

# ThermoFisher SCIENTIFIC

## Analysis of Amino Acids using the ECD-3000RS electrochemical detector



Ultimate 3000 HPLC with the  
ECD-3000RS Electrochemical  
Detector



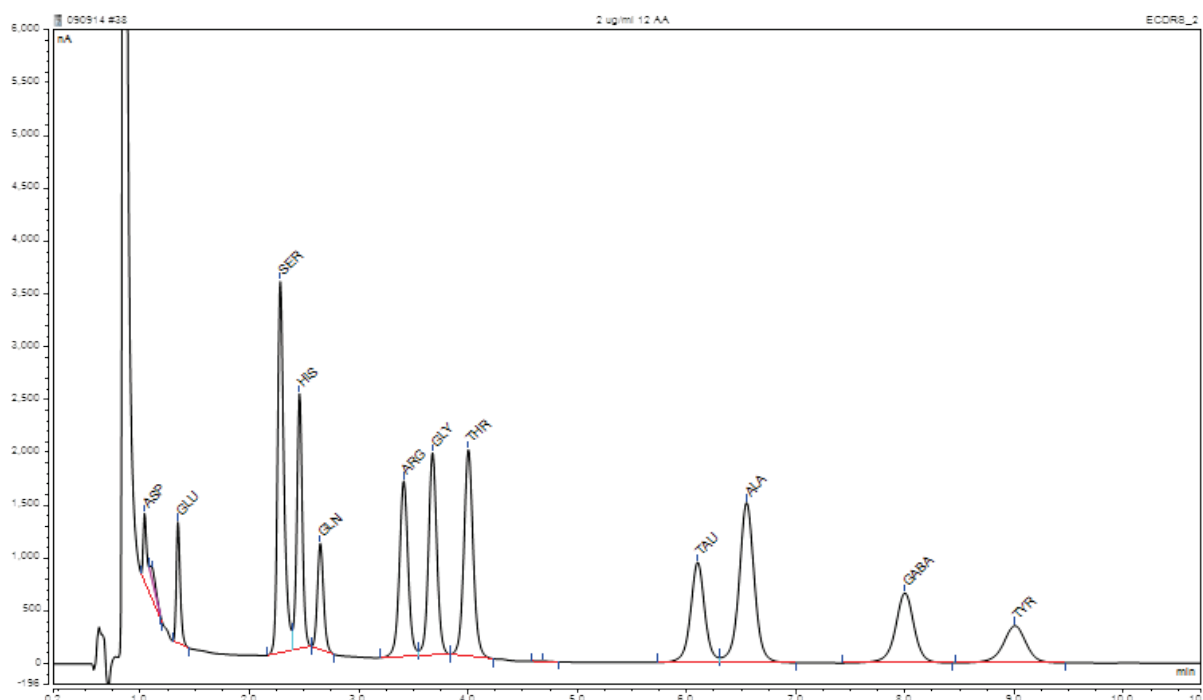
Coulometric Cell



Chromleon  
Chromatography Data Station

Thermo Fisher Scientific  
22 Alpha Road  
Chelmsford, MA 01824

A simple isocratic HPLC method for the analysis of neuroactive amino acids was used to analyze brain microdialysis samples.



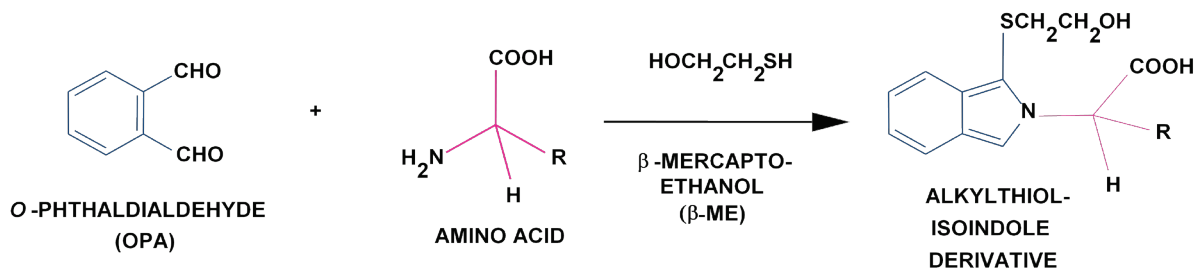
### **Instrumentation:**

Liquid Chromatography System:	UltiMate 3000
Pump:	ISO-3100BM
Autosampler:	WPS-300TBRS
Tray Temperature:	10 °C
Detector:	ECD 3000RS with 6011RS cell
E1 potential	+150 mV
E2 potential	+550 mV
Collection rate:	20 Hz
Chromatography Data System:	Chromeleon 7.2

### **Analytical conditions:**

Flow:	Isocratic at 0.750 mL/min.
Column:	Thermo Scientific Accucore PhenylHexyl, 2.6 micron, 3 mm X 100 mm, Accucore Ph/Hex 10 X 3.0 mm 2.6 micron Guard column and Uniguard Holder
Mobile Phase:	100 mM di-sodium hydrogen phosphate anhydrous 22% methanol, 3.5% acetonitrile, adjust to pH=6.75 with H <sub>3</sub> PO <sub>4</sub>
Inj. volume:	10 µL partial loop
Temperature:	40 °C
EC detector:	6011RS cell: E1 at +150 mV: E2 at +550 mV,

Standards and samples were placed on the autosampler tray and automatically pretreated with a solution containing OPA /  $\beta$ ME to form derivatives of amino acids as shown below.



**Stock OPA/ $\beta$ ME Solution:** (store at 4°C, usable over 5-day period)

- 27 mg OPA is dissolved in 1 mL methanol
- Add 5  $\mu$ L  $\beta$ ME
- Add 9 mL o-Phthalaldehyde (OPA) diluent

**Sample Preparation:**

- Place 15  $\mu$ L microdialysis samples (or standards) into glass microvials.
- Add 15  $\mu$ L of OPA/ $\beta$ ME solution to each sample.
- Mix 4 times and wait 1 minute.
- Add 7  $\mu$ L of 0.1N HCl solution to help lower the pH of the sample then inject 10  $\mu$ L and mix.