OIV-MA-AS315-04 Ethyl carbamate

Type II method

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 solvants 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[1] □ 0.25 mm □ 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[2]/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 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*

- 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 g/mL. Transfer 1 mL of the EC stock solution to a 100 mL volumetric flask and dilute with acetone to the mark.

5. *n-Propyl carbamate (PC), standard solutions*.

- 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.

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 *n*PC/mL,
- (2) 200 ng EC and 400 ng *n*PC/mL,
- (3) 400 ng EC and 400 ng *n*PC/mL,

- (4) 800 ng EC and 400 ng *n*PC/mL,
- (5) 1600 ng EC and 400 ng *n*PC/mL.

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.
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 *n*PC 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 *n*PC.

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/*n*PC 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 $\pm$ 0.01 mL.

4.2. Wines containing maximum 14% vol. of alcohol: 20.00 mL $\pm$ 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

(*N*ote: 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 x 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-*n*PC 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-*n*PC 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 -$C_2H_3)^+$ and (M - $CH_3)^+$ 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.

7. Method performance.

Sample	Mean EC found, ng/g	Recovery of added EC, %	s_r	S_R	RSD_r %	RSD_R%
Wine over 14 % alcohol (v/v)	40		1.59	4.77	4.01	12.02
	80	89	3.32	7.00	4.14	8.74
	162	90	8.20	11.11	5.05	6.84
Wine under 14% alcohol (v/v)	11		0.43	2.03	3.94	18.47
	25	93	1.67	2.67	6.73	10.73
	48	93	1.97	4.25	4.10	8.86

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

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