Determination of Fluoride in Wine by Fluoride Selective Ion Electrode, Standard Addition Method: Collaborative Study

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The accuracy, precision, and reproducibility of a rapid method for determination of fluoride in wine, using a fluoride selective ion electrode, were established by a collaborative study involving 12 laboratories, 5 in Europe and 7 in the United States. The laboratories assayed 6 Youden pairs of fluoride-fortified, red and white wine samples with fluoride concentrations ranging from 0.2 to 3.0 mg/L. The relative standard deviations of repeatability ranged from 1.94 to 4.88%; relative standard deviations of reproducibility ranged from 4.15 to 18.40%. HORRAT values ranged from 0.30 to 0.97. The average recovery was 99.97%. Based on the statistical results of this collaborative study, the Study Director recommends that this method be adopted First Action.

Although fluoride frequently occurs naturally in wine, usually at a concentration <1 mg/L, levels higher than this are occasionally seen. One source of introduction of fluoride into wine appears to be the use of the pesticide cryolite (Na3AlF6), which is registered in the United States for use in vineyards as prebloom or bloom control of various Lepidoptera larvae, such as the Omnivorous Leafroller and Grapeleaf Skeletonizer.

Some countries have set a maximum residue level (MRL) of fluoride for regulatory purposes. The level ranges from 0.5 to 7 mg/L. The Office International de la Vigne et du Vin (OIV; 1) recommends an MRL of 1.0 mg/L, except for wines treated with cryolite in conformity with national law, in which case the MRL is 3.0 mg/L. For regulatory purposes, it is therefore important to have an analytical method that is demonstrated to be accurate, precise, and reproducible. Search of the literature indicates the use of the fluoride selective electrode to measure the concentration of fluoride in wine as early as 1975 (2).

The Compendium of International Methods of Wine Analysis (3) of the OIV describes 2 methods of measuring fluoride in wine, using the fluoride ion-specific electrode. The methods describe a direct reading procedure as well as a known addition method. Our experience with the direct reading method is that it tends to give slightly higher results than those of the known addition method; ostensibly due to matrix effects. Gil Armentia et al. (4) compared the fluoride ion-specific electrode direct method, addition method, and Gran’s method (5), and found the addition method to give the best results.

Collaborative Study

The proposed method was submitted to 12 academic, government regulatory, industrial, and commercial laboratories. Five are European and 7 are located in the United States. The collaborative study was performed according to the guidelines prescribed by AOAC INTERNATIONAL (6), using the Youden protocol. Participating laboratories were sent a copy of the method and instructions plus a practice sample and 6 Youden pairs of samples composed of 3 spiked red wines and 3 spiked white wines. Each sample was randomly coded. A Microsoft Excel® spreadsheet that performed the necessary calculations, converting millivolt (mV) readings to fluoride concentrations was supplied to the participants. Collaborators were provided with 125 mL of the practice sample with a known fluoride concentration (2.2 mg/L) and instructed to use this sample to demonstrate method proficiency before proceeding with the analysis of the test samples. Participants were to make all standard solutions and buffer solution according to the method.

Test Sample Preparation

Six 2 L portions of a white wine and 6 of red wine, sufficient to provide 60 mL of each concentration of test wine to each of the collaborators, were filtered and then fortified with a 100 mg/L fluoride standard spiking solution to give the final concentrations of fluoride as follows: White wines: sample 1, 0.55 mg/L; sample 2, 0.85 mg/L; sample 3, 1.42 mg/L; sample 4, 1.70 mg/L; sample 5, 1.93 mg/L; and sample 6, 2.23 mg/L. Red wines: sample 7, 0.18 mg/L; sample 8, 0.46 mg/L; sample 9, 0.95 mg/L; sample 10, 1.22 mg/L; sample 11, 2.62 mg/L; sample 12, 3.00 mg/L.
mg/L; sample 12, 2.87 mg/L. The individual test sample solutions were sealed in 60 mL plastic bottles and randomly numbered before packaging one of each in sets to be shipped to the individual participants. The excess test samples were stored in 60 mL plastic bottles in a refrigerator set at approximately 4°C for possible replacement of samples lost or broken in shipment.

**Method of Analysis**

The development and initial testing is described elsewhere (2, 7, 8). We validated the proposed method by determining the linearity of the method at 6 concentrations: 0.1, 0.5, 1.0, 2.0, 3.0, and 4.0 mg/L. These standard concentrations were analyzed on 3 different days with an average correlation of 0.9983. The accuracy/bias of the method was determined by analyzing the linearity of the method at 6 concentrations: 0.1, 0.5, 1.0, 2.0, 3.0, and 3.0 mg/L, giving an average recovery of 103.8, 95.1, and 99.5%, respectively. The relative standard deviation of replicate determinations was determined by performing 5 replications of 3 fluoride standards: 0.5, 1.5, and 3.0 mg/L, giving an average recovery of 103.8, 95.1, and 99.5%, respectively. The relative standard deviation of repeatability (RSDr) was determined by 5 replications of these 3 standards on 5 different days. RSDr ranged from 3.0 to 9.2%. No difference was found between using a laboratory-made and a commercial buffer for adjusting total ionic strength.

**B. Apparatus**

(a) pH/Ion analyzer with standard addition capability.—Corning pH/ion Analyzer 455 (Cat. No. 75344), or pH/ion Analyzer with extended mV range, or equivalent.

(b) Fluoride selective ion electrode and single junction reference electrode or combination electrode.—Corning Fluoride Electrode (Cat. No. 34108-490), or equivalent.

(c) Beakers.—150 mL, plastic.

(d) Cylinder.—50 mL graduated, plastic, pouring.

(e) Magnetic stirrer.—With PTFE-coated stir bars.

(f) Plastic bottles with caps.—125 mL (Nalgene, or equivalent).

(g) Precision pipet.—Eppendorf pipet, 500 μL, or equivalent.

(h) Ultrasonic bath.

(i) Volumetric flasks.—Class A, 50 mL, 100 mL, and 1 L.

(j) Volumetric pipets.—Class A, 1, 2, 5, 10, 20, and 25 mL.

**C. Reagents**

Shelf life of solutions is 6 months.

(a) Total ionic strength adjustment buffer (TISAB).—TISAB III; Orion Research Inc. (Beverly, MA; Cat. No. 940911), or prepare as follows: To ca 700 mL deionized water in 1 L beaker, add 58.0 g sodium chloride and 29.4 g trisodium citrate·2H₂O. Dissolve 10.0 g CDTA (1,2-diaminocyclohexane-N,N,N',N''-tetraacetic acid) in a mixture of 6 mL 32% (w/v) sodium hydroxide and ca 50 mL deionized water. Mix the 2 solutions together, and then add 57 mL glacial acetic acid and adjust pH to 5.5 with 32% sodium hydroxide. Cool to room temperature, transfer to 1 L volumetric flask, and dilute to volume with deionized water.

(b) Fluoride stock standard solution.—100 mg/L. Weigh 221 mg NaF, dried at 105°C for 4 h, into 1 L volumetric flask, and dilute to volume with deionized water. Alternatively, 100 mg/L commercial fluoride standard stock solution (Orion Research) may be used.

(c) Wine blank.—A wine known to be fluoride-free is used as matrix blank.

(d) Spiked wine standard.—1 mg/L. Place 10 mL 100 mg/L fluoride stock standard solution, C(b)(1), into 1 L volumetric flask, and dilute to volume with fluoride-free wine.

**D. Preparation of Solutions**

(a) Calibration standards.—Place 25 mL 1.0, 2.0, and 5.0 mg/L standard solutions, C(b)(2), into 3 beakers, B(c), add 20 mL deionized water and 5 mL TISAB III, C(a), to each. Mix with magnetic stirrer.

(b) Wine.—Mix wine thoroughly before sampling. Degas sparkling wines by transferring to clean beaker and placing in an ultrasonic bath until gas no longer evolves. Place 25 mL wine into beaker with 20 mL deionized water and add 5 mL TISAB III, C(a), solution. Mix with magnetic stirrer. Dilution factor (DF) = 1.

**E. Determination**

(All standard and test solutions must be at the same temperature.)

(a) Calibration standards.—Measure the potential of each of the calibration solutions, using meter, B(a), fluoride selective electrode, B(b), and reference electrode, B(b). Take final reading when meter has stabilized (potential varies <0.2–0.3 mV/3 min). Record the readings for each of the calibration standards. Plot the log₁₀ of each of the standard concentrations vs the mV reading measured for each standard concentration on graph paper and determine the slope of the curve for that electrode.
### Table 2003.03A. Interlaboratory results for determination of fluoride in wine by fluoride selective ion electrode, standard addition

<table>
<thead>
<tr>
<th>Lab No.</th>
<th>Pair 1&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Pair 2&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Pair 3&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Pair 4&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Pair 5&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Pair 6&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.55</td>
<td>0.80</td>
<td>1.33</td>
<td>1.56</td>
<td>1.86</td>
<td>2.24</td>
</tr>
<tr>
<td>2</td>
<td>0.52</td>
<td>0.81</td>
<td>1.39</td>
<td>1.64</td>
<td>1.86</td>
<td>2.31</td>
</tr>
<tr>
<td>3</td>
<td>0.52</td>
<td>0.81</td>
<td>1.40</td>
<td>1.70</td>
<td>1.92</td>
<td>2.25</td>
</tr>
<tr>
<td>4</td>
<td>0.62</td>
<td>0.98</td>
<td>1.48</td>
<td>1.64</td>
<td>1.85</td>
<td>2.14</td>
</tr>
<tr>
<td>5</td>
<td>0.48</td>
<td>0.78</td>
<td>1.34</td>
<td>1.64</td>
<td>1.84</td>
<td>2.11</td>
</tr>
<tr>
<td>6</td>
<td>0.53</td>
<td>0.84</td>
<td>1.45</td>
<td>1.74</td>
<td>1.97</td>
<td>2.30</td>
</tr>
<tr>
<td>7</td>
<td>0.53</td>
<td>0.76</td>
<td>1.27</td>
<td>1.64</td>
<td>1.89</td>
<td>2.06</td>
</tr>
<tr>
<td>8</td>
<td>0.57</td>
<td>0.88</td>
<td>1.51</td>
<td>1.85</td>
<td>2.11</td>
<td>2.33</td>
</tr>
<tr>
<td>9</td>
<td>0.51</td>
<td>0.81</td>
<td>1.40</td>
<td>1.71</td>
<td>1.90</td>
<td>2.20</td>
</tr>
<tr>
<td>10</td>
<td>0.54</td>
<td>0.84</td>
<td>1.43</td>
<td>1.71</td>
<td>1.93</td>
<td>2.22</td>
</tr>
<tr>
<td>11</td>
<td>0.60</td>
<td>0.93</td>
<td>1.48</td>
<td>1.75</td>
<td>1.98</td>
<td>2.32</td>
</tr>
<tr>
<td>12</td>
<td>0.65</td>
<td>0.94</td>
<td>1.54</td>
<td>1.79</td>
<td>2.05</td>
<td>2.32</td>
</tr>
</tbody>
</table>

No. of cases: 12 12 12 12 12 12 11 11 12 12 12 12

Minimum: 0.48 0.76 1.27 1.56 1.84 2.06 0.12 0.39 0.88 1.12 2.44 2.72

Maximum: 0.65 0.98 1.54 1.85 2.11 2.33 0.28 0.57 1.06 1.32 2.81 3.08

Range: 0.17 0.22 0.27 0.29 0.27 0.27 0.16 0.18 0.18 0.20 0.37 0.36

Mean: 0.55 0.85 1.42 1.70 1.93 2.23 0.18 0.46 0.95 1.22 2.62 2.87

Median: 0.54 0.83 1.42 1.71 1.91 2.25 0.18 0.44 0.94 1.22 2.64 2.85

Std dev.: 0.050 0.069 0.079 0.079 0.084 0.091 0.052 0.063 0.061 0.065 0.090 0.114

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*Units are mg fluoride/L.
*Youden pairs.
*Value was deleted from data set by Cochran's test and was not included in the statistical analysis.

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### Table 2003.03B. Statistical data from interlaboratory study on analysis of fluoride in wine by fluoride selective ion electrode, standard addition method

<table>
<thead>
<tr>
<th>Statistic</th>
<th>White wine</th>
<th>Red wine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pair 1</td>
<td>Pair 2</td>
</tr>
<tr>
<td>Total No. of labs</td>
<td>12 12 12 11&lt;sup&gt;a&lt;/sup&gt;</td>
<td>12 12 12</td>
</tr>
<tr>
<td>No. of replicates per lab</td>
<td>2 2 2</td>
<td>2 2 2</td>
</tr>
<tr>
<td>Repeatability variance</td>
<td>0.0006 0.0015 0.0026</td>
<td>0.0002 0.0005 0.0049</td>
</tr>
<tr>
<td>Repeatability standard deviation</td>
<td>0.0235 0.0382 0.5106</td>
<td>0.0156 0.0211 0.0703</td>
</tr>
<tr>
<td>Relative standard deviation of repeatability (RSD&lt;sub&gt;r&lt;/sub&gt; %)</td>
<td>3.35 2.45 2.45</td>
<td>4.88 1.94 2.55</td>
</tr>
<tr>
<td>Reproducibility variance</td>
<td>0.0039 0.0070 0.0089</td>
<td>0.0034 0.0042 0.0130</td>
</tr>
<tr>
<td>Reproducibility standard deviation</td>
<td>0.0625 0.0835 0.0945</td>
<td>0.0587 0.0647 0.1141</td>
</tr>
<tr>
<td>Relative standard deviation of reproducibility (RSD&lt;sub&gt;r&lt;/sub&gt; %)</td>
<td>8.92 5.36 4.54</td>
<td>18.39 5.95 4.15</td>
</tr>
<tr>
<td>Horwitz equation applied (as RSD&lt;sub&gt;r&lt;/sub&gt;)</td>
<td>16.88 14.97 14.33</td>
<td>19.00 15.80 13.74</td>
</tr>
<tr>
<td>HORRAT value, RSD&lt;sub&gt;r&lt;/sub&gt;(measured)/RSD&lt;sub&gt;r&lt;/sub&gt;(Horwitz)</td>
<td>0.53 0.36 0.32</td>
<td>0.97 0.38 0.30</td>
</tr>
<tr>
<td>Average % recovery</td>
<td>100 100 99.9</td>
<td>99.8 100.3 99.8</td>
</tr>
</tbody>
</table>

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<sup>a</sup> One lab pair was deleted from data set by Cochran's test.
(b) Wine.—Measure and record the mV potential of the test sample after the readings have stabilized. Add 500 μL 100 mg/L fluoride standard, C(b)(1). After the readings have stabilized, read and record the mV potential.

If the fluoride concentration in the test sample is >2 mg/L, make a second determination after dilution of the test sample: Pipet 25 mL wine into 50 mL volumetric flask, and dilute to volume with deionized water. Place 25 mL diluted wine in 150 mL beaker, and add 20 mL deionized water and 5 mL TISAB III, C(a). Mix with magnetic stirrer and then proceed with measurement as above. DF = 2.

**F. Calculations**

Calculate the fluoride content of the test solution expressed in mg/L as:

$C_f = DF \times \frac{V_a \times C_a}{V_0} \times \frac{1}{((anti \log \Delta E / S) – 1)}$

if the standard addition volume ($V_{std}$) is <1% of the volume of the solution after standard addition, then $V_a = V_0$ and:

$C_f = DF \times C_a \times \frac{1}{((anti \log \Delta E / S) – 1)}$

where $C_f$ = fluoride concentration of the test solution (mg/L); $DF$ = dilution factor. If it is necessary to dilute the sample, use a $DF = 2$. If the sample is undiluted, then $DF = 1$; $V_0$ = initial volume of the test solution before standard addition (mL); $V_a$ = volume of the solution after standard addition (mL); $\Delta E$ = difference between potentials $E_1$ and $E_2$ obtained before and after standard addition in mV units; $S$ = slope of the curve for the electrode in the analysis solution, $E(a)$.

$C_a = \frac{V_{std} \times C_{std}}{V_{samp}}$

where $C_a$ = concentration (mg/L) of fluoride added to the test solution ($V_a$ is calculated by multiplying the volume ($V_{std}$) of standard added by the concentration ($C_{std}$) of the standard, and dividing by the volume of test sample (25 mL) used); $V_{std}$ = standard addition volume (0.5 mL); $V_{samp}$ = test sample volume used. In D(a) or D(b), $V_{samp} = 25$ mL; $C_{std}$ = concentration of addition standard, C(d)(1).

**Example of calculation.—(1)** For a sample prepared as in D(2)(b), and determined as in E(b):

$DF = 1$

$C_a = \frac{V_{std} \times C_{std}}{V_{samp}} = \frac{0.5 \text{mL} \times 100 \text{mg/L}}{25 \text{mL}} = 2 \text{mg/L}$

$\Delta E = 19.6 \text{ mV}$

$S = -58.342$

$C_f = DF \times C_a \times \frac{1}{((anti \log \Delta E / S) – 1)}$

$C_f = 1 \times 2 \text{ mg/L} \times \frac{1}{((anti \log 196/58342) – 1)}$

$C_f = 1 \times 2 \text{ mg/L} \times 0.856 = 1.71 \text{ mg/L fluoride}$

**G. Quality Control**

Analyze 1.0 mg/L standard, C(d)(2), as a continuing calibration verification at the beginning and end of each batch of wines. Results must be $1.0 \pm 0.1 \text{ mg/L}$.

Run a blank, C(e), and 1 mg/L spiked wine, C(d), as a laboratory control sample (LCS) at the beginning of each batch. The blank should not exceed $0.0 \pm 0.1 \text{ mg/L}$, and the LCS should not exceed $1.0 \pm 0.2 \text{ mg/L}$.


**Results and Discussion**

A summary of the results obtained by the 12 participants is given in Table 2003.03A. None of the laboratories reported any difficulties with the analysis. One Youden pair from one laboratory was determined to be an outlier, using the Cochran’s test. These results are noted in Table 2003.03A, and were not used in the statistical analysis.

Statistical analysis of the data reported by the collaborators indicates good precision within the laboratories and good agreement between the collaborators. The RSDr ranged from 1.94 to 4.88%; the RSDr ranged 4.15 to 18.40%. The HORRAT values (9), ranging from 0.30 to 0.97%, indicate very good reproducibility among participants. Average recovery ranged between 99.8 and 100.3% of the mean target. This agreement indicates good precision, accuracy, and reproducibility among the 12 laboratories.

**Conclusions**

The Study Director recommends that this method be adopted First Action in view of its demonstrated accuracy, precision, and reproducibility.

**Acknowledgments**

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- G. Andrade, Sutter Home Winery, St. Helena, CA
- G. Burns, ETS Labs, St. Helena, CA
- R. Eder, Höhere Bundeslehranstalt & Bundesamt für Wein und Obstbau, Klosterneuburg, Austria
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- A. Goriel, E&J Gallo Winery, Modesto, CA
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- A. Mabud, BATF, Rockville, MD
- J. Masschelin, BATF, Walnut Creek, CA
- D. Roberts, E&J Gallo Winery, Modesto, CA
- D. Tusseau, CIVC, Epernay, France
R. Wittkowski, Bundesinstitut für Gesundheitlichen Verbraucherschutz, Berlin, Germany

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