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# Higher Resolution Separation of Organic Acids and Common Inorganic Anions in Wine

## **INTRODUCTION**

The flavors imparted by wine are in part due to its organic acid composition. Tartaric, citric, and malic acids are the three major organic acids found naturally in wines. The maturation of a wine can be followed by changes in organic acid composition. For example, as many red wines age, the concentration of free tartaric acid decreases as it precipitates by binding with other components of the wine.

Organic acids also contribute to the overall acidity and tartness of a wine and can contribute flavors that are either pleasing or undesirable. For example, malic acid can impart a green apple flavor, whereas excessive acetic acid will impart an unwanted vinegar flavor. Malolactic fermentation is a winemaking technique popular for the production of some chardonnays. In this process, the malic acid is converted to lactic acid by bacteria, either naturally or by the specific introduction of the bacteria, to produce a wine with a lower acidity and different taste.

Although organic acids in wine can be determined by ion-exclusion chromatography, the peak capacity of most ion-exclusion columns is low and some organic acids are not well resolved, even when two columns are placed in series.<sup>1</sup> The ion-exclusion separation also does not allow the simultaneous determination of inorganic anions.

Ion chromatography (IC) with suppressed conductivity detection is an excellent way to separate a large variety of organic acids and detect them with high sensitivity along with inorganic anions. Masson used IC to determine organic acids and inorganic anions in grape juices used to make wine.<sup>2</sup> Dionex has also demonstrated that IC can be used to determine organic acids and inorganic anions in a variety of fruit juices, including grape juice.<sup>3,4</sup>

The method described here shows a higher resolution separation of the organic acids and inorganic acids found in wine than previously reported.<sup>1</sup> This approach is applied to the separation and detection of organic acids and inorganic anions in four red and white wine samples.

## **EQUIPMENT**

Dionex ICS-3000 or ICS-5000 system including:

DP or SP Pump

DC Detector/Chromatography module with dual-temperature zone equipped with 6-port valve (Injection valve)

AS Autosampler

AXP Pump

Chromeleon® Chromatography Data System (CDS) software Version 6.80 SR9 or higher

## REAGENTS AND STANDARDS

Deionized (DI) water, Type I reagent grade, 18 MΩ-cm resistivity or better

Sodium fluoride (NaF, Fluka)

Sodium acetate (C<sub>2</sub>H<sub>3</sub>O<sub>2</sub>Na, Fluka)

Lactic acid 85% (C<sub>3</sub>H<sub>6</sub>O<sub>3</sub>, APS)

Formic acid (C<sub>2</sub>H<sub>2</sub>O<sub>2</sub>, Merck)

Shikimic acid 99% (C<sub>7</sub>H<sub>10</sub>O<sub>5</sub>, Sigma)

Sodium chloride (NaCl, Fluka)

Sodium nitrite (NaNO<sub>2</sub>, Fluka)

Sodium bromide (NaBr, Fluka)

Sodium nitrate (NaNO<sub>3</sub>, Fluka)

Succinic acid ([C<sub>2</sub>H<sub>3</sub>O<sub>2</sub>]<sub>2</sub>, Ajax)

Malic acid (C<sub>6</sub>H<sub>6</sub>O<sub>5</sub>, Ajax)

Tartaric acid ([C<sub>2</sub>H<sub>3</sub>O<sub>3</sub>]<sub>2</sub>, Ajax)

Sodium sulfate (Na<sub>2</sub>SO<sub>4</sub>, Fluka)

Oxalic acid (C<sub>2</sub>H<sub>2</sub>O<sub>4</sub>\*2H<sub>2</sub>O, Merck)

Sodium hydrogen orthophosphate (Na<sub>2</sub>HPO<sub>4</sub>, Fluka)

Citric acid (C<sub>6</sub>H<sub>8</sub>O<sub>7</sub>, Ajax)

Sodium hydroxide solution (400 g/L) (NaOH, KANTO)

Methanol (CH<sub>3</sub>OH, RCI Labscan)

Ethanol (C<sub>2</sub>H<sub>5</sub>OH, RCI Labscan)

Sulfuric acid (98%) (H<sub>2</sub>SO<sub>4</sub>, RCI Labscan)

## PREPARATION OF SOLUTIONS AND REAGENTS

### Eluents

#### Sodium Hydroxide (0.1 M)

Dilute 10 mL of 400 g/L sodium hydroxide solution to 1 L in a 1 L volumetric flask with DI water and mix.

#### Sodium Hydroxide (1 M)

Dilute 100 mL of 400 g/L sodium hydroxide solution to 1 L in a 1 L volumetric flask with DI water and mix.

#### Methanol (12%)/Ethanol (16%) in DI water

Mix 120 mL of methanol, 160 mL of ethanol, and 720 mL of DI water in a 1L bottle and degas.

#### Sulfuric Acid Stock Solution (1 N)

Add approximately 700 mL of DI water into a 1 L volumetric flask and slowly add 50.04 g of 98% sulfuric acid into the same flask. Bring the volume to 1 L with DI water.

#### Sulfuric Acid (20 mN)

Dilute 20 mL of 1 N sulfuric acid stock solution in a 1 L volumetric flask with DI water.

### Standard Solutions

#### Stock Standard Solutions

For each stock standard, dissolve the weight of salt or acid solution shown in Table 1 in a 100 mL volumetric flask with DI water.

**Table 1. Masses of Compounds Used to Prepare 100 mL Stock Standard Solutions**

Anion	Compound	Stock Concentration (mg/L)	Weight (g)
Fluoride	Sodium fluoride	500	0.111
Acetate	Sodium acetate (C <sub>2</sub> H <sub>3</sub> O <sub>2</sub> Na)	1000	0.139
Lactate	Lactic acid 85% (C <sub>3</sub> H <sub>6</sub> O <sub>3</sub> )	2000	0.235
Formate	Formic acid (C <sub>2</sub> H <sub>2</sub> O <sub>2</sub> )	1000	0.100
Shikimate	Shikimic acid 99% (C <sub>7</sub> H <sub>10</sub> O <sub>5</sub> )	1000	0.101
Chloride	Sodium chloride (NaCl)	1000	0.165
Nitrite	Sodium nitrite (NaNO <sub>2</sub> )	1000	0.150
Bromide	Sodium bromide (NaBr)	1000	0.129
Nitrate	Sodium nitrate (NaNO <sub>3</sub> )	1000	0.137
Succinate	Succinic acid (C <sub>2</sub> H <sub>3</sub> O <sub>2</sub> ) <sub>2</sub>	1000	0.100
Malate	Malic acid (C <sub>6</sub> H <sub>6</sub> O <sub>5</sub> )	2000	0.200
Tartrate	Tartaric acid (C <sub>2</sub> H <sub>3</sub> O <sub>3</sub> ) <sub>2</sub>	2000	0.200
Sulfate	Sodium sulfate (Na <sub>2</sub> SO <sub>4</sub> )	1000	0.148
Oxalate	Oxalic acid (C <sub>2</sub> H <sub>2</sub> O <sub>4</sub> *2H <sub>2</sub> O)	1000	0.140
Phosphate	Sodium hydrogen phosphate (Na <sub>2</sub> HPO <sub>4</sub> )	2000	0.298
Citrate	Citric acid (C <sub>6</sub> H <sub>8</sub> O <sub>7</sub> )	1000	0.100

**Table 2. Concentrations of Working Standards and Their Preparation**

Anion	Calibration Standard Concentration (mg/L)					Volume of Stock Standard Solution (mL)				
	Level 1	Level 2	Level 3	Level 4	Level 5	Level 1	Level 2	Level 3	Level 4	Level 5
Fluoride	0.05	0.1	0.25	0.5	0.75	0.01	0.02	0.05	0.10	0.15
Acetate	0.6	1.2	3.0	6.0	9.0	0.06	0.12	0.30	0.60	0.90
Lactate	1.0	2.0	5.0	10	15	0.05	0.10	0.25	0.50	0.75
Formate	0.1	0.2	0.5	1.0	1.5	0.01	0.02	0.05	0.1	0.15
Shikimate	0.4	0.8	2.0	4.0	6.0	0.04	0.08	0.20	0.40	0.60
Chloride	0.2	0.4	1.0	2.0	3.0	0.02	0.04	0.10	0.20	0.30
Nitrite	0.2	0.4	1.0	2.0	3.0	0.02	0.10	0.20	0.20	0.30
Bromide	0.1	0.2	0.5	1.0	1.5	0.01	0.05	0.05	0.10	0.15
Nitrate	0.1	0.2	0.5	1.0	1.5	0.01	0.05	0.05	0.10	0.15
Succinate	0.6	1.2	3.0	6.0	9.0	0.06	0.12	0.30	0.60	0.90
Malate	1.0	2.0	5.0	10	15	0.05	0.10	0.25	0.50	0.75
Oxalate	0.1	0.2	0.5	1.0	1.5	0.01	0.02	0.05	0.10	0.15
Phosphate	1	2	5	10	15	0.05	0.10	0.25	0.50	0.75
Citrate	0.5	1	2.5	5	7.5	0.05	0.10	0.25	0.50	0.75

***Working Standard Stock Solutions***

For each calibration level, add the volumes of stock standard solutions listed in Table 2 to a 100 mL volumetric flask and bring to volume with DI water.

### *Spiking Standard Stock Solutions*

Prepare each spiking standard stock solution at 10× the spiked concentration by adding the volumes of stock standard solutions listed in Table 3 to a 100 mL volumetric flask, then bring to volume with DI water.

### **Sample Preparation**

Dilute a 1 mL wine sample with DI water in a 100 mL volumetric flask and treat the sample with an OnGuard® II RP cartridge. Discard the first 3 mL, then collect the sample in an autosampler vial. For more information about using the OnGuard II RP cartridge, please refer to the OnGuard II Cartridges Product Manual.<sup>5</sup>

### **Spiked Sample Preparation**

Prepare spiked sample in the same manner as in the Sample Preparation section. Add 10 mL of the appropriate spiking stock standard solution (Table 3) to the same volumetric flask as the wine before bringing the volume to 100 mL with DI water.

**Table 3. Concentrations and Preparation of the Spiking Stock Standard Solutions (100 mL)**

Anion	Spiking Stock Standard Concentration (mg/L)		Volume of Stock Standard Solution (mL)	
	Red Wine 1 and 2, and White Wine 1	White Wine 2	Red Wine 1 and 2, and White Wine 1	White Wine 2
Fluoride	1	1	0.20	0.20
Acetate	10	10	1.0	1.0
Lactate	20	20	1.0	1.0
Formate	1	1	0.10	0.10
Shikimate	5	5	0.50	0.50
Chloride	5	5	0.50	0.50
Nitrite	5	5	0.50	0.50
Bromide	2	2	0.20	0.20
Nitrate	1	1	0.10	0.10
Succinate	20	20	2.0	2.0
Malate	10	10	0.50	0.5
Oxalate	1	1	0.10	0.10
Phosphate	10	10	0.50	0.50
Citrate	5	20	0.50	2.0

## CONDITIONS

Columns:	OmniPac® PAX-100 Analytical, 4 × 250 mm (P/N 042150) OmniPac PAX-100 Guard, 4 × 50 mm (P/N 042151)
Trap Column:	IonPac® ATC-HC, 9 × 75 mm (P/N 059604)
Eluent:	A: DI water B: 12% Methanol/16% ethanol in DI water C: 0.1 M Sodium hydroxide D: 1.0 M Sodium hydroxide
Gradient:	See Table 4
Temperature:	30 °C
Injection Vol:	25 µL
Flow Rate:	1.0 mL/min
Detection:	Suppressed Conductivity
Suppressor:	AMMS® 300, 4 mm (P/N 064558)
Regenerant:	20 mN H <sub>2</sub> SO <sub>4</sub> , delivered by AXP pump

**Table 4. Gradient**

Time	%A	%B	%C	%D
-15.0	80	0	0	20
-10.0	80	0	0	20
-9.5	0	97	3	0
0.0	0	97	3	0
3.5	0	97	3	0
3.6	0	92	8	0
9.0	0	92	8	0
28.0	0	0	100 (Curve 7)	0
35.0	0	0	100	0

## RESULTS AND DISCUSSION

### Separation

The IonPac AS11 and AS11-HC columns are well known for their ability to separate organic acids and inorganic anions in a wide range of products including grape and other fruit juices.<sup>3,4</sup> A set of nine organic acids and seven common anions were chosen to represent anions most likely to be in a wine sample. Attempts to achieve adequate resolution of this set of analytes on the IonPac AS11 or AS11-HC columns, both with and without added methanol, were unsuccessful (Figure 1). In particular, it was difficult to resolve acetate, shikimate, and lactate as well as succinate and malate.

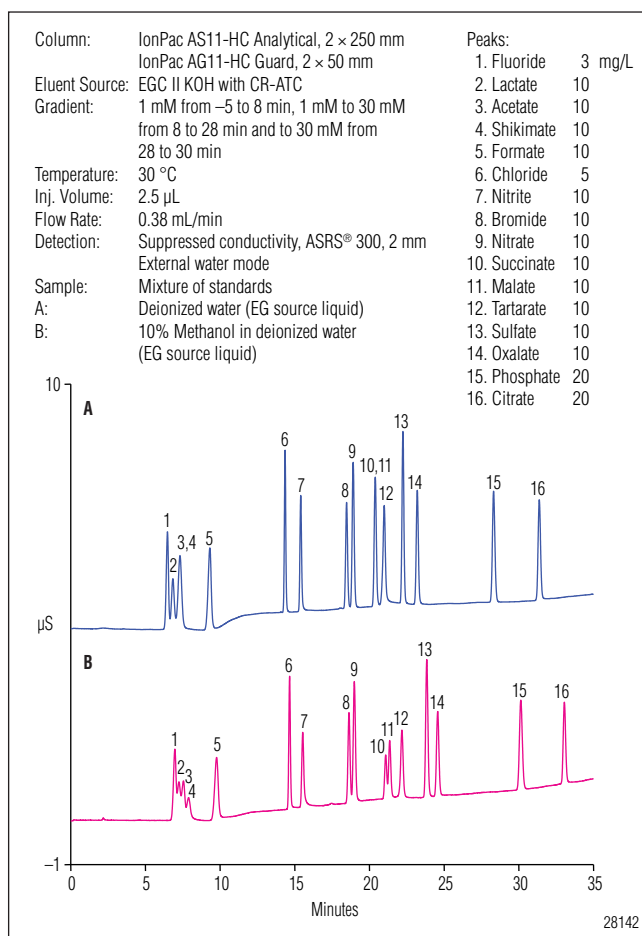


Figure 1. Chromatogram of a mixture of standards with and without adding methanol to the eluent.

The OmniPac PAX-100 column was then used in an attempt to achieve a better separation of the organic acids in wine. Using a sodium hydroxide eluent containing methanol and ethanol with gradient elution, the 16 compounds were resolved in 35 min (Figure 2). Applying these and similar conditions to the IonPac AS11 or 11-HC columns did not yield a similar separation. Because organic solvents were used in the separation on the OmniPac column, chemical regeneration was required for suppressed conductivity detection. For some applications, the separation without added organic solvent shown in Figure 1A may be adequate. That separation can be conveniently executed with a Reagent-Free™ IC (RFIC™) system where only DI water needs to be added to the system for the chromatography.

### Method Calibration

Before sample analysis, the method was calibrated using five mixed standards with different concentrations of each of the 16 anions. Concentrations were chosen based on a preliminary analysis of the samples. Three injections of each level were made to construct the calibration plot. Table 5 shows the calibration results.

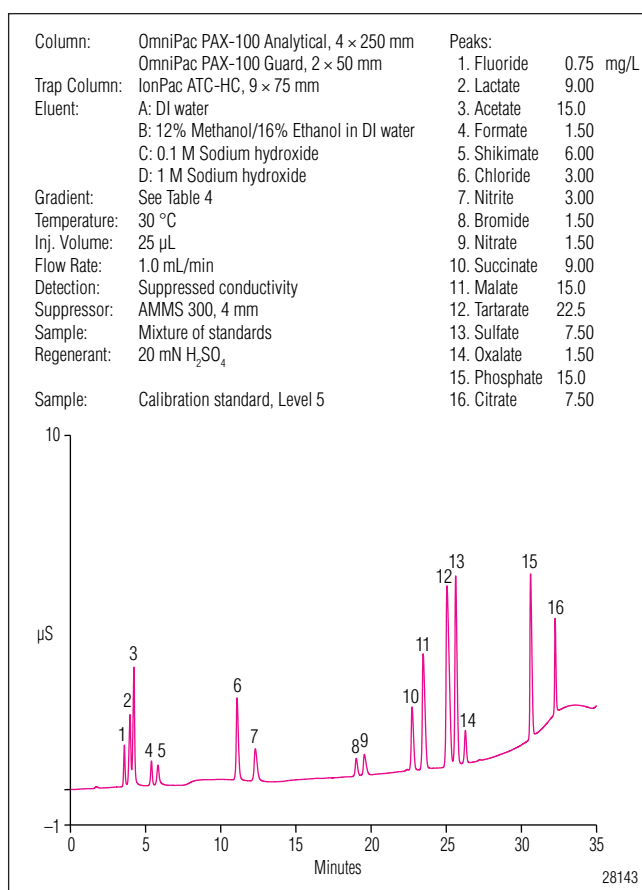


Figure 2. Chromatogram of the Level 5 Calibration Standard. Note: the rise and subsequent fall in the baseline between 7 and 12 min is present in the blank chromatogram (not shown).

Table 5. Calibration Standard Concentration and Calibration Results

Anion	Concentration (mg/L)					# Points	Calibration Result		
	Level 1	Level 2	Level 3	Level 4	Level 5		r <sup>2</sup>	Offset	Slope
Fluoride	0.05	0.1	0.25	0.5	0.75	15	0.9984	0.0000	0.1647
Acetate	0.6	1.2	3.0	6.0	9.0	15	0.9973	0.0101	0.0306
Lactate	1.0	2.0	5.0	10	15	15	0.9993	0.0042	0.0287
Formate	0.1	0.2	0.5	1.0	1.5	15	0.9977	-0.027	0.0591
Shikimate	0.4	0.8	2.0	4.0	6.0	15	0.9979	0.0004	0.0169
Chloride	0.2	0.4	1.0	2.0	3.0	15	0.9995	-0.0011	0.1261
Nitrite	0.2	0.4	1.0	2.0	3.0	15	0.9996	0.0029	0.0685
Bromide	0.1	0.2	0.5	1.0	1.5	15	0.9995	0.0002	0.0557
Nitrate	0.1	0.2	0.5	1.0	1.5	15	0.9991	-0.0019	0.0816
Succinate	0.6	1.2	3.0	6.0	9.0	15	0.9993	0.0153	0.0527
Malate	1.0	2.0	5.0	10	15	15	0.9998	-0.0040	0.1161
Oxalate	0.1	0.2	0.5	1.0	1.5	15	0.9996	-0.0007	0.0785
Phosphate	1	2	5	10	15	15	0.9995	-0.0133	0.0466
Citrate	0.5	1	2.5	5	7.5	15	0.9998	0.0006	0.0368

## Sample Analysis

Four wine samples were purchased at a local supermarket for this analysis. These included two different brands of red wine and two different brands of white wine (referred to as Red Wine 1, Red Wine 2, White Wine 1, and White Wine 2). Wine samples were diluted with DI water and treated with the OnGuard II RP cartridge before the analysis. This treatment removes hydrophobic components that could possibly foul the column. Figure 3 shows an overlay of the chromatograms of each of the four wine samples. Three injections of each wine sample were made to check the repeatability of the injection. The results of the wine sample analysis are shown in Table 6.

To judge the accuracy of this method, a spiking stock standard solution (Table 3) was added to the wine samples during sample preparation. Three injections of each spiked wine sample were made with the results shown in Table 7. The averaged value of three injections was used for recovery calculation. The recovery results of spiked sample analysis are shown in Table 8 and suggest the method is accurate. Overall, the data suggest that this is an effective method for determining the important organic acids and inorganic anions in wine.

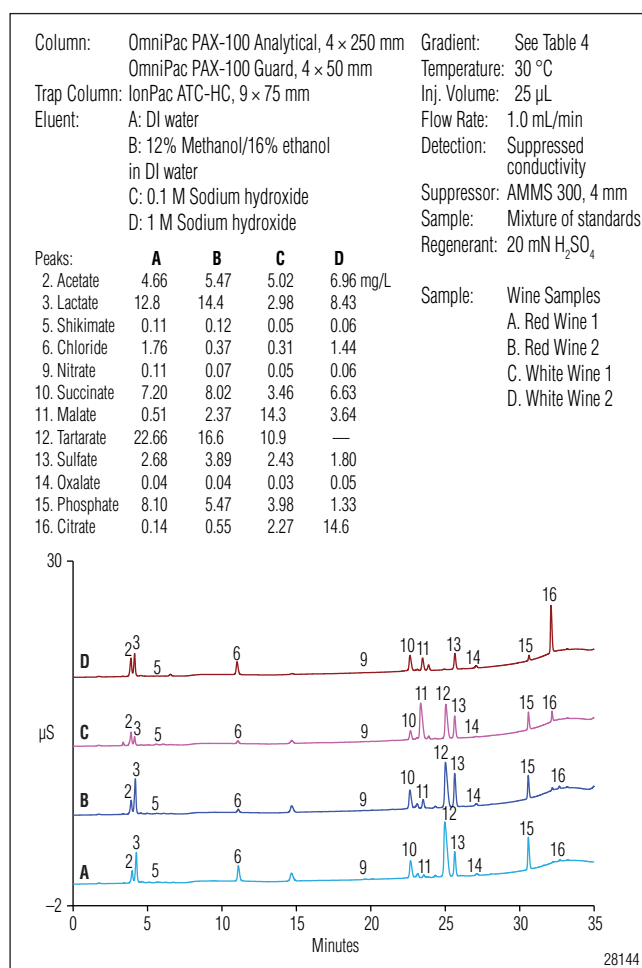


Figure 3. Chromatograms of the four wine samples.

**Table 6. Amount of Anions and Organic Acids in Wine Samples (100 × Dilution)**

Analyte	Red Wine 1		Red Wine 2		White Wine 1		White Wine 2	
	Average (mg/L)	RSD (n=3)	Average (mg/L)	RSD (n=3)	Average (mg/L)	RSD (n=3)	Average (mg/L)	RSD (n=3)
Fluoride	—	—	—	—	—	—	—	—
Acetate	4.66	0.91	5.47	0.52	5.02	1.51	6.96	0.60
Lactate	12.8	1.42	14.4	0.87	2.98	2.27	8.43	0.65
Formate	—	—	—	—	—	—	—	—
Shikimate	0.11	2.242	0.12	3.53	0.05	4.72	0.06	9.91
Chloride	1.76	1.20	0.37	0.45	0.31	2.05	1.44	0.80
Nitrite	—	—	—	—	—	—	—	—
Bromide	—	—	—	—	—	—	—	—
Nitrate	0.11	0.92	0.07	0.70	0.05	2.31	0.06	10.48
Succinate	7.20	1.07	8.02	0.35	3.46	1.41	6.63	1.22
Malate	0.51	1.41	2.37	0.57	14.3	1.35	3.64	1.37
Tartarate	22.6	0.96	16.6	0.34	10.9	1.47	—	—
Sulfate	2.68	1.09	3.89	0.45	2.43	1.80	1.80	1.34
Oxalate	0.04	0.77	0.04	1.79	0.03	4.77	0.05	1.98
Phosphate	8.10	0.95	5.47	0.50	3.98	1.68	1.33	2.16
Citrate	0.14	1.04	0.55	0.76	2.27	1.59	14.6	1.43

**Table 7. Amount of Anions and Organic Acids in Spiked Wine Samples (100 × Dilution)**

Analyte	Spiked Red Wine 1		Spiked Red Wine 2		Spiked White Wine 1		Spiked White Wine 2	
	Average (mg/L)	RSD n=3	Average (mg/L)	RSD n=3	Average (mg/L)	RSD n=3	Average (mg/L)	RSD n=3
Fluoride	0.11	1.81	0.11	2.19	0.10	1.42	0.08	5.04
Acetate	5.71	1.02	6.51	0.63	5.93	0.39	7.83	1.22
Lactate	14.4	0.75	16.2	0.90	4.85	0.07	10.1	0.57
Formate	0.10	3.18	0.09	1.12	0.08	0.59	0.12	2.34
Shikimate	0.68	2.80	0.59	9.35	0.63	2.54	0.44	8.71
Chloride	2.30	0.69	0.86	0.70	0.80	1.13	1.91	0.55
Nitrite	0.39	1.34	0.42	2.17	0.40	0.62	0.45	2.29
Bromide	0.21	2.07	0.20	2.35	0.20	1.08	0.19	1.43
Nitrate	0.22	2.38	0.17	2.83	0.14	0.87	0.15	2.01
Succinate	8.87	0.70	9.64	0.08	5.34	0.59	8.29	1.28
Malate	1.51	0.68	3.49	0.42	16.0	0.66	4.57	1.19
Tartarate	24.4	0.81	18.5	0.13	12.8	1.20	1.91	1.07
Sulfate	3.68	1.05	4.90	0.11	3.37	1.62	2.69	1.08
Oxalate	0.13	2.19	0.13	0.61	0.13	2.56	0.14	0.71
Phosphate	9.22	0.86	6.48	0.45	5.01	0.77	0.08	5.04
Citrate	0.60	1.27	1.10	5.28	2.83	2.68	7.83	1.22

Spiked concentration for all samples: Fluoride 0.1 mg/L, Acetate 1.0 mg/L, Lactate 2.0 mg/L, Formate 0.1 mg/L, Shikimate 0.5 mg/L, Chloride 0.5 mg/L, Nitrite 0.5 mg/L, Bromide 0.2 mg/L, Nitrate 0.1 mg/L, Succinate 2.0 mg/L, Malate 1.0 mg/L, Tartarate 2.0 mg/L, Sulfate 1.0 mg/L, Oxalate 0.1 mg/L, Phosphate 1.0 mg/L, Citrate 0.5 mg/L (2.0 mg/L for White Wine 2)

**Table 8. Recovery Results for Wine Samples**

Analyte	Red Wine 1 Recovery (%)	Red Wine 2 Recovery (%)	White Wine 1 Recovery (%)	White Wine 2 Recovery (%)
Fluoride	110	110	100	80.0
Acetate	105	104	91.0	87.0
Lactate	80.0	90.0	93.5	83.5
Formate	100	90.0	80.0	120
Shikimate	114	94.0	116	76.0
Chloride	108	98.0	98.0	94.0
Nitrite	78.0	84.0	90.0	90.0
Bromide	105	100	100	95.0
Nitrate	110	100	90.0	90.0
Succinate	84.5	81.0	94.0	83.0
Malate	100	112	85.0	93.0
Tartarate	90.0	95.0	95.0	95.5
Sulfate	100	101	94.0	89.0
Oxalate	90.0	90.0	100	90.0
Phosphate	112	101	103	83.0
Citrate	92.0	110	112	80.0

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