PURPOSE OF THE TEST

Acetaldehyde is one of the compounds formed during the process of alcoholic fermentation and reaches its maximum value at the beginning of it, when yeast reach the end of the exponential growth phase. Subsequently, acetaldehyde is partially consumed by the yeasts and bacteria present in the medium. At the end of the fermentation, the acetaldehyde contributes to the stabilization of the colour, as well as provides fruity and herbaceous tones to the flavour of the wine. It is able to bind sulfites in a very important way (up to 70% of the total), reducing the amount of free sulfite. Elevated concentrations of acetaldehyde can be harmful to health.

METHOD

Acetaldehyde is transformed into acetic acid by action of Aldehyde Dehydrogenase (ALDH) with NADH formation.

$$Acetaldehyde + NAD^+ \xrightarrow{ALDH} Acetic\ acid + NADH + H^+$$

The increase of absorbance at 340 nm due to NADH formation is directly proportional to the concentration of acetaldehyde in sample.

CONTENT

R1	2 x 25 mL	Buffer
R2	1 x 6.5 mL	Enzymatic diluent
R3	1 x 0.5 mL	ALDH

REAGENT PREPARATION

Reagent 1 is ready to use and is stable up to expire date stated on the label. Avoid contamination with other reagents. Do not freeze.

Reagent 2: Pour the content of R3 into R2 vial. Mix gently avoiding foam. This solution is stable up to one month at 2-8 ºC if contamination is avoided. Do not freeze.

Discard if blank absorbance is over 1.200 OD at 340 nm.

SAMPLES

The samples must be free of turbidity and particles. Centrifuge or filter if necessary. The presence of CO_2 introduces instability in the measure. Samples containing CO_2 must be degassed beforehand. In samples with very high colour intensity, the pigment may interfere with the measurement. Treat with polyvinylpolypyrrolidone (PVPP 0.1g for each 10 mL) to reduce the level of colour. Samples with concentration higher than the measurement range must be diluted accordingly with distilled water. Multiply the final result by the dilution factor.

Acetaldehyde is a very volatile compound. Keep samples/STD tightly closed until its use. Use immediately and do not return leftover calibrator to the vial.

PROCEDURE OVERVIEW

Treat standard, controls and samples as sample. Use distilled water as Blank.

Volumes stated below can be adjusted to other analytical procedures. Expected performance can vary if those ratios S:R1:R2 are not used exactly as stated.

Pipette into a cuvette:

	Blank reaction	Test Reaction
Reagent 1	880 µL	880 µL
Distilled water	12 µL	--
Sample/Standard	--	12 µL

Mix, incubate at 37ºC for 1 minutes and read absorbance at 340 nm (A_1). Then add into the cuvette:

	Blank reaction	Test Reaction
Reagent 2	120 µL	120 µL

Mix, incubate for 10 minutes at 37ºC and read absorbance at 340 nm (A_2).

Concentration of acetaldehyde is calculated as:

$$Acetaldehyde = \frac{(A_2 - 0.88xA_1)_{\text{sample}} - (A_2 - 0.88xA_1)_{\text{blank}}}{(A_2 - 0.88xA_1)_{\text{standard}} - (A_2 - 0.88xA_1)_{\text{blank}}}\ x\ C\ \text{g/L}$$

Factor 0.88 is used to correct absorbance for dilution after adding reagent 2. C is the value of concentration stated in the standard label for acetaldehyde.

ASSAY PARAMETERS FOR ANALYZER DIONYSOS®

Dionysos model	**150**	**240**
Name	ACETALDEHYDE	
Method	End Point A	
Direction	Increasing	
Main Wavelength	340	
Sec. Wavelength	--	
Sample	15	
Reagent 1	220	
Reagent 2	30	
Calibration	Linear	
Blank cycle [150 | 240]	3 - 4	3 - 4
Reading cycle [150 | 240]	20 - 21	31 - 32
Units	mg/L	
Decimals	0	
Measure range	5 ~ 300	
R1 Lim. Abs	12000	
Ratio Dil. Auto.	--	
Vol. Sample Dil. Auto	--	

Procedure is linear up to 300 mg/L. Calibrate with a single point using the highest concentration standard or with several points as per your quality procedures.

PERFORMANCE

Limit of Quantification (LoQ): 5 mg/L
Limit of linearity: 300 mg/L

NOTES

It is recommended to use wine controls to verify quality of calibration. Each laboratory should establish its own quality criteria for acceptance, as well as proper corrective action procedures in case of rejection.

REFERENCES

1. Compendium of International methods of analysis – OIV, Vol1&2 (2008)
2. Bermeyer, HU. Methods of Enzymatic Analysis, 2nd Ed. Vol. 1, p. 112-117. Academic Press, Inc. NY.