

Glutamate (Enzymatic) for Microdialysis

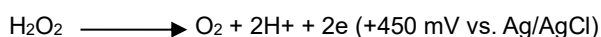
< Principal >

This analysis methodology combines HPLC–ECD with a glutamate oxidase immobilized column (E.C.1.4.3.11). The glutamate reaction takes place as follows:

At the glutamate oxidase immobilized column:



At the ECD:



< System Configuration >

A flow diagram of the system configuration is shown in Fig. 1.

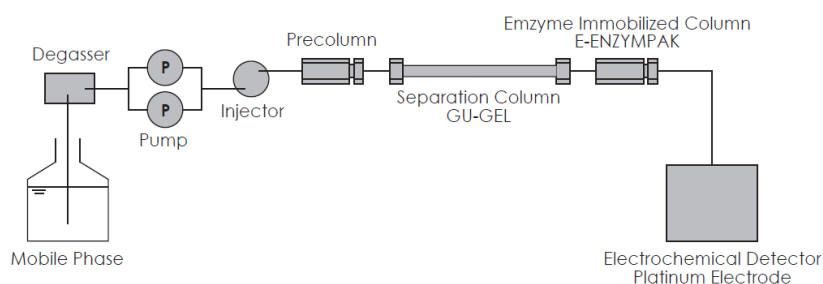


Fig. 1 System Configuration

< HPLC Conditions >

HPLC-ECD system	HTEC-510 (ONLINE:EAS-20S, OFFLINE:M-510)
Separation Column	GU-GEL (φ4.6 mm,i.d. x 150 mm)
Enzyme Column	E-ENZYMPAK (φ3 x 4 mm)
Precolumn for mobile phase	PC-04-CH (φ4.0 × 5.0 mm)
Mobile Phase	60 mM NH ₄ Cl-NH ₄ OH containing HDTA including 0.05 mg/L EDTA-2Na (hexadecyltrimethylammonium bromide)
Flow rate	370 μL/min
Column Temp.	33 °C
Working Electrode	WE-PT (Platinum)
Gasket	GS-25P
Applied potential	+450 mV vs. Ag/AgCl

Mobile phase preparation

Ammonium chloride	10% ammonium hydroxide*	H ₂ O	Hexadecyltrimethylammonium Bromide	EDTA · 2Na
3.20 g	168 µL	1000 mL	250 mg	0.5 mg

*If 10% ammonium is not available, dilute concentrated ammonium to a 10% solution before adding.

< Influence of pH on the Mobile Phase >

If the concentration of ammonium chloride in the mobile phase is constant, the retention time of glutamate will not change within a pH range of 7.0 to 7.5. On the other hand, the retention time of the other anions changes within the pH range mentioned above. The chromatogram shown in Fig. 3 was obtained when 10 µl of 10⁻⁶ M ringer's solution was injected into the analysis system. The negative peak at 12 minutes was generated by the difference in chloride ion concentration in the mobile phase and ringer's solution. The retention time of this negative peak is influenced by the pH of the mobile phase.

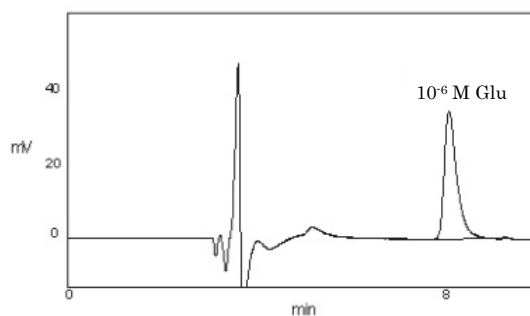


Fig. 3 Chromatogram of standard solution

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