High-throughput Determination of Neurotransmitters and Neuromodulators in Cerebrospinal Fluid by Ultra High Performance Liquid Chromatography Triple Quadrupole Mass Spectrometry

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Introduction

Neurotransmitters and neuromodulators play essential roles in informational & chemical transportation and cell functions in human. Analyzing important neurotransmitters and neuromodulators in bio-samples is crucial to both disease diagnosis and drug discovery. In this study, a high-throughput and sensitive UHPLC-MS/MS method was developed for simultaneous determination of the neurotransmitters and neuromodulators in cerebrospinal fluid. The analysis of all fourteen analytes can be rapidly achieved within 7 min . The method demonstrated the high sensitivity, excellent reproducibility and good linearity, providing a robust application for neurotransmitter and neuromodulator analysis in bio-samples. (see Fig.4 for the structures of targeted compounds).

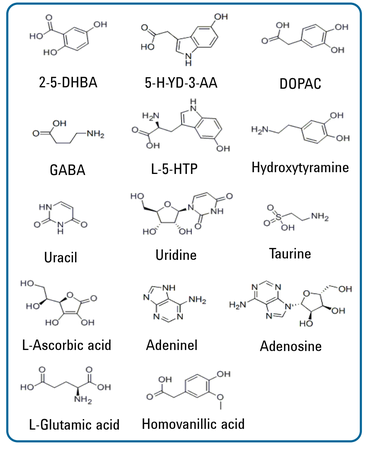

Fig. 1 Structures of the targeted compounds

Experimental

LC-MS conditions

The artificial cerebrospinal fluid samples containing neurotransmitters and neuromodulators were used for this study, which were provided by Shenyang Pharmacy University (China). MS/MS data were acquired on the 1260 UHPLC-6460QQQ system in the positive and negative modes with Jet Stream Electrospray, respectively,
Gradient elution was performed using mobile phase A containing (0.1% Formic acid) and B(acetonitrile), utilizing a Zorbax SB-Aq C18 column (2.1 x 100 mm, 1.8 µm; at 40 °C, flow rate of 0.3 mL/min; 10 µL injection).
Positive mode gradient(B%/min): 2/0, 2/1.0, 90/6.0, 90/7.0, and 2/7.1; Negative mode gradient(B%/min):2/0, 2/2.0, 30/4.0, 90/7.0, 90/7.9, and 2/8.0.
MRM transitions and corresponding collision energies for each analyte were optimized for quantitative analysis. Other MS parameters were also optimized including 60V Fragmentor, 300 °C drying gas temperature, 6L/min drying gas flow, 45 psi nebulizer pressure, 300°C sheath gas temperature, 12L /min sheath gas flow, and 1500V nozzle voltage. MRM conditions (Negative/Positive) were shown in Fig. 2

Compound Name	Precursor Ion	Product Ion	Dwell	Fragmentor	Collision Energy
Uridine	243	200.1	40	120	5
Uridine	243	110	40	120	12
L-Ascorbic acid	175	115	40	100	5
L-Ascorbic acid	175	87	40	100	15
DOPAC	167	123.1	40	60	4
Taurine	124	80	40	110	15
Uracil	111	42	40	80	10
Homovanillic acid	181	137	50	100	5
Homovanillic acid	181	122	50	100	10
2,5-Dihydroxybenzoic acid	153	109	50	100	10
2,5-Dihydroxybenzoic acid	153	108	50	100	20

Compound Name	Precursor Ion	Product Ion	Dwell	Fragmentor	Collision Energy
Adenosine	268.1	136.1	20	110	16
Adenosine	268.1	119.1	20	110	50
L-5-HTP	177.1	160.1	20	70	5
L-5-HTP	177.1	115.1	20	70	30
5-H-YD-3-AA	192.1	146.1	20	100	12
5-H-YD-3-AA	192.1	91	20	100	38
Hydroxytyramine	154.1	137.1	20	80	6
Hydroxytyramine	154.1	91.1	20	80	25
L-Glutamic acid	148.1	102.1	20	80	8
L-Glutamic acid	148.1	84.1	20	80	15
Adenine	136.1	119.1	20	110	25
Adenine	136.1	92.1	20	110	32
GABA	104.1	87	20	70	6
GABA	104.1	69	20	70	12

Fig. 2 MRM list of targeted compounds

Results and Discussion

LC optimization and EIC of the targeted analytes

The complexity of samples from brain is a key factor that needs to be considered prior to MS detection. However, many compounds such as most classical neurotransmitters or hydrophilic neuropeptides cannot be well retained on RPHPLC columns due to their high polarity. To solve this issue, low content organic mobile phase with added trifluoroacetic acid or ion pair agents can be used to increase their retention. Undoubtedly, such conditions lead to undesired low ionization efficiency for most of analytes when coupled to ESI-MS, reducing the sensitivity of the MS detection.

Experiments using a hydrophilic column (Agilent Zobax SB-Aq) to increase compounds retention and improve chromatographic separation.

Due to diverse structures of the targeted compounds, both positive and negative ion modes were used. Seven targeted analytes (uridine, L-ascorbic acid, DOPAC, taurine, uracil, homovanillic acid, and 2,5-dihydroxybenzoic acid) were analyzed in the positive ion mode, and others (adenosine, L-5-HTP, 5-H-YD-3-AA , hydroxytyramine , L-glutamic acid, adenine, and GABA) in the negative ion mode. The EICs of all analytes in real samples were shown in Fig. 3.

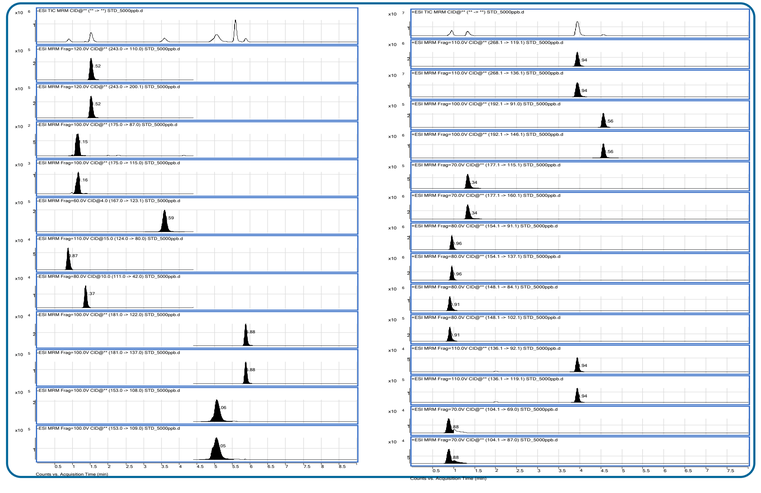

Fig3 MRM of all analytes in the real samples in both positive and negative modes

Results and Discussion

Linearity and LOQ

Typical linearity is shown in Fig. 4 with the good correlation coefficient ($R^2 \geq 0.997$). Except for L-ascorbic acid due to the easy oxidative damage, and the other 13 kinds of compounds can be achieved 1ng/mL (ppb) for LOQ.

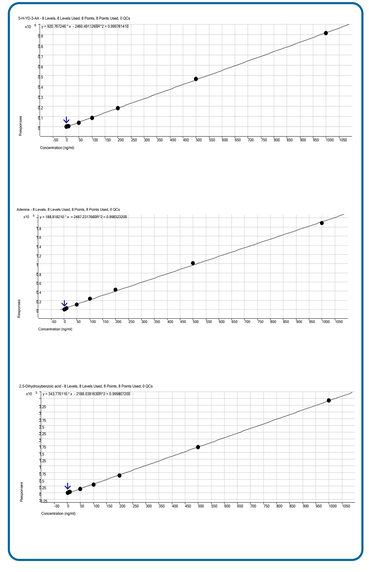

Fig. 4 Typical Linearity between concentration and MS abundance

Conclusions

Seven targeted analytes (uridine, L-ascorbic acid, DOPAC, taurine, uracil, homovanillic acid, and 2,5-dihydroxybenzoic acid) were analyzed in the positive ion mode, and others (adenosine, L-5-HTP, 5-H-YD-3-AA , hydroxytyramine , L-glutamic acid, adenine, and GABA) in the negative ion mode. The separation of all analytes was achieved within 7 min based on a series of optimization including column selection, column temperature, and LC gradient. Due to the polar property of most targeted analytes, SB-Aq C18 column that is stable in 100% water was used to achieve a better chromatographic separation. The quantitative method demonstrated reliable confirmation (accurate ratio of qualifier and quantifier ion abundances), good linearity (R>0.99), good reproducibility (RSD<3.0% and n=6 for all analytes) at the LOQ levels, and excellent sensitivity (< 0.1 ng/mL for all analytes). The method provided a robust technique for fast determination of neurotransmitters and neuromodulators in the complex sample such as cerebrospinal fluid.