Quality control of the analysis of histamine in fish by proficiency test

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Abstract: The analysis of histamine is required by the European Union for the importation of tuna and other Scombroid fish. The aim of this study was to investigate the quality of the analysis of histamine in fish, by means of reference material (RM) and of proficiency test (PT). Sample analysis carried out using RM provided 89.4% recovery. During the proficiency test, the histamine content of the sample was 311.9 mg/kg and the z-score was zero. These results assure the good performance of the laboratory in the analysis of histamine in fish, assuring reliability of results to clients.

1. Introduction

Accredited laboratories and non-accredited ones which care about the quality of the testing results, often participate in national and/or international quality control programs. The analytical quality control can be undertaken by means of Certified Reference Materials (CRM) or by means of proficiency testing programs. These activities are very important to obtain formal recognition of the technical competence of the laboratory and they are also essential to confirm reliability and accuracy of the results. These tests are required by a Brazilian Government Institute named Coordenação Geral de Acreditação do Instituto Nacional de Metrologia, Qualidade e Tecnologia (Cgcre/Inmetro) as a requirement for laboratory ISO 17025 accreditation by NIT DICLA – 026 [1, 2]. The proficiency tests are intended to provide an independent assessment of the competence of participating laboratories. Along with the use of validated methods, proficiency testing is an essential element to the laboratory as a control quality activity [3, 4].

In the academic environment, there is a great discussion on the applicability of the principles of Good Laboratory Practices (GLP), because to obtain the accreditation a series of detailed and costly, evaluations are required, making it difficult. However, the use of the concepts of GLP by universities can support in the quality of the generated data. The principles don’t need be implemented in its totality, but can guide the research. In theory, research and development, if properly documented and validated, can be accredited. Consequently, the research achieve higher reliability and data integrity through proper documentation [5].

Histamine is a neuro and vasoactive biogenic amine which, at high concentrations, can cause adverse effects to human health. The analysis of histamine in tuna is a requirement in the EU for the importation of fish. Recent studies indicated that tunas from the Brazilian coast which are used for
exportation are of excellent quality. However, histamine poisoning outbreaks have been reported in the country [6]. CRM for analysis of histamine in tuna are not available in the national and international market. In this case, Reference Materials produced by the Food Analysis Performance Assessment Scheme (FAPAS®) can be used in proficiency tests [4].

The aim of this study was to investigate the quality of the analysis of histamine in tuna by means of reference material and proficiency test.

2. Material and methods

2.1. Samples
To ensure the effectiveness of the method through the recovery percentage and check the levels of histamine, there were analyzed a RM (canned tuna, FAPAS®) with known amounts of histamine. A sample of canned tuna with unknown histamine content was sent by FAPAS® for the proficiency test.

2.2. Reagents and solvents
Histamine (HIM) dihydrochloride and o-phthalaldehyde were purchased from Sigma Chemical Co. (St. Louis, MO, USA). The reagents were of analytical grade, except for the HPLC solvents (acetonitrile and methanol), which were chromatographic grade. The solvents were filtered through 47 mm diameter and 0.45 mm pore size membrane (Millipore Corp., Milford, MA, USA) for aqueous and organic solvents (HAWP and HVLP, respectively). For the preparation of the solutions, ultrapure water from Milli-Q Plus System (Millipore Corp., Milford, MA, USA) was used.

2.3. Determination of histamine
Histamine was extracted from the sample (5 g) with 7 mL of trichloroacetic acid (TCA) 5%. The sample was homogenized on a vortex mixer (Biomatic, Brazil) for 70 sec and centrifuged (MR23i, Jouan SA, Saint Herblain, France) at 11,250 x g for 3 min at 0 °C. After centrifugation the supernatant was filtered on paper quality and the filtrate was collected in a 25 mL flask. The extraction was repeated two more times and the filtrates were combined. An aliquot of the extract was filtered through a HAWP membrane (13 mm, 0.45 mm, Millipore Corp., Milford, MA, USA) prior to HPLC analysis. Histamine was quantified by ion–pair HPLC using a reverse phase column (Nova-Pak® C18, 300 x 3.9 mm, 4 µm) and a guard column (Nova-Pak® C18, 20 x 3.9 mm, 4 mm) and fluorimetric detection at 340 nm excitation and 450 nm emission after post-column derivatization with OPA [7, 8].

The identification of histamine was possible by comparison of the retention time of the peaks found in the samples with that of standard solution of the amine in hydrochloric acid 0.1 mol/L. The quantification of histamine was performed by interpolation in an external calibration curve and multiplication by a correction factor [7, 8]. The analyzes were carried out in triplicate and the mean values and standard deviations were calculated.
3. Results
The mean levels of histamine found in RM and their respective percentages of recovery can be seen in table 1. The percent recovery ranged from 89.1% to 100.9%, since the mean concentration was certified 226.4 mg/kg. These results are within the acceptable range of recovery factor that is 80% to 110% for concentrations ≥ 10 µg/kg. Recovery aims to correct the result of the analysis of the systematic errors arising from the effects of extraction or digestion and losses from all stages of the analysis, performed by reading the instrumental response [9].

| Reference Material | Date            | HIM content (mg/kg) | Mean concentration certified (mg/kg) | Recovery (%) |
|---------------------|-----------------|---------------------|--------------------------------------|--------------|
| 1                   | January 10, 2013| 213.4               | 226.4                                | 94.2         |
| 2                   | January 23, 2013| 201.6               | 226.4                                | 89.1         |
| 3                   | April 03, 2013  | 202.4               | 226.4                                | 89.4         |
| 4                   | May 08, 2013    | 313.9               | 311.1                                | 100.9        |

Table 2 presents the mean level of histamine obtained in the analysis of the sample for proficiency test. This test was performed on April 3, 2012. The mean content was found to be 278.81 mg/kg. We also performed analysis of the RM. This had recovery of 89.4%. Considering this recovery, the histamine content of the sample corrected for proficiency test was 311.9 mg/kg.

The histamine content of the proficiency test sample was subsequently released by FAPAS® through report (FAPAS® Report 27101). According to the report, the expected result of the proficiency test was 311.1 mg/kg.

| Sample FAPAS® (Proficiency Test) | HIM Content (mg/kg) |
|----------------------------------|----------------------|
| 152/12 – Sample 1                | 282.06               |
| 152/12 – Sample 2                | 276.48               |
| 152/12 – Sample 3                | 277.88               |
| Mean value                       | 278.81               |

Among the participating laboratories, the Laboratório de Bioquímica de Alimentos (LBqA) had the best performance (-2 ≤ z ≤ 2), since the z-score obtained was 0.0. The z-score technique is adopted when you want to evaluate the performance of technical laboratories, especially in proficiency tests [10].

Of the 98 participating laboratories 56% achieved satisfactory performance (-2 ≤ z ≤ 2) and 44% had poor performance being: | z | > 2.

4. Conclusion
The Laboratorio de Bioquimica de Alimentos, LBqA - UFMG achieved satisfactory performance through RM test and had the best result in FAPAS® proficiency test for the analysis of histamine in fish. Therefore, the analysis of histamine in fish performed by LBqA is of excellent quality, ensuring confiability of results and customer confidence.
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