Rapid GABA Analysis by o-phthalaldehyde Derivatization

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Detection of GABA, one of the major inhibitory amino acids, is not possible using electrochemical detection. Thus, a precolumn derivatization method combined with HPLC-ECD (DC mode) is commonly used to detect GABA from biological samples. In this method, GABA is derivatized with *o*-phtalaldehyde (OPA) and 2-mercaptoethanol (2-ME) and injected into the HPLC system. The derivatization reagents, OPA and 2-ME, react with most amino acids which leads to multiple unknown peaks appearing after the GABA peak. These unknown peaks result in an extended chromatography time and suboptimal throughput. Recently, the identity of GABA has arisen in several papers. The identification of GABA using this method is sensitive to Ca^{2}+ concentrations.

Mobile Phase Switching

To improve these issues, Eicom suggests using a mobile phase switching valve at the low pressure zone before the HPLC pump. This enables the analysis time to become as short as 28 min without requiring the purchase of another HPLC pump and set-up of a high pressure gradient method. The switching valve is set upstream to the degasser. It allows a switch to be made between two types of mobile phase. These are the analytical mobile phase which contains a lower concentration of methanol and the other mobile phase which contains a higher concentration of methanol used to wash out the compounds that have a longer retention time on the column. The timing of the valve switching can be programmed by PowerChrom software, Eicom's standard data acquisition system.

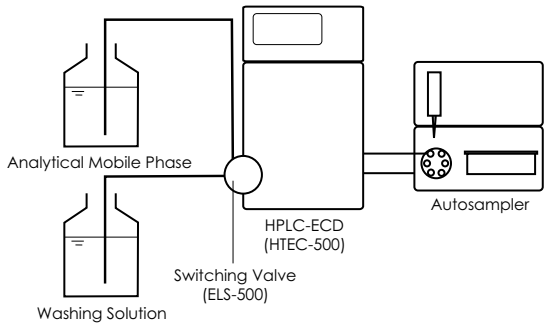

Fig 1. Analysis System Configuration

The system configuration is as shown in Fig. 1. GABA is derivatized with OPA and 2-ME using the autosampler at 4°C for 2.5 minutes followed by an automatic injection into the HPLC system with 30 fmol detection limit. Figure 2 illustrates typical chromatography for both standard solutions and dialysate from rat hippocampus samples. The specificity of the GABA peak was verified by calcium free perfusion in the frontal cortex (Fig. 3). A 70% decrease of the peak was observed 90 minutes following perfusing initiation.

Chromatographic Condition

HPLC-ECD	HTEC-500
Autosampler	AS-700
Switching Valve	ELS-500
Data processor	EPC-500
Column	SC-5ODS (3.0 ID x 150 mm)
Precolumn	AC-ODS
Temperature	33°C
M.P. flow rate	500 µl/min
Applied potential	+600 mV vs. Ag/AgCl
Working electrode	WE-GC (glassy carbon
Gasket	GS-25

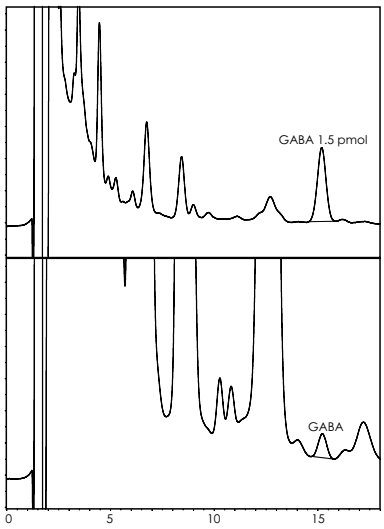

Fig 2. Sample Chromatograms, Top; 1.5 mpol Standard, Bottom; Rat Hippocampus Microdialysate (15 µL)

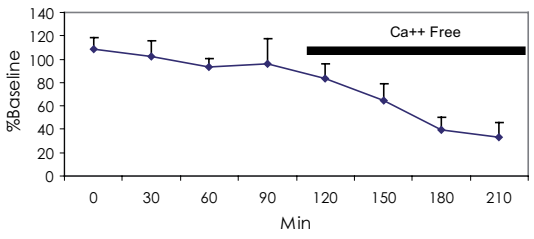

Fig 3. Calcium Free Perfusion in the Rat Prefrontal Cortex