



# Monitoring Malolactic Fermentation 3 Ways

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*The new 3 Ways series is designed to present the most common analysis methods for chemical parameters measured in the winery. Ranging from basic bench-top chemistry to big bucks lab equipment, this series should help you improve the way you run your analyses in-house, understand your options for upgrades, and learn what the service labs do for their fees.*

**Why run it:** To measure the progress of malolactic fermentation

**Basic bench-top:** Paper chromatography

Measures: Presence of malic, lactic, and tartaric acids

Materials:

- Chromatography paper
- Solvent (contains water, n-butanol, formic acid, and bromocresol green)
- Developing jar or tank
- Malic, lactic, and tartaric acid standards
- Micropipettes or hematocrit tubes
- Pencil, ruler, stapler, and clothes pins

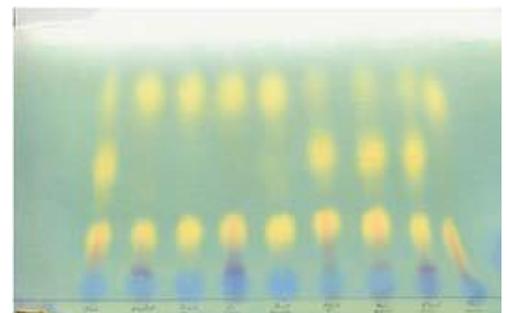
Analysis time: Approximately 6 hours for chromatography, plus 24 hours for drying before analysis

Lower limit of detection: 100 mg/L

Hazards: Chromatography solvent is flammable, and the fumes are somewhat toxic and should not be inhaled. Goggles, gloves, and a fume hood are recommended, and appropriate handling and disposal are necessary.

Cost: \$60-80 (inclusive kit; enough for approximately 150 analyses)

Theory and Practice: While paper chromatography is the cheapest analytical method, it's not very accurate and its lower limit of detection is rather high. Malolactic fermentation isn't considered safely complete until the malic acid concentration is below 30 mg/L. Because chromatography has a lower detection limit of 100 mg/L, it should be considered only a rough qualitative test rather than an accurate quantitative measure (i.e., it can detect if malic acid is present, but it doesn't measure the concentration of malic acid). Paper



*Paper Chromatography.*

*Image courtesy of Anna Katharine Mansfield.*

chromatography is generally more accurate than guessing, though, and coupled with careful observation, it can be a useful tool.

### **Introductory instrumental: Enzymatic Spectrometric Analysis**

Measures: Concentration of malic acid

Materials:

- Spectrophotometer
- Cuvettes
- Micropipettes
- Enzyme test kit (includes buffer solution, NAD, and other necessary reagents)

Analysis time: Approximately 30 minutes for sample prep, analysis, and data analysis

Lower limit of detection: 50 mg/L

Hazards: negligible (no special attire required)

Cost: \$2,000-5,000 for initial spectrophotometer purchase and approximately \$25 per test

Theory and Practice: Put simply, enzymatic analysis uses a series of chemical reactions to convert a compound of interest into one that can be measured via light absorbance on a spectrophotometer. For spectrometric analysis, NAD (nicotine-adenine dinucleotide) reacts with malic acid to produce an equivalent amount of NADH, which can be measured at 340 nm and compared to a standard curve to calculate the concentration of malic acid. This method is much more accurate than paper chromatography and is fairly simple to perform. Because of the flexibility of spectrophotometers for winery analyses, it's a good investment for those that would like to upgrade their in-house lab capabilities.

### **Chemistry geek: High Pressure Liquid Chromatography (HPLC)**

Measures: Concentrations of malic, lactic, tartaric, citric, and acetic acids

Materials:

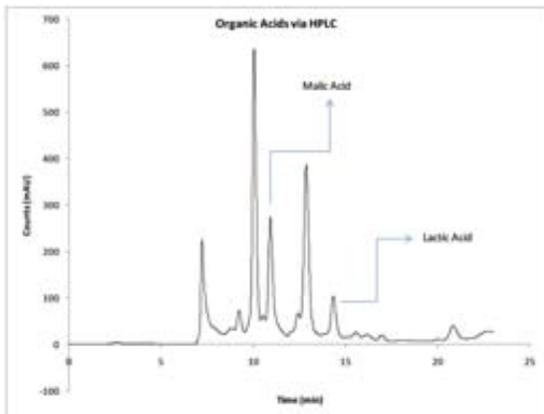
- HPLC, column, and assorted equipment
- Sample vials and caps
- Filtration membranes and syringes
- Acetonitrile, phosphoric acid, and distilled water
- Filtration unit for reagents
- 

Analysis time: 40 minutes, including sample prep, analysis, and data analysis

Lower limit of detection: 1-50 mg/L, depending on the method

Hazards: Acetonitrile is flammable and an irritant, and phosphoric acid is corrosive. Both require appropriate handling and disposal.

Cost: \$50,000-80,000 or more for the initial HPLC purchase and approximately \$30-50 per analysis for expendables and operator time



HPLC Chromatogram.  
Image courtesy of Dr. David C. Manns.

Theory and Practice: While potentially the most accurate method, the cost and knowledge required for HPLC analysis makes it better suited for the research lab than the winery. It is an appropriate tool, however, in cases where a MLF seems stuck or completion is questionable. HPLC is also useful for analysis of malic/tartaric ratios in juice to accurately assess ripeness and inform decisions about winemaking. Also, most HPLC analyses are sensitive to all organic acids in a wine, requiring only additional data analysis to provide a nearly complete acid work-up. Service labs generally base fees on analysis and operator time costs plus instrument depreciation. Subsequently, it's often cheaper to run multiple rather than single samples, as the time and expendables required to start and calibrate the instrument each time are the same, regardless of the number of samples analyzed.

#### **But what about...Accuvin malic test strips?**

Accuvin test strips work on the same premise as the enzymatic analyses, but with an additional step: the NADH participates in a diaphorase catalyzed reaction to produce a purple tetrazolium color indicator. The sample color is then compared to a color key to determine malic acid concentration. This method presents greater opportunities for human error, as assessment relies on the operator's ability to accurately compare colors, and the purple dye can oxidize and change color over time, so prompt reading is essential. In addition, red wine must be properly decolorized prior to analysis to produce accurate results. While the accuracy of this method has not been independently assessed, anecdotal evidence suggests that this method is usually more accurate than paper chromatography and is a good way to keep track of multiple fermentations (e.g., several barrels undergoing MLF). As with paper chromatography, the final analysis should probably be performed with a spec or an HPLC.

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