

Glutamate and GABA for Microdialysis

< HPLC Conditions >

HPLC-ECD system	HTEC-510 or 700 Series + ELS-500 (OFFLINE:M-510)
Separation Column	FA-3ODS (3 mm,i.d. x 75 mm)
Precolumn for sample	CA-ODS packing material in ID 3.0 × 4.0 mm
Precolumn for mobile phase	CA-ODS packing material in ID 4.0 × 5.0 mm
Mobile Phase A	100 mM phosphate buffer(pH 6.0) – Methanol – Acetonitrile (80 : 7 : 13, v/v) 5 mg/L EDTA · 2Na
Mobile Phase B	100 mM phosphate buffer(pH 6.0) – Acetonitrile (50 : 50, v/v) 5 mg/L EDTA · 2Na
Flow rate	500 µL/min
Column Temp.	40 °C
Working Electrode	WE-GC (Glassy Carbon)
Gasket	GS-25P
Applied potential	+600 mV vs. Ag/AgCl
Time Constant	1.0sec
Column wash	During 8 to 11 min

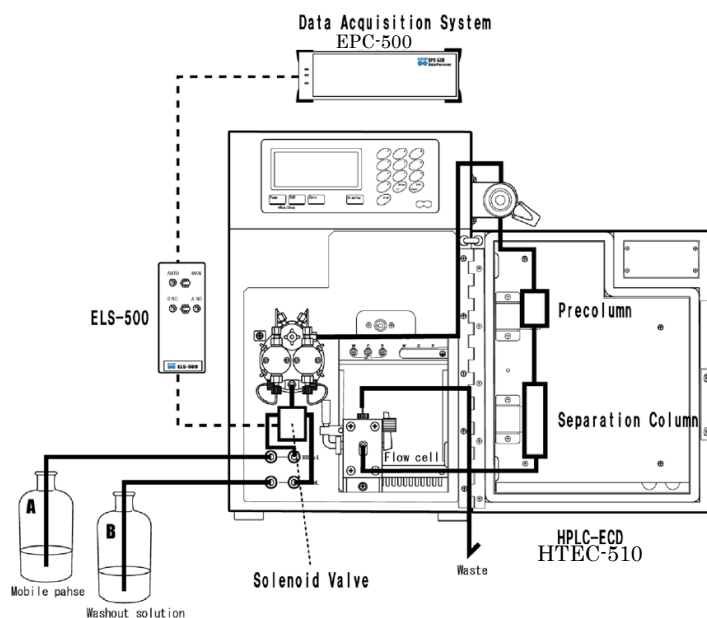


Fig. 1 Flow diagram of HPLC system. Low pressure switching valve can be used only with low dead volume systems such as Eicom's EP-700 or HTEC-510 that has pulse quenching technology. Otherwise, two different types of mobile phase will be mixed in a pulse damper.

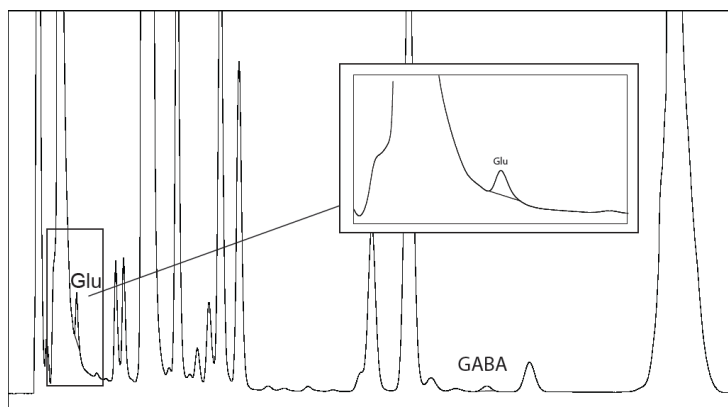


Fig. 2 Chromatogram of microdialysis sample injection which is obtained from rat hippocampus.

Typical Chromatograms

Figure 2 shows a typical chromatogram obtained from brain microdialysis samples. The Glu peak appears at 1.7 min and GABA peak appears at 12 min. There is a very large peak at about 17 min if there is no column wash process. The large peak can be removed and the total analysis run time can be shortened to 16 min with employing the column wash. The Glu peak appears just after the large front peak but it is still well separated.

Samples Types

This application works for brain tissue homogenates and brain microdialysis samples. It may also work for other type of samples.

Mobile Phase A 1 L

100 mM phosphate buffer (pH 6.0)	Methanol	Acetonitrile	EDTA · 2Na
800 mL	70 mL	130 mL	5 mg

100 mM phosphate buffer (pH 6.0) 1 L

NaH ₂ PO ₄ ·2H ₂ O	Na ₂ HPO ₄ ·12H ₂ O	H ₂ O
13.45 g	4.94 g	1000 mL

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