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Selective determination of aromatic amino acids by magnetic hydroxylated MWCNTs and MOFs based composite

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ABSTRACT

A novel magnetic solid-phase extraction (MSPE) based method was established for aromatic amino acids (AAAs) selective determination. Central to the method was the adopted extractant. An explored composite of metal organic frameworks (MOFs) and magnetic carbon nanotubes (CNTs) was employed for that, which exhibited superior adsorption affinity and selectivity to AAAs as compared to other amino acids with the mechanisms attributed to multiple hydrogen bonding and π-π electron-donor-acceptor (EDA) interactions. The morphology, structure and magnetic behavior of the composite were characterized and related MSPE procedure was established. Critical extraction conditions including pH, extraction time, temperature and salt addition were investigated and optimized. Subsequently the concentrations of three AAAs tryptophan (Trp), tyrosine (Tyr) and phenylalanine (Phe) in Lanzhou lily were determined by the composite based MSPE procedure coupled with high performance liquid chromatography-ultraviolet detection (HPLC-UV). The composite provided a superior sample clean-up function and many of the matrix interferences were eliminated, thus ensured AAAs were accurately and efficiently determined. The results showed that the method had good linearities with the linear coefficients above 0.99, desirable recoveries ranged from 88.0% to 96.8% with the RSD less than 5.1%, satisfactory precision and low limits of detection (LODs) which were respectively 0.04, 0.11, and 0.87 ng/g for Trp, Tyr and Phe. The composite based MSPE-HPLC-UV method has great potentials for the AAAs selective determination from complex matrix samples.

1. Introduction

Amino acids are life-critical nutrients for their roles as the key building blocks of proteins and the intermediates in the metabolism. They participate in the chemical reactions vital to life and are considered the basis of life [1]. The three proteinogenic aromatic amino acids (AAAs) tryptophan (Trp), tyrosine (Tyr) and phenylalanine (Phe) are essential for human body and must be supplemented from external sources just as foods or nutritional supplements. They are closely associated with many functions of the living cells and play important roles in diagnosis of liver, kidney and nervous diseases [2]. Phe and Tyr are generally recommended as antidepressants for mood elevating or appetite control, and Trp is used for promoting the release of neurotransmitter serotonin, which is favor of sleep regulation [3]. Whether Trp, Tyr and Phe intake are insufficiently or excessively, the normal functions and metabolism of the human body will be affected [4]. Therefore, quantification analysis of AAAs is of vital significance to foods evaluation.

Lanzhou lily (Lilium davidii var. unicolor), one of the best lilies in China with a 150-year cultivation history, is distributed in the Lanzhou area of northwestern China. It is well-known as an edible bulb crop as well as a traditional medicinal plant, and famous for its sweet and delicate taste, thick white flesh, superior quality and rich nutrients such as vitamins, minerals and proteins [5]. Quantitative analysis of AAAs in Lanzhou lily is significant to evaluate that popular food. However, the reported analytical methods for AAAs in Lanzhou lily were relatively rare thus far. High performance liquid chromatography-ultraviolet detection (HPLC-UV) is applicable for underivatized Trp, Tyr and Phe, yet an appropriate sample preparation procedure is indispensable prior to analysis in consideration of the complex matrix of the lily sample which will cause interferences to the target analytes determination and relatively low concentration of analytes which may not be accurately and efficiently detected.

Analyte extraction is the most basic procedure and feature of any sample preparation technique which aims for cleaning up, isolating,

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and concentrating the analyte of interest in a complex sample matrix [6]. For the purpose, solid-phase extraction (SPE) has been explored and further developed as a classical sample preparation technology throughout the years, which based on the sorption and desorption of target analyte through the selected sorbent. As the demands of sample extraction technology development and practical application, varieties of innovative sorbent-based approaches have been explored, such as solid-phase microextraction (SPME) [7], dispersive solid-phase extraction (DSPE) [8], stir-bar sorptive extraction (SBSE) [9] and magnetic solid-phase extraction (MSPE) [10]. MSPE was introduced for easy and rapid sample preparation, which employs magnetic sorbent for quickly extracting target analyte since it can be easily recovered by a magnet without the filtration or centrifugation procedure [11].Various magnetized materials have been explored for MSPE technique, such as magnetic metal organic frameworks (MOFs) [12], magnetic carbon nanomaterials (CNs) [13] and other coated or modified magnetic materials [14]. MOFs represent a class of inorganic-organic hybrid porous coordination polymers, which integrating the advantages of both metal (oxide) cations and organic electron donor linkers and exhibit unique properties including high surface area, diverse and tunable pore size, and easy to be functionalized [15]. Magnetized MOFs have been developed for efficient MSPE of target analytes in view of their excellent properties, such as magnetized MIL-101(Cr) [16], magnetic Co-MNPC [17], and magnetic MOF-5 [18]. Carbon nanotubes (CNTs) have been widely used in trapping and separating various compounds due to their unique structure which lead to excellent properties including large surface area, ability to establish π-π interactions and easy to be covalent or non-covalent functionalized [14]. Magnetized CNTs have been widely explored for tackling the issues faced in analytical science by the MSPE strategy [10,19]. More recently, the magnetic composites of MOFs and CNs have been explored for specific purpose and scope. A self-assembled magnetic graphene supported ZIF-67 composite has been developed for efficient adsorption of benzotriazole [20]. Our group has also explored a novel magnetic hydroxylated MWCNTs and MOFs based composite (Fe_3O_4-MWCNTs-OH@poly-ZIF67), which exhibits superior adsorption affinity to aromatic acids [21]. However, we found the composite has better adsorption capacity for AAAs due to their particular properties in our further study. The composite has great application potential for MSPE of AAAs in complex matrix samples.

In this work, we employed the composite of Fe_3O_4-MWCNTs-OH@poly-ZIF67 for AAAs determination from Lanzhou lily by the MSPE strategy prior to HPLC-UV analysis. The composite is sensitive to AAAs due to their benzene-ring structure as compared to other amino acids, and three targets AAAs (Trp, Tyr and Phe) just can be directly detected by the HPLC-UV without derivatization step due to their ultraviolet absorption capacities. Related MSPE parameters which can influence the extraction efficiency like pH, extraction time, temperature and salt addition were investigated and optimized, and extraction mechanism was evaluated for better application of the composite. The method for directly determination of AAAs from Lanzhou lily after MSPE procedure was established and validated, and subsequently the real samples were investigated and evaluated.

2. Experimental

2.1. Reagents and materials

Glutamic acid (Glu), Threonine (Thr), Valine (Val), Lysine (Lys), Trp, Tyr and Phe were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). MWCNTs-OH (OD, 10–20 nm; length, 10–30 μm; −OH content, 3.06 wt%; purity > 95 wt%) were gained from Chengdu Organic Chemical Co., Ltd. (Chengdu, China); Ferrous chloride tetrahydrate ($FeCl_2{\cdot}4H_2O$), ferric chloride hexahydrate ($FeCl_3{\cdot}6H_2O$) were both purchased from Beijing Chemicals Corporation (Beijing, China). Cobaltous nitrate hexahydrate ($Co(NO_3)_2{\cdot}6H_2O$), 2-methylimidazole (2-MIM) were both purchased from Aldrich Chemical Co., Ltd. (Shanghai, China). All the amino acids were of biological reagent and other reagents were of analytical reagent grade, all of which were used directly without further purification unless otherwise mentioned. Standard stock solutions of Glu, Thr, Val, Lys, Trp, Tyr and Phe (50.0 μg/mL) were prepared in brown flasks with ultrapure water then stored at 4 °C. All the working solutions were freshly prepared according to the required concentrations by appropriate diluting of the stock solutions.

2.2. Instruments

HPLC system included Waters 1525 HPLC system, 2998 diode array detection system and a reversed phase Redclassical C18 column (250 mm × 4.6 mm id, 5 μm). The mobile phase contained methanol and ultrapure water with 0.1% acetic acid (12:88 v/v) at a flow rate of 1.0 mL/min. The UV detection wavelengths were selected at 275, 257 and 278 nm for Tyr, Phe and Trp, respectively. In the selectivity evaluation, HPLC system was consisted of Agilent 1200 system, an evaporative light scattering detector (ELSD), and an Agilent C18 column (250 mm × 4.6 mm, 5 μm). Its mobile phase was made up of methanol and ultrapure water with 0.1% acetic acid (12:88, V/V). Scanning electron microscopy (SEM) image was observed on a MIRA3 SEM instrument (TESCAN, Czech Republic). Fourier transform infrared (FTIR) spectrum was performed on an IFS120HR spectrometer (Bruker, Germany). X-ray diffraction (XRD) measurement was performed on an X'pert PRO X-ray diffraction (Philips, Netherlands). Magnetic property was acquired by a 3473-70 vibrating sample magnetometer (VSM) (Gmw Associates, USA). Sample preparation was performed on a JY99-IIDN ultrasonic cell crusher (SCIENTZ, China).

2.3. Preparation of Fe_3O_4-MWCNTs-OH@poly-ZIF67

Fe_3O_4-MWCNTs-OH@poly-ZIF67 was synthesized easily according to a method we have developed [21]. First, Fe_3O_4-MWCNTs-OH was prepared as a chemical co-precipitation method. 500 mg MWCNTs-OH was dispersed homogeneously in a 250 mL three-necked round-bottomed flask with 60 mL of deoxygenated water by ultrasonication, and a 60 mL mixed solution consisted of 302 mg $FeCl_3{\cdot}6H_2O$ and 110 mg $FeCl_2{\cdot}4H_2O$ was added under mechanical stirring. Then 20 mL ammonia solution (5 wt%) was added dropwise at 70 °C and the mixture was vigorously stirred for 2 h. The above steps were performed under nitrogen atmosphere by a Schlenk line. Subsequently Fe_3O_4-MWCNTs-OH was collected by filtration and washing with ethanol and ultrapure water until pH of the eluent is ~7.0. Second, the target composite (Fe_3O_4-MWCNTs-OH@poly-ZIF67) was prepared by an in-situ synthesis strategy. 600 mg obtained Fe_3O_4-MWCNTs-OH was added to a uniform mixture solution consisted of 600 mg $Co(NO_3)_2{\cdot}6H_2O$ and 360 mg 2-MIM, subsequently the mixture was first stirred for 15 min at room temperature then sonicated for 2 h. The black precipitate was the target composite which was obtained by filtration, washing with ultrapure water and methanol for five times, and drying at 65 °C for 24 h.

2.4. MSPE and desorption procedures

9.0 mg of the prepared composite was dispersed into a 10 mL centrifuge tube with 3.0 mL of mixed analytes solution (each at 10.0 μg/mL). After the mixture was shaken on a shaker (25 °C, 500 rpm) for 20 min, the composite was separated by a magnet and the supernatant solution was collected and analyzed for the extraction evaluation. After that, the composite with the residual supernatant were transferred to an Eppendorf tube, then the composite was aggregated by the magnet and the residual solution was decanted. 0.2 mL of mixture consisted of 12% methanol and 88% water with the pH of 2.5, 3.0 and 3.5 were employed as the desorption solutions, respectively. The desorption condition was under sonication for 15 min at 45 °C and the desorption operation was performed for 3 times, finally