

# COLORIMETRIC METHOD FOR THE DETERMINATION OF O-DIPHENOLIC COMPOUND IN OLIVE OILS

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## 1.- Material and apparatus

- 1.1.- Equipment for Solid Phase Extracción (SPE)
- 1.2.- Diol-bonded phase cartridges of 6 mL (0,5g) (Waters or similar)
- 1,3.- Syringe filter of cellulose acetate (0.45 - $\mu$ m of pore size, (Alltech, Scharlab or similar)
- 1.4.- Rotary evaporator under vacuum
- 1.5.- UV-vis Spectrophotometer at  $\lambda=370$  nm, provided with quartz absorption cuvettes of 10.0 mm path length.

## 2.- Solvents and reactives

- 2.1.- n-Hexane AR
- 2.2.- Mixture n-hexane/ethyl acetate 90 :10 (v/v)
- 2.3.- Methanol for UV-spectrometry.
- 2.4.- Distilled or deionized water.
- 2.5.- Sodium molibdate dihydrate, AR (Merck or similar).
- 2.6.- Pyrocatechol (catechol) ( $\geq 99\%$ ) (Aldrich or similar).
- 2.7.- Solution methanol/water 1:1 (v/v).
- 2.8.- Solution A. Methanol/water 1:1 acidified with hydrochloric acid prepared by addition of 0,5 ml of 6N HCl to 100 ml of solution methanol/water 1:1 (2.7).
- 2.9.- Solution of 5% sodium molibdate dihydrate in methanol/water 1:1 (2.7).

## 3.- Calibration line

A calibration line (concentration, expressed in millimol/mL, vs absorbance) is obtained by measurement of solutions of pyrocatechol in solution A (2.8). Solutions of pyrocatechol are prepared in the range 0.02 – 0.07 mg/mL. These solutions must be freshly prepared and protected from light exposure. With the aid of a pipette, 2 mL of the phenolic solution are taken and poured into a glass tube. Then, 0,5 mL of sodium molybdate solution (2.9) are added, and the mixture is shaken. The absorbance at  $\lambda=370$  nm is measured. The absorbance of the phenolic solution is amended with the absorbance of a blank obtained by mixing 2 mL of the phenolic solution with 0.5 mL of methanol/water 1:1 (2.7).

Solutions of catechol, hydroxytyrosol, and hydroxytyrosyl acetate gave the following equation for an absorbance range of 0.2 – 0.8:

$$\text{Concentration of } o\text{-diphenols (mmol/mL)} = (-0.170 + 8.236 * \text{ABS}) * 10^{-4}$$

being ABS = absorbance phenolic solution – absorbance of the blank

#### **4.- Isolation of the phenolic extract**

A sample of olive oil ( $6 \pm 0.001$  g) is dissolved in 6 mL of hexane (2.1).

A diol-bonded phase 6-mL cartridge (1.2) is placed in a vacuum elution apparatus, and conditioned by the consecutive passing of 9 ml of methanol (2.3) and 9 ml of hexane (2.1). Then the vacuum is released to prevent drying of the column.

The oil solution is applied to the column and passed through the cartridge. The sample container is washed with two 4-ml portions of hexane that are run out of the cartridge. The sample container is washed again with 3 ml of the mixture hexane/ethyl acetate (90:10, v/v) (2.2) that are run out of the cartridge and discarded. Then, a 25-mL conical flask is placed and the column is eluted with 15 ml of methanol (2.3). The solvent is evaporated in a rotary evaporator at room temperature under vacuum until dryness. The residue is dissolved in 5 mL of acidified solution A (2.8).

#### **5.- Spectrophotometric determination**

With the aid of a pipette provided with a syringe filter of cellulose acetate, 2 mL of the phenolic extract are taken and poured into a glass tube. Then, 0,5 mL of sodium molybdate solution (2.9) are added, and the mixture is shaken. The absorbance at  $\lambda=370$  nm is measured. The absorbance of the phenolic solution is amended with the absorbance of a blank obtained by mixing 2 mL of the phenolic extract with 0.5 mL of methanol/water 1:1 (2.7).

If absorbance of phenolic solution is higher than 0.8, a new phenolic extract must be obtained and solved in 10 mL of solution A (2.8) instead of 5 mL. An alternative is the extraction from 3 g of oil.

If absorbance of phenolic solution is lower than 0.2, the phenolic extracts of two or more cartridges must be combined and solved in 5 mL of solution A (2.8). Using a single cartridge, the minimum concentration determined is 0.15 mmol/kg of oil.

## 5.- Calculations

The concentration of *o*-diphenols in oils, expressed in millimol/kg, is calculated by:

$$[C] \text{ in oil (mmol/kg)} = (C_{\text{extr}} * 2.5 * V_{\text{extr}} * 1000) / 2 * W_{\text{oil}}$$

Being:

$C_{\text{extr}}$  (mmol/mL) = concentration in the extract calculated by application of the absorbance to the calibration line

2.5 = volume (mL) of the reaction mixture

$V_{\text{extr}}$  = total volume (mL) of extract solution (usually 5 mL)

2 = Volume (mL) of extract solution taken for the reaction

$W_{\text{oil}}$  = weight (g) of oil sample.

The results must be expressed in millimol of *o*-diphenols / Kg of oil.