1. SCOPE AND APPLICATION
1.1 Principle: Histamine is extracted with methanol and derivatized with o-phthalaldehyde (OPT) to generate the fluorescent product (see figure below). This method is used to determine the histamine content in raw, precooked, and canned tuna.\(^1\)\(^4\)

1.2 Interference: All methods for histamine determination are overwhelmed with interfering substances which have to be removed in order to accurately measure the histamine present. The two naturally-occurring substances that cause the most interference are histidine and spermidine since they also react with OPT to form fluorescent products.\(^5\) However, spermidine, the major contaminant in extracts, can be separated from histamine on cellulose phosphate cation-exchange columns.\(^6\) There is also variability due to the pH and temperature sensitivity of the o-phthalaldehyde-histamine fluorophor.\(^5\)\(^7\) Because of the ubiquity of interfering fluorophors, all reagents used must be of the highest obtainable purity. Exposure of any of the materials involved to rubber or silicones may produce erratic results.\(^8\) It is recommended that polyethylene labware be used in place of glass, due to an observed loss of fluorescence.\(^9\) All labware should be acid washed and rinsed in distilled water. New solution must be prepared after four to seven days, due to an observed increase in blank readings.\(^8\)

2. SUMMARY OF METHOD
2.1 The histamine-containing materials are homogenized and extracted with methanol. The extract can then be passed through an anion exchange column to remove any remaining interfering substances. The eluant is reacted with the OPT reagent and allowed to stand for 4 minutes. The mixture is acidified with H\(_3\)PO\(_4\) and the corresponding fluorescence is read on a calibrated instrument.

<table>
<thead>
<tr>
<th>alkaline pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>o-phthalaldehyde</td>
</tr>
</tbody>
</table>

3. APPARATUS AND EQUIPMENT
- Turner Designs TD-700 Laboratory Fluorometer with standard photomultiplier tube (PMT) (P/N 7000-009)
- Near UV mercury-vapor lamp (P/N 10-049)
- 300 nm - 400 nm Excitation Filter (P/N 10-069R)
- 410 nm - 500 nm Emission Filters (P/N 10-059R and P/N10-061R)
- 10x10 mm Square Methacrylate Cuvettes (3.5 ml) (P/N 7000-959)

3.1 Labware. All reusable labware (glass, polyethylene, Teflon, etc.) should be cleaned by soaking in laboratory grade detergent and water for 4 hours, rinsed with tap water, deionized water, and methanol. It is recommended that polyethylene ware be used due to absorbance observed when using glass.

3.1.1 Assorted Class A calibrated pipettes.
3.1.2 Graduated cylinder, 100-mL.
3.1.3 Assorted volumetric flasks for preparing dilution standards.
3.2 Chromatographic columns (Kontes #K-422250).
4. REAGENTS AND STANDARDS

4.1 Ion exchange resin: Sigma 1X8-200, chloride form 100-200 mesh; or BioRad AG1-X8, 50-100 mesh, chloride form, Cat. No. 140-1431, or equivalent.

4.2 1.0N Sodium hydroxide: Dissolve 40g NaOH in 1 liter of distilled water.

4.3 2.0N Sodium hydroxide: Dissolve 80g NaOH in 1 liter of distilled water.

4.4 Histamine dihydrochloride: MCB #HX0440 or J.T. Baker #1-N330.

4.5 1.0N Hydrochloric acid: Add 83 ml concentrated HCl to about 500 ml distilled water. Cool and bring to 1 liter volume with distilled water.

4.6 0.1N Hydrochloric acid: Add 100ml 1N HCl to about 500 ml distilled water. Cool and bring to 1 liter volume with distilled water.

4.7 Methanol, reagent grade.

4.8 0.1% o-Phthalaldehyde(OPT reagent): Phthalic dicarboxaldehyde (Aldrich, Milwaukee, WI), or o-Phthaldialdehyde (Sigma, St. Louis, MO), C₆H₄(CHO)₂, F.W. 134.13. Dissolve 0.10 g OPT in 100 ml methanol. Store in an amber bottle and refrigerate when not in use. Prepare fresh weekly.

4.9 3.57N Phosphoric acid: Add 121.8 ml of 85% H₃PO₄ to about 500 ml distilled water. Bring to 1 liter volume with distilled water.

4.10 Histamine Standard Solution A, 1mg Hm/ml: Weigh 0.1656 g of histamine dihydrochloride into 100 ml volumetric flask. Dissolve in, and dilute to volume with 0.1N HCl.

4.11 Histamine Standard Solution B, 10 µg Hm/ml: Dilute 1.0 ml Solution A to 100 ml with 0.1N HCl.

4.12 Histamine Standard Solution A1(this is our control solution): Dilute 1.0 ml Solution A to 100 ml with methanol.

4.13 Histamine Standard Solution C, 0.1 µg Hm/ml: Dilute 1.0 ml Solution B to 100 ml with 0.1N HCl.

4.14 Histamine Standard Solution D, 0.2 µg Hm/ml: Dilute 2.0 ml Solution B to 100 ml with 0.1N HCl.

4.15 Histamine Standard Solution E, 0.3 µg Hm/ml: Dilute 3.0 ml Solution B to 100 ml with 0.1N HCl.

NOTE: Prepare Solutions A and B monthly and Solutions C, D, E, and A1 weekly. Refrigerate solutions when not in use.

5. PREPARATION

5.1 Resin Preparation:

5.1.1 Place 20 g of ion exchange resin in a beaker.

5.1.2 Add 2N sodium hydroxide to the resin in a ratio of 15 ml per gram of resin.

5.1.3 Mix well and allow the resin to settle for a minimum of 15 minutes, but no more than 30 minutes. Decant liquid and repeat with additional 2N sodium hydroxide.

5.1.4 Wash resin thoroughly with distilled water to remove traces of the sodium hydroxide until pH is less than or equal to 8.5.

5.1.5 Slurry resin with distilled water and transfer to a funnel containing a fluted filter paper. Thoroughly wash with distilled water.

5.1.6 Transfer resin to a suitable container and make sure the distilled water level is above the resin level at all times.

5.2 Column Preparation:

5.2.1 Slurry sufficient prepared resin into each column to form a bed 8 cm in height. Maintain a liquid level above the top of the resin at all times.

5.2.2 Refill columns with fresh resin at least twice per week.
6. INSTRUMENT SET-UP

6.1 Install the excitation and emission filters in the filter cylinder. Then, insert the filter cylinder into the sample chamber. For additional assistance, refer to Section III of your TD-700 Operating Manual.

6.2 Insert the lamp. Turn on the instrument and allow to warm-up for 10 minutes. For additional assistance, refer to Section IV of your TD-700 Operating Manual.

6.3 Calibrate instrument in the direct concentration mode with the prepared histamine standard solutions C, D, and E. Blank with a reagent blank.

7. PROCEDURE

7.1 Sample Preparation:

7.1.1 Blend fish in a Waring blender with an equal weight of deionized water to produce a 1:1 slurry.

7.1.2 Transfer 10.0 g of the slurry to a 150 ml beaker. Add 40.0 ml of methanol and mix thoroughly.

7.1.3 Using Whatman #1 filter paper, or equivalent, filter the mixture into a suitable container. If the filtrate is to be saved for later analysis, refrigerate in a closed container.

NOTE: Evaporation of methanol from the filtrate can cause erroneous results.

7.2 Histamine Extraction:

7.2.1 Pass 15-20 ml distilled water through the exchange column and discard.

7.2.2 Place a 50 ml volumetric flask containing 5 ml 1N HCl at the column outlet.

7.2.3 Pipet 1.0 ml of filtrate (methanol extract) onto the resin bed with 5-10 ml distilled water.

7.2.4 Immediately initiate column flow. Flow should be maintained at a rate greater than 3 ml/min.

7.2.5 When liquid level is slightly above the resin, add about 5 ml distilled water and allow it to flow through the resin. Repeat with distilled water in larger increments until total water through column is about 40 ml.

7.2.6 Discontinue column flow.

7.2.7 Remove volumetric flask and bring to 50 ml volume with distilled water. Store column effluent in the refrigerator if necessary to postpone determination for more than 2 hours.

7.3 Controls and Blanks:

7.3.1 At the beginning of a set of analyses, and again at the end, pass 1 ml of Solution A1 through one of the columns and proceed through the procedure as though it were a fish extract. Fluorescence readings should be very similar to Solution D reading. If readings are not within 20% of Solution D, all analyses performed at the same time are suspect and should be repeated.

7.3.2 After every 14 samples, 1 ml of methanol should be put through a column and run through the procedure as a fish extract. If a reading on one of these blanks is more than 5 units higher than the original blank reading, resin contamination is apparent and corrective action should be taken immediately.

7.4 Histamine Determination:

7.4.1 Into separate 25 ml glass stoppered flasks, pipet 5.0 ml of 0.1N HCl (Blank); Solutions C, D, and E; and each diluted column effluent.

7.4.2 Add 10 ml 0.1N HCl to each flask.

7.4.3 Add 3 ml 1N NaOH. Mix thoroughly.

7.4.4 Within 5 minutes, add 1 ml OPT solution and mix thoroughly.

7.4.5 After exactly 4 minutes, add 3 ml 3.57N H₃PO₄ and mix immediately.

7.4.6 Let solutions stand for 15-20 minutes and then determine the fluorescence intensities on the TD-700 Laboratory Fluorometer. If a sample reading is greater than that of Solution E, dilute 25
ml of the column effluent to 100 ml with 0.1N HCl and proceed from step 7.4.1.

CAUTION: Fish with high salt content may cause problems with the resin necessitating more frequent changing of columns.

7.4.7 If sample dilution was necessary in step 7.4.6, multiply the obtained result by 4.

8. REFERENCES


9. FURTHER READING
1. Tissue Extraction:

2. Blood Extraction:

3. Brain

4. Urine

5. Milk
6. **Skin**


7. **Improved Methods**


8. **General Information**


---

**Note:** This suggested method for the analysis of histamine is provided to us from Eddie Robalino, QC Manager of I.N.E.P.A.C.A (Industria Ecuatoriana Productora De Alimentos C.A.) in Manta, Ecuador. It has not been validated by Turner Designs.