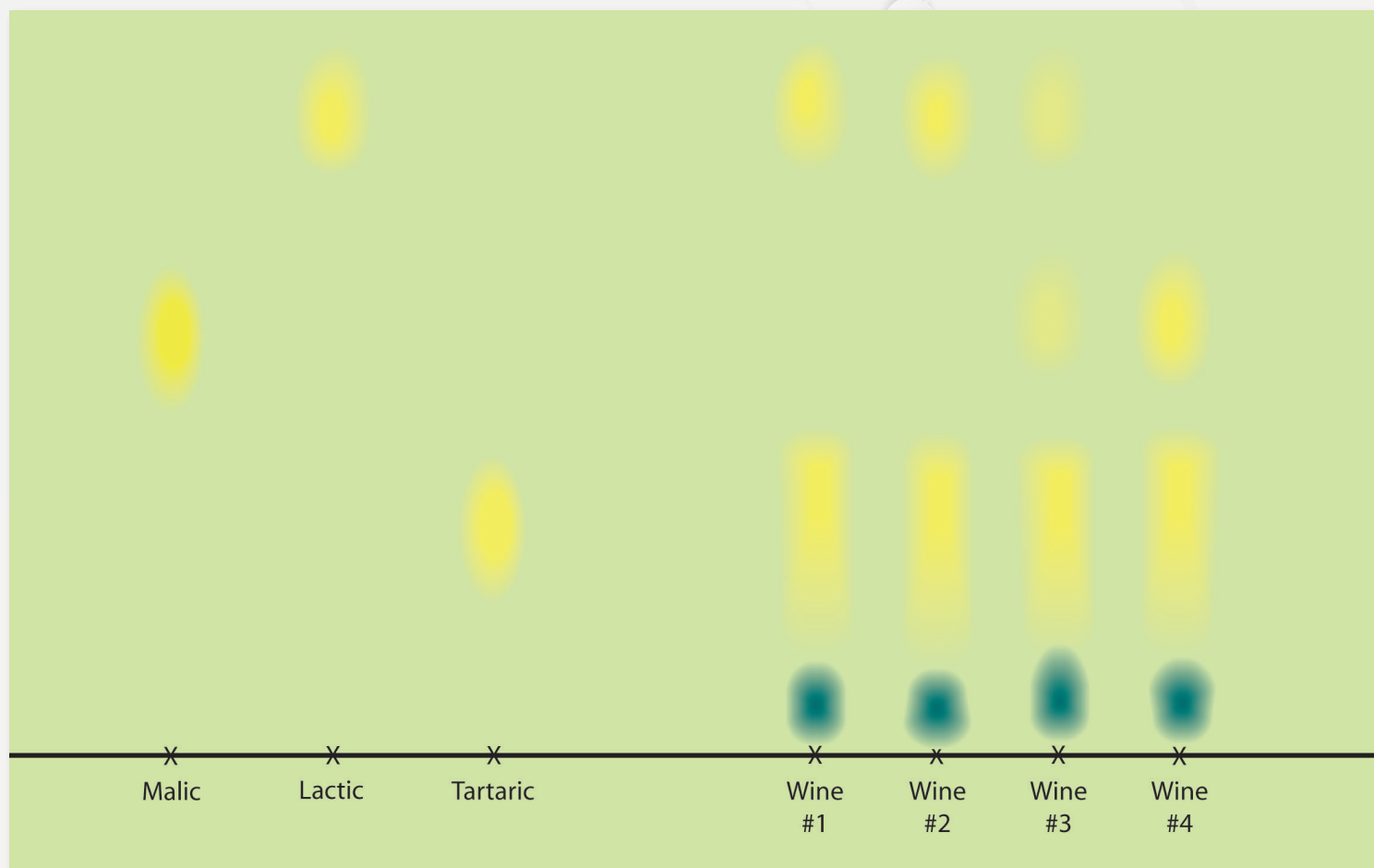
PREPARATION:

1. Using a pencil, draw a parallel line 1 inch from the long edge of the chromatography paper. Make a small “x” on the line for each of the three standard solutions and each wine to be tested. Do not place spots closer together than 1 inch. Keep paper clean. Label each “x” in pencil beneath the spotting line. Do not use a pen, as the ink is soluble in the solvent and will be carried away during the test.
2. Dip the clear end of a glass capillary pipette quickly into the solution and allow liquid to fill up $\frac{1}{4}$ ” to $\frac{1}{2}$ ” in the tube. It is helpful to have a paper towel handy so you can run some liquid out in case you draw up too much. Place the pipette tip carefully at an angle on the proper “x” and allow liquid to flow into the paper. You want to wind up with a spot on the paper about $\frac{1}{4}$ ” diameter. Changing the angle or tapping the top end of the pipette can start a stubborn one flowing. Use a fresh capillary tube for each standard and sample to prevent cross contamination.
3. When all spots are made, allow the chromatogram to dry for $\frac{1}{2}$ to 1 hour.
4. Pour solvent carefully into the chromatography jar until there is enough to cover the bottom $\frac{1}{4}$ to $\frac{1}{2}$ inch of the paper.
5. Staple the short ends of the chromatography paper together forming a cylinder. Do not overlap edges of the paper.
6. Lower the paper cylinder carefully into the jar and tightly seal the lid. The level of the solvent must be below the bottom edge of the spots.
7. Leave the jar sealed and undisturbed until the moving solvent line reaches the upper edge of the chromatogram. This will take 4–6 hours but can be safely extended to overnight even if solvent reaches the upper edge.
8. Using a white plastic clip, remove the cylinder from the jar and hang in a well-ventilated place to dry. It is very important that you not breathe the solvent fumes for more than a few minutes at a time, outside is the best place to hang your chromatogram.

- The solvent may be stored in the jar, but care should be taken to keep this out of reach of children or animals, and it should not be stored where it may be exposed to fire or extreme heat.
- When completely dry the chromatogram will have a blue-green background with yellow spots of acids. Excess humidity will delay or prevent full development of

blue-green background, however acid spots are easily visible. Drying in an air conditioned room or near a dehumidifier will complete color development. The presence of a particular acid can be confirmed by matching a spot above where you placed the wine sample with the height of one of the standard spots.

EXAMPLE CHROMATOGRAM:



READING A CHROMATOGRAM:

The standard acid solutions on the left of the Chromatogram leave a yellow spot that is used as a reference. Compare the yellow spots left by your wine samples with the yellow reference spots left by the standard acid solutions to determine what acids are in your wine. Note that if the yellow spots have blurred together you may need to further separate your samples or use a smaller amount of each sample. Drying the Chromatogram in a warm, low humidity, environment will enhance the colors and make it easier to read.

INTERPRETING WINE SAMPLES:

Use the above Chromatogram to help understand your readings.

- WINE#1 AND WINE#2** have both completed MLF since there is a yellow spot indicating the presence of Lactic Acid but no yellow spot indicating Malic Acid.
- WINE #3** is approximately half way through with MLF. Fainter yellow spots indicate the presence of both Lactic and Malic Acids.
- WINE #4** has not yet entered into MLF as indicated by the presence of a Malic Acid spot and the absence of a Lactic Acid spot.