Advances in Neurochemical Profiling of Brain Tissue Samples Using HPLC with a Novel Four-Channel Electrochemical Array Detector

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Overview

Purpose: In order to obtain the maximum information from biological samples, neuroscientists require a sensitive approach that can measure numerous key neurochemicals, simultaneously. 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.

Methods: Profiling of biogenic amines, their metabolites and precursor amino acids using HPLC chromatographic techniques with a multichannel electrochemical instrument and readily available column and mobile phase is described.

Results: The method enables the rapid separation of various neurochemical compounds at trace levels and without significant matrix interferences.

Introduction

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 so the use of electrochemical detection is routine for the analysis of these compounds. Chromatographic techniques have advanced over the years, however, even with the use of UHPLC columns, baseline resolution of many different analytes still remains difficult due to the constraints of isocratic HPLC mode for their separation. Although gradient elution would improve analyte resolution, electrochemical detection is typically only used with isocratic approaches due to adverse effects of changes in mobile phase composition on detector performance. A new modular electrochemical detector has been developed that uses multiple coulometric electrodes in series, with each electrode having a unique potential setting. This voltammetric approach provides additional resolution of analytes beyond their chromatographic separation. The detector is fully compatible with gradient HPLC techniques and provides an autoranging feature that enables the simultaneous measurement of low and high level analytes. Qualitative information is thereby enhanced while still maintaining quantitative sensitivity requirements for specific analytes at low concentrations. Examples illustrating the content of biogenic amines and acid metabolites in brain tissue samples are presented, using a four channel electrochemical array combined with UHPLC chromatographic separation.

Methods

Biogenic Amines and Metabolite Analytical Conditions

Flow:	Isocratic at 0.50 mL/min.
Temperature:	35 °C
Column:	Thermo Scientific™ Hypersil™ BDS C18 column, 3 μm, 3 × 150 mm; Thermo Scientific Hypersil BDS guard column (28103-013001); Thermo Scientific™ UniGuard™ guard cartridge holder (852-00)
Inj. Volume:	5 μL (standards) – 10 μL (tissue samples, partial loop)
Mobile Phase:	Thermo Scientific™ Dionex™ Test Phase (70-3829)
EC Cell:	Thermo Scientific™ Dionex™ model 6011RS ultra Coulometric Analytical cell: E1: +100 mV: E2: +250 mV, E3: +400 mV, E4: +550 mV vs. Pd reference electrode
Animals:	Male Sprague Dawley rats weighing 175–200 grams were administered vehicle (saline) via i.p. injection. One hour later animals were sacrificed by carbon dioxide asphyxiation and the brains rapidly removed, dissected, and frozen at -70 °C.
Sample Preparation:	Brain tissue samples (10–25 mg) were prepared in 0.3 N perchloric acid, sonicated to disrupt the tissue and centrifuged at 13,000 RPM for 10 min. The clear supernatant was transferred into an autosampler vial and placed on the autosampler at 10 °C.

FIGURE 1A. Inert HPLC system for trace neurochemical analysis.
FIGURE 1B. 6011RS ultra Coulometric Analytical Cell
FIGURE 1C. Specialized Thermo Scientific™ Dionex™ nanoViper™ capillaries

A

B

C

Thermo Scientific™ Dionex™ UltiMate™ 3000 SR-3000 Solvent Rack (without degasser). It is recommended that solvents should be degassed daily via vacuum degassing (this ensures highest possible sensitivity)
Thermo Scientific™ Dionex™ UltiMate™ 3000 ISO-3100BM Pump
Thermo Scientific™ Dionex™ UltiMate™ 3000 WPS-3000TBSL Analytical Autosampler
Thermo Scientific™ Dionex™ UltiMate™ 3000 ECD-3000RS Electrochemical Detector with integrated temperature controlled column compartment
Thermo Scientific™ Dionex™ Chromeleon™ CDS software, version 6.8

Results and Discussion

An instrumental prerequisite for trace analysis is that the HPLC system must be inert in order to achieve optimal sensitivity using an electrochemical detector. The system shown above in Figure 1A uses biocompatible materials in the flow path to reduce the influence of metal that can contribute to elevated background currents at the electrochemical cell. The recent introduction of the ECD-3000RS detector enables multiple electrodes to be attached in series after the HPLC column. Use of the 6011RS cell (Figure 1B) provides coulometric electrochemical efficiencies. This platform provides both chromatographic and voltammetric resolution of compounds. New nanoViper (Figure 1C) fingertight fittings were employed to cope with the higher pressures due to smaller column particles. These fingertight, virtually zero-dead-volume (ZDV) capillaries can operate at pressures up to 14,500 psi and are much safer to use than PEEK™ tubing which can slip when using elevated pressures. They are made of PeekSil™ tubing and are available in small internal dimensions to minimize chromatographic band spreading. Capillaries used on this system were 150 micron ID for all connections made prior to the autosampler valve and 100 micron ID for those made after the injector valve.

Analysis of Biogenic Amines and Acid Metabolites

A common assay used for brain tissue samples is the analysis of important biogenic amines norepinephrine (NE), dopamine (DA), and serotonin (5HT), amino acid precursors tyrosine and tryptophan and metabolites including dihydroxyphenyl acetic acid (DOPAC), 5-hydroxyindole acetic acid (5HIAA), kynurenine (KYN), homovanillic acid (HVA), and 3-methoxytyramine (3MT). A method is described which allows the complete separation of these compounds in less than 15 mins using a 3 micron column (Figure 2). Good linearity of response was obtained since the correlation coefficients ranged from R^2 = 0.9991–0.9999 for the 12 compounds evaluated (Table 1) over a concentration range of 5—500 ng/mL. These data used the signals obtained at the dominant channel for each compound. The percent relative standard deviation (%RSD) for the calibration curves (seven concentrations in duplicate) is also shown in Table 1. The RSD values ranged from 0.98% to 5.48%, indicating that the coulometric electrodes provided good stability during this analysis.

FIGURE 2. Neurochemical profiling with a four-channel electrochemical array detector (100 ng/mL)

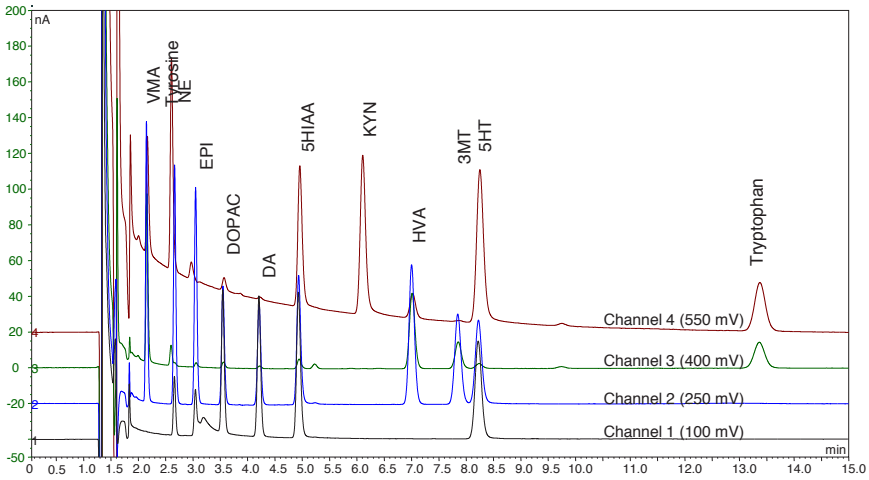
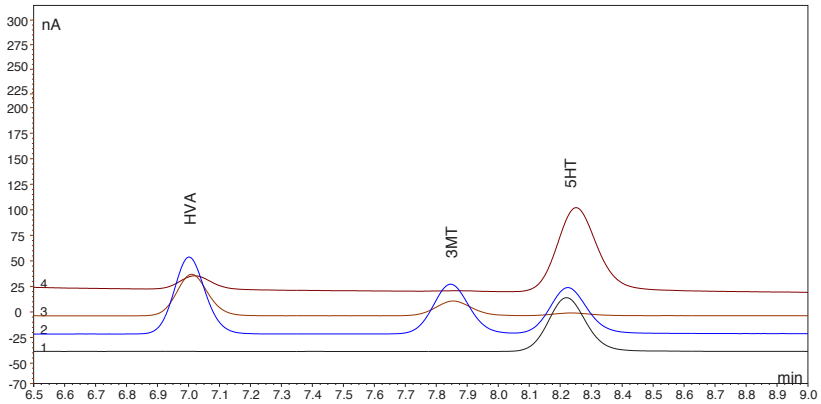


Table 1. Calibration data for standards ranging from 5–500 ng/mL

Peak	Name	RT (min)	Rel. Std. Dev %	Correlation Coeff. R^2	Dominant Channel
1	VMA	2.15	1.40	0.9999	2
2	Tyrosine	2.60	0.94	0.9999	4
3	NE	2.65	2.08	0.9998	2
4	EPI	2.98	1.89	0.9999	2
5	DOPAC	3.60	5.48	0.9991	1
6	DA	4.25	5.22	0.9992	1
7	5HIAA	5.00	3.73	0.9996	4
8	Kynurenine	6.10	1.15	0.9999	4
9	HVA	7.00	0.98	0.9999	3
10	3MT	7.85	3.81	0.9997	2
11	5HT	8.20	1.831	0.9999	4
12	Tryptophan	13.3	2.88	0.9997	4

In Figure 3, the section of the chromatographic trace shows both chromatographic and voltammetric resolution between the compounds 3MT and 5HT. Note that serotonin show a response at lower potentials of 100 and 250 mV, due to the oxidation of the 5-hydroxy group, and one at higher potentials of 550 mV due to the oxidation of the indole ring nitrogen. Voltammetric resolution offers superior insights into the proper identification of individual compounds since each will have a unique but reproducible pattern across the four electrode channels.

FIGURE 3. Chromatographic and voltammetric resolution of compounds (100 ng/mL)



The analysis of tissue samples is illustrated in Figures 4 and 5. These demonstrate that picogram sensitivity can be obtained using this technique. Brain tissues from various regions were analyzed using this method including the corpus striatum and frontal cortex.

FIGURE 4. Neurochemical profiling of brain tissue sample (corpus striatum)

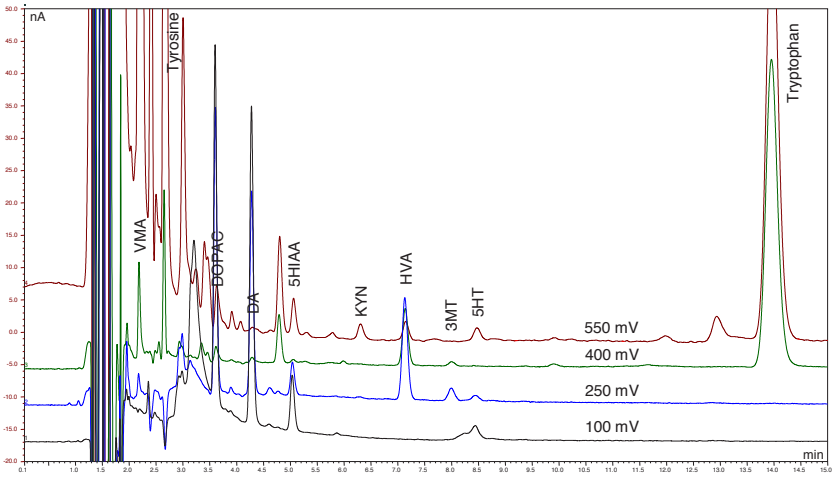
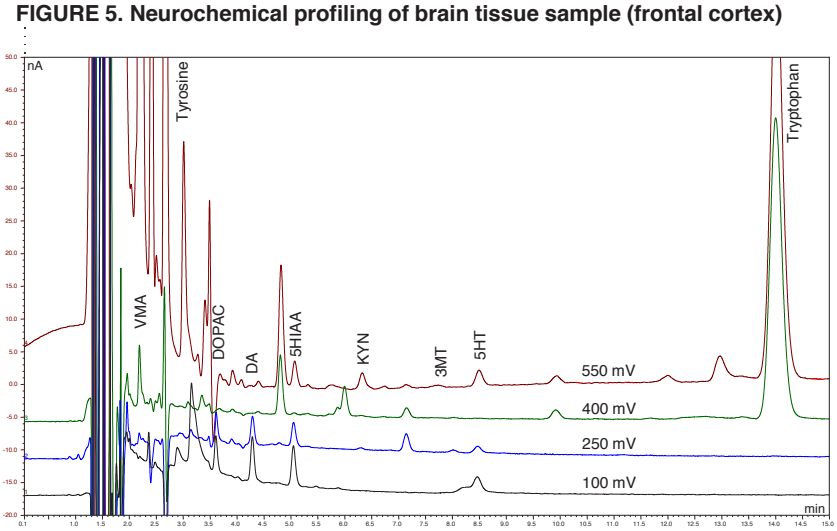


FIGURE 5. Neurochemical profiling of brain tissue sample (frontal cortex)



The levels of neurochemicals found in regional tissue samples are presented in Table 2. These data indicate that the corpus striatum has higher levels of the majority of neurochemicals measured, except for serotonin which was slightly elevated in the frontal cortex sample.

Table 2. Levels (ng/g tissue wet weight) of measured neurochemicals in regional brain tissues

Region	VMA	DOPAC	DA	5HIAA	KYN	HVA	3MT	5HT
Striatum	873	5235	4108	342	146	1086	202	115
Frontal Cortex	611	443	453	246	139	156	20	122

Region	Tyrosine	Tryptophan
Striatum	18472	14624
Frontal Cortex	13326	12158

FIGURE 6. Neurochemical concentrations of regional brain tissue samples (corpus striatum vs. frontal cortex region)

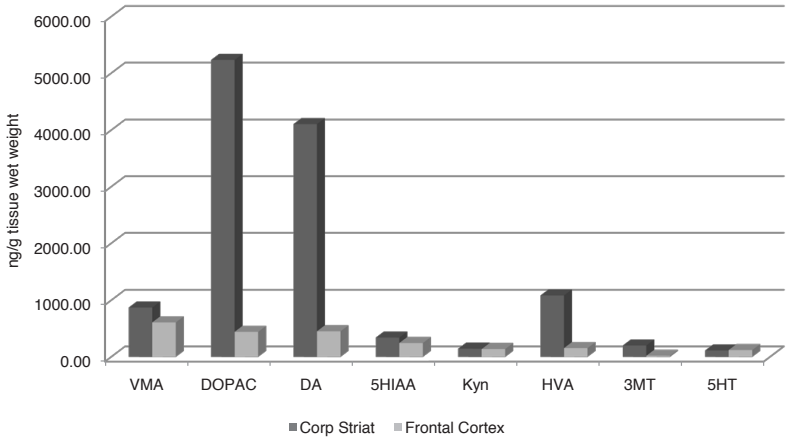
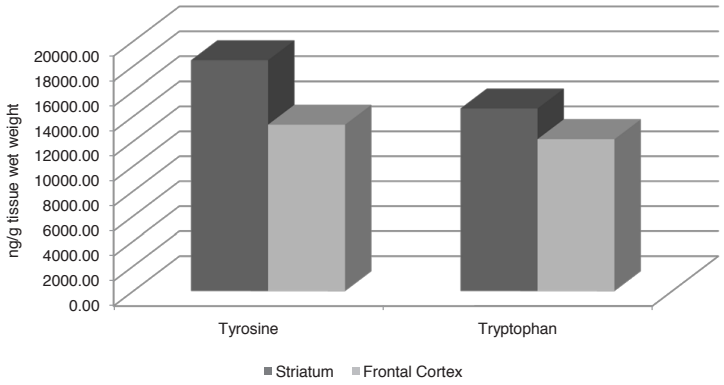


FIGURE 7. Amino acid precursor concentrations of brain tissue samples



Conclusions

• The method for biogenic amines, their metabolites, and precursor amino acids was both highly sensitive and rapid. All compounds were analyzed within 15 minutes and with limits of detection of less than 10 picograms on-column.

• Voltammetric resolution offers better insights into the proper identification of individual compounds since each will have a unique but reproducible pattern across the four electrode channels.

• Neurochemical profiles of brain tissue samples can be easily obtained using readily available instruments, columns, and mobile phases.

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