RESOLUTION OENO 8/98

DOSAGE OF ETHYL CARBAMATE IN ALCOHOLISED BEVERAGES

THE GENERAL ASSEMBLY,

READ Article 5, paragraph 4 of the International Convention of unification of methods of analysis and appreciation of wine of 13 October 1954,

ON THE PROPOSAL of the Sub-commission of methods of analysis and appreciation of wines,

DECIDES:

To replace in Annex A of the Compendium of international methods of wine analysis, the described method by the following method:

ETHYL CARBAMATE DOSAGE IN ALCOHOLISED BEVERAGES: SELECTIVE DETECTION METHOD BY GAS CHROMATOGRAPHY /MASS SPECTROMETRY

(Applicable to the determination of ethyl carbamate for concentrations comprised 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).

A. Principle of the method :

Propyl carbamate is added to a sample as an internal standard, the solution is diluted with water and placed in a solid phase extraction column of 50 ml. Ethyl carbamate and propyl carbamate are eluted with dichloromethane.

The eluate is concentrated with a rotative evaporator under vacuum. The concentrate is analysed is diluted by gas chromatography (GC), the detection is carried out by mass spectrometry by fragmentometry in SIM (Selected ions monitoring) mode.

B. Apparatus and chromatographic conditions (given as an example)

a. Gas chromatogramme/mass spectrometry(GC/MS) and if necessary a sample filter (passeur) and data treatment system or equivalent.

Certified in conformity Lisbon, 26th June 1998
The Director General of the OIV
Secretary of the General Assembly
GEORGES DUTRUC-ROSSET

Capillar column in grafted silicium 30m* x 0.25 mm * int., 0.25 µm of Carbowax 20M type.

Operational mode: injector 180°C, helium gas vector at 1 ml/min. and 25°C, injection according to the « Splitless / split » method.

Temperature programme: 40°C during 0.75 min., then program 10°C/min. up to 60°C, then 3°C/min. up to 150°C**, go up to 220°C and maintain for 4.25 min. at 220°C. The specific time of retention for ethyl carbamate is 23-27 min., that of propyle carbamate is 27-31 min.

---

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

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

Gas chromatograph/spectrometer (GC/MS) interface: transfer line 220°C. Mass spectrometer parameter set up manually with perfluorotributylamine and optimised for a lower mass sensitivity, SIM acquisition mode, solvent term and time for the start of acquisition 22 min., time of maintenance/ion 100 ms.

b. Rotative evaporator under vacuum or concentration system similar to Kuderna Danish. (NB. the recovery ratio of the ethyl carbamate test sample, C(g) must be comprised between 90-110% during the process).

c. Flask - pear-shaped, 300 ml, unique grinding in neck.

d. Concentration tube - 4 ml, graduated, with a teflon coated joint and a cork.

C. Reactives

a. Acetone - quality LC. NB.: Check each batch before use in GC/MS with regard to the absence of response for m/z 62, 74 et 89 ions.

b. Dichloromethane - NB.: Analyse each batch before use by GC/MS after 200 fold concentration, concentration to check the absence of response for m/z 62, 74 et 89 ions.

c. Ethanol – anhydric

d. Ethyl carbamate (CE) standard solutions

(1) « Mother » solution - 1.00 mg/ml. Weigh 100 mg EC (* 99% purity) in a volumetric flask of 100 ml and dilute with acetone.

(2) Standard working solution- 10.0 µg/ml. Transfer 1 ml of the EC « mother » solution in a volumetric flask of 100 ml and dilute with acetone up to the gauge mark.

e. Propyl carbamate (PC), standard solutions.

(1) « Mother » solution - 1.00 mg/ml. Weigh 100 mg PC (reactive quality) in a 100 ml volumetric flask and dilute with acetone up to the gauge mark.

(2) Standard working solution- 10.0 µg/ml. Transfer 1 ml of the PC « mother » solution in a volumetric flask of 100 ml and dilute with acetone up to the gauge mark.

(3) Internal standard solution PC - 400 ng/ml. Transfer 4 ml of the standard PC working solution in a volumetric flask of 100 ml and dilute with water up to the gauge mark.

f. Standard calibrated solutions EC - PC - Dilute the standard working solutions solutions of EC, (d) (2), and PC (e) (2), with dichloromethane in order to obtain:

(1) (100 ng EC and 400 ng PC)/ml,
(2) (200 ng EC and 400 ng PC)/ml,
(3) (400 ng EC and 400 ng PC)/ml,
(4) (800 ng EC and 400 ng PC)/ml,
(5) (1.600 ng EC and 400 ng PC)/ml.

g. Test sample - 100 ng EC/ml in 40 % of ethanol. Transfer 1 ml of the standard working solutions EC, (d) (2) in a 100 ml volumetric flask and dilute with 40 % of ethanol up to the gauge mark.

h. Solid phase extraction column - Disposable material, pre-packed with diatomed earth, capacity 50 ml.

NB. Before analysis, check each batch of extraction column for the recovery of EC and PC and the absence of response for ions of m/z 62,74 et 89. Prepare 100 ng EC/ml of test sample (g). Analyse 5.00 ml of the test sample as described in D(a), E et F. The recovery of 90-110 ng of EC/ml is satisfactory. Absorbents whose particle diameter is irregular can lead to a slow flow which affects the recovery of EC and 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 (f) by using 40 % ethanol instead of dichloromethane.

Analyse 1 ml of the standard calibration solution as described in D, E et F.

Establish a new standardization curveby using the EC/PC ratio of the extracted standards.

A. Preparation of the test sample

Place the test material in 2 separate bechers of 100 ml using the following quantities:

a. Wines containing over 14 % vol. of alcohol: 5.00 ml * 0,01 ml.

b. Wines containing maximum 14% vol. of alcohol: 20.00 ml * 0,01 ml.

In each becher, add 1 ml of internal standard PC solution, C(e) (3) and water, in order to obtain a total volume of 40 ml (or 40 g).

A. Extraction

Carry out the extraction under the "suction hood" with an appropriate ventilation.

Transfer the preparation carried out in D in the extraction column.

Rince the becher with 10 ml of water and transfer the rincing water in the column.

Let the liquid be absorbed in the column for 4 minutes - Eluate with 2 x 80 ml of dichloromethane.

Collect the eluate in a 300 ml cone-shaped flask.

Evaporate the eluate up to 2 to 3 ml with a water bath rotative evaporator at 30°C (NB.= do not let dry evaporate).

Transfer the concentrate residue in a 4 ml graduated tube, with a Pasteur pipette.

Rince the flask with 1 ml of dicloromethane and transfer the rincing liquid in the tube.

Concentrate the sample to 1 ml under a slight nitrogen stream.

Transfer, if need be, the concentrate in a flask from the sample filter (passeur) for CG/MS analysis.

F. GC/SM Analysis

a. Calibration curve - inject 1µl of each standard standardization solution C (f), in

GC/MS.

Draw the graph of the EC-PC area ratio for the response to m/z 62 ion on the ordinate axis and the quantity of EC in ng/ml on the ordinate axis (i.E. 100, 200, 400, 800, 1600 ng/ml).

b. CE quantification - inject 1µl of concentrated extract of E in the GC/MS system and calculate the EC-PC area ratio for m/z 62 ion. Establish 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 (g).

c. Verification of the EC purity.

Determine if the response for m/z 62, 74 and 89 ions appear during the time of EC retention. These responses are the characters respectively of the main fragments (M - C_2H_2)+ and (M - CH_3)+ and molecular ion (M). The presence of EC is confirmed if the relative proposrtions of these ions does not exceed 20% of the proportions of the EC standard. The extract may need to be reconcentrated in order to obtain a sufficient response for m/z 89 ion.

A. Collaborative Analysis.

Tables 1 to 3 show individual results for the practical training sample and for both types of wine.

The application of the Cochran test led to the elimination of one couple of results, for wine which alcoholic strength is superior to14 % vol. as well as wine which alcoholic strength is inferior or equal to 14 % vol., each coming from a different laboratory.

The relative reproducibility tends to decrease with the increase of ethyl carbamate concentration.

Table 1 : Performance of the method for the determination of ethyl carbamate EC in alcoholic beverages by CG/MS.

Sample	Average EC found ng/g	Recuperation of added EC %	S_r	S_R	$RSD_r\%$	$RSD_R\%$

Wines over 14% vol.	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
Wines below 14% vol.	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