Freshness determination of fish and fish products through total volatile basic nitrogen detection

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Abstract
Fish and fish products are considered suitable for human consumption provided that, following a chemical control, the value of the Total Volatile Basic Nitrogen (TVB-N) is appropriate. The reference method applied according to REG.CE 2074/2005 establishes the degree of freshness and is used for concentrations between 5 mg/100 g and at least 100 mg/100 g. The present study has aimed at the determining of the performance criteria for the method’s validation at the DSVSA laboratory – Brasov. The determinations were made by using chemically-certified re-actives and metrologically-tested equipment.

Keywords: TVB-N, degree of freshness, reference method, performance criteria, validation

Rezumat
Peştele şi produsele pescăreşti sunt considerate proprii consumului uman în cazul în care, în urma controlului chimic, valoarea atribuită Azotului basic volatil total (ABVT) este corespunzătoare. Considerată metodă de referință, în conformitate cu REG.CE 2074/2005, metoda stabilește gradului de prospetime și se aplică pentru concentrații cuprinse între 5 mg/100 g și cel puțin 100 mg/100 g. Studiul de față a urmărit stabilirea criteriilor de performanță pentru validarea metodei din cadrul laboratorului DSVSA – Brașov. Determinările au fost realizate utilizând reactivi certificați, chimic puri și aparatură verificată metrologic.

Cuvinte cheie: ABVT, grad de prospetime, metodă de referință, criterii de performanță, validare.

1. Introduction
Fish is a main food product in a healthy diet due its complex chemical compounds. The fish contains a lot of high-quality proteins, fats made up of polyunsaturated acids (Omega 3), vitamins (A and D) and minerals (phosphorus, iron, calcium etc.), and is recommended to both children and elderly, healthy or unhealthy persons (Banu & Ionescu, 2009).

In order to be consumed, a chemical control is obligatory for fish and fish products, so that its freshness degree can be determined. According to REG.CE 2074/2005 this is represented by the Total Volatile Basic Nitrogen (TVB-N). The method can be applied to concentrations between 5 mg-100 mg/100 g, and is a reference method.

According to the new regulations regarding the accreditation of laboratories, every analytical determination has to have a validation protocol. The aim of the validation protocol is to determine the method’s performance criteria, and the calculation of the measurement uncertainty.

2. Materials and methods
The present study for the method’s validation has been made in DSVSA laboratory – Brasov, at the Department of Food Chemistry. The working method is shown in Table 1 (work protocol).

The determinations have been made by using chemically-certified chemicals, and metrologically-tested equipment.
2.1. Chemicals used to perform measurements
- perchloric acid = 0.6g / 100 mL;
- KOH, 20 g / 100 mL;
- HCl 0.01n
- boric acid 4 %;
- 1% phenolphthalein in 95% ethanol.

<table>
<thead>
<tr>
<th>Table 1. – Work Protocol TVB-N</th>
</tr>
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<tbody>
<tr>
<td><strong>Stages / Procedures</strong></td>
</tr>
<tr>
<td>I. Extract obtaining</td>
</tr>
<tr>
<td>Weighing sample (m)</td>
</tr>
<tr>
<td>Mixing with perchloric acid solution</td>
</tr>
<tr>
<td>Mixture</td>
</tr>
<tr>
<td>Filtering</td>
</tr>
<tr>
<td>Extract conservation</td>
</tr>
<tr>
<td>II. Distillation</td>
</tr>
<tr>
<td>a) in the extraction tube</td>
</tr>
<tr>
<td>extract</td>
</tr>
<tr>
<td>KOH 20%</td>
</tr>
<tr>
<td>b) in the collecting recipient</td>
</tr>
<tr>
<td>boric acid 4%</td>
</tr>
<tr>
<td>Time distillation / collecting</td>
</tr>
<tr>
<td>III. Titration</td>
</tr>
<tr>
<td>HCl 0.01n</td>
</tr>
</tbody>
</table>

2.2. Materials and equipment
All chemical solutions have been prepared in type A flasks.

The distillation of the obtained extracts has been made in a VAPODEST distillation system, type Vap 30, with setting options for reaction and using time. The titration has been realised up to a value of pH = 5.0±0.1 units.

2.3. Statistical calculation
TVB-N = 0.14×(V – VM)×2×100 / m [mg/100g]
where:
VM is the volume of the blank sample used for titration; which must not exceed 0.2 mL;
2 – dilution factor;
m – mass of used sample.

2.3.1. Arithmetic mean
\[ X = \frac{\sum X_i}{n} \]
where:
X is the arithmetic mean;
Xᵢ are the results;
n is the number of determinations carried out

2.3.2. Standard deviation
Standard deviation is calculated using the equation:
\[ S_D = \sqrt{\frac{\sum (X_i - X)^2}{n-1}} \]
where:
SD is the standard deviation

2.3.3. Relative standard deviation
\[ \text{RSD}% = \frac{S_D}{X} \times 100 \]
where RSD is the relative standard deviation

2.3.4. Measurement uncertainty
\[ U = \sqrt{U_{c/m}^2 + U_{C}^2 + U_{R}^2 + U_{GR}^2} \]
\[ U_{c/m} = \sqrt{U_{C}^2 + U_{sol}^2 + U_{doz}^2} \]
\[ U_{GR} = \sqrt{U_{pur.subst.}^2 + U_{rec}^2 + U_{REC}^2} \]
\[ U_e = k \times U \]
where:
Uᵢ is the combined uncertainty;
U – the compounds of method uncertainty;
Uₑ – the expanded uncertainty;
Uᵢ – the weighing uncertainty;
Uᵢsol – the solution preparation uncertainty (boric acid; KOH, HCl, etc);
Uᵢdoz – is the solution dosage uncertainty (boric acid; KOH, HCl, etc);
Uᵢ – is the uncertainty according to the recurrence test / testului de repetabilitate;
Uᵢ – the uncertainty according to the internal reproductibility test;
UᵢGR – the uncertainty according to the retrievability degree;
Uᵢpur.subst. – the uncertainty given by the substance’s purity (NH₄)₂ SO₄;
Uᵢrec – the uncertainty given by the retrievability degree of the Vapodest system;
UᵢREC – the uncertainty given by the retrievability degree of the sample;
k – Student factor.

According to the tests: n = 1–10, the Student factor has the probability of 95%, k = 2.

2.3.5. Retrievability Degree
\[ \% \text{retrievability} = \frac{C_1 - C_3}{C_3} \times 100 \]
where:
C₁ is concentration measured in fortified sample;
C₂ – concentration measured in unfortified sample;
C₃ – fortifiable concentration.

3. Results and discussion

As working matrix we have chosen a hake sample. The average laboratory sample/average laboratory sample (homogenized) has been made from a quantity of 300 g. This has been delimited by working samples of ca. 10 g which were deposited in freezing conditions until their determination.

The validation is considered a process by which the performance criteria are established and a method’s limitations are traced. The validation’s purpose is to establish, by means of systematic laboratory studies, that the method is adequate, and that corresponding results can be obtained.

The performance criteria chosen for the method’s validation are:
– recurrence
– internal reproductibility
– retrievability degree
– expanded uncertainty

After the determination, the obtained results have been statistically processed and the following has been calculated: average (X), standard deviation (Sd), relative standard deviation (RSD).

3.1. Recurrence (r)

Ten concurrent determinations have been carried out by the same analyst from the work matrix. The results of two concurrent analysed samples have to be placed between ± 2 mg/100g (table 2)

3.2. Internal reproductibility (R)

Three different analysts have worked independently on different days of the week, on 5 determinations each taken from the same matrix (table 2).

Table 2. Statistical results for r and R

<table>
<thead>
<tr>
<th>Performance criterium</th>
<th>X</th>
<th>Sd</th>
<th>RSD%</th>
</tr>
</thead>
<tbody>
<tr>
<td>r</td>
<td>18,63</td>
<td>0,546</td>
<td>2,9</td>
</tr>
<tr>
<td>R</td>
<td>18,82</td>
<td>0,618</td>
<td>3,29</td>
</tr>
</tbody>
</table>

3.3. Retrievability degree (GR)

A) System retrievability: according to the ANSVSA nr. 51/2005 regulation this has to be placed between 95 – 105%. Should this be between 98–102% correction is not applicable.

The establishing of the system retrievability degree is done when the factors of average solutions are being prepared and verified within approximately two weeks.

Therefore, a stock solution (NH₄)₂ SO₄ (purity 99,0%) with a concentration of 17 mgNH₃/100 mL, from which five determinants have been taken as samples. The working time (for boiling) of the systems VAP 30 was programmed for: boiling time 645 and boiling power 90%.

The average results obtained are: 98,25%

B) Sample retrievability degree

It can be determined when the obtained results exceed the limit permitted by the standards in force, and when new solutions are being prepared (approximately once a month).

In order to determine the sample retrievability degree, ammonium has been added to the sample as a stock solution of ammonium sulphate so that, at the end of the determination, the added quantity of ammonium could be retrieved. Ten average samples and ten samples with an addition of (NH₄)₂ SO₄ have been used according to the method. For each determination the retrievability degree has been calculated, followed by the average.

The average results for the determinations carried out were: 98,84%.

3.4. Measurement uncertainty (Ue)

The components of the compound uncertainty are: combined uncertainty; uncertainty according to the recurrence test; uncertainty according to the internal reproductibility test; uncertainty according to the retrievability test. By applying the statistical calculation the following has resulted:

Compound Uncertainty – 0.0505
Expanded uncertainty – 0.101

4. Conclusions

According to REG.CE 2074/2005 the degree of freshness is determined by the value of the Total Volatile Basic Nitrogen (TVB-N)

The maximum value permitted for TVB-N is 35 mg nitrogen/100g fish for the Merlucciidae species, which means that the analysed sample is adequate from a freshness degree viewpoint with a
value of 18.63 mg nitrogen/100g (Reg. CE 2074/2005);
Following the statistical calculation, the obtained results correspond to the established performance criterion: $r, R, GR, U$. After the analysis of the results obtained for all the performance parameters mentioned above, it has been concluded that: the total volatile basic nitrogen (TVB-N) detection of fish and fish products can be successfully used in the application field as it fulfils all validation conditions.

References


* * * Commission Regulation (EC) no 2074/2005 - Annex II of Total Volatile Basic Nitrogen (TVB-N) limit values for certain categories of fishery products and analysis methods to be used.

* * * Regulamentul ANSVSA 51/2005 – Norma sanitary-veterinară de implementare a măsurilor de supraveghere și control al unor substanțe și a reziduurilor acestora la animalele văi și la produsele lor privind performanța metodelor analitice și interpretarea rezultatelor.