RESOLUTION OENO 12/2006

DETECTION OF SYNTHETIC ACETIC ACID IN WINE VINEGARS BY THE DETERMINATION OF BETA RADIOACTIVITY OF ^{14}C OF ACETIC ACID BY LIQUID SCINTILLATION

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 introduce into the Compendium of International Methods of Analysis of Wine Vinegars, the following method of type IV:

TITLE	Type of Method
Detection of synthetic acetic acid in wine vinegars by measuring the □ emissions of ^{14}C in the acetic acid using liquid scintillation	IV

1. Introduction

The concentration of the ^{14}C contained in natural vinegars is closely related to the year of production of the corresponding wines.

Levels which are lower than those for a given year indicate that synthetic acetic acid has been added or makes up the entire content.

2. Principle

The acetic acid is first extracted from the vinegar using diethyl ether then mixed with the Scintillation Fluid (SF) and “counted” for 400 minutes using liquid scintillation. Acetic acid of mineral origin (Control) is counted in the same way.

The result of the “net count”, obtained by subtracting the reading for the Control from that of the sample represents the □ emission value of the ^{14}C in the sample which, after taking statistical corrections into account, is then compared with the average value of the □ emissions of ^{14}C found in the ethanol in genuine late harvest wines.

3. Reagents

3.1. Sodium hydroxide pellets
3.2. 40% sodium hydroxide solution
3.3. Pure diethyl ether for analysis.
3.4. Sulphuric acid 10 M
3.5. Anhydrous sodium sulphate
3.6. Decolourising charcoal
3.7. Sodium hydroxide 0.1M
3.8. Scintilling mixture – example : Canberra Packard PicoFluor LLT (SF)
3.9. Hexadecane ^{14}C (Activity □ 1□ 10^6 dpm/gC)
3.10. Nitromethane
3.11. Synthetic acetic acid
3.12. Phenolphthalein:1% (m/v) solution in 95% vol. alcohol.

4. Equipment

4.1. Hood
4.2. Rotary vacuum evaporator
4.3. Nitrogen bottle with a regulator valve
4.4. Liquid scintillation spectrometer
4.5. Polyethylene counting flasks with plastic lids that can be hermetically sealed
4.6. Laboratory glassware
4.7. Ultrasound bath

5. Preparation of the sample

Homogenise the sample by shaking.

6. Procedure

6.1. Extraction of acetic acid

In a 2 l flask, place 450 ml of vinegar with a total acidity of 60 g/l in acetic acid (or the corresponding quantity if the vinegar presents a different total acidity); add (A□0.1□18) g of Sodium hydroxide pellets (A = number of ml of Sodium hydroxide 0.1M needed to neutralise 1.0 ml of vinegar) and if necessary, a few ml of 40% (3.2) sodium hydroxide solution to bring the pH to 9-10. Using the rotary evaporator, distil a volume of about 300 ml under vacuum at 50°C until the residue has reached a syrupy consistency and discard the distillate .

After cooling, transfer the residue to a 1 l stoppered flask and extract using diethyl ether (100 ml) in an ultrasound bath.

Transfer to a separatory funnel, separate the phases and discard the ether phase.

Repeat the extraction in an ultrasound bath, using a further 100 ml ether.

Transfer to a separatory funnel, separate the phases and discard the ether phase.

To the aqueous residue, add 25-30 ml sulphuric acid 10 M (pH 2-3) and extract using ether (3 aliquots of 100 ml), for 5 minutes in an ultrasound bath.

Join the ether phases, dry the phases with anhydrous sodium sulphate (approx. 2 g) (3.5), add decolourising charcoal (approx. 3 g) and filter with folded filter paper.

Evaporate the ether at 40°C and remove the last traces by bubbling cold nitrogen through. The acetic acid obtained, which has a slight yellow coloration, is dripped through a column filled with decolourising charcoal. (1,5 g).

The acetic acid obtained, approx. 15 ml, has a concentration ranging from 84 to 87%.

6.2. Assay of the concentration

- In a 20 ml graduated flask, use a pipette to drop 2 ml of the extracted acetic acid and add water up to the mark.
- Take 1 ml of the diluted solution and titrate using Sodium hydroxide 0.1M (3.7) in the presence of phenolphthalein (3.12).
- Calculate the concentration of acetic acid as an average percentage over 4 measurements, using the equation(1)

1. Acetic acid % = a x M x 60

Where the volume of Sodium hydroxide 0.1M used in titration; M is the molarity of

Sodium hydroxide (herein 0.1) and 60 is the weight of an equivalent of acetic acid.

7. Spectrometric measurements

7.1. Quenching curve

7.1.1. Standard solution

In a graduated flask, dissolve an exactly weighed quantity of hexadecane ^{14}C in the scintillation fluid so that the final solution has an activity of approx. 6000 dpm/ml: Standard Solution (S)

7.1.2. Preparation of the vials for the Quenching curve

Prepare 10 vials containing 16.5 ml scintillation fluid and 1 ml standard solution.

Count each vial for 20 minutes using the ET method (Efficiency Tracing) in order to exactly determine the activity.

Choose the 5 vials that have the most comparable activity and add to each vial the quantity of nitromethane indicated in the following table:

flask	Nitromethane µl	Quench level	Activity added
1	0.0	0.0	A_{aj}
2	20.0	+	A_{aj}
3	40.0	++	A_{aj}
4	80.0	+++	A_{aj}
5	100.0	++++	A_{aj}

7.1.3. Counting

Put the flasks in the instrument and count under the following conditions:

- Counting time: 10 minutes

- Cycles : 4
- Nuclide : Manual
- Counting window : Low Level (LL) = 1.5 kev; Upper Level (UL)= 29 kev
- Removal of background noise : no
- Counting method : Cpm
- Automatic correction of “quenching”: no

Calculate the average value of the activity measured A_m (Cpm) and calculate the percentage efficiency (E%) for each vial in relation to the activity added A_{aj} (Dpm):

- $E\% = A_m \times 100 / A_{aj}$

On a diagram, plot the efficiency values calculated (E%) and the corresponding quenching parameters given by the apparatus during the counting and calculate the equation of the curve.

The curve represents all the possible quenching situations which can occur during the course of a measurement for each E% value.

It is therefore possible to calculate the efficiency (E%) of a vial by introducing into the curve equation the quenching parameter given by the apparatus.

8. Counting of the sample

8.1. Preparation of the measurement vials

- In a vial (measurement vial) put 4.5 ml of extracted acetic acid and 17.5 ml of scintillation fluid (measuring flask).
- To determine the noise of each counting flask, prepare a flask with 4.5 ml of synthetic acetic acid and 17.5 ml of scintillating mixture (control flask).
- Put the two flasks in the apparatus and count for 400 minutes (100 minutes for 4 cycles). Cpm_{ef} and Cpm_{fe} are respectively obtained.

9. Results

9.1. Calculating the activity of the sample

The beta emission value of the ^{14}C in the sample (A_{sp}) is calculated from thefollowing:

- $A_{sp} = (Cpm_e - Cpm_f) \square 100 / E\% \square gC$

10. Presentation

The beta emission value of the ^{14}C (A_{sp}) is expressed in disintegrations per minute per gramme of carbon (Dpm/gC) or in centiBequerel per gramme of carbon (cBq/gC) and rounded down to one decimal place.

11. Interpretation of the results

The result obtained, taking into account statistical corrections, is compared with the average value of the □ emissions of ^{14}C found in the ethanol in genuine late harvest wines.

It should be pointed out, however, that to obtain a more accurate interpretation of the result, the measurement of the vinegar and that of the ethanol should be carried out by the same laboratory and using the same equipment.

12. Characteristics of the method

12.1. Internal validation procedure

Due to the difficulty in finding a suitable number of laboratories which owned the equipment necessary for taking part in the validation checks as set out in the procedures required by the OIV, an internal procedure has been prepared and implemented, as set out in Resolution OENO 8/2005;

12.1.1. Calculating the measurement uncertainty

12.1.2. Procedure:

- 10 samples of vinegar, 6 from the factory and 6 from sellers were extracted and counted twice.
- The standard deviation and uncertainty were calculated on all the counting parameters which contribute to obtaining the final result.
- The repeatability and repeatability uncertainty of the Quenching Curve (or Efficiency Curve) were also calculated.

The results are shown in Table 1.

Table 1

Sample	Cpm B	Gross Cpm	Net Cpm	tSIE	Eff%	Eff	EffCurve	G-H	(G-H)^2	Dpm	gC	Dpm/gC
A1	10.10	28.85	18.75	194.08	77.61	0.77609	0.78177	-0.00568	0.00003	24.16	1.67	14.47
A1	10.25	27.83	17.58	206.76	78.11	0.78112	0.79318	-0.01206	0.00015	22.51	1.54	14.64
A2	10.32	27.52	17.20	217.12	78.43	0.78428	0.80251	-0.01823	0.00033	21.93	1.57	14.01
A2	10.38	28.13	17.75	217.27	78.43	0.78431	0.80264	-0.01833	0.00034	22.63	1.62	13.96
A3	10.21	29.09	18.88	205.99	78.09	0.78085	0.79249	-0.01164	0.00014	24.18	1.62	14.93
A3	10.40	26.67	16.27	217.71	78.44	0.78443	0.80304	-0.01861	0.00035	20.74	1.48	14.01
A4	10.10	27.31	17.21	227.04	78.65	0.78649	0.81144	-0.02494	0.00062	21.88	1.54	14.21
A4	10.21	27.74	17.53	204.88	78.05	0.78046	0.79149	-0.01104	0.00012	22.46	1.62	13.86
A5	10.52	27.7	17.18	195.99	77.69	0.77693	0.78349	-0.00656	0.00004	22.11	1.56	14.17
A5	10.18	27.33	17.15	211.84	78.28	0.78278	0.79776	-0.01498	0.00022	21.91	1.54	14.23
A6	10.33	29.19	18.86	202.26	77.95	0.77948	0.78913	-0.00965	0.00009	24.20	1.66	14.58
A6	10.24	28.31	18.07	210.16	78.23	0.78225	0.79624	-0.01399	0.00020	23.10	1.61	14.35
A7(*)	9.95	27.82	17.87	198.27	77.79	0.77790	0.78554	-0.00765	0.00006	22.97	1.63	14.09
A7(*)	1.01	27.35	17.34	214.25	78.35	0.78349	0.79993	-0.01644	0.00027	22.13	1.59	13.92
A8(*)	1.35	27.63	17.28	204.1	78.02	0.78017	0.79079	-0.01062	0.00011	22.15	1.54	14.38
A8(*)	10.12	26.76	16.64	197.63	77.76	0.77763	0.78497	-0.00734	0.00005	21.40	1.48	14.46
A9(*)	10.18	28.54	18.36	211.14	78.26	0.78256	0.79713	-0.01457	0.00021	23.46	1.58	14.89
A9(*)	9.87	28.53	18.66	202.84	77.97	0.77970	0.78966	-0.00995	0.00010	23.93	1.62	14.77

A10(*)	10.21	28.67	18.46	189.48	77.39	0.77395	0.77763	-0.00368	0.00001	23.85	1.62	14.76
A10(*)	10.15	28.3	18.15	189.46	77.39	0.77394	0.77761	-0.00367	0.00001	23.45	1.67	14.04

(*) Commercial Samples

Cpm B = Background (disintegration per minute of synthetic acid)

Gross Cpm = Total number of Counts

Net Cpm = Gross Cpm - Cpm B

tSIE = Quench Parameter

Eff = Efficiency

The efficiency of the count (E) is calculated using equation (1)

(1) E = ax2+bx+c

Where **x** is a quench parameter (expressed as a tSIE) which is automatically calculated by the instrument.

Given the tSIE values obtained in a significant number of measurements (several hundred) fell within a very narrow range, it was decided to set the quenching curve as a straight line, represented therefore by equation (2)

(2) E = ax+b

The efficiency values, which were very similar when calculated using each of the two equations, proved the accuracy of the method adopted.

The following statistical parameters were calculated using the samples from Table 1.

Deviation = 0.04

Repeatability (r) = 0.65

Uncertainty (U) = 0.53 dpm/gC

12.2. Detection Limit (D.L.) and Quantification Limit (Q.L.)

The Detection Limit and the Quantification Limit were calculated by preparing in double 6 vials of synthetic acetic acid and then performing the count.

The results obtained are summarised in Table 2:

Cpm B	Average	St Dev	D.L. (Cpm)	Q.L. (Cpm)

10.48	10.60	0.14	0.43	1.40
10.62				
10.45				
10.87				
10.64				
10.65				
10.83				
10.61				
10.45				
10.65				
10.41				
10.57				

The following D.L. and Q.L. values were calculated on the basis of the counts in Table 2:

D.L.= 0.43

Q.L.= 1.40

12.3. Instrument Consistency

In order to check the consistency of the instruments, 6 vials of biogenic acetic acid were prepared, containing known variable quantities of between 0% and 80% of synthetic acetic acid.

The results of the count in Net Cpm are shown in Table 3

Table 3

%AcH (synth.)	Net Cpm
0.0	10.94
0.0	11.17
5.0	10.96
5.0	11.07
10.0	9.67
10.0	9.81
20.0	8.77
20.0	8.88
40.0	6.20
40.0	6.39
80.0	2.57
80.0	2.33

Fig 1. shows the curve obtained when the values from Table 3 are represented in graph form.

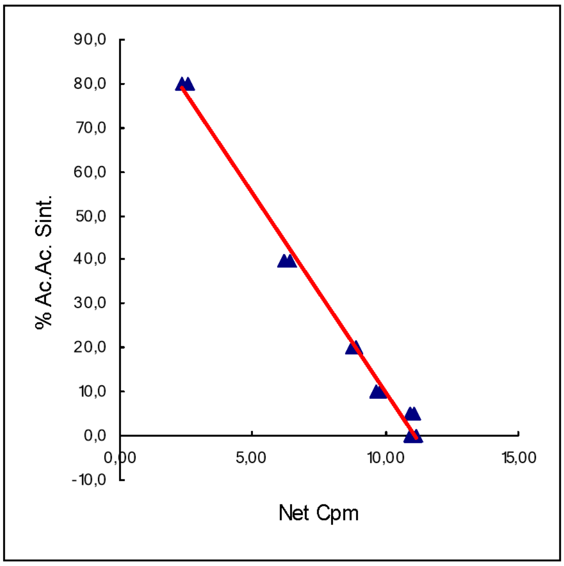

Fig. 1

Graph key: Ac.Ac.Sint = synthetic acetic acid

If the Q.L value calculated in 13.1 is introduced into the equation of the straight line in Fig. 1, the % of synthetic acetic acid which can be quantifies with certainty in the example in Table 3 = 13%.

Percentage values of acetic acid ≥ 13 % can therefore be determined in a predictable manner.

Certified in conformity Logrono, 28th July 2006
The Director General of the OIV
Secretary of the General Assembly
Frederico CASTELLUCCI