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Trace analysis of androgens and progestogens in environmental waters by ultra-performance liquid chromatography–electrospray tandem mass spectrometry

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ABSTRACT

A sensitive ultra-performance liquid chromatography–electrospray tandem mass spectrometry method, combined with solid-phase extraction and silica cartridge cleanup, was established for nine androgens (androstenedione, 19-nor-4-androstene-3,17-diol, androsterone, epiandrosterone, testosterone, methyltestosterone, trenbolone, nandrolone, stanozolol) and nine progestogens (progesterone, 17α-hydroxyprogesterone, 21α-hydroxyprogesterone, 6α-methyl-hydroxyprogesterone, 17α,20β-dihydroxy-4-pregnene-3-one, megestrol acetate, norethindrone, norgestrel, medroxyprogesterone acetate) in environmental waters. For the various water matrices considered, the overall method recoveries were from 78 to 100%, and no apparent signal suppression was found. The method detection limits for the eighteen analytes in the influent, effluent and surface water samples were 0.20–50, 0.04–20 and 0.01–12 ng/L, respectively. This method was used to analyze the residual androgens and progestogens in the wastewater and surface water samples from Japan, and ten analytes (0.03 (medroxyprogesterone acetate)–1441 ng/L (androsterone)) were detected in the wastewater samples, and four analytes (0.06 (progesterone)–0.46 ng/L (androstenedione)) were detected in the surface water samples.

1. Introduction

Considerable attention had been focused on the occurrence of estrogenic steroid hormones in the environment since an initial report showed that the exposure of fish to municipal wastewater effluents resulted in the feminization of fish at concentrations as low as 1 ng/L [1]. Recent studies have documented the masculinization of fish after their exposure to androgens at similarly low concentrations [2,3], and numerous progestogens, along with certain androgens as hormonal odorants and reproductive pheromones have also been shown to affect the reproductive physiology and behavior in many fish species at ng/L or even pg/L levels [4–6]. Therefore, the presence of androgens and progestogens in the environment should deserve greater attention.

A broad number of natural and synthetic androgens and progestogens have been used in human and veterinary therapy, or as growth promoters, and they can be discharged into the aqueous environment via sewage treatment plants (STPs). Therefore, there has been a need for developing a sensitive and reliable method to analyze the broad number of these compounds in various water matrices in order to assess their environmental risk. Gas chromatography–mass or tandem mass spectrometry [GC–MS(/MS)] has been used to analyze two androgens and one or two progestogens in the wastewater effluents [7] or surface water samples [8] after their derivatization. However, the sample derivatization for the wide range of androgens and progestogens proved complicated. Not all androgens and progestogens, such as stanozolol (an androgen), were able to be derivatized [9]. LC–MS(/MS) is an alternative method due to its sensitivity and specificity, without any need for derivatization, and it has been used to analyze androgens [10] or progestogens [11,12] in wastewater and surface water samples. However, both GC–MS(/MS) and LC–MS(/MS) methods were all targeted for less than four progestogens or five androgens. In addition, although LC–MS(/MS) has been viewed as a potential method for analyzing a broad range of these compounds, the matrix interference has proven to be a general problem even for the LC–MS/MS system, as exemplified by the signal suppression of progesterone (up to 38%) in the surface water samples [13].

In this study, we developed a sensitive and specific method for simultaneously analyzing nine androgens and nine progestogens in wastewater using solid-phase extraction (SPE) and ultra-

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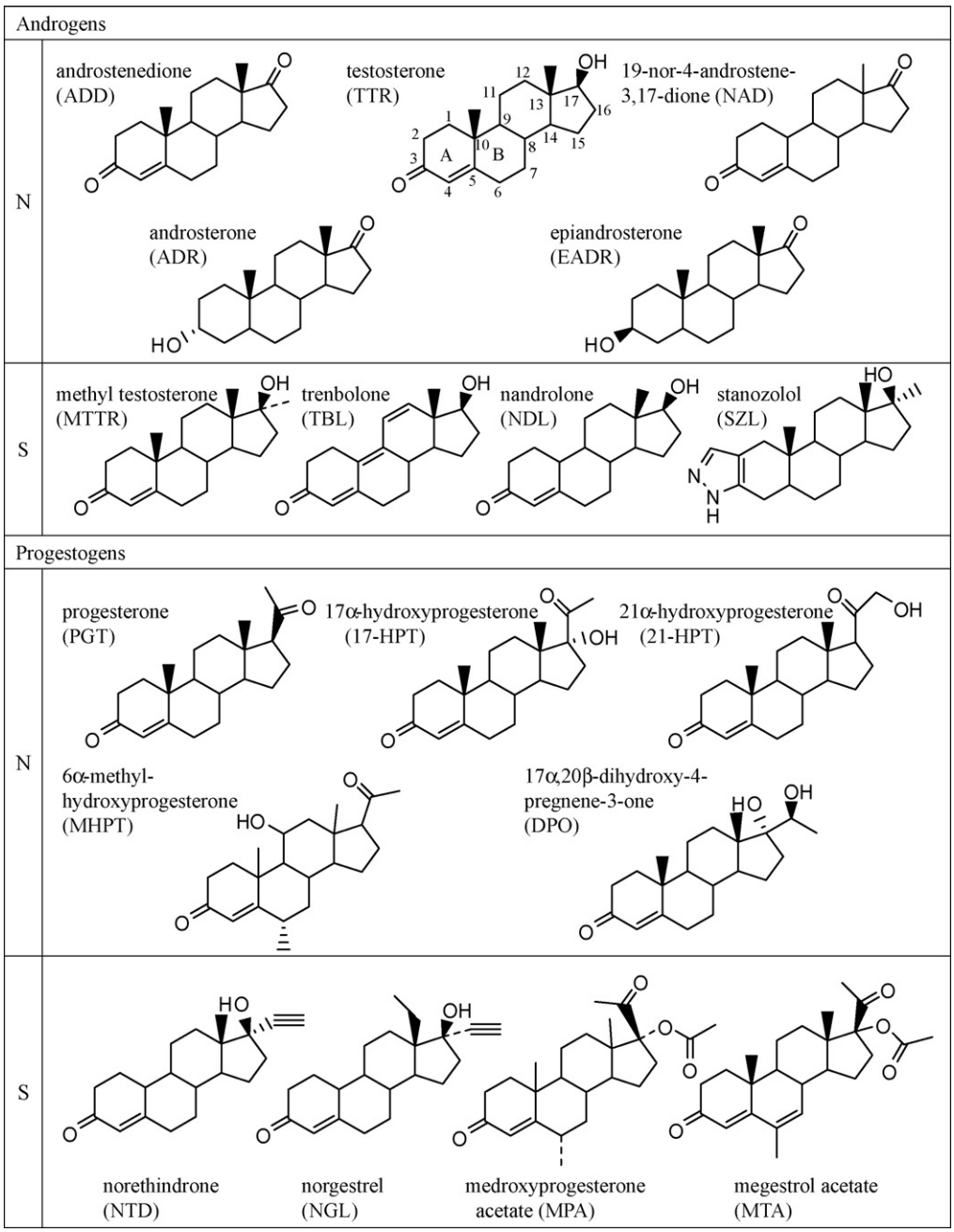

Fig. 1. Structure of target androgens and progestogens. N: natural steroid; S: synthetic steroid.

performance (UP)-LC–MS/MS analysis, where a silica cartridge was used in the sample cleanup. The target sex hormones (Fig. 1) were chosen from natural and synthetic androgens and progestogens which have been detected [7–13] or could be potentially present in the environment. Finally, this developed method was applied to the analysis of these compounds in wastewater and surface water samples.

2. Experimental

2.1. Materials

19-Nor-4-androstene-3,17-diol (NAD), trenbolone (TBL), nandrolone (NDL), androstenedione (ADD), norethindrone (NTD), 17α-hydroxyprogesterone (17-HPT), testosterone (TTR), 21α-hydroxyprogesterone (21-HPT), norgestrel (NGT), 17α,20β-dihydroxy-4-progegnen-3-one (DPO), methyltestosterone (MTTR), epiandrosterone (EADR), stanozolol (SZL), 6α-methylhydroxyprogesterone (MHPT), megestrol acetate (MTA), medroxyprogesterone acetate (MPA), progesterone (PGT), androsterone (ADR), [$^{13}C_2$]ethynyl-NTD, [$^{13}C_2$]TTR, [$^{2}H_6$]NGT (NGT-d_6) and [$^{2}H_9$]PGT (PGT-d_9) were purchased from Sigma (St Louis, MO, USA). Formic and acetic acids were of analytical grade (Wako, Saitama, Japan). Methanol, acetonitrile, ethyl acetate, hexane, and dichloromethane were all of HPLC grade purchased from Fisher Chemical (Japan). HPLC-grade water was prepared using a Milli-Q RC apparatus (Millipore, Bedford, MA, USA).