CAPSULES notes and applications from BASi
January 2002
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Histamine in Rat Microdialysates by LCEC

Purpose
Determination of histamine in microdialysates of rat anterior hypothalamus.

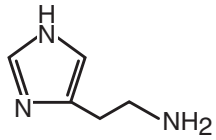

Figure 1. Structure of histamine.

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: BAS 592e liquid chromatograph
Electrode: Radial-flow 3 mm glassy carbon
Potential: +700 mV vs. Ag/AgCl
Column: 3 x 100 mm C_{18} (MF-8954)
Column Temperature: 35 °C
Mobile Phase: 5.9 g $NaH_2PO_4 \bullet H_20$, 1.1 g Na_2HPO_4, 186 mg EDTA brought to 500 mL H_20, 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 BAS 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.

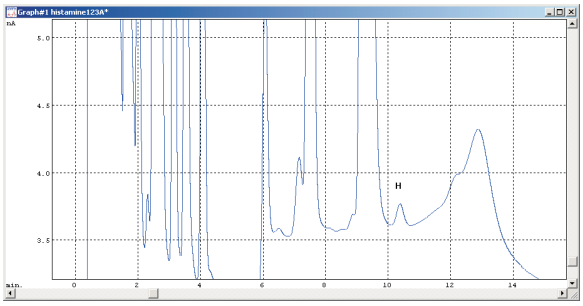

Figure 2. Separation of histamine (H) from rat anterior hypothalamus microdialysate. Peak corresponds to 50 nM histamine.

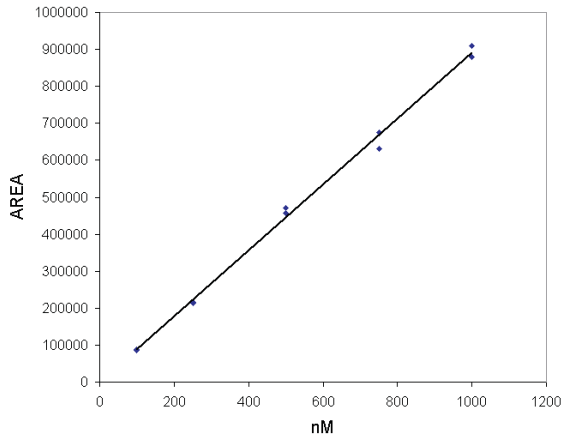

Figure 3. Linearity of histamine standards. R^2 = 0.997

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