CAPSULES
notes and applications from Bioanalytical Systems, Inc.

GABA in Rat Brain by Fluorescence Detection

Purpose

Gamma-aminobutyric acid (GABA) acts as an inhibitory amino acid in the brain. It has been implicated in Alzheimer's, Huntington's and Parkinson's diseases, as well as in epilepsy and schizophrenia. Naphthalenedialdehyde/cyanide* (NDA/CN, F1) was evaluated as a precolumn derivatization reagent for the detection of GABA in a brain homogenate sample.

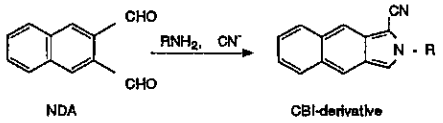

Figure 1. Reaction of NDA/CN with primary amines.

Existing Methods

GABA is normally detected by precolumn or postcolumn derivatization with orthophthalaldehyde (OPA). Naphthalenedialdehyde produces derivatives which have higher quantum efficiencies and are more stable than the corresponding OPA-mercaptoethanol derivatives (1,2).

Conditions

Mobile phase: 75% (v:v) 0.02 M sodium citrate (pH 7), 25% acetonitrile. Flow rate was 0.8 mL/min
Column: BAS phase II ODS, 3 µm column 100 X 3.2 mm (P/N MF-6213)
Detector: BAS FL-45 Fluorescence Detector: excitation wavelength = 420 nm, emission wavelength = 490 nm

Analysis of Brain Homogenate Sample

Approximately 0.3 mg of brain was placed in 5 mL of 20 mM sodium borate (pH 9.0) and sonicated for 15 min in an ice bath. The mixture was acidified with 25 µL of perchloric acid and centrifuged. The clarified supernatant was used in the derivatization procedure outlined above.

F2 shows the detection of GABA in a rat brain homogenate using NDA/CN. The method was linear between 200 femtomoles and 2 nanomoles injected. The CBI derivative of GABA was stable for over 10 hours if kept at 4°C and protected from light. The cooler temperature slows down the formation of additional side products.

Figure 2. Detection of GABA in a rat brain homogenate.

Typical Derivatization Conditions (BAS P/N CF-1045)

100 µL of brain homogenate
100 µL of 6 M NaOH
600 µL of 0.05 M sodium borate (pH 9)
100 µL of 10 mM NaCN
100 µL of 10 mM NDA
Reaction time 30 minutes (room temperature)

(This reaction can be scaled down by a factor of 100 with no adverse effects.)

Notes

The use of NaOH to adjust the pH of the sodium borate buffer may introduce impurities which lead to spurious peaks. We have found that using a 10-50 mM sodium borate solution (non-adjusted pH about 9.3) works well for the derivatization. (In this study, NaOH was added during the derivatization procedure to neutralize the acid of the brain homogenate.)

The NDA should always be added last to minimize side product formation.

The reaction mixture should be protected from light as it can undergo photodegradation.

References

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3. S.M. Lunte and O.S. Wong, Current Separations 10 (1990) 19-26.

* US PATENT #4837166