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TEST PROCEDURE

Monitoring Malo-Lactic Fermentation by Thin Layer Chromatography

This method is a guide to using Thin Layer Chromatography (TLC) to monitor the conversion of malic acid to lactic acid in wine. It is particularly useful in telling you whether malo-lactic fermentation has commenced, as indicated by the appearance of lactic acid on the plate, and if it is progressing satisfactorily towards completion. When the spot for malic acid is no longer visible on the plate, conversion may be complete. The method is a qualitative measure only of malic acid concentration, and as such cannot be relied upon to tell you whether malic acid has been totally converted. To confirm completion, more accurate procedures such as enzymatic analysis must be employed.

1. **General Safety Precautions**

The reagents used within this procedure are irritants, and should therefore be handled with care in a well-ventilated area. As a precaution, it is recommended that safety glasses be worn. The solvent mixture is also flammable, and should not be used in the vicinity of a flame.

2. **Reagents and Materials**

The reagents used in this technique are stable and will last in excess of one year if stored in a well sealed container. The TLC plates must be kept dry and clean. We recommend drying plates for 30 minutes at 110 degrees prior to use. Minimise all body contact with the plates by using tweezers or latex gloves.

3. **Apparatus (Vintessential MLF TLC Kit, Part No.4M510)**

3.1 Glass jar with lid, Part No. 9J010

3.2 Tubes, capillary, pack of 100, Part No. 2T310

3.3 Dish, plastic, Part No. 2P150

3.4 Plates, TLC, 2P550

3.5 Tweezers, stainless steel, 125mm, Part No. 2T910

3.6 MLF solvent, 125mL, Part No. 1S725

3.7 MLF indicator, 125mL, Part No. 1I 125

3.8 MLF standard, Lactic acid, 25mL, Part No. 1L025

3.9 MLF standard, Malic acid, 25mL, Part No. 1M025

4. **Spotting the Paper**

4.1 Take a TLC plate (hold top corner only by tweezers), carefully cut it to the required size, dry it with a warm hair dryer and place on a clean and dry surface. As a guide, a 4 x 8 cm plate is sufficient for about 3 determinations and a standard.

Caution! Silica coating is delicate. Handle carefully.

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4.2 Lay plate onto clean paper towelling on bench and with the assistance of a ruler and a soft lead pencil eg, 6B, carefully make a pencil line across the plate at a height of 1 cm from the bottom. Mark cross marks at 0.5 cm intervals along the pencil line, and at least 0.5 cm from the edge. In this way, up to 4 spots can be applied.

4.3 Using pencil mark the names of the standard and samples below each cross mark.

4.4 Use the capillary tube to collect sample. Drain the tube with the assistance of tissue paper so that sample height in the tube is only @ 1 mm. Quickly spot the sample exactly on the cross mark 2 times, drying the spot well between and after each application. Keep spots as small as possible (less than 2 mm diameter). To assist drying, a hairdryer should be used. It is important that the spots are dry otherwise the chromatogram will appear "streaky". The dryness of the spots can be assessed by observing the standard spot. Dryness is complete when this spot becomes invisible (about 1 minute). Use a fresh capillary tube for each sample.

4.5 Fill the glass jar to a depth of 0.5 cm with MLF Solvent mixture.

4.6 Using tweezers, carefully place the TLC plate into the beaker (spotted end down), ensuring that its sides do not touch or rest against the sides of the jar. Do not allow the solvent level to touch the spots.

4.7 Cover the jar, and allow the solvent front to advance to @ 0.5 cm from the top. This will take @ 10 minutes.

4.8 Remove TLC plate from beaker and dry thoroughly in well-ventilated area using a hair dryer on gentle heat. The plate is considered ready when you can no longer smell acetic acid.

4.9 Pour indicator into the plastic dish, and using tweezers, totally submerge TLC plate in one quick motion and then dry with hair dryer.

5. Interpretation of the Chromatogram

5.1 When the treated TLC plate is dry, the TLC plate will develop yellow spots on a green background.

5.2 Observe the position of each spot compared to that of the standard spot. Absence of a malic acid spot in the sample indicates that malo-lactic fermentation may be complete.

5.3 The order of spots from the baseline for a wine with their respective Rf values are as follows;

malic acid	0.43
lactic acid	0.64
succinic acid	0.78

If the spots appear too weak, intensity can be improved by ensuring spots are smaller, or by adding more sample to the TLC plate.

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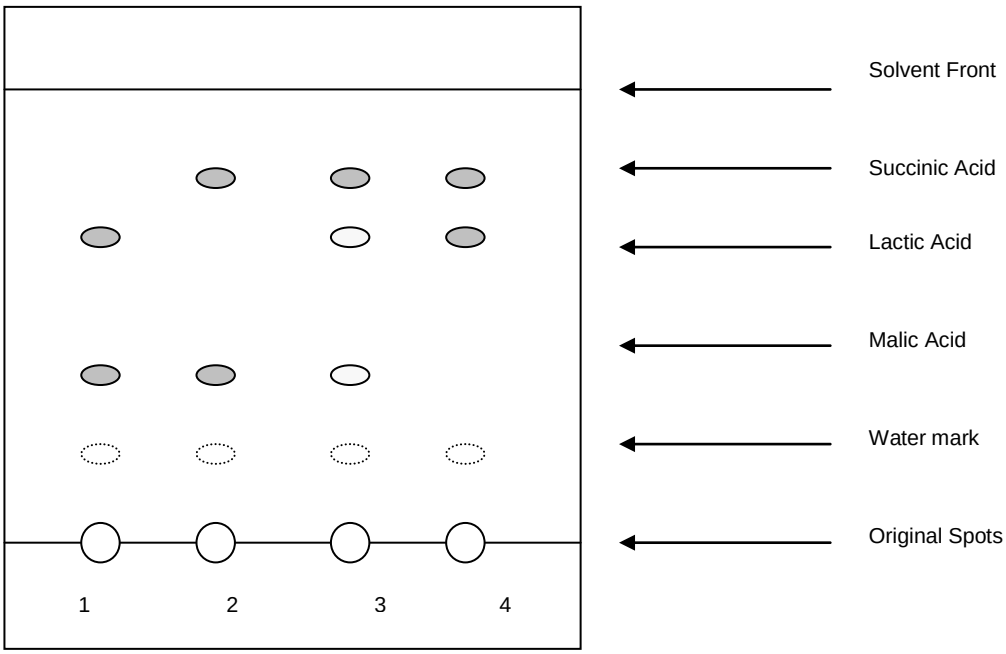
A typical TLC plate is shown in the figure below;



Legend:
Spot 1 Malic and Lactic Acid Standard
Spot 2 Wine sample not yet started MLF
Spot 3 Wine sample undergoing MLF
Spot 4 Wine sample completing MLF

6. Reference

Further information about the above procedure can be found in the following reference:

Harris, R.L.N; "Monitor your MLF's with TLC", The Australian Grapegrower and Winemaker, Technical Issue, 352, pp 13-15, April 1993.