Application Note: A Suggested Method for Histamine Analysis using AOAC Official Method 977.13

SCOPE AND APPLICATION

Product is extracted with 75% methanol. Extract is passed through ion exchange column. O-Phthaldialdehyde solution is added to eluate to form fluorescent histamine derivatives. Fluorescence intensity of derivatives is measured using fluorometer and histamine is quantified using external standards.

APPARATUS AND EQUIPMENT

- Trilogy Laboratory Fluorometer P/N 7200-000
- Histamine module - Ex: 350/80nm and Em: 410-490nm P/N 7200-049
- 10x10 mm Square Methacrylate Cuvettes (3.5 ml) P/N 7000-959

- *Chromatographic tube*.— 200 x 7 id mm polypropylene tube fitted with small plastic stopcocks and 45 cm Teflon tubing. Control flow rate at >3 mL/min by adjusting height of column relative to tubing outlet. Alternatively, use 2-way valve in place of tubing.

- Pipets 1& 5ml

Note: All labware including plastic and glass containers should be rinsed using HCl and Deionized (DI) water before use.

REAGENTS AND STANDARDS

- Ion-exchange resin.—Bio-Rad AG 1-X8, 50–100 mesh (Bio-Rad Laboratories, 1000 Alfred Nobel Dr, Hercules, CA 94547, USA; www.biorad.com) or Dowex 1-X8, 50–100 mesh.

  Preparation ion exchange resin:

  1. Convert to -OH form by adding 15 mL 2M NaOH/g resin to beaker. Swirl mixture and let stand approximately 30 minutes. Decant liquid and repeat with additional base.
  2. Thoroughly wash resin with DI water, pour the slurry into filter paper and wash again with DI water.
  3. Place glass wool plug in base of chromatographic tube and slurry in enough resin to form 8 cm bed. Maintain DI water level above top of resin bed at all times.
  4. Prepare resin fresh weekly and store submerged in DI water. Do not regenerate resin in packed column; rather, use batch regeneration in beaker when necessary. Wash column with 10 mL of DI water before applying each extract.

- 1.19M Phosphoric Acid.

  Preparation phosphoric acid:

  1. Dilute 79.33 mL 85% (15M H_3PO_4) to 1 L with DI water.
     For other concentrations of H_3PO_4, Dilute 17.493 mL (1.19M H_3PO_4) to 1 L with DI water.
  2. Standardize 5.00 mL by titration with 1.00M NaOH to phenolphthalein end point and adjust concentration if necessary.

- % O-Phthaldialdehyde (OPT) solution.

  Preparation O-Phthaldialdehyde (OPT) solution:

  1. Dissolve 100 mg OPT in 100 mL distilled-in-glass methanol.
  2. Store the OPT solution in amber bottle in refrigerator. Prepare fresh weekly.

- Histamine standard solutions

  ♦ Stock solution - 1 mg/mL - Prepare fresh weekly

  Preparation stock solution:

  1. Accurately weigh 169.1mg histamine.2HCl (98%) into 100 mL volumetric flask.
  2. Dissolve and dilute to volume with 0.1M HCl.

  ♦ Intermediate solution - 10µg/mL - Prepare fresh weekly

  Preparation intermediate solution:

  1. Pipet 1 mL stock solution into 100 mL volumetric flask and dilute to volume with 0.1M HCl.

  ♦ Working solution - 0.5, 1.0, and 1.5 mg/5 mL - Prepare fresh daily

  Preparation working solution:

  1. Pipet 1, 2, and 3 mL intermediate solution into separate 100 mL volumetric flasks and dilute each to volume with 0.1M HCl.

PROCEDURE

SAMPLE PREPARATION

1. Blend fish with an equal weight of DI water to produce a 1:1 slurry.
2. Transfer 10.0 g of the slurry to a 150 ml beaker. Add 40.0 ml of methanol and mix thoroughly.
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.

HISTAMINE EXTRACTION

1. Pass 4–5 mL DI water through column, and discard eluate.
2. Pipet 1 mL extract onto column and add 4–5 mL DI water. Immediately initiate column flow into 50 mL volumetric flask containing 5.00 mL 1.00M HCl. When liquid level is 2 mm above resin, add 5 mL DI water and let elute.
3. Add additional DI water until 35 mL has eluted. Stop column flow, dilute to volume with DI water, stopper, and mix.
4. Refrigerate eluate if necessary to postpone determination for more than 2 hours. .

HISTAMINE DETERMINATION

1. Pipet duplicate 5 mL aliquots of each working standard solution and each diluted column effluent into separate 50 mL glass or polypropylene Erlenmeyer's flask.
2. Pipet in 10 mL 0.1M HCl to each flask and mix.
3. Pipet in 3 mL 1M NaOH to each flask and mix.
4. Within 5 minutes, pipet in 1 mL OPT solution to each flask and mix immediately.
5. After exactly 4 min, pipet in 3 mL 1.19M H_3PO_4 to each flask and mix immediately. It is important to mix thoroughly after each addition and at least once during OPT reaction.
6. Prepare blank by substituting 5 mL 0.1M HCl for histamine solution.
7. Within 1.5 hours, record fluorescence intensity (I) of working standard solutions with H2O using Trilogy Laboratory Fluorometer in Raw Fluorescence Mode.
8. Plot I (corrected for blank) against µg histamine/5 mL aliquot.

CALCULATION

The plot of I - fluorescence measured by Trilogy Laboratory Fluorometer and corrected for blank - against mg histamine/5 mL test solution should be straight line passing through origin with slope = m = $[(I_a / 1.5) + I_b + 2I_c]/3$.

mg Histamine/100 g fish = (10)(F)(1/m)(I_s)

µg Histamine/g fish = 10 x (mg histamine/100 g fish)

where I_s, I_a, I_b and I_c = fluorescence from test solution, 1.5, 1.0, and 0.5 mg histamine standards, respectively; and F=dilution factor=(mL eluate + mL 0.1M HCl)/mL eluate. F=1 for undiluted eluate.

If calibration plot is not linear, use standard curve directly for quantitation. Each ordered pair (x, y) should be ≤0.1 µg histamine/5 mL test solution. Read all values from curve to nearest 0.05 µg histamine/5 mL test solution.

mg Histamine/100g test portion = (10)(F)(W)

µg Histamine/g test portion= 10 x (mg histamine/100g test portion)

where W = µg histamine/5 mL test solution as determined from standard curve.

REFERENCES

AOAC Official Method 977.13 Histamine in Seafood (35.1.32)