Highly Sensitive Acetylcholine Analysis - Cholinesterase Inhibitor Free

AN01-0815

Detection of acetylcholine (ACh) has historically been difficult for in-vivo monitoring via microdialysis due to the low levels present in the brain. To overcome this, a cholinesterase inhibitor has typically been added to the perfusate which increases the basal levels to an easily detectable range. This procedure has been accepted by the scientific community due to the difficult nature of ACh detection but not without controversy. Artificially modifying the physiological conditions changes the basal state and can result in confounding or misinterpreted results.

Eicom introduces a breakthrough technology coupling HPLC-ECD with a newly improved enzyme reactor, AC-ENZYM II 1.0 x 4.0 mm. This reactor has a seven times longer lifetime as our previous model reactor (AC-ENYMPAK, 3.0 x 4.0 mm). The combination of this reactor with our unique mobile phase conditions reduces chromatography noise and consequently increased our sensitivity two-fold. Our conventional enzyme reactor had 50% reduction in activity after one month of continuous mobile phase flow. The new AC-ENZYM II has only a 25% reduction of activity to ACh after 3 months of normal use without removal from the flow system.

The combination of the sensitive and stable HPLC-ECD system and the new enzyme reactor, AC-ENZYM II allows for precise detection of basal acetylcholine levels well above the limits of detection. This method reliably allows for a detection limit of 5 fmol in the 18 min analysis time.

Analytical Conditions

HPLC-ECD	Eicom HTEC-500
Separation Column	Eicompak AC-GEL (2.0 ID x 150 mm)
Precolumn	Precolumn CH-GEL
Enzyme Column	AC-ENZYM II (1.0 ID x 4 mm)
Flow Rate	150 µl/min
Column Temperature	33˚C
Applied Potential	450 mV vs. Ag/AgCl
Working Electrode	Platinum Electrode (WE-PT) with Gasket (GS-25P)

In-Vivo Microdialysis Study

Male Wistar rats (250-300 g) were used in the following studies. Rats were surgically implanted with guide cannulae (Eicom CXG-6) in the striatum. Eight hours after guide cannula implantation a microdialysis probe was slowly inserted (CX-I-6-03) and perfused with Ringer's solution at 1.0 µl/min. The dialysate was collected into an injector loop and mixed via a 3-way joint (JY-33) with internal standard (IS). The sample was then automatically injected into the HPLC-ECD for automated analysis with Eicom's online autoinjector (EAS-20). The results are shown next in figures 2 and 3.

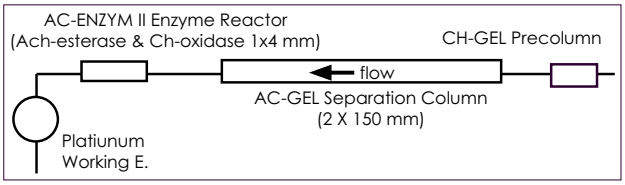

Fig. 1 Flow diagram of HPLC columns and detection. Enzyme immobilized column (reactor) and platinum working electrode can easily be used without chemical preparation such as enzyme modification of the working electrode.

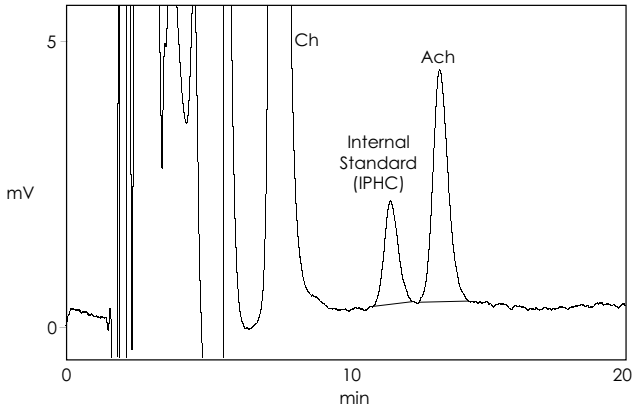

Fig. 2 Standard chromatogram obtained from a 20 µl injection of a 10 nM standard. The Choline (Ch) peak appears prior to the IPHC peak at 8 min.

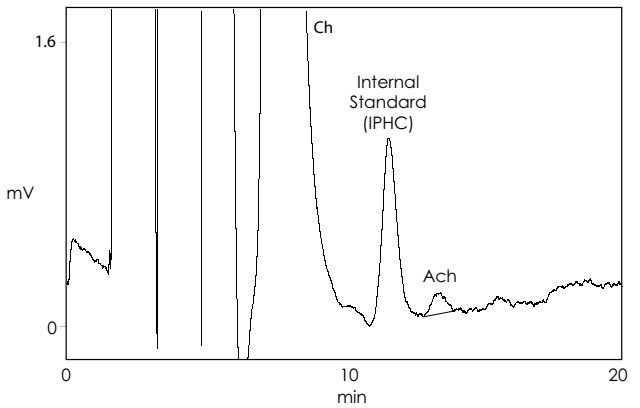

Fig. 3 ACh analysis obtained from a 20 µl striatum microdialysis sample. The ACh peak area corresponds to 10 fmol. This figure shows the basal condition following 3.5 hrs of aCSF perfusion without a cholinesterase inhibitor. The microdialysis probe used was Eicom CX-I-8-03, 3 mm active membrane length.