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Direct analysis of anabolic steroids in urine using Leidenfrost phenomenon assisted thermal desorption-dielectric barrier discharge ionization mass spectrometry

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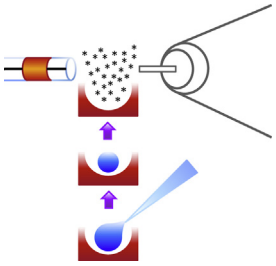
HIGHLIGHTS

- Urinary anabolic steroids were detected with high sensitivity using LPTD-DBDI-MS.
- Anabolic steroids were directly ionized using DBDI without any adduct formation.
- Analytical figures of merit are demonstrated using suitable internal standards.

GRAPHICAL ABSTRACT



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ABSTRACT

Rapid detection of trace level anabolic steroids in urine is highly desirable to monitor the consumption of performance enhancing anabolic steroids by athletes. The present article describes a novel strategy for identifying the trace anabolic steroids in urine using Leidenfrost phenomenon assisted thermal desorption (LPTD) coupled to dielectric barrier discharge (DBD) ionization mass spectrometry. Using this method the steroid molecules are enriched within a liquid droplet during the thermal desorption process and desorbed all-together at the last moment of droplet evaporation in a short time domain. The desorbed molecules were ionized using a dielectric barrier discharge ion-source in front of the mass spectrometer inlet at open atmosphere. This process facilitates the sensitivity enhancement with several orders of magnitude compared to the thermal desorption at a lower temperature. The limits of detection (LODs) of various steroid molecules were found to be in the range of 0.05–0.1 ng mL^{-1} for standard solutions and around two orders of magnitude higher for synthetic urine samples. The detection limits of urinary anabolic steroids could be lowered by using a simple and rapid dichloromethane extraction technique. The analytical figures of merit of this technique were evaluated at open atmosphere using suitable internal standards. The technique is simple and rapid for high sensitivity and high throughput screening of anabolic steroids in urine.

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1. Introduction

Anabolic steroids are considered as therapeutic agents used to promote the muscle growth and strength [1,2]. These compounds have wide applicability in the treatment of cancer and AIDS, where a rapid increase of muscle mass is highly desired [3]. However, administration of these compounds in a high dose is associated with adverse effects like permanent organ damage, reduced fertility, hypertension, psychiatric and behavioral disorders etc. [2,4–7]. Major concerns are the use of these compounds by athletes to increase their performance, which is popularly known as 'doping' [8]. These compounds stimulate the androgen receptors in brain and athletes enjoy a state of euphoria, increased aggressiveness and decreased fatigue [9,10]. Owing to the enhanced muscle power, doped athletes perform superiorly compared to their non-doped counterparts. In order to prohibit the illicit use of anabolic steroids and maintain a fair and healthy competition in sports ground, analytical strategies with high-throughput and high sensitivity spot detection of performance enhancing steroids in body fluids are highly desirable.

Analysis of urine is a convenient procedure to monitor the consumption of illicit compounds as urine can be obtained easily with large volumes [11,12]. However, challenges exist as the concentration levels of steroids in raw urine are very low [13]. Mass spectrometry is a dominant analytical tool for the analyses of doping compounds as it can accurately identify compounds based on their mass to charge ratios. Gas chromatography–mass spectrometry (GC–MS) is one of the most common and widely applied analytical techniques used for the detection of urinary steroids [13–16]. In order to enhance the volatility, ionization efficiency and detection limit, raw urinary samples need to undergo various sample pre-processing steps like hydrolyses, derivatization, pre-concentration etc. before injecting to the GC–MS system. These procedures are tedious, time consuming and need expert personnel to carry-on. Liquid chromatography–mass spectrometry (LC–MS) has also been used for the detection of steroids from urine [17–20]. But in this case also liquid–liquid extraction (LLE) or solid-phase extraction (SPE) is required for sample cleaning, pre-concentration and derivatization for increasing the ionization efficiency. On the other hand, ambient mass spectrometry (MS) is another choice where samples are being analyzed in high speed and specificity without or with minimal sample pre-treatment [21,22]. In 2007, reactive desorption electrospray ionization (DESI) was reported for the high throughput screening of anabolic steroids in urine [23]. Among various other mass spectrometric techniques, solvent assisted inlet ionization [24] and capillary photoionization [25] are significant for the high sensitivity screening of steroid molecules.

In this article, we report a new strategy for direct and high sensitivity analysis of anabolic steroids from urine with minimal pre-treatment using Leidenfrost phenomenon assisted thermal desorption (LPTD) coupled to dielectric barrier discharge ionization (DBDI) MS at open atmosphere. Earlier, we demonstrated that LPTD is a suitable desorption technique for high sensitivity detection of analytes based on their molecular ions and could be coupled with positive and negative mode MS operation [26,27]. In this technique a liquid droplet containing analyte is placed on a heated metallic sample holder situated in front of the inlet of mass spectrometer. The temperature of the sample holder must be higher than the Leidenfrost temperature of the liquid. Under this condition the liquid droplet levitates on the metallic surface for few seconds to minute based on the liquid's characteristic, metallic holder's temperature, and droplet volume and a slow solvent evaporation takes place. During the solvent evaporation process, the analyte molecules are enriched inside the liquid droplet and released at the final stage of droplet evaporation in a short time domain. Thus, the sensitivity of this technique becomes several times higher compared to the thermal desorption at a lower temperature. The desorbed neutral molecules are ionized in a post-ionization method at open atmosphere using DBD ion-source before entering the mass spectrometer inlet. The method was validated in terms of limit of detection (LOD), precision, linear range, correlation coefficient of linearity and recovery. Further, we coupled this whole process with a simple dichloromethane extraction technique for getting better sensitivity and reproducibility from urine sample. This is a rapid method and a single sample analysis time is around one minute.

2. Experimental

2.1. Reagents and solvents

Standard anabolic steroid samples (androstadienedione, androsterone hemisuccinate, 6-dehydrocholestenone, epitestoterone and stigmastadienone) were purchased from Steraloids Inc. (Newport, RI, USA). HPLC grade methanol and dichloromethane were purchased from Wako Pure Chemical Industries Ltd. (Osaka, Japan) and Cica-Reagent (Tokyo, Japan). The water was purified with a Milli-Q purification system (Millipore, Bedford, MA, USA). All of the chemicals used for the preparation of artificial urine were obtained from Cica-Reagent (Tokyo, Japan), except bilirubin, D-glucuronic acid and human albumin, which were obtained from Sigma–Aldrich (St. Louis, MO, USA).

2.2. Sample preparation

Stock solutions of all anabolic steroids (1 mg mL^{-1}) were separately prepared in methanol and stored in a refrigerator at −30 °C. Working standard solutions were prepared just before the experiments and dilutions were made using methanol. 'Artificial urine' was used to obtain the analysis data of anabolic steroids from urine. Artificial urine contains the major components present in human urine and prepared using the same procedure reported by Jacob et al. [28]. Water was used as a matrix for artificial urine and the concentrations of different components are as follows: ammonium sulfate (1.0 mg mL^{-1}), andosterone (0.02 mg mL^{-1}), ascorbic acid (0.02 mg mL^{-1}), bilirubin (0.005 mg mL^{-1}), citric acid (0.5 mg mL^{-1}), creatine (0.5 mg mL^{-1}), creatinine (1.5 mg mL^{-1}), cystine (0.1 mg mL^{-1}), glucuronic acid (0.5 mg mL^{-1}), hippuric acid (1.0 mg mL^{-1}), histidine (0.5 mg mL^{-1}), human albumin (0.1 mg mL^{-1}), magnesium sulfate (1.0 mg mL^{-1}), phenol (0.5 mg mL^{-1}), potassium phosphate monobasic (1.0 mg mL^{-1}), sodium chloride (10 mg mL^{-1}), urea (15 mg mL^{-1}), and uric acid (0.5 mg mL^{-1}). After preparation, the artificial urine was stored at −30 °C. The standard anabolic steroids were spiked in artificial urine just before the experiments. For the preparation of the working standard of urine, 10 μL of the diluted stock solutions of anabolic steroids were spiked in 1 mL of artificial urine. All of the experiments reported in this paper were performed using artificial urine.

2.3. Experimental setup

A home-made DBD ion-source was used for the ionization of the desorbed molecules. The ion source was placed in front of the mass spectrometer inlet in such a way that the desorbed molecules are ionized at open atmosphere before entering to the first vacuum chamber of the mass spectrometer. The detailed experimental set-up is illustrated in Fig. 1 and is similar to the reported one in our previous papers [26,27]. In short, the stainless steel sample holder was placed below the inlet of the mass spectrometer and the DBD ion-source made of glass tube with 3.0 mm o.d. and 1.5 mm i.d..