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Feasibility of desorption atmospheric pressure photoionization and desorption electrospray ionization mass spectrometry to monitor urinary steroid metabolites during pregnancy

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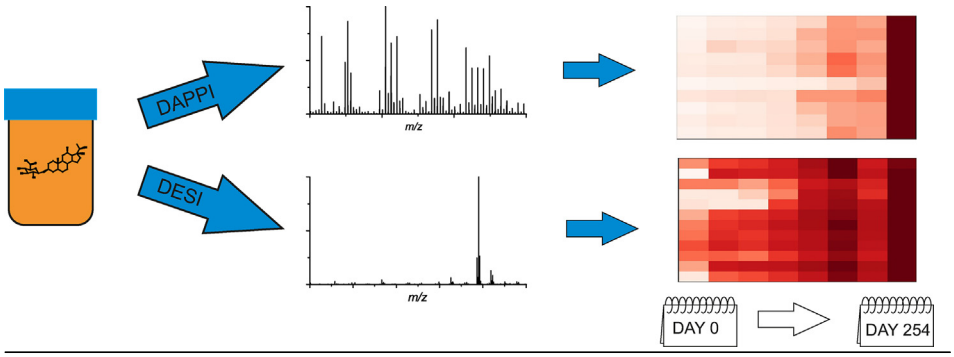
HIGHLIGHTS

- Fast analysis of urinary steroids of pregnant women by ambient MS.
- DAPPI showed increase of C19 and C21 steroids during the progress of pregnancy.
- DESI detected increase of C18 and C21 steroid glucuronide and sulfate conjugates.
- Similar steroid ion fingerprints were found for different women.
- Both techniques show promise for steroid marker screening in pregnancy.

GRAPHICAL ABSTRACT



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ABSTRACT

Steroids have important roles in the progress of pregnancy, and their study in maternal urine is a non-invasive method to monitor the steroid metabolome and its possible abnormalities. However, the current screening techniques of choice, namely immunoassays and gas and liquid chromatography–mass spectrometry, do not offer means for the rapid and non-targeted multi-analyte studies of large sample sets. In this study, we explore the feasibility of two ambient mass spectrometry methods in steroid fingerprinting. Urine samples from pregnant women were screened by desorption electrospray ionization (DESI) and desorption atmospheric pressure photoionization (DAPPI) Orbitrap high resolution mass spectrometry (HRMS). The urine samples were processed by solid phase extraction for the DESI measurements and by enzymatic hydrolysis and liquid–liquid-extraction for DAPPI. Consequently, steroid glucuronides and sulfates were detected by negative ion mode DESI–HRMS, and free steroids by positive ion mode DAPPI–HRMS. In DESI, signals of eleven steroid metabolite ions were found to increase as the pregnancy proceeded, and in DAPPI ten steroid ions showed at least an order of magnitude increase

Abbreviations: A-G, androsterone glucuronide; DAPPI, desorption atmospheric pressure photoionization; DESI, desorption electrospray ionization; D-S, DHEA sulfate; D_3-T, D_3-testosterone; HRMS, high resolution mass spectrometry; QqQ, triple quadrupole; T, testosterone; T-G, testosterone glucuronide.

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Steroid Metabolism

during pregnancy. In DESI, the increase was seen for ions corresponding to C18 and C21 steroid glucuronides, while DAPPI detected increased excretion of C19 and C21 steroids. Thus both techniques show promise for the steroid marker screening in pregnancy.

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1. Introduction

Steroids play important roles in pregnancy by supporting and maintaining the gestation [1,2] and participating in fetal development [1] and parturition. The placenta and fetal adrenal produce estrogen, progesterone, glucocorticoids, C19 steroids and their metabolites in large amounts, and the circulating and excreted levels of the steroids and their metabolites are increased in pregnant women [3–7]. Adequate circulating steroid levels are essential for successful pregnancy, as artificially induced changes of the steroid hormone concentrations and metabolism can lead to severe complications, including pre-term birth [8], and on the other hand, can also prevent pre-term births [9]. Thus it is of great interest to study if the steroid metabolome could be used to predict complications of pregnancy and the risk for pre-term birth. Due to the non-invasiveness and ease of collection, maternal urine represents a feasible matrix for large scale screening studies. For example, the steroid metabolite profile in maternal urine has been reported as a suitable diagnostic tool for identification of intrahepatic cholestasis of pregnancy [10], and Smith–Lemli–Opitz syndrome [11], as well as determining success of in vitro fertilization [12]. A recent study from our group gave also preliminary evidence that contractions may be predicted by studying steroid metabolites in urine [7].

Classically, steroid (metabolome) screening has relied on immunoassays (IA), but with the development of analytical systems, gas chromatography–mass spectrometry (GC–MS) [13] and liquid chromatography–mass spectrometry (LC–MS) have become tools of choice in steroid profiling and steroid biomarker discovery [14–17]. Direct IAs provide low cost and high throughput analyses and are therefore commonly used. Nevertheless, they are not always able to provide sufficient specificity [18–20], suffer from matrix effects [19], and are unable to monitor multiple compounds by a single analysis, and therefore they are ceasing to meet the improving standards of high quality clinical steroid analyses and research [21]. GC–MS and LC–MS on the other hand are selective, and can produce quantitative information on hundreds of molecules in a single run [13–16]. When GC or LC is combined with high resolution MS (HRMS) or, e.g., a neutral loss or precursor ion scan, they can also be used for non-targeted analyses to find previously unknown metabolites [14], which are unattainable for IAs. However, typical GC–MS analysis of steroids requires extensive sample preparation: clean-up, concentration, hydrolysis of conjugated steroids, and derivatization of non-volatiles. LC–MS also requires sample clean-up, but can be used for the separation of conjugated steroids in their native forms [22], and it does not necessarily require analyte derivatization making it faster compared to GC–MS. Taking also into account the runtime of the chromatographic separation, GC– and LC–MS methods are generally time consuming and laborious compared to IAs. When ultimate speed is needed, automated flow injection analysis (FIA) offers the benefits of mass spectrometric analyses. However, the automation relies typically on LC injectors, which are relatively slow due to wash cycles needed to prevent carry-over. Therefore, at present, there is demand for rapid multi-analyte screening methods for steroid fingerprinting for the identification of potential biomarkers and population level screening for abnormal steroid concentrations.

In this study, we test the feasibility of two ambient mass spectrometry methods for the rapid screening of urinary steroids during pregnancy to provide an alternative to typical steroid screening methods. Ambient mass spectrometry is a family of techniques, which enable mass spectrometric analyses lasting from a few to tens of seconds [23–25]. The techniques desorb analytes from a solid surface, such as a droplet of dried urine extract on a solid support, and ionize the desorbed molecules by atmospheric pressure ionization methods such as electrospray, chemical ionization or atmospheric pressure photoionization. Unlike IAs, ambient MS methods are able to detect many steroid species simultaneously in a single run and screen for untargeted species. However, isomeric and isobaric molecules can be resolved by ambient MS only if they have different fragmentation patterns in MS^n measurements or if MS is interfaced with ion mobility separation [26,27]. Ambient MS can be automated to provide extremely rapid analyses compared to LC–, GC– or even FIA–MS, and due to the very low sample consumption, the same samples can be subsequently re-analyzed using GC– or LC–MS to confirm the findings and to quantitate the detected metabolites. Previously, ambient MS methods have been tested in steroid analysis for doping control purposes [28–30]. However, to our best knowledge, the feasibility of ambient MS in the screening of endogenous steroids in urine has not been previously investigated. Here, we use desorption electrospray ionization (DESI) [31] to monitor steroid glucuronides and sulfates, and desorption atmospheric pressure photoionization (DAPPI) [32] to monitor total steroids (free + conjugated after hydrolysis) in pregnancy urine samples. The results are evaluated by comparing them with previously published LC–MS data from our laboratory [7] on the same sample set. Since DESI and DAPPI analysis of raw urine has been reported to result in MS instrument contamination [33], the samples were cleaned and concentrated with solid phase extraction (SPE) for the DESI analyses and liquid–liquid-extraction (LLE) for DAPPI following typical sample preparation procedures reported in literature for steroid conjugates and hydrolyzed steroids, respectively. An Orbitrap was used for the HRMS scan analyses, and the tentative identifications of DESI–HRMS were complemented by precursor ion scan measurements with a triple quadrupole (QqQ) MS.

2. Materials and methods

2.1. Chemicals

Acetone (≥99.8%), methanol (LC–MS Chromasolv®), ammonium hydroxide (28.0–30.0% NH_3 basis), D_3-testosterone (D_3-T, 17β-hydroxyandrost-4-en-3-one-16,16,17-D_3, 98 atom% D), pregnenolone-20,21-$^{13}C_2$-16,16-D_2 sulfate sodium salt ($^{13}C_2D_2$Preg-S, 98 atom% D, 99 atom% ^{13}C, 98% (CP)), acetic acid (≥99.85%), type HP-2 β-glucuronidase from *Helix pomatia* (with primary glucuronidase and secondary sulfatase activity), diethyl ether (≥99.8%), ammonium acetate (≥98%), DHEA sulfate (D-S, 5-androsten-3β-ol-17-one sulfate sodium salt dihydrate, ≥98%), testosterone glucuronide (T-G, 4-androsten-17β-ol-3-one 17-glucuronide), and androsterone glucuronide (A-G, 5α-androstan-3α-ol-17-one glucuronide) were purchased from Sigma–Aldrich (Steinheim, Germany). Stock solutions of the standards (1 mg mL^{-1}) were prepared in methanol. Potassium acetate and K_2CO_3 were from

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