Simultaneous quantification of biogenic amines as biomarkers for Parkinson's disease by combining ultraviolet and integrated pulsed amperometric detectors

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Abstract

We developed a method combining ultraviolet detection and integrated pulsed amperometric detection for Parkinson's disease diagnosis through the simultaneous quantification of dopamine, 5-hydroxyindolacetic acid, homovanillic acid, serotonin, 3,4-dihydroxyphenylacetic acid, norepinephrine, and epinephrine. All target components were completely separated within 40 min with 5% acetonitrile solution containing 8 mM HClO₄ and 0.20 mM - 1-octanesulfonic acid, and showed limits of detection of 0.03–0.10 ng and limits of quantification of 0.10–0.30 ng with linear regression coefficients of 0.9998–1.0000. All inter-day and intra-day precision values were below 9.58%, and the average recoveries were 93.71–109.82% for mouse striatum samples. In a clinical sample application, the levels of the seven components in striatal brain tissue in a mouse model of Parkinson's disease decreased significantly compared to those of a control group. It was also confirmed via orthogonal partial least squares discriminant analysis that the seven components are useful biomarkers. Our reversed phase–HPLC-UV-IPAD method is expected to be helpful as a simple and economic analytical method for biogenic amines and their metabolites as biomarkers of Parkinson's disease in clinical and biological labs.

Introduction

Parkinson's disease (PD) is a typical neurodegenerative disease causing motor disorders, such as tremors, bradykinesia, rigidity, and postural instability, and non-motile disorders, such as depression, anxiety, attention deficit, cognition, dementia, sleep disorders, bladder disturbances, fatigue, and weight change. In patients with PD, brain damage is observed in the substantia nigra pars compacta, striatum, and locus coeruleus, as follows. Damage to the substantia nigra pars compacta appears progressively as dopaminergic neuron damage in a posterolateral to anterior direction as PD progresses; dopaminergic neuron damage causes a decrease in dopamine (DA) in the brain. Serotonergic neuron damage in the striatum causes a decrease in serotonin (5-HT) receptor binding, which leads to a decrease in 5-HT. Noradrenergic neuron damage in the locus coeruleus causes a decrease in norepinephrine (NE). In addition, noradrenergic nerves are extensively deactivated in the prefrontal cortex, cerebellum, striatum, and hypothalamus, which also results in a decrease in NE. Judging from these pathological phenomena, it has been found that DA, 5-HT, and NE could be used as important biomarkers in the diagnosis of PD.

Because of their clinical and biological importance, numerous attempts have been made to measure neurotransmitters including the biogenic amines NE, 5-HT, DA, and epinephrine (E), metabolites of DA such as 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), and metabolites of 5-HT such as 5-hydroxyindoleacetic acid (5-HIAA). Measurement methods have included high-performance liquid chromatography with photodiode array detection (HPLC-PDA), HPLC-ultraviolet (HPLC-UV), HPLC-fluorescence detection (HPLC-FLD), HPLC-mass spectrometry (HPLC-MS/MS), and HPLC-electrochemical detection (HPLC-ECD). Among them, HPLC-FLD has good sensitivity and resolution but disadvantages in that a derivatization process is required and pretreatment steps are complicated. The HPLC-MS/MS method has the advantage of being highly sensitive and able to detect a wide range of compounds, but it remains unaffordable for many labs. Of the others, HPLC-PDA (or -UV) and HPLC-ECD are...
the most commonly used analytical methods, but these approaches also have some limitations in obtaining good sensitivity and selectivity for all biogenic amines because of the variety of chromophores or electroactive properties per compound.

We previously developed a reversed phase (RP)-HPLC-integrated pulsed amperometric detection (IPAD) method for improved HPLC-ECD to analyze biogenic amines and their metabolites. The RP-HPLC-IPAD method offered increased sensitivity and reproducibility by applying more than one potential in the potential cycle but still had the disadvantage of being less sensitive to HVA, a weak electroactive component. To compensate for this deficiency, we have developed an RP-HPLC-UV-IPAD method that can measure E, NE, and DA through IPAD and other components including HVA through the UV detector.

Here, we describe the process of simultaneously analyzing biogenic amines and their metabolites through the RP-HPLC-UV-IPAD system as a screening tool applied to the striatum (ST) of normal mice and a 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) mouse model of PD. While it is difficult to quantify biogenic amines and their metabolites simultaneously in biological matrix containing various interferences, significant improvement is shown to be gained by combining two detectors with different sensitivities and selectivities to each compound. This method represents a new attempt to analyze biogenic amines and their metabolites, and may be helpful as an accessible tool for researching and diagnosing PD.

**Materials And Methods**

**Chemicals and reagents.** NE, E, DOPAC, HVA, 5-HIAA, and dihydroxybenzoic acid (DHBA) were purchased from Sigma Aldrich (St. Louis, MO, USA). DA and 5-HT were bought from ChromaDex (Los Angeles, CA, USA). Sodium hydroxide (NaOH) 50.0% solution and HPLC-grade acetonitrile (ACN) were bought from Fisher Scientific (Fairlawn, NJ, USA). Perchloric acid (HClO₄, 70%) was purchased from Sigma Aldrich (St. Louis, MO, USA). Sodium 1-octanesulfonate (OSA) was purchased from TCI (Tokyo, Japan). Distilled water of 18 MΩ for the mobile phase was made with an Aquarius AW-1001 refining system (Top Trading, Seoul, Republic of Korea). Samples were weighed using a Mettler Toledo AX 105 (Greifensee, Switzerland). A nylon membrane filter (pore size 0.2 µm) was used for solvent filtration.

**HPLC-UV-IPAD system.** The HPLC equipment consisting of Nanospace SI-2/3201 and SI-2/3001 pumps was purchased from Shiseido Co. (Tokyo, Japan). A high pressure pulse damper was purchased from Dionex (Sunnyvale, CA, USA). The ICS-3000 series PAD from Dionex consisted of a Au-flow cell and a solvent-compatible cell. The PAD detector used a six-potential waveform: E₁ = 0.130 V (0.00–0.04 s); E₂ = 0.330 V (0.05–0.21 s); E₃ = 0.550 V (0.22–0.46 s); E₄ = 0.330 V (0.47–0.56 s); E₅ = −1.670 V (0.57–0.58 s); E₆ = 0.930V (0.59 s). The IPAD data were controlled via the Chromeleon program (Dionex). A Model Nanospace SI-2/UV detector was purchased from Shiseido. Data from the UV detector (set to 214 nm) were analyzed with the dsChrom program (Donam Instrument, Seoul, Korea). A Unison UK C-18 column (150.0×2.0 mm I.D., 3 µm, Imtakt, Kyoto, Japan) and a Unison UK C-18 guard column (5.0 mm×2.0 mm I.D., 3 µm, Imtakt) were used for chromatographic separation. The pre-column mobile phase consisted of a 5% ACN solution containing 8 mM HClO₄ and 0.20 mM OSA. The pre-column mobile phase was made on a daily basis, degassed by vacuum filtration, and then sonicated for 20 min before use. The pre-column flow rate was 0.20
mL/min. The column temperature was set to 40°C. The post-column eluent was 200 mM NaOH solution supplied at a flow rate of 0.8 mL/min using a pump with head of metal-free polyether ether ketone (PEEK) resin, which is compatible with alkaline solvents. The 200 mM NaOH was made via vacuum filtration on a daily basis and purged with helium throughout the experiment to minimize carbon dioxide absorption. The sample injection volume was 10 µL.

**Animals and ethical statement.** All experiments were approved by the Institutional Animal Care and Use Committee of Kyung Hee University (KHUASP(SE)-22-415). Animals were maintained according to the Use Guidelines and Animal Care of Kyung Hee University (Seoul, Republic of Korea). Male C57BL/6J mice (7 weeks old, 27–30 g) were purchased from Daehan Biolink Co. (Eumseong, Korea). The animals were housed six individuals per cage, and were accommodated under regulated conditions (12:12 h light/dark cycle). Water and food were available ad libitum. After acclimatization, mice were assigned to two groups, those without MPTP treatment (the control group, n = 6) and those with MPTP treatment (the MPTP group, n = 5); the latter group was established as the experimental PD model. In the MPTP group, each mouse was injected with MPTP (30 mg/kg, i.p.) once a day for 5 days. This study is performed in accordance with the ARRIVE guidelines.

**Preparation of standard solutions.** One milligram of each standard (NE, E, DA, DOPAC, 5-HT, 5-HIAA, and HVA) and internal standard (I.S., DHBA) was added to 1 ml of 0.2 M HClO₄. Each stock solution was 1000 µg/mL and was diluted with distilled water to prepare a calibration curve. The concentration of DHBA (I.S.) was 5.0 µg/mL for all solutions. All standard solutions were kept at 4°C before analysis.

**Preparation of sample solutions.** In order to measure the contents of metabolites, sections of the ST were prepared as samples. The ST sections were homogenized in 0.20 M HClO₄ solution. Homogenates were centrifuged for 10 min at 12,000g at 4°C, and the supernatant was injected into the HPLC. The levels of biogenic amines and their metabolites were expressed as ng per µg of total protein. Protein assay quantification was carried out through Bradford's protein assay (Bio-Rad, Hercules, CA, USA).

**Method validation.** Calibration curves were created to determine the linearity of all components. The regression equation used was $y = ax + b$, where $x$ and $y$ are the mass of the sample and the peak area ratio of the sample (component/internal standard), respectively. Inter- and intra-day assays were performed to estimate the precision of the analysis. Four injections (0.15, 10, 50, 200 ng for NE, 5-HT, and HIAA; 0.2, 10, 50, 200 ng for DA and HVA; 0.3, 10, 50, 200 ng for E; 2, 10, 20, 50 ng for DOPAC) were conducted three times a day for four consecutive days. A recovery test was performed by adding target components (12.5, 50, and 100 ng for NE; 20, 40, and 80 ng for E, DOPAC, and 5-HT; 6, 12, and 24 ng for DA; 10, 20, and 40 ng for HIAA and HVA) to the control mice ST samples. Three injections per sample were performed and measured.

**Results And Discussion**

**Establishment of the RP-HPLC-UV-IPAD method.** PAD is an electrochemical method that measures current changes by oxidation on a gold electrode via applied potential waveform, enabling the direct analysis of carbohydrates or amines at low pico-mole levels. It is essential to supply alkaline solution in PAD by mobile
phase or post-column eluent for the detection of carbohydrates or amines since they become electrochemically active under alkaline conditions (NaOH) (pH > 11). This study designed an RP-HPLC-UV-IPAD system, shown in Fig. 1, by combining RP-HPLC-UV with IPAD with an alkaline eluent system. After UV detection of target components in samples separated in the C18 column, the pre-column eluent is mixed with 200 mM NaOH solution, which is a post-column eluent, to convert the targets to electroactive forms in alkaline conditions for IPAD detection. By employing the hydroxide solution used as a pre-column eluent in anion-exchange chromatography as the post-column eluent, it was possible to apply an RP column, which is vulnerable to alkalis, and thus UV analysis could be completed without absorption band shift by alkaline conditions.

This system enables simultaneous UV detection and IPAD. In addition, our system has the advantage of being able to select between UV or PAD for each component in consideration of sensitivity and separation from the background components. Therefore, we used IPAD for NE, E, and DA, and UV detection for DOPAC, HIAA, HVA, and 5-HT. It has been reported that the greater the amount of acetonitrile in the mobile phase, the lower the sensitivity of PAD\textsuperscript{28}. The sensitivity of PAD in our method was maintained because the ACN concentration in our mobile phase was as low as 5% and also because the diameter of the column we used was 2.1 mm, which can reduce the flow of ACN contained in the mobile phase into PAD by one-fifth compared to the common 4.6 mm column. In addition, considering that the S/N ratio is the best when the flow rate of the NaOH solution is 0.80 ml/min, and the PAD signal is the most improved when the concentration of the NaOH solution is 100–200 mM\textsuperscript{28}, the flow rate and concentration of the NaOH solution were set in this work to 0.80 mL/min and 200 mM, respectively. We chose six-potential waveform, which has been shown to be highly sensitive to glycosides, bioamins and metabolites with hydroxyl group\textsuperscript{26}.

A tube with an internal diameter of 0.13 mm was used to connect the pre-column pump, column, and UV detector. Its use had the effect of not only significantly reducing the noise in the chromatogram by suppressing the pulse flow entering the column but also minimizing peak broadening after passing through the column. A 0.25 mm tube was used between the mixer and the IPAD detector so that the mobile phase and the NaOH solution were mixed well in the mixer and entered the IPAD detector. To reduce baseline noise, a high pressure pulse damper was installed between the post-column pump and the mixer.

**Optimized separation conditions.** The mobile phase conditions to completely separate the seven target components were investigated. Among the targets, NE, E, DA, and 5-HT are basic compounds, DOPAC and HVA are acidic compounds, and 5-HIAA is an amphoteric compound with both carboxyl and amine groups. Since the compounds have different properties, many difficulties have been encountered in establishing conditions for the complete separation of all seven target components. We determined the separation conditions by going through several steps, as follows.

Separation patterns according to ACN concentration were first examined (Fig. 2A). Three peaks for DOPAC (peak 3), 5-HIAA (peak 5), and 5-HT (peak 7) were isolated, but NE (peak 1), E (peak 2), DA (peak 4), and DHBA (I.S) were not isolated despite varying the concentration of ACN. HVA (peak 6) was not found at all. Considering the retention time and peak shape, the concentration of ACN was fixed to 5%.
Separation patterns according to HClO\textsubscript{4} concentration were then examined by adding HClO\textsubscript{4} to the 5% ACN solution (Fig. 2B). For DOPAC (peak 3), HVA (peak 7), and 5-HIAA (peak 5) with carboxyl groups, the components converted to molecular form by the addition of HClO\textsubscript{4}, so the peaks sharpened and completely separated from each other with much higher sensitivities. A very weak peak of HVA was also detected. However, despite changes in the concentration of HClO\textsubscript{4}, NE (peak 1) and E (peak 2) were not completely separated, and the retention times were almost unchanged. So the concentration of HClO\textsubscript{4} was arbitrarily fixed to 8 mM.

Next, separation patterns according to OSA concentration were examined by adding OSA to the 5% ACN solution containing 8 mM HClO\textsubscript{4} (Fig. 2C). OSA is an ion pair reagent. NE, E, DA, and 5-HT with amine groups formed ion pairs with OSA, thereby improving separation. At 0.05 mM OSA, all the components were completely separated. As the concentration of OSA increased, the retention times of NE (peak 1), E (peak 2), DA (peak 4), and 5-HT (peak 7) increased. Among them, the retention times of peaks 4 and 7 became quite long, so the order of peaks changed; for example, above 0.15 mM OSA, the order of peaks 3 and 4 and of peaks 6 and 7 changed. At 0.20 mM OSA, the resolution (Rs) between all components was very good at 2.0 or higher. Based on this result, we chose to add 0.20 mM OSA.

Lastly, separation patterns according to column temperature were examined with the 5% ACN solution containing 8 mM HClO\textsubscript{4} and 0.20 mM OSA (Fig. 2D). The higher the column temperature, the shorter the retention time of all peaks. At 30°C, the Rs between DHBA (I.S.) and DOPAC (peak 3) was not completely separated at 1.38, and the analysis time was too long and inefficient. At 40°C, the Rs of all peaks was 2.0 or higher, indicating complete separation, and the analysis time was shortened by 20 min. At 50°C and 60°C, the analysis time was further shortened but the overlapping of the peaks was severe. Accordingly, the column temperature was set at 40°C.

Figure 3 shows a chromatogram of the standard components from the RP-HPLC-UV-IPAD method with optimized conditions. NE, E, and DA were analyzed by the IPAD method, and DOPAC, 5-HIAA, HVA, and 5-HT were analyzed by the UV method. In particular, for HVA, the UV method was much more sensitive than IPAD, so the UV method was selected for this component. The RP-HPLC-UV-IPAD system has the advantage of being able to choose between UV and IPAD detectors in consideration of sensitivity and separation from the background peaks, making it suitable for the analysis of trace of clinical samples.

**Method validation.** The linearity of each component was determined using four concentrations: [DA, 0.10 ng; NE, 0.15 ng; E, 0.30 ng; 5-HT and 5-HIAA, 0.15 ng; HVA, 0.20 ng], 10.0 ng, 50.0 ng and 200.0 ng. Table 1 shows the linear ranges and equations. The linear regression coefficients ranged from 0.9998–1.0000, showing good linearity. The limits of quantitation (LOQs) (S/N = 10) by the IPAD method and the UV method were as follows, respectively: DA, 0.10 and 0.30 ng; NE, 0.15 and 0.20 ng; E, 0.30 and 0.40 ng; 5-HT, 0.40 and 0.15 ng; 5-HIAA, 0.50 and 0.15 ng; HVA, 50.00 and 0.20 ng; DOPAC, 1.00 and 0.20 ng. The LOQs for the IPAD method were 1.3–3.0 times higher for DA, NE, and E, and 2.6–250 times lower for 5-HT, 5-HIAA, HVA, and DOPAC than those of the UV method. The UV method here showed 3.0–32.9 times higher sensitivity in detecting these four components than other existing methods\textsuperscript{29–33}. Based on the sensitivity of the components, DA, NE, and E were analyzed via IPAD, and 5-HT, 5-HIAA, HVA, and DOPAC were analyzed via UV.
detection. These results indicate that our HPLC-UV-IPAD method offers good sensitivity in the analysis of target components under optimal conditions.

Table 1
Linearities, regression equations, correlation coefficients (R²), limits of detection (LOD) and limits of quantitation (LOQ) for DA, NE, E, 5-HT, 5-HIAA, HVA, and DOPAC

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Linear range (µg/mL)</th>
<th>Linear equation</th>
<th>R²</th>
<th>LOD (ng)</th>
<th>LOQ (ng)</th>
<th>UV</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>IPAD</td>
<td>UV</td>
<td>IPAD</td>
</tr>
<tr>
<td>DA</td>
<td>0.010-20.00</td>
<td>y = 0.196x + 0.0455</td>
<td>0.9999</td>
<td>0.03</td>
<td>0.10</td>
<td>0.10</td>
</tr>
<tr>
<td>NE</td>
<td>0.015-20.00</td>
<td>y = 0.1906x + 0.0572</td>
<td>0.9998</td>
<td>0.05</td>
<td>0.07</td>
<td>0.15</td>
</tr>
<tr>
<td>E</td>
<td>0.030-20.00</td>
<td>y = 0.122x + 0.0265</td>
<td>0.9999</td>
<td>0.10</td>
<td>0.13</td>
<td>0.30</td>
</tr>
<tr>
<td>5-HT</td>
<td>0.015-20.00</td>
<td>y = 0.2807x + 0.0365</td>
<td>0.9999</td>
<td>0.15</td>
<td>0.05</td>
<td>0.40</td>
</tr>
<tr>
<td>5-HIAA</td>
<td>0.015-20.00</td>
<td>y = 0.2913x − 0.0149</td>
<td>1.0000</td>
<td>0.16</td>
<td>0.05</td>
<td>0.50</td>
</tr>
<tr>
<td>HVA</td>
<td>0.020-20.00</td>
<td>y = 1.1600x − 0.1333</td>
<td>1.0000</td>
<td>15.00</td>
<td>0.06</td>
<td>50.00</td>
</tr>
<tr>
<td>DOPAC</td>
<td>0.020-5.00</td>
<td>y = 6.7266x − 2.8355</td>
<td>0.9998</td>
<td>0.35</td>
<td>0.07</td>
<td>1.00</td>
</tr>
</tbody>
</table>

a Data from Mikrochimica Acta. 2019, 186, 686–686
b Pharm. Biol. 2015, 53, 1539–1544
c J. Chromatogr. A 1984, 290, 247–262
d J. Chromatogr. B 2015, 998–999, 40–44

Since the simplicity and economic feasibility of analytical methods are important matters in clinical and biological labs, a simple preparation method of dissolving in 0.20 M HClO₄ solution and centrifuging was adopted. Thus, it was necessary to confirm the accuracy and precision of the developed method for complexed biological matrix. Inter- and intra-day precision were evaluated by analyzing samples each day for
four consecutive days. The relative standard deviations (%RSDs) ranged from 0.01–9.58% in the inter-day assay and 0.33–8.95% in the intra-day assay (Supplemental Table 1). The accuracy of this experimental method was evaluated by a recovery test (Supplemental Table 2). The mean recoveries and %RSD ranges were 93.71–109.82% and 0.46–7.74% for mouse ST samples. This demonstrates that the response of the detectors to the target samples was not influenced by the complexed components in biological matrix.

**Application to clinical samples.** To confirm whether the developed method is useful for biological sample analysis, we applied it to ST samples from five normal mice without treatment (the control group) and five PD model mice that underwent MPTP treatment (the MPTP group). Figure 4 shows chromatograms of the pretreated striatal tissue for each group. For the levels of biogenic amines and their metabolites in the striatal tissue, NE, E, and DA were measured by the IPAD method, and DOPAC, 5-HIAA, HVA, and 5-HT were measured by the UV method. The measured values were corrected by dividing by the total protein amount [each measured value (ng) / total protein amount (µg)], and the corrected values were regarded as the level of the measured component. The corrected values in the control and the MPTP groups were as follows (Fig. 5A), respectively: DA, 24.07 and 4.20 ng/µg; 5-HIAA, 2.86 and 0.23 ng/µg; HVA, 1.98 and 0.61 ng/µg; 5-HT, 1.45 and 0.64 ng/µg; DOPAC, 1.32 and 0.11 ng/µg; NE, 1.05 and 0.64 ng/µg; E, 0.15 and 0.05 ng/µg (Fig. 5A). The values for the MPTP group were 1.64–12.43 times lower than those of the control group, indicating a significant decrease in the values of the components in the MPTP group. This result is consistent with previous reports that the amounts of biogenic amines (DA, 5-HT, NE) and their metabolites (DOPAC, HVA, 5-HIAA) decrease due to dopaminergic, serotonergic, and noradrenergic neuron damage in PD patients.

We note that the RP-HPLC-UV-IPAD method has lower selectivity compared to the HPLC-MS/MS method capable of obtaining extracted ion chromatograms, and interference peaks in biological matrix made it difficult to achieve baseline separation of the target compounds in the present experiment. Nevertheless, the biogenic amines were successfully quantified in the ST samples at a level sufficient to distinguish the control and MPTP groups. Orthogonal partial least squares discriminant analysis (OPLS-DA), a statistical model suitable for diagnosing differences between two or more groups using multivariate data, is widely used to identify potential biomarkers. In this model, the closer the $R^2$ value is to 1, the more suitable the model is for diagnosing the difference between two groups. $Q^2$ is an indicator that can predict the stability of the model when some data is randomly added, meaning that the model is more stable and reliable as the $Q^2$ value approaches 1. We applied OPLS-DA to the clinical sample data analyzed by our method (Fig. 5B). The $R^2X$ and $R^2Y$ of our model were 0.928 and 0.918, respectively, indicating that our model is quite suitable for diagnosing the difference between the control group and the MPTP group. The $Q^2$ of our model was 0.813, which means that our model is rather stable and reliable as a PD model. Accordingly, the OPLS-DA results confirm that the normal and MPTP groups were clearly distinguished by the amounts of seven components in the tissues—DA, 5-HIAA, HVA, 5-HT, DOPAC, NE, and E.

**Conclusions**

We developed a simple and economical analytical method, RP-HPLC-UV-IPAD, for the simultaneous quantification of DA, 5-HIAA, HVA, 5-HT, DOPAC, NE, and E. All target components were completely separated within 40 min with a 5% ACN solution containing 8 mM HClO$_4$ and 0.20 mM OSA without complicated
pretreatment. Our method had good accuracy, precision, and sensitivity for all target components. It was confirmed by OPLS-DA that DA, 5-HIAA, HVA, 5-HT, DOPAC, NE, and E are useful biomarkers. In the future, we hope that RP-HPLC-UV-IPAD will be put into practical use as a diagnostic method for human as well as animal diseases.

**Declarations**

**Data availability**

The data used or analyzed during the current study are available from the corresponding author upon reasonable request.

**Conflicts of interest.** The authors have no conflicting financial interests to declare.

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**References**


**Figures**

**Figure 1**

Schematic diagram of the RP-HPLC-UV-IPAD method.
Figure 2

Chromatograms according to various mobile phase conditions: (A) ACN concentration, (B) HClO₄ concentration, (C) OSA concentration, and (D) column temperature. Peaks are as follows: 1, NE; 2, E; 3, DOPAC; 4, DA; 5, 5-HIAA; 6, HVA; 7, 5-HT; I.S., DHBA.
Figure 3

Chromatograms of UV (upper) and IPAD (lower) response for the standard components. Peaks are as follows: 1, NE; 2, E; 3, DOPAC; 4, DA; 5, 5-HIAA; 6, HVA; 7, 5-HT; I.S., DHBA.
Figure 4

UV and IPAD chromatograms of the pretreated mice ST samples for the control group (A and B) and MPTP group (C and D). Peaks are as follows: 1, NE; 2, E; 3, DOPAC; 4, DA; 5, 5-HIAA; 6, HVA; 7, 5-HT; I.S., DHBA.

Figure 5

(A) Measured levels of DA, 5-HIAA, HVA, 5-HT, DOPAC, NE, and E per total protein in mouse ST of the control and MPTP groups. (B) OPLS-DA score scatter plot for the control group and MPTP group.
Supplementary Files

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- Supportinginformation.docx