Vinmetrica's SC-50 MLF Analyzer: a Comparison of Methods for Measuring Malic Acid in Wines.

J. Richard Sportsman and Rachel Swanson

At Vinmetrica, our goal is to provide products for the accurate yet inexpensive analysis of wines. Recently we introduced the SC-50 MLF Analyzer that provides an accurate, simple and convenient method for measuring malic acid levels. This is of course vital information for the winemaker when deciding whether malolactic fermentation is complete.

The SC-50 MLF Analyzer kit, with the SC-300, -100, or -100A, can be used to determine malic acid concentration in wine. This kit relies on the biochemical MLF reaction caused by enzymes found in certain bacteria, including Lactobacilli and Oenococcus strains, and in the "Biopressure" agent component of the kit. These bacteria live on a variety of nutrients, but their production of CO_2 results almost entirely from the enzymatic transformation of malic acid to lactic acid:

Malic Acid		Lactic Acid
$HOOC–CH_2–CH(OH)–COOH$	$\rightarrow$	$CH_3–CH(OH)–COOH + CO_2$

The CO_2 creates pressure, which is converted by the MLF Analyzer to a signal for the SC-300 (or SC-100 or -100A). The CO_2 biopressure signal is directly proportional to the amount of malic acid in the sample. The level of malic acid can be calculated from the digital readout by a calibrator of malic acid provided with the kit. Detection limit is below 0.04 g/L (40 mg/L). The cost of the unit is around $120 and cost per test is about $3.00.

There are other methods available for analysis of MLF status. Methods like spectrophotometric enzymatic assays or HPLC analysis produce reliable results, but these have significant capital costs (in the thousands of dollars) and thus are outside the budgets of most small wineries and home winemakers. The old paper chromatography method can provide semi-quantitative assessment, but its sensitivity is inadequate to reliably determine levels below about 0.1 g/L malic acid. In addition the method is slow (24 hours typically to complete), and involves the use of noxious chemicals and solvents.

Another method that is available is the Accuvin™ system[1] that gives a colored spot on a test strip; the color intensity is matched visually to a chart to determine the approximate range of malic acid concentration. This method is semi-quantitative, but in principle its sensitivity should be adequate to make the call on completion of MLF. The cost of the kit comes to about $3 per test.

Since Accuvin and Vinmetrica's products are priced similarly in cost per test, and each gives similar claims of sensitivity, we decided to run a comparison of the two methods on some wine samples. For an

[1] Accuvin and Quick Test are trademarks of Accuvin LLC, P.O. Box 5328, Napa, CA 94581

independent assessment of the samples' malic acid concentration, we used HPLC and spectrophotometric enzyme assay as reference methods.

Methods:

The Vinmetrica SC-50™ kit was used as provided, following the kit's manual (version 1.6c). For some assays, an additional standard (0.10 g/L malic acid) was prepared. An SC-300 was used to read out the SC-50 signal.

Accuvin's Quick Test™ kits were purchased from Curds and Wine, LLC in San Diego and had an expiration date of 09/2015. They were used according to instructions in the kit. Twenty µL of sample was applied as directed. Reading was done by incandescent light. Two individuals performed the reading independently, and the assays were run on two separate occasions. Results were converted to units of g/L malic acid for consistency with the other techniques.

We used high performance liquid chromatography (HPLC) as one of two reference methods. The instrument was an Agilent 1120 Compact LC with binary solvent delivery and variable wavelength detector, interfaced to a computer running Chemstation software. The column was a 0.46 x 250 mm C18 (Allure Organic Acids 5 um, Restek) and mobile phase was 0.1% H_3PO_4 , pH adjusted to 2.9 with KOH, 0.7 mL/min. Detection was at 218 nm. Samples or standards were made to 1.0% H_3PO_4, centrifuged at 12,000 rpm 1 min., and then 20 µL injected. Concentrations of malic acid in samples were determined by ratioing the baseline-adjusted peak height to that of a 2g/L malic acid standard.

Enzymatic assays for malic acid were a second reference method. We followed the Compendium of International Methods of Wine and Must Analysis (Edition 2012), Method OIV-MA-AS313-11, using L-malate dehydrogenase and glutamate oxaloacetate transaminase from Sigma Chemical Co. We scaled down the liquid volumes proportionately to run the method in a total of 220 µL in a 96-well microplate format. Absorbance at 340 nm was measured using a Biotek Synergy H4 plate reader.

Samples:

Samples were three commercial wines - two cabernet sauvignons and a viognier, designated Red Q1, Red Q3 (also designated "FB" here), and White Q2, respectively. A spiked version of the Red Q1 sample (0.40 g/L malic acid added) was prepared by adding appropriate amounts of 1 M malic acid.

Results

Vinmetrica results. The SC-50 results are summarized in Table 1. Figure 1 shows a calibration curve based on this data that demonstrates the linearity of the Biopressure response.

Table 1. SC-50 Biopressure results.

Sample	P response	Malic g/L	Recovery
0.4 standard	47	0.40	0.40
0.1 standard	34.7	0.10	0.10
0 standard	30.7	0.00	---
White Q2 (-)	137	2.61	---
Red Q1 (-)	38.8	0.20	---
Red Q1 +0.4	57.1	0.65	0.45
Red Q3	32.5	0.04	---

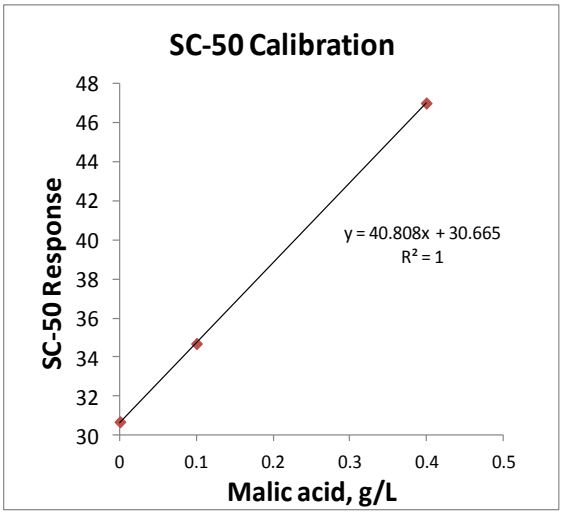

Figure 1. Calibration curve for the SC-50 bio-pressure assay, based on data from Table 1.

Results could be calculated from this calibration curve or from the single point ratio method described in the manual; the agreement between these methods was quite good (not shown). Note that recovery of 0.40 g/L spiked malic acid in sample RedQ1 was 0.45 or 112%.

Accuvin results. The test strip results are shown in Figure 2. Two of us read the color versus the chart and made the concentration assignments. Both assessments agreed closely. The assays were repeated on a second occasion with identical results. The concentration values assigned to these colors are shown in Table 2; all wine samples appeared to be less than 0.16 g/L. The 0.4 g/L malic acid standard was judged to be about the "300mg/L" level.

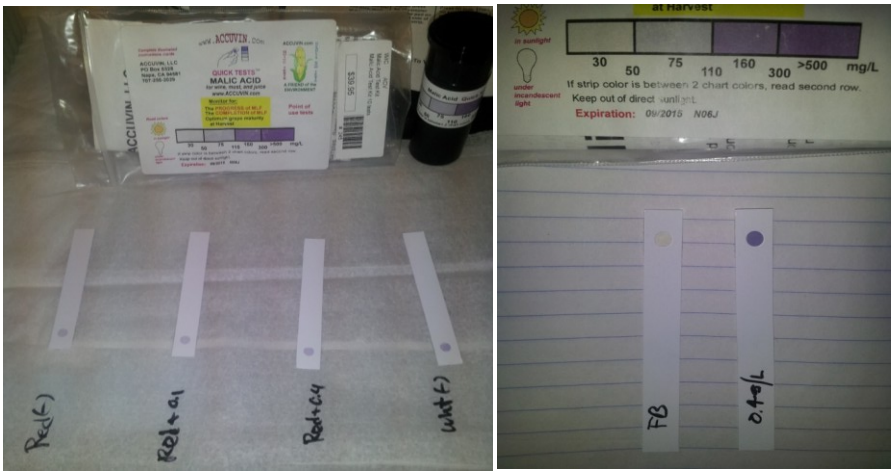

Figure 2. Photos of Accuvin test results. Left: RedQ1, RedQ1 + 0.1 g/L, RedQ1 + 0.4 g/L, and WhiteQ2. Right: Red Q3 ("FB") and the 0.4 g/L Malic Acid standard.

HPLC and enzyme assay results. The data from these standard procedures are included in Table 2. For reference, the HPLC chromatograms are shown in Figures 3 and 4.

Comparison of results. Table 2 summarizes the results of the four procedures. We observed that the SC-50 gives results that, though slightly higher on average, are in good agreement with those from HPLC or enzymatic assay. As expected, the white wine, having not undergone MLF, showed malic acid levels above 2 g/L, while the red wines, presumably having completed MLF, had low levels, below 0.2 g/L. Recovery of the deliberately spiked 0.4 g/L malic acid was 100% for HPLC method, 88% for the Enzyme assay and 112% for the SC-50 assay.

Table 2. Summary of malic acid results (in g/L) on three wine samples (one spiked with 0.4 g/L malic acid) comparing HPLC, Enzyme assay, SC-50 biopressure, and Accuvin Quick Tests.

Sample	HPLC	Enzyme assay	SC-50 Result	Accuvin
Red wine Q1	0.16	0.12	0.20	between .030 and .075
Red wine Q1 +0.4 g/L malic acid	0.56	0.47	0.65	between .075 and .160
Red Wine Q3 ("FB")	< 0.02	0.01	0.04	≤0.03
White wine Q2	2.3	2.5	2.6	about 0.160

In contrast, the Accuvin assessments of these samples were mostly considerably lower, although in the expected order; taking the maximum possible spread of the ranges for the two Red wine Q1 samples, spike recoveries were less than 0.16 - 0.03 = 0.13 or 32%. The Accuvin test should allow a maximum reponse of ">500 mg/L", but this level of color was not achieved in any of the wine samples. However, for sample Q3, where malic levels were ≤0.04 g/L by the other methods, the Accuvin result (≤ 0.03 g/L) was in agreement. In addition, the 0.4 g/L standard's result was about 0.3 by Accuvin, which we believe is within the expected error of reading the color of the assay strip.

In summary, the results of a comparison of four methods for determining malic acid in wine indicate that the Vinmetrica SC-50 is accurate relative to the standard procedures of HPLC and enzyme assay. The Accuvin strips showed varying degrees of accuracy in our hands. Part of this is no doubt due to the somewhat subjective nature of matching colors to a chart. It does seem that the Accuvin technique would allow an accurate assessment of completion of MLF, based on the lack of response to a wine sample that had clearly finished the process. It is less clear how easy it is to interpret Accuvin's results in samples that are midway through the MLF process, based on the results we see here.

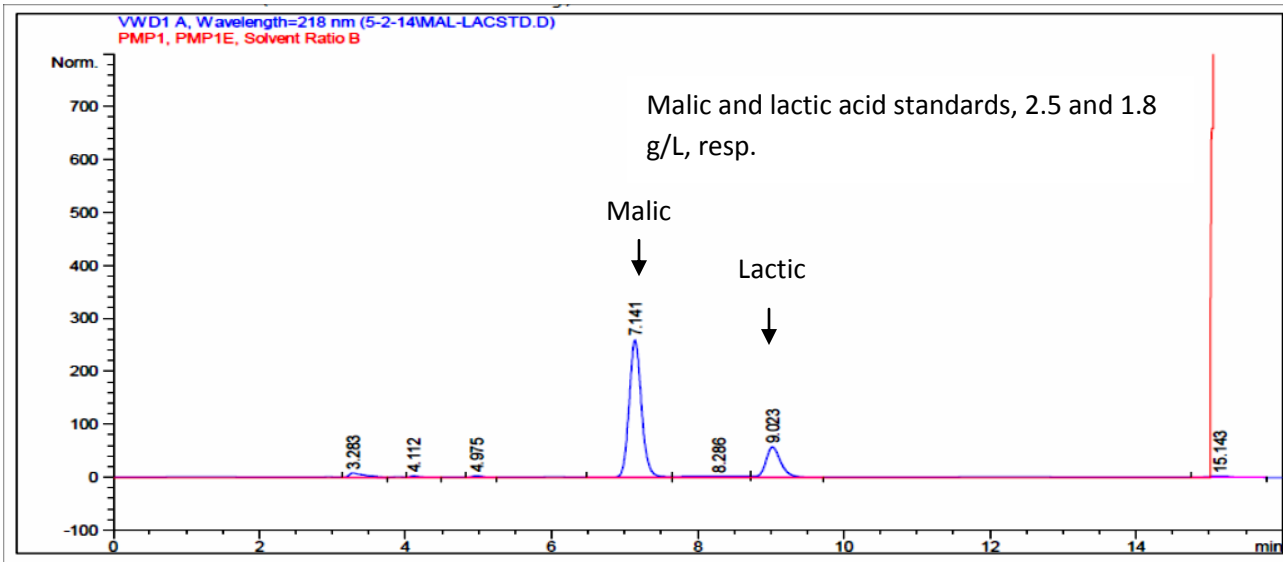

Figure 3. Chromatogram showing elution times of malic (7.1 min) and lactic (9.0 min) acids.

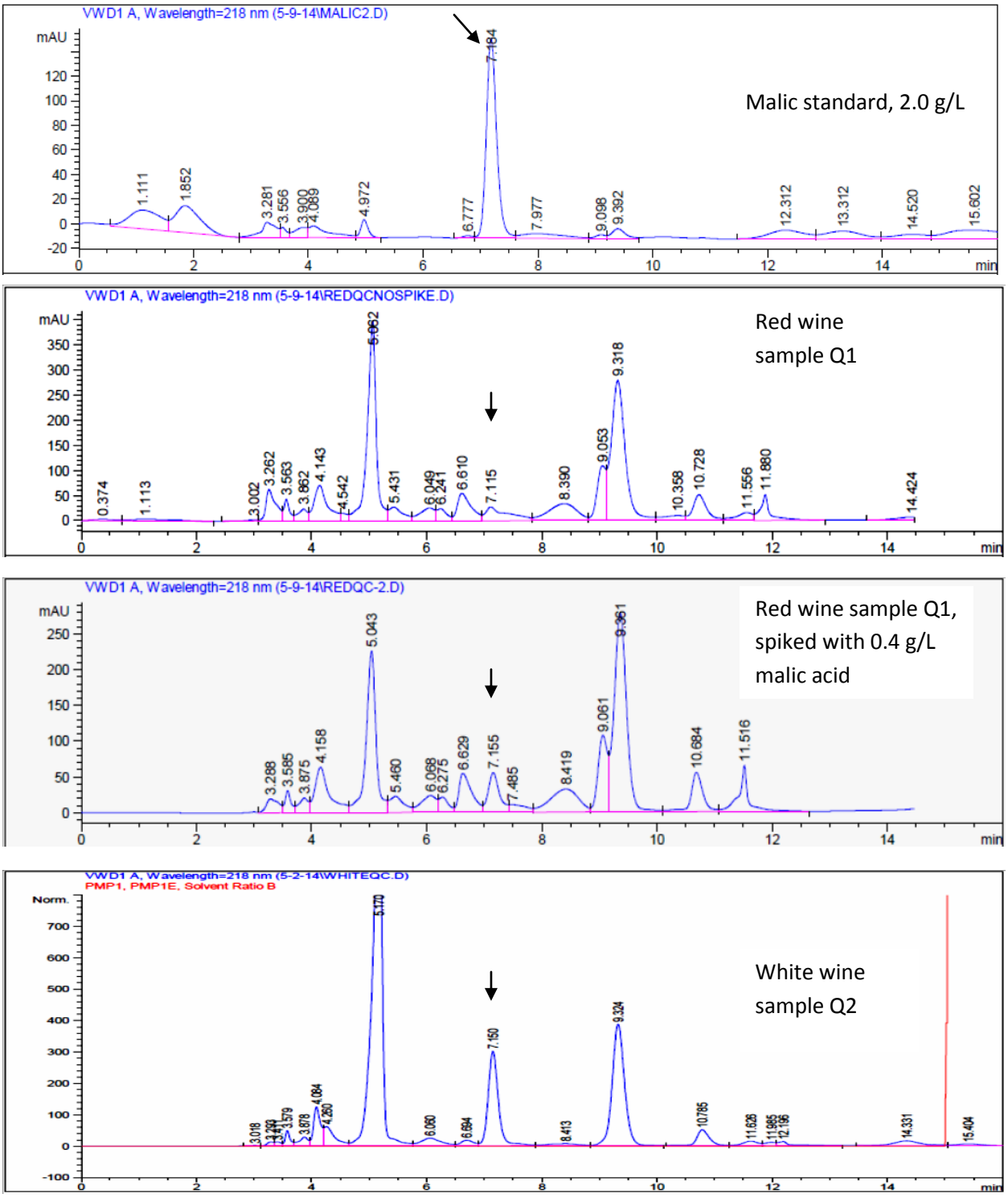

Figure 4. Chromatograms of Malic acid, RedQ1, RedQ1 spiked, and White Q2. Arrows show malic acid peak.

