RESOLUTION OIV-OENO 349-2011

COMPLEMENT TO THE METHOD FOR MEASURING OCHRATOXIN A BY IMMUNOAFFINITY COLUMN OENO 16/2001

THE GENERAL ASSEMBLY

IN VIEW OF Article 2 paragraph 2 iv of the Agreement of 3 April 2001 establishing the International Organisation of Vine and Wine;

IN VIEW OF the works of the OIV sub-commission of methods of analysis and appraisal of wine;

IN VIEW OF resolution OENO 16/2001 concerning the Determination of ochratoxin A by immunoaffinity column and HPLC with fluorescence detection;

IN VIEW OF the need to supplement this method particularly by detailing critical points to be considered;

A. DECIDES to supplement resolution OENO 16/2001 appearing in the Compendium of International Methods of Wine and Must Analysis, with the following points:Point 3 of the method, "REAGENTS", is reorganised as follows:

3. Reagents

13.1. Reagents for separation of the OTA on an immunoaffinity column

The reagents listed below are examples. Suppliers of immunoaffinity columns may offer dilution solutions and eluents suitable for their products. If so, it is preferable to use these products.

3.13.1. Sodium hydrogen phosphate dihydrate($NaH_2PO_4.H_2O$) CAS [10028-24-7]

3.13.2. Sodium dihydrogen phosphate monohydrate ($NaH_2PO_4.H_2O$) CAS [10049-21-5]

3.13.3. Sodium chloride (NaCl) CAS [7647-14-5]

3.13.4. Purified water for laboratories, for example EN ISO 3696 quality (water for analytical laboratory use – Specification and test method [ISO 3696:1987]).

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3.13.5. Phosphate buffer (dilution solution)

Dissolve 60g of Na2HPO4·2H2O (3.1.1) and 8.8g of $NaH_2PO_4.H_2O$ (3.1.2) in 950ml of water and add more water to make up to 1 litre.

3.13.6. Phosphate buffer saline (washing solution)

Dissolve 2.85g of Na2HPO4·2H2O (3.1.1), 0.55g of $NaH_2PO_4.H_2O$ (3.1.2) and 8.7g of NaCl in 950ml of water and add more water to make up to 1 litre.

3.13.7. Methanol (CH3OH) CAS [67-56-1]

13.1. Reagents for HPLC

3.14.1. Acetonitrile for HPLC (CH_3CN) CAS [75-05-8]

3.14.2. Glacial acetic acid (CH_3COOH) CAS [64-19-7]

3.14.3. Mobile phase: water: acetonitrile: glacial acetic acid, 99:99:2, v/v/v

Mix 990 ml of water with 990 ml of acetonitrile (3.2.2) and 20 ml of glacial acetic acid (3.2.3). In the presence of undissolved components, filter through a 0.45µm filter. Degas (with helium, for example) unless the HPLC equipment used includes a degassing step.

13.1. Reagents for the preparation of the OTA stock solution

3.15.1. Toluene ($C_6H_5CH_3$) CAS [108-88-3]

3.15.2. Mixture of solvents (toluene: glacial acetic acid, 99:1, v/v).

Mix 99 parts in volume of toluene (3.3.1) with one part volume of glacial acetic acid (3.2.2).

Points 3.13 and 3.14 are unchanged however their numbering has changed to 3.4 and 3.5.

B. Point 7 of the method, "CALCULATIONS", is modified as follows:

In the formula, the figure "2" is replaced by the variable "F", the dilution factor.

C. The following appendix outlining critical points of the method is appended to the

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method.

APPENDIX

Guide to the critical points of the method of measuring ochratoxin A by immunoaffinity column 16/2001, type II.

The critical points to observe are listed below for information purposes only and are a guide to applying the method. Numbering refers to paragraphs of the original resolution.

1. Field of application

For information purposes only the method can be applied to grape musts, partially fermented grape musts, and new wines still under fermentation. The validation parameters concern wines only.

2. Principle

The method is broken down into two steps. The first step involves purification and concentration of the OTA in the wine or the must by capture on an immunoaffinity column followed by elution. The second step involves quantification of the eluate by HPLC using fluorescence detection.

3. Reagent

3.13. OTA stock solution

The use of OTA in solid form in not recommended; it is recommended to use a standard solution of OTA (point 3.14)

3.14. Standard OTA solution

Use of a commercial solution of standard concentration (around 50 µg/ml) with an analysis certificate stating the reference value and uncertainty of the concentration.

In theory the volume of these solutions is not certified, and they must be sampled with certified pipettes to constitute stock solutions from 0.25 to 1 mg/l in pure ethanol or

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in the mobile phase of the HPLC method (see 3.2.3).

This solution is stable at -18°C for at least 4 years.

4. Equipment

4.13. Recommendations for assessment of the performance of immunoaffinity columns (optional)

The step of concentration on an immunoaffinity column is a major source of inaccuracy in the analysis method. Experience shows that the various columns offered on the market could have recovery rates of between 70 and 100%.

It is therefore recommended to check the performance of a batch of columns before use. This step is recommended where there has been a change in supplier or column references.

Characterisation of the batch of columns (measure of recovery rate):

Select around 10 columns representative of the types of column routinely used in the laboratory, and all from different batch numbers. Prepare the same number of wines representing different matrices, with zero OTA concentrations, with known additions xi of between 0.5 and

2 µg·kg^{-1}. After the known additions quickly analyse these n samples with the batch of selected columns. Let yi be the values found.

The recovery rate data are calculated, the rate being the measured quantity in relation to the known added quantity.

$$t_i = \frac{y_i}{x_i} \ (recovery\ rate\ with\ column\ i)$$

$$T = \frac{\sum t_i}{n} \ (average\ recovery\ rate)$$

$$S_t = \sqrt{\frac{\sum (t_i - T)^2}{n-1}} \ (srtandard\ deviation\ of\ the\ recovery\ rate)$$

The standard deviation of the recovery rate calculated in this way represents not only the variability of the recovery rate of the columns, but also the standard uncertainty of the measurement system used after use of the columns (HPLC). It is nevertheless

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possible to establish a reasonable estimate of the standard deviation of the recovery rate of the columns by deducting the standard uncertainty of the HPLC system from the calculated recovery error:

- Estimate the standard uncertainty S_v (expressed as the standard deviation) of the measurement system in the strict sense of the word (without considering the the immunoaffinity column step).

For this it is possible to use a fidelity study on the OTA solutions.
The standard deviation of the recovery rate Sp is estimated as follows:

$$S_p = \sqrt{S_t^2 - S_V^2}$$

For a fairly wide concentration range, it is preferable to express this value as the coefficient of variation of the standard deviation (RSDR).
CV% = Sp·100/ concentration of the addition

5. Procedure

The procedure outlined in point 5 is an example. The composition of dilution and washing solutions may differ from one column manufacturer to another. Likewise, the concentration of the diluted wine sample may be adjusted as needed.

6. Quantification of ochratoxine A (OTA)

6. 1. Calibration curve

Prepare a calibration curve daily or each time that the chromatographic conditions change. Prepare the curve using solutions produced by diluting the stock solution in the mobile phase (see 3.2.3). The values chosen must provide the working range taking into account the concentration factor of the wine.

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