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In situ derivatization-ultrasound-assisted dispersive liquid–liquid microextraction for the determination of neurotransmitters in Parkinson's rat brain microdialysates by ultra high performance liquid chromatography-tandem mass spectrometry

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ABSTRACT

Simultaneous monitoring of several neurotransmitters (NTs) linked to Parkinson's disease (PD) has important scientific significance for PD related pathology, pharmacology and drug screening. A new simple, fast and sensitive analytical method, based on in situ derivatization-ultrasound-assisted dispersive liquid–liquid microextraction (in situ DUADLLME) in a single step, has been proposed for the quantitative determination of catecholamines and their biosynthesis precursors and metabolites in rat brain microdialysates. The method involved the rapid injection of the mixture of low toxic bromobenzene (extractant) and acetonitrile (dispersant), which containing commercial Lissamine rhodamine B sulfonyl chloride (LRSC) as derivatization reagent, into the aqueous phase of sample and buffer, and the following in situ DUADLLME procedure. After centrifugation, 50 μL of the sedimented phase (bromobenzene) was directly injected for ultra high performance liquid chromatography-tandem mass spectrometry (UHPLC–MS/MS) detection in multiple reaction monitoring (MRM) mode. This interesting combination brought the advantages of speediness, simpleness, low matrix effects and high sensitivity in an effective way. Parameters of in situ DUADLLME and UHPLC–MS/MS conditions were all optimized in detail. The optimum conditions of in situ DUADLLME were found to be 30 μL of microdialysates, 150 μL of acetonitrile containing LRSC, 50 μL of bromobenzene and 800 μL of $NaHCO_3$-Na_2CO_3 buffer (pH 10.5) for 3.0 min at 37 °C. Under the optimized conditions, good linearity was observed with LODs (S/N > 3) and LOQs (S/N > 10) of LRSC derivatized-NTs in the range of 0.002-0.004 and 0.007-0.015 nmol/L, respectively. It also brought good precision (3.2-12.8%, peak area CVs%), accuracy (94.2-108.6%), recovery (94.5-105.5%) and stability (3.8-8.1%, peak area CVs%) results. Moreover, LRSC derivatization significantly improved chromatographic resolution and MS detection sensitivity of NTs when compared with the reported studies through the introduction of a permanent charged moiety from LRSC into NTs. Taken together, this in situ DUADLLME method was successfully applied for the simultaneous determination of six NTs in biological samples.

1. Introduction

Parkinson's disease (PD) is the second most common neurodegenerative disorder of the central nervous system's disease (CNS). PD is partially correlated with dopamine (DA) loss in the putamen and caudate nucleus of the striatum [1,2]. Catecholamines are important neurotransmitters (NTs) that mainly including DA,

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epinephrine (E) and norepinephrine (NE), which play crucial roles in the regulation of nervous system [3,4]. Furthermore, *L*-3, 4-dihydroxyphenylalanine (*L*-DOPA), the immediate metabolic precursor of DA, is used as the gold standard in the symptomatic treatment of PD [5], and 3, 4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA) are the major metabolites of dopamine [6]. In blood, *L*-DOPA is metabolized by the enzyme aromatic *L*-amino acid decarboxylase. *L*-DOPA is therefore usually given together with a systemic dopadecarboxylase inhibitor (carbidopa or benserazide) to give a larger fraction of *L*-DOPA to be converted to dopamine in the CNS [7]. DA has been reported as the principle biomarker for PD [8], and a number of potential biomarkers have also been reported. Scatton et al. discovered that the concentration of a series of NTs, such as DOPAC, HVA, NE, and serotonin (5-HT), changed from control subjects to parkinsonian patients in brain cortical areas [1]. Therefore, the quantitative determination of catecholamines and their biosynthesis precursors and metabolites in biological samples is of great significance. This is helpful for the biomarkers finding, treatment and drug screening of PD [1,6].

In vivo measurements of NTs concentration dynamics in the living brain enable study of the relationship between NT concentrations in relevant brain nuclei and behavior, drug effects, or disease states. Since its inception, in vivo microdialysis sampling has been the preeminent tool for making such measurements. The utility of this technique was first described in the field of neuroscience, although today the use of microdialysis is common in pharmacokinetic, pharmacodynamic and, now more recently, in clinical studies [9]. Moreover, it plays a prominent role in the pharmaceutical industry when screening novel neurological and psychiatric therapeutics [10].

In recent decades, many analytical methods have been reported for the quantification of amino acid neurotransmitters (AANTs) and monoamine neurotransmitters (MANTs) in the brain microdialysates. Among the earliest techniques are the radioenzymatic and immunological assays, which have been replaced by more sensitive and selective methods [11] such as capillary electrophoresis (CE) [12,13], electrochemical (ECD) [14,15], biosensors [16], fluorescence (FLD) [17], ultraviolet (UV) [18], and mass spectrometric (MS) detection [19–22]. However, the analysis of NTs still presents some challenges. NTs exist in biological samples at extremely low concentrations, which demanding specific and very sensitive bioanalytical methods. Moreover NTs are chemically unstable, prone to spontaneous oxdation, problems related to matrix interferences are also present and additionally, relatively few biosensor or microelectrode methods offer the ability to detect more than one analyte at a time [16]. Therefore, a simple, quick, reliable and sensitive analytical scheme is needed for determining and evaluating multi-NTs in brain microdialysates. Ultra high-performance liquid chromatography coupled with tandem mass spectrometry (UHPLC–MS/MS) using multiple reaction monitoring (MRM) detection allows a rapid, sensitive and specific quantification of multiple analytes in a single run [10]. Moreover, UHPLC–MS/MS detection can be used as a selective tool because the detection of the analytes correlates both the structural information and their molecular weight (and not only to the retention time) [23].

MS-based quantification of a large number of NTs in biological samples is not straightforward. Main challenges for the analysis of NTs in rat brain microdialysates by LC–MS are their low concentration levels, low sample volumes and high polarity. Additionally, the commonly used electrospray ionization (ESI) technique is prone to interference and ion suppression from matrix or co-eluting compounds during the LC–MS analysis [24]. A derivatization step by introducing a functional group into the analyte molecule is an efficient way to solve these problems. This facilitates the retentions of analytes in the chromatography separation, enhances ionization efficiency, decreases endogenous interference, and improves detectability [25]. Therefore, several derivatization methods coupled to LC–MS/MS were developed and applied. Using commercial dansyl chloride (DNS-Cl) as derivatization reagent, Cai et al. developed a LC–MS/MS method for the simultaneous determination of AANTs and MANTs in the plasma and urine [26]. S Greco et al. developed and validated a sensitive method for three MANTs using (5-*N*-succinimidoxy-5-oxopentyl) triphenylphosphonium bromide as derivatization reagent [23]. Recently, benzoyl chloride derivatization method was used for multiple NTs by LC–MS/MS for microdialysates monitoring by Song et al. [10]. Ji et al. developed and validated a HPLC–MS/MS method with diethyl labeling amine group for simultaneous measurement of a panel of MANTs in rat microdialysates [27]. However, these pre-column derivatization reagents have more or less limitations in their applications [28], such as poor stability, low detection sensitivity, operational inconvenience, difficult synthesis, or serious interferences in chromatogram. In this work, a commercial reagent with potential MS sensitizing effect, Lissamine rhodamine B sulfonylchloride (LRSC), was firstly used as derivatization reagent for NTs.

However, derivatization step may lead to mass matrix interference for the following UHPLC–MS/MS detection. Therefore, sample pretreatment is needed before instrumental analysis to obtain sensitive and reproducible results. Since dispersive liquid–liquid microextraction (DLLME) inception in 2006, the various modifications of primary DLLME reported in the literature so far can be categorized as microextraction coupled with derivatization [29,30], ionic liquid-based DLLME [31–33], ultrasound-assisted DLLME [34], low-toxicity dispersive liquid–liquid microextraction [35,36], Dual DLLME [37] and novel automated DLLME [38,39]. One-step DLLME coupled with derivatization are gaining more interest in DLLME related sample preparation methods due to its convenience, rapidity, simpleness and high efficiency.

In this work, a low toxic and in situ derivatization-ultrasound-assisted dispersive liquid–liquid microextraction (in situ DUADLLME) has been developed for the simultaneous determination of six NTs in rat brain microdialysates by UHPLC–MS/MS. Lissamine rhodamine B sulfonylchloride (LRSC) was employed as derivatization reagent for the first time. Fig. S1 is a schematic of in situ DUADLLME procedure of this work. The derivatization and microextraction of the analytes were performed in a single step, allowing the simplification of the procedure and a decrease in the time of sample handling. The proposed method also described the change of toxic chlorinated solvents with low volatility and low toxic bromobenzene, which can be directly and conveniently injected for UHPLC–MS/MS analysis. Various factors affecting derivatization, UADLLME and UHPLC–MS/MS conditions were evaluated and optimized. This developed and validated method was successfully applied to the simultaneous determination of catecholamines and their biosynthesis precursors and metabolites in rat brain microdialysates, which may facilitate the research of neurological diseases, especially for PD and related drug screening.

2. Experimental

2.1. Chemicals and materials

L-3,4-dihydroxyphenylalanine (*L*-DOPA), dopamine (DA), norepinephrine (NE), epinephrine (E), 3, 4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA) were purchased from Sigma (USA). Chloroform, tetrachloromethane, 1-bromo-3-methylbutane, bromocyclohexane, bromobenzene, 1-bromooctane, methanol, acetone, ethanol and acetonitrile were purchased from Shanghai Chemical Reagent (China). The internal