CAPSULES
preliminary notes and applications from Bioanalytical Systems, Inc.

Determination of Histamine in Plasma

Purpose

Determination of histamine levels in plasma. Histamine release from cells is stimulated by allergens, neurotransmitters, and various drugs. Histamine is rapidly cleared from the circulation once released, so methods of monitoring its levels in plasma must be sensitive and specific. Since histamine is only slightly electroactive, it must be modified before analysis.

Existing Methods

Fluorometry, HPLC-fluorometry, radioenzymatic assays, gas chromatography-mass spectrometry. These may be time consuming, expensive, and/or non-specific.

Reference

Determination of Histamine Concentrations in Plasma by Liquid Chromatography/Electrochemistry, L.G. Harsing, H. Nagashima, D. Duncalf, E.S.Vizi and P.L. Goldiner, Clin. Chem., 32(1986) 1823-1827.

Conditions

Detector: BAS LC-4B
Electrode: BAS glassy carbon
Potential: +0.40 V vs. Ag/AgCl
Column: 5 µm, Nucleosil C 18 (150 x 4.6 mm)
Mobile Phase: 0.07 M Na_2HPO_4/citric acid, pH 4.5, 0.26 mM Na_2EDTA, with 260 mL acetonitrile per L.
Flow rate: 0.8 mL/min.
Detection Limit: 50 pg of standard histamine

Sample Preparation

Blood samples were centrifuged to remove cells, and plasma proteins were precipitated with perchloric acid. Cleared plasma was further purified by passing through Amberlite CG 50 cation exchange resin. Histamine in the purified plasma was derivatized by the addition of o-phthalaldehyde and 2-mercaptoethanol. This mixture was injected onto the HPLC column.

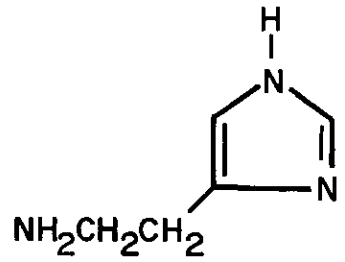

Figure 1. Structure of Histamine.

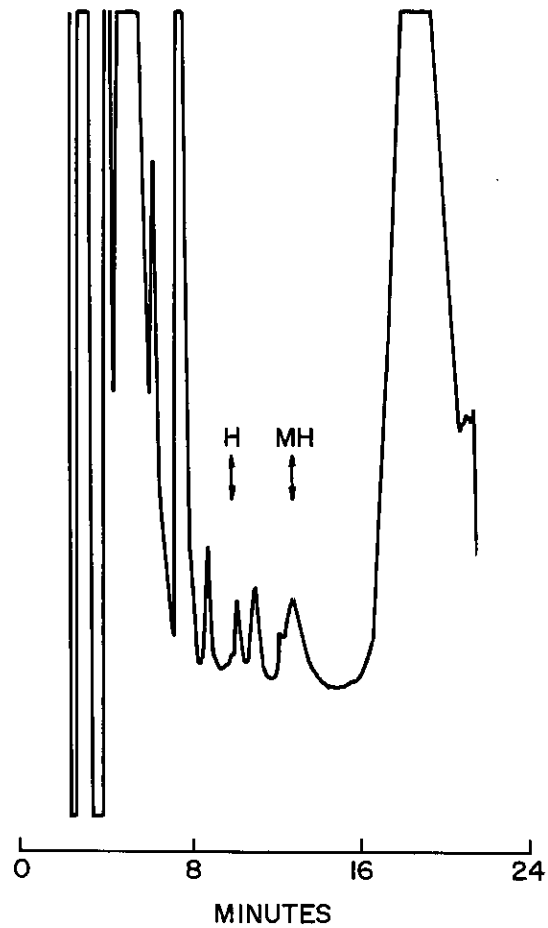

Figure 2. Chromatogram of histamine (H) and an internal standard, methylhistamine (MH)

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Notes

Recovery of histamine from the cation exchange column was 60%, and recovery from the protein-precipitation step was 77%.

Hydrodynamic voltammograms of standard histamine and histamine extracted from plasma were identical.

This detection procedure can be duplicated using the BAS 400 Liquid Chromatograph.

The information in this publication is supplied as a service to our customers. Performance of the methodology has not necessarily been verified by BAS technical staff.