STEROID ANALYSIS IN ENVIRONMENTAL WATER SAMPLES

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Introduction

The EDMAR Research Programme has been set up to investigate whether there is evidence of changes in the reproductive health of marine life and if so, seek to identify possible causes and assess the potential impacts on populations.

Certain steroids are known endocrine disrupting chemicals. They are the natural hormones produced and excreted by humans, and synthetic hormones, which are the active ingredients of the contraceptive pill.

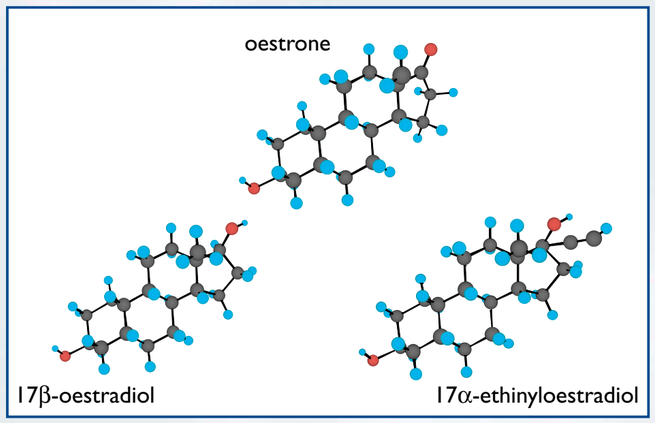
A sewage treatment works

These compounds can enter the aquatic environment via wastewater discharge from sewage treatment works.

Steroids in these discharges have been implicated in disrupting the endocrine systems of fish, causing male fish to exhibit female characteristics.

Previous work established that the oestrogenic activity found in predominately domestic sewage effluents could be attributed solely to the presence of three oestrogens.

These were the naturally occurring steroids, oestrone and 17β-oestradiol, and the synthetic contraceptive steroid 17α-ethinyloestradiol.



3D structures - oestrone, 17β-oestradiol and 17α-ethinyloestradiol

Method

Water samples are collected in silanised glass bottles in order to prevent adsorption of steroids to the inner glass surfaces.

These samples are extracted using C_{18} Solid Phase Extraction disks with depth filter.

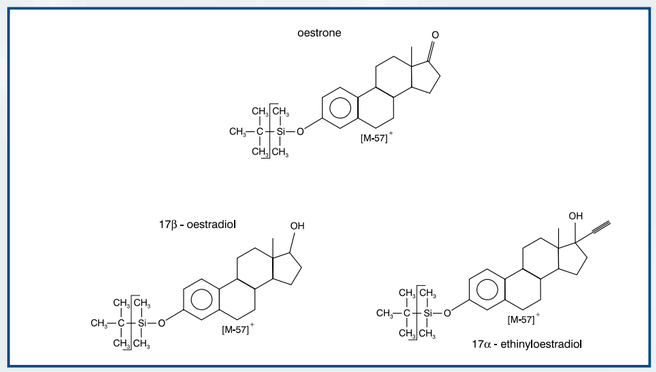
The experimental set-up for disk extraction manifold

The steroids are eluted from the C_{18} phase with 85% methanol in water.

The extracts are dried and reacted to form *tert*-butyldimethylsilyl derivatives.

The samples are analysed by GC-MS-MS using a ThermoQuest ion trap instrument.

Steroid analysis by Gas Chromatography-Mass Spectrometry



The internal standards used are the isotopically labelled analogues of the steroids to be determined, [$^2H_4$] oestrone, [$^2H_4$] 17β-oestradiol and [$^2H_4$] 17α-ethinyloestradiol

The method was validated using samples of river water spiked with 10 ng l^{-1} of each steroid. Five replicates were processed using C_{18} disks, and the results obtained are presented in Table 1.

Table 1. Concentrations of steroids determined and spiked values from the analysis of river water – results in ng l^{-1}

Sample	oestrone	17β-oestradiol	17α-ethinyloestradiol
Spiked concentration	12	10.8	13
Recovery	102 %	89 %	108 %
Mean	12.22	9.66	14.04
Standard deviation	0.661	0.498	0.713
Coefficient of variation	5.40 %	5.15 %	5.07 %

The concentrations of the three steroids in the procedural blanks were < 0.2 ng l^{-1} which allowed an operational LOD of 0.6 ng l^{-1}. The extractions are reproducible with CVs between 5.07 and 5.40 %.

Use of Method

This method has been employed to analyse samples of drinking water, reservoir water, treated wastewater and river water.

Wastewater samples from a mainly domestic treatment works were analysed to investigate the amount of oestrogens actually entering the environment , analysis of 30 samples demonstrated that the concentration ranges for the three oestrogens were;

oestrone 10 to 55 ng l^{-1},
17β-oestradiol 2 to 48 ng l^{-1},
and 17α-ethinyloestradiol <1 to 4 ng l^{-1}.

Dabholme Gut – on the River Tees

The EDMAR study involves analysis for the 3 oestrogens in estuarine waters. Samples have been collected from the River Tees, and the results obtained are shown in Table 2.

Table 2. Concentrations of steroids determined in samples from the River Tees – results in ng l^{-1}

Sample	oestrone	17β-oestradiol	17α-ethinyloestradiol
Dabholme Gut	5	2.6	2
Downstream Dabholme Gut	<0.6	<0.6	<0.6
Upstream Dabholme Gut	<0.6	<0.6	<0.6

The ability to link effects observed due to the presence of oestrogenic steroid compounds in the marine environment with concentrations of the causal compounds is essential to the EDMAR Programme and will help to ensure that any actions necessary to protect the marine environment are targeted appropriately.

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