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Review

Determination of steroid hormones in biological and environmental samples using green microextraction techniques: An overview

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ABSTRACT

Residues of steroid hormones have become a cause for concern because they can affect the biological activity of non-target organisms. Steroid hormones are a potential risk for wildlife and humans through the consumption of contaminated food or water. Their determination requires extraction and clean-up steps, prior to detection, to reach low concentration levels. In recent years, a great effort has been made to develop new analytical methodologies, such as microextraction techniques, that reduce environmental pollution. Researchers have modified old methods to incorporate procedures that use less-hazardous chemicals or that use smaller amounts of them. They are able to do direct analysis using miniaturised equipment and reduced amounts of solvents and wastes. These accomplishments are the main objectives of green analytical chemistry. In this overview, we focus on microextraction techniques for the determination of steroid hormones in biological (e.g., human urine, human serum, fish, shrimp and prawn tissue and milk) and environmental (e.g., wastewaters, surface waters, tap waters, river waters, sewage sludges, marine sediments and river sediments) samples. We comment on the most recent applications in sorptive-microextraction modes, such as solid phase microextraction (SPME) with molecularly imprinted polymers (MIPs), in-tube solid-phase microextraction (IT-SPME), stir-bar sorptive extraction (SBSE) and microextraction in packed sorbent (MEPS). We also describe liquid-phase microextraction (LPME) approaches reported in the literature that are applied to the determination of steroid hormones.

Jana Aufartová received her Master Degree in Pharmacy in 2008 at Charles University in Prague (Czech Republic). Since that year, she is working as a Ph.D. student under the guidance of Professor Solich and currently under the guidance of Professors Santana Rodríguez and Sosa-Ferrera at University of Las Palmas de Gran Canaria (ULPGC) (Spain). Her research is focused on development of new methodologies for the extraction, preconcentration and clean-up in the determination of pharmaceuticals (mainly fluoroquinolones and steroids) in environmental samples.

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Petr Solich is currently Professor and Head of the Analytical Chemistry Department at the Faculty of Pharmacy, Charles University in Hradec Králové, Czech Republic. His research interests are automation of analytical procedures; flow methods (flow injection analysis, sequential injection analysis and sequential injection chromatography); chromatographic methods (mainly UHPLC with sub-2-micron columns or use of monolithic columns) applied to environmental analysis of low concentrations of pharmaceuticals or bioanalytical analysis for determination of biomarkers and modern sample-preparation methods. He has published more than 140 research papers and has been responsible for more than 20 research grants from different disciplines (pharmaceutical, environmental, and bioanalytical).

1. Introduction

1.1. Background

In the last few decades, the amount of chemicals released into the environment has increased considerably. Among these compounds, hormone residues have become a cause for concern because they can affect the biological activity of non-target organisms. They are a potential risk for wildlife and humans through the consumption of contaminated food or water. The occurrence of chemical compounds influencing the sexual development of fish in English rivers was reported 15 years ago [1]. These exogenous substances that interfere with the endocrine system and disrupt the physiologic function of hormones are called endocrine-disruptor compounds (EDCs). The effects of natural and synthetic EDCs found in the environment include decreasing sperm count in human males, increasing breast cancer in women and causing reproductive abnormalities in humans [2,3]. The most potent active EDCs present in the environment belong to the chemical class of steroids, which are formed naturally by humans and wildlife or produced synthetically. Steroid hormones that are biosynthetically present in the body are called endogenous hormones, and exogenous steroids are foreign compounds, either naturally or synthetically produced. In addition to this classification, they can also be classified by their chemical structure and their pharmacological effects. Using these criteria, steroid hormones can be generally divided in three groups: estrogens, gestagens and androgens [4,5].

Regarding chemical structure, steroids are comprised of a skeleton of three cyclohexal carbon rings and one pentagonal carbon ring, which are generally arranged in a 6-6-6-5 structure to which various functional groups and side chains are attached. All steroids can be derived from cholesterol. Table 1 shows some examples of steroid hormones and their parent compound, cholesterol. The three main natural estrogens, estrone (E1), estradiol (E2) and estriol (E3), are C_{18} steroids that differ in the oxidation state of their rings. These C_{18} steroids stimulate the development of female reproductive structures and secondary sexual characteristics. Synthetic estrogens, such as ethinylestradiol (EE2) or mestranol (MES), are derived from E2. Because of their anabolic effects, estrogens have been used in animal fattening. Gestagens, also called progestagens, are derived from the C_{21}-steroid pregnane such as progesterone (P). Androgens are C_{19} steroids that stimulate or control the development of masculine characteristics. The most well-known androgen is testosterone (T). Natural and synthetic androgens, like all groups of steroids, have been used as growth promoters and in human and veterinary therapies. Because of their myotrophic action, anabolic androgenic steroids, including testosterone, have been widely used by athletes to improve athletic performance.

The occurrence of hormone residues has been increasingly reported in wastewater [6–9], surface waters and groundwaters [10–13] and even drinking water [14–16]. The occurrence is especially significant in places near influents and effluents of wastewater treatment plants (WWTPs). WWTPs are considered to be one of the principal sources of hormone contamination because they do not completely remove these compounds [17,18]. In general, the natural estrogenic steroids E1, E2 and E3 are often detected in water samples, while their conjugated forms or synthetic steroids, EE2 and MES, are detected only sporadically. Concentrations of estrogens in treated wastewater normally do not exceed a few $ng\,L^{-1}$, but values of $51\,ng\,L^{-1}$ for E1 have been reported [19]. The concentration of E1 in urine is approximately twice that of E2 or E3 [20]. This fact, combined with the biodegradation of E2 to E1 by oxidation (e.g., in WWTPs [21]), means that greater amounts of E1 can be expected in wastewater and surface water. The levels of gestagens in surface water, wastewater and sediments are in the same range as those of estrogenic steroids. Androgenic steroids in the aquatic environment originate from WWTPs effluents from paper mills and livestock-breeding operations. The androgenic steroids that are typically identified in WWTPs are testosterone and its metabolites or precursors of male and female hormones [22–24]. Influents of WWTPs can exhibit high levels of androgen steroids, but levels in effluents and surface water are usually several orders of magnitude lower.

Some of these compounds exhibit relatively low polarity; therefore, sorption to a solid phase is the expected behaviour. Human medications, including synthetic hormones, can enter the soil mainly through sewage sludge and spread into agricultural fields