Journal of Chromatography A, 1474 (2016) 32–39

Contents lists available at ScienceDirect

Journal of Chromatography A

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

Hollow fiber–stir bar sorptive extraction and microwave assisted derivatization of amino acids in biological matrices

Jia Li[a,*], Huan–Yang Qi[b], Yan–Bin Wang[a], Qiong Su[a], Shang Wu[a], Lan Wu[a,*]

[a] Chemical Engineering Institute, Key Laboratory of Environmental Friendly Composite Materials and Biomass Utilization, Northwest University for Nationalities, Lanzhou 730000, China
[b] Xiamen Medicine Research Institute, Xiamen 361008, China

ARTICLE INFO

Article history:
Received 30 June 2016
Received in revised form 29 October 2016
Accepted 31 October 2016
Available online 1 November 2016

Keywords:
Hollow fiber
Stir bar sorptive extraction
Amino acids
Microwave assisted derivatization
Biological matrices
Silylation

ABSTRACT

A kind of solid phase microextraction configuration combining the principles of hollow fiber solid phase microextraction (HF–SPME) and stir bar sorptive extraction (SBSE) is presented. The main feature of HF–SBSE is the use of microporous hollow fiber acting as the carrier and filter, while a thin stainless steel wire and silica microspheres in the lumen of hollow fiber respectively acting as the magnetic stirrer and the dispersed sorbents for the collection and extraction of the target analytes, thus affording extraction process like SBSE. Moreover, the prepared hollow fiber stir bar was applied to direct microextraction and microwave assisted derivatization with N,O–Bis(trimethylsilyl)trifluroacetamide (BSTFA) of four amino acids in rats' urine and cerebrospinal fluid followed by gas chromatography mass spectrometric analysis. The limits of detection for four amino acids were found to be in the range of 0.0003–0.017 μg mL^{-1}, and all the analytes did not exhibit any lack of fit. The extraction recoveries using HF–SBSE techniques ranged from 71.8% to 102.3%. The results indicated that hollow fiber stir bar sorptive extraction was a promising technique for the enrichment and direct derivatization of analytes extracted from biological matrices without sample clean–up.

1. Introduction

Amino acids play a vital role as intermediates in many important metabolic pathways, such as the biosynthesis of nucleotides, vitamins and secondary metabolites [1,2]. They are involved in many important metabolic processes that are vital to the health, growth, development and reproduction of organisms. The presence and levels of free amino acids in body fluids can be early indicators of neurological disease [3,4], and can be used to diagnose inborn errors of metabolism [5]. Some of the amino acids are also considered as potential biomarkers for some diseases [6]. Owing to the increasing role of amino acid detection in biomedical and pharmaceutical studies, several analytical devices, such as amino acid analyzer [7], high performance liquid chromatography (HPLC) [8–10], gas chromatography (GC) [11–14], capillary electrophoresis–mass spectrometry [15], and liquid chromatography–mass spectrometry (LC–MS) [16–18], have been described in the literature. Among these analytical techniques available for the detection of amino acids in biological matrices, gas chromatography mass spectrometry (GC–MS) with proper sample pretreatment, which enables separation, collection, detection, characterization and quantification of a wide variety of metabolites within a short time and allows the rapid analysis of liquid biological fluids, was widely used [19–21].

However, free amino acids need to be derivatized prior to GC–MS analysis due to their high polar and low volatile nature. Silylation is the most preferred derivatization for the analysis of amino acids using GC–MS [22]. Silylation involves the simultaneous reaction of the amino and carboxyl groups in a single step, and when a microwave is used for heating, the reaction time significantly shortens. In comparison with derivatization using conventional heating, derivatization with microwave radiation has a better relative response ratio and results in fewer artifacts in the analysis of compounds, such as amino acids, sugars, and fatty acids [23].

Stir bar sorptive extraction (SBSE), which was firstly developed in 1999, is another microextraction technique derived from SPME [24], and it has the merits of high recoveries and adsorption capacity. Thus, it has been rapidly developed and successfully applied to the trace analysis of various analytes in environmental, food and biological samples [25–29]. However, several commercialized SBSE coatings and most primary homemade SBSE coatings were organic polymers and modified polymers (mostly PDMS and PDMS–activated carbon) [25–29]. The extraction mechanisms of these coatings for the target analytes were mainly based on

* Corresponding authors.
E-mail addresses: lj5458325@126.com (J. Li), wulan@xbmu.edu.cn (L. Wu).

http://dx.doi.org/10.1016/j.chroma.2016.10.079
0021-9673/

the hydrophobic interactions, hence weak and medium polar compounds could be extracted with high efficiency, while polar compounds could be often extracted with the aid of derivatization. The further development of SBSE technique is highly dependent on the exploration of new extraction materials and innovative approaches, especially novel sorbents and supports with good affinities to polar compounds [30].

In order to meet ever–increasing needs, there is a tendency to combine different processes into single hybrid one [31,32]. A combination of procedures is a feasible way to develop new extraction approaches that may synergistically derive advantages from existing individual methods. Over the past few years, there have been many reports toward this end, mostly with the justification that the combination may achieve better matrix clean–up, higher analytical performance (such as sensitivity), and improved selectivity toward target analytes compared to the former individual methods [30]. Thus, researchers also tried to combine the advantages of stir bar with other configuration (such as hollow fiber) together [33–35].

Recently, our group carried out the preparation and application of oxide hollow fiber (SiO_2, ZrO_2, TiO_2) and Multi–Walled Carbon Nano Tubes (MWCNTs) reinforced oxide hollow fiber. Benefiting from our work of hollow fiber solid phase microextraction, a novel hollow fiber stir bar sorptive extraction (HF–SBSE) method was proposed in this study. The main feature of HF–SBSE is the use of microporous HF acting as the carrier and filter, while a thin stainless steel wire and silica microspheres in the lumen of HF respectively acting as the magnetic stirrer and the dispersed sorbents for the collection and extraction of the target analytes, thus affording extraction process like SBSE. Hollow fiber stir bar sorptive extraction is a new configuration of SPME, allowing direct immersion in complex biological matrices without centrifugation, filtration or precipitation, and even could be coupled with ultrasonic or microwave assisted derivatization. The application of microextraction combined with direct derivatization has been found very attractive due to enhancement of analyte recovery, improvement of separation, detectability and compound identification [36]. The sorbents in the lumen of hollow fiber can vary from self–made or commercialized materials in line with the affinities to the analytes with different polarity. HF–SBSE has opened the possibility to design new extraction devices by using materials with specific properties.

The aim of the present study was to propose a reliable and robust alternative GC–MS method based on hollow fiber–stir bar sorptive extraction and microwave assisted derivatization for the quantification of four amino acids in rats' cerebrospinal fluid and urine matrices. Since silica microspheres material has a relatively strong affinity for small molecular biological compounds when loaded in the HPLC chromatography column, they can be expected to extract and concentrate amino acids efficiently. After optimization of the extraction, derivatization and desorption conditions, the established method was applied to the rats' urine and CSF samples in combination with GC–MS analysis.

2. Materials and methods

2.1. Reagents and materials

Glycine (Gly), Methionine (Met), Tyrosine (Tyr), Tryptophan (Try), all of analytical grades (purity >99.9%), were purchased from National Institute for Control of Pharmaceutical and Biological Products (Beijing, China). N,O–Bis(trimethylsilyl)trifluroacetamide (BSTFA), purity >98%, was purchased from Aladdin Reagent Corporation (Shanghai, China). Methanol, ethanol, ethyl acetate, n–hexane, acetone, dimethylformamide (DMF), carbon tetrachloride, sodium chloride, disodium hydrogen phosphate and sodium dihydrogen phosphate, all of analytical grade, were purchased from Tianjin No.3 Chemical Reagent Factory (Tianjin, China). The polyvinylidene fluoride (PVDF) hollow fiber membrane (900 μm i.d., 300 μm wall thickness, and 0.02 μm pore size, pH: 2–11) was bought from Haike Membrana Co. Ltd (Guangzhou, China). YMC-ODS–A HG Silica microspheres (average particles size: 50 μm, average pore size: 12.8 nm, specific surface area: 345 m^2/g, carbon content: 17%) was purchased from YMC Co. Ltd. (Japan) High purity deionized water was used throughout the whole experiments. The urine and cerebrospinal fluid samples (CSF) of rats were provided by Key Laboratory of Chemistry of Northwestern Plant Resources and Key Laboratory for Natural Medicine of Gansu Province, Lanzhou Institute of Chemical Physics (Lanzhou, China).

2.2. Apparatus

Thermo Scientific DSQ TM II (Thermo Fishier Scientific, Assembled in China) equipped with splitless injector was employed for amino acids. A Thermo TR–5 MS column, 30 m × 0.25 mm i.d., 0.25 μm film thicknesses (Thermo, USA) was applied to analyze the extracted analytes. Helium (99.999%) was used as the carrier gas and kept at a flow rate of 1.0 mL min^{-1}. MS transfer line heater was 280 °C; ion source temperature was 250 °C; inlet temperature was 240 °C; EI was 70 eV. The GC–MS temperature program was as follows: initial temperature 80 °C, held for 1 min, first increased to 260 °C by 20 °C min^{-1} and held for 3 min. All the amino acids were derivizated with BSTFA under microwave irradiation in a domestic microwave oven (power: 700 W) for 2 min. Samples were injected in a splitless mode. Standard solutions and samples were analyzed in selective ion monitor (SIM) mode. Major fragment ions and molecular structures of the derivatives were separately listed in Table 1 and Fig. 1.

2.3. Preparation of standards solution

The standard stock solution was prepared by accurately weighting 100.0 mg of four amino acids into 100 mL volumetric flask and dissolved in water under sonication. The stock solution was diluted with water to obtain calibration standard solution, 0.001, 0.01, 0.05, 0.1, 1, 10, and 100 μg mL^{-1}, respectively.

One milliliter of the standard solution was added into the centrifuge tube and dried completely in N_2 at the temperature of 40 °C. BSTFA (0.1 mL) and derivative solvent (0.9 mL) were added into the centrifuge tube and reacted with analytes under microwave irradiation for several minutes. Of the 1.0 mL final derivative solution, 1 μL was directly injected into the GC–MS system for analysis.

2.4. Preparation of hollow fiber stir bar

PVDF hollow fiber was cut into 2 cm section by scissors and ultrasonically cleaned in acetone to remove any possible impurities in the fiber. One end of HF section was sealed by a lighter. A thin steel wire of 1 cm length was inserted in the lumen of HF. 0.1 g silica microspheres were dispersed in 1 mL absolute ethanol under sonication. Then the dispersion solution was injected slowly into the lumen of PVDF hollow fiber by a syringe. After ethanol was exuded and volatilized, the other end of HF was sealed by the lighter. The preparation process of the hollow fiber stir bar was illustrated in Fig. 2.

2.5. Extraction and derivatization mode

Fig. 2 also showed the schematic figure for the application of hollow fiber stir bar to extract amino acids in biological matrices and reacted with BSTFA. Prior to use, hollow fiber stir bar was pre–rinsed with acetone and dried in order to remove impurities.