OIV-MA-AS313-23 Identification of L-tartaric acid as being of plant or fossil origin by measuring its ^{14}C activity

Type IV method

1. Purpose and scope

The method can be used to identify tartaric acid as being of plant or fossil origin, and in cases of a mixture of the two, to determine the respective proportions of the two types. In these situations, the method enables the detection of fossil-derived L(+)-tartaric acid quantities below 10%.

2. Principle

In the majority of cases, commercially available tartaric acid of plant origin is a product of winemaking. The potassium hydrogénotartrate present in the lees is extracted and marketed in the form of L-tartaric acid. The ^{14}C concentration in the acid is therefore related, as with ethanol from wine, to the ^{14}C concentration in the carbon dioxide in wines from the same year of production. This concentration is relatively high as a result of the human activity involved.

Synthetic tartaric acid on the other hand, derived from fossil fuel by-products, has a much lower or even negligible concentration of ^{14}C.

Measuring the ^{14}C activity in DPM/gram of carbon (Disintegrations Per Minute) using liquid scintillation therefore allows the origin to be determined as well as any combination of the types.

3. Reagents and products

3.1. Reagents

3.1.1. Scintillation fluid such as Instagel Plus

3.1.2. ^{14}C toluene reference with activity certified by laboratory for callibration, for calculating the sensitivity and efficiency of the machine by the definition of a quench curve

3.1.3. ^{14}C and ^{3}H standards and ^{12}C toluene for the background noise, for calibrating the scintillation counter

3.1.4. Nitromethane 99%

3.1.5. Ultrapure water (>18 MΩ)

3.1.6. ^{14}C toluene solution with activity of approx. 430 DPM/ml obtained by diluting stock ^{14}C reference solution in ^{12}C toluene.

3.2. Standards

3.2.1. Defining the quench curve

Once the scintillator has been calibrated using the three certified ^{14}C, ^{3}H and ^{12}C toluene standards, plot a quench curve using the following procedure.

Prepare a dozen vials with 10 ml of a solution of 500 g/l of fossil-derived L-tartaric acid in water, then add the quantity of toluene ^{14}C standard needed for approx. 400-1000 DPM in total per vial (if necessary, make up an intermediate solution of standard solution in toluene), then add increasing quantities of nitromethane, e.g. for 12 vials: 0, 0, 0, 5, 10, 15, 20, 35, 50, 100, 200 and 400 µL followed by 10 m of scintillation fluid. There must be at least 3 samples containing no nitromethane.

Define a quench curve once a year, analysing the vials in increasing order of nitromethane content.

The quench curve can then be used to determine the sensitivity or mean efficiency.

3.2.2. Determination of background noise (test blank)

Using fossil-derived L-tartaric acid, such as that used for calculating the efficiency, determine the background noise, or test blank value. This test should be performed immediately after defining the quench curve, then roughly every three months.

3.2.3. Defining the calibration curve

The purity of the plant and fossil-derived L-tartaric acids must be checked using HPLC before the scintillation test is done.

Calibration using a mixture of tartaric acid (which is known with certainty to be of plant origin) containing between 0% and 100 % of this type in combination with the fossil-derived type.

Preparation of 500 g/l solutions			
	Blank or background noise	Standards	Internal standard
Weighing	respectively in 50 ml volumetric flasks		
	25 g fossil-derived L-tartaric acid	25 g known combinations of fossil and plant L-tartaric acid	Use the blank
Dissolution	Seal		
	Homogenise the mixture well by shaking and/or tumbling		

Preparation of scintillation mixtures			
	In plastic vials, add respectively		
Sample taken from the 500 g/l solutions	10 ml using volumetric pipettes		
Added concentration	//////////////////////////	//////////////////////////	100 µL
Added scintillation fluid	10 ml using an automatic burette		
	Screw the cap on		
	Wait 5 min. then analyse for 500 min.		

3.3. Internal control

3.3.1. Nature of product used for internal control

A 500 g/l solution of fossil-derived L-tartaric acid is enriched with a quantity of toluene ^{14}C (DPM<100)

The background noise should be determined using the same fossil-derived L-tartaric acid solution.

3.3.2. Nature of internal control

Measurement of the added concentration provides verification that there is no spectral interference in the medium being studied.

3.3.3. Internal control limits

The control limits depend on the equipment used: a 5% value is acceptable.

3.3.4. Inspection frequency and procedure

Once a month during frequent use, or at each analysis sequence, an internal control is performed on the scintillator. The same check is also carried out at every change of scintillation fluid batch or after a new quench curve has been defined.

3.3.5. Decision rules to be taken depending on the results of the internal control

If the results fall outside the internal control limits, calibrate the scintillator after checking the protocol, then repeat the internal control.

If the calibration is accurate but the new internal control measurement is not, make a new quench curve and carry out a new control.

4. APPARATUS

1. Liquid scintillation spectrometre with computer and printer previously calibrated with

quenching curve established with nitromethane

2. Low content potassium identical bottles (40K) with screw top stopper, and low background noise
3. 10 ml 2 graduations pipettes
4. Automatic distribution burette adapted to screw top for liquid scintillating bottle
5. Glass laboratory

5. Samples

The purity of the samples can be checked using HPLC if required, before running the scintillation analysis.

Make up a 500 g/solution of the sample to be analysed in ultra-pure water.

Preparation of 500 g/l solutions				
	Test blank or background noise	Standards	Internal standard	Sample
Weighing	respectively in 50 ml volumetric flasks			
	25 g fossil-derived L-tartaric acid	25 g known combinations of fossil-derived and plant L-tartaric acid	Use the blank	25 g
Dissolution	Seal			
	Homogenise the mixture well by shaking and/or tumbling			
Preparation of scintillation mixtures				
	In plastic vials, add respectively			
Sample taken from the 500 g/l solutions	10 ml using volumetric pipettes			
Added concentration	//////////////////////////	///////////////////	100 µL	/////////////////
Added scintillation fluid	10 ml using an automatic burette			
	Screw the cap on and shake hard			

	Wait 5 min. then analyse for 500 min.
Notes	Every 5 to 10 test samples, run a sample with 0 % plant tartaric acid, i.e. 10 ml fossil tartaric acid and 10 ml scintillation fluid.
	Measure the background noise at the end of each analysis sequence

6. Calculation

Measurements are given directly in Counts Per Minute CPM, but these must be converted to DPM/gram of carbon.

6.1. Results:

Calculation of the specific ^{14}C radioactivity of the sample in DPM/gram of carbon:

$$A = \frac{(X - X') \times 100 \times 3.125_{(1)}}{Rm \times m}$$

A: radioactivity in disintegrations/minute and per gram of carbon

X: CPM of the sample

X': CPM for the fossil L-tartaric acid used for the background noise

m: mass of the tartaric acid in the 10 ml sample from the 500 g/l solution, i.e. in 5 g of acid

Rm: the mean efficiency expressed as a percentage

(1) There are 3.125 grams of tartaric acid to each gram of carbon (ratio of the molar mass of the acid (150 g/mol) to the total mass of carbon (or 4 *x 12 = 48 g/mol)

The result is expressed to one decimal place.

6.2. Verification of the results using internal controls:

The check should be carried out by comparing the value obtained at § 3.5.1 with the result given by the added concentration method. If the difference is significant (> 5 %), recalculate the DPM value from the CPM value as below:

with the mean efficiency being obtained from the quench curve.

The two results must not differ by more than 5% from their mean value. If they do, repeat the analysis on the sample, doubling the quantity of the internal standard. Compare the 2 results obtained with the standards: if they do not differ by more than 5 % from the mean of the 2, give the mean result.

Note: in this case, that would mean that the quenching of the sample is so great that direct analysis cannot be used.

6.3. Uncertainty

The uncertainty value obtained under standard test conditions is +/- 0.7 DPM/gram of carbon.

7. Validation by comparison with a reference method

7.1. Principle

Tartaric acid is converted to CO_2 by combustion then converted to benzene;
Measurement is then carried out using liquid scintillation.
After undergoing a pre-treatment designed to eliminate any contamination, the CO_2 from the sample is converted to benzene following the reaction chain below:

$$C + O_2 \longrightarrow CO_2 (1)$$

$$CaCO_3 + 2HCl \longrightarrow CO_2 + H_2O + Ca^{2+} + 2Cl^- (2)$$

800°C

$$2CO_2 + 10Li \longrightarrow Li_2C_2 + 4Li_2O \ (3)$$

$$Li_2C_2 + H_2O \longrightarrow C_2H_2 + 2LiOH \ (4)$$

$$Al_2O_3 \ with \ Cr^{3+} \ and \ V$$

$$3C_2H_2 \longrightarrow C_6H_6 \ (5)$$

(1) Organic sample: the carbon flushed with oxygen plus a heat source (or by combustion in the presence of pressurised oxygen) produces carbon dioxide from the sample (CO_2).

(2) Mineral sample (marine or continental carbonates, water, etc.): The carbonate is attacked by pure hydrochloric acid (HC) to produce the carbon dioxide (CO_2) from the sample plus water and ionised calcium.

(3) The action of the CO_2 on lithium metal heated to between +600°C and +800°C produces lithium carbide and lithium oxide ($-Li_2$ O).

(4) The action of water (hydrolysis) on the lithium carbide produces acetylene (C_2H_2), lithium hydroxide,. Non-tritiated, radon-free water must be used.

(5) Trimerisation of the acetylene over a chrome-plated aluminium-based catalyst support at approx. 185 °C produces benzene (C_6H_6).

7.2. Procedure:

The carbon dioxide (CO_2) from a sample, obtained either by burning, combustion or acid attack, is preserved in a storage cylinder. The necessary quantity of lithium (lithium = catalyst for a chemical transformation) is placed in a nickel capsule, which is then placed at the bottom of a heat reaction chamber. A vacuum is created inside the chamber and its lower part is heated while its upper part is cooled at the sides with the help of a water circulation partition.

7.2.1. Carburisation.

After approximately one hour of heating, the temperature reaches 650°C. The CO_2 can then be brought into contact with melted lithium. The quantity of lithium is always higher in relation to the quantity of carbon in the sample. The excess amount of lithium to use in relation to the **stoechiometric** conditions varies from 20% to 100% according to different sources.

The chemical reaction (carburisation or "pickup") is almost instantaneous and the first few minutes of pickup are the most crucial in the carburisation process.

The reaction is **exothermic** (an increase of 200°C). Carburisation is quite rapid and is considered to be at the carburised stage after the first 20 minutes, but heating continues for 45 to 50 minutes in order to any eliminate traces of **radon** (a by-product of uranium), which could be mixed in with the carbon dioxide.

7.2.2. Cooling

Once the treatment period (heating) is complete, the reaction chambers are allowed to cool until they reach room temperature (25-30°C).

7.2.3. Hydrolysis of Lithium Carbide

Water is introduced into the reaction chambers, in a much higher quantity than that required by the reaction (1.5 L). The chemical reaction is instantaneous and the acetylene is released at the same time. This reaction is also exothermic (temperature increase between +80°C and +100°C).

The acetylene produced is then brought to a vapour state (sublimation) and trapped over the chrome-plated (Cr3+) aluminium catalyst support. This is previously air dried for a minimum of three hours, then vacuum dried for two hours under heat at +380°C. Drying is vital in order to eliminate any water remaining in the catalyst support balls.

7.2.4. Trimerisation - Polymerisation of acetylene to benzene by catalysis

Before trimerisation, the temperature of the catalyst support must have dropped to between +60°C and +70°C, and since this reaction is also exothermic, automatic temperature maintenance is needed. The catalyst support is then reheated to +180°C for 1½ hours and the vaporised **benzene** is desorbed then trapped in a trap tube surrounded by liquid nitrogen. Desorption takes place under dynamic vacuum. At the end of the experiment, the crystallised benzene is left to reheat to room temperature so that it regains its liquid state before being used for the counting.

7.3. Benchtop arrangement for the synthesis of Benzene

7.4. Reference Chemical solution for the Counting

A **solution volume set at 4 ml** is used as the reference for the liquid scintillation counting.

The solution comprises a target base of 3.52g benzene from the sample (solvent) + the scintillation fluid (solute) made up of 2 scintillation fluids, one main and one secondary.

Since the mass per volume of benzene is 0.88 g/litre, **0.88 x 4ml = 3.52 g**.

Main scintillation fluid	**Buthyl-PBD**
Chemical composition	**(2-(4-Biphenylyl)-5-(4-tert-buthyl-phenyl)-1,3,4-oxadiazole)**
Maximum wavelength fluorescence	**367 nanometers**
Secondary scintillation fluid	**bis-MSB**
Chemical composition	**1,4-Di-(2-Methylstyryl)-Benzene**
Maximum wavelength fluorescence	**415 nanometers**
Optical absorption and coupling emission of the two fluids:	
Maximum absorption wavelength	**409 nanometers**
Maximum absorption wavelength	**412 nanometers**

7.5. Delta ^{13}C correction for Isotope Fractionation

The measurement involves a correction for isotope fractionation using the standardisation procedure with a stand PDB ^{13}C **with a value of - 25 o/oo**.

8. Characteristics of the method

8.1. Procedure

One sample of wine-derived tartaric acid and one sample of synthetic acetic acid were used to prepare test tartaric acid solutions at 500 g/l.

The concentrations of the wine-derived tartaric acid in the solutions varied between

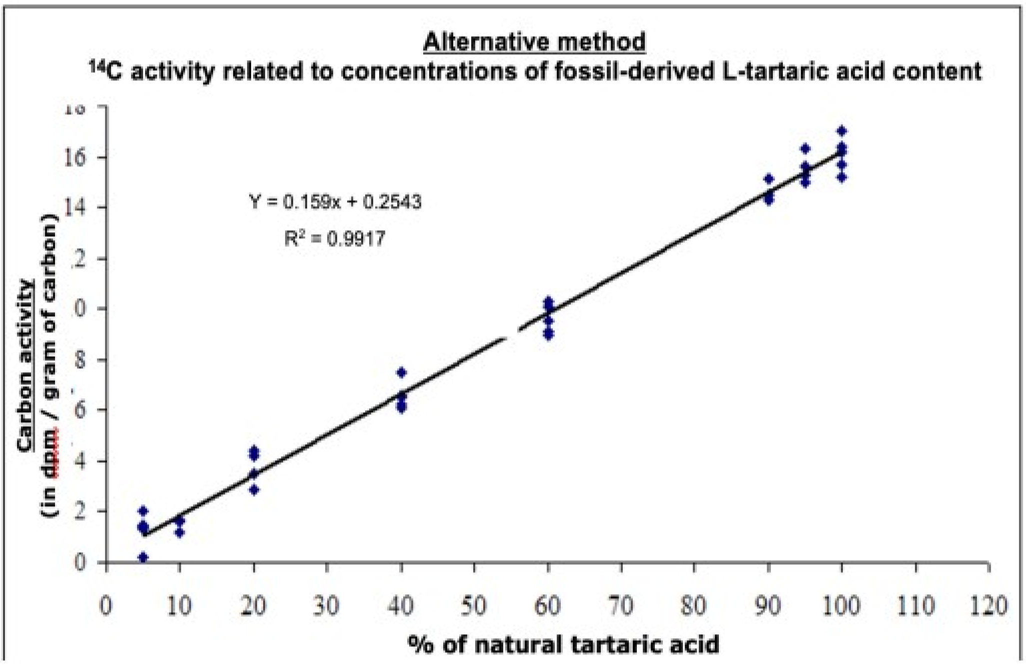
0°C and 100%.

The origin and purity of the two starter samples had been previously checked using the reference method.

8.2. Results:

The results are given in the table and diagram below:

% of wine-derived tartaric acid		
Actual concentrations	Results from the alternative method	Results from the reference method
0	0 and 0	0
10	3.5 and 6.0	12
20	11.4 and 12	22
30	24.6 and 25.4	31
40	34.7 and 38	40
50	41.4 and 50.6	50
60	57.8 and 58.8	63
70	60 and 63.3	70
80	81	81
85	84	86
90	88	91
95	94	96
100	100	100



8.3. Accuracy,trueness:

Accuracy is 6.9%.

The standard deviation of repeatability for the alternative method is: 2.86 % of plant tartaric acid.

9. Bibliography

- Compendium of international methods of analysis of spirits and alcohols and of the aromatic fraction of beverages, Office Internationale de la Vigne et du Vin, Edition officielle, juin 1994, page 201, 204, 210 et 307.
- Methods of analysis for neutral alcohol applicable to the wine sector, EEC Regulation no. 625/2003, 2 April 2003, Journal Officiel des communautés européennes 15 May 1992, n°L130, p18. (Journal Officiel, 8 April 2003, N° L90, p4).
- J. GUERAIN and S. TOURLIERE, Radioactivité carbone et tritium dans les alcools, Industries Alimentaires et Agricoles – 92nd year, July – August 1975, N° 7-8
- S. COHEN, B. CRESTO, S. NACHAMPASSAK, T. PAYOT, B. MEDINA, S.

CHAUVET, Détermination de l’origine de l’acide tartrique L(+): naturelle ou fossile par la détermination de son activité C^{14} - Document OIV FV 1238, 2006