Concise Glutamate Analysis by Using an Online Enzymatic Reaction

AN07-0815

In the central nervous system, glutamate plays a key role as a neurotransmitter and can be a toxic factor for neurons. Here we introduce a concise procedure for the analysis of glutamate levels in biological samples such as, tissue homogenate, tissue/cell culture media, blood serum/plasma and microdialysate. This is achieved by HPLC-ECD (DC mode) without sample derivatization.

This new technique employs an online immobilized enzyme column (glutamate oxidase) positioned after the separation column. After the glutamate is separated from other biogenic compounds, it is oxidized by the enzyme column to hydrogen peroxide which can then be detected by the platinum working electrode when the electrochemical detector is set to the DC mode. The detection limit for this methodology is <3 nM for any sample medium. Figure 1 illustrates the detection from brain tissue homogenate. One chromatogram can be completed in 10 min. The enzyme column life is more than 6 months for microdialysis samples.

Pulsed amperometric detection combined with ion exchange allows direct detection of amino acids but this separating condition cannot be applied to biological samples due to the complexity of the ion composition. One of the most common analysis methods used for glutamate measurements in biological samples is a biosensor technique. This is based on an enzymatic reaction coupled with the electrochemical detection of hydrogen peroxide using a platinum electrode. However, it is difficult with this method to detect the specific reaction to glutamate in biological samples because commercial glutamate oxidase has cross talk with other amino acids. In addition, an electrochemical detector responds not only to hydrogen peroxide but to other factors as well. In contrast, specific analysis can be achieved by applying the HPLC technique with a precolumn derivatization using o-phthaldialdehyde. However, this analysis requires intensive labor for liquid handling or an autosampler.

Chromatographic Conditions

HPLC-ECD	Eicom HTEC-500
Column	Eicom GU-GEL (4.6 ID x 150 mm)
Reactor	E-ENZ (3.0 ID x 40 mm)
M.P. flow rate	400 µl/min
Applied potential	+500 mV vs. Ag/AgCl
Working electrode	Eicom WE-PT
Time constant filter	3.0 sec
System Temperature	33°C

Available Application

- Microdialysis Samples
- Blood Plasma
- Tissue Homogenate
- Culture Media
- Others

The following is a representative example of an assay we have performed to demonstrate how our new methodology works. By using two online auto-injectors (Eicom EAS-20), the microdialysate was automatically injected followed by a standard glutamate solution. This standard was used as an internal standard (IS) in order to account for enzyme activity. However, the enzyme activity was stable enough to neglect the IS. Compared to the glutamate signal peak, the uric acid peak was relatively higher. By adjusting the injection time, the uric acid peak could be incorporated into the front-solvent peak and sequential 10 min injections are achievable (Fig. 2)

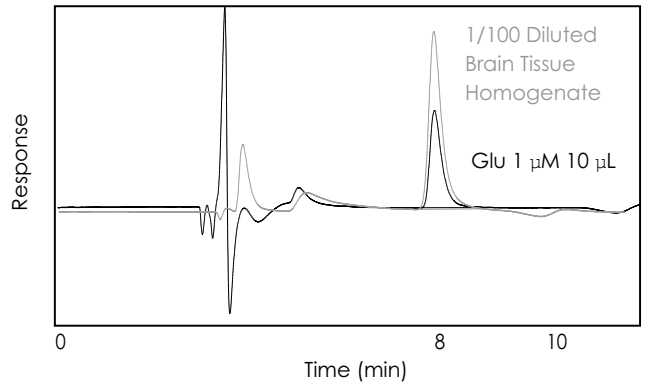

Fig. 1 The retention time of the glutamate peak is at 8 min, samples can be injected every 10 min.

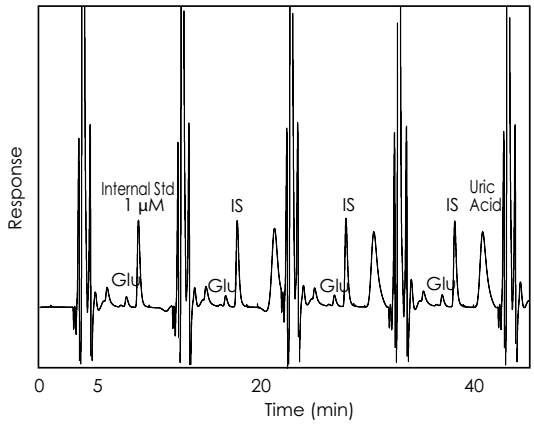

Fig. 2 Sequential Microdialysate Injections