Determination of 9 neurotransmitters in plasma by HPLC ESI-MS/MS

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ABSTRACT

Many neurological diseases are often accompanied by non-specific symptoms, such as headaches, cognitive impairment, and depression, which in severe cases can affect the diagnosis and treatment of the disease. Simultaneously detecting tyrosine, tryptophan, and their metabolites, combined with other cofactor assays, can help diagnose metabolic and neurological diseases. Therefore, it is important to develop a biomarker scheme for the simultaneous detection of multiple nervous system diseases and apply it to clinical diagnosis.
At present, there are still many difficulties in the detection of neurotransmitters in plasma/cerebrospinal fluid, such as low detection sensitivity, low extraction recovery rate, poor retention in reverse phase liquid chromatography, and severe matrix interferences. Therefore, establishing a high-throughput, wide coverage, and fast method for analyzing neurotransmitters in plasma is of great significance.
This protocol uses the SCIEX Citrine™ Triple Quad™ System and establishes a high-throughput LC-MS/MS detection method for neurotransmitters. The protein precipitation method is used to accurately quantify and analyze 9 neurotransmitters in plasma, and the sensitivity, linearity, accuracy, and precision of the method are systematically validated. This protocol can be used for clinical research on the pathological and physiological mechanisms of mental diseases. This protocol also provides reference testing methods and data support for evaluating treatment measures.

INTRODUCTION

Many nervous system diseases are accompanied by non-specific symptoms, such as headache, cognitive dysfunction, and depression, all of which can affect the diagnosis and treatment of the disease. The simultaneous detection of tyrosine and tryptophan, and their metabolites, combined with other cofactors, is helpful for diagnosing metabolic and neurological diseases. This protocol has established a high-throughput LC-MS/MS detection method for neurotransmitters. Precise quantification and analysis of 9 neurotransmitters in plasma was performed and systematic methodological validation was employed to evaluate the sensitivity, linearity, accuracy and precision of the method.

MATERIALS AND METHODS

Sample preparation:
Nine neurotransmitter standards (5-hydroxyindole acetic acid, 5-hydroxytryptamine, 2-amino-5-hydroxybenzoic acid, acetylcholine, γ-aminobutyric acid, glutamic acid, 3,4-dihydroxyphenylacetic acid, taurine and homovanillic acid) were diluted with water for analysis.
Accurately measured 200 µL plasma sample in a 1.5 mL centrifuge tube and added 20 µL internal standard mixed working solution and 600 µL contains acid methanol, the mixture was vortexed for 1 minute, placed at -20 ° C for 30 minutes, centrifuged at 14000 r/min for 10 minutes, and 350 µL of the supernatant after centrifugation was taken. After nitrogen blowing, 100 µL of the initial mobile phase was re-dissolved and analyzed by LC-MS/MS (Figure 1).

HPLC conditions:
A SCIEX Exion LC™ AD system equipped with HILIC (1.7 µm, 2.1 x 100 mm); T3 (3 µm, 2.1 x 150 mm) column was kept at 40° C with a gradient of eluent A water+0.1% fomic acid+ 10 mM ammonium formate and eluent B water/acetonitrile (10/90) +0.1% fomic acid+ 10 mM ammonium formate was used at a flow rate of 300 µL/min. The injection volume was set to 10 µL(Tables 1 and 2).

MS/MS conditions:
A SCIEX Citrine™ Triple Quad™ System with the Ion Drive™ Turbo V ion source and electrospray ionization (ESI) probe was used. Samples were analyzed on a triple quadrupole mass spectrometer using an MRM scan mode with positive/negative ion switching(Table 3).

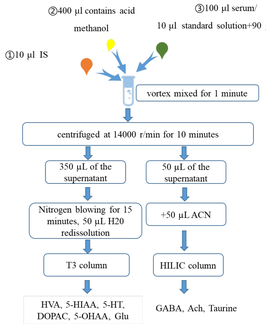

Figure 1. Sample preparation workflow.

Table 1. LC gradient for HILIC column.

Time (min)	A (%)	B (%)
0.0	0	100
2.0	0	100
4.0	50	50
8.5	50	50
8.6	0	100
10	0	100

Table 2. LC gradient for T3 column.

Time (min)	A (%)	B (%)
0.0	97	3
1.5	97	3
4.0	2	98
7.9	2	98
8.0	97	3
10	97	3

Table 3. MRM transitions of nine neurotransmitters assay.

Analyte	Q1	Q3	Dwell time	ID	DP	CE
5-Hydroxyindole acetic acid (5-HIAA)	192.2	146.2	15	5-HIAA-1*	170	25
	192.2	119.2	15	5-HIAA-2	170	40
	194	148.1	15	5-HIAA-IS	100	23
5-Hydroxytryptamine (5-HT)	177	160.2	15	5-HT-1*	100	23
	177	132	15	5-HT-2	100	28
	181	164	15	5-HT-IS	100	19
2-Amino-5-hydroxybenzoic Acid (5-OHAA)	154.1	136.2	15	5-OHAA-1*	100	16
	154.1	108.2	15	5-OHAA-2	100	27
Acetylcholine (Ach)	146.2	87.1	15	ACH-1*	170	20
	146.2	60.1	15	ACH-2	170	15
	155.1	87	15	ACH-IS	170	20
γ-Aminobutyric acid (GABA)	104	87.1	15	GABA-1*	70	16
	104	69.1	15	GABA-2	70	22
	110	93.1	15	GABA-IS	30	16
Glutamic acid (Glu)	148.1	84.1	10	GLU-1*	100	22
	148.1	102.1	10	GLU-2	100	16
	154.1	89.1	10	GLU-IS	40	20
3,4-Dihydroxyphenyl (DOPAC)	213	123.1	15	DOPAC-1*	-5	-18
	167	123	15	DOPAC-2	-10	-17
	172	123	15	DOPAC-IS	-10	-16
Taurine	124	80	15	Taurint-1*	-290	-28
	124	107	15	Taurine-2	-290	-19
	128	80	15	Taurine-IS	-290	-29
Homovanillic acid (HVA)	181	122	15	HVA-1*	-80	-21
	181	105	15	HVA-2	-80	-21
	184	140	15	HVA-IS	-50	-20

RESULTS

A method for the quantitation and identification of neurotransmitters was developed for 5-hydroxyindole acetic acid, 5-hydroxytryptamine, 2-amino-5-hydroxybenzoic acid, acetylcholine, γ-aminobutyric acid, glutamic acid, 3,4-dihydroxyphenylacetic acid, taurine and homovanillic acid was developed (Figure 2).

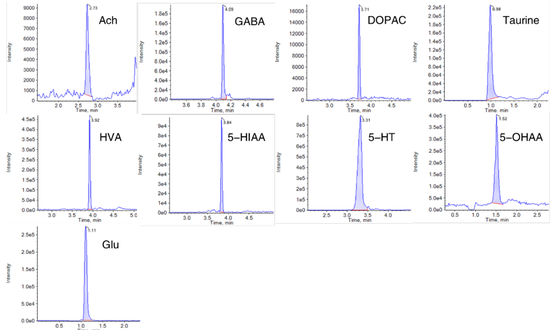

Figure 2. Chromatograms of 9 neurotransmitters.

In order to evaluate the accuracy of this detection method, 3 concentrations of working solutions(low, medium, and high) were added to the plasma to calculate the spiked recovery rate. Table 4 shows that the spiked recovery rates at different concentration levels were between 80.3% and 102.2%. The recovery rate meets the methodological requirements(Table 3).

Table 3. Experimental results of spiked recovery rate (%).

Recovery	DOPAC	Taurine	HVA	5-HIAA	5-HT	5-OHAA	GABA	Glu	Ach
Low	86.7	80.3	68.6	86.5	100.2	89.9	90.0	82.0	94.2
Medium	97.1	93.4	94.3	88.2	98.2	88.2	88.6	81.9	95.6
High	94.9	100.2	103.2	90.2	102.2	93.5	94.7	90.1	97.9

Ten plasma samples from different sources were selected and processed according to the above pre-processing steps for liquid quality analysis and detection. The results showed that the concentration values of DOPAC, taurine, HVA, 5-HIAA, 5-HT, 5-OHAA, and Glu in the plasma samples were within the linear range. The actual plasma and cerebrospinal fluid sample results are shown in Figure 3. According to relevant literature reports, Ach and GABA are suitable for detection in cerebrospinal fluid samples. It is recommended to use neostigmine, an acetylcholinesterase inhibitor, as the stabilizer of Ach in body fluid samples.

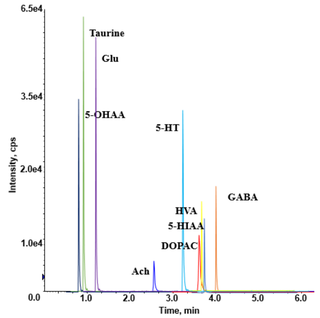

Figure 3. Chromatograms of 9 neurotransmitters in plasma and cerebrospinal fluid samples.

CONCLUSIONS

A fast, robust and reliable method was developed and validated for the detection of 9 neurotransmitters in plasma and cerebrospinal fluid matrix. In this method, 9 neurotransmitters were detected on 2 chromatographic columns to ensure lower matrix effect and to maximize recovery rate. This enables a robust method for the study of neurotransmitters. All 9 compounds had good reproducibility within the range of the standard curve (n=6).

REFERENCES

[1] Galla Z, Rajda C, Rácz G, Grecsó N, Baráth Á, Vécsei L, Bereczki C, Monostori P. Simultaneous determination of 30 neurologically and metabolically important molecules: A sensitive and selective way to measure tyrosine and tryptophan pathway metabolites and other biomarkers in human serum and cerebrospinal fluid. J Chromatogr A. 2021 Jan 4;1635:461775.
[2] Lamy E, Pilyser L, Paquet C, Bouaziz-Amar E, Grassin-Delyle S. High-sensitivity quantification of acetylcholine and choline in human cerebrospinal fluid with a validated LC-MS/MS method. Talanta. 2021 Mar 1;224:121881.

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