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Supported liquid extraction coupled to gas chromatography-selective mass spectrometric scan modes for serum steroid profiling

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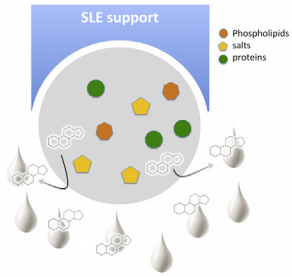
HIGHLIGHTS

- GC-MS-based analysis for different types of serum steroids was evaluated.
- All 37 steroids were measured in SIM and SRM simultaneously.
- Supported liquid extraction (SLE) ensured a good chromatographic selectivity.
- The validated method showed sex and age dependent changes of mouse serum steroids.

GRAPHICAL ABSTRACT



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ABSTRACT

Although, steroid profiling is being applied in clinical and biochemical studies, improvement of the technique is still needed for accurate quantification of steroids in case of limited biological sample volumes. To improve analytical sensitivity and selectivity in comparison to that of conventional methods, a method that comprises supported liquid extraction (SLE) and gas chromatography-mass spectrometry with a combination of selected-reaction and selected-ion monitoring modes (GC-SRM/SIM-MS) was developed. Here, this combination of SLE purification with GC-MS method was optimized with 37 different types of steroids and the results were compared to a solid-phase extraction (SPE) method. The devised assay led to an increase in extraction efficiency with the good chromatographic selectivity through a single extraction step. The limits of quantification of the serum steroids, ranged from 0.2 to 5 ng mL^{-1}, except for cholesterol (0.2 μg mL^{-1}), and the correlation coefficients for calibration curves were higher than 0.99. The precision and accuracy were 1.4%–10.5% and 82.7%–115.3%, respectively. The overall recoveries of 30 steroids ranged from 62.1% to 104.3%, while that of 7 sterols was 44.7%–75.7%. Then, this validated method was applied to monitor the serum steroid levels of mice, which showed significant sex and age dependent metabolic patterns. This technique can be used to evaluate the metabolic changes occurring in animal models as well as in clinical patients.

1. Introduction

Steroid hormones mediate a wide variety of important physiological roles, such as reproductive functions, aging process, inflammatory and stress response, as well as behavior, cognition, and

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mood [1,2]. They are synthesized in the adrenal glands, gonads, and some peripheral tissues, and are secreted into the bloodstream [3]. Steroid profiling provides quantitative information on the steroids that are involved in metabolic pathways (Fig. 1), and it has been applied to clinical and reproductive biological studies on humans as well as animal models [4–8]. Apart from humans, animal models, such as mouse, have been used for identification of the risk factors of diseases, and to monitor the effect of radiation exposure or brain injury, which cannot be achieved in humans [9–11]. However, very little has been known about the serum steroid levels in mouse models as compared to that in humans, as conventional analytical techniques require large volumes of blood samples. Therefore, technological improvements are necessary to accurately measure the low levels of serum steroids in animal experiments.

Owing to its high sensitivity and selectivity, mass spectrometry (MS) coupled to chromatographic separation method has been extensively used for steroid analysis in biological and clinical applications [4–14]. As the advancement of MS methods occurred, gas chromatography (GC) or liquid chromatography (LC) coupled to tandem mass spectrometry (MS/MS) have emerged for the analysis of steroids even at trace levels from complex biological specimens [4,7,11,15–17]. Although, GC methods need derivatization steps prior to instrumental analysis, the excellent chromatographic resolution makes it valuable [18]. In recent, this highly sensitive and specific GC-MS/MS method was used to evaluate steroids in small volumes of samples from humans [19,20] and experimental animals [21]. To expand the application of GC-MS technique in steroid profiling, we compared analytical sensitivity of all steroids analyzed in this study between selected-ion monitoring (SIM) and selected-reaction monitoring (SRM) modes to optimize the compound-selective MS scanning modes in a single run, that may provide the comprehensive screening assay [17].

In addition to the advancement of MS technologies, many extraction methods have been developed based on protein precipitation (PPT), liquid-liquid extraction (LLE), and solid-phase extraction (SPE) [22,23]. PPT is commonly used as a simple method for protein removal; however, phospholipids remain in the organic solvents [22]. In case of silica- or polymer-based SPE methods, such as C18 or Oasis HLB cartridges, they have a very broad range of application for steroid analysis [4–11,13,17,19–21]. However, conventional SPE methods are time-consuming and labor-intensive due to multiple steps and an additional LLE process is required prior to GC-MS analysis [5,6,10,13]. In recent studies, supported liquid extraction (SLE) that uses diatomaceous earth was introduced for aldosterone and vitamin D analysis [24,25]. SLE is a flow-through technique that has advantages such as rapid and simple purification as well as effective removal of proteins and phospholipids [22], which could be attained by performing only one-step purification.

Here, we developed the newly proposed SLE purification technique combined with selective GC-SIM/SRM-MS method in steroid profiling assay to allow comprehensive quantification of 37 various steroids, which include 6 androgens, 2 estrogens, 13 progestins, 9 corticoids, and 7 sterols from 100 μL of serum samples. Due to various analytical parameters in MS scan methods and sample purifications were considered and maximized for improved analytical efficiencies, the present study may provide valuable information in steroid profiling analysis and it was also successfully applied to quantify serum steroid levels between female and male mice.

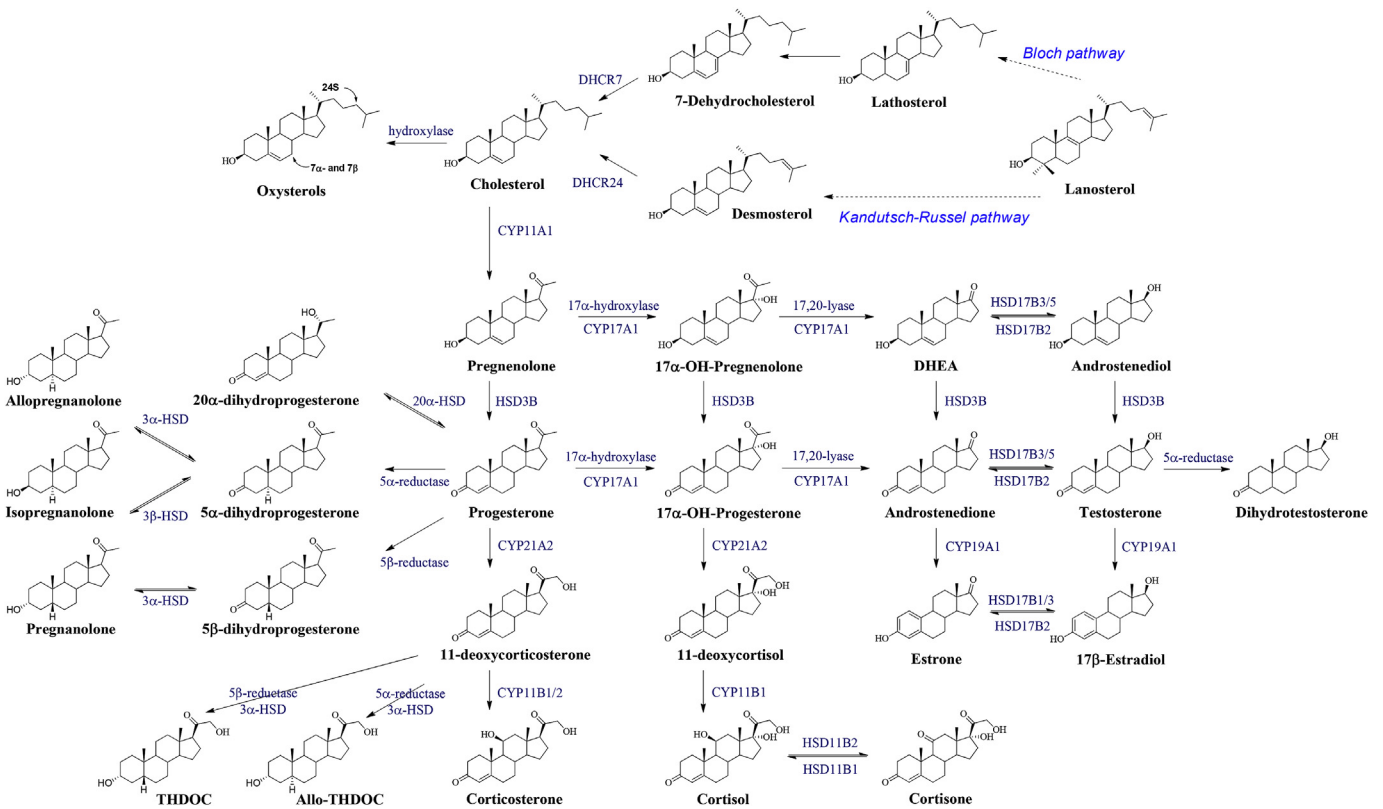

Fig. 1. Representative metabolic pathways of steroids studied.

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