Application Note: 436

Bioanalytical Assay for Neurotransmitters in Whole Blood by LC-MS/MS

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Key Words

- Aria TLX-1
- TSQ Quantum Ultra
- TurboFlow Technology
- Parkinson's Disease

Introduction

Taken orally in conjunction with Levodopa (L-DOPA), Carbidopa (C-DOPA) inhibits the metabolism of L-DOPA before it reaches the brain so that more is available to be converted into dopamine in the brain. 3-methoxy-L-tyrosine (3-OMD) is an important metabolite produced after L-DOPA administration. The following LC-MS/MS method using TurboFlow™ technology for on-line sample extraction using a Thermo Scientific Aria™ TLX-1 system coupled with Thermo Scientific TSQ Quantum Ultra™ triple quadrupole mass spectrometer demonstrates its suitability as a research method for these compounds in human whole blood.

Goal

To develop a quantitative, fast, automated LC-MS/MS method for analysis of neurotransmitters in human whole blood.

Method Information

These analytes were extracted on-line from crashed human whole blood. Calibration curves were analyzed using an Aria TLX-1 LC system coupled with a TSQ Quantum Ultra with heated electrospray ionization (H-ESI) source. Internal standards used were 4-chloro-L-phenylalanine and L-DOPA-d_3.

Experimental Conditions

Sample Preparation

A standard stock solution of 50 µg/mL L-Dopa, C-Dopa and 3-OMD in methanol was prepared. Methanol-quenched human whole blood (K_2 EDTA) was centrifuged at 10,000 RPM for 10 minutes. Calibrators were prepared in the supernatant. Analyte concentration ratio of spiking solution was 4 to 1 of L-DOPA and 3-OMD to C-DOPA. Final internal standard concentrations were 90 ng/mL for 4-chloro-L-phenylalanine and 225 ng/mL for L-DOPA-d_3, respectively. Injection volumes were 0.010 mL.

Aria TLX-1 System Parameters

Two 0.5 x 50 mm Thermo Scientific Cyclone™ MAX TurboFlow columns with a C18 HPLC column (4.6 x 150 mm, 5 µm particle size).

LC Method Mobile Phases

Loading Pump

Mobile Phase A:	10 mM Ammonium Acetate with 0.2% Ammonium Hydroxide (aq)
Mobile Phase B:	0.1% Formic Acid (aq)
Mobile Phase C:	50 mM Ammonium Acetate with 10% Formic Acid (aq)
Mobile Phase D:	50 mM Ammonium Acetate with 10% Formic Acid in Methanol

Elution Pump

Mobile Phase A:	0.1% Formic Acid (aq)
Mobile Phase B:	0.1% Formic Acid in Acetonitrile

Mass Spectrometer Parameters

Ion Polarity:	Positive ion mode
Vaporizer Temperature:	400 °C
Capillary Temperature:	300 °C
Sheath Gas Pressure (N_2):	60 units
Auxiliary Gas Pressure (N_2):	55 units
Scan Type:	Highly-selective reaction monitoring (H-SRM)
Scan Time:	0.050 s
Q1 (FWHM):	0.7
Q3 (FWHM):	0.7

Positive single reaction mode (+SRM) transitions and other MS parameters for test compounds are shown in Table 1. The whole experiment was controlled by Aria software.

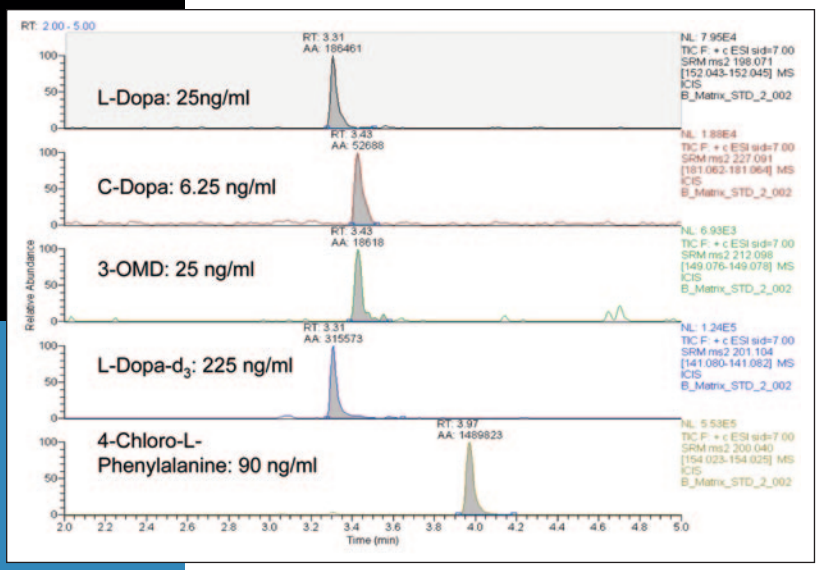

Figure 1: The representative chromatogram for the assay at the low end of the calibration curve

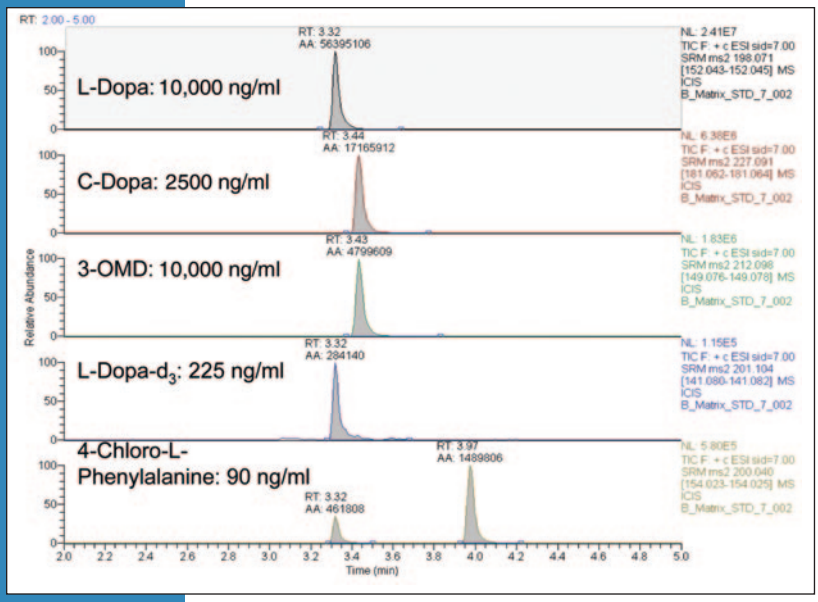

Figure 2: The representative chromatogram for the assay at the high end of the calibration curve

Results

Figures 1 shows a representative chromatogram for the assay at the low end of the curve. Figure 2 shows a representative chromatogram for the assay at the high end of the curve. Linearity of the calibration curves (N=3) ranged from 0.9942 to 0.9989 (with 1/x weighting). Figure 3 shows the representative linear calibration curves for all three test compounds. The excellent linear fits were over the range of 100-10000 ng/mL for L-Dopa and 3-OMD and 25-2500 ng/mL for C-Dopa. The limit of detection (LOD) levels were five-times lower for all compounds. The % CV values were less than 20% deviation for LLOQ and less than 15% deviation for all the other points on the calibration curve. Carryover was determined to be much less than 20% of lower limit of quantitation (LLOQ). A minimum of 85% recovery was achieved. The variability was determined by processing and analyzing five replicates of each of four QC samples. The test was repeated in three batches, Table 2. The results show that the %RSDs were well below the validation guideline of 15%.[1]

Table 1: Positive single reaction mode (+SRM) transitions and other MS parameters for test compounds

Compound	Parent Ion	Fragment Ion	Collision Energy (eV)	Tube Lens Offset
L-DOPA	198.071	152.044	14	72
C-DOPA	227.091	181.063	12	77
3-OMD	212.098	149.077	15	75
L-DOPA-d_3	201.104	141.081	16	87
4-Chloro-L-Phenyl-Alanine	200.040	154.024	14	61

Table 2: Low internal standard variability demonstrated the reliability of the method

L-Dopa-d_3 in QC Samples

	Batch #1	Batch #2	Batch #3
Number of Samples	20	20	20
RSD (%)	6.2	6.6	4.7

4-Chloro-L-Phenylalanine in QC Samples

	Batch #1	Batch #2	Batch #3
Number of Samples	20	20	20
RSD (%)	2.0	1.6	2.3

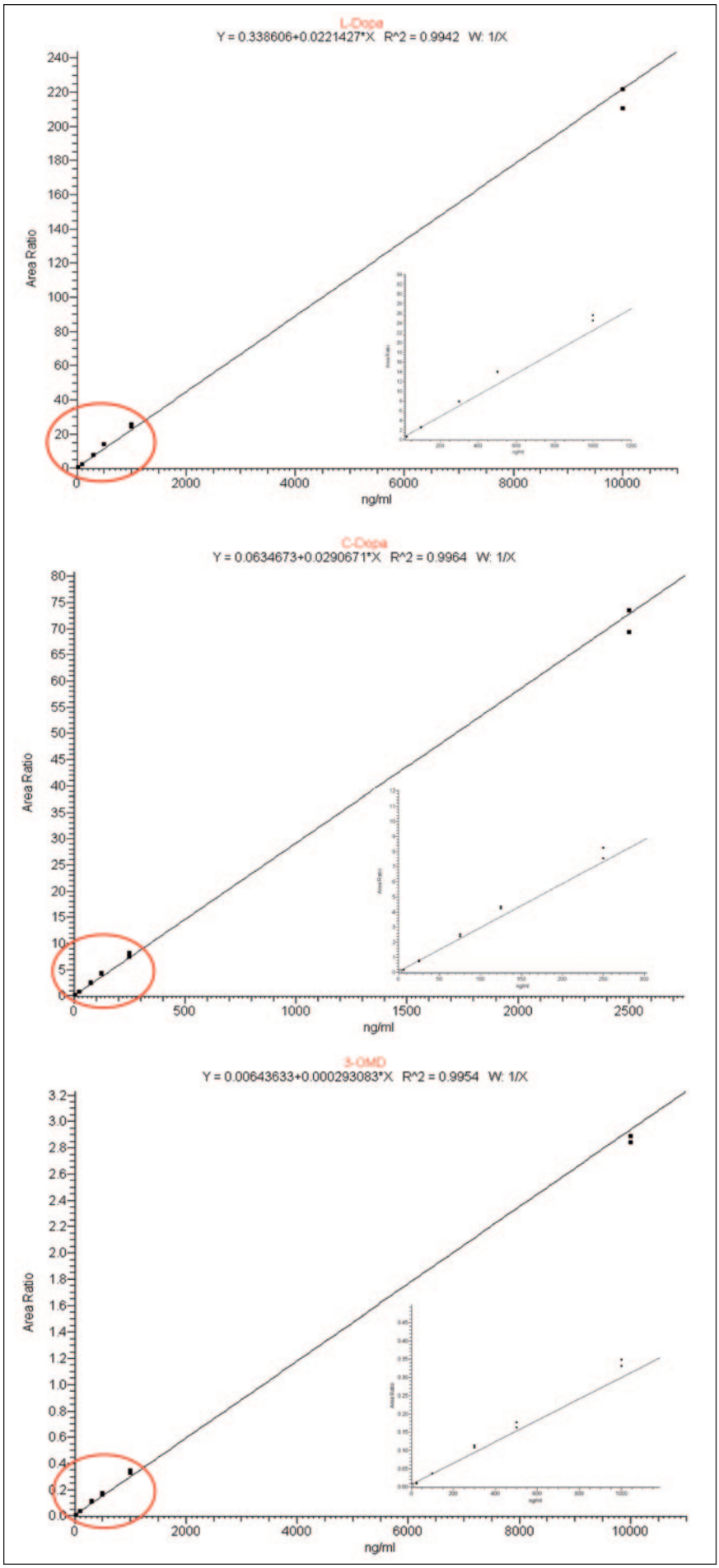

Figure 3: Representative linear calibration curves for all three test compounds

Conclusion

TurboFlow technology is a powerful technique for the direct analysis of drugs in biological fluids without the need for an extensive number of sample preparation steps. In this study, the use of an Aria TLX-1 LC system in front of a TSQ Quantum Ultra allows for low levels of detection (6.25 ng/mL for C-Dopa; 25 ng/mL for L-Dopa and 3-OMD) of each of these neurotransmitter compounds in human whole blood extract and yields results in less than 10 minutes per sample. With the Aria TLX-4 multiplexed system, the results will be available about every 2.5 minutes using only one mass spectrometer. The low variability of the results demonstrates the reliability of this research method.

Reference

1. Guidance for Industry Bioanalytical Method Validation, Food and Drug Administration, May 2001.

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