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Cyclodextrin based polymer sorbents for micro-solid phase extraction followed by liquid chromatography tandem mass spectrometry in determination of endogenous steroids

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ABSTRACT

Sorbents were prepared by cross-linking β-cyclodextrin (β-CD) using two different types of cross-linker units at variable reactant mole ratios. The resulting polymers containing β-CD were evaluated as sorbents in micro-solid phase extraction (μ-SPE) format for the extraction of the endogenous steroids testosterone (T), epitestosterone (E), androsterone (A), etiocholanolone (Etio), 5α-androstane-3α,17β-diol (5αAdiol) and 5β-androstane-3α,17β-diol (5βAdiol). The best sorbent (C1; cyclodextrin polymer) showed superior extraction characteristics compared with commercial sorbents (C18 and Bond Elut Plexa). Parameters influencing the extraction efficiency of the C1 sorbent such as extraction and desorption times, desorption solvent and volume of sample were investigated. The extracts were separated using a Hypersil Gold column (50 × 2.1 mm, 1.9 μm) under gradient elution coupled to a LC–MS/MS. The compounds were successfully separated within 8 min. The method offers good repeatability (RSD < 10%) and linearity ($r^2 > 0.995$) were within the range of 1–200 ng mL^{-1} for T and E, 250–4000 ng mL^{-1} for A and Etio and 25–500 ng mL^{-1} for 5αAdiol and 5βAdiol, respectively. The method was applied for the determination of steroid profile of urine from volunteers.

1. Introduction

Steroids are non-polar compounds that regulate metabolism via the endocrine system. They play important roles in physiological functions such as physical development, sexual maturation, metabolic homeostasis, etc [1–3]. "Steroid profile" in sports is composed of testosterone (T, 4-androstene-17β-ol-3-one), epitestosterone (E, 4-androstene-17α-ol-3-one), androsterone (A, 5α-androstane-3α-ol-17-one), etiocholanolone (Etio, 5β-androstane-3α-ol-17-one), 5α-androstane-3α,17β-diol (5αAdiol), 5β-androstane-3α,17β-diol (5βAdiol) and the ratio of T to E (T/E) (as free steroid content obtained from the free steroid fraction plus those released from the conjugated fraction on hydrolysis by glucuronidase) [4] (Fig. 1). This parameter is one of the World Anti-Doping Agency (WADA) requirements as routine testing of urine of athletes [5]. T and its precursors are one of the most abused classes of doping substances in sports. According to WADA Anti-Doping Violations Report in 2015, about 50% of positive tests for banned substances were related to AAS [6]. In the fight against drug abuse among elite athletes, since 2014, WADA has approved the so called "Steroid Module" of the Athlete Biological Passport, meant as a record over time of the steroid profile of each athlete. This approach, together with random testing and advancements in laboratory testing techniques appears to be effective in the prevention and detection of steroid use among athletes [6].

In clinical studies, measurement of steroids is useful for diagnosing endocrine disorders such as congenital adrenal hyperplasia, Cushing's syndrome, Alzheimer disease [7], inherited human dis-

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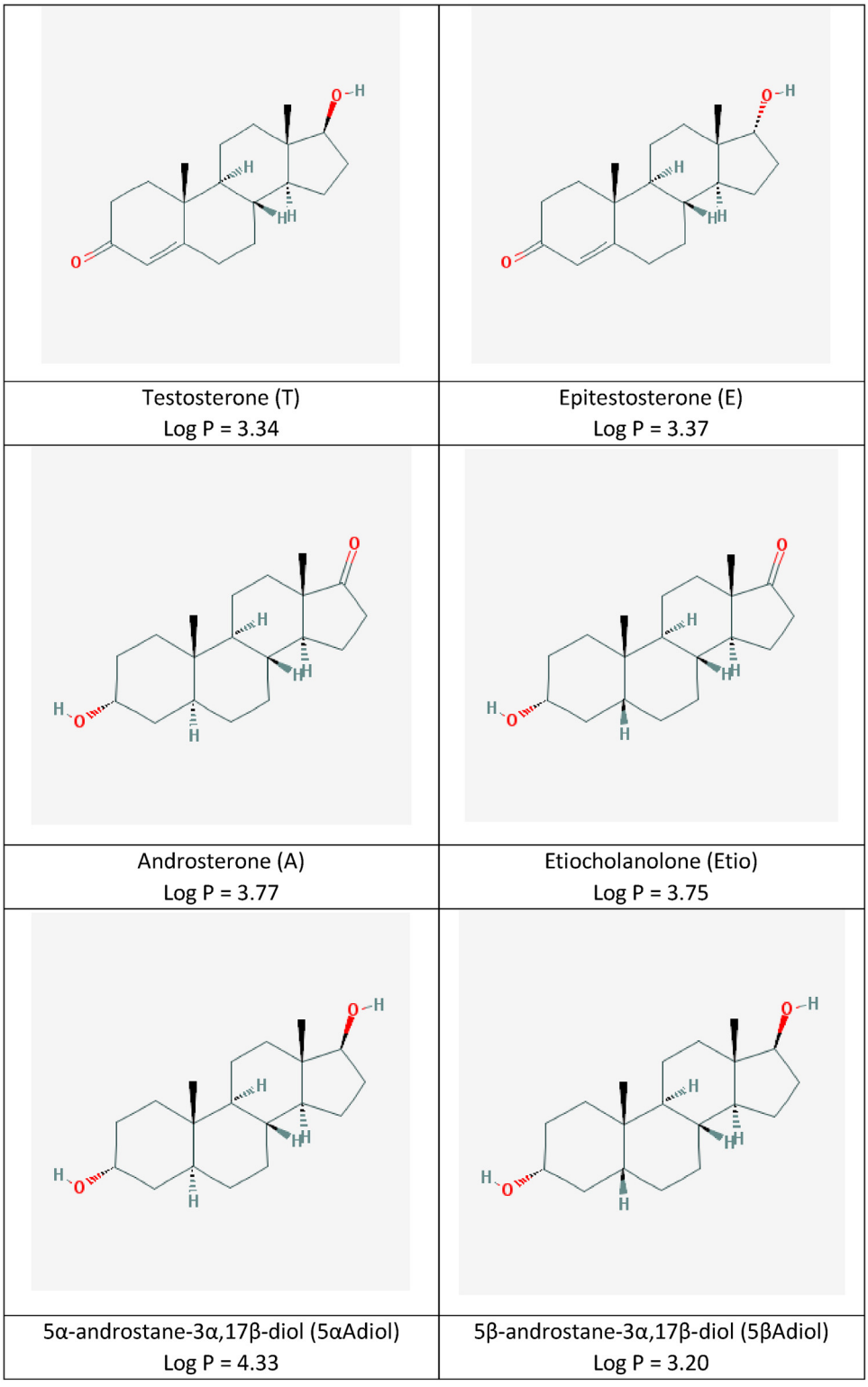

Fig. 1. Chemical structures of the steroids studied herein.

eases [8] and cancers [9,10]. Primary liver cancer occurrence and development are also closely linked to steroid hormones. The quantification of steroid hormones in patients with primary liver cancer is promising for early disease diagnosis [9]. Information on steroids is also required for veterinary growth promoter investigations [11], as well as for environmental studies [12–14]. Additionally, some members of the general public use steroids that are purchased through the internet and illicit sites to improve physical appearance or to enhance performance in recreational and non-competitive sports [6].

Due to the numerous areas that need steroid information, it is pertinent that suitable analytical methods are available. Radioimmunoassay (RIA) and enzyme linked immunoassay (ELISA) used to be the accepted standard techniques for the routine measure-