Analytica Chimica Acta xxx (2018) 1–7

Contents lists available at ScienceDirect

Analytica Chimica Acta

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

Rapid and accurate quantification of amphetamine and methamphetamine in human urine by antibody decorated magnetite nanoparticles coupled with matrix-assisted laser desorption ionization time-of-flight mass spectrometer analysis

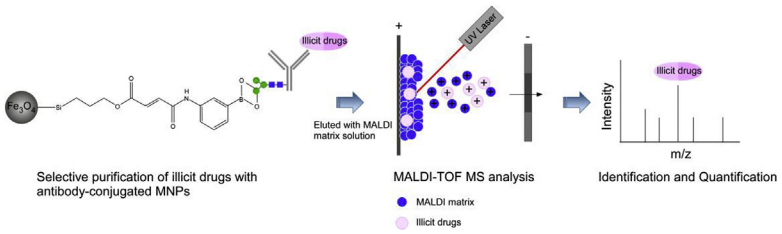
Kun-Ru Wu, He-Hsuan Hsiao*

Department of Chemistry, National Chung Hsing University, 250, Kuo Kuang Rd., Taichung, 402, Taiwan, ROC

HIGHLIGHTS

- Inexpensive antibody-conjugated boronic acid modified magnetite nanoparticles (MNPs) were facilely synthesized.
- AM- and MA-antibody conjugated MNPs were used for the selective purification of AM and MA, respectively.
- A method for screening and confirmation of AM and MA from human urine was developed with MALDI-TOF MS analysis.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 6 December 2017
Received in revised form
12 March 2018
Accepted 30 March 2018
Available online xxx

Keywords:
Antibody
Magnetite nanoparticles
Amphetamine
Methamphetamine
Mass spectrometry

ABSTRACT

In this study, a novel method for the simultaneous determination and accurate quantification of abused drugs in human urine was developed. Antibody conjugated boronic acid modified magnetite nanoparticles (Fe_3O_4, MNPs) were prepared for the selectively purification of illicit drugs in combination with high resolution matrix-assisted laser desorption ionization time-of-flight mass spectrometer (MALDI-TOF MS) analysis. Illicit drugs, amphetamine (AM) and methamphetamine (MA), were used as model analytes to demonstrate the feasibility of our strategy. Boronic acid functionalized MNPs were first prepared via one-pot synthesis to simplify and improve the efficiency of a chemical reaction. Anti-amphetamine antibody (anti-AM antibody) and anti-methamphetamine antibody (anti-MA antibody) was conjugated onto boronic acid modified MNPs, respectively, through the formation of boronate ester bond that could maintain the correct orientation to maximally capture their antigens. The capacity of antibody conjugation to boronic acid modified MNPs was at least 24 μg antibody/mg MNPs. Antibody-conjugated MNPs were designed to specifically capture AM and MA in human urine samples, both of which can be directly eluted to MALDI target plate by adding MALDI CHCA matrix solution for the following MALDI-MS analysis. The linear range of detection of the proposed method were 25–400 ng/mL and 25–1000 ng/mL with coefficients of determination between 0.9923 and 0.9997 for AM and MA, respectively. The lowest detectable concentrations of AM and MA were 1.87 and 3.75 ng/mL, respectively. More importantly, the proposed method allows rapid and accurate quantification of AM and MA from three suspects' urine samples. The obtained results are consistent with traditional GC/MS analysis.

* Corresponding author.
E-mail address: hhhsiao@dragon.nchu.edu.tw (H.-H. Hsiao).

https://doi.org/10.1016/j.aca.2018.03.057

Antibody-conjugated MNPs could thus prove to be powerful tools for important applications such as forensic science, food safety and clinical diagnosis of disease.

1. Introduction

Abused drug analysis plays an important role for the modern analytical chemistry with both legal and social respects in forensic investigations. A simple, rapid, sensitive, accurate and cost-effective method for the detection of abused drugs in the biological or other complex fluids would be of great value in the field of toxicology to serve the need for clinical diagnostics, drug trafficking, and law enforcements. Drugs of abuse testing are generally carried out in two ways, that is, screening and confirmation. As the presence of abused drugs are detected through commercial drug screening kits such as immunoassays [1–3], Marquis reagent test [4] or a similar colorimetric assay [5,6], the samples are subsequently collected and delivered to the laboratories for confirmation testing, which requires high selectivity, sensitivity and accuracy toward targeted drugs, and it is frequently implemented by chromatographic and spectroscopic techniques [7,8]. In the case of immunoassays, there is a risk for the misinterpretation of subjective color perception resulting in the false-positive or the false-negative results, and the degree of selectivity and sensitivity often depends on the specificity of the interaction between antibody and targeted molecules [9–11]. Furthermore, current methodology for the confirmation testing requires large amounts of biological samples, involving complicated and time-consuming sample preparation followed by gas chromatography-mass spectrometry (GC-MS) or liquid chromatography-mass spectrometry (LC-MS) analysis [7,12–14].

In the past few years, nanoparticles (NPs) based mass spectrometric assay have been extensively used in chemical and biomolecular analysis in the field of food safety, environmental pollutants, disease biomarkers, illicit drugs and more [15–18]. As the use of traditional procedures for the quantification of abused drugs is accompanied with the issues of long operation time, and sophisticated experimental procedures, nanotechnology is necessitated to simplify sample pretreatment for abused drugs analysis. Compared to other nanomaterials used as enrichment, magnetite nanoparticles (MNPs, Fe_3O_4) offer distinct advantages of (i) simple synthesis; (ii) easy surface modification and (iii) facile separation under external magnetic fields. Several reports have been demonstrated that low abundance analytes can be greatly isolated and enriched by the use of modified MNPs based on the properties of interaction regarding to hydrophobicity, hydrophilicity, electrostatic interaction, hydrogen bond, or π-π interaction between sorbents and analytes [19–21]. Due to the lack of specific interactions between modified MNPs and targeted molecules, co-extracted compounds will induce non-specific interference for the following spectroscopic measurement. Combination of these MNPs enriched methods with GC-MS or LC-MS approaches can improve the specificity and sensitivity. The signal of interfering substances were eliminated by chromatographic separation or mass isolation. However, the overall analysis time containing sample preparation followed by chromatographic-mass spectrometric analysis may take several hours to complete, depending on the complexity of samples and the instrument settings for the discrimination of targeted analytes. Nevertheless, the above mentioned drawbacks can be overcome utilizing affinity chromatography coupled with matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF MS) analysis. The antibody-conjugated MNPs possess robust and specific affinities for targeted antigen and MALDI-TOF MS offers excellent sensitivity, outstanding mass accuracy and high throughput for the targeted compounds [17,18,22].

Herein, a straightforward, fast and reliable method for the detection and quantification of abused drugs in human urine was developed by utilizing antibody decorated boronic acid modified MNPs coupled with MALDI-TOF MS analysis. Using amphetamine (AM) and methamphetamine (MA) as model targets, the effect of enrichment conditions as well as the performance of antibody-conjugated MNPs were investigated. Such abused drugs are of special importance as their use are rising globally at particularly rapid rates, especial in Taiwan, due to their low cost and ease of manufacture [23]. For sure, this is also a common problem in the world. To our knowledge, this novel strategy is first applied for comparative screening and confirmation of illicit drugs from human urine of suspects and healthy individuals. Site-specific antibody-conjugated MNPs with improved affinity and concomitant enhancement of sensitivity with MALDI-TOF MS detection can become a powerful drugs-of-abuse analysis platform.

2. Experimental section

2.1. Materials

Iron (III) chloride hexahydrate ($FeCl_3 \cdot 6H_2O$), iron (II) chloride tetrahydrate ($FeCl_2 \cdot 4H_2O$), tetraethyl orthosilicate (TEOS), 3-(Trimethoxysilyl)propyl methacrylate (γ-MAPS), 3-acrylamidophenylboronic acid, ammonium bicarbonate, α-cyano-4-hydroxycinnamic acid (CHCA) and trifluoroacetic acid (TFA) were obtained from Sigma-Aldrich (St. Louis, MO, US). N,N,N′,N′-tetramethylethylenediamine (TEMED) was obtained from Alfa Aesar (Heysham, England). Ammonium persulfate (APS) was obtained from Bio-Rad (Hercules, California, US). Acetonitrile (ACN) and ammonium solution (25%) were obtained from Merck (Darmstadt, Germany). Amphetamine, methamphetamine, amphetamine-D5 and methamphetamine-D5 were obtained from Cerilliant Corporation (Round Rock, Texas, US). Anti-amphetamine antibody (anti-AM antibody) was obtained from Fitzgerald (Acton, MA, US). Anti-methamphetamine antibody (anti-MA antibody) was obtained from abcam (Cambridge, MA, US).

2.2. Apparatus

The FT-IR spectrum of MNPs were measured by Tensor 27 FT-IR (Bruker, Germany). JEM-2100F transmission electron microscopy (JEOL, Japan) was used to record the average size of MNPs image. The MALDI-TOF MS and MS/MS detection were acquired with New ultrafleXtreme MALDI-TOF/TOF (Bruker, Germany) in reflectron mode equipped with smartbeam-II™ laser (355 nm wavelength, 2000 Hz and 1000 Hz repetition rate in MS and MS/MS mode, respectively), applying α-cyano-4-hydroxycinnamic acid (CHCA) as a matrix. The following ion source parameters were applied: ion source 1, 20.60 kV and ion source 2, 16.85 kV. Other parameter settings for MALDI-TOF MS analysis were as follows: pulsed ion extraction, 100 ns; lens, 6.0 kV; reflector 1, 20.90 kV, and reflector 2,

Please cite this article in press as: K.-R. Wu, H.-H. Hsiao, Rapid and accurate quantification of amphetamine and methamphetamine in human urine by antibody decorated magnetite nanoparticles coupled with matrix-assisted laser desorption ionization time-of-flight mass spectrometer analysis, Analytica Chimica Acta (2018), https://doi.org/10.1016/j.aca.2018.03.057