AOAC Official Method 999.01
Volatile Bases in Fish

Ammonia Ion Selective Electrode Method
First Action 1999

[Applicable to the determination 8–80 mg of volatile bases/100 g in seafood reported as NH_3.]

See Table **999.01** for the results of the interlaboratory study supporting acceptance of the method.

A. Principle

Ammonia and volatile amines (primarily trimethylamine) are liberated from homogenized fish tissues by addition of an alkaline ion strength adjusting (ISA) solution. The volatilized bases permeate a hydrophobic membrane until an equilibrium is reached with an ammonium ion selective electrode (SIE). The quantity of volatile bases is displayed in terms of the precalibrated ammonia response which is converted to mg (relative) ammonia/100 g based upon dilution factors.

B. Apparatus

(**a**) *pH meter*.—Orion Model 290A portable pH/ISE meter with British naval connector, or equivalent.

(**b**) *Ammonia electrode*.—Orion Model 95-12, or equivalent ammonia gas sensing electrode with ammonia sensor membranes.

(**c**) *Apparatus validation.*—Procedure to validate meter and ammonia electrode operation: (*1*) Verify standard calibration checks per manufacturer's instructions. Check troubleshooting section of meter and/or electrode manual when values are out of range. (*2*) Check repeatability relative standard deviation of instrumentation using the following (1): (*a*) Run replicate analysis of one test sample within the 8–80 mg NH_3/100 g range. Maximum allowable RSD_r is 14%. (*b*) For further confirmation of instrumentation RSD_r, run different test samples in duplicate at various ammonia concentrations within the levels 8–80 mg/100 g and calculate S_r as follows:

$$\sqrt{\frac{\Sigma(\quad)^2}{2}} \times$$

$$RSD_r = \Sigma(S_r/m)$$

where S_r = instrumentation standard deviation; 2 = from duplicate analyses; K = number of sets of duplicates (materials); D = difference between duplicate determinations; and m is the mean of all 2 k values. The maximum allowable RSD_r is 10% based on a 1-sided 95% upper confidence interval. This SD_r is more reflective of the true variation. (*3*) Check accuracy by spiking various test samples at the 10 mg NH_3/100 g (100 ppm) level. The acceptable recovery range is 85–110%.

Table 999.01 Results of interlaboratory study for volatile bases

Sample type	No. labs	No. outliers	Mean NH_3 mg/100 g	Mean % recovery	s_r	s_R	RSD_r	RSD_R	r[a]	R[b]	Horwitz ratio[c]
Cod[d]	9	0	15.4		1.28	2.20	8.3	14	3.58	6.15	1.9
Monkfish[d]	6	3	11.7	88.6	1.32	1.32	11	11	3.70	3.70	1.4
Sole[d]	8	1	8.43		0.87	0.87	10	10	2.44	2.44	1.2
Halibut[d]	9	0	28.0		3.56	3.56	13	13	9.97	9.97	1.9
Squid[d]	9	0	25.6	107	2.00	2.25	7.8	8.8	5.59	6.29	1.3
Cod[d]	8	1	62.3	128	2.58	6.02	4.2	9.7	7.24	16.9	1.6
Squid[d]	9	0	13.1		1.32	1.59	10	12	3.71	4.46	1.6
Monkfish[d]	9	0	20.5		1.41	2.14	6.9	10	3.96	5.99	1.4
Mackerel[e]	9	0	17.3		0.65[f]	1.94	3.8	11	1.82	5.43	1.5
Dog/squid[e]	9	0	23.3		3.98[f]	4.97	17	21	11.1	13.9	3.0
Monkfish	9	0	37.4			4.21		11			1.7
Squid	9	0	82.4			15.0		18			3.1
Dogfish	9	0	48.1			6.33		13			2.1

[a] $2.8 \times s_r$.

[b] $2.8 \times s_R$.

[c] Horwitz ratios between 0.5–2.0 indicate acceptable reproducibility precision.

[d] Blind duplicates.

[e] Youden matched pairs.

[f] s_r as calculated for Youden matched pairs.

Percent recovery (10 mg added NH_3/100 g fish tissue)

ID	1	2	3	4	5	6	7	8	9	Mean % recovery	s_R	RSD_R, %
Cod	118	104	94.1	94.0	156	178	136	Not done	140	128	30.4	23.8
Monkfish	92.0	84.0	92.0	97.0	78.0	104	91.0	Not done	71.0	88.6	10.6	12.0
Squid	110	88.0	106	96.0	118	128	96.0	Not done	110	107	13.0	12.1

C. Reagents

(**a**) *Ammonia standard solutions.*—(*1*) *1000 ppm stock solution*.—Weigh 0.315 g NH_4Cl into 100 mL volumetric flask and dilute to volume with distilled water. Alternatively, pipet 58.8 mL 0.1M NH_4Cl (1700 ppm NH_3), (**c**), into 100 mL volumetric flask. Dilute to volume with distilled water. Solution can be stored for 1 week refrigerated. Keep stock standard solutions refrigerated when not in use. (*2*) *Calibration solutions*.—(*a*) *50 ppm solution*.—Pipet 5 mL 1000 ppm NH_3 solution into 100 mL volumetric flask and dilute to volume with distilled water. (*b*) *20 ppm solution.*—Pipet 2 mL 1000 ppm NH_3 solution into 100 mL volumetric flask and dilute to volume with distilled water. (*c*) *5 ppm solution.*—Pipet 0.5 mL 1000 ppm NH_3 solution into 100 mL volumetric flask and dilute to volume with distilled water.

(**b**) *Ionic strength adjuster (ISA)*.—5M NaOH, 0.05M disodium EDTA, and 10% (v/v) methanol in distilled water. To prepare 200 mL ISA solution, weigh 3.72 g disodium EDTA into 200 mL volumetric flask and add 20 mL methanol. Weigh 40 g NaOH in 500 mL beaker and dissolve in 100 mL distilled water. Quantitatively transfer to 200 mL volumetric flask containing EDTA and methanol solution and dilute to volume with distilled water.

(**c**) *Electrode filling solution*.—0.1M NH_4Cl. Dissolve 5.349 g NH_4Cl in water and dilute to 1 L with distilled water.

D. Preparation of Ammonia Electrode

Prepare electrode according to the manual. Place membrane over outer tip of electrode casing similar to placing Parafilm over test tube opening. Avoid touching the membrane that will come in contact with the inner reference solution and the external test and standard solutions. Tweezers may help. Screw on external protector tip that holds membrane in place. Using Pasteur pipet, add NH_3 electrode filling solution, **C**(**c**), to inner electrode. Insert reference electrode into casing with membrane on the tip and screw electrode into casing. Some filling solution will overflow but this ensures enough solution has been added. Allow membrane to equilibrate overnight in 100 ppm NH_3 solution prepared by diluting 10 mL reagent, **C**(**a**)(*1*), to 100 mL with distilled H_2O in volumetric flask.

E. Calibration of ISE Meter

Calibrate ISE meter according to manufacturer's instruction using separate 100 mL aliquots of the 5 and 50 ppm calibration solutions, **C**(**a**)(*2*)(*b*), in 250 mL beakers followed by 100 mL of a 20 ppm control solution. Recalibrate the instrument if reading is outside 18–22 ppm.

F. Preparation of Test Material

Fillet whole fish without skin. Grind or blend a representative number of thawed fillets in a food processor or grinder until thoroughly minced (paste-like). If fish are frozen, run cold water over fillets placed in a plastic tray until no longer rigid, then grind. Weigh 5 g test portion for analysis or store refrigerated (3–5°C) in a sealable plastic bag for testing later that day. Freeze test portion in bag at –15°C if testing cannot be accomplished on same day.

G. Determination

Weigh 5.0 ± 0.1 g comminuted fish in 250 mL blender. Record weight to 0.1 g. Add 95 mL distilled water. Blend at high speed for 2 min. Transfer test mixture into 250 mL beaker and cover beaker with aluminum foil or Parafilm until measurement. Prepare 4–5 tests at a time for analysis. Run a 20 ppm control after every fifth test. If reading of control is between 18–22 ppm, more tests can be analyzed without recalibration. If reading is outside these values, recalibrate with freshly prepared 5 and 50 ppm standards and verify with fresh 20 ppm control. Add 2.0 mL ISA one test portion at a time before placing electrode in test solution. Do not add ISA to all blended suspensions prior to batch analysis; otherwise, NH_3 will be given off prematurely.

For routine percent recovery determination, weigh 5.0 ± 0.1 g comminuted fish in 250 mL blender jar with 94 mL deionized water, then pipet 1 mL of 1000 ppm NH_3 standard solution, **C**(**a**)(*1*), to represent a 20 mg/l00 g spike. Blend for 2 min at high speed and continue as above.

H. Calculations

$$\text{mg relative } NH_3/100 \text{ g} = (100/\text{Wt})(\text{ppm})(1 \text{ mg}/1000 \text{ ppm})(100) = 2 \text{ ppm if Wt} = 5.0 \text{ g comminuted fish tissue}$$

where Wt = weight of test portion in g (5.0); ppm = direct ISE meter reading; 1000 = conversion of ppm to mg; 100 = conversion to 100 g test portion.

$$\text{Recovery, \%} = -(\times 100$$

where S = spike equivalent in mg NH_3/100 g; S = 100/Wt when using 1 mL 1000 ppm spiking standard.

The term "relative" NH_3 indicates that the value reflects ammonia plus any TMA that permeates the membrane and is sensed by the electrode. Therefore, the electrode acts as a total volatile base probe measuring ammonia and trimethylamine.

References: (1) *J. AOAC Int*. **81**, 1011(1998).
J. AOAC Int. **83**, 933(2000).

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