Application Note – N°. 847/2024

Determination of Total Volatile Basic Nitrogen (TVB-N) in fish

Abstract: An easy and reliable method for the determination of the Total Volatile Basic Nitrogen (TVB-N) in fish sample is introduced. A protein-free extract of a certified, homogeneous fish sample is prepared with 0.6 N perchloric acid and an aliquot of the extract is taken. The distillation and boric acid titration are performed with the MultiKjel in combination with the Metrohm Eco Titrator. The obtained results of 49.96 mg/100 g correspond well to the certified reference values (52.49 ± 5.35 mg/100 g) with low relative standard deviations (0.27 %).

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1. Introduction

An easy and reliable method for the determination of the Total Volatile Basic Nitrogen (TVB-N) in fish sample is introduced below. This method is compliant with the EC guideline (EC No. 853/2004) [1] and ISO 19615 [2].

TVB-N compounds include ammonia, dimethylamine and trimethylamine and are a product of amine degradation by microorganisms. Therefore, they are used as an indicator for the freshness of a product. Naturally, these compounds increase with storage time and finally affect a product with the typical pungent smell of a spoiled product. The determination of TVB-N is of great importance to ensure freshness and therefore quality of a product.

In this application note, a protein-free extract of a certified, homogeneous fish sample is prepared with 0.6 N perchloric acid and an aliquot of the extract is taken. The distillation and boric acid titration are performed with the MultiKjel in combination with the Metrohm Eco Titrator.

2. Equipment

- MultiKjel with Eco Titrator and recirculating chiller F-314 (11K36531211).
- Mixer e.g. BUCHI Mixer B-400.
- Analytical balance (accuracy ± 0.1 mg).
- Beakers.
- Bulb pipettes – 20 mL, 50 mL.
- Single channel pipette e.g. Eppendorf, 1 mL.
- Volumetric flask 50 mL.

3. Chemicals and Materials

- Perchloric acid 60 %, VWR (20583.260).
- Hydrochloric acid 0.01 mol/L, volumetric solution, titer = 0.0969 Roth (N075.1).
- Ammonium dihydrogen phosphate (ADP) 99.99 %, Merck (1.01440.0050).
- Phenolphthalein solution 1 %, Riedel-de-Haën (34607).
- Sodium hydroxide 32 %, Brenntag (81980-452).
- BUCHI ready to use 2 % boric acid pH 4.65 with Sher indicator (11064972).

For a safe handling please pay attention to all corresponding MSDS!

When working with perchloric acid, necessary caution and preventive measures should be taken – e.g. use PVDF as sealing material for the Mixer B-400.

Reference solution (ADP):

- 0.8982 g ADP were weighed into a 50 mL volumetric flask and filled to mark with deionized water.

Certified reference material:

- Biogene Amine (2023) in Fischhomogenat (LVU, 79336 Herbolzheim), certified TVB-N content: 52.49 ± 5.35 mg/100 g.

4. Procedure

The determination of TVB-N in fish samples includes the following steps:

- Homogenization of the sample.
- Deproteinization of the sample using perchloric acid.
- Distillation and titration of the sample, using MultiKjel with Metrohm Eco Titrator.

4.1 Homogenization of the sample

1. Take a fish sample of at least 100 g (preferably a total fillet) and homogenize thoroughly by cutting.

   Note: From frozen fish, e.g. fillet blocks, cut a 100 – 200 g sample of approx. 2 cm thickness, place it in a water-tight plastic bag and thaw, e.g. by immersing the bag in a gently stirred water bath at about 20 °C but not more than 25 °C; thaw time: approx. 15 min.
   Bigger skin and bone pieces should be removed prior to homogenization.

4.2 Deproteinization of the sample using perchloric acid

2. Add 90.0 mL 0.6 N perchloric acid solution into the mixing vessel using bulb pipettes.
3. Weigh 10.0 g from the well-homogenized fish sample into the mixing vessel (indirect weighing technique). Note down the exact sample weight.
4. Blend for two minutes with a high-speed mixer, e.g. with the BUCHI B-400 Mixer.
5. Filter the suspension with a filter paper e.g. Whatman 595 1/2.

   Note: The extract can be kept for at least seven days if stored at 2 °C – 6 °C.

4.3 Distillation and titration

6. Add 50.0 mL extract, obtained from section 4.2, into a sample tube using a 50 mL bulb pipette.
7. Optionally, a few drops of phenolphthalein solution can be added to check for sufficient alkalization.
8. Blank preparation: 50 mL perchloric acid are used as blanks.
9. Reference: Add 1 mL reference solution and 50.0 mL perchloric acid into a sample tube.
10. Distil the samples according to the parameters listed in Table 1.

Table 1: Parameters for distillation and titration with MultiKjel and Eco Titrator.

Method parameters MultiKjel		Instrument settings	
Reaction detection	Off	MaxAccuracy mode	On
H_2O volume	0 mL	Chiller/tap water	Chiller F-314
NaOH volume	4 mL	Chiller temperature	10 °C
Reaction time	5 s	AutoDist mode	On
Steam steps	Fixed time		
Steam power	90 %	Automated Titration on Eco Titrator	
Level detection	Off	Eco Titrator Method	
Distillation time	300 s	Titrant	HCl 0.01 M
Stirrer Speed distillation	5	Sensor type	Potentiometric
Titration type	Boric acid titration	Method	Nitrogen (N)
H_3BO_3 volume	60 mL (2 %	Endpoint	pH = 4.65
Stirrer speed titration	8		
Titration start time	300 s		
Sample tube aspiration	20 s		
Reciever aspiration	20 s		

4.4 Calculation

TVB-N concentration is expressed in mg/100 g sample. The following equation (1) is used to calculate the results. For the reference substance, the following equations (2), (3) and (4) are used to calculate the recovery.

$$TVBN = \frac{(V_{Sample} - V_{Blank}) \cdot z \cdot c \cdot f \cdot MN \cdot 100}{m_{Sample} \cdot k_1} \quad (1)$$

$$\%N = \frac{(V_{Sample} - V_{Blank}) \cdot z \cdot c \cdot f \cdot MN \cdot 100}{m_{Ref}} \quad (2)$$

$$\%N_{ADP} = \frac{\%N}{P} \quad (3)$$

$$\%\mathrm{Rec} = \frac{\%N_{ADP}}{\%N_{Ref}} \quad (4)$$

TVBN: TVB-N concentration, expressed in mg/100 g sample.
V_{Sample}: Amount of titrant for the sample [mL].
V_{Blank}: Mean amount of titrant for the blank [mL].
z: Molar valence factor (1 for HCl, 2 for H_2SO_4).
c: Titrant concentration [mol/L].
f: Titrant factor (for commercial solutions normally 1.000).
MN: Molecular weight of nitrogen (14.007 g/mol).
m_{Sample}: Sample weight [g].
k_1: Correction factor of sample weight due to procedure (k_1 = 5/9).
m_{Ref}: Sample weight of reference material [mg].
%N: Percentage of weight of nitrogen.
$\%N_{ADP}$: Percentage of weight of nitrogen corrected for the purity of reference substance. ammonium dihydrogen phosphate [%].
P: Purity of the reference substance tryptophan [%].
$\%N_{Ref}$: Theoretical value of nitrogen for the reference substance (ADP: 12.18 %).
%Rec: Recovery of reference substance [%].

5. Result

5.1 Recovery of ammonium dihydrogen phosphate

The results of nitrogen determination and recovery for ammonium dihydrogen phosphate (ADP) analysis (assay > 99 %) are presented in Table 2. The nominal value of ADP is 12.18 % nitrogen. The recoveries are within the specification of 98 – 102 %.

Table 2: Results of the recovery of nitrogen in ammonium dihydrogen phosphate.

ADP	mSample [g]	V_{Sample} [mL]	%NTry	Recovery [%]
Sample 1	0.017964	16.576	12.32 %	101.17 %
Sample 2	0.017964	16.616	12.35 %	101.43 %
Average [%]	-	-	12.33 %	101.30 %
Rsd [%]	-	-	0.3	0.18

The mean blank volume (V_{Blank}) was 0.727 mL (n = 3).

5.2 TVB-N determination in fish

The results of the TVB-N determination in fish products are presented in table 3.

Table 3: Results of the determination of TVB-N in fish (certified content 52.49 ± 5.35 mg/100 g).

Fish (CRM)	m_{Sample} [g]	V_{Sample} [mL]	Nitrogen [mg]	TVB-N [mg/100 g]
Sample 1	10.018	20.6800	2.786	50.06
Sample 2	10.194	20.9530	2.824	49.87
Average [%]	-	-	2.805	49.96
Rsd [%]	-	-	0.27	0.27

The mean blank volume (V_{Blank}) was 0.727 mL (n = 3).

6. Comparison to Standard Methods

This application note is based on EC No. 853/2004 [1] with minor differences. These differences are shown in table 4.

Table 4: EC No. 853/2004 and ISO 19615.

	Application note	EC No. 853/2004	Notes/Impace
Antifoam agent	No antifoam agent used	Silicon oil	No impact, less working steps.
Sodium hydroxide	4 mL, 32 w %	6.5 mL, 20 w %	No impact, equivalent amount of NaOH.
Distillation	5 minutes at 90 % steam power	10 Minutes	No impact, low RSD indicates complete distillation.
Boric acid	60 mL, 2 w %	100 mL, 3 %	Less consumption of boric acid, no impact on the results.
Sodium hydroxide	60 mL (Conc. 32 %)	65 mL (Conc. 40 %)	No impact, same ratio of sodium hydroxide/sulfuric acid.
Rinse step after distillation	Omitted when using fully automated systems e.g. BUCHI MultiKjel with Metrohm Eco Titrator	Rinsing of outlet tube with water.	Less working steps, more automation.
pH endpoint	4.65	5.0	Endpoint must match the initial pH of the boric acid. In this application note, BUCHI's ready to use 2 % boric acid solution with a pH 4.65 was used. No impact on the end result.
Reference substance	Ammonium dihydrogen phosphate	Ammonium chloride	No impact. Release of ammonia when alkalized.

7. Conclusion

The determination of TVB-N in fish products using the Kjel Line systems (e.g. MultiKjel) with potentiometric titration provides reliable and reproducible results. These results correspond well to the certified reference values of 52.49 ± 5.35 mg/100 g with low relative standard deviations. The average recovery with ammonium dihydrogen phosphate was 101.30 %, which was within the specification of 98 – 102 %.

By coupling with Eco-Tirator to the distillation unit, MultiKjel systems offer easy automation without any manual handling after distillation and titration. Distillation with BasicKjel/EasyKjel followed by separate titration provides a cost-friendly alternative with equally quantitative results.

8. References

[1] Official Journal of the European Communities, No 853/2004.
[2] ISO 19615 Meat and fish products - Determination of volatile basic nitrogen.

Application Note K355-006, Determination of Total Volatile Basic Nitrogen (TVB-N) in fish and shrimps.
Operation Manual of K-365 Kjel Line