Journal of Chromatography B, 1002 (2015) 379–386

Contents lists available at ScienceDirect

Journal of Chromatography B

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

A rapid and simple method for the simultaneous determination of four endogenous monoamine neurotransmitters in rat brain using hydrophilic interaction liquid chromatography coupled with atmospheric-pressure chemical ionization tandem mass spectrometry

Wenbin Zhou[a,1], Bangjie Zhu[b,1], Feng Liu[b], Chunming Lyu[a], Shen Zhang[c], Chao Yan[b,*], Yu Cheng[b,*], Hai Wei[a,*]

[a] Center for Chinese Medical Therapy and Systems Biology, Shanghai University of Traditional Chinese Medicine, Shanghai 201203, China
[b] School of Pharmacy, Shanghai Jiao Tong University, Shanghai 200240, China
[c] Department of Rehabilitation, Changzheng Hospital affiliated to Second Military Medicine University, Shanghai 200003, China

ARTICLE INFO

Article history:
Received 29 June 2015
Received in revised form 25 August 2015
Accepted 27 August 2015
Available online 3 September 2015

Keywords:
Monoamine
Serotonin
Dopamine
Epinephrine
Norepinephrine
Hydrophilic interaction liquid chromatography
Tandem mass spectrometry
Brain

ABSTRACT

Endogenous monoamine neurotransmitters play an essential role in neural communication in mammalians. Many quantitative methods for endogenous monoamines have been developed during recent decades. Yet, matrix effect was usually a challenge in the quantification, in many cases asking for tedious sample preparation or sacrificing sensitivity. In this work, a simple, fast and sensitive method with no matrix effect was developed to simultaneously determine four endogenous monoamines including serotonin, dopamine, epinephrine and norepinephrine in rat brain tissues, using hydrophilic interaction liquid chromatography coupled with atmospheric-pressure chemical ionization tandem mass spectrometry. Various conditions, including columns, chromatographic conditions, ion source, MS/MS conditions, and brain tissue preparation methods, were optimized and validated. Pre-weighed 20 mg brain sample could be effectively and reproducibly homogenized and protein-precipitated by 20 times value of 0.2% formic acid in cold organic solvents (methanol–acetonitrile, 10:90, v/v). This method exhibited excellent linearity for all analytes (regression coefficients > 0.998 or 0.999). The precision, expressed as coefficients of variation, was less than 3.43% for intra-day analyses and ranged from 4.17% to 15.5% for inter-day analyses. Good performance was showed in limit of detection (between 0.3 nM and 3.0 nM for all analytes), recovery (90.8–120%), matrix effect (84.4–107%), accuracy (89.8–100%) and stability (88.3–104%). The validated method was well applied to simultaneously determine the endogenous serotonin, dopamine, epinephrine and norepinephrine in four brain sections of 18 Wistar rats. The quantification of four endogenous monoamines in rat brain performed excellently in the sensitivity, high throughput, simple sample preparation and matrix effect.

1. Introduction

Neurotransmitters (NTs), widely distributed in central nervous system and body fluids of mammals, play a significant role in triggering crucial component such as emotion, arousal and cognition [1–3]. Monoamines including Dopamine (DA), Epinephrine (E), Norepinephrine (NE) and Serotonin (5-HT) are essential in the family of NTs. 5-HT, biochemically derived from tryptophan, is primarily found in the gastrointestinal tract (GI tract), platelets, and the central nervous system of animals, including humans. It is popularly thought to be a contributor to feelings of well-being and happiness [4]. DA, NE, and E are also famously known as catecholamines, which are produced from phenylalanine and tyrosine. Dopamine is the first catecholamine synthesized from L-Dopa. In turn, norepinephrine and epinephrine are derived from further metabolic modification of dopamine. Dysregulation of monoamine NTs was frequently reported in the studies of monoamine neu-

Abbreviations: NTs, neurotransmitters; FD, fluorescent detection; SRM, selected reaction monitoring; DP, declustering potential; EP, entrance potential; CXP, collision cell exit potential; CAD, collision associated dissociation; 5-HT, serotonin; DA, dopamine; E, epinephrine; NE, norepinephrine; NH_4OAC, ammonium acetate; MeOH, methanol; ACN, acetonitrile.

* Corresponding authors.
E-mail addresses: chaoyan@unimicrotech.com (C. Yan), ycheng_16@163.com (Y. Cheng), wei_hai@hotmail.com (H. Wei).

[1] These authors equally contributed to this paper.

http://dx.doi.org/10.1016/j.jchromb.2015.08.042
1570-0232/

rotransmitter disorders [5] and psychiatric disorders, including depression [6], anxiety [7], and schizophrenia [8].

Many quantitative methods for endogenous monoamines, mainly based on high performance liquid chromatography (HPLC), have been developed during recent six or seven decades. Coupled with HPLC, various detectors including ultra violet detection (UV) [9], laser-induced fluorescence detection (LIF) [10], fluorescent detection (FD) [11–13], electrochemical detection (ECD) [14–16], and mass spectrometry (MS) [17] have been reported for the determination of monoamine NTs. In addition, HPLC coupled with tandem mass spectrometry (HPLC–MS/MS) is more powerful in its sensitivity and compound specificity [18–22]. Yet, analysis of monoamines is still challengeable, not only because of the low concentration in the biosamples, but also in the difficulty of increasing the hydrophobicity of the monoamines on the reversed LC column [23,24]. Matrix effect was another big problem in the quantification methods, in many cases asking for tedious sample preparation [23–25] such as solid-phase extraction (SPE) or derivatization process, sacrificing sensitivity [26], or even being ignored [20–27]. The previous literature showed poor matrix effect, usually below 70%, before the tedious sample preparation of solid-phase extraction or derivatization process [23–25].

Besides, the endogenous existence of the compounds in the biosamples is also a problem for the preparation of calibration solution in the quantitative determination. Boomsma et al. [28] reported that the catecholamines of DA, NE, and E were oxidized to the corresponding quinone forms and eliminated from untreated plasma and urine when kept at 37 °C for a few days. However, as what van de Merbel [29] pointed out, there is a risk associated with this approach. Next to the fact that the oxidized product might interfere with analyte detection, it might also be converted back to the analyte. Moreover, 5-HT is a stable compound, and cannot be eliminated with this method. To obtain analyte-free samples of the authentic biological matrix, a modified charcoal-stripped method can be a good alternative [30].

Hydrophilic interaction chromatography (HILIC), first suggested by Alpert in 1990 [31], has been defined as an alternative high performance liquid chromatograph mode for analyzing polar compounds [32]. HILIC allows high-resolution separation of highly polar substances with MS compatible mobile phase, with which ion-pair reagent or derivatization is no more necessary [33,34]. Because of its good performance in the separation of polar compounds [35], HILIC has been paid more attention to the analysis of polar drugs [36,37], metabolites [38,39], biologically important compounds [40,41] and etc., in recent years.

Up to now, endogenous monoamine NTs were still explored and highlighted in the studies of monoamine neurotransmitter disorders or psychiatric disorders, despite of their long existence in the neuroscience history. Yet, previous quantitative methods of these polar endogenous compounds had limitations in tedious preparation or matrix effect. In this work, we aimed at developing a simple, fast and sensitive method with no matrix effect to separate and quantify four endogenous monoamines in rat brain sections using hydrophilic interaction liquid chromatography coupled with tandem mass spectrometry (HILIC–UPLC–MS/MS).

2. Experimental

2.1. Chemicals and materials

Serotonin, Dopamine, Epinephrine, Norepinephrine and the internal standard of 4-chlorophenyl-alanine were purchased from Sigma–Aldrich (St. Louis, MO, USA). Another internal standard of L-methionine-$^{13}C_5$, ^{15}N (99%) was purchased from Cambridge Isotope Laboratories (Andover, MA, USA). Methanol (HPLC grade), acetonitrile (LC–MS grade), formic acid (99.5%, LC–MS), and ammonium acetate were purchased from Sigma–Aldrich. Pure water was produced by a Milli-Q purification system (Millipore, Bedford, MA, USA). Wistar rats (250–300 g, male, 18 rats) were purchased from the Vital River Laboratories, Beijing. The animals were housed a week at a controlled ambient temperature of 22–25 °C with 55–65% relative humidity and a 12 h light/12 h dark cycle (lights on at 8:00 AM) and were given food and water *ad libitum* at the Laboratory Animal Center in Shanghai University of Traditional Chinese Medicine, Shanghai, China.

2.2. Instrumentation

The UPLC–MS/MS analysis was performed on an ACQUITY ultra performance liquid chromatographic system (Waters, Milford, USA) coupled with an API 4000 triple quadrupole mass spectrometer (AB Sciex, Foster City, CA, USA). Instrument control was carried out with Analyst 1.5 software (AB Sciex, Foster City, CA, USA).

2.3. Solutions and standards

Stock solutions of 5-HT, DA, NE, E and two ISs were prepared by dissolving the respective compound in water to obtain drug concentration of 10 mM. Working solution was obtained by serial dilutions of stock solution with acetonitrile–ultrapure water (70:30, v/v) to desired concentrations of 60, 20, 6, 2, 0.6, 0.2, 0.06, and 0.02 μM. The endogenous NTs in the rat brain were removed by a charcoal-stripping technique for the preparation of standard calibrating solutions [30–42]. Endogenous-metabolite-released brain extract for calibration standards and QCs were prepared from brain tissue homogenate, which had been stripped of endogenous carbohydrates using neutral decolorizing carbon (Sigma–Aldrich). Charcoal was added to brain tissue homogenate at the concentration of 4 g/100 mL. The suspension was stirred at room temperature for 30 min, vortexed for 30 min, and then centrifuged at 4 °C, 13,000 rpm for 15 min. The supernatant was transferred to vials to be used as the endogenous-metabolite-released brain extract. Standard working solution containing all the analytes was spiked in the endogenous-NT-released brain extracts to prepare the calibrating solutions at concentrations of 3000, 1000, 300, 100, 30, 10, 3.0 and 1.0 nM. Quality control (QC) working solutions were prepared by spiking in endogenous-NT-released brain extracts with certain amounts of analytes at three concentration levels: 50, 100, and 500 nM. Stocking solutions were stored at −80 °C.

2.4. Preparation of brain sample

The fasting overnight animals were anesthetized with 3% pentobarbital sodium (Sigma–Aldrich). The rats were sacrificed and perfused intracardially with 50 mL physiological saline. Brains were quickly and carefully dissected by an experienced technician with reference to the rat brain atlas, and separated into four sections including cortex, hypophysis, brainstem, and hypothalamus on ice. The brain sections were immediately frozen by dropping in liquid nitrogen and then kept in −80 °C for storage. This study was approved by the Ethics Committee of Shanghai University of Traditional Chinese Medicine, Shanghai, China.

At analysis, 20 mg brain samples were pre-weighed and homogenized with 400 μL 0.2% formic acid in cold methanol–acetonitrile (10:90, v/v) with 0.5 μM 4-chlorophenyl-alanine (IS1) and 10 μM L-methionine-$^{13}C_5$, ^{15}N (IS2). The homogenized mixture was incubated at −20 °C for 20 min and centrifuged at 13,200 rpm for 15 min at 4 °C. The supernatant was transferred to 96-well plates for the HILIC–MS/MS analysis.