CAPSULES
notes and applications from Bioanalytical Systems, Inc.

Detection Of Pipecolic Acid In Rodent Brain

Purpose

Detection of endogenous levels of pipecolic acid (PA) in mg amounts of rodent brain tissue. The sensitivity and accuracy of the reported method was demonstrated in a study of the regional distribution of PA in rat and mouse brain.

Existing Methods

Thin-layer chromatography with or without spectrophotometric detection, and GC-MS. The former method lacks specificity and sensitivity for small amounts of brain tissue. The latter method has the required sensitivity but is not adaptable to routine analysis.

Reference

Quantitative Determination and Regional Distribution of Pipecolic Acid in Rodent Brain, J.S. Kim and E. Giacobini, Neurochem. Res., 1984, 9: 1559-1569.

Conditions

Liquid Chromatography: LC-304TD or BAS 200
Detector: BAS Dual Channel LC-4B Amperometric Controller
Electrode: Series Dual Glassy Carbon
Potential: Upstream -1.0 V, Downstream +0.85 V vs Ag/AgCl
Column: 5 µm C-18 Reverse-phase, 250 x 4.6 mm (Biophase ODS)
Temperature: 30°C
Mobile Phase: 80% (v/v) 50 mM sodium acetate, pH 5.0, 10% n- propanol, 10% acetonitrile
Detection Limit: 2 - 3 pmoles
Linear Range: 5 - 80 pmoles

Sample Preparation

A known amount of brain tissue was homogenized in 5% TCA and clarified by centrifugation. Nipecotic acid (NPA) was added as internal standard, and the TCA solution was washed with ethyl acetate. The remaining aqueous layer was passed through a Sep-pak C-18 cartridge (Waters Assoc., Milford, MA).

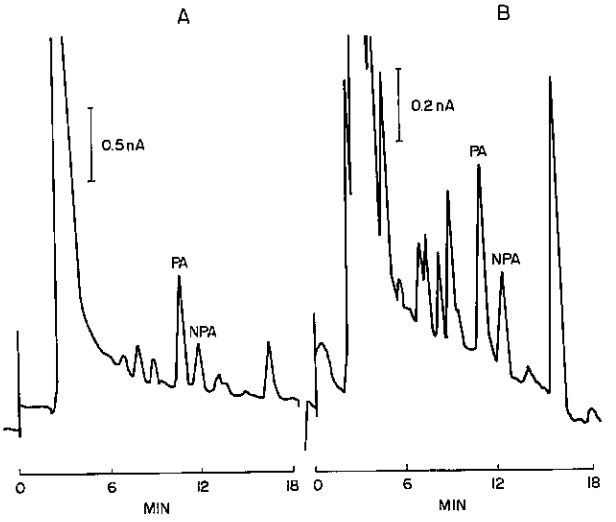

Figure 1. Chromatograms of A) whole brain homogenate and B) Pons- medulla oblongata. Redrawn from above stated reference.

The eluted amino acids were derivatized with dinitrofluorobenzene to their respective dinitrophenol (DNP) derivatives.

DNP-PA and DNP-NPA were isolated by thin-layer chromatography, using authentic compounds as reference, and injected.

Clinical Application

Pipecolic acid has been shown to be present in the mammalian brain. It is an intermediate in the major pathway of lysine metabolism in this tissue.

Pipecolic acid metabolism in brain has attracted attention because an elevation in this imino acid in the CNS, serum, urine, and CSF is associated with a severe metabolic disorder of the nervous system referred to as hyperpipecolic acidemia.

This capsule highlights a method to detect pipecolic acid in CSF, blood, or urine which may be of clinical value in the diagnosis of this condition.

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.

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