Megazyme

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TARTARIC ACID (TARTRATE)

(*Liquid Stable, Rapid*, Format)

ASSAY PROCEDURE

K-TART 12/19

(200 Manual Assays per Kit) or
(2000 Auto-Analyser Assays per Kit) or
(2000 Microplate Assays per Kit)

INTRODUCTION:

Tartaric acid occurs naturally in grapes and is one of the most prevalent organic acids, along with L-malic acid, present in wines.

Throughout the wine making industry it is assumed that tartaric acid is the only acid that contributes to total (titratable) acidity in wine and as such the measurement of tartaric acid is used as the key indicator of total acidity. The general levels of total acidity in wines range from approximately 0.4 to 1.0% (w/v). During the wine making process, if the total acidity of a wine is too low, tartaric acid can be added to the wine to increase the level of acidity and consequently decrease the pH level. This in turn acts as a preservative against microbial spoilage.

This kit (**K-TART**) is suitable for the specific measurement of tartaric acid, especially in wines and fruit juices.

SPECIFICITY, SENSITIVITY, LINEARITY AND PRECISION:

The assay is specific for tartaric acid in white wines, red wines and fruit juices. L-malic acid, D-malic acid, L-lactic acid and D-lactic acid do not react or interfere with the tartaric acid assay when present at concentrations of 2 g/L or less.

The smallest differentiating absorbance for the assay is 0.010 absorbance units. This corresponds to a tartaric acid concentration of ~ 54 mg/L of sample solution at a sample volume of 0.1 mL. The detection limit is ~ 108 mg/L, which is derived from an absorbance difference of 0.020 with a sample volume of 0.1 mL.

The assay is linear over the range of 0.15 to 11 g/L of tartaric acid per assay. In duplicate determinations using one sample solution, an absorbance difference of 0.005 to 0.010 may occur. With a sample volume of 0.1 mL, this corresponds to a tartaric acid concentration of ~ 27 to ~ 54 mg/L of sample solution. If the sample is diluted during sample preparation, the result is multiplied by the dilution factor, F.

SAFETY:

The general safety measures that apply to all chemical substances should be adhered to.

For more information regarding the safe usage and handling of this product please refer to the associated SDS that is available from the Megazyme website.

KITS:

Kits suitable for performing 200 assays in manual format (or 2000 assays in auto-analyser format or 2000 assays in microplate format) are available from Megazyme. The kits contain the full assay method plus:

Bottle 1: **Clarifying Agent (11 mL)**
Stable for > 2 years at room temperature.

Bottle 2: (x2) **Tartaric Acid Reagent 1 (44 mL)**
Stable for > 6 months at 4°C.

Bottle 3: (x2) **Tartaric Acid Reagent 2 (28 mL)**
Stable for > 6 months at 4°C.

Bottle 4: **Tartaric Acid Standard**
L-Tartaric acid (5 mL; 5 g/L).
Stable for > 5 years; store sealed at room temperature.

PREPARATION OF REAGENT SOLUTIONS (SUPPLIED):

1-4. Use the contents of bottles 1 to 4 as supplied.

EQUIPMENT (RECOMMENDED):

1. Disposable plastic cuvettes (1 cm light path, 3 mL).
2. Micro-pipettors, e.g. Gilson Pipetman® (200 µL and 1 mL).
3. Positive displacement pipettor, e.g. Eppendorf Multipette® - with 5 mL Combitip® (to dispense 0.1 mL, 0.25 mL and 0.4 mL aliquots of reagent solutions).
4. Stop clock.
5. Analytical balance.
6. Spectrophotometer set at 505 nm.
7. Vortex mixer (e.g. IKA® Yellowline Test Tube Shaker TTS2).
8. Whatman No. 1 (9 cm) filter papers.

A. MANUAL ASSAY PROCEDURE:

Wavelength: 505 nm
Cuvette: 1 cm light path (glass or plastic)
Temperature: ~ 25°C or 37°C
Final volume: 2.50 mL
Sample solution: 15-1100 µg of tartaric acid per cuvette (in 0.10 mL sample volume)

Read against air (without cuvette in the light path) or against water

Pipette into cuvettes	Sample	Standard	Blank
sample	0.10 mL	-	-
standard	-	0.10 mL	-
distilled water (at ~ 25°C)	1.75 mL	1.75 mL	1.85 mL
Tartaric Acid Reagent 1	0.40 mL	0.40 mL	0.40 mL
Mix* and read absorbances of the solutions (A_1) after exactly 1 min.			
Tartaric Acid Reagent 2	0.25 mL	0.25 mL	0.25 mL
Mix* and read absorbances of the solutions (A_2) after exactly 4 min.			

* for example with a plastic spatula or by gentle inversion after sealing the cuvette with a cuvette cap or Parafilm®.

MANUAL ASSAY PROCEDURE FOR RED WINES:

Pipette into cuvettes	Sample	Standard	Blank
solution 1 (Clarifying Agent)	0.05 mL	0.05 mL	0.05 mL
sample	0.10 mL	-	-
standard	-	0.10 mL	-
distilled water (at ~ 25°C)	1.70 mL	1.70 mL	1.80 mL
Mix* and incubate for 1 min. **Then add:**			
Tartaric Acid Reagent 1	0.40 mL	0.40 mL	0.40 mL
Mix* and read read absorbances of the solutions (A_1) after exactly 1 min.			
Tartaric Acid Reagent 2	0.25 mL	0.25 mL	0.25 mL
Mix* and read read absorbances of the solutions (A_2) after exactly 4 min.			

CALCULATION (Manual Assay Procedure):

Determine the $\Delta A_{Tartaric}$ for the sample, standard and blank. Subtract the $\Delta A_{Tartaric}$ of the blank from that of the sample and the standard, thereby obtaining $\Delta A_{Tartaric\text{-}Sample}$ and $\Delta A_{Tartaric\text{-}STD}$, respectively.

The value of $\Delta A_{Tartaric\text{-}Sample}$ and $\Delta A_{Tartaric\text{-}STD}$ should be at least 0.100 absorbance units to achieve sufficiently accurate results.

The concentration of tartaric acid can be calculated as follows:

$$\Delta A_{Tartaric} = A_2 - A_1$$

$$c = \frac{\Delta A_{Tartaric\text{-}Sample}}{\Delta A_{Tartaric\text{-}STD} / c_{Tartaric\text{-}STD}} \times F \quad [g/L]$$

where:

$c_{Tartaric\text{-}STD}$ = concentration of tartaric acid standard (g/L)
F = dilution factor

If the sample is diluted during preparation, the result must be multiplied by the dilution factor, F.

When analysing solid and semi-solid samples which are weighed out for sample preparation, the content (g/100 g) is calculated from the amount weighed as follows:

Content of tartaric acid

$$= \frac{c_{Tartaric}\ [g/L\ sample\ solution]}{weight_{sample}\ [g/L\ sample\ solution]} \times 100 \quad [g/100\ g]$$

NOTE: These calculations can be simplified by using the Megazyme ***Mega-Calc***™, downloadable from where the product appears on the Megazyme website (www.megazyme.com).

B. AUTO-ANALYSER ASSAY PROCEDURE:

Wavelength: 505 nm
Temperature: ~ 25°C or 37°C
Final volume: 0.250 mL
Sample solution: 1.5-110 µg of tartaric acid per cell (in 0.01 mL sample volume)

Pipette into cells	Sample	Standard
sample standard distilled water (at ~ 25°C) Tartaric Acid Reagent 1	0.010 mL - 0.175 mL 0.040 mL	- 0.010 mL 0.175 mL 0.040 mL
Mix and read absorbances of the solutions (A_1) after exactly 1 min.		
Tartaric Acid Reagent 2	0.025 mL	0.025 mL
Mix and read absorbances of the solutions (A_2) after exactly 4 min.		

AUTO-ANALYSER ASSAY PROCEDURE FOR RED WINES:

Pipette into cells	Sample	Standard
solution 1 (Clarifying Agent) sample standard distilled water (at ~ 25°C)	0.005 mL 0.010 mL - 0.170 mL	0.005 mL - 0.010 mL 0.170 mL
Mix* and incubate for 1 min. **Then add:**		
Tartaric Acid Reagent 1	0.040 mL	0.040 mL
Mix* and read absorbances of the solutions (A_1) after exactly 1 min.		
Tartaric Acid Reagent 2	0.025 mL	0.025 mL
Mix* and read absorbances of the solutions (A_2) after exactly 4 min.		

CALCULATION FORMULA (AUTO-ANALYSER):

$A_2 - (A_1 \times 225/250)$

C. MICROPLATE ASSAY PROCEDURE:

Wavelength: 505 nm
Microplate: 96-well (e.g. clear flat-bottomed, glass or plastic)
Temperature: ~ 25°C or 37°C
Final volume: 0.250 mL
Linearity: 1.5-110 µg of tartaric acid per well (in 0.01 mL sample volume)

Pipette into wells	Sample	Standard	Blank
sample	0.010 mL	-	-
standard	-	0.010 mL	-
distilled water (at ~ 25°C)	0.175 mL	0.175 mL	0.185 mL
Tartaric Acid Reagent 1	0.040 mL	0.040 mL	0.040 mL
Mix* and read absorbances of the solutions (A_1) after exactly 1 min.			
Tartaric Acid Reagent 2	0.025 mL	0.025 mL	0.025 mL
Mix* and read absorbances of the solutions (A_2) after exactly 4 min.			

* for example using microplate shaker, shake function on a microplate reader or repeated aspiration (e.g. using pipettor set at 50-100 µL volume).

MICROPLATE ASSAY PROCEDURE FOR RED WINES:

Pipette into wells	Sample	Standard	Blank
solution 1 (Clarifying Agent)	0.005 mL	0.005 mL	0.005 mL
sample	0.010 mL	-	-
standard	-	0.010 mL	-
distilled water (at ~ 25°C)	0.170 mL	0.170 mL	0.180 mL
Mix* and incubate for 1 min. **Then add:**			
Tartaric Acid Reagent 1	0.040 mL	0.040 mL	0.040 mL
Mix* and read absorbances of the solutions (A_1) after exactly 1 min.			
Tartaric Acid Reagent 2	0.025 mL	0.025 mL	0.025 mL
Mix* and read absorbances of the solutions (A_2) after exactly 4 min.			

NOTES:

1. For each batch of samples that is applied to the Microplate Assay Procedure a tartaric acid standard must be performed concurrently on the same plate using the same batch of reagents.
2. Calculation of tartaric acid content should be performed as described for the Manual Assay Procedure (page 4).

EQUIPMENT FOR MICROPLATE ASSAY PROCEDURE (RECOMMENDED):

1. Disposable 96 well polystyrene clear, flat bottom microplates, e.g. Matrix Technologies Corp. cat. no. 4915 (www.matrixtechcorp.com).
2. Disposable 25 mL reagent reservoirs, e.g. Matrix Technologies Corp. cat. no. 8093-11 (www.matrixtechcorp.com).
3. Micro-pipettors, e.g. Gilson Pipetman® (200 µL and 1 mL) and Multichannel Micro-pipettors, e.g. Gilson Pipetman® Ultra 8-channel (1-20 µL and 20-300 µL).
4. Stop clock.
5. Analytical balance.
6. Microplate shaker, e.g. Heidolph Titramax 100 or 1000 (www.heidolph-instruments.com).
7. Microplate reader set at 505 mn.
8. Vortex mixer (e.g. IKA® Yellowline Test Tube Shaker TTS2).
9. Whatman No. 1 (9 cm) filter papers.

SAMPLE PREPARATION:

Sample dilution.

The amount of tartaric acid present in the cuvette (i.e. in the 0.1 mL of sample being analysed) should range between 1.5 and 1100 µg. The sample solution must therefore be diluted sufficiently to yield a concentration of tartaric acid between 0.15 and 11 g/L.

Dilution table

Estimated concentration of tartaric acid (g/L)	Dilution with water	Dilution factor (F)
< 11	No dilution required	1
11-110	1 + 9	10
> 110	1 + 99	100

If the value of $\Delta A_{Tartaric}$ is too low (e.g. < 0.100), weigh out more sample or dilute less strongly.

SAMPLE PREPARATION EXAMPLES:

Determination of tartaric acid in fruit juice.

Clear, neutral solutions can generally be determined without any sample treatment (except dilution). Turbid liquids generally only require centrifuging or filtering through Whatman No. 1 filter paper before the dilution step. Coloured solutions are usually suitable for analysis after dilution to an appropriate tartaric acid concentration. However, coloured solutions can be analysed using the assay procedure for red wines.

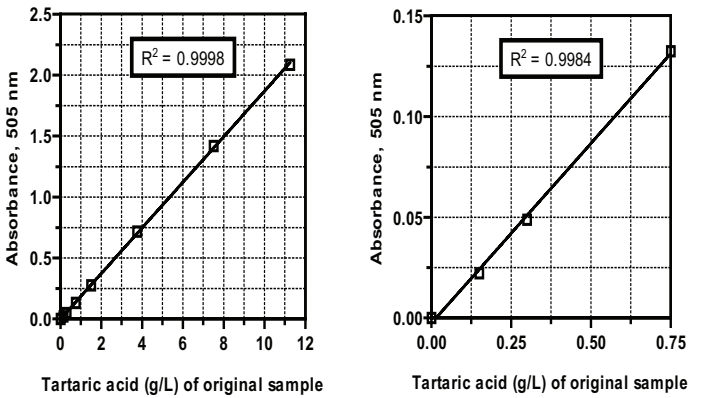

Figure 1. Calibration curve demonstrating the linearity of the colourimetric tartaric acid determination by **K-TART**. The tartaric acid standards were analysed using the standard procedure for the manual format in 1.0 cm light path cuvettes at 25°C.

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