CAPSULES
preliminary notes and applications from Bioanalytical Systems, Inc.

Aspartate and Glutamate in Rat Brain by Fluorescence Detection

Purpose

Aspartic acid and glutamic acid play important roles as excitatory amino acids in the brain. Naphthalenedialdehye/cyanide* (NDA/CN, F1) was evaluated as a precolumn derivatization reagent for the detection of aspartate and glutamate in both brain homogenates and microdialysates.

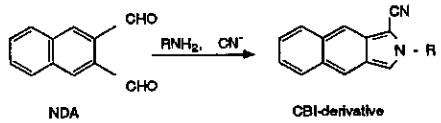

Figure 1. Reaction of NDA/CN with primary amines.

Existing methods

NDA/CN derivatives of amino acids are substantially more stable and possess higher quantum efficiencies than their ortho-phthalaldehyde-mercaptoethanol counterparts (1,2).

Conditions

Pump: BAS-200A Liquid Chromatograph
Mobile phase: 82%(v:v) 0.05 M sodium acetate pH 5, 18% acetonitrile. Flow rate was 1 mL/Min.
Detector: BAS FL-45 Fluorescence Detector: excitation wavelength= 420 nm, emission wavelength = 490 nm
Column: BAS phase II, C18, 3 µm 100 X 3.2 mm (BAS P/N MF-6213)
Injection Volume: 20 µL

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

10 µL dialysate or brain tissue homogenate
70 µL of 10 mM sodium borate (pH 9)
10 µL of 10 mM NaCN
10 µL of 10 mM NDA
Reaction time 30 minutes at room temperature.
Samples can then be stored a 4°C for up to 10 h.

(This procedure can be scaled down to a total volume of 10 µL with no adverse effect.)

Rat Brain Homogenate

Approximately 0.5 mg of brain was placed in 3 mL of 20 mM sodium borate (pH=9.0) and sonicated for 15 min in an ice bath. The mixture was acidified with 15 µL of perchloric acid and centrifuged. The clarified supernatant was used in the derivatization procedure outlined above.

F2 shows a separation of aspartic and glutamic acid in a rat brain homogenate. The method is linear between 200 femtomoles and 2 nanomoles injected, with detection limits of approximately 200 femtomoles. The compounds were stable for over ten hours if kept cold and protected from light.

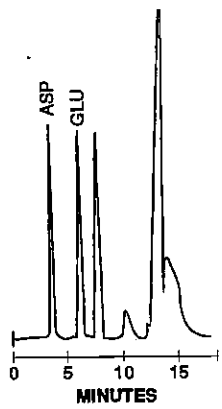

Figure 2. Detection of aspartic and glutamic acid in a rat brain homogenate.

Microdialysis

The microdialysis sample was obtained from the right anterior thalamus of the rat. A CMA microdialysis system with a 4.1 mm probe having a molecular weight cutoff of 5000 daltons was employed. The diameter of the probe was 300 µm. The flow rate was 2.2 µL/min. The perfusion buffer consisted of 140 mM NaCl, 3.4 mM KCl, 1.2 mM $MgCl_2$, 1.2 mM $CaCl_2$, 0.3 mM NaH_2PO_4, 1.2 mM Na_2HPO_4, pH 7.4.

F3 shows the separation of aspartic and glutamic acid in the microdialysis sample. In this case the mobile phase consisted of 54% 0.002 M sodium citrate (pH 7.5) and 46% methanol. A BAS phase II ODS, 5 µm, 250 cm X 4.6 mm column was employed. The peaks correspond to 52 picomoles of aspartic acid and 24 picomoles of glutamic acid, respectively. When electrochemical detection was employed, this mobile phase was brought to a final concentration of 0.05 M with respect to sodium perchlorate.

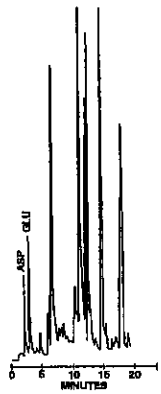

Figure 3. Microdialysate from rat hypothalamus. The following amino acids were identified and quantified: aspartic acid at 2.6 µm; glutamic acid at 1.2 µm. The remaining amino acids were tentatively identified based on retention time.

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.

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.

Keeping the samples cold (4°C) reduces the formation of side products due to the continued reaction of NDA and cyanide.

References

1. B.K. Matuszewski, R.S. Givens, K. Srinivasachar, R.G. Carlson and T. Higuchi, Anal. Chem. 59 (1987) 1102-1105.

2. P. de Montigny, J. F. Stobaugh, R.S. Givens, R.G. Carlson, K. Srinivasachar, L. A. Sternson and T. Higuchi, Anal. Chem., 59 (1987) 1096-1101.

* US PATENT # 4837166