Journal of Chromatography B 1054 (2017) 64–72

Contents lists available at ScienceDirect

Journal of Chromatography B

journal homepage: www.elsevier.com/locate/jchromb

A new combined method of stable isotope-labeling derivatization-ultrasound-assisted dispersive liquid–liquid microextraction for the determination of neurotransmitters in rat brain microdialysates by ultra high performance liquid chromatography tandem mass spectrometry

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ARTICLE INFO

Keywords:
In vivo microdialysis
Neurotransmitters
Triple quadrupole mass spectrometry
Sensitivity enhancement
Stable isotope-labeling derivatization
Targeted metabolomics

ABSTRACT

In this work, for the first time, a new hyphenated technique of stable isotope-labeling derivatization-ultrasound-assisted dispersive liquid–liquid microextraction has been developed for the simultaneous determination of monoamine neurotransmitters (MANTs) and their biosynthesis precursors and metabolites. The developed method was based on ultra high performance liquid chromatography tandem mass spectrometry detection using multiple-reaction monitoring mode. A pair of mass spectrometry sensitizing reagents, d_0-10-methyl-acridone-2-sulfonyl chloride and d_3-10-methyl-acridone-2-sulfonyl chloride, as stable isotope probes was utilized to facilely label neurotransmitters, respectively. The heavy labeled MANTs standards were prepared and used as internal standards for quantification to minimize the matrix effects in mass spectrometry analysis. Low toxic bromobenzene (extractant) and acetonitrile (dispersant) were utilized in microextraction procedure. Under the optimized conditions, good linearity was observed with the limits of detection (S/N > 3) and limits of quantification (S/N > 10) in the range of 0.002–0.010 and 0.015–0.040 nmol/L, respectively. Meanwhile, it also brought acceptable precision (4.2–8.8%, peak area RSDs %) and accuracy (recovery, 96.9–104.1%) results. This method was successfully applied to the simultaneous determination of monoamine neurotransmitters and their biosynthesis precursors and metabolites in rat brain microdialysates of Parkinson's disease and normal rats. This provided a new method for the neurotransmitters related studies in the future.

1. Introduction

Monoamine neurotransmitters (MANTs) and their biosynthesis precursors and metabolites are widely distributed in the central nervous system and the peripheral body fluids of mammals, including L-3,4-dihydroxyphenylalanine (L-DOPA), dopamine (DA), norepinephrine (NE), epinephrine (E), tryptophan (Trp), 5-hydroxy-tryptophan (5-HTP) and 5-hoxytryptamine (5-HT) and so on. These compounds play important functions in the peripheral and central nervous systems of the human and other mammal [1,2]. There are accumulating evidences that changes of these compounds within peripheral and central nervous systems have some direct associations with neurological disorders such as Parkinson's disease (PD) [3], Alzheimer's disease (AD) [4] and psychiatric illnesses such as depression [5] and so on. *In vivo* microdialysis is a reliable sampling technique that can be used to continuously monitor the concentrations of MANTs and their biosynthesis precursors and metabolites [6,7]. Monitoring the dynamics of neurotransmitters concentrations in the extracellular space of the brain by *in*

Abbreviations: ILD-UA-DLLME, stable isotope-labeling derivatization-ultrasound-assisted dispersive liquid–liquid microextraction; MANTs, monoamine neurotransmitters; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridin; MASC, 10-methyl-acridone-2-sulfonyl chloride

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http://dx.doi.org/10.1016/j.jchromb.2017.03.039
Received 18 January 2017; Received in revised form 18 March 2017; Accepted 31 March 2017
Available online 10 April 2017

vivo microdialysis sampling may play an important role in explaining disease mechanisms and developing new drugs [8,9]. Therefore, it is necessary to establish a sensitive and rapid method to accurately quantify MANTs and their biosynthesis precursors and metabolites in microdialysates.

Currently, many analytical methods have been developed for the analysis of neurotransmitters. Capillary electrophoresis (CE) or liquid chromatography (LC) with various detection techniques including fluorescence detection (FLD) [10], electrochemical detection (ECD) [11,12], ultraviolet (UV) detection [13], chemiluminescence (CL) [14,15], sensor [16], enzymelinked immunosorbent assay (ELISA) [17] and mass spectrometry (MS) [18,19], as well as microchip electrophoresis (MCE) coupled with laser induced fluorescence (LIF) detection [20] have been reported for the determination of neurotransmitters. These methods have more or less limitations, for instance, low sensitivity and selectivity for UV and CL, poor repeatability for ECD due to electrode degradation, interferences after derivatization for FLD, and the difficulty of simultaneously separating neurotransmitters that have similar electrophoretic behavior for ECD. MS detection provides better sensitivity and specificity which analytes are identified by both retention times and molecular masses, compared with UV, FLD, ECD and CL. In recent years, ultra high performance liquid chromatography coupled with tandem mass spectrometry (UHPLC–MS/MS) has aroused wide attention in analysis domain for the determination of multiple neurotransmitters in different biological samples matrices [21,22].

Whereas, the low physiological concentrations of neurotransmitters as well as the complexity of the biological matrices often result in unsatisfactory sensitivity, selectivity or matrix effect [23]. In order to accurately quantify and circumvent matrix effects, isotope internal standards are often used as reported in the works of Bergh and Hershey [22,24]. However, not all the isotope internal standards are commercially available, and the synthesis of isotope internal standards is typically difficult [25]. To improve these problems, the stable isotope-labeling derivatization reagents have been used as an alternative way to quantify many small molecules in LC–MS determination of complex samples [26]. The typical method of isotope-labeling derivatization normally introduces a light isotope tag to the analytes in one sample and a heavy isotope tag to another comparative sample, followed by mixing the light and heavy isotope-labeled samples for LC–MS detection. The heavy labeled standards can be used as the internal standards to solve the problems of matrix effects [27].

However, the sensitivity and accuracy are insufficient when isotope-labeling derivatization is used alone in biological samples because of the lower concentrations of neurotransmitters and more serious matrix interference. A new strategy of efficient extraction in combination with isotope-labeling derivatization is necessary, which can meet those requirements for the quantitative determination of neurotransmitters in biological samples. Recently, ultrasound-assisted dispersive liquid–liquid microextraction (UA-DLLME) has attracted much attention due to its speediness and efficiency, because ultrasonic radiation is an efficient method to accelerate the mass transfer process [28–30].

In this work, a new combined method of stable isotope-labeling derivatization with ultrasound-assisted dispersive liquid–liquid microextraction (ILD-UA-DLLME) has been developed for the simultaneous determination of MANTs and their biosynthesis precursors and metabolites by UHPLC–MS/MS. Different from our previous reports [31–35], 10-methyl-acridone-2-sulfonyl chloride (d_0-MASC) and its deuterated counterpart d_3-MASC, which were reported in our laboratory [36], were used as stable isotope-labeling derivatization reagent in this work to label analytes in real samples and standards, respectively. Various factors affecting isotope-labeling derivatization, UA-DLLME and UHPLC–MS/MS conditions were evaluated and optimized. This developed and validated method was successfully applied to the simultaneous determination of neurotransmitters in rat brain microdialysates of PD and normal rats.

2. Experimental

2.1. Chemicals and materials

L-3,4-dihydroxyphenylalanine (L-DOPA), dopamine (DA), norepinephrine (NE), epinephrine (E), tryptophan (Trp), 5-hydroxy-tryptophan (5-HTP), 5-hoxytryptamine (5-HT), acetonitrile (HPLC grade) and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridin (MPTP) were purchased from Sigma (St Louis, MO, USA). Chloroform, tetrachloromethane, 1-bromo-3-methylbutane, bromocyclohexane, bromobenzene, 1-bromooctane, methanol, acetone, ethanol and acetonitrile were purchased from Shanghai Experiment Reagent (Shanghai, China). Water was purified on a Milli-Q system (Millipore, Bedford, MA, USA). All other reagents used were of HPLC grade or at least of analytical grade obtained commercially. The labeling reagent of d_0-MASC and d_3-MASC were synthesized in authors' laboratory [36].

2.2. Instrumentations

The UHPLC–MS/MS instrumentation consisted of an Agilent 1290 series UHPLC system (Agilent, USA) equipped with an Agilent 6460 Triple Quadrupole MS/MS system (Agilent, USA). The chromatographic separation was carried out on an Agilent SB C18 column (2.1 mm × 50 mm, 1.8 μm) at 30 °C with a 5.0 min linear binary gradient elution. The flow rate was set at 0.2 mL/min. The injection volume was 2.0 μL. Eluent A was 5% acetonitrile/water containing 0.1% formic acid, and eluent B was acetonitrile containing 0.1% formic acid. The gradient elution was as follows: 58% B at injection time increased linearly to 98% B at 4.2 min and held for 0.8 min, and then the column was equilibrated with 42% A + 58% B for 1.5 min before the next run.

The mass spectrometer was operated in the positive ion electrospray mode with MRM. The optimal mass spectrometer conditions were as follows: drying gas temperature 300 °C; drying gas flow rate 10 L/min; nebulizer gas pressure 40 psi; sheath gas temperature 300 °C; sheath gas flow 11 L/min and capillary voltage 3.5 kV. The collision energy (CE) and fragmentor voltage (FV) were also optimized for the corresponding target compound. The MRM transitions, CEs and FVs are summarized in Table 1. Instrument conditions of direct detection method were set according to the literature [37].

In vivo microdialysis was accomplished by using a CMA 402 Syringe Pump, a CMA 120 System (Sweden) for freely moving animals, and a microdialysis probe (MAB6). The probe was perfused with Ringer's solution (5.0 mmol/L) at a flow rate of 2.0 μL/min and ASI stereotaxic flat skull coordinates. An automatic electronic water bath (China), a Xiangzhi TGL16M high-speed refrigerated centrifuge (China), a KQ2200E ultrasonic cleaner (China), and a VX-200 vortex mixer (Labnet, USA) were equipped for derivatization and UA-DLLME experiments.

2.3. In vivo microdialysis sampling

Male Sprague–Dawley rats (8 weeks, body weight 220 ± 20 g), provided by Shandong Lukang Pharmaceutical Co. Ltd. and were maintained in a germ-free environment and allowed free access to food and water. All animal experiments were performed in accordance with the principles of care and use of laboratory animals and were approved by the experiment animal administration committee of China. Sixteen rats were randomly divided into two groups (n = 8, Group I, normal rats; Group II, MPTP challenged rats). The establishment and behavioral assessment of PD rat model was the same as our report [32,33]. After the last behavioral assessment, rats were allowed to recover and *in vivo* microdialysis sampling experiments were performed later (CMA 120, Sweden). The brain microdialysis probe (MAB6) was perfused with 30% ethanol artificial cerebrospinal fluid at a rate of 2.0 μL/min using a microinjection pump (CMA 402, Sweden) and microsyringe (CMA,