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Chromatography for Neuroscience Applications Notebook

Sensitive, Selective, Proven Analytical Methods

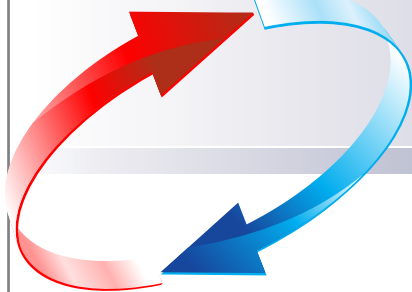


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Redefining HPLC and UHPLC to Give You More

The Thermo Scientific™ Dionex™ UltiMate™ 3000 platform is the most complete LC solution provided by a single chromatography powerhouse. By enabling all our UltiMate 3000 systems to be UHPLC compatible by design, we provide the market-leading system solution to all users, all laboratories and all analytes.

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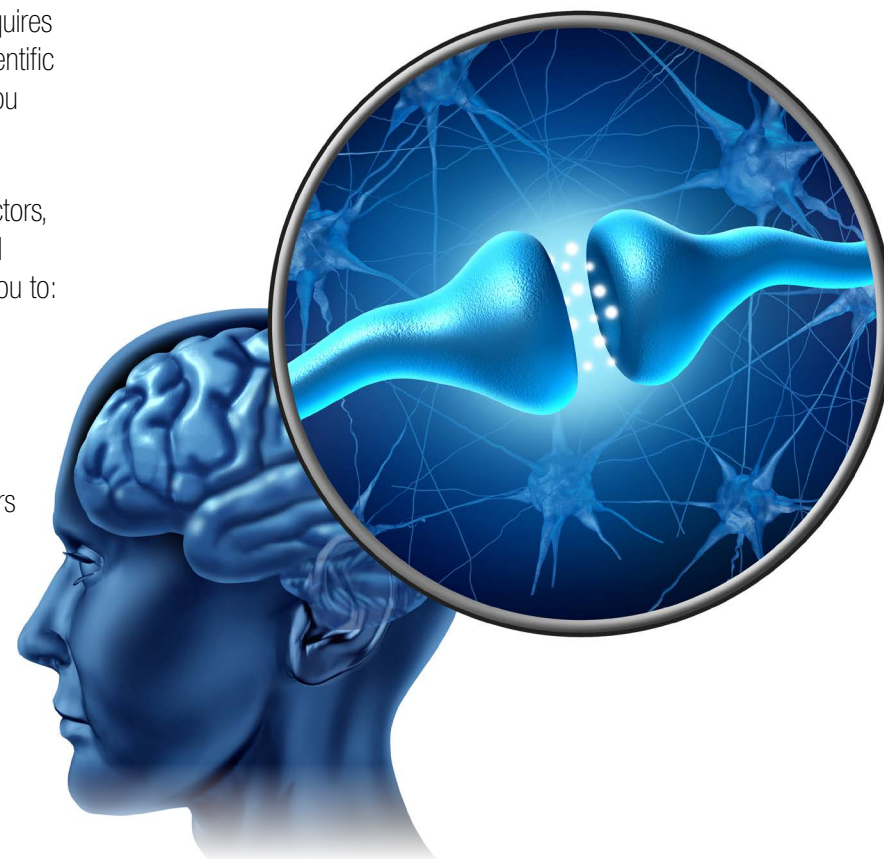
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When you are studying central neurotransmission and/or the effect of drugs and disease, you need precise, reproducible results, and you need them fast. The continuing need for greater temporal and spatial resolution—often with lower-volume microdialysis perfusate samples—requires the use of extremely sensitive analytical instrumentation. Thermo Scientific Dionex LC Systems for neuroscience offer distinct benefits to help you get the utmost information from your precious samples.

Using our UHPLC-ready systems, highly sensitive and selective detectors, and state of the art column technologies, along with proven analytical methods, precise automation and advanced data handling will help you to:

- Measure femtogram levels of analytes
- Analyze multiple neurotransmitters simultaneously
- Conserve precious samples and reagents using UHPLC
- Increase sample throughput with reliable, maintenance-free sensors



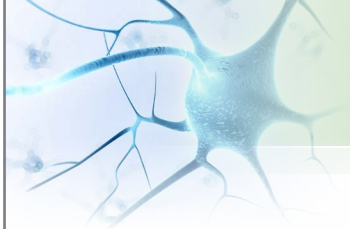


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Liquid Chromatography techniques combined with electrochemical detection (ECD) provide highly sensitive and selective analyses for a wide range of biological and pharmaceutical compounds. The Thermo Scientific UltiMate 3000 Electrochemical Detector is specifically designed to function along with our UltiMate 3000 UHPLC+ systems to provide superior sensitivity through minimizing background currents and noise, resulting in the best limits of detection. For monitoring biological processes, as in neuroscience, this enables greater spatial and temporal resolution. These capabilities extend well beyond neuroscience, from cardiovascular and cancer research to natural products, where high sensitivity and selectivity are critical.

This UltiMate 3000 system offers a turnkey solution for measurement of femtogram levels of oxidizable or reducible compounds, with full capabilities for analysis of neurotransmitters, drugs and metabolites, natural products and genotoxins from biological samples. The robustness and reliability of the system offers you the confidence and capability to achieve ultrasensitive results and maximum performance with minimum effort and downtime.

- The solution for analysis of neurotransmitters, thiols, and drug metabolites in biological systems
- A completely biocompatible flow path minimizes interference, assures exceptionally low backgrounds, and minimizes degradation of labile analytes
- Precision autosampler delivers high-performance analyses with zero sample carryover and accurate sampling from low-volume samples with minimal waste
- Our system configurations are designed for reliable operation and increased system longevity
- Advanced system control and monitoring using the Chromeleon CDS software



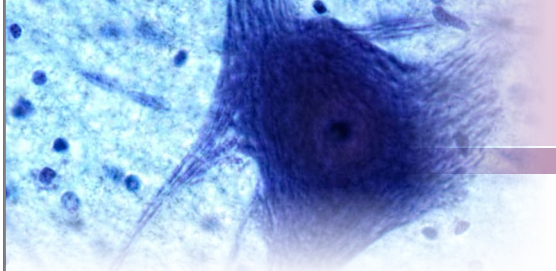


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In order to obtain the maximum information from biological samples, neuroscientists require a sensitive approach that can measure numerous key neurochemicals, simultaneously. The ability to measure low levels of many different neurochemicals simultaneously is challenging, due to detector sensitivity and the chromatographic issue of resolving analytes with similar chemical structures. Most of the biogenic amines and metabolites can be oxidized electrochemically, making the use of electrochemical detection routine for the analysis of these compounds. In this example, a simple, rapid, and accurate method was developed for the analysis of biogenic amines, their metabolites, and precursor amino acids using isocratic chromatography with a multichannel electrochemical detector. This enables both chromatographic and voltammetric resolution of many compounds, thereby enhancing the identification and accurate quantification of these compounds.

Conditions

Flow:	Isocratic at 0.50 mL/min.
Temperature:	35 °C
Column:	Thermo Scientific™ Hypersil™ BDS C18 column, 3 μm, 3 x 150 mm; Thermo Scientific Hypersil BDS guard column (28103-013001); Thermo Scientific™ UniGuard™ guard cartridge holder (852-00)
Injection Volume:	5 μL (standards) – 10 μL (tissue samples, partial loop)
Mobile Phase:	Thermo Scientific Dionex Test Mobile Phase (70-3829)
Detector:	Electrochemical—UltiMate 3000 Electrochemical Detector with two inline 6011RS ultra Coulometric Analytical Cells: E1: +100 mV; E2: +250 mV; E3: +400 mV, E4: +550 mV vs. Pd reference electrode

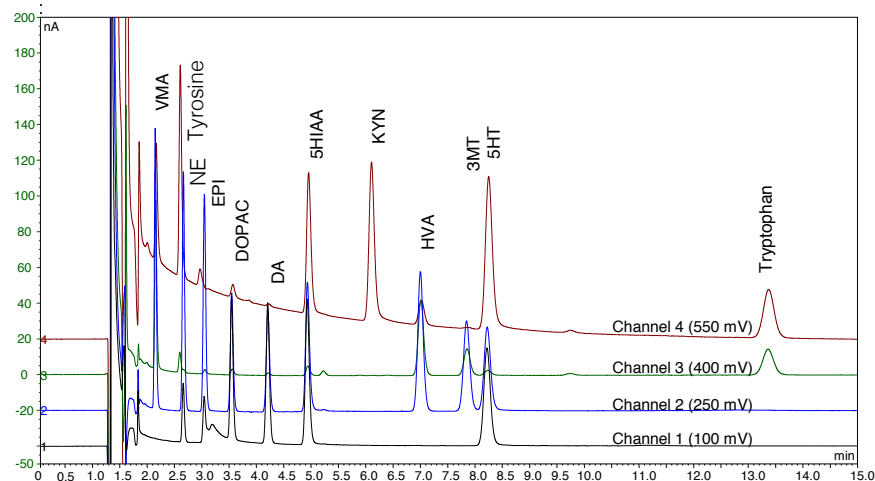


Figure 1. Neurochemical profiling with a four-channel electrochemical array detector.

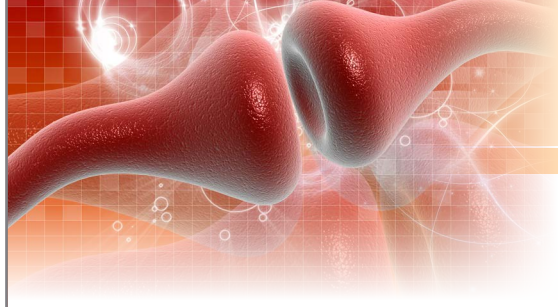


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A number of different compounds act as neurotransmitters, including monoamines such as norepinephrine (NE), dopamine (DA), and serotonin (5HT). This example showcases a high throughput, rapid and sensitive method for the analysis of dopamine and serotonin. DA and 5HT were analyzed in less than five minutes, as described using a short (50 mm) UHPLC column, and showed improved temporal resolution to assess possible neurochemical changes.

Conditions

Flow:	Isocratic at 0.40 mL/min.
Temperature:	32 °C
Column:	Thermo Scientific™ Acclaim™ RSLC PA2, 2.2 μm, 2.1 x 50 mm
Injection volume:	10 μL partial loop
Mobile Phase:	150 mM sodium dihydrogen phosphate, monohydrate, 4.76 mM citric acid, monohydrate, 3 mM sodium dodecyl sulfate (SDS), 50 μM EDTA, 15% acetonitrile, 10% methanol, adjust to pH=5.60 sodium hydroxide, 99.99%, semiconductor grade (14N solution)
Detector:	Electrochemical— Thermo Scientific™ Dionex™ Coulochem™ III detector with 5041A High Sensitivity Analytical cell with glassy carbon electrode; 12 μm BoPet gasket, E: +225 mV vs. Pd reference electrode
Sample:	Artificial cerebral spinal fluid (aCSF) was collected for 10 minutes at 1 μL/min from Collection a 3 mm microdialysis probe positioned in the prefrontal cortex of the rat brain.

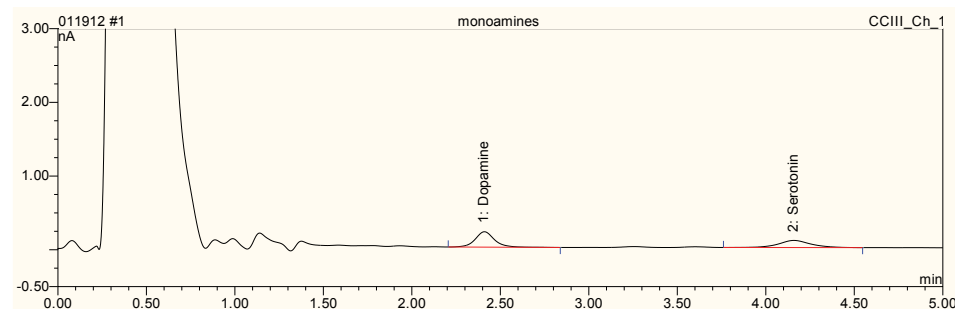


Figure 2. Rapid Analysis of dopamine and serotonin in a microdialysis sample.

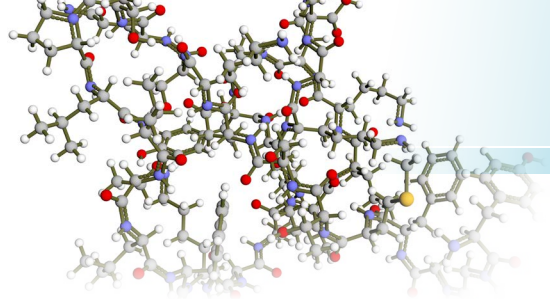


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Neuroactive amino acids act as excitatory and inhibitory molecules in the CNS. Measurement of low levels of these amino acids from basal striatal microdialysis perfusates can be accomplished very quickly using HPLC with fully automated in-line pre-column sample derivatization followed by separation and electrochemical detection. This example illustrates a fast and stable isocratic method for analysis of amino acid that act as neurotransmitters in the CNS. Here, this UHPLC analysis of microdialysis samples for their neuroactive amino acids was completed within 17 minutes with detection at low ng/mL levels, which is a 2 to 5 fold decrease over prior methods.

Conditions	
Flow:	Isocratic at 0.64 mL/min.
Temperature:	45 °C
Column:	Thermo Scientific™ Accucore™ PhenylHexyl, 2.6 μm, 3 x 100 mm, Accucore Ph/Hex, 2.6 μm, 3.0 x 10 mm Guard column and Uniguard Holder
Inj. volume:	5 μL partial loop
Mobile Phase:	100 mM di-sodium hydrogen phosphate anhydrous 22% methanol, 3.5% acetonitrile, adjust to pH=6.75 with H ₃ PO ₄
Detector:	Electrochemical—Coulchem III detector with 6011 ultra Coulometric Analytical cell: E1 at +150 mV; E2 at +550 mV vs. Pd reference electrode
Sample:	A sample of aCSF was collected for 15 minutes at 1 μL/min from a 3 mm microdialysis probe positioned in the corpus striatum of the rat brain.

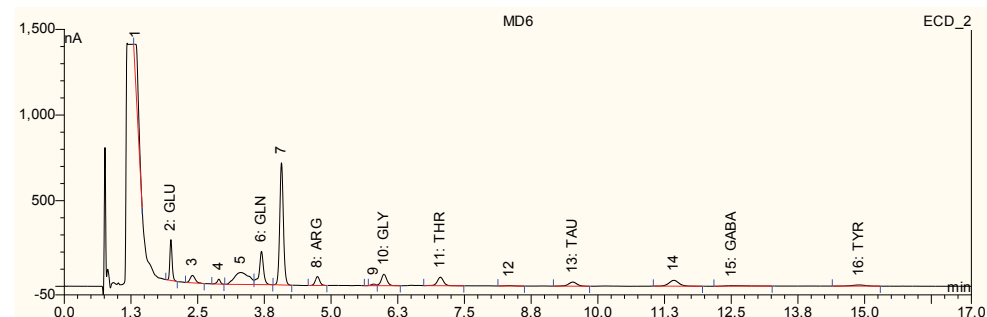


Figure 3. Analysis of neuroactive amino acids in a microdialysis sample from rat corpus striatum.



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A number of biochemically important sulfur containing compounds occur in vivo including: aminothiols such as cystine, glutathione (GSH) and homocystine. These aminothiols play numerous physiological roles. GSH is a major cellular antioxidant and a cofactor for glutathione peroxidase, an enzyme that detoxifies hydrogen peroxide and lipid hydroperoxides. The high ratio of GSH/GSSG keeps the cell in a reducing environment, which is essential for its survival. Decreases in this ratio are associated with cellular toxicity in numerous diseases including neurodegeneration (e.g., Parkinson's disease). This example shows a rapid and robust UPHLC method using a boron-doped diamond electrode for measuring various aminothiols.

Conditions	
Flow:	Isocratic at 0.500 mL/min
Column:	Accucore RP-MS column 2.6 μ m, 2.1 x 150 mm
Temperature:	Column: 50°C; Post-column: 25°C
Injection Volume:	2 μ L standards; 4 μ L samples
Mobile Phase:	0.1% pentafluoropropionic acid, 0.02% ammonium hydroxide, 2.5% acetonitrile, 97.4% water
Detector:	Electrochemical—UltiMate 3000 Electrochemical Detector with 6041RS ultra Amperometric Analytical Cell with BDD electrode; E: +1600 mV vs. Pd reference electrode
Sample:	5–20 μ L whole blood + 200 μ L 0.4 N PCA, mix and spin for 10 minutes at 13,000 RPM. The clear supernatant was transferred into an autosampler vial and placed on the autosampler at 10 °C.

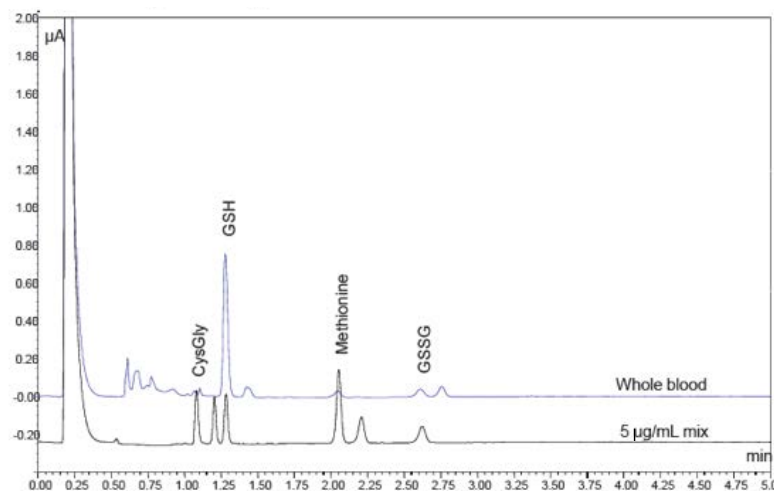


Figure 4. Aminothiol levels observed in whole blood using the BDD electrode.

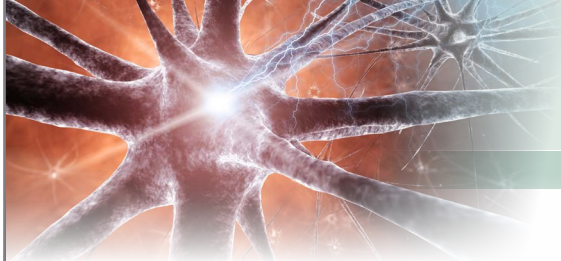


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Acetylcholine (ACh) is a critical neurotransmitter in the brain. Unfortunately, this substance occurs at very low levels in the extracellular space and is difficult to detect. In order to study cholinergic neurotransmission in vivo, a stable and easy-to-use approach is required. Although ACh is not electrochemically active, enzymes can be used to convert it to a product that is easily detectable.

This UHPLC example demonstrates a five-minute analysis time with improved separation and sharper peaks that provide enhanced sensitivity for this important analyte. Due to the high sensitivity, low noise performance of the UltiMate 3000 Electrochemical detector, the hydrogen peroxide produced through the use of our inline, solid-phase reactor (SPR) provides a suitable electrochemically-active moiety, that permits easy detection for correlation to active ACh levels in microdialysis samples at levels less than 20 fmol.

Conditions

Flow:	Isocratic at 0.30 mL/min.
Temperature:	40 °C
Column:	Hypersil BDS C18 column, 2.4 μm, 2.1 x 50 mm (28102-052130) ; Post-column Solid Phase Reactor for Acetylcholine: ACH-SPR (70-0640)
Inj. Volume:	10 μL
Mobile Phase:	100 mM Disodium hydrogen phosphate, 0.8 mM 1-Octanesulfonic Acid Sodium Salt, 0.005% Reagent MB (70-1025), pH 7.0 ±0.2 with H ₃ PO ₄
Detector:	Electrochemical—UltiMate 3000 Electrochemical Detector with 6041RS ultra Amperometric Analytical Cell with Pt electrode; E: +400 mV vs. Pd reference electrode

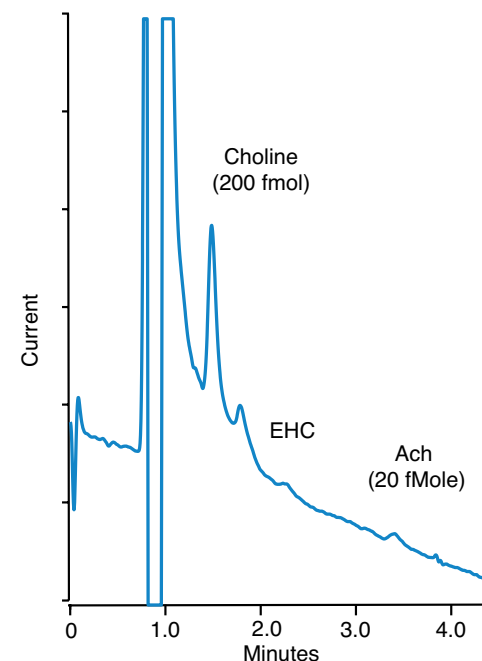


Figure 5: Rapid determination of acetylcholine levels at low femtomole levels.

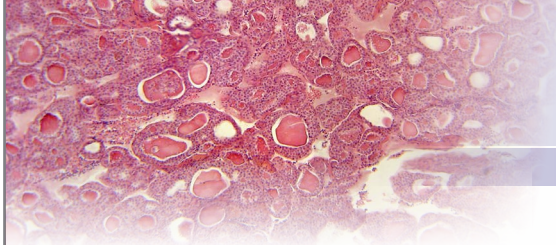


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Once thought only to be common in lower organisms, D amino acid enantiomers can now easily be quantitated from mammalian tissue homogenates, even in the presence of large amounts of their corresponding L-enantiomers. Our fast UHPLC method involves simple sample extraction, followed by automated precolumn derivatization for femtogram level detection, and provides enhanced resolution beyond other HPLC methods.

The example below showcases a method for the detection of D-Asp and D-Ser in the presence of a large amount of their corresponding L-enantiomers, with detection limits of 200 fg for D-Ser and 400 fg for D-Asp using a UltiMate 3000 fluorescence detector. This UHPLC method reduced the run time to under than 20 min versus 30–60 min using HPLC and enhanced the resolution between D- and L-Asp which allows more accurate determination of D-Asp levels in biological samples.

Conditions

Flow:	Gradient at 0.25 mL/min 0–6 min 3% B; 6.5–10 min 20% B; 11–14 min 80% B; equilibrate at 3% B for 7 min
Temperature:	35 °C
Column:	Thermo Scientific™ Hypersil GOLD™ 1.9 μm × 2.1 mm, P/N 25002-202130
Inj. Volume:	2 μL
Mobile Phase:	A: 50 mM Dibasic sodium phosphate, pH 6.5 ; B: Methanol
Temperature:	35 °C
Detector:	Fluorescence — Thermo Scientific Dionex UltiMate 3000 Fluorescence Detector (FLD-3400RS) Excitation: 340 nm, Emission: 450 nm, sensitivity 4

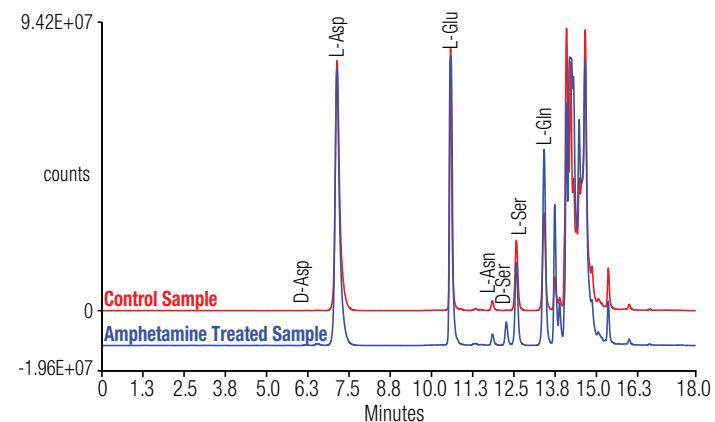


Figure 6. Comparison of amino acid levels found in rat brain stem tissue homogenates before and after treatment with amphetamine: upper red trace.

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Recommended System for High Sensitivity Isocratic Analyses of Neurotransmitters:

UHPLC⁺ Liquid Chromatography Modules & Accessories		
Pump:	UltiMate 3000 Biocompatible Isocratic Analytical Pump for Electrochemical Detection Systems (ISO-3100BM)	5042.0011
Autosampler:	UltiMate 3000 Analytical Biocompatible Split-Loop Thermostatted Well Plate Autosampler (WPS-3000TBRS)	5841.0020
Solvent Rack:	UltiMate 3000 Solvent Rack without built-in degassing (SR-3000)	5035.9200
Data System:	Chromeleon CDS	Chromeleon 7.2
Detector: Electrochemical		
UltiMate 3000 Electrochemical Detector (ECD-3000RS) <i>Add a DC potentiostat module for 1 or 2-channel operating capability or 2 DC potentiostat modules to expand for 4-channel operation with dual inline Coulometric sensors</i>		5070.0010
Required Accessories for UltiMate 3000 Electrochemical Detector:		
Potentiostat Module, DC, Dual channel, for ECD-3000RS detector		6070.1400
Electrochemical Sensors for UltiMate 3000 Electrochemical Detector		
Flow-through Dual electrode	ultra Coulometric analytical sensor (6011RS) for use with ECD-3000RS detector	6070.2400
Thin-layer single electrode	ultra Amperometric analytical sensor (6041RS) with accessories and gaskets <i>(choose a working electrode below)</i>	6070.3000
Working Electrodes for Amperometric Sensor (6041RS)		
For Neurotransmitters and related analytes	Working electrode, glassy carbon, high efficiency, for use with 6041RS sensor	6070.3200
For Thiols, disulfides and related analytes	Working electrode, boron-doped diamond, for use with 6041RS sensor	6070.3100
UHPLC Fingertight Fitting and Capillary Kits		
nanoViper Connection Kit	nanoViper Capillary Connection Kit for ECD-3000RS detector	6041.5105



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Recommended System for Gradient Analysis of Neuroactive Amino Acids:

UHPLC ⁺ Liquid Chromatography Modules & Accessories		
Pump:	UltiMate 3000 Quaternary Bio-RS Rapid Separation Pump, (LPG-3400RS)	5040.0036
Autosampler:	UltiMate 3000 Analytical Biocompatible Split-Loop Thermostatted Well Plate Autosampler (WPS-3000TBRS)	5841.0020
Solvent Rack:	UltiMate 3000 Solvent Rack without built-in degassing (SR-3000)	5035.9200
Column Compartment:	UltiMate 3000 Rapid Separation Thermostatted Column Compartment (TCC-3400RS)	5730.0000
Data System:	Chromeleon CDS	Chromeleon 7.2
Detector: Fluorescence		
UltiMate 3000 Rapid Separation Fluorescence Detector (FLD-3400RS) (<i>without Flow Cell</i>)		5078.0010
<i>Required Accessories for UltiMate 3000 Fluorescence Detector:</i>		
Flow cell:	Analytical Flow Cell for FLD-3000 Series detector, SST, 8 μ L Volume	6078.4230



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