RESOLUTION OIV/OENO 346/2009

ANALYSIS OF BIOGENIC AMINES IN MUSTS AND WINES USING HPLC

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,

FOLLOWING a proposal made by the "Methods of Analysis" Sub-commission

HAS HEREBY DECIDED to add the following type II method to Appendix A of the International Compendium of Methods of Analysis:

Title	Type of method
Analysis of biogenic amines in musts and wines using HPLC	II

1. SCOPE

This method can be applied for analysing biogenic amines in musts and wines:

Ethanolamine: up to 20 mg/l

Histamine: up to 15 mg/l

Methylamine: up to 10 mg/l

Serotonin: up to 20 mg/l

Ethylamine: up to 20 mg/l

Tyramine: up to 20 mg/l

Isopropylamine: up to 20 mg/l

Propylamine: normally absent

Isobutylamine: up to 15 mg/l

Butylamine: up to 10 mg/l

Tryptamine: up to 20 mg/l

Phenylethylamine: up to 20 mg/l

Putrescine or 1,4-diaminobutane: up to 40 mg/l

2-Methylbutylamine: up to 20 mg/l

3-Methylbutylamine: up to 20 mg/l

Cadaverine or 1,5-diaminopentane: up to 20 mg/l
Hexylamine: up to 10 mg/l

2. DEFINITION

The biogenic amines measured are:
Ethanolamine: C_2H_7NO– CAS [141 – 43 – 5]
Histamine: $C_5H_9N_3$ - CAS [51 – 45 – 6]
Methylamine: CH_5N– CAS [74 – 89 – 5]
Serotonin: $C_{10}H_{12}N_2O$– CAS [153 – 98 – 0]
Ethylamine: C_2H_7N C2H7N – CAS [557 – 66 – 4]
Tyramine: $C_8H_{11}NO$ - CAS [60 – 19 – 5]
Isopropylamine: C_3H_9N - CAS [75 – 31 – 0]
Propylamine: C_3H_9N C3H9N – CAS [107 – 10 – 8]
Isobutylamine: $C_4H_{11}N$– CAS [78 – 81 – 9]
Butylamine: $C_4H_{11}N$– CAS [109 – 73 – 9]
Tryptamine: $C_{10}H_{12}N_2$– CAS [61 – 54 – 1]
Phenylethylamine: $C_8H_{11}N$– CAS [64 – 04 – 0]
Putrescine or 1,4-diaminobutane: $C_4H_{12}N_2$– CAS [333 – 93 – 7]
2-Methylbutylamine: $C_5H_{13}N$ - CAS [96 – 15 – 1]
3-Methylbutylamine: $C_5H_{13}N$ - CAS [107 – 85 – 7]
Cadaverine or 1,5-diaminopentane: $C_5H_{14}N_2$– CAS [1476 – 39 – 7]
1,6-Diaminohexane: $C_6H_{16}N_2$– CAS [124 – 09 – 4]
Hexylamine: $C_6H_{15}N$– CAS [111 – 26 – 2]

3. PRINCIPLE

The biogenic amines are directly determined by HPLC using a C_{18} column after O-phthalaldehyde (OPA) derivatization and fluorimetric detection.

4. REAGENTS AND PRODUCTS

4.1. High purity resistivity water (18MΩ·cm)

4.2. Dihydrate disodium hydrogenophosphate - purity ≥ 99 %

4.3. Acetonitrile - Transmission minimum at 200 nm - purity ≥ 99 %

4.4. O-phthalaldehyde (OPA) - Application for fluorescence - purity ≥ 99 %

4.5. Disodium tetraborate decahydrate - purity ≥ 99 %

4.6. Methanol - purity ≥ 99 %

4.7. Hydrochloric acid 32 %

4.8. Sodium hydroxide pellets - purity ≥ 99 %

4.9. Ethanolamine - Purity ≥ 99 %

4.10. Histamine dichlorhydrate - Purity ≥ 99 %

4.11. Ethylamine chlorhydrate - Purity ≥ 99 %

4.12. Serotonin - Purity ≥ 99 %

4.13. Methylamine chlorhydrate – Purity ≥ 98 %

4.14. Tyramine chlorhydrate - Purity ≥ 99 %

4.15. Isopropylamine purity ≥ 99 %

4.16. Butylamine - Purity ≥ 99 %

4.17. Tryptamine chlorhydrate - purity ≥ 98 %

4.18. Phenylethylamine - Purity ≥ 99 %

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4.19. Putrescine dichlorhydrate - Purity $\geq$ 99 %

4.20. 2-Methylbutylamine - Purity $\geq$ 98 %

4.21. 3-Methylbutylamine - Purity $\geq$ 98 %

4.22. Cadaverine dichlorhydrate - Purity $\geq$ 99 %

4.23. 1-6-Diaminohexane - Purity $\geq$ 97 %

4.24. Hexylamine - Purity $\geq$ 99 %

4.25. Nitrogen (maximum impurities: H_2O $\geq$ 3 mg/l; O_2 $\geq$ 2 mg/L; CnHms $\geq$ 0.5 mg/l)

4.26. Helium (maximum impurities: H_2O $\geq$ 3 mg/l; O_2 $\geq$ 2 mg/L; CnHm $\geq$ 0.5 mg/l)

Preparation of reagent solutions:

4.27. Preparation of eluents

Phosphate solution A: Weigh 11.12 g $\pm$ 0.01 g of di-basic sodium phosphate (4.2) in a 50-ml beaker (5.5) on a balance (5.27). Transfer to a 2-litre volumetric flask (5.9) and make up to 2 litres with high purity water (4.1). Homogenize using a magnetic stirrer (5.30) and filter over a 0.45 µm membrane (5.17). Put in the 2-litre bottle (5.12).

Solution B: The acetonitrile (4.3) is used directly.

4.28. OPA solution – Daily preparation

Weigh 20 mg $\pm$ 0.1 mg of OPA (4.4) in a 50-ml flask (5.7) on the precision balance (5.27). Make up to 50 ml with methanol (4.6). Homogenize.

4.29. Preparation of the borate buffer (4.29) – Weekly preparation

Weigh 3.81 g $\pm$ 0.01 g of $Na_2B_4O_7.10H_2O$ (4.5) in a 25-ml beaker (5.6) on the precision balance (5.27). Transfer to a 100-ml volumetric flask (5.8) and make up to 100 ml with demineralised water (4.1). Homogenize with a magnetic stirrer (5.30), transfer to a 150-ml beaker (5.4) and adjust to pH 10.5 using a pH meter (5.28 and 5.29) with 10 N soda

(4.8).

4.30. 0.1 M hydrochloric acid solution: Put a little demineralised water (4.1) into a 2-litre volumetric flask (5.9). Add 20 ml of hydrochloric acid (4.7) using a 10-ml automatic pipette (5.24 and 5.25)

4.31. Calibration solution in 0.1 M hydrochloric acid

Guideline concentration of the calibration solution - weigh at ± 0.1 mg

	Indicative final concentration in the calibration mix in mg/l
Ethanolamine	5
Histamine	5
Methylamine	1
Serotonin	20
Ethylamine	2
Tyramine	7
Isopropylamine	4
Propylamine	2.5
Isobutylamine	5
Butylamine	5
Tryptamine	10
Phenylethylamine	2
Putrescine	12
2- Methylbutylamine	5
3- Methylbutylamine	6
Cadaverine	13
1.6 Diaminohexane	8

Hexylamine	5

The true concentration of the calibration solution is recorded with the batch number of the products used.

Certain biogenic amines being in salt form, the weight of the salt needs to be taken into account when determining the true weight of the biogenic amine.

The stock solution is made in a 100-ml volumetric flask (5.8).

The surrogate solution is made in a 250-ml volumetric flask (5.10).

4.32. 1,6 Diaminohexane internal standard

Weigh exactly 119 mg in a 25-ml Erlenmeyer flask (5.1) on a balance (5.26). Transfer to a 100-ml volumetric flask (5.8) and top up to the filling mark with 0.1 N hydrochloric acid (4.30).

4.33. 2-Mercaptoethanol - Purity $\geq$ 99 %.

5. APPARATUS

5.1. 25-ml Erlenmeyer flasks

5.2. 250-ml Erlenmeyer flasks

5.3. 100-ml beakers

5.4. 150-ml beakers

5.5. 50-ml beaker

5.6. 25-ml beaker

5.7. 50-ml volumetric flasks

5.8. 100-ml volumetric flasks

5.9. 2,000-ml volumetric flasks

5.10. 250-ml volumetric flask

5.11. 1-litre bottles

5.12. 2-litre bottle

5.13. 2-ml screw cap containers suitable for the sample changer

5.14. 50-ml syringe

5.15. Needle

5.16. Filter holder

5.17. 0.45 µm cellulose membrane

5.18. 0.8 µm cellulose membrane

5.19. 1.2 µm cellulose membrane

5.20. 5 µm cellulose membrane

5.21. Cellulose pre-filter

5.22. 1-ml automatic pipette

5.23. 5-ml automatic pipette

5.24. 10-ml automatic pipette

5.25. Cones for 10-ml, 5-ml and 1-ml automatic pipettes

5.26. Filtering system

5.27. Balances for weighing 0 to 205 g at ± 0.01 mg

5.28. pH meter

5.29. Electrode

5.30. Magnetic stirrer

5.31. HPLC pump

5.32. Changer-preparer equipped with an oven

Note: An oven is indispensable, if a changer-preparer is used for injecting several samples one after another. This operation may likewise be done manually) the results may be less precise;

5.33. Injection loop

5.34. 5 µm C18 column, 250 mm ´ 4 (which must lead to a similar chromatogram as presented in annex B);

5.35. Fluorimetric detector

5.36. Integrator

5.37. Borosilicic glass tube with a stopper and closure cap covered with PTFE

(ex Sovirel 15).

6. PREPARATION OF SAMPLES

Samples are previously purged of gas with nitrogen (4.25).

6.1. Filtering

Filter approximately 120 ml of the sample over membrane:

- for a wine: 0.45 µm (5.17),
- for a must or non-clarified wine: 0.45 (5.17) – 0.8 (5.18) – 1.2 (5.19) - 5 µm (5.20) + pre-filter (5.21), pile filters in the following order, the sample pushed by the top: 0.45 µm (5.17) + 0.8 µm (5.18) + 1.2 µm (5.19) + 5 µm (5.20) + prefiltered

(5.21)

6.2. Preparation of the sample

Put 100 ml of the sample (6.1) into a 100-ml volumetric flask (5.8);

Add 0.5 ml of 1-6-diaminohexane (4.32) at 119 mg/100 ml using a 1-ml automatic pipette (5.21 and 25);

Draw off 5 ml of the sample using the pipette (5.23 and 5.25); pour this into a 25-ml Erlenmeyer flask (5.1);

Add 5 ml of methanol to this (4.6) using the pipette (5.23 and 5.25);

Stir to homogenize;

Transfer to containers (5.13);

Start the HPLC pump (5.31), then inject 1 µl (5.32 and 5.33)

6.3. Derivatisation

In a borosilicic glass tune (5.37), pour 2 ml of OPA solution (4.28), 2 ml of borate buffer (4.29), 0,6 ml of 2-mercaptoethanol (4.33). Close, mix (5.30). Open and pour 0,4 ml of sample. Close, mix (5.30). Inject immediately, as the derivitive is not stable. Rinse recipient immediately after injection, due to odour.

Note: Derivatisation can be carried out by an automatic changer-preparer. In this case, the process will be programmed to come close to the proportion of manual derivisation

6.4. Routine cleaning

Syringe (5.13) and needle (5.14) rinsed with demineralised water (4.1) after each sample; filter holder (5.16) rinsed with hot water, then MeOH (4.6). Leave to drain and dry.

7. PROCEDURE

<u>Mobile phase (5.31)</u>

- A: phosphate buffer (4.2)
- B: acetonitrile (4.3)

Elution gradient:

time (in mins)	% A	% B
0	80	20
15	70	30
23	60	40
42	50	50
55	35	65
60	35	65
70	80	20
95	80	20

Note: The gradient can be adjusted to obtain a chromatogram close to the one presented in annex B

Flow rate: 1 ml/min;

Column temperature: 35 °C (5.32);

Detector (5.35): Exc = 356 nm, Em = 445 nm (5.30);

<u>Internal calibration</u>

The calibration solution is injected for each series;

Calibration by internal standard;

Calculation of response factors:

$$RF = Ccis \times area\ is \times Cci$$

Cci = concentration of the component in the calibration solution and

Ccis = concentration of the internal standard in the calibration solution (1-6-diaminohexane).

Area i = area of the product peak present in the sample

Area is = area of the internal standard peak in the sample

Calculation of concentrations:

$$Cci = (XF \times area\ i)/(area\ is \times RF)$$

Area i = area of the product peak present in the sample
Area is = area of the internal standard peak present in the sample
XF = quantity of internal calibration added to samples for analysis
XF = 119 x 0.5/100 = 5.95.

8. EXPRESSION OF RESULTS

Results are expressed in mg/l with one significant digit after the decimal point.

9. RELIABILITY

	r (mg/l)	R (mg/l)
Histamine	0.07x + 0.23	0.50x + 0.36
Methylamine	0.11x + 0.09	0.40x + 0.25
Ethylamine	0.34x - 0.08	0.33x + 0.18
Tyramine	0.06x + 0.15	0.54x + 0.13
Phenylethylamine	0.06x + 0.09	0.34x + 0.03
Diaminobutane	0.03x + 0.71	0.31x + 0.23
2-methylbutylamine et 3-methylbutylamine	0.38x + 0.03	0.38x + 0.03
Diaminopentane	0.14x + 0.09	0.36x + 0.12

The details of the interlaboratory trial with regard to reliability of the method are summarised in appendix A.

10. OTHER CHARACTERISTICS OF THE ANALYSIS

The influence of certain wine components: amino acids are released at the beginning of the analysis and do not impede in detection of biogenic amines.

The limit of detection (LOD) and limit of quantification (LOQ) according to an intralaboratory study

	LOD (in mg/l)	LOQ (in mg/l)
Histamine	0,01	0,03
Methylamine	0,01	0,02
Ethylamine	0,01	0,03
Tyramine	0,01	0,04
Phenylethylamine	0,02	0,06
Diaminobutane	0,02	0,06
2-methylbutylamine	0,01	0,03
3-methylbutylamine	0,03	0,10
Diaminopentane	0,01	0,03

11. QUALITY CONTROL

Quality controls may be carried out with certified reference materials, with wines the characteristics of which result from a consensus or spiked wines regularly inserted into analytical series and by following the corresponding control charts.

Annex A

Statistical data obtained from the results of interlaboratory trials

The following parameters were defined during an interlaboratory trial. This trial was carried out by the Oenology Institute of Bordeaux (France) under the supervision of the National Interprofessional Office of Wine (ONIVINS – France).

Year of interlaboratory trial: 1994

Number of laboratories: 7

Number of samples: 9 double blind samples

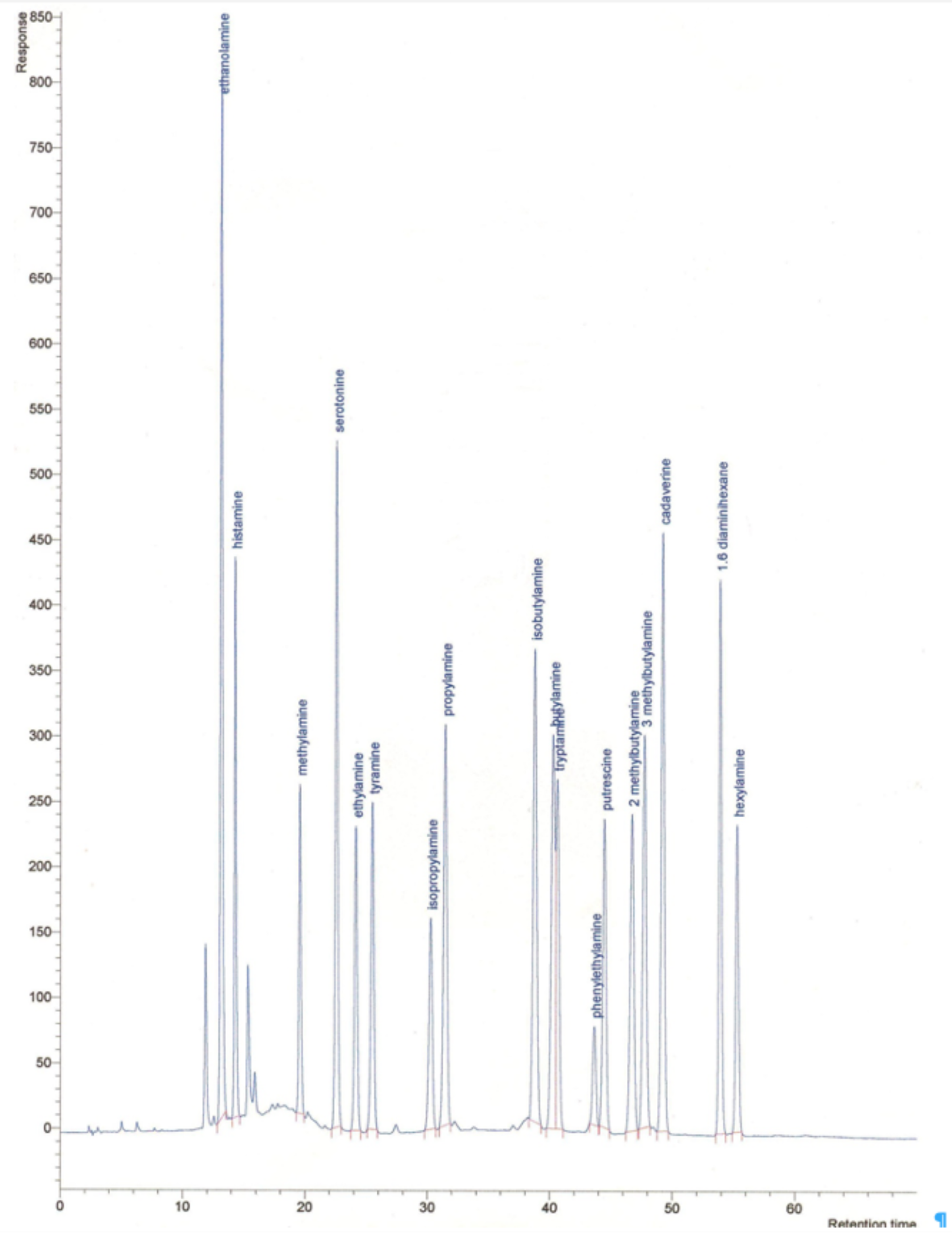
(Bulletin de l'O.I.V. November-December 1994, 765-766, p.916 to 962) numbers recalculated in compliance with ISO 5725-2:1994.

Types of samples: white wine (BT), white wine (BT) fortified = B1, white wine (BT) fortified = B2, red wine n°1 (RT), red wine fortified = R1, red wine (RT) fortified = R2, red wine n°2 (CT), red wine (CT) fortified = C1 and red wine (CT) fortified = C2. fortified in mg/l.

	HistN	MetN	EthN	TyrN	PhEtN	DiNbut	IsoamN	DiNpen
wine B1	wine BT + 0,5	vine BT + 0,12	wineBT + 0,13	wine BT + 0,36	vine BT + 0,15	wine BT + 0,5	wine BT + 0,28	wineBT + 0,25
wine B2	wine BT + 2	wine BT + 0,40	wine BT + 0,50	wine BT + 1,44	wine BT + 0,60	wine BT + 2	Wine BT + 0,1,74	wine BT + 1,04
wine C1	wine CT + 2	wine CT + 0,1	wine CT + 0,18	wine CT + 0,72	wine CT + 0,15	wine CT + 2	wine CT + 0,29	wine CT + 0,26
wineC2	wine CT + 4	wine CT + 0,41	wine CT + 0,50	wine CT + 2,90	wine CT + 0,58	wine CT + 8	wine CT + 1,14	wine CT + 1,04
wine R1	wine RT + 2	wine RT + 0,14	wine RT + 0,13	wine RT + 1,45	wine RT + 0,19	wine RT + 3	wine RT + 0,0,57	wine RT + 0,51
wine R2	wine RT + 5	wine RT + 0,41	wine RT + 0,50	wine RT + 2,88	wine RT + 0,59	wine RT + 10	wine RT + 2,28	wine RT + 2,08

HistN : histamine, MetN : methylamine, EthN : ethylamine, TyrN : tyramine, PhEtN : phenylethylamine, DiNbut : diaminobutane, IsoamN : isoamylamine and DiNpen : diaminopentane.

Annex B : Chromatogram model obtained by this method



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