

## PROCEDURE (TLC) FOR DETERMINING THE PRESENCE OF MALIC ACID IN WINE

1. The TLC Film sheet (20cm x 20cm) is marked with a pencil every 40mm along one edge and every 66 mm along the other edge. Use a ruler and pencil to draw parallel lines to both sides of the TLC sheet. The sheet is now divided into strips of dimensions 40mm x 66mm. The strips are now cut from the sheet using a sharp pair of scissors. (Note : Blunt scissors will tend to chip the silica gel coating on the strips)
2. Draw a faint line in pencil 10 mm from and parallel to the bottom edge of a strip of the TLC sheet of length 40mm  
Four samples can be analysed on a TLC sheet of width 40mm.
3. Place two or three drops of the Malic Acid Standard in a 100ml glass beaker then two or three drops of the Lactic Acid Standard in another 100ml beaker followed by a few drops of the sample in another 100ml glass beaker.
4. Place a capillary tube in each of the three(3) x 100ml glass beakers.  
Firstly, draw - up a small amount (approx 2- 3mm) of the Malic Acid Standard solution in the capillary tube, then tap the capillary tube containing the Std Malic Acid on the inside rim of the beaker in order to reduce the volume of liquid in the capillary tube to 1mm.
5. Place the capillary tube above the pencil line drawn on the TLC sheet and approx 5mm from the edge of the strip.
6. Now touch the end of the capillary tube (containing the liquid) quickly onto the pencil line so that only a small circle of sample remains on the TLC strip.  
NOTE : Only one application is required for both the standard Malic & Lactic acids and the sample.
7. A gentle stream of warm – hot air is now quickly applied to the spot using a hair dryer for approx.1 minute in order to limit or minimise the diameter of the spot.
8. This procedure is now used to spot the Standard Lactic Acid and Wine sample.  
Please Note : A separate capillary tube should be used for spotting each of the standard solutions and wine samples.
9. Now place 5ml of the TLC Solvent in the square chromatography jar using either a 10ml graduated pipette or 10ml graduated, glass measuring cylinder. Use a pair of tweezers to lower the TLC sheet into the jar so that the 40mm edge is resting on the bottom of the jar. The spots should be above the level of the solvent. Screw the lid on the jar.
10. Leave the jar to stand for approx 7 – 8 minutes while the solvent moves up the TLC strip to within 3mm from the top.

11. The TLC strip is now removed from the jar using tweezers and placed in a bull clip in order to secure the strip for drying. The hair dryer is now positioned behind the hand holding the bull clip to which is attached the TLC strip. The solvent is now removed by a stream of warm- hot air from the hair dryer directed at an angle down onto the TLC strip. The warm air from the dryer must be directed onto the TLC strip at an angle in order to remove the solvent via a shearing action that removes the solvent more effectively than applying the warm air at right angles to the TLC strip.
12. The strip is now checked for any smell of acetic acid (ie vinegar).  
If detected, continue air drying the strip. When the strip is free from any undesirable smells, use the tweezers to lower the strip into a 100ml glass beaker containing 100ml of the TLC Indicator solution.
13. Immediately remove the strip and air dry the strip with cold air from the hair dryer.  
The spots on the TLC sheet corresponding to standard Malic acid and the Malic acid present in the standard solution will each appear on the same level. The Malic acid spots will appear on a different level to the Lactic acid spots.

Using this simple procedure, the presence of Malic acid in the wine sample can be detected.

If the wine sample contains NO Malic acid, as indicated by no spot appearing for the wine sample alongside the spot for the Malic acid standard, then the wine has undergone a Malo-Lactic conversion.

In other words, the harsher, less stable malic acid has been converted to the more stable, milder, less acidic lactic acid.

Effectively, the wine has undergone a Deacidification Reaction

This test must be performed prior to bottling, since bottling wines containing malic acid may cause a secondary fermentation to occur in the bottle. Bottle breakage may therefore occur due to a build up of CO<sub>2</sub> pressure or the bottled wine may become cloudy and unsuitable for sale.

