

**COEI-2-HYDCAR Aromatic polycyclic hydrocarbons****Determination of benzo(a)pyrene in oenological charbons by HPLC****1. Principle**

Polycyclic aromatic hydrocarbons including benzo[a]pyrene are extracted by hexane; the solvent is evaporated and the residue is taken up by the methanol-tetrahydrofuran for analysis by HPLC.

**2. Apparatus and reagents****2.1. Reagents and calibrations**

- Acetonitrile for HPLC
- Hexane for pesticide residues
- Tetrahydrofuran for HPLC (THF)
- Deionised and microfiltered water
- Benzo[a]pyrene for HPLC.

**2. Apparatus and chromatographic conditions**

- octadecyl type HPLC column
- fluorimetric detector adjusted to the following detection conditions:
  - excitation wave length: 300 nm,
  - emission wave length: 416 nm.

Mobile phase:

- solvent A: Deionised and microfiltered water
- solvent B: acetonitrile

Variations in the composition of the solvent		
TIME in min	% solvent A	% solvent B

# INTERNATIONAL OENOLOGICAL CODEX

## Benzo[a]pyrene- Determination

---

0	50	50
15	20	80
40	0	100
45	50	50

Flow 1.0 ml/mn

### 2.3. Preparation of reference solutions

Benzo[a]pyrene reference solution at about 100 mg/l in a methanol/THF mixture (50/50) stored for 3 years maximum in cold conditions.

Daughter solution at about 20 µg/l, prepared extemporaneous (0.5 ml of reference solution in 50 ml of methanol/THF then 1 ml of this intermediate solution in 50 ml de methanol/THF).

### 2.4. Preparation of samples

2 g of oenological charbon are mixed in a 50 ml volumetric flask with 30 ml of hexane.

The polycyclic aromatic hydrocarbons are extracted for 5 min using a magnetic stirrer. The organic phase recovered by filtration is gathered in a evaporating flask and evaporated. The extract is taken up by 2 ml of a methanol/THF mixture (1/1, v/v) and injected.

## 3. Results

The benzo[a]pyrene content must not be higher than 1 µg/kg.

Remark: It is also possible to determine benzo[a]pyrene by chromatography in gaseous phase by an apolar capillary column with detection by mass spectrometry.