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Simultaneous determination of the repertoire of classical neurotransmitters released from embryonal carcinoma stem cells using online microdialysis coupled with hydrophilic interaction chromatography–tandem mass spectrometry

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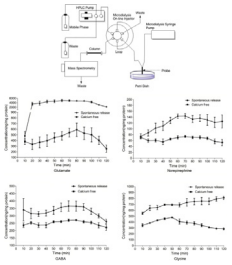
HIGHLIGHTS

- An online MD-HILIC–MS/MS method for simultaneously measuring the repertoire of classical transmitters was developed and validated.
- Hydrophilic interaction chromatography (HILIC) was successfully employed to online system.
- Stable isotope labeled internal standards and authentic matrix have been applied to guarantee reliable results.
- The method features simple procedure (no sample preparation), high recovery (≥73%), high accuracy (89.36% ≤ RE ≤ 116.89%), good reproducibility (2.18% ≤ RSD ≤ 14.56%), and sensitive limits of detection (2 pg for acetylcholine, serotonin, and glutamate, 10 pg for dopamine, norepinephrine, GABA, and glycine).

GRAPHICAL ABSTRACT



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ABSTRACT

Dynamic, continuous, and simultaneous multi-analysis of transmitters is important for the delineation of the complex interactions between the neuronal and intercellular communications. But the analysis of the whole repertoire of classical transmitters of diverse structure is challenging due to their different physico-chemical properties and to their high polarity feature which leads to poor retention in traditional reversed-phase columns during LC–MS analysis. Here, an online microdialysis coupled with hydrophilic

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interaction chromatography–tandem mass spectrometry (online MD-HILIC–MS/MS) detection method was developed for the simultaneous measurement of the repertoire of classical transmitters (acetylcholine, serotonin, dopamine, norepinephrine, glutamate, GABA, and glycine). Stable isotope labeled internal standards and authentic matrix have been applied to guarantee reliable results. The method was successfully employed to reveal the characteristics of transmitter release from embryonal carcinoma stem cells. The method features simple procedure (no sample preparation), high recovery ($\geq$73%), high accuracy (89.36% $\leq$ RE $\leq$ 116.89%), good reproducibility (2.18% $\leq$ RSD $\leq$ 14.56%), and sensitive limits of detection (2 pg for acetylcholine, serotonin, and glutamate, 10 pg for dopamine, norepinephrine, GABA, and glycine). It can be flexibly applied to determine the contents of the classical transmitters in other biological matrix samples with minor changes.

1. Introduction

Neurotransmitters are endogenous chemicals that are synthesized and released by neurons. They carry intercellular information and allow for the transmission of signals between neurons and target cells. Classical transmitters are small molecules that initiate primary neurotransmission functions. They are divided into acetylcholine, monoamines (serotonin, dopamine, and norepinephrine), and amino acids (glutamate, γ-aminobutyric acid (GABA), and glycine) based on structure and chemical properties (Fig. 1). Maintaining a balance among the concentrations of these multiple transmitters is of vital importance for neural communication and the well-being of the organism. Disruption of the balance leads to many disorders such as depression, schizophrenia, Parkinson's disease, Alzheimer's disease, and addiction [1–3]. Thus, dynamic, continuous, and simultaneous multi-analysis of the transmitters is important for the delineation of the complex interactions between the neuronal and intercellular communications. In contrast, the method used for the detection of a single transmitter provides no data on how transmitters influence each other or change simultaneously under certain physical or pathological conditions [4]. However, the analysis of the whole repertoire of classical transmitters of diverse structure is challenging due to their different physico-chemical properties.

Liquid separation techniques such as liquid chromatography (LC) and capillary electrophoresis (CE) coupled with electrochemical detection (ECD) [5–7] and laser-induced fluorescence detection (LIFD) [8–10] have been widely developed for the determination of the transmitters. However, these methods may be unsuitable for simultaneously determining multiple categories of transmitters because the analytes can only be identified by retention-time matching [11]. Moreover, the reliability of ECD is not enough owing to electrode fouling, whereas LIFD is time-consuming due to the derivatization process, which requires multiple steps for sample treatment and can result in derivatized impurities [12]. Compared with the ECD and the LIFD, mass spectrometry (MS) provides better sensitivity and specificity because analytes are identified by both retention time and molecular mass. Tandem mass spectrometry (MS/MS) further improves specificity and sensitivity because the specific dissociation pathways of analytes are based on their molecular structure in MS/MS detection [13,14]. Among MS techniques, the LC–MS is more common and less sophisticated than CE–MS. All classical transmitters are polar molecules, leading to poor retention in traditional reversed-phase (RP) columns during LC–MS/MS analysis [15]. Hydrophilic interaction liquid chromatography (HILIC) employing polar stationary phases, which was introduced by Alpert [16], has been used to achieve adequate retention and separation of polar compounds [17,18], including some classical transmitters [19–22].

HILIC–MS/MS detection needs to be coupled with sampling techniques to monitor the release of transmitters from biological tissue. Microdialysis (MD) is one such technique that is widely used by perfusing a fluid across a semipermeable membrane in a probe inserted into an area of interest [4,23]. Considering its ability for multi-analyte detection with continuous sampling and long-term measurements [24], the technique, coupled with HILIC–MS/MS, is compatible with monitoring transmitter release from non-neuronal cells because this kind of release is much slower but lasts longer compared with that from neuronal cells [25–27].

There have been several applications of online MD-(c)RPLC–MS system over the past decade for monitoring of transmitters or other important biomolecules. The narrow diameter of capillary LC columns offers the advantage of high mass sensitivity. However, its applicability was limited because sophisticated equipments are required and the robustness of cLC columns is less than that of conventional LC columns [28]. An online MD-RPLC–MS method for the analysis of melatonin has been developed based on external calibration in aqueous standard solutions [29]. However, internal standards are regarded to be indispensable in order to obtain reliable measures in LC–MS assays [30] and the surrogate matrix, the pure water, is regarded non-optimal for mimicking the actual biological matrix [31].

Herein, we have developed an online microdialysis coupled with HILIC–MS/MS (online MD-HILIC–MS/MS) method for the simultaneous determination of the repertoire of classical transmitters. The method has been successfully applied to monitor the transmitter repertoire released from embryonal carcinoma stem cell (ECSC) model which has the capacity to self-renew and contribute to various germ layers of the organism, including neural lineages, and act as a robust system for studying embryogenesis [32,33]. In the method development, stable isotope labeled internal standards and authentic matrix have been applied to guarantee reliable results. The method showed excellent performance in terms of simple procedure, high recovery, high accuracy, good reproducibility, and sensitive limits of detection.

2. Experimental

2.1. Chemicals and reagents

Acetylcholine chloride, serotonin hydrochloride, glutamate, dopamine hydrochloride, norepinephrine, GABA, and glycine were purchased from Sigma Chemical Co. (St. Louis, MO). The stable isotope-labeled internal standards (SIL–IS) used were acetylcholine-1,1,2,2-d_4 (acetylcholine-d_4), serotonin-α,α,β,β-d_4 creatinine sulfate complex (serotonin-d_4, 98 at.% D), L-glutamic-2,3,3,4,4-d_5 acid (glutamate-d_5, 98 at.% D), 2-(3,4-dihydroxyphenyl) ethyl-1,1,2,2-d_4-amine HCl (dopamine-d_4, 98 at.% D), norepinephrine-2,5,6,α,β,β-d_6 HCl (norepinephrine-d_6, 98 at.% D), 4-aminobutyric-2,2,3,3,4,4-d_6 acid (GABA-d_6, 99 at.% D), and glycine-d_2 (98 at.% D). All of the SIL–IS were purchased from C/D/N Isotopes (Quebec, Canada).