Journal of Chromatography A, 1325 (2014) 129–136

Contents lists available at ScienceDirect

Journal of Chromatography A

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

Strategies for quantitation of endogenous adenine nucleotides in human plasma using novel ion-pair hydrophilic interaction chromatography coupled with tandem mass spectrometry

Guodong Zhang [a,*], Annie D. Walker [b], Zhaosheng Lin [a], Xiaogang Han [a], Matthew Blatnik [a], Rick C. Steenwyk [a], Elizabeth A. Groeber [a]

[a] *Biomarker Research, Pharmacokinetics, Dynamics and Metabolism, Pfizer Global Research and Development, Groton, CT 06340, USA*
[b] *Cardiovascular, Metabolic, and Endocrine Diseases Research Unit, Pfizer Global Research and Development, Cambridge, MA 02139, USA*

ARTICLE INFO

Article history:
Received 30 September 2013
Received in revised form 3 December 2013
Accepted 4 December 2013
Available online 16 December 2013

Keywords:
Nucleotides
Organic Acids
IP–HILIC–MS/MS
AMP
ADP
ATP

ABSTRACT

We present here a novel and highly sensitive ion-pair hydrophilic interaction chromatography–tandem mass spectrometry (IP–HILIC–MS/MS) method for quantitation of highly polar acid metabolites like adenine nucleotides. A mobile phase based on diethylamine (DEA) and hexafluoro-2-isopropanol (HFIP) and an aminopropyl (NH2) column were applied for a novel chromatographic separation for the determination of AMP, ADP and ATP in biological matrices. This novel IP–HILIC mechanism could be hypothesized by the ion-pairing reagent (DEA) in the mobile phase forming neutral and hydrophilic complexes with the analytes of polar organic acids. The IP–HILIC–MS/MS assay for adenine nucleotides was successfully validated with satisfactory linearity, sensitivity, accuracy, reproducibility and matrix effects. The lower limit of quantitation (LLOQ) at 2.00 ng/mL obtained for ATP showed a least 10-fold higher sensitivity than previous LC–MS/MS assays except nano-LC–MS/MS assay. In summary, this novel IP–HILIC–MS/MS assay provides a sensitive method for nucleotides bioanalysis and shows great potential to determine a number of organic acids in biological matrices.

© 2013 Elsevier B.V. All rights reserved.

1. Introduction

The biological activities of adenine nucleotides such as AMP, ADP and ATP (Fig. 1) are extensive and varied. A variety of physiological and pathophysiological stimuli can lead to increased ATP release, including ischemia, hypoxia, platelet aggregation, sympathetic nerve stimulation and damage to virtually any cell type [1–5]. Plasma concentrations of AMP, ADP and ATP provide information on their relative physiological importance in regulatory mechanisms [6]. Reductions in oxygen tension equivalent to those seen in the normal coronary microcirculation have been shown to cause ATP release from red blood cells [7,8]. ATP has therefore been proposed as a possible physiologic mediator of changes in blood flow [9–11]. Since ATP-induced vasodilatation is mediated by endothelial cells, determination of plasma AMP, ADP and ATP will be indispensable [12,13]. Increased plasma ATP may be associated with chronic manifestations in cystic fibrosis patients [14]. There have been strong demands for analytical approaches of determining AMP, ADP and ATP in biological samples. Nevertheless, it has been one of the biggest challenges for bioanalysts to develop simple, sensitive and rapid assays for nucleotides quantification in plasma with respect to their high polarity [15–20]. ATP concentration in biological samples was mainly determined by the luciferase system in clinical labs [21,12,22–24]. Traditional reversed-phase liquid chromatography (RP-LC) with UV detection has been frequently applied for quantification of nucleotides utilizing phosphate buffer [25–27] or ion-pair reagents [28–30] in the mobile phases. Additionally, several authors have investigated strong anion exchange (SAX) columns using high concentrations of non-volatile salt buffer in the mobile phases [31,32], which are not compatible with mass spectrometry. During the last ten years, LC coupled with tandem mass spectrometry (LC–MS/MS) has proven to be a highly sensitive and selective technique. However, nucleotides, such as ATP, require special attention in terms of poor retention and peak tailing on chromatographic columns [19]. Peak tailing could result from the adsorption of the phosphate group of nucleotides to the residual hydroxyl group on the surface of reversed phase stationary phases. A volatile ion-pair agent is essential for most RP–LC–MS/MS assays to determine nucleotides[19,20,33–37,18]. Recently, the hydrophilic interaction chromatography (HILIC) technique was applied widely for the separation of highly polar compounds including biologically active compounds such as pharmaceutical drugs, neurotransmitters, nucleosides, nucleotides, and amino acids [38–40]. Several

* Corresponding author. Tel.: +1 860 686 3514.
E-mail address: Guodong.Zhang@pfizer.com (G. Zhang).

0021-9673/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.chroma.2013.12.017

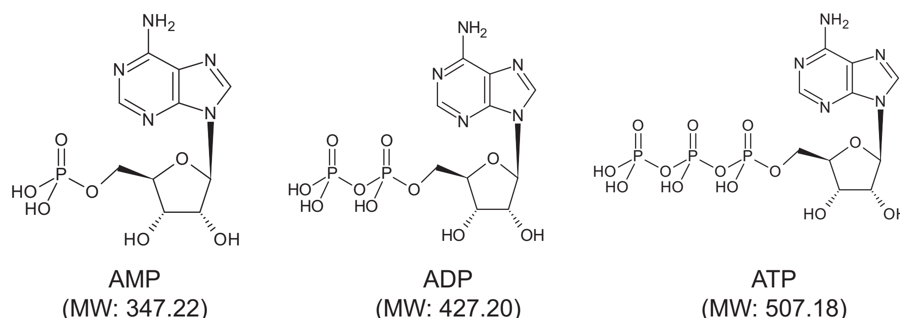

Fig. 1. Chemical structures and corresponding molecular weight (MW) of AMP, ADP and ATP.

HILIC–MS/MS assays were reported to determine nucleotides [41–43]. Nevertheless, most of the above LC–MS/MS assays only provided limited separation capacity and sensitivity with relative long run time (above 30 min).

Here we describe a novel, fast, highly sensitive, selective and validated ion-pair HILIC–MS/MS method utilizing diethylamine (DEA) and hexafluoro-2-isopropanol (HFIP) in the mobile phase and an aminopropyl (NH_2) chromatographic column. This new platform shows great potential to determine small organic acids in plasma.

2. Materials and methods

2.1. Materials

AMP, ADP, ATP, dGMP, dGDP, dGTP, and stable isotopically labeled isotope $^{15}N_5$-AMP, $^{15}N_5$-ADP, and $^{15}N_5$-ATP were obtained from Sigma-Aldrich Chemical Co. (St. Louis, MO, US). Ion-pairing reagents DEA and HFIP were provided by Sigma-Aldrich Chemical Co. HPLC grade water, methanol and acetonitrile were obtained from J.T. Baker, Inc. (Phillipsburg, NJ, US). All other reagents and solvents were analytical or HPLC grade. Human plasma and blood containing K_2EDTA were purchased from Bioreclamation Inc. (Westbury, NY, US) or internal (Pfizer) sources.

2.2. Instrumentation

The LC–MS/MS system used included two Shimadzu LC-20 AD binary pump systems (Columbia, MD), a Leap Technologies HTS PAL autosampler (Carrboro, NC), a Valco column switching valve (VICI, Huston, TX) and an API 4000 tandem quadrupole mass spectrometer (Concord, ON, Canada) with a TurboIonspray™ source operating in negative-ion multiple reaction monitoring mode. Data acquisition and analysis were performed with ABI/Sciex Analyst software, version 1.5.2.

2.3. Liquid chromatographic and mass spectrometric conditions

For the IP–HILIC–MS/MS method, chromatographic separations were performed at a flow rate of 0.35 mL/min on a Luna Amino (NH_2) column (Phenomenex, Torrance, CA) 50 mm × 2.0 mm, packed with 3.0 μm particles. Mobile phase A consisted of 100 mM HFIP and 0.5% DEA (v/v) (pH 8.9) in water, and mobile phase B consisted of 100 mM HFIP and 0.5% DEA (v/v) (pH 8.9) in acetonitrile. A binary gradient was used to perform the separations (see Table 1). A 5.0 μL injection of each sample was loaded onto the column.

The column eluent was directed into a Sciex API 4000 mass spectrometer. The LC flow was directed into the MS during the time interval of 0.8−3.5 min and diverted to waste before and after this time interval using a six-port switching valve (VICI, Houston, TX). The autosampler syringe and the injection valve were washed twice with 100 μL of water:acetonitrile (30:70, v/v), post sample injection, to reduce carryover. The system was operated in negative-ion multiple reaction monitoring (MRM) mode with unit mass resolution for Q_1 and a low resolution for Q_3. The MRM transition was established based on the formation of the most abundant product ion. The ESI (negative ionization mode) conditions were optimized as follows: entrance potential (EP) −10 V, curtain gas (CUR) 25, collision gas (CAD) 12, ionspray voltage (IS) −4000 V, nebulizer gas (GS1) 60, auxiliary gas (GS2) 60, and temperature (TEM) 600 °C. A summary of the declustering potential (DP), collision energy (CE),

Table 1
The gradient conditions for IP–HILIC–MS/MS and HILIC–MS/MS.

Time (min)	Mobile phase B (%; v/v)		
	IP–HILIC–MS/MS (NH_2 column)	IP–HILIC–MS/MS (Bare silica column[a])	HILIC–MS/MS[b] (NH_2 and bare silica column)
0.0	75.0	90.0	80.0
0.8	75.0	90.0	80.0
1.5	–	–	50.0
1.51	–	–	30.0
3.0	58.0	–	–
3.50	60.0	–	–
3.51	30.0	–	–
5.0	30.0	58.0	30.0
5.01	75.0	30.0	80.0
5.6	–	–	80.0
6.0	75.0	–	–
6.50	–	30.0	–
6.51	–	90.0	–
7.50	–	90.0	–

[a] Atlantis bare silica HILIC column (50 × 2.0 mm, 3 μm). The conditions were the same as NH_2 column described in experimental part.
[b] Mobile phase A was 50 mM ammonium formate containing 0.1% formic acid (v/v; pH 4.0) in water and mobile phase B was acetonitrile.

Download English Version:

https://daneshyari.com/en/article/1200426

Download Persian Version:

https://daneshyari.com/article/1200426

Daneshyari.com