RESOLUTION OIV-OENO 590-2017

DETERMINATION OF ETHYL CARBAMATE: REVISION OF METHOD OIV-MA-BS-25

THE GENERAL ASSEMBLY,

In view of Article 2, paragraph 2 iv of the Agreement establishing the International Organisation of Vine and Wine,

At the proposal of the “Methods of Analysis” Sub-Commission,

DECIDES to modify Method OIV-MA-BS-25 of the Compendium of International Methods of Analysis of Spirituous Beverages of Vitivinicultural Origin as follows:

Determination of ethyl carbamate

Type IV method

1. Title

Determination of ethyl carbamate in spirituous beverages by gas chromatography/mass spectrometry (GC/MS) coupling.

2. Scope of application

This method applies to different spirituous beverages and spirits of vitivinicultural origin.

3. Principle

The assay is performed by injection:

- of the spirituous beverage diluted to 40% vol. (dry extract less than 20 g/L) directly,
- of an ether extract ,
- of a dichloromethane extract after solid-phase adsorption using an extraction column,

into a chromatograph coupled to a mass spectrometer operating under electron impact, in "Selected Ion Monitoring" (SIM) or "Full Scan (FS)" acquisition mode.

4. Reagents and products

4.1. Reagents

4.1.1. Ethyl carbamate – CAS No.: 51-79-6

4.1.2. Internal standard: propyl carbamate (CAS No.: 627-12-3), butyl carbamate (CAS No.: 592-35-8) or deuterated ethyl-d5 carbamate (CAS No.: 73962-07-9)

4.1.3. Absolute ethanol – CAS No.: 64-17-5

4.1.4. Ultra-pure water

4.1.5. Solid-phase extraction column

4.1.6. Dichloromethane – CAS No.: 75-09-2

4.1.7. Ether – CAS No.: 60-29-7

4.1.8. Sodium sulphate – CAS No.: 7757-82-6

Note: A blank made from a 40% vol. aqueous-alcoholic solution should only have traces of ethyl carbamate below the limit of detection.

4.2. Solutions

4.2.1. Calibration solution (by way of example)

Stock solution: 1 g/L ethyl carbamate in absolute ethanol

Working solution: 10 mg/L dilution in absolute ethanol

Solutions for calibration: 400 µg/L or more, if necessary, in a 40% vol. aqueous-alcoholic mixture

Note: It is also possible to use additional concentration levels and a calibration curve.

4.2.2. Internal standard solution (to be adapted according to the internal standard)

Stock solution: 1 g/L propyl carbamate in absolute ethanol

Working solution: 10 mg/L dilution in absolute ethanol

5. Apparatus

5.1. Everyday laboratory glassware

5.2. Balance with precision of 0.1 mg

5.3. Gas chromatograph coupled to a mass spectrometer

6. Chromatographic conditions (by way of example)

- Injection: 1 or 2 µL in splitless mode (closure of valves for 20-30 sec)
- Injector temperature: 220 °C
- Carrier gas: H2 or He at a constant flow rate, 1 mL He/min for example, to be adapted to the characteristics of the column and carrier gas
- Wax-type polar capillary column (50 m x 0.22 mm), film thickness 0.2 µm or equivalent
- Programming of oven temperature to be adapted according to the matrix and internal standard:

	Increase (°C/min)	Temperature (°C)	Time (min)
Start		50	1.0
Ramp 1	5.0	150	
Ramp 2	20.0	220	10.5

- Transfer line temperature: 250 °C

7. Data acquisition method of the mass spectrometer

- Electron ionisation: 70 eV
- Source temperature: 230 °C
- Acquisition method:
- Selected Ion Monitoring (SIM): m/z = 62, 74 for ethyl, propyl and butyl carbamates, and 64 for deuterated ethyl-d5 carbamate,
- Full Scan (FS): full scanning of ions.

The chromatograms are re-processed with the single ion m/z = 62. The other ions are used to confirm peak purity by taking into account the ratio of their respective intensities.

Note: Certain NP or Hall detectors can be used.

8. Sample preparation

The samples to be analysed are diluted to 40% vol., through the addition of water or ethanol.

9. Procedure

9.1. Spirituous beverage with a dry extract of < 20 g/L

In a 10-mL flask, make up with the following:

- 200 µL of the 10 mg/L working internal standard solution,
- the calibration solution or the sample diluted to 40% vol.

The internal standard concentration of 200 µg/L may be modulated according to the ethyl carbamate content in the matrix to be analysed.

9.2. Spirituous beverage with a dry extract of > 20 g/L

By way of example, the following may be used:

- method (1) which consists of extracting the ethyl carbamate by ether after having saturated the sample using an excess of sodium sulphate intended to fix water,
- method (2,3) which involves fixing carbamates on a solid-phase extraction column followed by elution by dichloromethane and a concentration.

10. Calculations (example with propyl carbamate or butyl carbamate as an internal standard)

For quantification, m/z = 62 is used both for the internal standard and for ethyl carbamate.

10.1. Determination of the response factor

Quantification is carried out based on the response factor (RF) obtained through analysis of the reference solution:

$$RF = \frac{A_{IS}/C_{IS}}{A_{SS}/C_{SS}}$$

where:

A_{IS} is the peak area of the internal standard and CIS its concentration;

A_{SS} is the peak area of ethyl carbamate for the standard solution and CSS its concentration.

10.2. Calculation of the concentrations in the samples

Once the RF value is calculated:

$$C = Fconc. \times RF \times C_{IS} \times \frac{A}{A_{IS}}$$

where:

C is the concentration in the sample, A is the peak area and Fconc. is the concentration factor associated with any dilution.

10.3. Expression of results

The ethyl carbamate is expressed in µg/L, to the nearest whole number.

11. Bibliography

- (1) BERTRAND, A., BARROS, P., "Dosage du carbamate d'éthyle dans les vins et eaux de vie", Connaissance Vigne Vin, 1988, 22 (1) 39-47.
- (2) DENNIS, M. J., HOWARTH, N., MASSEY, R. C., PARKER, I., SCOTTER, M., STARTIN, J. R., "Method for the analysis of ethyl carbamate in alcoholic beverages by capillary gas chromatography", J. AOAC, 1986, 369 193.
- (3) COMPENDIUM OF INTERNATIONAL METHODS OF ANALYSIS OF WINES AND MUSTS – OIV. Ethyl carbamate. Type II Method OIV-MA-AS315-04.