Acetylcholine for Microdialysis

< HPLC Conditions >

HPLC-ECD system	HTEC-510
Separation Column	AC-GEL (φ2.0 x 150 mm)
Enzyme Reactor	AC-ENZYM1 (φ1.0 x 4 mm)
Precolumn for sample	PC-03-CH (φ3.0 x 4 mm)
Precolumn for mobile phase	PC-04-CH (φ4.0 x 5 mm)
Mobile Phase	5 g/L $KHCO_3$ including 50 mg/L EDTA・2Na and, 300 mg/L Sodium 1-Decanesulfonate(SDS)
Flow rate	150 µL/min
Column Temp.	33 ℃
Working Electrode	WE-PT (Platinum)
Gasket	GS-25P
Applied potential	+450 mV vs. Ag/AgCl
Time Constant	3.0 sec

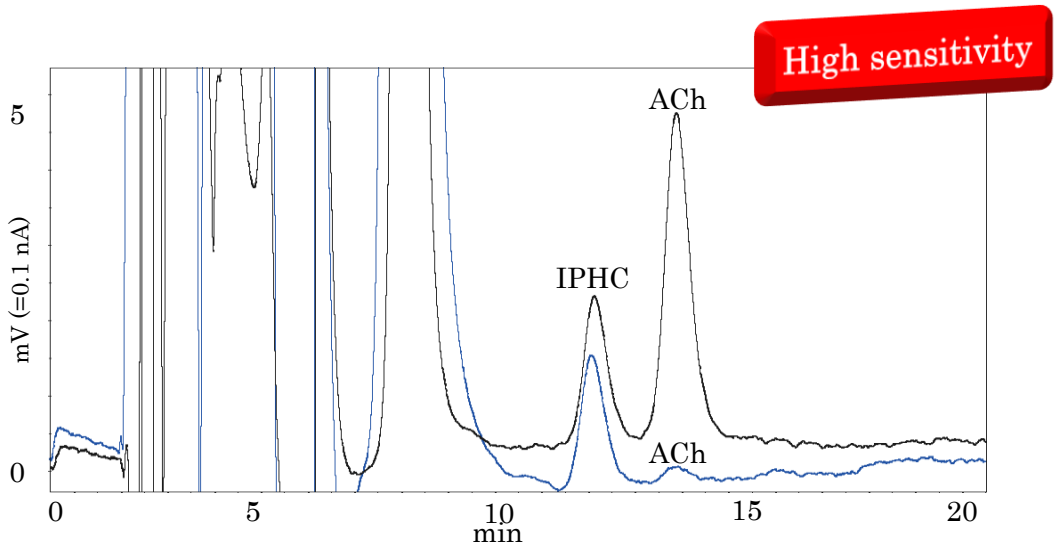

Fig. 1 The black chromatogram shows a standard chromatogram obtained from a 20 µl injection of 10 nM standard. The blue chromatogram was obtained from a 20 µl microdialysis sample. The peaks represent 3.9 fmol acetylcholine and 100 fmol IPHC. Microdialysis conditions; perfusate does not include acetylcholinesterase inhibitor.Obtained from the rat hippocumpus using 3 mm length membrane (Eicom CX-I probe). Flow rate was 1 µl/min and collected for 20 min started 3.5 hours after probe insertion.

< Normal Output Reading at these settings >

Column Pressure ： from 4.0 to 5.5 MPa.

Normal Peak Response ： About 1.8 mV (height) with 10 nM ACh x 10 µl injection

Normal Background Current ： 5 to 20 nA

< Mobile Phase Preparation (Microdialysis)>

Potassium hydrogen carbonate ($KHCO_3$)	H_2O	Sodium 1-Decanesulfonate	EDTA-2Na
5.0 g	1000 mL	300 mg	50 mg

< Preparing Reagents >

Water Quality

This requirement is strict.

To prepare mobile phase and other reagents for ACh analysis, please use ultrapure water. We highly recommend the MilliQ system or similar water purification system producing Type 1 water. Electric resistance of water needs to be 18.2 MΩ-cm or higher. Commercial HPLC grade water can be used but do not store for more than two weeks after opening the bottle.

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