Journal of Chromatography A, xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Journal of Chromatography A

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

An integrated liquid chromatography–tandem mass spectrometry approach for the ultra-sensitive determination of catecholamines in human peripheral blood mononuclear cells to assess neural-immune communication

Xiaoguang (Sunny) Li[a,*], Shu Li[a], Gottfried Kellermann[a,b]

[a] Pharmasan Labs, Inc., 373 280th Street, Osceola, WI 54020, USA
[b] NeuroScience, Inc., 373 280th Street, Osceola, WI 54020, USA

ARTICLE INFO

Article history:
Received 14 December 2015
Received in revised form 24 March 2016
Accepted 14 April 2016
Available online xxx

Keywords:
LC–MS/MS
Catecholamine
Peripheral blood mononuclear cells (PBMC)
HLB μElution
Phenylboronic acid (PBA)
MRM summation

ABSTRACT

Catecholamines play a vital role in the interactions between the nervous and immune systems and their dysfunctions are implicated in various autoimmune and neurological diseases. However, accurate quantitation of catecholamines in the immune system presents a special analytical challenge. We proposed the first LC–MS/MS method for the determination of catecholamines in human peripheral blood mononuclear cells (PBMC) with significantly improved sensitivity, selectivity and throughput without requiring derivatization, evaporation and ion-pairing reagent. PBMC were separated by density gradient centrifugation and lysed with 0.2 M acetic acid. The analytical novelty includes the first solid phase extraction on a 96-well hydrophilic-lipophilic-balanced (HLB) μElution plate upon complexation with phenylboronic acid (PBA), enabling specific clean-up and fivefold pre-concentration of catecholamines in a single extraction. LC chromatographic separation was obtained on a PFP column with 0.01% HCOOH as additive with enhanced signal response. Summation of five MRM transitions yielded three–four fold rise in sensitivity. The lower limit of quantification of 1 pg/mL for epinephrine (E) and 5 pg/mL for norepinephrine (NE) and dopamine (DA) represents a considerable sensitivity improvement over available methods. Less than 8.7% of intraday and interday precision, 91.8–111.3% of accuracy and successful assessment of reference intervals for 40 healthy donors suggested good reproducibility and reliability of the assay. The novel PBA-HLB-PFP-MRM summation approach allows rapid, sensitive and reliable determination of catecholamines in PBMC, which will facilitate better understanding of the new arena of neural-immune network. Additionally, the substantially improved method can be modified to quantify catecholamines and metabolites in other biological matrices.

© 2016 Elsevier B.V. All rights reserved.

1. Introduction

For a long time, the central nervous system has been considered immune privileged. A growing body of evidences has suggested an extensive cross-talk between the nervous and immune systems. The neurotransmitter's endogenous production and releasing mechanism, function in the immune system, and regulation of various immune-related diseases, have become an emerging new field of multidisciplinary research [1–4]. Catecholamines, including norepinephrine (NE), epinephrine (E) and dopamine (DA), one major category of neurotransmitters, have gained special attention. Extensive evidence [5–7] has suggested that dysfunction of sympathoadrenergic and dopaminergic pathways has been implicated in a wide range of autoimmune and neurological diseases, such as multiple sclerosis, rheumatoid arthritis, schizophrenia, Parkinson's disease, depression. The catecholamine levels are considered important to assess the dysfunctions of these two important pathways, monitor diverse diseases and facilitate new therapeutic drug development. However, compared to widely studied catecholamine quantitation in urine, plasma and tissues, detection of catecholamines in the immune system has rarely been explored [8–10].

The occurrence of catecholamine in the immune system was first proved by Bergquist et al. using capillary electrophoresis. In their work, catecholamines were detected in lymphocytes of human cerebrospinal fluid [11] and subsequently in human

* Corresponding author.
E-mail address: xiaoguang.li@pharmasan.com (X. Li).

http://dx.doi.org/10.1016/j.chroma.2016.04.039
0021-9673/© 2016 Elsevier B.V. All rights reserved.

Please cite this article in press as: X. Li, et al., An integrated liquid chromatography–tandem mass spectrometry approach for the ultra-sensitive determination of catecholamines in human peripheral blood mononuclear cells to assess neural-immune communication, J. Chromatogr. A (2016), http://dx.doi.org/10.1016/j.chroma.2016.04.039

peripheral blood mononuclear cells (PBMC) [12]. The existence of NE and DA in human PBMC was further confirmed by using high-performance liquid chromatography combined with electrochemical detection (HPLC-ECD). Endogenous levels of NE, E and DA in human PBMC were determined to be 0.206, below detection and 0.121 pmol/10 million cells by Musso et al. [13,14], whereas 4.7, 5.4 and 4.1 pmol/10 million cells were reported independently by Cosentino et al. [15,16]. The presence of catecholamines in the immune cells evidenced by these pioneer work clearly supported the communication between the nervous and immune systems. However, the analytical methods used for the catecholamine measurement had their drawbacks: high limit of detection is associated with capillary electrophoresis method; HPLC-ECD methodology generally requires laborious sample clean-up and long chromatographic run to minimize co-eluting interferences [8,10].

The determination of catecholamines in PBMC presents a difficult analytical challenge. First, the high polar nature of catecholamines makes them weakly retained on the traditional reverse-phase C18 column, thus substantially reduces the MS ionization efficiency; Second, the basal levels of catecholamines in PBMC are extremely low as indicated by the HPLC-ECD detection [13,15], thus demands an ultra-sensitive detection; Third, the instability derived from possible degradation of catechol limits the choices of sample treatment; Last but not the least, significantly larger volume of blood required for measurement of catecholamines in PBMC than other biological matrices [8,10] possesses another challenge for the analysis in PBMC (e.g., 150–180 mL blood for repeated experiments) [13]. Given the clinical significance of the endogenous catecholamines in human PBMC and limitations of the existing analytical methods, the need for an alternative method with high sensitivity and selectivity as well as an effective sample preparation is clear.

Tandem mass spectrometry (MS/MS) coupled with LC has been increasingly employed as an alternative analytical tool for the catecholamine measurement in biological matrices among a wide range of detection technologies in view of its superior sensitivity and specificity. Kushnir et al. demonstrated the superior specificity of LC–MS/MS by the determination of catecholamines in urine samples after phenylboronic acid (PBA)-based liquid–liquid extraction in the presence of drugs, which commonly interfere with HPLC analysis [17]. However, the sensitivity was relatively low (e.g., 2.5 ng/mL for E), and the liquid–liquid extraction was time-consuming.

Several different approaches have been reported to increase the sensitivity and selectivity for LC–MS/MS detection of catecholamines in biological matrices, including: derivatization [18,19], ion-pair reagents [20] and hydrophilic interaction chromatography (HILIC) column [21,22]. Chemical derivatization is a common strategy to improve chromatographic separation and increase mass spectrometric detection of analyte by converting high polar analyte to less polar and easier ionizable counterpart. An intriguing reductive ethylation labeling of catecholamines was reported for LC–MS/MS analysis of NE and E in plasma samples with considerably increased sensitivity to 20.0 pg/mL and 5.0 pg/mL, respectively [23]. However, the key reductive ethylation labeling was a laborious process involving a highly toxic chemical, and required to be performed in a vacuum hood thus hampered its application in general clinical laboratories. Additionally, the detection of another important catecholamine, DA, was not explored. Utilization of ion-pair reagents was another effective strategy to improve sensitivity, but at the expense of causing MS contamination and LC peak problems [20]. HILIC coupled with a sample clean-up on a 96-well weak cation exchange (WCX) μElution plate was applied as an alternative approach for catecholamine analysis in plasma [21]. Although better retention was obtained on the HILIC column, the sensitivity of 50 pg/mL for E was insufficient to detect low endogenous level of E in PBMC.

Due to the low concentrations of catecholamines in complicated biological matrices, an efficient sample pretreatment prior to analysis is necessary. Solid phase extraction (SPE) has been widely employed to remove interfering compounds and enrich the analytes with different types of sorbents, such as: C18, alumina, HLB, cation exchange and PBA [10,18–20,24,25]. However, the reported procedures typically resulted in low sensitivity and/or required laborious evaporation and reconstitution steps. Micro devices of alumina [26] and WCX [21] considerably simplified the sample clean-up procedures, but the extraction recovery was relatively low. Online extractions of catecholamines were reported to eliminate labor-intensive sample pretreatment, however, these methods required relatively long LC cycle time (14–30 min) and additional cost for the automation equipment [27,28]. Good recovery and selectivity were obtained for HPLC-ECD detection by using the combination of HLB sorbent and PBA complexation [25]. The effectiveness of the clean-up is based on the formation of a reversible covalent interaction between PBA and *cis*-hydroxyl groups of catecholamines under alkaline pH. Impurities in the biological matrix can be selectively removed by applying alkaline washes before the analytes of interest are eluted with an acidic elution. However, a complicated citric acid-based mobile phase as elution in this extraction protocol made it incompatible for MS detection. The SPE was modified using acidic elution for LC–MS/MS analysis, but the sensitivity was inadequate for the detection of catecholamines in PBMC [29]. In addition, a narrow pH range of 7.5–9.5 for the pre-treated mixture was crucial to achieve a good extraction recovery [24,29]. As such, the process became tedious if the pH had to be adjusted for each sample due to substantial pH variation of individual sample, which was one drawback of the PBA-based process. Alternatively, microextraction by packed sorbent (MEPS), a miniaturized form of SPE, has emerged as a new sample preparation technique with advantages of using reduced extraction sorbent and solvent [30,31]. For example, MEPS was applied for the determination of catecholamines in dried urine and plasma samples followed by HPLC-ECD detection, but the sensitivity was relatively low [32]. Therefore, despite notable progress has been achieved for the detection of catecholamines, the reported methods either required extensive sample preparation and/or lacked sensitivity, no methods are available for sensitive and fast detection of trace amount of catecholamines in human PBMC [8,10].

To address the limitations of the previously reported methods for catecholamine analysis, the primary goal of this work was to develop and validate a fast, highly sensitive and selective LC–MS/MS analysis incorporating a simple sample preparation for the simultaneous determination of catecholamines in human PBMC lysate. To the best of our knowledge, no MS/MS-based methods are available for this purpose. Another objective of this work was to assess the reference intervals of catecholamines in PBMC for a healthy population, which has not been reported yet. The proposed method and the reference ranges should be beneficial for the advance of the new emerging arena of neural-immune network.

2. Experimental

2.1. Chemicals and reagents

Catecholamine standard solutions were supplied by Cerilliant (Round Rock, Texas, USA). Deuterium-labeled internal standards (IS) d_6-NE, d_3-E and d_4-DA were obtained from CDN isotopes (Pointe-Claire, Quebec, Canada). Oasis HLB 96-well μElution plate (2 mg/30 μm) and positive pressure-96 processor were purchased from Waters (Milford, MA, USA). LC–MS grade of water, water with

Please cite this article in press as: X. Li, et al., An integrated liquid chromatography–tandem mass spectrometry approach for the ultra-sensitive determination of catecholamines in human peripheral blood mononuclear cells to assess neural-immune communication, J. Chromatogr. A (2016), http://dx.doi.org/10.1016/j.chroma.2016.04.039

Download English Version:

https://daneshyari.com/en/article/7610042

Download Persian Version:

https://daneshyari.com/article/7610042

Daneshyari.com