method OIV-MA-AS315-04  

**Ethyl Carbamate**  
(Resolution Oeno 8/98)

Ethyl carbamate analysis in alcoholic beverages: selective detection method by gas chromatography/mass spectrometry  
(Applicable to the determination of ethyl carbamate concentrations between 10 and 200 µg/l).

(Caution: respect safety measures when handling chemical products, ethanol, acetone and carcinogenic products: ethyl carbamate and dichloromethane. Get rid of used solvents in a suitable way, compatible with applicable environmental rules and regulations).

1. **Principle**  
Propyl carbamate is added to a sample as an internal standard, the solution is diluted with water and placed in a 50 mL solid phase extraction column. Ethyl carbamate and propyl carbamate are eluted with dichloromethane. The eluate is concentrated in a rotary evaporator under vacuum. The concentrate is analyzed by gas chromatography/mass spectrometry using selected ion monitoring mode.

2. **Apparatus**  
2.1 Gas chromatograph/mass spectrometer (GC/MS). With selected ion monitoring (SIM), and data handling system. An autosampler is desirable.

2.2 Capillary fused silica column: 30m* × 0.25 mm Ø int., 0.25 µm of Carbowax 20M type.

2.3 Operating conditions: injector 180°C, helium carrier gas at 1 mL/min at 25°C, splitless injection. Temperature program: 40°C for 0.75 min, then program 10°C/min to 60°C, then 3°C**/min to 150°C, post run: go up to 220°C and maintain for 4.25 min at 220°C. The retention time for ethyl carbamate is 23-27 min., that of propyl carbamate is 27-31 min.

GC/MS interface: transfer line 220°C. Mass spectrometer parameters set up manually with perfluorotributylamine and optimized for a lower mass

* For certain wines which are particularly rich, it may be desirable to use a 50m long capillary column.

** For certain wines which are particularly rich, it may be desirable to carry out a temperature program of 2°C per minute.

OIV-MA-AS315-04 : R2009
sensitivity, SIM acquisition mode, solvent delay and time for the start of acquisition 22 min., dwell time/ion 100 ms.

2.4 Rotary evaporator under vacuum or concentration system similar to Kuderna Danish. (Note: the recovery of the ethyl carbamate test sample, (3.7) must be between 90-110% during the process).

2.5 Flask - pear-shaped, 300 mL, single neck, 24/40 standard taper joint.

2.6 Concentrator tube - 4 mL, graduated, with a standard taper 19/22 Teflon coated joint and stopper.

3. Reagents

3.1 Acetone - HPLC quality. Note: Check each batch by GC/MS before use with regard to the absence of response for m/z 62, 74 and 89 ions.

3.2 Dichloromethane - Note: Analyze each batch before use by GC/MS after 200 fold concentration to check the absence of response for m/z 62, 74 and 89 ions.

3.3 Ethanol - anhydrous

3.4 Ethyl carbamate (EC) standard solutions

- (1) Stock solution - 1.00 mg/mL. Weigh 100 mg EC (≥ 99% purity) in a volumetric flask of 100 mL and dilute to mark with acetone.
- (2) Standard working solution - 10.0 µg/mL. Transfer 1 mL of the EC stock solution to a 100 mL volumetric flask and dilute with acetone to the mark.

3.5 n-Propyl carbamate (PC), standard solutions.

- (1) Stock solution - 1.00 mg/mL. Weigh 100 mg PC (reagent quality) in a 100 mL volumetric flask and dilute with acetone to the mark.
- (2) Standard working solution - 10.0 µg/mL. Transfer 1 mL of the PC stock solution to a volumetric flask of 100 mL and dilute with acetone to the mark.
- (3) Internal standard solution PC - 400 ng/mL. Transfer 4 mL of the standard PC working solution to a volumetric flask of 100 mL and dilute with water to the mark.

3.6 EC - nPC standard calibration solutions - Dilute the standard working solutions of EC, 3.4 (2), and PC 3.5 (2), with dichloromethane in order to obtain:

(1) 100 ng EC and 400 ng nPC/mL,
(2) 200 ng EC and 400 ng nPC/mL,
(3) 400 ng EC and 400 ng nPC/mL,
(4) 800 ng EC and 400 ng nPC/mL,
(5) 1600 ng EC and 400 ng nPC/mL.
3.7 Practice sample - 100 ng EC/mL in 40 % ethanol. Transfer 1 mL of the standard EC working solution, 3.4 (2) in a 100 mL volumetric flask and dilute with 40 % of ethanol to the mark.

3.8 Solid phase extraction column - Disposable material, pre-packed with diatomaceous earth, capacity 50 mL.

(Note: Before analysis, check each batch of extraction columns for the recovery of EC and nPC and the absence of response for ions of m/z 62,74 and 89.) Prepare 100 ng EC/mL of test sample 3.7.

Analyze 5.00 mL of the test sample as described in 4.1, 4.2, 5, and 6. The recovery of 90-110 ng of EC/mL is satisfactory. Adsorbents whose particle diameter is irregular can lead to a slow flow that affects the recovery of EC and nPC.

If, after several trials, 90-110 % of the test sample value is not obtained, change the column or use a corrected calibration recovery curve to quantify EC.

To obtain the corrected calibration curve, prepare standard solutions as described in 3.6 by using 40 % ethanol instead of dichloromethane.

Analyze 1 mL of the standard calibration solution as described in 4, 5, and 6.

Establish a new standardization curve by using the EC/nPC ratio of the extracted standards.

4. Preparation of the test sample

Place the test material in 2 separate 100 mL beakers using the following quantities:

4.1 Wines containing over 14 % vol. alcohol: 5.00 mL ± 0.01 mL.

4.2 Wines containing maximum 14% vol. of alcohol: 20.00 mL ± 0.01 mL.

In each beaker, add 1 mL of internal standard PC solution, 3.5 (3) and water, in order to obtain a total volume of 40 mL (or 40 g).

5. Extraction

(Note: Carry out the extraction under a fume hood with adequate ventilation.)

Transfer diluted test portion from 4 to the extraction column.

Rinse the beaker with 10 mL of water and transfer the rinsing water to the column. Let the liquid be absorbed in the column for 4 minutes. Elute with 2 - 80 mL of dichloromethane.

Collect the eluate in a 300 mL pear-shaped flask.

Evaporate the eluate to 2 to 3 mL in a rotary evaporator in a water bath at 30°C (Note: do not let extract evaporate to dryness).

Transfer the concentrated residue to a 4 mL graduated concentrator tube, with a 9 in Pasteur pipette.
Rinse the flask with 1 mL of dichloromethane and transfer the rinsing liquid to the tube.
Concentrate the sample to 1 mL under a slight nitrogen stream.
If an autosampler is used, transfer the concentrate to a vial for GC/MS analysis.

6. GC/MS Analysis

6.1 *Calibration curve* - Inject 1 µl of each calibration standard solution 3.6, into GC/MS. Plot the graph of the EC-nPC area ratio for the response to m/z 62 ion on the y-axis and the quantity of EC in ng/mL on the x-axis (i.e., 100, 200, 400, 800, 1600 ng/mL).

6.2 *EC quantification* - Inject 1 µl of concentrated extract from 5 in the GC/MS system and calculate the EC-nPC area ratio for m/z 62 ion. Determine the concentration of EC (ng/mL) in the extract by using the internal standard standardization curve. Calculate the EC concentration in the test sample (ng/mL) by dividing the quantity of EC (ng/mL) in the extract by the test sample volume 3.7.

6.3 *Confirmation of EC identity*. Determine if the response for m/z 62, 74 and 89 ions appear at the EC retention time. These responses characteristic respectively of the main fragments (M - C₄H₃)⁺ and (M - CH₃)⁺ and molecular ion (M). The presence of EC is confirmed if the relative ratio of these ions does not exceed 20% of the ratios of the EC standard. The extract may need to be further concentrated in order to obtain a sufficient response for the m/z 89 ion.


<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean EC found, ng/g</th>
<th>Recovery of added EC, %</th>
<th>Sᵣ</th>
<th>Sᵣ</th>
<th>RSDᵣ %</th>
<th>RSDᵣ %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wine over 14 % alcohol (v/v)</td>
<td>40</td>
<td>89</td>
<td>1.59</td>
<td>4.77</td>
<td>4.01</td>
<td>12.02</td>
</tr>
<tr>
<td></td>
<td>80</td>
<td>90</td>
<td>3.32</td>
<td>7.00</td>
<td>4.14</td>
<td>8.74</td>
</tr>
<tr>
<td></td>
<td>162</td>
<td>90</td>
<td>8.20</td>
<td>11.11</td>
<td>5.05</td>
<td>6.84</td>
</tr>
<tr>
<td>Wine under 14% alcohol (v/v)</td>
<td>11</td>
<td>93</td>
<td>0.43</td>
<td>2.03</td>
<td>3.94</td>
<td>18.47</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>93</td>
<td>1.67</td>
<td>2.67</td>
<td>6.73</td>
<td>10.73</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>93</td>
<td>1.97</td>
<td>4.25</td>
<td>4.10</td>
<td>8.86</td>
</tr>
</tbody>
</table>