Journal of Chromatography A, xxx (2018) xxx–xxx

Contents lists available at ScienceDirect

Journal of Chromatography A

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

Bioanalysis of underivatized amino acids in non-invasive exhaled breath condensate samples using liquid chromatography coupled with tandem mass spectrometry☆

Lucyna Konieczna[a,*], Magdalena Pyszka[a], Magdalena Okońska[a], Maciej Niedźwiecki[b], Tomasz Bączek[a]

[a] *Department of Pharmaceutical Chemistry, Medical University of Gdańsk, Gen. J. Hallera 107, 80-416 Gdańsk, Poland*
[b] *Department of Pediatrics, Hematology, Oncology and Endocrinology, Medical University of Gdańsk, Dębinki 7, 80-211 Gdańsk, Poland*

ARTICLE INFO

Article history:
Received 31 October 2017
Received in revised form 22 January 2018
Accepted 11 February 2018
Available online xxx

Keywords:
Exhaled breath condensate (EBC)
Amino acids
Leukemia
High-performance liquid chromatography coupled to tandem mass spectrometry (LC–MS/MS)
Sample preparation
Lyophilization
Vacuum evaporation

ABSTRACT

Exhaled breath condensate (EBC) is receiving increased attention as a novel, entirely non-invasive technique for collecting biomarker samples. This increased attention is due to the fact that EBC is simple, effort independent, rapid, can be repeated frequently, and can be performed on young children and patients suffering from a variety of diseases. By having a subject breathe tidally through a cooling system for 15–20 min, a sufficient amount of condensate is collected for analysis of biomarkers in clinical studies. However, bioanalysis of EBC involves an unavoidable sample preparation step due to the low concentration of its components. Thus, there is a need for a new and more sensitive analytical method of assessing EBC samples. While researchers have considered analyses of single and small quantities of amino acids – for example, those connected with leukemia – no one has previously attempted to simultaneously analyze a panel of 23 amino acids. Moreover, the present study is well-justified, as prior studies focusing on single amino acids and leukemia at the moment of diagnosis and during chemotherapy (33 days of treatment) are inconsistent. In the present study, amino acids were separated using an XBridge Amide column (3 mm × 100 mm, 3.5 μm). The mobile phase consisted of 10 mM of ammonium buffer in water with a pH of 3 (Phase A) and 10 mM ammonium buffer in acetonitrile (Phase B) under gradient program elution. The analytes were detected in electrospray positive ionization mode. Under optimal conditions, the proposed method exhibited limits of quantification (LOQ) in the range of 0.05–0.5 ng/mL, and good linearity, with the determination coefficient (R^2) falling between 0.9904 and 0.9998. The accuracy in human exhaled breath condensate samples ranged between 93.3–113.3% for the 23 studied amino acids, with intra- and inter-day coefficient of variation (CVs) of 0.13–9.92% and 0.17–10.53%, respectively. To demonstrate the liquid chromatography with hydrophilic interaction with electrospray source coupled to tandem mass spectrometry (LC-HILIC-ESI–MS/MS) method's applicability for biomedical investigations, it was verified and applied to determine amino acids in pediatric patients with leukemia. These tests confirmed that glutamine, arginine, homoarginine, asparagine, histidine, methionine, proline, hydroxyproline, threonine, tyrosine, and valine were present in significantly higher levels in pediatric leukemia patients than in the healthy control group. The developed assay is an attractive alternative to standard analytical methods, because it allows for the non-invasive, fast, sensitive, and reliable analysis of amino acids without derivatization in EBC.

© 2018 Elsevier B.V. All rights reserved.

1. Introduction

The acute lymphoblastic leukemia (ALL) is classified by the World Health Organization (WHO) as a form of cancer that arises from precursor lymphoid cells. Due to the heterogeneous nature of the disease, oncologists must perform numerous additional diagnostic tests, such as tests on BCR-ABL transcripts or immunological subtypes. Furthermore, given the possibility of recurrence

☆ Selected paper from 45th International Symposium on High Performance Liquid Phase Separations and Related Techniques (HPLC 2017), 18–22 June 2017, Prague, Czechia.

* Corresponding author.
E-mail address: tbaczek@gumed.edu.pl (T. Bączek).

https://doi.org/10.1016/j.chroma.2018.02.019
0021-9673/© 2018 Elsevier B.V. All rights reserved.

Please cite this article in press as: L. Konieczna, et al., Bioanalysis of underivatized amino acids in non-invasive exhaled breath condensate samples using liquid chromatography coupled with tandem mass spectrometry, J. Chromatogr. A (2018), https://doi.org/10.1016/j.chroma.2018.02.019

in some children and resistance to steroids used in therapy, it is important to develop new, more effective and less toxic therapeutic approaches to treating ALL. Similarly, it is also important to develop highly indicative, non-invasive, and inexpensive tests that can determine how effective these therapies are, especially in cases where the minimal residual disease is still unknown. Preliminary studies conducted on children with ALL show that the level of cerebrospinal glutamine at Day 0 is higher in this group than in pediatric patients without any type of leukemia. Thus, the authors hypothesize that increased baseline concentrations of glutamine in cerebrospinal fluid (CSF) can be used to identify patients with ALL [1]. Generally, the literature has shown that amino acid concentrations tend to diverge from one another in various physiological fluids under a different state of the body (normal and pathological) [1–4]. The findings of these studies are consistent with our recent results, wherein we differentiated nineteen amino acids in leukemia patients during a 33 day therapy period by measuring both plasma and CSF.

Changes in the levels of amino acids in body fluids are common indicators of numerous disorders. For example, branched-chain amino acids are known biomarkers of depression [5], and patients suffering from Huntington's Disease also exhibit lower levels of these amino acids in their plasma [6]. Additionally, disturbances in amino acid profiles have been discovered in some forms of cancers [7] and liver impairment [8]. Likewise, some changes in these profiles may also help in assessing the risk of diabetes [9]. Abnormalities in amino acid concentrations observed in examinations of other body fluids have also proven to be indicative of a number of other conditions. For example, patients with gastric cancer have been shown to have higher levels of amino acids in their gastric juice than in their blood [10]. Similarly, amino acid profiles are different in urine samples from patients with bladder cancer [11] and in CSF samples from patients suffering from preeclampsia [12]. Insofar as amino acids are considered to be biomarkers of obstructive sleep apnea (OSA), plasma samples from people suffering from OSA showed higher concentrations of homocysteine and cysteine [13,14], which are both seen as good biomarkers of cardiovascular risk among those with this condition [15]. Other articles connecting amino acids with OSA suggest that tryptophan metabolism plays a significant role in cardiovascular risk among OSA populations [16].

For the present study, we decided to examine EBC, as this collection method offers the critical advantage of being non-invasive. In the past few years, there has been a marked trend in biomedical research towards the development of more patient-friendly methods of sample collection. Consequently, many articles demonstrating EBC-based sample-collection methods and their potential application in every day clinical practice have been published. Some changes in EBC chemistry have been observed in patients with various conditions, including: asthma, Chronic Obstructive Pulmonary Disease (COPD), lung cancer, mechanical ventilation, Idiopathic Pulmonary Fibrosis (IPF), cystic fibrosis, Pulmonary Arterial Hypertension (PAH), sarcoidosis, Systemic Lupus Erythematosus (SLE), and Chronic Renal Disease (CRD). Furthermore, EBC may also be useful in pharmacokinetics. EBC analysis in OSA patients indicated higher levels of oxygen stress biomarkers [17].

Although some prior studies have analyzed particular amino acids concentrations in EBC, none have introduced a method for analyzing full amino acid profiles. It has been shown that EBC samples obtained from pediatric patients with asthma contain asymmetric levels of dimethylarginine (ADMA) [18]. However, EBC samples from patients with allergic asthma showed lower levels of arginine and no disturbances in tryptophan levels [19]. In addition, EBC samples obtained from asbestosis/silicosis patients showed elevated levels of tyrosine, which is considered to be an oxidative stress marker [20]. Other amino acids that have been analyzed using EBC include: lysine [21], tyrosine, hydroxyproline, proline [22], valine, leucine, aspartic acid, glutamic acid, phenylalanine, and citrulline [23].

The limited number of articles concerning EBC analysis in the research literature is likely attributable to the extremely low concentrations identified in EBC samples. Commercially available kits are not suitable for breath condensates as they are not designed to detect constituents present in the samples; consequently, they feature low sensitivity and specificity [18,23]. Other methods, such as immunoassay methods, which include ELISA and RIA [17], are susceptible to cross-reactivity reaction and false-positive results. Thus, the quantitative analysis of EBC samples requires new, highly sensitive methods. Some prior studies have used chromatographic assays for EBC analysis, including high-performance liquid chromatography (HPLC), gas chromatography (GC), and mass spectrometry (MS) [17,19]. Other studies have explored the use of hyphenated methods in conjunction with tandem mass spectrometry to analyze low-molecular substances of interest in EBC samples. Some of the methods considered in these studies include ultra-performance liquid chromatography-tandem mass spectrometry (UPLC–MS-MS) [20], LC-ESI–MS/MS [21], LC–MS–MS [22], and liquid chromatography quadrupole time-of-flight (LC–QTOF) [23]. However, all of these prior studies only focused a few amino acids. Thus, there is an urgent need to extend the list of the analyzed compounds, as doing so could lead to considerable improvements in diagnostic and monitoring techniques.

Therefore, the aim of this study was to develop an optimized, rapid, and sensitive analytical strategy based on LC–MS/MS in multiple reaction monitoring (MRM) mode. This method was then used to simultaneously determine 23 amino acids in EBC samples obtained from pediatric leukemia patients in order to assess the potential applicability of these amino acids as diagnostic biomarkers of disease.

2. Materials and methods

2.1. Chemicals

MS grade acetonitrile, ammonium formate, and formic acid were acquired from Sigma-Aldrich (St. Louis, MO, USA). Ultrapure water was obtained using a Milli-Q purification system (Millipore, Bedford, MA, USA) in the Department of Pharmaceutical Chemistry. All analyzed amino acids were analytical standards and were purchased from Sigma-Aldrich (St. Louis, MO, USA). Hydrochloric acid was acquired from POCH (Gliwice, Poland).

2.2. Equipment

LC Agilent 1260 Infinity system (Agilent Technologies, Santa Clara, CA, USA) was equipped with an autosampler, (G7129A) a quaternary pump (QuatPump, model G1311B), a degasser (G1322A), and a series multiple wavelength detector (model G13658). The chromatographic system was equipped with XBridge Amide analytical column (3 mm × 100 mm, 3.5 µm particle size (Waters, Milford, Massachusetts, USA), and the column was thermostated to 25 °C (1260 TCC G1316A). Separation was performed via an elution gradient of Mobile Phase A (10 mM ammonium formate, pH 3.0) and Mobile Phase B (10 mM ammonium formate in acetonitrile). The chromatographic system was coupled to an Agilent 6470 Triple Quadrupole mass spectrometer (Agilent Technologies Santa Clara, CA, USA) with an electrospray ion source (Jet Stream ESI). The mass spectrometer was operated in positive ion multiple reaction monitoring (MRM) mode. Data analysis was performed using MassHunter Workstation software (Agilent Technologies Santa Clara, CA, USA). Finally, a TurboDECCS condenser (Medivac, Parma,

Please cite this article in press as: L. Konieczna, et al., Bioanalysis of underivatized amino acids in non-invasive exhaled breath condensate samples using liquid chromatography coupled with tandem mass spectrometry, J. Chromatogr. A (2018), https://doi.org/10.1016/j.chroma.2018.02.019

Download English Version:

https://daneshyari.com/en/article/7608608

Download Persian Version:

https://daneshyari.com/article/7608608

Daneshyari.com