Analysis of biogenic amines in musts and wines HPLC (Type-II)

OIV-MA-AS315-18 Analysis of biogenic amines in musts and wines using HPLC

Type II method

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
- \bullet 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: C₂H₇N CAS [557 66 4]
- Tyramine: C₈H₁₁NO CAS [60 19 5]
- Isopropylamine: C_3H_9N CAS [75 31 0]
- Propylamine: C₃H₉N CAS [107 10 8]
- Isobutylamine: C₄H₁₁N CAS [78 81 9]
- Butylamine: C₄H₁₁N 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: C₆H₁₆N₂ 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 ($18M\Omega \cdot cm$)
- 4.2. Dihydrate disodium hydrogenophosphate purity \geq 99 %
- 4.3. Acetonitrile Transmission minimum at 200 nm purity \geq 99 %
- 4.4. O-phthalaldehyde (OPA) Application for fluorescence purity \geq 99 %

- 4.5. Disodium tetraborate decahydrate purity \geq 99 %
- 4.6. Methanol purity \geq 99 %
- 4.7. Hydrochloric acid 32 %
- 4.8. Sodium hydroxide pellets purity \geq 99 %

4.9. Ethanolamine - Purity \geq 99 %

- 4.10. Histamine dichlorhydrate Purity \geq 99 %
- 4.11. Ethylamine chlorhydrate Purity \geq 99 %
- 4.12. Serotonin Purity \geq 99 %
- 4.13. Methylamine chlorhydrate Purity \geq 98 %
- 4.14. Tyramine chlorhydrate Purity \geq 99 %
- 4.15. Isopropylamine purity \geq 99 %
- 4.16. Butylamine Purity \geq 99 %
- 4.17. Tryptamine chlorhydrate purity \geq 98 %
- 4.18. Phenylethylamine Purity \geq 99 %
- 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: $H_2O \le 3 \text{ mg/l}$; $O_2 \le 2 \text{ mg/L}$; $C_nH_ms \square 0.5 \text{ mg/l}$)
- 4.26. Helium (maximum impurities: $H_2O \leq 3 \text{ mg/l}$; $O_2 \leq 2 \text{ mg/L}$; $C_nH_m \square 0.5 \text{ mg/l}$)

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 0.01 g of $Na_2B_4O_7$ ·10H₂O (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 \pm 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 \square 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 \pm 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 μ m column, 250 mm \square 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 μ m (5.17) + 0.8 μ m (5.18) + 1.2 μ m (5.19) + 5 μ m (5.20) + prefiltered (5.21)

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

Mobile phase (5.31)

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

Elution gradient:

Time (in mins)	% A	% В
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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);

Internal calibration

The calibration solution is injected for each series;

Calibration by internal standard;

Calculation of response factors:

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

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 \square 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



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



Bibliography

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