# 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<br>in min | % solvent A | % solvent B |

| | | |
|---|---|---|
| 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.