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Review

Liquid chromatography methodologies for the determination of steroid hormones in aquatic environmental systems

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ABSTRACT

Steroid hormones are a diverse group of natural and synthetic compounds. Their wide use in human and veterinary medicine results in their continual introduction into the environment. In recent years, environmental concern over steroids that act as endocrine disruptors has increased because of their adverse effects on organisms or their progeny. Moreover, as these compounds are not totally removed from sewage in wastewater treatment plants, they can reach the aquatic environment and persist due to their physicochemical characteristics.

For this reason, a major trend in analytical chemistry is the development of rapid and efficient procedures for the extraction, determination and quantification of steroid hormones in environmental samples. Over the past few decades, the significant expansion of liquid chromatography technology utilizing mass spectrometry detection has led to applications with increased selectivity and sensitivity. Optimized extraction and microextraction techniques combined to these liquid chromatography techniques have lowered detection and quantification limits to the ng L^{-1}–μg L^{-1} range, which is the concentration of steroid hormones in liquid, solid and biota samples.

In this paper, the state-of-the-art techniques for the analysis of steroid hormones focused mainly in based liquid chromatography methods in liquid and aquatic solid and biota samples are reviewed. Handling, storage, extraction and detection methodologies are reviewed and compared for all families of steroid hormones.

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Abbreviations: ACN, acetonitrile; APCI, atmospheric pressure chemical ionization; APPI, atmospheric pressure photoionization; ASE, accelerate solvent extraction; BEH, bridge ethyl hybrid; CPE, cloud point extraction; DAD, diode array detection; DLLME, dispersive liquid–liquid microextraction; DMAE, dynamic microwave assisted extraction; EDCs, endocrine disruptor compounds; ESI, electrospray ionization; GC, gas chromatography; GH/IGF-I, growth hormone and insulin-like growth factor I; H2SO4, sulphuric acid; HCl, hydrochloric acid; LC, liquid chromatography; LLE, liquid–liquid extraction; MAE, microwave assisted extraction; MeOH, methanol; MS, mass spectrometer; MSPD, matrix solid phase dispersion; PLE, pressurised liquid extraction; QuEChERS, quick easy cheap effective rugged safe; SBSE, stir bar sorptive extraction; SFO, solidification of a floating drop; SPE, solid phase extraction; UAE, ultrasonic assisted extraction; UHPLC, ultra-high performance liquid chromatography; USEPA, United States Environmental Protection Agency; UV, ultraviolet–visible; WHO, World Health Organization; WWTP, wastewater treatment plant.

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1. Introduction

The quality of life on earth is intimately linked to the quality of the environment. In many countries, marine, terrestrial and aerial environmental problems; the increasing demand for water and; the constant discovery of potentially dangerous pollutants are serious issues. This situation necessitates research in all areas related to the protection of human health and the sustainable use of natural resources. The presence of emergent pollutants in the environment is one of the main issues concerning organizations committed to public and environmental health, such as the World Health Organization (WHO), the United States Environmental Protection Agency (USEPA) and the European Commission [1].

Many current pollution issues stem from intermittent or continuous leakage of chemical substances into the environment. The toxicity of these compounds to flora, fauna and humans has been demonstrated in several recent works [2,3]. Key amongst environmental contaminants are the endocrine disruptor compounds (EDCs). EDCs are a large group of natural and synthetic compounds that are defined as exogenous substances or mixtures that alter functions of the endocrine system and consequently cause adverse health effects in an intact organism, its progeny, or subpopulations [4,5].

Steroid hormones are a wide group of biologically active endocrine disruptor compounds that control many functions of endocrine systems. The main characteristic of steroid hormones is their cyclopentane-perhydro-phenanthrene structure, also known as sterane, derived from cholesterol. Partial removal of the cholesterol side chain results in the 21-carbon progestogens or gestagens and corticosteroids. The complete removal of the side chain provides 19-carbon products, called androgens. Finally, additional removal of the C-19 methyl group by aromatization produces 18-carbon compounds, known as estrogens. The names, acronyms and structures of the hormones reviewed in this paper are listed in Fig. 1a–d.

Steroid hormones can be divided into five subgroups based on structural characteristics: estrogens, androgens, progestogens, glucocorticoids and mineralcorticoids [6]. Within these groups, there are both natural and synthetic hormones. Natural steroids are mainly synthesized in the sex organs, adrenal glands and placenta.

Estrogens are female sex hormones, which are also present in males, and they control many physiological activities, such as growth and reproduction. The global consumption of estrogens has increased exponentially over the last 50 years due to their use as contraceptives, menopausal therapies, as treatment for some human cancers and as veterinary growth promoters. Estrogens are now considered to be the most prescribed drugs in the world [7]. Estrogens are subject to metabolic hydroxylation, oxidation and methylation and undergo biotransformation to sulphates and glucuronides. However, a significant amount of estrogens are excreted from the organism as free estrogens or conjugates. For example, after excretion, 17β-estradiol is rapidly oxidised to estrone, which can be transformed into estriol. Conversely, 17 α-ethynylestradiol is excreted as a conjugate in most cases [8]. There are several synthetic estrogens, such as diethylstilbestrol, that are not steroid hormones because they do not present the steroid structure. However, their characteristics and behaviors are similar to steroid estrogens and they are often studied together.

Progestogens, also known as gestagens, present a 21-carbon pregnane structure. They are called progestins because of their role in maintaining pregnancy, but they are also present in other phases of oestrous and menstrual cycles. As with estrogens, there are natural and synthetic progestogens. Gestagens are used in the treatment of several cancers and some menstrual disorders, but their main use is as an anticonceptive, sometimes in conjunction with estrogens. The observation that progesterone suppresses ovulation during pregnancy led to its development as a contraceptive. To minimize progesterone-related complications in contraceptive drugs, progestins were developed; norethisterone, was one of the first oral contraceptives sold in the United States [9].

Androgens are male sex hormones, and their main function is the control of male sexual characteristics. The three main androgens are testosterone, androsterone and dihydrotestosterone. Many analogues of these compounds have been developed as therapeutics and as anabolic substances, promoting muscular growth [10]. Their use as anabolic compounds has been highly controversial due to associated health problems. In children, anabolic compounds can result in growth retardation and precocious puberty. In veterinary medicine androgens, such as testosterone or trembolone acetate, have been widely used as growth promoters in cattle farming [11].

Glucocorticoids are a group of hormones excreted by the adrenal cortex (cortisol, cortisone) and are synthetically produced (dexamethasone, triamcinolone). They control the energy supply through gluconeogenesis and suppress stress-related inflammation and infection [12], making them useful in the treatment of several allergies, asthma, and arthritis or polymyalgia rheumatica [13]. Natural glucocorticoids are excreted as free glucocorticoids or conjugated glucocorticoids in both urine and dregs. In veterinary medicine, they are used as androgens to restore muscle strength and size.

Hormones can be of endogenous or exogenous origin. Biosynthetic steroid hormones are endogenous, while xenobiotic or exogenous steroids are foreign compounds, which are naturally or synthetically produced. Steroid hormones excreted by humans reach the aquatic environment daily via sewage systems, and several authors have stated that municipal wastewaters are the main source of contamination of aquatic environments [14,15]. This contamination is due to the stability, resistance to microbial degradation, and bio availability of hormonal compounds [16]. Moreover, industrial wastewaters are a source of hormonal contamination as well. Microbial degradation of phytosterols to androgens has been proposed as a natural source of androgenic steroids, which could explain elevated concentrations of androgenic activity downstream from paper mills [17].

Sumpter and Jobling's [18] finding in 1995, that municipal wastewater discharges induce physiological responses in fish, which is indicative of exposure to estrogens, has led to an increase in studies on the effects of hormonal compounds in biota [19]. Low concentrations ($50\,ng\,L^{-1}$) of ethynylestradiol, for example, have been linked to diminished reproduction in zebra fish [20], while changes in the production of vitellogenin in trout [21] has been observed at concentrations of $1\,ng\,L^{-1}$ of 17β-estradiol or its equivalents. Also, environmental concentrations of ethynylestradiol have been found to interfere with the GH/IGF-I system in bony fish [22].