# RESOLUTION OENO 22/2004

# DETERMINATION OF FLUORIDE CONTENT IN WINE USING A FLUORIDE SELECTIVE ION ELECTRODE

THE GENERAL ASSEMBLY,

CONSIDERING Article 2 paragraph 2 iv of the agreement establishing the International Organisation of Vine and Wine,

UPON THE PROPOSAL of the Sub-commission of Methods of Analysis and Appraisal of Wine,

DECIDES to complete Annex A of the Compendium of International Methods of Analysis of Wine and Must by the following type II method:

# DETERMINATION OF FLUORIDE CONTENT IN WINE USING A FLUORIDE SELECTIVE ION ELECTRODE, AND A STANDARD ADDITION METHOD

## 1. SCOPE

This method is applicable to the analysis of fluoride in all wines. With proper dilution, the range of detection is 0.1 mg/l to 10.0 mg/l.

## 2. PRINCIPLE

The concentration of fluoride in the sample is measured after addition of a buffer, using a fluoride ion selective electrode. The buffer provides a high, constant background ionic strength; complexes iron and aluminium (which would otherwise complex with fluoride); and adjusts the pH to a level that minimises the formation of a HF•HF complex. The matrix effects are then minimised using standard addition.

## 3. REAGENTS

3.1. Deionized or distilled water

3.2. Sodium chloride ≥ 99.0% purity

3.3. Trisodic citrate ≥ 99.0% purity

3.4. CDTA (1,2-diaminocyclohexane-N,N,N',N'- tetracetic hydrate acid) ≥ 98.0% purity



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3.5. Sodium hydroxide ≥ to 98.0% purity

3.6. Sodium hydroxide solution 32% (w/v) made from 3.5

3.7 Glacial acetic acid ≥ 99.0% purity

3.8. Sodium fluoride ≥ 99.0% purity

3.9. Commercial Total Ionic Strength Adjustment Buffer (TISAB) (i.e. III-Orion Research Inc. Cat. # 940911) or equivalent (See 4.2).

3.10. Alternative TISAB:

3.10.1. To ca. 700 ml water (3.1) in a 1 l beaker (4.3), add 58.0 g ± 0.1 g sodium chloride (3.2) and 29.4 g ± 0.1 g of tri-sodium citrate (3.3).

3.10.2. Dissolve 10.0 g ± 0.1 g of CDTA (1,2-diaminocyclohexane-N,N,N',N'-tetraacetic acid) (3.4) and 6 ml of 32% (w/v) sodium hydroxide (3.6) in approximately 50 ml of distilled water. (3.1)

3.10.3. Mix the two solutions together then add 57 ml of glacial acetic acid (3.7) and adjust pH to 5.5 with 32% (m/v) sodium hydroxide (3.6). Cool to room temperature, transfer to 1 l volumetric flask (4.10), and dilute to volume with water (3.1).

3.11. Fluoride standard solutions

3.11.1. Fluoride stock standard solution (100 mg/l):

Weigh 221 mg ± 1 mg of sodium fluoride (3.8) (dried at 105°C for 4 hours) into a 1 l polyethylene volumetric flask (4.10) and make to volume with water. (3.1)

3.11.2. Fluoride calibration standards at 1.0 mg/l, 2.0 mg/l and 5.0 mg/l : make 1.0 mg/l, 2.0 mg/l, and 5.0 mg/l calibration standards by pipetting 1 ml, 2 ml, and 5 ml of the 100 mg/l stock standard (3.11.1) into three polyethylene 100 ml volumetric flasks (4.10) respectively and diluting to volume with water (3.1).

3.12. Wine blank : a wine known to be fluoride free is used as a matrix blank

3.12.1. mg/l spiked wine standard - Place 10 ml (4.11) of 100 mg/l fluoride stock standard solution(3.11.1)into a 1 l volumetric flask (4.10) and bring to volume with fluoride free wine (3.12).

# 4. APPARATUS

4.1. pH/ion analyser with standard addition capability (e.g. Corning pH/ion Analyser 455, Cat. # 475344) or pH/ion analyser with extended mV range.

4.2. Fluoride ion selective electrode and single junction reference electrode or combination electrode (e.g., Corning Fluoride Electrode Cat. # 34108-490).

4.3. Beakers - 150 ml, 1 l, polyethylene



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4.4. Cylinder - 50 ml graduated, polyethylene,.pouring.

4.5. Magnetic stirrer

4.6. Magnetic stir bars, PTFE coated.

4.7. Plastic bottles with caps, 125 ml (Nalgene or equivalent)

4.8. Precision pipette, 500 µl

4.9. Ultrasonic bath

4.10. Volumetric flasks, Class A, 50 ml, 100 ml, and 1 l

4.11. Volumetric pipettes, Class A, 1 ml, 2ml, 5 ml, 10 ml, 20 ml, and 25 ml

# 5. PREPARATION OF CALIBRATION STANDARDS

5.1. Place 25 ml (4.11) of 1.0 1.0 mg/l, 2.0 mg/l, and 5.0 mg/l standard solutions (3.11.2) respectively into three 150 ml beakers (4.3), add 20 ml (4.11) of water (3.1) and (4.11) 5 ml of commercial TISAB (3.9) to each. Mix with a magnetic stirring. (4.5 and 4.6).

5.2. If using alternative TISAB reagent (3.10) : place 25 ml (4.11) of each standard solution (3.11.2) into three 150 ml beakers (4.3) and add 25 ml (4.11) of alternative TISAB reagent (3.10) to each. Mix with a magnetic stirrer. (4.5 and 4.6)

# 6. PREPARATION OF THE TEST SAMPLES

Mix the wine sample thoroughly before sampling. Sparkling wines should be degassed before sampling by transferring to a clean beaker and placing in an ultrasonic bath (4.9) until gas no longer evolves.

6.1. If using reagent (3.9), commercial TISAB : place 25 ml (4.11) of wine sample into a 150 ml beaker (4.3) with 20 ml (4.11) of water (3.1) and add 5 ml (4.11) of ommercial TISAB (3.9) solution. Mix with a magnetic stirrer (4.5 and 4.6). Dilution factor (DF) = 1.

6.2. If using alternative TISAB reagent (3.10) : place 25 ml (4.11) of wine sample in a 150 ml beaker (4.3) and add 25 ml (4.11) of alternative TISAB reagent (3.10). Mix with a magnetic stirrer (4.5 and 4.6). Dilution factor (DF) = 1.

# 7. PROCEDURE

Measurement (all standard and wine sample solutions must be at the same temperature).

## 7.1. Calibration standards

Measure the potential of each of the calibration solutions, using the meter (4.1),



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fluoride selective electrode (4.2), and reference electrode (4.2). The final reading must be taken when the readings have stabilised (stability is obtained when the potential varies by not more than 0.2 to 0.3 mV/ 3 minutes). Record the readings for each of the calibration standards.

The log10 of each of the standard concentrations versus the millivolt reading measured for each standard concentration is plotted on graph paper in order to determine the slope of the electrode.

### 7.2. Wine samples

Measure and record the potential expressed in mV (E1) of the sample (6.1 or 6.2) after the readings have stabilised. Add 500 µl (4.8) of 100 mg/l fluoride standard (3.11.1) to the sample (6.1 or 6.2). After the readings have stabilised, read and record the potential expressed in mV (E2) of the wine solution.

The final concentration must be at least double the fluoride concentration in the sample solution. To make sure, if the fluoride concentration in the test sample is above 2 mg/l on the first determination, a second determination must be made after dilution of the sample as follows (7.2.1 or 7.2.2).

7.2.1. When using the commercial TISAB buffer (3.9): pipette (4.11) 25 ml of wine sample in a 50 ml volumetric flask (4.10) and bring to volume with water. Take 25 ml (4.11) of this diluted wine in a 150 ml cylindrical beaker (4.3) and add 25 ml of commercial TISAB (3.9). Mix with a magnetic stirrer (4.5 and 4.6) and then proceed with measurement as in 7.02. Dilution factor (DF) = 2.

7.2.2. When using the alternative TISAB buffer (3.9): pipette (4.11) 25 ml of wine sample in a 50 ml volumetric flask (4.10) and bring to volume with water. Pour 25 ml (4.11) of this diluted wine in a 150 ml cylindrical beaker (4.3) and add 25 ml of alternative TISAB buffer (3.10). Mix with a magnetic stirrer (4.5 and 4.6) and then proceed with measurement as in 7.2. Dilution factor: (DF) = 2.

## 8. CALCULATION

The fluoride content of the sample solution expressed in mg/l is obtained by using the following formula:

$$C_f = \frac{V_a \times C_a}{V_o} \times \frac{1}{\left(\left(anti\frac{log\Delta E}{S}\right) - 1\right.}$$



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If the added standard solution V std is ‹ 1% of the volume of the solution after the addition, so $V_a = V_o$ and

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

$C_f$ = fluoride concentration of the sample solution (mg/l)

DF = dilution factor. If it is necessary to dilute the sample as in (7.2.1) or in (7.2.2), use the identical values for the dilution and the sample. That is to say, DF = 2 for a diluted sample (7.2.1) and (7.2.2) or DF = 1 if it is not as in (6.1) or (6.2)

$V_o$ = initial volume of the sample solution before standard addition (ml)

$V_a$ = volume of the solution after standard addition (ml)

$\Delta E$ = difference between potentials E1 and E2 obtained in (7.2) in mV.

S = slope of the calibration curve of the electrode.

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

where

$C_a$= concentration (in mg/l) of fluoride added to the sample volume (Vo ) obtained by multiplying the standard volume (3.11.1) added to the solution (Vstd) by the concentration (Cstd) of standard (3.11.1) and divided by the sample volume (25 ml) using (6.1) or (6.2)

$V_{std}$ = volume added standard (3.11.1) (0.5 ml)

$V_{samp}$ = sample volume used in (6.1) or (6.2), Vsamp = 25 ml

$C_{std}$ = standard concentration (3.11.1)

Calculation example:

(1) for a sample prepared as in (6.2) and measured as in (7.2)

$DF = 1$

$$C_a = \frac{V_{std} \times C_{std}}{V_{samp}} = \frac{0.5\ ml \times 100\ mg/l}{25\ ml} = 2\ mg/l$$



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□ E = 19.6 mV

S = -58.342

$$C_f = DF \times Ca \times \frac{1}{((anti\ log \Delta E/S - 1)} \quad C_f = 1 \times 2\ mg/l \times \frac{1}{((anti \log 19.6/58.342) - 1 -}$$

$C_f = 1 \times 2\ mg/l\ \times 0.856\ = 1.71\ mg/l$ of fluoride

(2) for a sample prepared as in (7.2.2), and measured as in (7.2)

*DF* = 2

$$C_a = \frac{V_{std} \times C_{std}}{V_{samp}} = \frac{0.5\ ml \times 100\ mg/l}{25\ ml} = 2\ mg/l$$

□E = 20.4 mV

S = -55.937

$$C_f = DF \times Ca \times \frac{1}{((anti\ log \Delta E/S) - 1} \quad C_f = 2 \times 2\ mg/L\ \times \frac{1}{((anti \log 20.4/55.937) - 1}$$

$C_f$ = 2 x 2 mg / l x 0.760 = 3.04 mg / l of fluoride

# 9. PRECISION

The details of inter laboratory study are given in Annex B. the Horrat (HoR) ranges from 0.30 to 0.97 and indicates a very good reproducibility among participants.

The results of the statistical calculations are given in Annex B table 2.

The standard deviation of repeatability (RDSr) ranges from 1.94% to 4.88%. The standard deviation of reproducibility (RDSR) ranges from 4.15% to 18.40%. Average % recovery ranged between 99.8% and 100.3% of the mean target.

# 10. QUALITY ASURANCE AND MANAGEMENT

10.1. Analyse a standard solution from 1.0 mg/l (3.11.2) at the beginning and end of each series of measurement. The results must be 1.0 ± 0.1 mg/l.



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10.2. Before each measurement series analyse a blank sample (3.12) and for the internal quality control (CQI) a overloaded wine (3.13). The blank sample must not be over 0.0 mg/l ± 0.1 mg/l. and the CQI must not be over 1.0 mg/l ± 0.2 mg/l.

# Annex A

# References


1. AOAC International, AOAC Official Methods Program, Associate Referee's Manual On Development, Study, Review, and Approval Process,1997
2. Postel, W.; Prasch, E., Wein-Wissenschoft, (1975) 30 (6), 320-326
3. Office International de la Vigne et du Vin, Compendium of International Methods of Wine Analysis, 5/10/94, 255–257
4. Gil Armentia, J. M.; Arranz, J. F.; Barrio, R. J.; Arranz, A., Anales de Bromatologia, (1988) 40 (1) 71-77
5. Gran, G; Analyst (1952) 77, 661
6. Corning fluoride ion selective electrode – Instruction Manual, 1994
7. Corning Instruction Manual pH/ion analyzer 455, 109121-1 Rev. A, 11/96
8. Horwitz,W.; Albert, R.; Journal of the Association of Official Analytical Chemists, (1991) 74 (5) 718


# Annex B

# Inter laboratory Study

# VALIDATION OF A FLUORIDE ION SELECTIVE ELECTRODE, STANDARD ADDITION METHOD FOR THE MEASUREMENT OF FLUORIDE IN WINE



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## B.1 Introduction

The validation by collaborative trial of a fluoride selective ion electrode, standard addition method for the determination of fluoride in wine is described. The collaborative trial involved a total of twelve participants, six European and six Americans, who took part in the study. The collaborative study was performed using the AOAC, Youden protocol(1).

## B2 Participants

The twelve participants of this validation consisted of laboratories from Austria, France, Germany, Spain, and the United States and comprised of the following: BATF Alcohol and Tobacco Laboratory—Alcohol Section, SF, Walnut Creek, CA., United States; BATF, National Laboratory Ctr., Rockville, MD, United States; Bundesinstitut für Gesundheitlichen Verbraucherschutz, Berlin, Germany; Canandaigua Winery, Madera, CA, United States; CIVC, Epernay, France; E. & J. Gallo Winery-Analytical Services Laboratory, Modesto, CA, United States; E. & J. Gallo Winery-Technical Analytical Services Laboratory, Modesto, CA, United States; ETS Labs, St. Helena, CA, United States; Höhere Bundeslehranstalt & Bundesamt für Wein und Obstbau, Klosterneuburg, Austria; Institut Catala de la Vinya i el Vi, Vilafranca del Penedes (Barcelona),Spain; Laboratorio Arbitral Agroalimentario, Madrid, Spain; and Sutter Home Winery, St. Helena, CA., United States.

## B3 Samples used in the trial

The samples used in the trial are given in Appendix I. They were distributed as twelve wine samples (six Youden pairs of samples comprised of three red wines and three white wines).

| Sample | Sample description |
|---|---|
| White wine with no fortification | (total of 0.6 mg/l F-) |
| White wine fortified with 0.3 mg /l | (total of 0.9 mg/l F-) |
| White wine fortified with 0.9 mg /l | (total de 1,5 mg/l F-) |



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| | |
|---|---|
| White wine fortified with 1.2 mg /l | (total de 1,8 mg/l F-) |
| White wine fortified with 1.4 mg /l | (total de 2,0 mg/l F-) |
| White wine fortified with 1.7 mg /l | (total de 2,3 mg/l F-) |
| Red wine with no fortification | (total de 0,2 mg/l F-) |
| Red wine fortified with 0.3 mg /l | (total de 0,5 mg/l F-) |
| Red wine fortified with 0.8 mg /l | (total de 1,0 mg/l F-) |
| Red wine fortified with 1.1 mg /l | (total de 1,3 mg/l F-) |
| Red wine fortified with 2.5 mg /l | (total de 2,7 mg/l F-) |
| Red wine fortified with 2.8 mg /l | (total de 3,0 mg/l F-) |

## B.4. Results

A summary of the results obtained by the twelve participants is given in Table I. 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(c) in Table I, and were not used in the statistical analysis.

*Table 1*

*Collaborative data for the determination of fluoride in wine by fluoride selective electrode, standard additiona*

| Lab | Pair 1[b] | | White Wine Pair 2[b] | | Pair 3[b] | | Pair 4[b] | | Red Wine Pair 5[b] | | Pair 6[b] | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Number | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| 1 | 0.55 | 0.80 | 1.33 | 1.56 | 1.86 | 2.24 | 0.19 | 0.45 | 0.89 | 1.17 | 2.54 | 2.77 |
| 2 | 0.52 | 0.81 | 1.39 | 1.64 | 1.86 | 2.31 | 0.19 | 0.46 | 0.92 | 1.20 | 2.58 | 2.77 |
| 3 | 0.52 | 0.81 | 1.40 | 1.70 | 1.92 | 2.25 | 0.14 | 0.42 | 0.96 | 1.22 | 2.64 | 2.95 |
| 4 | 0.62 | 0.98 | 1.48 | 1.64 | 1.85 | 2.14 | 0.28 | 0.56 | 1.00 | 1.32 | 2.64 | 2.72 |



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|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 5 | 0.48 | 0.78 | 1.34 | 1.64 | 1.84 | 2.11 | 0.12 | 0.39 | 0.88 | 1.16 | 2.56 | 2.82 |
| 6 | 0.53 | 0.84 | 1.45 | 1.74 | 1.97 | 2.30 | 0.13 | 0.43 | 0.92 | 1.21 | 2.66 | 2.93 |
| 7 | 0.53 | 0.76 | 1.27 | 1.64 | 1.89 | 2.06 | 0.14 | 0.40 | 0.88 | 1.12 | 2.44 | 2.83 |
| 8 | 0.57 | 0.88 | 1.51 | 1.85 | 2.11 | 2.33 | 0.48[c] | 0.48[c] | 1.01 | 1.32 | 2.64 | 3.08 |
| 9 | 0.51 | 0.81 | 1.40 | 1.71 | 1.90 | 2.20 | 0.13 | 0.42 | 0.90 | 1.19 | 2.60 | 2.86 |
| 10 | 0.54 | 0.84 | 1.43 | 1.71 | 1.93 | 2.22 | 0.18 | 0.44 | 0.96 | 1.23 | 2.66 | 2.87 |
| 11 | 0.60 | 0.93 | 1.48 | 1.75 | 1.98 | 2.32 | 0.25 | 0.57 | 1.06 | 1.31 | 2.68 | 2.82 |
| 12 | 0.65 | 0.94 | 1.54 | 1.79 | 2.05 | 2.32 | 0.21 | 0.52 | 1.03 | 1.24 | 2.81 | 3.07 |
| | | | | | | | | | | | | |
| N 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 |

*Table 2*

*Statistical data from the collaborative study on the analysis of fluoride in wine by fluoride selective ion electrode, standard addition method*

| STATISTIC | Pair 1 | White Wine Pair 2 | Pair 3 | pair 4 | Red Wine Pair 5 | Pair 6 |
|---|---|---|---|---|---|---|
| Total # of Labs | 12 | 12 | 12 | 11[d] | 12 | 12 |
| Number of "replicates" per lab | 2 | 2 | 2 | 2 | 2 | 2 |
| Mean (split levels) | 0.55<br>0.85 | 1.42<br>1.70 | 1.93<br>2.23 | 0.18<br>0.46 | 0.95<br>1.22 | 2.62<br>2.87 |
| Repeatability variance | 0.0006 | 0.0015 | 0.0026 | 0.0002 | 0.0005 | 0.0049 |





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|---|---|---|---|---|---|---|
| Repeatability Standard Deviation | 0.0235 | 0.0382 | 0.5106 | 0.0156 | 0.0211 | 0.0703 |
| Relative standard deviation RSDr, repeatability | 3.35 % | 2.45 % | 2.45 % | 4.88 % | 1.94 % | 2.55 % |
| Reproducibility variance | 0.0039 | 0.0070 | 0.0089 | 0.0034 | 0.0042 | 0.0130 |
| Reproducibility standard deviation | 0.0625 | 0.0835 | 0.0945 | 0.0587 | 0.0647 | 0.1141 |
| Relative standard deviation RSDR, reproducibility | 8.92 % | 5.36 % | 4.54 % | 18.39 % | 5.95 % | 4.15 % |
| Horwitz Equation Applied (as RSDR) | 16.88 | 14.97 | 14.33 | 19.00 | 15.80 | 13.74 |
| HORRAT Value HoR (RSDR (measured)/RSDR (Horwitz)) | 0.53 | 0.36 | 0.32 | 0.97 | 0.38 | 0.30 |
| Average % recovery | 93.1 | 94.6 | 96.7 | 91.0 | 94.4 | 96.4 |

[b] Youden pairs

[c] Value was deleted from data set by Cochran's Test and was not included in the statistical analysis

[c]

[d] One lab pair was deleted from data set by Cochran's Test





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