




Catechins are a group of flavonoid polyphenols found mainly in seeds and, to a lesser extent, in the skin. They occur naturally as a defence mechanism against berry infections, so their concentration is higher in varieties grown in humid climate. They are responsible for bitter notes in the taste of wine and, because their antioxidant properties, contribute to provide colour stability along the maturation process. The content of catechins in the sample is also directly related to the squeezing process and the period of contact with the grape skins.

## METHOD

In an acid medium, the catechins react specifically with the cinnamaldehyde to give a coloured compound.

The concentration of catechins is proportional to the absorbance at 620 nm.

## CONTENT

R1	1 x 30 mL  	Methanol (>60%), HCl 0.1 M  <i>WARNING: H226: Flammable liquid and vapour. H315: Causes skin irritation. H319: Causes serious eye irritation. P262: Do not get in eyes, on skin, or on clothing. P302+P352: IF ON SKIN: Wash with plenty of water. P305+P351+P338: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses if present and easy to do – continue rinsing.</i>
R2	1 x 30 mL 	Methanol (>60%), Cinnamaldehyde (DMACH)  <i>WARNING: H226: Flammable liquid and vapour. P262: Do not get in eyes, on skin, or on clothing. P305+P351+P338: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses if present and easy to do – continue rinsing.</i>
CTRL	1 x 3 mL	Catechins 100 mg/L (85 – 115 mg/L)
STD	1 x 3 mL	Catechins 250 mg/L

## REAGENT PREPARATION

Reagents are ready to use and are stable up to expiry date as supplied when stored at 2-8 °C. Do not freeze.

Discard if absorbance of blank is higher than 0.300 OD at 620 nm.

## SAMPLES

The samples must be free of turbidity and particles. Centrifuge or filter if necessary. The presence of CO<sub>2</sub> introduces instability in the measure. Samples containing CO<sub>2</sub> must be degassed beforehand. In samples with very high colour intensity, the pigment may interfere with the measurement. Samples with concentration higher than the measurement range must be diluted accordingly with distilled water. Multiply the final result by the dilution factor.

## 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	Sample/Std Reaction
Reagent 1	400 µL	400 µL
Distilled water	30 µL	--
Sample/Standard	--	30 µL

Mix, incubate at 37°C for 1 minutes and read absorbance at 620 nm (A<sub>1</sub>). Then add into the cuvette:

	Blank reaction	Sample/Std Reaction
Reagent 2	400 µL	400 µL

Mix, incubate for 2 minutes at 37°C and read absorbance at 620 nm (A<sub>2</sub>).

Concentration of catechins is calculated as:

$$Catechins = \frac{(A_2 - 0.52 \times A_1)_{sample} - (A_2 - 0.52 \times A_1)_{blank}}{(A_2 - 0.52 \times A_1)_{standard} - (A_2 - 0.52 \times A_1)_{blank}} \times C \text{ g/L}$$

Factor 0.52 is used to correct absorbance for dilution after adding reagent 2. C is the value of concentration stated in the label of standard.

## ASSAY PARAMETERS FOR ANALYZER DIONYSOS®

Dionysos model	150	240
Name	CATECHINS	
Method	End Point A	
Direction	Increasing	
Main Wavelength	620	
Sec. Wavelength	--	
Sample	15	
Reagent 1	200	
Reagent 2	200	
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	6 ~ 500	
R1 Lim. Abs	3000	
Ratio Dil. Auto.	--	
Vol. Sample Dil. Auto	--	

Procedure is linear up to 500 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): 6 mg/L

Limit of linearity: 500 mg/L

## NOTES

Using a control sample on a regular basis provides information on the calibration status and possible deterioration of the reagent. In case of deviations greater than 15% on the target value, it is advisable to check the calibration status of the test.

## REFERENCES

1. Compendium of International methods of analysis – OIV, Vol 1&2 (2008).
2. Bermejer, HU. Methods of Enzymatic Analysis, 2<sup>nd</sup> Ed. Vol. 1, p. 112-117. Academic Press, Inc. NY.
3. International Oenological Codex. OIV-OENO 554-2015

