



# Glutamate and GABA Analysis in Tissue Homogenate - 12 Minutes Analysis

AN06-0916

Detection of glutamate (Glu) and  $\gamma$ -aminobutyric acid (GABA) are in high demand, but current methods that depend on precolumn derivatization with *o*-phthaldialdehyde and  $\beta$ -mercapto ethanol (OPA-bME) can take as long as 40 min to 1 hour per sample to analyze. There are alternatives that use UHPLC or literally employ two systems connected with an online valve after the precolumn. However, both of these systems have high initial cost and require more maintenance than the system we present here.

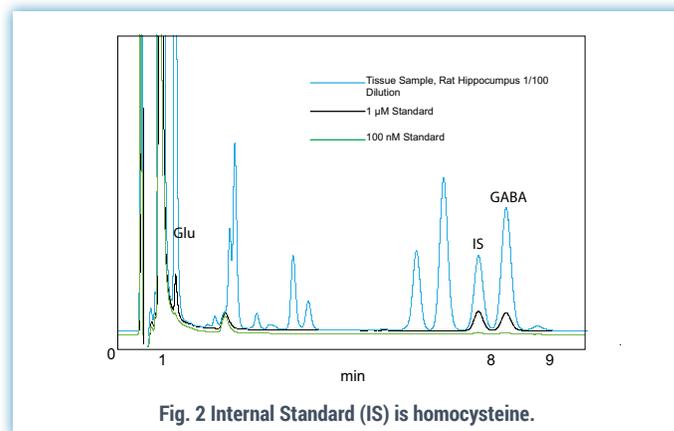
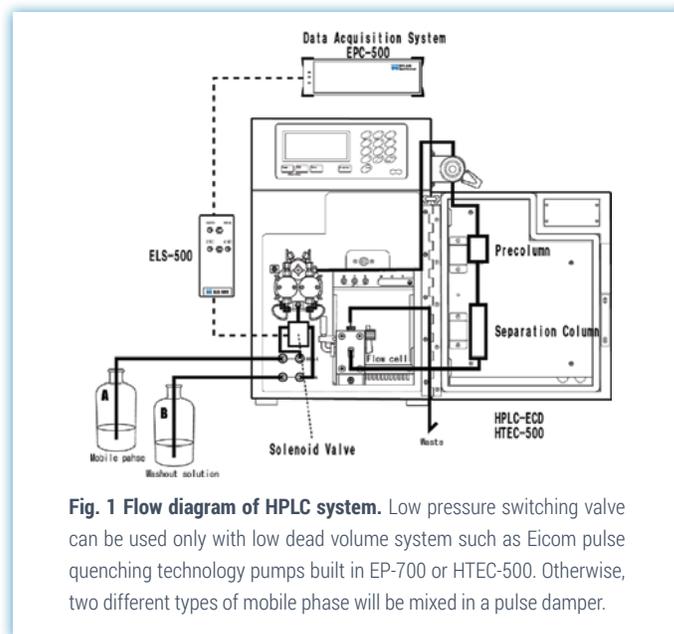
We have developed a concise method to analyze Glu and GABA in 12 minutes without requiring significant system upgrades. This application is available for use with the Eicom HTEC-500 or 700 Series after configuring with the low cost ELS-500 Liquid Switching Valve. This switching valve enables the mobile phase to be changed on the low pressure side of the pump. Peaks that elute long after the target peaks can be washed out of the column quickly with a second mobile phase. The use of such a switching system is only possible with Eicom Pulse Quenching Pumps because the elimination of a pulse damper from the system minimizes the volume of fluid between the pump and column.

While this procedure is available for both tissue and microdialysis samples, there are some restrictions with the use of the microdialysis samples. Please see the end of this note for more.

If you are only interested in detecting Glu, Eicom recommends using our online enzyme column method. Please refer to the application note which specifically discusses it.

## Analytical Conditions

HPLC-ECD	Eicom HTEC-500 or 700 Series + ELS-500 +Autosampler AS-700
Separation column	Eicompak FA-30DS (3.0 ID x 50 mm)
Precolumn	Eicompak CA-ODS
Flow rate	500 $\mu$ L/min
Column Temperature	40°C
Applied potential	+600 mV vs. Ag/AgCl
Working electrode	Glassy Carbon Electrode, WE-GC with Gasket (GS-25P)
Column Wash	During 8 to 11 min
Total Analysis Time	12 min



## Typical Chromatograms

GABA peak appears at 9 mins under these conditions. There is a very large peak at about 15 min (not shown) if column wash process is not initiated. The Glu peak is close to the large front peak. If the Glu level is less than 100 nM, the peak may be hard to see. Thus the lowest detection limit is about 100 nM for Glu and 10 nM for GABA with a 10  $\mu$ L injection.

## Analyzing Microdialysis Samples

If the sample contains more than 1  $\mu$ M glutamate and 10 nM GABA, as is usually the case in a microdialysis study, this application is sufficient. Work continues to further refine the method for optimal detection with both tissue and microdialysis samples. Please contact us for the latest method.