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Tech Notes

Histamine in Rat Microdialysates by Liquid Chromatography / Electrochemical Detection

1011

Purpose

Determination of histamine in microdialysates of rat anterior hypothalamus.

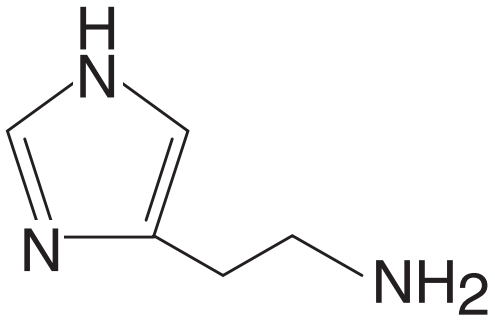

Figure 1. *Structure of histamine.*

Histamine (F1) is a heterocyclic primary amine known for its mediation of allergic response [1]. Stored in mast cells and peripheral blood basophils, its release plays a role in allergy, inflammation, gastric acid secretion, microcirculation and neurotransmission [2].

Existing Methods

Radioenzymatic assays, fluorometry, and LC with fluorescence or electrochemical detection (both require derivatization). The method below, modified from [3], relies on derivatization with o-phthalaldehyde (OPA).

Conditions

System: BASi Electrochemical Detector Package with a HPLC pump configured for microbore chromatography.
Electrochemical Detector Electrode: Radial-flow 3 mm glassy carbon (MF-1095)
Potential: +700 mV *vs*. Ag/AgCl
Column: 3 x 100 mm C_{18} (MF-8954)
Column Temperature: 35 °C
Mobile Phase: 5.9 g NaH_2PO_4 H20, 1.1 g Na_2HPO_4, 186 mg EDTA brought to 500 mL H20, adjusted to pH 6.4. Combine 480 mL with 240 mL acetonitrile and 280 mL methanol.
Flow Rate: 1 mL/min
Loop Size: 20 µL
Injection Volume: 10 µL

Notes

Samples were derivatized with OPA using Reagent A of the BASi Amino Acid Kit (MF-8905), using 20 µL sample and 4 µL Reagent A, with a 3-minute reaction time.

Separation of histamine in a rat microdialysis sample is shown in F2. A calibration curve for histamine standards in water is shown in F3. The limit of quantitation for real-world microdialysis samples was approximately 15 nM (0.15 pmoles injected).

References

1. *D. Egger, G. Reisbach, L. Hultner, J. Chromatogr. B 662 (1994) 103-107.*
2. *C.M.C.J. van Haaster, W. Engels, P.J.M.R. Lemmens, G. Hornstra, G.J. van der Vusse, J. Chromatogr. 617 (1993) 233-240.*
3. *T.B. Jensen, P.D. Marley, J. Chromatogr. B 670 (1995) 199-207.*

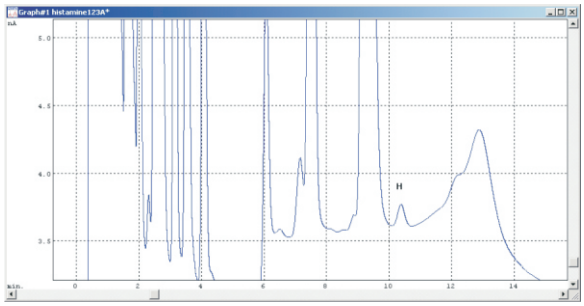

Figure 2. *Separation of histamine (H) from rat anterior hypothalamus microdialysate. Peak corresponds to 50 nM histamine.*

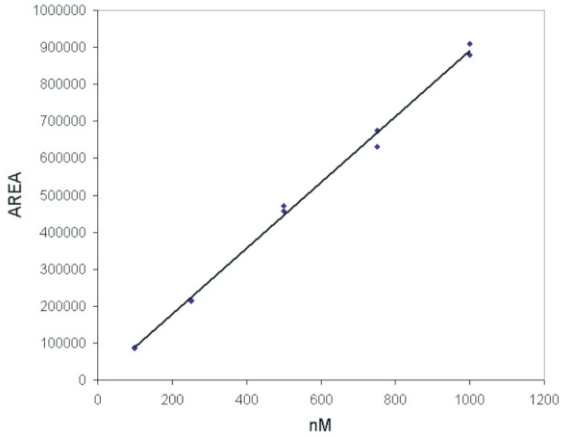

Figure 3. *Linearity of histamine standards. R2 = 0.997*

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