



**INTERNATIONAL
OLIVE OIL
COUNCIL**

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“RESOLUTION No. RES-1/93-IV/05

**DETECTION OF POLYCYCLIC AROMATIC HYDROCARBONS IN
EDIBLE OLIVE OILS AND OLIVE-POMACE OILS**

THE INTERNATIONAL OLIVE OIL COUNCIL,

Having regard to the recommendation made by the Committee on Olive Oil Chemistry and Standards Setting at its 6th meeting, held during the 85th session of the Council, pending the toxicological evaluation of polycyclic aromatic hydrocarbons by various international bodies,

Whereas a measure is needed to restore consumer confidence in olive-pomace oil and olive oil,

Whereas operators need a tool for the quality control of the olive-pomace oils that are traded internationally, pending the development of a standard method for the detection of polycyclic hydrocarbons that is applicable to olive oils and olive-pomace oils,

Having regard to the provisions adopted by certain countries on the limits and testing criteria for polycyclic aromatic hydrocarbons,

Whereas the Council chemists have made a recommendation,

DECIDES

To recommend to Members the application of the following for edible olive oils and olive-pomace oils:

- A maximum limit of 2 µg/kg for benzo(a)pyrene content, determined according to ISO standard 15302 “Animal and vegetable fats and oils – Determination of benzo(a)pyrene – Reverse-phase high performance liquid chromatography method”;

The type of method to be used must be sufficiently validated and must meet the following criteria:

Type 1

- Purification system by means of elution of the samples dissolved in the appropriate solvent, through a solid phase.
- Concentration of the extract for analysis.
- Analysis of the extract by reverse-phase high-performance liquid chromatography (HPLC), using a fluorescence detector that can preferably be programmed at excitation and emission wavelengths.
- Confirmation, where applicable, by an alternative, suitably validated method.^(*)

Type 2

- Saponification of the oil and extraction of possible residue with hexane or another suitable solvent.
- Concentration of the extract and re-dissolution in acetonitrile.
- Analysis of the extract by reverse-phase high-performance liquid chromatography, using a fluorescence detector that can preferably be programmed at excitation and emission wavelengths.
- Confirmation, where applicable, by an alternative, suitably validated method.^(*)

Madrid (Spain), 18 November 2005.”

^(*) **According to the parameters of the attached document entitled “Method of analysis to be used by the laboratory and laboratory control requirements“.**

METHOD OF ANALYSIS TO BE USED BY THE LABORATORY AND LABORATORY CONTROL REQUIREMENTS

1. Definitions

A number of the most commonly used definitions that the laboratory will be required to use are given below:

- $r =$ Repeatability, the value below which the absolute difference between two single test results obtained under repeatability conditions (i.e., same sample, same operator, same apparatus, same laboratory, and short interval of time) may be expected to lie within a specific probability (typically 95%) and hence $r = 1.428 \times s_r$.
- $s_r =$ Standard deviation, calculated from results generated under repeatability conditions.
- $RSD_r =$ Relative standard deviation, calculated from results generated under repeatability conditions $[(s_r/x) \times 100]$.
- $R =$ Reproducibility, the value below which the absolute difference between single test results obtained under reproducibility conditions (i.e., on identical material obtained by operators in different laboratories, using the standardised test method), may be expected to lie within a certain probability (typically 95%); $R = 2.8 \times s_R$.
- $s_R =$ Standard deviation, calculated from results under reproducibility conditions.
- $RSD_R =$ Relative standard deviation calculated from results generated under reproducibility conditions $[(s_R/x) \times 100]$, where x is the average of results over all laboratories and samples.

$HORRAT_r$ = the observed RSD_r divided by the RSD_r value estimated from the Horwitz equation (1) using the assumption $r = 0.66R$.

$HORRAT_R$ = the observed RSD_R value divided by the RSD_R value calculated from the Horwitz equation.

U = the expanded uncertainty, using a coverage factor of 2 which gives a level of confidence of approximately 95%.

2. Specific requirements

As no specific methods for the determination of benzo(a)pyrene in olive oils are prescribed at IOOC level, laboratories may select any validated method provided the selected method meets the performance criteria indicated in the Table. The validation should ideally include a certified reference material.

TABLE

Performance criteria for methods of analysis for benzo(a)pyrene

Parameter	Value/comment
Detection limit	No more than 0.3 $\mu\text{g}/\text{kg}$
Limit of quantification	No more than 0.9 $\mu\text{g}/\text{kg}$
Precision	$HORRAT_r$ or $HORRAT_R$ values of less than 1.5 in the validation collaborative trial
Recovery	5%-120%
Specificity	Free from matrix or spectral interferences, verification of positive detection

2.1. Performance criteria – Uncertainty function approach

However, an uncertainty approach may also be used to assess the suitability of the method of analysis to be used by the laboratory. The laboratory may use a method which will produce results within a maximum standard uncertainty. The maximum standard uncertainty can be calculated using the following formula:

$$U_f = \sqrt{[(LOD/2)^2 + (0.2C)^2]}$$

where:

U_f is the maximum standard uncertainty

LOD is the limit of detection of the method

C is the concentration of interest

If an analytical method provides results with uncertainty measurements less than the maximum standard uncertainty the method will be equally suitable to one which meets the performance characteristics given in the Table.

3. Recovery calculation and reporting of results

The analytical result is to be reported corrected or uncorrected for recovery. The manner of reporting and the level of recovery must be reported. The analytical result corrected for recovery is used for checking compliance.

The analytical result has to be reported as $x \pm U$ whereby x is the analytical result and U is the measurement uncertainty.

4. Laboratory quality standards

Proficiency testing

Participation in appropriate proficiency testing schemes which comply with the “International Harmonised Protocol for the Proficiency Testing of (Chemical) Analytical Laboratories” (2) developed under the auspices of IUPAC/ISO/AOAC.

Internal quality control

Laboratories should be able to demonstrate that they have internal quality control procedures in place. Examples of these are the “ISO/AOAC/IUPAC Guidelines on Internal Quality Control in Analytical Chemistry Laboratories” (3).